RESEARCH ARTICLES

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Systematics of the olive thrush *Turdus olivaceus* species complex with reference to the taxonomic status of the endangered Taita thrush *T. helleri*

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During the last 40 years, few species of African birds have undergone more taxonomic revision than the olive thrush *Turdus olivaceus.* This is due to disagreement on how to partition the striking phenotypic variation among allopatric populations. The current consensus is to recognise one species *T. olivaceus*, split into three assemblages: (1) the *olivaceus* group restricted to southern Africa, (2) the *swynnertoni* group of the Zimbabwean and southern Malawi highlands, and (3) the *abyssinicus* group of the montane highlands of eastern and central Africa. Mitochondrial DNA sequences from 63 individuals were analysed to investigate the phylogenetic relationships among 16 taxa (species and subspecies) in the *olivaceus* species complex (plus seven outgroup species), with, particular emphasis on the relationships and taxonomic status of the endangered Taita thrush (*helleri*). Phylogenetic hypotheses generated using parsimony, maximum likelihood, and Bayesian inference identified a number of discrete clades corresponding to recognised subspecies. Northern (*abyssinicus* clade) and southern populations (*olivaceus*+*swynnertoni* clade) of olive thrush differ by 9–10% in sequence divergence. Furthermore, all analytical methods suggested that *helleri* (Taita Hills) and *roehli* (Usambara and Pare Mountains) are reciprocally monophyletic with respect to mtDNA, and 2.5 to 10.5% divergent from all other forms of olive thrush. Both *helleri* and *roehli* are surrounded in adjacent highlands by populations of olive thrush that represent a more recent radiation, suggesting that *helleri* and *roehli* may be relict taxa which have been able to maintain their genetic integrity. The results of this study support previous arguments for recognizing the arid/woodland *T. smithi* as a species distinct from other southern African forest populations of *T. olivaceus* (including the *swynnertoni* group). Results further suggest that *T. abyssinicus*, *T. helleri*, and *T. roehli* be accorded species rank.

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Since the 1960s confusion has characterized the systematics of African thrushes in the genus *Turdus*. In particular, widespread disagreement has centred on the taxonomic status and species boundaries of the *Turdus olivaceus* species complex. Thrushes within this complex are characterised by having grey or olive-grey backs, underparts with varying amounts of matt orange, olive-grey and white, and streaking on the throat. Ripley (1964) recognised two species, the northern olive thrush (*abyssinicus*) and the southern olive thrush (*olivaceus*). Hall and Moreau (1970) retained Ripley's subspecific taxa, but lumped southern and northern olive thrush into one form (*olivaceus*), and reclassified some subspecies within the African thrush (*pelios*) (Table 1). Hall and Moreau (1970) further included *olivaceus* and *pelios* in a superspecies with the Kurricane thrush (*libonyanus*), bare-eyed thrush (*tephronotus*), the island-dwelling forms of Comoro (*bewsheri*) and Gulf of Guinea thrushes (*olivaceofuscus*) (Fig. 1).

Based on the discovery that *pelios* has a continuous type of song with a short repetitive motif, quite unlike the brief rhythmical phrases of *olivaceus*, Dowsett and Dowsett-Lemaire (1980) accorded *pelios* species rank. Further evidence supporting this suggestion is that *pelios* is confined to lowland evergreen forest (except on Mount Cameroon), whereas *olivaceus* occupies montane (high-altitude) rainforest in the northern portions of it's range and only inhabits drier country in western South Africa were *pelios* does not occur (Keith and Urban 1992, Dowsett and Dowsett-Lemaire 1993, Keith et al. 1997, Clement and Hathway 2000).

Recent taxonomic treatments of African thrushes (Sibley and Monroe 1990, Keith and Urban 1992, Dowsett and Dowsett-Lemaire 1993, Keith et al. 1997, Clement and Hathway 2000), have generally adopted the species boundaries suggested by Hall and Moreau (1970) for *olivaceus* and *pelios* (Table 1). The only major exceptions are that modern studies consider West African *nigrilorum* and *poensis* to be part of *pelios* and not *olivaceus* (Keith and Urban 1992, Keith et al. 1997, Clement and Hathway 2000; Table 1), and the central African races *bocagei* and *stromsi* are considered part of *pelios* by Keith et al. (1997), although not by Clement and Hathway (2000), who retain them in *olivaceus*.

Hall and Moreau (1970) used plumage characters to divide *Turdus olivaceus* populations into three assemblages: (1) the '*olivaceus*' group in South Africa, characterized by underparts that are rich in orange with a slight olive-grey wash on the breast, and a white throat with well marked streaking, they include *smithi*, which extends into the drier parts of central and western South Africa, and has grey underparts and a dusky throat, (2) the '*swynnertoni*' group extending from Zimbabwe to southern Malawi, which are similar to '*olivaceus*', but are smaller and have a darker bill, and (3) the '*abyssinicus*' group extending from northern Malawi through the Albertine Rift, Eastern Arc Mountains and the highlands of Kenyan and Ethiopia, characterized by having an olive-grey throat and breast (not white) with the rest of the underparts orange with some white around the vent.

Table 1. Two of the alternative classification schemes adopted for *Turdus olivaceus*: from 'Atlas of speciation in African passerine birds' (Hall and Moreau 1970) and 'Birds of Africa' volume V (Keith et al. 1997). * Indicate taxa sampled in this study.

Hall and Moreau (1970)			Keith et al. (1997)		
Species	Group	Subspecies	Species	Group	Subspecies
olivaceus	*olivaceus*	**olivaceus*	*olivaceus*	*olivaceus*	**olivaceus*
		**pondoensis*			**pondoensis*
					**transvaalensis*
					culminans
		**smithi*			**smithi*
	swynnertoni	*swynnertoni*		*swynnertoni*	*swynnertoni*
		**milanjensis*			**milanjensis*
	abyssinicus	**abyssinicus*		*abyssinicus*	**abyssinicus*
		**nyikae*			**nyikae*
		**roehli*			**roehli*
		**helleri*			**helleri*
		**bambuscicola*			**bambuscicola*
		oldeani			*oldeani*
		deckeni			*deckeni*
		**baraka*			**baraka*
		ludoviciae			*ludoviciae*
		bocagei	*pelios*		*bocagei*
		nigrilorum			*nigrilorum*
		poensis			*poensis*
pelios		*stormsi*			*stormsi*
		graueri			*graueri*
		**saturatus*			**saturatus*
		chiguancoides			*chiguancoides*
					**centralis*
		pelios			*pelios*

