Phylogenetic relationships of *Rhizoctonia* fungi within the Cantharellales

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Abstract

Phylogenetic relationships of *Rhizoctonia* fungi within the order Cantharellales were studied using sequence data from portions of the ribosomal DNA cluster regions ITS-LSU, *rpb2*, *tefl* and *atp6* for 50 taxa, and public sequence data from the *rpb2* locus for 165 taxa. Data sets were analyzed individually and combined using Maximum Parsimony, Maximum Likelihood and Bayesian Phylogenetic Inference methods. All analyses supported the monophyly of the family Ceratobasidiaceae, which comprises the genera *Ceratobasidium* and *Thanatephorus*. Multi-locus analysis revealed 10 well supported monophyletic groups that were consistent with previous separation into anastomosis groups based on hyphal fusion criteria. This analysis coupled with analyses of a larger sample of 165 *rpb2* sequences of fungi in the Cantharellales supported a sister relationship between the Botryobasidiaceae and Ceratobasidiaceae and a sister relationship of the Tulasnellaceae with the rest of the Cantharellales. The inclusion of additional sequence data did not clarify incongruences observed in previous studies of *Rhizoctonia* fungi in the Cantharellales based on analyses of a single or multiple genes. The diversity of ecological and morphological characters associated with these fungi requires further investigation on character evolution for re-evaluating homologous and homoplasious characters.

Keywords: *Rhizoctonia solani*; multi-locus phylogeny; *Rhizoctonia* anamorphs; fungal plant pathogens

1. Introduction

The systematics and taxonomy of anamorphic fungi classified as *Rhizoctonia* have been in a constant state of flux since the original description of the genus by DeCandolle in 1815, which was based primarily on the ability of the fungi to infect plants and form sclerotia (Stalpers and Andersen 1996). However, these fungi can also grow as saprobes or as beneficial endomycorrhizal symbionts of orchids (Masuhara et al. 1993; Cubeta and Vilgalys 2000; Jiang et al. 2015). The study of *Rhizoctonia* fungi is largely associated with their economic importance as pathogens on more than 500 species of plants (Farr et al. 2005). Since *R. solani* and other described species of *Rhizoctonia* do not produce asexual spores, morphological characteristics of vegetative cells (hyphae and sclerotia), such as the absence of clamp connections, patterns of branching and constriction, number of nuclei per cell, pigmentation, and hyphal width, were initially used to classify and identify them. With the discovery that the fungus could undergo sexual reproduction (Prillieux and Delacroix 1891; Rolfs 1903), color, shape, and size of the sexual fruiting structures have also been used as taxonomic characters. This discovery also established the connection of the anamorph (asexual) and teleomorph (sexual) stages of the fungi. Subsequently, several different genera of resupinate fungi that include *Botryobasidium* Donk, *Ceratobasidium* D.P. Rogers, *Thanatephorus* Donk, *Tulasnella* J. Schröter, and *Uthatabasidium* Donk were found to be associated with a *Rhizoctonia* anamorph (Talbot 1970; Stalpers and Andersen 1996). However, morphological characters of the anamorph and teleomorph are variable and of limited value in defining and delimiting species (Andersen and Stalpers 1994; Roberts 1999; Vilgalys and Cubeta 1994).

The single most important criterion for delineating species of *Rhizoctonia* is referred to as the “anastomosis group concept” (Matsumoto et al. 1932; Richter and Schneider 1953; Schultz

1936). This concept is based on the premise that hyphae of related isolates of the same species (independent of their capability to mate) have the ability to recognize and fuse (i.e. “anastomose”) with each other. The anastomosis group (AG) concept has been used extensively to examine *R. solani* (associated with a *Thanatephorus* teleomorph) and other species of *Rhizoctonia* (e.g. *Ceratorhiza*) associated with a *Ceratobasidium* teleomorph (Ogoshi et al. 1983; Parmeter et al. 1967; Carling 1996). At least 13 groups in *Thanatephorus* (designated as AG followed by a number, AG-1 to AG-13) and 21 groups in *Ceratobasidium* (designated as AG followed by a letter, AG-A to AG-U) have been described, but only 16 are currently used (Sharon et al. 2008). These AGs have been further divided into subgroups using additional biochemical, host association, nuclear condition of hyphal cells (binucleate or multinucleate) and molecular criteria or have been re-defined such as AG-bridging isolate (BI), now considered as a subgroup of AG-2 (=AG-2 BI, Carling et al. 2002). Although the formal taxonomic status of AG and subgroups has been the subject of considerable debate, recent sequence analyses of the internal transcribe spacer (ITS) and the large subunit (LSU) regions of the ribosomal DNA and β -tubulin genes have provided support for the monophyly of the majority of these groups (Cubeta et al. 1996; Kuninaga et al. 1997; González et al. 2001, 2006; Sharon et al. 2006, 2008).

More recently, higher phylogenies of the Kingdom Fungi have shown that *Tulasnella* (anamorph=*Epulorhiza*), *Botryobasidium* (anamorphs=*Allescheriella*, *Alysidium* and *Haplotrichum*), *Ceratobasidium*, *Thanatephorus*, and *Uthatabasidium* associated with the anamorph name *Rhizoctonia* cluster within the Cantharellales, a clade that includes a collection of taxa with extensive variation in lifestyles and morphology (Moncalvo et al. 2006, Hibbett et al. 2007; 2014). High variation is also observed in ribosomal RNA genes within the order. The first efforts to circumscribe the order and identify monophyletic groups were made using sequence

data from nuclear and mitochondrial rDNA (e.g. Hibbett and Thorn 2001, Binder et al. 2005, Moncalvo et al. 2006). These studies documented the accelerated evolutionary rate heterogeneity in these genes affecting phylogenetic reconstruction due to long-branch attraction. Other loci with less unequal evolutionary rates, particularly protein coding genes and alternative reconstruction methods were proposed for phylogenetic inferences within the Cantharellales (e.g. Moncalvo et al. 2006, Matheny et al. 2007, Buyck and Hofstetter 2011).

In the past two decades there has been an ample discussion on the circumscription and phylogenetic relationships within the Cantharellales, but problems still persist in the placement of some members of the clade (readers interested in this subject should refer to Hibbett and Thorn 2001, Larsson et al. 2004, Binder et al. 2005, Moncalvo et al. 2006, Hibbett et al. 2007, Matheny et al. 2007; Hibbett et al. 2014). For example, the *Ceratobasidiaceae* is currently considered a member of the order although its phylogenetic relationships are not well resolved (Moncalvo et al. 2006, Hibbett et al. 2007; 2014). This family was restricted to the core taxa *Ceratobasidium*, *Thanatephorus* and *Uthatabasidium*, but relationships among genera remain unresolved. All previous research within the *Ceratobasidiaceae* have displayed only a limited resolution not only for the scarce value of their morphological characters but because recognition of *Uthatabasidium* as a valid genus remains controversial.

