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Mitogenomic profiling and gut microbial analysis of the newly identified polystyrene-consuming lesser mealworm in Kenya

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Plastic waste has recently become a major global environmental concern and one of the biggest challenges has been seeking for alternative management options. Several studies have revealed the potential of several coleopteran species to degrade plastics, and this is the first research paper on plastic-degradation potential by lesser mealworms from Africa. This study evaluated the whole mitogenomic profile of the lesser mealworm to further identify the insect. The ability of the mealworm to consume Polystyrene (PS) was also evaluated alongside its associated gut microbiota diversity. Our results showed a complete circular mitochondrial genome which clustered closely to the *Alphitobius* genus but also suggested that our insect might be a new subspecies which require further identification. During the PS feeding trials, overall survival rates of the larvae decreased when fed a sole PS diet while PS intake was observed to increase over a 30-day period. The predominant bacteria observed in larvae fed PS diets were *Kluyvera*, *Lactococcus*, *Klebsiella*, *Enterobacter*, and *Enterococcus*, while *Stenotrophomonas* dominated the control diet. These findings demonstrated that the newly identified lesser mealworm can survive on a PS diet and has a consortium of important bacteria strongly associated with PS degradation. This work provides a better understanding of bioremediation applications, paving the way for further research into the metabolic pathways of plastic-degrading microbes and bringing hope to solving plastic waste pollution while providing high-value insect protein towards a circular economy.

Keywords Plastic biodegradation, Polystyrene, Lesser mealworm, Gut microbiota, Waste management

The accumulation of plastic waste has become a major global environmental concern that is rapidly escalating and Africa is no exception. The global plastics production is projected to reach 460 million tons, with production volumes expected to continue increasing over the next few decades[1]. By 2050, production is estimated to reach 590 million metric tons, indicating a growth of over 30%[2]. Africa has become the world's second most polluted continent, with over 500 containers of waste being imported each month, and this value is estimated to double in the next 5 years[3]. Additionally, The current recycling rate for plastic waste stands at a mere 14–18% and this low recycling rate, coupled with the enduring nature of plastics and microplastics, has led to detrimental effects on the environment, contributing to climate change, as well as posing risks to human health[4].

Polystyrene (PS) is an inexpensive and durable polymer whose annual production is estimated to be 20 million metric tons[5]. Polystyrene is one of the major microplastics that is accumulating both in terrestrial and aquatic ecosystems[6]. Polystyrene waste results from the commercial use of its common form, Styrofoam, which is used for various applications including food storage containers, packaging of equipment, disposable plates and cups, and insulation in construction[7]. To combat this growing crisis, more attention needs to be paid to various polystyrene degradation methods without secondary pollution. Different methods have been used to recycle PS including chemical, thermal, and mechanical methods[8]. Nevertheless, these methods are expensive and produce aromatic compounds that may magnify the negative environmental impacts[9]. Recent studies have refocused research interests on the ability of insects such as *T. molitor*, *G. mellonella*, and *Z. atratus* and their microbes to biodegrade PS as a promising sustainable strategy[10–13]. Recently, a new mealworm species termed the dark lesser mealworm has been reared at the International Center for Insect Physiology and Ecology (*icipe*), Kenya. The characteristics of this new species are similar to other mealworms in the Tenebrionidae family and specifically closely related to

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the *Alphitobiini* subfamily. The lesser mealworms are considered a major pest in poultry production and are easily observed in poultry litter, chicken coops, and manure[14]. This species causes damage to poultry houses and can be a reservoir for poultry pathogens. Nonetheless, a recent study on the lesser mealworm, *Alphitobius diaperinus*, concluded that this species is capable of ingesting and degrading PS, and identified *Cronobacter, Pseudomonas,* and *Kocuria* bacteria which have been proposed to have plastic-degradation capabilities[15].

Several Lepidopteran species such as the Indian meal moth (*Corcyra cephalonica*) and the lesser wax moth (*Achroia grisella)* have demonstrated the ability to degrade low-density plastics[16–18]. However, tenebroinids are one of the most characterized plastic-degrading insect species, and their gut bacteria have provided valuable insights into the roles gut microbes play in the degradation of plastic[11]. As recently described by[19], yellow mealworms were able to survive on PS-fed diets. The authors documented the ability of mealworms to degrade PS with approximately 47.7% of the Styrofoam ingested and converted into carbon dioxide while the residue was excreted as frass. In the subsequent experiments[20], demonstrated the distinct roles the gut microbiota plays in the biodegradation process where they established that *Exiguobacterium* sp. strain YT2 isolated from the mealworm gut was able to form a biofilm around the PS and degrade the PS. Other insect species such as the greater wax moth (*Galleria mellonella*) and super worms (*Zophobas morio*) have also been identified to have the same abilities as mealworms to degrade plastic and convert it into non-hazardous compounds[11]. In a study done by[12], PS degradation capabilities of *T. molitor*, *G. mellonella*, and *Z. atratus* were compared and reported that all three species were able to degrade PS at different degrees with the super worms having the highest PS consumption rates while the yellow mealworm depolymerized the PS strongly. In addition, they identified *Enterococcus* and *Enterobacteriaceae* bacteria in all three species which may play a role in plastic degradation.

However, information on characterizing and identifying potential PS-degrading bacteria and fungi in the gut of lesser mealworms, particularly in Africa is lacking. Therefore, the scope of the current research was to investigate the modulation of the gut microbiota of the newly identified dark lesser mealworm in response to PS-fed diets as well as identification through whole mitochondrial genome sequencing. This will shed light on the possibility of endemic mealworm to degrade PS and the associated microbes contributing to its effectiveness. This research lays the groundwork for future studies into the microbial upcycling of plastic waste into high-value insect protein-rich biomass for animal feeds.

Methodology

Mealworms rearing

The lesser mealworms' mother colony was reared at the animal rearing and containment unit (ARCU) of the International Centre of Insect Physiology and Ecology (*icipe*). The larvae were reared on trays (46W × 60L × 10H cm) provided with a bedding of wheat bran as a nutritional source. The wheat bran used in rearing and feeding trials was purchased from PEMBE Feeds LTD, Nairobi. The larvae were also provided with vegetable and fruit waste as a water source throughout the rearing period. The rearing room was maintained at a temperature of 26 ± 2 °C and 50–70 ± 2% humidity with normal lighting and proper aeration. From the mother colony, 6th instar larvae were collected for feeding trials and further molecular work as described below.

Mitogenome sequencing, assembly and annotation

Samples of two adult lesser mealworms were used to recover the complete mitogenomic profile and whole genomic DNA isolated from each sample using the Isolate II Genomic Extraction kit (Bioline, London, UK) following the manufacturer's instructions. The extracted DNA was then sent to BGI Genomics (BGI, Tai Po, N.T, Hong Kong) for whole genome sequencing using the DNBseq sequencing platform. The mapping and assembly of the mitogenomes from the sequenced contigs was done using the MitoZ v2.4 pipeline[21]. In brief, MitoZ filters out the raw reads then de-novo assembles the mitogenome based on a modified version of SOAPdenovo-Trans. We used the default quick mode assembly where the MitoZ filtered out the candidate mitogenome sequences using a confidence score and annotated the PCGs, tRNAs, and rRNAs. MITOS web server was used to compare the annotations to ensure correct annotation of all genes and also used to predict the tRNA structures. Nucleotide composition, AT, and GC skews were calculated using geneious prime. Phylogenetic relationship was inferred for 21 mitogenomes of Tenebrionidae family including our own dark mealworm and multiple sequence alignment was used to construct a bootstrapped neighbor-joining phylogenetic tree using Kimura 2 parameter in MEGA[22].

