Parasites & Vectors

RESEARCH

Open Access

Screening of natural *Wolbachia* infection in mosquitoes (Diptera: Culicidae) from the Cape Verde islands

Aires Januário Fernandes da Moura[1,2*], Vera Valadas[1], Silvania Da Veiga Leal[3], Eddyson Montalvo Sabino[1,4], Carla A. Sousa[1] and João Pinto[1]

Abstract

Background *Wolbachia pipientis* is an endosymbiont bacterium that induces cytoplasmic incompatibility and inhibits arboviral replication in mosquitoes. This study aimed to assess *Wolbachia* prevalence and genetic diversity in different mosquito species from Cape Verde.

Methods Mosquitoes were collected on six islands of Cape Verde and identified to species using morphological keys and PCR-based assays. *Wolbachia* was detected by amplifying a fragment of the surface protein gene (*wsp*). Multilocus sequence typing (MLST) was performed with five housekeeping genes (*coxA*, *gatB*, *ftsZ*, *hcpA*, and *fbpA*) and the *wsp* hypervariable region (HVR) for strain identification. Identification of *w*Pip groups (*w*Pip-I to *w*Pip-V) was performed using PCR–restriction fragment length polymorphism (RFLP) assay on the ankyrin domain gene *pk1*.

Results Nine mosquito species were collected, including the major vectors *Aedes aegypti*, *Anopheles arabiensis*, *Culex pipiens* sensu stricto, and *Culex quinquefasciatus*. *Wolbachia* was only detected in *Cx. pipiens* s.s. (100% prevalence), *Cx. quinquefasciatus* (98.3%), *Cx. pipiens/quinquefasciatus* hybrids (100%), and *Culex tigripes* (100%). Based on the results of MLST and *wsp* hypervariable region typing, *Wolbachia* from the *Cx. pipiens* complex was assigned to sequence type 9, *w*Pip clade, and supergroup B. PCR/RFLP analysis revealed three *w*Pip groups in Cape Verde, namely *w*Pip-II, *w*Pip-III, and *w*Pip-IV. *w*Pip-IV was the most prevalent, while *w*Pip-II and *w*Pip-III were found only on Maio and Fogo islands. *Wolbachia* detected in *Cx. tigripes* belongs to supergroup B, with no attributed MLST profile, indicating a new strain of *Wolbachia* in this mosquito species.

Conclusions A high prevalence and diversity of *Wolbachia* was found in species from the *Cx. pipiens* complex. This diversity may be related to the mosquito's colonization history on the Cape Verde islands. To the best of our knowledge, this is the first study to detect *Wolbachia* in *Cx. tigripes*, which may provide an additional opportunity for biocontrol initiatives.

Keywords *Wolbachia*, Genotyping, Mosquitoes, *Culex pipiens*, *Culex tigripes*, Cape Verde

*Correspondence:
Aires Januário Fernandes da Moura
a21001274@ihmt.unl.pt
Full list of author information is available at the end of the article

Background

Wolbachia pipientis (Alphaproteobacteria, *Rickettsiales*) is an obligate intracellular gram-negative bacterium and proteobacterial symbiont found in a variety of invertebrates, including insects, crustaceans, arachnids, and filarial nematodes [1]. Currently, the *Wolbachia* genus is subdivided into 17 supergroups (A–F; H–Q, and S), and most species known belong to supergroups A and B [2].

Wolbachia is transmitted vertically through host eggs and can influence longevity and reproduction, including feminization, parthenogenesis, and incompatibility between the female and male sex cells [3]. The best-known phenotype induced by *Wolbachia* in arthropods is cytoplasmic incompatibility (CI). It occurs when males harboring *Wolbachia* are crossed with uninfected females or between individuals infected with incompatible strains [4, 5]. The generally accepted model stipulates that cytoplasmic incompatibility results from a *Wolbachia* "modification" factor (mod; toxin) in the sperm that blocks early embryogenesis, and a *Wolbachia* "rescue" factor (resc; antitoxin) produced in the oocyte that allows the diploid zygote to develop if the cross is compatible [6, 7].

Besides cytoplasmic incompatibility, *Wolbachia* can inhibit viral replication in mosquitoes, including Zika, dengue, West Nile, and chikungunya arboviruses in *Aedes aegypti* [8, 9]. Other studies also suggest inhibition of pathogens such as *Plasmodium falciparum* in *Anopheles stephensi* and *Anopheles gambiae* and West Nile virus in *Culex quinquefasciatus* [1, 10, 11]. These abilities make *Wolbachia* a promising tool against mosquito-borne diseases and possibly an alternative to conventional vector control programs using insecticides. In fact, the release of males harboring incompatible *Wolbachia* into target populations has successfully decreased reproduction by sterilization [12, 13]. The release of *Ae. aegypti* transfected with the *Wolbachia w*Mel strain (derived from *Drosophila melanogaster*) led to the establishment of *Ae. aegypti* populations infected with *Wolbachia* and a proven decrease in dengue incidence in Australia [14] and Malaysia [15].

Cape Verde is threatened by several species of vector mosquitoes, including *Ae. aegypti*, *Anopheles arabiensis*, *Cx. quinquefasciatus*, and *Culex pipiens* sensu stricto (s.s.) [16]. Integrated vector control strategies are mainly directed against *An. arabiensis* and *Ae. aegypti,* using chemical insecticides, diesel, and biological control with *Gambusia* sp. fish [17]. However, despite control efforts, the country had its first dengue epidemic in 2009, followed by an outbreak of Zika in 2015–2016 [18] and a malaria outbreak in 2017 [19].

There is no data on the genetic diversity of *Wolbachia* infecting mosquitoes (Diptera: Culicidae) from the Cape Verde islands. This knowledge would be a first step for the design and implementation of programs to suppress mosquito populations through cytoplasmic incompatibility. In this context, the present study aims to detect and genetically characterize *Wolbachia* in populations of Culicidae from Cape Verde.

Methods

Study area and sample collection

An entomological survey was carried out in Cape Verde between February and June 2021. Larval and adult mosquito samples were collected on six islands (Santiago, Brava, Fogo, Maio, Santo Antão, and Boavista; Fig. 1) using BG-Sentinel and Centers for Disease Control and Prevention (CDC) light traps, dorsal aspirators, dippers, and pipettes. All collection sites were geo-referenced with a portable global positioning system (GPS) device (Garmin eTrex 10).

