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Original Article

Genetic Diversity of Bamboo (Yushinia alpina) Borer Larvae in the Mau Forest Complex, Kenya

Jackson Bwalya[1] & Dr. Calleb Olweny, PhD[1] & Dr. Donald Chungu, PhD[2]*

[1] Jaramogi Oginga Odinga University of Science and Technology, P. O. Box 210-40601, Bondo, Kenya.
[2] Cavendish University, P. O. Box 34625, Lusaka, Zambia.
[*] Author for Correspondence ORCID ID: https://orcid.org/0000-0001-9209-1883; email: jackson.bwalya31@gmail.com

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ABSTRACT

Bamboo borer larvae have caused major losses of bamboo cover in natural forests and plantations. Lack of information on the fauna of bamboo trees has been cited as the contributing factor to poor management of bamboo stands. Genetic diversity information helps understand the effects of different fauna in guiding management plans. Genetic diversity information has recently become an important tool in conservation science. This paper aimed to determine the genetic diversity of the bamboo borer larvae in the Mau Forest complex in order to generate information that could guide the management and conservation of bamboo trees (Yushinia Alpina) in the Mau Forest Complex. The mitochondrial C oxidase Subunit 1 (COI) of 12 isolates was sequenced and analyzed. A similarity search of the bamboo borer larvae was carried out using the National Center for Biotechnology Information (NCBI) BLAST search to identify the larvae species. The genetic diversity and genetic pairwise distances were determined, and Tajimas D and Nei's FU Fs statistics were calculated to estimate the population expansion that has occurred. The results showed genetic diversity (haplotype diversity 0.956) in the bamboo borer larvae population of the Mau Forest Complex. The nucleotide diversity (0.283) was found to be low. The similarity search showed that the bamboo borer larvae of Yushinia alpina belonged to four (4) species of noctuid larvae (Lepidoptera). The identity matches to the similar species scored an average of 94%. The Tajimas D (0.374) and FUs Fs (5.547) collectively indicated no rare excess mutations in the population. The results reveal high genetic diversity, which is key in the management of forest species.

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INTRODUCTION

Bamboo is increasingly becoming recognized as a natural resource with a remarkably high potential to contribute to environmental management and human development (Zhao et al., 2018a). Its properties of fast growth, renewability, strength, and the high number of applications derived from it provide mankind with a wide range of goods and services (Bystriakova et al., 2004). A particular benefit to forest ecosystems is bamboos ability to restore degraded land (Kirui & Obwoyere, 2018). Bamboo can bind soils, prevent water run-offs and survive fires in the case of above-ground biomass being burned (May et al., 2010). These benefits of bamboo have made it to be an important forest species. Bamboo can be an alternative source of biomass energy (Terefe, 2019), and the larvae of bamboo trees can be an alternative source of nutrition because of their high protein content (Sandhu et al., 2019). Bamboo helps reduce fossil fuel use by providing an alternative renewable biomass energy (Mukiri, 2015). Over the years, the potential of bamboo has been rooted in tradition -especially in Asia, South America, and Africa, where it has held an integral part in people's lives. Bamboo has over a thousand documented uses, from building materials to food (Rao et al., 1995). Bamboo forests are important for biodiversity conservation, from providing food and shelter to large animals (e.g., Giant Pandas and Mountain Gorillas) and birds to the level of the less studied soil organisms, insects, and other plants and shrubs that together make up the forest ecosystems (Jacobs et al., 2018a; May et al., 2010). The management of bamboo forests has in the recent past, focused on harvested bamboo (Mulatu et al., 2019) without calculating the economic losses that are caused by damage to standing bamboo (Varma & Sajeev, 2008). In Asia, an estimated 25% of bamboo stands are damaged by culm borers (Varma & Sajeev, 2008), while an estimated 10% of shoots are damaged due to a lack of effective management practices (Sulaeman et al., 2017; Varma & Sajeev, 2008).

