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RESEARCH ARTICLE

Molecular evidence of *Wolbachia* and *Orthoflavivirus* infection in field-collected mosquitoes in three provinces of Türkiye

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Abstract

Background: Mosquitoes transmit various pathogens causing diseases like Zika, Dengue, West Nile and Chikungunya. They also harbour insect-specific viruses (ISVs) and *Wolbachia*, which can block arbovirus transmission. This study investigated the prevalence of *Orthoflavivirus* and *Wolbachia* in mosquito populations from three provinces in Türkiye.

Methods: Mosquitoes were collected using CDC Miniature Light traps in 2022–2023. Morphologically identified specimens were pooled (1–10 individuals) and screened for *Orthoflavivirus* and *Wolbachia* via PCR and confirmed by Sanger sequencing. Infection prevalence was estimated using the maximum likelihood method. Mosquito taxa richness across provinces was estimated using the abundance-based, non-parametric Chao1 index.

Results: Among 8766 mosquitoes (11 taxa) collected, *Culex perexiguus*, *Ochlerotatus caspius* and *Anopheles claviger* were most abundant. *Anopheles flavivirus* (AnFV) detected in one *Oc. caspius* pool, while *Wolbachia* sequences belonging to supergroup B were detected in *An. claviger*, *Cx. pipiens* s.l., *Cx. perexiguus* and *Oc. caspius*, with an overall infection prevalence of 0.0119 (95% CI: 0.008–0.0161). The richest mosquito fauna was detected in Ankara, followed by Adana, and Çankırı.

Conclusion: This study provides new insights into mosquito richness and the prevalence of *Orthoflavivirus* and *Wolbachia* in Türkiye, contributing to vector surveillance and the potential use of *Wolbachia* in mosquito control strategies.

KEYWORDS
mosquito, *Orthoflavivirus*, Türkiye, vector surveillance, *Wolbachia*

INTRODUCTION

Mosquitoes play a critical role as vectors in transmitting numerous pathogens, including bacteria, parasites and arboviruses, resulting in significant morbidity and mortality in humans and animals globally [1]. Among arboviruses, orthoflaviviruses are a prominent group within the *Flaviviridae* family, characterised by a single-stranded, positive-sense RNA genome [2, 3]. Notable members of this group, such as *Orthoflavivirus denguei* (DENV), *Orthoflavivirus zikaense* (ZIKV), *Orthoflavivirus flavi* (YFV) and *Orthoflavivirus nilense* (WNV), are transmitted by mosquitoes, predominantly *Aedes* and *Culex* species. DENV alone affects approximately 390 million individuals annually, with 96 million presenting clinical symptoms, underscoring the global burden of these infections [4].

Besides pathogenic viruses, mosquitoes harbour non-pathogenic insect-specific viruses (ISVs), which are incapable of infecting vertebrates but have garnered interest due to their potential interactions with co-infecting arboviruses. Evidence of direct co-infections of insect-specific orthoflaviviruses (ISFs) with arboviruses highlights their possible influence on vector competence [5–8]. Furthermore, mosquitoes are hosts to diverse endosymbionts, including bacteria like *Wolbachia*, fungi, and other microorganisms, which can impact their biology and vectorial capacity [9].

Sustainable Development Goal: Good Health and Wellbeing

Wolbachia, an intracellular bacterium, has emerged as a promising biocontrol agent due to its ability to manipulate host reproduction through mechanisms like cytoplasmic incompatibility (CI) and to suppress the replication of arboviruses [10–12]. Studies exploring the interactions between *Wolbachia* and arboviruses have provided insights into their potential utility in reducing disease transmission [13, 14].

Türkiye's unique geography, located at the crossroads of Asia, Africa and Europe, and diverse ecological conditions makes it a hotspot for vector-borne diseases [15–19]. The country's varied ecosystems support the proliferation of 63 mosquito taxa [20] and provide ideal breeding sites for vectors. Moreover, Türkiye's position as a migratory bird stopover amplifies the risk of introducing and disseminating arboviruses such as WNV [21–26]. Rapid urbanisation and increased human encroachment into wildlife habitats further exacerbate the risk of zoonotic and arboviral infections [26].

Despite the increasing recognition of mosquito-borne diseases in Türkiye, research remains limited to case studies and localised investigations of transmission dynamics and control measures [27, 28]. Comprehensive surveillance efforts, including the identification of orthoflaviviruses, characterisation of their evolutionary relationships, and assessment of endosymbionts like *Wolbachia*, are sparse. Surveillance studies have been conducted in certain regions [29, 30]; however, a systematic approach to understanding the ecological and biocontrol potential of these interactions is lacking. Additionally, mosquito pathogen screenings in Türkiye target *Aedes* and *Culex* species, with a notable gap in our knowledge of *Orthoflavivirus-Wolbachia* interactions in other mosquito species.

We aimed to address these gaps by investigating mosquito species richness and screening for orthoflaviviruses and *Wolbachia* endosymbionts in field-collected mosquitoes from the Mediterranean and Central Anatolia regions of Türkiye. By identifying viral and endosymbiont associations and exploring their ecological roles, this work seeks to provide valuable insights into arbovirus transmission dynamics and potential biocontrol strategies.