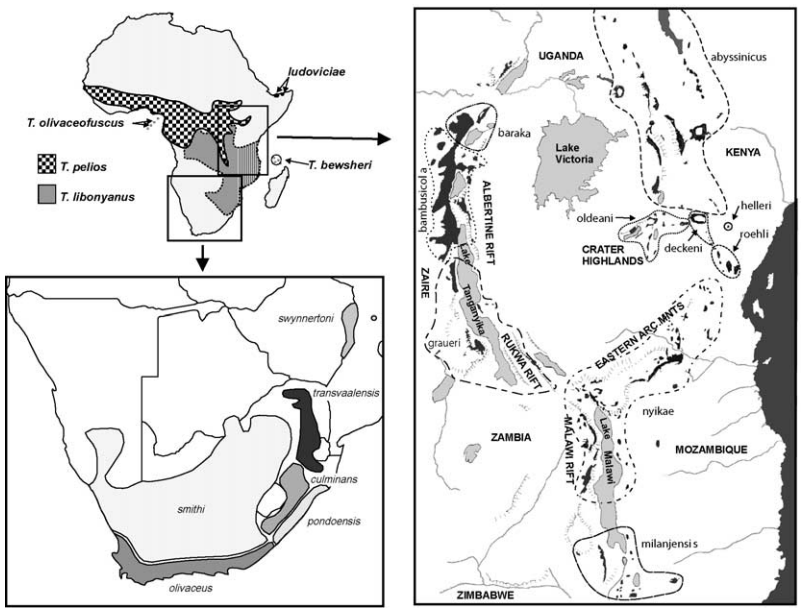

Fig. 1. Distribution ranges of most of the taxa in the olive thrush *Turdus olivaceus* species complex (ranges of the bare-eyed thrush *T. tephronotus* of arid Eastern Africa and the races *T. o. stromsi* and *bocagi* of Angola and central Africa are not depicted).

Although most authors accept the taxonomic recommendations of Hall and Moreau (1970; Table 1) in a broad sense, much debate has centred on the taxonomic status of thrush populations occupying the mountains on the Kenya-Tanzania border (Fig. 1). Most controversial is the Taita thrush (*helleri*), classified as a critically endangered taxon by BirdLife International (Collar et al. 1994, Birdlife International 2000). After the Somali blackbird (*ludoviciae*), *helleri* is phenotypically the most distinctive taxon among the montane forms of the '*abyssinicus*' group of *olivaceus*, with a blackish head, dark grey breast, white belly, and orange flanks. Hall and Moreau (1970), Sibley and Monroe (1990), Keith and Urban (1992), Dowsett and Dowsett-Lemaire (1993), Keith et al. (1997), and Clement and Hathway (2000), all recognise *helleri* as subspecifically distinct, but include it within *olivaceus*. This is due to a clinal taxon *roehli*, in the Pare and Usambara Mountains in northern Tanzania (Fig. 1), which by being duller and more olive than *helleri*, forms a 'clear link' (Keith and Urban 1992, Dowsett and Dowsett-Lemaire 1993) between *helleri* and other neighbouring populations of the '*abyssinicus*' group of *olivaceus*. Keith and Urban (1992) regard *helleri* and *roehli* as dull-coloured members of *olivaceus*, noting that all have orange underwing-coverts.

Collar and Stuart (1985), Collar et al. (1994), Brooks et al. (1998a,b), Bennun and Njoroge (1999), Zimmerman et al. (1999), Galbusera et al. (2000), BirdLife International (2000), and Stevenson and Fanshawe (2002) have persisted with recognizing *helleri* as a full species based on its distinct morphology. Whereas this view may have been influenced by the need for conservation attention due to the isolated distribution of *helleri* within the Taita Hills, it highlights the need for objective criteria on which to base taxonomic decisions. We therefore investigated the phylogenetic relationships of 16 taxa within the *olivaceus* species complex in an attempt to resolve some of the controversy surrounding species boundaries, particularly with respect to the endangered Taita thrush (*helleri*). Nucleotide sequence data from the NADH dehydrogenase subunit 2 (ND2) and subunit 3 genes (ND3) of the mitochondrial genome are used to help ascertain the phylogenetic relationships among taxa included in this study.

Methods

Collection of samples

In total 70 individuals from 23 taxa and 45 populations were analysed in this study (Appendix 1). Political and permitting issues precluded the collection of samples from the southern Albertine and Rukwa Rift areas, the crater highlands in Tanzania, and Zimbabwe. The entire *T. olivaceus* complex was rooted on the song thrush *T. philomelos* a broadly distributed Old World species (introduced to Australia and New Zealand) which forms one of the basal branches within *Turdus* (Voelker, Bowie and Rohwer unpublished data). Other outgroup taxa included were: *T. hortulorum*, *T. ruficollis*, *T. iliacus*, *T. grayi*, *T. plebejus* and *T. infuscatus*.

Laboratory procedures

The same methods of DNA extraction and PCR amplification of the mitochondrial NADH subunit 3 gene with flanking tRNAs (Glycine and Argenine) as described in Bowie et al. (2003) were employed. ND2 was PCR amplified using the same cycling conditions as in Bowie et al. (2003), with primers L5204 and H6312 (Cicero and Johnson 2001). Internal primers Turd-ND2L1 5′-CTGGCTTTCTCATCCATCTC and Turd-ND2H1 5′-AGGTGGGAGATGGATGAG were designed to facilitate double-stranded sequencing of the entire ND2 gene. PCR products were electrophoresed on 1.5% low-melting point agarose gels (FMC Bioproducts), stained with ethidium bromide and visualized under UV light. Amplicons of the appropriate length were cut out of the gel and purified using GELase™ (Epicentre Technologies, Madison, Wisconsin). The purified products were cycle-sequenced using Big Dye terminator chemistry (Applied Biosystems, Inc [ABI]), precipitated with 3M ammonium acetate or 100% isopropanol, rinsed in ethanol, dried and resuspended in formamide-EDTA solution, and run on an ABI 3100 automated DNA sequencer. Sequences were obtained from both strands of DNA for each individual, and some individuals were sequenced several times if any base ambiguity was encountered. All sequences were checked using the program Sequencher 3.0 (Gene Codes Corp) and aligned to the chicken *Gallus gallus* mtDNA sequence (Desjardins and Morais 1990) to test for the presence of any insertions or deletions, as well as to check that no stop-codons were present. All haplotypes from the *Turdus olivaceus* complex have been submitted to GenBank (accession numbers: NADH3 AY251556-AY251572 and DQ081016-DQ081062; NADH2 DQ080997-DQ081015).

Phylogenetic analyses

The heterogeneity of different gene regions was assessed using the incongruence length difference test (ILD test, Farris et al. 1995), as implemented in PAUP*. Invariant sites were removed, as suggested by Cunningham (1997), and 1,000 replicates, with five iterations per replicate were used to test whether the ND2 and ND3 data partitions were in significant conflict with each other (but see Yoder et al. 2001, Barker and Lutzoni 2002). We find that the ILD test is a good heuristic tool to assess whether there are problems with individual data sets or sequences rather than to assess phylogenetic conflict between gene regions.

