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# Identification of group I introns within the SSU rDNA gene in species of *Ceratocystiopsis* and related taxa

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ABSTRACT

During a recent phylogenetic study, group I introns were noted that interrupt the nuclear small subunit ribosomal RNA (SSU rDNA) gene in species of *Ceratocystiopsis*. Group I introns were found to be inserted at the following rDNA positions: S943, S989, and S1199. The introns have been characterized and phylogenetic analysis of the host gene and the corresponding intron data suggest that for S943 vertical transfer and frequent loss appear to be the most parsimonious explanation for the distribution of nuclear SSU rDNA introns among species of *Ceratocystiopsis*. The SSU rDNA data do suggest that a recent proposal of segregating the genus *Ophiostoma sensu lato* into *Ophiostoma sensu stricto*, *Grosmannia*, and *Ceratocystiopsis* has some merit but may need further amendments, as the SSU rDNA suggests that *Ophiostoma s. str.* may now represent a paraphyletic grouping.
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## Introduction

Group I introns are self-splicing elements that occur in bacteria, bacteriophages and in the organelles of fungi, plants, protists, and early branching metazoans (sea anemones, sponges, and soft corals) (Belfort *et al.* 2002; Hausner 2003; Gissi *et al.* 2008). Group I introns are also found in nuclear ribosomal genes (rDNA) in a wide variety of fungi, algae, and protists with lichen fungi being the richest source for group I introns (De Wachter *et al.*, 1992; Bhattacharya *et al.* 1996; Hibbett 1996; Bhattacharya *et al.* 2002; Lickey *et al.* 2003; Haugen *et al.* 2005; Feau *et al.* 2007; Gutiérrez *et al.* 2007; Hoshina & Imamura 2009).

Insertions within the nuclear rDNA usually occur at highly conserved sequences and they are relatively common among the fungi and have been reported from several rDNA positions (Gargas *et al.* 1995; Cannone *et al.* 2002); in addition spliceosomal introns have also been discovered in the rDNA of ascomycetes (Bhattacharya *et al.* 2000). Among the intron rich members of the lichen fungi examples of vertical transmission and horizontal spread of introns have been documented; introns also appear to move to new rDNA sites by reverse splicing into novel rRNA sites (Dujon 1989; Woodson & Cech 1989; Grube *et al.* 1999; Bhattacharya *et al.* 2000; Martín *et al.* 2003; Bhattacharya *et al.* 2005; Haugen *et al.* 2005). In addition rDNA introns can have a sporadic distribution among phylogenetically closely related fungi suggesting that introns can be gained and lost relatively rapidly (Nikoh & Fukatsu 2001; Haugen *et al.* 2005).

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Group I introns show minimal primary sequence conservation, but they do have conserved secondary and tertiary structures and these elements are autocatalytic by catalyzing their own excision from an RNA molecule; they can therefore be viewed as ribozymes. Almost all group I introns contain pairing regions referred to as P1 to P10, along with sequence segments that connect these helical regions. The P4–P6 and P3–P9 paired helical domains make up the catalytic core components and the P1 and P10 helices are the substrate domain (includes the internal guide sequence) wherein the 5′ and 3′ splice sites are juxtaposed to each other (Cech *et al.* 1994; Lindstrom & Pistolic 2005; Woodson 2005). The P3-P7-P9 helix contains the GTP binding pocket; here the 3′OH of an exogenous GTP initiates the splicing reaction that involves two transesterification reactions resulting in the splicing of the exons and the release of the intron RNA (Raghavan & Minnick 2009). Based on secondary structure characteristics, group I introns were classified initially into two subdivisions: I A and I B (Michel *et al.* 1982; Cech et al. 1994). However, based on both nucleotide sequences within the conserved core regions and variations within the secondary structure, group I intron classification has been further refined. Currently at least five classes are recognized (I A to I E), and these can be subdivided further e.g. I A1, I C3 etc. (Michel & Westhof 1990; Suh *et al.* 1999; Li & Zhang 2005). Over 20 000 group I introns have been identified in a variety of organisms, and the secondary structures of some group I introns and a list of rDNA intron insertions sites have been compiled in the Comparative RNA Web Site (R. Gutell; http://www.rna.ccbb.utexas.edu/, Cannone *et al.* 2002) and the Group I intron sequence and structure database (Zhou *et al.* 2008).

Nuclear group I introns usually lack potential open reading frames (ORFs). However, some group I introns belonging to the IC1 and IE subgroups have been noted in some fungi to encode homing endonucleases (HE) belonging to the HIS-CYS Box HE family (Haugen *et al.* 2004). Nuclear rDNA group I introns range in size from 250 to 600 nucleotides (nt), although there are examples that exceed 1000 nt (Haugen *et al.* 2004). There are also highly compact group I introns at 62–78 nt and these introns appear to be IC1 introns that only maintained the P1, P7, and P10 paired regions, however they still have self-splicing capacity (Harris & Rogers 2008).

During a recent study to investigate phylogenetic relationships among selected ophiostomatoid-like fungi, the SSU rDNA gene sequences were examined for members of the fungal genera *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr., *Grosmannia* Goid. and *Ophiostoma* Syd. & P. Syd. Among these fungi are many economically important tree pathogens and blue-stain fungi (see Wingfield *et al.* 1993; Hausner & Reid 2004; Hausner *et al.* 2005). Members of these genera lack forcible ascospore discharge, have deliquescent asci and form sticky ascospore droplets at the apex of their perithecial necks. These fungi also tend to produce their slimy/sticky conidia on long-stalked conidiophores. Many of these morphological features are adaptations for arthropod dispersal, and therefore these characters are under strong selection pressure for optimizing dispersal and competition for insect vectors and substrates (Harrington 1993; Farrell *et al.* 2001; Six & Wingfield 2011). Species assigned to *Ceratocystiopsis* in many ways resemble species of *Ophiostoma* except they tend to have small dark perithecia with short perithecial necks and falcate, sheathed ascospores (Upadhyay 1981; Zipfel *et al.* 2006; Plattner *et al.* 2009) and a lower tolerance to cycloheximide than *Ophiostoma* species (Harrington 1981; Hausner *et al.* 1993a, b). Species of *Grosmannia* can be distinguished from members of *Ophiostoma* and *Ceratocystiopsis* by the presence of a conidial state that can be assigned to the genus *Leptographium* Lagerb. & Melin. This SSU rDNA based study allowed as to investigate the evolutionary dynamics of nuclear SSU rDNA group I introns within the SSU rDNA gene of the ophiostomatoid fungi. So far no introns have been reported for this group of fungi thus this study is the first report on the occurrence and distribution of introns within this economically important group of fungi.

The objective of this study is to examine the group I intron variability in the nuclear SSU rDNA gene in species of *Ophiostoma*, *Grosmannia*, and *Ceratocystiopsis* and related taxa. This study will try to assess the stability of the introns found within this group of organisms and examine the evolutionary biology of the encountered group I introns and potentially provide insights into the mechanisms that are involved in their diversification and persistence within the SSU rDNA gene.

## Materials and methods

### *PCR amplification and sequencing of the SSU rDNA gene*

The fungal strains studied and their sources are listed in Table 1. Culturing conditions and DNA extraction protocols have been published previously (Hausner *et al.* 1992; Hausner & Reid 2004). Oligonucleotide primers utilized for both PCR amplification and DNA sequencing of the SSU rDNA gene were characterized in Hausner *et al.* (1993b), Gibb & Hausner (2003), and Hausner & Reid (2004). The primers SSU-J and SSU-T were initially used to screen our collection for the potential of SSU rDNA insertions. For some strains the sequences were extended across the ITS regions to examine group I introns located near the 3′ end of the SSU rDNA gene; here the SSU-Z and LSU-4 primers (described in Hausner & Wang 2005) were utilized to obtain the appropriate regions. PCR conditions for the primers above are described in Hausner & Reid (2004) and Hausner & Wang (2005). The PCR products were purified with the Wizard SV Gel and PCR clean-up system (Promega, 2800 Woods Hollow Road, Madison, WI 53711) in order to obtain DNAs suitable for DNA sequence analysis. The PCR products were sequenced using the cycle-sequencing protocols performed according to the manufacturer's recommendations (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404). Automated fluorescent DNA sequence analysis was performed using an ABI Prism 310 Genetic Analyzer (University of Calgary Core DNA service, Faculty of Medicine, University of Calgary, 3350 Hospital Drive NW, Calgary, AB, Canada T2 N 4N1). Initially the original forward and reverse primers utilized for the PCR reactions were used to start the sequences for the PCR products; from there primers were designed as needed to sequence both strands of the PCR amplicons.

