Endosymbiont diversity across native and invasive brown widow spider populations

Monica A. Mowery[1*], Laura C. Rosenwald[2], Eric Chapman[2], Yael Lubin[1], Michal Segoli[1], Thembile Khoza[3], Robin Lyle[4], Jennifer A. White[2]

1. Mitrani Department of Desert Ecology, Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, Israel
2. Department of Entomology, University of Kentucky, Lexington, KY, USA
3. South African National Biodiversity Institute, Biosystematics Division, Pretoria, South Africa
4. Agricultural Research Council – Plant Health and Protection, Biosystematics Division, Queenswood, South Africa

*Current affiliation: Department of Biology, York College, The City University of New York, Jamaica, NY, USA. Email: mmowery@york.cuny.edu

Abstract

The invasive brown widow spider, *Latrodectus geometricus* (Araneae: Theridiidae), has spread in multiple locations around the world and, along with it, brought associated organisms such as endosymbionts. We investigated endosymbiont diversity and prevalence across putative native and invasive populations of this spider, predicting lower endosymbiont diversity across the invasive range compared to the native range. First, we characterized the microbial community in the putative native (South Africa) and invasive (Israel and the United States) ranges via high throughput 16S sequencing of 103 adult females. All specimens were dominated by reads from only 1-3 amplicon sequence variants (ASV), and most individuals were infected with an apparently uniform strain of *Rhabdochlamydia*. We also found *Rhabdochlamydia* in spider eggs, indicating that it is a maternally-inherited endosymbiont. Relatively few other ASV were detected, but included two variant *Rhabdochlamydia* strains and several *Wolbachia*, *Spiroplasma* and Enterobacteriaceae strains. We then diagnostically screened 118 adult female spiders from native and invasive populations specifically for *Rhabdochlamydia* and *Wolbachia.* We found *Rhabdochlamydia* in 86% of individuals and represented in all populations, which suggests that

it is a consistent and potentially important associate of *L. geometricus. Wolbachia* was found at lower overall prevalence (14%) and was represented in all countries, but not all populations. In addition, we found evidence for geographic variation in endosymbiont prevalence: spiders from Israel were more likely to carry *Rhabdochlamydia* than those from the US and South Africa, and *Wolbachia* was geographically clustered in both Israel and South Africa. Characterizing endosymbiont prevalence and diversity is a first step in understanding their function inside the host and may shed light on the process of spread and population variability in cosmopolitan invasive species.

Introduction

When moving into new habitats, invasive species may bring along microbial associates that can influence the invasion process [1,2]. Some microbes are vertically inherited endosymbionts that are restricted to the invasive species, but may yet influence interactions between the invasive and native species. Maternally-inherited endosymbionts have been shown to affect traits important to host fitness such as dispersal [3], fecundity [4], and defenses against natural enemies [5], potentially providing an advantage to the invasive species [6]. Some endosymbionts affect the organism's reproductive biology, for example, by modifying offspring sex ratio in infected populations, which can affect the speed of invasive spread [7]. For example, acting as both a mutualist and reproductive manipulator, *Rickettsia* caused whiteflies to have higher fitness and a higher proportion of daughters, and quickly spread in invasive populations [8].

Assessing endosymbiont prevalence across geographically distant populations can provide a key to understanding the role of a symbiont. Widespread prevalence of a facultative endosymbiont suggests that the symbiont plays a functional role in its host, such as providing fitness benefits or manipulating reproduction [9,10]. The latter is often manifested by sex ratio

distortions, although the most common reproductive manipulation is cytoplasmic incompatibility (CI), which causes incompatibilities between infected males and uninfected females but does not alter the sex ratio of the population [11]. Our understanding of the dynamics and prevalence of facultative endosymbiont infection during invasive spread is limited, especially for non-insect arthropod endosymbionts.

Invasive populations are predicted to exhibit reduced endosymbiont prevalence and diversity compared to native populations. During founding events, often few individuals are initially introduced into the invasive range [12], in which case only a subset of endosymbionts found in the native range might be introduced to the new location [13]. However, in most biological invasions, multiple introductions are common [14], and so endosymbiont diversity might be lower initially, and then increase over time as more individuals arrive from various localities [15]. Comparing endosymbiont diversity across invasive and native populations can provide valuable insights into the gain and loss of microbial communities during the invasion process.

The brown widow spider, *Latrodectus geometricus* (Theridiidae), is a medically important spider with neurotoxic venom. *Latrodectus geometricus* has spread recently to multiple locations around the world from the putative native range in southern Africa, most likely via cargo shipments [16,17]. Evidence suggests that during invasion, establishment and spread, spider traits related to dispersal, fecundity, and body size shifted across populations that were established over different time periods [18]. In addition to these shifts in ecologically important traits, associations with other organisms, such as parasitoids [19] or endosymbionts, may have also changed during the invasion spread.

Endosymbionts of widow spiders (genus *Latrodectus*) are poorly known. A previous study on *L. geometricus* identified the endosymbiont *Rhabdochlamydia,* but only examined a few adult females in a single, inbred lab population in Florida, USA [20]. The same study did not detect *Rhabdochlamydia* in two other *Latrodectus* species. Hence, a further study across field-collected individuals worldwide is necessary to assess the presence of *Rhabdochlamydia* more broadly across populations of *L. geometricus*. The family Rhabdochlamydiaceae (Phylum: Chlamydiota) is predicted to be the most diverse chlamydial family [21]. It includes important vertebrate and human pathogens and is widespread across soil and aquatic ecosystems with many yet unknown hosts [22]. The genus *Rhabdochlamydia* has been found in a few distantly-related invertebrate hosts*,* including a cockroach [23], a tick [24], a dwarf spider [25], and a terrestrial isopod [26], although it was not found at a high prevalence within any of these species.

Also previously found in invasive populations of *L. geometricus* was *Wolbachia,* as a facultative associate in varying prevalence across populations [27]. *Wolbachia* infection is common in arthropods, with 40-60% of species infected [28], as well as in other invertebrates including nematodes [29]. *Wolbachia* is known to affect the fitness and reproduction of many of its hosts, which could have implications for successful invasive establishment and spread [30].

In this study, we compared endosymbiont presence and diversity across populations of the brown widow spider, *L. geometricus,* from the putative native range in South Africa to populations in the invasive range in the United States and Israel, using both high-throughput sequencing and diagnostic PCR screens. Our objectives were to 1) characterize the dominant endosymbionts in *L. geometricus,* 2) compare prevalence and diversity across purported native and known invasive ranges, and 3) investigate geographic patterns of endosymbiont infection within countries. We predicted that, due to founder effects, some endosymbiont would be lost

and infection rates would be lower in invasive populations in the U.S. and Israel compared to putative native populations in South Africa, and that geographic patterns of endosymbiont loss would reflect the proposed routes of invasive spread of *L. geometricus* within each country.

Methods

Study species

Latrodectus geometricus, the brown widow spider, is a globally invasive species that has established populations in parts of North and South America, the Middle East, Australia, and Asia [17]. In the United States, *L. geometricus* was first detected in Miami, Florida in 1936 [31], was confined to southern Florida until the late 1990s, and was subsequently detected in Texas and California in the 2000s [32]. In Israel, *L. geometricus* was first detected in the Tel Aviv area in 1980 [33], and in the Negev region after 2000 [34]. Throughout the global invasive range, *L. geometricus* is found in urban and settled habitats, and builds nests on and around buildings, on fences, garden furniture, trash bins, and in playgrounds [17].

