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Molecular Analysis of the Presence of *Wolbachia* in Indigenous Human Louse Populations in Iran

Samira Firooziyan[1,2], Mohammad Ali Oshaghi[3*], Saber Gholizadeh[1,2*]

[1]Pathogens and Vectors Research Center, Cellular and Molecular Medicine Research Institute, Urmia University of Medical Sciences, Urmia, Iran
[2]Department of Medical Entomology and Vector Control, School of Public Health, Urmia University of Medical Sciences, Urmia, Iran
[3]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

***Corresponding Authors:** Mohammad Ali Oshaghi, Email: moshaghi@tums.ac.ir, and Saber Gholizadeh, Email: saber@umsu.ac.ir

Abstract

Introduction: Human lice infestation continues to be a public health challenge. Head lice not only cause physical irritation, but also lead to social and psychological problems. Body lice are vectors of several infectious diseases. While chemical control remains the mainstay of management, increasing insecticide resistance highlights the need for alternative strategies. Investigating endosymbiotic bacteria such as *Wolbachia* may provide new ways to control lice populations.

Methods: Samples were collected from volunteers in four provinces of Iran. DNA extraction was performed according to the manufacturer's instructions with minor adjustments to improve results. The presence of *Wolbachia* was assessed using PCR and nested-PCR methods targeting the *Wolbachia* surface protein (wsp), glutamyl-tRNA amidotransferase B subunit (gatB), and cytochrome oxidase subunit 1 (coxA) genes. DNA isolated from *Wolbachia*-infected *Culex* mosquitoes was used as a positive control to confirm the effectiveness of the assay. Distilled deionized water was also used as a negative control to ensure no contamination or false positives.

Results: No *Wolbachia* DNA was found in any of the lice samples when the results were compared to the positive control. Amplification using the wsp, gatB, and coxA primer sets did not show any positive results in PCR or nested-PCR assays.

Conclusion: The absence of detectable *Wolbachia* in the louse samples of this study may be due to genetic variation in the primer binding regions or low bacterial density. Further molecular investigations using other markers and optimized primers are recommended to clarify the possible presence of *Wolbachia* in Iranian louse populations.

Keywords: *Pediculus*, *Wolbachia*, Iran

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Introduction

Human lice are obligate blood-sucking ectoparasites, meaning they cannot survive on any host other than humans. These parasites have long been a cause of medical and social concern worldwide (1). *Pediculus humanus capitis* causes head lice infestation. This parasite is transmitted through direct head-to-head contact, especially among children and adolescents in group settings (2). Although this species does not transmit serious disease to humans, clinical complications of infection ranging from itching and burning to secondary pyoderma are possible (1, 2). In contrast, *Pediculus humanus corporis* (body louse) lives in the seams of human clothing and attaches itself to the human body only to feed. This species is a vector of important infections such as epidemic typhus, trench fever, and relapsing fever (1). This discussion excludes *Pthirus pubis* infestations. Lice have been a persistent global public health issue since ancient times, with their widespread occurrence maintained by ineffective control measures and the increasing development of resistance to commonly used chemical pediculicides (3). Reports of resistance have emerged from the United States, United Kingdom, France, Argentina, Australia, and Iran (3–8). Given the sensitivity and resource demands of lice management, research into insect symbiotic microorganisms offers promising alternative approaches (9).

The genus *Wolbachia* is an obligate intracellular bacterium. *Wolbachia* belongs to the family Anaplasmataceae in the phylum Proteobacteria and is closely related to *Rickettsia* species (10). *Wolbachia* is one of the most common reproductive parasites of

arthropods worldwide. The bacterium exhibits complex interactions with its host, often ranging from parasitism to symbiosis (11, 12). Some hosts are obligately dependent on *Wolbachia* for survival or reproduction (13). Approximately 40–66% of arthropod species carry *Wolbachia* infections (2, 13–18). The bacteria localize mainly within reproductive tissues such as ovaries and testes and are maternally transmitted via egg cytoplasm (19). They induce cytoplasmic incompatibility, male killing, parthenogenesis, feminization, and can enhance fertility and fecundity in their hosts (20). These reproductive manipulations are hypothesized to involve interactions with host proteins (21).

