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# EVIDENCE OF THE PRESENCE OF INTRACELLULAR BACTERIAL

# WOLBACHIA IN INSECTS LIVING IN MANGROVE OF GUADELOUPE

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#### Introduction

Wolbachia is an intracellular bacterial symbiont that colonizes nearly 60% of Arthropods. This bacterium has the ability to manipulate the sex ratio of the infected host in several ways: production of infected females, feminization of males, destruction of infected male embryos, and cytoplasmic incompatibility between infected males and uninfected females. Transmission of the bacterium can be either vertical or horizontal (Werren, 1997).

Among insects of the superfamily Gerroidea (Hemiptera: Gerromorpha) from Guadeloupe in Lesser Antilles, Wolbachia was detected in three species: Limnogonus franciscanus (Stål, 1859), Rhagovalia plumbea Uhler, 1894, and Rheumatobates trinitatis (China, 1943). Two new strains of Wolbachia were detected in these Gerroidea. Limnogonus franciscanus and Rheumatobates trinitatis are infected by the same Wolbachia strain (WLfran) (Conjard et al., 2021), and Rhagovelia plumbea is infected with a strain previously observed in Scirtothrips perseae (RIGMAN-JONES et al., 2007). Gerroidea are skittering insects that live on the surface of water bodies, both salt, and fresh (Heckman, 2011). These insects have an opportunistic carnivorous diet, feeding on preys that fall into the water surface: springtails, copepods, flies, leafhoppers, ants or spiders (Andersen & Polhemus, 1976). By looking for Wolbachia in Arthorpods evolving in the same habitats as Gerroidea, if they share the same strain, it will be possible to validate the transmission of the bacterium in a horizontal way and thus by predation. Thus, we hope to have first indications of the interactions between these Arthropods.

### Materials and Methods

1° Sampling and collection of the insectes living in the marine frindge of the mangrove is done from the boat using a dip net. For the swamp forest and ponds, was done by foot from the shore. Were stored at RT before analysis in the laboratory within two hours.

2°The insects collected were identified by using determination keys: (Genaro & Juarrero, 2010; Marechal, 2011; Yokoyama, 2013; Meurgey et al, 2017; Meurgey & Ramage, 2020).

3° The presence of Wolbachia was done after whole DNA extraction on the insects by using the Wolbachia surface protein (wsp) universal primer set (wsp81F and wsp691R). The PCR products were directly sequenced by Eurofins (www.eurofinsgenomics.eu).

#### Results

#### Baie à Chat"

Description of the environment: Marine fringe of the mangrove coastline. Bay little sheltered from the swell. Mangrove with Rhizophora mangle.

Gerroidea Present: Rheumatobates trinitatis (Photo 8) infected with WLfran strain and *Rhagovelia plumbea* (Photo 7) infected with a strain observed in Scirtothrips perseae.

Description of the environment: Lagoon in the coastal mangrove. Space

Gerroidea Present: Rheumatobates trinitatis infected with WLfran strain

Ants Azteca delpini antillana; Camponotus sexguttatus Fabricius,

Spiders Alpaida sp. Pickard-Cambridge, 1889; Leucauge sp.

Insects tested against the presence of Wolbachia: 9 species

1793; Gnamptogenys striatula Mayr, 1884

sheltered from the sea swell and composed of Rhizophora mangle

Insects tested against the presence of Wolbachia: 3 species

Species collected and tested:

Ants *Azteca delpini antillana* (Photo 1)

Spiders Leucauge sp. (Photo 2)

Termite Nasutitermes sp. Dudley, 1890



Photo 1 *Azteca delpini antillana* 

"Manche à Eau"

Species collected and tested:

CRICKETS Gryllinae

Moths Lepidoptera

Sowbugs Isopoda

Fly Muscidae



Photo 2 *Leucauge* sp

#### THE BACTERIAL SYMBIONT STRAINS INVOLVED

Strain 6: New strain Wolbachia endosymbiont of Miridae

(The color code corresponds to each *Wolbachia* strain identified) Strain 1: Wolbachia endosymbiont of Limnogonus franciscanus WLfran MW114524.1 Strain 2: Wolbachia endosymbiont of Scirtothrips perseae DQ075191.1 Strain 3: Wolbachia endosymbiont of Sericomyrmex sp. LC027865.1 Strain 4: New strain Wolbachia endosymbiont of Leucauge sp. Strain 5: New strain Wolbachia endosymbiont of Brachycera