Study sites

We collected *L. geometricus* adult females from urban environments across the United States (Edisto Island, South Carolina $n = 10$; Gainesville, Florida $n = 10$; Austin, Texas $n = 6$; Los Angeles, California $n = 7$), Israel (Haifa $n = 7$, Tel Aviv $n = 10$, Be'er Sheva $n = 10$, Yeruham $n = 8$, Midreshet Ben-Gurion $n = 10$, Eilat $n = 1$), and South Africa (Modimolle $n = 10$, Pretoria $n = 5$, Johannesburg $n = 5$, Kimberley $n = 8$, Cape Town $n = 5$, Riebeeck-Kasteel $n = 6$, George $n = 7$). Spiders were deprived of food for one week before they were preserved in 100% ethanol. Starved individuals have minimal gut content and are less likely to result in false positives for endosymbionts found in the spider's prey [35]. To learn about the potential for vertical transmission, we also collected *L. geometricus* egg sacs from two sites in South Africa:

Kimberley (n = 1) and Riebeeck Kasteel (n = 2), and sampled egg sacs produced in the laboratory from Midreshet Ben-Gurion (n = 3) and Tel Aviv (n = 2), Israel.

Bacterial 16S sequencing

We surface-sterilized each adult female *L. geometricus* specimen (n = 125) with a series of bleach and ethanol rinses [36] before longitudinally dividing the abdomen in half and extracting DNA from one half using DNeasy Blood and Tissue extraction kits (Qiagen, Germantown, MD) according to manufacturer's instructions. In addition, we extracted DNA from the legs of two specimens, as well as from the eggs of 8 *L. geometricus* egg sacs to assess endosymbiont presence outside reproductive tissues and the potential for maternal transmission, respectively. Extraction quality for each sample was verified by PCR amplification of a ~650 bp segment of the COI gene (forward primer, lco1490: 5'-GGTCAACAAATCATAAAGATATTGG-3', reverse primer, hco2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'; cycling conditions: one cycle of 94ºC for 3 min, followed by 35 cycles of 95ºC for 30 s, 53ºC for 30 s, 72ºC for 1 min, final extension at 72ºC for 5 min [37]. If COI failed to amplify, we attempted a second extraction with the other half of the abdomen. If this extraction failed to amplify product as well, we assumed sample preservation had been poor and eliminated the specimen from the dataset entirely (7/125 specimens).

To investigate which endosymbionts were present in these specimens, we profiled the microbiomes using high-throughput sequencing of the bacterial community. We amplified the V4 region of bacterial 16S rRNA for each sample using dual indexed 515F/806R primers [38]. We visualized the resulting products, and multiplexed 1 µl aliquots from successful amplifications into one of two libraries that were purified with GenCatch PCR Cleanup Kits. Samples that failed to amplify (6/118 samples) were not included in the library. Each library also

145 included specimens from other projects that are not reported here, and received a PhiX spike to
146 increase sequence heterogeneity among the amplified sequences. Libraries were sequenced at the
147 University of Kentucky genomics core facility on an Illumina Miseq instrument using a paired-
148 end strategy and 250bp reads. Sequences from each run were demultiplexed, trimmed and
149 quality filtered within BaseSpace (Illumina, basespace.illumina.com), then imported into
150 QIIME2 (v2021.11, https://qiime2.org [39]) using a manifest. We conducted additional quality
151 control using deblur [40] implemented in QIIME2 using default parameters and a trim length of
152 251 bases. Resulting amplicon sequence variants (ASV) were taxonomically classified using a
153 naïve Bayes classifier that was trained on the 515F/806R V4 region of the Greengenes 13_8 99%
154 OTUs reference database [41]. We filtered out 15 ASV that originated from other specimens in
155 the sequencing run (e.g., obligate endosymbionts of other host taxa, see [42] for discussion of
156 index swapping), which collectively constituted only a small minority (0.14%) of the 3.57×10^6
157 reads associated with the *L. geometricus* samples. Following filtering, *L. geometricus* samples
158 with less than 1000 reads were excluded from further analysis (9/112 adult samples, 6/8 egg
159 samples). For the remaining samples, we blasted high prevalence ASV sequences (>1% of any *L.*
160 *geometricus* sample) against the NCBI nt database using the megablast algorithm, to identify
161 bacterial taxa that may not have been included in the reference database. For ASV that appeared
162 at very high prevalence or frequency (>90% of reads for any specimen, or found in multiple
163 specimens across multiple locations), we amplified a longer segment of 16S using universal
164 primers from specimen(s) dominated by that taxon, to aid in taxonomic placement (Forward
165 primer, 27F: 5'-AGAGTTTGATCMTGGCTCAG-3', reverse primer 1492R: 5'-
166 GGTTACCTTGTTACGACTT-3', cycling conditions: one cycle of 95ºC for 2 min, followed by
167 35 cycles of 92ºC for 30 s, 55ºC for 30 s, 72ºC for 30 s, final extension at 72ºC for 6 min [43].

Diagnostic PCR

We diagnostically screened all samples (all 118 adult female, 8 egg, and 2 leg samples) for the two bacterial genera previously identified from *L. geometricus*: *Wolbachia* (Class Alphaproteobacteria, Order Rickettsiales, Family Anaplasmataceae) and *Rhabdochlamydia* (Class Chlamydiia, Order Chlamydiales, Family Rhabdochlamydiaceae; Arrington, 2014; Dunaj et al., 2020). For *Wolbachia*, we followed previously published protocols (Baldo et al., 2006), using primers specific to the *Wolbachia* surface protein (*wsp*) gene (Forward primer, wspF1: 5'-GTCCAATARSTGATGARGAAAC-3', reverse primer wspR1: 5'-CYGCACCAAYAGYRCTRTAAA-3', cycling conditions: one cycle of 94ºC for 2 min, followed by 36 cycles of 94ºC for 30 s, 59ºC for 45 s, 72ºC for 1 min 30 s, final extension at 70ºC for 10 min [44]. For *Rhabdochlamydia*, we designed new primers in Primer3 [45] to amplify a ~540bp segment of 16S: Rhabdo_108F 5'-ACACTGCCCAAACTCCTACG-3' and Rhabdo_647R

5'-TTAGCTWCGACACAGCCAGG-3'. All reactions were run in 10µl volume; the *Rhabdochlamydia* reactions included 3 µL purified water, 1µL 10X Buffer (New England Biolabs), 1.2 µL 10mM dNTPs, 1.5 µL 25mM $MgCl_2$, 0.6µL each of forward and reverse primers at 5µM, and 0.1 µL of 5U New England Biolabs Taq Polymerase. PCR reactions received one cycle of 94ºC for 2 min, followed by 25 cycles of 95ºC for 15 s, 56ºC at 15 s, 68ºC for 45 s. We electrophoresed and visualized the products on 1% agarose gels stained with Gel Red (Biotium) alongside known positive and negative (reagents-only) controls. Samples with initial negative diagnoses were retested before being categorized as uninfected. For a subset of the samples with positive evidence of infection, we repeated the PCR at a 20µl volume and purified the PCR product with either GenCatch PCR Cleanup or Gel Extraction Kits (Epoch Life

191 Sciences, Missouri City, TX) according to manufacturer's instructions. Products were then
192 submitted for Sanger sequencing (Eurofins, Louisville, KY). Resulting sequences were
193 compared to the NCBI nucleotide database using the megablast algorithm, and specimens
194 returning a 97% or higher match to the expected bacterial genus were scored as positive. For
195 each strain of *Wolbachia*, we sequenced 5 MLST genes (*coxA*, *fbpA*, *ftsZ*, *gatB* and *hcpA*) and
196 the *Wolbachia* surface protein (*wsp*) according to [44].

