The occurrence of *Ophiostoma piliferum*-like fungi on pulpwood chips and other wood sources in South Africa

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THE OPHIOSTOMATOID FUNGI INCLUDE various economically important plant pathogens and sapstain fungi belonging to genera such as *Ophiostoma* and *Ceratocystis*. *Ophiostoma piliferum* is considered important because a white mutant of this fungus, marketed as Cartapip 97®, is applied on pulpwood chips of primarily softwoods to reduce pitch and prevent sapstain. The South African forestry industry could benefit greatly from a biological control product such as this. The importation of a product based on a living organism can, however, be a problem owing to quarantine regulations. The aim of this study was to determine whether *O. piliferum* occurs in South Africa. A literature survey revealed at least 20 confirmed reports of ophiostomatoid species having been present, but *O. piliferum* was not among them. We therefore conducted a survey during which isolates resembling *O. piliferum* were collected from pulpwood chips and other freshly cut wood sources, the typical niche of ophiostomatoid fungi. Based on morphology, the isolates collected could be divided into three groups, of which none represented *O. piliferum.* The first group resembled *O. stenoceras* and the second group *O. pluriannulatum.* The third group was identified as either *O. piceae* or *O. quercus,* two species that cannot be distinguished on the basis of morphology. We expect that the identity of all three groups will be confirmed by molecular studies. Although *O. piliferum* was not found in this investigation, the possibility that the fungus occurs in this country cannot be excluded. The isolates obtained in this study might also be considered in the development of a hardwood alternative to Cartapip 97®.

The ophiostomatoid fungi represent a phylogenetically diverse group of Ascomycetes that includes several economically important species from genera such as *Ophiostoma* H. & P. Sydow and *Ceratocystis* Ell. & Halst. The best-known species are probably the causal agents of Dutch elm disease, *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier.[1] Most ophiostomatoid species are not pathogenic, but are associated with sapstain or bluestain of timber that can greatly reduce the value of wood.[2] Not all ophiostomatoid species, however, are harmful to trees or detrimental to timber. For example, a white mutant of one of the sapstain species, *Ophiostoma piliferum* (Fries) H. & P. Sydow, is marketed as a pitch-removing and anti-sapstain agent, known as Cartapip 97®, in the United States and Europe.[3] 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.[4]

The South African forestry industry could benefit greatly from biological control agents such as Cartapip 97®. The Department of Agriculture, however, had to certify that Cartapip 97® was safe for general release before it could be imported. Preconditions for certification included showing that Cartapip 97® is non-pathogenic under South African conditions, and conducting a survey to determine whether *O. piliferum* occurs naturally in South Africa. Pathogenicity trials with the Cartapip 97® fungus demonstrated that it posed no threat to the forestry industry.[5]

The aims of the study reported here were to conduct a literature survey to determine which ophiostomatoid species have been reported from South Africa, and to collect and identify *O. piliferum*-like fungi occurring on wood in South Africa.

The literature review revealed that several ophiostomatoid fungi have been reported from South Africa (Table 1). Among these, *O. piliferum* was claimed in 1937 from pine in Mpumalanga.[6] No ascospores were observed in or around the perithecia, however, 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 cannot therefore be considered as positive evidence for the presence of *O. piliferum* in South Africa. Other reports included some *Ophiostoma* species, but none that resembles or is closely related to *O. piliferum.* Thus, there are no confirmed reports of *Ophiostoma* species from South Africa known to have potential in biological control.

Our survey of species resembling *O. piliferum* was conducted on woody substrates from niches where these fungi usually occur, such as wood chips in pulp mills. Hard- and softwood chips from three major pulp mills in South Africa (Sappi Kraft mill, Ngodwana, Mondi Kraft mill, Richards Bay, and Sappi Saiccor mill, 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 site. The sampling was repeated twice at each site within a year. From each bag, approximately 30 chips were randomly selected and incubated in ten 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 by transferring ascospore or conidial masses to 2% malt extract agar. Cultures resembling *O. piliferum,* obtained during previous studies from other wood sources in South Africa, were included in this study. All cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria.

All isolates were grown on water agar in the presence of sterilized pieces of wood (5 mm × 5 mm × 30 mm) representing the original host of the fungus. This was done to enhance the production of perithecia and ascospores. Cultures were incubated at 20–25°C until sexual structures formed. Fifty perithecia were removed from each of the isolates and mounted in lactophenol 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.