In a re-examination of the number of species belonging to the *Ceratobasidiales sensu lato*, Roberts (1999) stated that species of *Thanatephorus* and *Uthatabasidium* are morphologically similar and congeneric and should be synonymized, a taxonomic rearrangement that was accepted by later researchers (Oberwinkler et al. 2013, Veldre et al. 2013). Molecular studies have also shown that *Uthatabasidium* is not monophyletic and the distinction between *Uthatabasidium* and *Ceratobasidium* requires further investigation (Binder et al. 2005;

Moncalvo et al. 2006), as it is unclear whether the family contains two or three genera (Hibbett et al. 2014). Therefore, *Uthatabasidium fusisporum* here is considered to be *Thanatephorus fusisporum*. Phylogenies have also shown that the Ceratobasidiaceae represents a monophyletic group sister to both *Botryobasidium* and other members of this clade (Binder et al. 2005; Hibbett et al. 2007; Matheny et al. 2007; Moncalvo et al. 2006; Taylor and McCormick 2008). The primary objective of this research was to develop a comprehensive multi-locus gene phylogeny to understand the evolution and systematics of *Rhizoctonia* fungi and related resupinate taxa in the Cantharellales. The following hypotheses were developed and tested: 1) the family Ceratobasidiaceae is monophyletic; 2) *Ceratobasidium* and *Thanatephorus* represent well-supported monophyletic groups in the Ceratobasidiaceae; and 3) *Botryobasidium* is the sister group to the Ceratobasidiaceae.

2. Materials and Methods

2.1. Source of isolates

Fifty isolates were used in this study (Table 1). Isolates were obtained from Departamento de Produção Vegetal - Setor de Defesa Fitossanitária da Faculdade de Ciências Agronômicas -UNESP (SP, Brazil), the Centraalbureau voor Schimmelcultures – CBS- KNAW (Utrecht, The Netherlands) and the culture collections from the Cubeta and Vilgalys laboratories. All isolates were grown on potato dextrose agar (PDA) or malt extract agar (MEA) at 24 °C and stored at -80 °C in potato dextrose broth (PDB) with 50% glycerol.

2.2. DNA extraction and amplification

To extract fungal DNA, isolates were grown on PDB, followed by lyophilization at room temperature for 24-48 h. Lyophilized mycelium was ground in liquid nitrogen and nucleic acids were extracted using either the DNeasy Plant Mini Kit column (Qiagen, Valencia, CA, USA) or the extraction protocol published by Kuramae-Izioka (1997). PCR was used to amplify two nuclear encoded genes (the second largest subunit of RNA polymerase II (*rpb2*) and translation elongation factor 1 alpha (*tef1*); one mitochondrial encoded gene, ATP synthase subunit 6 (*atp6*); and two from the ribosomal DNA operon, the large subunit ribosomal DNA (LSU) and internal transcribed spacer region (ITS). The general PCR protocols applied to all markers are described in Table 2.

2.3. Amplicons sequencing

All PCR products were separated on agarose gel and purified prior to sequencing using either the QIAquick PCR purification Kit (Qiagen, Valencia, CA, USA) or the GFXTM PCR DNA gel band purification kit (Amersham Biosciences, Roosendaal, The Netherlands). When possible sequencing reactions were performed directly on purified PCR products by using one of the PCR primers used for amplification of a specific locus, but when necessary (due to the presence of polymorphism and introns) PCR products were cloned using the TOPO.TA Cloning Kit (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instructions. Clones were sequenced using the M13F and M13R primers. Sequencing reactions were performed using Big Dye chemistry v3.1 (Applied Biosystems, Foster City, CA USA) and analyzed on an Applied Biosystems 3730xl capillary sequencer. Sequence reads were trimmed and assembled using Sequencher (Version 4.6, Gene Codes Corporation, Ann Arbor, MI, USA). Individual

contigs were generated in Sequencher and BLAST (Altschul et al. 1990) was performed to confirm the identity of sequenced products.

2.4. Phylogenetic analysis of DNA sequence data

Phylogenetic analyses of all loci were performed independently and in combination with Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Phylogenetic Inference (BPI). Independent matrices consisted of loci ITS and LSU from the ribosomal DNA operon (50 and 22 taxa respectively), the nuclear encoded genes *rpb2* and *tef1* (49 and 45 taxa respectively), and the mitochondrial gene *atp6* (40 taxa). The combined matrix included some taxa for which a gene region is missing, however, for large alignments modest amounts of missing data generally did not have a negative affect on the results of phylogenetic analyses (Wiens 2006). The multi-locus data set emphasizes taxa within the Ceratobasidiaceae, whereas other members of the Cantharellales were under-represented for these loci. A separate analysis for testing the monophyly of the family was performed with only sequences of the *rpb2* locus available at GenBank. This gene has been useful at higher taxonomic levels because it has a higher phylogenetic informativeness compared with ribosomal genes and its conservation of amino acid sequence facilitates alignment (e.g. Matheny et al. 2005, 2007; Schoch et al. 2009). Nucleotide sequences were aligned using the MAFFT program (<http://www.ebi.ac.uk/Tools/msa/mafft/>), which allows rapid detection of homologous segments using fast Fourier transform (FFT) through an iterative refinement of an initial alignment. FASTA files were imported into Mesquite version 3.0 (Maddison and Maddison 2014) for concatenation and trimming of sequence data.

Heuristic searches for MP were conducted in PAUP* 4.0b10 (Swofford 2002). Analyses were executed with 1,000 random addition replicates and Tree-Bisection-Reconnection (TBR)

branch swapping after exclusion of uninformative characters. Statistical support for branches was calculated from 1,000 bootstrap replicates with TBR branch swapping and 10 heuristic searches per replica. Model parameters for ML were determined using jModelTest 2.1.4 (Darriba et al. 2012). Analyses were accomplished with GARLI v. 1.0 (Zwickl 2006) with model parameters fixed according values obtained with jModelTest (Supplementary Table S1). Searches consisted of 10 replicates to ensure that results were consistent and reproducible. Branch support for ML was determined simultaneously by doing 100 non-parametric bootstrap iterations in each of the 10 replicates. BPI analyses were conducted using MrBayes 3.1.2 (Altekar et al. 2004; Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Individual matrices were analyzed with model GTR + Γ (nst = 6; rates = gamma), because the specific models generated by jModelTest are not implemented in MrBayes. All trees were given equal weight *a priori*. For the concatenated matrix, sequences were partitioned by gene with a unique GTR + Γ model for each partition with unlinked parameters and allowing rates to vary across partitions. Each analysis comprised two independent 1 to 10-million generation runs, with four chains (one cold and three hot) each, until an average standard deviation of split frequencies of 0.01 or less was reached (combined matrix only reached 0.07 after 10-million generation run). We sampled trees every 100th generation and discarded initial samples applying a “burn-in” value of 25% before calculating the majority consensus tree and posterior probabilities (PP) for clades. Burn-in was assessed using the stability of likelihood values within and between the duplicate runs. The potential scale reduction factor (PSRF) was close to 1.000. For the concatenated and three individual matrices (ITS-LSU, *rpb2* and *tef1*) the taxon *Tulasnella pruinosa* (AFTOL ID 610) was used as the outgroup. Because of our inability to sequence locus *atp6* for *T. pruinosa*, *Clavulina* sp., was used as the outgroup in the analysis for this locus.

