Agriculture & Food Security

RESEARCH Open Access

# Variation among banana weevil *Cosmopolites sordidus* (Germar) populations in Uganda as revealed by AFLP markers and corm damage differences

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## Abstract

**Background:** The banana weevil *Cosmopolites sordidus* (Germar) is a major production constraint of bananas and plantains (*Musa* spp.) in the world. Differences in damage levels and pesticide response across regions led to the postulation that there might be considerable variation between banana weevil populations (biotypes) with varying levels of virulence. One of the most sustainable options for banana weevil control is the use of host plant resistance. While new resistant varieties are being developed through both conventional crossbreeding and biotechnology, there is a need to assess the genetic variation of banana weevil populations from eastern, central, southern, southwestern and midwest regions of Uganda to determine whether there are biotypes with different virulence levels. This would help guide new control strategies to target all the possible biotypes. The amplified fragment length polymorphism (AFLP) technique was used to analyze population genetic diversity using four primer combinations (EcoRI/MSeI).

**Results:** Analysis of molecular variance results presented no evidence to support significant genetic variability among the banana weevil populations from eastern, central, southern, southwestern and midwest regions. Practically, all the genetic variation was found to reside within populations (97% for sites and 98% for regions), with only approximately 3% and 2% residing among populations of sites and regions, respectively.

**Conclusions and recommendations:** AFLP markers clustered the banana weevils into two distinct populations consequently supporting the hypothesis of possible presence of banana weevil biotypes in Uganda. However, attempts should be made to make follow-up studies on the seemingly unique population of eastern Uganda using more robust molecular techniques to establish whether the eastern Uganda population constitutes a different biotype.

**Keywords:** AFLP markers, Banana weevil, Biotypes, *Cosmopolites sordidus*, Genetic variation

## Background

The banana weevil *Cosmopolites sordidus* (Germar) is the major production constraint of bananas and plantains (*Musa* spp.) in Uganda. Weevil damage in conjunction with low soil fertility, parasitic nematodes and diseases was implicated in the decline of banana productivity in many parts of Uganda [1]. The female weevil deposits eggs singly at the base of the banana plants at a rate of 1–4 eggs per week [2]. After hatching, the larvae tunnel into the corm and pseudostem of the plant causing stem damage that leads to plant stunting, delayed maturation, reduced bunch size, snapping and sometimes premature death of the affected plants [3].

Yield losses to banana weevil have been associated with sucker mortality, reduced bunch weights and shortened plantation life [3]. In the central region districts, Masaka and Rakai districts, yield losses of 20–60%, up to 100%

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and up to 100%, respectively, were reported by [4, 5], while in Mbarara, Bushenyi, Ntungamo and Kabale districts, there was negligible yield loss.

This has led to the postulation that there is a considerable variation between banana weevil populations from different regions of Uganda with varying levels of virulence. This is supported by recent research [5], which reported that the limited banana weevil mobility suggests the existence of discrete populations with limited gene flow and the likely evolution of local biotypes.

Although chemical pesticides are effective, their high cost, hazardous effects to health and the environment call for alternative control strategies to be developed or identified for the resource-limited small-scale farmers, who are the major banana producers. Several strategies, including habitat management (cultural control), host plant resistance and biological control, have been advocated for controlling this weevil borer [6]. Host plant resistance in particular is seen as the most promising and sustainable control strategy [7]. Available data indicate that all highland banana clones are susceptible to the weevil. However, screening trials suggest that many resistant Musa clones do exist mostly among non-cultivated wild diploids and that antibiosis is the predominant means of resistance in these clones.

According to [8], implementation of control measures and management programs for pest species relies on accurate, biologically meaningful identification. However, this process rests upon original taxonomic designations from which identifications are based. If the link between taxonomically defined and biological species is incongruous, management efforts may be misdirected, resulting in wastage of time, effort and resources.

This indicates that there is still a big task of characterizing the banana weevils in every agroecological zone of Uganda. In one trial in Uganda, 40% of newly planted weevil-free highland cooking suckers were killed by banana weevil remaining from an earlier trial [9]. Damage and yield loss due to banana weevil increase with time [5], and therefore to prevent total elimination of banana plantations in the near future, a sustainable control for this pest is of paramount importance.

Biotypes of the banana weevil have been reported to exist in Uganda [10]; unfortunately, the technique Random Amplified Polymorphic DNA (RAPDs) used in that previous study has got a number of limitations like problems with reproducibility of results and co-migration of equal-sized bands [11, 12].

This therefore calls for use of amplified fragment length polymorphism (AFLP) molecular marker technique that is more robust, highly reproducible and can generate a large number of polymorphic bands for resolving genetic relatedness among individual organisms, populations and species.

This study addressed the need to evaluate the variation among the banana weevil populations so as to develop control measures that will target all biotypes in the banana growing regions if confirmed to exist.

Through characterization of the banana weevil using highly sensitive AFLP markers, an understanding of the banana weevil and all its different forms generated will contribute toward the successful development of genetically modified bananas resistant to banana weevils. The overall objective of the study was to determine the genetic variation among populations of banana weevils from the different banana growing regions of Uganda.

## Methods

### Site description and source of materials

Molecular analysis on genetic variation of the different weevil populations was conducted in the biotechnology laboratories at the National Agricultural Research Laboratories, Kawanda. Sites were selected randomly from the various agroecological zones in every banana growing district in Uganda on the basis of a grid map (Fig. 1) produced for diagnostic surveys of banana-based cropping systems [13, 14]. From each district, one to three subcounties were selected for collection of banana weevils as shown in Table 1. Banana weevils were collected from farmers' fields using pseudostem traps and maintained in the laboratory in plastic containers.

### Maintenance of weevils

In molecular analysis, weevils from each site were randomly selected and preserved in 70% v/v ethanol, until DNA extraction.

### DNA isolation

Total genomic DNA was extracted from the wings, head and legs of female adult weevils to avoid extraction of DNA from ingested food materials using the Qiagen DNA extraction kit.

### AFLP amplification general methodology

The AFLP technique for DNA fingerprinting described in [11] was performed using commercial AFLP kit from Beckman Coulter, Inc. The sequences of oligo adapters, preselective amplification primers and selective amplification primers were obtained from Beckman Coulter, Inc.

