Biotechnology Reports 44 (2024) e00857

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Interaction and effects of temperature preference under a controlled environment on the diversity and abundance of the microbiome in *Lutzomyia longipalpis* (Diptera: Psychodidae)

Daniela Duque-Granda [a], Rafael José Vivero-Gómez [a,1,*], Howard Junca [b], Gloria Cadavid-Restrepo [a], Claudia Ximena Moreno-Herrera [a,1]

[a] *Grupo de Microbiodiversidad y Bioprospección, Laboratorio de Procesos Moleculares, Laboratorio de Biología Celular y Molecular, Universidad Nacional de Colombia sede Medellín, Street 59A #63-20, Medellín 050003, Colombia*

[b] *RG Microbial Ecology: Metabolism, Genomics & Evolution, Div. Ecogenomics & Holobionts, Microbiomas Foundation, LT11A, 250008, Chia, Colombia*

ARTICLE INFO

Keywords:
Sand flies
Thermocline-type device
Microbiota
Bacillus
Pseudomonas
Endosymbionts

ABSTRACT

Characterization of the temperature effects on the abundance and richness of the microbiota of *Lutzomyia longipalpis*, insect vector of *Leishmania infantum* in America, is an aspect of pivotal importance to understand the interactions between temperature, bacteria, and *Leishmania* infection. We developed and used a customized device with a temperature gradient (21–34 °C) to assess the temperature preferences of wild females of *Lu. longipalpis* collected in a rural area (Ricaurte, Cundinamarca, Colombia). Each replicate consisted of 50 females exposed to the gradient for an hour. At the end of the exposure time, insects were collected and separated by the temperature ranges selected varying from 21 °C to 34 °C. They were organized in 17 pools from which total DNA extracts were obtained, and samples were subjected to 16S rRNA amplicon sequencing analyzes. The most abundant phyla across the different temperature ranges were Proteobacteria (17.22–90.73 %), Firmicutes (5.99–77.21 %) and Actinobacteria (1.56–59.85 %). Results also showed an abundance (30 % to 57.36 %) of *Pseudomonas* (mainly at temperatures of 21–29 °C and 34 °C) that decreased to 6.55 %-13.20 % at temperatures of 31–33 °C, while *Bacillus* increase its abundance to 67.24 % at 29–33 °C. *Serratia* also had a greater representation (49.79 %), specifically in sand flies recovered at 25–27 °C. No significant differences were found at α-diversity level when comparing richness using the Shannon-Wiener, Simpson, and Chao1 indices, while *β*-diversity differences were found using the Bray-Curtis index (F-value of 3.5073, p-value < 0.013, R-squared of 0,4889), especially in the groups of *Lu. longipalpis* associated at higher temperatures (29–33 °C). It was also possible to detect the presence of endosymbionts such as *Spiroplasma* and *Arsenophonus* in the range of 29–33 °C. *Rickettsia* was only detected in *Lu. longipalpis* sand flies recovered between 25–27 °C. It was possible to characterize *Lu. longipalpis* microbiota in response to intraspecific temperature preferences and observe changes in bacterial communities and endosymbionts at different ranges of said environmental variable, which may be important in its vector competence and environmental plasticity to adapt to new climate change scenarios.

1. Introduction

Leishmaniasis is a group of disease forms caused by a diversity of parasites of the genus *Leishmania* that are transmitted by the bite of infected female sand flies of the subfamily Phlebotominae. Leishmaniasis epidemiology is influenced by characteristics of the parasite species and the sand fly vector, and also by the socioeconomic conditions of the population under exposure, such as poverty. In addition, the ecological characteristics and climatic conditions, current exacerbating changes as strong alterations in land use, human migration patterns, accelerated deforestation, and temperature variability, are quite important factors for its spreading [1–3]. In the year 2023, the total number of leishmaniasis cases in Colombia was 4162, where the calculated incidence was of 42.89 and 0.85 cases per 100,000 inhabitants for cutaneous and mucocutaneous leishmaniasis, respectively, while peasants and members of the military were the most affected population groups [4,5].

* Corresponding author.
E-mail addresses: rjviverog@unal.edu.co (R.J. Vivero-Gómez), cxmoreno@unal.edu.co (C.X. Moreno-Herrera).
[1] Claudia Ximena Moreno Herrera and Rafael José Vivero Gómez contributed equally as corresponding authors.

https://doi.org/10.1016/j.btre.2024.e00857
Received 15 January 2024; Received in revised form 5 September 2024; Accepted 8 September 2024
Available online 12 September 2024

Additionally, two species of phlebotomine sand flies, *Pintomyia evansi* and *Lutzomyia longipalpis*, have been reported in the country to be naturally infected with *Leishmania infantum*, a parasite associated with visceral leishmaniasis [6].

Lutzomyia longipalpis is a species complex [7] that is distributed in the Americas, from Mexico to Argentina, in arid and semi-arid areas, tropical dry forests, and very humid environments such as the Amazon River drainage, and is considered the main vector of *L. infantum* in the region [8,9]. As prevention and control strategies for this vector, and in general for phlebotomine sand flies, the World Health Organization recommends the use of protective clothing, repellents, insecticide-treated nets, control of reservoir hosts, collaboration among the stakeholders, and education of the community, while the implementation of such strategies in Colombia also include community education in the risk areas and community-based surveillance [4,10] as there are still no vaccines against leishmaniasis that are effective in humans [11].

Environmental impacts caused by climate change influence the distribution and survival of sand flies [12], and climate change can have a significant effect on the development of parasite promastigotes [13] which favors their transmission in new territories [10]. Temperature is a key factor for the extrinsic incubation period (EIP) required for parasites to develop within the vector, and in other insects, a simulation study by Kamiya et al. [14] suggests that dengue vectors with short EIPs enjoy a fitness advantage through increased incubation success even outside the expected temperature range of dengue transmission.

However, the relationship between temperature, parasites, and insect vectors is more complex, as insect microbiota also plays a role in the defense mechanisms against pathogens, affecting the vectorial capacity of insects. For example, there is a reduction in the number of *Lu. longipalpis* females infected with *Leishmania mexicana* when they are fed a sugar meal containing microorganisms isolated from the midgut [15].

Endosymbiont bacteria have the potential to modify temperature preferences in insects as observed for *Drosophila melanogaster* infected with various strains of *Wolbachia* [16,17] that may in turn influence the distribution of insects in a climate change scenario. Moghadam et al. [18] have shown that the developmental temperature had a significant influence on the richness of the microbiota in *D. melanogaster* subjected to low (13 °C) and high (23 °C and 31 °C) developmental temperatures. Onyango et al. [19] revealed a significant dissimilarity in mid-gut microbiome in *Aedes aegypti* mosquitoes reared at high (32 °C day/28 °C night) and low (30 °C day/26 °C night) diurnal temperature regimes, with an enrichment of *Bacillus subtilis, Acidovorax citrulli* and *Pseudomonas aeruginosa* associated with increase in temperature.

Studies on the effect of temperature *in Lu. longipalpis* have focused on the growth and longevity [20], and daily activity rhythms [21] in laboratory reared colonies suggest an optimum development for this species between 20–28 °C [20]. Martins et al., [22] also explored the interaction of *Lu. longipalpis* among temperature and the gene expression of heat shock proteins, blood ingestion, and infection across a thermal gradient (18–30 °C). The authors have suggested that the behavior of *Lu. longipalpis* is modulated to avoid temperature induced physiological damage during the gonotrophic cycle [22].

There are also studies depicting the microbiota associated with this sand fly species, specifically *Pseudomonadaceae, Enterobacteriaceae* and *Acetobacteraceae* [23,24]. Moreover, the diversity of bacterial genera encompasses from *Acinetobacter, Burkholderia, Pseudomonas, Enterobacter, Klebsiella, Serratia, Pantoea* to some endosymionts [25]. However, to the best of our knowledge there are no studies describing the relationship between temperature and *Lu. longipalpis* microbiota, and dynamics under fluctuations in ambient temperature, especially the microclimatic changes they undergo under natural conditions.

Studies on temperature variability, microbiomes and vectors are important in the current context. For example, the "tropicalization" of the climate in temperate regions promotes the emergence of Vector-borne diseases (VBDs) in areas previously unfavorable for them [26]. Climate variations and extreme events have profound impacts on infectious diseases. Infectious agents (protozoa, bacteria and viruses) and their associated vectors (as sand flies), lack thermostatic mechanisms and reproduction and survival rates are strongly influenced by variations in temperature [27].

Viewing the realistic temperature fluctuations in nature, those studies relying solely on a constant temperature may reflect inconsistent effects on the midgut microbiome. This gap in knowledge is highly important given the well documented role of the midgut microbiome in vectorial capacity. Therefore, we aimed to characterize the composition and structure of the bacterial microbiome in wild specimens of *Lu. longipalpis* associated with the temperature preferences of the sand flies using a custom device equipped with a temperature gradient and 16S rRNA amplicon sequencing profiling.

2. Materials and methods

2.1. Collection of samples

Lu. longipalpis used in the experiments were collected from the municipality of Ricaurte, Department of Cundinamarca, Colombia, during 2020–2021 using Shannon and CDC light traps at periods of high precipitation. The site is located in a rural area of the Andean region, at 248 m above sea level, with an average annual temperature of 27.0–29.9 °C, relative humidity of 70–75 %, and annual precipitation of 1321 to 1889 mm [28]. Identification of the sand flies was conducted using taxonomic keys [29].

2.2. Temperature preference assay

To investigate the associations of the bacterial microbiome and *Lu. longipalpis* under an environment with a dynamic temperature, we used a linear thermal gradient apparatus (Fig. 1, MB- Thermocline-type device) designed and elaborated by the MICROBIOP research group of the Universidad Nacional de Colombia, at Medellín [30]. The device is similar to other devices used to perform temperature preference assays mostly in *Drosophila* spp. [31].

