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A SNP BARCODE TO INFORM GENETIC VARIATION AND EVOLUTION PLASMODIUM FALCIPARUM MALARIA IN MALI

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ABSTRACT

Background: Despite of considerable advances in malaria control strategies, malaria continues to kill countless numbers of kids, mainly in sub-Saharan African countries. Mali is listed among the ten countries most suffering from malaria within the world in keeping with the quantity of cases and deaths... *Plasmodium falciparum* parasite is related to the foremost severe cases of malaria, especially in children. Malaria reports increasingly state drug resistance with severe cases, so investigating the genetic diversity and evolutionary history of *Plasmodium falciparum* are going to be crucial to understanding the evolution and variation of malarial drug resistance, and within the explore for potential vaccines. During this study, 186 samples were obtained from two sites in Mali: Koila Bamana in near of the town of Ségou and Nioro-du-Sahel situated within the Sahelian region. **Methods:** The 27-SNP barcode assay and phylogenetic tree (maximum likelihood method) were generated to research the variety and evolutionary relationship of the *Plasmodium falciparum* parasite population of 80 isolates in two varying patterns of malaria transmission in Mali. Excluding barcode sequences containing over four (4) missing SNPs, Minor allele frequencies (MAF), Genetic differentiation (GST= Genetic statistic), Average Minor allele frequencies (AMAF), the principal component analysis (PCA) were determined. **Results:** Fifty-seven of 80 isolates (71.3%) provided sufficient data quality for meeting the inclusion criteria of barcode. The parasite populations displayed a high degree of intra-population genetic diversity (MAF >1), the inter-population diversity was relatively low (AMAF = 0.34 and = 0.28 for Koila Bambara and Nioro du Sahel respectively) consort with barcode π value respectively 0.43 and 0.44 for Koila Bambara and Nioro. The populations failed to differ with relevance genetic diversity (GST=0.03). In both populations, the PCA sustained the genetic similarities of P. falciparum. Analysis of the phylogenetic tree revealed several groups of *P. falciparum* genetically close, which could have originated from Nioro du Sahel. **Conclusions:** The genetic diversity of *P.falciparum* populations is a major is a major consider in the parasite's ability to adapt to changes in its environment thus the measures observed here for Koila Bambara and Nioro du Sahel may partially explain the rising drug-resistance and growing severe cases in those regions in Mali.

KEYWORDS: SNP, Barcode, Evolution Malaria, *Plasmodium falciparum*, genetic diversity, Mali.

Abbreviations and Acronyms

1- ML= Maximum likelihood
2- SNP= Single Nucleotide Polymorphism
3- GST= Genetic statistic
4- NJ=Neighbor-joining
5- PCA = Principal component analysis
6- MAF= Minor Allele Frequency
7- Ts= Transition (Ts) to transversion (Tv)
8- Tv= Transversion

1- INTRODUCTION

I- Background of the study

Malaria is a fatal disease caused by the Plasmodium parasite and transmitted by the bite of an infected mosquito. Considered as tropical and subtropical disease, it's seen in 97 of those regions of the globe. In 2018, there have been 258 million cases and 4,050,000 deaths.[1] There are five species of parasites that cause malaria in humans, of which two *P. falciparum* and *P. vivax* are the foremost dangerous.[2] In line with the 2018 WHO Malaria Report, in 2018, P. falciparum was chargeable for 99.7% of estimated malaria cases within the WHO African Region, 50% of cases within the WHO region. South-East Asia Region, 71% in the Eastern Mediterranean Region and 65% in the Western Pacific Region.

In spite of giant advances in malaria control strategies, and monitor malaria transmission in sub-Saharan African countries, the disease are killing constantly incalculable children, (Diakité et al. Malar J).[3]

Mali is listed among the ten countries most plagued by malaria within the world in line with the amount of cases and deaths (3% of world cases and deaths 7% of cases in West Africa). In Mali from 2015 to 2018, malaria cases peaked between 387 and 391 per 1,000 of the population in danger (World Malaria Report 2019). Mali is split into three malaria transmission zones: Stable transmission areas covering the Soudano-Guinean part and rice cultivation areas. in these areas malaria is transmitted almost throughout the year.[1] Unstable malaria mainly occupying the Sahelo-Saharan zone where malaria transmission is intermittent.[1] Sporadic malaria zones are typical of the Saharan zone with more severe and complex malaria infections.[1]