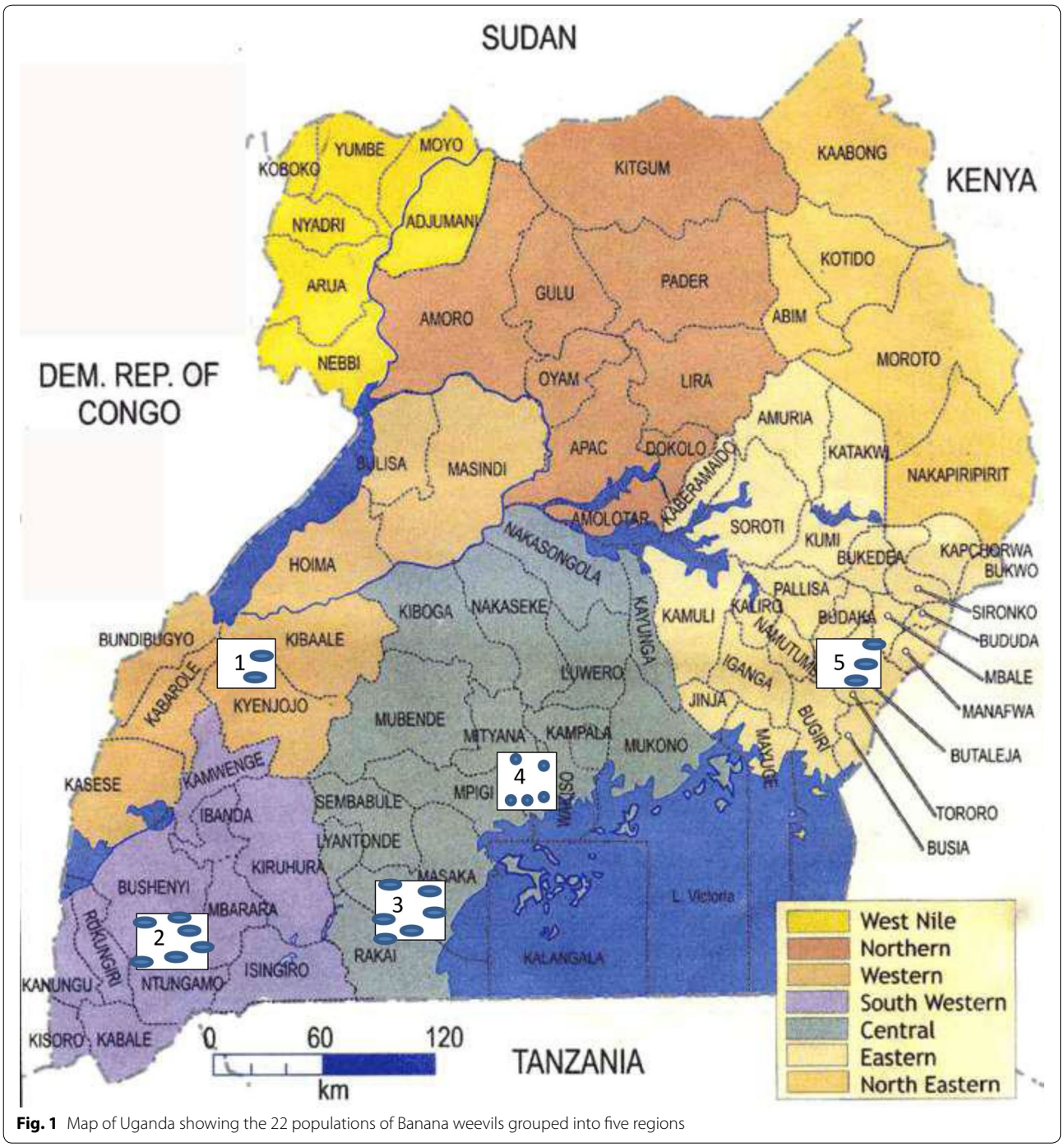


**Fig. 1** Map of Uganda showing the 22 populations of Banana weevils grouped into five regions

### Restriction of genomic DNA

Restriction digestion of genomic DNA was accomplished in 25 µl reaction mixture of 5 µl of 5X reaction buffer, 1 µl of 10 units/µl MseI and 1 µl of 10 units/µl EcoRI restriction enzymes, 10 µl of 100 ng weevil DNA and 8 µl of distilled deionized sterile water in 1.5 ml micro-centrifuge tube incubated at 370 °C

**Table 1 Sites (districts and sub-counties) sampled for banana weevil populations in Uganda and number of weevils sampled for DNA extraction**

| Region | District | Sub-county | No of weevils |
|---|---|---|---|
| Southwestern | Kabale | Kamwezi | 29 |
| | Ntungamo | Itojo | 10 |
| | | Kahuga | 12 |
| | | Ntungamo Town council | 14 |
| | Mbarara/Bushenyi | Bushenyi Town council | 9 |
| | | Bubaale | 13 |
| | | Kakika | 9 |
| Midwest | Kabarole | Kyenjojo and Fort Portal | 18 |
| Eastern | Mbale | Bumasike | 12 |
| | | Busiu | 9 |
| | | Mbale Municipality | 5 |
| | Sironko | Buwalasi | 19 |
| Central | Wakiso | Manyangwa | 25 |
| | | Nangabo | 10 |
| | Mukono | Nakifuma | 12 |
| | | Sonde | 14 |
| Southern | Rakai | Kilumba | 11 |
| | | Nabigasa | 10 |
| | | Dwaniro | 9 |
| | Masaka | Ndagwe | 11 |
| | | Bukoto | 10 |
| | | Kisseka | 11 |

for 3 h and then at 700 °C for 15 min to inactivate the endonucleases.

#### Ligation of oligonucleotide adapters

Ligation of adapters was achieved by adding 12 µl of adapter solution (containing 20 µM MseI Adaptor 5′-GACGATGAGTCCTGAG TACTCAGGACTCAT-5′ and 2 µM EcoRI Adaptor 5′-CTCGTAGACTGCGTACC CATCTGACGCATGGTTAA-5′) and 0.5 µl of 1 unit/µl T4 DNA-ligase to 12.5 µl of the digested product, and the mixture was incubated at 200C for 2 h. 1:10 dilution of the ligation mixture was performed using distilled deionized sterile water.

#### Preselective PCR amplification

Preselective PCR amplification was performed using the Beckman Coulter, Inc. AFLP kit contains 50 µl of the following: 5 µL of diluted restricted ligated DNA and 45 µl of a cocktail made with 40 µl of preamp primer mix (containing preselective 10 µM EcoRI and 10 µM MseI oligonucleotide primers complementary to the adapter and restriction sites (EcoRIoligo (E-5′-GACTGCGTACCA ATTCA 3′) and MseI oligo (M- 5′-GATGAGTCCTGA GTAAC)) plus 2 mM dNTPs, 5 µl 10X PCR buffer for AFLP containing 1 µ of 5 units/µl Taq polymerase. The PCR program for the preselective amplification was 20 repetitive cycles of 94 °C for 30 s, 56 °C for 60 s and 72 °C for 60 s. All samples were stored at 4 °C following amplification on a GeneAmp* 9700 PCR system (Applied Biosystems). 1:100 dilution of preselective amplification product was carried out using distilled deionized sterile water.

#### Selective PCR amplification

Selective PCR amplification of restriction fragments was performed using four primer pairs prepared for recognition of EcoRI and MseI adapters as displayed by Table 3. Fragments were visualized by attaching D2 WellRED™ dye to the 5′ end of each EcoRI selective amplification primer with no modification made to the MseI primer.

