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Genetic Diversity of the Fall Armyworm *Spodoptera frugiperda* (J.E. Smith) in the Democratic Republic of the Congo

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Abstract: In 2016, the fall armyworm (FAW), *Spodoptera frugiperda*, invaded western Africa and rapidly spread in sub-Saharan Africa, causing significant losses in yields of corn, a major food crop in Africa. Although the Democratic Republic of the Congo (DRC) is a large corn-growing country, the impact of FAW has not been investigated. This study was designed to expand investigations on the genetic diversity of FAW populations in the DRC. We collected FAW individuals from eight provinces across the country, for analysis of genetic variation. Based on the partial sequences of both mitochondrial *cytochrome oxidase subunit I* (*COI*) and nuclear *triosephosphate isomerase* (*Tpi*) genes, we compared polymorphic features of the *COI* haplotype and *Tpi* single nucleotide polymorphisms. The results revealed that most (84%) of the analyzed individuals were heterogeneous hybrids *Tpi*-corn/*COI*-rice (*Tpi*-C/*COI*-R), whereas 16% were homogenous *Tpi*-corn/*COI*-corn (*Tpi*-C/*COI*-C). Further analysis of the fourth exon/intron sequences of the *Tpi* gene identified two subgroups, *Tpi*Ca1 and *Tpi*Ca2, constituting 80% and 20%, respectively, of the collected individuals. Analysis of genetic variation among native and invasive populations indicated significant genetic differences (10.94%) between the native American and DRC populations, whereas both the DRC and African populations were genetically closer to Asian than American populations. This study provides important information on FAW genetic diversity in the DRC, which can be used for effective management of FAW.

Keywords: climate change; fall armyworm; genetic diversity; invasive pest; quarantine

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1. Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera, Noctuidae), is a devastating agricultural pest in the tropical and subtropical regions [1,2]. Although FAW is native to south America, since the first outbreak outside its native region in 2016, its global distribution range has swiftly expanded throughout Africa, Asia, and recently, Oceania [3–5]. This rapid range expansion is probably due to its capacity to adapt to a wide range of temperature conditions and its polyphagy [6,7]. FAW is a highly polyphagous species that can feed on at least 76 plant families, mainly Poaceae, Asteraceae, and Fabaceae [8]. However, FAW has a strong propensity for corn (Poaceae), which is the main food crop for more than 200 million African smallholder farmers [2]. FAW larvae damage corn plants by feeding on leaves, stems, and reproductive parts, thus destroying their growth potential [9]. When their population is large, they develop an "armyworm" behavior and disperse in large numbers, attacking almost all vegetation in their path [10].

According to host plant preference, FAW consists of two strains, namely, the corn strain and rice strain [11]. These strains are morphologically similar but genetically different with

2.09% genome divergence. They also exhibit variation in developmental, physiological, and ecological features such as host plant preference, and sex pheromones [11–13]. The rice strain is typically associated with rice *Oryza sativa* L., sugar cane *Saccharum officinarum* L., and grass species, such as Johnson grass *Sorghum halepense* (L.), Bermuda grass *Cynodon dactylon* (L.), whereas the corn strain is associated with corn *Zea mays* L. sorghum *Sorghum bicolor* (L.), soybean (*Glycine max*), and cotton *Gossypium hirsutum* (L.) [13,14].

Considering the intra- and interspecific variation among FAW strains, reliable strain identification is essential in field studies of FAW populations. The two strains of FAW are identified mainly based on polymorphisms in the mitochondrial gene *cytochrome c oxidase subunit 1* (*COI*) and the nuclear gene *triosephosphate isomerase* (*Tpi*) [15,16]. In the western hemisphere, the relationship between the *COI* and *Tpi* markers is important for identifying FAW strains [17]. However, in the invaded regions of Africa and Asia, strain identification using the two markers has shown discordant results. Overall, the host association in invasive populations was accurately predicted by *Tpi* but not *COI* [18,19]. The discordance between the *COI* and *Tpi* markers indicates the hybrid nature of FAW populations that invaded Africa [17]. This hybrid nature of invasive populations was lately confirmed by whole-genome sequencing studies [20,21]. The identification of two *COI*-based haplotypes and a small number of *Tpi* haplotypes showed that genetic diversity was low in the invasive populations of FAW [17,22,23]. The low genetic diversity and small number of haplotypes observed in most invaded locations indicate a possible recent introduction from a common source of the FAW population and could affect the monitoring of the crops at risk in these locations [17,19,23].

This study was conducted to expand investigations on the genetic diversity of FAW populations in the DRC. Thus, additional samples collected in new locations in the DRC were combined and compared with those from earlier studies [19,22] to provide a more representative picture of the country-wide genetic structure of FAW in the DRC. The genetic characterization of FAW in the DRC during the first six years of invasion can predict changes in the populations as they rebalance and respond to pest management measures. Additionally, the comparison of the populations of FAW in the DRC with those from both native and other invaded regions can provide the phylogeographic patterns and relationships of FAW haplotypes in the DRC and could be used in understanding the possible route of the FAW population that invaded the DRC.

2. Materials and Methods

2.1. Collection of FAW Samples

Samples were collected from corn fields at 21 locations in 8 provinces in the DRC during the five-year period from 2018 to 2022 (Table 1). After field collection, the larvae were preserved individually in 1.5 mL microcentrifuge tubes in 70% ethanol. The samples were transported to the plant clinic in Kinshasa, DRC and kept at −20 °C until they were sent to the Laboratory of Insect Molecular Physiology at Kyungpook National University, Republic of Korea, for DNA extraction and further genomic analysis.

2.2. DNA Extraction

Total genomic DNA was extracted from the head of single larva using a pure link genomic DNA mini kit (Invitrogen, Carlsbad, CA, USA) [22]. Each sample was homogenized in a 1.5 mL microcentrifuge tube containing 200 µL of digestion buffer and 20 µL of proteinase K (50 µg/mL) before it was incubated at 56 °C for 30 min. The DNA supernatant was collected in a genomic spin column and stored in a new 1.5 mL microcentrifuge tube at −20 °C until downstream analysis. DNA quality and concentration were assessed using a NanoPhotometer™ (Implen GmbH, Schatzbogen, Germany). The remaining portions of the samples were kept in 70% ethanol at −20 °C.

Table 1. List of fall armyworm *Spodoptera frugiperda* samples and the locations from which they were collected from the DRC.

