ISSN 2347-6893
Volume 9 Number 1
Journal of Advances in Biology

Based on the molecular phylogeny of the genus *Illadopsis*, a very great genetic difference between sedentary populations of *Illadopsis rufipennis* resurrects the name *Illadopsis extrema* Bates, 1930

Billy Nguembock[1,2,*], Corinne Cruaud[3]

[1]*Laboratory of Zoology, Ornithology Unit, Department of Animal Biology and Physiology, University of Yaounde I, P.O. Box 812 Yaounde, Cameroon;* [2]*Department of Biological Sciences, University of Montreal, C.P. 6128, Center-town, Montreal, Quebec, H3C 3J7, Canada.*

billyngue@yahoo.fr

[3]*Genoscope. National Sequencing Center. 2, rue Gaston Crémieux, CP5706, 91057 Evry Cedex, France.*
cruaud@genoscope.cns.fr

ABSTRACT

Illadopsis rufipennis is a sedentary member of the African jungle babblers and in our paper concerning this group, we noticed a strong divergence between its two subspecies. To check this divergence and leaning partially on our phylogram obtained in our paper already published, we investigated the genetic variation of the individuals of the two subspecies of *Illadopsis rufipennis* distributed in the west (Liberia) and the west (Central African Republic) of Africa. For this, we use two mitochondrial genes (ND2 and ND3) to calculate their genetic distances and to explore their mutational differentiation. We also used our phylogenetic and dating results already obtained and then some are already published. Thus with our ND2 and ND3, a genetic distance of 10.45% and 12.8% has been estimated respectively between individuals of *Illadopsis rufipennis extrema* (Liberia) and *Illadopsis rufipennis rufipennis* (Central African Republic) but it was, for the same markers, of 0.77% and 2.62% respectively between specimens of *I. r. rufipennis* caught in Central African Republic and Cameroon. For the mutational differentiation, a total of 153 different molecular characters were observed on the two markers investigated between these two subspecies. Leaning on our dating results, the split between the subspecies *Illadopsis rufipennis extrema* (Liberia) and the subclade *Illadopsis rufipennis rufipennis* (Central African Republic and Cameroon) and *Illadopsis pyrrhoptera* was estimated at 2.98 ± 0.88 Myrs. This time period slightly coincides with one of the three estimated peaks of global cooling occurred in Africa which created and maintained forest refuges during this most arid period and plays a key role in the speciation of several African taxa. Thus for these sedentary birds separated around 3260 km with several geographical barriers, marked by the variation of the color of their top and sides of the head as well as measures of their weight and for which no risk of recurrent gene flow is again possible, we suggest resurrecting name *Illadopsis extrema* Bates, 1930 for the individuals referred as *Illadopsis rufipennis extrema* of this current study.

Keywords: African Jungle Babblers; Divergence; Genetic Variation; Mutational Differentiation; Global Cooling; Speciation; Sedentary Birds; Geographic Barrier; Recurrent Gene Flow; Taxonomic Revision.

Academic Discipline and Sub-Discipline

Biology and Ornithology

SUBJECT

Phylogeny and Biogeography of the African Passeriformes (Speciation of the sedentary forest birds)

TYPE (METHOD/APPROACH)

Phylogeny analysis, mutational differentiation and molecular dating analysis

*Corresponding author

INTRODUCTION

African jungle babblers or illadopsises, genus *Illadopsis* Heine, 1859, are small shy babblers that occupy the undergrowth of humid African forest habitats. Before our molecular study [1], their taxonomy and their diversification were poorly known and their grouping was mainly based on their physical appearance although their morphological differentiation is not obvious [2, 3]. Since our study, their grouping is strongly supported and their relationships are clearly supported [1]. Otherwise also, their African diversification in time and space appeared more complex than the Pleistocene time frame which was traditionally associated with the diversification of African forest taxa [1, 4]. According to [5], *Illadopsis* comprises seven taxa among these, *Illadopsis rufipennis*.

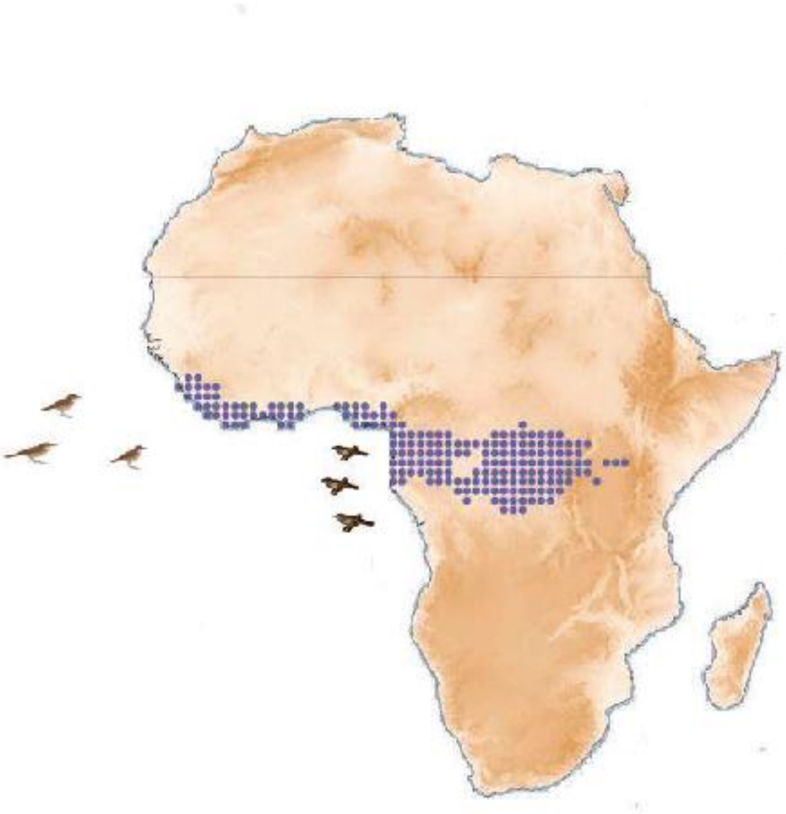

Figure 1. Distribution of the Pale-breasted Illadopsis. This map shows the simulated distribution of *Illadopsis rufipennis* based on the observed climate change and has highlighted its distribution between diverse parts of Africa (blue color).

