

ORIGINAL ARTICLE

Variation in the microbiota across different developmental stages of *Aedes albopictus* is affected by ampicillin exposure

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Abstract

The microbiota plays an important role in the growth of mosquitoes and the transmission of mosquito-borne pathogens. The effects of changes in aquatic habitats in which mosquitoes live, as one of the major factors closely associated with the microbial communities of mosquitoes, on the microbiota of different developmental stages remain to be elucidated. Here, we compared the microbiota of larvae and pupae of *Aedes albopictus* exposed to different ampicillin concentrations and investigated the bacterial composition of adult females. The results demonstrate that the microbial community differed substantially between developmental stages and that samples of the same stages shared similarities, whereas differences were observed between adult females. Based on all observations, we hypothesize that the use of ampicillin caused dysbiosis rather than excluding bacteria from mosquitoes and that the disturbing effect of ampicillin was obvious in adults. Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses revealed that most of the bacteria identified in this study were significantly associated with metabolism. Taken together, our results indicate that ampicillin can change the abundance of bacteria, while microbial communities of *Ae. albopictus* showed obvious stage-specific characteristics. Further investigations are needed to characterize specific bacterial components that are affected by ampicillin exposure and to quantify their functions, thereby providing a better understanding of the influence of antibiotics on microbial communities at different life stages.

KEYWORDS

16S rRNA gene, antibiotic, Asian tiger mosquito, stage-specific

1 | INTRODUCTION

The Asian tiger mosquito *Aedes albopictus* (Diptera, Culicidae) is an important vector for a number of arboviruses. It has been identified as a potentially competent vector for more than 22 viruses

in the laboratory and is responsible for the epidemic transmission of dengue, Zika, and chikungunya (Paupy, Delatte, Bagny, Corbel, & Fontenille, 2009). Originating in South-East Asia, *Ae. albopictus* has spread globally over the past three decades (Kraemer et al., 2015) and is considered one of the top 100 world's worst invasive

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alien species (Global Invasive Species Database, 2017). The international spread of *Ae. albopictus* has triggered numerous epidemics of dengue, Zika, chikungunya, and yellow fever (Bhatt et al., 2013; Leparc-Goffart, Nougairede, Cassadou, Prat, & Lamballerie, 2014; Lessler et al., 2016; Paules & Fauci, 2017). It is estimated that approximately 400 million people are infected with dengue viruses every year, and over half of the world's population is at risk of contracting the disease (Bhatt et al., 2013). Dengue has grown more than 30-fold during this three-decade period (Pang, Mak, & Gubler, 2017) and has become the most common mosquito-borne viral disease in the world. Zika virus has been proven to be associated with the occurrence of microcephaly and other congenital abnormalities. The South Pacific and the Americas had Zika epidemic outbreaks of in 2013 and 2015, respectively (Mayer, Tesh, & Vasilakis, 2017). Chikungunya virus emerged in the mid-2000s and spread from Africa into Asia and the Americas (Mayer et al., 2017). Although effective vaccines have been developed, yellow fever has spread to the Democratic Republic of Congo, Kenya, China, and Brazil (Bagchi, 2017; Zwizwai, 2015).

Due to the lack of effective vaccines for most of these pathogens (Flipse & Smit, 2015; Poland et al., 2018; Powers, 2018), mosquito control is relied upon to limit outbreaks and prevent the spread of these diseases. Chemical pesticides have shown high effectiveness in reducing mosquito populations and have long been used to date. However, the negative effects of this traditional approach have triggered an increase in insecticide resistance among mosquitoes and in environmental pollution, seriously impacting human/animal health and food safety and increasing the prices of the current insecticide interventions. The World Health Organization (WHO) has called for new vector control technologies (WHO, 2017).

Currently, innovative mosquito control strategies are desperately needed (Pang et al., 2017). Biological control, due to its effectiveness and environmental safety, has become an ideal strategy (Ricci et al., 2012). Moreover, the microbiota of mosquitoes is emerging as a potential tool in this effort (Wilke & Marrelli, 2015). Some members of the microbiome are known to sever functions essential for mosquito survival, development, fitness, immunity, reproduction, and vector competence (Cirimotich, Ramirez, & Dimopoulos, 2011; Coon, Brown, & Strand, 2016a; Coon, Vogel, Brown, & Strand, 2014; Hegde, Rasgon, & Hughes, 2015; Jupatanakul, Sim, & Dimopoulos, 2014; Ramirez et al., 2014). The development of malarial parasites was hampered after the microbiota of mosquitoes was evaluated, providing evidence for a potential strategy for malaria control. Some bacteria of mosquitoes have been shown to inhibit the transmission of dengue, chikungunya, and Zika viruses by reducing the mosquito life span (Aliota, Peinado, et al., 2016; Aliota, Walker, et al., 2016; McMeniman et al., 2009; Walker et al., 2011) or blocking pathogen proliferation (Moreira et al., 2009). These findings provide a promising avenue for the development of novel microbial-based vector control strategies to reduce vector competence and suppress outbreaks of mosquito-borne diseases.

The composition and structure of the mosquito microbiota are highly variable and are associated with species, breeding habitat, diet, and even microenvironmental factors (such as intestinal pH, oxygen status, and residence time of digesta; Belda et al., 2011; Hu, Lukasik, Moreau, & Russell, 2014; Mikaelyan, Meuser, & Brune, 2017; Pennington, Rivas, Prager, Walton, & Trumble, 2015). Previous studies have gathered sufficient information to demonstrate that the microbiota of mosquitoes is often dominated by relatively few genera (Boissière et al., 2012; Buck et al., 2016; Muturi, Ramirez, Rooney, & Kim, 2017; Osei-Poku, Mbogo, Palmer, & Jiggins, 2012; Wang, Gilbreath, Kukutla, Yan, & Xu, 2011; Yadav et al., 2015, 2016), which are principally acquired from their breeding habitats during immature developmental stages and from adult food sources. Therefore, changes in the microenvironment of aquatic habitats in which immature mosquitoes live are prone to alter the composition of the microbiota of mosquitoes.

Antibiotics in ingested human blood disturbed the microbiota of *Anopheles gambiae* and subsequently enhanced the susceptibility of mosquitoes to malaria (Gendrin et al., 2015). In the present study, using high-throughput 16S rRNA gene sequence analysis we explored the impact of antibiotics in aquatic habitats on the microbial assemblages of *Ae. albopictus*. Ampicillin was used alone instead of a cocktail of antibiotics to address its specific contribution to effect the microbiota of mosquitoes.

2 | METHODS AND MATERIALS

2.1 | Rearing of mosquitoes

The colony of *Ae. albopictus* was obtained from one maintained at the Chinese Center for Disease Control and Prevention (CDC) that was collected in Jiangsu Province. Mosquitoes were maintained at $27 \pm 1^\circ\text{C}$ and 65% relative humidity (RH) with a daily photoperiod of 14:10 hr (L:D). Larvae were fed a slurry of one part (5 g) pork liver powder (homemade) and one part (5 g) yeast per 100 ml of distilled water. Pupae were collected and placed into a beaker and then reared in insect nets (35 × 35 × 35 cm) until they hatched. Newly emerged adults were provided with 10% sugar solution ad libitum. Females 4–5 days old were supplied with fiber-free goat blood (Solarbio) using an artificial feeder (Hemotek Membrane Feeding System).