In the Mau Forest, Bamboo stand coverage has reduced due to human encroachment, animal grazing and bamboo degradation by dependent invertebrates (States et al., 2019). This reduction in bamboo stands in the Mau forest has affected the stability of the Mau Forest ecosystem and water towers (Kinyanjui et al., 2014). This discovery prompted the government of Kenya to come up with a national bamboo policy (Government of the Republic of Kenya, 2015, 2019) to introduce bamboo farming and control the harvest of bamboo from natural forest stands like the Mau Forest in order to benefit from the environmental benefits which have been recently discovered attributed to bamboo. The national bamboo policy was further developed to assist in the management of bamboo trees. The management of natural stands has proven difficult because of a lack of information to guide plans and methods of conservation (Darwall & Vié, 2005; Rao & Rao, 1995). Having adequate information on the fauna of Bamboo and other forest tree species and how they affect stands in the natural forest is important in conservation (Wily, 2002).

Bamboo has been placed as an important species for the environment as it helps with water retention and prevention of soil erosion (Jacobs & Islands, 2016). Lack of an information base to make reference in implementing management practices leads to unsustainable methods of management such as pest sprays and insecticides,

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which wipe out useful predator fauna and affect the environmental stability, indirectly contributing to the change in the composition of ecosystems (Sasahara & Shibata, 2020). Availability of information on fauna and the genetic diversity, biology and pest status of dependent fauna guides conservation plans (States et al., 2019). Genetic diversity is increasingly becoming a key component in the conservation and management of forest natural resources (Siago Kusia et al., 2021).

Genetic diversity information of species is used in selecting species with desired traits for breeding (Vicente et al., 2005) and conservation of species threatened with extinction (Schwartz et al., 2007). Genetic diversity information plays a major role in forest management as it helps to determine the adaptability of insects to changing climatic conditions, which is key in developing appropriate and sustainable forest management interventions. To sustainably manage and monitor standing bamboo in natural forests, there is a need to have adequate information available to allow the planning and implementation of appropriate management and monitoring tools.

The dynamism of bamboo forests is relatively well understood, but little is known about the structuring of animal communities associated with bamboo habitats and how they affect the status of bamboo (Jacobs et al., 2018). Having this information available would help forest managers implement sustainable methods of bamboo management that would improve the benefits derived from Bamboo trees in the Mau Forest Complex. This study aimed to determine the genetic diversity of the larvae that feed on standing bamboo trees (*Yushinia alpina*) to generate information on the bamboo borer larvae that would guide bamboo conservation and management in the Mau Forest Complex.

MATERIALS AND METHODS

Description of Study Site

The Mau Forest forms the largest closed-canopy forest ecosystem in East Africa and covers an estimated area of 417,000 hectares (States et al., 2019). and is the largest remaining indigenous montane forest in East Africa and forms the largest of the five water towers (Maureen, 2014). The Mau Forest is situated in the Rift Valley of Kenya, where it borders Nakuru County to the North, Narok County to the South, Bomet County to the South-West and Kericho County to the west and has a composition of 22 forest blocks (Kirui & Obwoyere, 2018). The Mau Forest is a catchment source for Lake Victoria and the White Nile (Kinyanjui et al., 2014). The Mau Forest hosts a diversity of vegetation with a host of indigenous montane trees. It is characterized by deep, fertile and volcanic soils (Sasahara & Shibata, 2020). The rainfall of the Mau Forest is among the highest in Kenya, with annual precipitation ranging from 1000 mm to 2000 mm (Kigomo, 2007). There is an altitudinal zonation in the way trees are arranged. The bamboo trees stand at an elevation of 2300 meters above sea level, bearing coordinates -0.646, 36.06 (Kinyanjui et al., 2014). The bamboo species occur in patches in a mixed stand covering an estimated area of 150,000 hectares (Zhao et al., 2018b).

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Plate 1: A picture of the Mau Forest from an elevation of 2300 m above sea level with GPS coordinates -0.646 and 36.06.

