Global Journal of Science Frontier Research: C
Biological Science
Volume 20 Issue 4 Version 1.0 Year 2020
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Phylogenetic, Demogenetic Evolution and Genetic Structure of *Sitophilus Zeamais* in Sahelo-Sudanian Climatic Zone of West Africa (Senegal, Mali, Niger, Burkina Faso, Guinea Conakry)

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This article aims to highlight a possible genetic structure of *S. zeamais* according to 5 countries of the semi-arid zone [23], to identify the types of demographic and phylogenetic evolution of the populations of the insect in these countries.

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GJSFR-C Classification: *FOR Code: 060601*

PHYLOGENETICDEMOGENETICEVOLUTIONANDGENETICSTRUCTUREOFSITOPHILUSZEAMAISINSAHELOSUDANIANCLIMATICZONEOWESTAFRICASENEGALMALINIGERBURKINAFASOGUINEACONAKRY

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Ngagne Demba Sarr [α], Mama Racky Ndiaye [σ] & Mbacké Sembène [ρ]

Abstract- Knowledge of the genetic distribution of *Sitophilus zeamais*, the main pest of maize stocks in West Africa, is a prerequisite for securing conserved maize crops. So far, most of the studies carried out to find a solution to the huge losses have been to identify bio-insecticides of vegetarian origin.

This article aims to highlight a possible genetic structure of *S. zeamais* according to 5 countries of the semi-arid zone [23], to identify the types of demographic and phylogenetic evolution of the populations of the insect in these countries.

This study will then make it possible to know the populations of the countries most prone to survival or extinction, through the evaluation of their genetic diversity and the identification of the type of selection that specifies them. To reach this objective, 60 insects were harvested in the countries. Exploitation of the Cytochrome B gene sequences corresponding to these individuals by population genetic study software (Bioedit, DNAsp, Mega, Harlequin, etc.) revealed a genetic structure of *S. zeamais* according to the 5 countries in the area, a close relationship between the populations of the insect which would be originating in Niger and at the end of the models of demographic evolution which are not confirmed however by the demogenetic tests.

Keywords: zeamais sitophilus, genetic structure, semi-arid zone, selection, cytochrome B.

I. Introduction

The maize weevil known scientifically as *Sitophilus zeamais* is a cosmopolitan insect particularly widespread in West Africa. The large losses of corn stocks with the significant socio-economic consequences that it causes have raised prospects for solutions. In semi-arid zones, most of these studies attempt to identify bio-insecticide products from plants [24], while genetic knowledge of the insect is also essential for an effective and sustainable solution. Thus this article aims on the one hand to highlight a possible genetic structure of *S. zeamais* according to 5 countries (Senegal, Niger, Mali, Burkina Faso, Guinea Conakry) of the semi-arid zone of Africa West and on the other hand to identify the types of demographic evolution of the populations of the insect in these countries and their degree of kinship.

The demonstration of a genetic differentiation of the insect according to these countries and the knowledge of the type of selection which characterizes the populations of the countries will make it possible to apprehend the capacities of survival of the insect in each country.

To achieve this objective, *Sitophilus Zeamais* insects were sampled in each of the 5 countries mentioned above. The 60 sequences corresponding to all of the individuals were exploited by software for studying population genetics (Bioedit, DNAsp, Mega, Harlequin, etc.), in relation to genetic structuring parameters (genetic distance, Fst, etc.), in relation to the objectives.

II. Materials and Methods

a) Sampling

i. Sampling locations

Harvesting of *zeamais Sitophilus* individuals was carried out in five (5) countries in the semi-arid zone [23]. These are Senegal, Mali, Burkina Faso, Guinea Conakry and Niger. (Table I).

