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Article

Comparative Analysis of Gut Microbiota in Laboratory Colony and Wild Populations of *Phlebotomus tobbi* and Molecular Surveillance of *Leishmania* Infection

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Abstract

Sand fly midgut microbiota plays a critical role in shaping *Leishmania* development and vector competence, yet functional evidence from natural vector populations remains limited. In this study, sand flies were collected between 2020 and 2022 in Cukurova region, Türkiye to characterize the gut bacterial composition of *Phlebotomus tobbi* and evaluate the anti-leishmanial potential of cultivable isolates. A total of 1,739 sand flies were captured (878 females, 861 males), of which *Ph. tobbi* was the predominant species (n = 1,312). 16S rRNA amplicon sequencing (V4–V6) showed that the gut microbiota was dominated by Proteobacteria, with *Erwinia aphidicola/persicina* representing the most abundant species across all analyzed groups. Fourteen cultivable bacterial species were identified by MALDI-TOF MS, including *Serratia liquefaciens*, *Pantoea agglomerans*, and *Micrococcus luteus*. Functional XTT assays against *Leishmania infantum* promastigotes demonstrated variable inhibitory activity among isolates. The strongest leishmanicidal effects were observed with *S. liquefaciens* (32.3%) and *M. luteus* (28.8%). Morphological examination confirmed promastigote rounding and cell death in isolates showing >25% activity. These findings define the gut bacterial landscape of *Ph. tobbi* in an endemic region and identify bacterial taxa with in vitro anti-leishmanial activity, highlighting their potential for future microbiota-based or paratransgenic control strategies.

Keywords: *Phlebotomus tobbi*; midgut microbiota; 16S rRNA sequencing; MALDI-TOF; leishmanicidal activity; *Leishmania infantum*; paratransgenesis

1. Introduction

Phlebotomine sand flies serve as vectors of several human diseases, including leishmaniasis, sand fly fever, and bartonellosis [1]. Among these, leishmaniasis remains a major public health problem in the Mediterranean Basin, largely due to high population density and sustained transmission [2]. Depending on the infecting *Leishmania* species and the host's immune status, the disease manifests primarily in two clinical forms: cutaneous leishmaniasis (CL), which is typically self-limiting and endemic in 85 countries, and visceral leishmaniasis (VL), a potentially fatal form endemic in 74 countries [3]. Sand fly vectors acquire the parasite during blood feeding, ingesting amastigote forms that transform into infective metacyclic promastigotes within the midgut, a process known as

metacyclogenesis. This critical developmental step is influenced not only by parasite genetics but also by the microbial environment of the sand fly gut [4,5].

Türkiye remains an endemic region for both VL and CL, reporting approximately 2,000 CL cases and 40 VL cases annually [6]. Nine *Phlebotomus* species are either confirmed or suspected vectors of Old World leishmaniasis in the country, with the highest burden concentrated in Anatolia and the Mediterranean regions [7–9]. *Phlebotomus tobbi* is one of the proven vectors of *L. infantum* in the Mediterranean region of Türkiye, with distribution extending to Cyprus, Albania, Greece, and Iran [10,11]. Recent studies indicate that in the Çukurova region, *Ph. tobbi* serves as the sole vector for both human CL and canine leishmaniasis (CanL) and is common across all three major biogeographical regions of Türkiye [8,10,12]. Although several vector control studies have been conducted in the region, interventions have largely been restricted to chemical methods, with no approaches targeting parasite development within the vector itself [8,13–15].

The life cycle of *Leishmania* involves multiple differentiation events enabling adaptation to distinct host environments. Increasing evidence highlights the pivotal role of sand fly gut microbiota in shaping parasite development, modulating immunity, and ultimately influencing vectorial capacity [4]. Stable associations between sand flies and bacterial symbionts, often maintained through vertical transmission, suggest long-term co-evolutionary interactions. These bacteria can impact larval survival, adult fitness, and susceptibility to *Leishmania* infection [4,16,17]. Experimental studies further indicate that bacterial metabolites secreted during gut colonization significantly influence *Leishmania* growth, differentiation, and survival [17,18]. Geographic variation in bacterial composition has also been reported in sand fly populations, including those from Türkiye, suggesting that local microbial communities may shape transmission dynamics [19,20]. Collectively, these findings emphasize a complex tripartite interaction among vector, parasite, and microbiota, with direct implications for disease epidemiology [21,22].

Despite these advances, our understanding of sand fly-associated microbiota remains limited compared with other vector systems such as mosquitoes and triatomines. While paratransgenesis has been successfully applied in *Rhodococcus rhodnii* of *Rhodnius prolixus* against *Trypanosoma cruzi*, applications in sand flies are still scarce [23,24]. Establishing paratransgenesis in new ecological settings first requires detailed characterization of the bacterial diversity associated with target sand fly species. The success of such approaches depends on understanding the composition and functional roles of midgut microbiota, as well as their interactions with *Leishmania*. However, the lack of baseline data on bacterial diversity and functional capacity continues to constrain the development of microbiota-based control strategies in leishmaniasis-endemic regions.

Türkiye harbors diverse ecological zones with multiple sand fly species and parasite lineages, yet no studies have systematically examined how bacterial communities vary across sand fly populations or influence vector competence. Filling this gap is critical not only for clarifying regional epidemiology but also for identifying bacterial taxa that may serve as candidates for paratransgenic interventions [23,25,26].

In this study, we address these gaps by: (i) characterizing the bacterial communities associated with field-collected *Phlebotomus tobbi* populations from endemic regions of Türkiye, (ii) evaluating the influence of these bacteria on *Leishmania* development and survival, and (iii) testing the leishmanicidal activity of selected bacterial isolates in vitro. By linking microbiota composition with functional assays, we aim to generate new insights into the role of sand fly-associated bacteria in parasite transmission and to explore their potential for paratransgenic applications

2. Materials and Methods

2.1. Study Area and Sand Fly Collection

Sand flies were collected from the Çukurova Basin in southern Türkiye, a region bordered by the Amanos Mountains to the east, the Western Taurus Mountains to the west, and the Mediterranean Sea to the south, covering an area of approximately 3,150 km². Sampling was conducted during the summers of 2020–2022 in four villages: Camili, Damyeri, Otluk, and Zerdali. Sites were selected based

on suitability for sand fly breeding and resting, including animal shelters (barns, poultry) and peridomestic environments, as described previously [12,15]. CDC miniature light traps were placed indoors, outdoors, and near vegetation, operating from dusk (~19:00) until dawn (~06:00). Captured sand flies were transported to the laboratory for morphological identification and sorting.

