

# Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*

Renate D. Zipfel<sup>1</sup>, Z. Wilhelm de Beer<sup>2\*</sup>, Karin Jacobs<sup>3</sup>, Brenda D. Wingfield<sup>1</sup> and Michael J. Wingfield<sup>2</sup>

<sup>1</sup>Department of Genetics, <sup>2</sup>Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa; <sup>3</sup>Department of Microbiology, University of Stellenbosch, Private Bag X1, Matieland, Stellenbosch, South Africa

\*Correspondence: Z. Wilhelm de Beer, wilhelm.debeer@fabi.up.ac.za

**Abstract:** *Ophiostoma* species have diverse morphological features and are found in a large variety of ecological niches. Many different classification schemes have been applied to these fungi in the past based on teleomorph and anamorph features. More recently, studies based on DNA sequence comparisons have shown that *Ophiostoma* consists of different phylogenetic groups, but the data have not been sufficient to define clear monophyletic lineages represented by practical taxonomic units. We used DNA sequence data from combined partial nuclear LSU and  $\beta$ -tubulin genes to consider the phylogenetic relationships of 50 *Ophiostoma* species, representing all the major morphological groups in the genus. Our data showed three well-supported, monophyletic lineages in *Ophiostoma*. Species with *Leptographium* anamorphs grouped together and to accommodate these species the teleomorph-genus *Grosmannia* (type species *G. penicillata*), including 27 species and 24 new combinations, is re-instated. Another well-defined lineage includes species that are cycloheximide-sensitive with short perithecal necks, falcate ascospores and *Hyalorhinochlaediella* anamorphs. For these species, the teleomorph-genus *Ceratocystiopsis* (type species *O. minuta*), including 11 species and three new combinations, is re-instated. A third group of species with either *Sporothrix* or *Pesotium* anamorphs includes species from various ecological niches such as *Protea* infructescences in South Africa. This group also includes *O. piliferum*, the type species of *Ophiostoma*, and these species are retained in that genus. *Ophiostoma* is redefined to reflect the changes resulting from new combinations in *Grosmannia* and *Ceratocystiopsis*. Our data have revealed additional lineages in *Ophiostoma* linked to morphological characters. However, these species are retained in *Ophiostoma* until further data for a larger number of species can be obtained to confirm monophyly of the apparent lineages.

**Taxonomic novelties:** *Ceratocystiopsis manitobensis* (J. Reid & Hausner) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *Cop. parva* (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *Cop. rollhanseniana* (J. Reid, Eydólfssd. & Hausner) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *Grosmannia abiocarpa* (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. aenigmatica* (K. Jacobs, M.J. Wingf. & Yamaoka) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. americana* (K. Jacobs & M.J. Wingf.) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. aurea* (R.C. Rob. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. cainii* (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. clavigera* (R.C. Rob. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. crassivaginata* (H.D. Griffin) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. cucullata* (H. Solheim) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. davidsonii* (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. dryocoetidis* (W.B. Kendr. & Molnar) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. europioides* (E.F. Wright & Cain) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. francke-grosmanniae* (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. galeiformis* (B.K. Bakshi) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. grandifoliae* (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. huntii* (R.C. Rob.) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. laricis* (K. van der Westh., Yamaoka & M.J. Wingf.) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. leptographioides* (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. olivacea* (Math.-Käärik) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. pseudoeuropioides* (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. radiicola* (J.-J. Kim, Seifert, & G.-H. Kim) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. robusta* (R.C. Rob. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. sagmatospora* (E.F. Wright & Cain) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. vesca* (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov., *G. wagneri* (Goheen & F.W. Cobb) Zipfel, Z.W. de Beer & M.J. Wingf. comb. nov.

**Key words:** *Ceratocystiopsis*, *Grosmannia*, *Ophiostoma*, phylogenetics.

## INTRODUCTION

Considerable confusion has surrounded the taxonomy of the so-called ophiostomatoid fungi ever since the first descriptions of *Ophiostoma* Syd. & P. Syd. and *Ceratocystis* Ellis & Halst. (Table 1). The majority of these fungi are specifically adapted for dispersal by insects, they resemble each other morphologically and they typically share similar niches linked to their biological characteristics. During the course of the last decade, phylogenetic studies based on DNA sequence comparisons have been applied to these fungi and they confirmed suggestions (De Hoog 1974, Von Arx 1974, Weijman & De Hoog 1975, Harrington 1981, 1984) that the two keystone genera, *Ophiostoma* and *Ceratocystis*, have polyphyletic origins (Hausner *et al.* 1992, 1993b,c, Spatafora & Blackwell 1994). Species sensitive to the antibiotic cycloheximide, and with *Thielaviopsis* Went anamorphs and endoconidia arising

from ring wall-building conidium development (Minter *et al.* 1983), clearly reside in *Ceratocystis* in the order *Microascales* Luttrell ex Benny & Kimbr. (Hausner *et al.* 1993b, Spatafora & Blackwell 1994, Paulin-Mahady *et al.* 2002). Species tolerant to cycloheximide, containing rhamnose and cellulose in their cell walls, and with anamorphs residing in *Sporothrix* Hektoen & C.F. Perkins, *Hyalorhinochlaediella* H.P. Upadhyay & W.B. Kendr., *Leptographium* Lagerb. & Melin, or *Pesotium* J.L. Crane & Schokn. emend. G. Okada & Seifert, reside in *Ophiostoma* in the *Ophiostomatales* Benny & Kimbr. (Hausner *et al.* 1993c, Spatafora & Blackwell 1994). Application of this definition for *Ophiostoma* results in more than 140 species, exhibiting a large variety of distinct teleomorph and anamorph features.

Teleomorph characters applied in taxonomic studies of *Ophiostoma* include the shape and size of the ascomata and ascospores, and the presence or absence of sheaths surrounding the ascospores.

Table 1. Definition of genera of the ophiostomatoid fungi as applied by different authors.

General/groups based on morphology		Ophiostoma	Ceratocystis	Grosmannia	Ceratocystiopsis	Europhium
<b>Teleomorph:</b>	<b>ascomata</b>	long to short-necked	long-necked	long to short-necked	short-necked	cleistothecia
	<b>ascospores</b>	various	various	various	falcate	hat-shaped
	<b>sheath around ascospores</b>	seldom	often	yes	yes	yes
<b>Anamorph(s)</b>		Sporothrix Hyalorhinochlaidiella Pesotum	Thielaviopsis	Leptographium Pesotum-like	Hyalorhinochlaidiella Sporothrix-like	Leptographium
(Halsted 1890)		*	Ceratocystis gen. nov.			
** (Hedgcock 1906)		Ceratostomella	Ceratostomella			
(Münc 1907)		Ceratostomella	Endoconidiophora gen. nov.			
(Sydow & Sydow 1919)		Ophiostoma gen. nov.	Ophiostoma			
(Nannfeldt 1932, Melin & Nannfeldt 1934)		Ophiostoma	Ophiostoma			
(Goidánich 1935, 1936)		Ophiostoma		Grosmannia gen. nov.		
(Siemaszko 1939)		Ophiostoma	Ophiostoma	Ophiostoma	Ophiostoma	
(Davidson 1942)		Ceratostomella	Ceratostomella	Ceratostomella	Ceratostomella	
(Bakshi 1951, Moreau 1952)		Ceratocystis	Ceratocystis	Ceratocystis		
(Von Arx 1952, Von Arx & Müller 1954)		Ophiostoma	Ophiostoma	Ophiostoma		
(Hunt 1956)		Ceratocystis	Ceratocystis	Ceratocystis	Ceratocystis	
(Parker 1957)						Europhium gen. nov.
(Wright & Cain 1961, Griffin 1968, Olchoweki & Reid 1973)		Ceratocystis	Ceratocystis	Ceratocystis	Ceratocystis	Europhium
(De Hoog 1974, Harrington 1987)		Ophiostoma	Ceratocystis	Ophiostoma	Ophiostoma	Ophiostoma
(Von Arx 1974)		Ophiostoma	Ceratocystis	Ophiostoma		Europhium
(Weijman & De Hoog 1975)		Ophiostoma	Ceratocystis			
(Upadhyay & Kendrick 1975)		Ceratocystis	Ceratocystis	Ceratocystis	Ceratocystiopsis gen. nov.	Ceratocystis
(Upadhyay 1981)		Ceratocystis	Ceratocystis	Ceratocystis	Ceratocystiopsis	Ceratocystis
(De Hoog & Scheffer 1984, Wingfield et al. 1993)		Ophiostoma	Ceratocystis	Ophiostoma	Ceratocystiopsis	Ophiostoma
(Von Arx & Van der Walt 1987)		Ophiostoma	Ceratocystis	Ophiostoma	Ceratocystiopsis	Europhium
(Hausner et al. 1992, 1993a–c, 2000, 2003, Hausner & Reid 2003)		Ophiostoma	Ceratocystis	Ophiostoma	Ophiostoma	Ophiostoma
(Jacobs & Wingfield 2001)		Ophiostoma	Ceratocystis	Ophiostoma	Ophiostoma	Ophiostoma
<b>This study</b>		Ophiostoma		Grosmannia	Ceratocystiopsis	Grosmannia

\* White spaces indicate that the particular author(s) did not include species from that specific group/genus in their study.

\*\* Example: Hedgcock (1906) included eight species in his study, seven of which fall according to morphology in the Ophiostoma group (column) and one in the Ceratocystis group (column), but he treated all in the genus *Ceratostomella*.

Table 2. Species and origin of strains included in this study.

Species	CBS no <sup>a</sup>	CMW no <sup>b</sup>	Substrate/Host	Origin	Collector	Anamorph	Ascospore shape	Sheath	GenBank no	
									LSU	$\beta$ -tubulin
<i>Leptographium lundbergii</i>	352.29	217	<i>Pinus</i> sp.	Europe	M. Lagerberg	<i>Leptographium</i> (Hedgcock 1906)	no teleomorph (Jacobs & Wingfield 2001)	-	DQ294388	DQ296108
<i>L. truncatum</i>	118584	29	<i>Pinus taeda</i>	South Africa	M.J. Wingfield	<i>Leptographium</i> (Jacobs & Wingfield 2001)	no teleomorph (Jacobs & Wingfield 2001)	-	DQ294390	DQ296110
<i>L. wageneri</i> var. <i>wageneri</i>	119492	1827	<i>Pinus monophylla</i>	U.S.A.	T. Harrington	<i>Leptographium</i> (Jacobs & Wingfield 2001)	bean-shaped (Jacobs & Wingfield 2001)	n	DQ294397	DQ296117
<i>Ophiostoma aenigmaticum</i> <sup>c</sup>	501.96	2199	<i>Picea jezoensis</i>	Japan	Y. Yamaoka	<i>Leptographium</i> (Jacobs & Wingfield 2001)	cucullate, hat-shaped (Jacobs & Wingfield 2001)	y	DQ294391	DQ296111
<i>O. africanum</i>	116571	823	<i>Protea gaguedi</i>	South Africa	M.J. Wingfield	<i>Sporothrix</i> (Jacobs & Wingfield 2001)	lunate (Marais & Wingfield 2001)	n	AF221015 <sup>d</sup>	DQ296073
<i>O. ainoae</i>	118672	1903	<i>Picea abies</i>	Norway	O. Olsen	<i>Pesotum</i> (Marais & Wingfield 2001)	cylindrical (Solheim 1986)	y	DQ294368	DQ296088
<i>O. aureum</i>	438.69 <sup>e</sup>	667	<i>Pinus contorta</i> var. <i>latifolia</i>	Canada	R.W. Davidson	<i>Leptographium</i> (Solheim 1986)	cucullate, hat-shaped (Jacobs & Wingfield 2001)	y	DQ294389	DQ296109
<i>O. araucariae</i>	114.68 <sup>e</sup>	671	<i>Araucaria</i> sp.	Chile	H. Butin	<i>Pesotum</i> (Robinson-Jeffrey & Davidson 1968)	ovoid to cylindrical (Okada <i>et al.</i> 1998)	n	DQ294373	DQ296093
<i>O. canum</i>	118668	5023	<i>Tomiscus minor</i>	Austria	T. Kirisits	<i>Pesotum</i> (Butin 1968)	orange section (Harrington <i>et al.</i> 2001)	n	DQ294372	DQ296092
<i>O. carpenteri</i>	118670	13793	<i>Trypodendron lineatum</i>	U.S.A.	S.E. Carpenter	<i>Sporothrix</i> -like (Mathiesen 1951)	narrowly clavate, straight or curved (Hausner <i>et al.</i> 2003)	n	DQ294363	DQ296083
<i>O. crassivaginatatum</i>	119144	134	unknown	unknown	T. Hinds	<i>Leptographium</i> (Hausner <i>et al.</i> 2003)	fusiform (Jacobs & Wingfield 2001)	y	DQ294386	DQ296106
<i>O. distortum</i>	397.77	467	ambrosia beetle gallery in <i>Picea engelmannii</i>	U.S.A.	R.W. Davidson	<i>Sporothrix</i> (Jacobs & Wingfield 2001)	orange section (Seifert <i>et al.</i> 1993)	n	DQ294369	DQ296089
<i>O. flexuosum</i>	208.83 <sup>e</sup>	907	<i>Picea abies</i>	Norway	H. Solheim	<i>Sporothrix</i> (Davidson 1971)	cylindrical to ossiform (Solheim 1986)	y	DQ294370	DQ296090
<i>O. floccosum</i>	1713	1713	<i>Pinus ponderosa</i>	U.S.A.	C. Bertagnole	<i>Pesotum</i> (Solheim 1986)	kidney-shaped (Harrington <i>et al.</i> 2001)	n	DQ294367	DQ296087
<i>O. francke-grosmanniae</i>	118671	2975	<i>Larix</i> sp.	U.S.A.	M.J. Wingfield	<i>Leptographium</i> (Mathiesen 1951)	hat-shaped, cucullate (Jacobs & Wingfield 2001)	y	DQ294395	DQ296115

Table 2. (Continued).

Species	CBS no <sup>a</sup>	CMW no <sup>b</sup>	Substrate/Host	Origin	Collector	Anamorph	Ascospore shape	Sheath	GenBank no	
									LSU	$\beta$ -tubulin
<i>O. fusiforme</i>	112912 <sup>e</sup>	9968	<i>Populus nigra</i>	Azerbaijan	D.N. Aghayeva	<i>Sporothrix</i> (Jacobs & Wingfield 2001)	allantoid (Aghayeva et al. 2004)	n	DQ294354	AY28461 <sup>d</sup>
<i>O. galeiforme</i>	115711	5290	<i>Pinus sylvestris</i>	Scotland	T. Kirisits	<i>Leptographium</i> (Aghayeva et al. 2004)	hat-shaped, bean-shaped (Zhou et al. 2004b)	y	DQ294383	DQ296103
<i>O. grandifoliae</i>	119679	703	<i>Fagus grandifolia</i>	U.S.A.	R.W. Davidson	<i>Leptographium</i> (Zhou et al. 2004b)	allantoid (Jacobs & Wingfield 2001)	y	DQ294399	DQ296119
<i>O. huntii</i>	153.65 <sup>e</sup>	2808	<i>Pinus contorta</i>	Canada	R.C. Robinson-Jeffrey	<i>Leptographium</i> (Jacobs & Wingfield 2001)	curved (Jacobs & Wingfield 2001)	y	DQ294387	DQ296107
<i>O. ips</i>	137.36 <sup>e</sup>	7075	<i>Ips integer</i>	U.S.A.	C.T. Rumbold	<i>Pesotum/Leptographium/Hyalorhinocladiella</i> (Jacobs & Wingfield 2001)	pillow-shaped (Seifert et al. 1993)	y	DQ294381	DQ296101
<i>O. laricis</i>		1913	<i>Larix</i> sp.	Japan	Y. Yamaoka	<i>Leptographium</i> (Rumbold 1936)	curved (Jacobs & Wingfield 2001)	y	DQ294393	DQ296113
<i>O. leptographioides</i>	144.59	481	unknown	U.S.A.	R.W. Davidson	<i>Leptographium</i> (Jacobs & Wingfield 2001)	hat-shaped, reniform (Jacobs & Wingfield 2001)	y	DQ294382	DQ296102
<i>O. lunatum</i>	112928	10564	<i>Larix decidua</i>	Austria	T. Kirisits	<i>Sporothrix</i> (Jacobs & Wingfield 2001)	allantoid (Aghayeva et al. 2004)	n	DQ294355	AY280467 <sup>d</sup>
<i>O. manitobense<sup>f</sup></i>	118838	13792	bark of <i>Pinus resinosa</i>	Canada	J. Reid	<i>Hyalorhinocladiella<sup>g</sup></i> (Aghayeva et al. 2004)	falcate (Hausner et al. 2003)	y	DQ294358	DQ296078
<i>O. minimum</i>	182.86	162	<i>Pinus banksiana</i>	U.S.A.	M.J. Wingfield	<i>Hyalorhinocladiella</i> (Hausner et al. 2003)	falcate (Upadhyay 1981)	y	DQ294361	DQ296081
<i>O. minutum</i>	119682	4586	<i>Ips cembrae</i>	Scotland	T. Kirisits	<i>Hyalorhinocladiella</i> (Upadhyay 1981)	falcate (Upadhyay 1981)	y	DQ294360	DQ296080
<i>O. minutum-bicolor</i>	393.77	1018	<i>Ips</i> gallery in <i>Pinus</i>	U.S.A.	R.W. Davidson	<i>Hyalorhinocladiella</i> (Siemaszko 1939)	falcate (Upadhyay & Kendrick 1975)	y	DQ294359	DQ296079
<i>O. montium</i>	151.78	13221	<i>Dendroctonus ponderosae</i> gallery in <i>P. ponderosa</i>	U.S.A.	R.W. Davidson	<i>Pesotum (= Graphilbum) / Hyalorhinocladiella</i> (Olchoweki & Reid 1973)	pillow-shaped (Upadhyay 1981)	y	DQ294379	DQ296099
<i>O. multiannulatum</i>	357.77	2567	<i>Pinus</i> sp.	U.S.A.	unknown	<i>Sporothrix</i> (Rumbold 1941)	reniform (De Hoog 1974)	n	DQ294366	DQ296086
<i>O. nigrocarpum</i>	638.66 <sup>e</sup>	651	<i>Pseudotsuga menziesii</i>	U.S.A.	R.W. Davidson	<i>Sporothrix</i> (Davidson 1935)	reniform (De Hoog 1974)	n	DQ294356	AY280480 <sup>d</sup>

Table 2. (Continued).

