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Characterization of Atypical Individuals of *Lannea* in Burkina Faso

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Abstract

Background and Objectives: The species to the *Lannea* genus are trees, shrubs with compound leaves. Thus, individuals called atypical *Lannea* with single leaves and of socio-economic interests have been identified in the central plateau region (Burkina Faso). This study aimed to contribute to the identification of atypical species. **Material and Methods:** The Polymerase Chain Reaction (PCR) method using specific primers was performed and consisted of extracting DNA from young leaves of *Lannea* individuals, amplifying and then sequencing portions of discriminating DNA (matK, rbcL and rps16). **Results:** It was shown that individuals belong to the *Lannea* genus, but are subdivided into three subgroups: a first subgroup containing *Lannea microcarpa* and two subgroups with no previously identified *Lannea* species. **Conclusion:** These atypical individuals are ecotypes or mutants of *Lannea microcarpa.*

Keywords

Characterization, *Lannea*, Atypical Individuals, Agrosystems, Polymerase Chain Reaction

1. Introduction

Genetic diversity of trees would be the factor optimizing the plant survival by enabling them to adapt to changing environmental conditions [1]. Under the influence of the forces of selection, animal or plant species evolve to adapt to their environment. Under, the influence of selective forces species whether animal or vegetable evolve to adapt to their environment [2] [3]. For plant species, this development occurs in changing from morphology to physiology. This seems to be

related to the environmental action on the genotype causing strong genetic mutations which create the emergence of new individuals [3] [4]. Indeed, in Burkina Faso, (Central Plateau region), individuals of trees newly encountered that seem to belong to the Anacardiaceae family with fruits that bear a strong resemblance to those of *Lannea microcarpa* and whose tree morphology is particularly different from that of known *Lannea* species. Indeed, the Anacardiaceae are a plant family that includes trees and shrubs, around 50 genera and more than 800 species have been recorded. According to NCBI 2022, about 16 species of the genus *Lannea* have been encountered worldwide (*Lannea antiscorbutica*, *Lannea coromandelica*, *Lannea discolor*, *Lannea edulis*, *Lannea nigritna*, *Lannea rivae*, *Lannea schimperi*, *Lannea schweinfurthii*, *Lannea velutina*, *Lannea welwitschia*, *Lannea microcarpa*, *Lannea egregia*, *Lannea fructicosa*, *Lannea acida*, *Lannea velutina*, *Lannea edulis*, *Lannea barteri*, *Lannea kerstingii*), including 8 in Burkina Faso (*Lannea microcarpa*, *Lannea acida*, *Lannea egregia*, *Lannea fructicosa*, *Lannea velutina*, *Lannea edulis*, *Lannea barteri*, *Lannea kerstingii*) [5].

These individuals are particularly popular with the indigenous population (who consume their fruits). According to surveys of residents, these trees are between 80 and 90 years old. However, these individuals exist in small numbers (4 trees) and do not seem to have been encountered elsewhere [5]. Studies have shown that these individuals come from the same ecological environment as *Lannea microcarpa* [6]. In the localities where they are present, people describe them as different trees from other *Lannea* species and find that the fruits have the same nutritional quality as those of *Lannea microcarpa* [6]. These same authors have shown that these atypical individuals still have a local name "Kankanm-wombsiba" (in Mooré) because of their physical characteristics and their mystical importance, with phenological stages that seem to coincide with those of *Lannea microcarpa.*

However, these individuals would present difficulties of natural regeneration (absence of seedlings naturally resulting from these trees) which in addition to their age add to the human pressure, are threatened of disappearance. Thus, we are interested in these individuals with the objective of characterizing them (morphological and molecular characterization) in order to identify and reproduce them in order to constitute a genetic conservatory of these individuals. The morphological characterization having already been done; the objective of this study is their molecular characterization.