### *Phylogenetic analysis*

The individual sequences were assembled manually into contigs using the GeneDoc program v2.5.010 (Nicholas *et al.* 1997). The online resource BLASTn (Altschul *et al.* 1990) was used to

**Table 1 – List of species and strains and SSU rDNA sequence GenBank accession numbers.**

| | Species | Strain number | Introns | | | | | Accession number |
|---|---|---|---|---|---|---|---|---|
| | | | S516 IE | S943 IC1 | S989 IE | S1199 E | 1512 ? | |
| 1 | *Ceratocystiopsis* sp. | WIN(M)1520[a] = MPB 1[b] | | | | | | HQ634836 |
| 2 | *Ceratocystiopsis* sp. | WIN(M)1521 = MPB 2 | | | | | | HQ634837 |
| 3 | *Ceratocystiopsis* sp. | WIN(M)1522 = MPB 3 | | | | | | HQ634838 |
| 4 | *Ceratocystiopsis brevicomi* Hsiau & T.C. Harr. | WIN(M)1452 = CBS 333.97[c] | | | | | | HQ202311 |
| 5 | *Ceratocystiopsis collifera* Marm. & Butin | WIN(M)908 = CBS 129.89 | | | | | | HQ634832 |
| 6 | *Ceratocystiopsis concentrica* (Olchow & J. Reid) H.P. Upadhyay | WIN(M)53 = JR71-21[d] | | X | | | | HQ634849 |
| 7 | *Ceratocystiopsis manitobensis* (J. Reid & Georg Hausner) Zipfel, Z.W. de Beer & M.J. Wingf. | WIN(M)237 = UAMH 9813[e] | | X | | | | HQ634850 |
| 8 | *Ceratocystiopsis minima* (Olchow. & J. Reid) H.P. Upadhyay | WIN(M)1501 | | X | | | | HQ202312 |
| 9 | *Ceratocystiopsis minima* | WIN(M)1462 = CBS 182.86 | | X | | | | HQ634851 |
| 10 | *Ceratocystiopsis minima* | WIN(M)61 = JR69-37 | | X | | | | HQ634852 |
| 11 | *Ceratocystiopsis minima* | WIN(M)85 = JR71-03 | | X | | | | HQ634856 |
| 12 | *Ceratocystiopsis minuta* (Siemaszko) H.P. Upadhyay & W.B. Kendr. | WIN(M)1453 = CBS 441.94 | | X | | | | HQ634853 |
| 13 | *Ceratocystiopsis minuta* | WIN(M)1533 = RJ 5095[e] | | | | | | HQ634826 |
| 14 | *Ceratocystiopsis minuta* | WIN(M)1532; RJ705 = UAMH 11218[f] | | | | | | HQ634827 |
| 15 | *Ceratocystiopsis minuta* | WIN(M)1535 = RJ191 | | | | | | HQ634828 |
| 16 | *Ceratocystiopsis minuta* | WIN(M)1534 = RJ689 | | | | | | HQ634829 |
| 17 | *Ceratocystiopsis minuta* | WIN(M)1537 = CBS 116963 | | X | | X | | HQ634854 |
| 18 | *Ceratocystiopsis minuta* | WIN(M)1536 = CBS 116796 | | | | | | HQ634830 |
| 19 | *Ceratocystiopsis minuta* | WIN(M)1523 = CBS 117042 | | X | | | | HQ634857 |
| 20 | *Ceratocystiopsis minuta-bicolor* (R.W. Davidson) H.P. Upadhyay | WIN(M)480 = JR87-6 | | X | | | | HQ634848 |
| 21 | *Ceratocystiopsis pallidobrunnea* (Olchowecki & J. Reid) H.P. Upadhyay | WIN(M)51 = JR69-14 | | X | X | X | | HQ634842 |
| 22 | *Ceratocystiopsis parva* (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf. | WIN(M)59 = JR71-21 | | X | | | | HQ595735 |
| 23 | *Ceratocystiopsis ranaculosus* J.R. Bridges & T.J. Perry | WIN(M)919 | | | | | | HQ634840 |
| 24 | *Ceratocystiopsis rollhanseniana* (J. Reid, Eyjólfsdóttir & Georg Hausner) Zipfel, Z.W. de Beer & M.J. Wingf. | WIN(M)113 | | X | | X | | HQ595736 |
| 25 | *Ceratocystiopsis rollhanseniana* | WIN(M)110 = UAMH 9797 | | | | | | HQ634834 |
| 26 | As *Ceratocystiopsis* crassivaginata | WIN(M)1458 = CBS 512.83 | | | | | | HQ595740 |
| 27 | *Exophiala calicioides* (Fr.) G. Okada & Seifert | WIN(M)717 = JR87-16 | | X | | | | HQ202314 |
| 28 | *Graphium pseudormiticum* M. Mouton & M.J. Wingf. | WIN(M)1569 = DAOM 234026[g] | | | | X | | HQ634858 |
| 29 | *Graphium pseudormiticum* | WIN(M)1571 = DAOM 234028 | | | | X | | HQ634859 |
| 30 | *Graphium pseudormiticum* | WIN(M)1570 = DAOM 234027 | | | | X | | HQ634860 |
| 31 | *Graphium* sp. *(novo)* | WIN(M)1490 = JR 87-3d | | | | | | HQ202315 |
| 32 | *Grosmannia crassivaginata* (H.D. Griffin) Zipfel, Z.W. de Beer & M.J. Wingf. | WIN(M)918 = UAMH 7004 | | | | | | HQ634833 |
| 33 | *Grosmannia crassivaginata* | WIN(M)184 | | | | | | HQ634835 |
| 34 | *Grosmannia davidsonii* (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf. | WIN(M)60 = JR71-30 | | | | | | HQ595732 |
| 35 | *Grosmannia davidsonii* | WIN(M)1495 = MCC 871[h] | | | | | | HQ634815 |
| 36 | *Grosmannia davidsonii* | WIN(M)1494 = MCC 870 | | | | | | HQ634816 |
| 37 | *Grosmannia davidsonii* | WIN(M)1132 | | | | | | HQ634819 |
| 38 | *Grosmannia penicillata* (Grosmann) Goid. | WIN(M)27 = NOR60-21[i] | | | | | | HQ634822 |
| 39 | *Grossmania piceaperda* (Rumbold) Goid. | WIN(M)980 = UAMH 9788 | | | | X | | HQ595733 |
| 40 | *Leptographium* sp. | WIN(M)984 | | | X | | | HQ595734 |
| 41 | *Leptographium* sp. | WIN(M)1429 | | | | | | HQ595737 |
| 42 | *Meria laricis* Vuill. | WIN(M)1525 = CBS 298.52 | | | | | | HQ634817 |
| 43 | *Meria laricis* | WIN(M)1526 = CBS 283.59 | | X | | | X | HQ634844 |
| 44 | *Meria laricis* | WIN(M)1527 = CBS 281.59 | | X | | | X | HQ634845 |
| 45 | *Meria laricis* | WIN(M)1528 = CBS 282.59 | | | | | | HQ634818 |
| 46 | *Ophiostoma brevicolle* (R.W. Davidson) de Hoog & R.J. Scheff. | WIN(M)811 = CBS 150.78 | | | | | | HQ634823 |

**Table 1 – (*continued*)**

| | Species | Strain number | Introns | | | | | Accession number |
|---|---|---|---|---|---|---|---|---|
| | | | S516 IE | S943 IC1 | S989 IE | S1199 E | 1512 ? | |
| 47 | *Ophiostoma crenulatum* (Olchow. & J. Reid) Georg Hausner & J. Reid | WIN(M)58 = JR 70-17 | | | | X | | HQ634855 |
| 48 | *Ophiostoma longisporum* (Olchow. & J. Reid) Georg Hausner, J. Reid & Klassen | WIN(M)48 | | | | | | HQ634831 |
| 49 | *Ophiostoma minus* (Hedgc.) Syd. & P. Syd. | WIN(M)861 = DAOM 29251 | | | | | | HQ634820 |
| 50 | *Ophiostoma minus* | WIN(M)871 = JR 23Rp3-367TA | | | | | | HQ634821 |
| 51 | *Ophiostoma retusum* (R.W. Davidson & T.E. Hinds) Georg Hausner, J. Reid & Klassen | ATCC 22324[j] | | | | | | HQ634841 |
| 52 | *Ophiostoma* sp. | WIN(M)1391 | | | | | | HQ634824 |
| 53 | *Pesotum fragrans* (Math.-Käärik) G. Okada & Seifert | WIN(M)1396 = JR87-4H | | | | X | | HQ634846 |
| 54 | *Pesotum* sp. | WIN(M)1426 = JR87-4C | | | | X | | HQ634847 |
| 55 | *Pesotum* sp. | WIN(M)1394 = JR87-10C | | | | | | HQ634839 |
| 56 | *Sarcotrochila macrospore* Ziller & A. Funk | WIN(M)1538 = CBS 274.74 | X | X | X | X | | HQ634843 |

a WIN(M) = University of Manitoba (Winnipeg) Collection.
b MPB = Mountain Pine Beetle collection, Department of Forestry, University of British Columbia, Canada.
c CBS = Centraal Bureau voor Schimmelcultures, Utrecht, The Netherlands.
d JR = J. Reid collection (in WIN(M) see above).
e RJ = R. Jankowiak collection (University of Agriculture in Cracow, Cracow, Poland).
f UAMH = University of Alberta Microfungus Collection & Herbarium, Devonian Botanic Garden, Edmonton, AB, Canada T6G 2E1.
g DAOM = Cereal and Oilseeds Research, Agriculture & Agri-Food Canada, Ottawa, Ont., Canada.
h MCC = Culture collection of H. Masuya, Forestry and Forest Products Research Institute, Ibaraki, Japan.
i NOR = NFRI, Norwegian Forest Research Institute, AS, Norway.
j ATCC = American Type Culture Collection, P.O. Box 1549, Manassas, VA 20108, USA.

retrieve sequences that were related to the SSU rDNA genes sequenced during this study (see Fig 1). Nucleotide sequences were aligned with Clustal-X (Thompson *et al.* 1997) and the alignments were refined manually with the aid of the GeneDoc program.