METHODS

Mosquito collection and identification

Mosquito specimens were collected in August 2022 in Çankırı and between May and November 2023 in Adana and Ankara (Figure 1) in seven locations (Kızılırmak, Alpagut, Güdül, Yeşilöz, Damyeri, Otluk, and Zerdali). Adult mosquitoes were captured using CDC Miniature Light traps, placed both indoors and outdoors near animal dwellings. The traps were set up in the morning and retrieved the next day between 10:00 AM and 12:00 PM.

Captured specimens were transferred to the laboratory alive and stored at −80°C. Morphological identification was performed on ice packs to preserve RNA viruses by using the MosKeyTool [31]. Mosquitoes were pooled by collection site, date, species, blood-feeding status and sex, with 1–10 individuals per pool, and stored at −80°C for molecular analysis.

Mosquito richness estimates and similarity across studied provinces

Mosquito taxa richness across provinces was estimated using the abundance-based, non-parametric Chao1 index,

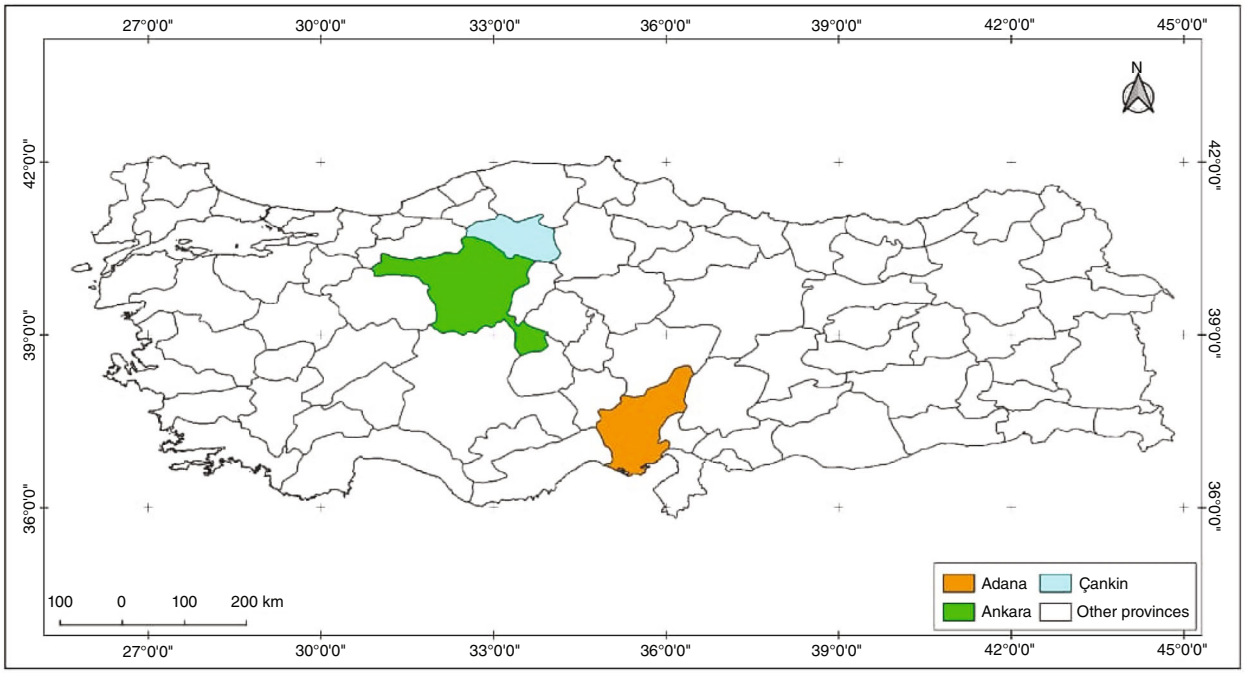

FIGURE 1 Map showing the provinces where mosquito collections were performed in 2022 and 2023.

implemented via the 'iNEXT' package [32] in the R software environment [33]. To compare mosquito taxa composition among provinces, Jaccard's similarity index from the 'vegan' package was applied [34].

Mosquito pool processing

Mosquito pool processing includes homogenisation, nucleic acid extraction and complementary DNA (cDNA) synthesis. Each pool was disrupted in 700 µL Dulbecco's Modified Eagle Medium (DMEM), vortexed and centrifuged at 8000*g* for 3 min and the supernatants were collected. RNA extraction and purification of the pool samples was performed according to the manufacturer's instructions for the High Pure Viral Nucleic Acid Kit (Roche, Germany). The reverse transcription into cDNA was performed using the iScript™ cDNA Synthesis Kit (BIO-RAD, USA), as recommended by the manufacturer. Obtained purified RNA was stored at −80°C for molecular analysis.

Orthoflavivirus screening

Orthoflavivirus screening was conducted using a pan-orthoflavivirus assay with degenerate primers designed to amplify a 960-base-pair (bp) region within the RNA-dependent RNA polymerase coding non-structural protein 5 (NS5) region of the *Orthoflavivirus* genome [35]. This assay detects both mosquito-borne pathogenic and ISFs. Each 30 µL reaction contained 10 µM of each primer, 10 mM dNTPs, 25 mM MgCl2 and 1 U/µL Taq polymerase. The thermocycler settings for both the first and second nested PCR were identical, with cycling parameters as follows: an initial denaturation at 94°C for 5 min; 40 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 3 min and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min. *Orthoflavivirus nilense* (WNV) served as the positive control, while water was used as the negative control. Amplification products were run on a 1.5% agarose gel, stained with ethidium bromide and visualised under UV light after electrophoresis.

