

## Bacteria in spiders? Presence of *Wolbachia* (Rickettsiales, Ehrlichiaeae) in Synspermiata spiders, including the first record for the family Sicariidae (Araneae)

### ¿Bacterias en arañas? Presencia de *Wolbachia* (Rickettsiales, Ehrlichiaeae) en arañas Synspermiata, incluyendo el primer registro para la familia Sicariidae (Araneae)



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**ABSTRACT.** Infection by the endosymbiotic bacteria *Wolbachia* (Ehrlichiaeae) has been recorded in a wide variety of arthropods, including spiders. Within the order Araneae, there are previous reports of *Wolbachia* infection in Synspermiata spiders. Herein, we assess the presence of *Wolbachia* in the genera *Physocyclus* (Pholcidae) and *Loxosceles* (Sicariidae) through molecular studies using the mitochondrial marker Cytochrome c oxidase 1 (CO1). We detected the presence of this bacteria in one *Physocyclus* species and seven *Loxosceles* species from Mexico. Our

findings represent the fifth record for the family Pholcidae and the first records for the family Sicariidae, being all first records of *Wolbachia* in spiders from Mexico. Combining these results with previous works, *Wolbachia* infection in spiders has been recorded in 19 families, 70 genera, and 122 species. The Linyphiidae family has the highest number of species reported with *Wolbachia* infections, with 43 species across 27 genera. Within Synspermiata spiders, *Wolbachia* has now been documented within: Dysderidae, Pholcidae, Telemidae, and Sicariidae families, being the last having the most records with seven species.

**Key words:** Endosymbiotic; infection; violin spiders; daddy long-legs spiders; Mexico

**RESUMEN.** La infección por la bacteria endosimbiótica *Wolbachia* (Ehrlichaceae) se ha registrado en una amplia variedad de artrópodos, incluyendo arañas. Dentro del orden Araneae, existen reportes previos de infección por *Wolbachia* en arañas Synspermiata. En este trabajo, evaluamos la presencia de *Wolbachia* en los géneros *Physocylus* (Pholcidae) y *Loxosceles* (Sicariidae) mediante estudios moleculares utilizando el marcador mitocondrial Citocromo c oxidasa 1 (CO1). Detectamos la presencia de esta bacteria en una especie de *Physocylus* y en siete especies de *Loxosceles* de México. Nuestros hallazgos representan el quinto registro para la familia Pholcidae y los primeros registros para la familia Sicariidae siendo los primeros registros de la bacteria *Wolbachia* en arañas de México. Combinando estos resultados con trabajos previos, la infección por *Wolbachia* en arañas se ha registrado en 19 familias, 70 géneros y 122 especies. La familia Linyphiidae tiene el mayor número de especies reportadas con infecciones por *Wolbachia*, con 43 especies en 27 géneros. Dentro del clado Synspermiata, *Wolbachia* ha sido documentada en las familias: Dysderidae, Pholcidae, Telemidae, y Sicariidae, siendo esta última la que tiene la mayoría de los registros con siete especies.

**Palabras clave:** Endosimbiótico; infección; arañas violinistas; arañas patonas; México

## INTRODUCTION

*Wolbachia* Hertig & Wolbach, 1924, is a common genus of intracellular endosymbiotic bacteria belonging to the order Rickettsiales (family Ehrlichaceae) that is known to infect a wide variety of animal groups including arthropods, such as insects, crustaceans, and arachnids (Hertig, 1936; Werren, 1997). The presence of *Wolbachia* has been reported in approximately 40% of terrestrial arthropods, mainly insects, likely making it the most widespread endosymbiont found on earth (Fenn et al., 2006; Zug et al., 2012; Yang et al., 2021). As was mentioned by Yang et al. (2021) within arthropods, *Wolbachia* has previously been found in isopods (Zimmermann et al., 2015), mites (Chen, 2016), scorpions (Baldo et al., 2007), spiders (Dunaj et al., 2020; Goodacre et al., 2006), and insects (Hilgenboecker et al., 2008; Yang et al., 2013, 2021). It is estimated that around 66% of insect species are infected with this bacterium (Hilgenboecker et al., 2008), making it one of the most reported reproductive parasites in spiders (Goodacre, 2011).

