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Full Length Research Paper

Several haplotypes of groundnut (*Arachis hypogaea* L.) seed-beetle, *Caryedon serratus* Ol. (Coleoptera: Chrysomelidae, Bruchinae), in West Africa: Genetic identification using 28S sequences

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Groundnut (*Arachis hypogaea* Linn.) is included among the crops which contribute efficiently to cover West African populations' nutritional needs. The groundnut seed infestation by *Caryedon serratus* (Coleoptera, Chrysomelidae, Bruchinae), whose larva develop within the seed by consuming the reserves contained in the cotyledon, brings about great losses from 70 to 83% between 4 and 6 months of storage. The purpose of this study was to identify the different haplotypes circulating within the West Africa sub-region. On the other hand, this study aimed at characterizing the genetical diversity and phylogenetical affinities between allopatric populations of the same host plant for the *C. serratus* species. As a result of the PCR-sequencing of 28S nuclear gene, struggling strategies are advocated later by taking into account the bio and agroecological parameters of these four countries. The obtained results allow the distinguished seven haplotypes (H) to be divided into four haplotype's groups (HG). The five individual haplotypes were composed of four haplotypes from Niger and one from Mali. It is the same *Piliostigma reticulatum* biotype which is adapted to groundnut that infests the sub-regional crops. The geographical isolation did not prevail over the genetical structuring of the populations of the same *C. serratus* given the host plant.

Key words: *Caryedon serratus*, *Arachis hypogaea*, 28S nuclear gene, haplotype, haplo-group, ecotype, West Africa, PCR-sequencing.

INTRODUCTION

Groundnut is among the vegetable productions which contribute more widely to the covering of nutritional needs (in particular, proteinic and calorific) of West African populations. However, the major ravager of this leguminous is a beetle, Bruchinae, whose larvae devastate the peasants' harvests (starvation and poisoning), and is also extremely expensive to the national economy. This groundnut's infestation depreciates the quality of its derived products because of the development of bacteria and mouldy bits which produce a toxic and carcinogenic substance (that is, aflatoxin). The products become dangerous for consumption, while cattle cakes become unusable (Thiaw and Sembène, 2010). According to the Inter-State Committee for the struggle against the Drought in Sahel (CILSS), the losses caused by the pillagers of agricultural stored products in the sub-region are estimated from 20 to 30%, which represent 1.3 up to 1.9 millions of tons in 2007. The losses intervene in all the phonological phases of the

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plant; from harvesting to consumption. The first *Caryedon serratus*'s attacks on groundnut worldwide was pointed out in Senegal in 1916 and aggravated in West African rural areas, as well as in some central Africa zones' great losses. Such losses reached 70% in 6 months of storage in Burkina Faso (Ouedraogo et al., 2010), 83% in four months of exposition in Senegal (Sembène et al., 2003), and 30 to 40% in Niger after several months of storage (Alzouma, 1995).

The *C. serratus*'s cosmopolitan and polyvoltine character assures its survival via alternative plants (Ouedraogo et al., 2010), thus entailing a structuring of its populations in biotypes or host-race depending on food spectrum. In fact, four biotypes subjugated to wild leguminosae of the *Piliostigma*, *Bauhinia*, *Tamarindus* and *Cassia* genres exist in Senegal (Sembène, 2000), with more or less genetically restricted flux (Sembène et al., 2010). So, it is commendable to evaluate the genetic diversity and phylogenetic affinities between allopatric populations of the same host plant for the *C. serratus* species. The aim of this work was to identify on one hand, the different haplotypes circulating, within the *Piliostigma* biotype adapted to the groundnut in the different agro-ecosystems of four countries (Senegal, Mali, Burkina Faso and Niger) in West African sub-region; while on the other hand, it assessed the phylogenetic affinities between allopatric populations of the same host plant in the *C. serratus* species, by using the PCR-sequencing of the 28S polymorphic nuclear gene. This approach would allow the study to advocate subsequently, the struggling strategies, by taking into account the bio and agro-ecologic parameters of the different countries of the sub-region.

MATERIALS AND METHODS

Sampling of *C. serratus*

The framework of this research was from north to south by a climatic diversity from Sahel to Guinean climate. It spreads out around Senegal, Mali, Burkina Faso and Niger. The sampling was made during the period of the year (November) when weevils are most abundant in nature, as all the host plants still bear pods. The collected individuals, either the groundnut or the *Piliostigma reticulatum*, were taken to the laboratory in plastic bags where they were put in bottles (16 cm high and 9 cm in diameter), the lid of which was supplied with an airing. The emergences were harvested every morning and conserved in 96% alcohol. The cocoons were isolated in plates containing 24 boreholes or moldy boxes until the adults' emergence. Some first generation adults were coupled with seeds of their host plant for 48 h. This allowed augmenting the number of the sampled population's individuals by a second generation.

The samples were specified according to the studied species, the country and the area where they stem from. Some Senegalese samples originated from the groundnut of Keur Ayip - CsSKa (13°57' 22.05''N 15°48' 46.68''W) and from Karang - CsSKg (13°35.597'N /16°25.330'W), which are located around the Gambian borders. Others were harvested from the *P. reticulatum* of Kawil - CsSKw (14°01.424'N/16°01.495'W), Samba Dia - CsSSd (14°07.765'N/16°42.344'W) and Kédougou - CsSK (12°33' 15.77''N/12°10' 23.63''W). These localities are exclusively situated in the groundnut basin, except Kédougou, which is in the Malian borders. *Piliostigma* infested pods since their host plant was collected from Mali in the following localities: Piama (CsMP) and Bawérékoro (CsMB). Burkina's samples originate from the zone of Tenkodogo [CsBT (11°47'N/ 0°22'O)], situated in the south-east of the country and in the suburb of Ouagadougou [CsB (12°21'N/1°32'O)]. These insects are subjugated to the groundnut. The samples of Niger (CsN) were harvested in Youri (13°17' 23.9 N/02 11' 31.5 E), a lateritic plateau located 26 km in the south-west of Niamey, and were subjugated to *P. reticulatum*.

