The coral symbiont *Candidatus* Aquarickettsia is variably abundant in threatened Caribbean acroporids and transmitted horizontally.

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Abstract

The aquatic symbiont "*Candidatus Aquarickettsia rohweri*" infects a diversity of non-bilaterian metazoan phyla. In the threatened coral *Acropora cervicornis*, *Aquarickettsia* proliferates in response to increased nutrient exposure, resulting in suppressed growth and increased disease susceptibility and mortality. This study evaluated the extent, as well as the ecology and evolution of *Aquarickettsia* infecting the Caribbean corals: *Ac. cervicornis* and *Ac. palmata* and their hybrid ('*Ac. prolifera*'). The bacterial parasite *Aquarickettsia* was found in all acroporids, with host and sampling location impacting infection magnitude. Phylogenomic and genome-wide single nucleotide variant analysis found *Aquarickettsia* clustering by region, not by coral taxon. Fixation analysis suggested within coral colonies, *Aquarickettsia* are genetically isolated to the extent that reinfection is unlikely. Relative to other Rickettsiales, *Aquarickettsia* is undergoing positive selection, with Florida populations experiencing greater positive selection relative to the other Caribbean locations. This may be due to *Aquarickettsia* response to increased nutrient stress in Florida, as indicated by greater *in situ* replication rates in these corals. *Aquarickettsia* did not significantly codiversify with either coral animal nor algal symbiont, and qPCR analysis of gametes and juveniles from susceptible coral genotypes indicated absence in early life stages. Thus, despite being an obligate parasite, *Aquarickettsia* must be horizontally transmitted via coral mucocytes, an unidentified secondary host, or a yet unexplored environmentally mediated mechanism. Importantly, the prevalence of *Aquarickettsia* in *Ac. cervicornis* and high abundance in Florida populations suggests that disease mitigation efforts in the US and Caribbean should focus on preventing early infection via horizontal transmission.

Introduction

The alpha-proteobacterium "*Ca.* Aquarickettsia *rohweri*" is a symbiont that infects a diversity of aquatic non-bilaterian metazoan phyla from around the world [1]. This includes reef-building corals

(scleractinians) (51), as well as other cnidarians (11), sponges (76), kelp mucus, and ctenophores [1]. Although fairly ubiquitous, *A. rohweri* may have a more pervasive interaction with Caribbean acroporids, as Rickettsiales-like organisms, likely to be *A. rohweri*, have been found in all histological examinations of these coral species since 1975 [2–5]. The genome of *A. rohweri* associated with Caribbean acroporids is significantly reduced (1.28 Mbp) and has limited metabolic capacities, including the inability to produce multiple amino acids and ATP [1]. Thus, *A. rohweri* is likely an obligate symbiont dependent on its host for nutrition and energy rather than free-living, but its transmission routes are not yet known. Within Caribbean acroporids, *A. rohweri* have been found in high concentrations in *Acropora cervicornis* exposed to rising nutrient concentrations [6] and in disease-susceptible genotypes [7] and *A. rohweri* has a possible role in the progression of white band disease (WBD) [6, 8, 9]. WBD has contributed significantly to the decline of the reef-building corals, *Ac. cervicornis*, and to a lesser extent, *Ac. palmata* [10, 11]. These two corals are now so rare that they have been listed as threatened under the US Endangered Species Act [12, 13]. Worldwide, coral diseases have contributed to regional losses of between 5-80% of coral cover [10, 14]. Therefore, it is of broad interest to gain a better understanding of how this parasitic symbiont is evolving and transmitted to inform disease management.

Transmission mode is a major determinant of symbiont population structure and evolution [15–17]. Symbionts can be transmitted either vertically via direct transfer from one host generation to the next, or horizontally from a secondary host or the environment. Vertically transmitted symbiont phylogenies are congruent with their host phylogenies, as has been observed in insects [17, 18] and inferred in deep sea clam symbionts [19, 20] and sponge symbionts [21]. Many Rickettsiales species closely related to *A. rohweri* are known to be transmitted vertically, including Wolbachia [16, 22]. In the absence of significant codiversification, symbionts are likely transmitted through an alternative host or through the environment [23, 24]. Close-proximity environmental transmission has been posited for symbionts associated with insect and nematode species [15] as well as flashlight fish [25, 26]. Because *A. rohweri* associates with evolutionarily distant hosts [1], this parasite may be transmitted horizontally similar to arthropod or plant mediated transmission of terrestrial Rickettsiales [27, 28]. Although secondary hosts

have not been identified yet, possible modes of transmission include the gastropod *Coralliophila abbreviata* [29], zooplankton [30], or other coral associates. In fact, microscopy of infected coral polyps cannot resolve whether *A. rohweri* is associated with the coral animal or the coral's obligate, intra-cellular algal mutualist, Symbiodiniaceae. Rickettsiales-like organisms were observed in the actinopharynx, cnidoglandular bands, gastrodermal mucocytes, oral disk, and tentacles of a healthy *Ac. cervicornis* [8], spaces also shared by the algal mutualist. All Caribbean acroporid species take up their algal symbionts anew from the environment upon larval settlement [31], and thus algal symbionts, and perhaps *A. rohweri* with them, are horizontally transmitted.

Regardless of transmission mode, *A. rohweri* populations may also be structured by host species and the environment, although the latter is difficult to disentangle, as one likely co-varies with the other [32]. For example, the location where the first genome of *A. rohweri* was characterized, the Florida Keys, has been exposed to increasing anthropogenic inputs [33, 34] and wide-spread bleaching events [35, 36]. Coral in this area have also experienced multi-year epizootics, including stony coral tissue loss, white-band, and white pox disease [11, 37, 38]. Differential exposure to these stressors may result in dissimilar disease resistance by location, with higher occurrences of disease resistance in Florida (27%) relative to similar populations found in Panama (6%) and USVI (8%) [39]. This in turn may influence the prevalence of infection of the nutrient-stress responsive *A. rohweri* [6, 40]. Although disentangling the impact of host and environment will require further sampling and experimental efforts, our comparative analysis of *A. rohweri* populations provides insight into how infection of this symbiont may be influenced by environmental conditions.

Though *A. rohweri* is capable of infecting a variety of non-bilaterian metazoan phyla, the present study focused on infection of *Acropora* coral species found in the Caribbean: *Ac. cervicornis*, *Ac. palmata*, and their hybrid, commonly referred to as *Ac. prolifera*. Our objective was to provide an in-depth analysis of *A. rohweri* population structure and acroporid infections in the Caribbean. We utilized genomic characterization of the three host taxa and their dominant symbiont, *Symbiodinium 'fitti'*, [32, 41, 42] to investigate possible strain-specific interactions between *A. rohweri* and members of the

holobiont. Additionally, we studied the diversity of *A. rohweri* infecting acroporids across the Caribbean to understand how quickly this parasite has evolved in this ecosystem [43–45]. Finally, we compared *A. rohweri* genomes to determine the degree of connectivity between populations and the likelihood of reinfection in the three host taxa.

Methods

Sample acquisition and sequencing

Coral samples were collected from across the geographic range of Caribbean acroporids by two research groups. A sample from the Florida Keys was collected as was described in Shaver et al., 2017; this sample was originally used to describe the *A. rohweri* reference genome (Acer44) (GCA 003953955). This sample was extracted and sequenced as described in Klinges et al, 2019. In summary, DNA was extracted using EZNA Tissue DNA Kit (Omega Bio-Tek) and the quality of the extraction was evaluated using the dsDNA HS assay using a Qubit 3.0 Fluorometer. Libraries were prepared using Nextera XT (Illumina) and sequenced using the v3 reagent kit on an Illumina Miseq at the OSU's Center for Genome Research and Biotechnology (CGRB).

All of the samples presented in Kitchen et al., 2019 and 2020 were evaluated for the presence of *A. rohweri*. Coral tissue samples collected by the Baums' laboratory originated from twelve reefs across the Caribbean, for a total of 23 *Ac. cervicornis*, 30 *Ac. palmata*, and 23 *Ac. prolifera*. Samples included in this study were collected between 2001 and 2017 (Table S1) and were extracted as described in Kitchen et al., 2019 and Reich et al., 2020, and are briefly summarized here. Sample DNA was extracted using the DNeasy kit (Qiagen, Valencia, CA) and assessed using gel electrophoresis and Qubit 2.0 fluorometry (Thermo Fisher, Waltham, MA) prior to library construction and sequencing by the Pennsylvania State University Genomics Core Facility. A single library from these samples (Acerv FL 14120) was prepared using the TruSeq DNA PCR-Free kit (Illumina, San Diego, CA) and the remaining samples were prepared using the TruSeq DNA Nano kit (Illumina). All Baums' laboratory samples were sequenced

using the Illumina HiSeq 2500 Rapid Run (Illumina, San Diego, CA) and were deposited under PRJNA473816.

