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Comparative Analysis of Gut Eukaryotic Communities in Three Laboratory-Reared Cockroach Species Using Metabarcoding

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ABSTRACT

Cockroaches are known reservoirs for diverse bacterial microbiomes. However, comprehensive analyses of the eukaryotic communities within cockroaches remain limited. In this study, we selected three long-term laboratory-reared cockroach species (*Blattella germanica*, *Periplaneta fuliginosa*, and *Periplaneta japonica*) and performed metabarcoding of the 18S rRNA V9 region using the iSeq 100 platform. The nematode *Blatticola blattae* was identified in *B. germanica*, and *Leidynema appendiculata* was found in both *P. fuliginosa* and *P. japonica*. The amplicon sequence variant (ASV) of *Nyctotherus* (Ciliophora) was detected in all three species of cockroach, while *Entamoeba* sp. was detected in *P. fuliginosa* and *P. japonica*. Compared with the other two species, *B. germanica* exhibited a higher prevalence of the fungus *Nephridiophaga*. Our findings showed that laboratory-reared cockroaches belonging to different species harbored distinct commensal and parasitic eukaryotic taxa. While most ASVs were of reduced clinical concern for humans as opposed to studies using wild-caught specimens, the detection of commensals, arthropod parasites and potential human pathogens illustrates the complexity of the cockroach-associated eukaryome and the influence of host identity. Our study emphasizes the importance of host-specificity in the eukaryotic community of laboratory-reared cockroach models.

1 | Introduction

Most microbiome studies in cockroaches have focused on bacterial species (Lee et al. 2020; Yun et al. 2024) and have documented bacterial contributions to nutrient acquisition, digestion, and interactions with host physiology (Domínguez-Santos et al. 2021; Tinker and Ottesen 2016). Bacterial symbionts such as *Blattabacterium* spp. are well-known for their roles in nitrogen fixation, vitamin synthesis, and uric acid recycling, contributing to the cockroach's ability to thrive in resource-poor environments (Sabree et al. 2009). Other studies have investigated the role of the microbiome in cockroaches as reservoirs of pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*, which can lead to foodborne illness (Memona et al. 2017; Moges et al. 2016; Musa et al. 2018; Ray et al. 2020; Rossato et al. 2018, 2019).

However, less attention has been paid to eukaryotic organisms such as protists, fungi, and helminths carried by common cockroaches such as *Blattella germanica, Periplaneta fuliginosa*, and *Periplaneta japonica*. Beyond metagenomic explorations, studies have investigated the eukaryome in different

cockroach species. The spore-forming protists *Nephridiophaga blaberi* and *Nephridiophaga blattellae* have been described from the malpighian tubules of *Blaberus craniifer* (Fabel et al. 2000) and *B. germanica*. From *B. germanica*, *Lophomonas blattarum* has been identified as a gut commensal of cockroaches. It has also been reported in human clinical samples, although its pathogenic role remains unproven and such reports have been questioned as possible misidentifications (Mewara et al. 2023). In addition, microscopy-based studies have reported *Nyctotherus*, *Gregarina*, and *Amoeba* from *B. germanica* (Martínez-Girón et al. 2017). Another common parasite of cockroaches is *Leidynema*, that parasitizes *P. fuliginosa, P. japonica, Pycnoscelus surinamensis*, and *Blattella nipponica* (Ozawa et al. 2014; Ozawa and Hasegawa 2018). *B. germanica* and *Periplaneta* cockroaches have been found to harbor the human parasites *Entamoeba histolytica* and *Entamoeba dispar*, as well as *Giardia lamblia*, *Ascaris lumbricoides* (Adenusi et al. 2018). Fungal species of medical importance such as *Candida*, *Aspergillus*, and *Penicillium* have been found to be disseminated by *Periplaneta americana* (Lemos et al. 2006), while *Aspergillus*, *Candida*, and *Nephridiophaga* have been reported from *B. germanica* (Merad et al. 2023; Strassert et al. 2022).

In this study, we conducted a characterization of the gut eukaryotic communities in three long-term laboratory-reared cockroach species using 18S rRNA metabarcoding. We aimed to identify and compare eukaryotic taxa present in each species, evaluate differences in community composition, and assess potential implications for their biology and their associated use as laboratory models.

2 | Materials and Methods

2.1 | Cockroach Species and DNA Extraction

Twelve cockroaches from three species that had been laboratory-reared for 6–29 years were used for the eukaryotic microbiome analysis: *B. germanica* (hereafter called BG, $n = 3$), *P. fuliginosa* (PF, $n = 5$), and *P. japonica* (PJ, $n = 4$). The BG population was reared for 29 years, PF for 24 years, and PJ for 6 years. *B. germanica* was obtained from the Korean National Institute of Health in 1995, *P. fuliginosa* was collected from Seoul in 2000, and *P. japonica* was obtained from Gyeonggi-do in 2018. The cockroaches were reared in plastic cages (27 × 34 × 19 cm; replaced every 4 months), incubated at 25°C and 50% relative humidity, and supplied with sterilized experimental mouse feed (Purina, St. Louis, MO, USA) and autoclaved tap water. All cockroaches were anesthetized in CO_2 for 1 min prior to preparation for DNA extraction.

