



On the base of molecular data, a great difference between sedentary subspecies of *Prinia subflava* Gmelin, 1789 suggests resurrecting name *Prinia mutatrix* Meise, 1936

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ABSTRACT

Prinia subflava is a sedentary member of the “open warblers cisticolid” clade and in our first papers, we noted a strong divergence between *Prinia subflava subflava* and *Prinia subflava mutatrix*. To confirm this divergence and leaning partially on our first obtained results, we investigated the genetic variation of the individuals of *P. s. subflava* (West Africa) and *P. s. mutatrix* (South East Africa). For the genetic variation, we used three mitochondrial genes (ATPase6, ND2 and ND3) to calculate their genetic distances within the cisticolid ingroup and to explore their mutational differentiation. With our ATPase6, ND2 and ND3, a genetic distance of 5.76%, 5.15% and 5.13% was estimated respectively between individuals of *P. s. subflava* (Cameroon) and *P. s. mutatrix* (Malawi) whereas it was, for the protein-coding gene ND2, only of 1.81% between the specimens of *P. s. subflava* caught in parts of West Africa (Gambia and Cameroon). For the mutational differentiation, a total of 113 different molecular characters were observed on the three markers investigated between *P. s. subflava* and *P. s. mutatrix*. Otherwise leaning on our dating results, *Prinia subflava subflava* diverged from *Prinia subflava mutatrix* during the Pliocene epoch. It is known that the East African Rift system whose the western branch formed the giant arc from Uganda to Malawi began its formation after a large uplift of East Africa during the Oligocene Epoch followed by climatic fluctuations in Africa with the global cooling during the Neogene period. So for these sedentary birds separated around 3162 km with several geographical barriers and for which no risk of recurrent gene flow is again possible, we suggest resurrecting the name *Prinia mutatrix* Meise, 1936 for individuals of the populations previously called *Prinia subflava mutatrix* in this study.

Keywords

Open warblers cisticolid clade; divergence; genetic variation; mutational differentiation; sedentary bird; geographical barrier; recurrent gene flow; taxonomic revision.

Academic Discipline And Sub-Discipline

Biology and Ornithology

SUBJECT

Phylogeny and Biogeography of the African Passeriformes (Process of speciation of the sedentary birds)

TYPE (METHOD/APPROACH)

Phylogeny analysis, mutational differentiation and molecular dating analysis

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Council for Innovative Research

Peer Review Research Publishing System

Journal of Advances in Biology

Vol. 8, No. 1

<https://rajpub.com>

editorsjab@gmail.com , editor@cirjab.com

INTRODUCTION

Prinia is one of the diverse lineages within the Cisticolidae avian family [1]. This genus is found in different biogeographical zones (Afrotropical, Indo-Malaysian and Palearctic regions). During long time, several genera were merged in *Prinia* (*Heliolais*, *Malcorus*, *Oreophilais*, *Phragmacia*, *Schistolais*, *Urolais* and *Urorhipis*) but now some of these genera are placed in their own separate genera [1, 2, 3, 4, 5, 6, 7, 8]. Until recently, the monophyly of this genus was supported [4, 7, 9] but *Prinia burnesii* has recently been retrieved in the family Pellorneidae [8]. According to [1], *Prinia* comprises twenty-five taxa among these *Prinia subflava*.



Figure 1a. Distribution of the Tawny-flanked Prinia. This map shows that the Tawny-flanked Prinia has a very large geographic range (blue color) and it is found in several countries of the African continent.

The Tawny-flanked Prinia is a polytypical warbler which has a very large geographical range (Figure 1a). It is a sedentary resident which lives in the West, East and South of Africa. *Prinia subflava* is found in Mauritania, Senegal, Gambia, Guinea-Bissau, Guinea, Mali, Sierra Leone, Liberia, Ivory Coast, Burkina Faso, Ghana, Togo, Benin, Nigeria, Niger, Chad, Cameroon, Central African Republic, Equatorial Guinea, Gabon, Democratic Republic of the Congo, Sudan, Eritrea, Ethiopia, Somalia, Kenya, Uganda, Rwanda, Burundi, Tanzania, Angola, Zambia, Mozambique, Malawi, Namibia, Botswana, Zimbabwe, South Africa, KwaZulu-Natal, Lesotho and Swaziland (see its distribution Map Figure 1a). It lives amongst shrubs, weeds, bushes, gardens, edges of old cultivated lands, rank grass in a variety of habitats comprising woodland and savanna [2, 10, 11].



Figure 1b. Distribution of the subspecies *Prinia subflava subflava*. This map shows that *P. s. subflava* has a limited geographical range (blue color).

According to [1], ten subspecies are now recognized. *Prinia subflava subflava* Gmelin, 1789, which is distributed from southern Mauritania and Senegal to South Sudan, central Ethiopia, southern Eritrea and northern Uganda; *P. s. melanorhyncha* Jardine & Fraser, 1852, which occurs from Sierra Leone in southwestern Nigeria and Cameroon to

southern Uganda, western Kenya and northwestern Tanzania; *P. s. pallescens* Madarasz, 1914, which is distributed from Mali to Sudan, west of Ethiopia and northwestern of Eritrea; *P. s. tenella* Cabanis, 1869, which is found in southern Somalia, Kenya and southern Tanzania; *P. s. graueri* Hartert, 1920, which occurs from Rwanda and Democratic Republic of the Congo to Angola; *P. s. kasokae* White, 1946, which is found only in Angola and western Zambia; *P. s. affinis* Smith, 1843, which is found in southeastern Democratic Republic of the Congo, southwestern Tanzania, central Zambia, Zimbabwe, eastern Botswana, western Mozambique and northeastern South Africa; *P. s. bechuanae* Macdonald, 1941, which is found in southwestern Angola, northern Namibia, western Botswana and extreme southwestern Zambia and northwestern Zimbabwe; *P. s. mutatrix* Meise, 1936, which occurs from southern Tanzania, Malawi, eastern Zambia, northern and central Mozambique and eastern Zimbabwe and *P. s. pondoensis* Roberts, 1922, which is found in southern Mozambique, eastern Swaziland and eastern South Africa [1, 2, 10, 11].