Identifying samples infected with A. rohweri

Sequences were filtered using BBMap version 36.20 to ensure sequences had a minimum length of 30 and a minimum average quality of 10. Reads were aligned to the reference genome (Acer44), using bowtie2 to quantify the proportion of reads that matched the reference sequence and identify candidates for assembly. The possible impact of host and location on the proportion of reads identified as Acer44 were evaluated using two-way analysis of variance (ANOVA) and post-hoc analysis using Tukey HDS within R [46]. The impact of reef location was also evaluated as a nested factor using one-way ANOVA and Tukey HDS. All samples with greater than 10,000 reads matching the Acer44 were *de novo* assembled using Spades version 3.13.1 with the single-cell option to account for possible PCR bias in assembly [47]. Contigs containing *A. rohweri* genes were identified using BLAST and binned as potential genomes; any contigs $\leq$ 200 bp were excluded from analysis. Genomes greater than 1.2 Mbp were evaluated using CheckM [48, 49].

Metagenome-assembled genomes (MAGs) were compared to the reference (Acer44) and to one another to evaluate commonalities and differences between samples. All samples were compared to one another to establish if there were additional species using pairwise average nucleotide identity (ANI) analysis using OrthoANI [48]. All *A. rohweri* genomes were annotated using Prokka (version 1.14.6) [50] and orthologs common to all samples were identified using orthofinder version 2.3.9 [51, 52]. 1528 orthologs were used to construct a pangenome and identify location-specific orthologs were plotted using the R-program upsetR [46]. Genes identified as being location-specific were further evaluated using the blastx search of the NCBI nr database limited to Rickettsiales [53].

Evaluating population structure and evolution of A. rohweri

Thirteen additional *A. rohweri* genomes plus the reference Acer44 taken from nine reefs across the Caribbean were used to evaluate potential differences in gene evolution. Phylogenomic trees were constructed with other well-characterized parasitic Rickettsiales species serving as outgroups. Rickettsiales species were selected on the criterium as being closely related to *A. rohweri* and having multiple completed strains with low percent contamination (Table S7); this analysis was performed both to characterize *A. rohweri* evolution and to identify possible shared functions. Rickettsiales genomes were annotated using Prokka (version 1.14.6) and orthologs were identified using orthofinder version 2.3.9 [51, 52]. A total of 143 single-copy orthologs were common to all samples. DNA sequences of orthologous genes were used to generate a phylogenomic tree. This tree was used both as the input for codiversification analysis as well as set the parameters for both evolutionary analysis and the root of the simplified and SNPs phylogenies. The DNA of each individual orthologous gene was aligned using MAFFT version v7.453 [54]. Genes were concatenated by sample and a tree was constructed using IQ-TREE [55, 56] with 1000 bootstrap replicates. Within IQ-Tree, J-modelTest determined the most likely model was the general time reversible model with empirical base and codon frequencies, allowing for a proportion of invariable sites, and a discrete Gamma model with default four rate categories (GTR+F+I+G4).

Whole-genome phylogenetic trends were also evaluated by identifying single-nucleotide polymorphism (SNP) relative to the reference genome *A. rohweri* Acer44. SNPs common to two or more samples that could be used to construct a SNPs phylogeny were found using the haploid SNP-caller, snippy [57], which implements bwa mem and freebayes to identify high-quality SNPs and assemble a core genome alignment. IQ-Tree was used to construct a tree from core-SNPs with 1000 bootstrap replicates; the model selected was jukes canter and ascertainment bias correction (JC+ASC). SNPs impacting annotated portions of the genome were annotated using SnpEFF version 4.3t to find the likely effects of variants on gene function.

Population structure and strain evolution were identified using both inter- and intra-sample diversity. SNPs were found by aligning sample reads to the prokka annotated reference genome (Acer44)

using bwa mem, with samclip processing allowing a maximum of a 10 clip length and the removal of duplicate alignments using samtools markdup. To avoid biases that effect variant detection, data was subsampled to the lowest number of reads ($2x10^4$) before realigning and then subsampled by the lowest median coverage found in a sample (3), similar to the normalizing protocol outlined in Romero Picazo et al., 2019 [24]. Variants were identified using lofreq with a minimum coverage of 10, a strand multiple testing correlation of $p > 0.001$, and a minimum SNP quality of 70 were used to find the fixation index (Fst) using the R packages seqinr [24, 58]. Fst estimates the degree of genetic variability between populations, where Fst closer to zero implies populations are mixing freely and Fst closer to 1 implies populations are genetically isolated. SNPs in functional regions were also used to find the intra-sample nucleotide diversity (π), which proves an estimate of the differences in genome diversity between samples. Scripts used to for F_{ST} and π analysis were are publicly available on github repositories (https://github.com/deropi/BathyBrooksiSymbionts). Differences between pairwise F_{ST} values were found within locations within host species was found using one-way with Tukey correction.

Trends in the evolution of functional genes in the *A. rohweri* genome as well as individual genes were evaluated using codeml to estimate ratio of nonsynonymous and synonymous substitutions (dN/dS). Lower values of dN/dS indicating stronger positive selection, that is selection against deleterious mutations to maintain function [59]. Values of dN/dS approaching 1 indicate positive selection or the selection of new mutations into the population. Individual orthologous proteins were aligned using MAFFT, and codon alignments were generated using pal2nal. Codon alignments were evaluated by codeml using a pairwise comparison, with all other parameters set to approximate the tree-building protocol described above. The result of this analysis was compared to the default parameters for codeml. Because the default parameters were less likely than our model parameters, only the results of our model parameters are presented. Only genes with a $dN > 0$, dS between 0.1 and 2, and $dN/dS > 10$ were used to find the average dN/dS. This is because pairwise comparisons where $dS < 0.01$ or $dN = 0$ are similar enough to be considered identical and $dS > 2$ are indicative that synonymous substitutions are near saturation. Similarly, values of $dN/dS > 10$ are considered largely artifactual [60]. From the single-copy

orthologs identified by orthofinder, 9% of the total pairwise comparisons are suitable for dN/dS analysis. Within-species and location comparisons were evaluated using two-way analysis of variance (ANOVA) and post-hock analysis using Tukey HDS within R [46].

Evaluating the impact of environment on A. rohweri replication rate

Because *A. rohweri* has been found in increased prevalence in response to nutrients, the *in-situ* replication rate is likely to be indicative of areas of sustained exposure to nutrients. Replication rate can be estimated for draft-quality genomes using the index of replication, iRep. iRep estimates the raw rate of replication of *A. rohweri* MAGs by realigning reads to the newly constructed genomes. Replication is estimated using an algorithum that assumes bi-directional replication from a single origin and accounts for the total change in coverage in genome fragments. A population where all cells are actively replicating would have an iRep of 2, and a population where only a quarter of the cells were replicating would have an iRep of 1.25 [61]. All genomes contructed had adaquit coverage to perform iRep analysis, but lacked the criterium to produce a filtered result (Table S8); the unfiltered result is presented as the genomes constructed are nearly identical (>99% ANI) and no not necessitate the filtering step to account for strain variation and/or integrated phage that can result in highly variable coverage [61]. Differences in the unfiltered rate of replication as a result of host or location sampled were evaluated using two-way ANOVA and the impact of location was evaluated as a nested factor using one-way ANOVA. All were evaluated using Tukey HDS post-hoc analysis within R [46].