An acrylic sheet was used to manufacture the lid and divisions within the prototype. The divisions were manufactured to partially restrict the passage of insects and thus ensure greater control over them. On the outside, ten digital temperature sensors were placed in pairs at 16.6 cm apart from each other and connected to a microcontroller to obtain real-time temperature readings (21–34 °C) using the Arduino software. It is worth noting that initially the temperature range was set from 18 °C to 36 °C, considering the lowest and highest temperatures in the different localities in Colombia where phlebotomine sand flies inhabit. However, we observed that temperature in the device was not stable at the two extremes of this range, and stability was only achieved when set from 21 °C to 34 °C.

Using an entomological aspirator, female *Lu. longipalpis* were introduced inside the device after detecting via the Arduino software stabilization of the temperature gradient throughout the channel. Temperature preferences of the sand flies were already seen after 1 h of exposure as sand fly movement between compartments with different temperature ranges was reduced when compared to the first minutes of the assay. After 1 h, the sand flies were anesthetized with ethyl acetate (99.8 %) vapor. Sand flies were sorted according to the temperature range in which the cadavers were found, then the whole bodies were recovered for DNA extraction, and conserved at -20 °C. Each replicate consisted of 50 females (800 females in total). All the specimens used for temperature exposure were female adults with different feeding statuses and unknown states of infection with *Leishmania* parasites given the nature of the field collection.

2.3. DNA extraction

A total of two or three pools per temperature range were processed

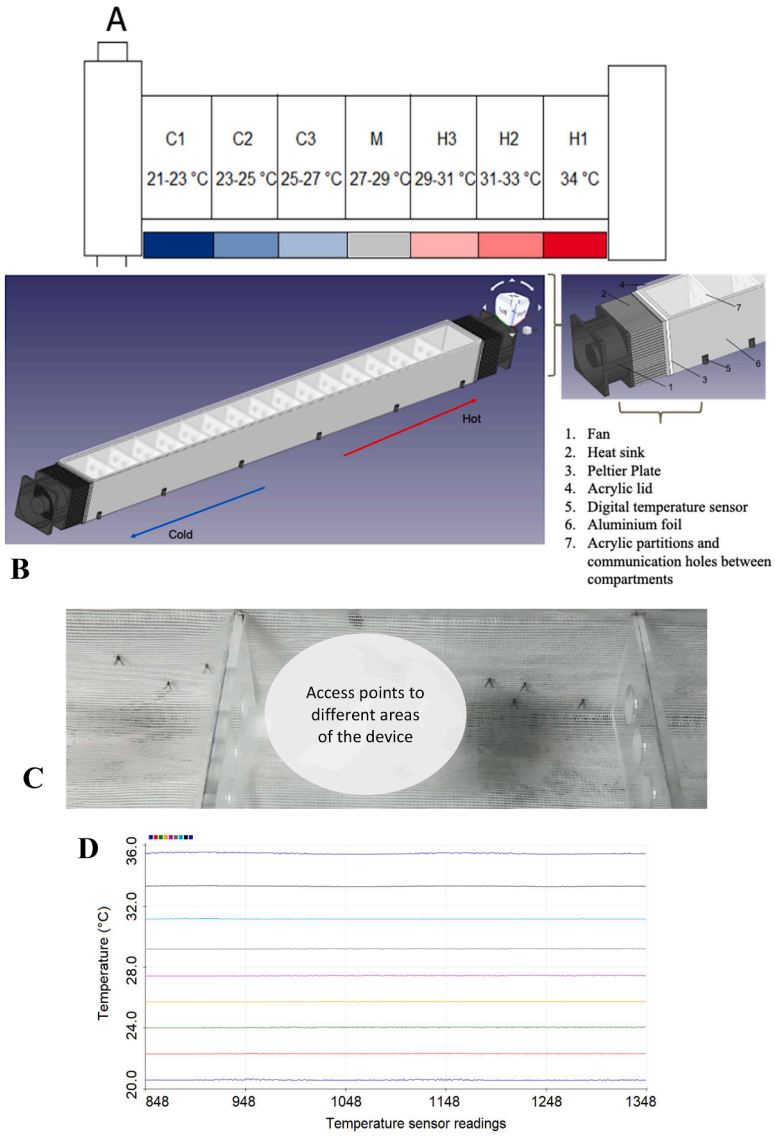

Fig. 1. Design and validation of the temperature gradient device. **A:** Temperature gradient device sketch and temperature ranges in each compartment of the apparatus. Letters C, M, and H describe the cold, medium, and high temperature compartments in the gradient, respectively. **B:** Structure and components of the device with temperature gradient. **C:** Assessment of temperature preferences in *Lu. longipalpis*. **D:** Number of real-time temperature readings (as counts) that were obtained from the Arduino software during the experiment.

for total genomic DNA isolation from whole sand flies (Table 1). Genomic DNA was isolated using Quick-DNA Tissue/Insect Miniprep Kit (Zymo Research, Irvine, CA, USA) and the concentration and quality (A260/A280 ratio) of each sample were measured using the Nanophotometer N60 (Implen GmbH, München, Germany).

2.4. NGS library preparation and sequencing of the DNA samples of Lu. longipalpis

The PCR amplification feasibility of the bacterial DNA extracted from all samples was initially assessed using the primer set 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′) and PCR conditions detailed in Espejo et al. [32]. A Biometra thermocycler (Analytik Jena, Jena, Germany) was used and the visualization of the resulting amplicons was achieved through an agarose gel electrophoresis at 1.2 % at 80 V for 45 min.

PCR amplicons using total DNA extracts as an initial template were generated utilizing PrimeStar HS DNA Polymerase (Takara, Kusatsu, Japan) cocktail mix with the primers 515F and 806R targeting the hypervariable region V4 of the 16S rRNA gene [33] in accordance with the instructions given by the manufacturer [34]. An initial step of 5 min at 95 °C was followed by 20 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 15 s, and extension at 72 °C for 45 s, and a final step at 4 °C until further processing.

From these reactions, 1 μl was added to new PCR cocktail mixes, each sample including a matching and overlapping primer with two unique indices and Illumina adapters [35], and the PCR was then run for 10 cycles at the same temperature and timing as the previous round. Agarose gel electrophoresis was used to determine whether PCR products of the predicted size were present. Using the SequalPrep Normalization Plate (Thermo Fisher Scientific, Santa Clara, CA, USA), the amplified products were purified, normalized, and pooled before being subjected to 250 bp paired-end Illumina MiSeq (Illumina Inc., San Diego, CA, USA) sequencing. The 16S ribosomal DNA sequencing based on DNA isolated from whole sand fly bodies allowed identification of the entire bacterial microbiome without distinguishing midgut from surface microbiota.

DADA2 package (1.18) [36] was used to assemble datasets for each

Table 1
Information from the total DNA samples of *Lu. longipalpis* according to the compartment from which they were retrieved. Letters C, M, and H describe the cold, medium, and high temperature compartments in the gradient device. The commercial control is composed of a mix of *Lysteria monocytogene, Pseudomonas aeruginosa, Bacillus subtillis, Escherichia coli, Salmonella enterica, Lactobacillus fermentum, Enterococcus faecalis, Staphylococcus aureus, Saccharomyces cerevisiae,* and *Cryptococcus neoformans.*

Code of pool	Compartment (Temperature)	Number of females per pool	DNA concentration (ng/μL)
1	C1 (21–23 °C)	10	6.70
2		10	6.35
4		10	6.90
5	C2 (23–25 °C)	7	4.50
6		10	8.25
7	C3 (25–27 °C)	5	4.80
8		10	6.10
9	M (27–29 °C)	7	4.90
10		4	3.10
11	H3 (29–31 °C)	5	3.85
12		4	3.45
13	H2 (31–33 °C)	4	4.75
14		5	2.05
16	H1 (34 °C)	8	4.90
17		7	7.00
Commercial control (Microbial community standard from ZYMO)	Control	–	9,15

amplicon, with trimmed reads based on quality, cuts of baseline noise due to primer sequences, and deletion of potentially chimeric sequences to be able to identify counts of each unique amplicon sequence variant (ASV) across all samples, and their classification was obtained using the RDP Naive Bayesian Classifier and Silva database (version 138.1) (Supplementary Table S1).

2.5. Statistical analysis

The online program MicrobiomeAnalyst (www.microbiomeanalyst.ca) was used to produce graphics representing abundances, box-and-whiskers plots of α-diversity including Shannon, Chao1, and Simpson indices using an analysis of variance (ANOVA) throughout the data set resulting from the temperature gradient device assays. Univariate Mann-Whitney tests were performed to find significant ASV abundance differences. Box-plots for visualization were generated using both the original and log-transformed data. PERMANOVA was used to assess differences in β-diversity using the Bray-Curtis dissimilarity distance. The latter distance measure was also used to perform principal coordinates analysis (PCoA) filtered as ASVs at the genus level and with 4 temperature categories (21–23 °C; 23–29 °C; 29–33 °C; and 34 °C), defined by the temperature preferences of sand flies within the device, and the significantly different groups were detected using Tukey's HSD test. Non-metric multidimensional scaling (NMDS) plot was also obtained with MicrobiomeAnalyst using the Bray-Curtis index. The diversity was also assessed through a hierarchical cluster analysis using the algorithm of Ward and Bray-Curtis index, a heatmap with Pearson's correlation coefficient for distance measures and the clustering algorithm of Ward, all with the same software [37]. In all cases the *P*-value cut-off was of 0.05.

The Random Forest classification algorithm along with the mean decrease in accuracy plot were used to uncover significant relationships between bacterial taxa and temperature, using the MicrobiomeAnalyst program, and taxa were organized in descending order of importance according to the strength of association, considering that the genus with the highest mean decrease in accuracy has the greatest association with the given classification, in this case, temperature range category.

