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RESEARCH ARTICLE

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Another tool in the toolbox: Aphid-specific *Wolbachia* protect against fungal pathogens

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Abstract

Aphids harbor nine common facultative symbionts, most mediating one or more ecological interactions. *Wolbachia pipientis*, well-studied in other arthropods, remains poorly characterized in aphids. In *Pentalonia nigronervosa* and *P. caladii*, global pests of banana, *Wolbachia* was initially hypothesized to function as a co-obligate nutritional symbiont alongside the traditional obligate *Buchnera*. However, genomic analyses failed to support this role. Our sampling across numerous populations revealed that more than 80% of *Pentalonia* aphids carried an M-supergroup strain of *Wolbachia* (*wPni*). The lack of fixation further supports a facultative status for *Wolbachia*, while high infection frequencies in these entirely asexual aphids strongly suggest *Wolbachia* confers net fitness benefits. Finding no correlation between *Wolbachia* presence and food plant use, we challenged *Wolbachia*-infected aphids with common natural enemies. Bioassays revealed that *Wolbachia* conferred significant protection against a specialized fungal pathogen (*Pandora neoaphidis*) but not against generalist pathogens or parasitoids. *Wolbachia* also improved aphid fitness in the absence of enemy challenge. Thus, we identified the first clear benefits for aphid-associated *Wolbachia* and M-supergroup strains specifically. Aphid-*Wolbachia* systems provide unique opportunities to merge key models of symbiosis to better understand infection dynamics and mechanisms underpinning symbiont-mediated phenotypes.

INTRODUCTION

Insects and other invertebrate animals frequently engage in heritable symbiosis with microbes (Douglas, 2015; Moran et al., 2008). Heritable symbionts are primarily transmitted maternally and spread within host populations by providing net fitness benefits or manipulating host reproduction to increase the proportion of infected matrilines at the expense of uninfected ones (Oliver et al., 2014; Pietri et al., 2016; Werren et al., 2008; Zug & Hammerstein, 2015). The most prevalent, taxonomically widespread, and versatile heritable symbiont on the planet is *Wolbachia pipientis* (Rickettsiales; α-Proteobacteria) (Landmann, 2019; Weinert et al., 2015; Zindel et al., 2011; Zug & Hammerstein, 2012). As a facultative symbiont, *Wolbachia* has been given the moniker 'master manipulator' as the only symbiont known to induce all four reproductive manipulations: parthenogenesis induction, cytoplasmic incompatibility, male-killing and feminization (Kaur et al., 2021; Werren et al., 2008). Facultative *Wolbachia* are also associated with providing hosts with resistance to a wide range of pathogens, including viruses, bacteria, eukaryotic microbes, and even nematodes (Correa & Ballard, 2016; Sanda, 2016; Saridaki & Bourtzis, 2010; Zug & Hammerstein, 2015). For instance, *Wolbachia*

infecting *Drosophila* protects a range of pathogens in native and novel dipteran hosts (Hamilton & Perlman, 2013; Hedges et al., 2008; Kaur et al., 2021; Pimentel et al., 2021). In some cases, *Wolbachia* has become essential for host development (Dedeine et al., 2001; Langworthy et al., 2000), including as the B vitamin-provisioning obligate symbiont of blood-feeding *Cimex* bedbugs (Hosokawa et al., 2010). It is further hypothesized that facultative *Wolbachia* strains may have more widespread roles as nutritional mutualists (Newton & Rice, 2020).

Wolbachia are grouped into at least 17 supergroups (A-F and H-R) (Kaur et al., 2021). Some supergroups are poorly characterized, indicating that novel roles for this symbiont are likely to be discovered. For example, *Wolbachia* appears common in aphids (Hemiptera: Aphididae) (Augustinos et al., 2011; Chen et al., 2019; Gauthier et al., 2015; Guo et al., 2019; Ren et al., 2020; Romanov et al., 2020; Wang et al., 2014; Zytynska & Weisser, 2016), which are important models for the study of symbiont-conferred phenotypes (Guo et al., 2017; Oliver et al., 2010). Yet, little is known about the roles of *Wolbachia* in this economically significant group. Moreover, *Wolbachia* strains from the M and N supergroups appear restricted to aphids, but little is known about their biology (Moreira et al., 2019; Romanov et al., 2020).

Wolbachia has been reported in two congeneric species of the banana aphid, *Pentalonia nigronervosa* and *P. caladii* (De Clerck et al., 2014; Jones et al., 2011), which vector *Banana bunchy top virus* (Nanoviridae); a significant disease of banana and other musaceous plants (Hu et al., 1996; Kumar et al., 2015; Qazi, 2016). These aphids are entirely asexual, indicating *Wolbachia* persistence likely results from mutualism rather than reproductive parasitism. As exclusive phloem feeders, these aphids require the obligate bacterial symbiont *Buchnera aphidicola* to provide nutrients absent in their diet (Moran et al., 2008). One study hypothesized that *Wolbachia* has developed into a nutrient-provisioning co-obligate symbiont, supporting decayed *Buchnera* function in *P. nigronervosa* (De Clerck et al., 2015). However, a genomic reanalysis found little support for this hypothesis (Manzano-Marín, 2020), leaving open the role of *Wolbachia* in *Pentalonia* aphids.

Here we used a multi-pronged approach to better understand the roles of *Wolbachia* in *Pentalonia* aphids. 16S rRNA amplicon sequencing and PCR-based diagnostics revealed that a specific strain (*wPni*) of M-supergroup *Wolbachia* was the only common facultative endosymbiont inhabiting these aphids. While infection frequencies of *wPni* were high across numerous populations, they were not fixed, further indicating that *Wolbachia* is unlikely to serve as a co-obligate symbiont in this system. We next created experimental lines of *P. nigronervosa* with and without *wPni* finding that *Wolbachia* conferred protection against the specialized fungal pathogen *Pandora neoaphidis*, while not protecting against the generalist fungal pathogen *Beauveria bassiana* or the parasitoid *Aphidius colemani*. *Wolbachia* also improved aphid fitness in the absence of enemy challenge. This study identifies fitness benefits of M-supergroup *Wolbachia* infection in aphids, including protective services, that allow for its spread and persistence in natural populations.

EXPERIMENTAL PROCEDURES

Specimen collection and species identification

A total of 289 field colonies of *Pentalonia* aphids were sampled across the main Hawaiian Islands and Gainesville, Florida between 2011 and 2017 (Table A1) from four food plants (banana, heliconia, taro, ginger). Wingless individuals occupying a colony cluster were sampled to maximize the likelihood of collecting clonally produced descendants. Aphids were placed into 1.5 mL tubes containing 95% ethanol and stored at −20°C until further use. Additional samples of *P. nigronervosa* collected on banana plants from Australia, Guam, and India were also obtained through collaborative efforts (see acknowledgments).

Given the morphological similarity of the two *Pentalonia* species, we amplified a diagnostic portion of the Cytochrome Oxidase subunit I (COI) gene to confirm species identity (accession numbers PQ375117-20). Total DNA was extracted from individual, field-collected adult aphids using a slightly modified Cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1990). Aphids were macerated in 200 μL of CTAB buffer and incubated at 65°C for ≥1 h, then an equal volume of chloroform was mixed in and samples were centrifuged at 16,000*g* for 15 min. The supernatant was transferred to new 1.5 mL centrifuge tubes and an equal volume of cold isopropanol was mixed in gently by inverting the tubes several times. After incubation at −20°C overnight, samples were thawed, then centrifuged for 20 min at 16,000*g* to pellet DNA. DNA was washed twice with 70% ethanol then a third time with 95% ethanol and allowed to air dry for ~4 h. Before rehydration in low EDTA TE buffer (Tris-EDTA; 10 mM Tris, 0.1 mM EDTA, pH 8.0). This DNA template was used in PCR reactions to amplify a portion of the COI gene using primers LepF1 and LepR1 following the protocol described in Foottit et al. (2010). We then Sanger sequenced this amplicon in both directions and distinguished the two *Pentalonia* species by comparing sequences of our field-collected aphids to reference sequences (Foottit et al., 2010) using a Kimura 2 parameter model and visualized with a neighbor-joining tree using MEGA 5.0 software (Tamura et al., 2011).

Assessment of the *Pentalonia* endosymbiont community using 16S rRNA amplicon sequencing

To identify the full suite of endosymbionts present in *P. nigronervosa* and *P. caladii*, we conducted 16S rRNA amplicon sequencing. Total DNA was extracted from 12 samples using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's protocols. Each sample consisted of 30 randomly chosen, field-collected aphids. We surveyed each *Pentalonia* species from the five Hawaiian Islands, as well as *P. nigronervosa* from Australia and Guam. Extracted DNA was used as a template in PCR to target/amplify the hypervariable V3f (5′-CCTACGGGAGGCAGCAG-3′) and V4r (5′-GGACTACHVGGGTWTCTAAT-3′) regions of the bacterial 16S rRNA gene. Initial PCR amplifications were carried out in a 30 μL mixture that included 5 μL of KAPA HiFi Fidelity Buffer (New England Biolabs, UK), 0.3 μM of forward and reverse primers, 0.3 mM each dNTPs, 0.5 U of KAPA HiFi HotStart DNA Polymerase, 10 ng of template DNA and nuclease-free water up to 30 μL. The PCR conditions were 98°C for 1 min (one cycle), 98°C for 20 s, 55°C for 15 s and 72°C for 45 s (30 cycles), followed by 72°C for 7 min. The amplified products were checked by electrophoresis in 1% agarose gel, purified using the Cycle Pure Kit (Omega Bio-Tek, USA), then quantified using a NanoDrop (Thermo Scientific, USA) spectrophotometer. We used a dual-index, paired read approach to sequence amplicons using the MiSeq 2 × 250 platform (Illumina, Inc. USA) at the University of Georgia Genomics and Bioinformatic Core (Athens, GA) using a previously described protocol (Caporaso et al., 2011; Kozich et al., 2013).

Paired-end raw sequences were demultiplexed and merged using PEAR v1.2.7 (Zhang et al., 2013), then denoised and truncated (250 bp forward; 190 reverse) in QIIME2 (Bolyen et al., 2019). Singletons and chimeras were discarded using the DADA2 plugin (Callahan et al., 2016). The remaining sequences (mean 5394 reads/sample) were clustered, with each unique sequence defined as an amplicon sequence variant (ASV). Contaminant ASVs were removed using the prevalence-based method in 'decontam' v1.14.0 (Davis et al., 2018). Taxonomy was assigned using feature-classifier-sklearn against the Greengenes database in QIIME2 (Bokulich et al., 2018; NCBI accession PRJNA1157374).

We confirmed facultative endosymbionts detected in 16S amplicon sequencing using diagnostic PCR. For *Serratia symbiotica*, we amplified portions of *accD*, *gyrB*, and *recJ* housekeeping genes (Henry et al., 2013) and a 23S rRNA gene fragment (Thao & Baumann, 2004). PCR amplicons were Sanger sequenced bidirectionally and analyzed with BLASTn to assess similarity to known symbiont strains (Altschul et al., 1990).

Estimating *Wolbachia* infection frequency

Diagnostic PCR was used to estimate the frequency of *Wolbachia* infection across *P*. sampled *nigronervosa and P. caladii* populations. Using the CTAB method described above, total DNA was extracted from either a single adult aphid (Florida samples) or two clonal adult aphids (Hawaiian samples). DNA was successfully extracted from 261 out of 282 aphids, as confirmed by PCR amplification of the CO1 gene using the previously described method. We then amplified fragments of three housekeeping genes using *Wolbachia*-specific PCR primers: W-specF and W-specR amplified a region of 16S rRNA (Werren & Windsor, 2000); gatB_F1/gatB_R amplified a portion of the glutamyl tRNA amidotransferase (*gatB*) gene, and fbpA_F1/fbpA_R1 amplified a portion of the fructose-bisphosphate aldolase (*fbpA*) gene, (Baldo et al., 2006; http://pubmlst.org/organisms/wolbachia-spp). Amplification conditions for the 16S rRNA primers included an initial pre-denaturation at 95°C for 30 s, followed by 30 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 2 min, and ending with a final extension for 5 min at 72°C, while those for *gat*B and *fbp*A followed published protocols (Baldo et al., 2006; http://pubmlst.org/organisms/wolbachia-spp). Samples that failed to amplify COI, potentially indicating poor template quality, were not screened for *Wolbachia*. All PCRs were performed in a final reaction volume of 20 μL including 4 μL of 5X reaction buffer (Promega, USA), 1.5 μL of dNTPs (2.5 mM), 1.5 μL of forward and reverse primers (10 pmol), 1.2 μL of $MgCl_2$ (25 mM) and 0.1 μL of Taq polymerase (Promega, USA, 1U/μL), 8.7 μL H_2O, and 2 μL of DNA. PCR products were visualized on 1.2% agarose gels. 2-tailed Fisher's exact test was used to compare *Wolbachia* infection frequencies between *Pentalonia* species or collection locations using a Bonferroni correction to account for multiple comparisons. Chi-square analysis was used to compare infection frequencies between food plant types.

Characterizing *wPni* strain diversity

We also characterized *Wolbachia* strain diversity by obtaining a near full-length sequence of the 16S rRNA gene, using *Wolbachia*-specific 16S primers (see above) combined pairwise with "universal" bacterial reverse and forward primers 1507R and 10F (Moran et al., 2003; Werren & Windsor, 2000). We also sequenced the five *Wolbachia* MLST typing loci *gatB*, *coxA, hcpA*, *ftsZ* and *fbpA*. Primers and PCR

conditions for MLST loci can be found in (Baldo et al., 2006; Werren & Windsor, 2000) and PubMLST (https://pubmlst.org/organisms/wolbachia-spp). The published *ftsZ* primer failed to amplify the target, so we designed a new reverse primer (ftsZ_FMR2 5′-TTCCY-GYGCRCCTTTCATTG-3′) that amplifies a ∼500 bp fragment that covers 100% of the typing region using the same reaction conditions. PCR amplicons for all loci were Sanger sequenced in both directions. All sequences generated were deposited in the GenBank database under accession numbers KJ786944–KJ786953. MLST sequences were compared with those in the PubMLST *Wolbachia* MLST database (http://pubmlst.org/organisms/wolbachia-spp/). New allele sequences were submitted directly to the database curator and were assigned a new allele number.

Phylogenetic analysis

16S rRNA sequences representing known super groups of *Wolbachia* were obtained from GenBank (accession numbers in Table A2), and sequences for *coxA*, *hcpA*, *fbpA*, *ftsZ*, and *gatB* were downloaded from the *Wolbachia* MLST website http://pubmlst.org/organisms/wolbachia-spp/ for use in phylogenetic analyses. Alignments were conducted using MUSCLE (Edgar, 2004), then visually inspected and manually adjusted as needed in Geneious v.10 (http://www.geneious.com/).

Maximum-Likelihood analyses were performed on the 16S rRNA sequence dataset and the concatenated MLST gene using the Randomized Axelerated Maximum Likelihood (RAxML) software v7.2.6 (Stamatakis, 2014) performed on the T-REX web server (Boc et al., 2012) (http://www.trex.uqam.ca/index.php?action=inference&project=trex). We selected the GTR CAT evolutionary model for both datasets and used the default Hill-Climbing algorithm available in RAxML. To analyze the inference robustness at the nodes of the trees we performed rapid alternative runs on 200 distinct starting trees with 1000 rapid bootstrap random seed.

