Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2024, 15, 556-572
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

A Comparison of Microbial Communities in Three Morphs of the *Rhynchophorus ferrugineus* (Olivier), a Key Pest of Date Palms

AbdulAziz M. A. Mohamed[1,2*], Malabika Roy Pathak[1], Muhammad Farooq[1], Jennifer A. White[3], Laura C. Rosenwald[3]

[1]Department of Life Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
[2]P.O. Box 32207, Isa Town, Kingdom of Bahrain
[3]Department of Entomology, University of Kentucky, Lexington, USA
Email: *amabdulkareem@gmail.com

How to cite this paper: Mohamed, A.M.A., Pathak, M.R., Farooq, M., White, J.A. and Rosenwald, L.C. (2024) A Comparison of Microbial Communities in Three Morphs of the *Rhynchophorus ferrugineus* (Olivier), a Key Pest of Date Palms. *Advances in Bioscience and Biotechnology*, **15**, 556-572.
https://doi.org/10.4236/abb.2024.1510035

Received: August 27, 2024
Accepted: October 22, 2024
Published: October 25, 2024

Abstract

The invasive insect pest, red palm weevil (RPW), *Rhynchophorus ferrugineus*, poses a significant threat to date production, causing substantial economic damage. If uncontrolled, RPW leads the severely infested host tree to collapse and eventually die. The symbiotic associations with microorganisms and RPW in their gut may help their host insects' establishment, development, nutrition assimilation, and survival. The objective of this research was the molecular characterization of the microbiome of RPW. In this study, the microbiome was compared among different tissues in females and males of RPW of three different morphs and larvae collected from date palm plantations in the Kingdom of Bahrain. A 251-bp segment of bacterial 16S rRNA was amplified by PCR, sequenced, and processed using the bioinformatics platform QIIME2. One ASV, corresponding to the obligate weevil symbiont *Nardonella*, predominated in adult female samples, constituting 56 ± 7% of total reads, but was less dominant in male samples (12 ± 3%) and larval samples (2.6 ± 1.9%). For females, samples that included reproductive tissues were almost entirely composed of *Nardonella* (88% - 99%). When *Nardonella* was excluded from analyses, there were no differences between adult females and adult males, but larval samples were more species-rich and differed in microbial composition from adults. There were no consistent differences in the microbiomes among morphs. Several specimens showed evidence of infection with host-specific strains of *Spiroplasma*-like members of the Entomoplasmatales, which are often pathogens or vertically transmitted symbionts. Such close microbial associates deserve additional attention as potential routes to control this destructive date palm pest.

Keywords

Date Palm, *Rhynchophorus ferruginous*, Gut, Endosymbionts, Bacteria, *Nardonella*

1. Introduction

The Red palm weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), is a widespread and invasive pest that attacks 40 palm species of 18 different palm genera across the globe [1] [2]. Date palm, *Phoenix dactylifera* L. (Arecales: Arecaceae), has experienced severe damage worldwide [3] [4]. The first report of RPW in 1906 vividly detailed its destructive impacts on the coconut palm trees, *Cocos nucifera*, in India [5]. In the mid-eighties of the last century, the RPW was discovered in the Arabian Gulf countries, posing a significant threat to the date palm trees in the region [6] [7]. Over time, the weevil has demonstrated a significant capacity for geographical expansion, establishing its presence in various Asian countries, the Middle East, North Africa, southern Europe, and the Caribbean Islands [8]. The significant spread of RPW in various regions is likely attributed to the international trade of palm trees coupled with the absence of reliable techniques for early detection of RPW infestation [3]. Moreover, clear visible symptoms of RPW infection in palm trees are typically absent until the advanced stages of infestation [5]. The damage of date palms by RPW is only visible when the trees are close to death by infestation [9] [10]. Red palm weevil larvae are the most destructive stage that feeds on the fibrous vascular bundles of the palms, producing wet fermenting chewed waste inside the trunk and creating a hollow tunnel [11] [12]. The palm tissues are rich in carbohydrates and contain 80% cellulose, hemicelluloses, lignin, sucrose, and glucose [13]. Therefore, palm tissue and tissue sap represent a non-easily digestible and nutrient-poor substrate for RPW [14]. Currently, it has been found that the RPW gut harbors a diverse bacterial community that helps in degrading polysaccharides and sucrose and significantly influences the nutrition metabolism of this pest [14]-[17].

In recent years, much evidence has accumulated to indicate that symbiotic microbiota has facilitated diverse metabolic activities, survival, and adaptive radiation of insects [18]-[20]. Many damaging wood-boring insects, including the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), the sirex wood wasp, *Sirex noctilio* Fabricius ((Hymenoptera: Siricidae), and the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), have shown symbiotic associations with microorganisms that play important roles in host nutrition through degradation of cellulose and hemicelluloses [21] [22] [23]. Some endosymbionts are obligate and are essential to the survival of the host [24] [25], such as *Buchnera* in aphids [26], *Wigglesworthia* in tsetse flies (Diptera: Glossinidae) [27], *Carsonella* in psyllids (Homoptera: Psyllidae) [28],

Blochmannia in carpenter ants, *Camponotus* sp. (Hymenoptera: Formacidae) [29], *Nardonella* in weevils [30] [31] and others. Weevils comprise a species-rich group of around 70,000 species in the Curculionidae [32] [33]. Most weevil species are infected by *Nardonella*, an ancient endosymbiont lineage found in specialized symbiotic organs [34] co-speciated with the host weevil for over 125 million years [35] [36]. The presence of *Nardenella* in the West Indian sweet potato weevil in *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) and several pest and non-pest weevils have been reported [36].

The substantial environmental and economic injury caused by RPW may be partially attributable to its associated endosymbionts [37]-[39]. Several studies have indicated that intestinal tracts and guts of RPW infesting palm trees harbor rich communities of nonpathogenic microbial species. These microbial species play a critical role in regulating the biological processes such as nutrient metabolism, growth, development, and immune activity of RPW life stages, including adults and larvae [40]-[44]. The findings have significant implications for developing novel and effective methods to control RPW populations and mitigate the impact of this invasive pest. A general analysis of the 16S ribosomal RNA (rRNA) genes was used to identify bacterial species [16] [45]-[47], but nothing is known about the microbial communities of the gut and other tissues of the RPW larvae and adults of different morphs from infested date palm trees of the Kingdom of Bahrain, which represents a significant threat to date palm production. We are therefore interested in identifying and characterizing the microbial communities in RPW adults of three morphs and larvae using high-throughput sequencing, which may help improve invasive pest bio-control management programs.