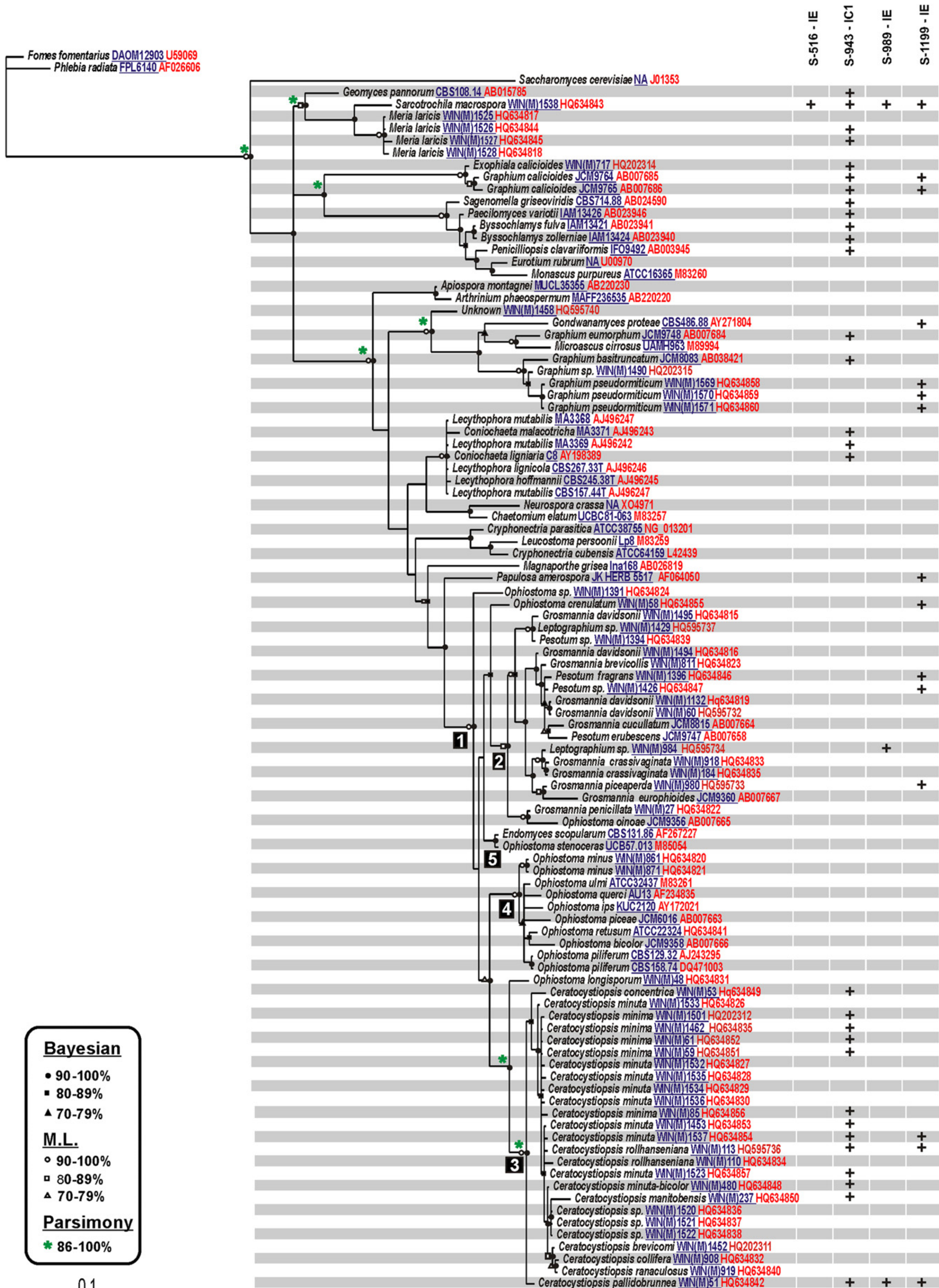
For phylogenetic analyses, only those segments of the alignment where all sequences could be aligned unambiguously were retained for the SSU rDNA data set, and intron sequences were deleted. Phylogenetic estimates were generated using Parsimony, likelihood-based and Bayesian approaches, by using components contained within the PHYLIP package (Felsenstein 2006, version 3.55c), PHYML (online web server, http://www.hiv.lanl.gov/content/sequence/PHYML/interface.html; Guindon *et al.* 2010), and the MrBayes program v3.1 (Ronquist & Huelsenbeck 2003; Ronquist 2004) respectively. In PHYLIP, phylogenetic trees were obtained by the DNAPARS programs in combination with bootstrap (BS) analysis (SEQBOOT) and CONSENSE. SEQBOOT was used to generate 1000 pseudoreplicates which were then analyzed with DNAPARS, the resulting treefile was submitted to CONSENSE. The latter program generates a majority rule consensus tree, which provides an estimate of the confidence levels for the major nodes present within the phylogenetic trees (Felsenstein 1985).

The models applied for Likelihood approaches were based on evaluating the nucleotide sequence alignments with the FindModel program (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html), which identified the GTR with gamma distribution as the best model. The SSU rDNA data set was analyzed with PHYML (Guindon & Gascuel 2003; Guindon *et al.* 2010) selecting the GTR option as implemented at http://www.hiv.lanl.gov/content/sequence/PHYML/interface.html and at http://www.atgc-montpellier.fr/phyml/ (Guindon *et al.* 2010). Branch support for ML trees was evaluated by applying the approximate likelihood-ratio test (aLRT; Anisimova & Gascuel 2006) and by BS analysis (1000 replicates).

The MrBayes program was used for Bayesian analysis applying the GTR model with gamma distribution to the SSU rDNA data set; four chains were run simultaneously for 1 000 000 generation with sample frequency of 100 and a 'burn-in' corresponding to the first 25 % of sampled trees. Phylogenetic trees were drawn with the TreeView program (Page 1996) using PHYLIP tree outfiles or PHYML and MrBayes tree files, and annotated with Corel Draw™ (Corel Corporation and Corel Corporation Limited, Ottawa, Canada).

### *Reverse transcriptase (RT)-PCR analysis*

RNA was isolated from *Ceratocystiopsis pallidobrunnea* WIN(M) 51, a strain that contained representatives of all three types of introns (S943, S989, & S1199) encountered within the *Ophiostoma/Grosmannia/Ceratocystiopsis* clade of fungi. About 50–100 mg of mycelium was used as the starting material for total RNA isolation using the RNeasy kit (Qiagen Sciences, 19300 Germantown Rd., Germantown, MD 20874). Initially the mycelium was ground in liquid nitrogen and then the RNA was purified following the yeast protocol of the RNeasy kit.


S-516 - IE
S-943 - IC1
S-989 - IE
S-1199 - IE
Bayesian
90-100%
80-89%
70-79%
M.L.
90-100%
80-89%
70-79%
Parsimony
86-100%
0.1

The RNA was treated with Ambion Inc. TURBO™ DNase (Invitrogen Life Technologies, 5250 Mainway, Burlington, ON, Canada L7L 5Z1) following the manufacturer's recommendation and about 1 μg of RNA was used as template for RT-PCR using the ThermoScript RT-PCR system (Invitrogen) according to manufacture's recommendations.

For investigating *in vivo* splicing of SSU rDNA introns the SSU-T primer (Hausner *et al*. 1993a), at a final concentration of 40 pmol/reaction, was used for first strand synthesis and subsequent PCR amplification was carried out with primers SSU-U and SSU-T (Hausner *et al*. 1993a). The PCR products generated by the RT-PCR reaction were cloned into the TOPO® TA cloning kit (Invitrogen) and sequenced.

### *RNA folding*

The secondary structure model of the putative group I introns was generated with the online program *mfold* (http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form; Zuker 2003). However, constraints were applied to the folds based on RNA secondary models of group I introns previously published by Cech (1990), Michel & Westhof (1990), Cech *et al*. (1994), Cannone *et al*. (2002), and Li & Zhang (2005). The flanking exon sequences were included in all models in order to identify the P1 and P10 helices (containing the internal guide sequence) for group I introns. The final structures were drawn with Corel Draw™. Designation of intron insertion sites is based on the insertion position with respect to the *Escherichia coli* SSU rDNA gene (accession number AB035922), and introns are named according to the proposed nomenclature by Johansen & Haugen (2001).

### *Ancestral state reconstruction*

Estimation of the ancestral character states for the presence and absence of the S943, S989, and S1199 introns within the *Ceratocystiopsis* clade were done with the MESQUITE program version 2.73 (Maddison & Maddison 2010). A 50 % majority rule tree generated by MrBayes program based on SSU rDNA sequences was used as the base for the reconstruction of the ancestral states. The phylogenetic tree included 30 different strains or species of *Ceratocystiopsis* and was rooted with *Ophiostoma ips*. The evolutionary history of each character was traced over the 50 % majority rule Bayesian tree using the Parsimony reconstruction method. The Mk1 setting was employed (Markov k-state 1 parameter model) which gives equal probability (or rate) for changes between any two character states that were used. In the analysis, each taxon was scored for presence (1) or absence (0) of each intron.

## Results

### *SSU rDNA introns*

Although we assayed for the presence or absence of introns using a PCR approach (see Fig 1) we have to admit that a 'minus' result does not unambiguously identify taxa that lack introns. PCR potentially amplifies smaller DNA fragments; thus, if there is heterogeneity within the rDNA tandem repeats rare intron-plus alleles could be missed (Simon *et al*. 2005a, b). So we are reporting the intron distribution for the 'dominant' version of the SSU rDNA gene for the taxa examined during this study.

Among the ophiostomatoid fungi studied we noted three nuclear SSU rDNA group I introns (Fig 1) inserted at positions 943, 989, and 1199 (Fig 2A and B). Based on structural features the S943 intron (Fig 3B) is an IC1 type and the S989 and S1199 introns (Fig 3C and D respectively) can be categorized as IE type group I introns. Among the ophiostomatoid fungi the SSU rDNA gene of *Ceratocystiopsis pallidobrunnea* WIN(M)51 had all three introns present. The same three introns were also found in the very distantly related species *Sarcotrochila macrospora* Ziller & A. Funk, belonging to the Helotiales. The S943 intron was the most frequently encountered intron, 13 times among our tested 25 *Ceratocystiopsis* strains, this compares to one instance of the S989 intron (in *Cop. pallidobrunnea*) and three examples of the S1199 intron [*Cop. pallidobrunnea*, *Ceratocystiopsis minuta* (WIN(M) 1537), and *Ceratocystiopsis rollhanseniana* (WIN(M)113)]. RT-PCR was performed on *Cop. pallidobrunnea* rRNA (Fig 4), as this offered an opportunity to examine representatives for all three introns with regards to their ability to splice *in vivo* and to define the intron/exon junctions. The latter confirmed the intron/exon junctions as defined by comparing SSU rDNA sequences that lacked introns with those that do (Figs 2B and 4).

