A Large Evaluation of Passerine Cisticolids (Aves: Passeriformes): More About Their Phylogeny and Diversification

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Abstract: African warblers (or Cisticolidae family) are small perching song birds with a large number of genera distributed throughout the World. The phylogeny of Cisticolidae is well-supported but their diversification is currently poorly known. To deepen their understanding of phylogeny and investigate their diversification, we sequenced four loci (mitochondrial ATPase 6, ND2 and ND3, and nuclear myoglobin intron 2) for several new cisticolid taxa and added several other sequences. Our analyses retrieve the monophyly of the African warblers and confirm *Neomixis* as their deepest branch. A group of taxa appear as their potential sister-taxa with our ND2 analyses but not with our combined analyses. New relationships are well-supported. Thus *Scotocerca inquieta* nests in the cettid clade whereas *Camaroptera superciliaris*, *Cisticola chubbi*, *Cisticola tinniens*, *Prinia flavicans* and *Poliolais lopezi* belong to the cisticolid clade. Our results support a splitting of the African warblers in two main clades. The first clade consists of genera *Orthotomus*, *Prinia*, *Cisticola*, *Scepomycter*, *Incana*, *Bathmocercus*, *Eminia*, *Hypergerus* and *Heliolais* while the second includes genera *Poliolais*, *Camaroptera*, *Urolais*, *Artisornis*, *Oreolais*, *Apalis*, *Schistolais*, *Calamonastes* and *Spiloptila*. Our results confirm the polyphyly of *Orthotomus* which generates a muddle between some *Cettia* species and other Asian tailorbirds. Waiting a study with *Orthotomus sepium* Horsf., 1821, to clarify their taxonomy, we suggest temporarily that the name *Phyllergates cucullatus* Temminck, 1836, refers to *Orthotomus cucullatus* of the present study. Our dating analysis reveals that cisticolid clades began their diversification during the transition Early-Middle Miocene epoch and at the beginning of the Pliocene epoch. The diversification of the "open cisticolid" clade would have occurred during the Middle Miocene but that of the "forest cisticolid" lineage would have taken place during the Upper Miocene.

Keywords: African warblers, maximum-likelihood, Bayesian inference, polyphyly, taxonomy, molecular dating, diversification, Miocene, Pliocene.

INTRODUCTION

African warblers represent an Old World passerine clade of tropical birds which has been established [1]. Several cisticolid genera are endemic to Africa or of obvious African origin. These warblers are small (around 9 cm) but some are medium size (19–20 cm). Cisticolid taxa often have underparts and head more distinctively patterned than their upperparts. On average, males are larger than females and the length of their tail is often more dimorphic [2]. The cisticolid bill is rather short, slender or long. Their wings are short and rounded but the number of tail feathers varies among the genera. Some cisticolid genera like *Orthotomus*, *Heliolais*, *Cisticola*, *Incana*, *Camaroptera*, *Calamonastes*, *Euryptila* and *Spiloptila* have twelve rectrices and others only ten or eight [2-4]. The cisticolid plumage colour varies from drab and cryptic to brightly coloured.

For many years, the cisticolid clade was included in the Old World Warblers (*Sylviidae*) but after the work of Sibley and Ahlquist [1], it has been erected to the family level. Afterwards, several molecular studies have strongly confirmed this clade [5-8]. Interestingly, considerable taxonomic advances have been achieved in keeping with the cisticolid clade. Several studies showed that *Rhopophilus pekinensis* did not belong to cisticolid clade but genera *Bathmocercus* and *Orthotomus* belonged to it [8, 3]. Concerning tailorbirds, Nguembock *et al*. [3] showed that African tailorbirds (*Artisornis*) were distantly related to Asian tailorbirds (*Orthotomus*). Otherwise, Nguembock *et al*. [3] found that the Moreau Warbler, which was always included in the genus *Bathmocercus*, was rather distantly related to it; they used this result to resurrect the genus *Scepomycter* [3].

In another study, Nguembock *et al*. [4] proposed a new generic name, *Oreolais*, for odd putative apalises *pulchra* and *ruwenzorii*. Johansson *et al*. [9] found that the ancient sylviid genus *Eremomela* nested within the cisticolid clade. Equally, Nguembock *et al*. [10] also found that *Scotocerca*

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inquieta nested within the cettid clade. Other subjects have been developed concerning this clade, for instance, the adaptation of the main basic nest type to their environment, the evolution of the stitching technique in "tailorbirds" but in return these studies did not shyly tackle subjects in keeping with their sister group, origin or diversification [3, 6].

According to diverse authors [3, 4, 8-11], the cisticolid clade constitutes a well diversified family which includes approximately 29 genera. These genera are distributed throughout the World and their origin is currently unknown. Our first results [3] suggested that some genera should have colonized the African mainland but we do not know how or when. In this study [3], we found that the insular genus *Neomixis* was their basal branch. Their closest relatives are currently poorly known. Beresford *et al*. [7] found that the cisticolid clade was close to some Pycnonotidae or Timaliidae taxa but this relationship was unresolved. Often regarded to be close to sylviid taxa, Alström *et al*. [8] failed to confirm this relationship while Nguembock *et al*. [3] obtained just a moderate support for the Yellow Longbill like their sister-taxon but only with the ND2 gene.

Aims of this study which includes several new cisticolid taxa were to enlarge a thorough knowledge of this group, to investigate their relationships and to obtain support for their sister-taxa. Otherwise leaning on our molecular dating, we aimed to discuss preliminary elements of their origin and diversification. We used 2716 bp of aligned sequenced data obtained from three mitochondrial protein-coding genes (ATPase 6, ND2 and ND3) and one intron (myoglobin intron 2).

MATERIALS AND METHODS

Taxon Sampling

Samples used comprised tissues from fresh specimens as well as Genbank and Embl-bank sequences. Fresh tissues came from banks of tissues (ZMUC, MNHN and FMNH) and others directly collected in Cameroonian highlands forests. We included 20 some 28 potential genera listed under Cisticolidae [12] and updated by recent molecular works [3, 4, 8, 9]. We added several taxa which are closely related to the cisticolid clade [3, 4, 7, 8] and other insular genera which could be closest relatives of this clade [5, 13]. To discuss the origin and the diversification of the cisticolid taxa within the Passerida, we additionally sampled 11 oscine passerine families recognized in Dickinson [12] and two recent suggested families (Stenostiridae and Picathartidae) [7, 14, 15] (Annexe). We selected one Monarchid, *Tersiphone rufiventer*, as an outgroup which was demonstrated outside the cisticolid clade [16]. Our molecular dataset was also increased by including ND2 and myoglobin intron 2 sequences taken in Genbank and Embl-bank (**Annexe**). A total of 123 Operational Taxonomic Units are included in our analyses (Annexe).

Laboratory Procedure

Genomic DNA was isolated from fresh tissues (blood or muscle) using a CTAB-based protocol [17]. We amplified and sequenced four gene regions which are known to have different evolutionary rates in birds, one evolving slowly (myoglobin intron 2) and others more rapidly (ATPase 6, ND2 and ND3). The mitochondrial ATPase 6 (684 bp) and ND3 (351 bp) were amplified as a single fragment [18, 19] whereas ND2 (1041 bp) as a single fragment [20] or as several smaller fragments. The nuclear myoglobin intron 2 (~ 624 bp) was amplified as a single fragment and conditions described by Heslewood *et al*. [21] and Slade *et al*. [22] or as several smaller fragments. All these primers are detailed in Table **1**.

