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Wolbachia superinfection in an Ecuadorian sample of the sand-flea *Tunga penetrans* (Preliminary note)

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Abstract

Wolbachia spp. are intracellular endosymbionts that cause reproductive alterations in their hosts. We here demonstrate the co-existence (superinfection) of both the arthropod- and the filarial-infecting strain in a sample of *Tunga penetrans* (L.) from Ecuador.

Key words: superinfection, *Tunga penetrans*, *Tunga trimamillata*, *Wolbachia*, *Wolbachia* 16S rDNA.

Obligate intracellular bacteria of the genus *Wolbachia* infect a wide variety of arthropods (insects, mites and isopods) and filarial nematodes. *Wolbachia* spp. are maternally inherited and can cause a number of reproductive alterations (phenotypes) in their hosts. These range from cytoplasmic incompatibility to sex-ratio distortion; the former determines the inviability of the offspring derived from crosses either between infected males and uninfected females, or between individuals infected by different *Wolbachia* strains; the latter produces a female-biased sex-ratio (Stouthamer *et al.*, 1999). Owing to the peculiar effects on its hosts, *Wolbachia* spp. have been recently involved in the control of arthropods of economic and public health interest (Beard *et al.*, 1998; Rasgon *et al.*, 2003).

From a phylogenetical point of view, six supergroups (A-F) have been so far detected: two of these (C and D) comprise *Wolbachia* strains from nematodes, the others embody strains pertaining to arthropods (Lo *et al.*, 2002).

In the tropical sand-flea *Tunga penetrans* (L., 1758) a filarial-related *Wolbachia* strain was identified (Fischer *et al.*, 2002; Heukelbach *et al.*, 2004). Through PCR amplification of the 16S rDNA gene and cell division protein gene *ftsZ*, we recently demonstrated the presence of an arthropod-infecting *Wolbachia* strain in *T. trimamillata* Pampiglione, Trentini, Fioravanti, Onore and Rivasi, 2002 and the complete absence of this bacterium in Ecuadorian populations of *T. penetrans* (Luchetti *et al.*, 2004).

The widening of our studies to other South American and African populations, led to the analyses of five *T. penetrans* specimens from Olmedo, Ecuador: these were neosomic females extracted from swine. DNA isolation and *Wolbachia* 16S rDNA amplification were performed as described in Luchetti et al (2004). PCR approach revealed the presence of a band of the expected size (1.0 kbp) in all analysed samples, thus indicating a 100% prevalence of *Wolbachia* infection.

The sequencing of the first 524 bp of one amplicon (TpOLMw1) was performed following standard procedures (Luchetti *et al.*, 2004) and then the obtained electropherogram was checked for the presence of nucleotide polymorphisms, as described in Feliciello *et al.* (2005). The final determined sequence has been submitted to Genbank as a "consensus sequence", with IUMB single letter code for multiple bases in the same position (A.N.: DQ015672).

BLAST search in public databases confirmed that we are dealing with a *Wolbachia* 16S rDNA sequence. This datum represents the first invention of *Wolbachia* in Ecuadorian *T. penetrans* and contrasts with a previous survey where no infection was reported (Luchetti *et al.*, 2004); however, it is in agreement with other literature data indicating a 100% prevalence of *Wolbachia* in *T. penetrans* (Fischer *et al.*, 2002; Heukelbach *et al.*, 2004). In our previous work, both general (O'Neill *et al.*, 1992) and specific (Fischer *et al.*, 2002) primers were used to evidence the presence of the endosymbiont, but no amplicons were ever obtained. Given the protocol followed, we think that previously analysed populations were uninfected. Obviously the possibility that they were infected with a very low prevalence in the population and/or with a low bacterial density in the organism cannot be at present ruled out.

The careful check of the electropherogram of presently analysed sequence revealed the presence of two bases in 15 sites out of 524 bp (table 1). The 60% of these variable sites showed contemporaneously the nucleotides diagnostic for the filarial-related strain from *T. penetrans* (A.N.: AY150558) and the arthropod-related strain from *T. trimamillata* (A.N.: AY350621 and AY350622) (table 1). This situation may be explained only considering the possibility that different strains of *Wolbachia* are present in this sample of *T. penetrans*. Furthermore, considering the other variable sites, it could be assumed the co-presence of more than two *Wolbachia* strains. These results support a superinfection of presently analysed sample and help to explain the divergence of detected *Wolbachia* strains in *T. penetrans* and *T. trimamillata* (Fischer *et al.*, 2002; Luchetti *et al.*, 2004). In fact, even if it is well known that *Wolbachia* phylogeny is usually incongruent with that of its hosts (Stouthamer *et al.*, 1999; Heath *et al.*, 1999; Vavre *et al.*, 1999), the presence of highly differentiated *Wolbachia* strains in *Tunga* species appears quite peculiar.

Table 1. Nucleotide variable positions, with single letter IUMB code (R = G or A; Y = C or T; W = A or T), of the *Wolbachia* 16S rDNA. Asterisks mark diagnostic sites for filarial- and arthropod-infecting strain.

37	46	61	70	116	122	123	126	128	164	199	254	298	409	426
R	R	Y	R	R	W	R	Y	R	R	Y	Y	Y	R	R
*	*			*	*	*	*	*					*	*

The condition of superinfection has been already demonstrated in several arthropods (Jeyaprakash and Hoy, 2000) and it represents an interesting aspect for evolutionary models dealing with *Wolbachia* dynamics. While endosymbiotic theory predicts a general trend toward clonality, *Wolbachia* constitutes an exception in which there is selection to maintain diversity with superinfection as a stage in which a novel *Wolbachia* variant will co-exist with the original infection type within a host (Dobson, 2004). This dynamics may explain the observation that *Wolbachia* phenotype can change frequently (Stouthamer *et al.*, 1999; Jiggins *et al.*, 2002), given that under this assumption additional pathways for the evolution of novel phenotypes are allowed.

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