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Analysis of Genetic Diversity of Banana Weevils (*Cosmopolites sordidus*) (Coleoptera: Curculionidae) Using Transcriptome-Derived Simple Sequence Repeat Markers

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Abstract

The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is an economically important insect pest of bananas. It causes up to 100% yield losses and substantial lifespan reduction in bananas. Advances in genomics, proteomics, and sequencing technologies have provided powerful pathways to genotyping disastrous pests such as *C. sordidus*. However, such technologies are often not available to the majority of rural subtropical African banana growers and pest control managers. This study was therefore motivated by the need to create cheap and easily accessible *C. sordidus* genotyping methods that could be deployed by banana pest control managers to the benefit of *C. sordidus* control programs in the tropics where such advanced technologies are not readily accessible. We used an in-house *C. sordidus* transcriptome from the an-ongoing study from which we mined an array of simple sequence repeat (SSR) markers. Of these, six highly polymorphic transcriptome-derived SSR markers were used to successfully genotype within and among banana weevil population genetic diversity of 12 *C. sordidus* populations collected from four banana-growing agro-ecological zones (AEZs) in Uganda. The developed transcriptome-derived SSR markers can be used by researchers in population genetics for characterization of the *C. sordidus* and identification of new genes that are linked to traits of particular interest. The significant genetic diversity revealed in *C. sordidus* provides pertinent information for integrated pest management strategies.

Key words: Musa, Cosmopolites *sordidus*, simple sequence repeat (SSR) marker, genetic diversity, Uganda

The Banana weevil (Fig. 1) is a monophagous insect pest of bananas causing devastating economic losses (Gold et al. 2001, Abera-Kalibata et al. 2006). The pest follows a *K*-selected life cycle (Uzakah and Odebiyi 2015), with a four-years life span and low fecundity (Gold and Messiaen 2000). The adult weevil lays eggs at the bottom sheath of the banana pseudostem and the emerging larvae tunnels through the corm as it feeds on the plant (Gold et al. 2001). This causes secondary rots which restrict water and nutrient uptake along with the weakened plant anchorage into the soil (Gold et al. 2001). Short banana lifespan reduced bunch weights, mat die-out, and 100% yield losses have been associated with larvae damage (Gold et al. 2001, Ocan et al. 2008). The level of weevil damage on banana plantations depends on the age of the banana plantation at infestation and the management practices followed (Masanza 2003).

C. sordidus is native to Southeast Asia and its occurrence in Uganda was first reported in 1960s in the traditional banana growing areas of the central region (Rukazambuga et al. 1998). Overtime, the pest has expanded to many other banana growing districts (Gold et al. 1999b, 2001). This is attributed to the transportation of planting materials from infested areas, climatic and farming systems changes (Erima et al. 2017, Murongo et al. 2019). *C. sordidus*-associated banana damage has been reported to vary

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Fig. 1. Banana weevil *cosmopolite sordidus* on the bench.

considerably across the banana growing districts. For example, yield losses of 20–60%, and up to 100% have been reported in the central districts of Masaka and Rakai respectively. On the other hand, negligible yield loss in banana yield due to *C. sordidus* has been reported in south-western Uganda districts (Gold et al. 1999b, Twesigye et al. 2018a). This yield loss difference is ascribed to local weevil populations (biotypes) with variable levels of virulence across the different banana growing areas (Ochieng 2001, Twesigye et al. 2018b).

Pesticide, biological, cultural, host resistance, and integrated pest management (IPM) studies have been intensified to devise means to control this rather economically important banana pest (Tumuhimbise et al. 2018, Twesigye et al. 2018a, Palanichamy et al. 2020). However, pesticide, biological and cultural control methods are not very effective and cost-effective due to the cryptic nature of *C. sordidus*, where the entire life cycle is almost completed within the corm, resulting into slow implementation of IPM control strategy (Gold et al. 2001). Integrated Pest Management (IPM) is the main *C. sordidus* control strategy involving cultural control practices, chemical use, biological control, and host resistance (Coyne et al. 2017). Current genetic diversity, population structure, gene flow, migration, and dispersal dynamics data sets are needed by pest control managers for targeted and effective IPM deployment for *C. sordidus* control (Agunbiade et al. 2013, Karsten et al. 2013). Information on population structure and genetic diversity of *C. sordidus* within a delimited geographic area can enhance integrated pest management programs by determining the logical locations of natural enemy releases or pesticides applications (Bergamo et al. 2018, Thangaraj et al. 2019). As such, time, costs, and resources are saved that could be misdirected without proper identification of the pest and management units.

C. sordidus genetic diversity and population structure have recently been studied using molecular markers such as random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), internal transcribed rDNA (ITS1+ITS2), and the mitochondrial COI $tRNA^{Leu}$-COII (Ochieng 2001, De Graaf 2008, Magaña et al. 2007, Kumar and Singh 2018, Twesigye et al. 2018a). However, these markers were only able to identify significant genetic diversity within but not among *C. sordidus* populations. This could be attributed to some drawbacks associated with these marker systems including poor reproducibility, dominance, errors borne with gel scoring, and maternal inheritance patterns (Shankar et al. 2015, Singh et al. 2017). Because of these limitations of current *C. sordidus* genotyping tools, there is a need to develop a robust marker system that can improve our knowledge of *C. sordidus* populations.

