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Genetic Characterization of *Corcyra cephalonica* Populations based on the Type of Food Substrate (Millet and Rice) and Two Agro-Ecological Zones in Senegal

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ABSTRACT

Millet and rice are two very essential bowls of cereal in the Senegalese daily food. Their storage gets a real problem because of the insects that depreciate those causing huge losses in terms of quantity and quality. The Lepidoptera *C. cephalonica* is one of these pests. The objective of this study is to characterize genetically the populations of *C. Cephalonica* on the millet and rice stocks in the Center of the Groundnut Basin and the Senegal River Delta thanks to the Cytochrome b gene to see if the environment and the food substrate affect the genetic structuration of these populations, (biotype and ecotype aspects). We have sequenced a portion of cytochrome b by the Next Generation Sequencing (NGS) method after extraction and amplification by Polymerase Chain Reaction (PCR). The genetic analyses (variability and genetic diversity indices, the populations' genetic structure, the demographic evolution, and the phylogenetic approach) have revealed a high genetic diversity in the global population with the presence of seventy-three (73) haplotypes for ninety-one (91) sequences. The populations of *C. cephalonica* might issue from a stable ancestral population (haplotypic diversities and strong nucleotide and non-significant Tajima D). The Molecular Variance Analysis (AMOVA) inferences and phylogenetic trees have shown that there is a significant genetic differentiation depending on the environment and the food support between the moth populations.

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INTRODUCTION

Knowledge of the temporal and spatial distribution of the genetic variability in and among the populations is one of the crucial steps in establishing the management strategies for natural populations. The flexible or fitness value of a character, a person, a population or even a species would depend on the genetic variability. It is seen as a guarantee that would allow people to adapt and sustain themselves in their changing environments **[31, 36, 17, 37, 23, 39]**. The characterization of this temporal element by establishing the progressive history of the genetic relations between populations/lineages is done by describing their family ties during their evolution thanks to phylogeny. This allows us to know their historical patterns and to establish hypotheses about the possible changing causes that have generated the dynamics of the observed diversity (spatial element) because the current structuration of the populations results in the past events **[24, 12]**. This would permit a better understanding of the mechanisms of this evolution. Besides, the study of the principles and processes that govern the geographical distribution of these genealogical lines (genetic lines) is the subject of phylogeography **[4, 2]**. The latter incorporates geographic and genetic information to infer the demographic history and progressive processes of species. It is interested in the genetic and demographic phenomena that have led to the current distribution and structuration of populations. From then, it attempts to reconstruct the changing scenarios taking into account the climate, geological and environmental events that have marked the history of the concerned regions. According to **[6]**, (thanks to the recent advances in DNA sequencing techniques), the phylogeographic analysis of molecular variation has become an increasingly important approach to find signs of interaction between the ecological factors, dispersion, and molecular evolution. Lepidopterans are the second largest order after beetles in Insecta and include moths. Most of them are agricultural and forest pests, pollinators, and resource insects **[32, 56]**. *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) belongs to a small subfamily of Galleriinae with 261 species of Pyralidae, which contains more than 330 species of 70 genera **[27, 56]**. The *Corcyra*genusonly contains two species, *C. nidicolella,* and *C. cephalonica*; the latter is known to be a pest of stored products and is controlled with botanical insecticides and trapped with sexual pheromones **[51, 1, 11, 56]**. Besides, it is recently used as an experimental model insect. A group of functional genes has been identified **[38, 8, 13, 25]** but information about the mitochondrial genome is lacking.

According to **[56]**, so far, there are four species within the Pyraloidea with a known mitogenome: *Diatraea saccharalis* **[32]**, *Ostrinia furnacalis*and *O. nubilalis* **[10]**, and *Chilo suppressalis* [unpublished, JF339041]. Thus, to better control the stocks'pests including *C. cephalonica*, a piece of better knowledge on the biology and the genetics of its populations is essential. The moth is found in different storage areas (attic, state stores, producers and traders etc.) located in different agro-ecological areas. In Senegal, the insect attacks a large number of food products, especially cereals and their transformed products. The question is how to realize the demographic evolution of the *C. cephalonica* populations and what effect would millet and rice grains and agro-ecological zones have on the genetic structuration of this insect? To answer this question, populations' genetics approaches have been applied and molecular markers have been used to characterize the populations of *C. cephalonica* according to the type of food substrate and two agro-ecological zones in Senegal, to establish whether there is a genetic structuration between these grains on the one hand, and between agro-ecological zones, on the other.

MATERIALS AND METHODS

Samples

Sampling took place in the storage sites in two agro-ecological zones in Senegal. The millet and rice samples were collected from the Center of the Groundnut Basin at a seed packing station located at 14° 39' 4.5" N; 16°15' 19.36" W belongs to the Sahelian-Sudanian Zone (SAS) and in the Senegal River Delta, at local producers and traders in Ross Bethio in Dagana department with the following geographical coordinates 16° 16' 41.95" N; 16 °8' 38.55" W belongs to the Sahelian zone. The sampling method used here is the same regardless of wherever you store. It is to collect randomly five kilograms (5 kg) of millet and rice grains from each storage location. The samples are then packaged in plastic bags and brought back to the laboratory. A mass breeding has been set up. It was carried out in the laboratory using the millet and rice samples. The harvested cereals were put in farmed jars placed in a room with controlled environments until the emergence of adults. These were collected using a test tube and stored in alcohol at 90 °C.

The samples were coded according to three criteria: the studied species, the type of food substrate, and the geographical origin; using in capital letters, the first letter of the food substrate,

the first letter of the insect's genus name, and the two last letters of the abbreviated word of the agro-ecological zone (see Table 1).

Table No. 1: The list of millet and rice samples, infested with *C. cephalonica*, by agro-ecological zone and associated acronyms.

A.E.Z	Geographical coordinates	FS	Codes	TSI	NIS
(CGB)	14° 39’ 4,50’’ N ; 16° 15’ 19,36’’ W	Mil	MCGB	States stores	22
		Riz	RCGB		26
(SRD)	16° 16’ 41,95’’ N ; 16° 8’ 38,55’’ W	Mil	MCSR	Producers stores	19
		Riz	RCSR		24

Caption: A.E.Z = Agro-Ecological Zone; CGB = Centre of the Groundnut Basin; SRD = Senegal River Delta; MCGB = Mil Corcyra of the centre of the Groundnut Basin; MCSR = Mil *Corcyra* of the Senegal River; RCGB =*Corcyra* Rice from the centre of the Groundnut Basin; RCSR = *Corcyra* Rice from the Senegal River; TSI = Type of Storage Infrastructure; NIS = Number of Individuals Sampled; FS = Food Substrate.

Choosing the gene cytochrome b (Cyt-b)

Cytochrome b is a region of mitochondrial DNA that is widely used for its particular characteristics. Choosing it as a marker for the genetic characterization of *C. cephalonica* populations on millet and rice is not fortuitous. Mitochondrial DNA is present in multiple copies in the cytoplasm of the cell but in a haploid form different from nuclear DNA **[21]**. Its reproduction does not occur in homologous recombination and then mtDNA is a marker particularly suitable for studies of genetic parentage in animals. Due to a relatively high mutation rate, mtDNA has been widely used as a phylogenetic marker. Cytochrome b is used to determine phylogenetic relationships due to its low sequence variability. In theory, the effective reproductive population for cytochrome b is low; this makes it more sensitive to stresses such as bottlenecks, genetic drift, and the founding effect of swarming **[15]**. It is easier to align between species because it has no gaps and easier to use to estimate the divergence of dates between taxa.