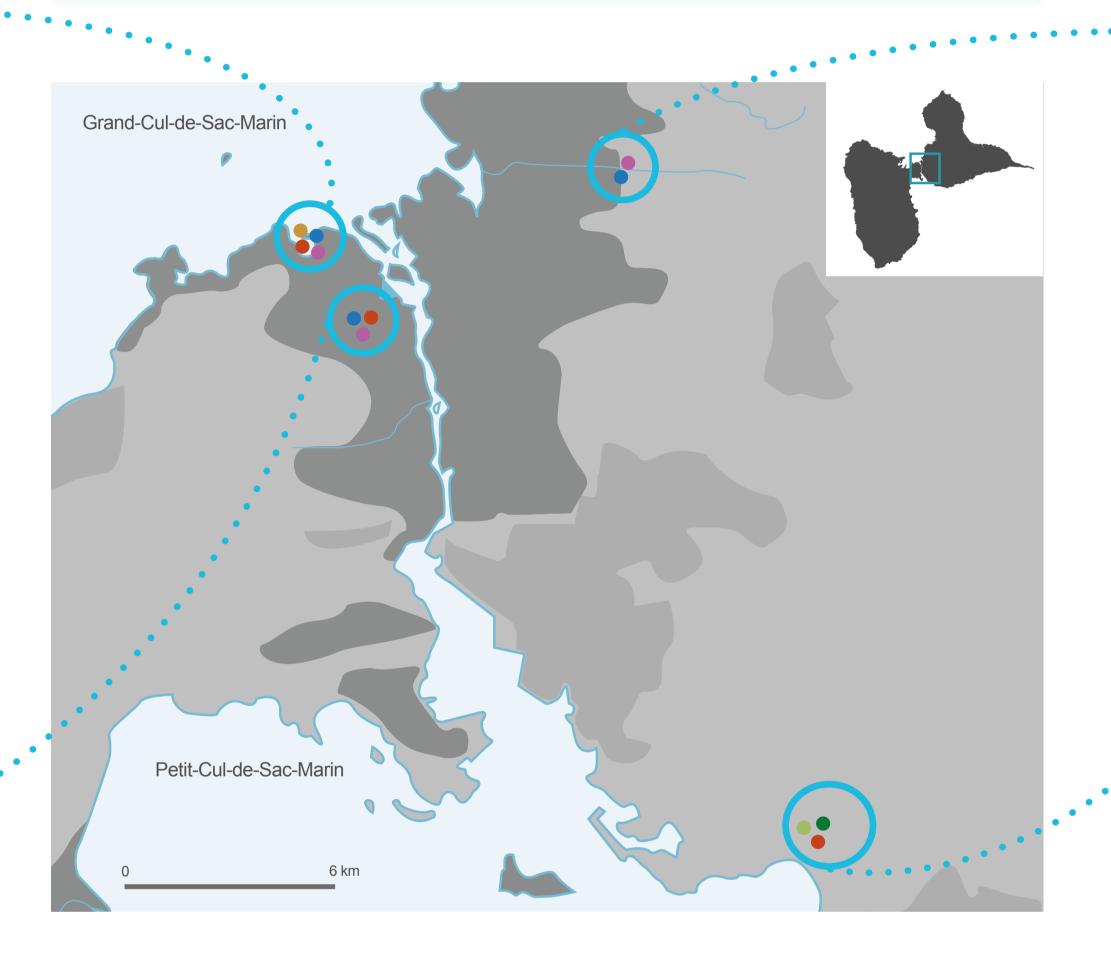


Photo 6 *Limnogonus franciscanus* 



Photo 7 *Rhagovelia plumbea* 



Photo 8 Rheumatobates trinitatis

### "Maison de la mangrove"

Description of the environment: Pterocarpus officinalis swampy backwater mangrove and wet grassland pond.

Gerroidea Present: Limnogonus franciscanus (Photo 6) infected with WLfran strain

Insects tested against the presence of Wolbachia: 12 species

Species collected and tested:

Ants Odontomachus bauri Emery, 1892; Nylanderia sp. Emery, 1906 **Bug Hemiptera** 

Dragonflies Ischnura ramburii (Selys, 1857); Micrathyria aequalis Hagen, 1861 Leafhopper *Hortensia similis* (Walker, 1851)

Spiders Alpaida sp. Pickard-Cambridge, 1889; Dolomedes sp. Latreille, 1804; Leucauge sp.; Lyssomanes michae Brignoli, 1984; Pisauridae sp. Simon, 1890 Termite Nasutitermes sp. Dudley, 1890



Photo 3 *Acromyrmex octospinosus* 



Photo 4 Miridae



Photo 5 Brachycera

## "ÉTANG FRÉCHOU"

Description of the environment: Pond with trees, Annona glabra, at the bottom of a gully surrounded by a grassy lawn.

Gerroidea present: Limnogonus franciscanus not infected, Microvelia pulchella Westwood, 1834, not infected

Insects tested against the presence of Wolbachia: 6 species

Species collected and tested:

Ants *Acromyrmex octospinosus* (Photo 3)

Bug Miridae (Photo 4)

CRICKET Orphulella sp. Giglio-Tos, 1894

FLY Brachycera (Photo 5)

Leafhopper Hortensia similis (Walker, 1851)

Termite Nasutitermes sp. Dudley, 1890

In this situation, L. franciscanus and R. trinitatis are infected with a single Wolbachia strain WLfran. However, in some cases, L. franciscanus may not be infected with Wolbachia, or may have a completely different, as yet unknown strain. Individuals of M. pulchella collected were tested negative for Wolbachia.

The same strain detected in the two mangrove Azteca delpini antillana Forel, 1899 and Acromyrmex octospinosus (Reich, 1793) was already described from *Sericomyrmex* sp. (LC027865.1). This strain must be specific to ants regardless of its habitat.

Whatever they are collected, *Leucauge* sp. White, 1841 individuals were positive for a new strain of *Wolbachia* that appears to be specific to this host species.

The two unidentified species that belong to the orders Miridae and Brachycera each have a new strain of Wolbachia observed in the "Étang à Fréchou".

# Conclusion

None of the Arthropods tested here had a *Wolbachia* strain in common with Gerroidea. Thus, even if these Arthropods are likely to be putative preys for Gerroidea, it does not seem that Wolbachia is transmitted through feeding in these insects. The Wolbachia endosymbiont must be transmitted vertically (from parent to child) and not horizontally (environmentally by food predation) (Werren & Windsor, 2000).

To improve the robustness of these data, it will be interesting to increase the number of samples taken at each site (especially at "Baie à Chat") or to try to collect the same species of preys at each site (Leucauges sp. or other ant species).

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