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RESEARCH ARTICLE

# Genetic Diversity of Common Bean Bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) From Different Bean Growing Regions of Tanzania

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## ABSTRACT

Bruchid beetles (Coleoptera: Bruchidae) are seed-eating insects; most of their species' feed on legumes. Bean crops around the world (especially *Phaseolus vulgaris*) are favorably attacked by the common bean weevil (*Acanthoscelides obtectus* (Say)) and Mexican bean weevil (*Zabrotes subfasciatus* (Boheman). *A. obtectus* and *Z. subfasciatus* are the main pests of beans. These pests are present in almost all bean-producing localities of Tanzania. This study aimed to identify the genetic diversity of bean bruchid weevils (*A. obtectus* and *Z. subfasciatus*) in Tanzania's bean-producing regions using molecular taxonomy (*12S rRNA* and *COI* markers). The results obtained did not show genetic diversity of *A. obtectus* present in Tanzania but showed 100% identity. *Z. subfasciatus* showed 80.2% identity. Differences in some sequence alignment explained the genetic diversity observed between *A. obtectus* and *Z. subfasciatus*. Better knowledge of bruchid diversity present in Tanzania will help breeders and farmers to propose effective management methods with an impact on environmental changes and human health.

***Keywords:*** Genetic diversity, Common bean bruchids, bean growing region

## INTRODUCTION

Common beans (Phaseolus vulgaris (L.)) are essential legume crops. Farmers grow it for protein, vitamins, minerals, and fibre. (Broughton et al., 2003; Castro-Guerrero et al., 2016). Dry seeds and bean stems are eaten. Tropical and subtropical cereal-based cropping methods grow it. Common bean pests like Acanthoscelides obtectus and Zabrotes subfasciatus harm crops in the field and during storage. (Hill, 1989; Paul, 2007). Bruchid infestation lowers grain's nutritional and market value and makes seeds unfit for human eating, agriculture, and commerce. These pests are mostly controlled by fumigation with toxic chemicals like carbon disulfide, phosphine, and methyl bromide or dusting with several insecticides, which leave residues on the grain and risk food safety. (Koona and Dom, 2005; Swella and Mushobozy, 2007).

Plant-based extracts can manage bruchids, but they are short-lived, degrade quickly, and may harm seed germination. (Yusuf et al., 2011). Morphological, biochemical, and molecular traits of host plants govern bruchids in common beans.

Resistant varieties are economically important, but ecotype variation in bruchids has made some common bean lines vulnerable to the pest in some regions. Breeders also face pest genetic diversity. Common bean bruchid resilience has decreased due to ecotypes. (Fox et al., 2010). Developing a cultivar resistant to numerous insect ecotypes is difficult. (Appleby and Credland, 2004). Understanding phylogeographic patterns in the species is of particular agronomic concern because knowledge of the innate range of a species may give new data on the climatic conditions in which the species evolved initially and can also otherwise guide the search for agents of biological control of *A. obtectus* and *Z. subfasciatus* in accordance of environmental variation. In developing countries, farmers who produce common bean experience significant losses because of the high reproductive rate of bean bruchids owing to their ability to reproduce in a broad range of ecological conditions (Alvarez et al., 2005). This modern expansion of the geographical and host range of common bean bruchids thus threatens the production of other crops, and there is an urgent need to control bruchid populations by other means than chemical methods. This research aimed to determine the genetic diversity of common bean bruchids (*A. obtectus* and *Z. subfasciatus*) from different bean-growing regions of Tanzania.

## MATERIALS AND METHODS

### Description of experimental site

Specimens of *A. obtectus* were collected in December 2020 from farmer's sites in Songwe, Morogoro, and Karatu, and *Z. subfasciatus* were collected from Kilimanjaro and Arusha in Tanzania and then brought to the African seed health laboratory at SUA for inoculation. The parent samples from each location were inoculated in bean genotypes AO-1012-29-3-3A and Njano gololi in a plastic container separately. The trial was left undisturbed for 14 days to allow bruchids to lay eggs under room temperature of 30 ± 5oC and relative humidity of 70 ± 5%. Then all parents removed from each plastic container were taken for DNA extraction. Similarly, the newly emerged adult F1 bruchids from AO-1012-29-3-3A and Njano gololi were extracted for DNA. Only 15 parents and 15 F1 bruchids from each location were used for DNA extraction.

### DNA extraction

The total genomic DNA was extracted using Quick-DNA Tissue/Insect kit (from ZYMO research). A total of 750 µL of bashing beadTM buffer was added to the bruchid sample and then ground. After grinding, the homogenized mixture was centrifuged at 13000rpm for 2min. Then 400µL supernatant was transferred to a Zymo-SpinTM III-F filter in a collection tube and centrifuge at 8000rpm for 1min. The cap was removed, and 1200µL genomic lysis buffer was added to the filtrate in the collection tube containing supernatant liquid and mixed well. After mixing, 800µL was transferred to Zymo-SpinTM IICR Column 1 in a collection tube and centrifuged at 10000rpm for 1min. After centrifuge, the flow was discarded from the collection tube, and the step was repeated for the remaining 800 µL. About 200µL of DNA pre-wash buffer was added to the Zymo-SpinTM IICR Column in a new collection tube and centrifuged at 10,000g for 1 minute. After centrifugation, 500µL of g-DNA Wash Buffer was added to the Zymo-SpinTM IICR Column and centrifuged at 10,000 g for 1 minute. In the final step, the Zymo-SpinTM IICR Column was transferred to a clean 1.5 ml microcentrifuge tube, and 100 µL of DNA Elution Buffer was added directly to the column matrix and centrifuge at 10,000 g for 1 minute to elute the DNA. The extracted DNA was stored at -20 °C.