For amplifications, cycling conditions were standard for these four markers [3, 4, 10]. Three microliters of the PCR-amplification product was electrophoresed on a 1.5% agarose gel and visualized under UV light with ethidium bromide to check the size of the fragment and hence ensure that the correct fragment had been amplified. We purified the PCR-amplification product using the 'QiaQuick PCR Purification Kit' (Qiagen, Holden, Germany). The purified products were cycle-sequenced using a 'Big Dye' (Applied Biosystems Inc, Forster City, CA, USA). Sequences were obtained on an ABI3100 sequencer. All sequences of ND2, ND3 and ATPase 6 were aligned using the algorithm in Sequencher 3.1. No insertions, deletions and stop-codons were detected in the reading frame of these three protein-coding genes suggesting that our sequences are of mitochondrial origin and not nuclear pseudogenes [23]. For the nuclear intron, sequences were aligned using Sequencher 3.1 and then exported to BioEdit v6.0.5 [24] where they were aligned more precisely by eye; these alignments indicated the presence of several insertion/deletion events. The occurrence of Single Nucleotide Polymorphisms (SNPs) in this locus was suggested by the presence of double peaks; theses double peaks were coded using the appropriate IUPAC codes.

Phylogenetic Analyses

Two methods were used to infer phylogenetic relationships. Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were conducted for each dataset separately (ND2-96 taxa; ATPase 6-79 taxa; ND3-80 taxa; myoglobin intron 2-103 taxa) and for the combined dataset (67 taxa). Gaps were treated as missing data. The topologies and parameters estimated were obtained for the ML and BI with PhyML v2.4 [25] and MrBayes v.3.1 [26], respectively. The model of sequence evolution was selected using the Akaike Information Criterion as implemented in the MrMODELTEST 2.0, running in conjunction with PAUP* 4.0b [27-29]. The selected models were then used for the separate ML analyses; these models and parameters estimated (base frequencies, rate matrix, shape parameter, proportion of invariable sites) are detailed in legends of the figures (Figs. **1-4**). Nodal supports in ML were estimated with 1000 bootstrap pseudo-replicates [30].

Bayesian inference was conducted for each dataset, and models as determined by MrMODELTEST 2.0 were applied to estimate optimal parameters. Four incrementally heated Metropolis-coupled Monte Carlo Markov chains were run for three million generations with trees sampled every 100 generations (30 001 trees sampled). With our sample, the stationarity was rapidly reached; the first 300 000 genera-

Fig. (1). Bayesian inference tree (mean log-likelihood value = 31103.21 SD = ± 2.89) obtained from the mitochondrial ND2 sequences under a GTR + I + G model of sequence evolution. Asterisks above the nodes indicate posterior probabilities (≥ 0.95) and those below, PhyML bootstrap values (≥0.70). Base frequencies: A = 0.40040, C = 0.36705, G = 0.06865, T = 0.16389, revmatrix = 0.20382, 6.06480, 0.32130, 0.21098, 3.24139, α = 0.688, I = 0.26 (estimated from PhyML) and Maximum likelihood tree (-ln = 24286.39) obtained from the mitochondrial ND2 gene is not shown.

tions (3000 trees) were discarded ('burn-in' period) and posterior probabilities (PP) were estimated from the remaining sampled generations. Four independent Bayesian runs initiated from random starting trees were performed for each dataset. Log-likelihood values and posterior probabilities across the four runs were also checked to warrant that chains had reached stationarity. For the combined Bayesian analysis, we used the selected models in a partitioned analysis (genes and codon positions) with the "unlink" command.

The topologies and nodal support obtained from different models were compared to detect incongruences. A node was considered to be supported if recovered with greater than 70% for bootstrap support [31] or with a posterior probability greater than 0.95 [32].

Analysis of Time of Divergence

For the diversification, we used the same method and calibration point already published in Zoologica Scripta [14] and in Journal of Avian Biology [33].

Thus, we used a Bayesian approach for estimates of divergence time using the MULTIDISTRIBUTE Software package [34, 35]. Our aim was to establish firstly the time of separation between other passeridan taxa and the cisticolid clade; secondly, we aimed to find time periods within the cisticolid clade in order to discuss the origin and the diversification of the cisticolid taxa in keeping with geological events which took place during the corresponding time periods. We performed dating analyses in a relative time frame
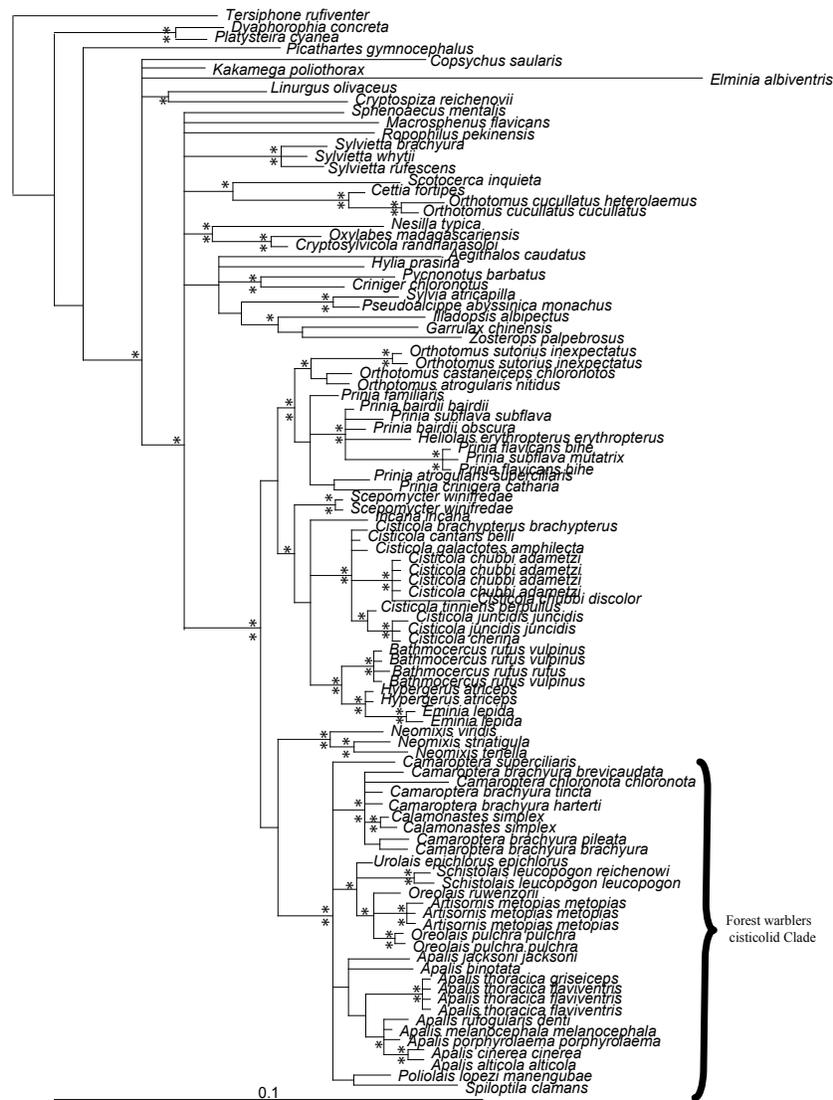
Fig. (2). Bayesian inference tree (mean log-likelihood value = 5552.09 sd = ± 0.78) obtained from the nuclear myoglobin intron 2 sequences under a GTR + I + G model of sequence evolution. Asterisks above the nodes indicate posterior probabilities (≥ 0.95) and those below, PhyML bootstrap values (≥0.70). Base frequencies: A =0.30032, C = 0.21796, G = 0.23139, T = 0.25033, revmatrix = 1.30146, 4.28906, 0.75131, 1.39321, 4.67474, α = 1.97, I = 0 (estimated from PhyML) and Maximum likelihood tree (-ln = 3997.52) obtained from the my-oglobin intron 2 is not shown.