Figure 1c. Distribution of the subspecies *Prinia subflava mutatrix*. This map shows that *P. s. mutatrix* has a very restricted geographical range (blue color) and is only found in the southern part of Africa.

Some authors have argued that *Prinia subflava* formed a superspecies with *P. inornata*, *P. Somalia* and *P. fluvialis* whereas for others, it can be considered conspecific to *P. inornata* even if it differs vocally and in plumage coloration [10, 11]. In our first studies [4, 5], we did not include these species but in our last study [7], we included two subspecies of *Prinia subflava* (*P. s. subflava* and *P. s. mutatrix*) as well as *Prinia flavicans*. Oddly in our combined tree with 2716 bp, these two subspecies of *P. subflava* are not sister-taxa but they have formed a clade with *Prinia flavicans bihe* in which *P. s. mutatrix* is sister-taxon of *P. f. bihe* with a high support [7]. Otherwise, the type subspecies of the species *Prinia subflava* Gmelin, 1789, is *Prinia subflava subflava*, initially described as *Motacilla subflava* Gmelin, 1789 (Figure 1b). This species includes ten (10) subspecies which in principle have a geographical variation. Among these, there is *Prinia subflava mutatrix* which has been described as *Prinia subflava mutatrix* Meise, 1936 (Figure 1c).

Based on our first results obtained for these two subspecies [4, 7], in this study which includes the type subspecies of the species *Prinia subflava* Gmelin, 1789, we examine the genetic variation of the individuals of the two subspecies of *Prinia subflava* caught in West Africa (Cameroon) and South Africa (Malawi). For this, we use three mitochondrial protein-coding genes evolving rapidly (ATPase6, ND2 and ND3) and divergence times in order to assess their real differential genetic structure and to provide more information about the diversification of these two sedentary subspecies of the Tawny-flanked *Prinia* which do not have a contact zone between these two African parts.

MATERIALS AND METHODS

For the taxon sampling, we kept the same biological material already used in our first publications [4, 5, 7]. And for the species taxonomy, we especially referred to our last study [7] as well as [1].

Genetic distances of the two subspecies of the Tawny-flanked *Prinia*

To examine the genetic distances between these two subspecies, we used nucleotide sequences of three mitochondrial genes (ATPase6, ND2 and ND3) and we selected as model "Number of difference" for the estimates of their genetic distances using MEGA v3.1. [12]. For this, we used the same taxa for which the Genbank accession numbers were already published in our first studies for a total of Operational Taxonomic Units of 54 (ND3), 53 (ATPase6) and 65 (ND2) (see Table 1 in Annexe).



Mutational differentiation between these two subspecies

In order to explore the main kinds of mutations between these two subspecies, we considered comparing the nucleotide sequences of their molecular markers. For this, we mainly used contigs of the three mitochondrial genes (ATPase6, ND2 and ND3) which were aligned using the algorithm in Sequencher 3.1 [13]; for each molecular marker, we compared the nucleotide sequences of these two subspecies in order to find diverse kinds of mutations.

Phylogenetic analysis

To examine the phylogenetic relationships of these two subspecies of *Prinia subflava*, we used the same methods (Maximum Likelihood and Bayesian Inference) which had been used in our last study [7]. As the results are the same, in this present study, we will lean on trees already obtained in our last study in order to analyze their relationships as well as their closely related with other ingroup taxa.

Divergence times

In order to investigate the diversification of the two subspecies of the Tawny-flanked *Prinia*, we used the same method with the Bayesian approach (estimate of the divergence time with MULTIDISTRIBUTE Software) [7]. And as the results are the same, we will lean on the same tree already obtained in our last study but we propose a new table with the estimated time divergence between these two subspecies of *Prinia subflava*.

RESULTS

Genetic distances results between these two subspecies

Results of the genetic distances are presented in Table 2. According to these estimates, *Prinia subflava subflava* appears to be quite different from *Prinia subflava mutatrix*. Thus, with the protein-coding gene ATPase6, the genetic distance obtained between these two subspecies was of 5.76% (Table 2). With the ND2 gene, the genetic distance obtained between *P. s. subflava* and *P. s. mutatrix* was of 5.15% whereas it was of 5.13% with the protein-coding gene ND3 (Table 2). At the same time, we noted that the genetic distance between specimens of *P. s. subflava* caught in various parts of West Africa (Gambia and Cameroon) estimated with the protein-coding gene ND2 was only of 1.81% (Table 2).

Table 2: Estimates of the genetic distance (values in %) obtained from the ADN mitochondrial among the representatives of the species *Prinia subflava* collected in several African forests following a “number of differences” model implemented in MEGA v.3.1. [12].

	ATPase 6	ND2	ND3
Nucleotide: number of differences between <i>P. s. subflava</i> (Cameroon) and <i>P. s. mutatrix</i> (Malawi)	5,76%	5,15%	5,13%
Nucleotide: number of differences between <i>P. s. subflava</i> (Cameroon) and <i>P. s. subflava</i> (Gambia)	-	1.81%	-

*: We used our largest cisticolid ingroup [7].