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Table I: Sampling country

Countries	Sample Code	Number of individuals	Geographic coordinates	
			Latitude	Longitude
Senegal	SzSn	20	14°29'51''N	14°27'09''W
Mali	SzMl	10	17°34'14''N	03°59'46'' W
Burkina Faso	SzBf	10	12°14'99''N	01°33'42''W
Guinea Conakry	SzG	10	09°56'44''N	09°41'48''W
Niger	SzNg	10	17°36'28''N	08°04'54''W

ii. *Harvest of individuals*

In each of the above countries, 250 g to 1 kg of infested corn were collected from storage locations, through project partners. The samples have been sent to the laboratory where they are kept in jars with mesh lids for mass breeding. The insects collected at the end of this breeding were kept in alcohol at 95°C, then transported to the laboratory for a genetic study. Each sample is identified by a code: the first 2 letters designate the binomial name of the species (S for Sitophilus and z for Zeamais), the 2 letters which follow indicate the country of origin (example: SzSn, with S = Sitophilus, z = zeamais, Sn = Senegal. SzBf, with S = Sitophilus, z = zeamais, Bf = Burkina Faso.

b) Molecular method of analysis

The cytochrome B gene was chosen to be amplified. The choice is explained by its particularity to keep very long without wear and it is used regularly in the studies of insects [7].

i. *DNA extraction*

Extraction is the technique of releasing DNA from the cell. It includes the individualization of cells (digestion) and the destruction of their plasma and nuclear membranes (lysis). The digestion of the cells consisted of placing their legs and prothorax in tubes containing ATL buffer and proteinases K. After incubation, the tubes were centrifuged to separate the supernatant from the cell debris. To destroy cell membranes, cell lysis buffer (LA) was added first, then ethanol (96%) after incubation, in the tubes. Then the tubes are passed through columns with a silica membrane. Finally the centrifugation of the tubes made it possible to retain DNA on the siliceous membranes of the columns because it was negatively charged.

ii. *DNA purification*

The DNA of the tubes was purified by adding 2 buffers AW1 and AW2 in each column. After centrifugation of the tubes and precipitation of the DNA at the bottom, the buffers and the contaminants are discarded. The columns are then replaced in other tubes in which AE buffer has been added to unhook the DNA. The DNA is thus removed and stored at -20°C.

iii. *PCR of the mitochondrial Cytochrome B gene*

The PCR of the mitochondrial Cyt.B gene was carried out by 2 primers defined by Simon et al [21]. For each sample (tube), the amplification was made from a total volume of 25 µl, including a mixed volume of 23 µl and a volume of 2 µl of DNA extract. The mixed volume was made up of : 18.3 µl of milli water, 2.5 µl of 10X buffer, 1 µl of additional Mgcl 2, 0.5 µl of Dntp, 0.25 µl of each primer and 0.2 µl of Taq polymerase. (Table II)

Table II: Identification of the primers used and programming of the PCR

Gene	Primer Names	Primer Sequences	PCR Program
Cyt.B	CB-J-10933(F)	5-TATGTACTACCATGAGGACAAATATC-3	1. Initial denaturation: 94°C, 3 min ; 35 denaturation cycles : 94°C, min 2. Hybrization: 47°C, 1 min 3. Elongation: 72°C, 2 min ; elongation finale: 72°C, 8 min
	CB-N-11367(R)	5-ATTACACCTCCTAATTTATTAGGAAT-3	

iv. *Bioinformatics analyzes*

The sequences were corrected and aligned by the Clustal software implemented in the Bioedit program version 7.2.5 [6].

The genetic structure of *Sitophilus zeamais* has been understood in relation to parameters of genetic differentiation. These are genetic distance, Fst, Nm and the Amova Test. The genetic distance between countries was calculated by the Mega 7 software version 7.0.14 [6], the global Nm and Fst indices by the DNAsp software, while the Fst values between populations were calculated by the Arlequin software version 3.5.1.3 [3]. The AMOVA test, on the other hand, made it possible to determine the share of populations and individuals in the genetic structure of the insect.

Using the mitochondrial Cyt.B gene, the Network software[1] made it possible to construct the haplotype network using the maximum parsimony method.

The demographic history of the populations sampled in the different countries was apprehended from a "mismatch distribution" analysis of the populations, correlated with the evaluation of the demographic tests of D de tajima, Fs de Fu [5], of R2 of Ramos and H of Fay and Wu. This analysis is accredited by the demographic indices SSD (sums of squares of deviations) and RAG, calculated between distributions observed and expected by the software Arlequin 3.5.13 [3]. The values of D of tajima, of Fs of Fu were calculated by the software Arlequin 3.5.13[3]. While those of R2 of Ramos and H of Fay and Wu were calculated by DNAsp software.