Mosquitoes were identified to species/complex using the Ribeiro et al. [20] identification key and stored individually in microtubes containing silica gel (for adults) or 80% ethanol (for larvae). For genetic analysis, DNA was extracted from single specimens using cetrimonium bromide (CTAB) 2% and proteinase K, according to Weeks et al. [21].

Species of the *An. gambiae* complex were identified by polymerase chain reaction (PCR) according to Scott et al. [22] using primer sequences described in Table S1 (Additional file 1: Table S1). PCR was performed using 12.5 µl of Xpert Taq^{PLUS} Mastermix (GriSP), 0.1 µM of ME and UN primers, 0.05 µM of GA primer, and 0.15 µM of AR primer, plus 1 µl of DNA template and water to a final volume of 25 µl. Cycling conditions were as follows: one cycle at 95 °C for 5 min, 30 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s; and a final cycle of 72 °C for 5 min.

For the *Cx. pipiens* complex, specimens were identified to species by PCR amplification of acetylcholinesterase-2 (*ace-2*) gene sequences using primers described by Smith & Fonseca [23] (Additional file 1: Table S1). PCR was performed using 12.5 µl of Xpert Taq^{PLUS} Mastermix (GriSP), 0.4 µM of ACEquin and B1246 primer, 0.2 µM of ACEpip primer, 1 µl of DNA template and water to a final volume of 25 µl. Cycling conditions were performed as follows: one cycle at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and one cycle at 72 °C for 5 min.

Whenever necessary, morphological identification of species other than the above was supported with the sequencing of a 710-base-pair (bp) fragment of cytochrome c oxidase subunit 1 mitochondrial gene (*COI*) with primers LCOI1490_F1 and HCOI2198_R1 (Additional file 1: Table S1) according to Folmer et al.

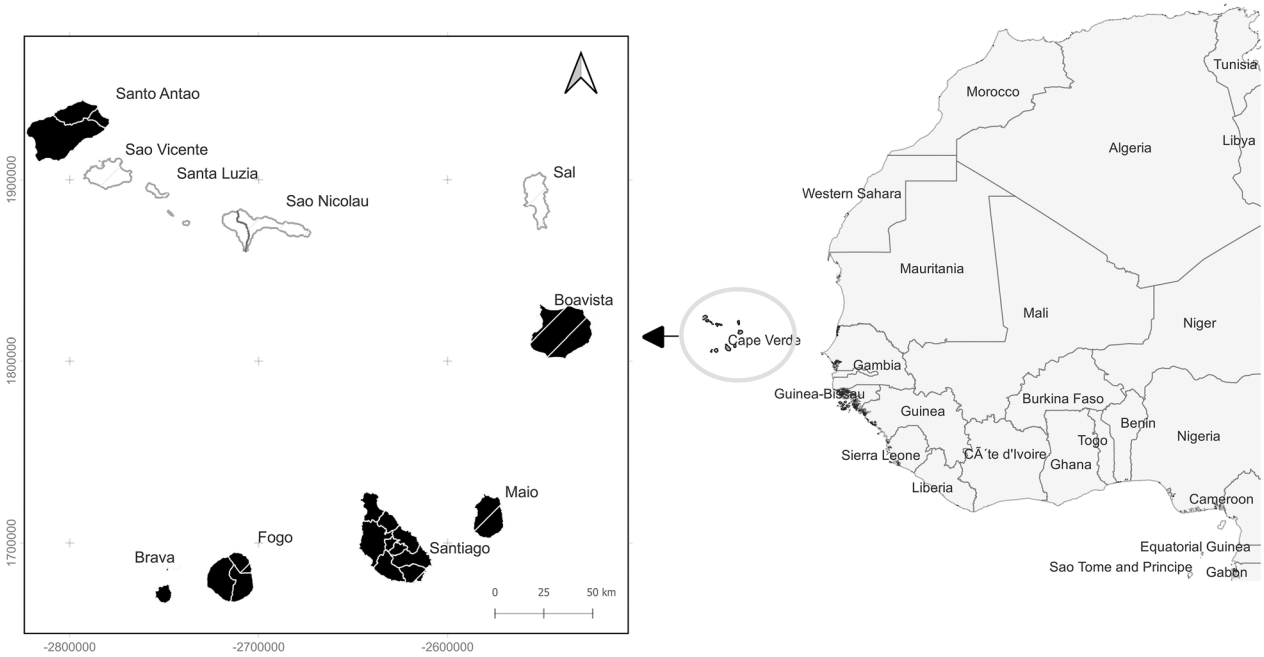

Fig. 1 Map of the North Atlantic region showing the geographic location of the Cape Verde islands. Mosquito samples were collected on the islands of Santo Antão, Boavista, Maio, Santiago, Fogo, and Brava (highlighted in black)

[24]. PCR was performed using 1X PCR buffer, 2 mM MgCl2, 0.2 mM dNTPs, 1 U Taq polymerase (Robust HotStart PCR Kit, Roche/Kapa Biosystems), 0.5 µM of each primer, 2 µl of DNA template, and water to a final volume of 20 µl. Cycling conditions were as follows: initial denaturation at 94 °C for 3 min; 40 cycles at 94 °C for 50 s; annealing at 45 °C during 30 s and 72 °C for 1 min; and final elongation at 72 °C for 5 min.

Screening of *Wolbachia*

Wolbachia detection in mosquito samples was performed by amplifying a 610-bp region of the *Wolbachia* surface protein gene (*wsp*) using primers 81F and 691R (Additional file 1: Table S2) described by Zhou et al. [25]. The amplification reaction comprised 12.5 µl of Xpert Taq^{PLUS} Mastermix (GriSP), 0.4 µM of each primer, 1 µl of DNA template, and water to a final volume of 25 µl. Cycling conditions were as follows: one cycle at 95 °C for 3 min, 35 cycles at 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and one cycle at 72 °C for 10 min.

All PCR products from the assays described above were analyzed by electrophoresis on a 1.5% agarose gel stained with GreenSafe Premium (NZYTech).

Wolbachia multilocus sequence typing (MLST) and wsp typing

Wolbachia genotyping was performed through amplification and sequencing of five MLST loci (*gatB*, *coxA*, *hcpA*, *ftsZ*, *fbpA*) and the *wsp* hypervariable region [26, 27]. The primer pairs for each locus and the size of amplified products are shown in supplemental materials (Additional file 1: Table S3).