No.	Sample ID	Province/Territory/Village	Location	Collection Date (Day/Month/Year)	Accession Number		Genetic Group	
					COI	*Tpi*	*COI*	*Tpi*
1	Congo11	Sud-Kivu/Kabare/ Katana	2°22′51″ N 28°82′35″ E	29 November 2018	MT103350	MT894220	*COI*-RS	*Tpi*-Ca1a
2	Congo42	Sud-Kivu/Walungu/Nduba	2°63′73″ N 28°69′63″ E	15 December 2018	MT103349	MT894225	*COI*-RS	*Tpi*-Ca1a
3	Congo3	Sud-Kivu/Kalehe/Bunyakiri	1°99′49″ N 28°54′62″ E	29 November 2018	OQ612484	OQ632453	*COI*-RS	*Tpi*-Ca1a
4	Congo41	Sud-Kivu/Uvira/Sange	3°06′10″ N 29°08′55″ E	15 December 2018	MT933055	MT894224	*COI*-RS	*Tpi*-Ca2b
5	Congo31	Sud-Kivu/Uvira/Luvungi	2°89′15″ N 28°97′12″ E	15 December 2018	MT933054	MT894223	*COI*-RS	*Tpi*-Ca2a
6	Congo21	Sud-Kivu/Kalehe/Minova	1°74′73″ N 28°98′78″ E	29 November 2018	MT933053	MT894222	*COI*-RS	*Tpi*-Ca2a
7	Congo12	Sud-Kivu/ Kabare/Miti	2°33′06″ N 28°76′69″ E	29 November 2018	MT933052	MT894221	*COI*-RS	*Tpi*-Ca2b
8	K1	Lomami/Kabinda/Kabinda	6°07′48″ S 24°28′48″ E	18 July 2020	OP132901	OQ468459	*COI*-RS	*Tpi*-Ca1a
9	Gem1	Sud-ubangi/Gemena/Gemena1	3°14′56″ N 19°46′36″ E	15 July 2020	OP132892	OQ468451	*COI*-RS	*Tpi*-Ca1a
10	Bkd	Sud-ubangi/Gemena/Bokunda	3°12′39″N 19°46′29″ E	15 July 2020	OP132899	OQ468460	*COI*-RS	*Tpi*-Ca1a
11	Bsg1	Sud-ubangi/Gemena/Bosengwen	3°13′50″N 19°42′57″ E	18 July 2020	OP132898	OQ468458	*COI*-CS	*Tpi*-Ca1a
12	Bbw1	Sud-ubangi/Gemena/Bombawuli	3°13′48″ N 19°53′51″ E	18 July 2020	OP132896	OQ468455	*COI*-RS	*Tpi*-Ca1a
13	Mtf1	Tanganyika/Kalemie/Kalemie	5°52′08″ S 29°10′14″ E	21 July 2020	OP132894	OQ468453	*COI*-RS	*Tpi*-Ca1a
14	Tshb1	Tshuapa/Boende/Boende1	0°17′13″ S 20°52′24″ E	18 July 2020	OP132895	OQ468454	*COI*-RS	*Tpi*-Ca1a
15	Blk1	Tshuapa/Boende/Baliko	0°18′05″ S 20°52′30″ E	18 July 2020	OP132897	OQ468456	*COI*-CS	*Tpi*-Ca1a
16	Bde1	Tshuapa/Boende/Boende3	0°16′39″ S 20°53′05″ E	15 July 2020	OP132898	OQ468457	*COI*-RS	*Tpi*-Ca1a
17	Isi1	Haut-Uélé/Isiro/Isiro	2°45′57″ N 27°36′32″ E	8 August 2020	OP132893	OQ468452	*COI*-RS	*Tpi*-Ca1a
18	M1	Kongo central/Matadi/Matadi	5°47′58″ S 13°26′26″ E	18 July 2020	OP132900	OQ632454	*COI*-RS	*Tpi*-Ca1a
19	Kst1	Kongo central/Kisantu/Kisantu1	5°13′82″ S, 15°09′08″ E	15 December 2022	OQ427278	OQ468462	*COI*-RS	*Tpi*-Ca2a
20	Kst2	Kongo central/Kisantu/Kisantu2	5°13′82″ S, 15°09′08″ E	15 December 2022	OQ427279	OQ468466	*COI*-CS	*Tpi*-Ca1a
21	Kst3	Kongo central/Kisantu/Kisantu3	5°13′82″ S, 15°09′08″ E	15 December 2022	OQ427280	OQ857569	COI-RS	Tpi-Ca1a
22	Plaba1	Kinshasa/Plateau de Bateke1	4°20′72″ S, 15°84′48″ E	20 December 2022	OQ427282	OQ468463	*COI*-RS	*Tpi*-Ca1a
23	Plaba2	Kinshasa/Plateau de Bateke2	4°20′72″ S, 15°84′48″ E	20 December 2022	OQ427284	OQ468464	*COI*-CS	*Tpi*-Ca1a
24	Kimw1	Kinshasa/Kimwenza1	4°47′11″ S, 15°30′14″ E	20 December 2022	OQ427281	OQ468461	*COI*-RS	*Tpi*-Ca1a
25	Kimw2	Kinshasa/Kimwenza2	4°47′11″ S, 15°30′14″ E	20 December 2022	OQ427283	OQ468465	*COI*-RS	*Tpi*-Ca1a

2.3. PCR Amplification and Sequence Analysis

DNA was subjected to PCR amplification using a SimpliAmp 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Each PCR reaction mixture of 30 µL contained 15 µL of Solg 2 × Taq PreMix (Solgent, Daejeon, Republic of Korea), 2 µL of each primer (10 pmol/µL), 2 µL of the DNA template, and 9 µL of sterile water. Partial *COI* (658 bp) and Tpi (444 bp) barcode regions of the FAW were amplified using the primer pairs LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGG TGACCAAAAAATCA-3′) for COI, and TPI412F (5′-CCGGACTGAAGG TTATCGCTTG-3′) and TPI1140R (5′-GCGGAAGCATTC GCTGACAACC-3′) for *Tpi* [23,24]. The thermo-cycling conditions for *COI* included an initial denaturation at 92 °C for 5 min, followed by 35 cycles of denaturation at 92 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. The *Tpi* gene thermo-cycling parameters included an initial denaturation at 94 °C for 1 min, followed by 33 cycles of denaturation at 92 °C for 30 s, annealing at 56 °C for 45 s, extension at 72 °C for 1 min, and final extension at 72 °C for 5 min. The amplified products were stained with ethidium bromide before they were visualized on 1% agarose gel under ultraviolet (UV) light. The amplified products were sequenced using the BigDye® Terminator Cycle Sequencing Kit and ABI Prism 3730XL DNA Analyzer (50 cm capillary) (DNA Sequencer) at the Celemics Sequencing Facility (Celemics, Seoul, Republic of Korea). The sequences generated in this study showed 100% similarity to those of FAW in the NCBI database. The sequences were submitted to the NCBI GenBank under accession numbers assigned to each specimen (Table 1).

2.4. DNA Polymorphism Analysis

COI sequences from our previous study [22] were aligned with the sequences generated in this study using ClustalW [24] and used for characterizing genetic diversity (Table 1). Furthermore, the diversity of the Congolese FAW population was compared with that from other geographic locations. The *COI* sequences reported from Africa (89), Asia (72), and America (126) (Table A1) were retrieved from GenBank database and trimmed to a length of 483 bp as this region was present in most the of sequences and was used for comparative polymorphism studies [25]. The dataset was classified into four main geographical categories: (1) Africa, (2) Asia, (3) America, and (4) the DRC. Descriptive statistics including nucleotide diversity, number of haplotypes (H), haplotype diversity (Hd), genetic neutrality, and mismatch distribution were generated using DnaSP ver. 6.12.03 [26]. Mismatch distribution curves which report the frequency of pairwise nucleotide-site differences between FAW sequences from the DRC, were generated using the constant population size model in DnaSP to further examine the demographic pattern of FAW in DRC.

The FAW *COI* and *Tpi* gene polymorphisms of the DRC samples were analyzed using previously published strain defining loci and polymorphic sites [27,28]. The single nucleotide polymorphisms (SNPs) (mCOI72, mCOI117, mCOI171, mCOI207, mCOI258, mCOI564, mCOI570, mCOI600, mCOI634, and mCOI663) that define the strain polymorphic sites of FAW found in the barcode region of the *COI* were used to distinguish between corn and rice strains of FAW in our previous study [22]. Additionally, the *Tpi* partial gene segment (444 bp), which contained 166 bp of the fourth exon (*Tpi*-E4) and 278 bp of the fourth intron (*Tpi*-I4), was used to identify the *S. frugiperda* host strain. The presence of the nucleotide base letters "C" or "T" at gTpi183, for the corn strain (*Tpi*-C) or rice strain (*Tpi*-R), respectively, allowed us to distinguish the FAW *Tpi*-based host strains.

2.5. Haplotype Network Plot and Phylogenetic Analysis

A haplotype network was constructed using the popART software ver. 1.7 [29]. Sequences were aligned and grouped within the four geographical regions using ClustalW [24] and Dnasp, respectively. The median joining network method was used to infer haplotype relationships. To generate the evolutionary relationship between the DRC FAW haplotypes, a phylogenetic tree for the *COI* gene was constructed using the maximum likelihood

method implemented in MEGA 6.0 [30], with other *Spodoptera* species and FAW corn and rice strains retrieved from NCBI [31,32]. For each phylogeny, 1000 bootstrap replicates were used to assess robustness using the Hasegawa–Kishino–Yano (HKY850) model and gamma distribution rate of variation between sites [33].

2.6. Analysis of Molecular Variance (AMOVA)

AMOVA was performed using Arlequin ver. 3.5.2.2 [34]. The analysis was conducted with four geographic groups including the rest of Africa, Asia, America, and the DR Congo. Apart from the overall AMOVA, the *COI* sequences from the four geographical regions were examined in six combinations comprising DRC vs. America, DRC vs. Africa, DRC vs. Asia, America vs. Africa, America vs. Asia, and Africa vs. Asia. We observed variance differences among groups by a randomized test with 1000 permutations in a haplotype-based standard AMOVA.

3. Results

3.1. PCR Amplification and Sequence Analysis

We recovered 25 nucleotide sequences of both *COI* and *Tpi* genes from the represented FAW samples collected from 21 different regions of the DRC (Table 1). Sequence analysis of the partial *COI* fragment (658 bp) showed that the *COI*-R constituted 84%, whereas the *COI*-C constituted 16% of the sequences (Figure 1b). Additionally, analysis of *Tpi* sequences on the polymorphic locus gTpi183 (which was used to identify the rice and corn strains) and at the *Tpi*-E4 was C but not T, which indicated that all the samples were from the *Tpi*-C genetic group. Furthermore, two *Tpi*-based haplotypes were identified in the DRC's FAW population, including the *Tpi*-Ca1 homozygous (in 80% of individuals), and *Tpi*-Ca2 homozygous (in 20% of individuals) (Figure 1c). The *Tpi*-R haplotype was not detected in any of the samples. Further analysis of the *Tpi*-Ca2 subgroup showed that the *Tpi*-Ca2a and *Tpi*-Ca2b genetic groups were detected in three and two individuals, respectively. Our results indicated that the nuclear *Tpi* marker consistently identifies the phenotypic feeding behavior of FAW on corn, which is the host plant of FAW in the DRC as well as in other African and Asian countries.