Source: Pic: Jackson Bwalya, Coordinates: My GPS coordinates app

Sample Preparation

Larvae were collected from the Mau Forest Complex from indigenous species of *Yushinia alpina* K. Schum (Sasahara & Shibata, 2020) standing at an elevation of 2300 meters above sea level at -0.656249, 36.05646. Approval to carry out the research and collect samples from the Mau Forest for the purpose of this research was obtained from the Jaramogi Oginga Odinga University of Science and Technology (JOOUST) Ethics Review Committee and the National Commission for Science, Technology, and Information (NACOSTI) and the Local Leadership and Forest Office in Narok County. The Mau Forest cover has reduced due to large parts of the forests being cleared for agricultural activities and domestic use (Kinyanjui et al., 2014). The bamboo tree cover in the Mau Forest has, in addition, reduced due to poor management and uncontrolled logging for domestic use (States et al., 2019). The bamboo tree cover has also reduced due to dependent fauna (Government of the Republic of Kenya, 2019). Bamboo in the Mau Forest covered an area of 301.96 $km^{2,}$ standing at an elevation of 2300 meters above sea level (Kirui & Obwoyere, 2018). The larvae samples used in this study were collected by dissecting infested culms of infested bamboo trees in the Mau Forest. The samples were collected and conserved in 70% ethanol and stored in airtight containers at room temperature for purposes of DNA extraction.

Plate 2: Infested bamboo culm was dissected, and larvae were found feeding on bamboo pulp.

Source: Jackson Bwalya

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DNA Extraction

Twelve (12) caterpillars were picked at Random from the Forty-three (43) that were collected. Genomic DNA was extracted using the Zymo Research DNA Mini Prep™ kit according to the manufacturer's specifications (Zymo Research Corp, South Africa). The concentration and purity of extracted DNA were estimated using a Nanodrop™ Lite Spectrophotometer (Thermo Scientific Inc, USA) at 260-280 nm and by horizontal gel electrophoresis (Thistle Scientific Ltd, USA) on a 0.8% (w/v) agarose gel at 100 V for 30 minutes and visualized under UV after staining with Gel Red™ (Thermo Scientific, USA) according to Emitaro *et al*. (2017).

Analysis of the Mitochondrial Gene Cytochrome C Oxidase Subunit I (COI)

A fragment of the COI mitochondrial gene was amplified by polymerase chain reaction (PCR) with the use of universal primers LCO 1490 (F) (5'—GGT CAA GET ATC ATA AAG ATA TTG G– 3') and HCO 2198 (R) (3'—TAA ACT TCA GGG TGA CCA AAA AAT CA– 5') (Former *et al.,* 1994). Bioneer Accu Power® PCR Premix (BioneerInc, USA) was used to perform PCR. To each 20 µl Bioneer reaction tube, 2 µl DNA, 2 µl Taq buffer, 1.4 µl Mgcl2, dNTPs 0.4 µl, Primers 2 µl, Taq DNA Polymerase 0.4 µl, Nuclease free water 11.8 µl was added. Amplification was performed in a programmable Master thermocycler (C1000-Bio Rad, USA). The PCR conditions included denaturation, annealing, and initial and final extension at temperatures of 94 ^{o}C for 30 sec, 55 ^{o}C for 1 minute, and 72 ^{o}C for 2 minutes, respectively before cooling off at 15 ^{o}C. PCR products were separated by horizontal gel electrophoresis on 1.5 % (w/v) agarose gel at 100V for 45 minutes and visualized under UV after staining with 2 µl Gel RedTM (Thermo Scientific). The quality of amplified PCR products recovered was assessed in horizontal gel electrophoresis on 1.5 % (w/v) agarose gel at 100 V for 45 minutes and visualized under UV after staining with 2 µl Gel RedTM (Thermo Scientific).

DNA Sequencing

The PCR products were sent to Macrogen Europe B.V. **(**Meibergdreef 311105 AZ, Amsterdam, Netherlands) for purification and sequencing. The sequences of all mitochondrial loci were assembled using Sanger. The Forward and reverse sequences were assembled and trimmed using Geneious Prime® 2020.0.4. Invalid end sequences were trimmed, and all sequences obtained in this study were deposited in the NCBI GenBank (www.ncbi.blast.lnm.nih.gov) for allocation of accession numbers of the isolates.

Data Analysis

The sequences were subjected to a similarity search using the National Centre for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST) to match the sequences to known species of larvae in the database. Polymorphic sites were determined on an intrapopulation level using DnaSP 6. Haplotype diversity and nucleotide diversity were determined. Phylogenetic analysis was determined using the MEGA version 11 software (Tamura et al., 2011; available at http://www.megasoftware.net/) and the tree was constructed using the Neighbour-Joining (NJ) algorithm (Tamura & Nei, 1993). Tajimas D test and Fu's F_s were determined using Alerquin software to estimate the expansion in the sequences.