Within arthropods, this intracellular bacteria can manipulate host reproduction in multiple ways in order to favor its own reproduction, such as: 1) inducing parthenogenesis, 2) increasing the proportion of females (killing male offspring), 3) feminization and cytoplasmic incompatibility, thus promoting its survival, and 4) spread (Durkin et al., 2021; Goodacre, 2011; Vanthournout et al., 2011; Yang et al., 2021). The main transmission strategy usually occurs through vertical transfer from the mother to the offspring through the cytoplasm of the ovule (Werren, 1997), the wide

distribution of this bacterium is difficult to explain solely by vertical transmission, there is also evidence of horizontal transfer through the food chain by sharing common food sources, as well as by parasites such as infected mites or wasps (Ahmed *et al.*, 2016; Le Clec'h *et al.*, 2013; Su *et al.*, 2019).

*Wolbachia* infections in spiders have been found in 99 species across 62 genera and 17 families (Yang *et al.*, 2021). This infection has been reported in both entelegynes spiders and Synspermiata spiders (= Haplogynae) (Goodacre, 2011). The most complete study of *Wolbachia* in spiders was carried out by Yang *et al.* (2021), who collected 1153 spiders from China and screened for *Wolbachia* in 975 individuals. However, to date there have been no records of *Wolbachia* in the spider family Sicariidae, including the genus *Loxosceles* that is medically important for humans due to their venomous bites (Vetter, 2015).

Although the bacteria *Wolbachia* has been recorded in wide spectrum of arthropods including arachnids worldwide, it has never been recorded previously for spiders from Mexico, which represent an opportunity to know its endosymbiotic interaction with spiders from Nearctic and Neotropical regions of the country.

In this study, we documented for the first time the presence of *Wolbachia* in the spider genus *Loxosceles* (Sicariidae) as well as in the genus *Physocyclus* (Pholcidae) based on molecular evidence from the mitochondrial Cytochrome c oxidase 1 (CO1) locus. These findings represent the first records of the bacteria *Wolbachia* in spiders from Mexico.

## MATERIALS AND METHODS

**Spider collection and DNA extraction.** This study was performed in parallel with a project on species delimitation in *Physocyclus* and *Loxosceles* spiders from Mexico using morphological and molecular data (Nolasco & Valdez-Mondragón, 2022; Valdez-Mondragón *et al.*, 2019; Navarro-Rodríguez & Valdez-Mondragón, 2024). Spiders were collected by hand between 2017 and 2019 from different localities and states across Mexico. Specimens were preserved in 80% ethanol for species identification and 96% ethanol for molecular studies, along with their collection data and identification to species level. Tissue selection for molecular analyses depended on the available material and the size of the specimens but was mainly isolated from whole legs (juveniles and adults), while the prosoma and opisthosoma were occasionally used in juveniles, following the protocols by Navarro-Rodríguez and Valdez-Mondragón (2020, 2024). Once the tissue was separated, it was stored in 96% ethanol under refrigeration at -20°C for subsequent extraction using a Qiagen DNeasy® extraction kit following the extraction protocols and modifications for pholcids spiders (*Physocyclus*) by Nolasco and Valdez-Mondragón (2022) and for violin spiders (*Loxosceles*) by Navarro-Rodríguez and Valdez-Mondragón (2020, 2024).

**Amplification and sequencing.** The mitochondrial marker Cytochrome c oxidase subunit I (CO1) was amplified using the following primer sets: LCO1490/HCO2198 and LCO1490-JJ/HCO2198-JJ (Folmer *et al.*, 1994; Astrin & Stüben, 2008) (Appendix 1). The PCR (Polymerase Chain Reaction) parameters for CO1 were performed following the protocols by Navarro-Rodríguez and Valdez-Mondragón (2024). The sequencing of both forward and reverse chains (5'–3' and 3'–5') of the PCR products was performed in a Sequencer Genetic Analyzer RUO Applied Biosystems Hitachi model 3750xL at the Laboratory of Molecular Biology and Health, IB-UNAM, Mexico City.

