Taxonomic revision of the banana Fusarium wilt TR4 pathogen is premature

Eliana Torres-Bedoya, Daniel P. Bebber, David J Studholme*

Corresponding author: DJS, email: d.j.studholme@exeter.ac.uk

Biosciences, University of Exeter, Exeter EX4 4QD. United Kingdom.

Abstract:

Taxonomic revisions for pathogens of crops should be based on robust underpinning evidence. Recently, a substantial revision was proposed for the taxonomy of the causative agent of *Fusarium* wilt on banana. We re-analysed the data on which this revision was based and discovered that the data do not robustly support the proposals. Several apparent discrepancies and errors in the published phylogenies cast further doubt on the conclusions drawn from them. Though we do not assert that the authors' conclusions are incorrect, we posit that the taxonomic changes are premature, given the data currently in the public domain.

Main text

The (unintended) consequences of taxonomic revisions

Taxonomy is not static; it must be updated in the light of new knowledge, especially new insights into evolutionary relationships. However, changes to taxonomy of phytopathogens can have adverse consequences; for example, they can make the taxonomy used in the legislation and regulation difficult to interpret and pose problems for its application (Lodovica, Peter, and Bonants Editors n.d.). This is problematic enough in a relatively wealthy territory, but financial consequences may be more dire in resource-poor countries. Revisions to the taxonomy of pathogens of tropical crops such as banana should not be imposed without strong justification and robust underpinning evidence. In our view, the recently proposed changes to the taxonomy of the causal agent of Fusarium wilt disease on banana, and their adoption by the *Index Fungorum* (www.indexfungorum.org) do not sufficiently stand up to scrutiny.

The importance of Fusarium wilt on banana

Bananas and plantains (*Musa* spp.) are enormously important for subsistence of many millions of smallholder and corporate growers in Africa, Asia and South and Central America, both for subsistence and export. The fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*) poses a global threat to banana production, causing a wilting disease formerly known as Panama Disease. In the twentieth century, decimation by *Foc* Race 1 led to the replacement of a near-monoculture of variety Gros Michel by the resistant Cavendish varieties. However, Cavendish is susceptible to *Foc* Race 4. Of particular concern are Race-4 strains known as *Foc* Tropical Race 4 (TR4) that are gaining a foothold from southeast Asia to sub-saharan Africa and recently established in Latin America, precipitating a state of national emergency in Colombia (Maymon et al. 2020; Zheng et al. 2018; Ordonez et al. 2015; Ploetz 2015; Butler 2013; Stokstad 2019; Dita et al. 2018; García-Bastidas et al. 2014; Ploetz et al. 2015; O'Neill et al. 2016; Ordoñez et al. 2016; Chittarath et al. 2018; Hung et al. 2018; Damodaran et al. 2019; Thangavelu et al. 2019; Aguayo et al. 2021; Hermanto et al. 2011).

Recent taxonomic revisions around the Fusarium wilt pathogen

It is against this backdrop of new and longstanding threats to banana production in low- and middle-income tropical nations that a substantial revision was proposed for the taxonomy of the causative agent, *Foc* (Maryani et al. 2019). It has long been known that *Foc* is not a single monophyletic group but rather a heterogeneous collection of lineages within the *Fusarium oxysporum* species complex that have independently converged upon pathogenicity in banana (Koenig, Ploetz, and Kistler 1997; Gordon and Martyn 1997; O'Donnell et al. 1998; Ploetz 2006; Fourie et al. 2009). The number of known independent lineages has been increased to nine following extensive sampling of isolates in Indonesia, a center of diversity of both host plant and pathogenic fungus (Maryani et al. 2019). The authors of that study went further than simply describing the lineages and formally proposed lineages as new species. The *Fusarium oyxsporum* species complex is conventionally divided into three major clades (O'Donnell et al. 1998). Maryani's lineages 1, 2 and 3 fall within O'Donnell's clade 1, while lineages 4 – 9 fall within clade 2. They also propose a clade 5 (that is distinct from lineage 5).

The authors of the recent *Foc* taxonomic revision did not explicitly state a rationale for proposing these new species. However, they used phylogeny and morphological characteristics as the basis and claimed that each new species represented a monophyletic lineage (Maryani et al. 2019). The supporting evidence for these monophyletic lineages consisted of phylogenetic trees based on molecular sequences for several genetic loci including *tef1a*, *rpb1* and *rpb2*. However, when we attempted to replicate these phylogenetic trees, we discovered that the data do not robustly support the monophyly of the proposed new species. We also identified several apparent discrepancies and errors in the published phylogenies that cast further doubt on the conclusions drawn from them.

