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First Report of Isolation of Two Endosymbiotic Symbiosis Bacteria " *Arsenophonus arthropodicus* and *Wolbachia*" from *Pseudolynchia canariensis* (Diptera: Hippoboscidae) Infesting Pigeons

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Abstract

ENDISYMBIOTIC bacteria are essential in the evolutionary ecology of insects as they act as both discrete parasites that affect host reproduction and partners that assist in host adaptability. Symbiotic microorganisms facilitate intimate connections between many species. *Pseudolynchia canariensis* is a significant ectoparasite of pigeons belonging to the hippoboscidae fly family. Methods: A total of 114 domestic pigeons in a herd situated in Al-Baha, a region in southwest Saudi Arabia, were found to be clinically affected by ectoparasites. The precise molecular mechanisms that govern the relationships between insects and these microbes are still not well comprehended, despite the breakthroughs in molecular tools that allow for the observation of endosymbiotic partnerships in living organisms. A symbiont-specific PCR amplification assay was utilized to assess the results of symbiont infection and determine the molecular phylogeny. Results: 20 individuals of *Pseudolynchia canariensis* were collected from pigeons. The specimens were discovered to be associated with two different types of endosymbiotic bacteria. Out of the 20 *Pseudolynchia canariensis,* 2% and 60% were positive for endosymbiotic bacteria. Wolbachia exhibited the highest prevalence, with an infection rate (wsp) of 60%, whereas Arsenophonus had a prevalence of 2%. The prevalence of Wolbachia infestation has exceeded that of Arsenophonus, reaching a rate of 60%. Molecular methods have shown the whole endosymbiotic community of *P. canariensis* flies, including endosymbionts, which may explain how many bacterial endosymbionts can live together in the host. Given the potential significance of Wolbachia and Arsenophonus in impeding disease transmission and controlling populations of detrimental insects.

Keywords: Ectoparasites, Pathogen, Pigeon fly, Phylogeny,

Introduction

Insect-bacterial symbioses encompass a wide range of kinds, varying from casual facultative symbioses to highly intimate and enduring obligatory symbioses. There is a close and regular interaction between bacterial symbionts transmitted by mothers and insects. Although numerous genetic symbionts are not essential for the survival of the host, they usually behave as conditional mutualists by offering protection against specific environmental stressors [1- 2]. The method by which symbionts are acquired is a crucial component that influences the symbiotic relationship. Vertical transmission, in particular, creates a situation where there is a limited number of individuals in the population. This not only decreases conflicts between different variants of the symbiont inside a host, but also ensures that the success of transmission for the microbe is closely tied to the reproductive capacity of the host [3]. To comprehend how evolution benefits from a change in transmission method, and the resulting evolutionary outcomes of these shifts, a thorough comprehension of clades that maintain diversity in this element of symbiosis is necessary. While previous studies in animal microbiology have primarily focused on identifiable pathogens and beneficial organisms in humans and domesticated ruminants (such as cattle), there is a growing fascination with the ecological aspects of microorganisms in many animals, including insects [4]. The primary ecological concerns encompass the diversity of microorganisms within individual animals, the allocation of microbial groups across different animal species, and the comparative

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significance of the animal's habitat and independent existence. During such instances, endosymbionts typically enhance the hosts' nutritional intake by providing vitamins or amino acids that are scarce or lacking in the available food sources. Their colonization by hosts may have occurred in the distant evolutionary past, dating back to the emergence of significant groups such as aphids, leafhoppers, or carpenter ants. This colonization is linked to a unique method of transmission from one generation to the next, which follows the speciation of the host. This process leads to a parallel diversification of both the host and the endosymbiont *Buchnera aphidicola*, as exemplified by aphids [5-6]. Flies belonging to the Hippoboscoidea superfamily (Diptera) are blood feeders that are dependent on blood for survival. They are associated with a wide range of bacterial endosymbionts. There are four well-established family-level groups within Hippoboscoidea: Glossinidae, which consists of 22 species of tsetse flies that primarily feed on mammals but also on reptiles and birds to a lesser extent; Hippoboscidae, which includes over 150 species of louse flies that feed on both birds and mammals; and two families of bat flies, Streblidae with over 230 species and Nycteribiidae with over 270 species, both exclusively feeding on bats [7] . In contrast, louse flies and bat flies have received significantly less attention. However, a recent discovery revealed that Arsenophonus, another type of endosymbiont, is unusually prevalent among louse flies and bat flies [9-10]. Arsenophonus has recently emerged as one of the most diversified symbiotic lineages known to date. It has been reported to infect a wide range of insect taxa, including aphids, parasitoid wasps, and triatomine bugs [11-12] Arsenophonus strains, formerly identified as Phlomobacter sp., were discovered in the phloem of plants that were fed upon by infected phytophagous insects. These strains were considered opportunistic plant diseases [13]. Additional studies have discovered that Arsenophonus belongs to a newly identified group of endosymbionts within the c-proteobacteria. These bacteria, known as Arsenophonus and similar organisms (ALOs), are phylogenetically related to Arsenophonus. In lice, they have been identified as Candidatus Riesia pediculicola [14]. Hippoboscidae, often known as louse flies, have been frequently observed in association with Arsenophonus bacteria. A new species of bacteria, named Arsenophonus apicola, was discovered from the honeybee species Apis mellifera [15-17]. The genus Wolbachia is a parasitic bacterium similar to rickettsia that is exclusively present in specific populations of tsetse flies, as well as some other insects, mites, crustaceans, and filarial worms. Arsenophonus is another intriguing bacterium [18-19]. We have identified a single species of *Wolbachia pipientis*, a commonly occurring intracellular bacteria that is transmitted vertically and is naturally found in the model organism *Drosophila melanogaster*. [20-22]. Remarkably, the infection of *W. pipientis* partially restores the fertility of female flies that have hypomorph mutations of bag of marbles (bam) in D. melanogaster. This gene is involved in the maintenance and development of germline stem cells (GSCs) [23]. The present study focuses on the characterization of two endosymbionts, Arsenophonus and Wolbachia pip, which inhabit the tissues of a pigeon species called *P.canariensis*. In this work, we want to examine the range of bacterial species associated with *P. canariensis* by utilizing species-specific PCR primers known as "Candidatus Arsenophonus endosymbionts" and "Wolbachia". This recently identified endosymbiont is characterized by its close phylogenetic association with other members of the Arsenophonus and *Wolbachia pipintis* genera.