Parsimony analyses (MP) were conducted in PAUP*4.0b10 (Swofford 2002) using a heuristic search, implementing stepwise addition with 10,000 random addition replicates, and TBR branch-swapping. Because transitions often accumulate at higher rates than transitions, MP analyses were conducted under several heuristic and empirical third position transition:transversion (ts:tv) weighting schemes, with first and second positions being weighted the same as third position transversions. In addition, codon-specific weighting schemes for ND2 and ND3 (when combined referred to here as 6-parameter MP, when separate as 3-parameter MP) were also implemented to evaluate the sensitivity of tree topology to saturation. Clade support was estimated using the non-parametric bootstrap (Felsenstein 1985) with 1,000 replicates, with each replicate containing five random addition replicates. Maximum likelihood analyses (ML) were conducted in PAUP*4.0b10 using a full heuristic search with 1,000 random addition replicates and TBR branch-swapping. Clade support was estimated using 250 bootstrap replicates.

MrBayes 3.0 (Ronquist and Huelsenbeck 2003) was used to conduct a Bayesian approach (BI) to phylogenetic inference. Four Metropolis-coupled MCMC chains (one cold and three heated chains) were run simultaneously to optimise efforts to find peaks in tree-space. To check that the log-likelihood distribution had become stationary, the fluctuating value of the log-likelihood was plotted. This search strategy was repeated three times (four runs of 2 million generations), with each run beginning from a random tree. The General-time-reversible model of nucleotide substitution with gamma and proportion of invariable sites ($GTR+I+\Gamma$) was used in the Bayesian analyses. A Dirichlet distribution was assumed for estimation of the base frequency parameters, and an uninformative (flat) prior was used for the topology. Trees were sampled every 1,000 generations, resulting in a sample of 2,001 trees per run. The number of cycles to discard (the burnin) was estimated empirically from the log-likelihood plots. Swapping among chains, and acceptance of proposed changes to model parameters were monitored to ensure that efficient mixing had occurred.

Results

Sequence variation

In ND2/ND3 (1,041 bp/351 bp), 383/130 sites (36.8%/37.0%) were variable and 255/82 (24.5%/23.4%) were parsimony informative, respectively. Three of the 32 tRNA bases flanking ND3 were parsimony informative. For ND2 and ND3 combined (1,392 bp), 62 first position sites (55.8% of the variable sites), 29 second position sites (56.9%) and 246 third position sites (70.1%), were parsimony informative. Four (12.5%) sites were variable among the 32 bp of tRNA sequenced.

The outgroup *Turdus philomelos* differed from other *Turdus* taxa by 15.8–18.3% sequence divergence (Table 2). Within the *olivaceus* complex (see Fig. 2), *bewsheri+libonyanus*, and *pelios* were most divergent

Table 2. Percentage pairwise sequence divergence (Kimura 2-parameter) between taxa within the *Turdus olivaceus* complex (1–16) and other thrushes (17–23). Taxa 1 to 5 represent members of the *abyssinicus* group, 6 to 9 the *olivaceus* group and 10 the *sywnnertoni* group of the olive thrush. Taxon reference numbers correspond to numbers listed in Appendix 1. Divergence estimates within each of these groups are highlighted in bold.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
abyssinicus #1 (1)																						
baraka #1 (2)	**0.8**																					
nyikae ZMUC #2 (3)	**2.7**	**2.1**																				
roehli #4 (4)	**3.9**	**3.1**	**4.2**																			
helleri #1 (5)	**3.2**	**2.5**	**3.6**	**3.9**																		
olivaceous #1 (6)	10.4	9.6	10.4	10.0	9.9																	
transvaalensis #1 (7)	10.3	9.5	10.4	10.3	9.8	**0.5**																
pondoensis #1 (8)	10.1	9.4	10.2	10.2	9.5	**0.6**	**0.3**															
smithi #1 (9)	10.2	9.6	10.3	9.8	9.8	**3.6**	**3.5**	**3.4**														
milanjensis #1 (10)	10.1	9.3	10.4	9.7	9.4	1.6	1.6	1.6	3.4													
olivaceofuscus #2 (11)	10.0	9.4	10.0	10.0	9.4	8.5	8.7	8.6	8.3	8.4												
pelios centralis #1 (12)	9.8	9.7	10.5	10.0	10.0	11.7	11.9	11.8	11.3	11.2	8.8											
pelios saturatus #1 (13)	9.5	9.3	10.1	9.7	9.5	11.6	11.6	11.7	11.3	11.4	9.1	4.6										
bewsheri #1 (14)	9.7	9.6	10.4	9.9	9.4	10.7	11.1	10.9	10.5	10.6	10.1	10.7	10.7									
bewsheri #2 (15)	9.1	9.0	9.8	9.3	8.8	10.6	11.0	10.7	10.8	10.7	9.4	9.8	10.5	3.3								
libonyanus #1 (16)	8.9	8.8	9.9	9.2	9.2	10.2	10.7	10.5	10.1	10.5	9.9	10.9	10.1	6.0	5.4							
grayi (17)	10.7	10.4	11.1	10.8	10.8	10.7	10.7	10.7	11.2	10.7	9.8	10.9	10.8	11.7	11.0	10.8						
hortulorum (18)	11.2	11.1	12.3	11.6	11.1	11.7	11.9	11.7	11.2	11.4	11.7	12.1	12.5	10.6	10.2	10.2	11.7					
iliacus (19)	10.3	9.7	10.6	9.6	9.8	10.7	10.9	10.8	10.1	10.7	10.1	12.0	11.7	10.3	10.3	9.9	11.2	11.0				
infuscatus (20)	10.1	9.5	10.6	10.1	10.1	9.9	10.3	9.9	9.8	9.8	9.5	11.4	11.4	9.6	9.2	9.8	9.9	11.0	10.1			
plebejus (21)	10.7	10.1	10.3	9.8	10.0	10.2	10.5	10.4	9.9	10.2	10.5	11.8	11.1	10.2	10.3	10.3	9.7	11.1	9.9	9.7		
ruficollis (22)	13.6	13.2	13.9	13.4	13.5	13.2	13.3	13.1	12.4	13.1	13.8	14.8	14.2	13.0	12.5	12.6	11.5	12.4	12.5	12.7	12.0	
philomelas (23)	17.4	17.2	17.3	17.6	17.8	16.7	16.9	16.7	16.8	17.6	16.7	17.0	16.2	18.8	16.8	17.0	17.0	17.2	16.9	16.8	15.8	18.3