DNA protocol

DNA extraction

C. serratus genome was entirely extracted with the aid of Qiagen (kit Qiagen Dneasy Tissue) standard method, through the insect's prothorax. The abdomen, the elytron and antennas were isolated to avoid a contamination and allow subsequently morphologic observations in case of specie confusion. After a first elution at 50 µl and a second at 30 µl, the DNA was conserved at -20°C. The polymerase chain reaction was realized either from the 1/10 dilution of DNA soaked in a volume of 50 µl or directly in 30 µl soaking.

PCR of 28S nuclear gene

It consists of an *in vitro* selective amplification of a particular sequence of DNA matrix via the extension of two primers: D2CF D45F (5' -TAC CGT GAG GGA AAG TTG AAA 3') and D2CR D45R (5' -AGA CTC CTT GGT CCG TGT TT3') by a polymerase DNA.

The amplification was performed by a repetition of cycles which assured a multiplication by 2 of the target DNA at every cycle (2^{35}). It was realized in a 25 µl volume of reaction, containing 18.525 µl ultra pure water, 2.5 µl of non colored tampon (10x), 1 µl $MgCl_2$, 0.5 µl dNTP, 0.175 µl of each primer, 0.125 µl of Taq and 2 µl of DNA extract. The PCR begins with a preliminary denaturing at 94°C (3 min), followed by a repetition of 35 cycles of initial denaturing at 92°C (30 s), after which hybridization occurred at 55°C (30 s) and pulled blades of complementary DNA at 72°C (1 min), ending in a final phase of extension at 72°C (10 min).

Genetic analyses

The 28S, coding gene for the 28S ribosomal RNA of the great sub-unity of the ribosome, was characterized on the same segment of chromosome by tandem repetitions, known as satellites.

The obtained 28S sequences were meticulously checked, corrected and aligned by BioEdit software, 7.0.5.3 version, so as to determine the sites' homologies and define the haplotypes. The individuals' nucleotides composition was calculated with BioEdit sequence editor. The standard clues of genetic variations (genetic distance intra/inter haplotypes, number of polymorphic sites, number of informing sites, the position and nature of the mutations) were detailed with the MEGA4 software (Molecular Evolutionary Genetics Analysis 4), 4.0.0.162 version. The relation between transversions and transitions and the frequency of nucleotides were also calculated with the software by the substitution pattern test.

The *C. serratus* ecotypes' phylogenetic reconstructions were estimated by the neighbor-joining methods, the maximum parsimony and the maximum likelihood. The neighbor-joining method (Saitou and Nei, 1987) was based on the ecotypes' matrix of genetic distance (the Kimura's distance 2-parameter) taken two by two in order to model the evolutionary processes. The Maximum Parsimony method (Fitch, 1971) considers that a tree is optimal

Table 1. Characteristics of the samples collected (country, locality, host plant, abbreviation and number).

Country	Locality	Host plant	Samples code	Number of sample
Sénégal	Karang	*A. hypogaea*	CsSKg	5
	Keur Ayip	*A. hypogaea*	CsSKa	5
	Kédougou	*P. reticulatum*	CsSK	5
	Samba Dia	*P. reticulatum*	CsSSd	5
Mali	Piama	*P. reticulatum*	CsMP	5
	Bawérékoro	*P. reticulatum*	CsMB	5
Burkina Faso	Tenckodogo	*A. hypogaea*	CsBT	5
Niger	Youri	*P. reticulatum*	CsN	10
Total				45

when its whole length (number of necessary paces to explain the game of analyzed data) is minimal. A consensus of all the retained trees was then realized. The Maximum Likelihood (ML) method (Felsenstein, 1981) allows testing of all the stories that could have engendered the game of the analyzed data. The Maximum Likelihood method was tested via the Phyml software.

The hardiness of the branches was evaluated for 1000 bootstrap repetitions and the reconstructions were deep-rooted (outgroup) with a consensus sequence between two homologue sequences (28S, F and R) of *Callosobruchus maculatus* of the Fouta-Senegal locality.

RESULTS

The sequences polymorphism

There were 45 sequences of 28S gene (465 pb) stemming from the individuals, scattered in the sampled countries. We obtain 20 in Senegal, 5 in Burkina Faso, and 10 individuals in Mali as well as in Niger. The alignment of the 45 sequences showed that only 39 sequences was aligned with either ambiguity or lacuna out of a portion of 465 bases pairs. Among the six equivocal individuals, in their alignment, three were from Niger (CsN3, CsN4 and CsN18), two from Bawérékoro (CsMB7 and CsMB12) and one from Samba Dia (CsSSd3), Senegal. Due to the fact that the alignment rate of the set formed by CsN3, CsMB7, CsMB12 and CsSSd3 was extremely weak, it rose to 34.02, 32.38, 35.24 and 34.22%, respectively. On top of this, non-conformity was added, in relation to others, on the migration gel of the sequence reaction. Besides, it is worth noting that CsN4 and CsN18 had alignment percentage of 47.34%, although they did not show any equivocation to the sequence reaction. Therefore, these six individuals were excluded from the list of the data exploitation.

