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Evolution and phylogenetic relationships of Senegalese populations of *Caryedon serratus* (Olivier, 1790) between 1998 and 2022

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Abstract

Groundnuts are an important food resource in Senegal, but post-harvest losses of this product are considerable and represent a major economic constraint for our country. Most often, these losses are due to beetles (pests) and among these, the one that causes the most damage to the stocks belongs to the Chrysomelidae family : *Caryedon serratus*, commonly known as groundnut seed-beetle. Faced with these losses, it is becoming urgent to look for new methods of control. The objective of this research is therefore to study the evolution and phylogenetic relationships of Senegalese populations of *C. serratus* associated with groundnuts (*Arachis hypogaea*) and wild host plants (*Piliostigma reticulatum*, *Tamarindus indica*, *Bauhinia rufescens* and *Cassia sieberiana*) between 1998 and 2022. For this, the Cytochrome B (Cyt-B) gene of different strains of *C. serratus* was sequenced. The results of demographic evolution suggests that, overall, populations of *C. serratus* may have originated from a stable ancestral population with a long evolutionary history. The network and phylogenetic trees reveal genetic affinities and proximity that result in gene flow between groundnut-infesting seed-beetles and those from *Cassia sieberiana*, meaning that the *C. sieberiana* was the source of the groundnut infestation in 2022.

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Introduction

Groundnuts are an important food resource in Senegal, but the post-harvest losses of this product are considerable and represent a major economic constraint for our country. Indeed, the contamination of groundnuts by insects greatly depreciates the nutrient content of this staple food. Among these insects, the one that causes the most damage to the stocks is a beetle belonging to the Chrysomelidae family : *Caryedon serratus* (Olivier, 1790), commonly known as groundnut seed-beetle. In Senegal, the damage caused by this seed-beetle to groundnuts can be up to 83% of quantitative loss over a storage period of 4 months (Ndiaye, 1991). Faced with these losses, it is becoming urgent to look for new methods of control.

In this perspective, different approaches have been used to study *Caryedon serratus* populations : morphometric studies, allozyme studies (Sembène and Delobel, 1996; 1998; Sembène *et al.*, 1998), and molecular: microsatellites (Sembène, 2000), Cytochrome B (Delobel *et al.*, 2003) and the Transcribed Internal Spacer, ITS1 (Sembène, 2004; Sembène *et al.*, 2010). In addition, it has been reported in numerous studies that *C. serratus* is an insect with a high level of diversity and genetic differentiation between its populations and biotypes (Delobel *et al.*, 2003; Sembène *et al.*, 2006; Diome *et al.*, 2011; Ndong *et al.*, 2011; Ndiaye, 2014). Thus, this work finds its originality in the fact that it is the first study to deal with the temporal aspect of the genetics of Senegalese populations of *C. serratus* (here,

between 1998 and 2022, i.e., a duration of 24 years) through the Cyt-B gene.

The objective of this research is therefore to study the evolution and phylogenetic relationships of different populations of *C. serratus* associated with groundnuts (*Arachis hypogaea*) and wild host plants (*Piliostigma reticulatum*, *Tamarindus indica*, *Bauhinia rufescens* and *Cassia sieberiana*) between the years 1998 and 2022. Specifically, it is a question of assessing genetic evolution of two (2) groups of *C. serratus* populations (groundnut populations and global populations) between the years 1998 and 2022 ; determine the phylogenetic affinities between groundnut populations of *C. serratus* and those from natural host plants in 2022; and to finally identify the wild strain of *C. serratus* responsible for the primary groundnut infestation in the fields in 2022. For this, the Cytochrome B (Cyt-B) gene of different strains of *C. serratus* was sequenced. The results obtained will undoubtedly contribute to the protection of groundnut seeds against *C. serratus* populations without the use of synthetic pesticides but rather by the definition of effective and non-polluting control strategies.

Materials and methods

Sampling

The samples studied come from different storage sites in the Senegalese groundnut basin (targeted agro-ecological zone) (Fig. 1).

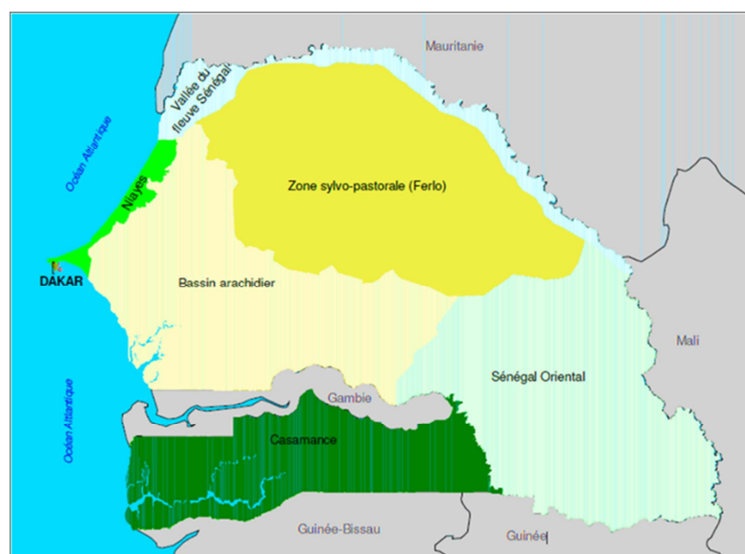


Fig. 1. Agro-ecological zones of Senegal (CSE, 2007)

