Wolbachia Infection of Neoceratitis asiatica (Diptera: Tephritidae)

Authors: Wang, Xiaoxue, Li, Zhihong, Zhang, Rong, He, Jia, Zhao, Zihua, et al.

Source: Florida Entomologist, 102(1) : 125-129

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.102.0120

Wolbachia infection of *Neoceratitis asiatica* (Diptera: Tephritidae)

Xiaoxue Wang[1], Zhihong Li[1], Rong Zhang[2], Jia He[2], Zihua Zhao[1], Shuhua Wei[2], and Lijun Liu[1,]*

Abstract

Neoceratitis asiatica (Becker) (Diptera: Tephritidae), known as wolfberry fruit fly, is a harmful pest of *Lycium barbarum* (Solanaceae). *Neoceratitis asiatica* female adults insert the ovipositor into the peel of *L. barbarum* and lay eggs, causing reductions in yield and economic loss. The symbiotic bacteria *Wolbachia* spp. have attracted considerable attention and interest by entomologists in recent years. *Wolbachia* infect many genera of tephritid fruit flies, such as *Anastrepha*, *Bactrocera*, *Rhagoletis*, *Dacus*, *Ceratitis*, and *Carypomya*. *Wolbachia* can induce complete cytoplasmic incompatibility in novel hosts, leading to complete suppression of laboratory populations by single releases of infected males, which potentially makes it a useful method for pest management. In this study, the infection of *Wolbachia* in *N. asiatica* from the Ningxia region in China was detected based on the *Wolbachia* surface protein gene sequence. The neighbor-joining tree showed *Wolbachia* in wolfberry fruit fly was *w*Ri strain. This research lays the foundation for further study about *Wolbachia* in Chinese wolfberry fruit fly, and also provides a basis for the prevention and control of other economically important fruit flies using *Wolbachia*.

Key Words: *Wolbachia w*Ri strain; *wsp* gene; phylogeny

Resumen

Neoceratitis asiatica (Becker) (Diptera: Tephritidae), conocida como la mosca de la fruta de Wolfberry, es una plaga nociva de *Lycium barbarum* (Solanaceae). Las adultas hembras de *Neoceratitis asiatica* insertan el ovipositor en la cascara de *L. barbarum* y ponen huevos, lo que provoca una reducción en el rendimiento y una pérdida económica. La bacteria simbiótica *Wolbachia* spp. ha atraído una considerable atención e interés por entomólogos en los últimos años. *Wolbachia* infecta muchos géneros de moscas de la fruta tefrítidas, como *Anastrepha*, *Bactrocera*, *Rhagoletis*, *Dacus*, *Ceratitis*, y *Carypomya*. *Wolbachia* puede inducir incompatibilidad citoplásmica completa en hospederos noveles, lo que lleva a la supresión completa de las poblaciones de laboratorio por liberaciones únicas de machos infectados, lo que le convierte potencialmente en un método útil para el manejo de plagas. En este estudio, se detectó la infección de *Wolbachia* en *N. asiatica* de Ningxia en China en base a la secuencia del gen de la proteína de la superficie de *Wolbachia*. El árbol filogenético conjuntando vecinos que se unió mostró que la *Wolbachia* en la mosca de la fruta de wolfberry es la cepa *w*Ri. Esta investigación sienta las bases para un estudio adicional sobre *Wolbachia* en la mosca de la fruta de wolfberry chino, y también proporciona una base para la prevención y el control de otras moscas de la fruta económicamente importantes utilizando la *Wolbachia*.