PS consumption and survival rates

The polystyrene (PS) materials used for feeding trials were collected from Styrofoam used in equipment packaging stored at *icipe*. To evaluate the PS consumption, the 6th instar larvae were placed in food storage containers (H110 × W208 × L208 mm) in a dark room maintained at a constant temperature of 26 ± 2 °C and 70 ± 2% humidity. Three experimental diets were compared for 30 days: Bran alone (3.6 g), PS alone (3.6 g), and Bran + PS (3.6 g:3.6 g). The cumulative ratio of bran to PS (B: PS) was 1:1 g/g. The experiments were replicated 3 times with 100 larvae in each treatment diet. The experiment was conducted until the onset of pupation. After every 5 days, the larvae were counted and dead larvae removed and the residual PS from PS diets was weighed. The survival rates were calculated as the percentage of the live larvae based on the initial number of each treatment diet (n = 100). The PS consumption rates were estimated as the weight of PS consumed divided by the initial weight of PS (3.6 g). This methodology was adapted from a previous study done by[20].

Microbial community analysis

After 30 days, 5 larvae from each container were randomly collected and the entire gut contents were harvested from each larva. The guts were separated into 3 parts (foregut, midgut and hindgut). Following the manufacturer's instructions, genomic DNA was extracted from each gut sample using the Isolate II Genomic Extraction

kit (Bioline, London, UK). A nanodrop 2000/2000c spectrophotometer (Thermo Fischer Scientific, Wilmington, USA) was used to determine the concentration and quality of the DNA, and those with good quality with an A260nm/A280nm range of 1.8–2.0 were chosen for metabarcoding analyses. The full-length bacterial 16S rRNA gene of ~ 1500 bp was sequenced using an Oxford Nanopore Technologies (ONT) MinION device with R9.4.1 flow cells. The libraries were prepared with the 16S barcoding kit SQK-16S024 as per the manufacturer's instructions. The following components were used in the library preparation with PCR step: 10 pmol L^{-1} of each 16S barcode, 10 ng L^{-1} of DNA template, 0.625 U L^{-1} MyTaq DNA polymerase (Bioline), and 5X MyTaq reaction buffer (5 mM dNTPs, 15 mM $MgCl_2$, stabilizer, and enhancer) (Bioline). The reactions were run in a Master cycler Nexus gradient thermal cycler (Eppendorf, Germany) under the following conditions: initial denaturation for 2 min at 95 °C, followed by 40 cycles of denaturation for 30 s at 95 °C, annealing for 45 s at 55 °C, extension for 1 min at 72 °C, and a final extension step of 10 min at 72 °C. The barcoded libraries were purified using AMPure XP beads (Agencourt Bioscience) and pooled together before loading on the flow cells for sequencing.

Sequencing and data analysis

Sequencing was done for 4 h with live base calling on the MinKNOW software (v 20.10.3) on the ONT cloud[23]. The reads that passed sequencing generated fastq files that were analyzed using the q2ONT pipeline (https://github.com/DeniRibicic/q2ONT) which uses features embedded in QIIME2[24]. In this pipeline, all the fastq files were concatenated and reads demultiplexed into their respective barcodes using the Porechop tool (v 0.2.4) (https://github.com/rrwick/Porechop). The demultiplexed reads were then trimmed and adapters removed to a minimum length of 1000 bp using the trimmomatic tool v 0.39[25]. Chimeric sequences were checked using VSEARCH and filtered out using the UCHIIME tool. The reads were then aligned to MAFFT to generate a rooted tree and taxonomy was then assigned using the SILVA132 database. The Operational Taxonomic Unit (OTU) clustering was performed using the open reference approach at 85% similarity. R software was then used for the downstream analysis and to achieve this, all the QIIME2 outputs were imported into R. OTUs not assigned to any taxonomy were filtered out and those that were assigned were used to generate cumulative bar plots of the most abundant genera present. Alpha and beta diversity parameters were calculated using the Phyloseq package in R[26]. Alpha diversity estimates were calculated using the Shannon index while the beta diversity was calculated using the Bray–Curtis dissimilarity distance method.

Statistical analysis

R software package (version 4.0.2) was used to execute all the statistical analyses[27]. ANOVA was used to analyze the differences in diet consumption and survival rates followed by pairwise comparison using Student's t-test with Tukey's correction to analyze differences between the diets[28]. PERMANOVA was used to compare the statistically significant differences between the microbial communities in the 3 diet groups using the Adonis function in R using 999 permutations.

Results

Sequencing, mapping and assembly of the African lesser mealworm complete mitogenome

Mitogenome DNBseq sequencing resulted to about 14 million reads which were used to assemble and annotate the complete mitochondrial genome of the dark lesser mealworm (Tenebrionidae: *Alphitobiini*). The circular mitogenome was 15,509 bp in and the gene orientation and order were identical to other mitogenomes publicly available for species from the Tenebrionidae family. The tenebrionid mitogenomes revealed highly conserved genome structures such as gene order, nucleotide content, and amino acid composition when compared to published studies on mealworms in literature. It consisted of 22 tRNAs, 13 Protein coding genes (PCGs), and 2 rRNAs (Table S1). The major strand (J strand) carried 23 genes (9 PCGs and 14 tRNAs) while the minor N strand carried 14 genes (4 PCGs, 8 tRNAs, and 2 rRNAs) (Fig. 1). The average combined length of all 13 PCGs was 10,964, which varied between 1798 (ND5) and 156 (ATP8) (Table S2).

The 16S rRNA (1307 bp) was located between the $tRNA^{L}$ and $tRNA^{V}$ and the 12S rRNA (767 bp) was located between the $tRNA^{V}$ and the AT-rich region. The largest non-coding region (886 bp) was annotated as the AT-rich region, located between the 12s rRNA and $tRNA^{Ile}$ (Fig. 1).

The 22 tRNA genes were interspaced throughout the coding region and the sizes varied between 60 and 70 bp (Table S1). All the tRNAs were folded into a typical clover-leaf structure except $tRNA^{Ser}$ which lacked a dihydroudine (DHU) arm (Fig. S1). The mitogenome had a high A + T content typical of insects.

The phylogenetic tree showed that the mitogenome clustered with *Alphitobius* sp. of the tribe Alphitobiini and showed that this tribe clustered closely with tribes Helopini and Blaptini (Fig. 2).

Polystyrene consumption and survival rates

The lesser mealworm was able to chew, burrow and successfully feed on the blocks of Styrofoam (Fig. 3). There was a progressive increase in the consumption of PS throughout the period of 30 days. From the initial 3.6 g of Styrofoam, the resulting total consumption at the end of the experiment was 11.7% by all the mealworms fed on the PS diets. Co-feeding of the mealworms on bran and PS increased the rates of PS consumption compared to those fed to sole PS diets (Fig. 4). The survival rates of mealworms on all three treatment groups were above 80% over the entire feeding period. However, the survival rate of mealworms decreased significantly when mealworms were fed solely on the PS diet (Fig. 5A). The PS consumption increased, as the survival rates decreased (Fig. 5B).

Gut microbial communities

After sequencing, a total of 300,062 reads and 428 OTUs were identified. The predominant phyla identified in the gut of mealworms subjected to different dietary treatments included Proteobacteria and Firmicutes, but to

