

Article III *Spatial and temporal turnovers of mycorrhizal communities in the host-tree specific epiphytic orchid* *Bulbophyllum variegatum*

Supplementary Material

Fig. S1 Hairy roots of *B. variegatum*

Fig. S2 Distribution of sampling sites on the island

Fig. S3 Phylogenetic tree of Sebaciniales

Fig. S4 Phylogenetic tree of Tulasnellaceae

Fig. S5 Temporal turnover of non-OMF fungi

Fig. S6 Fungal composition of seedlings and young seedlings

Fig. S7 NMDS stressing differences between 2006 and 2021 samplings

Fig. S8 Fungal sharing between 2006 and 2021

Fig. S9 Variance partitioning

Fig. S10 Germination experiment

Table S1 Statistics for differences in the distribution of main OMF OTUs between 2006 and 2021

Figure S1 (A) Hairy roots of *B. variegatum* cleaned in a petri dish. (B) Transversal section of a highly colonized root with root hairs. (C) Cotton-blue stained hair root with fungal colonization inside showing septate hyphae (*) with 90° branching (arrow). Bars: (A) 5 mm, (B) 2.5 mm, (C) 100 µm.



Figure S2 Sampling sites in La Réunion island (French overseas, Indian Ocean) and associated host tree of *Bulbophyllum variegatum*. All sites were sampled in 2021 except Lg which was sampled only in 2020, while only ML, Ed and BB were sampled in 2006.

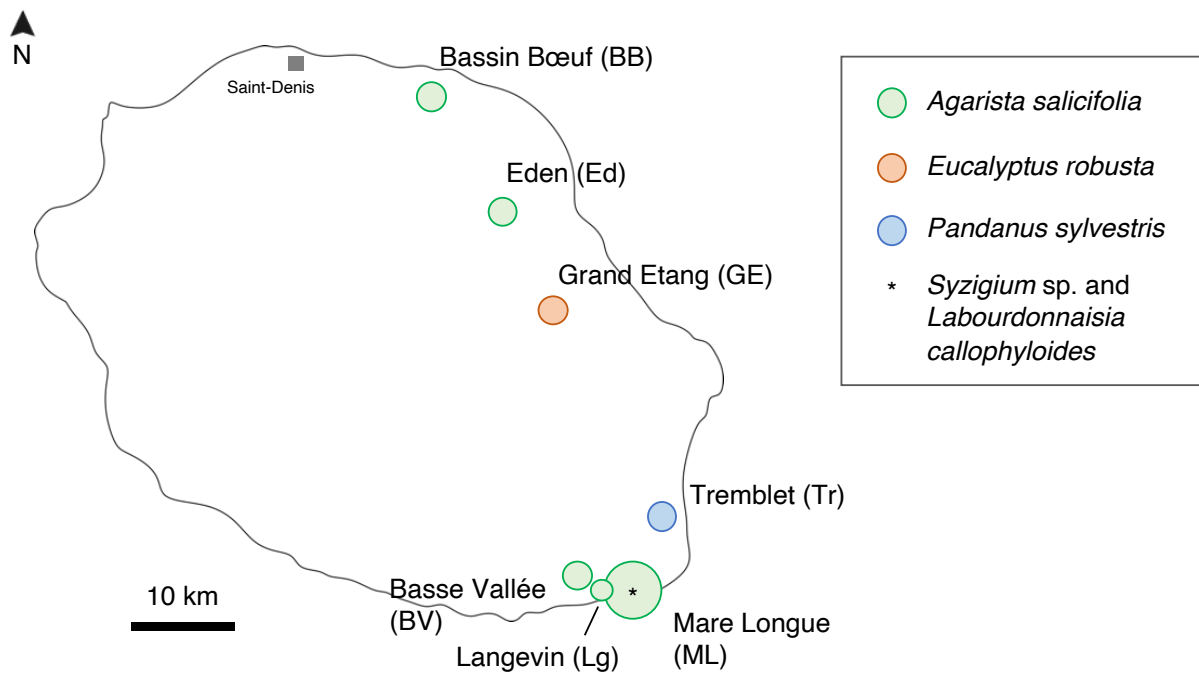
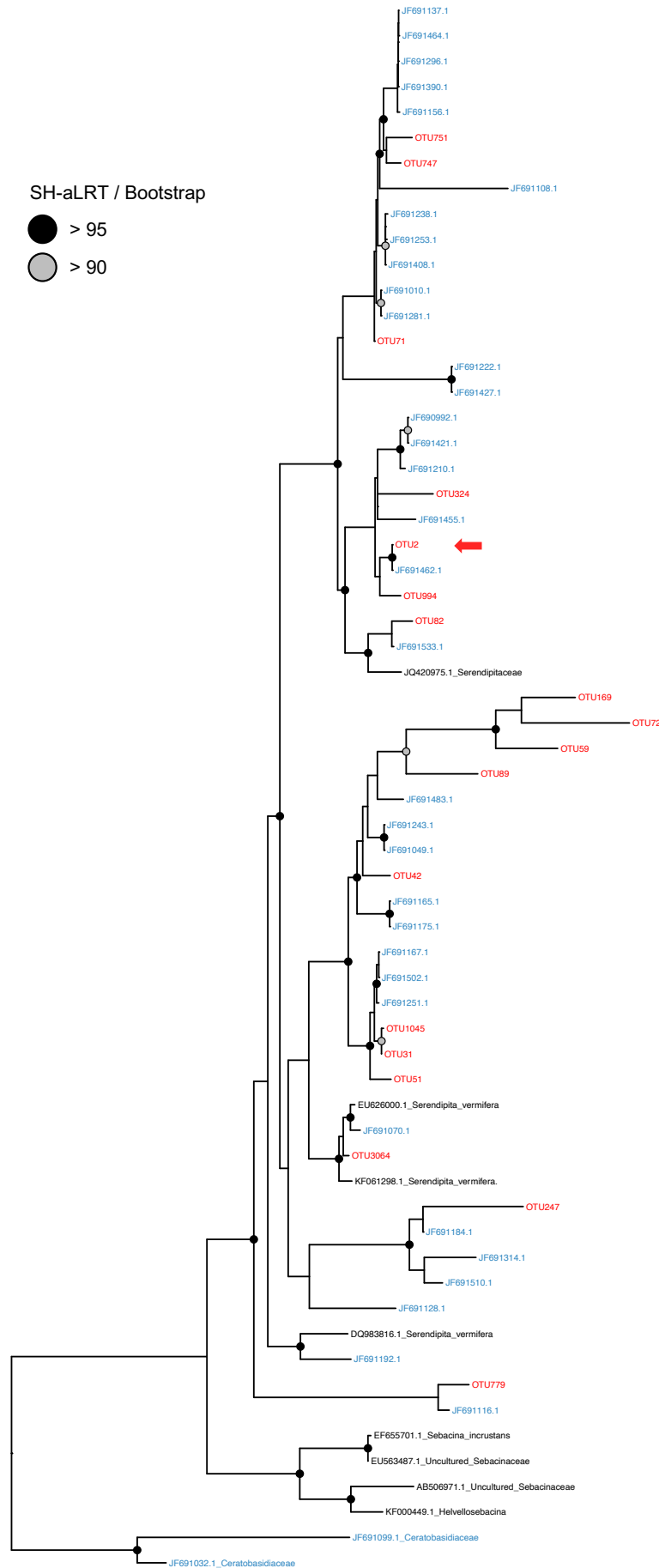


