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Microsatellite marker development and population genetic analysis of *Canarium schweinfurthii* (Burseraceae), an emblematic food tree of tropical Africa

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Abstract

Canarium schweinfurthii (Burseraceae), commonly known as African Elemi, is a culturally and economically significant food tree in tropical Africa, valued for its fruits, medicinal uses, and high-quality timber. Despite its importance and wide distribution, no prior studies have explored the species' genetic diversity, a crucial component for its conservation and sustainable management. This study aims to develop nuclear microsatellite (SSR) markers and use them to provide the first insights into the spatial genetic structure of *C. schweinfurthii* populations across West and Central Africa. We selected 48 primer pairs from a large SSR database and tested them for amplification and polymorphism. Thirteen polymorphic loci were retained and used to genotype 98 individuals sampled from four populations in three African countries (Cameroon, Côte d'Ivoire, and Burkina Faso), including both wild and cultivated trees. The 13 markers were highly polymorphic with a number of alleles per locus varying from 6 to 29 (average of 16.54 ± 7.81). The mean F_{IS} coefficient per population was 0.165 ± 0.070. The genetic differentiation (F_{ST}) was the lowest between geographically neighboring populations (between the population from Côte d'Ivoire and Burkina Faso, and between the two populations from Cameroon) and was the highest between West African populations and Central African populations. We have not observed any reduction in genetic diversity between the wild and cultivated populations in Cameroon. These new SSR markers provide effective tools to study gene flow and population structure in *C. schweinfurthii*. The observed genetic patterns offer a foundation for future genomic studies and support the development of targeted conservation strategies for this emblematic African tree.

Highlights

- Thirteen novel SSR markers were developed for *Canarium schweinfurthii*, a key African fruit tree species.
- High genetic diversity was revealed in both wild and cultivated populations across West and Central Africa.

- A clear genetic structure separates Upper and Lower Guinean populations, suggesting historical fragmentation.
- Cultivated populations maintain significant diversity, but show signs of inbreeding due to local management.
- New SSR tools enable future studies on gene flow, domestication and conservation of *C. schweinfurthii*.

Keywords Canarium schweinfurthii, African olive tree, SSR makers, Genetic diversity, Non timber forest product

1 Introduction

Genetic diversity in plant populations is a cornerstone of their capacity to adapt to environmental change, to withstand anthropogenic pressures, and to ensure their long-term survival [1–3]. *Canarium schweinfurthii*, commonly known as the 'bush candle tree', 'African olive tree' or 'African elemi' is also referred to in French as 'élémier d'Afrique', and locally as 'Aiélé' by foresters. It is an important pantropical species of the Burseraceae family, distributed from western Senegal to eastern Kenya [4–6]. The species is dioecious and can grow to over 50 m in height, with a canopy that can reach the upper layers of rainforests and riparian forests [7, 8]. While specific data on pollen dispersal are lacking, pollination is likely insect-mediated, as reported for related Burseraceae species. Seed dispersal is primarily zoochorous, with large mammals such as primates or ungulates being the main dispersal agents, attracted by the oily pulp of the fruits [4, 8, 9]. The species is valued for a variety of uses, covering socio-economic, medicinal and cultural dimensions. The edible fruits of *C. schweinfurthii* are primarily harvested for food and also serve as bait for large mammals in hunting (poaching) [8–10]. As a fruit tree, it produces oil-rich drupes consumed locally or processed into traditional cosmetics and medicinal preparations. Additionally, its resins, valued for their aromatic and antimicrobial properties, are widely used in local pharmacopoeia [11, 12]. Its wood is also highly valued and used in furniture manufactures because of its durability and aesthetic appeal. Despite its value, the tree is rarely cultivated. However, it is often selectively protected by local communities in agroforests and orchards [4, 8–10]. This selective conservation approach, whereby the most useful individuals species are maintained in anthropized landscapes, can be considered a form of incipient domestication [13]. Although *C. schweinfurthii* remains classified as 'Least Concern' by the IUCN Red List [14], the widespread overexploitation of its fruit and timber, deforestation trends and climate change are cause for serious concern. In fact, the use of unsustainable harvesting practices, combined with the ongoing degradation of its natural habitats due to rapid deforestation, exacerbated by climate change [15, 16], is putting considerable pressure on the species' populations, potentially leading to a decline in its genetic diversity and even local extinctions, as has been reported in several forest species affected by habitat degradation and overexploitation [17–19]. Such anthropogenic pressures are known to reduce effective population sizes, limit gene flow, and increase genetic drift, ultimately threatening the adaptive potential of tree populations [17, 19]. There is thus a need to develop sustainable management practices for this species to ensure its long-term contribution to the livelihoods of local small-scale farmers. This includes the sustainable management of its genetic resources, which can only be achieved if the spatial distribution of its genetic diversity is well characterised and documented beforehand. To date, no in-depth study

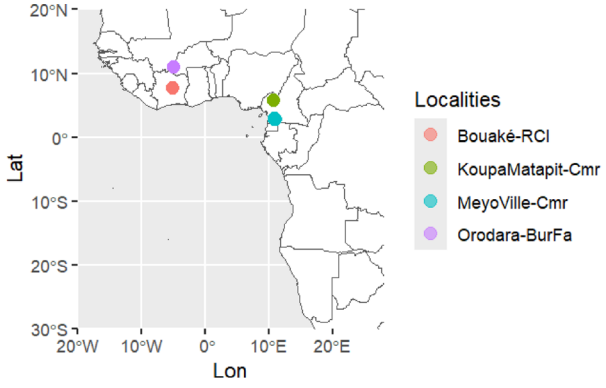
has been conducted on the distribution of *C. schweinfurthii* genetic diversity. This significant knowledge gap is a key factor in understanding how the species adapts to environmental change and remains resilient in the face of threats. Furthermore, unlike other related species with a reference genome, no genomic resources are available for *C. schweinfurthii*. Several genome sequences have been published for phylogenetically-related species, including *Boswellia sacra*, *Bursera bipinnata*, *Bursera cuneata* and *Bursera palmeri* [20, 21], but they appear to be too distant from a genetic point of view to envisage the development of a genomic approach on *C. schweinfurthii*, such as the study of nuclear single nucleotide polymorphisms (SNPs) [22, 23]. This gap highlights the need for alternative molecular tools to facilitate genetic studies and support *C. schweinfurthii* conservation planning. In this context, genetic markers, in particular nuclear microsatellites, also named SSRs (Simple Sequence Repeats), represent powerful tools that are appropriate for studying *C. schweinfurthii* genetic variability and population structure, due to their high level of polymorphism [24]. These markers are prized for their high polymorphism, co-dominance and wide distribution in the genome, making them particularly suitable for studies at the scale of individuals, populations and landscapes [25]. Their application not only makes it possible to patterns of genetic diversity distribution, but also to track gene flow between populations and detect genetic bottlenecks caused by anthropogenic pressures [26–28]. These markers are useful tools to acquire information on the spatial distribution of the genetic diversity of *C. schweinfurthii*. To this end, we have developed a set of new polymorphic SSRs and used them to characterise the genetic diversity and structure in *C. schweinfurthii*.