Bayesian analyses were performed on both 16S rRNA sequence and MLST datasets using MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001). The optimal evolutionary model for each sequence dataset selected using the Akaike Information Criterion in jModel Test v0.1.1 (Posada, 2008) resulting TVM + I + G for 16S rRNA, TVM + G for the *cox*A, GTR + I + G for the *fbp*A and the *gat*B, TIM3 + I + G for the *fts*Z, and GTR + G for the *hcp*A. Posterior probabilities were approximated by sampling the trees using a Markov Chain Monte Carlo method and model parameters were set as priors in MrBayes. We kept the default parameters provided by the software and ran six million generations for all the datasets. We discarded the first 25% of tree samples from the cold chain (burn in) when computing 50% majority-rule consensus trees.

Creating experimental aphid lines to examine the effects of *Wolbachia* infection

We attempted to establish isoclonal experimental lines with and without *wPni* to understand *Wolbachia's* role in banana aphids. While this method has been successful with other aphid species and symbionts (e.g., Higashi et al., 2020), we could not cure *P. nigronervosa* of *wPni* despite extensive efforts employing various tactics. These included antibiotic curing with variable exposure times (days) and concentrations of tetracycline or rifamycin by exposure to heat stress (≥28°C), which are commonly used to eliminate *Wolbachia* infections (Li et al., 2014). Earlier efforts to cure *wPni* were also unsuccessful (De Clerck et al., 2015). We also attempted microinjection of hemolymph from infected donors to uninfected recipients, but this resulted in high mortality of injected aphids and no successful transfers. Instead, we isolated and established eight clonal lines of *P. nigronervosa* collected from two locations (FL and HI) that varied in *Wolbachia* presence (4 *Wolbachia* infected (*wPni*+); 4 *Wolbachia-free* (*wPni*−)). To assess genetic variation, we conducted microsatellite genotyping among these eight lines, using five loci and conditions described in Galambao (2011). Based on the biology of this aphid, we expected the genotypes of aphid lines established from each locale to be highly similar, if not clonal. Samples were sent to the University of Georgia Genomics and Bioinformatic Core (Athens, GA) and University of Pennsylvania Genomic and Sequencing Core (Philadelphia, PA) for genotyping analysis on Applied Biosystems 3730xl and 3130xl DNA analyzer, respectively, using the ROX500 size standard, and analyzed using Geneious v.11 As predicted, we found few differences among amplified loci (Table A3) indicating our experimental lines shared highly similar genotypes. We also used diagnostic PCR (see above) to confirm that *wPni*+ aphids contained only *Wolbachia* (i.e., no other facultative endosymbionts) and that *wPni*− aphids harbored only *Buchnera* (i.e., no facultative symbionts). *Wolbachia* presence/absence was similarly confirmed prior to all bioassays. Colonies of experimental lines were maintained on whole banana plants in BugDorm nylon mesh cages (#4F2260) at 22 ± 1°C under natural light conditions.

Estimates of maternal transmission rates

Wolbachia inheritance was estimated using *P. nigronervosa* lines collected from Florida (PP3) and Hawaii

(Koloa). These lines were continuously maintained on banana leaf disks under laboratory conditions (20°C ± 1°C; 16 h day:8 h night light cycle). Individual offspring (in groups of 10–20 4th instar to adult aphids) produced from *Wolbachia*-infected mothers were screened for *Wolbachia* using the CTAB DNA extraction protocol and diagnostic PCR as described above. Aphids were sampled over a 6-month period, which is approximately 16 generations given time from birth to first reproduction is approximately 11 days and the average life span of *P. nigronervosa* is roughly 25 days (Higashi, Unpublished). The aphid COI gene (see above) was used as a positive control for template viability.

Evaluating *Wolbachia-mediated* protection against fungal pathogens

We were motivated to examine whether *wPni* protects banana aphids against *P. neoaphidis* because many aphid symbionts confer protection against this specialized fungal pathogen (Heyworth & Ferrari, 2015; Łukasik et al., 2013; Parker et al., 2013). To do this, *P. neoaphidis* (USDA strain #2588) previously preserved on *Acyrthosiphon pisum* cadavers were rehydrated on 1.5% agar plates (35 mm × 10 mm) in the dark at 20°C for at least 14 h to initiate sporulation. Petri dishes containing two *P. neoaphidis* sporulating cadavers were then inverted above a 35-mm petri dish containing 80 apterous 3rd–4th instar aphids from each of the eight *P. nigronervosa* lines. Aphids were exposed to *P. neoaphidis* for 2 h and fungal plates were rotated between replicates of the same treatment every ~30 min to normalize sporulation exposure of each aphid. Additionally, eight replicates ($n = 80$) of the *P. neoaphidis* susceptible *A. pisum* line, WI-48, were included as a control to ensure our enemy challenge assay had worked. After exposure, aphids were transferred in groups of ten (8 reps/line) onto healthy banana leaf disks (as previously described), and pea aphid cohorts were transferred onto fresh *Vicia faba* (fava bean) plants. Both leaf disks and plants were placed in Percival incubators at 20°C at 100% humidity for 24 h to induce sporulation.

We next determined whether *wPni* protects against the entomopathogenic generalist, *Beauveria bassiana*, a commercially available bioinsecticide used to control a variety of insect pests. *Beauveria* strain GHA (BotaniGard® 22WP) was mixed into solution using distilled water following the manufacturer's small volume rate. Individual 3rd–4th instar aphids were dipped and swirled in the *Beauveria* solution serval times for approximately 3–5 s and then immediately transferred onto a healthy banana leaf disk. A total of ten *Beauveria* exposed aphids were placed on a single healthy banana leaf disk (=1 rep) and a total of five replicates were established for each of our eight *P. nigronervosa* lines. Additionally, we exposed five replicates of 20 aphids ($n = 100$) of pea aphid line 5DAB to *Beauveria* using the method previously mentioned to ensure our infection procedure was successful.

For both fungal assays, we monitored aphids every 24 h for 10 days to assess three possible outcomes: aphid survival (aphid lives, fungus dies), fungal sporulation (fungus lives, aphid dies), and dual-mortality (both perish). Infected corpses were removed to prevent secondary fungal infections. We changed leaf disks and removed offspring as needed to minimize crowding. Comparisons within (*wPni*− or *wPni*+) and between (*wPni*− vs. *wPni*+) aphid groups were conducted using GzLM (Generalized Linear Model) with binomial distribution and logit link function (JMP Pro v.14, SAS Institute Inc., USA) to compare aphid survival, mortality and fungal sporulation. Pairwise contrasts between aphid lines were made using the contrast function after analysis with GzLM.

Evaluating *Wolbachia*-mediated protection against the parasitoid *Aphidius colemani*

Select aphid symbionts also confer protection against parasitoids (Heyworth & Ferrari, 2015; McLean et al., 2020; Oliver et al., 2003; Oliver & Higashi, 2019; Vorburger, 2014; Vorburger et al., 2010). Hence, we also examined whether *wPni* protects banana aphids against the wasp *Aphidius colemani* (Hymenoptera: Braconidae), which is a solitary parasitoid that attacks numerous aphid species, including *Pentalonia* (Ode et al., 2005; Stary, 1975; Völkl et al., 1990). Newly enclosed *A. colemani*, purchased from Beneficial Insectary© (Aphidiusforce C®), were provided honey and water and maintained in a biological incubator under 20°C and long light hours (16 light:8 dark). Eight groups of ten 3rd–4th instar aphids (8 reps/line; 80 total/line) from each of the eight *P. nigronervosa* aphid lines were placed onto separate banana leaf disks. Leaf disks were prepared by cutting a circular portion of banana leaf (~20 mm diameter) and embedding in 1% agar within a small petri dish (35 × 10mm). A single mated *A. colemani* female was allowed to parasitize each aphid once and was removed from the leaf disk once all aphids were attacked. Parasitized aphids were maintained at 24 ± 1°C and at 12 days post-parasitism, the proportion of aphid survival (aphid lives, wasp dies), mummification (wasp lives, aphid dies) and dual mortality (both perish) were recorded. A GzLM with binomial distribution and logit link function was used to compare parasitism outcomes across experimental lines (JMP Pro v.14 SAS Institute Inc., USA). Pairwise contrasts between aphid lines were made using the contrast function (likelihood ratio test) after GzLM.

Fitness effects associated with *Wolbachia* in the absence of enemy challenge

We measured aphid development time (birth to adulthood), cumulative fecundity, and longevity (measured as 50% survival) for our *wPni*− and *wPni*+ aphid lines to assess the constitutive fitness effects of harboring *Wolbachia*. Fifty to sixty adult aphids of each line were placed onto large banana leaf disks (100 mm × 15 mm) and allowed to reproduce for 24 h. Groups of 10 first-instar aphids from each line were placed onto small banana leaf disks (=1 replication). Leaf disks and aphids were maintained at 24°C ± 1°C and checked for molting every 2 days (an indication the aphid has transitioned to the next developmental stage) until adulthood. Aphids were transferred to fresh banana leaf disks approximately every 4–5 days as needed. A nonparametric Wilcoxon rank sum test was used to compare developmental times among lines with the same infection status and between *wPni*− and *wPni*+ aphids.

Cumulative fecundity (total offspring produced over the aphid lifespan) and 50% survivorship were assessed by allowing five newly developed *P. nigronervosa* adults from each of the *wPni*− and *wPni*+ lines to reproduce (=1 rep) on fresh banana leaf disks at 25°C ± 1. The number of offspring produced was counted every 2–3 days and then removed. Adult aphids were transferred to new banana leaf disks approximately every 4–5 days as needed. Aphid mortality was recorded and offspring counts continued until all aphid adults died. The fecundity data were normally distributed and compared within groups using analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) test. Fecundity between *wPni*− and *wPni*+ aphids was compared using a Student's 2-tailed *t*-test. Aphid survivorship was analyzed using a nonparametric Wilcoxon rank sum test. Aphid survival was fit to a Weibull distribution to estimate $\alpha = 50\%$ survival time.

RESULTS

Pentalonia* aphids have low endosymbiont diversity, but all examined populations harbored *Wolbachia. 16S amplicon sequencing recovered four heritable endosymbionts inhabiting *Pentalonia nigronervosa* and *P. caladii* aphids sampled across seven locations (Figure 1). As expected, the obligate symbiont *Buchnera* was present in all *Pentalonia* samples and often exhibited the greatest read abundance. *Wolbachia* was the only facultative symbiont detected across species and collection locations (Figure 1). *Serratia symbiotica* was also detected in both aphid species, but found on only three of the five island populations sampled. Diagnostic PCR corroborated the presence and distribution of *S. symbiotica* and *Arsenophonus* across our samples. We then sequenced fragments of three housekeeping genes *accD*, *gyrB*, and *recJ* from the *S. symbiotica* isolated from *Pentalonia*. BLASTn analyses (Altschul et al., 1990) of these sequences revealed 99% similarity to corresponding loci in *S. symbiotica* found in other aphid species (GenBank accession # OR283239–OR283241). The symbiont *Arsenophonus* was found only in *P. nigronervosa* collected from the island of Moloka'i. We sequenced a 500 bp portion of the 23S rRNA from this isolate (accession #OR271588). BLASTn analysis of this gene fragment revealed 99% sequence similarity to *Arsenophonus* previously isolated from honeybees (~99%) (see Table A4 for all loci and primers).

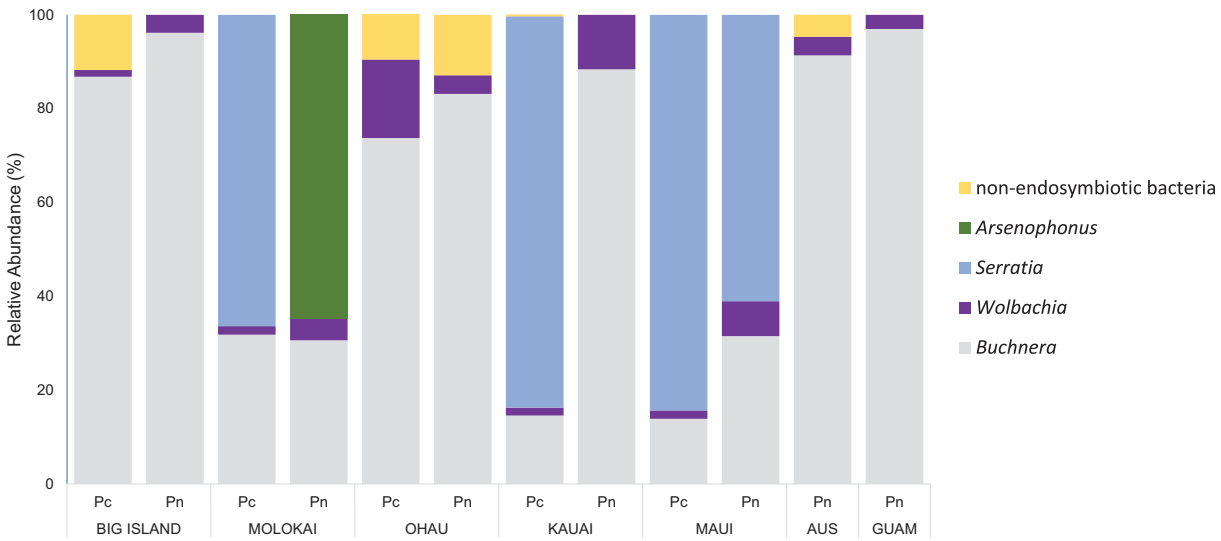

FIGURE 1 Relative read abundance (%) of endosymbiotic bacteria detected in pooled samples of *P. nigronervosa* (Pn) and *P. caladii* (Pc) collected across five Hawaiian Islands, Australia (AUS) and Guam (GU) using 16S rRNA amplicon sequencing. Non-endosymbiotic bacteria refers to lineages (4 total) in low read abundance predicted to be environmental contaminants.

***Wolbachia* infection frequencies are high but not fixed across natural populations**. Overall, we found that *Wolbachia* was present in 83% (217/261) of *Pentalonia* aphids examined across the five Hawaiian Islands. Rates of infection did not differ significantly between the two aphid species (ca 83%, 2-tailed Fisher's exact test; $P > 0.05$), nor within species across the five Hawaiian Islands (2-tailed Fisher's exact test with Bonferroni correction; $P > 0.01$; Table 1).

P. nigronervosa feeds primarily on plants in the Musaceae, including banana, while *P. caladii* feeds on plants from several families, including the Araceae (Taro, Elephant ear), Zingiberaceae (Ginger), Heliconiaceae (Heloconia), and Costaceae (Spiral Ginger) (Foottit et al., 2010; Rahmah et al., 2021). This food-plant association was observed in our field collections (Fisher's exact test; $P < 0.001$; Table A5). However, we did not observe a significant association between *Wolbachia* prevalence and food plant use (2-tailed Fisher's exact test with Bonferroni correction $P > 0.01$, Table 2).

TABLE 1 Incidence of *Wolbachia* infection across sampled *Pentalonia*.

Location	Colonies screened	Positive for *Wolbachia*	Avg. infection rate
P. nigronervosa[a]			
Kauai	23	19	82.6%
Oahu	7	6	85.7%
Maui	39	31	79.5%
Molokai	17	13	76.5%
Big Island	34	31	91.2%
Total	**120**	**100**	**83.3%**
P. caladii[b]			
Kauai	26	22	84.6%
Oahu	16	12	75.0%
Maui	44	39	88.6%
Molokai	17	10	58.8%
Big Island	38	34	89.5%
Total	**141**	**117**	**83.0%**
Overall[a]			
Kauai	49	41	83.7%
Oahu	23	18	78.3%
Maui	83	70	84.3%
Molokai	32	23	71.9%
Big Island	72	65	90.3%
Hawaii Total	**261**	**217**	**83.1%**

[a]Not significantly different by 2-tailed Fisher's exact test.
[b]Infection rates determined significantly different by 2-tailed Fisher's exact test.