197 For *Rhabdochlamydia,* we ran phylogenetic analyses to place the *L. geometricus* strains,
198 using a set of accessions across Chlamydiia with *Oligosphaera ethanolica* as an outgroup. For
199 each analysis, multiple alignments were assembled using the MAFFT server (v. 7;
200 https://mafft.cbrc.jp/alignment/server/ [46]) using the Q-INS-I alignment method that takes
201 secondary structure into account. Maximum likelihood phylogenetic analyses were conducted on
202 1576-character aligned datasets using Garli (v. 2.01 [47]). We applied the most complex model
203 available (GTR+I+G [48]) as per recommendations of Huelsenbock and Rannala [49] for
204 likelihood-based analyses. We conducted a 100-replicate ML search for the tree of highest log-
205 likelihood and a 500-replicate ML bootstrap analysis [50] with two search replicates per
206 individual bootstrap replicate. All analyses used the default settings.

207 We used the same approach to generate a *Wolbachia* phylogeny. We used a concatenated
208 data set containing 5 MLST genes (*coxA*, *fbpA*, *ftsZ*, *gatB* and *hcpA*; total of 2079 characters)
209 with 38 *Wolbachia* strains pulled from the Wolbachia PubMLST website
210 (pubmlst.org/organisms/wolbachia-spp [51]). Because rooting *Wolbachia* trees is challenging
211 [52], and our objective was only placement of our new strains within established *Wolbachia*
212 supergroups, we chose to simply root the tree within Supergroup A. Individual specimens were
213 scored for the presence of *Rhabdochlamydia* and *Wolbachia* based on the combination of

diagnostic, high-throughput, and Sanger sequencing data. For a sample to be scored positive, a positive diagnostic PCR needed to be corroborated by either high-throughput or Sanger sequencing validation. For a sample to be scored negative, consistent negative diagnostic PCRs needed to be accompanied by positive validation of spider COI and/or other bacterial taxa.

Statistical methods

All analyses were conducted in R version 4.0.2 [53]. To compare the prevalence of the dominant strains of *Rhabdochlamydia* and *Wolbachia* across South Africa, Israel, and the United States, we used a general linear model ("lme4" package [54]) with a binomial link function, with *Rhabdochlamydia1* or *Wolbachia1* presence or absence in an individual as the response variable, and country as the predictor. Maps showing collection localities in South Africa, Israel, and the United States, were generated using the R package ggspatial [55].

Results

Compared to most microbiomes in arthropods, *L. geometricus* spiders have a depauperate microbial fauna. Of 103 adult female spiders that produced sufficient read depth (mean ± SE of 33844 ± 2026 sequences per sample), all were dominated by one to three bacterial strains that accounted for greater than 90% of the reads (Figure 1). In 64 samples, a single strain accounted for greater than 99% of reads. In most samples, the most prevalent bacterial ASV was *Rhabdochlamydia* (83/103 samples) although a few samples each were dominated by ASVs corresponding to *Wolbachia* (6 samples), Enterobacteriaceae (10 samples), *Providencia* (2 samples), *Wohlfahrtimonas* (1 sample) and a bacteria that could not be placed by the Greengenes reference database, but which our analyses (see below) place within the Chlamydiales (Chlamydiales1, 3 samples, Figure 1).

Most samples had at least some *Rhabdochlamydia* representation. Nine samples from several locations in South Africa and the United States had negligible representation (<0.1% of reads) of *Rhabdochlamydia*. The number of *Rhabdochlamydia* reads in the latter samples ranged from 0 (out of 4222 reads) to 359 (out of 37618 reads), and most fell below the number of *Rhabdochlamydia* reads seen in blanks (9-81 reads). Two samples were diagnostically positive for *Rhabdochlamydia* despite low numbers of reads, and were additionally validated by Sanger sequencing of the diagnostic product, thus were counted as *Rhabdochlamydia* positive in the final dataset. In the remaining seven specimens, the low number of proportional reads and the diagnostic absence supports the genuine absence of *Rhabdochlamydia*. Of the additional 15 samples that were excluded from high throughput analysis due to poor initial amplification or insufficient read depth, six were validated to have *Rhabdochlamydia* and nine did not.

To gain insight into the occurrence of strains of the major endosymbionts found, we used Sanger sequencing data to distinguish among strains of the same symbiont clade. Most detected *Rhabdochlamydia* strains were identical (GenBank Accession #OP598824). Two variant strains were detected, each in one individual. The variant strain from a Modimolle, South Africa specimen (#OP598825) was 99.8% similar to the dominant strain, differing at only 1/480 bases of 16S. The variant strain from Eilat, Israel (#OP598826) was 98.8% similar, differing at 6/480 bases of 16S. Phylogenetically, all three strains were clustered together within the genus *Rhabdochlamydia* and family Rhabdochlamydiaceae (Figure 2).

Wolbachia was much less common than *Rhabdochlamydia*, found in 14% (17/118) of individuals, but represented in spiders collected from all three regions. We were able to sequence all MLST genes and *wsp* for all three strains of *Wolbachia* (accession numbers OP612314-OP612330), except *gatB* in *L. geometricus Wolbachia*3. The most widespread and characteristic

strain of *Wolbachia* in *L. geometricus*, *Wolbachia1*, was present in 13/118 specimens (11%), and phylogenetic analysis placed the strain in *Wolbachia* Supergroup F (Supplementary Figure 1). In contrast*, L. geometricus Wolbachia*2, which was found in four specimens across three localities in South Africa, belongs to a different *Wolbachia* clade, Supergroup B. A third *Wolbachia* strain, *L. geometricus Wolbachia3,* which was found in a single sample that had not been included in high throughput sequencing but was validated with diagnostic PCR and subsequent sequencing, was placed in Supergroup A.

Only 16 other ASV, besides *Rhabdochlamydia* and *Wolbachia,* were ever found at >1% prevalence in any sample, and the majority of these (nine) were each found in single specimens. Enterobacteriaceae1 represented a substantial proportion of reads in 12 individuals across several locations in South Africa and the United States, and was the dominant ASV in eight individuals. When blasted against the NCBI database, a 1359bp segment of 16S from this bacterium (#OP598828) was not closely aligned to any other accessions, bearing greatest resemblance to aphid secondary symbionts (e.g., EU348326 at 96.8%) or *Gilliamella*, a specialized honeybee gut symbiont (e.g., CP048265 at 95.84%). Enterobacteriaceae1 was absent from Israel, although a different Enterobacteriaceae ASV was detected from two individuals collected from one location in Israel. Two other gammaproteobacteria ASVs, *Providencia* and *Wohlfahrtiimonas*, were present in two and one specimens, respectively. One bacterial strain, which was found in four individuals across two locations in the southeast U.S., was not able to be placed against the Greengenes database in the QIIME2 pipeline, but a 498bp segment of 16S aligns most closely with other Chlamydiales in GenBank (e.g. FJ976094 at 87.2%). Our chlamydial phylogeny (Figure 2), also supports placement within this order, hence we have designated it Chlamydiales1. Other bacterial ASV were only found at a low percentage of reads across spiders

(two *Acenitobacter* ASV, two *Spiroplasma* ASV, and one each of *Entomoplasmatales, Sporosarcina, Bacillus, Enterococcus,* and *Lactococcus*).

Comparing across the three countries, a higher proportion of spiders collected in Israel were infected with the dominant strain of *Rhabdochlamydia, Rhabdochlamydia1,* than spiders from South Africa (GLM, z = -2.128, p = .033) or the U.S. (z = -2.538, p = .011). We found no differences in prevalence of the dominant *Wolbachia* strain, *Wolbachia1,* across countries (GLM, US-Israel, z = -0.689, p = .491; US-South Africa, z = -1.268, p = .205; Israel-South Africa, z = -0.669, p = .504). Using diagnostic PCR screening, we found evidence for *Rhabdochlamydia* in 100% (8/8) of *L. geometricus* eggs tested from South Africa and Israel. In contrast, only two out of eight egg sacs showed signal of *Wolbachia,* both from Tel Aviv, consistent with the proportional infection rate in adults from the source populations.