Genetic study

Extraction of *C. cephalonica* Genomic DNA

Genetic characterization of the rice borer cannot be done without firstly dissecting it and circumspectly extracting its genomic DNA. At first, *C.cephalonica* is dissected and its abdomen is eliminated. Its head, chest and legs are only used in extraction. These elements are crushed and placed in 1.5ml tubes. The extraction was done using the DNeasy Tissue kit (Qiagen Inc.) and its standard DNA extraction protocol done in four steps:

– The digestion stage during which 180 L of ATL digestion pad and 20el of K protease have put the tube containing the grinder to break the bonds between the tissues and individualize the cells after incubation at 55 °C for 3 hours. The mixture, after incubation, is centrifuged to 13000 rpm (rotation per minute) for one minute. The tissues debris is then removed and the supernatant is recovered in another tube.

– The stage of cell lysis consists of breaking the nuclear, cytoplasmic membrane and that of other cellular organelles for the DNA release. To do so, 200 L of AL cell lysis buffer are added to the previously collected supernatant. This solution is then vortexed and incubated at a 70-degree marie bath for 10 minutes.

– The purification stage that results in the formation of a precipitate isolating DNA molecules from contaminants (proteins, membranes, tampons, etc.). 200 l of ethanol 96-100% are added followed by a slight agitation of the mixture for a short time resulting in the formation of a nerve at the bottom of each tube and the release of a liquid remaining. Which liquid was collected and simultaneously placed in a 2ml silica column collector tube bearing the sample code. This tube is then centrifuged to 13000 rpm for one minute. After centrifugation, under ion interaction, the negatively charged DNA molecules are retained at the level of the positively charged silica membrane and the rest of this solution (proteins, lipids, polysaccharides) passes through the membrane and precipitates to the bottom of the collecting tube that is discarded. Finally, each column is placed in a new 1.5ml collection tube. Purifying grooming of DNA is followed by the use of two AW1 and AW2 washing pads. First, 500µlof AW1 is poured into the column and the resulting mixture is centrifuged to 13,000 rpm for one minute. After centrifugation, 500 µl of AW2 are secondly added and the mixture is centrifuged to 13000 rpm for three minutes. During

this last centrifuge, the two pads precipitate with contaminants at the bottom of the collector tubes that are discarded.

– The DNA elucidation stage during which the columns containing purified DNA are placed on other 1.5ml tubes. 50µl of the AE pad (provided by the Qiagen kit) previously incubated at 70 degrees are directly poured onto the silica membrane to pick up the silica's DNA. Incubation of the AE buffer at 70 °C increases the extraction yield by 15 to 20%. The DNA, once recovered, is stored at -20 °C.

Cytochrome b PCR

PCR is an *in vitro*amplification of a gene or a specific fragment DNA using a thermocycler. The amplification was carried out in a reaction volume of 25 µl containing 18.3 µl of water, 2.5 µl of 10x buffer, 1 µl of $MgCl_2$ additional, 0.5µl of dNTP, 0.25 µl of each primer, 0.2 µl of Taqpolymerase and 2 µl of DNA extract. It is a repetition of cycles that ensures a 2-fold multiplication of the target DNA in each cycle. It is a process that consists of three steps: initial denaturation at 94°C for three minutes, followed by 35 cycles of denaturation at 94 °C for one minute; hybridization at 47 °C for one minute characterized by the hooking of the primers and finally elongation at 72 degrees for 10 minutes. These steps thus listed constitute a cycle and ensure an x2 multiplication of the target DNA segment (the Cyt-b gene). The PCR technique may be based on the repetition of the same cycle several times: 35 cycles in this exercise, i.e. amplification of the gene 235 times. The primers CB1 (5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3') and CB2 (5'-ATT ACA CCT AAT TTA GGA AT-3') were used to amplify the cytochrome b gene.

Sequencing of the cytochrome gene b according to NGS

The purification of PCR products and the sequencing of the cytochrome b gene were performed with NGS. The sequencing was performed at National Institute of Agronomic Research (NIAR) with the NGS Illumina HiSeq X sequencer using the synthetic sequencing method. It consists of determining the nucleotide succession of a DNA fragment. Indeed, sequencing reveals point mutations by comparing the sequences of the same gene in different individuals. Illumina's NGS technologies inclue three common steps:

– the preparation of bookstores which consists of creating banks using random fragmentation of DNA followed by linking with small specific sequences. These banks are then amplified by using clonal amplification and PCR methods to enrich the library of fragments and increase the amount of DNA before sequencing,

– sequencing reaction cycles,

– sequencing which consists of taking an image after each of these cycles to determine the corresponding nucleotide.

Genetic Analysis

Genetic variability

Influenced by progressive forces such as mutations, natural selection, genetic drift and migration, ecological forces such as reproductive system, sex ratio, demography, geographic distance, etc., or bioclimatic and anthropogenic factors, genetic diversity is born and continues to evolve. Thus, through Cyt-b, different parameters were determined to explore genetic variability in this study. These are the number of Informative Polymorphic Sites (IPS), the number of Singleton Sites (SS), the average number of nucleotide differences (K), the number of Total Haplotypes (TH), and the number of single haplotype (SH). These parameters were released first on the basis of intra- and inter-middle food support and then based on the agro-ecological zone for a better comparative view of genetic diversity from one population to another. At first glance, the dataset was blasted to the Genbank database to see if our sequences matched those of the species we were supposed to be working on. The sequences were then manually cleaned and aligned through the Clustal-W algorithm and then carefully corrected with BioEdit 5.0.6 **[26]** software.

Genetic Diversity Indices: Pi and Hd and their standard deviations

These two parameters go together to inform us about the evolution of the population from which our samples are derived. By operating Pi and Hd, we will know whether it is an adaptive convergence or adaptive radiation. Pi or nucleotide diversity defines the probability that two randomly selected homologous nucleotide sites are different **[41]**. Below 10%, nucleotide diversity is low and zero at 0, while at more than 10% the divergence is deep **[42]**. Hd or haplotypic diversity defines the probability that two randomly selected haplotypes in a sample

are similar **[41]**. Unlike Pi, at 100, haplotypic diversity is zero and no divergence. The Pi and Hd were determined using the DnaSP v5.10.01 **[33, 45]** software. These two parameters were combined to inform us about the history of the populations originating from our samples as a prelude to analyses of demographic evolution.

– Low Hd (<50%) and low Pi (<50%), we expect an ancestral population that has drastically declined resulting in a recent bottleneck with a founding effect;

– In strong Hd (>50%) and strong Pi (>0.5%), the source population is stable with a long progressive history; this may be the result of a second contact between previously differentiated lineages.

– In strong Hd (>50%) and low Pi (<0,5%), the source population has undergone bottleneck followed by rapid population growth and an accumulation of mutations.

– In low Hd (<0,5%) and strong Pi (>0.5%), the source population is vast and has undergone an ephemeral bottleneck with a difference between geographically subdivided samples (relief aspect, climate, etc.) **[22]**.

Genetic structure of populations: genetic differentiation and molecular variance

For the genetic differentiation, only Nei's genetic distance D **[41]** has been determined to the detriment of F_{ST} because it is more suitable for loci than for sequences. Despite its limitations, we cannot do without the F_{ST} in AMOVA. Nei's genetic distance D **[41]** is the method used because of the high rate of mutations in Cyt-b. It was determined to measure the degree of parentage that exists between individuals based on the differences between homologous nucleotide sequences. Its intra- and inter-populations values, based on intra- and inter-middle-to-middle food support, and on the environment, were generated using MEGA v7.0.14 **[50]** with the Kimura-2-P model to take into account the non-equiprobability of transitions and transversions of mitochondrial DNA. The effect of distance on the genetic structuration of *C. cephalonica* populations was tested by performing a regression to examine the Isolation By Distance or IBD **[54]**. This regression was achieved using the R v1.1.447 software.