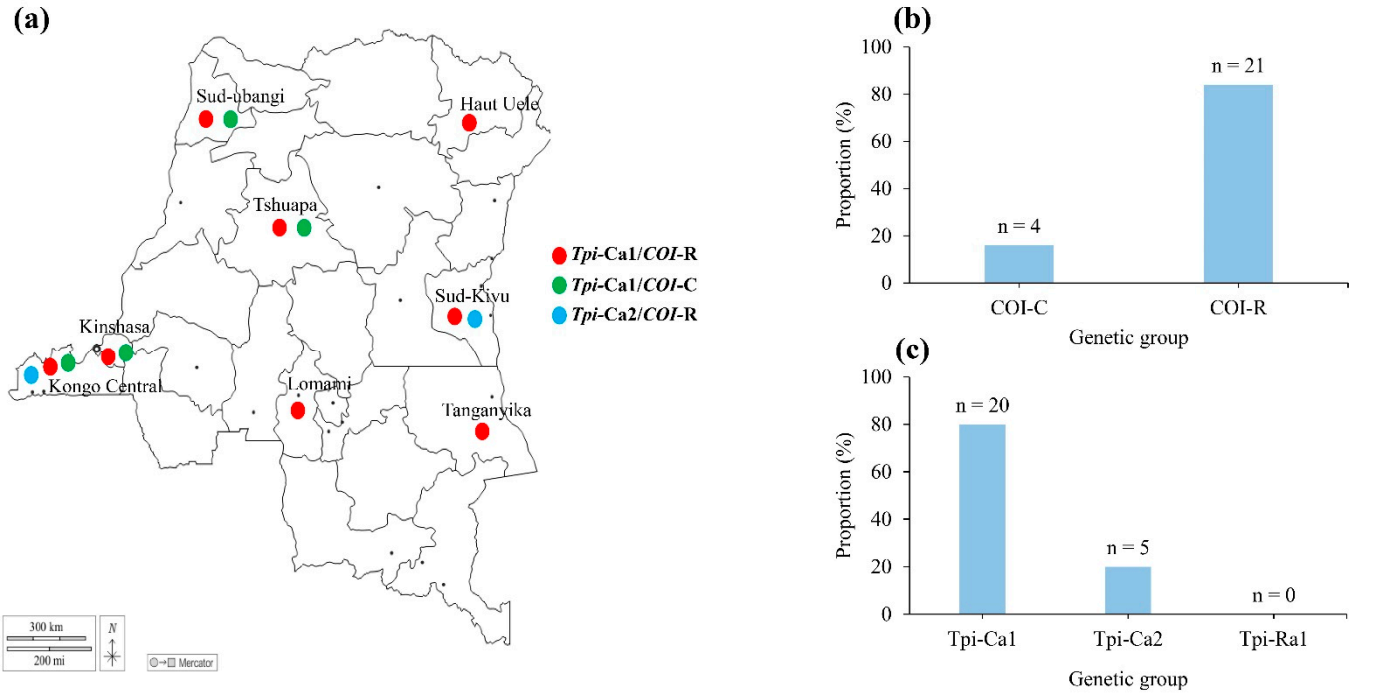

Figure 1. Distribution pattern of haplotypes of the fall armyworm *Spodoptera frugiperda* populations in the DRC based on collected locations (**a**), mitochondrial *COI* (**b**), and *Tpi* (**c**) partial gene markers.

3.2. Polymorphism Analysis

The haplotype diversity of FAW in the DRC was analyzed using 657 bp of the *COI* barcode region from individuals collected from eight provinces (Figure 1a). Our findings indicated seven polymorphic sites and a nucleotide diversity of 0.00469 (Table 2). Two distinct haplotypes (the corn and rice strains) were identified from the DRC's *COI* sequences with a haplotype diversity (Hd) of 0.324 (Table 2). Most (84%) of the *COI* sequences from this study belonged to a single rice haplotype (DRC_haplotype 1, submitted under GenBank accession number OP132901). The remaining 16% belonged to the corn strain haplotype (DRC_haplotye 2, submitted under GenBank accession number OP132898). Four sequences identified as corn strain were detected in specimens from four provinces of the DRC (Sud-ubangi, Tchuapa, Kongo central, and Kinshasa), and the rice strain sequences were detected throughout the country, suggesting that the distribution pattern of FAW haplotypes in the DRC was not region-specific.

The values of both the Fu's Fs and Tajima's D test statistic for the FAW population of the DRC were significantly positive (Table 2). Our results did not detect any nucleotide diversity within the strain populations. Genetic analysis of the FAW population in the DRC did not provide evidence of population expansion. Mismatch distribution analyses of the two strains indicated a bimodal curve, suggesting neutral evolution of FAW population in the DRC (Figure 2).

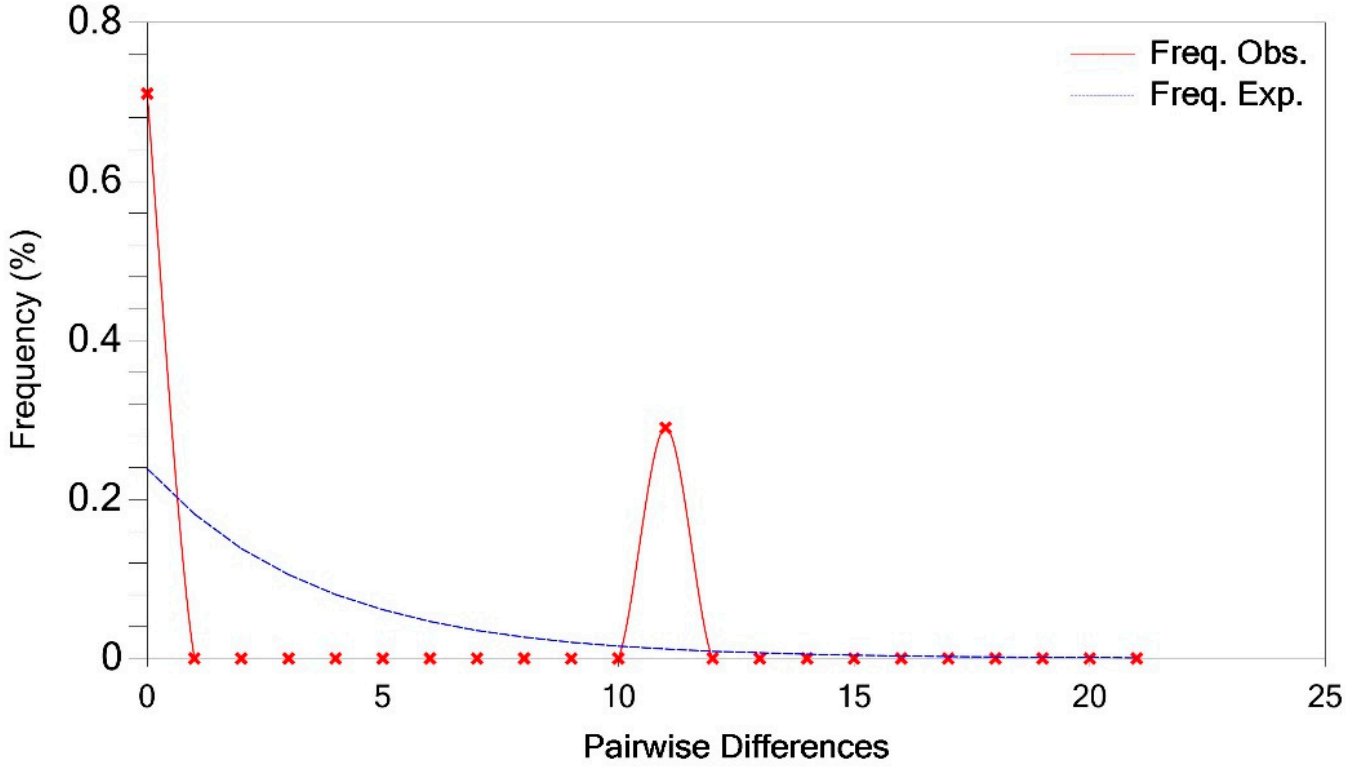

Figure 2. *COI* mismatch distribution curve indicating the observed (solid red line) and expected (dotted blue line) pairwise nucleotide site divergences computed with DnaSP.

Table 2. Summary of the genetic diversity of the fall armyworm *Spodoptera frugiperda* populations analyzed on the basis of partial *mtCOI* gene from four geographical locations.