By the end of the alignment, a comparison between the nucleotides enchainment of the 39 sequences allowed us to put at stake seven haplotypes, five individuals of which are from H3 to H7. The H1 haplotype mainly prevailed over other haplotypes and was composed of 32 individuals from all the sampling areas. A regrouping of two identical sequences, the one belonging to an individual from Kédougou (CsSK4) and the other from Bawérékoro (CsMB6), together formed H2 haplotype. The individual haplotypes H3 (CsN1), H4 (CsN6), H5 (CsN5) and H6 (CsN11) were very polymorphic. They also originated from Niger localities, whereas the H7 haplotype (CsMP6) was from Piama (Table 2). Furthermore, the H1 haplotype presented neither transition nor transversion, but it only presented a deletion (D) in the 9th position among all the individuals which constituted it. The H2 haplotype was characterized beyond this deletion by a transition between A and G in 114 position (Table 3). Thus, H2 haplotype was only different from H1 for just a basis as far as nucleotide composition was concerned (Table 2). As for H3 and H4, the common mutations and of discriminative values, were essentially noticed in these sites: 9, 51, 137 and 341, were respectively an insertion (A replaces a gap), a transversion (T replaces A), a tranversion (T replaces G) and a transition (A replaces G). Out of the four mutations, H5 admited the sense of an evolution in the 9th and 51st position of the basis pair. Finally, the H6 haplotype, endowed with a pretty high polymorphism was mainly characterized by transitions. Yet, it is worth mentioning that the ecotype (CsN11) embodying H6 was classified in the cases having ambiguous profile on the Macrogen's gel of migration. However, the common mutation (Figure 1 and Table 3) which linked it with the H6 Malian haplotype that happened between A and G, was a transition at the 341st position and its rate of alignment at 91.80% was very important in phylogenetic relationship.

The common fraction of 465 pb in 60 variables or 42 polymorphic sites was bore by an individual of Niger CsN11 that was aligned at 91.80%. It also admits 4 informing sites in parsimony, 53 single sites and 408 conserved sites. When the deletion was considered as a fifth state of character for the 28S, 3 sites were accounted for it (9, 375 and 376 pb). The nucleotide frequencies within the 465 pb were as follow: adenine A (0.161), guanine G (0.315), cytosine C (0.294) and thymine T (0.23). So, it can be noted that 80.95% of the mutations were of the transition type and 18.41% were

Table 2. Characteristics relating to the haplotypes diversity according to the genetic distances and the nucleotides composition.

Sequencing samples	Haplotypes	Genetic distances between haplotypes							Nucleotide composition				Percentage
		H1	H2	H3	H4	H5	H6	H7	A	G	C	T	
CsSKa1, CsSKa2 ,CsSKa3, CsSKa5, CsSKa6, CsSKg1, CsSKg13, CsSKg10, CsSKg5, CsSKg14, CsSK2, CsSK3, CsSK5, CsSK6, CsSSd1, CsSSd2, CsSSd4, CsSSd5, CsMP2, CsMP3, CsMP5, CsMB8, CsMP8,CsMB9, CsBT1, CsBT2, CsBT3, CsBT4, CsBT5, CsN2, CsN9, CsN10	H1	–							74	148	136	106	80,95% of Transition
CsSK4	H2	0.002	–						73	149	136	106	18,41% of Transversion
CsMB6									73	149	136	106	
CsN1	H3	0.035	0.038	–					78	137	137	113	
CsN6	H4	0.006	0.009	0.028	–				75	146	136	108	
CsN5	H5	0.004	0.006	0.035	0.006	–			75	148	135	107	0,64% of deletion
CsN11	H6	0.092	0.094	0.127	0.095	0.097	–		85	136	135	106	
CsMP6	H7	0.002	0.004	0.033	0.004	0.006	0.090	–	75	147	136	106	

of the transversion, versus 0.64% of deletion only (Table 3).

The ratio (R) transition/transversion was superior to 1%, showing thus a non saturation of the sites. This ratio was for the nuclear gene 28S which was exactly at 6.253.

Genetic distances

The genetic distance inside the H1 and H2 haplotypes was nil (0.000). Its value can not be given for H3, H4, H5, H6 and H7 haplotypes, since they were only composed of one individual each. The weakest genetic divergence (0.002) was observed between the H1/H2 and H1/H7 couples of the haplotypes. The genetic distance value (0.004) between twinned haplotype H7/H2 and H7/H4 indicated that H7 was closer to H1 and was also in the same distance that separated H1 and H5 haplotypes.

The H4 and H5 haplotypes from Niger were distinguished by a genetic divergence of 0.006. The 0.035 value was twice noted between the pairs of haplotypes H3/H1 and H3/H5. However, H3 was nearer to H4 (0.028). In fact, the haplotypes from Niger were particularly characterized by their genetic diversity. The greatest genetic distance was observed towards H6 and it increased from 0.090 (H6/H3) to 0.127 (H6/H3) (Table 2).

Phylogenetic trees

The haplotypes were grouped into clades called haplo-type's groups (HG) in the phylogenetic reconstructions based on the method used. The topology obtained with neighbor-joining (Figure 2) revealed four haplotype's groups. The haplotype's group HG1 was the exact phylogenetic representation of the haplotype H1 numerical

Table 3. Genetic description of haplotypes according to the nature and position of mutations.

