Ophiostoma species from hardwood sources in South Africa

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by

Zacharias Wilhelmus de Beer

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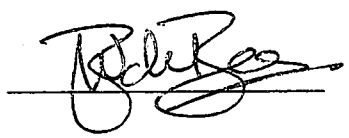
Study Leaders:

Prof. M.J. Wingfield
Prof. B.D. Wingfield

Declaration

I, the undersigned, hereby declare that the dissertation herewith submitted for the degree Magister Scientiae *to the University of the Free State, contains my own independent work and has hithertho not been submitted for any degree at any other university, and furthermore feed copyright of this dissertation in favour of the University.*



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Acknowledgements

Life is not so short but that there is always time enough for courtesy.

- Ralph Waldo Emerson

In the newsroom of the *Daily Express* in London, there used to be a notice that read *Avoid clichés like the plague*. It is difficult not to plague the acknowledgement section of a thesis with sentimentality or clichés. So, if what I wrote here has a clichéd ring to it, please believe and understand that it comes from the heart.

When I, as a taxonomist, started compiling the list of people who contributed to this thesis, I realised that the list can be roughly divided into two sections. First, those who contributed in an academic way, and then those who are part of my personal life. However, as within any good classification system, there are the borderline cases: people who can be classified in both groups.

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Preface

"INTRODUCTION

(to be read)"

- The Scofield Reference Bible, 1917

The ophiostomatoid fungi comprise several well-known genera of Ascomycetes such as *Ophiostoma* and *Ceratocystis*. The collective characteristic of all genera in this artificial group of fungi is the adaptation of their ascocarps, and in some cases conidiomata, for insect dispersal. Several species within the group cause tree diseases such as Dutch Elm Disease, and other species are known to degrade timber in the form of sapstain. The pathogenicity, ecology and taxonomy of many ophiostomatoid species have been intensively studied for more than a century. New developments in molecular biology are increasingly contributing to a better understanding of phylogenetic relationships between genera and species within the group. Most research on the group has, however, been conducted in the Northern Hemisphere. In contrast, little is known regarding the occurrence and distribution of ophiostomatoid fungi the Southern Hemisphere. The aim of this study was to gain a better understanding of the taxonomy and phylogeny of three *Ophiostoma* spp. occurring on hardwoods in South Africa and some other Southern Hemisphere countries.

It is only in the last decade that the potential value of certain *Ophiostoma* spp. has been recognised as biological control agents. A successful example of biological control is a product with the trade name Cartapip. It consists of a white mutant of *Ophiostoma piliferum*, and is applied as a pretreatment of softwood chips in pulp mills in Europe and the USA. The fungus removes pitch (which has detrimental effects on pulp) from the chips and, at the same time, prevents colonisation of the chips by sapstaining and rotting fungi. A product such as this might be of great benefit to the South African forestry industry, especially in pulp mills where extractives from *Eucalyptus* wood causes severe problems. Thus, the first chapter of this thesis represents a review of the literature dealing with the research and development of Cartapip, focusing on its pitch reducing abilities. Potential problems with the application of the product in South Africa are also discussed.

The importation of a product consisting of a living organism, such as Cartapip, can be problematic when the organism does not occur in the importing country. The South African quarantine authorities set certain requirements that had to be met before permission was granted for the importation of Cartapip. The first requirement was to determine whether *O. piliferum* occurs in South Africa. Chapter 2 represents the results of a preliminary study that aimed to answer this question. A list of all ophiostomatoid species reported from South Africa was compiled. Furthermore, all cultures resembling *O. piliferum* from the Tree Pathology Cooperative Programme (TPCP) culture collection, were studied. Additional isolates were also obtained from a limited survey conducted on wood chips from South African pulp mills. All isolates could be classified in one of three morphological groups that

resembled *Ophiostoma stenoceras*, *O. pluriannulatum* and *O. piceae*, respectively. The detailed taxonomic studies of these three groups of isolates are the topics of chapters 4, 5 and 6 of this thesis.

The taxonomic history of the ophiostomatoid fungi covers a century of conflicting points of view. To fully understand the taxonomy of the three *Ophiostoma* spp. that form the focus of this thesis, I found it necessary to compile a chronological review on the history of the genus *Ophiostoma*. This review forms the first part of Chapter 3. The second part is a summary of the higher classification of ophiostomatoid genera. The third part of the review is an attempt to clarify which of the 32 anamorph genus names associated with *Ophiostoma* in the past, are available for anamorphs of the genus.

The initial aim of Chapter 4 was to confirm the identity of the group of South African isolates resembling *O. stenoceras* with rDNA sequencing. The study, however, also provided the opportunity to investigate the phylogenetic relationships between *O. stenoceras* and the human pathogen, *Sporothrix schenckii*, which has previously been suggested by some authors to represent the anamorph of *O. stenoceras*. Authentic isolates of *Ophiostoma nigrocarpum*, *O. albidum* and *O. abietinum*, were also included in the study, since these species closely resemble *O. stenoceras*. The identity of isolates resembling *O. stenoceras* from some other Southern Hemisphere countries was, furthermore, confirmed.

Ophiostoma pluriannulatum closely resembles *O. piliferum* in all respects, apart from the fact that it is usually isolated from hardwoods and that annuli occur on the perithecial necks. The possibility that this fungus might be applied as a hardwood equivalent of Cartapip, is intriguing, and is currently being investigated in laboratories in New Zealand and the USA. Isolates resembling *O. pluriannulatum* from hardwoods in South Africa, Equador and Indonesia, however, present some morphological differences when compared with authentic *O. pluriannulatum* isolates. In Chapter 5, these fungi were compared and the phylogenetic relationships between them considered. We believe that the Southern Hemisphere group represents a new species and described it as such.

The third group of isolates obtained from the preliminary study of fungi occurring on wood chips from South Africa, resembled *O. piceae*, which is commonly isolated from softwoods in the Northern Hemisphere. An extensive phylogenetic study on the *O. piceae* complex, including nine species, has recentiy been published. Although some isolates from New Zealand were included, the study focused primarily on isolates from the Northern Hemisphere. In Chapter 6, the distribution of species from the *O. piceae* complex in the Southern Hemisphere is considered. Mating compatibility studies and rDNA sequencing

were, furthermore, employed to identify Southern Hemisphere isolates. Species identified included *Ophiostoma floccosum* from South Africa, and *Ophiostoma querci* from Brazil, New Zealand and South Africa.

For many years, *O. querci*, usually isolated from hardwoods, has been treated as a synonym of *O. piceae*. Recently, ecological, morphological, and mating type studies showed that *O. querci* represents a separate species. In the literature study conducted as a background for Chapter 6, it was noted that some contemporary authors use the name *O. querci*, while others prefer the name *O. quercus*. Chapter 7 presents a brief literature study that aims to resolve this confusion. Apart from the taxonomic literature, the International Code for Botanical Nomenclature was consulted, as well as experts on botanical Latin.

Three appendices compiled during the course of this study, are included at the end of the thesis. The appendices contain tabulated information regarding the morphology, distribution, host ranges, and insect vectors of the three groups of species studied in Chapters 4, 5 and 6 respectively. These appendices, as well as Chapters 1 and 3, were not written for publication purposes. However, since it contains useful information, it might be made available on the Internet. Chapters 2, 4, 5, 6 and 7 will, however, be submitted for publication. This explains why certain sections of these chapters might appear to be repetitive.

Chapter 1

Necessity is the mother of invention.

- Anonymous

Ophiostoma piliferum as biological control agent in the pulp industry: a review.

INTRODUCTION

The genus *Ophiostoma* H. & P. Syd. was established in 1919 when it replaced *Linostoma* Höhnel, which was a later homonym for a genus of flowering plants (Sydow & Sydow, 1919). At that time, species of *Ophiostoma* and related fungal genera such as *Ceratostomella* Sacc., were considered important only for the blue stain that they caused in lumber (Von Schrenk, 1903; Hedgcock, 1906; Münch, 1907, 1908a, b). It was, however, not long before the first pandemic of Dutch Elm disease, caused by *Ophiostoma ulmi* (Buism.) Nannf., swept through elm populations in both North America and Europe with devastating effects (Spierenberg, 1921; Wollenweber, 1927; Wollenweber & Stapp, 1928; Buisman, 1932). As a result of this disease, the attention of plant pathologists, and perhaps more importantly, mycologists, became focused on *Ophiostoma* species. Since the 1930's this genus, along with *Ceratocystis* Ell. & Halst., was intensively studied and much research has been published on the taxonomy, biology, and economic importance of many *Ophiostoma* species (Upadhyay, 1981; Wingfield *et al.*, 1993). The emphasis of this review, however, is not on the detrimental effects of the ophiostomatoid fungi, but on the possible benefits that some of these fungi could have as biological control agents in the pulp and paper industry.

Internationally, the pulp and paper industry is considered one of the largest consumers of roundwood timber. In 1994, the annual world-wide production of paper and board amounted to 260 million tons and it was expected to exceed 310 million tons in the year 2000 (Myréen, 1994). In South Africa, almost 60 % of all timber grown serves as raw material for pulping (Gerischer, 1994; Kruger & Dyer, 1995). Although two pulp mills in South Africa make use of bagasse (sugar-cane residue) (Gerischer, 1994), all the other mills, like the majority of pulp and paper mills world-wide, rely directly or indirectly (i.e. waste paper) on wood as their source of cellulose fibre (Smook, 1992).

Cellulose is one of the major structural components of wood, together with hemicellulose and lignin. The type and quantity of hemicellulose and lignin differs greatly in angiosperm and gymnosperm wood, and determines the main characteristics of these wood types

(Dadswell & Hillis, 1962; Blanchette, 1991a; Smook, 1992; Biermann, 1993). The cellulose fibre, which is the main constituent of the wood cell wall, is the most important component to the pulp industry (Dadswell & Hillis, 1962; Gerischer, 1994; Myréen, 1994). Lignin and hemicellulose serve as intercellular bonding material between these cellulose fibres (Dadswell & Hillis, 1962; Smook, 1992; Gerischer, 1994), but do not contribute to the mechanical strength of the fibres and reduce their bonding ability (Myréen, 1994). The purpose of pulping is, therefore, to separate the cellulose fibres by removing lignin (Gerischer, 1994). This can be accomplished either chemically, where the lignin is made soluble by chemical digestion, mechanically, where the wood chips are ground, or thermally, where elevated temperatures are used for softening and plasticising the lignin (Gardner & Hillis, 1962; Smook, 1992; Myréen, 1994). A combination of these treatments can also be applied to achieve the desired effect (Smook, 1992).

Wood extractives form another component of wood (Dadswell & Hillis, 1962; Smook, 1992; Biermann, 1993) and can be separated into three physiological categories: defensive resins, storage resins, and plant hormones (Brush *et al.*, 1994). Defensive resins comprise resin acids, terpenes, and phenolic compounds that protect trees against biological damage such as fungal and insect attacks (Blanchette, 1991b; Brush *et al.*, 1994; Morgan & Wyndham, 1996). Storage resins include fats, fatty acids, and waxes that serve as a reserve food supply to trees. Plant hormones are mainly phytosterols (Brush *et al.*, 1994).

The composition and concentration of wood extractives vary within the trees (Cohen, 1962; Farrell *et al.*, 1992), between trees (Cohen, 1962), wood species (Mutton, 1958, 1962; Braitberg, 1966; Biermann, 1993; Fischer *et al.*, 1996), geographical location (Farrell *et al.*, 1992; Brush *et al.*, 1994) and season of the year (Braitberg, 1966; Farrell *et al.*, 1993; Fischer *et al.*, 1996). As the chemical composition of extractives varies, so also do the physical and colloidal characteristics (Cohen, 1962). Generally, softwoods contain considerably more extractives than hardwoods, and among the commercial softwoods, the pines stand out as having by far the highest extractive content (Mutton, 1962; Smook, 1992).

Wood extractives, like lignin, can be the cause of production and paper quality problems in pulp and paper mills (Mutton, 1958, 1962; Hassler, 1988). Water-soluble extractives, such as soluble carbohydrates and phenolic compounds, do not cause serious problems during the pulping process (Brush *et al.*, 1994). Phenolic compounds that are chlorinated during the bleaching of pulp with chlorine are, however, toxic and can adversely affect the environment (Leuenberger *et al.*, 1985; Myréen, 1994; Annachhatre & Gheewala, 1996).

PITCH

Depositable pitch (Allen, 1980; Fischer & Messner, 1992a), often referred to as resin (Blanchette *et al.*, 1991), is considered one of the main problems in the pulping process. Pitch is a general term used for all low molecular weight, hydrophobic substances in wood that are soluble in neutral, non-polar organic solvents (Mutton, 1958; Blanchette, 1991c; Forde Kohler *et al.*, 1996) such as ethanol, methylene chloride, diethyl ether, benzene/alcohol mixtures, etc. (Mutton, 1962; Blanchette *et al.*, 1991). Pitch is composed of a large number of different components (**Table 1**). Some of these substances can be converted into new compounds during the pulping process and may be even more problematic than the original extractives (Fischer & Messner, 1992a; Brush *et al.*, 1994). Apart from the components listed in **Table 1**, pitch can also contain other compounds that have not been fully characterised (Allen, 1975; Brush *et al.*, 1994).

Pitch and the pulping process

Although pitch constitutes less than 10 % of the total weight of wood (Leopold & Mutton, 1959; Farrell *et al.*, 1992; Smook, 1992), it is responsible for a range of problems throughout the entire pulping process (Farrell *et al.*, 1992; Brush *et al.*, 1994). The nature of these problems depends on the type of wood (Burggraaf *et al.*, 1996), the type of processing (Farrell *et al.*, 1993; Burggraaf *et al.*, 1996), as well as the sequence of chemical conditions in the mill (Fischer & Messner, 1992a; Brush *et al.*, 1994).

Pitch located inside the parenchyma cells or on the surfaces of fibres and parenchyma cells in wood pulps, has little tendency to be deposited on processing equipment (Allen, 1975; Fischer & Messner, 1992c). Pitch is, however, released from the fibres at different times during pulping. It is this freely suspended colloidal pitch that appears to be the most troublesome form of pitch (Allen, 1975). The deposition of colloidal pitch usually occurs when there is a change of temperature and/or pH (Farrell *et al.*, 1989, 1992), or when triglycerides in the pulp are chlorinated and polymerised during bleaching to form sticky components (Leopold & Mutton, 1959; Fischer & Messner, 1992a, b, c). Deposition can take place alone or with fibres, fillers, defoamer components, coating binders from broke, insoluble inorganic salts (Farrell *et al.*, 1989, 1993; Nelson & Hemingway, 1971), sand, small stones, talc, asbestos, and in some cases dye (Nelson & Hemingway, 1971). Pitch deposited on the exposed parts of the paper machines, can impair the production process and

degrade product quality in various ways (Dreisbach & Michalopoulos, 1989; Fischer & Messner, 1992c), the most important of which are:

- Reduced wettability as a result of ether-soluble extractives present in ray cells (Mutton, 1958; Gardner & Hillis, 1962).
- Reduced penetrability of wood chips (Gardner & Hillis, 1962; Tay *et al.*, 1996).
- Increased chemical consumption (Gardner & Hillis, 1962).
- Reduced lignin solubility (Mutton, 1958; Gardner & Hillis, 1962).
- Increased amounts of bleaching chemicals needed (Hillis & Swain, 1962).
- High viscosity and weak burning properties of black liquor (Gardner & Hillis, 1962).
- Accumulation on mill equipment, leading to shut down while affected parts are cleaned or replaced (Cohen, 1962; Allen, 1980; Fischer & Messner, 1992a, b; Fujita *et al.*, 1992a, b; Smook, 1992; Iverson, 1994; Tay *et al.*, 1996).
- Equipment corrosion as a result of polyphenols (Gardner & Hillis, 1962).
- Reduced pulp washing efficiency due to foam formation (Gardner & Hillis, 1962; Dreisbach & Michalopoulos, 1989).
- Sticking of pulp at the press (Cohen, 1962; Gardner & Hillis, 1962; Allen, 1980; Smook, 1992).
- Reduced permeability of the press felt (Smook, 1992).
- A reduction in yield (Gardner & Hillis, 1962).
- Loss of brightness (Hillis & Swain, 1962).
- Reduced pulp and paper strength (Brandal & Lindheim, 1966; Farrell *et al.*, 1992; Blanchette, 1991c).
- Resin specks in the pulp and on paper produced from it (Cohen, 1962; Allen, 1980; Tay *et al.*, 1996).
- Breakage of paper on paper machines (Farrell *et al.*, 1989; Blanchette, 1991c).
- Holes and scabs in the final sheet (Dreisbach & Michalopoulos, 1989; Allen, 1980; Smook, 1992).

All these factors inevitably influence the quality and price of the finished product adversely (Mutton, 1958, 1962; Blanchette *et al.*, 1991, 1992; Fujita *et al.*, 1992a). Future prospects are that these problems are likely to become more severe because high speed machines induce pitch deposition and higher production rates overload washing equipment resulting in a dirtier, more pitch laden stock. In spite of these adverse trends, the market will still demand high quality products that are virtually free from pitch related defects (Dreisbach & Michalopoulos, 1989).

Pitch and the environment

Apart from the problems that pitch causes during pulping, another major driving force for new developments in pitch control is the impact that pitch has on the environment. A massive attack by environmental interest groups against the pulp industry was launched after polychlorinated dioxins and furans were discovered in the effluent of pulp mills (Myréen, 1994). Dioxins are formed during bleaching when elemental chlorine is used (Biermann, 1993), but may also originate from pentachlorophenol-based fungicides (Luthe, 1996; Elliott & Martin, 1998) applied to wood to control blue stain (Behrendt *et al.*, 1995a; Grönberg, 1996). The formation of measurable amounts of chlorinated dioxins in bleach plants can easily be eliminated by replacing chlorine with chlorine dioxide (Biermann, 1993; Elliott & Martin, 1998), but the campaign against chlorinated compounds is still active and is now focused on bleaching with chlorine-containing compounds in general (Myréen, 1994). The implementation of external waste water treatment plants at pulp mills world-wide has reduced the discharge of suspended solids and biologically oxygen-consuming substances considerably (Biermann, 1993; Myréen, 1994), but since the pulp mills are becoming larger, the contamination of receiving water by organic substances, nutrients and organo-chlorides poses an ever growing problem to the mills (Leuenberger *et al.*, 1985; Myréen, 1994; Elliott & Martin, 1998).

A significant fraction of these contaminating substances originate from pitch in the wood (Myréen, 1994; Hall & Liver, 1996; Liver & Hall, 1996). Free, chlorinated and decarboxylated resin acids, together with fatty acids, are among the most common components in the effluents and have been shown to contribute a major part of the acute lethal toxicity of these effluents to aquatic organisms (Leuenberger *et al.*, 1985; Biermann, 1993; Wang *et al.*, 1994, 1995; Burggraaf *et al.*, 1996; Morgan & Wyndham, 1996; Roy-Arcand & Archibald, 1996). Although laboratory trials have shown that toxic resin and fatty acids can be destroyed by activated sludge treatment (Kahmark & Unwin, 1996) and ozonation of the effluent, these methods, especially the latter one, would be quite expensive to apply at industrial level (Roy-Arcand & Archibald, 1996).

Apart from waste waters, air pollution at pulp and paper mills is also a major cause for concern (Biermann, 1993; Juuti *et al.*, 1996). Again, pitch contributes significantly to the problem. In this instance, it is in the form of volatile organic chemicals (Biermann, 1993), of which chloroform is considered to be the most important (Juuti *et al.*, 1996). It is, therefore, expected that a reduction in the pitch content of wood chips prior to pulping, will substantially

lower the detrimental effects of both types of pollution on the environment (Myréen, 1994). This would also be less expensive than attempting to treat effluents or to reduce air pollution.

PITCH CONTROL

1. Non-chemical control

A wide variety of non-chemical and chemical methods are applied in the paper industry to reduce problematic pitch in mills. Non-chemical solutions to pitch problems comprise the use of wood species with low pitch content (Farrell *et al.*, 1992, 1993; Allen, 1980), the felling of trees in the season when resin content is lowest (Cohen, 1962), the ageing or seasoning of wood chips or logs (Mutton, 1962; Blanchette *et al.*, 1991; Smook, 1992; Biermann, 1993; Tay *et al.*, 1996), the removal of fines, as well as the cooking (Mutton, 1958) and washing of pulp (Allen, 1980). During seasoning, the extractives in wood can be decreased through a number of proposed mechanisms, including microbial activity (Blanchette *et al.*, 1991), oxidative processes (Biermann, 1993), hydrolysis of the glyceride content (Mutton, 1958), and the activity of viable wood cells after felling (Brush *et al.*, 1994). However, certain components of resin, such as waxes, are only partially or not at all degraded by these mechanisms, and generally persist in the pulp (Blanchette *et al.*, 1991). Furthermore, outdoor storage or seasoning may induce pitch removal, but it usually adversely affects the quality of pulp, especially during prolonged storage, because of bacterial and fungal degradation (Cohen, 1962; Björkman & Haeger, 1963; Farrell *et al.*, 1992). These organisms can cause staining and discoloration of the wood and might even be responsible for a certain degree of cellulose degradation (Blanchette *et al.*, 1991).

2. Chemical control

Pitch can be chemically controlled by coagulation with alum (Farrell *et al.*, 1992, 1993; Biermann, 1993), stabilisation with dispersants (Braitberg, 1966; Allen, 1980) or adsorption onto talc (Hassler, 1988; Fischer & Messner, 1992c; Fujita *et al.*, 1992a) and other mineral surfaces (Allen, 1975; Smook, 1992). Other chemical control measures include chelating agents (Braitberg, 1966; Smook, 1992), surface active agents (Mutton, 1958; Biermann, 1993), retention aids (Allen, 1980; Farrell *et al.*, 1993), cationic polymers (Hassler, 1988; Biermann, 1993), anionic polymers (Hassler, 1988) and fractionation (Allen, 1980). Chemical control measures are sometimes combined with mechanical treatments (e.g. high-

compression screw pressing) to remove resin from pulp (Tay *et al.*, 1996). Although these conventional methods reduce the problems caused by pitch, they are costly and do not fully resolve the problems (Braitberg, 1966; Fischer & Messner, 1992a; Fujita *et al.*, 1992).

3. A biochemical approach

In recent years, biotechnology has offered new options for pitch control, the first of which is a biochemical approach. This involves the treatment of wood chips with enzymes, specifically lipases, prior to pulping, or the modification of mechanical or sulfite pulp with similar enzymes (Fischer & Messner, 1992b, c; Fujita *et al.*, 1992b; Iverson, 1994; Grönberg & Dunlop-Jones, 1996). In both cases triglycerides are hydrolysed and the liberated fatty acids are extracted with sodium hydroxide solution in a washing stage. The enzyme treated pitch is much less adhesive than native pitch (Fischer & Messner, 1992a, b; Fujita *et al.*, 1992a; Brush *et al.*, 1994; Iverson, 1994; Wang *et al.*, 1995). This approach was the first successful application of biotechnology in a paper making process, and the technology has been applied routinely at two paper mills in Japan since early 1990. It is, however, not yet commercially applied since more research is being conducted to develop a thermostable lipase (Fujita *et al.*, 1992a, b).

4. Non-specific biological control

The biological detoxification of chlorinated and non-chlorinated resin acids in the secondary treatment of pulp mill effluents, is widely applied to reduce the impact of pitch on the environment. The results of this process, however, depend on a range of environmental factors and are, therefore, inconsistent. Furthermore, the sustained presence of resin acids in receiving waters and sediments downstream from pulp mills remains a concern (Wang *et al.*, 1995). Although there are currently researchers working on the possibility of utilising specific resin acid degrading bacteria in the treatment of effluents (Morgan & Wyndham, 1996), the work is in a very preliminary stage and far from being applied at industrial level. This type of research is aimed at treating the consequences, rather than the cause, of pitch related pollution in the effluent.

5. A biological approach: Cartapip

A second biotechnological option for pitch control is a biological approach where a living fungus, *Ophiostoma piliferum* (Fr.) H. & P. Syd., is applied to reduce pitch by metabolising it (Farrell *et al.*, 1992; Fischer *et al.*, 1994; Iverson, 1994). Although other fungi, including

white-rot (lignin-degrading) fungi such as *Ceriporiopsis subvermispora* (Pil.) Gilbn. & Ryv. and *Phlebiopsis gigantea* (Fr.) Jül., lower the resin content of wood chips (Fischer *et al.*, 1994, 1996; Blanchette *et al.*, 1997), *O. piliferum* was the first fungus to be commercially applied in a biopulping process (Farrell *et al.*, 1992, 1993). Since the application of this approach is the topic of this review, it is appropriate to briefly consider the development of the product and process.

5.1 Origin of the fungus. In Virginia, U.S.A., Bear Island Paper Company (B.I.P. Co.) operates a thermomechanical pulp mill where newsprint is produced for the Washington Post and Wall Street Journal (Farrell *et al.* 1989, 1993). For many years the company applied the practice of ageing pulpwood to attain a certain level of pitch reduction. Although this was achieved to some extent, pitch remained a serious problem at the mill (Farrell *et al.*, 1992, 1993).

Over the years, it was recognised that during summer months, a pulp containing less pitch was produced and that the paper machines ran much more efficiently. Paper strength also improved during this time (Farrell *et al.*, 1989, 1992, 1993). However, at the time when pitch reduction was optimal, a darkening of wood chips, associated with blue stain, occurred with a significant decrease in pulp brightness (Farrell *et al.*, 1989; Blanchette *et al.*, 1991).

In 1987, Sandoz Chemicals Biotech Research Corporation (SCBRC) of Lexington, Massachusetts, (now Biotech Division of Clariant Corporation) set up a biological screening program at the B.I.P. Co. mill to determine whether a living organism was responsible for the reduction of pitch (Farrell *et al.*, 1989, 1993, 1998). By 1988, SCBRC had identified several naturally occurring fungi from the southern yellow pine wood chip pile. These fungi reduced pitch by more than 50 % in less than two weeks in laboratory trials (Blanchette, 1991c; Farrell *et al.*, 1993). One of the fungi was a well known blue staining ascomycete, *O. piliferum*, that is commonly found throughout the U.S.A. (Upadhyay, 1981; Farrell *et al.*, 1989, 1992; Blanchette *et al.*, 1992). Various other species of *Ophiostoma* and other fungal genera were also isolated from the chip piles. *Ophiostoma piliferum*, however, displays several characteristics that makes it suitable for application as a biological control agent (Blanchette, 1991c; Farrell *et al.*, 1992, 1993). These are as follows:

- It is a member of the genus *Ophiostoma,* which is known for its aggressive pioneer species that are able to colonise freshly cut wood (Blanchette, 1991c; Blanchette *et al.*, 1992, 1994, 1997; Farrell *et al.*, 1993; Behrendt *et al.*, 1995a).

- The fungus has the ability to grow on different types of wood, and naturally infects most of the wood species in need of treatment (Blanchette *et al.*, 1991; Wall *et al.*, 1995).
- *O. piliferum* can grow strongly and rapidly in a competitive non-sterile environment (Blanchette *et al.*, 1991; Wall *et al.*, 1994, 1995; Behrendt *et al.*, 1995a).
- It is not associated with bark beetles and is considered to be exclusively saprophytic (Farrell *et al.*, 1989; Blanchette *et al.*, 1991, 1992; Grönberg, 1996), although there has been one report that it is pathogenic to *Pinus taeda* (Basham, 1970).
- The cellulosic content of wood is not substantially degraded by *O. piliferum* (Blanchette *et al.*, 1991; Anonymous, 1992a; Wall *et al.*, 1994, 1995; Behrendt *et al.*, 1995a) and it also does not produce ligninases (Wall *et al.*, 1994).
- *O. piliferum* can be grown in liquid culture, which implies that production can be carried out in existing fermentation facilities (Farrell *et al.*, 1989, 1992).
- The fungus can grow at temperatures from 4 to 40 °C, with optimal growth at 20 to 30 °C (Wall *et al.*, 1994), enabling it to survive and compete at varying temperatures in chip piles.
- *O. piliferum* is classified in the subphylum Ascomycotina because it produces homokaryotic ascospores (Farrell *et al.*, 1989; Kendrick, 1992). This ability makes it possible to select for strains of the fungus with specific characteristics (Farrell *et al.*, 1989). A homokaryotic strain is also preferred for the process because the characteristics of such a strain are stable (Blanchette *et al.*, 1991).

5.2 ***Development of the product.*** Due to its positive biocontrol characteristics, *O. piliferum* was identified as the most suitable candidate for pitch control. The next objective was to develop a product which could be manufactured cost effectively at an appropriate scale for application in the pulp and paper industry. The product had to reduce pitch effectively without affecting brightness, and it had to be easy to apply. Such a product was produced in mid 1990 by crossing two homokaryotic strains of *O. piliferum*: one a strong pitch remover and the other a white strain that did not produce the typical dark mycelium (Farrell *et al.*, 1989, 1992; Wall *et al.*, 1995). From the offspring, a non-pigmented isolate that grows rapidly while degrading substantial quantities of pitch in laboratory trials, was selected (Blanchette *et al.*, 1992; Anonymous, 1992a; Farrell *et al.*, 1992, 1993; Grönberg & Dunlop-Jones, 1996). It appears as if there is a connection between the inability to produce pigment (melanin) and the fact that this strain is unable to produce mature perithecia, because melanin

plays a role in perithecial development. It is, therefore, unlikely that this strain would mutate back to a pigment producing, blue-staining fungus (Zimmerman *et al.*, 1995).

Another consideration was to determine whether the selected strain was safe to humans. Through extensive animal studies, it was shown to be non-pathogenic and non-toxic, and thus unable to cause disease in animals and humans (Blanchette *et al.*, 1991; Anonymous, 1992a). At this stage Sandoz filed patent applications for the product world-wide (Farrell *et al.*, 1989; Blanchette *et al.*, 1991).

The first field trials with the new product were conducted on southern yellow pine chip piles 6-10 wet tons in size. The product proved to be easy to apply, and complete colonisation of the chip piles took place within 4-10 days. Pitch was again reduced by more than 50 % (Farrell *et al.*, 1989; Blanchette *et al.*, 1994). Subsequent field trials confirmed these results (Blanchette *et al.*, 1992; Forde Kohler *et al.*, 1997).

Following the field trials, large scale mill trials were conducted on 1000-7000 wet tons of chips. The resulting thermomechanical pulp had significantly reduced pitch levels, leading to a reduction of traditional pitch controlling agents. Paper machine efficiency and paper strength improved, insolubles in waste water treatment systems were reduced and there was an overall increase in yield at the mill (Farrell *et al.*, 1989).

This new product was called Cartapip™, which is an acronym for Pulp Improvement Product (Farrell *et al.*, 1989). Three strains of *O. piliferum* were originally registered, namely Cartapip™ 28, Cartapip™ 58 and Cartapip™ 97, but the only one to reach the market was Cartapip™ 97 (Farrell *et al.*, 1993). The product proved to serve its purpose and the first sales were made in December 1990 (Farrell *et al.*, 1989, 1993). It is currently sold under the trade name of Cartapip™ (Anonymous, 1994; Behrendt *et al.*, 1995a; White-McDougall *et al.*, 1998) and is available from Clariant Chemicals Corporation, Charlotte, North Carolina (Blanchette *et al.*, 1997). Ongoing research is being conducted to develop even more aggressive albino strains, also from other *Ophiostoma* species such as *O. piceae* (Münch) H. & P. Syd. and *O. pluriannulatum* (Hedgc.) H. & P. Syd. (Blanchette *et al.*, 1997; Farrell *et al.*, 1998; White-McDougall *et al.*, 1998).

5.3 Application. Cartapip is marketed as a dry, light brown powder consisting of lyophilised fungal biomass with additives such as preservatives and stabilising agents (Farrell *et al.*, 1989, 1992; Blanchette, 1991c; Blanchette *et al.*, 1991, 1994). It can be diluted in any proportion with fresh water (Blanchette *et al.*, 1991; Anonymous, 1992b; Farrell *et al.*, 1992; Grönberg, 1996), but a 1-3 % solids solution is advisable (Farrell *et al.*, 1989). A dosage of

1 kg/100 tons of wet chips is recommended (Anonymous, 1992b). When sprayed onto freshly cut wood, the living fungal material in Cartapip is activated and rapidly colonises the wood (Farrell *et al.*, 1989; Blanchette, 1991c; Blanchette *et al.*, 1991, 1994; Anonymous, 1992a; Grönberg, 1996). The dosage and storage time in chip piles may vary considerably and depends on a number of factors including the wood species, wood age, the temperature and moisture conditions, geographical location, the original microbial conditions of the wood, and the amount of pitch desired to be removed (Blanchette, 1991c; Anonymous, 1992a, b; Farrell *et al.*, 1993). Satisfactory results are generally obtained after a period of time extending from 7 to 35 days (Blanchette *et al.*, 1991; Wall *et al.*, 1994), and the treatment seems to be effective for at least two to three months (Grönberg & Dunlop-Jones, 1996). Wood treated with Cartapip is suitable for use in any conventional pulping system, including mechanical, thermomechanical, chemimechanical, chemithermomechanical and chemical processes (Blanchette *et al.*, 1991).

A Cartapip treatment programme is easy to implement (Anonymous, 1994) and can be applied on a wide variety of pulpwood (**Table 2**). Although it was initially tested on southern yellow pine (*Pinus taeda*), it has since been shown to effectively remove pitch from many other softwoods. Certain benefits have also been observed for some hardwoods (Blanchette *et al.*, 1991; Anonymous, 1992a).

Cartapip has not only been applied successfully as biological control agent of pitch and blue stain fungi on pulpwood chips and mechanical pulps, but also on debarked and undebarked cut timbers (Blanchette *et al.*, 1991, 1994; Behrendt *et al.*, 1994, 1995a, b; Grönberg, 1996; Grönberg & Dunlop-Jones, 1996; Blanchette *et al.*, 1997; Uzunovic *et al.*, 1999). Treatment of cut timbers is, however, of a longer duration than that of refined pulpwood and may extend for two months or more (Blanchette *et al.*, 1991). This application of Cartapip might lead to a reduction in the use of expensive and toxic fungicides to control sapstain on timber in future (Behrendt *et al.*, 1995a).

5.4 Effects of Cartapip on wood. According to the developers of the product, Cartapip metabolises resin or pitch in the wood while growing in the tracheids, ray parenchyma cells and resin ducts (Farrell *et al.*, 1992, 1993; Anonymous, 1992a, 1994; Wall *et al.*, 1994). Furthermore, the levels of steryl esters (Wall *et al.*, 1995; Rocheleau *et al.*, 1999), triglycerides, fatty acids and resin acids in the wood are reduced by the fungus prior to pulping (Farrell *et al.*, 1989, 1992; Blanchette, 1991c; Anonymous, 1994; Brush *et al.*, 1994; Dorado *et al.*, 2000). However, independent studies on the effects of Cartapip on

Pinus sylvestris (Scots pine), produced some contradictory results. While the triglyceride and free fatty acid content was reduced by Cartapip, it did not reduce the sterol and resin acid content of the wood (Grönberg, 1996; Grönberg & Dunlop-Jones, 1996; Dorado *et al.*, 2000). Various isolates of *O. piliferum* were also tested on *Eucalyptus globulus* wood chips. The fungi hydrolyzed the sterol esters and triglycerides in the wood, but increased the content of free sitosterol, a major compound in pitch deposits. *Ophiostoma piliferum*, therefore, does not seem to be suitable for the treatment of *Eucalyptus* wood (Gutiérrez *et al.* 1999).

Apart from removing the pitch, Cartapip also affects the ray parenchyma cell walls, but tracheid cell walls are not degraded by the fungus and no loss of wood strength occurs (Blanchette *et al.*, 1992; Breuil *et al.*, 1994; Brush *et al.*, 1994). The disruption of ray parenchyma cells does, however, weaken the binding of tracheids, allowing for easier separation during the mechanical refining process, which could reduce energy requirements. Rather than breaking the fibres, the refining process detaches longer tracheids more readily. The result of weakened ray cells is longer fibres, which improve paper strength (Blanchette *et al.*, 1992).

The disruption of ray parenchyma cells and the perforation of pit membranes also increase the porosity of wood. Pitch removal together with increased porosity enables cooking chemicals to diffuse into the wood so that improved impregnation is achieved. Lower amounts of chemicals and shorter cooking times are, therefore, needed for sulphate (kraft) and sulphite processes (Blanchette, 1991c; Blanchette *et al.*, 1992; Anonymous, 1992a; Wall *et al.*, 1994).

Another advantage of Cartapip is that it is a primary coloniser. When it is applied on especially freshly chipped wood, it competes strongly with other fungi occurring naturally on chip piles (Blanchette, 1991c; Anonymous, 1992a; Farrell *et al.*, 1993; Zimmerman *et al.*, 1995; White-McDougall *et al.*, 1998). These can be staining fungi, including other *Ophiostoma* species, and/or wood degrading fungi, especially the soft (or white) rot and brown rot fungi (Lindgren & Eslyn, 1961; Blanchette, 1991a, c; Blanchette *et al.*, 1992). Cartapip, therefore, reduces staining and enhances pulp brightness, resulting in a reduction of chemicals needed for bleaching (Araki & Lee, 1991; Blanchette, 1991c; Blanchette *et al.*, 1992; Farrell *et al.*, 1992; Behrendt *et al.*, 1995b). An increase in yield should also be possible where cellulose degrading fungi are out-competed by Cartapip (Anonymous, 1992a).

5.5 Benefits. Since Cartapip was first tested at the B.I.P. Company thermomechanical mill, is has been applied regularly at the mill. Scientific studies conducted over a period of several

years confirmed that the application of Cartapip is beneficial for thermomechanical pulping processes (Wall *et al.*, 1995; Grönberg, 1996; Grönberg & Dunlop-Jones, 1996). These benefits, including those for chemical pulping, can be summarised in three categories (Anonymous, 1992a):

Quality:

- Overall improvement of chip quality (reduction of background organisms) (Anonymous, 1992a, 1994; Wall *et al.*, 1994; Grönberg, 1996).
- Reduction of rejects (Anonymous, 1992a, 1994; Wall *et al.*, 1994, 1996).
- Decrease in fines content (Anonymous, 1994; Forde Kohler *et al.*, 1996; Wall *et al.*, 1994, 1995).
- Reduced extractives (Anonymous, 1992a, 1994; Wall *et al.*, 1994, 1995, 1996; Forde Kohler *et al.*, 1995, 1997; Grönberg & Dunlop-Jones, 1996; White-McDougall *et al.*, 1998; Rocheleau *et al.*, 1999; Dorado *et al.*, 2000).
- Improved penetration of cooking chemicals (Anonymous, 1994; Wall *et al.*, 1996).
- Lignin removal is facilitated (Anonymous, 1994).
- Chips and pulp are easier to wash (Anonymous, 1992a, 1994).
- Increased viscosity (Anonymous, 1992a; Wall *et al.*, 1994, 1996; Grönberg & Dunlop-Jones, 1996).
- Reduction in Kappa number (Wall *et al.*, 1994, 1995, 1996; Grönberg & Dunlop-Jones, 1996).
- Longer fibre lengths (Forde Kohler *et al.*, 1995, 1996, 1997).
- Increased brightness (Anonymous, 1992a, 1994; Wall *et al.*, 1994, 1995, 1996; Grönberg & Dunlop-Jones, 1996).
- Improved tensile, tear, burst and overall paper strength (Anonymous, 1992a, 1994; Forde Kohler *et al.*, 1995, 1996, 1997; Wall *et al.*, 1993, 1994, 1995, 1996; Grönberg, 1996; Grönberg & Dunlop-Jones, 1996).

Economic:

- Reduced seasoning time for chips (Anonymous, 1992a, 1994).
- Shorter cooking times (Anonymous, 1992a; Wall *et al.*, 1994, 1996).
- Reduced pitch control agents (Wall *et al.*, 1993, 1995).
- Reduced alum usage (Anonymous, 1992a; Wall *et al.*, 1995).
- Reduced active alkali requirement (Wall *et al.*, 1994, 1996).

- Reduced expensive bleaching chemicals (Anonymous, 1992a, 1994; Wall *et al.*, 1994, 1995, 1996; Grönberg, 1996; Grönberg & Dunlop-Jones, 1996).
- Reduced black liquor solids (Anonymous, 1992a).
- Reduced pitch build-up, less downtime on cleaning machines (Anonymous, 1992a; Forde Kohler *et al.*, 1996; Wall *et al.*, 1994, 1995, 1996).
- Improved paper machine speed/runnability (Wall *et al.*, 1994, 1995; Grönberg, 1996).
- Reduced load on recovery system (Wall *et al.*, 1994).
- Higher yield (Anonymous, 1992a; Anonymous, 1994).

Environment:

- Classified by the EPA and USDA as non-pathogenic and non-toxic to plants, animals and humans (Anonymous, 1992a, 1994; Wall *et al.*, 1994; Grönberg, 1996).
- Allows for reduction of chlorinated organics (Anonymous, 1992a, 1994).
- Reduced toxicity of pulp mill effluent (Anonymous, 1992a).

5.6 Potential problems. Cartapip, in theory, has a large number of benefits for the pulp and timber industries. However, there might be problems with its implementation on a large scale. *Ophiostoma piliferum* grows best between 19 and 35 °C (Anonymous, 1992a). The differences in temperature within large chip piles may cause an uneven colonisation pattern by the fungus (Björkman & Haeger, 1963) and, therefore, an uneven removal of pitch. Furthermore, the strain currently marketed as Cartapip originated from much cooler climatic conditions than the almost sub-tropical conditions at most of South Africa's pulp mills and the major export harbour for wood and wood products, Richards Bay. The fungus might also not compete effectively with sapstain fungi adapted to these conditions.

5.7 Obstacles in the market place. At present, Cartapip is applied in only a limited number of mechanical pulp mills in the USA and Europe. Since little has been published on the industry's reaction to Cartapip, possible reasons for it not being applied more widely must be considered. From personal conversations with people in the pulp industry, some reasons for the reluctance to implement the biological approach encompassed by Cartapip are:

- A lack of knowledge about the product. This is despite the fact that it has been extensively advertised in journals such as *Tappi,* and at pulp and paper technology congresses.

- Many pulp mills would have to be completely re-arranged to accommodate a longer storage time of wood chips.
- Limited storage space might be a problem at many mills.
- At a large South African pulp mill, it has recently taken two years to optimise conditions for optimum brightness and yield. Considering the number of variables in the pulping process, the operators of such a mill will be extremely hesitant to experiment with a new product such as Cartapip.
- A part of the extractive content of wood is recovered as black liquor which is utilised at many mills, including mills of important companies like Weyerhaeuser, to produce electricity (Gardner & Hillis, 1962; Smook, 1992; Biermann, 1993; Myréen, 1994; Raymond, 1996). Removing pitch from the wood may have a negative effect on this important alternative energy source.
- The production of turpentine (Smook, 1992; Biermann, 1993) and its secondary products such as camphor, solvents and insecticides (Smook, 1992) at certain mills, might be negatively influenced.
- The production of tall oil (Gardner & Hillis, 1962; Anonymous, 1992a), which is refined into useful products such as soaps and lubricants (Smook, 1992; Biermann, 1993), might also be reduced.

Rapid changes in the pulp and paper industry are not conceivable since it is one of the most capital-intensive of the large-scale manufacturing industries. Modernisation takes place depending on the technical state of the actual mill and on the financial situation of the company, which depends on the fluctuating market situation (Myréen, 1994). Although Cartapip is completely non-toxic, has no health risk to humans, and is not damaging to the environment, the primary consideration for its application will, for many companies, be financial, rather than environmental (Farrell *et al.*, 1989; Anonymous, 1992a).

CONCLUSIONS

Until recently, very little interest has been shown in the utilization of microorganisms by the paper and pulp industry. Opportunities to improve the pulping process through the use of various microbial treatments are, however, beginning to attract serious attention. In addition, pressure from environmental groups to reduce levels of toxic substances used in pulp and

paper production, are also influencing the major pulp companies to consider changes in the production processes.

At the Third Paper Industry Research Needs Workshop, held in 1996 at North Carolina State University, delegates compiled a list of the overall top ten research needs for the international pulp and paper industry. According to this list, the first priority for the industry should be 'to develop techniques for increased closure of mill systems, reducing liquid effluent discharges, moving toward the minimum-environmental-impact mill' (Edwards, 1997). Coming from representatives of the paper industry, this clearly illustrates the growing environmental awareness of the industry.

The introduction of Cartapip to the pulp industry world-wide has emerged at a good time, also for the South African industry. Although the industrial application of Cartapip might imply adaptation and some reorganisation of existent practices at most mills, the promising results of large scale mill trials should encourage the South African pulp and paper industry to, at the least, consider Cartapip and similar products, and investigate the possibilities of applying them.

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Table 1. A classification of the components of pitch according to Mutton (1962). Other references mentioning some of the individual components of pitch are also listed.