### DNA amplification

Polymerase chain reaction (PCR) amplifications were performed in a final volume of 25 µL, which contained 1µL of extracted DNA, 12.5µL of 2x Taq-

Master mix, 9.5 of nuclease-free water, and 1µL of each forward and reverse primers. PCR conditions were set corresponding to particular primers. Sample for *A. obtectus* and *Z. subfasciatus* were amplified using *12Sbi* and *12Sai* for 12s *rRNA*; *C1-J-2183* and modified *TL2-N-3014* for *COI* obtained from Inqaba Biotech with their specific PCR conditions as shown in Table 1 below.

**Table 1.** Polymerase chain reaction description of the primer used (Simon et al., 1994)

| Primer | Primer sequences | Primer base pair |
|---|---|---|
| *12Sbi*-F;*12Sai*-R | F-5´- AAGAGCGACGGGCGATGTGT-3´; R-5´- AAACTAGGATTAGATACCCTATTAT-3´ | 379 |
| *C1-J-2183*-F; Modified *TL2-N-3014* f | F-5´- CAACATTTATTTTGATTTTTTGG -3´; R-5´- TCCATTGCACTAATCTGCCATATTA -3´ | 736 |

PCR reactions were performed for each primer pair on a Gene Amp® PCR System 9700 using the following cycling conditions: initial denaturation at 92°C for 1min 30s; 30 cycles of 92°C for 30s, annealing temperature at 55°C for 1 min 30s; and final elongation at 72°C for 10 min. Annealing temperatures were 55°C for both *12s rRNA* and *COI*.

## DNA visualization of Amplicons

To visualize the amplicons, 8µl of the PCR product was separated by electrophoresis in 1% agarose gel (1g of agarose dissolved in 100ml of 1 x TAE) with subsequent staining by 3µl of ethidium bromide, then electrophoresed at 100V for 50min until dye markers have migrated to an appropriate distance. The gel was photographed using a smartphone Samsung Galaxy A20s (0916) without a UV sensor.

## Preparation of sample and Mitochondrial DNA sequencing

The integrity of the PCR amplicons was visualized on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd.) stained with EZ-vision® Bluelight DNA Dye. In order to identify nucleotide sequences from PCR products, single bands of PCR amplified DNA fragments generated by each of the 12 *Sbi* forward primer and *12Sai* reverse primer for *12s rRNA* and *C1-J-2183* forward primer and *TL2-N-3014* reverse primer for COI were excised from the agarose gels and recovered into Agarose purification column using ExoSAP Protocol.

The fragments were sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, and BRD3-100/1000 according to manufacturer's instructions. The products were labeled and then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053). Purified PCR products were used for mitochondrial DNA sequencing at Inqaba biotechnology in South Africa using an applied Bio system ABI 3730XL Genetic Analyser with a 50cm array, using POP7. Forward and reverse primers produced gene-specific nucleotide sequences in separate reactions. BLAST was performed on parental and F1 bean bruchid mitochondrial DNA sequences from Njano gololi and AO-1012-29-3-3A bean lines. Clustal-W aligned NCBI database and sequences to identify sequence identity.

## Sequence alignment and Phylogenetic analysis

The sequences were aligned using the CLUSTAL W multiple alignment procedure in Macvector version 18.0.2 (Thompson et al., 1994), and a phylogenetic analysis was performed in MEGA v.7 (Kumar et al., 2016) using the maximum likelihood (ML) model. For *12s RNA* and COI, there were a total of 366 and 749 nucleotide positions, respectively, in the final dataset. Finally, the distance matrices for 12S rRNA and COI were constructed using a General Time Reversible model (Tavare, 1986).

A Nearest-Neighbour-Interchange analysis was used to draw a tree with bootstrap analysis of 1000 replicates/ bootstraps (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances, computed using the General Time Reversible model (Tavare, 1986), are expressed as the number of uniform rates per site. Only bruchid sequences were included in the analysis in the General Time Reversible method (Tavare, 1986). A sequence of the species KP682959 *Spermophagus* sp. and KP682946 *S. decellei* (plant host *Porana racemose*) were added as an outgroup species. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016). For *A. obtectus* out of the group were HQ178007 *Bruchidius lutescens* and HQ178006 *B. kiliwaensis.*

## RESULTS

**PCR amplicons:** Data from DNA analysis of bruchid species based on Mitochondria DNA have demonstrated the similarity of bruchid ecotypes. No polymorphic bands of bean bruchids were observed in the progenies that emerged from resistant and susceptible bean genotype and their parents (Figure. 1).