Selective PCR amplification reaction was carried out by adding diluted DNA template from the preselective PCR reaction, 5 units/1 µl Taq polymerase, 2 mM dNTPs, a dye-labeled 1 µM EcoRI primer, 5 µM MseI primer and the standard buffers. The PCR reaction mixture consists of 15 µl of a mixture of primers, dNTPs, Taq DNA polymerase, PCR buffer and 5 µl of diluted amplified product from preselective amplification in 96-well plates. The PCR program for the selective amplification consisted of an initial warm-up at 94 °C for 2 min, one cycle of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 2 min, followed by 12 subsequent cycles, each with a 0.7 °C lowering of the annealing temperature, followed by 23 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 2 min and finally a hold at 60 °C for 30 min and samples stored at 4 °C (Table 2).

#### Fragment separation by capillary electrophoresis

Products of selective amplification were sent to North Carolina State University, College of Agriculture and Life

**Table 2 Sequence of primers used for selective amplification**

| Primer pair | *Eco*RI fluorescent dye-labeled primers | *Mse*I primer |
|---|---|---|
| 1st primer pair | 5′-GACTGCGTACCAATTCAAG-3′ | 5′-GATGAGTCCTGAGTAACAA-3′ |
| 2nd primer pair | 5′-GACTGCGTACCAATTCACG-3′ | 5′-GATGAGTCCTGAGTAACAG-3′ |
| 3rd primer pair | 5′-GACTGCGTACCAATTCAGC-3′ | 5′-GATGAGTCCTGAGTAACTG-3′ |
| 4th primer pair | 5′-GACTGCGTACCAATTCAGG-3′ | 5 -GATGAGTCCTGAGTAACAT-3′ |

**Table 3 AMOVA for 22 populations of banana weevils from Uganda**

| Source of variation | *df* | Sum of squares | MS | Est. Var. | Percentage of variation (%) | *P* value | *F* statistics |
|---|---|---|---|---|---|---|---|
| Among pops | 21 | 1130.536 | 53.835 | 0.667 | 3 | 0.897 | 0.032 |
| Within pops | 1107 | 22,018.466 | 19.890 | 19.890 | 97 | 0.036 | 0.010 |
| Total | 1128 | 230,149.00 | | 20.557 | 100 | | |

Sciences Genomic Sciences Laboratory in the USA for separation of amplified DNA fragments to identify the polymorphic alleles by capillary electrophoresis using the ABI model fragment analyzer. For any given sample, allele present was scored as 1 and allele absent was recorded as 0 to generate binary data.

### Analysis of molecular variance

Binary data produced by ABI model fragment analyzer were transferred to GenAIEx 6.5 [15] for analysis of molecular variance (AMOVA) to determine the genetic structure and genetic diversity present within and among populations. Total variation of the AFLP dataset was subdivided into two constituents, namely among populations and within population. One thousand permutations were done by AMOVA to determine the significance of these two constituents. Pairwise comparisons were conducted to test genetic divergence among populations (FST).

### Phylogenetic analysis

Nei's genetic distance matrix was generated in GeneAIEx 6.5 [15]. This matrix was then transferred to Free Tree, and a phylogenetic tree was produced using unweighted pair group method analysis (UPGMA). After 1000 bootstraps, a consensus tree was constructed and was then visualized and printed using Tree view.

### Principle component analysis (PCoA)

Principle component analysis was conducted using GenAIEx 6.5 to obtain a three-dimensional plot of data for superior graphic illustration using the Nei's genetic distance matrix.

## Results

### Genetic variation analysis

#### *Fragment separation by capillary electrophoresis*

Four AFLP selective primer combinations yielded 387 polymorphic alleles. For any given sample, polymorphic allele present was scored as 1 and allele absent was recorded as 0 by ABI.

#### *Analysis of molecular variance (AMOVA)*

The results of AMOVA indicated that the difference among groups was not significant ($P=0.897$) for the 22 populations and ($P=0.894$) for the five regions while the amounts of genetic variation within population were significant at $P=0.036$ for the 22 populations and $P=0.023$ for the five regions (Tables 5, 6). Very low genetic divergence values $F_{ST}$ 0.032 and 0.010 for among populations and within population, respectively, 0.021 and 0.010 for among agroecological zones and within banana growing regions, respectively, were attained (Tables 3, 4).

AMOVA showed high within-population variation of 97.0% of the total variation and high within banana growing regions population variation of 98.0%. Only 3% was credited to differences among populations within regions, whereas 2% was a result of genotypic variations (Tables 3, 4).

The mean unbiased heterozygosity (uHe) for populations ranged from 0.018 in Manyangwa to 0.085 in Bumasike, while in regions it ranged from 0.036 for central region to 0.072 for southwestern region (Tables 5, 6).

The percentage of polymorphic loci P revealed a substantial amount of variation within populations and within banana growing regions. Percentage of

**Table 4 AMOVA for 22 populations of banana weevils grouped into five regions of Uganda**

| Source of variation | *df* | Sum of squares | MS | Est. Var. | Percentage of variation (%) | *P* value | *F* statistics |
|---|---|---|---|---|---|---|---|
| Among regions | 4 | 488.005 | 122.001 | 0.421 | 2 | 0.894 | 0.021 |
| Within regions | 1244 | 24,661.836 | 19.825 | 19.825 | 98 | 0.023 | 0.010 |
| Total | 1248 | 25,149.841 | | 20.245 | 100 | | |

**Table 5 Genetic diversity indices for 22 banana weevil populations in Uganda (band frequencies, estimated allele frequencies and estimated heterozygosity)**