Main group	Subgroup	References
OLEORESIN (mainly in conifers)	1. Terpenes:	(Allen, 1975; Blanchette, 1991c; Smook, 1992)
	- monoterpenes	(Biermann, 1993)
	- diterpenes	(Leuenberger *et al.*, 1985; Biermann, 1993)
	- sesquiterpenes	(Biermann, 1993)
	- triterpenes	(Cohen, 1962)
	2. Resin acids:	(Mutton, 1958; Cohen, 1962; Nelson & Hemingway, 1971; Allen, 1975; Blanchette, 1991c; Smook, 1992; Biermann, 1993; Myréen, 1994; Gao *et al.* 1995; Burggraaf *et al.*, 1996; Morgan & Wyndham, 1996)
	- diterpene acids	(Biermann, 1993; Wang *et al.*, 1995; Burggraaf *et al.*, 1996)
	3. Neutral materials:	
	- esters of resin acids	
	- aldehydes	
	- tricyclic diterpenes	
	- bicyclic sesquiterpene alcohols	
	- diterpene alcohols	
FATTY RESIN (hard- and softwoods)	1. Fatty acids:	(Mutton, 1958; Cohen, 1962; Nelson & Hemingway, 1971; Allen, 1975; Blanchette, 1991c; Smook, 1992; Biermann, 1993; Myréen, 1994; Gao *et al.* 1995)
	- glycerolesters of fatty acids (fats and oils)	(Mutton, 1958; Cohen, 1962; Nelson & Hemingway, 1971; Allen, 1975; Blanchette, 1991c; Biermann, 1993; Gao *et al.* 1995; White-McDougall *et al.*, 1998)
	- esters of higher monohydric alcohols (waxes)	(Cohen, 1962; Blanchette, 1991c; Gao *et al.* 1995; White-McDougall *et al.*, 1998)
	2. Unsaponifiables:	(Mutton, 1958; Cohen, 1962; Nelson & Hemingway, 1971)
	- sterols	(Cohen, 1962; Allen, 1975; Blanchette, 1991c; Smook, 1992; Biermann, 1993; Gao *et al.* 1995; White-McDougall *et al.*, 1998)
	- alcohols	(Cohen, 1962; Smook, 1992; Biermann, 1993; Gao *et al.* 1995)
	- saturated aliphatic hydrocarbons	(White-McDougall *et al.*, 1998)
	- phenolic compounds	(Leuenberger *et al.*, 1985; Biermann, 1993; Gao *et al.* 1995)
	~ flavonoids	(Allen, 1975; Biermann, 1993)
	~ tannins	(Biermann, 1993)

Table 2. Wood types on which Cartapip can be applied.

Genus or specie of tree	Common name	References
- *Abies concolor* (Gord. & Glend.) Lindl.	white fir	(Wall *et al.*, 1994)
- *Acer* species	Maple	(Wall *et al.*, 1994, 1995)
- *Betula* species	Birch	(Blanchette, 1991c; Blanchette *et al.*, 1991)
- *Cedrus* species	Cedar	(Blanchette *et al.*, 1991)
- *Cupressus* species	Cypress	(Blanchette *et al.*, 1991)
- *Eucalyptus* species	Gum	(Blanchette, 1991c; Farrell *et al.*, 1993)
- *Fagus* species	Beech	(Blanchette *et al.*, 1991)
- *Larix* species	Larch	(Blanchette *et al.*, 1991)
- *Picea* species	Spruce	(Blanchette, 1991c; Blanchette *et al.*, 1991)
- *Picea abies* (L.) Karst	Norway spruce	(Fischer *et al.*, 1994)
- *Picea glauca* (Moench) Voss.	White spruce	(Grönberg, 1996; Grönberg & Dunlop-Jones, 1996)
- *Pinus* species	Pine	(Farrell *et al.*, 1989; Blanchette, 1991c; Blanchette *et al.*, 1991)
- *Pinus banksiana* Lamb.	Jack pine	(Wall *et al.*, 1994, 1995)
- *Pinus contorta* Dougl. var. *latifolia* Engelm.	lodgepole pine	(Blanchette *et al.*, 1991)
- *Pinus nigra* Arnold	Austrian Corsican pine	(Jones *et al.*, 1998)
- *Pinus radiata* D. Don	Monterey/radiata pine	(Wall *et al.*, 1995; Farrell *et al.*, 1998)
- *Pinus resinosa* Ait.	red pine	(Behrendt *et al.*, 1994, 1995b; Blanchette *et al.*, 1997)
- *Pinus sylvestris* L.	Scots pine	(Grönberg, 1996; Grönberg & Dunlop-Jones, 1996; Jones *et al.*, 1998; Dorado *et al.*, 2000)
- *Pinus taeda* L.	loblolly pine (southern yellow pine)	(Blanchette, 1991c; Blanchette *et al.*, 1992; Farrell *et al.* 1993; Brush *et al.*, 1994; Fischer *et al.*, 1994; Forde Kohler *et al.*, 1997)
- *Pinus virginiana* Mill.	Virginia pine (southern yellow pine)	(Farrell *et al.*, 1989; Brush *et al.*, 1994; Forde Kohler *et al.*, 1997)
- *Populus* species	aspen and poplar	(Farrell *et al.*, 1989; Blanchette, 1991c; Blanchette *et al.*, 1991)
- *Populus grandidentata* Michx.	bigtooth aspen	(Grönberg, 1996; Grönberg & Dunlop-Jones, 1996)
- *Populus tremuloides* Michx.	quaking aspen	(Blanchette *et al.*, 1997; White-McDougall *et al.*, 1998; Rocheleau *et al.*, 1999)
- *Pseudotsuga menziesii* (Mirb.) Franco	Douglas fir	(Blanchette *et al.*, 1991; Farrell *et al.*, 1993)
- *Quercus* species	Oak	(Blanchette *et al.*, 1991)
- *Taxus baccata* L.	Yew	(Blanchette *et al.*, 1991)
- *Nyssa* species	Tupelo	(Blanchette *et al.*, 1991)
- other northern hardwoods		(Farrell *et al.*, 1993)
- mixed tropical hardwoods		(Farrell *et al.*, 1989, 1993)

Chapter 2

A classification is merely a hypothesis that will be confirmed or rejected by testing.

- Luttrell, 1977

Ophiostomatoid fungi resembling *Ophiostoma piliferum* on pulpwood chips and other hardwood substrates in South Africa

ABSTRACT

The ophiostomatoid fungi include some economically important plant pathogens and sapstain fungi belonging to genera such as *Ophiostoma* and *Ceratocystis*. *Ophiostoma piliferum*, however, is considered important because a white mutant of this fungus, marketed as Cartapip®, is applied on pulpwood chips to reduce pitch and prevent sapstain. The South African forestry industry could benefit from biological control products such as these. The importation of a product based on a living organism can, however, be problematic due to quarantine regulations, if the organism does not already occur in the importing country. Although several ophiostomatoid fungi have been reported from South Africa, *O. piliferum* has not been found in this country. The aim of this study was to collect and identify South African isolates resembling *O. piliferum* from pulpwood chips and other exotic and native wood substrates. Wood chips were incubated under moist conditions and isolations were made once perithecia were produced. Structures were investigated microscopically. A total of 32 isolates resembling *O. piliferum* were obtained, of which six were from indigenous hosts. Based on morphology, the isolates could be divided into three groups, of which none represented *O. piliferum*. The first group had smaller ascospores than *O. piliferum* and resembled *O. stenoceras*, while the second group had annuli on the perithecial necks and resembled *O. pluriannulatum*. The third group had both a *Sporothrix* and a *Pesotum* anamorph, and were identified as either *O. piceae* or *O. querci*. The identity of all three groups of isolates must be confirmed by comparing them with herbarium material and authenticated isolates from other parts of the world. The possible application of these isolates as biological control agents in the pulp and timber industry also deserved investigation.

INTRODUCTION

The ophiostomatoid fungi represent a phylogenetically diverse group that includes several well-known genera of Ascomycetes such as *Ophiostoma* H. & P. Sydow and *Ceratocystis* Ell. & Halst. The most prominent character shared by all ophiostomatoid species is long-necked ascocarps producing spores in sticky masses that are adapted for insect dispersal. Similarly, many of their associated anamorph genera have conidiophores that are adapted for dispersal by insects (Upadhyay, 1981; Wingfield *et al.*, 1993b). These anamorphs include genera such as *Sporothrix* Hektoen & Perkins ex Nicot & Mariat, *Leptographium* Lag. & Melin, *Pesotum* Crane & Schoknecht, and *Chalara* (Corda) Rabenh. (Mouton *et al.*, 1994a).

The insect-associated ophiostomatoid fungi include various economically important pathogens. Well-known examples are the causal agents of Dutch Elm Disease, *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier (Hubbes, 1999), the three varieties of *Leptographium wageneri* (Goheen & F.W. Cobb) T.C. Harr. responsible for black-stain root disease of conifers in the United States (Cobb, 1988), and *Ceratocystis fagacearum* (Bretz) Hunt, associated with vascular wilt of oak in the United States (Appel, 1995). Many ophiostomatoid species are also associated with sapstain or bluestain of timber (Seifert, 1993).

Not all ophiostomatoid species are harmful to trees or detrimental to timber. For example, a white mutant of one of the bluestain species, *Ophiostoma piliferum* (Fries) H. & P. Sydow, is marketed as a pitch-removing and anti-sapstain agent, known as Cartapip®, in the United States and Europe (Farrell *et al.*, 1993). *Ophiostoma piliferum* is characterised by orange section shaped ascospores and a *Sporothrix* anamorph (Upadhyay, 1981). Other species that are morphologically and ecologically similar to *O. piliferum*, have also been reported as potential candidates for the control of pitch and sapstain. These include *O. pluriannulatum* (Hedgcock) H. & P. Sydow, *O. piceae* (Münch) H. & P. Sydow and *O. floccosum* Mathiesen (Kay, 1997; Blanchette *et al.*, 1997). The South African forestry industry could benefit greatly from biological control agents such as these. The importation of products based on living organisms, however, can be problematic due to quarantine regulations, especially if the organisms do not already occur in the importing country (Palm, 1999).

Several ophiostomatoid fungi have been reported from South Africa (**Table 1**). Among these, *O. piliferum* was reported in 1937 from pine in Mpumalanga (Laughton, 1937). However, no ascospores were observed in or around the perithecia, and no associated anamorphs were mentioned. This implies that the perithecia could have belonged to any of a

number of other *Ophiostoma*, or even *Ceratocystis*, species. The report can, therefore, not be considered as positive evidence for the occurrence of *O. piliferum* in South Africa.

In 1947, another report mentioned a species of *Graphium* Corda associated with a *Sporotrichum* Link ex Fr. state, producing perithecia in culture (Brown *et al.*, 1947). The teleomorph was not assigned a name in this case. *Ophiostoma* anamorphs previously assigned to *Graphium* and *Sporotrichum*, are at present considered species of *Pesotum* and *Sporothrix* respectively (Okada *et al.*, 1998; De Hoog, 1974). Several *Ophiostoma* species produce both *Pesotum* and *Sporothrix* anamorphs and most reside in the *O. piceae* complex (Harrington *et al.*, 2001). The isolate reported from timber in a South African mine (Brown *et al.*, 1947), could therefore, have belonged to any of the species in this complex. Unfortunately, no cultures were preserved from either the study of Laughton (1937) or Brown *et al.* (1947), and confirmation of the identity of the fungi is not possible. Thus, there are no clear reports of *Ophiostoma* spp. from South Africa known to have potential in biological control. The objective of this study was, therefore, to collect and identify South African isolates resembling *O. piliferum* from woody substrates from niches where these fungi are known to occur.

METHODS

Collection and isolation

Hard- and softwood chips from three major pulp mills in South Africa (Sappi Ngodwana; Mondi Kraft Richards Bay; Sappi Saiccor Umkomaas) were screened for the presence of fungi resembling *O. piliferum*. Two 1 kg bags of hardwood (*Eucalyptus* and/or *Acacia mearnsii*) chips, and two 1 kg bags of pine chips, were collected from different locations in the chip piles at each of these sites. The sampling was repeated twice at each site within a period of one year. From each bag, approximately 30 chips were randomly selected and incubated in 10 Petri dishes with moist tissue paper at room temperature. The appearance of sexual and asexual structures was noted during the incubation period and cultures were made on 2% Malt Extract Agar (MEA) by transferring ascospore or conidial masses to the growth medium.

Cultures resembling *O. piliferum*, originating from other wood sources within South Africa, were also included in this study. Some of these cultures originated from samples received by the diagnostic clinic of the Tree Pathology Cooperative Programme (TPCP)

during the past 12 years. Other isolates, from both exotic and indigenous hosts, were collected during field extention work of the TPCP in forestry areas. All cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.

Identification of isolates

All isolates were cultured on Water Agar in the presence of sterilised pieces of wood (5 mm x 5 mm x 30 mm) representing the original host of the fungus. This was done to enhance the production of perithecia and ascospores. Cultures were incubated at 20 to 25 °C until sexual structures formed. Fifty perithecia were removed from each of the isolates and mounted in lacto-phenol stained with cotton blue. The structures were then studied microscopically and 25 measurements were made of important characters of the perithecia and ascospores. Microscope slides of the anamorph states were also prepared and studied in a similar way.

RESULTS

Collection and isolation

In total, 32 isolates of fungi resembling *O. piliferum* were included in the study (**Table 2**). Ophiostomatoid fungi resembling *O. piliferum* were not particularly common on wood chips from South African pulp mills. Only two isolates resembling *O. piliferum* were obtained from *Eucalyptus* chips, two from *Acacia mearnsii* chips, and none from pine chips. From the remaining 28 isolates, six originated from native hosts, while the remaining 22 came from exotic hardwoods.

Identification of isolates

The period required for the production of perithecia in culture varied from one week to three months. Some of the cultures had apparently lost the ability to produce perithecia, which complicated identification. A total of 21 of the 32 isolates under investigation produced perithecia (**Table 2**). Only those that produced perithecia could be identified with any certainty. Examination of ascospores, perithecia and anamorphs of isolates collected, led us to conclude that all isolates belonged to one of three morphological groups, none of which was *O. piliferum*. On the basis of morphology, we believe that these represent three distinct taxa (**Table 3**), which are referred to as Groups A, B and C. The remaining 11 isolates, not

producing perithecia, were placed in the three groups based on cultural and anamorph characters.

The average length of ascospores of isolates from Group A was notably shorter than that of *O. piliferum*. This group might represent *O. stenoceras* (Robak) Nannf. Isolates from Group B had perithecia with light brown bases, whereas the bases reported for *O. piliferum* have always been reported as black. Many isolates from this group had annuli on their perithecial necks, which is not typical of *O. piliferum*. The second group, therefore, more closely resembled *O. pluriannulatum*. Light brown perithecial bases have, however, not been reported for *O. pluriannulatum*. Isolates from Group C had perithecia and ascospores similar to those of *O. piliferum*, but apart from the *Sporothrix* anamorph that is typical for *O. piliferum*, also produced a *Pesotum*-like anamorph in culture. This corresponds with descriptions of both *O. piceae* and *O. querci* (Georgévitch) Nannf.

DISCUSSION

Twenty-one isolates of *Ophiostoma* spp. from hardwood sources in South Africa were identified in this study. Comparison with descriptions of similar fungi led us to conclude that none of these represented *O. piliferum*. All these isolates could be assigned to one of three morphological groups. Group A resembled *Ophiostoma stenoceras*, Group B *O. pluriannulatum* and Group C either *O. piceae* or *O. querci*. The identity of all these isolates will need to be confirmed based on comparisons with type specimens and, in some cases, DNA sequence data.

Ophiostoma stenoceras has often been treated as the teleomorph of *Sporothrix schenckii* Hektoen & Perkins ex Nicot & Mariat, the causal agent of sporotrichosis in humans and other mammals (Mariat *et al.*, 1968; Nicot & Mariat, 1973; De Hoog, 1974; Upadhyay, 1981). The validity of this association has been disputed and several biochemical studies indicated that the two species might be distinct (Mendonça-Hagler *et al.*, 1974; Travassos *et al.*, 1974; Suzuki *et al.*, 1981). Berbee & Taylor (1992) confirmed with rDNA sequencing that *S. schenckii* is phylogenetically related to the genus *Ophiostoma*. The phylogenetic relationship between *S. schenckii* and *O. stenoceras*, however, remains to be clarified.

Ophiostoma stenoceras has been reported from various hard- and softwood hosts in the Northern Hemisphere (Griffin, 1968; Kåårik, 1980; Upadhyay, 1981) and New Zealand (Farrell *et al.*, 1997), but has not been reported from other Southern Hemisphere countries.

Sporothrix schenckii has often been isolated in South Africa, as the fungus was responsible for several outbreaks of disease in South African mines during the previous century (Vismer & Hull, 1997). The fungus grows on *Eucalyptus* and wattle wood props used in the mines and infects the workers through small wounds (Vismer & Eicker, 1994). The prevalence of isolates from this study resembling *O. stenoceras* (Group A) on *Eucalyptus* spp. and wattle, is therefore of particular significance. A phylogenetic study, comparing these isolates with authentic isolates of *O. stenoceras* and *S. schenckii*, might provide the opportunity to clarify the relationship between the two species.

The South African isolates assigned to Group B, resemble *O. pluriannulatum* in almost all respects. The perithecial bases of this species, however, have consistently been reported as black (Hedgcock, 1906; Hunt, 1956; Upadhyay, 1981), whereas those of the South African isolates were predominantly light brown in colour. The South African isolates could, therefore, represent a distinct taxon. As is the case with *O. stenoceras*, *O. pluriannulatum* has been reported almost exclusively from hardwoods from the Northern Hemisphere (Hedgcock, 1906; Lagerberg *et al.*, 1927; Hunt, 1956; Upadhyay, 1981). The only report of the fungus south of the equator was from New Zealand (Farrell *et al.*, 1997).

The third group of isolates in our study (Group C), resembles *O. piceae*. These isolates, however, could represent any of a number of species in the *O. piceae* complex. Confusion in the taxonomy of this complex has only recently been addressed (Harrington *et al.*, 2001). Based on morphology, the South African isolates most closely resemble *O. querci*. This fungus occurs almost exclusively on hardwoods in the Northern Hemisphere, and has been reported only from New Zealand (Farrell *et al.*, 1997) and Australia (Harrington *et al.*, 2001) in the Southern Hemisphere.

Results of this study serve as a foundation for further studies, which will be aimed at identifying the most common *Ophiostoma* spp. occurring on wood chips and freshly cut wood in South Africa. In order to determine names for these fungi, comparisons with herbarium specimens and authenticated isolates will be necessary. Due to the very similar morphology of these fungi, comparisons based on DNA sequence data should also be undertaken.

Once reliable species names have been determined, South African isolates might be considered as possible biological control agents of pitch and bluestain, especially for application on hardwood species. The fact that *O. pluriannulatum* is reportedly heterothallic (Gregor, 1932) and has only a *Sporothrix* anamorph, makes it the most suitable candidate for further work on biological control. The thallism of the South African isolates resembling

O. pluriannulatum, must therefore, be determined. Furthermore, its pitch removing and competitive growth abilities will need careful consideration.

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Table 1. Ophiostomatoid fungi previously reported from South Africa.

Year	Species [1]	Host	References
1927	*Sporotrichum beurmanii* Matr. & Ramond [= *Sporothrix schenckii* Hektoen & Perkins]	human	Doidge, 1950
1931	[2] *Thielaviopsis basicola* (Berk. & Br.) Ferraris	*Nicotiana tabacum*	Gorter, 1977
1937	[3] *Ophiostoma piliferum* (Fries) H. & P. Sydow [= *Ceratostomella pilifera* (Fries) Winter]	logs of *Pinus radiata*	Laughton, 1937
1937	*Ceratocystis paradoxa* (Dade) Moreau [= *Thielaviopsis paradoxa* (de Seyn) v. Höhn.]	*Saccharum officinarum*	Doidge, 1950
1947	[2] *Graphium* sp. associated with *Sporotrichum* sp., producing perithecia.	Timber and air	Brown *et al.*, 1947
1956	*Ceratocystis adiposa* (Butler) Moreau	shoots of *Pinus* sp.	Talbot, 1956
1965	*Chalara terrestris* Agnihothrudu & Barna	*Eucalyptus saligna*	Marasas *et al.*, 1966
1974	[2, 4] *Graphium putredinis* (Corda) Hughes	Soil	Eicker, 1974
1974	*Ceratocystis fimbriata* Ellis & Halstead	*Protea gigantea*	Gorter, 1979
1978	*Leptographium reconditum* Jooste	*Triticum* rhizosphere	Jooste, 1978
1980	*Ophiostoma ips* (Rumbold) Nannf. [= *Ceratocystis ips* (Rumbold) Moreau]	*Orthotomicus erosus* *ex Pinus* spp.	Wingfield & Marasas, 1980a
1980	*Ophiostoma serpens* (Goidánich) von Arx [= *Verticicladiella alacris* M.J. Wingfield & Marasas]	roots of *Pinus pinaster* and *P. radiata*	Wingfield & Marasas, 1980b
1983	*Leptographium lundbergii* Lagerberg & Melin [= *Verticicladiella truncata* M.J. Wingfield & Marasas]	roots of *Pinus taeda*	Wingfield & Marasas, 1983
1985	*Ophiostoma* spp. *Leptographium* spp. [2] *Graphium* spp.	*Orthotomicus erosus* *Hylurgus ligniperda* *Hylastes angustatus*	Wingfield *et al.*, 1985; Wingfield & Swart, 1989
1988	*Gondwanamyces proteae* (M.J. Wingf., Van Wyk & Marasas) Marais & M.J. Wingf. [= *Ceratocystiopsis proteae* M.J. Wingf., Van Wyk & Marasas]	*Protea repens*	Wingfield *et al.*, 1988a
1988	[2, 5] *Graphium* sp.	*Orthotomicus erosus*	Wingfield *et al.*, 1988b
1993	*Sporothrix eucalypti* M.J. Wingfield, Crous & Swart	*Eucalyptus grandis*	Wingfield *et al.*, 1993
1993	*Gondwanamyces capensis* (M.J. Wingfield & Van Wyk) Marais & M.J. Wingfield [= *Ophiostoma capense* M.J. Wingfield & van Wyk]	*Protea* spp.	Wingfield & Van Wyk, 1993
1993	*Ceratocystis fimbriata* Ellis & Halstead	*Acacia mearnsii*	Morris *et al.*, 1993
1994	*Ophiostoma splendens* Marais & M.J. Wingfield	*Protea* spp.	Marais & Wingfield, 1994
1994	[2, 5] *Graphium pseudormiticum* M. Mouton & M.J. Wingfield	*Orthotomicus erosus*	Mouton *et al.*, 1994b
1996	*Ceratocystis albofundus* M.J. Wingfield, De Beer & Morris	*Protea* sp.	Wingfield *et al.*, 1996
1997	*Ophiostoma protearum* Marais & M.J. Wingfield	*Protea* sp.	Marais & Wingfield, 1997
2001	*Ophiostoma africanum* Marais & M.J. Wingfield	*Protea gaguedi*	Marais & Wingfield, 2001

[1] The species name currently in use is listed first. The name used in the original report is in parenthesis.
[2] Species from genera treated in the past as anamorphs of *Ceratocystis* or *Ophiostoma*, but which is not considered as such at present.
[3] Doubtful identification.
[4] *Graphium putredinis* is currently classified in the Microascales and is associated with teleomorph genera such as *Petriella* and *Pseudallescheria* (Okada *et al.*, 1998).
[5] In 1994 described as a new species (Mouton *et al.*, 1994b). At present classified in the Mircroascales as part of the *Graphium penicilliodes* Corda complex (Okada *et al.*, 1998).

Table 2. Ophiostomatoid isolates resembling *Ophiostoma piliferum* from South Africa.

Group	Isolate No[1]	Host	Area	Collector
A	CMW 2342[2]	*Eucalyptus fastigata* Deane & Maid.	Lothair, Mpumalanga	G.H.J. Kemp
	CMW 2343[2]	*E. grandis*	Kwambonambi, KwaZulu-Natal	"
	CMW 2344[2]	*Eucalyptus smithii* R.T. Bak.	Richmond, KwaZulu-Natal	"
	CMW 2345	*E. grandis*	Barberton, Mpumalanga	"
	CMW 2346	*E. grandis*	Tzaneen, Northern Province	"
	CMW 2347[2]	*E. fastigata*	Lothair, Mpumalanga	"
	CMW 2348[2]	*E. smithii*	Paulpietersburg, KwaZulu-Natal	"
	CMW 2349[2]	*E. grandis*	White River, Mpumalanga	"
	CMW 2523[2]	*Acacia mearnsii* De Wild. (chips)	Sappi Saiccor, Umkomaas, KwaZulu-Natal	Z.W. de Beer
	CMW 2524[2]	*A. mearnsii* (chips)	"	"
	CMW 2525[2]	*E. grandis*	Kwambonambi, KwaZulu-Natal	I. v. d. Westhuizen
B	CMW0368[2]	*Ocotea bullata* (Burch.) Mey.	Saasveld, Western Cape	M.J. Wingfield
	CMW 0931	*Macaranga capensis* (Baillon) Benth.	Kwambonambi, KwaZulu-Natal	"
	CMW 0932[2]	*M. capensis*	"	"
	CMW 0935	*M. capensis*	"	"
	CMW 1207[2]	*Jacaranda mimosifolia* D. Don.	Sabie, Mpumalanga	"
	CMW 1211[2]	*Eucalyptus* sp.	Lydenburg, Mpumalanga	"
	CMW 1213	*J. mimosifolia*	Sabie, Mpumalanga	"
	CMW 1235[2]	*Eucalyptus* sp.	Lydenburg, Mpumalanga	"
	CMW 1251[2]	*Eucalyptus grandis* Hill. ex Maid.	Howick, KwaZulu-Natal	"
C	CMW 0864[2]	*Quercus robur* L.	Stellenbosch, Western Cape	"
	CMW 0866[2]	*Q. robur*	"	"
	CMW 0867[2]	*Q. robur*	"	"
	CMW 1044[2]	*Olinia ventosa* (L.) Cufod.	George, Western Cape	"
	CMW 1045	*O. ventosa*	"	"
	CMW 1249	*Psidium guajava* L.	Kwambonambi, KwaZulu-Natal	"
	CMW 1255[2]	*E. grandis*	"	"
	CMW 1257	*E. grandis*	"	"
	CMW 2105	*E. grandis*	Sabie, Mpumalanga	P.W. Crous
	CMW 2519[2]	*Eucalyptus* sp. (chips)	Mondi Kraft Richards Bay, KwaZulu-Natal	Z.W. de Beer
	CMW 2522	*Eucalyptus* sp. (chips)	"	"
	CMW 2534	*E. grandis*	White River, Mpumalanga	G.H.J. Kemp

1 All numbers refer to the culture collection (CMW) of the Forestry and Biotechnology Institute (FABI), Pretoria, South Africa.

2 Isolates producing perithecia in culture.

Table 3. Morphological characteristics of ophiostomatoid isolates resembling *Ophiostoma piliferum* from South Africa. Bold type indicates the major differences between the three groups and *O. piliferum*.

			O. piliferum[1]	Group A	Group B	Group C
Anamorph			*Sporothrix*	*Sporothrix*	*Sporothrix*	*Sporothrix* ***Pesotum***
Ascospores:	**Length**		3 - 5 µ	**3 µ**	5 µ	4 µ
	Width		1.5 - 2 µ	1.5 µ	1.5 µ	1.5 µ
Perithecia:	**Base**	**colour**	black	black	**light brown**	black
		diameter	75 - 250 µ	135 µ	150 µ	160 µ
	Neck	**length**	300 - 3000 µ	680 µ	1470 µ	900 µ
		width: base	18 - 40 µ	26 µ	25 µ	25 µ
		width: neck	8 - 15 µ	10 µ	12 µ	9 µ
		annuli	0	0	**0 - 3**	0

[1] Characteristics are those published by Upadhyay, 1981.

Chapter 3

The grouping of species into genera and other taxonomical categories of a higher or lower rank serves two purposes. One is, to express our opinion of affinities and relationships. The other, to facilitate identification. Both purposes are equally important but often very difficult to combine.

- Elias Melin & J.A. Nannfeldt, 1932

The family Ceratostomataceae, which includes most of the important staining fungi is a very unified group. The species have constant differences and may be easily identified when obtained in pure culture.

- Ross W. Davidson, 1935

Taxonomy of the genus *Ophiostoma* and its associated anamorphs: a review.

THE GENUS *OPHIOSTOMA*

The taxonomy of the genus *Ophiostoma* H. & P. Sydow, together with that of the morphologically similar genus *Ceratocystis* Ell. & Halst.[1], has been the topic of many research papers and reviews during the past century (Lagerberg *et al.*, 1927; Melin & Nannfeldt, 1934; Hunt, 1956; Wright & Cain, 1961; Griffin, 1968; Olchowecki & Reid, 1973; De Hoog, 1974; Upadhyay, 1981, 1993; De Hoog & Scheffer, 1984; Perry, 1991; Samuels, 1993). The majority of these reviews have tended to ignore some aspects of the taxonomic history of *Ophiostoma* and have focused more sharply on *Ceratocystis*. The reason for this is that *Ophiostoma* has, for many years, been considered a synonym of *Ceratocystis*, and the latter genus has been given taxonomic preference. To fully understand the taxonomy of the *Ophiostoma* spp. that form the focus of this thesis, it is necessary to consider the chronology of events that have lead to the current status of *Ophiostoma*.

The era of morphology

The first 150 years of research on ophiostomatoid taxonomy was dominated by morphological studies under the light microscope. This started in 1817, when the type species of the genus *Ophiostoma*, presently known as *O. piliferum* (Fr.) H. & P. Sydow, was described as *Sphaeria pilifera* Fr. (Fries, 1822; Sydow & Sydow, 1919). At that stage, the genus *Sphaeria* Haller ex Fr. accommodated most fungi with either perithecia or pycnidia (Fries, 1822; Hawksworth *et al.*, 1983). In 1869, Fuckel transferred *S. pilifera* to *Ceratostoma* Fuckel (Fuckel, 1869). Saccardo had established the genus *Ceratostomella* Sacc. in 1878 for *Ceratostoma* spp. with colourless ascospores (Saccardo, 1878), but it was

[1] The fungal genus *Ceratocystis* Ell. & Halst. should not be confused with the genus *Ceratocystis* Jaekel (Echinodermata, Stylophora), which was erected in 1901 to accommodate invertebrate fossils (Jaekel, 1901). Since the taxonomy of these organisms is governed by the International Code of Zoological Nomenclature, which functions independantly from the International Code of Botanical Nomenclature, both genus names are valid.

Winter, in his 1887 revision of the genus *Ceratostoma*, who transferred *Ceratostoma piliferum* (Fr.) Fuckel to *Ceratostomella* (Hedgcock, 1906; Münch, 1907).

Hedgcock (1906) retained the name *Ceratostomella pilifera* (Fr.) Winter for the most prevalent cause of sapstain in his extensive study on 'wood-bluing fungi' of North America. He, furthermore, described six new *Ceratostomella* spp. and redescribed one existing specie. This was based on the morphology of the conidial states as well as the perithecia (Hedgcock, 1906).

In 1907, Münch showed that *Ceratostomella pilifera* represented a complex of species. He divided *Cer. pilifera* into four new species, the so-called *piliferum*-group of species, which could be distinguished from each other primarily on conidial morphology. He also recognised the differences between the exogenous and endogenous conidial stages of *Ceratostomella* spp., and erected the genus *Endoconidiophora* Münch for species with endoconidial asexual states (Münch, 1907).

Von Höhnel, in 1918, identified two groups of species within the genus *Ceratostomella*. The name *Ceratostomella* was maintained for the two cotypes, *Cer. vestita* Sacc. and *Cer. cirrhosa* (Pers.) Sacc., with hyaline ascospores formed in persistent asci and perithecia that lacked ostiolar hyphae. The second group had hyaline ascospores produced in evanescent asci with ostiolar hyphae on the perithecia. This group was assigned to a new genus, *Linostoma* Höhnel (Von Höhnel, 1918). The name *Linostoma* was, however, not available, because it was already used for a genus of flowering plants described in 1831 by Wallich (Sydow & Sydow, 1919; Upadhyay, 1981). In 1919, Von H. and P. Sydow, therefore, erected the genus *Ophiostoma* to accommodate all the species erroneously assigned to *Linostoma*. *O. piliferum* was named as the type species for the new genus (Sydow & Sydow, 1919). For some reason, perhaps because it was published in German, both the works of Von Höhnel and the Sydows were ignored by the majority of their colleagues, who continued to use the generic name *Ceratostomella* for the next fifteen years (Hubert, 1921, 1929; MacCallum, 1922; Taylor, 1922; Yeates, 1924; Boyce, 1925; Georgévitch, 1926, 1927; Lagerberg *et al.*, 1927; Sartoris, 1927; Zach, 1927, 1929; Dade, 1928; Wollenweber & Stapp, 1928; Buisman, 1929, 1932, 1933a, b; Lebedeff, 1929, 1930; Grosmann, 1930, 1932; Rumbold, 1930, 1931, 1934; Münch, 1931; Mittman, 1932; Robak, 1932, 1934; Hedgcock, 1933; Nisikado & Yamauti, 1933, 1934; Leach *et al.*, 1934; Davidson, 1935).

In 1932, Nannfeldt became the first to accept the generic concept of Von Höhnel and the name *Ophiostoma*. He erected the family Ophiostomataceae for *Ophiostoma*, with *Endoconidiophora* as synonym (Nannfeldt, 1932). Another paper followed in 1934, in

which Nannfeldt, together with Melin, transferred 11 species to *Ophiostoma* and divided the genus into two sections for which they provided a Latin diagnosis (Melin & Nannfeldt, 1934). The first section, *Brevirostrata* Nannf., was characterised by short, broad perithecial necks. The second section, *Longirostrata* Nannf., could be distinguished by long perithecial necks. The second section was subdivided into two groups. The first group encompassed species with 'exogenous *Cephalosporium*- or *Cladosporium*-like conidia,' including species with *Graphium* Corda[2] conidial stages, and the second group comprised species with 'endoconidia of the *Chalara* type.' The species referred to the second group included *Endoconidiophora coerulescens* Münch, *Ceratostomella paradoxa* Dade and *Cer. adiposa* (Butl.) Sartoris. The fourth species transferred to this group, was *Cer. fimbriata* (Ell. & Halst.) Elliot (Melin & Nannfeldt, 1934).

Ceratostomella fimbriata was described in 1890 by Halsted as *Ceratocystis fimbriata* Ell. & Halst., the type for the newly erected genus, *Ceratocystis* (Halsted, 1890). Halsted, however, considered the ascospores to be pycnospores[3], because no asci were observed (Halsted, 1890; Elliott, 1923). Saccardo (1892) also considered the perithecia to be pycnidia, and thus discarded *Ceratocystis* and transferred *C. fimbriata* to *Sphaeronaema* Fr. Elliott (1923) discovered that the spores were produced in evanescent asci and placed the fungus in the genus *Ceratostomella*. The subsequent new combination, *Ophiostoma fimbriatum* (Ell. & Halst.) Nannf. (Melin & Nannfeldt, 1934), became the first connection between *Ophiostoma* and *Ceratocystis*.

The first author after Melin and Nannfeldt to acknowledge the genus *Ophiostoma*, was Goidànich (1935). He transferred more *Ceratostomella* spp. to *Ophiostoma*, and erected a new genus, *Grosmannia* Goid., to accommodate species with *Leptographium* Lag. & Melin conidial states (Goidànich, 1935, 1936). While the erection of the genus *Grosmannia* was viewed with scepticism (Siemaszko, 1939), the generic name *Ophiostoma* became widely used in Europe during the following decade (Goidànich, 1936; Rennerfelt, 1937; Siemaszko, 1939; Bisby & Mason, 1940; Erdtmann & Rennerfelt, 1949; Mathiesen, 1950; Rennerfelt, 1950).

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[2] It is interesting to note that *Graphium* Scopoli is the name for a genus of swallowtailed butterflies in the family Papilionidae (Dickson, 1978). The genus was described in 1777 (Dickson, 1978), 60 years before *Graphium* Corda (Carmichael *et al.*, 1980), but since the taxonomy of butterflies is ruled by the International Code of Zoological Nomenclature, the fungal genus is perfectly valid.

[3] Pycnospores is an obsolete term previously used for conidia formed in a pycnidium (Hawksworth *et al.*, 1995).

Scientists from the New World were reluctant to accept the work of the European (Von Höhnel, 1918; Sydow & Sydow, 1919; Goidànich, 1935, 1936) and Scandinavian (Melin & Nannfeldt, 1934) authors. Davidson (1942) did not agree with the classification of Melin and Nannfeldt, and maintained that no perithecial characters should be applied to separate groups within *Ophiostoma*. He did agree that 'the species placed in *Ophiostoma* by Melin and Nannfeldt are probably generically distinct from *Ceratostomella vestita* and related species.' However, he continued to give preference to the name *Ceratostomella* above *Ophiostoma*, and was supported in this approach by other North American mycologists (Andrus, 1936; Rumbold, 1936, 1941; Swingle, 1936; Ellis, 1939; Verrall 1939, 1941a, b; Bramble & Holst, 1940; Chapman & Scheffer, 1940; Craighead & George, 1940; Davidson, 1940, 1942; Leach, 1940; Taylor-Vinje, 1940; Robbins & Ma, 1942; Shafer & Liming, 1950). The only exception was Donald Limber who accepted Melin and Nannfeldt's treatment (Melin & Nannfeldt, 1934), and described *Ophiostoma narcissi* Limber from *Narcissus* bulbs (Limber, 1950).

Bakshi (1951), in a very influential paper, suggested that *Ophiostoma*, *Endoconidiophora*, and *Rostrella* Zimmerm., should be treated as synonyms of *Ceratocystis*, since *Ceratocystis* was described before *Ophiostoma*. Like Siemaszko (1939), he did not accept the genus *Grosmannia*. He retained *Ceratostomella* for species with persistent asci and monostichous ascospores, and argued that the genus *Ceratocystis* is the 'most acceptable' for species of *Ceratostomella* with deliquescent asci and unicellular ascospores. He referred two *Ophiostoma* spp. to *Ceratocystis* and described three new species for the genus (Bakshi, 1950, 1951).

Apparently unaware of the work of Bakshi, Von Arx, in 1952, declared *Rostrella*, *Endoconidiophora*, *Linostoma*, *Grosmannia* and *Ceratostomella* auct. non Sacc., synonyms of *Ophiostoma*, and transferred thirteen species of *Ceratostomella*, *Grosmannia* and *Endoconidiophora* to *Ophiostoma* (Von Arx, 1952). In collaboration with Müller, he added *Ceratocystis* to the list of synonyms in 1954, and transferred five more species to *Ophiostoma* (Von Arx & Müller, 1954), including two of the newly described *Ceratocystis* spp. of Bakshi (1951). It was, however, Bakshi's treatment that became widely accepted, and the transfer of species from the other genera, including *Ophiostoma*, to *Ceratocystis*, was completed by C. Moreau (1952), M. and F. Moreau (1952), and Hunt (1956).

The statement by Perry (1991), that Hunt 'synonymized *Sphaeria* Haller ex Fr. and *Sphaeronaemella* Karsten ex Seeler under *Ceratocystis*,' is incorrect. Hunt (1956) categorically excluded *Sphaeronaemella*, *Ceratostomella* and *Ceratostoma* from synonymy with *Ceratocystis*. He did, however, reduce *Ophiostoma*, *Endoconidiophora*,

Grosmannia and *Sphaeria* to synonymy with *Ceratocystis*. He also developed a key to 39 species and provided sections for them based on conidial development. The first section contained species with endoconidial imperfect states, the second comprised all species with *Leptographium* or *Graphium* stages, and the third section included species with mycelial conidia only (Hunt, 1956).

In the 18 years following the work of Bakshi, Moreau and Hunt, many new *Ceratocystis* spp. were described. During this period, Davidson, in collaboration with various authors, described 30 species of *Ceratocystis* sensu lato, of which 29 were new and one was a new combination (Davidson, 1955, 1958, 1966, 1971; Davidson *et al.*, 1964; DeVay *et al.*, 1968; Robinson-Jeffrey & Davidson, 1968). Griffin (1968) reported 32 species from Ontario, including 11 new species, and produced a key to 60 species. Olchowecki and Reid (1973) followed this trend with an even more extensive study, reporting 50 species from Manitoba, of which 25 were new. They included a key to 70 species and subdivided the genus *Ceratocystis* into four groups based on ascospore morphology (Olchowecki & Reid, 1973). Various other authors also contributed to the list of new species during this period (Wright & Cain, 1961; Robinson-Jeffrey & Grinchenko, 1964; Kendrick & Molnar, 1965; Butin, 1968; Guerrero, 1971; Butin & Zimmerman, 1972).

Von Arx (1970), in the first edition of *The Genera of Fungi Sporulating in Pure Culture*, treated *Endoconidiophora*, *Grosmannia*, *Linostoma*, *Ophiostoma* and *Rostrella*, as synonyms of *Ceratocystis*. He thus changed his original point of view (Von Arx, 1952). In 1973, together with Müller (Müller & Von Arx, 1973), he continued to treat *Ophiostoma* as synonym of *Ceratocystis* in a key to important genera of the Ophiostomataceae. *Europhium* Parker, erected for species with closed ascocarps and no necks (Parker, 1957), together with *Sphaeronaemella*, were treated as genera distinct from *Ceratocystis* (Müller & Von Arx, 1973).

A season of biochemistry

In all the treatments of *Ophiostoma* and *Ceratocystis* prior to 1967, only morphological characters were considered. During the 1960's, however, biochemical characters became an increasingly important aid to morphology in delineating taxa (Bartnicki-Garcia, 1968). When Rosinski and Campana discovered cellulose in the hyphal walls of *Ceratocystis ulmi* (Buism.) Moreau, it was viewed upon as an anomaly, since cellulose had never been found in any Ascomycete before that time (Rosinski & Campana, 1964). The taxonomic

importance of the discovery became evident when cellulose was discovered in cell walls of other *Ceratocystis* spp. with exoconidial states (Rosinksi, 1965; Smith *et al.*, 1967; Jewell, 1974). Species with endoconidial states did not have cellulose in their cell walls. The biochemical differences between the endo- and exoconidial species within *Ceratocystis* sensu Bakshi, were further supported by proton magnetic resonance data from Spencer and Gorin (1971). According to this study, the main sugar components of the first group were glucose and mannose, while those of the second group were rhamnose and mannose.

Acknowledging the importance of the biochemical characters, the first serious criticism on Bakshi and Hunt's taxonomic arrangement came in 1974 from De Hoog (1974). He separated the genus *Ceratocystis* sensu lato into two distinct genera based on morphology of the conidial states. Firstly, according to his classification, *Ceratocystis* sensu stricto had to accommodate species with anamorphs in *Chalara* (Corda) Rabenh., *Chalaropsis* Peyr., and *Thielaviopsis* Went. He proposed *Rostrella*, *Endoconidiophora* and Nannfeldt's *Longirostrata* section of *Ophiostoma,* as synonyms of *Ceratocystis*. Secondly, species with anamorphs in *Sporothrix* Hekt. & Perkins ex Nicot & Mariat, *Verticicladiella* S. Hughes, *Leptographium*, and *Graphium*, were placed in the genus *Ophiostoma*. Synonyms for *Ophiostoma* were *Linostoma*, *Grosmannia*, *Europhium* and the sections *Longirostrata* and *Brevirostrata* of *Ophiostoma* (De Hoog, 1974). In the second edition of *The Genera of Fungi Sporulating in Pure Culture* (Von Arx, 1974), Von Arx, in support of De Hoog (1974), reverted to his original treatment (Von Arx, 1952). He proposed essentially the same approach as De Hoog, but maintained *Europhium* as a distinct genus (Von Arx, 1974).

Upadhyay and Kendrick (1975), without acknowledging the work by De Hoog (1974) and Von Arx (1974), transferred 19 species of *Ceratocystis* sensu lato to a new genus, *Ceratocystiopsis* Upadhyay & Kendr., based on the presence of falcate ascospores and short perithecial necks. *Europhium* was synonymized with *Ceratocystis*, and four new anamorph genera were described for *Ceratocystis* sensu lato: *Hyalorhinocladiella* Upadh. & Kendr., *Graphilbum* Upadh. & Kendr., *Hyalopesotum* Upadh. & Kendr., and *Pachnodium* Upadh. & Kendr. (Upadhyay & Kendrick, 1975).

Biochemical data obtained by Weijman and De Hoog (1975), supported the suggested bipartition of *Ceratocystis* sensu lato by Von Arx (1974) and De Hoog (1974), and also corresponded with earlier biochemical studies (Rosinksi, 1965; Smith *et al.*, 1967; Spencer & Gorin, 1971; Jewell, 1974). The species without cellulose and rhamnose and with *Chalara* anamorphs, were treated as *Ceratocystis* sensu stricto. Species in which both

compounds were present and with *Sporothrix* and/or *Graphium*-like conidial states, were placed in *Ophiostoma* (Weijman & De Hoog, 1975).

In 1978, Upadhyay declared *Sphaeronaemella* a synonym of *Ceratocystis* sensu lato, discounting the importance of pigments in ascocarps as an important generic character. Although *Sphaeronaemella,* according to the International Code of Botanical Nomenclature, should have been given preference above *Ceratocystis*, Upadhyay proposed the conservation of *Ceratocystis* against *Sphaeronaemella*. He also retained *Ophiostoma* as a synonym of *Ceratocystis* (Upadhyay, 1978).

The results of a study by Dabinett and Wellman (1978), supported the separation of *Ophiostoma* and *Ceratocystis* based on conidium ontogeny. Investigating the value of numerical taxonomy in the classification of certain Hyphomycetes and Ascomycetes, they used 98 characters representing morphology, conidium ontogeny and physiology (Dabinett & Wellman, 1978). Samuels and Müller (1978) also considered the type of conidiogenesis, together with the presence or absence of cellulose, more important than teleomorph morphology, and endorsed the separation of *Ophiostoma* and *Ceratocystis* (Samuels & Müller, 1978).

Despite all the evidence supporting the separation of the two genera, Upadhyay, in his 1981 monograph, treated *Ophiostoma*, together with *Sphaeronaemella*, *Rostrella*, *Endoconidiophora*, *Linostoma*, *Grosmannia*, and *Europhium*, as synonyms of *Ceratocystis*. *Ceratocystis* sensu lato was subdivided into four sections (*Ophiostoma*, *Ips*, *Ceratocystis* and *Endoconidiophora)*, while *Ceratocystiopsis* was maintained as a separate genus (Upadhyay, 1981).