To examine the extent of genomic support and source of phylogenetic signal in the concatenated matrix four approaches were used. First, we conducted a Bayesian Concordance Analysis (BCA) using BUCKy 1.4.3 (Larget et al. 2010). In this analysis, individual gene trees are summarized with mbsum (distributed within the BUCKy package) to provide a concordance factor (CF) per clade that represents the proportion of gene trees that are in agreement (Wielstra et al. 2014). Individual gene trees with all taxa (including with missing sequences) were generated with MrBayes using a single eight-chain 50,000 generations run, sampled every 100 generations and applying a burn-in value of 25%. The output of mbsum was further processed in BUCKy to create a primary concordance tree with CFs for clades. Concordance factors are reported for the default prior number (α) of 1 since we did not have any evidence for *a priori* level of discordance among loci. Second, sensitivity analyses involving the inclusion or exclusion of different locus were performed to determine the relative contribution of each gene on the CF. Third, for determining conflict and congruence of each gene tree in the ML concatenated tree topology, we compared manually each bipartition and recorded whether the bipartition was concordant with or conflicted with each clade in the combined tree (Smith et al. 2015). Lastly, we calculate the Internode Certainty (IC), Internode Certainty All (ICA), Tree Certainty (TC), and Tree Certainty All (TCA) adjusted for partial gene trees (Kobert et al. 2015), by considering the frequency of all conflicting bipartitions (0= maximum conflict; 1= strong certainty) in the RAxMLHPC-AVX version. These measures have been proposed for quantifying the degree of incongruence for a given internode, or for an entire tree in phylogenies inferred from different data matrices (Kobert et al. 2015; Salichos and Rokas 2013; Salichos et al. 2014; Stamatakis 2006). For performing this analysis, the unresolved branch (AG-10, AG-8,(AG-F, AG-4 HGIII) in the ML concatenated tree had to be rearranged to the branch (AG-10, AG-

8),(AG-F, AG-4 HGIII) for having a fully resolved bifurcated tree.

For testing the monophyly of the Ceratobasidiaceae, we conducted additional analyses with 165 sequences of *rpb2* from representative taxa in the Cantharellales (*Clavulina*, *Cantharellus*, *Craterellus*, *Hydnum*, *Botryobasidium*, and *Tulasnella*) including representatives from Sebaciniales and Auriculariales. Sequences selected were only those that were complete for the segment sequenced for the multi-locus analyses. Analyses were as follow: a) Parsimony Ratchet in NONA (PR; Goloboff, 1998) with 1000 iterations, holding 100 trees per iteration and with 10% of the characters perturbed; b) Maximum Likelihood with RAxML with default parameters and the GTRGAMMA substitution model; and c) BPI with two independent 4-million generation runs, with four chains (one cold and three hot) each, sampling trees every 100th generation and discarding a burn-in value of 25%. Statistical support for the ML analysis was calculated from 1,000 bootstrap replicates. To explore the history of trophic behavior and nuclear division, maximum parsimony reconstruction of ancestral states were performed using Mesquite v. 3.0 (Maddison and Maddison 2014). Characters were coded with all transformations unordered and equally weighted from descriptions from the literature and personal communication from the authors. Trees were subsequently edited using Adobe illustrator V 16.0.0. The alignment and resulting trees from multi-locus gene analyses are deposited in TreeBASE (<http://purl.org/phylo/treebase/phylows/study/TB2:S15006>)

3. Results

3.1. Single locus analyses

A comparison of general features for phylogenetic analyses on nucleotide sequences from single and combined regions is summarized in Table 3. Individual gene topologies showed that

groups associated with the genera *Ceratobasidium* and *Thanatephorus* were consistently included in one large well-supported monophyletic group regardless of the method employed for phylogenetic reconstruction. However, *Ceratobasidium* and *Thanatephorus* were not monophyletic. Several small clades were consistent with previous anastomosis grouping based on hyphal fusion (anastomosis) criteria. In most analyses, AG-1-IC, AG-2-2, AG-3, AG-9, AG-A, AG-E, and AG-D were monophyletic. Topologies differed among individual gene phylogenies. For example, *Thanatephorus* sp. CBS 293.31 was placed related to AG-4, AG-2-2 or AG-5 depending the locus analyzed; or *Ceratobasidium* sp. CBS 476.82 in some analyses was related to *Thanatephorus*, while in others to isolates of *Ceratobasidium* (Supplementary Fig S1 A-D). The phylogenetic position of other representative members of the Cantharellales was also uncertain in single locus analyses. For example, data from the ITS-LSU region clustered *Clavulina* sp., *Hydnum albomagnum* and *Botryobasidium simile* in a small clade sister to the Ceratobasidiaceae, while data from the *rpb2* gene grouped *Cantharellus cibarius*, *Clavulina* sp. and *Hydnum albomagnum*.

Analyses for testing the monophyly of the family was performed with 165 sequences and 487 characters from which 318 were informative for parsimony ratchet. This analysis generated 33627 trees of 3727 steps. Maximum Likelihood analysis with RAxML and the GTRGAMMA substitution model resulted in a loglikelihood (–ln) score of -15822.8677. The consensus tree from the Parsimony Ratchet (PR) analysis was unresolved on early-diverging lineages within the Cantharellales (topology not shown). However, Maximum Likelihood and Bayesian Phylogenetic Inference recovered all families within the Cantharellales as monophyletic with significant posterior probabilities, but lower bootstrap support. These analyses, with a more extensive sampling of taxa also placed *Botryobasidium* (Botryobasidiaceae) as a sister group of

the Ceratobasidiaceae as did single and multi-locus analyses, and moderately supported the sister relationship of Tulasnellaceae with the rest of the Cantharellales (Fig 1).

3.2. Multi-locus gene phylogeny

Phylogenetic analyses of the concatenated data set employing three different methods for phylogenetic reconstruction supported the hypothesis that the family Ceratobasidiaceae is monophyletic and includes the genera *Ceratobasidium* and *Thanatephorus*. But as with individual analyses, *Ceratobasidium* and *Thanatephorus* were not recovered as monophyletic. *Thanatephorus* (syn. *Uthatabasidium*) *fusisporum* was closely related to species of *Ceratobasidium*, contrasting with the proposition of the taxonomic rearrangement for transferring it into *Thanatephorus* (Hauerslev and Roberts in Oberwinkler et al. 2013). Although very similar, the clades recovered within the Ceratobasidiaceae have discrepancies depending on the reconstruction method used (Fig 2). For example, all three phylogenetic reconstruction methods placed *T. fusisporum* in distinct locations within the tree, and only MP analysis recovered *Thanatephorus praticola* Kotila (= *Rhizoctonia solani* AG-4) as monophyletic with low support (Fig 2). Only isolates from AG-4 HGI were recovered as monophyletic in most analyses (Supplementary Table S2).

The concatenated matrix (ITS-LSU, *rpb2*, *tef1* and *atp6*) included 50 taxa and 3318 characters from which 1339 were informative for MP analysis. This analysis generated two trees of 6079 steps (CI = 0.380; RI = 0.575, Fig 3). The best-fit substitution model for ML analysis under the Akaike information criterion (AIC) was TIM3+I+G. Nucleotide frequencies and substitution rates values were as follow: “Lset base=(0.2522 0.2474 0.2170 0.2835) nst=6 rmat=(0.7542 3.1616 1.0000 0.7542 4.2313) rates=gamma shape= 0.3300 ncat=4

pinvar=0.0000". Analyses with these model parameters fixed resulted in a loglikelihood ($-\ln$) score of -33315.3131. The primary concordance tree generated with BCA using the independent posterior probabilities of the individual gene trees is similar to the Bayesian tree generated with the concatenated data set (Supplementary Fig S2). However, CF values were highly variable. Values obtained go from as low as 0.001 up to 0.936. Only 10 out of 46 clades had CF values above 0.5 (Fig 3), reflecting discordance among individual gene trees. The CF values were in general, lower on the branches representing earlier divergences.