Sequencing samples	Haplo-types	Substitution	Mutations	Positions
CsSKa1, CsSKa2 ,CsSKa3, CsSKa5, CsSKa6, CsSKg1, CsSKg13, CsSKg10, CsSKg5, CsSKg14, CsSK2, CsSK3, CsSK5, CsSK6, CsSSd1, CsSSd2, CsSSd4, CsSSd5, CsMP2, CsMP3, CsMP5, CsMB8, CsMP8,CsMB9, CsBT1, CsBT2, CsBT3, CsBT4, CsBT5, CsN2, CsN9, CsN10	H1	Absence of the base Adenine	Deletion	9pb
CsSK4	H2	G → A	Transition	114pb
CsMB6		G → A	Transition	114pb
CsN1	H3	A → D T → A T → C A → C	Insertion/Transversion/Transvers./ Transit.	9/51/137/341pb
CsN6	H4	A → D T → A T → C A → C	Insertion/Transversion/Transvers./ Transit.	9/51/137/341pb
CsN5	H5	A → D T → A	Insertion/Transversion	9/51pb
CsN11	H6	A → G	Transition	341pb
CsMP6	H7	A → C	Transition	341pb

majority. The haplotype's group HG2, solely made of the H2 haplotype, brought together two individuals from two neighboring countries: CsSK4 Kédougou from Senegal and CsMB6 from Bawérékoro of Mali. It was characterized in other parts by its basal level of all the methods of phylogenetic reconstruction. The haplotype's group HG3 included, in a single clade, the individual haplotypes H3, H4 and H5. It was supposed as the feature group in Niger because it consisted entirely of individuals from Niger: CsN1, CsN6 and CsN5. Haplotypes H6 and H7 united under the name haplo-type's group HG4 were respectively individual CsN11 of Niger and CsMP6 of Piama from Mali. In the study's entire data set, a country was not noted where its people were only present in one haplo-type's group.

The consensus tree (Figure 3) obtained, after consensus value 50, by the method of Maximum Parsimony (MP) confirmed the exact topology of the tree obtained with the Neighbour-Joining method in satisfactory bootstrap values (89, 90 and 100%). Since a bootstrap is considered significant if its value is greater than 70%, the bootstrap value associated with the consensus subgroup formed by CsN1 and CsN6 was 100% (90% NJ), while the latter were related to CsN5, with whom the haplotype's group HG3, (59%) (55% NJ) was formed.

Haplo-types H6 and H7 were formed by the haplotype's group HG4 value of 89% (66% NJ) and the haplotype's group HG2 value of 90% (64% NJ). The method of Maximum Parsimony (Figure 4) having the same composition as the cladistic Neighbour-Joining method considered that all the haplotypes formed two haplotype's groups: HG1 and HG2. The haplotype's groups HG3 and HG4 always consisted the same individuals by this method considered as the sub-groups of the haplotype's group HG1. HG3 matched SH3 and HG4 to SH4. Meanwhile, the haplotype's group HG2 still maintained its position near the basal outgroups, *Callosobrochus maculatus*, with 64% bootstrap. However, the phylogenetic relationships between individuals, obtained by the method of Maximum Likelihood (Figure 5), considered the haplotype's of group HG2 as a sub-group (Sh2) in the haplotype's group HG1 that differs on the basis of a bootstrap value equal to 90% (Figure 4). Thus, a fundamental observation based on the premise that the likelihood has removed the CsN5 of the HG3 to insert its basal position in HG1, occurred. The bootstrap value (55% NJ and 43% MP)

Figure 1. Alignment of DNA sequences obtained and the characteristic of the seven haplotypes of 28S nuclear gene of ecotypes from Senegal, Mali, Burkina Faso and Niger. The CsSKa1 individual was taken as the reference of the 32 individuals of H1 haplotype since it was totally identical to them as far as the nucleotide enchainment of its DNA is concerned. All the possible numbers of both ends indicate the beginning and end of each line. The number inside shows the position of mutations.