work because no reliable fossil calibration point is available within this group; for this, we used a secondary calibration point derived from the previous study of Barker *et al*. [16] as well as a scale of time to convert time periods obtained. As a calibration point, we used the dispersal from Australasia (Picathartidae) (47.6 - 42.4). We set the basal node to time units and the distance between the tip and the root to 82 Myrs (± 41 Myrs); this date corresponds to the estimation of the split between Acanthisittidae and other passerines [16]. And as priors, we adopted 0.0089 ± 0.0089 substitution per site per time unit for the rate at the root node (estimated using the procedure described in the multidivtime.readme file). The Bayesian topology obtained with the combined dataset (ND2 and myoglobin intron 2) was specified for dating analyses and we used lower and upper constraints to account for the standard deviation.

RESULTS

Individual and Concatenated Datasets

We obtained between 617 (*Scotocerca inquieta* MNHN) and 1041 bp (majority of samples) for the ND2. Out of the full alignment of 1041 characters, 628 (60.33%) were parsi-mony informative and 718 (68.97%) variable. MrMODELTEST selected the GTR + G + I as the best-fit model. For Bayesian inference, the average of the four runs resulted in –ln = 31103.21 with a SD = ± 2.89. Both BI and ML recovered the same topology which received high boot-strap and posterior probabilities (Fig. **1**).

With the myoglobin intron 2, we obtained between 634 (*Prinia flavicans bihe* MNHN) and 640 bp (majority of sam-ples). Indels were observed of which the most noteworthy

Table 1. **Primers Sequences Used for Amplification and Sequencing**

Primers	Sequence	References
L5219Met (ND2)	5' CCC ATA CCC CGA AAA TGA TG 3'	Sorenson *et al.* (1999)
INTL (ND2)	5' TGR ATR GGV CTN AAY CAR AC 3'	Nguembock *et al.* (2007)
INTH (ND2)	5' GNG MGM GAT RAK GAG AAG GTC A 3'	Nguembock *et al.* (2007)
H6313Trp (ND2)	5' CTC TTA TTT AAG GCT TTG AAG GC 3'	Sorenson *et al.* (1999)
ATPase 6 (A8PWL)	5' CCTGAACCTGACCATGAAC 3'	Eberhard and Bermingham (2004)
ATPase 6 (CO3HMH)	5' CACATAGTRGACCCCAGCCCATG 3'	Eberhard and Bermingham (2004)
L10755 (ND3)	5' GACTTCCAATCTTTAAAATCTGG 3'	Chesser (1999)
H11151 (ND3)	5' GATTTGTTGAGCCGAAATCAAC 3'	Chesser (1999)
MYO2 (Myoglobin)	5' GCCACCAGGCACAAGAAGATCCC 3'	Slade *et al.* (1993)
MYO2IntF	5' TRA GAC CCA TAA AAC TAA GTG 3'	Nguembock *et al.* (2007)
MYO3IntR	5' TGA TCT GCT TCA TGA CCT T 3'	Nguembock *et al.* (2007)
MYO3F (Myoglobin)	5' GCAAGGACCTTGATAATGACTT 3'	Heslewood *et al.* (1998), modified

Table 2. **The Most Noteworthy Indels Observed in the Myoglobin Intron 2**

Types of indel identified	Total length of the indels	Taxa sharing each of the different indel
1 – autapomorphic deletion	seven bases	representatives of *Orthotomus*
2 – autapomorphic insertion	one base	representatives of *Orthotomus sutorius*
3 – synapomorphic deletion	seven bases	*Prinia bairdii, Prinia subflava* and *Heliolais erythropterus*
4 – autapomorphic deletion	seven bases	representatives of *Prinia flavicans*
5 – autapomorphic insertion	one base	representatives of *Prinia flavicans*
6 – synapomorphic deletion	four bases	members of the Asiatic prinias
7 – autapomorphic deletion	two bases	samples of *Eminia lepida*
8 – autapomorphic deletion	one base	samples of *Hypergerus atriceps*
9 – synapomorphic insertion	three bases	*Oreolais* and *Artisornis*
10 – synapomorphic deletion	two bases	*Oreolais* and *Artisornis*
11 – synapomorphic deletion	four bases	*Urolais, Schistolais, Oreolais* and *Artisornis*
12 – autapomorphic insertion	one base	members of *Artisornis metopias*

are indicated in the Table **2**. The aligned myoglobin sequences comprised 640 characters, of which 220 (34.37%) and 406 (63.44%) were parsimony informative and variable, respectively. MrMODELTEST designated the GTR + G + I as the best-fit model. Parameters were estimated by PhyML and are given in detail in the legend of the Fig. (**2**). For Bayesian inference, the average of the four runs resulted in –ln = 5552.09 (sd = ± 0.78). Both ML and BI recovered the same topology which received moderate bootstrap and posterior probability support (Fig. **2**).

We obtained between 485 (*Bathmocercus rufus vulpinus* ZMUC/CDI) and 684 bp (majority of samples) for the ATPase 6. Out of the full alignment of 684 characters, 350 (51.17%) and 381 (55.7%) were parsimony informative and

variable, respectively. MrMODELTEST selected the GTR + G + I as the best-fit model. For Bayesian inference, the average of the four runs resulted in –ln = 15183.45 with a SD = ± 5.8. Both BI and ML recovered the same topology which received moderate bootstrap and posterior probability support (Fig. **3**).