The Pale-breasted Illadopsis is a polytypical Illadopsis which has a very large geographical range. It is a sedentary resident which lives in the West, East and South of Africa. *Illadopsis rufipennis* is found in Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Nigeria, Cameroon, Central African Republic, Gabon, Equatorial Guinea, Republic of the Congo, Democratic Republic of the Congo, Uganda, Kenya, Tanzania and Angola (Figures 1, 2 and 3). It lives in tropical and subtropical moist lowland forests and mountain forests, but also in various other areas such as forest patches in grassland sometimes between 500 and 2200 meters [3].

Figure 2. Distribution of *Illadopsis extrema*. This map shows that this subspecies of *Illadopsis rufipennis* has a very restricted geographical range and is found only from Sierra Leone to Ghana (blue color).

According to [5], four subspecies are now recognized, *Illadopsis rufipennis rufipennis* Sharpe, 1872; *I. r. bocagei* Salvadori, 1903; *I. r. distans* Friedmann, 1928 and *I. r. extrema* Bates, 1930. *Illadopsis rufipennis rufipennis* is distributed from southern Nigeria to Angola, Uganda, western Kenya and northwestern Tanzania; *I. r. bocagei* to Bioko island; *I. r. distans* from southern Kenya to northeastern Tanzania and Zanzibar island and finally, *I. r. extrema* from Sierra Leone to Ghana [2, 3, 5].

Figure 3. Distribution of *Illadopsis rufipennis rufipennis*. This map shows a view of the geographical range of this subspecies of *Illadopsis rufipennis* which can be found in several countries including Nigeria, Angola, Uganda, Kenya and Tanzania (blue color).

In our study concerning the genus *Illadopsis* [1], we had included two (2) subspecies of *Illadopsis rufipennis*, *I. r. rufipennis* and *I. r. extrema*. Curiously, we found that these two subspecies were not sister-taxa [1]. Only, these subspecies strongly formed a clade with *I. pyrrhoptera* in which *I. r. rufipennis* appeared strongly as the sister-taxon of *I. pyrrhoptera* [1]. Otherwise in this study, we also noted the basal position of *I. r. extrema* of the clade comprising *I. r. rufipennis* and *I. pyrrhoptera* [1].

The type subspecies of the species *Illadopsis rufipennis* Sharpe, 1872 is *Illadopsis rufipennis rufipennis*. *Illadopsis rufipennis rufipennis* has been described as *Trichastoma rufipennis* Sharpe, 1872 and during a little time as *Turdinus albipectus minutus* van Someren, 1915 but it is since recognized as *Illadopsis rufipennis rufipennis* Sharpe, 1872. This species includes four (4) subspecies which in principle have a geographic variation. Among these, there is *Illadopsis rufipennis extrema* Bates, 1930 which has been described as such and which has a very restricted geographical range (Figure 2).

Leaning on our first results obtained regarding the *Illadopsis* genus, in this study which includes the type subspecies of the species *Illadopsis rufipennis* Sharpe, 1872, we examine the great genetic variation of the two subspecies of *Illadopsis rufipennis* caught on the one hand in a part of West Africa (Liberia), and on the other hand in another part of this West Africa (Cameroon and Central African Republic). For this, we use two mitochondrial protein-coding genes evolving rapidly (ND2 and ND3) in order to examine, for these two sedentary subspecies of the Pale-breasted Illadopsis having no contact zone, part of their current genetic heritage and divergence times to provide more information about their diversification.

MATERIALS AND METHODS

For the taxon sampling, we kept the same biological material already used in our first publication [1]. Thus for the taxonomy of the species, we will refer to our last study [1] as well as to the new designation name of this family [5].

Genetic distances of the two subspecies of the Pale-breasted Illadopsis

To examine the genetic distances between these two subspecies, we used nucleotide sequences of two mitochondrial genes (ND2 and ND3) and we selected as model "Number of differences" for the estimates of their genetic distances using MEGA v3.1. [6]. For this, we used taxa already published in our first study for a total of Operational Taxonomic Units of 41 (ND2 and ND3) (see Table 1 in Annexe).

Mutational differentiation between the two subspecies of the Pale-breasted Illadopsis

In order to explore the main differences between these two subspecies, we considered comparing the nucleotide sequences of their molecular markers. For this, we mainly used contigs of the two mitochondrial genes (ND2 and ND3) which were aligned using the algorithm in Sequencher 3.1 [7]; for each molecular marker, we compared the nucleotide sequences of these two subspecies to find mutations.

Phylogenetic analysis

To examine the phylogenetic relationships of these two subspecies of *Illadopsis rufipennis*, two methods (Maximum Likelihood and Bayesian Inference) were used in our first study [1]. In this present study, we lean on trees already obtained in our last study [1] in order to analyze on the one hand their relationships and on the other hand, their closed related with other ingroup taxa.

Divergence times

In order to investigate the diversification of the two subspecies of the Pale-breasted Illadopsis, we rely on the tree obtained with the Bayesian approach (estimate of the divergence time with MULTIDISTRIBUTE Software) and already published in our last study [1].

RESULTS

Genetic distances results between these two subspecies

Results of the genetic distances are presented in Table 2. According to these estimates, the subspecies *Illadopsis rufipennis rufipennis* appears to be different from the subspecies *Illadopsis rufipennis extrema*. Thus, with the protein-coding gene ND2, the genetic distance obtained between these two subspecies was of 10.45% (Table 2). With the ND3 gene, the genetic distance obtained between *I. r. extrema* and *I. r. rufipennis* was of 12.8% (Table 2). At the same time, we noted that the genetic distances between specimens of *I. r. rufipennis* caught in the Central African Republic and Cameroon with protein-coding genes ND2 and ND3 were only 0.77% and 2.62%, respectively (Table 2).