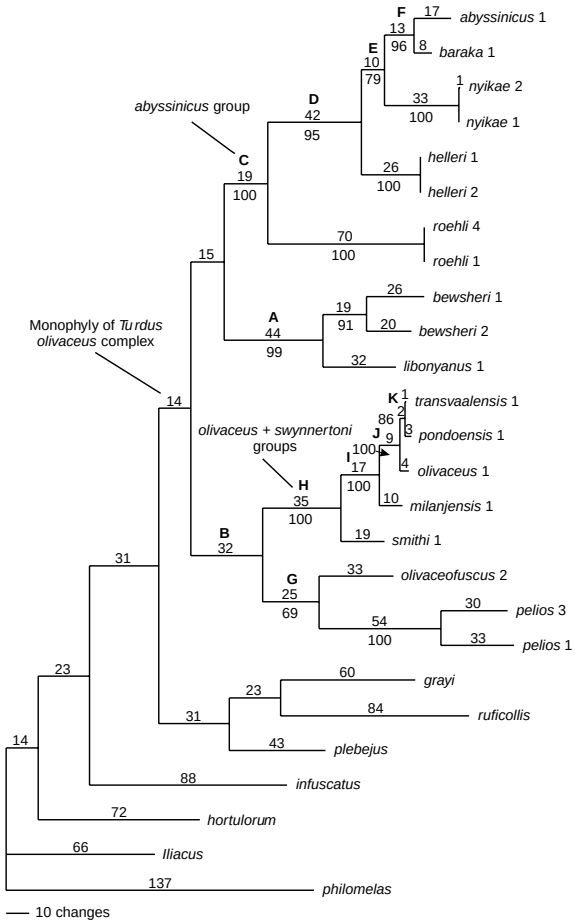

Fig. 2. Parsimony analyses of the combined dataset (1,424 bp) using a 10 ts:tv ratio recovered two trees (L = 4,980, CI = 0.587, RI = 0.647), one of which is presented. Values above the branches represent absolute number of changes along a branch (not weighted) and values below are bootstrap values from 1,000 replicates (5 random addition iterations per replicate). Note for members of the *Turdus olivaceus* complex we use subspecific names for uniformity. See Table 1 and text for additional details of traditional and proposed taxonomic changes.

from other members of the complex (8–11%). *Turdus olivaceofuscus* was well separated from all remaining members of the complex (8.4–10.1%), and approximately equidistant between the '*olivaceus*' clade and *pelios*. Both the '*olivaceus*' and '*abyssinicus*' clades were 9.5–10.4% divergent from each other and 9.3–11.9% divergent from *pelios*. Sequence divergence within the '*abyssinicus*' clade varied. There was little variation between *abyssinicus*, *baraka* and *bambuscicola*, but divergence was relatively large (2.5–3.9%) between *helleri* and other members of the clade. Furthermore, *roehli* was 3.1–4.2% divergent from other members of the '*abyssinicus*' clade, and *nyikae* differed by 2.1–4.2%. Within the '*olivaceus*' clade, little variation existed between *olivaceus*, *pondoensis* and *transvaalensis*. However, *smithi* was comparatively relatively distinct (ca. 3.5%) from other members of the '*olivaceus*' clade. The '*swynnertoni*' group, represented here by *milanjensis* (see Table 1), was 9.3–10.4% divergent from the '*abyssinicus*' clade, but 1.6–3.4% divergent from other members of the '*olivaceus*' clade.

Phylogenetic analyses

Parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted on a 26 taxon dataset, representing all sampled taxa except for *bambuscicola* which was identical to *baraka*. All data were combined (1,424 bp) due to a non-significant (P = 0.304) ILD test between the ND2 and ND3 data partitions. However, to gain a perspective on the relative contribution of the different gene regions, parsimony analyses were also conducted for each gene region independently (Table 3).

Saturation plots for both ND2 and ND3 (not shown) indicated that only third position transitions showed the levelling off associated with saturation. This occurred by the 10% Kimura-2-parameter distance estimate. To take into account apparent saturation of third position transitions, step matrices were constructed that down-weighted third position transitions over transversions by varying degrees (empirical estimate = 10.7 ts:tv ratio, Table 3). Weighting transitions down relative to transversions resulted in a topology (Fig. 2) with a greater number of nodes resolved relative to equal weights, higher bootstrap values, and was congruent to a greater degree with the topologies obtained using model based methods (ML and BI, Fig. 3, Table 3). Six parameter parsimony analyses were also conducted where a ts:tv ratio was determined for each codon position for each gene region (ND2pos1 8.3:1; ND2pos2 32.5:1; ND2pos3 13.1:1; ND3pos1 12.1:1; ND3pos2 2.7:1 and ND3pos3 9.1:1), but analyzed simultaneously. The topologies obtained were similar to the topology obtained under a 10:1 ts:tv ratio when applied to only third position transitions (Fig. 2), however the codon-specific topology differs in that *bewsheri* and *libonyanus* form a clade which is sister to the remainder of the ingroup (although with < 50% bootstrap support). Incorporating a codon-specific approach did not consistently increase bootstrap support at nodes, rather nodes that were well supported in all analyses (MP, ML and BI) remained well supported, whereas nodes poorly supported in the ML and BI analyses, but well supported in earlier MP analyses, tended to be less well supported in the codon-specific MP analyses (Table 3).

The dataset was analysed using Modeltest 3.06 (Posada and Crandall 1998) to help ascertain which of 56 nucleotide substitution models best described the dataset. Using log-likelihood ratio tests a TrN model of

Table 3. Summary of topological arrangements resulting from combined and partitioned analyses of the two mtDNA gene fragments. Nodes refer to those labelled in Fig. 2 (nodes A to K) and Fig. 3. M_{phy} designates monophyly of the *Turdus olivaceus* complex (see Fig. 2). "X" indicates that the node was not recovered, "√" indicates the presence of a node and the values above represent the degree of support. The percentage of times a node was obtained and the percentage of times the node received significant support (>70% bootstrap; >0.95 posterior probability) are also indicated. # Designates the number of trees recovered (MP and ML) or sampled (BI).

