

Supplementary Materials

Table S1. Prevalence of bacterial endosymbionts screened in populations of barley stem gall midge.

| Population | Gender | Sample Size | <i>Wolbachia</i> | <i>Spiroplasma</i> | <i>Cardinium</i> | <i>Arsenophonus</i> |
|------------|--------|-------------|------------------|--------------------|------------------|---------------------|
| Rabat | Larvae | 22 | +(5) | - | - | - |
| | Male | 11 | +(3) | - | - | - |
| | Female | 11 | +(2) | - | - | - |
| Fes-Meknes | Larvae | 30 | +(1) | - | - | - |
| | Male | 11 | +(1) | - | - | - |
| | Female | 11 | +(1) | - | - | - |
| Doukkala | Larvae | 38 | - | - | - | - |

+ infected individuals (the number of infected samples per location), – uninfected individuals.

Table S2. List of bacterial primers and annealing temperatures used.

| Gene | Primer | Sequence (5'-3') | Tm °C | Product size |
|-----------------------|--------|------------------------------|-------|--------------|
| <i>Wolbachia</i> | WspecF | YATACCTATTCGAAGGGATAG | 54 °C | 438 bp |
| | WspecR | AGCTTCGAGTGAAACCAATTC | | |
| <i>Spiroplasma</i> | 63F | GCCTAATACATGCAAGTCGAACGG | 57 °C | 450 bp |
| | TKSS | TAGCCGTGGCTTTCTGGTAA | | |
| <i>Cardinium</i> | CloF1 | GGAACCTTACCTGGGCTAGAATGTATT | 55 °C | 466 bp |
| | CloR1 | GCCACTGTCTTCAAGCTCTACCAAC | | |
| <i>Arsenophonus</i> | ArsF | GGGTTGTAAAGTACTTTTCAGTCGT | 56 °C | 600 bp |
| | ArsR3 | CCTYTATCTCTAAAGGTTTCGCTGGATG | | |
| 12S rRNA | 12SCFR | GAGAGTGACGGGCGATATGT | 54 °C | 377 bp |
| | 12SCRR | AAACCAGGATTAGATACCCTATTAT | | |
| 16S rRNA V3-V4 region | U341F | CCTACGGGRCAGCAG | 53 °C | 464 bp |
| | 805R | GACTACCAGGGTATCTAAT | | |
| 16S rRNA | 27F | AGAGTTTGATCCTGGCTCAG | 54 °C | 1500bp |
| | 1429R | GGTACCTTGTTACGACTT | | |

Table S3. Distribution of Dependencia family (Otu 45)

| Region | Doukkala | | | | | | Abda | | | | | | Rabat | | | | | Fes-Meknes | | | | | | | | Total |
|-------------|----------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-------|-----|-----|-----|-----|------------|-----|-----|-----|-----|-----|-----|--|-------|
| Sample code | I13 | I14 | I15 | I17 | I4 | I8 | I24 | I29 | I31 | I32 | I33 | I34 | I40 | I41 | I44 | I46 | I47 | I59 | I61 | I63 | I67 | I69 | I73 | I76 | | 24 |
| Reads | 285 | 99 | 134 | 33 | 382 | 429 | 429 | 38 | 143 | 125 | 703 | 45 | 32 | 37 | 228 | 101 | 122 | 922 | 296 | 331 | 25 | 73 | 148 | 42 | | 5202 |

Table S4. Distribution of Negativicutes class (Otu 106)

| Region | Doukkala | | | | | Abda | Fes-Meknes | Total |
|-------------|----------|-----|------|-----|-----|------|------------|-------|
| Sample code | I7 | I9 | I16 | I17 | I18 | I23 | I67 | 7 |
| Reads | 214 | 300 | 1190 | 933 | 534 | 933 | 150 | 4254 |