For the ND3, sequences length ranged from 226 bp (*Platysteira cyanea* MNHN) to 351 bp (majority of samples). Out of the full alignment of 351 characters, 192 (54.7%) were parsimony informative and 218 (62.11%) variable. MrMODELTEST designated the GTR + G + I as the best-fit model. For Bayesian inference, the average of the four runs resulted in –ln = 7909.51 (sd = ± 5.14). Both BI and ML
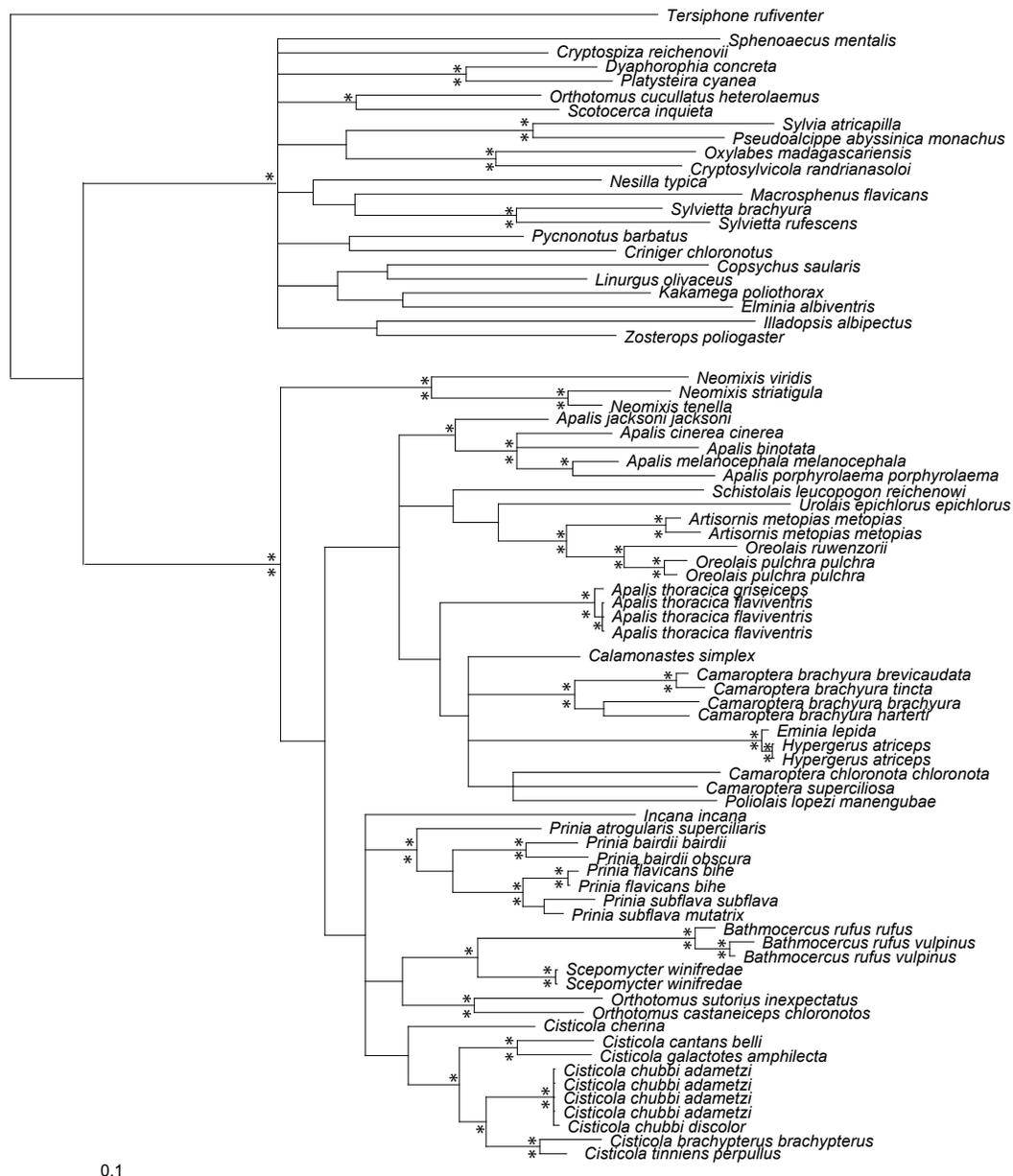
Fig. (3). Bayesian inference tree (mean log-likelihood value = 15183.45 sd = ± 5.8) obtained from the mitochondrial ATPase 6 sequences under a GTR + I + G model of sequence evolution. Asterisks above the nodes indicate posterior probabilities (≥ 0.95) and those below, PhyML bootstrap values (≥0.70). Base frequencies: A = 0.37148, C = 0.40665, G = 0.06987, T = 0.15200, revmatrix = 0.16713, 6.49400, 0.40647, 0.27873, 4.11502, α = 0.496, I = 0.37 (estimated from PhyML) and Maximum likelihood tree (-ln = 12336.75279) obtained from the mitochondrial ATPase 6 gene is not shown.

recovered the same topology which received low bootstrap and posterior probability (tree not shown).

The concatenated dataset included 67 OTUs and 2723 bp. In Bayesian analyses, the concatenated dataset was partitioned by gene and codon position (ten partitions). The four independent runs with the concatenated dataset partitioned yielded a 50% majority consensus rule tree (-ln = 49395.72 ± 1.64, Fig. **4**) where 36 out of 43 nodes of the ingroup taxa were supported by posterior probabilities of greater than 0.95. Not surprisingly our results suggest that the tree based on mitochondrial protein-coding data (ATPase 6, ND2 and ND3) are more resolved than the tree based on nuclear intron sequences (myoglobin intron 2) but the topology based on

the combined dataset is the best resolved. As a consequence, we focus the interpretation of our results almost mainly on the combined analysis.

Phylogenetic Results

No major conflict was observed between four individual genes used, or when compared to the concatenated dataset. The only incongruence involves the position of the basal insular *Neomixis*; ND2 and concatenated dataset suggest that *Neomixis* is basal within the cisticolid clade (Figs. **1** and **4**) whereas myoglobin intron 2 also recovers a basal position of this genus but only for the "forest cisticolid" clade (Fig. **2**).

Table 3.   Inferred Dates of Cisticolidae Divergences. Shown are the Average, Minimum, Maximum and Confidence Interval Values from Relaxed Molecular Clock Analysis Using the MULTIDISTRIBUTE Software Package from the Combined Dataset ND2 Gene and Myoglobin Intron 2. Numbered Nodes are Labelled on Fig. 5

Node	Description	Minimum	Mean	Maximum	Confidence Interval (95%)
1	Other passeridan taxa/Cisticolidae	14.06	15.14	16.22	13.14 – 17.38
2	Basal branch (Neomixis)/other cisticolid taxa	12.51	13.55	14.59	11.63 – 15.7
3	Open warblers clade/other cisticolid taxa	11.9	12.92	13.94	11.02 – 15.01
4	Orthotomus and Prinia/other taxa of the open warblers clade	10.45	11.43	12.4	9.58 – 13.41
5	Forest warblers clade/other cisticolid taxa	10.03	10.99	11.95	9.2 – 12.98
6	Orthotomus/Prinia	8.42	9.34	10.26	7.65 – 11.24
7	Poliolais, Calamonastes and Camaroptera/Urolais, Oreolais, Artisornis and Schistolais	7.92	8.84	9.76	7.16 – 10.74
8	Incana, Scepomycter and Bathmocercus/Cisticola	7.2	8.01	8.82	6.52 – 9.71
9	Schistolais/Urolais, Oreolais and Artisornis	7.06	7.95	8.84	6.31 – 9.83
10	Prinia (Asia)/Prinia (Africa)	6.39	7.21	8.03	5.72 – 8.93

According to our analyses, African warblers can be divided into two main clades, very well delimited with the most informative character partitions, ND2 (Fig. **1**) as well as the combined analysis (Fig. **4**) but differently recovered with other markers (Figs. **2** and **3**). The DNA data give high support for the monophyly of the African warblers (Figs. **1**-**4**). Of the eight multi-specific genera studied, only *Orthotomus* appears polyphyletic (Figs. **1-4**). We will lean on the combined tree for the presentation of the two main clades except for some particular cases where we will use individual trees.

