



Preliminary Study of Bacterial Communities Associated with Native Blowflies in a Primary Malaysian Tropical Rainforest

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Abstract

Blowflies (Diptera: Calliphoridae) fill key ecological niches in carrion decomposition and are widely utilised as indicators in forensic investigations; however, their associations with bacterial communities remain poorly documented in tropical rainforest ecosystems. This preliminary study investigated blowfly diversity and surface-associated bacterial communities in Kuala Keniam, Pahang National Park, Malaysia. Modified minnow traps baited with fish were deployed along a forest transect over six days, yielding 2,276 adult blowflies representing eight calliphorid taxa, predominantly *Chrysomya* species. Bacterial communities associated with fresh and advanced-decay bait, as well as with adult *Chrysomya nigripes* and *Chrysomya villeneuvei*, were characterised using 16S rRNA gene sequencing. Distinct microbial succession patterns were observed across decomposition stages. *Shewanella algae* were prevalent in fresh bait and anaerobic taxa, such as *Bacteroides coprosuis*, becoming more abundant as decay advanced. Blowfly-associated bacterial communities differed from those of the bait and varied among species, with *Providencia rettgeri* dominating in *Chrysomya nigripes* and *Klebsiella oxytoca* prevalent in *Chrysomya villeneuvei*. These results highlight the dynamic insect–microbe interactions of carrion decomposition and provide baseline data for understanding blowfly ecology and associated microbiomes in tropical rainforest environments.

Keywords: Blowfly, Calliphoridae, *Chrysomya nigripes*, *Chrysomya villeneuvei*, microbiome, 16S rRNA, Tropical Rainforest.

1. Introduction

Blowflies (Diptera: Calliphoridae) are universally recognised as the primary colonisers of decomposing remains. They play essential roles in ecology, public health, and forensic science [1]. In forensic entomology, the predictable succession and developmental rates of blowflies make them critical tools for legal investigations. Larval colonisation typically occurs within hours of death, rendering them valuable indicators of the postmortem interval (PMI) [2, 3], while adults are frequently observed on wounds, faeces, and flowers. The accuracy of forensic estimates often depends on understanding the biological and environmental factors that drive insect behaviour. Blowflies inhabit septic environments and have intimate associations with bacterial communities that significantly influence their attraction to, oviposition on, development in, and survival on carrion [4, 5]. Consequently, investigating the specific microbiomes associated with native blowflies not only establishes baseline ecological data but also holds the potential to refine forensic practices by revealing how microbial markers might influence PMI estimations.

Volatile organic compounds (VOCs) produced by bacterial metabolic activity are the primary drivers of blowfly attraction. Gram-negative bacteria, including *Proteus mirabilis*, *Providencia rettgeri*, and *Morganella morganii*, are known to release VOCs that are highly attractive to gravid females, with *Providencia rettgeri*

eliciting stronger responses compared to sterile substrates [6, 7]. Similar findings have been reported for *Lucilia sericata* and *Cochliomyia macellaria*, demonstrating that bacterial cues are critical across diverse blowfly taxa [8, 9]. Furthermore, certain bacteria function as mutualists. For instance, *Proteus mirabilis* can persist throughout blowfly development and produce antimicrobial compounds (mirabilicides) which suppress competing microbes, suggesting a co-adapted relationship between blowflies and bacteria within carrion habitats [10, 11].

Blowflies are also recognised vectors of pathogenic bacteria. In Malaysia, *Chrysomya megacephala* has been reported to harbour coagulase-negative staphylococci (80.27%) and Group D non-enterococcal streptococci (69.05%) [12]. Pathogenic enteric bacteria, such as *Escherichia coli* O157:H7 and *Salmonella typhi*, have been detected at prevalence rates of 3.06% and 20.88%, respectively, alongside other human pathogens, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These complex bacterial communities vary according to geographic location, decomposition stage, and food source. Conventional culture-based methods fail to capture this full diversity; conversely, metagenomic approaches, particularly 16S rRNA gene sequencing, facilitate comprehensive profiling of blowfly microbiomes, including uncultivable taxa and their functional potential [11, 13, 14].

Although blowfly–microbe interactions have been extensively studied in temperate regions, knowledge regarding bacterial communities carried by blowflies in tropical rainforests remains limited. Specifically, data concerning blowflies attracted to decomposing substrates in remote Malaysian rainforest habitats are absent. With the advent of next-generation sequencing technologies, it is now possible to obtain rapid, cost-effective insights into these bacterial populations via culture-independent approaches [15]. Currently, there is no evidence suggesting that blowflies in Kuala Keniam, Pahang National Park, transmit pathogens to humans; however, these native species may serve as vital bioindicators of rainforest ecosystem health. Consequently, this preliminary study seeks to characterise the bacterial communities associated with native blowflies in a Malaysian rainforest, providing novel insights into insect–microbe interactions within a biodiversity hotspot and establishing a foundation for future ecological, forensic, and public health applications.