Table 1. Agro-ecological zones and geographic coordinates of the four (4) locations sampled in the groundnut basin between 2020 and 2022

Regions	Localities	Storage sites	Agro-ecological zones	Geographic coordinates	
				Latitude	Longitude
Diourbel	Diourbel	Stockroom	North groundnut basin	14° 39' 21.438" N	16° 13' 31.089" W
	Touba	Stockroom	North groundnut basin	14° 54' 23.850" N	15° 51' 55.806" W
Fatick	Passy	Granary	South groundnut basin	13° 58' 42.369" N	16° 15' 52.136" W
Kaolack	Keur Ayib	Granary	South groundnut basin	13° 35' 45.800" N	15° 36' 02.100" W

Table 2. Summary of the different sampling parameters between 1998 and 2022

Host plants	Harvest years	Sample codes	Number of individuals sequenced
<i>Arachis hypogaea</i>	2022	CsA-2022	52
	1998	CsA-1998	34
<i>Piliostigma reticulatum</i>	2022	CsP-2022	13
	1998	CsP-1998	9
<i>Tamarindus indica</i>	2022	CsT-2022	9
	1998	CsT-1998	6
<i>Bauhinia rufescens</i>	2022	CsB-2022	11
	1998	CsB-1998	5
<i>Cassia sieberiana</i>	2022	CsC-2022	8
	1998	CsC-1998	6
Total			153

Indeed, they were taken from four (04) localities in three (03) regions of Senegal : Diourbel (in Diourbel and Touba), Fatick (in Passy) and Kaolack (in Keur Ayib). These regions can be divided into two sub-zones : the North Groundnut Basin (Diourbel) and the South Groundnut Basin (Fatick and Kaolack) (Table 1).

In this study, the sampling method used is the same regardless of the storage site. Indeed, it consists of taking a random sample of four kilograms (04 Kg) of groundnuts (about 1000 pods) from each locality. The pods are then placed in jars with wire lids and placed in a ventilated room in the laboratory where the ambient temperature follows the fluctuations of the outside temperature. Samples are kept for a minimum of 75 days to control the level of *C. serratus* infestation. Daily monitoring of this mass culture made it possible to collect the adults of *C. serratus* that emerge from the samples. The individuals collected in this way are preserved in 95° alcohol for the realization of the molecular protocol.

The *Caryedon serratus* specimens that were the subject of this study come from pods sampled in 1998 and between 2020 and 2022 from different host plant

species. Pods collected between 2020 and 2022 are coded as 2022 (Table 2).

Genetic studies

Choice of cytochrome B (Cyt-B)

The Cyt-B gene is considered to be one of the most widely used genes for determining phylogenetic relationships due to its low sequence variability and probably the best known mitochondrial gene in terms of its structure and the function of the protein produced (Esposti *et al.*, 1993). It is distinguished by a high intra-specific variability (Avisé, 1994) especially in insects (Simmons and Weller, 2001). It is easier to align between species because it does not have gaps and easier to use to estimate the divergence of dates between taxa.

Extraction of genomic DNA from *C. serratus*

The extraction of DNA from *C. serratus* was done using the standard Qiagen method (Qiagen Dneasy Tissue kit). The insect is first dissected to carefully remove the abdomen, elytra and fins. The other parts of the body (the head, thorax and legs) are crushed and then placed in a 1.5 ml tube. These three parts are therefore the only ones to be used in the extraction protocol which took place in 4 steps : digestion, cell lysis, purification and elution.

Table 3. Identification of the sequences of the different primers used in PCR

Gene	Primer names	Primer sequences
Cyt-B	CB-J-10933 (F)	5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3'
	CB-N-11367 (R)	5'-ATT ACA CCT CCT AAT TTA TTA GGA AT-3'

Table 4. Summary of PCR conditions

Gene	PCR conditions
Cyt-B	(1) Initial denaturation : 94°C, 3 min ; 35 denaturation cycles : 94°C, 1 min ; (2) Hybridization : 47°C, 1 min ; (3) Elongation : 72°C, 1 min ; final elongation : 72°C, 10 min.

Amplification by PCR (Polymerase Chain Reaction)

PCR is a specific in vitro of a target DNA sequence ; that is to say, a repetition of cycles that ensures a doubling (02) of the target DNA at each cycle. In reality, PCR is based on a repetition of three (03) phases : DNA denaturation, hybridization of primers on templates, and elongation of the 3' ends of the neosynthesized strands. The primers used were defined by Simon *et al.* (1994) (Table 3) and the conditions summarized in Table 4.