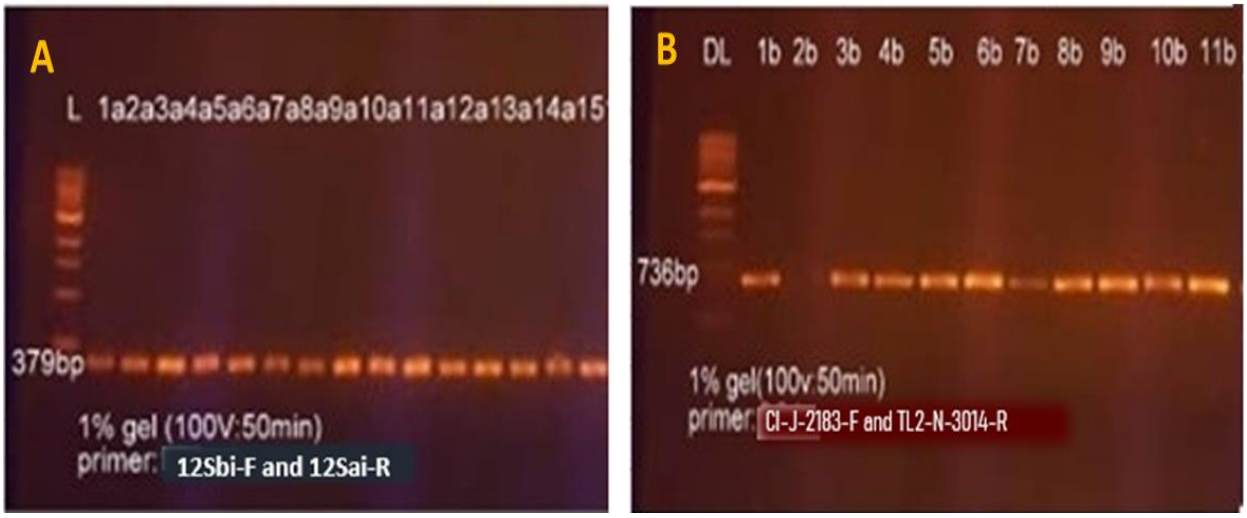


**Figure 1.** PCR amplicons of the bean bruchid samples collected from different bean growing region.

Note: Picture A, PCR product amplifying the *COI* region. Picture B: PCR product amplifying the *12s rRNA.* DL= DNA Ladder (1kb; N3232S), 1b to 11b and 1a to 15a=bean bruchids mtRNA samples; water as a negative control was maintained for PCR analysis.

### Mitochondrial DNA sequencing *12S rRNA*

Mitochondrial DNA sequences were obtained from PCR-amplified products. The nucleotides were compared to published sequences in the database for the genes originally used to design primers. DNA sequences for parents in Morogoro, Songwe, and Karatu, as well as F1 bean bruchids that emerged from Njano gololi of Morogoro and Karatu showed a 100% identity to the published A. obtectus sequence from Brazil (KF157282), Sweden (MF925724) and Switzerland (AY676676). The published nucleotide sequence from China (KX825864), France (AY945998), and Republic of the Congo (MN420800) exhibited 99.7% nucleotide sequence similarities.

Bean bruchids from Kilimanjaro and Arusha were identified as Z. subfasciatus. Parents' bruchids from Arusha, F1 emerged from Njano gololi, and F1 from A0 29-3-3A of the Arusha region exhibited 82.8%, 82.8%, and 82.2% sequence identity, respectively. Arusha parents, $F_1$ from Njano gololi and Kilimanjaro, showed 82.8% similarity to the published Zabrotes subfasciatus from France (AY945994). Emerged F1 in AO 10-12-29-3-3A from Arusha showed 82.2% identity to Z. obliteratus from France (AY945993).

### Mitochondrial DNA sequencing *COI*

Mitochondrial DNA sequences were obtained from PCR-amplified products. The nucleotides were compared to published sequences in the database for the genes originally used to design primers. DNA sequences for parents bruchids in Morogoro, F1 bean bruchids emerged from Njano gololi in Morogoro region and F1 adults emerged from AO 29−3A−3 in Morogoro, Songwe parents, Songwe F1 adults emerged from Njano gololi, Karatu parents and Karatu F1 adults bruchids emerged from A0 29 3A 3 showed 100% nucleotide sequence similarities to the published *A. obtectus* sequence from China (KJ909879 and KX825864). Also, there was 99.7% and 99.5% similarity to the published from Sweden (MF925724) and India (MN120831), respectively.

For *Z. subfasciatus* species in Arusha region, parents and F1 adults emerged from Njano gololi showed 80.2% identity to *Z. subfasciatus* population from China (KJ909880).