Genetic structuration was also evaluated by a Molecular Variance Analysis **[19]** to detect the source of variance at the molecular level. AMOVA estimates genetic structure indices using the information on the allelic content of haplotypes and their frequencies **[19]**.

This molecular variance between the different sources of variation was computed through the ARLEQUIN v3.1 **[18]** program. The fixation index F_{ST} that expresses a decrease in heterozygous related to the divergence between the subpopulation and the total population was determined. F_{ST} - (TH - SH) / TH. This index is therefore used as an index of genetic differentiation between subpopulations **[55, 52]**. If the F_{ST} is equal or very close to 0, it means that there are many genetic exchanges between populations (little genetic differentiation, panmictic population). Conversely, if the F_{ST} is close to 1, this results in a strong genetic differentiation between populations, suggesting very little or no gene flow between populations. According to **[53]**, an F_{ST} between 0 and 0.05 reveals low differentiation; a F_{ST} between 0.05 and 0.15 reflects moderate differentiation; an F_{ST} between 0.15 and 0.25 suggests a significant differentiation, and beyond 0.25, the F_{ST} illustrates a very important one.

Demographic Evolution

To trace the demographic history, first, demogenetic tests were carried out using the D statistics of Tajima **[49]** and Fs of Fu **[20]** using the software ARLEQUIN v3.1 **[18]**. The aim of these tests is to distinguish sequences that evolve according to a neutral progressive model from those that evolve according to a deterministic process.

The values of these two significantly positive or negative statistics reflect, respectively, a population suffering from a bottleneck and a population that is rapidly expanding, while in the case of non-significance, neutral mutations accumulate, translating a population into demographic balance. Finally, zero Tajima D and Fu F_S values suggest a population of constant size. As a complement, R^2 by Ramos-Onsins and Rosas **[43]** was computed with the ARLEQUIN v3.1 **[18]** program. This is a statistic based on the difference between the singleton number and the average number of nucleotide differences. We then analyzed the Pairwise difference (Mismatch distribution), which is the graphic representation of the distribution of genetic distances existing between individuals of a population taken two to two. Mismatch's analysis combines two indices that test the quality of distribution adjustment. These indices are

the SSD (the sum of squares of deviations) and the Rag (irregularity index). Mismatch curves were obtained by DnaSP v5.10.01 software **[33, 45]** and the SSD and Rag indices were executed with the ARLEQUIN v3.1 **[18]** program. A multimodal Mismatch curve reflects a stable population and when the curve is unimodal the population is expanding population **[44]**.

Phylogenetic Approach

The phylogenetic approach provides information on the kinship relationships between different populations of *C. cephalonica*. First, to explore phylogenetic relationships, a network of haplotypes was built using the Network v5.0.0 **[5]** program to look for associations between haplotypes. Then, for phylogenetic trees, we chose the distance method of Neighbor-Joining. The distance-based tree was then constructed using the MEGA 5 software **[50]** using Kimura-2-P as a model that assumes that transitions and transversions are not equiprobable during the evolution of sequences, especially for mitochondrial DNA where transitions can account for 90% of mutations **[30, 39]** and the bootstrapping method has been calibrated to 1000 replications. Moreover, 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 method of maximum likelihood does not evaluate all possible trees because they are numerous; the likelihood then gives only a partial exploration of all the trees made. The smallest Akaike Information Criterion (AIC) was used to retain the best progressive model; the substitution matrix GTR+G (Gamma Time Reversible + G): if the substitution rate often changes from one site to another within a sequence, a G law and if the sites remain invariable, a Law I.This AIC index is determined by the software Jmodeltest v2.1.7 **[14]** for Bayesian inference. The Bayesian tree is built by MrBayes software **[28]**. The distribution of later tree probabilities was estimated by 4 MCMC chains (three of which were gradually "heated" and one cold). 1,100,000 generations were realized for each of the chains by sampling the different parameters every 1,000 generations and the ignition period can be determined by examining, during the "cold" chain, the evolution of the likelihood function because the generations made during the ignition period are eliminated from the analyses. So, conservatively, the first 275,000 generations were eliminated (25%) and the inferences are then made over the next 825,000 generations. The two (02) constructions were all rooted in a consensus sequence of the species *Sitotroga cerealella* as an outgroup.

RESULTS

Genetic variability of Cyt-b

Animal mitogenomes are generally closed circular molecules 14 to 20 kb in length with 37 genes, 13 protein-coding genes (PCG), 22 transfer RNAs (tRNA) and two ribosomal RNA (rRNA). It also contains a non-codingrich area in A-T (also known as the control region) responsible for regulating the transcription and the mitogenome replication **[7, 48]**. *Corcyra* mitogenoma is a circular molecule of 15,273 bps in length; data was uploaded to Genbank (HQ897685). *Corcyra* mitogenoma showed standard genetic supplement containing 13 PCG (protein-coding gene), 2 rRNA, 22 tRNA and non-coding typical regions for lepidopterans **[56]**. The cytochrome b gene has a length of 1143 nucleotides, located between 10489 and 11631 bp of the *C. cephalonica* mitogenome (HQ897685.1 Position: 10,956; Gene position: 468; Genbank). The region of cytochrome b consisting of 730 bps was sequenced. Ninety-one (91) Cyt-b sequences from four populations are the data and to be analyzed. Each sequence has 730 base pairs (bp). In the overall population, there are 73 haplotypes, 652 (89.32%) invariable sites, 78 (10.68%) polymorphic sites including 57 (73%) information sites in parsimony, and 21 (27%) singletons sites. The number of nucleotide differences is high depending on the food substrate but does not show a significant difference between millet and rice. On the other hand, depending on the environment, this K number is small but reveals a clear difference between the populations of the CGB and SRD moths. All SRD haplotypes are unique (Table 2). The singleton variable sites (two variants) (21) are located in position 29, 49, 100, 145, 156, 234, 244, 275, 281, 341, 349, 403, 444, 527, 535, 593, 623, 636, 654, 702 and 724 bp. Informational parsimonious sites (two variants) (numbering 54) in positions 18, 21, 24, 43, 53, 85, 90, 93, 94, 96, 98, 99, 131, 135, 146, 183, 197, 216, 232, 233, 235, 269, 305, 307, 321, 334, 338, 357, 372, 373, 377, 407, 428, 430, 443, 445, 468, 469, 490, 491, 531, 542, 559, 581, 604, 624, 634, 637, 655 674, 678, 681, 683 and 713 bp. Variable singleton sites (three variants) (0) and informational parsimonious sites (three variants) (number 3) in positions 91, 95, and 582 of cytochrome b.

Table No. 2: Genetic polymorphism of Cyt-b depending on food support and environment.

Settings	CGB		SRD		MIL	RIZ	CGB	SRD	Total
	Mil	Riz	Mil	Riz					
Effective	22	26	19	24	41	50	48	43	91
IPS	4	7	15	21	45	49	13	28	57
SS	17	15	6	10	16	18	23	5	21
K	2,385	2,834	5,298	6,403	20,807	21,34	2,702	6,003	20,982
TH	12	19	19	24	31	42	31	43	73
UH	10	16	19	24	29	39	26	43	68

Caption: IPS: informative polymorphic sites, SS: singleton sites, K: number of nucleotide differences, TH: total number of haplotypes, UH: unique haplotypes, CGB: the center of the groundnut basin, SRD: Senegal River Delta.