Wolbachia prevalence was too low for formal spatial analysis, but visually appeared to have some level of clustering (Figure 3). In South Africa, both *Wolbachia1* and *Wolbachia2* were found in northeastern populations (Johannesburg, Pretoria, and Modimolle) but were not detected elsewhere in the country. Likewise, in Israel, *Wolbachia1* was present in central and northern populations (Tel Aviv and Haifa), but was not detected in the southern Negev populations (Beer Sheva, Yeruham, Sede Boqer, Eilat). Among the four U.S. populations, *Wolbachia1* was found in spiders collected from Florida and Texas, *Wolbachia3* was in South Carolina, but no *Wolbachia* was detected in spiders from California, the most recently detected invasive population.

Discussion

Latrodectus geometricus spiders have maintained a characteristic microbiome throughout their global spread. We identified one predominant endosymbiont, *Rhabdochlamydia1* in almost all

305 spiders (86%), and represented in all collection locations. We also found a characteristic
306 Supergroup F *Wolbachia* (*Wolbachia1*) represented in all countries, albeit in fewer individuals
307 (11% of spiders). We detected both *Rhabdochlamydia1* and *Wolbachia1* in *L. geometricus* eggs,
308 indicating that both are vertically transmitted endosymbionts.

309 The widespread presence of *Rhabdochlamydia* suggests that it might be important
310 functionally for the host. In other arthropods, endosymbionts found at consistently high
311 frequency across wide geographic ranges have often subsequently been found to have important
312 fitness or reproductive consequences for their hosts [56,57]. Little is known about the functional
313 role of *Rhabdochlamydia* in arthropods. It was described from a variety of mostly non-insect
314 arthropods and was generally found at low prevalence in the tested populations [23,24,26]. In a
315 terrestrial isopod, *Rhabdochlamydia* had pathogenic effects [26]. The high prevalence (86%)
316 and vertical transmission of *Rhabdochlamydia* in *L. geometricus* argue against a strongly
317 pathogenic role for this bacterial strain within our system. Genomic analysis of
318 *Rhabdochlamydia* found in other arthropod hosts, an isopod and a tick, found pathways for
319 polyamine synthesis [22], which are relevant for virulence and stress responses, suggesting that
320 some strains of this bacteria are potentially beneficial in their host.

321 We also detected *Rhabdochlamydia* in *L. geometricus* legs, consistent with the work of
322 Dunaj et al. [20], which indicated that the bacteria is found throughout the body and not just
323 restricted to reproductive tissue. Dunaj et al. [20] also found that the bacterial community of *L.*
324 *geometricus* was dominated by *Rhabdochlamydia*, lacking the microbial diversity of the other
325 spider species they examined, and speculated that this result may have been an artifact of
326 laboratory-reared, inbred *L. geometricus* spiders. Our field collected spiders from locations
327 around the world suggest that their result was not an artifact, but a genuine representation of a

328 characteristic and depauperate bacterial community in *L. geometricus*. Vertically transmitted
329 bacterial symbionts often dominate the sampled microbiomes of their hosts, overwhelming the
330 signal from more casual bacterial associates [11,25,35].

331 Importantly, maternal transmission of *Rhabdochlamydia* suggests the possibility of
332 reproductive manipulation of host by symbiont. Reproductive manipulation is extremely
333 common in vertically transmitted symbionts, and the list of bacteria that have been demonstrated
334 to induce such manipulations is rapidly expanding [11,58]. *Rhabdochlamydia* has not yet been
335 tested for host reproductive manipulation. The widespread prevalence and vertical transmission
336 of *Rhabdochlamydia* in *L. geometricus* would make this system an excellent prospect for such
337 investigations.

338 *Latrodectus geometricus* was host to several strains of *Wolbachia*, a bacterial clade well
339 known for reproductive manipulation. *Wolbachia* is common in spiders, but most strains belong
340 to Supergroup A or B, as is the case in insects [30]. In contrast, the dominant *Wolbachia* strain in
341 *L. geometricus* belongs to Supergroup F, which has rarely been reported for spiders. Supergroup
342 F has been found sporadically in arthropods, including South African scorpions [59], termites
343 [60], quill mites [61], and nematodes [62]. Preliminary work on *L. geometricus* suggested that
344 *Wolbachia* might induce mild CI in this species [63], but the strain of *Wolbachia* was not
345 characterized, and additional experiments will be necessary to fully validate CI in this system.

346 Although symbiont communities were largely similar across our sampled regions, we did
347 find some subtle differences between the likely native and invasive ranges. *Rhabdochlamydia*
348 was found at highest prevalence in Israel compared to populations in the U.S. and South Africa.
349 Multiple strains of *Rhabdochlamydia, Wolbachia,* and the Enterobacteriaceae were found in
350 South Africa, the putative native population. The dominant strain of Enterobacteriaceae was

found in South Africa and the U.S., but absent in Israel, the newest invasive region that we sampled. From a previous study, *Wolbachia* prevalence in *L. geometricus* in the U.S. was highest near the initial site of introduction in Florida [27]. In comparison, we found lower *Wolbachia* prevalence in other locations in the southeastern and central U.S, and absence in spiders from California, the most recently established population. Similarly, in Israel, *Wolbachia* was absent in recently established populations in southern Israel. These patterns are consistent with the loss of endosymbionts during the invasion process, but more localities, specimens, and more knowledge of the invasion route is needed. Climatic differences such as hotter, dryer conditions in the Negev Desert in southern Israel could also contribute to reduction of *Wolbachia* [64], although deeper sampling effort would be needed to assess whether *Wolbachia* is entirely absent from these locations.

Further work will test the functional role and fitness effects of endosymbiont presence in *L. geometricus*, as well as compare patterns of host-endosymbiont diversity during invasive spread. Invasive *L. geometricus* are highly dispersive [18], and are less susceptible to parasitism by parasitoids compared to native widow species in the invasive range [19]. It would be valuable to test whether these advantages and others during invasion are related to interactions with endosymbionts. In particular, the dominance and high prevalence of *Rhabdochlamydia* across global populations of *L. geometricus* suggests an important role of this endosymbiont. Characterizing potentially important and widespread endosymbionts is a step towards understanding their relevance to ecological interactions and responses to rapid environmental changes.

Acknowledgements: We thank Ofir Altstein, Ishai Hoffmann, Cayley Buckner, Madison Heisey, Catherine Scott, Nishant Singh, Alyssa Fuller, Astri Leroy, Annari van der Merwe,

Fiona Hellmann, Joh Henschel, Theresa Henschel, and Colin Ralston for assistance collecting spiders. This work was supported by a Zuckerman STEM Leadership Postdoctoral Fellowship to MAM. This material is based upon work supported by the National Science Foundation under Grant No. 1953223 and the National Institute of Food and Agriculture, U.S. Department of Agriculture (Hatch No. 1020740).