3. Results

3.1. Relative abundance of bacteria in total DNA of Lu. longipalpis in relation to temperature preference of the sand flies.

Once the data were filtered, the number of reads obtained was 287,453 with a range of reads from 4924 to 61,601 among all samples (Supplementary Figure S1). Rarefaction showed that near-complete coverage of sequence diversity was achieved from the 16S amplicons (Supplementary Figure S1).

Three phyla were the most abundant throughout the temperature gradient groups: Proteobacteria (17.22–90.73 %), Firmicutes (5.99–77.21 %) and Actinobacteria (1.56–59.85 %) (Fig. 2). Specifically, there was an overall increase in the relative abundance of Firmicutes along with a decrease in Proteobacteria in sand flies at the temperature range of 29–33 °C (Supplementary Table S2).

In general, the core microbial community identified in *Lu. longipalpis* consisted of 13 genera: *Pseudomonas, Staphylococcus, Klebsiella, Enterococcus, Stenotrophomonas, Bacillus, Acinetobacter, Aquabacterium, Acidovorax, Ralstonia, Corynebacterium, Morganella, Lawsonella* (Fig. 3.)

The most abundant genera in *Lu. longipalpis* exposed to the temperature gradient were *Pseudomonas* (mainly at temperatures of 21–29 °C and 34 °C) and *Bacillus* (mainly at temperatures of 25 °C and 29–33 °C) followed by *Staphylococcus, Klebsiella, Stenotrophomonas, Enterococcus*, and *Ralstonia* (Fig. 4). Additionally, *Serratia* bacteria had a greater representation (49.79 %), specifically in sand flies recovered at 25–27 °C (Supplementary Table S3).

The identification of significant features or potential biomarkers via statistical analyzes, considering the variation in microbiota abundance, was assessed through a univariate analysis (Mann-Whitney test) and box plot visualization. On the genus level, the univariate test showed that *Bacillus* were more abundant mainly in the ranges 29–33 °C (p-value of 0.096; FDR = 0.50635), and other genera such as *Pseudomonas* and *Ralstonia* (P-value > 0.05 in all cases) were better represented in sand flies at lower temperatures (23–29 °C) (Supplementary Figure S2). It was possible to calculate abundances related to endosymbionts of interest such as *Arsenophonus,* with a significantly statistical representation (p-value of 0.029; FDR = 0.43579) at high temperatures (29–33 °C) (Supplementary Figure S2), similar to *Spiroplasma* (*P*-value of 0.15) that also was found at 34 °C (Supplementary Figure S2). Infections with *Rickettsia* were too low and only detected in sand flies recovered between 25–27 °C (Table S1).

Pattern search using Pearson's correlation coefficient (*r*) allowed the observation of the association between bacterial genera according to the temperature ranges. The bacteria that presented the highest number of interactions were selected, that is, they can be decisive in the modulation of the microbiota. In this case, a strong negative correlation was observed between *Bacillus* and *Pseudomonas* bacteria (r = -0.84, P = 0.00007, and FDR = 0.015) as well as a tendency of a negative but non-significant association of the first with *Acinetobacter* and *Acidovorax* (Figs. 5A, 6). The greatest positive association and correlation of *Bacillus* was with *Arsenophonus* (r = 0.77, P = 0.006, FDR = 0.015), specifically at temperatures of 29–33 °C, in addition to a tendency of a positive association with other bacteria such as *Massilia* and *Citrobacter* at this temperature range (Figs. 5A, 6).

Although with low relative abundance, but with multiple interactions of bacteria at different temperatures, we found that *Pantoea* (Figs. 5B, 6) was strongly positively correlated to *Brevibacterium* and *Pseudonocardia* (P < 0.05 in all cases, FDR range 0.004–0.035) at 34 °C, and with *Streptomyces* and *Dietzia* as well, whereas a strong association with *Staphylococcus* was observed at 21–23 °C (P < 0.05, FDR =0.012). At the same temperature, *Pantoea* exhibited strong positive associations with several other genera as *Olligela* (Fig. 5B), *Legionella,* and *Timonella* (P < 0.05 in all cases) (Figs. 5B, 6).

In general, it was possible to observe with the Random Forest algorithm (Figure S3) that the relative abundance of *Arsenophonus* and

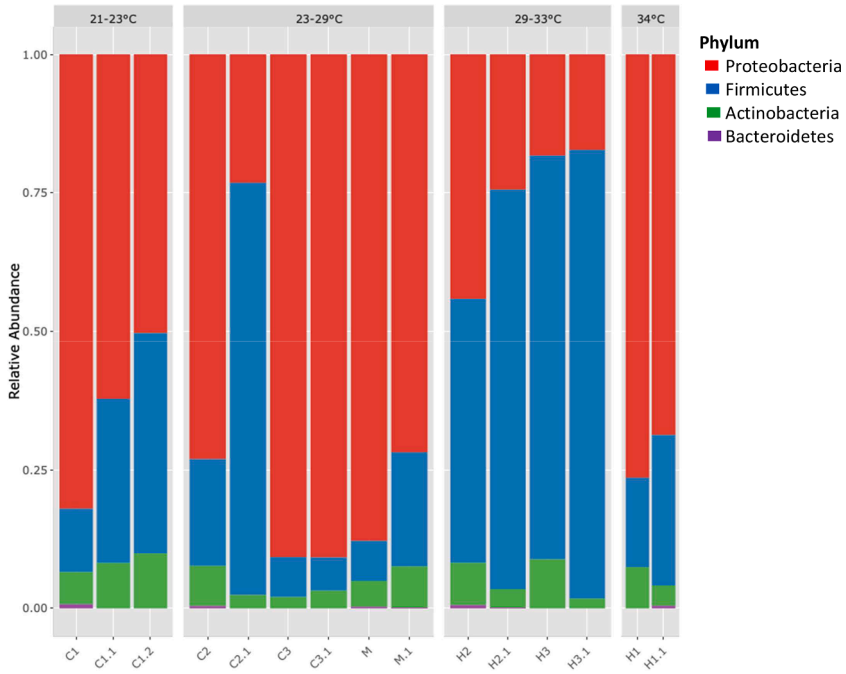

Fig. 2. A stacked bar chart of phyla abundance across samples of total DNA from female *Lu. longipalpis* exposed to the temperature gradient: 21–23 °C (C1, C1.1, C1.2), 23–25 °C (C2, C2.1), 25–27 °C (C3, C3.1), 27–29 °C (M, M.1), 29–31 °C (H3, H3.1), 31–33 °C (H2, H2.1), and 34 °C (H1, H1.1). Letters C, M, and H describe the cold, medium, and high temperature compartments in the gradient from where the sand flies were recovered, respectively. C1, C1.1, C1.2, C2, C2.1, C3, C3.1, M, M.1, H3, H3.1, H2, H2.1, H1, and H1.1 represent the samples recovered in each compartment and its replicates.

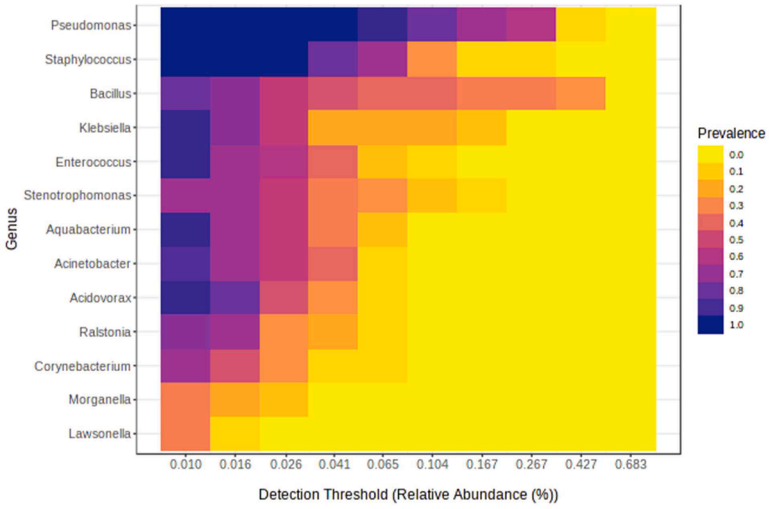

Fig. 3. Core microbiome from all samples of total DNA from *Lu. longipalpis* females, where a higher prevalence at the genus level is shown in blue, while its lowest in yellow.

Bacillus are important in predicting groups of *Lu. longipalpis* with temperature preference at 29–33 °C, while bacteria such as *Timonella,* are predictive for low temperatures between 21–23 °C (Figure S3). Other bacteria also registered highly relative abundance in different temperature ranges as *Enterococcus, Carnobacterium, Staphylococcus, Morganella,* and *Legionella* that were associated as indicators of temperatures between 21–23 °C (Figure S3). At 23–29 °C there was a higher association with *Pseudomonas, Curtobacterium,* and *Stenotrophomonas,* while at 34 °C the association was greater with *Paracoccus, Salinicoccus,* and *Ralstonia* (Figure S3).

3.2. Richness and β-diversity of the microbiome in Lu. longipalpis exposed to the temperature gradient

Microbial richness in samples of wild-caught female specimens of *Lu. longipalpis* was measured using the Shannon, Simpson, and Chao1 indices (Fig. 7). There were no significant differences in the diversity between the groups within the temperature range studied. However, Shannon and Simpson indices for the groups at the lowest (21–23 °C) and the highest (34 °C) temperature had somewhat higher diversity when compared to the groups at 23–29 °C and 29–33 °C, whereas the values of the Chao1 index were more homogenous along the temperature gradient, with a slightly higher values at 29–33 °C (Fig. 7).