In the same year, Harrington (1981) applied another taxonomic test to distinguish between *Ceratocystis* sensu stricto and *Ophiostoma*. He showed that growth of *Ceratocystis* sensu stricto spp. (with *Chalara*-like anamorphs) were inhibited by cycloheximide, an antibiotic that prevent protein synthesis, while growth of *Ophiostoma* spp. with other anamorphs, including species of *Europhium*, were generally unaffected by the substance (Harrington, 1981). The uncertainty concerning the status of *Ophiostoma* versus *Ceratocystis* was acknowledged in the 1983 edition of *Ainsworth and Bisby's Dictionary of the Fungi.* The authors noted that the order Ophiostomatales might comprise *Ceratocystiopsis*, *Ceratocystis* and possibly *Ophiostoma* (Hawksworth *et al.*, 1983).

De Hoog and Scheffer (1984), in an important publication, proposed the official separation of *Ceratocystis* sensu lato into *Ophiostoma* and *Ceratocystis* sensu stricto. They considered all the published data available and recommended that the genus *Ophiostoma*

should accomodate species with conidial anamorphs other than *Chalara*, with rhamnose in their cell walls and resistance to cycloheximide. Fourteen new combinations were proposed for *Ophiostoma* (De Hoog & Scheffer, 1984).

Solheim (1986), was one of the first to follow the recommendations by De Hoog and Scheffer (1984). He described three new species and and proposed three new combinations for *Ophiostoma* (Solheim, 1986). Livingston and Davidson (1987) followed with another new species in 1987. In the same year Harrington proposed 11 new combinations for *Ophiostoma* (Harrington, 1987).

In 1989, Kowalski and Butin described six species, of which two were new, in *Ceratocystis*. However, five of these, including the two new ones, should have been described in *Ophiostoma* according to De Hoog and Scheffer's treatment (Kowalski & Butin, 1989). These were transferred to *Ophiostoma* by Rulamort the following year (Seifert *et al.*, 1993).

An International Symposium on the taxonomy and biology of the Ophiostomatales took place at Bad Windsheim in Germany in August 1990. This became one of the most important events in the taxonomic history of ophiostomatoid fungi. It was the first time that virtually all the researchers working on this group of fungi were brought together to discuss the taxonomy, ecology and pathogenicity of these fungi. The complete proceedings were compiled, with additions, and printed in book form in 1993 (Wingfield *et al.*, 1993). All the contributors to the symposium and subsequent book, except Upadhyay, regarded *Ophiostoma* and *Ceratocystis* as two discrete genera (Seifert *et al.*, 1993; Upadhyay, 1993; Samuels, 1993; Seifert & Okada, 1993; De Hoog, 1993; Van.Wyk *et al.*, 1993). Upadhyay, in the first chapter of the book, upheld his 1981 point of view that *Ophiostoma* is a synonym of *Ceratocystis* (Upadhyay, 1993). Also included in the book was a nomenclator for all the described species of *Ophiostoma*, *Ceratocystis*, *Ceratocystiopsis* and *Sphaeronaemella*, with complete synonymies (Seifert *et al.*, 1993). This was an attempt to present an alternative classification to that of Upadhyay (1981). Some of the 85 species listed under *Ophiostoma* remain to be transferred to the genus (Seifert *et al.*, 1993).

The molecular age

Up to the 1990's, all arguments in the debate concerning *Ceratocystis* versus *Ophiostoma* arose because different authors emphasised different characters. These were mainly morphological characters of either the teleomorph or the anamorph states, sometimes

accompanied by host specificity. During the past thirty years, the morphological characters used have been supplemented with biochemical data. The age of molecular taxonomy, with the focus on phylogenetic relationships, has however, brought a new dimension to the debate.

The first molecular study to investigate the phylogenetic relationship between *Ophiostoma* and *Ceratocystis*, was conducted by Hausner *et al.* (1992). Small subunit ribosomal DNA (ssrDNA) from species of *Ophiostoma* and *Ceratocystis* with galeate ascospores was analysed. They concluded that these two genera were not closely related, and that galeate ascospores should not be considered as an indicator of evolutionary relatedness (Hausner *et al.*, 1992; Hausner, 1994), as had previously been suggested (Cain, 1972; Redhead & Malloch, 1977). They also showed that *Europhium* spp. are closely related to *Ophiostoma*, and supported the synonymy of *Europhium* and *Ophiostoma* (Hausner *et al.*, 1992, 1993a).

Following this work, the same authors analysed partial sequences of both small and large subunit rDNA, and confirmed that *Ceratocystis* and *Ophiostoma* are polyphyletic (Hausner *et al.*, 1993a, b). Although morphological criteria might suggest that the genus *Ophiostoma* is heterogeneous (Hunt, 1956; Griffin 1968; Olchowecki & Reid, 1973; Upadhyay & Kendrick, 1975; Upadhyay, 1981), analyses of rDNA sequences of 21 species of *Ophiostoma* failed to support the strict subdivision of the genus based on either ascospore characters or the nature of the anamorph (Hausner *et al.*, 1993b). Studies by Spatafora and Blackwell, also analysing ssrDNA, confirmed that *Ceratocystis* and *Ophiostoma* are not closely related (Blackwell, 1994; Spatafora & Blackwell, 1993, 1994a, b). With the new molecular information at their disposal (Berbee & Taylor, 1992b; Spatafora & Blackwell, 1994b; Hausner *et al.*, 1992, 1993a), the authors of the 1995 edition of *Ainsworth and Bisby's Dictionary of the Fungi*, emended their neutral position from the 1983 edition and endorsed the separation of *Ophiostoma* and *Ceratocystis* (Hawksworth *et al.*, 1995).

In a review on the status of *Ceratocystiopsis*, Wingfield (1993) suggested that the genus should be reduced to synonymy with *Ophiostoma* based on ascospore and anamorph morphology. Hausner *et al.* (1993c) endorsed this point of view and formally reduced *Ceratocystiopsis* to synonymy with *Ophiostoma* based on partial rDNA sequences from both the small and large subunit genes. They transferred ten *Ceratocystiopsis* spp. to *Ophiostoma*. Of these, eight were new combinations (Hausner *et al.*, 1993c). One species, *O. crassivaginatum* (Griffin) Harrington, had been transferred to *Ophiostoma* already in

1987 (Harrington, 1987). For the type species of *Ceratocystiopsis,* the original name of *Ophiostoma minutum* Siemaszko (Siemaszko, 1939), was reinstated (Hausner *et al.*, 1993c).

Viljoen (1996), using RFLP and DNA hybridisation data, confirmed the relatedness between *Ophiostoma* and most of the *Ceratocystiopsis* spp. transferred by Hausner *et al.* (1993c). The synonymy, however, has not been accepted by Marais *et al.* (1998), since three of the species included in their study were phylogenetically distinct from the others, and could not be transferred to *Ophiostoma.* Marais *et al.* (1998) erected a new genus, *Gondwanamyces* Marais & M.J. Wingf., to accommodate one of these species, *Ceratocystiopsis proteae* M.J. Wingf., P.S. van Wyk & Marasas, together with *Ophiostoma capense* M.J. Wingf. & P.S. van Wyk. Both species are characterised by *Knoxdaviesia* M.J. Wingf., P.S. van Wyk & Marasas anamorphs. RFLP analyses of the rRNA operon regions showed that these species are phylogenetically distinct from *Ceratocystiopsis*, *Ophiostoma* and *Ceratocystis* (Marais *et al.*, 1998). This was confirmed with sequences from the large subunit ribosomal RNA gene (Viljoen *et al.*, 1999). *Ceratocystiopsis falcata* Wright and Cain, another of the species not related to *Ophiostoma* according to Hausner *et al.* (1993c), was designated the type of another new ophiostomatoid genus, *Cornuvesica* Viljoen, M.J. Wingf. and Jacobs, which is characterized by a *Chalara*-like anamorph (Viljoen *et al.*, 2000). The status of several other *Ceratocystiopsis* spp. remains uncertain.

The ascospore-based treatment of *Ceratocystis* sensu lato by Bakshi (1951) and Upadhyay (1981), where *Ophiostoma* was considered a synonym of *Ceratocystis*, was followed until recently by many authors (Balder, 1989, 1990; Eisenhauer, 1989, 1991; Frisullo *et al.*, 1989; Kowalski & Butin, 1989; Kryukova, 1989; Ziegler *et al.*, 1989; Appel *et al.*, 1990; Kowalski & Bartnik, 1990; Croan & Highly, 1992; Degreef & Malaisse, 1992; Upadhyay, 1993). The majority of contemporary papers, however, regard the differences in conidium ontogeny, together with biochemical and molecular data, as more important, and treat *Ophiostoma* and *Ceratocystis* as phylogenetically distinct (Brasier & Kirk, 1989a, b; Farr *et al.*, 1989; Levieux *et al.*, 1989; Moser *et al.*, 1989; Przybyl & De Hoog, 1989; Webber & Gibbs, 1989; Wingfield & Swart, 1989; Barr, 1990; Cech *et al.*, 1990; Seifert & Grylls 1990; Vannini & Luisi, 1990; Wulf, 1990; Yamaoka *et al.*, 1990; Yde-Anderson, 1990; Berbee & Taylor, 1992a, 1993; Hausner *et al.*, 1992; Samuels, 1993; Seifert *et al.*, 1993; Taylor, 1993; Wingfield & Van Wyk, 1993; Blackwell, 1994; Marias & Wingfield, 1994; Mitchell & Brasier, 1994; Spatafora & Blackwell, 1994b, c; Viljoen *et al.*, 1994;

Wingfield *et al.*, 1994; Wulf & Kowalski, 1994; Brasier & Mehrotra, 1995; Hawksworth *et al.*, 1995; Samuels & Seifert, 1995; Zimmerman *et al.*, 1995; Kirisits, 1996; Blanchette *et al.*, 1997; Farrell *et al.*, 1998; Marais *et al.*, 1998; Okada *et al.*, 1998; White-McDougall *et al.*, 1998; Uzunovic *et al.*, 2000; Wingfield, B.D., *et al.*, 1999; Harrington *et al.*, 2001; Schroeder *et al.*, 2001). Since the status of several *Ceratocystiopsis* spp. is undetermined, the genus should still be considered distinct from *Ophiostoma*. The following summary represents the current status of *Ophiostoma* and its associated genera.

Sphaeronaemella Karsten, Hedwigia 23: 17. 1884.

Ceratocystis Ell. & Halst., Bull. New Jers. Agric. Exp. Stn 76: 14. 1890.

= *Rostrella* Zimmerm., Meded. Lds. Pl.Tuin, Batavia 37:24. 1900 (non Fabre, Annls. Sci. Nat., Sér 6, 9: 66. 1878).

= *Endoconidiophora* Münch, Naturw. Z. Forst- u. Landw. 6: 564. 1907.

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

Ophiostoma H. & P. Syd., Annls Mycol. 17: 43. 1919.

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

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

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

= *Grosmannia* Goid., Boll. Staz. Patol. veg. Roma 16: 26. 1936.

= *Europhium* Parker, Can. J. Bot. 35: 175. 1957.

Ceratocystiopsis Upadh. & Kendr., Mycologia 67: 799. 1975.

Gondwanamyces Marais & M.J. Wingf., Mycologia 90: 139. 1998.

Cornuvesica Viljoen, M.J. Wingf. & Jacobs, Mycological Research 104: 366. 2000.

HIGHER CLASSIFICATION OF *OPHIOSTOMA*

Both *Ophiostoma* and *Ceratocystis* are placed in the Phylum (Division) Ascomycota (Hawksworth *et al.*, 1995). The classification of these genera, especially at ordinal level, has, however, been entangled in the disagreement over generic concepts throughout its taxonomic history. The different classifications of the ophiostomatoid genera at family and ordinal levels are summarised in **Table 1**. Also at these higher levels of taxonomy,

molecular data are providing us with a clearer understanding of the phylogenetic relationships between the genera. At present, it is accepted that *Ophiostoma* is classified in the Ophiostomatales, and *Ceratocystis* in the Microascales (Barr & Cannon, 1994; Blackwell & Spatafora, 1994; Spatafora & Blackwell, 1994c; Hawksworth *et al.*, 1995; Wingfield, B.D., *et al.*, 1999).

ANAMORPH GENERA ASSOCIATED WITH *OPHIOSTOMA*

A total of 32 hyphomycetous anamorph genera have, at various times in the past, been associated with species of *Ophiostoma* (**Table 2**). The nature of the associations between *Ophiostoma* and these anamorphs varies from formal species descriptions, where both the anamorph and teleomorph states were given binomials, to cases where anamorphs were assigned to a form genus, but without an epithet. There are also instances where the association between these two morphs was based merely on suggestion, and where these connections were not proved.

The high number of anamorphs associated with *Ophiostoma* led to considerable confusion in ophiostomatoid taxonomy. Various other factors contributed to this confusion over the years. Many of the genera with which *Ophiostoma* anamorphs were associated, were not valid. There are, furthermore, a number of *Ophiostoma* spp. that produce more than one anamorph at the same time. These synanamorphs often exist alongside each other and a continuum of modes of conidiogenesis can occur between them (Münch, 1907; Hunt, 1956; Travassos & Lloyd, 1980; De Hoog & Scheffer, 1984; Maekawa *et al.*, 1987; Wingfield *et al.*, 1991). The synanamorphs of *O. ips* (Rumbold) Nannf., for example, have been assigned to a total of eight different genera (Leach *et al.*, 1934; Melin & Nannfeldt, 1934; Goidánich, 1935; Moreau, 1952; Upadhyay, 1981; Harrington, 1988; Hutchison & Reid, 1988a; Seifert *et al.*, 1993; Benadé *et al.*, 1995; Okada *et al.*, 1998). As a result of the difficulties in assigning anamorph states to specific genera, some authors, in papers dealing with large numbers of *Ophiostoma* spp., refrained from assigning genus names to conidial states (Hunt, 1956; Griffin, 1968; Olchowecki & Reid, 1973). These apparent attempts to simplify, however, eventually added to the taxonomic perplexity.

At present, only four genera (**Table 2**) are considered appropriate for anamorphs of *Ophiostoma* (Seifert *et al.*, 1993). Since some species of *Ceratocystis* sensu stricto were treated in *Ophiostoma* in the past, the anamorphs associated with those species are also

listed here and discussed. The reasons why other listed genera are not available to anamorphs of *Ophiostoma* at present, are considered briefly. For the purpose of this discussion, the anamorphs associated with *Ophiostoma* are treated in four groups (**Table 2**) based on conidium ontogeny and morphology. In many cases, however, a continuum in patterns of conidium development may exist for some of these genera (Benadé *et al.*, 1996, 1997). The four groups are, therefore, not necessarily of taxonomic significance. **Group A** contains genera characterized by phialides (Nag Raj & Kendrick, 1975; Upadhyay, 1981; De Hoog & Scheffer, 1984) from which conidia develop through ring wall building (Minter *et al.*, 1983). In the genera of **Groups B, C** and **D**, conidium development takes place through apical wall building (Minter *et al.*, 1983). The differences between these three groups are found in the morphology of the conidiophores.

Group A:* Chalara-*like anamorphs

Conidial development in this group takes place through a ring wall building process (Minter *et al.*, 1983). ***Chalara*** was described in 1838 as a subgenus of *Torula* (Pers.) Link, but was raised to generic rank in 1844 (Nag Raj & Kendrick, 1975). When Saccardo (1892) transferred *Ceratocystis fimbriata* to *Sphaeronaema*, it was mentioned that one of the conidial forms was a *Chalara* (Saccardo, 1892). Only after Elliot (1923) discovered that the spores were produced in asci and referred the species to the ascomycete genus *Ceratostomella*, the *Chalara* state was considered the anamorph of an ascomycete.

Thielaviopsis and ***Chalaropsis***, two genera morphologically similar to *Chalara*, were established in 1893 and 1916 respectively (Nag Raj & Kendrick, 1975). The anamorph of *Ceratocystis paradoxa* (Dade) C. Moreau, was designated as the type of *Thielaviopsis* (Nag Raj & Kendrick, 1975). Although some other authors also referred ophiostomatoid anamorphs to these genera (Moreau, 1952; Melin & Nannfeldt, 1934; Riedl, 1962; Bliss, 1941; Von Arx, 1970), both were reduced to synonymy with *Chalara* by Nag Raj and Kendrick in (1975). A recent phylogenetic study on 46 *Chalara* spp., including teleomorph species with *Chalara*-like anamorphs, showed that *Ceratocystis* spp. with *Chalara* anamorphs form a distinct group, separate from other species of *Chalara* (Paulin & Harrington, 2000). It was suggested that anamorphs of *Ceratocystis* should be treated as *Thielaviopsis*, rather than *Chalara* (Paulin & Harrington, 2000). The type species of *Chalara*, *Chalara fusioides* (Corda) Rabenh., was, however, not included in the phylogenetic part of the study (Paulin & Harrington, 2000) and I, therefore, prefer to treat *Ceratocystis* anamorphs as *Chalara* until further work has been done. Thus, all

ophiostomatoid teleomorphs with *Chalara* anamorphs are currently placed in the genus *Ceratocystis* sensu stricto (De Hoog & Scheffer, 1984; Samuels, 1993), apart from *Ceratocystiopsis falcata.* This species has an anamorph unlike typical species of *Chalara* (Nag Raj & Kendrick, 1975), and was recently placed in a new ascomycete genus, *Cornuvesica* (Viljoen *et al.*, 2000). It was suggested that the anamorph of *Cop. falcata* might represent a genus distinct from *Chalara* (Viljoen *et al.*, 2000).

Ceratocystis autographa Bakshi, is another species which is unusual because the two anamorph states reported for it comes from phylogenetically distinct genera, namely *Chalara* (Nag Raj & Kendrick, 1975) and *Sporothrix* (Wingfield *et al.*, 1995). Scanning electron microscopy, however, showed that conidiogenesis of the so-called *Chalara*-state differs distinctly from that of typical *Chalara* isolates (Wingfield *et al.*, 1995). A new genus, *Xenochalar*a M.J. Wingf. & Crous, was recently described to accomodate an isolate similar to the anamorph present in the type material of *C. autographa* (Coetzee *et al.*, 2000). *Ceratocystis autographa* was, however, treated as a species of dubious status due to the poor state of the type material and the absence of more isolates (Coetzee *et al.*, 2000).

The genus ***Gabarnaudia*** Samson & Gams was erected for the anamorphs of two *Sphaeronaemella* spp. (Samson, 1974) which were treated in *Ceratocystis* by Upadhyay (Upadhyay, 1981). Upadhyay's treatment of *Sphaeronaemella* was, however, not accepted and it is currently considered a genus distinct from *Ceratocystis* sensu stricto (Hutchison & Reid, 1988a; Cannon & Hawksworth, 1982; Seifert *et al.*, 1993). The genus *Gabarnaudia* is, therefore, not linked to species of *Ophiostoma, Ceratocystis* or *Ceratocystiopsis*.

Group B: **Sporothrix-*like anamorphs***

The genera that I treat as **Group B** consists of species with simple conidiophores (Wolfaardt *et al.*, 1992), bearing conidia described by Münch (1907) and Hunt (1956) as 'mycelial conidia.' Upadhyay (1981), described these genera as having 'conidiophores that are simple, reduced, or hardly distinguishable from the vegetative hyphae.'

Hedgcock (1906) was the first to mention an anamorph genus from this group in association with an ophiostomatoid fungus. He stated that the anamorphs of *Ceratostomella pilifera* [= *Ophiostoma piliferum*], *Cer. pluriannulata* Hedgc. [= *O. pluriannulatum* (Hedgc.) H. & P. Sydow] and *Cer. exigua* Hedgc. [= *O. minus* (Hedgc.) H. & P. Sydow] resembled ***Cephalosporium*** auct. non Corda (Hedgcock, 1906). Many subsequent authors also mentioned this genus in anamorph descriptions of other

Ophiostoma spp. (Lagerberg *et al.*, 1927; Loos, 1932; Melin & Nannfeldt, 1934; Nisikado & Yamauti, 1935; Davidson, 1942; Bakshi, 1950, 1951; Mathiesen, 1951; Moreau, 1952; Mathiesen-Kåårik, 1953; Sczerbin-Parfenenko, 1953; Hunt, 1956; Guerrero, 1971; Butin & Zimmerman, 1972), but *Cephalosporium* was eventually reduced to synonymy with ***Acremonium*** Link ex Fr. (Gams, 1968, 1971). The type species of *Acremonium, A. alternatum* Link : Fr. is, however, placed in the Ascomycete order Hypocreales (Glenn *et al.*, 1996), which is only distantly related to the Ophiostomatales (Berbee & Taylor, 1994). These molecular data support the suggestion by Gams (1971) that *Ophiostoma* anamorphs described as *Cephalosporium*-like should not be placed in the genus *Acremonium*. Furthermore, the *Cephalosporium*-like conidial states of *Ophiostoma* spp. have indeterminate conidiogenous cells and proliferate sympodially or percurrently, producing holoblastic conidia (Gams, 1971; Upadhyay, 1981; Benadé *et al.*, 1996). De Hoog (1974), for this reason, treated the *Cephalosporium* anamorphs of *O. tetropii* Mathiesen (Mathiesen, 1951), *O. epigloeum* (Guerrero) de Hoog (Guerrero, 1971), *O. piliferum* (Hedgcock, 1906), *O. ulmi* (Buisman) Nannf. (Melin & Nannfeldt, 1934) and *O. longirostellatum* (Bakshi) v. Arx & Müller (Von Arx & Müller, 1954) as *Sporothrix*, rather than *Acremonium*. Upadhyay (1981) transferred the *Cephalosporium* anamorphs of these and other *Ophiostoma* spp. to three genera: *Sporothrix*, *Hyalorhinocladiella* and/or *Hyalodendron* Diddens. He, however, assigned one of the synanamorphs of *Ceratocystis nigra* Davids. [= *O. nigrum* (Davids.) De Hoog & Scheffer], to *Acremonium* (Upadhyay, 1981). Mouton *et al.* (1994) considered this *Acremonium* anamorph of *O. nigrum* a reduced form of *Hyalorhinocladiella,* the other anamorph state assigned to *O. nigrum* by Upadhyay (1981). Upadhyay (1981), furthermore, stated that the *Graphilbum* state of *Ceratocystis ips* (Rumbold) Moreau [= *O. ips*] at times resembles *Acremonium*. Hutchison and Reid (1988a) proceeded to officially assign a third synanamorph, besides the *Graphilbum* and *Hyalorhinocladiella* states, to *O. ips,* and placed it in *Acremonium*. Benadé *et al.* (1995) disagreed and concluded that the anamorph of *O. ips* would best be accommodated in *Hyalorhinocladiella*.

After Hedgcock introduced *Cephalosporium* as anamorph genus for *Ceratostomella* spp. (Hedgcock, 1906), Münch (1907) described three conidial stages with 'mycelial conidia' in association with *Ceratostomella* spp. He recognised that the three stages are linked to each other with intermediate forms and might be less pronounced in nature (Münch, 1907). Münch (1907) did not assign the first of these stages to a specific genus, but it corresponds with Hedgcock's *Cephalosporium* type (Hedgcock, 1906; Lagerberg *et*

al., 1927). The second conidial stage was described as 'resembling ***Cladosporium*** Link' (Münch, 1907). He associated this genus with the anamorphs of three *Ceratostomella* spp., including *Cer. piceae* Münch [= *O. piceae* (Münch) H. & P. Sydow] (Münch, 1907). Many subsequent papers mentioned *Cladosporium,* often together with *Cephalosporium*, as anamorph genus for species of *Ophiostoma* (MacCallum, 1922; Georgévitch, 1926, 1927; Lagerberg *et al.*, 1927; Loos, 1932; Melin & Nannfeldt, 1934; Nisikado & Yamauti, 1935; Bakshi, 1950, 1951; Mathiesen, 1951; Moreau, 1952; Hunt, 1956; Hinds & Davidson, 1967; DeVay *et al.*, 1968; Butin & Zimmerman, 1972). Several of these *Cladosporium* states had since been transferred to *Sporothrix* (Crane & Schoknecht, 1973; De Hoog, 1974; Kowalski & Butin, 1989; Przybyl & De Hoog, 1989; Solheim, 1986; Upadhyay, 1981; Seifert *et al.,* 1993; Hutchison & Reid, 1988a), because *Cladosporium* spp., by definition, have dark coloured conidia (Barron, 1968; Ellis, 1971; Carmichael *et al.*, 1980), while the genus *Sporothrix* is characterised by hyaline conidia produced on conspicuous denticles (De Hoog, 1974).

Münch, in his discussion of the third conidial stage associated with *Ceratostomella*, referred to the genus ***Sporotrichum*** Link ex Fr. (Münch, 1907). The genus was, however, not mentioned in any of his species descriptions (Münch, 1907). Before Münch, Boulanger described an association between *Chaetomium* Kunze, *Graphium* and *Sporotrichum* (Hedgcock, 1906; Münch, 1907; Lagerberg *et al.*, 1927; Melin & Nannfeldt, 1934; Upadhyay, 1981). Lagerberg *et al.* (1927) were of the opinion that the ascocarps of the so-called *Chaetomium* were in fact perithecia of which the 'necks had been broken off', and that it could have belonged to *Ceratostomella piceae* [= *O. piceae*]. Despite these reports, the first *Ophiostoma* anamorph formally assigned to the genus *Sporotrichum*, was only published in 1968 (De Hoog, 1974). Mariat and De Bièvre suggested *Sporotrichum schenckii* (Hekt. & Perkins) de Beurmann & Gougerot [= *Sporothrix schenckii* Hekt. & Perkins] to be the anamorph of a species of *Ceratocystis* [= *Ophiostoma*] (Nicot & Mariat, 1973; De Hoog, 1974), later specified as *O. stenoceras* (Robak) Nannf. (Andrieu *et al.*, 1971; Mariat, 1971).

The genus *Sporotrichum* was erected in 1809 by Link, but none of the 13 species described was designated as type (Carmichael, 1962). Hughes (1958) examined 12 of these species and assigned *S. aureum* Link as type. The genus ***Sporothrix*** was established in 1900 (Hektoen & Perkins, 1900) for *S. schenckii*, but was considered invalid because no generic diagnosis was provided (De Hoog, 1974). Most subsequent workers referred to the fungus as *Sporotrichum schenckii* (Carmichael, 1962). Carmichael (1962), however, stated

that what was referred to as *Sporotrichum schenckii,* did 'not in the least resemble *S. aureum.*' He, therefore, referred *Sporotrichum schenckii* back to *Sporothrix* (Carmichael, 1962), but neglected to supply a Latin diagnosis for the genus and did not differentiate adequately between the two genera (Taylor, 1970a). Von Arx (1971) redescribed both the genus *Sporotrichum* and its type, *S. aureum.* He recognised clamp connections in the hyphae (Von Arx, 1971), indicative of its Basidiomycete nature (Von Arx, 1973). Stalpers (1978) later confirmed that *S. aureum* is the anamorph of a basidiomycete (Stalpers, 1978; Von Arx, 1973). This implies that the genus *Sporotrichum* is not available for Ascomycete anamorphs (Stalpers, 1978).

Barron (1968) supported Carmichael and distinguished clearly between *Sporothrix* and *Sporotrichum.* He suggested that 'the so-called *Sporotrichum* states described for certain *Ceratocystis* spp. should be referred to as *Sporothrix*' (Barron, 1968). According to Taylor (1970a), however, the name *Sporothrix* remained invalid. Nicot and Mariat (1973) eventually validated *Sporothrix* with *S. schenckii* as type, which made the genus available for the mycelial anamorphs of *Ophiostoma* spp. Domsch *et al.* (1993) did not regard this as a validation of the genus because they considered the genus to be valid 'in view of the rather exhaustive descriptio generico-specifica (Art. 42) by Hektoen and Perkins' (Yamaoka *et al.*, 1998). Accepting the genus as valid, De Hoog (1974) referred the anamorphs of 12 *Ophiostoma* spp. to *Sporothrix.* Most subsequent authors accepted the name *Sporothrix* and the genus, at present, accommodates anamorphs previously ascribed to *Cladosporium*, *Cephalosporium* and *Hyalodendron* (Robinson-Jeffrey & Davidson, 1968; Hinds & Davidson, 1975; Sameuls & Müller, 1978; Davidson, 1979; Upadhyay, 1981; Butin & Aquilar, 1984; Solheim, 1986; Livingston & Davidson, 1987; Hutchison & Reid, 1988a; Constantinescu & Ryman, 1989; Kowalski & Butin, 1989; Marmolejo & Butin, 1990; Brasier, 1991; Seifert *et al.,* 1993; Samuels & Seifert, 1995; Wingfield *et al.*, 1995).

Apart from the anamorphs of *Ophiostoma,* the genus *Sporothrix* also accomodates various species with other affinities (De Hoog, 1974, 1993). Weijman and De Hoog (1985), for practical reasons, supported De Hoog (1974) in maintaining *Sporothrix* as an artificial form genus for morphologically similar species. They, however, proposed that the three known phylogenetic groups should be treated as three sections (Weijman & De Hoog, 1985). Section *Sporothrix* for spp. with teleomorphs in *Ophiostoma* and similar genera, section *Farinosa* for species with teleomorphs in the Endomycetes, and section *Luteoalba* for species with teleomorphs in the Phragmobasidiomycetes, especially the

Dacrymycetales (Smith & Batenburg-Van der Vegte, 1985; Weijman & De Hoog, 1985; Suzuki & Nakase, 1986; De Hoog, 1993). The type species of *Sporothrix, S. schenckii,* is phylogenetically related to *Ophiostoma* (Berbee & Taylor, 1992b). *Sporothrix* should, therefore, be reserved for anamorphs associated with teleomorph genera in the Ophiostomatales. Species from the latter two sections should be transferred to appropriate genera, as was already done with *S. cyanescens* de Hoog & de Vries and *S. luteoalba* de Hoog, both of which were referred to a new genus, *Cerinosterus* R.T. Moore (1987). *Cerinosterus luteoalba* (de Hoog) R.T. Moore is the anamorph *of Ditiola pezizaeformis* (Lév.) Reid (Reid, 1974), which belongs to the Dacrymycetales (Middelhoven *et al.,* 2000).

After Münch (1907), Robak (1932) was the first to mention two other anamorph genera in association with *Ceratostomella* [= *Ophiostoma*]: ***Cylindrocephalum*** Bon. and ***Hormodendron***. He suggested that the *Cephalosporium* type conidiophores of *O. stenoceras* (Robak) Melin & Nannf. should rather be described as *Cylindrocephalum*. Hughes, however, reduced *Cylindrocephalum* to synonymy with *Chalara* (Hughes, 1958), which means that *Cylindrocephalum* is not available for anamorphs of *Ophiostoma*. Apart from Robak (1932) and Melin and Nannfeldt (1934), no other authors associated *Cylindrocephalum* with *Ophiostoma*. In the descriptions of the *Cladosporium* type conidiophores of *O. stenoceras* and *O. piceae*, Robak stated that it would be more correct to refer to these as 'of a ***Hormodendron*** type' (Robak, 1932). The name *Hormodendron* is an orthographic variant of ***Hormodendrum*** Bon. (Carmichael *et al.*, 1980), and the only author who connected this genus with an ophiostomatoid genus was Howard in 1961 (Taylor, 1970b). He suggested that secondary conidia of *S. schenckii* 'arose by a budding process similar to that which produces the blastospores of *Hormodendrum'* (Taylor, 1970b). Taylor (1970b), however, later showed that secondary conidia of *S. schenckii* are produced sympodially. *Hormodendrum* was later reduced to synonymy with *Cladosporium* (Hughes, 1958; Barron, 1968; Von Arx, 1974).

The genus ***Hyalodendron***, established in 1934 as a hyaline analogue of *Cladosporium* (Diddens, 1934; Barron, 1968), was another anamorph genus to be connected to *Ophiostoma*. Goidànich (1935) described one of the two synanamorphs of *Ophiostoma catonianum* Goid. as *Hyalodendron pirinum* Goid. Georgescu *et al.* were the first to support Goidánich, and described one of the anamorphs of *Ophiostoma roboris* Georgescu & Teodoru [= *O. quercus* (Georgév.) Nannf.] as *Hyalodendron roboris* Georgescu & Teodoru (Sczerbin-Parfenenko, 1953; De Hoog, 1979; Przybyl & De Hoog, 1989).

Upadhyay (1981) assigned the *Cladosporium* anamorphs of four more *Ophiostoma* spp. to *Hyalodendron*, and was followed by various other authors (Ivanchenko, 1957; Maekawa *et al.*, 1987; Hutchison & Reid, 1988a; Kowalski & Butin, 1989; Degreef & Malaisse, 1992; Marmolejo & Butin, 1993). *Hyalodendron,* however, had never been available for *Ophiostoma* anamorphs since the type species is the anamorph of a Basidiomycete (De Hoog, 1979, 1993). Some *Ophiostoma* anamorphs previously assigned to *Hyalodendron* were, therefore, transferred to *Sporothrix* (De Hoog, 1974; Butin & Aquilar, 1984; Solheim, 1986; Przybyl & De Hoog, 1989; Benadé *et al.*, 1998), while others still need to be reconsidered (De Hoog, 1993; Seifert *et al.,* 1993; Mouton *et al.*, 1994).

In 1948, Georgescu *et al.* described *Ophiostoma valachicum* Georgescu & Teodoru [= *O. piceae*], based on the presence of an anamorph placed in the genus ***Rhinotrichum*** Corda (Sczerbin-Parfenenko, 1953; Przybyl & De Hoog, 1989). It appears as if only a few authors supported the placement of *Ophiostoma* anamorphs in *Rhinotrichum* (Sczerbin-Parfenenko, 1953; Ivanchenko, 1957; Uroševic, 1983). Potlajchuk distinguished between three basic types of mononematous anamorphs of *Ophiostoma* (Ivanchenko, 1957). The first type, with 'lateral conidia,' was placed in *Rhinotrichum*, the second, with catenulate conidia, in *Hyalodendron*, and the third, with 'dense conidial heads,' in *Cephalosporium* (Przybyl & De Hoog, 1989). Przybyl and De Hoog (1989) were, however, of the opinion that all three could be accomodated within the single genus *Sporothrix. Rhinotrichum* was, in any case, not validly published (Hawksworth *et al.*, 1983), and is, therefore, not available for *Ophiostoma* anamorphs.

Sczerbin-Parfenenko (1953) described a new *Ophiostoma* species, *O. kubanicum* Sczerb.-Parf., with three synanamorphs. The first of these was assigned to *Cephalosporium*, the second to was named *Graphium kubanicum* Sczerb.-Parf., and the third was *Verticillium kubanicum* Sczerb.-Parf. (Sczerbin-Parfenenko, 1953). This became the first and only association between the anamorph genus ***Verticillium*** Nees, and *Ophiostoma*. Already in 1957, Ivanchencko (1957) regarded this anamorph as identical to the *Cephalosporium* synanamorph, which is currently treated in the genus *Sporothrix* (Przybyl & De Hoog, 1989).

De Hoog (1974), in a discussion of the *Sporothrix* anamorph of *O. piliferum*, mentioned that the conidial states of *C. ambrosia* Bakshi and *C. alba* DeVay, Davids. & Moller, resembled ***Raffaelea*** v. Arx & Henneb. The anamorph of *O. triangulosporum* Butin, was also described as *Raffaelea*-like in the original description of the species (Butin, 1978). Upadhyay (1981) treated *C. ambrosia* as a synonym of *O. piliferum* and assigned the

anamorph to *Hyalodendron,* but it is currently considered to be a species of *Sporothrix* (Seifert *et al.,* 1993). Upadhyay (1981), furthermore, referred *O. triangulosporum* and *C. alba* to *Ceratocystis* and *Ceratocystiopsis* respectively. The anamorphs of both species were placed in *Hyalorhinocladiella* (Upadhyay, 1981), where they still reside (Seifert *et al.,* 1993). At present, no *Ophiostoma* anamorphs are associated with *Raffaelea*, although the genus is phylogenetically related to *Ophiostoma* and was placed in the Ophiostomatales based on 18S rDNA sequences (Jones *et al.*, 1994; Jones & Blackwell, 1998). *Raffaelea* differs from *Sporothrix* in that it has larger structures and blastoconidia that are abstricted with a broader base (Domsch *et al.,* 1993).

The genus ***Hyalorhinocladiella*** was erected to accommodate the anamorph of *Ceratocystiopsis minuta-bicolor* (Davidson) Upadh. & Kendr. (Upadhyay & Kendrick, 1975). Anamorphs of more than 20 *Ceratocystiopsis* and *Ophiostoma* spp. have since been assigned to *Hyalorhinocladiella* (Upadhyay, 1981; Seifert *et al.,* 1993; Yamaoka *et al.*, 1997; Kirschner & Oberwinkler, 1999). A continuum in conidium development exists between *Hyalorhinocladiella* and its macronematous analogues *Leptographium* and *Pesotum* Crane & Schoknecht, and also between *Sporothrix* and *Hyalorhinocladiella* (Benadé *et al.*, 1996, 1997). The latter two genera can, however, be distinguished from each other on careful examination (Benadé *et al.*, 1996). Conidia in *Sporothrix* are produced through sympodial proliferation on peglike denticles, whereas percurrent proliferation in *Hyalorhinocladiella* leaves flat, low-profile, ring-like scars on the surface of conidiogenous cells (Mouton *et al.*, 1994; Benadé *et al.*, 1996). Conidia of the latter genus are, furthermore, cylindrical, clavate or Y-shaped, sessile with truncate bases (De Hoog, 1993), and usually produced in gloeoid masses (Benadé *et al.*, 1997). *Sporothrix* spp. produce conidia that are lacrymoid to fusiform, basally acuminate with narrow scars (De Hoog, 1993), physically separated from each other, relatively dry and tend to be larger than those of *Hyalorhinocladiella* (De Hoog, 1974; Benadé *et al.*, 1997).

When *Ceratocystis retusi* Davidson & Hinds was described, the anamorph state was not assigned to any genus (Benadé *et al.*, 1995). In his monograph, Upadhyay referred the species to *Ceratocystiopsis* and assigned the anamorph to ***Allescheriella*** Hennings (Upadhyay, 1981). This was, however, a mistake since *Allescheriella* accommodates aleurosporic anamorphs of Basidiomycetes (Hughes, 1953; Carmichael *et al.*, 1980). A study of conidiogenesis in some *Ophiostoma* anamorphs by Benadé *et al.* (1998) confirmed that this anamorph should have been placed in the genus *Sporothrix.*

Group C:* Leptographium-*like anamorphs

In **Group C**, in inlcude anamorph genera where conidiophores are both macronematous and mononematous (Upadhyay, 1981; Wolfaardt *et al.*, 1992). The most prominent genus in this category is ***Leptographium***, which was established in 1927 for *L. lundbergii* Lag. & Melin (Lagerberg *et al.*, 1927). The first connection between *Leptographium* and an ascomycete followed in 1932 when Grosmann assigned a teleomorph, *Ceratostomella penicillata* Grosm. [= *O. penicillatum* (Grosm.) Siem.] (Grosmann, 1932), to the newly described *L. penicillatum* Grosm. (Grosmann, 1930). Melin and Nannfeldt (Melin & Nannfeldt, 1934) considered the genus ***Hantzschia*** Auersw. as a possible alternative to accommodate the *Leptographium* anamorph of *Cer. penicillatum,* but *L. penicillatum* was never actually transferred to *Hantzchia.* Goidànich (Goidànich, 1936) proceeded to transfer *Cer. penicillata* to his newly erected genus, *Grosmannia,* and referred the anamorph to ***Scopularia*** Preuss. *Scopularia* Preuss, together with *Hantzschia*, were, however, later shown to be invalid (Shaw & Hubert, 1952; Hughes, 1958; Kendrick, 1964a, b; Jacobs & Wingfield, 2001). All anamorphs of *Ophiostoma* spp. described as *Scopularia* (Goidànich, 1936; Mathiesen 1951) are currently accommodated in *Leptographium* (Hunt, 1956; Jacobs *et al.*, 2001). A comprehensive review on the taxonomy of *Leptographium* will be published soon in a new monograph on the genus, which will include 46 species, most of which are phylogenetically connected to *Ophiostoma* (Jacobs *et al.*, 2001).

Miller and Cernzow, in 1934, assigned the *Leptographium*-like anamorphs of *Ceratostomella imperfecta* Miller & Cernzow and *Cer. comata* Miller & Cernzow to the genus ***Haplographium*** Berk. & Broome (Moreau, 1952; Hunt, 1956). Since the conidia of *H. delicatum* Berk. & Broome, the type species, are described as dark in colour, Shaw and Hubert (1952) suggested that *Haplographium* spp. with hyaline conidia should be transferred to *Leptographium.*

Another genus with mononematous conidiophores to be associated with *Ophiostoma* was ***Verticicladiella*** (Kendrick, 1962). Hughes (1953) erected the genus for species with sympodial conidium development, as opposed to annellidic conidiogenesis in *Leptographium.* Wingfield (1985), however, showed that some species of *Leptographium* and *Verticicladiella* exhibit both sympodial and annellidic proliferation on a single conidiogenous cell, making it impossible to separate them on the basis of conidium development. *Verticicladiella* was, therefore, synonymized with *Leptographium* (Wingfield, 1985).

The only *Ophiostoma* anamorph ever placed in ***Phialocephala*** Kendr., was the anamorph of *O. francke-grosmanniae* Davids. (Upadhyay, 1981). The anamorph is currently accommodated in *Leptographium* (Wingfield *et al.*, 1987; Harrington, 1988; Mouton *et al.*, 1992), to which it was referred to initially (Davidson, 1971). *Phialocephala* is distinguished from *Leptographium* and *Verticicladiella* in that it produces conidia phialidically (Kendrick, 1961, 1962; Wingfield, 1985). The genus *Phialocephala* is currently being reviewed, and molecular comparisons shall hopefully provide a clearer view of its relatedness to the ophiostomatoid fungi (R. Jacobs, personal communication).

The last genus in this group is ***Knoxdaviesia***. It resembles *Leptographium,* but is distinct in its absence of a series of metulae and the presence of conidiogenous cells directly on the stipe (Wingfield *et al.*, 1988). *Knoxdaviesia* was erected to accommodate the anamorph of *Ceratocystiopsis proteae* (Wingfield *et al.*, 1988). The first and only *Ophiostoma* anamorph to be assigned to *Knoxdaviesia* was that of *Ophiostoma capense* (Wingfield & Van Wyk, 1993). Both species have since been referred to *Gondwanamyces* (Marais *et al.*, 1998), which means that, at present, no *Ophiostoma* spp. are associated with *Knoxdaviesia*.

Group D:* Graphium-*like anamorphs

Genera with synnematal conidiophores constitutes **Group D**, the fourth group of *Ophiostoma* anamorphs. The first association of such an anamorph genus with an ophiostomatoid teleomorph was made by Münch (1907), when he placed the anamorphs of *Ceratostomella piceae* [= *O. piceae*] and *Cer. cana* Münch [= *O. canum* H. & P. Sydow] in ***Graphium***. The genus, erected in 1837 by Corda, became widely used for the synnematal anamorphs of these and other *Ophiostoma* spp. (Buisman, 1932; Leach, 1934; Melin & Nannfeldt, 1934; Goidànich, 1935, 1936; Mathiesen, 1951; Moreau, 1952; Mathiesen-Kåårik, 1953; Hunt, 1956; Davidson, 1971; Olchowecki & Reid, 1973; De Hoog, 1974; Upadhyay, 1981; Harrington, 1988; Wingfield *et al.*, 1991, 1995; Seifert & Okada, 1993; Seifert *et al.*, 1993; Brasier & Mehrotra, 1995; Yamaoka *et al.*, 1998).

In 1973, Crane and Schoknecht showed that *Graphium penicillioides* Corda, the type species of *Graphium*, has monoblastic conidiogenous cells, and that holoblastic conidia are produced by percurrent proliferation resulting in a series of annellations (Crane & Schoknecht, 1973). The synnematal anamorphs of *Ceratocystis piceae* [= *O. piceae*] and *C. ulmi* [= *O. ulmi*] differ from *G. penicillioides* in having polyblastic conidiogenous cells and holoblastic conidia produced on denticles. A new genus, ***Pesotum***, was therefore

established for these two *Ophiostoma* anamorphs (Crane & Schoknecht, 1973). Some authors accepted the new genus (Upadhyay, 1981; Butin & Aquilar, 1984; Solheim, 1986; Maekawa *et al.*, 1987; Hutchison & Reid, 1988a; Kowalski & Butin, 1989; Brasier & Mehrotra, 1995), while others preferred the use of *Graphium* (Olchowecki & Reid, 1973; De Hoog, 1974; Harrington, 1988; Wingfield *et al.*, 1991, 1995; Seifert *et al.*, 1993; Seifert & Okada, 1993; Brasier & Mehrotra, 1995; Yamaoka *et al.*, 1997, 1998).

Upadhyay and Kendrick (1974) followed the rationale of Crane and Schoknecht and proposed another new genus, ***Phialographium*** Upadh. & Kendr., for the synnematal conidial states of *Ceratocystis* [= *Ophiostoma*] with phialides producing enteroblastic-phialidic conidia in mucilage. The anamorph of *Ceratocystis sagmatospora* Wright & Cain [= *O. sagmatospora* (Wright & Cain) Solheim] was designated as type species (Upadhyay & Kendrick, 1974). The anamorphs of four other *Ophiostoma* spp. were eventually assigned to *Phialographium* (Upadhyay, 1981; Solheim, 1986; Constantinescu & Ryman, 1989).