Palabras Clave: *Wolbachia w*Ri strain; gen *wsp*; filogenia

Neoceratitis asiatica (Becker) (Diptera: Tephritidae), known as wolfberry fruit fly, became the most harmful pest to *Lycium barbarum* (Solanaceae) during the 1950s through the 1970s in the Ningxia region of China (Wu et al. 1963), and yield loss was more than 20%, sometimes as high as 55%. From the 1990s to the beginning of the 21st century, it was a consistently damaging pest (Ren & Hu 2004; Ren 2010). *Lycium barbarum*, which is called Chinese wolfberry in English, is a source of Chinese herbal medicines. Its dried ripe fruit has beneficial effects on the liver, kidneys, and eyes (Zhao et al. 2009; Xu et al. 2014). Berries, leaves, and roots of *L. barbarum* contain polysaccharides, amino acids, vitamins, and trace elements that are of high medicinal and nutritional value (Xu et al. 2014). *Lycium barbarum* is an important economic crop in the northwest region of China, especially in Ningxia, Xinjiang, and Gansu. Inner Mongolia, Qinghai, Hebei, Xizang, Shaanxi, Shanxi, Liaoning, Jiangsu, Zhejiang, and Guangdong Provinces also have this species (Wu & Gao 1964; Hu et al. 2009; Xue & Lin 2009; Xu et al. 2014).

The infestation rate of wolfberry trees has attained 70% in severely affected areas (Zheng 2015). *Neoceratitis asiatica* female adults insert the ovipositor into the peel of *L. barbarum* and lay eggs. Initially, the exterior surface of damaged *L. barbarum* shows no difference from healthy fruit, but in the late stage, white curved stripes appear on the peel. Beneath the peel of infested fruit, the flesh is consumed by *N. asiatica* and the fruit is full of frass. Under these circumstances, the fruit cannot be used as a commodity or medicine, and thus has no economic value (Wu et al. 1963; Guo et al. 2017; Li et al. 2017).

Neoceratitis asiatica occurs during May to Sep, producing 3 generations per yr. It has 3 fairly unique characteristics. First, *N. asiatica* is monophagous, feeding only on *L. barbarum*. Second, the

[1]Department of Entomology, College of Plant Protection, China Agricultural University, Beijing 100193, China; E-mails: wangxiaoxue94@163.com (X. W.), lizh@cau.edu.cn (Z. L.), zhzhao@cau.edu.cn (Z. Z.), ljliu@cau.edu.cn (L. L.)
[2]The Institute of Plant Protection, Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan, 750002, China; E-mails: yczhrnx@163.com (R. Z.), hejiayc@126.com (J. H.), weishuhua666@163.com (S. W.)
*Corresponding author; Email: ljliu@cau.edu.cn

adult female generally lays only 1 egg per fruit. If 2 or 3 eggs are laid in a fruit, only 1 larva survives. Third, adults have no phototaxis, and cannot be baited with sweet or sour wine (Wu & Meng 1963). These traits make it difficult to capture and culture *N. asiatica*. Investigations into *N. asiatica* began in the 1960s, and in the nearly half century following, research principally focused on the identification, pattern of occurrence, and control of the pest. Methods combining morphological characteristics and DNA barcoding have been used for its identification (Guo et al. 2017). The mitochondrial genome of this fruit fly also has been studied to determine its phylogenetic status (Su et al. 2017).