Ampicillin (Solarbio) was added to distilled water for a final antibiotic concentration of 50, 100, 150, and 200 $\mu\text{g/ml}$; distilled water was used as a negative control (0 $\mu\text{g/ml}$; Figure A1). Two hundred eggs were placed into each group, and all of them were reared under the same conditions to eliminate potential dissimilarity that could lead to biases in environmental factors. As ampicillin was added to water, the larval and pupal stages were the main stages that could be influenced by the antibiotics. The microbiota of adult females hatched from all five groups was also tested to investigate the potential prolonged effect of ampicillin on variation in the *Ae. albopictus* microbiome.

2.2 | Microbial DNA extraction and sequencing

A total of 18 third-instar larvae, 18 pupae (1-day-old), and 18 unfed females (2-days-old virgin females) collected from each treatment group were washed twice in 70% ethanol for 5 min and three times in sterile deionized water in preparation for DNA extraction. Then, the samples from each treatment were divided into three pools with six larvae, six pupae, and six female adults per pool. DNA was extracted using a Bacterial DNA Kit (Omega). The extraction procedures were performed in a biosafety cabinet to ensure that the samples were protected from environmental contamination. A Nanodrop 2000 system (Thermo Fisher Scientific) was used to determine the DNA concentration. The amplicon library preparation was performed by polymerase chain reaction (PCR) amplification of the V3-V4 region of the 16S rRNA gene using the primer sets 338F (5'-ACT CCT ACG GGA GGC AGC A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3') with adaptor and barcode sequences (Mori et al., 2014).

Polymerase chain reaction was performed with the following program: an initial denaturation at 95°C for 5 min, followed by 15 cycles at 95°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 7 min. DNA libraries were validated using an Agilent 2100 Bioanalyzer (Agilent Technologies) and quantified with a Qubit 2.0 fluorometer. Library sequencing was performed using the Illumina HiSeq 2500 platform (2 × 250 paired ends) according to the manufacturer's instructions at Biomarker Technologies. All raw sequences were deposited into the NCBI Sequence Read Archive with the accession number PRJNA572589.

2.3 | Statistical analysis

Raw Illumina fastq files were merged and quality-filtered using QIIME 1.9.1 (Caporaso et al., 2010). Sequences with ambiguous nucleotides and with lengths less than 200 bp were trimmed out. The remaining sequences were then classified into taxa by BLAST search with the ribosomal database project (RDP) database at a 97% pairwise identity threshold. The operational taxonomic units (OTUs) were sorted using the clustering program Vsearch (1.9.6) (Rognes, Flouri, Nichols, Quince, & Mahé, 2016) against the Silva 132 database (<http://www.arb-silva.de>) using the default 97% identity threshold. The relative abundances at the phylum, family, and genus levels are shown in the provided bar graphs.

Alpha diversity was measured using the Shannon evenness index for community diversity and the Chao1 index for community richness, and the sequencing depth (Good's coverage) was calculated by Mothur (version 1.30 <http://www.mothur.org>) and visualized with R software v3.2.4 (R Core Team, 2014). Beta diversity was calculated using weighted and unweighted UniFrac distances and visualized via principal coordinate analysis (PCoA). The influence of environmental variables on dominant bacterial communities was investigated by modeling redundancy analysis (RDA) or

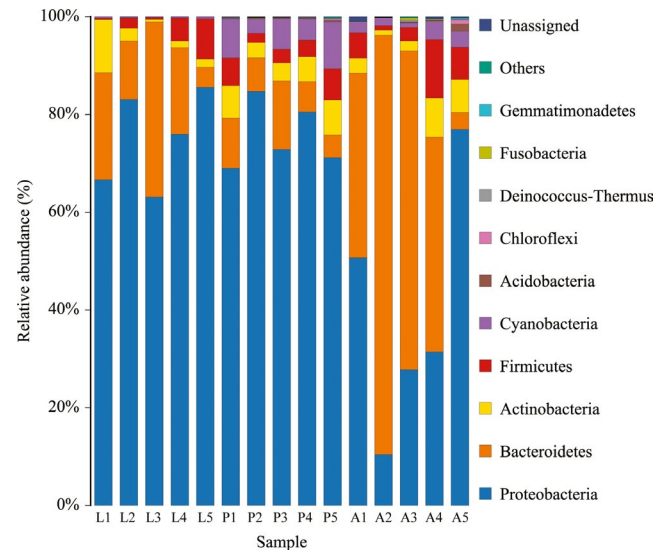


FIGURE 1 Relative abundance of bacterial phyla detected in mosquitoes exposed to different ampicillin concentrations across developmental stages of *Ae. albopictus*

canonical correspondence analysis (CCA), which was performed according to detrended correspondence analysis (DCA). The independent influences of antibiotics and developmental stages on the bacterial community composition were assessed by Mantel's test in PC-ORD 5.

The linear discriminant analysis (LDA) effect size (LEfSe) was used to detect significant alterations in the bacterial composition associated with all samples. In the LEfSe method, the Kruskal–Wallis test ($\alpha = .05$) was applied to identify taxa with significantly different abundances between groups, and an LDA score of >3.0 was used as a threshold to estimate the effect size of each differentially abundant feature. Putative microbiota functions were predicted by annotating pathways of OTUs against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa & Goto, 2000) using PICRUST (Langille et al., 2013). The bacterial taxonomic or KEGG modules were compared using the Wilcoxon rank-sum test, and the p values were corrected using the Benjamini–Hochberg method.

3 | RESULTS

3.1 | Microbiome diversity

A total of 2,454,524 reads were obtained from the 45 samples after 16S rRNA high-throughput sequencing analysis. After quality filtering, 2,149,911 reads were assigned to 431 OTUs at a 97% identity threshold, and there were 48,852 reads per mosquito sample on average. Rarefaction curve analysis indicated that the sequencing depth was sufficient to observe all OTUs in the mosquito samples (Figure A2).

