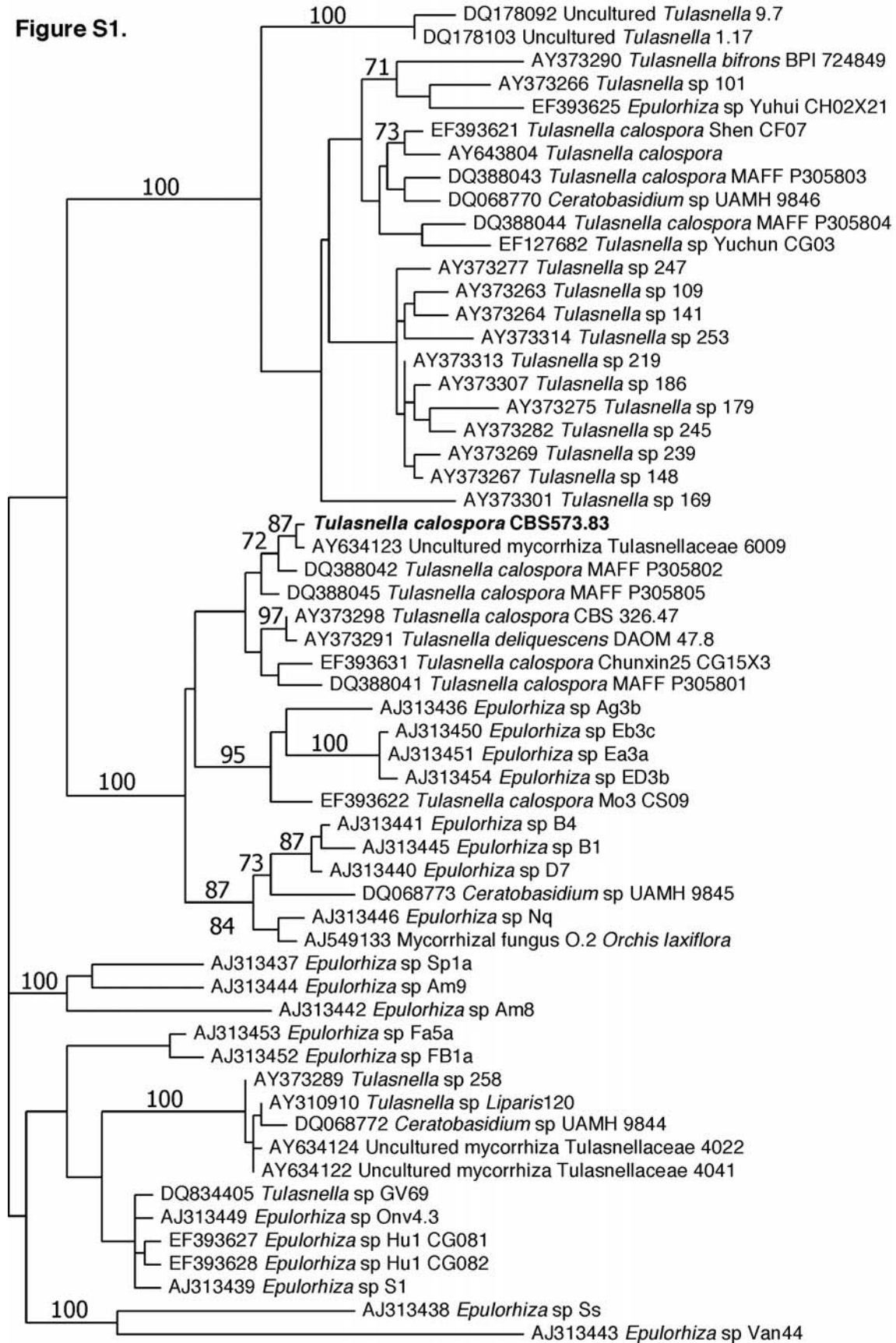


Figure S1.