Mutational differentiation results between these two subspecies

Results of the mutational differentiation are presented in Tables 3a, 3b, 3c and we note that only our mitochondrial genome permitted to observe the mutational differentiation between these two subspecies of *Prinia subflava*. According to these tables, the DNA sequences of *Prinia subflava subflava* appeared to be very different from the DNA sequences of *Prinia subflava mutatrix*. A total of 113 different molecular characters were thus observed on the three genes of the mitochondrial genome investigated between these two subspecies of *Prinia subflava* (Tables 3a; 3b; 3c). With the protein-coding gene ND2, we noted 61 different molecular characters between these two subspecies (Table 3a). With the ATPase6 gene, 34 molecular characters were found different between *P. s. subflava* and *P. s. mutatrix* (Table 3b) and 18 different substitution mutations were observed between these two subspecies with the ND3 gene (Table 3c). In our tables, we indicate precisely the number of substitution positions as observed in the cisticolid alignment of the three mitochondrial genes examined (Tables 3a; 3b; 3c).

Table 3a: Molecular characters (substitution mutations) observed in the alignment of ND2 sequences among the representatives of the species *Prinia subflava* collected from several African forests.

Different molecular characters between <i>Prinia subflava subflava</i> * and <i>P. s. mutatrix</i> ** (number of substitution position in the cisticolid alignment of ND2 sequences)	Total of different molecular characters observed for each kind of substitution
Transition A-G	
N°174, N°188, N°189, N°237, N°399, N°492, N°501, N°522, N°585, N°681, N°693, N°738, N°865, N°891,	15



N°912	
Transition C-T	
N°108, N°144, N°183, N°273, N°278, N°291, N°333, N°345, N°372, N°374, N°450, N°483, N°513, N°525, N°558, N°575, N°615, N°813, N°852, N°862, N°876, N°879, N°951, N°970, N°986, N°1035	26
Transversion A-C	
N°367, N°457, N°756, N°762, N°783, N°858, N°950, N°958, N°1017, N°1020, N°1030	11
Transversion G-T	
N°943	1
Transversion C-G	
N°123, N°448, N°1010	3
Transversion A-T	
N°732, N°841, N°952, N°1007, N°1033	5
Total of molecular characters between two subspecies <i>P. s. subflava</i> and <i>P. s. mutatrix</i>	61

*: Sequence DQ871367 already published in Genbank [4]. **: Sequence JX259202 already published in Genbank [7].

Phylogenetic results

On the basis of our last publication on the cisticolid group [7], the genus *Prinia* was found in our “open warblers cisticolid” clade. Leaning on this publication, we noted that *Prinia subflava subflava* (Cameroon) was not close to *Prinia subflava mutatrix* (Malawi) (Figures 2 and 3). The subspecies *Prinia subflava mutatrix* appeared to be strongly close to *Prinia flavicans bihe* than to *Prinia subflava subflava* with high bootstrap values (posterior probabilities ≥ 0.95 and PhyML bootstrap values ≥ 0.70) (Figures 2 and 3). Otherwise, all the phylograms obtained with ND2 as well as the concatenated datasets showed that *Prinia subflava subflava* (Cameroon) diverged deeply from the group which includes *Prinia subflava mutatrix* (Malawi) and this divergence was strongly supported with high bootstrap values (posterior probabilities ≥ 0.95 and PhyML bootstrap values ≥ 0.70) (Figures 2 and 3). We note that the nuclear marker used in our last study (myoglobin intron 2) did not resolve the relationship between the subspecies *P. s. subflava* and *P. s. mutatrix* (tree not shown).

Table 3b: Molecular characters (substitution mutations) observed in the alignment of ATPase6 sequences among the representatives of *Prinia subflava* collected from several African forests.

Different molecular characters between <i>Prinia subflava subflava</i> * and <i>P. s. mutatrix</i> ** (number of substitution position in the cisticolid alignment of ATPase6 sequences)	Total of different molecular characters observed for each kind of substitution
Transition A-G	
N°90, N°103, N°117, N°120, N°129, N°138, N°150, N°171, N°198, N°243, N°303, N°381, N°387, N°390, N°486, N°519, N°570	17
Transition C-T	
N°126, N°345, N°360, N°375, N°410, N°450, N°462, N°465, N°489, N°543, N°561	11
Transversion A-C	
N°159, N°234, N°300, N°324, N°342, N°594	6
Total of molecular characters between two subspecies <i>P. s. subflava</i> and <i>P. s. mutatrix</i>	34

*: Sequence JX259102 already published in Genbank [7]. **: Sequence JX259144 already published in Genbank [7].