2. Material and Methods

2.1. Material

Study activities were carried out in two sites located at Ziniare (12˚35'01"N, 1˚17'48"O), which covers an area of 8605 km^2, or 3.1% of the national territory with an estimated population of 33,301 inhabitants; and Zorgho (12˚14'49"N, 0˚36'55"O) with an area of 15,300 km^2 and a population of approximately 76,423 inhabitants. Zorgho and Ziniaré are municipalities located in the Central Plateau region, where settlements of *Lannea* are found. The choice of these sites is justified by the fact that atypical individuals of *Lannea* were only encountered in

these localities [7]. Thus, in the villages of Naab-Mayooghin and Mamousyorgo in which the leaves of studied morphotypes were collected. Both villages belong to the Central Plateau region within the North Sudanuan phytogeographical zone of Burkina Faso. The increasing population (2.4% annually) is mainly rural and nearly 80% of the population are farmers. Agriculture is the dominant land use surrounding the study sites. The climate is that of a typical Sudano-Sahelian Zone, with two distinct seasons: a dry season from October to May and a rainy season from June to September. The annual long-term rainfall for the region ranged from 701 to 900 mm over a period of 120 to 140 days [7]. Rainfall is variable, both temporally and spatially. The mean annual potential for evapotranspiration exceeds 2000 mm per year.

In this study, the specimen consisted of seven trees of which four were defined newly encountered individuals and named as follows: 1) NLA (atypical *Lannea* Naab-Mayooghin); 2) MLa (Atypical *Lannea* of Mamousyorgo 1); 3) MaLa (Atypical *Lannea* of Mamousyorgo 2); 4) ZLa (Atypical *Lannea* of Ziniare). The three remaining trees were *Lannea microcarpa* individuals and named: Lm1, Lm2 and R6 which is branched to give MaLa. Lm1 is *L. microcarpa* used as a control plant at Zorgho and Lm2 is that of Ziniare. Number of tree samples was limited to 7 because the atypical individuals available were 3 in number and for each individual a control (*L. microcarpa*) was used. Number of tree samples was limited to 7 because the atypical individuals available were 3 in number and for each individual a control (*L. microcarpa*) was used. The 7th tree was also a *L. microcarpa* tree from which the 2nd atypical individual was born following a ramification at the level of the trunk.

2.2. Methods

Sampling consisted of randomly collecting 50 leaves per individual. Specimens were labelled, and dried in the shade. They were sent to specimens of young leaves collected from all atypical individuals and from *Lannea microcarpa* trees (control). All leaf samples were sent to Genoscreen for sequence reaction (extraction/amplification/sequencing).

2.2.1. DNA Extraction

The collected young leaves from each individual were dried in the laboratory (room temperature: 37˚C) and crushed into fine powder. One hundred milligrams (100 mg) of the powder were used for DNA extraction. The extraction technique followed the NucleoSpin Plant II kit protocol [8] A buffer named PL1 buffer (Power leveling or power level) was used for cell lysis for 1 hour at 65˚C.

2.2.2. Control of DNA Quality

The quality of the extracted DNA was checked following two steps: the optical density (OD) of the DNA was measured by using a spectrometer at 260 nm and also after migration, by using agarose gel electrophoresis. For that, 10 µL of DNA solution was mixed with 2 µL of buffer 2 µL in the gel wells, and then sub-

jected to an electric charge in TBE buffer (Tris Borate EDTA) for 35 minutes at 60 volts. After coloration of gel in a bath of ethidium bromide (0.1%), is visualized under UV rays.

2.2.3. Amplification of matK, rbcL and rps16 Regions by PCR (Polymerase Chain Reaction)

The following regions: matK, rbcL and rps16 were chosen for amplification and sequencing because of their level of variation. The DNA Amplification allows obtaining from a small amount of DNA, large quantities of a specific DNA fragment and length defined following a chain of reactions [9]. This chain comprises DNA denaturation, hybridization and polymerization.

The amplification concerned the matK, rbcL and rps16 genes and the primers used are presented in **Table 1**.