Data availability statement: The datasets generated and/or analyzed during the current study are available via NCBI SRA, Bioproject PRJNA1068539: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1068539

References

1. Chalkowski, K., Lepczyk, C. A. & Zohdy, S. Parasite ecology of invasive species: conceptual framework and new hypotheses. *Trends in Parasitology* **34**, 655–663 (2018). https://doi.org/10.1016/j.pt.2018.05.008
2. Sepúlveda, D. A., Zepeda-Paulo, F., Ramírez, C. C., Lavandero, B., & Figueroa, C. C. Diversity, frequency, and geographic distribution of facultative bacterial endosymbionts in introduced aphid pests. *Insect Science*, **24**(3), 511–521. (2017). https://doi.org/10.1111/1744-7917.12313
3. Leonardo, T. E. & Mondor, E. B. Symbiont modifies host life-history traits that affect gene flow. *Proceedings of the Royal Society B: Biological Sciences* **273**, 1079–1084 (2006). https://doi.org/10.1098/rspb.2005.3408
4. Vorburger, C. & Gouskov, A. Only helpful when required: a longevity cost of harbouring defensive symbionts. *Journal of Evolutionary Biology* **24**, 1611–1617 (2011). https://doi.org/10.1111/j.1420-9101.2011.02292.x
5. Oliver, K. M. & Martinez, A. J. How resident microbes modulate ecologically-important traits of insects. *Current Opinion in Insect Science* **4**, 1–7 (2014). https://doi.org/10.1016/j.cois.2014.08.001
6. Jaenike, J. Population genetics of beneficial heritable symbionts. *Trends in Ecology & Evolution* **27**, 226–232 (2012). https://doi.org/10.1016/j.tree.2011.10.005

7. Rey, O. *et al.* Distribution of endosymbiotic reproductive manipulators reflects invasion process and not reproductive system polymorphism in the little fire ant *Wasmannia auropunctata*. *PLOS ONE* **8**, e58467 (2013). https://doi.org/10.1371/journal.pone.0058467
8. Himler, A. G. *et al.* Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. *Science* **332**, 254–256 (2011). https://doi.org/10.1126/science.1199410
9. Duron, O., Hurst, G. D. D., Hornett, E. A., Josling, J. A. & Engelstädter, J. High incidence of the maternally inherited bacterium *Cardinium* in spiders. *Molecular Ecology* **17**, 1427–1437 (2008). https://doi.org/10.1111/j.1365-294X.2008.03689.x
10. Łukasik, P., van Asch, M., Guo, H., Ferrari, J. & Charles J. Godfray, H. Unrelated facultative endosymbionts protect aphids against a fungal pathogen. *Ecology Letters* **16**, 214–218 (2013). https://doi.org/10.1111/ele.12031
11. Rosenwald, L. C., Sitvarin, M. I. & White, J. A. Endosymbiotic *Rickettsiella* causes cytoplasmic incompatibility in a spider host. *Proc Biol Sci* **287**, 20201107 (2020). https://doi.org/10.1098/rspb.2020.1107
12. Dlugosch, K. M. & Parker, I. M. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* **17**, 431–449 (2008). https://doi.org/10.1111/j.1365-294X.2007.03538.x
13. Shoemaker, D. D., Ross, K. G., Keller, L., Vargo, E. L. & Werren, J. H. *Wolbachia* infections in native and introduced populations of fire ants (*Solenopsis spp.*). *Insect Molecular Biology* **9**, 661–673 (2000). https://doi.org/10.1046/j.1365-2583.2000.00233.x
14. Bertelsmeier, C. & Keller, L. Bridgehead effects and role of adaptive evolution in invasive populations. *Trends in Ecology & Evolution* **33**, 527–534 (2018). https://doi.org/10.1016/j.tree.2018.04.014
15. Desneux, N. *et al.* Intraspecific variation in facultative symbiont infection among native and exotic pest populations: Potential implications for biological control. *Biological Control* **116**, 27–35 (2018). https://doi.org/10.1016/j.biocontrol.2017.06.007
16. Garb, J. E., González, A. & Gillespie, R. G. The black widow spider genus *Latrodectus* (Araneae: Theridiidae): phylogeny, biogeography, and invasion history. *Molecular Phylogenetics and Evolution* **31**, 1127–1142 (2004). https://doi.org/10.1016/j.ympev.2003.10.012

17. Sadir, M. & Marske, K. A. Urban environments aid invasion of brown widows (Theridiidae: *Latrodectus geometricus*) in North America, constraining regions of overlap and mitigating potential impact on native widows. *Frontiers in Ecology and Evolution* **9**, (2021). https://www.frontiersin.org/articles/10.3389/fevo.2021.757902
18. Mowery, M. A., Lubin, Y. & Segoli, M. Invasive brown widow spiders disperse aerially under a broad range of environmental conditions. *Ethology* **128**, 564–571 (2022). https://doi.org/10.1111/eth.13314
19. Mowery, M. A., Arabesky, V., Lubin, Y. & Segoli, M. Differential parasitism of native and invasive widow spider egg sacs. *Behavioral Ecology* **33**, 565–572 (2022). https://doi.org/10.1093/beheco/arac017
20. Dunaj, S. J., Bettencourt, B. R., Garb, J. E. & Brucker, R. M. Spider phylosymbiosis: divergence of widow spider species and their tissues' microbiomes. *BMC Evolutionary Biology* **20**, 104 (2020). https://doi.org/10.1186/s12862-020-01664-x
21. Halter, T. *et al.* Ecology and evolution of chlamydial symbionts of arthropods. *ISME COMMUN.* **2**, 1–11 (2022). https://doi.org/10.1038/s43705-022-00124-5
22. Halter T, Köstlbacher S, Rattei T, Hendrickx F, Manzano-Marín A, Horn M. One to host them all: genomics of the diverse bacterial endosymbionts of the spider *Oedothorax gibbosus*. Microb Genom. 2023 Feb;9(2):mgen000943. doi: 10.1099/mgen.0.000943.
23. Corsaro, D. *et al.* 'Candidatus *Rhabdochlamydia crassificans*', an intracellular bacterial pathogen of the cockroach *Blatta orientalis* (Insecta: Blattodea). *Systematic and Applied Microbiology* **30**, 221–228 (2007). https://doi.org/10.1016/j.syapm.2006.06.001
24. Pillonel, T. *et al.* Sequencing the obligate intracellular *Rhabdochlamydia helvetica* within its tick host *Ixodes ricinus* to investigate their symbiotic relationship. *Genome Biology and Evolution* **11**, 1334–1344 (2019). https://doi.org/10.1093/gbe/evz072
25. Vanthournout, B. & Hendrickx, F. Endosymbiont dominated bacterial communities in a dwarf spider. *PLOS ONE* **10**, e0117297 (2015). https://doi.org/10.1371/journal.pone.0117297
26. Kostanjšek, R., Štrus, J., Drobne, D. & Avguštin, G. 'Candidatus *Rhabdochlamydia porcellionis*', an intracellular bacterium from the hepatopancreas of the terrestrial isopod *Porcellio scaber* (Crustacea: Isopoda). *International Journal of Systematic and Evolutionary Microbiology* **54**, 543–549 (2004). https://doi.org/10.1099/ijs.0.02802-0