2. Materials and methods

2.1 Study site

We carried out fieldwork at the UiTM–PERHILITAN Research Station in Kuala Keniam, Pahang National Park, Malaysia (4°31'07"N, 102°28'31"E) (Figure 1). The research permit has been awarded by the Headquarters, Department of Wildlife and National Parks (PERHILITAN), Peninsular Malaysia (Reference number [JPHLTN.600-6/1/4 JLD2 (171)]). The study area is situated at the estuary of the Keniam River (Sungai Keniam), within a lowland dipterocarp forest at elevations ranging from 120 to 300 metres above sea level. The region experiences a tropical rainforest climate, with approximately 2,260 mm of annual rainfall; mean monthly temperatures between 25°C and 37°C; and relative humidity consistently exceeding 80%. These pristine conditions provide an optimal setting for investigating insect–microbe interactions in a high-biodiversity environment.

2.2 Sampling Design

Nine sampling plots were established along a 1-km transect within the Kuala Keniam rainforest (Figure 1C). The transect was positioned along existing ranger and tourist trails in order to facilitate accessibility and ensure safety. All selected plots were located in areas devoid of active human disturbance. A fishbone design was employed for trap placement, with plots spaced at 100-m intervals to minimise overlap in insect activity and ensure adequate spatial coverage. One insect trap was installed in each of the 9 plots. This setup was replicated 3 times to enhance reproducibility and account for local variations in fly activity. The study was conducted over six days, corresponding to the complete decomposition of the 300–350 g fish bait. The objective was to establish a baseline of insect–microbe interactions during one full decomposition cycle, rather than to examine longer-term or seasonal variation, as this was the first preliminary record of blowfly captures using fish-baited traps in the Kuala Keniam Rainforest.

2.3 Blowfly sampling procedures

Modified minnow traps were deployed in each sampling plot (Figure 2). Fresh mackerel (*Scomber scombrus*) weighing 300 to 350 grams was used as bait. Day 0 was designated as the installation date, and sampling was conducted daily from 09:00 to 12:00 until Day 6, by which time the bait had decomposed. Captured flies were carefully removed and immobilised using dry ice in an insulated container for approximately one minute. Following immobilisation, specimens were transferred into sterile plastic bags to prevent contamination. Sampling occurred during the fourth week of October 2021. All procedures adhered to strict aseptic techniques. Sterile

gloves and equipment were used during handling, and traps were disinfected with 70% ethanol after each collection before being returned to their original positions. Specimens were transported on ice to the laboratory and stored at 4°C for further analysis.

2.4 Environmental data collection

Environmental parameters, including ambient temperature (°C) and relative humidity (RH%) (Digital Hygro-Thermo Meter (DHT-1), Daeyoon Scale Industrial Co., Ltd., Seoul, South Korea), along with geographical coordinates (Garmin™ eTrex Handheld GPS), were recorded for each site.