Thanks to the development of new polymorphic SSRs and by relying on a sampling covering wild and cultivated populations (see below for details) from the Upper and the Lower Guinean regions [29], we were able to test the following hypotheses: (i) a pattern of genetic differentiation between the Upper and the Lower Guinean populations as already observed in other species [30]; (ii) a reduction in genetic diversity in cultivated populations from Cameroon compared to wild populations from the same region, due to a genetic bottleneck effect related to the species' cultivation history [31].

2 Materials and methods

2.1 Plant material and DNA extraction

The plant materials collections were made by various researchers with considerable expertise in botany (Dr Armel Chakocha in Cameroon, Dr Beda Innocent Adji in Côte d'Ivoire and Ir Guibien Zerbo in Burkina Faso). In Cameroon, Dr Armel Chakocha collected mainly leaves and a small amount of cambium; in Côte d'Ivoire, Dr Beda Innocent Adji collected mainly cambium and a small amount of leaves; and in Burkina Faso, Ir Guibien Zerbo collected cambium and leaves from each individual at the same time. The type of plant material collected (leaves or cambium) depends on the accessibility of the tree crown. As a result, leaves are collected from shorter trees, while cambium is collected instead of leaves from very tall trees where easy access to the crown is impossible. Dried leaves and/or cambium dried using silica gel from 98 *C. schweinfurthii* individuals from four populations (localities) spread across three countries were sampled and collected: Meyo-Ville (N = 31; wild individuals) and Koupa-Matapit (N = 25; cultivated individuals) in Cameroon, Orodara in Burkina Faso (N = 23; wild), and Bouaké in Côte d'Ivoire (N = 19; wild; see Fig. 1). DNA was extracted using an adapted version of the



RCI= Republic of Côte d'Ivoire ; Cmr=Cameroon ; BurFa=Burkina Faso ; Lat= Latitude ; Lon=Longitude

Fig. 1 Geographic origin of sampled populations

protocol by Mariac et al. [32]. To ensure DNA purity and overcome difficulties associated with high levels of phenolic, polysaccharidic and glycol-lipidic compounds, the homogenate from each sample was pre-treated with a sorbitol-based extraction buffer solution before following the standard extraction protocol [32] (see the detailed composition of the buffer in Supplementary Material S1). Three washing series, each involving 1.5mL of buffer solution per sample and per wash were performed.

2.2 Microsatellite development

A genomic library was prepared for two out of the 98 individuals (ARM0325 and ARM0326, Supplementary Information 1) from Cameroon following the protocol by Mariac et al. [33], and sequenced in paired-end using Illumina MiSeq v2 reagents with 2 × 250 bp reads at Novogene (Munich, Germany). We obtained 1,463,279 and 1,458,372 raw reads for individuals ARM325 and ARM326, respectively. Reads were joined using FLASH v1.2.11 [34], resulting in 1,035,067 and 1,010,583 joined reads for individuals ARM325 and ARM326, respectively.

The software QDD v3.1.2 [35] was used with default settings to identify microsatellite repeat units (nuclear SSR motifs) and design primers. We identified 8,432 and 9,647 reads with an SSR motifs for ARM325 and ARM326 respectively. These raw sequencing data and reads with SSRs have been deposited in GenBank SRA under accession PRJNA1125310 (available to https://www.ncbi.nlm.nih.gov/sra/PRJNA1125310). Forty eight loci per individual were selected based on the following criteria: [1] perfect di- or trinucleotide microsatellite repeat units (i.e., non-composite); [2] motif repetitions with a minimum threshold of 10; [3] primers located at least 20 bp away from the microsatellite region; [4] PCR product size between 90 and 250 bp. For each primer pair, a tail with one out of four fluorochromes was added to the 5' end of the Reverse or Forward primer (Q1-6-FAM, Q2-NED, Q3-VIC, Q4-PET [35, 36] ; see Table 1).

The presence of self-dimers and cross primer dimers for each of the 48 primer pairs selected for each individual was checked on the ThermoFisher Multiple Primer Analyzer web page (https://www.thermofisher.com/fr/fr/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/multiple-primer-analyzer.html). For each of the 48 primer pairs selected, an amplification test was first carried out on four individuals in a simplex reaction (see below for details of the PCR conditions). Following this step, the polymorphism

Table 1 Characterization of 13 microsatellites loci developed for *Canarium schweinfurthii*