Molecular detection of *Wolbachia* in mosquito pools

The detection of *Wolbachia* was carried out by performing PCR on cDNA, targeting the *Wolbachia* surface protein (*wsp*) gene using primers wsp81F and wsp691R [36]. The PCR procedure followed a specific thermal cycling profile: an initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing of primers at 55°C for 1 min and elongation at 72°C for 1 min, with a final elongation step at 72°C for 5 min. *Drosophila melanogaster* DGRP-RAL-100 genotype served as the positive control, while water was used as the negative control. The amplified cDNA products were then visualised on a 1.5% agarose gel to confirm their presence and size.

Sequence data analysis

Purified amplification products were subjected to bidirectional sequencing analysis utilising the primers used for PCR amplification. The sequences were manually checked and edited by using BioEdit (v.2.7.5). *Orthoflavivirus* and *Wolbachia* datasets were constructed using sequences obtained in this study, complemented by the most identical sequences identified through the BLAST search algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [37]. A taxon identity tree was obtained for the *Wolbachia* dataset by conducting a neighbour-joining (NJ) analysis under the assumption of Kimura's two-parameter (K2P) substitution model, whereas the phylogenetic relationships between different *Orthoflavivirus* sequences were evaluated through maximum-likelihood (ML) analysis with the K2P substitution model. The reliability of both trees was assessed through 1000 bootstrap replicates for each sequence. Both NJ and ML trees were constructed using MEGA v6.0 [38].

Wolbachia infection rate estimates

The *Wolbachia* infection rate of pooled mosquito data was estimated using the 'PooledInfRate' package [39], implemented in the R environment, which accounts for different pool sizes and provides confidence intervals that reflect the sample size. A logistic regression model was employed to examine the relationship between infection rate and factors such as mosquito species, sex and location. The significance of each predictor was evaluated using the Analysis of Deviance Table method.

RESULTS

Mosquito composition and richness

A total of 8766 mosquito specimens from 11 taxa were collected in 2022 and 2023. The most abundant species was *Ochlerotatus caspius* ($n = 3606$, 41.90%), followed by *Anopheles claviger* ($n = 3148$, 35.91%) and *Culex perexiguus* ($n = 494$, 5.74%). In contrast, *Aedes vexans* ($n = 2$, 0.02%) and *Anopheles superpictus* ($n = 8$, 0.09%) were the least common taxa. The Chao1 richness estimate indicated that the sampling effort for Çankırı was complete, with the lowest richness value recorded in this province (Chao1 estimator = 6.00, 95% CI: 6.00–6.00), where most of the specimens (87.92%) were collected. In contrast, the lowest number of specimens was collected in Ankara (2.59%), and the Chao1 richness estimate suggested a higher number of taxa (Chao1 estimator = 7.49, 95% CI: 7.00–10.78). The number of mosquito taxa recorded in Adana was seven,

which is in line with the results obtained from the Chao1 estimator (Chao1 estimator = 7.00, 95% CI: 7.00–7.42), indicating that the sampling effort for this province was nearly complete. Sharing five taxa (*An. claviger*, *An. maculipennis* s.l., *Cx. perexiguus*, *Cx. pipiens* s.l. and *Oc. caspius*), Ankara and Çankırı were the most similar provinces in terms of mosquito composition (Jaccard index = 0.6250). In contrast, Adana and Çankırı shared only three taxa (*Cx. perexiguus*, *Cx. pipiens* s.l. and *Oc. caspius*) with a Jaccard index of 0.4000. The number of shared taxa between Adana and Ankara was four (*An. claviger*, *Cx. perexiguus*, *Cx. pipiens* s.l. and *Oc. caspius*) with a similarity index of 0.4444. Unique taxa were also identified in each province. *An. hyrcanus* was exclusive to Çankırı, while *Aedes vexans* and *Aedes cretinus* were found only in Ankara. In Adana, *An. superpictus*, *Culex tritaeniorhynchus* and *Culiseta longiareolata* were collected exclusively (Table 1).

Orthoflavivirus detection and typing in mosquito pools

Except for the pools generated from *An. claviger*, *An. maculipennis* and *Oc. caspius* collected from Çankırı province, all the pools generated for all other taxa collected from the three provinces were screened for virus presence. For the Çankırı samples, 46, 27 and 78 pools of *An. claviger*, *An. maculipennis* and *Oc. caspius*, respectively, were randomly selected for virus detection analysis. Among the 397 mosquito pools screened for *Orthoflavivirus* detection, only one pool tested positive (0.025%).

The positive pool consisted of 10 *Oc. caspius* females collected from Çankırı, yielding a 243 bp amplicon after editing (GenBank accession no: PV021283). BLAST search showed the sequence had 97.94% similarity to *Anopheles flavivirus* (AnFV) identified in *An. hyrcanus* from Austria (GenBank accession no: MF678433) and 97.16% similarity to AnFV detected in *An. maculipennis* from Türkiye (GenBank accession no: MF361276). Consistent with the BLAST results, maximum-likelihood (ML) analysis grouped the *Orthoflavivirus* sequence from the *Oc. caspius* pool within the same clade as AnFV identified in *An. hyrcanus* from Austria. The closest group to this clade was formed by *Orthoflavivirus* sequences identified in *An. squamosus* individuals sampled from South Africa and Mozambique (Figure 2).