Identifying likelihood of codiversification of A. rohweri *with coral or algal symbiont*

To evaluate the various possible methods of transmission, multiple codiversification analyses were performed to compare *A. rohweri* evolution with the two common eukaryotic members of the holobiont, *Acropora* and the algal symbiont, the Symbiodiniaceae*, Symbiodinium 'fitti'*. Both gene-based and SNP-phylogenies were constructed. Coral phylogenies were constructed using mitochondrial control region [62] and full mitochondrial genomes used to identify the parentage of the hybrid species *Acropora*

prolifera. The mitochondrial genomes for each individual were assembled using two approaches. In the first approach, filtered and trimmed short-read sequences were mapped to the *A. digitifera* mitochondrial genome sequence (NCBI: NC_022830.1, Shinzato et al. (2011)) using Bowtie 2 v2.3.4.1 (Langmead et al., 2012) with the parameters for the --sensitive mode. Reads were extracted using bamtofastq in bedtools v2.26.0 (Quinlan et al., 2010) and then assembled using SPAdes v3.10.1 (Bankevich et al., 2012) with various kmer sizes (-k 21, 33, 55, 77 and 99). In the second approach, we used the *de novo* organelle genome assembler NOVOplasty (Dierckxsens et al., 2016). The *A. digitifera* mitochondrial genome was used as the seed sequence to extract similar sequences from the original, unfiltered reads for each individual. Consensus sequences of the mitochondrial genomes for each individual were created after manual alignment of the sequences from the two approaches using MEGAX (Kumar et al., 2018). The consensus sequences were run through the web server MITOS (Bernt et al., 2013) to predict genes, tRNAs and rRNAs and non-coding regions. The phylogenetic relationship of the mitochondrial genomes was inferred with the Maximum Likelihood method using RAxML v8.2.12 (Stamatakis, 2014). We included eight acroporid mitochondrial genomes from the Indo-Pacific as outgroups (Liu et al., 2015; van Oppen et al., 2002). Because the mitochondrial genome can undergo different models of evolution among sites, we ran the genome alignment through PartitionFinder v2.1.1 (Lanfear et al., 2016) to determine the best partitioning scheme and substitution models using the greedy algorithm with estimated braches set as linked. In the ML analyses, we used the GTRGAMMA substitution model for the nine partitions. The tree topology with the highest-likelihood based on AIC criterion was reconstructed from 200 replicate trees and nodal support was take from 1,000 bootstrap replicates.

Symbiodinium gene trees were constructed using genes described in Pochon et al., 2014 and whole genome SNPs were found as described in Reich et al., 2020, excluding all samples identified as having multiple symbiont infections. All genes were identified using a BLAST search of the aforementioned *de novo* Spades assemblies. Gene trees were aligned using MAFFT and all phylogenies were constructed using IQ-Tree with 1000 bootstrap replicates; genes and model parameters are described in Table S8. Bacterial and eukaryotic phylogenies were evaluated for significant codiversification using

the Procrustes Approach to Cophylogeny (PACo), which uses ultrametric rooted trees to create cophenetic matrices that are evaluated for codivergence 10^5 times in R [63]. This global fit method evaluates phylogenies that are not fully resolved to evaluate if there is significant codiversification between a host and symbiont species. To evaluate transmission by the coral host, bacterial phylogenomic and SNPs trees were compared to coral phylogenies (whole genome SNPs trees and mitochondrial SNPs and genes). To evaluate transmission with a *Symbiodinium* host, bacterial phylogenomic and SNPs phylogenies were compared to *Symbiodinium* gene and SNPs trees. Only comparisons with $p < 0.05$ are presented along with the residual sum of squares (m^2) to provide a context for how well the data fit the codiversification model. Significant codiversification analysis were evaluated using a jackknife sum of squares to find those members contributing to the significant association, as they will have values are below the 95% residual sum of squares.

Evaluating vertical transmission via qPCR of susceptible coral genotypes at early life stages

A few days preceding the annual *Acropora cervicornis* spawning event in 2019 and 2020, sexually mature, adult colonies of genotypes 13 and 50 were brought into Mote Marine Laboratory's Elizabeth Moore International Center for Coral Reef Research & Restoration from their offshore coral nursery in the lower Florida Keys. On land, corals were isolated to keep replicate colonies and genotypes separated for conducting 2-parent controlled crosses. Spawning, fertilization, settlement, and grow-out were conducted following published protocols and under standardized conditions [64–72]. *A. cervicornis* is a simultaneous hermaphrodite and broadcast spawning activity was observed in August during the predicted peak spawning window for this species [73]. Gamete bundles of eggs and sperm were collected from each genotype, and after bundle dissolution, sperm was isolated from the eggs by filtration using a sieve (100 µm mesh). Triplicate subsamples were concentrated via centrifugation and then snap-frozen and stored at -80°C. Triplicate subsamples of 50-100 eggs per genotype were also snap-frozen and stored at -80°C. The remaining egg stock from genotype 13 and sperm stock from genotype 50 were combined and allowed to undergo fertilization for one hour (cross '13e x 50s'). Embryos were reared in replicate

cultures with filtered seawater at room temperature (27°C). Approximately one week later, triplicate subsamples of 50 larvae were snap-frozen and stored at -80°C. The remaining larvae were settled in 5-gallon glass tanks using unconditioned ceramic settlement substrates and live, crushed up crustose coralline algae (CCA) as the settlement cue. After settlement, sexual recruits were reared under common garden conditions in Mote's land-based coral nursery. Corals were maintained in flow-through mesocosms ('raceways') with running seawater, from which algal endosymbionts were naturally acquired. Fouling algae was mitigated using intertidal herbivorous snails (*Batillaria spp.)*, and the daily husbandry regime consisted of raceway siphoning and basting of the substrates to remove snail detritus. Flow rates were maintained between 4 and 6 L/min depending on season and outdoor weather conditions.

Coral hybrid genotypes were produced from a cross of two disease-susceptible *A. cervicornis* parents (genotypes 13 and 50, Muller et al., 2018) collected from the Mote Marine Laboratory *in situ* coral nursery in 2019 and 2020. The hybrid genotype was sampled at gamete (egg and sperm), larval (<1 week of age), recruit (~2 months), and juvenile (~1 year) stages. DNA was extracted from early life stage coral samples using the Omega E.Z.N.A.® DNA/RNA Isolation Kit. Extracted nucleic acids were stored at -80°C until further processing. Quantitative polymerase chain reaction (qPCR) was performed on 7 early life stage samples (in triplicate) using primers designed to target the *Acropora cervicornis* actin gene (as an endogenous control) and an *Aquarickettsia*-specific gene, *tlc1*, using iQ SYBR Green Supermix (Bio-Rad). An 149bp section of the *tlc1* gene of *Aquarickettsia rohweri* was amplified using sequence-specific primers (F: 5'-GGCACCTATTGTAGTTGCGG-3', R: 5'-CATCAGCTGCTGCCTTACCT-3'), and the actin gene of *Acropora cervicornis* was amplified as in Wright et al., 2018 [74] as an endogenous control. A sample of *Acropora hyacinthus* (collected from Mo'orea, French Polynesia in 2017) was used as a calibrator, as this species expresses actin but lacks *Aquarickettsia rohweri*. A positive control (a sample of adult genotype 50 with known quantity of *A. rohweri*) and a no-template control (molecular grade water) were prepared using the same methods and quantified simultaneously. A 35-cycle qPCR was performed on an Applied Biosystems 7500 Fast Real-Time PCR System, using cycling parameters selected to minimize mispriming: An initial denaturation

311 step of 3 min at 95°C, followed by 35 cycles of 95°C for 15s, 56°C for 30s, and 72°C for 30s. Melt curve
312 analysis was performed to identify any off-target products. Results were confirmed through non-
313 quantitative PCR of the *tlc1* and 16S rRNA genes (515F-806R primer set, Apprill et al., 2015) using
314 AccuStart™ II PCR ToughMix (QuantaBio) and subsequent gel electrophoresis on a 1.5% agarose gel
315 with Invitrogen 100 bp DNA Ladder (ThermoFisher Scientific).
316
317 **Results**
318 *Metagenome Assembled Genomes (MAGs) generated from multiple locations and hosts suggest that A.*
319 *rohweri associates with many Carribean* Acropora
320 *Ca. Aquaricketssia rohweri* sequences were found in all *Acropora* specimen collected from
321 across the Caribbean as part of the Kitchen et al. 2019, 2020 and Reich et al. 2020 studies (Table 1).
322 Samples had between 38 and 1,219,071 reads that aligned to the reference genome, *A. rohweri* Acer44
323 (GCA 003953955) (Table S1). The *A. rohweri* reads were normalized to the total coral host reads as an
324 intrinsic measure of microbial load [75–77]; using this method, reads varied according to coral host and
325 sampling location (Fig. S1), but not source of collection (Tukey adjusted p=0.5; data not shown). *A.*
326 *rohweri* made up a greater proportion of the total reads in *Ac. cervicornis* samples relative to *Ac. prolifera*
327 (on average, 4x more reads, Tukey adjusted p=0.0002) and *Ac. palmata* (86x more reads, p<0.0001)
328 samples. Additionally, *A. rohweri* made up a greater proportion of the total reads from all samples
329 collected in Belize relative to Curacao (46x more, p= 0.007). Although limited to *Ac. cervicornis* samples,
330 a greater proportion of *A. rohweri* reads were found in Belize samples relative to those collected from
331 Florida (1.6x more, p<0.00001) and the USVI (1.8x more, p=0.017).
332 Only samples of *Ac. cervicornis* and *Ac. prolifera* from Florida, USVI, and Belize contained
333 sufficient reads to construct *A. rohweri* Metagenome Assembled Genomes (MAGs) (deposited under
334 PRJNA666461). These MAGs were used to compare *A. rohweri* from different locations (Belize: 7,
335 Florida: 4, USVI: 3) and different host-taxa (*Ac. cervicornis*: 11, *Ac. prolifera*: 3). Six of the MAGs
336 constructed were less complete than the original *A. rohweri* genome (<98.9% complete), but four from

