Supplementary Materials

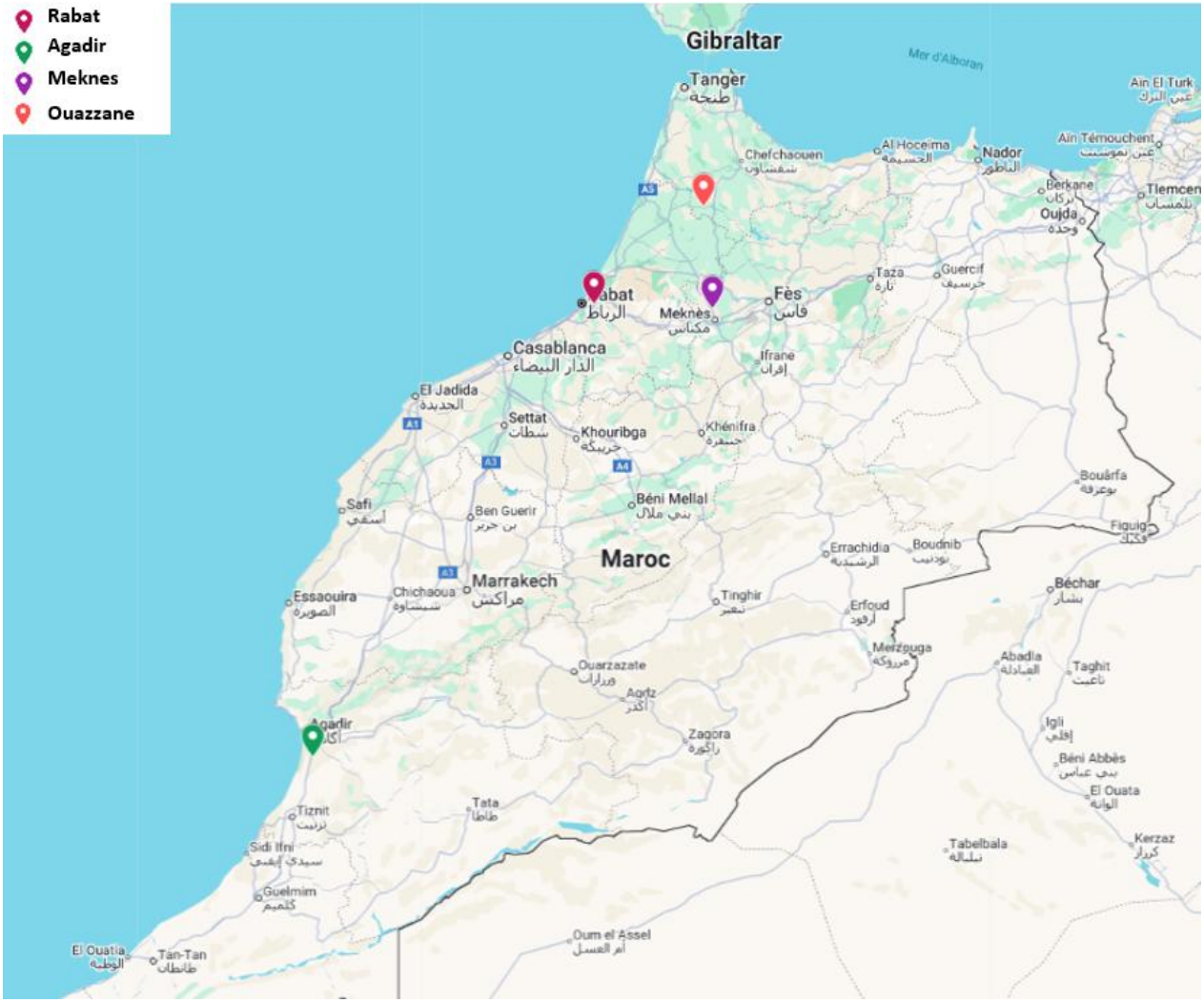


Figure S1. Graphical map of the regions

Table S1. List of bacterial primers and annealing temperatures

Gene	Primer	Sequence (5′-3′)	Tm (°C)	Product size
Wolbachia	WspecF	YATACCTATTCGAAGGGATAG	54 °C	438 bp
	WspecR	AGCTTCGAGTGAAACCAATTC		
Spiroplasma	SpouR1	CTTCAGTGTGCAGCTAACGC	55 °C	400 bp
	SpouF1	AAATCTTGTTAAGCAAGAAG		
16S rRNA	27F	AGAGTTTGATCCTGGCTCAG	54 °C	1500bp
	1429R	GGTTACCTTGTTACGACTT		

Table S2. Prevalence of bacterial endosymbionts screened in populations of *D. opuntiae*. + infected individuals (the number of infected samples per location), − uninfected individuals.