The cockroaches were washed in sterile distilled water and then 70% ethanol before DNA extraction. Each anesthetized cockroach was immersed in sterilized PBS, and the entire gut (foregut, midgut, and hindgut) was separated from the chitinous cuticle using a syringe needle. The separated guts were used for DNA extraction using the NucleoSpin DNA Insect Mini kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) as described previously (Lee et al. 2023).

2.2 | Illumina Sequencing

For the eukaryotic microbiome study, the 18S rRNA gene V9 region was amplified via polymerase chain reaction (PCR) using the primers 1391f (5′-GTA CAC ACC GCC CGT C-3′) and EukBr (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′). Illumina adapters were synthesized upstream of the respective primers for NGS library preparation (forward primer adapter: 5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG-3′, reverse primer adapter: 5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3′) (Choi et al. 2024). KAPA HiFi HotStart ReadyMix (Roche Sequencing Solutions, Pleasanton, CA, USA) was used for PCR amplification, and the conditions were as follows: one cycle at 95°C for 5 min, followed by 30 cycles of 98°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final cycle of 72°C for 5 min. AMPure XP (Beckman Colter, Brea, CA, USA) was used for DNA purification. A limited cycling amplification step (eight cycles) was performed to add the multiplexing indices and Illumina sequencing adapters. Mixed amplicons were pooled and sequenced on an Illumina iSeq 100 sequencing system using an Illumina iSeq 100 i1 Reagent v2 kit (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions (Kim et al. 2021).

2.3 | Bioinformatics Analysis

The sequencing data were processed using Quantitative Insights into Microbial Ecology version 2 (QIIME 2; version 2023.5; https://qiime2.org) and subsequently denoised using the divisive amplicon denoising algorithm 2 (DADA2) method (Callahan et al. 2016). After taxonomic assignment, host-associated and uncultured sequences were excluded from the feature table for further analyses using the qiime taxa filter-table command. The amplicon sequence variants (ASVs) were then compared to a dereplicated NCBI RefSeq 18S rRNA database (v.220513) compiled in-house clustered at 100% identity. Taxonomic classification was performed using the classify-consensus-blast method from the QIIME2 feature-classifier plugin. Using the following parameters: maxaccepts 1, minimum sequence identity of 0.95, minimum coverage 0.5, minimum consensus threshold 0.75, and maximum e-value 0.001. Alpha and beta diversity analyses across the species were calculated using reads normalized to relative abundances and rarefied to 1000 reads. To determine the differences in species richness and Shannon index among the groups, statistical analyses were conducted using the Wilcoxon rank-sum test. For beta diversity analysis, principal coordinate analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA) were carried out based on the Bray–Curtis distance metric. Linear discriminant analysis effect size (LEfSe) analysis was performed in R using the microbiomeMarker package to identify differentially abundant taxa, with an LDA score cut-off of 3 and $p < 0.05$ considered statistically significant.

2.4 | Microscopic Imaging of the Parasites

Cockroaches were anesthetized with CO_2 gas and washed with 70% ethanol and triple distilled water. Whole guts were dissected using the needle of a 5-mL syringe in a Petri dish

containing sterilized PBS. After dissection, the guts were opened and the contents were allowed to be passively released into PBS for 20 min to isolate parasites without damaging them. To remove debris, parasites were transferred to a cell-culture dish and a glass slide containing fresh PBS to obtain images of the putative species detected by metabarcoding for further validation using Sanger sequencing. Nematodes were imaged at 60× magnification using an SZX10 microscope (Olympus, Tokyo, Japan), and other relatively large protists such as *Nyctotherus* sp. (Ciliophora) were visualized at 200× magnification using a BX43 microscope (Olympus, Tokyo, Japan). The images were taken with an eXcope microscope camera and software (Olympus, Japan). The isolated parasites were used for PCR identification.

