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ORIGINAL PAPER

Supergroup F *Wolbachia* in terrestrial isopods: Horizontal transmission from termites?

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Abstract

Horizontal transmission between distantly related species has been used to explain how *Wolbachia* infect multiple species at astonishing rates despite the selection for resistance. Recently, a terrestrial isopod species was found to be infected by an unusual strain of supergroup F *Wolbachia*. However, only *Wolbachia* of supergroup B is typically found in isopods. One possibility is that these isopods acquired the infection because of their recurrent contact with termites—a group with strong evidence of infection by supergroup F *Wolbachia*. Thus, our goals were: (1) check if the infection was an isolated case in isopods, or if it revealed a broader pattern; (2) search for *Wolbachia* infection in the termites within Brazil; and (3) look for evidence consistent with horizontal transmission between isopods and termites. We collected *Neotroponiscus* terrestrial isopods and termites along the Brazilian coastal Atlantic forest. We sequenced and identified the *Wolbachia* strains found in these groups using *coxA*, *dnaA*, and *fpbA* genes. We constructed phylogenies for both bacteria and host taxa and tested for coevolution. We found the supergroup F *Wolbachia* in other species and populations of *Neotroponiscus*, and also in *Nasutitermes* and *Procornitermes* termites. The phylogenies showed that, despite the phylogenetic distance between isopods and termites, the *Wolbachia* strains clustered together. Furthermore, cophylogenetic analyses showed significant jumps of *Wolbachia* between terrestrial isopods and termites. Thus, our study suggests that the horizontal transmission of supergroup F *Wolbachia* between termites and terrestrial isopods is likely. Our study also helps understanding the success and worldwide distribution of this symbiont.

Keywords Horizontal transmission · Isoptera · Isopoda · Neotropical fauna · Symbiosis

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Introduction

Wolbachia is a bacterial genus that includes obligate-intracellular bacteria that occur in a high proportion of arthropod species and many filarial nematodes (Werren et al. 2008). This evolutionary success that elevated *Wolbachia* to pandemic status was made possible by the efficient vertical transmission and the reproductive manipulation of hosts (Gerth et al. 2014). Despite the seeming success, several host lineages show a 'loss' of *Wolbachia* infection probably due to a selection for resistance (Zug and Hammerstein 2012). In fact, according to estimates, 0.14 loss events and 0.11 acquisition events are expected per million years (Bailly-Bechet et al. 2017). Nevertheless, *Wolbachia* maintain their infection prevalence at very high rates, particularly in arthropods, and this does not seem constrained by host phylogenetics (Zug and Hammerstein 2012). One possibility for the high prevalence rates might thus lie in the diversity of *Wolbachia* bacteria. To assess *Wolbachia* diversity, researchers used phylogenetic approaches that lead to their classification in 'supergroups' (Werren et al. 1995; Bandi et al. 1998; Casiraghi et al. 2005; Baldo et al. 2006b). To some extent, each *Wolbachia* 'supergroup' seems to associate with particular host groups; and the host groups differ among the supergroups. For example, supergroup A and B strains are the most abundant ones, being found in ~ 40% of all terrestrial arthropods' species (Zug and Hammerstein 2012). *Wolbachia* of supergroups C and D are found exclusively in co-evolutionary associations consistent with their mutualistic dependency with some filarial nematodes (Taylor et al. 2013). Supergroup F, on the other hand, can infect both arthropods and filarial hosts (Casiraghi et al. 2005). Thus, supergroup F is relatively atypical because of its ability to infect groups that are not phylogenetically close; hence, understanding what makes supergroup F atypical can allow us to understand the evolution of bacterial infection.

The mechanisms proposed to explain the phylogenetic widespread pattern of *Wolbachia* rely on horizontal transmission between distant species with close interactions. These close interactions approximate distant related species physically, creating an opportunity for horizontal transmission, as can be seen in interactions such as parasitoids and phoretic vectors (Covacin and Barker 2006; Gehrer and Vorburger 2012; Ahmed et al. 2015; Qi et al. 2019), use of common food sources (Sintupachee et al. 2006; Oliver et al. 2010; Stahlhut et al. 2010; Li et al. 2017), and predation and cannibalism (Yun et al. 2011; Le Clec'h et al. 2013; Brown and Lloyd 2015; Faria et al. 2016). As *Wolbachia* appears to transmit easily through hemolymph or the gut, the simplest route of transmission seems to be contacting the uninfected host through excretions or injuries (Rigaud and Juchault 1995). After entering the new host, *Wolbachia* can use cell-to-cell transfer to establish both somatic and germline infection (Frydman et al. 2006). Alternatively, *Wolbachia* could infect new hosts without cell-to-cell transfer. By using the host phagocytic and endocytic machinery, they can be transferred successfully to new hosts (White et al. 2017). Thus, one might expect that host groups infected with supergroup F can be infected by and infect any species in which they are in close contact with.

Terrestrial isopods are an interesting case of *Wolbachia* infection because, until recently, infections were almost exclusively from supergroup B (Rousset et al. 1992; Juchault et al. 1994; Bouchon et al. 1998, 2008; Nyirõ; et al. 2002; Ben Afia Hatira et al. 2008; Wiwatanaratanabutr et al. 2009; Almerão et al. 2012; Cordaux et al. 2012; Zimmermann et al. 2015). However, the Neotropical isopod *Neotroponiscus littoralis* from Northeast Brazil was infected with *Wolbachia* of supergroup F, a lineage that was never detected in crustaceans before (Zimmermann et al. 2015). *Neotroponiscus* is composed of ten small-sized and poorly studied species, endemic of South American Atlantic coastal forests (Cardoso et al. 2017). Contrary to most species of terrestrial isopods that live in leaf litter (Richardson and Araujo 2015), *Neotroponiscus* are generally found on banana and bromeliad leaves, decaying wood, and—most importantly—in termite nests (Lemos de Castro 1970a, b; Lenko 1971; Lisboa et al. 2013).