In this study, the PCR was performed using template extracted from young leaves of plants as described above. Thus, the PCR amplification volume was 25 µL. This was composed of 5 µL of DNA, 0.1 µL of Taq (Go Taq), 5 µL of buffer, 1 µL of $MgCl_2$ mM, 0.5 µL of dNTP, 1 µL of each primer and 12.4 µL of ultrapure water. The amplification process was performed as follows: Initial denaturation of the DNA at 94˚C for 5 min, 35 amplification cycles including denaturation at 94˚C for 30 sec, primer annealing at 55˚C for 30 sec and extension at 72˚C for 1 min, a final extension at 72˚C for 7 mins.

2.2.4. Sequencing of Amplified Products

The sequencing of the amplified products was performed by Genoscreen laboratory in Lille (France). It consists of determining the order of sequence of nucleotides for the DNA fragments.

2.3. Analysis of Sequencing Data

All obtained sequences were corrected using Chromas Lite software 2.6. The search for similarity with other sequences was carried out by comparing the sequences of our samples with those listed in NCBI (National Center for Biotechnology, http://www.ncbi.nlm.nih.gov) GenBank using Blast software 2.2.31. To estimate the relationship between individuals, a phylogenetic tree was constructed with Seaview version 4.5.4 [13] from DNA sequences (matK, rbcL and rps16)

Table 1. Genes and primer pairs used for DNA amplification.

Genes	Amorces	Amorces sequence	Direction	Size of amplified DNA	References
Rps16	rps-F	5'-GGAATGAATGGGCTCTTGGC-3'	Sens	847 pb	[10]
	rps-R2	5'-TCGATAAACGGCTCATTGGG-3'	Non-sens		
matK	matKpkF4	5'-ACGGTTCTTTCTACACGAGTATT-3'	Sens	700 pb	[11]
	matKpkR1	5'-TCTGCATATACGCACAAATCGG-3'	Non-sens		
rbcL	rbcLa-F	5'-ATCTTGGCAGCATTCCGAGT-3'	Sens	600 pb	[12]
	rbcLa-R	5'-AATTTCACCTGTTTCAGCCTGC-3'	Non-sens		

using the PhyML (Maximum-Likelihood Phylogenies) method. For the outgroups, the sequences of *Sclerocarya birrea* were used for the MatK region, *Anacardium occidental* for rbcL and *Entrophospora colombiana* for rps16 [14].

3. Results

3.1. Sequencing of the Region matK

MatK gene sequences were obtained for all individuals. Figure 1 shows the phylogenetic tree made from 7 MatK gene sequences and its related reference sequences from GenBank. The phylogenetic analysis reveals that all the individuals