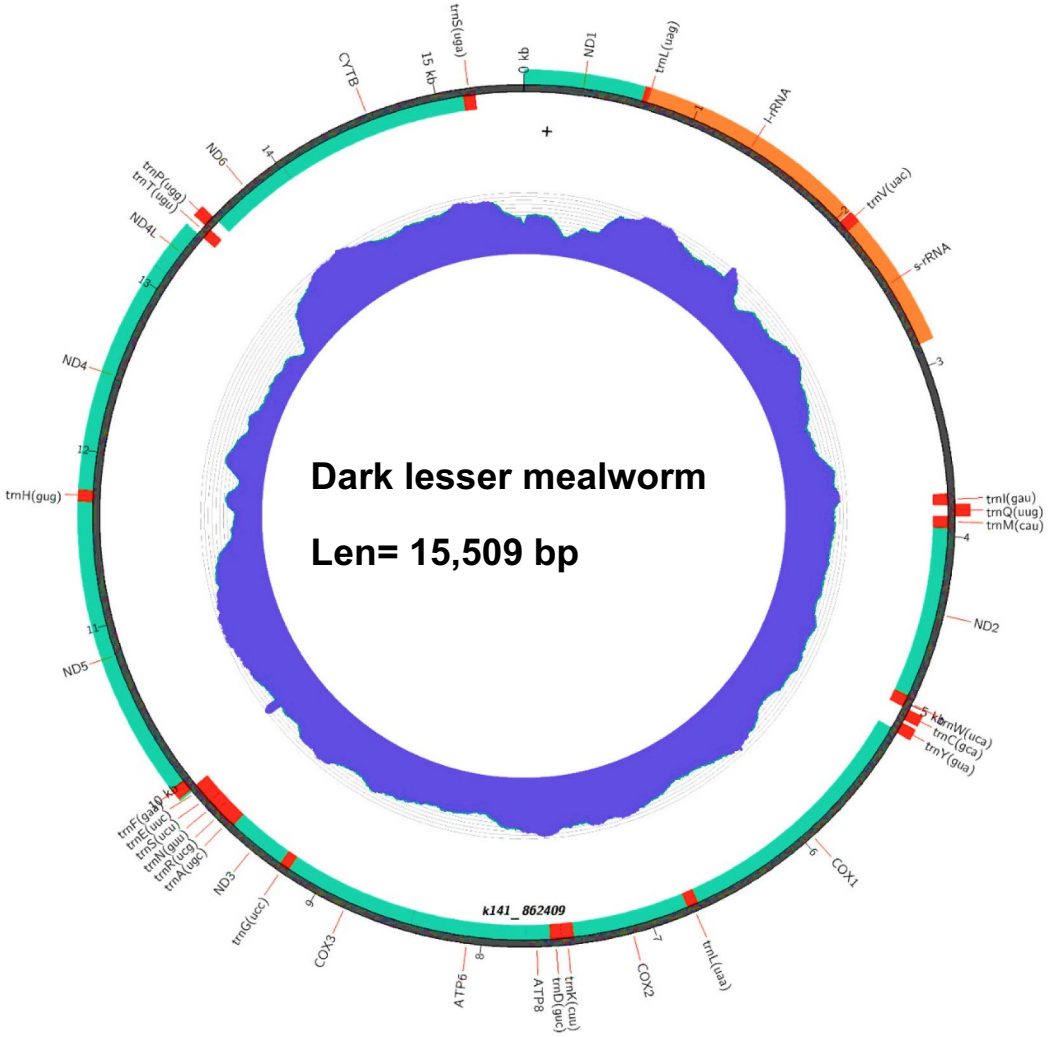


Fig. 1. A circular map illustrating the gene order of the lesser mealworm mitogenome.

a lesser extent Cyanobacteria, Actinobacteria, and Bacteroidetes. At the class level, γ-proteobacteria and Bacilli were the most abundant. OTUs clustering at the family level showed 4 predominant families: Enterobacteriaceae, Streptococcaceae, Xanthomonadaceae, and Enterococcaceae (Fig S2). Further comparative analysis showed that bacteria from the Genus *Kluyvera*, *Lactococcus, Stenotrophomonas, Klebsiella, Enterococcus,* and *Citrobacter* were dominant across all the 3 diet groups (Fig. 6A). *Kluyvera, Lactococcus* and *Klebsiella* were more abundant in the gut samples of mealworms fed solely on PS. The predominant bacteria from mealworms provided with a sole wheat bran diet were *Staphylococcus* and *Stenotrophomonas* (Fig. 6B). The upper and midgut sections of the mealworms fed on sole wheat bran were dominated by *Stenotrophomonas* while the hindgut was dominated by *Staphylococcus.* Larvae fed on combined PS and wheat bran diets were dominated by *Lactococcus* in the upper gut sections and *Kluyvera* in the hindgut. For the larvae fed solely on the PS diet, the hindgut section was dominated by *Enterococcus* and *Citrobacter* while *Stenotrophomonas* was common in the upper gut (Fig. 6C).

The Venn diagram revealed unique OTUs of the mealworm when fed on the three diet treatments (Fig. 7). The exposure of mealworms to PS-based diets enhanced the richness of bacterial diversity of the gut compared to those on control diets. From the 428 OTUs identified, only 49 taxa were shared amongst the 3 diet groups. The PS diets had 58 shared OTUs which were high compared to those shared with the control diet. The PS and wheat bran diet had the highest number of unique taxa, followed by the PS only diet. The control which had bran only had the least number of OTUs identified (Fig. 7).

The Alpha diversity as measured using the Shannon index did not vary significantly between the various dietary treatments. Those fed on PS-based feeds showed a more diverse community than those subjected to sole wheat bran diets (Fig. 8). PCoA plot based on Bray Curtis dissimilarity matrix and PERMANOVA analysis confirmed this similarity. As shown in Fig. 9 below, the control samples grouped very closely to those fed on PS and bran while the sole PS diets were grouped closely together.

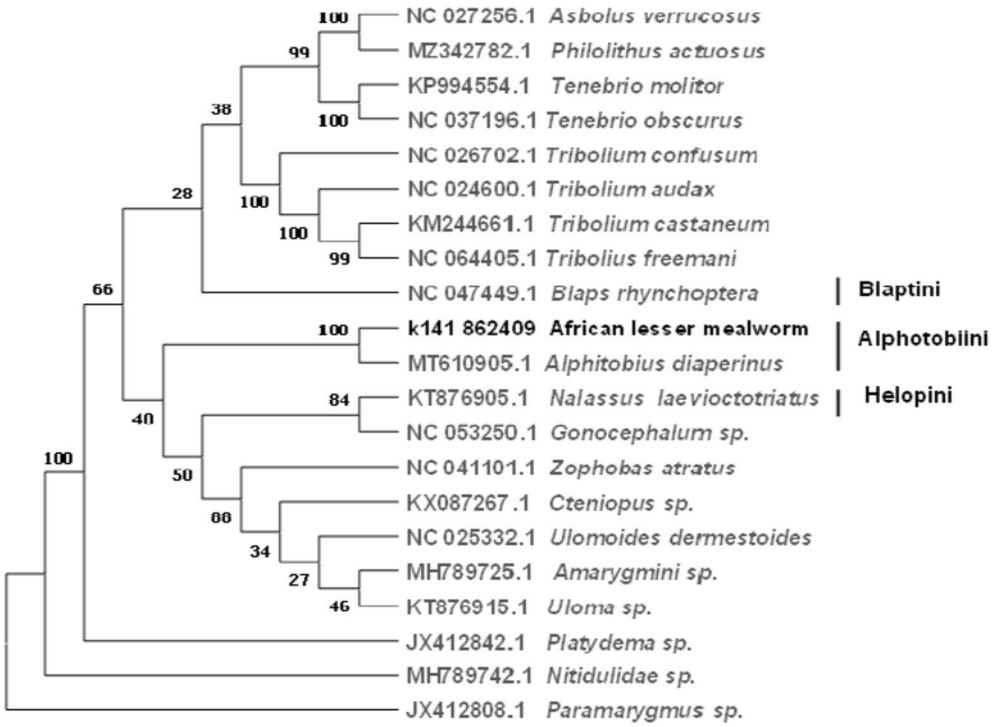


Fig. 2. Maximum-likelihood phylogenetic tree of 21 Tenebrionidae mitochondrial genomes. The phylogenetic tree was based on Kimura 2 parameter with 1000 bootstrap replications.