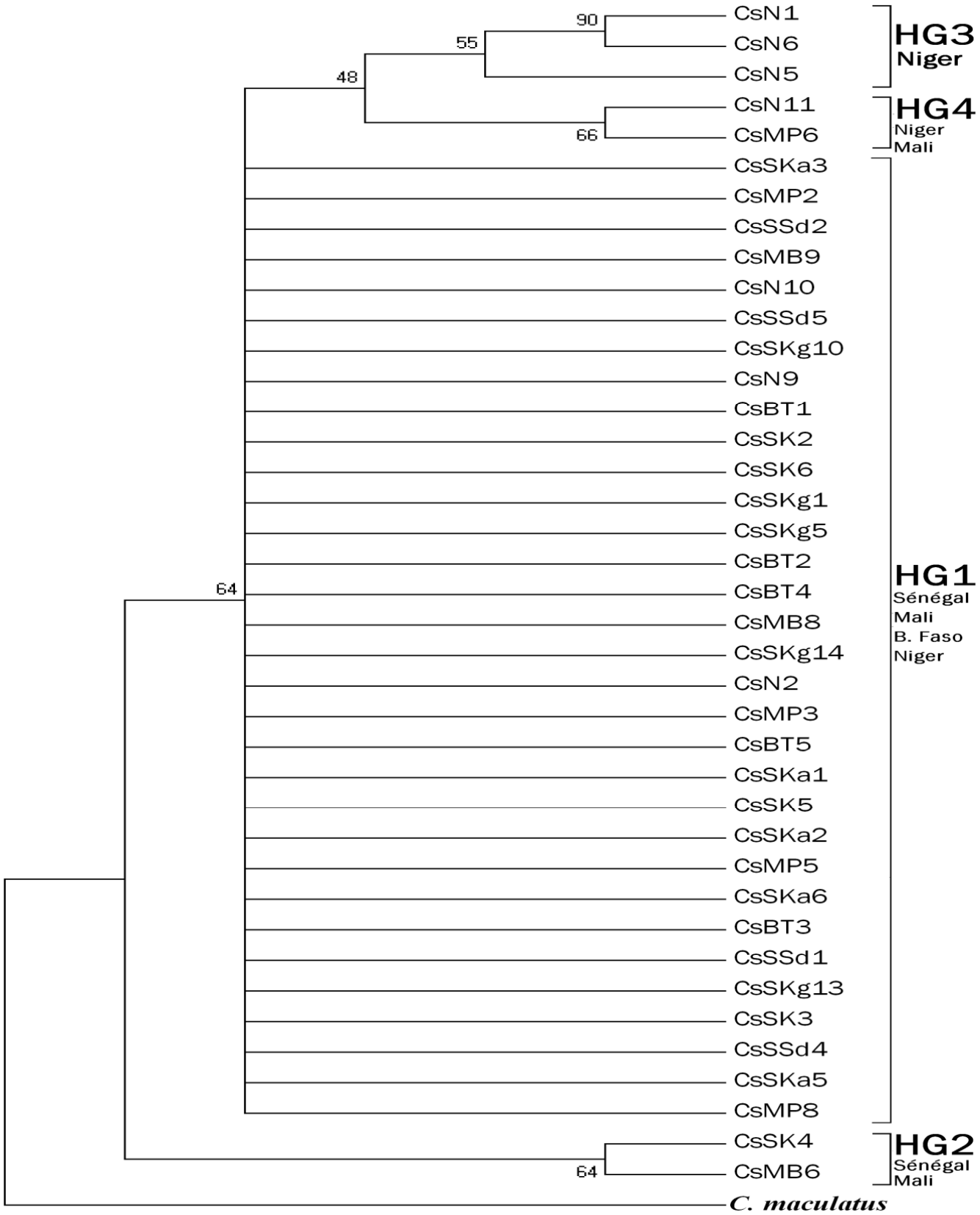

Figure 2. Phylogenetic tree obtained using the neighbour-joining method for the 28S gene (465 pb). The hardiness of the branches was evaluated for 1000 bootstrap repetitions. The reconstructions was deep-rooted (outgroup) with a consensus sequence between two homologue sequences (28S, F and R) of *C. maculatus* of the Fouta-Senegal locality. HG, Haplotype's group.