Species	CBS no <sup>a</sup>	CMW no <sup>b</sup>	Substrate/Host	Origin	Collector	Anamorph	Ascospore shape	Sheath	GenBank no	
									LSU	$\beta$ -tubulin
<i>O. novo-ulmi</i>	119476	10573	<i>Picea abies</i>	Austria	Neumuller	<i>Pesotum</i> (Davidson 1966)	orange section (Harrington et al. 2001)	n	DQ294375	DQ296095
<i>O. penicillatum</i>	140.36 <sup>e</sup>	470	<i>Picea abies</i>	Germany	H. Grossmann	<i>Leptographium</i> (Brasier 1991)	allantoid (Jacobs & Wingfield 2001)	y	DQ294385	DQ296105
<i>O. penicillatum</i>	116008	2644	wood from <i>Picea abies</i>	Norway	H. Solheim	<i>Leptographium</i> (Jacobs & Wingfield 2001)	allantoid (Jacobs & Wingfield 2001)	y	DQ294384	DQ296104
<i>O. piceae</i>	119678	8093	<i>Teitropium</i> sp.	Canada	K. Harrison	<i>Pesotum</i> (Jacobs & Wingfield 2001)	lunate (Harrington et al. 2001)	n	DQ294371	DQ296091
<i>O. piceiperdum</i>	366.75	660	<i>Picea abies</i>	Finland	A.M. Hallakselä	<i>Leptographium</i> (Harrington et al. 2001)	cucullate (Jacobs & Wingfield 2001)	y	DQ294392	DQ296112
<i>O. piliferum</i>	129.32	7879	<i>Pinus sylvestris</i>	unknown	H. Diddens	<i>Sporothrix</i> (Jacobs & Wingfield 2001)	orange section (De Hoog 1974)	n	DQ294377	DQ296097
<i>O. piliferum</i>	118835	7877	unknown	unknown	unknown	<i>Sporothrix</i> (Upadhyay 1981)	orange section (De Hoog 1974)	n	DQ294378	DQ296098
<i>O. pluriannulatum</i>	118684	75	unknown	unknown	R.W. Davidson	<i>Sporothrix</i> (Upadhyay 1981)	reniform (Seifert et al. 1993)	n	DQ294365	DQ296085
<i>O. protearum</i>	116568	1102	<i>Protea caffra</i>	South Africa	M.J. Wingfield	<i>Sporothrix</i> (Hedgcock 1906)	lunate (Marais & Wingfield 1997)	n	AF221014 <sup>d</sup>	DQ296072
<i>O. pulvinisporum</i>	118673 <sup>e</sup>	9022	<i>Pinus pseudostrabus</i>	Mexico	X. Zhou	<i>Hyalorhinocladiella</i> / <i>Leptographium</i> / <i>Pesotum</i> (Marais & Wingfield 1997)	pillow-shaped (Zhou et al. 2004a)	y	DQ294380	DQ296100
<i>O. quercus</i>	118713	3110	<i>Juglans cinerea</i>	U.S.A.	M.J. Wingfield	<i>Pesotum</i> (Zhou et al. 2004a)	reniform (Harrington et al. 2001)	n	DQ294376	DQ296096
<i>O. ranaculosum</i>	119683	13940	<i>Pinus echinata</i>	U.S.A.	F. Hains	<i>Sporothrix</i> (Georgévitch 1926)	falcate (Bridges & Perry 1987)	y	DQ294357	DQ296077
<i>O. robustum</i>	119480	2805	unknown	unknown	T. Hinds	<i>Leptographium</i> (Bridges & Perry 1987)	hat-shaped, reniform (Jacobs & Wingfield 2001)	y	DQ294398	DQ296118
<i>O. rollhanseni</i>	118669	13791	beetle galleries in <i>Pinus sylvestris</i>	Norway	J. Reid	<i>Hyalorhinocladiella</i> <sup>e</sup> (Jacobs & Wingfield 2001)	falcate (Hausner et al. 2003)	y	DQ294362	DQ296082
<i>O. serpens</i>	641.76	290	<i>Pinus pinea</i>	Italy	Gambogi	<i>Leptographium</i> (Hausner et al. 2003)	ellipsoid (Jacobs & Wingfield 2001)	y	DQ294394	DQ296114
<i>O. splendens</i>	116569	872	<i>Protea repens</i>	South Africa	M.J. Wingfield	<i>Sporothrix</i> (Jacobs & Wingfield 2001)	lunate (Marais & Wingfield 1994)	n	AF221013 <sup>d</sup>	DQ296071

80 Table 2. (Continued).

Species	CBS no <sup>a</sup>	CMW no <sup>b</sup>	Substrate/Host	Origin	Collector	Anamorph	Ascospore shape	Sheath	GenBank no	
									LSU	$\beta$ -tubulin
<i>O. stenoceras</i>	237.32 <sup>e</sup>	3202	pine pulp	Norway	H. Robak	<i>Sporothrix</i> (Marais & Wingfield 1994)	orange section (De Hoog 1974)	n	DQ294350	DQ296074
<i>O. subannulatum</i>	118667	518	<i>Pinus ponderosa</i>	unknown	W. Livingston	<i>Sporothrix</i> (Robak 1932)	allantoid to broadly lunate (Livingston & Davidson 1987)	n	DQ294364	DQ296084
<i>O. ulmi</i>	119479	1462	<i>Ulmus procera</i>	U.S.A.	C. Brasier	<i>Pesotum</i> (Livingston & Davidson 1987)	elongate orange section (Harrington et al. 2001)	n	DQ294374	DQ296094
<i>O. wagneri</i>	118845	491	<i>Pinus jeffreyi</i>	unknown	T. Harrington	<i>Leptographium</i> (Buisman 1932)	bean-shaped (Jacobs & Wingfield 2001)	n	DQ294396	DQ296116
<i>Sporothrix inflata</i>	239.68 <sup>e</sup>	12527	soil	Germany	W. Gams	<i>Sporothrix</i> (Jacobs & Wingfield 2001)	no teleomorph	-	DQ294351	DQ296075
<i>S. schenckii</i>	117842	7614	human sporotrichosis	South Africa	H. Vismer	<i>Sporothrix</i> (De Hoog 1974)	no teleomorph	-	DQ294352	AY280477 <sup>d</sup>
	119145	17137	horse	South Africa	J.A. Picard	<i>Sporothrix</i> (De Hoog 1974)	no teleomorph	-	DQ294353	DQ296076

<sup>a</sup>CBS = Culture Collection of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

<sup>b</sup>CMW = Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

<sup>c</sup>Species names in bold type are species transferred to *Grosmannia* in this study.

<sup>d</sup>DNA sequence was obtained from GenBank.

<sup>e</sup>Ex-type cultures.

<sup>f</sup>Underlined species names are species transferred to *Ceratocystiopsis* in this study.

<sup>g</sup>No anamorph-genus mentioned in the original description. We assign a genus here based on our interpretation of the original species description.



The majority of *Ophiostoma* spp. have ascomata with long necks giving rise to masses of sticky ascospores adapted for dispersal by insects (Upadhyay 1981, Harrington 1987, Jacobs & Wingfield 2001). In 1957, Parker described the genus *Europhium* A.K. Parker for a species that exhibits all the characters of *Ophiostoma*, but with ascocarps cleistothecial, lacking necks and ostioles (Parker 1957). Subsequently three additional species were described in *Europhium* (Robinson-Jeffrey & Davidson 1968). All four of the species were eventually transferred to *Ophiostoma* (Harrington 1987) because the formation or length of necks, and the presence of an ostiole, might be affected by the environment and were considered 'less reliable' taxonomic characters (Upadhyay 1981). A number of phylogenetic studies confirmed that these species are closely related to *Ophiostoma* spp. with *Leptographium* anamorphs (Hausner *et al.* 2000, Lim *et al.* 2004).

*Ophiostoma* spp. have ascospores with unusual shapes. Several studies have applied this characteristic to define groups within the genus, which at the time of these studies was treated as a synonym of *Ceratocystis* (Wright & Cain 1961, Griffin 1968, Olchowecki & Reid 1973, Upadhyay 1981). For species that have falcate ascospores with sheaths and short perithecial necks, Upadhyay & Kendrick (1975) established *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. However, Wingfield (1993) argued that ascospore shape should not be the sole character to delineate genera, and that it was illogical to maintain *Ceratocystiopsis* as a separate genus because *Ophiostoma* contained many species with a variety of other, distinct ascospore forms. He thus suggested that *Ceratocystiopsis* should be treated as a synonym of *Ophiostoma* and that ascospore morphology should only be one of several characteristics on which to base further subdivisions in the genus. Hausner *et al.* (1993a) proceeded to formally reduce *Ceratocystiopsis* to synonymy with *Ophiostoma*, based on partial SSU and LSU rDNA sequences. These authors included 10 *Ceratocystiopsis* spp., but only one *Ophiostoma* (*O. ips* (Rumbold) Nannf.) and one *Ceratocystis* (*C. fimbriata* Ellis & Halst.) species in the phylogenetic analysis of the data. Phylogenetic studies involving other ascomycete genera confirmed that ascospore morphology should not be used as the only character for taxonomic grouping as similar ascospore shapes often originated more than once in a genus (Hausner *et al.* 1992, Wingfield *et al.* 1994).

The diversity of the anamorphs associated with *Ophiostoma* established anamorph morphology as a preferred characteristic to group species in the genus (Münch 1907, Melin & Nannfeldt 1934, Hunt 1956, Davidson 1958, Mathiesen-Käärik 1960). However, this approach is complicated by the fact that a significant number of *Ophiostoma* spp. produce not only one, but combinations of up to three of the four possible anamorph states associated with the genus (De Hoog 1974, Okada *et al.* 1998). *Ophiostoma ips*, for example, has a continuum of synanamorph states, which based on current definitions range from *Hyalorhinocladiella*-like and *Leptographium*-like to *Pesotum* (Seifert *et al.* 1993).

The anamorphs of just this one species have previously been classified in *Graphium* (Leach *et al.* 1934), *Scopularia* Preuss (Goidánich 1936), *Cephalosporium auct. non* Corda (Moreau 1952), *Leptographium* (Moreau 1952), *Hyalorhinocladiella* (Upadhyay 1981), *Graphillum* H.P. Upadhyay & W.B. Kendr. (Upadhyay 1981), *Acremonium* Link : Fr. (Hutchison & Reid 1988), and *Pesotum* (Okada *et al.* 1998).

The only case where a teleomorph-genus has specifically been erected to accommodate *Ophiostoma* spp. based on a common anamorph, was when Goidánich (1936) established *Grosmannia* Goid. for four species with *Leptographium* anamorphs. He first described *Grosmannia* invalidly, without a Latin description (Goidánich 1935). Later Goidánich validated the genus and at the same time corrected the spelling to *Grosmannia* (Goidánich 1936). Siemaszko (1939) reduced *Grosmannia* to synonymy with *Ophiostoma* on the basis of teleomorph morphology. *Grosmannia* has been treated in all subsequent studies as synonym of either *Ophiostoma* (Mathiesen 1951, Von Arx 1952, De Hoog 1974, Von Arx 1974, Seifert *et al.* 1993, Jacobs & Wingfield 2001) or *Ceratocystis* (Bakshi 1951, Moreau 1952, Hunt 1956, Davidson 1958, Griffin 1968, Upadhyay 1981). Phylogenetic studies have placed three of the original four *Grosmannia* species, *G. serpens* Goid., *G. penicillata* (Grosmann) Goid. and *G. ips* (Rumbold) Goid., in *Ophiostoma* (Hausner *et al.* 2000, Jacobs *et al.* 2001, Zhou *et al.* 2004a, 2005). The fourth species, *G. pini* (Münch) Goid., has been treated as a synonym of *O. minus* (Hedgc.) Syd. & P. Syd. (Moreau 1952, Hunt 1956, Griffin 1968, Olchowecki & Reid 1973, Upadhyay 1981) which, based on phylogeny, also resides in *Ophiostoma* (Gorton *et al.* 2004).

Amongst the four anamorph-genera associated with *Ophiostoma* spp., *Sporothrix* appears to be the most common form, with conidia produced sympodially on denticles arising from undifferentiated hyphae (De Hoog 1974). This is also the form that occurs most often as a synanamorph of *Pesotum* spp. (Crane & Schoknecht 1973, De Hoog 1974, Okada *et al.* 1998, Harrington *et al.* 2001). The original description of *Pesotum* included mononematous conidiophores and conidiogenous cells with prominent denticles, thus the *Sporothrix*-like component of the anamorph (Crane & Schoknecht 1973). In a study showing that *Graphium* is phylogenetically distinct from the synnematal anamorphs of *Ophiostoma* spp., and where *Pesotum* was redefined to encompass all synnematal anamorphs of *Ophiostoma*, only the synnematal form was described (Okada *et al.* 1998). The *Sporothrix* form was thus treated as a distinct synanamorph of *Pesotum* (Okada *et al.* 1998). However, Harrington *et al.* (2001) accepted the original description of *Pesotum*, which included the *Sporothrix*-like forms, but restricted the genus to anamorphs with affinities to the *O. piceae* complex. Harrington *et al.* (2001) also stated that the synnemata of *Ophiostoma* spp. outside the *O. piceae* complex are loose aggregates of *Leptographium* conidiophores, without the fused stipe cells that are characteristic of the *O. piceae* complex.

These synnematosous species outside the *O. piceae* complex often lack a *Sporothrix* anamorph, although some species produce a mononematous form without prominent denticles, resembling *Hyalorhinocladiella*. *Hyalorhinocladiella* was described for the mononematous anamorphs of *Ceratocystiopsis* and *Ophiostoma* (Upadhyay & Kendrick 1975), where conidia are produced through sympodial proliferation, leaving flat, ring-like scars on the surface of conidiogenous cells, as opposed to the denticles visible in *Sporothrix* spp. (Mouton *et al.* 1994, Benade *et al.* 1996). Although these anamorph-genera can be defined broadly, the delimitation of species groups based on anamorph morphology remains problematic, especially because of intermediate and overlapping forms.

Phylogenetic studies have substantially improved the ability to delimit species within almost all the morphological groups (based on ascospores and anamorphs) of the genus *Ophiostoma* (Harrington *et al.* 2001, De Beer *et al.* 2003, Jacobs & Kirisits 2003, Kim *et al.* 2003, Gorton *et al.* 2004, Lim *et al.* 2004, Zhou *et al.* 2004b). In the more recent of these studies, multi-gene approaches employing ribosomal together with protein-coding genetic data have become the norm. The morphological divergence in *Ophiostoma* strongly suggests that some of the morphological traits must be represented by monophyletic lineages. However, phylogenetic studies that have all been based on partial ribosomal DNA data have failed to support the definition of monophyletic lineages in *Ophiostoma* (Hausner *et al.* 1993b, 2000, Jacobs *et al.* 2001, Hausner & Reid 2003). In this investigation we reconsider the view that *Ophiostoma* might be logically subdivided based on monophyly. This is achieved using DNA sequences from domains 1 and 2 of the 5' end of the nuclear LSU gene, together with partial sequences for the  $\beta$ -tubulin gene region. Fifty species of *Ophiostoma* representing all the ascospore forms and anamorph shapes associated with the genus are included in the study.