A year after *Phialographium* was established, Upadhyay and Kendrick (1975), erected three more genera for synnematal anamorphs of *Ophiostoma* and *Ceratocystiopsis*: ***Graphilbum*** Upadh. & Kendr. and ***Hyalopesotum*** Upadh. & Kendr. as the hyaline analogues for *Graphium* and *Pesotum* respectively (Upadhyay & Kendrick, 1975), and ***Pachnodium*** Upadh. & Kendr. as the synnematal analogue of *Sporothrix* with single or catenate holoblastic conidia (Upadhyay & Kendrick, 1975; Mouton *et al.*, 1994). These genera did not gain much recognition. Apart from the type species of each genus, the anamorphs of only two other species were assigned to *Graphilbum* (Upadhyay, 1981; Hutchison & Reid, 1988a; Yamaoka *et al.*, 1997), and another two to *Hyalopesotum* (Upadhyay, 1981). Hutchison and Reid (1988b), furthermore, assigned a species without a known teleomorph to *Hyalopesotum.* No other anamorphs were assigned to *Pachnodium* apart from the type.

Upadhyay (1981) established the genus ***Graphiocladiella*** Upadh. for the anamorph of *Ceratocystis clavigera* Robinson-Jeffrey & Davids. [= *O. clavigerum* (Robinson-Jeffrey & Davids.) Harrington], which has both mononematous and synnematous conidiophores. *Graphiocladiella clavigerum* Upadh. has septate conidia on annellated or apparently sympodially proliferating conidiogenous cells (Seifert & Okada, 1993).

In 1991, Wingfield *et al.* transferred both *Pesotum* and *Phialographium* back to *Graphium*, arguing that 'conidiogenous cells of both genera proliferated percurrently, a process supposedly diagnostic of the related genus, *Graphium*' (Wingfield *et al.*, 1991).

Mouton *et al.* (1993), Seifert *et al.* (1993), as well as Seifert and Okada (1993), supported the recommendation of Wingfield *et al.* (1991). Seifert and Okada (1993), furthermore, suggested that *Graphilbum*, *Hyalopesotum*, *Pachnodium* and *Graphiocladiella* should also be considered synonyms of *Graphium,* and that *Graphium* should be emended to include all the anamorph species previously assigned to the six other synnematal genera.

A recent phylogenetic reappraisal of the *Graphium* complex, based on 18S rDNA sequences (Okada *et al.*, 1998), contradicted the suggested amalgamation of the synnematal anamorph genera of *Ophiostoma* into a single *Graphium* with an enlarged generic concept. This study showed that both *Graphium penicillioides* (the lectotype species of *Graphium*; Hughes, 1958) and *G. putredinis* (Corda) Hughes, are species aggregates and are related to the Microascales, and not to the Ophiostomatales, as was previously believed. It was, therefore, suggested that *Graphium* should no longer be used for anamorphs of *Ophiostoma*. According to Okada *et al.* (1998), the next available generic name for synnematous anamorphs of *Ophiostoma* was *Pesotum*. The other segregate genera proposed earlier as synonyms for *Graphium*, had a wider variety of conidium ontogeny than the original concept of *Pesotum* (Crane & Schoknecht, 1973). Okada *et al.* (1998) consequently emended the generic description of *Pesotum* to include *Graphilbum, Pachnodium, Graphiocladiella, Hyalopesotum* and *Phialographium* (Okada *et al.*, 1998). A nomenclator for the 26 known species of *Pesotum* was compiled, which included eight new combinations (Okada *et al.*, 1998). Three *Ceratocystis* spp. with *Pesotum* anamorphs were also transferred to *Ophiostoma* (Okada *et al.*, 1998). Uzunovic *et al.* (2000) supported Okada *et al.* (1998) and placed the synnematous anamorph of *O. setosum* Uzunovic, Seifert, S.H. Kim & C. Breuil, in *Pesotum*.

Harrington *et al.* (2001) accepted the definition for *Pesotum* of Okada *et al.* (1998), but proposed that *Pesotum* should be restricted for anamorphs with affinities to the *O. piceae* complex. They, furthermore, suggested that synnematous anamorphs of *Ophiostoma* spp., with phialidic coniodiogenous cells, should be placed in the genus *Phialographium* (e.g. the anamorph of *O. cucculatum* Solheim) (Harrington *et al.*, 2001). Wingfield *et al.* (1989) had, however, shown that the synnematous anamorph of *O. cucculatum* exhibits more than one kind of conidiogenesis. I, therefore, agree with Wingfield *et al.* (1991) that the segregation of synnematous anamorphs of *Ophiostoma* spp. among different genera is inappropriate (1991), and support Okada *et al.* (1998) who treated *Phialographium* as a synonym of *Pesotum*.

Conclusions

The anamorph genera currently associated with *Ophiostoma* and its related genera can be summarized as follows:

Sphaeronaemella - ***Gabarnaudia*** Samson & Gams, CBS Studies 6:88-92. 1974.

Ceratocystis - ***Chalara*** (Corda) Rabenh., Deutschl. Krypt.-Flora 1:38. 1844.

Ophiostoma - ***Sporothrix*** Hekt. & Perkins ex Nicot & Mariat, Mycopath. 49:53-65. 1973.

- ***Hyalorhinocladiella*** Upadh. & Kendr., Mycologia 67:798-805. 1975.
- ***Leptographium*** Lag. & Melin, Svensk Skogsv. Tidskr. 25:145-272. 1927.
- ***Pesotum*** Cranc & Schoknecht, Amer. J. Bot. 60:346-354. 1973.
- ***?Xenochalara*** M.J. Wingf. & Crous, S.A.J. Bot. 66:101-102. 2000.

Ceratocystiopsis - ***Sporothrix*** Hekt. & Perkins ex Nicot & Mariat, Mycopath. 49:53-65. 1973.

- ***Hyalorhinocladiella*** Upadh. & Kendr., Mycologia 67:798-805. 1975.

Gondwanamyces - ***Knoxdaviesia*** M.J. Wingf., P.S. van Wyk & Marasas, Mycologia 80:23-30. 1988.

Cornuvesica - ***Chalara*** (Corda) Rabenh., Deutschl. Krypt.-Flora 1:38. 1844.

- ***?Xenochalara*** M.J. Wingf. & Crous, S.A.J. Bot. 66:101-102. 2000.

THE FUTURE

Advances in molecular techniques are changing the way we think about taxonomy and promises to provide answers to some century old questions. Multigene analyses have brought about the prospect to clarify phylogenetic relationships within the genus *Ophiostoma*. These data will make it possible to determine the evolutionary significance of characters such as ascospore shape, anamorph morphology, or pathogenicity and host range. The question as to where the line should be drawn between phylogenetic relationships and practical identification techniques, is just as relevant now as it was in 1932 when Melin & Nannfeldt stated that 'both purposes are equally important but very difficult to combine' (Melin & Nannfeldt, 1934). The definitions of populations, species and genera will be changed and adapted as we gain more knowledge, but ultimately the opinions remain subjective. The aim should, however, be to select molecular and morphological traits that correspond with each other in order to simplify, rather than complicate, classification.

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Table 1. The higher classification of *Ophiostoma* and allied genera.

Year	Supraordinal	Order	Family	Genus / Genera	References
1897	Pyrenomycetineae	Sphaeriales	Ceratostomataceae	*Ceratostomella* *Ceratostoma*	Lindau, 1897
1906	Pyrenomycetes	-	-	*Ceratostomella* *Endoconidiophora*	Münch, 1907
1927	-	-	Ceratostomataceae	*Ceratostomella* *Endoconidiophora*	Lagerberg *et al.*, 1927; Davidson, 1935
1932	- Series: Ascohymeniales	Plectascales Sphaeriales	Ophiostomataceae -	*Ophiostoma* *Ceratostomella*	Nannfeldt, 1932
1935	-	-	Ceratostomataceae	*Ceratostomella*	Davidson, 1935
1935	-	Plectascales	Ophiostomataceae	*Ophiostoma* *Grosmannia* *Ophiostomella* *Chaetoceratostoma*	Goidànich, 1935, 1936
1945	Euascomycetes	Sphaeriales	Ceratostomataceae	*Ceratostomella* *Ophiostoma*	Ainsworth & Bisby, 1945
1951	Subclass: Euascomycetes Series: Unitunicate Subseries: Plectomycetes	Microascales[1]	Ophiostomataceae	*Ophiostoma*	Luttrell, 1951, 1955
1952	Subclass: Euascomycetes	Plectascales	Ophiostomataceae	*Ophiostoma* *Microascus*	Gäumann, 1952
1954	Euascomycetes	Sphaeriales	Ceratostomataceae	*Ceratostomella* *Ceratocystis*	Ainsworth & Bisby, 1954
1954	-	Plectascales	-	*Ophiostoma*	Von Arx & Müller, 1954
1956	-	Plectascales	-	*Ceratocystis*	Hunt, 1956
1958	-	-	Ophiostomataceae	*Ceratocystis*	Davidson, 1958
1961	Ascohymeniales	Ophiostomatales	Ophiostomataceae	*Ceratocystis*	Rosinski, 1961
1963	Euascomycetidae	Microascales	Ophiostomataceae	*Ceratocystis*	Ainsworth, 1963
1965	Euascomycetidae Plectomycetes	Microascales	Ophiostomataceae	*Ceratocystis*	Kendrick & Molnar, 1965
1970	Ascomycetes	Sphaeriales	-	*Ceratocystis* *Sphaeronaemella*	Von Arx, 1970
1971	Ascomycotina Plectomycetes	Microascales	Ophiostomataceae	*Ceratocystis*	Ainsworth, 1971
1973	Pyrenomycetes	Sphaeriales	Ophiostomataceae	*Ceratocystis* *Europhium* *Sphaeronaemella*	Müller & Von Arx, 1973; Ainsworth *et al.*, 1973
1974	Ascomycetes	Sphaeriales	-	*Ceratocystis* *Ophiostoma* *Europhium* *Sphaeronaemella*	Von Arx, 1974
1975	Plectomycetes	Microascales	Ophiostomataceae	*Ceratocystis* *Ceratocystiopsis*	Upadhyay & Kendrick, 1975
1975	Euascomycetes (Unitunicate)	Melanosporales	Ceratocystaceae	*Ceratocystis* *Sphaeronaemella*	Wehmeyer, 1975
1977	Pyrenomycetes	Sphaeriales	Ophiostomataceae	*Ceratocystis*	Hallaksela, 1977
1977	-	Endomycetales	Endomycetaceae	*Ceratocystis* *Ceratocystiopsis* *Europhium* *Ophiostoma*	Redhead & Malloch, 1977
1979	Plectomycetes	Diaporthales	Endomycetaceae	*Ceratocystis* *Ceratocystiopsis* *Europhium*	Malloch, 1979

[1] A *nomen nudum* validated by Benny & Kimbrough (1980).

Table 1 (continued). The higher classification of *Ophiostoma* and allied genera.

Year	Supraordinal	Order	Family	Genus / Genera	References
1979	Pyrenomycetes	Sphaeriales	Sphaeriaceae Ophiostomataceae	*Ceratocystis* *Ophiostoma*	Von Arx, 1979
1980	Plectomycetes	Ophiostomatales	Ophiostomataceae	*Sphaeronaemella* *Ceratocystiopsis* *Ceratocystis* *Ophiostoma*	Benny & Kimbrough, 1980
1981	Plectomycetes	Microascales	Ophiostomataceae	*Ceratocystis* *Ceratocystiopsis*	Upadhyay, 1981
1981	-	-	Ophiostomataceae Nectriaceae	*Ophiostoma* *Europhium* *Ceratocystiopsis* *Ceratocystis* *Sphaeronaemella*	Von Arx, 1981
1983	-	Ophiostomatales	Ophiostomataceae	*Ceratocystis* = *Ophiostoma?* *Ceratocystiopsis*	Hawksworth *et al.*, 1983
1985	-	Ophiostomatales	-	*Ophiostoma* *Ceratocystis*	Kendrick, 1992
1986	-	Ophiostomatales	Ophiostomataceae	*Ophiostoma* *Europhium* *Ceratocystiopsis* *Ceratocystis* *Sphaeronaemella*	Solheim, 1986; Von Arx & Van der Walt, 1986
1987	-	Ophiostomatales	Ophiostomataceae Pyxidiophoraceae	*Ophiostoma* *Ceratocystiopsis* *Europhium* *Ceratocystis* *Pyxidiophora*	Von Arx & Van der Walt, 1987
1988	Plectomycetes	Ophiostomatales	Ophiostomataceae	*Ceratocystis*	Otani, 1988
1989	Pyrenomycetes	Ophiostomatales	-	*Ceratocystiopsis* *Ceratocystis* *Ceratostomella* *Ophiostoma*	Farr *et al.*, 1989
1990	Hymenoascomycetes	Sordariales Microascales	Lasiosphaeriaceae Ophiostomataceae	*Ceratocystis* *Ophiostoma* *Ceratocystiopsis* *Europhium*	Barr, 1990
1993	Plectomycetes	Microascales	Ophiostomataceae	*Ceratocystis* *Ceratocystiopsis*	Upadhyay, 1993
1993	Hymenoascomycetes	Sordariales Xylariales	Lasiosphaeriaceae Ophiostomataceae	*Ceratocystis* *Ophiostoma* *Ceratocystiopsis*	Samuels, 1993
1994	-	Microascales Ophiostomatales	- Ophiostomataceae	*Ceratocystis* *Ophiostoma*	Blackwell & Spatafora, 1994; Barr & Cannon, 1994
1995	-	Microascales Ophiostomatales	*incertae sedis* Ophiostomataceae	*Ceratocystis* *Sphaeronaemella* *Ophiostoma* *Klasterskya* *Spumatoria* *Subbaromyces*	Hawksworth *et al.*, 1995
1999	Pyrenomycetes	Microascales Ophiostomatales	- -	*Ceratocystis* *Gondwanamyces* *Ophiostoma*	Wingfield, B.D., *et al.*, 1999

Table 2. Anamorph genera associated with the ascomycete genus *Ophiostoma*. Genera currently associated with *Ophiostoma* are printed in bold type.

Group	Anamorph genera	Description of genus		First association with ophiostomatoid teleomorph	
		Year	References	Year	References
A	*Chalara* (Corda) Rabenh.	1844	[1] (Carmichael *et al.*, 1980)	1892	Saccardo, 1892; Elliott, 1923
	= *Thielaviopsis* Went	1893	(Carmichael *et al.*, 1980)	1928	Dade, 1928
	= *Chalaropsis* Peyr.	1916	(Carmichael *et al.*, 1980)	1941	Bliss, 1941
	Gabarnaudia Samson & Gams	1974	Samson, 1974	1974	Samson, 1974
	[2] ?***Xenochalara*** M.J. Wingf. & Crous	2000	Coetzee *et al.*, 2000	2000	Coetzee *et al.*, 2000
B	*Acremonium* Link ex Fr.	1809	(Carmichael *et al.*, 1980)	1975	Upadhyay & Kendrick, 1975; Upadhyay, 1981
	= *Cephalosporium* auct. non Corda	1839	(Carmichael *et al.*, 1980)	1906	Hedgcock, 1906
	Cladosporium Link	1815	(Carmichael *et al.*, 1980)	1907	Münch, 1907
	Sporotrichum Link ex Fr.	1809	(De Hoog, 1974; Fries, 1821)	1907	Münch, 1907
	Cylindrocephalum Bon.	1851	(Hughes, 1958)	1932	Robak, 1932
	[3] *Hormodendron*	-	(Carmichael *et al.*, 1980)	1932	Robak, 1932
	Hyalodendron Diddens	1934	Diddens, 1934	1935	Goidànich, 1935
	Rhinotrichum Corda	1837	(Hawksworth *et al.*, 1993)	1948	(Przybyl & De Hoog, 1989)
	Sporothrix Hekt. & Perkins ex Nicot & Mariat	[4] 1900	Hektoen & Perkins, 1900; Nicot & Mariat, 1973	1968	Mariat *et al.*, 1968
	Raffaelea v. Arx & Henneb.	1965	Von Arx & Hennebert, 1965	1974	De Hoog, 1974
	Hyalorhinocladiella Upadh. & Kendr.	1975	Upadhyay & Kendrick, 1975	1975	Upadhyay & Kendrick, 1975
	Allescheriella Hennings	1897	(Ellis, 1971)	1981	Upadhyay, 1981
	Verticillium Nees	1817	(Carmichael *et al.*, 1980)	1953	(Przybyl & De Hoog, 1989)
C	***Leptographium*** Lag. & Melin	1928	Lagerberg *et al.*, 1927	1932	Grosmann, 1932
	= *Verticicladiella* S. Hughes	1953	Hughes, 1953	1962	Kendrick, 1962
	Hantzschia Auersw.	1862	(Carmichael *et al.*, 1980)	1934	Melin & Nannfeldt, 1934
	Haplographium Berk. & Broome	1859	(Carmichael *et al.*, 1980)	1934	(Hunt, 1956; Moreau 1952)
	Scopularia Preuss	1851	Preuss, 1851	1936	Goidànich, 1936
	Phialocephala Kendr.	1961	Kendrick, 1961	1981	Upadhyay, 1981
	Knoxdaviesia M.J. Wingf., P.S. van Wyk & Marasas	1988	Wingfield *et al.*, 1988	1988	Wingfield *et al.*, 1988
D	*Graphium* Corda	1837	Corda, 1837	1907	Münch, 1907
	Pesotum Crane & Schocknecht ex Okada & Seifert	1973	Crane & Schoknecht, 1973	1973	Crane & Schoknecht, 1973
	= *Phialographium* Upadh. & Kendr.	1974	Upadhyay & Kendrick, 1974	1974	Upadhyay & Kendrick, 1974
	= *Graphilbum* Upadh. & Kendr.	1975	Upadhyay & Kendrick, 1975	1975	Upadhyay & Kendrick, 1975
	= *Hyalopesotum* Upadh. & Kendr.	1975	Upadhyay & Kendrick, 1975	1975	Upadhyay & Kendrick, 1975
	= *Pachnodium* Upadh. & Kendr.	1975	Upadhyay & Kendrick, 1975	1975	Upadhyay & Kendrick, 1975
	= *Graphiocladiella* Upadh.	1981	Upadhyay, 1981	1981	Upadhyay, 1981

[1] Parentheses indicate that the original publication in which the genus was described, was not available.
[2] The connection between *Xenochalara* and *Ophiostoma* has yet to be confirmed (Coetzee *et al.*, 2000).
[3] An orthographic variant of *Hormodendrum* Bon., described in 1851 (Carmichael *et al.*, 1980).
[4] Validated by Nicot and Mariat, 1973.

Chapter 4

When drinking wine amongst the roses
Or guzzling beer while throwing bricks
Or playing games in bales of hay
Where lurks the tricky Sporothrix,
Beware the price you pay for play,
When you get struck by dread mycoses.

- S. Vaisrub, 1971

Phylogeny of the *Ophiostoma stenoceras - Sporothrix schenckii* complex[1]

ABSTRACT

Ophiostoma stenoceras is a well-known sapstaining fungus occurring on various coniferous and some hardwood hosts in the Northern Hemisphere. In the Southern Hemisphere, the fungus has only been described from New Zealand. The human pathogen, *Sporothrix schenckii*, has been suggested to be the anamorph of *O. stenoceras*. The aim of this study was to gain a better understanding of the phylogenetic relationship between these two species. The study also provided the opportunity to confirm the identity of some *Sporothrix* and *O. stenoceras*-like isolates recently collected from wood and soil around the world. For this purpose, the DNA sequence of Internal Transcribed Spacer (ITS) regions of the ribosomal DNA operon was determined. Isolates of *O. albidum, O. abietinum* and *O. nigrocarpum,* all morphologically similar to *O. stenoceras*, were included in the study. From phylogenetic analyses of the sequence data four main clades were observed. These represented *O. stenoceras*, *O. nigrocarpum* and two separate groups containing isolates of *S. schenckii*. Our results confirm earlier suggestions that *S. schenckii* should be classified within the teleomorph genus *Ophiostoma*, but support studies separating *O. stenoceras* and *S. schenckii*. *O. albidum* should be considered a synonym of *O. stenoceras*, and *O. abietinum* a synonym of *O. nigrocarpum*. The two groups within *S. schenckii* might represent two species, but this needs further confirmation. This study represents the first reports of *O. stenoceras* from Colombia, Kenya, Uruguay and South Africa, as well as the first reports of *O. nigrocarpum* from Austria, Canada, Japan and South Africa.

[1] See Appendix 1 for more information about the distribution, host range, insect vectors and morphology of *Ophiostoma* spp. treated in this chapter.

INTRODUCTION

Ophiostoma stenoceras (Robak) Nannf. is a sapstaining fungus that was first described from ground wood pulp in Norway (Robak, 1932). It has since been isolated from many other coniferous hosts, as well as some hardwood trees from the Northern Hemisphere (Davidson, 1942; Griffin, 1968; Otani, 1988). In the Southern Hemisphere, *O. stenoceras* has been reported only from New Zealand (Farrell *et al.*, 1997; Schirp *et al.*, 1999). The fungus causes a slight grey stain on pine and spruce (Kåårik, 1980), but is not considered economically important (Davidson, 1942; Griffin, 1968).

The first suggestion that *O. stenoceras* might represent the teleomorph of *Sporothrix schenckii* Hektoen & Perkins, the causative agent of human sporotrichosis, was made by Mariat (Mariat *et al.*, 1968; Mariat, 1971a, b; Nicot & Mariat, 1973). The relationship between *O. stenoceras* and *S. schenckii* has since been the subject of many research papers. A wide variety of taxonomic criteria were employed in these studies, including conidium morphology, vitamin requirements, starch degradation, resistance to digestion by macrophage cells, immunological studies, cell wall components, neutral and polar lipid composition, carbohydrate composition, acid phosphatase isoenzyme patterns, and pathogenicity studies (De Hoog, 1974; Travassos & Lloyd, 1980; Summerbell *et al.*, 1993). Molecular investigations included techniques such as DNA-DNA hybridisation, GC content (Mendonça-Hagler *et al.*, 1974) and mitochondrial restriction fragments (Suzuki *et al.*, 1988). The results of these studies were often contradictory, some suggesting that *S. schenckii* was the anamorph of *O. stenoceras* (Taylor, 1970; De Hoog, 1974), and others showing differences between the two species (Mendonça-Hagler *et al.*, 1974; Travassos *et al.*, 1974; Suzuki *et al.*, 1988). All these investigations were reviewed by Travassos and Lloyd (1980), as well as Summerbell *et al.* (1993). Although Travassos and Lloyd concluded that *S. schenckii* "bears little relation" to *O. stenoceras*, and a list of suggested criteria to distinguish between the two species was compiled (Summerbell *et al.*, 1993), the phylogenetic relationship between the two fungi was never clarified.

Berbee and Taylor (Berbee & Taylor, 1992) confirmed with DNA sequencing that *S. schenckii* is phylogenetically related to *Ophiostoma*. The 18S rDNA gene, sequenced in their study is highly conserved and does not exhibit sufficient variability to allow for distinction between closely related species. The aim of our study was, therefore, to gain a better understanding of the phylogenetic relationships between *O. stenoceras* and *S. schenckii*. To achieve this goal, we sequenced both the Internal Transcribed Spacer (ITS) regions,

including the 5.8S gene, of the ribosomal RNA operon. Isolates of *O. nigrocarpum* (Davidson) De Hoog, *O. albidum* Mathiesen-Kåårik, and *O. abietinum* Marmolejo & Butin, all three species that are morphologically similar to *O. stenoceras,* were also included in the phylogenetic study. The study also provided us with the opportunity to confirm the identity of some *Sporothrix* and *O. stenoceras*-like isolates from wood and soil that have recently been collected from various Southern Hemisphere countries.

MATERIALS & METHODS

Isolates

Isolates resembling *O. stenoceras* and *O. nigrocarpum* (**Table 1**) were collected from wood, bark beetles and soil from various countries, world-wide. Authenticated isolates of both these species, as well as one isolate each of *O. abietinum* and *O. albidum*, were obtained from the CBS and ATCC. The isolate of *O. abietinum* (C696) is associated with the type of the species. The *O. albidum* isolate (C1190) was one of the isolates examined by Mathiesen-Kåårik (1953) when she described the species. Unfortunately no type material was designated for the species (Hunt, 1956), and no culture representing type material exists.

Sporothrix schenckii isolates (**Table 1**) were obtained from wood, soil and human patients. The rDNA sequence for the *S. schenckii* isolate from the USA was obtained from GenBank.

Where isolations were made from wood samples, these were initially incubated in Petri dishes with moist tissue paper at room temperature. After the appearance of either perithecia or conidiophores, spore masses were transferred from these structures to 2 % Biolab Malt Extract Agar (MEA), and the cultures were purified.

For isolations from soil, 1 g of each sample was diluted in 100 ml sterile water. A dilution series with five dilutions was made. Of each of the dilutions, 1 ml was plated onto 2 % Malt- and 0.2 % Yeast Extract Agar (MYA). The plates were incubated at 20 °C for one to three days. Colonies with a *Sporothrix*-like appearance were transferred to clean MEA plates and purified. Isolates from bark beetles and humans were obtained following the methods described by Hsiau and Harrington (1997), and Vismer and Hull (1997), respectively.

The *O. ulmi* (Buisman) Nannf. and *O. ips* (Rumbold) Nannf. isolates included as outgroups in the phylogenetic analysis, have been sequenced as part of an earlier study (Harrington *et al.*, 2001). These sequences are also available from GenBank. The relevant accession numbers are provided in **Table 1**.

All isolates used in this study are maintained on MEA slants at 4 °C in either the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, or in the culture collection (C) of T.C. Harrington, Department of Plant Pathology, Iowa State University, USA.

Preparation of single spore cultures

One single spore culture was prepared from each of the isolates for DNA extraction. Single ascospore cultures were made from isolates producing perithecia in culture (**Table 1**). Some isolates did not produce viable perithecia in culture. Single conidium cultures were prepared for these isolates.

To induce sporulation, each isolate was grown on Water Agar (WA: 30 g agar, 1000 ml distilled water) at room temperature. The cultures were inoculated onto the WA alongside a sterilised piece of pine (for isolates from softwoods) or eucalyptus (for isolates from hardwoods). Cultures were incubated at 20 °C until perithecia formed.

Single ascospore cultures were prepared by transferring a drop of ascospores to a 2.5 ml Eppendorf tube containing 1 ml of sterile water. The tube was incubated for 10 minutes and shaken for 20 seconds using a vortex mixer. The last two steps were repeated once and the diluted ascospores spread onto the surface of WA in Petri dishes. After 10 minutes, water on the surface of the first plate was poured over and spread onto a second Petri dish containing 1.5 % MEA (15 g/l Difco Malt Extract, 30 g/l Agar). Both Petri dishes were incubated at 25 °C until germination occurred, usually within 12 to 48 hours. The germ tube was allowed to extend. Before branching or spore formation occurred, a hyphal tip was carefully dissected from the agar. This was transferred to 2 % MEA and incubated at 20 °C for one week. Hyphal tips from five separate spores were transferred for each isolate. One culture representing the most typical colony morphology from the five single ascospore cultures, was selected for DNA extraction.

Some of the isolates produced no perithecia, or only protoperithecia without any ascospores, in culture. All these isolates did, however, produce a *Sporothrix* state. Five single conidial cultures were made from each of these isolates, following the same procedure as for producing single ascospore cultures. Conidia were obtained for this purpose by scraping the surface of the agar bearing sporulating cultures.

Fertility tests

Sixteen isolates producing perithecia in culture (**Table 1**) were selected to determine whether these isolates were self-fertile or self-sterile. For each of these isolates, 40 single ascospore cultures were produced as described above. The single spore cultures were incubated at 20 °C until perithecia formed. Perithecia from five representative cultures of the 40 single spore cultures, were mounted in lactophenol blue. Light microscopy was used to confirm the presence of typical ascospores. An isolate was considered self-fertile if more than 32 of the 40 (> 80 %) single spore cultures produced perithecia with typical ascospores.

In four of the 16 sporulating isolates, none of the 40 single ascospore cultures produced perithecia. This indicated that these isolates were probably self-sterile. To confirm this, crosses were made between single spore cultures from each isolate. At least 10 single spore cultures per isolate were selected and crossed with each other in five pairs. Two agar plugs with mycelium, repesenting two of the 10 single spore cultures from the same isolate, were inoculated 5 mm apart on WA in close proximity of a piece of sterilised pine wood. As a control, each culture was crossed against itself on separate Petri dishes. Petri dishes with crosses were incubated at 20 °C until perithecia formed. Where both control crosses did not produce any perithecia, and the cross between the two different single spore cultures produced perithecia, the isolates from which these single spore cultures originated, were considered self sterile. Crossings between single spore cultures from different isolates were not conducted.

DNA sequencing and sequence analysis

For the phylogenetic analyses, isolates were grown for 10 days in a liquid medium containing 2 % Malt Extract. DNA was extracted using the method of DeScenzo and Harrington (1994). A part of the ribosomal DNA operon, including the 3' end of the small subunit (SSU) rDNA gene, internal transcribed spacer (ITS) region 1, the 5.8S gene, ITS region 2 and the 5' end of the large subunit (LSU), was amplified using PCR with the primers ITS1-F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990). The reaction mixture (50 µl final volume) contained 2.6 U Expand™ High Fidelity Taq Polymerase mixture (Boehringer Mannheim, South Africa), 5 µl PCR reaction buffer, 3 mM $MgCl_2$, 5 µl DNTPs, and 10 µM of each primer. PCR reactions were performed in a Hybaid Touchdown PCR machine (Hybaid, Middlesex, UK). PCR conditions were as follows: one cycle of 2 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C, followed by one cycle of 8 min at 72 °C. PCR products were visualised on a 1 % (w/v) agarose gel, stained with ethidium bromide.

PCR fragments were purified using the QIAquick PCR purification kit. Both strands of the PCR fragments were sequenced using the primers ITS1-F, ITS4, CS2 and CS3 (Wingfield *et al.,* 1996) and the Thermo Sequenase Dye Terminator Cycle Sequencing Pre-mix Kit (Amersham Life Science). Sequences were determined using an ABI Prism 377 Automatic DNA sequencer (Perkin Elmer).

The nucleotide sequences were aligned manually and the phylogenetic relationship determined using PAUP (Phylogenetic Analysis Using Parsimony) 4.0b2a (Swofford, 1998). Uninformative characters were excluded and a heuristic search, using TBR (Tree Bisection and Reconstruction) branch swapping (MULPAR on), was conducted to determine the most parsimonious trees. Trees were rooted using sequences of *O. ulmi* and *O. ips*. Due to capacity constraints on the computer, 100 bootstrap analyses were run on 1000 saved trees to determine confidence intervals at the branching points.

RESULTS

Fertility tests

The 16 isolates that were subjected to fertility tests could be divided in two distinct groups based on the results. The first group of 12 isolates was clearly self-fertile (**Table 1**). For each of these isolates, more than 32 of 40 single ascospore cultures produced perithecia with viable ascospores. The second group consisted of four isolates that were self-sterile (**Table 1**). Not one of the single ascospore cultures obtained from these four isolates produced perithecia with ascospores. In a small number of cases, protoperithecia without any ascospores were formed. When single spore cultures obtained from the same original isolate, were crossed with each other, perithecia with viable ascospores were produced. Positive crosses were thus obtained for each of the four isolates.

Sequence analysis

PCR products were approximately 560 bp in size. Within the ITS 1 region of all isolates, there was a GC-rich area of approximately 70 bases. This area proved difficult to sequence, probably due to G-C folding, resulting in secondary structures which would be difficult for the polymerase to read through. The *S. schenckii* isolates provedto be the most difficult to sequence. Although it was possible to get the full sequence for most of the isolates, there were 5 isolates for which around 25 bases could not be determined. Manual alignment resulted in a

total alignment of 617 characters, including gaps, for each of the 54 taxa. 468 parsimony uniformative characters were excluded, leaving 149 characters in the analyses. Most of the variation in the sequence was found within the ITS 1 region.

Using *O. ulmi* and *O. ips* as the outgroup taxa, 14 400 most parsimonious trees (CI = 0.844, HI = 0.156, RI = 0.949) of 392 steps were produced. The topologies of the different trees were the same and a single tree was chosen for presentation (**Figure 1**). Four main clades were resolved in all trees. Variation between the different trees resulted from changes within the main clades. Bootstrap values supporting the branches of the *O. stenoceras* and *O. nigrocarpum* groups were 94 % and 97 % respectively. *S. schenckii* isolates were separated from these species with a 92 % confidence interval, while the two *S. schenckii* groups were separated from each other with a 100 % confidence interval.

DISCUSSION

In this study, we could show that *O. stenoceras*, *O. nigrocarpum* and *S. schenckii* are phylogenetically closely related fungi. The rDNA sequence data, however, clearly separated these three species. Our results endorse earlier suggestions (Berbee & Taylor, 1992) that *S. schenckii* should be classified within the teleomorph genus *Ophiostoma*. They also support previous morphological, biochemical and molecular studies that have separated *O. stenoceras* and *S. schenckii* (Travassos & Lloyd, 1980; Summerbell *et al.*, 1993). Another interesting outcome of this study was that, although distinct, *O. nigrocarpum* and *O. stenoceras* appear to be more closely related to each other than to *S. schenckii*. It furthermore, appears as if *S. schenckii* represent more than one species.

The four main clades in the phylogenetic tree (**Figure 1**) represent *O. stenoceras*, *O. nigrocarpum* and two separate groups containing isolates of *S. schenckii*. Further discussion will treat each clade independently.

The Ophiostoma stenoceras *clade*

The *O. stenoceras* clade includes 26 isolates, including the strain representing the type of the species (CMW3202) from Norway. Other *O. stenoceras* isolates from Europe (Italy, the Netherlands, and France), the USA and New Zealand, grouped together in this clade. The *O. albidum* isolate (C1190) also grouped within the *O. stenoceras* clade. This species was originally described from bark beetle galleries in Sweden and was distinguished from

O. stenoceras by its smaller perithecia (Mathiesen-Kåårik, 1953). Although other slight morphological differences between the two species have been reported (Kåårik, 1960; Mathiesen-Kåårik, 1960; Aoshima, 1965; Griffin, 1968), De Hoog (1974) and Upadhyay (1981) treated *O. albidum* as a synonym of *O. stenoceras*. Our results support this synonymy.

Isolates resembling *O. stenoceras* from Africa, South America and Indonesia were confirmed to represent this species. This study thus represents the first report of *O. stenoceras* from Colombia, Kenya, Uruguay, Indonesia and South Africa. In South Africa the fungus is distributed widely on a variety of hardwood hosts. Two *Sporothrix* isolates from New Zealand (C966 and C982) which resembled the anamorph of *O. stenoceras*, also grouped in this clade. They could, therefore, be positively identified as *O. stenoceras*.

The fact that three of the *O. stenoceras* isolates in the study came from humans, is of particular significance. The isolate of Mariat (C1189) from healthy human scalp and which was suggested to represent the teleomorph of *S. schenckii* (Mariat, 1971a), grouped clearly within the *O. stenoceras* clade. Our data, therefore, confirm previous studies showing that this isolate cannot be considered the teleomorph of *S. schenckii* (Mendonça-Hagler *et al.*, 1974; Suzuki *et al.*, 1988).

The* Ophiostoma nigrocarpum *clade

This clade contained 14 isolates, which included seven authenticated isolates of *O. nigrocarpum* from conifers in the USA. These seven isolates form the core of the *O. nigrocarpum* clade, and grouped closely together. Two *Sporothrix* isolates from New Zealand also grouped within the main *O. nigrocarpum* clade. Although the sequences of these two isolates differed slightly from the other *O. nigrocarpum* isolates, we believe they represent the anamorph of this species.

The strain associated with the type of *O. abietinum*, also grouped with the authentic *O. nigrocarpum* isolates. *O. abietinum* was originally described from *Abies* in Mexico (Marmolejo & Butin, 1990). Based on perithecium morphology, *O. abietinum* can be considered intermediate between *O. stenoceras* and *O. nigrocarpum* (Robak, 1932; Davidson, 1966; Marmolejo & Butin, 1990). Our results suggest that *O. abietinum* should be considered a synonym of *O. nigrocarpum*.

Three of the remaining four isolates in the larger *O. nigrocarpum* clade, originated from hardwoods. The exception is the Canadian isolate from the bark beetle *Dendroctonus ponderosae* that infests *Pinus* spp. All four these isolates were originally identified as *O. stenoceras*. Although there is some sequence variation among these four isolates, as well

as between them and the remaining *O. nigrocarpum* isolates, all the isolates in this clade are strongly separated from *O. stenoceras*. We, therefore, believe that they can be assigned to *O. nigrocarpum* with some confidence. These isolates would then represent the first reports of *O. nigrocarpum* from Austria, Canada, Japan and South Africa. The host range of *O. nigrocarpum* would thus also be extended to include hardwoods.

Results of this study showed a conspicuous difference between the *O. stenoceras* and *O. nigrocarpum* clades. This relates to the variability within each clade. The rDNA sequences of isolates in the *O. stenoceras* clade are visibly more conserved than those in the *O. nigrocarpum* clade, which shows much more variation. Results from the fertility studies conducted on 16 isolates from the two groups might elucidate this. The 12 isolates tested from the *O. stenoceras* group were all self-fertile, which corresponds with earlier reports that both *O. stenoceras* and *O. albidum* are homothallic (Kåårik, 1960). In contrast, the four isolates from the *O. nigrocarpum* group, all proved to be self-sterile. This might lead to a greater degree of outcrossing amongst individuals in the *O. nigrocarpum* group. A higher level of genetic recombination would occur, resulting in more variability. It is known that in other groups of fungi, far less variation occurs within homothallic species than within heterothallic species (Glass *et al.*, 1990).

The Sporothrix schenckii *clades*

Within the larger *S. schenckii* clade, there are two strongly supported groups of isolates. All isolates from the first group originated from diseased human tissue. This includes the American isolate from a human (ATCC14284), which groups together with the South African isolates from humans. With one exception, all the isolates from the second group originated from either soil or plant material. This confirms previous observations where morphological and physiological differences were observed between isolates of *S. schenckii* from human tissue and those from other sources (Travassos & Lloyd, 1980). Isolates from wood and soil also tend to be less pathogenic to mice (Travassos & Lloyd, 1980), suggesting some differences between them. Whether these two groups of isolates represent distinct species needs further evaluation. It also raises questions as to the origin of the human pathogen.

In this study the phylogenetic relationships between *O. stenoceras* and *S. schenckii* were resolved. The study also highlighted the need for additional investigations of *O. nigrocarpum*. Additional isolates and other regions of the genome should be included in such studies. The two clades that are resolved in the *S. schenckii* group are intriguing and need further investigation both with more sequences and clinical trials.

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Table 1. Isolates used in this study.

Species	Isolate[1]	Other numbers	GenBank	Collector or Supplier	Substrate	Origin	Perithecia produced	Fertility[2]
Ophiostoma abietinum	C696[3]	CBS125.89	-	J.G. Marmolejo	*Abies vejari*	Mexico	No	NT
O. albidum	C1190[4]	CBS798.73	-	A. Mathiesen-Kåårik	wood	Sweden	No	NT
O. ips	C327	-	AF198244	T.C. Harrington	-	USA, New York	NT	NT
O. nigrocarpum	CMW7619	C140	-	D. Owen	*Pinus ponderosa*	USA, California	Yes	SS
	CMW7620	C190	-	D. Owen	*Pinus ponderosa*	USA, California	Yes	SS
	CMW7621	C201; ATCC22391; RWD237	-	R.W. Davidson	*Dendroctonus* sp.	USA, California	Yes	SS
	C314	CBS408.77; RWD873	-	H.S. Whitney	*Pinus ponderosa*	USA, California	Yes	NT
	C349	-	-	T.C. Harrington	*Pinus taeda*	USA, Louisiana	Yes	SS
	C558	-	-	T.C. Harrington	*Dendroctonus frontalis*	USA, Minnesota	No	NT
	C818[5]	-	-	Y. Masuya	*Quercus serrata*	Japan	No	NT
	C946[6]	-	-	R. Blanchette	-	New Zealand	No	NT
	C1142[6]	13NZ-493	-	R. Farrell	-	New Zealand	No	NT
	C1302	-	-	D. Hofstra	*Pinus radiata*	USA, California	Yes	NT
	CMW1468[5]	C1211	-	Y. Hiratsuka, Y. Yamaoka	*Dendroctonus ponderosae*	Canada, British Columbia	No	NT
	CMW2543[5]	-	-	P.W. Crous	*Eucalyptus* leaves	South Africa, Western Cape	Yes	NT
	CMW7131[5]	HA206	-	E. Halmschlager, T. Kirisits	*Quercus petraea*	Austria	Yes	NT
O. stenoceras	C962	UFV177	-	A. Alfenas	*Eucalyptus globulus*	Uruguay	Yes	SF
	C965	3NZ-35	-	R. Farrell	sap	New Zealand	Yes	SF
	C966[6]	3NZ-38b	-	R. Farrell	wood	New Zealand	No	NT
	C982[6]	-	-	R. Farrell	leaves of conifer	New Zealand	No	NT
	C1189	CBS360.71; UAHM5131	-	F. Mariat	skin of human head	France	No	NT

(Continued on following page)

Table 1. Continued.

Species	Isolate[1]	Other numbers	GenBank	Collector or Supplier	Substrate	Origin	Perithecia produced	Fertility[2]
Ophiostoma stenoceras	C1191	CBS208.75	-	D. Herderschee	skin of human	Netherlands	Yes	NT
	C1192	CBS103.78	-	R.W. Davidson	human	USA, Chicago	No	NT
	C1193	CBS470.92	-	F. Marziano	soil	Italy	No	NT
	CMW101	C459	-	T.E. Hinds	-	USA	No	NT
	CMW129	C80; RWD905	-	R.W. Davidson	-	USA	Yes	SF
	CMW2344	-	-	G.H.J. Kemp	*Eucalyptus smithii*	South Africa, Kwazulu-Natal	Yes	SF
	CMW2347	-	-	G.H.J. Kemp	*Eucalyptus fastigata*	South Africa, Mpumalanga	Yes	SF
	CMW2348	C703	-	G.H.J. Kemp	*Eucalyptus smithii*	South Africa, Kwazulu-Natal	Yes	NT
	CMW2349	-	-	G.H.J. Kemp	*Eucalyptus grandis*	South Africa, Mpumalanga	Yes	NT
	CMW2524	-	-	Z.W. de Beer	*Acacia mearnsii*	South Africa, Kwazulu-Natal	Yes	SF
	CMW2530	-	-	Z.W. de Beer	*Eucalyptus grandis*	Colombia	Yes	NT
	CMW2533	-	-	Z.W. de Beer	*Eucalyptus grandis*	Colombia	Yes	SF
	CMW2625	C447; UCB57.013	-	M.L. Berbee, J.W. Taylor	-	USA	Yes	SF
	CMW3202[7]	C1188; CBS237.32	-	H. Robak	pine pulp	Norway	Yes	NT
	CMW3998	-	-	V.N. Thanh	soil ex. *Euc.* plantation	Kenya	Yes	SF
	CMW4003	-	-	V.N. Thanh	soil ex. *Euc.* plantation	Kenya	Yes	SF
	CMW4007	-	-	V.N. Thanh	soil ex. *Euc.* plantation	Colombia	Yes	SF
	CMW4020	-	-	V.N. Thanh	soil ex. *Euc.* plantation	Colombia	Yes	SF
	CMW4031	-	-	Z.W. de Beer	indigenous hardwood	Indonesia	Yes	NT
	CMW5346	-	-	A. Smit	canker on apple tree	South Africa, Western Cape	Yes	NT
	CMW5347	-	-	A. Smit	canker on apple tree	South Africa, Western Cape	Yes	NT

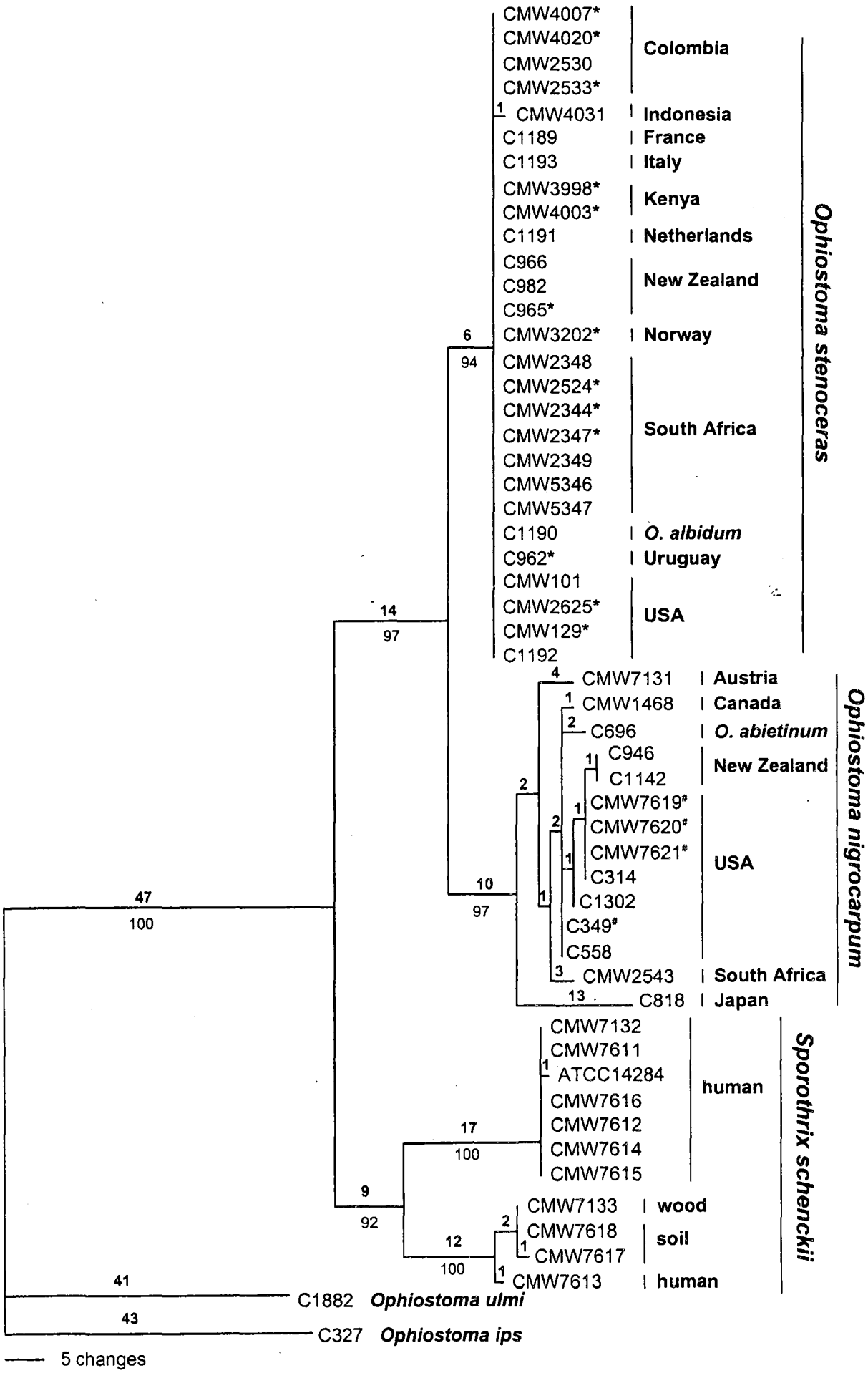
(Continued on following page)

Table 1. Continued.