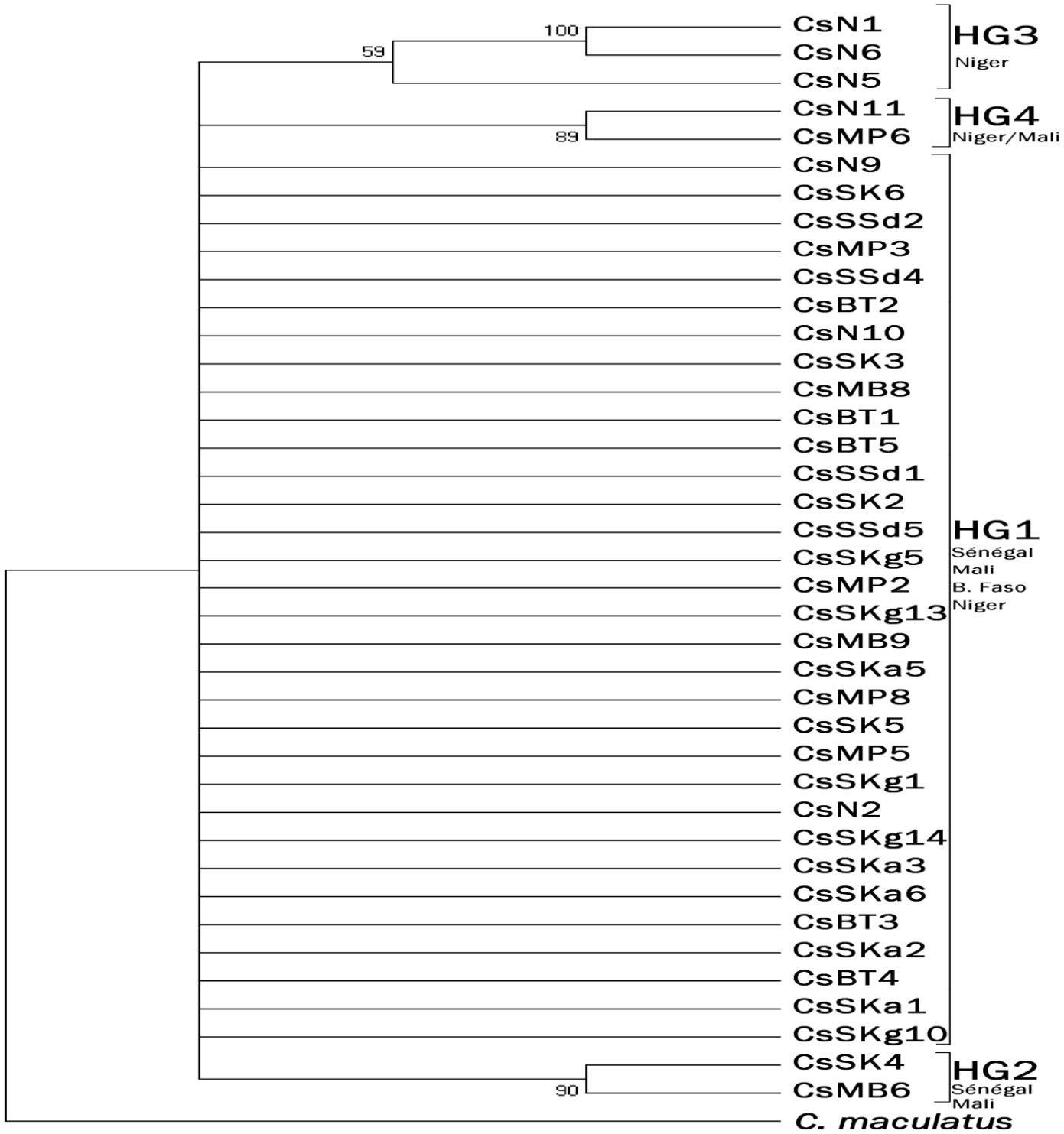

Figure 3. Phylogenetic tree of the consensus method of maximum parsimony for the 28S gene. The reconstructions was deep-rooted (outgroup) with a consensus sequence between two homologue sequences (28S, F and R) of *C. maculatus* of the Fouta-Senegal locality. HG, Haplotype's group.

connecting CsN5 to the subgroup formed by CsN1 and CsN6 was fairly low. This was confirmed by the methods of Neighbor-joining (NJ) and Maximum Parsimony (MP) by a closer relationship between CsN1 and CsN6 in a dynamic of bootstrap from 90 to 100% (Figures 2 and 4).

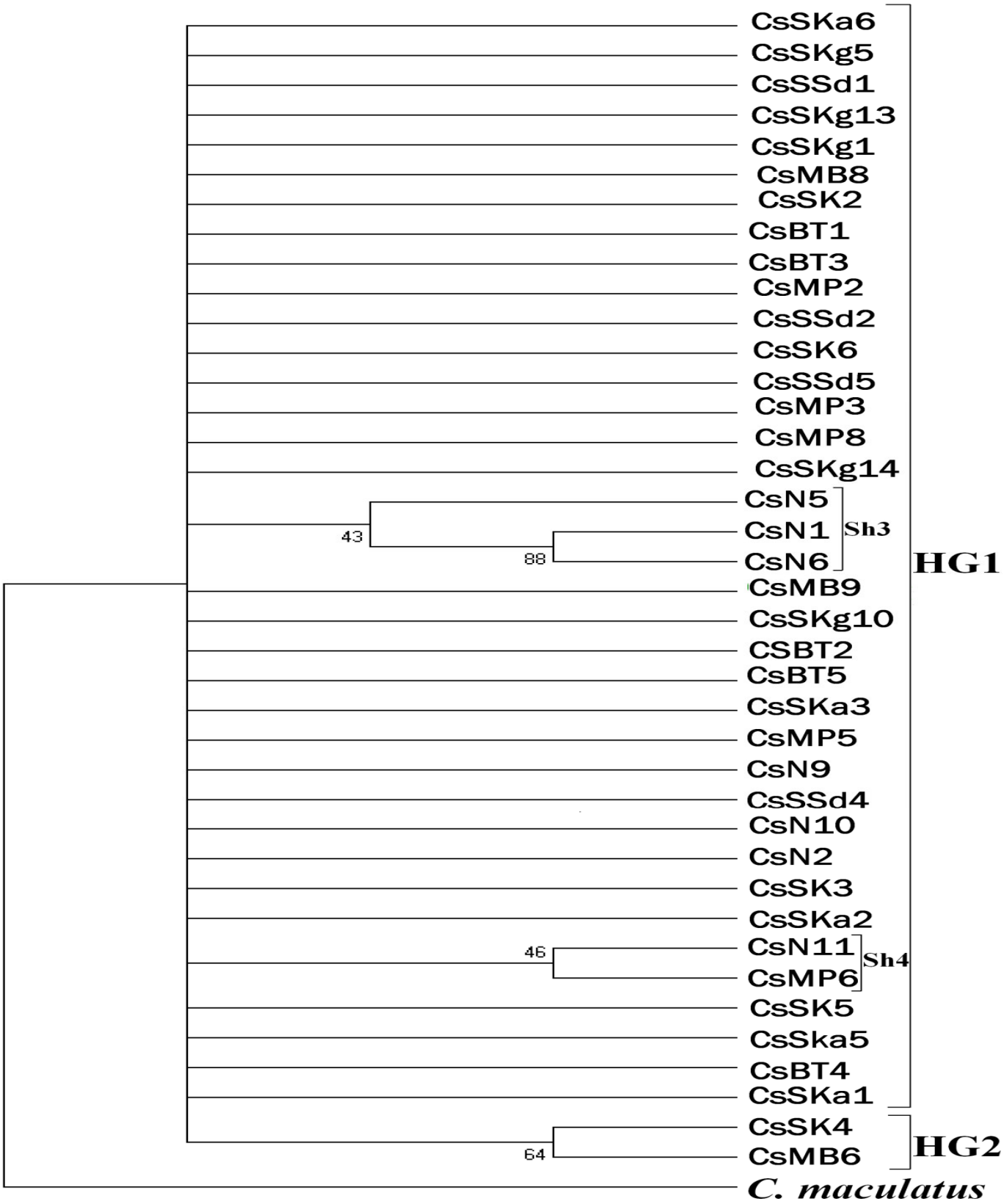

Figure 4. Phylogenetic tree obtained using the method of maximum parsimony for the 28S gene. The reconstructions was deep-rooted (outgroup) with a consensus sequence between two homologue sequences (28S, F and R) of *C. maculatus* of the Fouta-Senegal locality. HG, Haplotype's group.