Mix	Microsatellite marker	Primer sequences (5′–3′) [a]	Fluorescent label [b]	Repeat motif [c]	PCR product size	Allele size range (bp)	Genebank accession No
Multiplex 1	CS02	F : *TGTAAAACGACGGCCAGT*TTCTCATCTCCACAAATTCCACA	Q1-6-FAM	$(AT)_{10}$	123	130–185	PP933780
		R : GGCCATCCAAGACCAGAACA					
	CS11	F : *TAGGAGTGCAGCAAGCAT*CAGGAGGCCACATCGTCTAT	Q2-NED	$(AG)_{12}$	140	125–170	PP933781
		R : ATTTGTCCTGCTCCTGTGCT					
	CS36	F : *CTAGTTATTGCTCAGCGGT*TGCTGGTTACGCAGTTGCTA	Q4-PET	$(AG)_{17}$	222	220–270	PP933782
		R : TTCCAGAGGATTTCTTGATGTCT					
	CS37	F : *CACTGCTTAGAGCGATGC*AGAGTTCCTTACATAGTGTCACCA	Q3-VIC	$(AT)_{11}$	222	220–270	PP933783
		R : CTGCTCAGCTTACAATGACCA					
	CS50	F : TAACCATCTGCCGGTGTTCC	Q1-6-FAM	$(AC)_{10}$	231	236–272	PP933784
		R : *TGTAAAACGACGGCCAGT*TTCTGGCAAGGCAAGCAGAT					
Multiplex 2	CS05	F : *TGTAAAACGACGGCCAGT*AACCAATACCGTCATCACCC	Q1-6-FAM	$(AG)_{10}$	129	120–160	PP933785
		R : CGAGAGGTGGTGTTGACATT					
	CS19	F : *CTAGTTATTGCTCAGCGGT*AGGTCAGCAGTCCTTTGACA	Q4-PET	$(AG)_{23}$	151	130–185	PP933786
		R : GGGTGGCATCTGGAACTCAT					
	CS20	F : *CACTGCTTAGAGCGATGC*TGAGAAACTGAGCAGTCGAGA	Q3-VIC	$(AG)_{12}$	152	155–200	PP933787
		R : TGGCATTTGAAACAGAAATCCTTCA					
	CS34	F : *TAGGAGTGCAGCAAGCAT*CGTTGACACTCTCACTCGCT	Q2-NED	$(AG)_{12}$	204	205–240	PP933788
		R : GCAAGATTTAGCTGACCCAACA					
	CS40	F : *CTAGTTATTGCTCAGCGGT*AGAGTGGCTGATAAAGATCTATGCA	Q4-PET	$(AT)_{21}$	131	210–295	PP933789
		R : GGGCTGAATGTTGTCAAGGC					
	CS42	F : *CACTGCTTAGAGCGATGC*AGGGTATTGAAGATAAGTAGATTAGCA	Q3-VIC	$(AT)_{15}$	240	225–285	PP933790
		R : TGTTACTAGCATTCTCTTGCAGTG					
	CS49	F : AGAATCCCAGATGTAACACCTGT	Q1-6-FAM	$(AG)_{11}$	241	245–290	PP933791
		R : *TGTAAAACGACGGCCAGT*TGTTAGTCCGTGCGTGTCAC					
	CS52	F : CATCCAGACAGTATGAATGGCA	Q2-NED	$(AG)_{17}$	143	135–187	PP933792
		R : *TAGGAGTGCAGCAAGCAT*CTGACCTAATTGTTCAAAGCAGCT					

[a] The universal linkers attached to the forward primers are underlined

[b] Q1 = TGTAAAACGACGGCCAGT; Q2 = TAGGAGTGCAGCAAGCAT; Q3 = CACTGCTTAGAGCGATGC; Q4 = CTAGTT ATTGCT CAG CGG T [36, 37]

[c] Number of repeats found in the clone that corresponds to the accession number

[d] Optimal annealing temperature was 57° C for all loci

of 18 SSR markers that amplified well was assessed on 16 individuals using multiplex reactions (see below for details of PCR conditions). Finally, 13 markers were found to be polymorphic and were grouped into two multiplexes (Table 1) using Multiplex Manager 1.0 software [38]. The nucleotide sequences developed for these 13 SSRs were deposited in GenBank and the accession numbers are shown in Table 1.

2.3 PCR conditions and microsatellite amplification tests

Simplex PCRs, reactions were performed in a final volume of 10 µL containing DNA template, Type-it Microsatellite PCR Master Mix (Qiagen), forward and reverse primers, and fluorescently labelled tails (details in Supplementary Material S2). The 13 selected markers were amplified in two multiplex reactions of five and eight SSRs respectively (Table 1). Multiplex PCRs were carried out in a final volume of 15 µL (as detailed in Supplementary Material S2). Thermocycling conditions were identical for simplex and multiplex reactions, following a two-step annealing protocol (57 °C then 53 °C). Full details of reagent volumes and primer concentrations are provided in Supplementary Material S2. Genotyping of all samples was performed using an ABI 3500 XL sequencer (Applied Biosystems, Foster City, California, USA) at the CIRAD genotyping platform in Montpellier. Electropherograms were visualized and scored with Geneious Prime® version 2023.2.1 [39].

2.4 Microsatellite diversity analysis

Genotypes were analyzed for the 98 individuals locus by locus using the automated procedure implemented in Geneious Prime® 2023.2 [39]. and manually corrected. Genetic diversity was assessed in each population by estimating the number of alleles per locus (*A*), rarefied allelic richness for a subsample size of k (k = 38) gene copies (*AR*), observed heterozygosity (H_O), expected heterozygosity (H_E) and the fixation index (F_{IS}) using the program SPAGeDi 1.5 [40]. We tested whether the frequency of F_{IS} was significantly different from zero after permuting gene copies among individuals 999 times. We used INEST 2.3 [41, 42] to jointly estimate the frequencies of null alleles (*r*) and $F_{(null)}$, an estimator of the inbreeding coefficient (F_{ISc}) that removes the bias caused by null alleles, under a population inbreeding model (PIM) accounting for inbreeding and genotyping failures. Differences in the level of diversity between populations were tested using the Student-Newman-Keuls (*SNK*) post-hoc test implemented in the 'agricolae' package in R Studio. Principal component analysis for visualizing individuals according to their genetic assignments on principal axes 1 (PC1) and 2 (PC2) was performed using the 'adegenet, LEA, dplyr, ggplot2 in snmf' package implemented in R studio.

In order to further characterize patterns of genetic differentiation within our dataset, we used a Bayesian clustering algorithm using Markov Chain Monte Carlo (*MCMC*) as implemented in STRUCTURE 2.3.4 software [43, 44], under the admixture model with correlated allele frequencies and without any prior information on the spatial origin of individuals. Each run consisted of 1,000,000 *MCMC* iterations following a burn-in of 100,000 iterations. We tested K = 2 and K = 3 to evaluate the main patterns of population structure across the 98 individuals. K = 2 revealed a clear and biologically interpretable division between West and Central African populations, while K = 3 allowed further resolution between the two Cameroonian populations.