Termite nests are ideal places for horizontal transmission of bacteria because they provide shelter and food for many species (Costa et al. 2009). Unfortunately, we know very little about *Wolbachia* infection on Neotropical termite fauna. We know that *Cavitermes tuberosus*, a termite species from Northern Brazil, is infected by *Wolbachia* belonging to supergroup F (Hellemans et al. 2019) and another species, *Serritermes serrifer,* from Central Brazil, is infected by an unidentified *Wolbachia* supergroup (Lo and Evans 2007). *Wolbachia* of supergroup F are already found in symbiosis with ~ 20 species of termites belonging to three different families and distributed worldwide (Lo and Evans 2007; Roy and Harry 2007; Salunke et al. 2010; Hellemans et al. 2019; Yashiro and Lo 2019). Besides, supergroup F *Wolbachia* are generally rare, with phylogenetic analyses suggesting a recent emergence of this supergroup through frequent horizontal transmission (Panaram and Marshall 2007; Lefoulon et al. 2012, 2016). Given the proximity between *Neotroponiscus* isopods and termites that can be infected by supergroup F, we hypothesize that the supergroup F found on *Neotroponiscus littoralis* was acquired by horizontal transmission from the termite nests in which they inhabit.

Considering this scenario, our goal is: (i) to investigate the presence of *Wolbachia* in other species of *Neotroponiscus* and test if the initial finding was an isolated event; (ii) to test for *Wolbachia* infection in the termites found in the Brazilian coastal forests; (iii) to test the phylogenetic and cophylogenetic relationships between the *Wolbachia* strains found in both taxa to ascertain if horizontal transmission is possible.

Materials and methods

Sample collection, DNA extraction and Wolbachia identification

We sampled terrestrial isopod populations and termite nests in eight states along the Brazilian Atlantic Forest between the years 2013–2014 (Fig. 1) and stored in 100% ethanol at − 20 °C. We identified *Neotroponiscus* adults based on morphological criteria (Lemos de Castro 1970a, b). We also sampled the termites (soldiers and workers) and sent to an expert for identification. The number of sampled individuals of each species and the sampling sites are listed in Table 1.

From these samples, total DNA was extracted with PureLink Genomic DNA Kit (Invitrogen/K1820-01). For detection of *Wolbachia*, three protein-coding genes were used: *dnaA*, *coxA* and *fbpA* (as previously described by Baldo et al. 2006a, b). We decided to use these genetic markers because they showed the best amplification results based on the study of Zimmerman et al. (2015), thus providing clear and unambiguous sequences. DNA from isopods and termites was also amplified with universal primers of the mitochondrial gene Cytochrome Oxidase I (COI) (Folmer et al. 1994). Polymerase chain reaction (PCR) products were electrophoresed on a 1.0% agarose gel and sequences were obtained using BigDye technology by Macrogen Inc., South Korea.

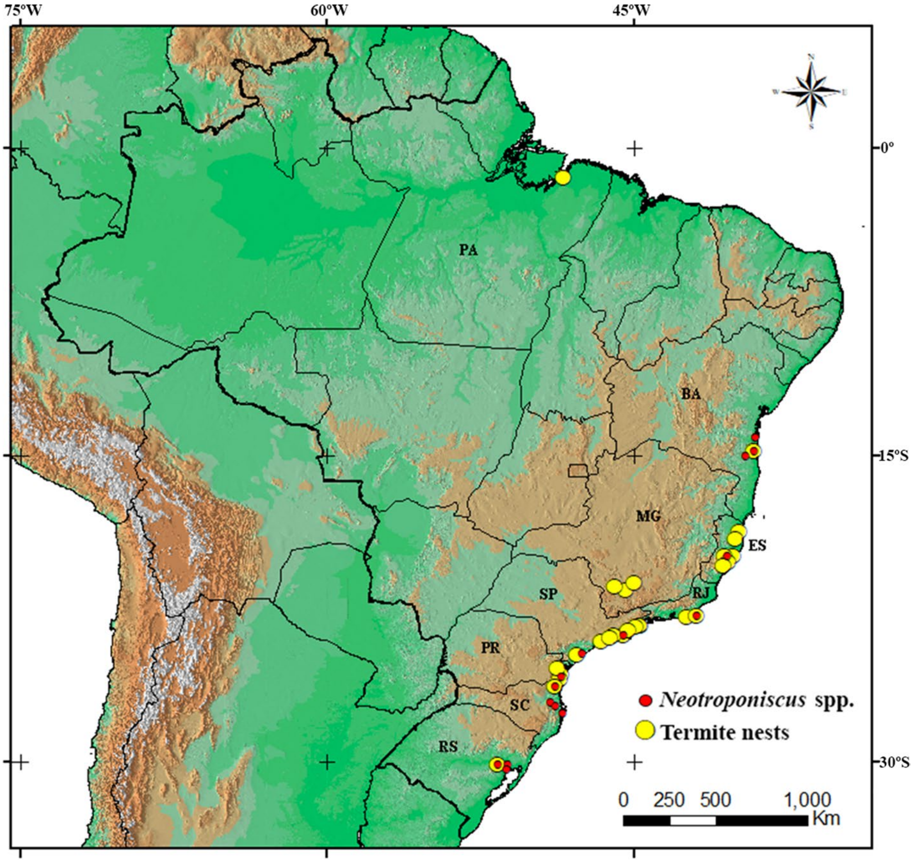

Fig. 1 Collection sites of *Neotroponiscus* isopods and termite nests examined for *Wolbachia* infection in Brazil