The symbiotic bacteria *Wolbachia* spp. (Anaplasmataceae) have attracted a great deal of attention by entomologists in recent years. *Wolbachia* were first detected in *Culex pipiens* (L.) (Diptera: Culicidae) (Hertig 1936). They are thought to have potentially important roles in genetic control of pests because of their effects on the reproduction of their hosts. *Wolbachia* can induce cytoplasmic incompatibility, parthenogenesis, and feminization of their host (Werren 1997). Cytoplasmic incompatibility provides a reproductive advantage to infected females over uninfected females, resulting in the invasion of *Wolbachia* into a population (Pan et al. 2018). *Wolbachia* can interfere with pathogen infection and inhibit some human pathogens, such as dengue and Zika viruses, malaria parasites, and filarial worms (Kambris et al. 2009; Moreira et al. 2009; Bian et al. 2010, 2013; Dutra et al. 2016). *Wolbachia* also infect many genera of tephritid fruit flies, including *Anastrepha*, *Bactrocera*, *Rhagoletis*, *Dacus*, *Ceratitis*, and *Carypomya* (Jamnongluk et al. 2002; Riegler & Stauffer 2002; Arthofer et al. 2009; Coscrato et al. 2009; Schuler et al. 2011, 2013; Augustinos et al. 2013, 2015; Coats et al. 2013; Karimi & Darsouei 2014; Morrow et al. 2015). Zabalou et al. (2004) transinfected cytoplasmic incompatibility-*Wolbachia* from *Rhagoletis cerasi* (L.) to *Ceratitis capitata* (Wiedemann) (both Diptera: Tephritidae), and *Wolbachia* induced complete cytoplasmic incompatibility in the novel host, leading to complete suppression of laboratory populations by single releases of infected males. Several studies also reported occurrence of *Wolbachia* in Chinese populations of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). However, there were no infections of *B. dorsalis* by *Wolbachia* in Yunnan, Fujian, and Wuhan populations (Augustinos et al. 2015). Similar to this result, Liu et al. (2016) also found the absence of *Wolbachia* in 16 collection sites in Thailand, China (Yunnan, Guizhou, Guangxi, Guangdong, Hainan, Fujian, Zhejiang, Shanghai Provinces), and the lab population. In contrast, *Wolbachia* had been positively detected in the Fujian, Guangdong, Hainan, Yunnan, and Guangxi Provinces, but the infection rates were very low (0.7–3%) (Sun et al. 2007). That may be the reason why the cytoplasmic incompatibility-*Wolbachia* based method has not been used in *B. dorsalis* successfully. However, if we can find *Wolbachia* strains that can cause cytoplasmic incompatibility in other fruit flies, such as *Wolbachia* in *N. asiatica*, we can try to transinfect this specific strain to *B. dorsalis* to reduce its population. In brief, *Wolbachia*-induced cytoplasmic incompatibility may be used as an environmentally friendly tool for the biological control of fruit flies. However, lack of a suitable strain prevented the application of *Wolbachia* in fruit fly management. We believe that assessment of *Wolbachia* in different species of fruit flies can provide more candidate strains for pest management.

In this study, *Wolbachia* infection in different developmental stages of *N. asiatica* from Ningxia Province in China was detected based on *wsp* gene sequence, and then the phylogenetic relationship of the *Wolbachia* strain in this fruit fly was analyzed via neighbor-joining tree building. This research will help us in screening more putative strains with good potential for future applications in pest management, and thus provide a basis for the prevention and control of other economically important fruit flies.

Materials and Methods

SAMPLE COLLECTION

Larvae, pupae, and adults were collected in wolfberry trees from Zhongning County, Ningxia (1.090166°E, 37.190000°E) on 30 Sep 2017 (Table 1). All samples were preserved in 100% ethanol and stored at −4 °C before DNA extraction.

DNA EXTRACTION, WSP GENE AMPLIFICATION, AND SEQUENCING

Total genomic DNA was isolated from whole individuals of *N. asiatica* using the commercial TIANamp Genomic DNA Kit (TIANGEN Biotech Co. Ltd., Beijing, China) following the manufacturer's protocol. Polymerase chain reaction (PCR) was completed in a final volume of 25 µL containing 12.5 µL 2 × Taq PCR MasterMix, 9.5 µL sterilized distilled water, 1 µL DNA as a template, 1 µL forward and 1 µL reverse primer, respectively. Amplifications were performed with general primers (Table 2) for *wsp* gene (Braig et al. 1998) used the following thermal cycling profile: 95 °C for 3 min; followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min; then 72 °C for 10 min. The reaction was performed on Veriti TM 96-well Thermal Cycler (Applied Biosystems Inc., Waltham, Massachusetts, USA). After amplification, 5 µL of PCR products were separated in 1.5% (w/v) agarose gels (1 × Tris Acetate-EDTA buffer) and stained with GeneGreen Nucleic Acid Dye (TIANGEN Biotech Co. Ltd., Beijing, China) and visualized under UV light. The PCR products were purified and bi-directional sequenced using the same amplification primers used commercially by the Sangon Biotech Co. Ltd., Shanghai, China.

DATA ANALYSES

The sequences obtained from *N. asiatica* were Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information, Bethesda, Maryland, USA. The *wsp* gene sequences from this study were aligned using MEGA 7.0 (Temple University, Philadelphia, Pennsylvania, USA) (Kumar et al. 2016), and 3 representative sequences were selected. The 3 sequences then were compared with 24 reference *wsp* sequences from GenBank representing different *Wolbachia* strains infecting various hosts (Table 3). We used Gblocks Server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) to eliminate poorly aligned positions and divergent regions so that it became more suitable for phylogenetic analysis. The neighbor-joining tree was conducted in MEGA version 7.0, distances were calculated using the Kimura 2-parameter (K2P) model (Kimura 1980) in the bootstrap test (1,000 replications) (Felsenstein 1985). All 3 sequences were submitted to GenBank; the accession numbers MG950140, MG940141, MG950142 were *wsp* genes in larvae, pupae, and adults of *N. asiatica*, respectively (Table 1).