Sequencing

The sequencing of the Cyt-B gene was performed by the South Korean sequencing company Macrogen. This technique consists of determining the nucleotide sequence of a DNA fragment. It therefore makes it possible, by comparing the sequences of the same gene in different individuals of the same species or of different species, to highlight point mutations.

Genetic analyses

Sequence cleaning and alignment

Sequence cleaning is the visual verification of the correct automatic interpretation of chromatograms. First, the dataset was blasted to the Genbank to check if our sequences actually match those of the *Caryedon serratus* species. Subsequently, the resulting raw sequences were manually corrected and aligned with the BioEdit ver software. 5.0.6 (Hall, 2001) which uses the Clustal-W algorithm (Thompson *et al.*, 1997).

Demographic evolution

To trace the demographic history of *C. serratus* populations, demogenetic tests were carried out using statistics from Tajima's D (Tajima, 1989), Fu's Fs (Fu, 1997) and R2 (Ramos-Onsins and Rozas, 2002). In

addition, the demographic indices (SSD and Rag) of mismatch distribution (Harpending, 1994) have been combined with mismatch distribution curves (Rogers and Harpending, 1992).

Demogenetic tests

These are exactly tests of selective neutrality and population balance. The significance of the statistics of D, Fs and R2 was tested by generating random samples under the assumption of selective neutrality and population equilibrium, using a coalescence simulation algorithm adapted from Hudson (1990) using the ARLEQUIN ver software. 3.1 (D and Fs statistics) and DNAsp ver. 5.10.01 (R2 statistics).

Mismatch distributions and demographic indices

Mismatch curves are obtained using the DnaSP ver software. 5.10.01 (Librado and Rozas, 2009). The Mismatch analysis combines two indices that test the fit quality of the distribution : these are the SSD (sum of squares of deviations) and the Rag (irregularity index). These indices are calculated using the ARLEQUIN ver. 3.1 (Excoffier and Heckel, 2006).

Phylogenetic approach

Phylogenetic analysis allows us to gather information on the relationships that exist between the different populations studied.

Haplotype networks

Haplotype networks represent the relationships between different non-recombinant DNA sequences (haplotypes). With the Cyt-B sequences, a network of haplotypes of the 2022 populations was built using the Network ver program. 5.0.0.0 (Bandelt *et al.*,

1999) in order to detect phylogenetic relationships between the different haplotypes.

Reconstruction of phylogenetic trees

Concerning phylogenetic trees, the method of genetic distances of neighbor-joining was chosen on the one hand. Thus, the trees by the method of genetic distances were built via the MEGA 7 worm software. 7.0.14 (Tamura *et al.*, 2016) using the p-distance model. On the other hand, in probabilistic methods, it is much more relevant to infer with the Bayesian method (Bayesian Inference, IB) despite its tendency to frequently overestimate Bayesian supports because the maximum likelihood method does not evaluate all possible trees because they are numerous ; the likelihood then gives only a partial exploration of all the trees made (Lô, 2021).

Thus, the Bayesian tree was built using MrBayes ver software. 3.2.5 (Huelsenbeck and Ronquist, 2001) and FigTree ver. 1.4.4. Finally, the reconstructions were rooted with a homologous sequence of the

species *Corcyra cephalonica* (Stainton) because the credibility of the phylogenetic relationships of the trees depends essentially on the out-group sequence.

Results

Demographic trends

Neutrality tests and demographic indices of *Cyt-B*

In the total population, the neutrality estimators reveal positive and non-significant values for D of Tajima and Fs of Fu but significantly positive for R2 of Ramos-Onsins and Rozas. In this population, the demographic indices (SSD and Rag) are not significant (Table 5).

For the global populations (Pop-2022 and Pop-1998), the results of the neutrality tests are positive and not significant for D of Tajima and Fs of Fu but significantly positive for R2 of Ramos-Onsins and Rozas. For these populations, all demographic indices (SSD and Rag) are non-significant with an exception at the level of the 2022 overall population (Pop-2022) where the Rag is significant (Table 5).

Table 5. Demogenetic tests and demographic indices of *Cyt-B*

Parameters studied	Groundnut populations		Global populations		Total
	CsA-2022	CsA-1998	Pop-2022	Pop-1998	
Tajima's D	0.38031 (P = 0.73400)	2.78756 (P = 1.00000)	1.03813 (P = 0.89900)	3.20278 (P = 1.00000)	1.85220 (P = 0.90825)
Fu's Fs	13.83478 (P = 0.99600)	33.36950 (P = 1.00000)	17.71866 (P = 0.99600)	14.39379 (P = 0.99500)	19.82919 (P = 0.99675)
R2	0.10478 (P = 0.00000) ***	0.11406 (P = 0.00000) ***	0.09295 (P = 0.00000) ***	0.10231 (P = 0.00000) ***	0.08578 (P = 0.00000) ***
SSD	0.05661 (P = 0.02000) **	0.48735 (P = 0.00000) ***	0.02758 (P = 0.11000)	0.09004 (P = 0.34000)	0.16540 (P = 0.11750)
Rag	0.05510 (P = 0.01000) **	0.49947 (P = 0.94000)	0.02248 (P = 0.00000) ***	0.09160 (P = 0.27000)	0.16716 (P = 0.30500)

For groundnut populations (CsA-2022 and CsA-1998), the results of demogenetic tests are also positive and not significant for D from Tajima and Fs from Fu but significantly positive for R2 from Ramos-Onsins and Rozas. In these populations, all demographic indices (SSD and Rag) are significant except for the 1998 groundnut population Rag (CsA-1998) which is non-significant (Table 5).