2. Material and Methods

2.1. Field Collection of Red Palm Weevil

The adult and larval red palm weevils were hand-picked from infested date palm trees that were 7 to 10 years old in the Kingdom of Bahrain from September 2017 to March 2018. The collections were made at three different locations: Hoarat A'ali, Maqabah, and Zallaq (**Table S1**). Both stages of the weevils were found to coexist within the same feeding sites in the middle of the palm trunks at a height of 0.8 - 1.5 m from the ground. Specimens of each developmental stage were placed in covered cylinder plastic containers (1500 ml) supplied with freshly cut palm petiole pieces and then transferred to the laboratory. In the laboratory, the adult weevils were categorized into three distinct color morphs based on their black pronotum markings, as shown in **Figure 1**. Furthermore, each morph's female and male adult weevils were sexed based on their rostrum. Female weevils possess a longer, curved, hairless rostrum, while male weevils have a shorter rostrum with thick, erect setae at the apex. All specimens of both adults and larval weevils were preserved then in 95% ethanol for endosymbiont analysis.

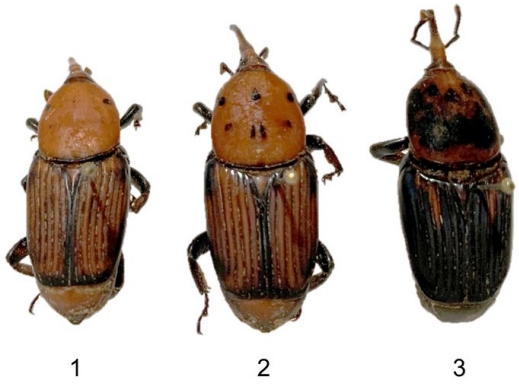

Figure 1. Three distinct red palm weevil morphs distinguished by their pronotal markings were collected from the Kingdom of Bahrain.

2.2. Red Palm Weevil Sample Preparation, Dissection, and Isolation of Microbiome

Individual RPW specimens were surface sterilized using a series of washes: first with PCR water, followed by 0.5% bleach, and finished with PCR water. Specimens were then dissected with sterile instruments. Due to specimen preservation, the abdominal contents were friable and not easily dissected from one another. For several specimens, reproductive organs and the hindgut were separated for individual analysis, but for most adult specimens and all larval specimens, generalized abdominal contents were homogenized for subsequent analysis. Dissected tissues were rewashed using PCR water and 0.5% bleach before homogenization and DNA extraction.

2.3. DNA Isolation, PCR, and Sequencing

DNA was extracted from RPW specimens (*i.e.*, one or more subsamples) using a DNA extraction kit (Qiagen DNEasy Extraction Kit, Germantown, MD) according to manufacturer's instructions. The microbiomes of the RPW tissue samples were profiled using metabarcoding and high-throughput sequencing of the bacterial community. Each sample was individually labeled with a unique combination of indexed forward and reverse primers that amplified the V4 region of bacterial 16S rRNA [48]. A subsample of each reaction was electrophoresed on a 1% agarose gel stained with GelRed (Biotium); samples that resulted in strong amplification of bacterial products were included in the library. For samples with weak or no amplification, a second PCR was attempted, and if the second PCR result was also weak, the sample was excluded. Included samples were multiplexed into one library that was purified using a GenCatch PCR Cleanup Kit (Epoch Life Sciences, Missouri City, TX). Several non-weevil samples were also included in the library as positive controls to evaluate within library cross-sample contamination. These samples (an *Aphis craccivora* aphid and a *Mermessus fradeorum* spider) are dominated by a few highly specialized endosymbionts [49] [50] that could be readily filtered out from the final result. The combined sample quality was assessed at the B-CELL sequencing facility (Bluegrass Community & Technical College, Lexington

Kentucky) before being sequenced on an Illumina MiSeq 2500 instrument using Miseq Reagent Kit v2 250PE chemistry.

2.4. Sequence Analysis

Sequences were demultiplexed, trimmed and quality filtered in BaseSpace (Illumina, https://basespace.illumina.com/), then they were imported into QIIME2 (v2017.11, https://qiime2.org) using a manifest [51]. Additional quality control was conducted using deblur [52], implemented in QIIME2 using default parameters and a trim length of 251 bases. This procedure allowed distinction among Amplicon Sequence Variants (ASVs) that differed by even one base pair over the sequenced length [53]. Resulting sequences were taxonomically classified using a naïve Bayes classifier that was trained on the 515F/806R V4 region of the Greengenes 13_8 99% OTUs reference database [54]. Additionally, high-prevalence sequences were queried against the NCBI nucleotide database using the Megablast algorithm to identify symbiotic taxa.

2.5. Functional Profiling of Bacterial Community

To analyze the bacterial community, ASVs corresponding to mitochondria, chloroplasts, and characteristic symbionts for other specimens included in the library (e.g., *Buchnera* for aphids) were filtered out, along with any ASVs with less than 10 reads across all samples in the run. Individual weevil samples that totaled less than 1000 reads were also removed (N = 2 samples). The community characteristics across different tissue samples (ovary, gut, general abdominal) in female specimens (N = 29 samples) were first compared. The rarified sampling depth was set to 1160 reads (corresponding to the sample with the lowest read number in the set), then QIIME2's core Phylogenetics package was used to calculate bacterial community richness using Shannon's Index and observed ASVs for samples, comparing values among tissues using nonparametric Kruskal-Wallis tests. Bacterial community composition among tissue samples was then compared via PERMANOVA of both weighted and unweighted UniFrac values, with 999 permutations per test. The process was repeated to compare among life stages (larva, male, female), using only general abdominal samples (N = 39), as that was the most consistent sample type across all life stages. For this contrast, samples were rarified to 2100 reads per sample. Due to the dominance of a single ASV corresponding to *Nardonella*, particularly within female specimens, this ASV was filtered out, and the analyses were repeated, with rarification set to 940 reads per sample. Finally, to determine whether there were differences among morphs of the RPW, we further restricted the dataset to just male abdominal specimens (N = 28) and repeated the bacterial community analyses, again using a rarification of 940 reads per sample.

3. Results

The microbiomes of the RPW specimens collected from infested trunks of *P. dactylifera* in the Kingdom of Bahrain differed among tissues in adult female

specimens due to the prevalence of a single ASV corresponding to the obligate weevil symbiont *Nardonella* (Identical to NCBI accession #LC014984.1; Figure 2A, Table S2). *Nardonella* constituted 98% or more of all reads from ovaries and 88% or more of reads from generalized abdominal contents of adult females. Samples that were restricted to the hindgut had a lower but variable *Nardonella* presence, allowing some resolution of the remaining bacterial community. Driven by *Nardonella*, the observed ASVs in rarified subsamples yielded a median of only 4 ASVs for ovary samples, 7 for general abdominal samples, and 44 for gut samples (Kruskal Wallis H = 18.2, P = 0.0001). The Shannon index of diversity was also different (Kruskal Wallis H = 18.4, P < 0.0001), as were both weighted UniFrac (Pseudo F = 9.33 P = 0.002) and unweighted UniFrac (Pseudo F = 4.35 P < 0.001) measures for bacterial community similarity among tissues. In the gut samples where *Nardonella* presence was not so overwhelming, prevalent ASVs included a bacterial strain in the *Entomoplasmatales*, with 96% similar to strains of *Spiroplasma* (Figure 2B, Table S2). Other abundant ASVs included strains of *Enterobacteriaceae* (*Erwinia*), *Lactobacillaceae* (*Lactobacillus, Lactococcus*), *Leuconostocaceae* (*Leuconostoc*), *Enterococcaceae* (*Enterococcus*), *Comomonadaceae*, *Streptococcaceae* (*Lactococcus*), and other *Entomoplasmataceae* (*Entomoplasma*) (Figure 2B, Table S2).