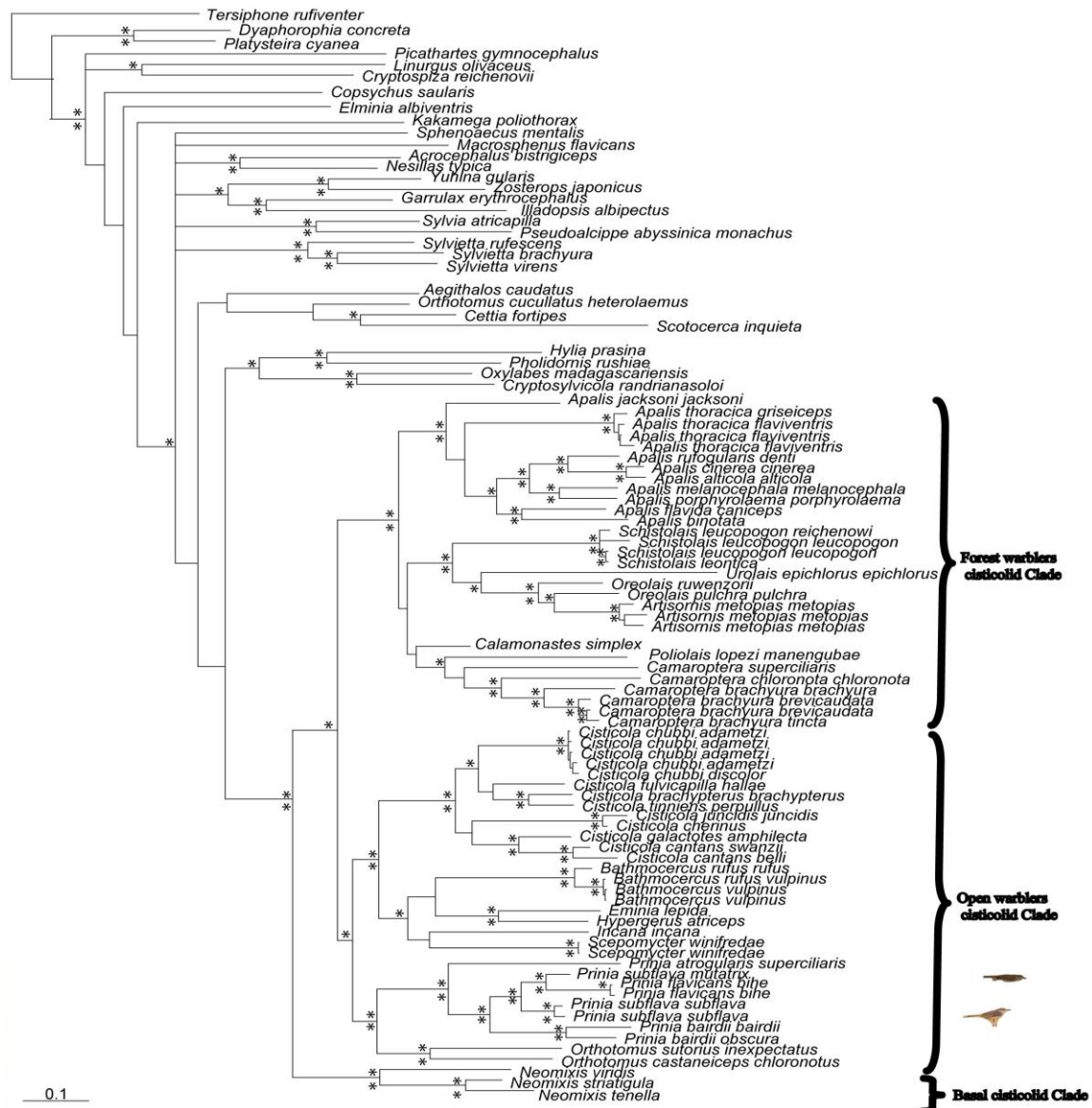


Figure 2. Pictures of *Prinia subflava subflava* and *Prinia subflava mutatrix* on the ND2 tree obtained in [7] supporting the strong divergence between the individuals of the populations of the subspecies *Prinia subflava subflava* (Cameroon) and *Prinia subflava mutatrix* (Malawi).

Table 3c: Molecular characters (substitution mutations) observed in the alignment of ND3 sequences among the representatives of the species *Prinia subflava* collected from several African forests.

Different molecular characters between <i>Prinia subflava subflava</i> * and <i>P. s. mutatrix</i> ** (number of substitution position in the cisticolid alignment of ND3 sequences)	Total of different molecular characters observed for each kind of substitution
Transition A-G	
N°25, N°54, N°177, N°219, N°243, N°250, N°303	7
Transition C-T	
N°61, N°83, N°87, N°129, N°133, N°168, N°210, N°225, N°307	9
Transversion A-C	
N°246, N°282	2
Total of molecular characters between two subspecies <i>P. s. subflava</i> and <i>P. s. mutatrix</i>	18

*: Sequence EU247915 already published in Genbank [5]. **: Sequence JX259243 already published in Genbank [7].