**DNA sequence editing and BLAST.** BioEdit v. 7.0.9.0 (Hall 1999) and Geneious v. 8.1.9 (Rozen & Skaletsky, 2000) were used for molecular sequence editing. Once the sequences were edited, they were verified using the BLAST tools ([www.ncbi.nih.gov/Blast.cgi](http://www.ncbi.nih.gov/Blast.cgi)) to find regions of similarity between the newly obtained sequences and those deposited in the GenBank database ([www.ncbi.nih.gov](http://www.ncbi.nih.gov)), as well as to identify possible contamination. The parameters used under BLAST were as follows: Choose Search Set: Database (Standard databases (nr etc.), nucleotide collection (nr/nt); Program selection: Optimize for Highly similar sequences (megablast); Algorithm parameters (General parameters, Scoring Parameters, Filters and Masking): standard.

## RESULTS

According to the best matches from the BLAST nucleotide search, *Wolbachia* bacteria was identified in a total of 29 specimens belonging to eight species of spiders within the families Pholcidae and Sicariidae. Specifically, *Wolbachia* was found in four sequences from two individuals of *Physocyclus hoogstrali* Gertsch & Davis, 1942 (Pholcidae) collected from two different populations in Nuevo León in northeast Mexico; as well as in 25 sequences from 17 individuals of seven different *Loxosceles* species from different localities and states across Mexico (Table 1). Of the samples sequenced, seven corresponded to adult male spiders, five to adult females, and seven to juvenile specimens. *Wolbachia* was mainly found in tissues from males, females, and juveniles, but also from complete juvenile specimens.

BLAST searches of CO1 and CO1-JJ found high Query Coverage matches with *Wolbachia* bacteria (>90%) for 19 sequences, and low Query Coverage matches (<90%) in 9 sequences (Table 2). Regarding the Highest percent identity, 15 sequences had >90% identity, whereas 15 sequences showed <90% identity (Table 2). Fourteen sequences corresponded to *Wolbachia pipientis* Hertig, 1936, including all sequences of *P. hoogstraali*, whereas 15 sequences from *Loxosceles* corresponded to *Wolbachia* spp.

Considering the reports of spiders infected by *Wolbachia* presented in Yang *et al.* (2021: supplementary information), the results presented herein increase this number to 19 families, 70 genera, and 122 species (Fig. 1). Linyphiidae contains the highest number of species reported to be infected by *Wolbachia* to date, with 27 genera and 43 species (Fig. 1).

## DISCUSSION

Knowledge of the presence of *Wolbachia* in arthropods has increased in recent decades, as *Wolbachia* infection has been recorded in 66% of insect species (Hilgenboecker *et al.*, 2008; Zug *et al.*, 2012), 45% of mite species (Breeuwer & Jacobs, 1996; Chen, 2016), and 35% of terrestrial isopod species (Bouchon *et al.*, 1998; Cordaux *et al.*, 2001; Zimmermann *et al.*, 2015). In the case of spiders, the work by Yang *et al.* (2021) from Mangshan, China, found *Wolbachia* in the 84.6% of 1153 individuals which represents 68 spider species in 45 genera and 16 families in this study.

According to Yang *et al.* (2021), exists a phylogenetic incongruence between the phylogenies of the host spiders and their corresponding *Wolbachia* species, indicating that horizontal transmission may occur between spider species. The four known mechanisms for horizontal transmission are blood contact (Rigaud & Juchault, 1995), host-parasitoid association (Ahmed *et al.*, 2015; Batista *et al.*, 2010), feeding relationship (Kittayapong *et al.*, 2003), and common usage of the same plant tissues (Li *et al.*, 2017; Yang *et al.*, 2013).

Based on our results from studying Synspermiata species, the presence of *Wolbachia* in *P. hoogstraali* and seven species of *Loxosceles* from Mexico is likely due to horizontal transfer and no differences were found between sexes or populations in the studied species, as *Wolbachia* was

**Table 1.** Spider species of *Physocyclus* (Pholcidae) and *Loxosceles* (Sicariidae), sequence vouchers, and localities in Mexico used for DNA extraction and PCR of the molecular marker CO1, indicating the tissue used for molecular studies. The samples include only those recorded with the bacteria *Wolbachia*. HCO= reverse, LCO= forward.