In the sensitivity analyses, most clades were recovered in separate analyses with mitochondrial (*atp6*) and nuclear markers (ITS-LSU, *rpb2* and *tef1*) irrespective of the reconstruction method used (Supplementary Table S2). However, the combination of *rpb2* and *tef1* provided the highest concordance values (Supplementary Table S3). Analysis of concordant bipartitions in the combined ML topology for *Rhizoctonia* fungi is presented in Fig 4. The numbers of clades concordant with the concatenated tree at that node are mapped on branches. Only eight small terminal clades were supported with more than two genes; the IC and ICA measurements also showed that the highest values correspond to those clades. Several nodes had values less than 1.0 in the concatenated phylogeny reflecting conflicts. Most had negative values showing that the internode conflicted with one or more bipartitions having a higher frequency. Values at or near -1 indicated absence of support for the bipartition defined by a given internode. The TC and TCA values for the concatenated ML tree under uniform bipartition adjustment were -11.352130 and 1.984236, respectively.

Tree topologies from all analyses showed shorter branch lengths of taxa within the family when compared to those produced by other taxa of the Cantharellales included in the analyses. Bootstrap and PP values provided consistent topology and statistical support for several higher

relationships within the Ceratobasidiaceae. Ten strongly supported clades with the three metrics of support above 80% were recovered (Fig 3, Clades I to X). These clades were recovered in most analyses irrespective of the gene or combination of genes or the reconstruction method used (Supplementary Table S2), but its position differed in all individual analyses (Supplementary Fig S1 A-D). Clade I included all isolates of *Thanatephorus sasakii* AG-1-IC. This group was accommodated in a larger clade supported by two of three measures containing *T. sasakii* (AG-1-IB), *T. pendulus* (AG-1-IA) and *Ceratobasidium* sp. (CBS 476.82). Clades II and III consisted of two isolates of *Ceratobasidium* AG-E and AG-P with AG-U, respectively. These three clades were included in a larger clade with less support as clades II and III. Clade IV grouped two isolates of *Ceratobasidium* (AG-R and AG-S). Clades V to VIII contained only species of *Thanatephorus*, while clades IX and X clustered only species of *Ceratobasidium*. Eight isolates of *Ceratobasidium* assembled with all but one isolate of *Thanatephorus*, within a strongly supported clade that we designate as the thanatephoroid clade (Fig 3). *Thanatephorus* (syn. *Uthatabasidium*) *fusisporum* was grouped with low support with species of *Ceratobasidium* *globisporum* and *C. sphaerosporum*.

4. Discussion

The main objective of this study was to develop a comprehensive multi-locus gene phylogeny to better understand the evolution and systematics of *Rhizoctonia* fungi and related resupinate taxa in the Cantharellales. Although, several molecular studies have delimited this clade (e.g., Binder et al. 2005; Moncalvo et al. 2006) none of these previous studies have provided an inclusive systematic treatment of *Rhizoctonia* fungi with other resupinate taxa. Several new sequences were produced and combined with those available in public databases to

perform multiple phylogenetic analyses from both separate and concatenated data sets. All of our analyses indicated that the family Ceratobasidiaceae is monophyletic and supports hypothesis 1. The monophyly of this family was also resolved in a recent analysis using publicly available ITS sequences (Veldre et al. 2013). Their study also revealed that *Ceratobasidium* was paraphyletic with at least three groups (AG-E, AG-P with AG-U and AG-R with AG-S) clustering together with isolates of *Thanatephorus*. These results are consistent with our findings. Therefore, hypothesis 2 that *Ceratobasidium* and *Thanatephorus* each represent a well-supported monophyletic group was rejected. Our multi-locus analyses also indicate that *Botryobasidium* (Botryobasidiaceae) is a sister group to the Ceratobasidiaceae and these findings provide support for hypothesis 3. The position of Botryobasidiaceae as a sister group to the Ceratobasidiaceae is also supported in our ML and BPI analyses with 165 sequences of the *rpb2* locus (Fig 1). However, PR analysis did not resolve relationships among the families within the Cantharellales. A multilocus phylogeny with protein coding genes and multiple representatives of each family would be necessary to confirm these relationships.

4.1. Phylogenetic relationships within Ceratobasidiaceae

Within the Ceratobasidiaceae, 10 inclusive well-supported monophyletic groups were revealed (Fig 3 and Supplementary Table S2). In general, the groups were consistent with previous separation based on hyphal fusion criteria and placement of isolates into anastomosis groups (i.e., the AG concept). Clade I included only isolates of *Thanatephorus* belonging to AG-1-IC. They were related to isolates from AG-1-IA, AG-1-IB and one isolate of *Ceratobasidium* sp. CBS 476.82. All are plant pathogens associated with monocots that include rice, corn, sorghum, turfgrass, and several dicots hosts such as peanut and soybean. This CBS number is

associated to isolate BN 38 (CAG-4 anastomosis tester isolate, Burpee 1980) (CBS-KNAW Fungal Biodiversity Centre, <http://www.cbs.knaw.nl/>) and is considered to belong to a subgroup of AG-F (Sharon et al. 2007).

Clades II to IV grouped two isolates of *Ceratobasidium* AG-E, AG-P with AG-U and AG-R and AG-S respectively (Fig 3). The close relationship existing within these isolates has been previously recognized (Hyakumachi et al. 2005; Rinehart et al. 2007; Sharon et al. 2008; Veldre et al. 2013). Isolates of AG-U have been found to recognize and undergo hyphal fusion (anastomose) with certain isolates of AG-P, and Sharon et al. (2008) have suggested that AG-U is a subgroup of AG-P. In their study, they recovered a cluster consisting of same AGs including AG-R and AG-S plus a subgroup of AG-F. Similar to our results, this group of *Ceratobasidium* spp., was located inside a major clade composed for only species of *Thanatephorus* (Sharon et al. 2008; Veldre et al. 2013). Related to clade IV but with no support was *Thanatephorus* sp. (AG-6 HGI). Although, Yokoyama and Ogoshi (1986) reported hyphal fusion among some isolates of *Ceratobasidium* AG-F and *Thanatephorus* AG-6 suggesting a possible genetic relationship based on somatic recognition, this connection of AG-6 HGI with AG-R and AG-S is ambiguous since the placement of AG-6 HGI changed in different analyses.

Clades V to VIII contained only species of *Thanatephorus*. Internal relationships within these clades are in general agreement with previous phylogenetic studies, but one novel supported relationship was revealed in Clade V between AG-5 and AG-7 (Fig 3). These relationships have not been previously observed or reported and AGs of additional isolates should be included to better justify their relationship.

Clade IX clustered only species of *Ceratobasidium* spp., two isolates of AG-A one binucleate species (DAOM 138188) that does not anastomose with others, and AG-G. Under the

AG system developed in Japan, AG-A corresponds to CAG-2 (Ogoshi et al. 1983). Since the teleomorph of CAG-2 was identified as *Ceratobasidium cornigerum* (Burpee et al. 1980), isolates in this clade appear to belong to the same teleomorph species. The binucleate species (DAOM 138188) has also been associated to AG-A (Ogoshi et al. 1983) and linked to *C. cornigerum*, which is considered a genetically variable species (Burpee et al. 1980). It was named *Rhizoctonia endophytica* (Saksena and Vaartaja 1960), but according to Andersen and Stalpers (1994) is not a valid name. A relationship between *Ceratobasidium* AG-A and AG-G has also been observed from pathogenic isolates from strawberry in Israel (Sharon et al. 2007), and both AGs have been reported to cause similar symptoms on other plant species (e.g., Mazzola 1997; Ogoshi et al. 1983).