	DRC	Africa	America	Asia	Total
No. of sequences	25	89	126	72	308
No. of sites	483	483	482	483	482
No. of polymorphic sites	7	8	34	9	37
No. of mutations	7	8	38	9	41

Table 2. *Cont.*

	DRC	Africa	America	Asia	Total
No. of haplotypes	2	3	29	4	32
Haplotype diversity	0.324	0.344	0.742	0.378	0.562
Nucleotides diversity	0.00469	0.00478	0.00855	0.00520	0.00735
Fu's Fs statistic	6.012	6.837	−9.966	5.134	−9.841
Fu and Li's D × test statistic	1.29627	0.47452	−3.82406 **	−0.08303	−5.46527 **
Fu and Li's F × test statistic	1.14734	0.79287	−3.33095 **	0.30287	−4.28883 **
Tajima's D	0.53489	1.13421	−1.25518	0.92310	−1.28326

**: significant at $p < 0.02$.

3.3. Comparative Genetic Analyses of the FAW Population in the DRC and Three Geographic Regions

Comparative analysis of the *COI* partial gene region (483 bp) common to all the sequences, revealed haplotype numbers of 29, 3, and 4 in FAW populations from America, Africa, and Asia, respectively (Table 2). The two DRC haplotypes (rice and corn strains) were identical to the predominant rice and corn haplotypes from America (GenBank Accession: U72977.1 and U72975.1, respectively), which are most likely to be the ancestors of all haplotypes in the invaded regions. The neutrality test statistics for the DRC and African FAW populations revealed that FAW populations in these regions are still evolving neutrally relative to the American and Asian FAW populations (Table 2).

3.4. Comparative Phylogenetic and Haplotype Network Analysis

The phylogenetic analysis, based on the maximum likelihood method, indicated that both the rice and corn strain haplotypes from the DRC were identical to haplotypes from American, Asian and other African regions (Figure 3). Haplotype network analysis showed that there were two ancestral strain haplotypes (DRC-RS and DRC-CS) in the FAW populations of the DRC (Figure 4). The network showed the presence of the two ancestral haplotypes in the four geographical regions, with the *Tpi*-C/*COI*-R group being the dominant haplotype in the invaded regions (Africa, Asia, and the DRC). However, the distribution of novel haplotypes in America, Africa, and Asia differed significantly. Our findings suggest the 29 distinct haplotypes in America with the corn strain (*Tpi*-C/*COI*-C) as the most prevalent haplotype, whereas in the two invaded regions, the rice strain haplotype (*Tpi*-C/*COI*-R) was the most prevalent, with 3 and 4 haplotypes in Africa and Asia, respectively (Figure 3). The *COI* marker information indicated that there was no evidence of multiple introductions in the DRC.

3.5. Population Structure of FAW

We performed seven single AMOVA analyses, including one comparing all the geographical regions and six different combinations of groups (DRC and Africa, DRC and America, DRC and Asia, Africa and America, Africa and Asia, and America and Asia) (Table 3). The findings showed significant genetic differences among all the geographical regions (12.70%). The analysis of genetic variation among native and invasive populations indicated significant genetic differences between the native American and DRC populations (10.94%), whereas both DRC populations and those from other parts of Africa were genetically closer to the Asian populations than to American populations (Table 3).

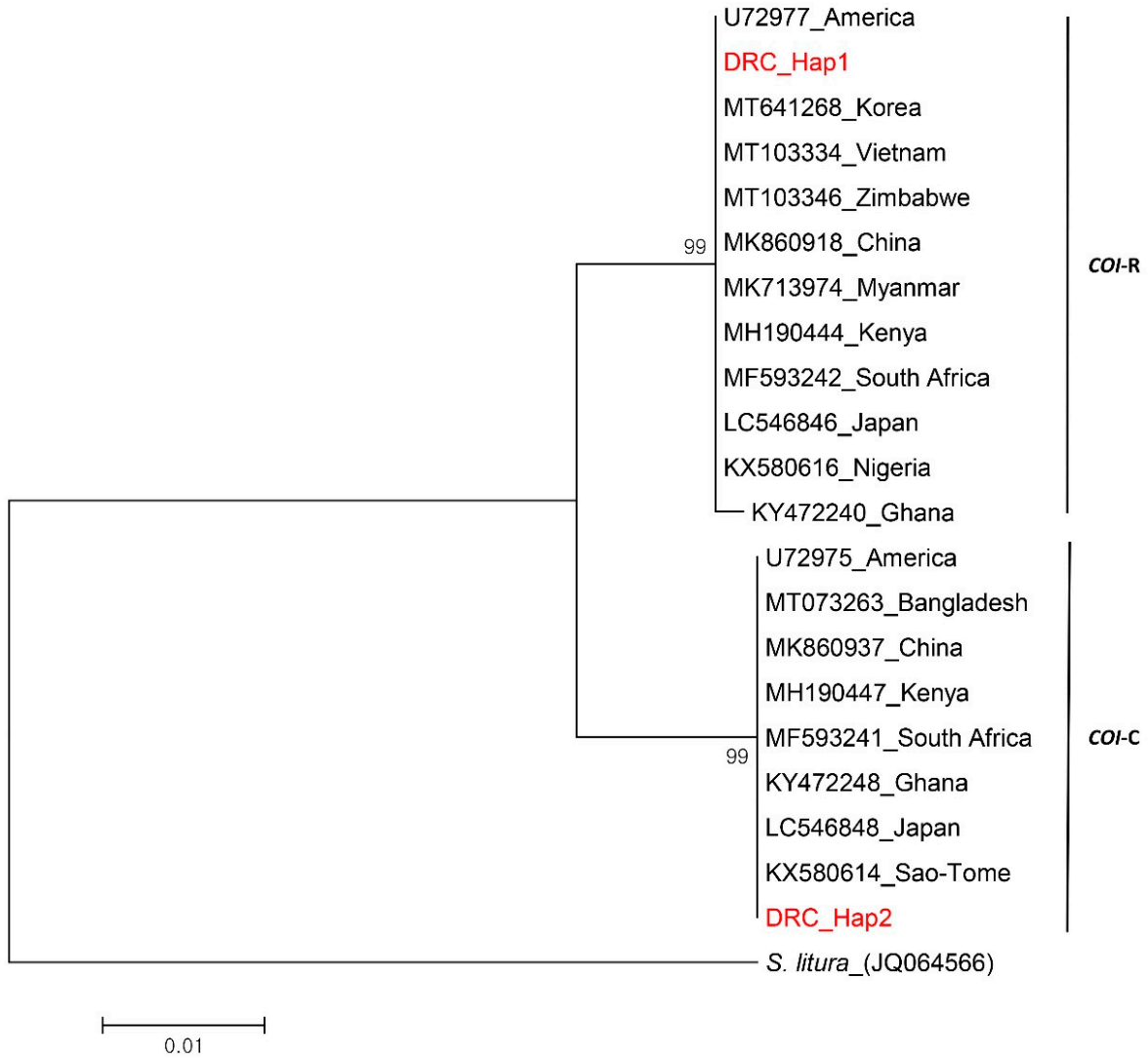

Figure 3. A phylogenetic tree derived from a maximum likelihood analysis comparing the two DRC *COI* haplotypes with those from *Spodoptera frugiperda* host strains of other invaded and native regions. For each phylogeny, 1000 bootstrap replicates were used to assess robustness using the Hasegawa–Kishino–Yano (HKY850) model, and gamma distribution rates of variation between sites were used to construct the phylogenetic tree in MEGA6.

Table 3. Results of analysis of molecular variance (AMOVA) among the four fall armyworm *Spodoptera frugiperda* geographical groups.

Group	Source	df	SS	Variance Component	Total Variance	*p*-Value
All	Among groups	3	71.411	0.2369	12.70	0.0001
	Among populations within groups	22	94.082	0.2900	15.54	
	Within populations	283	379.008	1.3392	71.76	
	Total	308	544.502	1.86629		
DRC and Africa	Among groups	1	0.019	−0.04839	−4.33	0.17595
	Among populations within groups	12	19.429	0.07543	6.75	
	Within populations	96	104.744	1.09108	97.58	
	Total	109	124.191	1.11811		

Table 3. *Cont.*

Group	Source	df	SS	Variance Component	Total Variance	*p*-Value
America and DRC	Among groups	1	18.325	0.25957	10.94	0.0001
	Among populations within groups	11	86.942	0.63554	26.79	
	Within populations	142	209.759	1.47718	62.27	
	Total	154	315.026	2.37228		
Asia and DRC	Among groups	1	0.154	−0.06807	−4.44	0.1700
	Among populations within groups	10	22.870	0.11554	7.54	
	Within populations	81	120.245	1.48451	96.90	
	Total	92	143.269	1.53197		
Africa and America	Among groups	1	5.473	0.03769	11.17	0.0001
	Among populations within groups	12	10.107	0.04366	12.94	
	Within populations	206	52.757	0.25610	75.89	
	Total	219	68.336	0.33745		
America and Asia	Among groups	1	38.821	0.25114	11.51	0.0001
	Among populations within groups	11	94.217	0.54236	24.85	
	Within populations	190	263.859	1.38873	63.64	
	Total	202	396.897	2.18223		
Africa and Asia	Among groups	1	0.132	−0.04126	−3.48	0.0400
	Among populations within groups	12	26.895	0.11284	9.52	
	Within populations	147	163.694	1.11356	93.96	
	Total	160	190.720	1.18514		