Population	Gender	Sample Size	*Wolbachia*	*Spiroplasma*
Agadir	Female	10	+(1)	**-**
	Male	10	-	**3**
	Nymph	10	-	**-**
Rabat	Female	10	-	**-**
	Male	10	-	**-**
	Nymph	10	-	**-**
Meknes	Female	10	+(1)	**-**
	Male	10	-	**-**
	Nymph	10	-	**-**
Ouazzane	Female	10	+(4)	**-**
	Male	10	+(6)	**1**
	Nymph	10	+(6)	**-**
Total		120	+(18)	+(4)

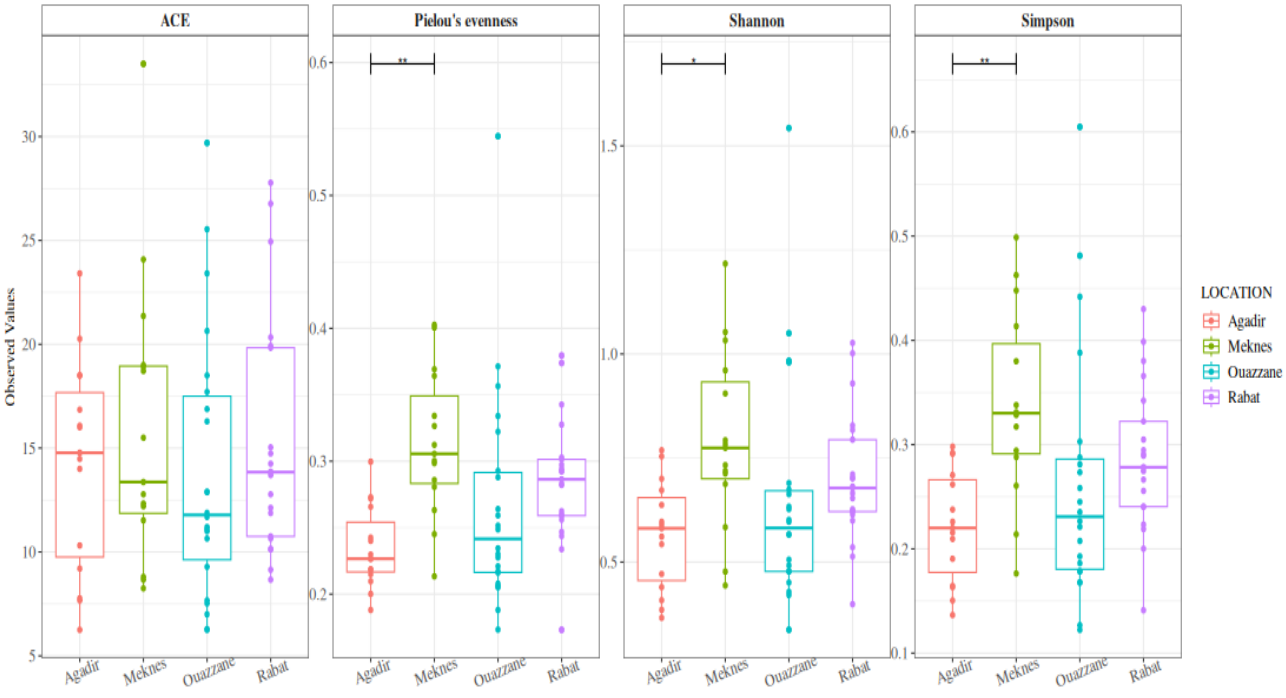


Figure S2. Species richness and diversity indices of *D. opuntiae* samples based on geographical location. Boxes indicate the inter-quartile range (IQR), the line within the boxes indicates the median, and the dots signify samples. (* $0.01 < p < 0.05$, ** $p \leq 0.01$).

