

Gross morphology betrays phylogeny: the Scrub Warbler *Scotocerca inquieta* is not a cisticolid

PER ALSTRÖM,^{1*} JON FJELDSÅ,² SILKE FREGIN³ & URBAN OLSSON⁴

¹Swedish Species Information Centre, Swedish University of Agricultural Sciences, Box 7007, SE-750 07 Uppsala, Sweden

²Zoological Museum, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

³Vogelwarte Hiddensee, Zoological Institute and Museum, Ernst Moritz Arndt University of Greifswald, 17489 Greifswald, Germany

⁴Department of Zoology, University of Gothenburg, Box 463, SE-405 30 Göteborg, Sweden

The Scrub Warbler, which inhabits arid areas from North Africa to western Asia, has long been thought to be closely related to cisticolid warblers. However, analyses based on two mitochondrial and four nuclear loci place this species sister to the mainly Asian Cettiidae (bush warblers, tesias, etc.). Superficial morphological similarity to cisticolid warblers has previously clouded the species true relationship. Detailed morphology, such as facial bristles and claw and footpad structure, also supports a closer relationship to Cettiidae and some other non-cisticolid warblers.

Keywords: Cettiidae, Cisticolidae, mitochondrial DNA, morphology, nuclear DNA, Passeriformes, phylogeny, Sylvioidea.

The Scrub Warbler *Scotocerca inquieta* is a distinctive warbler of arid landscapes from North Africa through Arabia and into western Asia. It is generally scarce and patchily distributed in tracts of sand or firm soil, wadi beds and rocky slopes, where there is usually some cover of low shrubs (Cramp 1992, Ryan 2006). Sand-coloured and faintly striped, with short, rounded wings and a fairly long dark tail incessantly raised and jerked from side to side, it resembles some oriental species of *Prinia* warblers, notably Graceful *Prinia gracilis*, which inhabits the same geographical region but more mesic habitats. Most classifications place the Scrub Warbler in the monotypic genus *Scotocerca* (e.g. Sharpe 1883, Vaurie 1955, 1959, Watson *et al.* 1986, Urban *et al.* 1997, Sibley & Monroe 1990, Dickinson 2003, Ryan 2006), although it has also been lumped with *Prinia* in the past (according to Vaurie 1955 and Urban *et al.* 1997). Taxonomic lists usually place it close to *Prinia*, in the group of Old World warblers now typically recognized as the family Cisticolidae (Watson *et al.* 1986, Urban *et al.* 1987, Sibley & Monroe 1990,

Dickinson 2003, Ryan 2006). It has also been placed close to the superficially similar monotypic East Asian genus *Rhopophilus* (e.g. Dickinson 2003), which we now know to be a babbler (Timaliidae *sensu* Alström *et al.* 2006, Sylviidae *sensu* Gelang *et al.* 2009). Several recognized subspecies differ mainly in colour hues and the degree of the streaking on the plumage, as well as in iris colour (Cramp 1992, Ryan 2006).

Although the Scrub Warbler is found in shrubby areas, and never occurs far from cover, it usually forages on the ground, searching for food underneath bushes and around their edges. Meinertzhagen (1949) pointed out that it is 'peculiar in having its nostrils concealed by plumelets, which could be seen as an adaptation to exclude blown sand'.

Panov (2003) described the behaviour of *Scotocerca* to be similar to that of *Prinia* and distinct from that of other major warbler lineages. However, others have emphasized its rather different, wren-like, appearance (e.g. Rasmussen & Anderton 2005). The only phylogenetic analysis including *Scotocerca* (Barhoum & Burns 2002) used an unpublished mitochondrial cytochrome *b* sequence from GenBank in a study aiming to evaluate the

*Corresponding author.
Email: per.alstrom@artdata.slu.se

relationships of the Wren tit *Chamaea fasciata*. In this tree, *Scotocerca* was placed in a clade together with *Cettia*, *Phylloscopus* and members of the Acrocephalidae, being sister to the latter, although with no statistical support.

Here we present an assessment of the phylogenetic position of *Scotocerca*, based on molecular and morphological evidence. We analyse sequence data of two mitochondrial genes and four nuclear introns of a wide range of warbler-like birds, Sylvioidea (*sensu* Alström *et al.* 2006), and discuss morphological traits in light of the molecular phylogeny.

METHODS

Taxonomic sampling

For a balanced taxon sampling we scrutinized the results of several comprehensive oscine phylogenies that included a good selection of Sylvioidea species (Beresford *et al.* 2005, Alström *et al.* 2006, Jönsson & Fjeldså 2006, Johansson *et al.* 2008, Gelang *et al.* 2009) and selected representatives of relevant major lineages. Including *Scotocerca*, the analysis comprised 33 species of Sylvioidea plus two members of Passeroidea as outgroup taxa (see Johansson *et al.* 2008). Scientific species names follow Dickinson (2003), and family names follow Alström *et al.* (2006), Johansson *et al.* (2008), Gelang *et al.* (2009) and Aleixo and Pacheco (2006: Donacobiidae). Approximately half of the sequences were obtained from GenBank.

DNA extraction, PCR amplification and sequencing

DNA was extracted using the QIA Quick DNEasy kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's instructions. We sequenced the mitochondrial genes cytochrome *b* (hereafter *cyt b*) and nicotinamide dehydrogenase subunit 2 (ND2), and the nuclear introns glyceraldehyde-3-phosphodehydrogenase intron 11 (GAPDH), ornithine decarboxylase exon 6 (partial), intron 6, exon 7, intron 7 and exon 8 (partial) (ODC), the entire nuclear myoglobin intron 2 (*myo*), and the complete nuclear lactate dehydrogenase (LDH) intron 3. Amplification and sequencing of *cyt b* and *myo* followed the protocols described in Olsson *et al.* (2005), ND2 those of Sorenson *et al.* (1999), ODC those of Allen and Omland (2003), GAPDH (G3PDH) those of Fjeldså *et al.* (2003)

and LDH those of Fregin *et al.* (2009). The *cyt b* and ND2 sequences were amplified as one fragment to decrease the risk of amplifying nuclear copies (pseudogenes) of coding genes (e.g. Sorenson & Quinn 1998). The sequences have been deposited in GenBank (accession numbers are detailed in Table 1).

Phylogenetic analyses

Sequences were aligned using MEGALIGN 4.03 in the DNASTAR package (DNASTAR Inc., Madison, WI, USA); some manual adjustment was necessary for the nuclear sequences. Molecular phylogenies were estimated by Bayesian inference (BI) using MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Posterior probabilities (PP) were calculated for the mitochondrial loci under a general time-reversible (GTR) model (Lanave *et al.* 1984, Tavaré 1986, Rodríguez *et al.* 1990), assuming rate variation across sites according to a discrete gamma distribution with four rate categories (Γ ; Yang 1994) and an estimated proportion of invariant sites (I; Gu *et al.* 1995). For the nuclear introns, the HKY model (Hasegawa *et al.* 1985), assuming rate variation across sites according to a discrete gamma distribution with four rate categories (Γ ; Yang 1994), was suggested. The choice of model was determined based on the Bayesian information criterion calculated by JMODELTEST version 0.1.1 (Posada 2008).