	Node				M_{phy}	A	B	C	D	E	F	G	H	I	J	K
Gene	Analyses	#	CI	RI												
All data	MP 10 ts:tv	2	.587	.647	√ <50	√ 99	√ <50	√ 100	√ 95	√ 79	√ 96	√ 69	√ 100	√ 100	√ 100	√ 86
(1,424 bp)	MP 0 ts:tv	1	.505	.618	X	√ 100	X	√ 100	√ 77	√ 94	√ 98	X	√ 100	√ 100	√ 100	√ 68
	MP 2 ts:tv	1	.533	.626	X	√ 100	X	√ 100	√ 90	√ 95	√ 99	X	√ 100	√ 100	√ 100	√ 82
	MP 5 ts:tv	1	.578	.649	√ <50	√ 100	√ <50	√ 100	√ 88	√ 95	√ 99	√ <50	√ 100	√ 100	√ 100	√ 82
	MP 50 ts:tv	1	.609	.658	X	√ 99	X	√ 99	√ 95	√ 79	√ 95	√ 69	√ 99	√ 98	√ 100	√ 86
	MP 6 para.	1	.678	.677	√ <50	√ 99	√ <50	√ 100	√ 72	√ 96	√ 98	√ <50	√ 100	√ 100	√ 100	√ 78
	ML	1			X	√ 99	√ <50	√ 100	X	√ 94	√ 98	√ <50	√ 99	√ 96	√ 98	√ 70
	BI	5700			X	√ 1.0	X	√ 1.0	√ .61	√ 1.0	√ 1.0	√ .71	√ 1.0	√ 1.0	√ 1.0	√ .99
	% Obtained				3	8	4	8	7	8	8	6	8	8	8	8
	% Supported				0	100	0	100	75	100	100	0	100	100	100	100
ND2 only	MP 10 ts:tv	3	.577	.638	X	√ 92	√ <50	√ 99	√ 80	√ 79	√ 80	√ 58	√ 99	√ 97	√ 93	√ 85
(1,041 bp)	MP 0 ts:tv	4	.491	.615	X	√ 96	X	√ 100	√ 69	√ 91	√ 91	X	√ 100	√ 100	√ 89	√ 65
	MP 2 ts:tv	1	.518	.620	X	√ 98	X	√ 100	√ 83	√ 89	√ 91	X	√ 100	√ 100	√ 92	√ 82
	MP 5 ts:tv	6	.555	.629	X	√ 96	√ <50	√ 100	√ 84	√ 82	√ 84	√ 58	√ 100	√ 100	√ 93	√ 85
	MP 50 ts:tv	3	.600	.648	X	√ 90	√ <50	√ 93	√ 80	√ 74	√ 77	√ 58	√ 94	√ 92	√ 94	√ 87
	MP 3 para.	2	.680	.681	X	√ 93	X	√ 94	√ 67	√ 92	√ 92	X	√ 100	√ 100	√ 91	√ 77
	% Obtained				0	6	3	6	6	6	6	3	6	6	6	6
	% Supported				0	100	0	100	67	100	100	0	100	100	100	83
ND3 only	MP 10 ts:tv	1	.655	.723	X	√ 93	X	√ 93	√ 70	√ 65	√ 79	X	√ 92	√ 83	√ 99	X
(351 bp)	MP 0 ts:tv	7	.580	.670	X	√ 94	X	√ 95	√ <50	√ <50	√ 77	X	√ 98	√ 89	√ 99	X
	MP 2 ts:tv	11	.601	.681	X	√ 97	X	√ 96	√ 60	√ 57	√ 79	X	√ 97	√ 88	√ 100	X
	MP 5 ts:tv	1	.635	.707	X	√ 96	X	√ 94	√ 70	√ 65	√ 82	X	√ 95	√ 85	√ 99	X
	MP 50 ts:tv	1	.676	.740	X	√ 91	X	√ 91	√ 71	√ 67	√ 79	X	√ 89	√ 84	√ 100	X
	MP 3 para.	5	.739	.756	X	√ 94	X	√ 97	X	√ 67	√ 81	X	√ 99	√ 90	√ 100	X
	% Obtained				0	6	0	6	5	6	6	0	6	6	6	0
	% Supported				0	100	0	100	20	0	100	0	100	100	100	0

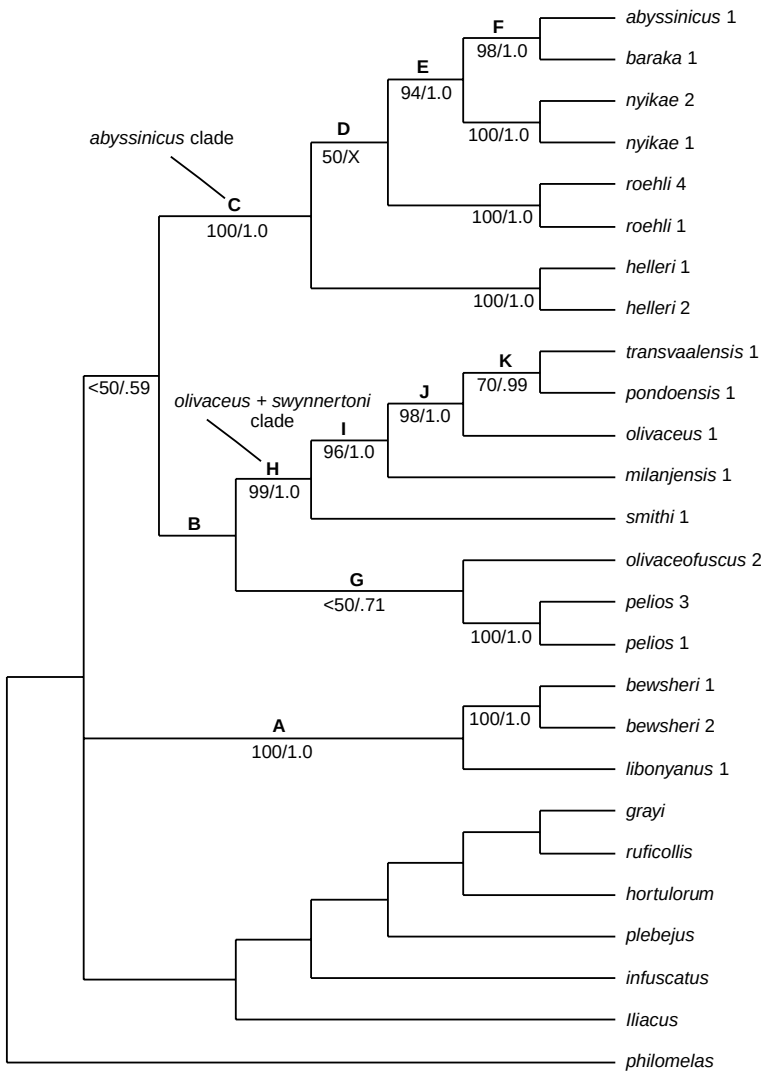

Fig. 3. Maximum likelihood analyses using the combined dataset (1,424 bp) and a TrN+Γ model of nucleotide substitution recovered one tree of length –ln7153.31. Bootstrap support values from 250 replicates are indicated below branches alongside posterior probabilities for specific nodes (splits) calculated from a posterior distribution of 5,700 trees (see text for details).

nucleotide substitution with gamma shape parameter (Γ) was selected, and then incorporated into PAUP*. Parameters for the model were as follows: base frequencies, A = 0.295, C = 0.369, G = 0.119 and T = 0.217; rate matrix, [A–C] = 1.0, [A–G] = 36.9, [A–T] = 1.0, [C–G] = 1.0, [C–T] = 18.6 and [G–T] = 1.0; gamma shape parameter = 0.223. During each run the Markov chains reached a stable negative log-likelihood score after ca. 25,000 generations. Being conservative with post-burnin estimates, only trees sampled after 100,000 generations (n = 1,900 trees per run) were used in determining the posterior probabilities of the model parameters, branch lengths and splits (nodes) within the tree. The marginal probabilities of the BI rate matrix were, [A–C] = 1.2, [A–G] = 41.6, [A–T] = 1.2, [C–G] = 1.0, [C–T] = 21.8 and [G–T] = 1.0. The marginal probabilities of the nucleotide frequencies were A = 0.295, C = 0.366, G = 0.123 and T = 0.216, the marginal probability of the proportion of invariable sites was 0.450, and the shape of the gamma parameter was 1.381.