Simple sequence repeat marker development using conventional methods such as screening of SSR enriched or nonenriched genomic libraries with repetitive probes is time consuming, laborious, and expensive (Zane et al. 2002, Chen et al. 2016). These problems can be overcome by the use of Next-generation sequencing (NGS) and mining existing genome and transcriptome datasets (Li et al. 2019, Tian et al. 2019). Transcriptome sequences are sets of genes containing functional information (Shiel et al. 2015, Walker et al. 2019). Transcriptome-derived microsatellite markers are therefore situated close to or within functional genes (Duan et al. 2020) which enables the identification of new genes that are linked to traits of particular interest (Kim et al. 2008). As such, transcriptomic sequencing for mining SSR markers has been applied to study the genetic diversity of several insect species, such as *Rhagoletis pomonella* (Diptera: Tephritidae) (Schwarz et al. 2009), *Aphis glycines* (Hemiptera: Aphididae) (Bai et al. 2010), *Dolerus aeneus* (Hymenoptera: Symphyta) (Cook et al. 2011), *Maruca vitrata* (Margam et al. 2011), *Spodoptera exigua* (Lepidoptera: Noctuidae) (Pascual et al. 2012) and *Rhopalosiphum padi* (Hemiptera: Aphididae) (Duan et al. 2017), but not *C. sordidus*.

Advances in genomics, proteomics, and sequencing technologies offer powerful pathways to genotyping *C. sordidus* populations and helping pest control managers to devise effective means for their control. However, such technologies are often not available to most rural African banana growers and pest control managers. To contribute to the creation of a repertoire of genotyping techniques that could be used by pest control managers in low technology settings in the control of *C. sordidus,* we sought to develop an array of SSR markers. SSR markers are robust at genotyping because they are information-rich, co-dominant, and are highly polymorphic. Much as other sets of markers and sequencing techniques have been on the rise, SSRs are still one of the leading genotyping options based on their methodological simplicity that requires low DNA amounts for amplification making it quick, efficient, and cost-effective (Souza et al. 2018, Wang et al. 2019). Despite the inherent advantages of SSR markers for genotyping, they have largely not been used for genotyping *C. sordidus* populations due to limited publicly available *C. sordidus* genomic and transcriptomic datasets (Valencia et al. 2016). We, therefore, used an in-house *C. sordidus* transcriptome from the an-ongoing study from which we mined an array of SSR markers that we used to genotype *C. sordidus* populations from different banana growing agro-ecological zones (AEZs) of Uganda.

Materials and Methods

C. sordidus Sampling and DNA Extraction

With consent from farmers, *C. sordidus* samples were collected from banana fields using pseudostem traps (Jallow 2013). Three to four pseudostem traps were laid 7 km from each farmer's field to avoid sampling multiple members of the same maternal clone. A total of 807 *C. sordidus* beetles were collected from 12 populations (designated by districts of Uganda) (Table 1, Fig. 2). The 12 geographically distinct *C. sordidus* populations represent four banana growing agro-ecological zones (AEZs) of Uganda; Southwestern, Western, Central (Lake Victoria crescent), and Eastern (highlands) (Okurut et al. 2012). The weevils were then transported in aerated containers to the entomology laboratory. A sample of 100 *C. sordidus* were selected, sexed; thus 35 and 65 female and male adult *C. sordidus* specimens respectively. The female weevils were then preserved in absolute alcohol awaiting DNA extraction. The

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Table 1. Sampling details of 12 *C. sordidus* population from the four banana agro ecological zones of Uganda

Data collector: Ali Milton, Juliet Kemigisha and Betty Nyangwire

Sampling site	Populations (District)	Agro ecological zones	Sampling date	Number of weevils	Latitude (N)	Longitude (E)
Bukiyi	Sironko	Eastern	12 April 2018	21	N 01.17201	E 034.19905
Mafudu	Sironko	Eastern	12 April 2018	25	N 01.18558	E 034.19398
Bukhulo	Sironko	Eastern	12 April 2018	15	N 01.21120	E 034.19552
Butansi	Kamuli	Eastern	16 April 2018	27	N 00.9172	E 033.08117
Kitayungwa	Kamuli	Eastern	16 April 2018	13	N 00.87586	E 033.11296
Southern division	Kamuli	Eastern	16 April 2018	17	N 00.93662	E 033.09026
Mutoto	Mbale	Eastern	13 April 2018	10	N 01.05203	E 034.19897
Bukasakya	Mbale	Eastern	13 April 2018	28	N 01.03812	E 034.16894
Bungokho	Mbale	Eastern	13 April 2018	22	N 01.05203	E 034.19897
Karambi	Kabarole	Mid -western	6 June 2018	24	N 00.64501	E 030.23503
Mugusu	Kabarole	Mid- western	6 June 2018	14	N 00.62393	E 030.20544
Karago TC	Kabarole	Mid- western	6 June 2018	17	N 00.66937	E 030.20826
Karusandara	Kasese	Mid -western	9 June 2018	13	N 00.24211	E 030.16277
Maliba	Kasese	Mid -western	9 June 2018	30	N 00.32640	E 030.10347
Rwanyamehembe	Mbarara	Southwestern	15 June 2018	22	N 00.50357	E 030.60797
Rubindi	Mbarara	Southwestern	15 June 2018	17	S 00.37210	E 030.52991
Kakiika	Mbarara	Southwestern	15 June 2018	20	S 00.52241	E 030.62044
Kebisoni	Rukungiri	Southwestern	19 June 2019	23	S 00.85281	E 030.00897
Buyanja	Rukungiri	Southwestern	19 June 2018	27	S 00.86145	E 029.98670
Nyakageme	Rukungiri	Southwestern	19 June 2018	16	S 00.80057	E 029.91953
Nyimbwa	Luwero	Central	4 Aug. 2018	19	N 00.65197	E 032.51985
Kalagala	Luwero	Central	4 Aug. 2018	30	N 00.64952	E 032.556435
Katikamu	Luwero	Central	4 Aug. 2018	35	N 007.73876	E 032.505882
Kasangombe	Nakaseke	Central	7 Aug. 2018	29	N 00.71005	E 032.46515
Semuto	Nakaseke	Central	7 Aug. 2018	30	N 00.62223	E 032.31886
Nakaseke	Nakaseke	Central	7 Aug. 2018	27	N 00.70138	E 032.33825
Busimbi	Mityana	Central	11 Aug. 2018	29	N 00.43177	E 032.01482
Bulera	Mityana	Central	11 Aug. 2018	38	N 00.43890	E 031.97557
Central division	Mityana	Central	11 Aug. 2018	32	N 00.41286	E 032.01109
Kabalinga	Mubende	Central	15 Aug. 2018	24	N 00.54046	E 0.31.31439
Kitenga	Mubende	Central	15 Aug. 2018	23	N 00.497555	E 031.53137
Bagezzi	Mubende	Central	15 Aug. 2018	40	N 00.54294	E 031.35150
Namulonge	Wakiso	Central	30 Aug. 2018	50	N 00. 0.530	E 32.583421