PCR for each locus was performed using 1X PCR buffer, 0.2 mM dNTPs, 1.5 mM MgCl2, 0.5U Taq polymerase (Robust HotStart PCR Kit, Roche/Kapa Biosystems), 1 µM of each primer, 2 µl of DNA template, and water to a final volume of 40 µl. Cycling conditions were as follows: initial denaturation at 94 °C for 2 min; 37 cycles at 94 °C for 30 s, annealing at 54 °C (for *hcpA*, *gatB*, *ftsZ*, and *coxA*), and 59 °C (*fbpA* and *wsp*) for 45 s, and 72 °C for 90 s; and final elongation at 72 °C for 10 min.

Five microliters of PCR product from each locus was used in electrophoresis to confirm amplification. The remaining 35 µl was purified using an Exo/SAP Go PCR purification kit (GriSP) and sent for direct DNA sequencing at STAB Vida (Oeiras, Portugal) using forward and reverse primers.

Wolbachia MLST and hypervariable *wsp* sequences were edited and aligned using BioEdit (version 7.0.9.0). Consensus and concatenated sequences (*gatB*, *coxA*, *fbpA*, *ftsZ*, *hcpA*, and *wsp* hypervariable region [HVR]) were queried in the *Wolbachia* MLST database (https://pubmlst.org/bigsdb?db=pubmlst_wolbachia_seqdef) for strain characterization. Sequences were also subjected to the nucleotide Basic Local Alignment Search Tool (BLAST) to verify the similarity with deposited

sequences in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic analysis was conducted using the gamma-distributed Tamura 3-parameter nucleotide substitution model, and a neighbor-joining tree was generated employing 1000 bootstraps in Molecular Evolutionary Genetics Analysis version 11 (MEGA11) [28].

Identification of wPip groups by PCR–RFLP

Identification of *w*Pip groups (*w*Pip-I to *w*Pip-V) was performed using a PCR–restriction fragment length polymorphism (RFLP) assay based on the ankyrin (ANK) *Wolbachia* marker *pk1* [29–31]. A PCR that amplifies a 1300-bp fragment of the ANK domain gene (*pk1*) was performed with primers pk1_For and pk1_Rev (Additional file 1: Table S2) [32]. The reaction components included 10 µl of Xpert Taq^{PLUS} Mastermix (GriSP), 0.4 µM of each primer, 2 µl of DNA template, and water to a final volume of 20 µl. Cycling conditions were as follows: one cycle at 94ºC for 5 min; 35 cycles at 94 °C for 30 s, 52ºC for 30 s, and 72 °C for 90 s; and a final cycle of 72 °C for 5 min. PCR product was analyzed by electrophoresis on a 2% agarose gel stained with GreenSafe Premium (NZYTech).

The *pk1* PCR product was digested with restriction enzymes *Taq*αI and *Pst*I to identify different *w*Pip groups [29]. Digestion with *Taq*αI was performed with the following reaction mixture: 2 µl of Buffer C (NZYTech), 10 µl of the PCR product, 18 µl of water, and 2 µl *Taq*αI enzyme (NZYTech) at 10U/µl. The mixture was placed in a thermal cycler at 65ºC for 90 min. The reaction was stopped by adding 0.02 mM of ethylenediaminetetraacetic acid (EDTA) (pH = 8) to each tube, and the digestion product was visualized by electrophoresis on a 2% agarose gel. Each allele (*w*Pip group) was detected according to the size of the resulting fragments: allele "a" or "e" (*w*Pip-I or *w*Pip-V; 991, 251, 107 bp); "b" (*w*Pip-III; 669, 665 bp); "c" (*w*Pip-II; 851, 498 bp); "d" (*w*Pip-IV; 497, 251, 107 bp) [29].

If alleles "a" or "e" (*w*Pip-I or *w*Pip-V) were present, the two were differentiated by digesting the *pk1* PCR product with the *Pst*I restriction enzyme. For this purpose, a reaction mixture was prepared with 2 µl of Buffer A (NZYTech), 12 µl pk1 PCR product, 1 µl *Pst*I enzyme (NZYTech) at 10U/µl, and 5 µl of water. The mixture was incubated at 37 °C for 1 h, and the reaction stopped by incubating at 80 °C for 20 min. Digested DNA fragments were separated by electrophoresis on a 2% agarose gel. *w*Pip alleles resulting from *pstI* digestion included "a" (*w*Pip- I; 903, 303, 141 bp) and "e" (*w*Pip-V; 903, 430 bp) [29, 30].

Sequencing of *pk1* PCR products was performed to confirm the RFLP profile. For this purpose, the *pk1* PCR product was purified as described above for the MLST and sent for direct sequencing using reverse and forward primers. Sequences were subjected to the nucleotide BLAST, and phylogenetic analysis was performed using the gamma-distributed Tamura 3-parameter nucleotide substitution model, and a neighbor-joining tree was generated using 1000 bootstraps in MEGA software version 11.0.11.

Results

Mosquito species identification

A total of 1648 mosquitoes (303 larvae and 1345 adults) were collected (Additional file 2: Table S4 for details). Species identification by morphological characters revealed the presence of *Ae. aegypti* ($n = 663$, 40.2%), *Aedes caspius* ($n = 39$, 2.4%), *An. gambiae* sensu lato (s.l.) ($n = 49$, 3.0%), *Anopheles pretoriensis* ($n = 275$, 16.7%), *Cx. pipiens* s.l. ($n = 584$, 35.4%), *Culex thalassius* ($n = 7$, 0.4%), *Culex tigripes* ($n = 3$, 0.2%), and *Culiseta longiareolata* ($n = 28$, 1.7%).

Ribosomal DNA PCR for identifying species of the *An. gambiae* complex revealed that all collected specimens from this complex belonged to *An. arabiensis*. For the *Cx. pipiens* complex, specimens were identified by *ace-2* PCR as *Cx. pipiens* s.s. ($n = 10$, 1.7%), *Cx. quinquefasciatus* ($n = 545$, 93.3%), and *Cx. pipiens/Cx. quinquefasciatus* hybrids ($n = 29$, 5.0%).