Species	Isolate[1]	Other numbers	GenBank	Collector or Supplier	Substrate	Origin	Perithecia produced	Fertility[2]
Ophiostoma ulmi	C1882	CBS102.63	AF198232	F.W. Holmes, H.M. Heybroek	*Ulmus hollandica*	Netherlands	NT	NT
Sporothrix schenckii	CMW7132	-	-	J.J. van der Merwe	human	South Africa	No	NT
	CMW7133	-	-	Z.W. de Beer	*Rosa* sp.	South Africa	No	NT
	CMW7611	MRC6856	-	H.F. Vismer	human sporotrichosis	South Africa	No	NT
	CMW7612	MRC6862	-	H.F. Vismer	human sporotrichosis	South Africa	No	NT
	CMW7613	MRC6864	-	H.F. Vismer	human sporotrichosis	South Africa	No	NT
	CMW7614	MRC6867	-	H.F. Vismer	human sporotrichosis	South Africa	No	NT
	CMW7615	MRC6956	-	H.F. Vismer	human sporotrichosis	South Africa	No	NT
	CMW7616	MRC6957	-	H.F. Vismer	human sporotrichosis	South Africa	No	NT
	CMW7617	MRC6963	-	H.F. Vismer	soil	South Africa	No	NT
	CMW7618	MRC6965	-	H.F. Vismer	soil	South Africa	No	NT
	-	ATCC14284	AF117945	C.W. Emmons	human	USA, Maryland	NT	NT

[1] C = Culture Collection of T.C. Harrington, Department of Plant Pathology, Iowa State University, Iowa, USA.
CMW = Culture Collection Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.
[2] NT = Not tested; SS = Self-sterile; SF = Self-fertile.
[3] Isolate C696 from Mexico is associated with the holotype of *O. abietinum*.
[4] Isolate C1190 from Sweden was one of the isolates studied by Mathiesen-Kåårik (1953) for the original species description of *O. albidum*.
[5] Isolates originally identified as *O. stenoceras*.
[6] Isolates originally identified as *Sporothrix* spp.
[7] Isolate CMW3202 from Sweden is associated with type of *Ceratostomella stenoceras* = *O. stenoceras*.

Figure 1. One of the most parsimonious trees obtained by Heuristic searches of the partial ribosomal RNA operon (including partial Small Subunit, Internal Transcribed Spacer (ITS1) region, 5.8S gene, ITS2, and partial Large Subunit). Bootstrap values are given below, and branch lengths above the lines at branching points. (#self-fertile; * self-sterile).


CMW4007*
CMW4020*
CMW2530
CMW2533*
Colombia
1 CMW4031
Indonesia
C1189
France
C1193
Italy
CMW3998*
CMW4003*
Kenya
C1191
Netherlands
C966
C982
C965*
New Zealand
6
94
CMW3202*
Norway
CMW2348
CMW2524*
CMW2344*
CMW2347*
CMW2349
CMW5346
CMW5347
South Africa
C1190
O. albidum
C962*
Uruguay
CMW101
CMW2625*
CMW129*
C1192
USA
Ophiostoma stenoceras
14
97
4 CMW7131
Austria
1 CMW1468
Canada
2 C696
O. abietinum
1 C946
C1142
New Zealand
2
CMW7619#
1
CMW7620#
2
CMW7621#
1
C314
C1302
C349#
C558
USA
10
97
1
3 CMW2543
South Africa
13 C818
Japan
Ophiostoma nigrocarpum
47
100
CMW7132
CMW7611
1 ATCC14284
CMW7616
CMW7612
CMW7614
CMW7615
human
17
100
9
92
CMW7133
wood
2
CMW7618
12
1 CMW7617
soil
100
1 CMW7613
human
Sporothrix schenckii
41
C1882 Ophiostoma ulmi
43
C327 Ophiostoma ips
5 changes

Chapter 5

Ex Africa semper aliquid novi.

- Pliny the Elder, AD 23-79

Die onopsigtelike voëls wat tussen die takke kwetter en fluit sonder dat ek hulle kan waarneem en die skaars sigbare kruipende dierelewe tussen die wortels en klippe is vir my eweneens onbekend, en in 'n streek wat hom skynbaar skaars uit die skeppingsproses losgeworstel het, lyk dit trouens moontlik dat plante en diere onbenoem gebly het, wagtend op 'n naamgewer om hulle identiteit te gee.

- Karel Schoeman, uit *Verkenning*

A new *Ophiostoma* species from hardwoods in the Southern Hemisphere[1]

ABSTRACT

Ophiostoma pluriannulatum is a well-known agent of sapstain of especially hardwoods in the Northern Hemisphere, and of *Pinus radiata* in New Zealand. Albino strains of the fungus have also been tested as potential biocontrol agents against other sapstaining fungi, and for pitch removal in wood chips prior to pulping. In this study, isolates resembling *O. pluriannulatum*, but with some distinct characters, were collected from hardwood sources in South Africa, Equador and Indonesia. The aim was to confirm the identity of these isolates by comparing them with *O. pluriannulatum* isolates and the type material from the Northern Hemisphere. Morphological characters, rDNA sequences and thallism of isolates were used as criteria for comparisons. The Southern Hemisphere isolates have brown perithecial bases with longer necks and longer ascospores than the Northern Hemisphere isolates, which have black perithecial bases. The most conspicious difference between the two groups was the presence of club-shaped ornamental hyphae on the perithecial bases of Southern Hemisphere isolates. The Northern Hemisphere isolates were all heterothallic, while both heterothallic and homothallic isolates were present in the Southern Hemisphere group. ITS sequencing confirmed that there are sufficient genetic differences to distinguish between the groups. A new species, *Ophiostoma tropicale,* is, therefore, described.

[1] See Appendix 2 for more information about the distribution, host range, insect vectors and morphology of *Ophiostoma* spp. treated in this chapter.

INTRODUCTION

Ophiostoma pluriannulatum (Hedgcock) H. & P. Sydow was described in 1906 as *Ceratostomella pluriannulata* Hedgcock, one of the causal agents of sapstain on *Quercus borealis* in the USA (Hedgcock, 1906). In 1919 it was transferred to the genus *Ophiostoma* H. & P. Sydow (Sydow & Sydow, 1919), and in 1952, Moreau assigned it to the genus *Ceratocystis* Ell. & Halst. (Moreau, 1952). De Hoog and Scheffer (1984), however, suggested that all species of *Ceratocystis* sensu lato resistant to cycloheximide and with rhamnose in their cell walls, should reside in *Ophiostoma.* It is, therefore, generally accepted that the fungus resides in this genus (Seifert *et al.,* 1993).

Ophiostoma pluriannulatum is an economically important fungus causing sapstain of hardwoods such as *Fagus, Populus, Quercus, Ulmus* and *Liquidambar* (Hedgcock, 1933; Verrall, 1939; Campbell, 1960; Marmolejo & García-Ocañas, 1993). It is occasionally also found on softwood species such as *Abies*, *Picea* and *Pinus* (Mathiesen-Kåårik, 1960; Marmolejo & García-Ocañas, 1993; Farrell *et al.,* 1997). The fungus is widely distributed in North America (Davidson, 1935; Verrall, 1939; Hunt, 1956; Campbell, 1960; Griffin, 1968; Appel *et al.,* 1990; Marmolejo & García-Ocañas, 1993) and has been reported from Europe (Lagerberg *et al.*, 1927; Mathiesen-Kåårik, 1960; Griffin, 1968) and Japan (Otani, 1988). The only report of *O. pluriannulatum* from the Southern Hemisphere is from New Zealand, where it is considered one of the most important agents of sapstain on *Pinus radiata* (Farrell *et al.,* 1997).

Biological control is becoming increasingly important as an alternative to chemical control in the management of sapstain. Albino strains of *O. piliferum* (Fries) H. & P. Sydow have been registered and are used as biological control agents against other sapstain fungi in the Northern Hemisphere (Farrell *et al.*, 1993; Behrendt *et al.*, 1995). If applied to pulpwood, these non-staining strains out-compete staining fungi and remove pitch from the wood chips prior to pulping (Dorado *et al.,* 2000). It has been shown that the *O. piliferum* strains compete less effectively on hardwoods such as *Eucalyptus* (Gutiérrez *et al.,* 1999). Albino strains of other *Ophiostoma* spp., including *O. pluriannulatum,* are, therefore, being evaluated as alternative biocontrol agents for application on hardwoods in warmer climates. Most of these strains have originated from softwood hosts in temperate climates (Blanchette *et al.,* 1997; Kay, 1997).

In this study, a survey was conducted to collect and identify *Ophiostoma* spp. occurring on hardwoods in South Africa. Some of the isolates resembled *O. pluriannulatum.* Similar

isolates were also obtained from indigenous hardwoods from Equador and Indonesia. All these isolates were compared with Northern Hemisphere isolates and the type material of *O. pluriannulatum*, based on morphology and rDNA data.

MATERIALS & METHODS

Isolates

Ten isolates resembling *O. pluriannulatum* from the Southern Hemisphere were compared with six *O. pluriannulatum* isolates from the Northern Hemisphere (**Table 1**), using morphology and phylogeny. All isolates are maintained on 2 % Malt Extract Agar (MEA: 20 g/l Malt Extract; 20 g/l Agar; 1000 ml distilled water) in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. The type specimen and other herbarium material of *O. pluriannulatum* were obtained from the US National Fungus Collections, Beltsville, Maryland. The Dried specimens examined were: BPI596081, BPI596082, BPI596083 (Holotype), BPI596084, BPI596085, BPI596086, all from *Quercus borealis*, Indiana, USA.

Morphology

Isolates were induced to sporulate on water agar (WA: 20 g/l Agar; 1000 ml distilled water) amended with sterilised wood of the original host. Cultures were incubated at 25 °C and exposed to 12 hour cycles of darkness and near UV light. For each isolate, morphological characters of 25 perithecia and 10 ascospores were documented using a light microscope. For the anamorph state of each isolate, 25 conidia and 25 conidiophores were studied.

Growth of isolates in culture, was compared using a technique similar to that described by Marais and Wingfield (2001). For each isolate, 18 Petri-dishes with 20 ml 2 % MEA were inoculated with the test fungus. Three cultures of each isolate were incubated at temperatures ranging from 10 to 35 °C, at 5 °C intervals. Colony diameters were measured after 8 days and averages calculated.

Tests for thallism

Single spore cultures were prepared as described in **Chapter 4,** and the thallism of selected isolates (**Table 1**) was determined. Sixty to 65 single ascospore cultures, and 25 to 40 single conidium cultures, were prepared for each of the selected isolates. An isolate was treated as

homothallic when perithecia were produced in single ascospore and single conidium cultures obtained from that isolate. When neither single ascospore, nor single conidium cultures, gave rise to perithecia, single spore cultures, originating from the same isolate, were paired against each other in different combinations as described previously (**Chapter 4**). Where these crosses gave rise to perithecia, isolates were considered heterothallic.

DNA sequencing and sequence analysis

A part of the ribosomal DNA operon, including the internal transcribed spacer (ITS) region 1, the 5.8S gene, and the ITS region 2, was sequenced for all isolates (**Table 1**). DNA extraction, PCR reactions and sequencing were conducted following the same procedures and using the same primers as described in **Chapter 4**. The nucleotide sequences were manually aligned and the data set was analysed using PAUP (Phylogenetic Analysis Using Parsimony) 4.0b2a (Swofford, 1999). A maximum parsimony heuristic search, using simple stepwise addition and Tree Bisection and Reconstruction (TBR) as branch swopping algorithm (MULPARS on) was performed. Trees were rooted using sequence data obtained from GenBank for isolates of *O. ulmi* (Buism.) Nannf. and *O. piliferum* as outgroups (**Table 1**). One thousand bootstrap analyses were done to determine confidence intervals at the branching points.

RESULTS

Morphology

All the Southern Hemisphere isolates, and two of the six Northern Hemisphere isolates, produced perithecia in culture (**Table 1**). Several perithecia with ascospores were also present in the herbarium specimens examined. Perithecial bases of the Southern Hemisphere isolates were generally light brown in colour (**Figure 1**), whereas those from the Northern Hemisphere cultures, as well as the type material, were dark brown to black. Ornamental hyphae on the perithecial bases of the Southern Hemisphere isolates had a unique club-shaped morphology, and were significantly shorter than the hair-like ornamental hyphae present in *O. pluriannulatum* isolates (**Figure 1**). Perithecial neck lengths of Southern Hemisphere isolates varied between 220 and 5000 µm, while those of Northern Hemisphere isolates varied between 540 and 3100 µm. Annuli were present on the necks of more than 50% of perithecia in all cultures. Up to 10 annuli were present on the necks of some perithecia. A crown of

hyphae, similar to ostiolar hyphae, was commonly present on the annuli. These were present in all cultures and herbarium specimens. Ascospores of the Southern Hemisphere isolates varied between 5 and 6 μm in length, whereas those from the Northern Hemisphere isolates and herbarium material were between 4 and 5.5 μm long.

The diameter of perithecial bases, width of perithecial necks, ostiolar hyphae, anamorph morphology and colony morphology, fell within the same ranges for both groups of isolates. No anamorph structures were observed on the herbarium specimens, but the structures produced in cultures from this study (**Figure 1**) were similar to those in the description of Hedgcock (1906). Conspicuous denticles were present on the condiogenous cells of all isolates. The morphology and colour of the cultures varied, but were in accordance with previous descriptions (Hedgcock, 1906; Lagerberg *et al.*, 1927; Hunt, 1956; Upadhyay, 1981). The Southern Hemisphere isolates generally presented less white mycelium on the agar surface than *O. pluriannulatum* isolates.

Tests for thallism

Sixty-one of 65 single ascospore cultures from one of the South African isolates (CMW368) originating on a native host, produced perithecia. Of 25 single conidial cultures obtained from this isolate, 24 produced perithecia. For another South African isolate (CMW932) from an indigenous host, 59 of 60 single ascospore cultures produced perithecia, while none of the 40 single conidial isolates gave rise to sexual fruiting structures. The single conidium cultures derived from this isolate also did not produce perithecia when paired against each other. None of the single spore cultures from any of the other isolates, produced perithecia (**Table 1**).

DNA sequencing and sequence analysis

The amplified rDNA fragment were approximately 610 bp in size in all isolates considered in this study. After alignment, the total data set consisted of 624 characters, including gaps. Of these, 425 characters were constant. Of the variable characters, 170 were parsimony-uninformative and 47 parsimony informative. Two most parsimonious trees of 283 steps (CI = 0.979, HI = 0.021, RI = 0.947) were retained. Variation between the trees was within the main clades and not between them, thus, resulting in the same interpretation.

All isolates grouped into one clade (**Figure 2**), separate from the closely related species used as outgroups. Within this major clade, two smaller clades (A and B) were evident, and these were supported by bootstrap values of 66 and 97 % respectively. Clade A included only isolates from the Southern Hemisphere, and clade B was comprised of *O. pluriannulatum*

isolates from the Northern Hemisphere. More variation was evident amongst isolates in clade A than those in clade B.

TAXONOMY

THE FOLLOWING DESCRIPTION SHOULD BE SEEN AS A DRAFT FOR THE PURPOSES OF THIS THESIS AND SHOULD NOT BE CITED. THE COMPLETE DESCRIPTION WILL BE PUBLISHED ELSEWHERE.

Ophiostoma tropicale Z.W. De Beer, B.D. Wingf. & M.J. Wingf., **sp. nov.** **Figs 1A-1G**

Etym.: tropicale, derived from the Greek ΤΡΟΠΙΚΌς [tropeekos], referring to the origin of this fungus in tropical to subtropical environments.

Anamorph: *Sporothrix*

Perithecia produced on host tissue and on agar plates. *Perithecial bases* light brown to dark brown, globose, (65-)125(-167) µm in diameter, ornamented with subhyaline to dark brown, smooth, aseptate hyphae, up to 50 µm long, 3-5 µm wide, often with swollen, rounded ends. *Perithecial necks* dark brown to black, becoming light brown to the apex, cylindrical, tapered, (350-)1350(-5000) µm long, (12-)23(-37) µm wide at base, (7-)11(-23) µm wide at apex. *Ostiolar hyphae* divergent, (5-)23(-62) µm long; 0-10 annuli present at irregular intervals along neck, sometimes without hyphae, or with 1-20 hyphae similar to the ostiolar hyphae. *Asci* not observed. *Ascospores* hyaline, aseptate, allantoid to orange section shaped in side view, cylindrical with obtuse ends in face view, nearly globose in end view, (5-)5.5(-6) x (1-)1.5(-1.7) µm, lacking gelatinous sheath.

Conidiophores mononematous, hyaline, simple or loosely branched, <120 µm in length, (1.5-)3.5(-5) µm wide. *Conidiogenous cells* terminal or intercalary, denticulate, proliferating sympodially, (5-)6.1(-9) µm long, (1.5-)1.8(-2.3) µm wide. *Conidia* hyaline, aseptate, elongate ellipsoid, fusiform or cylindrical to clavate, (4.5-)7(18) x (1.5-)2.2(3) µm; ramoconidia cylindrical up to 33 x 4.5 µm.

Colonies with optimal growth at 25 °C on 2 % MEA, reaching 41 mm in 8 days. No growth below 5 °C and little growth above 35 °C, flocculose to floccose, with tufts of mycelium often adhering to the agar, white, reverse pale brown to grey to black. Superficial

hyphae hyaline, 1-4 μm wide; immersed hyphae mostly pigmented, turning dark with age, 2-8 μm wide.

SPECIMENS EXAMINED: HOLOTYPE: CMW4124[2] from *Shizolobium parahybum*, Ecuador, collected by M.J. Wingfield, 1997. PARATYPES: **South Africa:** CMW368 from *Ocotea bullata*, CMW932 from *Macaranga capensis*, CMW1207 from *Jacaranda mimosifolia*. **Indonesia:** CMW4026 from Indigenous hardwood. CULTURES: **South Africa:** CMW1251 from *Eucalyptus grandis*. **Indonesia:** CMW4028 from Indigenous hardwood. **Ecuador:** CMW4139 from *Shizolobium parahybum*, CMW7744 and CMW7745 from Indigenous hardwood.

DISCUSSION

Morphological and molecular data separates the Northern and Southern Hemisphere groups of isolates resembling *O. pluriannulatum*. The Northern Hemisphere group is represented by *O. pluriannulatum*, and a new species, *Ophiostoma tropicale*, is described to accommodate isolates from the Southern Hemisphere. This is, furthermore, the first conclusive report of the presence of both homo- and heterothallic strains within a single *Ophiostoma* species.

Morphological features of teleomorph structures of *O. tropicale* were in most respects similar to those of the isolates and herbarium specimens of *O. pluriannulatum*. The most typical characteristic of *O. pluriannulatum*, the presence of annuli on the perithecial necks (Hedgcock, 1906; Lagerberg *et al.*, 1927; Hunt, 1956; Upadhyay, 1981), was also present in *O. tropicale*. There were, however, significant differences between *O. pluriannulatum* and the Southern Hemisphere isolates. Typically, perithecial bases of *O. pluriannulatum* have been reported as black (Hedgcock, 1906; Hunt, 1956; Upadhyay, 1981), which was also true for the isolates from the Northern Hemisphere, included in this study. All the *O. tropicale* isolates, however, presented light brown to dark brown bases. It is interesting to note that Marmolejo and García-Ocañas (1993), in a first report of *O. pluriannulatum* from Mexico, described the perithecial bases as being black. In an accompanying diagram of the species, however, its perithecia were illustrated with light coloured bases, similar to those of the

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[2] Herbarium material currently bearing the CMW culture numbers, will be deposited in the National Collection of Fungi (PREM), PPRI, ICSR, Pretoria.

Southern Hemisphere isolates in our study. This is unlikely to have been a printing error, since the perithecial bases of other species, for example *O. ips* (Rumbold) Nannf., also described as black in their study, were drawn much darker.

The relatively short, club-shaped ornamental hyphae on the bases of isolates of *O. tropicale* were unusual. This distinguished them from hair-like ornamental hyphae observed on the bases of *O. pluriannulatum* isolates, which have been described in several reports (Lagerberg *et al.*, 1927; Hunt, 1956). The range of perithecial neck lengths of the Southern Hemisphere isolates, was much greater than that observed for the Northern Hemisphere isolates included in this study. Lagerberg *et al.* (1927), however, reported neck lengths of up to 4800 µm and Gregor (1932) of up to 5000 µm in the same fungus. This corresponds with the neck lengths in some of our Southern Hemisphere isolates. The range of ascospore lengths observed for *O. pluriannulatum*, corresponded well with the data from previous studies (Hedgcock, 1906; Lagerberg *et al.*, 1927; Hunt, 1956; Upadhyay, 1981). The average ascospore length presented by the *O. tropicale* isolates, were 1 µm longer than that of *O. pluriannulatum*.

Hedgcock (1906) noted that the anamorph of *O. pluriannulatum* resembled *Cephalosporium* auct. non Corda. Other authors assigned the asexual state of *O. pluriannulatum* to *Cladosporium* Link (Lagerberg *et al.*, 1927; Gregor, 1932; Hunt, 1956) and *Hyalodendron* Diddens (Upadhyay, 1981; Otani, 1988; Marmolejo & García-Ocañas, 1993). The morphology of the anamorphs of isolates in this study, however, fits well within the generic description of the genus *Sporothrix* Hektoen & Perkins *ex* Nicot and Mariat (De Hoog, 1974). We, therefore, treat the anamorph of *O. pluriannulatum* in this genus.

Gregor (1932) showed that *O. pluriannulatum* is heterothallic. Results of the present study confirmed this observation. All the Northern Hemisphere isolates, and all but two of the Southern Hemisphere isolates, were heterothallic. Heterothallism has been described for many *Ophiostoma* spp. closely related to *O. pluriannulatum*. These include *O. multiannulatum* (Hedgcock & Davidson) N. Fries (Davidson, 1940), *O. piliferum* (Mittman, 1932; Zimmerman *et al.*, 1995), *O. ulmi* (Buisman, 1932), *O. novo-ulmi* Brasier (Brasier, 1991), *O. himal-ulmi* Brasier & M.D. Mehrotra (Brasier & Mehrotra, 1995), *O. piceae* (Münch) H. & P. Sydow (Brasier & Kirk, 1993), *O. querci* (Georgèvitch) Nannf. (Brasier & Kirk, 1993), *O. setosum* Uzunovic, Seifert, S.H. Kim & C. Breuil (Uzunovic *et al.*, 2000) and *O. floccosum* Mathiesen (Harrington *et al.*, 2000). The South African isolate from *Ocotea* was, however, homothallic. Homothallism has also been reported for several species of *Ophiostoma*: *O. stenoceras* (Rumbold) Nannf. (Kåårik, 1960), *O. ips* (Leach, 1934),

O. galeiformis (Bakshi) Mathiesen-Kåårik (Bakshi, 1951), and *O. longirostellatum* (Bakshi) Arx. & E. Müller (Bakshi, 1951).

The presence of heterothallic and homothallic strains in a single species is not an uncommon phenomenon for Ascomycetes (Webster & Butler, 1967; Nauta & Hoekstra, 1992; Harrington *et al.*, 1993). Gregor (1932) suggested that homothallic and heterothallic forms of *O. pluriannulatum* might exist, although all the strains in her study were heterothallic. Her suggestion was based on the 'fact that neither Hedgcock (1906), nor Lagerberg *et al.* (1927), recorded heterothallism in the species, although these investigators worked with monospore cultures' (Gregor, 1932). Our results for *O. tropicale* might indicate that her conclusion for *O. pluriannulatum* was correct, although it was based on conjecture.

The South African isolate from *Macaranga* could not be classified as either heterothallic or homothallic. Similarly, Loos (1932) reported that single ascospore cultures derived from an isolate of *Ceratostomella fagi* Loos (= *O. querci*), gave rise to perithecia. Single conidium cultures of the same isolate, however, did not produce perithecia. *Ophiostoma querci* is generally regarded as heterothallic (Brasier & Kirk, 1993). Although improbable, these isolates might be pseudohomothallic (Turgeon *et al.*, 1993) or display secondary homothallism (Elliot, 1994). Studies to confirm that two nuclei of opposite mating types are present in the ascospores, would need to be conducted to confirm this. Crosses where male and female sterility is tested, might also clarify the apparent lack of fertility of the single conidium cultures.

Ophiostoma tropicale seemingly occupies the hardwood niche in the tropics and Southern Hemisphere that is occupied by the closely related *O. pluriannulatum* in the Northern Hemisphere. The host range of *O. tropicale* includes genera from plant families not regularly associated with species of *Ophiostoma.* These include genera such as *Ocotea* (Lauraceae), *Macaranga* (Euphorbiaceae), *Jacaranda* (Bignoniaceae), *Eucalyptus* (Myrtaceae) and *Schizolobium* (Leguminosae). The possibility that this fungus can be applied in future as the hardwood equivalent of a product like Cartapip (= *O. piliferum*), deserves further investigation.

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Table 1. Isolates and herbarium specimens used in this study.

Species	Culture no.	Other numbers	GenBank	Collector or Supplier	Substrate	Origin	Perithecia produced	Thallism[1]
O. pluriannulatum	CMW75	-	-	R.W. Davidson	-	USA	No	NT
	CMW108	C416	-	T. Hinds	-	USA	No	NT
	CMW2481	-	-	B.T. Grylls	*Picea* sp.	Canada	Yes	HE
	CMW2618	-	-	Z.W. de Beer	*Liquidambar styraciflua*	USA, Alabama	Yes	HE
	CMW7746	C691	-	T.C. Harrington	-	-	No	NT
	CMW7747	C931	-	T.C. Harrington	*Populus* sp.	USA	No	NT
O. tropicale	CMW368	-	-	M.J. Wingfield	*Ocotea bullata*	South Africa, Southern Cape	Yes	HO
	CMW932	-	-	M.J. Wingfield	*Macaranga capensis*	South Africa, KwaZulu-Natal	Yes	PS
	CMW1207	-	-	M.J. Wingfield	*Jacaranda mimosifolia*	South Africa, Mpumalanga	Yes	NT
	CMW1251	-	-	M.J. Wingfield	*Eucalyptus grandis*	South Africa, KwaZulu-Natal	Yes	NT
	CMW4026	-	-	Z.W. de Beer	Indigenous hardwood	Indonesia	Yes	HE
	CMW4028	-	-	Z.W. de Beer	Indigenous hardwood	Indonesia	Yes	HE
	CMW4124[2]	-	-	M.J. Wingfield	*Schizolobium parahybum*	Ecuador	Yes	HE
	CMW4139	-	-	M.J. Wingfield	*Schizolobium parahybum*	Ecuador	Yes	HE
	CMW7744	-	-	Z.W. de Beer	Indigenous hardwood	Ecuador	Yes	HE
	CMW7745	-	-	Z.W. de Beer	Indigenous hardwood	Ecuador	Yes	HE
O. piliferum	-	CBS129.32	AF221070	H. Diddens	*Pinus sylvestris*	-	-	-
O. ulmi	-	CBS102.63; C1882	AF198232	F.W. Holmes, H.M. Heybroek	*Ulmus hollandica*	Netherlands	-	-

[1] NT = Not Tested; HO = Homothallic; HE = Heterothallic; PS = Pseudohomothallic.

[2] Culture associated with the type material of *Ophiostoma tropicale*.

Figure 1. *Ophiostoma tropicale.* **A.** Ascospores (Bar = 10 μm). **B.** Annuli on perithecial necks (Bar = 50 μm). **C.** Light brown base of perithecium (Bar = 50 μm). **D.** Club-shaped ornamental hyphae on perithecial base (Bar = 10 μm). **E.** Divergent ostiolar hyphae (Bar = 10 μm). **F.** Conidia of *Sporothrix* anamorph (Bar = 10 μm). **G.** Denticulate conidiophore (Bar = 10 μm).

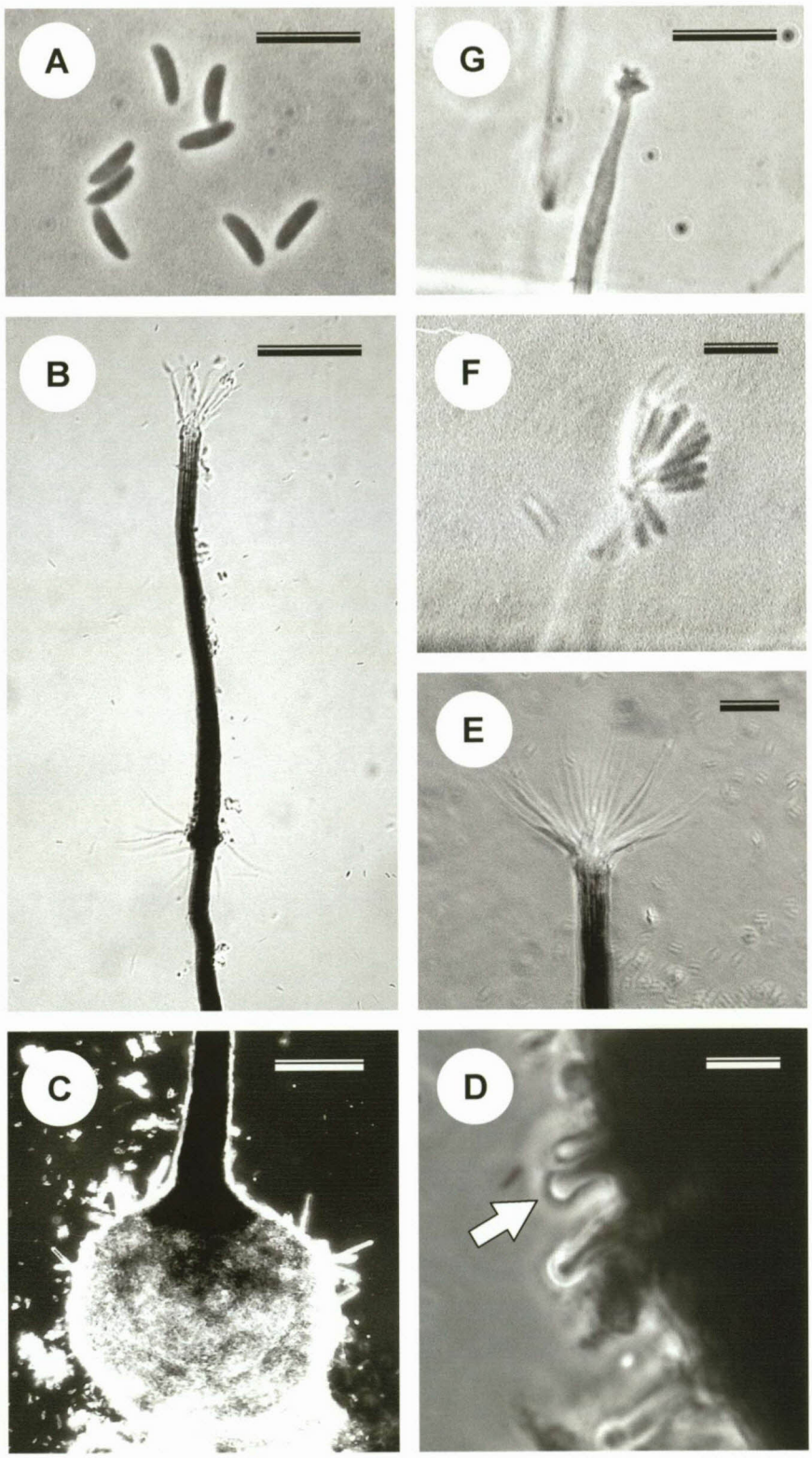
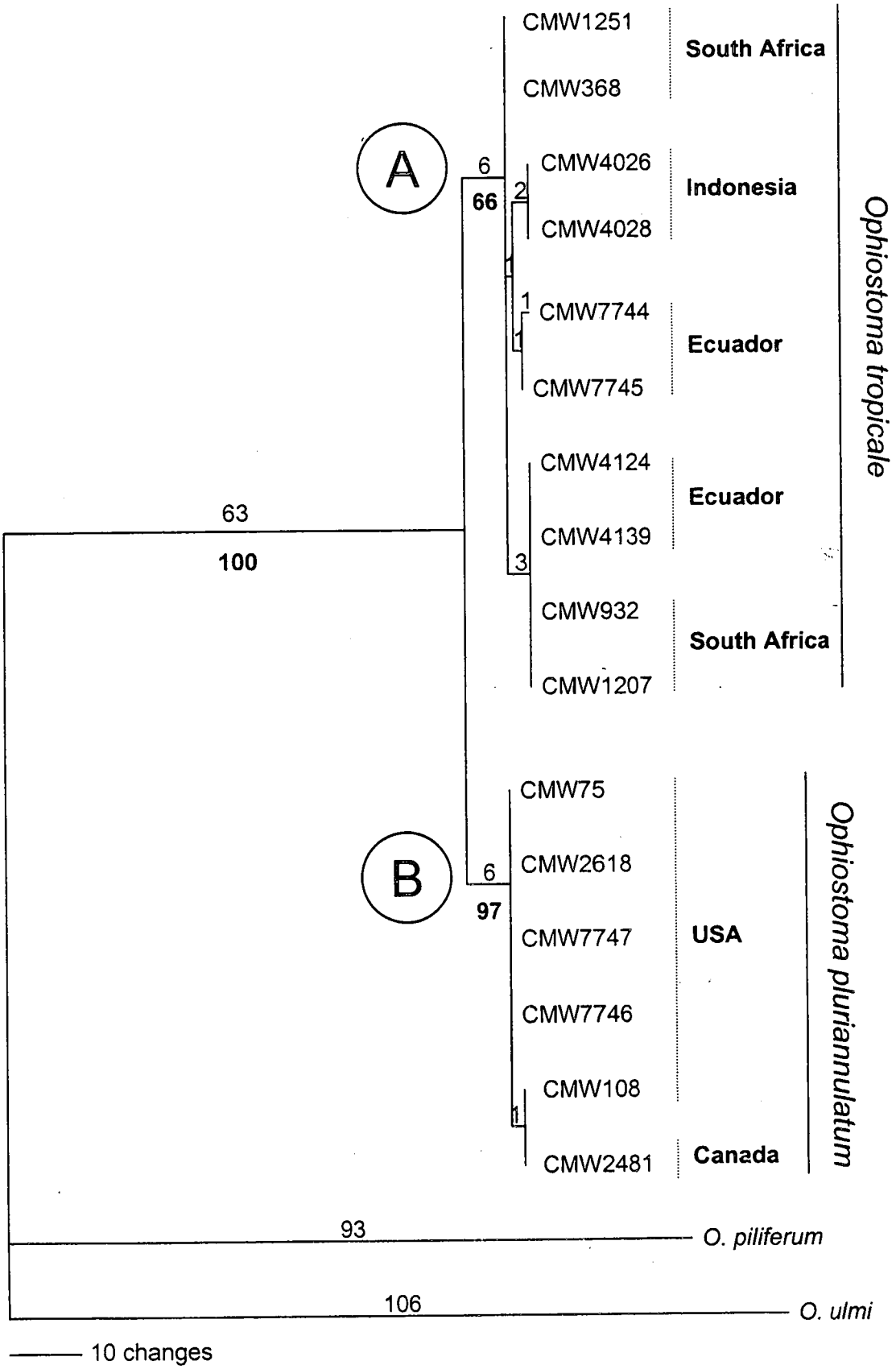

A
G
B
F
E
C
D

Figure 2. One of the most parsimonious trees obtained by Heuristic searches of the partial Ribosomal DNA operon (including partial Small Subunit, Internal Transcribed Spacer (ITS1) region, 5.8S gene, ITS2, and partial Large Subunit). Bootstrap values are given below, and branch lengths above the lines at branching points.


CMW1251
South Africa
CMW368
A
6
66
2
CMW4026
Indonesia
CMW4028
1
1
1
CMW7744
Ecuador
1
CMW7745
Ophiostoma tropicale
CMW4124
Ecuador
63
100
CMW4139
3
CMW932
South Africa
CMW1207
CMW75
B
6
97
CMW2618
CMW7747
USA
CMW7746
CMW108
1
CMW2481
Canada
Ophiostoma pluriannulatum
93
O. piliferum
106
O. ulmi
10 changes

Chapter 6

I have remonstrated [to T.H. Huxley] ... against the impression that he would leave, that sterility was a universal and infallible criterion of species.

- Charles Darwin to C. Lyell, 1860

The *Ophiostoma piceae* complex in South Africa: a phylogenetic study[1]

ABSTRACT

The *Ophiostoma piceae* species complex incorporates several economically important species, including serious tree pathogens and agents of bluestain. The species in the complex are morphologically similar, but can be distinguished from each other based on morphology, biology, mating type studies, and molecular data. At present, all the species in the complex are considered to be native to the Northern Hemisphere, most of them with a very wide distribution. Only a few sporadic reports of members of the complex are available from the Southern Hemisphere, where they are believed to have been introduced. These include countries like New Zealand, Australia, Chile, Brazil and Uruguay. The aim of this study was to confirm the identity of isolates resembling *O. piceae* originating from three Southern Hemisphere countries, using mating compatibility and rDNA sequencing. Our results show that *O. querci* is widely distributed throughout South Africa on both native and exotic hardwoods. *Ophiostoma querci* is also reported for the first time from Brazil, again from a native host. *Ophiostoma floccosum* is reported for the first time from South Africa, but from an exotic *Pinus* species. These results suggest that species of the *O. piceae* complex are common in the Southern Hemisphere, and that current views on the origins of especially *O. piceae* and *O. querci*, need to be reconsidered.

[1] See Appendix 3 for more information about the distribution, host range, insect vectors and morphology of *Ophiostoma* spp. treated in this chapter.

INTRODUCTION

The *Ophiostoma piceae* species complex comprises nine species of fungi (Harrington *et al.*, 2001). These include agents of serious tree diseases (Brasier, 1990; Hubbes, 1999) and degradation of timber (Seifert & Grylls, 1990; Schirp *et al.*, 1999). Some of the better known fungi in the complex are *O. ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier that caused the two Dutch Elm Disease pandemics that swept across Europe and North America during the past century (Brasier, 1990, 1991). Both *O. ulmi* and *O. novo-ulmi*, together with the less pathogenic *O. himal-ulmi* (Brasier & Mehrotra, 1995), are phylogenetically closely related to *O. querci* (Georgévitch) Nannf. (Harrington *et al.*, 2001). *Ophiostoma querci* and its suggested synonyms [*O. fagi* (Loos) Nannf., *O. roboris* Georgescu & Teodoru, *O. valachicum* Georgescu & Teodoru and *O. kubanicum* Sczerbin-Parfenenko] (Harrington *et al.*, 2001), have often been associated with Oak decline in central and eastern Europe (Anonymous, 1990). The remaining four species in the *O. piceae* complex [i.e. *O. piceae* (Münch) H. & P. Sydow, *O. canum* (Münch) H. & P. Sydow, *O. floccosum* Mathiesen and *O. setosum* Uzunovic] are not considered pathogens, and are primarily associated with bluestain of conifer timber (Seifert & Grylls, 1990; Schirp *et al.*, 1999).

The taxonomy of the *O. piceae* complex has been controversial, virtually since *O. piceae* was first described (Münch, 1907). Many of the species in the complex are morphologically almost indistinguishable from each other (Przybyl & De Hoog, 1989). The debates surrounding the generic concepts of *Ceratocystis* and *Ophiostoma,* have added to the taxonomic disorder (Samuels, 1993; Upadhyay, 1993). However, recent studies have resolved much of the confusion within the complex. Apart from studies distinguishing the Dutch Elm Disease fungi (Brasier, 1991; Jeng *et al.*, 1996; Brasier & Mehrotra, 1995), the taxonomic relationship between *O. piceae* and *O. querci* has also been intensively investigated, combining morphological (Morelet, 1992; Przybyl & Morelet, 1993; Halmschlager *et al.*, 1994) and biological characters (Brasier & Stephens, 1993; Przybyl & Morelet, 1993), mating compatibility studies (Brasier & Kirk, 1993), as well as molecular data (Halmschlager *et al.*, 1994; Pipe *et al.*, 1995; Kim *et al.*, 1999; Harrington *et al.*, 2001). These studies confirmed that the two species are phylogenetically and biologically distinct. *Ophiostoma piceae*, furthermore, occurs almost exclusively on coniferous hosts (Brasier & Kirk, 1993; Przybyl & Morelet, 1993; Halmschlager *et al.*, 1994; Kim *et al.*, 1999). *Ophiostoma querci,* although occasionally found on conifers, occurs primarily on hardwoods

(Brasier & Kirk, 1993; Przybyl & Morelet, 1993; Halmschlager *et al.*, 1994; Kim *et al.*, 1999).

Ophiostoma piceae, and to a lesser extent *O. querci*, have been reported often during the past century from Northern Hemisphere countries (Seifert & Grylls, 1990; Przybyl & Morelet, 1993; Halmschlager *et al.*, 1994). Together with the other species in the complex, they are considered native to the Northern Hemisphere (Harrington *et al.*, 2001). In addition to this, suggestions have been made that *O. querci* is native to Europe, and that it was introduced from there into North America and the Southern Hemisphere (Brasier & Kirk, 1993; Harrington *et al.*, 2001).

Only a few reports on species in the *O. piceae* complex are available from the Southern Hemisphere (**Table 1**). These include reports of *O. piceae* from hardwood hosts native to Chile (Butin & Aquilar, 1984; Billings, 1993), and from native species of the Podocarpaceae in New Zealand (Hutchison & Reid, 1988). Most of the other reports are from *Pinus* spp. introduced into the Southern Hemisphere (Butin & Peredo, 1986; Hutchison & Reid, 1988; Schirp *et al.*, 1999; Harrington *et al.*, 2001).

It is probable that in the past, *O. querci* was often reported as *O. piceae* (Harrington *et al.*, 2001), since *O querci* was treated as a synonym of *O. piceae* for more than 30 years (Hunt, 1956; Upadhyay, 1981; Przybyl & De Hoog, 1989). For many of the reports from both the Northern and Southern Hemispheres, no associated cultures or herbarium material available for study. This makes it difficult to confirm the accuracy of past identifications and to determine the origin of these fungi.

With the aid of the new diagnostic tools, it is now possible to study the distribution and reconsider views on the origins of *O. piceae* and *O. querci*. This aim of this study was, therefore, to confirm the identity of isolates resembling *O. piceae* collected in three Southern Hemisphere countries. This goal was achieved by employing mating compatibility of isolates and through analysis of rDNA sequence data.

MATERIALS & METHODS

Isolates

Nine isolates resembling *O. piceae* (referred to as *Ophiostoma* isolates) and five isolates resembling its *Pesotum* anamorph, were included in the study. Twelve of these isolates were

collected on different hosts from various locations in South Africa. One *Pesotum* isolate from Brazil, and one from New Zealand, were also included (**Table 2**).

Wood samples were initially incubated in Petri dishes with moist tissue paper at room temperature. After the appearance of perithecia or conidiophores, spore masses were transferred from these structures to 2% Biolab Malt Extract Agar (MEA), and the cultures were purified.

Two isolates identified as *O. piceae* from Japan, one from *Picea* (CMW2318) and one from *Betula* (CMW1564), were included to determine whether the *Betula* isolate did not perhaps represent *O. querci*, not previously found in Japan. For comparative purposes, 12 authentic isolates of *O. piceae* and *O. querci* from various parts of the world were obtained from other culture collections (**Table 3**).

All isolates used in this study are maintained on MEA slants at 4 °C in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Representative isolates have also been deposited in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands.

Mating compatibility studies

One single spore culture from each of the isolates was used for mating compatibility studies and DNA extraction. Where isolates produced perithecia, single ascospore cultures were made. Likewise, single conidium cultures were prepared for those cultures that produced only a *Pesotum* state.

For mating reactions, cultures were placed next to each other alongside a piece of wood (5 x 5 x 25 mm) from its original host, on Water Agar (WA: 30 g agar, 1000 ml distilled water). Cultures were incubated at room temperature until perithecia appeared. Any perithecia produced in a mating reaction, with viable ascospores, were scored as a positive (+). No perithecia, or perithecia without ascospores (protoperithecia), were scored as a negative (-).