Table 1. Sample information of collection site and accession number.

Location information	Accession number in NCBI
Zhongning County, Ningxia (37.324040°N, 105.688220°E)	MG950140 (larvae) MG940141 (pupa) MG950142 (adult)

Table 2. General primers used to amplify wsp gene.

Primer	5'-sequence-3'
81 F (forward)	5'-TGG TCC AAT AAG TGA TGA AGA AAC-3'
691 R (reverse)	5'-AAA AAT TAA ACG CTA CTC CA-3'

Results

BLAST RESULT

The BLAST result from National Center for Biotechnology Information showed that all the sequences from the total DNA of *Neoceratitis asiatica* were the *wsp* gene of *Wolbachia*. The sequences of the *wsp* gene of *Wolbachia* in different stages of *N. asiatica* showed that they were identical, and were similar to the *w*Ri strain.

PERCENTAGE INFECTION

Using the universal primers (81F, 691R) for *Wolbachia*, we screened 50 larvae, 50 pupae, and 32 adults. Thirteen larvae, 26 pupae, and 32 adults were positively detected in agarose gels.

PHYLOGENETIC ANALYSIS

Phylogenetic analysis of partial sequences of the *wsp* gene identified in *N. asiatica*, other fruit flies, and several other arthropod species are shown in Figure 1. In this neighbor-joining tree, 3 *wsp* gene sequences of *Wolbachia* in this study were in A Group, and together with the *w*Ri strain in *Drosophila simulans*. However, the infection of *Wolbachia* in females and males has not been compared, and should to be the focus of future research.