2.5 | Taxon-Specific PCR

Nematodes isolated from cockroach guts were individually separated, placed in Type D Nucleospin Bead Tubes (Macherey-Nagel, Germany), added with 0.5 U of liquid protease K (Macherey-Nagel, Germany), and homogenized in an MN Bead Tube Holder in combination with a Vortex-Genie 2 (Macherey-Nagel, Germany) at maximum speed for 30 min. The disrupted samples were used for PCR using a QIAamp DNA Mini Kit (QIAGEN, Germany). To identify protists, the same DNA samples used for next-generation sequencing were utilized for PCR. PCR, followed by Sanger sequencing, was performed using specific primers (Table 1) with AccuPower Taq PCR PreMix & Master Mix (Bioneer, Daejeon, South Korea). Maximum likelihood phylogenetic trees were constructed in IQ-TREE with automatic model selection and ultrafast analysis of 10,000 bootstrap replicates to validate the taxonomic assignment of the amplicon sequences. Additional Bayesian posterior probabilities supports were calculated in BEAST X v10.X using default priors with 10 million MCMC generations, sampling every 1000 steps and discarding 10% as burnin.

3 | Results

3.1 | Identification of the Eukaryotic Community Through 18S rRNA Gene Sequencing

The average count of assigned reads was 229,914 ± 82,360 (mean ± standard deviation). An average of 110,110 ± 117,372 counts belonged to the host and were filtered out before ASV calculations. The range of reads varied from a minimum of 69,286 to a maximum of 365,256. Figure 1A shows the relative abundances of protists, helminths, and fungi in individual cockroach specimens. A total of four protist genera, two helminth genera, and 10 fungal genera were identified. Fungal taxa generally exhibited higher relative abundances compared to helminths and protists in the *B. germanica* samples, whereas protist taxa showed higher relative abundances in the majority of the *P. japonica* and *P. fuliginosa* samples.

Among the protist taxa, *Nyctotherus* sp. was predominantly observed in most cockroach specimens (average relative abundance: 90.89%) (Figure 1B). *Blastocystis* sp. (Bigyra) was detected only in the *P. fuliginosa* samples (average relative abundance: 14.47%). Meanwhile, *Entamoeba* sp. (Amoebozoa) was identified in both *P. fuliginosa* (average relative abundance: 5.67%) and *P. japonica* (average relative abundance: 10.70%). *Metopus* sp. (Ciliophora) was found in only one *P. japonica* sample, with a read count of 27.

Helminths were detected in all cockroach samples (Figure 1C). *Blatticola* sp. (Nematoda) was identified only in *B. germanica*, with read counts ranging from 39 to 108. However, its relative abundance was only about 1.2% compared to fungi (Figure 1A,C). In contrast, *Leidynema* sp. (Nematoda) was found exclusively in *P. fuliginosa* and *P. japonica*, with read counts ranging from 124 to 838.

Fungi were also detected in the cockroach samples (Figure 1D). In *B. germanica*, *Nephridiophaga* sp. (Chytridiomycota) was

TABLE 1 | Primers for specific eukaryotic samples.

Target	Primer		Sequence (5′–3′)	TM (°C)	Product size	References
Thelastomatidae	D2A		ACA AGT ACC GTG AGG GAA AGT TG	57	751 bp	Vogt et al. (2014)
	D3B		TCG GAA GGA ACC AGC TAC TA	55		
Nephridiophaga	Nephbla3 fw		CAG TTG GGG GCG TCA GTA TT	58	939 bp	Radek et al. (2017)
	EukB		TGA TCC TTC TGC AGG TTC ACC TAC	60		
Entamoeba	1st PCR	EukA	AAC CTG GTT GAT CCT GCC AGT	60	1950 bp	Kawano et al. (2017)
		EukB	TGA TCC TTC TGC AGG TTC ACC TAC	60		
	2nd PCR	01F	GCC AGT ATT ATA TGC TGA	46	1900 bp	
		01R	CCT TGT TAC GAC TTC TCC TT	52		