3 Results

From 2,045,650 reads produced by NGS sequencing 18,079 reads containing SSR motifs were identified, 48 of which were selected for PCR testing. Loci exhibiting non-specific or no amplification were excluded. The 13 final selected loci were those that amplified consistently in all the populations evaluated and were polymorphic. The level of polymorphism of these markers (number of alleles per locus) assessed in 98 individuals

ranged from 6 to 29, with an average of 16.54 ± 7.81 per locus (Table 2 ; Appendices: Table. S1). Missing data per locus ranged from 0 to 3% (3% for CS37, CS40, CS42 and CS49) and were randomly distributed across individuals. The majority of loci with a relatively lower amplification success were from multiplex 2, which contained eight loci (see Table 1), compared with multiplex 1 (5 loci, see Table 1).

The number of alleles per locus varied from 5 to 22 with an average of 11.34 ± 4.66 in Meyo-Ville (Table 2), from 3 to 17 with an average of 9.85 ± 4.88 in Koupa-Matapit, from 4 to 15 with an average of 8.38 ± 3.38 in Orodara, and from 3 to 15 with an average of 8.46 ± 3.33 in Bouaké. The analysis of variance test of allelic richness (*AR*) by population indicated that statistically, there were no significant differences between populations evaluated at the 5% threshold (Fig. 1, $P > 0.05$). The multilocus expected heterozygosity (H_E) was the lowest in Orodara (H_E = 0.750) and the highest in Meyo-Ville (H_E=0.805). The observed heterozygosity (H_O), was the lowest in Koupa-Matapit (H_O = 0.559) and the highest in Meyo-Ville (H_O =0.684) (Fig. 2).

Significant deviations from Hardy-Weinberg equilibrium (see Table 2) were identified in Meyo-Ville population for five loci (CS11, CS05, CS20, CS40 and CS42), in Koupa-Matapit population for eight loci (CS02, CS36, CS37, CS05, CS20, CS40, CS42 and CS49), in Orodara population for three loci (CS02, CS36 and CS42) and in Bouaké population for four loci (CS11, CS36, CS20 and CS49). The multi-locus inbreeding index (F_{IS}) for each population ranged from − 0.100 to 0.403 with a multilocus value of 0.153 for Meyo-Ville, from − 0.042 to 0.633 with a multilocus value of 0.270 for Koupa-Matapit, from − 0.006 to 0.295 with a multilocus value of 0.093 for Orodara, and from − 0.014 to 0.415 with a multilocus value of 0.143 for Bouaké (Table 2). The null allele frequency, which indicates the proportion of alleles not detected by standard methods due to mutations in the binding sites of the primer used, varied from 0 to 0.180 for Meyo-Ville, from 0 to 0.270 for Koupa-Matapit, from 0 to 0.110 for Orodara, and from 0 to 0.180 for Bouaké (Table 2).

The corrected mean value of the inbreeding coefficient F_{ISc} for each of the four populations, after taking into account the effects of null alleles was F_{ISc} = 0.036 for Meyo-Ville, (95% highest posterior density interval [0-0.091]), F_{ISc} = 0.190 for Koupa-Matapit (95% HPD [0.051–0.313]), F_{ISc} = 0.041 for Orodara (95% HPD [0-0.094]) and F_{ISc} = 0.101 for Bouaké (95% HPD [0-0.181]).

Overall, the genetic differentiation among populations (multilocus F_{ST}) was 0.080. The pairwise F_{ST} values were between 0.035 (between Bouaké and Orodara; Table 3) and 0.123 (between Koupa-Matapit and Orodara; Table 3).

At $K = 2$, the genetic differentiation observed through the STRUCTURE analysis grouped individuals from West Africa from one side (Bouaké and Orodara; Fig. 3a) and those from Central Africa from another side (Meyo-Ville and Koupa-Matapit). At $K = 3$, a further subdivision separated the two Cameroonian populations (Fig. 3b).

Figure 4 shows the visualization of individuals according to their genetic assignments to different geographical groups. The graph below (Fig. 4a) shows the genetic separation of individuals in the three countries surveyed: Burkina Faso, Cameroon and Côte d'Ivoire. The principal components PC1 and PC2 show the main variation between groups, with a marked distinction between West African and Central African populations. In Fig. 4b, the colors correspond to countries: Burkina Faso (green), Cameroon (orange) and Côte d'Ivoire (black), and the shapes show geographical groupings. The

circles represent individuals from Central Africa, the triangles represent individuals from West Africa, and the rectangles represent mixed or intermediate individuals. This categorization reflects a clear genetic distinction based on the geography of the individuals sampled.

4 Discussion

We have here developed 13 new SSR markers for *Canarium schweinfurthii* and evaluated the distribution of genetic diversity in the species across four populations from Cameroon, Côte d'Ivoire and Burkina Faso. The 13 loci were highly polymorphic as shown by the mean number of alleles per locus (16.54 ± 7.81). The typically high level of polymorphism found for SSRs in this study is comparable to that found in other tree species [27, 28, 45–47]. Analysis of variance showed that the rarefied allele richness (the only genetic index that can be compared between populations thanks to the rarefaction procedure) were not statistically different from one population to another ($P > 0.05$, Fig. 1). With regard to the Cameroonian populations, the absence of information on the origin of the cultivated population makes it difficult to interpret this lack of difference in genetic diversity. Archaeobotanical remains of the species were found in the Shum Laka rock shelter, around 100 km West of Koupa-Matapit (both sites are located in the Grassfields region of Cameroon), ca. 6000 years BP [48]. This might either indicate that the species is naturally distributed in the Grassfields region, or that it has been introduced from forest areas a number of millennia ago using seed from the forest. As there is no difference in the level of genetic diversity between the cultivated (Koupa-Matapit) and wild (Meyo Ville) populations of *C. schweinfurthii* in Cameroon, this suggests that the history of cultivation and/or the management practices by farmers have not yet had a negative effect on the species' genetic diversity.