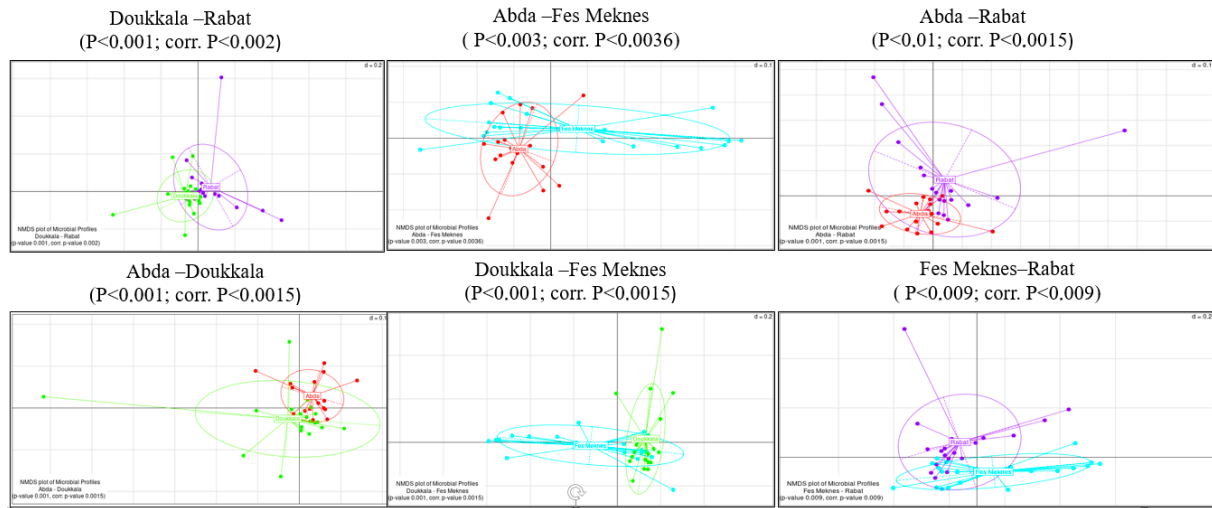


Figure S1. Pairwise non-metric multidimensional scaling (NMDS) plot of bacterial communities in Barley stem gall midge samples collected from Rabat (purple), Fes-Meknes (blue), Abda (red), and Doukkala (green) ($p < 0.001$). The 'd' stands for the grid's dissimilarity scale ($d = 0.2$ implies that the distance between two grid lines represents approximately 20% dissimilarity between the regions).

Table S6: Distribution of Wolbachia genus (Otu 156)

| Region | Abda | Rabat | | | | | | | Total |
|-------------|------|-------|-----|-----|-----|-----|-----|-----|-------|
| Sample code | I22 | I41 | I43 | I49 | I50 | I54 | I55 | I56 | 8 |
| Reads | 4 | 7 | 10 | 11 | 451 | 125 | 260 | 10 | 878 |

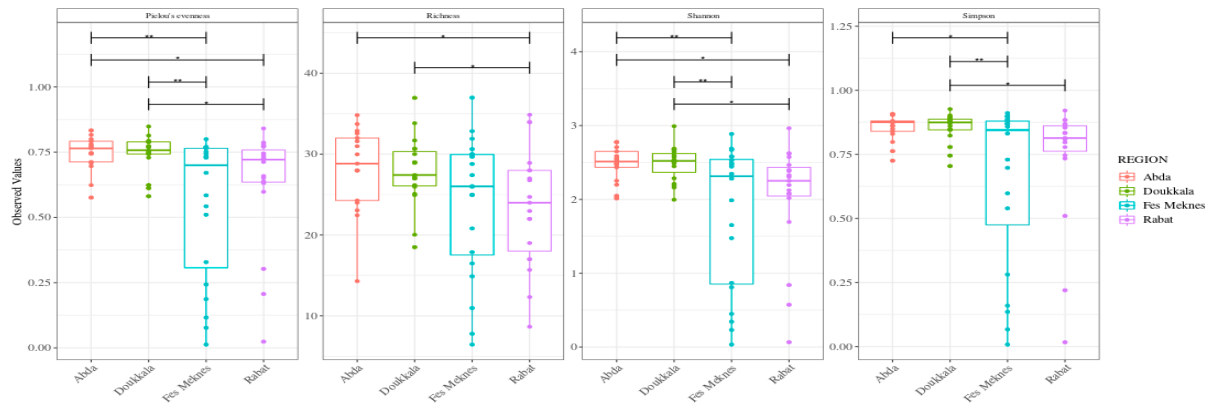


Figure S2. Species richness and diversity indices with significant differences in natural populations of Barley steam gall midge samples collected from the regions of Rabat, Fes Meknes, Abda, and Doukkala. The interquartile range (IQR) is shown by boxes, a line within the boxes represents the median, and samples are represented by dots. (* 0.01 < p < 0.05, ** p ≤ 0.01).