Genetic diversity within the genus is well documented, with eight subgroups (A–H) identified through sequencing of 16S rDNA and ftsZ genes (22–26). In lice, infections have been reported exclusively from *Wolbachia* subgroup F (27). The *Wolbachia* surface protein (wsp), an octa-beta barrel membrane protein, is a key target for detection and phylogenetic analysis (28). This protein plays a significant role in modulating the host's immune responses, regulating cell growth, influencing pathogenicity, and triggering programmed cell death. *Wolbachia* infections may interfere with the reproductive processes of lice, potentially leading to reduced population levels (28), and may interfere with their vectorial capacity (29). Consequently, *Wolbachia* is being researched as a potential biocontrol agent against lice as an alternative to traditional insecticides.

Research has identified *Wolbachia* in lice populations from Canada, Argentina, the United States, and Australia using the wsp gene as a molecular marker (29). In contrast, lice collected from the United States, Russia, and Madagascar showed no evidence of *Wolbachia* infection using the same marker (30–32). This study aimed to investigate the presence of *Wolbachia* in head and body lice populations gathered from Iran.

Methods

This study received ethical approval from Urmia university of medical sciences (Ethical code: IR.UMSU.REC.1404.168). A total of 123 human lice specimens were collected from volunteers in four provinces of Iran (Khorasan, West Azerbaijan, Zanjan, and Qom), including 97 head lice and 26 body lice (Table 1, Figure 1).

Head lice were collected mainly from school students during screening. Body lice samples were obtained from individuals residing in drug rehabilitation camps by exchanging contaminated clothing for new clothing. Informed consent was obtained from all participants or their legal guardians, in accordance with ethical guidelines.

The samples were stored in 70% ethanol until molecular analysis. Genomic DNA was isolated from lice using the YTA Genomic DNA Extraction Kit (Yekta Tajhiz Azma, Tehran, Iran). To increase the yield, slight adjustments were made to the protocol of the DNA extraction kit manufacturer. To crush the lice cuticle, each louse was individually frozen in liquid nitrogen in a microtube and homogenized in 200 µl of FATG1 lysis buffer using a micropestle. Proteinase K (20 µL) was then added, and the samples were incubated at 60°C for one hour to digest proteins. Subsequent steps followed the manufacturer's instructions precisely.

The wsp gene was used to investigate *Wolbachia* infection using a nested PCR method. The first round of amplification of a 632 bp fragment was performed using primers 81F and 691R, followed by a second round with primers 183F and 691R to produce a 501 bp nested product. To increase the robustness of detection and confirm the results, two additional *Wolbachia*-specific molecular markers—glutamyl-tRNA amidotransferase subunit B (gatB) and cytochrome oxidase subunit 1 (coxA)—were co-amplified using the corresponding primers to produce 500 bp and 650 bp products (Table 2). PCR amplification conditions, including annealing temperature and cycle time, were optimized according to the established protocols (Table 3).

Positive controls consisted of *Wolbachia*-infected *Culex* mosquito DNA, while negative controls used nuclease-free distilled water to monitor contamination. PCR products were visualized by gel electrophoresis, and selected amplicons were subjected to sequencing for confirmation.

Results

In this study, 75 lice samples, including 49 head lice and 26 body lice, were collected from four provinces of Iran. Each specimen was thoroughly tested for *Wolbachia* presence using both nested PCR and conventional PCR methods, targeting the wsp, gatB and coxA, genes. Despite the comprehensive screening and inclusion of positive controls validating the assays, none of the lice samples tested positive for *Wolbachia* DNA. These results indicate an absence of *Wolbachia* infection in the analyzed lice populations from these regions of Iran.