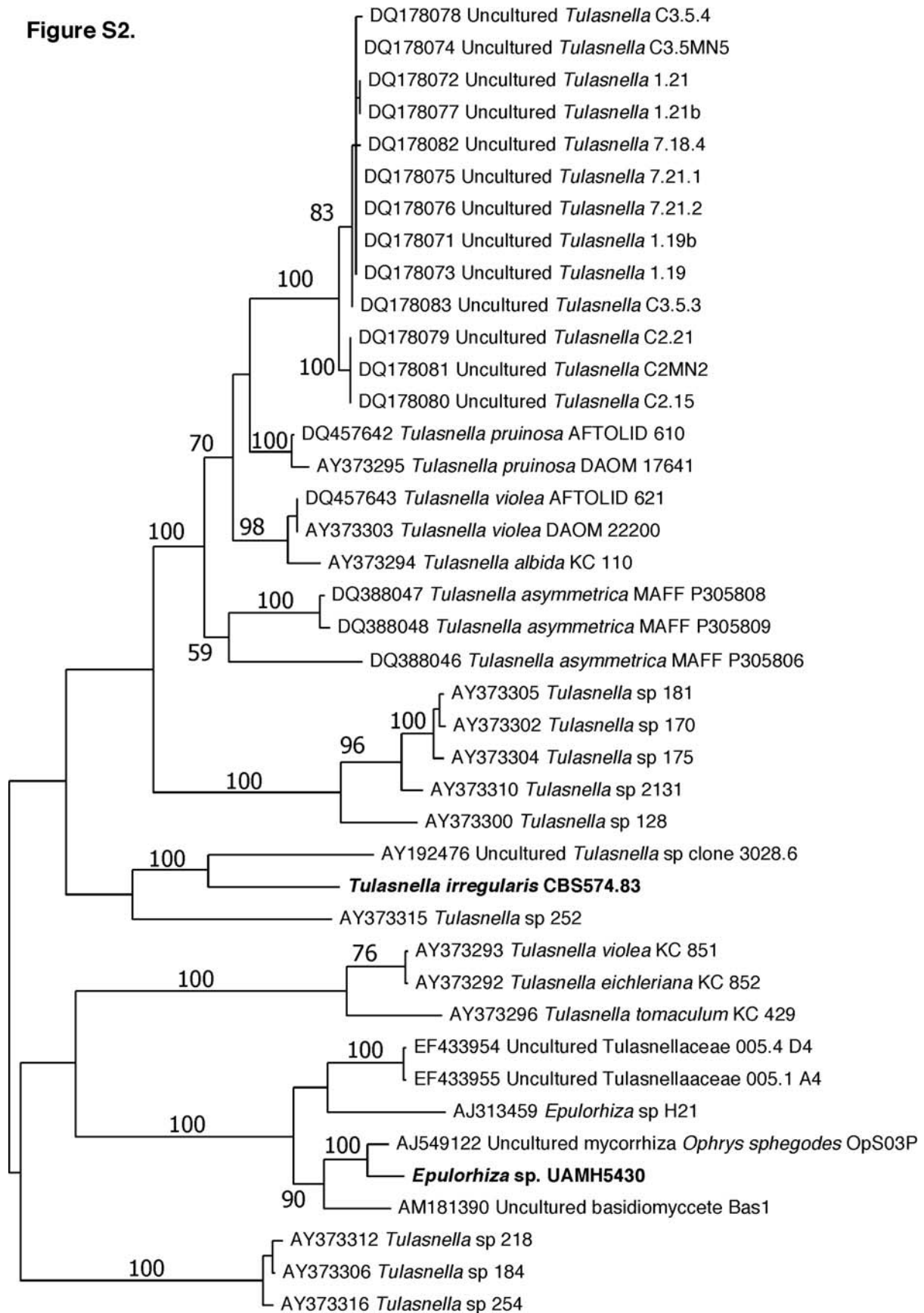


**Fig. S1** Maximum likelihood tree of the first Tulasnellaceae clade.

Sequences downloaded from GenBank are shown with accession numbers. New sequences from this study are shown in bold. The full alignment comprised 55 taxa and 651 positions and required 36,400 generations to reach likelihood search termination under default settings in GARLI (Zwickl, 2006). The maximum likelihood was  $-\ln L = 3638.24$ . Values near branches indicate support from 100 likelihood bootstrap replicates. There were 214 variable positions, of which 130 were parsimony informative. The midpoint rooted ML tree required 506 steps under the parsimony criterion, while 100,000 most parsimonious trees of 502 steps were obtained in a restricted heuristic search in PAUP\*4.0b10. When pruned using Gblocks (Castresana, 2000), 10% of the positions were removed, which had no significant impact on the topology.