The "open cisticolid" clade, which includes nine currently recognized genera, is moderately supported with the ND2 and concatenated dataset (Figs. **1** and **4**); this clade can be subdivided into two subclades. The first subclade consists of two genera, *Orthotomus* and *Prinia*, but *Heliolais* nests within the genus *Prinia* (Fig. **2**). Within this subclade, *Prinia flavicans* appears close to *P. subflava* and *P. bairdii* is in a basal position to this group (*P. flavicans-P. subflava*); within all prinias, the Asiatic sample always appears in a basal position. *Orthotomus* is polyphyletic by the separate position of *O. cucullatus*; *O. sutorius*, *O. castaneiceps* and *O. atrogularis* are grouped together and always appear closely related to *Prinia* (Figs. **1-4**). The second subclade includes genera *Cisticola*, *Scepomycter*, *Incana* and *Bathmocercus*, but also *Eminia* and *Hypergeris* which are sister-taxa (Figs. **1-4**). *Cisticola* always appears monophyletic but positions of *Bathmocercus*, *Incana* and *Scepomycter* remain unresolved in this study.

The "forest cisticolid" clade, which also comprises nine currently recognized genera, appears strongly supported (Figs. **1**, **2** and **4**). Within this clade, the montane genera *Urolais*, *Schistolais*, *Oreolais* and *Artisornis* form a subclade which is highly supported (Figs. **1** and **2**); always within this subclade, *Artisornis* is close to *Oreolais* and these two genera are closely related to *Urolais* (Figs. **1** and **4**). *Schistolais*

appears in a basal position within this subclade (Fig. **1**). *Poliolais* appears close to *Camaroptera* with the ND2 but not with the concatenated dataset (Figs. **1** and **4**). *Apalis* is monophyletic in this study and *A. thoracica* appears in a basal position (Fig. **4**).

Dating Analysis Results

Results of the dating analyses are presented in Table **3** and Fig. (**5**). The divergence of the cisticolid clade took place during the lower Miocene and the diversification of several genera within this group also took place during the same Miocene epoch. Four nodes are retrieved in the medium Miocene; they include diversifications of the basal branch (*Neomixis*), *Orthotomus* and *Prinia* genera, the "open cisticolid" clade as well as that of the "forest cisticolid" clade. Five nodes are retrieved in the upper Miocene; they include diversifications between taxa recognized as distinct genera [node 6 which represents the split between *Orthotomus*/*Prinia*; node 7 which represents the split between *Schistolais*/*Urolais*/*Oreolais*/*Artisornis* and *Camaroptera*/*Calamonastes*/*Poliolais*; node 8 which represents the split between *Cisticola*/*Bathmocercus*, *Incana* and *Scepomycter*; node 9 which represents the split between *Schistolais*/*Urolais*, *Oreolais* and *Artisornis*; node 10 which represents the split between *Prinia* (Asia)/*Prinia* (Africa)].

DISCUSSION

Phylogeny of the African Warblers

The phylogram obtained represents the best estimate of the relationships within the cisticolid clade and two main clades appear to be strongly supported.

In their phylogeny, *Neomixis* appears to be in a basal position and this result agrees with Nguembock *et al*. [3]. With regard to their sister-group neither Yellow Longbill nor Pycnonotidae nor Timaliidae taxa were found close to Afri-

Fig. (4). Bayesian inference tree (mean log-likelihood value = 49395.72 ± 1.64) obtained from the combined dataset. The optimal parameterization as estimated by MrMODELTEST was assumed for each of ten partitions. Asterisks above the nodes indicate posterior probabilities (≥ 0.95) and those below, PhyML bootstrap values (≥0.70).

can warblers. In return two insular genera, *Oxylabes* and *Cryptosylvicola*, and two other taxa, *Hylia prasina* and *Pholidornis rushiae*, are well-supported as their sister-taxa in our ND2 tree but these two last taxa were lacking in our combined tree. Otherwise, some sylviid taxa, such as the genus *Sylvietta,* were lacking. Thus, their sister-group can be best considered as unresolved and we prefer to wait a further study to confidentially define them.

According to our main results, two clades of the African warblers are well-supported (Figs. **1-4**). These two clades have been suggested by some authors [2, 11] but only this splitting was not based on molecular results. With regard to these authors, the cisticolid clade should be divided into two lineages and our results virtually retrieve their suggestion. The first includes the largest genus, *Cisticola*, a combination of African genera, *Scepomycter*, *Bathmocercus*, *Incana*, *Heliolais*, *Eminia* and *Hypergerus*, an Asian-African genus, *Prinia*, and an Asian genus, *Orthotomus*. The second in-

cludes mainly African genera, *Apalis*, *Schistolais*, *Urolais*, *Poliolais*, *Camaroptera*, *Calamonastes*, *Artisornis* and *Oreolais*.

In reflection to our results, the splitting of the African warblers is almost in agreement with their living environment. Thus, African warblers included in the "open cisticolid" clade are almost all or less birds living in an open environment. These taxa often present brightly plumage colour [2, 11, 36]. Many of their members also show some common characteristics such as the nest structure which is usually enclosed, typically a ball with a side entrance at the top [2, 11, 36].

Also, for taxa in the "forest cisticolid" clade, practically all these birds live in forest, in dense woodland or in more dense areas [2, 11, 36]. This "forest cisticolid" clade was already obtained by Sefc *et al*. [6], Alström *et al*. [8], Nguembock *et al*. [3] and Nguembock *et al*. [4]. This "forest

cisticolid" clade appears as a natural lineage (Figs. **1-2**). Most birds of this second clade generally present drab and cryptic plumage colour except for some taxa [2, 11, 36].

Some Novel Relationships

Prior to this study and in accordance to several others [3-7, 8, 10, 13, 37], only eight of the 25 potential cisticolid genera from Dickinson [12] (*Malcorus*, *Phragmacia*, *Oreophilais*, *Drymocichla*, *Phyllolais*, *Scotocerca*, *Urorhipis* and *Poliolais*) have not been included in a very large molecular study including several other cisticolid taxa. Two of these, in this case *Scotocerca* and *Poliolais*, were included in this study.

The Streaked Scrub-warbler, *Scotocerca inquieta*, is a Palearctic monotypic species. Molecularly, it would have been studied by Sokolov [38] and has been formally studied by Nguembock *et al*. [10]. Preliminary results obtained by these authors have been confirmed by Alström *et al*. [37]. This species was previously placed in the cisticolid clade but according to our combined results (with 2716 bp), the Streaked Scrub-warbler is a cettid taxon (Fig. **4**). Its isolated position was surprising at first glance but it is consistent with several morphological and ecological traits particularly in their juveniles.