It is possible that the proposed new species do in fact correspond to a biological reality; after all, some of the species appear to bear some distinct characteristic phenotypic features. However, the taxonomic revisions are not strongly and robustly supported by the molecular sequence data that are currently in the public domain. Therefore, considering the administrative burden imposed on those attempting to manage the disease and the potential for confusion in the research community, we oppose the adoption of these taxonomic revisions until more incontrovertible evidence is published. Several previous studies have recognised that *F. oxysporum* contains at least two or three biologically meaningful species. A useful species concept for fungi is one in which recombination occurs within a species but not between different species (Taylor et al. 2000). Phylogenetic analyses implementing this concept supported the existence of two (Laurence et al. 2014) or three (Brankovics et al. 2017) phylogenetic species corresponding to O'Donnell's clades 1, 2 and 3. We also note that previous studies of the *F. oxysporum* genetic diversity did not propose to elevate the various clades and subclades to the status of separate species.

Lack of phylogenetic support for *F. odoratissimum* and *F. purpurascens*

The most impactful aspect of the recent taxonomic revision (Maryani et al. 2019) is the proposal of a new species, *Fusarium odoratissimum*, which includes strains informally dubbed TR4. We were unable to replicate the phylogeny on which that proposal is based. This new species is proposed to comprise lineage 1 in Figure 6 of Maryani and colleagues' paper (Maryani et al. 2019). That figure consists of a phylogenetic tree based on concatenated sequences of *tef1* and/or *rpb1* and/or *rpb2* depending on availability of sequence data for each isolate. Each of their nine lineages, including lineage 1 (i.e., *F. odoratissimum*), had less than 70 % bootstrap support.

We attempted to replicate their phylogenetic analysis and failed to recover a clade corresponding to their lineage 1; rather, we found that members of species *F. odoratissimum* and *F. purpurascens* are intermingled, with *F. tardichlamydosporum* NRRL 36108 and *F. phialophorum* NRRL 36110 also falling within the *F. odoratissimum* – *F. purpurascens* clade (Figure 1).

We next generated a phylogenetic tree based solely on the *tef1* locus (Figure 2), which also has *F. odoratissimum* and *F. purpurascens* are intermingled, suggesting a lack of robust support for these two proposed species as monophyletic entities. Isolates NRRL 36111, 36105, 36113, 36117, 36106, 36115, 36120, 36116, 36118, 36108, FocCNPMFR2 and FocMal43 fall into clade 2 and NRRL 36101 fall into clade 3 according to Maryani and colleagues but they fall into clade 1 (*F. odoratissimum*) in our phylogenetic reconstruction. This throws further doubt on the monophyly of *F. odoratissimum*.

It is important to emphasize that we do not claim that our phylogeny is more correct than theirs; rather, we are pointing out that the underlying sequence data do not unequivocally support either phylogeny. Unfortunately, the multiple sequence alignments that underlie the phylogeny are not readily available to allow scrutiny by peer reviewers and interested readers (Vihinen 2020). Maryani and colleagues state that they submitted trees to TreeBASE (Sanderson et al. 1994) but no accession numbers were provided and we were unable to find the trees in TreeBASE.

Further concerns about the published phylogeny

There are further important ambiguities and discrepancies in Maryani and colleagues' Figure 6 (Maryani et al. 2019) that undermine their proposed taxonomic changes. For example, lineage 3 is paraphyletic, its last common ancestor being also an ancestor of lineages 1 and 2. This error might be explained by a trivial oversight, which could be remedied by exclusion of isolates InaCC F869 and NRRL 36110 from Lineage 3. The inclusion of InaCC F820 in lineage 4 seems to be similarly erroneous. Another, more serious error arises where Figure 6 falls across the page break between pages 175 and 176 (Maryani et al. 2019). At the bottom of page 175, two limbs of the tree are indicated as joining to three limbs at the top of page 176. This might be explained by part of the tree having been accidentally omitted from the figure, leaving clade 2 unconnected with the rest of the tree. In any case, confidence in the phylogenetic tree is compromised.