462 27. Arrington, B. D. The prevalence and effect of *Wolbachia* infection on the brown widow
463 spider (*Latrodectus geometricus*). (Georgia Southern University, 2014).
464 28. Zug, R. & Hammerstein, P. Still a host of hosts for *Wolbachia*: Analysis of recent data
465 suggests that 40% of terrestrial arthropod species are infected. *PLOS ONE* **7**, e38544 (2012).
466 https://doi.org/10.1371/journal.pone.0038544
467 29. Fenn, K. *et al.* Phylogenetic relationships of the *Wolbachia* of nematodes and arthropods.
468 *PLOS Pathogens* **2**, e94 (2006). https://doi.org/10.1371/journal.ppat.0020094
469 30. Kaur, R. *et al.* Living in the endosymbiotic world of *Wolbachia*: A centennial review. *Cell*
470 *Host & Microbe* **29**, 879–893 (2021). https://doi.org/10.1016/j.chom.2021.03.006
471 31. Pearson, J. F. W. *Latrodectus geometricus* Koch in southern Florida. *Science* **83**, 522–523
472 (1936).
473 32. Vincent, L. S., Vetter, R. S., Wrenn, W. J., Kempf, J. K. & Berrian, J. E. The brown widow
474 spider *Latrodectus geometricus* C. L. Koch, 1841, in southern California. *The Pan-Pacific*
475 *Entomologist* **84**, 344–349 (2009). https://doi.org/10.3956/2008-07.1
476 33. Levy, G. & Amitai, P. Revision of the widow-spider genus *Latrodectus* (Araneae:
477 Theridiidae) in Israel. *Zool J Linn Soc* **77**, 39–63 (1983). https://doi.org/10.1111/j.1096-
478 3642.1983.tb01720.x
479 34. Mowery, M. A., Lubin, Y., Harari, A., Mason, A. C. & Andrade, M. C. B. Dispersal and life
480 history of brown widow spiders in dated invasive populations on two continents. *Animal*
481 *Behaviour* (2022) doi:https://doi.org/10.1016/j.anbehav.2022.02.006.
482 35. White, J. A. *et al.* Endosymbiotic bacteria are prevalent and diverse in agricultural spiders.
483 *Microb Ecol* **79**, 472–481 (2020). https://doi.org/10.1007/s00248-019-01411-w
484 36. Curry, M. M., Paliulis, L. V., Welch, K. D., Harwood, J. D. & White, J. A. Multiple
485 endosymbiont infections and reproductive manipulations in a linyphiid spider population.
486 *Heredity* **115**, 146–152 (2015). https://doi.org/10.1038/hdy.2015.2
487 37. Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. DNA primers for amplification
488 of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol*
489 *Mar Biol Biotechnol* **3**, 294–299 (1994).
490 38. Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D. Development
491 of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence

492 data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**, 5112–5120
493 (2013). https://doi.org/10.1128/AEM.01043-13

494 39. Caporaso, J. G. *et al.* QIIME allows analysis of high-throughput community sequencing data.
495 *Nat Methods* **7**, 335–336 (2010). https://doi.org/10.1038/nmeth.f.303
496 40. Bokulich, N. A. *et al.* Quality-filtering vastly improves diversity estimates from Illumina
497 amplicon sequencing. *Nat Methods* **10**, 57–59 (2013). https://doi.org/10.1038/nmeth.2276
498 41. DeSantis, T. Z. *et al.* Greengenes, a chimera-checked 16S rRNA gene database and
499 workbench compatible with ARB. *Appl Environ Microbiol* **72**, 5069–5072 (2006).
500 https://doi.org/10.1128/AEM.03006-05
501 42. Larsson, A., Stanley, G. M., Sinha, R., Weissman, I. & Sandberg, R. Computational
502 correction of index switching in multiplexed sequencing libraries. *Nature Methods* (2018)
503 doi:10.1038/nmeth.4666. https://doi.org/10.1038/nmeth.4666
504 43. Hongoh, Y., Ohkuma, M. & Kudo, T. Molecular analysis of bacterial microbiota in the gut
505 of the termite *Reticulitermes speratus* (Isoptera; Rhinotermitidae). *FEMS Microbiology*
506 *Ecology* **44**, 231–242 (2003). https://doi.org/10.1016/S0168-6496(03)00026-6
507 44. Baldo, L. *et al.* Multilocus sequence typing system for the endosymbiont *Wolbachia*
508 *pipientis*. *Applied and Environmental Microbiology* **72**, 7098–7110 (2006).
509 https://doi.org/10.1007/s00284-007-9009-4
510 45. Untergasser, A. *et al.* Primer3—new capabilities and interfaces. *Nucleic Acids Research* **40**,
511 e115 (2012). https://doi.org/10.1093/nar/gks596
512 46. Katoh, K., Rozewicki, J. & Yamada, K. D. MAFFT online service: multiple sequence
513 alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**,
514 1160–1166 (2019). https://doi.org/10.1093/bib/bbx108
515 47. Zwickl, D. J. Genetic algorithm approaches for the phylogenetic analysis of large biological
516 sequence datasets under the maximum likelihood criterion. (The University of Texas at
517 Austin, United States -- Texas, 2006).
518 48. Rodríguez, F., Oliver, J. L., Marín, A. & Medina, J. R. The general stochastic model of
519 nucleotide substitution. *Journal of Theoretical Biology* **142**, 485–501 (1990).
520 https://doi.org/10.1016/S0022-5193(05)80104-3

49. Huelsenbeck, J. P. & Rannala, B. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology* **53**, 904–913 (2004). https://doi.org/10.1080/10635150490522629
50. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783–791 (1985). https://doi.org/10.2307/2408678
51. Jolley, K. A., Bray, J. E. & Maiden, M. C. J. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* **3**, 124 (2018). https://doi.org/10.12688/wellcomeopenres.14826.1
52. Rodrigues, J. *et al. Wolbachia* springs eternal: symbiosis in *Collembola* is associated with host ecology. *Royal Society Open Science* **10**, 230288 (2023). https://doi.org/10/1098/rsos.230288.
53. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing (2023).
54. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models using lme4. *Journal of Statistical Software* **67**, 1–48 (2015). https://doi.org/10.18637/jss.v067.i01
55. Dunnington, D., Thorne, B. & Hernangómez, D. ggspatial: Spatial Data Framework for ggplot2. (2023). https://github.com/paleolimbot/ggspatial.
56. Arif, S. *et al.* Evidence for multiple colonisations and *Wolbachia* infections shaping the genetic structure of the widespread butterfly *Polyommatus icarus* in the British Isles. *Molecular Ecology* **30**, 5196–5213 (2021). https://doi.org/10.1111/mec.16126
57. Cornwell, B. H. & Hernández, L. Genetic structure in the endosymbiont *Breviolum 'muscatinei'* is correlated with geographical location, environment and host species. *Proceedings of the Royal Society B: Biological Sciences* **288**, 20202896 (2021). https://doi.org/10.1098/rspb.2020.2896
58. Pollmann, M. *et al.* Highly transmissible cytoplasmic incompatibility by the extracellular insect symbiont *Spiroplasma*. *iScience* **25**, 104335 (2022). https://doi.org/10.1016/j.isci.2022.104335
59. Baldo, L., Prendini, L., Corthals, A. & Werren, J. H. *Wolbachia* are present in southern African scorpions and cluster with Supergroup F. *Curr Microbiol* **55**, 367–373 (2007). https://doi.org/10.1007/s00284-007-9009-4

60. Lo, N. & Evans, T. A. Phylogenetic diversity of the intracellular symbiont *Wolbachia* in termites. *Mol Phylogenet Evol* **44**, 461–466 (2007). https://doi.org/10.1016/j.ympev.2006.10.028
61. Glowska, E., Dragun-Damian, A., Dabert, M. & Gerth, M. New *Wolbachia* supergroups detected in quill mites (Acari: Syringophilidae). *Infection, Genetics and Evolution* **30**, 140–146 (2015). https://doi.org/10.1016/j.meegid.2014.12.019
62. Casiraghi, M. *et al.* Phylogeny of *Wolbachia pipientis* based on gltA, groEL and ftsZ gene sequences: clustering of arthropod and nematode symbionts in the F supergroup, and evidence for further diversity in the *Wolbachia* tree. *Microbiology* **151**, 4015–4022 (2005). https://doi.org/10.1099/mic.0.28313-0
63. Knight, E. Characterizing the complex relationship between the brown widow spider and Its bacterial endosymbiont, *Wolbachia*. *Electronic Theses and Dissertations* (2018). https://digitalcommons.georgiasouthern.edu/etd/1825
64. Charlesworth, J., Weinert, L. A., Araujo, E. V. & Welch, J. J. *Wolbachia*, *Cardinium* and climate: an analysis of global data. *Biology Letters* **15**, 20190273 (2019). https://doi.org/10.1098/rsbl.2019.0273