Furthermore, the White-tailed Warbler, *Poliolais lopezi*, is a cisticolid taxon (Figs. **1**, **2** and **4**). This taxon is a monospecific genus endemic to the south-east of Nigeria, Cameroon highlands and the Bioko Island. It is a tiny forest warbler with a noteworthy fine and longish bill (B. Nguembock personal observation) and it inhabits mountain forest. According to [2], its affinities are uncertain and our study fails to find its sister-taxon.

Otherwise, for cisticolid taxa newly included in a molecular study (for instance *Camaroptera superciliaris*, *Cisticola tinniens*, *Prinia flavicans*, etc.), several new subspecific, inter-specific and inter-generic relationships appeared strongly supported (Figs. **1**, **2**, **3** and **4**). Our study also confirms a strong relationship between the new cisticolid genus *Oreolais sensu* Nguembock *et al*. [4] and the genus *Artisornis*.

Suggestion of the Taxonomic Change

Our study included two tailorbirds (*Artisornis metopias* and *Orthtotomus sutorius*) which belong to the Cisticolidae family (Figs. **1-4**). But it also includes an Asian species, *Orthotomus cucullatus*, which belongs to the Cettidae family. In the past, this species was included in the cisticolid clade but Alström *et al*. [8] had shown that it was close to *Cettia*. Nguembock *et al*. [3] had confirmed that this taxon was outgroup to a cisticolid clade. To avoid a muddle between this *Cettia* species and other Asian tailorbirds and waiting a large study including the type species of *Orthotomus*, *O. sepium* Horsf., 1821, we propose temporarily that the name *Phyllergates cucullatus* Temminck, 1836, be used for samples hitherto referred to *Orthotomus cucullatus* of the present study.

Diversification of the Cisticolid Taxa

Within the Passerida, Barker *et al*. [16] suggested that the diversification of most groups took place during the Miocene epoch. Like Barker *et al*. [16] concerning the two clades of the African warblers, our data suggest that they began their diversification during the transition Early-Middle Miocene epoch (Table **3**). As a result, we think that it is not careful to suggest a hypothesis on the origin of the cisticolid clade because we did not confidentially resolve their sister-taxa.

Currently, cisticolid taxa are throughout the World but mainly in the African mainland. Their phylogram gives high support for their basal branch (the insular *Neomixis*). We estimated the time period of the insular basal divergence from other remaining continental cisticolid taxa at 13.55 Ma and this date virtually corresponds to that estimated for the arrival of Carnivora to Madagascar [39]. These authors noted that these mammals probably emigrated from Africa. In accordance, the diversification of insular African warblers from continental Africa seems to be likely due to the fact that interchanges between some mammals ranging from Madagascar and Africa have equally been proved during this time frame [39]. The diversification of other cisticolid taxa would have involved dispersal and vicariance processes inside the African mainland.

The diversification of the "open cisticolid" clade would have occurred during the Middle Miocene. As for the diversification of the "forest cisticolid" lineage, it would have taken place during the Upper Miocene (Table **3**). Within this "open cisticolid" clade, at least two intercontinental dispersal events, involving African and Asian mainlands, took place during the Upper Miocene epoch (Fig. **5**). We note that these two intercontinental events coincide with our estimation of the divergence between the Asian *Orthotomus* and the African-Asian *Prinia*. Similarly, the diversification of other endemic African taxa took place during the same Neogene period (Fig. **5**). According to [40], the mammalian faunal interchange between Afro-arabia and Eurasia took place less than 27 Myrs during the Neogene period. We think that the dispersal of some African taxa from Asia would have likely taken place during the same period.

For the diversification of endemic African taxa of the cisticolid clade during the Neogene period, it is well documented that a few geological changes have affected African biota. Particularly, there is a spread of grasslands corresponding to a contraction of the forest cover in the early Miocene, and an uplift of rift shoulders [41, 42]. Moreover, during the Neogene age, the uplift caused the isolation of eastern lowland forests from the main Guinea-Congolian rainforest block [43-46]. Furthermore, some palynological evidence suggests high rainforest biodiversity during the Miocene epoch in Africa [47, 48]. Due to the geological and climatic deterioration, the dispersal of cisticolid clades in Africa was very likely by the break-up of tropical forests during the Neogene period. Within the "forest cisticolid" lineage, our data suggest an early dispersal of genera *Camaroptera*, *Calamonastes* and *Poliolais* during the Late Miocene but the diversification of the genus *Schistolais* took place during the Upper Miocene (Fig. **5**).

Fig. (5). Chronogram obtained from the Bayesian relaxed-clock method of Thorne et al. [35] and Thorne and Kishino [35] and using the Bayesian topology obtained with the combined dataset partitioned by gene and codon position. Numbered nodes labelled on figure correspond to the description given in Table **3**.

CONCLUSION

The phylogeny of the African warblers appears more structured with our study. Firstly their monophyly as well as their basal branch are strongly supported. Secondly this group, which was included during long time in the paraphyletic Old World Warblers, comprises henceforth two main clades (the "open cisticolid" and the "forest cisticolid") with the "forest cisticolid" clade which appears to be a natural lineage. Within these clades, several new relationships are strongly supported.

The diversification of the African warblers in time and in space aligns oneself with previous results [16, 39, 40]. Thus,

the diversification of their two clades would have taken place during the Neogene period. Otherwise in our study, we observe that dispersal and vicariance processes inside the African mainland would have influenced the diversification within the two cisticolid clades.

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CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ABBREVIATIONS

Bp	=	base pairs
ATPase 6	=	ATP synthase subunit 6
ND2	=	NADH dehydrogenase subunit 2
ND3	=	NADH dehydrogenase subunit 3
BI	=	Bayesian Inference
ML	=	Maximum Likelihood
Myrs/Ma	=	Millions years
SD	=	Standard Deviation
OUT	=	Operational Taxonomic Unit

Annexe

Annexe. Names of Taxa (Following Dickinson [12] and Several Recent Molecular Works), Family and Country for Individual Specimens, Museum Voucher or Tissue Numbers and the Length of DNA Sequences Used in This Study. Acronyms are: AMNH, American Museum of Natural History; FMNH, Field Museum of Natural History; NRM, Swedish Museum of Natural History; UMMZ, University of Michigan Museum of Zoology; MNHN, Museum National d'Histoire Naturelle; ZMUC, Zoological Museum of the University (Copenhagen)