Figure S3 Phylogenetic tree of the Sebacinales. OTUs from Illumina sequencing are coloured in red, sequences from Martos *et al.*, 2012 are coloured in blue, and other sequences from GenBank are in black. Circles at nodes indicate sh-aLRT and bootstrap values both > 90% (grey) or >95% (black). The red arrow points at the OTU2.



Serendipitaceae

Sebacinaceae

Figure S4 Phylogenetic tree of the Tulasnellaceae. OTUs from Illumina sequencing are coloured in red, sequences of fungal strains isolated from *B. variegatum* or other orchid species are in black, and sequences from Martos *et al.*, 2012 are coloured in blue. Circles at nodes indicate sh-aLRT and bootstrap values both > 90% (grey) or >95% (black). Red and black arrows point at OTU1 and OTU3, and at the two isolated Tulasnellaceae strains, respectively.

SH-aLRT / Bootstrap

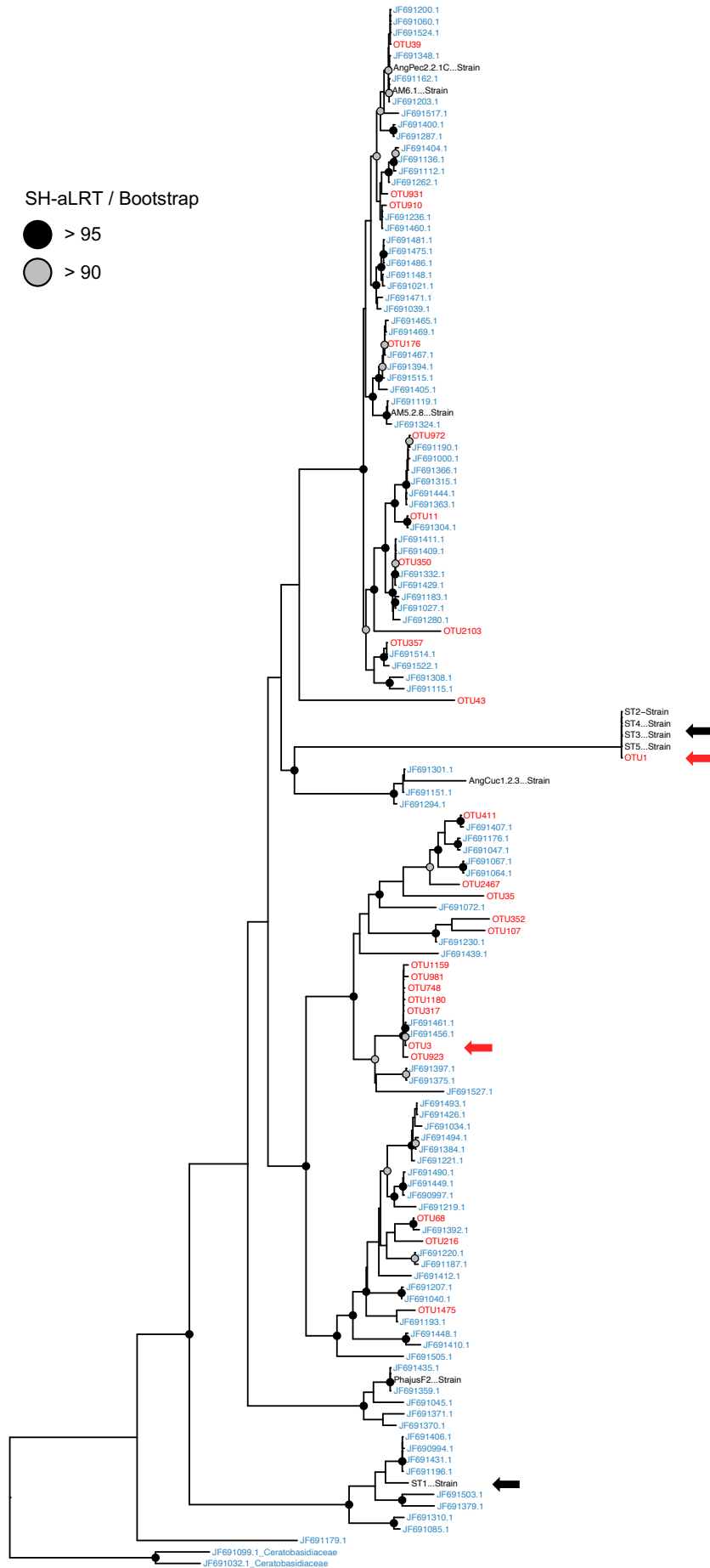
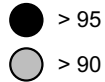


Figure S5 Turnover of endophytic fungi during *Bulbophyllum variegatum* lifecycle as estimated by leaf length. (A-J) The abundances of each OTU in root samples are plotted against the length of the longest leaf of the sampled orchid individual. Blue lines and associated 95% intervals represent the significant (p -value < 0.05) linear tendency according to a linear model ($y \sim x$).