<u>Table 2:</u> Estimates of the genetic distance (values in %) obtained from the ADN mitochondrial among the subspecies of the species *Illadopsis rufipennis* collected in several African forests following a "number of differences" model implemented in MEGA v3.1. [6].

	ND2	**ND3**
Nucleotide: number of differences between *I. rufipennis rufipennis* (Central African Republic and Cameroon)/ *I. rufipennis extrema* (Liberia)	10.45	12.8
Nucleotide: number of differences between *I. rufipennis rufipennis* (Central African Republic)/*I. rufipennis rufipennis* (Cameroon)	0.77	2.62

*: We used our largest *Illadopsis* ingroup [1].

Mutational differentiation results between these two subspecies

Results of the mutational differentiation are presented in Tables 3a and 3b. According to our investigation, the nuclear genome did not permit to find the mutational differentiation between these two subspecies and only our mitochondrial genome permitted to observe it. According to these tables, the subspecies *Illadopsis rufipennis extrema* has several different molecular characters (substitution mutations) compared to *Illadopsis rufipennis rufipennis*. With the two mitochondrial genes investigated, a total of 153 different molecular characters were observed between these two subspecies of *Illadopsis rufipennis* (Tables 3a; 3b). Thus, with the protein-coding gene ND3, we noted 37 different molecular characters between *I. r. extrema* and *I. r. rufipennis* (Table 3b). However, up to 116 different substitution mutations have been recovered between these two subspecies with the ND2 gene (Table 3a). In our tables, we indicate accurately the number of substitution positions as observed in the *Illadopsis* alignment of the two mitochondrial genes investigated (Tables 3a; 3b).

<u>Table 3a:</u> Molecular characters (substitution mutations) observed in the alignment of ND2 sequences among the subspecies of the species *Illadopsis rufipennis* collected from several African forests.

Different molecular characters between *Illadopsis rufipennis rufipennis and *I. r. extrema*** (number of substitution position in the *Illadopsis* alignment of ND2 sequences)**	**Total of different molecular characters observed for each kind of substitution**
Transition A-G	
N°31, N°66, N°79, N°90, N°189, N°192, N°246, N°267, N°288, N°300, N°312, N°397, N°423, N°432, N°438, N°448, N°471, N°478, N°484, N°519, N°523, N°561, N°570, N°571, N°609, N°625, N°630, N°693, N°703, N°735, N°759, N°783, N°819, N°826, N°864, N°870, N°891, N°901, N°903, N°979, N°991, N°1032	42
Transition C-T	
N°48, N°60, N°63, N°75, N°120, N°126, N°150, N°171, N°174, N°186, N°205, N°219, N°222, N°231, N°243, N°261, N°270, N°286, N°306, N°348, N°363, N°370, N°378, N°387, N°450, N°469, N°477, N°479, N°480, N°490, N°525, N°543, N°564, N°573, N°576, N°594, N°602, N°636, N°655, N°696, N°750, N°753, N°780, N°786, N°795, N°801, N°853, N°879, N°880, N°888, N°900, N°912, N°915, N°921, N°924, N°948, N°969, N°971, N°978, N°982, N°1011, N°1020, N°1031, N°1034	64
Transversion A-C	
N°57, N°129, N°330, N°726, N°733, N°967	6
Transversion A-T	
N°42, N°354, N°744, N°1030	4
Total of molecular characters between two subspecies *I. r. rufipennis* and *I. r. extrema*	116

*: Sequence EU686326 already published in Genbank [1].**: Sequence EU686328 already published in Genbank [1].

Phylogenetic results

In our last publication on the *Illadopsis* genus [1], all members of this genus strongly formed a monophyletic group; within this clade, *Illadopsis rufipennis* appeared strongly related to *Illadopsis pyrrhoptera* with our ND2, ß-fibrinogen intron5 (tree not shown) and the concatenated datasets and with high bootstrap values (posterior probabilities 1.00) (Figures 4 and 5).