## MATERIALS AND METHODS

### Isolates

Isolates used in this study (Table 2) are maintained at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, as well as in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa. Cultures were grown on malt extract agar (MEA, 2 % malt extract [Biolab, Merck] and 2 % agar [Biolab, Merck]) at 21–24 °C for DNA extraction.

A large variety of potential outgroups were initially tested for suitability in the phylogenetic analysis used in this study. From these tests three species of *Cryphonectria* were selected as being the most appropriate and these include: *Cry. cubensis* (CBS 101281; LSU = AF408338;  $\beta$ -tubulin = DQ246580), *Cry. havanensis* (CBS 505.63; LSU = AF408339;  $\beta$ -tubulin = AY063478), and *Cry. nitschkei* (CBS 109776; LSU = AF408341;  $\beta$ -tubulin = DQ120768). The names used here are those published by Castlebury *et al.* (2002)

in GenBank although we recognize that Gryzenhout *et al.* (2005) have shown that *Cry. havanensis* (CBS 505.63) is incorrectly identified and also represents *Cry. cubensis*.

### DNA extraction and PCR

DNA was extracted from mycelium grown on 2 % MEA using the DNA extraction method described by Aghayeva *et al.* (2004). Two genes were amplified for sequencing and phylogenetic analysis. The 5' end of the nuclear large subunit rDNA was amplified using the primers LR0R (5' ACCCGCTGAACTTAAGC 3') and LR5 (5' TCCTGAGGGAACTTCG 3') (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>). Part of the  $\beta$ -tubulin gene was amplified with primers T10 (5' ACGATAGGTTACCTCCAGAGAC 3') (O'Donnell & Cigelnik 1997) or Bt2a (5' GGTAACCAAATCGGTGC GCTTTC 3') in combination with Bt2b (5' GGTAACCAAATCGGTGCTGCTTTC 3') (Glass & Donaldson 1995). Reaction volumes for the PCR amplification were 50  $\mu$ L and contained 5  $\mu$ L 10  $\times$  PCR reaction buffer (Super-Therm, JMR Holdings, U.S.A.), 2.5 mM MgCl<sub>2</sub>, 10 mM dNTP, 10  $\mu$ M of each primer, 2  $\mu$ L DNA and 2.5 U Super-Therm Taq polymerase (JMR Holdings, U.S.A.). The PCR conditions for the amplification of both the LSU and  $\beta$ -tubulin genes included denaturing for 3 min at 94 °C, annealing at 47–52 °C for 1 min, and elongation at 72 °C for 1 min. This was repeated for 35 cycles ending with a final elongation step at 72 °C for 5 min. Success of the PCR amplification was confirmed on a 1 % (w/v) agarose gel stained with ethidium bromide. DNA was visualized under UV light. The PCR fragments were purified with QIAquick® PCR purification kit (Quiagen®) eluting the DNA in water.

### DNA sequencing

Sequencing of the purified PCR fragments was performed using the primers noted above and the Big Dye™ Terminator v. 3.0 cycle sequencing premix kit (Applied Biosystems, Foster City, CA, U.S.A.). The fragments were analyzed on an ABI PRISIM™ 377 or ABI PRISIM™ 3100 Genetic Analyzer (Applied Biosystems). DNA Sequence data were edited using Sequence Navigator (Applied Biosystems) and aligned in CLUSTAL-X (Thompson *et al.* 1997) and then in T-Coffee (Notredame *et al.* 2000) using multiple alignment algorithms. T-Coffee was used to combine the alignment results of Clustal X with the local and global pairwise alignments obtained in T-Coffee, to produce a multiple sequence alignment with the best agreement of these methods. The default parameters in T-Coffee were used for the analysis. Manual adjustments of the dataset were performed in PAUP v. 4.0b8 (Phylogenetic Analysis Using Parsimony) (Swofford 2001) as follows: for the analysis of the partial LSU gene, sequences were trimmed at the 5' and 3' ends to align with DNA sequences from GenBank used for the outgroups. For the partial  $\beta$ -tubulin gene the sequences were trimmed on the 5' end to correspond with the beginning of exon 4 of the  $\beta$ -tubulin gene. Analyses were carried out using parsimony, neighbour-joining and maximum likelihood



(Swofford 2001) and Bayesian inference (MrBayes 3.0b4) (Huelsenbeck & Ronquist 2001).

### Phylogenetic analysis

**Maximum parsimony:** For parsimony analysis, ambiguous and missing nucleotides were eliminated and the remaining characters were weighted according to the consistency index (CI). A heuristic search was performed with tree-bisection-reconnection (TBR) branch swapping. The resulting trees were used to obtain a majority rule consensus tree. Confidence values were estimated using Bootstrap analysis (1000 replicates) with the full consensus option.

**Bayesian inference:** Data were analysed using a Bayesian approach based on a Markov chain Monte Carlo (MCMC) analysis. A general time reversal (GTR+I+G) model as determined by AIC criteria of Modeltest (Posada & Crandall 1998) was used for the analysis. The proportion of sites was assumed to be invariable, while the rate of the remaining sites was drawn from a gamma distribution with six categories. All parameters were inferred from the data. Four Markov chains were initiated at random and the program was allowed to run for 2000000 generations with a sample frequency of 100. The analysis was repeated six times and consensus trees obtained from the six independent analyses were examined for consistency. One of the six analyses was used to calculate a consensus tree with mean branch lengths. The likelihood convergence was determined and these sampled trees were discarded as burn in. The following trees with their branch lengths were used to generate a consensus tree based on 50 % majority rule with mean branch lengths and posterior probabilities for the nodes using PAUP (Swofford 2001).

**Neighbour-joining:** A distance tree was calculated using Neighbour-joining analysis based on the evolutionary model that was determined as GTR+I+G based on AIC criteria using the Modeltest 3.06 (Posada & Crandall 1998). Distance settings were adjusted according to the Akaike information criteria (AIC) model: proportion invariable sites were assumed to be 0.4369 and the rates for variable sites were assumed to follow a gamma distribution with shape parameter of 0.5593. Confidence was determined by 1000 bootstrap replicates. The starting tree was obtained from the Neighbour-joining tree, the branch swap algorithm set to TBR (tree bisection reconnection).

**Maximum likelihood:** Likelihood settings were set according to GTR+I+G model as determined by AIC criteria in Modeltest 3.06 (Posada & Crandall 1998). Assumed proportion invariable sites were set to 0.4369. The variable sites were assumed to have a gamma distribution with a 0.5593 shape parameter. The search was performed heuristically with random stepwise addition and TBR branch swapping. Confidence values were estimated using bootstrap analysis (1000 replicates) determined by heuristic search and TBR branch swapping.

## RESULTS

### DNA sequence comparisons

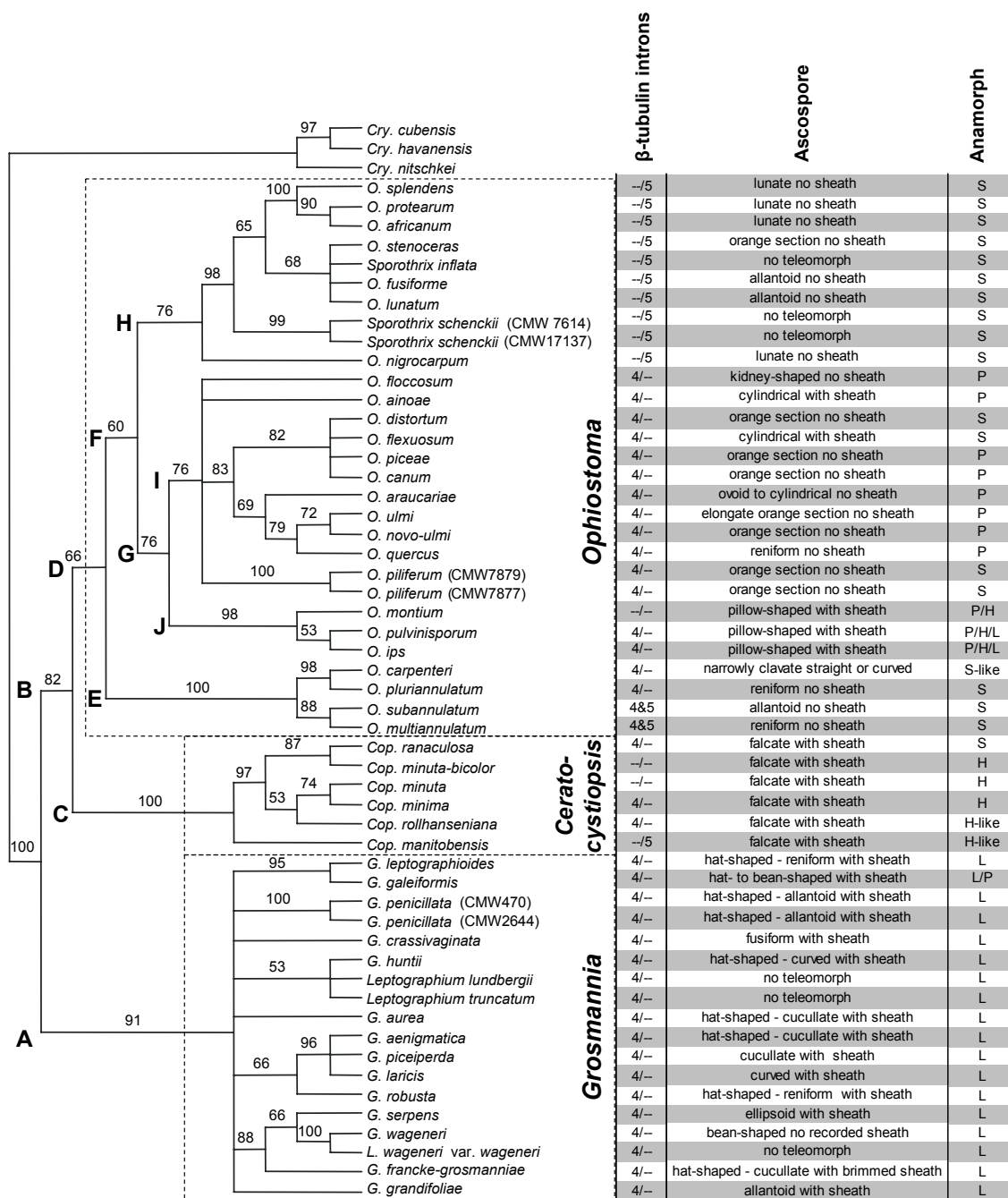
The 5' region of the LSU gene resulted in amplicons in the range of 697–702 nucleotides. This amplicon included the D1 and D2 region of the LSU gene. Amplicons in the range of 218–334 nucleotides in length were obtained from the partial  $\beta$ -tubulin gene. This region included exon 4, exon 5 and the 5' end of exon 6, as well as intron 4 situated between exons 4 and 5, and intron 5 between exons 5 and 6. DNA sequences of the three exons were of equal length for all taxa studied. However introns 4 and 5 were highly variable in both nucleotide length and DNA sequence. Some taxa lacked either intron 4 or intron 5 or both introns. The high level of variability of the DNA sequence observed in the introns, and the presence or absence of the introns (Fig. 1), accounted for the large difference in  $\beta$ -tubulin sequence lengths.

DNA sequence alignments resulted in 714 characters for the partial LSU gene and 402 characters for the partial  $\beta$ -tubulin gene. However, due to the high level of variability of the introns found in the  $\beta$ -tubulin region, the intron sequences were excluded from further analysis, resulting in 220 characters of the  $\beta$ -tubulin gene used in the analysis.

### Phylogenetic analysis

Preliminary cladistic analysis based on parsimony showed that trees generated for both LSU (data not shown) and combined LSU/ $\beta$ -tubulin gene regions had similar topologies. Furthermore, the combined data set resulted in higher confidence values for the obtained groupings. Combined LSU and  $\beta$ -tubulin data sets (excluding  $\beta$ -tubulin introns), which consisted of a total of 934 characters, were thus used. Congruence of the LSU and  $\beta$ -tubulin datasets was not supported by the partition homogeneity test (PHT). This was most probably due to the highly conserved nature of the  $\beta$ -tubulin gene resulting in poor resolution of the terminal branches. The similar node topology of the trees obtained from the LSU and LSU/ $\beta$ -tubulin genes, and the increased bootstrap support for the groups obtained using the combined data set, justified that the data of these two genes should be considered together, irrespective of the incongruence of the loci.

**Maximum parsimony:** For the cladistic analysis of the combined data set, 39 missing and ambiguous characters were excluded from the analysis. Of the remaining 895 characters, 608 characters were constant, 48 variable characters were parsimony-uninformative and 239 characters were parsimony-informative. Characters were re-weighted according to the maximum consistency index. This resulted in 751 characters with a weight of 1, and 144 characters with a weight other than 1. Four trees with similar topologies were obtained using maximum parsimony analysis with the tree bisection reconnection (TBR) branch swapping algorithm. The deeper nodes were consistent in all four trees, with slight variations in the topology of the terminal nodes. From the four trees a 50 % majority rule consensus tree

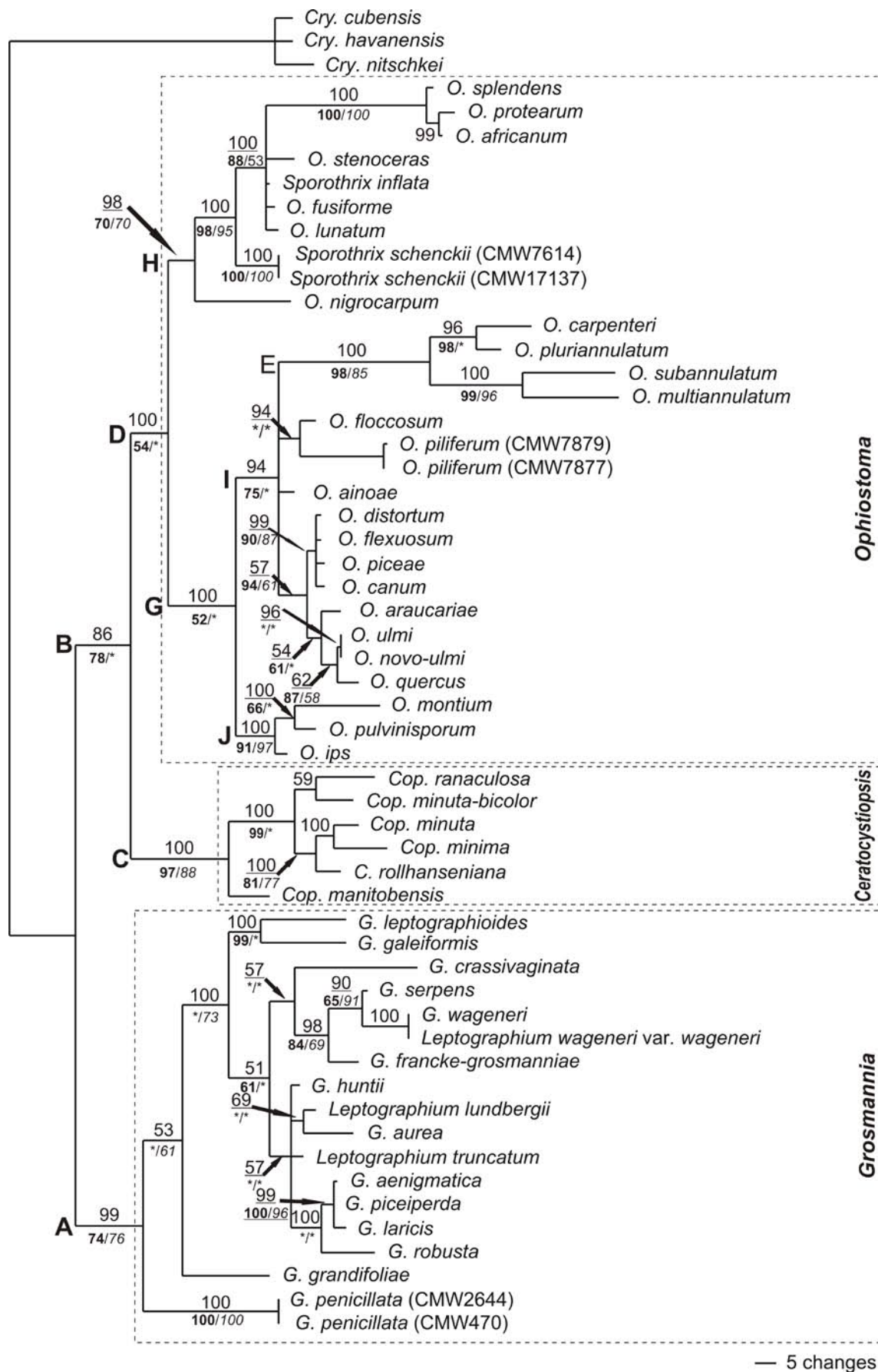


**Fig. 1.** Cladogram based on 50 % majority rule consensus tree (tree length = 383 steps; CI = 0.656; RI = 0.860) obtained from four trees produced by maximum parsimony analysis with the TBR algorithm, using a heuristic search on the combined data set of partial nuclear LSU and  $\beta$ -tubulin DNA sequence. Data was weighted according to consistency index. Bootstrap support values (1000 replicates) above 50 % are indicated at the branches. The tree was rooted to the outgroup consisting of three *Cryphonectria* spp. The following information is indicated in columns next to the taxa:  $\beta$ -tubulin introns (4 = intron 4 present; 5 = intron 5 present). Ascospore shapes are described, and the presence or absence of sheaths indicated. Anamorphs associated with each taxon (H = *Hyalorhinocladiella*; L = *Leptographium*; P = *Pesotum*, S = *Sporothrix*).

was compiled with the TBR algorithm. The tree length was 383 steps, CI = 0.656, and the retention index (RI) = 0.860. A consensus cladogram was obtained (Fig. 1).