Malaria reports increasingly state drug resistance with severe cases, so investigating the genetic diversity and evolutionary history of *Plasmodium falciparum* are going to be crucial to understanding the evolution and variation. Anti-malarial resistance, and within the explore for potential vaccines.[3][5]

In 2003, Paul Hebert of the University of Guelph in Canada and his collaborators proposed the "DNA barcode" as a technique of identifying species. Other biologists have followed suit using this method to analyze the small-scale taxonomic of various species. DNA sequence and phylogenetic linkage analyzes were have been use to understand the range within a species and their classification.[6] Paul Hebert declared that in the future we'd be ready to follow the evolution of life on the world as we follow the weather today.[6]

Here, as a part of an ongoing effort to beat malaria eradication in Mali. From 186 malaria patients, we investigated the range and evolution of the *P. falciparum* using 101 SNPs (Single Nucleotide Polymorphism) barcode of 80 isolates. Samples were collected during 2016 from two different malaria zones: Kola Bamana a stable transmission area localized within the northeast of Ségou city the fourth region of Mali (Niger River). Nioro-du-Sahel within the northeast of the town of Kayes, near the Mauritanian border with an unstable transmission.

2- MATERIALS AND METHODS

I- Study site description

The study site covers Koila Bamana within the northeast of Ségou city the fourth region of Mali (Niger River) (Lat = 13.5719, Long = -5.6895) and Nioro-du-Sahel (Lat = 15.224674, Long = −9.583888) (see fig N° 01) within the Kayes region 241 km northeast of the town of Kayes, near the Mauritanian border [3]. (Figure N°1)

Figure N°1

Showing in Map of Mali the study sites (read dot Nioro du Sahel and read star Koila Bamanan) where Plasmodium falciparum samples were collected from malaria patients.

II- Sample collection

In this study, we used the rapid diagnostic test (RDT) to detect *P. falciparum* contamination. Then we took from the patients whose tests were possible with RDT by the veins four (4) ml of blood to form a dried bloodstain (DBS) by depositing 50 µl of blood on filter paper.[3] To possess information on the genomics of *P. falciparum*, we used DBS obtained previously to extract DNA. We used the 101 SNPs selected on the *P. falciparum* genome for the sample barcodes by filling them with the genotypes .[3,7]

III- DNA extraction

With the Qiagen DNA Investigator kit, we used the blood stains dried on filter paper to extract the DNA by then subjecting it to whole genome amplification (WGA) by pre-amplification, by primer extension[9] or by selective WGA before genotyping.[8,3]

IV- Genotype determination

Genotyping was prepared through Mass ARRAY platform integrating iPLEX and Mass ARRAY tools (Agena Bioscience, Hamburg, Germany).We did Mass ARRAY on the DNA pulling out from 186 samples and focused on the 101 SNPs earlier described (Diakite et all). Entirely genotypes were called from background adjusted topmost intensities, which were regularized and called by batch. In short-term, batches underwent calling employing a heuristic algorithm, which recognizes intensity ranges for every SNP in single infection samples, and called mixed base loci ("heterozygous") maintained those range thresholds, correcting for background intensity.[3] Batches were plate grounded and limited between 96 and 384 samples. These are needed to get for each assay ranges of intensities to determine background intensity levels.[3]

V- Sample barcodes

We used the 101 SNPs selected from the *P. falciparum* genome for the sample barcodes by filling each position with the genotypes from 80 isolates. The sample barcodes are formed by concatenated genotypes at 101 SNPs across the *P. falciparum* genome. These SNPs are all biallelic, i.e. only two alleles are detected. They were chosen for their usefulness in analyses of relationship between parasites and host, and do not seem to be associated to drug resistance. SNPs within the barcode are represented by the observed nucleotide (A, T, C and G). If the genotype is missing (could not be detected), the symbol "X" is used; the symbol "N" indicated that both alleles were observed (heterozygous call). (Diakite et all).