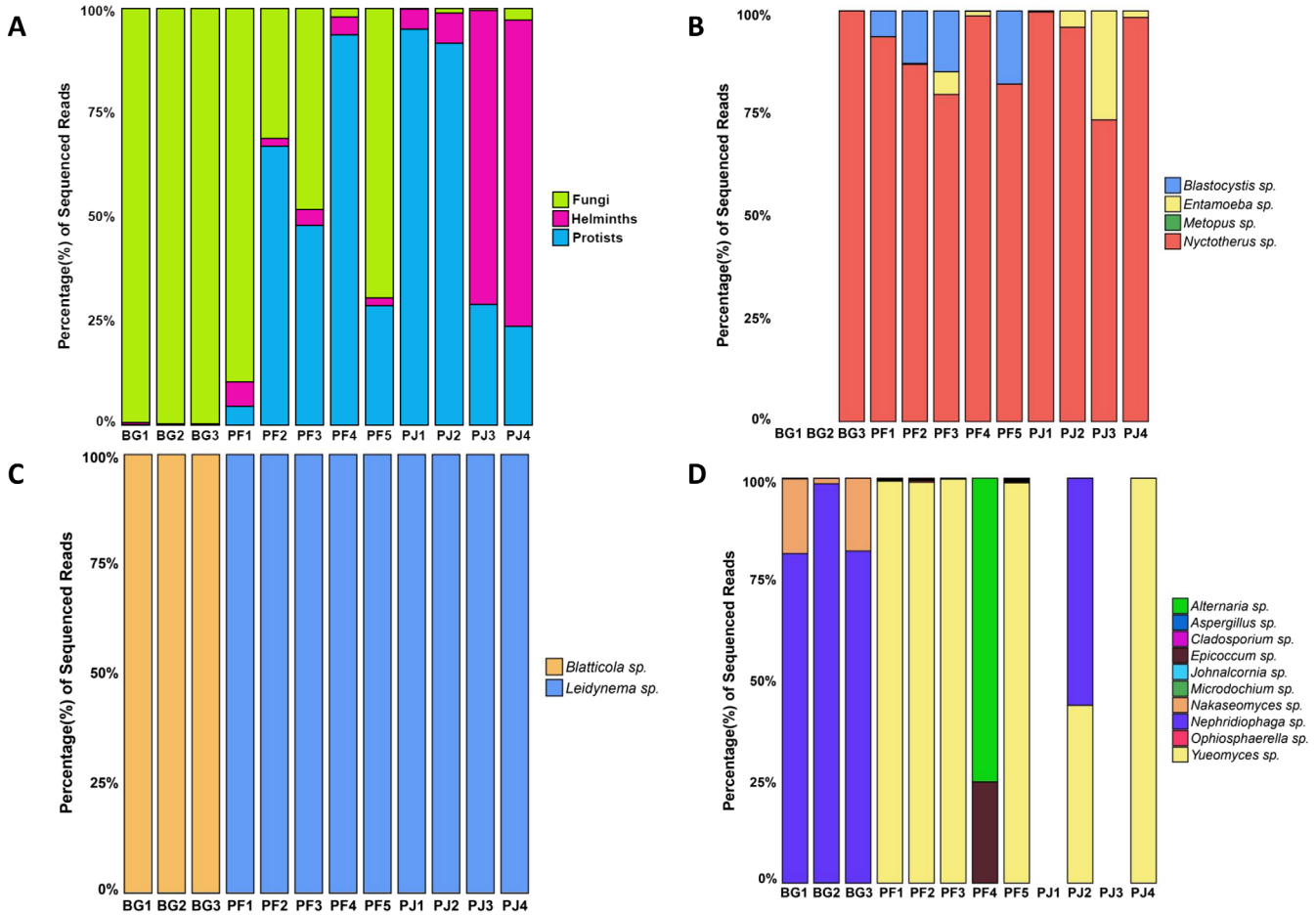

FIGURE 1 | Composition of eukaryotic taxa in the gut microbiota of *Blattella germanica* (BG, $n=3$), *Periplaneta fuliginosa* (PF, $n=5$), and *Periplaneta japonica* (PJ, $n=4$). (A) Composition of the taxa of protists, helminths, fungi, and based on 18S rRNA. (B) Composition of protists found in the samples. (C) Composition of helminths found in the samples. (D) Composition of fungi found in the samples.

dominant (average relative abundance: 87.34%), followed by *Nakaseomyces* sp. (Ascomycota) (average relative abundance: 12.57%), which was also detected in all *B. germanica* samples. *Yueomyces* sp. (Ascomycota) exhibited a relatively high abundance in the majority of *P. fuliginosa* samples (average relative abundance: 99.24%) and occurred in one *B. germanica* sample and two *P. japonica* samples. Detailed read counts of each eukaryotic taxon detected in individual cockroach specimens are provided in Tables S1–S3.

3.2 | Specific PCR of Cockroach Parasites and Fungi

Nematodes were visualized and individual parasites were recovered for PCR validation and Sanger sequencing. Representative images of the nematodes and protists observed in the laboratory-reared cockroaches are presented in Figure 2. *Blatticola* sp. (Figure 2A) was observed in *B. germanica*, *Leidynema* sp. (Figure 2B) was observed in *P. fuliginosa* and *P. japonica*, and *Nyctotherus* sp. (Figure 2C) was observed in all cockroach samples.

We then extracted DNA from these nematodes and performed PCR amplification using primers specific for nematode species, followed by Sanger sequencing. The resulting sequences were compared to the GenBank database, and they showed 100% identity with *Blatticola blattae* (GenBank accession: GQ368472.1) and *Leidynema appendiculata* (GenBank accession: KC540759.1), respectively. To confirm the presence of *Nephridiophaga* sp. and *Entamoeba*, we used specific primers targeting each taxon. The resulting sequences showed a 99.78% match with *N. blattellae* (GenBank accession: AY603958.1) and a 96.75% match with uncultured *Entamoeba* (GenBank accession: LC259432.1), respectively. PCR targeting *Nyctotherus* sp. using specific primers, however, resulted in nonspecific amplification. These taxonomic assignments were validated with maximum likelihood phylogenetic trees. *Blatticola* and *Leidynema* sequences found here were grouped with sequences of *B. blattae* and *L. appendiculata* in the phylogenetic tree of the Thelastomatidae family, while the *Nephridiophaga* sequences grouped with *N. blattellae* in the tree of the Nephridiophagaceae family as shown in Figure 3.