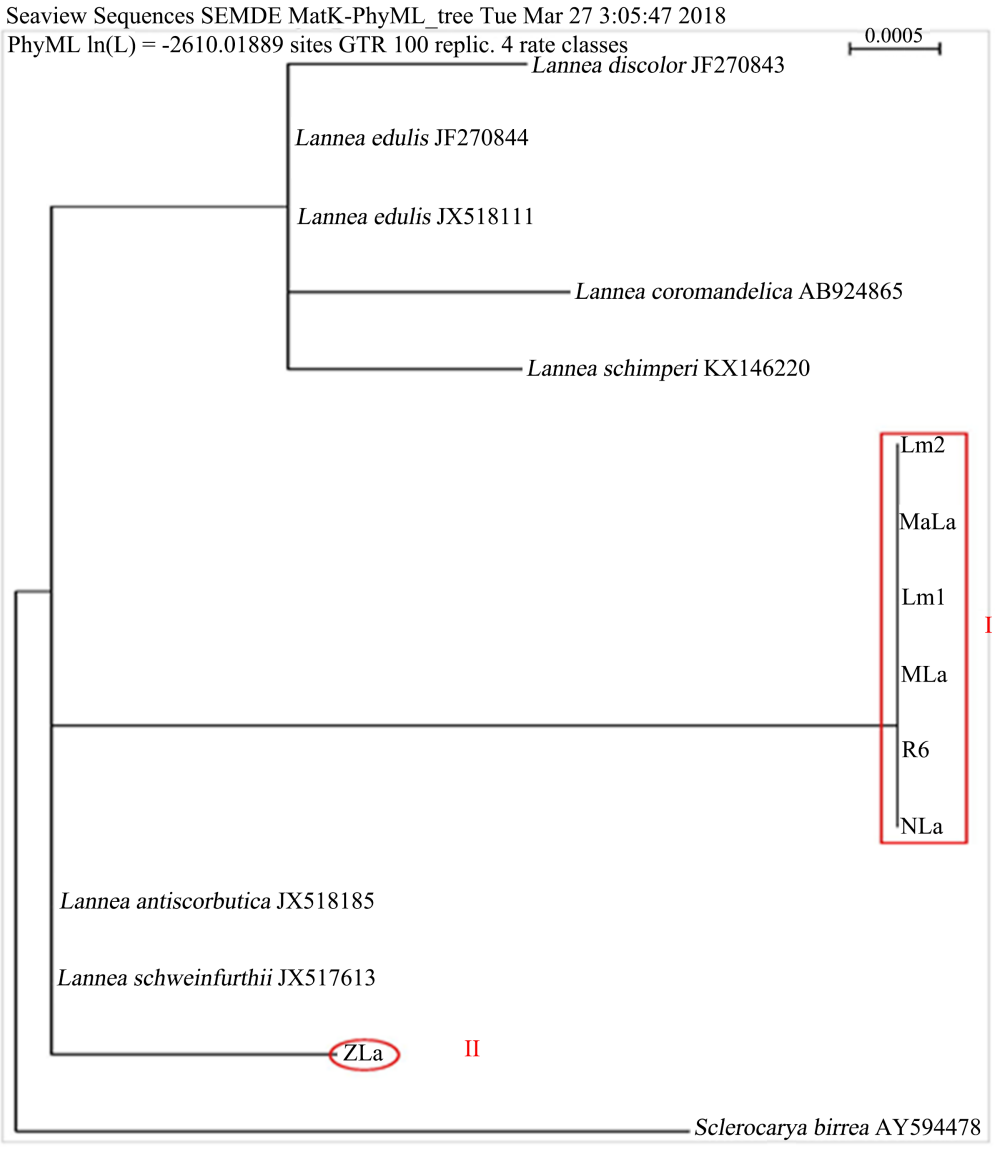

Figure 1. Phylogenetic tree PhyML established using matK sequences of the seven atypical *Lannea* plant species and their related reference species in GenBank. ZLa: Atypical *Lannea* from Ziniare; NLa: Atypical *Lannea* from Naab-Mayooghin; MLa: Atypical *Lannea* from Mamousyorgo 1; MaLa: Atypical *Lannea* from Mamousyorgo 2; Lm1 = *L. microcarpa* from Zorgho; Lm2: *L. microcarpa* from Ziniare; R6: *L. microcarpa* branched.

studied are grouped in a single genus (*Lannea*) divided into two subgroups. The first subgroup includes *L. microcarpa* (Lm1, Lm2 and R6) and the atypical individuals (NLa, MLa and MaLa). The second subgroup includes a single individual Zla. However, all other individuals in both subgroups were not related to any species previously identified in GenBank.

3.2. Sequencing of rbcL Region

A phylogenetic tree was constructed from seven (07) rbcL sequences and the corresponding GenBank reference sequences, as shown in Figure 2. This phylogenetic tree shows that all the individuals studied are grouped into a single genus (*Lannea*). As with the MatK gene, no individuals were close to previously identified species in GenBank.