A novel M-supergroup *Wolbachia* strain named *wPni* infects *P. nigronervosa* and *P. caladii* populations and is vertically transmitted at high rates. A single strain of *Wolbachia*, identical in all individuals tested ($n = 214$) across five loci, was recovered from both *Pentalonia* species sampled across the five Hawaiian Islands. This novel strain was submitted to the MLST database and assigned a new strain type (ST#402; Table 3). Sequences for *gatB*, *coxA*, *hcpA*, and *ftsZ* genes were assigned new allele numbers, while locus *fbpA* matched an existing allele #344 (Table 3). ST#402 was also found in *P. nigronervosa* from Australia and Florida. *wPni* isolates from India and Guam were designated as different strain types, ST#405 and ST#406, respectively, yet shared allelic identity with ST#402 at four of the five loci (Table 3).

Maximum likelihood phylogenies based on ~1400 bp of the16S rRNA gene showed *wPni* clustered within the M-supergroup (Figure 2), a clade that, to date, is associated only with *Wolbachia* isolated from aphids (Augustinos et al., 2011; Wang et al., 2014). An unrooted maximum likelihood phylogeny constructed from concatenated MLST sequences showed all three distinct *wPni* isolated clustered together within the M-supergroup (Figure A1).

Bioassays indicated that *Wolbachia* ST#402 was maternally transmitted at high rates in the laboratory. *P. nigronervosa* lines collected from Florida ($N = 102$ aphids) and Hawaii ($N = 104$) both exhibited 100% maternal transmission across approximately 16 generations (Table A6).

Wolbachia* provides protection against the specialized fungal pathogen *Pandora neoaphidis*, but not against the generalist *Beauveria bassiana. We observed low rates of aphid survival (10%) and high rates of fungal sporulation (46%) in our susceptible pea aphid 'positive' control line (WI-48) following exposure to the pathogen *P. neoaphidis* (Figure 3A), demonstrating that our enemy challenge assay exhibited pathogenicity levels similar to those of past experiments (Doremus & Oliver, 2017). When we challenged the focal aphid *P. nigronervosa* with the same pathogen, those that carried *Wolbachia wPni* exhibited significantly higher rates of aphid survival (ca. 24%; $\chi^2 = 45.43$, $P < 0.0001$; Figure 3A), and lower rates of fungal sporulation (ca. 27%; $\chi^2 = 51.80$, $P < 0.0001$) compared to controls lacking the symbiont (Figure 3A, Table A7). Aphid survival (GzLM; $\chi^2 = 9.65$, $df = 3$, $P = 0.022$) and fungal sporulation varied significantly (GzLM; $\chi^2 = 36.61$, $df = 3$, $P < 0.0001$) among the four *wPni*+ lines (Figure 3C), suggesting the potential for strain diversity not picked up by our strain typing method. In contrast, we found no variation in aphid survival and sporulation (GzLM, $df = 3$, $P > 0.05$; Figure 3B) among the four *wPni*− aphid lines, consistent with high clonal similarity.

TABLE 2 Incidence of *wPni* infection in *Pentalonia* aphids found on various food plants.

Plant family	Pn (# of W+/# screened)	Pc (# of W+/# screened)	Pn + Pc (# of W+/# screened[c])	%
Musaceae[a]	**90**/110	**1**/1	**91**/111	82.0
Araceae[b]	**7**/7	**11**/13	**18**/20	88.9
Zingiberaceae[b]	**2**/2	**92**/111	**94**/113	83.2
Heliconiaceae[b]	**1**/1	**8**/10	**9**/11	81.1
Costaceae[b]	**0**/0	**5**/6	**5**/6	83.3
%	**83.3**	**83.0**	**83.1**	
P value (Chi-sq)	**0.54** (2.18)	**0.99** (0.29)	**0.94** (0.79)	

Abbreviations: Pn = *P. nigronervosa*; Pc = *P. caladii*.
[a]Banana plants mainly colonized by *P. nigronervosa*.
[b]Plant hosts mainly colonized by *P. caladii*.
[c]DNA extracted from two aphids sampled from a single colony on a single host plant.

TABLE 3 *Wolbachia* MLST allele profiles for infected *Pentalonia* populations.

			***Wolbachia* allele #**					
Aphid species	**Sample origin**	**# of colonies screened**	**gatB**	**coxA**	**hcpA**	**ftsZ**	**fbpA**	**ST #**
P. nigronervosa	Hawaii	25	218	202	219	183	344	402
P. caladii	Hawaii	25	218	202	219	183	344	402
P. nigronervosa	Guam	1	164	202	219	183	344	406
P. nigronervosa	Australia	2	218	202	219	183	344	402
P. nigronervosa	India	1	218	202	219	186	344	405
P. nigronervosa	Florida	1	218	202	219	183	344	402

When we exposed *P. nigronervosa* aphids to the generalist pathogen *B. bassiana*, we observed similar rates of survival (GzLM; $\chi^2 = 0.165$, $df = 1$, $P = 0.685$) and fungal sporulation rates (GzLM; $\chi^2 = 0.724$, $df = 1$, $P = 0.395$) between lines with and without *wPni* (Figure 3D, Table A8). We also found no variation in aphid survival or fungal sporulation among examined aphid genotypes or *wPni* isolates (GzLM, $df = 3$, $P > 0.05$) (Figure 3E,F). Together, these results indicate that *wPni* has little impact on interactions with this generalist pathogen.

Wolbachia* failed to protect banana aphids against the parasitoid *A. colemani. In our parasitoid challenge assays, rates of mummification, a common proxy for successful parasitism (Oliver et al., 2012), did not vary between *P. nigronervosa* aphid lines with and without *wPni* (Figure 4; GzLM $P > 0.05$). There was a small (~5%) marginally significant (GzLM; $\chi^2 = 3.88$, $df = 1$, $P = 0.05$) increase in aphid survival following parasitism, which could indicate anti-parasitoid benefits of *Wolbachia* infection. However, unlike the anti-fungal protection, these effects were not consistent and varied significantly across both *Wolbachia*-free (Figure 4B) and *wPni*+ (Figure 4C) aphids (Table A9). Similar variation was observed in dual-mortality (both aphid and wasp perish) ($\chi^2 = 8.67$, $df = 1$, $P = 0.003$) for *wPni*+ aphids post-parasitism exposure (Figure 4B,C).

***Wolbachia* confers net fitness benefits for banana aphids in the absence of enemy challenge**. On average, *wPni*+ *P. nigronervosa* developed faster ($\chi^2 = 12.8$, $df = 1$, $P = 0.0003$), produced more offspring ($t = 1.74$, $df = 1$, $P = 0.043$) and lived significantly longer ($\chi^2 = 16.3$, $df = 1$, $P < 0.0001$; Figure A2) than *P. nigronervosa* without *wPni* (Table 4). Fitness performance varied little among the four *wPni*− *P. nigronervosa* lines except for line 'Oahu-W' which showed a small but significant increase in survival relative to the other *wPni*− lines (Table 4). We observed differences in development time and fecundity between the four *wPni*+ *P. nigronervosa* lines ($\chi^2 = 13.89$, $df = 3$, $P = 0.0031$) (Table 4). The *wPni*+ *P. nigronervosa* line 'PP3 (FL)' performed best in these metrics, while the 'Koloa' exhibited the poorest performance. Interestingly, line PP3 (FL) also provided the most effective protection against *P. neoaphidis*, whereas 'Koloa' offered the least. This pattern suggests no apparent trade-off between anti-fungal protection and fitness effects in the absence of enemy challenge.

DISCUSSION

Aphids are leading models for characterizing symbiont-conferred phenotypes (Brandt et al., 2017; Brisson & Stern, 2006; Huang & Qiao, 2014; Oliver et al., 2010). *Wolbachia* is a frequent passenger of this economically important insect group, with strains from supergroups M and N appearing restricted to aphids (Augustinos et

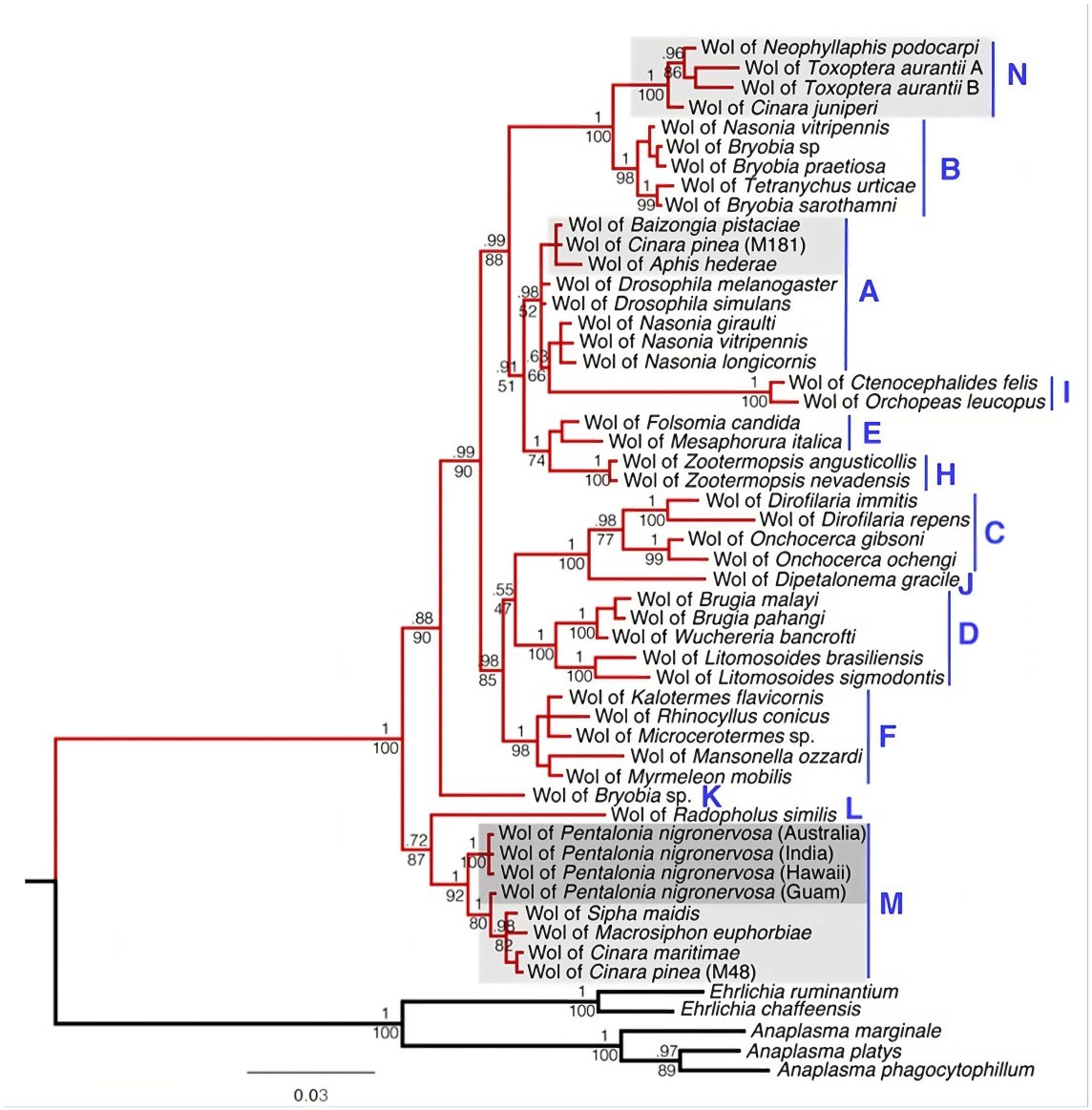

FIGURE 2 Maximum likelihood phylogeny of *Wolbachia* (red branches) based on 1400 bp of the 16S rRNA gene. The dark grey box presents isolates colonizing aphids of the genus *Pentalonia* (with strain type indicated to the right), and light grey indicating isolates from other aphid species. Two Rickettsiales, *Anaplasma* and *Ehlirichia*, were used as outgroups (black branches). Capital blue letters and the vertical blue bars indicate *Wolbachia* supergroups. Values below the nodes are bootstrap values from the Maximum Likelihood analysis (ML for the tree: −6297.33). Values above the nodes are for the posterior probabilities generated from the Bayesian analyses which shared similar topology. Refer to Table A2 for accession numbers.

al., 2011, De Clerck et al., 2014, Wang et al., 2014, Zytynska & Weisser, 2016, Chen et al., 2019, Moreira et al., 2019). However, studies elucidating the roles of aphid-associated *Wolbachia* have been conspicuously absent. In this study, we examined an M-supergroup strain of *Wolbachia* (*wPni*) carried by the entirely parthenogenetic aphids *Pentalonia*, an important pest of bananas. Our findings indicate that *wPni* provides antifungal defenses and improves host fitness in the absence of natural enemies. This study represents one of only two documented cases where this widespread symbiont confers protection against fungal pathogens in any arthropod host (Panteleev et al., 2007; Perlmutter et al., 2023). Symbiont-mediated defenses provide the potential for rapid host adaptation that may be important in natural and agricultural systems (Ives et al., 2020; Jaenike et al., 2010; Oliver et al., 2008; Smith et al., 2015).