Phylogenetic analysis

A BLAST search at the NCBI (http://www.ncbi.nlm.nih.gov) was used to identify the *Wolbachia* supergroup of the infected individuals in our study. Once we confirmed that all belonged to supergroup F, we included in our alignments other sequences from supergroup F available in GenBank (www.ncbi.nlm.nih.gov/genbank/) and *Wolbachia* MLST database (pubmlst.org/wolbachia/). *Wolbachia* sequences from supergroups A, B, C, D and E were used as out-group (Table S1). The *dnaA*, *coxA* and *fbpA* sequences were aligned using Muscle (Edgar 2004), implemented in MEGA version 7.0 (Kumar et al. 2016). Recombination analyses were conducted on single and concatenated alignments using the RDP3 package (Martin et al. 2010) with default settings. Sequences were considered linear and the highest acceptable *P* value cutoff was 0.05. The best-fit models of nucleotide substitution for each gene were selected with JModeltest 2.1.10 (Darriba et al. 2012). This approach suggested the following models: HKY + G for *coxA*, HKY + I + G for *fbpA* and HKY + I + G for *dnaA*. Phylogenetic reconstructions of *Wolbachia* were performed with Bayesian inference in BEAST version 1.8 (Drummond et al. 2012) using concatenated

Table 1 Terrestrial isopods and termites tested for *Wolbachia* infection and their collection sites

Species	State	Municipality	Soldiers tested (infected)	Workers tested (infected)	Latitude	Longitude
Termites						
Coptotermes sp.	Espirito Santo	Santa Tereza	4	0	– 19,9416	– 40,5824
Diversitermes castaniceps	São Paulo	Maresias	1	6	– 23,8247	– 45,5286
Diversitermes castaniceps	Espirito Santo	Sooterama	5	2	– 19,1164	– 40,0733
Diversitermes cf. *castaniceps*	São Paulo	Ubatuba	14	0	– 23,3542	– 44,7652
Microcerotermes sp.	Rio de Janeiro	Trindade	0	7	– 23,3299	– 44,7057
Nasutitermes cf. ephratae	Espirito Santo	Aracruz	11 (5)	3	– 19,9434	– 40,1628
Nasutitermes cf. itapocuensis	Rio grande do Sul	Eldorado do Sul	8 (3)	6 (2)	– 30,1004	– 51,6946
Nasutitermes cf. jaraguae	São Paulo	Guarujá	3	11 (1)	– 23,9066	– 46,1639
Nasutitermes corniger	Espirito Santo	São Mateus	7	0	– 18,7483	– 39,8648
Nasutitermes corniger	Bahia	Ilhéus	10	6	– 14,7977	– 39,1727
Nasutitermes gaigei	Pará	Belém	3	11	– 1,4537	– 48,4768
Nasutitermes jaraguae	Paraná	Matinhos	0	7	– 25,7060	– 48,5720
Nasutitermes jaraguae	São Paulo	Itamambuca	8	6	– 23,3822	– 44,9660
Nasutitermes jaraguae	São Paulo	Caraguatatuba	0	14	– 23,5702	– 45,2971
Nasutitermes jaraguae	Minas Gerais	Varginha	7	7	– 21,6226	– 45,4305
Nasutitermes jaraguae	Santa Catarina	Joinvile	10	4	– 26,3219	– 48,8636
Nasutitermes jaraguae	São Paulo	Bertioga	14 (1)	0	– 23,7849	– 46,0196
Nasutitermes macrocephalus	Rio de Janeiro	Saquarema	14	0	– 22,9246	– 42,4402
Nasutitermes morphotype A	São Paulo	Iguapé	9	5	– 24,7636	– 47,7859
Nasutitermes morphotype A	Santa Catarina	Itapoá	8	6	– 25,9690	– 48,6392
Nasutitermes morphotype A	Paraná	Morretes	10	4	– 25,4135	– 48,7714
*Nasutiterme*s morphotype A	São Paulo	Mongaguá	3	4	– 24,0844	– 46,6103
Nasutitermes sp. 1	Espirito Santo	Cariacica	9	5	– 20,2415	– 40,4173
Nasutitermes sp. 2	Rio de Janeiro	Cabo Frio	3 (3)	4 (3)	– 22,8671	– 41,9850
Neocapritermes opacus	Espirito Santo	Domingos Martins	4	10	– 20,3803	– 40,6302

Table 1 (continued)

