

Supplementary material – File S1

Community Richness and Diversity of Endophytic Fungi Associated with the Orchid *Guarianthe skinneri* Infested with “Black Blotch” in the Soconusco Region, Chiapas, Mexico

F. Hernández-Ramírez, A. Damon, S. P. Fernández-Pavía, K. Guillén-Navarro, L. Iracheta-Donjuan, E. Zarza and R. Castro-Chan

Table S1. Sample ID, barcode, plant number, condition, tissue, sample, number of raw sequences, number of filtered sequences, number of sequences without chimeras. The samples correspond to leaves (L), pseudobulbs (P) and roots (R) of five (1-5) asymptomatic plants (AP) and five (6-10) symptomatic plants (SP) of *Guarianthe skinneri*.

| SampleID | Barcode | Plant | Condition | Tissue | Sample | Raw sequences | Dada-ITSxpress | Dada-ITSx-UCHIME |
|----------|----------|-------|-----------|--------|---------|---------------|----------------|------------------|
| T4.R1 | ATCGATGC | 1 | AP | L | 1-AP-L | 215758 | 65453 | 59969 |
| T4.R2 | ATCGCCAA | 2 | AP | L | 2-AP-L | 538225 | 123752 | 119845 |
| T4.R3 | ATCGCCTT | 3 | AP | L | 3-AP-L | 213764 | 68544 | 65410 |
| T4.R4 | ATCGCGAT | 4 | AP | L | 4-AP-L | 269370 | 62528 | 62346 |
| T4.R5 | ATCGCGTA | 5 | AP | L | 5-AP-L | 151471 | 110646 | 110288 |
| T5.R1 | ATCGGCAT | 1 | AP | P | 1-AP-P | 330069 | 116802 | 106055 |
| T5.R2 | ATCGGCTA | 2 | AP | P | 2-AP-P | 393540 | 109719 | 106447 |
| T5.R3 | ATCGTACG | 3 | AP | P | 3-AP-P | 298451 | 78061 | 67188 |
| T5.R4 | ATCGTAGC | 4 | AP | P | 4-AP-P | 462846 | 127246 | 108754 |
| T5.R5 | ATCGTTCC | 5 | AP | P | 5-AP-P | 424253 | 98466 | 93898 |
| T6.R1 | ATCGTTGG | 1 | AP | R | 1-AP-R | 439851 | 113361 | 108528 |
| T6.R2 | ATGCAACC | 2 | AP | R | 2-AP-R | 444652 | 123270 | 120874 |
| T6.R3 | ATGCAAGG | 3 | AP | R | 3-AP-R | 330528 | 63125 | 60504 |
| T6.R4 | ATGCATCG | 4 | AP | R | 4-AP-R | 281130 | 142881 | 139537 |
| T6.R5 | ATGCATGC | 5 | AP | R | 5-AP-R | 663244 | 164519 | 160632 |
| T10.R1 | ATGCCGAT | 6 | SP | L | 6-SP-L | 469070 | 63394 | 59103 |
| T10.R2 | ATGCCGTA | 7 | SP | L | 7-SP-L | 313260 | 83043 | 82702 |
| T10.R3 | ATGCGCAT | 8 | SP | L | 8- SP-L | 377838 | 80204 | 77666 |

| | | | | | | | |
|--------|----------|-------|---|----------|--------|--------|--------|
| T10.R4 | ATGCGCTA | 9 SP | L | 9- SP-L | 717045 | 237646 | 236501 |
| T10.R5 | ATGCGGAA | 10 SP | L | 10-SP-L | 484045 | 91548 | 88882 |
| T11.R1 | ATGCGGTT | 6 SP | P | 6- SP-P | 301400 | 56875 | 52475 |
| T11.R2 | ATGCTACG | 7 SP | P | 7- SP-P | 579654 | 63218 | 62069 |
| T11.R3 | ATGCTAGC | 8 SP | P | 8- SP-P | 554395 | 154851 | 153397 |
| T11.R4 | ATGCTTCC | 9 SP | P | 9- SP-P | 579339 | 102917 | 95973 |
| T11.R5 | ATGCTTGG | 10 SP | P | 10- SP-P | 512337 | 139075 | 136506 |
| T12.R1 | ATGGAACG | 6 SP | R | 6- SP-R | 408895 | 10463 | 9346 |
| T12.R2 | ATGGAAGC | 7 SP | R | 7- SP-R | 762220 | 138784 | 131957 |
| T12.R3 | ATGGATCC | 8 SP | R | 8- SP-R | 316132 | 20515 | 18077 |
| T12.R4 | ATGGATGG | 9 SP | R | 9- SP-R | 656609 | 170886 | 166532 |
| T12.R5 | ATGCCCAT | 10 SP | R | 10-SP-R | 757706 | 175860 | 172269 |