Wolbachia detection and typing in mosquito pools

Of the 397 mosquito pools screened for *Orthoflavivirus*, 9.57% ($n = 38$) were found to be infected with *Wolbachia*. No *Wolbachia* infection was detected in pools derived from *Ae. cretinus*, *Ae. vexans*, *An. hyrcanus*, *An. maculipennis* s.l., *An. superpictus*, *Cs. longiareolata* or *Cx. tritaeniorhynchus*. Infections were identified exclusively in *An. claviger* (1/82, 1.22%), *Oc. caspius* (2/81, 2.47%), *Cx. perexiguus* (24/62, 38.71%) and *Cx. pipiens* s.l. (11/23, 47.83%).

The ML estimate of pooled prevalence revealed an overall *Wolbachia* infection prevalence of 0.0119. Among taxa, *Cx. pipiens* s.l. exhibited the highest prevalence (0.1008), followed by *Cx. perexiguus* (0.0059), *Oc. caspius* (0.0031) and *An. claviger* (0.0013) (Table 2). Among the 216 pools created from mosquito specimens collected in Çankırı province, 12.5% ($n = 27$) tested positive for *Wolbachia*. In comparison, 7.54% of the mosquito pools from Adana (8/106) and 4.54% (2/44) of the pools from Ankara were found to be infected with *Wolbachia*. Detailed information regarding the positive pools was provided in Table S1.

The logistic regression results revealed that *Cx. pipiens* s.l. (Estimate = 3.9260, $p < 0.0001$) and *Cx. perexiguus* (Estimate = 3.5580, $p < 0.0001$) had a significantly higher likelihood of being infected with *Wolbachia*. Additionally,

TABLE 1 Mosquito taxa collected from the studied provinces and information regarding the pools generated for viral and bacterial screening.

	Çankırı		Ankara		Adana		Total		
	♀	♂	♀	♂	♀	♂	#	%	No of pools
Ae. cretinus	0	0	52	0	0	0	52	0.59	8
Ae. vexans	0	0	2	0	0	0	2	0.02	1
An. claviger	2754	390	1	0	3	0	3148	35.91	323
An. maculipennis s.l.	377	0	52	0	0	0	429	4.89	51
An. hyrcanus	406	0	0	0	0	0	406	4.63	47
An. superpictus	0	0	0	0	8	0	8	0.09	4
Cx. perexiguus	259	3	25	5	191	11	494	5.64	62
Cx. pipiens s.l.	101	4	4	0	37	0	146	1.67	23
Cx. triaeteniorynchus	0	0	0	0	415	2	417	4.76	45
Cs. longiareolata	0	0	0	0	60	0	60	0.68	6
Oc. caspius	3410	3	57	0	136	0	3606	41.14	387
Total	7307	400	193	5	849	12	8766		957