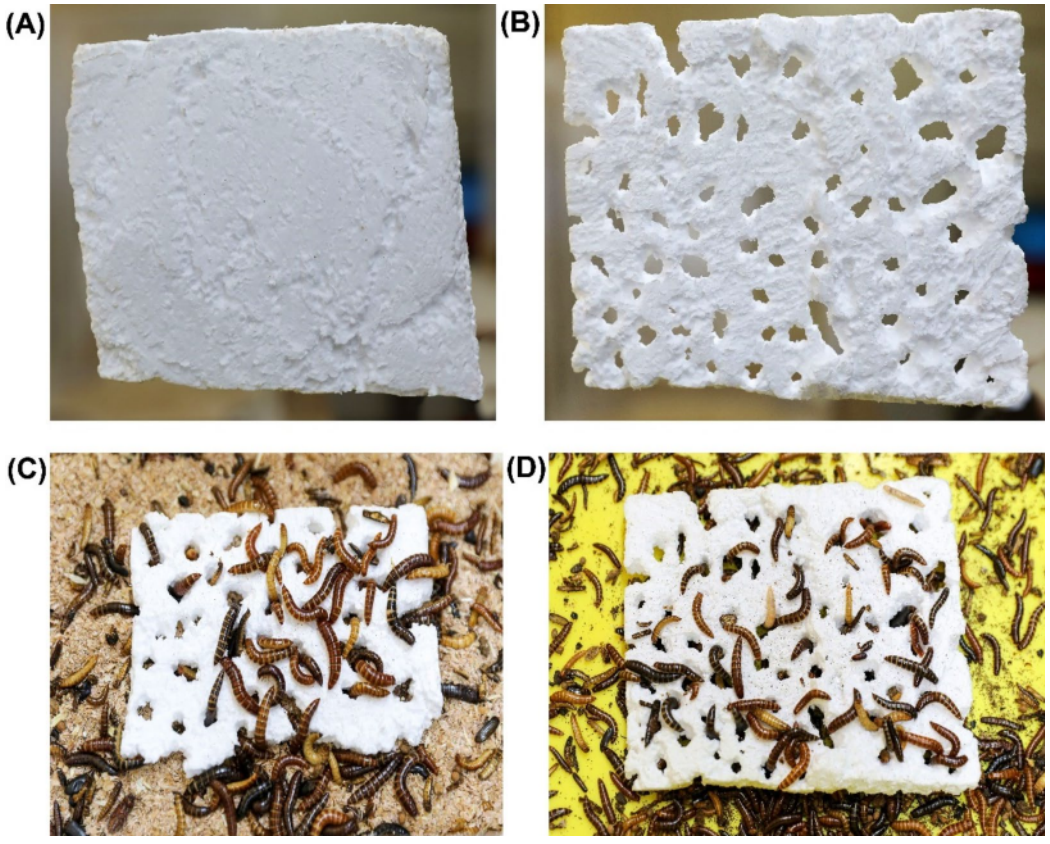


Fig. 3. Styrofoam block before feeding (**A**), Styrofoam block after 30 day feeding and consumption of the polystyrene (PS) evident by the holes and tunnels formed (**B**), mealworms feeding on polystyrene and bran diet (**C**), mealworms feeding on sole Polystyrene diet (**D**).

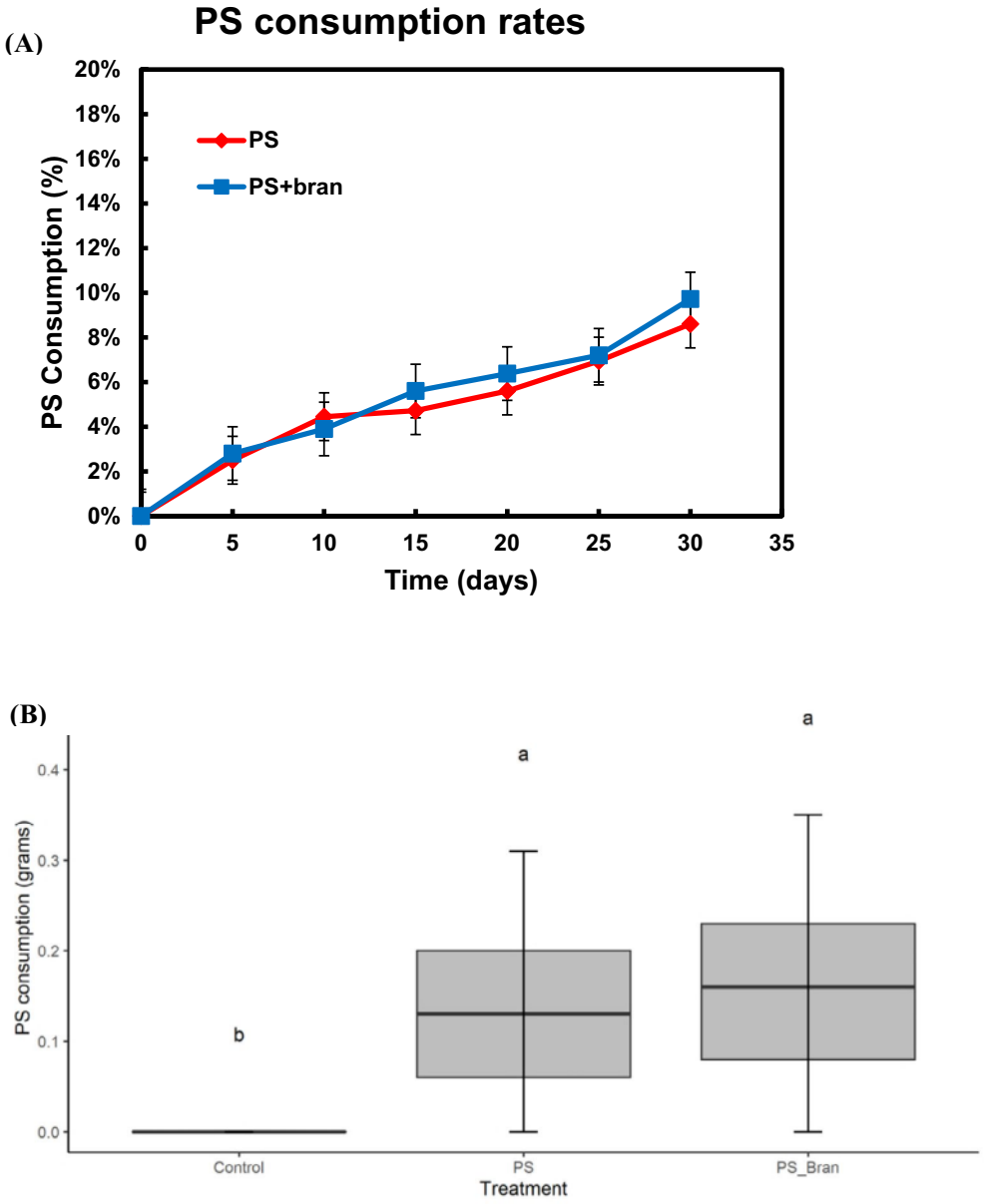


Fig. 4. The PS consumption rates over the 30-day feeding period (**A**), Box plot showing that there were no significant differences in the PS consumption rates between the PS diets (**B**).

Discussion

Polystyrene is one of the most common types of plastic used globally but its resilience to natural degradation has resulted in its accumulation in the environment[7,8,29]. Therefore, identifying alternative sustainable methods to accelerate biodegradation would be effective as a plastic waste management strategy. Insects have been proven to have the ability to consume, degrade and mineralize plastics and their gut microbiome has been suggested to play an important role in their digestion processes[10,11,30,31]. Here, we present a report study in Africa on the potential ability of a newly identified lesser mealworm larvae (Coleoptera: Tenebrionidae) to consume polystyrene and the associated microbial communities when fed PS diets.

The first stage of this research study was to provide an identification report using the mitogenomic profile of the lesser mealworm collected in Kenya and present its phylogenetic relationship. The circular mitogenome was 15,509 bp in length which was slightly shorter than previously published *Alphitobius* mitogenomes[32]. These differences between the published *Alphitobius* mitogenomes and the newly identified lesser mealworm suggest that it could be a new subspecies or a new species altogether which requires further identification. The gene orientation and order were identical to other mitogenomes publicly available for species from the Tenebrionidae family and also to the common type suggested as ancestral to insects[33,34]. The size of the protein-coding genes was similar to other members of the family Tenebrionidae. All the tRNAs were folded into a typical clover-leaf

