



A Better Look at *Fusarium* Species Living in Mexican Vanilla Orchids Using RPB2

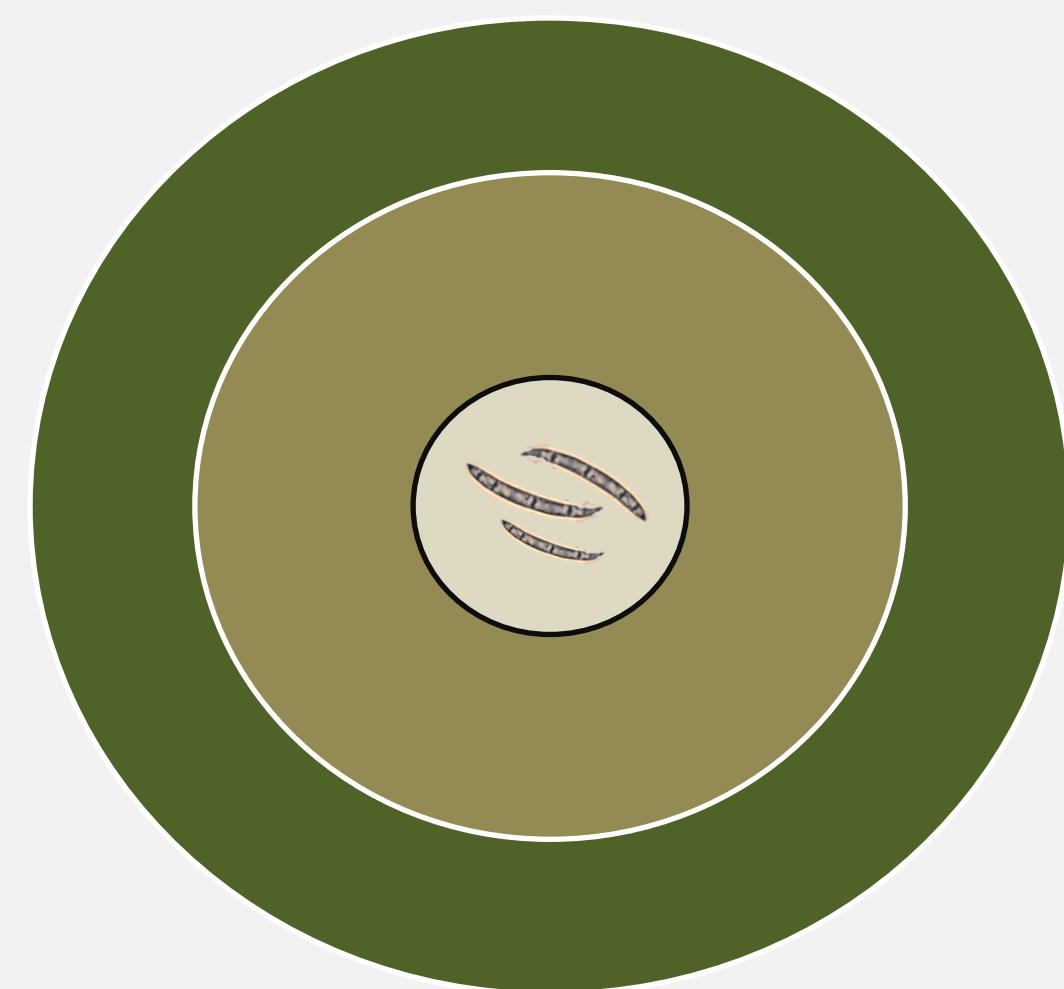


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A Brief Introduction:

- Vanilla planifolia* orchid species are commercially valuable hemi-epiphytes that sustain high mortality rates when infected with pathogenic *Fusarium oxysporum* (Hernandez-Hernandez et al. 2004).
- Most orchids depend on mycorrhizal symbionts such as *Tulasnella sp.* and *Ceratobasidium sp.* for germination and nutrient exchange, and it is largely hypothesized that *Fusarium* species exist within *Vanilla* roots as latent pathogens rather than mutualists (Porras et al. 2007, Porras et al. 2011).
- Recent studies of cultures grown from healthy *V. planifolia* root clippings from specimen in Mexico have revealed the presence of various *Fusarium sp.*, known for their pathogenic qualities in *Vanilla* plants (Johnson, unpublished data).
- To better identify cultured *Fusarium*, sequencing of the cultured DNA at the ITS marker was completed, with ambiguous results.
- This research sought to improve the resolution of species identification by sequencing the entire RPB2 marker in order to better understand which *Fusarium* species might occupy a healthy specimen of *Vanilla planifolia*.