Table S2. Taxonomy of the composition of the fungal community. The samples correspond to leaves (L), pseudobulbs (P) and roots (R) of five (1-5) asymptomatic plants (AP) and five (6-10) symptomatic plants (SP) of *Guarianthe skinneri*.

| Sample | Phylum | Class | Order | Family | Genus | Species |
|----------|--------|-------|-------|--------|-------|---------|
| 1-AP-L | 2 | 6 | 22 | 32 | 38 | 32 |
| 2-AP-L | 2 | 7 | 17 | 19 | 20 | 20 |
| 3-AP-L | 2 | 7 | 21 | 25 | 33 | 26 |
| 4-AP-L | 2 | 6 | 15 | 19 | 17 | 16 |
| 5-AP-L | 2 | 6 | 18 | 19 | 19 | 19 |
| 1-AP-P | 2 | 6 | 20 | 28 | 28 | 26 |
| 2-AP-P | 3 | 7 | 20 | 27 | 26 | 21 |
| 3-AP-P | 2 | 11 | 27 | 32 | 35 | 25 |
| 4-AP-P | 3 | 11 | 30 | 44 | 51 | 41 |
| 5-AP-P | 3 | 6 | 27 | 40 | 43 | 35 |
| 1-AP-R | 2 | 8 | 26 | 44 | 53 | 33 |
| 2-AP-R | 2 | 7 | 24 | 27 | 34 | 30 |
| 3-AP-R | 3 | 7 | 24 | 35 | 36 | 21 |
| 4-AP-R | 2 | 10 | 22 | 28 | 27 | 19 |
| 5-AP-R | 3 | 10 | 30 | 32 | 35 | 31 |
| 6-SP-L | 2 | 7 | 21 | 25 | 27 | 26 |
| 7-SP-L | 2 | 6 | 18 | 21 | 24 | 23 |
| 8- SP-L | 2 | 6 | 23 | 28 | 32 | 33 |
| 9- SP-L | 2 | 8 | 19 | 20 | 21 | 20 |
| 10- SP-L | 5 | 17 | 45 | 57 | 76 | 49 |
| 6- SP-P | 2 | 7 | 21 | 25 | 23 | 19 |

| | | | | | | |
|----------|---|----|----|-----|-----|-----|
| 7- SP-P | 3 | 7 | 26 | 37 | 42 | 40 |
| 8- SP-P | 2 | 12 | 34 | 39 | 44 | 34 |
| 9- SP-P | 2 | 6 | 18 | 20 | 21 | 22 |
| 10- SP-P | 2 | 7 | 25 | 35 | 43 | 35 |
| 6- SP-R | 3 | 9 | 27 | 35 | 35 | 20 |
| 7- SP-R | 2 | 11 | 32 | 44 | 51 | 42 |
| 8- SP-R | 2 | 7 | 20 | 25 | 24 | 17 |
| 9- SP-R | 4 | 13 | 37 | 44 | 49 | 39 |
| 10- SP-R | 3 | 9 | 30 | 45 | 48 | 35 |
| Total | 5 | 28 | 70 | 156 | 234 | 164 |