The S943 intron has been described from a wide variety of fungi (Gargas *et al*. 1995; Perotto *et al*. 2000; Feau *et al*. 2007). Additional examples of the S943 intron were noted among members of the *Eurotiomycetes*, two members of the Microascales, and three strains belonging to the *Coniochaetales* (*Sordariomycetes*); however, none were noted among the tested members of *Grosmannia* and *Ophiostoma*. The S989 intron was only encountered three times during this study with examples in *Cop. pallidobrunnea*, *Leptographium* sp. WIN(M)984 (*Grosmannia* clade) and *S. macrospora*. The S1199 intron also has a spotty distribution but it was observed in three *Ceratocystiopsis* species, three members of the *Grosmannia* clade, in *Ophiostoma crenulatum*, in *Papulosa amerospora*, three members of the Microascales, two members of the *Chaetothyriales* (*Eurotiomycetes*) and this intron is also present in *S. macrospora*. Finally the S516 group IE (Fig 3A) and S1512 introns were only

**Fig 1 – Phylogenetic tree based on the SSU rDNA data set for *Ceratocystiopsis* and related ascomycete taxa. The tree topology is based on Bayesian analysis, solid circles, squares, and triangles represents Posterior Probability (PP) supportive values (90–100 %), (80–89 %), and (70–79 %) respectively as obtained from a 50 % majority Bayesian consensus tree. Open circles, squares, and triangles represent BS support (90–100 %), (80–89 %), and (70–79 %) respectively based on ML analysis. Nodes supported with BS ≥ 86 % based on Parsimony analysis are marked with asterisks. Nodes that received less than 70 % support (BS or PP) were collapsed. The branch lengths are based on Bayesian analysis and are proportional to the number of substitutions per site. Strain numbers (underlined) and Genbank accession numbers are listed next to species names. The tree is rooted with SSU rDNA sequences from the *Basidiomycetes* fungi *Fomes fomentarius* (L.) Fr. and *Phlebia radiata* Fr.**

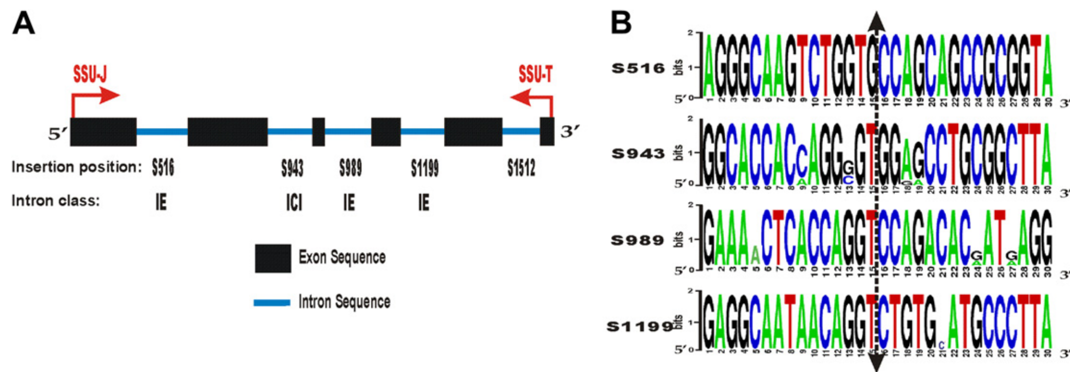



**Fig 2 – (A) A schematic representation of the SSU rDNA showing the initial primers (SSU-J and SSU-T) used to amplify the gene and the various types of group I introns found during this study. The introns are located at positions S516, S943, S989, S1199, and S1512 with reference to the *E. coli* SSU rDNA sequence. (B) Nucleotide sequence logos are shown for the exon sequences that flank the intron insertion sites (15 upstream and 15 downstream nucleotides). The double headed arrow indicates the insertion position for the following introns: S516, S943, S989, and S1199. The logos were generated by the online program WebLogo version 2.8.2 (Crooks *et al.* 2004; http://weblogo.berkeley.edu/logo.cgi).**

encountered within *S. macrospore* and two strains of *Meria laricis* respectively. The S1512 intron did not appear to follow any of the conventional group I intron folds so we could not assign it to any category of group I intron. Typically group I introns inserted into the S1512 position belong to the IC1 category (http://www.rna.ccbb.utexas.edu/). The S1512 intron had been previously noted by Gutiérrez *et al.* (2007) in members of the lichen family *Parmeliaceae*.

### *Intron* versus *host gene phylogenies*

We focused on the evolutionary dynamics of the S943 and S1199 group I introns, as several examples were found in the SSU rDNA in some members of the ophiostomatoid fungi. The other introns were only noted in a few instances, thus, not providing enough of a data set to warrant further analysis. Phylogenetic trees for the host SSU rDNA sequences when mirrored with the phylogeny based on the S943 intron data set (Fig 5A & B) had similar topologies. For the S943 intron we focussed on those found within *Ceratocystiopsis* species, as we could not generate unambiguous alignments for the intron core sequences when nonophiostomatoid sequences were included. The S943 intron has an extremely wide distribution (Nikoh & Fukatsu 2001; Haugen *et al.* 2005) and that would suggest that it is an ancient intron. The presence of the S943 intron in the basal branching member of *Ceratocystiopsis* (*Ceratocystiopsis pallidobrunnea*) combined with the SSU rDNA tree being similar to the S943 intron tree suggests that this intron most likely is vertically transferred among species of *Ceratocystiopsis* and the random distribution noted (Fig 1) is most likely due to frequent loss.

The S1199 intron is widely distributed among the ascomycetes fungi (Gibb & Hausner 2003) and we noted several examples of this intron among the sequences examined (Fig 1). When the host gene tree was mirrored with the intron-based tree (Fig 5C and D), the topologies were essentially similar, failing to support strong arguments for horizontal movement of this intron among the taxa examined in this study. Ancestral state analysis (Fig 6) appears to confirm the above results for the S943 intron, however it fails to support the notion that the presence of S1199 can be explained solely by vertical transmission and frequent loss.

### *SSU rDNA phylogenetic analysis*

The phylogenetic analysis showed some well-supported clades with regards to fungi that are commonly referred to as ophiostomatoid fungi (i.e. members of *Ophiostoma*, *Ceratocystiopsis*, and *Grosmannia*) (Fig 1, node 1). Species that can be assigned to *Grosmannia* based on the presence of a *Leptographium* anamorph and *Leptographium* species can be included within one clade (Fig 1, node 2), although this grouping also includes *Pesotum* sp. (WIN(M) 1426 and 1394), *Pesotum erubescens* (Math.-Käärik) G. Okada, and *Pesotum fragrans* (Math.-Käärik) M. Morelet.

Species that can be assigned to *Ceratocystiopsis* (Fig 1, node 3) formed a monophyletic group that received strong node support. The SSU rDNA sequence of *Ophiostoma longisporum* was placed outside of the node that joins all *Ceratocystiopsis* species. Another well-supported clade (Fig 1, node 4), referred to from now on as the '*Ophiostoma* group', includes representatives of *Ophiostoma minus*, *Ophiostoma ips*, (Rumbold) Nannf., *Ophiostoma piliferum* (Fr.) Syd. & P. Syd., *Ophiostoma retusum*, *Ophiostoma bicolor* R.W. Davidson & D.E. Wells.

The SSU rDNA sequences of *Ophiostoma stenoceras* (Robak) Melin & Nannf. and *Ophiostoma crenulatum* and an undescribed species of *Ophiostoma* (WIN(M)1391; James Reid and Georg Hausner.,_unpubl. data) do not appear to have a close link to the '*Ophiostoma* group' (Fig 1). A strain of *Endomyces scopularum* Helfer, a yeast-like fungus, appears to be monophyletic with *O. stenoceras* (Fig 1, node 5); however, it should be noted that *E. scopularum* should be viewed as a *Sporothrix* sp. (see Suh *et al.* 2001).

## Discussion

### *SSU rDNA intron distribution among the ophiostomatoid fungi*

As less is known about the occurrence of rDNA group I introns in nonlichenized fungi we focussed on the ophiostomatoid