Material and Methods

Description of the study area, collection, identification and DNA extraction of fly

From November 2021 to September 2022, a total of 114 pigeon specimens were taken and subjected to examination at Al-Baha City, located in southwest Saudi Arabia at coordinates 19° 51′ 34″ N and 41° 33′ 26″ E. The region has a semi-arid climate. Every individual of *P. canariensis* was gathered by hand from the host and transferred into Eppendorf tubes containing 70% ethanol. The identification of *P. canariensis* was conducted in the Laboratory of Parasitology at Al-Baha University using a stereomicroscope, as described in other research [24-25]. The *P. canariensis* samples were homogenized using a mini-prep DNA isolation kit called GeneAll® Exgene™ Clinic SV DNA Isolation Kit, manufactured by Biotechnology Co., Ltd. in Seoul, Republic of Korea. The entire DNA was extracted and collected in a 30 µL solution of elution buffer, which consisted of Tris buffer with a pH of 8.5 and was preheated to a temperature of 70 °C.

PCR amplification for Screening and sequencing for bacterial endosymbionts

PCR amplification was performed using DNA samples collected from each *P. canariensis* as a template. The first set of PCR primers, CAIF (5-GCC TGA TGC AGC CAT GCC GCG TGT ATG-3') and CAIR (5-GTC ATC CCC ACC TTC C-3'), were initially created to amplify a 500-bp segment of the "Candidatus Arsenophonus arthropodicus" [26]. The identification of "*Candidatus Arsenophonus arthropodicus*" was carried out using polymerase chain reactions (PCRs) with 5µl of DNA template, 1 µl (10 pmol) of each primer (tootling 30 µl reaction volume), and 12.5 units of Taq DNA polymerase (Promega) per microliter of reaction mixture. The cycling conditions comprised an initial denaturation step at 95°C for 4 minutes, followed by 30 cycles of

amplification involving denaturation at 95°C for 30 seconds, annealing at 65°C for 1 minute, and extension at 72°C for 1 minute. The process concluded with a final extension step at 72°C for 4 minutes.

Further PCR screening identified endosymbionts other than those found in the Arsenophonus-positive samples. *P. canariensis* was shown to be infected with Wolbachia. The species-specific primer setswsp81F(TGGTCCAATAAGTGATGAAGAAA C-3) and wsp691R (AAAAATTAAACGCTACTCCA-3) were used to perform conventional PCR [27]. Wsp PCRs were conducted using a 40 µL reaction mixture consisting of 20 µL 2X BioMix Red BIOTAQ DNA polymerase (Bioline, UK), 1 µl each primer (final concentration, 0.4 µM), and 2 µl of extracted DNA. The reaction mixtures underwent a thermal cycle consisting of an initial activation step at 94°C for 2 minutes, followed by 35 cycles at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. The final step involved an extension at 72°C for 5 minutes. The PCR products were observed using 1.5% agarose gel electrophoresis with SYBR Safe dye (Invitrogen) at a voltage of 150 V for a duration of 30 minutes. The PCR products were separated by electrophoresis using a 1.5% agarose gel. The amplification products were sequenced bidirectionally at Macrogen in South Korea .

Phylogenetic analyses

Based on the neighbor-joining analysis conducted in this work (Fig. 1), it has been determined that the Arsenophonus and Wolbachia group of arthropod endosymbionts forms a strong clade, which is supported by 100% of bootstrap resamples. The raw sequence data was processed and aligned using the BIOEDIT software with the CLUSTALW algorithm [28], both of which were implemented in MEGA [29]. Subsequently, the data was evaluated using BLAST, a tool provided by the National Centre for Biotechnology Information (NCBI). Recently, a set of fresh genetic information has been provided to GenBank, specifically pertaining to Wolbachia (with Accession Numbers OR636836-OR636846) and Arsenophonus (OR982690- OR982692).