The cladogram (Fig. 1) showed that the taxa are grouped in distinct, well-supported clades. Clade A (91 % bootstrap) included only taxa that have *Leptographium* anamorph states. All the species in this group had intron 4 and lacked intron 5 in the  $\beta$ -tubulin gene (Fig. 1). Clade B (82 % bootstrap) consisted of several distinct groups. Within Clade B, Clade C (100 % bootstrap) formed a monophyletic group including the recently described species *O. rollhansenianum* J. Reid, Eyjólfsson & Hausner and *O. manitobense* J.

Reid & Hausner, as well as taxa previously residing in the genus *Ceratocystiopsis*. These taxa all have short perithecial necks and falcate ascospores, and are sensitive to cycloheximide. Two anamorph states are associated with taxa in this group. They are *Sporothrix*, the anamorph of *O. ranaculosum* (J.R. Bridges & T.J. Perry) Hausner, J. Reid & Klassen, and *Hyalorhinocladiella*, associated with *O. minuta-bicolor* (R.W. Davidson) Hausner, J. Reid & Klassen, *O. minutum* Siemaszko, *O. minimum* (Olchowecki & J. Reid) Hausner, J. Reid & Klassen, *O. rollhansenianum* and *O. manitobense*.



**Fig. 2.** Phylogram resulting from a Bayesian Monte Carlo Markov chain (MCMC) analyses of 934 nucleotides of partial LSU and  $\beta$ -tubulin sequences. The 50 % Majority rule consensus tree was obtained from 18000 trees. The numbers above each node indicate posterior probabilities obtained from Bayesian analyses. Bootstrap values (1000 replicates) obtained for Neighbour-joining and Maximum likelihood analyses are indicated below each node in **bold** and *italic*, respectively. A support less than 50 % is represented by \*. In Neighbour-joining analysis group E is situated basal to groups I and J, and not part of group I. In Maximum likelihood analysis groups D and G are not supported, and group E forms a separate clade not linked to any other clade.



Clade D (Fig. 1) had a relatively low bootstrap support of 66 %. The taxa in this clade were subdivided in numerous smaller clades with various levels of confidence support. Clade E (100 % bootstrap) included four taxa with *Sporothrix* anamorphs producing secondary conidia. *Ophiostoma pluriannulatum* (Hedgc.) Syd. & P. Syd., *O. subannulatum* Livingston & R.W. Davidson, and *O. multiannulatum* (Hedgc. & R.W. Davidson) Hendr. have naked (no sheath) reniform ascospores, and *O. carpenteri* J. Reid & Hausner has naked, narrowly clavate ascospores. Clade F had poor bootstrap support (60 %) and consists of two subclades (G and H). Within Clade G, *O. ips*, *O. pulvinisporum* X.D. Zhou & M.J. Wingf., and *O. montium* (Rumbold) Arx, formed one well-supported group (Clade J, 98 % bootstrap support). These species have pillow-shaped ascospores protected by a sheath and a continuum of anamorphs including *Hyalorhinochlaediella* and *Pesotum*. The second subclade (I, with bootstrap 76 %) was less well defined and consisted of members of the *O. piceae* complex with *Pesotum* anamorphs and *O. piliferum* (Fr.) Syd. & P. Syd. Other species in this clade were *O. ainoae* H. Solheim and *O. araucariae* (Butin) de Hoog & R.J. Scheff. with *Pesotum*-like anamorphs, and *O. distortum* (Davidson) de Hoog & R.J. Scheff., *O. flexuosum* H. Solheim, and *O. piliferum* with *Sporothrix* anamorphs. All species in this clade had intron 4 and lacked intron 5 in the  $\beta$ -tubulin gene (Fig. 1).

Clade H (76 % bootstrap) consisted only of taxa with *Sporothrix* anamorphs and ascospores varying from orange section to allantoid in shape. The species in this clade all lacked intron 4 and had intron 5 in the  $\beta$ -tubulin gene (Fig. 1). In this clade, *O. nigrocarpum* (R.W. Davidson) de Hoog grouped separately from the other taxa that formed a clade with 98 % bootstrap support. Species in this clade include *Sporothrix schenckii* Hektoen & C.F. Perkins, the type species for the anamorph-genus *Sporothrix*, *O. stenoceras* (Robak) Nannf., *S. inflata* de Hoog, *O. fusiforme* D.N. Aghayeva & M.J. Wingf. and *O. lunatum* D.N. Aghayeva & M.J. Wingf. The three species of *Ophiostoma* found within infructescences of *Protea* spp. in South Africa, *O. splendens* G.J. Marias & M.J. Wingf., *O. protearum* G.J. Marias & M.J. Wingf., and *O. africanum* G.J. Marias & M.J. Wingf., constituted a well-defined, smaller clade with strong bootstrap support within Clade H.

**Bayesian inference:** Consistent results were obtained in the six runs of the Bayesian phylogenetic analysis (Model GTR+I+G). The topologies of the obtained trees differed only slightly in the terminal nodes where low confidence values were obtained. No variations were observed in the deeper nodes supported by high confidence values. The stationary phase of the Markov chains was observed after 33000 generations. The first 2000 trees (representing 200000 generations) were thus discarded and 18000 trees were included to calculate the 50 % rule consensus tree for each run. One of the phylogenetic trees obtained is presented in Fig. 2. The calculated confidence values (posterior probabilities) are indicated above the relevant nodes where support exceeded 50 %.

The deeper nodes obtained from the Bayesian analysis (MB) were identical to those obtained with maximum parsimony (MP). Support for the groups was, however, higher for Bayesian inference in the deeper branches than the Bootstrap support obtained for MP: group A (MB = 99 %; MP = 91 %), group B (MB = 86 %; MP = 82 %), group C (MB = 100 %; MP = 100 %), group D (MB = 100 %; MP = 66 %). Group D consisted of several subgroups. Groups E (MB = 100 %; MP = 100 %), H (MB = 98 %; MP = 76 %), and J (MB = 100 %; MP = 98 %) remain clustered together with high statistical support. However, the topology of the groups found within group D, obtained from Bayesian inference, differ in structure from the topology obtained in MP analysis. One major difference in topology is that group E forms as a separate group basal to group H and G in the MP analysis, while Bayesian inference resulted in group E forming part of group G. However, group E remained a separate entity with high posterior probability support.

**Neighbour-joining:** Phylogenetic distance was determined by Neighbour-joining (NJ) analyses based on the general time reversal model. Statistical support for the nodes was calculated using 1000 NJ bootstrap repeats. NJ support values for nodes obtained are indicated bold (Fig. 2). The topology obtained from NJ is similar to that obtained from Bayesian inference. With the exception of group E, clustering basal to group J and I closest to group G and not basal to groupings G and H or within group F as observed on MP and Bayesian analysis respectively.

**Maximum likelihood:** For the phylogenetic relationship estimated using maximum likelihood (ML), the GTR+I+G evolutionary model determined by Model Test based on Akaike Information Criteria (AIC) was applied. Estimated proportion invariable sites (I) was set to 0.4369 and the shape parameter for gamma distribution (G) was set to 0.5595 and no molecular clock was enforced on the data set. Bootstrap values for the groupings were determined by 1000 bootstrap repeats. ML support (50 % or higher) for groups obtained are indicated in italics (Fig. 2) on the phylogenetic tree obtained by Bayesian inference. Groups A–C, E, and H–J were supported by ML. However the deeper node resolution of these groups differs significantly from MP and Bayesian inference. Groupings D and G were not supported and group B had poor ML statistical support.

For consistency in the discussion we refer to the clades obtained in parsimony analysis, and the groups obtained in Bayesian, NJ and ML analysis, as groups.

## TAXONOMY

Analyses of phylogenetic data obtained in this study provided strong support for the hypothesis that the genus *Ophiostoma* includes at least three monophyletic lineages. Two of these lineages correspond clearly with a combination of both anamorph and teleomorph

characters. These characters have also previously been recognised as taxonomically informative and have been employed to define the two genera, *Grosmannia* and *Ceratocystiopsis*. Based on robust phylogenetic support as well as clearly defined morphological characters, we re-instate these genera with emended descriptions and establish the necessary new combinations. The description of the genus *Ophiostoma* is emended to reflect these taxonomic changes.

***Ophiostoma*** Syd. & P. Syd., Ann. Mycol. 17: 43. 1919. emend. Z.W. de Beer, Zipfel & M.J. Wingf.

= *Linostoma* Höhn., Ann. Mycol. 16: 91. 1918. (*non* Wallich, Cat. East Indies Comp., London. 1828).

= *Ophiostoma* Syd. & P. Syd. section *Brevirostrata* Nannf., Svenska SkogsvFör. Tidskr. 32: 407. 1934.

= *Ophiostoma* Syd. & P. Syd. section *Longirostrata* Nannf. *pro parte*, Svenska SkogsvFör. Tidskr. 32: 407. 1934.

*Ascocarps* subhyaline to dark brown to black, bases globose; necks straight or flexuous, cylindrical, brown to black; ostiole often surrounded by ostiolar hyphae. *Asci* 8-spored, evanescent, globose to broadly clavate. *Ascospores* hyaline, aseptate, cylindrical, lunate, allantoid, reniform, orange section- or pillow-shaped, sometimes with a hyaline, gelatinous sheath. *Anamorphs* most commonly *Sporothrix* and/or *Pesotum*, occasionally *Hyalorhinocladiella*-like, rarely *Leptographium*-like. Phylogenetically classified in the *Ophiostomatales*.

**Type species: *Ophiostoma piliferum*** Fr. : Fr. Syd. & P. Syd., Ann. Mycol. 17: 43. 1919.

**Basionym:** *Sphaeria pilifera* Fr., Syst Mycol. 2: 472. 1822.

≡ *Ceratostoma piliferum* (Fr.) Fuckel, Symb. Mycol. p. 128. 1869.

≡ *Ceratostomella pilifera* (Fr.) G. Winter, Rabenh. Kryptogamen-Flora 1: 252. 1887.

≡ *Linostoma piliferum* (Fr.) Höhn., Ann. Mycol. 16: 91. 1918.

≡ *Ceratocystis pilifera* (Fr.) C. Moreau, Rev. Mycol. (Paris), Suppl. Colon. 17: 22. 1952.

**Anamorph:** *Sporothrix* (De Hoog 1974).

***Ceratocystiopsis*** H.P. Upadhyay & W.B. Kendr., Mycologia 67: 799. 1975. emend. Z.W. de Beer, Zipfel & M.J. Wingf.

*Ascocarps* subhyaline to dark brown to black, bases globose to subglobose; necks relatively short, mostly tapered toward the apex, sometimes surrounded by a collar-like structure; ostiolar hyphae convergent or lacking. *Asci* 8-spored, evanescent, fusiform, clavate or ellipsoidal, hyaline. *Ascospores* hyaline, aseptate, elongate, falcate, or slender with obtuse ends, sometimes with bulbous swelling, most often with a hyaline sheath. Sensitive to cycloheximide. *Anamorphs* *Hyalorhinocladiella* or *Sporothrix*-like. Phylogenetically classified in the *Ophiostomatales* within a monophyletic lineage including *Ceratocystiopsis minuta*.

(1) Type species: ***Ceratocystiopsis minuta*** (Siemaszko) H.P. Upadhyay & W.B. Kendr., Mycologia 67: 800. 1975.

**Basionym:** *Ophiostoma minutum* Siemaszko, Planta Pol. 7: 23. 1939.

≡ *Ceratostomella minuta* (Siemaszko) R.W. Davidson, Mycologia

34: 655. 1942.

≡ *Ceratocystis minuta* (Siemaszko) J. Hunt, Lloydia 19: 49. 1956.

= *Ceratocystis dolominuta* H.D. Griffin, Canad. J. Bot. 46: 702. 1968.

**Anamorph:** *Hyalorhinocladiella* (Upadhyay 1981).

**Note:** Synonymy of *C. dolominuta* and *Cop. minuta* suggested by Upadhyay (1981).

(2) ***Ceratocystiopsis brevicomi*** Hsiau & T.C. Harr., Mycologia 89: 661. 1997.

**Anamorph:** not assigned to a genus (Hsiau & Harrington 1997).

**Phylogenetic information:** *Ceratocystiopsis brevicomi* is distinct from, but close to *Cop. ranaculosa* and *Cop. collifera* (Hsiau & Harrington 1997, Six & Paine 1999).

(3) ***Ceratocystiopsis collifera*** Marm. & Butin, Sydowia 42: 197. 1990.

**Basionym:** *Ophiostoma colliferum* (Marm. & Butin) Hausner, J. Reid & Klassen, Mycol. Res. 97: 631. 1993.

**Anamorph:** *Sporothrix* (Marmolejo & Butin 1990).

**Phylogenetic information:** *Ceratocystiopsis collifera* is closely related to *Cop. minima* and *Cop. parva* (Hausner *et al.* 1993a).

(4) ***Ceratocystiopsis concentrica*** (Olchow. & J. Reid) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 121. 1981.

**Basionym:** *Ceratocystis concentrica* Olchow. & J. Reid, Canad. J. Bot. 52: 1679. 1974.

≡ *Ophiostoma concentricum* (Olchow. & J. Reid) Hausner & J. Reid, Canad. J. Bot. 81: 874. 2003.

**Anamorph:** *Hyalorhinocladiella* (De Hoog 1993).

**Phylogenetic information:** *Ceratocystiopsis concentrica* is part of the *Minuta* complex *sensu* Hausner & Reid (2003).

(5) ***Ceratocystiopsis manitobensis*** (J. Reid & Hausner) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500805.

**Basionym:** *Ophiostoma manitobense* J. Reid & Hausner, Canad. J. Bot. 81: 46. 2003.

**Anamorph:** Not assigned to a genus (Hausner *et al.* 2003), but morphologically similar to *Hyalorhinocladiella*.

(6) ***Ceratocystiopsis minima*** (Olchow. & J. Reid) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 129. 1981.

**Basionym:** *Ceratocystis minima* Olchow. & J. Reid, Canad. J. Bot. 52: 1684. 1974.

≡ *Ophiostoma minimum* (Olchow. & J. Reid) Hausner, J. Reid & Klassen, Mycol. Res. 97: 631. 1993.

**Anamorph:** *Hyalorhinocladiella* (Upadhyay 1981).

(7) ***Ceratocystiopsis minuta-bicolor*** (R.W. Davidson) H.P. Upadhyay & W.B. Kendr., Mycologia 67: 800. 1975.

**Basionym:** *Ceratocystis minuta-bicolor* R.W. Davidson, Mycopath. Mycologia Appl. 28: 280. 1966.

≡ *Ophiostoma minutum-bicolor* (R.W. Davidson) Hausner, J. Reid & Klassen, Mycol. Res. 97: 631. 1993.

= *Ceratocystis pallida* H.D. Griffin, Canad. J. Bot. 46: 708. 1968.



*Anamorph: Hyalorhinocladiella minuta-bicolor* H.P. Upadhyay & W.B. Kendr., *Mycologia* 67: 800. 1975.

*Note:* Synonymy of *C. pallida* with *Cop. minuta-bicolor* suggested by Upadhyay (1981).

(8) ***Ceratocystiopsis pallidobrunnea*** (Olchow. & J. Reid) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 133. 1981.

*Basionym: Ceratocystis pallidobrunnea* Olchow. & J. Reid, *Canad. J. Bot.* 52: 1685. 1974.

≡ *Ophiostoma pallidobrunneum* (Olchow. & J. Reid) Hausner & J. Reid, *Canad. J. Bot.* 81: 875. 2003.

*Anamorph: Hyalorhinocladiella* (De Hoog 1993).

*Phylogenetic information:* Part of the *Minuta* complex *sensu* Hausner & Reid (2003).

(9) ***Ceratocystiopsis parva*** (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500806.