Discussion

Wolbachia, an intracellular bacterium found in the class Insecta, shows a heterogeneous distribution among

Table 1. Geographical Distribution and Sample Size of Lice Collected from the Selected Cities

Province	District	Geographic coordinates	Lice counts	Collection dates
Khorasan	Mashhad	36°17′49.92″N and 59°36′24.08″E	21	2023
Qom	Qom	34°38′29.67″N and 50°52′28.57″E	15	2022
Zanjan	Zanjan	36°40′58.82″N and 48°30′31.40″E	10	2021
West Azerbaijan	Urmia	37°32′59.30″N and 45°04′43.06″E	77	2021- 2023

different insect orders. The presence of this bacterium has been studied and identified in some species. However, others have not been sufficiently studied, and no definitive or documented diagnosis is available (33). This highlights the need for extensive research across a wider range of insect species. In Iran, several studies have focused on determining the prevalence of *Wolbachia* infections in insect populations and have shown infection rates in mosquitoes, Pedrous beetles, several species of fruit flies, and various species of sand flies to be very variable (34–37).

These differences indicate complex interactions between *Wolbachia* and their insect hosts, influenced by host genetics, environmental variables, and geographic factors. Further evidence for the presence of this bacterium in Iranian insect populations comes from unpublished genetic sequences deposited in GenBank by Jadid et al. These sequences identified *Wolbachia* strains in *Culex pipiens* mosquitoes during 2006–2007 (GenBank accessions EF539841-52, DQ900650-54). The present study provided the first comprehensive report on the occurrence and distribution of *Wolbachia* in human lice in Iran, representing a significant advance in understanding the epidemiology of this bacterium in the region. Given the unique ecological niche of lice as a major health priority, this study aimed to determine the presence of *Wolbachia* in the vector.

Recently, numerous studies from around the world have investigated the detection of endosymbiont bacteria, including *Wolbachia*, in human lice populations from diverse geographical locations (38–42). Such research has been conducted to elucidate the prevalence, diversity, and effects of symbionts on host biology. However, the detection of *Wolbachia* in lice has been inconsistent across studies, reflecting complex host-symbiont dynamics. For example, Veracx et al. (2005) reported no presence of *Wolbachia* in body lice using the wsp marker, while head lice were positive (43). Similarly, *Wolbachia* was undetectable in head lice samples from the USA, Russia, and Madagascar, using wsp primers (30), implying either absence or challenges in detection linked to low bacterial load or gene variation.

In this study, molecular screening employed three genetic markers—wsp, gatB, and coxA— to maximize *Wolbachia* detection sensitivity in lice; nevertheless, all samples tested negative. The validity of negative results was confirmed by positive control assays, indicating methodological reliability. Therefore, the absence of *Wolbachia* in these lice is unlikely due to technical shortcomings such as primer inefficiency or sample quality.

Resistance to liceicides is a major concern in lice management, but linking the absence of *Wolbachia* to resistance to liceicides is premature and potentially misleading. Alternative explanations offered by previous studies include host physiological incompatibility, altered host tissue environment, and microbiome interactions that affect *Wolbachia* survival (43–45).

To more fully understand the prevalence and distribution of *Wolbachia* in Iranian lice, future research should expand sampling to other areas and use alternative molecular markers such as 16S rRNA, ftsz, and ARM-targeted endpoint PCR (46,47). Next-generation sequencing (NGS) technologies can provide comprehensive profiles of lice microbial communities and even reveal low-abundance *Wolbachia* or other endosymbionts undetectable by conventional PCR.