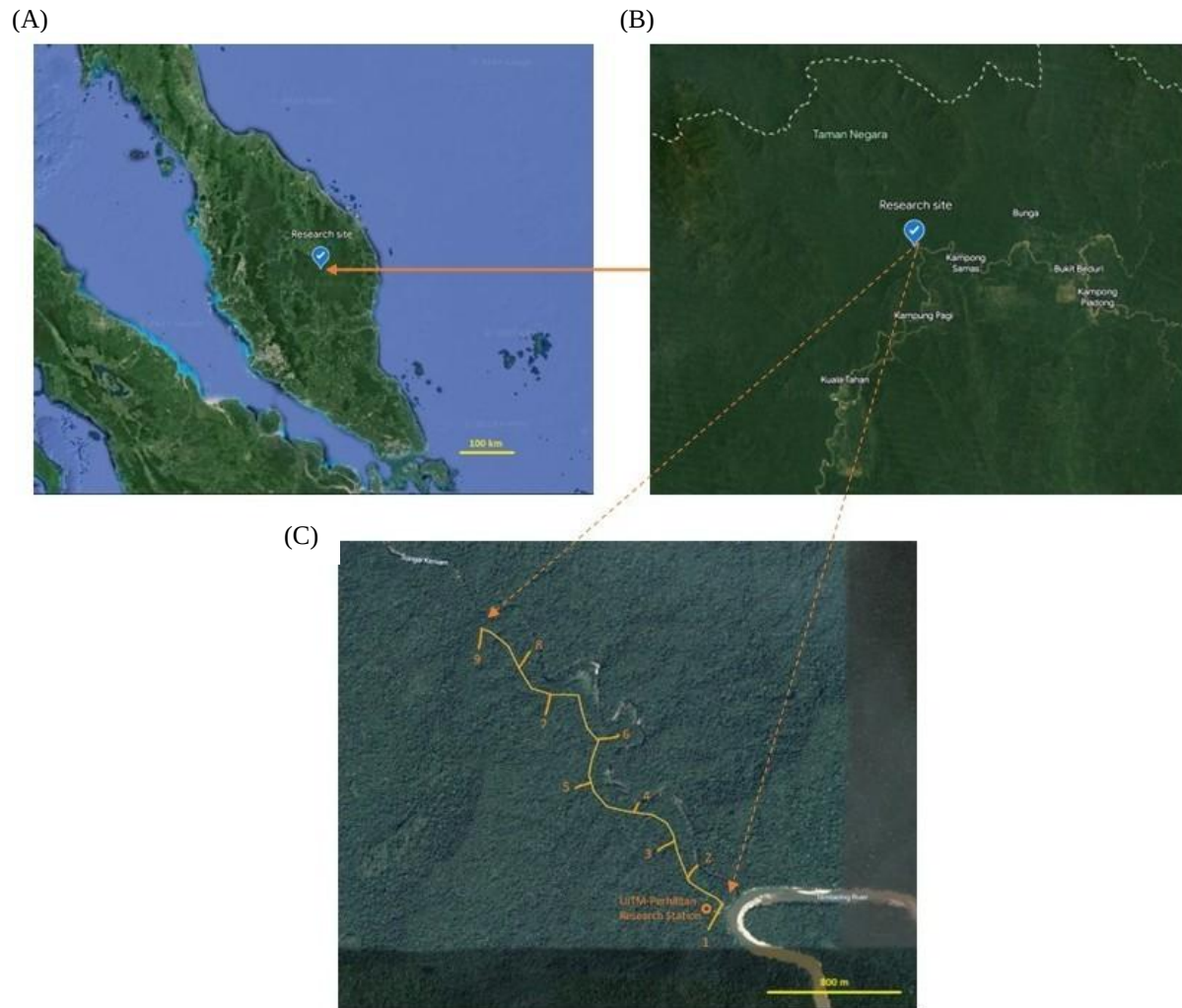


Figure 1 The arrangement of sampling sites and plots at Kuala Keniam Rainforest, Pahang National Park, Malaysia. (A) approximate location in Malaysia, (B) the location of the sampling site, and (C) the distribution of plots for sample collection.



Figure 2 (A) Modified minnow trap suspended and covered with a plastic bag to prevent rain from destroying the sample. (B) A modified minnow trap containing captured blowflies. (C) Schematic diagram of a modified minnow trap.

2.5 Blowfly identification

Blowfly specimens were identified using a dichotomous key adapted from Kurahashi et al. [16]. Uncommon specimens were pinned, dried, and forwarded to experts for taxonomic verification.

2.6 Sample collection for 16S rRNA sequencing

Microbial samples were collected from the surface of fresh fish bait on Day 0 (labelled FB) using sterile, pre-moistened cotton swabs. The bait surface was swabbed firmly for 5 seconds in a unidirectional manner, and the swab was immediately transferred to a vial containing 1 ml of stabilising solution (DNA/RNA Shield™, Zymo Research, USA). To avoid cross-contamination, fresh gloves and swabs were used for each sample. Three adult *Chrysomya nigripes* specimens collected on Day 1 (labelled CNFB) and three adult *Chrysomya villeneuvi* specimens collected on Day 4 (labelled CVADB) were transferred into sterile 2.0 mL microcentrifuge tubes containing 1.5 mL of DNA/RNA Shield™. To specifically acquire the surface microbiome, intact flies were fully submerged in the reagent. This soaking method effectively washes microbial cells from the fly's exoskeleton directly into the solution, where the reagent immediately lyses them and stabilises the released nucleic acids. This

preservation method ensured the recovery of high-quality DNA without requiring immediate refrigeration. All tubes were maintained at temperatures below 20°C until delivery to the biotechnology facility.

2.7 Metagenomics 16S rRNA sequencing

Specimens were submitted to AGTC Genomics Sdn. Bhd. (Kuala Lumpur) for metagenomic analysis. Genomic DNA was extracted using the Quick-DNA™ MagBead Plus Kit (Zymo Research, USA). The 16S rRNA gene was amplified using universal bacterial primers targeting the V3–V4 region, incorporating Illumina overhang adapters [17]. PCR amplicons were purified using AMPure XP beads (Beckman Coulter, USA), followed by a second PCR step to attach Illumina indices. Libraries were normalised to 4 nM, pooled, and sequenced on the Illumina MiSeq platform (paired-end 250 bp reads), yielding approximately 100,000 sequences per sample. Raw reads were processed using the CLC Genomics Workbench pipeline (Qiagen, Germany). Operational Taxonomic Unit (OTU) clustering was performed at 97% identity. The resulting OTU table was analysed against the Greengenes database [18].

2.8 Decomposition Stages of Fish Bait

Decomposition phases were defined using indicators adapted from standard forensic entomology frameworks [15]. These stages (fresh, bloated, active decay, and advanced decay) have been adapted for use with fish bait to ensure consistent sampling and to provide a reproducible framework for categorising bait condition. Table 1 summarises the stages.