Clade X contained all three isolates of AG-D. Within this clade isolates CBS 132.82 and CBS 223.51 from US and Japan, respectively, also formed a strongly supported smaller clade, which may indicate a differentiation of AG-D into subgroups, as has been proposed (Priyatmojo et al. 2001; Toda et al. 1999). *Ceratobasidium* sp. AG-Q was related to Clade X but with limited support (Fig 3). The relationship of *Ceratobasidium* spp. AG-D and AG-Q agree with previous molecular phylogenetic analyses that found strong support for a clade including both AGs (González et al. 2001, 2006). This relationship has been also shown based on similarities in RFLPs, RAPD and fatty acid analyses (Hyakumachi et al. 2005). A moderately supported small clade grouped *C. globisporum* (AG-Bb, syn. *Ceratobasidium oryzae-sativae*) with *C. sphaerosporum*. Warcup and Talbot (1971, 1980) described these taxa as the sexual stage for isolates of *Rhizoctonia* sampled from orchids roots in Queensland. However, Roberts (1999) suggested that several characters of these species overlap and may be morphological extremes of a single taxon. *C. oryzae-sativae* is a less aggressive and damaging pathogen on rice than *R.*

solani AG1-IA, but can produce similar lesions on the leaf sheath (Johanson et al. 1998).
Thanatephorus (syn. *Uthatabasidium*) *fusisporum* was also included in this clade but with no support and the taxonomic position of this isolate within the family was ambiguous. There were disagreements in its placement depending on the gene and reconstruction method used. Therefore, the inclusion of additional isolates of this species is required to improve their phylogenetic relationships and to clarify if the family contains two or three genera.

4.2. Gene discordance

The BCA analysis revealed concordance factors less than 1 for several clades. Thirty-six of 46 clades had CF values below 0.5 reflecting discordance among individual gene trees. Although, discordance has been observed in rapidly and recently derived taxa (Nosenko et al. 2013; Wielstra et al. 2014), in the *Rhizoctonia* species complex part of the discordance is likely due to those taxa with missing data in individual gene matrices (Table 3). BCA analyses must be performed with same number of taxa. Therefore, missing data were coded with interrogation points (e.g., ?), which influenced the concordance value. Nevertheless, the resulting concordance tree provides an estimate of the discordance and divergence at various points in the history of the Cantharellales (Supplementary Fig S2). Although internal lineages in the Ceratobasidiaceae still deserve careful scrutiny, there were several terminal clades with high concordance values, regardless of the combination of genes analyzed (Supplementary Table S3). As in bootstrap or BPI support values, low concordance was mainly on branches representing earlier divergences. Therefore, processes including hybridization, introgression, recombination, horizontal/lateral gene transfer, incomplete lineage sorting, etc., may be influencing the low gene concordance values in the *Rhizoctonia* species complex. However, assemblage of sequence data from more

loci with higher concordance, and inclusion of more taxa are needed to establish which evolutionary processes have shaped the observed gene discordance. Not surprisingly, the number of gene regions that support a specific clade is related to the concordance factor (Fig 4 and Supplementary Fig S2). However, there were clades with low concordance factors that received high support in the analysis of concatenated data (Fig 3), emphasizing the distinction between concordance factors and support values such as bootstrap (Salichos and Rokas 2013).

Fontenot et al. (2011) has observed that species affected by natural hybridization often demonstrate patterns of discordance between phylogenies generated using nuclear and mitochondrial markers, but in our sensitivity analyses inclusion or exclusion of mitochondrial data (*atp6*) had limited impact irrespective of the reconstruction method used (Supplementary Table S2, columns E and G). However, many important deep relationships remained unresolved despite our increased sampling of gene regions and inclusion of more sophisticated analyses compared with previous studies (González et al. 2001).

The IC, ICA, TC and TCA values reflected that the genetic loci sampled had conflicted histories. They were incongruent at internodes in the set of gene trees and between the individual gene trees across the entire phylogeny. Overall, concatenated trees did not reflect the evolutionary histories of these loci. There was only a small set of relationships within the *Rhizoctonia* fungi that appeared to be robustly supported by this data.

In general, previous research with these taxa have displayed only a limited resolution or unsupported branches; causing their phylogenetic relationships to remain unresolved. Therefore, it is important for future studies to design strategies to distinguish the causes that may be influencing the low concordance values of genes in this group. This experimental approach will contribute to understanding the evolutionary relationships and evolutionary processes involved in

the diversification of this complex group of fungi.

4.3. Ecological roles in the Ceratobasidiaceae

Among the teleomorphs of *Rhizoctonia* fungi, *Ceratobasidium* and *Thanatephorus* represent the most studied genera because of their economic importance as plant pathogens. However, they are also ecologically important saprobes capable of degrading organic matter and functioning as beneficial endomycorrhizal symbionts of orchids. In this study, ancestral states of trophic behavior could not be reconstructed with certainty within the Cantharellales. Early-divergent lineages were unresolved or unsupported. However, most analyses placed *Botryobasidium* (Botryobasidiaceae) as a sister group of the Ceratobasidiaceae. In the context of the concatenated phylogeny, trophic behavior represents a challenging homology problem since many taxa can have multiple modes of nutrition (Supplementary Fig S3) and we were very cautious in interpreting this data. Almost all isolates can exist as saprobes, even the species that associate with orchids. However, some isolates in different AGs are better competitive saprobes than others. There are also differences in aggressiveness (virulence) of isolates that belong to different AG. On some plant hosts *Ceratobasidium* and *Thanatephorus* can cause severe disease and on others they cause limited to no disease. Consequently, the ecological role and trophic behavior of *Rhizoctonia* fungi are challenging to discern because of their overlapping niches and ability to associate with many plant hosts. Nonetheless, an examination of the ecological habitat and trophic behavior associated with isolates and AG groups comprising each of the lineages identified in this study provided a glimpse into the lifestyles common to each of these clades.

Isolates of Clade I represent the most important pathogens of major agricultural crops worldwide, such as rice, sorghum, corn, peanut, cabbage, and soybean (Fig 3). In contrast,

isolates grouped into Clades II to IV are most often associated with woody plant hosts and have been previously shown to cause root and stem diseases of ornamental plant species (Hietala and Sen 1996; Hyakumachi et al. 2005). Clade V is composed of isolates of *Thanatephorus* spp., AG-5 and AG-7 that are predominantly described as non-aggressive pathogens of apple, legumes, sugar beet, and potato (e.g., Lehtonen et al. 2008; Mazzola et al. 1996, 1997; Sneh et al. 1991; Windels et al. 1997). However, isolates belonging to AG-5 have also been found to be beneficial to orchids (Andersen and Rasmussen 1996; Carling et al. 1999).

Clade VI grouped isolates belonging to *Thanatephorus* spp., AG-2-2 IIIB and AG-2-2 IV. These fungi cannot be distinguished based on hyphal fusion criteria and mainly cause sheath blight of grasses and root rot of sugar beet, respectively (Hyakumachi et al. 1998; Ogoshi 1987). In a recent study with publicly available ITS sequences, Veldre et al. (2013) found that AG-2 was polyphyletic. This result was similar to what we observed in this study, since subgroup AG-2-1 did not cluster with AG-2-2 (Fig 3). Clade VII is composed of isolates belonging to *Thanatephorus* AG-9, AG-2-1 and AG-3 that have been predominantly reported as pathogens of plants in the family Solanaceae (e.g., Bartz et al. 2010; Lehtonen et al. 2008; Woodhall et al. 2007; Yanar et al. 2005).