As for the structural differences of the microbiome (β-diversity) in

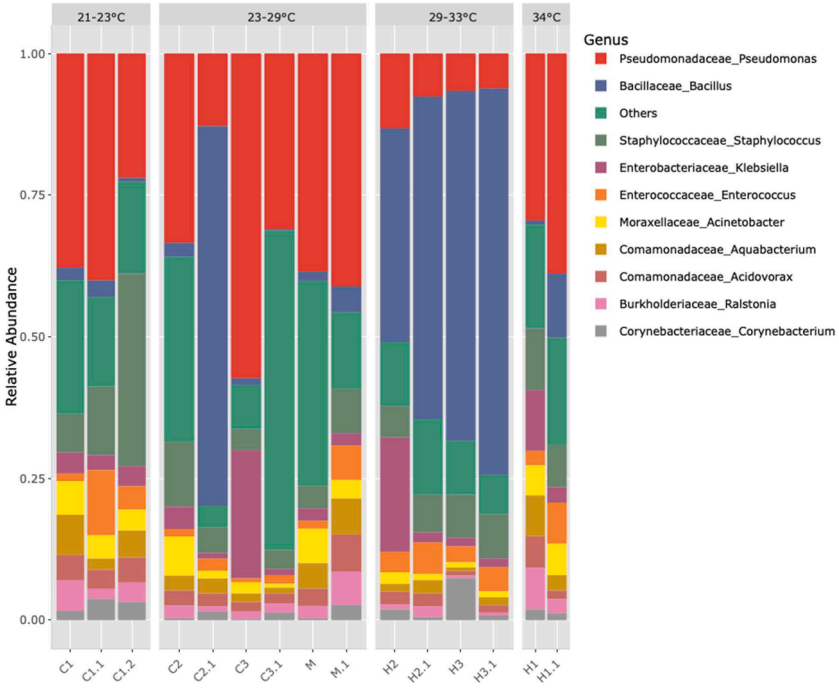

Fig. 4. A stacked bar chart of genus level abundance across samples of total DNA from female *Lu. longipalpis* exposed to the temperature gradient: 21–23 °C (C1, C1.1, C1.2); 23–25 °C (C2, C2.1); 25–27 °C (C3, C3.1); 27–29 °C (M, M.1); 29–31 °C (H3, H3.1); 31–33 °C (H2, H2.1) and 34 °C (H1, H1.1). Letters C, M, and H describe the cold, medium, and high temperature compartments in the gradient from where the sand flies were recovered, respectively. C1, C1.1, C1.2, C2, C2.1, C3, C3.1, M, M.1, H3, H3.1, H2, H2.1, H1, and H1.1 represent the samples recovered in each compartment and its replicates.

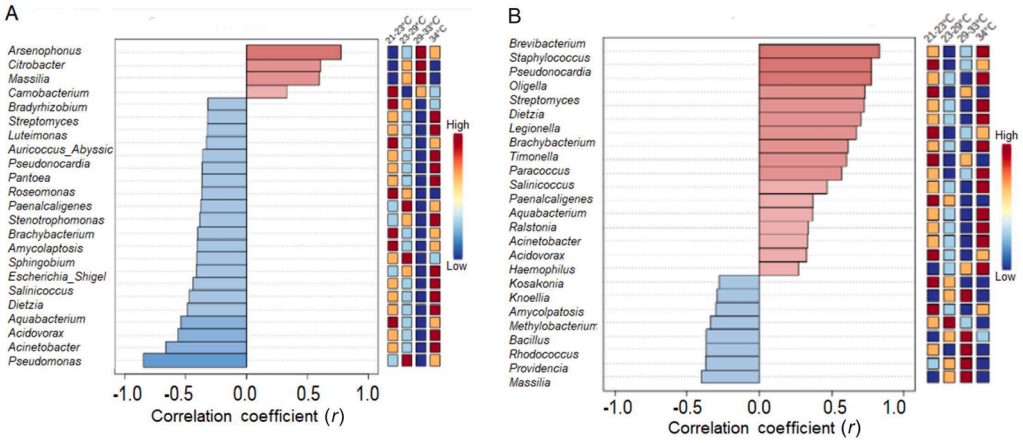

Fig. 5. Pearson's correlation coefficient (*r*) at the genus level of the total samples showing the bacteria most positively and negatively associated with *Bacillus* (**A**); *Pantoea* (**B**). Squares in red symbolize the strongest association between taxa and temperature, while those in dark blue symbolize the weakest. Orange and light blue squares symbolize a moderately strong and weak associations, respectively.

Lu. longipalpis samples subjected to the temperature gradient, there were differences between bacterial communities at 29–33 °C in relation to 21–23 °C (Fig. 8a) due to the high relative abundance of *Bacilllus* (F-value of 3.5073, p-value < 0.013., R-squared of 0,4889). Groups of individuals present in temperatures below 29 °C were located in a different cluster in the analysis of Principal Coordinates Analysis using Bray-Curtis Index (Fig. 8a). The Non-metric Multidimensional Scaling Analysis (NMDS) for the evaluation of the differences in the microbial composition according to temperature ranges (cold, medium and hot) showed no statistically significant differences (F-value of 1.8237, p-value < 0.086., R-squared of 0,2331, NMDS stress = 0.19232) (Fig 8b).

In summary, the heatmap shows mainly variations in the abundance and structures of bacterial communities from *Lu. longipalpis* females, between the cold (21–23 °C) and hot (29–34 °C) ends (Fig 9). The mean temperature range (23–29 °C) presents the least variation in the structure and composition of the microbiota, and bacteria such as *Pseudomonas, Serratia*, and *Shigella* that have a significant abundance are not determinant bacteria in the modulation of the microbiota (Fig 9).

4. Discussion

Recently, the impact of temperature on insects started to attract more attention in those with vectorial capacity, especially because modifications in the epidemiology of vector-borne diseases have been strongly associated with climate change. This study allowed to evidence that temperature had an effect on the microbiota structure of wild-caught specimens of *Lu. longipalpis* exposed to a temperature gradient device that better depicts the environmental conditions these insects endure

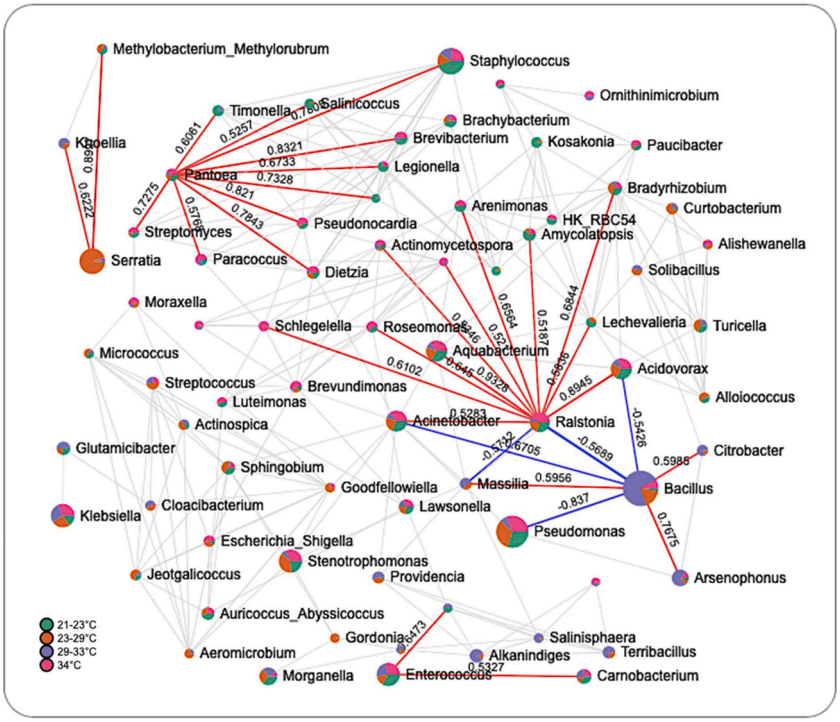

Fig. 6. Correlation and interaction network analysis of the microbiota in groups from female *Lu. longipalpis* exposed to the temperature gradient. Network was built using Pearson correlations from log-transformed abundance. Node colors represent unique taxonomy identifiers. Blue lines represent co-exclusion/negative correlation; red lines represent co-occurrence/positive correlation interactions between relative abundance profiles. Multiple edges connecting the same nodes indicate significance from more than one metric (Bray-Curtis dissimilarity and Pearson correlation).

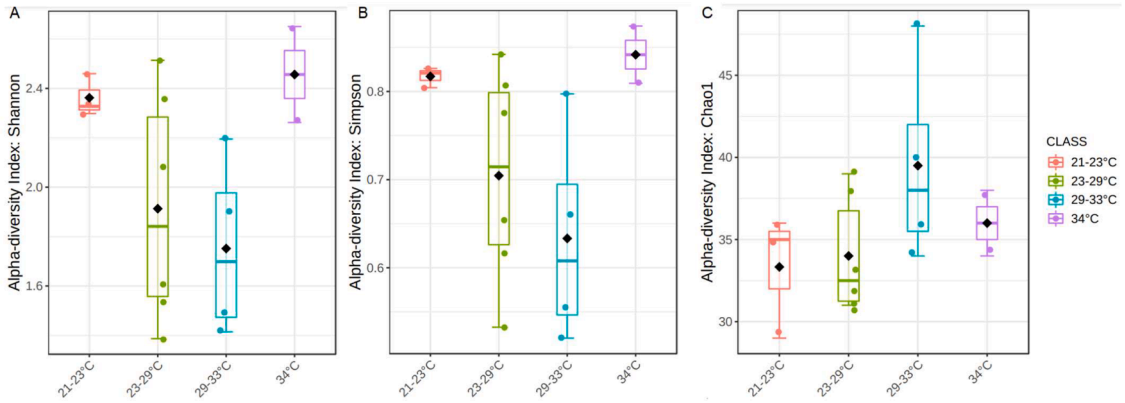

Fig. 7. Alpha-diversity box-and-whiskers plots of bacterial composition using amplicon sequence variants in *Lu. longipalpis* exposed to the temperature gradient. **A:** Richness assessed with Shannon-Wiener index (p-value of 0.1122, F-value of 2.5156). **B:** Simpson index (p-value of 0.1010, F-value of 2.6472). **C:** Chao1 index (p-value of 0.25383, F-value of 1.563).

during the day than working with steady temperatures, given that changes in this variable and in the microbiota have an implication on the potential transmission of *Leishmania* parasites under fluctuating temperatures [38].