2.2. Establishment of Laboratory Colonies

A colony of *Phlebotomus tobbi* was established from field-collected specimens and maintained at the VERG Laboratories, Department of Biology, Hacettepe University, following established procedures [27,28]. Both male and female progeny from the laboratory-reared F1 generation obtained from parental females were used in the bacterial colonization and vertical transmission experiments.

2.3. Midgut Dissections and Bacterial Diversity

Sand flies were sorted by sex and physiological status prior to dissection. To minimize contamination, specimens were washed in antimicrobial solution (Gibco™ Pen-Strep 10,000 U/mL) before processing. Midguts were dissected under sterile conditions as described previously, and each was examined microscopically to detect natural *Leishmania* infections [19]. Dissected gut material was divided into two portions: one was inoculated on various culture media (TSA, PCA, MHB, NB), while the other was preserved for molecular analysis of bacterial diversity.

Cultivable bacterial isolates were identified using MALDI-TOF mass spectrometry. For culture-independent analysis, sand flies were pooled by sex and collection site (≤ 10 individuals per pool) and preserved in RNA/DNA Shield (Zymo Research) [29]. DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research) according to the manufacturer's instructions. The V4–V6 regions of the bacterial 16S rRNA gene were amplified and sequenced by next-generation sequencing (NGS). Taxonomic classification and relative abundance profiles were generated using Geneious v9 (Biomatters). Library preparation and sequencing were performed commercially by Zymo Research.

2.4. Leishmanicidal Activity of Bacterial Isolates

The leishmanicidal activity of bacterial isolates was evaluated using the XTT viability assay. *Leishmania infantum* (MHOM/TN/80/IPT-1) promastigotes were thawed from cryopreservation, cultured in RPMI-1640 medium supplemented with 15% fetal bovine serum (FBS), and maintained until reaching the plateau growth phase, which was confirmed by Thoma chamber counts. Bacterial isolates were prepared at different CFU/ml concentrations in RPMI-1640 and co-cultured with promastigotes (1×10^6 /ml) in 96-well plates (100 μ l/well each). Plates were incubated at 24 °C for 48 h, after which parasite viability was assessed. Pentostam was used as the positive control and RPMI-1640 medium as the negative control. All experimental conditions, including each bacterial isolate and control group, were prepared and tested in triplicate independent replicates. XTT normalization formula [(%) = $[100 \times (\text{sample absorbance}) / (\text{control absorbance})]$] applied as described previously [30]. Mortality values were calculated as the mean of three replicate measurements, and variability among replicates was expressed as standard deviation (SD), which was represented in the graphical analyses.

2.5. Molecular Surveillance of *Leishmania* Infections in Field-Collected Sand Flies

Female *Ph. tobbi* specimens were pooled (5–10 individuals per pool) in ZR Bashing Bead™ tubes (Zymo Research). Positive control DNA was obtained from reference isolates (MHOM/TN/80/IPT-1), while male sand flies served as negative controls. Pools were homogenized in a Magna Lyser (Roche) at 7,000 g for 50 s, resuspended in 200 μ l Qiagen tissue lysis buffer, and incubated overnight at 56 °C. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) and eluted in 50 μ l of buffer. *Leishmania* DNA was detected using kinetoplast DNA (kDNA)-specific primers (JW11/JW12) with the SYBR Green I Master Kit (Roche), following the protocol described recently [31].

3. Results

3.1. Sand Fly Collections and Species Composition

A total of 1,739 sand flies were collected from four field campaigns conducted in the İmamoğlu and Kozan districts (Adana Province) between 2020–2022. Specimens comprised 878 females and 861 males. Specimen identification revealed dominance of *Ph. tobbi* (n = 1,312; 75.5%), followed by *Ph. papatasi* (n = 338; 19.4%), *Sergentomyia dentata* (n = 44; 2.5%), *Ph. major* s.l. (n = 23; 1.3%), *Ph. perfiliewi* s.l. (n = 8; 0.5%), and *Ph. sergenti* (n = 8; 0.5%). Two specimens of *Adlerius* sp. were also recorded. Sampling sites were located at 176–214 m elevation, mainly within enclosed animal shelters with sheep, goats, dogs, and cattle as potential blood sources.

Table 1. Species composition of sand flies collected from İmamopu and Kozan districts (2020–2022).

Species	Female (n)	Male (n)	Total (n)	% of total
<i>Phlebotomus tobbi</i>	660	652	1.312	75.5 %
<i>Ph. papatasi</i>	176	162	338	19.4 %
<i>Sergentomyia dentata</i>	22	22	44	2.5 %
<i>Ph. major</i> s.l.	12	11	23	1.3 %
<i>Ph. perfiliewi</i> s.l.	4	4	8	0.5 %
<i>Ph. sergenti</i>	4	4	8	0.5 %
<i>Adlerius</i> sp.	-	2	2	0.1 %

3.2. Surveillance of Leishmania Infection

Midgut dissections were performed on all 878 female specimens. No promastigote infections were detected microscopically. Subsequently, molecular screening was conducted on 84 pools (67 *Ph. tobbi*, 17 *Ph. papatasi*) using kDNA-targeted real-time PCR, and no *Leishmania* DNA was detected.

3.3. Bacterial Diversity in Field and Colony Specimens

Metagenomic profiling of *Ph. tobbi* midguts revealed 15 bacterial taxa spanning *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. Community composition varied by sand fly sex and feeding status. Field-collected females were dominated by *Erwinia aphidicola/persicina* (46.3%), *Serratia marcescens/nematodiphila* (20.8%), and *Ralstonia pickettii* (7.9%). Males showed higher prevalence of *Erwinia aphidicola/persicina* (63.6%) and *Pantoea ananatis* (13.9%). Blood-fed females were enriched for *Burkholderia (Paraburkholderia) fungorum* (18.5%), *Stenotrophomonas maltophilia* (14.9%), and *Serratia marcescens/nematodiphila* (17.4%).

Colony females were dominated by *Erwinia aphidicola/persicina* (41.4%) and *S. maltophilia* (30.1%), whereas colony-derived males showed dominance of *E. aphidicola/persicina* (46.3%) and *S. maltophilia* (23.4%). Alpha and beta-diversity analyses indicated reduced richness in colony populations compared to field specimens. Rare taxa (<0.1%) were excluded from graphical analyses to avoid distribution bias. All metagenomic data was presented as Supplementary file (S1).

Table 2. Relative abundance of dominant bacterial taxa in *Ph. tobbi* midguts.