Results

The DNA templates obtained from samples of *P. canariensis* contained Arsenophonus bacteria. Arsenophonus symbiotic bacteria were detected using a normal PCR method on a DNA template, and the quality of the DNA was evaluated by CIA PCR amplification. Out of the 20 DNA templates analyzed, four of them were found to be positive with the Arsenophonus bacteria in the Pseudolynchia species known as *Pseudolynchia canariensis*. Genomic analysis of Arsenophonus DNA in *Pseudolynchia canariensis*. Analyzed were the DNA samples taken from each screened endosymbiont of *Pseudolynchia canariensis*. The CIAF/CIAR primers yielded amplified products with a length of 500 base pairs. The gene segments were directly sequenced and then matched with the CIA genes present in the gene bank for Arsenophonus endosymbiosis (accession number OR982690 -OR982692). The analysis revealed that the four samples isolates exhibited complete nucleotide similarity (100%) with sequences originating from USA, South Bohemia (DQ115536.1, MF429875, AY264673, and MF429868). Further PCR screening detected Wolbachia, in addition to Arsenophonus, in twelve samples. The DNA templates obtained from *P. canariensis* samples were found to include Wolbachia bacteria. The traditional PCR approach was used to detect the presence of Wolbachia symbionts bacteria in the DNA template. The quality of the DNA template was tested by performing wsp PCR amplification. Out of the 20 DNA templates analyzed, 12 of them were found to be infected with Wolbachia in the *Pseudolynchia* species known as *P. canariensis*. Analyzed was the DNA isolated from each screened endosymbiont of *P. canariensis*. The wsp (81F/ 691R) primers yielded 500 basepair amplification products. The gene fragments were directly sequenced and then aligned with the wsp genes from the gene bank, namely those with accession numbers OR636835-OR636846, which are associated with Wolbachia endosymbiosis. The BLASTn tests revealed that all four samples isolates had 99%-100% similarity with an identical strain of *Wolbachia pipientis,* which is comparable to the *Candidatus Wolbachia arthropodicus* found in Finland.

Phylogenetic analyses

The present study involved analyzing the amplified DNA sequences and conducting database searches to identify similarities between the CIA and wsp sequences and Arsenophonus. The phylogenetic trees in Figure 1 provide further details on the CIA gene. Furthermore, the evolutionary trees in Figure 2 demonstrate the affiliation of Wolbachia to the wsp gene.

Discussion

The presence of Arsenophonus and Wolbachia was examined in 20 samples using the CAIF&R primers and a Wolbachia-specific wsp-based PCR test. The screen displayed that the infection rate of Wolbachia was 60%, while the infection rate of Arsenophonus was just 2%. This is new symbiont occupies a distinct from the bacteria that house domestic pigeons in *P. canariensis*.

The current investigation focused on *P. canariensis*, specifically examining the presence of Arsenophonus and Wolbachia in various geographic areas and host species. Previous studies have identified the presence of two distinct endosymbiotic

bacteria in various arthropods through targeted surveys. These arthropods include *triatomine bugs* [30], ticks [31], whiteflies [32], aphids [33-34], soybean [35] and psyllids [36]. The specific molecular connections between hosts and symbionts that have conferred insects with their exceptional resistance to a particular type of endosymbionts associated with bacteria remain mostly ambiguous. In this study, we present evidence demonstrating that the blood-feeding hippoboscid *P. canariensis* has an endosymbiotic bacteria that is closely linked to the wolbachia spp. and Arsenophonus group.

Based on the phylogenetic study, the size of "*Candidatus* Arsenophonus arthropodicus" is most similar to the *Arsenophonus spp*. that have been described Prior to this, there have been little research publications on this subject in Saudi Arabia [37]. Despite the identification of Arsenophonus group members in several arthropod species, our understanding of their role in symbiosis remains limited. Nevertheless, there have been proposals indicating that Arsenophonus might serve a protective function [38]. Phylogenetic investigations suggest a close relationship between Arsenophonus arthropodicus and the Arsenophonus endosymbionts discovered in fruit flies, whiteflies, psyllids, aphids, and mealybugs [39-43]. In addition, we focused our attention on Arsenophonus symbionts and *Wolbachia* spp., both of which are commonly found in louse flies.