Regarding phyla, it was possible to show that there is a high abundance of Proteobacteria and Firmicutes in all temperature ranges, finding greater representativeness of Proteobacteria in cold temperatures (21–23 °C) that is maintained in increasing temperatures up to 29 °C. However, the percentage of Firmicutes increases between 29–33 °C, while at 34 °C the abundance of Proteobacteria increase again. The pattern observed for Proteobacteria and Firmicutes has also been reported in other studies with *Gryllus veletis* where crickets were exposed to different breeding temperatures associated with seasonal changes, and it was observed that in the transition from summer to winter (from warm to colder temperatures) the relative abundance of Proteobacteria increased from 7 % to 26 % while for the same period, the relative abundance of Firmicutes decreased from 50 % to 38 % [39]. In general, there is evidence that environmental variables have an impact on microbial communities being these results in accordance with what has been observed in other sand flies of the *Phlebotomus* genus [38].

At a molecular level, Martins et al., [22] analyzed the vector-parasite system, evidencing an increase of HSP90 in sand flies after blood ingestion and infected with *Leishmania* at 40 °C, also noticing that heat shock protein levels of expression decreased slower when the parasite was present, however its relation with microbiota was not considered. It is worth noting that both Proteobacteria and Firmicutes are known for displaying mechanisms of cell to cell communication as the Quorum Sensing system, deploying signal molecules such as N-Acyl-Homoserine

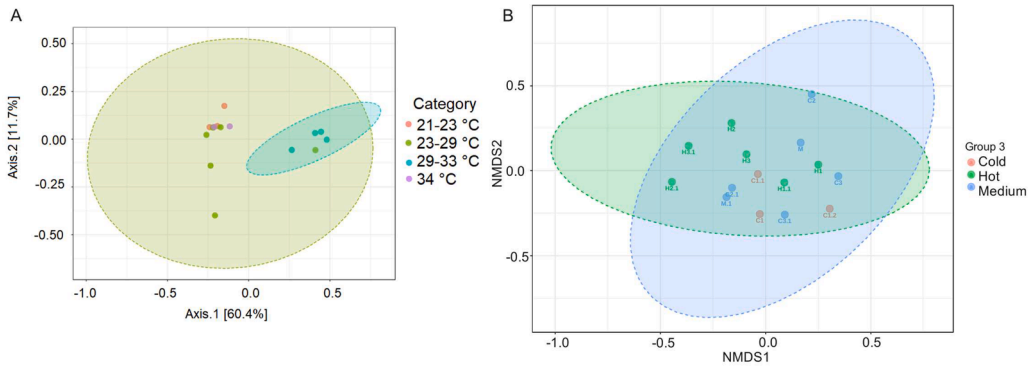

Fig. 8. Beta-diversity of amplicon sequence variants in *Lu. longipalpis* exposed to the temperature gradient. **A:** Principal Coordinates Analysis (PCoA) plot generated using Bray-Curtis Index (PERMANOVA, F-value of 3.5073, p-value < 0.013., R-squared of 0,4889. **B:** The Non-metric Multidimensional Scaling Analysis (NMDS) for the evaluation of the differences in the microbial composition according to temperature ranges (cold, medium and hot) showed no statistically significant differences (F-value of 1.8237, p-value < 0.086., R-squared of 0,2331, NMDS stress = 0.19232).

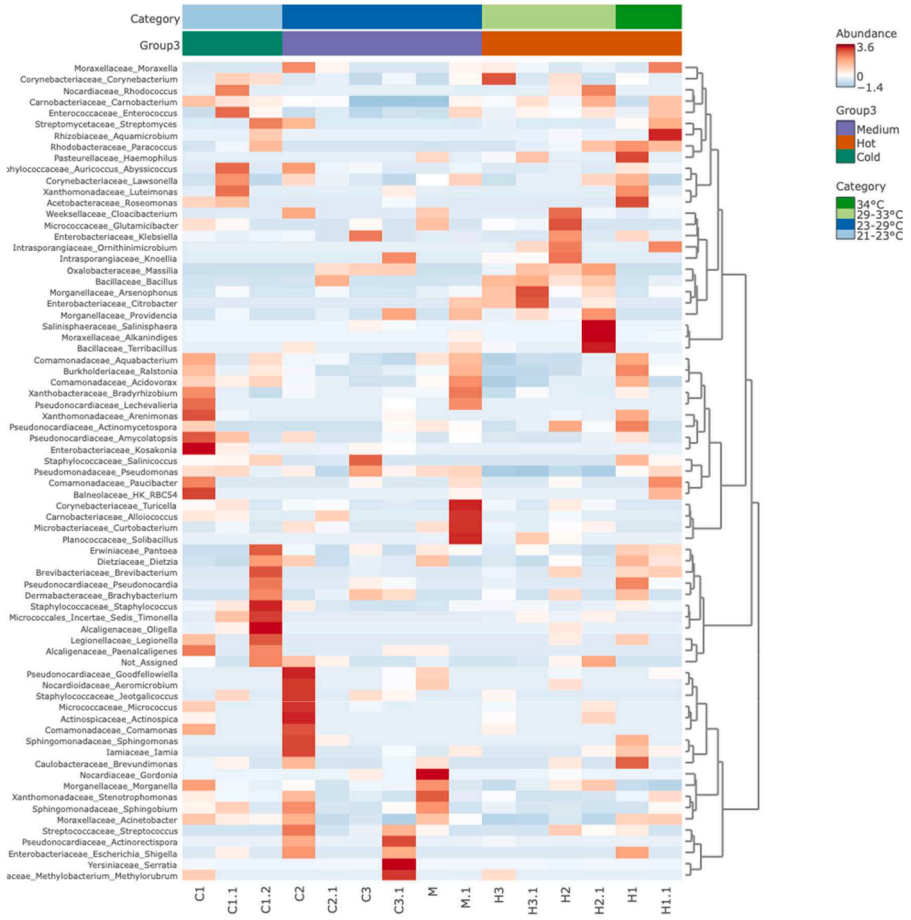

Fig 9. β-diversity of microbial communities in *Lu. longipalpis* after a temperature gradient exposure. Hierarchical cluster analysis using the algorithm of Ward and Bray-Curtis Index with a Heatmap with Pearson Correlation Coefficient for distance measure and the clustering algorithm of Ward.

Lactones (AHL) and small oligopeptides, respectively [40,41], and in Proteobacteria, AHL and 2-Alky-4-Quinolones have been detected in bacteria of *Acinetobacter, Enterobacter, Pseudomonas,* and *Pantoea* genera isolated from phlebotomine sand flies of *Pi. evansi* [42], however, its relationship with environmental stressors such as temperature is yet to be elucidated.

Specifically, in this study, it was possible to detect infection of *Arsenophonus* in wild females of *Lu. longipalpis*. It is known that these bacteria can transfer both horizontally and vertically, and in aphids it has been seen how the transmission and infection rates of this genus decrease in insect populations exposed to temperatures of 30 °C and increment in breeding environmental conditions with temperatures of 20 °C [43]. On the contrary, in this case, we found that in wild specimens of *Lu. longipalpis* there are important associations of this microorganism in temperatures between 29–33 °C and to a lesser extent at 34 °C, observing that the abundance of *Arsenophonus* is higher in warmer environments than in lower temperature ranges. The findings of these bacteria are in agreement with previous reports for *Pi. evansi*,

phlebotomine sand fly vector of L. *infantum* alongside *Lu. longipalpis* in America, where *Arsenophonus* was detected in a sample treated with antibiotics that also had a high load of *Leishmania* infection [44].

In general, *Arsenophonus* is characterized by its reproductive manipulation, where the male-killing phenotype is expressed causing the death of approximately 80 % of males [45]. Additionally, its infection has been identified in various insects with hematophagous habits, including sand flies of *Pi. evansi* and *Psychodopygus panamensis* species in Colombia [46,44]. *Arsenophonus spp.* genomes are known to contain sets of genes related to the synthesis of vitamin B [47,48], and some of its strains are susceptible or resistant to chlorate neonicotinoid insecticides [49]. Its role as an obligate or facultative endosymbiont in sand fly vectors is still unknown, this being the first approach to understand the infection in terms of abundances modulated by fluctuations of abiotic factors such as environmental temperature in phlebotomines.

It was found that *Arsenophonus* was highly related to other groups such as *Citrobacter, Bacillus,* and *Massilia* that were found in the microbiota of the *Lu. longipalpis* that preferred temperatures of 29–33 °C and, to a lesser extent, 34 °C. Of these bacteria, *Bacillus* spp. are known for the mechanism to cope and persist after heat stress [50]. Regarding *Massilia*, these bacteria have been associated with extreme climates such as desert soils [51], have been found as endophytes in plants that grow in these types of ecosystems [52] in dry and low relative humidity sites, and in this case, in high abundance in *Lu. longipalpis* recovered from compartments between 29–33 °C, possibly indicating *Lu. longipalpis* as a new natural reservoir for such genus.

Similar to *Bacillus, Pseudomonas* also has mechanisms that allow it to undergo stress from both cold [53] and warm environmental conditions, the latter through the expression of the genes RNA polymerase *rpoE, rpoS* and *rpoH* and has an easy adaptability to different temperature ranges [50].