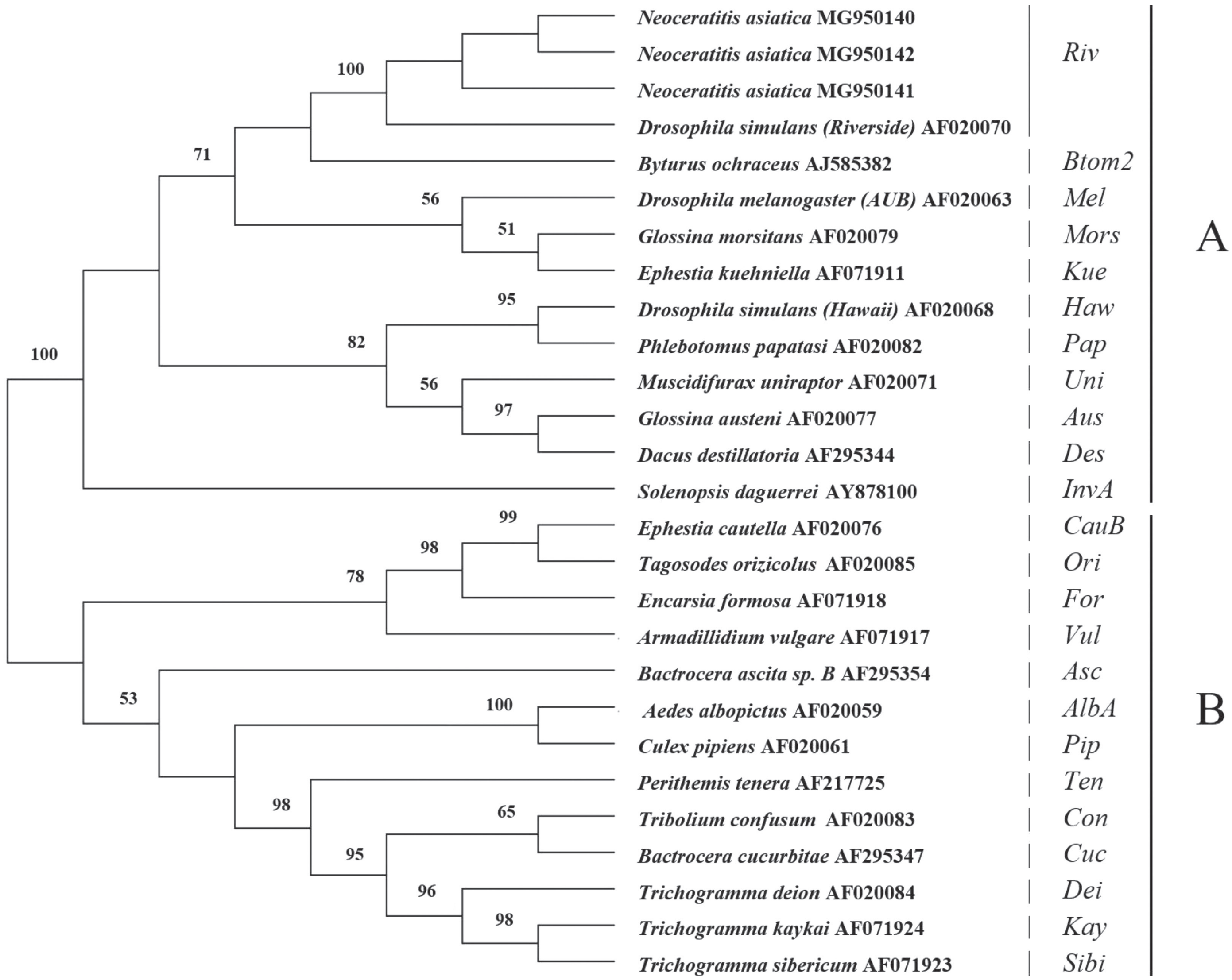
Discussion

Wolbachia are one of the most abundant symbiotic microbes in arthropods (Werren 1997). In 1 species, when a male is infected by 1 or more kinds of *Wolbachia*, mating with an uninfected female or carrying a different strain, cytoplasmic incompatibility results in embryonic mortality (Bourtzis et al. 2003). The cytoplasmic incompatibility caused by *Wolbachia* can be used as an environmentally benign tool for the biological control of fruit flies. Most *Wolbachia* in fruit flies belonged to group A. Sun et al. (2007) found that *B. dorsalis* in Yunnan and Hainan provinces carried strain *Cuc* and *Mel* of *Wolbachia*, respectively. *Rhagoletis cerasi* (L.), *Anastrepha* spp., and *C. capitata* (all Diptera: Tephritidae) also had the strain *Mel*; *Dacus destillatoria* (Bezzi) contained the strain *Des* (Coscrato et al. 2009). *Ceratitis vesuviana* Costa showed infection with *Wolbachia* between *Mors* and *Riv* in the phylogenetic tree (Karimi & Darsouei 2014).

This research confirmed that different development stages of wolfberry fruit fly can be infected with the *w*Ri strain of *Wolbachia*. *w*Ri have a significant influence on cytoplasmic incompatibility in their natural host *D. simulans* (Hoffmann et al. 1986; Weeks et al. 2007), which may contribute to the pest management of economically important fruit flies by *Wolbachia* transmission. Bian et al. (2010) used *Wolbachia* strain *w*AlbB to control the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae), the vector of dengue and Zika viruses. They found this strain could inhibit viral replication and dissemination in *Ae. aegypti*; thus, the spread of dengue and Zika viruses can be blocked through the control of the insect. They also used *Wolbachia* strain *w*AlbB to increase resistance in the mosquito *Anopheles stephensi* Liston (Diptera: Culicidae) to the human malaria parasite *Plasmodium falciparum* (Bian et al. 2013). *Wolbachia* can change the symbiotic relationship and can boost the immune system response to enhance the host's resistance to pathogens (Pan et al.

Table 3. Wolbachia strains used to construct the phylogenetic tree.