Mismatch distributions

Under the assumption of a population expanding demonstratorically, the analysis of the mismatch distribution curves (or distribution of disparities)

reflects a multimodal pattern for both global populations (Pop-2022 and Pop-1998) and groundnut populations (CsA-2022 and CsA-1998) ; the same is true for the total population. This distribution is different from that expected and reflects stable populations, which are therefore not in a phase of demographic expansion (Fig. 2).

Phylogenetic approach

Haplotype networks of *C. serratus* populations in 2022

The network shows that the haplotypes have been distributed according to the five (05) host plants, which reminds us of the five (05) populations of *C.*

serratus that are the subject of our study. Each haplotype corresponds to a disk whose dimensions are proportional to the number of individuals it contains. Of the 93 individuals sequenced in 2022, there are nineteen (19) haplotypes, including 11 (H1 to H11) on the 52 individuals from Groundnut, 01

(H12) on the 13 individuals from *Piliostigma reticulatum*, 01 (H13) on the 09 individuals originating from *Tamarindus indica*, 05 (H14 to H18) on the 11 individuals collected from *Bauhinia rufescens* and 01 (H19) on the 08 individuals of *Cassia sieberiana* (Fig. 3).

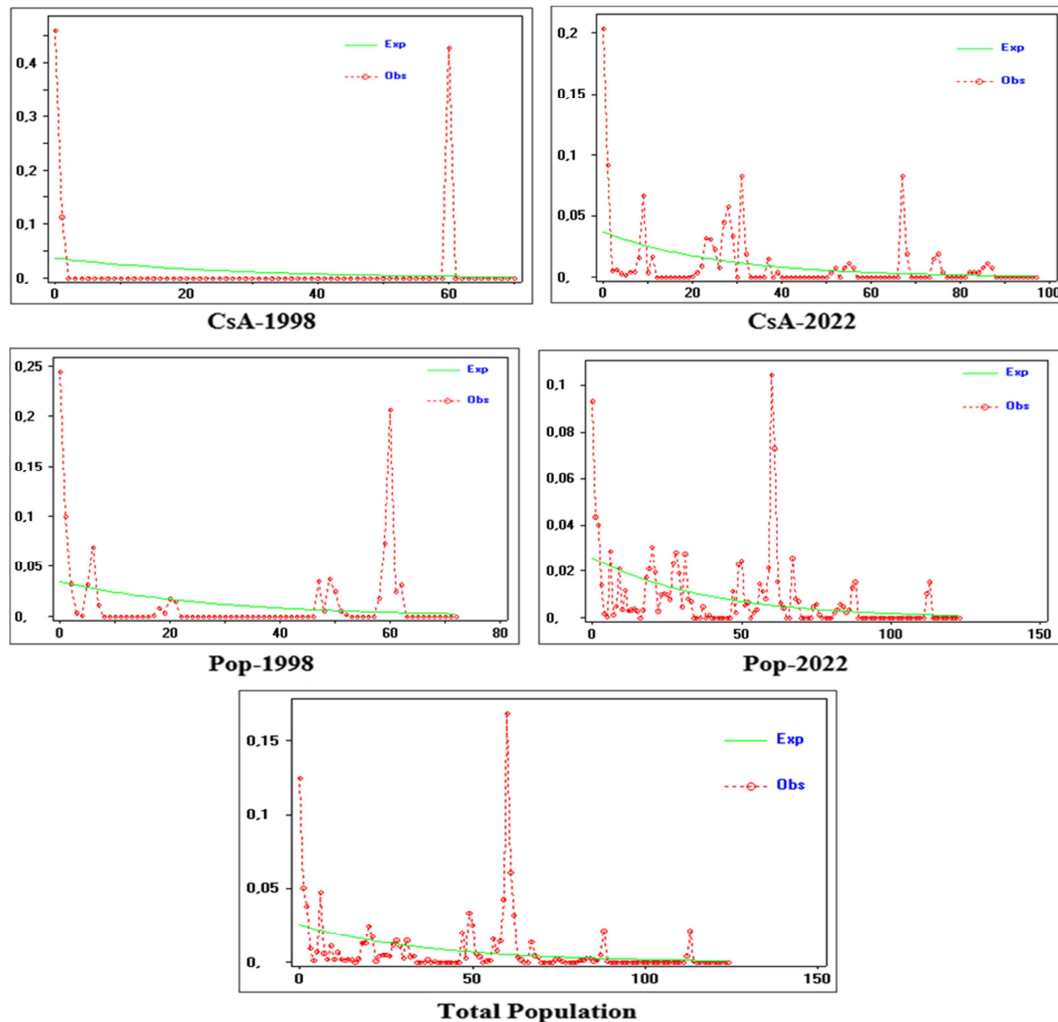


Fig. 2. Mismatch distribution of Cyt-B sequences as a function of harvest years of individuals from groundnut populations, overall (global) populations and the total population