Monophyly of the *olivaceus* complex was almost never recovered (Table 3), usually due to *bewsheri* and *libonyanus* falling among an unresolved polytomy with the outgroup *Turdus* species sampled in this study (Fig. 3). However, in all analyses, a sister relationship between *bewsheri* and *libonyanus* was strongly supported (node A, Figs 2 and 3, Table 3). The phylogenetic position of the other island member of the complex, *olivaceofuscus*, remains largely speculative, with this species sister to *pelios* in some analyses, but never with any degree of support (nodes B and G, Figs 2 and 3, Table 3). In all analyses the '*abyssinicus*' clade of the *olivaceus* complex was recovered as monophyletic with strong support (node C, Figs 2 and 3, Table 3). In MP analyses of the combined and partitioned datasets within

the '*abyssinicus*' clade *roehli* formed the basal branch with high support (node D, Figs 2 and 3, Table 3). In contrast, *helleri* formed the basal branch when the data were analysed using ML (Fig. 3 50% bootstrap), and although BI recovered *roehli* as basal (as in MP) this was with little statistical support (P = 0.61). Either *roehli* (MP and BI) or *helleri* (ML) were sister to a clade containing *nyikae*, *baraka*, *bambusicola* and *abyssinicus* (node E, Figs 2 and 3, Table 3) with *nyikae* always sister to, *abyssinicus*, *baraka* and *bambusicola* (node F, Figs 2 and 3, Table 3).

Although the '*abyssinicus*' group (Table 1) is here supported as a monophyletic clade, the '*olivaceus*' group is polyphyletic (node H, Figs 2 and 3, Table 3). The member of the '*swynnertoni*' group sampled, *milanjensis* from the southern Malawi Rift was sister to the South African forest forms of the '*olivaceus*' group (*olivaceus*, *pondoensis* and *transvaalensis*; nodes J and K, Figs 2 and 3, Table 3) to the exclusion of the arid *smithi*, which was the basal member of the clade encompassing the '*olivaceus* plus *swynnertoni*' groups (node H, Figs 2 and 3, Table 3).

Parsimony (MP) analyses of a larger ND3 dataset (70 individuals) resulted in 24 most parsimonious trees (10 ts:tv; length = 1,437, CI = 0.630, RI = 0.882, Fig. 4). Despite the smaller number of base pairs (383) the same well-supported clades are recovered as from the 26 taxon dataset (Figs 2 and 3, Table 3). Thus, more widespread sampling within taxa did not lead to the identification of any secondary contact or hybridization events among members within the '*abyssinicus*' or '*olivaceus*' clades of the olive thrush.

The main features of the above analyses are: (1) support for the monophyly of the '*abyssinicus*' group, with thrushes from (a) the Taita Hills (*helleri*), (b) the Usambara Mountains (*roehli*), (c) the southern and central Eastern Arc Mountains (*nyikae*), and (d) the Kenyan Highlands (*abyssinicus*) and the northern Albertine rift (*baraka* and *bambusicola*) each forming reciprocally monophyletic clades, (2) the polyphyly of the

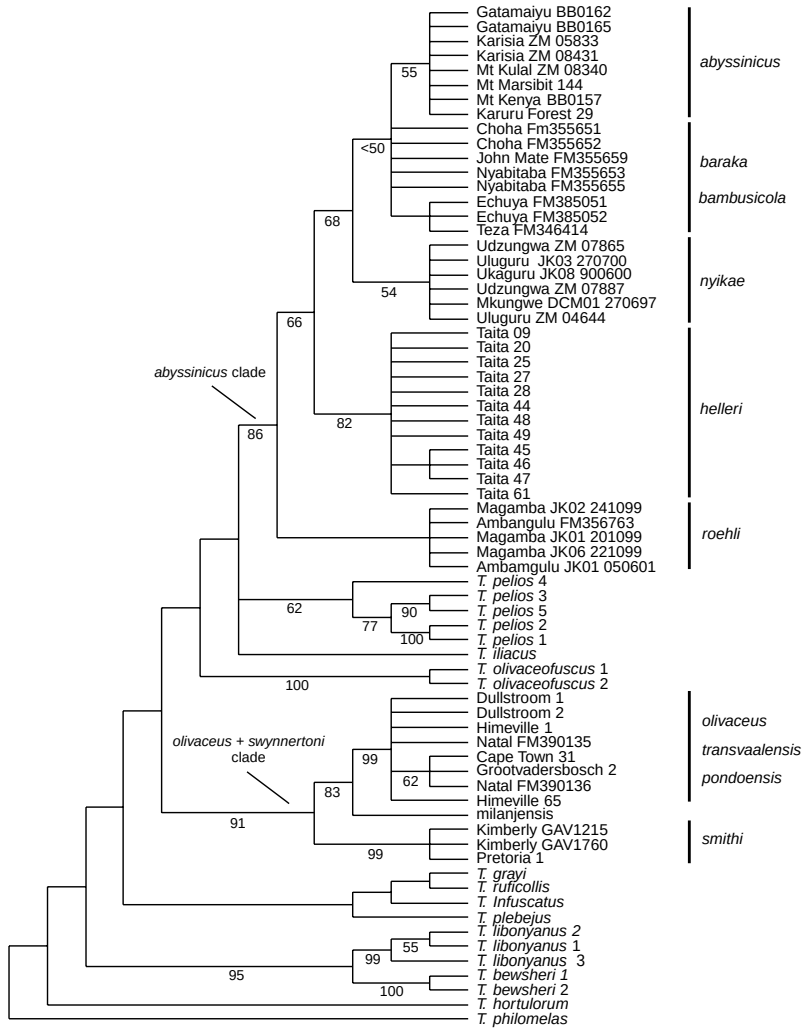

Fig. 4. Parsimony analyses of the ND3 dataset alone (383 bp) using a 10 ts:tv ratio, but with much greater geographical sampling recovered 24 trees (L = 1,437, CI = 0.630, RI = 0.882), the strict consensus of which is presented. Values below branches represent bootstrap values from 1,000 replicates. Increased geographical sampling did not alter the conclusions drawn from analyses of the smaller 26 taxa dataset.

'*olivaceus*' group with *milanjensis* ('*swynnertoni*' group) sister to other forest taxa of South Africa (*olivaceus*, *pondoensis* and *transvaalensis*), with (3) *smithi* from the arid shrublands of coastal and west-central South Africa forming a clade sister to *olivaceus*, *pondoensis*, *transvaalensis* and *milanjensis*, and (4) poor support for relationships among deeper nodes.