Bacterial taxon*	Field Female	Field Male	Colony Female	Colony Male
<i>E. aphidicola/persicina</i>	46.3 %	63.6 %	41.4 %	46.3 %
<i>S. marcescens/nematodiphila</i>	20.8 %	9.7 %	5.6 %	6.4 %
<i>P. ananatis</i>	2.9 %	13.9 %	—	—
<i>B. fungorum</i>	18.5 %	—	—	—
<i>S. maltophilia</i>	14.9 %	—	30.1 %	23.4 %

<i>R. pickettii</i>	7.9 %	4.7 %	—	—
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* Only taxa with ≥ 0.1 % abundance in at least one group are shown.

3.4. Cultivable Bacteria and MALDI-TOF Identification

Field-derived isolates grown on TSA, PCA, MHA, and broth media were Gram-characterized and identified via MALDI-TOF. Fourteen species were confirmed: *Staphylococcus cohnii*, *Pantoea agglomerans*, *Bacillus simplex*, *Serratia liquefaciens*, *Micrococcus luteus*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, *Bacillus pumilus*, *Streptococcus equinus*, *Bacillus clausii*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Paenibacillus cookii*, and *Lactobacillus paracasei*.

3.5. Leishmanicidal Activity of Bacterial Isolates

All cultured bacterial isolates were tested against *L. infantum* promastigotes using the XTT assay, and the results were expressed as the mean mortality percentages obtained from three independent replicate experiments. Mortality rates varied among species: *S. liquefaciens* (32.3%), *M. luteus* (28.8%), and *S. equinus* (19.7%) exhibited the highest inhibitory effects. Moderate activity was observed for *B. simplex* (17.5%), *B. pumilus* (16.2%), and *S. cohnii* (14.2%), whereas several isolates, including *E. faecium*, *L. casei*, *L. rhamnosus*, *P. cookii*, and *L. paracasei*, showed negligible activity (<5%). Variability among replicate measurements was represented in the graphical presentation, allowing visualization of reproducibility across triplicate assays. Morphological examination further confirmed promastigote rounding and cell death in isolates demonstrating >25% inhibitory activity (Figure 1).

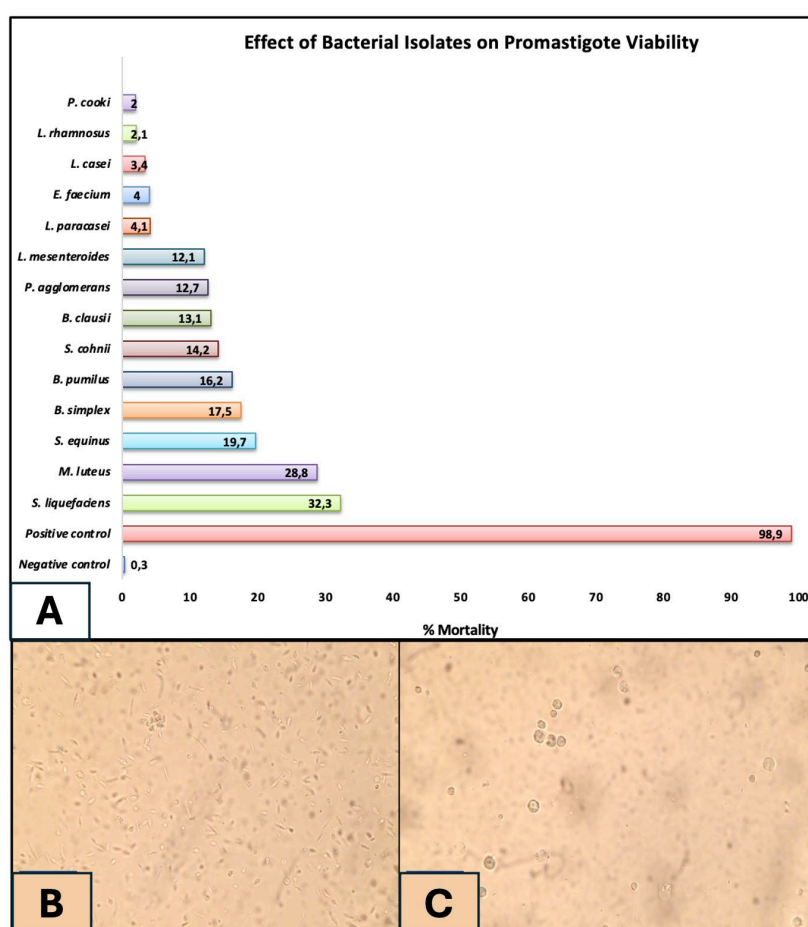


Figure 1. Leishmanicidal effects of cultured bacterial isolates on *L. infantum* promastigotes A) Percent mortality of *L. infantum* promastigotes after 24 h co-incubation with bacterial supernatants (XTT assay). Mean \pm SD of three independent experiments. B) Control group promastigotes C) Treatment group promastigotes (*Serratia liquefaciens*).

4. Discussion

This study provides the first comprehensive characterization of the midgut microbiota of *Phlebotomus tobbi*, both in field-collected and laboratory-colonized populations, and evaluates the functional impact of bacterial isolates on *Leishmania infantum* promastigotes. To our knowledge, this is the first such study conducted in Türkiye to define the bacterial community composition of *Ph. tobbi* midguts using high-throughput 16S based sequencing in combination with cultivation-based approaches and functional assays. The findings therefore represent a critical baseline dataset that enhances our understanding of vector biology and the ecological determinants of leishmaniasis transmission.

One of the major questions of this study was to determine the midgut bacterial composition of field-collected *Ph. tobbi* specimens and their dynamics in such hyperendemic region for leishmaniasis. Sand fly-associated microbiota have been examined in several vector species, most notably *Ph. papatasi*, *Ph. argentipes*, and *Lutzomyia longipalpis*, and very different bacterial profiles were reported [19,32,33]. These data highlight that sand fly-associated microbiota is influenced not solely by the vector species but also by geographic determinants. Thus, vector microbiome studies should also include geographical determinants and should be evaluated in this context. Baseline microbiota characterizations are indispensable since they provide the foundation for hypothesis-driven research on host–microbe–parasite interactions and enable future longitudinal or intervention studies to contextualize their results. By establishing baseline microbial profiles for *Ph. tobbi* in such hyperendemic region, we believe that our study will support future paratransgenesis studies.