Four Arsenophonus endosymbionts (2%) were identified in this investigation. Unlike the previous investigation, they successfully got a pure culture isolation of *Candidatus* Arsenophonus arthropodicus, a recently identified species found in the louse fly *P. canariensis* [26]. *Arsenophonus nasoniae*, a bacterium found in the parasitoid wasp *Nasonia vitripennis*, is known to cause a sex ratio distortion that favors females. This phenomenon is comparable to what is observed in certain strains of Wolbachia bacteria. Their occurrence and dispersion can be elucidated by ecological and climatic factors that are conducive or detrimental to the proliferation of flies in specific nations. Recent studies have shown that Wolbachia Cif (CI factor) proteins modify the host sperms, leading to embryonic lethality that defines CI [44]. Our results indicate that *Wolbachia pipientis*, a type of endosymbiotic bacteria, was the most prevalent, accounting for nearly 60% of the observed cases. Specifically, *Wolbachia pipientis* was detected in *P. canariensis*. This may lead to the integration of Wolbachia's capacity to proliferate arthropod populations, which has generated curiosity in utilizing them as a means to transmit advantageous traits (such as disease resistance) into populations of insect vectors. This species exhibits a combination of mutually beneficial symbiotic relationships and genetically altered variations. Additionally, it naturally carries internal bacteria that are transferred from one generation to the next. *Drosophila melanogaster* is the scientific name for a species of fruit fly [45]. An assessment has been conducted to determine the impact of temperature on maternal transmission and the underlying patterns of Wolbachia localization in 10 different strains of Wolbachia. These strains have diverged up to 50 million years and include strains that are closely related to wMel, as well as their natural hosts, Drosophila. Despite the variability and often low quantities of Wolbachia in the ovaries and developing germline in late-stage oocytes, many Wolbachia are able to sustain high transmission rates across different temperatures [46]. Moreover, Wolbachia may be intentionally introduced across different groups of organisms by the process of microinjection into eggs. This demonstrates their ability to successfully inhabit several types of arthropod cells. Nevertheless, there is variation in the permissiveness of hosts and Wolbachia strains might exhibit dissimilarities in their capacity to infect various host species. These impacts require systematic investigation.

Previous investigations have revealed two recent findings that are highly pertinent to the issue of inter taxon transmission. Wolbachia, like to all Rickettsiales, are bacteria that must live inside host cells and it has been presumed that they are unable to thrive outside of these cells. However, in the tissue culture tests, it was demonstrated that Wolbachia continued to exist for a significant period of time even after the host cells had perished [47,48]. An evident anomaly to this principle arises with Wolbachia, the symbiotic organism that is most extensively prevalent in arthropods and worms [19]. The Arsenophonus groups are the only other closely related arthropod endosymbionts that are found in a wide range of hosts. These bacteria have a sequence similarity of over 99% to their 16S rRNA and wasp genes. Crucially, it has never been identified in arthropods or mammalian bloodstream. This paper is the inaugural investigation conducted on *P. canariensis*. Thus, these two categories of bacteria offer a fresh opportunity to investigate the connections between Arsenophonus and Wolbachia in the Hippoboscid Louse Fly *P. canariensis* in Al-Baha city.

Conclusion

Symbiosis creates opportunities for hosts to occupy previously unexploited ecological niches, perhaps leading to an increased rate of speciation. Our analysis using PCR-based screening and sequencing techniques on *P. canariensis* from Saudi Arabia has provided new and unique information on the early stages of the shift from an independent existence to a reliance on a host in this bacterial genome. In this study we demonstrated that

Wolbachia was the most prevalent bacteria compared to the other detected bacteria, Arsenophonus. Through the use of molecular methods, the entire endosymbiotic consortium of *P. canariensis* flies has been revealed. These endosymbionts may help to reveal the scientific reason behind the co-existence of bacterial endosymbionts within the *P. canariensis* host. This information adds to the knowledge that Wolbachia and Arsenophonus may be beneficial for preventing the spread of pathogens and controlling insect pests as well.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of Al-Baha University, (ethics approval number; 8/1442).

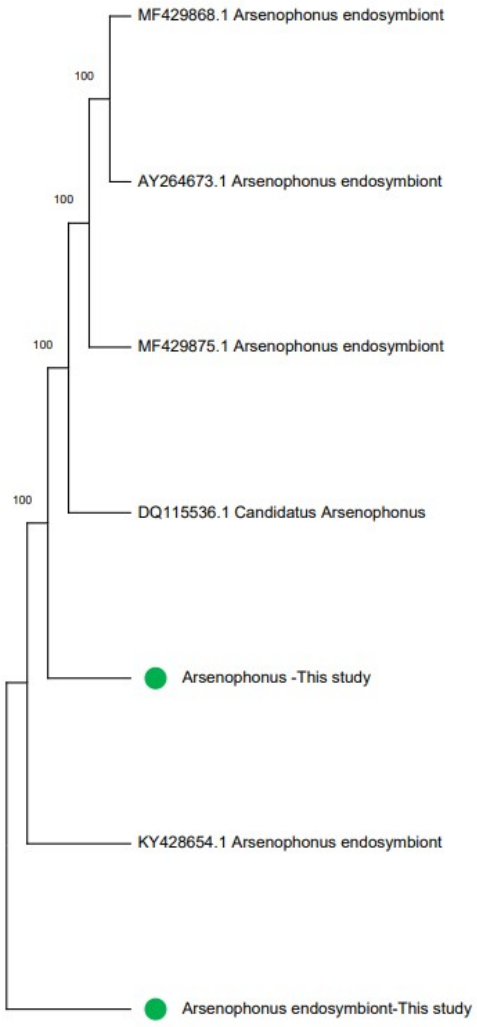

Fig.1. Phylogenetic relationships based on the (CIA) gene. Neighbor-joining phylogenetic tree of Arsenophonus-infected *P. canariensis* based on 500 bp of (CIA) gene sequences. Bootstrap values are shown on the branches. Sequences generated from the present study are indicated in green color with other related sequences retrieved from GenBank.