*Basionym: Ceratocystis parva* Olchow. & J. Reid, *Canad. J. Bot.* 52: 1686. 1974.

≡ *Ophiostoma parvum* (Olchow. & J. Reid) Hausner, J. Reid & Klassen, *Mycol. Res.* 97: 631. 1993.

*Anamorph:* not assigned to an anamorph-genus (Olchowecki & Reid 1973), but similar to *Hyalorhinocladiella*.

*Phylogenetic information:* Upadhyay treated this species as synonym of *Cop. minima*, but Hausner, Reid & Klassen (1993a) showed that *Cop. parva* is closely related to, but distinct from *Cop. minima* and *Cop. minuta*.

(10) ***Ceratocystiopsis ranaculosa*** J.R. Bridges & T.J. Perry, *Mycologia* 79: 631. 1987.

≡ *Ophiostoma ranaculosum* (J.R. Bridges & T.J. Perry) Hausner, J. Reid & Klassen, *Mycol. Res.* 97: 631. 1993.

*Anamorph: Sporothrix* (Bridges & Perry 1987).

(11) ***Ceratocystiopsis rollhanseniana*** (J. Reid, Eyjólfssd. & Hausner) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500807.

*Basionym: Ophiostoma rollhansenianum* J. Reid, Eyjólfssd. & Hausner, *Canad. J. Bot.* 81: 44. 2003.

*Anamorph:* not assigned to a genus (Hausner *et al.* 2003), but morphologically similar to *Hyalorhino-cladiella*.

#### Status of other species linked to *Ceratocystiopsis*

(a) ***Ceratocystiopsis alba*** (DeVay, R.W. Davidson & W.J. Moller) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 120. 1981.

*Basionym: Ceratocystis alba* DeVay, R.W. Davidson & W.J. Moller, *Mycologia* 60: 636. 1968.

*Anamorph: Hyalorhinocladiella* (Upadhyay 1981).

*Phylogenetic information:* *Ceratocystiopsis alba* is phylogenetically unrelated to any of the genera in the *Ophiostomatales* (Hausner *et al.* 1993a).

(b) ***Ophiostoma carpenteri*** J. Reid & Hausner, *Canad. J. Bot.* 81: 42. 2003.

*Anamorph:* not assigned to a genus (Hausner *et al.*

2003), but morphologically similar to *Hyalorhino-cladiella*.

*Phylogenetic information:* Outside the *Minuta* complex, appears to be related to *O. retusum* (Hausner *et al.* 1993a, Hausner & Reid 2003).

(c) ***Ceratocystiopsis conicicollis*** (Olchow. & J. Reid) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 122. 1981.

*Basionym: Ceratocystis conicicollis* Olchow. & J. Reid, *Canad. J. Bot.* 52: 1680. 1974.

*Anamorph: Hyalorhinocladiella* (Upadhyay 1981).

*Phylogenetic information:* none – status uncertain.

(d) ***Grosmannia crassivaginata*** (H.D. Griffin) Zipfel, Z.W. de Beer & M.J. Wingf. (see under *Grosmannia*, this study).

(e) ***Ophiostoma crenulatum*** (Olchow. & J. Reid) Hausner & J. Reid, *Canad. J. Bot.* 81: 875. 2003.

*Basionym: Ceratocystis crenulata* Olchow. & J. Reid, *Canad. J. Bot.* 52: 1681. 1974.

≡ *Ceratocystiopsis crenulata* (Olchow. & J. Reid) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 124. 1981.

*Anamorph: Hyalorhinocladiella* (Upadhyay 1981).

*Phylogenetic information:* Outside the *Minuta* complex, appears to be related to *O. fasciatum* (Hausner & Reid 2003).

(f) ***Cornuvesica falcata*** (E.F. Wright & Cain) C.D. Viljoen, M.J. Wingf. & K. Jacobs, *Mycol. Res.* 104: 366.

*Basionym: Ceratocystis falcata* E.F. Wright & Cain, *Canad. J. Bot.* 39: 1226. 1961.

≡ *Ceratocystiopsis falcata* (E.F. Wright & Cain) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 125. 1981.

*Anamorph: Chalara*-like (Viljoen *et al.* 2000).

*Phylogenetic information:* *Cornuvesica falcata* is phylogenetically unrelated to the *Ophiostomatales* (Hausner *et al.* 2000).

(g) ***Ophiostoma fasciatum*** (Olchow. & J. Reid) Hausner, J. Reid & Klassen, *Mycol. Res.* 97: 631. 1993.

*Basionym: Ceratocystis fasciata* Olchow. & J. Reid, *Canad. J. Bot.* 52: 1682. 1974.

= *Ceratocystis spinifera* Olchow. & J. Reid, *Canad. J. Bot.* 52: 1686. 1974.

*Anamorph: Hyalorhinocladiella* (Upadhyay 1981).

*Phylogenetic information:* *Ophiostoma fasciatum* is not part of the *Minuta* complex, and related to *O. crenulatum* and *O. ips* (Hausner *et al.* 1993a, Hausner & Reid 2003).

(h) ***Ophiostoma longisporum*** (Olchow. & J. Reid) Hausner, J. Reid & Klassen, *Mycol. Res.* 97: 631. 1993.

*Basionym: Ceratocystis longispora* Olchow. & J. Reid, *Canad. J. Bot.* 52: 1683. 1974.

≡ *Ceratocystiopsis longispora* (Olchow. & J. Reid) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 128. 1981.

Anamorph: *Sporothrix* (Upadhyay 1981).

*Phylogenetic information:* *Ophiostoma longisporum* is not part of the *Minuta* complex, and falls basal to *O. ips* within *Ophiostoma* (Hausner *et al.* 1993a, Hausner & Reid 2003).

(i) ***Ceratocystiopsis ochracea*** (H.D. Griffin) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 132. 1981.

*Basionym:* *Ceratocystis ochracea* H.D. Griffin, *Canad. J. Bot.* 46: 706. 1968.

*Anamorph:* no anamorph on type material and no description of anamorph in Griffin (1968).

*Phylogenetic information:* none – status uncertain.

(j) ***Gondwanamyces proteae*** (M.J. Wingf., P.S. van Wyk & Marasas) G.J. Marais & M.J. Wingf., *Mycologia* 90: 139. 1998.

*Basionym:* *Ceratocystiopsis proteae* M.J. Wingf., P.S. van Wyk & Marasas, *Mycologia* 80: 24. 1988.

*Anamorph:* *Knoxdaviesia proteae* M.J. Wingf., P.S. van Wyk & Marasas, *Mycologia* 80: 26. 1988.

*Phylogenetic information:* *Gondwanamyces proteae* has been placed in the order *Microascales*, and is unrelated to the *Ophiostomatales* (Viljoen *et al.* 1999).

(k) ***Ophiostoma retusum*** (R.W. Davidson & T.E. Hinds) Hausner, J. Reid & Klassen, *Mycol. Res.* 97: 631. 1993.

*Basionym:* *Ceratocystis retusi* R.W. Davidson & T.E. Hinds, *Mycologia* 64: 407. 1972.

≡ *Ceratocystiopsis retusi* (R.W. Davidson & T.E. Hinds) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 135. 1981.

*Anamorph:* *Sporothrix* (Seifert *et al.* 1993, Benade *et al.* 1998).

*Phylogenetic information:* *Ophiostoma retusum* is not part of the *Minuta* complex, but closer to *O. ips* and *O. carpenteri* (Hausner *et al.* 1993a, Hausner & Reid 2003).

(l) ***Ceratocystiopsis spinulosa*** (H.D. Griffin) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 136. 1981.

*Basionym:* *Ceratocystis spinulosa* H.D. Griffin, *Canad. J. Bot.* 46: 713. 1968.

*Anamorph:* *Hyalorhinocladia* (De Hoog 1993).

*Phylogenetic information:* none – status uncertain.

***Grosmannia*** Goid., *Boll. Staz. Patol. Veg.* 16: 27. 1936. emend. Z.W. de Beer, Zipfel & M.J. Wingf.

= *Europhium* A.K. Parker, *Canad. J. Bot.* 35: 175. 1957.

*Ascomata* black, bases globose, seldom ornamented; necks absent or present, pigmented, tapered toward apex; ostiolar hyphae mostly absent, when present, convergent or divergent. *Asci* 8-spored, evanescent. *Ascospores* hyaline, aseptate, reniform, curved, allantoid, fusiform, orange section- or hat-shaped, often invested in a sheath. *Anamorph* *Leptographium*, or with synnemata appearing as a loose aggregation of *Leptographium* conidiophores. Phylogenetically

classified in the *Ophiostomatales* within a monophyletic group containing *Grosmannia penicillata*.  $\beta$ -tubulin gene contains intron 4 and lacks intron 5.

(1) Type species: ***Grosmannia penicillata*** (Grosmann) Goid., *Boll. Staz. Patol. Veg.* 16: 27. 1936.

*Basionym:* *Ceratosomella penicillata* Grosmann, *Hedwigia* 72: 190. 1932.

≡ *Ophiostoma penicillatum* (Grosmann) Siemaszko, *Planta Pol.* 7: 24. 1939.

≡ *Ceratocystis penicillata* (Grosmann) C. Moreau, *Rev. Mycol. (Paris), Suppl. Colon.* 17: 22. 1952.

*Anamorph:* *Leptographium penicillatum* Grosmann, *Z. Parasitenk.* 3: 94. 1931.

≡ *Scopularia penicillata* (Grosmann) Goid., *Boll. Staz. Patol. Veg.* 16: 39. 1936.

≡ *Verticicladiella penicillata* (Grosmann) W.B. Kendr., *Canad. J. Bot.* 40: 776. 1962.

(2) ***Grosmannia abiocarpa*** (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500808.

*Basionym:* *Ceratocystis abiocarpa* R.W. Davidson, *Mycopathol. Mycol. Appl.* 28: 273. 1966.

≡ *Ophiostoma abiocarum* (R.W. Davidson) T.C. Harr., *Mycotaxon* 28: 41. 1987.

*Anamorph:* *Leptographium* (Upadhyay 1981).

*Phylogenetic information:* *Grosmannia abiocarpa* is closely related to *G. penicillata* and *G. huntii* (Jacobs *et al.* 2001).

(3) ***Grosmannia aenigmatica*** (K. Jacobs, M.J. Wingf. & Yamaoka) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500809.

*Basionym:* *Ophiostoma aenigmaticum* K. Jacobs, M.J. Wingf. & Yamaoka, *Mycol. Res.* 102: 291. 1998.

*Anamorph:* *Leptographium aenigmaticum* M.J. Wingf. & Yamaoka, *Mycol. Res.* 102: 291. 1998.

(4) ***Grosmannia americana*** (K. Jacobs & M.J. Wingf.) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500810.

*Basionym:* *Ophiostoma americanum* K. Jacobs & M.J. Wingf., *Canad. J. Bot.* 75: 1318. 1997.

*Anamorph:* *Leptographium americanum* K. Jacobs & M.J. Wingf., *Canad. J. Bot.* 75: 1318. 1997.

*Phylogenetic information:* *Grosmannia americana* is closely related to *G. penicillata* (Jacobs *et al.* 2001) and *G. huntii* (Jacobs *et al.* 2001, Kim *et al.* 2004).

(5) ***Grosmannia aurea*** (R.C. Rob.-Jeffr. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500811.

*Basionym:* *Europhium aureum* R.C. Rob. & R.W. Davidson, *Canad. J. Bot.* 46: 1525. 1968.

≡ *Ceratocystis aurea* (R.C. Rob.-Jeffr. & R.W. Davidson) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 37. 1981.

≡ *Ophiostoma aureum* (R.C. Rob.-Jeffr. & R.W. Davidson) T.C. Harr., *Mycotaxon* 28: 41. 1987.

*Anamorph:* *Leptographium aureum* M.J. Wingf., *Trans. Brit. Mycol. Soc.* 85: 92. 1985.

(6) ***Grosmannia cainii*** (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500812.

*Basionym:* *Ceratocystis cainii* Olchow. & J. Reid, *Canad. J. Bot.* 52: 1697. 1974.

≡ *Ophiostoma cainii* (Olchow. & J. Reid) T.C. Harr., *Mycotaxon* 28: 41. 1987.

*Anamorph:* *Pesotum* (Okada *et al.* 1998, Kim *et al.* 2005).

*Phylogenetic information:* *Grosmannia cainii* is closely related to *G. leptographioides* (Kim *et al.* 2005).

(7) *Grosmannia clavigera* (R.C. Rob.-Jeffr. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500813.

*Basionym:* *Europhium clavigerum* R.C. Rob.-Jeffr. & R.W. Davidson, *Canad. J. Bot.* 46: 1523. 1968.

≡ *Ceratocystis clavigera* (R.C. Rob.-Jeffr. & R.W. Davidson) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 40. 1981.

≡ *Ophiostoma clavigerum* (R.C. Rob.-Jeffr. & R.W. Davidson) T.C. Harr., *Mycotaxon* 28: 41. 1987.

*Anamorph:* *Leptographium clavigerum* (H.P. Upadhyay) T.C. Harr., Six & McNew, *Mycologia* 95: 791. 2003.

≡ *Graphiocladiella clavigera* H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 40. 1981.

≡ *Pesotum clavigerum* (H.P. Upadhyay) G. Okada & Seifert, *Canad. J. Bot.* 76: 1503. 1998.

*Phylogenetic information:* *Grosmannia clavigera* is closely related to *G. robusta* and *G. aurea* (Kim *et al.* 2004, Lim *et al.* 2004, Kim *et al.* 2005).

(8) *Grosmannia crassivaginata* (H.D. Griffin) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500814.

*Basionym:* *Ceratocystis crassivaginata* H.D. Griffin, *Canad. J. Bot.* 46: 701. 1968.

≡ *Ceratocystiopsis crassivaginata* (H.D. Griffin) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 123. 1981.

≡ *Ophiostoma crassivaginatum* (H.D. Griffin) T.C. Harr., *Mycotaxon* 28: 41. 1987.

*Anamorph:* *Leptographium crassivaginatum* M.J. Wingf., *Trans. Brit. Mycol. Soc.* 85: 92. 1985.

(9) *Grosmannia cucullata* (H. Solheim) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500815.

*Basionym:* *Ophiostoma cucullatum* H. Solheim, *Nordic J. Bot.* 6: 202–203. 1986.

*Anamorph:* *Pesotum* (Okada *et al.* 1998).

*Phylogenetic information:* *Grosmannia cucullata* groups with several other *Leptographium* spp. (Hausner *et al.* 2000).

(10) *Grosmannia davidsonii* (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500816.

*Basionym:* *Ceratocystis davidsonii* Olchow. & J. Reid, *Canad. J. Bot.* 52: 1698. 1974.

≡ *Ophiostoma davidsonii* (Olchow. & J. Reid) H. Solheim, *Nordic J. Bot.* 6: 203. 1986.

*Anamorph:* *Pesotum* (Okada *et al.* 1998).

*Phylogenetic information:* *Grosmannia davidsonii* groups within the *Leptographium* clade (Hausner *et al.* 2000).

(11) *Grosmannia dryocoetidis* (W.B. Kendr. & Molnar) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.**

MycoBank MB500817.

*Basionym:* *Ceratocystis dryocoetidis* W.B. Kendr. & Molnar, *Canad. J. Bot.* 43: 39. 1965.

≡ *Ophiostoma dryocoetidis* (W.B. Kendr. & Molnar) de Hoog & R.J. Scheff., *Mycologia* 76: 297. 1984.

*Anamorph:* *Leptographium dryocoetidis* M.J. Wingf., *Trans. Brit. Mycol. Soc.* 85: 92. 1985.

≡ *Verticicladiella dryocoetidis* W.B. Kendr. & Molnar, *Canad. J. Bot.* 43: 39. 1965.

*Phylogenetic information:* *Grosmannia dryocoetidis* is related to *G. huntii*, *G. francke-grosmanniae* and *G. penicillata* among other species with *Leptographium* anamorphs (Jacobs *et al.* 2001, Kim *et al.* 2005).

(12) *Grosmannia europhioides* (E.F. Wright & Cain) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500818.

*Basionym:* *Ceratocystis europhioides* E.F. Wright & Cain, *Canad. J. Bot.* 39: 1222. 1961.

≡ *Ophiostoma europhioides* (E.F. Wright & Cain) H. Solheim, *Nordic J. Bot.* 6: 203. 1986.

*Anamorph:* *Leptographium* (Solheim 1986).

*Phylogenetic information:* Upadhyay (1981) treated *G. europhioides* as synonym of *G. piceiperda*, but Solheim (1986), Harrington (1988), Yamaoka (1997) and Jacobs *et al.* (1998) treated the two species as distinct. However, Harrington (1988) considered *G. pseudoeurophioides* a synonym of *G. europhioides*. Jacobs & Wingfield (2001) treated these species as synonyms of *G. piceiperda*. Phylogenetic data of Hausner *et al.* (1993b, 2000) suggest that these represent three distinct species.