The authors proposed a new species *F. grosmichelli* composed of *Foc* lineage 4, but there were several issues apparent with this clade and taxon. According to their Table 3, Isolate InaCC F820 belongs to this new species, yet in their Figure 6 it is quite clear that it does not fall within lineage 4; rather it seems to be an early-branching member of lineage 3 (*F. phialophorum*). Another problem concerns isolates InaCC F824, F988 and F938; each of these appears at two different locations in the FOSC clade in Figure 4 of Maryani *et al*. study, without explanation. Similarly, InaCC F839 appears twice in clade 1 in their Figure 5. Isolates InaCC F856, InaCC F929 and InaCC F983 are also duplicated in Figure 6 of Maryani et al., InaCC F983 even falls in two completely different lineages L3 (*F. phialophorum*) and L7 (*F. cugenangense*). Isolate NRRL 34939 appears in the phylogenetic tree in Figure 4 of Maryani *et al*., though not listed in the accompanying Table 3. Similarly, isolate NRRL 36104 is included in a phylogenetic tree, but is not included in the corresponding table. The most likely explanation for these latter discrepancies is a simple typographical error. Nevertheless, taken together, the constellation of errors and inconsistencies in this study combine to erode confidence in its conclusions and the taxonomic proposals based upon them.

What is TR4?

Given the threat posed by this pathogen and the ambiguity around its taxonomy, it is worthwhile to ask the question: what is *Foc* tropical race 4 (TR4)? Different authors have defined the term TR4 as "a new race" (Maymon et al. 2020), a synonym for vegetative compatibility group VCG01213 (Maryani et al. 2019; Zheng et al. 2018), a "unique genotype" (Maryani et al. 2019), a synonym for the species *F. odoratissimum* (Warmington et al. 2019) and as those isolates of Race 4 that cause disease on Cavendish banana in tropical conditions (Czislowski et al. 2018). Clearly, *F. odoratissimum* is not synonymous with *Foc* TR4 since included within this species is at least one isolate (CBS 794.70) that belongs to special form *perniciosum* rather than *cubense* (Lombard et al. 2019). So, TR4 has been used to describe such diverse entities as species, race, vegetative compatibility group, genotype and set of isolates. Most of these definitions are problematic, but the most coherent is "those isolates of race 4 that cause disease on Cavendish in tropical conditions" (Czislowski et al. 2018). That is, TR4 is a subset of race 4, which in turn is defined as comprising strains pathogenic to all race 1- and 2-susceptible cultivars plus the Cavendish subgroup (Czislowski et al. 2018; Ji Su 1986; Bourne 2007). TR4 isolates are members of the *F. oxysporum* species complex, and appear to be mostly if not entirely restricted to Clade 1 *sensu* O'Donnell (O'Donnell et al. 1998). Ultimately, however, TR4 is a phenotype, not a taxonomic unit. If further data emerge that confirm *F. odoratissimum* as a discrete species, then it is very likely that strains designated as TR4 will indeed fall within that species.

Concluding Discussion

In summary, given the multiple issues undermining confidence in the study that underlies recent taxonomic revision (Maryani et al. 2019), we counsel against its adoption, yet. It is important to emphasise that we are not saying that those authors' conclusions are incorrect. Maybe future publication of existing but as-yet-unavailable data (Maryani 2018) and subsequent research will confirm the monophyly of the proposed new species. Rather, we are concerned that the taxonomic changes are premature, based on the data currently in the public domain and the body of currently published knowledge. It is unclear how the newly proposed species (Maryani et al. 2019) integrate with the previous framework proposed by some of the same authors that divided the species complex into 15 species (Lombard et al. 2019). There continues to be active debate and controversy around the taxonomy of *Fusarium* species; recently a letter co-authored by many prominent *Fusarium* researchers rejected a proposal to split the genus into seven genera (Geiser et al. 2020). Morphology of asexual reproductive structures was previously used to distinguish ten species within the *Elegans* division of *Fusarium*; however, these were collapsed into a single species *F. oxysporum* on the grounds that these differences are small and morphology is variable and susceptible to environmental influence (Snyder and Hansen 1940; Nelson 1991). Re-splitting would be unwise without significant improvement in our ability to distinguish the proposed species morphologically and/or genetically. The existence of monophyletic lineages is not itself sufficient justification for taxonomic revision; acceptable rationales for revision might include greater clarity or taxonomic stability, neither of which is achieved in Maryani's proposal.