We observed relatively high F_{IS} values in all populations. For Koupa-Matapit and Bouaké, these high values were not only explained by the presence of null alleles as attested by F_{ISc} values. This suggests either the presence of a substructure within these two populations (Wahlund effect) or a pattern of inbreeding (selfing and/or biparental inbreeding). No substructure was detected through the STRUCTURE analyses. Also, as the species is dioecious, selfing cannot happen. Biparental inbreeding remains a possible explanation. In Koupa-Matapit, the species is managed by farmers. Trees that are not productive (i.e. male trees) are frequently cut [49]. This management practice implies a strong reduction of the male population size (diminution of the number of pollen donors), with possible inbreeding consequences. Such a reduction in the number of potential fathers increases the probability that the seeds are the result of crosses between a limited number of males, thereby promoting biparental inbreeding and explaining the high value of F_{ISc} (0.190). Concerning the Bouake population, fortunately, the recent demographic history of the *Canarium* species in this population is quite well documented. Individuals were collected in a control plot of an old experimental site, which was established in Kokondékro forest in 1936 by A. Aubréville and collaborators to test the influence of fire on the evolution of species composition [50, 51]. At the time of the establishment of the experiment (1936), only one individual of *C. schweinfurthii* was inventoried in the control plot [50]. In 1994, 31 individuals were inventoried in the same plot [51]. These inventories suggest that the current population of Bouaké has passed through a severe recent bottleneck, and that trees sampled are thus the descendants of

Table 2 Genetic properties of the 13 newly developed polymorphic nuclear microsatellites markers in four populations of *Canarium schweinfurthii*

Multiplex	Locus	Cameroon (Meyo-Ville, *N* = 31)						Cameroon (Koupa-Matapit, *N* = 25)					
		A	AR	H_O	H_E	F_{IS}[a]	*r*	A	AR	H_O	H_E	F_{IS}[a]	*r*
Multiplex-1	CS02	12	10.18	0.839	0.856	0.020	0 ± 0.00	14	12.74	0.640	0.868	0.266***	0.11 ± 0.05
	CS11	6	5.45	0.484	0.654	0.264*	0.09 ± 0.06	5	4.69	0.560	0.612	0.087	0.001 ± 0.03
	CS36	9	8.63	0.700	0.834	0.163	0.05 ± 0.06	8	7.37	0.6250	0.808	0.230*	0.12 ± 0.08
	CS37	14	12.14	0.767	0.859	0.109	0.03 ± 0.04	10	9.27	0.6090	0.821	0.263**	0.13 ± 0.09
	CS50	6	5.65	0.742	0.675	-0.100	0.04 ± 0.04	3	2.99	0.280	0.286	0.023	0 ± 0.00
Multiplex-2	CS05	5	4.61	0.419	0.698	0.403**	0.16 ± 0.05	4	4.00	0.280	0.711	0.633***	0.25 ± 0.06
	CS19	13	11.27	0.871	0.865	0.007	0 ± 0.00	12	10.68	0.840	0.806	-0.042	0 ± 0.00
	CS20	13	11.80	0.548	0.905	0.398***	0.18 ± 0.05	11	10.26	0.360	0.884	0.598***	0.27 ± 0.05
	CS34	10	7.99	0.645	0.701	0.081	0 ± 0.00	11	9.92	0.680	0.805	0.158	0.05 ± 0.05
	CS40	13	10.83	0.633	0.826	0.236*	0.12 ± 0.07	14	12.16	0.680	0.861	0.214*	0.09 ± 0.05
	CS42	16	13.07	0.667	0.888	0.252**	0.13 ± 0.07	17	15.51	0.480	0.934	0.491***	0.23 ± 0.05
	CS49	22	17.25	0.900	0.923	0.025	0.006 ± 0.02	16	14.04	0.680	0.878	0.229**	0.08 ± 0.06
	CS52	9	7.67	0.677	0.784	0.138	0.07 ± 0.05	3	3.00	0.560	0.638	0.124	0.04 ± 0.07
Multilocus mean		11.38	9.74	0.684	0.805	0.153***	–	9.85	8.97	0.559	0.763	0.270***	–
Multiplex	**Locus**	**Burkina Faso (Orodara, *N* = 23)**						**Côte d'Ivoire (Bouaké, *N* = 19)**					
		A	AR	H_0	H_E	F_{IS}[a]	*r*	A	AR	H_0	H_E	F_{IS}[a]	*r*
Multiplex-1	CS02	15	14.16	0.783	0.906	0.139*	0.07 ± 0.05	15	15	0.842	0.94	0.107	0.05 ± 0.05
	CS11	5	4.80	0.348	0.490	0.295	0.03 ± 0.05	6	6	0.263	0.376	0.305*	0.08 ± 0.08
	CS36	7	6.79	0.478	0.634	0.250*	0.05 ± 0.05	7	7	0.579	0.869	0.340***	0.15 ± 0.06
	CS37	8	7.62	0.826	0.830	0.004	0 ± 0.00	9	9	0.737	0.828	0.113	0.04 ± 0.06
	CS50	4	4.00	0.565	0.712	0.210	0.04 ± 0.07	3	3	0.526	0.519	-0.014	0 ± 0.00

Table 2 (continued)

Multiplex	Locus	Burkina Faso (Orodara, *N* = 23)						Côte d'Ivoire (Bouaké, *N* = 19)					
		A	AR	H_0	H_E	F_{IS}[a]	*r*	A	AR	H_0	H_E	F_{IS}[a]	*r*
Multiplex-2	CS05	5	4.85	0.455	0.591	0.235	0.07 ± 0.08	4	4	0.632	0.741	0.151	0.06 ± 0.07
	CS19	11	10.30	0.818	0.800	-0.023	0 ± 0.00	12	12	0.842	0.91	0.075	0.01 ± 0.03
	CS20	8	7.71	0.667	0.803	0.173	0.09 ± 0.06	10	10	0.526	0.89	0.415***	0.18 ± 0.06
	CS34	7	6.85	0.682	0.787	0.136	0.002 ± 0.02	7	7	0.684	0.673	-0.017	0 ± 0.00
	CS40	9	8.70	0.762	0.757	-0.006	0.02 ± 0.02	7	7	0.632	0.68	0.073	0.05 ± 0.05
	CS42	14	13.32	1.000	0.873	-0.149*	0 ± 0.00	12	12	0.789	0.898	0.123	0.05 ± 0.05
	CS49	10	9.80	0.857	0.884	0.031	0 ± 0.01	9	9	0.632	0.838	0.251*	0.12 ± 0.06
	CS52	6	5.90	0.619	0.681	0.092	0.005 ± 0.04	9	9	0.737	0.624	-0.186	0 ± 0.00
Multilocus mean		8.38	8.06	0.681	0.750	0.093***	–	8.46	8.46	0.648	0.753	0.143***	–