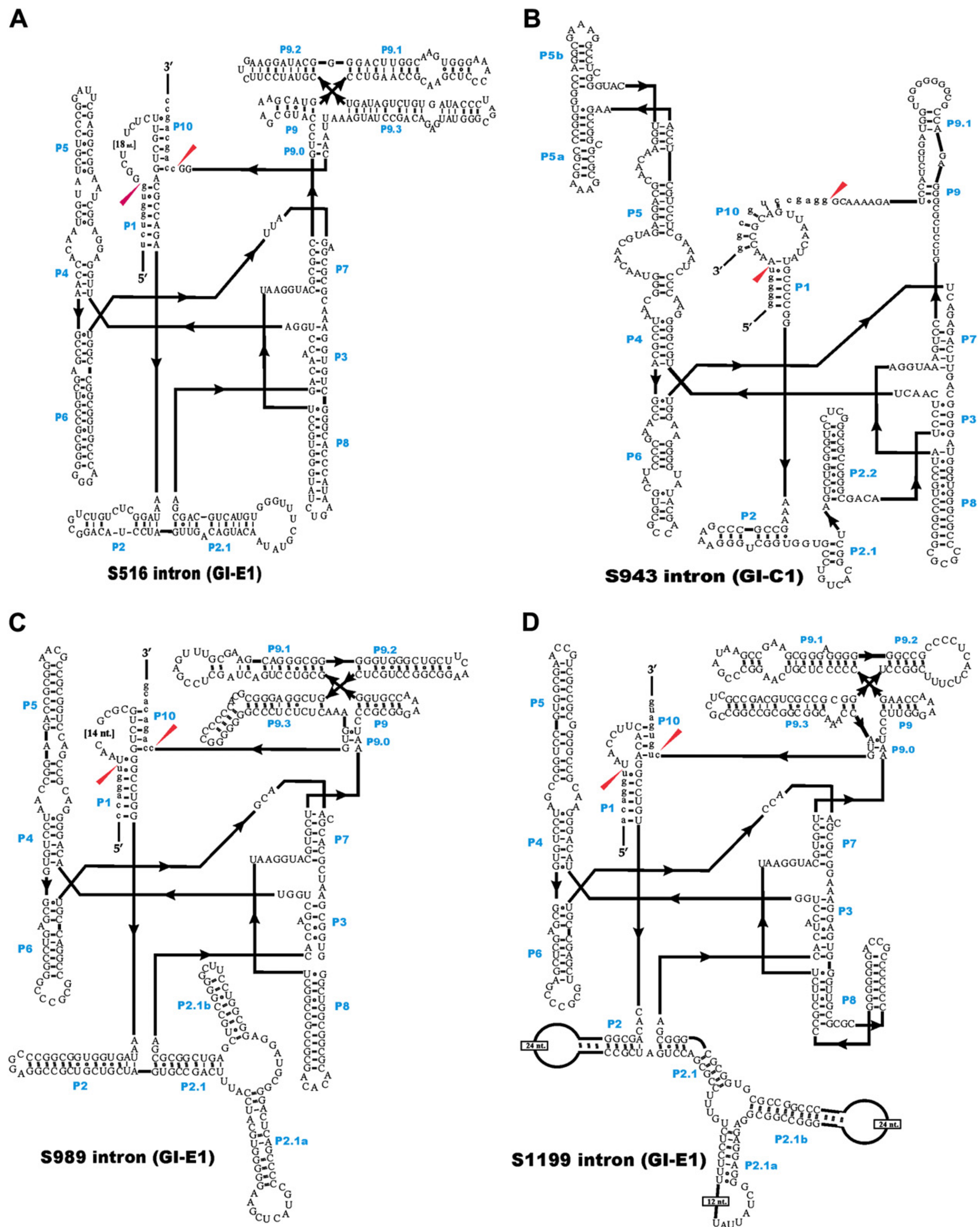



**Fig 3 – Secondary structures for the following group I introns: [A] S516, [B] S943, [C] S989, and [D] S1199. Intron sequences are in upper-case letters and exon sequences are in lower-case letters. The ten pairing regions (P1–P10) are indicated. The solid red arrowheads indicate the intron–exon junctions (putative 5′ and 3′ splicing sites). Designation of intron types is based on previous reports by Michel & Westhof (1990) and Suh *et al.* (1999).**

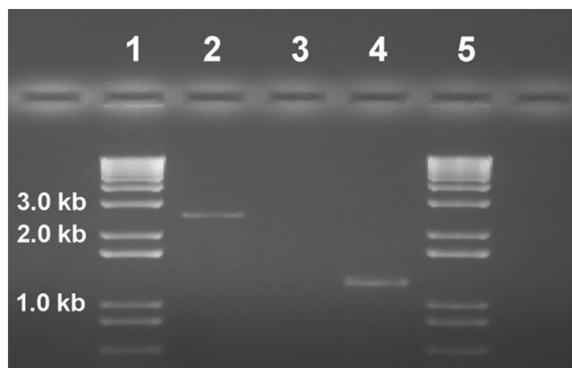



**Fig 4 – RT-PCR analysis of the SSU rDNA for *Cop. pallidobrunnea* WIN(M)51 to demonstrate *in vivo* splicing of the S943, S989, and S1199 introns and to confirm the exon/intron junctions (see Fig 2B). Lanes 1 and 5 contain the 1 kb plus DNA ladder (Invitrogen), lane 2 represents a standard PCR reaction using primers SSU-U and SSU-T with whole DNA as a template, while lane 4 contains amplicons derived from the RT generated cDNA template. The negative control in lane 3 (standard PCR using whole cell RNA as a template) yielded no bands confirming that the RNA samples were DNA-free. The genomic DNA generated a 2.7 kb PCR fragment indicating the presence of the S943, S989, and S1199 introns, while the cDNA template generated a 1.2 kb PCR fragment indicating that all introns were spliced out.**

fungi, and based on the taxon sampling represented in this study, we can conclude that species of *Ceratocystiopsis* appear to have more group I introns within their SSU rDNA gene compared to species of *Ophiostoma* and *Grosmannia*.

In general the introns are not stable markers that could be used in taxonomic applications, for example, among eight strains identified as *Ceratocystiopsis minuta* three stains [WIN(M)1453, 1537, 1523] had the S943 introns but five strains lacked introns (WIN(M) 1532, 1533, 1534, 1535, 1536) and one strain of *Cop. minuta* (Win(M) 1537), in addition to the S943 intron, had the S1199 intron. However, as reported previously *Cop. minuta* could represent a species complex (Plattner *et al.* 2009). All *Cop. minima* strains had the S943 intron but one strain of *Ceratocystiopsis rollhanseniana* (WIN(M)113) had both the S943 and S1199 introns whereas the second strain of this species WIN(110) had no introns.

The presence of multiple introns (S943, S989, S1199) within *Ceratocystiopsis pallidobrunnea* is of interest as: (1) it is the only examined member of the *Ceratocystiopsis* clade that has three introns including the S989 intron; and (2) it appears to be a basal member of the genus *Ceratocystiopis* (this paper Fig 1 and Plattner *et al.* 2009). In this study the only other fungus allied with the ophiostomatoid fungi that has the S989 intron is a strain of *Leptographium* (WIN(M) 984). This rather spotty distribution of some group I introns does raise questions with regards to lateral transfers but at this stage we need to examine more members with *Grosmannia* to get a better understanding of the evolutionary dynamics of this particular group I intron.

For both comparisons between the SSU rDNA tree and S943 or S1199 trees the topologies between the host tree and the intron trees were somewhat discrepant (Fig 5). Considering how closely related species of *Ceratocystiopsis* are (see Plattner *et al.* 2009) and the weak statistical support obtained for many nodes within the intron trees we feel the discrepancies noted were more likely the result of the limited resolution within the intron sequence phylogenetic analysis than evidence for lateral transfer of group I introns among species of *Ceratocystiopsis*. However, we cannot rule out the possibility of these introns moving laterally among members of this genus. Using the S943 and S1199 introns as discreet characters in ancestral state analysis provides support that S943 is an ancestral character for the members of the *Ceratocystiopsis* clade, however for the S1199 intron this analysis failed to provide any support for the hypothesis that this intron is ancestral to this group. The latter therefore suggests that the S1199 intron could have been gained independently by horizontal transfers by members of this genus.

Overall, our data appear to be similar to what have been observed in some other systems (Nikoh & Fukatsu 2001; Bhattacharya *et al.* 2002), which is, that the evolutionary history of group I introns within the SSU rDNA gene among ophiostomatoid fungi is driven by rare gains (such as lateral transfers) followed by rapid loss(es) in descendant lineages, thus resulting in what appears to be sporadic intron distributions (Fig 1). However, it appears that for some introns (S1199) frequent gain cannot be excluded as a possibility of the spotty distribution of some introns.

The distribution of nuclear group I introns can be the result of a variety of processes such as intron gain by horizontal and/or vertical transmission and intron loss due to precise excision. Reverse splicing allows a free group I intron RNA to insert into a homologous or heterologous RNA; this mode of mobility requires complementary base pairing between the intron and the exon RNA sequences. This model of transposition would explain how group I introns, as encountered in this study, that lack intron-encoded proteins can persist in populations. This mechanism requires the additional steps of reverse transcription of the RNA and the integration of the cDNA into the rDNA locus by a recombination step that replaces the intron-less copy with the intron-plus cDNA. Although reverse splicing has not yet been demonstrated in genetic crosses, circumstantial evidence would suggest that nuclear rDNA group I introns can move by this mechanism (Bhattacharya *et al.* 2002, 2005). As reverse splicing requires less homology (4–6 nucleotides), this mechanism allows introns to spread more easily into heterologous sites, although in this study we did not see any examples of ectopic integration.

A possible scenario for precise intron loss can be envisioned by the reverse transcription of a processed (i.e. spliced) version of the SSU rRNA followed by a recombination event whereby the intron-minus cDNA replaces the intron-plus genomic version of the SSU rDNA. This mechanism could remove introns from one rDNA unit, but the subsequent mechanism(s) whereby the now rDNA-intron-minus unit replaces the other intron hosting rDNA units within the repetitive rDNA gene family are unknown. One would assume that genetic processes that drive concerted evolution would be involved in this transition.