In a climate change scenario, with changing temperature and humidity, environmental conditions are creating and favoring niches for the migration and spread of insect vectors of human disease. The microbiota of *Lu. longipalpis* sand flies shows temperature-mediated diversity, with notable changes in the abundance and composition of bacterial groups at different temperature ranges. This variation is particularly pronounced between 29–33 °C, suggesting that temperature plays a crucial role in shaping the microbial community within these insects vectors. Such shifts in microbiota composition under varying temperatures could influence the physiology and overall fitness of *Lu. longipalpis,* particularly in the context of climate change.

Furthermore, the application of pyrethroid insecticides, such as alpha-cypermethrin, beta-cypermethrin, deltamethrin, and lambda-cyhalothrin, under rising temperatures may contribute to changes in bacterial diversity within the sand fly microbiome. Studies have identified bacteria like *Serratia, Pseudomonas,* and *Bacillus,* as capable of degrading pyrethoids [54,55],. In co-culture with *Streptomyces aureus, Bacillus subtillis* is capable of degrading cypermethrin by hidrolases acting on the ester linkage to produce α-hydroxy-3-phenoxy-beneneacetonitrile, and in isolation its shown to biodegrade the compound evidenced by the presence of metabolites such as 4-propylbenzoate, 4-propylbenzaldehyde, phenol M-tert-butyl, and 1-dodecanol, while confirmed laccase and esterase genes may indicate its involvement in the pyrethroid metabolism [56–58]. This metabolic activity could lead to the selection of specific bacterial strains that enhance insecticide resistance, as observed with lambda-cyhalothrin resistance in insect vectors mediated by gut microbiota [59]. The enzymatic activity provided by symbiotic bacteria plays a significant role in this degradation process, highlighting the complex interaction between temperature, microbiota diversity, and insecticide resistance [60].

Moreover, temperature influenced not only the abundance of bacteria involved in biodegradation and insecticide resistance but also affected genera known to elicit immune responses in *Lu. longipalpis* cell lines, such as *Serratia* and *Staphylococcus* [61]. Additionally, bacteria with leishmanicidal potential, like *Pantoea* sp. [62,63] showed increased interaction at the highest temperature (34 °C) and a positive association with *Brevibacterium,* a proposed candidate for paratransgenic blockage of *Leishmania* transmission [64].

Wolbachia, Cardinium, and *Microsporidia* were not present in any of the *Lu. longipalpis* sampled across the different temperature ranges, however, the infection by these microorganisms have been identified in other species of phlebotomine sand flies as in the case of *Wolbachia* in *Pi. evansi, Micropigomyia cayennensis,* and *Evandromyia dubitans* [65], and *Cardinium* and *Microsporidia* in *Pi. evansi* [66,67]. Nevertheless, *Asaia, Spiroplasma,* and *Rickettsia* genera were present, where it is interesting to note that *Asaia* could not be detected in temperatures below 27 °C, this being a genus that has shown potential in impacting the number of promastigotes of *Leishmania mexicana* in *Lu. longipalpis* [15]. Similarly, *Spiroplasma* had a greater representativeness in temperatures above 27 °C, and although its role in phlebotomines is not clear, it has been suggested that it has the potential to express the phenotype of male-killing in *Phlebotomus* sp. species. [68].

As for bacteria of the genus *Rickettsia*, it was only possible to detect them in females who preferred temperatures between 25–27 °C. These bacteria have previously been detected in phlebotomines of *Psathyromyia aclydifera* with a low prevalence [69], similar to what was found in *Lu. longipalpis* in this study. However, in other insects, such as *Bemisia tabaci,* it has been observed that prevalence of the bacterium fluctuates depending on the whitefly population. For instance, in populations of cucumber whiteflies, the infection rate by *Rickettsia* decreases from 64.47 % to 35.29 % during the transition from summer to winter. In contrast, in populations of cotton whiteflies, while the infection frequency remains low, it appears to be unaffected by seasonal changes [70].

Overall, we found low prevalence of endosymbionts as *Rickettsia, Asaia,* and even *Spiroplasma*, that did not allow a detailed analysis of the behavior based on temperature preferences in wild specimens of *Lu. longipalpis*. Considering that fluctuations in temperature due to climate change may affect the distribution of sand flies causing an increase in the number of Leishmaniasis cases worldwide and the transmission of *Leishmania* parasites in areas where it was not present before [10], the insect vector-parasite-microbiota-temperature system gains complexity when planning biological control strategies, as the variation in the representativeness of different bacterial groups of biological control interest changes with temperature.

5. Conclusions

In female *Lu. longipalpis* sand flies, collected in Ricaurte, Cundinamarca, variations in microbiota structure were observed based on temperature preferences. These variations were most pronounced between 29–33 °C. Fluctuations in the abundances of some bacterial groups were identified across all temperature ranges evaluated. These findings contribute to understanding the dynamics of the bacterial microbiota in this insect vector within the context of climate change.

Ethical approval

Collection of *Lu. longipalpis* specimens was conducted in a private property with the correspondent permission of landowners, under the Colombian Decree N° 1376 of 2013 of the Ministry of Environment and Sustainable Development (MADS).

Funding

This work was supported by the projects Hermes 47050 and 50500 of the Universidad Nacional de Colombia Sede Medellín.

CRediT authorship contribution statement

Daniela Duque-Granda: Writing – review & editing, Writing –

original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rafael José Vivero-Gómez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Howard Junca:** Writing – review & editing, Methodology, Investigation, Data curation. **Gloria Cadavid-Restrepo:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Claudia Ximena Moreno-Herrera:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

There are no conflicts of interest that may affect the content, results or conclusions of the article.

Data availability

Data will be made available on request.

Acknowledgments

The authors would like to thank the Universidad Nacional de Colombia Sede Medellín for the permission granted for the use of laboratory installations and equipment during the COVID-19 pandemic as well as to the research group MICROBIOP for all the suggestions made throughout this investigation. A special thanks to Biological Engineer Ricardo Santander Gualdrón, who made the operation with the temperature gradient device possible and to Pablo Emilio Góngora for his aid at collection sites.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.btre.2024.e00857.

References

[1] M.A. Contreras-Gutiérrez, I.D. Vélez, C. Porter, S.I. Uribe, Lista actualizada de flebotomíneos (Diptera: Psychodidae: Phlebotominae) de la región cafetera colombiana, Biomédica 34 (2014) 483–492, https://doi.org/10.7705/biomedica.v34i3.2121.

[2] P.F.P. Pimenta, V.C. de Freitas, C.C. Monteiro, A.C.M.A. Pires, N.F.C. Secundino, Biology of the *Leishmania*−sand fly interaction, in: F.E. Rangel, J.J. Shaw (Eds.), Brazilian Sand Flies, Ed., Springer, Cham, 2018, pp. 319–339.

[3] World Health Organization. (2021). Retrieved from: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis.

[4] Instituto Nacional de Salud, Protocolo De Vigilancia en Salud Pública de Leishmaniasis, 6, 2023, https://doi.org/10.33610/IMYH4569. Versión.

[5] Ministerio del interior (2021). Boletín epidemiológico. Retrieved from: https://www.mininterior.gov.co/wp-content/uploads/2021/12/3.16-Boletin-Epidemiologico-Noviembre-2021-2.pdf.

[6] C. González, O.L Cabrera, L.E. Munstermann, C. Ferro, Distribución de los vectores de Leishmania infantum (Kinetoplastida: Trypanosomatidae) en Colombia, BioMedica 1 (2006) 64–72.

[7] M.A.C. Gutierrez, R.O.H. Lopez, A.T. Ramos, I.D. Vélez, R.V. Gomez, J. Arrivillaga-Henríquez, S. Uribe, DNA barcoding of Lutzomyia longipalpis species complex (Diptera: Psychodidae), suggests the existence of 8 candidate species, Acta Tropica 221 (2021) 105983, https://doi.org/10.1016/j.actatropica.2021.105983.

[8] M. Flórez, J.P. Martínez, R. Gutiérrez, K.P. Luna, V.H. Serrano, C. Ferro, et al., *Lutzomyia longipalpis* (Diptera: Psychodidae) en un foco suburbano de leishmaniosis visceral en el Cañón del Chicamocha en Santander, Colombia, Biomédica 26 (Suppl. 1) (2006) 109–120. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-41572006000500013&lng=en&tlng=es.

[9] L.C.de Sousa-Paula, D. Otranto, F Dantas-Torres, *Lutzomyia longipalpis* (Sand Fly), Trends. Parasitol. (2020), https://doi.org/10.1016/j.pt.2020.05.007.

[10] World Health Organization. (2023). Leishmaniasis. Retrieved from: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis.

[11] D.P. Lage, P.A.F. Ribeiro, D.S. Dias, D.V.C. Mendonça, F.F. Ramos, L.M. Carvalho, D. de Oliveira, B.T. Steiner, V.T. Martins, L. Perin, A.S. Machado, T.T.O. Santos, G. S.V. Tavares, J.A. Oliveira-da-Silva, J.S. Oliveira, B.M. Roatt, M.A. Machado-de-Ávila, A.L Texeira, M.V. Humbert, E.A.F. Coehlo, M. Christodoulides, A candidate vaccine for human visceral leishmaniasis based on a specific T cell epitope-containing chimeric protein protects mice against *Leishmania infantum* infection, NPJ. Vaccines. 5 (1) (2020), https://doi.org/10.1038/s41541-020-00224-0.

[12] L.K Koch, J. Kochmann, S. Klimpel, S. Cunze, Modeling the climatic suitability of leishmaniasis vector species in Europe, Sci. Rep. 7 (1) (2017) 13325, https://doi.org/10.1038/s41598-017-13822-1.

[13] J. Hlavacova, J. Votypka, P. Volf, The Effect of temperature on *Leishmania* (Kineto plastida: Trypanosomatidae) development in sand flies, J. Med. Entomol. 50 (5) (2013) 955–958, https://doi.org/10.1603/ME13053.