N individuals number sampled, *A* number of alleles, *AR* rarefied allelic richness *for k* = 38 gene copies, H_0 observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient or fixation index, *r* frequency of null alleles, [a]Significance of deviation from Hardy–Weinberg equilibrium (*HWE*): *$P < 0.05$, **$P < 0.01$,***$P < 0.001$, Total : total observed among all four populations

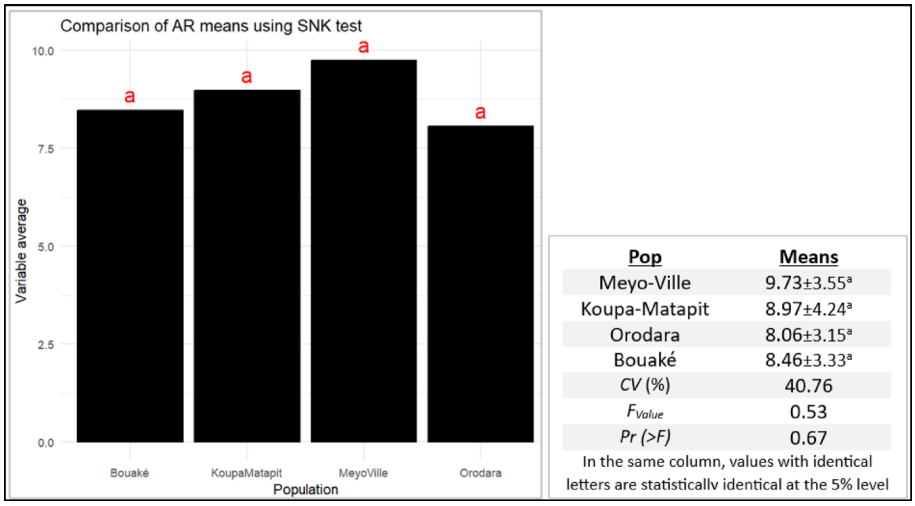
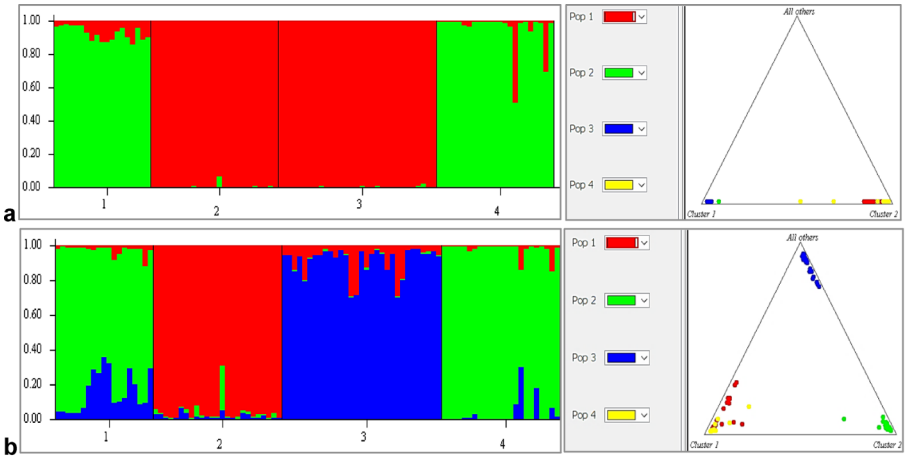


Fig. 2 Comparison of allelic richness (*AR*) between populations at the 5% threshold

Table 3 The F_{ST} distribution matrix between population pairs

F_{ST}	Meyo-Ville	Koupa-Matapit	Orodara	Bouaké
Meyo-Ville	–			
Koupa-Matapit	0.057	–		
Orodara	0.092	0.123	–	
Bouaké	0.070	0.100	0.035	–

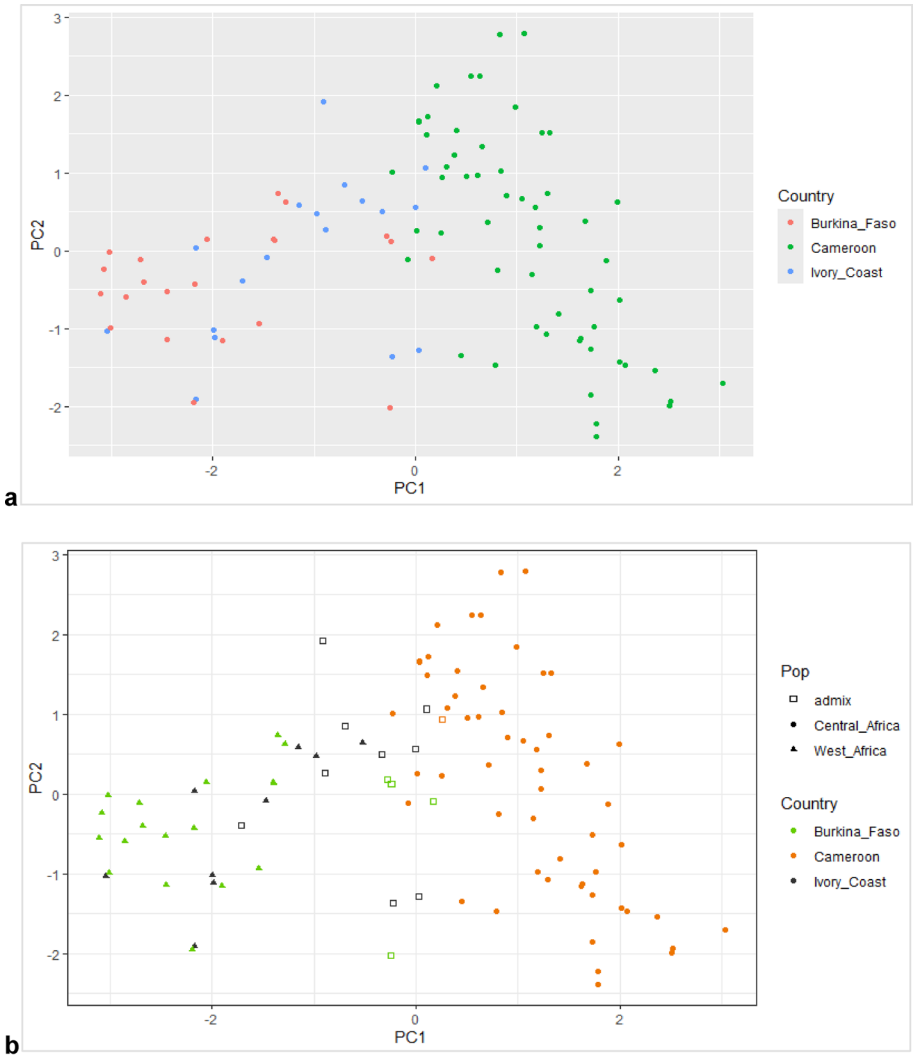


(1)= Pop 1= Population 1= Bouaké_Côte d'Ivoire ; (2)= Pop 2= population 2= Koupa-Matapit_Cameroon ; (3)= Pop 3= population 3 =Meyo-Ville_Cameroon ; (4)= Pop 4= population 4= Orodara_Burkina Faso ; **a : K=2 ; b : K=3**