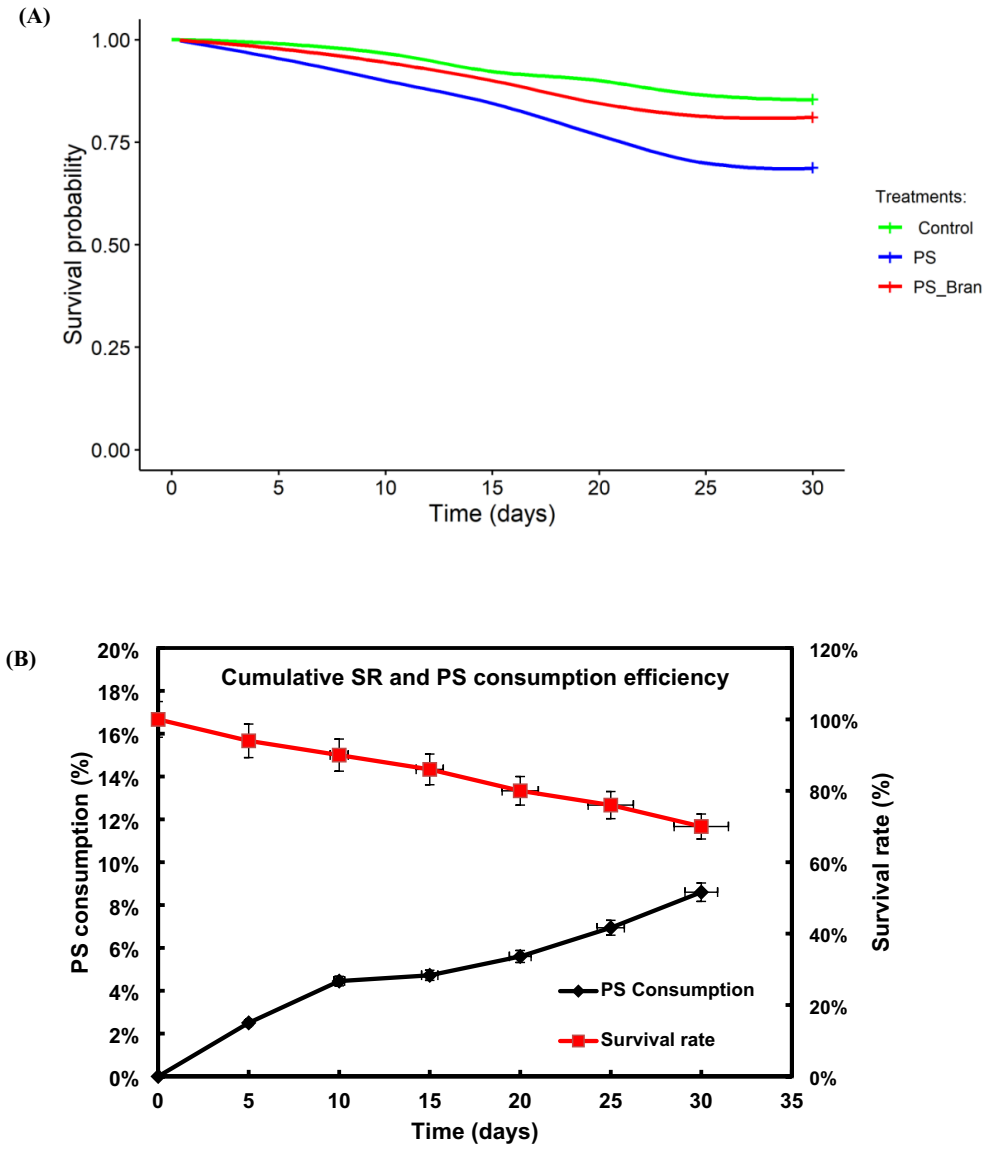


Fig. 5. The survival probability (**A**) and (**B**) PS consumption efficiency compared to survival rates over the period of 30 days.

structure except $tRNA^{Ser}$ which lacked a dihydroudine (DHU) arm and this incomplete structure has also been detected in other insect groups[35–37].

Our study revealed that the lesser mealworm may be capable of consuming polystyrene as evidenced by the PS consumption and chewing of the Styrofoam. The PS consumption rates increased over the 30-day period more so when a co-diet of bran was added. These results compare to those presented previously on other common mealworms (*T. molitor* and *T. obscurus*) in that the addition of a nutrient-rich co-diet such as bran enhances PS consumption[38,39]. The survival rates in all the three diet groups in our study ranged between 80 and 100% over the entire feeding period. A study done by[13] on the ability of super worms to degrade PS showed a survival rate of 95% when fed PS diets while[12] compared the survival rates of yellow mealworms and greater wax moth-fed PS diets and found the survival rates to be less than 75%. This variability in survival rates in previous studies and even our study may be due to environmental, rearing conditions, and other factors. From our study, the survival rates in larvae fed sole PS decreased over time suggesting that PS alone is a poor diet and a co-feed with bran is more feasible. The microbial diversities in larvae fed PS and bran co-feed were more compared to those fed solely on PS. This also demonstrates that the gut microbial diversity is greatly influenced by the diet.

Relative microbial abundance analysis revealed that the gut of the lesser mealworm was dominated by the phyla Proteobacteria and Firmicutes. Other phyla including Cyanobacteria, Actinobacteria, and Bacteroidetes were also present but in lesser portions. This observation aligns with previous studies on common mealworms with Firmicutes and Proteobacteria being the dominant phyla[40–42], although in our study the Proteobacteria were more dominant than the Firmicutes. The families Enterobacteriaceae, Streptococcaceae, Xanthomonadaceae, and Enterococcaceae were predominant across all diet groups and these families have also been observed in other

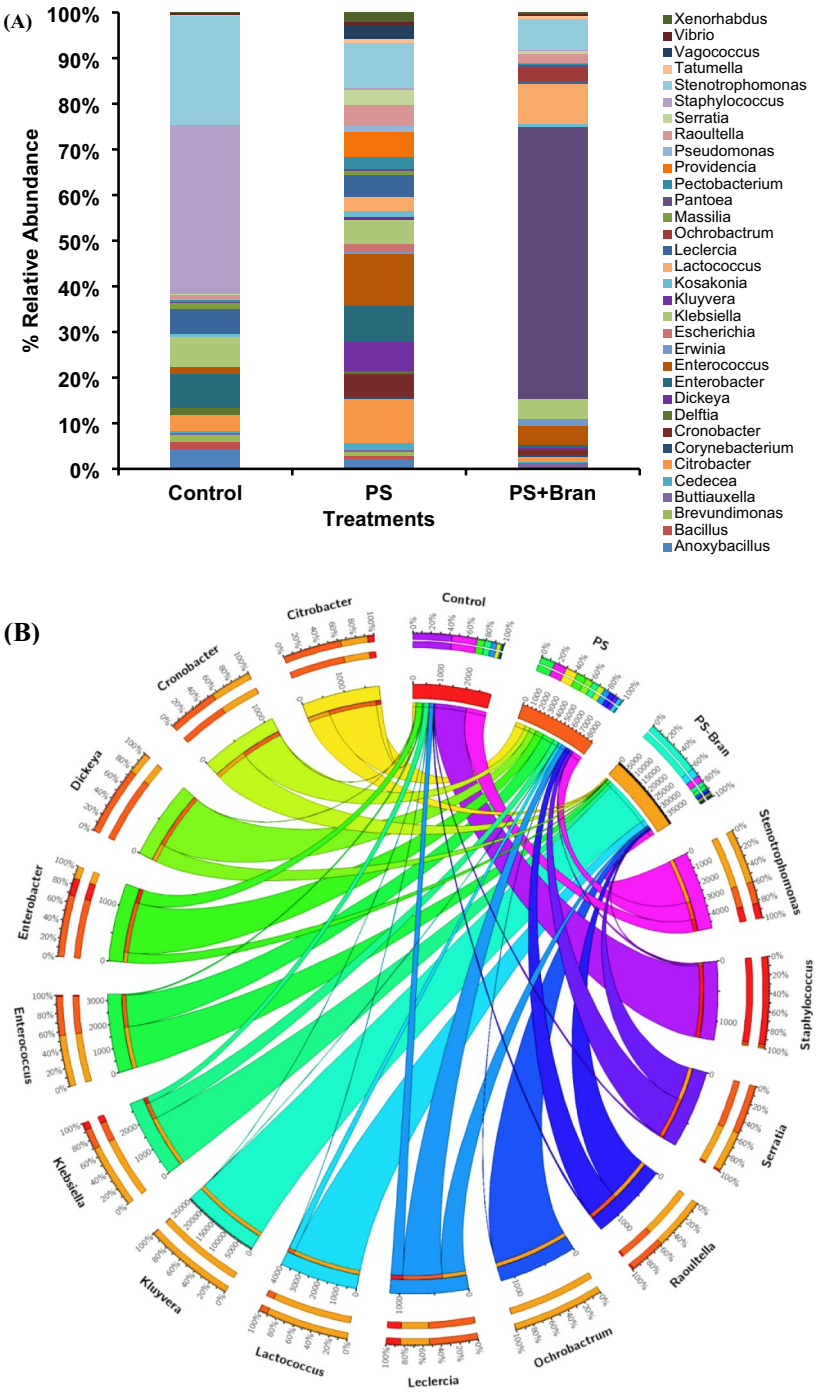
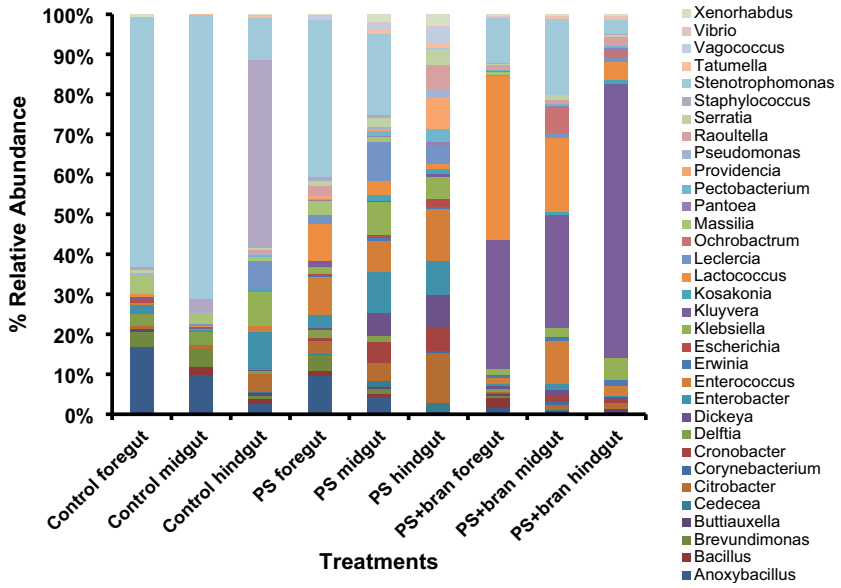