Screening of *Wolbachia*

The *wsp* fragment was amplified only in *Cx. pipiens* s.s. (10/10 = 100% prevalence), *Cx. quinquefasciatus* (536/545 = 98.3%), *Cx. pipiens/Cx. quinquefasciatus* hybrids (29/29 = 100%), and *Cx. tigripes* (3/3 = 100%). The remaining species were negative for *Wolbachia.*

Wolbachia MLST and *wsp* typing

We analyzed 80 mosquitoes that were positive for *wsp* for *Wolbachia* MLST and *wsp* typing. Allelic profiles resulting from MLST loci and the *wsp* hypervariable region sequencing revealed that *Wolbachia* from *Cx. pipiens* s.s., *Cx. quinquefasciatus*, and *Cx. pipiens/Cx. quinquefasciatus* hybrids belong to sequence type 9, *w*Pip clade, and supergroup B *Wolbachia* (Table 1). The same result was obtained from phylogenetic analysis using concatenated sequences of MLST loci (*coxA*, *gatB*, *ftsZ*, *fbpA*, *hcpA*) and the *wsp* hypervariable region (Fig. 2).

For *Cx. tigripes*, the allelic profile obtained was unavailable in the MLST database, thus not allowing the determination of a sequence type. However, the phylogenetic analysis indicates that *Wolbachia* from *Cx. tigripes* also belongs to supergroup B but integrates a distinct clade from *w*Pip (Fig. 2).

Table 1 Allelic profile of MLST genes and *wsp* hypervariable region for different species of Culicidae collected in Cape Verde islands

Host species	Island (*n*)	*gatB*	*coxA*	*hcpA*	*ftsZ*	*fbpA*	*wsp*	*HVR1*	*HVR2*	*HVR3*	*HVR4*	*ST*
Cx. quinquefasciatus	Santiago (*n* = 15) Brava (*n* = 15) Boavista (*n* = 12) Maio (*n* = 7) Fogo (*n* = 1) S. Antão (*n* = 10)	4	3	3	22	4	10	10	8	10	8	9
Cx. pipiens s.s.	Maio (*n* = 2) S. Antão (*n* = 2)	4	3	3	22	4	10	10	8	10	8	9
Hybrids pip/qui	Maio (*n* = 1) Fogo (*n* = 2) S. Antão (*n* = 10)	4	3	3	22	4	10	10	8	10	8	9
Cx. tigripes	Santiago (*n* = 3)	9	182[b]	12	117	203[b]	NA[a]	NA[a]	232	222	84	NA[a]

[a] NA represents allelic profile or sequence type not available in the *Wolbachia* MLST database

[b] Sequences with partial match in the *Wolbachia* MLST database

*w*Pip groups and their distribution in the archipelago

Results from *pk1* PCR–RFLP showed the occurrence of three different *w*Pip groups in Cape Verde, namely *w*Pip-IV (88.9%), *w*Pip-II (7.4%), and *w*Pip-III (3.7%) (Table 2). The *w*Pip-IV group was detected in *Cx. quinquefasciatus* from five islands (Santiago, Brava, Santo Antão, Maio and Boavista) and in *Cx. pipiens* s.s. from Santo Antão. The *w*Pip-II group was detected only in *Cx. pipiens* s.s. from Maio, while *w*Pip-III was found exclusively in *Cx. quinquefasciatus* and *Cx. pipiens*/*quinquefasciatus* hybrids from the island of Fogo (Table 2).

Sequencing of *pk1* PCR products confirmed the observed RFLP profiles and similarity with *pk1* sequences deposited in GenBank (Fig. 3).

Discussion

Wolbachia has garnered substantial attention for its ability to control diseases transmitted by mosquitoes. This study represents the first assessment of *Wolbachia*'s prevalence and genetic diversity in mosquitoes from Cape Verde. Our objective is to expand knowledge of this bacterium through our findings and illustrate its potential for controlling mosquito-borne diseases in the archipelago.

The MLST and *wsp* typing results revealed that *Wolbachia* from *Cx. pipiens* and *Cx. quinquefasciatus* and their hybrids belong to the *w*Pip clade and share a monophyletic origin within *Wolbachia* group B. The same results were obtained by Atyame et al. [33] and Dumas et al. [30] when studying *Wolbachia* genetic diversity from Cx. *pipiens* s.l. populations originating from different regions of the world. According to the authors, these findings suggest that *w*Pip strains comprise a recent clade of the *Wolbachia* supergroup B [30, 33].

The analysis of the fast-evolving *pk1* gene revealed further variation within the *w*Pip strain, indicating the presence of *w*Pip-II, *w*Pip-III, and *w*Pip-IV groups in Cape Verde. The occurrence of different *w*Pip groups suggests multiple introduction events into the archipelago. In the past, Cape Verde was a maritime hub between Europe and mainland Africa, and the intense movement of ships may explain the diversity of *w*Pip found on the islands. This result contrasts with that of the southwestern Indian Ocean islands, in which *Wolbachia* infecting *Cx. quinquefasciatus* all belonged to the *w*Pip-I group [13]. It is noteworthy that *w*Pip-I was the only group found in mainland Sub-Saharan Africa, South America, and Southeast Asia, whereas only *w*Pip-III was detected in North America [30, 33]. Europe shows the highest diversity, with all five groups of the *w*Pip clade being found in this continent [30]. The presence of *w*Pip-II, *w*Pip-III, and *w*Pip-IV groups in Cape Verde islands suggests at least three introduction events of *Wolbachia* possibly originating from Europe. However, a North American origin for the *w*Pip-III group in Fogo Island cannot be excluded. Interestingly, the differences found in the genetic composition of the *w*Pip clade among islands agree with the genetic structure of the *Cx. pipiens* complex in Cape Verde. Previous microsatellite-based analysis suggested that *Cx. quinquefasciatus* from Fogo Island may comprise a genetic ancestry cluster distinct from the other islands [34]. What was previously considered an admixed *Cx. quinquefasciatus* population in Fogo Island [34] may, in fact, represent a genetically differentiated population originating from a *w*Pip-III group source population.