| Population | N | Na | Ne | I | He | uHe |
|---|---|---|---|---|---|---|
| *Kyenjojo* | | | | | | |
| Mean | 72.000 | 1.276 | 1.091 | 0.133 | 0.072 | 0.072 |
| SD | 0.000 | 0.049 | 0.007 | 0.008 | 0.005 | 0.005 |
| *Bushenyi* | | | | | | |
| Mean | 36.000 | 1.121 | 1.103 | 0.140 | 0.078 | 0.079 |
| SD | 0.000 | 0.051 | 0.008 | 0.009 | 0.005 | 0.006 |
| *Kamwezi* | | | | | | |
| Mean | 116.000 | 1.271 | 1.076 | 0.117 | 0.062 | 0.062 |
| SD | 0.000 | 0.049 | 0.006 | 0.007 | 0.004 | 0.004 |
| *Kahuga* | | | | | | |
| Mean | 49.000 | 1.282 | 1.100 | 0.144 | 0.078 | 0.079 |
| SD | 0.000 | 0.049 | 0.007 | 0.008 | 0.005 | 0.005 |
| *Itojo* | | | | | | |
| Mean | 40.000 | 1.075 | 1.087 | 0.127 | 0.069 | 0.070 |
| SD | 0.000 | 0.051 | 0.007 | 0.008 | 0.005 | 0.005 |
| *Ntungamo-tc* | | | | | | |
| Mean | 57.000 | 1.168 | 1.089 | 0.131 | 0.070 | 0.071 |
| SD | 0.000 | 0.050 | 0.007 | 0.008 | 0.005 | 0.005 |
| *Bubaale* | | | | | | |
| Mean | 48.000 | 1.147 | 1.101 | 0.140 | 0.077 | 0.078 |
| SD | 0.000 | 0.050 | 0.008 | 0.009 | 0.005 | 0.005 |
| *Kakika* | | | | | | |
| Mean | 36.000 | 1.003 | 1.108 | 0.141 | 0.080 | 0.081 |
| SD | 0.000 | 0.051 | 0.009 | 0.009 | 0.006 | 0.006 |
| *Dwaniro* | | | | | | |
| Mean | 36.000 | 1.121 | 1.095 | 0.136 | 0.074 | 0.075 |
| SD | 0.000 | 0.051 | 0.007 | 0.008 | 0.005 | 0.005 |
| *Nabigasa* | | | | | | |
| Mean | 40.000 | 1.003 | 1.075 | 0.112 | 0.060 | 0.061 |
| SD | 0.000 | 0.051 | 0.006 | 0.007 | 0.004 | 0.005 |
| *Kilumba* | | | | | | |
| Mean | 44.000 | 1.152 | 1.103 | 0.142 | 0.079 | 0.079 |
| SD | 0.000 | 0.050 | 0.008 | 0.009 | 0.005 | 0.005 |
| *Ndagwe* | | | | | | |
| Mean | 44.000 | 1.018 | 1.080 | 0.118 | 0.064 | 0.064 |
| SD | 0.000 | 0.051 | 0.007 | 0.008 | 0.005 | 0.005 |
| *Bukoto* | | | | | | |
| Mean | 41.000 | 1.096 | 1.099 | 0.138 | 0.076 | 0.077 |
| SD | 0.000 | 0.051 | 0.008 | 0.008 | 0.005 | 0.005 |
| *Kisekka* | | | | | | |
| Mean | 44.000 | 1.080 | 1.078 | 0.118 | 0.063 | 0.064 |
| SD | 0.000 | 0.051 | 0.006 | 0.007 | 0.004 | 0.004 |
| *Manyangwa* | | | | | | |
| Mean | 101.000 | 0.791 | 1.020 | 0.040 | 0.018 | 0.018 |
| SD | 0.000 | 0.050 | 0.002 | 0.004 | 0.002 | 0.002 |
| *Nangabo* | | | | | | |
| Mean | 41.000 | 0.708 | 1.022 | 0.045 | 0.020 | 0.020 |
| SD | 0.000 | 0.049 | 0.002 | 0.004 | 0.002 | 0.002 |

**Table 5 (continued)**

| Population | N | Na | Ne | I | He | uHe |
|---|---|---|---|---|---|---|
| *Nakifuma* | | | | | | |
| Mean | 48.000 | 1.106 | 1.084 | 0.122 | 0.066 | 0.067 |
| SD | 0.000 | 0.051 | 0.007 | 0.008 | 0.005 | 0.005 |
| *Sonde* | | | | | | |
| Mean | 56.000 | 0.899 | 1.035 | 0.064 | 0.030 | 0.031 |
| SD | 0.000 | 0.051 | 0.003 | 0.005 | 0.003 | 0.003 |
| *Mbale Municipality* | | | | | | |
| Mean | 20.000 | 0.822 | 1.102 | 0.130 | 0.075 | 0.077 |
| SD | 0.000 | 0.050 | 0.009 | 0.009 | 0.006 | 0.006 |
| *Busiu* | | | | | | |
| Mean | 36.000 | 1.282 | 1.101 | 0.143 | 0.078 | 0.079 |
| SD | 0.000 | 0.049 | 0.008 | 0.008 | 0.005 | 0.005 |
| *Bumasike* | | | | | | |
| Mean | 48.000 | 1.276 | 1.109 | 0.153 | 0.084 | 0.085 |
| SD | 0.000 | 0.049 | 0.008 | 0.008 | 0.005 | 0.005 |
| *Buwalasi* | | | | | | |
| Mean | 76 | 1.101 | 1.046 | 0.079 | 0.039 | 0.039 |
| SD | 0.000 | 0.051 | 0.004 | 0.006 | 0.003 | 0.003 |
| *Grand total over loci and populations* | | | | | | |
| Mean | 51.318 | 1.081 | 1.081 | 0.119 | 0.064 | 0.065 |
| SD | 0.235 | 0.011 | 0.001 | 0.002 | 0.001 | 0.001 |

**Table 6 Genetic diversity indices for 22 populations of banana weevils grouped as regions (band frequencies, estimated allele frequencies and estimated heterozygosity)**

| Population | N | Na | Ne | I | He | uHe |
|---|---|---|---|---|---|---|
| *Midwest* | | | | | | |
| Mean | 148.000 | 1.473 | 1.066 | 0.109 | 0.055 | 0.055 |
| SD | 0.000 | 0.045 | 0.005 | 0.006 | 0.004 | 0.004 |
| *Southwest* | | | | | | |
| Mean | 382.000 | 1.633 | 1.090 | 0.135 | 0.072 | 0.072 |
| SD | 0.000 | 0.039 | 0.007 | 0.008 | 0.005 | 0.005 |
| *South* | | | | | | |
| Mean | 249.000 | 1.488 | 1.087 | 0.131 | 0.070 | 0.070 |
| SD | 0.000 | 0.044 | 0.007 | 0.008 | 0.005 | 0.005 |
| *Central* | | | | | | |
| Mean | 290.000 | 1.380 | 1.041 | 0.076 | 0.036 | 0.036 |
| SD | 0.000 | 0.047 | 0.003 | 0.005 | 0.003 | 0.003 |
| *East* | | | | | | |
| Mean | 180.000 | 1.556 | 1.078 | 0.123 | 0.064 | 0.064 |
| SD | 0.000 | 0.042 | 0.006 | 0.007 | 0.004 | 0.004 |
| *Grand total over loci and populations* | | | | | | |
| Mean | 249.800 | 1.506 | 1.073 | 0.115 | 0.059 | 0.059 |
| SD | 1.884 | 0.020 | 0.003 | 0.003 | 0.002 | 0.002 |

*N* no. of weevil DNA samples, *Na* no. of different alleles, *Ne* no. of effective alleles, *I* Shannon's information index, *He* expected heterozygosity, *uHe* unbiased expected heterozygosity