Phylogenetic reconstruction makes it possible to clarify the relationships of kinship existing between haplotypes identified in the different agroecological zones. So in our study, we built 2 phylogenetic trees, one using the maximum parsimony (MP) method and the other using the maximum likelihood (MC) method, using the software Mega vesion7.0.14 [21]. The comparison of these 2 trees allowed to check the coherence of the interpretation of the phylogeny of the populations.

III. Results and Discussion

a) Results

i. Genetic structuring parameters

The Malian population of *S. zeamais* is homogeneous. The individuals of Burkina Faso and Guinea Conakry are genetically close (small genetic distance). On the other hand, the populations of Senegal and Niger which are characterized by high values of genetic distance, are made up individually of divergent insects. (Table III).

Table III: Genetic distance of *S. zeamais* within countries (all values are significant)

Countries	Genetic Distance	Standard deviation
Senegal	0,027	0,014
Mali	0,000	0,000
Niger	0,027	0,014
Burkina Faso	0,014	0,011
Guinea Conakry	0,011	0,006

The genetic distance between populations in the countries indicates that individuals from Mali and on the one hand from Guinea Conakry and on the other hand from Burkina Faso are genetically very close. The pairs of populations Burkina Faso-Guinea Conakry, Niger-Mali and to a lesser extent Mali-Senegal, have low genetic distance values. However, the values are high for the pairs Burkina Faso-Niger, Burkina Faso-Senegal, Senegal-Guinea Conakry and Niger-Guinea Conakry and very high between Niger and Senegal (Table IV).

Table IV: Genetic distance between countries (all values are significant)

Genetic Distance	Senegal	Mali	Niger	Burkina Faso	Guinea Conakry
Senegal		0,012	0,007	0,013	0,009
Mali	0,022		0,008	0,014	0,010
Niger	0,008	0,015		0,011	0,004
Burkina Faso	0,024	0,032	0,019		0,012
Guinea Conakry	0,014	0,022	0,007	0,024	

The values of Fst are relatively low for the pairs of populations Senegal-Mali, Senegal-Guinea Conakry and Mali-Niger. They are relatively high between Senegal and Niger and between Senegal and Guinea Conakry. But the values are very high between the pairs of countries Senegal-Burkina Faso, Guinea-Burkina Faso and Mali-Guinea Conakry. (Table V).

Table V: The values of Fst between the countries (the values in bold are not significant)

Fst	Senegal	Mali	Niger	Burkina Faso	Guinea Conakry
Senegal					
Mali	0,1736				
Niger	0,2216	0,1944			
Burkina Faso	0,2848	0,3282	**0,0455**		
Guinea Conakry	0,1427	0,2963	0,2106	0,3140	

Even if the Nm is more suitable for microsatellites, its values here attest those of Fst. Indeed, pairs of countries which are characterized by very high Nm values (Nm greater than 1) have low Fst values, (Table VI).

Table VI: Values of Nm between countries (all values are significant)

Country1	Country 2	Nm
Burkina Faso	Niger	5,24
Burkina Faso	Mali	0,51
Burkina Faso	Senegal	0,67
Burkina Faso	Guinea Conakry	0,55
Niger	Mali	1,04
Niger	Senegal	1,00
Niger	Guinea Conakry	0,94
Mali	Senegal	0,79
Mali	Guinea Conakry	0,59
Senegal	Guinea Conakry	1,17
Population Globale		0,90

The AMOVA Test corroborates the genetic structure of S. zeamais according to the 5 countries of the semi-arid zone, with a high Fst is significant. But the share of genetic variation in countries' populations in genetic differentiation is smaller than that of individuals within a population. (Table VII).

Table VII: AMOVA test (all values in gray are significant).