Evolution of genetic divergence was calculated according to Tamura, Nei., and Kumar. (2004). Analyses were conducted using the Maximum Composite Likelihood model [1]. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 802 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2].

RESULTS AND DISCUSSION

Species delineation Results based on Nucleotide BLAST using the National Institute of

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Biotechnology Basic Local Alignment Search Tool (BLAST)

The sequences subjected to the NCBI BLAST search tool for similarity search showed species that showed close identification to the isolates of larvae in this study. The table below (*Table 1*) shows the results of the similarity search. It shows the species in the NCBI gene bank that each isolate identified closely with and the percentage of identity to the species. The p values in the table are a representation of the level of background noise in each Sequence. 0.00 is an indication of no background noise in the Sequence.

Table 1: Similarity Search, Percentage Identity and E-Value

Sample Id	Collected At	Similar Species	Gen Bank Accession No. of Similar Species	P Value	Identity Match Percentage
JB1	Mau Complex Forest	*Apamea acera*	HM868321.1	0.00	94.37
JB2	Mau Complex Forest	*Zomariana doxasticana*	KC314462.1	0.00	91.59
JB3	Mau Complex Forest	*Apamea acera*	HM868321.1	0.00	94.42
JB4	Mau Complex Forest	*Apamea acera*	HM868321.1	0.00	94.22
JB5	Mau Complex Forest	*Apamea aceera*	HM868321.1	0.00	94.42
JB7	Mau Complex Forest	*Apamea acera*	HM868321.1	0.00	94.42
JB9	Mau Complex Forest	*Euros proprius*	JN301443.1	$2e^{-95}$	80.37
JB10	Mau Complex Forest	*Apamea acera*	HM868321.1	0.00	90.46
JB11	Mau Complex Forest	*Hedya nubiferana*	KT144269.1	0.00	91.23
JB12	Mau Complex Forest	*Apamea acera*	HM868321.1	0.00	90.40

A similarity search of the NCBI database for the 10 samples that were successfully sequenced and aligned in the current study showed no relationship of the isolates to the known documented bamboo borer larvae *O. fuscidentalis* or *D. minutus*. The similarity search revealed seven (7) of the specimen sequences showed identity match percentages with a mean value of 94% with sequences of *Apamea ascera* (Gene Bank Accession no. HM868321.1), the sample bearing ID JB2 scored a 91.59% identity to *Zomariana doxasticana* (Gene Bank Accession no. KC314462.1), the sample with ID JB9 scored 80.37% similarity to *Euros proprius* (Gene Bank Accession no. JN301443.1) and the sample bearing the ID JB11 scored a 91,23% similarity to *Hedya nubiferyana* (Gene Bank Accession no. KT144269.1). The identity match percentages in the table were the highest recorded identity match percentages for each isolate in the study. None of the sequences of the isolates in this study scored an identity percentage match of 97% or above. The e-values of the sequences showed a value of 0.00 except for one Sequence, which showed an e-value of $2e^{-95}$. The E-values confirm the sequences lack random background noise except for the sample bearing the ID JB9, which exhibited traces of background noise.

Assigning of Accession Numbers.

All the samples were submitted to the National Center for Biotechnology Information (NCBI) Gene Bank under submission number SUB11707291, and 7 of the 10 sequences have since been allocated accession numbers and published in the NCBI gene bank database. Table

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2 shows the Sequences and the assigned accession numbers by the National Center for Biotechnology Information (NCBI).

Table 2: The accession numbers assigned to the sequences collected from the Mau Forest by the National Center for Biotechnology Information (NCBI).

Sample ID	Voucher Number/Name	Accession Number in NCBI
JB1	*Apamea sp*	ON898545
JB2	*Apamea sp*	ON898544
JB3	*Apamea Sp*	ON898543
JB4	*Apamea Sp*	ON898542
JB5	*Apamea Sp*	ON898541
JB7	*Apamea Sp*	ON898540
JB9	*Euros Sp*	Unallocated
JB10	*Apamea Sp*	Unallocated
JB11	*Hedya Sp*	Unallocated
JB12	*Apamea Sp*	ON898539

Phylogenetic Tree

Figure 1 is a Phylogenetic tree produced by maximum likelihood using 10 isolates of larvae specimens collected from standing bamboo (*Yushinia alpina*) from the Mau Forest. Four (4) sequences of Lepidoptera species that identified closely to the isolates and an outgroup coleoptera species were obtained from the NCBI Genbank database in order to determine the phylogenetic relationship of Isolates. The four lepidoptera species are *Hedya nubiferana* (Gene Bank accession number KT244269.1), *Oletherutin sp* (Gene Bank accession number KJ982230.1), *Omphisa fuscidentalis* Hampson (Gene Bank accession number ON869464.1) and *Apamea acera* (Gene Bank accession number HM868321.1). The outgroup used to root the tree is *Gonimbresia cytherea* (NC 061331.1:1484-3014).