The loci were analysed separately as well as combined. Concatenated sequences were divided into six partitions, which were allowed to have partition-specific model parameters (Ronquist & Huelsenbeck 2003, Nylander *et al.* 2004). Default priors in MRBAYES were used. Four Metropolis-coupled MCMC chains with an incremental heating temperature of 0.2 were run for 50 million generations, and sampled every 1000 generations. Two runs were run simultaneously, starting from random trees, and the results compared to ascertain that the chains had reached the same target distribution. Stationarity of chain likelihood values and average standard deviation of split frequencies were inspected manually in MRBAYES; other parameter values and effective sample size were monitored in TRACER 1.5.0 (Rambaut & Drummond 2009a). The first 25% of the sampled iterations were discarded as the burnin, well after stationarity of chain likelihood values had been established.

Table 1. Samples used in the present study (in alphabetical order).

Species	Museum no.	Locality	GenBank no.
<i>Acrocephalus dumetorum</i>	NRM 569279 ^a	India: Punjab	Cytb: AJ004773 ¹ , ND2: FJ358016 ² , GAPDH: FJ357911 ² , Myo: AY887682 ¹ , ODC: EF625338 ³
<i>Alauda arvensis arvensis</i>	NRM 966614 ^b	Kazakhstan Sweden	LDH: AY136597 ³ Cytb: AY228047 ¹ , ND2: HQ333078 , GAPDH: FJ357913 ² , LDH: HQ333047 , Myo: AY228284 ¹ , ODC: EF625336 ⁴
<i>Andropadus masukuensis roehli</i>	ZMUC 117572 ^b	Tanzania	Cytb: HQ333035 , ND2: AF003450 ⁵ , GAPDH: –, LDH: HQ333048 , Myo: EF625238 ⁴ , ODC: EF625287 ⁴
<i>Apalis flavida florisuga</i>	DZUG U2204; VH B0745 (LDH)	KwaZulu-Natal, South Africa Gambia	Cytb: HQ333036 , GAPDH: HQ333097 , LDH: HQ333049 , Myo: HQ333069 , ODC: HQ333083
<i>Bradypterus baboecala tongensis/transvaalensis</i>	NRM 20046782 ^c	South Africa: Natal	ND2: AY136597 ⁶ Cytb: DQ008473 ¹ , ND2: AY382343 ⁷ , GAPDH: HQ333098 , LDH: HQ333050 , Myo: DQ008525 ¹ , ODC: HQ333084
<i>Bradypterus tacsanowskius</i>	UWBM 57938	Mongolia	Cytb: HQ333037 , LDH: HQ333051 , ODC: HQ333085
	UWBM 51699	Russia: Irkutsk	ND2: AY382352 ⁷ , Myo: HQ333070
	NRM 20046783	China: Hebei	GAPDH: HQ333099
<i>Bernieria madagascariensis inceleber</i>	FMNH 431202	Madagascar: Toliara	Cytb: HQ333038 , ND2: HQ333079 , GAPDH: HQ333100 , LDH: HQ333052 , Myo: HQ333071 , ODC: HQ333086
<i>Cettia cetti orientalis</i>	DZUG U1037	Armenia	Cytb: HQ333039 , GAPDH: HQ333101 , Myo: HQ333072 , ODC: HQ333087
		Russia: Astrakhan	ND2: AY382353 ⁷
<i>C. c. cetti</i>	VH A1579	Germany	LDH: HQ333053
<i>Cettia fortipes davidiana</i>	DZUG U2207	Vietnam: Tonkin	Cytb: HQ333040 , GAPDH: HQ333102 , LDH: –, Myo: HQ333073 , ODC: HQ333088
		China	ND2: DQ125976 ⁸
<i>Donacobius atricapilla atricapilla</i>	NRM 966966 ^b	Paraguay: Dpto. Concepcion	Cytb: DQ008481 ¹ , ND2: FJ358018 ² , GAPDH: FJ357915 ² , LDH: HQ333054 , Myo: DQ008533 ¹ , ODC: EU680723 ⁹
<i>Delichon urbicum urbicum</i>	NRM 20046816 ^d	Spain	Cytb: DQ008517 ¹ , GAPDH: HQ333103 , LDH: HQ333055 , Myo: DQ008568 ¹
		Sweden	ODC: EU680721 ⁹
		South Africa	ND2: AY826043 ¹⁰
<i>Erythrocercus mcallii mcallii</i>		? (unpublished)	Cytb: AF096465
		Cameroon	ND2: DQ125982 ⁸ , GAPDH: –, LDH: –, Myo: DQ125954 ⁸ , ODC: EU680727 ⁹
<i>Hippolais icterina</i>	NRM 20046788	Greece Ukraine	Cytb: FJ883046 ³ LDH: FJ883078 ³ , Myo: FJ883120 ³ , ODC: FJ883153 ³
		Sweden	ND2: FJ178357 ⁹ , GAPDH: HQ333104
<i>Hirundo rustica rustica</i>	VH A1574	Sweden	Cytb: DQ008516 ¹ , Myo: AY064258 ¹ , ODC: EF625337 ⁹
		Germany	GAPDH: HQ333105 , LDH: HQ333056
		?	ND2: DQ176512 ¹¹
<i>Hylia prasina prasina</i>	ZMUC 119098	Uganda	Cytb: HQ333041 , ND2: AY136606 ⁶ , GAPDH: –, LDH: HQ333057 , Myo: EU680583 ⁹ , ODC: EU680732 ⁹
<i>Mirafra javanica williamsoni</i>	NRM 20046819 ^b	Thailand	Cytb: DQ008520 ¹ , ND2: HQ333080 , GAPDH: HQ333106 , LDH: HQ333058 , Myo: DQ008571 ¹ , ODC: HQ333089
<i>Melocichla mentalis mentalis</i>	NRM 20046804 ^e	Nigeria	Cytb: DQ008500 ¹ , GAPDH: HQ333107 , Myo: DQ008551 ¹ , ODC: HQ333090
	VH A1550	Ivory Coast Cameroon	LDH: HQ333059

Table 1. (Continued).