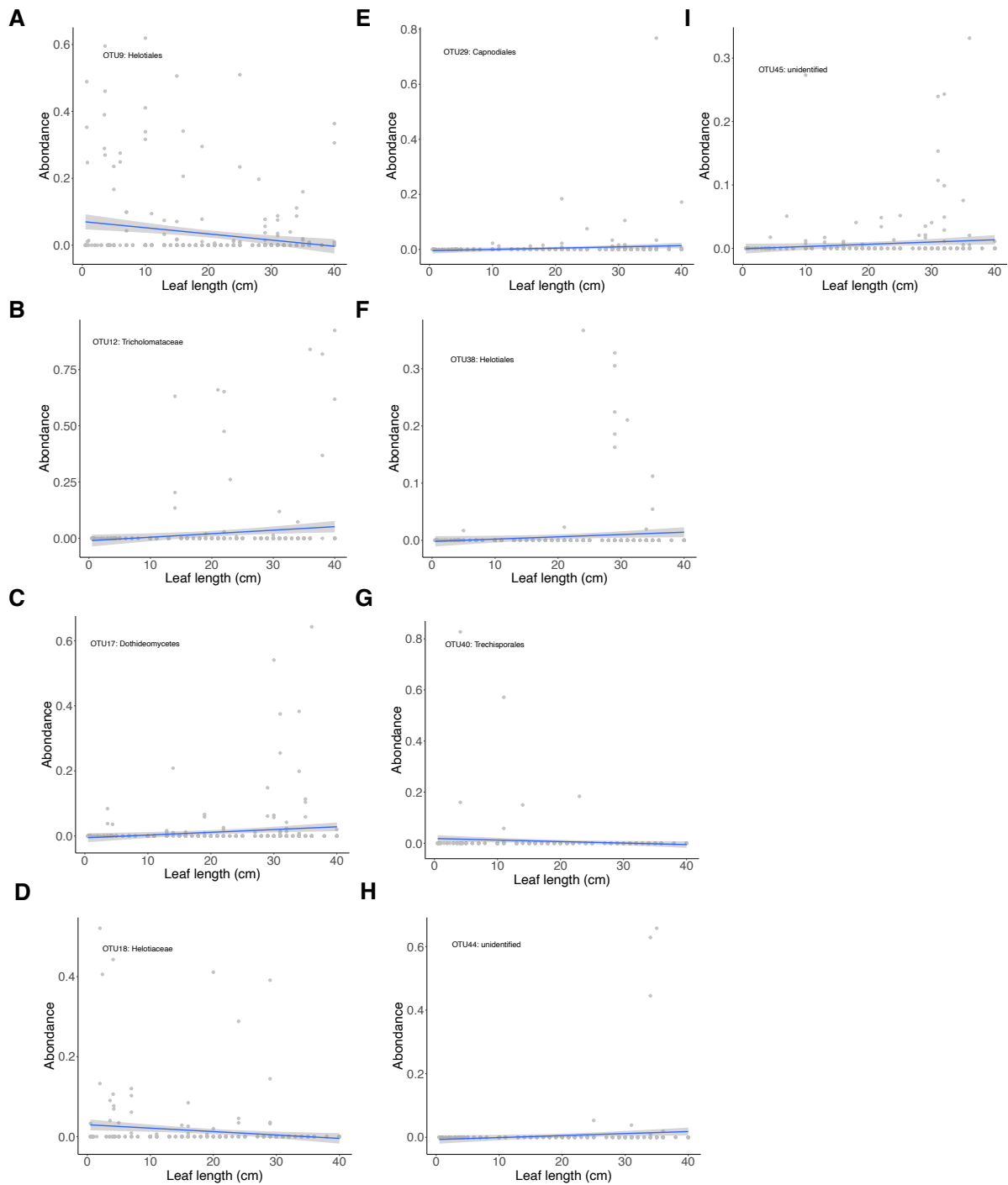


Figure S6 Fungal composition in (A-B) young seedlings and (C-F) seedlings with primers (A, C, E) ITS86-F/ITS4 and (B, D, F) 5.8S-OF/ITS4-Tul. Each bar represents one sample (root section of seedling or entire young seedling). (A-B) All six young seedlings were found on the same tree at Eden (Ed) site. (C-D) Seedlings were found at two sites (Ed or BB) on one tree each (the same as young seedlings at Ed site). (E-F) All seedlings were found on one tree. Helotiales were represented by several OTUs.