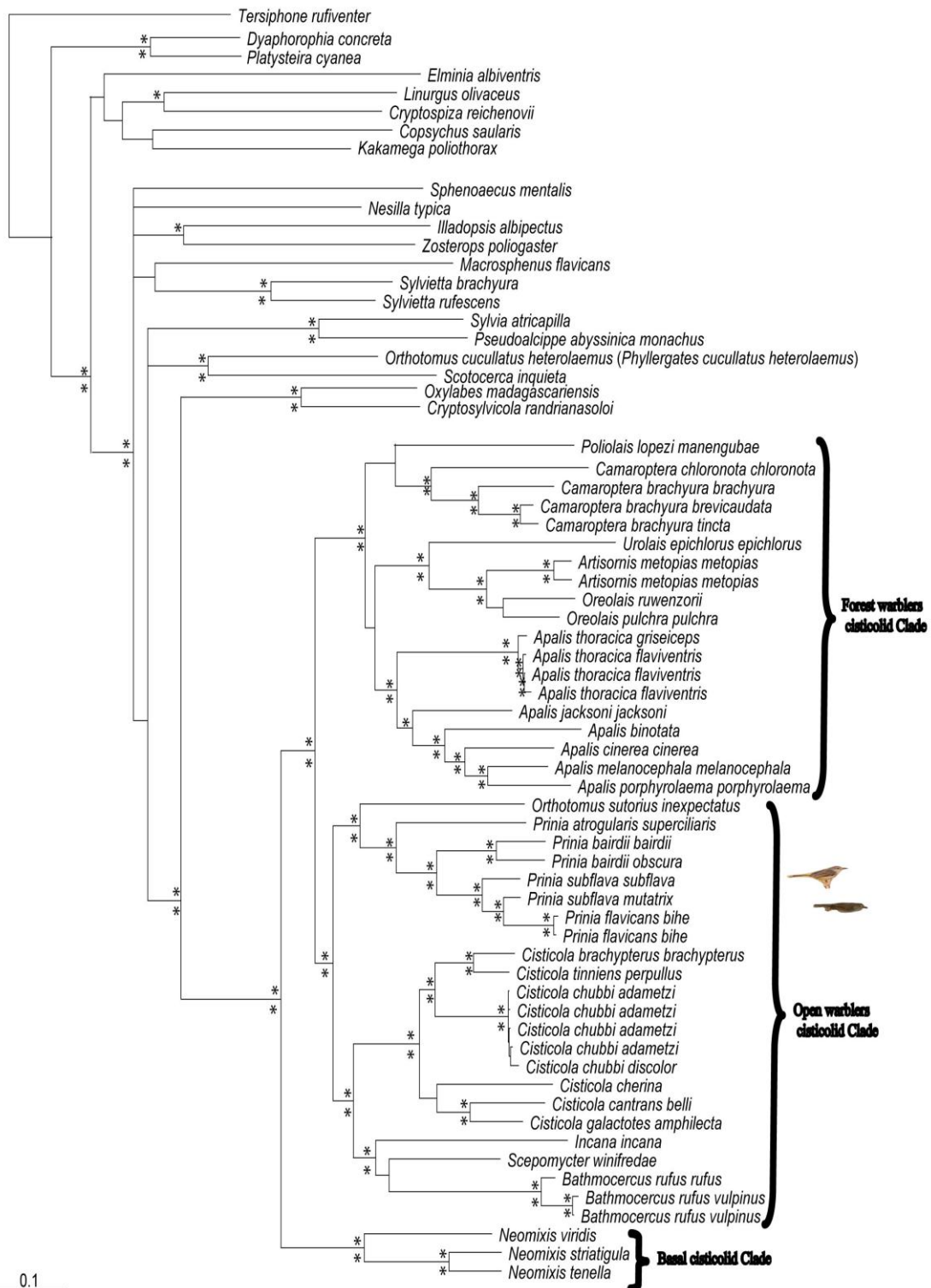


Figure 3. Pictures of *Prinia subflava subflava* and *Prinia subflava mutatrix* on the combined dataset tree obtained in [7] confirming the strong divergence between the individuals of the populations of the subspecies *Prinia subflava subflava* (Cameroon) and *Prinia subflava mutatrix* (Malawi).

Dating analysis results

On the basis of our dating results already published in our last publication [7], the diversification of the “open warblers cisticolid” clade took place during the Neogene period (Figure 4; Table 4). Within this period, the diversification of the genus *Prinia* took place during the Miocene epoch (Figure 4) but the subspecies *Prinia subflava subflava* (Cameroon) diverged from the subclade including *Prinia subflava mutatrix* (Malawi) during the Pliocene epoch (Figure 4; Table 4).



Table 4: Some dates inferred within the cisticolid lineage obtained from a relaxed molecular clock analysis using the MULTIDISTRIBUTE Software package in which the time divergence of the two subspecies of *Prinia subflava* has been estimated. Some of these dates have already been suggested in [7].

Description	minimum	mean	Maximum	Confidence Interval (95%)
Open warblers clade/other cisticolid taxa	11.9	12.92	13.94	11.02 – 15.01
<i>Orthotomus/Prinia</i>	8.42	9.34	10.26	7.65 – 11.24
<i>Prinia</i> (Asia)/ <i>Prinia</i> (Africa)	6.39	7.21	8.03	5.72 – 8.93
<i>P. subflava subflava</i> / <i>P. subflava mutatrix</i> and <i>P. flavicans bihe</i>	2	2.2	2.5	1.33 – 3.4

DISCUSSION

Several phylogenetic aspects of African warblers are already discussed in our last publication (see [7]). In this paper, we will focus on the geological (plate tectonics) and climatic changes that have occurred on the African continent and subsequently their potential results on the avian diversification of some African taxa as well as possibly their speciation.

Some geological change within the *Prinia subflava* geographic range

It is well known that until the Mesozoic Era, the African continent was linked to other southern continents in the Gondwanaland and following the intense volcanic activity during this Era, the African continent was separated from the others during the Cretaceous system. This continent includes several mountains such as Cameroon Highlands Forests or East African Montane Forests for which according to [14], the distension of the African plate during the Cenozoic Era would be the main reason for the separation between the Cameroon Highlands Forests and the mountain forests of East Africa. Only, the East African Rift system is composed of two main segments including the western branch which formed a giant arc from Uganda to Malawi. A recent study [15] found that around 25 to 30 Myrs a large uplift of East Africa occurred and created the distinct landscapes and climates that marked this part of Africa.

Otherwise, it is documented that the climate of Africa has fluctuated a lot over the course of history and especially the Pleistocene climate variation is often cited in order to explain the biogeographical pattern in Africa [16, 17, 18, 19, 20]. Thus during periods of the global cooling, estimated at a peak close to 1.0, 1.7 and 2.8 Myrs [21, 22], the African continent has been subjected to climatic fluctuations involving a succession of more humid and arid phases. This increase in aridity would have facilitated the expansion of savannah and semi-arid biomes, and the contraction of forest biomes [16, 17, 18, 19, 23, 24, 25].