The absence of the African *w*Pip-I group from Cape Verde *Cx. quinquefasciatus* is not easily explained. Mainland Africa would be the natural candidate for a source population of *w*Pip-I *Cx. quinquefasciatus* that would have colonized the Cape Verdean islands, as suggested for the southwestern Indian Ocean islands [30]. However, *Cx. quinquefasciatus* was predominantly infected by the *w*Pip-IV group. This result may suggest that *Cx.*

Fig. 2 Phylogenetic tree generated from concatenated sequences of MLST loci (*coxA*, *gatB*, *ftsZ*, *fbpA*, *hcpA*) and the wsp hypervariable region. Numbers on branches indicate percentage bootstrap support (1000 replicates). Reference sequences were obtained from the *Wolbachia* MLST database and are marked by full circles. Each *Wolbachia* supergroup is marked with a different color: yellow, supergroup B; black, supergroup A; red, supergroup D; and green, supergroup F. The scale bar indicates the number of substitutions

Table 2 *w*Pip groups detected in *Cx. pipiens* s.l. from Cape Verde islands according to *pk1* PCR–RFLP

Islands	Species	*n*	*w*Pip group
Santiago	*Cx. quinquefasciatus*	15	*w*Pip- IV
Brava	*Cx. quinquefasciatus*	15	*w*Pip- IV
Santo Antão	*Cx. pipiens*	2	*w*Pip- IV
	Cx. quinquefasciatus	10	*w*Pip- IV
	Hybrids *Cx. pipiens/quinquefasciatus*	10	*w*Pip- IV
Maio	*Cx. pipiens*	6	*w*Pip- II
	Cx. quinquefasciatus	7	*w*Pip- IV
	Hybrids *Cx. pipiens/quinquefasciatus*	1	*w*Pip- IV
Fogo	*Cx. quinquefasciatus*	1	*w*Pip- III
	Hybrids *Cx. pipiens/quinquefasciatus*	2	*w*Pip- III
Boavista	*Cx. quinquefasciatus*	12	*w*Pip- IV

quinquefasciatus from Cape Verde may have derived from a yet to be sampled *w*Pip-IV population of mainland Africa. Another explanation would involve the cytoplasmic transfer of *w*Pip-IV from European *Cx. pipiens* s.s. to *w*Pip-I *Cx. quinquefasciatus* via hybridization, followed by the latter's replacement through cytoplasmic incompatibility (CI). High levels of CI have been reported in crosses between *w*Pip-II and *w*Pip-IV, as well as between *w*Pip-III- and *w*Pip-IV-infected mosquitoes [7, 29]. Studies involving experimental crosses would be required to assess CI between *w*Pip-I and *w*Pip-IV and whether this CI would confer an adaptive advantage to *w*Pip-IV-infected mosquitoes.

Wolbachia was not detected in *Ae. aegypti* from Cape Verde islands, which is consistent with most surveys on this species where no evidence of *Wolbachia* natural infection was found [35–38]. The presence of *Wolbachia* in *Ae. aegypti* has been reported on only a few occasions, including those from New Mexico, the USA [39], and Kuala Lumpur, Malaysia [40]. However, the possibility of *Wolbachia* detection in *Ae. aegypti* being the result of an infection with a *Wolbachia*-carrying nematode or of environmental contamination during field collections could not be excluded [36]. *Wolbachia* was also not detected in *An. arabiensis* and *An. pretoriensis* from Cape Verde. While this result is in line with most studies that screened for *Wolbachia* in *Anopheles* species [41, 42], there have been a few reports on the presence of the endosymbiont in *An. gambiae* and *An. coluzzii* from Mali [43], *An. gambiae* from the Democratic Republic of Congo, and *An. coluzzii* in Ghana [44]. Shaw et al. [45] concluded that *Wolbachia* natural *Anopheles* infections do not induce cytoplasmic incompatibility or sex ratio distortion but show a negative correlation with *Plasmodium* infection, suggesting that *Wolbachia* may interfere with malaria transmission.

This study reports for the first time the presence of *Wolbachia* in *Cx. tigripes*. Phylogenetic analyses indicate that *Wolbachia* isolated from this mosquito belongs to supergroup B, with no attributed MLST profile. This result suggests the presence of a new strain of *Wolbachia* infecting *Cx. tigripes* in Santiago Island. *Culex tigripes* is the only predatory mosquito in Cape Verde [46], and on the island of Santiago, its larvae are often found in breeding sites associated with *Cx. pipiens* s.l. species [47]. Our results exclude environmental contamination by *Cx. pipiens* s.l. *Wolbachia* since we detected *Wolbachia* in both larvae and an adult male of *Cx. tigripes* (Additional file 2: Table S4). More importantly, the concatenated sequences of the MLST loci and the *wsp* HVR region clearly showed that the strain detected in *Cx. tigripes* forms a monophyletic group separate from the *w*Pip clade. Our phylogenetic analyses also exclude contamination with *Wolbachia* from supergroups D and F, which are generally found in nematodes [2, 48].

The use of *Wolbachia*-based methods in vector management holds significant promise. The newly detected *Wolbachia* strain in *Cx. tigripes* from Cape Verde encourages further research to assess their ability to be firmly established in major vector trans-infected lines, induce cytoplasmic incompatibility, or reduce the ability to transmit pathogens. Proof of these abilities may offer an additional opportunity for biocontrol initiatives.

The incompatible insect technique (IIT), a variation of the sterile insect technique (SIT), can be performed by taking advantage of the *w*Pip-induced cytoplasmic incompatibility. Studies have indicated that *Cx. pipiens* s.l. mosquitoes infected with identical *Wolbachia* *w*Pip groups tend to exhibit cytoplasmic compatibility, while crossing between mosquitoes carrying different *w*Pip groups is often incompatible [13, 31]. As a result, our findings regarding the natural occurrence of *w*Pip groups in Cape Verde can provide valuable insights for implementing control programs for *Cx. pipiens* s.l. in the archipelago.