Thus, the highest haplotypic diversity is obtained with groundnuts, which have 11 haplotypes. Indeed, the majority haplotype (CsA1) contains 22 individuals originating from the Groundnut and represents 23.66% of the 2022 sequences. It should also be noted that, on the whole, the biotypes do not have haplotypes in common ; they are all characterized by endemic (private) haplotypes, including 05 individual haplotypes found exclusively in groundnuts : these are CsA23, CsA43, CsA46, CsA49 and CsA52. The

presence of about ten median vectors and a very large number of mutational steps in *C. serratus* populations was also noted ; there is only one mutation step from CsA1 (groundnut haplotype) to CsC3 (*C. sieberiana* haplotype), about ten to go from CsA23 (groundnut haplotype) to CsB5 (*B. rufescens* haplotype) and about twenty to go from CsB2 (*B. rufescens* haplotype) to CsP1 (*P. reticulatum* haplotype) or to CsT1 (*T. indica* haplotype). Therefore, three (3) groups of haplotypes can be

identified in this network : a first group consisting of the groundnut and *C. sieberiana* haplotypes (blue and brown discs), a second group containing the *P. reticulatum* and *T. indica* haplotypes (green and purple discs), and a third grouping only the *B. rufescens* haplotypes (red discs). However, the haplotypes of *B. rufescens* (group 3) are intermediate between the haplotypes of groups 1 and 2, but closer to those of the former (Fig. 3).

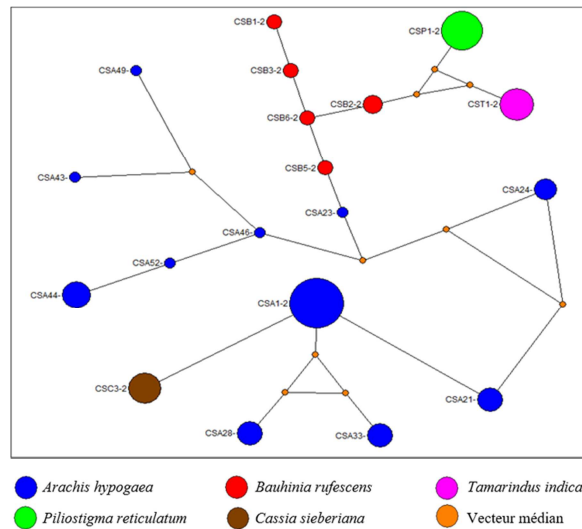


Fig. 3. Cyt-B gene haplotype networks of *C. serratus* populations in 2022

Legend : Distribution of haplotypes of *C. serratus* populations according to host plants. The haplotype(s) of each host plant is represented by a colour.

Phylogenetic trees of C. serratus populations in 2022

The phylogenetic trees obtained with the distance method (Neighbor-Joining) and the probabilistic method (Bayesian Inference) are practically similar. On trees, the leaves (individuals) of each haplogroup are colored according to the infested host plants.

Thus, the topology of the Bayesian tree (Fig. 4) reveals that it is made up of three (03) haplogroups (HG1, HG2 and HG3) that are very solid and significantly supported by a high posterior probability (100%). The first haplogroup (HG1) is shared ; it contains two (02) individuals of *B. rufescens* (CsB5 and CsB10) and all individuals from Groundnut and *C. sieberiana*. The second (HG2) is also shared ; it includes three (03)

individuals of *B. rufescens* (CsB2, CsB4 and CsB9) and all individuals from *T. indica* and *P. reticulatum*. The third (HG3) on the other hand is private and isolates only four (04) individuals of *B. rufescens* (CsB1, CsB3, CsB7 and CsB8). It should also be noted that this tree has two (02) non-resolving individuals, all collected from *B. rufescens* (CsB6 and CsB11).