Isolates belonging to AG-4 (Clade VIII) are generalist pathogens of plants and cause seed rot, and pre- and post emergence damping-off of seedlings (Kuramae et al. 2003; Liao et al. 2012; Polizzi et al. 2011). Isolates linked to *C. cornigerum* and *Ceratobasidium* sp., AG-G found in Clade IX are also plant pathogens of a variety of host plants, but in contrast to isolates of AG-4, they can grow endophytically in roots without causing disease symptoms. Isolates grouped within Clade X (*Ceratobasidium* sp. AG-D) are pathogens of cereals and turfgrasses, and several other species of plants outside of the order Poales, such as sugar beet, cotton, bean and mat rush.

Inference of ancestral state suggests that plant pathogenic groups of *Rhizoctonia* may possibly be derived from a mycorrhizal ancestor since the Ceratobasidiaceae represents the only recognized lineage in the Cantharellales that contain plant pathogenic species. Recent studies with public ITS sequences of *Rhizoctonia* fungi in the family Ceratobasidiaceae have also suggested that pathogenic groups were derived from putative soil saprobes (Sharon et al. 2006, Veldre et al. 2013). The discovery that the lichen associated fungus *Burgoa* clusters near *Ceratobasidium* and *Thanatephorus* in the Cantharellales is intriguing (Lawrey et al. 2007), since these species are parasites of the green algal component of lichens. Future analysis of the position of this taxon within the Cantharellales could provide novel insight about the evolution of parasitic lifestyles on plants. Taken collectively, these circumstantial pieces of evidence suggest that pathogenicity might be rapidly and recently derived in *Rhizoctonia* fungi. Our current phylogenies support this hypothesis, as they revealed the presence of many short branches in nearly all of the isolates analyzed (Fig 2), and individual gene phylogenies have increased support for terminal nodes. Therefore, the rapid divergence in the presence of agricultural practices may have contributed to the expansion and diverse ecology of the pathogenic species in the *Rhizoctonia* complex. However, some lineages within the Ceratobasidiaceae are also known to establish beneficial endomycorrhizal associations with orchids (Jiang et al. 2015; Rasmussen 1995; Taylor and McCormick 2008; Warcup 1985, 1991). Endomycorrhizal associations of orchids and liverworts are also commonly found in another member of the Cantharellales, the resupinate genus *Tulasnella* (Andersen and Rasmussen 1996; Bidartondo et al. 2003; Kottke et al. 2003; Sikaroodi et al. 2001). Interestingly, Yagame and colleagues (2008, 2012) have reported that isolates of *Ceratobasidium* sampled from rhizomes of achlorophyllous orchids can also form ectomycorrhizae with pines suggesting that the orchid is able to access photosynthates from

adjacent trees.

The diversity in ecology, life history and trophic behavior coupled with a worldwide distribution suggests that *Rhizoctonia* fungi may represent an ancient lineage that has evolved complex relationships with soil microorganisms and plants, ultimately contributing to its success in both time and space (Baker 1970). The phylogenetic framework generated in this study shows that for a better understanding of adaptation of these fungi, further work is needed on saprobic and endomycorrhizal groups within each AG on distribution, population genetic diversity and host plant interactions. Furthermore, as this study and others have revealed *Rhizoctonia* fungi appear to contain a great diversity of cryptic species (Taylor and McCormick 2008; Veldre et al. 2013). Therefore, additional taxonomic and molecular systematics research that includes specific isolates of *Rhizoctonia* fungi with previously well-documented trophic behavior coupled with functional genes associated with this behavior are warranted.

As stated previously, ancestral states could not be reconstructed with certainty within the Cantharellales in this study. All methods for inferring the states at the ancestral nodes assume a specific tree and set of branch lengths when estimating the ancestral character state (Royer-Carenzi et al. 2013). Therefore, inferences of ancestral states are conditioned on the tree and branch lengths being true. Uncertainty in the phylogenies contributes to making ancestral state reconstruction ambiguous. On the other hand, it has also been observed that even for cases where the phylogeny is well supported, the uncertainty in parameters of the phylogenetic model, such as the branch lengths on the tree and the substitution parameters, can be large, making ancestral state reconstruction also unreliable (Huelsenbeck and Bollback 2001). Therefore, in the context of our concatenated phylogeny the reconstruction of ancestral states for any character represents

a homology problem (Supplementary Fig S3) since many taxa can have multiple lifestyles and modes of nutrition.

4.4 Morphological complexity in the Cantharellales

The phylogenies presented in Figures 1 to 3, agree with previous conclusions of a variable mixture of genetically divergent taxa within the Cantharellales (Binder et al. 2005; Moncalvo et al. 2006). This diversity of taxa creates challenges for inferring the pattern of morphological transformations. For example, the recent analyses of orchid endomycorrhizal species with molecular data placed a species of the wood-associate fungus *Sistotrema* with clamp connections within the Ceratobasidiaceae (Kristiansen et al. 2001; Taylor and McCormick 2008), a group of fungi that do not usually produce these hyphal structures. However, this finding that a species of *Sistotrema*, a highly polyphyletic genus, clusters near *Ceratobasidium* and *Thanatephorus* is not well supported and needs further investigation.

The Cantharellales includes cantharelloid to agaricoid, hydroid, coralloid, clavarioid, and corticioid fungi (Hibbett and Thorn 2001). The basidium morphology and number of sterigmata is also highly variable. Basidial forms range from short cylindrical or subcylindrical as in *Botryobasidium* to elongate cylindrical as in *Clavulina*, and with sterigmata as variable as 2 to 8 (Binder et al. 2005), and basidia may be free or occur in clusters as in *Botryobasidium* (Langer et al. 2000). The ample variation observed in this trait indicates that there have been numerous morphological transformations within the Cantharellales, which creates challenges for using these characters for delimiting genera in taxonomic analyses. However, within Ceratobasidiaceae basidial morphology is more homogeneous as most species have short uniform basidia with 2-8 sterigmata.

One character that appears to be a synapomorphy within the morphologically diverse Cantharellales is stichic (rather than chiasitic) nuclear division (Larsson et al. 2004; Pine et al. 1999). This character appears to generally support the derived status (Supplementary Fig S3). However, information related to this nuclear division character within the Botryobasidiaceae and Ceratobasidiaceae is still lacking (Moncalvo et al. 2006) and in need of revision since originally the genus considered *Uthatabasidium* placed in synonymy to *Thanatephorus* by Roberts (1999), was described as having non-stichic nuclear division (Tu et al. in Langer 2001). *Tulasnella* occurring as a sister group to the rest of the Cantharellales has also been reported to display non-stichic nuclear division (Penancier in Moncalvo et al. 2006). Consequently, these observations suggest that the transformations between characters states of this cytological character have occurred more than once. Therefore, further cytological studies are needed to investigate nuclear division in members of the Botryobasidiaceae and Ceratobasidiaceae to gain more insight into the evolution of this character.