Diversification of the genus *Prinia*

According to our dating results, the diversification of the genus *Prinia* took place during the Miocene epoch and the estimated divergence between the subspecies *Prinia subflava subflava* and *Prinia subflava mutatrix* dated from 2 to 2.5 Myrs (Figure 4 and Table 4); these periods appear younger than those estimated approximately for the separation between the forests of the Cameroon Highlands and the mountain forests of East Africa [14] and the broad uplift has occurred and created distinct landscapes and climates within the East African Rift such as suggested by [15]. On the other hand, these putative dates correspond slightly to one of the three estimated peaks of global cooling that has occurred in Africa, 2.8 Myrs BP [21, 22]. According to several authors [16, 17, 18, 19, 21, 25], the fragmentation of the forest block as well as the creation and maintenance of forest refuges during the most arid period are the main origin of many African forest birds.

The genus *Prinia* includes exclusively sedentary birds. According to [26], these birds rarely disperse more than a few kilometres from their natal sites contrary to migratory birds. For these sedentary birds, Highlands forests, oceans, seas and sometimes a very long river constitute geographical barriers [27] and in the past, ornithologists assessed the motility capabilities of taxa before defining their vicariance or dispersal hypothesis and often explained biogeographical patterns [28].

Based on our results, the genetic structure and diversity within the investigated individuals of the subspecies of *Prinia subflava* between parts of West and South-East Africa seem to show that they are too divergent. According to our dating results, the event that separated these two subspecies took place during the Plio-Pleistocene transition (Figure 4) and corresponds to a stop of recurrent gene flow between populations of the subspecies *Prinia subflava subflava* (Cameroon) and those of the subspecies *Prinia subflava mutatrix* (Malawi). According to the countries taken, these two African parts (West and South-East Africa) are globally separated by about 5000 km. The speciation process, which has started, is highlighted by the different genetic structure observed between individuals of the two subspecies of *Prinia subflava*.

Thus, with the mitochondrial markers used, we found a total of 113 different molecular characters between these two subspecies (see tables 3a, 3b and 3c). It is widely documented that mutations are raw materials of evolution and that they play a key role in speciation as well as can constitute a great indicator. It is important to note that these mutations are



Figure 4. Pictures of *Prinia subflava subflava* and *Prinia subflava mutatrix* on the chronogram obtained in [7] showing that the diversification between the subspecies *Prinia subflava subflava* (Cameroon) and the subclade which includes *Prinia subflava mutatrix* (Malawi) took place during the Pliocene epoch.

those which are generally transmitted to descendants. [29] have shown that the synonymous substitution rate, and therefore the mutation rate, is correlated to the net diversification and in some cases, this diversification often leads to speciation of several birds. So, the high mutational differentiation observed between the subspecies *Prinia subflava subflava* and *Prinia subflava mutatrix* correlated to their diversification dated from 2 to 2.5 Myrs has certainly led to their speciation, at least that of *Prinia subflava mutatrix* which no longer shares the same genetic heritage as *Prinia subflava subflava*, two sedentary subspecies separated around 3162 km and for which no risk of recurrent flow is again possible.

We think that the use of several individuals from the populations of these two subspecies of *Prinia subflava* would have been better but we are confident that it would not have modified our biogeographical as well as mutational results for which our phylogenetic results have already been published [7] because the genetic structure and the diversity within the investigated individuals of the West and South-East African populations of *Prinia subflava* is strongly highlighted in this study (Figures 2, 3 and 4; Tables 2, 3a, 3b and 3c).

Taxonomic implications and revisions

The significant result of our study is the vicariant speciation of the two subspecies of *Prinia subflava* highlighted in this study, probably initiated by the main vicariance events. For a long time these two taxa have been considered the same

species [1, 2, 10], on the basis of an overall similarity in shape and pattern but probably also reflecting limited information on these two subspecies. No previous molecular study included both of these two subspecies before our last study of the cisticolid group [7]. We can now conclude that similarities such as the general color and size as well as the natural habitat conditions of these birds were of course acquired in their common ancestor, but currently their genetic heritage is totally different and make them without no doubt two distinct taxa.



Figure 5a. The back of the species *Prinia mutatrix* Meise, 1936, name newly resurrected after this current study.

Leaning on our results in this current study, we suggest resurrecting the name regarding the name of the current subspecies *Prinia subflava mutatrix*. Molecularly, *P. s. mutatrix* appears quite different to *P. s. subflava* in this study and this result corroborates morphologically. In fact, the breeding plumage in *P. s. mutatrix* is darker and browner above, with greyish crown whereas in *P. s. subflava*, the breeding plumage is dark grey-brown, slightly paler on nape, faintly washed russet on lower back and rump, with brownish-grey crown; concerning the non-breeding plumage, it is paler on forehead and crown, mantle and back with variable tawny wash, rump tawny in *P. s. mutatrix* whereas it is rufous-tinged light brown above in the male of *P. s. subflava* [2, 11].

For *Prinia subflava mutatrix* Meise, 1936; this taxon was initially described as such *Prinia subflava mutatrix*. As in this study we include the type subspecies of the species *Prinia subflava*, we propose to resurrect the name *Prinia mutatrix* Meise, 1936, refers to *Prinia subflava mutatrix* of the current study (Figures 5a, 5b and 5c).



Figure 5b. The belly of the species *Prinia mutatrix* Meise, 1936, name newly resurrected.