females were used because they are often larger and easier to recover genomic DNA from. Total genomic DNA was extracted from the heads, wings, and legs of the female weevils to avoid contamination with ingesta using the modified CTAB procedure (Lienhard and Schäffer 2019). The standard CTAB protocol was adapted by adding 3µl RNase after the lysis step followed by incubation at 22°C for 10 min and precipitation at 13,000 rpm. The integrity and quality of *C. sordidus genomic* DNA were evaluated by resolving such genomic DNA on 0.8% agarose gels and inspection under an ultraviolet spectrometer.

Construction of *C. sordidus* Transcriptome Datasets by *de novo* Assembly

The *C. sordidus* transcriptome was obtained from the ongoing study at the National Agricultural Research Laboratories Kawanda (NARL), Uganda. The *C. sordidus* larvae were obtained from the corms of a banana plantation close to NARL (1210 m; 32° 36′ E and 0° 25′ N). The transcriptome sequence data were obtained from the complementary DNA of the *C. sordidus* larvae isolated and sequenced using 454 pyro-sequencing technology platforms. The sequences were trimmed to remove low-quality reads and assembled de novo using QIAGEN CLC Genomic Workbench v11.0 (Qiagen Manchester, M15 6SH, UK) set to default parameters as previously described (Annadurai et al. 2012).

Development of *C. sordidus* Transcriptome-Derived SSR Markers

GMATA v2.2 was used to mine microsatellites from *C. sordidus* transcriptome (Wang and Wang 2016). Primers were designed within the regions flanking each SSR locus using default GMATA v2.2 parameters of product size120–400 bp, Tm 60°C (57–62°C), primer target length of 20 nucleotides (18–25 nt), GC percentage minimum GC of 40%, maximum GC of 65% and optimized GC at 50%, maximum self-complementarity 6, maximum 3′self-complementarity 2, and maximum Ns 1. The designed primer pairs were computed as potential SSR markers. All designed primers were investigated for sequence identity. Unique SSR markers were assigned an ID with the marker identity (MK) prefix for each unique primer pair.

In Silico e-PCR Amplification Analysis

A virtual e-PCR amplification was carried out in the GMATA v2.2 package using the e-PCR algorithm (Schuler 1997). The parameters were set thus; margin 3,000, no gap and mismatch in primer sequence, allowed amplicon size range of 100–1,000, word size (–w) 12, and contiguous word (–f) 1 on unique markers to investigate polymorphism in the *C. sordidus* transcriptome sequences. The markers with a high amplicon size difference of 10 bp were selected from total *in silico* polymorphic SSRs markers as these could easily be resolved and scored on agarose gels.