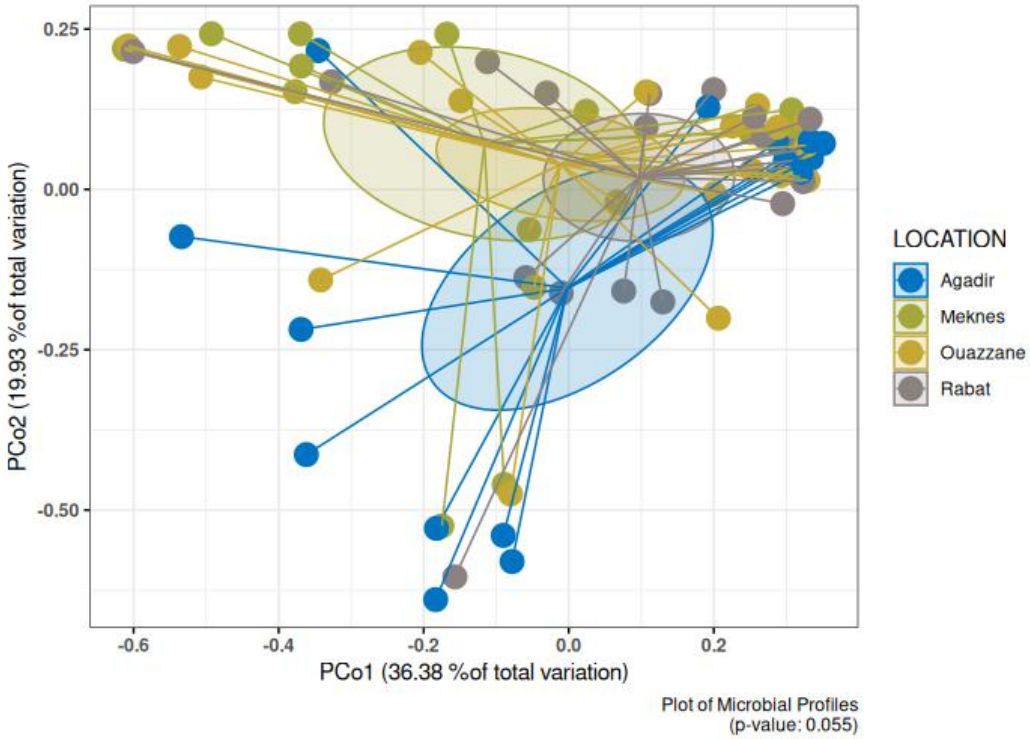


Figure S3. PCoA of *D. opuntiae*-associated bacterial communities, excluding *Candidatus* Dactylopiibacterium, according to the Bray-Curtis metric.