(13) *Grosmannia francke-grosmanniae* (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500819.

*Basionym:* *Ceratocystis francke-grosmanniae* R.W. Davidson, *Mycologia* 63: 6. 1971.

≡ *Ophiostoma francke-grosmanniae* (R.W. Davidson) de Hoog & R.J. Scheff., *Mycologia* 76: 297. 1984.

*Anamorph:* *Leptographium francke-grosmanniae* K. Jacobs & M.J. Wingf., In Jacobs & Wingfield, *Leptographium species*: 99. 2001.

(14) *Grosmannia galeiformis* (B.K. Bakshi) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500820.

*Basionym:* *Ceratocystis galeiformis* Bakshi, *Mycol. Pap.* 35: 13. 1951.

≡ *Ophiostoma galeiforme* (B.K. Bakshi) Math.-Käärik, *Medd. Skogsforskningsinst.* 43: 47. 1953.

*Anamorph:* *Leptographium* (Harrington *et al.* 2001, Zhou *et al.* 2004b).

*Note:* The anamorph of *G. galeiformis* exhibits predominantly synnematous structures in culture (Zhou *et al.* 2004b). However, these might be viewed as a loose aggregation of mononematous conidiophores, and the anamorph of *G. galeiformis* was attributed to *Leptographium* based on phylogenetic association by Zhou *et al.* (2004b).

(15) *Grosmannia grandifoliae* (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500821.



*Basionym: Ceratocystis grandifoliae* R.W. Davidson, Mem. N.Y. Bot. Gard. 28: 45. 1976.

≡ *Ophiostoma grandifoliae* (R.W. Davidson) T.C. Harr., Mycotaxon 28: 41. 1987.

*Anamorph: Leptographium grandifoliae* M.J. Wingf., Trans. Brit. Mycol. Soc. 85: 92. 1985.

(16) **Grosmannia huntii** (R.C. Rob.-Jeffer.) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500822.

*Basionym: Ceratocystis huntii* R.C. Rob.-Jeffer., Canad. J. Bot. 42: 528. 1964.

≡ *Ophiostoma huntii* (R.C. Rob.-Jeffer.) de Hoog & R.J. Scheff., Mycologia 76: 297. 1984.

*Anamorph: Leptographium huntii* M.J. Wingf., Trans. Brit. Mycol. Soc. 85: 92. 1985.

(17) **Grosmannia laricis** (K. van der Westh., Yamaoka & M.J. Wingf.) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500823.

*Basionym: Ophiostoma laricis* K. van der Westh., Yamaoka & M.J. Wingf., Mycol. Res. 99: 1336. 1995.

*Anamorph: Leptographium laricis* K. van der Westh., Yamaoka & M.J. Wingf., Mycol. Res. 99: 1336. 1995.

(18) **Grosmannia leptographioides** (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500824.

*Basionym: Ceratostomella leptographioides* R.W. Davidson, Mycologia 34: 657. 1942.

≡ *Ophiostoma leptographioides* (R.W. Davidson) Arx, Antonie van Leeuwenhoek 18: 211. 1952.

≡ *Ceratocystis leptographioides* (R.W. Davidson) J. Hunt, Lloydia 19: 28. 1956.

*Anamorph: Leptographium leptographioides* K. Jacobs & M.J. Wingf., In Jacobs & Wingfield, *Leptographium species*: 118. 2001.

(19) **Grosmannia olivacea** (Mathiesen) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500825.

*Basionym: Ophiostoma olivaceum* Mathiesen, Svensk. Bot. Tidskr. 45: 212. 1951.

≡ *Ceratocystis olivacea* (Mathiesen) J. Hunt, Lloydia 19: 29. 1956.

*Anamorph: Pesotum* (Okada *et al.* 1998).

*Phylogenetic information: Grosmannia olivacea* groups within the *Leptographium* group (Kim *et al.* 2005).

(20) **Grosmannia piceiperda** (Rumbold) Goid., Boll. Staz. Patol. Veg. 16: 255. 1936.

*Basionym: Ceratostomella piceiperda* Rumbold, J. Agric. Res. 52: 436. 1936. [as 'piceaperda']

≡ *Ophiostoma piceiperdum* (Rumbold) Arx, Antonie van Leeuwenhoek 18: 211. 1952.

≡ *Ceratocystis piceiperdum* (Rumbold) C. Moreau, Rev. Mycol. (Paris), Suppl. Colon. 17: 22. 1952.

*Anamorph: Leptographium piceiperdum* K. Jacobs, M.J. Wingf. & Crous, Mycol. Res. 104: 240. 2000. [as 'piceaperdum']

(21) **Grosmannia pseudoeurophioides** (Olchow. & J. Reid) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500826.

*Basionym: Ceratocystis pseudoeurophioides* Olchow. & J. Reid, Canad. J. Bot. 52: 1700. 1974.

≡ *Ophiostoma pseudoeurophioides* (Olchow. & J. Reid) Hausner, J. Reid & Klassen, Canad. J. Bot. 71: 1264. 1993.

*Anamorph: Leptographium* (Hausner *et al.* 1993b).

*Phylogenetic information:* This species was considered a synonym of *G. penicillata* (Upadhyay 1981), of *G. europhioides* (Harrington 1988) and of *G. piceiperda* (Jacobs *et al.* 1998, Jacobs & Wingfield 2001). However, phylogenetic data of Hausner *et al.* (1993b, 2000), showed that *G. pseudoeurophioides* is distinct from all three of the above-mentioned species.

(22) **Grosmannia radiaticola** (J.-J. Kim, Seifert, & G.-H. Kim) Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500827.

*Basionym: Ophiostoma radiaticola* J.-J. Kim, Seifert, & G.-H. Kim, Mycotaxon 91: 486. 2005.

*Anamorph: Pesotum pini* (L.J. Hutchison & J. Reid) G. Okada & Seifert, Canad. J. Bot. 76: 1504. 1998.

≡ *Hyalopesotum pini* L.J. Hutchison & J. Reid, N.Z. J. Bot. 26: 90. 1988.

*Phylogenetic information:* This fungus is closely related to *G. galeiformis* (Kim *et al.* 2005).

(23) **Grosmannia robusta** (R.C. Rob.-Jeffer. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500828.

*Basionym: Europhium robustum* R.C. Rob.-Jeffer. & R.W. Davidson, Canad. J. Bot. 46: 1525. 1968.

≡ *Ceratocystis robusta* (R.C. Rob.-Jeffer. & R.W. Davidson) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 58. 1981.

≡ *Ophiostoma robustum* (R.C. Rob.-Jeffer. & R.W. Davidson) T.C. Harr., Mycotaxon 28: 42. 1987.

*Anamorph: Leptographium robustum* M.J. Wingf., Trans. Brit. Mycol. Soc. 85: 92. 1985.

(24) **Grosmannia sagmatospora** (E.F. Wright & Cain) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500829.

*Basionym: Ceratocystis sagmatospora* E.F. Wright & Cain, Canad. J. Bot. 39: 1226. 1961.

≡ *Ophiostoma sagmatosporum* (E.F. Wright & Cain) H. Solheim, Nordic J. Bot. 6: 203. 1986.

*Anamorph: Pesotum sagmatosporum* (H.P. Upadhyay & W.B. Kendr.) G. Okada & Seifert, Canad. J. Bot. 76: 1504.

≡ *Phialographium sagmatosporae* H.P. Upadhyay & W.B. Kendr., Mycologia 66: 183. 1974.

≡ *Graphium sagmatosporae* (H.P. Upadhyay & W.B. Kendr.) M.J. Wingf. & W.B. Kendr., Mycol. Res. 95: 1332. 1991.

*Phylogenetic information: Grosmannia sagmatospora* falls within the *Leptographium* group (Kim *et al.* 2005).

(25) **Grosmannia serpens** Goid., Goidánich, Boll. Staz. Patol. Veg. 16: 27. 1936.

≡ *Ophiostoma serpens* (Goid.) Arx, Antonie van Leeuwenhoek 18: 211. 1952.

≡ *Ceratocystis serpens* (Goid.) C. Moreau, Rev. Mycol. (Paris), Suppl. Colon. 17: 22. 1952.

*Anamorph: Leptographium serpens* (Goid.) M.J. Wingf., Trans. Brit. Mycol. Soc. 85: 92. 1985.

≡ *Scopularia serpens* Goid., Boll. Staz. Patol. Veg. 16: 42. 1936.

≡ *Verticicladiella serpens* (Goid.) W.B. Kendr., Canad. J. Bot. 40: 781. 1962.

= *Verticicladiella alacris* M.J. Wingf. & Marasas, Trans. Brit. Mycol. Soc. 75: 22. 1980.

≡ *Leptographium alacre* (M.J. Wingf. & Marasas) M. Morelet, Ann. Soc. Sci. Nat. Archéol. Toulon Var 40: 44. 1988.

= *Leptographium gallaeciae* F. Magan (*nom. inval.*).

(26) **Grosmannia vesca** (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500830.

*Basionym:* *Ceratocystis vesca* R.W. Davidson, *Mycologia* 50: 666. 1958.

≡ *Ophiostoma vescum* (R.W. Davidson) Hausner, J. Reid & Klassen. *Can. J. Bot.* 71: 1264. 1993.

*Anamorph:* *Pesotum* (Okada *et al.* 1998).

*Phylogenetic information:* *Grosmannia vesca* was treated as a synonym of *G. olivacea* (Griffin 1968, Olchowecki & Reid 1973, Upadhyay 1981). However, *G. vesca* groups close to but distinct from *G. olivacea*, *G. crassivaginata*, *G. francke-grosmanniae* and *G. cucullata* (Hausner *et al.* 1993b, 2000).

(27) **Grosmannia wageneri** (Goheen & F.W. Cobb) Zipfel, Z.W. de Beer & M.J. Wingf., **comb. nov.** MycoBank MB500831.

*Basionym:* *Ceratocystis wageneri* Goheen & F.W. Cobb, *Phytopathology* 68: 1193. 1978.

≡ *Ophiostoma wageneri* (Goheen & F.W. Cobb) T.C. Harr., *Mycotaxon* 28: 42. 1987.

*Anamorph:* *Leptographium wageneri* var. *ponderosae* (T.C. Harr. & F.W. Cobb) T.C. Harr. & F.W. Cobb, *Mycotaxon* 30: 505. 1987.

≡ *Verticicladiella wageneri* var. *ponderosae* T.C. Harr. & F.W. Cobb, *Mycol.* 78: 568. 1986.

*Note:* Teleomorph structures for *G. wageneri* have been observed only once and these were associated with *Leptographium wageneri* var. *ponderosae* (Jacobs & Wingfield 2001), of which an isolate was included in the present study. Teleomorphs have never been observed for *L. wageneri* var. *wageneri* (also included in this study) and *L. wageneri* var. *pseudotsugae* (Jacobs & Wingfield 2001).

#### Status of other species linked to *Leptographium*

(a) **Ophiostoma brevicolle** (R.W. Davidson) de Hoog & R.J. Scheff., *Mycologia* 76: 297. 1984.

*Basionym:* *Ceratocystis brevicollis* R.W. Davidson, *Mycologia* 50: 667. 1958.

*Anamorph:* *Leptographium brevicolle* K. Jacobs & M.J. Wingf., In Jacobs & Wingfield, *Leptographium species*: 72. 2001.

*Phylogenetic information:* Sequence data for *O. brevicolle* from previous studies are contradictory. According to Hausner *et al.* (2000) *O. brevicolle* (CBS 150.78 = CMW 474) is closely related to *G. francke-grosmanniae*. However, Jacobs *et al.* (2001) showed that *O. brevicolle* (CBS 795.73 = CMW 447) groups with *O. trinacriforme* (CBS 210.58 = CMW 670). The GenBank sequences of the two *O. brevicolle* isolates differ significantly from each other. We have thus chosen to treat *O. brevicolle* as a species of *Ophiostoma* until the confusion regarding the species has been resolved.

(b) **Ceratostomella imperfecta** V.V. Mill. & Tcherntz., *State For. Tech. Publ. Off. Moscow*. p. 123. 1934.

≡ *Ceratocystis imperfecta* (V.V. Miller & Tcherntz.) C. Moreau, *Rev. Mycol. (Paris)*, *Suppl. Colon.* 17: 22. 1952.

*Anamorph:* *Leptographium* (Hunt 1956).

*Phylogenetic information:* none.

*Note:* Hunt (1956) suggested, based only on the original description, that this species could be a synonym of *G. penicillata*. Upadhyay (1981) also lists *C. imperfecta* as synonym of *G. penicillata*, apparently based only on the suggestion of Hunt (1956).

(c) **Ophiostoma obscurum** (R.W. Davidson) Hendr., *Ann. Gembloux* 43: 99. 1937.

*Basionym:* *Ceratostomella obscura* R.W. Davidson, *J. Agric. Res.* 50: 798. 1935.

≡ *Ophiostoma obscurum* (R.W. Davidson) Arx, *Antonie van Leeuwenhoek* 18: 211. 1952. (superfluous combination).

≡ *Ceratocystis obscura* (R.W. Davidson) J. Hunt, *Lloydia* 19: 30. 1956.

*Anamorph:* 'transitional form between *Leptographium* and *Graphium*' (Hunt 1956).

*Phylogenetic information:* none.

*Note:* Hunt (1956) treated *O. obscurum* as a valid species. However, Upadhyay (1981) did not find the teleomorph on the type specimen and treated it as a doubtful species. Its status remains uncertain.

(d) **Ophiostoma pini** (Münch) Syd. & P. Syd., *Ann. Mycol.* 17: 43. 1917.

*Basionym:* *Ceratostomella pini* Münch, *Naturwiss. Z. Forst-Landw.* 5: 541. 1907.

≡ *Grosmannia pini* (Münch) Goid., *Boll. Staz. Patol. Veg.* 16: 27. 1936.

≡ *Ceratocystis pini* (Münch) C. Moreau, *Rev. Mycol. (Paris)*, *Suppl. Colon.* 17: 22. 1952.

*Anamorph:* *Leptographium* (Moreau 1952).

*Phylogenetic information:* none.

*Note:* *Ophiostoma pini* has been treated as synonym of *O. minus* (Hedgcock) H. & P. Sydow by Hunt (1956), Griffin (1968), Olchowecki & Reid (1973), and Upadhyay (1981). Goidánich (1936) placed *O. pini* in *Grosmannia*. We have chosen to consider *O. pini* a synonym of *O. minus* until phylogenetic data are available to resolve its status more clearly.

(e) **Ophiostoma rostrocylicum** (R.W. Davidson) Arx, *Antonie van Leeuwenhoek* 18: 212. 1952.

*Basionym:* *Ceratostomella rostrocylicum* R.W. Davidson, *Mycologia* 34: 658. 1942.

≡ *Ceratocystis rostrocylicum* (R.W. Davidson) J. Hunt, *Lloydia* 19: 26. 1956.

*Anamorph:* *Leptographium* (Hunt 1956).

*Phylogenetic information:* none.

*Note:* Hunt (1956) and Upadhyay (1981) considered this a distinct species, but Jacobs & Wingfield (2001) treated it as doubtful because no type material was designated for it.

(f) **Ophiostoma trinacriforme** (A.K. Parker) T.C. Harr., *Mycotaxon* 28: 42. 1987.

*Basionym:* *Europhium trinacriforme* A.K. Parker, *Canad. J. Bot.* 35: 175. 1957.

≡ *Ceratocystis trinacriformis* (A.K. Parker) H.P. Upadhyay, In Upadhyay, *Monograph of Ceratocystis and Ceratocystiopsis*: 63. 1981.

*Anamorph:* *Leptographium trinacriforme* K. Jacobs &



M.J. Wingf., In Jacobs & Wingfield, *Leptographium species*: 167. 2001

*Phylogenetic information*: Hausner *et al.* (2000) showed that *O. trinacriforme* (CFB 527) grouped close to *O. ips* and *O. longirostellatum*. In the study by Jacobs *et al.* (2001), *O. trinacriforme* (CBS 210.58 = CMW 670) grouped close to *O. brevicolle*, in a clade separate from the two main clades accommodating *Leptographium* spp. The GenBank sequences for these two *O. trinacriforme* isolates differ significantly. We have thus chosen to treat it as a species of *Ophiostoma* until its taxonomic status has been resolved.

(g) ***Ophiostoma truncicolor*** R.W. Davidson, *Mycologia* 47: 63. 1955.

≡ *Ceratocystis truncicola* (R.W. Davidson) H.D. Griffin, *Canad. J. Bot.* 46: 710. 1968.

*Anamorph*: *Graphium*-like (Davidson 1955).

*Phylogenetic information*: none.