**Table 7 Percentage of polymorphic loci within sites and within banana growing regions**

| Population (site) | %*P* | Population (banana growing regions) | %*P* |
|---|---|---|---|
| Kyenjojo | 63.82 | Mid-Western | 73.64 |
| Kamwezi | 63.57 | | |
| Kahuga | 64.08 | | |
| Itojo | 53.75 | | |
| Ntungamo-TC | 58.40 | | |
| Bubaale | 57.36 | Southwestern | 81.65 |
| Dwaniro | 56.07 | | |
| Nabigasa | 50.13 | | |
| Kilumba | 57.62 | | |
| Ndagwe | 50.90 | | |
| Bukoto | 54.78 | | |
| Kisekka | 54.01 | Southern | 74.42 |
| Manyangwa | 39.53 | | |
| Nangabo | 35.40 | | |
| Nakifuma | 55.30 | | |
| Sonde | 44.96 | Central | 68.99 |
| Municipality | 41.09 | | |
| Busiu | 64.08 | | |
| Bumasike | 63.82 | | |
| Buwalasi | 55.04 | Eastern | 77.78 |
| Mean | 54.09 | | 75.30 |
| SD | 1.73 | | 2.12 |

polymorphic loci *P* for localities and regions extended from 35.40% in Nangabo to 64.08% in Busiu and Kahuga with a mean of 54.09% and 68.99% in central to 81.65% in southwestern with a mean of 75.30%, respectively (Table 7).

For localities, banana weevils from Busiu and Kahuga exhibited the highest degree of genetic diversity ($P=64.08\%$, uHe = 0.079) while for regions, the uppermost level of genetic variability of $P=81.65\%$, uHe = 0.072 was revealed in banana weevils collected from southwestern Uganda. Banana weevils from Manyangwa displayed the lowest degree of genetic diversity ($P=39.53\%$, uHe = 0.018) while for regions, those from central Uganda harbored the lowest ($P=68.99\%$, uHe = 0.036) (Tables 5, 6 and 7).

The total number of polymorphic bands within locations varied from 137 in Nangabo to 248 in Kahuga, and 267 in central to 316 in southwestern within regions, respectively. The aggregate number of bands unique to a single population within locations varied from 0 in Bushenyi, Kakika, Bukoto, Kisseka, Sonde, Mbale Municipality to 9 in Busiu. In regions, it varied from 4 in central to 15 in eastern region (Tables 8, 9). Genetic distances [16] measured averaged between 0.000 and 0.004 (*D* values) for most of the comparisons between banana weevil populations. The highest genetic distances of 0.005 were recorded for four pairs: Nangabo and municipality; Kakika and Munyangwa; Kakika and Nangabo; and Busiu and Manyangwa as exhibited by Table 10.

### Phylogenetic analysis

The UPGMA phylogenetic tree (Fig. 2) constructed from *D* values among populations demonstrated that the 22 populations were clustered into two main groups. Group 1 was comprised of Kyenjojo, Bushenyi, Kahuga, Kamwezi, Ntungamo town council, Itojo, Kakika, Bubaale, Kyenjojo, Bukoto, Kisseka, Kilumba, Ndagwe, Dwaniro, Nabigasa, Manyangwa, Nakifuma and Nangabo Banana weevil populations. These populations are representative of the central, midwest, south and southwest regions while Group 2 consisted of Sonde, Buwalasi, Bumasike, Busiu and Mbale Municipality banana weevil populations which are illustrative of the East region except for Sonde. The UPGMA phylogenetic tree among individuals illustrated that the 1129 individuals from 22 different localities/populations were congregated into two clusters (Fig. 4) supported by the bootstrap values 0.70–0.67. The results of UPGMA phylogenetic tree (Fig. 3) for the 22 banana weevil population were consistent with those of the UPGMA phylogenetic tree (Fig. 4) for the five regions. Figure 4 indicates that banana weevils collected from the eastern region are genetically distant from those collected from central, midwest, south and southwest reg ions.

### Principle component analysis (PCoA)

When principle component analysis (PCoA) was conducted based on the mean pairwise genetic distance for the 22 populations and the populations of banana weevils grouped into the five regions, three-dimensional Eigen

**Table 8 Descriptive statistical analysis of AFLP markers across 22 populations of banana weevils belonging to five agroecological zones showing total band patterns**

| Population | Kye | Bus | Kam | Kah | Ito | Ntu | Bub | Kak | Dwa | Nab | Kil | Nda | Buk | Kis | Man | Nan | Nak | Son | Mun | Bui | Bum | Buw |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. bands | 247 | 217 | 246 | 248 | 208 | 226 | 222 | 194 | 217 | 194 | 223 | 197 | 212 | 209 | 153 | 137 | 214 | 174 | 159 | 248 | 247 | 213 |
| No. bands Freq. | 150 | 169 | 145 | 160 | 167 | 162 | 156 | 161 | 172 | 147 | 158 | 136 | 149 | 139 | 39 | 44 | 139 | 98 | 159 | 174 | 182 | 99 |
| No. private bands | 2 | 0 | 5 | 1 | 1 | 1 | 2 | 0 | 2 | 1 | 3 | 1 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 9 | 2 | 3 |
| No. of LComm bands | 32 | 30 | 30 | 33 | 20 | 33 | 27 | 19 | 23 | 20 | 27 | 18 | 23 | 21 | 11 | 6 | 25 | 14 | 16 | 32 | 32 | 25 |
| No. of LComm bands | 59 | 55 | 59 | 62 | 45 | 63 | 54 | 40 | 50 | 44 | 58 | 44 | 51 | 44 | 26 | 18 | 49 | 32 | 30 | 60 | 63 | 52 |
| Mean He | 0.072 | 0.078 | 0.062 | 0.078 | 0.069 | 0.070 | 0.077 | 0.080 | 0.074 | 0.060 | 0.079 | 0.064 | 0.076 | 0.063 | 0.018 | 0.020 | 0.066 | 0.030 | 0.075 | 0.078 | 0.084 | 0.039 |
| SE of mean He | 0.005 | 0.005 | 0.004 | 0.005 | 0.005 | 0.005 | 0.005 | 0.006 | 0.005 | 0.004 | 0.005 | 0.005 | 0.005 | 0.004 | 0.002 | 0.002 | 0.005 | 0.003 | 0.006 | 0.005 | 0.005 | 0.003 |
| Mean uHe | 0.072 | 0.079 | 0.062 | 0.079 | 0.070 | 0.071 | 0.078 | 0.081 | 0.075 | 0.061 | 0.079 | 0.064 | 0.077 | 0.064 | 0.018 | 0.020 | 0.067 | 0.031 | 0.077 | 0.079 | 0.085 | 0.039 |
| SE of mean uHe | 0.005 | 0.006 | 0.004 | 0.005 | 0.005 | 0.005 | 0.005 | 0.006 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.004 | 0.002 | 0.002 | 0.005 | 0.003 | 0.006 | 0.005 | 0.005 | 0.003 |