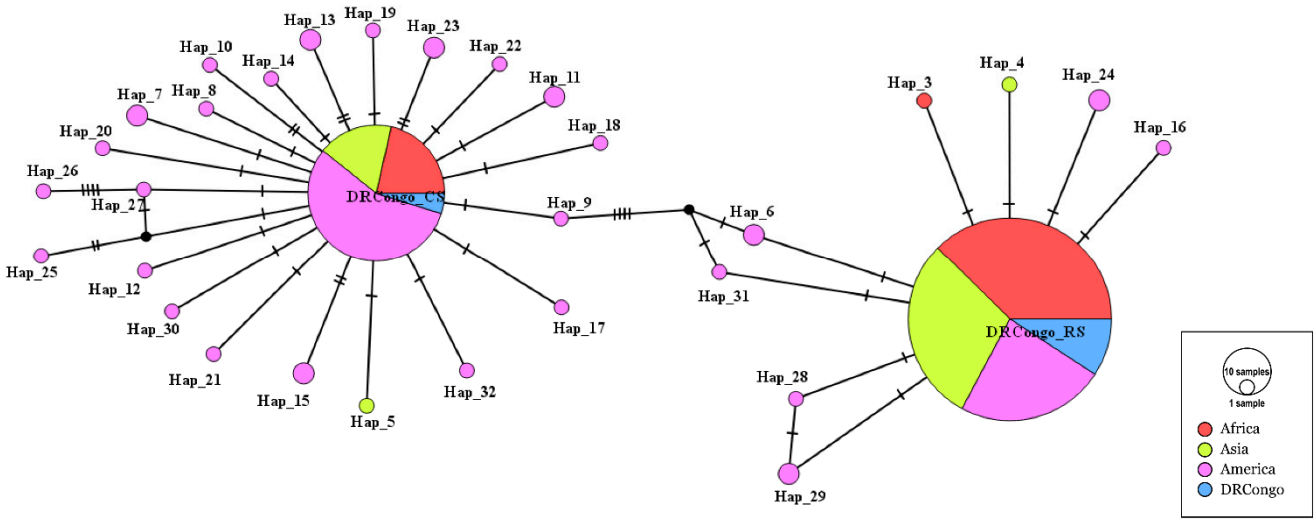

Figure 4. Median-joining haplotype network of the fall armyworm *Spodoptera frugiperda* mt*COI* gene partial sequences from four geographical groups (DRC, Africa, America, and Asia). Each pie represents a distinct haplotype, with the radius equal to the number of sequences belonging to that haplotype.

4. Discussion

This study aimed to analyze the genetic diversity and distribution of the FAW population that invaded the DRC. The findings suggest low genetic variability in the Congolese FAW population as only two haplotypes from each of the genes (*COI* and *Tpi*) were recovered. Most (84%) of the samples were *COI*-R, whereas *COI*-C occurred in 16%. These findings were consistent with those of a recent study conducted in Uganda, a neighboring country [32], and a previous report from 11 sub-Saharan African countries, including two provinces of the DRC [19]. Based on the *COI* marker, both corn and rice strains were detected in FAW specimens collected from corn fields, despite the known association of

strains to their host plant [35,36]. Similar findings have been reported in several African and Asian countries [19,22,32]. These findings suggested that the discordance between the *COI* marker and host plants may be due to FAW's plasticity in plant choice or the inability of the marker to reliably discriminate between the corn and rice strains of FAW.

The *COI*-based analysis of population genetics test statistics revealed that the FAW populations in the DRC, like those from the rest of Africa, are evolving in a neutral pattern. This neutral pattern was further supported by the absence of novel haplotypes and the low genetic diversity in FAW populations from the DRC. In contrast, the haplotype network of FAW populations in America indicates that the populations are still expanding. Thus, our findings indicate that America is still the main point of FAW population expansion. Our findings corroborate those of previous studies which also recorded that the FAW populations in Africa and America were still evolving neutrally and expanding, respectively [25,32].

Analysis of the partial sex-linked *Tpi* nuclear gene showed an 84% detection discrepancy between the *COI* and *Tpi* markers in the DRC FAW population, an observation that corroborated previous findings from other invaded regions of Africa and Asia [19,22]. In this study, we observed a dominance of the hybrid *Tpi*-C/*COI*-R individuals over the homogeneous *Tpi*-C/*COI*-C individuals among specimens collected from the corn host plant, suggesting that the *Tpi* marker is more accurate in determining the FAW–host strain association than the *COI* marker. Previously, Nagoshi et al. [17] and Nayer et al. [25] found that the hybrid *Tpi*-C/*COI*-R and the homogenous *Tpi*-C/*COI*-C were equally distributed in Central Africa, whereas in eastern and southern Africa and India, the hybrid strain predominated. Our study did not detect *Tpi*-R/*COI*-R homogenous individuals in the DRC FAW population, which occur in the western hemisphere but are rare in Africa [37]. These results are similar to those of previous studies showing that the homogeneous strain was more marginally distributed in invaded regions than the hybrid strain [19,25,32]. This hybrid strain is expected to result from the small initial interstrain mating populations explained by the admixture regularly seen during invasive events [38]. However, interstrain hybrids may have a large fitness advantage and become more prevalent in the invasive population, including in the FAW population from the DRC, eventually leading to the extinction of one or both strains in favor of more unique hybrid genotypes. These findings, combined with those of Nagoshi et al. [19] suggest that the unique African rice strain *Tpi* haplotype of the FAW found in several African regions may be rare in the DRC. In fact, Nagoshi et al. [19] detected the *Tpi*-Ra1 in the FAW specimens from the Haut-Katanga region of the DRC but not in those from the Sud-Ubangi region, which is in line with our results. These results have implications for the assessment of the crops at risk and the design of FAW management measures in the DRC. Further assessments are needed in other regions of the DRC.

Analysis of the fourth exon and intron regions of the nuclear *Tpi* gene showed the existence of two subgroups of the *Tpi*-Ca genetic group, including the predominant *Tpi*-Ca1, and minor *Tpi*-Ca2 subgroups. Further analysis showed the presence of two polymorphic variants, *Tpi*-Ca2a and *Tpi*-Ca2b, which have A or C at nucleotide 148 of Tpi-I4. The predominance of *Tpi*-Ca1a over *Tpi*-Ca2a and *Tpi*-Ca2b in invading FAW populations was also observed in Uganda [32], India [25], and several African and Asian regions [19,22].

As a highly polyphagous crop pest with a larval stage able to feed on the aerial parts of a wide range of plants, FAW can develop and establish for several generations in the DRC owing to its favorable biodiversity [39]. However, our findings combined with those of previous studies indicate that the FAW population in the DRC feeds mainly on corn plants and rarely on other plants [17,22]. This observation calls into question the nature of the hybrid genotype (*Tpi*-C/*COI*-R) detected in this study. Thus, at the molecular level, it seems that corn preference is more associated with the *Tpi* gene marker than the *COI* gene marker. This finding is not completely new because previous studies in invaded regions of Africa and Asia recorded the same genetic pattern in FAW [17,32]. These similarities in genotype features between invading populations of FAW provide evidence of a common origin

and should be used for further evolutionary studies that include the FAW whole genome sequence to better understand FAW population dynamics and subsequent dissemination in the DRC.

In summary, this study aimed to analyze the genetic diversity and distribution of the FAW population six years after the first reports of FAW invasion in the DRC. Our findings suggest that the FAW population that invaded the DRC is still evolving neutrally with a low number of haplotypes based on the *COI* gene marker. The observed low numbers of haplotypes and the hybrid nature of the FAW population in the DRC could be explained by a single introduction followed by a rapid dispersion through natural and trade-related processes. This finding combined with further studies on the migration dynamics may serve as important tools for the management of this crop pest in the DRC. Additionally, our findings showed that the nuclear *Tpi* gene marker was more accurate in determining the host association of FAW than the *COI* gene marker. Based on both the *COI* and *Tpi* markers, our study detected three genetic groups in the DRC's FAW populations, including the hybrids *Tpi*-Ca1/*COI*-R, *Tpi*-Ca2/*COI*-R, and the homogeneous *Tpi*-Ca1/*COI*-C. These results will serve as baseline resource for future studies on how the invading FAW population may change to adapt to the DRC's bio-environment and in the design of management measures. However, additional genotype surveys in other regions of the country combined with more evolutionary studies are required to refine knowledge of the FAW population dynamics and subsequent spreading routes of the pest.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Details of FAW COI gene sequences used in the present study.