Next, the microbial community was compared among life stages (larva, male, female), using only general abdominal samples (N = 39), as that was the only consistent sample type across all life stages. In different samples rarified to 2100 reads, a median of only 12 ASVs was observed in adult female abdominal samples versus 50 ASVs in male abdomens and 88 ASVs in larval abdomens (H = 17, P = 0.0002). This again corresponded to very different Shannon Indices (Kruskal Wallis H = 14.8, = 0.0006), as well as weighted UniFrac (Pseudo F = 8.86 P = 0.001) and unweighted UniFrac (Pseudo F = 2.99 P = 0.001) measures. These differences among microbial communities for different life stages were driven by *Nardonella*, which had lower and variable prevalence in adult male samples, and much lower prevalence in larval samples than adult females (Figure 2A). When *Nardonella* was excluded from the microbial community analyses, the differences in bacterial communities among life stages diminished, with differences only between larvae and adults. When rarified to 940 reads, 62 ASVs were observed in larval samples versus 41 in adult females and 39 in adult males (H = 7.85, P = 0.019). The Shannon diversity index did not differ among life stages (H = 5.22, P = 0.07), but both weighted (Pseudo F = 1.87, P = 0.05) and unweighted UniFrac weighted (Pseudo F = 1.38, P = 0.042) measures differed significantly between larvae and adults, but not between males and females. The *Spiroplasma*-like ASV was observed in some individuals of each life stage and for some specimens composed the majority of reads (Figure 2B).

When the microbial communities were compared among weevil morphs (restricted only to male abdominal samples [N = 28] of three different morphs), no differences were observed in the median number of ASVs (H = 4.32, P = 0.12), or

community diversity as measured by the Shannon index (H = 1.55, P = 0.46) the weighted unifrac (Pseudo F = 0.77, P = 0.62) or unweighted unifrac (Pseudo F = 1.22, P = 0.13; Figure 2B).

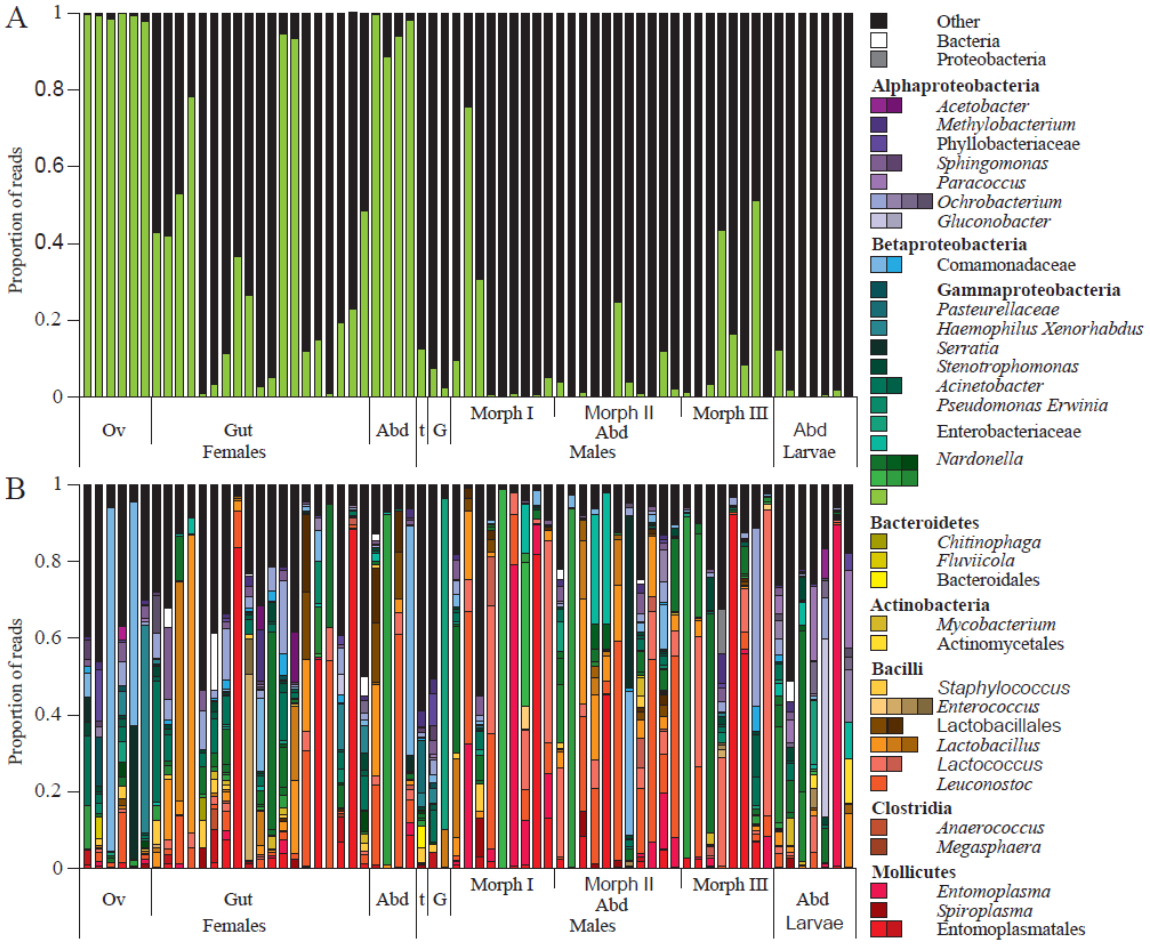

Figure 2. Proportional microbiome composition of *R. ferrugineus*, including the obligate symbiont *Nardonella* (A) or excluding Nardonella (B). Samples originated from weevil ovaries (Ov), testes (t), gut (G), or generalized abdomen (Abd), which included reproductive and digestive systems. All bacterial sequence variants that exceeded 0.1% of total non-*Nardonella* reads or exceeded 5% of reads from any individual sample are depicted as different shades as indicated. Corresponding sequences and read counts are available in Table S2.