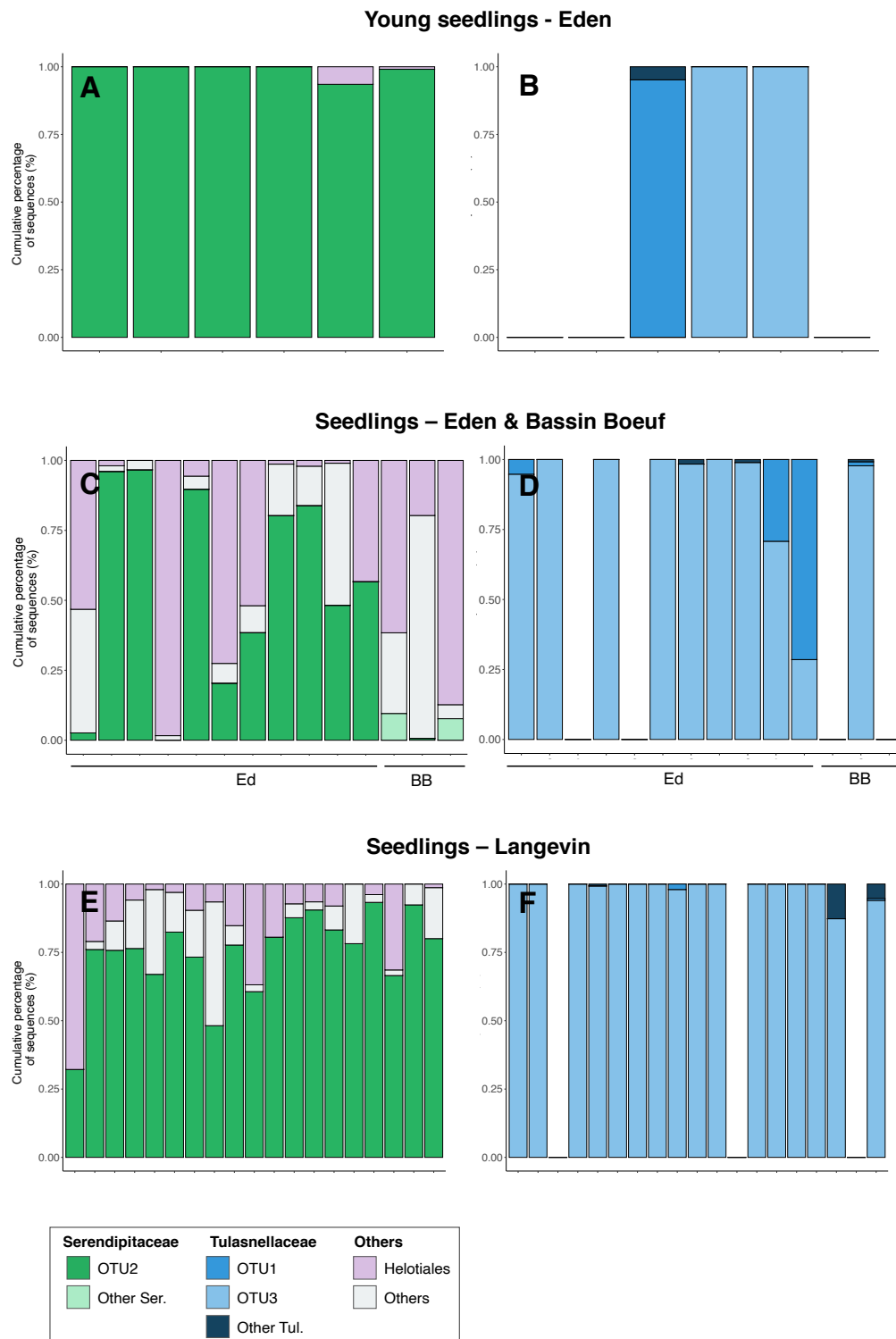


Figure S7 Non-metric multidimensional scaling (NMDS) of total fungal communities on the whole dataset. The same NMDS distribution as in Fig. 4 is shown, with different symbols' colours.