Belize were 100% complete with no contamination and a larger N_{50} value than the reference assembly (Table 1). All *A. rohweri* MAGs were at least 1.21 Mbp and had a >99% Average Nucleotide Identity (ANI) in pairwise comparisons to the reference genome and one another (Table 1; Table S2).

From the assembled bacterial genomes, between 1184 and 1343 genes were annotated per sample and 98.4% of sequences were identified as belonging to 1528 orthogroups. No orthologs were found to be exclusive to either coral host. Greater than 30% of these sequences were identified as single-copy orthologs shared by all samples. Location-specific orthologs were all single-copy; Florida had 8 unique orthologs, Belize 40, and USVI 21 (Fig. 1). The majority of location-specific genes were annotated by prokka as hypothetical proteins; however, searching for the function of these genes against the NCBI nr database resulted in additional annotation. Functional genes specific to locales include: the protein transfer gene *secA* in Florida, as well as multiple transport genes in Belize and USVI, as well as two genes involved in the type II toxin-antitoxin system in Belize (Table S3).

The coral parasite, A. rohwerii, differentiates by location, not by host

Phylogenomic analysis of the orthologous genes of the *de novo* assembled *A. rohweri* MAGs showed variation among samples collected from different locations, irrespective of host. Comparison to well-studied host-associated Rickettsiales species (*Ehrlichia chaffeensis*, *Rickettsia prowazekii*, *Rickettsia rickettsii*, and *Wolbachia pipientis*) resulted in 71 orthologous genes. The phylogenetic tree constructed from DNA of these orthologs resulted in all newly constructed *A. rohweri* genomes tightly clustered near the reference genome Acer44 (Fig. S2). Limiting phylogenomic analysis to *A. rohweri* samples results in clear differentiation between samples collected across the Caribbean and north-west Atlantic; Belize *A. rohweri* are distinct from those isolated in the Virgin Islands and Florida, regardless of host identity (Fig. 2A). Further, the *A. rohweri* isolated from *Ac. prolifera* does not form a separate clade from those isolated from *Ac. cervicornis* even when collected from the same location. This is especially evident in Belize samples; *A. rohweri* genomes constructed from *Ac. prolifera* and *Ac. cervicornis* taken from the same reef are more closely related than *A. rohweri* collected from *Ac. prolifera* from a neighboring reef. Clustering

by location was similar in the SNP analysis (Fig. 2B), despite there being a surprisingly small number of SNPs per sample (*n*=11 to 2345), at a minimum read depth of 10, minimum fraction of 0.9, and a minimum mapping quality of 100 (Table S4). Relative to the reference genome Acer44, samples had low levels of genetic polymorphism (0.63 ± 0.69 SNPs/kilobase) (Table S4). This resulted in few SNPs that are shared by multiple samples (*n*=15), i.e. those that are informative in phylogenetic analysis.

Analysis of inter- and intrasample SNPs within annotated portions of the genome indicates that although samples have similar levels of genetic diversity, the bacteria found within individual coral colonies are relatively genetically isolated from one another. This is even true of samples collected from the same reef (Fig. 3). Intrasample nucleotide diversity (π) did not differ among sampling locations (reefs) and was on average $1.85 \pm 0.8 \times 10^{5}$. However, pairwise comparison of intrasample *Ac. rowheri* SNPs between coral samples mostly resulted in an fixation index (F_{ST}) of 0.86 or greater, suggesting *A. rowheri* infections are genetically isolated from one another by coral sample. This level of genetic isolation was found in all pairwise comparisions in USVI and Belize, and was even observed in pairwise comparisions of samples taken from the same reef. Samples from Florida had significantly lower fixation indices relative to USVI and Belize (F_{ST} =0.65-0.83; Tukey adjusted $p<0.0001$) Although these values still imply *A. rowheri* populations in Florida coral colonies are somewhat genetically isolated from each other, these populations may be less fixed than those found in USVI or Belize. The aforementioned account for *A. rohweri* found in both *Acropora* taxa; host identity did not affect the level of genetic isolation (Tukey adjusted p=0.9).

All samples had high-quality, intersample SNPs identified using snippy [57] with annotated functional genes relative to the reference genome *A. rohweri* Acer44. Of those SNPs that impacted functional genes, a majority of the SNPs (on average 62%) were identified as missense mutations (Table S4). Three samples from Belize (Aprol BE 13843, Acer BE 13797, and Acer BE 13792) were found to have a nonsense stop gain mutation in the IS66 family transposase ISDpr4. Four additional transposases were identified as having missense mutations, including IS6 family transposase ISCca2, which was found

388 to have a missense mutation in all Belize and USVI samples. These SNPs were annotated by SnpEFF as
389 having a moderate impact on function (Table S5).
390
391 *A. rohweri undergoing positive selection*
392 Samples were compared to well-studied Rickettsiales relatives of *A. rohweri* to evaluate whether
393 populations of *A. rohweri* are undergoing neutral or positive selection relative to other closely related
394 parasites. *A. rohweri* had the highest mean dN/dS, with a much broader distribution of dN/dS values
395 relative to all other Rickettsiales species (36-89% greater dN/dS, $p<0.0001$) (Fig. 4A; Table). This
396 indicates *A. rohweri* is undergoing greater positive selection than closely related Rickettsiales.
397 Comparison of dN/dS of *A. rohweri* from the two *Acropora* host taxa did not result in significantly
398 different dN/dS ($p=0.06$; data not shown), but location did affect dN/dS. There was a higher median
399 dN/dS for *A. rohweri* from Florida relative to those from USVI (65% higher, $p=0.048$) (Fig. 4B).
400 However, all populations had some genes undergoing positive selection (Table S5). Most of these genes
401 were identified by prokka as hypothetical. Of those that were not hypothetical, GTPase Era, DUF2312
402 domain-containing protein, and the Bifunctional protein FolD were consistently undergoing some level of
403 positive selection (0.5-0.9 dN/dS) in all samples taken from Belize. The strongest positive selection (≥ 1.0
404 dN/dS) were 50S ribosomal protein L13 and the type IV secretion system protein VirD4, although
405 selection was only observed in a subset of comparisons between *A. rohweri* samples (Table S7).
406
407 *A. rohweri from Florida samples had higher replication rates*
408 Samples collected from Florida had consistantly higher estimated unfiltered rates of replication
409 (iRep), with the identity of the coral taxon having no impact (Tukey adjusted, $p=0.3$) (Fig. 5). The
410 unfiltered iRep of samples from Flordia was 19% higher than samples from Belize and 30% higher than
411 samples from USVI. A single sample from Belize had as high an iRep as Florida samples (Acer BE
412 13786); this sample was taken alongside five other Belize samples and also from the same reef as an
413 additional sample (Aprol BE 13778 from South Carrie Bow Cay). Florida samples had a significantly