4. Discussion

The comparison among different tissue samples in female RPWs showed that one ASV, corresponding to the obligate weevil endosymbiont *Nardonella*, was higher in ovaries than in gut and generalized abdominal samples. *Nardonella* is found in most, but not all clades of weevils, and has previously been reported from RPW [55]. This maternally inherited Gammaproteobacteria has an exceptionally long evolutionary history with weevils [55] and is responsible for the hardness of the cuticle due to its extreme metabolic capacity for tyrosine synthesis [31] [44]. Feeding studies have shown that other weevils, including RPW, that were fed antibiotic–supplemented diet as larvae, frequently suffered morphological abnormalities

as *Nardonella*-suppressed adults [56]. In larvae, *Nardonella* is housed in a specialized bacteriome at the juncture of the foregut and midgut but must also be provisioned to developing eggs for maternal transmission, thus explaining the very strong *Nardonella* signal from the ovaries, and the somewhat less strong signal from the generalized abdominal samples, which included reproductive tissues [55]. This strong *Nardonella* signal from the female reproductive tissues was largely responsible for differences in the microbial community among females, males, and larvae. Once *Nardonella* was excluded from the analyses, it became clear that the remaining differences among life stages occurred between larvae and adults: larvae had greater microbial species richness than adults, and community composition also shifted, tending to include greater representation by Alphaproteobacteria such as *Ochrobacterium*, *Paracoccus*, and *Gluconbacter*, and less representation by Mollicutes and Bacilli, such as *Leuconostoc* and *Lactococcus* (**Figure 2B**).

After *Nardonella*, the next most prevalent ASV was a *Spiroplasma*-like member of the Entomoplasmatales. *Spiroplasmas* are wall-less bacteria that are frequent associates of arthropods. They can be commensal, pathogenic, or mutualistic and are sometimes transmitted transovarially from mother to offspring [57]. Other studies have found strains of *Spiroplasma* to be present in some weevil species as endosymbiont lineages [58] [59]. In RPW, the *Spiroplasma*-like ASV was the predominant signal from several adult male and female specimens (**Figure 2B**). Still, it was not detected in ovary samples, suggesting it is not maternally transmitted. However, relatively few ovaries-only samples were examined, and the extraordinarily strong signal from *Nardonella* in the ovaries could have swamped out signal from any other bacteria; thus, the presence of *Spiroplasma* in reproductive tissue cannot be completely excluded. *Spiroplasmas* often manipulate the reproduction of host arthropods [57] but can also be pathogenic [60] or mutualistic for insect hosts [61] [62]. *Spiroplasma* and other endosymbionts can confer condition-dependent fitness advantages to several insect hosts [61] [63] [64]. Whether this microbe is pathogenic or mutualistic in nature, it is worth additional investigation for potential utility for control of the RPW [65] [66].

The effect of a diverse array of endosymbiotic bacteria is a matter of concern in research interest and may have applied relevance [56] [67]. The abundant gut bacteria in RPW belong primarily to families in Enterobacteriaceae, *Lactobacillaceae*, and *Entomoplasmataceae*, which have previously been found to be members of the highly stable microbial community across different life stages in the guts of RPW [16] [47]. This complex gut microbiota of Proteobacteria, Bacteroidetes, and Firmicutes helps to degrade plant polysaccharides, ferment palm tissues, and modulate insect nutrition and metabolism [16] [47].

The microbiota in insect guts potentially provides many beneficial services to their hosts [68] [69]. The presence of *Enterobacter* and other *Enterobacteriaceae*, as in other insects, play a beneficial role in nutrition by degradation of many plant polymers, soluble plant polysaccharides, and fermentation of sap sugar [47] [70].

Some members of Enterobacteriaceae were also identified as intracellular symbionts of grain weevils, *Sitophilus* spp. (Coleoptera: *Curculionidae*) [71], and some of the isolates fix nitrogen in natural populations of the fruit fly, *Ceratitis capitate* (Diptera: Tephritidae) [72]. *Entomoplasma*, *Lactobacillus*, and *Lactococcus* were most frequently present in RPW gut and helped in cellulose degradation and glucose fermentation [16]. Acidification caused by *Lactococcus*, *Lactobacillus*, and *Leuconostoc* promotes fermentation and confers advantages to insect host nutrition [14] [15] [42]. Most of these bacteria were reported in the gut of RPW and other insects, which help in cellulose degradation, mixed acid fermentation, and nutrient assimilation in insect metabolism [14]-[16] [42] [73].

In conclusion, culture-independent studies by PCR amplification of 16S rRNA have allowed microbial identification among different life stages and tissue samples of three different morphs of RPW, which were collected from the Kingdom of Bahrain. One ASV, corresponding to *Nardonella*, was particularly prevalent in adult female samples that included reproductive tissue. Males and larvae had much less *Nardonella*, which is not surprising for a bacterial symbiont that is maternally inherited. After *Nardonella* was excluded, the distribution of the other major bacterial taxa showed that the bacterial community in larvae was different than in adults but did not differ significantly among morphs. Overall bacterial communities resembled those characterized in previous studies of RPW, but the present study identified a *Spiroplasma*-like ASV that was dominant in several specimens and should be further investigated for phenotypic effects on the weevil host.

Acknowledgements

The authors would like to thank Mr. Ahmed Ali Al-Asfoor, Plant Wealth Directorate, Agriculture Affairs, Ministry of Municipalities Affairs and Agriculture, for the field assistance and collection of red palm weevil. We also thank Prof. Abdulrahman Saad Aldawood, Department of Plant Protection, King Saud University, Saudi Arabia, for the valuable comments on the first manuscript draft. The research was supported by Research Grant # "LS_AA_2017" from the College of Graduate Studies, Agricultural Biotechnology Program, Department of Life Sciences, Arabian Gulf University. It was also partly supported by the National Institute of Food and Agriculture, United States Department of Agriculture (Hatch No. 1020740).

Disclosure

AbdulAziz M.A. Mohamed and Malabika Roy Pathak were formerly affiliated with the Department of Life Sciences at Arabian Gulf University.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Anonymous (2013) Save Algarve Palms. http://www.savealgarvepalms.com/en/weevil-facts/host-palm-trees

[2] Abdel Farag El-Shafie, H. and Romeno Faleiro, J. (2020) Red Palm Weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae): Global Invasion, Current Management Options, Challenges and Future Prospects. In: El-Shafie, H.A. Ed., *Invasive Species—Introduction Pathways, Economic Impact, and Possible Management Options*, IntechOpen, 1-170. https://doi.org/10.5772/intechopen.93391

[3] Murphy, S. and Briscoe, B. (1999) The Red Palm Weevil as an Alien Invasive: Biology and the Prospects for Biological Control as a Component of IPM. *Biocontrol News and Information*, **20**, 35-46.