### *Group I introns in nuclear rDNA*

Ribosomal RNA genes are under functional constraints and thus are expected to evolve slowly, ideal for ribozyme

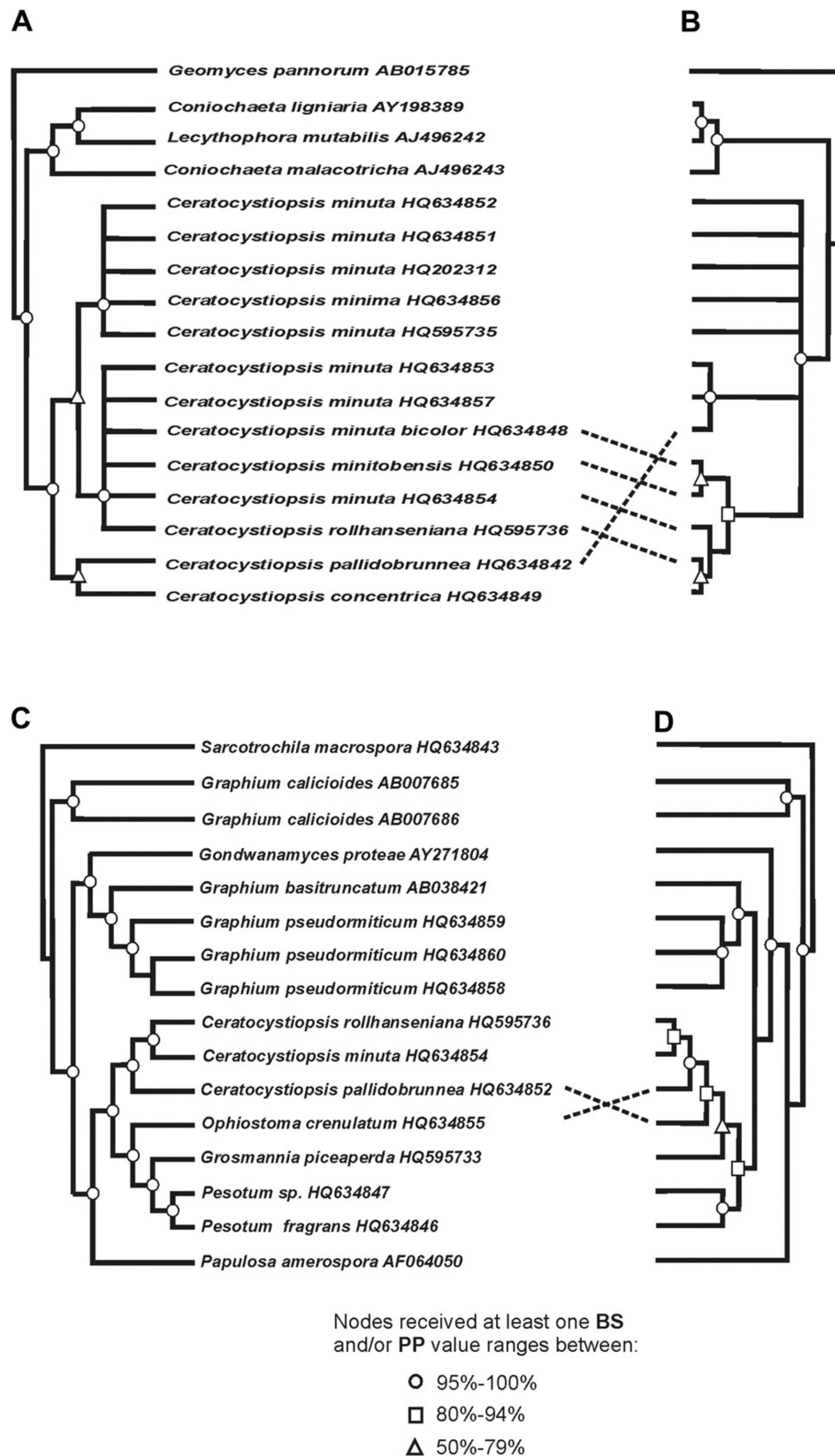



**Fig 5 – Phylogenetic relationships between group I introns and their corresponding host SSU rDNAs. [A] SSU rDNA based phylogeny *versus* the S943 group I intron phylogeny [B]; based on the P1 to P8 conserved stem sequences of the S943 intron. [C] SSU rDNA phylogeny compared to the S1199 group I intron phylogeny [D]; the intron alignment consists of the P1 to P8 conserved stem sequences. For the group I intron alignments the P9 stem sequences were excluded because they could not be aligned unambiguously. The tree topology [A, B, C, & D] is based on the majority rule consensus tree generated by Parsimony analysis (DNAPARS), open circles, squares, and triangles represents nodes received BS and/or PP values (95–100 %), (80–94 %), and (50–79 %) respectively as obtained from a 50 % consensus tree. Genbank accession numbers for the SSU rDNA sequences are listed along with the species name.**

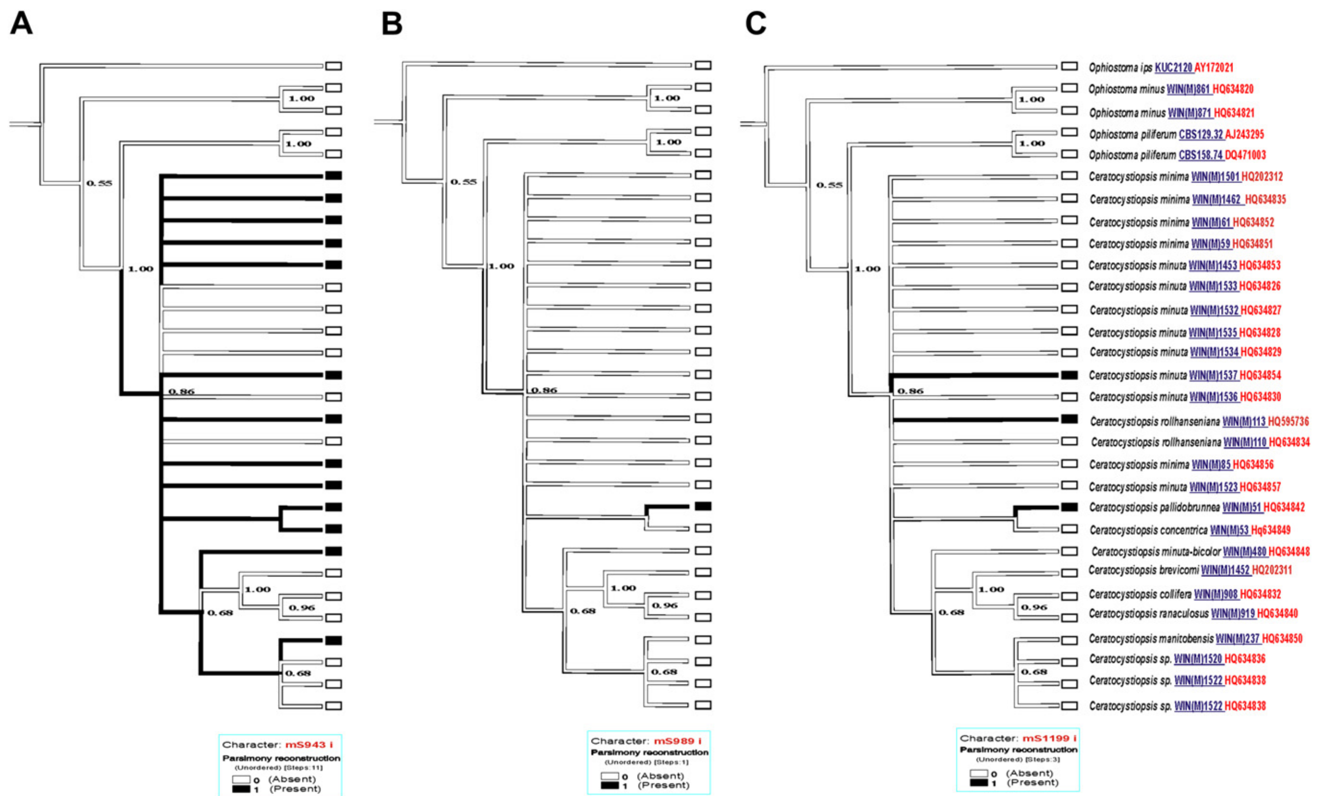



**Fig 6 – The most parsimonious ancestral character mapping for the presence and absence of SSU rDNA introns [A] S943, [B] S989, and [C] S1199 on a 50 % Bayesian majority consensus tree for members of the genus *Ceratocystiopsis*. The numbers at the nodes represent PP supportive values. Character states are: 0 = absence of intron (indicated by white lines and blank squares); 1 = presence of introns, indicated by black lines and filled in squares.**

elements that need to maintain their core sequences to maintain auto-splicing abilities or potentially face extinction, as they might become toxic to the host gene (Goddard *et al.* 2006; Lynch *et al.* 2006; Hausner 2012). Also repetitive DNA is ideal for mobile introns as intron-minus and intron-plus alleles could exist within the same space; thus rDNA offers the presence of multiple targets for group I introns to invade.

In fungi, like most eukaryotes, ribosomal RNA genes are organized in tandem repeats with copy numbers for the repeats varying from 45 (*Aspergillus nidulans*) to 180 (*Saccharomyces paradoxus*) in taxa examined so far (Gerbi 1985; Ganley & Kobayashi 2007). A recent study showed that among members of the *Saccharomycetaceae*, *A. nidulans* and *Cryptococcus neoformans* rDNA repeats showed remarkably low levels of sequence variation, strongly suggesting that concerted evolution operates within this repetitive gene family, leading to rapid homogenization among its individual repeats (Ganley & Kobayashi 2007). Homogenization in part is thought to be accomplished by a continual turnover of repeat copies by unequal recombination (reviewed in Elder & Turner 1995; Liao 1999; Eickbush & Eickbush 2007). The spread or loss of rDNA introns within the rDNA tandem repeats may be accelerated because of concerted evolution. An intron may gain a foothold within rDNA not because it has a highly efficient way to rapidly invade all available sites but because by chance one rDNA copy that gained an intron eventually becomes the dominant version. Conversely, an intron might be lost as an intron-less version over time replaces rDNA repeats with introns.

The presence of nuclear rDNA group I introns raises some interesting questions as to why they are found in fungi, protozoans, and algae but so far have not been noted in plant and metazoan nuclear rDNA. Fungi are organisms that represent an 'open system' (no true cell walls separating cells) with limited separation between somatic and germ line tissue, thus, there are few barriers separating somatic compartments from those involved in generating meiotic or mitotic spores (Goddard & Burt 1999). Such a system may allow for transmitting mobile genetic agents by transient hyphal contacts or *via* intermediates such as viruses or genetic transfer systems similar to those within *Agrobacterium tumefaciens* (Andersson 2009; reviewed in Hausner 2012). Also as discussed previously concerted evolution may be a mechanism that allows introns to spread through the rDNA repeats. One can also speculate that rDNA tolerates group I introns, as rDNA is transcribed by RNA polymerase I, which must be tolerant to transcribing structural RNAs (such as rRNAs). Finally, in fungi repeat-induced point mutations (RIP) is a mechanism that guards against mobile elements spreading within the genome; however, RIP does not appear to operate in the nucleolus (specifically the nuclear organizer regions; reviewed in Hane & Oliver 2010). The rDNA tandem repeats are therefore not affected by this mechanism and mobile elements such as group I introns have a refuge within this genomic niche. In other regions of

the genome RIP mutates duplicated DNA sequences during sexual reproduction, potentially inactivating copies of repeated sequences and thus limiting the spread of mobile elements (Selker 2002; Diguistini *et al.* 2011). All the above factors probably contribute towards the evolutionary dynamics of nuclear rDNA group I introns within the fungi.