Species	State	Municipality	Soldiers tested (infected)	Workers tested (infected)	Latitude	Longitude
Procornitermes araujoi	Minas Gerais	Alfenas	4 (3)	6 (5)	− 21,4250	− 45,9460
Procornitermes lespesii	Minas Gerais	Lavras	7	7 (3)	− 21,2306	− 44,9801
Species	State	Municipality	Male tested (infected)	Female tested (infected)	Latitude	Longitude
Terrestrial isopods						
Neotroponsicus argentinus	Bahia	Maraiúvis	0	1	− 14,1672	− 39,0936
Neotroponsicus argentinus	Paraná	Matinhos	0	1	− 25,8356	− 48,5707
Neotroponsicus argentinus	Santa Catarina	Joinville	0	1	− 26,3219	− 48,8636
Neotroponsicus argentinus	Santa Catarina	Florianópolis	1	1	− 27,6107	− 48,5057
Neotroponsicus argentinus	São Paulo	Iguape	0	1	− 24,7051	− 47,5465
Neotroponsicus carolii	Bahia	Itajú	1 (1)	0	− 15,0328	− 39,6042
Neotroponsicus daguerri	Rio Grande do Sul	Eldorado do Sul	2	9	− 30,1004	− 51,6946
Neotroponsicus daguerri	Rio Grande do Sul	Porto Alegre	1	1	− 30,0967	− 51,1860
Neotroponsicus daguerri	Rio Grande do Sul	Barra do Ribeiro	0	1	− 30,3472	− 51,2431
Neotroponsicus lenkoi	São Paulo	Maresias	0	1	− 23,8247	− 45,5286
Neotroponsicus littoralis	Rio de Janeiro	Cabo Frio	2 (2)	3 (3)	− 22,8671	− 41,9850
Neotroponsicus littoralis	Bahia	Itabuna	2	0	− 14,7989	− 39,1722
Neotroponsicus perlatus	Espírito Santo	Santa Tereza	1	1	− 19,9347	− 40,4705
Neotroponsicus plaumani	Santa Catarina	Blumenau	1	1	− 27,0575	− 49,0861
Neotroponsicus plaumani	Santa Catarina	São João Batista	0	1	− 27,2644	− 48,8533

Infected species are in bold

(*coxA* + *fbpA* and *dnaA* + *coxA* + *fbpA*) genes sequences. We used *coxA* + *fbpA* and *dnaA* + *coxA* + *fbpA* reconstructions to assess the robustness of the reconstructions because there are few *dnaA* sequences available on databases. Four simultaneous Markov chains were run for 50 million generations with a sampling frequency of 1,000 generations and an initial burn-in of 10%. Concatenated alignments were run with the alignments partitioned so that the specific model of evolution corresponded to each gene fragment. The runs were checked for convergence using Tracer version 1.6 (Rambaut et al., 2014). The results were visualized and checked with FigTree 1.4.2 (Rambaut 2014). All *dnaA*, *coxA*, *fbpA* and COI gene sequences generated in this study were deposited into GenBank under accession numbers KX024833 to KX024843 for *coxA*, KX036766 to KX036775 for *fbpA*, KX036776 to KX036786 for *COI* and KX036787 to KX036796 for *dnaA*.

Comparative phylogenetic analysis

For the cophylogenetic analysis, we generated a *Wolbachia* tree of representative strains from A, B, C, D, E and F supergroups using *coxA*, *dnaA* and *fbpA* sequences available in GenBank (Table S1) and the sequences from the present study. The closest relative taxa to *Wolbachia*, *Anaplasma marginale* and *Ehrlichia canis*, were used as outgroups. Similarly, host phylogeny was reconstructed using the corresponding COI mitochondrial sequences of host taxa (Table S2). To visualize host-bacteria associations, tanglegrams were generated in TreeMap 3.0, using the default settings (Charleston and Robertson 2002). Since we were interested in both the evolutionary history of the infection (i.e., who infected whom), and whether the phylogenies of hosts and *Wolbachia* were congruent, we used both event-based methods (for the reconstruction of phylogenies) and global fit (for congruency tests). Statistical congruence between host and *Wolbachia* phylogenies was performed using both the Procrustean Approach to Cophylogeny (PACo) program (Balbuena et al. 2013) and the ParaFit function (Legendre et al. 2002) from the ape R package (Paradis and Schliep 2019). The significance of the global goodness of fit of PACo and the Parafit global test was estimated using 10,000 permutations of the association matrix. The level of statistical significance of cospeciation was determined using reconciliation analyses implemented in the program JANE 4.0 (Conow et al. 2010). As recommended by Conow et al. (2010), the genetic algorithm parameters were set at the default values for selection strength and mutation rate, and number of generations. We thus performed analyses with 100 generations, population sizes of 100, and a default cost setting matrix of 0 for cospeciation, 1 for duplication of parasites, 2 for duplication and host switch, 1 for loss of parasite, and 1 for failure to diverge. In a conservative manner, we minimized the total cost results by maximizing the cospeciation events while allowing host switching.

Results

Infection detections

Thirty-six terrestrial isopods belonging to six species of *Neotroponiscus* were analyzed (Fig. 2). Besides the already known infected population of *N. littoralis* from Northeast Brazil, the presence of *Wolbachia* was also observed in individuals of this same species sampled in Southeast Brazil. We also detected *Wolbachia* infection in another species, *N.*