**Table 9 Total band pattern in the populations regions**

| Population | Midwest | Southwest | South | Central | East |
|---|---|---|---|---|---|
| No. bands | 285 | 316 | 288 | 267 | 301 |
| No. bands Freq. ≥ 5% | 133 | 155 | 153 | 99 | 150 |
| No. private bands | 7 | 5 | 8 | 4 | 15 |
| No. of LComm bands (≤ 25%) | 0 | 0 | 0 | 0 | 0 |
| No. of LComm bands (≤ 50%) | 15 | 15 | 14 | 14 | 15 |
| Mean He | 0.055 | 0.072 | 0.070 | 0.036 | 0.064 |
| SE of mean He | 0.004 | 0.005 | 0.005 | 0.003 | 0.004 |
| Mean uHe | 0.055 | 0.072 | 0.070 | 0.036 | 0.064 |
| SE of mean uHe | 0.004 | 0.005 | 0.005 | 0.003 | 0.004 |

No. bands, no. of different bands; no. bands freq. ≥ 5%, no. of different bands with a frequency ≥ 5%; no. private bands, no. of bands unique to a single population; no. of LComm bands (≤ 25%), no. of locally common bands (freq. ≥ 5%) found in 25% or fewer populations; no. of LComm bands (≤ 50%), no. of locally common bands (freq. ≥ 5%) found in 50% or fewer populations; He, expected heterozygosity

plots were obtained (Fig. 5). PCoA showed that individuals from Bumasike, Mbale Municipality and Busiu all from the eastern region were clustered in the same plane except for Buwalasi (Tables 11, 12).

## Discussion

The banana weevil, *C. sordidus*, is a chief biotic constraint in Uganda and Africa at large. However, the extent of genetic variation in *C. sordidus* populations and possible relationship to corm damage are yet to be fully documented. This study provides an insight about the genetic diversity among banana weevils from the different banana growing regions in Uganda and how it correlates with corm damage levels.

### Genetic diversity

Results from molecular analysis demonstrated that genetic diversity among banana weevil populations collected from the different regions was not significantly diverse while within-population variation was substantial.

Very high within-population variation and insignificant among-population variation in insects were also reported in [16, 17] using AFLP markers. Analysis of molecular variance indicated that the genetic differences among groups were not significant; $P = 0.897$ for the 22 populations studied and $P = 0.894$ when they are grouped into five regions. It has been stated that genetic divergence ($F_{ST}$) values less than 0.2 are indicative of high gene flow and in this study $F_{ST}$ values for the 22 populations were 0.032 and 0.010 for among populations and within populations, respectively, and 0.021 and 0.010 for among regions and within regions, respectively [16].

As predicted, genetic variability measured as mean unbiased heterozygosity (uHe) within locations/sites (uHe = 0.018 to 0.085, grand mean uHe = 0.065) and regions (uHe = 0.036 to 0.072, grand mean uHe = 0.059) of banana weevils collection indicated a low degree of genetic variability among populations thereby agreeing with $F_{ST}$ values, AMOVA *P* values and Nei's genetic distance (*D*) values.

This low level of genetic variability between populations supports the possibility of gene flow among all the examined populations. This sufficient gene flow typically in the form of migration occurring across populations counteracts with any effects of selection or genetic drift. Accordingly, this high gene flow suggests that populations among species will become genetically homogeneous in the absence of stabilizing forces such as strong variance selection [18].

It has further been reported in [19] that banana weevils have a limited mobility, and consequently, gene flow due to movement of banana weevils on their own from one region to another is rendered impossible. Therefore, gene flow may be as a result of farmers transporting banana planting materials possibly infested with banana weevils from one region to another as they are trying to look for better varieties.

AMOVA also demonstrated that 97% (in locations) and 98% (in regions) of the variation in the dataset were from genotypic variation within population. High percentage of polymorphic loci (*P*) within locations/sites ($P = 54.09\%$) and banana growing regions ($P = 75.30\%$) of collection of banana weevils indicated a high degree of genetic variability within population. This within site and regions variation may be as a result of selection pressures such as application of pesticides and different environmental conditions which force the genome of individual banana weevils to evolve in different directions, thereby causing a high degree of variation within banana weevils found in a given locality or region.

Contrary to sufficient gene flow from one location/region to another developing homogeneous genome at region level/large geographical surface area, sufficient gene flow can lead to variation within smaller locations/zones due to the introduction of new genes. Therefore, in this study high gene flow is in part a contributing factor to very high within-population genetic diversity. The phylogenetic tree (UPGMA) (Fig. 3) constructed based on the *D* values among the 22 populations demonstrated that all banana weevils from the eastern region, Buwalasi, Mbale Municipality, Busiu and Bumasike formed one distinct branch with a bootstrap value of 100% with the exception of Sonde. This may be as a result of a farmer in Sonde transporting banana planting materials infested

**Table 10 Pairwise population matrix of Nei's [15] genetic distance for the 22 banana weevil population studied across Uganda**

| Kye | Bus | Kam | Kah | Ito | Ntu | Bub | Kak | Dwa | Nabi | Kil | Nda | Buk | Kis | Man | Nang | Nak | Son | Mun | Bui | Bum | Buw | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | | | | | | | | | | | | | | | | | | | | | | Kyenjojo |
| 0.001 | 0.000 | | | | | | | | | | | | | | | | | | | | | Bushenyi |
| 0.001 | 0.002 | 0.000 | | | | | | | | | | | | | | | | | | | | Kamwezi |
| 0.001 | 0.001 | 0.001 | 0.000 | | | | | | | | | | | | | | | | | | | Kahuga |
| 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | | | | | | | | | | | | | | | | | | Itojo |
| 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | | | | | | | | | | | | | | | | | Ntungamo |
| 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | | | | | | | | | | | | | | | | Bubaale |
| 0.001 | 0.001 | 0.002 | 0.001 | 0.002 | 0.001 | 0.001 | 0.000 | | | | | | | | | | | | | | | Kakika |
| 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | | | | | | | | | | | | | | Dwaniro |
| 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | 0.000 | | | | | | | | | | | | | Nabigasa |
| 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | | | | | | | | | | | | Kilumba |
| 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.000 | | | | | | | | | | | Ndagwe |
| 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | | | | | | | | | | Bukoto |
| 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | | | | | | | | | Kisekka |
| 0.003 | 0.005 | 0.002 | 0.004 | 0.003 | 0.003 | 0.004 | 0.005 | 0.003 | 0.002 | 0.004 | 0.002 | 0.004 | 0.002 | 0.000 | | | | | | | | Manyangwa |
| 0.003 | 0.004 | 0.002 | 0.003 | 0.003 | 0.003 | 0.004 | 0.005 | 0.003 | 0.002 | 0.004 | 0.002 | 0.004 | 0.002 | 0.000 | 0.000 | | | | | | | Nangabo |
| 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.003 | 0.003 | 0.000 | | | | | | Nakifuma |
| 0.002 | 0.003 | 0.001 | 0.003 | 0.002 | 0.002 | 0.003 | 0.004 | 0.002 | 0.001 | 0.003 | 0.002 | 0.003 | 0.002 | 0.001 | 0.000 | 0.002 | 0.000 | | | | | Sonde |
| 0.002 | 0.003 | 0.001 | 0.002 | 0.002 | 0.002 | 0.002 | 0.003 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.005 | 0.005 | 0.002 | 0.004 | 0.000 | | | | Mun |
| 0.002 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.001 | 0.001 | 0.001 | 0.004 | 0.004 | 0.002 | 0.003 | 0.001 | 0.000 | | | Busiu |
| 0.002 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | 0.002 | 0.001 | 0.002 | 0.001 | 0.002 | 0.004 | 0.004 | 0.002 | 0.003 | 0.002 | 0.001 | 0.000 | | Bumasike |
| 0.002 | 0.003 | 0.001 | 0.002 | 0.002 | 0.002 | 0.003 | 0.004 | 0.002 | 0.001 | 0.002 | 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.003 | 0.002 | 0.002 | 0.000 | Buwalasi |