What We're Aiming For:



Which *Fusarium* species exist mutually within healthy vanilla?

How diverse are the species?

Are these species known pathogens?

How It Was Done:

PCR was performed using two sets of RPB2 primers on previously cultured *Fusarium* DNA extract.

Presence of desired amplicon was noted using gel electrophoresis. The samples were washed and special ddNTP terminators were attached.

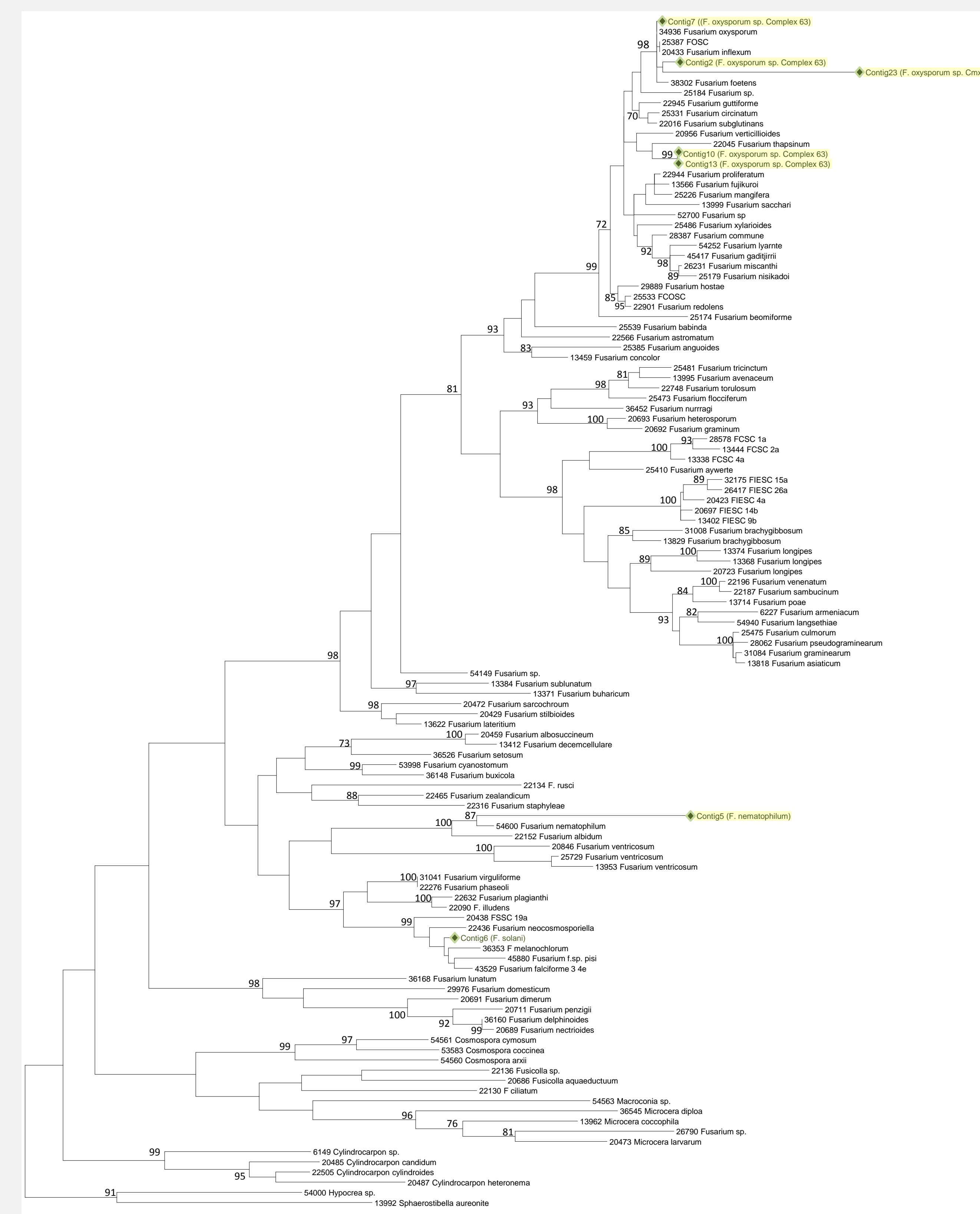
Sanger Sequencing was performed at the Field Museum.