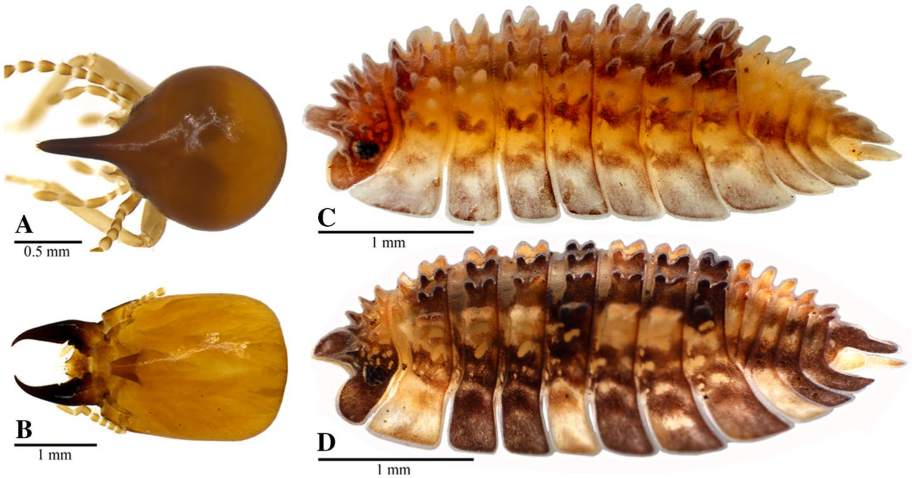

Fig. 2 Representatives of terrestrial isopods and termite species infected by *Wolbachia* strains of supergroup F. **a** *Nasutitermes itapocuensis*; **b** *Procornitermes araujoi*; **c** *Neotroponiscus littoralis*; **d** *Neotroponicus carolii*

carolii also sampled in Northeast Brazil (Table 1) representing the first *Wolbachia* infection record for this species.

Regarding the termites, 317 individuals belonging to 17 species were analyzed, which represents the largest survey of *Wolbachia* infection in Neotropical termites. Seven species from two genera, *Nasutitermes* and *Procornitermes* (Fig. 2), sampled in five Brazilian states were *Wolbachia* positive. The infection status of each species and the number of individuals screened are listed in Table 1. This is the first time that the infection is recorded in the termite species studied here.

Wolbachia identification

All strains found both in isopods and termites belong to the supergroup F. Two strains were found in the terrestrial isopods, one on each *Neotroponiscus* species. Both strains clustered together in the phylogenies (Figs. 3 and 4). In termites, two strains of the supergroup F were found in *Procornitermes* (one in *P. araujoi* and one in *P. lespesii*). On the other hand, six strains were found in *Nasutitermes*: two in *N.* cf. *ephratae* and one on each of the following taxa: *N.* cf. *itapocuensis*, *N. jaraguae*, *N.* cf. *jaraguae* and *Nasutitermes* sp. As in the case of terrestrial isopods, *Procornitermes* and *Nasutitermes* sequences were very similar despite the large geographical distance among the sample locations (Figs. 3 and 4).

Phylogenetic correlation between isopods and termites

The *Wolbachia* strains found in terrestrial isopods are closely related to those found in termites, regardless of how the phylogeny was generated. *Wolbachia* sequences found in termites and terrestrial isopods were always grouped in the same clade regardless of the number of host species and sequences used to build the tree (i.e., *fbpA* + *coxA* or

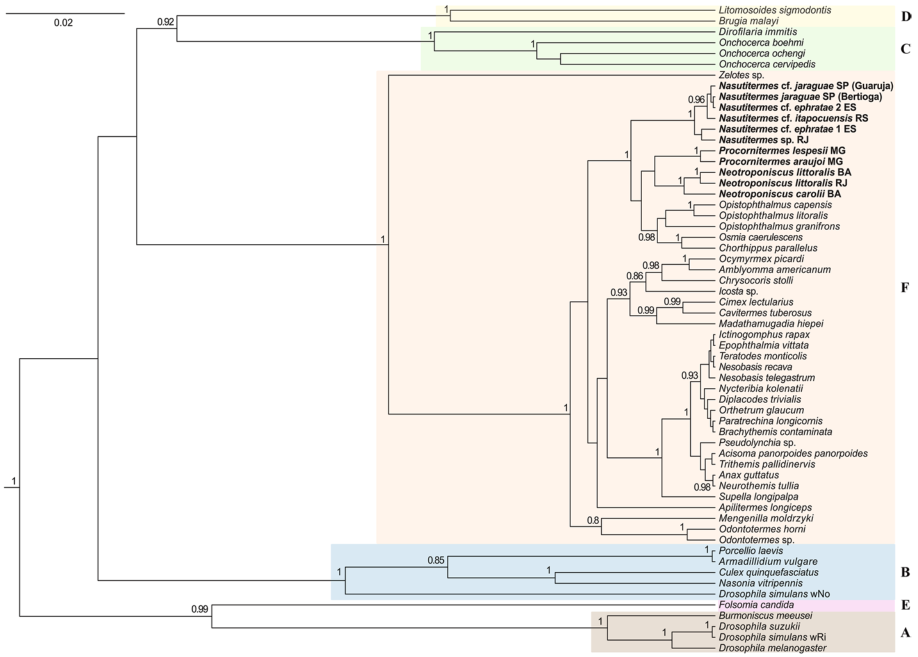

Fig. 3 Unrooted phylogeny of *Wolbachia* based on concatenated datasets of *coxA* and *fbpA* sequences. Numbers above branches represent Bayesian posterior probabilities based on one run of 50 million generations. Posterior probabilities < 0.75 are not shown. Sequences were obtained from GenBank and MLST *Wolbachia* databases except those from species in bold. Letters represent *Wolbachia* supergroups A, B, C, D, E and F

dnaA + *fbpA* + *coxA* sequences; Figs. 3 and 4), which denotes the robustness of the reconstructed phylogenies.