VI- SNP assay design

For our assay development, we examined the previous selected 101 SNPs for MAF in four populations: Mali, Gambia, Senegal and Thailand on "PlasmoDB" to make sure those only common variants of *P.falciparum* genome were used for further analysis. SNPs having a mean MAF of a minimum of 35% in tree of the four populations were maintain for final assay. We moved some SNPs tightly linked with another. A number of them were retained to maximise coverage across chromosomes.[11]

3- Statistics analysis

I- Data cleaning

To ensure that only top quality data were considered for further analysis, proper clean up measures were taken. All samples having barcodes with over four (4) missing SNPs (X) were expelled from the ultimate assay.

II- Evolution of the single nucleotide polymorphic loci

Mutations represent not only the essential element in populations' evolution but also its guideline.[12] Investigating mutation directions in Koila Bambara and Nioro du sahel we used Maximum Likelihood to estimate the substitution matrix on MEGA-X.[13]

III- Minor allele frequency

We estimated minor allelic frequency manually by counting the quantity of alleles for every SNP in each population. We estimate the polymorphic genotypes contribution by considering each of them with half compared to the monomorphism genotypes.[15,16,17] We also designated the typical MAF (AMAF) as being the unweighted mean of the MAF values for the two populations for every SNP.[15]

IV- Population diversity

Genetic diversity is a vital component of population diversity. a standard known measure of population diversity is barcode π. We calculated barcode π from both *P. falciparum* populations on MEGA-X software. Barcode π was estimate as mean of the pair-wise differences at assayed SNP between all members of a population divided by the entire number of assayed SNPs.[12] This parameter provides a decent estimate of the genetic variability of the population.

V- Population divergence

a- Genetic differentiation

Using MEGA-X software with a bootstrap procedure (1000 replicates), we measured the divergence of populations by estimate the average between parasite populations from the quantity of base differences per site.[13]

VI- Principal component analysis

We performed PCA on our two parasite populations with PAST software from Nioro du Sahel and Koïla Bambana all 57 samples together that were successfully barcoded.

VII- Phylogenetic

In order to well understand, P. falciparum genetic evolution between our two sites, we reconstruct a phylogenetic tree by observing the barcode sequences using maximum likelihood method and HKY85 substitution. The web program PhyML (v3.1/3.0 aLRT) was used with bootstrap procedure (1000 replicates).[18,19,20]

3- RESULTS AND DISCUSSION

I- Data cleaning

We used 80 samples, (40 each from Nioro Sahel and Koila Bambara) to comprehend a full 27 SNP barcode. Excluding all samples with over four (4) missing SNPs, 57 samples, 31 and 26 samples from Nioro du Sahel and Koïla Bambara, respectively, have passed control.(see Table N°1 a. and b.)