414 higher estimated rate of replication than those taken from the USVI (p=0.04); no difference in rate was
415 seen when comparing iRep among reefs (p=0.44).
416
417 *Codiversification analysis and qPCR of coral offspring suggests horizontal transmission*
418 All phylogenetic methods used to analyze the coral diversity of samples infected with *A. rohweri*
419 resulted in differentation between *Ac. cervicornis* and *Ac. prolifera* (Fig. S3, S4). Codiversification
420 analysis among *A. rohweri* and coral phylogenies largely were not signficant. Although coral phylogenies
421 constructed using the mitochondrial control region identified by Vollmer et al., 2002, were signficantly
422 codiversifying with bacterial phylogenomic and SNPs trees (p=0.02), the m^2 values indicate the data does
423 not fit the codiversification model and are therefore highly unlikely (>800). Coral phylogenies contructed
424 using the whole coral mitochondrial genome also did not codiversify with either *A. rohweri*
425 phylogenomic or SNP phylogenies ($p<0.01$ and $m^2>330$). This was recapitulated in the jackknife sum of
426 squares of both Procrustes Approach to Cophylogeny (PACo) analysis, which did not identify any
427 samples as significantly contributing to codiversification (Table S8).
428 Analysis of the *S. 'fitti'* gene trees and *A. rohweri* SNP phylyogenies were also incongruous. The
429 phylogeny constructed using the genes recommended in Pochon et al., 2014 resulted in placement of all
430 samples as *Symbiodinium 'fitti'* (ITS2 type A3, formerly "Clade A"; Fig. S5). The gene tree also showed
431 a clear differentiation between the samples collected from Florida relative to the USVI and Belize
432 samples. However, USVI and Belize samples formed a single clade, which is dissimilar from the
433 clustering that occurred in either *A. rohweri* phylogeny (Fig. S5). As was previously described in Reich et
434 al., 2020, the *S. 'fitti'* SNPs phylogeny clustered by host species and not location (Fig. S6). Similar to the
435 coral analysis, codiversification analysis of *S. 'fitti'* with *A. rohweri* phylogenies were mostly
436 insignificant. Only the *S. 'fitti'* gene tree had significant codivergence with *A. rohweri* phylogenomic and
437 SNPs phylogenies (p=0.02), however the m^2 values were also prohibitively high for this analysis
438 ($m^2>380$), and jackknife analysis identified 0-2 samples contributing to this trend (Table S7).

Quantitative PCR was utilized to detect *A. rohweri* in early life stages of *Acropora cervicornis*. qPCR was performed on samples of gametes (egg and sperm), larvae (<1 week of age), recruits (~2 months), and juveniles (~1 year) of a hybrid genotype produced by crossing two disease-susceptible *Acropora cervicornis* parents (genotypes 13 and 50, Muller et al., 2018). A calibrator sample (*Acropora hyacinthus*, known to lack *A. rohweri*) and a positive control (adult genotype 50 *A. cervicornis*, with an average relative abundance of *A. rohweri* greater than 70%) were also quantified. While adult *A. cervicornis* collected from the Mote *in-situ* coral nursery had high fold change of *tlc1* relative to the calibrator sample, amplification of *tlc1* across all early life stage samples was not significantly different from the calibrator sample and led to the failure of the thresholding algorithm, indicating an absence or undetectable level of *A. rohweri* infection in these early stages of *A. cervicornis* ontogeny.

Discussion

Ca. Aquaricketssia rohweri infection was found in every sample of acroporid corals taken from across their Caribbean and north-west Atlantic geographic range, with *Ac. cervicornis* samples consistently yielding more *A. rowheri* reads relative to *Ac. prolifera* and *Ac. palmata* (Fig S1A). This coincides with relatively higher disease susceptiblity of *Ac. cerivornis* [6, 8, 9]. Assuming the proportion of *A. rohweri* to host reads are indeed reflective of infection status [75–77], relative resistance of *Ac. palmata* may be attributed to environmental factors such as depth, innate host immunity, or defenses mounted by the host microbiome [78–80]. Determining which factors may be leading to resistance in *Ac. palmata* is a valuable area of further research.

The coral *Ac. cervicornis* yielded higher read numbers of *A. rohweri*, but both *Ac. cervicornis* and *Ac. prolifera* hosted sufficient reads to construct *A. rohweri* genomes of similar length and quality as the reference genome Acer44. Phylogenomic analysis using orthologous genes and SNPs indicate the bacteria infecting Caribbean acroporids are specific to the collection location and not the host. This is in contrast to the only other acroporid symbiont with population genetic information, the dinoflagellate *S. fitti*. Strains of *S. fitti* preferentially associate with each host, regardless of reef location. These contrasting

patterns of population structure indicate that the forces shaping the two coral symbionts are different despite their shared host. Differing population structures have also been reported in terrestrial symbionts, including those populating aphids and whiteflies [27, 81, 82].

A. rohweri populations from the three sampling locations form separate clades in phylogenomic and SNP phylogenies, with Florida and USVI samples likely sharing a closer ancestral lineage than Florida and Belize. However, USVI is not basal to Florida populations, suggesting that *A. rohweri* from Florida did not originate from the US Virgin Islands. A barrier to gene flow has been identified between the eastern and western Caribbean for the coral host [83, 84], suggesting there is a barrier to planktonic genflow between these environments. Similar genetic differentiation by location has been observed for the *Ac. cervicornis* sequences of the same samples used in this analysis [41]. Even though seasonally variable surface currents connect all sampling locations [84, 85], and all samples are genetically similar (relative to the reference genome, all samples were >99% ANI), the differences found reveal clear differentiation between Florida, the US Virgin Islands, and Belize *A. rohweri* populations.

A. rohweri collected across the Caribbean have low levels of genetic polymorphism with <2500 SNPs relative to reference genome of 1.28 Mbp. Thus, *A. rohweri* may be considered monomorphic (*sensu*, 54-56). Although the rate of genetic polymorphisms in *A. rohweri* is similar to that of bioluminescent mutualists *Ca*. Photodemus katoptron and *Ca*. Photodemus blepaharus, it is also comparable to that seen in pathogens *Yersinia pestis* and *Bacillus anthracis*, [86–88]. Lower levels of genetic polymorphism are correlated with virulence in some bacteria [88]. The role of *A. rohweri* in coral disease is an active area of research, and thus it is difficult to interpret the low levels of genetic polymorphisms in *A. rohweri*, but it is notably low for a symbiont spanning such a large geographic range.

Of the subset of SNPs that impact functional regions, most (62%) resulted in a change to the amino acid identity and therefore likely affect protein function. Although the majority of genes impacted by missense mutations were hypothetical proteins, some gene annotations were identified as transposases. Moreover, the single gene found to have acquired a stop codon in all USVI and Belize samples was

within the transposase ISDpr4. The loss of transposases and the eventual loss of these gene regions is characteristic of long-term obligate symbionts [16, 89]; therefore, the *A. rohweri* genome may still be actively reducing through the loss of mobile genetic elements.

Although the bacteria were closely related to each other and are phylogenetically clustering by sampling location, there is surprisingly little genetic mixing, even within a single reef. Pairwise comparisons of the fixation indices between all samples had such high values that genetic mixing among samples and thus reinfection is unlikely to occur between or even within a reef. The most extreme case was observed in comparing samples collected from the same reef in Belize, as all pairwise comparisions had an F_{ST} of 0.95 or greater. The lowest level of pairwise genetic differences were observed in Florida, which implies a slightly higher probability of reinfection relative to the other populations sampled, but laboratory studies would be needed to evaluate whether this is due to host or environmental factors. Thus, despite low genetic diversity observed overall, genetic diversity was distributed such that locations and samples were highly differentiated suggesting that *A. rohweri* infection may occur earlier in the coral lifespan and propagate within the host with little to no genetic mixing occuring thereafter.

Our work also shows that *A. rohweri* is undergoing greater positive selection relative to closely related parasitic Rickettsiales species, with genes involved in speciating and virulence being most impacted. While the identity of the coral host did not matter, sampling location did impact did impact the degree of positive selection. The average dN/dS of Florida samples is, on average, 2.7 times greater than samples from USVI and 2.8 times greater than those in Belize. Although differences in dN/dS were not observed for all samples at each location, these trends were observed in a subset of the comparisons between all sampling locations. Genes that were associated with ribosomal assembly, L13 and GTPase ERA, which assemble 50S and 30S ribosomal proteins respectively, were undergoing positive selection in a subset of the samples. The consequence of ribosomal-associated genes undergoing positive selection is unknown, but it may be indicative of speciation occurring between the different sampling locations. Another gene undergoing positive selection in a subset of samples across locations was the Type IV secretion system-coupling protein VirD4. VirD is essential to T4SS and is involved in substrate

recruitment, which plays a role in oncogenic DNA transfer and virulence in Agrobacterium [90–93]. Thus, positive selection in VirD may be affecting how *A. rohweri* in Florida populations interact with their host species.

