Orchid mycorrhizal association among three co-occurring *Vanilla* species in Costa Rica

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Introduction

- Genus ***Vanilla*** is recognized as a group of **hemi-epiphytic** orchids.
- Like in other orchids, growth, distribution patterns, and abundance of ***Vanilla*** species can be **heavily dependent on** the **orchid mycorrhizal fungi (OMF)**, also termed as **orchid-mycorrhizal association**.
- **The orchid mycorrhizal association** of **wild *Vanilla*** species in Costa Rica are **poorly understood**.
- **Three *Vanilla* species**, *V. hartii*, *V. pompona*, and *V. trigonocarpa* have been found **to co-occur** in the wild in Costa Rica.

Research Hypotheses

1. The most common host orchid species will associate with a wider range of OMF communities.
2. Across the three host orchid species, OMF communities within the roots attached to phorophytes ('bark roots') and the below-ground roots ('soil roots') will be dissimilar.

Methods

- Surface sterilization of roots, and inspection for pelotons
- DNA extraction
- Fungal amplification of the ITS region: ITS 3 / ITS 4-OF [1]
- NGS Library preparation
- Paired end sequencing on MiSeq Desktop Sequencer
- Bioinformatics and Biostatistics

Figure 1. Map of sampling sites across Costa Rica
- A mixed population: Vaha-po-tr_p
- Single populations: Vaha_S1, Vaha_S2, Vapo_S, Vatr_C, Vatr_M, and Vatr_P

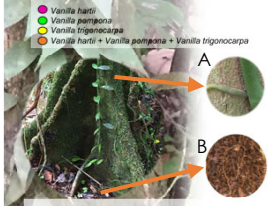

Figure 2. General growth habit of *Vanilla* vine. (A) Bark roots and (B) Soil roots.

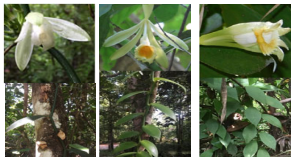

Figure 3. Study species

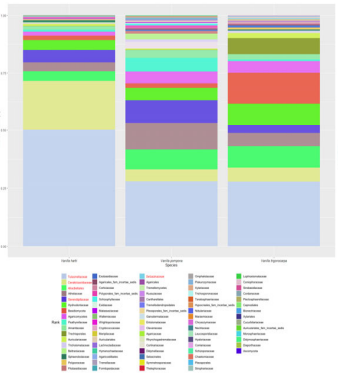

Figure 4. The relative abundance (in the total fungal read abundances) of the endophytic fungi (including the OMF family that are highlighted in red), detected in the three *Vanilla* species.

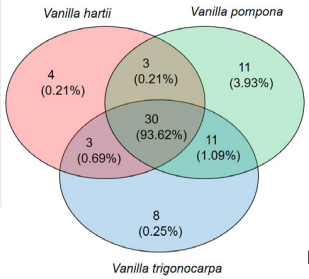

Figure 5. Venn diagram showing the number of OTUs and the relative abundances of OMF that are shared among the three *Vanilla* species.

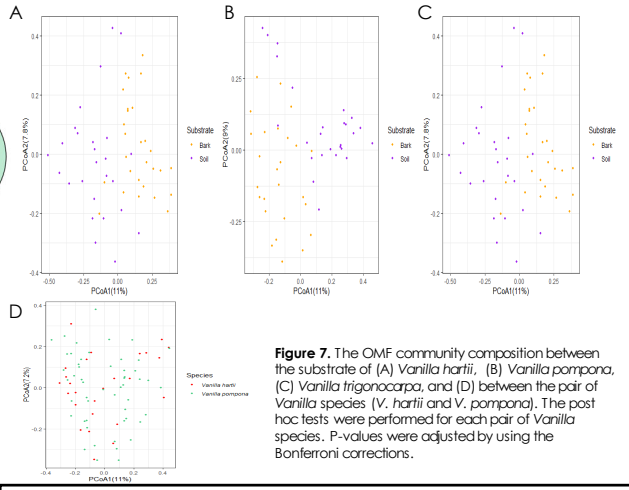

Figure 7. The OMF community composition between the substrate of (A) *Vanilla hartii*, (B) *Vanilla pompona*, (C) *Vanilla trigonocarpa*, and (D) between the pair of *Vanilla* species (*V. hartii* and *V. pompona*). The post hoc tests were performed for each pair of *Vanilla* species. P-values were adjusted by using the Bonferroni corrections.

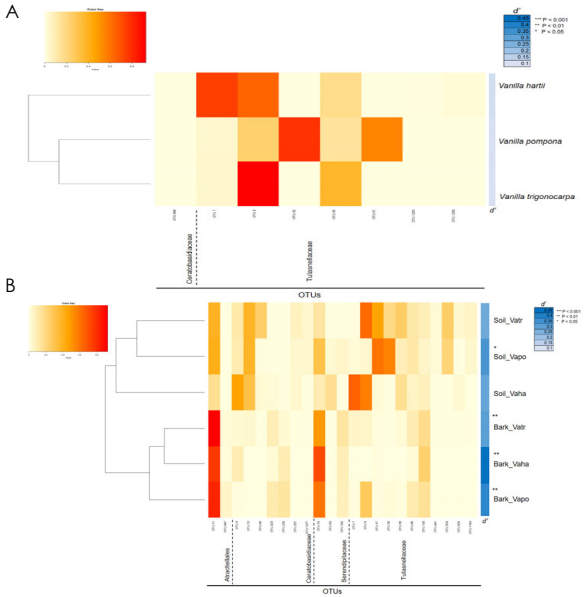

Figure 6. Hierarchical clustering of (A) the three *Vanilla* species and (B) the substrate with weighted Bray-Curtis measured by β using the Bray-Curtis index. The color intensity of heat map indicated that the relative abundance of OTUs (A) among the three *Vanilla* species and (B) between the substrate as shown in the legend. The blue box represents the association specificity measured by the d' index of the association specificity. P-values were adjusted by using the Bonferroni corrections.

Results

- Obtained 533,181 sequences and 299 fungal OTUs
- The **mean OTU abundance** showed a **significant difference** among the three *Vanilla* species. P-value < 0.05 (Fig. 4)
- OMF communities within the roots of *Vanilla hartii* were **dissimilar** to the OMF communities within roots of *Vanilla pompona* and *Vanilla trigonocarpa* (Fig. 6A)
- OMF communities were **distinct** between both substrates (Fig. 6B)
- The OMF community composition of both substrate was **significant difference**. P-value is < 0.05 (Fig. 7A - C)
- The OMF community compositions among three *Vanilla* species were **significant different**, especially in between *V. hartii* and *V. pompona*. P- value is < 0.05. (Fig. 7D)

Conclusions

- **The OMF community composition and the mean OTU abundance** showed **significant difference** among the three *Vanilla* species.
- The **significant difference** in the OMF community composition between the substrate.

References

[1] Waud, M., Busschaert, P., Ruyter, S., Jacquemyn, H. & Lievens B. 2014. Impact of primer choice on characterization of orchid mycorrhizal communities using 454 pyrosequencing. *Molecular Ecology Resources*, **14**: 679 – 699.

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