The limited confidence in Maryani's phylogenetic analysis arises in part from the sparsity of the data. The phylogeny is based on just three loci, fewer for some isolates. Increasing the number of sampled loci might strengthen robustness of phylogenetic inferences, as seen in recent studies that considered the whole mitochondrial genome (Brankovics et al. 2017) or the entire nuclear and mitochondrial genomes (Achari et al. 2020). The latter confirmed the existence of five well-supported clades corresponding to three distinct species within the *F. oxysporum* complex. Genome-scale sequencing data for Maryani's collection of diverse Indonesian isolates may well resolve the current ambiguities.

Finally, we draw attention to the various conflicting uses of the term TR4 and recommend that it be used in the sense of Czislowski and colleagues (2018) and as a phenotypic rather than taxonomic designation. We look forward to publication of further research in this area that will resolve the phylogenetic and taxonomic ambiguities.

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Figures

Figure 1. Maximum likelihood tree inferred from a combined dataset of *rpb1*, *rpb2* and *tef1a* from 215 *Fusarium* spp. isolates. Taxa are labelled and coloured according to the species attributed by Maryani et al. (2019). Isolates mentioned in the main text are indicated by text labels. *Fusarium fujikuroi* (CBS 221.76) served as the outgroup to root the tree. Sequences were obtained from the NCBI Entrez portal (Sayers et al. 2019) via the accession numbers provided by Maryani et al. (2019). Sequences were aligned using MAFFT (Katoh 2002) and manually trimmed in Seaview (Gouy, Guindon, and Gascuel 2010). Phylogenetic trees were generated using PhyML (Guindon and Gascuel 2003) from using the command lines documented in the Extra Files. Graphics were rendered using the Interactive Tree of Life (Letunic and Bork 2021). Bootstrap support is indicated by thickness of branches. Species designations are coloured as blue for *duoseptatum*, brown for *grosmichelii*, green for *odoratissimum*, white for *oxysporum*, *magenta* for phialophorum, red for *purpurascens*, cyan for

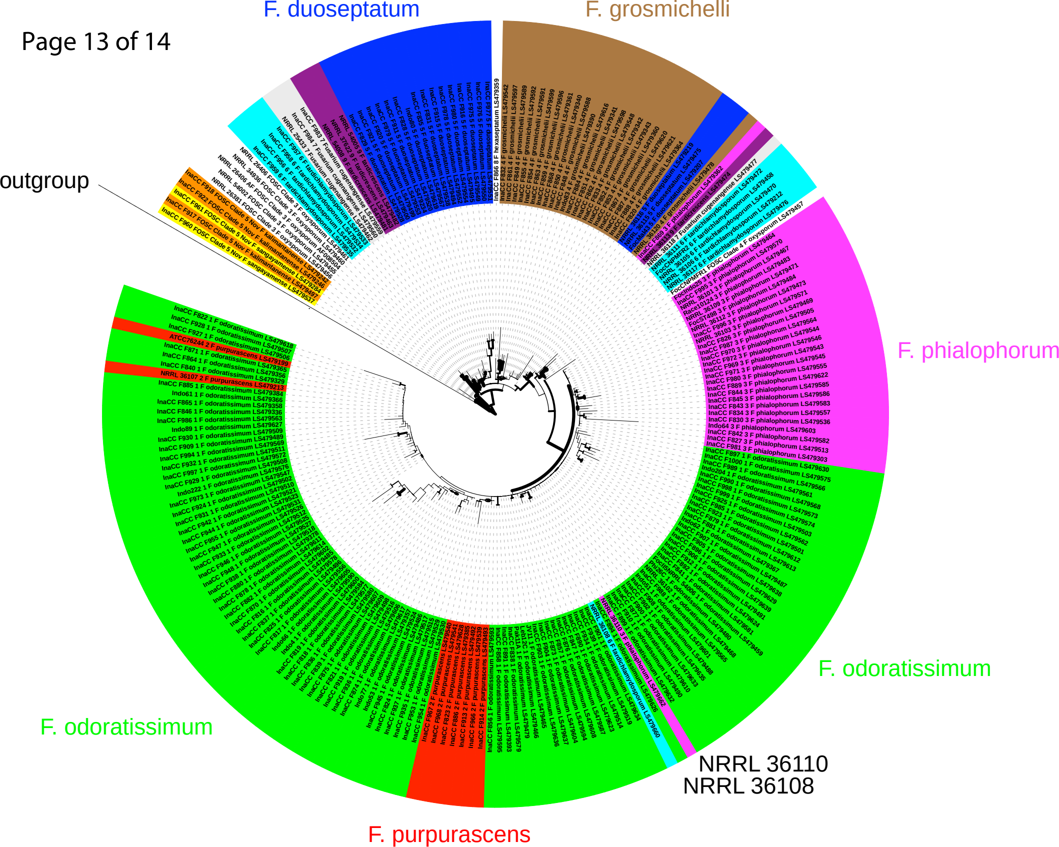
tardichlamydosporum, purple for *tardiscrescens*, orange for *kalimantense*, yellow for *sangayamense* and mercury for *cugenangense*.