Experiments conducted in semi-field conditions on La Réunion showed that the mating between local *Cx. quinquefasciatus* *w*Pip-I females and non-native males carrying the *w*Pip-IV (Istanbul strain) resulted in 100% embryonic mortality [13]. Altinli et al. [29] demonstrated naturally occurring CI patterns between *w*Pip-IV-harboring males and *w*Pip-I- or *w*Pip-II-harboring females in *Cx. pipiens* s.l. populations from Turkey. These observations reveal that IIT based on *w*Pip-inducing IC could be employed to control *Cx. pipiens* populations. The same methodology can be implemented in Cape Verde considering the data we gathered on the prevalence and distribution of *w*Pip groups in the archipelago. It would be worthwhile to

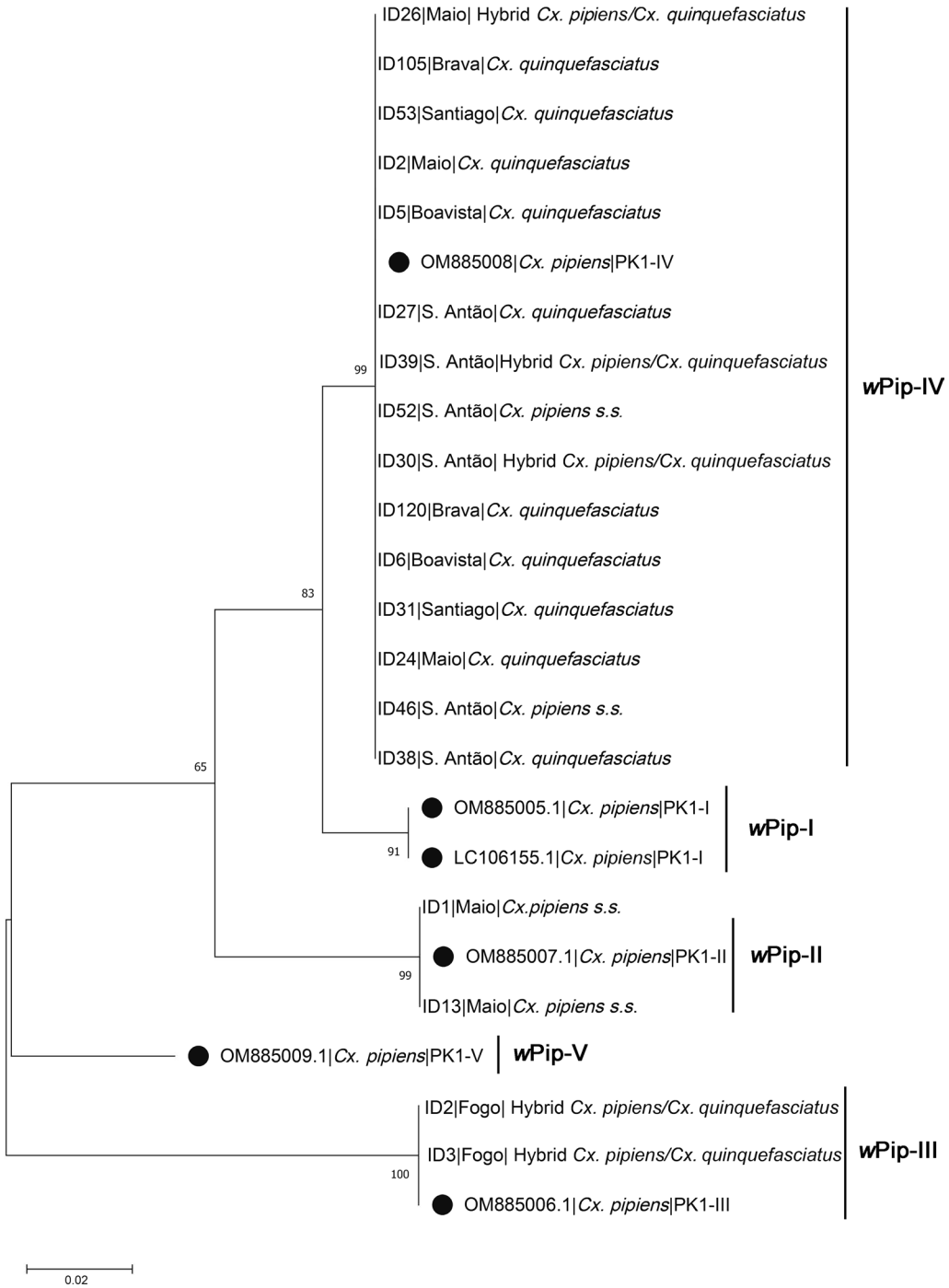

Fig. 3 Phylogenetic tree generated from pk1 sequences by Bayesian analysis. Known *w*Pip group pk1 sequences are marked by full circles. Numbers on branches indicate percentage bootstrap support (1000 replicates). The scale bar indicates the number of substitutions

analyze the pattern of cytoplasmic incompatibility among the different *w*Pip groups in Cape Verde and determine whether an island-specific *w*Pip group could be used to regulate *Cx. pipiens* s.l. populations on another island. As an alternative, male *Cx. pipiens* from other regions of the world carrying *w*Pip groups not present in Cape Verde, could be introduced into the archipelago to sterilize local females. It is noteworthy that IIT based on *w*Pip-inducing IC could be a favorable alternative to the costly radiation and genetic

manipulation methods, and its implementation would provide a more advantageous solution for low-income nations.

Conclusion

Our study revealed that *Wolbachia* is widespread in *Cx. pipiens* s.l. from the Cape Verde islands but absent from other mosquito species except for *Cx. tigripes,*, where a novel *Wolbachia* strain was unveiled. The three distinct *w*Pip groups circulating in *Cx. pipiens* s.l. suggest multiple introduction events in the archipelago, possibly of non-African origin. The finding of a novel *Wolbachia* strain in *Cx. tigripes* may provide an additional candidate to be used in biocontrol approaches. Further studies would be required to isolate this new *Wolbachia* strain to be used in transfection studies with major mosquito vectors in order to assess its potential impact on mosquito fitness and vector competence.

Abbreviations

CI	Cytoplasmic incompatibility
MLST	Multilocus sequence typing
RFLP	Restriction fragment length polymorphism
Wsp	*Wolbachia* surface protein

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-023-05745-w.

Additional file 1: Table S1. Primer sequences used for molecular identification of mosquito species collected in Cape Verde islands. **Table S2.** Primers used for PCR detection of *Wolbachia* and genotyping of *w*Pip I–V groups by PCR-RFLP. **Table S3.** Primers used for *Wolbachia* MLST loci and wsp hypervariable region amplification and sequence analysis.

Additional file 2: Table S4. Mosquito species collected on each island and tested for *Wolbachia* using *wsp*.

