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Identification, molecular characterization, and evolution of group I introns at the expansion segment D11 of 28S rDNA in *Rhizoctonia* species

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ARTICLE INFO

Article history:
Received 22 November 2012
Received in revised form
3 June 2013
Accepted 19 June 2013
Available online 28 June 2013
Corresponding Editor:
Joseph W. Spatafora

Keywords:
Anamorph
LSU rDNA
Phytopathogenic fungi
RNA secondary structure
Thanatephorus

ABSTRACT

The nuclear ribosomal DNA of *Rhizoctonia* species is polymorphic in terms of the nucleotide composition and length. Insertions of 349–410 nucleotides in length with characteristics of group I introns were detected at a single insertion point at the expansion segment D11 of 28S rDNA in 12 out of 64 isolates. Eleven corresponded to *Rhizoctonia solani* (teleomorph: *Thanatephorous*) and one (AG-Q) to *Rhizoctonia* spp. (teleomorph: *Ceratobasidium*). Sequence data showed that all but AG-Q contained conserved DNA catalytic core regions (P, Q, R, and S) essential for selfsplicing. The predicted secondary structure revealed that base-paired helices corresponded to subgroup IC1. Isolates from same anastomosis group and even subgroups within *R. solani* were variable with regard to possession of introns. Phylogenetic analyses indicated that introns were vertically transmitted. Unfortunately, sequence data from the conserved region from all 64 isolates were not useful for delimiting species. Analyses with IC1 introns at same insertion point, of both *Ascomycota* and *Basidiomycota* indicated the possibility of horizontal transfer at this site. The present study uncovered new questions on evolutionary pattern of change of these introns within *Rhizoctonia* species.

Introduction

Anamorphic fungi in the genus *Rhizoctonia* make up a complex taxonomic group. Members of this genus have been found to be important pathogens associated with roots of plants and soil. The most widely studied species is *Rhizoctonia solani* (teleomorph: *Thanatephorous*), which affects many agricultural and horticultural crops and is composed of genetically isolated groups distributed worldwide (Ogoshi 1987). Identification and classification of this group are based on hyphal anastomosis, a method developed by Matsumoto *et al.* more than 90 y ago. This process implies that isolates having the ability to fuse are genetically related. To date, 14 anastomosis groups (AGs) and several intraspecific groups have been recognized within *R. solani* on the basis of cultural morphology, host range, and biochemical or molecular characteristics (e.g. Ogoshi 1987; Carling 1996; Carling *et al.* 2002). However, phylogenetic analyses with sequence data of either or both the ITS region and the 5′ end of the 28S rDNA in *Rhizoctonia* species have demonstrated that some AGs are not monophyletic (González *et al.* 2001, 2006). Nuclear rDNA has been useful for phylogenetic analyses at several taxonomic ranks because it presents different mutation rates along the molecule (e.g. Hillis & Dixon 1991; Hibbett 1992; Gillespie *et al.* 2005b). The ITS region is composed by the

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1878-6146/$ – see front matter
http://dx.doi.org/10.1016/j.funbio.2013.06.006

conserved 5.8S rRNA-encoding gene and two variable regions, ITS1 and ITS2. The large and small ribosomal subunits (28S and 18S) are composed of conserved regions and expansion segments (ES) or divergent domains (DD). The conserved regions are maintained across all domains of life while the ES can vary greatly, even across recently diverged lineages (e.g. Hassouna *et al.* 1984; Michot & Bachellerie 1987; Hancock & Dover 1988; Kuzoff *et al.* 1998; Hopple & Vilgalys 1999; Gillespie *et al.* 2005a, b). Several studies of nuclear rDNA have revealed that variation within the ES is often due to the presence of group I introns (e.g. Takizawa *et al.* 2011).

Group I introns have been found in genes encoding mRNA, tRNA or rRNA in nuclear and chloroplast genome of plants and green algae, in nuclear and mitochondria genome of fungi and opisthokonts, in nuclear genomes of several protist and algae, and in genomes of bacteria and bacteriophages (http://www.rna.icmb.utexas.edu/). The manner in which group I introns spread in DNA has been attributed to mechanisms of homing and reverse splicing (Bhattacharya *et al.* 2005; Haugen *et al.* 2005), but their distribution is irregular. Introns may be present in some specimens and absent from others (Dujon 1989; Mavridou *et al.* 2000; Creer 2007). The transfer pathways recognize assigned sequences even when introns are transferred beyond the species level. Consequently, introns at homologous gene sites between different host organisms are almost always more phylogenetically related than those at heterologous sites within an organism (e.g. Nikoh & Fukatsu 2001; Del Campo *et al.* 2009; Hoshina & Imamura 2009).

In eukaryotic nuclear genome, group I introns are found exclusively in the rDNA. Within this molecule, species have displayed variability useful for reconstructing phylogenies attributed in part to the presence of this type of introns in the 28S and 18S rRNA genes (Nikoh & Fukatsu 2001; Wang *et al.* 2003; Garrido-Jurado *et al.* 2011). Group I introns within the rDNA are highly diverse in their lengths and primary sequences, but are defined as a group by their common core secondary structure and their common mechanism of selfsplicing (Doudna & Cech 2002). Secondary structure is composed by the presence of conserved sequence elements, termed P, Q, R, and S and a series of base-paired helices numbered P1 through P9 organized into three domains (P1–P2, P4–P6, and P3–P9). In the first step of selfsplicing, exogenous guanosine or GTP binds in the G-site (at P7) and cleaves the 5′ splice site at P1. The 3′-terminal guanosine of the intron then occupies the G-site for the second step of splicing (Cech & Golden 1999). Paired helices form the framework of the secondary structure, which is used for the inference and analysis of all putative intron secondary structures (Lilley & Eckstein 2008). Group I introns are classified into at least ten subgroups (IA1, IA2, IA3, IB1, IB2, IB3, IB4, IC1, IC2, and IC3) within five main groups (IA, IB, IC, ID, and IE) based on distinct primary sequence motifs, genomic location, and characteristic structural features in peripheral regions (Einvik *et al.* 1998; Li & Zhang 2005; Mitra *et al.* 2011). The database of RNA introns (http://www.rna.icmb.utexas.edu/) has documented more than 100 group I introns at over 15 unique sites in the 28S rRNA genes since the year 2002. However, the precise statistics are unknown since new rDNA sequences are continually deposited in GenBank (Cannone *et al.* 2002; Jackson *et al.* 2002; Bhattacharya *et al.* 2005; Hoshina & Imamura 2009; Garrido-Jurado *et al.* 2011). At the 3′ region of the fungal 28S rRNA genes, there are usually four recognition sites at which group I introns may be inserted corresponding to positions 1921, 2066, 2449, and 2563, with reference to homologous position in *Escherichia coli* (Ec) 23S rRNA gene (Jackson *et al.* 2009). In any given strain, all, some or none of these sites may contain intron. When they are present, it has been observed that they belong to subgroups IC1 and IE; polymorphism may also be present in the P helices of introns of the same subgroup within the same species (Jackson *et al.* 2009; Takizawa *et al.* 2011).

This study was undertaken to investigate firstly, the variability existing in an ES located at position Ec2449 in the 3′ region of the 28S rRNA genes from *Rhizoctonia* species with characteristics of group I intron and secondly, to assess its contribution to phylogenetic analyses and its evolutionary implications with other introns inserted at same position. To address the first issue comparative sequence analysis was used to develop models of RNA secondary structure. To examine the second issue, phylogenetic analyses using parsimony, maximum likelihood (ML), and Bayesian posterior probabilities (BPPs) were performed to contrast the hypotheses of evolutionary relationships.

Materials and methods

DNA amplification and sequencing

DNA from a previous study (González *et al.* 2001) was used for amplifying the 3′-terminal region of 28S rDNA from 47 isolates of *Rhizoctonia solani* and 17 isolates of *Rhizoctonia* spp. (teleomorph: *Ceratobasidium*, Table 1). Primers used for amplification were LR11R (5′-GAAAAGTTACCACAGGGATAACTG-3′, reverse and modified from primer LR11; http://www.biology.duke.edu/fungi/mycolab/primers), and one newly designed called 28SRhizo (5′-CATTCAAGTCGTCTGCAAAGGATTCA-3′). Reactions were performed in a 25 μl mixture containing 50 mM KCl, 20 mM Tris–HCl (pH 8.4), 3.0 mM $MgCl_2$, 200 μM of each of the four deoxynucleoside triphosphates, 0.2 μM of each primer, and 2.5 units of *Taq* polymerase-recombinant (Invitrogen, Carlsbad, CA, USA). The amplifications were performed on a thermocycler (Mastercycler, Eppendorf, Hamburg, Germany). The amplification program included an initial denaturation at 94 °C for 5 min, followed by 35 cycles with denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 2 min, and a final extension for 7 min at 72 °C. Amplified DNA was purified prior sequencing with the Wizard SV gel and PCR clean-up system kit as described by manufacturers (Promega, Madison, WI, USA). Amplified DNA was sequenced using ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer instructions. Cycle sequence products were cleaned with an isopropanol precipitation and electrophoresed using an ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Sequence analysis

Alignment of resulting sequences was performed using ClustalW (Thompson *et al.* 1994) included in the BioEdit software version 7.0.9.0 (Hall 1999) with default parameters for a gap open and gap extended penalties. To test whether base

Table 1 – AGs and subgroups of *Rhizoctonia* spp., used in this study along with GenBank accession numbers of the 3′-terminal region of 28S rDNA.