Finally, the lack of detection of *Wolbachia* in Iranian lice suggests biological limitations. The diversity in symbiont-host relationships was highlighted, and the need for further and extensive studies to clarify the ecological roles

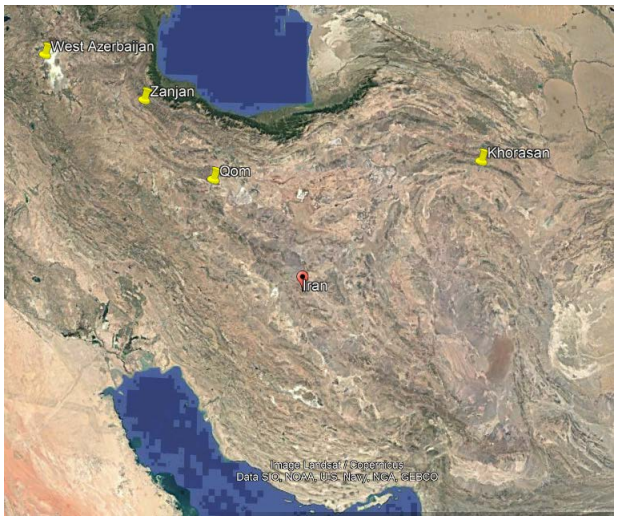

Figure 1. Google Map of study areas for collection of lice specimens in Iran

Table 2. Primer information for wsp, coxA, and gatB genes used in *Wolbachia* detection from lice collected in Iran

Primer	Sequence 5′ to 3′	References
wsp 81F wsp 691R	5'–TGGTCCA ATAAGTGATGAAGAAAC–3' 5'– AAAAATTAAACGCTACTCCA–3'	(7)
wsp 183F wsp 691R	5'–AAGGAACCG AAGTTCATG–3' 5'–AAAAATTAAACGCTACTCCA–3'	(7)
gatBF gatBR	5'–GAK TTA AAY CGY GCA GGB GTT–3' 5'–TGG YAA YTC RGG YAA AGA TGA–3'	(11)
coxAF coxAR	5'–TTG GRG CRA TYA ACT TTA TAG–3' 5'–CTA AAG ACT TTK ACR CCA GT–3'	(11)

Table 3. PCR and Nested-PCR amplification parameters. Steps 2–4 were cycled 35 times.

Nested-PCR/ PCR	Primer	Step 1 (Initial denaturation)		Step 2 (Denaturation)		Step 3 (Annealing)		Step 4 (Extension)		Step 5 (Final Extension)	
		Temperature	Time	Temperature	Time	Temperature	Time	Temperature	Time	Temperature	Time
Nested-PCR	wsp	94˚c	4′	94˚c	2′	54.5˚c	1′30″	70˚c	1′20″	71˚c	10′
PCR	gatB	95˚c	1′30″	95˚c	45″	56˚c	1′15″	72˚c	1′30″	70˚c	10′
PCR	coxA	95˚c	4′45″	95˚c	15″	54.5˚c	30″	72˚c	30″	70˚c	10′

of *Wolbachia* is emphasized.

Body lice collection was challenged by concealing the contamination among residents of drug rehabilitation camps; incentives such as the provision of new clothes and free medication facilitated their cooperation.

Conclusion

In this study, *Wolbachia* was not detected in head and body lice samples. It can be due to mutations in the regions where the wsp primers bind. To definitively rule out the presence of *Wolbachia*, it is recommended to use additional primer sets.

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The authors declare that they have used Grammarly AI to improve readability and proofread. After using this tool/service, the author has reviewed and edited the content.

Authors' Contribution

Conceptualization: Samira Firooziyan.
Data curation: Samira Firooziyan.
Formal analysis: Mohammad Ali Oshaghi.
Investigation: Samira Firooziyan.
Methodology: Saber Gholizadeh.
Project administration: Saber Gholizadeh and Mohammad Ali Oshaghi.
Resources: Samira Firooziyan.
Software: Saber Gholizadeh.
Supervision: Mohammad Ali Oshaghi.
Validation: Saber Gholizadeh.
Visualization: Mohammad Ali Oshaghi.
Writing–original draft: Samira Firooziyan.

Competing Interests

The authors declare no competing interests.

Data Availability Statement

The data are available from the authors upon reasonable request.