Fig. 6. Bacterial relative abundance across the 3 diet treatments. (**A**) Stacked bar plot showed bacterial abundance at the genus level, (**B**) circos plot showing the top 14 abundant bacteria genera and (**C**) cumulative bacterial abundance at genus level from the fore, mid, and hindgut sections of the mealworms fed on the 3 diets.

previous studies involving PS degradation by mealworms[9,39]. In our current study, we observed that ingesting PS increased the abundance of the Enterobacteriaceae family rendering this group of bacteria the most probable candidates to be involved in the initial PS breakdown in the lesser mealworm gut. The Enterobacteriaceae family is known for its diverse metabolic capabilities, including the degradation of various complex organic compounds[43]. Bacteria from this family including the genera *Citrobacter, Kosakonia*, *Enterobacter*, *Lactococcus* and *Kluyvera* have been reported previously to be associated with degradation of PS and are known to harbour enzymes capable of breaking down synthetic polymers[38,44,45]. The increase in the Enterobacteriaceae family observed in our study could be attributed to the presence of potential PS-degrading microbes such as *Kluyvera,*



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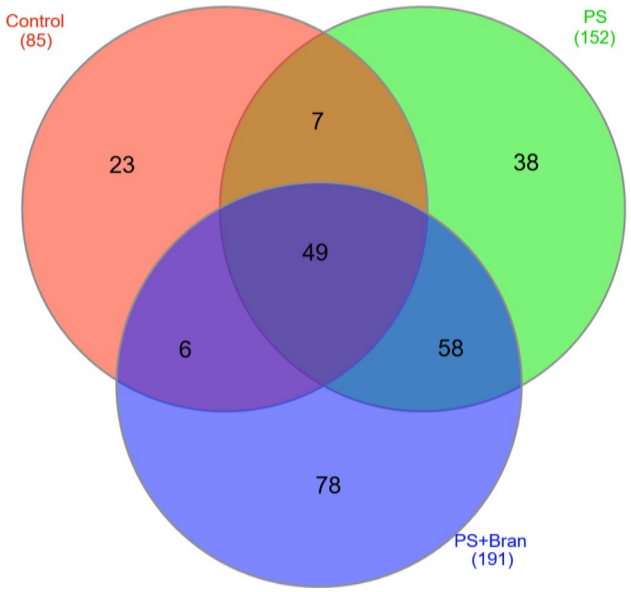

Fig. 7. Venn diagram representing the shared bacteria taxa of mealworm fed on the 3 diets with the unique taxa shown in each group.

Klebsiella, and *Citrobacter,* indicating an adaptive response to polystyrene aiding its breakdown and possibly further degradation.

Further relative analysis at genus level in our study indicated that facultative anaerobes, γ-proteobacteria, from the family Enterobacteriaceae, including the genera *Kluyvera, Klebsiella*, and *Citrobacter* were the most abundant bacteria taxa in the PS diet groups. These taxa are some of the common gut-associated bacteria in mealworms and insects in general. *Kluyvera* which was the predominant genus in the larvae fed solely on PS diets has been identified to be a potential PS and polypropylene-degrading microbe although it has not been commonly reported as a plastic-degrading bacteria[20]. The genus *Lactococcus* from the Streptococcaceae family was also observed in high abundance in the PS diets as compared to the control diets which contained wheat bran only. This gram-positive bacterium is primarily known for its use as a probiotic and even though its role in plastic degradation has not been extensively studied, some studies have reported the presence of *Lactococcus* as a PS- degrading bacteria in the guts of *Tenebrio molitor*[46,47]. The control (wheat bran) on the other hand was dominated by the genus *Stenotrophomonas* of the Xanthomonadaceae family and this has not been reported in previous studies especially those that have used bran as the nutritional source. This genus is an uncommon gram-negative bacterium that has been denoted as a potential pathogen or commensal of certain species including

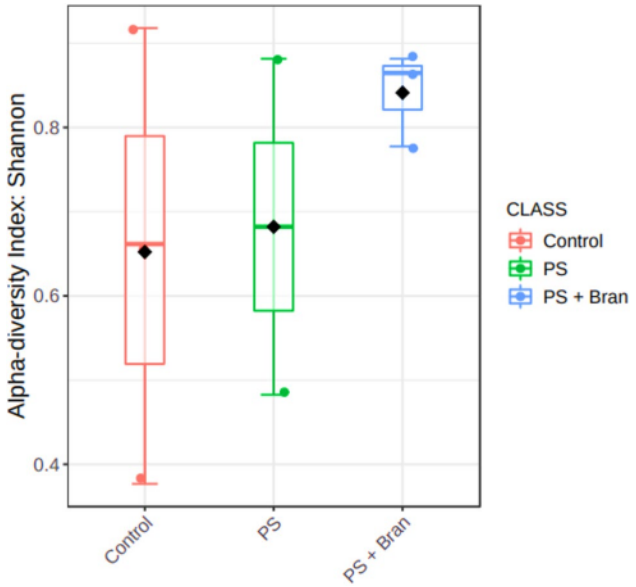

Fig. 8. Alpha diversity measure using Shannon index for mealworms fed on the different dietary treatments.