Though microscopy found *A. rohweri* living in close proximity to coral and S. '*fitti'*, neither are likely transmitting the parasite vertically. Coral larvae seemed the most likely method for transmission across the Caribbean, as larvae can travel long distances as plankton (>500 km) [84]. Similarly, algal symbionts could provide the necessary nutrients to *A. rohweri* and facilitate parasitic infection when *S. 'fitti'* is acquired by juvenile coral hosts [94–97]. It is also possible that the parasite could be carried alongside either member of the coral holobiont as they reproduce asexually. However, either sexual or asexual reproduction would have resulted in congruence between the parasite and host phylogeny. Yet, codiversification analysis of both SNPs and gene-based phylogenies resulted in neither coral nor *S. 'fitti'* having clear codivergence with *A. rohweri*. Additionally, qPCR evaluation of early life phases (<1 week to 1 year) from disease-susceptible coral genotypes known to harbor *A. rohweri* as adults did not have detectable *A. rohweri*. The reduced metabolic capabilities of *A. rohweri* [1] and the lack of evidence for a dormancy pathway also suggests that the bacteria is unlikely to survive long periods in the environment as free-living bacteria. It is therefore most likely that the bacteria are transmitted *via* an alternative method that would provide the necessary nutrients, such as through the movement of coral mucocytes coupled with some abrasion or inoculation event or through an as yet unidentified intermediate host.

Overall, the results of this study show that *A. rohweri* infection differs among coral hosts and locations, is evolving at different rates across its host's range, and is horizontally transmitted. These findings suggest new pathways to the study of *A. rohweri* and its potential contribution to coral diseases in the Caribbean. For example, exploring possible host or microbiome-based deterrents of *A. rohweri* infection of *Ac. palmata* [98] may be valuable to the preservation of Caribbean acroporids. Additionally, Florida may be a unique focal point for the study of how *A. rohweri* infection impacts coral disease progression. Several *Ac. cervicornis* and an *Ac. prolifera* from the Florida Keys host high concentrations of *A. rohweri* that tend to be less isolated, undergo greater selection in speciation and virulence genes, and

543 are propagating faster in Florida than in other sampling locations. Thus, further research into
544 environmental stressors and host responses in this population will be invaluable to our understanding
545 pathogen evolution, its role in coral disease, and the restoration and recovery of this fragile ecosystem.
546
547 **Data accessibility**
548 All sequences used are available in the SRA in PRJNA473816, assembled *A. rohweri* genomes are
549 accessible under PRJNA66646, and coral mitochondrial genomes are MW246489-MW246565.
550
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Table and Figure Legends

Table 1: Collection data and genome quality information for *A. rohweri* constructed from PRJNA473816, including the reference genome from Klinges et al., 2019. Coral host taxa include *Ac. cervicornis* (Acer) and *Ac. prolifera* (Aprol). *Symbiodinium 'fitti'* infection status, whether by a single strain or multiple strains, were evaluated in Reich et al., 2020. Completeness, contamination, length,

803 percent GC, and N50 were found using checkm, genes are the total number of prokka annotations,
804 and the ANI to the reference sequence *A. rohweri* Acer44 was found using orthoANI.
805
806 Fig. 1: The number of orthologous genes in *A. rohweri* identified using orthofinder for each sample.
807 Location specific genes are highlighted in the bar plot by location color: Florida (yellow, 8
808 orthologs), USVI (pink, 21 orthologs), and Belize (blue, 40 orthologs). Host identity is noted in
809 blue text for *Ac. prolifera* (Aprol) and black text for *Ac. cervicornis* (Acer), although no orthologs
810 are exclusive to either host taxon.
811
812 Fig. 2: *A. rohweri* phylogenies for all MAG generated using (A) orthofinder to identify orthologs to
813 construct a phylogenomic tree, rooting based on comparison to other Rickettsiales (Fig. S1) (B)
814 Phylogenetic tree of core-SNPs (15) generated by snippy and rooted based on the outcome of
815 orthofinder tree. Bootstrap values greater than 70 are shown.
816
817 Fig. 3: Pairwise comparisons of the fixation indices (F_{ST}) and intrasample nucleotide diversity (π) for each
818 sample from each location, generated using methods outlined in Romero Picazo et al., 2019 [24].
819 Samples originating from Belize shown in blue, Florida in yellow, and the USVI in orange. All
820 comparisons between USVI and Belize samples resulted in an F_{ST} of > 0.86 whereas samples with
821 the Florida populations were between 0.64-0.83.
822
823 Fig. 4: Plots of average dN/dS values for whole genome comparisons of prokka annotated genes. (A)
824 Average dN/dS of closely related well studied Rickettsiales species were all lower relative to *A.*
825 *rohweri* ($p<0.0001$). (B) Average dN/dS of *A. rohweri* is significantly greater in Florida than
826 USVI ($p = 0.048$).
827
828 Fig. 5: The average unfiltered estimates for the rate of replication for (A) sampling locations and (B) coral

829 host.

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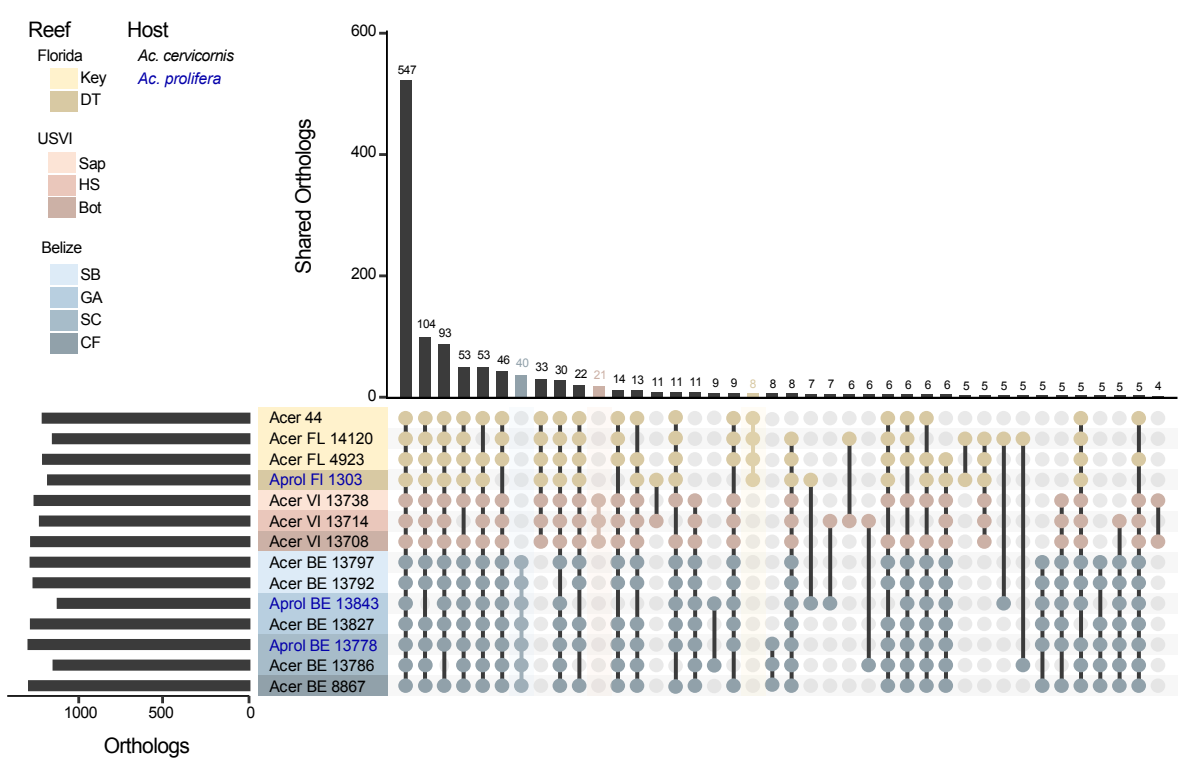
832