Ethical Approval

This study has been ethically approved (Ethical code: IR.UMSU.REC.1404.168), and since this research did not involve human study, therefore, the informed consent form was not used.

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References

1. Badiaga S, Brouqui P. Human louse-transmitted infectious diseases. Clin Microbiol Infect 2012;18(4):332-7. doi:10.1111/j.1469-0691.2012.03778.x
2. Firooziyan S, Sadaghianifar A, Taghilou B, Galavani H, Ghaffari E, Gholizadeh S. Identification of novel voltage-gated sodium channel mutations in human head and body lice (Phthiraptera: Pediculidae). J Med Entomol 2017;54(5):1337-43. doi:10.1093/jme/tjx107
3. Aliverdi F, Mahmoodi Z, Mehdizadeh Tourzani Z, Amini L, Mohamadi F. The relationship between social network use and students' quality of life. J Kerman Univ Med Sci 2024;31(6):299-304. doi:10.34172/jkmu.2024.46
4. Mumcuoglu KY. Prevention and treatment of head lice in children. Paediatr Drugs 1999;1(3):211-8. doi:10.2165/00128072-199901030-00005
5. Clark JM. Determination, mechanism and monitoring of knockdown resistance in permethrin-resistant human head lice, *Pediculus humanus capitis*. J Asia Pac Entomol 2009;12(1):1-7. doi:10.1016/j.aspen.2008.10.007
6. Burkhart CG, Burkhart CN. Head lice therapies revisited. Dermatol Online J 2006;12(6):3. doi:10.5070/d35dz638vq
7. Burgess IF, Burgess NA. Dimeticone 4% liquid gel found to kill all lice and eggs with a single 15 minute application. BMC Res Notes 2011;4:15. doi:10.1186/1756-0500-4-15
8. Kurt O, Balcioğlu IC, Burgess IF, Limoncu ME, Girginkardeşler N, Tabak T, et al. Treatment of head lice with dimeticone 4% lotion: comparison of two formulations in a randomised controlled trial in rural Turkey. BMC Public Health 2009;9:441. doi:10.1186/1471-2458-9-441
9. Kamalinejad M, Sharif A, Gachkar L, Ehsani A, Ahmadian-Atta M, Torabi MR, et al. Investigating the efficacy of sumac topical solution against permethrin-resistant human head lice. Int J Med Toxicol Forensic Med 2019;9(3):117-24. doi: 10.32598/ijmtfm.v9i3.25296.
10. Sazama EJ, Ouellette SP, Wesner JS. Bacterial endosymbionts are common among, but not necessarily within, insect species. Environ Entomol 2019;48(1):127-33. doi:10.1093/ee/nvy188
11. O'Neill SL, Giordano R, Colbert AM, Karr TL, Robertson HM. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc Natl Acad Sci U S A 1992;89(7):2699-702. doi:10.1073/pnas.89.7.2699
12. Rousset F, Bouchon D, Pintureau B, Juchault P, Solignac M. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. Proc Biol Sci 1992;250(1328):91-8. doi:10.1098/rspb.1992.0135
13. Stouthamer R, Breeuwert JA, Luck RF, Werren JH. Molecular identification of microorganisms associated with parthenogenesis. Nature 1993;361(6407):66-8. doi:10.1038/361066a0
14. Simsek S, Ciftci AT. Serological and molecular detection of *Dirofilaria* species in stray dogs and investigation of *Wolbachia* DNA by PCR in Turkey. J Arthropod Borne Dis 2016;10(4):445-53.
15. Sazama EJ, Bosch MJ, Shouldis CS, Ouellette SP, Wesner JS. Incidence of *Wolbachia* in aquatic insects. Ecol Evol 2017;7(4):1165-9. doi:10.1002/ece3.2742
16. Werren JH, Windsor D, Guo LR. Distribution of Wolbachia among neotropical arthropods. Proc R Soc Lond B Biol Sci 1995;262(1364):197-204. doi:10.1098/rspb.1995.0196
17. Kozek WJ, Rao RU. The discovery of *Wolbachia* in arthropods and nematodes–a historical perspective. In: Hoerauf A, Rao RU, eds. *Wolbachia*: A Bug's Life in Another Bug. Karger Medical and Scientific Publishers; 2007. p. 1-14. doi:10.1159/000104228
18. Werren JH, Windsor DM. *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? Proc Biol Sci 2000;267(1450):1277-85. doi:10.1098/rspb.2000.1139
19. Kittayapong P, Jamnongluk W, Thipaksorn A, Milne JR, Sindhusake C. *Wolbachia* infection complexity among insects in the tropical rice-field community. Mol Ecol 2003;12(4):1049-60. doi:10.1046/j.1365-294x.2003.01793.x
20. Werren JH, Zhang W, Guo LR. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. Proc Biol Sci 1995;261(1360):55-63. doi:10.1098/rspb.1995.0117
21. Bensaadi-Merchermek N, Salvado JC, Cagnon C, Karama S, Mouchès C. Characterization of the unlinked 16S rDNA and 23S-5S rRNA operon of *Wolbachia pipientis*, a prokaryotic parasite of insect gonads. Gene 1995;165(1):81-6. doi:10.1016/0378-1119(95)00539-i
22. Kirkness EF, Haas BJ, Sun W, Braig HR, Perotti MA, Clark JM, et al. Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. Proc Natl Acad Sci U S A 2010;107(27):12168-73. doi:10.1073/pnas.1003379107
23. Werren JH. *Wolbachia* and speciation. In: Endless Forms: Species and Speciation. Oxford University Press; 1998. p.