We used 16S rRNA amplicon sequencing to uncover the endosymbiont communities of more than 360 *Pentalonia* aphids pooled across 12 populations and found that only three (*Wolbachia*, *Serratia symbiotica* and *Arsenophonus*) of the nine facultative symbionts that commonly infect aphids (Guo et al., 2017) were present in one or both *Pentalonia* species (Figure 1). This low diversity heritable microbiome contrasts

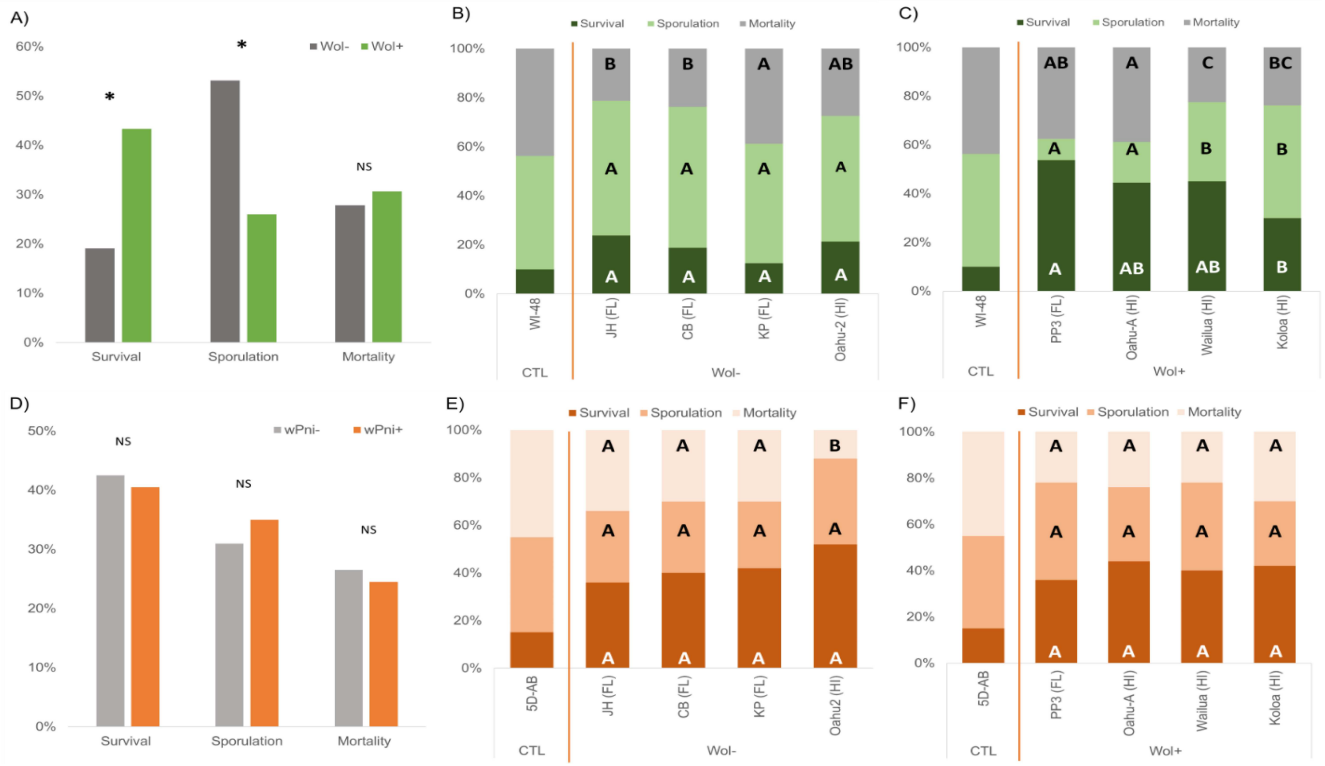

FIGURE 3 Aphid survival, fungal sporulation, and dual-mortality rates of *P. nigronervosa* aphid lines with (*wPni*+) and without (*wPni*−) 10 days post exposure to the entomopathogen *P. neoaphidis* (panels A–C) or *B. bassiana* (panels D–F). The asterisks above bars in panel A indicate a significant difference ($P \leq 0.05$), and 'NS' indicates no significant difference ($P > 0.05$). Letters in panels B, C, E, & F indicate significant differences for each of the exposure outcomes among *wPni*− (B, E) and *wPni*+ (C, F) aphids by pairwise contrasts after GzLM (NS = $P > 0.05$). Results from pea aphid control lines WI-48 (for *P. neoaphidis*) and 5DAB (*B. bassiana*) are included only as a visual reference.

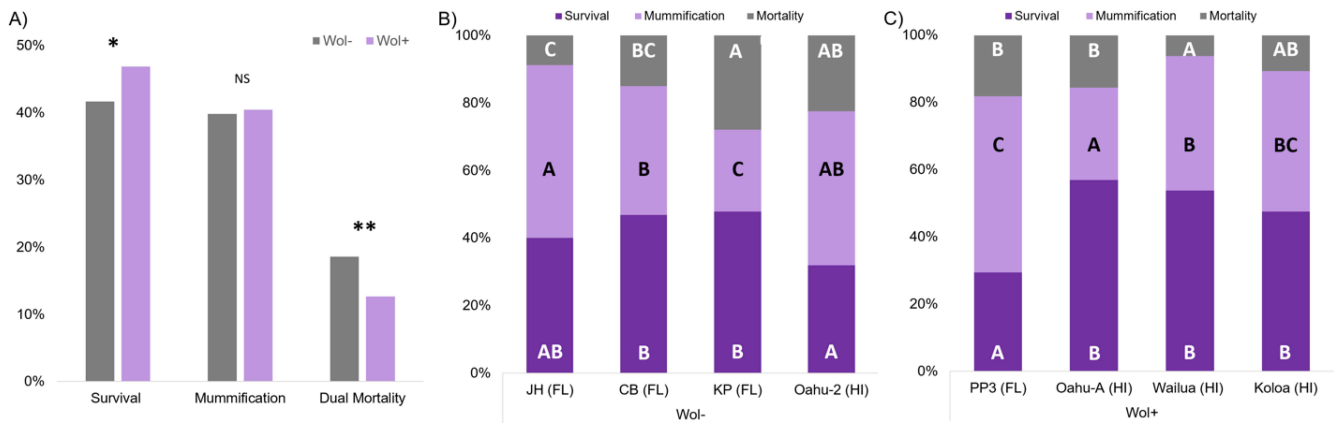

FIGURE 4 Outcomes 12 days after an enemy challenge by the parasitoid *A. colemani*. (A) % of aphids surviving, % of aphids mummifying, and dual mortality (both aphid and wasp perish) between *wPni*+ and *wPni*− lines. (NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$). Also presented are within-group comparisons of outcomes among (B) *wPni*− and (C) *wPni*+ aphids with letters indicating significant differences.

with, for instance, the seven facultative symbionts routinely found in most sampled pea aphid populations (Russell et al., 2013; Rock et al., 2018). The asexuality of *Pentalonia* may reduce opportunities for acquiring symbionts (Moran & Dunbar, 2006).

Wolbachia was the only facultative symbiont present in all populations (Figure 1). MLST and phylogenetic analysis identified a single, widespread *Wolbachia* strain (ST#402) from the M-supergroup (Table 3, Figure 2) present in all Hawaiian *P. nigronervosa and P. caladii* samples, and in limited sampling of *P. nigronervosa* from Australia and Florida. This is consistent with the widespread dispersal of *Pentalonia* aphids via the banana and ornamental plant trades

TABLE 4 Mean (±SE) development time, cumulative fecundity and aphid longevity (50% survival) for each aphid line.

Line (loc)	*wPni* +/−	Development time (h) ± SE	Cumulative fecundity offspring ± SE	50% Survival days
Within group				
JH (FL)	*wPni* (−)	278.3 ± 3.7 a	53.5 ± 3.9 a	19.7 a
CB (FL)	*wPni* (−)	271.0 ± 3.3 a	53.5 ± 11.4 a	21.1 a
KP (FL)	*wPni* (−)	273.0 ± 3.6 a	60.1 ± 14.3 a	24.2 ab
Oahu-W (HI)	*wPni* (−)	277.6 ± 2.1 a	56.4 ± 13.2 a	25.1 b
Within group				
PP3 (FL)	*wPni* +	260.1 ± 2.9 c	71.9 ± 11.2 a	24.2 a
Oahu-A (HI)	*wPni* +	265.6 ± 2.8 bc	66.0 ± 10.3 a	27.4 a
Wailua (HI)	*wPni* +	268.8 ± 3.2 ab	60.3 ± 17.3 a	27.3 a
Koloa (HI)	*wPni* +	272.5 ± 2.7 a	47.6 ± 1.8 b	22.3 a
Between groups	*wPni* (−)	275.1 ± 1.6 b	55.88 ± 2.0 b	21.8 b
	wPni +	266.5 ± 1.5 a	61.45 ± 15.8 a	25.2 a

Note: Development time was analyzed using multiple Wilcoxon Rank Sum tests. Within-group fecundity was compared using a Tukey's post hoc test. A one-tail *t*-test was used to compare *wPni*− and *wPni*+ groups ($P > t$). The letter after the value denotes a significant difference at $P < 0.05$. Survival data were fit to Weibull distribution for estimates of 50% survival. The letter after the value indicates a significant difference ($P < 0.05$) by the Wilcoxon test.

(Halbert & Baker, 2015). Similar, but distinct, *wPni* strains were found in *P. nigronervosa* from Guam (ST# 406) and India (ST# 405) indicating some strain variability is present (see below). Anholocyclic aphids, such as *Pentalonia*, are often represented by a few dominant clonal genotypes (Galambao, 2011; Harrison & Mondor, 2011), which in turn may limit the diversity of their inherited symbionts (Dykstra et al., 2014). Fitness benefits and efficient maternal transfer (100% in our lab-based assays; Table A6) drive symbiont spread, but occasional horizontal transmission via shared food plants or parasitoids may also contribute (Caspi-Fluger et al., 2012; Gehrer & Vorburger, 2012).

Wolbachia was initially hypothesized to be a co-obligate symbiont supplying specific vitamins that a decaying *Buchnera* could no longer produce (De Clerck et al., 2015). A subsequent re-analysis, however, indicated the putative missing B2 (riboflavin) genes were present (Manzano-Marín, 2020), as did our own PCR-based assays of these loci. A more conclusive nail in the coffin for the co-obligate hypothesis is that our survey clearly indicates that *Wolbachia* is a facultative symbiont in banana aphids. Infection frequencies of *Wolbachia* in *P. nigronervosa* across the Hawaiian Islands and other populations were high ($\bar{x} = 83\%$; $N = 120$; Table 1), but never fixed; a requirement for co-obligate status. We found similar infection frequencies ($\bar{x} = 83\%$; $N = 141$; Table 1) in *P. caladii*, consistent with an earlier report for this species (Jones et al., 2011) and suggestive of similar roles for *Wolbachia* in these related aphids. It is possible that *Wolbachia*-supplied nutrition is conditionally required, and may, for example, be particularly beneficial on specific food plants (Wagner et al., 2015). However, our surveys showed no association between *Wolbachia* presence and food-plant usage (Table 2).

The high infection frequencies, combined with high rates of maternal transmission in these exclusively parthenogenetic aphids strongly suggested that *Wolbachia* confers net fitness benefits. Given that the other common aphid symbionts confer protection against specialized natural enemies (Asplen et al., 2014; Heyworth & Ferrari, 2015; Higashi et al., 2023; Łukasik et al., 2013; Oliver et al., 2003; Parker et al., 2013; Parker et al., 2021; Scarborough et al., 2005; Schmid et al., 2012), we investigated whether *wPni* protects *P. nigronervosa* against two fungal entomopathogens and a common parasitoid. When challenged with the specialist fungal pathogen *P. neoaphidis*, *P. nigronervosa* aphids carrying *wPni* experienced higher rates of survival and lower fungal sporulation compared to those without the symbiont. This study is the first to identify a role for M-supergroup *Wolbachia*. In contrast, *P. nigronervosa* with and without *wPni* were equally susceptible to the generalist *B. bassiana*, highlighting the specificity of *wPni* anti-fungal defenses (Figure 3). Such specialization in anti-fungal protection is seen for other aphid symbionts, including *Regiella insecticola* (e.g., Parker et al., 2013).

While protective roles against numerous pathogens have been identified for *Wolbachia* (Brownlie & Johnson, 2009; Fenton et al., 2011; Hedges et al., 2008; Hughes et al., 2011; Teixeira et al., 2008; Zélé et al., 2012), considerably less attention has been paid to *Wolbachia* defenses against fungal pathogens, which are major insect threats (Vega et al., 2012; Wang & Wang, 2017; Ye et al., 2013). This is only report, outside of wMel (A supergroup) in *Drosophila melanogaster* demonstrating *Wolbachia*-mediated protection against fungal entomopathogens (Panteleev et al., 2007; Perlmutter et al., 2023). The discovery of the anti-fungal defenses in isolates residing in two

Wolbachia supergroups suggests that this phenotype is potentially widespread across arthropods. Given that at least six of the nine aphid facultative symbionts perform anti-fungal services this is among the most common symbiont-mediated phenotypes (Heyworth & Ferrari, 2015; Łukasik et al., 2013, Parker et al., 2013). Despite this, little is known about the mechanisms underlying anti-fungal protection. The frequent occurrence of this phenotype among diverse symbiont lineages suggests that 'immune priming' or competition for resources shared between host and symbiont may be the most likely general mechanisms, although the production of anti-fungal peptides remains a possibility (Gerardo et al., 2020).

Symbiont-based defenses often exhibit extensive phenotypic variation owing to strain-level differences (Cayetano & Vorburger, 2015; Gimmi & Vorburger, 2021; Higashi and Oliver 2019; Martinez, Weldon, & Oliver, 2014b; Mathé-Hubert et al., 2019, McLean et al., 2020; Oliver et al., 2005; Parker et al., 2017) including for *Wolbachia*-conferred phenotypes (Bordenstein & Werren, 2007; Chrostek et al., 2013; Graham et al., 2012; Hoffmann et al., 2015; Martinez et al., 2017; Martinez, Longdon, et al., 2014c). While all *Wolbachia*-carrying *P. nigronervosa* lines received protection against *Pandora* relative to symbiont-free ones in our study, line PP3 exhibited higher survival and lower sporulation than the others (Figure 3C). And the lack of variability in *Wolbachia*-free aphid clones following *Pandora* challenge, suggests that symbiont strain, or symbiont genotype × host genotype interactions are more likely than aphid genotype to contribute to this variation (Gimmi & Vorburger, 2021; Parker et al., 2017; Weldon et al., 2020). The factors responsible for phenotypic differences in protective symbioses are often on mobile elements (Chevignon et al., 2018; Lindsey et al., 2018; Oliver et al., 2009; Patel et al., 2023), and our multi-locus typing protocols would not have captured this variation. To resolve this, we plan to sequence relevant *wPni* genomes. Ultimately, our inability to manipulate *wPni* infections in the banana aphid system limits inferences about the sources of variation. While experimental manipulation among shared genotypes is the gold standard for isolating effects of symbiont infection (Oliver et al., 2010), the consistent anti-*Pandora* protection observed across multiple, highly similar aphid genotypes provides clear support for *Wolbachia*-mediated protection against specialist fungal pathogens. Nonetheless, it is possible that variation among aphid lines, including that arising from mitochondria or *Buchnera* impacted contributed to phenotypic variation. Overall, more work is needed to understand strain variation in this system and how strains interact with a presumably limited set of aphid genotypes (Parker et al., 2021; Peng et al., 2023).

While other heritable symbionts are known to protect against parasitoids, there is only one unconfirmed report of *Wolbachia* protecting weevils against parasitoids (Hsiao, 1996; Oliver & Moran, 2009). In our study, *wPni* did not protect *P. nigronervosa* against attack by the parasitoid, *A. colemani* (Figure 4), suggesting this may be an uncommon phenotype for *Wolbachia*. Several parasitoid species have been evaluated for control of natural populations of *Pentalonia* banana aphids (Völkl et al., 1990; Wellings et al., 1994), and it remains possible that *wPni* protects against other wasp species. The symbionts *Hamiltonella* and *Spiroplasma*, for example, protect against some parasitoid species but not others (Asplen et al., 2014; Cayetano & Vorburger, 2015; Hopper et al., 2018; Kraft et al., 2017; Martinez et al., 2016; McLean et al., 2020; McLean & Godfray, 2015). Symbiont-free aphids also exhibited modest endogenous defenses against *A. colemani* (Figure 4B), a phenomenon observed in other aphids (e.g., Martinez, Ritter, et al., 2014a).

Wolbachia also improved *P. nigronervosa* fitness in the absence of enemy challenge. *Pentalonia nigronervosa* infected with *wPni* were more fecund, lived longer, and developed into adults faster than those without *wPni* (Table 4). Thus, not only did *wPni* protect *P. nigronervosa* against a common enemy, but also improved host fitness under permissive conditions. The mechanism underlying improved fitness is unclear, but could involve nutritional supplementation (Newton & Rice, 2020). Previous studies indicate that protective symbionts in other aphid systems exert variable effects on hosts absent natural enemies, some imposing strong constitutive costs while others are relatively benign (Doremus & Oliver, 2017; Polin et al., 2014; Russell & Moran, 2006; Vorburger et al., 2013; Weldon et al., 2013; Weldon et al., 2020). Another facultative symbiont, *Rickettsia*, in whiteflies, can manipulate reproduction and provide protection against bacterial pathogens, but also improves fitness in the absence of enemy challenge; although the latter varies with host genotype (Cass et al., 2016; Hendry et al., 2014; Himler et al., 2011).