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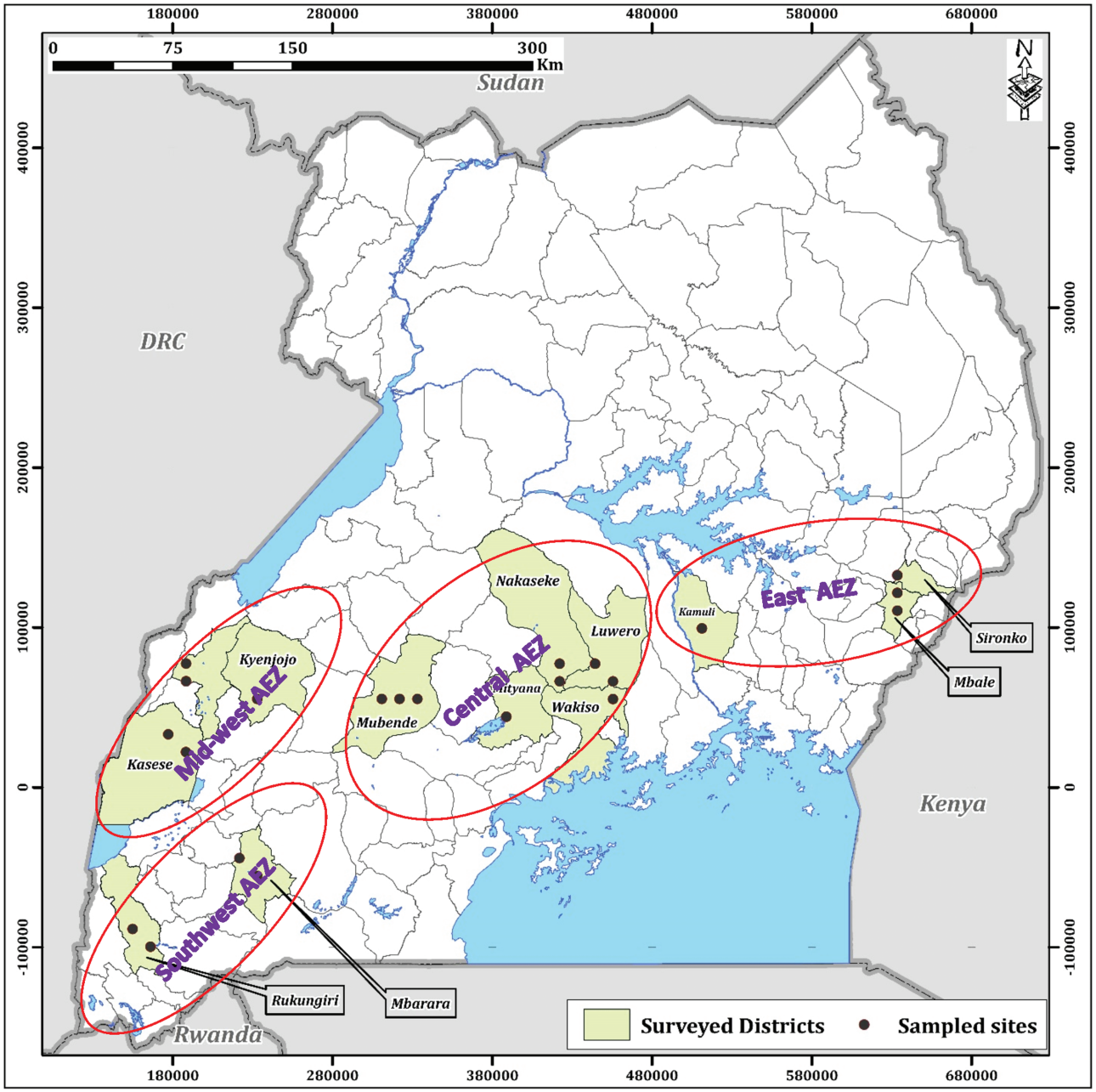

Fig. 2. Geographic location of 12 *C. sordidus* populations in four banana agro-ecological zones (AEZs) of Uganda.

PCR Amplification and Validation of Selected SSRs

SSR primer pairs ($n = 27$) were used to determine the genetic diversity of the 12 *C. sordidus* populations. Each PCR reaction was carried out in a total reaction volume of 25 µl containing 3.0 µl (25 ng) genomic DNA, 20 µl (1X) AccuPower Hot Start PCR premix (Bioneer Corporation, Daejeon, Republic of Korea), SSR forward primer 1 µl (10 µM) and SSR reverse primer 1 µl (10 µM). PCR was performed in Bio Rad C1000 touch thermal cycler (Hercules, CA 94547, USA) using the following thermal cycling conditions; 5 min at 95°C for initial denaturation and 32 cycles of 30 s at 95°C for denaturation, 30 s at the optimized annealing temperature (Ta) for each primer-pair, and 2 min at 72°C for an extension step. An additional cycle of 5 min at 72°C was also used for primer extension. The PCR products were resolved on 6% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE). The SSR allele sizes were scored at each locus as fragment size in visual comparison with a standard O'gene 100 bp DNA ladder (Thermo Scientific, Waltham, MA, USA). The band sizes (the allele in the same row) were scored and transformed to a 1 when they were present or to a 0 when absent (De Vicente et al. 2004).

Data Analysis

Data were grouped into districts and agro-ecological zones (AEZs). The datasets were then analyzed for; the number of alleles observed (Na), effective number of alleles (Ne), Shannon's information index (I), observed heterozygosity (Ho), expected heterozygosity (He), genetic differentiation coefficient (Fst), gene flow, Nei's genetic distance, polymorphic information content and percentage polymorphic loci using GenAlEx 6.5 (Smouse and Peakall 2012). The unweighted pair group method of arithmetic averages (UPGMA) dendrogram was constructed from Nei's genetic distance matrix using Free Tree

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(Thankachan et al. 2016) and visualized in Tree view (Kuznetsova et al. 2018). To determine the amount of genetic variation within and among the twelve *C. sordidus* populations, analysis of molecular variance (AMOVA) was carried out using GenAlEx 6.5 (Smouse and Peakall 2012). Principal Coordinate Analysis (PCoA) was computed from Nei's genetic distance to obtain a three-dimensional plot of patterns in the data to highlight similarities and differences between and among *C. sordidus* populations using GenAlEx 6.5 (Smouse and Peakall 2012).