The ten (10) COI gene sequences of bamboo borer larvae isolated in this study and the four (4) species of noctuid sequences obtained from the National Center for Biotechnology Information (NCBI) database produced a phylogenetic tree with three (3) distinct clades (Figure 1). Isolates JB 1 (ON898545), JB2 (ON898544), JB4 (ON898542) and JB7 (ON898540) clustered into one clade which shared a recent common ancestor with the species *Zomariana doxasticana* (KC314462.1). Isolates JB10 (Accession number unassigned), JB9 (Accession number unassigned), JB3 (ON898543), JB5 (ON898541) and JB 12 (ON898539) cluster into another clade which shares common ancestry with the noctuid species *Apamea acera* (Gene Bank accession number HM868321.1) and *Apamea acera* (KJ387546.1). The isolate JB11 clustered in a clade with two noctuid species *Omphisa fuscidentalis* Hampson (Gene Bank accession number ON869464.1) and *Zomariana doxasticana* (GU688777.1). The phylogenetic tree produced in this study showed a variation of close relationships. The species that matched closely to the isolates in this study (Species-level) have little record of their economic importance on bamboo trees (Huang et al., 2022; Luo et al., 2018; Management, 2021; Varma & Sajeev, 2008). The existing mentions their economic importance in wheat as the affected wheat field in North America (Oppenheim et al., 2018).

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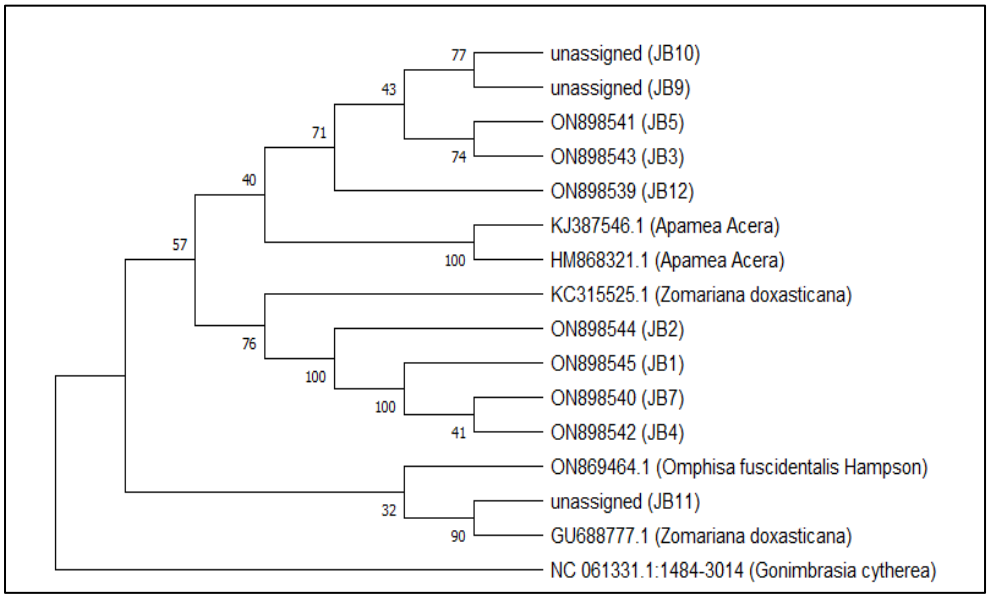
Figure 1: Phylogenetic tree by Maximum Likelihood showing the relationship between the samples collected from the Mau Forest based on the neighbour-joining method.



Genetic Pairwise Divergence

Genetic distance based on Mitochondria Cytochrome Oxidase Subunit gene sequence using Kimura 2 Parameter calculations. The genetic distance gives an idea of how much breeding occurs between the isolates.