AG–subgroup	Isolate	Substrate, Origin[1/(Source)]	3′-Terminal region of 28S rDNA GenBank accession number
***Rhizoctonia solani* (teleomorph = *Thanatephorus*)**			
AG-1-IA	2Rs	Rice, US[(13)]	JX988977[a]
AG-1-IA	48Rs	Rice, US	JX988978
AG-1-IA	54Rs	Soybean, US	JX988979
AG-1-IB	P-18	Dry beans, Panama[(7)]	JX988980
AG-1-IB	SFBV-1	Sugar beet, Japan[(8)]	JX988981
AG-1-IC	91087	Australia[(10)]	JX988982
AG-1-IC	M34	*Pinus resinosa*, Quebec[(1)]	JX988983
AG-2-1	95Rs	Alaska[(5)]	JX988984
AG-2-1	96Rs	Alaska[(5)]	JX988985
AG-2-1	100Rs	Alaska[(5)]	JX988986
AG-2-2	9Rs	Carrot, US[(1)]	JX988987
AG-2-2 IIIB	15Rs	Mat rush, Japan[(12)]	JX988989
AG-2-2 IV	16Rs	Sugar beet, Japan[(12)]	JX988988[a]
AG-2	27Rs	*Phaseolus vulgaris*, US	JX988990
AG-3	4Rs	Potato, US[(14)]	JX988991[a]
AG-3	5Rs	Potato, US[(1)]	JX988992[a]
AG-3	42Rs	Potato, US[(2)]	JX988993[a]
AG-4 HGI	AH-1	Peanut, Japan[(8)]	JX988994
AG-4 HGII	7Rs	Alfalfa, US[(1)]	JX988995
AG-4 HGII	18Rs	Sugar beet, Japan[(12)]	JX988996
AG-4 HGII	30Rs	Unknown, Canada[(4)]	JX988999
AG-4 HGIII	6Rs	Conifer, US[(4)]	JX988997
AG-4	25Rs	Unknown	JX988998
AG-5	19Rs	Soybean Japan[(12)]	JX989000
AG-5	31Rs	Sugar beet, Japan[(11)]	JX989001
AG-6 HGI	70Rs	Soil, Japan[(12)]	JX989002
AG-6 HGI	AT2-1	Soil, Japan[(8)]	JX989003
AG-6 HGI	UBU-1-A	Soil, Japan[(8)]	JX989004[a]
AG-6 GV	HN1-1	Soil, Japan[(8)]	JX989005[a]
AG-6 GV	75Rs	Soil, Japan[(12)]	JX989006
AG-7	91ST8057-2A-RSA	Soil, US[(15)]	JX989007
AG-7	1535	Soil, Japan[(8)]	JX989008
AG-7	63Rs	Soil, Japan[(5)]	JX989009
AG-8	(ZG1-1)91784	*Lupinus angustifolius*, Australia[(8)]	JX989010[a]
AG-8	(ZG1-4)88351	*Hordeum vulgare*, Australia[(10)]	JX989011
AG-8	(ZG1-2)SA50	Oats, Australia[(6)]	JX989012
AG-9	111Rs	Potato, US[(5)]	JX989013
AG-9	114Rs	Alaska[(5)]	JX989014
AG-9	116Rs	Potato, US[(5)]	JX989015
AG-9	117Rs	Alaska[(5)]	JX989016
AG-9	119Rs	Alaska[(5)]	JX989017
AG-10	W45b3	Soil, US[(12)]	JX989018[a]
AG-10	(ZG9)91614	Barley, Australia[(10)]	JX989019[a]
AG-11	Roth16	Soybean, US[(15)]	JX989020[a]
AG-11	(ZG-3)R1013	*L. angustifolius*, Australia[(16)]	JX989021
AG-BI	80Rs	Japan[(12)]	JX989022
AG-BI	66Rs	Japan[(12)]	JX989023
Binucleate *Rhizoctonia* spp. (teleomorph = *Ceratobasidium*)			
AG-Bo	SIR-2	Sweetpotato, Japan[(12)]	JX989024
AG-D	C-610	Unknown, Japan[(12)]	JX989025
AG-F	SIR-1	Sweetpotato, Japan[(12)]	JX989026
AG-H	STC-9	Soil, Japan[(12)]	JX989027
AG-L	FK02-1	Soil, Japan[(12)]	JX989028
AG-Q	C-620	Soil, Japan[(12)]	JX989029[a]
CAG-1	89Rs	Turfgrass, US[(9)]	JX989030
CAG-1	BN244	Soil, US[(9)]	JX989031
CAG-1	91Rs	Soil, US[(9)]	JX989032
CAG-3	39Rs	*Arachis* sp., US[(3)]	JX989033
CAG-3	BN31	Peanut, US[(3)]	JX989034
CAG-4	BN38	Soybean, US[(3)]	JX989035

(*continued on next page*)

Table 1 – (continued)

AG–subgroup	Isolate	Substrate, Origin[1/(Source)]	3′-Terminal region of 28S rDNA GenBank accession number
CAG-4	41Rs	*Glycine* sp., US	JX989036
CAG-4		Unknown	JX989037
CAG-5	38Rs	*Cucumis* sp., US[(3)]	JX989038
CAG-5	BN37	Cucumber, US[(3)]	JX989039
CAG-6	BN74	*Erigeron* sp., US[(3)]	JX989040

[1/]Isolates provided by; 1 = N. Anderson; 2 = K. Barker; 3 = L. Burpee; 4 = E. Butler; 5 = D. Carling; 6 = A. Dube; 7 = Godoy-Lutz; 8 = S. Kuninaga; 9 = L.T. Lucas; 10 = G. MacNish; 11 = S. Naito; 12 = A. Ogoshi; 13 = N. O'Neill; 14 = G. Papavizas; 15 = C. Rothrock, and 16 = M. Sweetingham.
a Isolates with an insertion corresponding to a putative group IC1 intron.

composition was biased within each ES (Hancock & Dover 1988) a Chi-square test of homogeneity of base frequencies across taxa was conducted using the program PAUP* version 4.0b8 (Swofford 2001). The BioEdit software was also used to construct an entropy plot to have an idea of the information content or how well the nucleotide for a new sequence could be predicted at each position in the alignment. The entropy at a column position is independent of the total information possible at a given position, and depends only upon the frequencies of characters that appear in that column. BioEdit uses Shannon's entropy for assessing levels of entropy at each position in the alignment (measured in nits). Sequences with ES were inspected visually to detect distinctive features of group I introns such as conserved DNA catalytic core regions (P, Q, R, and S), and stem-loop constructs P1–P9 (e.g. Cech 1988; Zhou *et al.* 2007) and compared with similar sequences from fungi (e.g. Mavridou *et al.* 2000; Márquez *et al.* 2006). The Basic Local Alignment Search Tool (BLAST) was used to determine the maximum identity between ES sequences from this study and the GenBank sequence database (Altschul *et al.* 1990).

Secondary structure prediction of group I introns from Rhizoctonia *species*

The secondary structure of each unique ES was obtained in the Vienna RNA webservers with the new RNAalifold version with better gap character handling (Bernhart *et al.* 2008, http://rna.tbi.univie.ac.at/cgi-bin/RNAalifold.cgi). Fold algorithms and basic functions were as follows: minimum free energy (MFE) only; no GU pairs at the end of the helices and avoiding isolate base pairs. The MFE structures were saved as connect format (ct) with the purpose of having the nucleic acid sequence together with its secondary structure (Markham & Zuker 2008). Ct files were used to draw the models of secondary structure for each unique ES with the program RNAViz 2.0 (De Rijk *et al.* 2003). Manual adjustments were done at catalytic core P and Q to obey the presentation of a group I intron structure.

Phylogenetic analyses

Phylogenetic analyses with and without group I introns were performed using maximum parsimony (MP; WINCLADA-ASADO (WinClada extended functionality) [Goloboff 1994; Nixon 1999; Goloboff *et al.* 2000]), ML (GARLI v. 0.951 [Zwickl 2006]), and BPP (message passing interface (MPI)-enabled version of MrBayes v. 3.1.2 [Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; Altekar *et al.* 2004]). MP analyses were conducted with the NONA ratchet algorithm with 500 iterations and holding 50 trees per iteration. The program jModelTest v. 0.1.1 (Guindon & Gascuel 2003; Felsenstein 2005; Posada 2008) was used for finding the optimal model of DNA substitution for ML and BPP analyses. The Akaike information criterion was used to evaluate the fit of competing models. ML analyses were performed with model parameters fixed according to the values obtained with jModelTest in order to reduce total runtimes. Searches consisted of ten replicates to ensure that results were consistent. Branch support for ML was determined simultaneously by doing 100 nonparametric bootstrap iterations in each of the ten replicates. Each BPP analysis comprised two independent five-milliongeneration 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. We sampled trees every 100th generation and discarded initial samples applying a 'burnin' value of 12 500 generations before calculating the majority consensus tree and posterior probabilities for clades. All analyses were performed with characters weighted equally and gaps treated as missing data. Three data matrices were constructed for analyses. Matrix 'A' included 64 *Rhizoctonia* isolates plus *Candida valdiviana* and *Arxula terrestris* as outgroup taxa. Last two species had maximum identity with *Rhizoctonia* isolates after the BLAST search for each ES. Matrix 'B' included only *Rhizoctonia* isolates and matrix 'C' only *Rhizoctonia* isolates but without intron's sequences. In last two matrices AG-Q (teleomorph: *Ceratobasidium*), was used to polarize the tree. Branch support for MP analysis was determined with jackknife values. One thousand replicates were performed with ten searches per replicate (MULT*N) and holding ten trees per search. BPP values were also included as measures of level of support. The phylogenetic trees were visualized with TreeGraph ver.2.0.4 (Stöver & Müller 2010). Pattern of intron evolution was explored using the character-state optimization function in Mesquite version 2.75 (Maddison & Maddison 2011). Alignments and resulting trees are deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S13653).