Discussion

The genus *Turdus* is one of the largest and most widespread of all bird genera (Bock and Farrand 1980, Clement and Hathway 2000). Its cosmopolitan distribution combined with the great plumage diversity shown within the genus has made taxonomic treatments of many species difficult, as evidenced by the continued contention regarding the taxonomic status of components of the olive thrush complex. In particular, species limits among *libonyanus* (Kurricane thrush), *pelios* (African thrush) and *olivaceus* (olive thrush), have varied extensively (Keith and Urban 1992, Clement and Hathway 2000 for reviews). However, these same treatments have tended to agree that the island restricted forms *bewsheri* (Comoro thrush) and *olivaceofuscus* (Gulf of Guinea thrush), as well as the distinctive *tephronotus* (bare-eyed thrush, northeast Africa), are best regarded as full species.

Phylogenetic hypotheses generated in this study (Table 2, Figs 2–4) all support according species status to *bewsheri*, *pelios* and *libonyanus*. There is significant genetic diversity within *bewsheri* and *pelios* (Fig. 2), and further study may reveal that these species represent species complexes. Phylogenetic hypotheses also suggest that the *olivaceus* complex is polyphyletic (Table 2). Southern African taxa (*olivaceus*, *pondoensis*, *transvaalensis*, *milanjensis* and *smithi*) are split from the northern forms (*baraka*, *bambusicola*, *abyssinicus*, *helleri*, *roehli* and *nyikae*) by 9–10.5% sequence divergence, but in most analyses these two groups do not form sister clades (Table 3).

The considerable sequence divergence among members currently classified under the umbrella of the *olivaceus* complex is not unexpected. Traditionally, the '*abyssinicus*' group of eastern and central Africa was considered a distinct species from the southern African taxa (White 1960, 1962a,b, Ripley 1964, Mackworth-Praed and Grant 1973 onwards). Phylogenetic analyses of mtDNA in this study strongly support according the '*abyssinicus*' clade and '*olivaceus*' clade species rank, *Turdus abyssinicus* Gmelin 1789, the northern or mountain olive thrush, and *Turdus olivaceus* Linnaeus 1766, the southern olive thrush.

The Turdus olivaceus/swynnertoni clade

In a recent study using both morphological (plumage and biometric data) and molecular data (1,058 bp of mtDNA), Bowie et al. (2003) suggested that *smithi* warrants specific status. The present study with extensive sampling of northern taxa supports these conclusions. However, with the inclusion of *milanjensis*, a member of the centrally distributed '*swynnertoni*' group (Fig. 1), the '*olivaceus*' group (*olivaceus*, *transvaalensis*, *pondoensis*, *culminans* and *smithi*) is rendered polyphyletic. The two members of the '*swynnertoni*' group, *swynnertoni* and *milanjensis* are very similar to the South African forest populations of olive thrush being only slightly smaller and having a darker bill (Clancey 1982).

The Turdus abyssinicus clade

The recognition of *T. abyssinicus* refuels the much-debated question of whether some of the more well marked 'races' of the '*abyssinicus*' clade, such as *helleri* (Taita thrush), *ludoviciae* (Somali blackbird), and *menachensis* (Yemen thrush), warrant full species rank (Keith and Urban 1992, Clement and Hathway 2000 for a review of opinions). *Helleri* with its distinctive blackish head and breast is one of four divergent subspecies (*deckeni*, *oldeani*, and *roehli* being the others), which geographically separate central Kenyan birds (*abyssinicus*), from those of central Tanzania (*nyikae*, Fig. 1). The controversy surrounding the taxonomic status of the distinctive *helleri*, stems from some authors (Keith and Urban 1992, Dowsett and Dowsett-Lemaire 1993) considering *roehli* to be intermediate between *helleri* and *nyikae* both in geography (Fig. 1), and plumage, with the white belly and reddish flanks being suggestive of *helleri* and the olive-grey head reminiscent of *nyikae*. Consequently, most modern systematic treatments of African thrushes have adopted the conservative approach of suggesting that these divergent races be considered part of *Turdus olivaceus* (including *abyssinicus*, Keith and Urban 1992, Dowsett and Dowsett-Lemaire 1993, Keith et al. 1997, Clement and Hathway 2000).

The sampled individuals of *helleri* always clustered together with strong support (Figs 2–4). Monophyly is also observed for the five individuals from the two populations of *roehli* (Fig. 4), and *roehli* is ca. 4% divergent from *helleri* and *nyikae* (Table 2). All three analytical methods suggest that either *helleri* (MP and BI) or *roehli* (ML) are sister to all other forms (*abyssinicus*, *baraka* and *nyikae*) of the '*abyssinicus*' clade. The central and southern Eastern Arc and northern Malawi Rift taxon *nyikae* is sister to an Albertine Rift (*baraka* and *bambusicola*) and Kenyan Highland (*abyssinicus*) clade in all analyses. This suggests that southwestern East Africa in the past has been more

closely linked biogeographically with central Africa and the Kenyan Highlands to the exclusion of the northern Eastern Arc. Thus, *abyssinicus* (including *baraka* and *bambusicola*) and *nyikae* have been able to spread to all montane forests in eastern/central Africa, even extending to the highlands of Ethiopia. In contrast, the more morphologically distinct and geographically restricted *helleri* and *roehli* appear to have maintained their genetic integrity, with no evidence to suggest introgression is or has taken place. Both *helleri* and *roehli* can be defined by discrete plumage characters (see Introduction and Clement and Hathway 2000) and are well supported in all the molecular analyses. Using either a biological (Mayr 1942) or phylogenetic (Cracraft 1983) species definition, both taxa warrant species rank: *Turdus helleri* Mearns 1913 (Taita thrush), and *Turdus roehli* Reichenow 1905, for which we suggest the common name Usambara thrush, based on the locality of the type specimen.

The Taita Hills in southeastern Kenya are a series of isolated montane forests, geographically considered to be the northernmost range of the Eastern Arc Mountains. *Turdus helleri* occupies four of the remaining fragments: Yale (2 ha), Chawia (ca. 94 ha), Ngangao (136 ha), and the largest fragment Mbololo (ca. 179 ha) (Lens et al. 2002). A study of 155 individuals using microsatellite markers (Galbusera et al. 2000), suggested that the three largest remaining populations are genetically isolated, with the largest difference existing between populations in Mbololo and Chawia. With respect to mtDNA only two haplotypes were detected among the 12 individuals analysed (Fig. 4). The two haplotypes differed from each other by only one nucleotide substitution, with one haplotype restricted to Mbololo, whereas Chawia and Ngangao were genetically identical for mtDNA. The Mbololo massif is separated from the rest of the Taita Hills by a valley at about 900m (see Fig. 1 in Brooks et al. 1998a). Our data do not support the recognition of potential races within *helleri*.