Table 1 Description of the decomposition stages of fish bait

Stages	Description
Fresh	The initial stage is immediately after the fish defrosts. The skin is glossy, the scales are intact, and the eyes are clear.
Bloated	As bacterial activity increases, gases, mainly carbon dioxide and methane, are produced as byproducts of microbial metabolism. These gases cause the fish's body to bloat.
Active decay	The fish's body begins to break down rapidly. The abdomen may rupture due to gas accumulation, and the skin may become discoloured and slough off. The fish's flesh softens, and a strong odour of decay becomes noticeable.
Advanced decay	During this stage, the fish's tissues continue to degrade. The flesh becomes mushy and disintegrates, bones become more exposed, and the internal organs may be barely recognisable. The pungent odour of decay persists.

3. Results

3.1 Decomposition stages of fish

Fish decomposition progressed through four distinct stages: fresh, bloated, active decay, and advanced decay. The duration of each stage was observed as follows: fresh (day 0), bloated (day 1), active decay (days 2–3), and advanced decay (days 4–6) (Figure 3). The absence of odour, glossy skin, and clear eyes characterised the fresh stage. In the bloated stage, bait size increased slightly due to the production of fermentation gases. During active decay, the abdomen ruptured, and the skin became discoloured. A persistent, strong odour, soft disintegrating flesh, and exposed bones identified advanced decay.

3.2 Weather data

Between Oct 29 and Nov 2, 2021, rainfall was intermittent at the UiTM–PERHILITAN Research Station in Kuala Keniam, Pahang National Park, Malaysia, with 7.5 mm on Oct 31 and 6.8 mm on Nov 1. On other days, rainfall was minimal. Mean daily temperatures ranged from 26.5–27.8°C, and relative humidity stayed high (85–95%). These conditions reflect a warm, moist environment favourable for decomposition processes and insect colonisation.



Figure 3 Decomposition stages of fish bait: (A) Fresh (Day 0); intact morphology, clear eyes, no odour; (B) Bloated (Day 1); abdominal distension from fermentation gases; (C) Active decay (Days 2-3); abdomen ruptures, skin discolours and sloughs, strong odour; (D) Advanced decay (Days 4-6); mushy flesh, exposed bones, loss of internal organs.

3.3 Blowfly abundance during the decomposition of fish

A total of 2,276 adult blowflies were collected across the nine plots (Table 2). This dataset represents the first record of blowfly captures using fish-baited traps in this location. The assemblage was dominated by *Chrysomya defixa* (1,841 individuals; ~81%), which was present across all decomposition stages. *Phumosiya promittens* (203; 9%) and *Chrysomya villeneuvi* (130; 6%) were also regularly associated with the bait. Smaller numbers of *Hemipyrellia ligurriens* (55; 2%), *Chrysomya nigripes* (27; 1%), and *Chrysomya rufifacies* (17; <1%) were recorded. Rare taxa included *Hypopigiopsis fumipennis* and *Silbomyia* sp. Daily abundance patterns revealed low captures during the bloated stage (Day 1); a sharp increase during active decay (Days 2–3); a peak on Day 5 (advanced decay); and a subsequent decline on Day 6. These findings underscore the dominance of *Chrysomya defixa* in colonising decomposing fish bait in the Kuala Keniam Rainforest.

Table 2 List of blowflies (Calliphoridae) species collected at nine plots in Kuala Keniam Rainforest, Pahang National Park, Malaysia.

Species	Frequency
<i>Chrysomya defixa</i>	1,841
<i>Phumosiya promittens</i>	203
<i>Chrysomya villeneuvi</i>	130
<i>Chrysomya nigripes</i>	27
<i>Chrysomya rufifacies</i>	17
<i>Hypopigiopsis fumipennis</i>	2
<i>Hemipyrellia ligurriens</i>	55
<i>Silbomyia</i> sp.	1
Total	2,276

Daily abundance patterns (Figure 4) showed low captures during the bloated stage (Day 1) and a sharp increase during active decay (Days 2–3). A sudden decline in captures was observed on Day 4, coinciding with a heavy rain downpour on the night of Day 3 that temporarily suppressed blowfly flight activity and foraging behaviour. Abundance subsequently peaked on Day 5 during advanced decay, then declined on Day 6. The variance observed between the three replicates is likely attributable to localised differences in canopy cover across the plots, which influenced how heavily the rain impacted individual traps and how quickly volatile organic compound (VOC) plumes recovered the following day. These findings highlight the dominance of *Chrysomya defixa* in colonising decomposing fish bait. This is first entomological baseline for blowfly community structure in the Kuala Keniam Rainforest.