Results and discussion

LSU insertions and sequence analysis

PCR amplification products from the 3′-terminal region of 28S rDNA from *Rhizoctonia* species revealed an insertion in 12 out of 64 isolates. Eleven isolates of *Rhizoctonia solani* (teleomorph: *Thanatephorous*) and one from *Rhizoctonia* spp. (teleomorph:

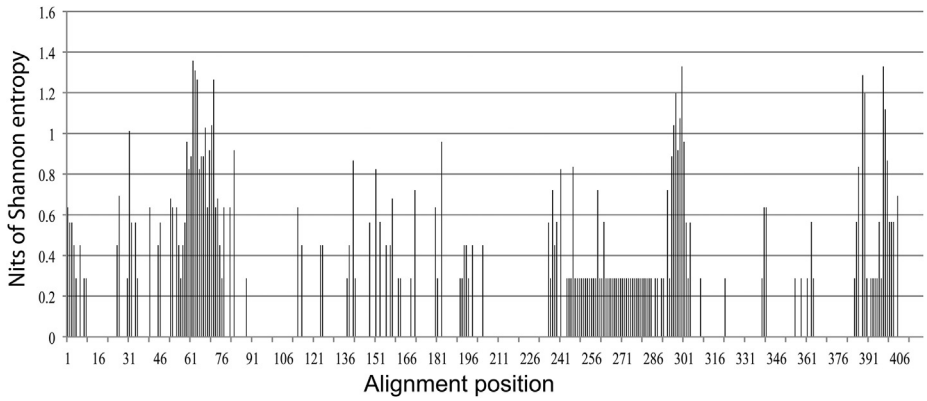
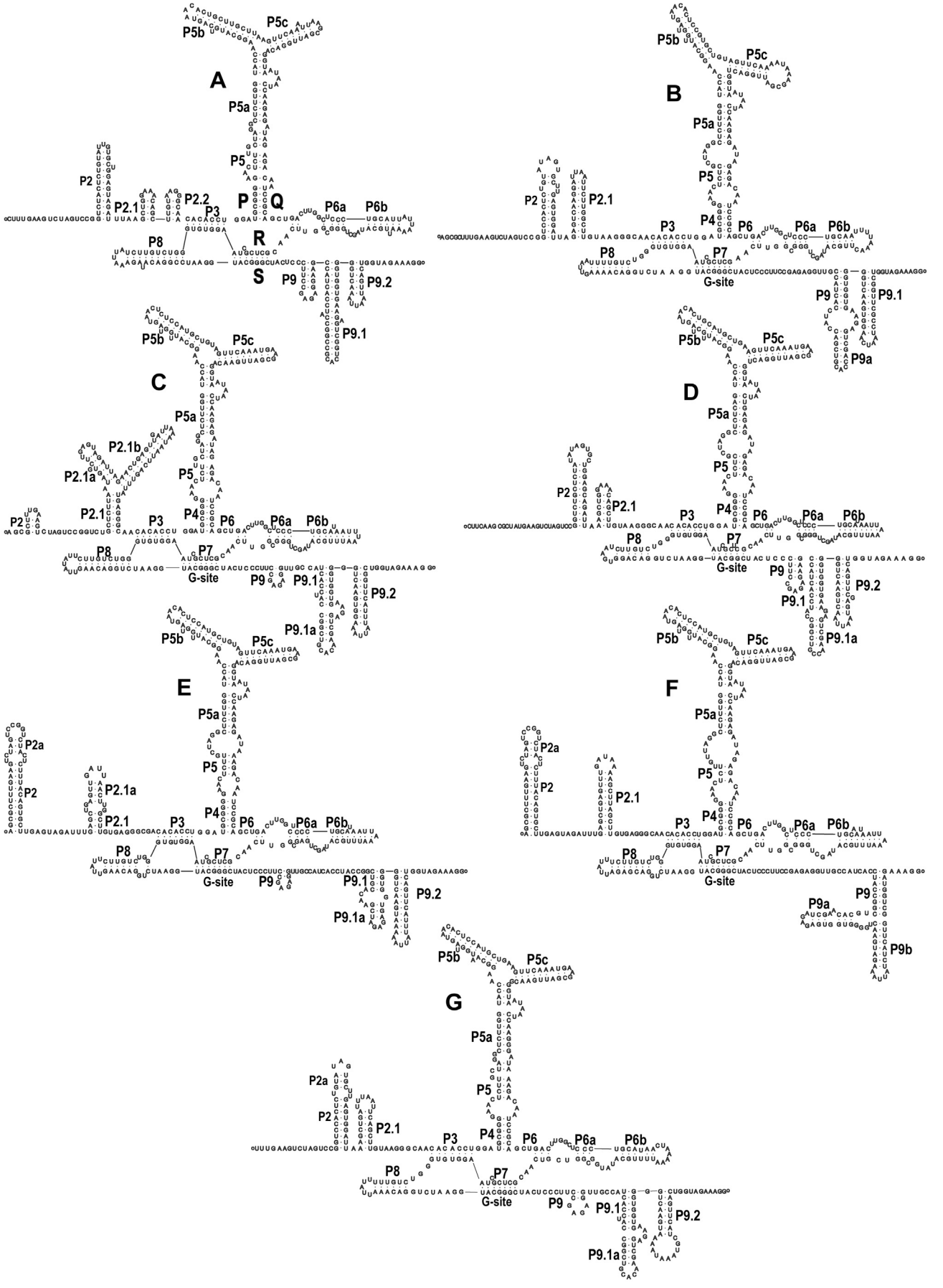


Fig 1 – Entropy plot of variable and conserved nucleotide positions estimated along the alignment of the ES D11 of 28S rDNA from 12 isolates of *Rhizoctonia* species.

Ceratobasidium) had the insertion (Table 1). Two PCR amplification products were obtained from isolates AG-1-IA(2Rs) and AG-Q, corresponding apparently to presence and absence of the insertion respectively, implying that the sequence of the LSU rDNA was from different genotypes/rDNA copies. These isolates showed low amplification stability. Two classes of alleles, with differences in the frequency of amplified product were obtained in repeated amplifications (data not shown). This result indicates that gene conversion has homogenized most, but not all rDNA repeats to either possess or lack

Table 2 – Sequence comparison of intron structural core regions P, Q, R, and S from *Rhizoctonia solani* and sequences with maximum score identity after a BLAST search.

Species with highest identity in BLAST search	Catalytic core elements intron subgroup IC1			
	P	Q	R	S
Candida valdiviana (DQ438220.1), *Candida petrohuensis* (DQ442703.1), *Candida castrensis* (DQ438195.1), *Candida tartarivorans* (DQ438226.1), *Candida santjacobensis* (DQ442701.1), *Arxula terrestris* (DQ442683.1), *Lipomyces kockii* (DQ518976.1), *Lipomyces doorenjongii* (DQ518974.1), *Lipomyces starkeyi* (DQ518981.1), *Lipomyces mesembrius* (DQ518979.1), *Myxozyma melibiosi* (DQ518988.1), *Myxozyma monticola* (DQ518989.1), *Myxozyma neotropica* (DQ518992.1), *Beauveria bassiana* (EU334678.1), *Cordyceps bassiana* (EF115313.1), *Cordyceps kanzashiana* (AB044639.1), *Cordyceps prolifica* (AB044640.1), *Blastobotrys* sp. (DQ442698.1), *Arxula adeninivorans* (DQ442697.1; Z50840.2), *Beauveria bassiana* (AF430701.1; AF293966.1; AF391117.1; AF322938.1; AF322937.1; AF391118.1; AF430700.1) *Rhizoctonia solani* AG-1-IA(2Rs), AG-2-2 IV(16Rs), AG-3(4RS), AG-3(5RS), AG-3(42RS), AG-6 HG1(UBU-1-A), AG-8([ZG1-1]91784), AG-10(W45b3), AG-10([ZG9]91614), AG-11(Roth16)	TGCGGG	TCCGCA	GCTCGCTA	TACGGGC
Rhizoctonia solani AG-6 GV(HN1-1)	TGCGGG	TCCGCA	GCTCGCTA	TACGG–C
Beauveria bassiana (AF363481.1)	TGCGGG	TCCGCA	GCTCGCTA	TTACG––
Trigonopsis sp. (DQ442706.1), *Candida cantarellii* (DQ442705.1)	TGCGGG	CCCGCA	GCTCGCCA	TACGGGC
Trigonopsis variabilis (DQ442707.1), *Candida vinaria* (DQ442708.1), *Lipomyces tetrasporus* (DQ518982.1)	TGCGGG	TCCGCA	GCTCGGAA	TACGGGC
Penicillium chrysogenum (AM920431.1); *Cordyceps* sp. (AB044641.1)	TGCGGG	TCCGCA	GCTCGGTA	TACGGGC
Gaeumannomyces graminis var. *tritici* (U17160.1), *Gaeumannomyces graminis* var. *avenae* (U17161.1)	TGCTGG	TCAGCA	GCTCGCTA	TACGGGC
Metarhizium anisopliae var. *anisopliae* (AF197124.1)	TGCGGG	TCCG–A	GCTCGCTA	TACG–––
Lipomyces spencermartinisiae (DQ518980.1)	TGCGGG	TCCGCA	GCTCGGTA	TACGGGC
	Catalytic core elements intron group IE			
	P	**Q**	**R**	**S**
Cordyceps prolifica (AB044640.1), *Cordyceps kanzashiana* (AB044639.1)	TCGAGG	TCTCGA	GAGCGCG	CGTGCC
Cordyceps sp. (AB044641.1)	TCGGGG	TCCCGA	GAGCGCG	CGTGCC


A
B
C
D
E
F
G
P5b
P5c
P5a
P5
P2
P2.1
P2.2
P3
P
Q
R
S
P6a
P6b
P8
P9
P9.1
P9.2
P2.1b
P2.1a
P4
P6
P7
G-site
P2a
P9.1a
P9a
P9b

introns. If this proves to be true, it opens the possibility that in *Rhizoctonia* isolates previously thought to lack introns they might actually be present in such small number of copies, but they go undetected by the PCR assay, or that the variable presence of the insertion could produce anomalous amplicons (e.g. Shinohara *et al.* 1996; Holst-Jensen *et al.* 1999). *Rhizoctonia* species rarely produce sexual structures. Therefore, it is difficult to establish single spore isolates in this group of fungi, for discarding different genotypes. However, it will be required in the future, cloning the rDNA for testing different rDNA copies. For the remaining isolates, a single PCR amplification product was obtained of about 477 nucleotides.