Table N°1

A

Sample	Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
K-23039	Kola	T	X	X	T	A	A	T	T	A	A	A	C	G	A	T	C	G	C	G	A	A	G	T	A	T	A	G
K-23040	Kola	T	G	G	X	X	A	C	A	T	T	A	C	A	A	T	C	A	C	G	G	A	G	C	A	A	A	G
K-23043	Kola	T	G	G	T	G	A	T	A	T	N	G	A	G	A	T	N	G	T	N	G	T	C	T	G	X	A	A
K-23044	Kola	T	G	G	T	G	A	T	T	A	A	N	A	G	G	T	C	G	T	G	G	T	C	N	G	X	A	A
K-23045	Kola	T	G	G	C	N	A	T	T	A	A	A	C	G	N	G	C	A	T	A	A	N	X	X	A	X	A	G
K-23047	Kola	T	A	G	C	A	A	C	T	A	T	G	C	A	A	N	C	A	T	G	G	A	G	T	G	A	A	G
K-23052	Kola	T	G	A	T	A	N	N	T	N	T	N	A	G	A	T	C	A	T	G	G	T	N	T	G	A	A	G
K-23056	Kola	T	G	G	C	A	T	T	A	N	A	A	C	A	A	N	C	G	T	A	G	T	G	T	G	A	G	A
K-23059	Kola	T	G	A	T	A	T	T	T	A	T	A	C	G	A	G	T	G	T	A	A	T	C	T	A	A	A	G
K-23065	Kola	T	G	A	N	A	N	N	T	A	N	G	A	N	A	G	N	A	T	N	N	T	G	T	G	X	A	A
K-23067	Kola	T	G	G	T	G	T	T	T	T	A	A	C	A	A	N	C	G	T	G	G	T	C	C	A	T	N	G
K-23068	Kola	T	X	G	X	A	A	T	T	A	T	G	C	G	A	T	T	G	T	A	X	T	G	C	A	X	A	G
K-23069	Kola	T	G	A	T	A	N	C	A	N	A	N	A	N	A	T	N	N	N	N	N	N	N	C	N	A	N	A
K-23071	Kola	T	G	G	N	A	N	T	A	N	A	A	A	N	A	N	C	A	C	A	N	N	C	T	A	T	G	A
K-23073	Kola	T	G	N	T	A	A	N	T	T	A	N	A	G	A	G	N	N	C	G	N	A	C	C	G	T	N	G
K-23075	Kola	T	X	G	X	A	A	T	T	T	T	G	A	A	A	G	T	A	C	A	A	T	X	T	A	A	X	A
K-23076	Kola	T	N	A	T	A	T	C	T	T	A	G	X	G	A	G	C	A	C	G	G	A	C	C	G	A	A	A
K-23077	Kola	T	X	G	T	G	T	T	T	N	N	G	C	G	A	T	C	G	X	N	A	T	N	N	G	T	A	A
K-23078	Kola	T	X	G	C	X	T	C	A	T	A	A	A	G	G	T	T	A	C	A	G	T	C	C	A	T	G	G
K-23079	Kola	T	G	N	N	G	N	N	A	N	A	G	C	N	A	N	C	G	N	N	A	N	G	C	A	A	A	N
K-23081	Kola	T	G	G	C	N	N	C	N	N	T	A	A	X	A	T	C	N	C	A	G	N	G	N	N	X	A	N
K-23082	Kola	T	G	N	T	A	N	C	A	T	A	G	A	G	A	T	C	A	T	A	N	N	G	T	G	T	A	A
K-2308	Kola	T	X	N	N	N	N	N	N	T	N	G	C	A	A	T	N	A	T	N	N	T	X	T	X	A	A	N
K-23086	Kola	T	X	A	C	G	N	C	A	N	A	G	A	A	A	T	T	G	T	G	A	T	G	T	N	A	A	A
K-23087	Kola	T	X	G	T	G	N	C	T	A	A	A	A	A	A	T	C	A	T	A	A	T	G	T	G	T	G	A
K-23091	Kola	T	X	G	C	A	A	T	N	T	A	A	A	G	G	T	C	A	C	A	G	T	G	T	X	A	A	G
K-23095	Kola	T	N	N	C	A	A	C	T	T	A	G	C	A	G	G	C	A	T	G	G	A	C	T	G	A	G	A
K-23098	Kola	T	G	A	C	G	A	T	T	A	A	G	A	G	G	T	C	A	T	A	N	N	C	T	A	A	A	A
K-23105	Kola	T	G	G	C	A	A	C	A	T	T	N	C	A	A	T	C	N	T	G	A	T	C	T	A	A	G	G
K-23106	Kola	T	X	N	C	A	A	T	A	T	A	G	A	A	G	T	C	N	T	A	A	N	G	N	G	T	G	A
K-23108	Kola	T	A	N	C	A	T	T	A	T	A	A	A	A	A	G	T	A	C	N	G	A	G	C	A	A	A	G