833

Sample ID	Location	Reef	Coll date	Symb infect	Comp (%)	Contam (%)	Length (Mbp)	GC. (%)	N50	Genes	ANI
Acer44	Florida	Key Largo	1-Jun-13	unknown	98.9	1.20	1.28	0.28	10,860	1,321	NA
Acer FL 4923	Florida	Key Largo	22-Nov-11	solo	97.6	0.10	1.21	0.28	4,669	1,243	99.9
Acer FL 14120	Florida	Key Largo	1-Mar-16	solo	100.0	0.00	1.25	0.28	12,616	1,255	99.93
Aprol FI 1303	Florida	DryTortugas	29-Jul-03	multi	97.8	1.32	1.23	0.28	3,890	1,343	99.91
Acer VI 13738	USVI	Sapphire	30-Oct-15	multi	98.9	0.00	1.27	0.28	24,610	1,303	99.74
Acer VI 13714	USVI	Hans Lollik 2	29-Oct-15	solo	96.1	1.53	1.26	0.28	3,725	1,299	99.66
Acer VI 13708	USVI	Botany 2	28-Oct-15	multi	98.9	0.00	1.29	0.28	45,966	1,302	99.75
Acer BE 13797	Belize	Sandbores 2	7-Nov-15	solo	100.0	0.00	1.28	0.28	22,023	1,305	99.59
Acer BE 13792	Belize	Sandbores 2	7-Nov-15	solo	96.7	0.28	1.26	0.28	8,464	1,295	99.61
Aprol BE 13843	Belize	Glovers Atoll	8-Nov-15	solo	84.3	0.75	1.21	0.28	2,686	1,288	99.42
Acer BE 13827	Belize	Glovers Atoll	8-Nov-15	solo	100.0	0.00	1.29	0.28	17,891	1,302	99.61
Aprol BE 13778	Belize	S.Carrie Bow Cay	5-Nov-15	solo	100.0	0.00	1.29	0.28	27,657	1,208	99.65
Acer BE 13786	Belize	S.Carrie Bow Cay	6-Nov-15	multi	91.2	1.95	1.25	0.28	3,035	1,215	99.58
Acer BE 8867	Belize	Curfew	13-Sep-12	solo	100.0	0.00	1.28	0.28	27,658	1,184	99.6


Reef
Florida
Key
DT
USVI
Sap
HS
Bot
Belize
SB
GA
SC
CF
Host
Ac. cervicornis
Ac. prolifera
Shared Orthologs
600
400
200
0
547
104
93
53
53
46
40
33
30
22
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Acer 44
Acer FL 14120
Acer FL 4923
Aprol FI 1303
Acer VI 13738
Acer VI 13714
Acer VI 13708
Acer BE 13797
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Acer BE 8867
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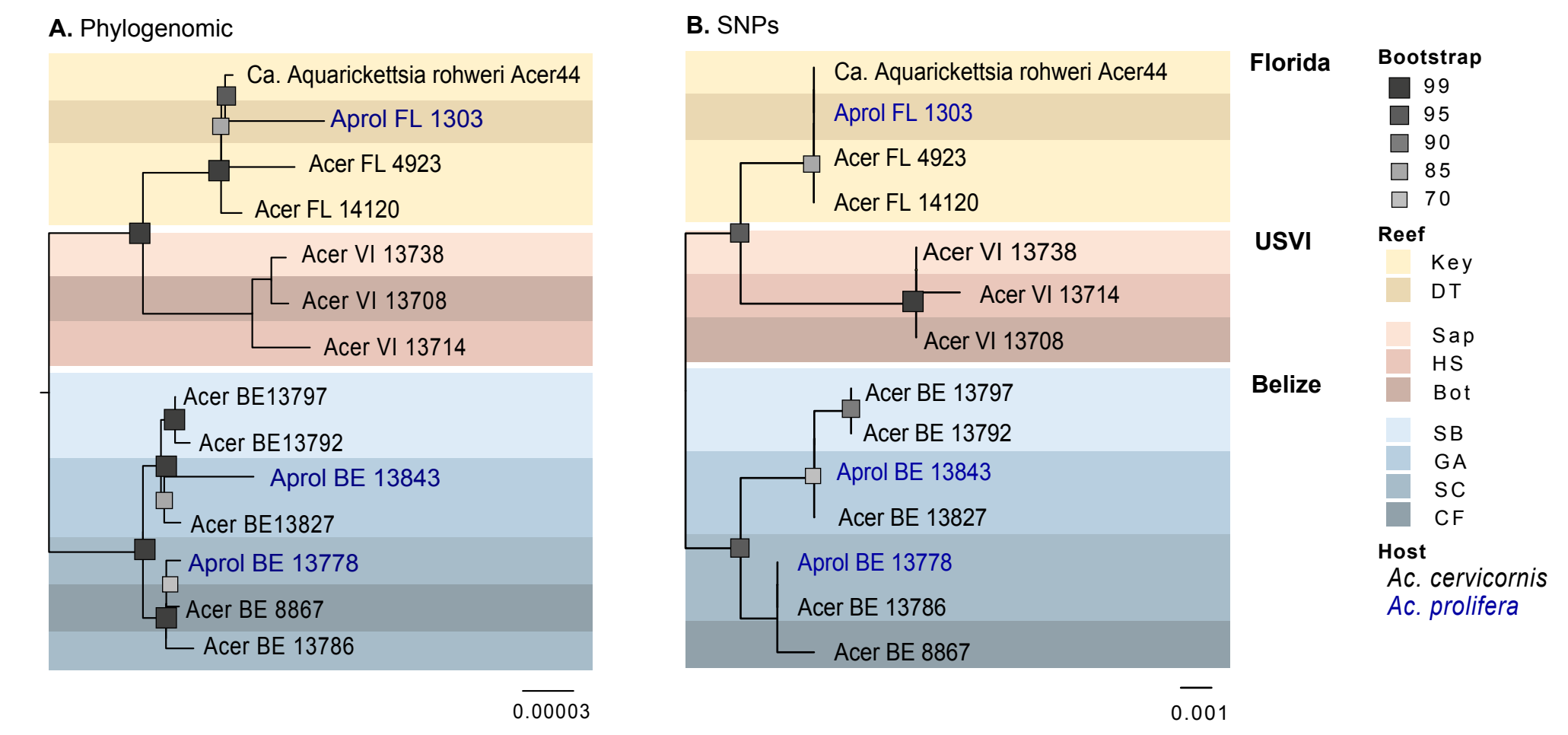
Orthologs


A. Phylogenomic
Ca. Aquarickettsia rohweri Acer44
Aprol FL 1303
Acer FL 4923
Acer FL 14120
Acer VI 13738
Acer VI 13708
Acer VI 13714
Acer BE13797
Acer BE13792
Aprol BE 13843
Acer BE13827
Aprol BE 13778
Acer BE 8867
Acer BE 13786
0.00003
B. SNPs
Ca. Aquarickettsia rohweri Acer44
Aprol FL 1303
Acer FL 4923
Acer FL 14120
Acer VI 13738
Acer VI 13714
Acer VI 13708
Acer BE 13797
Acer BE 13792
Aprol BE 13843
Acer BE 13827
Aprol BE 13778
Acer BE 13786
Acer BE 8867
0.001
Florida
USVI
Belize
Bootstrap
99
95
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Reef
Key
DT
Sap
HS
Bot
SB
GA
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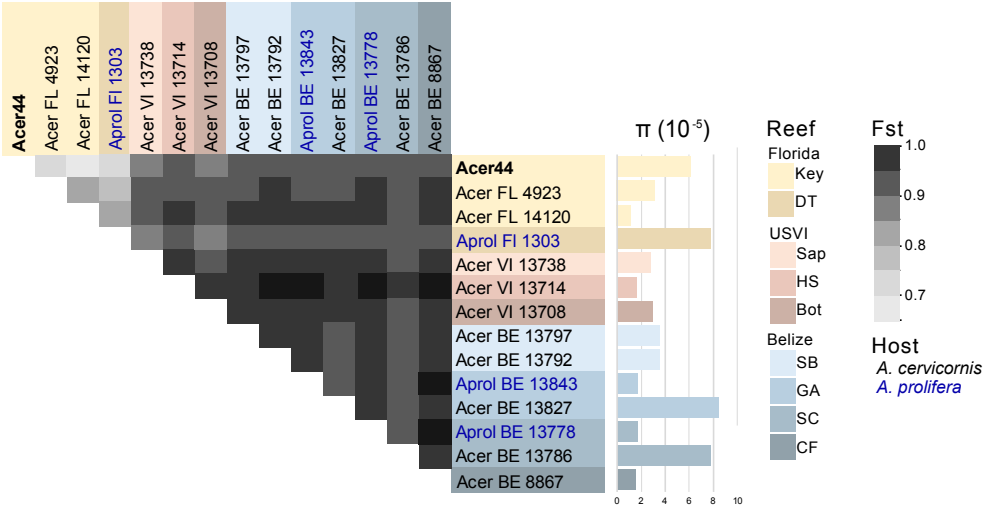
Host
Ac. cervicornis
Ac. prolifera