[14] T. Kamiya, M.A. Greischar, K. Wadhawan, b. Gilbert, K. Paaijmans, N Mideo, Temperature-dependent variation in the extrinsic incubation period elevates the risk of vector-borne disease emergence, Epidemics. (2019), https://doi.org/10.1016/j.epidem.2019.100382.

[15] M.R. Sant'Anna, H. Diaz-Albiter, K. Aguiar-Martins, W.S. Al Salem, R. R. Cavalcante, V.M. Dillon, P.A. Bates, F.A. Genta, R.J Dillon, Colonisation resistance in the sand fly gut: Leishmania protects Lutzomyia longipalpis from bacterial infection, Parasit. Vectors. 7 (1) (2014) 329, https://doi.org/10.1186/1756-3305-7-329.

[16] P.A. Arnold, S.C. Levin, A.L. Stevanovic, K.N. Johnson, *Drosophila melanogaster* infected with *Wolbachia* strain *w*MelCS prefer cooler temperatures, Ecol. Entomol. (2018), https://doi.org/10.1111/ccn.12696.

[17] A.M. Truitt, M. Kapun, R. Kaur, W.J. Miller, *Wolbachia* modifies thermal preference in *Drosophila melanogaster*, Environ. Microbiol. (2018), https://doi.org/10.1111/1462-2920.14347.

[18] N.N. Moghadam, P.M. Thorshauge, T.N. Kristensen, N. de Jonge, S. Bahrndorff, H. Kjeldal, J.L. Nielsen, Strong responses of *Drosophila melanogaster* microbiota to developmental temperature, Fly. (Austin) 12 (1) (2018) 1–12, https://doi.org/10.1080/19336934.2017.1394558.

[19] G.M. Onyango, M.S. Bialosuknia, F.A. Payne, N. Mathias, T.A. Ciota, D.L. Kramer, Increase in temperature enriches heat tolerant taxa in *Aedes aegypti* midguts, Sci. Rep. 10 (2020) 19135, https://doi.org/10.1038/s41598-020-76188-x.

[20] H. Guzmán, R.B. Tesh, Effects of temperature and diet on the growth and longevity of phlebotomine sand flies (Diptera: Psychodidae), Biomédica 20 (2000) 190–199.

[21] G.B. Rivas, N.A. de Souza, A.A. Peixoto, R. Bruno, Effects of temperature and photoperiod on daily activity rhythms of *Lutzomyia longipalpis* (Diptera: Psychodidae), Parasites Vectors 7 (2014) 278, https://doi.org/10.1186/1756-3305-7-278.

[22] K.A. Martins, C.S. Morais, S.J. Broughton, C.R. Lazzari, P.A. Bates, M.H. Pereira, R. J. Dillon, Response to thermal and infection stresses in an American vector of visceral leishmaniasis, Med. Vet. Entomol. 37 (2) (2023) 238–251, https://doi.org/10.1111/mve.12626.

[23] P.H. Kelly, S.M. Bahr, T.D. Serafim, N.J. Ajami, J.F. Petrosino, C. Meneses, J. R. Kirby, J.G. Valenzuela, S. Kamhawi, M.E. Wilson, The Gut Microbiome of the vector Lutzomyia longipalpis is essential for survival of *Leishmania infantum*, mBio 8 (1) (2017), https://doi.org/10.1128/mBio.01121-16 e01121-16.

[24] A.C.A.M. Pires, L.E.M. Villegas, T.B. Campolina, A.S. Orfanó, P.F.P. Pimenta, N.F. C. Secundino, Bacterial diversity of wild-caught *Lutzomyia longipalpis* (a vector of zoonotic visceral leishmaniasis in Brazil) under distinct physiological conditions by metagenomics analysis, Parasit. Vectors. 10 (1) (2017), https://doi.org/10.1186/s13071-017-2593-7.

[25] C. Gouveia, M.D. Asensi, V. Zahner, E.F. Rangel, S.M. Oliveira, Study on the bacterial midgut microbiota associated to different Brazilian populations of Lutzomyia longipalpis (Lutz & Neiva) (Diptera: Psychodidae), Neotrop 37 (5) (2008) 597–601, https://doi.org/10.1590/s1519-566x2008000500016.

[26] M.J. Osland, P.W. Stevens, M.M. Lamont, R.C. Brusca, K.M. Hart, J.H. Waddle, C. A. Langtimm, C.M. Williams, B.D. Keim, A.J. Terando, E.A. Reyier, K.E. Marshall, M.E. Loik, R.E. Boucek, A.B. Lewis, J.A. Seminoff, Tropicalization of temperate ecosystems in North America: the northward range expansion of tropical organisms in response to warming winter temperatures, Glob. Chang. Biol. 27 (13) (2021) 3009–3034, https://doi.org/10.1111/gcb.15563.

[27] World Health Organization. (2003). Climate change and human health: risks and responses. Editors: McMichael, A. J., Campbell-Lendrum, D. H., Corvalán, C. F., Ebi, K. L., Githeko, A. K., Scheraga, J. D. et al. Retrieved from: https://iris.who.int/handle/10665/42742.

[28] IDEAM - UNAL, Variabilidad Climática y Cambio Climático en Colombia, Bogotá, D.C., 2018.

[29] E.A.B. Galati, J.D. Andrade-Filho, A.C.L. Silva, A.L. Falcao, Description of a new genus and a new species of new world Phlebotominae (Diptera, Psychodidae), Rev. Brasil Entomol. 47 (2003) 63–70. https://www.scielo.br/j/rbent/a/zStmf9b9BcPS9JFmBvrCfpJ/?lang=en.

[30] R. Vivero-Gomez, D. Duque-Granda, J.A. Rader, A. Stuckert, R. Santander-Gualdron, G. Cadavid-Restrepo, C.X. Moreno-Herrera, D.R. Matute, Humidity and temperature preference in two Neotropical species of sand flies, Parasit. Vectors. 17 (1) (2024) 246, https://doi.org/10.1186/s13071-024-06325-2.

[31] T. Goda, F.N. Hamada, Drosophila temperature preference rhythms: an innovative model to understand body temperature rhythms, Int. J. Mol. Sci. 20 (8) (2019) 1988, https://doi.org/10.3390/ijms20081988.

[32] R.T. Espejo, C.G. Feijóo, J. Romero, M. Vásquez, PAGE analysis of the heteroduplexes formed between PCR-amplified 16S rRNA genes: estimation of sequence similarity and rDNA complexity, Microbiology (N. Y) 144 (1998) 1611–1617.

[33] Earth Microbiome Project, 2021. https://earthmicrobiome.org/protocols-and-standards/16s/. Accessed on february 2, 2021.