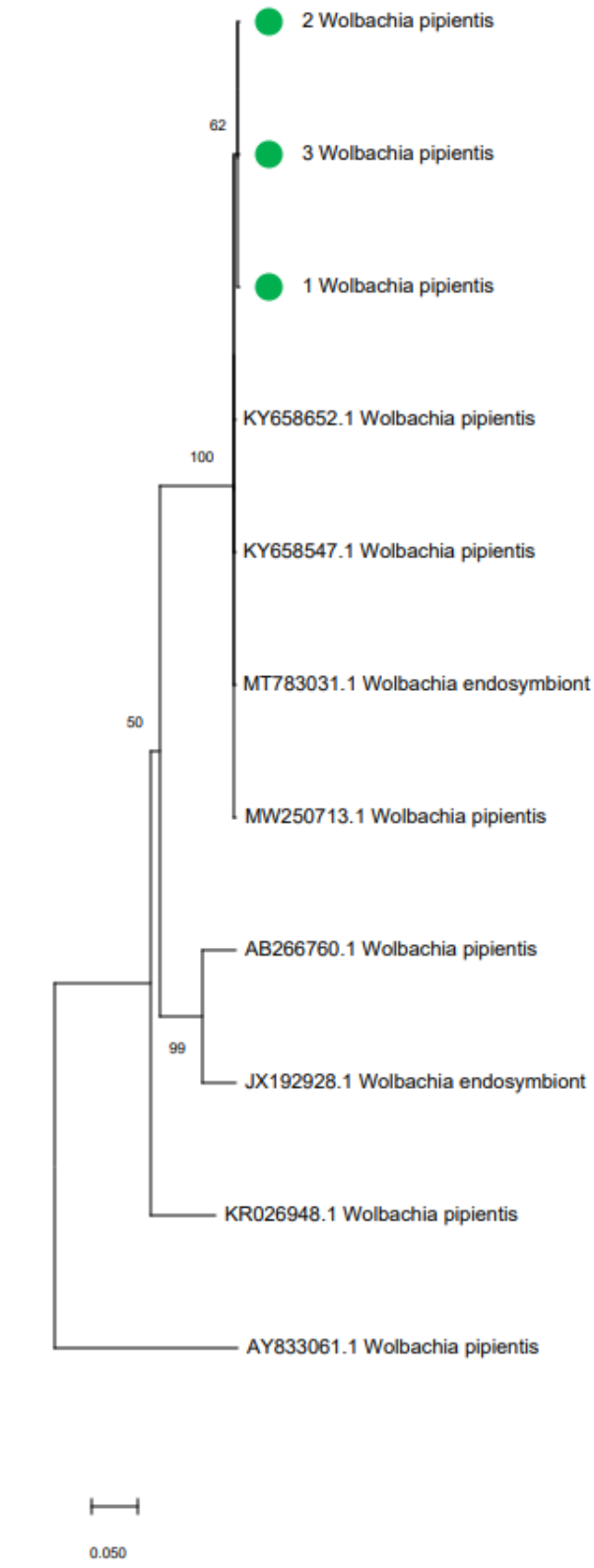

Fig. 2. Phylogenetic relationships based on the (wsp) gene. Neighbor-joining phylogenetic tree of Wolbachia-infected *P. canariensis* based on 500 bp of (wsp) gene sequences. Bootstrap values are shown on the branches. Sequences generated from the present study are indicated in green color with other related sequences retrieved from GenBank.

References

1. Werren, J. H., Baldo, L. and Clark, M.E. Wolbachia: Master manipulators of invertebrate biology. *Nature Reviews Microbiology*, **6** (10), 741-751 (2008). Doi.org/10.1038/nrmicro1969.

2. Wilkes, T. E., Duron, O., Darby, A. C., Hypša, V., Nováková, E. and Hurst, G. D. 12 the genus arsenophonus. *Manipulative tenants: bacteria associated with arthropods*, Book, P 225-241 (2011).

3. Leeks, A., Dos Santos, M. and West, S. A. Transmission, relatedness, and the evolution of cooperative symbionts. *Journal of Evolutionary Biology*, **32**(10), 1036-1045 (2019). Doi: 10.1111/jeb.13505

4. Douglas, A. E. The ecology of symbiotic micro-organisms. *Advances in Ecological Research*, **26**, 69-103 (1995). Doi: 10.1016/S0065-2504(08)60064-1

5. Michalik, A., Franco, D. C., Deng, J., Szklarzewicz, T., Stroiński, A., Kobiałka, M. and Łukasik, P. Variable organization of symbiont-containing tissue across planthoppers hosting different heritable endosymbionts. *Frontiers in Physiology*, **14**, 1135346 (2023). DOI: 10.3389/fphys.2023.1135346.