[4] Giblin-Davis, R.M. and Roda, A.L. (2013) Real Time Internet Invasive Pest Identification Training: A Case Study with *Rhynchophorus* Weevils. *Florida Entomologist*, **96**, 741-745. https://doi.org/10.1653/024.096.0306

[5] AlDosary, N., AlDobai, S. and Faleiro, J. (2016) Review on the Management of Red Palm Weevil *Rhynchophorus ferrugineus* Olivier in Date Palm *Phoenix dactylifera* L. *Emirates Journal of Food and Agriculture*, **28**, 34-44. https://doi.org/10.9755/ejfa.2015-10-897

[6] Abraham, V.A., Faleiro, J.R., Shuaibi, M.A. and Kumar, T.P. (2000) A Strategy to Manage Red Palm Weevil *Rhynchophorus ferrugineus* Oliv. On Date Palm *Phoenix dactylifera* L.—Its Successful Implementation in Al-Hassa, Kingdom of Saudi Arabia. *Pestology*, **24**, 23-30.

[7] Faleiro, J.R. (2006) A Review of the Issues and Management of the Red Palm Weevil *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in Coconut and Date during the Last One Hundred Years. *International Journal of Tropical Insect Science*, **26**, 135-154.

[8] EPPO (2022) EPPO Global Database. *Rhynchophorus ferrugineus*. https://gd.eppo.int/taxon/RHYCFE/distribution

[9] Faleiro, J.R. and Ashok Kumar, J. (2008) A Rapid Decision Sampling Plan for Implementing Area—Wide Management of the Red Palm Weevil, *Rhynchophorus ferrugineus*, in Coconut Plantations of India. *Journal of Insect Science*, **8**, 15. https://doi.org/10.1673/031.008.1501

[10] Dembilio, Ó. and Jacas, J.A. (2010) Basic Bio-Ecological Parameters of the Invasive Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), in *Phoenix canariensis* under Mediterranean Climate. *Bulletin of Entomological Research*, **101**, 153-163. https://doi.org/10.1017/s0007485310000283

[11] Butera, G., Ferraro, C., Colazza, S., Alonzo, G. and Quatrini, P. (2012) The Culturable Bacterial Community of Frass Produced by Larvae of *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) in the Canary Island Date Palm. *Letters in Applied Microbiology*, **54**, 530-536. https://doi.org/10.1111/j.1472-765x.2012.03238.x

[12] Mohamed, A.M.A., Farooq, M. and Pathak, M.R. (2020) Identification and Characterization of Bacterial Community Associated with the Chewed Feeding Waste of Red Palm Weevil in Infested Date Palm Trees. *Advances in Bioscience and Biotechnology*, **11**, 80-93. https://doi.org/10.4236/abb.2020.113007

[13] Souli, A., Sebai, H., Rtibi, K., Chehimi, L., Sakly, M., Amri, M., *et al*. (2014) Effects of Dates Pulp Extract and Palm Sap (*Phoenix dactylifera* L.) on Gastrointestinal Transit Activity in Healthy Rats. *Journal of Medicinal Food*, **17**, 782-786. https://doi.org/10.1089/jmf.2013.0112

[14] Montagna, M., Chouaia, B., Mazza, G., Prosdocimi, E.M., Crotti, E., Mereghetti, V., *et al*. (2015) Effects of the Diet on the Microbiota of the Red Palm Weevil (Coleoptera: Dryophthoridae). *PLOS ONE*, **10**, e0117439. https://doi.org/10.1371/journal.pone.0117439

[15] Jia, S., Zhang, X., Zhang, G., Yin, A., Zhang, S., Li, F., *et al*. (2013) Seasonally Variable Intestinal Metagenomes of the Red Palm Weevil (*Rhynchophorus ferrugineus*). *Environmental Microbiology*, **15**, 3020-3029. https://doi.org/10.1111/1462-2920.12262

[16] Muhammad, A., Fang, Y., Hou, Y. and Shi, Z. (2017) The Gut Entomotype of Red Palm Weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae) and Their Effect on Host Nutrition Metabolism. *Frontiers in Microbiology*, **8**, Article No. 2291. https://doi.org/10.3389/fmicb.2017.02291

[17] Farzane Basavand,, Khani, A., Yaghubi, S., Najimi, M. and Sahebzadeh, N. (2024) Investigation on Gut Microbiota Diversity of *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) Larvae. *Biology Bulletin*, **51**, 294-301. https://doi.org/10.1134/s106235902360352x

[18] Douglas, A.E. (2009) The Microbial Dimension in Insect Nutritional Ecology. *Functional Ecology*, **23**, 38-47. https://doi.org/10.1111/j.1365-2435.2008.01442.x

[19] Sugio, A., Dubreuil, G., Giron, D. and Simon, J. (2014) Plant-Insect Interactions under Bacterial Influence: Ecological Implications and Underlying Mechanisms. *Journal of Experimental Botany*, **66**, 467-478. https://doi.org/10.1093/jxb/eru435

[20] Xu, L., Lu, M. and Sun, J. (2015) Invasive Bark Beetle-Associated Microbes Degrade a Host Defensive Monoterpene. *Insect Science*, **23**, 183-190. https://doi.org/10.1111/1744-7917.12255

[21] Delalibera, I., Handelsman, J. and Raffa, K.F. (2005) Contrasts in Cellulolytic Activities of Gut Microorganisms between the Wood Borer, *Saperda vestita* (Coleoptera: Cerambycidae), and the Bark Beetles, *Ips pini* and *Dendroctonus frontalis* (Coleoptera: Curculionidae). *Environmental Entomology*, **34**, 541-547. https://doi.org/10.1603/0046-225x-34.3.541

[22] Vasanthakumar, A., Handelsman, J., Schloss, P.D., Bauer, L.S. and Raffa, K.F. (2008) Gut Microbiota of an Invasive Subcortical Beetle, *Agrilus planipennis* Fairmaire, across Various Life Stages. *Environmental Entomology*, **37**, 1344-1353. https://doi.org/10.1093/ee/37.5.1344

[23] Adams, A.S., Jordan, M.S., Adams, S.M., Suen, G., Goodwin, L.A., Davenport, K.W., *et al*. (2011) Cellulose-Degrading Bacteria Associated with the Invasive Woodwasp *Sirex noctilio*. *The ISME Journal*, **5**, 1323-1331. https://doi.org/10.1038/ismej.2011.14

[24] Moran, N.A., McCutcheon, J.P. and Nakabachi, A. (2008) Genomics and Evolution of Heritable Bacterial Symbionts. *Annual Review of Genetics*, **42**, 165-190. https://doi.org/10.1146/annurev.genet.41.110306.130119

[25] Bourtzis, K. and Miller, T.A. (2009) Insect Symbiosis. Contemporary Topics in Entomology Series. *Florida Entomologist*, **92**, 409-411.