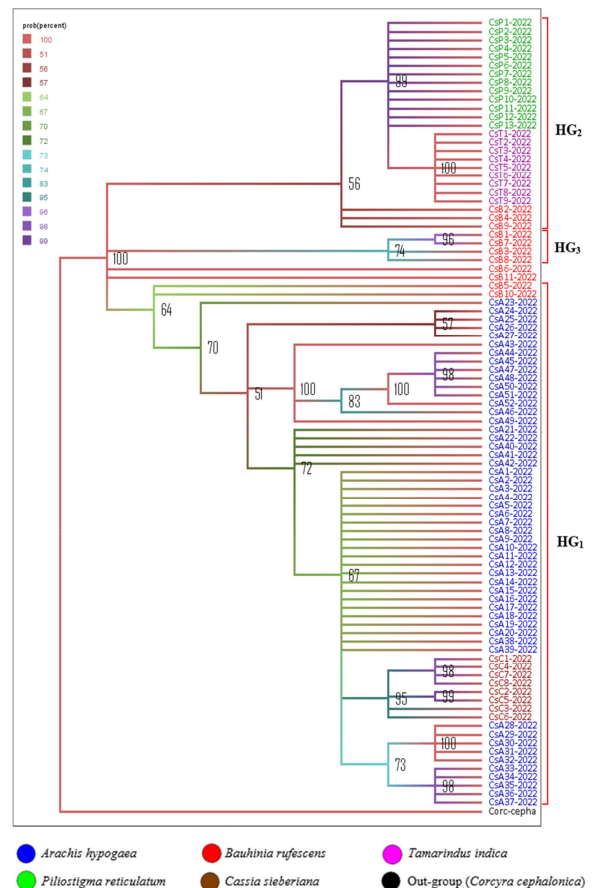


Fig. 4. Bayesian inference of the Cyt-B gene of 2022 individuals as a function of host plants

Legend : Distribution of individuals in *C. serratus* populations according to host plants. The individuals of each host plant are represented by a colour.

In addition, the appearance of the Neighbor-Joining tree (Fig. 5) also reflects three (03) haplogroups (HG1, HG2 and HG3) that are quite distinct and supported by sufficiently significant Bootstrap values (79% for HG1 and 100% for HG2 and HG3). Haplogroup 1 (HG1) is shared ; it contains almost all the individuals from the groundnut and all the individuals from *C. sieberiana* and *B. rufescens*.

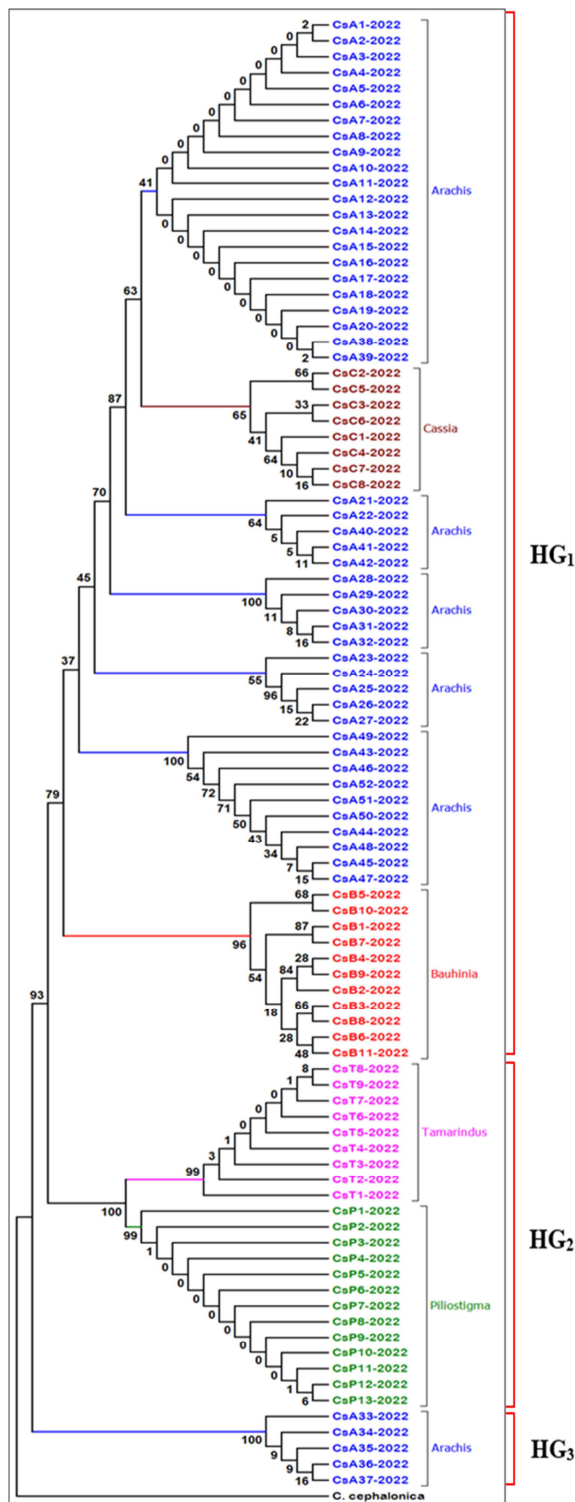


Fig. 5. Neighbor-Joining of the Cyt-B gene of 2022 individuals based on host plants

Haplogroup 2 (HG2) is also shared and includes all individuals originating from *T. indica* and *P. reticulatum*. On the other hand, haplogroup 3 (HG3) is private and isolates only five (05) individuals dependent on groundnuts (CsA33 to CsA37). It

should also be noted that unlike the Bayesian tree which has two (02) non-resolving individuals, all the individuals of this tree are classified.