6. Liang, Y., Dikow, R. B., Su, X., Wen, J. and Ren, Z. Comparative genomics of the primary endosymbiont Buchnera aphidicola in aphid hosts and their coevolutionary relationships. *BMC Biology*, **22**(1), 137 (2024). Doi:10.1186/s12915-024-01934-w

7. Petersen, F. T., Meier, R., Kutty, S. N. and Wiegmann, B. M. The phylogeny and evolution of host choice in the hippoboscoidea (diptera) as reconstructed using four molecular markers. *Molecular Phylogenetics and Evolution*, **45**(1), 111-122 (2007). Doi: 10.1016/j.ympev.2007.04.023

8. Dittmar, K., Porter, M. L., Murray, S. and Whiting, M. F. Molecular phylogenetic analysis of nycteribiid and streblid bat flies (diptera: Brachycera, calyptratae): Implications for host associations and phylogeographic origins. *Molecular Phylogenetics and Evolution*, **38**(1), 155-170 (2006). Doi: 10.1016/j.ympev.2005.06.008

9. Vogel, K. J. and Kerri L. C. "Functions and mechanisms of symbionts of insect disease vectors." In *Advances in Insect Physiology*, **58**, 233-275. Academic Press, (2020). Doi: 10.1016/bs.aiip.2020.03.004

10. Trowbridge, R. E., Dittmar, K. and Whiting, M. F. Identification and phylogenetic analysis of arsenophonus and photorhabdus-type bacteria from adult hippoboscidae and streblidae (hippoboscoidea). *Journal of Invertebrate Pathology*, **91**(1), 64-68 (2006). Doi: 10.1016/j.jip.2005.08.009

11. Nováková, E., Hypša, V. and Moran, N. A. Arsenophonus, an emerging clade of intracellular symbionts with a broad host distribution. *BMC Microbiology*, **9**, 1-14 (2009). Doi: 10.1186/1471-2180-9-143

12. Jousselin, E., Cœur d'Acier, A., Vanlerberghe-Masutti, F. and Duron, O. Evolution and diversity of A rsenophonus endosymbionts in aphids. *Molecular Ecology*, **22**(1), 260-270 (2013). Doi: org/10.1111/mec.12092

13. Bojan, D., Stepanović, J., Fránová, J., Zwolińska, A., Rekanović, E., Stepanović, M., Vučković, N., Duduk, N., and Vico, I. Geographical variations, prevalence, and molecular dynamics of fastidious phloem-limited pathogens infecting sugar beet across Central Europe. *PloS One*, **19**(7), e0306136 (2024). Doi: 10.1371/journal.pone.0306136.

14. Allen, J. M., Reed, D. L., Perotti, M. A. and Braig, H. R. Evolutionary relationships of "*Candidatus riesia spp*.," endosymbiotic enterobacteriaceae living within hematophagous primate lice. *Applied and Environmental Microbiology*, **73**(5), 1659-1664 (2007). Doi: 10.1128/AEM.01877-06

15. Duron, O., Schneppat, U. E., Berthomieu, A., Goodman, S. Droz, M. and Origin, B., acquisition and diversification of heritable bacterial endosymbionts in louse flies and bat flies. *Molecular Ecology*, **23**(8), 2105-2117 (2014). Doi: 10.1111/mec.12704

16. Gaggìa, F., Jakobsen, R. R., Alberoni, D., Baffoni, L., Cutajar, S., Mifsud, D., Nielsen, D. S. and Di Gioia, D. Environment or genetic isolation? An atypical intestinal microbiota in the Maltese honey bee Apis mellifera spp. ruttneri. *Frontiers in Microbiology*, **14**, 1127717 (2023). Doi: 10.3389/fmicb.2023.1127717.

17. Trowbridge, R.E., Dittmar. K. and Whiting, M.F. Identification and phylogenetic analysis of arsenophonus-and photorhabdus-type bacteria from adult hippoboscidae and streblidae (hippoboscoidea). *J. Invertebr. Pathol*., **91** (1), 64-68 (2006). Doi: 10.1016/j.jip.2005.08.009

18. Werren, J.H. Biology of wolbachia. *Annu Rev Entomol*., **42** (1), 587-609 (1997). Doi: 10.1146/annurev.ento.42.1.587

19. Bandi, C., Anderson, T.J., Genchi, C. and Blaxter, M.L. Phylogeny of wolbachia in filarial nematodes. *Proceedings of the Royal Society of London.Series B: Biological Sciences*, **265** (1413), 2407-2413 (1998). Doi: 10.1098/rspb.1998.0591

20. Vavre, F., Fleury, F., Lepetit, D., Fouillet , P. and Boulétreau, M. Phylogenetic evidence for horizontal transmission of wolbachia in host-parasitoid associations. *Mol. Biol. Evol*., **16** (12), 1711-1723 (1999). Doi: 10.1093/oxfordjournals.molbev.a026084

21. Nevalainen, L.B. and Newton, I.L. Detection and assessment of wolbachia pipientis infection. In: *Drosophila oogenesis: Methods and protocols.* Springer, 291-307 (2023). Doi: 10.1007/978-1-0716-2970-3_15

22. Gerth, M., Gansauge, M., Weigert, A. and Bleidorn, C. Phylogenomic analyses uncover origin and spread of the wolbachia pandemic. *Nature Communications*, **5**(1), 5117 (2014). Doi: 10.1038/ncomms6117

23. Kagemann, C.H., Colocho, G.M. and Aquadro, C.F. Gene expression changes in drosophila melanogaster females associated with the rescue of the bag of marbles (bam) hypomorph fertility defect by wolbachia pipientis. *bioRxiv*., **22**, 558898 (2023). Doi.10.1101/2023.09.22.558898