Figure 2. Maximum likelihood tree of the FOSC inferred from *tef1a* from 234 *Fusarium* spp. isolates. Taxa are labelled and coloured according to the species attributed by Maryani et al. (2019) using the same colour coding as in Figure 1. *Fusarium fujikuroi* (CBS 221.76) served as the outgroup to root the tree. Sequences were obtained from the NCBI Entrez portal (Sayers et al. 2019) via the accession numbers provided by Maryani et al. (2019). Sequences were aligned using MAFFT (Katoh 2002) and manually trimmed in Seaview (Gouy, Guindon, and Gascuel 2010). Phylogenetic trees were generated using PhyML (Guindon and Gascuel 2003) using the command lines documented in the Extra Files. Graphics were rendered using the Interactive Tree of Life (Letunic and Bork 2021). Bootstrap support is indicated by thickness of branches.

Extra files

- **Multiple sequence alignment of concatenated *rpb1*, *rpb2* and *tef1a* from 215 *Fusarium* spp. isolates.** This is the alignment from which the phylogeny in Figure 1 is derived. Sequences were obtained from the NCBI Entrez portal (Sayers et al. 2019) via the accession numbers provided by Maryani et al. (2019). Sequences were aligned using MAFFT (Katoh 2002) and manually trimmed in Seaview (Gouy, Guindon, and Gascuel 2010). Phylogenetic trees were generated using PhyML (Guindon and Gascuel 2003). This file serves as input for phylogenetic analysis tools such as RAxML. Filename: concatenated_fna.txt.
- **Multiple sequence alignment of *tef1a* from 234 *Fusarium* spp. isolates.** This is the alignment from which the phylogeny in Figure 2 is derived. Sequences were obtained from the NCBI Entrez portal (Sayers et al. 2019) via the accession numbers provided by Maryani et al. (2019). Sequences were aligned using MAFFT (Katoh 2002) and manually trimmed in Seaview (Gouy, Guindon, and Gascuel 2010). Phylogenetic trees were generated using PhyML (Guindon and Gascuel 2003). This file serves as input for phylogenetic analysis tools such as RAxML. Filename: tef1_fna.txt.
- **RAxML commands used to generate the trees illustrated in Figures 1 and 2.** Filename: run_RAxML.pdf.

- **RAxML bipartitions tree file for concatenated *rpb1*, *rpb2* and *tef1a* from 215 *Fusarium* spp. isolates.** This is the output from RAxML in Newick format and serves as input into tree visualisation tools. Filename: RAxML_bipartitions.concatenated_partitions.txt.
- **RAxML bipartitions tree file for concatenated *tef1a* from 234 *Fusarium* spp. isolates.** This is the output from RAxML in Newick format and serves as input into tree visualisation tools. Filename: RAxML_bipartitions.tef1_partitions.txt.
- **Nexus tree file for concatenated *rpb1*, *rpb2* and *tef1a* from 215 *Fusarium* spp. isolates.** This is the output from RAxML converted into Nexus format (Maddison, Swofford, and Maddison 1997). Filename: concatenated-nexus.txt.
- **Nexus tree file for concatenated *tef1a* from 234 *Fusarium* spp. isolates.** This is the output from RAxML converted into Nexus format (Maddison, Swofford, and Maddison 1997). Filename: tef1-nexus.txt.