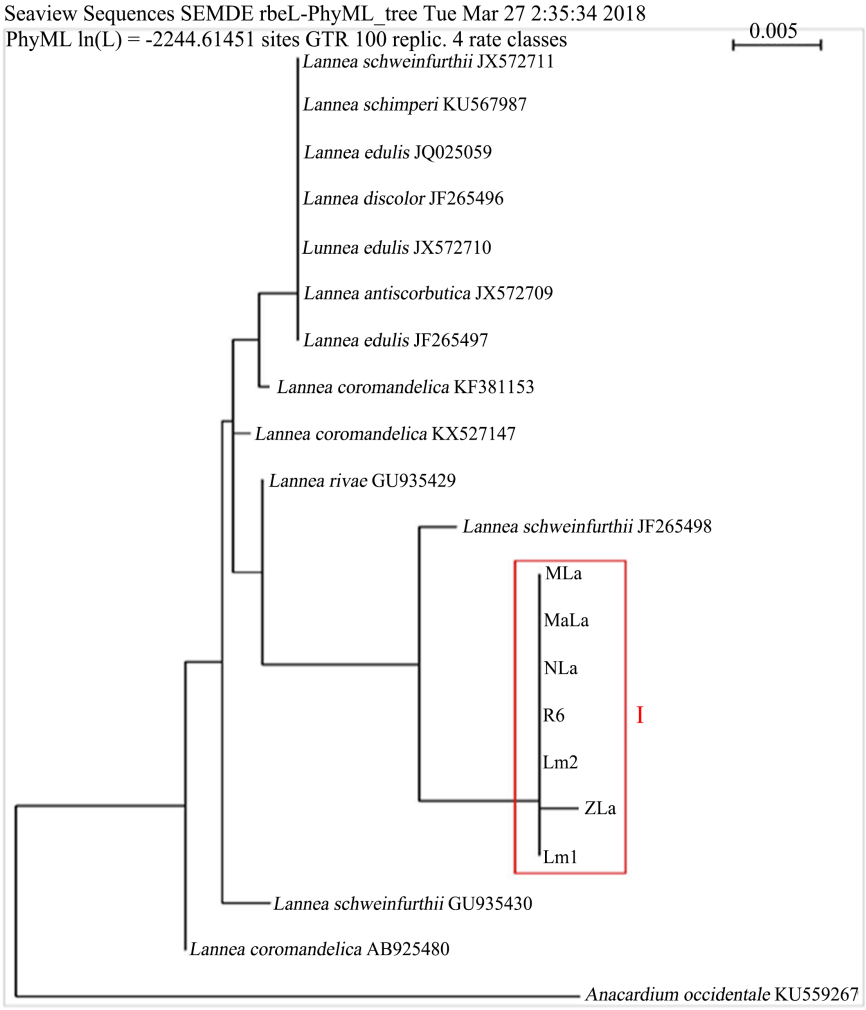

Figure 2. Phylogenetic tree PhyML established using rbcL sequences of six individuals from atypical *Lannea* plants species and their related reference sequences in GenBank. ZLa: Atypical *Lannea* from Ziniare; NLa: Atypical *Lannea* from Naab-Mayooghin; MLa: Atypical *Lannea* from Mamousyorgo 1; MaLa: Atypical *Lannea* from Mamousyorgo 2; Lm1 = *L. microcarpa* from Zorgho; Lm2: *L. microcarpa* from Ziniare; R6: *L. microcarpa* branched.

3.3. Sequencing of rps16 Region

Concerning the rps16 gene, results showed that all individuals were classified in a single group belonging to the *Lannea* genus with three subgroups (**Figure 3**). These subgroups (SG) were as follow: The SG I, composed of MLa, MaLa Lm1 and Lm2 and SG II with R6 and NLa had individuals which were not close to any existing species in GenBank. Thus, it was noted from these two SG that *L. microcarpa* was present. With regards to the SG III, it was represented by ZLa which appeared to be close to *Lannea coromandelica* but different from other atypical individuals and from *L. microcarpa*. It was also shown that all these individuals belong to the *Lannea* genus.