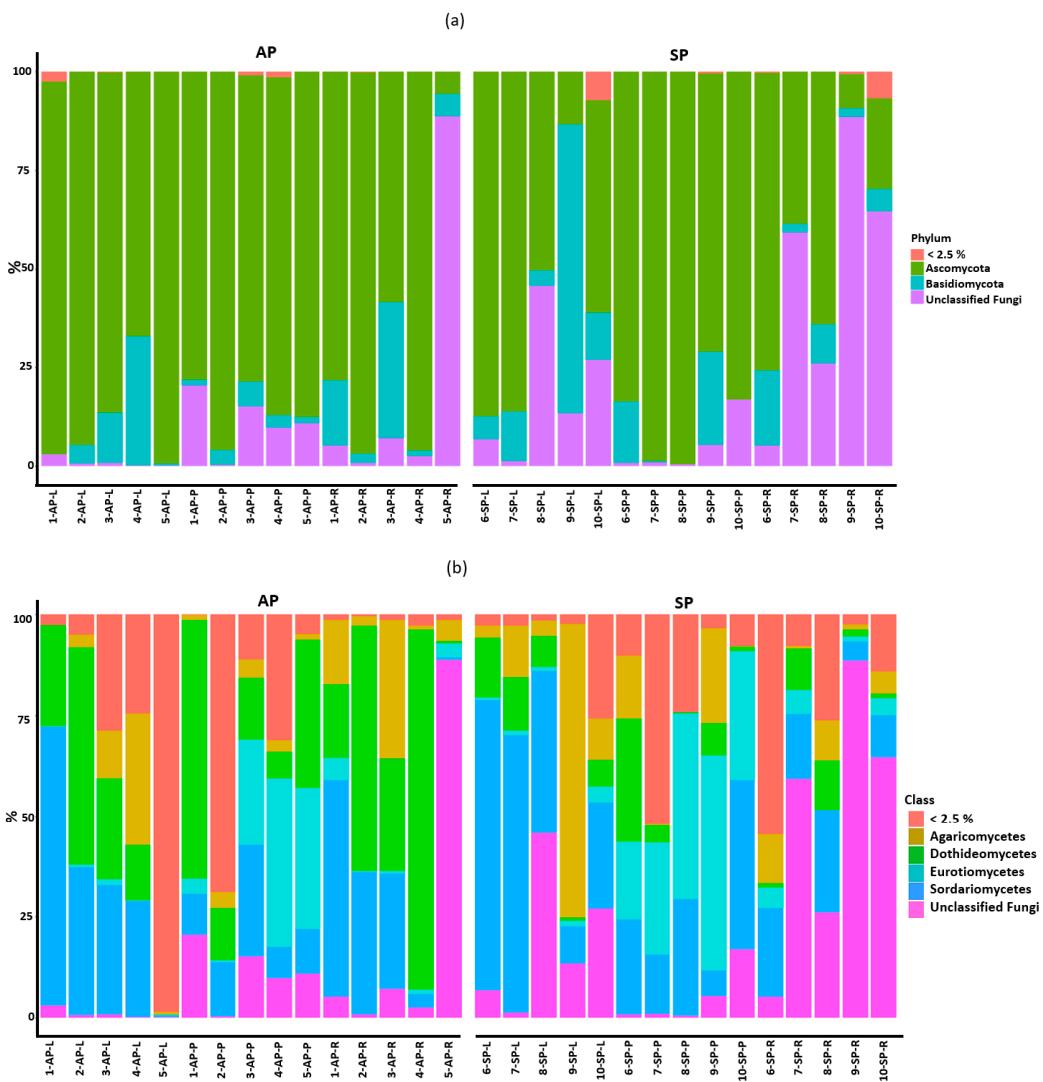


Figure S1. Relative frequency of Phylum (a) and Class (b) assigned to endophytic fungi associated with *Guarianthe skinneri* asymptomatic and symptomatic plants. The samples correspond to the leaves (L), pseudobulb (P) and roots (R) of five (1-5) asymptomatic plants (AP) and five (6-10) symptomatic plants (SP).