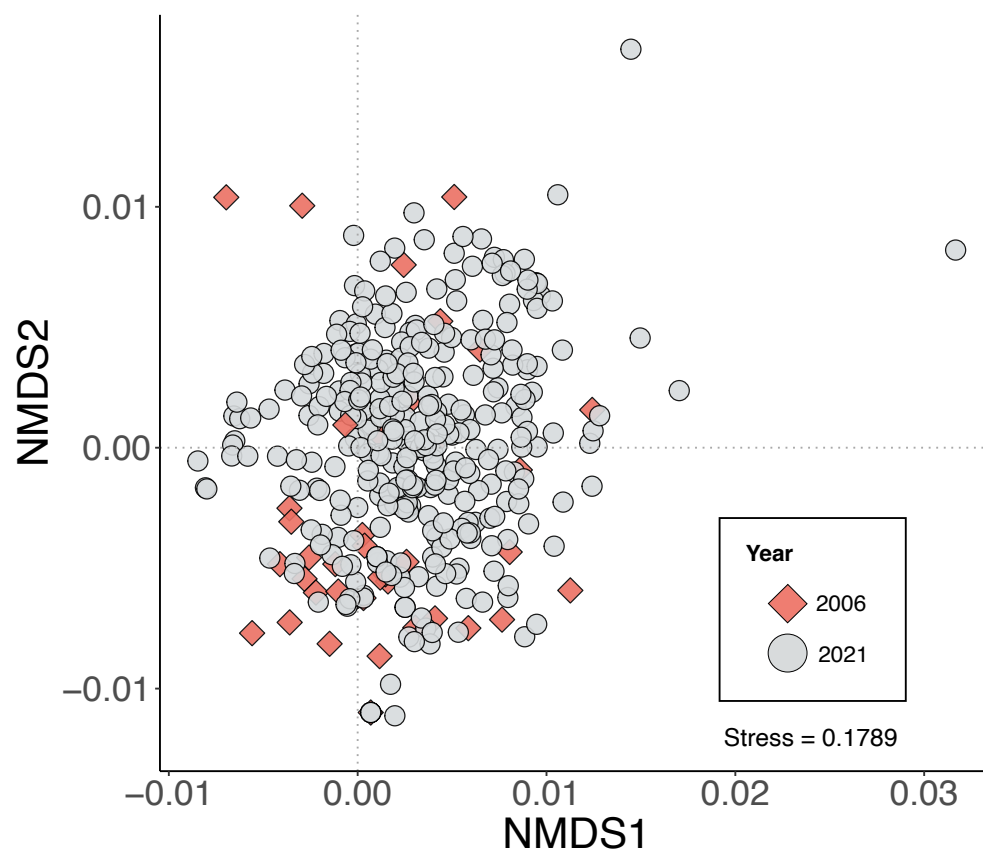


Figure S8 Fungal sharing between 2006 and 2021 at (A) all three sites, (B) Mare Longue, (C) Bassin Boeuf and (D) Eden. The Venn diagrams show the number of fungi (whole community) shared between 2006 (red) and 2021 (blue). At the left (resp. right) of the Venn diagram, histograms show the frequency (%) in roots of *Bulbophyllum variegatum* of the three most frequent OMF OTUs (*i.e.*, OTU1 and OTU3 in the Tulasnellaceae and OTU2 in the Serendipitaceae) in 2006 (resp. 2021). N, number of samples.