Moreover, the phylogenetic comparison of *Wolbachia* and their hosts showed no congruence in a substantial part of the tanglegram (Fig. 5). Overall, both ParaFit and PACo analyses provided no evidence for significant coevolution between *Wolbachia* and hosts (ParaFitGlobal = 76.06896, $P = 0.3425657$; m2 global value = 187.3399, $P = 0.1905$; 10,000 permutations). Apart from the outgroups, only six of the 33 individual host-bacteria links are significant (based on ParaFit1 or Parafit2, $P \leq 0.05$). These six links are between *Wolbachia* supergroups C and D and their long-lasting associations with filarial nematodes. As can be seen in Fig. S1, these links contributed with most of the cophylogenetic structure (i.e., high squared residuals; Fig. S1). On the other hand, the less contributing host-*Wolbachia* associations to the cophylogenetic structure are those belonging to supergroups A, B and F. Indeed, terrestrial isopods and termites with their respective *Wolbachia* strains were among the associations that contributed least to the phylogenetic congruence between hosts and bacteria.

The events-based method suggested significant horizontal transfers between termites and isopods when reconstructing the evolutionary history of the phylogeny. In the Treemap analysis, 22 optimal reconstructions were found. The optimal solutions postulated 22 cospeciations, 24 losses, 18 host switches, and 25 duplication events. However, the lower cost solutions were found in the JANE 4.0 analysis with 115 isomorphic solutions for a

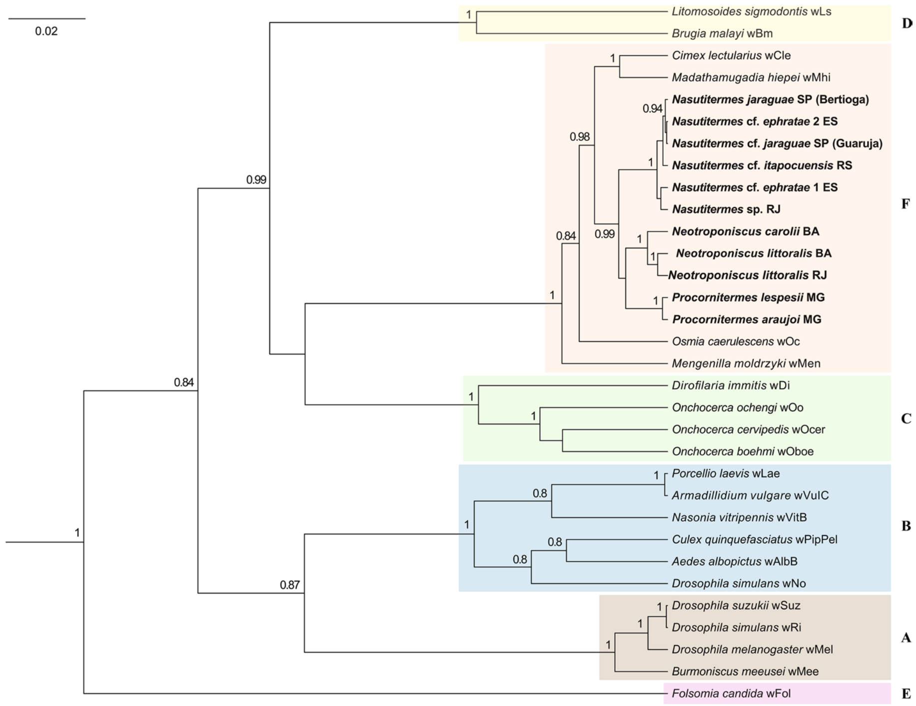

Fig. 4 Unrooted phylogeny of *Wolbachia* based on concatenated datasets of *coxA*, *dnaA* and *fbpA* sequences. Numbers above branches represent Bayesian posterior probabilities based on one run of 50 million generations. Posterior probabilities < 0.75 are not shown. Sequences were obtained from GenBank and MLST *Wolbachia* databases except those from species in bold. Letters represent *Wolbachia* supergroups A, B, C, D, E and F

total cost value of 42. The optimal solutions resulted in 15 cospeciations, 8 losses, 17 host switches, and no duplication events (Fig. 6). Accordingly, most of the host switches (*Wolbachia* horizontal transmissions) occurred in the F supergroup. At the highest phylogenetic level, we found host switches between isopods and termites: between *N. carolii* and the termite *Nasutitermes* sp. RJ, and between *N. littoralis* and *P. araujoi*. At a lower phylogenetic level, we found also several transfers within termites and within isopods as well (Fig. 6).

Discussion

Horizontal transmission events are supposed to be recurrent in *Wolbachia*. However, the probability of successful likely is highest between closely related hosts and decreases with increasing phylogenetic distance (Zug and Hammerstein 2012). Thus, horizontal transmission of *Wolbachia* between distantly related arthropods is relatively rare. This study presents evidence for the horizontal transmission between unrelated hosts involving strains of supergroup F, an atypical supergroup of *Wolbachia* that infects a large variety of arthropods hosts and nematodes (Casiraghi et al. 2005). In this study, we corroborate our hypothesis that the unexpected discovery of supergroup F *Wolbachia* in Neotropical terrestrial isopods could be the result of horizontal transmission from termites. Interestingly,