Source of variance	Degrees of liberty	Sum of squares of deviation	Variance components	Pourcentage of variance	Fixation indix
Between countries	4	34.817	0,5699 Va (Fst)	21,72	**Fst= 0,217**
Within countries	55	113,000	2,0545 Vb	78,28	**Fst= 0,783**
Total	59	147,817	2,6245	100	1,000

The Mantel Test reveals a negative correlation between genetic distance and geographic distance because r is negative and significant. (r = -0.534, P = 0.005). In other words when the geographic distance increases the genetic distance decreases. (Figure I).

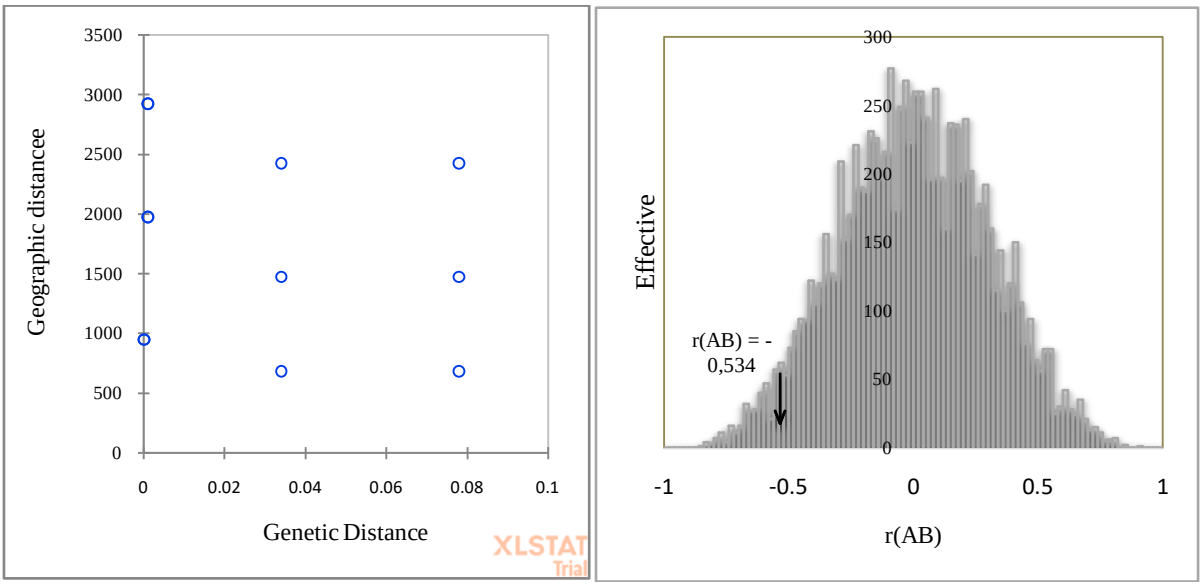

Figure I: Mantel Test : Correlation between geographic distance and genetic distance

ii. *Demogenetic and phylogenetic evolution parameters*

With the exception of Mali, all countries have high haplotypic diversity values and low nucleotide diversity values.

The populations of Senegal, Niger and Guinea Conakry present negative values of D of Tajima, values of Fs of Fu, SSD, Rg and R2 of Ramos positive. Burkina Faso is characterized by a negative Fs of Fu, values of D of Tajima, R2 of Ramos, SSD and Rg positive. The demogenetic test values are zero for the population of Mali. However, none of the parameter values is significant for all populations. (Table VIII).

Table VIII: Demogenetic tests

Countries	Demographic Parameters						Genetic Diversity	
	D of Tajima	Fs of Fu	R^2	H of Fay and Wu	SSD	Rg	Hd	Pi
Senegal	-0,781 (0.217)	0,717 (0.668)	0,162 (0,50)	-0,170 (0,34)	0,056 (0.120)	0,118 (0,090)	0,784 (0,083)	0.011 (0.002)
Mali	0,000 (1,000)	0,000 (1,000)	----	----	0,000 (0,000)	0,000 (0,000)	0,000 (0,000)	0,000 (0,000)
Niger	-0,418 (0,341)	1,232 (0.720)	0,161 (0,53)	0,041 (0,33)	0,049 (0.500)	0,080 (0,530)	0,844 (0,103)	0,011 (0,002)
Burkina Faso	0,528 (0.730)	-0,341 (0.375)	0,162 (0,23)	----	0,077 (0.150)	0,218 (0,060)	0,867 (0,007)	0,013 (0,003)
Guinea Conakry	-0,842 (0.215)	0,223 (0.552)	0,161 (0,53)	-0,007 (0,32)	0,037 (0.180)	0,134 (0,530)	0,711 (0,117)	0,003 (0,001)

Hd= Haplotypic diversity, Pi= Nucleotidic diversity, SSD= Sum of Squared Deviation, Rg=Happending's Raggedness.