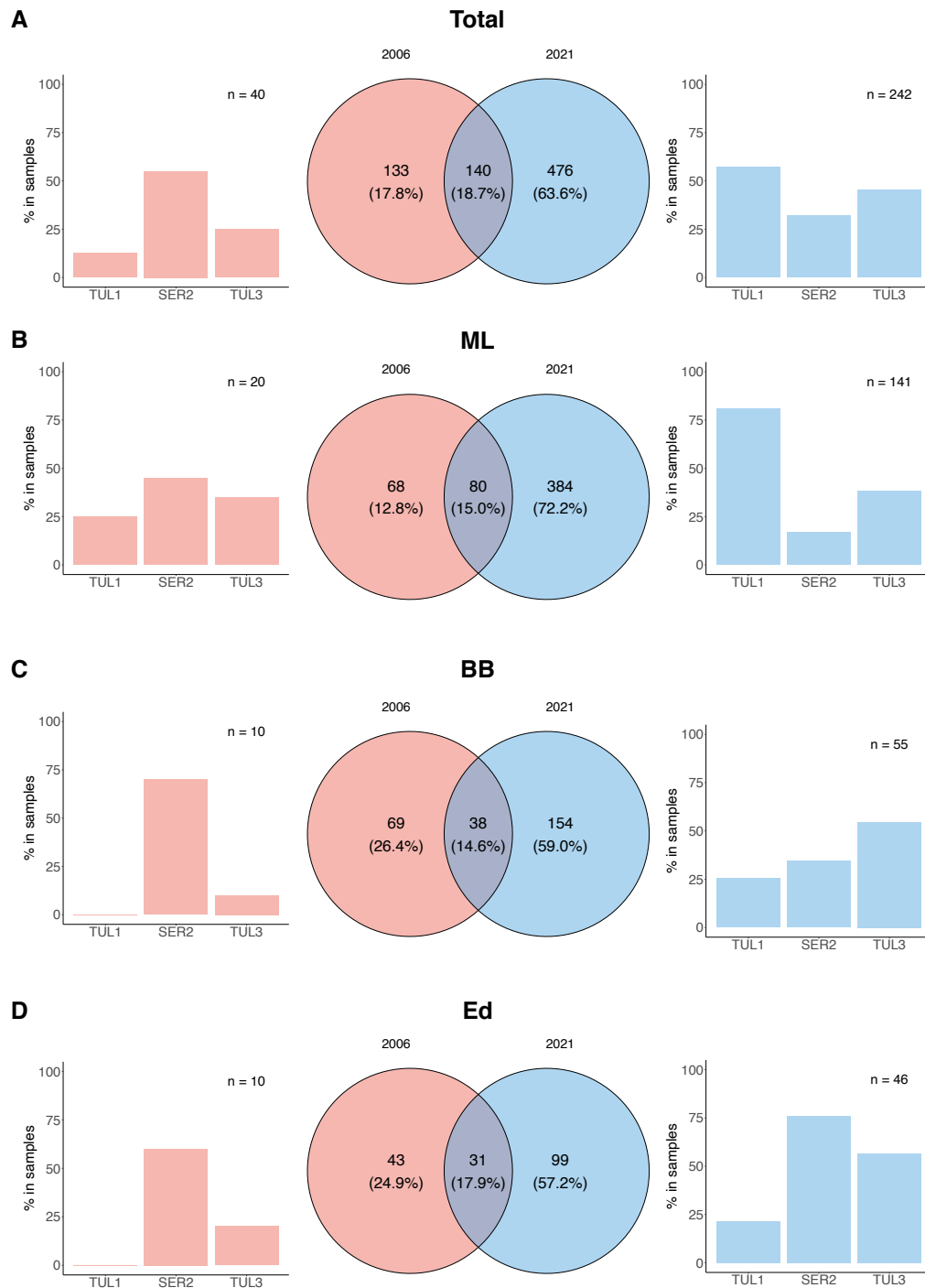


Figure S9 Variance partitioning of (A) total fungal communities and (B) OMF communities between the age of *Bulbophyllum variegatum* (as estimated by the length of the longest leaf, green), the site (yellow) and the height on the tree (Height, blue).

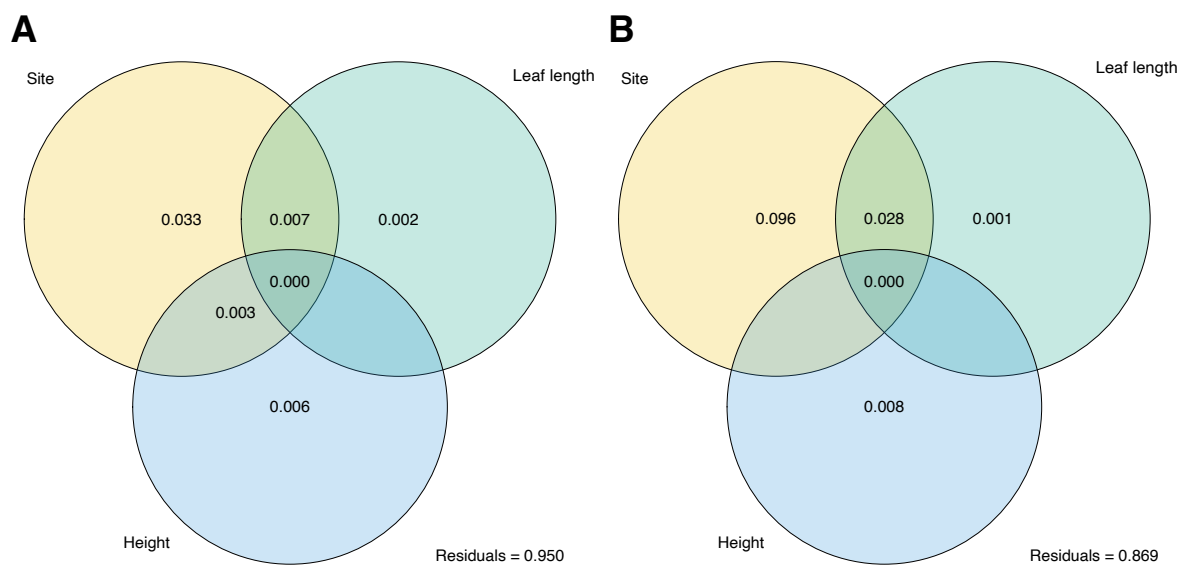


Figure S10 Germination experiment. Thirteen fungal strains (x-axis) have been tested for their capacity to enhance seed germination (y-axis). Strains were either Tulasnellaceae (Tul.) or other fungi, isolated from *Bulbophyllum variegatum* or other orchid species (AngCuc, *Angraecum cucullatum*; AM, *A. mauritianum*; AngPec, *A. pectinatum*; Phaius, *Phaius tetragonum*). For strains isolated from *B. variegatum*, names refer to Table 2. T+, P668 medium; T-, OAT medium without fungal strain. Stage 0, no germination; stage 1, swelling of the embryo. Error bars represent standard deviation (n = 3, except ST5 and T- for which n = 1).