[26] Clark, M.A., Moran, N.A., Baumann, P. and Wernegreen, J.J. (2000) Cospeciation between Bacterial Endosymbionts (*Buchnera*) and a Recent Radiation of Aphids (*Uroleucon*) and Pitfalls of Testing for Phylogenetic Congruence. *Evolution*, **54**, 517-525. https://doi.org/10.1111/j.0014-3820.2000.tb00054.x

[27] Chen, X., Li, S. and Aksoy, S. (1999) Concordant Evolution of a Symbiont with Its Host Insect Species: Molecular Phylogeny of Genus Glossina and Its Bacteriome-Associated Endosymbiont, *Wigglesworthia glossinidia*. *Journal of Molecular Evolution*, **48**, 49-58. https://doi.org/10.1007/pl00006444

[28] Thao, M.L., Moran, N.A., Abbot, P., Brennan, E.B., Burckhardt, D.H. and Baumann, P. (2000) Cospeciation of Psyllids and Their Primary Prokaryotic Endosymbionts. *Applied and Environmental Microbiology*, **66**, 2898-2905. https://doi.org/10.1128/aem.66.7.2898-2905.2000

[29] Sauer, C., Stackebrandt, E., Gadau, J., Hölldobler, B. and Gross, R. (2000) Systematic Relationships and Cospeciation of Bacterial Endosymbionts and Their Carpenter Ant Host Species: Proposal of the New Taxon *Candidatus* Blochmannia Gen. Nov. *International Journal of Systematic and Evolutionary Microbiology*, **50**, 1877-1886. https://doi.org/10.1099/00207713-50-5-1877

[30] Conord, C., Despres, L., Vallier, A., Balmand, S., Miquel, C., Zundel, S., *et al*. (2008) Long-Term Evolutionary Stability of Bacterial Endosymbiosis in Curculionoidea: Additional Evidence of Symbiont Replacement in the Dryophthoridae Family. *Molecular Biology and Evolution*, **25**, 859-868. https://doi.org/10.1093/molbev/msn027

[31] Anbutsu, H., Moriyama, M., Nikoh, N., Hosokawa, T., Futahashi, R., Tanahashi, M., *et al*. (2017) Small Genome Symbiont Underlies Cuticle Hardness in Beetles. *Proceedings of the National Academy of Sciences*, **114**, E8382-E8391. https://doi.org/10.1073/pnas.1712857114

[32] Oberprieler, R.G., Marvaldi, A.E. and Anderson, R.S. (2007) Weevils, Weevils, Weevils Everywhere. *Zootaxa*, **1668**, 491-520. https://doi.org/10.11646/zootaxa.1668.1.24

[33] McKenna, D.D., Sequeira, A.S., Marvaldi, A.E. and Farrell, B.D. (2009) Temporal Lags and Overlap in the Diversification of Weevils and Flowering Plants. *Proceedings of the National Academy of Sciences*, **106**, 7083-7088. https://doi.org/10.1073/pnas.0810618106

[34] Nardon, P., Lefèvre, C., Delobel, B., Charles, H. and Heddi, A. (2002) Occurrence of Endosymbiosis in Dryophtoridae Weevils: Cytological Insights into Bacterial Symbiotic Structures. *Symbiosis*, **33**, 227-241.

[35] Lefèvre, C., Charles, H., Vallier, A., Delobel, B., Farrell, B. and Heddi, A. (2004) Endosymbiont Phylogenesis in the Dryophthoridae Weevils: Evidence for Bacterial Replacement. *Molecular Biology and Evolution*, **21**, 965-973. https://doi.org/10.1093/molbev/msh063

[36] Hosokawa, T. and Fukatsu, T. (2010) Nardonella Endosymbiont in the West Indian Sweet Potato Weevil *Euscepes postfasciatus* (Coleoptera: Curculionidae). *Applied Entomology and Zoology*, **45**, 115-120. https://doi.org/10.1303/aez.2010.115

[37] Zchori-Fein, E. and Bourtzis, K. (2011) Manipulative Tenants: Bacteria Associated with Arthropods. CRC Press.

[38] Weinert, L.A., Araujo-Jnr, E.V., Ahmed, M.Z. and Welch, J.J. (2015) The Incidence of Bacterial Endosymbionts in Terrestrial Arthropods. *Proceedings of the Royal Society B: Biological Sciences*, **282**, Article ID: 20150249. https://doi.org/10.1098/rspb.2015.0249

[39] Dohet, L., Grégoire, J., Berasategui, A., Kaltenpoth, M. and Biedermann, P.H.W. (2016) Bacterial and Fungal Symbionts of Parasitic Dendroctonus Bark Beetles. *FEMS Microbiology Ecology*, **92**, fiw129. https://doi.org/10.1093/femsec/fiw129

[40] Salama, H.S., Foda, M.S., El-Bendary, M.A. and Abdel-Razek, A. (2004) Infection of Red Palm Weevil, *Rhynchophorus ferrugineus*, by Spore-Forming Bacilli Indigenous to Its Natural Habitat in Egypt. *Journal of Pest Science*, **77**, 27-31. https://doi.org/10.1007/s10340-003-0023-4

[41] Khiyami, M. and Alyamani, E. (2008) Aerobic and Facultative Anaerobic Bacteria from Gut of Red Palm Weevil (*Rhynchophorus ferrugineus*). *African Journal of*

Biotechnology, **7**, 1432-1437.

[42] Habineza, P., Muhammad, A., Ji, T., Xiao, R., Yin, X., Hou, Y., *et al*. (2019) The Promoting Effect of Gut Microbiota on Growth and Development of Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae) by Modulating Its Nutritional Metabolism. *Frontiers in Microbiology*, **10**, Article No. 1212. https://doi.org/10.3389/fmicb.2019.01212

[43] Liu, Q., Su, Z., Liu, H., Lu, S., Ma, B., Zhao, Y., *et al*. (2021) The Effect of Gut Bacteria on the Physiology of Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier and Their Potential for the Control of This Pest. *Insects*, **12**, Article No. 594. https://doi.org/10.3390/insects12070594

[44] Huang, Y., Feng, Z., Li, F. and Hou, Y. (2024) Host-Encoded Aminotransferase Import into the Endosymbiotic Bacteria *Nardonella* of Red Palm Weevil. *Insects*, **15**, Article No. 35. https://doi.org/10.3390/insects15010035

[45] Clarridge, J.E. (2004) Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases. *Clinical Microbiology Reviews*, **17**, 840-862. https://doi.org/10.1128/cmr.17.4.840-862.2004

[46] Petti, C.A., Polage, C.R. and Schreckenberger, P. (2005) The Role of 16S rRNA Gene Sequencing in Identification of Microorganisms Misidentified by Conventional Methods. *Journal of Clinical Microbiology*, **43**, 6123-6125. https://doi.org/10.1128/jcm.43.12.6123-6125.2005

[47] Tagliavia, M., Messina, E., Manachini, B., Cappello, S. and Quatrini, P. (2014) The Gut Microbiota of Larvae of *Rhynchophorus ferrugineus* Oliver (Coleoptera: Curculionidae). *BMC Microbiology*, **14**, Article No. 136. https://doi.org/10.1186/1471-2180-14-136