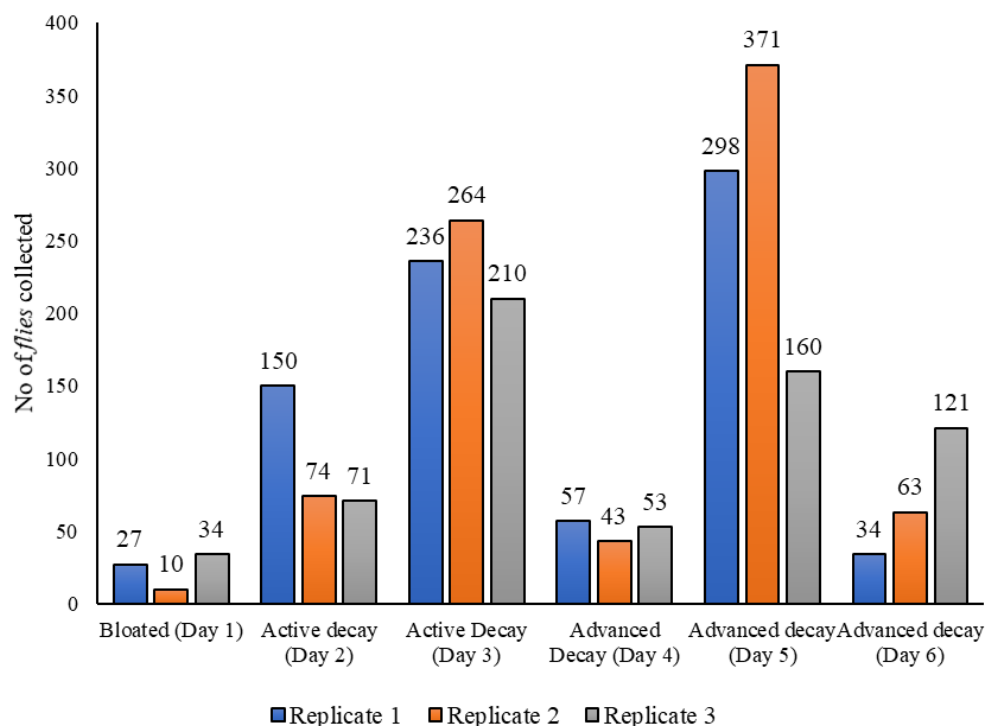


Figure 4 Blowfly abundance over six days of fish bait decomposition in the Kuala Keniam Rainforest, Pahang National Park, Malaysia. Daily numbers of blowflies captured across three replicates, showing peaks during active decay (Day 3) and advanced decay (Day 5)

3.4 Surface-associated bacterial communities of *Chrysomya nigripes*, *Chrysomya villeneuvei*, and fish bait

On Day 1 (Fresh Bait), *Chrysomya nigripes* was the most frequent species collected at Plot 7. It was thus selected to represent the early coloniser. By Day 4 (Advanced Decay) (Table 3), the catch shifted, with *Chrysomya villeneuvei* first appearing. This species was selected to represent the advanced decay stage due to its documented association with decomposed tissues [20]. Bacterial community profiles revealed clear distinctions between the bait samples and blowfly specimens. Concurrent bacterial species were analysed. *Providencia rettgeri* was prevalent in both fresh and advanced-decomposition bait and was also dominant on *Chrysomya nigripes* during the fresh phase. In the advanced phase, *Klebsiella oxytoca* was the dominant species on *Chrysomya villeneuvei* but was not detected in other samples. Venn diagram analysis indicates partial transfer of bacteria from the bait to the insect. For *Chrysomya villeneuvei*, only 12% of bacterial species were shared with the bait, suggesting that it carries a distinct community which is potentially influenced by its own microbiota or environmental exposure.

Table 3 Counts of adult blowflies captured at Plot 7 during fresh bait (Day 1, FB) and advanced decay bait (Day 4, ADB)

Species	Day 1 (FB)	Day 4 (ADB)
<i>Phumosi promittens</i> .	2	13
<i>Chrysomya villeneuvei</i>	0	7
<i>Chrysomya nigripes</i>	17	1
<i>Chrysomya rufifacies</i>	14	0
<i>Hypopigiopsis fumipennis</i>	1	0
Total	34	21

The selection of Plot 7 and these two species reflects how blowflies' bacterial communities respond to decomposition stages in a tropical forest setting, with *Chrysomya nigripes* marking early colonisation and *Chrysomya villeneuvei* linked to advanced decay. The diversity levels of bacteria on each of *Chrysomya villeneuvei* and *Chrysomya nigripes*, during both the fresh and advanced decay phases, are shown in Figure 5.