The relative abundance of the bacterial community at the phylum level is shown in Figure 1. Bacteria found in *Ae. albopictus*

were classified into 14 phyla (Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Deinococcus–Thermus, Epsilonbacteraeota, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospirae, Patescibacteria, Proteobacteria, and Verrucomicrobia), with the phylum Proteobacteria being predominant in the microbiota of most groups. Bacteroidetes was the second most prevalent phylum across these samples. At the genus level, the relative abundance of bacterial taxa varied markedly between samples (Figure 2). Along with the developmental stages of *Ae. albopictus*, the relative abundance of *Acinetobacter* obviously decreased, whereas *Delftia* and *Elizabethkingia* were predominant in pupae

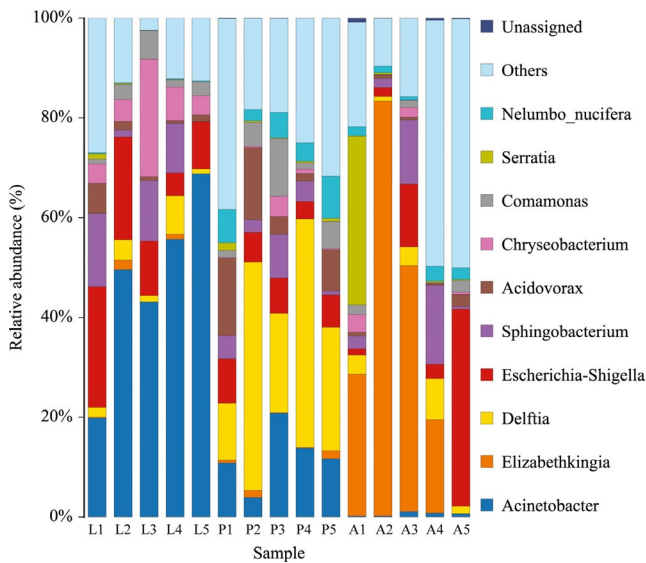


FIGURE 2 Relative abundance of bacterial genera detected in mosquitoes exposed to different ampicillin concentrations across developmental stages of *Ae. albopictus*

and adults, respectively. In addition, the relative abundance of *Escherichia-Shigella* and *Chryseobacterium* was much higher in larvae than in other stages, and the proportion of *Serratia* was drastically increased in group A1. *Elizabethkingia* was replaced by *Escherichia-Shigella* in group A5. Moreover, an unclassified genus was detected in all groups and accounted for a substantial proportion of many groups (Figure 2).

Good's coverage ranged from 99.9% to 100% for each sample, indicating that the identified 16S rDNA sequences were sufficient to fully represent the bacterial diversity of samples in this study (Table 1). The Chao1 indexes demonstrated that the richness of adult females was slightly higher than that of larvae and pupae, while Shannon's diversity index was lower for larvae and adult females than pupae. Both the richness and diversity of larvae were the lowest. This difference was also supported by the PCoA results (Figure 3). The weighted UniFrac PCoA showed that 54.26% (axis 1) and 17.48% (axis 2) of the variation among the developmental stages were explained (Figure 3). Clear clustering of the communities hosted by the larvae and pupae revealed an evident pattern of specialization, while the communities hosted by the adult females were more similar to those hosted by the pupae.

Based on the DCA results, RDA was chosen to investigate the influences of antibiotics and developmental stages on the bacterial community of *Ae. albopictus*. Altogether, 23.9% of the total variability in the bacterial community at the genus level was explained based on RDA (Figure 4). The first ordination RDA axis (RDA 1, horizontal) was positively correlated with developmental stage and explained 15.41% of the variability in the bacterial community, while the second ordination RDA axis (RDA 2, vertical) exhibited a negative correlation with antibiotics use and explained 8.49% of the total variability in the bacterial community.

Sample I	Ampicillin concentration (µg/ml)	Chao1	Shannon	Coverage
L1	50	292.17	2.5328	0.999
L2	100	315.28	1.9149	0.999
L3	150	291.30	1.7201	0.999
L4	200	295.33	1.8274	0.999
L5	0	341.22	1.427	0.999
P1	50	359	3.3541	0.999
P2	100	312.25	2.4114	0.999
P3	150	301.76	2.8386	0.999
P4	200	366.5	2.5454	0.999
P5	0	392.5	3.315	0.999
A1	50	359.15	2.5838	0.999
A2	100	320.24	1.0011	0.999
A3	150	381.53	2.2645	0.999
A4	200	377.15	3.4921	0.999
A5	0	332.07	3.0713	0.999

Note: A, adult female; L, larva; P, pupae.

TABLE 1 Samples and alpha diversity indices of *Aedes albopictus* used in this study

FIGURE 3 Principal coordinate analysis (PCoA) of the microbial communities from mosquitoes exposed to different ampicillin concentrations across developmental stages of *Ae. albopictus*. A, adult female; L, larva; P, pupae

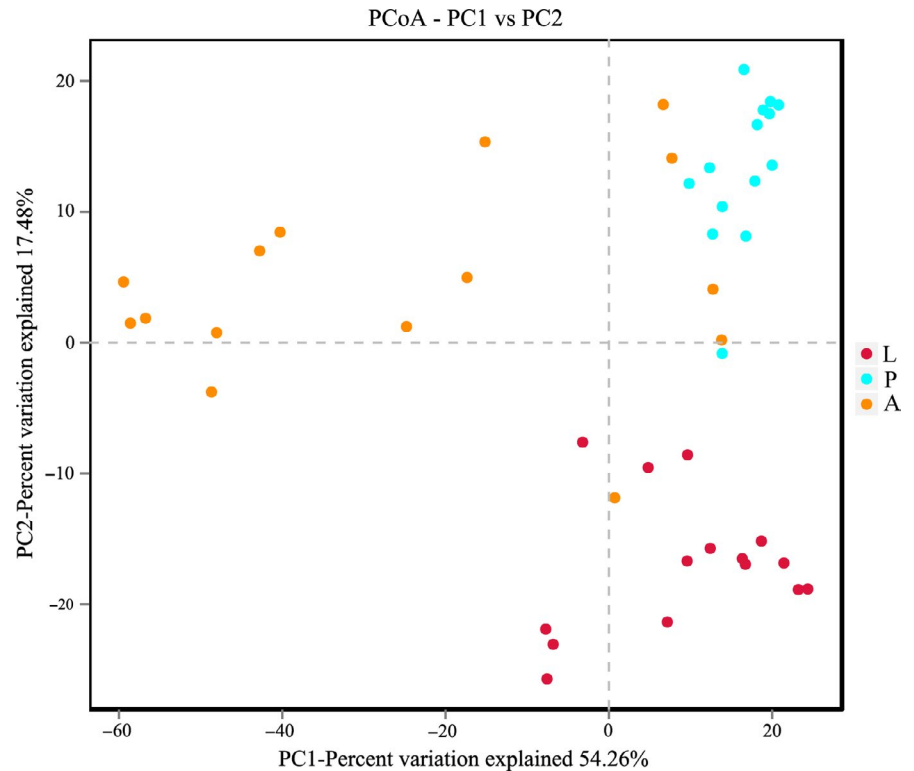
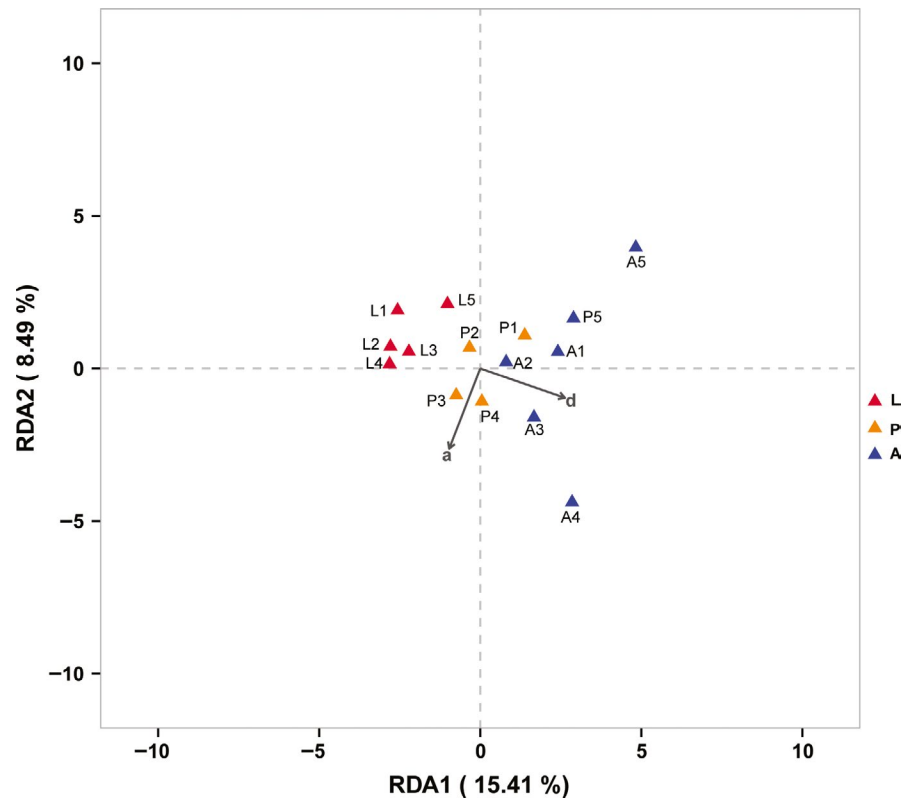