3.3 | Eukaryotic Diversity in the Three Cockroach Species

To investigate eukaryotic diversity in the gut communities of the three cockroach species, we performed alpha and beta diversity analyses. The Shannon index did not differ significantly

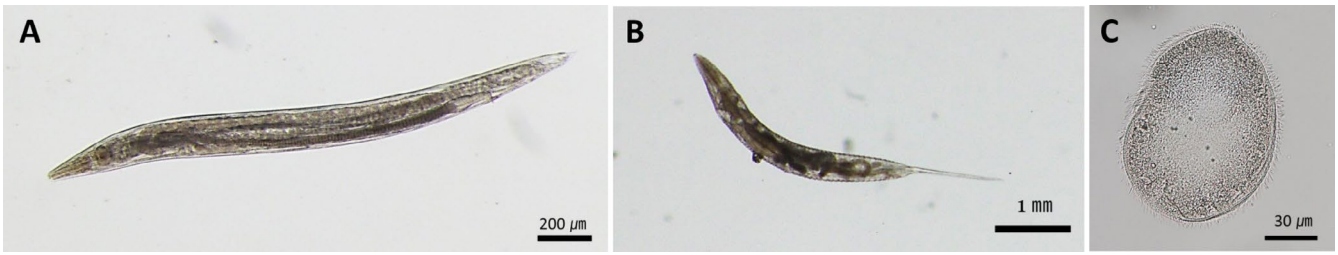

FIGURE 2 | Images of some nematodes and protists found in this study through bright-field microscopy. (A) *Blatticola* sp. (nematode). (B) *Leidynema* sp. (nematode). (C) *Nyctotherus* sp. (ciliate).

among the three species (Figure 4A, $p = 0.13$). However, PCoA based on Bray–Curtis distances showed clear differences in gut eukaryotic community composition among species (Figure 4B). PERMANOVA confirmed that community composition differed significantly among species ($R^2 = 0.542$, $F = 5.322$, $p = 0.001$). LEfSe analysis further identified differentially enriched taxa across species (Figure 4C). Specifically, *Nephridiophaga*, *Nakaseomyces*, and *Blatticola* were enriched in *B. germanica*, *Alternaria* was enriched in *P. fuliginosa*, and *Nyctotherus* was more enriched in *P. japonica*.

4 | Discussion

This study provides a comprehensive analysis of the eukaryotic community within the gut of three cockroach species, *B. germanica*, *P. fuliginosa*, and *P. japonica*, reared under the same laboratory conditions. Using the metabarcoding of the 18S rRNA gene, we identified a diverse community of eukaryotic taxa that included protists, nematodes, and fungi.

Shannon diversity index comparisons revealed no significant differences among species in alpha diversity. However, beta diversity analysis indicated distinct eukaryotic community compositions for each cockroach species. These differences emerged despite all cockroach species being reared under identical conditions, suggesting that host-associated factors such as physiology or other species-related traits may contribute to shaping the gut eukaryotic community.

Although beta diversity analyses provide insights into the community composition of different species, they may be affected by amplification biases inherent to metabarcoding, particularly in eukaryotic communities. Such biases may be introduced due to differences in genome copy number between protists or fungi and multicellular eukaryotes such as nematodes.

Here, *Nyctotherus* was the most abundant protist across all samples, present in 83.3% (10 of 12) of the samples. This genus is known to be responsible for cellulolytic and methanogenic activity in the hindgut of *P. americana* (Gijzen et al. 1994) and contributes to the host's hindgut metabolism via their symbiosis with methanogenic bacteria. Notably, certain cockroach lineages such as *Cryptocercus*, as well as termites, both within Blattodea, harbor complex communities of cellulolytic hindgut protists. The dominance of this taxon suggests that mutualistic protists play roles in cockroach gut function and reflect patterns of symbiosis within Blattodea. In addition, *Nyctotherus* is known to inhabit the hindgut of cockroaches and other insects, as well as other hosts such as arthropods, reptiles, and fish (Piñeiro et al. 2023; Satbige et al. 2017).