Species	Museum no.	Locality	GenBank no.
<i>Megalurus gramineus goulburni</i>	ANWC D224	Australia: South Australia	ND2: DQ125998 ⁸ Cytb: HQ333042 , GAPDH: HQ333108 , LDH: HQ333060 , Myo: HQ333074 , ODC: HQ333091
<i>Orthotomus sutorius inexpectatus</i>	NRM 20046795 ^f	Australia: New South Wales NW Thailand	ND2: AY382397 ⁷ Cytb: DQ008491 ¹ , GAPDH: HQ333109 , Myo: DQ008542 ¹ , ODC: HQ333092
<i>O. s. guzuratus</i>	VH A1581	China: Yunnan	ND2: DQ871365 ¹²
<i>Pycnonotus barbatus inornatus</i>	DZUG U2047	India: Himachal Pradesh Mauretania	LDH: HQ333061 Cytb: HQ333043 , GAPDH: HQ333110 , LDH: HQ333062 , Myo: HQ333075 , ODC: HQ333093
<i>P. b. ssp.</i>		Cameroon	ND2: AF407054 ¹³
<i>Phylloscopus collybita abietinus</i>		Sweden	Cytb: HQ121526 ¹⁵ , GAPDH: FJ357920 ² , LDH: –, Myo: DQ125966 ⁸ , ODC: FJ358084 ²
<i>Proeopyga albiventer albiventer</i>	NRM 20086723	W Russia China: Sichuan	ND2: EU481500 ¹⁴ Cytb: HQ121521 ² , ND2: FJ358001 ¹⁵ , GAPDH: FJ357889 ² , LDH: –, Myo: FJ357959 ² , ODC: FJ358058 ²
<i>Prinia familiaris</i>	NRM 20046794 ^b	Indonesia: Java	Cytb: DQ008490 ¹ , ND2: HQ333081 , GAPDH: HQ121547 ¹⁵ , LDH: HQ333063 , Myo: DQ008541 ¹ , ODC: HQ121557 ¹⁵
<i>Sylvia atricapilla atricapilla</i>		Germany Sweden	Cytb: Z73494 ¹⁶ GAPDH: EF441232 ¹⁷ , Myo: AY887727 ¹⁷ , ODC: EF441254 ¹⁷
		France	ND2: DQ125994 ⁸
<i>Scotocerca inquieta saharae</i>	VH A0364 DZUG U1775	Germany Tunisia	LDH: HQ333067 Cytb: HQ333044 , ND2: HQ333082 , GAPDH: HQ333111 , LDH: HQ333064 , Myo: HQ333076 , ODC: HQ333094
<i>Stachyris nigriceps yunnanensis/rileyi</i>	NRM 20026627	Vietnam: Ha Tinh province	Cytb: HQ333045 , GAPDH: HQ333112 , LDH: HQ333065 , ODC: HQ333095
<i>S. n. yunnanensis</i>		Vietnam: Tonkin ⁹	Myo: AY228321 ¹⁸
<i>S. n. ssp.</i>		Thailand	ND2: FJ358009 ²
<i>Sphenoeacus afer</i>	VH B0898	South Africa	Cytb: ND2: DQ125991 ⁸ , GAPDH: –, Myo: EU680601 ⁹ , ODC: EU680766 ⁹
<i>Thamnornis chloropetoides</i>	FMNH 436448	Madagascar	LDH: HQ333066 Cytb: HQ333046 , ND2: DQ125995 ⁸ , GAPDH: FJ357923 , LDH: HQ333068 , Myo: HQ333077 , ODC: HQ333096

^aAll from India concern same sample.

^bAll concern same sample.

^cAll except ND2 concern same sample.

^dAll from Spain concern same sample.

^eAll from Nigeria concern same sample.

^fCytb, Myo, ODC and GAPDH are from the same sample.

⁹Communicated by Peter Nilsson (not given in paper).

References: ¹Alström et al. (2006), ²Gelang et al. (2009), ³Fregin et al. (2009), ⁴Johansson et al. (2007), ⁵Roy (1997), ⁶Sefc et al. (2003), ⁷Drovetski et al. (2004), ⁸Fuchs et al. (2006), ⁹Johansson et al. (2008), ¹⁰Sheldon et al. (2005), ¹¹Zink et al. (2006), ¹²Nguembock et al. (2007), ¹³Sorenson and Payne (2001), ¹⁴Reeves et al. (2008), ¹⁵Irestedt et al. (2011), ¹⁶Helbig et al. (1995), ¹⁷Jønsson et al. (2007), ¹⁸Ericson and Johansson (2003).

ANWC, Australian National Wildlife Collection (CSIRO), Canberra, Australia; DZUG, Department of Zoology, University of Gothenburg, Göteborg, Sweden; FMNH, Field Museum of Natural History, Chicago, USA; NRM, Swedish Museum of Natural History, Stockholm, Sweden; UWBM, University of Washington Burke Museum, Seattle, USA; VH, Vogelwarte Hiddensee, Zoological Institute and Museum, Ernst Moritz Arndt University of Greifswald, Greifswald, Germany; ZMUC, Zoological Museum of the University of Copenhagen, Copenhagen, Denmark. Sequences that are new to this study are in bold.

The data were also analysed using BEAST version 1.5.4 (Drummond & Rambaut 2007, 2009). XML files for analysis in BEAST were generated in BEAUTI version 1.5.4 (Rambaut *et al.* 2009), applying the same substitution models as in the MRBAYES analyses and a log-normal uncorrelated relaxed clock prior (Drummond *et al.* 2006) with a fixed mean rate of 1.0 under a birth-death prior. For the remaining parameters the default prior settings were used. Fifty million generations were run, sampled every 1000th generation. The MCMC output was analysed in TRACER version 1.5.0 (Rambaut & Drummond 2009a) to evaluate whether valid estimates of the posterior distribution of the parameters had been obtained. The first 25% of the generations were discarded as burnin. Trees were summarized using TREEANNOTATOR version 1.5.4 (Rambaut & Drummond 2009b), choosing 'Maximum clade credibility tree' and 'Mean heights', and displayed in FIGTREE version 1.3.1 (Rambaut 2009).

Maximum likelihood bootstrapping (1000 replicates) was performed in TREEFINDER (Jobb *et al.* 2004, Jobb 2008) using default settings and the same nucleotide substitution models as in the Bayesian inferences, except for the combined analysis, which was done under the GTR + Γ model, as proposed by TREEFINDER.

Morphological analysis

For morphological evidence, J.F. examined 10 specimens (museum skins) of *Scotocerca inquieta* and a broad selection of other warblers in the Zoological Museum of the University of Copenhagen, Denmark. The specimens were examined under a stereo microscope or through a 10 \times magnifying hand lens, with specific focus on the structural characters of the bill, head and feet. In addition, 30 specimens of *S. inquieta* from throughout the species' range, and a wide selection of other genera were studied by P.A. in The Natural History Museum, Tring, UK. These were examined through a 10 \times magnifying hand lens, focusing on the characters of the head.

RESULTS

Sequence characteristics

We obtained a dataset comprising 4541 base pairs (bp), of which 1439 (32%) were parsimony infor-

mative. Including all outgroup taxa, the aligned ODC sequences comprised 761 bp, of which 162 (21%) were parsimony informative: myo 763 bp, 130 (17%) parsimony informative; LDH 536 bp, 131 (24%) parsimony informative; GAPDH 399 bp, 82 (21%) parsimony informative; ND2 1041 bp, 520 (50%) parsimony informative; and cyt *b* 1041 bp, 414 (40%) parsimony informative. No unexpected start or stop codons or indels that could indicate the presence of nuclear paralogues were present in either the ND2 or cyt *b* sequences. Including all outgroups, the combined ODC, myo, LDH and GAPDH (hereafter nuclear) dataset consisted of 2459 bp, of which 505 (21%) were parsimony informative. The combined mitochondrial dataset consisted of 2082 bp, of which 934 (45%) were parsimony informative.