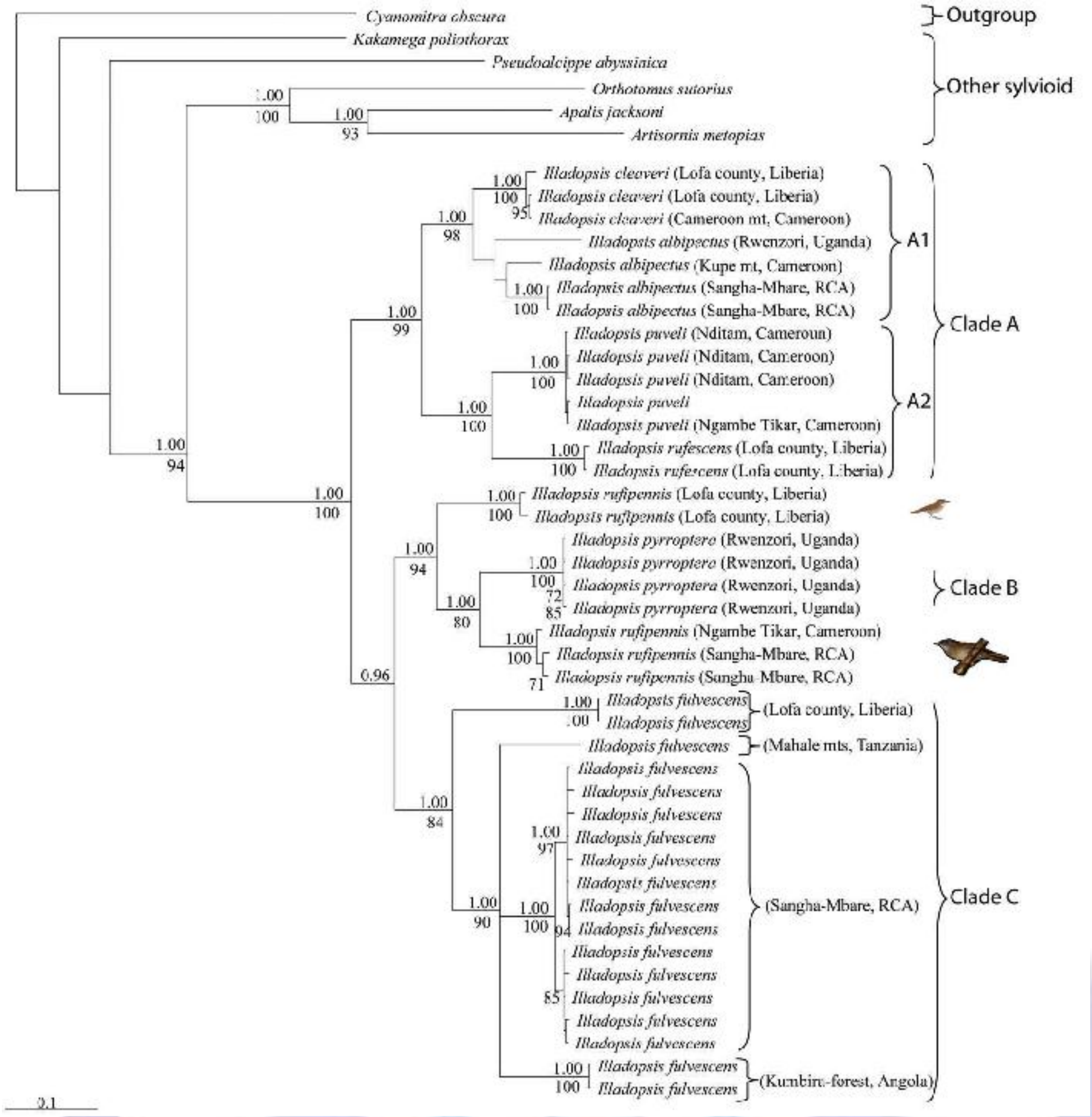

Figure 4. Pictures of *Illadopsis rufipennis extrema* and *Illadopsis rufipennis rufipennis* on the ND2 tree obtained in [1] confirming the strong divergence between individuals of the populations of Pale-breasted Illadopsis from the same part of West Africa, *I. r. extrema* (Liberia) and *I. r. rufipennis* (Central African Republic).

On the basis of on our first results [1], we note that the subspecies *Illadopsis rufipennis extrema* (Liberia) is not closely related to the subspecies *Illadopsis rufipennis rufipennis* (Central African Republic) which appeared strongly close to *Illadopsis pyrrhoptera* with high bootstrap values (posterior probabilities 1.00 and PhyML bootstrap values 100) (Figures 4 and 5). Otherwise, all the phylograms obtained with ND2, ß-fibrinogen intron5 (not shown) as well as the concatenated datasets clearly show that *I. r. extrema* (Liberia) diverged profoundly from the subclade which includes *I. r. rufipennis* (Central African Republic) and *I. pyrrhoptera*, and this divergence is strongly supported by high bootstrap values (posterior probabilities 1.00 and PhyML bootstrap values ≥ 0.80) (Figures 4 and 5).

Table 3b: **Molecular characters (substitution mutations) observed in the alignment of ND3 sequences among the subspecies of the species *Illadopsis rufipennis* collected from several African forests.**

Different molecular characters between *Illadopsis rufipennis rufipennis and *I. r. extrema*** (number of substitution position in the *Illadopsis* alignment of ND3 sequences)**	**Total of different molecular characters observed for each kind of substitution**
Transition A-G	
N°46, N°75, N°80, N°111, N°204, N°324, N°333, N°348	8
Transition C-T	
N°14, N°18, N°26, N°47, N°52, N°79, N°120, N°123, N°126, N°141, N°156, N°172, N°189, N°190, N°198, N°213, N°228, N°257, N°261, N°289, N°292, N°315	22
Transversion A-C	
N°72, N°85, N°153, N°285, N°286	5
Transversion A-T	
N°68, N°291	2
Total of molecular characters between two subspecies *I. r. rufipennis* and *I. r. extrema*	37

*: Sequence EU686369 already published in Genbank [1].**: Sequence EU686371 already published in Genbank [1].