Detailed inspection of sequences revealed that insertion starts at positions: 2449 of *Escherichia coli* 23S (Ec2449, GenBank J01695); 2815 of *Saccharomyces cerevisiae* 25S ribosomal RNA gene (Georgiev *et al.* 1981; or position 2928 in *S. cerevisiae*, GenBank J01355.1), and corresponds to the ES D11 in *S. cerevisiae* (Hassouna *et al.* 1984; Neuvéglise *et al.* 1997). Insertion site was located inside the primer LR11R used for amplification (5′-GAAAAGTTACCACAGGGATAACTG-3′) at position 20 after the third thymine (underline). Primer position within *E. coli* 23S goes from position 2430 to 2453, and within *S. cerevisiae* goes from position 2796 to 2820 (or 2910–2933, GenBank J01355.1).

Sequence length and composition of the insertion varied among and within AGs. Sizes were between 349 (AG-Q) and 410 (AG-3 and AG-10) nucleotides approximately. After alignment there were 425 nucleotide positions for this insertion. Aligned data matrix for phylogenetic analyses consisted of 12 isolates with 902 nucleotides positions and 52 with 477. There were no nucleotide composition biases for the 12 sequences from the insertion. The Chi-square test of homogeneity of base frequencies across taxa with insertion indicated that there was no significant difference in the frequency of bases. Mean base frequencies were as follows: 0.260 for A, 0.204 for C, 0.272 for G, and 0.263 for T. Similar results were found for all 64 sequences after position 425. Not all isolates of a same AG had the insertion nor were all insertions, when present in several isolates from same AG, identical. For instance, six isolates from AG-1 were sequenced and only one had insertion. In contrast, all isolates sequenced from AG-3 and AG-10 had the insertion. Within these, the proportion of identical nucleotides in the insertion also varied. Identity value for the three isolates from AG-3 was 1.00 whereas identity for the two isolates from AG-10 was 0.97. These two isolates differed by a single transition (G/A). Proportion of identical nucleotides among insertions from all 12 isolates ranged from 0.62 between AG-Q and AG-6 HGI (UBU-1-A) to 1.00 among isolates from AG-3. Based on this new genetic information, an entropy plot for the ES of *Rhizoctonia* species was constructed. The entropy plot showed a clear distribution of both variable and conserved nucleotide positions along this region (Fig 1). Overall variation was more frequent at the two ends of sequences even after excluding incomplete data at the beginning of the sequences for some AGs.

BLAST analyses for all 12 ES sequences from *Rhizoctonia* species showed maximum sequence identity with introns from fungal species within phylum *Ascomycota*. Identity was between 72 and 74 % with *Candida valdiviana* (DQ438220.1) and/or *Arxula terrestris* (DQ442683.1). Only isolate AG-Q had maximum identity (79 %) with *Trigonopsis variabilis* (DQ442707.1). Some interesting structural features were noticed after a careful inspection of the alignment of this novel ES for *Rhizoctonia* species and sequences from GeneBank. All the insertions detected in *R. solani*'s isolates presented characteristics of selfsplicing group IC1, such as the catalytic core regions P (TGCGGG), Q (TCCGCA), R (GCTCGCTA), and S (TACGGGC). BLAST analyses revealed that 27 fungal species from an ample phylogenetic scale within *Ascomycota* (e.g. Mavridou *et al.* 2000; Wang *et al.* 2003; Márquez *et al.* 2006) had exactly the same catalytic core sequences than *R. solani*'s isolates (teleomorph: *Thanatephorous*) and only 12 differed by one or two mutations in any of them (Table 2). The isolate AG-Q (teleomorph: *Ceratobasidium*) had catalytic core regions P, Q, and S but region R was not evident. All introns were inserted at exactly the same position (Ec2449) than other introns such as intron 33-int3 from a *Metarhizium anisopliae* isolate from Madagascar (Mavridou *et al.* 2000), and intron Bb17 and Bb2 from *Beauveria bassiana* (Pantou *et al.* 2003; Wang *et al.* 2003). To my knowledge, the present study provides the first report of group I introns and intraspecific sequence polymorphisms in the ES D11 of 28S rDNA within *Rhizoctonia* species.

Secondary structure prediction of group I introns from Rhizoctonia *species*

The ES in the LSU rDNA of *Rhizoctonia* spp., were characterized as putative group I introns according to primary and secondary structural analyses, as follows: (a) the occurrence of group I introns in a positions similar as in other organisms (Mavridou *et al.* 2000; Pantou *et al.* 2003; Wang *et al.* 2003; Garrido-Jurado *et al.* 2011); (b) distinctive paired elements in secondary structure (Burke *et al.* 1987; Michel & Westhof 1990; Cech *et al.* 1994), with the exception of AG-Q that lacked paired elements P3 and P7, and (c) guanine as the last intron base (Pantou *et al.* 2003). Structural elements characteristic of group IC1 introns were the presence of P2.1, a large P5abc domain, and an A-rich bulge in the P5a. The large P5abc domain, functions as a scaffold to stabilize the final ribozyme structure that includes the P3–P9 stack of paired elements and P1–P2 substrate domain (Haugen *et al.* 2004). For instance, the loop at P5b is bound by a receptor sequence located between P6a and P6b in the other half of the molecule. It was not possible to identify clearly paired element P1, nor the last exon base (uracil), due to some missing data at the

Fig 2 – Predicted RNA secondary structures of group I introns found in isolates of *Rhizoctonia solani*. (A) AG-1-IA(2Rs), (B) AG-2-2 IV(16Rs), (C) AG-3(4Rs, 5Rs, and 42Rs), (D) AG-6 HGI(UBU-1-A) and AG-6 GV(HN1-1), (E) AG-8([ZG1-1]91784), (F) AG-10(W45b3 and [ZG9]91614), (G) AG-11(Roth16). Catalytic core elements (P, Q, R, S) are indicated on model A. Number of paired elements are specified except P4, P6, and P7 for model A. Paired element P1 is not indicated due to some missing data at the beginning of sequences.

beginning of sequences after the priming site. However, this last element might also be present since the insertion starts at position 20 of the primer LR11R used for sequencing (5′-GAAAAGTTACCACAGGGATAACTG-3′) after the third thymine ([underlined], uracil in RNA). Secondary structure models were accomplished with the complete *Tetrahymena thermophila* group I intron sequence skeleton within the software RNAViz (Fig 2). Manual adjustment was necessary in stem loop P6 due to one interaction present in all introns, except AG-2-2 IV and AG-6 HGI that involved the P element (underlined) and other sequence inside P6 (AUGCGGGGA-UCCCUG-CAU). This interaction had to be disrupted to obey the proper pairing of P and Q elements (UGCGGG-UCCGCA, Fig 2). A distinct feature for secondary structure models C and F (isolates AG-3 and Ag-6) is a bifurcated helix at P2 and P9 respectively, replacing the typical single helix present in IC1 introns. Three different nucleotide interactions were obtained at the beginning of P8, despite being the same nucleotides in all introns and folding parameters in RNAViz. The program generated an extra helix in models E and F, while in models A and C paired more nucleotides than in models B, D, and G (Fig 2). All introns show low variation at the nucleotide level with the exception of AG-Q. A difference between secondary structure model for AG-Q and the remaining ones is the absence of P3 and P7 structural elements (not shown). Although these segments are essential for selfsplicing in group IC1 introns it has been observed that some may lack one or more structural elements (Wilmotte *et al.* 1993; Zhou *et al.* 2007; Harris & Rogers 2011). This suggests that some portions of the intron are less structurally constrained and their absence does not affect its ability to selfsplice in order to produce a functional long subunit of rRNA (Myllys *et al.* 1999). Selfsplicing has been demonstrated in small introns lacking most of the supporting scaffolds (Grube *et al.* 1996; Harris & Rogers 2008). These introns have only retained P1, P10, as well as part of P9, which has a site that is similar to the P7 region that holds the initiating guanosine in larger group I introns. It has been suggested that part of the SSU rRNAs where they were found, may help to support these introns so that they can splice (Harris & Rogers 2008; Rogers 2012). However the unusual secondary structure for isolate AG-Q that apparently lacks the catalytic core vital for splicing makes necessary verify intron excision from mature rRNA. The fact that this isolate presented two PCR amplification products indicates that more than one rDNA repeat coexists in the same genome and that they are variable in their sequence and structure. Therefore, cloning the rDNA repeats is required for elucidating clearly how many and how different the repeats are.

Phylogenetic analysis

To assess the contribution of group I introns in the phylogeny of *Rhizoctonia* species parsimony, ML and Bayesian analyses were carried out. Matrix 'A' included 66 taxa and 1131 characters from which 209 were informative. MP analysis with this matrix generated 21 058 trees of 432 steps (Ci = 0.67; Ri = 0.76). Topology of consensus tree was mostly unresolved with only nine monophyletic groups: six formed by two isolates each, one with three, and two with five (Fig 3). Matrix 'B' included 64 taxa, 902 characters, and 102 informative. MP analysis with this matrix yielded 20 362 trees of 229 steps (Ci = 0.61; Ri = 0.78). Tree topology (not shown) was similar to the one generated with matrix 'A' but with less resolution. There were five monophyletic groups, two formed by two isolates each [AG-6 GV(75Rs) with CAG-5(38Rs); and AG-L with CAG-1(89Rs)], two with five [AG-2-2(9Rs), AG-2-2 IV(16Rs), AG-2-2 IIIB(15Rs), AG-2(27Rs), and AG-9(116Rs)] and [AG-BI(66Rs), CAG-1(RSBN244), CAG-1(91Rs), and AG-6 HGI(AT2-1) with AG-8([ZG1-2]SA50)]; and one with ten that included smaller clades as follows: AG-5(19Rs) with AG-5(31Rs); AG-10(W45b) with AG-10([ZG9]91614); AG-8([ZG1-1]91784) with AG-BI(80Rs), one with the three isolates of AG-3(4Rs, 5Rs, and 42Rs); and AG-11(Roth16). Matrix 'C' incorporated 64 taxa, 477 characters, and 45 informative. MP analyses produced 23 338 trees of 111 steps (Ci = 0.54; Ri = 0.81). Tree topology resolution improved with this analysis (Fig 3).