Species	DNA voucher LATLAX	State	Tissue
<i>Physocyclus hoogstraali</i>	Ara0520-HCO	Nuevo León:	Male: 3 legs
	Ara0520-LCO	Grutas de San Bartolo	
<i>Physocyclus hoogstraali</i>	Ara0522-HCO	Nuevo León:	Male: 3 legs
	Ara0522-LCO	"La Boca" cave	
<i>Loxosceles colima</i>	Ara-0114-HCO Ara- 0114-LCO	Jalisco: Camichines, Cocula	Juvenile complete specimen
<i>Loxosceles colima</i>	Ara-0655-JJ-HCO	Jalisco: 1km al E de Amacuatitlan, Mpio. Tonaya	Female: 1 leg
<i>Loxosceles colima</i>	Ara-0763-JJ-LCO	Guerrero: 2km al NO del Cayacal. Mpio. Petatlán	Female: 1 leg
<i>Loxosceles colima</i>	Ara-0650-HCO	Jalisco: 6km al E de Tonaya	Female: 1 leg
<i>Loxosceles colima</i>	Ara-0651-HCO	Jalisco:	Juvenile complete
	Ara-0651-LCO	6km al E de Tonaya	specimen
<i>Loxosceles colima</i>	Ara-0652-HCO	Jalisco:	Female: 1 leg
	Ara-0652-LCO	1 km E of Amacuatitlan	
<i>Loxosceles colima</i>	Ara-0281-JJ-HCO	Colima:	Juvenile: 2 legs 2019
	Ara-0281-JJ-LCO	Coquimatlan	
<i>Loxosceles francisca</i>	Ara-0321-JJ-LCO	Baja California: Ensenada	Male: 2 legs 2019
<i>Loxosceles francisca</i>	Ara-0325-JJ-LCO	Baja California: Ensenada	Juvenile: 4 legs 2019
<i>Loxosceles tenango</i>	Ara-0188-JJ-HCO	Hidalgo:	Juvenile: 1 leg 2018
	Ara-0188-JJ-LCO	Chapulhuacan	
<i>Loxosceles tenango</i>	Ara-0193-JJ-HCO	Hidalgo:	Juvenile: Prosome and 4
	Ara-0193-JJ-LCO	Pisaflores	legs 2018
<i>Loxosceles tolantongo</i>	Ara-0174-JJ-LCO	Hidalgo: Cardonal	Juvenile: 4 legs 2018
<i>Loxosceles valdosa</i>	Ara-0620-JJ-HCO	Tamaulipas:	Male: 2 legs 2019
	Ara-0620-JJ-LCO	Mante	
<i>Loxosceles valdosa</i>	Ara-0622-JJ-HCO	Tamaulipas:	Female: 4 legs 2019
	Ara-0622-JJ-LCO	Mante	
<i>Loxosceles</i> sp. 1	Ara-0215-JJ-LCO	Guerrero: Quechultenango	Male: 1 leg 2018
<i>Loxosceles</i> sp. 1	Ara-0218-JJ-LCO	Guerrero: Mochitlan	Male: 1 leg 2018
<i>Loxosceles</i> sp. 2	Ara0277-JJ-LCO	San Luis Potosí: Ciudad Valles	Male: 1 leg 2017

**Table 2.** Summary of the sequences from *Physocyclus* (Pholcidae) and *Loxosceles* (Sicariidae) found with the highest significant alignment identities and similarities with the bacteria *Wolbachia* using the BLAST search tools. HCO= reverse, LCO= forward.