– The H1 haplotype contains 14 individuals, H21: 4 individuals, H3 and H15: 2 individuals each on, H2, H5, H6, H7, H8, H9, H10, H11, H13, H14, H16, H17, H18, H19, H20, and H22 to H73 each contains an individual (see Table 3). Five groups of haplotypes are obtained: Haplotypes H13 to H30 are only found in individuals raised in rice and originating from the center of the groundnut basin (RCGB); H2 to H12: made up of moth from the millet of the center of the groundnut basin (MCGB); H21 to H49: observed in insects emerging from the millet of the Senegal River Delta (MSRD); H50 to H73: found in insects that come from the rice of the river delta; H1: observed on both cereals and only in the centre of the groundnut basin is the majority haplotype.

Table No. 3:The haplotypes, the individuals who make them up and their number

Haplotype	Individual	Number	Haplotype	Individual	Number
H1	[MCGB1 MCGB2 MCGB3 MCGB8 MCGB11 MCGB13 MCGB16 MCGB19 MCGB20 MCGB21 RCGB11 RCGB21 RCGB23 RCGB25]	14	H39	[MCSR9]	1
H2	[MCGB4]	1	H40	[MCSR10]	1
H3	[MCGB5 MCGB9]	2	H41	[MCSR11]	1
H4	[MCGB6]	1	H42	[MCSR12]	1
H5	[MCGB7]	1	H43	[MCSR13]	1
H6	[MCGB10]	1	H44	[MCSR14]	1
H7	[MCGB12]	1	H45	[MCSR15]	1
H8	[MCGB14]	1	H46	[MCSR16]	1
H9	[MCGB15]	1	H47	[MCSR17]	1
H10	[MCGB17]	1	H48	[MCSR18]	1
H11	[MCGB18]	1	H49	[MCSR19]	1
H12	[MCGB22]	1	H50	[RCSR1]	1
H13	[RCGB1]	1	H51	[RCSR2]	1
H14	[RCGB2]	1	H52	[RCSR3]	1
H15	[RCGB3 RCGB26]	2	H53	[RCSR4]	1
H16	[RCGB4]	1	H54	[RCSR5]	1
H17	[RCGB5]	1	H55	[RCSR6]	1
H18	[RCGB6]	1	H56	[RCSR7]	1
H19	[RCGB7]	1	H57	[RCSR8]	1
H20	[RCGB8]	1	H58	[RCSR9]	1
H21	[RCGB9 RCGB19 RGCB22 RCGB24]	4	H59	[RCSR10]	1
H22	[RCGB10]	1	H60	[RCSR11]	1
H23	[RCGB12]	1	H61	[RCSR12]	1
H24	[RCGB13]	1	H62	[RCSR13]	1
H25	[RCGB14]	1	H63	[RCSR14]	1
H26	[RCGB15]	1	H64	[RCSR15]	1
H27	[RCGB16]	1	H65	[RCSR16]	1

H28	[RCGB17]	1	H66	[RCSR17]	1
H29	[RCGB18]	1	H67	[RCSR17]	1
H30	[RCGB20]	1	H68	[RCSR18]	1
H31	[MCSR1]	1	H69	[RCSR19]	1
H32	[MCSR2]	1	H70	[RCSR20]	1
H33	[MCSR3]	1	H71	[RCSR21]	1
H34	[MCSR4]	1	H72	[RCSR22]	1
H35	[MCSR5]	1	H73	[RCSR23]	1
H36	[MCSR6]	1			
H37	[MCSR7]	1			
H38	[MCSR8]	1			

Genetic diversity Indices: Hd, Pi, and their standard deviations

These two indices are first determined in each of the four original populations, then in populations based on food support and environment, and finally in the overall population. Cyt-b shows strong haplotypic diversity (Hd>50%) and relatively low nucleotide diversity (Pi<0,5%) original populations: MCGB, RCGB and in the population function of the environment: CGB. On the other hand, the cyt-b gene reveals a strong haplotypic diversity (Hd>50%) and strong nucleotide diversity (Pi>0.5%) in the original MCSR and RCSR populations, the overall population, in the environmental function population (SRD) and in both populations based on the food substrate (Table 4).

Table No. 4: Standard Genetic Diversity Indexes: Hd and Pi.

Populations	Hd	Pi
MCGB	0,801 +/- 0,00773	0,00327 +/- 0.00206
RCGB	0,960 +/- 00062	0,00388 +/- 0.00235
MCSR	1,000 +/- 0,00029	0,00726 +/- 0.00409
RCSR	1,000 +/- 0,00016	0,00877 +/- 0.00480
Mil	0,944 +/- 0,00092	0,0285 +/- 0.01426
Riz	0,989 +/- 0,00007	0,02923 +/- 0.01458
CGB	0,912 +/- 0;00126	0,0037 +/- 0.00222
SRD	1 +/- 0,00003	0,00822 +/- 0.00443
Total	0,976 +/- 0,00012	0,02874 +/- 0.01418

Population's genetic structure: genetic differentiation and molecular variance:

Nei's Genetic Distance

Nei's values of D reveal that genetic distance is low between the original populations of the same medium although they come from different food substrates: MCGB and RCGB (0.00379 ± 0.0008), MCSR, and RCSR (0.00834 ± 0.00161). However, this genetic distance is significant between populations of different environments: CGB and SRD (0.05329 ± 0.00774). It is not as negligible among populations of different food substrates as a whole (Table 5).

Table No. 5: Genetic distance D of Nei intra and inter-middle.

	RCGB		RCSR
MCGB	0,00379 ± 0,0008	MCSR	0,00834 ± 0,00161
	MIL		SRD
RIZ	0,02942 ± 0,00408	CGB	0,05329 ± 0,00774

The regression between genetic distance and geographic distance shows a significantly high R^2 coefficient of determination. The R^2 reflects more than 99% of the variance in the genetic distance that disappears when the geographic distance is fixed. The original order is negatively significant, while the slope is positively significant. Thus the hypothesis zero (H0) stipulating the two dies have no link must be rejected and the alternative hypothesis H1 is accepted. The genetic distance of different populations of *C. cephalonica* is then correlated with geographic distance (Table 6).

Table No. 6: Regression between genetic distance and geographic distance.

Coefficient of determination	R^2	0,9949
	p-value	9,96E-05
Y-intercept	β0	-23,462
	p-value	0,0279
Slope of the right	β1	3958,57
	p-value	9,96E-05

Model Equation: Genetic Distance = -23,462 - 3958.57 Geographical Distance

Molecular Variance (AMOVA)

Molecular variance analysis focuses on three criteria (environment, food support and population). Taking into account the agro-ecological zone and food support, 4 groups were defined: the group of populations the Center of the Groundnut Basin (CGB), the population group of the Senegal River Delta (SRD), the group of populations of millet and the group of populations of rice. The analysis reveals that the source of molecular variance is mainly due to genetic diversity between individuals in the same population that represents 95 to 98% of the total variance. However, genetic diversity between groups, despite its lowness, is significant and accumulates 4.41% of the total variation for the first two groups (CGB and SRD) and 1.4% for the other two (mil and rice) (Table 7).

Table No. 7: Source and percentage of molecular variance.