B

Sample	Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
N-18948	Nioro	T	A	A	T	A	A	T	A	X	T	G	A	A	G	G	C	A	T	A	G	T	C	C	A	X	A	A
N-18964	Nioro	X	A	A	T	A	A	T	T	A	T	G	A	A	A	T	C	G	C	A	G	T	C	T	G	A	A	A
N-18955	Nioro	T	A	A	C	A	T	N	T	A	T	A	A	G	G	N	N	N	C	A	G	T	C	C	A	A	A	A
N-18958	Nioro	T	G	A	T	A	T	N	T	A	N	N	A	G	N	T	C	A	N	G	A	T	C	C	A	X	N	N
N-18961	Nioro	T	X	A	T	N	T	C	N	N	N	A	A	G	A	T	N	G	C	G	G	N	C	C	A	X	N	N
N-18960	Nioro	T	A	G	N	N	N	N	N	A	N	N	N	N	N	N	N	A	N	A	N	N	C	C	A	X	A	N
N-18968	Nioro	T	A	A	T	A	N	C	A	A	N	G	C	G	G	N	N	N	C	A	A	A	G	T	G	A	G	N
N-18972	Nioro	T	A	A	N	A	N	T	N	A	N	N	A	X	A	G	N	A	C	G	G	A	C	T	G	X	G	A
N-18953	Nioro	T	G	G	C	A	T	T	T	A	T	G	C	A	A	T	T	A	T	A	A	T	G	C	A	T	A	A
N-18969	Nioro	X	A	A	C	A	T	T	T	A	T	G	C	A	G	T	T	A	T	A	A	T	G	T	G	X	A	A
N-18977	Nioro	T	G	A	C	A	T	C	A	A	T	A	C	A	G	T	C	G	T	A	G	T	C	C	G	X	A	A
N-18954	Nioro	T	G	A	N	G	A	N	T	A	N	G	X	A	A	T	T	N	T	A	A	T	N	C	N	X	A	A
N-18974	Nioro	T	A	A	N	N	T	C	T	A	T	G	C	G	A	T	C	A	T	G	A	A	G	T	G	X	G	G
N-18951	Nioro	T	A	A	T	A	T	T	A	T	A	A	A	A	A	T	T	A	C	A	A	A	N	C	A	A	G	A
N-18956	Nioro	T	A	G	T	A	T	T	A	T	A	A	C	A	A	T	T	G	C	A	A	A	C	C	G	A	A	G
N-18949	Nioro	T	A	G	T	A	A	T	A	T	A	A	A	G	A	T	C	G	T	A	A	A	C	T	A	T	G	G
N-18950	Nioro	T	G	G	C	A	A	T	A	A	T	A	A	A	A	G	T	A	C	A	G	A	G	C	A	A	A	A
N-18975	Nioro	T	A	A	C	A	A	T	A	T	A	G	A	A	A	T	T	G	C	G	G	T	G	C	G	X	G	G
N-18976	Nioro	T	G	A	C	A	T	C	A	T	T	G	A	A	A	T	C	A	C	A	A	T	G	T	G	X	G	G
N-18966	Nioro	T	A	A	C	G	T	C	T	T	T	G	A	G	G	G	C	A	C	G	A	T	G	C	A	T	G	G
N-18971	Nioro	T	A	G	C	G	T	T	T	T	T	A	C	A	G	G	C	A	A	A	A	T	G	C	A	A	A	A
N-18959	Nioro	T	G	G	C	G	N	T	T	T	T	A	A	G	A	T	C	A	C	A	N	T	G	T	G	X	A	A
N-18957	Nioro	T	A	N	C	G	T	N	N	T	N	G	N	G	A	N	C	N	T	N	A	T	G	N	N	T	G	A
N-18962	Nioro	T	G	G	C	N	N	N	N	T	T	N	A	G	N	N	C	A	C	A	N	N	G	N	G	X	N	N
N-18978	Nioro	T	G	A	N	N	T	N	N	T	N	N	N	N	A	N	N	A	C	N	N	N	N	N	N	A	N	N
N-18970	Nioro	T	A	A	N	A	N	C	N	N	T	G	A	G	A	T	C	A	C	G	G	T	G	N	A	X	X	N

Table N°1: Showing 27-SNP Barcodes for 57 *Plasmodium falciparum* isolates found from malaria patients in Koïla Bambara (A) and Nioro du Sahel (B).