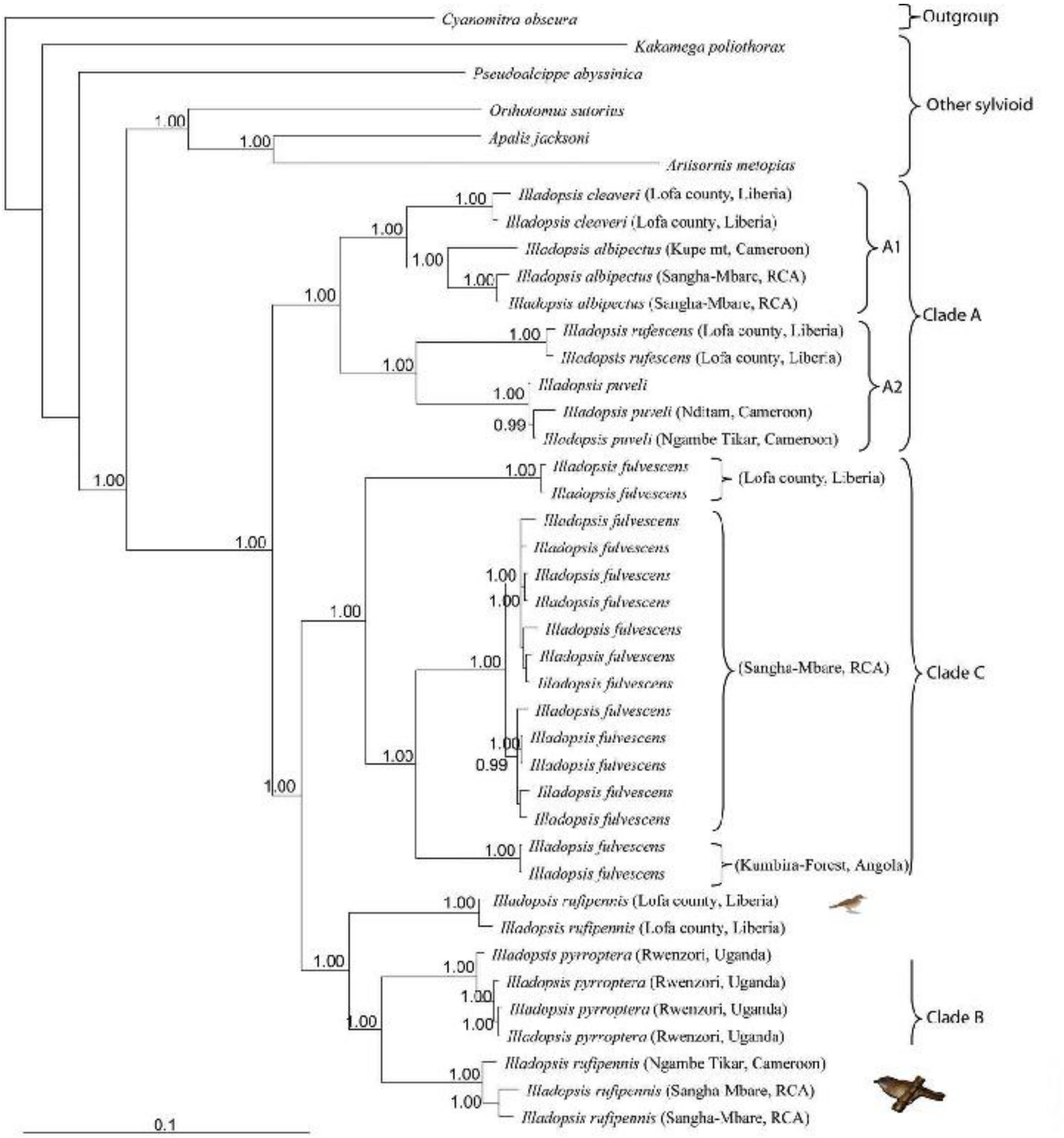

Figure 5. Pictures of *Illadopsis rufipennis extrema* and *Illadopsis rufipennis rufipennis* on the concatenated dataset tree obtained in [1] supporting the strong divergence between individuals of the populations of Pale-breasted Illadopsis from the same part of West Africa, *I. r. extrema* (Liberia) and *I. r. rufipennis* (Central African Republic).

Dating analysis results

Leaning on our dating results already published [1], we note that the diversification of the genus *Illadopsis* took place during the Neogene period (Figure 6; Table 4).

Table 4: **Some dates inferred within the genus *Illadopsis* suggested in [1] from a relaxed molecular clock analysis using the MULTIDISTRIBUTE Software package in which the time divergence between individuals of *Illadopsis rufipennis extrema* (Liberia) and the subclade which includes the subspecies *Illadopsis rufipennis rufipennis* (Central African Republic) and the species *Illadopsis pyrrhoptera* has been estimated.**

Split	**Date**	**CI (95%)**
Split between *I. rufipennis* and *I. pyrrhoptera*/*I. fulvescens*	3.07 ± 0.89	1.65 – 5.11
I. rufipennis (Lofa county)/ *I. pyrrhoptera* and other *rufipennis*	2.98 ± 0.88	1.58 – 5.03
I. rufipennis (Sangha-Mbare) and *I. pyrrhoptera*	2.14 ± 0.76	0.97 – 3.97

During this Neogene period, *Illadopsis fulvescens* diverged from the clade which includes *Illadopsis rufipennis* and *Illadopsis pyrrhoptera* during the Pliocene epoch (Figure 6); within this clade, the subspecies *Illadopsis rufipennis extrema* (Liberia) diverged from the subclade which includes the subspecies *Illadopsis rufipennis rufipennis* (Central African Republic) and the species *Illadopsis pyrrhoptera* during the Neogene period (Figure 6).