Acknowledgements

We are grateful to the National Institute of Public Health for the laboratory support in Cape Verde, and to technicians from the Ministry of Health for their assistance in field work. We would like to thank Prof. Paulo Almeida for providing DNA controls of *Cx. pipiens* and *Cx. quinquefasciatus* used in the species identification PCR assay.

Author contributions

AJFM, CAS, and JP designed the study. AJFM, EMS, and SLV performed field work and specimen identification. AJFM and VV performed the molecular laboratory work. AJFM, SLV, CAS, and JP drafted the manuscript. All authors read and approved the final manuscript.

Funding

This work was funded by national funds through FCT—Fundação para a Ciência e Tecnologia, I.P., within the framework of the project ARBOMONITOR (PTDC/BIA-OUT/29477/2017. Aires da Moura was funded by the Ph.D. fellowship program of Camões I.P.

Availability of data and materials

Sequences generated in this study are available in the GenBank database: *pk1* sequences (OQ223307-OQ223325); *ftsZ* (OQ223326-OQ223348); *hcpA* (OQ223349-OQ223371); *fbpA* (OQ223372-OQ223394); *coxA* (OQ225016-OQ225038); *gatB* (OQ225039-OQ225061); and *wsp* (OQ236526-OQ236548). All reference sequence accession numbers (GenBank) and MLST database IDs are included in the article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL., Rua da Junqueira 100, 1349-008, Lisboa, Portugal. [2]Unidade de Ciências da Natureza, da Vida E Do Ambiente, Universidade Jean Piaget de Cabo Verde, Praia, Cape Verde. [3]Laboratório de Entomologia Médica, Instituto Nacional de Saúde Pública, Praia, Cape Verde. [4]Laboratório de Simulidos, Universidad Nacional Hermilio Valdizan, Huánuco, Peru.

Received: 29 December 2022 Accepted: 17 March 2023
Published online: 25 April 2023

References

1. Glaser RL, Meola MA. The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to West Nile virus infection. PLoS ONE. 2010;5:e11977.
2. Kaur R, Shropshire JD, Cross KL, Leigh B, Mansueto AJ, Stewart V, et al. Living in the endosymbiotic world of *Wolbachia*: a centennial review. Cell Host Microbe. 2021;29:879–93.
3. Werren JH, Baldo L, Clark ME. *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol. 2008;6:741–51.
4. Flores HA, O'Neill SL. Controlling vector-borne diseases by releasing modified mosquitoes. Nat Rev Microbiol. 2018;16:508–18.
5. Duron O, Bernard C, Unal S, Berthomieu A, Berticat C, Weill M. Tracking factors modulating cytoplasmic incompatibilities in the mosquito *Culex pipiens*. Mol Ecol. 2006;15:3061–71.
6. Bonneau M, Atyame C, Beji M, Justy F, Cohen-Gonsaud M, Sicard M, et al. *Culex pipiens* crossing type diversity is governed by an amplified and polymorphic operon of *Wolbachia*. Nat Commun. 2018;9:1491.
7. Atyame C, Labbé P, Dumas E, Milesi P, Charlat S, Fort P, et al. *Wolbachia* divergence and the evolution of cytoplasmic incompatibility in *Culex pipiens*. PLoS ONE. 2014;9:e87336.
8. Kittayapong P, Chansang C, Chansang U, Mongkalangoon P, Ninphanomchai S, Limohpasmanee W. Combined sterile insect technique and incompatible insect technique: The first proof-of-concept to suppress *Aedes aegypti* vector populations in semi-rural settings in Thailand. PLoS Negl Trop Dis. 2019;13:e0007771.
9. van den Hurk AF, Hall-Mendelin S, Pyke AT, Frentiu FD, McElroy K, Day A, et al. Impact of *Wolbachia* on Infection with Chikungunya and Yellow Fever Viruses in the Mosquito Vector *Aedes aegypti*. PLoS Negl Trop Dis. 2012;6:e1892.
10. Hughes GL, Koga R, Xue P, Fukatsu T, Rasgon JL. *Wolbachia* infections are virulent and inhibit the human malaria parasite *Plasmodium falciparum* in *Anopheles gambiae*. PLoS Pathog. 2011;7:e1002043.
11. Bian G, Joshi D, Dong Y, Lu P, Zhou G, Pan X, et al. *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. Science. 2013;340:748–51.
12. Atyame CM, Pasteur N, Dumas E, Tortosa P, Tantely ML, Pocquet N, et al. Cytoplasmic incompatibility as a means of controlling *Culex pipiens quinquefasciatus* mosquito in the islands of the south-western indian ocean. PLoS Negl Trop Dis. 2011;5:e1440.
13. Atyame CM, Cattel J, Lebon C, Flores O, Dehecq JS, Weill M, et al. *Wolbachia*-based population control strategy targeting *Culex*