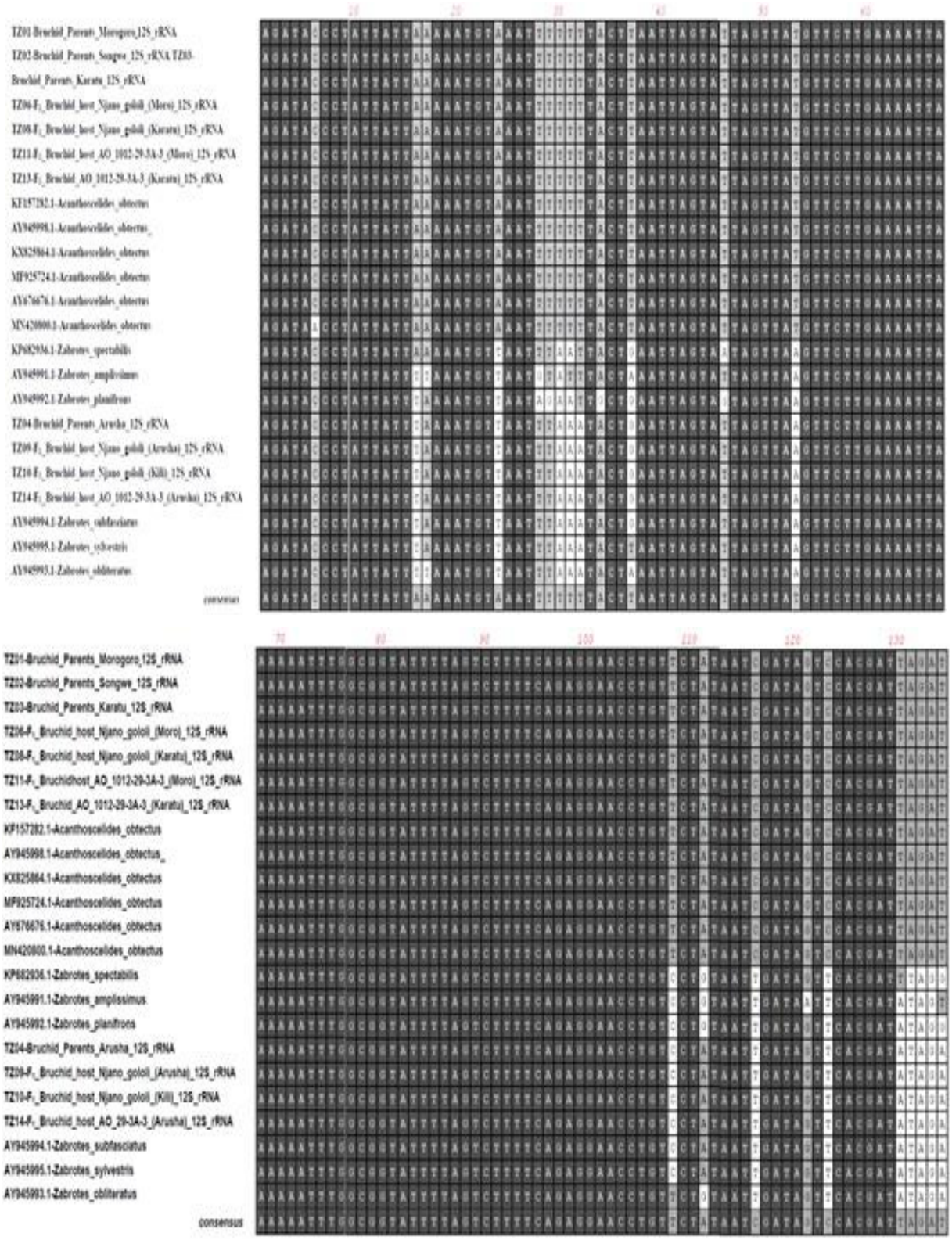


**Figure 2.** Sequence alignment for mitochondrial gene (12S rRNA) of bean bruchids and the out groups.

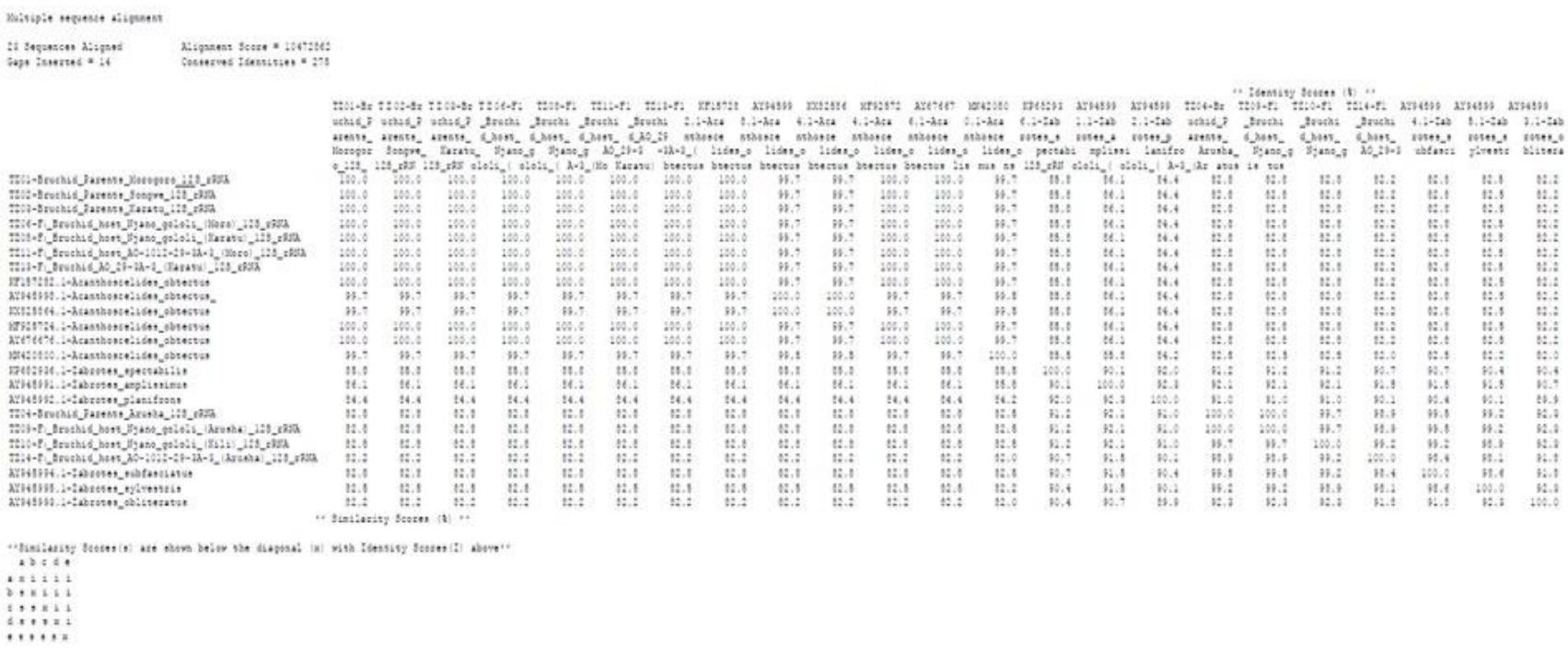


**Figure 3.** Identity matrix of Bean bruchids (*A. obtectus* and *Z. subfasciatus*) from mitochondrial Gene *12s rRNA*

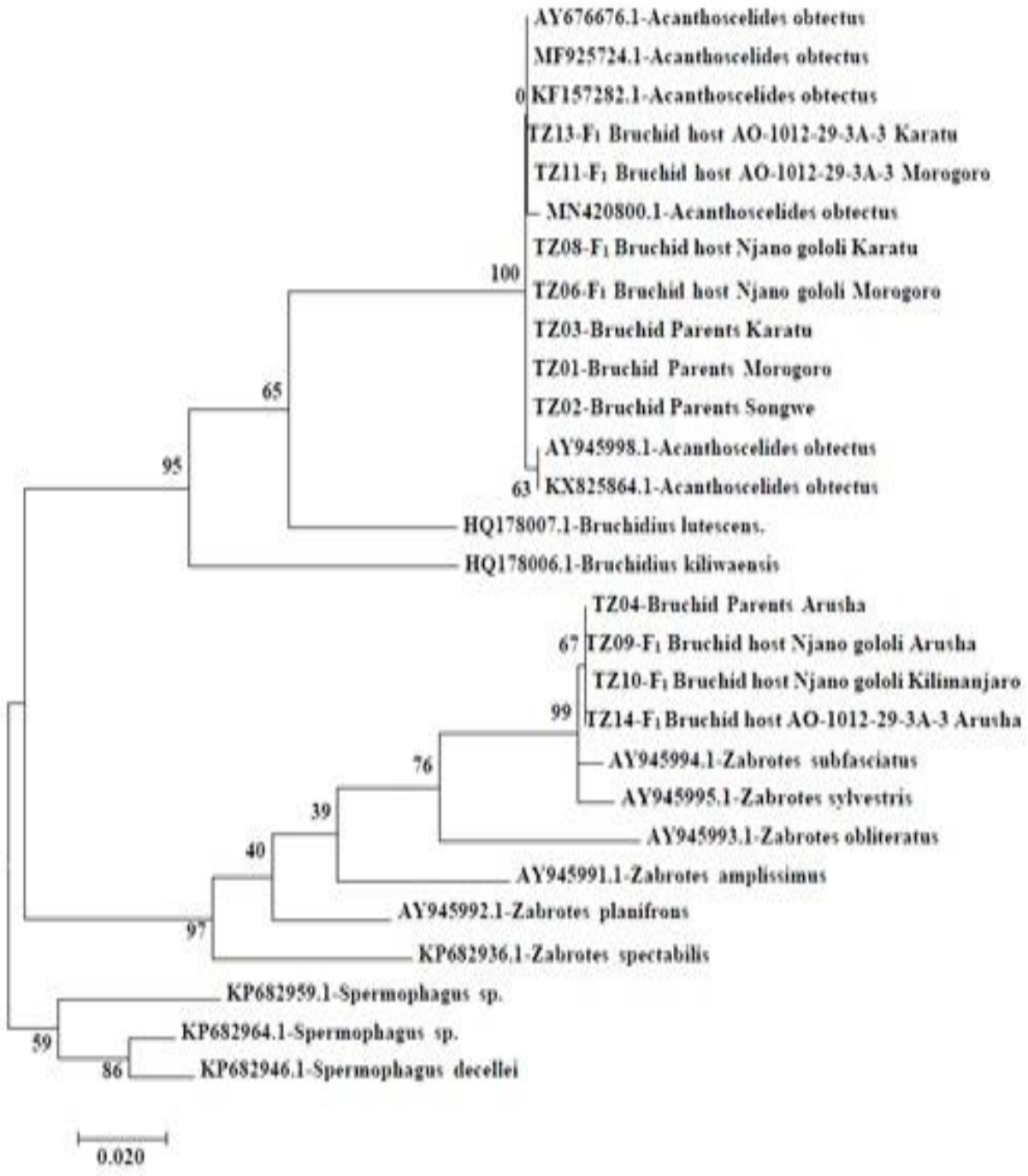


**Figure 4.** Phylogenetic tree developed from PAUP program with bootstrap values calculated based on the nucleotide sequences of mitochondrial genes from bean bruchids (*A. obtectus* and *Z. subfasciatus*).

Note: HQ178007 *Bruchidius lutescens* and HQ178006 *B. kiliwaensis* were used as an out-group for *A. obtectus* and AY945992 *Z. planifrons,* KP682936 *Z. spectabilis*, AY945991 *Z. amplissimus*, KP682959 *Spermophagus* sp, KP682964 *Spermophagus* sp and KP682946 *Spermophagus decellei* were used as an out-group for *Z. subfasciatus.* Sequence genes for out-group were taken from NCBI database.