245-60. doi:10.1093/oso/9780195109009.003.0018
24. Lo N, Casiraghi M, Salati E, Bazzocchi C, Bandi C. How many *Wolbachia* supergroups exist? Mol Biol Evol 2002;19(3):341-6. doi:10.1093/oxfordjournals.molbev.a004087
25. Bordenstein S, Rosengaus RB. Discovery of a novel *Wolbachia* super group in Isoptera. Curr Microbiol 2005;51(6):393-8. doi:10.1007/s00284-005-0084-0
26. Lo N, Paraskevopoulos C, Bourtzis K, O'Neill SL, Werren JH, Bordenstein SR, et al. Taxonomic status of the intracellular bacterium *Wolbachia pipientis*. Int J Syst Evol Microbiol 2007;57(Pt 3):654-7. doi:10.1099/ijs.0.64515-0
27. Ros VI, Fleming VM, Feil EJ, Breeuwer JA. How diverse is the genus *Wolbachia*? Multiple-gene sequencing reveals a putatively new *Wolbachia* supergroup recovered from spider mites (Acari: Tetranychidae). Appl Environ Microbiol 2009;75(4):1036-43. doi:10.1128/aem.01109-08
28. Covacin C, Barker SC. Supergroup F *Wolbachia* bacteria parasitise lice (Insecta: Phthiraptera). Parasitol Res 2007;100(3):479-85. doi:10.1007/s00436-006-0309-6
29. Zhou W, Rousset F, O'Neil S. Phylogeny and PCR-based classification of *Wolbachia* strains using wsp gene sequences. Proc Biol Sci 1998;265(1395):509-15. doi:10.1098/rspb.1998.0324
30. Baldo L, Desjardins CA, Russell JA, Stahlhut JK, Werren JH. Accelerated microevolution in an outer membrane protein (OMP) of the intracellular bacteria *Wolbachia*. BMC Evol Biol 2010;10:48. doi:10.1186/1471-2148-10-48
31. Anderson M. Detection of *Wolbachia* in Human Lice. GS4 Georgia Southern Student Scholars Symposium. Georgia Southern University; 2016. doi:10.20429/gs4.2016.009
32. Bleidorn C, Gerth M. A critical re-evaluation of multilocus sequence typing (MLST) efforts in Wolbachia. FEMS Microbiol. Ecol 2018;94(1):163. doi:10.1093/femsec/fix163
33. Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, et al. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Appl Environ Microbiol 2006;72(11):7098-110. doi:10.1128/aem.00731-06
34. Werren JH. Biology of *Wolbachia*. Annu Rev Entomol 1997;42:587-609. doi:10.1146/annurev.ento.42.1.587
35. Bozorg-Omid F, Oshaghi MA, Vahedi M, Karimian F, Seyyed-Zadeh SJ, Chavshin AR. *Wolbachia* infection in West Nile virus vectors of northwest Iran. Appl Entomol Zool 2020;55(1):105-13. doi:10.1007/s13355-019-00658-6