Acer44
Acer FL 4923
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Acer VI 13714
Acer VI 13708
Acer BE 13797
Acer BE 13792
Aprol BE 13843
Acer BE 13827
Aprol BE 13778
Acer BE 13786
Acer BE 8867
π (10-5)
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2
4
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Reef
Florida
Key
DT
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HS
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1.0
0.9
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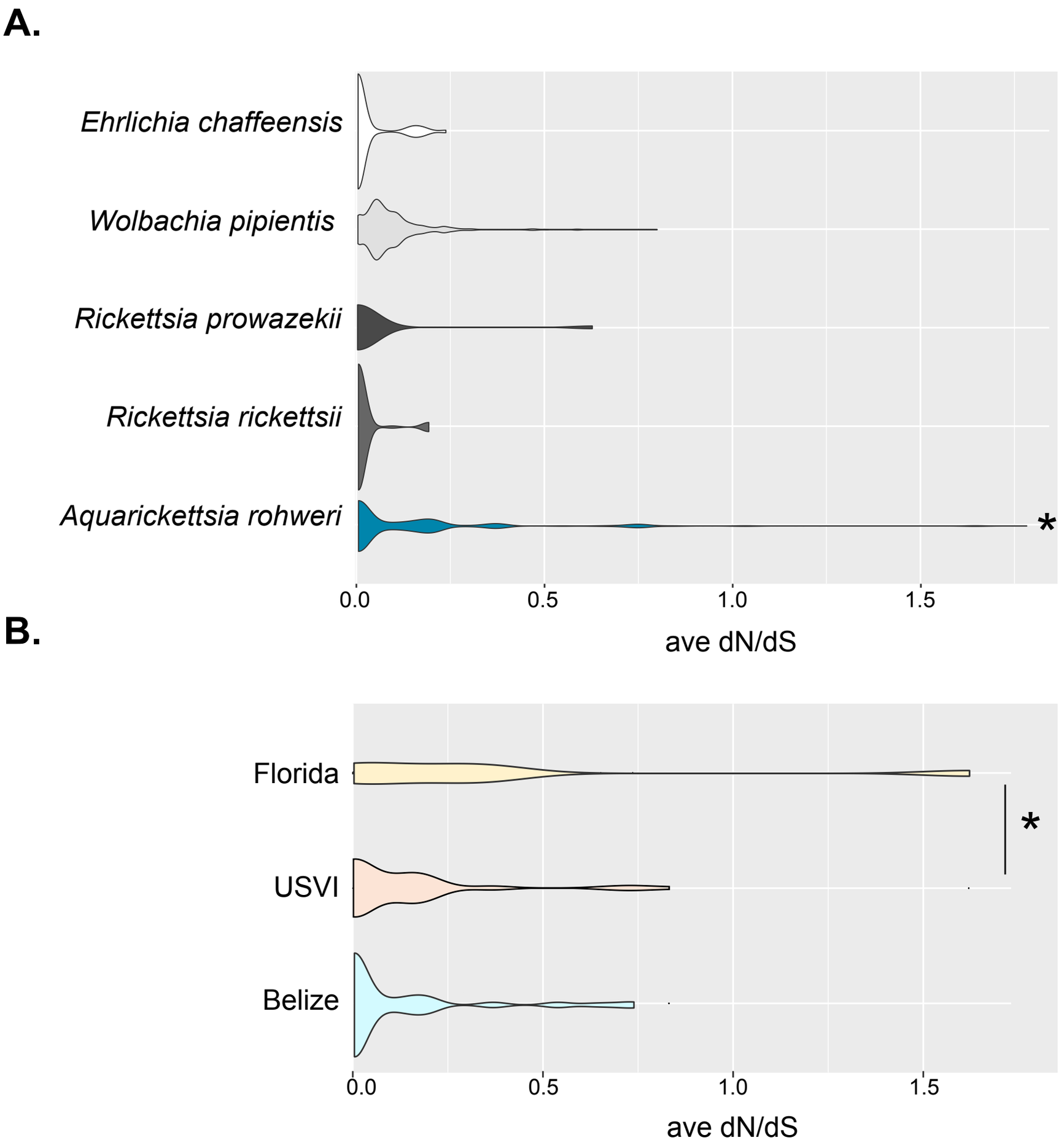
Host
A. cervicornis
A. prolifera


A.
Ehrlichia chaffeensis
Wolbachia pipientis
Rickettsia prowazekii
Rickettsia rickettsii
Aquarickettsia rohweri
*
0.0
0.5
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Florida
USVI
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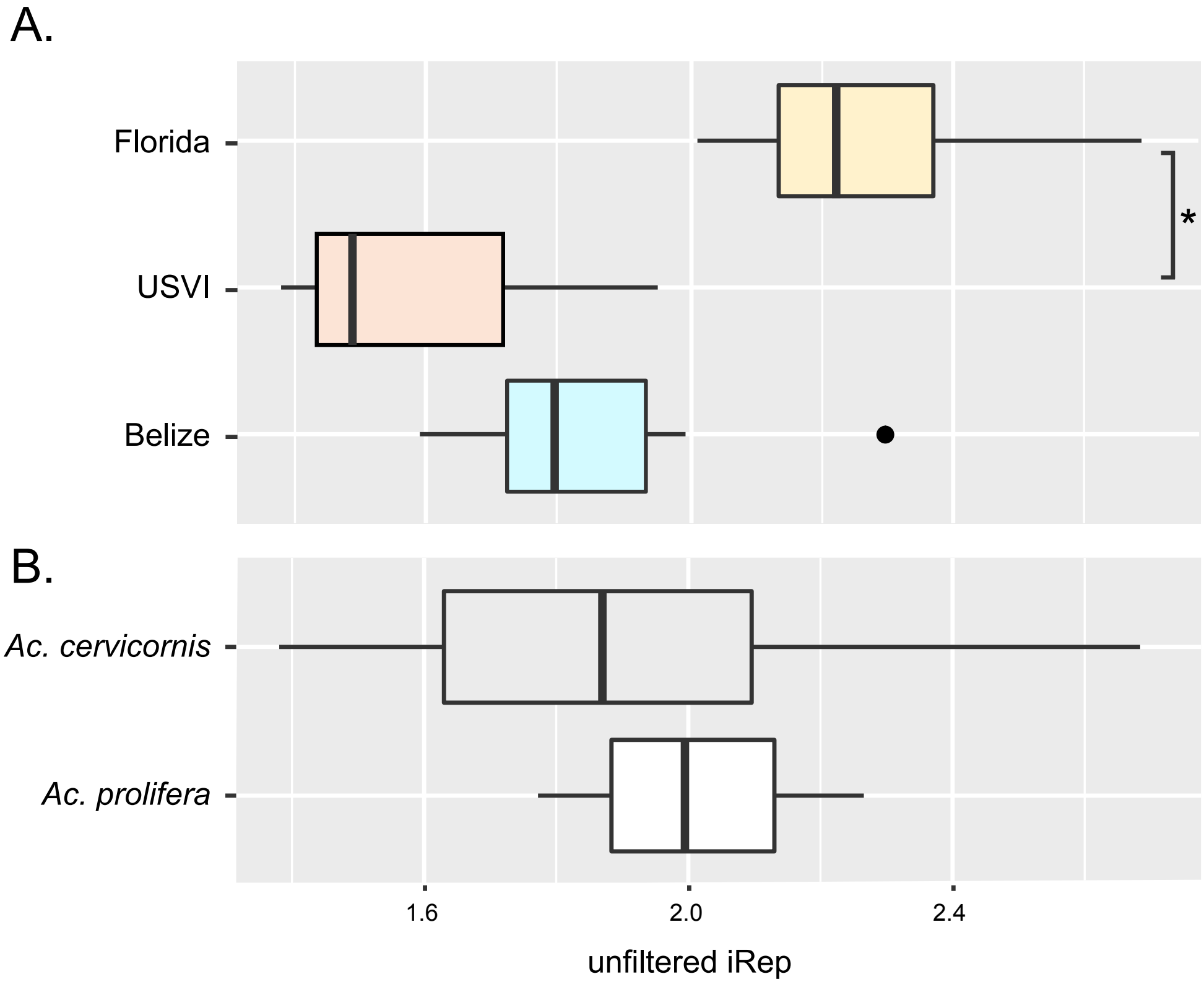
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Ac. cervicornis
Ac. prolifera
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