(A) COI gene sequences from America			
No.	**GenBank Accession**	**Location**	**Year Submitted**
1.	KX281221.1	Canada	2017
2.	U72978.1	USA	1996
3.	U72977.1	USA	1996
4.	U72976.1	USA	1996
5.	U72975.1	USA	1996
6.	U72974.1	USA	1996
7.	KT809294.1	Brazil	2018
8.	KT809293.1	Brazil	2018
9.	KT809292.1	Brazil	2018
10.	KT809291.1	Brazil	2018
11.	KT809290.1	Brazil	2018
12.	KT809289.1	Brazil	2018
13.	KT809288.1	Brazil	2018
14.	KT809287.1	Brazil	2018
15.	KT809286.1	Brazil	2018

Table A1. *Cont.*

16.	KT809285.1	Brazil	2018
17.	KT809284.1	Brazil	2018
18.	KT809283.1	Brazil	2018
19.	KT809282.1	Brazil	2018
20.	KT809281.1	Brazil	2018
21	KT809280.1	Brazil	2018
22.	KT809279.1	Brazil	2018
23.	KT809278.1	Brazil	2018
24.	KT809277.1	Brazil	2018
25.	KT809276.1	Brazil	2018
26.	KT809275.1	Brazil	2018
27.	KT809274.1	Brazil	2018
28.	KT809273.1	Brazil	2018
29.	KT809272.1	Brazil	2018
30.	KT809271.1	Brazil	2018
31.	KT809270.1	Brazil	2018
32.	KT809269.1	Brazil	2018
33.	KT809268.1	Brazil	2018
34.	KT809267.1	Brazil	2018
35.	KT809266.1	Brazil	2018
36.	KT809265.1	Brazil	2018
37.	KT809264.1	Brazil	2018
38.	KT809263.1	Brazil	2018
39.	KT809262.1	Brazil	2018
40.	KT809261.1	Brazil	2018
41.	KT809260.1	Brazil	2018
42.	KT809259.1	Brazil	2018
43.	KT809258.1	Brazil	2018
44.	KT809257.1	Brazil	2018
45.	KT809256.1	Brazil	2018
46.	KT809255.1	Brazil	2018
47.	KT809254.1	Brazil	2018
48.	KT809253.1	Brazil	2018
49.	KT809252.1	Brazil	2018
50.	KT809251.1	Brazil	2018
51.	KT809250.1	Brazil	2018
52.	KT809249.1	Brazil	2018
53.	KT809248.1	Brazil	2018
54.	KT809247.1	Brazil	2018
55.	KT809246.1	Brazil	2018
56.	KT809245.1	Brazil	2018
57.	KT809244.1	Brazil	2018
58.	KT809243.1	Brazil	2018
59.	KT809242.1	Brazil	2018
60.	KT809241.1	Brazil	2018
61.	KT809240.1	Brazil	2018
62.	KT809239.1	Brazil	2018
63.	KT809238.1	Brazil	2018
64.	KT809237.1	Brazil	2018
65.	KT809236.1	Brazil	2018
66.	KT809235.1	Brazil	2018
67.	KJ634298.1	Suriname	2014
68.	KJ634297.1	Honduras	2014
69.	MK318422.1	Mexico	2019
70.	MK318420.1	Mexico	2019
71.	MK318377.1	Puerto Rico	2019
72.	MK318373.1	Puerto Rico	2019
73.	MK318372.1	Mexico	2019
74.	MK318311.1	Mexico	2019
75.	MK318297.1	Dominican	2019

Table A1. *Cont.*

76.	GU439151.1	Ontario	2018
77.	GU439150.1	Puslinch	2018
78.	GU439149.1	Puslinch	2018
79.	GU439148.1	Puslinch	2018
80.	GU439147.1	Puslinch	2018
81.	GU090724.1	Puslinch	2018
82.	GU090723.1	Puslinch	2018
83.	GU095403.1	New Brunswick	2018
84.	GU094756.1	Puslinch	2018
85.	GU094755.1	Puslinch	2018
86.	GU094754.1	Puslinch	2018
87.	KJ388147.1	Quebec	2018
88.	HM102314.1	USA	2016
89.	KJ641998.1	Guano	2015
90.	KJ641997.1	Guano	2015
91.	KF624877.1	Roraima	2014
92.	KF624876.1	Roraima	2014
93.	JQ559528.1	Costa Rica	2012
94.	JQ554012.1	Costa Rica	2012
95.	JQ572603.1	Costa Rica	2012
96.	JQ571459.1	Costa Rica	2012
97.	JQ547900.1	Costa rica	2012
98.	JQ577923.1	Costa Rica	2012
99.	JF854747.1	Campina Grande	2012
100.	JF854746.1	Morretes	2012
101.	JF854745.1	Morretes	2012
102.	JF854744.1	Campina Grande	2012
103.	JF854743.1	Morretes	2012
104.	JF854741.1	Morretes	2012
105.	JF854740.1	Morretes	2012
106.	HQ964527.1	Massachusetts	2012
107.	HQ964487.1	Massachusetts	2012
108.	HQ964486.1	Massachusetts	2012
109.	HQ964485.1	Massachusetts	2012
110.	HQ964443.1	Massachusetts	2012
111.	HQ964441.1	Massachusetts	2012
112.	HQ964442.1	Massachusetts	2012
113.	HQ964440.1	Massachusetts	2012
114.	HQ964439.1	Massachusetts	2012
115.	HQ964394.1	Massachusetts	2012
116.	HQ964393.1	Massachusetts	2012
117.	HQ964352.1	Massachusetts	2012
118.	HQ964351.1	Massachusetts	2012
119.	GU159435.1	Costa Rica	2012
120.	GU159434.1	Costa Rica	2012
121.	GU159433.1	Costa Rica	2012
122.	GU159432.1	Costa Rica	2012
123.	GU159431.1	Costa Rica	2012
124.	GU159430.1	Costa Rica	2012
125.	GU159429.1	Costa Rica	2012
126.	GU658451.1	Alvaro Obregon	2019
(B) COI gene sequences from Africa			
No.	**GenBank Accession**	**Location**	**Year Submitted**
1.	MF593258.1	South Africa	2018
2.	MF593257.1	South Africa	2018
3.	MF593256.1	South Africa	2018
4.	MF593255.1	South Africa	2018
5.	MF593254.1	South Africa	2018
6.	MF593253.1	South Africa	2018

Table A1. *Cont.*

7.	MF593252.1	South Africa	2018
8.	MF593251.1	South Africa	2018
9.	MF593250.1	South Africa	2018
10.	MF593249.1	South Africa	2018
11.	MF593248.1	South Africa	2018
12.	MF593247.1	South Africa	2018
13.	MF593246.1	South Africa	2018
14.	MF593245.1	South Africa	2018
15.	MF593244.1	South Africa	2018
16.	MF593243.1	South Africa	2018
17.	MF593242.1	South Africa	2018
18.	MF593241.1	South Africa	2018
19	MK493020.1	South Africa	2019
20.	MK493019.1	South Africa	2019
21.	MK493018.1	South Africa	2019
22.	MK493017.1	South Africa	2019
23.	MK493016.1	South Africa	2019
24.	MT933058	Tanzania	2020
	MT103348	Tanzania	
25.	MT103346.1	Zimbabwe	2020
	MT103347	Zimbabwe	
26.	KX580619.1	Nigeria	2016
27.	KX580618.1	Nigeria	2016
28.	KX580617.1	Nigeria	2016
29.	KX580616.1	Nigeria	2016
30.	KX580615.1	Sao-Tome,	2016
31.	KX580614.1	Sao-Tome	2016
32.	MT641267.1	Uganda	2020
33.	MF278659.1	Tanzania	2018
34.	MF278658.1	Tanzania	2018
35.	MF278657.1	Tanzania	2018
36.	MH190448.1	Kenya	2018
37.	MH190447.1	Kenya	2018
38.	MH190446.1	Kenya	2018
39.	MH190445.1	Kenya	2018
40.	MH190444.1	Kenya	2018
41.	KY472255.1	Ghana	2017
42.	KY472254.1	Ghana	2017
43.	KY472253.1	Ghana	2017
44.	KY472252.1	Ghana	2017
45.	KY472251.1	Ghana	2017
46.	KY472250.1	Ghana	2017
47.	KY472249.1	Ghana	2017
48.	KY472248.1	Ghana	2017
49.	KY472245.1	Ghana	2017
50.	KY472244.1	Ghana	2017
51.	KY472242.1	Ghana	2017
52.	KY472241.1	Ghana	2017
53.	KY472240.1	Ghana	2017
54.	MG993205.1	Malawi: Sande	2018
55.	MF197867.1	Uganda	2018
56.	MK493006.1	Kenya	2019
57.	MK493000.1	Kenya	2019
58.	MK492996.1	Kenya	2019
59.	MK493010.1	Kenya	2019
60.	MK493009.1	Kenya	2019
61.	MK493008.1	Kenya	2019
62.	MK493007.1	Kenya	2019
63.	MK493004.1	Kenya	2019
64.	MK493003.1	Kenya	2019