Figure 5c. The flank of the species *Prinia mutatrix* Meise, 1936, name newly resurrected.



ACKNOWLEDGMENTS

We are grateful to various researchers and institutions that caught and provided several samples or those whose sequences and pictures have been used in this study. We thank Mrs. Christelle Leung of the University of Montreal for her technical support as well as all my staff from the Ornithology Unit of the Laboratory of Zoology of the University of Yaounde I (Cameroon) for their constant assistance. We also thank all my new staff from the Southwest Cameroon Region (Cameroon) and we appreciate greatly several anonymous referees for their comments on an earlier version of this manuscript.

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Author' biography with Photo



Dr. Billy Nguembock is born 1975 in Eseka, a town of the Centre Region of Cameroon. He received his master's degree of Biodiversity: Genetics, History and mechanisms of the Evolutionary at the Paris VI University, France, 2004 and his Ph.D's degree of Diversity of Living Organisms in the same University Paris VI, 2008 with *Very Honorable Mention*. He is a Lecturer in the Department of Animal Biology and Physiology at the University of Yaounde I (Cameroon) since 2010 and his interesting fields are Phylogeny, Biogeography and Phylogeography of the African Passeriformes (Aves). He has published several papers in the more prestigious scientific journals around the World and he often acts as an Invited Researcher at the University of Montreal (Canada).

ANNEXE

Table 1: Operational Taxonomic Units (OTUs) used to estimate directly genetic distances within the cisticolid ingroup between subspecies of the species *Prinia subflava* and to explore mutational differentiation after making contigs between these two subspecies. Genbank and Embl-bank accession numbers of these OTUs have been already published in other publications concerning the cisticolid clade.

Species	Country	Family	Voucher	ATPase6	ND2	ND3
Ingroup						
<i>Apalis alticola alticola</i>	Tanzania	Cisticolidae	ZMUC 123398	-	EU239793	EU247893
<i>Apalis binotata</i>	Tanzania	Cisticolidae	ZMUC 133492	JX259140	EU239794	EU247894
<i>Apalis cinerea cinerea</i>	Burundi	Cisticolidae	FMNH 358088	JX259106	DQ871370	EU247904
<i>Apalis jacksoni jacksoni</i>	Burundi	Cisticolidae	FMNH 358081	-	DQ871371	EU247905
<i>Apalis melanocephala melanocephala</i>	Tanzania	Cisticolidae	ZMUC 129285	JX259138	EU239795	EU247895
<i>Apalis porphyrolaema porphyrolaema</i>	Tanzania	Cisticolidae	ZMUC 123196	JX259141	EU239796	EU247896
<i>Apalis rufogularis denti</i>	Kenya	Cisticolidae	MNHN68-1139	-	DQ871387	EU247907



<i>Apalis thoracica griseiceps</i>	Tanzania	Cisticolidae	ZMUC 136606	JX259139	EU239797	EU247897
<i>Apalis thoracica flaviventris</i>	Malawi	Cisticolidae	GA94334	JX259146	EU239798	EU247898
<i>Apalis thoracica flaviventris</i>	Malawi	Cisticolidae	GA94354	JX259147	EU239799	EU247899
<i>Apalis thoracica flaviventris</i>	Malawi	Cisticolidae	GA94346	JX259148	EU239800	EU247900
<i>Artisornis metopias metopias</i>	Tanzania	Cisticolidae	ZMUC 119714	-	DQ871363	EU247910
<i>Artisornis metopias metopias</i>	Tanzania	Cisticolidae	ZMUC 119915	JX259099	DQ871364	EU247911
<i>Artisornis metopias metopias</i>	Tanzania	Cisticolidae	FMNH 356778	JX259115	DQ871380	EU247909
<i>Bathmocercus rufus vulpinus</i>	Uganda	Cisticolidae	ZMUC/CDI	JX259096	DQ871359	JX259214
<i>Bathmocercus rufus vulpinus</i>	Uganda	Cisticolidae	ZMUC/CD9	-	DQ871360	JX259215
<i>Bathmocercus rufus vulpinus</i>	Uganda	Cisticolidae	FMNH 385102	JX259108	DQ871373	JX259219
<i>Bathmocercus rufus rufus</i>	Gabon	Cisticolidae	FMNH 389391	JX259109	DQ871374	JX259220
<i>Calamonastes simplex</i>	Kenya	Cisticolidae	ZMUC	JX259119	JX259192	-
<i>Camaroptera brachyura brachyuran</i>	South Africa	Cisticolidae	FMNH 390141	JX259110	DQ871375	JX259221
<i>Camaroptera brachyura tincta</i>	Guinea	Cisticolidae	MNHN 40-14	JX259137	JX259199	JX259240
<i>Camaroptera brachyura brevicaudata</i>	Cameroon	Cisticolidae	MNHN	JX259090	DQ871357	EU247912
<i>Camaroptera chloronota chloronota</i>	Cameroon	Cisticolidae	MNHN	JX259104	DQ871369	EU247913
<i>Camaroptera brachyura harterti</i>	Angola	Cisticolidae	GA59033	JX259150	-	EU247901
<i>Camaroptera superciliaris</i>	-	Cisticolidae	MNHN	JX259105	JX259191	-
<i>Cisticola brachypterus brachypterus</i>	Cameroon	Cisticolidae	MNHN	JX259092	JX259189	JX259211