FIGURE 4 Redundancy analysis (RDA) ordination plots for the relationships between antibiotic concentrations and the bacterial communities. a, antibiotic; d, developmental stage



LefSe analysis revealed the bacterial taxa with differential abundances according to an LDA value higher than 4.0 for the different growth stages of *Ae. albopictus* (Figure 5; Figures A3 and A4). The results revealed that at the genus level there were 26 and 19 major taxa

in L1 and L5, while L2, L3, and L4 harbored only 4, 6, and 4 major taxa, respectively. Similar results were also detected in the pupae and adults, except that there were no biomarkers in P5 and A3. A low abundance of bacterial taxa was found in groups P2, P3, P4, A2, and A4.

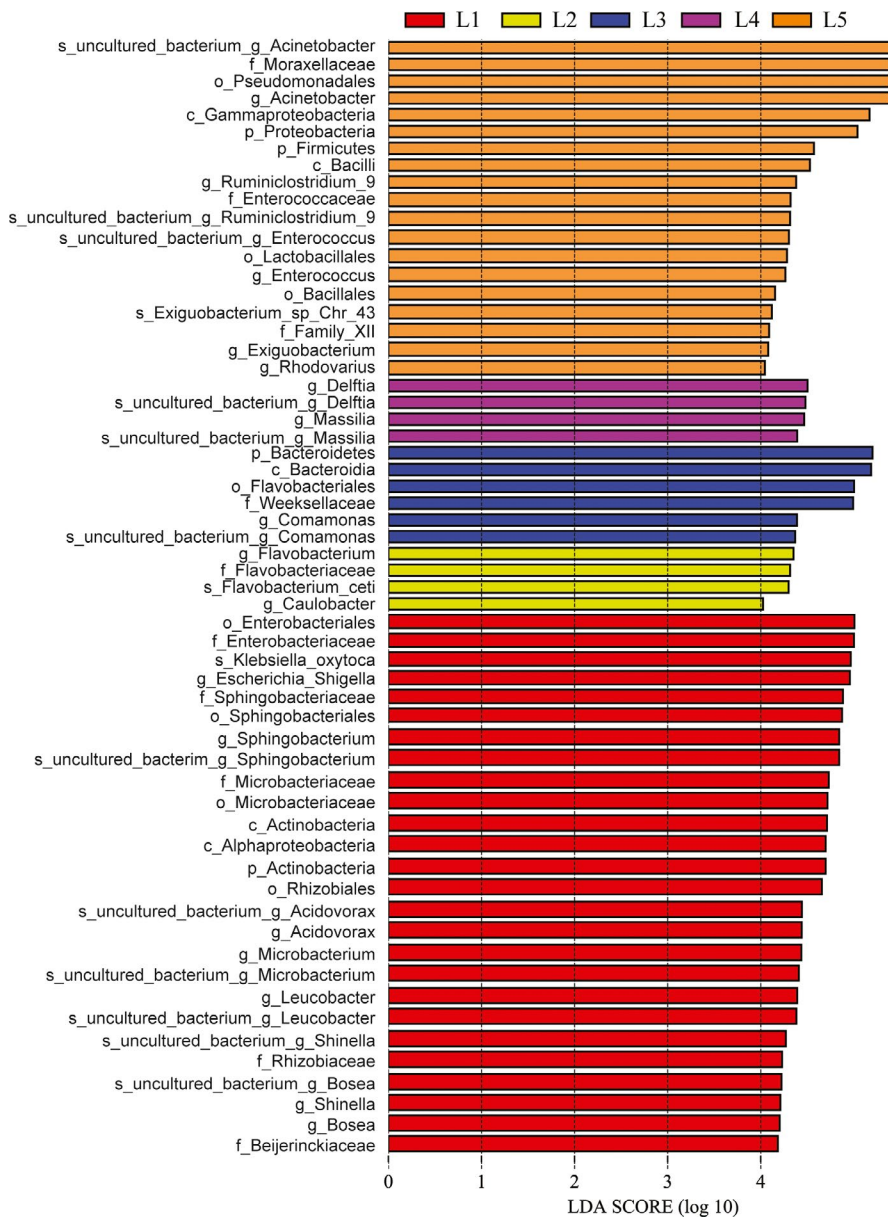


FIGURE 5 Bacterial taxa significantly differentiated between different groups identified by linear discriminant analysis effect size (LEfSe) using the default parameters. Histogram of the LDA scores (>4) computed for bacterial taxa differentially abundant among different groups. The length of the bar column represents the LDA score. larva; pupae; and adult female

To investigate the functional divergence among the bacterial taxa hosted by *Ae. albopictus*, metagenomic functions were predicted using PICRUSt. The KEGG Orthology (KO) abundances were calculated for each sample, and the abundances of functional categories on level III of the KEGG database were quantified. A total of 43 KEGG pathways were predicted (Table S1: <https://doi.org/10.5281/zenodo.3666935>). KEGG pathway analysis revealed a predominance of pathways related to metabolism (carbohydrates, lipid, energy, and amino acid metabolism), organismal systems (endocrine system, nervous system, and environmental adaptation), and human diseases (infectious diseases: bacterial, parasitic, and viral). Specifically, ABC transporters (environmental information processing), biosynthesis of amino acids (metabolism), and carbon metabolism (metabolism) were three major pathways that were enriched in all groups and displayed variation with the effects of both antibiotic use and developmental stage (Figure 6; Figures A5 and A6).