In addition to protists, *B. blattae* was observed in *B. germanica* (Kobayashi et al. 2021). *B. blattae* has been reported to improve cockroach survival during starvation, but this effect was only observed in combination with fecal feeding (Kobayashi and Taira 2024). However, it has also been associated with reduced adult lifespan and lower offspring number (when co-infected with fungi like *Nephridiophaga*) (Strassert et al. 2022). Furthermore, *Leidynema* sp. was identified in both *P. fuliginosa* and *P. japonica* in this study (Ozawa and Hasegawa 2018; Vicente et al. 2018). This nematode naturally and experimentally infects multiple cockroach species, exhibiting similar infection patterns (Ozawa and Hasegawa 2018).

N. blattellae, predominantly found in *B. germanica*, is generally considered a symbiotic organism (Radek and Herth 1999; Radek et al. 2011). However, it may reduce host fitness in co-infection with nematodes (Strassert et al. 2022). Although *Yueomyces* was reported in patients with severe alcohol-associated hepatitis (Philips et al. 2024), its relevance for human health remains unclear and requires further study (Nenciarini et al. 2024). In a hospital setting, fungal species isolated from cockroaches included *Rhizopus* sp., *Candida* spp., *Aspergillus niger*, and *Lichtheimia* sp. (Merad et al. 2023). In our study, *Aspergillus* spp. were identified in both *B. germanica* and *P. fuliginosa*.

In a study conducted in Nigeria, two cockroach species (*P. americana* and *B. germanica*) were found to harbor various protists and helminths (Adenusi et al. 2018). The identified protists included *E. histolytica/dispar*, *Entamoeba coli*, *G. lamblia*, and *Cryptosporidium* sp., while the helminths consisted of *A. lumbricoides*, *Trichuris trichiura*, hookworms, *Enterobius vermicularis*, *Strongyloides stercoralis*, *Taenia* spp., *Echinococcus* sp., and *Hymenolepis nana*. These parasites were detected on both the body surface and in the gut of *P. americana* and *B. germanica*. Similar findings of parasite presence in both the gut and on the body surface of cockroaches were also reported in studies from Ethiopia and Cameroon (Atiokeng Tatang et al. 2017; Haile et al. 2018; Kinfu and Erko 2008). In cockroaches from Ethiopia, *E. coli*, *E. histolytica/dispar*, *A. lumbricoides*, *E. vermicularis*, *T. trichiura*, and *Taenia* spp. were identified (Haile et al. 2018; Kinfu and Erko 2008). In cockroaches from Cameroon, *Ascaris* sp., *Trichuris* sp., *Capillaria* sp., hookworms, and *Eimeria* sp. were identified (Atiokeng Tatang et al. 2017). The absence of human pathogens in laboratory-reared cockroaches as opposed