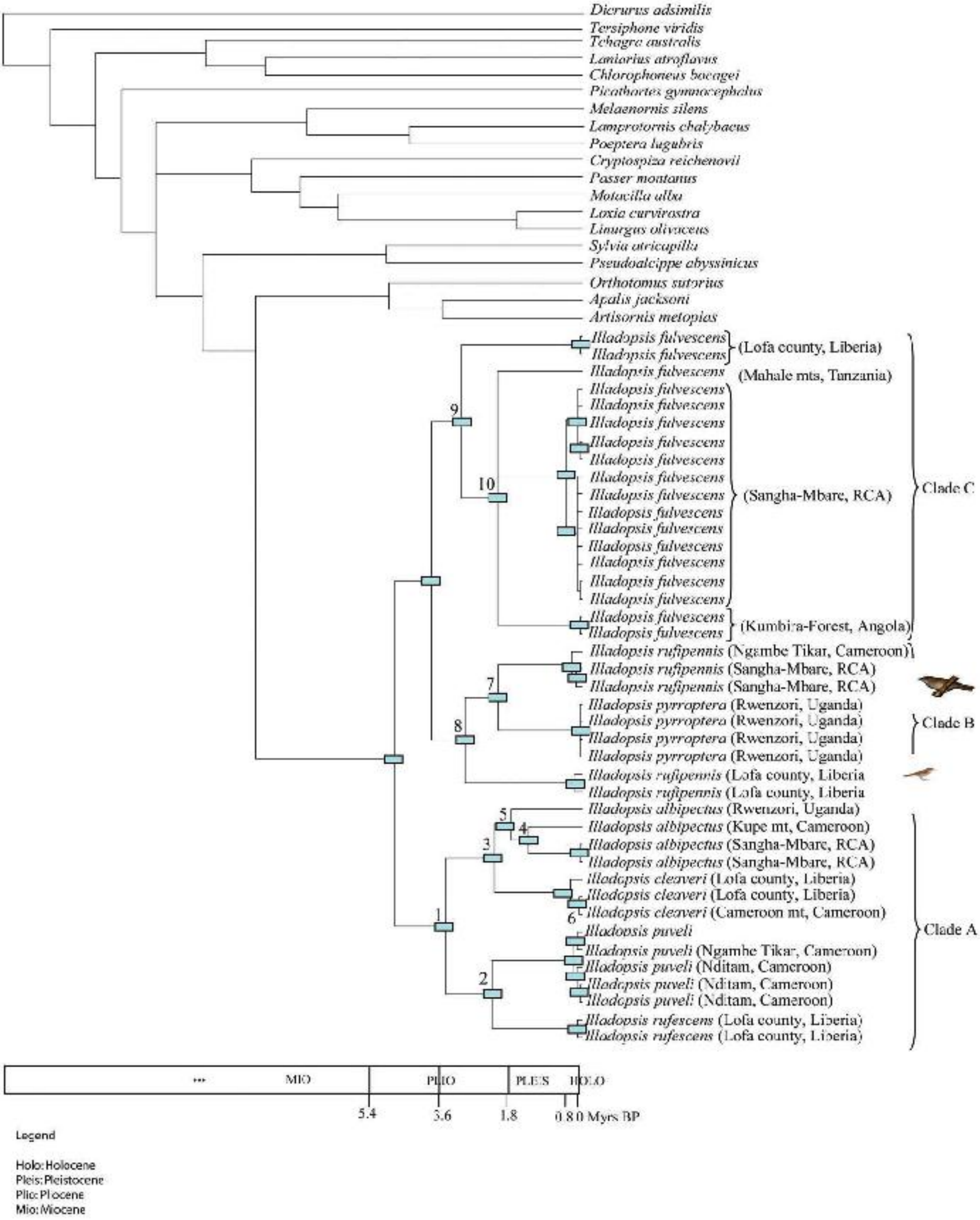

Figure 6. Pictures of *Illadopsis rufipennis extrema* and *Illadopsis rufipennis rufipennis* on the chronogram obtained in [1] illustrating the time divergence during the Neogene period between individuals of *I. r. extrema* (Liberia) and the subclade which includes the subspecies *I. r. rufipennis* (Central African Republic) and the species *I. pyrrhoptera*.

DISCUSSION

Several phylogenetic aspects of the African jungle babblers are already addressed in our last publication which revealed the complexity of the diversification of African taxa in time and space [1]. In our current study on the *Illadopsis rufipennis* species, we will focus primarily on the climatic and geological changes that occurred during the Neogene period in certain parts of the African continent and their possible consequences on the diversification of certain African lowland birds as well as possibly their speciation. The species *Illadopsis rufipennis* has a very large geographical range and is distributed from the extreme west to the extreme east of Africa (Figures 1, 2 and 3).

Climate and geological changes within the *Illadopsis rufipennis* geographic range

It is now well known that the climate of Africa has fluctuated greatly throughtout history and in particular the climatic variation of the Pleistocene which is often quoted to explain the pattern of biogeography in Africa [8, 9, 10, 11, 12]. And during periods of the global cooling which are estimated close to 1.0, 1.7 and 2.8 Myrs [13, 14], the continent of Africa has undergone climatic fluctuations implying a succession of wetter and arid phases. Otherwise, this great aridity would have facilitated the expansion of the biomes between the savannah and those semi-arid, and the contraction of forest biomes as observed in some parts which offer favorable conditions for speciation [8, 9, 10, 11, 15, 16, 17].

Otherwise, the Lofa County range (Liberia) shares the same area as the Guinean highlands which are linked to the Jos Plateau in Nigeria, and in the Adamawa region and the Cameroon highlands of Cameroon. According to [18], the forests of the Cameroonian Highlands were linked to the forests of the East African Highlands by the Adamawa Plateau and they would have been separated after the distension of the African plate during the Cenozoic Era. And among the Highlands of Guinea, there is the Mount Nimba. This mountain is a very old mountain which would probably be shaped until the end of the Eocene epoch [19] and this period coincides relatively with that suggested by [18] concerning the distension of the African plate.

Diversification of the species *Illadopsis rufipennis*

According to our dating results, the diversification of the genus *Illadopsis* took place during the Pliocene epoch or the Plio-Pleistocene transition (Figure 6; Table 4). The estimated divergence between the *Illadopsis rufipennis* and *Illadopsis pyrrhoptera* clade and the species *Illadopsis fulvescens* was dated to 3.07 ± 0.89 Myrs and the split between the subspecies *Illadopsis rufipennis extrema* (Liberia) and the subclade *Illadopsis rufipennis rufipennis* (Central African Republic and Cameroon) and *Illadopsis pyrrhoptera* has been estimated at 2.98 ± 0.88 Myrs (Table 4). These periods seem younger than those estimated approximately for the formation of one of the main mountains of the Guinean Highlands (Mount Nimba) by [19] and for the distension of the African plate which would have separated the forests of the Camerooonian Highlands and the forests of the Highlands of East Africa by [18]. On the other hand, these periods slightly coincide with one of the three estimated peaks of global cooling in Africa, 2.8 Myrs BP, suggested by [13, 14]. Several authors [8, 9, 10, 11, 13, 17] have postulated that the main origin of several forest birds in Africa results from the fragmentation of the forest block associated with the creation and maintenance of forest refuges during the most arid period in this continent.

The genus *Illadopsis* consists exclusively of sedentary forest birds. According to [20], contrary to migratory birds which have great dispersing abilities and can thus disperse very far from their natal sites, sedentary birds rarely disperse more than a few kilometres from their natal sites. For sedentary forest birds, several types of topography such as Highlands forests and various kinds of watercourse such as oceans, seas and sometimes a very long river constitute geographical barriers [21]. Based on our results, the genetic structure and diversity within individuals of the *Illadopsis rufipennis* species between parts of West Africa (Liberia) and West (Central African Republic) seem to indicate that these occupy distinct biotopes (Tables 2, 3a and 3b). According to our dating results, the event that separated these two subspecies took place during the Pliocene epoch (Figure 6) and corresponds to a stop of recurrent gene flow between populations of the subspecies *Illadopsis rufipennis rufipennis* (Central African Republic) and those of the subspecies *Illadopsis rufipennis extrema* (Liberia). These two African parts (West and West Africa) are globally separated around 3260 km according to these two countries.