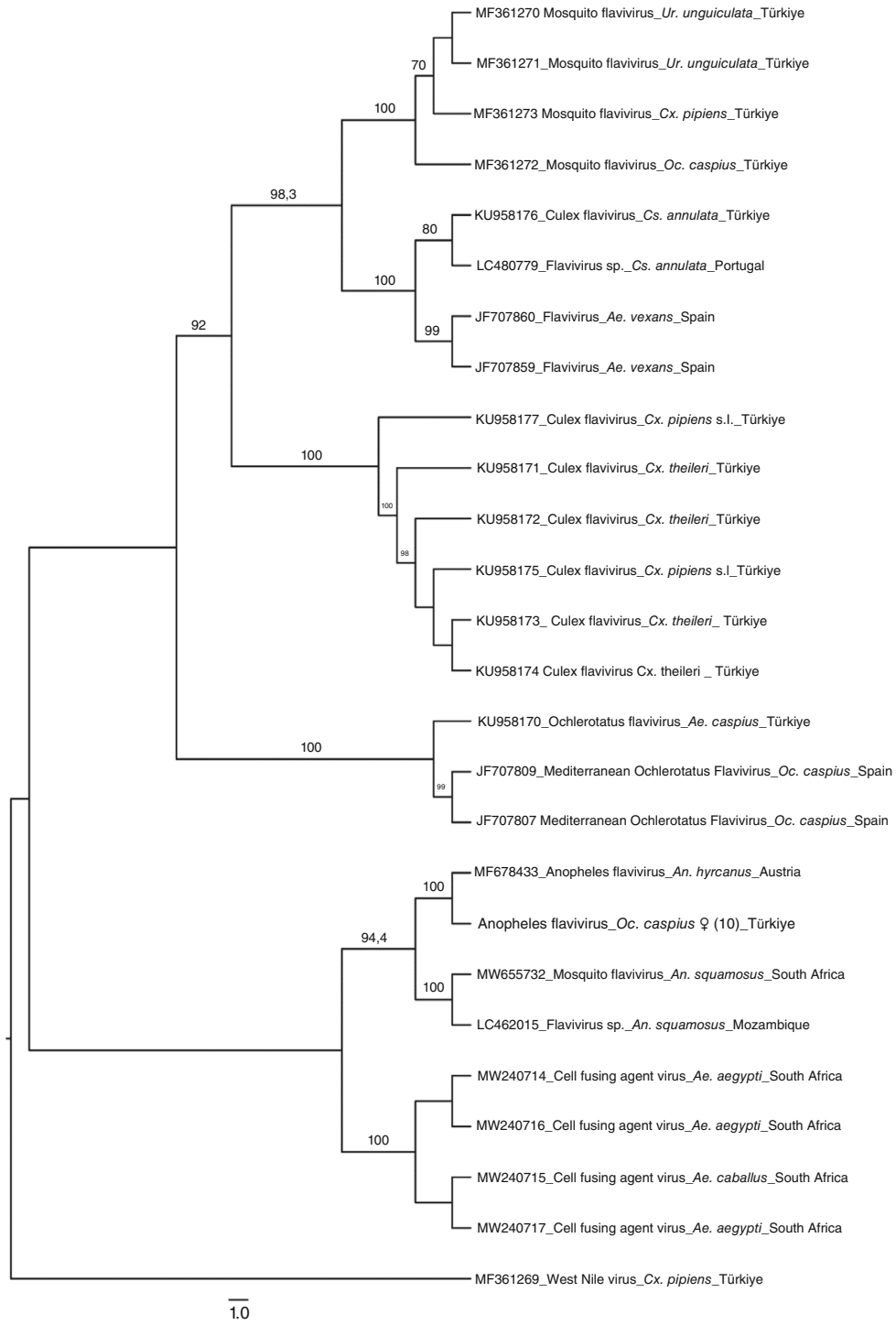

FIGURE 2 Maximum Likelihood tree for *Orthoflavivirus* NS5 sequences of ISFs. The sequences retrieved from GenBank are provided with their accession numbers. The *Anopheles flavivirus* sequence obtained in this study is highlighted in bold, and the pool size for *Oc. caspius* is indicated in parentheses. Only the bootstrap values higher than 70 were shown.

being sampled from Çankırı (Estimate = 1.357, $p = 0.00633$) significantly increased the likelihood of *Wolbachia* infection compared to the reference location, Adana. However, the Analysis of Deviance Table test did not show a significant effect for the overall location ($p = 0.2156$) and sex ($p = 0.2379$) variables. In contrast, mosquito taxon was a significant predictor of the variation observed in infection rates ($p < 0.0001$).

The sequencing of the *wsp* gene region was successful for 19 *Wolbachia* infected pools (GenBank accession numbers: PV021264-PV021282). The NJ analysis of these sequences, along with the other *wsp* sequences retrieved

TABLE 2 Estimates of infection prevalence for *Wolbachia* in mosquito pools using the maximum-likelihood estimate of pooled prevalence method.

	Prevalence	Lower CI (95%)	Upper CI (95%)	No. of mosquito specimens	No. of pools	No. of positive pools
All taxa	0.0119	0.008	0.0161	3306	397	38
An. claviger	0.0013	7.3317×10^{-5}	0.0062	784	82	1
Oc. caspius	0.0031	0.0005	0.0010	653	81	2
Cx. perexiguus	0.0059	0.0395	0.0865	496	62	24
Cx. pipiens s.l.	0.1008	0.0559	0.1720	145	23	11

Abbreviations: CI, confidence interval.

from GenBank, revealed that *An. claviger* ($n = 1$), *Cx. perexiguus* ($n = 13$) and *Cx. pipiens* s.l. ($n = 5$) specimens collected from three provinces were infected with *Wolbachia* strains belonging to the Supergroup B (Figure 3).

DISCUSSION

Monitoring studies are vital for public health as they serve as key tools for the early detection of pathogens, helping predict their spread and enabling effective response strategies. They allow researchers to evaluate the prevalence and intensity of infections in mosquito populations, assess disease transmission risks to humans and animals, and explore factors influencing pathogen spread within these communities. This study was conducted in selected locations across three provinces: Ankara, Çankırı and Adana. A total of 8766 mosquitoes from 11 taxa were recorded in these areas. The dominant taxon was *Oc. caspius*, followed by *An. claviger* and *Cx. perexiguus*, while *Ae. vexans* and *An. superpictus* were the least common. Our findings revealed that the sampling effort (224 trap-nights) in the surveyed localities (Damyeri, Otluk and Zerdali) in Adana is nearly complete. The mosquito fauna included seven taxa, aligning partially with previous surveillance in a larger region encompassing our sampling sites. Although we recorded *Oc. caspius* for the first time in these three villages, it is known to be distributed in other distinct regions of Adana [40, 41]. The Chao1 richness estimate indicated complete sampling in Çankırı, where we collected the most specimens with minimal trapping effort (9 trap-nights). *An. claviger* (40.79%) and *Oc. caspius* (44.28%) were the dominant taxa and were also recorded for the first time in this province. However, *Coquillettidia richardii* and *Culex modestus*, previously documented in the same area [41], were not found during our study. In Ankara, our sampling (133 trap-nights) was incomplete (Chao1 estimator = 7.49, 95% CI: 7.00–10.78), but we did record *Ae. cretinus*, *Ae. vexans*, *Cx. perexiguus* and *Oc. caspius* for the first time. Previous surveys in urban Ankara also identified *An. claviger*, *An. maculipennis* s.l. and *Cx. pipiens* s.l. and noted the absence of three taxa we could not detect: *Cq. richardii*, *Culiseta annulata* and *Cx. theileri* [15, 41].