Source of variation	Percentage of variation	Fixation indices
Molecular variation betweenarea		
Between area	4,41	F_{ST}=0,04409*** (P-value = 0,0000)
Within an area	95,59	
Molecular variation between food medium		
Between food support	1,4	F_{ST}=0,01398** (P-value = 0,01931)
Within a food support	98,6	
Molecular variation between populations		
Between populations	4,75	F_{ST}=0,04747*** (P-value = 0,0000)
Within a population	95,25	

Demographic Evolution

Analysis of mismatch distribution curves under the assumption of an expanding population gives a bimodal look for the overall population and that of the CGB, although the SSDs are insignificant. The mismatch distribution curve for the Population of the SRD showed a unimodal distribution (Figure 1). Considering the overall population, the value of Tajima's demogenetic test D is positive and not significant and that of the F_S test of $F_U$is negative and significant; R^2, however, is not significantly positive and finally the SSD and Rag demographic indices are

insignificant (Table 8). Yet, these demogenetic values (Tajima D and F_U F_S) are negative and significant for the centre of the groundnut basin with insignificant SSD and Rag indices and a non-significant positive R^2 value. For the SRD, these demogenetic tests reveal negative but only significant values with F_U F_S. Its SSD and Rag indices are insignificant and finally its R^2 value is positive and not significant (Table 8).

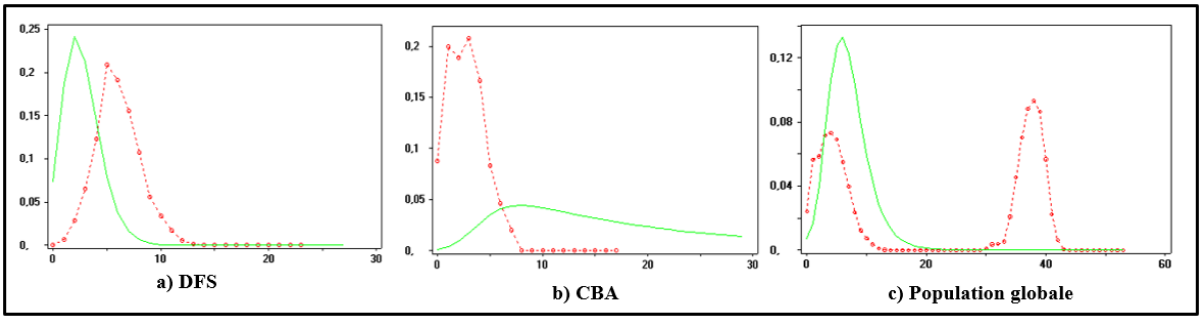


Figure No. 1: Mismatch distributions per pair of individuals from the global population (c), the CGB population (b) and the SRD population (a).

Table No. 8: Demogenetic tests of neutrality and demographic statistics of the past.

	CGB	SRD	Total
Tajima's test D			
Tajima's D	-2,2655	-0,73262	1,20796
P-value	0,002***	0,257	0,922
F_U's test F_S			
F_U'sF_S	-26,54885	-25,27605	-23,99518
P-value	0***	0***	0,001***
SSD Index			
SSD	0,00216	0,00496	0,04005
P-value	0,68	0,126	0,325
Rag index			
Rag	0,02401	0,02017	0,00631
P-value	0,757	0,101	0,652
R^2	0,09283	0,09472	0,09484
P-value	0,923	0,891	0,895

Phylogenetic Approach

Haplotypical Network

This network (Figure 2) reveals that haplotypes are distributed according to the agro-ecological zone; all individuals in the CGB form a group on the one side (blue box) and those of the SRD a group on the other side (red box). RCSR10 is the transition haplotype and *C. cephalonica* first infested the SRD; the CGB was not infested until later.

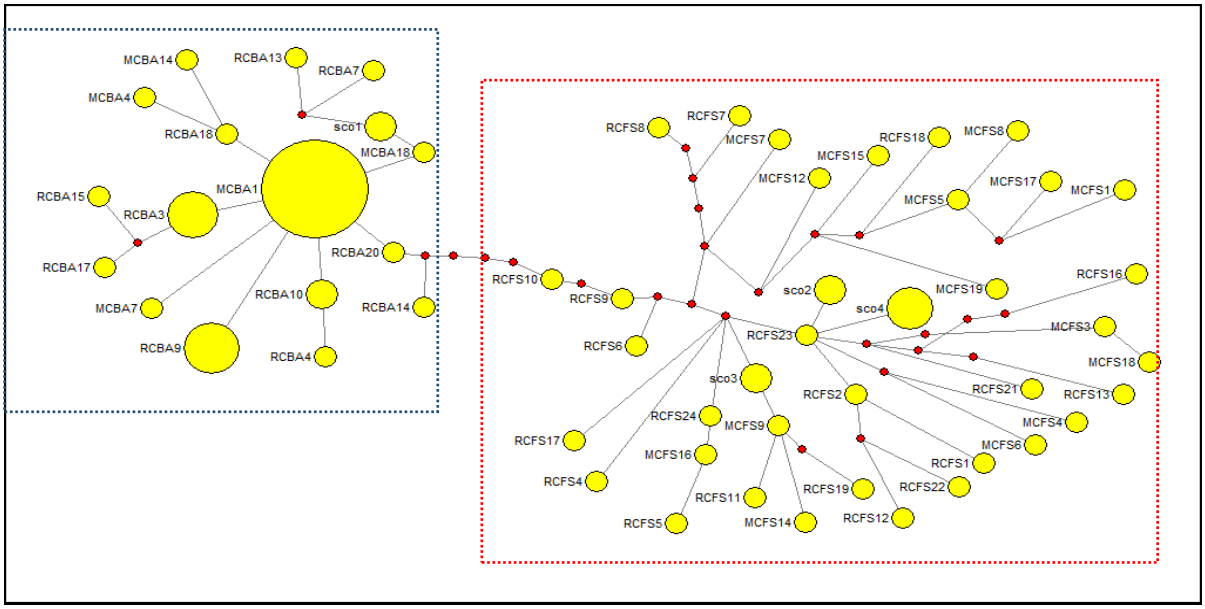


Figure No. 2: Haplotypic network of C. cephalonica populations

Phylogenetic trees of haplotypes

The topologies of the *C. cephalonica* phylogenetic trees obtained are similar between the Neighbor-Joining method and the Bayesian one. These two methods revealed phylogenetic trees consisting of two distinct clades and supported significantly by a strong bootstrap (99%) for Neighbor-Joining (Figure 4) and a strong posterior probability (100%) Bayesian inference (Figure 3). The first clade isolates all individuals from the CGB and constitutes the HG1 haplogroup and the other clade groups all the individuals of the SRD into the HG2 haplogroup; this corroborates the existing correlation between genetic and geographic distances and the pattern of the haplotypic network (Figure 2); this corroborates the existing correlation between

genetic and geographic distances and the pattern of the haplotypic network.The trees were rooted with *Sitotroga cerealella.*