For Türkiye, the importance of this dataset is amplified by the country's unique ecological setting. The Çukurova Basin, from which the specimens were collected, represents one of the most active transmission foci for both cutaneous and visceral leishmaniasis. The study area exhibits high *Leishmania* positivity, which is influenced by various ecological, social, and epidemiological factors [14,34]. Cross-sectional analysis revealed a CanL prevalence of 27.18% using IFAT and 41.74% through other diagnostic methods, indicating significant infection rates in these areas [8]. Understanding the microbiota of local sand fly populations is therefore not only an academic exercise but a step toward developing targeted strategies for leishmaniasis control in endemic foci. The data may also be extrapolated to neighboring Mediterranean and Middle Eastern regions, where *Ph. tobbi* co-occurs and contributes to transmission cycles.

Additional contribution of this study is the comparison between field-derived and laboratory-colonized *Ph. tobbi* populations. The gut microbiota of *Ph. tobbi* demonstrates notable differences between laboratory-reared colonies and wild populations. Such reasons like controlled diets and environmental factors, colony flies were found to host a lower diversity of gut bacteria. As also found in this study, previous researchers have likewise reported reduced bacterial richness, compared to the broader diversity observed in wild populations [19,35]. This reduction raises two important considerations: (i) colony-based experimental systems may underestimate the complexity of natural microbiota and thus provide an incomplete picture of vector–parasite interactions, and (ii) certain bacteria with potential functional relevance in parasite development or inhibition may be lost under laboratory conditions [36]. The persistence of *Erwinia* in both environments suggest strong host dependence, whereas the enrichment of *Stenotrophomonas maltophilia* in colonies may reflect adaptation to laboratory conditions [37]. These insights highlight the importance of validating laboratory findings against field-derived baselines.

Globally, the gut microbiota of sand flies is predominantly composed of *Proteobacteria* and *Firmicutes*, with *Proteobacteria* often representing the majority in wild populations [35]. This bacterial composition plays a crucial role in supporting the development of parasites within the sand fly gut lumen, where the presence of symbiotic and commensal bacteria influences parasite survival and maturation [36,38]. Studies also suggest that certain microbial symbionts may either enhance or inhibit activity, depending on the bacterial species present, further highlighting the microbiota's role in vector competence [37]. These findings align with broader observations in other sand fly species, where the microbiota of wild populations displays greater richness and functional diversity

compared to laboratory-reared counterparts. Such differences are important to consider in efforts to manipulate sand fly microbiota as part of integrated control strategies for leishmaniasis [38–40].

Across both field-collected and laboratory-reared populations of *Ph. tobbi*, *Erwinia aphidicola/persicina* emerged as the dominant bacterial taxon, accounting for up to 63% of sequences in males and more than 40% in females, indicating a stable and potentially well-adapted association between *Ph. tobbi* and *Erwinia* spp. This persistent dominance may reflect vertical transmission, efficient colonization of the sand fly gut, or strong ecological adaptation to the host intestinal environment. Although *Erwinia* is widely recognized as a plant-associated bacterium, accumulating evidence from other arthropod systems suggests that certain *Erwinia* species can support host nutrition by supplying essential amino acids and vitamins and may persist throughout metamorphic stages, highlighting their broader symbiotic potential [16,41,42]. In sand flies, however, the functional significance of this association remains unresolved. It is plausible that *Erwinia* contributes to nutrient provisioning, modulation of gut physiology, immune homeostasis, or competitive exclusion of other microbial taxa, all of which may indirectly influence vector competence. Given that gut-associated bacteria can affect parasite establishment by altering nutrient availability, immune responses, or facilitating attachment of *Leishmania* to the gut epithelium, the high abundance of *Erwinia* raises the possibility that it may either promote or inhibit parasite development depending on its ecological interactions within the gut microbiome [38,43]. Similar observations in related bacteria, such as *Delftia tsuruhatensis*, which can interfere with parasite colonization, further support the relevance of exploring these microbial interactions [44]. Taken together, the ubiquity and abundance of *Erwinia* in *Ph. tobbi* make it a compelling target for deeper functional studies and a promising candidate for microbiota-based vector control approaches, including paratransgenic manipulation or selective microbial disruption.

The second notable finding was the recurrent detection of *Serratia* species, particularly *Serratia marcescens/nematodiphila* in sequencing data and *Serratia liquefaciens* among cultivable isolates. *Serratia* has been repeatedly associated with inhibitory effects on protozoan parasites, including *Leishmania* [19,37,45]. Our functional assays confirmed that *S. liquefaciens* exhibited the highest leishmanicidal activity, killing over 30% of promastigotes in vitro. This corroborates findings in other sand fly vectors, where *Serratia* spp. have been implicated in reducing parasite development through production of antimicrobial peptides, proteases, or secondary metabolites [46–48]. The consistent isolation of *Serratia* from *Ph. tobbi* and its demonstrated inhibitory capacity highlights its promise as a candidate for paratransgenic interventions aimed at reducing *Leishmania* transmission. *Micrococcus luteus*, which showed nearly 29% mortality in our assays, has been reported to produce carotenoids and antimicrobial metabolites with broad inhibitory potential [49]. *Streptococcus equinus*, *Bacillus simplex*, and *Bacillus pumilus* showed intermediate leishmanicidal activity, aligning with prior observations that *Bacillus* spp. can generate lipopeptides and bacteriocins with antiprotozoal properties [48]. Conversely, lactic acid bacteria such as *Lactobacillus casei*, *L. rhamnosus*, and *L. mesenteroides* showed negligible activity, though their probiotic potential and influence on gut homeostasis cannot be discounted. Collectively, these findings underscore the taxonomic and functional heterogeneity of the *Ph. tobbi* microbiota and the necessity of evaluating both dominant and rare taxa for their role in shaping vector competence.

Our molecular surveillance did not detect *Leishmania* DNA in any of the 878 dissected females or 84 pooled samples tested. The results obtained in this study are surprising when compared with the high *Leishmania* positivity rates reported in previous studies conducted in the region, both in humans and reservoir hosts [8,50,51]. While this may reflect true low prevalence during the sampling period, it is also possible that infection rates in the İmamoğlu and Kozan districts are temporally variable. The fact that the field studies were conducted during a period of strict COVID-19 precautions may have influenced the negative *Leishmania* results. Reduced human activity, travel restrictions, reduced outdoor activity, and social distancing, during lockdowns might have directly or indirectly influenced vector-pathogen dynamics, though evidence on this is still limited. In addition, it is also possible that certain personal hygiene and protective measures adopted during that period exerted a negative impact on vector–host interactions. These factors likely contributed to

the negative results, highlighting the need to account for pandemic-related disruptions in similar studies. In India, reported cases of malaria, dengue, chikungunya, Japanese encephalitis, and kala-azar declined markedly during the 2020 lockdown period compared with the previous year, suggesting that movement restrictions may have reduced exposure opportunities [52]. The absence of infections, however, provides a cleaner baseline for microbiota characterization, free from confounding effects of parasite colonization. In future studies, comparative analyses of infected versus uninfected *Ph. tobbi* midguts will be crucial to identify bacterial taxa that either facilitate or inhibit parasite establishment.