In total, 33 isolates of fungi resembling *O. piliferum* were obtained (Table 2). Ophiostomatoid fungi resembling *O. piliferum* were not particularly common on wood chips from the pulp mills. Only two isolates resembling *O. piliferum* were found on *Eucalyptus* chips, two on *Acacia mearnsii* (wattle) chips, and none from pine chips. Of the remaining 29 isolates,

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Table 1. Ophiostomatoid fungi previously reported from South Africa.

Year	Species[a]	Host	References
1927	*Sporotrichum beurmanii* Matr. & Ramond [= *Sporothrix schenckii* Hektoen & Perkins]	Human	15
1931	[b]*Thielaviopsis basicola* (Berk. & Br.) Ferraris	*Nicotiana tabacum*	16
1937	[c]*Ophiostoma piliferum* (Fries) H. & P. Sydow [= *Ceratostomella pilifera* (Fries) Winter]	Logs of *Pinus radiata*	6
1937	*Ceratocystis paradoxa* (Dade) Moreau [= *Thielaviopsis paradoxa* (de Seyn) v. Höhn]	*Saccharum officinarum*	15
1947	[b]*Graphium* sp. associated with *Sporotrichum* sp., producing perithecia.	Timber and air	17
1956	*Ceratocystis adiposa* (Butler) Moreau	Shoots of *Pinus* sp.	18
1965	*Chalara terrestris* Agnihothrudu & Barna	*Eucalyptus saligna*	19
1974	[b]*Graphium putredinis* (Corda) Hughes	Soil	20
1974	*Ceratocystis fimbriata* Ell. & Halst.	*Protea gigantea*	21
1978	*Leptographium reconditum* Jooste	*Triticum* rhizosphere	22
1980	*Ophiostoma ips* (Rumbold) Nannf. [= *Ceratocystis ips* (Rumbold) Moreau]	*Orthotomicus erosus*	23
1980	*Ophiostoma serpens* (Goid.) v. Arx [= *Verticicladiella alacris* M.J. Wingf. & Marasas]	Roots of *P. pinaster* & *P. radiata*	24
1983	*Leptographium lundbergii* Lag. & Melin [= *Verticicladiella truncata* M.J. Wingf. & Marasas]	Roots of *P. taeda*	25
1985	*Ophiostoma* spp. *Leptographium* spp. [b]*Graphium* spp.	*Orthotomicus erosus* *Hylurgus ligniperda* *Hylastes angustatus*	26, 27
1988	*Gondwanamyces proteae* (M.J. Wingf., Van Wyk & Marasas) Marais & M.J. Wingf. [= *Ceratocystiopsis proteae* M.J. Wingf., Van Wyk & Marasas]	*Protea repens*	28
1988	[b,d]*Graphium* sp.	*Orthotomicus erosus*	29
1993	*Sporothrix eucalypti* M.J. Wingf., Crous & Swart	*Eucalyptus grandis*	30
1993	*Gondwanamyces capensis* (M.J. Wingf. & Van Wyk) Marais & M.J. Wingf. [= *Ophiostoma capense* M.J. Wingf. & Van Wyk]	*Protea* spp.	31
1993	*Ceratocystis fimbriata* Ell. & Halst.	*Acacia mearnsii*	32
1994	*Ophiostoma splendens* Marais & M.J. Wingf.	*Protea* spp.	33
1994	[b,d]*Graphium pseudormiticum* M. Mouton & M.J. Wingf.	*Orthotomicus erosus*	34
1996	*Ceratocystis albofundus* M.J. Wingf., De Beer & Morris	*Protea* sp.	35
1997	*Ophiostoma protearum* Marais & M.J. Wingf.	*Protea* sp.	36
2001	*Ophiostoma africanum* Marais & M.J. Wingf.	*Protea gaguedi*	37

[a]The species name currently accepted is listed first. The name used in the original report is in brackets.
[b]These species are from genera treated in the past as anamorphs of *Ceratocystis* or *Ophiostoma*, but which are not associated with these teleomorph genera at present.
[c]Doubtful identification, see text.
[d]In 1994 described as a new species.[34] At present classified in the Microascales as part of the *Graphium penicilliodes* Corda complex.[38]

six originated from native hosts, one from a pine log, and 21 from exotic hardwoods.