Results

A total of 2,098 potential SSR markers were generated. Of these, 904 (43.2%) were unique and 882 (42%) were in *silico* polymorphic. From the in *silico* polymorphic markers, 27 primer pairs were selected based on amplicon size difference and 16 generated amplification products of the expected sizes in one of the *C. sordidus* populations. Finally, six primer pairs showed polymorphism and were used in the analysis (Supp Table S1 [online only]). Marker MK566 and MK665 showed the highest and lowest polymorphic information content values of 0.58 and 0.10 respectively. The most abundant repeat motif distribution among the SSR markers were; 1,514 (72.2%) dinucleotides, 515 (24.5%) trinucleotides, 65 (3.1%) tetranucleotides, 2 (0.1%) pentanucleotides and 2 (0.1%) hexanucleotides. The most frequent motif combinations included 439 (29%) of AT/AT among dinucleotides and 16 (3.1%) of TTA/TAA among trinucleotides. The highest SSR repeat length frequency was 10–15, adding up to 58.6% of total SSRs, followed by the 18, 20, and 14 repeat unit categories adding up to 19.4 % while other repeat categories of 16, 21, 22, and 24 together added up to 9.4% (Supp Fig. S1 [online only]).

Genetic Diversity Within and Among Banana Weevil Populations

The highest and the lowest genetic diversity within the *C. sordidus* populations was observed in Mityana (He 0.39) and Wakiso (He 0.00) with an overall average gene diversity (He) of 0.18. The Shannon Information Index within the populations varied between 0.000 and 0.60 in Wakiso and Mityana populations. The percentage loci polymorphism within the populations varied between 0.00 and 75% for Wakiso and Mityana, with an overall average percentage loci polymorphism of 36.8% within the twelve populations. The fixation index (F) values of the 12 weevil populations varied from −0.056 to 0.175 (Table 2). The highest and lowest genetic diversity among *C. sordidus* populations was observed in Central AEZ; (He 0.39) and Eastern AEZ (He 0.18) with an overall average gene diversity of (He 0.22) among the populations. The Shannon information index among the populations varied from 0.26 (East) to 0.66 (Central). The percentage loci polymorphism varied from 33.3% (East) to 75 % (Central), with an overall average polymorphism of 50% among the 4 AEZs. The fixation index (F) values of the 4 AEZs populations varied from −0.074 to 0.272 (Table 3).

Population Genetic Diversity and Gene Flow

The total genetic divergence coefficient (F_{ST}) and the gene flow (New York State Department of Environmental Conservation) within the *C. sordidus* populations were 0.41 and 0.85 while the F_{ST} and Nm among the populations were 0.39 and 0.92. Analysis of molecular variance (AMOVA) revealed a significant genetic variation (72%) within and among (28%) the 12 banana growing districts. A genetic variation of 79% was observed within and 21% among *C. sordidus* populations in four Uganda banana-growing AEZs (Table 4).

Genetic Distance Between Banana Weevil Populations

The genetic distance within the 12 *C. sordidus* populations taken from the four AEZs varied greatly between populations and AEZs; 0.007 (Mbale and Sironko), 2.71 (Luwero and Wakiso) (Table 5), 0.17 (Mid-west and South-west) and 1.08 (Midwest and Central) AEZs (Table 6).

UPGMA Dendrogram Analysis

The 12 *C. sordidus* populations clustered into three main groups. Group one was comprised of *C. sordidus* populations from Kasese, Kabarole, Rukungiri, and Mbarara which represent South-west and Mid-west AEZs. Group two was comprised of *C. sordidus* populations from Sironko, Mbale, Kamuli, and Wakiso representing the Eastern AEZ except for Wakiso [Central AEZ]. The third group comprised Mityana, Mubende, Luwero, and Nakaseke *C. sordidus* populations belonging to Central AEZ (Fig. 3).

Table 2. SSR-based genetic diversity among twelve *C. sordidus* (Germar) populations from Uganda

Populations	*N*	Na	Ne	I	Ho	He	uHe	Fst	Nm	PPL	F
Mbarara	6	1.333	1.286	0.255	0.250	0.172	0.199	0.357	0.450	33.33%	−0.500
Rukungiri	6	0.833	0.782	0.038	0.028	0.023	0.028	0.319	0.534	8.33%	−0.200
Kabarole	5	1.500	1.397	0.306	0.389	0.213	0.306	0.455	0.300	50.00%	−0.733
Kasese	6	1.000	0.933	0.151	0.167	0.104	0.167	0.617	0.155	25.00%	−0.556
Sironko	5	1.333	1.272	0.249	0.250	0.167	0.250	0.867	0.038	33.33%	−0.533
Mbale	5	1.333	1.316	0.261	0.292	0.177	0.205	0.881	0.034	33.33%	−0.686
Kamuli	6	1.250	1.183	0.209	0.250	0.146	0.189	0.404	0.369	33.33%	−0.667
Wakiso	3	0.250	0.250	0.000	0.000	0.000	0.000	0.862	0.040	0.00%	0.000
Mubende	5	1.583	1.397	0.378	0.417	0.255	0.406	0.729	0.093	58.33%	−0.581
Mityana	6	2.083	1.864	0.602	0.417	0.385	0.525	0.896	0.029	75.00%	−0.056
Luwero	6	1.833	1.695	0.472	0.278	0.306	0.367	1.000	0.000	58.33%	0.175
Nakaseke	6	1.417	1.328	0.278	0.208	0.177	0.231	1.000	0.000	33.33%	−0.233
Grand mean	65	1.313	1.225	0.267	0.245	0.177	0.239	0.699	0.170	36.81%	−0.379

N, Number of individual weevils in a population; Na, Number of alleles; Ne, Effective number of alleles; I, Shannon's Information Index; Ho, Observed heterozygosity; He, Expected heterozygosity; uHe, unbiased Expected heterozygosity; Fst, genetic differentiation among subpopulations; Nm, Gene flow; PPL, percentage of polymorphic loci; F, Fixation Index.