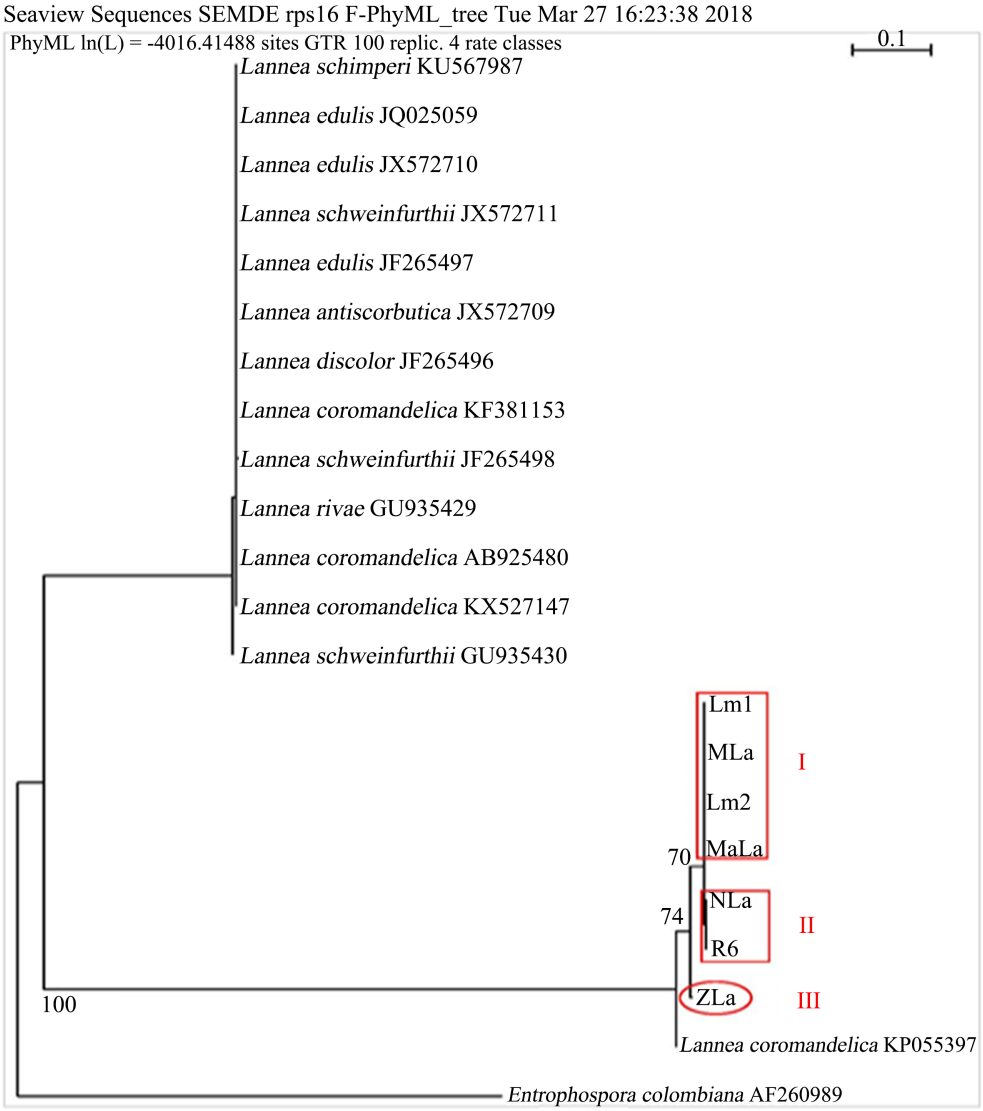

Figure 3. Phylogenetic tree PhyML established from rps16 sequences of six individuals of atypical *Lannea* plants species and their related reference sequences in GenBank. ZLa: Atypical *Lannea* from Ziniare; NLa: Atypical *Lannea* from Naab-Mayooghin; MLa: Atypical *Lannea* from Mamousyorgo 1; MaLa: Atypical *Lannea* from Mamousyorgo 2; Lm1 = *L. microcarpa* from Zorgho; Lm2: *L. microcarpa* from Ziniare; R6: *L. microcarpa* branched.