II- Evolution of the single nucleotide polymorphic loci

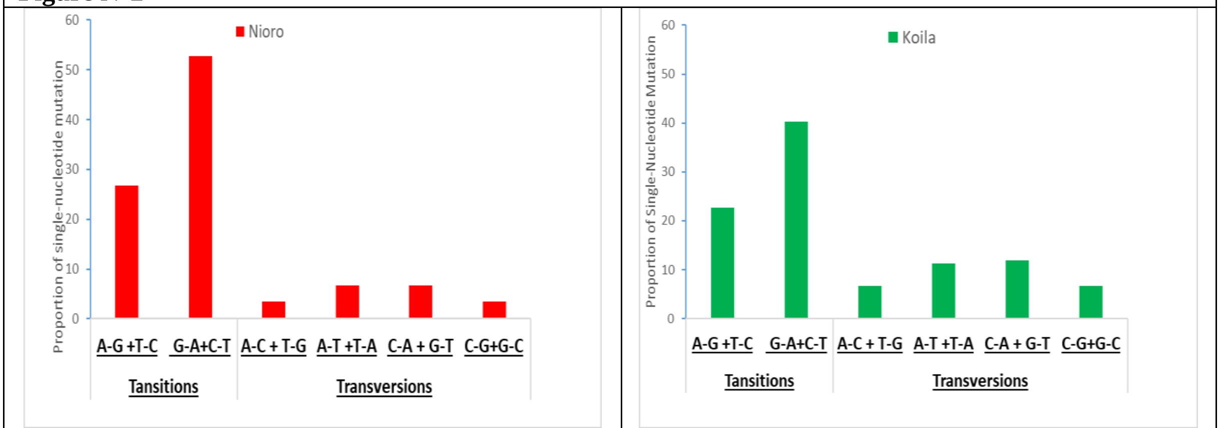
Of the 837 sites for Koila Bamanan, 702 for Nioro du Sahel the mutation directions of respectly 695 sites and 598 were deducible. The proportions of Transition /Tansversion (Ts/Tv) were elevated at the 2 sites but particularly in Nioro du Sahel (1.71 and 4.21, respectively), with an overall value of two.91. (See Figure N°3).

Results show that the proportions of transitional mutations and transversional mutations observed at the 2 population were considerably different. In transversion mutations C-T and G –A mutations were much frequent compare to others.

In transitional mutations, A-C+T-G and G- C+C-G were much fewer. (See Figure N°2).

There was a tendency that the SNP-barcode sequences of *Plasmodium falciparum* in Koila Bamanan and Nioro had a high nucleotide substitution ration of transition to transversasion with the best in Nioro (Ts/Tv = 4.21), indicating that *Plasmodium falciparum* genes were under high selection pressure in these populations.

Figure N°2



A B

Displaying the proportion of each substitution variety in SNP barcode sequences of *Plasmodium falciparum* genomes from Koila Bamanan (A) and Nioro du Sahel (B) in Mali.

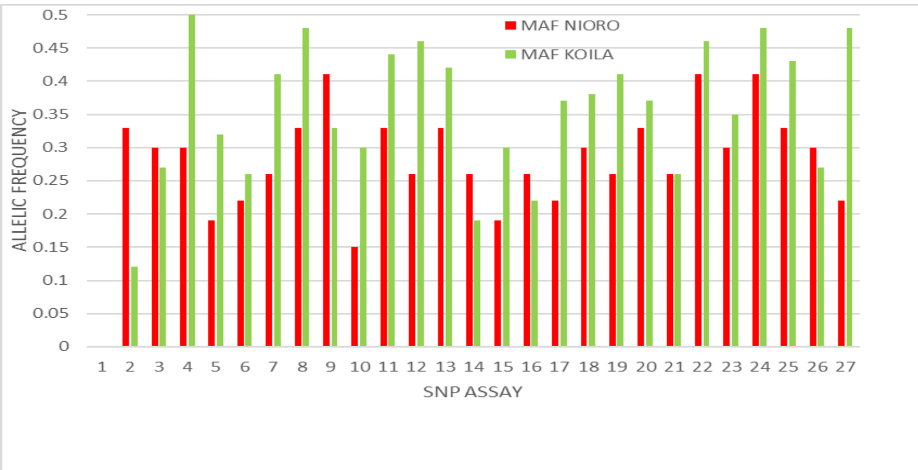
Figure N°3

Presenting the quotients of Transition/Transversion in SNP barcode sequences of *Plasmodium falciparum* genomes from Koila Bamanan (A) and Nioro du Sahel (B) in Mali.

III- Minor allele frequency

In both populations, samples minor allelic frequency values reveal a significant diversity index. MAF in any respect SNPs in both population was superior to 0.1 (See figure N°4). once we compare MAF, value of our two population to one another there's not big difference (See figure N°4).