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Table 3. SSR-based genetic diversity among twelve *C. sordidus* (Germar) populations from four banana growing 4 AEZS of Uganda

AEZs	*N*	Na	Ne	I	Ho	He	uHe	PPL	F
South-west	12	1.500	1.325	0.272	0.190	0.178	0.202	41.67%	−0.074
Mid-west	11	1.667	1.490	0.361	0.275	0.230	0.270	50.00%	−0.239
East	16	1.333	1.306	0.259	0.267	0.175	0.188	33.33%	−0.570
Central	26	2.417	1.942	0.655	0.300	0.390	0.413	75.00%	0.272
Grand mean	65	1.729	1.516	0.387	0.258	0.244	0.268	50.00%	−0.068

N, Number of individual weevils in a AEZs; Na, Number of alleles; Ne, Effective number of alleles; I, Shannon's Information Index; Ho, Observed heterozygosity; He, Expected heterozygosity; uHe, unbiased Expected heterozygosity; PPL, percentage of polymorphic loci; F, Fixation Index.

Table 4. Analysis of molecular variance (AMOVA) for *C. sordidus* (Germar) populations (n = 12) in four different banana growing AEZs of Uganda

Source Variance	Df	Sum of squares	Mean of squares	Est. Variance	Total variance	Fst	Nm	*P* (rand >= data)
Among Pops	11	177.951	17.795	3.119	28%	0.226	0.854	0.001
Within Pops	23	188.167	8.181	8.181	72%	0.536		0.001
Total	34	366.118		11.300	100%			
Among AEZs	3	91.652	30.551	2.522	21%	0.213	0.923	0.001
Within AEZs	31	290.234	9.362	9.362	79%	0.559		0.001
Total	34	381.886		11.885	100%			

Df, degree of freedom; Nm, gene flow; Fst, genetic divergence and Probability; *P* (rand >= data), for Fst based on standard permutation across the full data set where $P = 0.001$ is significant.

Table 5. Pairwise Population Matrix of Nei's standard genetic distance for *C. sordidus* (Germar) populations (n = 12)

Mbarara	Rukungiri	Kabarole	Kasese	Sironko	Mbale	Kamuli	Wakiso	Mubende	Mityana	Luwero	Nakaseke	
0.000												Mbarara
0.436	0.000											Rukungiri
0.419	0.799	0.000										Kabarole
0.155	0.433	0.473	0.000									Kasese
0.332	0.838	0.755	0.310	0.000								Sironko
0.301	0.799	0.701	0.273	0.007	0.000							Mbale
0.289	0.729	0.642	0.245	0.042	0.021	0.000						Kamuli
0.834	0.534	0.691	0.657	1.088	1.012	0.851	0.000					Wakiso
0.505	0.634	0.947	0.451	0.154	0.153	0.156	0.738	0.000				Mubende
0.838	0.790	1.330	0.776	0.428	0.463	0.527	1.548	0.338	0.000			Mityana
1.182	0.982	2.270	1.184	0.609	0.625	0.687	2.708	0.487	0.220	0.000		Luwero
1.099	1.225	2.301	1.127	0.463	0.490	0.534	2.334	0.322	0.469	0.142	0.000	Nakaseke
0.532	0.706	1.011	0.558	0.349	0.395	0.459	1.466	0.287	0.230	0.071	0.000	Mean

Table 6. Pairwise group's matrix of the genetic distance of *C. sordidus* (Germar) populations (n = 12) in different banana growing AEZs (N = 4) of Uganda

South-west	Mid-west	East	Central	
0.000				South-west
0.173	0.000			Mid-west
0.361	0.504	0.000		East
0.752	1.082	0.355	0.000	Central

PCoA for Within or Among Population Genetic Diversity of Banana Weevils

Principal Coordinate Analysis (PCoA) analysis conducted on the mean pair-wise genetic distance of 12 *C. sordidus* populations revealed that the majority of *C. sordidus* population clustered around the AEZs from which they were isolated except those from Wakiso district (one of the Central AEZ districts). The genetic variation is explained by the first 3 axes (1, 2, and 3) 20.66, 17.83, and 16.58%, respectively (Fig. 4).

Discussion

PCR-based markers like RAPDS, AFLPs have been developed and applied to genotype the genetic diversity and structure of the banana weevils (De Graaf 2008, Magaña et al. 2007, Ochieng 2001, Kumar and Singh 2018, Twesigye et al. 2018a). However, these marker systems are dominant, require labor-intensive electrophoresis, with only a few of such markers available, which suggests the difficulty in covering the entire genome (Shankar et al. 2015, Singh et al. 2017). With the low costs involved in NGS, many insect genomes and transcriptomes have been sequenced. As such, large datasets are publicly available in the databases and these have allowed for cheap mining of robust and cheap molecular markers that can easily be accessed by pest control managers in low technology settings (Li et al. 2019, Tian et al. 2019).