Spider species	DNA voucher LATLAX	Sequence length (bp)	BLAST Description	<i>Wolbachia</i> species	Query Coverage (%)	Highest percent identity (%)	GenBank Accession number
<i>P. hoogstraali</i>	Ara-0520-HCO	680	<i>Wolbachia pipientis</i> isolate ant7 chromosome, complete genome	<i>Wolbachia pipientis</i>	94	98.76	CP095495.1
<i>P. hoogstraali</i>	Ara-0520-LCO	585	<i>Wolbachia pipientis</i> isolate ant7 chromosome, complete genome	<i>Wolbachia pipientis</i>	94	95.64	CP095495.1
<i>P. hoogstraali</i>	Ara-0522-HCO	611	<i>Wolbachia pipientis</i> isolate ant7 chromosome, complete genome	<i>Wolbachia pipientis</i>	82	89.96	CP095495.1
<i>P. hoogstraali</i>	Ara-0522-LCO	681	<i>Wolbachia pipientis</i> isolate ant7 chromosome, complete genome	<i>Wolbachia pipientis</i>	94	99.07	CP095495.1
<i>L. colima</i>	Ara-0114-HCO	682	<i>Wolbachia pipientis</i> isolate wStv chromosome	<i>Wolbachia pipientis</i>	94	98.76	CP050531.1
<i>L. colima</i>	Ara-0114-LCO	693	<i>Wolbachia pipientis</i> isolate wStv chromosome	<i>Wolbachia pipientis</i>	94	92.59	CP050531.1
<i>L. colima</i>	Ara-0655-JJ-HCO	637	<i>Wolbachia pipientis</i> isolate wStv chromosome	<i>Wolbachia pipientis</i>	95	97.36	CP050531.1
<i>L. colima</i>	Ara-0763-JJ-LCO	546	<i>Wolbachia pipientis</i> isolate ant7 chromosome, complete genome	<i>Wolbachia pipientis</i>	92	76.95	CP095495.1

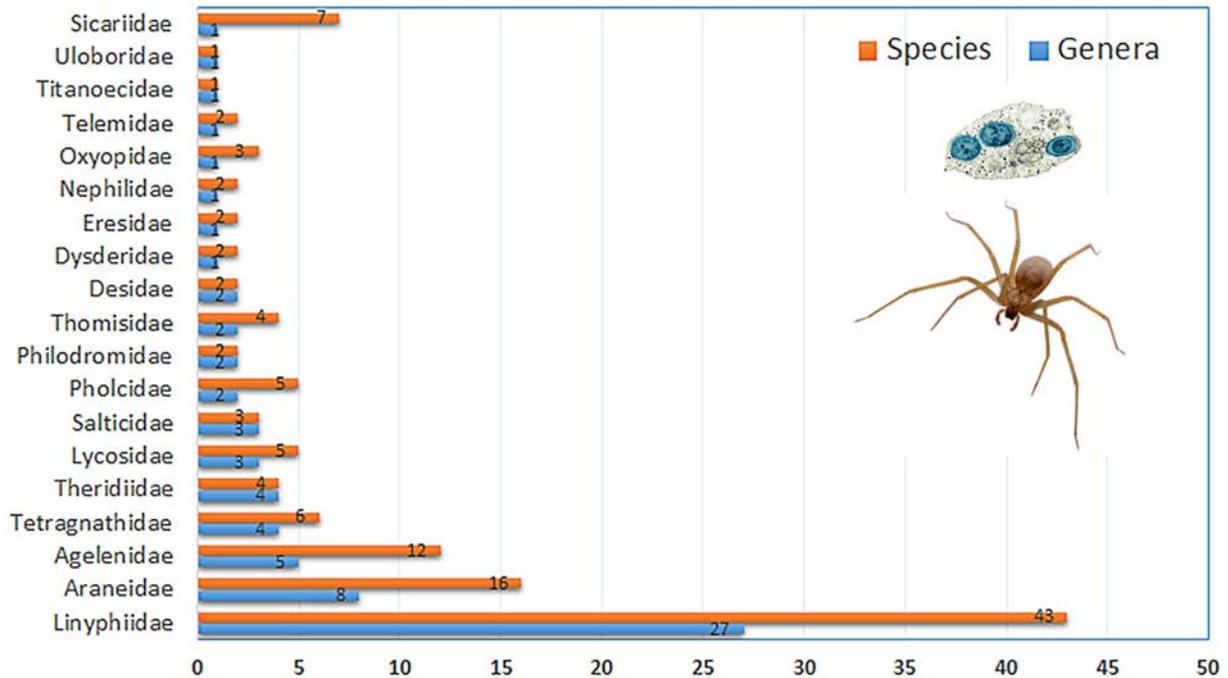
<i>L. colima</i>	Ara-0650-HCO	683	<i>Wolbachia pipientis</i> isolate wStv chromosome	<i>Wolbachia pipientis</i>	89	99.51	CP050531.1
<i>L. colima</i>	Ara-0651-LCO	688	<i>Wolbachia pipientis</i> isolate wStv chromosome	<i>Wolbachia pipientis</i>	90	98.39	CP050531.1
<i>L. colima</i>	Ara-0651-HCO	681	<i>Wolbachia pipientis</i> isolate wStv chromosome	<i>Wolbachia pipientis</i>	89	99.51	CP050531.1
<i>L. colima</i>	Ara-0652-HCO	702	<i>Wolbachia pipientis</i> isolate wStv chromosome	<i>Wolbachia pipientis</i>	93	87.23	CP050531.1
<i>L. colima</i>	Ara-0652-LCO	684	<i>Wolbachia</i> endosymbiont (group A) of <i>Cerceris ruficornis</i>	<i>Wolbachia</i> sp. endosymbiont	95	84.04	OZ035014.1
<i>L. colima</i>	Ara-0281-JJ-HCO	675	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i> isolate NDT 795.1 chromosome	<i>Wolbachia</i> sp. endosymbiont	82	91.09	CP158586.1
<i>L. colima</i>	Ara-0281-JJ-LCO	682	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i> isolate NDT 795.1 chromosome	<i>Wolbachia</i> sp. endosymbiont	93	96.54	CP158586.1
<i>L. francisca</i>	Ara-0321-JJ-LCO	592	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	95	90.19	CP158586.1
<i>L. francisca</i>	Ara-0325-JJ-LCO	750	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	81	81.85	CP158586.1
<i>L. tenango</i>	Ara0188-JJ-HCO	682	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	92	98.57	CP158586.1
<i>L. tenango</i>	Ara0188-JJ-LCO	674	<i>Wolbachia</i> endosymbiont	<i>Wolbachia</i> sp. endosymbiont	92	99.04	CP158586.1