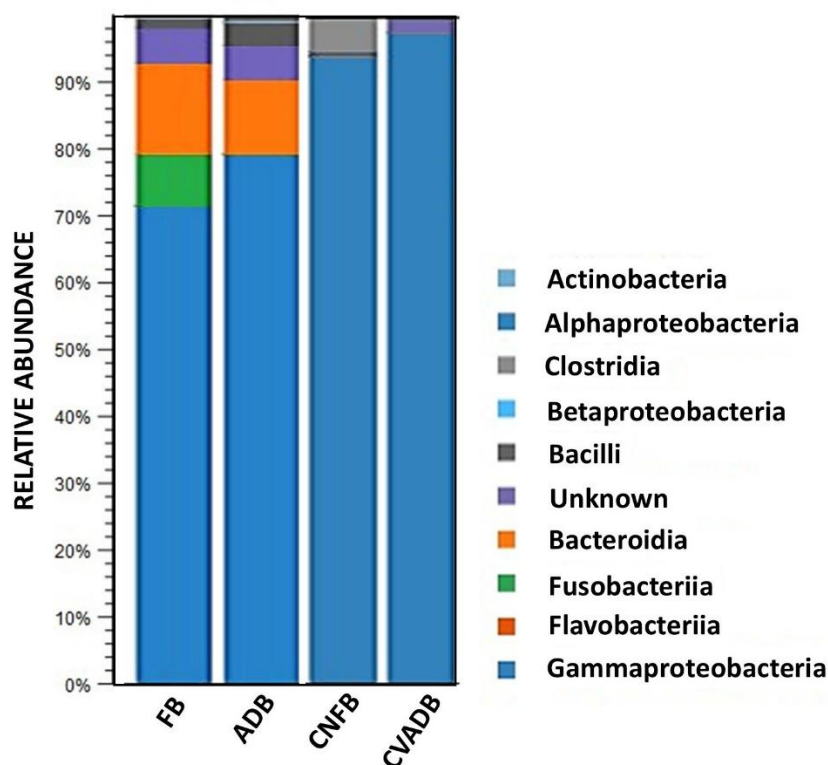


Figure 5 Relative abundance (%) of bacterial classes identified through 16S rRNA sequencing across four sample types: fresh bait (FB), advanced decay bait (ADB), *Chrysomya nigripes* collected from fresh bait (CNFB), and *Chrysomya villeneuvei* collected from advanced decay bait (CVADB). Colour variations correspond to distinct bacterial classes as indicated in the legend, highlighting the dominance of Gammaproteobacteria under all conditions.

The bacterial community profiles from 16S rRNA sequencing showed clear differences between bait samples and blowfly specimens. The bacterial community diversity on flies' surfaces is lower than that on bait samples. In the fresh bait (FB) sample, the dominant bacterial class was Gammaproteobacteria, followed by Fusobacteria, Bacteroidia, and Bacilli. In the advanced decay bait (ADB), Gammaproteobacteria remained dominant, but there was a noticeable increase in Bacteroidia and Bacilli. These are commonly associated with organic decomposition and tissue breakdown.

The bacterial classes identified through 16S rRNA sequencing for *Chrysomya nigripes* collected from fresh bait (CNFB) and *Chrysomya villeneuvei* collected from advanced decay bait (CVADB), represented by the third and fourth bars from the left, respectively, show reduced taxonomic richness compared to the diverse communities found in bait alone. Both the CNFB and CVADB samples are overwhelmingly dominated by a single bacterial class: Gammaproteobacteria. In the CNFB profile, Gammaproteobacteria constitute approximately 95% of the total microbial community, with a small secondary presence of the class Clostridia. Similarly, the CVADB profile shows an even higher relative abundance of Gammaproteobacteria (exceeding 95%), with the remaining minimal fraction categorised as an unknown Class. Notably, the taxa primarily responsible for active tissue breakdown and anaerobic putrefaction in the bait samples, such as Bacteroidia, Fusobacteriia, and Bacilli, are entirely absent or below the threshold of detection in the insect samples.

The list of concurrent bacteria species in the bait and blowfly species was tabulated in Table 4. *Providencia rettgeri* was the most prevalent species in both fresh and advanced-decomposition fish bait. The same species was shown to be prevalent in bait and in the external parts of *Chrysomya nigripes* during the fresh decomposition phase. In the advanced decomposition phase, three unknown bacteria were found on the bait and on the exterior of *Chrysomya villeneuvei*, among which an unidentified Enterobacteriaceae was the most dominant species. Meanwhile, *Klebsiella oxytoca* was the dominant species on the external surface of *Chrysomya villeneuvei*, but was not detected in other samples.

Table 4 List of overlapping bacterial taxa. The species are ordered from most abundant to least abundant.

FB $\cap$ ADB	FB $\cap$ CNFB	ADB $\cap$ CVADB
1. <i>Providencia rettgeri</i>	1. <i>Providencia rettgeri</i>	1. <i>Enterobacteriaceae</i> (Unidentified)
2. <i>Bacteroides coprosuis</i>	2. <i>Proteus columbae</i>	2. <i>Bacteria</i> (Unidentified)
3. <i>Enterococcus timonensis</i>	3. <i>Providencia alcalifaciens</i>	3. <i>Firmicutes</i> (Unidentified)
4. <i>Proteus columbae</i>	4. <i>Enterobacteriaceae</i> (Unidentified)	
5. <i>Enterobacteriaceae</i> (Unidentified)	5. <i>Bacteria</i> (Unidentified)	
6. <i>Bacteria</i> (Unidentified)		
7. <i>Streptococcus pneumoniae</i>		

In Plot 7, bacterial species detected on fresh bait (FB) and advanced decay bait (ADB) were compared using a Venn diagram. Each bait type harboured six unique bacterial species (31.6%), while seven species (36.8%) were shared between the two conditions. This overlap suggests that certain bacteria persist throughout the decomposition process, while others are specific to either the fresh or advanced decay stage (Figure 6A). A second comparison was made between fresh bait (FB) and *Chrysomya nigripes* collected from the same bait, CNFB. The bait had seven unique species (41.2%), while the blowfly had four unique species (23.5%), and six species (35.3%) were shared. This indicates partial transfer of bacteria from bait to insect, with some species remaining specific to either the bait surface or the blowfly (Figure 6B).