Sequence data was analyzed using Codon Code to correct base pairs and form "contigs" or alignments of forward and reverse DNA strands of each sample.

High quality contigs were placed into alignment with known sequences from *Fusarium*-ID database using Mega6 and a phylogenetic tree was generated.

What We Found:

The Big Picture: RPB2 Phylogeny From Alignment



This tree is based upon a maximum-likelihood algorithm of an alignment of 110 *Fusarium sp.* reference sequences and 7 samples sequenced at the RPB2 marker (O'Donnell, USDA). Experimental data are marked by green diamonds on the branch tips. 1000 bootstraps were run with values above 70 reported.

Comparing *Fusarium*-ID Database Results for ITS and RPB2 markers

Sample ID	ITS <i>Fusarium</i> -ID Result	RPB2 <i>Fusarium</i> -ID Result
Contig2	<i>Fusarium oxysporum</i>	<i>Fusarium oxysporum</i> species Complex 63
Contig5	<i>Fusarium nematophilum</i>	<i>Fusarium nematophilum</i>
Contig6	<i>Fusarium solani</i>	<i>Fusarium solani</i>
Contig7	<i>Fusarium oxysporum</i>	<i>Fusarium oxysporum</i> species Complex 63
Contig10	<i>Fusarium oxysporum</i>	<i>Fusarium oxysporum</i> species Complex 63
Contig13	<i>Fusarium oxysporum</i>	<i>Fusarium oxysporum</i> species Complex 63
Contig23	<i>Fusarium solani</i>	<i>Fusarium oxysporum</i> species Complex 63

Search results from *Fusarium*-ID database returned very similar results for the majority of species, with one disagreement concerning Contig23.

What It Means:

- When comparing results of *Fusarium*-ID search queries from ITS and RPB2 markers, it appears that both markers offer similar levels of resolution at the species level regarding database results.
- Findings from both markers implicate that the majority of *Fusarium* living as endophytes in *Vanilla planifolia* were likely either *F. oxysporum*, *F. nematophilum*, *F. solani*, or part of a cryptic *F. oxysporum* species complex. Both *F. solani* and *F. oxysporum* have history in causing various vascular plant disease (Michielse CB et al. 2009; Zhang N., O'Donnell K. et al. 2006)
- The phylogenetic tree created by aligning sample data with verified RPB2 sequences of known *Fusarium* suggests that the tree-building method may yield more informative results than database query. Using a phylogenetic technique that relies on relative alignment rather than database-matching reveals both subtle and large differences between the samples that is visually accessible as well. Moreover, databases at this juncture may be lacking in sufficient capacity to provide comprehensive identification of *Fusarium*.
- Overall, it is clear that pathogenic *Fusarium* occupy root space with important mycorrhizal fungi. These endophytes are likely opportunists waiting for a depression in the immune systems of the healthy *Vanilla planifolia* (Porras et al. 2011).

Moving Forward:

- A main priority should be the successful sequencing of both sets of RPB2 primer, comprised of "Set A" and "Set B". At present all DNA is represented by "Set B". To complete the process of successfully sequencing and aligning data for as many of the *Fusarium* DNA samples, more work is necessary to improve PCR results and troubleshoot contamination problems that were evident in many samples. A sequence of the special strain of *F. oxysporum* f. sp. *Vanillae*—the specific culture of pathogen responsible for Vanilla root rot—would also give further phylogenetic insight pertaining to pathogenicity (Alconero R. 1968).
- Sequencing the DNA at the TEF1 α marker may provide additional insight into the precise identities of extant *Fusarium*, if suitable reference sequences of this marker are available in the future.
- A thorough in-vivo assay of cultured *Fusarium* and carefully controlled *Vanilla planifolia* plants would provided a definitive answer to the questions of pathogenicity of the samples examined in this research.

References:

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