Note that our ITS sequence from Warcup's *Tulasnella calospora* isolate CBS 573.83 falls into this first clade, within a large cluster of relatively closely related sequences from GenBank. Most of the sequences from teleomorphic isolates were also identified as *T. calospora*, which indicates that the European, Australian and Asian concepts of this cosmopolitan taxon are in agreement. CBS 573.83 was isolated from *Caladenia reticulata* in southern Australia by Warcup and Talbot (isolate 062; 1967) and this species was one of the most commonly encountered in their extensive studies of diverse Australian orchids (Warcup, 1981). However, a number of collections identified as *T. calospora* from Europe, North America, Australia (including other isolates from Warcup, (Suarez *et al.*, 2006) and Asia fall into a distinct subclade within this clade of the Tulasnellaceae. The two major clades of *T. calospora* isolates were recovered in all most parsimonious trees as well as ML analyses. Thus, *T. calospora* has either been misidentified in some cases or belongs to a species complex. Similar observations were recently reported by Suárez *et al.* (2006). Despite the large number of sequences already available for this clade, our sequence from CBS 573.83 is sister to an uncultured, environmental sequence obtained directly from the North American orchid *Epipactis gigantea* by Bidartondo *et al.* (2004). The first clade containing *T. calospora* sequences, including CBS573.83, also contains a sequence from GenBank labeled *T. deliquescens*, a name which Roberts (1999) suggested is synonymous with *T. calospora*.

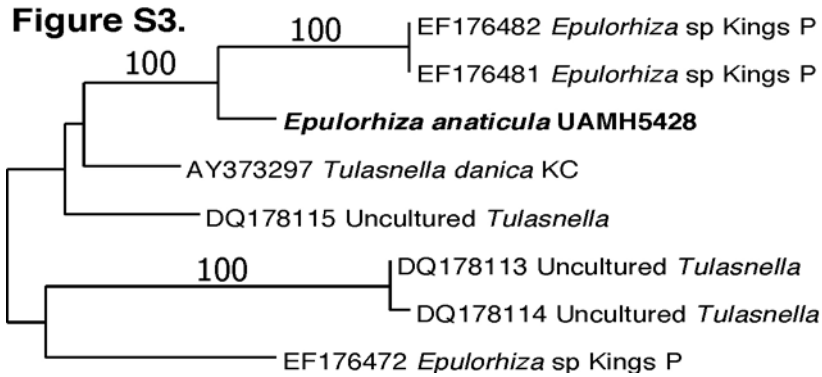
Figure S2.



**Fig. S2** Maximum likelihood tree of the second Tulasnellaceae clade.

The alignment comprised 41 taxa and 798 positions. The likelihood search required 18,900 generations to complete and found a tree with  $-\ln L = 8698.25$ . There were 635 variable positions, of which 555 were parsimony informative. The midpoint rooted ML tree required 2001 steps under the parsimony criterion, while 100,000 most parsimonious trees of 1989 steps were obtained in a restricted heuristic search in PAUP\*4.0b10. The topologies of the parsimony majority rule consensus tree and the likelihood tree were nearly identical. When pruned using Gblocks, 42% of the positions in the full alignment were excluded, but the resulting tree did not conflict with the tree from the full alignment.

As can be seen in the tree, this second clade encompasses diverse ITS sequences spanning six named teleomorphs, a number of environmental sequences, and our sequences from the holotype strain *Tulasnella irregularis* CBS 574.83 and ‘*Epulorhiza repens*’ UAMH 5430. The named taxa *T. pruinosa*, *T. violea*, *T. albida* and *T. asymmetrica* come out in a cluster together (with the exception of one putative *T. violea* sequence), while our *T. irregularis* sequence falls into a distinct cluster containing only environmental sequences. The most closely related environmental sequence was obtained from mycorrhizae of the myco-heterotrophic liverwort *Cryptothallus mirabilis* (Bidartondo *et al.*, 2003). It resides on a fairly long branch, meaning that very closely related fungi have not been sequenced. Our sequence from ‘*Epulorhiza repens*’ UAMH 5430 falls out in a second cluster previously represented only by environmental sequences and an isolate designated only as ‘*Epulorhiza* sp.’. The teleomorphic stage of *Epulorhiza repens* was reported by Warcup & Talbot (1967) to be *Tulasnella calospora*. Clearly, the fungus described by Currah *et al.* (1987) is distantly related to the array of *Tulasnella calospora* collections shown in Fig. S1. Hence we have labeled UAMH 5430 simply as ‘*Epulorhiza* sp.’ in this paper.