[34] J.G. Caporaso, C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J.A. Gilbert, G. Smith, R Knight, Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms, ISMe J. 6 (2012) 1621–1624, https://doi.org/10.1038/ismej.2012.8.
[35] S. Rath, B. Heidrich, D.H. Pieper, M. Vital, Uncovering the trimethylamine-producing bacteria of the human gut microbiota, Microbiome (54) (2017) 5, https://doi.org/10.1186/s40168-017-0271-9.
[36] B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. Holmes, DADA2: High-resolution sample inference from Illumina amplicon data, Nat. Methods 13 (7) (2016) 581–583, https://doi.org/10.1038/nmeth.3869.
[37] J. Chong, P. Liu, G. Zhou, J. Xia, Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data, Nat. Protoc. 15 (2020) 799–821, https://doi.org/10.1038/s41596-019-0264-1.
[38] F. Karimian, M. Koosha, N. Choubdar, M.A. Oshaghi, Comparative analysis of the gut microbiota of sand fly vectors of zoonotic visceral leishmaniasis (ZVL) in Iran; host-environment interplay shapes diversity, PLoS. Negl. Trop. Dis. 16 (7) (2022) e0010609, https://doi.org/10.1371/journal.pntd.0010609.
[39] L.V. Ferguson, P. Dhakal, J.E. Lebenzon, D.E. Heinrichs, C. Bucking, B.J. Sinclair, Seasonal shifts in the insect gut microbiome are concurrent with changes in cold tolerance and immunity, Funct. Ecol. 32 (2018) 2357–2368, https://doi.org/10.1111/1365-2435.13153.
[40] R.G. Abisado, S. Benomar, J.R. Klaus, A.A. Dandekar, J.R. Chandler, Bacterial quorum sensing and microbial community interactions, mBio 9 (3) (2018), https://doi.org/10.1128/mBio.02331-17 e02331-17.
[41] Y. Su, T. Ding, Targeting microbial quorum sensing: the next frontier to hinder bacterial driven gastrointestinal infections, Gut. Microbes. 15 (2) (2023) 2252780, https://doi.org/10.1080/19490976.2023.2252780.
[42] R.J. Vivero-Gomez, G. Mesa Bedoya, J. Higuita-Castro, S.M. Robledo, C.X. Moreno-Herrera, Gloria. Cadavid-Restrepo, Detection of quorum sensing signal molecules, particularly N-Acyl homoserine lactones, 2-Alky-4-Quinolones, and Diketopiperazines, in gram-negative bacteria isolated from insect vector of Leishmaniasis, Front. Trop. Dis. 2 (2021), https://doi.org/10.3389/fitd.2021.760228.
[43] C.-Y. Chang, X.-W. Sun, P.-P. Tian, N.-H. Miao, Y.-L. Zhang, X.-D. Liu, Plant secondary metabolite and temperature determine the prevalence of *Arsenophonus* endosymbionts in aphid populations, Environ. Microbiol. 24 (2022) 3764–3776, https://doi.org/10.1111/1462-2920.15929.
[44] R.J. Vivero-Gómez, Castañeda-Monsalve, M.C. Atencia, R. Hoyos-López, G. Hurst, G. Cadavid-Restrepo, C.X. Moreno-Herrera, Molecular phylogeny of heritable symbionts and microbiota diversity analysis in phlebotominae sand flies and *Culex nigripalpus* from Colombia, PLoS. Negl. Trop. Dis. (2021), https://doi.org/10.1371/journal.pntd.0009942.
[45] M. Bohacsova, O. Mediannikov, M. Kazimirova, D. Raoult, Z. Sekeyova, *Arsenophonus nasoniae* and Rickettsiae infection of *Ixodes ricinus* due to parasitic wasp *Ixodiphagus hookeri*, PLoS. One 11 (2016) e0149950, https://doi.org/10.1371/journal.pone.0149950.
[46] D. Duque-Granda, C.X. Moreno-Herrera, G.E. Cadavid-Restrepo, R.J Vivero-Gómez, Molecular detection and phylogenetic analyses of *Arsenophonus* endosymbiont in wild specimens of phlebotomine sand flies from Colombia, J. Asia. Pac. Entomol. 26 (1) (2023), https://doi.org/10.1016/j.aspen.2022.102023.
[47] E. Nováková, V. Hypša, P. Nguyen, F. Husník, A.C. Darby, Genome sequence of Candidatus Arsenophonus lipopteni, the exclusive symbiont of a blood sucking fly Lipoptena cervi (Diptera: Hippoboscidae), Stand. Genomic. Sci. 11 (2016) 72, https://doi.org/10.1186/s40793-016-0195-1.
[48] J. Xue, X. Zhou, C.X. Zhang, L.L. Yu, H.W. Fan, Z. Wang, H.J. Xu, Y. Xi, Z.R. Zhu, W.W. Zhou, P.L. Pan, B.L. Li, J.K. Colbourne, H. Noda, Y. Suetsugu, T. Kobayashi, Y. Zheng, S. Liu, R. Zhang, Y. Liu, J.A. Cheng, Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation, Genome Biol. 15 (12) (2014) 521, https://doi.org/10.1186/s13059-014-0521-0.
[49] R. Pang, M. Chen, L. Yue, K. Xing, T. Li, K. Kang, Z. Liang, L. Yuan, W. Zhang, A distinct strain of *Arsenophonus* symbiont decreases insecticide resistance in its insect host, PLoS. Genet. 14 (10) (2018) e1007725, https://doi.org/10.1371/journal.pgen.1007725.
[50] M. Sakil Munna, J. Tahera, M. Mohibul Hassan Afrad, I.T. Nur, R. Noor, Survival of Bacillus spp. SUBB01 at high temperatures and a preliminary assessment of its ability to protect heat-stressed Escherichia coli cells, BMC. Res. Notes. 8 (2015) 637, https://doi.org/10.1186/s13104-015-1631-9.
[51] A.A. Belov, V.S. Cheptsov, E.A. Vorobyova, N.A. Manucharova, Z.S. Ezhelev, Stress-tolerance and taxonomy of culturable bacterial communities isolated from a Central Mojave Desert soil sample, Geosciences. (Basel) 9 (2019) 166, https://doi.org/10.3390/geosciences9040166.
[52] P.M. Chimwamurombe, J.L. Grönemeyer, B. Reinhold-Hurek, Isolation and characterization of culturable seed-associated bacterial endophytes from gnotobiotically grown Marama bean seedlings, FEMS. Microbiol. Ecol. 92 (2016) fiw083, https://doi.org/10.1093/femsec/fiw083.
[53] R.A. Arango, S.D. Schoville, C.R. Currie, C. Carlos-Shanley, Experimental warming reduces survival, cold tolerance, and gut prokaryotic diversity of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), Front. Microbiol. 12 (2021) 632715, https://doi.org/10.3389/fmicb.2021.632715.
[54] M. Cycoń, Z. Piotrowska-Seget, Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: a review, Front. Microbiol. 7 (2016) 1463, https://doi.org/10.3389/fmicb.2016.01463.
[55] P. Bhatt, Y. Huang, H. Zhan, S. Chen, Insight into microbial applications for the biodegradation of pyrethroid insecticides, Front. Microbiol. 10 (2019) 1778, https://doi.org/10.3389/fmicb.2019.01778.
[56] S. Chen, J. Luo, M. Hu, K. Lai, P. Geng, H. Huang, Enhancement of cypermethrin degradation by a coculture of *Bacillus cereus* ZH-3 and *Streptomyces aureus* HP-S-01, Bioresour. Technol. 110 (2012) 97–104, https://doi.org/10.1016/j.biortech.2012.01.106.
[57] S. Gangola, A. Sharma, P. Bhatt, P. Khati, P. Chaudhary, Presence of esterase and laccase in Bacillus subtilis facilitates biodegradation and detoxification of cypermethrin, Sci. Rep. 8 (1) (2018), https://doi.org/10.1038/s41598-018-31082-5.
[58] Pankaj, A. Sharma, S. Gangola, P. Khati, G. Kumar, A Srivastava, Novel pathway of cypermethrin biodegradation in a *Bacillus* sp. strain SG2 isolated from cypermethrin-contaminated agriculture field, 3. Biotech. 6 (1) (2016) 45, https://doi.org/10.1007/s13205-016-0372-3.
[59] A. Arévalo-Cortés, A.M. Mejia-Jaramillo, Y. Granada, H. Coatsworth, C. Lowenberger, O. Triana-Chavez, The midgut microbiota of Colombian *Aedes aegypti* populations with different levels of resistance to the insecticide lambda-cyhalothrin, Insects. 11 (2020) 584, https://doi.org/10.3390/insects11090584.
[60] A. Soltani, H. Vatandoost, M.A. Oshaghi, A.A. Enayati, A.R. Chavshin, The role of midgut symbiotic bacteria in resistance of *Anopheles stephensi* (Diptera: Culicidae) to organophosphate insecticides, Pathog. Glob. Health 111 (6) (2017) 289–296, https://doi.org/10.1080/20477724.2017.1356052.
[61] E.L. Telleria, A. Martins-da-Silva, A.J. Tempone, Y.M. Traub-Csekö, *Leishmania*, microbiota and sand fly immunity, Parasitology. 145 (10) (2018) 1336–1353, https://doi.org/10.1017/S0031182018001014.
[62] S. Al-Qaysi, H. Al-Haideri, S.M. Al-Shimmary, J.M. Abdulhameed, O.I. Alajrawy, M.M. Al-Halbosiy, et al., Bioactive levan-type exopolysaccharide produced by *Pantoea agglomerans* ZMR7: characterization and optimization for enhanced production, J. Microbiol. Biotechnol. 31 (2021) 696–704, https://doi.org/10.4014/jmb.2101.01025.
[63] R.J. Vivero, G.B. Mesa, S.M. Robledo, C.X.M. Herrera, G. Cadavid-Restrepo, Enzymatic, antimicrobial, and leishmanicidal bioactivity of Gram-negative bacteria strains from the midgut of *Lutzomyia evansi,* an insect vector of leishmaniasis in Colombia, Biotechnol. Rep. (2019) e00379, https://doi.org/10.1016/j.btre.2019.e00379.
[64] H. Hillesland, A. Read, B. Subhadra, I. Hurwitz, R. McKelvey, K. Ghosh, P. Das, R. Durvasula, Identification of aerobic gut bacteria from the kala azar vector, Phlebotomus argentipes: a platform for potential paratransgenic manipulation of sand flies, Am. J. Trop. Med. Hyg. 79 (2008) 881–886.
[65] R.J. Vivero, G. Cadavid-Restrepo, C.X. Herrera, S.I. Soto, Molecular detection and identification of *Wolbachia* in three species of the genus *Lutzomyia* on the Colombian Caribbean coast, Parasit. Vectors. 10 (1) (2017) 110, https://doi.org/10.1186/s13071-017-2031-x.
[66] R.J. Vivero, V.A Castañeda-Monsalve, L.R. Romero, G. D Hurst, G. Cadavid-Restrepo, C.X. Moreno-Herrera, Gut microbiota dynamics in natural populations of *Pintomyia evansi* under experimental infection with *Leishmania infantum*, Microorganisms. 9 (6) (2021) 1214, https://doi.org/10.3390/microorganisms9061214. *4*.
[67] R.J. Vivero, M. Villegas-Plazas, G.E. Cadavid-Restrepo, C. Herrera, S.I. Uribe, H. Junca, Wild specimens of sand fly phlebotomine *Lutzomyia evansi,* vector of leishmaniasis, show high abundance of *Methylobacterium* and natural carriage of *Wolbachia* and *Cardinium* types in the midgut microbiome, Sci. Rep. 9 (1) (2019) 17746, https://doi.org/10.1038/s41598-019-53769-z.
[68] B. Karatepe, S. Aksoy, M. Karatepe, Investigation of *Wolbachia spp*. and *Spiroplasma* spp. in *Phlebotomus* species by molecular methods, Sci. Rep. 8 (2018) 10616, https://doi.org/10.1038/s41598-018-29031-3.
[69] Y.N. Lozano-Sardaneta, A. Valderrama, S. Sánchez-Montes, E. Grostieta, P. Colunga-Salas, V. Sánchez-Cordero, I. Becker, Rickettsial agents detected in the genus *Psathyromyia* (Diptera:Phlebotominae) from a biosphere reserve of Veracruz, Mexico, Parasitol. Int. 82 (2021) 102286, https://doi.org/10.1016/j.parint.2021.102286. Epub Jan 21. PMID: 33486127.
[70] D. Zhao, Z. Zhang, H. Niu, H. Guo, Win by quantity: a Striking Rickettsia-Bias Symbiont community revealed by seasonal tracking in the Whitefly *Bemisia tabaci*, Microb. Ecol. 81 (2) (2021) 523–534, https://doi.org/10.1007/s00248-020-01607-5.