24. Rahola, N.S. M. Goodman, and Vincent, R. "The Hippoboscidae (Insecta: Diptera) from Madagascar, with new records from the "Parc National de Midongy Befotaka"." *Parasite: journal de la Société Française de Parasitologie,* **18**(2), 82127 (2011). Doi: 10.1051/parasite/2011182127

25. Erdem, ˙ I., Zerek, A. andYaman, M. The first record Pseudolynchia canariensis (Diptera: Hippoboscidae) in an Eurasian eagle owl (Bubo bubo Linnaeus, 1758) in Turkey. *Kafkas Univ. Vet. Fak. Derg.,* **25**, 887–888 (2019). Doi: 10.9775/ kvfd.2019.22882

26. Dale, C., Beeton, M., Harbison, C., Jones, T. and Pontes, M. Isolation, pure culture, and characterization of "*Candidatus arsenophonus arthropodicus,*" an intracellular secondary endosymbiont from the hippoboscid louse fly *Pseudolynchia canariensis*. *Applied and Environmental Microbiology*, **72**(4), 2997-3004 (2006). Doi: 10.1128/AEM.72.4.2997-3004.2006

27. Plichart, C. and Legrand, A.M. Detection and characterization of Wolbachia infections in Wuchereria bancrofti (Spirurida: Onchocercidae) var. pacifica and Aedes (Stegomyia) polynesiensis (Diptera: Culicidae). *Am. J. Trop. Med. Hyg*., **73**(2), 354-358 (2005)

28. Thompson, J. D., Toby, J. G. and Des, G. H. "Multiple sequence alignment using ClustalW and ClustalX. *Curr Protoc. Bioinformatics,* **1**(Chapter 2:Unit 2.3), s00 (2003). Doi: 10.1002/0471250953.bi0203s00

29. Kumar Sudhir, Koichiro Tamura and Masatoshi Nei. "MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment." *Briefings in bioinformatics,* **5**(2), 150-163(2004). Doi: 10.1093/bib/5.2.150

30. Hypša, V. and Dale, C. In vitro culture and phylogenetic analysis of "Candidatus arsenophonus triatominarum," an intracellular bacterium from the triatomine bug, triatoma infestans. *Int. J. Syst. Evol. Microbiol*., **47** (4), 1140-1144 (1997). Doi: 10.1099/00207713-47-4-1140

31. Grindle, N., Tyner, J.J., Clay, K. and Fuqua, C. Identification of arsenophonus-type bacteria from the dog tick dermacentor variabilis. *J. Invertebr. Pathol*., **83** (3), 264-266 (2003). Doi: 10.1016/s0022-2011(03)00080-6

32. Thao, M.L. and Baumann, P. Evidence for multiple acquisition of arsenophonus by whitefly species (sternorrhyncha: Aleyrodidae). *Curr. Microbiol*., **48**, 140-144 (2004). Doi: 10.1007/s00284-003-4157-7

33. Russell, J.A., Latorre, A., Sabater-Muñoz, B., Moya, A. and Moran, N.A. Side-stepping secondary symbionts: Widespread horizontal transfer across and beyond the aphidoidea. *Mol. Ecol*., **12** (4), 061-1075 (2003). Doi: 10.1046/j.1365-294x.2003.01780.x

34. Tsuchida, T., Koga, R., Shibao H., Matsumoto, T. and Fukatsu, T. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, acyrthosiphon pisum. *Mol. Ecol*., **11** (10), 2123-2135 (2002). Doi: 10.1046/j.1365-294x.2002.01606.x

35. Wulff, J.A., Buckman, K.A., Wu, K., Heimpel, G.E. and White, J.A. The endosymbiont arsenophonus is widespread in soybean aphid, aphis glycines, but does not provide protection from parasitoids or a fungal pathogen. *PloS one*., **8** (4), e62145 (2013). Doi: 10.1371/journal.pone.0062145

36. Subandiyah, S., Nikoh, N., Tsuyumu, S., Somowiyarjo, S. and Fukatsu, T. Complex endosymbiotic microbiota of the citrus psyllid diaphorina citri (homoptera: Psylloidea). *Zool Sci*., **17** (7), 983-989 (2000). Doi:10.2108/zsj.17.983

37. Perveen, N., Muzaffar, S.B., Vijayan, R. and Al-Deeb, M.A. Microbial communities associated with the camel tick, hyalomma dromedarii: 16S rRNA gene-based analysis. *Scientific Reports*., **10** (1), 17035 (2020). Doi: 10.1038/s41598-020-74116-7

38. Ragab, A.I. Genetic variability of the whitefly Bemisia tabaci and its secondary endosymbionts in the Arabian Peninsula. Theses and Dissertations.MS Theses. *KAUST Research Repository*(2013). Doi: 10.25781/KAUST-78UQH

39. Russell, S.L., Castillo, J.R. and Sullivan, W.T. Wolbachia endosymbionts manipulate the self-renewal and differentiation of germline stem cells to reinforce fertility of their fruit fly host. *Plos Biology*, **21** (10), e3002335 (2023). Doi: 10.1371/journal.pbio.3002335