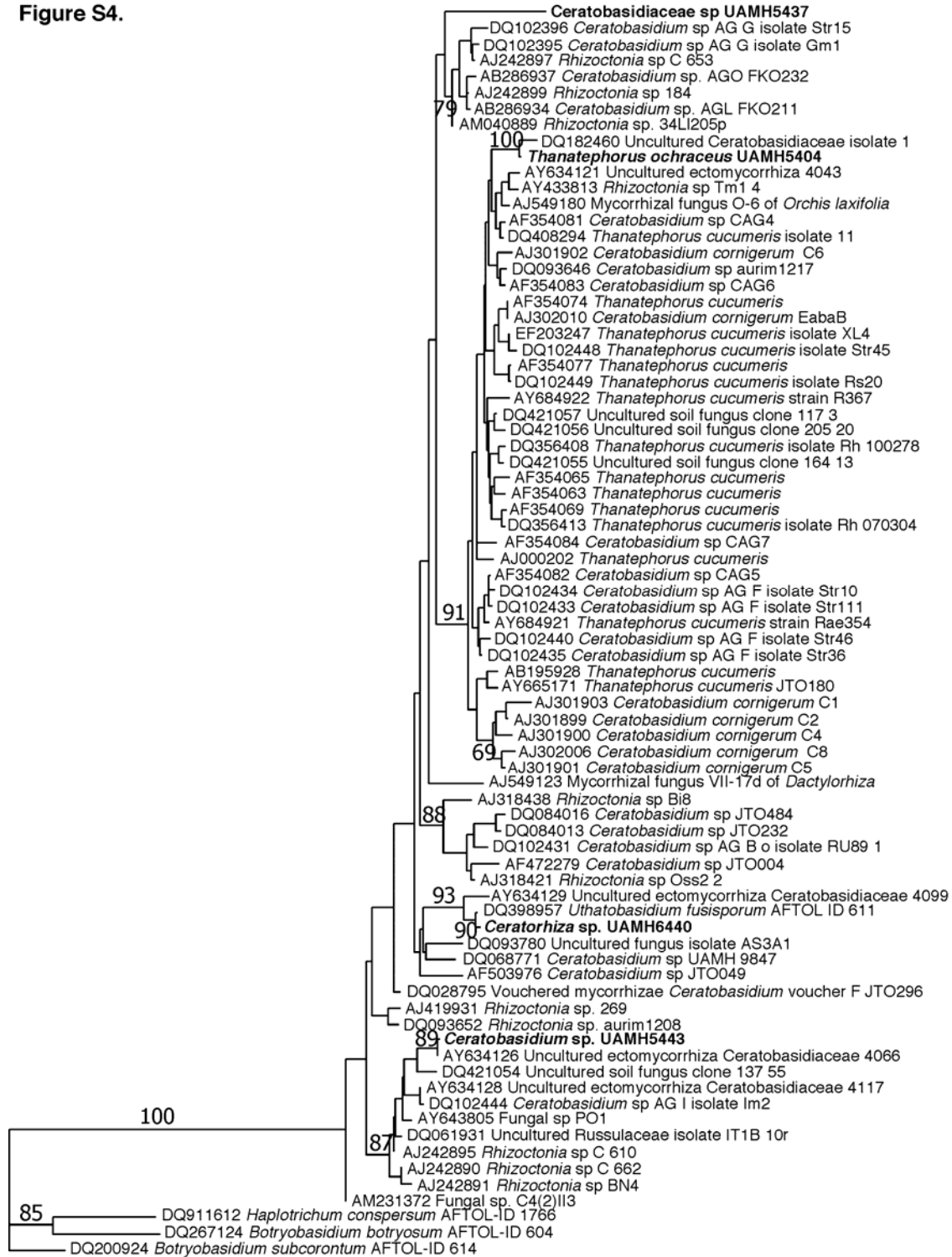


**Fig. S3** Maximum likelihood tree of the third Tulasnellaceae clade.

Because the most closely related BLAST matches included only the ITS 2 region, the alignment was restricted to 228 positions and included eight taxa. The likelihood search required 20,200 generations and found a tree with  $-\ln L = 634.29$ . There were 47 variable positions, of which 35 were parsimony informative. The midpoint rooted ML tree required 68 steps under the parsimony criterion, as did the single most parsimonious tree obtained in a complete heuristic search in PAUP\*4.0b10. The likelihood and parsimony trees had identical topologies, as did the single parsimony tree obtained from analysis of a full ITS alignment spanning 579 characters (data not shown). For the short alignment, analysis with Gblocks suggested the exclusion of 2% of the characters, and produced an identical tree.

This third clade comprises a relatively small group with divergent ITS sequences which were difficult to align to one another, much less to other *Tulasnella* species. Our sequence from *Epulorhiza anaticula* UAMH 5428 falls into this clade. Although UAMH 5428 is not the type strain (which was unavailable), it was among the strains used to develop the description of *E. anaticula* (Currah *et al.*, 1987). The anamorphic stage of *Epulorhiza anaticula* features unique isthmus-like bridges between its monilioid cells. Our ITS sequence analyses confirm that it belongs in the Tulasnellaceae and falls within a clade containing *T. danica* and several unidentified Australian isolates.