Figure N°4



Displaying Minor allele frequencies (MAF) of *Plasmodium falciparum* isolates obtained from Malaria patients in Koila Bamana and Nioro du Saheh

IV- Population diversity

The calculated π statistic for both site Koïla Bamara and Nioro du sahel respectively 0.43 and 0.44 denote that these populations are rather diverse.

V- Population divergence

The net average value between parasite populations from the quantity of base differences per site of 0.03 insinuate that there's a significant genetic similarity between our two *P. falciparum* populations. This result confirms the little difference observed by comparing MAF values between two populations.

VI- Principal component analysis

The outcomes of the PCA show that distinctly in each of the parasite populations the genetic multiplicity is important, the points showing more divergence (Fig. 5a, b). By putting all the samples obtained and having satisfied the standard control criteria within the two sites together, we were able to see that the parasites are grouped round the same region on the plot (Fig. 4c). These results confirms the high level of intra-population diversity and also the small degree of inter-population consistent with the MAF values.

Figure N°5

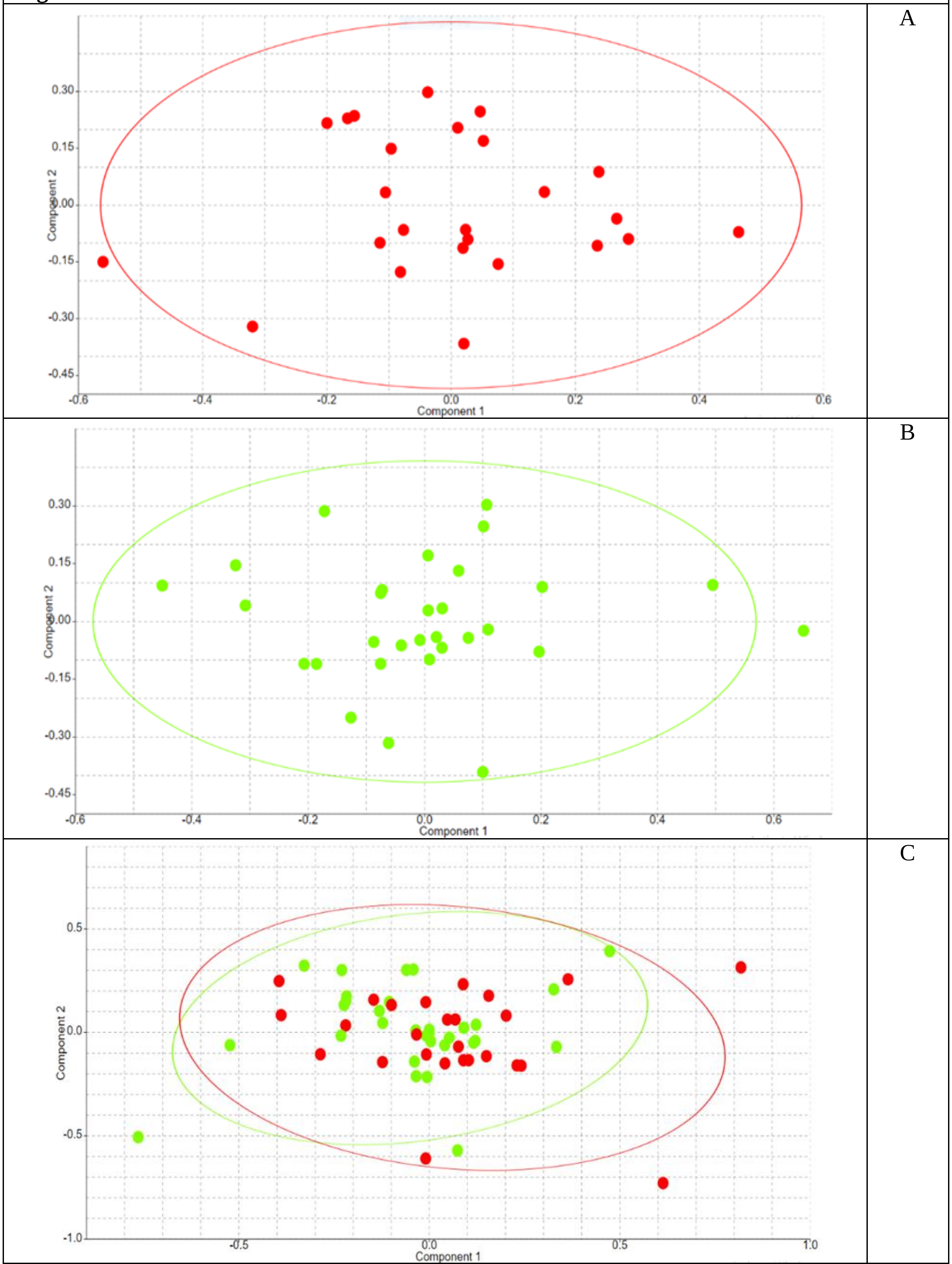


Figure N°5: Showing Principal component analysis of *P. falciparum* isolates obtained from malaria patients in Koila Bamana (read point) and Nioro du sahel (B, Green point) combined (C)

VII- Phylogenetic analysis

We generated phylogenetic tree with information from nucleotide sequences of the 27-SNP barcode formed by 57 *Plasmodium falciparum* obtained from malaria patients in Koïla Bambara and Nioro du Sahel. Looking to the phylogenetic tree, we are able to see that there are several distinct groups of *plasmodium falciparum* with a quit genetic distance from one another and grouped together in five important clades (See figure N°6).