The third comparison involved advanced decay bait (ADB) and *Chrysomya villeneuvei* collected from the same bait, namely CVADB. The bait sample contained 10 unique bacterial species (40%), while the blowfly contained 12 unique species (48%). Only three species (12%) were shared. This low overlap suggests that *Chrysomya villeneuvei* harbours a distinct bacterial community from the bait surface, as possibly influenced by its own microbiota or environmental exposure (Figure 6C). The diagrams highlight shared and unique bacterial species, reflecting microbial succession across bait conditions and partial transfer or divergence of bacterial communities between bait and blowflies. The study revealed that *Shewanella algae* was the dominant species in the early stage of fish decomposition, whereas *Bacteroides coprosuis* predominated in the advanced decay stage.

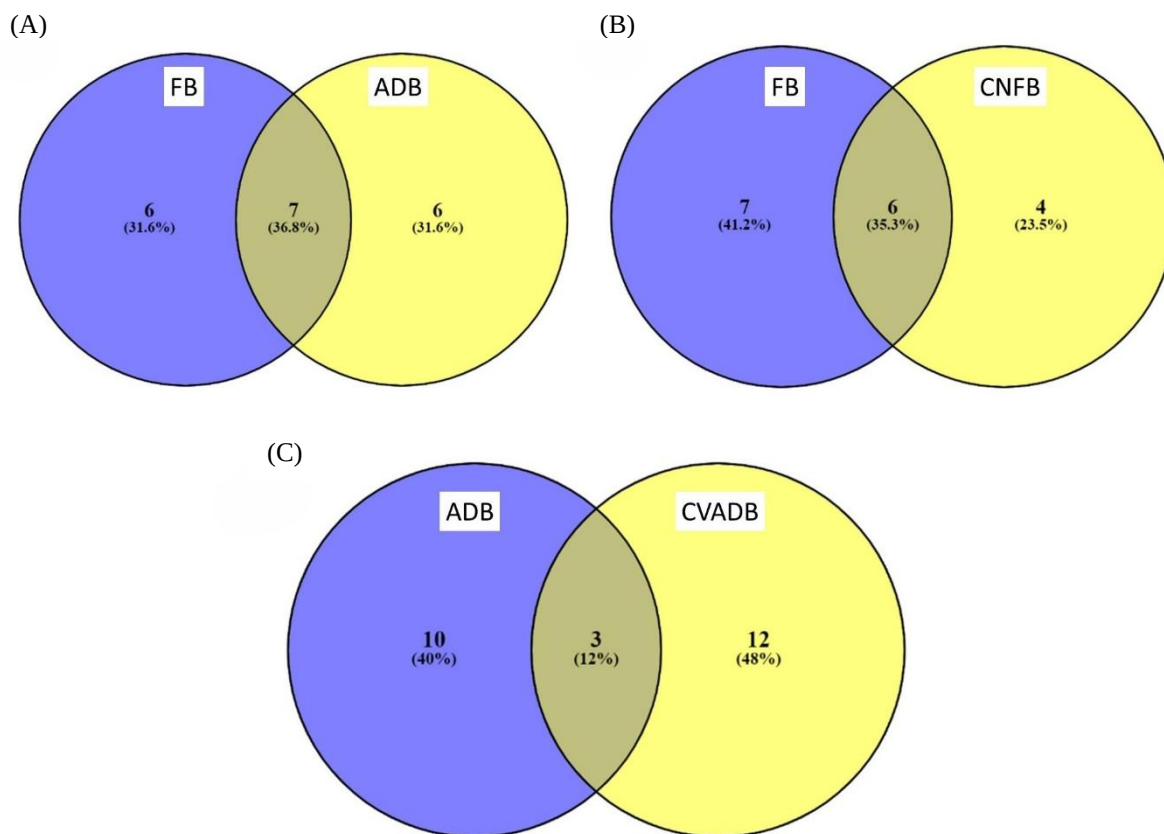


Figure 6. Venn diagrams showing the commonalities and the number of bacteria species detected. A: fresh bait (FB) and advanced decay bait (ADB); B: fresh bait (FB) and *Chrysomya nigripes* collected from fresh bait (CNFB) C: advanced decay bait (ADB) and *Chrysomya villeneuvei* collected from advanced decay bait (CVADB). The diagrams highlight shared and unique bacterial species, reflecting microbial succession across bait conditions and partial transfer or divergence of bacterial communities between bait and blowflies.

4. Discussion

This study provides integrative insights into the ecological relationships between blowfly assemblages and their surface-associated bacterial communities in a low-disturbance tropical rainforest. The results demonstrate that blowfly species composition and bacterial community structure are jointly shaped by the decomposition stage, reflecting dynamic insect–microbe interactions [21, 22].