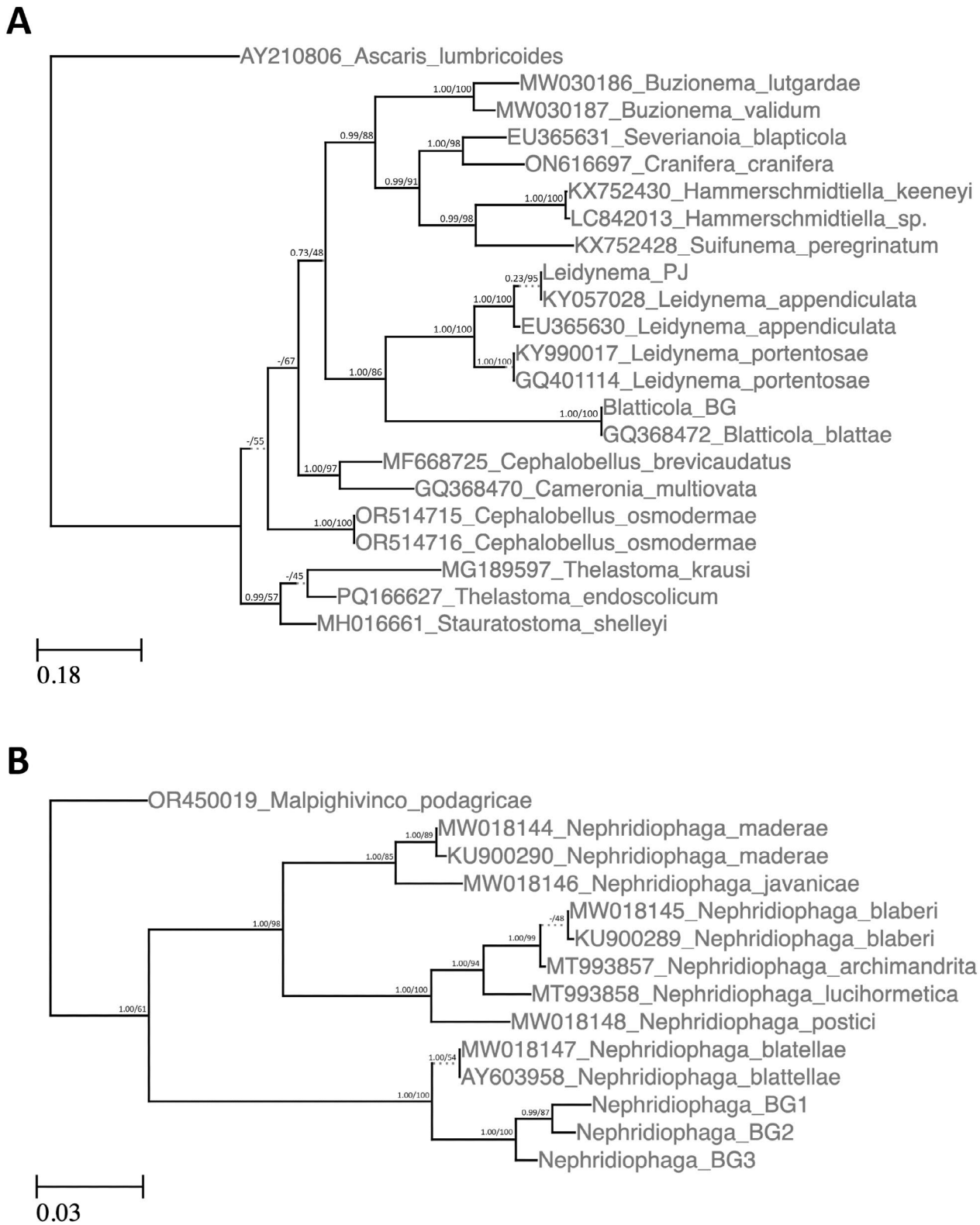

FIGURE 3 | Maximum likelihood phylogenetic trees of some eukaryotes identified in this study. (A) *Blatticola* and *Leidynema* sequences grouped with *Blatticola blattae* and *Leidynema appendiculata* within Thelastomatidae family. (B) *Nephridiophaga* sequences grouped with *Nephridiophaga blattellae* within Nephridiophagaceae family. Bayesian posterior probabilities and maximum likelihood bootstrap values are shown at nodes (posterior/bootstrap).

to those found in wild-caught specimens reported in other studies may indicate that wild specimens that interact with humans and the environment pose a heightened concern to human health.

While our study offers valuable insights, it also had several limitations. A key limitation of this study is that only a single laboratory colony was examined for each cockroach species. Therefore, the observed differences in gut eukaryotic communities may reflect

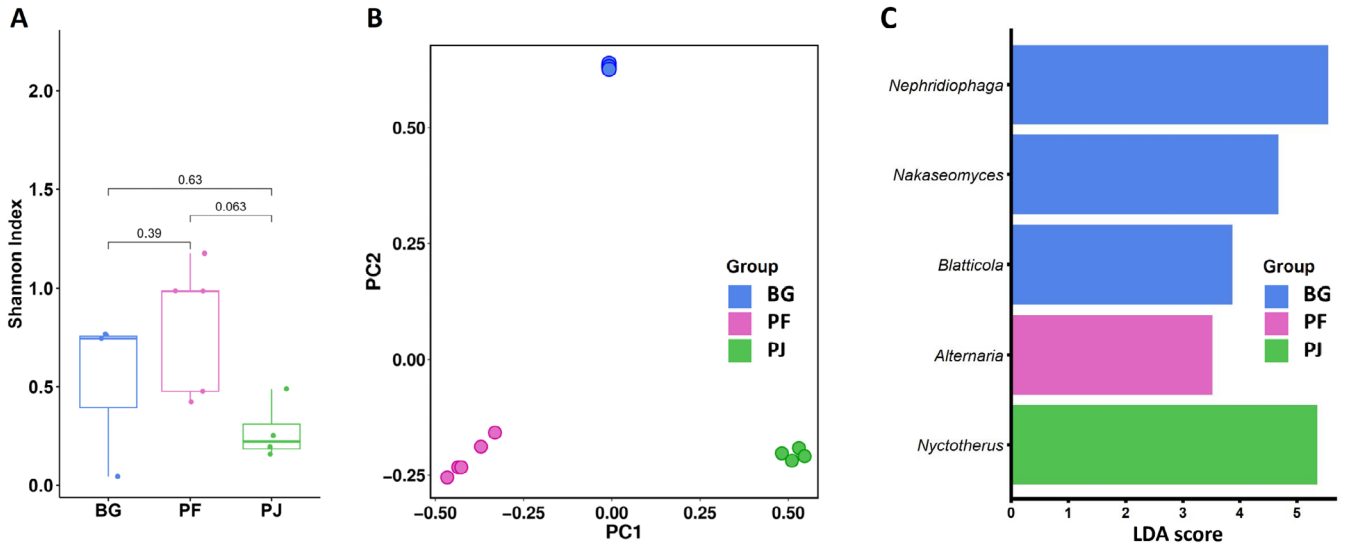

FIGURE 4 | Alpha and beta diversities of gut eukaryotic communities of laboratory-reared cockroaches after sequence filtering. (A) Box plots of the Shannon diversity index for *Blattella germanica* (BG), *Periplaneta fuliginosa* (PF), and *Periplaneta japonica* (PJ) samples. (B) Principal coordinate analysis (PCoA) representing the distribution of the 12 cockroach samples using the Bray–Curtis dissimilarity index. (C) LEfSe Bar Plot: Bar plot showing taxa with LDA scores greater than 3, indicating significant differential abundance.