Table S3. PERMANOVA analysis. The samples correspond to leaves (L), pseudobulbs (P) and roots (R) of symptomatic and asymptomatic plants of *Guarianthe skinneri*.

| Method name | PERMANOVA | | |
|------------------------|---------------------|----------|---------|
| | Test statistic name | Pseudo-F | |
| Group | L vs P | L vs R | P vs R |
| Simple size | 20 | 19 | 19 |
| Number of groups | 2 | 2 | 2 |
| Test statistic | 1.62578 | 1.54544 | 1.11884 |
| p-value | 0.001 | 0.001 | 0.079 |
| q-value | 0.0015 | 0.0015 | 0.079 |
| Number of permutations | 999 | 999 | 999 |

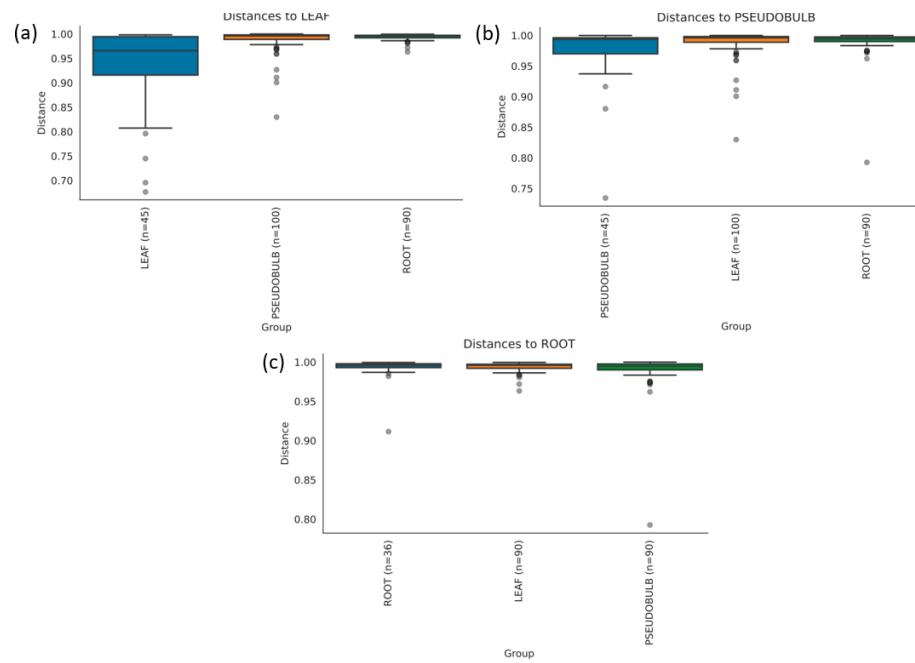


Figure S2. Associations by tissue using Bray-Curtis distances, calculated with Qiime2, based on the data set including all taxa detected in leaves (a), pseudobulbs (b) and roots (c) of symptomatic and asymptomatic plants of *Guarianthe skinneri*.

Table S4. PERMANOVA analysis of asymptomatic plants of *Guarianthe skinneri*. The samples correspond to leaves (L), pseudobulbs (P) and roots (R).

| Method name | | PERMANOVA | | |
|------------------------|--------|-----------|--------|--|
| Test statistic name | | Pseudo-F | | |
| Group | L vs P | L vs R | P vs R | |
| Simple size | 10 | 10 | 10 | |
| Number of groups | 2 | 2 | 2 | |
| Test statistic | 1.52 | 1.36 | 1.08 | |
| p-value | 0.005 | 0.097 | 0.105 | |
| q-value | 0.015 | 0.015 | 0.015 | |
| Number of permutations | 999 | 999 | 999 | |

Table S5. PERMANOVA analysis of symptomatic plants of *Guarianthe skinneri*. The samples correspond to leaves (L), pseudobulbs (P) and roots (R).

| Method name | | PERMANOVA | | |
|------------------------|--------|-----------|--------|--|
| Test statistic name | | Pseudo-F | | |
| Group | L vs P | L vs R | P vs R | |
| Simple size | 10 | 9 | 9 | |
| Number of groups | 2 | 2 | 2 | |
| Test statistic | 1.35 | 1.34 | 1.28 | |
| p-value | 0.02 | 0.01 | 0.02 | |
| q-value | 0.02 | 0.02 | 0.02 | |
| Number of permutations | 999 | 999 | 999 | |