The best-fit substitution model for data matrix 'A' under Akaike and Bayesian information criterion was TPM2uf + G. Nucleotide frequencies and substitution rate values were as follows: 'Lset base = (0.258 0.185 0.253 0.303) nst = 6 rmat = (1.965 5.530 1.965 1.000 5.531 1.000) rates = gamma shape = 0.255 ncat = 4 pinvar = 0'. Model for matrix 'B' was GTR + G with nucleotide frequencies and substitution rate values as follows: 'Lset base = (0.242 0.199 0.269 0.290) nst = 6 rmat = (0.651 5.581 3.223 1.220 5.939 1.000) rates = gamma shape = 0.154 ncat = 4 pinvar = 0'. Model for matrix 'C' was SYM + I + G and nucleotide frequencies and substitution rate values as follows: 'Lset base = equal nst = 6 rmat = (0.619 3.002 1.500 1.138 4.662 1.000) rates = gamma shape = 0.519 ncat = 4 pinvar = 0.582'. ML analyses with these model parameters fixed resulted in the following loglikelihood (−ln) scores: Matrix 'A' produced a tree with a loglikelihood of −4640.562. Matrix 'B' a tree with a loglikelihood of −3003.604, and matrix 'C' a tree with loglikelihood of −1740.181. Topologies of ML analyses (not shown) contained all monophyletic groups obtained with MP analyses. Majority-rule consensus trees with posterior probability distribution obtained with MrBayes are presented in Fig 3. Posterior probabilities are above branches. Jackknife and bootstrap values are placed below the corresponding branches separated by a forward slash.

The variabilities in group I introns and conserved regions from LSU rDNA genes have been used as molecular tool for the identification of different fungi or as molecular characters to reconstruct phylogenetic relationships. However, the polymorphism observed in intron and conserved region within *Rhizoctonia* species, was not enough for delimiting species unambiguously. As shown in Fig 3, introns were generally not phylogenetically restricted to a particular lineage, but rather, sporadically distributed among distinct AGs. Among the 12 isolates that presented intron there were 102 phylogenetic informative characters, while the conserved region for the same taxa had 45 informative characters. Overall, consensus tree was more resolved when analysis was performed with sequences only from the conserved region than when the intron sequences were also incorporated (Fig 3). However, the few monophyletic groups resolved were in agreement with previous phylogenetic hypothesis for *Rhizoctonia* spp. (González *et al.* 2001, 2006). These analyses confirm that: *Rhizoctonia*

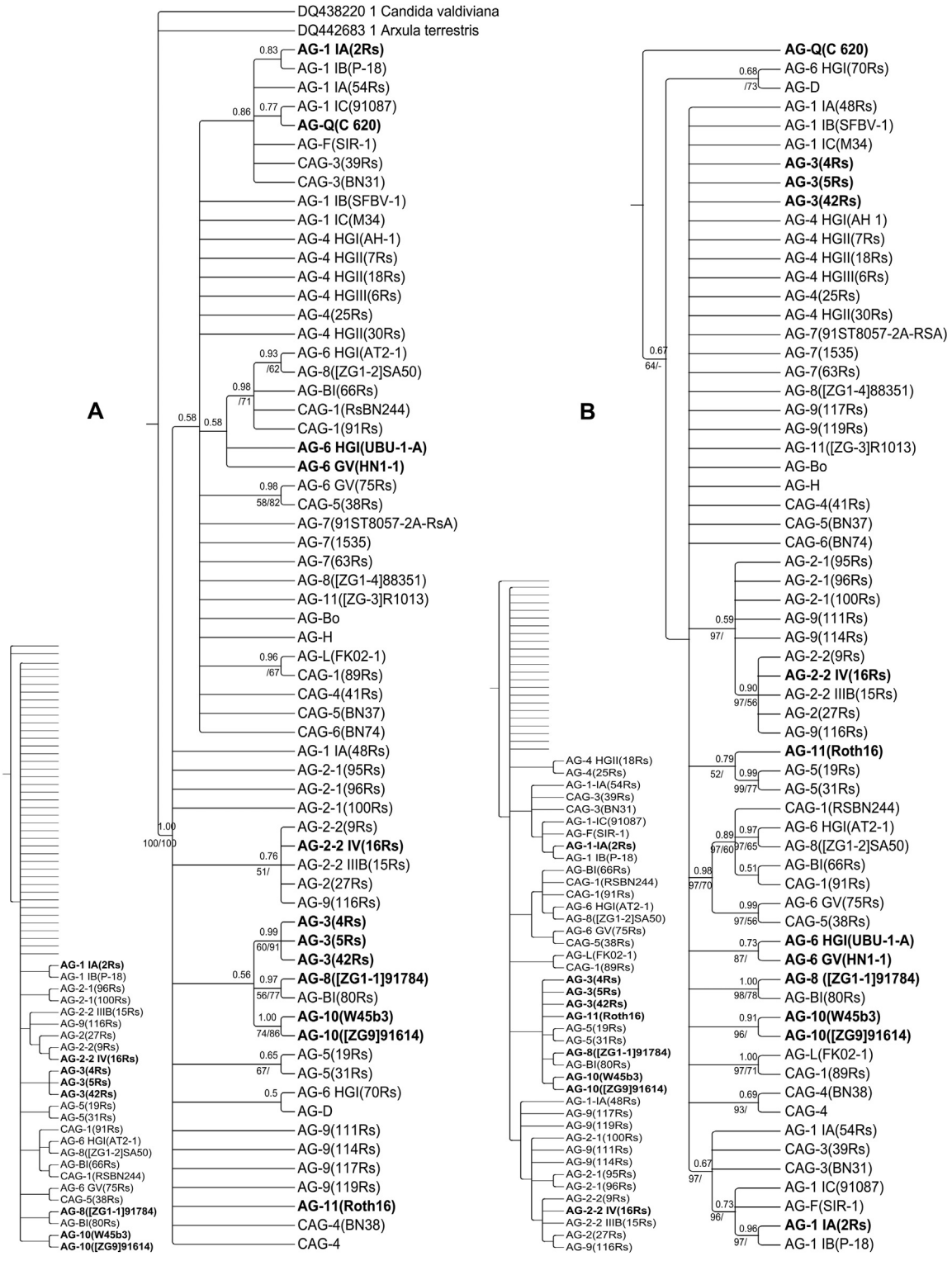


Fig 3 – Phylogeny of *Rhizoctonia* species obtained with sequences from the 3′-terminal region of 28S rDNA. (A) Analyses derived from data matrix 'A'. (B) Analyses derived from data matrix 'C'. Strict consensus tree of MP analysis showing only resolved clades and Bayesian majority-rule consensus tree respectively. Numbers above branches show posterior probabilities. Numbers below branches are jackknife/bootstrap estimates from the ML analysis. In some clades there is no jackknife, bootstrap or posterior probabilities support. Isolates with group I introns are in bold.

solani is not monophyletic; all isolates from AG-3, AG-5, and AG-10 are, and AG-2 and AG-9 are closely related. Nonetheless, some reconstructed relationships were not observed in previous hypothesis such as the close relationship between isolates from CAG-1 with AG-1. The same monophyletic groups were found with ML analyses (topology not shown) indicating that the relationships obtained in these groups are independent of the analytical strategy or model used.

The complex distribution of group I introns within eukaryotes suggests that the process of intron loss and intron acquisition is in a dynamic flux in the genetic systems in which they reside, and it has been proposed that both lateral transfer and vertical transmission have played important roles in the evolutionary history not only of these elements but they may have been more important in the evolution of fungi that in other eukaryotes (Bhattacharya *et al.* 1994, 2005; Tan 1997; Rosewich & Kistler 2000; Wikmark *et al.* 2007). A possible lateral transmission of group I introns by viruses has been suggested as a mechanism of intron acquisition even between distantly related organisms (Nozaki *et al.* 1998; Bhattacharya *et al.* 2005). Viruses are not uncommon among fungi and among *Rhizoctonia* species (Finkler *et al.* 1985, 1988; Charlton *et al.* 2008). Therefore it may be possible that transmission of group I introns by viruses to some isolates of *Rhizoctonia* species was the mechanism of intron acquisition as has been observed in other fungi (Yokoyama *et al.* 2002) but an exhaustive study of naturally occurring viruses in this group is required to explore this hypothesis. In phytopathogenic fungi, vertical inheritance has been proposed to be intimately linked with the evolution of the host organism as well and has been suggested that plants infected by fungus with group I introns may contain similar elements in their genomes (Nishida & Sugiyama 1995). After BLAST searches none of the intron sequences from *Rhizoctonia* species were present in plants naturally infected by this fungus such as rice, barley, wheat or potato. All hits in searches corresponded to species of *Pezizomycotina* and *Saccharomycotina* within *Ascomycota*.

Many phylogenetic studies with introns from SSU rDNA or domains D1 and D2 in LSU rDNA or both have contributed to solve phylogenetic relationships in many fungal species. However, only few phylogenetic studies have been performed with domain D11 (Wang *et al.* 2003; Wikmark *et al.* 2007; Jackson *et al.* 2009; Garrido-Jurado *et al.* 2011). In *Rhizoctonia* species, domain D11 resulted interesting due to the discovery of introns in 12 out of 64 isolates. Unfortunately, sequences from this domain were of little value for phylogenetic

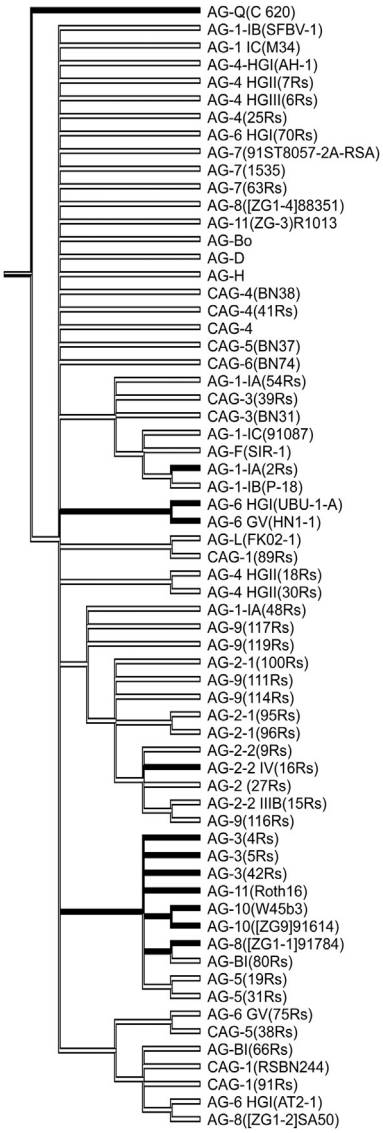


Fig 4 – Hypothesis of intron evolution in *Rhizoctonia* species. This MP tree was constructed with matrix 'C' and introns coded as presence/absence, onto which intron has been mapped.