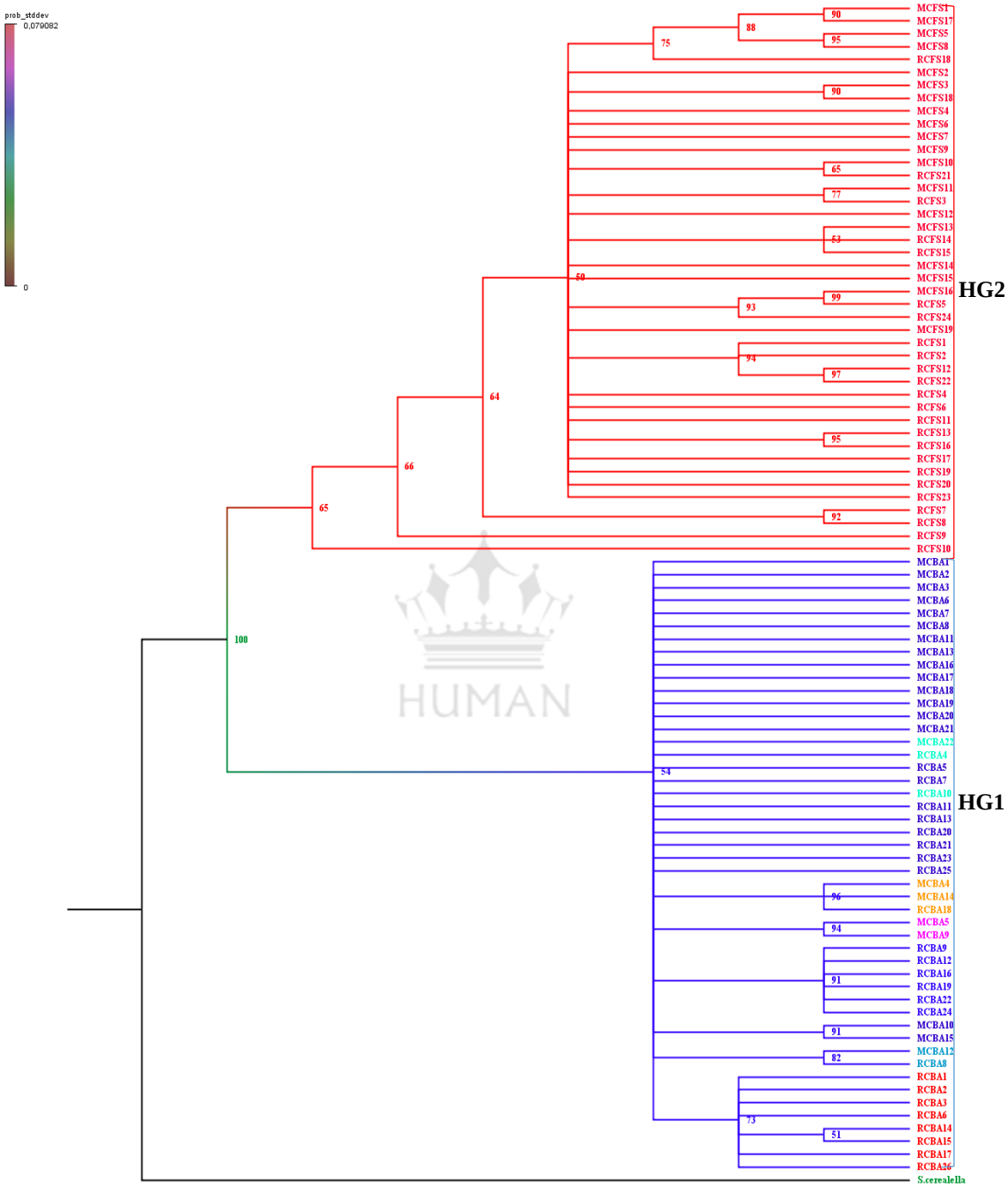


Figure No. 3: *C. cephalonica* phylogenetic tree by Bayesian inference depending on the agro-ecological zone.

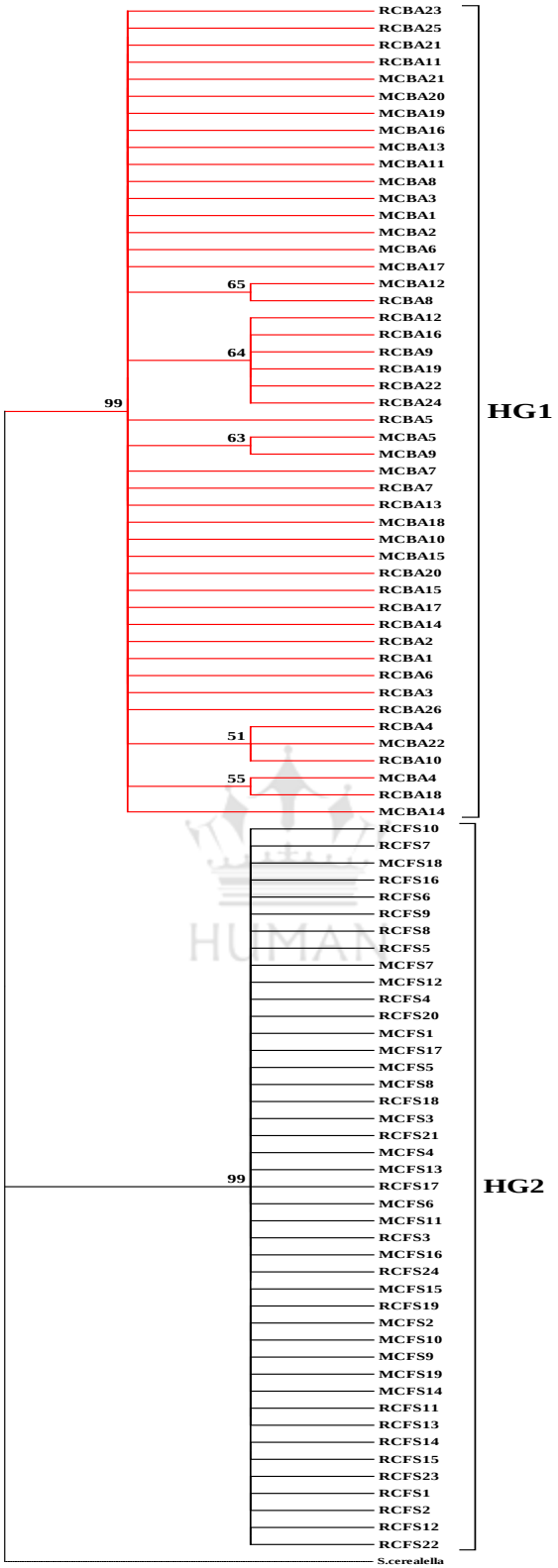
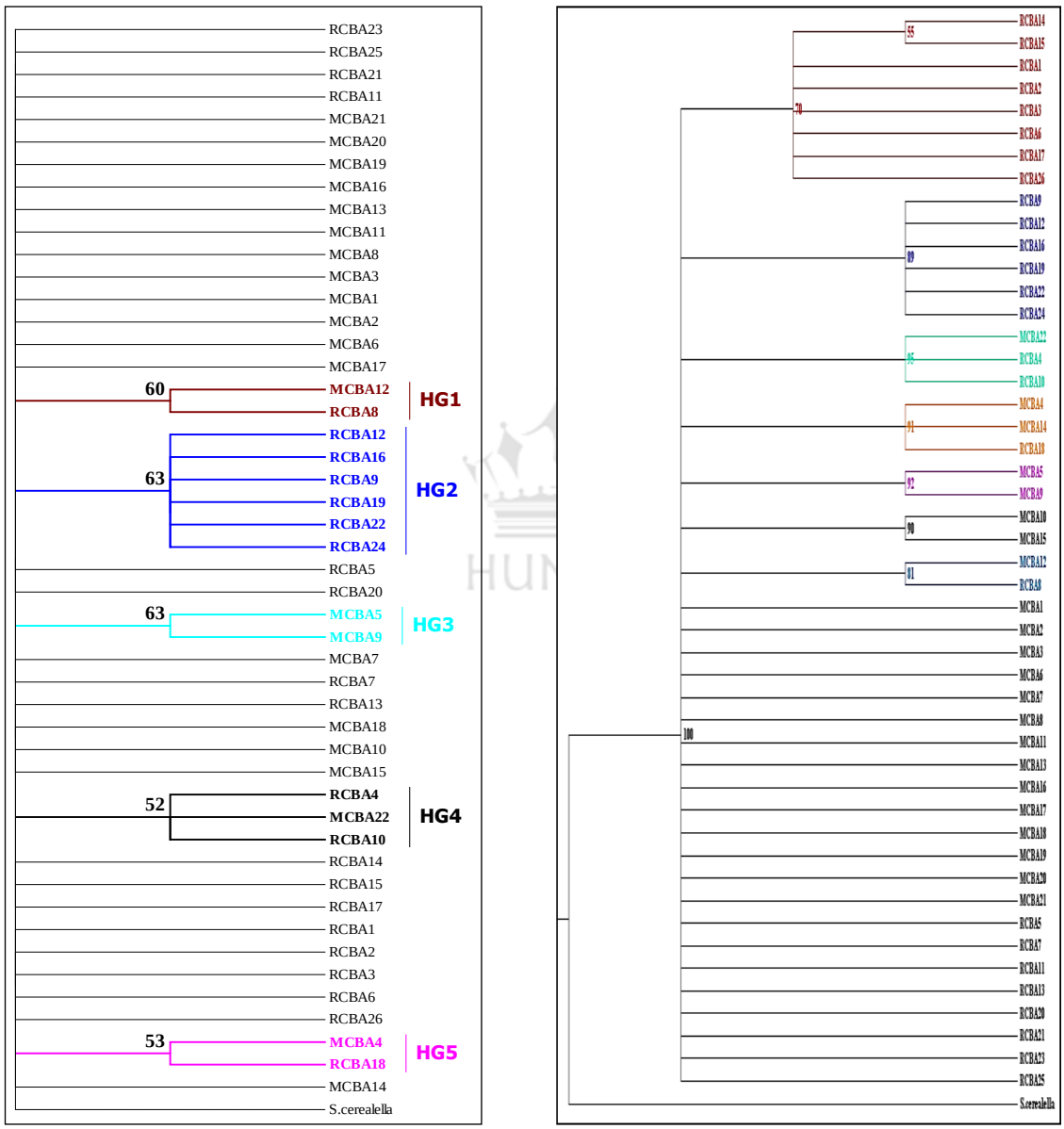