36. Maleki-Ravasan N, Akhavan N, Raz A, Jafari M, Zakeri S, Dinparast Djadid N. Co-occurrence of pederin-producing and *Wolbachia endobacteria* in *Paederus fuscipes* Curtis, 1840 (Coleoptera: Staphilinidae) and its evolutionary consequences. Microbiologyopen 2019;8(7):e00777. doi:10.1002/mbo3.777
37. Karimi J, Darsouei R. Presence of the endosymbiont *Wolbachia* among some fruit flies (Diptera: Tephritidae) from Iran: a multilocus sequence typing approach. J Asia Pac Entomol 2014;17(1):105-12. doi:10.1016/j.aspen.2013.11.002
38. Bordbar A, Soleimani S, Fardid F, Zolfaghari MR, Parvizi P. Three strains of *Wolbachia pipientis* and high rates of infection in Iranian sandfly species. Bull Entomol Res 2014;104(2):195-202. doi:10.1017/s0007485313000631
39. Allen JM, Reed DL, Perotti MA, Braig HR. Evolutionary relationships of "*Candidatus* Riesia spp.," endosymbiotic enterobacteriaceae living within hematophagous primate lice. Appl Environ Microbiol 2007;73(5):1659-64. doi:10.1128/aem.01877-06
40. Sasaki-Fukatsu K, Koga R, Nikoh N, Yoshizawa K, Kasai S, Mihara M, et al. Symbiotic bacteria associated with stomach discs of human lice. Appl Environ Microbiol 2006;72(11):7349-52. doi:10.1128/aem.01429-06
41. Perotti MA, Kirkness EF, Reed DL, Braig HR. Endosymbionts of lice. In: Insect Symbiosis. Vol 3. CRC Press; 2008. p. 205-19. doi:10.1201/9781420064117.ch9
42. Kyei-Poku GK, Colwell DD, Coghlin P, Benkel B, Floate KD. On the ubiquity and phylogeny of *Wolbachia* in lice. Mol Ecol 2005;14(1):285-94. doi:10.1111/j.1365-294X.2004.02409.x
43. Veracx A, Raoult D. Biology and genetics of human head and body lice. Trends Parasitol 2012;28(12):563-71. doi:10.1016/j.pt.2012.09.003
44. Kittayapong P, Baisley KJ, Baimai V, O'Neill SL. Distribution and diversity of *Wolbachia* infections in Southeast Asian mosquitoes (Diptera: Culicidae). J Med Entomol 2000;37(3):340-5. doi:10.1093/jmedent/37.3.340
45. Osei-Poku J, Han C, Mbogo CM, Jiggins FM. Identification of *Wolbachia* strains in mosquito disease vectors. PLoS One 2012;7(11):e49922. doi:10.1371/journal.pone.0049922
46. Johnson KN. The impact of *Wolbachia* on virus infection in mosquitoes. Viruses 2015;7(11):5705-17. doi:10.3390/v7112903
47. Schneider DI, Klasson L, Lind AE, Miller WJ. More than fishing in the dark: PCR of a dispersed sequence produces simple but ultrasensitive *Wolbachia* detection. BMC Microbiol 2014;14:121. doi:10.1186/1471-2180-14-121