Table 3: Genetic distance based on Mitochondria Cytochrome Oxidase Subunit gene sequence using Kimura 2 Parameter calculations.

	JB12	JB11	JB10	JB9	JB7	JB5	JB4	JB3	JB2	JB1
JB12										
JB11	0.16									
JB10	0.05	0.20								
JB9	0.14	0.30	0.15							
JB7	0.70	0.74	0.69	0.81						
JB5	0.00	0.16	0.07	0.14	0.70					
JB4	0.70	0.74	0.69	0.82	0.00	0.71				
JB3	0.00	0.16	0.05	0.14	0.70	0.00	0.70			
JB2	0.80	0.85	0.77	0.89	0.17	0.80	0.18	0.80		
JB1	0.68	0.74	0.70	0.81	0.00	0.68	0.00	0.68	0.16	

The genetic distances in the current study were calculated for the sequences to estimate the mutation that has occurred in the population. From the results, we see that there is a high genetic distance between JB12 and 4 other sequences JB1 (0.68), JB2 (0.80), JB4 (0.70) and JB7 (0.70), with the highest distance being between JB12 and JB2 (0.80). This shows the level of mutation that has occurred in the sequences. JB2 is the Sequence that has the most sites that have mutated from the other sequences obtained in this study. The smallest genetic distance in the current study is noted between JB12 and JB5 (0.00) and JB3 and JB12 (0.00) and JB5 and JB3 (0.00). The genetic distances observed confirm the phylogenetic tree clustering and the similarity search findings. The level of differences in the sites is an indication of a level of genetic divergence (Joshi et al., 2013).

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Genetic Diversity

Table 4: A table showing calculated genetic diversity indices of the bamboo borer larvae isolates collected from the Mau Forest

Number of Individuals	Number of Haplotypes	Haplotype Diversity	Nucleotide Diversity	Tajimas D test (p-Value)	FUs Fs test (p-Value)
10	8	0.956	0.283	0.374	5.547

The haplotype diversity, nucleotide diversity, Tajima's D test and Fu's Fs test are presented in *Table 4*, including associated simulated p-values.

The haplotype diversity, which is also known as the genetic diversity in this study, was found to be 0.956. Haplotype diversity represents the probability that two randomly selected alleles are different. In the current study, the probability of genetic diversity is high. The high haplotype diversity can be an explanation for the unique breeding history of a population (Zhang et al., 2021). The nucleotide diversity in this study was relatively low (0.283), as confirmed by the pairwise genetic divergencies. This could indicate a small long-term effective population under the theory of natural evolution (Liu et al., 2014).

Tajima's D value in this study was positive (0.374) but statistically not significant at p>0.10. The positive result of the Tajimas D is an indication of a balance in the selection of the population as expected under the neutrality theory and no excess rare nucleotide site variants as would be expected under a neutral model of evolution. The result of Fu's FS test was positive (5.547), indicating a deficiency of rare haplotypes over what would be expected under neutrality. Following Fu's Fs test, the hypothesis of neutral evolution was significantly rejected at p>0.10 for the bamboo borer larvae. The positive value of the Tajimas D and the positive value of the FUs Fs collectively is evidence of there being no excess rare mutations in the population. The calculated genetic indices support the findings of the similarity search and the subsequent sequence analyses that have been carried out in this study.

The Haplotype network below (*Figure 2*) shows the relationship and number of mutations that have occurred between the sequences.