4 | DISCUSSION

Studies have revealed that the microbiota of mosquitoes is typically composed of 10–70 bacterial taxa and that Proteobacteria is the predominant phylum (Minard et al., 2013; Osei-Poku et al., 2012). It has been proposed that mosquitoes acquire bacteria largely from the aquatic environment in which they live as larvae (Wang et al., 2011), and some others are acquired from food sources and include transovarially transmitted bacteria from females to the next generation (Coon et al., 2014; Duguma et al., 2015; Lindh, Borg-Karlson, & Faye, 2008). It is plausible that many factors (internal and external changes) can influence the composition and structure of the mosquito microbiota.

We characterized not only the microbiota of *Ae. albopictus* at immature stages (larvae, pupae) while inhabiting water with different ampicillin concentrations but also the variations in the microbiome composition of unfed adult females hatched from the aforementioned

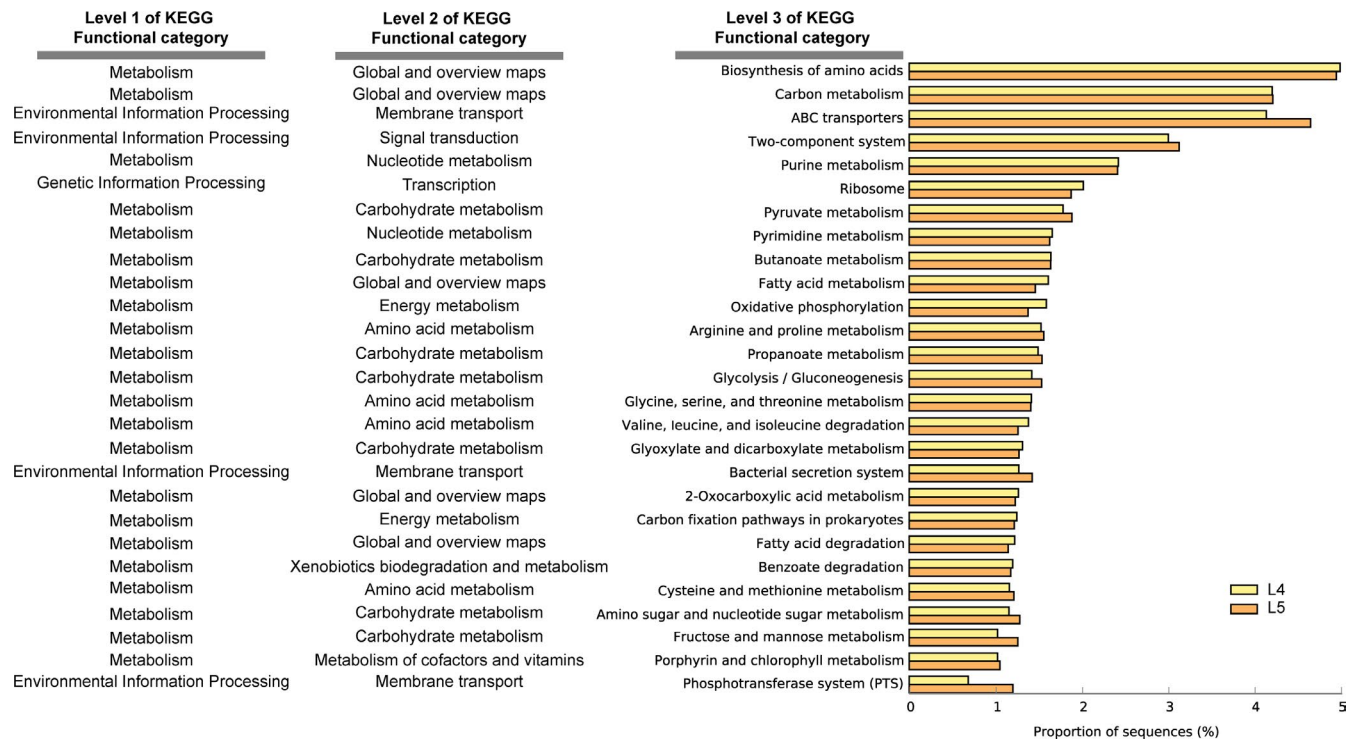


FIGURE 6 Relative abundance of KEGG functional categories and pathways that were significantly enriched in larvae

pupae. The predominance of Proteobacteria in most immature stage groups was consistent with the findings of previous studies (Audsley et al., 2017; David, Santos, Vicente, & Maciel-de-Freitas, 2016; Muturi, Kim, Bara, Bach, & Siddappaji, 2016; Osei-Poku et al., 2012). However, divergence was observed in the dominant phylum between adult females that were originating from immature stages in the ampicillin-treated groups (A1–A4) and those originating from immature stages in the control group (A5) (Figure 1). The high abundance of Bacteroidetes in groups A1–A4 was probably due to the delayed effect of ampicillin; while it did not significantly change the bacterial composition of larvae or pupae, the influence accumulated and was present in adults. The predominant genera exhibited obvious stage specificity, with *Acinetobacter*, *Delftia*, and *Elizabethkingia* being predominant in larvae, pupae, and adult females, respectively. Changes in the composition of the microbiota along with developmental stages have been reported in several other mosquitoes (Gimonneau et al., 2014; Kim, Lampman, & Muturi, 2015; Wang et al., 2011). These variations in terms of the diversity and composition of the microbiome are closely related to the biological and ecological dynamic interactions throughout the life cycle of mosquitoes (Coon et al., 2014; Wang et al., 2011). Moreover, the shift from larvae to adults is accompanied by changes in habitat from aquatic to terrestrial environments, most likely leading to a significant variation in the microbiota between immature and adult mosquitoes (Coon et al., 2014; Minard et al., 2013; Moll, Romoser, Modrakowski, Moncayo, & Lerdthusnee, 2001; Romoser, Moll, Moncayo, & Lerdthusnee, 2000; Wang et al., 2011). Corresponding to the divergence at the phylum level, a differential in the microbial composition was also detected

at the genus level in adult females. The dominance of *Elizabethkingia* in groups A1–A4, together with the high abundance of *Escherichia-Shigella* in the control group (A5), further illustrated the prolonged effect of ampicillin. Furthermore, *Elizabethkingia* has been reported to produce antimicrobial compounds (Ngwa et al., 2013), which might contribute to its colonization in the ampicillin-treated groups. Similar results were also found in *Ae. albopictus* samples exposed to an antibiotic cocktail (gentamicin and penicillin-streptomycin), and the abundance of *Elizabethkingia* was negatively correlated with the antibiotic concentration used (Guégan, Zouache, et al., 2018).