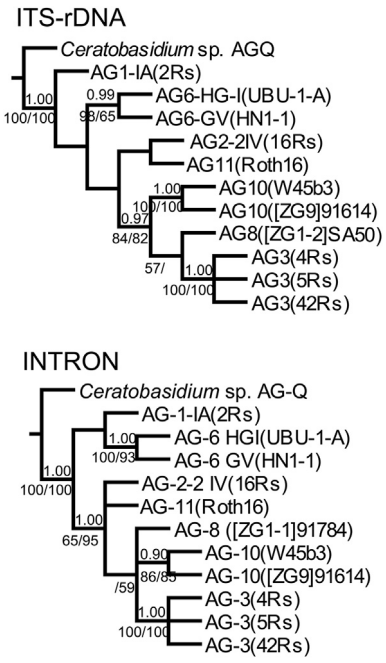


Fig 5 – Phylogeny of ITS-rDNA and group I introns from *Rhizoctonia* species. Numbers above branches show posterior probabilities. Numbers below branches are jackknife/bootstrap estimates from the ML analysis. In some clades there is no jackknife, bootstrap or posterior probabilities support.

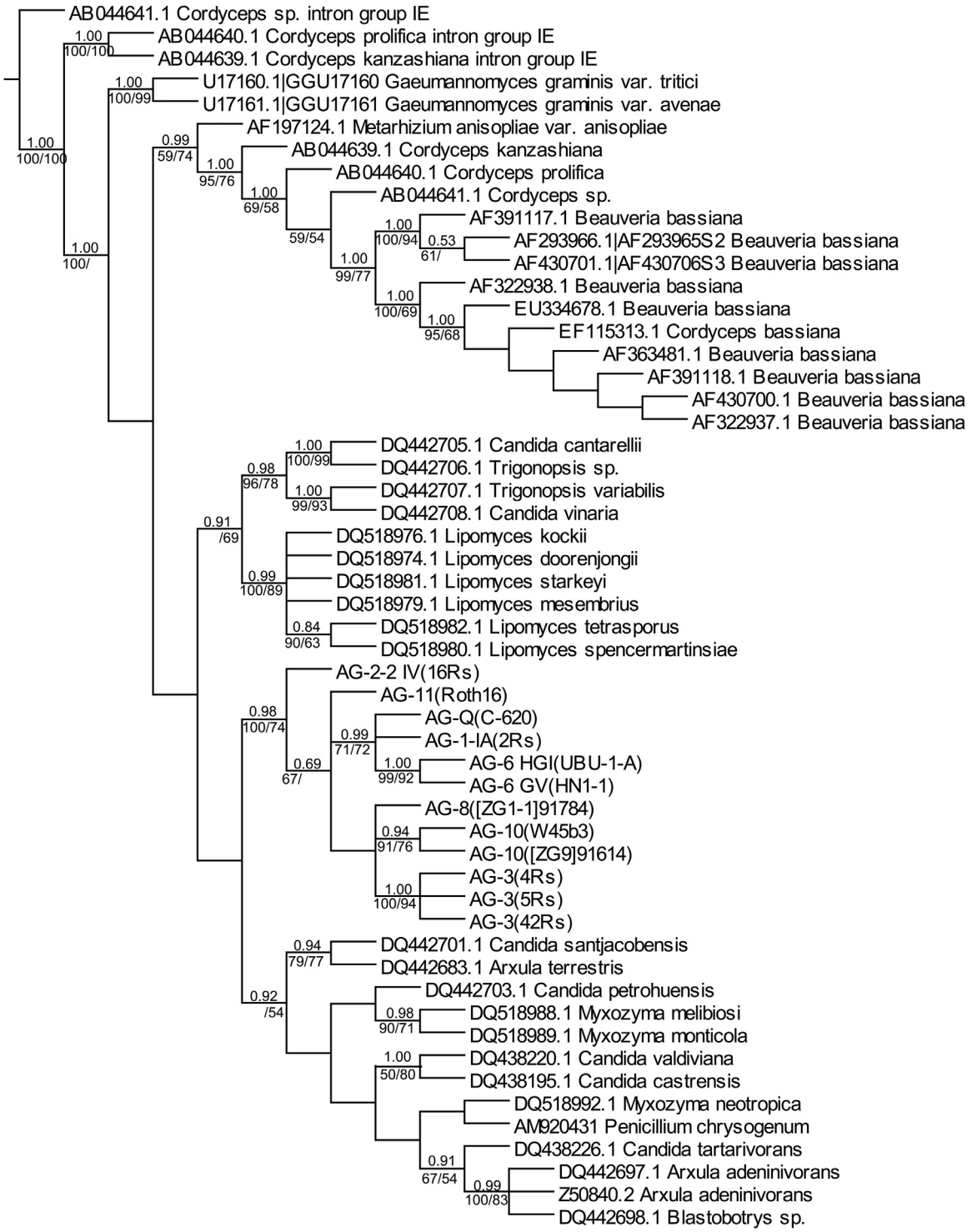


Fig 6 – Phylogeny of IC1 introns at position Ec2449. Numbers above branches show posterior probabilities. Numbers below branches are jackknife/bootstrap estimates from the ML analysis. In some clades there is no jackknife, bootstrap or posterior probabilities support. IE introns at position 2563 from *Cordyceps* spp., are used as outgroup.

reconstruction. One would expect that all isolates from a same AG or at least the same subgroup within *R. solani* would possess the intron because they are closely related taxa. This was true only for the isolates tested from AG-3 and AG-10 (Table 1) but only one isolate from at least two tested had the intron from AG-8 and 11 and subgroups AG-1-IA, AG-2-2 IV, AG-6 HGI, AG-6 GV. The high sequence similarity found within introns from AG-3 and AG-10 is consistent with the hypothesis of descent from an ancestral intron that was initially acquired and vertically inherited (Müller *et al.* 2001). It has been proposed that introns occupying the same insertion site are phylogenetically related and are transmitted vertically

(Nikoh & Fukatsu 2001; Garrido-Jurado *et al.* 2011), and when the same insertion site is not always occupied by introns this could be related to intron loss events throughout the evolution of the species (Niwa *et al.* 2011). If it is the case for *Rhizoctonia* species then, it is reasonable to expect that during its evolution, there were many independent intron loss–gain events resulting in the apparent erratic distribution that is observed today (Fig 4). This irregular distribution in several taxonomic ranks has been addressed previously (e.g. Rosewich & Kistler 2000). Intron evolution patterns in *Rhizoctonia* species revealed minimal congruency between clades and geographic origin of isolates (Fig 4, Table 1). Only the three isolates from AG-3 sampled, had intron and were from the same geographic origin. In contrast, five isolates were sequenced from AG-6 from same geographic origin and only two had intron. Also, the two isolates sequenced from AG-10 had intron and were from different geographic origins (US and Australia). The rest of the isolates with introns are dispersed in the cladogram.

An additional parsimony analysis was performed to test if intron sequences recovered same relationships obtained with sequences from the ITS region (ITS1-5.8S-ITS2) from rDNA for identical isolates from a previous study (González *et al.* 2001, GenBank accession numbers: AF354095.1, AF354097.1, AF354103.1, AF354101.1, AB000014.1, AF354114.1, AF354111.2, AF354071.1, AF354067.1, AF354064.1, AF354107.1, and AF354106.1). Analysis with ITS sequences (94 informative characters) generated two trees of 200 steps (Ci = 0.67; Ri = 0.69). Analysis with intron sequences (64 informative characters) produced four trees of 131 steps (Ci = 0.66; Ri = 0.69). Topology of consensus tree was similar (Fig 5) and congruent with overall phylogeny, indicating that introns were gained early in the evolution of *Rhizoctonia* species with subsequent vertical inheritance but were lost in the majority of isolates (81 %) leaving a scattered distribution pattern. Therefore the lack of resolution when analyses are performed with matrix 'A' (all sequences included, Fig 3) is mainly due to the absence of intron sequences for 52 isolates.

Nonetheless, it has been proposed that even if species phylogenies are congruent with intron phylogenies horizontal transfer cannot be ruled out (Bhattacharya *et al.* 1996; Hibbett 1996). If there is only vertical transmission of introns it had to be assume that the rate of intron loss was several times higher than intron gain to explain irregular intron distribution within organisms. Also, it would be expected that when sequences from homologous introns from distant organisms are included in an analysis they should be monophyletic and this is not always the case (Hibbett 1996; Holst-Jensen *et al.* 1999). Therefore there is a need for further research on the origin and transmission of introns within *Rhizoctonia* species.