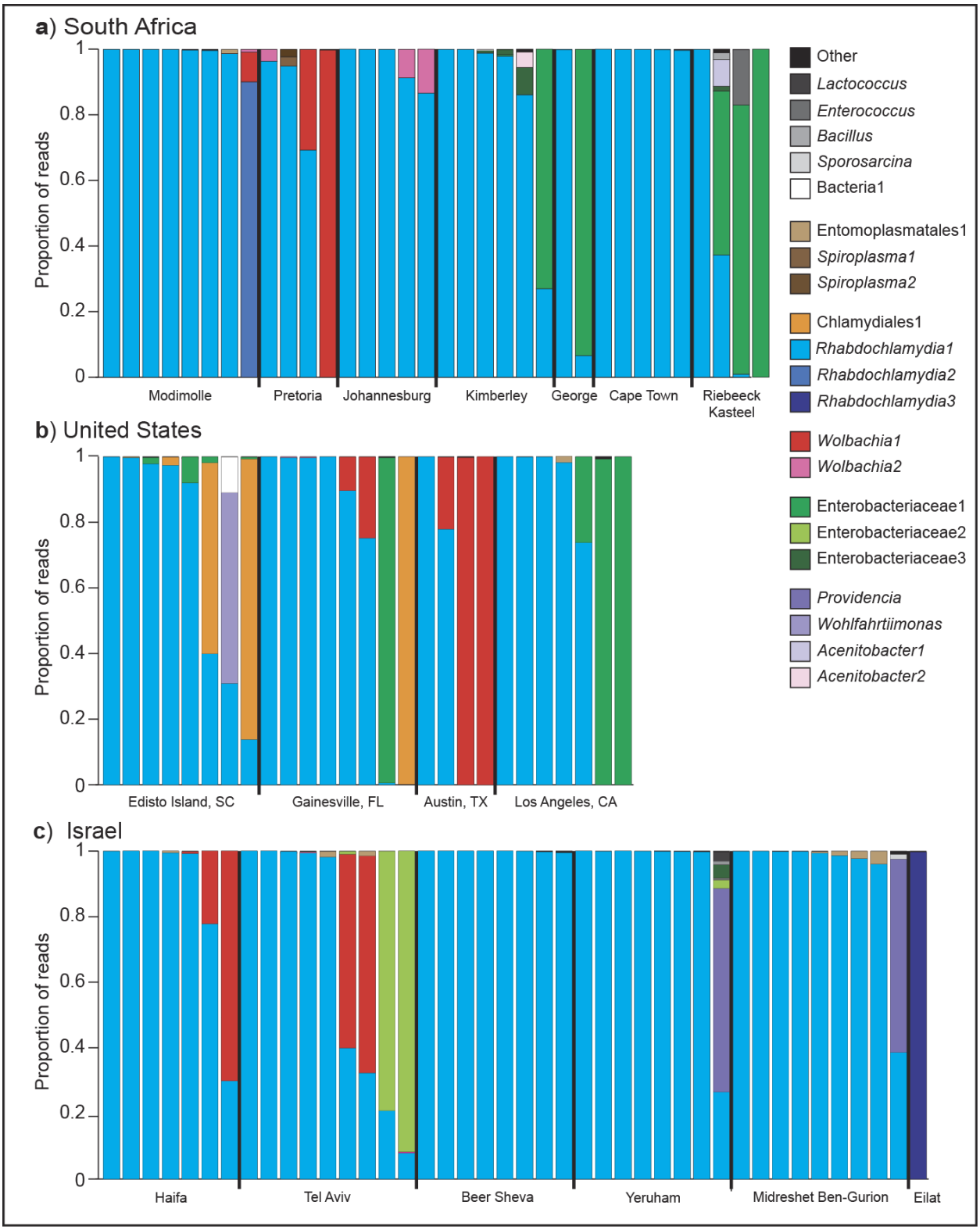
571 Figure legends.

572 **Figure 1.** High throughput analysis of bacterial associates in *Latrodectus geometricus*.
573 Proportional distribution of 16S sequencing reads from *L. geometricus* adult females collected
574 from South Africa (a), the United States (b), and Israel (c). All bacterial strain types that
575 exceeded 1% of reads in any sample are depicted. All remaining strains are collected within the
576 "other" category.

577 **Figure 2.** Phylogenetic placement of Chlamydial bacterial associates of *Latrodectus*
578 *geometricus.* Tree of highest log likelihood from 500 maximum likelihood searches of a 35 OTU
579 16S data set containing 1576 characters conducted with Garli (v. 2.01) using the default settings.
580 Taxa in bold are the new strains from *L. geometricus* (labeled Rhabdochlamydia1, 2, 3 and
581 Chlamydiales1). Numbers above the nodes are bootstrap values above 50 (500 bootstrap
582 replicates with 2 searches per replicate).

583 **Figure 3.** Proportion of adult female *L. geometricus* infected with *Rhabdochlamydia1* and/or
584 *Wolbachia1* detected through PCR screening across 17 localities in a) South Africa, b) Israel,
585 and c) the United States. Blue represents individuals infected with just *Rhabdochlamydia1,*
586 purple represents individuals infected with both *Rhabdochlamydia1* and *Wolbachia1,* red
587 represents individuals infected with just *Wolbachia1,* and white represents individuals infected
588 with neither *Wolbachia1* nor *Rhabdochlamydia1.* Size of pie charts corresponds to the number of
589 individual spiders screened from each site (range = one specimen from Eilat, Israel to 10
590 specimens from Edisto Island, SC, USA, see Supplementary table 1 for sample sizes and
591 collection localities).