CONCLUSIONS

Wolbachia is the most extensively studied arthropod endosymbiont, with an ever expanding repertoire of functions that support its proliferation across diverse host populations. Our study provides additional support for *Wolbachia*-mediated defenses against fungal entomopathogens, expanding this symbiont's toolbox. The functions of *Wolbachia* remain uncharacterized for most host associations, with defensive roles likely underestimated due to their cryptic nature, only becoming apparent through targeted investigation. The *Pentalonia* aphid model with *wPni* as the single, dominant facultative symbiont, provides opportunities to merge two important models of symbiosis (aphids and *Wolbachia*) to understand the biology of each better. This

system also provides unique opportunities to expand symbiont-assisted control of agents that threaten human interests. Entomopathogenic fungi can be used as biological control agents against aphids and other pests (Goettel et al., 2005; Shah & Pell, 2003), and defensive symbionts have the potential to disrupt these efforts (Desneux et al., 2018; Ives et al., 2020; Käch et al., 2018; Nell et al., 2024; Vorburger, 2018). *Pentalonia* aphids are also economically important vectors of *Banana bunchy top virus* (BBTV), the causative agent of Banana bunchy top disease; a serious threat impacting banana production. Given that *Wolbachia* is particularly known for its anti-viral capabilities, including those associated with arthropod-vectored human diseases (Chrostek et al., 2013; Fenton et al., 2011; Hedges et al., 2008; Hoffmann et al., 2011; Moreira et al., 2009), investigating whether *wPni* impacts the transmission dynamics of BBTV may have important implications for pest management strategies and sustainable food production.

AUTHOR CONTRIBUTIONS

Clesson H. V. Higashi: Conceptualization; investigation; funding acquisition; writing – original draft; methodology; writing – review and editing; data curation; supervision; formal analysis; validation; visualization; project administration. **Vilas Patel:** Writing – review and editing; investigation; supervision; methodology; validation; formal analysis. **Bryan Kamalaker:** Investigation; writing – review and editing. **Rahul Inaganti:** Investigation. **Alberto Bressan:** Conceptualization; writing – review and editing; supervision. **Jacob A. Russell:** Writing – review and editing; conceptualization; supervision; funding acquisition. **Kerry M. Oliver:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing; methodology; project administration; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available in the Appendix and the figshare repository: https://dx.doi.org/10.6084/m9.figshare.27110728.

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APPENDIX A

TABLE A1 Sample information of *Pentalonia* aphids collected from populations across the Hawaiian Islands.

Sample ID	Region	Locality	Family	Host plant	Latitude	Longitude
1PNBI-22	Big Island	St. Benedict, Honaunau	Zingiberaceae	Red ginger	19.43496667	−155.88835
1PNBI-45	Big Island	Keaukaha Park	Musaceae	Apple Banana	19.73283333	−155.04445
1PNBI-53	Big Island	World Botanical Garden, Honomu	Musaceae	Apple Banana	19.9034	−155.1461667
1PNBI-58	Big Island	Hiiakea Garden, Keeau	Musaceae	Banana	19.60576667	−154.9536
1PNBI-73	Big Island	Keolamauloa Farm, Puuello	Musaceae	Banana	20.02808333	-155.406
1PNBI-83	Big Island	Keeau Hwy, Keeau	Musaceae	Banana	19.61791667	−155.04415
1PNH106	Big Island	Onomea Scenic Drive, Papaiko	Musaceae	Banana	19.80203333	−155.09355
1PNH108	Big Island	Onomea Scenic Drive, Papaiko	Zingiberaceae	Idianhead ginger	19.80815	−155.0953
1PNH124	Big Island	Donkey Mill Road, Kona	Musaceae	Banana	19.57231667	−155.9388667
1PNH151	Big Island	Honaunau	Musaceae	Banana	19.46273333	−155.8777833
1PNH178	Big Island	Transfer Station, Kapaau	Musaceae	Banana	20.2183	−155.82665
1PNH181	Big Island	Farmers Market, Hawi	Musaceae	Banana	20.2363	−155.8305167
1PNH212	Big Island	Alaili Rd., Pahoa	Musaceae	Banana	19.44311667	−154.9570167
1PNH229	Big Island	Lauoni St., Pahoa	Musaceae	Banana	19.4721	−154.8869333
1PNH240	Big Island	Pohiki Organic Farm, Pahoa	Musaceae	Banana	19.46643333	−154.8584
1PNH249	Big Island	Papaikou	Musaceae	Banana	19.8318	−155.0977833
1PNH261	Big Island	Waipio Rd., Waipio	Musaceae	Banana	20.11636667	−155.5792167
1PNH266	Big Island	Waipio Rd., Waipio	Musaceae	Banana	20.11603333	−155.5631333
1PNH267	Big Island	Waipio Rd., Waipio	Musaceae	Banana	20.11161667	−155.55355
1PNH268	Big Island	Waipio Rd., Waipio	Musaceae	Banana	20.1035	−155.5298167
1PNH284	Big Island	Mark Twain Rd., Na'alehu	Musaceae	Banana	19.04213333	−155.6158167
1PNH289	Big Island	Na'alehu	Musaceae	Banana	19.0634	−155.5856667
1PNH292	Big Island	Ohia St., Honoka'a	Musaceae	Banana	20.07108333	−155.4526667
1PNH295	Big Island	Pa'auhau	Musaceae	Banana	20.0812	−155.4355833
1PNH299	Big Island	Ho'omau St., Pa'auhau	Musaceae	Banana	20.08235	−155.4353667
1PNH306	Big Island	Alakahi Rd., Punalu'u	Musaceae	Banana	19.1405	−155.51695
1PNH310	Big Island	Hawaii Belt Rd., Punalu'u	Musaceae	Banana	19.15321667	−155.5082667
1PNH314	Big Island	Lower Kane Rd., Puuello	Musaceae	Banana	20.05116667	−155.3691667
1PNH317	Big Island	Kaohe Place, Puuello	Musaceae	Banana	20.03446667	−155.3599333
1PNH318	Big Island	Ke'ehia Place, Hawaii Belt Road	Musaceae	Banana	20.01601667	−155.3106333
1PNH326	Big Island	Wakea Avenue, Discovery Harbor	Musaceae	Banana	19.03285	−155.6270667
1PNH332	Big Island	Sunrise Nursery, Kailua Kona	Musaceae	Banana	19.68858333	−156.0166833
1PNH345	Big Island	Four Seasons, Kaupulehu, Waikaloa	Musaceae	Banana	19.82921667	−155.9894833
1PNH360	Big Island	Laupahoehoe Point Road, Laupahoehoe	Musaceae	Banana	19.99206667	−155.24145
1PNH381	Big Island	Puianako St., Hilo	Musaceae	Banana	19.69446667	−155.0725333
1PNH388	Big Island	Hoomana St., Hilo	Musaceae	Banana	19.71625	−155.1005833
1PNH392	Big Island	Kukuau St., Hilo	Musaceae	Banana	19.69918333	−155.1034167
1PNH396	Big Island	Waimea	Araceae	Taro	20.01713333	−155.6534
1PNH400	Big Island	Waiakea Uka, Hilo	Musaceae	Banana	20.0196	−155.6453167
1PNH413	Big Island	Ocean View, HI, Hawaii	Musaceae	Banana	19.13026	−155.839218
1PNH90	Big Island	Hwy, Kurtistown	Musaceae	Banana	19.5915	−155.0748333
1PNK108	Kauai	Kuhio Hwy, Hanalei	Musaceae	Banana	22.2035	−159.49255
1PNK110	Kauai	Hanalei Beach Park, Hanalei	Musaceae	Banana	22.21358333	−159.4962333
1PNK129	Kauai	Koolau Rd., Moloaa	Musaceae	Banana	22.1944	−159.35565
1PNK136	Kauai	Wainiha Powerhouse Rd, Hanalei	Musaceae	Banana	22.21093333	−159.5499

TABLE A1 (Continued)

Sample ID	Region	Locality	Family	Host plant	Latitude	Longitude
1PNK142	Kauai	Waipa Gardens, Waipa	Musaceae	Banana	22.20191667	−159.5118167
1PNK159	Kauai	Hauiki Rd., Kapaa	Musaceae	Banana	22.08525	−159.3509
1PNK177	Kauai	Ohiki Rd., Hanalei	Musaceae	Banana	22.19646667	−159.46905
1PNK185	Kauai	Anahulu Rd., Hanalei	Musaceae	Banana	22.22061667	−159.5483333
1PNK19	Kauai	Aukona St., Hanamaulu	Musaceae	Banana	21.99358333	−159.36605
1PNK197	Kauai	Kipu Rd., Kipu	Musaceae	Banana	21.9508	−159.4223667
1PNK213	Kauai	Omao Rd., Koloa	Musaceae	Banana	21.91831667	−159.48255
1PNK216	Kauai	Akemama, Lawai	Musaceae	Banana	21.93101667	−159.5029833
1PNK221	Kauai	Lae Rd., Kaleho	Musaceae	Banana	21.93055	−159.5262167
1PNK222	Kauai	Mehana Rd., Elelee	Musaceae	Banana	21.90631667	−159.5797333
1PNK231	Kauai	Pee Rd., Poipu	Musaceae	Banana	21.87336667	−159.4445167
1PNK271	Kauai	No Name St., (Makia) Makaweli	Musaceae	Banana	21.94213333	−159.6296667
1PNK28	Kauai	Kekaha Rd., Waimea	Musaceae	Banana	21.96221667	−159.6959833
1PNK56	Kauai	Puhi Rd., Puhi	Musaceae	Banana	21.96138333	−159.3875333
1PNK59	Kauai	Immaculate Concepcion Church, Lihue	Musaceae	Banana	21.99018333	−159.3638667
1PNK64	Kauai	Kukui Rd., Kapaa	Musaceae	Banana	22.07563333	−159.3199833
1PNK77	Kauai	Anahola Rd., Anahola	Araceae	Taro	22.1421	−159.3094167
1PNK78	Kauai	Anahola Rd., Anahola	Araceae	Black magic Taro	22.1421	−159.3094333
1PNK87	Kauai	Kaupea Rd., Kilauea	Musaceae	Banana	22.22031667	−159.4077333
1PNM104	Maui	Hana Herbs, Hana	Musaceae	Banana	20.71321667	−156.0079833
1PNM11	Maui	Aina Lani Farm, Makawao	Musaceae	Banana	20.81451667	−156.3217167
1PNM115	Maui	Aloha Cottages, Hana	Musaceae	Banana	20.7561	−155.9857
1PNM121	Maui	Hana Hwy, past Hana	Musaceae	Banana	20.67615	−156.0327167
1PNM122	Maui	Hana Hwy, past Hana	Zingiberaceae	Yellow ginger	20.6734	−156.0410333
1PNM128	Maui	Laulima Farm, Kipahulu	Musaceae	Banana	20.65171667	−156.0597167
1PNM134	Maui	Evonuk Farm, Kula	Musaceae	Banana	20.741878	−156.326407
1PNM140	Maui	Enchanting Floral Gardens, Kula	Musaceae	Banana	20.741878	−156.326407
1PNM143	Maui	Enchanting Floral Gardens, Kula	Araceae	Taro	20.79371667	−156.32585
1PNM149	Maui	Sanctuary Farms, Kamaole	Musaceae	Banana	20.69526667	−156.3848667
1PNM153	Maui	Lokelani Farm, Waihe'e	Musaceae	Banana	20.93931667	−156.5156167
1PNM158	Maui	Waihee Beach Park, Waihee	Musaceae	Banana	20.9315	−156.4987
1PNM16	Maui	Haile Maile community garden	Musaceae	Banana	20.87361667	−156.3368333
1PNM161	Maui	Iao valley park, Iao valley	Musaceae	Banana	20.88256667	−156.5356167
1PNM162	Maui	Iao valley park, Iao valley	Araceae	Xanthosoma	20.88236667	−156.5355333
1PNM17	Maui	Haile Maile community garden	Araceae	Taro	20.8737	−156.3369667
1PNM178	Maui	Park, Wailea	Musaceae	Banana	20.7047	−156.4374167
1PNM182	Maui	Resort Management Group, Kihei	Musaceae	Banana	20.7875	−156.4641667
1PNM191	Maui	Mokuhau park, Happy Valley	Musaceae	Banana	20.88871667	−156.5099
1PNM200	Maui	DT Fleming Beach Park, Honokahua	Musaceae	Banana	21.00321667	−156.6513833
1PNM202	Maui	Akoni Place, Paia	Musaceae	Banana	20.91555	−156.3806333
1PNM204	Maui	Poho Place, Paia	Musaceae	Banana	20.9291	−156.3667
1PNM214	Maui	Paia Garden, Paia	Musaceae	Banana	20.91491667	−156.3824667
1PNM233	Maui	St. Anne Catholic Church, Waihee	Musaceae	Banana	20.93028333	−156.5118833
1PNM234	Maui	Malaihi Rd., Waihu	Musaceae	Banana	20.92006667	−156.49895
1PNM32	Maui	Hoolawa Garden, Huelo	Musaceae	Banana	20.93123333	−156.3179833
1PNM35	Maui	Maui Community College ag farm	Musaceae	Banana	20.88931667	−156.4749833

(Continues)

TABLE A1 (Continued)