Figure No. 4: *C. cephalonica* phylogenetic trees according to the agro-ecological zone by the Neighbor-Joining method.

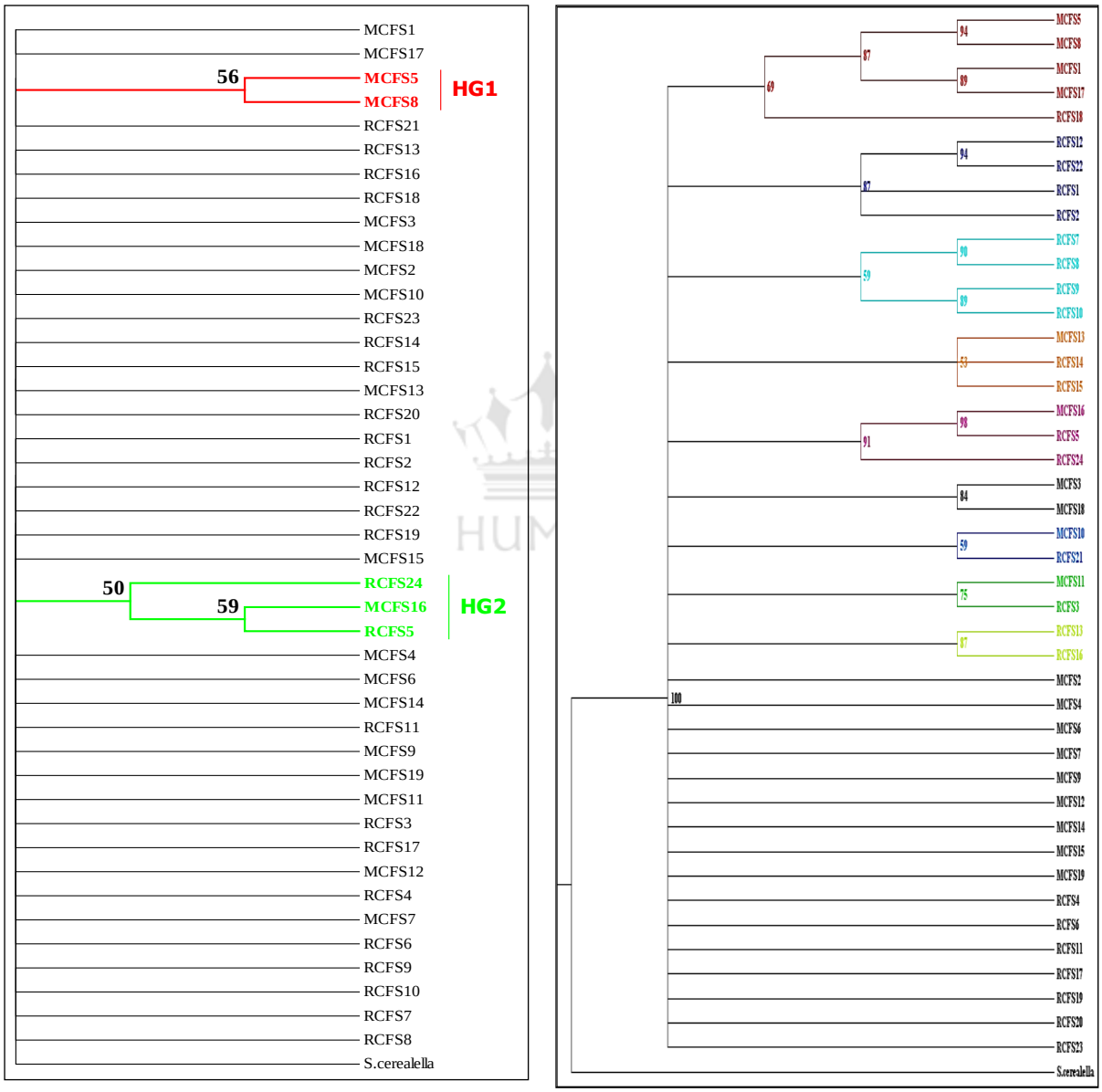
In the CGB, the phylogenetic trees, for the two methods used, consist of a single clade. This clade, for each method, consists of sub-clades that showed some phylogenetic affinities between individuals of different food support and between individuals of the same food support. These sub-clades are supported by medium bootstraps (50-63%) for the Neighbor-Joining method (Figure 5A) and by high Bayesian supports (78-95%) for Bayesian inference (Figure 5B). Individuals from rice are more related.



A : Neighbor-Joining method. B : Bayesian inference

Figure 5: *C. cephalonica* phylogenetic trees based on the food substrate in the CGB.

In the DFS, *C. cephalonica*'s two phylogenetic trees are each made up of a single clade. The latter consists of sub-clades supported by medium bootstraps (50 to 56%) for the Neighbor-Joining method (Figure 6A) and sub-clades supported by medium to high posterior probabilities (53-91%) Bayesian inference (Figure 6B). The Bayesian method showed more phylogenetic links that are of two types: phylogenetic affinities between individuals of the same food substrate and affinities between individuals of different food substrates.