Both the Taita thrush (*helleri*) and the Usambara thrush (*roehli*) are highly restricted endemics. The Taita Hills have experienced severe fragmentation, and further degradation of its fragments warrant a need for immediate management. It is hoped that the data presented in this paper firmly settles the taxonomic dispute that has surrounded the recognition of species status for the Taita thrush and that the recognition of this 'new' endemic species for Kenya provides further impetus for the continued development of a species conservation plan for this species (Lens et al. 1998, Galbusera et al. 2000) and the Taita Hills as a whole (Brooks et al. 1998a).

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Appendix 1. *Turdus* outgroups, taxa and populations of the *Turdus olivaceus* species complex analysed in this study. *Refers to the location of voucher specimens: LSUMZ – Louisiana State University Museum of Natural Science, ZMUC – Zoological Museum of the University of Copenhagen, FMNH – The Field Museum of Natural History, MBM – Barrick Museum at the University of Nevada Las Vegas, UWBM – University of Washington Burke Museum, and the Percy FitzPatrick Institute, University of Cape Town.

Taxon		Inst./Indiv.*	Accession no.	Country	General collecting locality
Turdus philomelos		LSUMZ	B13468	Germany	Schelswig-Holstein
T. hortulorum		UWBM	51161	Russia	Primorskiy Kray
T. ruficollis		UWBM	46282	Russia	Avt. Rep. Gorno-Altay
T. iliacus		LSUMZ	B13478	Germany	Schelswig-Holstein
T. grayi		MBM	6620	Honduras	Dpto. Copan
T. plebejus		MBM	4322	Nicaragua	Matagalpa
T. infuscatus		MBM	13588	Guatemala	Quetzaltenango
T. libonyanus	1	UWBM	52923	South Africa	KwaZulu Natal
	2	FitzPatrick	#61	South Africa	Tzaneen
	3	Peter Ryan	4A19951	Malawi	Mount Zomba
T. bewsheri	1	Ben Warren	GC159	Comoro Is.	Grande Comore
	2	Ben Warren	GC193	Comoro Is.	Mohéli
T. olivaceofuscus	1	Martim de Melo	#1	Rep. of São Tomé & Príncipe Is.	São Tomé
	2	Martim de Melo	#2	Rep. of São Tomé & Príncipe Is.	São Tomé

Appendix 1 (*Continued*)

Taxon		Inst./Indiv.*	Accession no.	Country	General collecting locality
T. pelios saturatus	1	FMNH	396636	Ghana	Gonja Triange, 2 km N of Buipe
	2	FMNH	396634	Ghana	Gonja Triange, 2 km N of Buipe
T. p. centralis	1	FMNH	429758	Demo. Rep. of the Congo	Lwiro
	2	ZMUC	RK16-280501	Demo. Rep. of the Congo	Lwiro
	3	FMNH	385054	Uganda	Ngoto Swamp
T. olivaceus olivaceus	1	FitzPatrick	#31	South Africa	Cape Town
	2	Rauri Bowie	#2	South Africa	Grootvadersbosch
T. o. pondoensis	1	FitzPatrick	#65	South Africa	Himeville
	2	FitzPatrick	#1	South Africa	Himeville
	3	FMNH	390135	South Africa	Boston
	4	FMNH	390136	South Africa	Boston
T. o. transvaalensis	1	FitzPatrick	#1	South Africa	Dullstroom
	2	FitzPatrick	#2	South Africa	Dullstroom
T. o. smithi	1	MBM	5877	South Africa	Kimberley
	2	MBM	7883	South Africa	Kimberley
	3	FitzPatrick	#1	South Africa	Pretoria
T. o. milanjensis		Martim de Melo	#1	Mozambique	Mount Namuli
T. o. helleri	1	Luc Lens	09	Kenya	Chawia, Taita Hills
	2	Luc Lens	20	Kenya	Chawia, Taita Hills
	3	Luc Lens	25	Kenya	Chawia, Taita Hills
	4	Luc Lens	27	Kenya	Chawia, Taita Hills
	5	Luc Lens	28	Kenya	Ngangao, Taita Hills
	6	Luc Lens	44	Kenya	Ngangao, Taita Hills
	7	Luc Lens	45	Kenya	Mbololo, Taita Hills
	8	Luc Lens	46	Kenya	Mbololo, Taita Hills
	9	Luc Lens	47	Kenya	Mbololo, Taita Hills
	10	Luc Lens	48	Kenya	Ngangao, Taita Hills
	11	Luc Lens	49	Kenya	Ngangao, Taita Hills
	12	Luc Lens	61	Kenya	Ngangao, Taita Hills
T. o. roehli	1	ZMUC	JK01-201099	Tanzania	Magamba, W. Usambara Mnts
	2	ZMUC	JK06-221099	Tanzania	Magamba, W. Usambara Mnts
	3	ZMUC	JK02-241099	Tanzania	Magamba, W. Usambara Mnts
	4	FMNH	356763	Tanzania	Ambangulu, W. Usambara Mnts
	5	ZMUC	JK01-050601	Tanzania	Ambangulu, W. Usambara Mnts
T. o. nyikae	1	ZMUC	O7887	Tanzania	Udzungwa Scarp Forest
	2	ZMUC	O7865	Tanzania	Udzungwa Scarp Forest
	3	ZMUC	JK03-270700	Tanzania	Uluguru Mnts
	4	ZMUC	DCM01-270697	Tanzania	Kungwe
	5	ZMUC	JK08-900600	Tanzania	Ukaguru Mnts
	6	ZMUC	O4644	Tanzania	Uluguru Mnts

Appendix 1 (*Continued*)

Taxon		Inst./Indiv.*	Accession no.	Country	General collecting locality
T. o. bambusicola	1	FMNH	346414	Burundi	Teza, Muramuya District
T. o. baraka	1	FMNH	385051	Uganda	Echuya Forest Reserve, Kabale
	2	FMNH	385052	Uganda	Echuya Forest Reserve, Kabale
	3	FMNH	355653	Uganda	Nyabitaba, Ruwenzori Mnts
	4	FMNH	355655	Uganda	Nyabitaba, Ruwenzori Mnts
	5	FMNH	355659	Uganda	John Mate, Ruwenzori Mnts
	6	FMNH	355651	Uganda	Choha, Ruwenzori Mnts
	7	FMNH	355652	Uganda	Choha, Ruwenzori Mnts
T. o. abyssinicus	1	Rauri Bowie	BB0165	Kenya	Gatamaiyu Forest, S. Aberdares
	2	Rauri Bowie	BB0162	Kenya	Gatamaiyu Forest, S. Aberdares
	3	ZMUC	O5833	Kenya	Karisia Hill, N. Aberdares
	4	ZMUC	O8431	Kenya	Karisia Hill, N. Aberdares
	5	ZMUC	O8340	Kenya	Mount Kulal
	6	ZMUC	144	Kenya	Mount Marsibit
	7	Rauri Bowie	BB0157	Kenya	Mount Kenya
	8	FitzPatrick	#29	Kenya	Karuru Forest