Figure S4.



**Fig. S4** Maximum likelihood tree of the Ceratobasidiaceae.

The pruned alignment comprised 78 taxa and 482 positions (58% of the positions in the full alignment). The alignment has 258 variable characters of which 196 were parsimony informative. Stationarity of the likelihood was reached in 14826 generations, resulting in a best tree of  $-\ln L$  5984.5000. The tree was rooted to *Botryobasidium* based on Moncalvo *et al.* (2006). The ML tree required 1110 steps under the parsimony criterion, while 86 most parsimonious trees of 1092 steps were obtained in an unrestricted heuristic search in PAUP\*4.0b10.

Our sequence from Currah's '*C. obscurum*' isolate UAMH 5443 falls out in a clade composed entirely of *Ceratobasidium* strains, but in a clade distinct from all the *C. cornigerum* strains available on GenBank. Both Warcup & Talbot (1967) and Currah (1987) have referred certain isolates from orchids to the taxon *Ceratobasidium obscurum* Rogers. Upon re-examination of the type specimen of *C. obscurum*, characters that better align with *Thanatephorus* were observed (Roberts, 1998a). The new combination *Thanatephorus obscurus* was proposed for the type specimen, while Currah's isolate was retained in *Ceratobasidium*, possibly falling within the *C. cornigerum* complex (Roberts, 1998a). We have therefore labeled this isolate as *Ceratobasidium* sp. pending further examination. Interestingly, UAMH 5443 belongs to a subclade lacking identified species and containing several environmental sequences attributed to ectomycorrhizas.

Our ITS sequence indicates that '*Thanatephorus pennatus*', UAMH 5404 falls within a clade containing some isolates ascribed to *Thanatephorus cucumeris* as well as the majority of strains identified as *Ceratobasidium cornigerum*. However, UAMH 5404 is separated from other members of this clade by a long branch, so it is unlikely to be con-specific with other sequenced strains. *Thanatephorus pennatus* was described as a new species when an isolate from *Calypso bulbosa* fruited in culture (Currah, 1987). Roberts (1998b) re-examined UAMH 5404 as well as *Thanatephorus orchidicola* Warcup and Talbot (1966), and synonymized both with *Thanatephorus ochraceus* (originally described as *Botryobasidium ochraceum* by Masee). It was said to be the only *Thanatephorus* species in the United Kingdom with such wide, unswollen hyphae; the hyphae are also brown and slow-growing (Roberts, 1998b).

The anamorphic strain *Ceratorhiza goodyerae-repentis* UAMH 6440 was isolated from *Platanthera obtusata* and described in Currah *et al.* (1990) and Currah & Sherburne (1992). The species was first isolated from *Goodyera repens* and described by Constantin (Constantin & Dufour, 1920). Many orchid isolates have been ascribed to this taxon since then, and Warcup & Talbot (1967) obtained the teleomorph *Ceratobasidium cornigerum* from one of their isolates. As can be seen in the tree, our sequence from UAMH 6440 does not appear to be closely related to the many *Ceratobasidium cornigerum* sequences available in GenBank, despite the fact that this name appears several places in the tree.

UAMH 5437, '*Sistotrema* sp.' appears to be much more closely allied with the Ceratobasidiaceae than other sequenced *Sistotrema* species. Currah *et al.* (1990) were the first to report a clamp-bearing, anamorphic isolate referred to the genus *Sistotrema* as an orchid mycorrhizal fungus. Most species in the genus are considered to be saprophytic. Several recent studies (Binder *et al.*, 2005; Moncalvo *et al.*, 2006; Nilsson *et al.*, 2006) have shown that *Sistotrema* is highly polyphyletic, with species belonging to several lineages within the cantharelloid clade, which also contains the Ceratobasidiaceae. Some of these lineages are ectomycorrhizal. However, none fall within the Ceratobasidiaceae. With *Botryobasidium subcoronatum* designated as outgroup, UAMH 5437 fell within the *Ceratobasidium-Thanatephorus* complex with 100% bootstrap support, though on a relatively long branch. This result agrees with our previous placement of this particular strain within the Ceratobasidiaceae based upon analysis of a fragment of the mitochondrial large subunit gene (Kristiansen *et al.*, 2001). This result is somewhat surprising, given that UAMH has clamp connections and *Rhizoctonia* species are generally considered to lack clamps. In other words, it is no longer safe to assume that the observation of clamp connections in an orchid mycorrhizal peloton indicates association with non-*Rhizoctonia* fungi.

## Supplementary References (Figs S1–S4)

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