Table S8. Total occurrence of ASVs associated with *Guarianthe skinneri*, classified by Trophic Mode and Guild. Bold type corresponds to guild labeled with endophyte type.

| Trophic Mode | Total ASVs | Percentaje % | Guild | ASVs | Percentaje % |
|----------------------|------------|--------------|---|------|--------------|
| Saprotoph | 304 | 16.37 | Undefined Saprotoph | 244 | 13.1 |
| | | | Wood Saprotoph | 26 | 1.4 |
| | | | Soil Saprotoph | 10 | 0.5 |
| | | | Plant Pathogen-Undefined Saprotoph | 6 | 0.3 |
| | | | Dung Saprotoph | 4 | 0.2 |
| | | | Dung Saprotoph-Plant Saprotoph-Wood Saprotoph | 4 | 0.2 |
| | | | Dung Saprotoph-Undefined Saprotoph-Wood Saprotoph | 4 | 0.2 |
| | | | Dung Saprotoph-Wood Saprotoph | 2 | 0.1 |
| | | | Litter Saprotoph-Soil Saprotoph-Wood Saprotoph | 2 | 0.1 |
| | | | Litter Saprotoph | 1 | 0.1 |
| | | | Plant Saprotoph-Wood Saprotoph | 1 | 0.1 |
| Pathotroph-Saprotoph | 170 | 9.15 | Animal Pathogen-Undefined Saprotoph | 39 | 2.1 |
| | | | Animal Pathogen-Plant Pathogen-Undefined Saprotoph | 36 | 1.9 |
| | | | Plant Pathogen-Undefined Saprotoph | 28 | 1.5 |
| | | | Endophyte-Plant Pathogen-Wood Saprotoph | 22 | 1.2 |
| | | | Animal Pathogen-Fungal Parasite-Undefined Saprotoph | 18 | 1.0 |
| | | | Plant Pathogen-Wood Saprotoph | 14 | 0.8 |
| | | | Plant Pathogen | 8 | 0.4 |
| | | | Endophyte-Lichen Parasite-Plant Pathogen-Undefined Saprotoph | 2 | 0.1 |

| | | | | | |
|-------------|-----|------|---------------------------|----|-----|
| | | | Fungal Parasite-Plant | 2 | 0.1 |
| | | | Pathogen-Plant Saprotoph | | |
| Pathotroph- | 168 | 9.05 | Animal Pathogen- | 47 | 2.5 |
| Saprotoph- | | | Endophyte-Fungal | | |
| Symbiotroph | | | Parasite-Lichen Parasite- | | |
| | | | Plant Pathogen-Wood | | |
| | | | Saprotoph | | |
| | | | Animal Pathogen- | 30 | 1.6 |
| | | | Endophyte-Lichen | | |
| | | | Parasite-Plant Pathogen- | | |
| | | | Soil Saprotoph-Wood | | |
| | | | Saprotoph | | |
| | | | Animal Pathogen- | 23 | 1.2 |
| | | | Endophyte-Endosymbiont- | | |
| | | | Epiphyte-Soil Saprotoph- | | |
| | | | Undefined Saprotoph | | |
| | | | Dung Saprotoph- | 21 | 1.1 |
| | | | Endophyte-Plant | | |
| | | | Pathogen-Undefined | | |
| | | | Saprotoph | | |
| | | | Animal Pathogen- | 11 | 0.6 |
| | | | Endophyte-Fungal | | |
| | | | Parasite-Plant Pathogen- | | |
| | | | Wood Saprotoph | | |
| | | | Endophyte-Lichen | 5 | 0.3 |
| | | | Parasite-Plant Pathogen- | | |
| | | | Undefined Saprotoph | | |
| | | | Animal Pathogen-Dung | 5 | 0.3 |
| | | | Saprotoph-Endophyte- | | |
| | | | Lichen Parasite-Plant | | |
| | | | Pathogen-Undefined | | |
| | | | Saprotoph | | |
| | | | Animal Pathogen- | 4 | 0.2 |
| | | | Endophyte-Epiphyte-Plant | | |
| | | | Pathogen-Undefined | | |
| | | | Saprotoph | | |
| | | | Animal Pathogen- | 3 | 0.2 |
| | | | Endophyte-Plant | | |
| | | | Pathogen-Wood | | |
| | | | Saprotoph | | |
| | | | Endophyte-Plant | 3 | 0.2 |
| | | | Pathogen-Wood | | |
| | | | Saprotoph | | |
| | | | Animal Pathogen- | 2 | 0.1 |
| | | | Endophyte-Epiphyte- | | |
| | | | Fungal Parasite-Plant | | |
| | | | Pathogen-Wood | | |
| | | | Saprotoph | | |