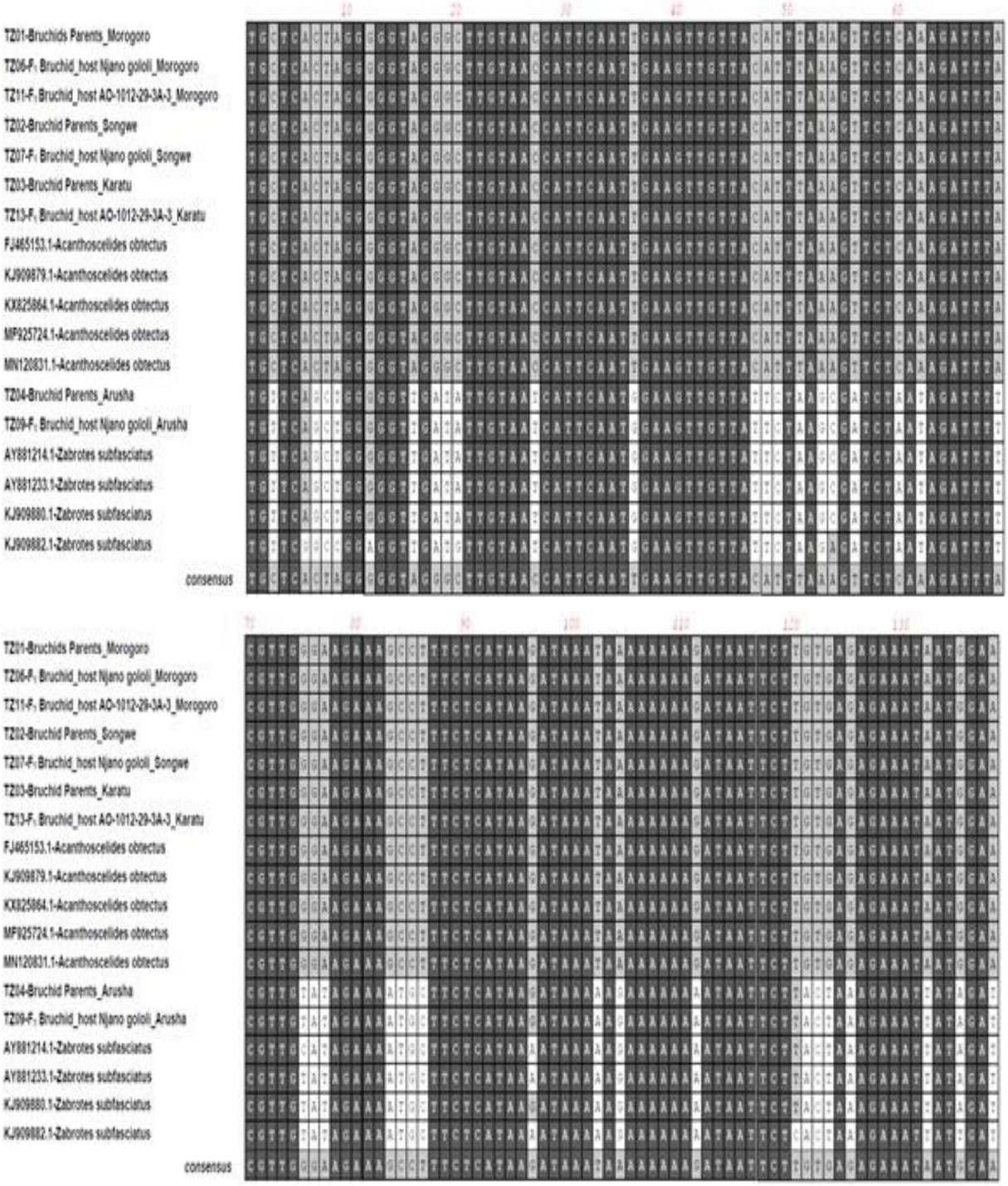


**Figure 4.** Sequence alignment for mitochondrial gene (COI) of bean bruchids and the out groups.

ClustalW multiple sequence alignment

10 Sequences Aligned  Processing time: 3.6 seconds
Gaps Inserted = 0  Conserved Identities = 570
score = 6902704

Pairwise Alignment Mode: Slow
Pairwise Alignment Parameters:
    Open Gap Penalty = 15.0 E x t e n d  Gap Penalty = 6.7

Multiple Alignment Parameters:
    Open Gap Penalty = 15.0  Extend Gap Penalty = 6.7
    Delay Divergent = 40%  Transitions: Weighted