The speciation process, which began, is highlighted by the different genetic structure noted between individuals of the two subspecies of the species *Illadopsis rufipennis* of the same African part, West (*I. r. rufipennis*) and West (*I. r. extrema*) Africa. Thus with the mitochondrial markers used, we found a total of 153 different molecular characters between these two subspecies of *Illadopsis rufipennis* only for two markers (Tables 3a and 3b). It is well known that mutations are the raw tools of evolution. They play a crucial role in speciation and can be an important indicator. Usually these mutations are the ones that can be passed on to the offspring. Thus in order to highlight the northern and southern lineages of the medaka *Oryzias latipes* after estimation of their divergence (4 Myrs), [22] have implemented a method which has made it possible to find several accumulated mutations since this time of divergence which indicate a strict genetic separation between the two populations of medaka. In the same vein, the mutational differentiation observed between these two subspecies of *Illadopsis rufipennis* appears as the occurrence of the vicariant speciation between these two sedentary forest subspecies separated around 3260 km and for which no risk of recurrent flow is again possible.

We note that the use of individuals of the populations of these two subspecies of the species *Illadopsis rufipennis* would have been better but seriously we are convinced that this would not have altered our biogeographical and mutational results for which our phylogenetic results have been already published [1] because the genetic structure as well as the diversity within the examined individuals of the populations of the same West African part of *Illadopsis rufipennis* are strongly highlighted in this current study (Figures 4 and 5; Tables 2, 3a and 3b).

Taxonomic revision

The main result of our study is the vicariant speciation of the two sedentary forest subspecies of *Illadopsis rufipennis* highlighted in this study with the genetic structure as well as the mutational differentiation within individuals of the populations of the same West African part, population of *I. r. extrema* (Liberia) and population of *I. r. rufipennis* (Central African Republic). For many years, these two taxa have been regarded as same species [2, 3, 5], based on an overall similarity in form and pattern but certainly in relation to the insufficient information on these two subspecies. Thus until our first study concerning the African jungle babblers, no earlier molecular study had included these two subspecies in the same study [1]. We can now conclude that the similarities between these two sedentary forest birds were certainly acquired in their common ancestor but no longer enough to be able to characterize these two completely distinct taxa.

Based on the results of the current study, we suggest resurrecting the name regarding the present name of the subspecies of *Illadopsis rufipennis*:

For *Illadopsis rufipennis extrema* Bates, 1930; this taxon has been described as *Illadopsis extrema* Bates, 1930 but which has since been recognized as *Illadopsis rufipennis extrema* Bates, 1930. Molecularly, this taxon appeared very different to *Illadopsis rufipennis rufipennis* in this study. Morphologically, *Illadopsis rufipennis rufipennis* differs from *Illadopsis rufipennis extrema* on the general coloration particularly *I. r. rufipennis* has head-sides distinctly greyish [3, 23]. Thus somewhat more rufous (less olive) than *Illadopsis rufipennis rufipennis*, *Illadopsis rufipennis extrema* shows the top and sides of head richly brown, not grey, and concolorous with the mantle [3]. Otherwise, according to [3], the weight of the males of *Illadopsis rufipennis rufipennis* caught in the Central African Republic was on average of 28 grams while that of the males of *Illadopsis rufipennis extrema* caught in Liberia was on average of 22 to 25 grams. As in this study, we include the type subspecies of the species *Illadopsis rufipennis*, *Illadopsis rufipennis rufipennis* Sharpe, 1872, we propose to resurrect the name *Illadopsis extrema* Bates, 1930, refers to *Illadopsis rufipennis extrema* from the present study.

ACKNOWLEDGMENTS

We are grateful to various researchers and institutions that caught and provided samples or those whose sequences and pictures have been used in this study. We thank all my new staff from the Southwest Cameroon Region as well as several anonymous referees for their comments on an earlier version of this manuscript, in particular some English-speaking researchers and students from the University of Yaounde I (Cameroon). We also thank all my staff from the Ornithology Unit of the Laboratory of Zoology of the University of Yaounde I (Cameroon) for their technical assistance.

REFERENCES

1. Nguembock, B., Cibois, A., Bowie, R. C. K., Cruaud, C. and Pasquet, E. (2009). Phylogeny and biogeography of the genus *Illadopsis* (Passeriformes: Timaliidae) reveal the complexity of diversification of some African taxa. J. Avian. Biol. 40: 113-125.
2. Hall, B. P. and Moreau, R. E. (1970). An atlas of speciation in African passerine birds. London: Trustees of the British Museum (Natural History).
3. Fry, C. H., Keith, S. and Urban, E. K. (2000). The Birds of Africa. Volume 6, Academic Press, London.
4. Moyle, R. G., Andersen, M. J., Oliveros, C. H., Steinheimer, F. D., Reddy, S. (2012). Phylogeny and biogeography of the core Babblers (Aves: Timaliidae). Syst. Biol. 61: 631-651.
5. International Ornithologist Congress. 2012. The World Bird List. Version 3.1. http://www.worldbirdnames.org/n-warblers.html
6. Kumar, S., Tamura, K. and Nei, M. (2004). MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignement. Brief. Bioinform. 5: 150-163.
7. Gene Codes Corporations (1998). Sequencher. Version 3.1. Ann. Arbor, MI.

8. Diamond, A. W. and Hamilton, A. C. (1980). The distribution of forest passerine birds and Quaternary climate change in Africa. J. Zool. 191: 379-402.

9. Crowe, T. M. and Crowe, A. A. (1982). Patterns of distribution, diversity and endemism in Afro-tropical birds. J. Zool. 198: 417-442.

10. Mayr, E. and O'Hara, R. J. (1986). The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. Evolution 40: 55-67.

11. Hamilton, A., Taylor, D. and Howard, P. (2001). Hotspots in African Forest as Quaternary Refugia. In African rain forest ecology and conservation: An interdisciplinary perspective (Weber W, White L, Vedder A, Naughton-Treves L, eds). Yale University Press, New Haven, pp. 233-262.

12. Maley, J. (2001). The Impact of Arid Phases on the African Rain Forest Through Geological History. In African rain forest ecology and conservation: An interdisciplinary perspective (Weber W, White L, Vedder A, Naughton-Treves L, eds). Yale University Press, New Haven, pp. 68-87.