*Note*: Upadhyay (1981) and Seifert *et al.* (1993) listed *O. truncicolor* as a synonym of *O. penicillatum*. The species was not included in the monograph of *Leptographium* (Jacobs & Wingfield 2001).

(h) ***Ophiostoma valdivianum*** (Butin) Rulamort, *Bull. Soc. Bot. Centre-Ouest, N.S.* 17: 192. 1986.

*Basionym*: *Ceratocystis valdiviana* Butin, *Phytopathol. Z.* 109: 86. 1984.

≡ *Ophiostoma valdivianum* (Butin) T.C. Harr., *Mycotaxon* 28: 42. 1987 (superfluous combination).

*Anamorph*: *Sporothrix* and *Leptographium* (Butin & Aquilar 1984, Seifert *et al.* 1993).

*Phylogenetic information*: none.

*Note*: Jacobs & Wingfield (2001) treated this as a dubious species since no type material or cultures were available for study.

## DISCUSSION

In this study we have produced robust phylogenetic data showing that the genus *Ophiostoma* consists of at least three groups representing separate genera. Based on this phylogenetic evidence and clear morphological characteristics, we have re-instated the teleomorph-genera *Ceratocystiopsis* and *Grosmannia*. The former genus now incorporates 11 species including three new combinations, and the latter 27 species including 24 new combinations. The remaining taxa are retained in *Ophiostoma* even though some monophyletic groups are evident in the larger genus. Because data derived in this study did not provide consistent evidence to support these subgroups amongst the species retained in *Ophiostoma*, we have chosen not to subdivide the genus further at the present time.

The genus *Ceratocystiopsis* has been re-instated to accommodate taxa that have short ascomatal necks, produce falcate ascospores with sheaths and have *Hyalorhinocladiella* (occasionally *Sporothrix*-like) anamorphs. Upadhyay & Kendrick (1975) established

*Ceratocystiopsis* to separate taxa having these distinct characteristics from taxa residing in the aggregate genus *Ceratocystis*. Our data revealed a strongly supported, monophyletic lineage with *Cop. minuta* central to it, and with morphological characters consistent with the original description of *Ceratocystiopsis*. All species in this group have  $\beta$ -tubulin intron 4 and lack intron 5. This monophyletic group was previously recognised and described as the *Minuta* complex by Hausner *et al.* (2003), and the nine species in the complex were characterised by sensitivity to cycloheximide. Amalgamating the data from this study and other published phylogenetic data, *Ceratocystiopsis* accommodates 11 species.

Hausner *et al.* (2003) retained their earlier view (Hausner *et al.* 1993a) that the group treated as *Ceratocystiopsis* in this study, could not constitute a genus because some species with falcate ascospores did not form part of this lineage. The view here would be that falcate ascospores evolved more than once in the *Ophiostomatales*. Amongst the species not monophyletic with *Cop. minuta*, two (*Cop. alba*, *Cornuvesica falcata*) are completely unrelated to the *Ophiostomatales*, no phylogenetic data exist for three species (*Cop. conicicollis*, *Cop. ochracea*, *Cop. spinulosa*), and one has a *Leptographium* anamorph and resides in *Grosmannia* (*G. crassivaginata*). The remaining five species (*O. carpenteri*, *O. crenulatum*, *O. fasciatum*, *O. longisporum*, *O. retusum*) are all more closely related to *Ophiostoma* spp. than to *Cop. minuta*, and we treat these as species of *Ophiostoma*. Results of the present study have shown that there is substantial, consistent phylogenetic evidence to support a distinct generic taxon for *Ceratocystiopsis*.

*Grosmannia* has been reinstated to accommodate teleomorph taxa that form a monophyletic group including both *G. penicillata* (type species of *Grosmannia*) and *Leptographium lundbergii* (type species of *Leptographium*). Species in this genus are also characterized by the presence of intron 4 and absence of intron 5 in the  $\beta$ -tubulin gene. Goidánich (1936) established *Grosmannia* for four species with *Scopularia* (= *Leptographium*) anamorphs. However, the genus was not widely recognised and most teleomorph species with *Leptographium* anamorphs were treated as *Ceratostomella*, *Ceratocystis*, *Europhium*, and more recently, *Ophiostoma* (Table 1).

Hausner *et al.* (2000) indicated that *Ophiostoma* spp. with *Leptographium* anamorphs appear to group together. However, they interpreted the separation of these species from other *Ophiostoma* spp. that are related to the type of the genus, *O. piliferum*, as artificial. Their conclusions were based on sequences of the partial ribosomal SSU and LSU regions. Results of the present study arose from the 5' region of the nuclear LSU gene, including the variable D1 and D2 regions, and partial DNA sequence data for  $\beta$ -tubulin, a coding gene. These regions are more variable than those used by Hausner *et al.* (2000). We thus found consistently strong support for the group of species that incorporates *G. penicillata* and *L. lundbergii*, as well as 13 other species with *Leptographium* anamorphs.

Nine of the species that we have accommodated in *Grosmannia* produce synnematosous synanamorphs together with a *Leptographium* state, or a continuum of forms between the two states. The synnematosous anamorphs of seven of the nine species (*G. cainii*, *G. clavigera*, *G. cucullata*, *G. davidsonii*, *G. olivacea*, *G. sagmatospora*, *G. vesca*) were assigned to the genus *Pesotum* by Okada *et al.* (1998), applying their inclusive definition of *Pesotum*. The anamorphic fungus, *Pesotum pini*, was also included in their list of new combinations (Okada *et al.* 1998). The teleomorph for this species, *G. radiaticola*, was discovered only recently (Kim *et al.* 2005) and represents one of the nine species that we have assigned to *Grosmannia*. The other *Grosmannia* species that forms a synnematosous anamorph is *G. galeiformis*. This species was not included in the study of Okada *et al.* (1998). Zhou *et al.* (2004b) recognized that the synnematosous anamorph of *G. galeiformis* dominates in culture, but accepted the suggestion of Harrington *et al.* (2001) to retain *Pesotum* for anamorphs of the *O. piceae* complex. Zhou *et al.* (2004), therefore, recommended that the *Leptographium* state be treated as the primary anamorph of *O. galeiforme*.

Harrington *et al.* (2001) suggested that synnemata evolved more than once in *Ophiostoma* (*sensu* Harrington, including *Grosmannia*). They suggested that synnemata with fused stipe cells and a *Sporothrix* synanamorph were only formed by species in the *O. piceae* complex. The synnemata of the nine *Grosmannia* spp. with synnematosous anamorphs treated in this study, are best viewed as a 'loose aggregation of *Leptographium* conidiophores' (Harrington *et al.* 2001). Furthermore, none of the nine species have micronematous conidiophores such as those defining *Sporothrix* (Harrington *et al.* 2001). Upadhyay (1981) described *Graphiocladiella* Upadhyay, with the anamorph of *G. clavigera* as type species, for species with both mononematous (*Leptographium*-like) and synnematosous anamorphs. This could then be the appropriate genus in which to accommodate the anamorphs of *Grosmannia* spp. exhibiting both conidiophore types, and anamorph species producing synnematosous anamorphs that phylogenetically reside in *Grosmannia*.

The only *Grosmannia* species that has been reported to produce a *Sporothrix* synanamorph together with a *Leptographium* state, is *G. francke-grosmanniae* (Mouton *et al.* 1992). However, the *Sporothrix* state was not mentioned in the descriptions of the species by Upadhyay (1981) and Jacobs & Wingfield (2001), possibly indicating that this form is produced only rarely. Two *Leptographium* spp. without known teleomorphs, *L. elegans* M.J. Wingf., Crous & Tzean, and *L. bistatum* J.-J. Kim & G.-H. Kim, also produce *Sporothrix*-like synanamorphs (Jacobs & Wingfield 2001, Kim *et al.* 2004). Illustrations of *L. elegans* (Jacobs & Wingfield 2001) and *L. bistatum* (Kim *et al.* 2004) shows that conidiophores bearing denticulate conidiogenous cells, become pigmented towards the base. This is in contrast with species of *Sporothrix s. str.* (with *S. schenckii* as type), defined as having hyaline conidiophores (De Hoog 1974). Both these *Leptographium* spp. have

been shown to be phylogenetically related to the fungi that we now treat in *Grosmannia* (Jacobs *et al.* 2001, Kim *et al.* 2004), but they do not consistently group in a monophyletic clade with each other or with *G. francke-grosmanniae* (Kim *et al.* 2004). Even though some *Grosmannia* and/or *Leptographium* spp. might produce *Sporothrix*-like conidiophores, it is our view that this character is rare and inconsistent with the definition of *Sporothrix s. str.*

The *Ophiostoma* spp. included in the present study formed a monophyletic group (Group D in Figs 1–2) that consisted of a number of strongly supported subgroups. Group J was supported consistently with high bootstrap values and included *O. ips*, *O. montium* and *O. pulvinisporum*. These species all have pillow-shaped ascospores with distinct sheaths that distinguish them from all other species in *Ophiostoma* (Rumbold 1936, Olchowecki & Reid 1973, Zhou *et al.* 2004a). All three species exhibit a continuum of anamorph structures described as *Hyalorhinocladiella*, *Leptographium*- and *Pesotum*-like (Table 2). Our data distinctly separate these taxa from species in *Grosmannia* with *Leptographium* anamorphs. Harrington *et al.* (2001) also argued that the synnematosous anamorph of *O. ips* should not be referred to as *Pesotum*, since *O. ips* does not have a *Sporothrix* synanamorph, which is also true for the other two species. The notion of De Hoog (1993) that only *Ophiostoma* spp. with pillow-shaped or falcate ascospores (thus *Ceratocystiopsis* spp.) have *Hyalorhinocladiella* anamorphs, is supported by our data.

Another group of *Ophiostoma* spp. with relatively high bootstrap support (Group I) included the type species for the genus, *O. piliferum*, together with *O. distortum* and *O. flexuosum* that have *Sporothrix* anamorphs (Seifert *et al.* 1993). The group also includes *O. ainoae* and *O. araucariae* that have *Pesotum*-like anamorphs but no recorded *Sporothrix* synanamorphs (Harrington *et al.* 2001). The remaining taxa in Group I are members of the *O. piceae* complex (*sensu* Harrington *et al.* 2001) that have *Pesotum* anamorphs. The group thus represents species spanning the entire spectrum of the anamorph continuum; those that have only a *Sporothrix* anamorph, those with anamorphs in *Pesotum sensu* Harrington *et al.* (2001) (synnematal structures as well as *Sporothrix* states), and those that have synnemata lacking the *Sporothrix* state. All the species residing in Group I have ascospores without sheaths that vary from cylindrical to orange section-shaped. All the species in this group also have intron 4 and lack intron 5 in the  $\beta$ -tubulin gene (Fig. 1). Harrington *et al.* (2001) defined the *O. piceae* complex as a well-resolved monophyletic group containing nine species with *Pesotum* anamorphs. However, our results show that other species without *Pesotum* anamorphs group in between species of the so-called complex. Resolution in our data is poor, most probably because of the conserved nature of the genes in our analyses. ITS and  $\beta$ -tubulin sequence data including the introns, will be necessary to resolve the phylogeny of the species in this group.



A subgroup (Group E) of the *Ophiostoma* group (D), with consistently high statistical support includes *O. pluriannulatum*, *O. multiannulatum*, *O. subannulatum*, and *O. carpenteri*. The first three species have long ascomatal necks with annuli and reniform ascospores without sheaths (Hedgcock 1906, Davidson 1935, Livingston & Davidson 1987). *Ophiostoma carpenteri* has a relatively short perithecial neck with no annuli and elongated clavate ascospores without sheath (Hausner *et al.* 2003). All four species have prominent ostiolar hyphae and *Sporothrix* anamorphs producing secondary conidia (Hedgcock 1906, Davidson 1935, Livingston & Davidson 1987).

Group H in the *Ophiostoma* group (D) consists of species that have only *Sporothrix* anamorphs. This group included *Sporothrix schenckii*, the type species of the genus. All species in the group lack intron 4 and have intron 5 of the  $\beta$ -tubulin gene (Fig. 1). Where teleomorphs are known, ascospores are more or less reniform and not protected by a sheath (Table 2). The taxa in this group are found in a diverse range of ecological niches. For example: *S. schenckii* occurs on wood and in soil, and causes human sporotrichosis (De Hoog 1993, De Beer *et al.* 2003), *S. inflata* occurs in soil (De Hoog 1974), and *O. nigrocarpum*, *O. stenoceras*, *O. fusiforme* and *O. lunatum* are wood-inhabiting (Robak 1932, Davidson 1966, Aghayeva *et al.* 2004). Three species, *O. splendens*, *O. protearum* and *O. africanum* have been reported only from *Protea* infructescences in South Africa (Marais & Wingfield 2001). Our data suggest that the species from *Protea* might form a monophyletic lineage within *Ophiostoma*. However, this hypothesis was not supported where greater numbers of species from proteas were included (Roets *et al.* 2006).

Data derived from this study provide strong support for the separation of *Grosmannia* and *Ceratocystiopsis* from *Ophiostoma*. This separation has been implemented and it will hopefully simplify the application of names for the large number of species occurring in *Ophiostoma sensu lato*. Although our definition of *Ophiostoma sensu stricto* treats this genus as if it is a unified group of species, our data provide relatively strong support for the view that it contains a number of groups, supported by morphological and possibly ecological characters. At the present time we believe that there is insufficient data to further subdivide *Ophiostoma* in a meaningful way. However, we are convinced that addition of taxa and consideration of DNA sequence data for additional gene regions will result in the emergence of further genera in *Ophiostoma sensu lato*.

## ACKNOWLEDGEMENTS

We thank Grace Nakabonge, Marieke Gryzenhout and Elsie de Meyer for making sequences available to us. We are indebted to Dr Fourie Joubert and Wayne Delpont of the Bioinformatics and Computational Biology Unit, Pretoria Node of the National Bioinformatics Network, for high throughput computational infrastructure and technical assistance in running the larger sequence analyses. For their contributions and advice, we thank Drs W. Gams and H.F. Glen. We acknowledge the financial support of the members of the Tree

Protection Co-operative Programme (TCP), the department of Trade and Industry (DTI) THRIP initiative, the National Research Foundation (NRF) and the NRF/DST Centre of Excellence in Tree Health Biotechnology (CTHB).

## REFERENCES

- Aghayeva DN, Wingfield MJ, De Beer ZW, Kirisits T (2004). Two new *Ophiostoma* species with *Sporothrix* anamorphs from Austria and Azerbaijan. *Mycologia* **96**: 866–878.
- Bakshi BK (1951). Studies on four species of *Ceratocystis*, with a discussion on fungi causing sap-stain in Britain. *Mycological Papers* **35**: 1–16.
- Benade E, Wingfield MJ, Van Wyk PS (1996). Conidium development in the *Hyalorhinochlaeniella* anamorph of *Ceratocystiopsis minuta-bicolor* and *Ophiostoma minus*. *Canadian Journal of Botany* **74**: 891–897.
- Benade E, Wingfield MJ, Van Wyk PS (1998). Conidium development in *Hyalodendron* and *Allescheriella* anamorphs of *Ophiostoma* and *Ceratocystiopsis*. *Mycotaxon* **68**: 251–263.
- Brasier CM (1991). *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. *Mycopathologia* **115**: 151–161.
- Bridges JR, Perry TJ (1987). *Ceratocystiopsis ranunculosis* sp. nov. associated with the southern pine beetle. *Mycologia* **79**: 630–633.
- Buisman C (1932). *Ceratostomella ulmi*, de geslachtelijke vorm van *Graphium ulmi* Schwartz. *Tijdschrift over Plantenziekten* **38**: 1–5.
- Butin H (1968). A new species of *Ceratocystis* causing blue-stain in *Araucaria araucana*. *Canadian Journal of Botany* **46**: 61–63.
- Butin H, Aquilar AM (1984). Blue-stain fungi on *Nothofagus* from Chile - including new species of *Ceratocystis* Ellis & Halst. *Phytopathologische Zeitschrift* **109**: 80–89.
- Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN (2002). A preliminary overview of the *Diaportheales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.
- Crane JL, Schoknecht JD (1973). Conidiogenesis in *Ceratocystis ulmi*, *Ceratocystis piceae* and *Graphium penicillioides*. *American Journal of Botany* **60**: 346–354.
- Davidson RW (1935). Fungi causing stain in logs and lumber in the Southern States, including five new species. *Journal of Agricultural Research* **50**: 789–807.
- Davidson RW (1942). Some additional species of *Ceratostomella* in the United States. *Mycologia* **34**: 650–662.
- Davidson RW (1955). Wood-staining fungi associated with bark beetles in Engelmann spruce in Colorado. *Mycologia* **47**: 58–67.
- Davidson RW (1958). Additional species of *Ophiostomataceae* from Colorado. *Mycologia* **50**: 661–670.
- Davidson RW (1966). New species of *Ceratocystis* from conifers. *Mycopathologia et Mycologia applicata* **28**: 273–286.
- Davidson RW (1971). New species of *Ceratocystis*. *Mycologia* **63**: 5–15.
- De Beer ZW, Harrington TC, Vismar HF, Wingfield BD, Wingfield MJ (2003). Phylogeny of the *Ophiostoma stenoceras* – *Sporothrix schenckii* complex. *Mycologia* **95**: 434–441.
- De Hoog GS (1974). The genera *Blastobotrys*, *Sporothrix*, *Calcarisporium* and *Calcarisporiella* gen. nov. *Studies in Mycology* **7**: 1–84.
- De Hoog GS (1993). *Sporothrix*-like anamorphs of *Ophiostoma* species and other fungi. In: *Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity* (Wingfield MJ, Seifert KA, Webber J, eds). American Phytopathological Society, St. Paul, Minnesota: 53–60.
- De Hoog GS, Scheffer RJ (1984). *Ceratocystis* versus *Ophiostoma*: a reappraisal. *Mycologia* **76**: 292–299.
- Georgévitch P (1926). *Ceratostomella querci* n. sp. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* **183**: 759–761.
- Glass NL, Donaldson GC (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. *Applied and Environmental Microbiology* **61**: 1323–1330.
- Goidánich G (1935). Una nuova species di "*Ophiostoma*" vivente sul pero ed alcune osservazioni sull'esatta posizione sistematica