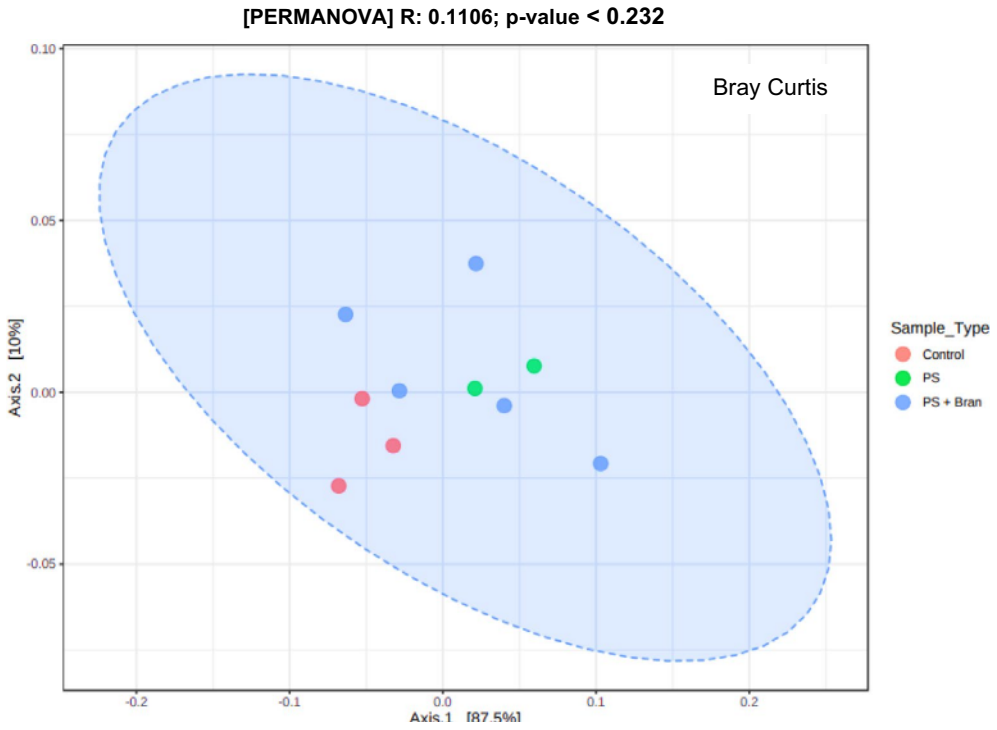

Fig. 9. Two-dimensional PCoA plot of beta diversity based on Bray Curtis dissimilarity index across the bacteria genera among mealworm fed on the three dietary groups.

insects[48]. However, a study done by[49] who isolated *S. maltophilia* from plastic debris found in soil suggested that this genus could also have the potential to degrade polyethylene.

Numerous research studies have so far identified PS-degrading microbes from the gut of insects. Yang et al.[19] was among the first studies to identify the PS- degrading bacteria *Exiguobacterium* sp. YT2 from the guts of *T. molitor*. A study done by[38] identified gut bacteria species associated with PS- degradation including *Citrobacter*, *Klebsiella* and *Serratia*. Wang et al.[50] also investigated gut microbes from the gut of *T. castaneum* fed PS diets and was able to identify *Acinetobacter* sp. to be highly associated with PS-degradation. Furthermore[44], isolated PE degrading *Enterobacter absuriae* YT1 from the guts of the Indian meal moth and[51] isolated PS degrading *Pseudomonas* sp. from the gut of the *Zophobas atratus* larvae. The contrast in the microbial communities identified in the different studies highlights the effects of the diet compositions and the co-diets mainly used on the insects.

It also suggests that insects may contain digestive enzymes and bioreagents for plastic degradation in their guts and that plastic degradation by insects is either a synergistic or symbiotic reaction between the gut microbes and the host. Therefore, research on the gut microbiome of plastic-degrading insects is an important approach to understanding the insights of plastic biodegradation by insects. The role of the gut microbes in PS degradation and plastic bioremediation is not yet fully understood and this should further be investigated through isolation and enzymatic tests to determine the microbial pathways involved in depolymerization of the plastics.

Conclusion

Our study was able to identify microbial communities from the gut of the newly identified lesser mealworm when fed on Polystyrene diets and the complete mitogenome analysis provided valuable insights into the genetic makeup shedding light on its phylogenetic relationships. The presence of gut microbes such as *Kluyvera, Klebsiella* and *Lactococcus* possibly associated with PS degradation supports reports made by previous studies on the capability of mealworms to degrade Polystyrene. Our study sparked new questions such as which genes are expressed during PS degradation and what are the complete pathways used by the microbes. Which members of the gut community are involved throughout the degradation process? As such questions arise, employing transcriptomics to quantify gene expression levels and functions will contribute to addressing them. A comprehensive mitogenome sequence will serve as a crucial resource for future studies that will help in identifying specific genes and metabolic pathways involved in plastic degradation. Moreover, comparative mitogenomics can help in understanding the evolutionary adaptations of mealworms to synthetic polymers, providing a broader perspective on the ecological and functional significance of plastic degradation.

While the diverse capabilities of lesser mealworms offer promising possibilities in developing sustainable waste management strategies, challenges such as optimization, scaling up for practical application and ensuring the safety of the end products needs to be addressed. Additionally, the efficacy of the lesser mealworm in degrading different types of plastics and the potential impacts on their health and behaviour requires further investigation as well as their ability to convert waste into high-value insect protein-rich biomass for animal feeds. Continued research and collaborative efforts between scientists, policymakers, and industries will be instrumental in realizing the full potential of lesser mealworms and other similar organisms. These combined efforts hold the key to addressing plastic waste while providing high-value insect protein towards fostering a circular economy framework.

Data availability

Sequence data that support the findings of this study have been deposited in NCBI with the BioProject number PRJNA1102172 and can be accessed using the link below: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1102172?reviewer=11bfm31da9eru12f48c2iben8h.

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References

1. Global Plastics Outlook. Global Plastics Outlook (OECD, 2022). https://doi.org/10.1787/de747aef-en.
2. Balabantaray, S. R., Singh, P. K., Pandey, A. K., Chaturvedi, B. K. & Sharma, A. K. Forecasting global plastic production and microplastic emission using advanced optimised discrete grey model. *Environ. Sci. Pollut. Res.* **30**(59), 123039–123054 (2023).
3. Amankwa, M. O., Tetteh, E. K., Mohale, G. T., Dagba, G. & Opoku, P. The production of valuable products and fuel from plastic waste in Africa. *Discov. Sustain.* https://doi.org/10.1007/s43621-021-00040-z (2021).
4. Africa, S. Identifying policy gaps and opportunities plastic pollution in Africa. www.wwf.org.za/report/plastic_pollution_policy_in_africa (2022).
5. Association of Plastics Manufacturers. Plastics – the Facts. *Plastics Europe* 38 (2018).
6. Europe, P. Plastics Europe Association of Plastics Manufacturers Plastics—the facts 2021 an analysis of European plastics production, demand and waste data. *Plastics - the Facts* **2021**, 34 (2021).
7. Ho, B. T., Roberts, T. K. & Lucas, S. An overview on biodegradation of polystyrene and modified polystyrene: The microbial approach. *Crit. Rev. Biotechnol.* **38**, 308–320 (2018).
8. Maharana, T., Negi, Y. S. & Mohanty, B. Review article: Recycling of polystyrene. *Polym. Plast. Technol. Eng.* **46**, 729–736 (2007).
9. Peña-Pascagaza, P. M., López-Ramírez, N. A. & Ballen-Segura, M. A. Tenebrio molitor and its gut bacteria growth in polystyrene (PS) presence as the sole source carbon. *Univ. Sci.* **25**, 37–53 (2020).
10. Mallar, C. N. R. A review on the role of insects and their gut microbiota in plastic biodegradation. 79 (2020).
11. Bilal, H., Raza, H., Bibi, H. & Bibi, T. Plastic biodegradation through insects and their symbionts microbes: A review. *J. Bioresour. Manag.* **8**, 95–103 (2021).
12. Jiang, S., Su, T., Zhao, J. & Wang, Z. Biodegradation of polystyrene by *Tenebrio molitor*, *Galleria mellonella*, and *Zophobas atratus* larvae and comparison of their degradation effects. *Polymers* **13**, 3539 (2021).
13. Sun, J., Prabhu, A., Aroney, S. T. N. & Rinke, C. Insights into plastic biodegradation: Community composition and functional capabilities of the superworm (Zophobas morio) microbiome in styrofoam feeding trials. *Microb. Genom.* **8**, 1–19 (2022).
14. Sammarco, B. C., Hinkle, N. C. & Crossley, M. S. Biology and management of lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in broiler houses. *J. Integr. Pest. Manag.* https://doi.org/10.1093/jipm/pmad003 (2023).
15. Cucini, C. et al. Bacterial and fungal diversity in the gut of polystyrene-fed *Alphitobius diaperinus* (Insecta: Coleoptera). *Anim. Gene* **17–18**, 200109 (2020).
16. Chalup, A. et al. First report of the lesser wax moth *Achroia grisella* F. (Lepidoptera: Pyralidae) consuming polyethylene (silo-bag) in northwestern Argentina. *J. Apic Res.* **57**, 569–571 (2018).
17. Suresh Kesti, S. & Chandrabanda Thimmappa, S. First report on biodegradation of low density polyethylene by rice moth larvae, *Corcyra cephalonica* (stainton). *Holist. Approach Environ.* **9**, 79–83 (2019).
18. Mahmoud, E. A., Al-Hagar, O. E. A. & El-Aziz, M. F. A. Gamma radiation effect on the midgut bacteria of *Plodia interpunctella* and its role in organic wastes biodegradation. *Int. J. Trop. Insect Sci.* **41**, 261–272. https://doi.org/10.1007/s42690-020-00203-x (2021).