| | | | | | |
|------------------------|-----|------|----------------------------------|-----|-----|
| | | | Bryophyte Parasite-Dung | 2 | 0.1 |
| | | | Saprotrhop- | | |
| | | | Ectomycorrhizal-Fungal | | |
| | | | Parasite-Leaf Saprotrhop- | | |
| | | | Plant Parasite-Undefined | | |
| | | | Saprotrhop-Wood | | |
| | | | Saprotrhop | | |
| | | | Animal Pathogen- | 2 | 0.1 |
| | | | Endophyte-Ericoid | | |
| | | | Mycorrhizal-Plant | | |
| | | | Pathogen-Wood | | |
| | | | Saprotrhop | | |
| | | | Endophyte-Lichen | 2 | 0.1 |
| | | | Parasite-Undefined | | |
| | | | Saprotrhop | | |
| | | | Endophyte-Plant | 2 | 0.1 |
| | | | Pathogen-Plant Saprotrhop | | |
| | | | Animal Pathogen- | 1 | 0.1 |
| | | | Endophyte-Epiphyte- | | |
| | | | Undefined Saprotrhop | | |
| | | | Animal Pathogen-Soil | 1 | 0.1 |
| | | | Saprotrhop | | |
| | | | Animal Endosymbiont- | 1 | 0.1 |
| | | | Animal Pathogen-Plant | | |
| | | | Pathogen-Undefined | | |
| | | | Saprotrhop | | |
| | | | Ectomycorrhizal- | 1 | 0.1 |
| | | | Endophyte-Plant | | |
| | | | Pathogen-Wood | | |
| | | | Saprotrhop | | |
| | | | Endomycorrhizal-Plant | 1 | 0.1 |
| | | | Pathogen-Undefined | | |
| | | | Saprotrhop | | |
| | | | Endophyte-Fungal | 1 | 0.1 |
| | | | Parasite-Lichen Parasite- | | |
| | | | Plant Pathogen-Wood | | |
| | | | Saprotrhop | | |
| Pathotroph-Symbiotroph | 117 | 6.30 | Endophyte-Plant Pathogen | 112 | 6.0 |
| | | | Endophyte-Epiphyte- | 3 | 0.2 |
| | | | Fungal Parasite-Insect | | |
| | | | Parasite | | |
| | | | Animal Pathogen-Animal | 2 | 0.1 |
| | | | Endosymbiont | | |
| Pathotroph | 104 | 5.60 | Plant Pathogen | 94 | 5.1 |
| | | | Animal Pathogen | 5 | 0.3 |
| | | | Fungal Parasite | 4 | 0.2 |

| | | | | | |
|-----------------------|-----|------|---|------------------|--------------------------|
| | | | Litter Saprotoph-Plant Pathogen | 1 | 0.1 |
| Saprotoph-Symbiotroph | 67 | 3.61 | Endophyte-Uncertain Saprotoph-Wood Saprotoph Endophyte-Litter Saprotoph-Soil Saprotoph-Uncertain Saprotoph Dung Saprotoph- Endophyte-Litter Saprotoph-Uncertain Saprotoph Dung Saprotoph- Ectomycorrhizal-Litter Saprotoph-Uncertain Saprotoph Endophyte-Uncertain Saprotoph | 54 | 2.9 |
| Symbiotroph | 12 | 0.65 | Epiphyte Arbuscular Mycorrhizal Ectomycorrhizal Endophyte | 5 3 3 1 | 0.3 0.2 0.2 0.1 |
| Total | 942 | 50.7 | | 942 | 50.7 |