4.1 Blowfly assemblage structure and ecological implications

The overwhelming dominance of *Chrysomya* species, particularly *Chrysomya defixa*, aligns with patterns reported in other tropical forest systems [16, 23]. While *Chrysomya megacephala* and *Chrysomya rufifacies* are frequently highlighted in regional studies [24], the prevalence of *Chrysomya defixa* in this study suggests that it plays a critical ecological role in nutrient recycling of rainforest carrion. The peak in abundance during active and early advanced decay aligns with classical successional models [2, 20] and is likely driven by the release of VOCs from intense microbial metabolism [8, 25].

4.2 Blowfly-associated bacterial communities and insect–microbe interactions

Bacterial communities on adult blowflies differed from those found on bait surfaces, indicating that adults are not merely passive vectors [1]. The extreme dominance of Gammaproteobacteria on the external surfaces of both CNFB and CVADB, as depicted in Figure 5, challenges the assumption that a blowfly's exterior microbiome is merely a random, passive reflection of the environment it lands on.

While the bait sources host a highly diverse necrobiome, the blowfly exoskeleton acts as a highly selective interface. Members of the class Gammaproteobacteria, which includes genera like *Ignatzschineria*, *Providencia*, and *Psychrobacter*, possess a well-documented, specific affinity for the blowfly exoskeleton. These bacteria are highly adapted to adhere to the complex morphological structures of the fly's surface, such as microvilli, setae, and sensory hairs on the legs and wings, which facilitate intense microbial retention. Conversely, *Chrysomya villeneuvei* carried a distinct assemblage dominated by *Klebsiella oxytoca*. The exclusive presence of *Klebsiella oxytoca* on the flies suggests acquisition from alternative reservoirs or maintenance within a selective microbiome. Similar patterns of independent bacterial persistence have been observed in other calliphorids [10, 13].

The study identified *Shewanella algae* as dominant in fresh bait and *Bacteroides coprosuis* in advanced decay. *Shewanella algae* is common in aquatic environments [26], while *Bacteroides coprosuis*, an anaerobe, is associated with late-stage decomposition [27]. *Providencia rettgeri*, an opportunistic pathogen [28], was dominant on *Chrysomya nigripes*. *Klebsiella oxytoca*, found on *Chrysomya villeneuvei*, is a known nosocomial pathogen [29] and may play a role in larval development [30].

4.3 Forensic and public health relevance

The observed coupling between blowfly species and bacterial communities underscores the importance of microbial partners in shaping insect behaviour. These reciprocal interactions are critical to forensic applications, where incorporating microbial data may enhance Post Mortem Interval (PMI) estimation [6, 25]. Furthermore, the detection of opportunistic pathogens such as *Providencia* and *Klebsiella* highlights the potential role of blowflies as mechanical vectors at the wildlife–human interface [1, 15]. These concerns may increase as human activity in these ecosystems increases.

4.4 Limitations and future research directions

This preliminary investigation has been limited by sample size, its short temporal scope (a single six-day decomposition event), and its focus on surface-associated bacteria. Consequently, these findings may not fully represent blowfly and microbial community dynamics across different seasons or periods of extreme weather. Future research should include greater temporal replication across wet and dry seasons and analysis of the internal microbiome. Nevertheless, this study does represent the first combined assessment of blowfly assemblages and bacteria in an undisturbed Malaysian rainforest. It establishes a vital baseline for regional forensic and public health modelling.

5. Conclusions

This study provides the first baseline data on blowfly-associated bacterial communities in tropical rainforest environments, particularly in Kuala Keniam, Pahang National Park, Malaysia. We demonstrated that blowfly species differ in their microbiota across decomposition stages, with *Chrysomya nigripes* associated with fresh bait

and *Chrysomya villeneuvei* with advanced decay. Successional changes in bait microbiota, from *Shewanella algae* in early stages to *Bacteroides coprosuis* in advanced decay, were mirrored by shifts in blowfly-associated bacteria. The consistent presence of *Providencia rettgeri* across bait and blowflies highlights its ecological importance in carrion breakdown and insect attraction. By integrating 16S rRNA sequencing, this preliminary study has revealed that dominant blowfly species in the Kuala Keniam rainforest harbour diverse, stage-dependent bacterial successions, including niche-specific pathogens. These findings underscore the intricate insect–microbe synergy driving tropical decomposition and establish a critical baseline for regional forensic and public health modelling. Future longitudinal research across diverse habitats and seasons To strengthen these reference datasets and elucidate the complex dynamics of native blowfly microbiomes in Malaysia’s primary rainforests.

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7. Author Contributions

Azwandi Ahmad: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualisation, Writing – original draft, Writing – review and editing.; Ahmad Azani Othman: Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review and editing.; Muhammad Syafiq bin Mohd Nasarudin: Formal analysis, Investigation, Software, Writing – review and editing.; Mohamad Faiz Foong Abdullah: Investigation, Methodology, Supervision, Writing – review and editing.; Mohamad Azizi: Investigation, Resources.

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