Fig. 3 Mean individual assignment probabilities for the 98 *C. schweinfurthii* individuals for: $K=2$ (**a**) and $K=3$ (**b**)

a limited number of genitors. Such a strong bottleneck inevitably led to high inbreeding in the offspring and explains the F_{ISc} value of 0.101. In comparison, the low inbreeding coefficient values observed in the populations of Meyo-Ville (F_{ISc} = 0.036) and Orodara (F_{ISc} = 0.041) could be attributed to their more wild and indigenous nature compared to the other two populations and to a relatively undisturbed patterns of gene flow among individuals.

The overall genetic differentiation among all populations (multilocus F_{ST} = 0.080) is relatively low considering the geographical distribution of the populations studied here



PC=principal components; admix=admixture or interbreed or intermediary

Fig. 4 Distribution of 98 *C. schweinfurthii* individuals according to principal components (PC1 and PC2) (**a**) and visualisation with additional symbols for populations and geographical groups (**b**)

(two from West Africa, two from Central Africa). Moreover, the genetic differentiation between geographically adjacent populations was relatively low (between Orodara and Bouaké and between Meyo-Ville and Koupa-Matapit), whereas it was relatively higher when comparing populations from West Africa and populations from Central Africa. The level of genetic differentiation between West and Central Africa is comparable to that obtained for *Erythrophleum ivorense* (F_{ST} =0.080; [44]) of for *Distemonanthus benthamianus* (F_{ST} =0.074; [52]); two species from tropical rainforests. In contrast, studies on *Milicia excelsa*, a widespread dioecious wind-pollinated tree, revealed a much deeper divergence between Benin populations (within the Dahomey Gap) and Lower Guinean populations (Cameroon, Gabon, Congo), interpreted as resulting from long-term isolation in distinct Pleistocene forest refugia [53]. In that study, pairwise nuclear F_{ST} values between Benin and Lower Guinean populations reached up to 0.230, while within Lower Guinea the structure was much weaker (F_{ST} = 0.035). Such contrasting patterns highlight that the relatively low genetic differentiation observed in *C. schweinfurthii* may

reflect more recent or less pronounced historical fragmentation between West and Central African forests, or a more efficient seed-mediated gene flow across these regions, compared to species like *M. excelsa* whose seed dispersal is predominantly done by bats. In addition to natural dispersal mechanisms, the extensive historical use of the species by human populations may have played a role in promoting long-distance seed dispersal. Archaeobotanical evidence supports the ancient and widespread exploitation of *C. schweinfurthii* in Central Africa, as documented in Shum Laka [48] and in West Africa [5, 54–56]. This low differentiation could reflect a relatively recent separation between West and Central African populations, as suggested by palaeoecological reconstructions of forest habitats [57]. The STRUCTURE analysis (Fig. 3) confirms this differentiation by distinguishing two main groups: one grouping populations from West Africa (Bouaké and Orodara; Fig. 3a) and another grouping those from Central Africa (Meyo-Ville and Koupa-Matapit). Furthermore, Bayesian clustering analysis (STRUCTURE) confirmed this pattern by assigning most individuals from Upper Guinea to a distinct genetic cluster, clearly separated from the cluster predominantly comprising individuals from Lower Guinea. This supports the presence of two main genetic groups corresponding to the Upper and Lower Guinean regions, consistent with the F_{ST} estimates. The presence of these two main genetic groups, separating populations from Upper Guinea (West Africa) and Lower Guinea (Central Africa), points to a historical disruption of gene flow across the species' range. This pattern is consistent with palaeoecological scenarios of forest contraction and expansion during the Pleistocene, as observed in other tropical tree species [22, 58]. Leaving aside the cultivated population of Cameroon, and focusing only on wild populations, several hypotheses can be envisaged to explain the observed genetic structure between West and Central Africa. The first hypothesis is that of a historical differentiation between the forest (Central Africa) and savannah (West Africa) domains, which would have limited gene flow between these habitats and led to the current structuring of the populations. It would be interesting adding individuals from the West African forest domain, to further test this hypothesis.

The Bayesian clustering analysis revealed a structure within Cameroon (Central Africa), with a separation between the wild (forest) population (Meyo-Ville) and the cultivated (savannah) population (Koupa-Matapit; Fig. 3). As already discussed, it is probable that farmers' management practices have a genetic impact. A recent ethnoecological study in the Koupa-Matapit region [49] reports that farmers tend to remove unproductive trees, i.e. male individuals. This practice significantly reduces the number of pollen donors, which decreases the effective population size and increases the likelihood of inbreeding (biparental inbreeding). This could explain the high inbreeding signal (F_{ISc} = 0.190) observed in Koupa-Matapit, even in the absence of a loss of allelic diversity. The reduction of the effective population size of the population will also increase the drift effect on allele frequencies, which might lead to a signal of genetic differentiation. We should also bear in mind that we have no information on the origin of this cultivated population. The observed patterns of genetic differentiation may reveal that the cultivated material in Koupa-Matapit originates from a different forest region to Meyo-Ville, such as the rainforests in the south-west and north-west of Cameroon. A similar pattern of genetic distribution have been observed in other emblematic food tree species from the region, *Garcinia kola* [27] and *Cola nitida* (unpublished data), which raises questions about the origin of the food trees grown in the savannah zone of Cameroon, particularly

in the western region. Alternatively, the species may be naturally present in this savannah ecosystem. The presence of the species in Sudanian forest formations, for example in Bouaké and Orodorara, supports the latter point. The influence of farmers' practices and the different origins of the cultivated material may have acted synergistically. In order to refine these hypotheses, additional analyses using chloroplast markers (cpDNA) or more in-depth genomic approaches will enable us to explore gene flow and the evolutionary processes involved in greater detail. In addition, a broader sampling effort, incorporating other populations spread across the entire range of *C. schweinfurthii*, would provide a more complete picture of genetic diversity and differentiation dynamics on a continental scale. Because the sampling is limited to four populations, this may not reflect all the genetic variability of the target species in its actual distribution.