Arboviruses, which are transmitted by arthropods like mosquitoes, ticks and sand flies, are prevalent in various regions worldwide, including Türkiye. Among these, *Orthoflavivirus nilense* (WNV) is recognised as the most common mosquito-borne virus circulating in the country. *Orthoflavivirus denguei* (DENV) and *Orthoflavivirus zikaense* (ZIKV) have been reported as imported cases [26]. WNV was first identified in 2007 from human serum samples in Şanlıurfa Province [42]. Since then, the virus has spread to other areas via various vertebrate hosts, being detected not only in human sera [16, 21] but also in donkeys in Iğdır and corvids in Istanbul [43, 44]. In a comprehensive bio-surveillance study conducted between 2014 and 2015 across Türkiye, WNV was identified alongside several ISVs in Sakarya Province, with a human case reported. Additionally, WNV was found in *Ae. albopictus* and *Cx. pipiens* from the Black Sea Region, as well as in *Cx. pipiens* s.l. pools from Thrace [22, 25, 45]. ZIKV and DENV were also identified as imported cases from travellers with clinical symptoms [46, 47]. Although the screening assay used in our study effectively detects flaviviruses [35], we did not detect any pathogenic flaviviruses in mosquito pools from Adana, Ankara and Çankırı. However, WNV cases have been documented in horses and humans in Ankara [48, 49]. Furthermore, WNV exposure among humans and animals, along with the detection of WNV RNA in mosquitoes from several locations in the Mediterranean and Eastern Thrace regions [21], highlights the virus's circulation in different regions of Turkey and emphasises the need for continued WNV monitoring for public health.

Controlling arboviral diseases is increasingly challenging due to vector behaviours and environmental changes, driving interest in alternative control methods [50]. Identifying natural symbionts in mosquitoes that reduce arbovirus transmission is a key step for these efforts. For instance, the Nhumirim virus inhibited *Orthoflavivirus nilense* replication [6], while dual ISV infections in *Aedes* cells limited arbovirus replication and altered cell susceptibility [51]. Novel ISVs have also been linked to interactions with chikungunya [52], underscoring their potential to influence arbovirus dynamics.

The current study detected *Anopheles flavivirus* (AnFV) in female *Oc. caspius* pools collected from Çankırı, showing high similarity to AnFV identified in *An. hyrcanus* from Austria and an ISV found in *An. maculipennis* s.l. from Türkiye [24]. This reports the first identification of AnFV in *Oc. caspius* in Türkiye. While this finding is new for this mosquito genus, another study had previously reported ISVs,