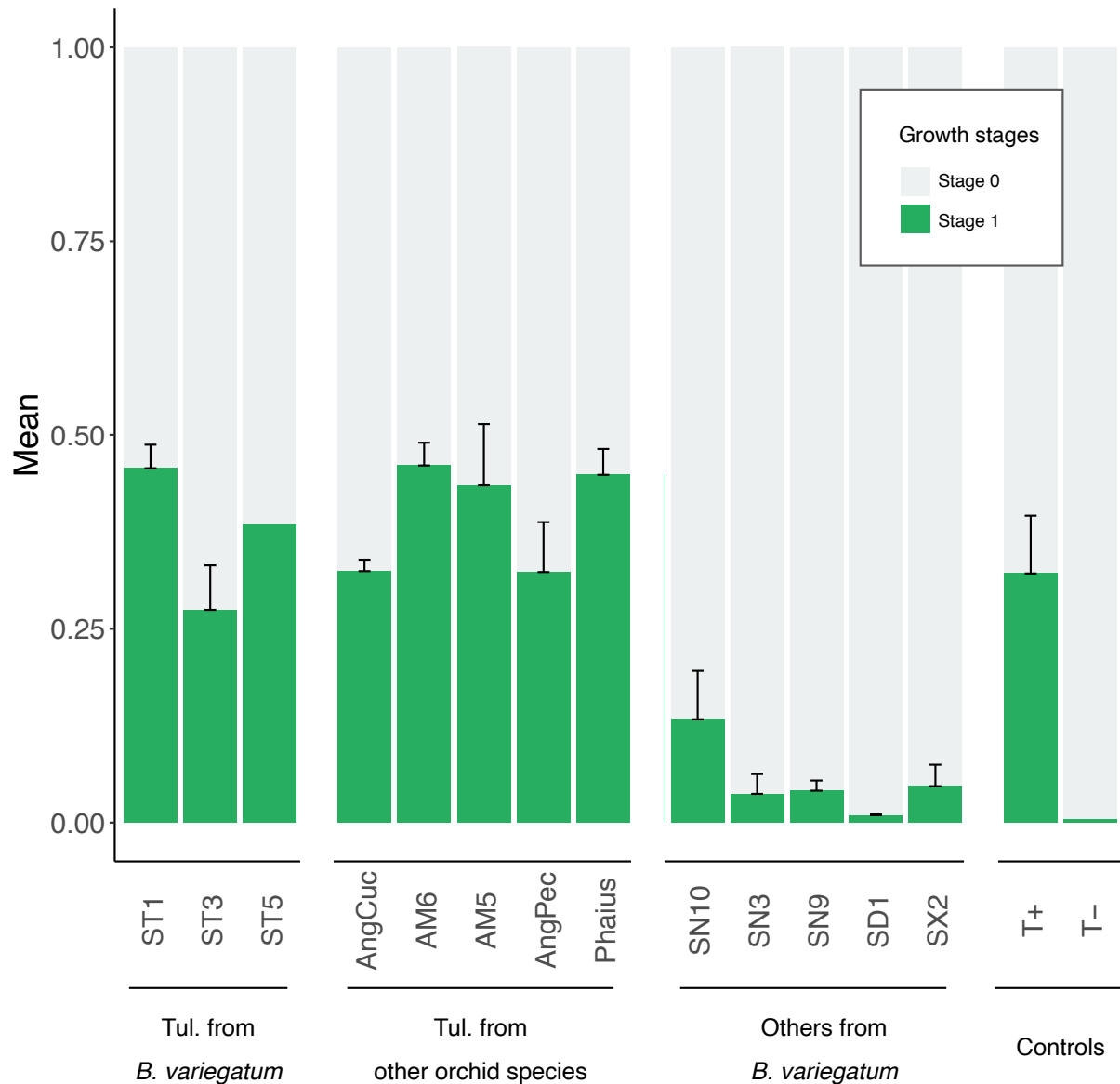


Table S1 Differences in OTU's frequency between 2006 and 2021 for TUL1, SER2 and TUL3. The first two columns show the % of root colonized by these OTUs at each site (ML, BB and Ed) or at all sites (Total). Chi², chi-squared value or odd ratio from Fisher test when necessary (in italics). P-value, associated p-value for both tests. Lines with corresponding p-values < 0.05 are bolded. See also Fig. S8.

	% 2006	% 2021	Chi ²	p-value
Total				
TUL1	12.5	57.0	25.48	< 0.001
SER2	55.0	32.2	6.81	0.009
TUL3	25.0	45.5	5.07	0.024
ML				
TUL1	25.0	80.9	25.5	< 0.001
SER2	45.0	17.0	3.94	0.007
TUL3	35.0	38.3	0.00	0.969
BB				
TUL1	0.0	25.5	<i>0.00</i>	<i>0.102</i>
SER2	70.0	34.6	<i>4.31</i>	<i>0.075</i>
TUL3	10.0	54.6	0.10	0.014
Ed				
TUL1	0.0	21.7	<i>0.00</i>	<i>0.180</i>
SER2	60.0	76.1	<i>0.48</i>	<i>0.431</i>
TUL3	20.0	56.5	3.04	0.081