** Identity Scores (%) **

| | TZ01-Br uchids Parents _Morogo ro | TZ06-F1 Bruchi d_host Njano g ololi_M | TZ11-F1 Bruchi d_host AO-1012- 29-3 A-3_Mor | TZ02-Br uchid P arents_ Songwe | TZ07-F1 Bruchi d_host Njano g ololi_S | TZ03-Br uchid P arents_ Karatu | TZ13-F1 Bruchi d_host AO-1012- 29-3 A-3_Kar | FJ46515 3.1-Aca nthosce lides o btectus | KJ90987 9.1-Aca nthosce lides o btectus | KX82586 4.1-Aca nthosce lides o btectus | MF92672 4.1-Aca nthosce lides o btectus | MN12083 1.1-Aca nthosce lides o btectus | TZ04-Br uchid P arents_ Arusha | TZ09-F1 Bruchi d_host Njano g ololi_A | AY88121 4.1-Zab rotes s ubfasci atus | AY88123 3.1-Zab rotes s ubfasci atus | KJ90988 0.1-Zab rotes s ubfasci atus | KJ90988 2.1-Zab rotes s ubfasci atus |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TZ01-Bruchids Parents_Morogoro | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 100.0 | 100.0 | 99.7 | 99.5 | 80.2 | 80.2 | 80.1 | 80.0 | 80.2 | 78.8 |
| TZ06-F1 Bruchid_host Njano gololi_Morogoro | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 100.0 | 100.0 | 99.7 | 99.5 | 80.2 | 80.2 | 80.1 | 80.0 | 80.2 | 78.8 |
| TZ11-F1 Bruchid_host AO-1012-29-3A-3_Morogoro | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 100.0 | 100.0 | 99.7 | 99.5 | 80.2 | 80.2 | 80.1 | 80.0 | 80.2 | 78.8 |
| TZ02-Bruchid Parents_Songwe | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 100.0 | 100.0 | 99.7 | 99.5 | 80.2 | 80.2 | 80.1 | 80.0 | 80.2 | 78.8 |
| TZ07-F1 Bruchid_host Njano gololi_Songwe | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 100.0 | 100.0 | 99.7 | 99.5 | 80.2 | 80.2 | 80.1 | 80.0 | 80.2 | 78.8 |
| TZ03-Bruchid Parents_Karatu | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 100.0 | 100.0 | 99.7 | 99.5 | 80.2 | 80.2 | 80.1 | 80.0 | 80.2 | 78.8 |
| TZ13-F1 Bruchid_host AO-1012-29-3A-3_Karatu | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 100.0 | 100.0 | 99.7 | 99.5 | 80.2 | 80.2 | 80.1 | 80.0 | 80.2 | 78.8 |
| FJ465153.1-Acanthoscelides obtectus | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 100.0 | 99.5 | 99.5 | 99.7 | 100.0 | 80.6 | 80.6 | 80.5 | 80.4 | 80.6 | 79.2 |
| KJ909879.1-Acanthoscelides obtectus | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 100.0 | 100.0 | 99.7 | 99.5 | 80.2 | 80.2 | 80.1 | 80.0 | 80.2 | 78.8 |
| KX828646.1-Acanthoscelides obtectus | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.5 | 100.0 | 100.0 | 99.7 | 99.5 | 80.2 | 80.2 | 80.1 | 80.0 | 80.2 | 78.8 |
| MF926724.1-Acanthoscelides obtectus | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 100.0 | 99.7 | 80.5 | 80.5 | 80.4 | 80.2 | 80.5 | 79.0 |
| MN120831.1-Acanthoscelides obtectus | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 100.0 | 99.5 | 99.5 | 99.7 | 100.0 | 80.6 | 80.6 | 80.5 | 80.4 | 80.6 | 79.2 |
| TZ04-Bruchid Parents_Arusha | 80.2 | 80.2 | 80.2 | 80.2 | 80.2 | 80.2 | 80.2 | 80.6 | 80.2 | 80.2 | 80.5 | 80.6 | 100.0 | 100.0 | 99.2 | 99.5 | 100.0 | 91.6 |
| TZ09-F1 Bruchid_host Njano gololi_Arusha | 80.2 | 80.2 | 80.2 | 80.2 | 80.2 | 80.2 | 80.2 | 80.6 | 80.2 | 80.2 | 80.5 | 80.6 | 100.0 | 100.0 | 99.2 | 99.5 | 100.0 | 91.6 |
| AY881214.1-Zabrotes subfasciatus | 80.1 | 80.1 | 80.1 | 80.1 | 80.1 | 80.1 | 80.1 | 80.5 | 80.1 | 80.1 | 80.4 | 80.5 | 99.2 | 99.2 | 100.0 | 99.7 | 99.2 | 91.7 |
| AY881233.1-Zabrotes subfasciatus | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.0 | 80.4 | 80.0 | 80.0 | 80.2 | 80.4 | 99.5 | 99.5 | 99.7 | 100.0 | 99.5 | 92.0 |
| KJ909880.1-Zabrotes subfasciatus | 80.2 | 80.2 | 80.2 | 80.2 | 80.2 | 80.2 | 80.2 | 80.6 | 80.2 | 80.2 | 80.5 | 80.6 | 100.0 | 100.0 | 99.2 | 99.5 | 100.0 | 91.6 |
| KJ909882.1-Zabrotes subfasciatus | 78.8 | 78.8 | 78.8 | 78.8 | 78.8 | 78.8 | 78.8 | 79.2 | 78.8 | 78.8 | 79.0 | 79.2 | 91.6 | 91.6 | 91.7 | 92.0 | 91.6 | 100.0 |

** Similarity Scores (%) **

**Similarity Scores(s) are shown below the diagonal (x) with Identity Scores(I) above**

| | a | b | c | d | e |
|---|---|---|---|---|---|
| a | x | i | i | i | i |
| b | s | x | i | i | i |
| c | s | s | x | i | i |
| d | s | s | s | x | i |
| e | s | s | s | s | x |