			of <i>Polyergus mexicanus</i>					
<i>L. tenango</i>	Ara-0193-JJ-HCO	684	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	95	81.09	CP158586.1	
<i>L. tenango</i>	Ara-0193-JJ-LCO	713	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	92	85.50	CP158586.1	
<i>L. tolantongo</i>	Ara-0174-JJ-LCO	688	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	87	86.70	CP158586.1	
<i>L. valdosa</i>	Ara-0620-JJ-HCO	706	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	95	85.21	CP158586.1	
<i>L. valdosa</i>	Ara-0620-JJ-LCO	718	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	92	89.10	CP158586.1	
<i>L. valdosa</i>	Ara-0622-JJ-HCO	723	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	79	81.55	CP158586.1	
<i>L. valdosa</i>	Ara-0622-JJ-LCO	724	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	92	91.36	CP158586.1	
<i>Loxosceles</i> sp. 1	Ara-0215-JJ-LCO	735	<i>Wolbachia pipientis</i> isolate wStv chromosome	<i>Wolbachia pipientis</i>	66	87.76	CP050531.1	
<i>Loxosceles</i> sp. 1	Ara-0218-JJ-LCO	708	<i>Wolbachia pipientis</i> isolate wStv chromosome	<i>Wolbachia pipientis</i>	96	88.64	CP050531.1	
<i>Loxosceles</i> sp. 2	Ara0277-JJ-LCO	739	<i>Wolbachia</i> endosymbiont of <i>Polyergus mexicanus</i>	<i>Wolbachia</i> sp. endosymbiont	71	86.39	CP158586.1	

recording in adult males and females, as well as juvenile specimens. Although the exact mechanism of horizontal transmission between spiders remains unknown and requires further research, the blood contact, host-parasitoid association, and feeding relationship are plausible pathways that would allow for horizontal transfer of *Wolbachia* between spiders (Yang *et al.*, 2021).

According to the results of the BLAST search performed in this study, 14 sequences of CO1 matched with *W. pipientis*, the type species of the *Wolbachia* genus that was originally discovered

within the ovaries of the mosquito *Culex pipiens* (Hertig & Wolbach, 1924). The other 15 sequences from *Loxosceles* corresponded to *Wolbachia* sp., an endosymbiotic bacterium of *Polyergus mexicanus* Forel, 1899, a species of slave-making ant in the subfamily Formicinae.