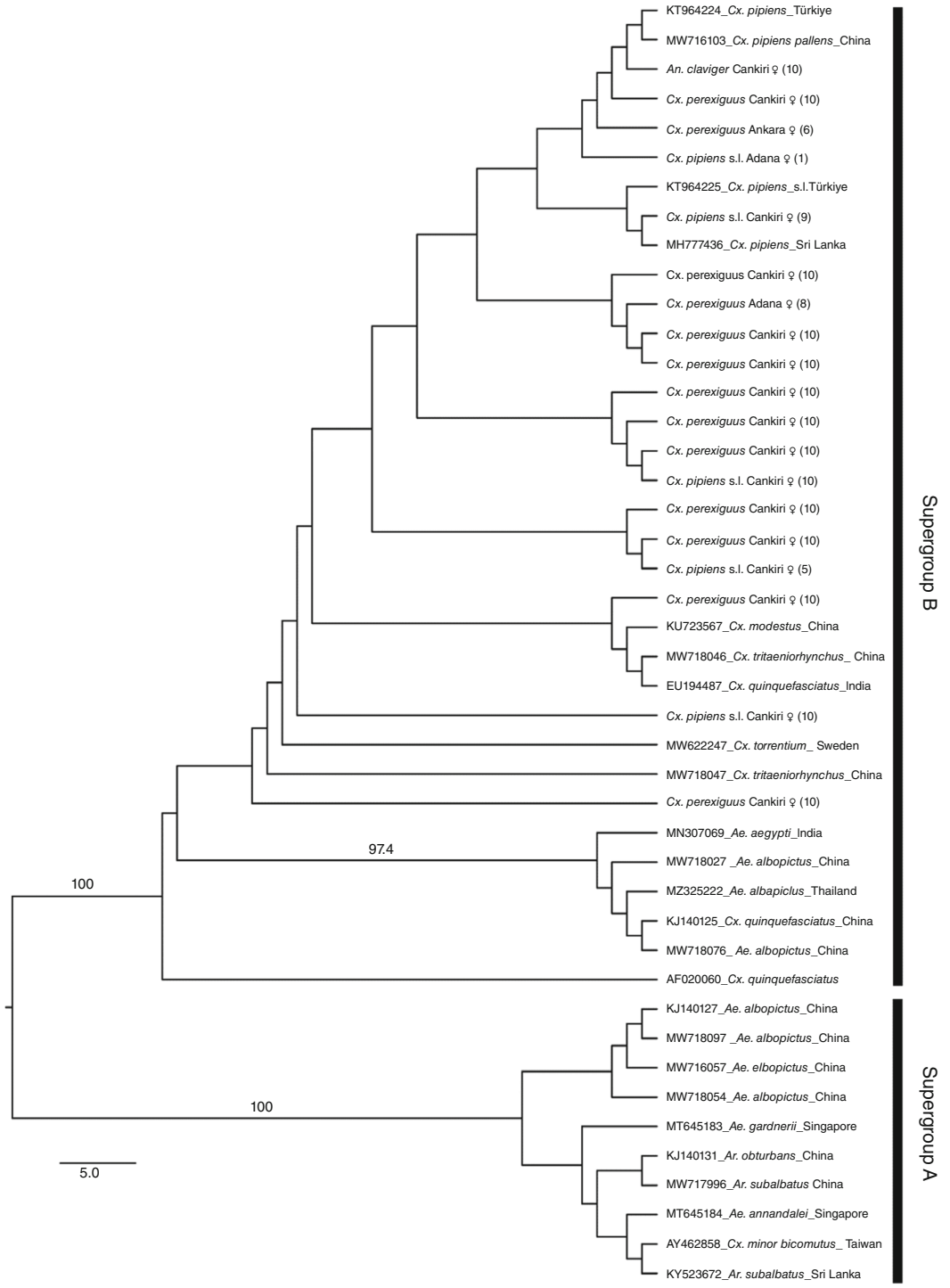

FIGURE 3 Neighbour-Joining tree for the *Wolbachia wsp* gene sequences. Sequences retrieved from GenBank are labelled with their accession numbers. Sequences obtained from the mosquito pools in this study include corresponding location and sex information. Pool sizes are indicated in parentheses. Only bootstrap values higher than 70 are shown.

including *Culex*-specific viruses in *Anopheles* mosquitoes [53]. In China, *Culex flavivirus* was found to infect several mosquito species, including *An. sinensis* [54]. Additionally, AnFV has been detected in *Aedes* mosquitoes, which are typically known vectors for diseases like DENV and ZIKV [54]. AnFV belongs to the *Flaviviridae* family, similar to other orthoflaviviruses such as WNV, ZIKV and DENV. Its presence in *Aedes* mosquitoes suggests possible ecological interactions or shared habitats between these species. This underscores the complexity of viral ecology and the necessity for ongoing surveillance to grasp vector competence and disease transmission dynamics among different

mosquito species. Phylogenetic analysis indicates that the novel strain found in *Oc. caspius* in Türkiye is closely related to strains from Mozambique and South Africa, suggesting either geographical spread or a common ancestry of the virus across various regions. These findings highlight the global distribution and evolutionary connections of orthoflaviviruses within mosquito populations [55]. Ergünay et al., 2017, conducted an extensive bio-surveillance study across Türkiye, identifying multiple distinct ISVs co-circulating with WNV in mosquito populations in the Mediterranean, Aegean and Thrace regions. These interactions could have significant implications for disease epidemiology. Although research on viruses in *Anopheles* species is limited, high similarities have been noted between ISFs from Hungary and the USA and the novel strain identified in this study. In France, Lequime and Lambrechts (2017) reported endogenous viral components related to orthoflaviviruses in the genomes of *Anopheles* mosquitoes, indicating the presence of rare orthoflaviviruses associated with this genus [56]. Similarly, Colmant et al., 2017 found a new lineage of ISVs in Australian *Anopheles* mosquitoes, which aligns with previous findings [57]. However, some research has not found evidence that cell-fusing agent virus (CFAV) affects WNV replication, transmission, or dissemination in *Cx. quinquefasciatus* mosquitoes [58]. Overall, these results enhance our understanding of the diversity and host interactions of orthoflaviviruses, highlighting the importance of studying vector-specific viral dynamics for effective disease control.

Detecting symbiotic *Wolbachia* in mosquitoes is crucial for disease surveillance, understanding transmission dynamics and informing vector control strategies. In this study, we identified *Wolbachia* infections in various vectors, including *Cx. pipiens* s.l., *Cx. perexiguus*, *Oc. caspius* and the malaria vector *An. claviger*, across the surveyed provinces. Sequencing of the *wsp* gene region from *Wolbachia-infected* pools revealed that all strains belong to Supergroup B, with the highest infection rate in *Cx. pipiens* s.l. (0.1008), followed by *Cx. perexiguus* (0.0059), *Oc. caspius* (0.0031) and *An. claviger* (0.0013). These findings constitute the first documented report of *Wolbachia* symbionts in Ankara, Çankırı and Adana, as well as the first evidence of natural infections in *Cx. perexiguus* and *An. claviger*. The initial evidence of natural *Wolbachia* infection in Türkiye was reported in Kayseri in *Cx. pipiens*, with a low infection rate (5.08%) [59, 60]. Similar evidence has also been documented in western Türkiye, indicating a wider presence of *Wolbachia* infections in *Cx. pipiens* populations across different regions [29]. Furthermore, another investigation delved into the diversity of *Wolbachia* strains and their linked CI in *Cx. pipiens* s.l. populations in Türkiye. This research holds significance as *Wolbachia* can trigger CI, where infected males can only mate successfully with infected females, impacting mosquito population dynamics. Various *Wolbachia* strains were identified in a considerable number of Turkish *Cx. pipiens* s.l. populations, examining how these strains affect CI patterns [61]. The strain identified in *Culex* species in our investigation showed a high similarity to the previously described strain in Türkiye. Additionally, this particular *Wolbachia* strain has also been reported in several other countries, including India and Sri Lanka [62, 63]. Importantly, this study represents the first documentation of *Wolbachia* in three new provinces in the Mediterranean region and Central Anatolia, marking a significant advancement in understanding the circulation of these bacteria in Türkiye. This underscores the need for further research to map the distribution of *Wolbachia* and assist policymakers and researchers in the timely implementation of control measures. Our findings indicated that none of the mosquito species were co-infected with *Wolbachia* and arboviruses. Previous studies have shown that *Wolbachia* presence in mosquitoes is associated with reduced arboviral replication and transmission [46]. Additionally, *Wolbachia* has been found to enhance ISF infection in *Ae. aegypti*, potentially influencing disease dynamics, while simultaneously inhibiting DENV infection [12]. Co-infection with *Wolbachia* and DENV has also been shown to reduce virus transmission [13]. These results emphasise the intricate relationships between *Wolbachia*, arboviruses and mosquito vectors, highlighting *Wolbachia* ‘s potential as a tool for controlling arboviral diseases. Additionally, *Ae. aegypti* mosquitoes infected with *Wolbachia* have demonstrated the ability to block pathogens like DENV and ZIKV [14]. This could explain the absence of arboviruses in *Wolbachia-infected* mosquito pools, illustrating how *Wolbachia* may interfere with arbovirus replication or transmission within the host mosquitoes in Çankırı, Ankara and Adana. To deepen our understanding, it is vital to broaden this surveillance study or conduct experimental infections in the surveyed areas, providing valuable insights into transmission dynamics and potential control strategies related to *Wolbachia* and arboviral diseases.