- della forma ascofora e delle forme metagenetiche del genere. *Bollettino della Stazione di Patologia Vegetale di Roma* **15**: 122–168.
- Goidánich G (1936). Il genere di Ascomiceti *Grosmannia* G. Goid. *Bollettino della Stazione di Patologia Vegetale di Roma* **16**: 26–60.
- Gorton C, Kim SH, Henricot B, Webber J, Breuil C (2004). Phylogenetic analysis of the bluestain fungus *Ophiostoma minus* based on partial ITS rDNA and beta-tubulin gene sequences. *Mycological Research* **108**: 759–765.
- Griffin HD (1968). The genus *Ceratocystis* in Ontario. *Canadian Journal of Botany* **46**: 689–718.
- Grynhout M, Myburg H, Wingfield BD, Wingfield MJ (2006). *Cryphonectriaceae* (*Diaporthales*), a new family including *Chrysosporthe*, *Cryphonectria*, *Endothia* and allied genera. *Mycologia*: In Press.
- Halsted BD (1890). Some fungous diseases of sweet potato. *New Jersey Agricultural College Experiment Station Bulletin* **76**: 1–32.
- Harrington TC (1981). Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. *Mycologia* **73**: 1123–1129.
- Harrington TC (1987). New combinations in *Ophiostoma* of *Ceratocystis* species with *Leptographium* anamorphs. *Mycotaxon* **28**: 39–43.
- Harrington TC (1988). *Leptographium* species, their distributions, hosts and insect vectors. In: *Leptographium root diseases of conifers* (Harrington TC, Cobb FW, eds). APS Press, St. Paul, Minnesota: 1–39.
- Harrington TC, McNew D, Steimel J, Hofstra D, Farrell R (2001). Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. *Mycologia* **93**: 111–136.
- Hausner G, Eydólfsson GG, Reid J (2003). Three new species of *Ophiostoma* and notes on *Cornuvesica falcata*. *Canadian Journal of Botany* **81**: 40–48.
- Hausner G, Reid J (2003). Notes on *Ceratocystis brunnea* and some other *Ophiostoma* species based on partial ribosomal DNA sequence analysis. *Canadian Journal of Botany* **81**: 865–876.
- Hausner G, Reid J, Klassen GR (1992). Do galeate-ascospore members of the *Cephalosascaceae*, *Endomycetaceae* and *Ophiostomataceae* share a common phylogeny? *Mycologia* **84**: 870–881.
- Hausner G, Reid J, Klassen GR (1993a). *Ceratocystiopsis*: a reappraisal based on molecular criteria. *Mycological Research* **97**: 625–633.
- Hausner G, Reid J, Klassen GR (1993b). 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 (1993c). On the subdivision of *Ceratocystis* s.l., based on partial ribosomal DNA sequences. *Canadian Journal of Botany* **71**: 52–63.
- Hausner G, Reid J, Klassen GR (2000). On the phylogeny of members of *Ceratocystis* s.s. and *Ophiostoma* that possess different anamorphic states, with emphasis on the anamorph genus *Leptographium*, based on partial ribosomal DNA sequences. *Canadian Journal of Botany* **78**: 903–916.
- Hedgcock GG (1906). Studies upon some chromogenic fungi which discolor wood. *Missouri Botanical Garden Annual Report* **17**: 59–114.
- Hsiau PT-W, Harrington TC (1997). *Ceratocystiopsis brevicomis* sp. nov., a mycangial fungus from *Dendroctonus brevicomis* (Coleoptera: Scolytidae). *Mycologia* **89**: 661–669.
- Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Hunt J (1956). Taxonomy of the genus *Ceratocystis*. *Lloydia* **19**: 1–58.
- Hutchison LJ, Reid J (1988). Taxonomy of some potential wood-staining fungi from New Zealand 1. *Ophiostomataceae*. *New Zealand Journal of Botany* **26**: 63–81.
- Jacobs K, Kirisits T (2003). *Ophiostoma kryptum* sp. nov. from *Larix decidua* and *Picea abies* in Europe, similar to *O. minus*. *Mycological Research* **107**: 1231–1242.
- Jacobs K, Solheim H, Wingfield BD, Wingfield MJ (2005). Taxonomic re-evaluation of *Leptographium lundbergii* based on DNA sequence comparisons and morphology. *Mycological Research* **109**: 1149–1161.
- Jacobs K, Wingfield BD, Wingfield MJ (2001). Phylogenetic relationships in *Leptographium* based on morphological and molecular characters. *Canadian Journal of Botany* **79**: 719–732.
- Jacobs K, Wingfield MJ (2001). *Leptographium* species - Tree Pathogens, Insect Associates, and agents of blue-stain. The American Phytopathological Society, St. Paul, Minnesota.
- Jacobs K, Wingfield MJ, Wingfield BD, Yamaoka Y (1998). Comparison of *Ophiostoma huntii* and *O. europhioides* and description of *O. aenigmaticum* sp. nov. *Mycological Research* **102**: 289–294.
- Kim J-J, Kim SH, Lee S, Breuil C (2003). Distinguishing *Ophiostoma ips* and *Ophiostoma montium*, two bark beetle-associated sapstain fungi. *FEMS Microbiology Letters* **222**: 187–192.
- Kim J-J, Lim YW, Seifert KA, Kim SH, Breuil C, Kim G-H (2005). Taxonomy of *Ophiostoma radiaticola* sp. nov. (*Ophiostomatales*, *Ascomycetes*), the teleomorph of *Pesotum pini*, isolates from logs of *Pinus radiata*. *Mycotaxon* **91**: 481–496.
- Kim J-J, Lim YW, Wingfield MJ, Breuil C, Kim GH (2004). *Leptographium bistatum* sp. nov., a new species with a *Sporothrix* synanamorph from *Pinus radiata* in Korea. *Mycological Research* **108**: 699–706.
- Leach JG, Orr LW, Christensen C (1934). The interrelationships of bark beetles and blue-staining fungi in felled Norway pine timber. *Journal of Agricultural Research* **49**: 315–341.
- Lim YW, Alamouti S, Kim J-J, Lee S, Breuil C (2004). Multigene phylogenies of *Ophiostoma clavigerum* and closely related species from bark beetle-attacked *Pinus* in North America. *FEMS Microbiology Letters* **237**: 89–96.
- Livingston WH, Davidson RW (1987). *Ophiostoma subannulatum*, a new fungal species pathogenic to grand fir roots. *Mycologia* **79**: 144–147.
- Marais GJ, Wingfield MJ (1994). Fungi associated with infructescences of *Protea* species in South Africa, including a new species of *Ophiostoma*. *Mycological Research* **98**: 369–374.
- Marais GJ, Wingfield MJ (1997). *Ophiostoma protearum* sp. nov. associated with *Protea caffra* infructescences. *Canadian Journal of Botany* **75**: 362–367.
- Marais GJ, Wingfield MJ (2001). *Ophiostoma africanum* sp. nov., and a key to ophiostomatoid species from *Protea* infructescences. *Mycological Research* **105**: 240–246.
- Marmolejo JG, Butin H (1990). New conifer-inhabiting species of *Ophiostoma* and *Ceratocystiopsis* (*Ascomycetes*, *Microascales*) from Mexico. *Sydowia* **42**: 193–199.
- Mathiesen-Käärik A (1960). Studies on the ecology, taxonomy and physiology of Swedish insect-associated blue stain fungi. *Oikos* **11**: 1–25.
- Mathiesen A (1951). Einige neue *Ophiostoma*-Arten in Schweden. *Svensk Botanisk Tidskrift* **45**: 203–232.
- Melin E, Nannfeldt JA (1934). Researches into the blueing of ground wood-pulp. *Svenska Skogsvårdsföreningens Tidskrift* **32**: 397–616.
- Minter DW, Kirk PM, Sutton BC (1983). Thallic phialides. *Transactions British Mycological Society* **80**: 39–66.
- Moreau C (1952). Coexistence des formes *Thielaviopsis* et *Graphium* chez une souche de *Ceratocystis major* (van Beyma) nov. comb. *Revue de Mycologie (Paris)* **17**: 17–25.
- Mouton M, Wingfield MJ, Van Wyk PS (1992). The anamorph of *Ophiostoma francke-grosmanniae* is a *Leptographium*. *Mycologia* **84**: 857–862.
- Mouton M, Wingfield MJ, Van Wyk PS (1994). Conidium development in anamorphs of *Ceratocystis* sensu lato: a review. *South African Journal of Science* **90**: 293–298.
- Münch E (1907). Die Blaufäule des Nadelholzes. I–II. *Naturwissenschaftliche Zeitschrift für Forst- und Landwirtschaft* **5**: 531–573.
- Nannfeldt JA (1932). Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. *Nova Acta Regiae Societatis Scientiarum Upsaliensis, Series IV* **8**: 1–369.
- Notredame C, Higgins DG, Heringa J (2000). T-coffee: a novel method for fast and accurate multiple sequence alignment. *Journal of Molecular Biology* **302**: 205–217.
- O'Donnell K, Cigelnik E (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**: 103–116.
- Okada G, Seifert KA, Takematsu A, Yamaoka Y, Miyazaki S, Tubaki K (1998). A molecular phylogenetic reappraisal of the *Graphium* complex based on 18S rDNA sequences. *Canadian Journal of Botany* **76**: 1495–1506.

- Olchowecki A, Reid J (1973). Taxonomy of the genus *Ceratocystis* in Manitoba. *Canadian Journal of Botany* **52**: 1675–1711.
- Parker AK (1957). *Europhium*, a new genus of the Ascomycetes with a *Leptographium* imperfect state. *Canadian Journal of Botany* **35**: 173–179.
- Paulin-Mahady AE, Harrington TC, McNew D (2002). Phylogenetic and taxonomic evaluation of *Chalara*, *Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*. *Mycologia* **94**: 62–72.
- Posada D, Crandall KA (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Robak H (1932). Investigations regarding fungi on Norwegian ground wood pulp and fungal infection at wood pulp mills. *Nyt Magazin for Naturvidenskaberne* **71**: 185–330.
- Robinson-Jeffrey RC, Davidson RW (1968). Three new *Europhium* species with *Verticicladiella* imperfect states on blue-stained pine. *Canadian Journal of Botany* **46**: 1523–1527.
- Roets F, De Beer ZW, Dreyer LL, Crous PW, Zipfel R, Wingfield MJ (2006). Multi-gene phylogeny of *Ophiostoma* spp. associated with *Protea* infructescences including two new species. *Studies in Mycology* **55**: 199–212.
- Rumbold CT (1936). Three blue-staining fungi, including two new species, associated with bark beetles. *Journal of Agricultural Research* **52**: 419–437.
- Rumbold CT (1941). A blue stain fungus, *Ceratostomella montium* n. sp., and some yeasts associated with two species of *Dendroctonus*. *Journal of Agricultural Research* **62**: 589–601.
- Seifert KA, Wingfield MJ, Kendrick WB (1993). A nomenclator for described species of *Ceratocystis*, *Ophiostoma*, *Ceratocystiopsis*, *Ceratostomella* and *Sphaeronaemella*. In: *Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity* (Wingfield MJ, Seifert KA, Webber J, eds). American Phytopathological Society, St. Paul, Minnesota, U.S.A.: 269–287.
- Siemaszko W (1939). [Fungi associated with bark beetles in Poland.] [In Polish]. *Planta Polonica* **7**: 1–54.
- Six DL, Paine TD (1999). Allozyme diversity and gene flow in *Ophiostoma clavigerum* (*Ophiostomatales: Ophiostomataceae*), the mycangial fungus of the Jeffrey pine beetle, *Dendroctonus jeffreyi* (Coleoptera: Scolytidae). *Canadian Journal of Forest Research* **29**: 324–331.
- Solheim H (1986). Species of *Ophiostomataceae* isolated from *Picea abies* infested by the bark beetle *Ips typographus*. *Nordic Journal of Botany* **6**: 199–207.
- Spatafora JW, Blackwell M (1994). The polyphyletic origins of ophiostomatoid fungi. *Mycological Research* **98**: 1–9.
- Swofford DL (2001). PAUP\*. Phylogenetic analysis using parsimony (\*and other methods), version 4.0b1. Sinauer Associates, Sunderland, Massachusetts.
- Sydow H, Sydow P (1919). Mykologische Mitteilungen. *Annales Mycologici* **17**: 33–47.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876–4882.
- Upadhyay HP (1981). *A monograph of Ceratocystis and Ceratocystiopsis*. The University of Georgia Press, Athens, GA, U.S.A.
- Upadhyay HP, Kendrick WB (1975). Prodrum for a revision of *Ceratocystis* (*Microascales, Ascomycetes*) and its conidial states. *Mycologia* **67**: 798–805.
- Viljoen CD, Wingfield BD, Wingfield MJ (1999). Relatedness of *Custingophora olivaceae* to *Gondwanamyces* spp. from *Protea* spp. *Mycological Research* **103**: 497–500.
- Viljoen CD, Wingfield MJ, Jacobs K, Wingfield BD (2000). *Cornuvesica*, a new genus to accommodate *Ceratocystiopsis falcata*. *Mycological Research* **104**: 365–367.
- Von Arx JA (1952). Ueber die Ascomycetengattungen *Ceratostomella* Sacc., *Ophiostoma* Syd. und *Rostrella* Zimmerman. *Antonie van Leeuwenhoek* **18**: 13–213.
- Von Arx JA (1974). *The genera of fungi sporulating in pure culture*. 2nd edition. J. Cramer, Lehre, Germany.
- Von Arx JA, Müller E (1954). Die Gattungen der amerosporen Pyrenomyceten. *Beiträge zur Kryptogamenflora der Schweiz* **11**: 1–134.
- Von Arx JA, Van der Walt JP (1987). *Ophiostomatales* and *Endomycetales*. *Studies in Mycology* **30**: 167–176.
- Weijman ACM, De Hoog GS (1975). On the subdivision of the genus *Ceratocystis*. *Antonie van Leeuwenhoek* **41**: 353–360.
- Wingfield BD, Grant WS, Wolfaardt JF, Wingfield MJ (1994). Ribosomal RNA sequence phylogeny is not congruent with ascospore morphology among species in *Ceratocystis sensu stricto*. *Molecular Biology and Evolution* **11**: 376–383.
- Wingfield MJ (1993). Problems in delineating the genus *Ceratocystiopsis*. In: *Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity* (Wingfield MJ, Seifert KA, Webber J, eds). American Phytopathological Society, St. Paul, Minnesota: 21–26.
- Wingfield MJ, Seifert KA, Webber J (1993). *Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity*. American Phytopathological Society, St. Paul, Minnesota.
- Wright EF, Cain RF (1961). New species of the genus *Ceratocystis*. *Canadian Journal of Botany* **39**: 1215–1230.
- Yamaoka Y, Wingfield MJ, Takahashi I, Solheim H (1997). Ophiostomatoid fungi associated with the spruce bark beetle *Ips typographus* f. *japonicus* in Japan. *Mycological Research* **101**: 1215–1227.
- Zhou XD, De Beer ZW, Cibrian D, Wingfield BD, Wingfield MJ (2004a). Characterization of *Ophiostoma* species associated with pine bark beetles from Mexico, including *O. pulvinisporum* sp. nov. *Mycological Research* **108**: 690–698.
- Zhou XD, De Beer ZW, Harrington TC, McNew D, Kirisits T, Wingfield MJ (2004b). Epitypification of *Ophiostoma galeiforme* and phylogeny of species in the *O. galeiforme* complex. *Mycologia* **96**: 1306–1315.