**Figure 1.** Total number of families, genera, and species of spiders with reports of *Wolbachia* infections. Updated from Yang *et al.* (2021: supplementary information) and based on the present work.

A wide range of bacterial species have been identified to infect different species of spiders (Goodacre, 2011), even within the Synspermiata spiders (Cordaux *et al.*, 2001; Rowley *et al.*, (2004); despite this, this study represents the first record of *Wolbachia* infection in the genus *Loxosceles* and more broadly in the family Sicariidae. While most studies exhibit a predilection for recognizing the effects of this bacterium in females (Vanthournout *et al.*, 2011; Vanthournout & Hendrickx, 2015), we found *Wolbachia* to be present mostly in male tissues. The implications that this infection carries in both males and females remains to be fully understood, and the carriers of this bacterium may convey certain advantages, as mentioned in previous works (Correa & Ballard, 2016). Similarly, an in-depth study on the types of bacteria that could infect *Physocyclus* and *Loxosceles* species is needed, since at least four other endosymbionts have been found alongside *Wolbachia* in other spider species (Vanthournout *et al.*, 2011).

Finally, combining our results with the list presented in Yang *et al.* (2021: supplementary information) increases the number of spider families within the Synspermiata spiders for which *Wolbachia* infections are known to four families: Dysderidae, Pholcidae, Telemidae, and Sicariidae, now with Pholcidae. The last having the largest number of known infected species in this group, found in four species of the genus *Pholcus* C. L. Koch, 1850, as well as *Physocyclus hoogstraali* (Appendix 2). Although Yang *et al.* (2021) included *Atypus suiningensis* Zhang, 1985 (Atypidae) in their study on the presence of *Wolbachia* in different spiders from Mangshan, China, only araneomorphs (Araneomorphae) spiders species have been reported with *Wolbachia* infections so far.

In conclusion, although *Wolbachia* was found in 29 specimens belonging to eight spider species of Synspermiata, more additional samples, and even reproductive, ecological and molecular studies are needed to establish whether of such as populations the infections by horizontal transfer is due to feeding relationships or host-parasitoid associations, this last commonly found in spiders (Foelix, 2011; Takasuka & Broad, 2024), without discard the vertical transfer in spiders.

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## Appendix

**Appendix 1.** Primer sets used in this work for PCR amplification of the CO1 gene region.

Gene	Primer name	Primer sequence (5'–3')	Reference
CO1	LCO1490	5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'	Folmer <i>et al.</i> , (1994), Astrin & Stüben (2008)
	HCO2198	5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'	
	LCO1490-JJ	5'-CHA CWA AYC ATA AAG ATA TYG G-3'	
	HCO2198-JJ	5'-AWA CTT CVG GRT GCV CAA ARA ATC A-3'	

**Appendix 2.** Families, genera, and species of Synspermiata spiders with reports of *Wolbachia* infection. \*= indicates first record of infection by *Wolbachia*.

Family	Genus	Species	Reference
Dysderidae	<i>Dysdera</i>	<i>Dysdera erythrina</i>	Cordaux <i>et al.</i> , 2001
Pholcidae	<i>Pholcus</i>	<i>Pholcus phalangioides</i>	Duron <i>et al.</i> , 2008
		<i>Pholcus crypticolens</i>	Wang <i>et al.</i> , 2010; Yan <i>et al.</i> , 2015
		<i>Pholcus phalangioides</i>	Rowley <i>et al.</i> , 2004
		<i>Pholcus affinis</i>	Wang <i>et al.</i> , 2007
Telemidae	<i>Physocyclus</i> *	<i>Physocyclus hoogstraali</i> *	This work
	<i>Telema</i>	<i>Telema cordata</i>	Wang <i>et al.</i> , 2016
		<i>Telema cucurbitina</i>	Wang <i>et al.</i> , 2016
Sicariidae	<i>Loxosceles</i> *	<i>Loxosceles colima</i> *	This work
		<i>Loxosceles francisca</i> *	This work
		<i>Loxosceles tenango</i> *	This work
		<i>Loxosceles tolantongo</i> *	This work
		<i>Loxosceles valdosa</i> *	This work
		<i>Loxosceles</i> sp. 1*	This work
		<i>Loxosceles</i> sp. 2*	This work



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