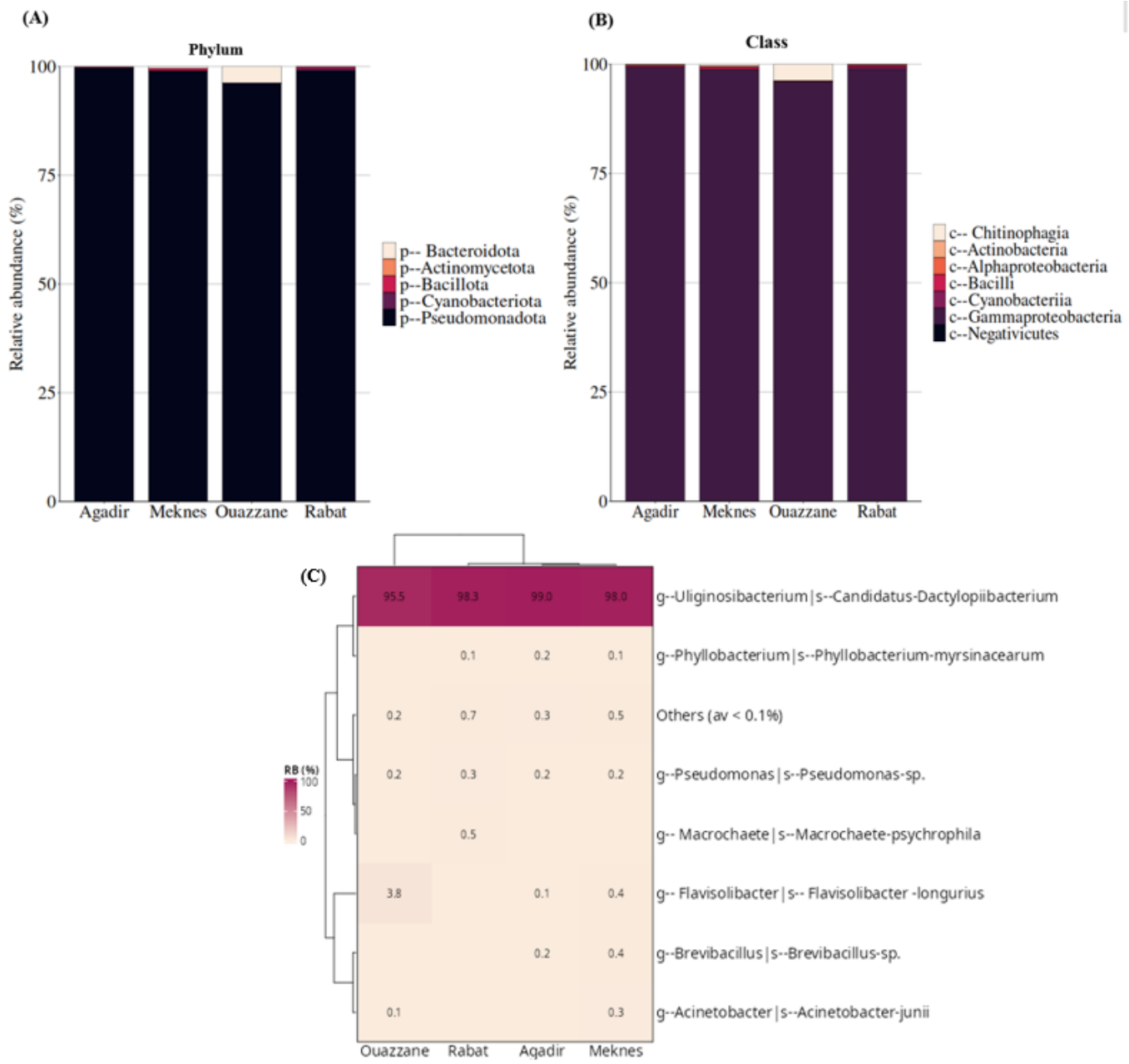


Figure S4. Relative abundance of natural *D. opuntiae* population microbiota at the phylum (A), class (B), and genus/species (C) levels.