Table A1. *Cont.*

65.	MK493002.1	Kenya	2019
66.	MK493001.1	Kenya	2019
67.	MK492999.1	Kenya	2019
68.	MK492998.1	Kenya	2019
69.	MK492997.1	Kenya	2019
70.	MK492995.1	Kenya	2019
71.	MK492994.1	Kenya	2019
72.	MK492993.1	Kenya	2019
73.	MK492992.1	Kenya	2019
74.	MK492991.1	Kenya	2019
75.	MK492990.1	Kenya	2019
76.	MK492989.1	Kenya	2019
77.	MK492988.1	Kenya	2019
78.	MK492987.1	Kenya	2019
79.	MK492986.1	Kenya	2019
80.	MK492985.1	Kenya	2019
81.	MK492984.1	Kenya	2019
82.	MK492983.1	Kenya	2019
83.	MK492982.1	Kenya	2019
84.	MK492981.1	Kenya	2019
85	MK492972.1	Uganda	2018
86	MK492971.1	Uganda	
87	MK492970.1	Uganda	2022
88	MK492969.1	Uganda	2022
89	MK492958.1	Tanzania	2020

(C) COI gene sequences from Asia

No.	GenBank Accession	Location	Year Submitted
1.	MT103344.1	Bangladesh: Dhaka	2020
2.	MT103343.1	Bangladesh: Dhaka	2020
3.	MT103342.1	South Korea: Gyeongsan	2020
4.	MT103341.1	Viet Nam: Ninh binh	2020
5.	MT103340.1	Viet Nam: Ninh binh	2020
6.	MT103339.1	Viet Nam: Ha noi	2020
7.	MT103338.1	Viet Nam: Vinh phuc	2020
8.	MT103336.1	Viet Nam: Hanoi	2020
9.	MT103335.1	Viet Nam: Vinh Phuc	2020
10.	MT103334.1	Viet Nam: Ninh Binh	2020
11.	MT641270.1	South Korea: Gyeongsan	2020
12.	MT641269.1	South Korea: Jeju	2020
13.	MT641268.1	South Korea: Campus	2020
14.	LC546868.1	Japan: Aomori	2020
15.	LC546867.1	Japan: Aomori	2020
16.	LC546866.1	Japan: Iwate	2020
17.	LC546865.1	Japan: Kanagawa	2020
18.	LC546864.1	Japan: Chiba	2020
19.	LC546863.1	Japan: Fukushima	2020
20.	LC546862.1	Japan: Ibaraki	2020
21	LC546861.1	Japan: Ibaraki	2020
22.	LC546860.1	Japan: Miyazaki	2020
23.	LC546859.1	Japan: Miyazaki	2020
24.	LC546858.1	Japan: Miyazaki	2020
25.	LC546857.1	Japan: Okinawa	2020
26.	LC546856.1	Japan: Okinawa	2020
27.	LC546855.1	Japan: Okinawa	2020
28.	LC546854.1	Japan: Kagoshima	2020
29.	LC546853.1	Japan: Kagoshima	2020
30.	LC546852.1	Japan: Kagoshima	2020
31.	LC546851.1	Japan: Kagoshima	2020
32.	LC546850.1	Japan: Kagoshima	2020

Table A1. *Cont.*

33.	LC546849.1	Japan: Kagoshima	2020
34.	LC546848.1	Japan: Kagoshima	2020
35.	LC546847.1	Japan: Kagoshima	2020
36.	LC546846.1	Japan: Kagoshima	2020
37.	MK913648.1	Viet Nam: Nghe An	2019
38.	MK913647.1	Viet Nam: Nghe An	2019
39.	MK913646.1	Viet Nam: Ha Noi	2019
40.	MK860942.1	China: Tengchong, Yunnan	2019
41.	MK860941.1	China: Tengchong, Yunnan	2019
42.	MK860940.1	China: Tengchong, Yunnan	2019
43.	MK860939.1	China: Tengchong, Yunnan	2019
44.	MK860938.1	China: Tengchong, Yunnan	2019
45.	MK860937.1	China: Tengchong, Yunnan	2019
46.	MK860936.1	China: Ruili, Yunnan	2019
47.	MK860935.1	China: Ruili, Yunnan	2019
48.	MK860934.1	China: Ruili, Yunnan	2019
49.	MK860933.1	China: Ruili, Yunnan	2019
50.	MK860932.1	China: Ruili, Yunnan	2019
51.	MK860931.1	China: Ruili, Yunnan	2019
52.	MK860930.1	China: Ruili, Yunnan	2019
53.	MK860927.1	China: Ruili, Yunnan	2019
54.	MK860926.1	China: Ruili, Yunnan	2019
55.	MK860925.1	China: Ruili, Yunnan	2019
56.	MK860924.1	China: Ruili, Yunnan	2019
57.	MK860923.1	China: Mangshi, Yunnan	2019
58.	MK860922.1	China: Mangshi, Yunnan	2019
59.	MK860921.1	China: Mangshi, Yunnan	2019
60.	MK860920.1	China: Mangshi, Yunnan	2019
61.	MK860919.1	China: Mangshi, Yunnan	2019
62.	MK860918.1	China: Mangshi, Yunnan	2019
63.	MK713974.1	Myanmar	2019
64.	MN075831.1	China	2019
65.	MN075830.1	China	2019
66.	MK913645.1	Viet Nam: Ninh Binh	2019
67.	MT073263.1	Bangladesh: Gazipur	2020
68.	MT180097.1	Pakistan	2020
69.	OP132904.1	South Korea	2020
70.	MT073264.1	Bangladesh: Bogura	2020
71.	MT073266.1	Bangladesh: Jamalpur	2020
72.	MT073265.1	Bangladesh: Rangpur	2020

References

1. Navik, O.; Shylesha, A.N.; Patil, J.; Venkatesan, T.; Lalitha, Y.; Ashika, T.R. Damage, distribution and natural enemies of invasive fall armyworm *Spodoptera frugiperda* (J.E. smith) under rainfed maize in Karnataka, India. *Crop Prot.* **2021**, *143*, 105536. [CrossRef]
2. Day, R.; Abrahams, P.; Bateman, M.; Beale, T.; Clottey, V.; Cock, M.; Colmenarez, Y.; Corniani, N.; Early, R.; Godwin, J.; et al. Fall armyworm: Impacts and implications for Africa. *Outlooks Pest Manag.* **2017**, *28*, 196–201. [CrossRef]
3. Goergen, G.; Kumar, P.L.; Sankung, S.B.; Togola, A.; Tamò, M. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS ONE* **2016**, *11*, e0165632. [CrossRef] [PubMed]
4. Qi, G.J.; Ma, J.; Wan, J.; Ren, Y.L.; McKirdy, S.; Hu, G.; Zhang, Z.F. Source regions of the first immigration of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) invading Australia. *Insects* **2021**, *12*, 1104. [CrossRef] [PubMed]
5. Rane, R.; Walsh, T.K.; Lenancker, P.; Gock, A.; Dao, T.H.; Nguyen, V.L.; Khin, T.N.; Amalin, D.; Chittarath, K.; Faheem, M.; et al. Complex multiple introductions drive fall armyworm invasions into Asia and Australia. *Sci. Rep.* **2023**, *13*, 660. [CrossRef] [PubMed]
6. Malekera, M.J.; Acharya, R.; Mostafiz, M.; Hwang, H.; Bhusal, N. Temperature-dependent development models describing the effects of temperature on the development of the fall armyworm *Spodoptera frugiperda* (J.E. Smith). *Insects* **2022**, *13*, 1084. [CrossRef] [PubMed]
7. Du Plessis, H.; Schlemmer, M.L.; Van den Berg, J. The effect of temperature on the development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insects* **2020**, *11*, 228. [CrossRef]