Antibiotics have frequently been used to disturb mosquito-associated microbiota, and several antibiotic cocktails have been proven to impact the survival and fecundity of mosquitoes and even alter the efficiency of mosquito-borne disease transmission (Dong, Manfredini, & Dimopoulos, 2009; Gendrin et al., 2015; Rossolini, Arena, Pecile, & Pollini, 2014; Upton, Povelones, & Christophides, 2015). Guégan, Zouache, et al. (2018) reported that both the community structure and bacterial diversity of *Ae. albopictus* were influenced by the antibiotic cocktail of gentamicin and penicillin-streptomycin in the early ingestion stage. However, as one of the antibiotics frequently used in agriculture and human medicine, the role played by ampicillin in microbiota changes has rarely been explored. In this study, our results showed fluctuating bacterial diversity when comparing ampicillin-treated and untreated *Ae. albopictus* samples. Meanwhile, the bacterial composition also displayed differences among individuals; *Serratia* was especially enriched in A1, which can probably be attributed to individual differences among samples. The relative abundances of taxonomic groups within the

bacterial community varied among samples, which might be attributed to the extent of bacterial disturbance caused by different concentrations of ampicillin. With the limitation of dominant bacteria, ecological niches change accordingly and allow other bacteria to colonize and/or promote the growth of rare bacteria (Guégan, Zouache, et al., 2018), as demonstrated by the high abundance of *Elizabethkingia* in adult females originating from ampicillin-treated pupae (A1–A4) compared to those originating from untreated pupae in the control groups (A5) (Figure 2). Furthermore, mosquitoes harbor some antibiotic-resistant bacteria (Coon, Brown, & Strand, 2016b); thus, antibiotics do not fully clear bacteria. The variation in the abundance and composition of the microbiota could indicate dysbiosis in the mosquitoes treated with antibiotics (Hughes et al., 2014; Pennington, Prager, Walton, & Trumble, 2016).

Inconsistent with previous studies suggesting that larvae possess the highest bacterial diversity (Wang et al., 2018), our results reveal that both the bacterial richness and diversity of larvae were lower than those of other stages (Table 1). Taking the sample that was not exposed to the antibiotic as a reference, some unique bacteria in the ampicillin-treated samples changed in abundance as a result of different concentrations, as detected by the LEfSe analysis (Figure 5; Figures A3 and A4). We speculate that these differences could be caused by ampicillin inhibiting the growth of some bacteria and accelerating the proliferation of others, such as bacteria capable of metabolism under the effects of the antibiotic. Moreover, studies carried out in mosquitoes have shown that the habitat is an important driver of the structure of the mosquito bacterial community. For example, a recent study identified similar bacterial communities in *Aedes aegypti* and *Anopheles gambiae* reared under the same laboratory conditions (Coon et al., 2014). Sequence analysis of bacterial 16S rRNA gene amplicons showed that the bacterial community composition of *Ae. aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* differed substantially in larvae from different collection sites, whereas the community composition of larvae from the same site was similar (Coon et al., 2016b). Therefore, the influence of both different ampicillin concentrations and distinct mosquito populations might be responsible for the differences in bacterial diversity in different studies. Regardless of the extent to which ampicillin alters the microbiota of *Ae. albopictus*, the microbial community displayed stage-specific features on PCoA (Figure 3). Furthermore, the impact of developmental stages on the microbiota of *Ae. albopictus* was also supported by the RDA results. The separation of samples, especially that of the adult females, was clearly positively related to developmental stage. Hence, the developmental stage was a major factor that influenced the structure of the microbial community in these samples.

Microbiota have been shown to play critical roles in the fitness of their hosts, and some are involved in the metabolism of antibiotics and contribute to host antibiotic resistance (Coon et al., 2016b). Detailed information regarding the effect of antibiotic-resistant bacteria on metabolism has not yet been described. Based on the different bacterial compositions of the antibiotic-exposed groups, we identified their potential function. Altogether, 43 KEGG pathways were predicted and most pathways were significantly

associated with metabolism. The underlying mechanisms by which ampicillin influences the microbiota remain poorly understood, and we currently cannot verify the role of specific bacteria in different pathways; however, KEGG analysis produced a functional view of the differential expression profile. We propose that metabolism could be a contributing factor that is initially influenced by ampicillin and consequently shapes the host physiology (Minard et al., 2013).

The results presented here highlight the different effects of ampicillin exposure on the microbiota across *Ae. albopictus* developmental stages. The bacterial taxa that were more abundant in the ampicillin-exposed samples than in the untreated samples will be a focus of future work to characterize specific bacterial components that are affected by ampicillin exposure; additionally, the contribution of these bacterial taxa to resistance in mosquitoes will be quantified. Based on these observations, it could be interesting to assess the selection pressure from the extensive use of antibiotics in agriculture and medicine and to evaluate the influence of this pressure on the transmission efficiency of mosquito-borne pathogens. Although there are still many unanswered questions, these preliminary results pave the way for further characterization of the mosquito microbiota to enable the selection of promising bacterial candidates for potential use in an innovative microbial-based vector control approach to reduce the burden of mosquito-borne diseases.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

ZRL and ZZ conceived and designed the experiment. WQ, XZJ, and YGF performed laboratory work, interpreted results, and compiled tables and figures. ZRL and WQ wrote the first draft of the manuscript, and MF and LQY contributed to finalizing the paper. All authors contributed critically to the drafts and gave final approval for publication.

ETHICS STATEMENT

None required.

DATA AVAILABILITY STATEMENT

All sequences have been deposited into the NCBI Sequence Read Archive under the BioProject accession number PRJNA572589. Supplementary material has been deposited in Zenodo at <https://doi.org/10.5281/zenodo.3666935> (Table S1: KEGG Orthology groups predicted for bacteria of different groups).

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APPENDIX 1

FIGURE A1 Experimental design for microbiota diversity study of *Aedes albopictus* mosquitoes