On the basis of anamorph and teleomorph morphology, we believe that the isolates represent three distinct taxa that we refer to as Groups A, B and C (Table 2). The average length of ascospores of isolates from Group A was notably shorter than that of *O. piliferum,* and these isolates resembled *O. stenoceras* (Robak) Nannfeldt. This fungus has been treated as the teleomorph of *Sporothrix schenckii* Hektoen & Perkins ex Nicot & Mariat, the causal agent of sporotrichosis in humans and other mammals. The validity of this teleomorph-anamorph association has been disputed and several biochemical studies indicated that the two species might be distinct.[7] Berbee and Taylor[8] 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[9] and New Zealand,[10] 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 local mines during the previous century.[11] The fungus grows on *Eucalyptus* and wattle wood props used in the mines, and infects mine workers through small wounds.[12] The prevalence of *O. stenoceras*-like isolates on *Eucalyptus* species and wattle is therefore of particular significance. A phylogenetic study, comparing these isolates with authentic examples of *O. stenoceras* and *S. schenckii,* will provide the opportunity to clarify the relationship between the two species.

Isolates from Group B had annuli on their perithecial necks, which is not typical for *O. piliferum* but more characteristic of *O. pluriannulatum.* The perithecial bases of this species, however, have consistently been reported as black,[9] 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 in the northern hemisphere,[9] apart from one claimed from New Zealand.[10]

Isolates from Group C had perithecia and ascospores similar to those of *O. piliferum*; apart from the *Sporothrix* anamorph that is typical for *O. piliferum,* they also produced a *Pesotum* anamorph in culture, and thus resembled *O. piceae.* These isolates could, however, represent any of a number of species in the *O. piceae* complex, which are morphologically difficult to distinguish. Based on the host range, the South African isolates may represent *O. quercus* (Georgévitch) Nannf., considered to be the hardwood equivalent of *O. piceae,* which occurs primarily on conifers.[13] *Ophiostoma quercus* occurs almost exclusively on hardwoods in the northern hemisphere, and has been reported only from New Zealand[10] and Australia[13] in the southern.

All three groups resemble *O. piliferum.* The morphological differences, however, led us to conclude that none of these represented this species. The possibility that *O. piliferum* occurs in South Africa cannot, however, be excluded. The identities of the isolates investigated will have to be confirmed by comparison with type specimens, authenticated isolates, and DNA sequence data. Once the identities have been confirmed, South African isolates from Groups B and C may be considered as possible biological control agents of pitch and bluestain. Although Cartapip 97® is effective on various softwoods and selected hardwoods, it is less effective on hardwoods such as *Eucalyptus.*[14] Most isolates examined in this study

Table 2. Morphological characteristics of ophiostomatoid isolates resembling *Ophiostoma piliferum* from South Africa. Bold type indicates the major differences between the three groups and *O. piliferum*. Species names of hosts native to South Africa are underlined.

		*O. piliferum**	Group A	Group B	Group C
Anamorph:		*Sporothrix*	*Sporothrix*	*Sporothrix*	*Sporothrix* **Pesotum**
Ascospores					
length (μm):		3–5	**3**	5	4
width (μm):		1.5–2	1.5	1.5	1.5
Perithecia					
base:	colour	Black	Black	**Light brown**	Black
	diameter (μm)	75–250	135	150	160
neck:	length (μm)	300–3000	680	1470	900
	width: base (μm)	18–40	26	25	25
	width: neck (μm)	8–15	10	12	9
	annuli	0	0	**0–9**	0
Isolate numbers:		—	CMW2342–CMW2349; CMW2523–CMW2525	CMW0368 CMW0931 CMW0932 CMW0935 CMW1207 CMW1211 CMW1213 CMW1235 CMW1251	CMW0864 CMW0866 CMW0867 CMW1044 CMW1045 CMW1249 CMW1255 CMW1257 CMW2105 CMW2519 CMW2522 CMW2534 CMW7661
Hosts:		*Pinus* spp. *Populus* sp. *Platanus* sp.	*Eucalyptus* spp. *Acacia mearnsii*	*Eucalyptus* spp. *Jacaranda mimosifolia* *Ocotea bullata* *Macaranga capensis*	*Eucalyptus* spp. *Pinus* sp. *Quercus robur* *Psidium guajava* *Olinia ventosa*

*Characteristics according to Upadhyay.[9]

are common to hardwood species, which makes them more suitable candidates for application on hardwoods. Further studies on the efficacy of these isolates, however, as well as pathogenicity trials, will have to be conducted before any of these isolates can be used in field trials or on an industrial scale.

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