A: by Neighbor-Joining method.

B: by Bayesian inference (BI).

Figure No. 6: *C. cephalonica* phylogenetic tree based on food substrate in the SRD.

DISCUSSION

The objective of this research was to study the genetic structuration of *C. cephalonica* populations on millet and rice stocks in two agro-ecologically different areas of Senegal: the CGB and the SRD to see if there is a biotype and ecotype aspect between these populations. This would probably allow the development of different methods of control between zone on the one hand and between food substrates on the other hand against *C. cephalonica*. Ninety-one (91) sequences of the Mitochondrial gene Cyt-b constitute the data and the nucleotide changes made over all of these sequences that have detected seventy-three (73) haplotypes of which sixty-eight (68) are individual; which shows enormous genetic diversity for a sample of only 91 sequences.This high number of haplotypes could be explained by the important length of sequences (730bp) obtained, for the first time, with NGS technology. According to **[4]**, several factors probably contribute to the rate of rapid change in mtDNA. The high number of haplotypes is certainly surprising compared to the 18 haplotypes of **[46]** for 78 individuals or 9 haplotypes of **[16]** for 60 individuals but it may be due to the length of the sequences that almost split that of the two mentioned studies above. In the SRD, the high number of unique haplotypes could be explained by the fact that this area borders Mauritania and imports millet to other regions of the country; that is to say there is a lot of trade that brings new haplotypes.

In addition, this environment is experiencing a high production of rice which is exported to other parts of the country, which would mean that rice is not infested with haplotypes from other agro-ecological areas. The number of nucleotide differences K shows that there is more homogeneity although it is less in the CGB (K=2,702) than in the SRD (K=6,003). These K values are close to those of **[40]** between the Sahel-Sudanese and Guinea-Congolese populations of *Sitophilus zeamaïs*. From the centre to the north of the country, in agro-ecological zones, there are different climate factors that could explain the genetic differentiation observed between zones. The CGB has a strong haplotypic diversity and low nucleotide diversity, so its populations would issue from an ancestral population that has undergone a bottleneck followed by a rapid population growth with an accumulation of mutations. However, in the SRD, where haplotypic and nucleotide diversities are strong, which falls within the standard ranges of genetic diversity of insect pests **[29]**; populations of *C. cephalonica* are thought to come from a stable ancestral population with a long changing history. This may be due to the second contact of *C.*

cephalonica lineages that had previously separated but Tajima D and F_U's F_S significantly negative and mismatch curve with its unimodal and non-significant SSD appearance disproving this origin.

However, the total population has strong haplotypic and nucleotide diversities; this means that the four populations of the sampled rice moth would come from a stable ancestral population with a large effect size. The non-significant Tajima D confirms this total population of *C. cephalonica* stable. To date, very little information is available on the genetic structure of *C. cephalonica*. The small genetic distance between populations of the same emerging area from different food substrate (MCGB and RCGB, RCSR and MCSR) shows that there is very little or no genetic difference between these populations. This is different from the results of many entomological works such as those of **[9]**, **[35]** depending on which the food substrate affects the biological parameters of *Corcyra cephalonica*. This effect should first be felt at the molecular level so on DNA which should show a fairly significant genetic distance between millet and rice. This small genetic difference may be due to the insect's polyphage behaviour. Elsewhere, AMOVA inferences, although it is low, would confirm these entomologist authors because molecular variance is significantly explained at 1.4% by the food substrate and the F_{ST} = 0.01398. According to **[53]**, an F_{ST} between 0 and 0.05 reveals a low genetic differentiation. However, the effect of the latter is very small compared to the effect of host plants on the genetic structuration of *Caryedon serratus* populations **[47]** where the genetic distance is thirty times those in this study. Between agro-ecological environments or areas, there is a fairly important genetic distance that shows a clear difference in the genetic structuration of populations from the CGB to those of the SRD. In the same vein, Molecular Variance Analysis (AMOVA) shows that 4.41% of the genetic variation, between these *Corcyra* populations, are due to the environment with an F_{ST}= 0.04409 which is significant. This partition, though it is significant, is twice as small as that obtained by **[40]** in the study of the effect of biogeographical zones on the genetic structuration of populations of *Sitophilus zeamaïs*. Moreover, this genetic differentiation between populations of *C. cephalonica* from the CGB and those of the SRD is explained by distance isolation **[54]** through regression that shows the existence of a strong link between genetic distance and geographic distance. This confirms the conclusion of the work of **[34]** according to which these two agro-ecological zones have a base on the bio ecology of the different

populations of *C. cephalonica*. There would then be an ecotype aspect between these populations that would be linked to the ecological factors, particularly in climate one, of these two sites that belong to different agro-ecological zones and which would be a genetic differentiation. However, in all proportion, this result is the opposite of that of **[46]** according to which, there is no genetic differentiation between *Sitophilus* populations according to the agro-ecological zone for Cyt-b.

The research hypothesis is that there would be a genetic structuration of *C. cephalonica* populations according to the agro-ecological zones on the one hand and the food substrate on the other. This hypothesis seems plausible from the haplotypic network and the two phylogenetic trees that show two clades supported by a strong bootstrapping (Neighbor-Joining) and a high posterior probability (Bayesian inference). The first clade or haplogroup1 (HG1) consists only of individuals from the CGB while the other (HG2) groups the individuals of the SRD; this confirms the isolation by the distance and effect of agro-ecological zones on the genetic structuration of the rice moth. The origin of the infestations would be at the level of the rice of the river delta which would contaminate first the millet of the same area and then the rice of the CGB. This makes sense since the stored rice in the packing station in the CGB comes mostly from the river delta. This result could be explained by different abiotic factors between areas, more particularly climate factors (temperature, illumination, wind, relative air humidity and rainfall); the area of the river delta presents a more favorable climate for the development of the moth because it is located close to the Atlantic Ocean. The effect of the food substrate on the bio-ecology of the insect is proven according to the work of **[35]**, but the obtained phylogenetic trees according to the food substrate especially on the Bayesian tree show that this state of affairs would not be linked to a genetic base since groups of individuals of different food supports showed phylogenetic affinities in the CGB as in the SRD.

This is easy to understand from the fact that the nutritional composition of the two bowls of cereal in protein, mineral salts, and vitamins is different and would act more morphologically and physiologically than genetically of the insect. The example of athletes who modify their diets for physical transformation or to boost their performance, the use of fertilizing drugs to increase the chances of fertilization in certain individuals, among others, and which does not change their genomes, can be perfect illustrations of this food substrate effect.

CONCLUSION AND PERSPECTIVES

In this study, populations of *C. cephalonica* on millet and rice stocks were genetically characterized by the environment and food support through the Cyt-b gene. The various analyses have shown that the overall population is in demographic equilibrium (stable) and that the existence of the ecotype aspect is proven with distance isolation (IBD); producers should avoid mixing the populations of the moth with millet and rice grains from different agro-ecological areas to reduce crossbreeding between populations and thus decrease genetic diversity. However, a low genetic differentiation, although it is significant, exists according to the AMOVA test between populations according to food support; which pushes us to relativize the existence of the biotype aspect.

The hypothesis that the zone effect is related to the molecular biology of the moth seems to be plausible. Further studies need to be done to remove ambiguity on this biotype aspect and at the same time further confirm the ecotype aspect with nuclear markers such as microsatellites or ITS to take into account all information coming from the matrilineal and patrilineal sides.

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