Species	Country	Family	Voucher	ATPase6	ND2	ND3	Myoglobin
Cisticolidae							
Apalis alticola alticola	Tanzania	Cisticolidae	ZMUC 123398	-	EU239793	EU247893	EU247917
Apalis binotata	Tanzania	Cisticolidae	ZMUC 133492	JX259140	EU239794	EU247894	EU247920
Apalis cinerea cinerea	Burundi	Cisticolidae	FMNH 358088	JX259106	DQ871370	EU247904	DQ871408
Apalis jacksoni jacksoni	Burundi	Cisticolidae	FMNH 358081	-	DQ871371	EU247905	DQ871409
Apalis melanocephala melanocephala	Tanzania	Cisticolidae	ZMUC 129285	JX259138	EU239795	EU247895	EU247918
Apalis porphyrolaema porphyrolaema	Tanzania	Cisticolidae	ZMUC 123196	JX259141	EU239796	EU247896	EU247921
Apalis rufogularis denti	Kenya	Cisticolidae	MNHN68-1139	-	DQ871387	EU247907	DQ871431
Apalis thoracica griseiceps	Tanzania	Cisticolidae	ZMUC 136606	JX259139	EU239797	EU247897	EU247919
Apalis thoracica flaviventris	Malawi	Cisticolidae	GA94334	JX259146	EU239798	EU247898	EU247922
Apalis thoracica flaviventris	Malawi	Cisticolidae	GA94354	JX259147	EU239799	EU247899	EU247923
Apalis thoracica flaviventris	Malawi	Cisticolidae	GA94346	JX259148	EU239800	EU247900	EU247924
Artisornis metopias metopias	Tanzania	Cisticolidae	ZMUC 119714	-	DQ871363	EU247910	DQ871399
Artisornis metopias metopias	Tanzania	Cisticolidae	ZMUC 119915	JX259099	DQ871364	EU247911	DQ87140
Artisornis metopias metopias	Tanzania	Cisticolidae	FMNH 356778	JX259115	DQ871380	EU247909	DQ871418
Bathmocercus rufus vulpinus	Uganda	Cisticolidae	ZMUC/CDI	JX259096	DQ871359	JX259214	DQ871395
Bathmocercus rufus vulpinus	Uganda	Cisticolidae	ZMUC/CD9	-	DQ871360	JX259215	DQ871396
Bathmocercus rufus vulpinus	Uganda	Cisticolidae	FMNH 385102	JX259108	DQ871373	JX259219	DQ871411
Bathmocercus rufus rufus	Gabon	Cisticolidae	FMNH 389391	JX259109	DQ871374	JX259220	DQ871412
Calamonastes simplex	Kenya	Cisticolidae	ZMUC	JX259119	JX259192	-	DQ871404
Calamonastes simplex	Kenya	Cisticolidae	ZMUC	-	-	-	DQ871422

Camaroptera brachyura brachyura	South Africa	Cisticolidae	FMNH 390141	JX259110	DQ871375	JX259221	DQ871413
Camaroptera brachyura tincta	Guinea	Cisticolidae	MNHN 40-14	JX259137	JX259199	JX259240	JX259173
Camaroptera brachyura brevicaudata	Cameroon	Cisticolidae	MNHN	JX259090	DQ871357	EU247912	DQ871389
Camaroptera chloronota chloronota	Cameroon	Cisticolidae	MNHN	JX259104	DQ871369	EU247913	DQ871407
Camaroptera brachyura harterti	Angola	Cisticolidae	GA59033	JX259150	-	EU247901	EU247925
Camaroptera superciliaris	-	Cisticolidae	MNHN	JX259105	JX259191	-	JX259163
Cisticola brachypterus brachypterus	Cameroon	Cisticolidae	MNHN	JX259092	JX259189	JX259211	DQ871391
Cisticola cantans belli	Uganda	Cisticolidae	FMNH 391762	JX259111	DQ871376	JX259222	DQ871414
Cisticola cherina	Madagascar	Cisticolidae	FMNH 352929	JX259112	DQ871377	JX259223	DQ871415
Cisticola chubbi adametzi	Cameroon	Cisticolidae	MNHN 40-6	JX259132	JX259194	JX259235	JX259168
Cisticola chubbi adametzi	Cameroon	Cisticolidae	MNHN 40-10	JX259133	JX259195	JX259236	JX259169
Cisticola chubbi adametzi	Cameroon	Cisticolidae	MNHN 40-11	JX259134	JX259196	JX259237	JX259170
Cisticola chubbi adametzi	Cameroon	Cisticolidae	MNHN 40-12	JX259135	JX259197	JX259238	JX259171
Cisticola chubbi discolor	Cameroon	Cisticolidae	MNHN	JX259159	JX259207	JX259252	JX259186
Cisticola galactotes amphilecta	Uganda	Cisticolidae	FMNH 346443	JX259113	DQ871378	JX259224	DQ871416
Cisticola tinniens perpullus	Angola	Cisticolidae	GA59061	JX259149	-	JX259245	JX259178
Eminia lepida	Kenya	Cisticolidae	ZMUC	JX259122	-	JX259229	DQ871425
Eminia lepida	-	Cisticolidae	MNHN	-	-	JX259227	JX259164
Hypergerus atriceps	Cameroon	Cisticolidae	MNHN	JX259120	AF407053	-	DQ871426
Hypergerus atriceps	-	Cisticolidae	-	JX259123	-	-	JX259165
Incana incana	Socotra	Cisticolidae	MNHN	JX259103	DQ871368	JX259218	DQ871406
Neomixis striatigula	Madagascar	Cisticolidae	FMNH	JX259124	DQ871383	JX259230	DQ871427
Neomixis tenella	Madagascar	Cisticolidae	FMNH	JX259125	DQ871384	JX259231	DQ871428
Neomixis viridis	Madagascar	Cisticolidae	FMNH	JX259126	DQ871385	JX259232	DQ871429
Oreolais pulchra pulchra	Kenya	Cisticolidae	MNHN	JX259129	DQ871388	EU247906	DQ871432
Oreolais pulchra pulchra	Cameroon	Cisticolidae	MNHN 40-95	JX259155	-	JX259248	JX259182
Oreolais ruwenzorii	Uganda	Cisticolidae	FMNH 355837	JX259107	DQ871372	EU247908	DQ871410
Orthotomus castaneiceps chloronotus	Philippines	Cisticolidae	FMNH 350975	JX259114	DQ871379	-	DQ871417
Orthotomus sutorius inexpectatus	China, Yunnan	Cisticolidae	MNHN	JX259100	DQ871365	EU686375	DQ871401
Poliolais lopezi manengubae	Cameroon	Cisticolidae	MNHN 40-13	JX259136	JX259198	JX259239	JX259172
Prinia atrogularis superciliaris	China, Yunnan	Cisticolidae	MNHN	JX259101	DQ871366	JX259217	DQ871402
Prinia bairdii bairdii	Cameroon	Cisticolidae	MNHN	JX259093	DQ871358	JX259212	DQ871392
Prinia bairdii obscura	Uganda	Cisticolidae	FMNH 355824	JX259117	DQ871381	JX259226	DQ871420
Prinia flavicans bihe	Angola	Cisticolidae	W17420	JX259142	JX259200	JX259241	JX259174
Prinia flavicans bihe	Angola	Cisticolidae	W7418	JX259143	JX259201	JX259242	JX259175
Prinia subflava subflava	Cameroon	Cisticolidae	MNHN	JX259102	DQ871367	EU247915	DQ871405
Prinia subflava mutatrix	Malawi	Cisticolidae	GA84821	JX259144	JX259202	JX259243	JX259176
Scepomycter winifredae	Tanzania	Cisticolidae	ZMUC/JK2	JX259097	DQ871361	JX259216	DQ871397
Scepomycter winifredae	Tanzania	Cisticolidae	ZMUC/LHA1	JX259098	DQ871362	-	DQ871398
Schistolais leucopogon reichenowi	Uganda	Cisticolidae	FMNH 391767	JX259118	DQ871382	-	DQ871421