8. Montezano, D.G.; Specht, A.; Sosa-Gómez, D.R.; Roque-Specht, V.F.; Sousa-Silva, J.C.; Paula-Moraes, S.V.; Peterson, J.A.; Hunt, T.E. Host Plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. *African Entomol.* **2018**, *26*, 286–300. [CrossRef]
9. Vickery, R.A. *Studies on the Fall Army Worm in the Gulf Coast District of Texas*; United States Department of Agriculture: Washington, DC, USA, 1929.
10. Capinera, J.L. *Fall Armyworm, Spodoptera frugiperda (J.E. Smith) (Insecta: Lepidoptera: Noctuidae)*; University of Florida IFAS Extension: Gainesville, FL, USA, 2002.
11. Prowell, D.P.; McMichael, M.; Silvain, J.F. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* **2004**, *97*, 1034–1044. [CrossRef]
12. Kergoat, G.J.; Prowell, D.P.; Le Ru, B.P.; Mitchell, A.; Dumas, P.; Clamens, A.L.; Condamine, F.L.; Silvain, J.F. Disentangling dispersal, vicariance and adaptive radiation patterns: A case study using armyworms in the pest genus *Spodoptera* (Lepidoptera: Noctuidae). *Mol. Phylogenet. Evol.* **2012**, *65*, 855–870. [CrossRef]
13. Kenis, M.; Benelli, G.; Biondi, A.; Calatayud, P.-A.; Day, R.; Desneux, N.; Harrison, R.D.; Kriticos, D.; Rwomushana, I.; van den Berg, J.; et al. Invasiveness, biology, ecology, and management of the fall armyworm, *Spodoptera frugiperda*. *Entomol. Gen.* **2022**, *43*, 187–241. [CrossRef]
14. Meagher, R.L.; Nagoshi, R.N.; Armstrong, J.S.; Niogret, J.; Epsky, N.D.; Flanders, K.L. Captures and host strains of fall armyworm (Lepidoptera: Noctuidae) males in traps baited with different commercial pheromone blends. *Florida Entomol.* **2013**, *96*, 729–740. [CrossRef]
15. Nagoshi, R.N. The fall armyworm *triose phosphate isomerase* (*Tpi*) gene as a marker of strain identity and interstrain mating. *Ann. Entomol. Soc. Am.* **2010**, *103*, 283–292. [CrossRef]
16. Levy, H.C.; Garcia-Maruniak, A.; Maruniak, J.E. Strain identification of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) insects and cell line: PCR-RFLP of cytochrome oxidase C subunit I gene. *Florida Entomol.* **2003**, *85*, 186–190. [CrossRef]
17. Nagoshi, R.N.; Goergen, G.; Tounou, K.A.; Agboka, K.; Koffi, D.; Meagher, R.L. Analysis of strain distribution, migratory potential, and invasion history of fall armyworm populations in northern sub-Saharan Africa. *Sci. Rep.* **2018**, *8*, 3710. [CrossRef] [PubMed]
18. Nagoshi, R.N.; Htain, N.N.; Boughton, D.; Zhang, L.; Xiao, Y.; Nagoshi, B.Y.; Mota-Sanchez, D. Southeastern Asia fall armyworms are closely related to populations in Africa and India, consistent with common origin and recent migration. *Sci. Rep.* **2020**, *10*, 1421. [CrossRef]
19. Nagoshi, R.N.; Goergen, G.; Plessis, H.D.; van den Berg, J.; Meagher, R. Genetic comparisons of fall armyworm populations from 11 countries spanning sub-Saharan Africa provide insights into strain composition and migratory behaviors. *Sci. Rep.* **2019**, *9*, 8311. [CrossRef]
20. Tay, W.T.; Rane, R.; Padovan, A.; Walsh, T.; Elfekih, S.; Downes, S.; Nam, K.; d'Alençon, E.; Zhang, J.; Wu, Y.; et al. Whole genome sequencing of global *Spodoptera frugiperda* populations: Evidence for complex, multiple introductions across the Old World. *bioRxiv* **2020**, bioRxiv:2020.06.12.147660.
21. Yainna, S.; Tay, W.T.; Fiteni, E.; Legeai, F.; Clamens, A.L.; Gimenez, S.; Frayssinet, M.; Asokan, R.; Kalleshwaraswamy, C.M.; Deshmukh, S.; et al. Genomic balancing selection is key to the invasive success of the fall armyworm. *bioRxiv* **2020**. [CrossRef]
22. Acharya, R.; Akintola, A.A.; Malekera, M.J.; Kamulegeya, P.; Nyakunga, K.B.; Mutimbu, M.K.; Shrestha, Y.K.; Hemayet, J.S.M.; Hoat, T.X.; Dao, H.T.; et al. Genetic relationship of fall armyworm (*Spodoptera frugiperda*) populations that invaded Africa and Asia. *Insects* **2021**, *12*, 439. [CrossRef] [PubMed]
23. Nagoshi, R.N.; Dhanani, I.; Asokan, R.; Mahadevaswamy, H.M.; Kalleshwaraswamy, C.M.; Sharanabasappa; Meagher, R.L. Genetic characterization of fall armyworm infesting South Africa and India indicate recent introduction from a common source population. *PLoS ONE* **2019**, *14*, e0217755. [CrossRef] [PubMed]
24. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]
25. Nayyar, N.; Gracy, R.G.; Ashika, T.R.; Mohan, G.; Swathi, R.S.; Mohan, M.; Chaudhary, M.; Bakthavatsalam, N.; Venkatesan, T. Population structure and genetic diversity of invasive fall armyworm after 2 years of introduction in India. *Sci. Rep.* **2021**, *11*, 7760. [CrossRef] [PubMed]
26. Rozas, J.; Ferrer-Mata, A.; Sanchez-DelBarrio, J.C.; Guirao-Rico, S.; Librado, P.; Ramos-Onsins, S.E.; Sanchez-Gracia, A. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* **2017**, *34*, 3299–3302. [CrossRef] [PubMed]
27. Laura Juárez, M.; Gabriela Murúa, M.; Gabriela García, M.; Ontivero, M.; Teresa Vera, M.; Vilardi, J.C.; Groot, A.T.; Castagnaro, A.P.; Gastaminza, G.; Willink, E. Host association of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn and rice strains in Argentina, Brazil, and Paraguay. *J. Econ. Entomol.* **2012**, *105*, 573–582. [CrossRef] [PubMed]
28. Nagoshi, R.N.; Fleischer, S.; Meagher, R.L.; Hay-Roe, M.; Khan, A.; Murúa, M.G.; Silvie, P.; Vergara, C.; Westbrook, J. Fall armyworm migration across the lesser antilles and the potential for genetic exchanges between north and south American populations. *PLoS ONE* **2017**, *12*, e0171743. [CrossRef]
29. Leigh, J.W.; Bryant, D. POPART: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* **2015**, *6*, 1110–1116. [CrossRef]
30. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [CrossRef]
31. Sayers, E.W.; Bolton, E.E.; Brister, J.R.; Canese, K.; Chan, J.; Comeau, D.C.; Connor, R.; Funk, K.; Kelly, C.; Kim, S.; et al. Database resources of the national center for biotechnology information. *Nucleic Acids Res.* **2022**, *50*, D20–D26. [CrossRef]

32. Omuut, G.; Mollel, H.G.; Kanyesigye, D.; Akohoue, F.; Adumo Aropet, S.; Wagaba, H.; Otim, M.H. Genetic analyses and detection of point mutations in the *acetylcholinesterase-1* gene associated with organophosphate insecticide resistance in fall armyworm (*Spodoptera frugiperda*) populations from Uganda. *BMC Genom.* **2023**, *24*, 22. [CrossRef]
33. Felsenstein, J. Confidence Limits on Phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783. [CrossRef]
34. Excoffier, L.; Laval, G.; Schneider, S. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol. Bioinforma.* **2005**, *1*, 117693430500100. [CrossRef]
35. Nagoshi, R.N.; Meagher, R.L.; Flanders, K.; Gore, J.; Jackson, R.; Lopez, J.; Armstrong, J.S.; Buntin, G.D.; Sansone, C.; Leonard, B.R. Using haplotypes to monitor the migration of fall armyworm (Lepidoptera: Noctuidae) corn-strain populations from Texas and Florida. *J. Econ. Entomol.* **2008**, *101*, 742–749. [CrossRef] [PubMed]
36. Murúa, M.G.; Nagoshi, R.N.; Santos, D.A.D.; Hay-Roe, M.M.; Meagher, R.L.; Vilardi, J.C. Demonstration using field collections that Argentina fall armyworm populations exhibit strain-specific host plant preferences. *J. Econ. Entomol.* **2015**, *108*, 2305–2315. [CrossRef] [PubMed]
37. Nagoshi, R.N. Evidence that a major subpopulation of fall armyworm found in the Western Hemisphere is rare or absent in Africa, which may limit the range of crops at risk of infestation. *PLoS ONE* **2019**, *14*, e0208966. [CrossRef]
38. Kolbe, J.J.; Glor, R.E.; Schettino, L.R.; Lara, A.C.; Larson, A.; Losos, J.B. Genetic variation increases during biological invasion by a Cuban lizard. *Nature* **2004**, *431*, 177–181. [CrossRef] [PubMed]
39. The World Bank Group. *Climate Risk Profile: Congo, Democratic Republic*; The World Bank Group: Washington, DC, USA, 2021.