<i>Cisticola cantans belli</i>	Uganda	Cisticolidae	FMNH 391762	JX259111	DQ871376	JX259222
<i>Cisticola cherina</i>	Madagascar	Cisticolidae	FMNH 352929	JX259112	DQ871377	JX259223
<i>Cisticola chubbi adametzi</i>	Cameroon	Cisticolidae	MNHN 40-6	JX259132	JX259194	JX259235
<i>Cisticola chubbi adametzi</i>	Cameroon	Cisticolidae	MNHN 40-10	JX259133	JX259195	JX259236
<i>Cisticola chubbi adametzi</i>	Cameroon	Cisticolidae	MNHN 40-11	JX259134	JX259196	JX259237
<i>Cisticola chubbi adametzi</i>	Cameroon	Cisticolidae	MNHN 40-12	JX259135	JX259197	JX259238
<i>Cisticola chubbi discolor</i>	Cameroon	Cisticolidae	MNHN	JX259159	JX259207	JX259252
<i>Cisticola galactotes amphilecta</i>	Uganda	Cisticolidae	FMNH 346443	JX259113	DQ871378	JX259224
<i>Cisticola tinniens perpallus</i>	Angola	Cisticolidae	GA59061	JX259149	-	JX259245
<i>Eminia lepida</i>	Kenya	Cisticolidae	ZMUC	JX259122	-	JX259229
<i>Eminia lepida</i>	-	Cisticolidae	MNHN	-	-	JX259227
<i>Hypergerus atriceps</i>	Cameroon	Cisticolidae	MNHN	JX259120	AF407053	-
<i>Hypergerus atriceps</i>	-	Cisticolidae	-	JX259123	-	-
<i>Incana incana</i>	Socotra	Cisticolidae	MNHN	JX259103	DQ871368	JX259218
<i>Neomixis striatigula</i>	Madagascar	Cisticolidae	FMNH	JX259124	DQ871383	JX259230
<i>Neomixis tenella</i>	Madagascar	Cisticolidae	FMNH	JX259125	DQ871384	JX259231
<i>Neomixis viridis</i>	Madagascar	Cisticolidae	FMNH	JX259126	DQ871385	JX259232
<i>Oreolais pulchra pulchra</i>	Kenya	Cisticolidae	MNHN	JX259129	DQ871388	EU247906



<i>Oreolais pulchra pulchra</i>	Cameroon	Cisticolidae	MNHN 40-95	JX259155	-	JX259248
<i>Oreolais ruwenzorii</i>	Uganda	Cisticolidae	FMNH 355837	JX259107	DQ871372	EU247908
<i>Orthotomus castaneiceps chloronotus</i>	Philippines	Cisticolidae	FMNH 350975	JX259114	DQ871379	-
<i>Orthotomus sutorius inexpectatus</i>	China, Yunnan	Cisticolidae	MNHN	JX259100	DQ871365	EU686375
<i>Poliolais lopezi manengubae</i>	Cameroon	Cisticolidae	MNHN 40-13	JX259136	JX259198	JX259239
<i>Prinia atrogularis superciliaris</i>	China, Yunnan	Cisticolidae	MNHN	JX259101	DQ871366	JX259217
<i>Prinia bairdii bairdii</i>	Cameroon	Cisticolidae	MNHN	JX259093	DQ871358	JX259212
<i>Prinia bairdii obscura</i>	Uganda	Cisticolidae	FMNH 355824	JX259117	DQ871381	JX259226
<i>Prinia flavicans bihe</i>	Angola	Cisticolidae	W17420	JX259142	JX259200	JX259241
<i>Prinia flavicans bihe</i>	Angola	Cisticolidae	W7418	JX259143	JX259201	JX259242
<i>Prinia subflava subflava</i>	Cameroon	Cisticolidae	MNHN	JX259102	DQ871367	EU247915
<i>Prinia subflava mutatrix</i>	Malawi	Cisticolidae	GA84821	JX259144	JX259202	JX259243
<i>Scepomycter winifredae</i>	Tanzania	Cisticolidae	ZMUC/JK2	JX259097	DQ871361	JX259216
<i>Scepomycter winifredae</i>	Tanzania	Cisticolidae	ZMUC/LHA1	JX259098	DQ871362	-
<i>Schistolais leucopogon reichenowi</i>	Uganda	Cisticolidae	FMNH 391767	JX259118	DQ871382	-
<i>Schistolais leucopogon leucopogon</i>	Cameroon	Cisticolidae	MNHN 40-7	-	EU239801	EU247902
<i>Urolais epichlorus epichlorus</i>	Cameroon	Cisticolidae	MNHN 40-5	-	EU239802	EU247903
<i>Apalis flavida caniceps</i>	Gambia	Cisticolidae	UMMZ235.837	AY136597		
<i>Camaroptera brachyura brevicaudata</i>	Gambia	Cisticolidae	UMMZ A339	AF407052		



<i>Cisticola cantans swanzii</i>	Gambia	Cisticolidae	UMMZ235.853	AY136593		
<i>Cisticola fulvicapilla hallae</i>	Zimbabwe	Cisticolidae	UMMZ A761	AF407051		
<i>Cisticola juncidis juncidis</i>	France	Cisticolidae	MNHN	JX259190		
<i>Eminia lepida</i>	Kenya	Cisticolidae	UMMZ 211635	AY136598		
<i>Hypergerus atriceps</i>	Gambia	Cisticolidae	UMMZ A345	AF407053		
<i>Prinia subflava subflava</i>	Gambia	Cisticolidae	UMMZ235.854	AY136594		
<i>Schistolais leontica</i>	Guinea	Cisticolidae	UMMZ235.855	AY136595		
<i>Schistolais leucopogon leucopogon</i>	Cameroon	Cisticolidae	UMMZ232.418	AY136596		