Paratransgenic strategies have emerged as one of the most promising microbiota-based approaches for controlling vector-borne diseases by exploiting symbiotic or commensal bacteria naturally associated with insect vectors and engineering them to express molecules that interfere with pathogen development [23,53]. In sand flies, several bacterial taxa have already been proposed as suitable candidates for such interventions, including *Serratia* AS1, *Enterobacter cloacae*, *Bacillus subtilis*, and *Ochrobactrum* spp., all of which possess characteristics favorable for stable colonization, genetic manipulation, and persistence across developmental stages [24,26,46]. In this context, our demonstration that *Ph. tobbi*-derived bacteria, particularly *Serratia liquefaciens* and *Micrococcus luteus*, exert measurable leishmanicidal activity is especially significant, as these taxa represent promising local candidates for paratransgenic development. Their natural association with *Ph. tobbi* suggests that they could potentially be engineered to deliver anti-*Leishmania* effector molecules directly within the sand fly midgut, analogous to successful paratransgenic systems developed in triatomines targeting *T. cruzi* [54]. Given Türkiye's substantial leishmaniasis burden and the epidemiological importance of *Ph. tobbi* as a proven vector, the country provides an ideal ecological and public health setting for piloting such microbiota-driven interventions. Beyond these bacterial candidates, *Wolbachia* represents another highly attractive yet underexplored avenue for sand fly control. Previous studies conducted in Türkiye have documented the presence of *Wolbachia* in sand fly populations, while infection rates can reach as high as 90% in certain taxa, the overall positivity rates are reported at approximately 16%, indicating that this endosymbiont is naturally established in local vector populations [55]. This relatively notable prevalence suggests that *Wolbachia*-based strategies, which have already demonstrated success in mosquito-borne disease control programs, may hold considerable promise for future sand fly-targeted biocontrol applications in the region [56–58]. More broadly, these findings reinforce the concept that gut microbiota are central determinants of vector competence, shaping parasite survival through complex networks involving microbial competition, metabolite exchange, colonization resistance, and host immune modulation. The present dataset therefore provides a critical ecological baseline for designing future paratransgenic platforms in local vector populations. To advance this field, future studies should integrate metagenomic, metatranscriptomic, and metabolomic approaches to resolve the functional landscape of the *Ph. tobbi* gut ecosystem, complemented by field-based manipulative experiments involving targeted introduction, depletion, or engineering of specific microbial taxa to establish causal links between microbiota composition and *Leishmania* development.

From a public health perspective, baseline microbiota surveys such as this are invaluable. They not only advance fundamental science but also open applied avenues for sustainable vector control. Given the limitations of chemical insecticides and the lack of effective vaccines against leishmaniasis, microbiota-based strategies may represent one of the few scalable innovations available for interrupting transmission. The prominence of *Erwinia* and *Serratia* in *Ph. tobbi* populations across different conditions suggests these bacteria should be prioritized for functional and applied research.

5. Conclusions

In summary, this study delivers the first global characterization of *Ph. tobbi* midgut microbiota and provides baseline data that are critical for understanding the ecology of this important vector. By identifying dominant taxa such as *Erwinia aphidicola/persicina* and *Serratia liquefaciens*, demonstrating their persistence across field and colony populations, and uncovering their functional potential against *Leishmania* parasites, we establish a new foundation for both basic and applied

research. These findings reinforce the concept that vector competence is not determined by the parasite alone but is the outcome of a complex tripartite interaction between sand flies, parasites, and their associated microbiota. Building on this baseline, future research can explore microbiota manipulation and paratransgenesis as novel, sustainable tools in the fight against leishmaniasis.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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Abbreviations

The following abbreviations are used in this manuscript:

TSA	Tryptic Soy Agar
PCA	Plate Count Agar
MHB	Mueller-Hinton Broth
NB	Nutrient Broth
CL	Cutaneous Leishmaniasis
VL	Visceral Leishmaniasis
CanL	Canine Leishmaniasis
CDC	Centers for Disease Control and Prevention
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight

References

1. WHO Global Leishmaniasis Updates 2023: 3 Years of the NTD Road Map; 2023;
2. Alvar, J.; Yactayo, S.; Bern, C. Leishmaniasis and Poverty. *Trends Parasitol.* **2006**, *22*, 552–557, doi:10.1016/j.pt.2006.09.004.
3. Mann, S.; Frasca, K.; Scherrer, S.; Henao-Martínez, A.F.; Newman, S.; Ramanan, P.; Suarez, J.A. A Review of Leishmaniasis: Current Knowledge and Future Directions. *Curr. Trop. Med. Rep.* **2021**, *8*, 121–132, doi:10.1007/s40475-021-00232-7.
4. Kelly, P.H.; Bahr, S.M.; Serafim, T.D.; Ajami, N.J.; Petrosino, J.F.; Meneses, C.; Kirby, J.R.; Valenzuela, J.G.; Kamhawi, S.; Wilson, M.E. The Gut Microbiome of the Vector *Lutzomyia Longipalpis* Is Essential for Survival of *Leishmania Infantum*. *mBio* **2017**, *8*, doi:10.1128/mBio.01121-16.
5. McGwire, B.S.; Satoskar, A.R. Leishmaniasis: Clinical Syndromes and Treatment. *QJM* **2014**, *107*, 7–14, doi:10.1093/qjmed/hct116.