Molecular phylogeny

The trees recovered by MRBAYES and BEAST were essentially the same, with most nodes having high posterior probability (Fig. 1). Only two of the nodes that differ between these two methods are strongly supported by one of them (MRBAYES) and poorly supported by the other one; both these cases involve very short internal branches. Maximum likelihood bootstrapping supports all of the families, but generally fails to resolve the relationships among them.

Scotocerca is inferred to be sister to the essentially Eurasian Cettiidae, with the Chestnut-capped Flycatcher *Erythrocerus mccallii*, representing a genus endemic to sub-Saharan Africa, being sister to these, although with mixed support (BI PP = 0.99, ML bootstrap < 50%). The mainly Eurasian Aegithalidae is sister to the *Scotocerca*/*Erythrocerus*/Cettiidae clade, but also with mixed support (BI PP = 0.99, ML bootstrap < 50%). The position of *Scotocerca* is strongly supported by three single-locus analyses (BI PP > 0.95; cyt *b* + ND2, ODC, LDH). The clade containing Cettiidae, *Scotocerca* and *Erythrocerus* is further supported by a unique 15-bp deletion in the ODC alignment (Fig. 1).

In GenBank, there is a cyt *b* sequence of *S. inquieta* (AF139826) from an unpublished study, presumably collected somewhere in the former Soviet Union, submitted by E. P. Sokolov. This sequence is sister to our North African sample (BI PP 1.0) but differs from it by the substantial margin of 10.8% (uncorrected).

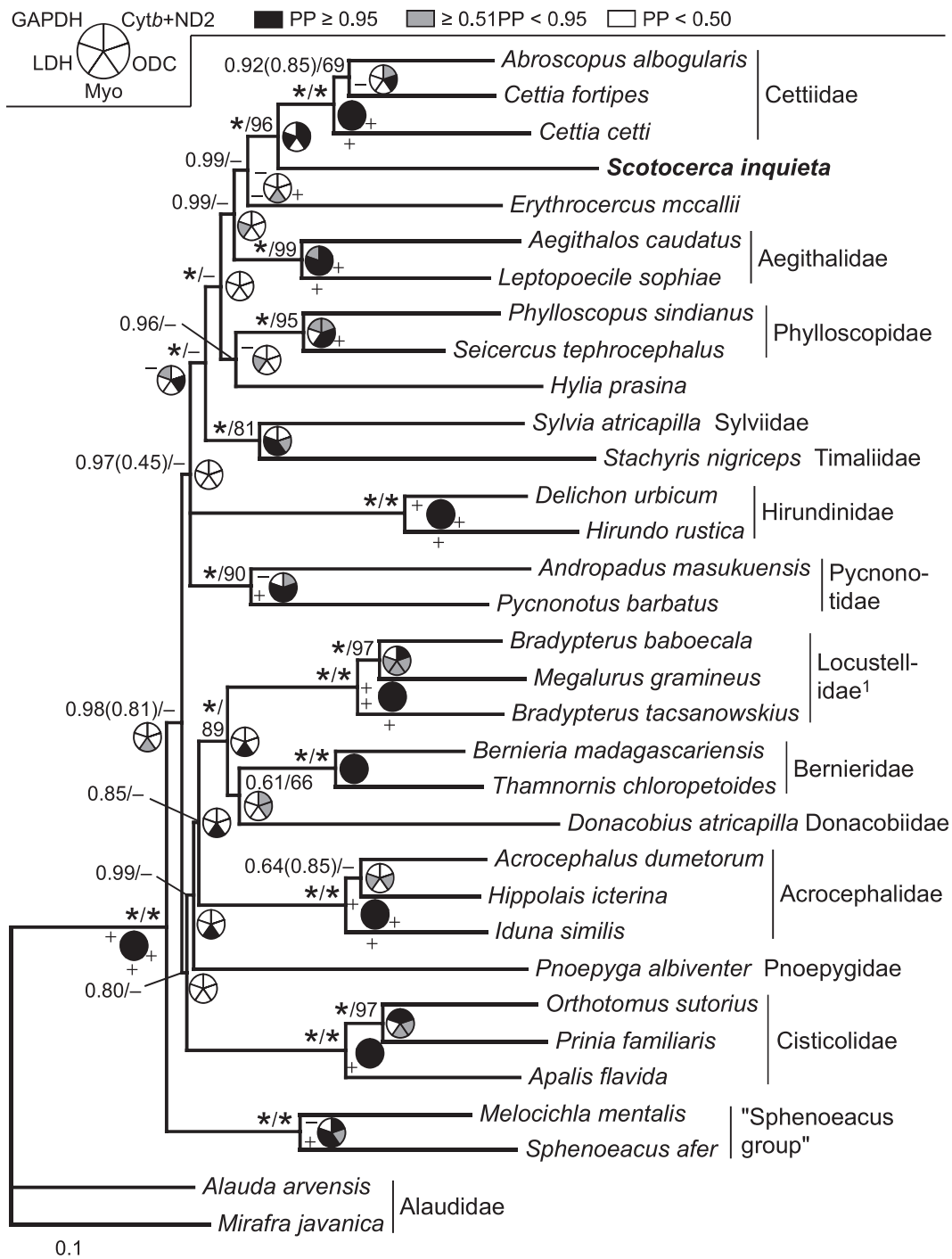


Figure 1. Majority rule (50%) consensus tree of *Scotocerca inquieta* (highlighted in bold) and representatives of other families in Sylvioidea, based on the total DNA data set (mitochondrial *cyt b* and ND2; nuclear ODC, *myo*, LDH and GAPDH intron sequences) inferred using a mixed-model Bayesian analysis (MRBAYES). Posterior probabilities and maximum likelihood bootstrap values are indicated at the nodes in this order; an asterisk represents posterior probability of 1.0 or a bootstrap value of 100%. Values in parentheses represent posterior probabilities from the Bayesian BEAST analysis, when these deviate by > 0.05 from the MRBAYES analysis. Pie charts at nodes denote support in single-locus analyses (see explanation at top of figure); + at a pie indicates further support for the clade by one or more indels (in the locus whose pie is adjacent to the +); - at a pie indicates that no sequence is available for this locus for at least one of the species in the pertinent clade (see Table 1). ¹Usually called Megaluridae.

Morphology

As mentioned in some earlier descriptions, the head and body feathers of the Scrub Warbler show a faint pink to violet tinge, visible at some angles and in certain lighting and therefore probably an effect of the feather microstructure rather than of pigments. A similar tinge is seen in long-tailed tits *Aegithalos* spp. (more in some taxa than in others), American Bushtit *Psaltriparus minimus* and as a more intense violet in tit-warblers *Leptopoecile* spp.