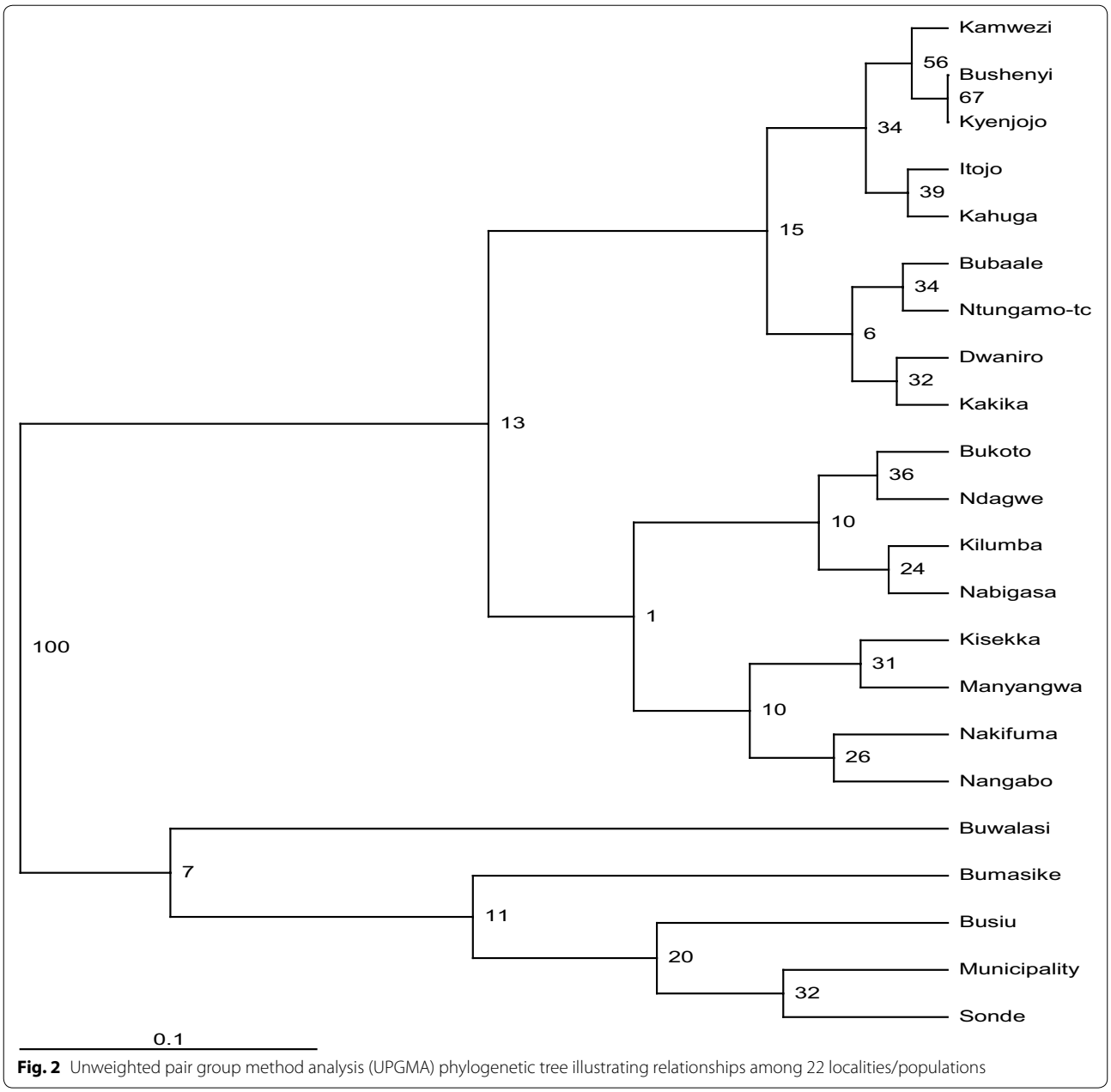


**Fig. 2** Unweighted pair group method analysis (UPGMA) phylogenetic tree illustrating relationships among 22 localities/populations

with banana weevils from the eastern region, thereby introducing those variants in the central region.

Banana weevils from the central, midwest, south and southwest regions designed a common branch in which the representatives of all populations were intermixed. This branch again had a bootstrap value of 100%. The phylogenetic tree (Fig. 4) created from the *D* values among the five-region weevil populations is in agreement

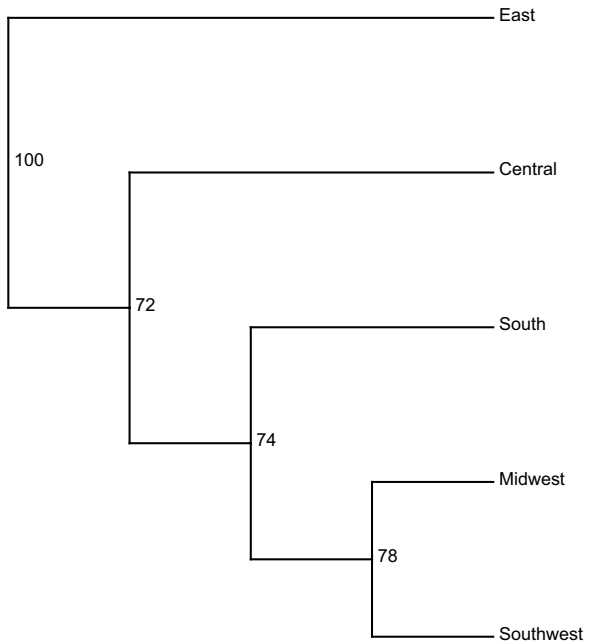


**Fig. 3** UPGMA for five regions populations of banana weevils based on Nei's [15] genetic distances calculated from 397 polymorphic bands. A number of branches indicate bootstraps with 100 replicates

with UPGMA tree (Figure) designed from the *D* values among the 22 banana weevil populations. Figure 4 reveals that East region weevil population is farther from other populations.