Wolbachia group	Supergroup	Host and associated *Wolbachia* strain (reference strain)	GenBank accession number
	A Group		
*Me*l		*Drosophila melanogaster* (AUB)	AF020063
*Alb*A		*Aedes albopictus*	AF020059
*Mor*s		*Glossina morsitans*	AF020079
Riv		*Drosophila simulans* (Riverside)	AF020070
*Un*i		*Muscidifurax uniraptor*	AF020071
*Ha*w		*Drosophila simulans* (Hawaii)	AF020068
*Pa*p		*Phlebotomus papatasi*	AF020082
*Au*s		*Glossina austeni*	AF020077
*De*s		*Dacus destillatoria*	AF295344
*Ku*e		*Ephestia kuehniella*	AF071911
*Btom*2		*Byturus ochraceus*	AJ585382
*Inv*A		*Solenopsis daguerrei*	AY878100
	B Group		
*Co*n		*Tribolium confusum*	AF020083
*De*i		*Trichogramma deion*	AF020084
*Pi*p		*Culex pipiens*	AF020061
*Cau*B		*Ephestia cautella*	AF020076
*Cu*c		*Bactrocera cucurbitae*	AF295347
*Ka*y		*Trichogramma kaykai*	AF071924
*Sib*i		*Trichogramma sibericum*	AF071923
*Te*n		*Perithemis tenera*	AF217725
*Fo*r		*Encarsia formosa*	AF071918
*Vu*l		*Armadillidium vulgare*	AF071917
*As*c		*Bactrocera ascita* sp. B	AF295354
*Or*i		*Tagosodes orizicolus*	AF020085



. Phylogenetic tree of *Wolbachia* strains based on the *wsp* gene. Neighbor-joining based on *wsp* gene sequences showing the relationships among arthropod hosts and *Wolbachia* strains. Distances were calculated using the K2P model with a bootstrap test (1,000 replications). The number at each branch point is the percentage supported by the bootstrap. Only numbers greater than 50 are shown.

2018). However, there is still an absence of population control of *B. dorsalis* using *Wolbachia*. The low infection rate of *Wolbachia* in *B. dorsalis* is the biggest obstacle. Transinfecting *Wolbachia* strains from other fruit flies maybe a good solution to this problem. This research provided a candidate strain, isolated from wolfberry fruit fly, with the potential for controlling *B. dorsalis*. In further research, we will focus on the suitability of *Wolbachia* for population control in *B. dorsalis*. The potential exists to control not only *B. dorsalis*, but also other economically important fruit flies.

Our study was the first to document the existence of *Wolbachia* in Chinese wolfberry fruit fly. We suggest that the *w*Ri strain of *Neoceratitis asiatica* has the potential to assist in the prevention and control of other economically important fruit flies.

Acknowledgments

Financial support was provided by the National Key Research and Development Project of China (No. 2016YFC1200605), and the Science and Technology Innovation Pilot Fund Project of the Ningxia Academy of Agriculture and Forestry Sciences (NKYZ-16–0502). We are grateful to Jing Wei of China Agricultural University for help with collecting larvae from wolfberry fruit.

References Cited

Arthofer W, Riegler M, Schneider D, Krammer M, Miller WJ, Stauffer C. 2009. Hidden *Wolbachia* diversity in field populations of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera, Tephritidae). Molecular Ecology 18: 3816–3830.

Augustinos AA, Asimakopoulou AK, Moraiti CA, Mavragani-Tsipidou P, Papadopoulos NT, Bourtzis K. 2013. Microsatellite and *Wolbachia* analysis in *Rhagoletis cerasi* natural populations: population structuring and multiple infections. Ecology and Evolution 4: 1943–1962.

Augustinos AA, Drosopoulou E, Gariou-Papalexiou A, Asimakis ED, Cáceres C, Tsiamis G, Bourtzis K, Mavragani-Tsipidou P, Zacharopoulou A. 2015. Cytogenetic and symbiont analysis of five members of the *B. dorsalis* complex (Diptera, Tephritidae): no evidence of chromosomal or symbiont-based speciation events. Zookeys 540: 273–298.

Bian G, Joshi D, Dong YM, Lu P, Zhou GL, Pan XL, Xu Y, Dimopoulos G, Xi ZY. 2013. *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. Science 340: 748–751.

Bian G, Xu Y, Lu P, Xie Y, Xi ZY. 2010. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. PLoS Pathogens 6: e1000833. doi: [10.1371/journal.ppat.1000833]

Bourtzis K, Braig HR, Karr TL. 2003. Cytoplasmic incompatibility, pp. 217–246 *In* Bourtzis K, Miller TA [eds.], Insect Symbiosis, 1st edition. CRC Press, Boca Raton, Florida, USA.