Finally, the haplogroups of the two trees reflect visible phylogenetic affinities between individuals from Groundnut and *C. sieberiana* (see HG1 of the two trees) and between individuals from *P. reticulatum* and *T. indica* (see HG2 of the two trees). However, individuals of *B. rufescens*, intermediate between these 2 haplogroups, are more closely related to individuals from Groundnut and *C. sieberiana* than to individuals originating from *P. reticulatum* and *T. indica* (see HG1 and HG2 of the Bayesian tree and HG1 of the Neighbor-Joining tree). These phylogenetic affinities are therefore of two types : kinship links between individuals of the same host plant (see HG3 of the 2 trees) and phylogenetic links between individuals of different host plants (see HG1 and HG2 of the 2 trees).

Discussion

Demographic evolution

The high values of the genetic diversity indices (Hd and Pi) reveal that the total population and the global populations (Pop-2022 and Pop-1998) would have originated from a stable ancestral population with a large effective size, which may be a signal of admixture from populations of *C. serratus* that have been isolated from each other. This is supported by the mismatch distribution curves (they show a multimodal appearance indicating demographic stability) and the demogenetic tests of Tajima's D and Fu's Fs (Tajima's D and Fu's Fs are non-significant and reflect a population in demographic equilibrium or in moderate expansion). However, the significantly positive neutrality tests of the R2 of Ramos-Onsins and Rozas and the non-significant demographic indices of the SSD and the Rag invalidate this origin except for the significant Rag of the overall population of 2022 (Pop-2022) ; indeed, if R2 is weakly positive and significant, then there is a recent population expansion, and if the p-values of the SSD and the Rag are non-significant, then the population in question is in demographic expansion.

In addition, according to the strong indices of genetic diversity (Hd and Pi), groundnut populations (CsA-2022 and CsA-1998) come from a stable ancestral population with a long evolutionary history. This could be the result of a second contact between previously differentiated *C. serratus* lineages. This origin is also confirmed by the "mismatch distribution" curves (they reflect a multimodal distribution reflecting a population of constant size) and the demogenetic tests of Tajima's D and Fu's Fs (Tajima's D and Fu's Fs are insignificant and indicate a population in demographic equilibrium or in moderate expansion).

Similarly, this origin is validated by the significant demographic indices of the SSD and the Rag except for the non-significant Rag of the 1998 groundnut population (CsA-1998) ; indeed, if the p-values of the SSD and the Rag are significant, then the population under consideration is in demographic stability or at the beginning of demographic expansion.

However, the significantly positive neutrality tests of the R2 of Ramos-Onsins and Rozas invalidate this origin because if R2 is weakly positive and significant, then there is a recent demographic expansion.

Based on these results, we can expect two threats : the beginning of a demographic expansion of insects and the appearance of strains resistant to different control methods, in this case chemical methods. These threats are thought to be favoured by the existence of high genetic diversity within populations (Ndiaye, 2018). In addition, high genetic diversity is correlated with life history traits, such as species characterized by an R strategy (MacArthur and Wilson, 1967; Galtier and Romiguier, 2015), producing many descendants, such as insects. In addition, genetic diversity is positively correlated with population size : the greater the genetic diversity, the larger the population size (Frankham, 1996).

Phylogenetic relationships

The Cyt-B network consists of 19 haplotypes distributed according to the host plants : 11 (H1 to

H11) for groundnuts, 01 (H12) for *Piliostigma reticulatum*, 01 (H13) for *Tamarindus indica*, 05 (H14 to H18) for *Bauhinia rufescens* and 01 (H19) for *Cassia sieberiana*. This allows us to confirm that the structuring of groundnut seed-beetle at the expense of the specific food spectrum has been in force since 1998. In other words, the 5 biotypes reported by Sembène *et al.* (1998) are always specific to a plant or a group of these 5 host plants ; according to Sembène (2000), these biotypes were revealed first by the morphometric method and then by allozyme markers and sequencing of ITS1. In addition, it is known that Caesalpiniaceae hosts of *C. serratus* have seeds that differ in both the form and the chemical composition of the nutritional substances (Robert, 1985), substances that can modify the pheromone composition of insects; which could constitute a prezygotic reproductive barrier.

This would likely result in preferential reproduction and mating would preferably be with partners that have developed in seeds of the same host plant (Sembène, 2000).

In addition, the network reveals that the 19 haplotypes can be divided into three (03) groups : the group of groundnut and *C. sieberiana* haplotypes (group 1: blue and brown discs), the group of *P. reticulatum* and *T. indica* haplotypes (group 2 : green and purple discs) and the group of *B. rufescens* haplotypes (group 3: discs in red). The haplotypes of the 3rd group (*B. rufescens* haplotypes) are intermediate between the haplotypes of groups 1 and 2, but closer to those of group 1. These results therefore lead us to believe that in 2022 there is a visible genetic proximity between seed-beetles dependent on Groundnut and *C. sieberiana* on the one hand and between seed-beetles from *P. reticulatum* and *T. indica* on the other. This proximity, reflecting the existence of gene flow between the seed-beetles infesting the groundnut and *C. sieberiana*, is not in line with that reported by Sembène *et al.* (1998) (the latter revealed in 1998 a proximity between the seed-beetles from the groundnut and *P. reticulatum*). However, this

dissimilarity can be corroborated by the work of Sembène, which has shown since the year 2000 that the seed-beetles subservient to *P. reticulatum* and those infesting groundnut have begun to diverge, although genetically very close. Indeed, thanks to the use of hypervariable markers (microsatellites), Sembène (2000) succeeded in genetically separating the populations of *C. serratus* originating from these two host plants. In addition, the highest mutation rates are known to be associated with microsatellites with a large number of repeats (Weber, 1990).