Schistolais leucopogon leucopogon	Cameroon	Cisticolidae	MNHN 40-7	-	EU239801	EU247902	EU247926
Urolais epichlorus epichlorus	Cameroon	Cisticolidae	MNHN 40-5	-	EU239802	EU247903	EU247927
Cisticolidae (Genbank and Embl-bank sequences)							
Apalis flavida caniceps	Gambia	Cisticolidae	UMMZ 235.837	AY136597			
Camaroptera brachyura pileata	Tanzania	Cisticolidae	ZMUC 01986		DQ008549		
Camaroptera brachyura brevicaudata	Gambia	Cisticolidae	UMMZ A339		AF407052		
Cisticola cantans swanzii	Gambia	Cisticolidae	UMMZ 235.853	AY136593			
Cisticola fulvicapilla hallae	Zimbabwe	Cisticolidae	UMMZ A761		AF407051		
Cisticola juncidis juncidis	France	Cisticolidae	MNHN			JX259190	DQ871403
Cisticola juncidis juncidis	Italia	Cisticolidae	NRM		DQ008544		
Eminia lepida	Kenya	Cisticolidae	UMMZ 211635		AY136598		
Heliolais erythropterus erythropterus	Niger	Cisticolidae	NRM		DQ008550		
Hypergerus atriceps	Gambia	Cisticolidae	UMMZ A345		AF407053		
Orthotomus atrogularis nitidus	Vietnam	Cisticolidae	NRM		DQ008543		
Orthotomus sutorius inexpectatus	Thailand	Cisticolidae	NRM		DQ008542		
Prinia crinigera catharia	Myanmar	Cisticolidae	NRM		DQ008540		
Prinia familiaris	Indonesia	Cisticolidae	NRM 20046794	DQ008541			
Prinia subflava subflava	Gambia	Cisticolidae	UMMZ 235.854	AY136594			
Schistolais leontica	Guinea	Cisticolidae	UMMZ 235.855	AY136595			
Schistolais leucopogon leucopogon	Cameroon	Cisticolidae	UMMZ 232.418	AY136596			
Spiloptila clamans	Mauritania	Cisticolidae	NRM	DQ008546			
Other groups included in this study							
Aegithalos caudatus	-	Aegithalidae	UMMZ A571		AY136588		
Aegithalos caudatus	Sweden	Aegithalidae	NRM 976089		AY228281		
Cettia fortipes	-	Cettidae	-		DQ125976	DQ125948	
Cettia fortipes	-	Cettidae	KIZ05353		DQ837490		
Copsychus saularis	Thailand	Muscicapidae	MNHN	JX259121	DQ125977	JX259228	DQ871424
Criniger chloronotus	Cameroon	Pycnonotidae	MNHN	JX259157	-	JX259250	JX259184
Cryptospiza reichenovii	Cameroon	Estrildidae	MNHN	JX259158	JX259206	JX259251	JX259185
Dyaphorophyia concreta	Cameroon	Platysteiridae	MNHN	JX259160	JX259208	JX259253	JX259187
Elminia albiventris	Cameroon	Stenostiridae	MNHN 40-63	JX259151	EU652712	JX259246	JX259179
Hylia prasina	-	Cettidae	LSUMZ 163337	AY136606	DQ125955		
Illadopsis albipectus	Cameroon	Timaliidae	MNHN	JX259153	JX259205	EU686336	JX259181
Kakamega poliothorax	Cameroon	Timaliidae	MNHN 40-4	JX259131	EU686330	EU686374	EU686289
Linurgus olivaceus	Cameroon	Fringillidae	MNHN 40-8	EU880940	EU652706	EU881006	EU669964
Orthotomus cucullatus cucullatus	Indonesia	Cettidae	NRM		DQ008566		
Orthotomus cucullatus heterolaemus	Philippines	Cettidae	FMNH 357483	JX259116	DQ125999	JX259225	DQ871419
Picathartes gymnocephalus	Liberia	Picathartidae	AMNH AC350		DQ125989	AY228314	
Pholidornis rushiae	-	Paridae	ZFMK 61.1422		AY136586		
Platysteira cyanea	Cameroon	Platysteiridae	MNHN	JX259161	JX259209	JX259254	JX259188

Pseudoalcippe abyssinica monachus	Cameroon	Timaliidae	MNHN 40-75	JX259154	EU652717	EU686376	EU669975
Pycnonotus barbatus inornatus	Cameroon	Pycnonotidae	MNHN	JX259156	-	JX259249	JX259183
Rhopophilus pekinensis	China	Timaliidae	NRM		DQ008538		
Scotocerca inquieta	-	Cettidae	MNHN	JX259145	JX259203	JX259244	JX259177
Sylvia atricapilla	Israel	Timaliidae	-		NC_010228	NC_010228	NC_010228
Sylvia atricapilla	Sweden	Timaliidae	NRM 976380		AY887727		
Zosterops japonicus	-	Zosteropidae	GLGS2247		DQ837491		
Zosterops japonicus	-	Zosteropidae	MNHN		DQ861968		
Zosterops palpebrosus	-	Zosteropidae	MNHN		DQ125973		
Zosterops poliogaster	Kenya	Zosteropidae	ZMUC 08629	DQ328433			
Sylviidae							
Acrocephalus bistrigiceps	Japan	Sylviidae	UMMZ 234.837	AY136601			
Cryptosylvicola randrianasoloi	-	Sylviidae	MNHN	JX259128	-	JX259233	JX259166
Garrulax chinensis	-	Sylviidae	NRM 20046790	DQ008536			
Garrulax erythrocephalus	-	Sylviidae	MNHN		DQ861987		
Garrulax squamatus	-	Sylviidae	GLGS1833		DQ837502		
Macrosphenus flavicans	Cameroon	Sylviidae	MNHN	JX259094	DQ125997	JX259213	DQ871393
Nesilla typica	-	Sylviidae	MNHN	JX259130	JX259193	JX259234	JX259167
Oxylabes madagascariensis	-	Sylviidae	MNHN	JX259089	-	JX259210	JX259162
Sphenoaecus mentalis	Cameroon	Sylviidae	MNHN	JX259091	DQ125998	EU247916	DQ871390
Sylvietta brachyura	-	Sylviidae	MNHN	JX259095	DQ126001	-	DQ871394
Sylvietta rufescens	Malawi	Sylviidae	MNHN	JX259127	DQ871386	-	DQ871430
Sylvietta virens	Gambia	Sylviidae	UMMZ 235.840	AY136602			
Sylvietta whytii	Kenya	Sylviidae	ZMUC		DQ871423		
Yuhina brunneiceps	-	Sylviidae	NTNUT0620		DQ837501		
Yuhina gularis	-	Sylviidae	MNHN		DQ861973		
Outgroup							
Tersiphone rufiventer	Cameroon	Monarchidae	MNHN 40-64	JX259152	JX259204	JX259247	JX259180

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