Mismatch distribution curves are multimodal for all countries. But it is particularly bimodal for Guinea Conakry. (Figure II).

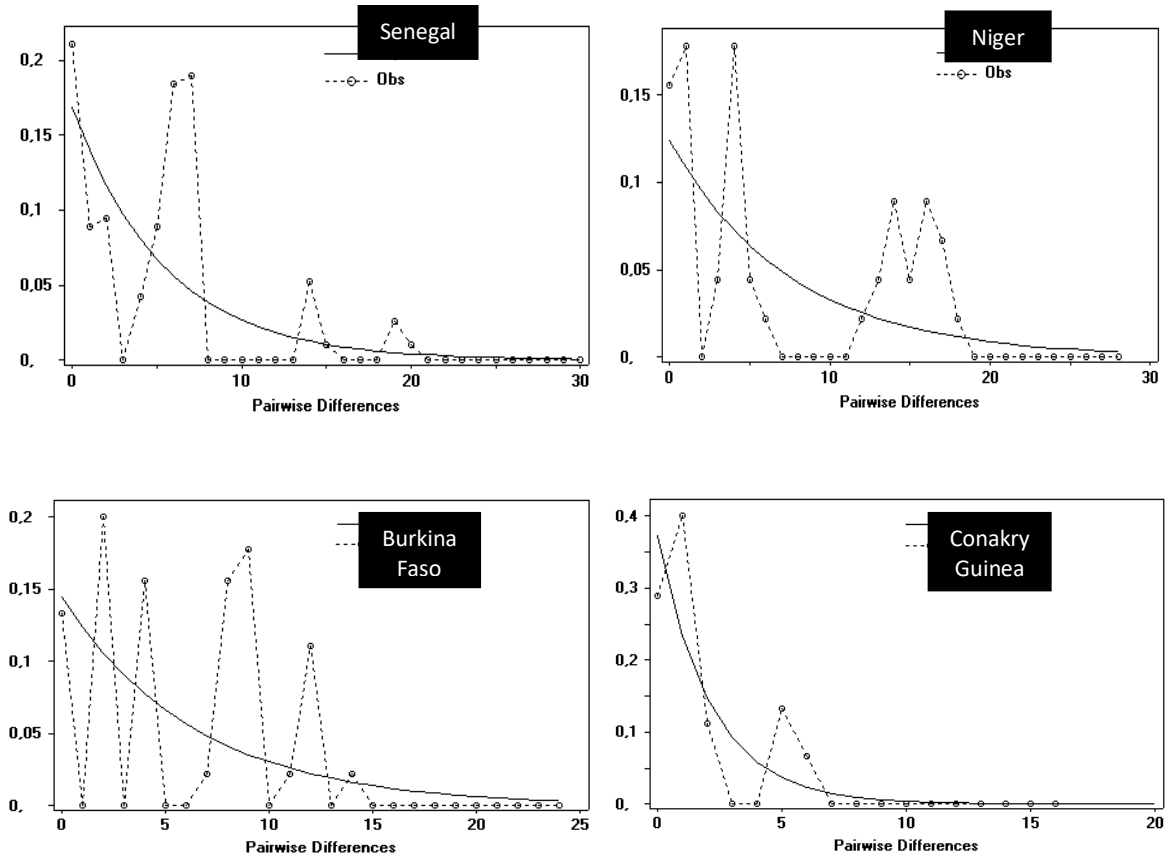

Figure II: Mismatch distribution

Phylogenetic trees constructed according to the maximum likelihood method and the maximum parsimony method mainly highlighted a single clade supported by strong Bootstrap values (57% and 74%). (Figure III). This clade includes all the haplotypes encountered in the countries of the semi-arid zone, except 2 haplotypes deprived of Niger (H11, H9).