6. WHO Global Leishmaniasis Surveillance Updates 2024: Consolidating Gains and New Initiatives; 2025;
7. Özbilgin, A.; Harman, M.; Karakuş, M.; Bart, A.; Töz, S.; Kurt, Ö.; Çavuş, İ.; Polat, E.; Gündüz, C.; Van Gool, T.; et al. Leishmaniasis in Turkey: Visceral and Cutaneous Leishmaniasis Caused by *Leishmania Donovanii* in Turkey. *Acta Trop.* **2017**, *173*, 90–96, doi:10.1016/j.actatropica.2017.05.032.
8. Karakuş, M.; Töz, S.; Ertabaklar, H.; Paşa, S.; Atasoy, A.; Arserim, S.K.; Ölgün, M.K.; Ziya Alkan, M.; Durrant, C.; Özbel, Y. Evaluation of Conjunctival Swab Sampling in the Diagnosis of Canine Leishmaniasis: A Two-Year Follow-up Study in Çukurova Plain, Turkey. *Vet. Parasitol.* **2015**, *214*, 295–302, doi:10.1016/j.vetpar.2015.09.009.
9. Ok, Ü.Z.; Balcıoğlu, İ.C.; Taylan Özkan, A.; Özensoy, S.; Özbel, Y. Leishmaniasis in Turkey. *Acta Trop.* **2002**, *84*, 43–48, doi:10.1016/S0001-706X(02)00134-1.
10. Erisoz Kasap, O.; Linton, Y.M.; Karakus, M.; Ozbel, Y.; Alten, B. Revision of the Species Composition and Distribution of Turkish Sand Flies Using DNA Barcodes. *Parasit. Vectors* **2019**, *12*, doi:10.1186/s13071-019-3669-3.
11. Alten, B.; Maia, C.; Afonso, M.O.; Campino, L.; Jiménez, M.; González, E.; Molina, R.; Bañuls, A.L.; Prudhomme, J.; Vergnes, B.; et al. Seasonal Dynamics of Phlebotomine Sand Fly Species Proven Vectors of Mediterranean Leishmaniasis Caused by *Leishmania Infantum*. *PLoS Negl. Trop. Dis.* **2016**, *10*, doi:10.1371/journal.pntd.0004458.
12. Kasap, O.E.; Belen, A.; Kaynas, S.; Simsek, F.M.; Biler, L.; Ata, N.; Alten, B. Activity Patterns of Sand Fly (Diptera: Psychodidae) Species and Comparative Performance of Different Traps in an Endemic Cutaneous Leishmaniasis Focus in Cukurova Plain, Southern Anatolia, Turkey. *Acta Veterinaria Brno* **2009**, *78*, 327–335, doi:10.2754/avb200978020327.
13. Votýpka, J.; Kasap, O.E.; Volf, P.; Kodym, P.; Alten, B. Risk Factors for Cutaneous Leishmaniasis in Cukurova Region, Turkey. *Trans. R. Soc. Trop. Med. Hyg.* **2012**, *106*, 186–190, doi:10.1016/j.trstmh.2011.12.004.
14. Gunay, F.; Karakus, M.; Oguz, G.; Dogan, M.; Karakaya, Y.; Ergun, G.; Kaynas, S.; Kasap, O.E.; Ozbel, Y.; Alten, B. Evaluation of the Efficacy of Olyset® Plus in a Village-Based Cohort Study in the Cukurova Plain, Turkey, in an Area of Hyperendemic Cutaneous Leishmaniasis; 2014; Vol. 39;.
15. Karakuş, M.; Sarıkaya, Y.; Oğuz, G.; Doğan, M.; Ergun, G.; Günay, F.; Kasap, Ö.E.; Özbel, Y.; Alten, B. Assessment of Diagnostic Doses for Widely Used Synthetic Pyrethroids (Deltamethrin & Permethrin) in an Endemic Focus of Leishmaniasis in Turkey. *Parasit. Vectors* **2016**, *9*, 526, doi:10.1186/s13071-016-1812-y.
16. Telleria, E.L.; Martins-da-Silva, A.; Tempone, A.J.; Traub-Csekö, Y.M. *Leishmania*, Microbiota and Sand Fly Immunity. *Parasitology* **2018**, *145*, 1336–1353, doi:10.1017/S0031182018001014.
17. Tabbabi, A.; Mizushima, D.; Yamamoto, D.S.; Kato, H. Effects of Host Species on Microbiota Composition in *Phlebotomus* and *Lutzomyia* Sand Flies. *Parasit. Vectors* **2023**, *16*, 310, doi:10.1186/s13071-023-05939-2.
18. Volf, P.; Kiewegová, A.; Nemeč, A. Bacterial Colonisation in the Gut of *Phlebotomus Duboscqi* (Diptera: Psychodidae): Transtadial Passage and the Role of Female Diet. *Folia Parasitol. (Praha)*. **2002**, *49*, 73–77, doi:10.14411/fp.2002.014.
19. Karakuş, M.; Karabey, B.; Orçun Kalkan, Ş.; Özdemir, G.; Oğuz, G.; Erişöz Kasap, Ö.; Alten, B.; Töz, S.; Özbel, Y. Midgut Bacterial Diversity of Wild Populations of *Phlebotomus* (P.) *Papatasi*, the Vector of Zoonotic Cutaneous Leishmaniasis (ZCL) in Turkey. *Sci. Rep.* **2017**, *7*, doi:10.1038/s41598-017-13948-2.
20. Monteiro, C.C.; Villegas, L.E.M.; Campolina, T.B.; Pires, A.C.M.A.; Miranda, J.C.; Pimenta, P.F.P.; Secundino, N.F.C. Bacterial Diversity of the American Sand Fly *Lutzomyia Intermedia* Using High-Throughput Metagenomic Sequencing. *Parasit. Vectors* **2016**, *9*, 480, doi:10.1186/s13071-016-1767-z.
21. De Pablos, L.; Ferreira, T.; Walrad, P. Developmental Differentiation in *Leishmania* Lifecycle Progression: Post-Transcriptional Control Conducts the Orchestra. *Curr. Opin. Microbiol.* **2016**, *34*, 82–89, doi:10.1016/j.mib.2016.08.004.
22. Kraeva, N.; Butenko, A.; Hlaváčová, J.; Kostygov, A.; Myškova, J.; Grybchuk, D.; Leštinová, T.; Votýpka, J.; Volf, P.; Opperdoes, F.; et al. *Leptomonas Seymouri*: Adaptations to the Dixenous Life Cycle Analyzed by Genome Sequencing, Transcriptome Profiling and Co-Infection with *Leishmania Donovanii*. *PLoS Pathog.* **2015**, *11*, e1005127, doi:10.1371/journal.ppat.1005127.