13. deMenocal, P. B. (1995). Plio-Pleistocene African climate. Science 270: 53-58.

14. deMenocal, P. B. (2004). African climate change and faunal evolution during the Pliocene-Pleistocene. Ear. Planet. Sci. Lett. 220: 2-24.

15. Hamilton, A. C. (1992). History of forests and climate. In The Conservation Atlas of Tropical Forests: Africa (Sayer JA, Harcourt CS, Collins NM, eds). IUCN and MacMillan Publishers, United Kingdom, pp. 17-25.

16. Jenkins, M. and Hamilton, A. C. (1992). Biological diversity. In The Conservation Atlas of Tropical Forests: Africa (Sayer JA, Harcourt CS, Collins NM, eds). IUCN and MacMillan Publishers, United Kingdom, pp. 26-32.

17. Partridge, T. C., Wood, B. A. and deMenocal, P. B. (1995). The influence of global climatic change and regional uplift in large-mammalian evolution in East and Southern Africa. In Paleoclimate and Evolution with Emphasis on Human Origins (Vrba ES, Denton GH, Partridge TC, Burckle LH, eds). Yale University Press, New Haven, pp. 331-335.

18. Moreau, R. E. (1966). The bird faunas of Africa and its islands. Volume 8. New York and London: Academic Press.

19. Lévêque, C. (1997). Biodiversity dynamics and conservation: the freshwater fish of tropical Africa. Cambridge University Press, 432p.

20. Borrow, N. and Demey, R. (2004). Field Guide to the Birds of Western Africa. Christopher Helm. London.

21. Nguembock, B. (2008). Etude phylogénétique et biogéographique de l'avifaune forestière des montagnes de l'Ouest Cameroun. Ph.D Thesis, University of Paris VI, France.

22. Takeda, H. (2008). Draft genome of the medaka fish: a comprehensive resource for medaka developmental genetics and vertebrate evolutionary biology. Dev. Growth Differ. 50 Suppl 1: S157-S166.

23. Borrow, N. and Demey, R. (2001). A Guide to the Birds of Western Africa. Princeton University Press, Princeton and Oxford.

Author's 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 PhD'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 *Illadopsis* ingroup between subspecies of the species *Illadopsis rufipennis* and to explore mutational differentiation after making contigs between these two subspecies. Genbank accession numbers of these OTUs have been already published in our first publication concerning the genus *Illadopsis* [1].

Species	Country	Family	N° Accession	ND2	ND3
Ingroup					
Illadopsis albipectus	Uganda	Pellorneidae	ZMC 122295	EU686291	EU686333
Illadopsis albipectus	Central African Republic	Pellorneidae	AMNH 51	EU686292	EU686334
Illadopsis albipectus	Central African Republic	Pellorneidae	AMNH 2031	EU686293	EU686335
Illadopsis albipectus	Cameroon	Pellorneidae	MNHN 40-74	EU686294	EU686336
Illadopsis cleaveri	Liberia	Pellorneidae	AMNH 5191	EU686295	EU686337
Illadopsis cleaveri	Liberia	Pellorneidae	AMNH 5209	EU686296	EU686338
Illadopsis cleaveri	Cameroon	Pellorneidae	ZMC 73066	EU686297	EU686339
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 017	EU686298	EU686340
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 38	EU686299	EU686341
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 101	EU686300	EU686342
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 126	EU686301	EU686343
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 127	EU686302	EU686344
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 207	EU686303	EU686345
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 2011	-	EU686346
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 2165	EU686304	EU686347
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 27724	EU686305	EU686348
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 23743	EU686306	EU686349
Illadopsis fulvescens	Liberia	Pellorneidae	AMNH 5203	EU686307	EU686350
Illadopsis fulvescens	Liberia	Pellorneidae	AMNH 243	EU686308	EU686351
Illadopsis fulvescens	Tanzania	Pellorneidae	ZMC 135223	EU686309	EU686352
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 022	EU686310	EU686353
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 026	EU686311	EU686354
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 047	EU686312	EU686355
Illadopsis fulvescens	Central African Republic	Pellorneidae	AMNH 071	EU686313	EU686356
Illadopsis fulvescens	Angola	Pellorneidae	SW 36-C02	EU686314	EU686357
Illadopsis fulvescens	Angola	Pellorneidae	SW 36-C01	EU686315	EU686358
Illadopsis puveli	-	Pellorneidae	ZMC b	EU686316	EU686359
Illadopsis puveli	Cameroon	Pellorneidae	MNHN1998-775	EU686317	EU686360
Illadopsis puveli	Cameroon	Pellorneidae	MNHN1998-759	EU686318	EU686361
Illadopsis puveli	Cameroon	Pellorneidae	MNHN1998-760	EU686319	-
Illadopsis puveli	Cameroon	Pellorneidae	MNHN	EU686320	EU686362
Illadopsis pyrroptera	Uganda	Pellorneidae	ZMC 123065	EU686321	EU686363
Illadopsis pyrroptera	Uganda	Pellorneidae	ZMC 123068	EU652715	EU686364
Illadopsis pyrroptera	Uganda	Pellorneidae	ZMC 123071	EU686322	EU686365
Illadopsis pyrroptera	Uganda	Pellorneidae	ZMC 123073	EU686323	EU686366
Illadopsis rufescens	Liberia	Pellorneidae	AMNH 451	EU686324	EU686367
Illadopsis rufescens	Liberia	Pellorneidae	AMNH 5202	EU686325	EU686368
Illadopsis rufipennis	Central African Republic	Pellorneidae	AMNH 012	EU686326	EU686369
Illadopsis rufipennis	Central African Republic	Pellorneidae	AMNH 2100	EU686327	EU686370
Illadopsis rufipennis	Liberia	Pellorneidae	AMNH 5187	EU686328	EU686371
Illadopsis rufipennis	Liberia	Pellorneidae	AMNH 5192	EU686329	EU686372
Illadopsis rufipennis	Cameroon	Pellorneidae	MNHN1998-772	EU652716	EU686373