F. duoseptatum
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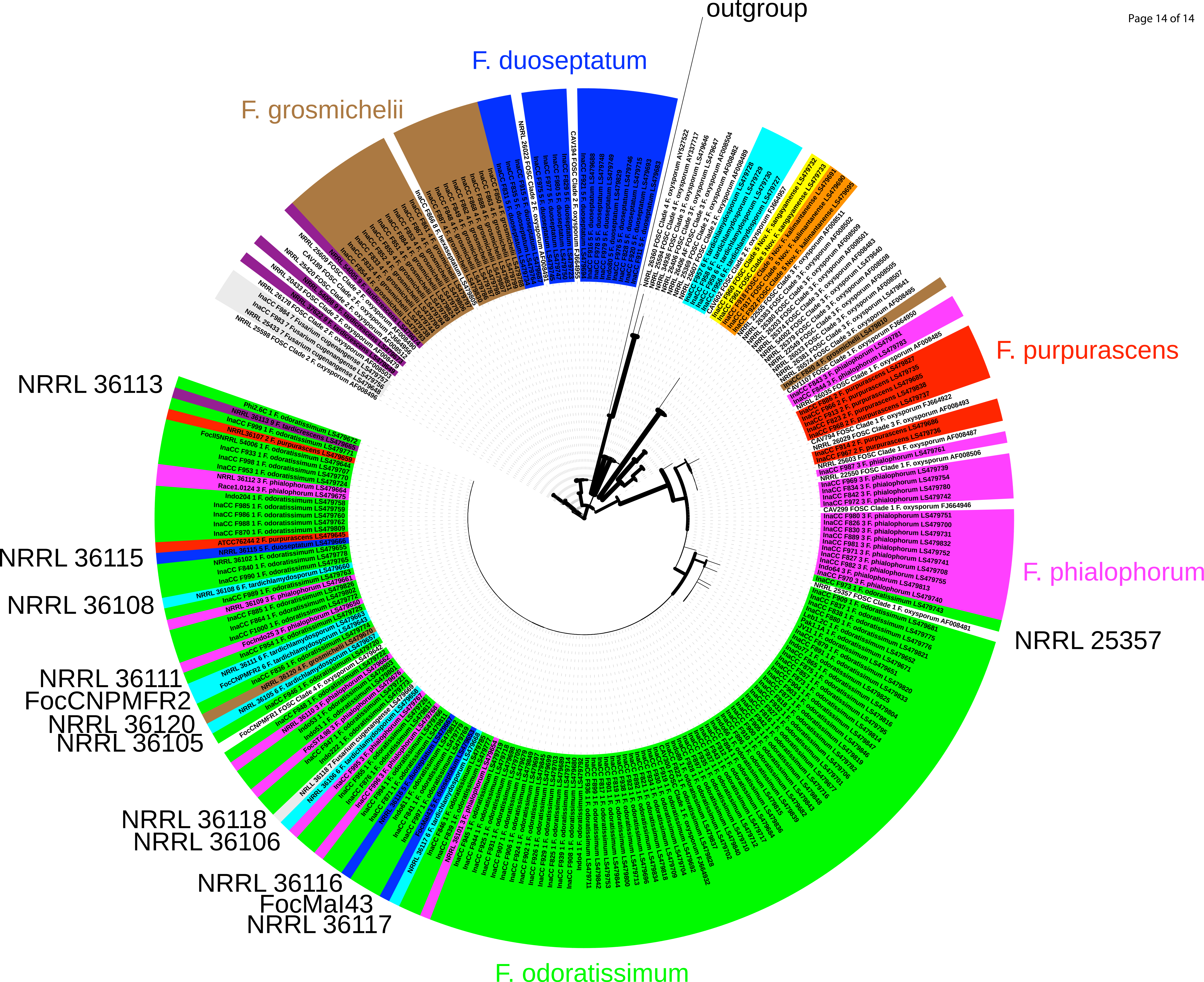
F. phialophorum
F. odoratissimum
F. odoratissimum
NRRL 36110
NRRL 36108
F. purpurascens


outgroup
F. duoseptatum
F. grosmichelii
F. purpurascens
F. phialophorum
NRRL 25357
F. odoratissimum
NRRL 36113
NRRL 36115
NRRL 36108
NRRL 36111
FocCNPMFR2
NRRL 36120
NRRL 36105
NRRL 36118
NRRL 36106
NRRL 36116
FocMal43
NRRL 36117