These results highlight the importance of taking into account the genetic structuring of populations in order to develop appropriate conservation strategies. Forest populations, such as those in Meyo-Ville and Orodara, could play a key role as genetic reservoirs for restoring populations. On the other hand, the Koupa-Matapit and Bouaké populations would require specific strategies aimed at limiting inbreeding and encouraging gene flow, even if this is worth noting that their current levels of genetic diversity are not significantly lower than the other two populations. Moreover, the transfer of genetic material between these regions should be considered with caution, as it could introduce alleles that are poorly adapted to certain ecological conditions.

Finally, this study is a crucial first step towards understanding the evolutionary history of *C. schweinfurthii*. The 13 microsatellite markers developed open the way for new perspectives and complementary studies on differentiation mechanisms, thereby enhancing conservation efforts and the sustainable management of *C. schweinfurthii.*

Although these results are preliminary, they have some implications for the conservation of *C. schweinfurthii*. Firstly, wild populations such as those in Meyo-Ville and Orodara, which have lower levels of inbreeding, should be considered as priority genetic reservoirs for potential restoration or reinforcement programmes. Secondly, the populations of Koupa-Matapit and Bouaké, although not showing reduced allelic diversity, suffer from a deficit in heterozygotes, suggesting risks related to inbreeding and genetic drift. For these populations, specific management strategies could include enrichment with diverse genetic material to increase gene flow and counteract the effects of small effective population size. Finally, the genetic differentiation observed between West and Central Africa highlights the importance of considering these two regions as separate management units, avoiding large-scale transfers of genetic material that could introduce alleles that are poorly adapted to local ecological conditions.

5 Conclusion

This study led to the development of 13 highly polymorphic microsatellite markers for the African olive tree, *C. schweinfurthii*, a prominent agroforestry fruit tree highly valued in Africa, which revealed a high but unevenly distributed genetic diversity. We observed a slight genetic structure between the populations of West and Central Africa, and a pattern of genetic differentiation between the cultivated (savannah) and the wild (forest) population from Cameroon possibly resulting from ecological and/or human factors. The observed deficit in heterozygotes, particularly in Koupa-Matapit and Bouaké, suggests a drift effect due to the reduction of their population size. In contrast, Meyo-Ville

and Orodara seem to be at a better genetic equilibrium, as expected for undisturbed wild populations. These results underline the importance to tailor conservation strategies to each population, limiting inbreeding and preserving genetic diversity. In-depth genomic analyses, including the use of chloroplast markers (cpDNA) and wider sampling, will be needed to refine these preliminary results on the evolutionary history of the species and effectively guide the management of the species.

Supplementary Information

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Supplementary Material 1

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Author contributions
All authors contributed to the study. B.I.A., A.F.C., G.C.Z. and M.L.A.T. collected the field samples. Material preparation and data collection were performed by B.I.A., M.H.S. and J.D. B.IA. and M.H.S. undertook the experiment. B.I.A., M.H.S., M.C. and J.D analyzed the data. The original manuscript draft was written by B.I.A. M.C and J.D corrected the manuscript. All authors read and approved the manuscript.

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Data availability

The datasets produced and/or analysed and all additional data in the current study are available from: 1. The raw sequencing data (NGS) used for the development of SSR markers from two individuals are deposited in GenBank SRA under accession number PRJNA1125310 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1125310) ; 2. The DNA sequences of the 13 microsatellites loci described in this paper have been deposited in GenBank (see Table 1) ; 3. Genotyping data for the 98 individuals and 13 SSR loci, as well as associated documentation, are available in the DataSuds repository (IRD, France) at https://doi.org/10.23708/GWRGBC. Data reuse is granted under CC-BY license.

Declarations

Ethical approval
Canarium schweinfurthii is a common fruit species in Central and West Africa. It is found either in the wild or cultivated, and is the only representative of the genus in the Guineo-Sudanese domain. Due to its uses, the species is very well known by local populations, and as it is the only representative of the *Canarium* genus in this region, it cannot be confused taxonomically with any other species. For this reasons, the collection of leaves and cambium for genetic analyses was carried out with a high degree of confidence, without the need to collect herbarium specimens. The good amplification of SSR markers in all individuals sampled further support this statement as SSR do not amplify in taxonomically-distant species. It is therefore also unnecessary to indicate the reference specimens of wild individuals collected and the identification/deposit number of the reference specimen. All samples of plant material (leaves and/or cambium) for each individual are well preserved in the laboratory of the UMR DIADE (Joint Research Unit for Plant Diversity, Adaptation and Development) of the IRD (Institute of Research for Development) in Montpellier, France. Permits for Collection and Access : Permits for fieldwork and the collection of plant samples were authorised by SODEFOR's General Management (Cel: +225 22483000, infos@sodefor.ci) under reference No. 03484-23 CT06/KA/CAK, Mr Mamadou SANGARE (Managing Director of SODEFOR), Dr Cyrille N'Gouan Kouassi (CNRA Regional Director in Bouaké, cyrille.kouassi@cnra.ci, Cel: +225 05 55 23 77 27) and Dr Brahima Coulibaly (Head of CNRA's Forest-Environment Programme, coulibaly.brahima@cnra.ci, Cel: +225 07 09 13 92 38). This research complies with local and international guidelines on plant research and biodiversity conservation, ensuring minimal impact on natural habitats. Applications for access and research permits (ABS Agreement of the Nagoya Protocol) have been submitted by the authors from the various countries to the Nagoya Focal Points of the countries concerned and are currently being processed.

Consent to participate
Not applicable.

Consent to publish
Not applicable.

Human and animal participants
No human participants and/or Animals were involved in this study.

Competing interests
The authors declare no competing interests.

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