quinquefasciatus mosquitoes proves efficient under semi-field conditions. PLoS ONE. 2015;10:e0119288.
14. Ryan PA, Turley AP, Wilson G, Hurst TP, Retzki K, Brown-Kenyon J, et al. Establishment of *w*Mel *Wolbachia* in *Aedes aegypti* mosquitoes and reduction of local dengue transmission in Cairns and surrounding locations in northern Queensland. Australia Gates Open Res. 2020;3:1547.
15. Nazni WA, Hoffmann AA, NoorAfizah A, Cheong YL, Mancini MV, Golding N, et al. Establishment of *Wolbachia* Strain *w*AlbB in Malaysian Populations of *Aedes aegypti* for Dengue Control. Curr Biol. 2019;29:4241-4248.e5.
16. Alves J, Gomes B, Rodrigues R, Silva J, Arez AP, Pinto J, et al. Mosquito fauna on the Cape Verde islands (West Africa): an update on species distribution and a new finding. J Vector Ecol. 2010;35:307–12.
17. Salgueiro P, Serrano C, Gomes B, Alves J, Sousa CA, Abecasis A, et al. Phylogeography and invasion history of *Aedes aegypti*, the Dengue and Zika mosquito vector in Cape Verde islands (West Africa). Evol Appl. 2019;12:1797–811.
18. Faye O, de Lourdes MM, Vrancken B, Prot M, Lequime S, Diarra M, et al. Genomic epidemiology of 2015–2016 Zika virus outbreak in Cape Verde. Emerg Infect Dis. 2020;26:1084–90.
19. DePina AdJ, Dia AK, de Ascenção A, Martins S, Ferreira MC, Moreira AL, et al. Knowledge, attitudes and practices about malaria in Cabo Verde: a country in the pre-elimination context. BMC Public Health. 2019;19:850.
20. Ribeiro H, Ramos HC. Guia ilustrado para a identificação dos mosquitos de Angola. 4th ed. Lisboa: Soc. Portuguesa Entomologia; 1995.
21. Weeks AR, van Opijnen T, Breeuwer JAJ. AFLP fingerprinting for assessing intraspecific variation and genome mapping in mites. Exp Appl Acarol. 2000;24:775–93.
22. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am J Trop Med Hyg. 1993;49:520–9.
23. Smith JL, Fonseca DM. Rapid assays for identification of members of the *Culex (Culex) pipiens* complex, their hybrids, and other sibling species (*Diptera: Culicidae*). Am J Trop Med Hyg. 2004;70:339–45.
24. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994;3:294–9.
25. Zhou W, Rousset F, O'Neill S. Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. Proc R Soc B Biol Sci. 1998;265:509–15.
26. Baldo L, Hotopp JCD, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, et al. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Appl Environ Microbiol. 2006;72:7098–110.
27. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST. org website and their applications. Wellcome Open Res. 2018;3:1–20.
28. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38:3022–7.
29. Altinli M, Gunay F, Alten B, Weill M, Sicard M. *Wolbachia* diversity and cytoplasmic incompatibility patterns in *Culex pipiens* populations in Turkey. Parasit Vectors. 2018;11:1–9.
30. Dumas E, Atyame CM, Milesi P, Fonseca DM, Shaikevich EV, Unal S, et al. Population structure of *Wolbachia* and cytoplasmic introgression in a complex of mosquito species. BMC Evol Biol. 2013;13:181.
31. Sicard M, Namias A, Perriat-Sanguinet M, Carron E, Unal S, Altinli M, et al. Cytoplasmic incompatibility variations in relation with *Wolbachia cid* genes divergence in *Culex pipiens*. mBio. 2021;12:e02797-20.
32. Duron O, Boureux A, Echaubard P, Berthomieu A, Berticat C, Fort P, et al. Variability and expression of ankyrin domain genes in *Wolbachia* variants infecting the mosquito *Culex pipiens*. J Bacteriol. 2007;189:4442–8.
33. Atyame C, Delsuc F, Pasteur N, Weill M, Duron O. Diversification of *Wolbachia* endosymbiont in the *Culex pipiens* mosquito. Mol Biol Evol. 2011;28:2761–72.
34. Gomes B, Alves J, Sousa CA, Santa-Ana M, Vieira I, Silva TL, et al. Hybridization and population structure of the *Culex pipiens* complex in the islands of Macaronesia. Ecol Evol. 2012;2:1889–902.
35. Goindin D, Cannet A, Delannay C, Ramdini C, Gustave J, Atyame C, et al. Screening of natural *Wolbachia* infection in *Aedes aegypti*, *Aedes taeniorhynchus* and *Culex quinquefasciatus* from Guadeloupe (French West Indies). Acta Trop. 2018;185:314–7.
36. Ross PA, Callahan AG, Yang Q, Jasper M, Arif MAK, Afizah AN, et al. An elusive endosymbiont: does *Wolbachia* occur naturally in *Aedes aegypti*? Ecol Evol. 2020;10:1581–91.
37. Gloria-Soria A, Chiodo TG, Powell JR. Lack of evidence for natural *Wolbachia* infections in *Aedes aegypti* (*Diptera: Culicidae*). J Med Entomol. 2018;55:1354–6.
38. Torres R, Hernandez E, Flores V, Ramirez JL, Joyce AL. *Wolbachia* in mosquitoes from the Central Valley of California, USA. Parasit Vectors. 2020;13:558.
39. Kulkarni A, Yu W, Jiang J, Sanchez C, Karna AK, Martinez KJL, et al. *Wolbachia* pipientis occurs in *Aedes aegypti* populations in New Mexico and Florida, USA. Ecol Evol. 2019;9:6148–56.
40. Teo CHJ, Lim PKC, Voon K, Mak JW. Detection of dengue viruses and *Wolbachia* in *Aedes aegypti* and *Aedes albopictus* larvae from four urban localities in Kuala Lumpur. Malaysia Trop Biomed. 2017;34:583–97.
41. Chrostek E, Gerth M. Is *Anopheles gambiae* a natural host of *wolbachia*? MBio. 2019;10:1–10.
42. Sawadogo SP, Kabore DA, Tibiri EB, Hughes A, Gnankine O, Quek S, et al. Lack of robust evidence for a *Wolbachia* infection in *Anopheles gambiae* from Burkina Faso. Med Vet Entomol. 2022;36:301–8.
43. Gomes FM, Hixson BL, Tyner MDW, Ramirez JL, Canepa GE, Alves Silva TL, et al. Effect of naturally occurring Wolbachia in *Anopheles gambiae s.l* mosquitoes from Mali on Plasmodium falciparum malaria transmission. Proc Natl Acad Sci USA. 2017;114:12566–71.
44. Jeffries CL, Lawrence GG, Golovko G, Kristan M, Orsborne J, Spence K, et al. Novel *Wolbachia* strains in *Anopheles* malaria vectors from Sub-Saharan Africa. Wellcome Open Res. 2018;3:113.
45. Shaw WR, Marcenac P, Childs LM, Buckee CO, Baldini F, Sawadogo SP, et al. *Wolbachia* infections in natural *Anopheles* populations affect egg laying and negatively correlate with *Plasmodium* development. Nat Commun. 2016;7:11772.
46. Duarte EH. The haematophagous arthropods (*Animalia*: *Arthropoda*) of the Cape Verde Islands: a review. Zool Caboverdiana. 2014;4:31–42.
47. Correia W, Varela I, Spencer H, Alves J, Heleno DE. Characterization of mosquito breeding sites in the Cape Verde islands with emphasis on major vectors. Int J Mosq Res. 2015;2:192–9.
48. Bandi C, Anderson TJC, Genchi C, Blaxter ML. Phylogeny of *Wolbachia* in filarial nematodes. Proc R Soc B Biol Sci. 1998;265:2407–13.

Publisher's Note