Braig HR, Zhou W, Dobson S, O'Neill SL. 1998. Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia*. Journal of Bacteriology 180: 2373–2378.

Coats VC, Stack PA, Rumpho ME. 2013. Japanese barberry seed predation by *Rhagoletis meigenii* fruit flies harboring *Wolbachia* endosymbionts. Symbiosis 59: 145–156.

Coscrato VE, Braz ASK, Perondini ALP, Selivon D, Marino CL. 2009. *Wolbachia* in *Anastrepha* fruit flies (Diptera: Tephritidae). Current Microbiology 59: 295–301.

Dutra HL, Rocha MN, Dias FB, Mansur SB, Caragata EP, Moreira LA. 2016. *Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. Cell Host & Microbe 19: 771–774.

Felsenstein J. 1985. Confidence intervals on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Guo SK, He J, Zhao ZH, Liu LJ, Gao LY, Wei SH, Guo XY, Zhang R, Li ZH. 2017. Identification of *Neoceratitis asiatica* (Becker) (Diptera: Tephritidae) based on morphological characteristics and DNA barcode. Zootaxa 4363: 553–560.

Hertig M. 1936. The *rickettsia*, *Wolbachia pipientis* and associated inclusions of the mosquito *Culex pipiens*. Parasitology 28: 453–486.

Hoffmann AA, Turelli M, Simmons GM. 1986. Unidirectional incompatibility between populations of *Drosophila simulans*. Evolution 40: 692–701.

Hu JF, Miu K, Dong ZH. 2009. Study on the occurrence and prevention of the disease and insect pests of Chinese wolfberry. Modern Agricultural Science and Technology 4: 101–102. (in Chinese with English summary)

Jamnongluk W, Kittayapong P, Baimai V, O'Neill SL. 2002. *Wolbachia* infections of tephritid fruit flies: molecular evidence for five distinct strains in a single host species. Current Microbiology 45: 255–260.

Kambris Z, Cook PE, Phuc HK, Sinkins SP. 2009. Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. Science 326: 134–136.

Karimi J, Darsouei R. 2014. Presence of the endosymbiont *Wolbachia* among some fruit flies (Diptera: Tephritidae) from Iran: a multilocus sequence typing approach. Journal of Asia-Pacific Entomology 17: 105–112.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. Journal of Molecular Evolution 16: 111–120.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874.

Li F, Liu XL, Ma JG. 2017. Study on the occurrence and prevention of wolfberry trypetid in Ningxia. Ningxia Journal of Agriculture and Forestry Science and Technology 58: 35–36. (in Chinese with English summary)

Liu LJ, Martinez-Sañudo I, Mazzon L, Prabhakar CS, Girolami V, Deng YL, Dai Y, Li ZH. 2016. Bacterial communities associated with invasive populations of *Bactrocera dorsalis* (Diptera: Tephritidae) in China. Bulletin of Entomological Research 106: 1–11.

Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu GJ, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin S, Day A, Riegler M, Hugo LE, Johnson KN, Kay BH, McGraw EA, van den Hurk AF, Ryan PA, O'Neill SL. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and plasmodium. Cell 139: 1268–1278.

Morrow JL, Frommer M, Royer JE, Shearman DCA, Riegler M. 2015. *Wolbachia* pseudogenes and low prevalence infections in tropical but not temperate Australian tephritid fruit flies: manifestations of lateral gene transfer and endosymbiont spillover? BMC Evolutionary Biology 15: 202. doi: [10.1186/s12862-015-0474-2]

Pan XL, Pike A, Joshi D, Bian GW, McFadden MJ, Lu P, Liang X, Zhang FR, Raikhel AS, Xi ZY. 2018. The bacterium *Wolbachia* exploits host innate immunity to establish a symbiotic relationship with the dengue vector mosquito *Aedes aegypti*. The ISME Journal 12: 277–288.