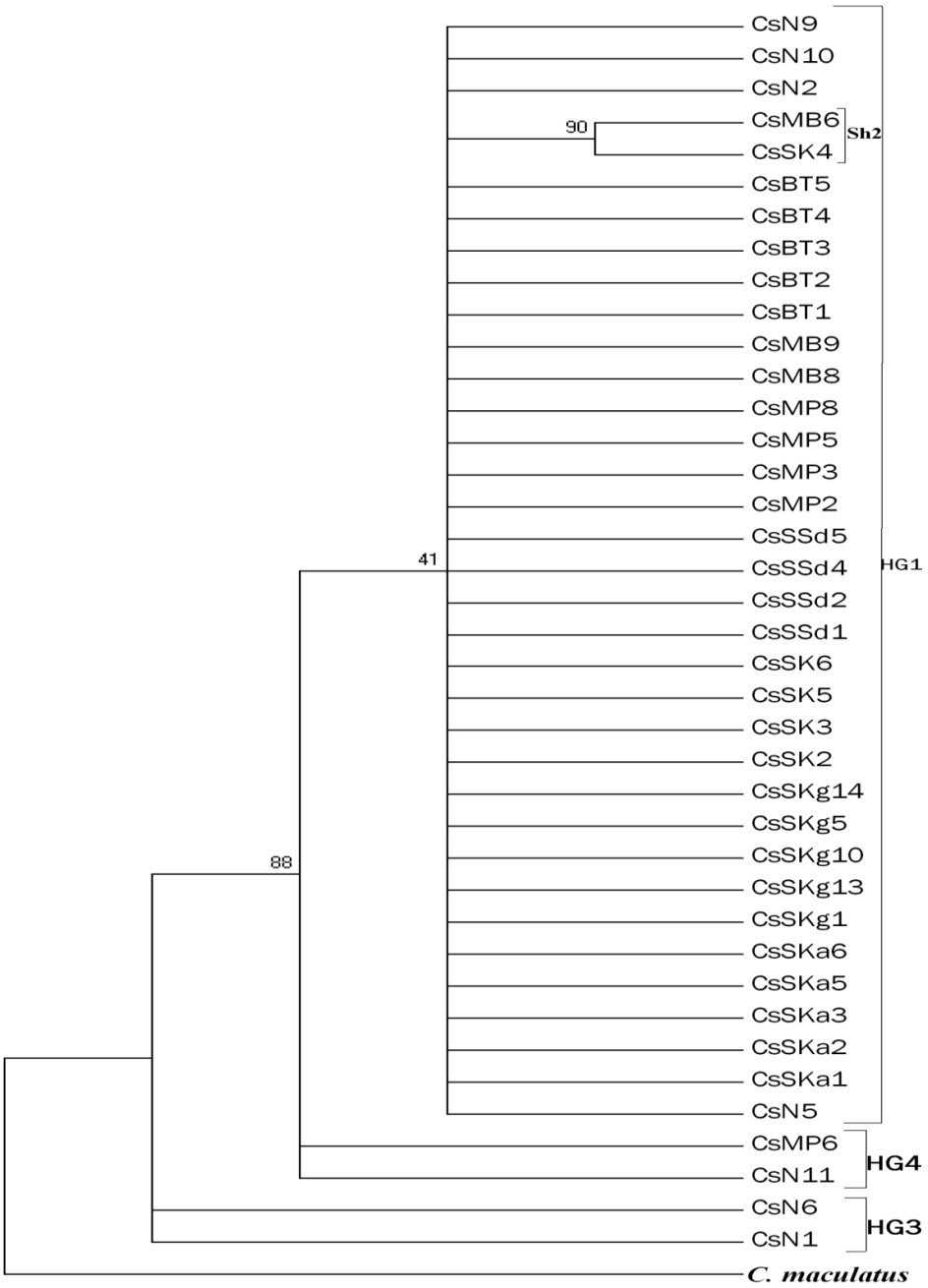

Figure 5. Phylogenetic tree obtained using the method of maximum likelihood for the 28S gene. The reconstructions was deep-rooted (outgroup) with a consensus sequence between two homologue sequences (28S, F and R) of *C. maculatus* of the Fouta-Senegal locality. HG, Haplotype's group.

DISCUSSION

The objective of this work was to identify on one hand, the different haplotypes circulating within the *Piliostigma* biotype adapted to the groundnut, in the different agro-ecosystems of four countries in West African sub-region (Senegal, Mali, Burkina Faso and Niger). On the other hand, it was to assess the phylogenetic affinities between allopatric populations of the same host plant in the*C. serratus* species; the PCR-sequencing of the 28S polymorphic nuclear gene was used.

The 28S nuclear gene's amplification by polymerase chain reaction (PCR) showed the reference size marker (600 pb) fragment in all the individuals. Ndiaye (2009) assigned the same value for the very gene by characterizing genetically two bruchidae insects and pests of the

bean storage: *Callosobruchus maculatus* and *Bruchidius atrolineatus*.

Among the samples that showed a band above the reaction of 600 pb sequence, four (CsN3, CsN4, CsMB7 and CsSSd3) showed a small percentage of alignment equal to 34.02, 32.38, 35.24 and 34.22%, respectively. Besides, two individuals from Niger (CsN4 and CsN18), unidentified in the case of the incorrect sequence reaction, were aligned to 47.34%. These findings remind us that these individuals belong to other species (*Caryedon gonagra* or *Caryedon crempelli*) that are sympatric and sharing the same host plants as C. *serratus*. These individuals were excluded in the subsequent data analysis.