Our study has contributed to a better and more comprehensive understanding of the phylogenetic relatedness of fungi with *Rhizoctonia* anamorphs in the Cantharellales. The analyses presented in this study provide a robust hypothesis for the monophyly of the Ceratobasidiaceae. However, neither *Ceratobasidium* or *Thanatephorus* are monophyletic. Our phylogenetic hypotheses also indicated that the Botryobasidiaceae is a sister group of Ceratobasidiaceae, and that the Tulasnellaceae is sister to the rest of the Cantharellales. However, these relationships need further investigation since some discrepancies were found depending on the reconstruction method used. Our results also showed that the multi-locus gene phylogeny was advantageous in resolving some relationships by combining the corresponding support for different nodes from different gene partitions in terminal nodes. Unfortunately, additional data

did not recover all incongruences observed in previous studies. The shorter branches found in recently derived taxa within the Ceratobasidiaceae help in part to explain disagreements in the taxonomy of *Rhizoctonia* fungi. Nonetheless, it is important for future studies to use more concordant loci for avoiding gene conflict topologies in early-diverging lineages, and to determine which evolutionary processes are causing discordance. A more robust phylogeny would also be useful to test alternative analytic methods more recently developed for reconstructing ancestral states of characters. The diversity of morphological and ecological characters observed within these fungi requires further research on character evolution for re-evaluating homologous and homoplasious characters within *Rhizoctonia* anamorphs in this clade.

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- 1 Table 1 - Anastomosis group (AG) affinity, plant host, geographic origin, and GenBank accession numbers of *Rhizoctonia* fungi used
- 2 in this study.

Isolate	AG	Host	Geographic origin	Other name(s)	GenBank accession number				
					ITS-LSU		<i>rpb2</i>	<i>tef1</i> α	<i>atp6</i>
CBS 700.82	AG-1-IA	<i>Eichhornia crassipes</i>	Panama	<i>R. solani</i> , <i>Thanatephorus pendulus</i> , <i>Aquathanatephorus pendulus</i>	KP171634	-	DQ301727	DQ301660	DQ301596
CBS 206.84	AG-1-IB	<i>Phaseolus vulgaris</i>	Japan	<i>T. sasakii</i> , <i>R. solani</i>	DQ279038	KP171642	DQ301747	DQ301680	DQ301616
CBS 140.82	AG-1-IC	<i>Glycine max</i>	Canada		DQ279062	-	DQ301730	DQ301677	DQ301613
CBS 207.84	AG-1-IC	<i>Phaseolus vulgaris</i>	Japan		DQ278991	-	DQ301748	DQ301681	DQ301617
CBS 523.96	AG-1-IC	<i>Pinus</i> sp.	Canada		DQ279032	-	DQ301751	DQ301685	DQ301621
SJ07	AG-2-2 IIB	<i>Glycine max</i>	Brazil	<i>Thanatephorus cucumeris</i>	AY270015	-	DQ301755	DQ301689	DQ301625
SA1-1	AG-2-2 IV	<i>Glycine max</i>	Japan		KP171635	KP171643	DQ301734	DQ301666	DQ301602
CBS 208.84	AG-2-1	<i>Linum usitatissimum</i>	Japan	<i>R. solani</i>	EU244841	-	DQ301749	DQ301682	DQ301618
Rhs1AP*	AG-3 PT	<i>Solanum tuberosum</i>	USA, Maine	<i>T. cucumeris</i>	KP171636	KP171644	DQ301735	DQ301667	DQ301603
CBS 200.25	AG-3 PT	<i>Solanum tuberosum</i>	NA		DQ278994	KP171645	DQ301746	DQ301679	DQ301615
SJ02	AG-4 HGI	<i>Glycine max</i>	Puerto Rico	<i>T. praticola</i>	AY270003	-	DQ301754	DQ301688	DQ301624
AH-1	AG-4 HGI	<i>Arachis hypogea</i>	Japan		AY154307	-	DQ301736	DQ301668	DQ301604
187-Rs	AG-4 HGIII	<i>Arachis hypogea</i>	USA, Georgia		AY154309	-	DQ301738	DQ301670	DQ301606
Rh-165	AG-4 HGII	<i>Beta vulgaris</i>	Japan	<i>T. praticola</i>	AB000033	-	DQ301737	DQ301669	DQ301605
GM 10	AG-5	<i>Glycine max</i>	Japan	<i>Rhizoctonia</i> sp.	KP171637	KP171646	DQ301739	DQ301671	DQ301607
OHT-1-1	AG-6 HGI	Soil	Japan		AF153779	-	DQ301740	DQ301672	DQ301608
76Rs	AG-7	Soil	Japan		AF354096	AF354096	DQ301741	DQ301673	DQ301609
CBS 101782	AG-8	<i>Triticum aestivum</i>	Australia	<i>Thanatephorus</i> sp.	DQ279008	KP171647	DQ301744	DQ301676	DQ301612
S-21	AG-9	Soil	USA, Alaska	<i>R. solani</i>	KP171638	-	DQ301742	DQ301674	DQ301610
CBS 970.96	AG-9	<i>Solanum tuberosum</i>	USA, Alaska		DQ279005	KP171648	DQ301752	DQ301686	DQ301622

CBS 346.84	AG-10	<i>Spinacia oleracea</i>	The Netherlands	<i>R. solani</i>	DQ278947	-	DQ301750	DQ301684	DQ301588
C-538	AG-A	<i>Solanum tuberosum</i>	Japan	<i>C. cornigerum</i> , <i>C. raminicola</i> , CAG 2	DQ279052	-	DQ301695	DQ301631	DQ301565
C-484	AG-Ba	<i>Oryzae sativae</i>	Japan	<i>Ceratobasidium</i> sp.	AB196641	KP171649	DQ301696	DQ301632	DQ301566
CBS 569.83	AG-Bb	<i>Trichoglottis australiensis</i>	Australia	<i>Ceratobasidium globisporum</i>	DQ278942	-	DQ301723	DQ301644	DQ301592
SIR-2	AG-A	<i>Ipomoea batatis</i>	Japan	<i>C. cornigerum</i>	AF354091	AF354091	DQ301697	DQ301633	DQ301567
CBS 132.82	AG-D	<i>Festuca</i> sp.	USA	<i>C. cornigerum</i> , <i>C. cereale</i> , <i>C. graminearum</i> , <i>C. oryzae-sativae</i> , <i>R. cerealis</i> , CAG 1	DQ278930	-	DQ301707	DQ301645	DQ301576
CBS 223.51	AG-D	Unknown	Japan		DQ278939	-	-	DQ301655	DQ301586
CBS 438.80	AG-D	<i>Juncus</i> sp.	Japan		KP171639	KP171650	DQ301719	DQ301656	DQ301589
F-18	AG-E	<i>Linum usitatissimum</i>	Japan	<i>Ceratobasidium</i> sp., CAG 6	DQ279013	-	DQ301699	DQ301635	DQ301569
CBS 137.82	AG-E	<i>Conyza canadensis</i>	USA	<i>Ceratobasidium cornigerum</i> , CAG6	DQ278934	KP171651	DQ301711	DQ301649	DQ301580
AH-6	AG-F	<i>Arachis hypogea</i>	Japan	<i>Ceratobasidium</i> sp., CAG 4	DQ279014	-	DQ301700	-	-
AH-9	AG-G	<i>Arachis hypogea</i>	Japan	<i>Ceratobasidium</i> sp.	AB196646	KP171652	DQ301701	DQ301636	DQ301570
C-158	AG-P	<i>Camillia sinensis</i>	Japan	<i>Ceratobasidium</i> sp.	DQ279015	-	DQ301705	DQ301640	DQ301573
C-620	AG-Q	<i>Cynodon dactylon</i>	Japan	<i>Ceratobasidium</i> sp.	AF354095	AF354095	KP171657	DQ301641	DQ301574
CBS 136.82	AG-R	<i>Taxus</i> sp.	USA	<i>C. cornigerum</i> , CAG 5	DQ278933	KP171653	DQ301710	DQ301648	DQ301579
CBS 139.82	AG-S	<i>Pittosporum</i> sp.	USA	<i>C. cornigerum</i> , CAG 7	DQ278936	-	DQ301713	DQ301650	DQ301581
CBS 135.82	AG-U	<i>Juniperus</i> sp.	USA	<i>C. cornigerum</i> , <i>C. raminicola</i> , CAG 3	DQ278932	-	DQ301709	DQ301647	DQ301578
CBS 476.82	ND	<i>Glycine max</i>	USA	<i>C. cornigerum</i> , CAG 4	DQ278941	KP171654	DQ301720	DQ301643	-
CBS 293.31	ND	<i>Gossypium</i> sp.	Turkey	<i>Thanatephorus</i> sp.	DQ278959	-	DQ301729	DQ301683	DQ301619
CBS 148.54	ND	Unknown	France	<i>C. cornigerum</i>	DQ278937	KP171656	DQ301714	DQ301651	-
CBS 570.83	ND	<i>Sarcophilus dilatatus</i>	Australia	<i>C. cornigerum</i> , <i>C. papillatum</i>	AJ427401	-	DQ301724	-	-
CBS 154.35	ND	<i>Coffea</i> sp.	India	<i>Ceratobasidium noxium</i> , <i>Corticium koleroga</i>	DQ278938	-	DQ301715	DQ301653	DQ301584