The BLAST search optimized for somewhat similar sequences, found the maximum score identity with sequences from *Ascomycota*, some previously identified as introns. The fact that the search detected only sequences from these fungi might be due to unequal sampling of sequences at the 3′ region of the 28S rRNA genes for other organisms. Alignment of these sequences indicates that other than the regions of the highest sequence similarity within the core of the intron secondary structure (P, Q, R, and S), these introns do not have extensive regions of sequence similarity. Pairwise distance values obtained with PAUP* version 4.0b8 (Swofford 2001), ranged from 0.0 for isolates from AG-3 to 0.797 between DQ518981.1 *Lipomyces starkeyi* and AF322938.1 *Beauveria bassiana*. Alignment was optimized with the MAFFT program (http://toolkit.tuebingen.mpg.de/mafft/), which allows rapid detection of homologous segments using fast Fourier transform (FFT) through an iterative optimization. This allowed for the finding of homology at peripheral regions of conserved elements P, Q, R, and S of the introns. If these introns were also transmitted vertically it would be expected that they reflect the *Ascomycota* phylogeny. Fig 6 shows a MP analysis with intron sequences from *Rhizoctonia* and *Ascomycota* species. The evolutionarily distantly related IE subgroup introns (Suh *et al.* 1999; Bhattacharya *et al.* 2005) were used as outgroup for rooting the phylogeny.

The phylogenetic analysis of IC1 introns at position Ec2449 of both, *Ascomycota* and *Basidiomycota* yielded 29 equally most parsimonious trees (Fig 6). The length of the strict consensus trees is, 2538 steps with a CI of 44, and RI of 73. Consensus tree indicates that IC1 introns of *Rhizoctonia* species formed a monophyletic clade that is clustered within *Ascomycota* IC1 lineages, which is inconsistent with the fungal phylogeny. Within *Ascomycota*, all *Pezizomycotina* species are clustered in the upper part of the tree with the exception of *Penicillium chrysogenum*. In the lower part of the tree, all *Saccharomycetes* species are clustered with one exception (*P. chrysogenum*). Mixed with *Saccharomycetes* is the clade with all introns from *Rhizoctonia* species. However, basal lineages are not statistically supported (Fig 6). The MP analysis does not support the hypothesis that fungal IC1 introns at position Ec2449 have only been inherited from a common ancestor. This analysis also indicated the possibility of horizontal transfer of introns at this site between *Ascomycota* and *Basidiomycota*.

Concluding remarks

The focus of this study was to investigate the variability existing in an ES located at the 3′-terminal region of 28S rDNA from *Rhizoctonia* species with characteristics of group I intron and to assess its contribution to phylogenetic analyses. This study found that sequences in domain D11 within this region presented a newly found group IC1 introns for 12 isolates of *Rhizoctonia* species. Interestingly, isolates from the same AG and even subgroups from other studies (e.g. AG-6, González *et al.* 2001), varied with regard to possession of introns. Secondary structure of introns within this fungi revealed that some might lack structural elements, but it is essential to test their ability to selfsplice in order to produce a functional long subunit rRNA. The present study also uncovered a number of new questions for *Rhizoctonia*'s systematics, e.g. the question about the close relationship between some isolates from CAG-1 with some from AG-1 and some isolates from AG-6 with CAG-5. It is evident from this study that there is a need for more information to gain additional phylogenetic resolution. The comparative analyses of *Rhizoctonia* intron sequences and a phylogeny obtained with sequences from the ITS region revealed that introns were gained early in the evolution of *Rhizoctonia* species with subsequent vertical inheritance but were lost in the majority of isolates. However, analyses of IC1 introns at position Ec2449 of both,

Ascomycota and *Basidiomycota* indicated the possibility of horizontal transfer at this position.

Acknowledgements

This study was financially supported by Consejo Nacional de Ciencia y Tecnología (CONACyT No. 103158). The author expresses sincere gratitude to Dr Rytas Vilgalys (Department of Biology, Duke University) and Dr Marc Cubeta (Department of Plant Pathology, NC State University), for generously supplying mycelium and/or samples of DNA, and Rafael Ortega and Elsa Utrera for their partial contribution on generating some sequence data. I also want to acknowledge two anonymous reviewers for their valuable comments to improve this manuscript.

REFERENCES

Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F, 2004. Parallel Metropolis-coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. *Bioinformatics* **20**: 407–415.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215**: 403–410.

Bernhart SH, Hofacker IL, Will S, Gruber AR, Stadler PF, 2008. RNAalifold: improved consensus structure prediction for RNA alignments. *BMC Bioinformatics* **9**: 474. http://dx.doi.org/10.1186/1471-2105-9-474.

Bhattacharya D, Surek B, Rüsing M, Damberger S, Melkonian M, 1994. Group I introns are inherited through common ancestry in the nuclear-encoded rRNA of Zygnematales (Charophyceae). *Proceedings of the National Academy of Sciences USA* **91**: 9916–9920.

Bhattacharya D, Friedl T, Damberger S, 1996. Nuclear-encoded rDNA group I introns: origin and phylogenetic relationships of insertion site lineages in the green algae. *Molecular Biology and Evolution* **13**: 978–989.

Bhattacharya D, Reeb V, Simon DM, Lutzoni F, 2005. Phylogenetic analyses suggest reverse splicing spread of group I introns in fungal ribosomal DNA. *BMC Evolutionary Biology* **5**: 68. http://dx.doi.org/10.1186/1471-2148-5-68.

Burke JM, Belfort M, Cech TR, Davies RW, Schweyen RJ, Shub DA, Szostak JW, Tabak HE, 1987. Structural conventions for group I introns. *Nucleic Acids Research* **15**: 7217–7221.

Cannone JJ, Subramanian S, Schnare MN, Collet JR, D'Souza LM, Du Y, Feng B, Lin N, Madabusi LV, Müller KM, Pande N, Shang Z, Yu N, Gutell RR, 2002. The comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. *BMC Bioinformatics* **3**: 2. http://dx.doi.org/10.1186/1471-2105-3-2.

Carling DE, 1996. Grouping in *Rhizoctonia solani* by hyphal anastomosis reaction. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds), *Rhizoctonia Species: taxonomy, molecular biology, ecology, pathology and disease control*. Kluwer Academic Publishers, Dordrecht, pp. 37–47.

Carling DE, Baird RE, Gitaitits RD, Brainard KA, Kuninaga S, 2002. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathology* **92**: 893–899.

Cech TR, 1988. Conserved sequences and structures of group I introns: building an active site for RNA catalysis – a review. *Gene* **73**: 259–271.

Cech TR, Damberger SH, Gutell RR, 1994. Representation of the secondary and tertiary structure of group I introns. *Nature Structural Biology* **1**: 273–280.

Cech TR, Golden BL, 1999. Building a catalytic active site using only RNA. In: Gesteland RF, Cech TR, Atkins JF (eds), *The RNA World*. CSHL Press, Cold Spring Harbor, NY, pp. 321–349.

Charlton ND, Carbone I, Tavantzi SM, Cubeta MA, 2008. Phylogenetic relatedness of the M2 double-stranded RNA in *Rhizoctonia* fungi. *Mycologia* **100**: 555–564.

Creer S, 2007. Choosing and using introns in molecular phylogenetics. *Evolutionary Bioinformatics* **3**: 99–108.

De Rijk P, Wuyts J, Wachter RD, 2003. RnaViz2: an improved representation of RNA secondary structure. *Bioinformatics* **19**: 299–300.

Del Campo EM, Casano LM, Gasulla F, Barreno E, 2009. Presence of multiple group I introns closely related to bacteria and fungi in plastid 23S rRNAs of lichen-forming *Trebouxia*. *International Microbiology* **12**: 59–67.

Doudna JA, Cech TR, 2002. The chemical repertoire of natural ribozymes. *Nature* **418**: 222–228.

Dujon B, 1989. Group I introns as mobile genetic elements: facts and mechanistic speculations – a review. *Gene* **82**: 91–114.

Einvik C, Nielsen H, Westhof E, Michel F, Johansen S, 1998. Group I-like ribozymes with a novel core organization perform obligate sequential hydrolytic cleavages at two processing sites. *RNA* **4**: 530–541.

Felsenstein J, 2005. *PHYLIP (Phylogeny Inference Package), Version 3.6*. University of Washington, Seattle, USA.

Finkler A, Koltin Y, Barash I, Sneh B, Pozniak D, 1985. Isolation of a virus from virulent strains of *Rhizoctonia solani*. *Journal of General Virology* **66**: 1221–1232.

Finkler A, Ben-Zvi BS, Koltin Y, Barash I, 1988. Transcription and *in vitro* translation of the dsRNA virus isolated from *Rhizoctonia solani*. *Virus Genes* **1**: 205–219.

Garrido-Jurado I, Márquez M, Ortiz-Urquiza A, Santiago-Álvarez C, Iturriaga EA, Quesada-Moraga E, Monte E, Hermosa R, 2011. Genetic analyses place most Spanish isolates of *Beauveria bassiana* in a molecular group with worldwide distribution. *BMC Microbiology* **11**: 84. http://dx.doi.org/10.1186/1471-2180-11-84.

Georgiev OI, Nikolaev N, Hadjiolov AA, Skryabin KG, Zakharyev VM, Bayev AA, 1981. The structure of the yeast ribosomal RNA genes. 4. Complete sequence of the 25S rRNA gene from *Saccharomyces cerevisiae*. *Nucleic Acids Research* **9**: 6953–6958.

Gillespie JJ, Munro JB, Heraty JM, Yoder MJ, Owen AK, Carmichael AE, 2005a. A secondary structural model of the 28S rRNA expansion segments D2 and D3 for chalcidoid wasps (Hymenoptera: Chalcidoidea). *Molecular Biology and Evolution* **22**: 1593–1608.

Gillespie JJ, Yoder MJ, Wharton RA, 2005b. Predicted secondary structure for 28S and 18S rRNA from Ichneumonoidea (Insecta: Hymenoptera: Apocrita): impact on sequence alignment and phylogeny estimation. *Journal of Molecular Evolution* **61**: 114–137.

Goloboff PA, 1994. Character optimization and calculation of tree lengths. *Cladistics* **9**: 433–436.

Goloboff PA, Farris JS, Nixon KC, 2000. *TNT. (Tree Analysis Using New Technology. Program and Documentation)* Published by the authors. Tucumán, Argentina.

González D, Carling DE, Kuninaga S, Vilgalys R, Cubeta MA, 2001. Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorphs. *Mycologia* **93**: 1138–1150.