Sample ID	Region	Locality	Family	Host plant	Latitude	Longitude
1PNM37	Maui	Kula Ag Park experimental substation	Musaceae	Banana	20.79513333	−156.3596667
1PNM43	Maui	Kapalua Farms, Lahaina	Musaceae	Banana	20.99156667	−156.6635333
1PNM45	Maui	Kapalua Farms, Lahaina	Araceae	Taro	20.99126667	−156.6635167
1PNM54	Maui	Honokahau Rd., Kahikeli Hwy	Musaceae	Banana	21.01551667	−156.6072667
1PNM56	Maui	Kahikeli Hwy	Musaceae	Banana	20.97603333	−156.5338833
1PNM61	Maui	Honopiilani Hwy	Musaceae	Banana	20.8121	−156.6231
1PNM71	Maui	Theo's Farm, Lahaina	Musaceae	Banana	20.84773333	−156.6393333
1PNM75	Maui	DikensonS, Lahaina	Musaceae	Banana	20.87675	−156.6754667
1PNM80	Maui	Luau, Lahaina	Musaceae	Banana	20.88548333	−156.6855
1PNM86	Maui	Road to Hana	Musaceae	Banana	20.92321667	−156.2858333
1PNM92	Maui	Keanae Rd., Keanae	Musaceae	Banana	20.8641	−156.1440667
1PNM95	Maui	Hamoa Rd., Hana	Musaceae	Banana	20.721	−155.9855167
1PNM96	Maui	Hana Hwy, past Hana	Musaceae	Banana	20.70515	−155.9965667
1PNF11	Molokai	Halawa Valley Farm, Halawa	Musaceae	Banana	21.157339	−156.737976
1PNF20	Molokai	Wailea Perma Farm, Wailea	Araceae	Taro	21.13516	−157.01033
1PNF22	Molokai	Wailea Perma Farm, Wailea	Musaceae	Banana	21.13516	−157.01033
1PNF25	Molokai	Phyllis White, Kalae	Musaceae	Banana	21.161631	−157.005707
1PNF26	Molokai	Kumu Farms, Hoolehua	Musaceae	Banana	21.16935	−157.076447
1PNF32	Molokai	Hui Ho Farm, Kipu	Musaceae	Banana	21.16061	−157.022263
1PNF40	Molokai	Puunana St., Maunaloa	Musaceae	Banana	21.134145	−157.212054
1PNF42	Molokai	Puunana St., Maunaloa	Musaceae	Banana	21.134145	−157.212054
1PNF43	Molokai	Bills Farm, Hoolehua	Musaceae	Banana	21.16935	−157.076447
1PNF44	Molokai	Perma Farm, Hoolehua	Musaceae	Banana	21.16935	−157.076447
1PNF51	Molokai	Molokai Shores, Kamiloloa	Musaceae	Banana	21.080999	−156.999863
1PNF65	Molokai	Elementary School, Kaluaaha	Musaceae	Banana	21.058451	−156.834885
1PNF72	Molokai	Makaena St., Kaunakakai	Musaceae	Banana	21.094245	−157.025154
1PNF73	Molokai	Kahiwa St., Kaunakakai	Musaceae	Banana	21.100285	−157.036261
1PNF77	Molokai	Molokai Middle School, Kualapuu	Heliconiaceae	Heliconia sp.	21.151911	−157.036606
1PNF8	Molokai	PuuO Hoku Ranch, Halawa	Musaceae	Banana	21.157339	−156.737976
1PNF86	Molokai	USDA, Hoolehua	Musaceae	Banana	21.16935	−157.076447
1PNO1	Oahu	Nenue St., Wailupe	Musaceae	Banana	21.28091667	−157.7531333
1PNO105	Oahu	Iliani St., Kailua bay	Musaceae	Banana	21.4163	−157.7515
1PNO12	Oahu	Liliukolani Park, Honolulu	Musaceae	Banana	21.3298	−157.8663833
1PNO13	Oahu	Liliukolani Park, Honolulu	Musaceae	Banana	21.3307	−157.86605
1PNO24	Oahu	Manoa Heritage Garden, Honolulu	Musaceae	Banana	21.31188333	−157.8145167
1PNO36	Oahu	Au St., Waialua	Musaceae	Banana	21.58158333	−158.1395167
1PNO37	Oahu	Au St., Waialua	Musaceae	Banana	21.58158333	−158.1395
1PNO46	Oahu	Urban Garden Center, Pearl City	Musaceae	Banana	21.39286667	−157.9740333
1PNO71	Oahu	Olamana Farm, Waimanalo	Musaceae	Banana	21.34136667	−157.7447833
1PNO82	Oahu	Helemano Plantation, Wahiawa	Musaceae	Banana	21.52768333	−158.0392167
1PNO83	Oahu	Helemano Plantation, Wahiawa	Musaceae	Banana	21.52796667	−158.0391
1PNPaf1	Oahu	Aloun Farm	Musaceae	Banana	21.36225	−158.0551099
1PNPoa1	Oahu	Poamoho Research Station	Musaceae	Banana	21.483601	−157.964783
2PNBI-35	Big Island	Waiakea Uka, Hilo	Zingiberaceae	Red ginger	19.6659	−155.1052333
2PNBI-38	Big Island	Banyan Drive, Hilo	Heliconiaceae	Heliconia sp.	19.72716667	−155.06335
2PNBI-49	Big Island	World Botanical Garden, Honomu	Zingiberaceae	Pink ginger	19.90365	−155.13675
2PNBI-51	Big Island	World Botanical Garden, Honomu	Zingiberaceae	Yellow ginger	19.90333333	−155.1353167

TABLE A1 (Continued)

Sample ID	Region	Locality	Family	Host plant	Latitude	Longitude
2PNBI-56	Big Island	Hiiakea Garden, Keeau	Zingiberaceae	Pink ginger	19.60576667	−154.9536
2PNBI-74	Big Island	Hilo Industrial Park, Hilo	Zingiberaceae	Red ginger	20.03925	−155.4392
2PNBI-77	Big Island	Keeau Loop, Keeau	Zingiberaceae	Pink ginger	19.62736667	−155.03795
2PNBI-84	Big Island	Keeau Hwy, Keeau	Zingiberaceae	Pink ginger	19.61791667	−155.04415
2PNBI-86	Big Island	Keeau Hwy, Keeau	Zingiberaceae	Idianhead ginger	19.5806	−155.0119667
2PNBI-89	Big Island	Hwy, Kurtistown	Zingiberaceae	Red ginger	19.5992	−155.0543
2PNH100	Big Island	Prince Kuhio Plaza, Hilo	Zingiberaceae	Red ginger	19.69785	−155.0649833
2PNH112	Big Island	Greenwell Farms, Kealakekua	Zingiberaceae	Red ginger	19.5113	−155.92335
2PNH127	Big Island	Kailua Kona	Zingiberaceae	Red ginger	19.62443333	−155.942
2PNH136	Big Island	Chez Marquiz Farm, Kona	Zingiberaceae	Red ginger	19.6618	−155.9436
2PNH183	Big Island	Farmers Market, Hawi	Zingiberaceae	Red ginger	20.2372	−155.8309833
2PNH203	Big Island	Pahoa Hwy, Pahoa	Zingiberaceae	White ginger	19.44941667	−154.9435667
2PNH214	Big Island	Alaili Rd., Pahoa	Zingiberaceae	White ginger	19.44253333	−154.9560167
2PNH223	Big Island	Vernas, Kalapana	Zingiberaceae	Red ginger	19.4882	−154.92905
2PNH225	Big Island	Lava Tree Park, Pahoa	Zingiberaceae	Pineapple ginger	19.48303333	−154.9036333
2PNH228	Big Island	Pahoa	Zingiberaceae	Pineapple ginger	19.472	−154.8869
2PNH230	Big Island	Lauoni St., Pahoa	Zingiberaceae	white/green ginger	19.47215	−154.8868333
2PNH235	Big Island	Pohiki Hwy, Pahoa	Zingiberaceae	Red ginger	19.4697	−154.8845167
2PNH250	Big Island	Papaikou	Zingiberaceae	Red ginger	19.82998333	−155.1092
2PNH265	Big Island	Kukuihaele Rd., Waipio	Zingiberaceae	Pink ginger	20.1173	−155.5758667
2PNH281	Big Island	Greensand Community, Na'alehu	Zingiberaceae	Bell ginger	19.0444	−155.61045
2PNH283	Big Island	Mark Twai Rd., Na'alehu	Araceae	Taro	19.04198333	−155.61575
2PNH300	Big Island	Ho'omau St., Pa'auhau	Araceae	Taro	20.08245	−155.4354167
2PNH309	Big Island	Alakahi Rd., Punalu'u	Zingiberaceae	Red ginger	19.1406	−155.5172167
2PNH322	Big Island	Pinao St., Waiohinu	Zingiberaceae	Red ginger	19.06738333	−155.6124833
2PNH327	Big Island	Wakea Ave., Discovery Harbor	Zingiberaceae	Pink ginger	19.03308333	−155.6271333
2PNH338	Big Island	Kona Resort Village, Kailua Kona	Zingiberaceae	Variegated ginger	19.82648333	−155.9731833
2PNH342	Big Island	Four Seasons, Kaupuluhehu, Waikaloa	Zingiberaceae	Red ginger	19.8274	−155.9923667
2PNH352	Big Island	Hawaii Belt Rd., Glenwood	Zingiberaceae	Ginger spp.	19.51186667	−155.1396333
2PNH357	Big Island	Laupahoehoe Point RD, Laupahoehoe	Zingiberaceae	Yellow ginger	19.98985	−155.2452833
2PNH358	Big Island	Laupahoehoe Point RD, Laupahoehoe	Zingiberaceae	Red ginger	19.9918	−155.2415167
2PNH361	Big Island	Laupahoehoe Point RD, Laupahoehoe	Araceae	Taro	19.98963333	−155.2439167
2PNH384	Big Island	Aalalani Place, Hilo	Zingiberaceae	Red ginger	19.68768333	−155.0832333
2PNH390	Big Island	Hele Mana St., Hilo	Zingiberaceae	Ginger	19.70388333	−155.1176333
2PNH91	Big Island	Hwy, Kurtistown	Zingiberaceae	Red ginger	19.5915	−155.0748333
2PNH94	Big Island	Kurtistown	Zingiberaceae	Kahili ginger	19.5822	−155.061
2PNH99	Big Island	Mauna Loa Macnut, Hilo	Zingiberaceae	Pink ginger	19.6573	−155.0089333
2PNK100	Kauai	Anini Rd., Princeville	Araceae	Taro	22.22583333	−159.45475
2PNK101	Kauai	Anini Rd., Princeville	Zingiberaceae	Red ginger	22.22568333	−159.4547833
2PNK107	Kauai	Kuhio Hwy, Hanalei	Zingiberaceae	Idianhead ginger	22.20348333	−159.4926333
2PNK130	Kauai	Koolau Rd., Moloaa	Zingiberaceae	Red ginger	22.19425	−159.3558833
2PNK137	Kauai	Wainiha Powerhouse Rd., Hanalei	Zingiberaceae	White ginger	22.21	−159.5488333
2PNK141	Kauai	Wainiha Powerhouse Rd., Hanalei	Araceae	Zanthosoma sp.	22.21081667	−159.5494
2PNK16	Kauai	Aukoona St., Hanamaulu	Zingiberaceae	Red ginger	21.99243333	−159.3609833

(Continues)

TABLE A1 (Continued)

Sample ID	Region	Locality	Family	Host plant	Latitude	Longitude
2PNK172	Kauai	Kolo Rd., Kilauea	Zingiberaceae	Olena	22.20343333	−159.40235
2PNK18	Kauai	Aukona St., Hanamaulu	Zingiberaceae	Variegated ginger	21.99366667	−159.3661
2PNK190	Kauai	Kuhio Hwy, Hanalei	Zingiberaceae	Red ginger	22.222451	−159.551109
2PNK194	Kauai	Grove Farm, Lihue	Zingiberaceae	Yellow ginger	21.96485	−159.3701
2PNK199	Kauai	Kipu Rd., Kipu	Zingiberaceae	Red ginger	21.95115	−159.4239833
2PNK200	Kauai	Kipu Rd., Kipu	Zingiberaceae	Idianhead ginger	21.95115	−159.424
2PNK209	Kauai	Naulu Rd., Koloa	Zingiberaceae	Red ginger	21.91695	−159.48025
2PNK22	Kauai	Jarves St., Lihue	Zingiberaceae	Red ginger	21.97835	−159.3700333
2PNK227	Kauai	Kaumualii Hwy, Puhi	Zingiberaceae	Shell ginger	21.96428333	−159.4302667
2PNK23	Kauai	Kukio Grove, Lihue	Zingiberaceae	Red ginger	21.97073333	−159.3797167
2PNK237	Kauai	Poipu Rd., Poipu	Zingiberaceae	Red ginger	21.88821667	−159.4673167
2PNK246	Kauai	Kauai Coffee Camp, Numila	Musaceae	Banana	21.89851667	−159.5572833
2PNK267	Kauai	Awawa Rd., Hanapepe	Zingiberaceae	Red ginger	21.91215	−159.5900333
2PNK268	Kauai	Kaumualii Hwy, Makaweli	Zingiberaceae	Red ginger	21.93486667	−159.6443333
2PNK274	Kauai	No Name St. (Mauka), Makaweli	Zingiberaceae	Red ginger	21.942	−159.6307667
2PNK33	Kauai	Pacific Missle Range Facility, Kekaha	Zingiberaceae	Red ginger	22.00146667	−159.7614333
2PNK35	Kauai	Waimea Canyon Road, Waimea	Zingiberaceae	Red ginger	21.96428333	−159.6636
2PNK4	Kauai	Kaulualii Rd., Lihue	Heliconiaceae	Heliconia	21.96321667	−159.4040333
2PNK55	Kauai	Puhi Rd., Puhi	Zingiberaceae	Red ginger	21.96131667	−159.3874833
2PNK58	Kauai	Immaculate Concepcion Church Lihue	Zingiberaceae	Red ginger	21.99018333	−159.36385
2PNK99	Kauai	Anini Rd., Princeville	Araceae	Dieffenbachia	22.22575	−159.4547833
2PNM103	Maui	Hana Herbs, Hana	Zingiberaceae	Red ginger	20.71343333	−156.0081333
2PNM105	Maui	Hana Herbs, Hana	Costaceae	Costus sp.	20.71313333	−156.0082167
2PNM106	Maui	Vibrant Farm, Hana	Zingiberaceae	Pink ginger	20.7093	−156.0096167
2PNM108	Maui	Helani Farms, Hana	Costaceae	Costus sp.	20.76355	−155.9989333
2PNM109	Maui	Keanae Arboretum, Keanae	Zingiberaceae	Yellow ginger	20.85506667	−156.1501667
2PNM112	Maui	Honokalani Rd., Hana	Zingiberaceae	White ginger	20.78431667	−156.0064167
2PNM114	Maui	Honokalani Rd., Hana	Zingiberaceae	Red ginger	20.78558333	−156.0042167
2PNM117	Maui	Hana Maui Botanical Garden, Hana	Zingiberaceae	Pink ginger	20.79341667	−156.0318833
2PNM127	Maui	Laulima Farm, Kipahulu	Araceae	Xanthosoma	20.65183333	−156.06
2PNM135	Maui	Evonuk Farm, Kula	Araceae	Dieffenbachia	20.80465	−156.3452833
2PNM142	Maui	Enchanting Floral Gardens, Kula	Heliconiaceae	Heliconia sp	20.79421667	−156.32625
2PNM15	Maui	Maui Farm, Kula	Zingiberaceae	Pink ginger	20.88486667	−156.3410833
2PNM152	Maui	Lokelani Farm, Waihe'e	Heliconiaceae	Heliconia sp	20.93918333	−156.5156
2PNM167	Maui	Ike Drive, Wailea	Zingiberaceae	Red ginger	20.68625	−156.4411
2PNM170	Maui	Four Seasons, Wailea	Zingiberaceae	Red ginger	20.68058333	−156.4424333
2PNM171	Maui	Four Seasons, Wailea	Zingiberaceae	White ginger	20.68056667	−156.4424167
2PNM181	Maui	Ki hana Nursery, Kihei	Costaceae	Costus sp.	20.73636667	−156.4536
2PNM189	Maui	Heritage Gardens, Iao Valley	Araceae	Taro	20.88001667	−156.5462667
2PNM192	Maui	Main St., Wailuku	Zingiberaceae	Red ginger	20.8865	−156.5048167
2PNM194	Maui	Hauli St., Maalea	Zingiberaceae	Red ginger	20.79603333	−156.5065667
2PNM198	Maui	Mcdonalds, Kaanapali	Zingiberaceae	Red ginger	20.97191667	−156.6775833
2PNM203	Maui	Baldwin Ave., Paia	Zingiberaceae	White ginger	20.91343333	−156.3788833
2PNM207	Maui	The Plant Lady Farm, Haiku	Zingiberaceae	Shell ginger	20.92723333	−156.3323
2PNM212	Maui	Puniawa Rd., Huelo	Zingiberaceae	White ginger	20.91666667	−156.2495833
2PNM213	Maui	Puniawa Rd., Huelo	Araceae	Ape	20.91665	−156.2496

TABLE A1 (Continued)