The subjugated bruchids to groundnut and those that infest *Piliostigma reticulatum* have the same nucleotide sequences in their 28S sequences and were aligned to 100%. This similarity between the two strains was previously reported inmorphometric allozymic studies (Sembène and Delobel, 1996; Sembene et al., 1998), Cytochrome B (Delobel et al., 2003; Sembene et al., 2003) and gene internal transcribed spacer ITS1 (Sembène, 2004) of the groundnut bruchid. At this level of variability within species, can we say that the nuclear 28S gene is a pretty good marker for characterizing *Caryedon serratus* strains? This gene is known as a very slow marker. The fact is that in groundnut bruchid, 18 variable sites were found on a portion of 465 base pairs for 38 sequences.

In total, the study distinguished seven haplotypes divided into four haplotype's groups, whereas Diome (2010) identified 37 haplotypes divided into 19 haplotype's groups with the cytochrome B gene. This difference is explained by the fact that cytochrome B is highly mutational and its mutations are preserved unlike the 28S, which is not only less mutational but does not retain its mutations and presents more deletions.

The genetic distance based on the DNA matrix (Kimura 2-parameter), within haplotypes H1 and H2, was zero (0.000). The rest are individual haplotypes and could not be calculated (Table 1). Genetic divergence between haplotypes H1, H2, H3, H4, H5 and H7 varied from 0.002 to 0.038. The H1 haplotype numerical majority, and those whose intrahaplotype genetic distance was zero, was taken as a reference. This is supported by the fact that haplotypes H2, H4, H5 and H7 were closest to it with a genetic distance ranging from 0.002 to 0.006. This is the reason why the H1 haplotype is a majority in the historyof "Groundnut–Bruche Association" which was scattered in the West African zone from Senegal, that is, the original point of infestation in the twentieth century and was later given in Mali and Niger divergent haplotypes based on agro-ecological settings. The haplotype H3, nearest to its neighbor H4 of the same country (Niger), was distant from other haplotypes by a maximum value of 0.038. This result seemed to enter the same logic as that obtained by Sembene et al. (2010), in that the genetic distances derived from the combination of data from mitochondrial cytochrome B gene and nuclear internal transcribed spacer (ITS1) for *C. serratus* subjugated to groundnut varied from 0.000 to 0.037. Genetic distances within and between haplotypes showed homogeneity in the range except the characteristic of intraspecific haplo-type H6 where the greatest genetic distances obtained against it ranged from 0.090 to 0.127. The distance between biotypes infesting *C. sieberiana* from Sembene (2010), ranged from 0.186 to 0.195. Thus, H6 was almost equidistant between the other haplotypes and individualspledged to *C. sieberiana*. The characteristics of the Nigerien haplotypes H3, H4, H5 and H6 can be approved in the context as that significant gene flow exists with weevils of tamarind or *C. gonagra* from India in this area of Niger that is a crossroad of trade between West Africa and North Asia. Anthropogenic dispersal of species in this sub-regional scope would entail a co-existence between the populations subjugated to *P. reticulatum* and those from other horizons. This observation is the cause of the disruption of biological communities in this area, thus causing a restructuring by increasing genetic diversity and haplotype flows of migrants.

Four groupings have emerged from those phylogenetic reconstructions, some of which have a value of haplotype's group or sub haplotype's group. HG1 included 32 individuals, absolutely identical in every respect, from all sampled countries namely: Senegal, Gambian border, Mali, Burkina Faso and Niger. This is consistent with Sembène et al. (1998) who stated that geographical distances below 400 km are not determinative of the genetic structuring of *C. serratus* populations of the same given plant. The work of Diome et al. (2011) on the genetic characterization of ecotypes of *C. serratus* with the cytochrome B gene in the West African sub-region is in phases and it showed 19 haplotype groups, whose haplotype composition was independent of the geographical origin of samples. The HG2, including an individual of Kédougou (CsSK4) and another individual (CsMB6) of Bawérékoro (Mali), is justified by the high proportion of migrant flows due to the geographical proximity between the two communities that share similar ecological conditions. The HG3 was exclusively Nigerien and it had more mutations. Despite their genetic divergence, we believe that the haplotypes of Niger are not yet reproductively isolated from the populations subjugated to groundnuts, because the genetic distance that separates their groups mentioned above was in the range of variability within species. However, the H5 haplotype evolving towards H3 and H4 haplotypes, at benchmark of the shared mutations, confirms a mutational trend of *Caryedon serratus* populations in Niger. The HG4 haplotype group, composed of CsMP6 of PIAM (Mali) and CsN11 (Niger), particularly depended on the ecological conditions prevailing in these countries.

Conclusion

The singularity of the groundnut bruchid, *C. serratus*, lies in almost perfect identity with that which exists in this species between the fundamental spectrum (all plants from which the development is supposed to be) and the spectrum that is realized. This study distinguished in the same host plant seven haplotypes, in which five individual haplotypes were composed of four haplotypes from Niger and one from Mali.

However, the ecotypes of Niger showed an evolutionary trend quite different from those of Senegal, Mali and Burkina Faso. That is the reason why these preliminary results must be deepened by studying the genetic diversity of populations of *C. serratus* subjugated to different host plants in Niger or in holding populations of *C. serratus* to develop rational control methods other than killing insects by chemical (hazardous and expensive).

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