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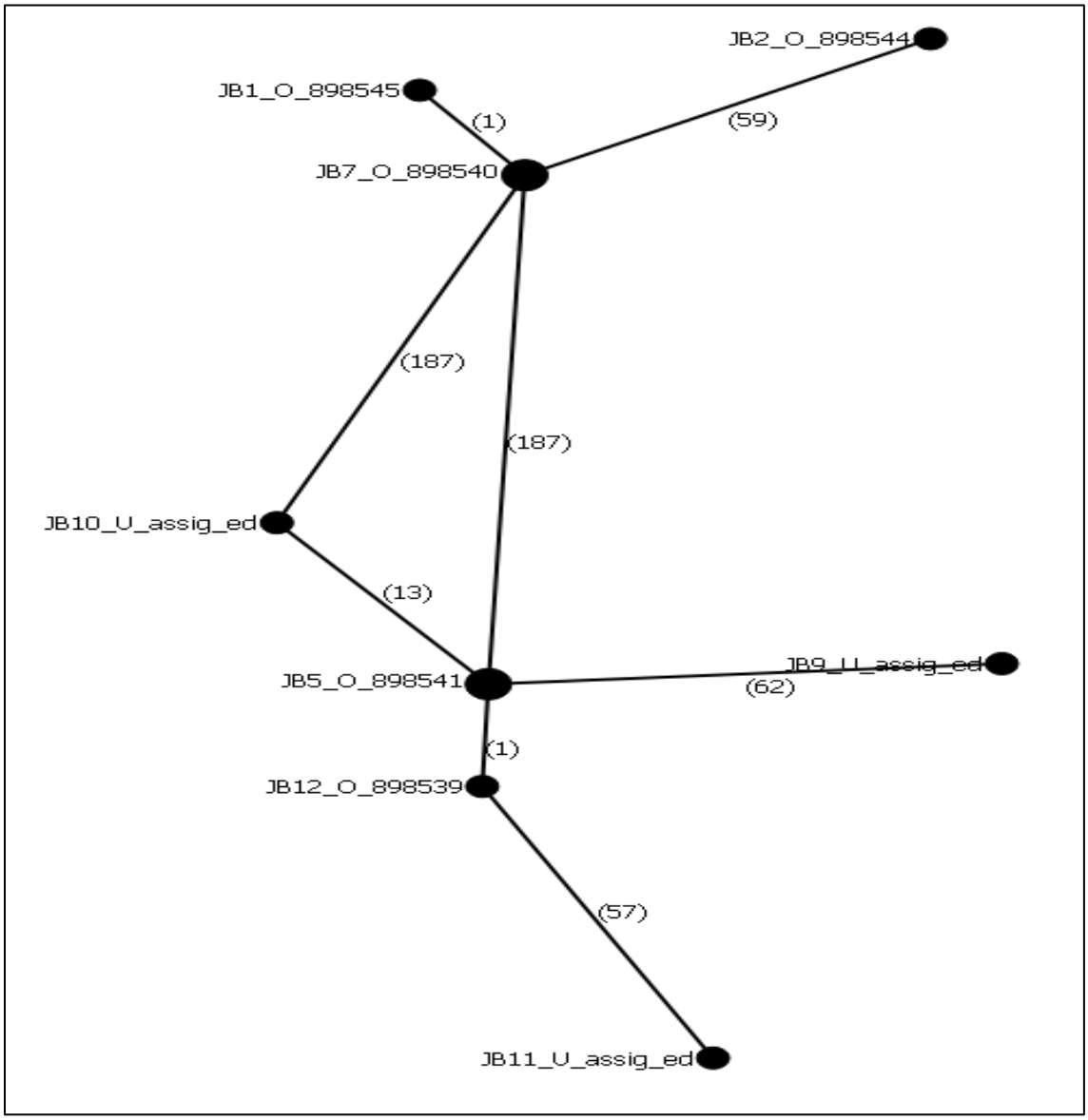
Figure 2: A Haplotype network of the isolates in this study developed by minimum spanning method.



The circle size corresponds to the number of haplotypes with the branches representing a nucleotide change and the numbers on the branches indicating the number of mutations that have occurred between the sequences.

DISCUSSION

Taxonomic Assignment

Correct identification and classification of dependent fauna and understanding of the genetic makeup and variation are essential in the development of new technologies that allow for sustainable use and conservation of natural resources (Schwartz et al., 2007). Molecular markers are proving to be an important tool in species identification because of their efficiency in identification (Jehle et al., 2006; Office & Mussels, 2014) particularly the use of the COI gene in identification and genetic diversity studies has become widely used as it is cost-effective (Arnemann et al., 2016; Nong et al., 2019). This study aimed to identify and assess the genetic diversity of bamboo borer of the Mau Forest using Cytochrome Oxidase Subunit I (COI) genes. Hendricks *et al.* (2021) argue that DNA barcoding using the gene COI is not an efficient method for the identification and cataloguing of insect species because the standard COI region may not offer adequate resolution into species identification. However, COI gene sequences have been proven to be suitable for the identification of forensically

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important insect species as they provide clear differentiation and identification of insect species (Henaish & Elmetwaly, 2021).