Two tester strains of opposite mating types were selected from 40 single ascospore cultures prepared for one of the South African isolates (CMW2519) from *Eucalyptus*. The 40 cultures were paired against each other in different combinations. Some positive reactions produced more perithecia than others. It was assumed that these cultures were more fertile. From the 40 cultures tested, three of the more fertile cultures were selected for each mating type. These six cultures were then paired against each other in all possible combinations. From these

reactions, the most fertile A and B mating type cultures were selected as tester strains (CMW 2520 and CMW2521).

Two more sets of tester strains, representing the A and B mating types of *O. piceae* and *O. querci*, were obtained from T.C. Harrington (**Table 3**). These strains were originally developed from isolates described from the UK (Brasier & Kirk 1993). The six tester strains were paired against each other in all combinations, and with single spore cultures of the unidentified isolates from different hosts and geographical origins (**Table 4**). As a control, all isolates were paired against themselves. Mating types were designated to all cultures tested, based on the mating types of the tester strains from the UK.

DNA sequencing and sequence analysis

DNA extraction, PCR and sequencing reactions were all conducted following the same procedures and using the same primers as described in **Chapter 4**. The complete Internal Transcribed Spacer (ITS) regions, including the complete 5.8S gene, were sequenced. rDNA sequences of a further 12 isolates, representing *O. ips* (Rumbold) Nannf., *O. floccosum*, *O. himal-ulmi*, *O. novo-ulmi*, and *O. ulmi*, were obtained from GenBank (**Table 3**). The nucleotide sequences were manually aligned. The aligned DNA sequence data were analysed using PAUP (Phylogenetic Analysis Using Parsimony) 4.0b2a (Swofford, 1998). Uninformative characters were excluded and a heuristic search with Tree Bisection and Reconstruction (TBR) (MULPARS on) was conducted. Trees were rooted using *O. ips* as outgroup. One thousand bootstrap analyses were run to determine confidence intervals at the branching points.

RESULTS

Mating compatibility studies

The *O. piceae* A and B tester strains paired with each other, but not with any of the other tester strains (**Table 4**). The *O. querci* tester strains from the UK gave positive crosses with each other and with the South African tester strains from *Eucalyptus*. None of the control crosses gave rise to perithecia with viable ascospores.

All the unidentified *Ophiostoma* isolates from South Africa gave positive results when paired against the *O. querci* tester strains from the UK and South Africa (**Table 4**). Two of the *Pesotum* isolates from South Africa, as well as the *Pesotum* isolates from Brazil and New

Zealand, paired with the *O. querci* tester strains. One *Pesotum* isolate from South Africa (CMW7661), however, did not give any positive reactions. None of the South African, Brazilian or New Zealand isolates reacted positively with the *O. piceae* tester strains.

The *Picea* isolate from Japan (CMW2318) was the only isolate resulting in a positive cross with the *O. piceae* tester strains. The *Betula* isolate (CMW1564), however, gave positive results with the *O. querci* tester strains. When the two Japanese isolates was crossed against each other (data not shown), no perithecia were formed.

DNA sequencing and sequence analysis

PCR products were approximately 600 basepairs in size. This included the 3' end of the Small Subunit, ITS 1, the 5.8S gene, ITS 2 and the 5' end of the Large Subunit. Manual alignment resulted in a total of 606 unordered characters, including gaps. Of these, only the 66 parsimony informative characters were used in the analysis, while 467 constant and 73 uninformative characters were excluded. With *O. ips* as the outgroup taxon, four most parsimonious trees (CI = 0.860, HI = 0.140, RI = 0.941) of 193 steps were produced. Variation between the trees were within the main clades and not between them, thus, resulting in the same conclusion.

Three main clades (**Figure 1**) were evident in all the phylogenetic trees. The first clade, with bootstrap support of 100 %, represented the *O. querci* - *O. ulmi* group. All isolates in this clade, apart from four, originated from hardwood hosts. We refer to this as the hardwood clade. This clade was comprised of four sub-clades, the first of which included all authentic *O. querci* isolates. The Dutch Elm Disease fungi resided in three separate clades. The *O. ulmi* and *O. novo-ulmi* clades were closely related, while *O. himal-ulmi* was more closely related to *O. querci* than to the other two species.

The second major clade represented *O. piceae* and contained all the isolates of this species identified from previous studies (**Table 3**). The culture from Germany, linked to the type of *O. piceae,* was also included in this clade.

The third clade, representing *O. floccosum,* contained three isolates. These included the culture linked to the type specimen from Sweden, the unidentified *Pesotum* isolate from the USA, as well as a *Pesotum* isolate from South Africa. The hosts of all three isolates belong to the Pinaceae.

DISCUSSION

Results of this study have shown that members of the *O. piceae* species complex are widely distributed in South Africa and throughout the Southern Hemisphere on a variety of native and introduced trees. Mating compatibility studies confirmed the identity of ten *O. querci* isolates from South Africa, and one each from New Zealand, Brazil and Japan. These findings were verified with rDNA sequencing, as was the identity of an *O. floccosum* isolate from South Africa and one from the USA. *Ophiostoma querci* is reported for the first time from Brazil and Japan, and *O. floccosum* for the first time from South Africa.

The Ophiostoma querci *clade*

Northern Hemisphere isolates. Previous studies have shown that in the Northern Hemisphere, *O. querci* occurs preferentially on hardwoods such as *Quercus* and *Fagus* (Morelet, 1992; Brasier & Kirk, 1993; Przybyl & Morelet, 1993; Halmschlager *et al.*, 1994; Pipe *et al.*, 1995; Harrington *et al.*, 2001). In the *O. querci* clade, only the Canadian isolate originated from a conifer. All other Northern Hemisphere isolates were from hardwood hosts native to the countries where they were isolated. These included an isolate from Japan, previously identified as *O. piceae*.

Sequencing data and the mating compatibility studies confirmed that the Japanese isolate from *Betula* was *O. querci*. *Ophiostoma piceae* has been described from several other hardwood hosts in Japan, including *Acer*, *Prunus*, *Quercus*, *Magnolia* and *Kalopanax* (Nisikado & Yamauti, 1935). It is possible that these reports of *O. piceae* might represent *O. querci*.

Southern Hemisphere isolates. In our phylogenetic study, all the South African isolates from hardwoods grouped closely with *O. querci* isolates from Europe, Canada and Japan. This supports the results from the mating compatibility studies where the South African isolates all mated with the tester strains from *Quercus* in the United Kingdom.

The sequence of the one tester strain (CMW7651) from an unidentified *Quercus* sp. in the UK was identical to that of the South African isolate from *Q. robur* (CMW7656). This isolate grouped separately from the other South African *O. querci* isolates, which suggests a different origin. *Quercus robur* was introduced into South Africa from Europe by the early Dutch settlers in the 16th century. The South African wine industry has, furthermore, imported oak timber from Europe for centuries to produce wine barrels. It is, therefore, not surprising that

the South African *O. querci* isolate from oak comes from Stellenbosch, the centre of the wine producing area.

Two South African isolates in the *O. querci* clade originated in KwaZulu-Natal and Mpumalanga respectively. Both were isolated from plantation *Eucalyptus* spp., introduced from Australia. It is possible that this fungus is the same as that described with "*Graphium*" and "*Sporotrichum*" states, which was collected on *Eucalyptus* timber in South African gold mines during the early 1940's (Brown *et al.* 1947). This cannot be confirmed as material was never stored. The only other report of *O. querci* on *Eucalyptus* comes from Uruguay (Harrington *et al.,* 2001), where these trees are also exotic. *Ophiostoma piceae* has been reported from *Eucalyptus* in New Zealand (Hutchison & Reid, 1988), but this could have been *O. querci*. Interestingly, *O. querci* has not yet been reported from *Eucalyptus* or any other native hardwood in Australia.

A South African isolate from *Olinia ventosa,* native to montane forests in the Southern Cape coastal region, also formed part of the *O. querci* clade. This isolate mated with the *O. querci* tester strains, confirming its identity.

The *Pesotum* isolate from a native hardwood in Brazil appears to represent the only report of *O. querci* from that country. *Ophiostoma piceae*, however, has been reported from *Grevillea robusta* (Mendes *et al*, 1998), a proteaceous hardwood introduced into Brazil from Australia. This fungus might have been *O. querci*, and not *O. piceae*. *Grevillea robusta* is planted throughout South Africa as an ornamental and occurs in close proximity of many native *Protea* spp. Several ophiostomatoid fungi have been described from South African proteas in recent years (Marais & Wingfield, 2001). However, neither *O. querci*, nor any other species from the *O. piceae* complex, were reported from these native hardwoods in South Africa.

Our results confirmed the identity of two *O. querci* isolates from *Pinus* in New Zealand, as well as one from *Pinus* in South Africa. *Ophiostoma querci* has been reported in Australia from exotic pines (Harrington *et al.,* 2001), and from exotic and native conifers in New Zealand (Farrell *et al.,* 1998; Schirp *et al.,* 1999). *Ophiostoma piceae* has been reported on introduced and native conifers in New Zealand (Hutchison & Reid, 1988; Schirp *et al.*, 1999) and Chile (Butin & Peredo, 1986; Billings, 1993). Again, some of these reports might represent *O. querci.*

Our results and other recent studies have shown that *O. querci* occurs not only on hardwoods, but also on the sapwood of introduced and native conifers in the Southern Hemisphere. In North America and Europe *O. querci* is common on hardwoods but occurs

only occasionally on native conifers (Brasier & Kirk, 1993; Pipe *et al.*, 1995; Kim *et al.*, 1999; Harrington *et al.*, 2001). It has been suggested that the preponderance of a single mating type of *O. querci* on oak in North America might indicate that the fungus was introduced from Europe (Brasier & Kirk, 1993).

The origin of *O. querci* in the Southern Hemisphere remains uncertain. It is possible that *O. querci* is native to the Northern Hemisphere, and was introduced into the Southern Hemisphere (Brasier & Kirk, 1993; Harrington *et al.*, 2001). However, the widespread occurrence of *O. querci* on native hosts in the Southern Hemisphere suggests that the fungus might be native in some of these countries. One of the differences between *O. piceae* and *O. querci* is that isolates from *O. querci* grow at 32 °C, while isolates from *O. piceae* do not (Brasier & Stephens, 1993). This supports the idea that the fungus originated in a warmer climate. It is clear that, at this stage, all these scenarios are based on limited information. Much more research, including population studies, will be necessary to raise these theories above the level of conjecture and determine the true origin of *O. querci*.

The Dutch Elm Disease fungi

All three the Dutch Elm disease species have been reported from the Northern Hemisphere, with only *O. novo-ulmi* reported from New Zealand in the Southern Hemisphere (Ridley *et al.*, 2000). The possible origins of the three Dutch Elm Disease fungi have been studied intensively (Brasier, 1991, 2001; Brasier & Mehrotra, 1995). One hypothesis to explain the sudden outbreak of disease in eastern Europe in the 1940's (Brasier, 1990, 1991) is as follows: Occasional host crossovers by the insect vectors, or non-specific vectors such as diptera or mites, might introduce an oak fungus such as *O. querci* to elm (Brasier, 1990). Such an event could have resulted in *O. querci* evolving into the aggressive Eurasian race of *O. novo-ulmi,* which could have caused a sudden outbreak of disease (Brasier, 1990, 1991). Another hypothesis is that the pathogen could have resulted from a hybridisation event between *O. querci* and the resident non-aggressive *O. ulmi* (Brasier, 1990, 2001; Brasier & Stephens, 1993; Brasier & Mehrotra, 1995). A proper knowledge of the distribution and origin of other species in the complex, including *O. querci*, will, therefore, serve to elucidate the evolutionary processes involved in the creation of new diseases or disease outbreaks associated with these species.

The Ophiostoma piceae *and* O. floccosum *clades*

All the *O. piceae* isolates included in this study originated from conifers in the Northern Hemisphere. Included were the isolates from spruce in Japan, France, Austria and Poland. DNA sequences of the *O. piceae* isolates from these countries have not been published previously.

There are several reports of *O. piceae* from the Southern Hemisphere, but only two of these have been confirmed based on sequence data. Both were from *P. radiata*, in Chile (Harrington *et al.*, 2001) and New Zealand (Schirp *et al.*, 1999). Our data suggest that most other reports of *O. piceae* in the Southern Hemisphere probably represented *O. querci*.

In the phylogenetic study of Harrington *et al.* (2001), *O. canum*, *O. setosum* and *O. floccosum* were shown to be more closely related to *O. piceae* than to *O. querci* or the Dutch Elm Disease fungi. No *O. canum* or *O. setosum* isolates were included in our study since none of the unidentified isolates resembled these species. The *O. floccosum* culture from *Pinus* in South Africa, identified in this study, represents only the third report of the fungus from the Southern Hemisphere. Previous reports of the fungus were from *Pinus* in Australia and New Zealand (Schirp *et al.*, 1999; Harrington *et al.*, 2001). *Ophiostoma floccosum* was originally described from Sweden (Mathiesen, 1951) and has since also been reported from the UK, North America and Korea (Harrington *et al.*, 2001). The fungus appears to be restricted to conifer hosts (Mathiesen, 1951; Harrington *et al.*, 2001).

Ophiostoma piceae and *O. floccosum* have a limited distribution in the Southern Hemisphere, and have only been reported from conifers. In the Northern Hemisphere, both these species also occur primarily on conifers. A recent population study of *O. piceae* in Canada revealed a high level of genetic diversity within the Canadian populations, suggesting that the fungus can be considered indigenous to North America (Gagné *et al.*, 2001). Evidence generated in our and other recent studies suggest strongly that *O. piceae* and *O. floccosum* have been introduced into the Southern Hemisphere. The situation with *O. querci* is less certain and deserves further study of the genetic diversity of populations.

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Table 1. Species of the *Ophiostoma piceae* complex reported from the Southern Hemisphere.

Country	Species	Host			Reference(s)
		Family	Genus or species	[1]Native/ Introduced	
Australia	*O. floccosum*	Pinaceae	*Pinus* sp.	I	Harrington *et al.*, 2001
	O. querci	"	*Pinus radiata*	I	Harrington *et al.*, 2001
Brazil	*O. piceae*	Proteaceae	*Grevillea robusta*	I	[2]Mendes *et al.*, 1998
Chile	*O. piceae*	Nothofagaceae	*Nothofagus dombeyi*	N	[2]Butin & Aquilar, 1984; Billings, 1993
		"	*Nothofagus pumilio*	N	[2]Butin & Aquilar, 1984; Billings, 1993
		Monimiaceae	*Laurelia philippiana*	N	Billings, 1993
		"	*Laurelia sempervirems*	N	Billings, 1993
		Pinaceae	*Pinus radiata*	I	Billings, 1993; Butin & Peredo, 1986
		"	*Pinus ponderosa*	I	Butin & Peredo, 1986
		"	*Pinus elliottii*	I	Butin & Peredo, 1986
New Zealand	*O. floccosum*	Pinaceae	*Pinus radiata*	I	Schirp *et al.*, 1999
	O. novo-ulmi	Ulmaceae?	?	I	Ridley *et al.*, 2000
	O. piceae	Nothofagaceae	*Nothofagus fusca*	N	[2]Butcher, 1968
		"	*Nothofagus menziesii*	N	[2]Hutchison & Reid, 1988
		Myrtaceae	*Eucalyptus* sp.	I	[2]Hutchison & Reid, 1988
		Pinaceae	*Larix* sp.	I	[2]Hutchison & Reid, 1988
		"	*Pinus radiata*	I	[2]Hutchison & Reid, 1988; Schirp *et al.*, 1999
		"	*Pseudotsuga menziesii*	I	[2]Hutchison & Reid, 1988
		Podocarpaceae	*Dacrydium cupressinum*	N	[2]Hutchison & Reid, 1988
		"	*Podocarpus* sp.	N	[2]Hutchison & Reid, 1988
		"	*Prymnopitys spicata* [=*Podocarpus spicatus*]	N	[2]Hutchison & Reid, 1988
	O. querci	Pinaceae	*Pinus radiata*	I	Schirp *et al.*, 1999
		"	*Cupressocyparis macrocarpa*	N	Farrell *et al.*, 1998
	O. setosum	"	*Pinus radiata*	I	Harrington *et al.*, 2001
South Africa	*O. querci*	Oliniaceae	*Olinia* sp.	N	De Beer *et al.*, 1995
		Myrtaceae	*Eucalyptus grandis*	I	De Beer *et al.*, 1995
		Fagaceae	*Quercus robur*	I	De Beer *et al.*, 1995
Uruguay	*O. querci*	Myrtaceae	*Eucalyptus* sp.	I	Harrington *et al.*, 2001

[1] I = Host tree introduced in the specific country.
N= Host tree native to the specific country.
[2] *O. querci* treated as a synonym of *O. piceae*.

Table 2. Isolates of *Ophiostoma* spp. with *Pesotum* anamorphs from the Southern Hemisphere identified in this study.

Previous identification	Current identification	[1]Isolate	Collector	Host			Origin	rDNA sequence	Used in mating tests
				Genus or species	Family	[2]Native / Introduced			
Ophiostoma sp.	*O. querci*	[3]**CMW 2520**	Z.W. de Beer	*Eucalyptus* chips	Myrtaceae	I	South Africa, Kwazulu-Natal	Yes	Yes
		[3]**CMW 2521**	Z.W. de Beer	*Eucalyptus* chips	"	I	South Africa, Kwazulu-Natal	No	Yes
		CMW 7653	Z.W. de Beer	*Eucalyptus* chips	"	I	South Africa, Kwazulu-Natal	No	Yes
		CMW 7654	M.J. Wingfield	*Eucalyptus grandis*	"	I	South Africa, Kwazulu-Natal	No	Yes
		CMW 7655	M.J. Wingfield	*Quercus robur*	Fagaceae	I	South Africa, Western Cape	No	Yes
		CMW 7656	M.J. Wingfield	*Quercus robur*	"	I	South Africa, Western Cape	Yes	Yes
		CMW 7657	M.J. Wingfield	*Quercus robur*	"	I	South Africa, Western Cape	No	Yes
		CMW 7658	M.J. Wingfield	*Olinia ventosa*	Oliniaceae	N	South Africa, Southern Cape	Yes	Yes
		CMW 7659	M.J. Wingfield	*Olinia ventosa*	"	N	South Africa, Southern Cape	No	Yes
Pesotum sp.	*O. querci*	CMW 2534	G.H.J. Kemp	*Eucalyptus grandis*	Myrtaceae	I	South Africa, Mpumalanga	Yes	Yes
		CMW 3119	Z.W. de Beer	*Pinus* chips	Pinaceae	I	South Africa, Kwazulu-Natal	Yes	Yes
		CMW 2542	M.J. Wingfield	Indigenous hardwood	?	N	Brazil	Yes	Yes
		CMW 7660	Z.W. de Beer	*Pinus* chips	Pinaceae	I	New Zealand	Yes	Yes
	O. floccosum	CMW 7661	Z.W. de Beer	*Pinus elliottii*	"	I	South Africa, Kwazulu-Natal	Yes	Yes

[1] Isolate numbers of tester strains used in the mating compatibility studies are printed in bold type.

[2] I = Host tree introduced in the specific country.
N= Host tree native to the specific country.

[3] Two single ascospore cultures of opposite mating types, obtained from the same sexually reproducing isolate (CMW 2519).

Table 3. Isolates of selected species from the *Ophiostoma piceae* complex from the Northern Hemisphere used as reference material in this study.

Species	Isolate[1]	GenBank	Other numbers	Collector or Supplier	Host	Origin	References Sequencing[2]	References Other[3]
O. ips	-	[4]AF198244	C 327	T.C. Harrington	-	USA, New York	Harrington *et al.*, 2001	-
O. piceae	CMW 2318	-	YCC 066	Y. Yamaoka	*Picea jezoensis*	Japan	-	Yamaoka *et al.*, 1997
	CMW 2468	-	0.95	M. Morelet	*Picea abies*	France	-	-
	CMW 7644	-	W 5; HA 378	T. Kirisits & E. Halmschlager	*Picea abies*	Austria	Kim *et al.*, 1999	Halmschlager *et al.*, 1994
	CMW 7646	-	HMIPC 14445	T. Kowalski	*Picea abies*	Poland	-	Halmschlager *et al.*, 1994
	[5]CMW 7648	-	C 967; H 2181	D.B. Redfern & J.F. Webber	*Picea sitchensis*	UK, Scotland	Harrington *et al.*, 2001	Brasier & Kirk, 1993
	[5]CMW 7649	AF081130	C 968; H 2009	J.N. Gibbs	*Pinus sylvestris*	UK	Harrington *et al.*, 2001	Brasier & Kirk, 1993b
	-	AF081129	AU 100-1	S.H. Kim *et al.*	*Picea mariana*	Canada	Kim *et al.*, 1999	-
	-	[6]AF198226	C 1087; CBS 108.21	E. Münch	*Abies* or *Picea*	Germany	Harrington *et al.*, 2001	Münch, 1907
	-	AF198227	C 1246; CBS 102356	J. Worrall	*Pseudotsuga menziesii*	USA, New York	Harrington *et al.*, 2001	-
O. querci	[7]CMW 1564	-	JCM 6016	G. Okada	*Betula platyphylla*	Japan	-	-
	CMW 2463	-	0.96	M. Morelet	*Fagus sylvatica*	France	-	-
	CMW 7645	-	W 3; HA 367	T. Kirisits & E. Halmschlager	*Quercus robur*	Austria	-	Halmschlager *et al.*, 1994
	CMW 7647	-	HMIPC 15807	T. Kowalski	*Quercus robur*	Poland	Kim *et al.*, 1999	Halmschlager *et al.*, 1994
	[5]CMW 7650	AF198238	C 969; CBS 102352; H1042	P.T. Scard & J.F. Webber	*Quercus* sp.	UK, England	Harrington *et al.*, 2001	Brasier & Kirk, 1993
	[5]CMW 7651	AF198239	C 970; CBS 102353; H1039	P.T. Scard & J.F. Webber	*Quercus* sp.	UK, England	Harrington *et al.*, 2001	Brasier & Kirk, 1993
	CMW 7652	-	C 934-9	R.A. Blanchette	*Pinus radiata*	New Zealand	Harrington *et al.*, 2001	-
	-	AF081132	AU 160-9	S.H. Kim *et al.*	*Tsuga* sp.	Canada	Kim *et al.*, 1999	-
	-	[8]AF198237	C 1085; CBS 236.32	W. Loos	*Fagus sylvatica*	Germany	Harrington *et al.*, 2001	Loos, 1932

(Continued)

Table 3. Continued.

Species	Isolate[1]	GenBank	Other numbers	Collector or Supplier	Substrate	Origin	References Sequencing[2]	References Other[3]
O. floccosum	-	[9]AF198231	C 1086; CBS 799.73	A. Kåårik	*Picea* or *Pinus*	Sweden	Harrington *et al.*, 2001	Mathiesen, 1951
	[10]CMW 1731	-	8806130	C. Bertagnole	*Pinus ponderosa*	USA, Idaho	-	-
O. himal-ulmi	-	AF198233	C 1183; CBS 374.67	H.M. Heybroek	*Ulmus wallichiana*	India	Harrington *et al.*, 2001	-
	-	AF 198234	C 1306; HP27	C.M. Brasier	*Ulmus wallichiana*	India	Harrington *et al.*, 2001	Brasier & Mehrotra, 1995
O. novo-ulmi	-	AF198236	C 510	T.C. Harrington	*Ulmus* sp.	USA, Iowa	Harrington *et al.*, 2001	-
	-	AF198235	C 1185; CBS 298.87	H.M. Heybroek	*Ulmus* sp.	Russia	Harrington *et al.*, 2001	-
O. ulmi	-	AF198232	C 1182; CBS 102.63	F.W. Holmes & H.M. Heybroek	*Ulmus hollandica*	Netherlands	Harrington *et al.*, 2001	-

[1] CMW isolates used in this study are maintained in the Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Where CMW numbers are absent in column 2, DNA sequences were obtained from GenBank.
[2] References to papers where rDNA from these particular isolates were sequenced.
[3] References to papers where these particular isolates were used in morphological and/or mating compatibility studies.
[4] *Ophiostoma ips* does not form part of the *O. piceae* complex, but was included as outgroup taxon in the phylogenetic analysis.
[5] Isolate numbers of tester strains used in the mating compatibility studies are printed in bold type.
[6] Culture representing the type of *O. piceae* (Münch, 1907).
[7] Culture previously identified as *O. piceae*.
[8] Culture representing the type of *O. fagi*, which is now considered a synonym of *O. querci* (Harrington *et al.*, 2001).
[9] Culture representing the type of *O. floccosum* (Mathiesen, 1951).
[10] Culture deposited in our collection as a *Graphium* (= *Pesotum*) species.

Table 4. Results of mating compatibility studies between tester strains of *Ophiostoma piceae* and *O. querci* from the UK and unidentified *Ophiostoma* and *Pesotum* isolates from the Southern Hemisphere.

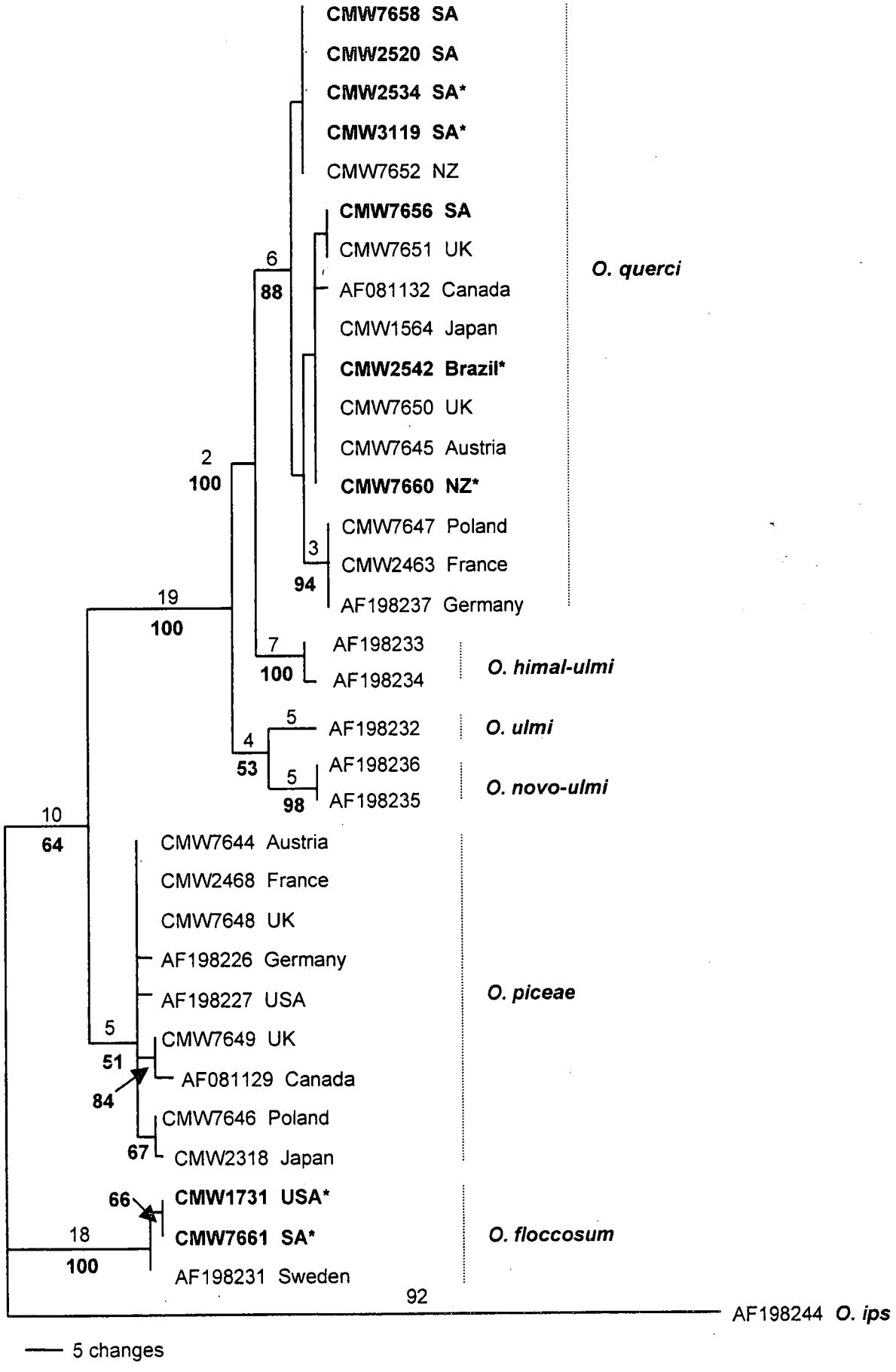
			O. piceae		***O. querci***		***Ophiostoma***									***Pesotum***					***O. piceae***	
			UK		**UK**		**South Africa**									**South Africa**			**NZ**	**Brazil**	**Japan**	
			CMW 7648	CMW 7649	CMW 7651	CMW 7650	CMW 2520	CMW 2521	CMW 7653	CMW 7654	CMW 7655	CMW 7656	CMW 7657	CMW 7658	CMW 7659	CMW 2534	CMW 3119	CMW 7661	CMW 7660	CMW 2542	CMW 2318	CMW 1564
	[1]Mating type↓→		A	B	A	B	a	b	a	a	b	b	a	b	a	b	a	-	b	a	b	a
O. piceae	CMW 7648	A	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	CMW 7649	B		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O. querci	CMW 7651	A			-	+	-	+	-	-	+	+	-	+	-	+	-	-	+	-	-	-
	CMW 7650	B				-	+	-	+	+	-	-	+	-	+	-	+	-	-	+	-	+
SA	CMW 2520	a					-	+	-	-	+	+	-	+	-	+	-	-	+	-	-	-
	CMW 2521	b						-	+	+	-	-	+	-	+	-	+	-	-	+	-	+

[1] Mating types of tester strains were designated as A and B (Harrington *et al.*, 2001; Brasier & Kirk, 1993). Based on compatibility with these, mating types (a or b) were assigned to the other isolates.

+ Positive reaction: mating of two strains resulted in the formation of perithecia with viable ascospores.

- Negative reaction: no perithecia produced, or perithecia without viable ascospores.

Figure 1. One of the four most parsimonious trees obtained by Heuristic searches of the partial Ribosomal DNA operon (including partial Small Subunit, Internal Transcribed Spacer (ITS1) region, 5.8S gene, ITS2, and partial Large Subunit). Bootstrap values are given below, and branch lengths above the lines at branching points. Isolate numbers in bold type were identified in this study. Isolates producing only the *Pesotum* anamorph in culture are indicated with *.


CMW7658 SA
CMW2520 SA
CMW2534 SA*
CMW3119 SA*
CMW7652 NZ
CMW7656 SA
CMW7651 UK
AF081132 Canada
CMW1564 Japan
CMW2542 Brazil*
CMW7650 UK
CMW7645 Austria
CMW7660 NZ*
CMW7647 Poland
CMW2463 France
AF198237 Germany
O. querci
6
88
2
100
3
94
19
100
7
100
AF198233
AF198234
O. himal-ulmi
5
AF198232
O. ulmi
4
53
5
98
AF198236
AF198235
O. novo-ulmi
10
64
CMW7644 Austria
CMW2468 France
CMW7648 UK
AF198226 Germany
AF198227 USA
CMW7649 UK
AF081129 Canada
CMW7646 Poland
CMW2318 Japan
O. piceae
5
51
84
67
66
CMW1731 USA*
CMW7661 SA*
AF198231 Sweden
O. floccosum
18
100
92
AF198244 O. ips
5 changes

Chapter 7

Tommorrow you will receive an optice about some small corrections which I made during the performance of the symphonies. When I gave these works to you, I had not heard either of them performed - and one should not want to be so like a god as not to have to correct something here and there in one's created works.

- Ludwig von Beethoven in a letter to Breitkopf and Härtel, 4 March 1809.

Ophiostoma quercus or *Ophiostoma querci*?[1]

ABSTRACT

Ophiostoma querci is a well-known sapstaining fungus occurring world-wide on hardwoods. Over the past decade confusion persisted in the literature as to the correct spelling of the specific epithet. Some authors referred to the fungus as *O. querci,* while others preferred to use the name *O. quercus.* The aim of this paper was to clarify the uncertainty. The taxonomic history of the species, the guidelines for species names as presented in the International Code of Botanical Nomenclature, and grammatical rules of classical Latin, were considered. We conclude that correct name for the species is *Ophiostoma querci.*

Ophiostoma querci (Georgévitch) Nannfeldt is a sapstaining fungus occurring on various hardwoods. The fungus has often been associated with Oak Decline in central and eastern Europe (Anonymous, 1990), and is closely related to the Dutch Elm Disease pathogens *O. ulmi* and *O. novo-ulmi* (Harrington *et al.,* 2001). *Ophiostoma querci* has been reported from many Northern Hemisphere countries, and more recently also from Southern Hemisphere countries such as South Africa (De Beer *et al.,* 1995), Australia, New Zealand and Uruguay (Harrington *et al.,* 2001). In recent literature some authors referred to the fungus as *O. querci*, while others used the name *O. quercus*. The aim of this paper is to clarify the uncertainty surrounding the spelling of the specific epithet of the species name of this Ascomycete. To achieve this, it is necessary to briefly review the taxonomic history of the species. We also consider requirements and recommendations pertaining to species names in the International Code of Botanical Nomenclature.

[1] See Appendix 3 for more information about the distribution, host range, insect vectors and morphology of *Ophiostoma* spp. treated in this chapter.

Ophiostoma querci was first described in 1926 as *Ceratostomella Querci* Georgévitch[2] (Georgévitch, 1926). The following year, the same author changed the name to *Ceratostomella quercus* Georgevitch (Georgevitch, 1927). Melin and Nannfeldt (1934) transferred the species to the genus *Ophiostoma* H. & P. Sydow, and the new combination was referred to as *Ophiostoma quercus* (Georgév.) Nannf. Moreau (1952) placed the species in yet another genus, and named it *Ceratocystis querci* (Georgevitch) Moreau. Hunt (1956), without reference to Moreau's paper, synonymized what he called *Ceratostomella querci* Georgew. with *Ceratocystis piceae* (Münch) Bakshi.

In 1989, Przybyl & de Hoog referred *C. piceae,* including *Cer. querci* as synonym, back to the genus *Ophiostoma*, following de Hoog and Scheffer's distinction between *Ophiostoma* and *Ceratocystis* (De Hoog & Scheffer, 1984). In the same year, Brasier and Kirk (1989) suggested that the hardwood and conifer forms of *O. piceae* may be distinct species. For the previous 33 years, the hardwood form was treated by most authors as a synonym of *O. piceae* (Hunt, 1956; Griffin, 1968; Olchowecki & Reid, 1973; Upadhyay, 1981; Hutchison & Reid, 1988; Przybyl & de Hoog, 1989). In 1990, it was suggested that 'the name *O. querci* may need to be re-established for the hardwood taxon' (Brasier & Webber, 1990). This was formally done in 1992 by Morelet, who referred to it as *Ophiostoma querci* (Georgévitch) Nannfeldt. The separation of the two species was confirmed by several studies (Brasier & Kirk, 1993; Brasier & Stephens, 1993; Przybyl & Morelet, 1993; Delatour *et al.,* 1994; Halmschlager *et al.,* 1994; Pipe *et al.,* 1995; Kim *et al.,* 1999; Harrington *et al.,* 2001). However, confusion persisted in these and other publications about the correct name for the hardwood species. Most authors followed the suggestions of Brasier and Webber (1990) and Morelet (1992), referring to the hardwood species as *O. querci* (Brasier & Kirk, 1993; Brasier & Stephens, 1993; Przybyl & Morelet, 1993; Delatour *et al.,* 1994; Farrell *et al.,* 1997, 1998; Okada *et al.,* 1998; Harrington *et al.,* 2001). Some authors, however, chose to use *O. quercus* (Georg.) Nannf. (Halmschlager *et al.,* 1994; Pipe *et al.,* 1995; Kim *et al.,* 1999; Schirp *et al.,* 1999; Xiao *et al.,* 1999; Brasier, 2001; Gagné *et al.,* 2001).

In only two papers, the choice of the name is motivated. The first was Halmschlager *et al.* (1994), who pointed out that '*Ophiostoma quercus* (Georgév.) Nannf. is the name Nannfeldt used in the original paper (Melin & Nannfeldt, 1934), when he placed *Ceratostomella quercus* in the genus *Ophiostoma.*' Furthermore, they stated that 'the name *O. querci* is not correct

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[2] The spelling of generic names, species epithets, and authorities are cited exactly as they were printed in the original publications, which might give the impression of inconsistency.

because in Latin the genitive of *Quercus* is *Quercus* ("U"-declension).' Without referring to the paper of Halmschlager *et al.* (1994), Pipe *et al.* (1995) came to the same conclusion, stating that 'the genitive case of the Latin noun *quercus* (= oak), which is in the fourth declension, is *quercus*, not *querci*.' However, according to Lewis and Short (1879) *Quercus* / *-us* is the usual form, but the alternative genitive form *querci,* is known from reputable sources, although much more rarely. Both *querci* and *quercus* may thus be used in the genitive case.

The basionym for the species is *Ceratostomella Querci* (Georgévitch, 1926). It is not certain why Georgévitch changed the epithet from *Querci* to *quercus* a year after the initial description (Georgévitch, 1927). If it was for grammatical reasons, it is now clear that this was not necessary. The new combination proposed by Melin and Nannfeldt (1934), *Ophiostoma quercus*, was gramatically correct. However, in accordance with Article 49 of the International Code of Botanical Nomenclature (Greuter *et al.*, 2000), Melin and Nannfeldt should have used the epithet of the basionym, which was *Querci*, with a capital *Q*. According to the Code, the new combination had an incorrect Latin termination, but it was validly published. Article 32.6 of the Code allows for correction of such an error, 'without change of the author's name or date of publication.' Although the use of the capital initial letter for an epithet derived from a generic name (in this case *Querci* derived from *Quercus*) is allowed by the Code, the Code recommends that 'all specific and infraspecific epithets should be written with a small initial letter' (Recommendation 60F). In conclusion, we suggest that the original epithet, *querci*, should be maintained, but without the capital *Q*, and that the species name should, therefore, be referred to as follows:

Ophiostoma querci (Georgévitch) Nannfeldt, Svenska Skogsvårdsföringens Tidskrift 3-4: 397-616.

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Appendix 1

We must never forget that a good classification incorporates and reflects everything we know about the organisms.

- Bryce Kendrick, 1993

Ophiostoma stenoceras and related species: a tabulated review

CONTENTS

NOTE: References are listed according to number, starting on page 204.

Table 1.1. Currently accepted synonyms of *Ophiostoma stenoceras.*

Species name	Reference[1]
Ophiostoma stenoceras (Robak) Nannf., in Melin & Nannfeldt, Svenska SkogsvFör. Tidskr. 32: 408. 1932.[2]	
≡ *Ceratostomella stenoceras* Robak, Nyt Mag. Naturvid. 71: 207. 1932.	
≡ *Ceratocystis stenoceras* (Robak) C. Moreau, Revue Mycol. 17: 22. 1952.[3]	
= *Ophiostoma albidum* Mathiesen-Kaärik, Meddr. St. SkogsvFör. Inst. 43: 50. 1953.	21
≡ *Ceratocystis albida* (Mathiesen-Kaärik) Hunt, Lloydia 19: 48. 1956.	
= *Ceratocystis gossypina* Davids., Mycologia 63:12. 1971.	88
≡ *Ophiostoma gossypinum* (Davids.) J. Taylor, Mycopath. Mycol. Appl. 38:112. 1976.	(88)[4]
= *Ceratocystis gossypina* Davids. var. *robusta* Davids., Mycologia 63:13. 1971.	(88)
= *Ceratocystis eucastanea* Davids., Mycologia 70:856. 1978.	88
=? *Ophiostoma valachicum* Georgescu & Teodoru, Anal. Inst. Cerc. Exp. For., Ser. 1, 11:198. 1948.[5]	50
≡ *Ceratocystis valachicum* (Georgescu & Teodoru) Potlajczuk, Novosti Sist. niz. Rast. 22:155. 1985.	1103

1 Reference suggesting synonymy or new combination.

2 Elias Melin and J.A. Nannfeldt were the authors of the paper in which *Ceratostomella stenoceras* was placed in the genus *Ophiostoma*. Melin collected the isolates, and Nannfeldt was responsible for the taxonomy. In most subsequent papers, the species name was cited as *O. stenoceras* (Robak) Melin & Nannf. (88;97;28;21;71;361). On page 408 of the original paper, however, only Nannfeldt was given as authority of the new combination. According to Article 46.2 of the Code of Botanical Nomenclature, the correct name and authority for the species is as cited in the table above.

3 Some confusion was created when Mathiesen-Kaärik referred to the species as *Ceratocystis stenoceras* (Robak) Hunt (60;902), instead of *C. stenoceras* (Robak) C. Moreau. Hunt, however, considered it an imperfectly known species, apparently as a result of a typographical error in Robak's Latin diagnosis (see footnote 1 of **Table 1.5**), and referred to it as *Ceratostomella stenoceras* Robak (45).

4 Parenthesis indicate that the original reference suggesting synonymy was not available for this study. The reference listed here is the one from which the information was obtained.

5 Upadhyay (88) considered *O. valachicum* a doubtful species since he could not obtain material for his study. Kowalski & Butin (50) suggested that it is closely related to *O. stenoceras*. Przybyl and De Hoog (72), however, considered it a 'likely' synonym of *O. piceae*, based on ascospore shape and size, although they could not obtain material. Seifert *et al.* (97), also listed it as a synonym of *O. piceae*.

Table 1.2. Distribution of *Ophiostoma stenoceras* and its synonyms.

Origin	Reference(s)
Canada	49
Canada, British Columbia	474
Canada, Manitoba	69[1]
Canada, New Brunswick	77[1]
Canada, Nova Scotia	77[1]
Canada, Ontario	35
Canada, Quebec	35;77[1]
Corsica	156
Cosmopolitain	28
France	118;156
Germany	163
Guatemala	874;156
Japan	49[1];671;902[1];1048
Mexico	156
Netherlands	899
New Zealand	814;1126
Norway	49;179
Poland	50;259;362
Sweden	49;59[1];60;179;288;407[1+];1059
USA	49[1+]
USA, Arizona	18[2]
USA, California	18[2]
USA, Colorado	18[2]
USA, Kentucky	74[4]
USA, New Hampshire	18[2]
USA, New Jersey	15
USA, New Mexico	18[2,3];88
USA, New York	15;18[2]
USA, North Carolina	19[4];88
USA, Oregon	18[2]
USA, Virginia	15;19[4]
USA, Washington	18[2]

1 Reported as *O. albidum.*
2 Reported as *O. gossypinum.*
3 Reported as *Ceratocystis gossypina* var. *robusta.*
4 Reported as *C. eucastaneae.*
\+ *O. stenoceras* reported as a separate species together with the other species.

Table 1.3. Hosts of *Ophiostoma stenoceras* and its synonyms.

Host	Reference(s)
Abies spp.	28
Abies alba	118
Abies balsamea	77[1]
Abies sachalinensis	902[1]
Apodemus sp. (wild mammal, tail)	156
Betula spp.	28
Betula populifolia	15
Castanea spp.	28
Castanea dentata	19[4];88
Clethrionomus glareolus	156
Cricetus cricetus	156;899
Erica gracilis (in greenhouse)	899
Eucalyptus sp.	156
Fagus crenata	671;902;1048;1049
Human (hair)	21
Picea spp.	28;49;59[1];60[1+];77[1];407[1+]
Picea abies [= *Picea excelsa*]	902[1];1124[4]
Picea glehnii	902[1]
Picea jezoensis	902[1]
Picea pungens	18[2]
Pinus spp.	28;49;59[1];60[1+];77[1];407[1+]
Pinus contorta var. *latifolia*	18[2];474
Pinus flexilis	18[2]
Pinus monticola	18[2]
Pinus ponderosa	18[2,3];188
Pinus pungens	18[2]
Pinus strobus	18[2]
Pinus sylvestris	901[1];1124[4]
Quercus spp.	15;28;50;163;1124[4]
Quercus robur	259;362
Sea water	899
Soil	899
Sorex araneus (wild mammal, tail)	156
Ulmus americana	69[1]
White water	288
Wood pulp	179;1059

[1] Reported as *O. albidum*.
[3] Reported as *Ceratocystis gossypina* var. *robusta*.
[2] Reported as *O. gossypinum*.
[4] Reported as *C. eucastaneae*.
[+] *O. stenoceras* reported as a separate species together with the other species.

Table 1.4. Insects associated with *Ophiostoma stenoceras* and its synonyms.

Insect	Reference(s)
Acanthocinus aedilis	59;407
Bark beetles	1048
Crypturgus cinereus	1124[4]
Crypturgus pusillus	1124[4]
Dendroctonus spp.	18[2]
Dendroctonus adjunctus	71;240[3]
Dendroctonus convexifrons	18[3]
Dendroctonus ponderosae	474
Dryocoetus autographus	1124[4]
Hylurgops palliatus	1124[4]
Ips spp.	18[2]
Ips typographus	59;1124[4]
Other bark insects	18[2]
Pissodes pini	59[1]; 407 [1+]
Pityogenes chalcographus	1124[4]
Rhagium inquisitor	59[1]
Scolytus intricatus	1124[4]
Tetropium sp.	59[1]
Xyleborus monographus	1124[4]

1 Reported as *O. albidum.*
2 Reported as *O. gossypinum.*
3 Reported as *Ceratocystis gossypina* var. *robusta.*
4 Referred to as the *O. stenoceras* group, which might, according to the drawings of the ascospores include species like *O. piliferum, O. pluriannulatum*, or others.
\+ *O. stenoceras* reported as a separate species together with the other species.