This scenario is clearer when principle component analysis (PCoA) was conducted. PCoA (Fig. 5) clustered all the weevil populations from the East region in the same plane/coordinates with the exception of Buwalasi population which is a bit farther, and the Sonde population is placed by PCoA in the Manyangwa and Nangabo plane presumably where it belongs based on geographic distance. The limited banana weevil mobility suggests the existence of discrete populations with limited gene flow and the likely evolution of local biotypes [19].

Epidemiologically, this explains the presence of a unique population in the eastern region. River Nile most likely acts as a physical barrier preventing migration of banana weevils from central, south, southwest and midwest regions to the East region. This leaves movement of banana weevils by farmers as they would be route through which banana weevils from other regions are imported to the eastern region; apparently, this does not seem to play a big role given the observation in the study. This situation has led to a speculation that the genetically isolated weevil populations are following their own unique evolutionary pathways. Each cluster is accumulating different mutations as well as being subjected to different selective pressures. The accumulated genetic changes have created a new unique weevil population in the eastern region.

On the other hand, there is no main geographic barrier except for the expansive cattle corridor in western Uganda between the central, south, southwest and midwest regions which can prevent gene migration between them. To support this assumption of sufficient gene migration between these regions, there are very large banana plantations running from one region to another, especially for south, midwest and southwest regions with very small strips of land separating these banana plantations. There is also a very high level of exchange of planting materials between these four regions where some of them may be infested with banana weevils, hence promoting gene flow.


**Fig. 4** Principle coordinate analysis based on mean pairwise genetic distance for 22 different banana weevil populations

## Conclusions and recommendations

### Conclusions

AMOVA and $F_{ST}$ values established a significant variation within population and a nonsignificant genetic variability among populations. Principle component analysis and phylogenetic analysis clustered the banana weevil populations into two groups: the eastern region population and the central, south, southwest and midwest regions populations, consequently proving the hypothesis of possible presence of significant genetic variation among the banana weevil populations of Uganda. Therefore, robust AFLP markers for characterization of banana weevils in Uganda have been successfully developed.

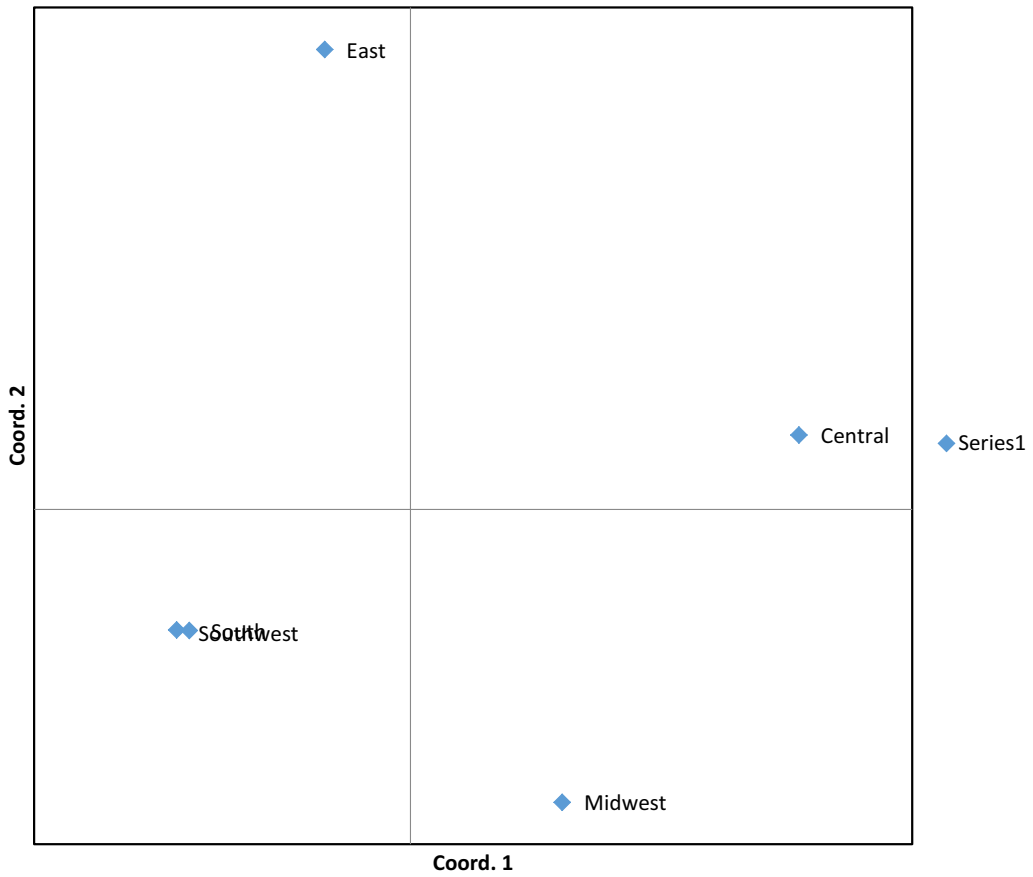


**Fig. 5** Principle coordinate analysis based on mean pairwise genetic distance for 22 different banana weevil populations collected from five regions

**Table 11 Pairwise population matrix of Nei's [15] genetic distance for the five regions**

| Midwest | Southwest | South | Central | East | |
|---|---|---|---|---|---|
| 0.000 | | | | | Midwest |
| 0.001 | 0.000 | | | | Southwest |
| 0.001 | 0.000 | 0.000 | | | South |
| 0.001 | 0.001 | 0.001 | 0.000 | | Central |
| 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | East |

**Table 12 Pairwise population matrix of Nei's [15] genetic identity for the five regions**

| Midwest | Southwest | South | Central | East | |
|---|---|---|---|---|---|
| 1.000 | | | | | Midwest |
| 0.999 | 1.000 | | | | Southwest |
| 0.999 | 1.000 | 1.000 | | | South |
| 0.999 | 0.999 | 0.999 | 1.000 | | Central |
| 0.999 | 0.999 | 0.999 | 0.999 | 1.000 | East |

## Recommendations

Attempts should be made to make follow-up studies on the seemingly unique population of eastern Uganda using other molecular techniques such as microsatellites, mitochondrial DNA (mtDNA) sequence variation and single-nucleotide polymorphism to establish whether the eastern Uganda population constitutes a different biotype.

**Authors' contributions**

KS, AK and CT designed the study, KS conducted the experiments and CT, KS, AK and WT analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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**Acknowledgements**
The authors would like to thank Bioversity International for funding the research and National Agricultural Laboratories, Kawanda, for providing laboratory facilities and office space.

**Competing interests**
The authors declare that they have no competing interests.

**Availability of data and material**
All data generated or analyzed during this study are included in this published article.

**Consent for publication**
Not applicable.

**Ethics approval and consent to participate**
Not applicable.

**Funding**
This project was funded by Bioversity International through the National Banana Research Program.

## Publisher's Note



Received: 1 September 2017 Accepted: 16 October 2018
Published online: 23 October 2018

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