The plumage is long and loose, but the feathers of the lower forehead, lores and chin, i.e. mainly around the base of the bill, include many dark hair-like bristles formed by elongated, thick, stiff, bare and heavily pigmented shafts and terminal barbs. Many of these extend beyond the nostrils, including three prominent rictal bristles above the gape (Fig. 2c). Within the Cettiidae, most of the species of *Cettia* bush warblers and the monotypic *Tickellia* (Broad-billed Warbler) are very similar to *Scotocerca* with respect to bristles, including the rictal bristles, whereas a few other *Cettia*, all *Urosphena* (stubtails) and *Tesia* (tesias) and the monotypic *Oligura* (Chestnut-headed Tesia) lack bristles other than three (shorter) rictal bristles. Mountain Tailorbird *Phyllergates cucullatus* (previously in the cisticolid genus *Orthotomus* (tailorbirds), but shown to belong in the Cettiidae by Alström *et al.* 2006) has very few indistinct bristles except for three rictal bristles. The three species of *Abroscopus* warblers, also in the Cettiidae, have prominent bristles around the base of the bill, but have four instead of three rictal bristles.

All three species of *Erythrocerus* have a facial bristle structure resembling that of *Scotocerca*, but also have additional and markedly stronger bristles above and below the gape (Fig. 2b), as in monarch flycatchers, Monarchidae. In Aegithalidae, *Leptopoecile* has similar bristles as *Scotocerca* (Fig. 2a), *Psaltriparus* has much shorter bristles, and most *Aegithalos* (long-tailed tits) species have three short rictal bristles (none in Long-tailed Tit *Aegithalos caudatus*) in addition to the stiff terminal feather barbs. The examined representatives of the Cisticolidae (several species of the larger genera examined, as well as *Schistolais*, *Heliolais*, *Urolais*, *Drymochila*, *Spiloptila*, *Hypergerus* and *Eminia*) have a different feather structure with only two rictal bristles (Fig. 2d). Apart from this, several Cisticolidae species have some thick and dark terminal barbs on the throat, mainly on the sides,

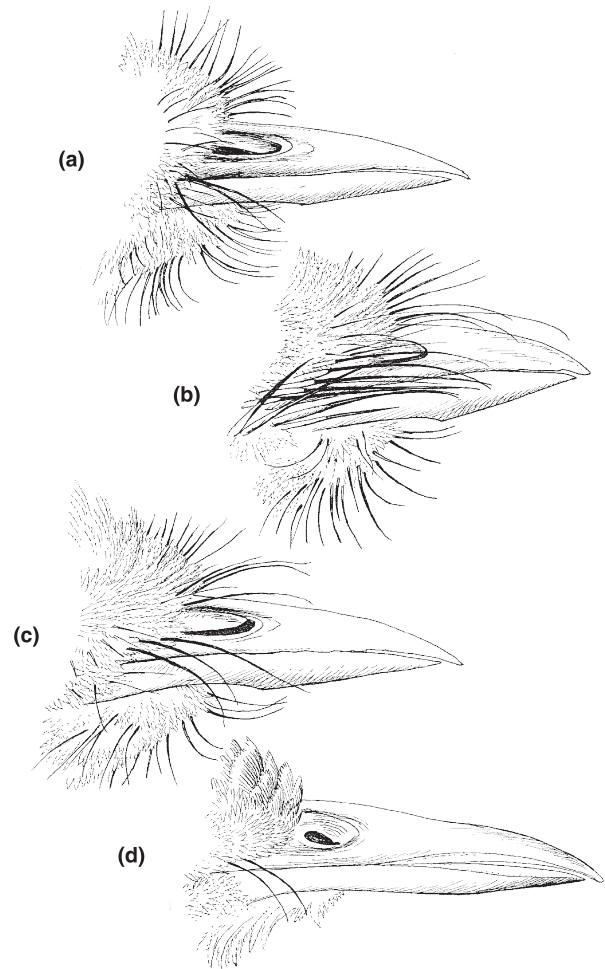


Figure 2. Feather structure on the anterior face of White-browed Tit-warbler *Leptopoecile sophiae* (a), Livingstone's Flycatcher *Erythrocerus livingstoni* (b), Scrub Warbler *Scotocerca inquieta* (c) and a desert cisticolid Cricket Longtail *Spiloptila clamans* (d). Drawings by Jon Fjeldså.

unlike in *Scotocerca*, which has them around the entire base of the bill.

The tail of *Scotocerca* is slightly rounded distally, with the outermost rectrices < 10 mm shorter than the longest. *Erythrocerus* spp. and most Cettiidae species (except those with extremely short tails) also have moderately rounded tails. Aegithalidae and most Cisticolidae have more strongly graduated tails. Moreover, *Scotocerca* has ten rectrices in common with the Cettiidae (with the exception of *Tesia* spp., which have only eight rectrices in their extremely short tails). In contrast, *Erythrocerus* spp., all Aegithalidae and the majority of Cisticolidae have 12 rectrices (cf. Urban *et al.* 1997, Ryan 2006, Nguembock *et al.* 2008, our own studies).

The feet of *Scotocerca* have rather short and thick toes with thick brown claws that have a subterminal notch and a prominent lateral groove (Fig. 3d). Most other warblers have pale, longer and more slender claws with narrower lateral grooves, but *Leptopoeile*, *Psaltriparus*, *Aegithalos*, *Erythrocerus* and some *Cettia* have similar claw shapes as in *Scotocerca*, although with variable development of the subterminal notch (Fig. 3). *Leptopoeile* and *Erythrocerus* also have short and

thick toes (Fig. 3b,c), and these, as well as *Scotocerca* and most Cettiidae, differ from typical oscines as the papillae of the toe-pads are large and flat, unlike the typical rugose structure of the swollen toe-pads in most oscines (Fig. 3e and Lennerstedt 1973). In general, the toe-pads are rather restricted as much of the soles are composed of folds and intervening furrows. However, large and swollen toe-pads are seen in *Psaltriparus* and notably in *Aegithalos*, which has smooth toe-pads with no obvious papillar structure (Fig. 3a).