Table 1.5. Morphology of the teleomorph of *Ophiostoma stenoceras*.

			Robak 1932 (179)	Davidson 1942 (15)	Aoshima 1965 (902)	Mariat & Diez 1974 (874)	Nishimura *et al.* 1976 (261)	Upadhyay 1981 (88)	Kowalski & Butin 1989 (50)
Species			*Cer. stenoceras*	*Cer. stenoceras*	*C. stenoceras*	*O. stenoceras*	*C. stenoceras*	*C. stenoceras*	*C. stenoceras*
Perithecia:	**Base**	**colour**	-	black	black	black	-	black	black
		diameter	132-248 µ	95-140 µ	70-110 µ	120-200 µ	96-231 µ	(65-)80-180(-200) µ	90-160 µ
		ornamentation	covered with hairs, 2 µ wide, < 125 µ long	-	-	dark pointed hairs 2-4 µ wide, < 110 µ long,	covered with hyphae	brown, septate, smooth, hyphal hairs, 1.5-3.5 µ wide	olive brown 2-3 µ wide < 240 µ long
	Neck	**length**	420-1500 µ[1]	370-650 µ	250-700 µ	270-700(-1500) µ	870 µ	400-1400(-2200)µ	600-1400(1800) µ
		width: base	20 µ	25 µ	18-40 µ	17-30 µ	38-54 µ	25-55 µ	25-45 µ
		width: tip	8-12 µ	13 µ	10-25 µ		10-15 µ	10-15 µ	9-15 µ
		ostiolar hyphae	hyaline, unicellular, vary in number, 20-48(-62) x 2.6-2.7 µ	hyaline, slender, flexuous, 30-40 x 1.2 µ	4-10 hyaline, 10-20 µ long, 0.7-2.5 µ at base, 0.5-2 µ at tip	10-25 straight, ostiolar hairs, 20-45(-60) µ	hyaline	numerous, hyaline, septate, divergent, 18-35(-55) µ long, 1.5-2.5 µ wide, tapering	20-25 in number 25-45 x 2.5 µ
Ascospores:	**Colour**		-	hyaline	hyaline	hyaline	-	hyaline	hyaline
	Septation		-	-	-	-	-	1-celled	1-celled
	Shape:	**face view**	-	-	-	-	-	elliptical	elliptical
	Shape:	**side view**	orange section	-	kidney-shaped	orange section	orange section	orange section	orange section
	Shape:	**end view**	-	-	-	-	-	globose	-
	Length		2-2.9 µ	4-4.8 µ	3.5-5.5 µ	2.7(-3.3) µ	2 µ	(2-)2.5-4.5 µ	3.1-3.7(-4) µ
	Width		1.3-1.4 µ	1.5-1.8 µ	1-2 µ	1.4(-1.9) µ	1 µ	1-1.5 µ	1.8-2.2 µ
Figures[2]			D p.211	D p. 652	-	-	SEM P p.126	P Fig. 403-407	D p. 243

[1] In the Latin diagnosis of Robak's paper, the length was given as 120-150 µ, which was a typographical error. The correct values, as cited here, were given on p. 211 of Robak's paper.

[2] D = Drawing(s); P = Photograph(s)

Morphology of teleomorph also measured and described in: (671;902); Other illustrations: (35;261).

Table 1.6. Morphology of the teleomorph of synonyms of *Ophiostoma stenoceras*.

			Mathiesen 1953 (59)	Hunt 1956 (45)	Aoshima 1965 (902)	Davidson 1971 (18)	Davidson 1971 (18)	Davidson 1978 (19)
Species			*O. albidum*	*C. albida*	*C. albida*	*C. gossypina*	*C. gossypina* var. *robusta*	*C. eucastanea*
Perithecia:	**Base**	**colour**	brown-black	black	black	black	black	black
		diameter	(66-)80(-85) µ	< 140 µ	70-120 µ	110-175 µ	180-260 µ	75-110 µ
		ornamentation	-	few undifferentiated, brown hyphae	-	-	short brown hyphae	-
	Neck	**length**	(340-)428(-482) µ	< 400 µ	120-250 µ	400-1200 µ	800-1200 µ	950-1300 µ
		width: base	(20-)25(-28) µ	11-27 µ	35-50 µ	35-55 µ	38-55 µ	25-30 µ
		width: tip	10 µ	8-11 µ	30-45 µ	12-15 µ	10-15 µ	9.6-13 µ
		ostiolar hyphae	hyaline, 1 to 2 celled, 11-23 µ	hyaline, spreading, 10-15 in number, < 20 x 1.5 µ	0-15 in number, hyaline, < 50 µ long, 3 µ at base, 1 µ at tip	15-25, hyaline, straight, outward, 1.3 µ at base, 0.7-0.9 µ at tip	hyaline, outspreading, 6-15 µ long	hyaline, 25-35 µ, 2.5-3 µ at base, 1.2 µ at tip
Ascospores:	**Colour**		-	-	hyaline	-	-	hyaline
	Shape: face view		-	cylindrical	-	-	-	-
	Shape: side view		reniform	bean-shaped	kidney-shaped	curved	orange section	slightly curved
	Length		3.5 µ	3.5-4 µ	3-4 µ	3-4 µ	3-4 µ	3.5-4.5 µ
	Width		1.3 µ	1-1.5 µ	1.6-2.2 µ	1-1.2 µ	1-1.3 µ	1.2-1.5 µ
Figures[1]			D p.51	-	-	D p.9	D p.9	P p. 857

[1] D = Drawing(s); P = Photograph(s)
Other illustrations: (69)

Table 1.7. Morphology of the *Sporothrix* anamorph of *Ophiostoma stenoceras*.

		Robak 1932 (179)	Davidson 1942 (15)	Upadhyay, 1981 (88)[1]	Kowalski & Butin 1989 (50)
Genus/Species		*Cephalosporium, Cylindrocephalum, Hormodendron*	*Cephalosporium*-like	*Sporothrix schenckii*	*Sporothrix*
Conidiophores:	**length**	-	-	-	< 300 µ
	width: base	-	-	1.5-4 µ	-
Conidiogenous cells:	**denticles: length**	-	-	0.5-1 µ	-
	position	terminal, subterminal	-	-	apical, intercalary
Conidia:	**colour**	-	hyaline	hyaline	hyaline
	septation	-	one-celled	1-celled	-
	shape	elongated ellipsoidical to ovoid	elongate or nearly spherical	clavate to ellipsoid, ovoid or globose	elliptic, somewhat bent
	secondary conidia	conidia swell, propagate by budding	-	-	-
	length	3.4-6.9 µ	4-8 µ	2-7(-9) µ	3.5-6 µ
	width	2-3.4 µ	1.4-2 or 2.8-5 µ	1-2.5(-3) µ	2-3 µ
Colony:	**growth rate**	-	5.5 mm in 5 days	25-30 mm in 12 days	25 mm in 12 days
	colour	greyish-white	white, often become dark brown	hyaline to white or dull gray brown, with dark patches	white to cream
	morphology	slightly domed. later flat, aerial hyphae rare	sparse growth of aerial mycelium	appressed to effuse, mycelium superficial and submerged	floccose to slimy
Figures[2]		D p.207; P p.209	D p.652.	P Figs. 403-407.	D p. 243

[1] *Sporothrix schenckii* treated as anamorph of *Ophiostoma stenoceras*.
[2] D = Drawing(s); P = Photograph(s)
Anamorphs also described: as *S. schenckii* (671); as *Cladosporium* (902).
Colonies described in detail: *O. albidum* & *O. stenoceras* (407).

Table 2.1. Currently accepted synonyms of *Sporothrix schenckii* (21).

Species name
Sporothrix schenckii Hekt. & Perkins, J. exp. Med. 5: 77. 1900.
≡ *Sporotrichum schenckii* (Hekt. & Perkins) de Beurmann & Gougerot, Archs Parasit. 15: 5. 1911.
≡? *Sporotrichum schenckii-beurmanii* Greco var. *schenckii* (Hekt. & Perkins) de Beurmann & Gougerot, Archs Parasit. 15: 5. 1911.
≡ *Rhinocladium schenckii* (Hekt. & Perkins) Verdun, Précis Parasitol., éd. 2. 1913.
≡ *Rhinotrichum schenckii* (Hekt. & Perkins) Ota, Jap. J. Dermatol. Urol. 27: 921. 1927.
≡? *Sporotrichum beurmanii* Matr. & Ramond var. *schenckii* (Hekt. & Perkins) Red. & Cif., Tratt. Micropat. umana 5: 452. 1942.
≡? *Sporothrix schenckii* var. *luriei* Ajello & Kaplan 1969
= *Sporotrichum beurmanii* Matr. & Ramond, C. r. hebd. Séanc. Mém. Soc. Biol. 2: 380. 1905.
≡ *Trichosporium beurmanii* (Matr. & Ramond) Lutz & Splendore, Annali Ig. sper. 17: 581. 1907.
≡ *Rhinocladium beurmanii* (Matr. & Ramond) Vuill., Bull. Séanc. Soc. Sci. Nancy 11: 138. 1910.
≡? *Sporotrichum schenckii-beurmanii* Greco var. *beurmanii* (Matr. & Ramond) de Beurmann & Gougerot, Archs Parasit. 15: 39. 1911.
≡ *Sporotrichopsis beurmanii* (Matr. & Ramond) Gueguen, in de Beurmann & Gougerot, Archs Parasit. 15: 39. 1911.
≡ *Sporothrix beurmanii* (Matr. & Ramond) Meyer & Aird., J. infect Dis. 16: 399. 1915.
≡ *Rhinotrichum beurmanii* (Matr. & Ramond) Ota, Jap. J. Dermatol. Urol. 28: 4. 1928.
≡ *Sporotrichum schenckii* (Hekt. & Perkins) de Beurmann & Gougerot var. *beurmanii* (Matr. & Ramond) C.W. Dodge, Med. Mycol. p. 805. 1935.
= *Sporotrichum schenckii-beurmanii* Greco, Argent. Med. 45: 699. 1907 (nomen nudum).
≡ *Sporothrix schenckii-beurmanii* (Greco) Meyer & Aird, J. infect. Dis. 16: 407. 1915 (incidentally mentioned).
= *Sporotrichum asteroides* Splendore, Revta Soc. scient S. Paulo 3: 62. 1908.
≡ *Sporotrichum beurmanii* Matr. & Ramond var. *asteroides* (Splendore) de Beurmann & Gougerot, Rev. med. Trop. Hyg. 7: 185. 1910.
≡ *Rhinocladium asteroides* (Splendore) Verdun, Précis Parasitol., éd. 2. 1913.
≡ *Sporothrix asteroides* (Splendore) J. Davis, J. infect. Dis. 12: 453. 1913.
≡ *Rhinocladium beurmanii* (Matr. & Ramond) Vuill. var. *asteroides* (Splendore) C.W. Dodge (as 'Vuill.'), Med. Mycol. p. 802. 1935.
≡ *Rhinotrichum asteroides* (Splendore) C.W. Dodge (as 'Verdun'), Med. Mycol. p. 802. 1935.
= *Sporotrichum indicum* Castell., J. trop. Med. Hyg. 11: 261. 1908 (without diagnosis).
≡ *Sporotrichum beurmanii* Matr. & Ramond var. *indicum* (Castell.) de Beurmann & Gougerot, Les Sporotrichosis p. 179. 1912 (nomen provisorium).
≡ *Rhinocladium indicum* (Castell.) Verdun, Précis Parasitol, éd. 2. 1913.
≡ *Rhinotrichum indicum* (Castell.) Ota, Jap. J. Dermatol. Urol. 27: 928. 1927.

(Continued on following page.)

Table 2.1. Currently accepted synonyms of *Sporothrix schenckii* (continued).

Species name
= *Sporotrichum beurmanii* Matr. & Ramond, C. r. hebd. Séanc. Mém. Soc. Biol. 2: 380. 1905.
= *Sporotrichum equi* Carougeau, J. méd. vét. Zootechn. 60:80. 1909.
≡ *Rhinocladium equi* (Carougeau) Lurie, Mycologia 40: 107. 1948 (as 'equinum').
= *Sporotrichum* sp., Jeanselme & Chevallier, Bull. Mém. Soc. méd. Hop., Paris 29: 792. 1910.
≡ *Sporotrichum jeanselmei* Brumpt & Langer., Précis Parasitol., éd. 1. 1910.
≡ *Sporotrichum beurmanii* Matr. & Ramond var. *jeanselmei* (Brumpt & Langer) de Beurmann & Gougerot, Archs Parasit. 15: 51. 1911.
≡ *Rhinocladium jeanselmei* (Brumpt & Langer) Verdun, Précis Parasitol, éd. 2. 1913.
≡ *Rhinotrichum jeanselmei* (Brumpt & Langer) Ota, Jap. J. Dermatol. Urol. 28: 5. 1928.
= *Sporotrichum fonsecae* Filho, Revta med.-cirurg. Braz. 37: 265. 1929.
≡ *Rhinocladium fonsecae* (Filho) Filho, Revta med.-cirurg. Braz. 38: 163. 1930.
=? *Sporotrichum grigsbyi* C.W. Dodge, Med. Mycol. p. 801. 1935 (without Latin diagnosis).
= *Rhinocladium* sp., MacKinnon, Archos Soc. biol. Montev. 5: 1325. 1931.
≡ *Sporotrichum schenckii* (Hekt. & Perkins) de Beurmann & Gougerot var. *greconis* C.W. Dodge, Med. Mycol. p. 808. 1935 (without Latin diagnosis).
≡ *Sporotrichum greconis* Gougerot, Ann. N.Y. Acad. Sci. 50: 1348. 1950 (incidentally mentioned).
= *Sporotrichum* (*Rhinocladium*) *verticillioides* A. Sartory et al., C. r. hebd. Séanc. Acad. Sci., Paris 201: 1501. 1935.
= *Rhinocladium pereirae* Miranda, Um novo Esporotricado. 1936 (without Latin diagnosis).
≡ *Sporotrichum pereirae* (Miranda) Red. & Cif., Tratt. Micopat. umana 5: 460. 1942.
= *Sporotrichum* (*Rhinocladium*) *tropicale* Panja et al., Indian med. Gaz. 82: 202. 1947 (without Latin diagnosis).
= *Sporotrichum acuminatum* Lurie, Mycologia 43: 120. 1951 (without diagnosis).
= *Calcarisporium pallidum* Tubaki, Nagaoa 5: 13. 1955.
≡ *Sporothrix pallidum* (Tubaki) Matsushima.
= *Sporothrix albicans* S.B. Saksena, Curr. Sci. 34: 318. 1965.
=? *Dolichoascus schenckii* Thibaut & Ansel, 1970

Table 2.2. Distribution of *Sporothrix schenckii.*

Origin	Reference(s)
Argentina	383
Australia	383
Botswana	772;881
Brazil	156;874
Canada	156
Chile	156
Colombia	156
Corsica (France)	874
Cuba	1118
France	899
Germany	899;934[1]
Guatamala	156
Israel	874
Italy	934[1]
Japan	667
Mexico	156
Mozambique	881;899
Netherlands	899
Nigeria	772
Peru	156
Poland	1127
Portugal	156
South Africa	772;881;990a;750;751;990
Sudan	772
UK	899;934[1]
USA	934[1];156
USA, California	874
USA, Hawaii	874
USA, Vermont	156
USA, Wisconsin	156
Taiwan	874
Uruguay	156;874
Venezuela	156
Worldwide	874
Zimbabwe	772

[1] Reported as *Sporotrichum beurmanii.*

Table 2.3. Hosts of *Sporothrix schenckii*.

Host	Reference(s)
Acacia mearnsii wood	751;772
Aechmea sp. (Bromeliaceae)	156
Air	156
Armadillos	156
Barberry	750
Beech stems	156;874
Cacti	156
Carnations	750
Cats	899
Conifer wood	874
Corn	156
Dogs	156
Equisetum	874
Eucalyptus wood	772;874;750
Grass	21;156
Hair	156
Horse	751;881
Humans	21;772;874;881;934;990a
Insects	156
Mammals	156
Meadow hay	750
Meat (cold stored products)	874
Palm tree (rotten trunks)	156
Pinus laricio var. *corsicana*	156
Prairie hay	156
Quercus robur	1127
Rat dung	156
Rodents	156
Roses	772
Salt	750
Spartina paens (Salt marsh hay)	156
Sphagnum moss	874
Soil	21;156;874
Straw	156
Tilapia mozambica (fish)	156;874
Timber/wood	21;156;750;990a
Water	156

Table 2.4. Morphology of *Sporothrix schenckii.*

		Nicot & Mariat 1973 (770) [1]	De Hoog 1974 (21) [1]
Conidiophores:	**length**	-	10-40 µ
	width: base	-	0.7-1.5 (-2) µ
Conidiogenous cells:	**description**	solitary, scattered, erect	-
	measurements	10-40 µ long, 0.7-1.5 µ wide	-
	denticles	in clusters, 0.5-1 µ long	0.5-1 µ
Conidia:	**colour**	hyaline	hyaline
	septation	-	-
	shape	ovoid to fusiform with rounded tip, straight, sometimes obconical or campanulate in older cultures	guttuliform to fusiform with pointed base
	length	2.5-5.5 µ	2.5-5.5 (-8) µ
	width	1.5-2.5 µ	1.5-2.5 (-3) µ
Colony:	**growth rate**	6-22 mm in 10 days	6-22 mm/ 10 days
	colour	hyaline, later greyish to dull brown	hyaline to greyish to dull brown
	morphology	finely floccose, velvety or flanulose	smooth to finely floccose, velvety, lanose or funiculose
Figures[2]		D p. 55, 57	D p. 36

[1] Treated as anamorph of *O. stenoceras.*
[2] D = Drawing(s); P = Photograph(s).
More decriptions: as *Sporotrichum schenckii* & *S. beurmanii* (934).

Table 3.1. Currently accepted synonyms of *Ophiostoma nigrocarpum*.

Species name	Reference[1]
Ophiostoma nigrocarpum (Davids.) de Hoog, Stud. Mycol. 7: 62. 1974.	523
≡ *Ceratocystis nigrocarpa* Davids., Mycopath. Mycol. appl. 28: 276. 1966.	
= *Ophiostoma abietinum* Marmolejo & Butin, Sydowia 42: 194. 1990.	Chapter 4 of this thesis.

[1] Reference suggesting synonymy or new combination.

Table 3.2. Distribution of *Ophiostoma nigrocarpum*.

Origin	Reference(s)
Australia	1111
Mexico	55[1];1110[1]
New Zealand	1111
North America	28
USA	562
USA, California	88;171[2];523;1080
USA, Idaho	21;49;88;171[2];523

[1] Reported as *O. abietinum*.
[2] Identity of *O. nigrocarpum* not confirmed (171).

Table 3.3. Hosts of *Ophiostoma nigrocarpum*.

Host	Reference(s)
Abies spp.	21;28;88;523
Abies vejari	55[1];1110[1]
Pinus spp.	27;28;49
Pinus coulteri	171[2]
Pinus ponderosa	88;171[2];523;562;1080
Pinus radiata	814
Pseudotsuga spp.	28;49;523
Pseudotsuga douglasii	21
Pseudotsuga menziesii	171[2]
Quercus spp.	28
Tsuga spp.	49;523
Tsuga canadensis	523

[1] Reported as *O. abietinum*.
[2] Identity of *O. nigrocarpum* not confirmed.

Table 3.4. Insects associated with *Ophiostoma nigrocarpum*.

Insect	Reference(s)
Dendroctonus brevicomis	71;171[1];240;523;562;1080
Dendroctonus frontalis	71
Dendroctonus pseudotsugae	71;171[1]
Ips paraconfuses	171[1]
Ips pini	384;689
Pseudohylesinus sp.	55[2];1110[2]
Scolytus sp.	523
Tarsonemus endophloeus(mite)	171[1]
Tarsonemus ips (mite)	171[1]

[1] Identity of *O. nigrocarpum* not confirmed.
[2] Reported as *O. abietinum*.

Table 3.5. Morphology of the teleomorph of *Ophiostoma nigrocarpum*.

			Davidson 1966 (523)	Upadhyay 1981 (88)	Marmolejo & Butin 1990 (55)[1]
Perithecia:	**Base**	**colour**	black	black	black to dark brown
		diameter	50-80 µ	15-108 µ	105-170 µ
		ornamentation	-	sometimes, brown, smooth, 1.5-3.5 µ wide	-
	Neck	**length**	120-160 µ	(115-)145-200 µ	450-650 µ
		width: base	15-25 µ	(17-)20-25 µ	19-24.5 µ
		width: neck	10-12 µ	10-20 µ	9.5-11.5 µ
		ostiolar hyphae	absent or very short, hyaline, curved outward	usually absent, when present, hyaline, curved or straight, divergent, < 22 µ long, 1.5 µ wide	present, sometimes absent, hyaline, septate, 7-10 in number, 13-19 µ long, 2-3 µ wide
Ascospores:	**Colour**		hyaline	hyaline	hyaline
	Septation		-	1-celled	1-celled
	Shape:	**face view**	-	fusiform to elliptical	-
	Shape:	**side view**	curved or crescent shaped	crescent shaped or lunate with obtuse ends	orange section shaped
	Shape:	**end view**	-	nearly globose	-
	Length		3-4 µ	3-5 µ	3-4.5 µ
	Width		1-1.3 µ	1-1.5 µ	2-2.5 µ
Figures[2]			P p.277	P Figs. 378-381	D Fig.1

[1] Reported as *O. abietinum*.
[2] P = Photograph(s).

Table 3.6. Morphology of the *Sporothrix* anamorph of *Ophiostoma nigrocarpum*.

		Davidson 1966 (523)	**De Hoog 1974 (21)**	**Upadhyay 1981 (88)**	**Marmolejo & Butin 1990 (55)**
Genus		-	*Sporothrix*	*Sporothrix*	*Sporothrix*
Conidiophores:	**length**	-	10-25 µ	5.5-35 µ	5-50 µ
	width: base	-	1.5-2.5 µ	0.5-2 µ	1.5-2.5 µ
Conidiogenous cells:	**denticles: length**	-	0.5-2.5 µ	0.5-2 µ	-
	position	-	-	terminal or lateral, intercalary or integrated	-
Conidia:	**colour**	hyaline	hyaline	hyaline	hyaline
	septation	-	-	1-celled	1-celled
	shape	broadly ovoid	obovoidal to broadly ellipsoidal	obovoid, broadly ellipsoidal, clavate or cylindrical	slightly curved, clavate or cylindrical
	length	3-5 µ	3.5-5 µ	(2-)2.5-5.5(-7)µ	4-7.5 µ
	width	1.5-3 µ	1.5-2.5 µ	1-2.2 µ	1-2 µ
Colony:	**growth rate**	30 mm in 10 days	20-25 mm/ 10 days	35 mm in 12 days	25 mm in 10 days
	colour	hyaline, turning light grey to dark grey	grey, often locally grey to blackish	dingy white to gray with dark gray to blackish patches	hyphae hyaline
	morphology	appressed at first, few aerial white strands, often yeast-like	smooth, flat,	appressed to flocculose, mycelium mostly immersed	appressed
Figures[2]		P. p.277	D p. 62	P Figs. 378-381.	D Fig. 1

[1] Reported as *O. abietinum*.
[2] D = Drawing(s); P = Photograph(s).

Appendix 2

detail *noun* [di táyl, dee tàyl] (plural **details**)

1. an individual part of something, especially one of several items of information
2. a small element of a work of art or building structure, considered separately

- Encarta Dictionary

Ophiostoma pluriannulatum: a tabulated review

CONTENTS

NOTE: References are listed according to number, starting on page 204.

Table 1. Currently accepted synonyms of *Ophiostoma pluriannulatum*.

Species name	Reference[1]
Ophiostoma pluriannulatum (Hedgc.) H. & P. Sydow, Ann. Mycol. 17: 43. 1919.	87
≡ *Ceratostomella pluriannulata* Hedgc., Mo. Bot. Gard. Ann. Rep. 17: 72. 1906.	
≡ *Ceratocystis pluriannulata* (Hedgc.) C. Moreau, Revue Mycol., Suppl. Colon. 17:22. 1952.	61

[1] Reference suggesting new combination.

Table 2. Distribution of *Ophiostoma pluriannulatum.*

Origin	Reference(s)
Canada, British Columbia	45
England	225
Europe	29
Mexico	1110
New Zealand	814
North America	29
Japan	1048;671;902
Sweden	51;57;59;60
United Kingdom	206
United Kingdom, England	35
USA	41
USA, Southern	140
USA, District of Columbia	35
USA, Florida	14
USA, Georgia	140
USA, Indiana	45
USA, Louisiana	12;14;140;141;326;
USA, Maryland	35
USA, Minnesota	1056;323
USA, Mississippi	14;140;141
USA, New York	45
USA, North Carolina	35
USA, Tennessee	35
USA, Texas	623
USA, Virginia	131
USA, West Virginia	45
USA, Wisconsin	27

Table 3. Hosts of *Ophiostoma pluriannulatum*.

Host	Plant family	Reference(s)
Abies spp.	Pinaceae	49
Abies firma Sieb. et Zucc.	"	671;902
Abies sachalensis	"	902
Aesculus spp.	Hippocastanaceae	29
Aesculus glabra	"	35
Carya spp.	Juglandaceae	29
Carya glabra	"	35
Conifers	-	45
Fagus sp.	Fagaceae	35
Fagus crenata Blume.	"	671;902;1048
Hardwoods	-	14;45
Liquidambar spp.	Hamamelidaceae	29
Liquidambar styraciflua L.	"	35;326;619
Liriodendron spp.	Magnoliaceae	29
Liriodendron tulipifera	"	35
Picea spp.	Pinaceae	51;60
Picea abies (L.) Karst. [= *Picea excelsa* (Lam.) Link.]	"	902
Picea glehnii Mast.	"	902
Picea jezoensis (Sieb. et Zucc) Carr.	"	671;902
Pinus spp.	"	14;29;60;140;326;902
Pinus caribea Morelet	"	140
Pinus echinata Mill.	"	140
Pinus densiflora Sieb. et Zucc.	"	671
Pinus palustris Mill.	"	140
Pinus pseudostrbus Lindl	"	1110
*Pinus radiata*D. Don.	"	814
Pinus taeda L.	"	140
Populus spp.	Salicaceae	27;29;1056
Populus tremuloides	"	323
Populus trichocarpa	"	35
Quercus spp.	Fagaceae	29
Quercus affinis	"	1110
Quercus borealis	"	35
Quercus crispula	"	902
Quercus ellipsoidalis	"	323

(Continued on following page.)

Table 3. Hosts of *Ophiostoma pluriannulatum* (continued).

Host	Plant family	Reference
Quercus fusiformis Small.	Fagaceae	623
Quercus marilandica Muenchh.	"	623
Quercus mongolica Fischer. var. *grosseserrata* (Blume) Rehd. et Wils.	"	671
Quercus rubra	"	41
Quercus texana Buckl.	"	623
Quercus virginiana Mill.	"	623
Ulmus sp.	Ulmaceae	225
Ulmus americana L.	"	315

Table 4. Insects associated with *Ophiostoma pluriannulatum*.

Vector	Reference
Ambrosia beetles	141
Ants	141
Blastophagus minor	57;59
Colopterus maculatus Erich. (Nitidulidae)	623
Colopterus truncatus Rand. (Nitidulidae)	623
Cryptarcha concinna Melsh (Nitidulidae)	623
Dipterous insects	141
Ips typographus	59
Lobiopa undulata Say (Nitidulidae)	623
Nitidulidae	409
Other beetles	141
Other insects	141
Pissodes pini	59
Platypus compositus	141
Pterocyclon mali	141
Tenebrionids	141
Xyloborus affinis	141
Xylobiops basiliaris	141

Table 5. Morphology of the teleomorph of *Ophiostoma pluriannulatum*.

			Hedgcock, 1906 (41)	Lagerberg *et al.*, 1927 (51)	Hunt, 1956 (45)	Upadhyay, 1981 (88)
Perithecia:	**Base**	**colour**	black	-	black	black
		diameter	90-120-200 µ	112-224 µ	80-250 µ	75-247 µ
		ornamentation	-	thickly covered with hairs	none or hyaline to pale brown to dark brown	none or pale brown, 1.5-3 µ thick
		length of orn.	-	-	< 25 x 3 µ	-
	Neck	**length**	900-1500-2000 µ	1500-4800 µ	< 3000 µ	560-2800(-3300) µ
		width: base	30 µ	-	20-40 µ	(15-)18-35(-45) µ
		width: apex	10 µ	16 µ	7-15 µ	7.5-20 µ
		ostiolar hyphae	tapering, 20 µ long	tapering, 21-58 µ	tapered, < 60 µ long 1 µ at tip	tapered 10-75 µ 0.5-2 µ at tip
		annuli	0-2	2-5	3-6	3-8
Ascospores:	**Colour**		hyaline	-	-	hyaline
	Septation		-	-	-	1-celled
	Shape:	**face view**	-	-	-	elliptical
	Shape:	**side view**	reniform	-	bean or orange section	orange section
	Length		4-4.5-5 µ	4.0-4.4 µ	3.5-5 µ	3-5-(5.5) µ
	Width		1.5-1.7 µ	1.5 µ	1-2 µ	1-1.5 µ
Figures[1]			D pl. 3, 4	D p. 186,187	-	P Figs. 387-392

[1] D = Drawing(s); P = Photograph(s)
Morphology of teleomorph also measured and described in: (671); (902); [(1110) D p.169]

Table 6. Morphology of the *Sporothrix*[1] anamorph of *Ophiostoma pluriannulatum*.

		Hedgcock, 1906 (41)	**Lagerberg *et al.*, 1927 (51)**	**Hunt, 1956 (45)**	**Upadhyay, 1981 (88)**
Genus		*Cephalosporium*	*Cladosporium*	*Cladosporium*	*Hyalodendron*
Conidiophores:	**length**	-	-	150 µ	-
	width: base	-	-	1.5 µ	2-4(05) µ
Conidiogenous cells:	**length**	-	5-6.6 µ	-	-
	width	-	1.8-2.3 µ	-	-
Conidia:	**colour**	hyaline	-	hyaline	hyaline
	septation	unicellular	-	-	1-celled
	shape	-	-	elongate-ellipsoid	elongate ellipsoid, fusiform or cylindrical to clavate
	length	(5)-6-(8)	-	5-7.5 µ	4.6-18 µ
	width	(2)-1.5-(3) µ	-	15.-2.5 µ	1.5-3 µ
Ramoconidia:	**length x width**	-	-	-	33 x 4.5 µ
Colony:	**growth rate**	-	20 mm in 10 days	20 mm in 10 days	85 mm in 12 days
	colour	white	snowy-white	white	white to dingy white
	colour under surface	-	dark ```olive-green	pale brown or black	pale brown to grayish black
	morphology	floccose	compact air-mycelium	tufted	flocculose to floccose
Figures[2]		D pl. 5	P. p. 185; D p. 187	D p. 15	P Figs. 387-392

[1] The anamorph of *O. pluriannulatum* is presently treated as a *Sporothrix* species (88;940).

[2] D = Drawing(s); P = Photograph(s)

Morphology of anamorph also measured and described in: (671); [(1110) Dp.169 as *Hyalodendron*]; [(902) as *Cladosporium*]

Appendix 3

I have made this letter longer than usual because I lack the time to make it shorter.

- Blaise Pascal

Ophiostoma piceae and *Ophiostoma querci*: a tabulated review

CONTENTS

NOTE: **References are listed according to number, starting on page 204.**

Table 1.1. Present taxonomic status of *Ophiostoma piceae* and related species.[1]

Species name	Reference[2]
Ophiostoma piceae (Münch) H. & P. Sydow, Ann. Mycol. 17: 43. 1919.	87
≡ *Ceratostomella piceae* Münch, Naturw. Ztschr. Land. Forstw. 5: 547. 1907.	
≡ *Ceratocystis piceae* (Münch) Bakshi, Trans. Br. Mycol. Soc. 33: 113. 1950.	1
[3]*Ophiostoma catonianum* (Goid.) Goid., Boll. Staz. Patol. veg. Roma, N. Ser., 15: 132. 1935.	274
≡ *Ceratostomella catoniana* Goid., Atti Accad. naz. Lincei Rc., Ser 6, 21: 199. 1935.	
≡ *Ceratocystis catoniana* (Goid.) C. Moreau, Revue Mycol., Suppl. Colon. 17: 22. 1952.	61
[3]*Ophiostoma floccosum* Mathiesen, Svenska bot. Tidskr. 45: 219. 1951.	
≡ *Ceratocystis floccosa* (Mathiesen) Hunt, Lloydia 19: 36. 1956.	45
[4]*Ophiostoma perfectum* (Davids.) de Hoog, Stud. Mycol. 7: 54. 1974.	21
≡ *Ceratocystis perfecta* Davids., Mycologia 50: 665. 1958.	

[1] *O. querci* and its synonyms are not included here as they are considered in **Table 2.1.**

[2] Reference suggesting new combination.

[3] *O. catonianum* and *O. floccosum* were treated as a synonyms of *O. piceae* by De Hoog (21), Upadhyay (88), Przybyl & De Hoog (72) and Seifert *et al.* (97). Harrington *et al.* (1096), however, considers both as species distinct from *O. piceae* and *O. querci* based on rDNA sequences.

[4] Seifert *et al.* (97) list *O. perfectum* as a synonym of *O. piceae*, mentioning that Przybyl and De Hoog (72) 'declared it a synonym of *O. piceae*.' This, however, is a mistake, since the species is not discussed in the paper by Przybyl and De Hoog (72). In the original description of *O. perfectum*, only a mycelial anamorph was described - no mention was made of a synnematous anamorph (17). De Hoog (21), however, described a *Graphium*-like conidial state for this species, but treated it as a species separately from *O. piceae*, as did Olchowecki and Reid (69), and Upadhyay (88). I prefer not to treat *O. perfectum* as a synonym of *O. piceae*, until the phylogenetic relatedness of the two species have been investigated.

Table 1.2. Distribution of *Ophiostoma piceae* and related species.[1]

Origin	Reference(s)
Australia	545[2];1096[7]
Austria	359;400a;807;892;944
Belgium	365[3];408
Brazil	1116c
Canada	72[3];806;944
Canada, Eastern	265[3]
Canada, Western	265[3]
Canada, Alberta	1115
Canada, British Columbia	8;558;1115
Canada, Manitoba	69[3]
Canada, New Brunswick	8;77;1115
Canada, Nova Scotia	8;77
Canada, Ontario	35[3];1115
Canada, Quebec	8;77;577;582;1115
Canada, Saskatchewan	1115
Chile	11[3];1096;1116d;1116a
China	171
Czech Republic	367;400;408;1074;1085
Europe	29;35[3]
Finland	1077;1125
France	8;181;558
Japan	293;389[4];671[3];796;902;1048;1049
Germany	13;25[4];66; 150;162[3];163[3];287;407;408;606[4];644; 1073;1096;1124
Hungary	400e[4];602[4]
Ireland	88
Italy	274[5];400f[4];570[4];1106[5]
Japan	125;843;944
Korea	1096[7]
Mexico	1110[6]
Netherlands	400g[4]
New Zealand	176;574a;594;814;861;1096;1096[7];1099;

[1] The origin of *O. querci* and its synonyms are not included here, as they are considered in **Table 2.2.**
[2] Reported as *Graphium piceae.*
[3] *O. querci* treated as synonym of *O. piceae.*
[4] Reported as *O. piceae,* but possibly *O. querci.*
[5] *Ophiostoma catonianum.*
[6] It is possible that this report represents both *O. piceae* and *O. querci.*
[7] *Ophiostoma floccosum.*
[8] *Ophiostoma perfectum.*

Table 1.2. Distribution of *Ophiostoma piceae* and related species (continued).[1]

Origin	Reference(s)
North America	29;35[3];249
Norway	80;81;82;179;191;194;603;604;665;689
Poland	8;50[3]; 247;259[3];359;362[3];408;644
Romania	576
Russia	238;283;306;424;607
Russia, Siberia	902
Spain	8;558;580;1060
Sweden	51;57;58[2];59;60;63;72;73;288;407;407[7];359;944;1055;1062;1066;1069;1096[7]
Switzerland	276
UK	8;558;855;944;1018;1096;1096[7]
UK, England	8;35[3]; 231;357
UK, Northern Ireland	13;367
UK, Scotland	1;8;53;1061; 807
UK, Wales	8;1057
USA	72[3]
USA, Arizona	13
USA, California	1096
USA, Colorado	17[8]
USA, Idaho	1096[7]
USA, Maine	27[3]
USA, Minnesota	187[3];323
USA, New York	1096
USA, Oregon	16
USA, Texas	623
USA, Virginia	652
USA, Washington	16;1096;1096[7]
USA, West Virginia	168

1 The origin of *O. querci* and its synonyms are not included here, as they are considered in **Table 2.3.**
2 Reported as *Graphium piceae.*
3 *O. querci* treated as synonym of *O. piceae.*
4 Reported as *O. piceae,* but possibly *O. querci.*
5 *Ophiostoma catonianum.*
6 It is possible that this report represents both *O. piceae* and *O. querci.*
7 *Ophiostoma floccosum.*
8 *Ophiostoma perfectum.*

Table 1.3. Hosts of *Ophiostoma piceae* and related species[1].

Host	Family	Reference(s)
Abies spp.	Pinaceae	8;29;306
Abies alba Mill.	"	66;359;644;1124
Abies balsamea (L.) Mill.	"	29;35[2];77;577;1115
Abies firma Sieb. & Zucc.	"	902
Abies lasiocarpa Nutt. var. *arizonica* Lemm.	"	13
Abies procera Rehd.	"	16;619
Abies sachalensis	"	902
Acer spp.	Aceraceae	29
Acer mono Maxim.	"	671[2]
Acer pictum Thunb.	"	293
Acer pseudoplatanus	"	8;558
Acer rubrum	"	35[2]
Acer saccharum	"	27[2]
Air	-	1066
Betula japonica Sieb.	Betulaceae	293
Betula papyrifera Marsh.	"	35[2]
Betula pendula Roth	"	1116b
Betula platyphylla Sukatchev var. *japonica* (Miq.) Hara	"	671[2]
Betula pubescens Ehrh.	"	191
Cat hair	-	1096[3]
Chamaecyparis obtusa (Sieb. et Zucc.) Endl.	Cupressaceae	293;671[2]
Chamaecyparis pisifera (Sieb. et Zucc.) Endl.	"	293;671[2]
Conifers	-	35[2];1077
Crataegus sp.	Rosaceae	1073
Cyclobalanopsis myrsinaefolia	?	902
Dacrydium cupressinum Lamb.	Podocarpaceae	176[2]
Eucalyptus sp.	Myrtaceae	176[2]
Fagus spp.	Fagaceae	29
Fagus crenata Blume	"	902;1048;1049
Fagus grandifolia	"	27[2]
Fagus sylvatica L.	"	188k;408
Grevillea robusta	Proteaceae	1116c

[1] The hosts of *O. querci* and its synonyms are not included here, as they are considered in **Table 2.3.**
[2] *O. querci* treated as synonym of *O. piceae.*
[3] *Ophiostoma perfectum.*
[4] *Ophiostoma floccosum.*
[5] *Ophiostoma catonianum.*
[6] Reported as *O. piceae,* but possibly *O. querci.*
[7] Reported as *Graphium piceae.*

Table 1.3. Hosts of *Ophiostoma piceae* and related species (continued)[1].

Host	Family	Reference(s)
Hardwoods	-	35[2]
Human	-	383
Kalopanax pictus (Thunb.) Nakai	Araliaceae	671[2]
Kalopanax ricinifolium Miq.	"	293
Larix sp.	Pinaceae	176[2]
Larix decidua Mill.	"	644;807;1124
Larix leptolepis Murr. [= *Larix kaempferi* (Lamb.) Carr.]	"	1;796;902
Laurelia philippiana	?	1116a
Laurelia sempervirens	?	1116a
Magnolia hypoleuca S. et Z. [= *Magnolia obovata* Thunb.]	Magnoliaceae	293;671[2]
Nothofagus dombeyi (Mirb.) Oerst.	Fagaceae	11[2];1116a
Nothofagus fusca (Hook) Oerst.	"	176[2];574a
Nothofagus menziesii (Hook) Oerst.	"	176[2]
Nothofagus pumilio (Poepp. & Endl.) Krasser	"	11[2];1116a
Picea spp.	Pinaceae	8;29;51;58[4];60;66;73;77;265[2];283;404;407[2];607; 944;1062;1069;1096
Picea abies (L.) Karst. [= *P.icea excelsa* (Lam.) Link.]	"	1;8;13;21;53;57;72[2];80;81;82;150;194;231;247;359; 367;408;558;603;604;665;689;739;892;902;1057; 1085;1124;1125
Picea asperata Masters	"	171
Picea engelmannii Perry	"	17[3]
Picea glauca (Moench) Voss	"	35[2];619
Picea glehnii Mast.	"	293;902
Picea hondoensis Mast.	"	902
Picea jezoensis (Sieb. et Zucc.) Carr.	"	293;671;843;902;944
Picea mariana (Mill.) B.S.P.	"	35[2];69[2];944;1115
Picea sitchensis (Bong.) Carr.	"	8;13;16;35[2];72[2];367;558;1057;
Pinus spp.	Pinaceae	8;45[2];51;58[4];60;265[2];283;306;404;407;407[2];576; 902;944;1096;1096[4]
Pinus banksiana Lamb.	"	69;1115
Pinus contorta Loud. var. *latifolia* S. Wats.	"	944;1115
Pinus densiflora Sieb. et Zucc.	"	293;671[2]

1 The hosts of *O. querci* and its synonyms are not included here, as they are considered in **Table 2.3.**
2 *O. querci* treated as synonym of *O. piceae.*
3 *Ophiostoma perfectum.*
4 *Ophiostoma floccosum.*
5 *Ophiostoma catonianum.*
6 Reported as *O. piceae,* but possibly *O. querci.*
7 Reported as *Graphium piceae.*

Table 1.3. Hosts of *Ophiostoma piceae* and related species (continued)[1].

Host	Family	Reference(s)
Pinus eliottii	Pinaceae	1116d
Pinus hartwegii	"	1110
Pinus koraiensis	"	902
Pinus laricio Poir. [= *Pinu nigra* Arn.]	"	8;1085
Pinus nigra var. *maritima* (Aiton) Melville	"	855;1018
Pinus parviflora Sieb. et Zucc.	"	293
Pinus ponderosa Laws.	"	1101[3];1116d
Pinus radiata D. Don.	"	176[2];594;814;861;1099;1116d
Pinus strobus L.	"	8;53;558;652;1061
Pinus sylvestris L.	"	8;181;247;357;367;408;558;944;1060;1085
Pinus thunbergii Parlat.	"	293;671[2]
Podocarpus sp.	Taxaceae	176[2]
Populus spp.	Salicaceae	29;265[2]
Populus tremuloides	"	8;250;323
Prumnopitys spicata [= *Podocarpus spicatus* R.Br. ex Mirb.]	Taxaceae	176[2]
Prunus incisa Thunb.	Rosaceae	293;671[2]
Pseudotsuga spp.	Pinaceae	29;1096;1096[4]
Pseudotsuga menziesii (Mirb.) Franco	"	16;176[2];265[2]
Pyrus communis L.	Rosaceae	72[5];274[5];1106[5]
Quercus spp.	Fagaceae	25[6];29; 72[2];187[2];163[2];400; 1074;
Quercus affinis	"	1110[6]
Quercus alba L.	"	168
Quercus cerris L.	"	570[6]
Oercus crispula	"	902
Quercus ellipsoidalis	"	31
Quercus fusiformis Small.	"	623
Quercus longipes	"	424
Quercus marilandica Muenchh.	"	623
Quercus pedunculata	"	247
Quercus petraea	"	72[2];162[2];400e[6];602[6];606[6]
Quercus pubescens Willd.	"	570[6]
Quercus robur L.	"	162[2];259[2];362[2];365[2];400g[6];408

1 The hosts of *O. querci* and its synonyms are not included here, as they are considered in **Table 2.3.**
2 *O. querci* treated as synonym of *O. piceae.*
3 *Ophiostoma perfectum.*
4 *Ophiostoma floccosum.*
5 *Ophiostoma catonianum.*
6 Reported as *O. piceae,* but possibly *O. querci.*
7 Reported as *Graphium piceae.*

Table 1.3. Hosts of *Ophiostoma piceae* and related species (continued)[1].

Host	Family	Reference(s)
Quercus rubra L.	Fagaceae	408
Quercus serrata Thunb.	"	293;671[2]
Quercus spicata	"	1116b
Quercus texana Buckl.	"	623
Quercus virginiana Mill.	"	623
Softwood	-	901
Thuja sp.	Cupressaceae	1116e
Thuja occidentalis L.	"	1116b
Tropical hardwoods	-	545[7]
Tsuga spp.	Pinaceae	8;29;265[2];558;944;1096;1096[4]
Tsuga heterophylla (Raf.) Sarg.	"	16;72[2]
Ulmus carpinifolia.	Ulmaceae	8;558
Water	-	288
White water	-	288
Wood pulp	-	179;288;296

1 The hosts of *O. querci* and its synonyms are not included here, as they are considered in **Table 2.3**.
2 *O. querci* treated as synonym of *O. piceae.*
3 *Ophiostoma perfectum.*
4 *Ophiostoma floccosum.*
5 *Ophiostoma catonianum.*
6 Reported as *O. piceae,* but possibly *O. querci.*
7 Reported as *Graphium piceae.*

Table 1.4. Insects associated with *Ophiostoma piceae* and related species[1].