González D, Cubeta MA, Vilgalys R, 2006. Phylogenetic utility of indels within ribosomal DNA and beta-tubulin sequences from fungi in the *Rhizoctonia solani* species complex. *Molecular Phylogenetics and Evolution* **40**: 459–470.

Grube M, Gargas A, DePriest PT, 1996. A small insertion in the SSU rDNA of the lichen fungus *Arthonia lapidicola* is a degenerate group-I intron. *Current Genetics* **29**: 582–586.

Guindon S, Gascuel O, 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* **52**: 696–704.

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Hancock JM, Dover GA, 1988. Molecular coevolution among cryptically simple expansion segments of eukaryotic 26S/28S rRNAs. *Molecular Biology and Evolution* **5**: 377–391.

Harris LB, Rogers SO, 2008. Splicing and evolution of an unusually small group I intron. *Current Genetics* **54**: 213–222.

Harris LB, Rogers SO, 2011. Evolution of small putative group I introns in the SSU rRNA gene locus of *Phialophora* species. *BMC Research Notes* **4**: 258. http://dx.doi.org/10.1186/1756-0500-4-258.

Hassouna N, Michot B, Bachellerie J-P, 1984. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Research* **12**: 3562–3583.

Haugen P, Runge HJ, Bhattacharya D, 2004. Long-term evolution of the S788 fungal nuclear small subunit rRNA group I introns. *RNA* **10**: 1084–1096.

Haugen P, Simon DM, Bhattacharya D, 2005. The natural history of group I introns. *Trends in Genetics* **21**: 111–119.

Hibbett DS, 1992. Ribosomal RNA and fungal systematics. *Transactions of the Mycological Society of Japan* **33**: 533–556.

Hibbett DS, 1996. Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi. *Molecular Biology and Evolution* **13**: 903–917.

Hillis DM, Dixon MT, 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *The Quarterly Review of Biology* **66**: 411–453.

Holst-Jensen A, Vaage M, Schumacher T, Johansen S, 1999. Structural characteristics and possible horizontal transfer of group I introns between closely related plant pathogenic fungi. *Molecular Biology and Evolution* **16**: 114–126.

Hoshina R, Imamura N, 2009. Phylogenetically close group I introns with different positions among *Paramecium bursaria* photobionts imply a primitive stage of intron diversification. *Molecular Biology and Evolution* **26**: 1309–1319.

Hopple JS, Vilgalys R, 1999. Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophyly. *Molecular Phylogenetics and Evolution* **13**: 1–19.

Huelsenbeck JP, Ronquist F, 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Jackson CJ, Barton RC, Clark CG, Kelly SL, 2009. Molecular characterization of a subgroup IE intron with wide distribution in the large subunit rRNA genes of dermatophyte fungi. *Medical Mycology* **47**: 609–617.

Jackson SA, Cannone JJ, Lee JC, Gutell RR, Woodson SA, 2002. Distribution of rRNA introns in the three-dimensional structure of ribosome. *Journal of Molecular Biology* **323**: 35–52.

Kuzoff RK, Sweere JA, Soltis DE, Soltis PS, Zimmer EA, 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. *Molecular Biology and Evolution* **15**: 251–263.

Li Z, Zhang Y, 2005. Predicting the secondary structures and tertiary interactions of 211 group I introns in IE subgroup. *Nucleic Acids Research* **33**: 2118–2128.

Lilley MJD, Eckstein F (eds), 2008. *Ribozymes and RNA Catalysis*. RSC Publishing, Cambridge, UK.

Maddison WP, Maddison DR, 2011. *Mesquite: a modular system for evolutionary analysis* Version 2.75. http://mesquiteproject.org.

Markham NR, Zuker M, 2008. UNAFold: software for nucleic acid folding and hybridization. *Methods in Molecular Biology* **453**: 3–31.

Márquez M, Iturriaga EA, Quesada-Moraga E, Santiago-Álvarez C, Monte E, Hermosa R, 2006. Detection of potentially valuable polymorphism in four group I intron insertion sites at the 3′-end of the LSU rDNA genes in biocontrol isolates of *Metarhizium anisopliae*. *BMC Microbiology* **6**: 77. http://dx.doi.org/10.1186/1471-2180-6-77.

Mavridou A, Cannone J, Typas MA, 2000. Identification of group-I introns at three different positions within the 28S rDNA gene of the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae*. *Fungal Genetics and Biology* **31**: 79–90.

Michel E, Westhof E, 1990. Modeling of the three-dimensional architecture of group I catalytic introns based on comparative sequence analysis. *Journal of Molecular Biology* **216**: 585–610.

Michot B, Bachellerie J-P, 1987. Comparison of large subunit rRNAs reveal some eukaryotic-specific elements of secondary structure. *Biochimie* **69**: 11–23.

Mitra S, Laederach A, Golden BL, Altman RB, Brenowitz M, 2011. RNA molecules with conserved catalytic cores but variable peripheries fold along unique energetically optimized pathways. *RNA* **17**: 1589–1603. http://dx.doi.org/10.1261/rna.2694811.

Müller KM, Cannone JJ, Gutell RR, Sheath RG, 2001. A structural and phylogenetic analysis of the group IC1 introns in the order Bangiales (Rodophyta). *Molecular Biology and Evolution* **18**: 1654–1667.

Myllys L, Källersjö M, Tehler A, 1999. Variable sizes of introns in the SSU rDNA in three species of *Roccella* (Arthoniales, Euascomycetes). *Current Genetics* **36**.

Neuvéglise C, Brygoo Y, Riba G, 1997. 28s rDNA group-1 introns: a powerful tool for identifying strains of *Beauveria brongniartii*. *Molecular Ecology* **6**: 373–381.

Nikoh N, Fukatsu T, 2001. Evolutionary dynamics of multiple group I introns in nuclear ribosomal RNA genes of endoparasitic fungi of the genus *Cordyceps*. *Molecular Biology and Evolution* **18**: 1631–1642.

Nishida H, Sugiyama J, 1995. A common group I intron between a plant parasitic fungus and its host. *Molecular Biology and Evolution* **12**: 883–886.

Niwa R, Kawahara A, Murakami H, Tanaka S, Ezawa T, 2011. Complete structure of nuclear rDNA of the obligate plant parasite *Plasmodiophora brassicae*: intraspecific polymorphisms in the exon and group I intron of the large subunit rDNA. *Protist* **162**: 423–434.

Nixon K, 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* **15**: 407–414.

Nozaki H, Ohta N, Yamada T, Takano H, 1998. Characterization of *rbc*L group IA introns from two colonial volvocalean species (Chlorophyceae). *Plant Molecular Biology* **37**: 77–85.

Ogoshi A, 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. *Annual Review of Phytopathology* **25**: 125–143.

Pantou M, Mavridou A, Typas MA, 2003. IGS sequence variation, group I introns and the complete nuclear ribosomal DNA of the entomopathogenic fungus *Metarhizium*: excellent tools for isolate detection and phylogenetic analysis. *Fungal Genetics and Biology* **38**: 159–174.

Posada D, 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**: 1253–1256.

Rogers SO, 2012. *Integrated Molecular Evolution*. CRC Press, Boca Raton, Florida.

Ronquist F, Huelsenbeck J, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Rosewich UL, Kistler HC, 2000. Role of horizontal gene transfer in the evolution of fungi. *Annual Review of Phytopathology* **38**: 325–363.

Shinohara ML, LoBuglio KF, Rogers SO, 1996. Group-I intron family in the nuclear ribosomal RNA small subunit genes of *Cenococcum geophilum* isolates. *Current Genetics* **29**: 377–387.

Stöver BC, Müller KF, 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**: 7. http://dx.doi.org/10.1186/1471-2105-11-7.

Suh S-O, Jones KG, Blackwell M, 1999. A group I intron in the nuclear small subunit rRNA gene of *Cryptendoxyla hypophloia*, an Ascomycetous fungus: evidence for a new major class of group I introns. *Journal of Molecular Evolution* **48**: 493–500.

Swofford DL, 2001. *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods)* version 4.0b8. Sinauer Associates, Sunderland, Massachusetts.

Takizawa K, Hashizume T, Kamei K, 2011. Occurrence and characteristics of group 1 introns found at three different positions within the 28S ribosomal RNA gene of the dematiaceous *Phialophora verrucosa*: phylogenetic and secondary structural implications. *BMC Microbiology* **11**: 94. http://dx.doi.org/10.1186/1471-2180-11-94.

Tan MK, 1997. Origin and inheritance of group I introns in 26S rRNA genes of *Gaeumannomyces graminis*. *Journal of Molecular Evolution* **44**: 637–645.

Thompson JD, Higgins DG, Gibson TJ, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.

Wang CS, Li Z, Typas MA, Butt TM, 2003. Nuclear large subunit rDNA group I intron distribution in a population of *Beauveria bassiana* strains: phylogenetic implications. *Mycological Research* **107**: 1189–1200.

Wikmark O-G, Haugen P, Haugli K, Johansen SD, 2007. Obligatory group I introns with unusual features at positions 1949 and 2449 in nuclear LSU rDNA of *Didymiaceae myxomycetes*. *Molecular Phylogenetics and Evolution* **43**: 596–604.

Wilmotte A, Peer YVD, Goris A, Chapelle S, Baere RD, Nelissen B, Neefs J-M, Hennebert GL, Wachter RD, 1993. Evolutionary relationships among higher fungi inferred from small ribosomal subunit RNA sequence analysis. *Systematic and Applied Microbiology* **16**: 436–444.

Yokoyama E, Yamagishi K, Hara A, 2002. Group-I intron containing a putative homing endonuclease gene in the small subunit ribosomal DNA of *Beauveria bassiana* IFO 31676. *Molecular Biology and Evolution* **19**: 2022–2025.

Zhou Y, Lu C, Wu Q-J, Wang Y, Sun Z-T, Deng JC, Zhang Y, 2007. GISSD: group I intron sequence and structure database. *Nucleic Acids Research* **36**: D-31–D-37.

Zwickl D, 2006. *Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion* [Doctoral Dissertation]. University of Texas at Austin, Austin, Texas.