CBS 571.83	ND	<i>Pomatocalpa macphersonii</i>	Australia	<i>Ceratobasidium sphaerosporum</i>	DQ278943	-	DQ301725	DQ301658	DQ301594
DAOM 138188	ND	<i>Pinus banksiana</i>	Canada, Saskatchewan	<i>Rhizoctonia endophytica</i> , <i>Ceratobasidium</i> sp., <i>C. cornigerum</i>	KP171640	KP171655	KP171658	-	-
AFTOL ID 611	ND			<i>Thanatephorus</i> (synonym <i>Uthatabasidium</i>) <i>fusisporum</i>	DQ398957	AF518664	DQ381842	-	-
AFTOL ID 610 DAOM 17641	ND	<i>Populus</i> sp.	Canada, Ontario	<i>Tulasnella pruinosa</i>	DQ457642	-	DQ381839	DQ061274	-
GEL2348	ND		Germany	<i>Botryobasidium simile</i>	KP171641	DQ898730	DQ898770	-	-
AFTOL ID 667	ND			<i>Clavulina</i> sp.	DQ202266	AY745694	DQ366286	DQ028589	DQ120947
AFTOL ID 607	ND			<i>Cantharellus cibarius</i>	DQ200926	-	DQ366285	DQ059050	-
AFTOL ID 471	ND			<i>Hydnum albomagnum</i>	DQ218305	AY700199	DQ234553	DQ234568	-

3 ND = not determined

4 - = no sequence data

5 Strain Rhs1AP denoted by the asterisk represents a strain used in this study for which the genome sequence is available (Cubeta et al.

6 2014)

Table 2. Primers sequence and PCR conditions applied for each of the genes employed to analyze relationships among *Rhizoctonia* fungi. An asterisk (*) indicates 35 cycles for PCR. All other PCRs were 30 cycles.

Gene	Primer name	Reference	Sequence (5'-3')	Cycle conditions
				annealing/ extension
<i>rpb2</i>	bRPB2-6F	Matheny 2005	TGGGGYATGGNTTGYCCYGC	60 °C, 1 min
	bRPB2-7.1R		CCCATRGCYTGyTTMCCCAT	72 °C, 2 min
	RPB2-980F	Reeb et al. 2004	TGYCCIGCIGARACICCHGARGG	52 °C, 1 min
	fRPB2-7cR		CCCATRGCCTTGyTTRCCCAT	72 °C, 2 min
<i>tef1</i>	TEF1-F	Litvintseva et al. 2006	AATCGTCAAGGAGACCAACG	60 °C, 1 min
	TEF1-R		CGTCACCAGACTTGACGAAC	72 °C, 2 min
<i>atp6</i>	ATP61	Kretzer and Bruns 1999	ATTAATTSWCCWTTAGAWCAATT	touch down: * 37 °C, 55 sec 72 °C, 1 min then 45 °C, 55 sec 72 °C, 1 min (+4 sec/cycle)
	ATP62		TAATTCTANWGCATCTTTAATRT A	
ITS	ITS4	White et al. 1990	TCCTCCGCTTATTGATATGC	55 °C, 1 min
	ITS5		GGAAGTAAAAGTCGTAACAAGG	72 °C, 2 min
LSU	LROR	Vilgalys and Hester 1990	GTACCCGCTGAACTTAAGC	51 °C, 45 sec
	LR5		ATCCTGAGGGAACTTC	72 °C, 2.5 min

Table 3 - Sequence data, their properties, and estimated models of sequence evolution for each locus and combined (Figs 2 and Supplementary S1)

Locus	No. of isolates in matrix	Length alignment	MP analyses				ML analyses	
			Informative characters	No. of trees	Length	Indices	Best-fit model	–ln scores
ITS-LSU	50	1462	418	2	1567	CI=0.459 RI=0.579 RC=0.266	TIM2+G	10466.4260
<i>rpb2</i>	49	674	367	18	2016	CI=0.330 RI=0.563 RC=0.186	TVM+G	8862.3926
<i>tef1</i>	45	514	372	71	1371	CI=0.468 RI=0.682 RC=0.319	TrN+G	6427.2248
<i>atp6</i>	40	668	182	48	630	CI=0.448 RI=0.763 RC=0.341	TPM3uf+G	4515.7805
Combined	50	3318	1339	2	6079	CI=0.380 RI=0.575 RC=0.218	TIM3+I+G	33315.3131

Fig 1 – Phylogenetic hypothesis of the Cantharellales based on nucleotide sequences of *rpb2*. The tree is a Bayesian 50% majority-rule consensus tree and produced from a data set of 165 sequences and 487 nucleotide characters. Only support values for main clades and the thanatephoroid clade within the Ceratobasidiaceae are shown. Posterior probabilities are indicated to the left, and ML bootstrap values to the right. Taxa marked in bold were those used in multi-locus analyses.

Fig 2 - Hypotheses of inferred relationships of *Rhizoctonia* fungi obtained with: i) Maximum Parsimony (MP), ii) Maximum Likelihood (ML) and iii) Bayesian Phylogenetic Inference (BPI) for concatenated data set of four genomic regions (ITS-LSU, *rpb2*, *tef1* and *atp6*). Taxa shaded in grey are the members of the family Ceratobasidiaceae. Nodes that collapsed in the strict consensus tree are marked with an asterisk. Scale bar for ML and BPI represents nucleotide substitution per site, and for MP the number of steps. Values for support above 80% for two of three metrics (MP bootstrap, ML bootstrap or PP) are shown as thick branches.

Fig 3 - Phylogenetic relationships of *Rhizoctonia* fungi based on parsimony analysis of the concatenated data set of four genomic regions (ITS-LSU, *rpb2*, *tef1* and *atp6*). Values for support above 80% for at least two metrics (MP bootstrap/ML bootstrap/PP) are reported along the branches. Roman numerals (I-X) indicate supported clades (>80%) with all three metrics. Circles below branches indicate concordance factors above 0.5.

Fig 4 - ML phylogeny of the Cantharellales showing genomic support. Left tree shows internode certainty (IC and ICA values respectively). Right tree shows the number of

genomic regions (ITS-LSU, *rpb2*, *tef1*, *atp6*) that support a specific clade. Numbers at the left are concordant genes, at right those that are in conflict.

Figure(s)

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