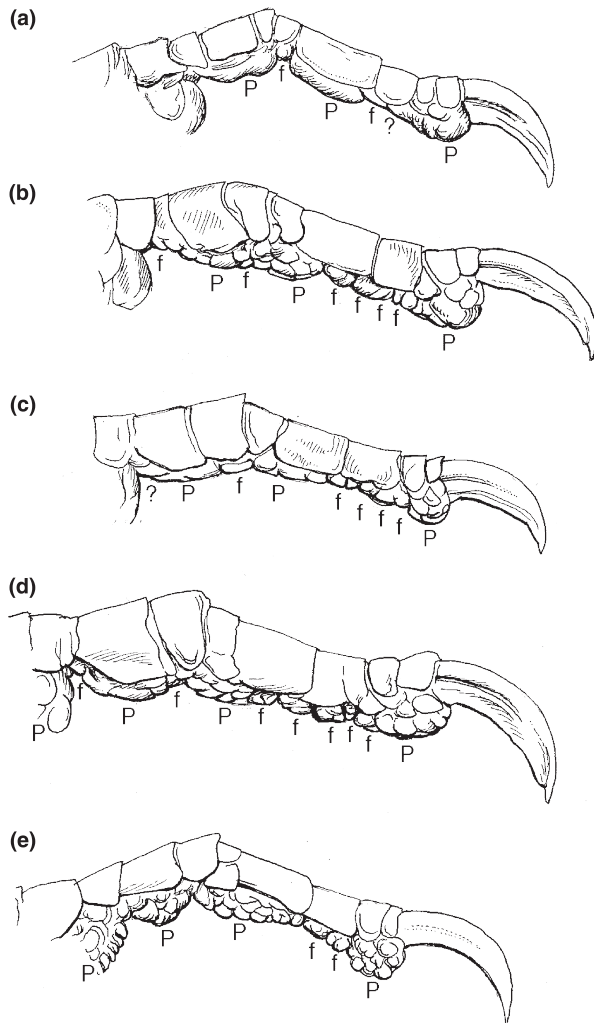


Figure 3. Lateral view of digit II of: Black-throated Tit *Aegithalos concinnus* (a), White-browed Tit-warbler *Leptopoeile sophiae* (b), Chestnut-capped Flycatcher *Erythrocerus mccallii* (c), Scrub Warbler *Scotocerca inquieta* (d) and Cricket Longtail *Spiloptila clamans* (e). The first three species forage primarily in trees and/or scrub, whereas the two latter species are desert birds, which feed mainly on the ground. P marks toe-pads, f marks folds with transverse rows of papillae separated by furrows. Drawings by Jon Fjeldså.

DISCUSSION

Scotocerca's sister relationships to Cettiidae is well supported by the molecular data, and the close affinity to species now placed in Cisticolidae, which has been assumed by most previous authors (e.g. Vaurie 1959, Watson *et al.* 1986, Urban *et al.* 1987, Sibley & Monroe 1990, Dickinson 2003, Ryan 2006), is strongly refuted. However, the molecular evidence for a close relationship between the *Scotocerca*/Cettiidae clade and *Erythrocerus* and Aegithalidae is best considered as tentative. It is apparent that *Scotocerca* was misplaced in the cisticolid group because of the widespread practice of earlier taxonomists to embed aberrant and poorly known birds in larger groups based on superficial resemblance and geographical proximity. Molecular systematics have now demonstrated that many of the large, traditional passerine groups, such as 'warblers', 'chats' and 'flycatchers', are functional groups rather than clades (e.g. Cibois & Cracraft 2004, Voelker & Spellman 2004, Alström *et al.* 2006, Outlaw *et al.* 2010, Sangster *et al.* 2010, Zuccon & Ericson 2010), and need to be re-defined before we can start meaningful analyses of biogeography and patterns of biological diversification.

Although too few morphological characters were assessed to allow for rigorous character analysis, it is noteworthy that the morphological traits characterized are indeed concordant with the molecular phylogeny. This applies to structural features such as facial bristles, number of rectrices, shape of the tail, and structure of the claws and toe-pads, where *Scotocerca* resembles the Cettiidae rather than members of the Cisticolidae. We note, however, that *Scotocerca* also resembles the Aegithalidae, especially the genus *Leptopoeile* and *Erythrocerus*, which are more distantly related to *Scotocerca*. Accordingly, these shared traits might

represent ancestral traits. The facial bristles of *Scotocerca*, described by Meinertzhagen (1949) as an adaptation to exclude blown sand, are in fact shared by species inhabiting non-desert habitats, so a sensory function or aid in capturing insects would seem more probable.

Scotocerca, Aegithalidae, *Erythrocerus* and most Cettiidae differ from most other oscines in details of their feet. Typical oscines have 13 well-developed toe-pads with a rough surface of dense wart-like papillae (Fig. 3f), assumed to facilitate perching on slender twigs (Lennerstedt 1973). However, this structure is not very prominent in most Cettiidae, *Scotocerca*, Aegithalidae and *Erythrocerus*, where much of the soles are composed of transverse rows of papillae ('folds') separated by furrows, and where the remaining pads have rather flat papillae. Such structures have been described for terrestrial oscines (e.g. Alaudidae, Passeridae), and also for some species which move on tree trunks (*Certhia*, treecreepers) or on terminal branches of coniferous trees (*Regulus*, goldcrests/kinglets) (Lennerstedt 1973, Fjeldså *et al.* 2010). The short and thick toes with strong and blunt claws in *Scotocerca* could be thought to be adaptations for foraging on the ground, but the similar condition across several genera that are here suggested to be part of the same clade may instead suggest that this could be a shared ancestral adaptation to allow for greater flexibility in vegetation where perching on thin twigs is often difficult, because of spines, lichens and tiny sclerophyllic leaves (as in shrubs or conifers, preferred by *Leptopoeile*, or a dense foliage of small leaves of Mimosaceae and Caesalpinaceae trees, preferred by *Erythrocerus*). Moreover, White-browed Tit-warbler *Leptopoeile sophiae* and several Cettiidae often feed on the ground. *Aegithalos* may secondarily have developed large, swollen toe-pads (of a distinctive, smooth type) to climb among the thin twigs of deciduous trees and shrubs.

In general, the results of the molecular analyses are congruent with those of other recent studies, which have had a more comprehensive taxon sampling, but have been based on fewer or partly different loci (Beresford *et al.* 2005, Alström *et al.* 2006, Jönsson & Fjeldså 2006, Johansson *et al.* 2008, Gelang *et al.* 2009). Although most internal nodes are strongly supported by the Bayesian analysis, several lack maximum likelihood bootstrap support, and these are invariably associated with very short branch lengths. It seems likely that the Bayesian posterior probabilities have been

spuriously inflated in these cases (cf. Lewis *et al.* 2005, Yang 2007). Since these nodes are only recovered by one or even none of the single-locus analyses, they need to be corroborated by additional data.

Detailed studies of the phylogeography and vocalizations of the Scrub Warbler are warranted, as the large genetic distance between our North African *cyt b* sequence and a *cyt b* sequence in GenBank of unknown geographical origin (probably Asia) suggests that more than one species may be present.

We are grateful to all who generously provided samples: the Australian National Wildlife Collection (CSIRO), Canberra, Australia; David Willard and The Field Museum of Natural History, Chicago, USA; Göran Frisk, Ulf Johansson and Peter Nilsson and the Swedish Museum of Natural History, Stockholm, Sweden; Sharon Birks and the University of Washington Burke Museum, Seattle, USA; Jan Bolding Kristensen and the Zoological Museum of the University of Copenhagen, Copenhagen, Denmark; as well as Geoff Carey and Paul Leader. Mark Adams and Robert Prys-Jones are thanked for granting access to the collection at the Natural History Museum, Tring, and for providing general assistance. Joel Edwartz and Mikael Edwartz provided valuable help in the field. Hadoram Shirihai and Lars Svensson are acknowledged for letting us look at the texts on Scrub Warbler for their forthcoming Handbook of Western Palearctic Birds. The study was financed by the Swedish Research Council grant no. 621-2006-3194 (to U.O.), and P.A. gratefully acknowledges the Riksmusei Vänners Linnaeus Award, which allowed him to devote time to this study.