Despite the fact of those sites are over 800 km far from each other, the results of the net average divergence shows a low level of divergence (0.02) between the two populations. This lower genetic variance observed between these two sites with a difference epidemiological condition in our study may well be because of similar selective pressure from the varied control and management measures within the country at large.

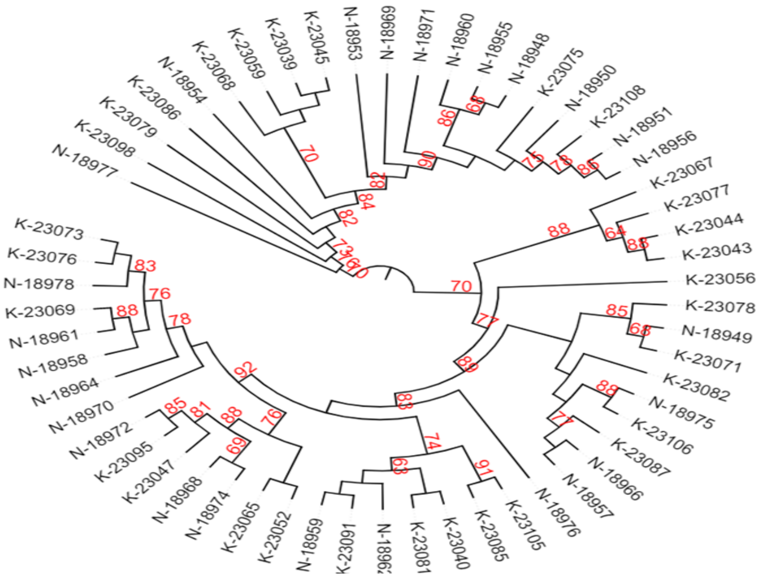


Figure N°6: Showing Maximum likelihood (ML) tree of P. falciparum isolates collected from Koila Bamana (K*) 31 isolates and Nioro du sahel (N*) with 26 isolates using the 27 SNP barcode.

4- CONCLUSION

These results reveal a significant degree of P. falciparum diversity in Koila Bamana and Nioro du Sahel. The genetic diversity of *P.falciparum* populations may be a major consider the parasite's ability to adapt to changes in its environment thus the measures observed here for Koila Bambara and Nioro du Sahel may partially explain the rising drug-resistance and growing severe cases in those regions in Mali. Our results is in step with the results of a previously large study on genetic diversity of *Plasmodium falciparum* in Mali. SNP-barcode technology provides a strong, low-cost method to research the range within the parasites genome, which may be used to explore *Plasmodium falciparum* population changing aspects.

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Conflicts of interest: The authors of this paper declearing No conflict of interest.

5- Ethical approvals

Beforehand of our study, we gained a community permission from traditional and customary commanders. The ethics commission of the faculty of medicine and Pharmacy of the University of Sciences, Technics and Technologies of Bamako (USTTB), Mali has permitted this study. On paper, informed accord was gotten from a parental or caretaker of each registered kid.

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