[48] Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. and Schloss, P.D. (2013) Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the Miseq Illumina Sequencing Platform. *Applied and Environmental Microbiology*, **79**, 5112-5120. https://doi.org/10.1128/aem.01043-13

[49] Brady, C.M. and White, J.A. (2013) Cowpea Aphid (*Aphis craccivora*) Associated with Different Host Plants Has Different Facultative Endosymbionts. *Ecological Entomology*, **38**, 433-437. https://doi.org/10.1111/een.12020

[50] Rosenwald, L.C., Sitvarin, M.I. and White, J.A. (2020) Endosymbiotic *Rickettsiella* Causes Cytoplasmic Incompatibility in a Spider Host. *Proceedings of the Royal Society B: Biological Sciences*, **287**, Article ID: 20201107. https://doi.org/10.1098/rspb.2020.1107

[51] Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al*. (2010) QIIME Allows Analysis of High-Throughput Community Sequencing Data. *Nature Methods*, **7**, 335-336. https://doi.org/10.1038/nmeth.f.303

[52] Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., *et al*. (2013) Quality-Filtering Vastly Improves Diversity Estimates from Illumina Amplicon Sequencing. *Nature Methods*, **10**, 57-59. https://doi.org/10.1038/nmeth.2276

[53] Amir, A., McDonald, D., Navas-Molina, J.A., Kopylova, E., Morton, J.T., Zech Xu, Z., *et al*. (2017) Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. *mSystems*, **2**, e00191-e00116. https://doi.org/10.1128/msystems.00191-16

[54] DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., *et al*. (2006) Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with Arb. *Applied and Environmental Microbiology*, **72**, 5069-5072. https://doi.org/10.1128/aem.03006-05

[55] Hosokawa, T., Koga, R., Tanaka, K., Moriyama, M., Anbutsu, H. and Fukatsu, T. (2015) *Nardonella* Endosymbionts of Japanese Pest and Non-Pest Weevils (Coleoptera: Curculionidae). *Applied Entomology and Zoology*, **50**, 223-229. https://doi.org/10.1007/s13355-015-0326-y

[56] Kuriwada, T., Hosokawa, T., Kumano, N., Shiromoto, K., Haraguchi, D. and Fukatsu, T. (2010) Biological Role of *Nardonella* Endosymbiont in Its Weevil Host. *PLOS ONE*, **5**, e13101. https://doi.org/10.1371/journal.pone.0013101

[57] Anbutsu, H. and Fukatsu, T. (2011) *Spiroplasma* as a Model Insect Endosymbiont. *Environmental Microbiology Reports*, **3**, 144-153. https://doi.org/10.1111/j.1758-2229.2010.00240.x

[58] Toju, H. and Fukatsu, T. (2010) Diversity and Infection Prevalence of Endosymbionts in Natural Populations of the Chestnut Weevil: Relevance of Local Climate and Host Plants. *Molecular Ecology*, **20**, 853-868. https://doi.org/10.1111/j.1365-294x.2010.04980.x

[59] White, J.A., Richards, N.K., Laugraud, A., Saeed, A., Curry, M.M. and McNeill, M.R. (2015) Endosymbiotic Candidates for Parasitoid Defense in Exotic and Native New Zealand Weevils. *Microbial Ecology*, **70**, 274-286. https://doi.org/10.1007/s00248-014-0561-8

[60] Regassa, L.B. (2006) Spiroplasmas: Evolutionary Relationships and Biodiversity. *Frontiers in Bioscience*, **11**, Article No. 2983. https://doi.org/10.2741/2027

[61] Jaenike, J., Unckless, R., Cockburn, S.N., Boelio, L.M. and Perlman, S.J. (2010) Adaptation via Symbiosis: Recent Spread of a *drosophila* Defensive Symbiont. *Science*, **329**, 212-215. https://doi.org/10.1126/science.1188235

[62] Xie, J., Vilchez, I. and Mateos, M. (2010) *Spiroplasma* Bacteria Enhance Survival of *Drosophila hydei* Attacked by the Parasitic Wasp *Leptopilina heterotoma*. *PLOS ONE*, **5**, e12149. https://doi.org/10.1371/journal.pone.0012149

[63] Hedges, L.M., Brownlie, J.C., O'Neill, S.L. and Johnson, K.N. (2008) Wolbachia and Virus Protection in Insects. *Science*, **322**, 702. https://doi.org/10.1126/science.1162418

[64] Ballinger, M.J. and Perlman, S.J. (2019) The Defensive *Spiroplasma*. *Current Opinion in Insect Science*, **32**, 36-41. https://doi.org/10.1016/j.cois.2018.10.004

[65] Bourtzis, K. (2008) Wolbachia-Based Technologies for Insect Pest Population Control. In: Aksoy, S., Ed., *Transgenesis and the Management of Vector-Borne Disease*, Springer, 104-113. https://doi.org/10.1007/978-0-387-78225-6_9

[66] Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M. and Goettel, M.S. (2015) Insect Pathogens as Biological Control Agents: Back to the Future. *Journal of Invertebrate Pathology*, **132**, 1-41. https://doi.org/10.1016/j.jip.2015.07.009

[67] Heddi, A. and Nardon, P. (2005) *Sitophilus oryzae* L.: A Model for Intracellular Symbiosis in the Dryophthoridae Weevils (Coleoptera). *Symbiosis*, **39**, 1-11.

[68] Colman, D.R., Toolson, E.C. and Takacs-Vesbach, C.D. (2012) Do Diet and Taxonomy Influence Insect Gut Bacterial Communities? *Molecular Ecology*, **21**, 5124-5137. https://doi.org/10.1111/j.1365-294x.2012.05752.x

[69] Engel, P. and Moran, N.A. (2013) The Gut Microbiota of Insects—Diversity in Structure and Function. *FEMS Microbiology Reviews*, **37**, 699-735. https://doi.org/10.1111/1574-6976.12025

[70] Dillon, R.J. and Dillon, V.M. (2004) The Gut Bacteria of Insects: Nonpathogenic Interactions. *Annual Review of Entomology*, **49**, 71-92.

https://doi.org/10.1146/annurev.ento.49.061802.123416

[71] Campbell, B.C., Bragg, T.S. and Turner, C.E. (1992) Phylogeny of Symbiotic Bacteria of Four Weevil Species (Coleoptera: Curculionidae) Based on Analysis of 16S Ribosomal DNA. *Insect Biochemistry and Molecular Biology*, **22**, 415-421. https://doi.org/10.1016/0965-1748(92)90136-3

[72] Behar, A., Yuval, B. and Jurkevitch, E. (2005) Enterobacteria-Mediated Nitrogen Fixation in Natural Populations of the Fruit Fly *Ceratitis capitata*. *Molecular Ecology*, **14**, 2637-2643. https://doi.org/10.1111/j.1365-294x.2005.02615.x

[73] Rinke, R., Costa, A.S., Fonseca, F.P.P., Almeida, L.C., Delalibera Júnior, I. and Henrique-Silva, F. (2011) Microbial Diversity in the Larval Gut of Field and Laboratory Populations of the Sugarcane Weevil *Sphenophorus Levis* (Coleoptera, Curculionidae). *Genetics and Molecular Research*, **10**, 2679-2691. https://doi.org/10.4238/2011.november.1.1

Appendix

Table S1. Specimen metadata.