592



593

594 Figure 1

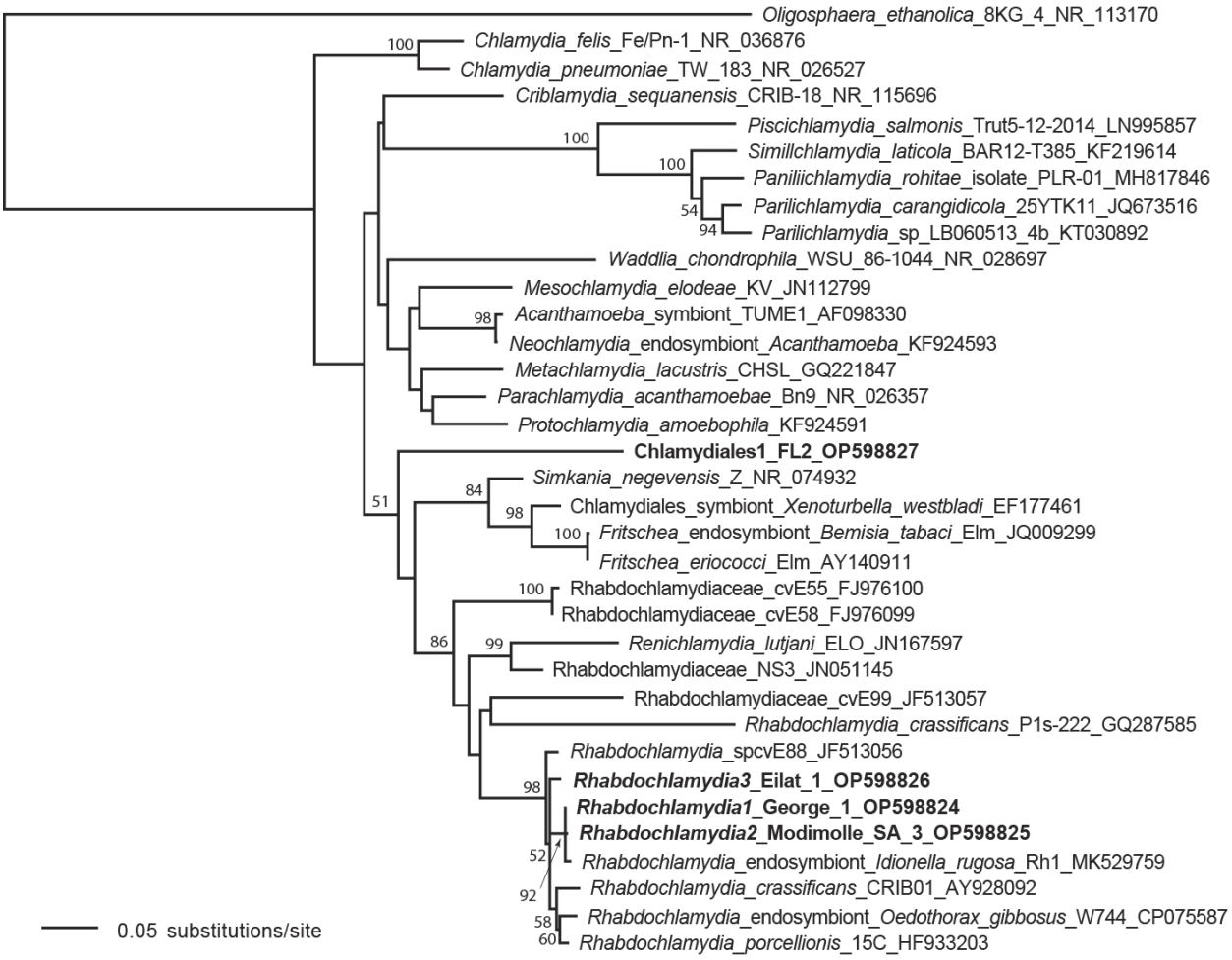
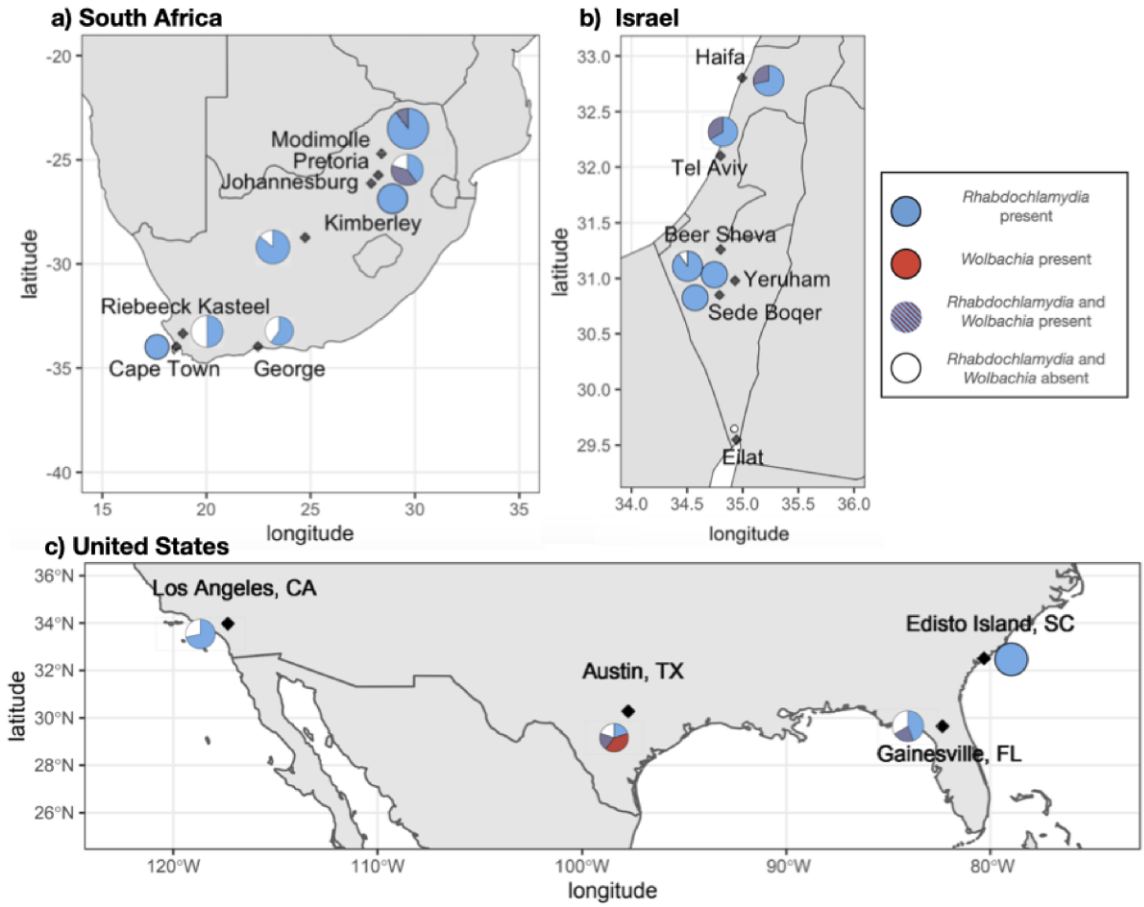

Oligosphaera_ethanolica_8KG_4_NR_113170
Chlamydia_felis_Fe/Pn-1_NR_036876
Chlamydia_pneumoniae_TW_183_NR_026527
Criblamydia_sequanensis_CRIB-18_NR_115696
Piscichlamydia_salmonis_Trut5-12-2014_LN995857
Simillchlamydia_laticola_BAR12-T385_KF219614
Paniliichlamydia_rohitae_isolate_PLR-01_MH817846
Parilichlamydia_carangidicola_25YTK11_JQ673516
Parilichlamydia_sp_LB060513_4b_KT030892
Waddlia_chondrophila_WSU_86-1044_NR_028697
Mesochlamydia_elodeae_KV_JN112799
Acanthamoeba_symbiont_TUME1_AF098330
Neochlamydia_endosymbiont_Acanthamoeba_KF924593
Metachlamydia_lacustris_CHSL_GQ221847
Parachlamydia_acanthamoebae_Bn9_NR_026357
Protochlamydia_amoebophila_KF924591
Chlamydiales1_FL2_OP598827
Simkania_negevensis_Z_NR_074932
Chlamydiales_symbiont_Xenoturbella_westbladi_EF177461
Fritschea_endosymbiont_Bemisia_tabaci_Elm_JQ009299
Fritschea_eriococci_Elm_AY140911
Rhabdochlamydiaceae_cvE55_FJ976100
Rhabdochlamydiaceae_cvE58_FJ976099
Renichlamydia_lutjani_ELO_JN167597
Rhabdochlamydiaceae_NS3_JN051145
Rhabdochlamydiaceae_cvE99_JF513057
Rhabdochlamydia_crassificans_P1s-222_GQ287585
Rhabdochlamydia_spcvE88_JF513056
Rhabdochlamydia3_Eilat_1_OP598826
Rhabdochlamydia1_George_1_OP598824
Rhabdochlamydia2_Modimolle_SA_3_OP598825
Rhabdochlamydia_endosymbiont_Idionella_rugosa_Rh1_MK529759
Rhabdochlamydia_crassificans_CRIB01_AY928092
Rhabdochlamydia_endosymbiont_Oedothorax_gibbosus_W744_CP075587
Rhabdochlamydia_porcellionis_15C_HF933203
100
100
100
54
94
98
51
84
98
100
100
86
99
98
52
92
58
60
0.05 substitutions/site

Figure 2



598

599 Figure 3

600