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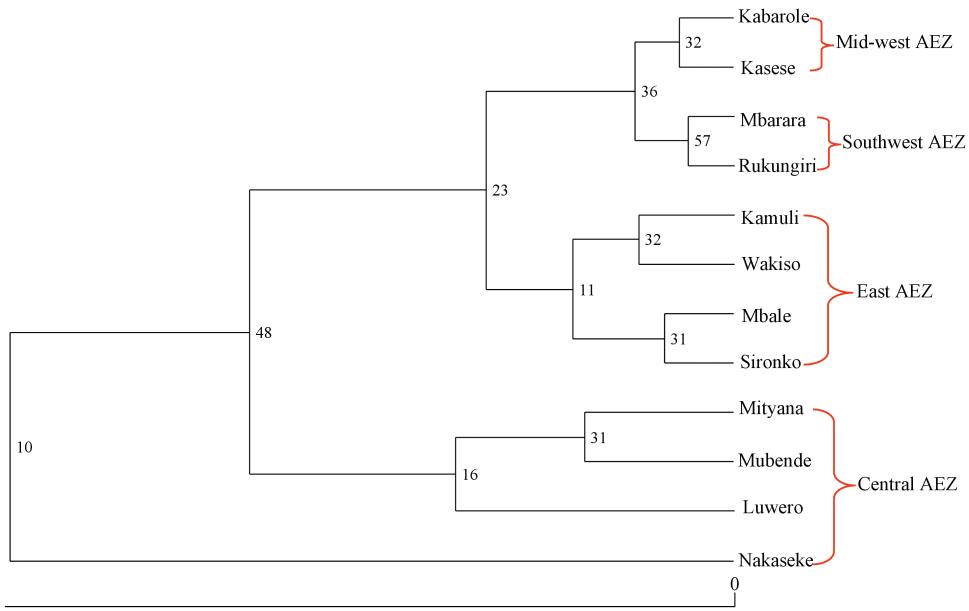

Fig. 3. *C. sordidus* dendrogram of 12 *C. sordidus* populations grouped into four AEZs. This dendrogram was constructed from the genetic distance matrix estimated from the SSR data and clustered using UPGMA. The bootstrap test (1,000 replicates) representing the percentage of replicate trees in which the associated taxa clustered together in is shown next to the branches.

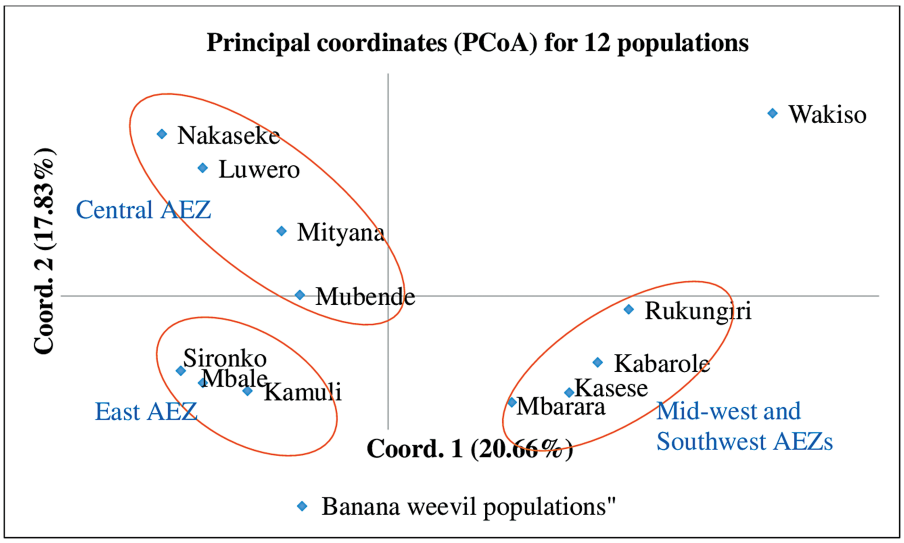

Fig. 4. Principal coordinate analysis (PCoA) of genetic variation of 12 and four AEZs C. *sordidus* populations explained by (20.66, 17.83, and 16.58% variation explained by axis 1, 2, and 3.

Simple sequence repeat marker development using conventional methods such as screening of SSR enriched or nonenriched genomic libraries are slow, laborious, and costly thereby rendering this approach difficult (Zane et al. 2002, Chen et al. 2016). These can be overcome by the mining of existing NGS data sets from publicly available databases (Li et al. 2019, Tian et al. 2019). A total of, 2,098 SSR markers were identified from existing transcriptomic resources. This is higher than those of other insect pests, such as *Diabrotica virgifera* (Coleoptera: Chrysomelidae) (305) (Kim et al. 2008), *Agrilus planipennis* (Coleoptera: Buprestidae) (317 in midgut and 571 in fat body) (Mittapalli et al. 2010), *Liposcelis entomophila* (Enderlein) (Wei et al. 2013), and *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) (1,228) (Luo et al. 2014). The most common microsatellite loci repeats identified were dinucleotide repeats 1,514 (72.2%) followed by trinucleotide repeats 515 (24.5%). This is in agreement with previous studies showing the same abundance of dinucleotide and trinucleotide motifs (Pannebakker et al. 2010, Zhai et al. 2014). The most abundant SSR repeat motifs in *C. sordidus* transcriptome were AT/TA and AAT/ATT. These motifs were also observed in *Rhopalosiphum padi* (Duan et al. 2017), *Schistosoma mansoni* (Trematoda Schistosomatidae) (Yuanju et al. 2007), *Tenebrio molitor* (Coleoptera: Tenebrionidae) (Zhu et al. 2013), and *P. solenopsis* (Luo et al. 2014). The higher proportion of AT/TA and AAT/ATT could be due to a higher proportion of A/T-rich SSRs in the poly-A tails of retro-transposed sequences and processed pseudogenes. The low GC/CG motif (0.5%) frequency can be explained by the low C/G content of the transcriptome (46%) (Meglécz et al. 2012, Duan et al. 2017, Wang et al. 2019).