	Location	Collection date	Morph	RPW sex	Host	Collector	Samples
RPW 1	Hoarat A'ali	24-IV-2017	I	M	*Phoenix dactylifera*	Khalid Alhudaib	Abdomen
RPW 2	Hoarat A'ali	24-IV-2017	II	M	*P. dactylifera*	Khalid Alhudaib	Gut, Testes
RPW 3	Hoarat A'ali	24-IV-2017	II	M	*P. dactylifera*	Khalid Alhudaib	Gut
RPW 5	Hoarat A'ali	24-IV-2017	II	F	*P. dactylifera*	Khalid Alhudaib	Gut
RPW 6	Hoarat A'ali	24-IV-2017	II	F	*P. dactylifera*	Khalid Alhudaib	Gut
RPW 7	Hoarat A'ali	24-IV-2017	II	F	*P. dactylifera*	Khalid Alhudaib	Gut
RPW 8	Hoarat A'ali	24-IV-2017	III	M	*P. dactylifera*	Khalid Alhudaib	Abdomen
RPW 9	Hoarat A'ali	24-IV-2017	III	M	*P. dactylifera*	Khalid Alhudaib	Abdomen
RPW 10	Hoarat A'ali	14-II-2018	I	M	*Whashingtonia spp.*	A.M.A. Mohamed	Abdomen
RPW 12	Hoarat A'ali	14-II-2018	I	F	*Whashingtonia spp.*	A.M.A. Mohamed	Gut
RPW 14	Hoarat A'ali	14-II-2018	I	F	*Whashingtonia spp.*	A.M.A. Mohamed	Abdomen
RPW 20	Hoarat A'ali	14-II-2018	III	F	*Whashingtonia spp.*	A.M.A. Mohamed	Abdomen
RPW 21	Maqabah	18-IV-2017	I	M	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPW 22	Maqabah	18-IV-2017	IIa	M	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPW 23	Maqabah	18-IV-2017	IIa	M	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPW 24	Maqabah	18-IV-2017	IIa	F	*P. dactylifera*	A.M.A. Mohamed	Gut
RPW 26	Maqabah	18-IV-2017	IIa	F	*P. dactylifera*	A.M.A. Mohamed	Gut
RPW 28	Maqabah	18-IV-2017	II	M	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPW 29	Maqabah	18-IV-2017	II	M	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPW 30	Maqabah	18-IV-2017	II	M	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPW 31	Maqabah	18-IV-2017	II	F	*P. dactylifera*	A.M.A. Mohamed	Gut
RPW 33	Maqabah	18-IV-2017	II	F	*P. dactylifera*	A.M.A. Mohamed	Gut
RPW 36	Maqabah	18-IV-2017	III	M	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPW 38	Maqabah	18-IV-2017	III	M	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPW 39	Maqabah	18-IV-2017	III	F	*P. dactylifera*	A.M.A. Mohamed	Gut
RPW 40	Maqabah	18-IV-2017	III	F	*P. dactylifera*	A.M.A. Mohamed	Gut
RPW 41	Maqabah	18-IV-2017	III	F	*P. dactylifera*	A.M.A. Mohamed	Gut
RPW 42	Maqabah	18-IV-2017	III	F	*P. dactylifera*	A.M.A. Mohamed	Gut
RPW 43	Maqabah	18-IV-2017	III	F	*P. dactylifera*	A.M.A. Mohamed	Gut
RPW 44	Maqabah	18-IV-2017	II	M	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPW 45	Zallaq	23-XII-2017	I	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 46	Zallaq	23-XII-2017	I	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 47	Zallaq	23-XII-2017	I	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 48	Zallaq	23-XII-2017	I	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 49	Zallaq	23-XII-2017	I	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 50	Zallaq	23-XII-2017	I	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 51	Zallaq	23-XII-2017	I	F	*P. dactylifera*	Sayed Hameed	Gut, Ovaries
RPW 52	Zallaq	23-XII-2017	I	F	*P. dactylifera*	Sayed Hameed	Gut, Ovaries
RPW 53	Zallaq	23-XII-2017	I	F	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 54	Zallaq	23-XII-2017	I	F	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 55	Zallaq	23-XII-2017	II	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 56	Zallaq	23-XII-2017	II	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 57	Zallaq	23-XII-2017	II	M	*P. dactylifera*	Sayed Hameed	Abdomen

Continued

RPW 58	Zallaq	23-XII-2017	II	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 59	Zallaq	23-XII-2017	II	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 60	Zallaq	23-XII-2017	II	F	*P. dactylifera*	Sayed Hameed	Gut, Ovaries
RPW 61	Zallaq	23-XII-2017	II	F	*P. dactylifera*	Sayed Hameed	Gut, Ovaries
RPW 62	Zallaq	23-XII-2017	II	F	*P. dactylifera*	Sayed Hameed	Gut, Ovaries
RPW 63	Zallaq	23-XII-2017	II	F	*P. dactylifera*	Sayed Hameed	Gut, Ovaries
RPW 64	Zallaq	23-XII-2017	III	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 66	Zallaq	23-XII-2017	III	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 67	Zallaq	23-XII-2017	III	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPW 68	Zallaq	23-XII-2017	III	M	*P. dactylifera*	Sayed Hameed	Abdomen
RPWL4	Hoarat A'ali	28-XII-2017	N/A	Larva	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPWL6	Hoarat A'ali	28-XII-2017	N/A	Larva	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPWL9	Hoarat A'ali	28-XII-2017	N/A	Larva	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPWL16	Hoarat A'ali	28-XII-2017	N/A	Larva	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPWL17	Hoarat A'ali	28-XII-2017	N/A	Larva	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPWL19	Hoarat A'ali	28-XII-2017	N/A	Larva	*P. dactylifera*	A.M.A. Mohamed	Abdomen
RPWL24	Hoarat A'ali	28-XII-2017	N/A	Larva	*P. dactylifera*	A.M.A. Mohamed	Abdomen

Table S2. ASV taxonomic identity, sequences, and frequency distribution across *R. ferrugineus* samples.

https://docs.google.com/spreadsheets/d/19gx16ZOKixWNjyJU95PBJBlMkimNmAuppdzqTfeib_8/edit?usp=sharing