40. Kwak, Y. and Hansen, A.K. Unveiling metabolic integration in psyllids and their nutritional endosymbionts through comparative transcriptomics analysis. *I Sience*, **26** (10), 107930 (2023). Doi: 10.1016/j.isci.2023.107930

41. Kareem, A.A., Al-Zurfi, S. and Lahuf, A.A. Diversity and molecular identification of endosymbionts of the white-flies bemisia tabaci and trialeurodes vaporariorum. *Agricultural Sciences (JKAS)*, **10** (3), 1-17 (2023). Doi:10.59658/jkas.v10i3.1235

42. Tomaz, J.P., Cobianchi, J.V.L., Lima, L.S., de Oliveira, L.M., Hoshino, A.T. and Androcioli, H.G. Whitefly distribution and interaction with endosymbionts in the state of paraná. *Semina: Ciências Agrárias*, **44** (5), 1661-1681(2023). Doi: 10.5433/1679-0359.2023v44n5p1661

43. Gherna, R.L., Werren, J.H. and Weisburg, W. Arsenophonus nasoniae gen. nov., sp. nov., the causative agent of the son-killer trait in the parasitic wasp nasonia vitripennis. *Int. J. Syst. Evol. Microbiol*., **41** (4), 563-565 (1991). Doi: 10.1016/j.jip.2024.108073

44. Kaur, R., Leigh, B. A., Ritchie, I. T. and Bordenstein, S. R. The Cif proteins from Wolbachia prophage WO modify sperm genome integrity to establish cytoplasmic incompatibility. *PLoS Biology*, **20**(5), e3001584 (2022).Doi:10.1371/journal.pbio.3001584.

45. Nevalainen, L. B. and Newton, I. L. Detection and assessment of wolbachia pipientis infection. In: *Drosophila oogenesis: Methods and protocols.* Springer, 291-307 (2023). Doi: 10.1007/978-1-0716-2970-3_15

46. Hague, M. T., Wheeler, T. B. and Cooper, B. S. Comparative analysis of Wolbachia maternal transmission and localization in host ovaries. *Communications Biology,* **7**(1), 727 (2024). Doi:10.1038/s42003-024-06431-y

47.Fallon, A.M. Cytological properties of an *Aedes albopictus* mosquito cell line infected with *Wolbachia* strain *w*AlbB. *InVitro Cell. Dev. Biol.-Animal,* **44**,154–161(2008). Doi.10.1007/s11626-008-9090-4

48. Pais R, Lohs C, Wu Y, Wang J, Aksoy S. The obligate mutualist Wigglesworthia glossinidia influences reproduction, digestion, and immunity processes of its host, the tsetse fly. Appl. Environ. Microbiol., **74**(19), 5965-74. 2008. Doi: 10.1128/AEM.00741-08

دراسة اوليه تم فيها عزل نوعين من البكتيريا التكافلية من نوع الولابكيه والاريسونوفونا التي تصيب ذباب الحمام

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الملخص

تعتبر البكتيريا التكافلية ضرورية في البيئة التطورية للحشرات حيث انها تعمل كطفيليات منفصلة تؤثر على تكاثر المضيف وقدرته على التكيف. تسهل الكائنات الحية الدقيقة التكافلية الارتباط بين العديد من الأنواع. *P. canariensis* هو طفيل خارجي يتطفل على الحمام والذي ينتمي إلى عائلة ذبابة hippoboscidae.

طرق البحث: تم 114 حماماً منزلياً من منطقة الباحة، وهي منطقة في جنوب غرب المملكة العربية السعودية، متأثرة سريرياً بالطفيليات الخارجية. لا تزال الطرق الجزيئية الدقيقة التي تحكم العلاقات بين الحشرات وهذه البكتيريا غير مفهومة جيدًا، على الرغم من توافر التقنيات الحديثة و الأدوات الجزيئية التي تسمح بتشخيص البكتيريا التكافلية الداخلية في الكائنات الحية. تم استخدام تقنيه تفاعل البوليمرز المتسلسل PCR لتشخيص البكتيريا التكافليه وتحديد السلالة الجزيئية. النتائج: تم اكتشاف وجود نوعين مختلفين من البكتيريا التكافلية في 20 عينه من *P. canariensis* . أظهرت النتائج ان بكتيريا الولابكيه وجدت بنسبه عاليه ، حيث بلغ معدل الإصابة 60% (wsp)، في حين بلغ معدل انتشار الإصابة بـ Arsenophonus 2%. لقد تجاوز معدل انتشار الإصابة ببكتيريا Wolbachia انتشار بكتيريا Arsenophonus، حيث وصل إلى معدل 60%. أظهرت الطرق الحديثه الجزيئية مجتمع تعايش داخلي كامل لذباب *P. canariensis*، بما في ذلك التكافل الداخلي، وهو ما قد يفسر عدد التعايش الداخلي البكتيري الذي يمكن أن يعيش معًا في المضيف. نظرا للأهمية المحتملة لبكتيريا Wolbachia وArsenophonus في إعاقة انتقال الأمراض والسيطرة على أعداد الحشرات الضارة.

الكلمات الدالة: الطفيليات الخارجية ، مسببات الأمراض ، ذبابة الحمام ، تطورالسلالة.