Insect	Reference(s)
Acanthocinus aedilis	58[2];59
Blastophagus minor	57;59
Blastophagus piniperda	57;59
Calvolia sp. (Mite)	181
Colopterus maculatus Erich. (Nitidulidae)	623
Colopterus truncatus Rand. (Coleoptera: Nitidulidae)	186;623
Corthylus columbianus Hopk. (Coleoptera: Scolytidae)	94;168;241[3]
Cryphalus abietis (Ratz.)	1124
Cryptarcha concinna Melsh (Nitidulidae)	623
Crypturgus cinereus (Hrbst.)	1124
Crypturgus pusilluss (Gyll.)	1124
Dendroctonus rufipennis Kirby	249;502;689
Dendrolaelaps quadrisetus (Berlese)	63
Dryocoetes autographus Ratzeb. (Bark beetle)	1;1124
Epuraea sp. (Coleoptera: Nitidulidae)	186
Glischrochilus fasciatus (Coleoptera: Nitidulidae)	186
Glischrochilus obtusus (Coleoptera: Nitidulidae)	186
Glischrochilus quadrisignatus Say (Coleoptera: Nitidulidae)	186
Glischrochilus sanguinolentus (Coleoptera: Nitidulidae)	186
Glischrochilus siepmanni (Coleoptera: Nitidulidae)	186
Gnathotrichus materiarius (Fitch)	1124
Histiostoma ovalis (Mite)	181
Hylastes ater Paykull	57;59;357
Hylastes cunicularius	59
Hylesinus piniperda	53
Hylobius pales (Hbst) (Coleoptera: Curculionidae)	652
Hylurgops glabratus Zett. (Bark beetle)	892
Hylurgops palliatus Gyll. (Bark beetle)	1;57;59;407;689;739;1124
Ips acuminatus	57;59
Ips cembrae (Heer)	796;807
Ips duplicatus Sahlb.	689;739
Ips pilifrons	17[4]

1 The insects associated with *O. querci* and its synonyms are not included here, as they are considered in **Table 2.4.**

2 *Ophiostoma floccosum.*

3 Reported as *O. pluriannulatum*, but doubtful since synnemata were present. Possibly *O. piceae* or *O. querci* according to the description.

4 *Ophiostoma perfectum.*

5 Reported as *O. piceae*, but possibly *O. querci.*

6 *Ophiostoma catonianum.*

7 *O. querci* treated as synonym of *O. piceae.*

Table 1.4. Insects associated with *Ophiostoma piceae* and related species (continued)[1].

Insect	Reference(s)
Ips typographus L. (Bark beetle)	57;58[2];59;63;73;80;81;82;247;276;407;407[2];689;739;892;1124;1125
Ips typographus L. f. *japonicus* Niijima	796;843
Ips sexdentatus Boerner (Bark beetle)	57;58[2];59;63;73;80;81;82;181
Lobiopa undulata Say (Nitidulidae)	623
Monochamus sutor	57;59
Myelophilus piniperda L.	357
Orthotomicus laricis	1124
Orthotomicus proximus	57;59;407
Pissodes pini	59
Pissodes nemorensis Germar (Coleoptera: Curculionidae)	652
Pityogenes chalcographus L.	57;58[2];59;689;739;1124
Pityogenes quadridens	57;59
Platypodidae	1124
Polygraphus poligraphus (L.)	384
Scolytidae	384
Scolytus intricatus	606[5]
Sirex sp.	57
Tarsonemus sp. (phoretic mite on *Ips nitidis*)	171
Tetropium sp.	57;59
Thomasiniana crataegi	1073
Tomicus piniperda	1124
Trichouropoda polytrichasimilis (Mite)	181
Trypodendron lineatum (Ambrosia beetle)	1
Trypodendron domesticum	88;1124
Uroobovella ipidis (Vitzthum) Mite	63;181
Xyloborus dispar F.	274[6];1106[6]
Xyloborus monographus F.(det. Kubisz)	259[7]
Xyloterus domesticus	188k
Xyloterus lineatus Oll.	49;59;88;1085

1 The insects associated with *O. querci* and its synonyms are not included here, as they are considered in **Table 2.4.**
2 *Ophiostoma floccosum.*
3 Reported as *O. pluriannulatum*, but doubtful since synnemata were present. Possibly *O. piceae* or *O. querci* according to the description.
4 *Ophiostoma perfectum.*
5 Reported as *O. piceae,* but possibly *O. querci.*
6 *Ophiostoma catonianum.*
7 *O. querci* treated as synonym of *O. piceae.*

Table 1.5. Morphology of the teleomorph *Ophiostoma piceae.*

			Münch, 1907 (66)	MacCallum, 1922 (53)	Lagerberg *et al.*, 1927 (51)	Nisikado *et al.*, 1935 (293)	Siemaszko, 1939 (247)	Frisullo *et al.*, 1989 (570)	Przybyl & Morelet, 1993 (367)
Perithecia:	**Base**	**colour**	black	black	-	dark brown or black	-	black	dark
		diameter	160-240 µ	150-250 µ	192-224 µ	(105-)157(-225) µ	130-240 µ	(70-)80(-120) µ	100-180(-220) µ
		ornamentation	sometimes covered with hair-like hyphae	fine, light brown hair-like hyphae	straight, distended hairs	sometimes covered with mycelial strands	-	-	-
	Neck	**length**	800-1200 µ	850-1000(-1200)µ	1060-1500-1970 µ	(650-)1247(-1950) µ	600-1600 µ	< 1100 µ	600-1600 µ
		width: base	20-30 µ	20-40 µ	32 µ	(5-)26(-55) µ	20-40 µ	< 60 µ	-
		width: apex	-	-	14-16 µ	(3-)10(-18) µ	7.8-17.5 µ	> 15 µ	-
		ostiolar hyphae	present, 20-50 µ	-	present, 10.7-21.4 x 2.4-3.2 µ	10-15 colorless cilia, 20-30 µ	- 10-30 µ	14-28	about 25, 38(-55) µ
Ascospores:	**Colour**		colorless	-	-	colorless	-	hyaline	-
	Septation		-	-	-	-	-	-	-
	Shape:	**end view**	-	-	-	-	-	-	-
	Shape:	**face view**	cylindrical	-	-	long elliptical	-	-	-
	Shape:	**side view**	slightly curved	slightly curved	-	reniform	-	reniform	reniform
	Length		3.5-4.5 µ	3.4.5 µ	2.3-4.6 µ	(2.8-)3.7(-4.8) µ	2.5-3.5-4 µ	(2-)3(-5) µ	(3.5-)2.5 µ
	Width		1.5-2 µ	-	1.6 µ	(0.8-)1.4(-2.3) µ	1-1.5 µ	(1-)1.5(-2) µ	1.5 µ
Figures[1]			-	D pl. IX	D p. 177	P & D pl. XXV, XXVIII	P pl.III	P p.83	-

[1] D = Drawing(s); P = Photograph(s)

Morphology of teleomorph also measured and described in: [(1085) D p. 50].

Other illustrations: [(16) D. p.580].

Table 1.6. Morphology of the teleomorph *Ophiostoma piceae* in cases where *O. querci* was treated as its synonym.

			Hunt, 1956 (45)	Upadhyay, 1981 (88)	Butin & Aquilar, 1984 (11)	Hutchsion & Reid, 1988 (176)	Kowalski & Butin, 1989 (50)	Przybyl & De Hoog, 1989 (72)	Eisenhauer, 1991 (25)
Perithecia:	**Base**	**colour**	black	black	black	black	black	-	black
		diameter	80-180 µ	77-192 µ	100-230 µ	100-195 µ	80-100 µ	70-145 µ	81-115-200 µ
		ornamentation	ornamented with few ventral hairs up to 120 x 3.5 µ	ornamented with brown hyphal hairs, < 130 x 5 µ	rigid dark bown hyphal hairs, < 75 µ long	ornamented or smooth	a few straight brown hyphal hairs, < 150 µ long	< 85 µ long	-
	Neck	**length**	<1000 µ	500-1500(-2900) µ	900-1800 µ	530-1860 µ	400-1100 µ	380-1000 µ	167-1570 µ
		width: base	20-50 µ	18-45(-52) µ	25-40 µ	22-40 µ	15-45 µ	25-35 µ	12-36 µ
		width: apex	5-25 µ	4.5-23 µ	10-20 µ	7.5-16.0 µ	9-20 µ	7-12 µ	6-18(20) µ
		ostiolar hyphae	tapered, 15-25 in number, 10-25(-40) x 2-3 µ	hyaline, cylindrical, blunt, divergent, sparsely septate	divergent	hyaline, straight or divergent, 6-25 x 1.2-2.5 µ	divergent, 25 x 1 µ,	hyaline, cylindrical, 6-20 in number, < 62 µ	divergent
Ascospores:	**Colour**		hyaline	hyaline	Hyaline	hyaline	Hyaline	hyaline	-
	Septation		1-celled	1-celled	1-celled	1-celled	1-celled	1-celled	-
	Shape:	**end view**	-	globose	-	spherical	-	-	-
	Shape:	**face view**	-	broadly elliptical	broad elliptical	ellipsoid	elliptical	-	-
	Shape:	**side view**	bean-shaped	lunate or orange section shaped	slightly curved	allantoid	orange section	allantoid	3.5-6 µ
	Length		3-4.5 µ	(2-)2.5-4.5(-5) µ	3.5-4.5 µ	2.8-4.5 µ	3.1-3.7 µ	2-6 µ	1.5-2.5 µ
	Width		1.5-2 µ	1.5-2 µ	1-2 µ	1-2.2 µ	2 µ	1-2 µ	P p.270
Figures[1]			-	-	D p. 82	P p. 70-71	D p. 241	-	-

[1] D = Drawing(s); P = Photograph(s)

Morphology of teleomorph also measured and described in: (671); [as *O. perfectum* (17);(892)].

Other illustrations: [as *O. floccosum* (58) D. p. 216].

Table 1.7. Morphology of the *Pesotum* anamorph of *Ophiostoma piceae*.

		Münch, 1907 (66)	MacCallum, 1922 (53)	Lagerberg *et al.*, 1927 (51)	Nisikado *et al.*, 1935 (293)	Frisullo *et al.*, 1989 (570)	Przybyl & Morelet, 1993 (367)	Halmschlager *et al.*, 1994 (359)
Genus/species		-	-	-	*Graphium*	*Graphium*	*Graphium piceae*	*Graphium*
Conidiophores (synnemata):	**length**	500-100 µ	-	-	(120-)403(-740) µ	(1300-)1400(-1600) µ	200-700(-800) µ	(228-)849(-1946) µ
	width: head	-	-	-	-	-	-	(65-)157(-334) µ
	width: base	-	-	-	(8-)26(-55) µ	(27-)32(-35) µ	-	(10-)33(-106) µ
	width: apex	-	-	-	-	-	-	(16-)37(-106) µ
	colour: apex	-	hyaline	-	colorless	-	-	-
	colour: stalk	black-brown	black	-	brown or dark brown	-	-	-
Conidiogenous cells:		-	-	-	-	holoblastc, annelidic	(8-)10(-13) µ	-
Conidia:	**colour**	hyaline	-	-	colorless	hyaline	-	-
	shape	-	-	-	elliptical	ovoid to ellipsoidal	oblong	-
	length	3.5-4 µ	3-4 µ	3.2-4.8 µ	3-8 µ	(2.5-)4(-6) µ	(4.5-)2.5 µ	-
	width	1.7 µ	1.5-1.75 µ	16.-1.9 µ	2-4 µ	(1-)1.5(-3) µ	1(-1.5) µ	-
Figures[1]		D p. 548, 549, 550, 553, 554	D pl. IX	P p. 180-181	P pl.XXV	P p. 83	-	-

[1] D = Drawing(s); P = Photograph(s)

Morphology of *Pesotum* anamorph also measured and described in: [1085 (D p.48, P p.49)]; [as *G. piceae* (892)]; [(1110) D p.168].

Other illustrations: [(16) D. p.580]; [(49) Fig. 1]; [(179) D. p.205]; [(1) P pl. 9-10]; [(389) SEM p. 11].

Table 1.8. Morphology of the *Pesotum* anamorph of *Ophiostoma piceae* in cases where *O. querci* was treated as its synonym.

		Hunt, 1956 (45)	Crane & Schocknecht, 1973 (13)	Upadhyay, 1981 (88)	Butin & Aquilar, 1984 (11)
Genus/species		-	*Pesotum piceae*	*Pesotum piceae*	-
Conidiophores(synnemata):	**length**	< 1500 µ	500-1022 µ	(300-)470-1200(-1500) µ	300-800 µ
	width: base	< 30 µ	20-30 µ	12-30 µ	30-50 µ
	colour: apex	hyaline	subhyaline	pale brown or subhyaline	brown - subhyaline
	colour: stalk	brown to black	dark brown to black	dark brown to black	-
Conidiogenous cells:		-	hyaline to subhyaline, filiform, simple or branched, terminal, polyblastic, sympodial conidiogenesis, nodules or denticles12.4-20(-23.8) x1µ	penicillately branched, sympodial, aseptate, simple, hyaline, 9-21(-25) long, 1-2 µ at base	-
Conidia:	**colour**	hyaline	hyaline	hyaline	hyaline
	septation	-	1-celled	1-celled	1-celled
	shape	ellipsoid to ovoid	oblong to cylindrical or obovate, reniform to allantoid	ellipsoidal to ovoid, oblong to cylindrical, curved or allantoid	elliptical - ovoid, oblong - cylindrical
	length	3-5 µ	3-4.5(-5.5) µ	2.5-5(-5.5) µ	4-6 µ
	width	1.2.5 µ	1.5(-2) µ	1-2.5 µ	2.5 µ
Figures[1]		D pl.3 p.15	P p. 350-351.	-	D p. 82

(Continue on following page.)

[1] D = Drawing(s); P = Photograph(s)

Table 1.8. Morphology of the *Pesotum* anamorph of *Ophiostoma piceae* in cases where *O. querci* was treated as its synonym (continued).

		Hutchison & Reid, 1988 (176)	Kowalski & Butin, 1989 (50)	Przybyl & De Hoog, 1989 (72)	Degreef & Malaisse, 1992 (365)
Genus/species		-	-	-	*Pesotum*
Conidiophores(synnemata):	**length**	165-1930 µ	360-800 (-1400) µ	(150-)450-600(-700) µ	250-750 µ
	width: base	11-130 µ	-	-	45-60 µ
	colour: apex	-	-	pale brown	hyaline
	colour: stalk	-	black	dark brown	-
Conidiogenous cells:		hyaline, polyblastic, sympodial, slghtly tapering, terminal to intercalary	-	slender with short, sympodial rachis, few inconspicious scars	-
Conidia:	**colour**	hyaline	hyaline	subhyaline	hyaline
	septation	1-celled	1-celled	-	unicellular
	shape	ellipsoidal, ovoid to oblong, slightly curved	cylindrical - allantoid	oblong	ellipsoid to ovoid
	length	3-6 µ	3.1-3.7 µ	2.5-5.5 µ	3-4 µ
	width	1-2 µ	1.5-2 µ	2.0-2.5 µ	1-2 µ
Figures[1]		P p.70-71	D p. 241	D p. 181-2	P p.110

[1] D = Drawing(s); P = Photograph(s)

Table 1.9. Morphology of the *Sporothrix* anamorph of *Ophiostoma piceae*.

		Münch, 1907 (66)	MacCallum, 1922 (53)	Lagerberg *et al.*, 1927 (51)	Nisikado *et al.*, 1935 (293)	Przybyl & Morelet, 1993 (367)
Genus		*Cladosporium* (3 types)	*Cladosporium*	*Cephalosporium*	*Cephalosporium* to *Cladosporium*	*Sporothrix*
Conidiogenous cells:	**denticles**	-	-	-	2 or 3 or many protuberances verticillately	-
Conidia:	**colour**	-	-	-	(4-)8(-22) µ	hyaline
	septation	-	-	-	2-4 µ	0-2
	shape	ellipsoid	-	-	colorless, spindle-shaped, elliptical or long elliptical with round or pointed ends	clavate
	length	6-15 µ	4-15 µ	8-12.8 µ	-	(7-)16(-28) µ
	width	4 µ	2.5-3.5 µ	3.2-4 µ	-	-
Colony:	**growth rate**	-	-	30 mm in 10 days	35 mm in 7 days	(67-)72(-79) mm in 10 days
	colour	variable from black to white	-	light grey, turning dark olive-green	colorless to dark olive	grey, reverse pale brownish
	morphology	-	-	compact air-mycelium	thin with aerial mycelium	scant - floccose, felty
Figures[1]		D p. 551, 552, 553	D pl. IX	P p. 176; D p. 179	D pl. XXVII	-

[1] D = Drawing(s); P = Photograph(s)

Morphology of *Sporothrix* anamorph also described in: [(1) as '*Cladosporium, & Cephalosporium*]; [(892) as *Sporothrix* and *Hyalodendron*]; [(1085) as *Cladosporium*]; [(1110) D p.168].
Colony morphology of *O. piceae* and *O. floccosum* described in (407).
Other illustrations: [(1) P pl. 9-10]; [(16) D. p.580]; [(49) Fig. 1]; [(179) D. p.205]; [(389) as *Sporothrix, Hyalodendron & Acremonium,* SEM p. 11].

Table 1.10. Morphology of the *Sporothrix* anamorph of *Ophiostoma piceae* in cases where *O. querci* was treated as its synonym.

		Hunt, 1956 (45)	Crane & Schocknecht, 1973 (13)	Upadhyay, 1981 (88)	Butin & Aquilar, 1984 (11)
Genus		*Cladosporium* to *Cephalosporium*	*Pesotum piceae*[1]	*Sporothrix* to *Hyalodenrdon*	*Sporothrix* to *Hyalodendron*
Conidiophores:	**length**	10-25 µ	(12-)24-166(-175) µ	(10-)22-115(-175) µ	10-20-80 µ
	width: base	1-2 µ	1-2.2 µ	1-2.5(-3.5) µ	2-4 µ
	description	-	hyaline to light brown, septate simple or branched, terminal or lateral	hyaline, simple, loosely branched, septate at base,	-
Conidiogenous cells:		-	hyaline to subhyaline, elongated or inflated, polyblastic	terminal. sympodial, denticulate	denticulate
	denticles	-	prominent	cylindrical or inflated, < 2 µ long	-
Conidia:	**colour**	hyaline	hyaline	hyaline	hyaline
	septation	-	0-1	1-celled	1-celled
	shape	ellipsoid, often pointed at one end	cylindrical, fusiform-elliptical, rounded at apex, tapering, holoblastic	cylindrical, elliptical or fusiform,	elliptical or fusiform with pointed base
	length	3-9 µ	(3.5-)5-16(-20) µ	(3-)5-15 µ	4-10 µ
	width	1-3 µ	1-2 µ	1-3.5 µ	2 µ
Colony:	**growth rate**	25 mm/ 10 days (22-24 °C)	-	20-25 mm in 12 days (22°C)	40 mm/ 10 days (rtemp)
	colour	grayish white to gray to brown	white to grey-white to brown	white to grayish brown	white to whitegrey to greyish brown
	morphology	aerial mycelial mat, suppressed when older	immersed or floccose	effuse, superficial and immersed	effuse, dense mycelium
Figures[2]		-	P p.350-351.	-	D p. 82

(Continued on following page).

[1] The species description of *P. piceae* includes both the synnematous and mononematous anamorphs of *O. piceae*.

[2] D = Drawing(s); P = Photograph(s)

Table 1.10. Morphology of the *Sporothrix* anamorph of *Ophiostoma piceae* in cases where *O. querci* was treated as its synonym (continued).

		Hutchison & Reid, 1988 (176)	Kowalski & Butin, 1989 (50)	Przybyl & De Hoog, 1989 (72)	Degreef & Malaisse, 1992 (365)
Genus		*Sporothrix* to *Hyalodendron*	*Sporothrix* to *Hyalodendron*	*Sporothrix*	*Sporothrix* to *Hyalodendron*
Conidiophores:	**length**	-	20-80 µ	(10-)20-55(-70) µ	10-70 µ
	width: base	-	2-4 µ	-	2 µ
	description	-	-	-	hyaline, ramified
Conidiogenous cells:		terminal, integrated	denticulate	terminal or lateral	hyaline with ramifications
	denticles	-	-	0.5-1.5(-2) µ	-
Conidia:	**colour**	hyaline	hyaline	hyaline	hyaline
	septation	0-1	1-celled	0-1(-2)	unicellular
	shape	cylindrical, fusiform to clavate, tapering	elliptical	-	elliptical to globose
	length	4-10 µ	3.5-6 µ	(7-)10-25(-34) µ	8-70 µ
	width	1-2 µ	2-3 µ	2.0-3.5 µ	1-2 µ
Colony:	**growth rate**	44-50 mm in 12 days	45 mm in 12 days	15-20(-35) mm in 10 days	-
	colour	variable, brownish-yellow with grey, finally black	white to grey-brown	pale to dark brown	-
	morphology	floccose to funiculose, often sectored	flocculose	farinose to floccose, vague concentric zones	-
Figures[1]		P. p.70-71	D p. 241	-	-

[1] D = Drawing(s); P = Photograph(s)

Table 2.1. Present taxonomic status of *Ophiostoma querci.*

Species name	Ref.[1]
Ophiostoma querci (Georgév.) Nannf., in Melin & Nannfeldt, Sven. Skogsvardsforen. Tidskr. 32: 408. 1934.[2,3]	
≡ *Ceratostomella querci* Georgév., C. R. Acad. Sci. Paris 183: 759. 1926.	
≡ *Ceratostomella quercus* Georgév., Biol. Gen. 3: 245. 1927.	
≡ *Ceratocystis querci* (Georgév.) C. Moreau, Revue Mycol., Suppl. Colon. 17: 22. 1952.	
= ?*Sphaeria dryina* Pers., Syn. Meth. Fung. 2:58. 1801. - Syst. Mycol. 2:473. 1923.[4]	72
≡ *Sphaeria pilifera* Fr. var. *dryina* (Pers.) Fr., Syst. Mycol. 2:473. 1922.	
≡ *Ceratostoma piliferum* (Fr.) Fuckel var. *dryinum* (Pers.) Sacc., Syll. Fung. 1:219. 1882.	
= *Ophiostoma fagi* (Loos) Nannf., in Melin & Nannfeldt, Svensk SkogsFör. Tidskr. 32: 408. 1934.[2,6]	1096
≡ *Ceratostomella fagi* Loos, Arch. Mikrobiol. 3: 376. 1932.	
≡ *Ceratocystis fagi* (Loos) C. Moreau, Revue Mycol., Suppl. Colon. 17: 22. 1952.	
≡ ?*Ceratocystis fagi* (Loos) Paclt, Ces Mykol. 8: 80. 1954.[5]	
= ?*Ophiostoma roboris* Georgescu & Teodoru, Anal. Inst. Cerc. Exp. For., Ser. 1, 11: 198. 1948.[4]	72
≡ *Ceratocystis roboris* (Georgescu & Teodoru) Potlajchuk, in Potlajchuk & Schekunova, Nov. Sist. Niz. Rast. 22: 154. 1985.	
= ?*Ophiostoma valachicum* Georgescu & Teodoru, Anal. Inst. Cerc. Exp. For., Ser. 1, 11: 198. 1948.[4]	72
≡ *Ceratocystis valachicum* (Georgescu & Teodoru) Potlajchuk, in Potlajchuk & Schekunova, Nov. Sist. Niz. Rast. 22: 155. 1985.	
= ?*Ophiostoma kubanicum* (Sczerbin-Parfenenko) Potlajchuk, in Potlajchuk & Schekunova, Nov. Sist. niz. Rast. 22:153. 1985.[4]	72
≡ *Ceratocystis kubanicum* Sczerbin-Parfenenko, Goslesbumizgat p.49. 1953.	
= ?*Ceratostomella quercus* Santos & Camara, Agron. lusit. 17:136. 1955.[6]	

1 Reference suggesting synonymy.

2 Elias Melin and J.A. Nannfeldt were the authors of the paper in which *Ceratostomella quercus* and *Ceratostomella fagi* were placed in the genus *Ophiostoma* (288). In some subsequent papers, the new combinations were cited as *O. quercus* (Georgev.) Melin & Nannf. and *O. fagi* (Loos) Melin & Nannf. (e.g. in 97). On page 408 of the original paper, however, only Nannfeldt was given as authority of the new combinations. According to Article 46.2 of the Code of Botanical Nomenclature, the correct names and authorities for these species are as cited in the table above.

3 Although Melin and Nannfeldt called the new combination *O. quercus* (Georgév.) Nannf., Chapter 7 of this thesis explains why it is more correct to refer to *O. querci* (Georgév.) Nannf.

4 No type material exist for these species, but based on the original descriptions, they are considered synonyms of *O. querci* (72).

5 *Ceratostomella fagi* Loos was transferred to the genus *Ceratocystis* by Paclt in 1954 (105), but the new combination was already made by Moreau in 1952 (61). The name *Cer. fagi* (Loos) Paclt is thus not valid.

6 The name *Cer. quercus* Santos & Camara can be confused with *Cer. quercus* Georgév. The first was described from *Quercus* leaves in Portugal (107). The original description could, however, not be obtained. If the species has persistent asci, it might be a valid species of *Ceratostomella*. However, if it has dehiscent asci and is a species of *Ophiostoma*, the name will not be valid and the taxonomy will have to be reconsidered.

Table 2.2. Distribution of *Ophiostoma querci* and its synonyms.

Origin	Reference(s)
Australia	1096
Austria	400a[1,2];359;944
Azerbaijan	8;558
Belgium	408[1]
Bulgaria	164[1,2]
Canada	558;944
Canada, Ontario	8
Canada, Quebec	8
Czech Republic	50;408[1];669[1,2]
Europe	50
France	8;62;361;367;
Germany	101[3];287[3];289;364;645;1096[3];1124
Hungary	8
Korea	1096
Moldova (then Moldavia in USSR)	1097[1,2]
New Zealand	814;1096;1099
Poland	8;359;367;408[1];533;558;944
Portugal	107[4]
Romania	8;106[1,2]
Russia	579[1,2,5];601[1];1033[1,2,3,5];1098[1,2,5];1104[1,2,5]
South Africa	990a[6];1096
Sweden	359;1096
Switzerland	359;431;
Tadjikistan	8;558;
UK	8;268;408[1];558; 944;1096
UK, England	8
Uruguay	1096
USA	899;944
USA, Washington	1096
Yugoslavia	50;101;224;236

1 Reported as *O. roboris*.
2 Reported as *O. valachicum*.
3 Reported as *O. fagi*.
4 Reported as *Ceratostomella quercus* Santos & Camara.
5 Reported as *O. kubanicum*.
6 Reported as a *Graphium* species, associated with a *Sporotrichum* species, producing perithecia in culture. Possibly *O. querci*.

Table 2.3. Hosts of *Ophiostoma querci* and its synonyms.

Host	Family	Reference(s)
Abies sp.	Pinaceae	1096
Betula sp.	Betulaceae	421[1]
Cupressocyparis macrocarpa	Pinaceae	814
Eucalyptus sp.	Myrtaceae	1096
Fagus spp.	Fagaceae	8;287[2];400;558;1096
Fagus orientalis Lipsky	"	1103[2]
Fagus sylvatica L.	"	72[2];101[2];1124
Fraxinus excelsior L.	Oleaceae	1124
Malus domestica	Rosaceae	1103[1]
Olinia sp.	Oliniaceae	433
Pinus spp.	Pinaceae	8;558;1096
Pinus radiata D. Don	"	814;861;1099;
Pseudotsuga sp.	"	1096
Quercus spp.	Fagaceae	8;62;107[3];164[1,4];289;1097[1,4];1103[1,4,5]; 367; 400;404;408[1];558;579[1,4,5];601[1];944;1096; 1104[1,4,5];1124
Quercus longipes	"	8;558
Quercus pedunculata Ehrh.	"	101;224;236;645
Quercus petraea (Matt) Liebl	"	8;62;359;361;367
Quercus robur L.	"	8;106[1];268;359;364;367;408[1];431;533;669[4]
Quercus robur L. var. *tardissima*	"	669[1]
Quercus rubra L.	"	8
Quercus serrata Thunb.	"	389[4]
Tsuga spp.	Pinaceae	944
Ulmus carpinifolia	Ulmaceae	8;558

[1] Reported as *O. roboris*.
[2] Reported as *O. fagi*.
[3] Reported as *Ceratostomella quercus* Santos & Camara.
[4] Reported as *O. valachicum*.
[5] Reported as *O. kubanicum*.

Table 2.4. Insects associated with *Ophiostoma querci* and its synonyms.

Insect	Reference(s)
Ips typographus	268
Leperisinus varius	1124
Mesosa myops Dalm (yellow spotted longhorn beetle)	421[1]
Scolytus intricatus Ratz. (oak bark beetle)	421[1];1098[1,2,3]; 1124
Taphrorychus bicolor	1124

[1] Reported as *O. roboris*.
[2] Reported as *O. kubanicum*.
[3] Reported as *O. valachicum*.

Table 2.5. Morphology of the teleomorph of *Ophiostoma querci*.

			Georgévitch, 1926 (236)	Georgévitch, 1927 (224)	Lehmann, 1932 (645)	Sczerbin-Parfenenko, 1953 (1104)	Morelet, 1992 (62)	Przybyl, 1992 (408) [1]	Pzybyl & Morelet, 1993 (367)
Perithecia:	**Base**	**colour**	-	-	black	-	black	-	brown
		diameter	(129) 150-240 µ	(129)150-240 µ	115-130 µ	150-240 µ	80-185 µ	96-165 µ	90-160(-190) µ
		ornamentation	-	-	none	-	-	-	-
	Neck	**length**	970-990 µ	970-990 µ	630-850 µ	700-800 µ	1000-2000 µ	480-2220 µ	1100-1900 µ
		width: base	(22) 27 µ	22 µ	21-24 µ	-	- µ	15-33 µ	-
		width: apex	14 µ	14-17 µ	8-10 µ	-	-µ	8-22 µ	-
		ostiolar hyphae	< 31,5 x 5 µ	31,5 x 2 µ	12-20 in number, 23-23 µ	-	septate, < 33 µ long	7.45 x 1 µ	15 in number, 21(-37) µ
Ascospores:	**Colour**		-	-	-	-	hyaline	-	-
	Septation		-	-	-	-	1-celled	-	-
	Shape:	**side view**	reniform	reniform	kidney-shaped	-	reniform	allantoid	allantoid
	Length		4 µ	4 µ	3.2 µ	4 µ	2.8-4.3 µ	3-4.5 µ	(3.5-)2.5 µ
	Width		2 µ	2 µ	1.9 µ	2 µ	1.4-2 µ	-	1.5 µ
Figures[2]			-	P pl. VII	-	-	-	-	-

[1] Described as *O. roboris*.

[2] D = Drawing(s); P = Photograph(s)

Table 2.6. Morphology of the *Pesotum* anamorph of *Ophiostoma querci*.

		Georgévitch, 1926 (236)	Georgévitch, 1927 (224)	Lehmann, 1932 (645)	Wilson, 1967 (421) [1]
Genus		*Graphium*	*Graphium*	*Graphium*	*Graphium*
Conidiophores (synnemata):	**length**	260-285(-619) µ	- µ	(300-)425(-600) µ	(320-)522(-1110) µ
	width: head	-	-	130-300 µ	-
	width: base	-	- µ	> 20 µ	41 µ
	width: apex	120 µ	120 µ	< 90 µ	8 µ
	colour: apex	-	-	-	colorless
	colour: stalk	brown	brown	-	-
Conidiogenous cells:		-	1.7-3 µ	-	-
Conidia:	**colour**	hyaline	hyaline	-	colorless
	septation	-	-	-	single-celled
	shape	elliptical to oval	elliptical to oval	elliptical	oval, rarely pear-shaped & curved
	length	4 µ	4 µ	3.6 µ	2.8-5 µ
	width	2 µ	2 µ	2.3 µ	1.3-1.7 µ
Figures[2]		-	P Tafel VII	-	-

(Continued on following page.)

[1] Described as *O. roboris*.

[2] D = Drawing(s); P = Photograph(s)

Table 2.6. Morphology of the *Pesotum* anamorph of *Ophiostoma querci* (continued).

		Eisenhauer, 1991 (25) [1]	Morelet, 1992 (62)	Przybyl, 1992 (408) [2]	Pzybyl & Morelet, 1993 (367)	Halmschlager *et al.*, 1994 (359)
Genus/species		*Pesotum*	*Graphium pirinum*	*Pesotum*	*Graphium pirinum*	*Graphium*
Conidiophores (synnemata):	**length**	(400)600-750 µ	300-1200 µ	200-700 µ	(130-)350-500(-600) µ	(228-)523(-1097) µ
	width: head	-	-	-	-	(52-)117(-272) µ
	width: base	(20)30-60 µ	-	-	-	(8-)24(-70) µ
	width: apex	(20)30-60 µ	-	-	-	(11-)26(-65) µ
	colour: apex	subhyaline	lighter	-	-	-
	colour: stalk	dark brown	dark brown to black	-	-	-
Conidiogenous cells:		12-20 x 1.5-3µ	sympodial, penicillately branched	-	(8-)13-14(-16) µ	-
Conidia:	**colour**	subhyaline	hyaline	-	-	-
	septation	-	1-celled	-	-	-
	shape	-	ellipsoid tp ovoid	-	oblong	-
	length	3-5.5 µ	2.8-4.7(-5.7) µ	2.5-5.5 µ	(4.5-)2.5 µ	-
	width	1.5-2.5 µ	1.4-2(-2.4) µ	2-2.5 µ	1.5(-2.5) µ	-
Figures[3]		P p.272	-	-	-	-

[1] Described as *O. piceae*, but most likely *O. querci*.
[2] Described as *O. roboris*.
[3] D = Drawing(s); P = Photograph(s)

Table 2.7. Morphology of the *Sporothrix* anamorph of *Ophiostoma querci*.

		Wilson, 1967 (421) [1]	Eisenhauer, 1991 (25) [2]	Morelet, 1992 (62)	Przybyl, 1992 (408) [1]	Pzybyl & Morelet, 1993 (367)
Genus/species		*Hyalodendron*	*Sporothrix*	*Sporothrix pirinum*	*Sporothrix*	*Sporothrix pirinum*
Conidiophores:	**length**	19.6-64.8 µ	10-15 µ	-	-	-
	width: base	-	0.5-1 µ	-	-	-
Conidiogenous cells:		-	hyaline	terminal or intercalary	-	-
		-		denticulate	-	-
Conidia:	**colour**	-	subhyaline	hyaline	-	hyaline
	septation	sometimes	-	0-2	0-3	-
	shape	diverse in form	-	cylindric to fusiforme	-	0-2
	length	4.5-24.2 µ	1-1.5 µ	4-20 µ	3-45 µ	clavate
	width	1.7-2.2 µ	0.25-0.5 µ	1.4-2.8 µ	1-3 µ	(7-)15(-27) µ
Colony:	**growth rate**	-	-	-	-	(55-)57(-67) mm in 10 days
	colour	-	-	-	-	whitish grey, reverse pale brownish
	morphology	-	-	-	-	scant - floccose, felty
Figures[3]		-	P p. 273	-	-	-

[1] Described as *O. roboris*.
[2] Described as *O. piceae*, but most likely *O. querci*.
[3] D = Drawing(s); P = Photograph(s)

References to Appendices 1, 2 and 3

Take it from an erstwhile librarian: there are far more mistakes in the references section of a paper than anywhere else.

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Summary / Opsomming

Sermons on brevity and chastity are about equally effective. Verbal promiscuity flows from poverty of language and obesity of thought, and from an unseemly haste to reach print.

- Eli Chernin

SUMMARY

The ophiostomatoid fungi are an economically important group of fungi, known for their ability to stain sapwood and cause tree diseases. In recent years, certain species in the group have also been considered as potential biological control agents in the pulp and paper industry. White mutants of species like *Ophiostoma piliferum* utilize pitch, which can cause problems in the pulping process, in freshly cut pulpwood chips. At the same time, other degrading fungi are out-competed. The first chapter of the thesis reviews the development, application, benefits and possible problems, of these biological control products. The possible application of such products in the South African pulp industry is also considered.

One of the major concerns for the application of a biological control product such *O. piliferum* in South Africa, is the fact that it consists of a living fungus originating in the USA. A survey was, therefore, conducted to determine whether the fungus occurs in South Africa. The typical niche for *O. piliferum* is stained logs, lumber, and pulpwood chips. Isolates resembling *O. piliferum* were obtained from both exotic and indigenous wood sources. Based on morphology, these isolates could be separated into three groups, which resembled the descriptions of *O. stenoceras*, *O. pluriannulatum*, and *O. piceae*, respectively. For correct identification, the South African isolates had to be compared with herbarium material and authentic isolates from other parts of the world. The comparative taxonomic studies for the three groups of fungi form the basis of Chapters 4, 5 and 6 of this thesis.

The taxonomic history of the genera *Ophiostoma* and *Ceratocystis* is complicated and confused. Published literature on the two genera, with the emphasis on *Ophiostoma* and its associated anamorph genera, is reviewed in Chapter 3. This serves as a background for the four following chapters of the thesis.

Ribosomal DNA sequencing confirmed that one group of South African isolates is the same as *O. stenoceras* isolates from the Northern Hemisphere. Similar isolates from Colombia, Uruguay, and Kenya, were also included in the study and represent the first reports of *O. stenoceras* from these countries. *Ophiostoma albidum*, *O. abietinum* and *O. nigrocarpum*, all closely resemble *O. stenoceras* morphologically. Our sequence data show that *O. albidum* should be considered a synonym of *O. stenoceras*, and that *O. abietinum* is a synonym of *O. nigrocarpum*, which is a species distinct from *O. stenoceras*. For the past three decades, *O. stenoceras* has been considered the teleomorph of *Sporothrix schenckii*, the human pathogen. Our results, however, showed that rDNA sequences of the two species are significantly different, confirming that *S. schenckii* is a distinct species.

The group of South African isolates resembling *O. pluriannulatum*, differed from this Northern Hemisphere species in that isolates have light brown perithecial bases and club-shaped ornamental hyphae on the perithecial bases. Similar isolates were obtained from Equador and Indonesia. Ribosomal DNA sequence data made it possible to distinguish between the two groups, and the Southern Hemisphere fungus is, therefore, described as a new species, *Ophiostoma tropicale*.

Ophiostoma piceae and *O. querci* are virtually indistinguishable based on morphology, but hosts, rDNA sequences, and mating compatibility, can be used to separate the two species. By applying these criteria, the South African isolates resembling *O. piceae* obtained in the survey (Chapter 2), grouped with *O. querci*. Also included in the *O. querci* group were isolates from Brazil and Japan. One South African isolate, however, were identified as *O. floccosum*, representing the first report of this fungus from South Africa. The presence and distribution of species of the *O. piceae* complex in the Southern Hemisphere, are also discussed in Chapter 6.

In recent literature, some confusion has emerged regarding the use of the name *O. querci* as opposed to *O. quercus*. The last chapter of the thesis presents a brief review of the Latin and nomenclatural guidelines applicable in this particular case. The conclusion is that both names are grammatically acceptable. However, following the Code of Botanical Nomenclature, *O. querci* should be given preference.

The work presented in this thesis contributes significantly to our understanding of the ophiostomatoid fungi, and in the greater context, biodiversity, in South Africa. Ribosomal DNA sequencing was successfully applied in conjunction with traditional taxonomic criteria to distinguish between species. However, many new questions arose from these results, especially regarding the phylogeny of *Ophiostoma* spp. with *Sporothrix* anamorphs. The results obtained from this study, will serve as the foundation for future research addressing these questions.

KEYWORDS: ophiostomatoid, *Ophiostoma, Ceratocystis, Ophiostoma pluriannulatum, Ophiostoma tropicale, Ophiostoma piceae, Ophiostoma querci, Ophiostoma stenoceras, Sporothrix schenckii*

OPSOMMING

Die ophiostomatoïde fungi is 'n ekonomiese belangrike groep swamme wat bekend is vir hulle vermoë om vars hout te verkleur en boomsiektes te veroorsaak. Gedurende die afgelope dekade is sekere spesies in die groep ook oorweeg as potensiële biologiese beheeragente in die pulp- en papierindustrie. Wit mutante van spesies soos *Ophiostoma piliferum,* benut en verwyder hars, wat probleme in die verpulpingsproses veroorsaak, uit vars gesnipperde pulphout. Terselfdertyd word ander verkleurings- en verrottingsswamme d.m.v. kompetisie geïnhibeer. Die eerste hoofstuk van hierdie tesis is 'n oorsig oor die ontwikkeling, aanwending, voordele en moontlike probleme met hierdie biologiese beheeragente. Die potensiële aanwending van sulke produkte in Suid-Afrikaanse pulpmeulens word ook bespreek.

Een van die belangrikste vrese rondom die aanwending van 'n biologiese beheeragent soos *O. piliferum* in Suid-Afrika, is die feit dat dit bestaan uit 'n lewende fungus wat oorspronklik uit die VSA kom. 'n Opname is daarom gedoen om vas te stel of die swam in Suid-Afrika voorkom. Die tipiese nis vir *O. piliferum* is verkleurde stompe, planke en pulphoutsnippers. Isolate wat ooreenstem met *O. piliferum* is verkry van beide uitheemse en inheemse houtsoorte. Op grond van morfologie kon die isolate in drie groepe verdeel word, wat onderskeidelik ooreenstem met die beskrywings van *O. stenoceras, O. pluriannulatum* en *O. piceae.* Vir korrekte identifikasie moes hierdie isolate met herbariummateriaal en oorspronklike isolate van ander wêrelddele vergelyk word. Die vergelykende taksonomiese studies van hierdie drie groepe fungi vorm die basis vir Hoofstukke 4,5 en 6 van hierdie tesis.

Die taksonomiese geskiedenis van *Ophiostoma* en *Ceratocystis* is ingewikkeld en verwarrend. Die literatuur wat handel oor die twee genera, met die klem op *Ophiostoma* en die geassosieerde anamorf genera, word oorsigtelik behandel in Hoofstuk 3. Hierdie oorsig dien as agtergrond vir die daaropvolgende vier hoofstukke van die tesis.

Ribosomale DNS basisopeenvolging het bevestig dat een groep Suid-Afrikaanse isolate dieselfde is as *O. stenoceras* isolate afkomstig van die Noordelike Halfrond. Soortgelyke isolate van Colombia, Uruguay, en Kenia, is ook ingesluit in die studie. Dit is die eerste keer dat *O. stenoceras* in hierdie lande gerapporteer word. *Ophiostoma albidum, O. abietinum* en *O. nigrocarpum* toon morfologiese ooreenkomste met *O. stenoceras.* Resultate van basisopeenvolgingbepalings bevestig dat *O. albidum* 'n sinoniem is van *O. stenoceras*, en dat *O. abietinum* 'n sinoniem is van *O. nigrocarpum*, wat 'n aparte spesie van *O. stenoceras* is. Vir die afgelope dertig jaar is *O. stenoceras* beskou as die teleomorf van die menslike

patogeen, *Sporothrix schenckii*. Ons resultate wys egter dat ribosomale DNS basisopeenvolgings van die twee spesies beduidend verskil, wat bevestig dat *S. schenckii* 'n aparte spesie is.

Die groep Suid-Afrikaanse isolate wat morfologies grotendeels ooreenstem met *O. pluriannulatum*, verskil in sekere opsigte van dié spesie uit die Noordelike Halfrond. Perithecia van die Suid-Afrikaanse isolate het ligte bruin basisse en knuppelvormige ornamentele hifes op die perithecium basisse. Soortgelyke isolate is ook verkry uit Equador en Indonesia. Ribosomale DNS basisopeenvolgingsdata het dit moontlik gemaak om tussen die twee groepe te onderskei en die fungus uit die Suidelike halfrond word dus as 'n nuwe spesie, *Ophiostoma tropicale,* beskryf.

Ophiostoma piceae en *O. querci* kan feitlik nie van mekaar onderskei word op grond van morfologie nie, maar gashere, rDNS basisopeenvolgings, en paringstipes kan gebruik word om die twee spesies te skei. Op grond van hierdie kriteria, het die Suid-Afrikaanse isolate wat vergelykbaar is met *O. piceae* (Hoofstuk 2), duidelik saam met *O. querci* gegroepeer. Isolate van Japan en Brasilië het ook in die groep geval. Een Suid-Afrikaanse isolaat, is egter geïdentifiseer as *O. floccosum,* wat vir die eerste keer in Suid-Afrika gerapporteer word. Die teenwoordigheid en verspreiding van spesies uit die *O. piceae* kompleks in die Suidelike Halfrond word ook bespreek in Hoofstuk 6.

In onlangse artikels, heers daar heelwat verwarring rondom die gebruik van die naam *O. querci* teenoor *O. quercus*. Die laaste hoofstuk van die tesis is 'n kort oorsig van die Latynse en nomenklatuur riglyne wat van toepassing is in hierdie geval. Die gevolgtrekking is dat albei name grammaties korrek is. Ooreenkomstig die Kode vir Botaniese Nomenklatuur, moet *O. querci* egter voorkeur kry.

Die navorsing wat in hierdie tesis aangebied word, dra beduidend by tot 'n beter begrip van die ophiostomatoïde fungi, en in die groter konteks, biodiversiteit in Suid-Afrika. Ribosomale DNS basisopeenvolging is suksesvol aangewend, aanvullend tot tradisionele taksonomiese kriteria, om te onderskei tussen spesies. Baie nuwe vraagstukke ontstaan egter uit hierdie resultate, veral rakende die filogenie van *Ophiostoma* spp. met *Sporothrix* anamorwe. Die resultate van hierdie studie sal dien as die fondament vir verdere navorsing wat die bogenoemde vraagstukke sal aanspreek.