Sample ID	Region	Locality	Family	Host plant	Latitude	Longitude
2PNM216	Maui	Alakapa Place, Paia	Zingiberaceae	Red ginger	20.90646667	−156.41165
2PNM217	Maui	Sugar cane museum, Puunene	Zingiberaceae	Red ginger	20.86738333	−156.4555667
2PNM22	Maui	Yellow bamboo seed nursery, Haiku	Costaceae	Variagated costus	20.92925	−156.3105667
2PNM228	Maui	Maui Storage, Kahuluhi	Zingiberaceae	Red ginger	20.88253333	−156.4533833
2PNM235	Maui	Malaihi Rd., Waihu	Zingiberaceae	Pink ginger	20.91913333	−156.5029667
2PNM26	Maui	Yellow bamboo seed nursery, Haiku	Heliconiaceae	Heliconia sp	20.9296	−156.3105333
2PNM27	Maui	Yellow bamboo seed nursery, Haiku	Zingiberaceae	Red ginger	20.92961667	−156.3106667
2PNM28	Maui	HaleAkua Garden, Haiku	Heliconiaceae	Heliconia sp	20.9091	−156.21955
2PNM29	Maui	HaleAkua Garden, Haiku	Zingiberaceae	Red ginger	20.90911667	−156.2196167
2PNM49	Maui	Kapalua Farms, Lahaina	Zingiberaceae	Red ginger	20.87816	−156.677673
2PNM50	Maui	Lower Honopililani Hwy, Kapalua	Heliconiaceae	Heliconia sp	20.99635	−156.6644
2PNM57	Maui	Kahikeli Hwy	Zingiberaceae	Red ginger	20.97613333	−156.5341333
2PNM59	Maui	Ma Aleaea Bay, Maalaea	Zingiberaceae	Yellow ginger	20.79221667	−156.5117333
2PNM6	Maui	Maui Tropical Plantation, Wailuku	Zingiberaceae	White ginger	20.84855	−156.5073667
2PNM60	Maui	Honopiilani Hwy	Zingiberaceae	Yellow ginger	20.8115	−156.6219167
2PNM7	Maui	Maui Tropical Plantation, Wailuku	Zingiberaceae	Variagated ginger	20.84991667	−156.5068167
2PNM73	Maui	Kumu niu place, Lahaina	Zingiberaceae	Red ginger	20.85683333	−156.6378333
2PNM79	Maui	Luau, Lahaina	Zingiberaceae	Red ginger	20.88596667	−156.6849833
2PNM88	Maui	Road to Hana	Zingiberaceae	Yellow ginger	20.8721	−156.1874
2PNM89	Maui	Garden of Eden, Hana Hwy	Zingiberaceae	Red ginger	20.86855	−156.1798667
2PNM99	Maui	Hana Hwy, past Hana	Zingiberaceae	Red ginger	20.68376667	−156.0220833
2PNF12	Molokai	Halawa Valley Farm, Halawa	Zingiberaceae	Pink ginger	21.157339	−156.737976
2PNF27	Molokai	Kumu Farms, Hoolehua	Zingiberaceae	Red ginger	21.16935	−157.076447
2PNF33	Molokai	Hui Ho Farm, Kipu	Zingiberaceae	White ginger	21.16061	−157.022263
2PNF35	Molokai	Nani Kai, Kaluakoi	Zingiberaceae	Red ginger	21.13516	−157.01033
2PNF38	Molokai	Maunaloa Rd., Maunaloa	Zingiberaceae	Red ginger	21.13284	−157.214274
2PNF49	Molokai	Molokai Shores, Kamiloloa	Zingiberaceae	Red ginger	21.080999	−156.999863
2PNF54	Molokai	Molokai Shores, Kamiloloa	Araceae	Taro	21.080999	−156.999863
2PNF57	Molokai	Wavecrest, Ualapue	Zingiberaceae	Red ginger	21.06216	−156.829849
2PNF59	Molokai	Wavecrest, Ualapue	Zingiberaceae	Yellow ginger	21.06216	−156.829849
2PNF6	Molokai	Puuo Hoku Ranch, Halawa	Zingiberaceae	Pink ginger	21.157339	−156.737976
2PNF61	Molokai	Elementary School, Kaluaaha	Zingiberaceae	Red ginger	21.058451	−156.834885
2PNF63	Molokai	Elementary School, Kaluaaha	Heliconiaceae	Heliconia sp.	21.058451	−156.834885
2PNF64	Molokai	Elementary School, Kaluaaha	Heliconiaceae	Heliconia sp.	21.058451	−156.834885
2PNF75	Molokai	Lihi Pali Ave., Kualapuu	Zingiberaceae	White ginger	21.151911	−157.036606
2PNF76	Molokai	Molokai Middle School, Kualapuu	Zingiberaceae	White ginger	21.151911	−157.036606
2PNF82	Molokai	Kualapuu Highschool, Kualapuu	Zingiberaceae	Red ginger	21.15191	−157.036599
2PNF93	Molokai	Beach Boy Farm, Hoolehua	Zingiberaceae	Red ginger	21.169355	−157.076449
2PNO106	Oahu	Iliani St., Kailua Bay	Zingiberaceae	Red ginger	21.4163	−157.7515167
2PNO117	Oahu	Okana Place, Kaneohe	Costaceae	Costus sp.	21.44456667	−157.8310667
2PNO120	Oahu	Ahuimanu Place, Kahaluu	Zingiberaceae	Red ginger	21.44911667	−157.8336
2PNO19	Oahu	Kapahulu Ave., Honolulu	Zingiberaceae	Variegated ginger	21.28605	−157.81225
2PNO32	Oahu	Keeaumoku St., Honolulu	Zingiberaceae	Ginger sp.	21.29848333	−157.8393667
2PNO4	Oahu	Kahawai St., Manoa valley	Zingiberaceae	Red ginger	21.31025	−157.8118833
2PNO41	Oahu	Achiu In, Haliewa	Zingiberaceae	Red ginger	21.58576667	−158.1034167
2PNO44	Oahu	Achiu In, Haliewa	Araceae	Zanthosoma	21.58626667	−158.1029667
2PNO7	Oahu	Liliukolani Park, Honolulu	Zingiberaceae	Ginger sp.	21.31921667	−157.8561

(Continues)

TABLE A1 (Continued)

Sample ID	Region	Locality	Family	Host plant	Latitude	Longitude
2PNO73	Oahu	Olamana Farm, Waimanalo	Heliconiaceae	Orange beak heliconia	21.34111667	−157.7449333
2PNO74	Oahu	Olamana Farm, Waimanalo	Costaceae	Costus sp.	21.34115	−157.745
2PNO89	Oahu	Helemano Plantation, Wahiawa	Zingiberaceae	Red ginger	21.5282	−158.0383
2PNO95	Oahu	Hoomaluhia Garden, Kaneohe	Zingiberaceae	Red ginger	21.39288333	−157.8094667
2PNO97	Oahu	Castel Medical Center, Maunawila	Zingiberaceae	Beehive ginger	21.3802	−157.7577667
2PNO99	Oahu	Castel Medical Center, Maunawila	Zingiberaceae	Red ginger	21.3802	−157.7577667
GuamPN	Guam	UOG Station, Mangilao	Musaceae	Banana	13.432606	144.802441
AussiPN	Australia	Brisbane, Queensland	Musaceae	Banana	−27.473455	153.028899
IndiaPN	India	University of Calcutta	Musaceae	Banana	22.563561	88.34703
KP	Florida	Kanapaha Botanical Gardens	Musaceae	Banana	29.612105	−82.408906
CB	Florida	Casablanca Housing	Musaceae	Banana	29.619138	−82.364262
JH	Florida	Jennings Hall, Univ. of Florida	Musaceae	Banana	29.644208	29.644208
PP3	Florida	Fifeild Hall, Univ. of Florida	Musaceae	Banana	29.638776	−82.360450
SG	Florida	Field and Fork Farm and Gardens	Musaceae	Banana	29.644750	−82.363204
SE	Florida	Tree House Village	Musaceae	Banana	29.630829	−82.325303
FDA	Florida	Florida Dept. of Agriculture	Musaceae	Banana	29.634263	−82.371527

Note: Bolded Sample IDs indicate those used to characterize *Wolbachia* strains with multi-locus typing.

TABLE A2 Taxonomic details of invertebrate host animals and *Wolbachia* supergroup designations.

Host species	Phylum	Class	Order	16S rRNA GenBank accession number	Supergroup
Aphis hederae	Arthropoda	Insecta	Hemiptera	JN384066	A
Baizongia pistaciae	Arthropoda	Insecta	Hemiptera	JN384067	
Cinara pinea (M181)	Arthropoda	Insecta	Hemiptera	JN384072	
Drosophila melanogaster	Arthropoda	Insecta	Diptera	DQ412083	
Drosophila simulans	Arthropoda	Insecta	Diptera	NC012416/DQ412085.1	
Nasonia giraulti	Arthropoda	Insecta	Hymenoptera	M84690	
Nasonia vitripennis	Arthropoda	Insecta	Hymenoptera	M84688	
Nasonia longicornis	Arthropoda	Insecta	Hymenoptera	M84691	
Nasonia vitripennis	Arthropoda	Insecta	Hymenoptera	M84686	B
Cinara juniperi	Arthropoda	Insecta	Hemiptera	JN384071	
Bryobia sp. I	Arthropoda	Prostigmata	Acarina	EU499318	
Bryobia praetiosa	Arthropoda	Prostigmata	Acarina	EU499317	
Tetranychus urticae	Arthropoda	Prostigmata	Acarina	EU499319	
Bryobia sarothamni	Arthropoda	Prostigmata	Acarina	EU499315	
Dirofilaria immitis	Nematoda	Secernentea	Spirurida	Z49261	C
Dirofilaria repens	Nematoda	Secernentea	Spirurida	AJ276500	
Onchocerca gibsoni	Nematoda	Secernentea	Spirurida	AJ276499	
Onchocerca ochengi	Nematoda	Secernentea	Spirurida	AJ010276	
Brugia malayi	Nematoda	Secernentea	Spirurida	AF051145	D
Brugia pahangi	Nematoda	Secernentea	Spirurida	AJ012646	
Wuchereria bancrofti	Nematoda	Secernentea	Spirurida	AF093510	
Litomosoides brasiliensis	Nematoda	Secernentea	Spirurida	AJ548799	
Litomosoides sigmodontis	Nematoda	Secernentea	Spirurida	AF069068	
Folsomia candida	Arthropoda	Collembola	Collembola	AF179630	E
Mesaphorura italica	Arthropoda	Collembola	Collembola	AJ575104	
Ceratozetella thienemanni	Arthropoda	Arachnida	Sarcoptiformes	MH716233.1	
Kalotermes flavicollis	Arthropoda	Insecta	Blattodea	Y11377	F
Mansonella ozzardi.	Nematoda	Secernentea	Spirurida	AJ279034	
Rhinocyllus conicus	Arthropoda	Insecta	Coleoptera	M85267	
*Microcerotermes*sp.	Arthropoda	Insecta	Blattodea	AJ292347	
Myrmeleon mobilis	Arthropoda	Insecta	Neuroptera	DQ068882	
Zootermopsis angusticollis	Arthropoda	Insecta	Blattodea	AY764279	H
Zootermopsis nevadensis	Arthropoda	Insecta	Blattodea	AY764280	
Ctenocephalides felis	Arthropoda	Insecta	Siphonaptera	AY335923	I
Orchopeas leucopus	Arthropoda	Insecta	Siphonaptera	AY335924	
Dipetalonema gracile	Nematoda	Secernentea	Spirurida	AJ548802	J
Bryobia sp.	Arthropoda	Arachnida	Prostigmata	EU499316	K
Radopholus similis	Nematoda	Chromadorea	Rhabditida	EU833482	L
Cinara pinea (M48)	Arthropoda	Insecta	Hemiptera	JN384075	M
Macrosiphum euphorbiae	Arthropoda	Insecta	Hemiptera	JN384089	
Cinara maritimae	Arthropoda	Insecta	Hemiptera	JN384076	
Sipha maydis	Arthropoda	Insecta	Hemiptera	JN384068	
P. nigronervosa (Hawaii)	Arthropoda	Insecta	Hemiptera	KJ786949	
P. nigronervosa (Guam)	Arthropoda	Insecta	Hemiptera	KJ786950	
P. nigronervosa (India)	Arthropoda	Insecta	Hemiptera	KJ786951	
P. nigronervosa (Australia)	Arthropoda	Insecta	Hemiptera	KJ786952	

(Continues)

TABLE A2 (Continued)

Host species	Phylum	Class	Order	16S rRNA GenBank accession number	Supergroup
Toxoptera aurantii B	Arthropoda	Insecta	Hemiptera	JN384095	N
Toxoptera aurantii A	Arthropoda	Insecta	Hemiptera	JN384094	
Neophyllaphis podocarpi	Arthropoda	Insecta	Hemiptera	JN384096	
Bemisia tabaci	Arthropoda	Insecta	Hemiptera	KF454771	O
Syringophilopsis Turdus	Arthropoda	Arachnida	Trombidiformes	KP114103.1	P
Torotrogla merulae	Arthropoda	Arachnida	Trombidiformes	KP114099.1	
Torotrogla cardueli	Arthropoda	Arachnida	Trombidiformes	KP114101.1	Q
Atemnus politus	Arthropoda	Arachnida	Pseudoscorpiones	MN931248.1	S
Chthonius ischnocheles	Arthropoda	Arachnida	Pseudoscorpiones	MN931247.1	

Note: GenBank accession numbers of 16S rRNA sequences are also included.

TABLE A3 Five microsatellite loci used to genotype *P. nigronervosa* lines used in this study.

Loci name			s17b		S23		Ago66		S24		AF-4	
Aphid line	Collection location	Infection status	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Oahu2	Oahu, HI	No Wol	135	141	99	113	97	150	160		140	206
JH	Gainesville, FL	No Wol	135	141	89	113	97	150	160		140	206
KP	Gainesville, FL	No Wol	135	141	89	113	97	150	160		140	206
CB	Gainesville, FL	No Wol	135	141	99	113	97	150	160		140	206
Wailua	Kauai, HI	Wol+	135	141	89	113	97	150	160		140	
Koloa	Kauai, HI	Wol+	135	141	89	113	97	150	160		140	
Oahu-A	Oahu, HI	Wol+	135	141	89	113	97	150	160		140	206
PP3	Gainesville, FL	Wol+	135	141	89	113	97	150	160		140	206

TABLE A4 Primers used to confirm *Serratia symbiotica* and *Arsenophonus* infection in pooled *Pentalonia* samples used in MiSeq analysis.

Gene	Sequence	Reference
accD[a]	For—ACACCCTACTGGATAAAGGC	Henry et al., 2013
	Rev—GATGTTGATGTCGCCCAGC	
gyrB[a]	For—TGACATTCTGGCCAAGCGCC	Henry et al., 2013
	Rev—ACTACCGCGATCAGCCCCTC	
recJ[a]	For—GAAGCAATCGTTAATCCC	Henry et al., 2013
	Rev—TATCGAGATCGTTAGCCAGC	
23S[b]	For—CGTTTGATGAATTCATAGTCAAA	Thao & Baumann, 2004
	Rev—GGTCCTCCAGTTAGTGTTACCCAAC	

[a]Used to ID *Serratia symbiotica*.
[b]Used to ID *Aresenophonus*.

TABLE A5 The prevalence of *P. nigronervosa* (Pn) and *P. caladii* (Pc) on banana and non-banana food plants across Hawaii.