4. Discussion

The sequence analysis showed that the atypical individuals belong to the same group as the existing *Lannea* species in Genbank. This highlighted that these individuals are of the same kind. These results confirmed those obtained from the morphological characterization previously carried out by [5]. The sequencing of the various genes allowed the grouping of the individuals into several subgroups. We noted for these results that for considering one or other sequence, Lm1, Lm2 and R6 belong to the same subgroup as atypical individuals. This means that atypical individuals are ecotypes or morphotypes of *L. microcarpa*, thus justifying results of [15]. Furthermore, studies of [16] by using an approach for plants identification, showed that when an unknown individual belongs to the same group as a known species, then it is an ecotype of this species. Thus, atypical individuals in this study could be considered as ecotypes of *L. microcarpa.*

It is also shown from the sequence analysis of the three genes (matK, rbcL and rps16) a divergence of the phylogenic positions of individuals which are gene dependent. Indeed, for each given gene, different subgroups of individuals were obtained, suggesting that the phylogenetic positions of individuals in this study were unstable. This could be explained by the fact that some eukaryotic genes can sometimes undergo mutations and genetic recombination under environmental effect to generate new individuals called morphotypes or mutants [16]. Atypical individuals of *Lannea* in the present study can then be considered as ecotypes of *L. microcarpa* and could be originated from mutations that might occur in some genes of the species. Similar results were found by [17] who showed that in eukaryotic plants, the recombination rates vary among species. These studies demonstrated that the mutation rate is increasing from prokaryotes to eukaryotes and is maximal for multicellular eukaryotes (Atypical individuals). The mutation phenomenon is a fundamental source for the emergence of atypical individuals which were found to be new type of individuals for *L. microcarpa* or ecotypes. This is important because it could provide richness through the creation of new forms of individuals within plants [18]. These results corroborate with those of Yi *et al.* [19], Bieniek *et al.* [20] who showed that most terrestrial plants often undergo mutations creating new individuals. Similar results were found by Samuel *et al.* [21] in species from the Phyllanthaceae family. Indeed, for species of *Lannea* genus being terrestrial plants Yang, *et al.* [22] supported these phenomena in these types of plants. Also, atypical individuals having emerged within the population of *Lannea microcarpa*, could be originated from gene flow movements. Since these movements create mostly the mutations within species, this led to the emergence of new individuals. Therefore, these mutations and recombination were responsible for the appearance of atypical individuals. Similar results were found by [23] who showed that the recombination rate modulated the efficiency of natural selection. The sequencing of various genes enabled to divide individuals into several subgroups. However, apart from ZLa which seems to be very close to *Lannea coromandelica* with re-

gards to the analysis of rps16 sequences, other individuals belong to the subgroups that were clearly distinct and not closely related to the reference sequences of previously identified species in GenBank. The results showed that for one or the other sequence, Lm1, Lm2 and R6 which are *L. microcarpa* species, belong to the same subgroup as the atypical individuals. This means that atypical individuals are ecotypes or morphotypes of *L. microcarpa*.

5. Conclusion

This study presents the first molecular characterization of atypical individuals and *L. microcarpa* which are exploited plants in Burkina Faso. It confirmed that these individuals belong to the *Lannea* genus with the existence of a genetic polymorphism between them and the other existing species of the *Lannea* genus in Genbank. Analysis of the phylogenetic trees permitted to classification of individuals from the same group and a subgroup of *L. microcarpa*, indicating that these mutant atypical individuals are ecotypes of *L. microcarpa*. Mutations have important benefits as they provide richness to genetic diversity by creating new individuals. This study will contribute to the protection of resources through concrete actions.

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Conflicts of Interest

The authors state that this research was conducted in the absence of any commercial or financial relationship that could be interpreted as a potential conflict of interest.

Contributions of Authors

Souleymane Ganaba contributed to the scientific supervision, and Hadou HARO to the data analyses and writing of the manuscript, Kadidia SEMDE was responsible for data collection and of the writing manuscript.

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