**Figure 6.** Identity matrix of Bean bruchids (*A. obtectus* and *Z. subfasciatus*) from mitochondrial gene *COI*

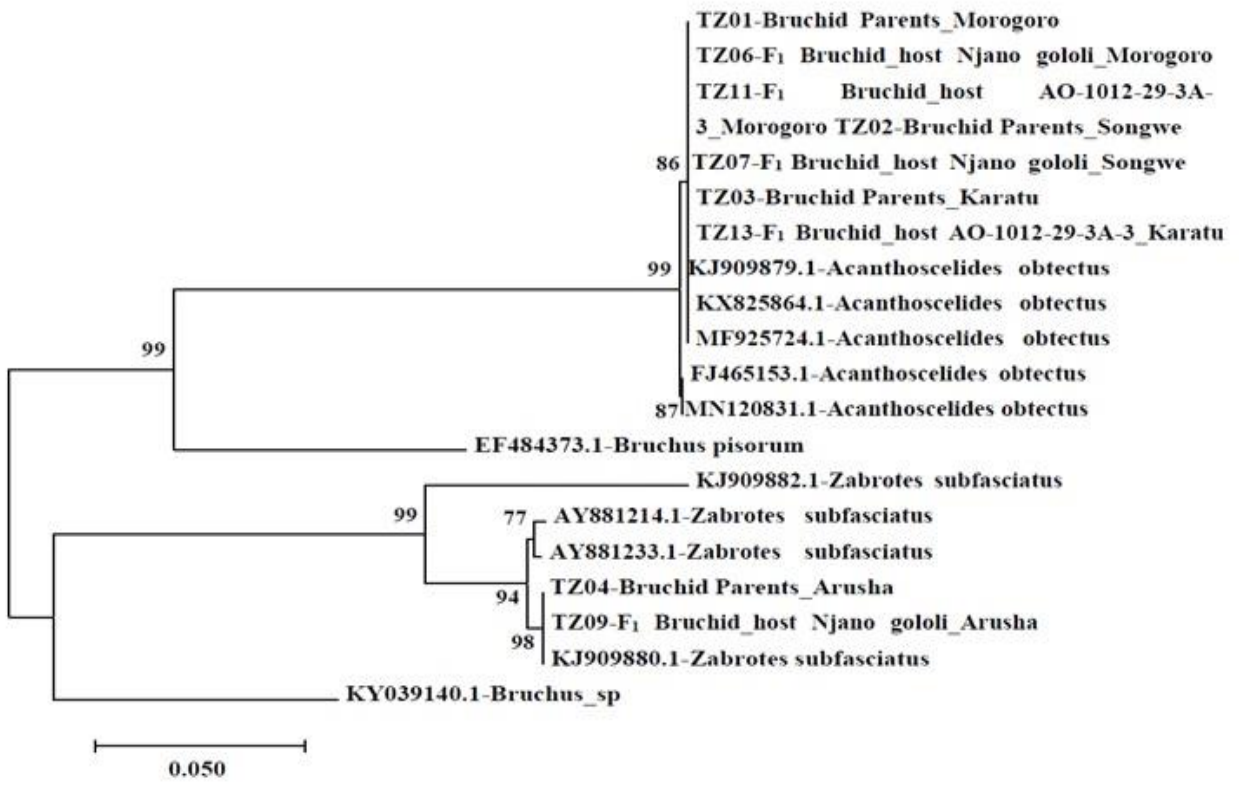


**Figure 5.** Phylogenetic tree developed from PAUP program with bootstrap values calculated based on the nucleotide sequences of mitochondrial genes from bean bruchids (*A. obtectus* and *Z. subfasciatus*).

*Note:* EF484373 *Bruchus pisorum* was used as an out-group for *A. obtectus* and KY039140 *Bruchus* sp was used as an out-group for *Z. subfasciatus*. Sequence genes for out-group were taken from NCBI database.

## DISCUSSION

Bean bruchids are destructive storage pests that adapt strongly to many environmental conditions. In Tanzania, bean bruchids are found in all bean-growing regions. If uncontrolled, populations of *A. obtectus* and *Z. subfasciatus* can grow exponentially and cause significant losses of bean seeds (Southgate, 1979). Genetic diversity analysis helps understand the genetic structure, differentiation, and relationship among bean bruchids populations, which makes sense when developing pest management approaches and enacting resistance screening and resistance breeding strategies.

The findings from this study showed no intra-specific genetic variation observed in *A. obtectus* species despite the resistant bean genotype's geographical location and feeding effect. The results agree with studies of Ong'amo (2012), who did a survey of genetic diversity and population structure of *Busseola segeta* and confirmed that *B. segeta* moths did not exhibit genetic variation in terms of host use. *B. segeta* presence in a wide range of hosts in different fields without genetic variation strongly suggests the existence of host use plasticity. Some insects can overcome plant defense without undergoing genetic variation.

Similarities of nucleotide sequence of *A. obtectus* in geographical location of Tanzania may be due to human trade of host bean. Since bean weevils move with bean seeds the sequence identity of *A. obtectus* may be due to transfer and exchange of common bean from one region to another for marketing within the country. This is in agreement with (Duan *et al.,* 2017), who reported that absence or very low genetic variation between bean weevils is apt to communicate and diffuse along with bean seed using egg, larva, pupa, or adult, which likely results in transfer and expansion of this pest between regions and identical host selection pressures in both populations.

A very low genetic variation of 0.6% observed in *Z. subfasciatus* emerged from resistant bean genotypes due to feeding effects of AO 10-12-29-3-3A bean genotypes to bean bruchids. This was the same as Gaete-Eastman et al. (2004), who did a study on phenotypical and genetic variation

of *Neuquenaphis edwardsi* and *N. staryi* and reported a slightly intraspecific genetic variation of *N. edwardsi* species due to the outcome of environmental factors and/or host features affecting aphid morphology.

This study observed interspecific genetic diversity between *A. obtectus* and *Z. subfasciatus*. These two species were observed to have different nucleotide sequencing. The phylogenetic tree clustering plot based on genetic distance between populations of *A. obtectus* from different regions were clustered into one genetic group and the population of *Z. subfasciatus* into another group, suggesting the existence of distinct genetic differentiation among the two bruchid species

## CONCLUSION

The results from this study did not show within/intra-species genetic diversity of bean bruchids, and the F1 progeny emerged due to feeding on resistant or susceptible bean genotypes. This concludes that the environment has nothing to do with a genetic change in insects, perhaps for a very long period since the mutation is a gradual change. Hence virulence of an insect on attacking beans can change according to environmental conditions without undergoing genetic variations.

Variations between *A. obtectus* and *Z. subfasciatus* have been validated in this experiment, confirming the genetic variability and virulence of each bean during storage. Of more importance is that bruchid resistance should essentially focus on addressing resistance to two bruchid species and not resistance to both *A. obtectus* and *Z. subfasciatus.*

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## CONFLICT OF INTERESTS

The authors declare no conflict of interests.

## AUTHORS' CONTRIBUTIONS

EM designed, collected, analyzed, interpreted, and wrote the paper. PK and LC critically revised the article, commenting on results, conclusions, and suggestions. All authors reviewed and accepted the manuscript.

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