The highest haplotypic diversity is obtained with the Groundnut, which has 11 haplotypes. Indeed, the majority haplotype (CsA1) contains 22 individuals from the Groundnut and represents 23.66% of the 2022 sequences ; From this majority haplotype derive the others by one to several mutational steps and about ten median vectors. Therefore, the CsC3 haplotype of *C. sieberiana* individuals derives from the majority haplotype (CsA1) by a single mutational step, which further confirms the genetic proximity between groundnut and *C. sieberiana* dependent seed-beetles. Overall, the biotypes do not have haplotypes in common ; they are all characterized by private haplotypes, 05 of which are individual from groundnuts only : these are CsA23, CsA43, CsA46, CsA49 and CsA52. This lack of common haplotypes therefore leads us to affirm that the genetic isolation between the different biotypes of *C. serratus* has become total in 2022; according to Sembène *et al.* (1998), this isolation was partial in 1998.

The phylogenetic trees obtained with the distance method (Neighbor-Joining) and the probabilistic method (Bayesian Inference) are almost identical for Cyt-B. But, compared to the haplotype network, the most resolving phylogenetic reconstruction is obtained with the Bayesian method which has only two (02) non-resolving individuals (CsB6 and CsB11). This method reveals the existence of 3 haplogroups (HG1, HG2 and HG3) that are very solid and significantly supported by a high posterior probability (100%) ; Individuals in haplogroups are grouped according to host plants. Haplogroup 1 (HG1) is

shared and contains two (02) elements of *B. rufescens* (CsB5 and CsB10) and all individuals from Groundnut and *C. sieberiana*. Haplogroup 2 (HG2) is also shared and includes three (03) elements of *B. rufescens* (CsB2, CsB4 and CsB9) and all individuals from *T. indica* and *P. reticulatum*. On the other hand, haplogroup 3 (HG3) is private and isolates only four (04) individuals of *B. rufescens* (CsB1, CsB3, CsB7 and CsB8). Thus, the presence of *B. rufescens* individuals in all three (03) haplogroups can be explained by the fact that *B. rufescens* is a tree that bears fruit all year round.

The haplogroups of this tree therefore reflect visible phylogenetic affinities between individuals from Groundnut and *C. sieberiana* (see HG1) and between individuals from *P. reticulatum* and *T. indica* (see HG2). Individuals of *B. rufescens*, present in all three (03) haplogroups, are more closely related to individuals from Groundnut and *C. sieberiana* than to individuals from *P. reticulatum* and *T. indica* (see HG1 and HG2). These relationships always remind us that in 2022, seed-beetles dependent on Groundnut and *C. sieberiana* are genetically close and the same is true for seed-beetles from *P. reticulatum* and *T. indica*. This proximity also makes us think of a higher number of *C. sieberiana* than that of other wild hosts in the vicinity and within the areas sampled in 2022, which would favor the passage of the insect from other natural hosts to *C. sieberiana* and therefore its navigation between the Groundnut and *C. sieberiana* depending on whether it is in winter or in the dry season. Thus, according to the affinities and genetic proximity reflected in the network and phylogenetic trees, it is clear that the strain dependent on *C. sieberiana* was at the origin of the groundnut infestation in 2022, by allotrophy. In addition, according to Sembène *et al.* (1998), the morphological, allozymic and gene similarities, as well as the importance of the calculated gene flow between the seed-beetles from *P. reticulatum* and those dependent on groundnuts, allow us to affirm that *C. serratus* from *P. reticulatum* were the source of the groundnut infestation in 1998.

Conclusion

This part of the research is oriented towards the study of the evolution and phylogenetic relationships of different populations of *C. serratus* associated with groundnut and wild host plants between the years 1998 and 2022. The results of demographic evolution suggests that, overall, *C. serratus* populations come from a stable ancestral population (in demographic equilibrium) with a long evolutionary history, which could be the result of a second contact between previously differentiated *C. serratus* lineages. The network and phylogenetic trees reveal that in 2022, the seed-beetles dependent on Groundnut and *C. sieberiana* are genetically close and the same is true for the seed-beetles from *P. reticulatum* and *T. indica*. The seed-beetles from *B. rufescens* are more related to those from Groundnut and *C. sieberiana*. These phylogenetic affinities result in gene flow between groundnut-infesting seed-beetles and those from *C. sieberiana*, which allows us to affirm that the strain dependent on *C. sieberiana* was the source of the groundnut infestation in 2022.

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