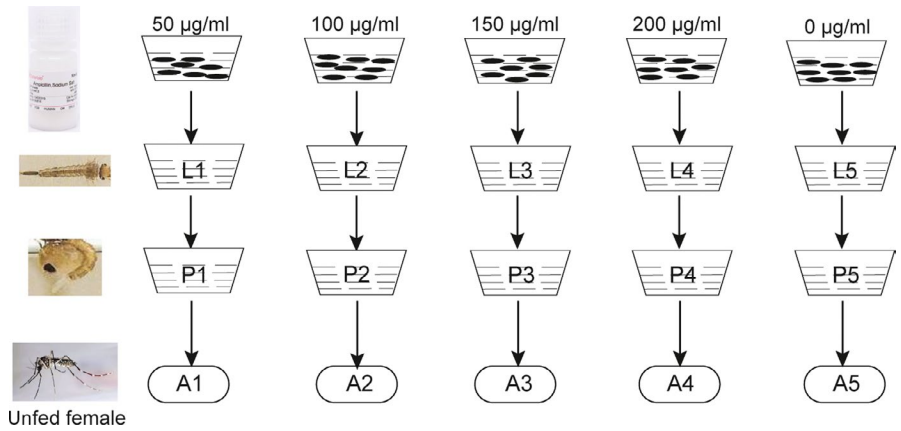
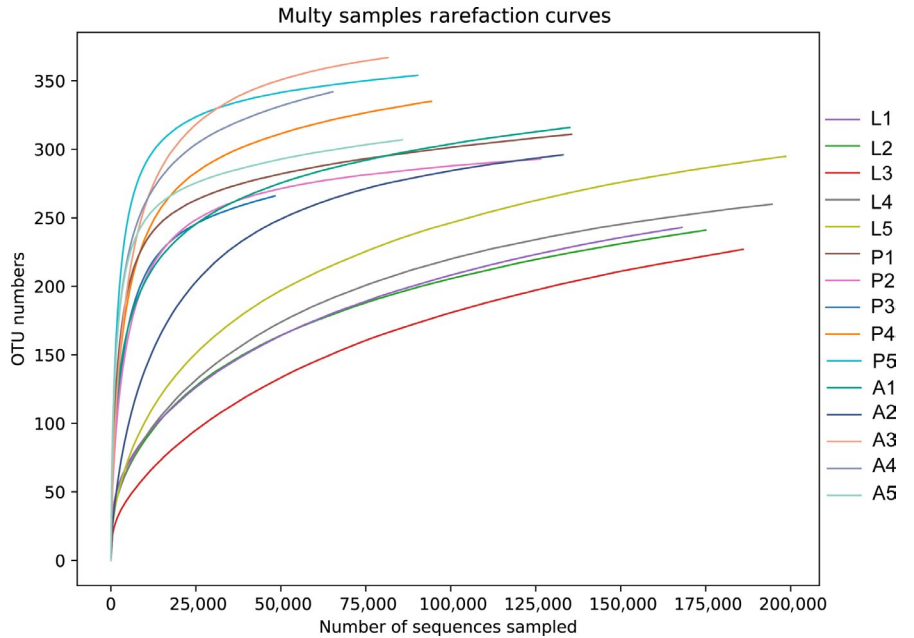


FIGURE A2 Rarefaction curves of all samples used in this study



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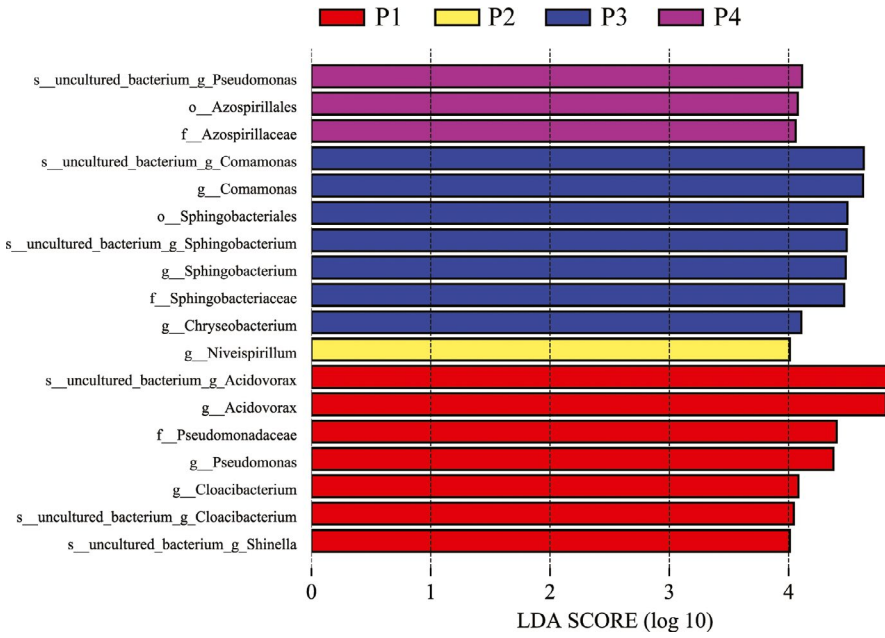


FIGURE A3 Relative abundance of KEGG functional categories and pathways that were significantly enriched in pupae

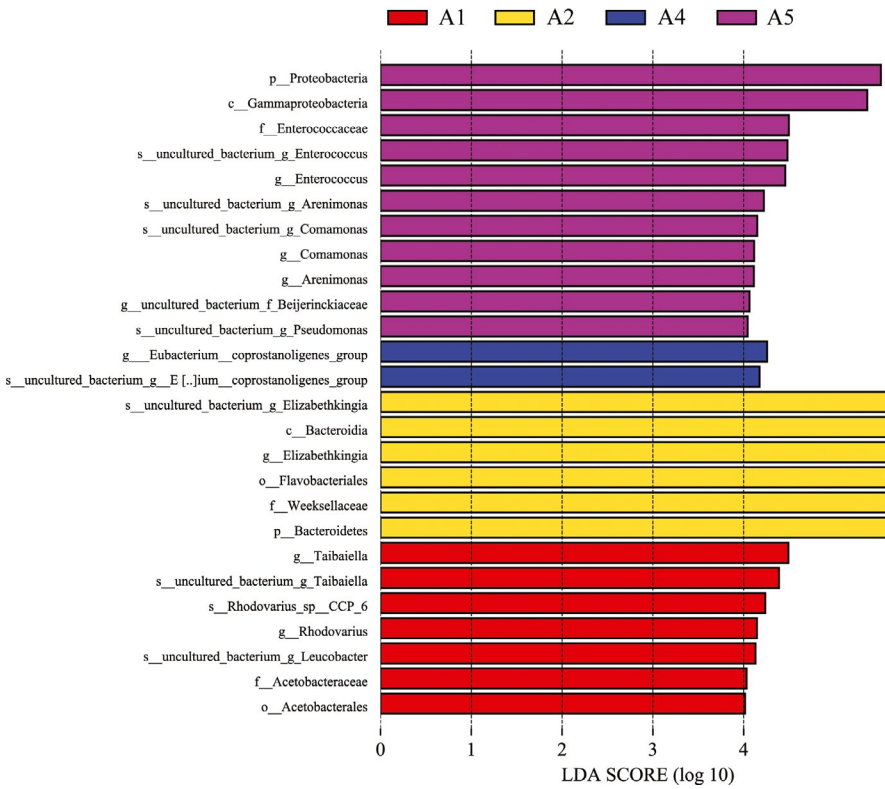


FIGURE A4 Relative abundance of KEGG functional categories and pathways that were significantly enriched in adult female