Aphid species	Banana	Non-banana	Total	*P* value
Pn	110	10	120	>0.001
Pc	1	140	141	
Total	111	150		

TABLE A6 Maternal transmission rate of *Wolbachia* in banana aphids approaches 100% in the lab.

	No. of aphids infected/no. screened	
Generation	PP3 (FL)	Wailua (HI)
1	10/10	10/10
2	8/8	10/10
3	10/10	10/10
4	19/19	16/16
5	19/19	20/20
6	18/18	19/19
7	18/18	19/19
Total	102/102	104/104

TABLE A7 Logistic regression-based likelihood ratio tests (LRT) for specialist *P. neoaphidus* fungal assays.

Fungal enemy	Contrast	Variable	LRT (L–R ChiSq; Prob > ChiSq)
P. neoaphidus	wPni− vs. wPni+	Survival	$F_{wPni+} = 45.432$, $P < 0.001$
		Sporulation	$F_{wPni+} = 51.802$, $P = < 0.001$
		Dual mortality	$F_{wPni+} = 0.751$, $P = 0.386$
P. neoaphidus	CB vs. JH	Survival	$F_{JH} = 0.599$, $P = 0.439$
		Sporulation	$F_{JH} = 0.102$, $P = 0.750$
		Dual mortality	$F_{JH} = 0.143$, $P = 0.705$
P. neoaphidus	CB vs. KP	Survival	$F_{KP} = 1.192$, $P = 0.275$
		Sporulation	$F_{KP} = 1.231$, $P = 0.267$
		Dual mortality	$F_{KP} = 4.220$, $P = 0.040$
P. neoaphidus	CB vs. Oahu2	Survival	$F_{Oahu2} = 0.156$, $P = 0.693$
		Sporulation	$F_{Oahu2} = 0.630$, $P = 0.427$
		Dual mortality	$F_{Oahu2} = 0.295$, $P = 0.587$
P. neoaphidus	JH vs. KP	Survival	$F_{KP} = 3.459$, $P = 0.063$
		Sporulation	$F_{KP} = 0.627$, $P = 0.429$
		Dual mortality	$F_{KP} = 5.898$, $P = 0.015$
P. neoaphidus	JH vs. Oahu2	Survival	$F_{Oahu2} = 0.143$, $P = 0.705$
		Sporulation	$F_{Oahu2} = 0.226$, $P = 0.635$
		Dual mortality	$F_{Oahu2} = 0.849$, $P = 0.357$
P. neoaphidus	Oahu2 vs. KP	Survival	$F_{KP} = 2.204$, $P = 0.138$
		Sporulation	$F_{KP} = 0.100$, $P = 0.752$
		Dual mortality	$F_{KP} = 2.294$, $P = 0.130$
P. neoaphidus	OahuAB vs. Koloa	Survival	$F_{Koloa} = 3.794$, $P = 0.051$
		Sporulation	$F_{Koloa} = 17.806$, $P < 0.001$
		Dual mortality	$F_{Koloa} = 4.535$, $P = 0.033$
P. neoaphidus	OahuAB vs. PP3	Survival	$F_{PP3} = 1.470$, $P = 0.225$
		Sporulation	$F_{PP3} = 2.415$, $P = 0.120$
		Dual mortality	$F_{PP3} = 0.035$, $P = 0.852$
P. neoaphidus	OahuAB vs. Wailua	Survival	$F_{Wailua} = 0.005$, $P = 0.942$
		Sporulation	$F_{Wailua} = 5.833$, $P = 0.016$
		Dual mortality	$F_{Wailua} = 5.381$, $P = 0.020$
P. neoaphidus	Koloa vs. PP3	Survival	$F_{PP3} = 9.372$, $P = 0.002$
		Sporulation	$F_{PP3} = 30.286$, $P < 0.001$
		Dual mortality	$F_{PP3} = 3.582$, $P = 3.582$
P. neoaphidus	Koloa vs. Wailua	Survival	$F_{Wailua} = 3.860$, $P = 0.049$
		Sporulation	$F_{Wailua} = 3.181$, $P = 0.075$
		Dual mortality	$F_{Wailua} = 0.035$, $P = 0.851$
P. neoaphidus	PP3 vs. Wailua	Survival	$F_{Wailua} = 1.227$, $P = 0.268$
		Sporulation	$F_{Wailua} = 14.495$, $P = 0.0001$
		Dual mortality	$F_{Wailua} = 4.320$, $P = 0.038$

TABLE A8 Logistic regression-based likelihood ratio tests (LRT) for generalist *B. bassiana* fungal assays.

Fungal Enemy	Contrast	Variable	LRT (L-R ChiSq; Prob > ChiSq)
B. bassiana	*wPni*− vs. wPni+	Survival	$F_{wPni+} = 0.165, P = 0.685$
		Sporulation	$F_{wPni+} = 0.724, P = 0.395$
		Dual Mortality	$F_{wPni+} = 0.000, P = 1.000$
B. bassiana	CB vs. JH	Survival	$F_{JH} = 0.170, P = 0.680$
		Sporulation	$F_{JH} = 2.842, P = 0.999$
		Dual Mortality	$F_{JH} = 0.184, P = 0.668$
B. bassiana	CB vs. KP	Survival	$F_{KP} = 0.041, P = 0.839$
		Sporulation	$F_{KP} = 0.049, P = 0.826$
		Dual Mortality	$F_{KP} = 0.000, P = 1.000$
B. bassiana	CB vs. Oahu2	Survival	$F_{Oahu2} = 1.452, P = 0.228$
		Sporulation	$F_{Oahu2} = 0.407, P = 0.523$
		Dual Mortality	$F_{Oahu2} = 5.012, P = 0.0.25$
B. bassiana	JH vs. KP	Survival	$F_{KP} = 0.379, P = 0.538$
		Sporulation	$F_{KP} = 0.049, P = 0.826$
		Dual Mortality	$F_{KP} = 0.184, P = 0.668$
B. bassiana	JH vs. Oahu2	Survival	$F_{Oahu2} = 2.609, P = 0.106$
		Sporulation	$F_{Oahu2} = 0.407, P = 0.523$
		Dual Mortality	$F_{Oahu2} = 7.059, P = 0.008$
B. bassiana	Oahu2 vs. KP	Survival	$F_{KP} = 1.005, P = 0.316$
		Sporulation	$F_{KP} = 0.738, P = 0.391$
		Dual Mortality	$F_{KP} = 5.012, P = 0.025$
B. bassiana	OahuAB vs. Koloa	Survival	$F_{Koloa} = 0.041, P = 0.840$
		Sporulation	$F_{Koloa} = 0.191, P = 0.662$
		Dual Mortality	$F_{Koloa} = 0.047, P = 0.829$
B. bassiana	OahuAB vs. PP3	Survival	$F_{PP3} = 0.668, P = 0.414$
		Sporulation	$F_{PP3} = 1.075, P = 0.300$
		Dual Mortality	$F_{PP3} = 1.274, P = 0.259$
B. bassiana	OahuAB vs. Wailua	Survival	$F_{Wailua} = 0.164, P = 0.685$
		Sporulation	$F_{Wailua} = 0.396, P = 0.529$
		Dual Mortality	$F_{Wailua} = 1.274, P = 00259$
B. bassiana	Koloa vs. PP3	Survival	$F_{PP3} = 0.379, P = 0.538$
		Sporulation	$F_{PP3} = 2.165, P = 0.141$
		Dual Mortality	$F_{PP3} = 0.834, P = 0.361$
B. bassiana	Koloa vs. Wailua	Survival	$F_{Wailua} = 0.041, P = 0.839$
		Sporulation	$F_{Wailua} = 1.134, P = 0.287$
		Dual Mortality	$F_{Wailua} = 0.834, P = 0.361$
B. bassiana	PP3 vs. Wailua	Survival	$F_{Wailua} = 0.170, P = 0.680$
		Sporulation	$F_{Wailua} = 0.167, P = 0.683$
		Dual Mortality	$F_{Wailua} = 0.000, P = 1.000$

TABLE A9 Logistic regression-based likelihood ratio tests (LRT) for *A. colmanii* parasitism assays.

Contrast	Variable	LRT (L-R ChiSq; Prob > ChiSq)
wPni− vs. wPni+	Survival	$F_{wPni+} = 3.881$, $P = 0.049$
	Sporulation	$F_{wPni+} = 0.013$, $P = 0.909$
	Dual Mortality	$F_{wPni+} = 8.673$, $P = 0.003$
CB vs. JH	Survival	$F_{JH} = 1.837$, $P = 0.175$
	Sporulation	$F_{JH} = 6.695$, $P = 0.010$
	Dual Mortality	$F_{JH} = 3.748$, $P = 0.053$
CB vs. KP	Survival	$F_{KP} = 0.050$, $P = 0.823$
	Sporulation	$F_{KP} = 7.084$, $P = 0.008$
	Dual Mortality	$F_{KP} = 8.253$, $P = 0.004$
CB vs. Oahu2	Survival	$F_{Oahu2} = 7.576$, $P = 0.006$
	Sporulation	$F_{Oahu2} = 1.851$, $P = 0.174$
	Dual Mortality	$F_{Oahu2} = 2.970$, $P = 0.085$
JH vs. KP	Survival	$F_{KP} = 2.492$, $P = 0.114$
	Sporulation	$F_{KP} = 27.222$, $P < 0.001$
	Dual Mortality	$F_{KP} = 22.600$, $P < 0.001$
JH vs. Oahu2	Survival	$F_{Oahu2} = 1.965$, $P = 0.161$
	Sporulation	$F_{Oahu2} = 1.514$, $P = 0.218$
	Dual Mortality	$F_{Oahu2} = 13.186$, $P < 0.001$
Oahu2 vs. KP	Survival	$F_{KP} = 8.850$, $P = 0.003$
	Sporulation	$F_{KP} = 16.069$, $P < 0.001$
	Dual Mortality	$F_{KP} = 1.341$, $P = 0.247$
OahuAB vs. Koloa	Survival	$F_{Koloa} = 3.213$, $P = 0.073$
	Sporulation	$F_{Koloa} = 7.337$, $P = 0.007$
	Dual Mortality	$F_{Koloa} = 1.377$, $P = 0.241$
OahuAB vs. PP3	Survival	$F_{PP3} = 26.140$, $P < 0.001$
	Sporulation	$F_{PP3} = 21.107$, $P < 0.001$
	Dual Mortality	$F_{PP3} = 0.566$, $P = 0.452$
OahuAB vs. Wailua	Survival	$F_{Wailua} = 0.456$, $P = 0.500$
	Sporulation	$F_{Wailua} = 5.615$, $P = 0.018$
	Dual Mortality	$F_{Wailua} = 6.625$, $P = 0.010$
Koloa vs. PP3	Survival	$F_{PP\ 3} = 11.187$, $P < 0.001$
	Sporulation	$F_{PP3} = 3.631$, $P = 0.057$
	Dual Mortality	$F_{PP3} = 3.693$, $P = 0.055$
Koloa vs. Wailua	Survival	$F_{Wailua} = 1.251$, $P = 0.263$
	Sporulation	$F_{Wailua} = 0.116$, $P = 0.733$
	Dual Mortality	$F_{Wailua} = 2.001$, $P = 0.157$
PP3 vs. Wailua	Survival	$F_{Wailua} = 19.800$, $P < 0.001$
	Sporulation	$F_{Wailua} = 5.042$, $P = 0.025$
	Dual Mortality	$F_{Wailua} = 10.948$, $P < 0.001$

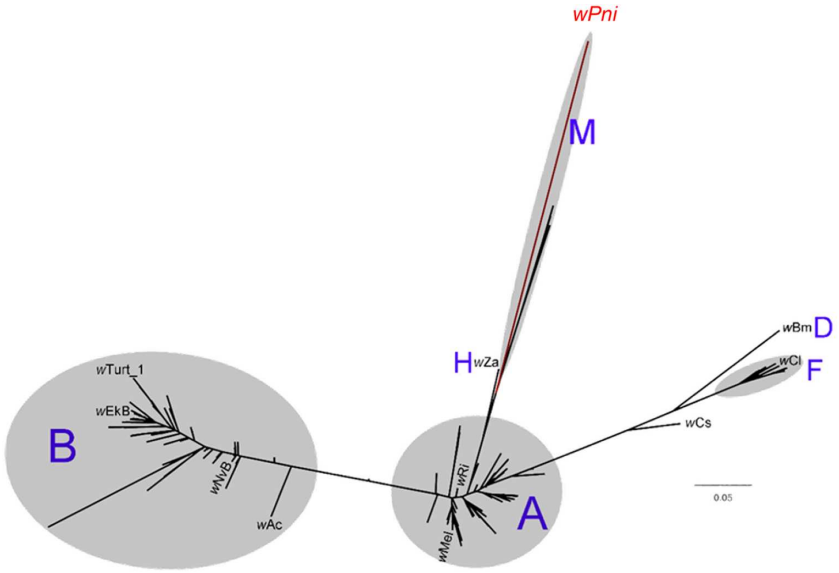

FIGURE A1 Maximum Likelihood phylogeny of *Wolbachia* derived from allele sequences: *gat*B, *cox*A, *hcp*A, *fts*Z, and *fbp*A (2085bp) of 277 allelic profiles available in the *Wolbachia* MLST website (Baldo et al., 2006). Maximum Likelihood for the tree: −31,265.28. Bootstrap values were omitted to allow readability of the tree. *Wolbachia* allelic profiles (*wPni*) identified in *Pentalonia nigronervosa* have been labeled as red (STs 402, 405, and 406). *Wolbachia* super-groups are labeled with capital blue letters and grey shaded areas when multiple strains were available. Representative strains have been mapped on the branches using the following acronyms: *w*Ri of *Drosophila simulans* (ST#17), *w*Bm of *Brugia malayi* (ST#35), *w*Cl of *Cimex lectularis* (ST#8) *w*Mel of *Drosophila melanogaster* (ST#1), *w*Cs of *Cordylochernes scorpioides* (ST#167 and #168), *w*Za of *Zootermes angusticollis* (ST#90), *w*Ac of *Apanteles chilonis* (ST#271), *w*NvB of *Nasonia vitripennis* B (ST#26), wEkB of *Ephestia kuehniella* B (ST#20), *w*Turt_1 of *Tetranychus urticae* (ST#279).

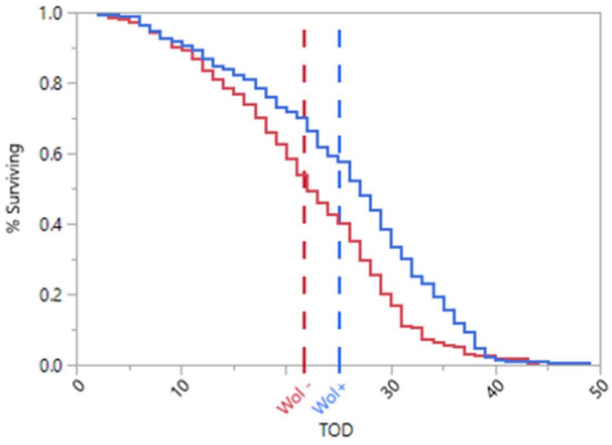

FIGURE A2 Survival curves for *wPni*− (red) and *wPni*+ (blue) aphids in the absence of natural enemies compared using Kaplan–Meier plots. The probability of 50% survival (vertical dashed line) was calculated after the Weibull fit ($\alpha = 0.05$). TOD = time of death.