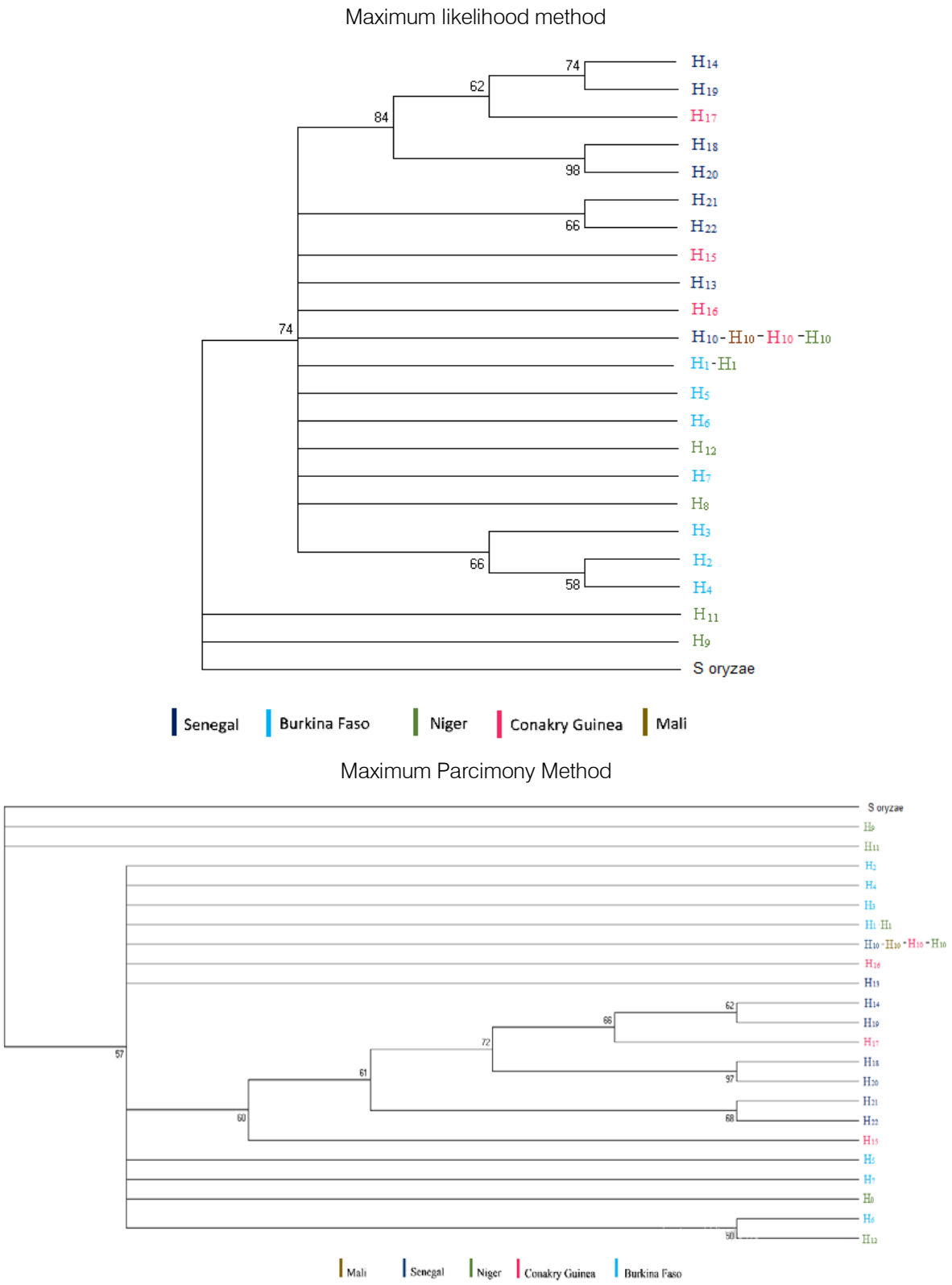

Figure III: Phylogenetic Trees

b) Discussion

The objective of the article was to study the demographic and phylogenetic evolution of the populations of *S. zeamais* in 5 countries of West Africa sharing the same climate (semi-arid zone), but also to research a possible genetic structure of the insect according to these countries.