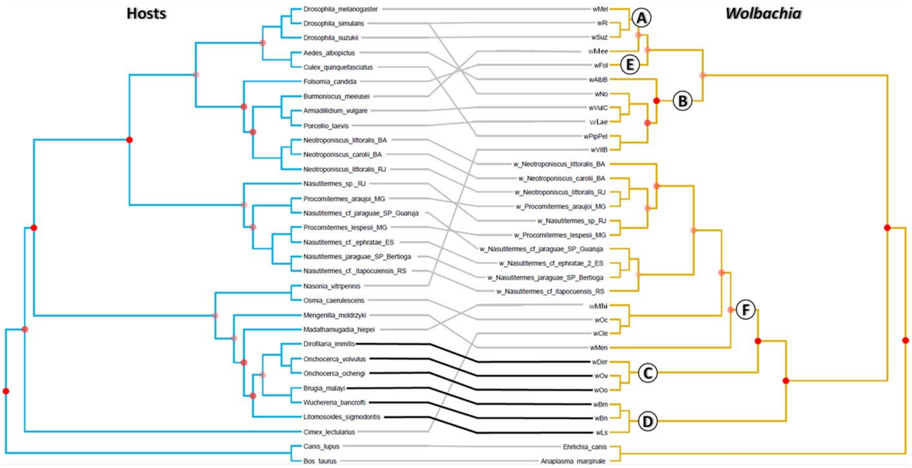

Fig. 5 Tanglegram of cophylogenetic relationships between hosts (left) and *Wolbachia* strains (right). Maximum likelihood phylogenies for *Wolbachia* bacteria (yellow) and their hosts (blue) rooted with *A. marginale* and *E. canis* as outgroups. All host-symbiont associations are shown in the tanglegram as grey and black connecting lines. Black lines indicate significant individual cospeciation links between *Wolbachia* and the host as indicated by ParaFit ($P \leq 0.01$), while gray lines represent non-significant links. Significance of the congruence of the nodes to their counterpart subtree in the other tree as indicated by the Treemap z-scores are indicated by red dots. The opacity of the dot is related to the significance of the test: opaque dot for $P < 0.001$; 50% opacity for $P \leq 0.01$, and 25% opacity for $P \leq 0.05$. A, B, C, D, E and F *Wolbachia* supergroups are indicated by circled letters. The following codes represent the *Wolbachia* strains according to the host species: wAlbB = *Aedes albopictus,* wBm = *Brugia malayi*, wBn = *Wuchereria bancrofti,* wCle = *Cimex lectularius*, wDim = *Dirofilaria immitis,* wFol = *Folsomia candida*, wLae = *Porcellio laevis*, wLs = *Litomosoides sigmodontis*, wMee = *Burmoniscus meeusei,* wMel = *Drosophila melanogaster*, wMen = *Mengenilla moldrzyki,* wMhi = *Madathamugadia hiepei*, wNo = *Drosophila simulans*, wOc = *Osmia caerulescens*, wOo = *Onchocerca ochengi*, wOv = *Onchocerca volvulus*, wPipPel = *Culex quinquefasciatus*, wRi = *Drosophila simulans*, wSuz = *Drosophila suzukii,* wVitB = *Nasonia vitripennis*, wVulC = *Armadillidium vulgare*

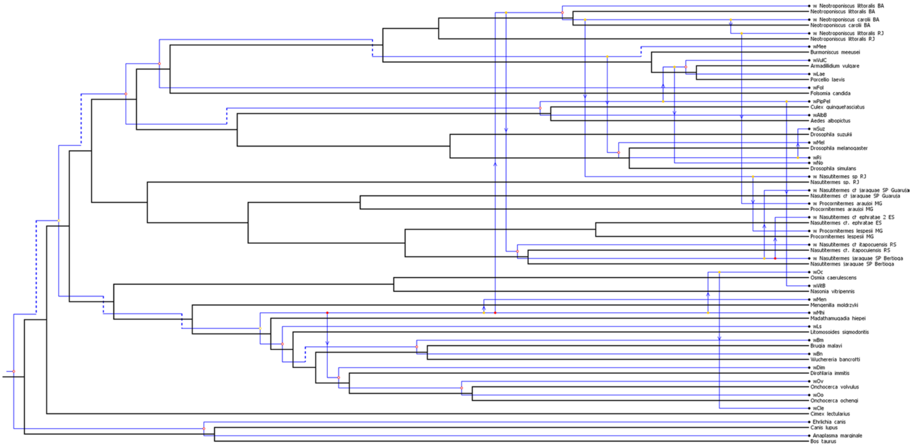

Fig. 6 Optimal cophylogeny reconstruction of hosts and *Wolbachia* strains produced by JANE 4.0. One of 115 isomorphic solutions with 15 cospeciations, 17 host switches, and eight losses (total cost = 42). Blue and black lines indicate the phylogenies of *Wolbachia* and hosts, respectively. Empty circles represent cospeciations; arrows represent host switches; and dashed lines represent loss events. See Fig. 5 legend for abbreviation of *Wolbachia* strains

Wolbachia strains found in *Neotroponiscus* isopods and termite species clustered together in phylogenies regardless of the relatedness of the host taxa. We also detected horizontal transfers between isopods and termites when reconstructing the evolutionary history of *Wolbachia*, confirming that the supergroup F *Wolbachia* and host taxa phylogenies did not have an underlying structure resembling coevolution. Thus, our results suggest that a horizontal transmission of supergroup F *Wolbachia* endosymbionts between termites and terrestrial isopods is likely.

The *Wolbachia* strains found in the termites *Nasutitermes* and *Procornitermes* are phylogenetically close to each other. *Nasutitermes* is the most common and abundant of all termite genera in Brazil. They usually build arboreal nests and are abundant in the same areas where *Neotroponiscus* occurs (Thorne et al. 1996; Constantino and Acioli 2006; Souza et al. 2012). *Procornitermes*, on the other hand, is a poorly studied genus composed of species that build subterranean and epigean nests (Cancello 1986; Moreira et al. 2018). However, *Procornitermes* can occur with *Nasutitermes* spp. in the soil and under wood material over the soil (Amaral-Castro et al. 2004). When we add the close phylogenetic relationship between the termites, it is not surprising that the *Wolbachia* strains are closely related. Indeed, *Wolbachia* is pre-adapted to infect closely related hosts due to their similar physiologies (Russell et al. 2009).

