**Background**

To gain a better understanding of the placement of *Monosporascus* within the Xylariales, a separate five-gene phylogenetic analysis was performed using the genes beta-tubulin, MCM7, EF1α, RPB1 and RPB2 (Figure S1), which are commonly used to examine phylogenetic relationships in fungi at various levels of divergence (James et al. 2006). The inclusion of several additional members of the Xylariales in this analysis suggests the *Monosporascus* isolates were most closely related to *Eutypa lata,* which is commonly associated with disease in grape vines and has been placed in the family Diatrypaceae (Acero et al. 2004).

**Methodology**

Relationships among *Monosporascus* isolates relative to other members of the Xylariales were examined with a five-gene phylogenetic analysis employing beta-tubulin, minichromosome maintenance complex component 7 (MCM7), translation elongation factor 1 alpha (EF1*a*), DNA-directed RNA polymerase II subunit RPB1 and DNA-directed RNA polymerase II subunit RPB2. Sequences were obtained from our *Monosporascus* assemblies and genome assemblies of other members of the Xylariales, identified based on blastn and blastp alignments generated using queries from members of the Xylariales and Sordariales (Table 2). Sequences were aligned and trees constructed as described above for ribosomal ITS sequences.

**Supplementary Table S1.** Sordariales and Xylariales sequences used as queries in multi-gene phylogenetic analysis

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Species (strain)** | **Sequence type** | **NCBI Accession** |
| Beta-tubulin | *Pestalotiopsis fici*(W106-1) | Nucleotide | NW\_006917102.1:1335824-1337715 |
| EF1α | *Neurospora crassa*(OR74A) | Protein | EAA35632.2 |
| MCM7 | *Neurospora crassa*(OR74A) | Protein | EAA34642.1 |
| RPB1 | *Pestalotiopsis fici*(W106-1) | Protein | ETS81956.1 |
| RPB2 | *Biscogniauxia marginata*(CBS 124505) | Protein | AMQ10338.1 |

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**Figure S1.** Five-gene maximum-likelihood tree. Bootstrap values (percent of 1000 replicates) are shown for branches with greater than 65% support. Note that among the non-*Monosporascus* Xylariales species examined *E. lata* is the most closely related to members of the genus *Monosporascus*.