REFERENCES

- Aleixo, A. & Pacheco, J.F. 2006. A family name for the monotypic oscine passerine genus *Donacobius*. *Rev. Bras. Ornitol.* **14**: 172–173.
- Allen, E.S. & Omland, K.E. 2003. Novel intron phylogeny supports plumage convergence in orioles (*Icterus*). *Auk* **120**: 961–969.
- Alström, P., Ericson, P.G.P., Olsson, U. & Sundberg, P. 2006. Phylogeny and classification of the avian superfamily Sylvioidea. *Mol. Phylogenet. Evol.* **38**: 381–397.
- Barhoum, D.N. & Burns, K.J. 2002. Phylogenetic relationships of the Wrentit based on mitochondrial cytochrome *b* sequences. *Condor* **104**: 740–749.
- Beresford, P., Barker, F.K., Ryan, P.G. & Crowe, T.M. 2005. African endemics span the tree of songbirds (Passeri): molecular systematics of several evolutionary 'enigmas.' *Proc. Proc. R. Soc. Lond., B, Biol. Sci.* **272**: 849–858.
- Cibois, A. & Cracraft, J. 2004. Assessing the passerine "Tap-stry": phylogenetic relationships of the Muscicapoidae inferred from nuclear DNA sequences. *Mol. Phylogenet. Evol.* **32**: 264–273.

- Cramp, S.** (ed.) 1992. *The Birds of the Western Palearctic*, Vol. 6 Oxford: Oxford University Press.
- Dickinson, E.C.** 2003. *The Howard and Moore Complete Checklist of the Birds of the World*. London: Christopher Helm.
- Drovetski, S.V., Zink, R.M., Fadeev, I.V., Nesterov, E.V., Koblik, E.A., Red'kin, Y.A. & Rohwer, S.** 2004. Mitochondrial phylogeny of *Locustella* and related genera. *J. Avian Biol.* **35**: 105–110.
- Drummond, A.J. & Rambaut, A.** 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**: 214.
- Drummond, A.J. & Rambaut, A.** 2009. BEAST. Version 1.5.4. <http://beast.bio.ed.ac.uk>.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A.** 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* **4**: e88.
- Ericson, P.G.P. & Johansson, U.S.** 2003. Phylogeny of Passerida (Aves: Passeriformes) based on nuclear and mitochondrial sequence data. *Mol. Phylogenet. Evol.* **29**: 126–138.
- Fjeldså, J., Zuccon, D., Irestedt, M., Johansson, U.S. & Ericson, P.G.P.** 2003. *Sapayoa aenigma*: a new world representative of “Old World suboscines”. *Proc. Biol. Sci.* **27**: S238–S241.
- Fjeldså, J., Zuccon, D., Irestedt, M. & Ericson, P.G.P.** 2010. The Cinnamon Ibon *Hypocryptadius cinnamomeus* is a forest canopy sparrow. *Ibis* **152**: 747–760.
- Fregin, S., Haase, M., Olsson, U. & Alström, P.** 2009. Multi-locus phylogeny of the family Acrocephalidae (Aves: Passeriformes) – The traditional taxonomy overthrown. *Mol. Phylogenet. Evol.* **52**: 866–878.
- Fuchs, J., Fjeldså, J., Bowie, R.C.K., Voelker, G. & Pasquet, E.** 2006. The African warbler genus *Hyliota* as a lost lineage in the Oscine songbird tree: molecular support for an African origin of the Passerida. *Mol. Phylogenet. Evol.* **36**: 186–197.
- Gelang, M., Cibois, A., Pasquet, E., Olsson, U., Alström, P. & Ericson, P.G.P.** 2009. Phylogeny of babblers (Aves, Passeriformes): major lineages, family limits and classifications. *Zool. Scr.* **38**: 225–236.
- Gu, X., Fu, Y.-X. & Li, W.-H.** 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Mol. Biol. Evol.* **12**: 546–557.
- Hasegawa, M., Kishino, H. & Yano, T.** 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**: 160–174.
- Helbig, A.J., Seibold, I., Martens, J. & Wink, M.** 1995. Genetic differentiation and phylogenetic relationships of Bonelli's Warbler *Phylloscopus bonelli* and Green Warbler *Phylloscopus nitidus*. *J. Avian Biol.* **26**: 139–153.
- Huelsenbeck, J.P. & Ronquist, F.** 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754–755.
- Irestedt, M., Gelang, M., Sangster, G., Olsson, U., Ericson, P.G.P. & Alström, P.** 2011. Neumann's Warbler *Hemitesia neumanni* (Sylviidae): the sole African member of a Palearctic Miocene avifauna. *Ibis* **153**: 78–86.
- Jobb, G.** 2008. *Treefinder*. Munich: Germany. Distributed by the author at <http://www.treefinder.de>. (version June 2008).
- Jobb, G., von Haeseler, A. & Strimmer, K.** 2004. Treefinder: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evol. Biol.* **4**: 18.
- Johansson, U.S., Fjeldså, J., Lokugalappatti, L.G.S. & Bowie, R.C.K.** 2007. A nuclear DNA phylogeny and proposed taxonomic revision of African greenbul (Aves, Passeriformes, Pycnonotidae). *Zool. Scr.* **36**: 417–427.
- Johansson, U.S., Fjeldså, J. & Bowie, R.C.K.** 2008. Phylogenetic relationships within Passerida (Aves: Passeriformes): a review and a new molecular phylogeny based on three nuclear intron markers. *Mol. Phylogenet. Evol.* **48**: 858–876.
- Jönsson, K.A. & Fjeldså, J.** 2006. A phylogenetic supertree of oscine passerine birds (Aves: Passeri). *Zool. Scr.* **35**: 149–186.
- Jönsson, K.A., Fjeldså, J., Ericson, P.G.P. & Irestedt, M.** 2007. Systematic placement of an enigmatic Southeast Asian taxon *Eupetes macrocerus* and implications for the biogeography of a main songbird radiation, the Passerida. *Biol. Lett.* **3**: 323–326.
- Lanave, C., Preparata, C., Saccone, C. & Serio, G.** 1984. A new method for calculating evolutionary substitution rates. *J. Mol. Evol.* **20**: 86–93.
- Lennerstedt, I.** 1973. *Pads, furrows and p0061pillae in the foot of birds*. PhD thesis, University of Lund, Sweden.
- Lewis, P.O., Holder, M.T. & Holsinger, K.E.** 2005. Polytomies and Bayesian phylogenetic inference. *Syst. Biol.* **54**: 241–253.
- Meinertzhagen, R.** 1949. Notes on Saudi Arabian birds. *Ibis* **91**: 465–482.
- Nguembock, B., Fjeldså, J., Tillier, A. & Pasquet, E.** 2007. A phylogeny for the Cisticolidae (Aves: Passeriformes) based on nuclear and mitochondrial DNA sequence data, and a re-interpretation of a unique nest-building specialization. *Mol. Phylogenet. Evol.* **42**: 272–286.
- Nguembock, B., Fjeldså, J., Couloux, A., Cruaud, C. & Pasquet, E.** 2008. Polyphyly of the genus *Apalis* and a new generic name for the species *pulchra* and *ruwenzorii*. *Ibis* **150**: 756–765.
- Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L.** 2004. Bayesian phylogenetic analysis of combined data. *Syst. Biol.* **53**: 47–67.
- Olsson, U., Alström, P., Ericson, P.G.P. & Sundberg, P.** 2005. Non-monophyletic taxa and cryptic species – Evidence from a molecular phylogeny of leaf-warblers (*Phylloscopus*, Aves). *Mol. Phylogenet. Evol.* **36**: 261–276.
- Outlaw, R.K., Voelker, G. & Bowie, R.C.K.** 2010. Shall we chat? Evolutionary relationships in the genus *Cercomela* (Muscicapidae) and its relation to *Oenanthe* reveals extensive polyphyly among chats distributed in Africa, India and the Palearctic. *Mol. Phylogenet. Evol.* **55**: 284–292.
- Panov, E.N.** 2003. Ethology of the shrub warbler *Scotocerca inquieta* and the graceful prinia *Prinia gracilis* related to the problem of the family Sylviidae (Sylviidae, Passeriformes). *Zool. Zhurnal* **82**: 402–412.
- Posada, D.** 2008. jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**: 1253–1256.
- Rambaut, A.** 2009. *FigTree*. Version 1.3.1. Available from <http://tree.bio.ed.ac.uk/software/figtree>.
- Rambaut, A. & Drummond, A.J.** 2009a. *Tracer*. Version 1.5. Available at <http://beast.bio.ed.ac.uk>.
- Rambaut, A. & Drummond, A.J.** 2009b. *TreeAnnotator*. Version 1.5.4. 2009. Available at <http://beast.bio.ed.ac.uk>.
- Rambaut, A., Drummond, A.J. & Xie, W.** 2009. *BEAUti*. Version 1.5.4. 2009. Available at <http://beast.bio.ed.ac.uk>.