19. Yang, Y. et al. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 1. Chemical and physical characterization and isotopic tests. *Environ. Sci. Technol.* **49**, 12080–12086 (2015).
20. Yang, S. S. et al. Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor* Linnaeus): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle. *Chemosphere* **191**, 979–989 (2018).
21. Meng, G., Li, Y., Yang, C. & Liu, S. MitoZ: A toolkit for animal mitochondrial genome assembly, annotation and visualization. *Nucleic Acids Res.* **47**, e63 (2019).
22. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549 (2018).
23. Wang, Y., Zhao, Y., Bollas, A., Wang, Y. & Au, K. F. Nanopore sequencing technology, bioinformatics and applications. *Nat. Biotechnol.* **39**, 1348–1365. https://doi.org/10.1038/s41587-021-01108-x (2021).
24. Bolyen, E. et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857 (2019).
25. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
26. McMurdie, P. J. & Holmes, S. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**, e61217 (2013).
27. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2023).
28. Kaufmann, J. & Schering, A. G. *Analysis of Variance ANOVA*.
29. Lear, G. et al. Plastics and the microbiome: Impacts and solutions. *Environ. Microbiomes* **16**, 1–19 (2021).
30. Kesti, S. S. & Shivasharana, C. T. The role of insects and microorganisms in plastic biodegradation. A comprehensive review. *Int. J. Sci. Res. Biol. Sci.* **5**, 75–79 (2019).
31. Liaqat, S. Microbial ecology: A new perspective of plastic degradation. *Pure Appl. Biol.* **9**, 2138–2150 (2020).
32. Hong, K. J., Ki, W., Lee, H., Park, J. & Lee, W. The second complete mitochondrial genome of *Alphitobius diaperinus* Panzer, 1797 (Coleoptera: Tenebrionidae): Investigation of intraspecific variations on mitochondrial genome. *Mitochondrial DNA B Resour.* **5**, 2997–2999 (2020).
33. Taanman, J. W. The mitochondrial genome: Structure, transcription, translation and replication. *Biochim. Biophys. Acta Bioenerg.* **1410**, 103–123 (1999).
34. Tomberlin, J. K. et al. A survey of bacterial diversity from successive life stages of black soldier fly (Diptera: Stratiomyidae) by using 16S rDNA pyrosequencing. *J. Med. Entomol.* **50**, 647–658 (2013).
35. Liao, F. et al. The complete mitochondrial genome of the fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae). *Int. J. Biol. Sci.* **6**, 172–186 (2010).
36. Li, H. et al. The architecture and complete sequence of mitochondrial genome of an assassin bug *Agriosphodrus dohrni* (Hemiptera: Reduviidae). *Int. J. Biol. Sci.* **7**, 792–804 (2011).
37. Liu, L. N. & Wang, C. Y. Complete mitochondrial genome of yellow meal worm (*Tenebrio molitor*). *Dong wu xue yan jiu= Zoological research / 'Dong wu xue yan jiu' bian ji wei yuan hui bian ji* **35**, 537–545 (2014).
38. Brandon, A. M. et al. Biodegradation of polyethylene and plastic mixtures in mealworms (larvae of *Tenebrio molitor*) and effects on the gut microbiome. *Environ. Sci. Technol.* **52**, 6526–6533 (2018).
39. Peng, B. Y. et al. Biodegradation of polystyrene by dark (*Tenebrio obscurus*) and yellow (*Tenebrio molitor*) mealworms (Coleoptera: Tenebrionidae). *Environ. Sci. Technol.* **53**, 5256–5265 (2019).
40. Wang, Y. & Zhang, Y. Investigation of gut-associated bacteria in tenebrio molitor (Coleoptera: Tenebrionidae) larvae using culture-dependent and DGGE methods. *Ann. Entomol. Soc. Am.* **108**, 941–949 (2015).
41. Cassone, B. J., Grove, H. C., Elebute, O., Villanueva, S. M. P. & LeMoine, C. M. R. Role of the intestinal microbiome in low-density polyethylene degradation by caterpillar larvae of the greater wax moth, *Galleria mellonella*. *Proc. R. Soc. B Biol. Sci.* **287**, 9–11 (2020).
42. Jiang, S., Su, T., Zhao, J. & Wang, Z. Isolation, identification, and characterization of polystyrene-degrading bacteria from the gut of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae. *Front. Bioeng. Biotechnol.* **9**, 1–9 (2021).
43. Octavia, S. & Lan, R. The family Enterobacteriaceae. In *The Prokaryotes* (eds Rosenberg, E. *et al.*) 225–286 (Springer, 2014).
44. Yang, J., Yang, Y., Wu, W. M., Zhao, J. & Jiang, L. Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. *Environ. Sci. Technol.* **48**, 13776–13784 (2014).
45. Yang, Y. et al. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 2. Role of gut microorganisms. *Environ. Sci. Technol.* **49**, 12087–12093 (2015).
46. Urbanek, A. K., Rybak, J., Wróbel, M., Leluk, K. & Mirończuk, A. M. A comprehensive assessment of microbiome diversity in *Tenebrio molitor* fed with polystyrene waste. *Environ. Pollut.* **262**, 114281 (2020).
47. Przemieniecki, S. W., Kosewska, A., Ciesielski, S. & Kosewska, O. Changes in the gut microbiome and enzymatic profile of *Tenebrio molitor* larvae biodegrading cellulose, polyethylene and polystyrene waste. *Environ. Pollut.* **256**, 113265 (2020).
48. Brooke, J. S. Advances in the microbiology of *Stenotrophomonas maltophilia*. *Clin. Microbiol. Rev.* https://doi.org/10.1128/CMR.00030-19 (2021).
49. Peixoto, J., Silva, L. P. & Krüger, R. H. Brazilian Cerrado soil reveals an untapped microbial potential for unpretreated polyethylene biodegradation. *J. Hazard Mater.* **324**, 634–644 (2017).
50. Wang, Z., Xin, X., Shi, X. & Zhang, Y. A polystyrene-degrading *Acinetobacter bacterium* isolated from the larvae of *Tribolium castaneum*. *Sci. Total Environ.* **726**, 138564 (2020).
51. Kim, H. R. et al. Biodegradation of polystyrene by *Pseudomonas* sp. Isolated from the gut of superworms (larvae of *Zophobas atratus*). *Environ. Sci. Technol.* **54**, 6987–6996 (2020).

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Author contributions

C.M.T., E.W.N. S.K and F.M.K. conceived the experiment; C.M.T., E.W.N., S.K and F.M.K. designed the experiment; software; E.W.N. and F.M.K.; investigation; C.M.T., E.W.N., S.K and F.M.K.; resources, C.M.T.; writing—original draft preparation, C.M.T., E.W.N., and F.M.K.; writing—review and editing, C.M.T., E.W.N. and F.M.K.; visualization, C.M.T., E.W.N. and F.M.K.; supervision, C.M.T. and F.M.K.; project administration, C.M.T.; funding acquisition, C.M.T. All authors have read and agreed to the published version of the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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