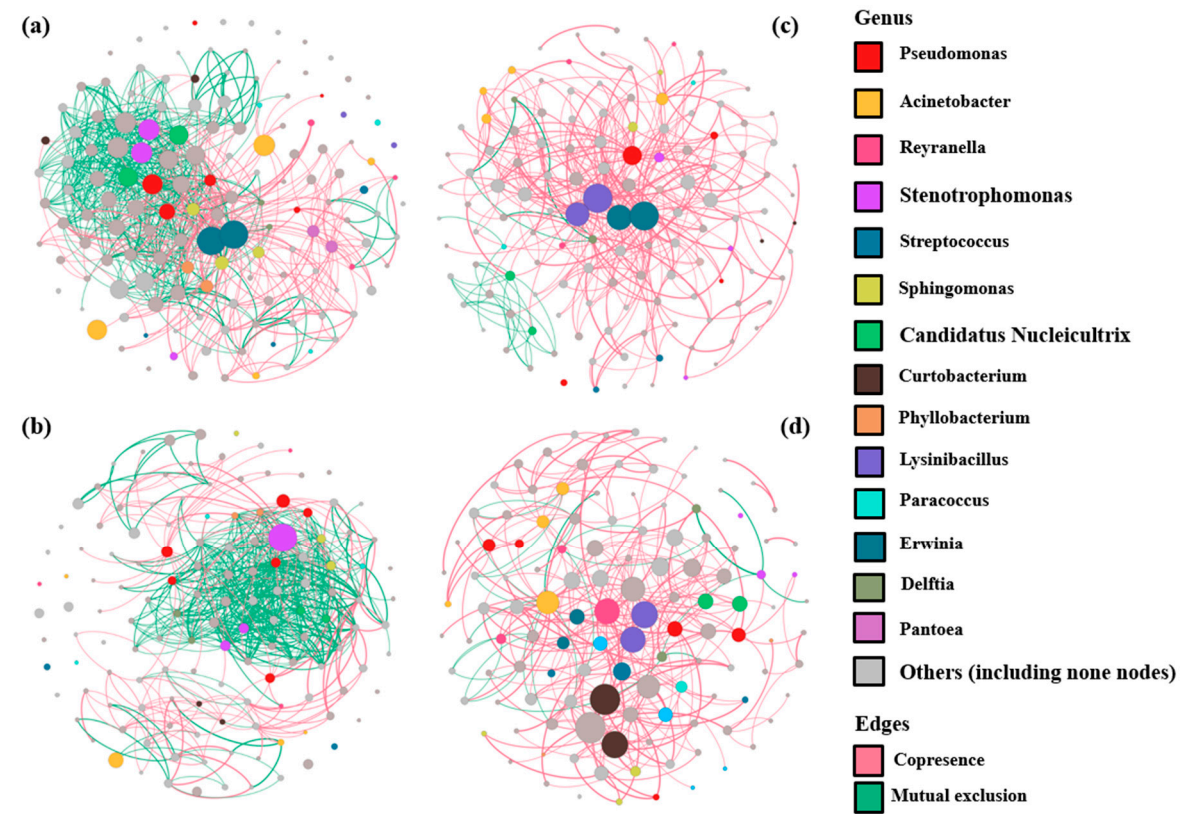


Figure S3. Co-occurrence and mutual exclusion networks at the genus level for the OTUs that compose the bacterial communities of *Mayetiola hordei* natural populations from the four regions, (a) Fes-Meknes, (b) Rabat, (c) Doukkala, and (d) Abda. The degree of interaction determines the size of each node. Cases of copresence are shown by green edges, while mutual exclusion is represented by red edges.