colony-specific characteristics rather than true species-level differences. In addition, the colonies differed substantially in their duration of laboratory rearing, which may have further influenced community composition. As a result, it was not possible to disentangle the effects of host species from those of colony history or rearing conditions. We focused exclusively on laboratory-reared cockroaches, which harbored a different eukaryotic community compared with wild-caught populations reported in other studies. The cockroach eukaryotic community may be influenced by the duration of laboratory rearing due to the potential for the microbiome to shift over time. In this study, beta diversity differed by species under the same rearing conditions. The distinct communities could be attributed to biotic factors related to the cockroach species and abiotic factors such as original collection site and differences in rearing duration (Tchouassi et al. 2019). While our data do not allow us to isolate the effects of rearing duration from species identity, this factor should be considered in future comparative works. Although our primer set was designed to target all eukaryotes, it may still fail to detect certain taxa due to inherent amplification biases. For example, gregarines, which are common in cockroaches (Martínez-Girón et al. 2017; Yahaya et al. 2017), were not detected in this study. It will be useful to assess in future studies whether this is due to primer biases against gregarine sequences or the effect of rearing conditions. Our dataset includes both unicellular and multicellular organisms that differ in biomass and number of cells per individual. Although nematodes are multicellular, they showed the highest relative abundance in only two out of 12 samples. Microscopic examination revealed substantial variation in worm burden among individual hosts, with some cockroaches harboring more than 20 nematodes while others contained fewer than five. Such large differences among samples, together with inefficient lysis during DNA extraction and amplification biases inherent to metabarcoding, may have contributed to the marked variation in nematode read abundance across samples. Future studies may benefit from separate analyses of taxonomic groups to reduce the impact of amplification biases.

5 | Conclusions

In conclusion, our study revealed the gut eukaryotic communities of *B. germanica, P. fuliginosa*, and *P. japonica* differ significantly in composition and dominant taxa even under standardized laboratory rearing conditions for long periods. Our findings highlight the influence of host species in shaping the eukaryome. The detection of commensals, arthropod parasites, and potential human pathogens also suggests a complex nature of the cockroach-associated eukaryotic community. Our study establishes a baseline for understanding the development of different eukaryomes in experimental cockroach models as opposed to wild-caught specimens and emphasizes the importance of considering host-specific microbiome differences when interpreting results obtained from laboratory-reared cockroach models.

Author Contributions

D.K. conceived the study. D.K. and X.C. wrote the manuscript. J.H.C. and M.K. extracted the DNA from all the samples. X.C., S.O., D.-Y.C, A.S. designed, supervised, and coordinated the study. S.Y., M.-H.Y. and Y.S.J. prepared the samples for metabarcoding and ran the iSeq100 instrument. T.-S.Y. and S.C. designed, supervised, and coordinated the study. J.Y.K. formulated the research aims, developed the methodology, supervised the fieldwork, and coordinated the study, analyzed samples and data, and reviewed and edited the manuscript.

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The authors have nothing to report.

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Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in NCBI Sequence Read Archive at https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1203370, reference number PRJNA1203370.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Read counts of protist taxa in laboratory-reared cockroaches. Read counts of protist taxa detected in the gut samples of three cockroach species, *Blattella germanica* (BG, $n = 3$), *Periplaneta japonica* (PJ, $n = 4$), and *Periplaneta fuliginosa* (PF, $n = 5$). based on 18S rRNA gene metabarcoding. **Table S2:** Read counts of nematode taxa in laboratory-reared cockroaches. Read counts of nematode taxa detected in the gut samples of three cockroach species, *Blattella germanica* (BG, $n = 3$), *Periplaneta japonica* (PJ, $n = 4$), and *Periplaneta fuliginosa* (PF, $n = 5$) based on 18S rRNA gene metabarcoding. **Table S3:** Read counts of fungi taxa in laboratory-reared cockroaches. Read counts of nematode fungi detected in the gut samples of three cockroach species, *Blattella germanica* (BG, $n = 3$), *Periplaneta japonica* (PJ, $n = 4$), and *Periplaneta fuliginosa* (PF, $n = 5$). based on 18S rRNA gene metabarcoding.