Ren YP. 2010. Evolution of disease and insect pest population of cultivated medlar in different periods and their chemical control method. Journal of Anhui Agriculture Science 38: 2443–2445. (in Chinese with English summary)

Ren YP, Hu ZQ. 2004. The progress of research on chemical control for main diseases and insect pests of Chinese wolfberry in Ningxia. Journal of Ningxia Agricultural College 25: 88–91. (in Chinese with English summary)

Riegler M, Stauffer C. 2002. *Wolbachia* infections and superinfections in cytoplasmically incompatible populations of the European cherry fruit fly *Rhagoletis cerasi* (Diptera, Tephritidae). Molecular Ecology 11: 2425–2434.

Schuler H, Arthofer W, Riegler M, Bertheau C, Krumböck S, Köppler K, Vogt H, Teixeira LAF, Stauffer C. 2011. Multiple *Wolbachia* infections in *Rhagoletis pomonella*. Entomologia Experimentalis et Applicata 139: 138–144.

Schuler H, Bertheau C, Egan SP, Feder JL, Riegler M, Schlick-Steiner BC, Steiner FM, Johannesen J, Kern P, Tuba K, Lakatos F, Köppler K, Arthofer W, Stauffer C. 2013. Evidence for a recent horizontal transmission and spatial spread of *Wolbachia* from endemic *Rhagoletis cerasi* (Diptera: Tephritidae) to invasive *Rhagoletis cingulata* in Europe. Molecular Ecology 22: 4101–4111.

Su Y, Zhang Y, Feng SQ, He J, Zhao ZH, Bai ZZ, Liu LJ, Zhang R, Li ZH. 2017. The mitochondrial genome of the wolfberry fruit fly, *Neoceratitis asiatica* (Becker) (Diptera: Tephritidae) and the phylogeny of *Neoceratitis* Hendel genus. Scientific Reports 7: 16612. doi: 10.1038/s41598-017-16929-7

Sun X, Cui LW, Li ZH. 2007. Diversity and phylogeny of *Wolbachia* infecting *Bactrocera dorsalis* (Diptera: Tephritidae). Molecular Ecology and Evolution 36: 1283–1289.

Weeks AR, Turelli M, Harcombe WR, Reynolds KT, Hoffmann AA. 2007. From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. PLoS Biology 5: e114. doi: [10.1371/journal.pbio.0050114]

Werren JH. 1997. Biology of Wolbachia. Annual Review of Entomology 42: 587–609.

Wu FZ, Gao ZN. 1964. A preliminary report on the fauna of agricultural insects of Ningxia autonomous region, China. Acta Entomologica Sinica 13: 572–580.

Wu FZ, Huang RX, Meng QX, Liang ZQ. 1963. Studies on the life history and the control of *Lycium* fruit fly *Neoceratitis asiatica* (Becker) (Diptera: Tephritidae). Journal of Plant Protection 2: 387–398. (in Chinese with English summary)

Wu FZ, Meng QX. 1963. Distribution, damage, life habit and prevention and control method of *Neoceratitis asiatica* (Becker). Ningxia Agricultural Science Communication 6: 35–37. (in Chinese with English summary)

Xu CQ, Liu S, Xu R, Chen J, Qiao HL, Jin HY, Lin C, Guo K, Cheng HZ. 2014. Investigation of production status in major wolfberry producing areas of China and some suggestions. China Journal of Chinese Materia Medica 39: 1979–1984. (in Chinese with English summary)

Xue FX, Lin HM. 2009. The prevention and control strategy of *Lycium barbarum*. Scientific and Technical Information of Gansu 38: 57–58. (in Chinese with English summary)

Zabalou S, Riegler M, Theodorakopoulou M, Stauffer C, Savakis C, Bourtzis K. 2004. *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. Proceedings of National Academy of Sciences of the USA 101: 15042–15045.

Zhao ZH, Zhang R, He DH, Wang F, Zhang TT, Zhang ZS. 2009. Risk assessment and control strategies of pest in *Lycium barbarum* fields under different managements. Chinese Journal of Applied Ecology 20: 843–850. (in Chinese with English summary)

Zheng QW. 2015. Preliminary progress has been made in the development of the control technology of Chinese wolfberry in Ningxia. Pesticide Market News 26: 66. doi: 10.13378/j.cnki.pmn.2015.26.064. (in Chinese with English summary)