23. Ratcliffe, N.A.; Furtado Pacheco, J.P.; Dyson, P.; Castro, H.C.; Gonzalez, M.S.; Azambuja, P.; Mello, C.B. Overview of Paratransgenesis as a Strategy to Control Pathogen Transmission by Insect Vectors. *Parasit. Vectors* **2022**, *15*, 112, doi:10.1186/s13071-021-05132-3.
24. Hurwitz, I.; Hillesland, H.; Fieck, A.; Das, P.; Durvasula, R. The Paratransgenic Sand Fly: A Platform for Control of Leishmania Transmission. *Parasit. Vectors* **2011**, *4*, doi:10.1186/1756-3305-4-82.
25. Vivero, R.J.; Castañeda-Monsalve, V.A.; Romero, L.R.; Hurst, G.; Cadavid-Restrepo, G.; Moreno-Herrera, C.X. Gut Microbiota Dynamics in Natural Populations of *Pintomyia Evansi* under Experimental Infection with *Leishmania Infantum*. *Microorganisms* **2021**, *9*, 1214, doi:10.3390/microorganisms9061214.
26. Vaselek, S.; Sarac, B.E.; Uzunkaya, A.D.; Yilmaz, A.; Karaaslan, C.; Alten, B. Identification of *Ochrobactrum* as a Bacteria with Transstadial Transmission and Potential for Application in Paratransgenic Control of Leishmaniasis. *Parasitol. Res.* **2024**, *123*, 82, doi:10.1007/s00436-023-08087-9.
27. Lawyer, P.; Killick-Kendrick, M.; Rowland, T.; Rowton, E.; Volf, P. Laboratory Colonization and Mass Rearing of Phlebotomine Sand Flies (Diptera, Psychodidae). *Parasite* **2017**, *24*, 42, doi:10.1051/parasite/2017041.
28. Volf, P.; Volfova, V. Establishment and Maintenance of Sand Fly Colonies. *Journal of Vector Ecology* **2011**, *36*, S1–S9, doi:10.1111/j.1948-7134.2011.00106.x.
29. Dingle, T.C.; Butler-Wu, S.M. MALDI-TOF Mass Spectrometry for Microorganism Identification. *Clin. Lab. Med.* **2013**, *33*, 589–609, doi:10.1016/j.cll.2013.03.001.
30. Yıldırım, A.; Aksoy, T.; Balcıoğlu, İ.C. Comparative Assessment of Colorimetric Assays in Evaluating Intracellular Drug Susceptibility of *Leishmania Tropica* against Conventional Antileishmanial Drugs. *Parasitol. Int.* **2025**, *106*, 103021, doi:10.1016/j.parint.2024.103021.
31. Karakuş, M.; Pekağırbaş, M.; Demir, S.; Eren, H.; Töz, S.; Özbek, Y. Molecular Screening of *Leishmania* Spp. Infection and Bloodmeals in Sandflies from a Leishmaniasis Focus in Southwestern Turkey. *Med. Vet. Entomol.* **2017**, *31*, 224–229, doi:10.1111/mve.12216.
32. Gunathilaka, N.; Perera, H.; Wijerathna, T.; Rodrigo, W.; Wijegunawardana, N.D.A.D. The Diversity of Midgut Bacteria among Wild-Caught *Phlebotomus Argentipes* (Psychodidae: Phlebotominae), the Vector of Leishmaniasis in Sri Lanka. *Biomed Res. Int.* **2020**, *2020*, doi:10.1155/2020/5458063.
33. Heerman, M.; Weng, J.L.; Hurwitz, I.; Durvasula, R.; Ramalho-Ortigao, M. Bacterial Infection and Immune Responses in *Lutzomyia Longipalpis* Sand Fly Larvae Midgut. *PLoS Negl. Trop. Dis.* **2015**, *9*, doi:10.1371/journal.pntd.0003923.
34. Svobodová, M.; Alten, B.; Zídková, L.; Dvořák, V.; Hlavačková, J.; Myšková, J.; Šeblová, V.; Kasap, O.E.; Belen, A.; Votýpka, J.; et al. Cutaneous Leishmaniasis Caused by *Leishmania Infantum* Transmitted by *Phlebotomus Tobbi*. *Int. J. Parasitol.* **2009**, *39*, 251–256, doi:10.1016/j.ijpara.2008.06.016.
35. Karimian, F.; Vatandoost, H.; Rassi, Y.; Maleki-Ravasan, N.; Mohebbali, M.; Shirazi, M.H.; Koosha, M.; Choubdar, N.; Oshaghi, M.A. Aerobic Midgut Microbiota of Sand Fly Vectors of Zoonotic Visceral Leishmaniasis from Northern Iran, a Step toward Finding Potential Paratransgenic Candidates. *Parasit. Vectors* **2019**, *12*, 10, doi:10.1186/s13071-018-3273-y.
36. Karimian, F.; Koosha, M.; Choubdar, N.; Oshaghi, M.A. 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.* **2022**, *16*, e0010609, doi:10.1371/journal.pntd.0010609.
37. Tang, K.; Zhang, Y.; Meneses, C.; Rogerio, L.A.; Willen, L.; Iniguez, E.; Kamhawi, S.; Valenzuela, J.G.; Oliveira, F.; Cecilio, P. *Phlebotomus Duboscqi* Gut Microbiota Dynamics in the Context of *Leishmania* Infection. *Front. Immunol.* **2026**, *16*, doi:10.3389/fimmu.2025.1717935.
38. Tabbabi, A.; Mizushima, D.; Yamamoto, D.S.; Kato, H. Sand Flies and Their Microbiota. *Parasitologia* **2022**, *2*, 71–87, doi:10.3390/parasitologia2020008.
39. Vaselek, S. Overview of Microbial Studies in Sandflies and Their Progress toward Development of Paratransgenic Approach for the Control of *Leishmania* Sp. *Frontiers in Tropical Diseases* **2024**, *5*, doi:10.3389/fitd.2024.1369077.
40. Akhoundi, M.; Bakhtiari, R.; Guillard, T.; Baghaei, A.; Tolouei, R.; Sereno, D.; Toubas, D.; Depaquit, J.; Abyaneh, M.R. Diversity of the Bacterial and Fungal Microflora from the Midgut and Cuticle of