The horizontal transmission reported here likely occurred from micro-habitat sharing. Horizontal transmission of *Wolbachia* involves species living in close association, such as being connected via shared physical space or belonging to similar trophic levels (Stahlhut et al. 2010). These two prerequisites are met by termites and isopods and can explain how *Wolbachia* jumped hosts. First, *Neotroponiscus* species are frequently found in decaying wood and tree barks, where termites build arboreal nests, especially *Nasutitermes* (Boulogne et al. 2017). In fact, *N. carolii* isopods were already found occupying abandoned arboreal nests of *Nasutitermes* spp. termites (Lisboa et al. 2013). Such sites would be ideal for terrestrial isopods as they are rich in organic matter and provide a moist and shaded shelter (Costa et al. 2009). Therefore, it is relatively easy for the isopods to find the termite nests in these environments.

Second, terrestrial isopods and termites occupy the same ecological guild. Terrestrial isopods feed on litter, decomposing tree trunks, and nearly all kind of detritus which makes them keystone decomposers in many terrestrial ecosystems (Hornung 2011). It has been recently demonstrated a close cooperation between microbiota and terrestrial isopods for the lignocellulose degradation (Bredon et al. 2018, 2019, 2020). Similarly, due to the symbiotic organisms in the termites' hindgut, termites are also important cellulose decomposers wherever they inhabit (Hongoh 2010; Brune 2014). Thus, both groups are decomposers of plant-derived organic matter. Being of the same guild (and having relatively similar roles) might thus account for the high bacterial diversity found in these animals, including the presence of *Wolbachia* (Dittmer et al. 2016; Diouf et al. 2018). Additionally, occupying similar ecological guilds can also make the gut environment relatively similar, facilitating the horizontal transmission of *Wolbachia*.

Although terrestrial isopods are primarily herbivores, they are occasionally found eating non-plant items which suggest a possible route of transmission. Although rarely, terrestrial isopods can act as predators, preying on several kinds of small living invertebrates (Edney et al. 1974), including eggs and their own young (Warburg 1994). The isopods that prey become prone to be infected by the *Wolbachia* bacteria found on their food items (Le Clec'h et al. 2013). It is unlikely that the small-sized *Neotroponiscus* prey on soldiers or worker termites, but they could prey on termite eggs or larvae. In fact, *Wolbachia* is the most abundant bacterial group in the early stages of life—particularly the eggs—of

Nasutitermes arborum termites (Diouf et al. 2018), which could increase the likelihood of horizontal transmission. If we add that termites occur in high densities (e.g., nests of the arboreal termite *N. corniger* contain up to 800,000 individuals Thorne and Noirot 1982), it is thus possible that isopods consume anything left by the termites when abandoning the nest, being of decaying eggs or larvae, which increases the likelihood of horizontal transmission.

Supergroup F strains are known for being geographically and phylogenetically widespread among host taxa (Ros et al. 2009). However, they are also a relatively uncommon strain of *Wolbachia*. Thus, finding that the first supergroup F strain in isopods was not an isolated event already suggested that the evolutionary history of *Wolbachia* supergroup F is more complex. Indeed, supergroup F was also found in several species of termites, including other species of *Nasutitermes* termites that occur in distant regions, such as Japan, Malaysia, Australia, and Venezuela (Lo and Evans 2007). It was thus rather unsurprising when we found that *Wolbachia* strains found in scorpions, bees, and locusts nested within the monophyletic group of termites and isopods *Wolbachia* (Fig. 3). The horizontal transmission of *Wolbachia* might thus be more widespread in arthropods than we initially thought. Unfortunately, tests on how the supergroup F can transfer horizontally are still scarce (not to mention inexistent). Our study was the first to assess if horizontal transmission between isopods and termites was possible, but we still lack proper investigation. We are unsure why the scorpions, bees, and locusts that do not inhabit South America were included within the monophyletic group of South American strains. But, but the same pattern has been found in other studies, in which strains of supergroup F from hosts occurring in distinct continents grouped together (Baldo et al. 2007; Lefoulon et al. 2012). It thus seems a promising venue for further research.

The evolution of endosymbiotic bacteria such as *Wolbachia* is shaped by the ecology and evolution of many arthropods (Hellemans et al. 2019). Despite the importance of horizontal transmission for *Wolbachia*, the routes of transmission are not yet fully known (Gerth et al. 2013). Our study is one of the few that provides evidence for horizontal transmission based on in situ studies—especially on such distant related taxa. Our results suggest that the horizontal transmission of *Wolbachia* strains of supergroup F between termites and terrestrial isopods occurred. We hope more studies will be carried out to clarify the mechanisms governing horizontal transmission of *Wolbachia* and allow the understanding of the success and worldwide distribution of this symbiont.

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Author contributions BLZ, GMC and PBA conceived the idea and designed methodology. GMC collected the samples. BLZ and PHP generated the molecular data. BLZ and DB analyzed the molecular data. BLZ and AVP led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability All gene sequences were submitted to NCBI database (https://www.ncbi.nlm.nih.gov/genbank/) under Accession Numbers KX024833 to KX024843, KX036766 to KX036775, KX036776 to KX036786 and KX036787 to KX036796.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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