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Genetic diversity indices among *C. sordidus* (Germar) in four banana-growing AEZs of Uganda demonstrated that the genetic diversity of *C. sordidus* populations was highest in the Central AEZ and lowest for Eastern AEZ (He 0.390 and 0.175 respectively) (Table 6). The central regions are the oldest banana-growing AEZ with well-established weevil populations with large gene pools that can thrive in these environments (Gold et al. 1999b). As such, incidence of shortening banana stand duration and disappearance of the East African highland banana have been reported in this region (Rukazambuga et al. 1998, Gold et al. 2004). The Eastern AEZ had the lowest genetic diversity due to the small *C. sordidus* population size. These were introduced by the transportation of planting materials creating genetic bottlenecks with small gene pools leading to low genetic diversity. This is in agreement with studies conducted by Twesigye et al. (2018a) and Kumar and Singh (2018) where the *C. sordidus* populations structured into geographical locations. With these markers, time, costs, and resources can be saved that otherwise would be misdirected and wasted in trying to identify this pest (Bergamo et al. 2018).

Geographical barriers such as man-made trenches and natural barriers such as rivers like the Nile, swamps, and mountainous slopes of Elgon have created two genetically distinct groups (the Eastern and Central AEZ subpopulations) (Storfer et al. 2010, da Silveira Queiroga et al. 2019). These populations are divided according to geography and habitat fragmentation. This is due to the limited self-dispersal ability of the *C. sordidus* (Rannestad et al. 2011). As such, transportation of infested planting material is a major mode of dispersal (Gold et al. 1999c). The movement of adult *C. sordidus* has been reported to be 60 m in 5 months within their natural habitat (Gold et al. 2001). Geographical barriers have fragmented these habitats restricting the rate of gene flow between populations resulting into unique populations. This is in agreement with studies conducted by Duan et al. (2013) on *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) using simple sequence repeat markers and mitochondrial loci that identified two populations (western and Northern) respectively. This information is vital for the effective deployment of IPM control strategies as delimited geographical management units have been identified. *C. sordidus* populations of Mid-west and South-west AEZs were clustered together into a common branch. This can be explained by restricted migration/ movement or gene flow of infected suckers or rhizomes, by farmers between regions (Gold et al. 1999b). Similar observations of clustering in *C. sordidus* populations diversity analyses have also been obtained with AFLPs markers (Twesigye et al. 2018a) suggesting no expansion of the pest in these regions.

Principal Coordinate Analysis (PCoA) clustered all the weevil populations from the Eastern, Central AEZs, and Wakiso population separately in different clades. However, the Wakiso *C. sordidus* population did not cluster with Central AEZ most likely due to the importation or transportation of infected banana suckers from different AEZs (Twesigye et al. 2018a). This is in agreement with a recent *C. sordidus* genetic diversity study using AFLP markers (Twesigye et al. 2018a); the south-west and mid-west *C. sordidus* populations were clustered together on the same clade probably as a result of limited *C. sordidus* dispersal (Gold et al. 2001).

The AMOVA analysis presented significant genetic diversity within and among *C. sordidus* populations (P = 0.001). However, the observed genetic diversity was highest within the *C. sordidus* populations and had little but significant levels of diversity among the populations. The high genetic diversity within the *C. sordidus* populations is probably due to high selection pressures such as the application of pesticides. For example, Collins et al. (1991) and Gold et al. (1999a) applied organophosphorus, carbofuran, and dieldrin insecticides to control the banana weevils which resulted into insecticide-resistant weevil populations. Our study also identified a little significant genetic diversity among the population suggesting some variation attributed to sex-biased gene flow (Shankar et al. 2015). This differed from previous studies that used RAPDs (Yadav et al. 2017), AFLPs (Yadav et al. 2017, Twesigye et al. 2018a) that identified only diversity within the populations. These differences could be attributed to the different inheritance patterns of the markers used, as they could be sex-biased gene flow among these populations. There is a need to further study the species at different geographic scales to establish this phenomenon (Torres et al. 2007, Shankar et al. 2015).

Though these markers have been able to genotype the genetic diversity of *C. sordidus* populations, they are known to be less polymorphic compared to noncoding SSR markers. This is due to sequence redundancy, resulting in multiple sets of markers at the same locus explaining the low number of polymorphic markers (Meglécz et al. 2013). There is, therefore, a need to further analyze putatively polymorphic SSR markers using more sensitive methods like capillary electrophoresis and Next-generation sequencing (NGS). These methods could reduce the errors associated with scoring fragment length that can cause size homoplasy leading to underestimation of genetic variability (Šarhanová et al. 2018).

Conclusion

The study identified 6 highly polymorphic transcriptome-derived SSR markers which were used to successfully genotype within and among banana weevil population genetic diversity of 12 *C. sordidus* populations taken from four Uganda banana-growing AEZs. These markers identified a broad 'within and among' *C. sordidus* population genetic diversity which other dominant markers were not able to resolve previously. The high genetic diversity could be explained by several factors including genetic drift, natural and artificial barriers that regulate the rate of gene flow, level of transportation of infected plantlets between different AEZs. This provides key information for IPM control strategies as populations were distinct between different AEZs.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

Supp Fig. S1. Selected statistical graphics by GMATA for *C.sordidus*. (A) the major length distribution of the repeated motif, (B) the distribution of the repeated motif nucleotides, and (C) the length distribution of the SSRs.

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