- Phlebotomine Sand Flies Collected in North-Western Iran. *PLoS One* **2012**, *7*, doi:10.1371/journal.pone.0050259.
41. Louradour, I.; Monteiro, C.C.; Inbar, E.; Ghosh, K.; Merkhofer, R.; Lawyer, P.; Paun, A.; Smelkinson, M.; Secundino, N.; Lewis, M.; et al. The Midgut Microbiota Plays an Essential Role in Sand Fly Vector Competence for *Leishmania Major*. *Cell. Microbiol.* **2017**, *19*, e12755, doi:10.1111/cmi.12755.
 42. Campolina, T.B.; Villegas, L.E.M.; Monteiro, C.C.; Pimenta, P.F.P.; Secundino, N.F.C. Tripartite Interactions: *Leishmania*, Microbiota and *Lutzomyia Longipalpis*. *PLoS Negl. Trop. Dis.* **2020**, *14*, e0008666, doi:10.1371/journal.pntd.0008666.
 43. Sabbahi, R.; Hock, V.; Azzaoui, K.; Hammouti, B. *Leishmania*–Sand Fly Interactions: Exploring the Role of the Immune Response and Potential Strategies for Leishmaniasis Control. *Journal of Parasitic Diseases* **2024**, *48*, 655–670, doi:10.1007/s12639-024-01684-0.
 44. Cecilio, P.; Rogerio, L.A.; D. Serafim, T.; Tang, K.; Willen, L.; Iniguez, E.; Meneses, C.; Chaves, L.F.; Zhang, Y.; dos Santos Felix, L.; et al. *Leishmania* Sand Fly-Transmission Is Disrupted by *Delftia Tsuruhatensis* TC1 Bacteria. *Nat. Commun.* **2025**, *16*, 3571, doi:10.1038/s41467-025-58769-4.
 45. Diaz-Albiter, H.; Sant'Anna, M.R.V.; Genta, F.A.; Dillon, R.J. Reactive Oxygen Species-Mediated Immunity against *Leishmania Mexicana* and *Serratia Marcescens* in the Phlebotomine Sand Fly *Lutzomyia Longipalpis*. *Journal of Biological Chemistry* **2012**, *287*, 23995–24003, doi:10.1074/jbc.M112.376095.
 46. Ghassemi, M.; Akhavan, A.A.; Zahraei-Ramezani, A.; Yakhchali, B.; Zarean, M.R.; Jafari, R.; Oshaghi, M.A. Assessing Survival of Transgenic Bacteria, *Serratia AS1* and *Enterobacter Cloacae*, in Sugar Bait, White Saxaul Plant (*Haloxylon Persicum*) and Rodent Barrow's Soil, A Contained-Field Study for Paratransgenesis Approach. *J. Arthropod. Borne. Dis.* **2024**, doi:10.18502/jad.v18i1.15668.
 47. Duque-Granda, D.; Vivero-Gómez, R.J.; Junca, H.; Cadavid-Restrepo, G.; Moreno-Herrera, C.X. Interaction and Effects of Temperature Preference under a Controlled Environment on the Diversity and Abundance of the Microbiome in *Lutzomyia Longipalpis* (Diptera: Psychodidae). *Biotechnology Reports* **2024**, *44*, e00857, doi:10.1016/j.btre.2024.e00857.
 48. Daoudi, M.; Outamassine, A.; Redouane, E.; Loqman, S.; Hafidi, M.; Boumezzough, A.; Olivier, M.; Boussaa, S.; Ndao, M. Characterization of Gut Bacteria in Natural Populations of Sand Flies (Diptera: Psychodidae) from Endemic and Non-Endemic Areas of Leishmaniasis in Morocco. *Microorganisms* **2025**, *13*, 2279, doi:10.3390/microorganisms13102279.
 49. Yaghoobi, M.; Moridi Farimani, M.; Khan, A.; Asadollahi, M.; Omrani, M.; Luyten, W.; Hu, H. Investigation of Phytochemical Profiling and Biological Activities of Methanol Extract from *Eryngium Billardieri*: Antimicrobial, Antibiofilm, and Anthelmintic Properties. *Front. Plant Sci.* **2025**, *16*, doi:10.3389/fpls.2025.1667335.
 50. Alabaz, D.; Eroğlu, F.; Elçi, H.; Çay, Ü. Identification of *Leishmania Tropica* from Pediatric Visceral Leishmaniasis in Southern Mediterranean Region of Turkey. *Mediterr. J. Hematol. Infect. Dis.* **2022**, *14*, e2022053, doi:10.4084/MJHID.2022.053.
 51. Özbilgin, A.; Töz, S.; Harman, M.; Günaştı Topal, S.; Uzun, S.; Okudan, F.; Güngör, D.; Erat, A.; Ertabaklar, H.; Ertuğ, S.; et al. The Current Clinical and Geographical Situation of Cutaneous Leishmaniasis Based on Species Identification in Turkey. *Acta Trop.* **2019**, *190*, 59–67, doi:10.1016/j.actatropica.2018.11.001.
 52. Mayilsamy, M.; Vijayakumar, A.; Veeramanocharan, R.; Rajaiah, P.; Balakrishnan, V.; Kumar, A. Impact of COVID-19 Lockdown during 2020 on the Occurrence of Vector-Borne Diseases in India. *J. Vector Borne Dis.* **2023**, *60*, 207, doi:10.4103/0972-9062.364762.
 53. Cleanclay, W.D.; Kernyuy, F.B.; Kintung, I.F.; Yensii, N.G.; Chick, J.A.; Obi, A.M.M. Evaluating Paratransgenesis Using Engineered Symbiotic Bacteria for Plasmodium Inhibition in Mosquito Vectors: A Systematic Review. *PLoS Negl. Trop. Dis.* **2026**, *20*, e0013654, doi:10.1371/journal.pntd.0013654.
 54. Sasser, D.; Epis, S.; Pajoro, M.; Bandi, C. Microbial Symbiosis and the Control of Vector-Borne Pathogens in Tsetse Flies, Human Lice, and Triatomine Bugs. *Pathog. Glob. Health* **2013**, *107*, 285–292, doi:10.1179/2047773213Y.0000000109.
 55. Yilmaz, A.; Kasap, O.E. Prevalence of *Wolbachia* in Natural Sand Fly (Diptera: Psychodidae) Populations from Türkiye and Its Potential Role in Mitochondrial Divergence. *Parasit. Vectors* **2025**, *19*, 16, doi:10.1186/s13071-025-07157-4.

56. Karami, M.; Hassan Moosa-Kazemi, S.; Ali Oshaghi, M.; Vatandoost, H.; Mehdi Sedaghat, M.; Rajabnia, R.; Hosseini, M.; Maleki-Ravasan, N.; Yahyapour, Y.; Ferdosi-Shahandashti, E. *Wolbachia Endobacteria in Natural Populations of Culex Pipiens of Iran and Its Phylogenetic Congruence*; 2016; Vol. 10;.
57. Brelsfoard, C.; Tsiamis, G.; Falchetto, M.; Gomulski, L.M.; Telleria, E.; Alam, U.; Doudoumis, V.; Scolari, F.; Benoit, J.B.; Swain, M.; et al. Presence of Extensive Wolbachia Symbiont Insertions Discovered in the Genome of Its Host *Glossina Morsitans Morsitans*. *PLoS Negl. Trop. Dis.* **2014**, *8*, doi:10.1371/journal.pntd.0002728.
58. Kiplagat, S.; Matoke-Muhia, D.; Owino, B.O.; Tchouassi, D.P.; Masiga, D.K.; Hurst, G.D.D.; Villinger, J. Sand Fly Endosymbionts in Kenya: Rickettsia and Wolbachia Associations with Leishmania and Detection of Rickettsia Africae. *Parasit. Vectors* **2026**, *19*, 117, doi:10.1186/s13071-026-07283-7.

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