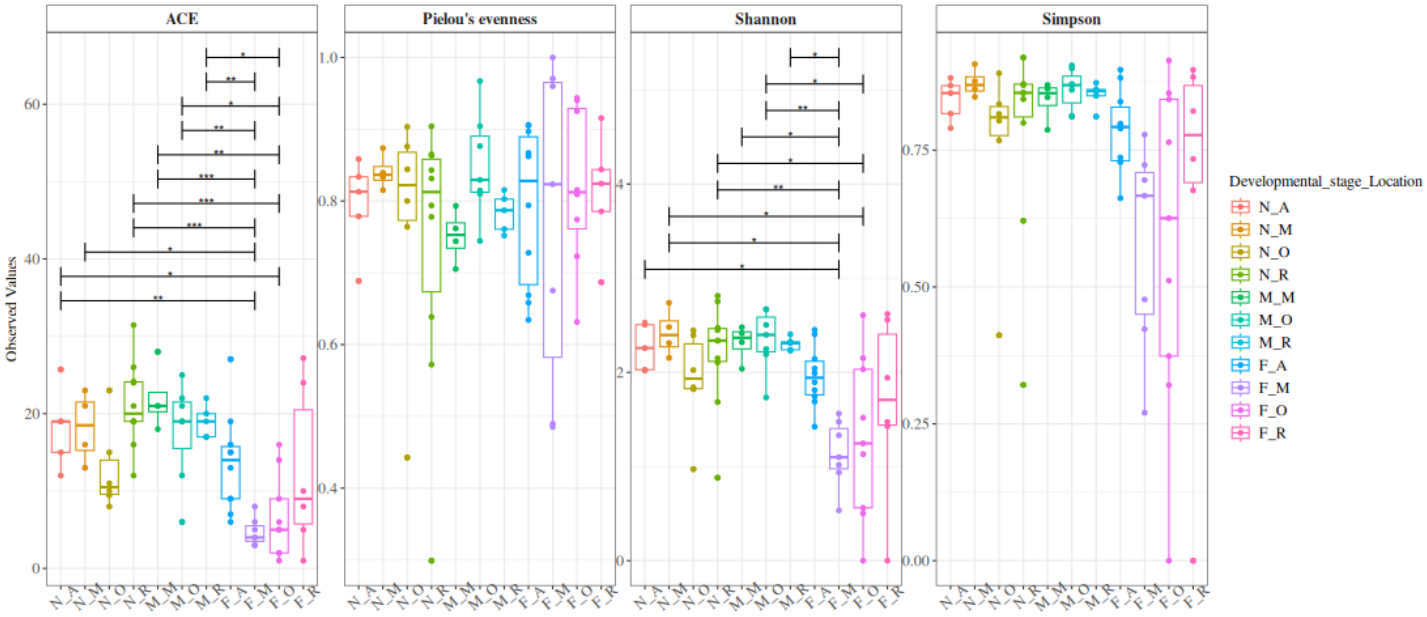


Figure S5. Species richness and diversity indices for *D. opuntiae* samples showed significant variations after excluding *Candidatus* Dactylopiibacterium based on their developmental stages and locations. The inter-quartile range (IQR) is shown by boxes, the median is shown by the line inside the boxes, and samples are shown by the dots. ** p<0.01, *** p<0.001, and * p<0.05.

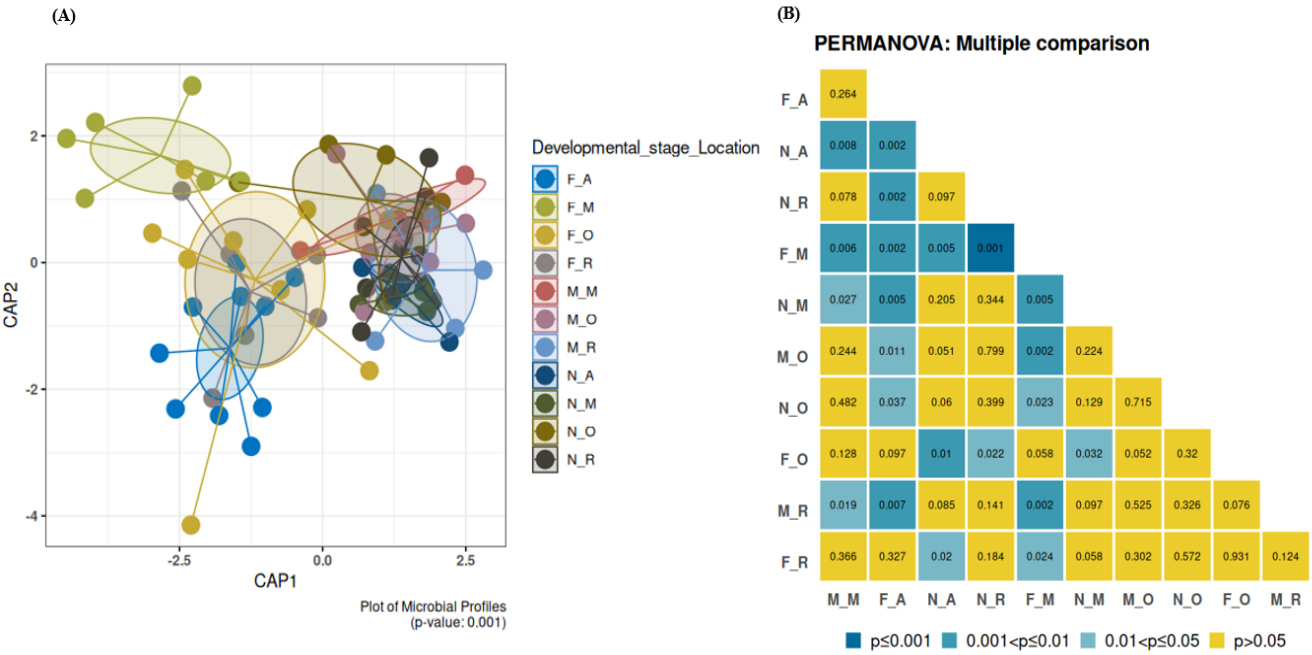


Figure S6. Diversity of *D. opuntiae*-associated bacterial communities based on all developmental stages and locations. Constrained Analysis of Principal Coordinates (CAP) plot based on the Bray-Curtis metric (A) and the pairwise PERMANOVA comparison (B).