To utilise *Wolbachia* as a strategy against mosquito-borne diseases such as through sterile insect techniques or pathogen-blocking methods it’s essential to induce CI to integrate the bacteria into populations of naturally occurring arthropods [64]. While the endosymbiont *Wolbachia* has been shown to inhibit arbovirus replication and spread, prior research indicates that the *Wolbachia* strains identified in this study confer CI [65], making them suitable for control applications. Our study showed that *Cx. pipiens* s.l. exhibited a high average infection rate compared to *Cx. perexiguus*. These results are consistent with earlier investigations by Morçiçek et al. (2018), which reported a strong association between *Wolbachia* and *Cx. pipiens* s.l., with infection rates of 50% in Aydın, 75% in İzmir and 100% in Çanakkale, Türkiye. Notably, *Cx. perexiguus* was not found to be infected in the six Aegean provinces surveyed. The findings also corroborate research by Yang et al., 2021 [47] and Bergman et al., 2021 [66], who documented high *Wolbachia* infection rates in *Cx. pipiens* in China (97%) and Sweden (87%), highlighting the capacity of these arboviral vectors to harbour the bacteria. Although *Oc. caspius* and *An. claviger* showed low *Wolbachia* infection rates in our study, their infection levels varied in other regions [47].

A pertinent question for future research is whether *Wolbachia* remains less susceptible to infection in other provinces of Türkiye, and whether *Aedes* and *Anopheles* species are more likely to harbour *Wolbachia* compared to *Culex*. Literature indicates that *Culex* species, particularly *Cx. quinquefasciatus*, demonstrates a high prevalence of natural *Wolbachia* infection, with nearly all populations approaching 100% infection rates [67]. Conversely, *Wolbachia* infections in *Aedes* mosquitoes have been primarily observed in experimental settings, while they are rare in *Anopheles* populations [68]. The overall low prevalence of *Wolbachia* infections in the studied area may be attributed to limitations in detection methods, which can only identify low-frequency *Wolbachia* signals and may overlook low-level infections [47]. Variability among host species could also play a role, as some species may exhibit natural resistance or low compatibility with the bacteria [69]. A negative infection rate for *Wolbachia* in certain vectors should not be taken to mean that this endosymbiont is absent. Collectively, these findings indicate that *Wolbachia* infections in mosquitoes may occur in a variable manner across Türkiye. Logistic regression analysis revealed that mosquito taxon is a significant predictor of *Wolbachia* infection rates, emphasising the strong influence of mosquito species on *Wolbachia* prevalence. This information is vital for understanding and managing mosquito-borne diseases, as different species have varying abilities to transmit pathogens [70]. By targeting specific mosquito taxa with *Wolbachia*-based control strategies such as those that alter vector competence or population dynamics [71] researchers could potentially reduce disease transmission more effectively. The Analysis of Deviance Table test did not indicate a significant effect for overall location variables, and sex had no influence on *Wolbachia* infection rates, suggesting that both males and females are equally likely to be infected. This implies that *Wolbachia* infection rates are independent of the host's sex, indicating that other factors, such as genetic background, environmental conditions, or specific behaviours, may have a more substantial impact on infection rates than sex alone [72].

CONCLUSIONS

This study reveals the presence of bacterial and insect-specific viral symbionts in natural populations of *Cx. perexiguus*, *Cx. pipiens* s.l., *Oc. caspius* and *An. claviger* mosquitoes in Türkiye. It also reports the first identification of natural *Wolbachia* infection in the provinces of Çankırı, Ankara and Adana. This finding involves several disease vector species, especially those associated with arboviruses and malaria, which have not been previously investigated. It is crucial to continue exploring a wider range of symbionts in mosquitoes to develop control methods based on bacterial and viral symbionts. These approaches have the potential to significantly impact the transmission of arboviral diseases in Türkiye and globally, representing a significant advancement in vector management.

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SUPPORTING INFORMATION

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