### *SSU rDNA and taxonomic implications for* Ophiostoma sensu lato

The taxonomy of the *Ophiostomatales* and the family *Ophiostomataceae* is very complex (Hausner *et al.* 2000; Hausner & Reid 2004; Zipfel *et al.* 2006) and historically has been subject of vigorous debates (Upadhyay 1981; Hausner *et al.* 1993a, b; Spatafora & Blackwell 1994). Recently Zipfel *et al.* (2006), based on partial LSU rDNA and β-tubulin sequences, provided some justification to subdivide the genus *Ophiostoma* by resurrecting the genus *Grosmannia* for '*Ophiostoma* species' with *Leptographium* anamorphs and for maintaining the genus *Ceratocystiopsis* for ophiostomatoid species with short perithecial necks and falcate ascospores. What now remains in *Ophiostoma sensu* Zipfel *et al.* (2006) could broadly be defined as members of the 'ips' and 'pilifera' spore groups, as defined by Olchowecki & Reid (1974).

Our study showed that some *Pesotum* species are allied with members of *Grosmannia*. Okada *et al.* (2000), based on SSU rDNA data, placed *Pesotum fragrans* next to *Ophiostoma penicillatum* (Grosmann) Siemaszko (now *Grosmannia penicillata* (Grosmann) Goid.). Zipfel *et al.* (2006) showed that *Grosmannia galeiformis* (B.K. Bakshi) Zipfel, Z.W. de Beer & M.J. Wingf., a species with both synnematous and mononematous anamorphs (i.e. *Pesotum*-like and *Leptographium*-like conidial states), belongs to the *Grosmannia* clade, so in the future more species with *Pesotum* conidial states might be assigned to this genus. Previous studies that included SSU rDNA sequences representing different *Raffaelea* Arx & Hennebert emend. T.C. Harr. species (Gebhardt *et al.* 2005; Kolařík & Hulcr 2008; Harrington *et al.* 2010) suggest, that these asexual species are also phylogenetically linked to species of *Grosmannia*.

*Ceratocystiopsis*, a genus that includes species with falcate ascospores, short perithecial necks and, if present, anamorphs assignable to *Hyalorhinocladiella*, formed a monophyletic clade. It has to be stated that the taxonomy of *Cop. minuta*, the type species for *Ceratocystiopsis*, is rather complex and, as currently circumscribed, probably includes several cryptic species (Plattner *et al.* 2009). To help resolve this situations strain WIN(M) 1532 [=UAMH 11218, =R. Jankowiak 705] was grown and dried on wood chips and has then been designated as the epitype (Reid & Hausner 2010). *Ophiostoma longisporum*, based on ITS, partial β-tubulin, and LSU rDNA sequences, was excluded from *Ceratocystiopsis* by Plattner *et al.* (2009) but in that study this species did group basally to other members of this genus. This study, albeit based on SSU rDNA sequences only, again confirms a basal position for *Ophiostoma longisporum* next to the *Ceratocystiopsis* clade.

The SSU rDNA data suggest that there is a problem with *Ophiostoma sensu* Zipfel *et al.* (2006). Although our analysis shows strains centred around *Ophiostoma ips* and *Ophiostoma piceae* (Münch) Syd. & P. Syd., including *Ophiostoma minus* and *Ophiostoma piliferum* (referred to as the *Ophiostoma* group), form a monophyletic grouping but several *Ophiostoma* species failed to group with the three currently accepted genera that comprise what used to be *Ophiostoma sensu lato*. Kolařík & Hulcr (2008) also showed in their SSU rDNA analysis that *Ophiostoma stenoceras* groups apart from both *Grosmannia* species and *Ophiostoma* species, forming a monophyletic group with *Sporothrix schenckii* Hektoen & C.F. Perkins and *Endomyces scopularum*. The SSU rDNA data do suggest that the proposal by Zipfel *et al.* (2006) has some merit but it also shows that the status of *Ophiostoma* s.s. may need more consideration and additional genera may have to be proposed to accommodate those species not allied to the ips/pilifera/piceae group.

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REFERENCES

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

Andersson JO, 2009. Horizontal gene transfer between microbial eukaryotes. *Methods in Molecular Biology* **532**: 473–487.

Anisimova M, Gascuel O, 2006. Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Systematic Biology* **55**: 539–552.

Belfort M, Derbyshire V, Parker MM, Cousineau B, Lambowitz AM, 2002. Mobile introns: pathways and proteins. In: Craig NL, Craigie R, Gellert M, Lambowitz AM (eds), *Mobile DNA II*. ASM Press, Washington DC, pp. 761–783.

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, Friedl T, Helms G, 2002. Vertical evolution and intragenic spread of lichen-fungal group I introns. *Journal of Molecular Evolution* **55**: 74–84.

Bhattacharya D, Reeb V, Simon DM, Lutzoni F, 2005. Phylogenetic analysis suggests reverse splicing spread of group I introns in fungal ribosomal DNA. *BMC Evolutionary Biology* **5**: 68.

Bhattacharya D, Lutzoni F, Reeb V, Simon D, Nason J, Fernandez F, 2000. Widespread occurrence of spliceosomal introns in the rDNA genes of ascomycetes. *Molecular Biology and Evolution* **17**: 1971–1984.

Cannone JJ, Subramanian S, Schnare MN, Collett 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. *BioMed Central Bioinformatics* **3**: 2 [Correction: BioMed Central Bioinformatics. 3:15].

Cech TR, 1990. Self-splicing of group-I introns. *Annual Review of Biochemistry* **55**: 599–629.

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

Crooks GE, Hon G, Chandonia JM, Brenner SE, 2004. WebLogo: a sequence logo generator. *Genome Research* **14**: 1188–1190.

De Wachter R, Neefs JM, Goris A, Van de Peer Y, 1992. The gene coding for small ribosomal subunit RNA in the basidiomycete *Ustilago maydis* contains a group I intron. *Nucleic Acids Research* **20**: 1251–1257.

Diguistini S, Wang Y, Liao NY, Taylor G, Tanguay P, Feau N, Henrissat B, Chan SK, Hesse-Orce U, Alamouti SM, Tsui CK, Docking RT, Levasseur A, Haridas S, Robertson G, Birol I, Holt RA, Marra MA, Hamelin RC, Hirst M, Jones SJ, Bohlmann J, Breuil C, 2011. Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont *Grosmannia clavigera*, a lodgepole pine pathogen. *Proceedings of the National Academy of Sciences USA* **108**: 2504–2509.

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

Eickbush TH, Eickbush DG, 2007. Finely orchestrated movements: evolution of the ribosomal RNA genes. *Genetics* **175**: 477–485.

Elder JF, Turner BJ, 1995. Concerted evolution of repetitive DNA-sequences in eukaryotes. *Quarterly Review of Biology* **70**: 297–320.

Farrell B, Sequeira A, O'Meara BC, Normark B, Chung J, Jordal B, 2001. The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodini). *Evolution* **55**: 2011–2027.

Feau N, Hamelin RC, Bernier L, 2007. Variability of nuclear SSU-rDNA group introns within *Septoria* species: incongruence with host sequence phylogenies. *Journal of Molecular Evolution* **64**: 489–499.

Felsenstein FJ, 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Felsenstein FJ, 2006. *PHYLIP (Phylogeny Inference Package). Version 3.6a* Distributed by the author. Department of Genetics, University of Washington, Seattle.

Ganley ARD, Kobayashi T, 2007. Highly efficient concerted evolution in the ribosomal DNA repeats: total rDNA repeat variation revealed by whole-genome shotgun sequence data. *Genome Research* **17**: 184–191.

Gargas A, DePriest PT, Taylor JW, 1995. Positions of multiple insertions in SSU rDNA of lichen-forming fungi. *Molecular Biology and Evolution* **12**: 208–218.

Gebhardt H, Weiss M, Oberwinkler F, 2005. *Dryadomyces amasae*: a nutritional fungus associated with ambrosia beetles of the genus *Amasa* (*Coleoptera: Curculionidae*, *Scolytinae*). *Mycological Research* **109**: 687–696.

Gerbi SA, 1985. The evolution of eukaryotic ribosomal DNA. *BioSystems* **19**: 247–258.

Gibb EA, Hausner G, 2003. A group I intron-like sequence in the nuclear SSU gene of the ophiostomatoid fungus *Gondwanamyces proteae*. *Mycological Research* **107**: 1442–1450.

Gissi C, Iannelli F, Pesole G, 2008. Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. *Heredity* **101**: 301–320.

Goddard MR, Burt A, 1999. Recurrent invasion and extinction of a selfish gene. *Proceedings of the National Academy of Sciences USA* **96**: 13880–13885.

Goddard MR, Leigh J, Roger AJ, Pemberton AJ, 2006. Invasion and persistence of a selfish gene in the Cnidaria. *PLoS ONE* **1**: e3.

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

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O, 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.

Gutiérrez G, Blanco O, Divakar PK, Lumbsch HT, Crespo A, 2007. Patterns of group I intron presence in nuclear SSU rDNA of the lichen family Parmeliaceae. *Journal of Molecular Evolution* **64**: 181–195.

Grube M, Gutmann B, Arup U, de los Rios A, Mattsson J, Wedin M, 1999. An exceptional group-I intron-like insertion in the SSU rDNA of lichen mycobionts. *Current Genetics* **35**: 536–541.