Genetic differentiation parameters (DG, Fst) and the AMOVA Test have highlighted a genetic distribution of individuals of *S. zeamais* according to these countries. A genetic structure of this insect in Senegal and Guinea Conakry has already been highlighted with fairly similar values by Ndong et al [14]. Other insect pests have also been the subject of genetic differentiation according to localities. This is the case of the main millet pest in Senegal, *Tribolium Castaneum*[9], the corn stalk pest in Benin *Busseola Fusca* (Fuller) [19].

The share of genetic variation of individuals within populations is greater than that of populations between countries. This state of affairs indicates that anthropogenic activities such as agricultural practices, systems of conservation of harvest stocks, commercial activities would influence the genetic structure of the insect more than the intrinsic ecological characteristics of the countries. The work of Ndong et al [14]is likely to confirm this hypothesis. Indeed, they revealed in an agroclimatic zone a genetic structuring of the insect according to storage means. The genetic differentiation here is not due to the geographic distance between countries based on the results of the Mantel Test, as has been the case in other studies. Bossart and Prowell[2] suggest that genetic isolation may be due to a different factor than geographic distance. These researchers state that the ecological heterogeneity of a species' habitat often involves dispersion barriers.

The demographic evolution of *S. zeamais* populations in the countries was apprehended by demographic parameters. The multimodality of the mismatch distribution curves of the populations of Senegal, Niger and Burkina Fasso, which is not however confirmed, due to the non-significance of the SSD and Rg values, indicates that the corresponding populations are in demographic expansion. In Senegal and Niger, this demographic expansion is in line with negative Tajima's D values for these populations, which are not, however, significant. Thus the non-significance of the values of D of Tajima and Fs of Fu and the high values of haplotypic diversity and low of nucleotide diversity in Senegal, Niger and Burkina Fasso suggest stability or moderate demographic expansion. In fact, a high haplotypic diversity and a low nucleotide diversity can be the result of a rapid population growth from an ancestral population with a small population and for which there is not enough time elapsed to find a high diversity between haplotypic[4]. The bimodal distribution of Guinea Conakry not confirmed by the values of SSD and Rg and the non-significance of the values of D of Tajima and Fs of Fu suggest a demographic stability of this population. The negative values of H of Fay and Fu, of D of Tajima of Guinea de Conakry and of Senegal even if they are not significant suggest that the populations of these countries underwent a positive selection. On the other hand, the very homogeneous population of Mali has undergone negative selection or a bottleneck.

Phylogenetic trees according to the maximum likelihood and maximum parsimony methods highlighted a single clade grouping together almost all the insects of the semi-arid zone. These insects would come from Niger according to the high genetic diversity which characterizes this country compared to others and the percentage (100%) occupied by the ancestral and sub-regional H10 haplotype in this country. Indeed, there is a direct probabilistic relationship between the frequency of an allele and its age [22]because the most frequent haplotype is on average the oldest. The transversality of this haplotype in the 5 countries is the fruit of trade. Indeed, the rural populations of the West African sub-region are characterized by a commercial dynamism through grain exchanges. This transfer of grain from one country to another could be accompanied by the transfer of larvae, egg-laying cocoons or even adults [8]. But this transfer did not have a substantial impact on genetic isolation because it involved a single haplotype.

IV. Conclusion

The study highlighted a genetic structure of *S. zeamais* according to the 5 countries of the Sahelo-Sudanian zone of West Africa, a common origin of insects which would be the Niger and models of demographic evolution mixed. Since the countries present genetically different individuals, studies can relate to the genetic diversity of *S. zeamais* in each of them, to apprehend the capacities of adaptation of the insects in the countries, because the genetic diversity of an individual is positively linked to its adaptive potential.

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