- Rasmussen, P.C. & Anderton, J.C. 2005. *Birds of Southern Asia: The Ripley Guide*. Barcelona: Lynx Edicions.
- Reeves, A.B., Drovetski, S.V. & Fadeev, V. 2008. Mitochondrial DNA data imply a stepping-stone colonization of Beringia by Arctic Warbler *Phylloscopus borealis*. *J. Avian Biol.* **39**: 567–575.
- Rodríguez, J., Oliver, L., Marín, A. & Medina, R. 1990. The general stochastic model of nucleotide substitution. *J. Theor. Biol.* **142**: 485–501.
- Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Roy, M.S. 1997. Recent diversification in African greenbulbs (Pycnonotidae: *Andropadus*) supports a montane speciation model. *Proc. R. Soc. Lond. B* **264**: 1337–1344.
- Ryan, P.G. 2006. Family Cisticolidae. In del Hoyo, J., Elliott, A. & Sargatal, J. (eds) *Handbook of the Birds of the World*, Vol. 11: 378–490. Barcelona: Lynx Edicions.
- Sangster, G., Alström, P., Forsmark, E. & Olsson, U. 2010. Multilocus phylogenetic analysis of Old World chats and flycatchers reveals extensive paraphyly at family, subfamily and genus level (Aves: Muscicapidae). *Mol. Phylogenet. Evol.* **57**: 380–392.
- Sefc, K.M., Payne, R.B. & Sorenson, M.D. 2003. Phylogenetic relationships of African Sunbird-like Warblers: Moho (*Hypergus atriceps*), Green Hylia (*Hylia prasina*) and Tit-hylia (*Pholidornis rushiae*). *Ostrich* **74**: 8–17.
- Sharpe, R.B. 1883. *Catalogue of the Passeriformes, or Perching Birds, in the Collection of the British Museum, Part 4*. London: Trustees of the British Museum.
- Sheldon, F.H., Whittigham, L.A., Moyle, R.G., Slikas, B. & Winkler, D.W. 2005. Phylogeny of swallows (Aves: Hirundinidae) estimated from nuclear and mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **35**: 254–270.
- Sibley, C.G. & Monroe, B.L. Jr 1990. *Distribution and Taxonomy of Birds of the World*. New Haven: Yale University Press.
- Sorenson, M.D. & Payne, R.B. 2001. A single ancient origin of brood parasitism in African finches: implications for host-parasite coevolution. *Evolution* **55**: 2550–2567.
- Sorenson, M.D. & Quinn, T.W. 1998. Numts: a challenge for avian systematics and population biology. *Auk* **115**: 214–221.
- Sorenson, M.D., Ast, J.C., Dimchev, D.E., Yuri, T. & Mindell, D.P. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Mol. Phylogenet. Evol.* **12**: 105–114.
- Tavaré, S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. *Lec. Math. Life Sci.* **17**: 57–86.
- Urban, E.K., Fry, C.H. & Keith, S. 1997. *The Birds of Africa*, Vol. 5. London: Academic Press.
- Vaurie, C. 1955. Systematic notes on Palearctic birds. No. 18. Supplementary notes on Corvidae, Timaliinae, Alaudidae, Sylviinae, Hirundinidae, and Turdinae. *Am. Mus. Novit.* **1753**: 1–19.
- Vaurie, C. 1959. *The Birds of the Palearctic Fauna. A Systematic Reference. Order Passeriformes*. London: Witherby.
- Voelker, G. & Spellman, G.M. 2004. Nuclear and mitochondrial DNA evidence of polyphyly in the avian superfamily Muscicapidae. *Mol. Phylogenet. Evol.* **30**: 386–394.
- Watson, G.E., Traylor, M.A. Jr & Mayr, E. 1986. Family Sylviidae. In Mayr, E. & Cottrell, G.W. (eds) *Checklist of Birds of the World*, Vol 11: 3–292. Cambridge, MA: Museum of Comparative Zoology.
- Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J. Mol. Evol.* **39**: 306–314.
- Yang, Z. 2007. Fair-balance paradox, star-tree paradox, and Bayesian phylogenetics. *Mol. Biol. Evol.* **24**: 1639–1655.
- Zink, R.M., Pavlova, A., Rohwer, S. & Drovetski, S. 2006. Barn swallows before barns: population histories and intercontinental colonization. *Proc. R. Soc. Lond. B* **273**: 1245–1251.
- Zuccon, D. & Ericson, P.G.P. 2010. A multi-gene phylogeny disentangles the chat-flycatcher complex (Aves: Muscicapidae). *Zool. Scr.* **39**: 213–224.

Received 19 August 2010;
 revision accepted 27 October 2010.
 Associate Editor: Martin Collinson.