Hane JK, Oliver RP, 2010. *In silico* reversal of repeat-induced point mutation (RIP) identifies the origins of repeat families and uncovers obscured duplicated genes. *BMC Genomics* **11**: 655.

Harrington TC, 1981. Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. *Mycologia* **73**: 1123–1129.

Harrington TC, 1993. Biology and taxonomy of fungi associated with bark beetles. In: Schowalter TD, Filip GM (eds), *Beetle-Pathogen Interactions in Conifer Forests*. Academic Press, pp. 37–58.

Harrington TC, Aghayeva DN, Fraedrich SW, 2010. New combinations in *Raffaelea*, *Ambrosiella*, *and Hyalorhinocladiella*, and four new species from the redbay ambrosia beetle, *Xyleborus glabratus*. *Mycotaxon* **111**: 337–361.

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

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

Haugen P, Reeb V, Lutzoni F, Bhattacharya D, 2004. The evolution of homing endonuclease genes and group I introns in nuclear rDNA. *Molecular Biology and Evolution* **21**: 129–140.

Hausner G, 2003. Fungal mitochondrial genomes, introns and plasmids. In: Arora DK, Khachatourians GG (eds), *Applied Mycology and Biotechnology. Volume III: Fungal Genomics*. Elsevier Science, New York.

Hausner G, 2012. Introns, mobile elements and plasmids. In: Bullerwell CE (ed.), *Organelle Genetics: evolution of organelle genomes and gene expression*. Springer Verlag, Berlin, pp. 329–358.

Hausner G, Reid J, 2004. The nuclear small subunit ribosomal genes of *Sphaeronaemella helvellae*, *Sphaeronaemella fimicola*, *Gabarnaudia betae* and *Cornuvesica falcata*: phylogenetic implications. *Canadian Journal of Botany* **82**: 752–762.

Hausner G, Wang X, 2005. Unusual compact rDNA gene arrangements within some members of the Ascomycota: evidence for molecular co-evolution between ITS1 and ITS2. *Genome* **48**: 648–660.

Hausner G, Reid J, Klassen GR, 1992. Do galeate-ascospore members of the Cephaloascaceae, Endomycetaceae and Ophiostomataceae share a common phylogeny? *Mycologia* **84**: 870–881.

Hausner G, Reid J, Klassen GR, 1993a. On the phylogeny of *Ophiostoma*, *Ceratocystis s.s.*, and *Microascus*, and relationships within *Ophiostoma* based on partial ribosomal DNA sequences. *Canadian Journal of Botany* **71**: 1249–1265.

Hausner G, Reid J, Klassen GR, 1993b. *Ceratocystiopsis*: a reappraisal based on molecular criteria. *Mycological Research* **97**: 625–633.

Hausner G, Reid J, Klassen GR, 2000. On the phylogeny of the members of *Ceratocystis s.l.* that possess different anamorphic states, with emphasis on the asexual genus *Leptographium*, based on partial ribosomal sequences. *Canadian Journal of Botany* **78**: 903–916.

Hausner G, Iranpour M, Kim J-J, Breuil C, Davis CN, Gibb EA, Reid J, Loewen PC, Hopkin AA, 2005. Fungi vectored by the introduced bark beetle *Tomicus piniperda* in Ontario, Canada and comments on the taxonomy of *Leptographium lundbergii*, *L. terebrantis*, *L. truncatum* and *L. wingfieldii*. *Canadian Journal of Botany* **83**: 1222–1237.

Hibbett DS, 1996. Phylogenetic evidence for horizontal transmission of group 1 introns in the nuclear ribosomal DNA of mushroom-forming fungi. *Molecular Biology and Evolution* **13**: 903–917.

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.

Johansen JS, Haugen P, 2001. A new nomenclature of group I introns in ribosomal DNA. *RNA* **7**: 935–936.
Kolařík M, Hulcr J, 2008. Mycobiota associated with the ambrosia beetle *Scolytodes unipunctatus* (Coleoptera: Curculionidae, Scolytinae). *Mycological Research* **113**: 44–60.
Li ZJ, Zhang Y, 2005. Predicting the secondary structures and tertiary interactions of 211 group I introns in IE subgroup. *Nucleic Acids Research* **33**: 2118–2128.
Liao D, 1999. Concerted evolution: molecular mechanism and biological implications. *American Journal of Human Genetics* **64**: 24–30.
Lickey EB, Hughes KW, Petersen RH, 2003. Variability and phylogenetic incongruence of an SSU nrDNA group I intron in *Artomyces*, *Auriscalpium*, and *Lentinellus* (Auriscalpiaceae: Homobasidiomycetes). *Molecular Biology and Evolution* **20**: 1909–1916.
Lindstrom SC, Pistolic J, 2005. Detection of a group I (IE) fungal intron in the green algal genus *Urospora* (Ulvophyceae). *Journal of Phycology* **41**: 359–365.
Lynch M, Koskella B, Schaack S, 2006. Mutation pressure and the evolution of organelle genomic architecture. *Science* **311**: 1727–1730.
Maddison WP, Maddison DR, 2010. Mesquite: a modular system for evolutionary analysis. Version 2.73. http://mesquiteproject.org.
Martín MP, Coucheron DH, Johansen S, 2003. Structural features and evolutionary considerations of group IB introns in SSU rDNA of the lichen fungus *Teloschistes*. *Fungal Genetics and Biology* **40**: 252–260.
Michel F, 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.
Michel F, Jacquier A, Dujon B, 1982. Comparison of fungal mitochondrial introns reveals extensive homologies in RNA secondary structure. *Biochimie* **64**: 867–881.
Nicholas KB, Nicholas HB, Deerfield II DW, 1997. GeneDoc: analysis and visualization of genetic variation. *EMB News* **4**: 14.
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.
Okada G, Jacobs K, Kirisits T, Louis-Seize GW, Seifert KA, Sugita T, Takematsu A, Wingfield MJ, 2000. Epitypification of *Graphium penicillioides* Corda, with comments on the phylogeny and taxonomy of *Graphium*-like synnematous fungi. *Studies in Mycology* **45**: 169–188.
Olchowecki A, Reid J, 1974. Taxonomy of the genus *Ceratocystis* in Manitoba. *Canadian Journal of Botany* **52**: 1675–1711.
Page RDM, 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357–358.
Perotto S, Nepote-Fus P, Saletta L, Bandi C, Young JP, 2000. A diverse population of introns in the nuclear ribosomal genes of ericoid mycorrhizal fungi includes elements with sequence similarity to endonuclease-coding genes. *Molecular Biology and Evolution* **17**: 44–59.
Plattner A, Kim J-J, Reid J, Hausner G, Woon YL, Yamaoka Y, Breuil C, 2009. Resolving taxonomic and phylogenetic incongruence within the species *Ceratocystiopsis minuta*. *Mycologia* **101**: 878–887.
Raghavan R, Minnick MF, 2009. Group I introns and inteins: disparate origins but convergent parasitic strategies. *Journal of Bacteriology* **191**: 6193–6202.
Reid J, Hausner G, 2010. The epitypification of *Ophiostoma minutum*, now *Ceratocystiopsis minuta*. *Mycotaxon* **113**: 463–474.
Ronquist F, 2004. Bayesian inference of character evolution. *Trends in Ecology and Evolution* **19**: 475–481.
Ronquist F, Huelsenbeck JP, 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
Selker EU, 2002. Repeat-induced gene silencing in fungi. *Advances in Genetics* **46**: 439–450.
Simon DM, Hummel CL, Sheely SL, Bhattacharya D, 2005a. Heterogeneity of intron presence or absence in rDNA genes of lichen species *Physcia aipolia* and *P. stellaris*. *Current Genetics* **47**: 389–399.
Simon D, Moline J, Helms G, Friedl T, Bhattacharya D, 2005b. Divergent histories of rRNA group I introns in the lichen family Physciaceae. *Journal of Molecular Evolution* **60**: 434–446.
Six DL, Wingfield MJ, 2011. The role of phytopathogenicity in bark beetle-fungus symbioses: a challenge to the classic paradigm. *Annual Review of Entomology* **56**: 255–272.
Spatafora JW, Blackwell M, 1994. Polyphyletic origins of ophiostomatoid fungi. *Mycological Research* **98**: 1–9.
Suh S-Q, Jones KG, Blackwell M, 1999. A group I intron in the nuclear small subunit rRNA gene of *Cryptendoxyla hypophloia*, an ascomycetes fungus: evidence for a new major class of group I introns. *Journal of Molecular Evolution* **48**: 493–500.
Suh S-O, Kurtzman CP, Blackwell M, 2001. The status of*Endomyces scopularum* – a filamentous fungus and two yeasts. *Mycologia* **93**: 317–322.
Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**: 4876–4882.
Upadhyay HP, 1981. *A Monograph of Ceratocystis and Ceratocystiopsis*. University of Georgia Press, Athens, G.A.
Wingfield MJ, Seifert KA, Webber JF (eds), 1993. *Ceratocystis and Ophiostoma: biology, taxonomy and ecology*. American Phytopathological Society Press, St. Paul.
Woodson SA, 2005. Structure and assembly of group I introns. *Current Opinion in Structural Biology* **15**: 324–330.
Woodson SA, Cech TR, 1989. Reverse self-splicing of the *Tetrahymena* group I intron: implication for the directionality of splicing and for intron transposition. *Cell* **157**: 335–345.
Zhou Y, Lu C, Wu Q-J, Wang Y, Sun Z-T, Deng J-C, Zhang Y, 2008. GISSD: group I intron sequence and structure database. *Nucleic Acids Research* **36**: D31–D37.
Zipfel RD, de Beer ZW, Jacobs K, Wingfield BD, Wingfield MJ, 2006. Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. *Studies in Mycology* **55**: 75–97.
Zuker M, 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* **31**: 3406–3415.