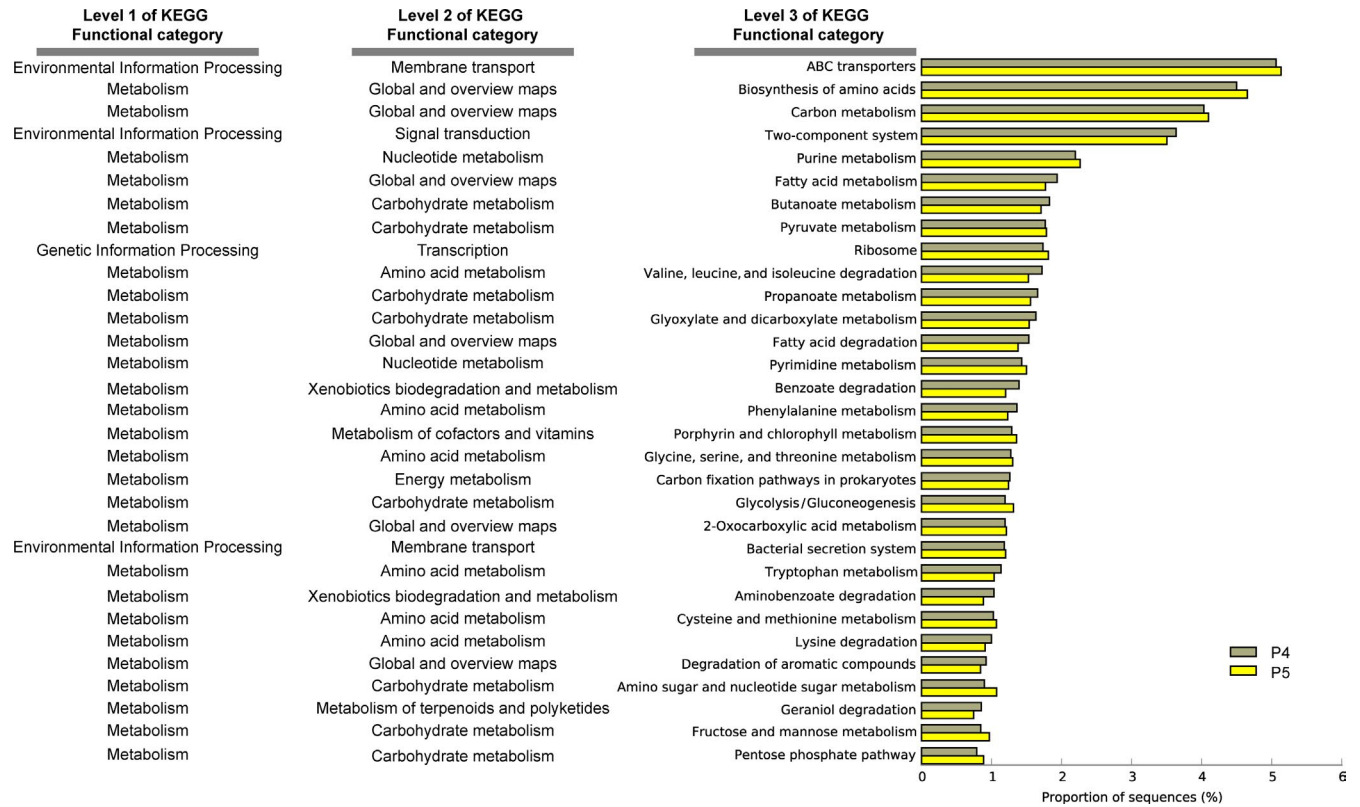


FIGURE A5 Relative abundance of KEGG functional categories and pathways that were significantly enriched in pupae

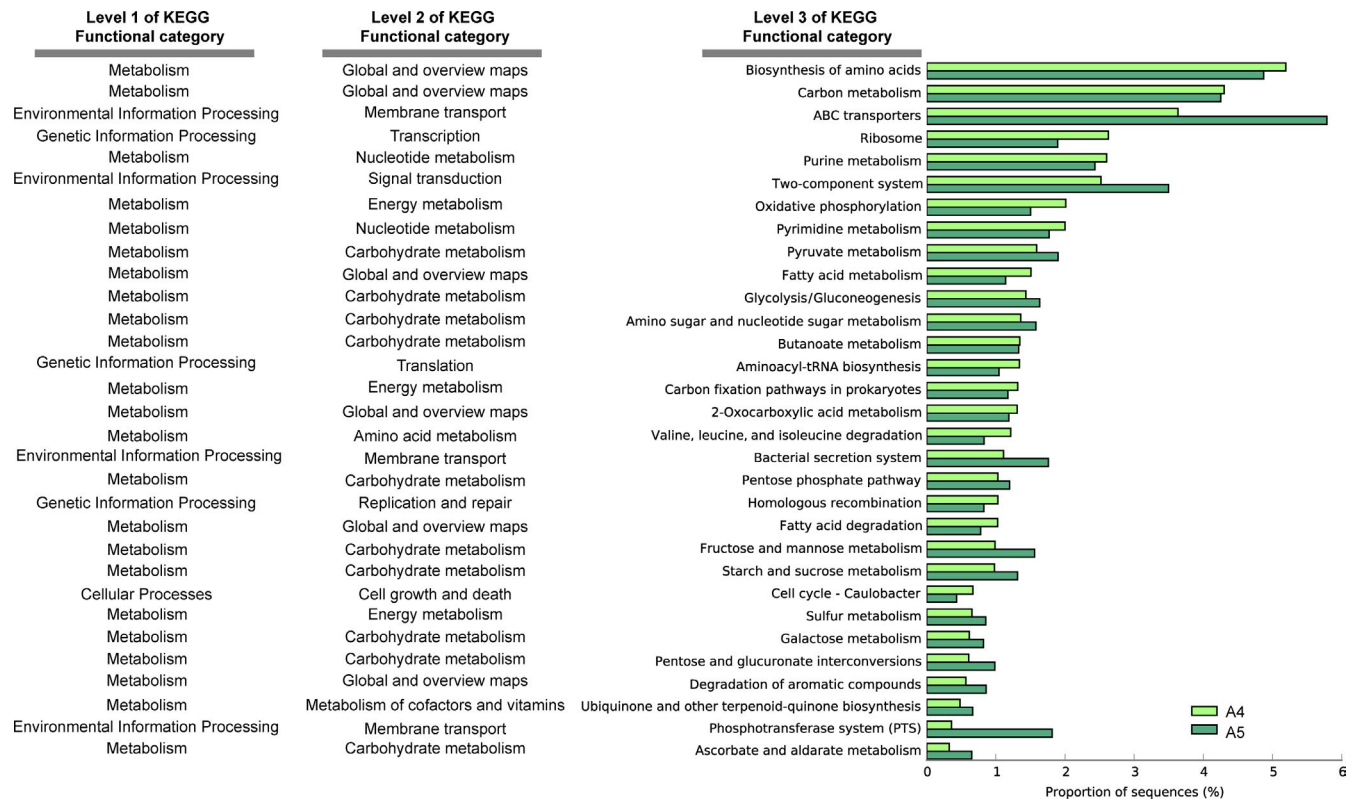


FIGURE A6 Relative abundance of KEGG functional categories and pathways that were significantly enriched in adult female