The use of COI in identification and genetic diversity studies has recently become widely used, especially in the implementation of forest management plans (Oppenheim et al., 2018). Zhang et al. (2019) carried out a study to identify and assess the genetic variation of morphologically similar bamboo wireworms. The conclusion was that the approach of using DNA Barcoding for identification advanced the understanding of bamboo shoot wireworms. This aided in the monitoring of the bamboo wireworms and in the management of bamboo stands. The use of the COI mitochondria gene helped to distinguish morphologically similar larvae which could not easily be identified morphologically (Zhang et al., 2019). The conclusion of Zhang et al. (2019) agrees with the findings of the similarity search in this study. The isolates which were morphologically identical were identified as four different noctuid species in the NCBI blast search. Four (4) species of bamboo borer larvae (Euros, Hedya, Zomariana and Apamea) were revealed to feed on Y. alpine trees in the Mau Forest. *Apamea sp* showed dominance as out of the twelve (12) sampled individuals, seven (7) were apamea. The dominance of *Apamea sp* in the findings of this study suggests underlying differences in the host preference of the identified larvae. This study represents the first documented identification of bamboo borer larvae in Kenya.

Phylogenetic Analysis

It is plausible with the findings in this study to conclude that there is a considerable level of genetic variation in the bamboo borer larvae of the isolates in this study and therefore, agree with the hypothesis that bamboo can host different species of borer larvae (Seifert et al., 2016). Different studies have confirmed bamboo as a host for different species of larvae (Jacobs et al., 2018; Luo et al., 2018; Sittichaya et al., 2013). Bamboo as a plant is becoming recognized as an important plant for the conservation of water towers, especially in natural forests (Terefe, 2019). It therefore, begs research that will aid in developing and implementing monitoring and management technologies for bamboo forest management.

Genetic Diversity Assessment

The haplotype diversity in the current study was found to be 0.956. This haplotype diversity is high compared to the genetic diversity of other insects (Hole & Hole, 2009). A high haplotype diversity (also referred to as genetic diversity) is an indication of the ability of a species to withstand diseases and adapt to changes in climates and other environmental risks (FAO et al., 2001). The high haplotype diversity of the bamboo borer larvae species identified in this study is an indication of its ability to survive the changing climatic dynamics. Genetic diversity has been described as the bridge that lies between extinction and survival of many species (Mondini et al., 2009). The post-2020 global biodiversity framework has set out to safeguard the genetic diversity of species (Hoban et al., 2020). The importance of genetic diversity can be extended to the management of forests. The findings in this study argue the conclusion that bamboo borers of standing bamboo are of the same species (Leela Kayikananta, 2000). The combination of a high haplotype diversity and a low nucleotide diversity in this study is an indication of rapid demographic expansion from a small, effective population (Grapputo et al., 2005).

The results of Tajimas D in the current study were found to be positive, an indication of a lack of expansion in the population resulting from a recent bottleneck (Alvi et al., 2020). The population bottleneck could be attributed to different reasons, one of which could be the destruction of the habitat (Martins et al., 2007). The FUs Fs statistics value for the current study was positive, an indication of past population expansion which occurred (Siago Kusia et al., 2021). The findings of the current study agree with the theory of neutrality test, which dictates that most mutations are none significant as they can be removed by natural selection (Mackintosh et al., 2019). The results of the current study indicate that the population is evolving under

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natural conditions (Mukhopadhyay & Bhattacharjee, 2016). The values of the neutrality tests of Tajimas D (Tajima, 1989) and FUs Fs statistics (FU, 1997) are used to estimate the population history of clades (Mukhopadhyay & Bhattacharjee, 2016), which can also be confirmed by the phylogenetic tree. Both the Tajimas D and FU F statistics were not significant.

CONCLUSION

We successfully sequenced the mitochondria C Oxidase subunit I (COI) of ten (10) isolates from the Mau Forest complex bamboo and carried out a similarity search to identify the isolates to a species. Genetic diversity was determined showing high levels of genetic diversity, indicating the ability of the species to survive changes in climate. The results of the Tajimas D and FUs Fs are evidence of natural evolution occurring in the population.

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