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DNA sequence comparisons of *Ophiostoma* spp., including *Ophiostoma aurorae* sp. nov., associated with pine bark beetles in South Africa

Xudong Zhou[1*], Z. Wilhelm de Beer[2] and Michael J. Wingfield[1]

[1]*Tree Protection Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI);* [2]*Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, Republic of South Africa*

**Correspondence*: Xudong Zhou, Xu.Zhou@fabi.up.ac.za

Abstract: Bark beetles (Coleoptera: Scolytinae) are well-recognized vectors of *Ophiostoma* species. Three non-native bark beetle species infest various *Pinus* species in South Africa, and they are known to carry at least 12 different species of ophiostomatoid fungi. Some of these fungi have not been identified to species level. The aim of this study was to determine or confirm the identities of *Ophiostoma* species associated with bark beetles in South Africa using comparisons of DNA sequence data. Identities of *Ophiostoma ips*, *O. floccosum*, *O. pluriannulatum, O. quercus* and *O. stenoceras* were confirmed. *Ophiostoma abietinum*, *O. piliferum* and *Pesotum fragrans* are recognised for the first time and the new species, *O. aurorae* sp. nov., is described from pine-infesting bark beetles in South Africa.

Taxonomic novelty: *Ophiostoma aurorae* X.D. Zhou & M.J. Wingf. sp. nov.
Key words: Bark beetle, *Ophiostoma*, phylogeny, taxonomy.

INTRODUCTION

Conifer-infesting bark beetles (Coleoptera: Scolytinae) are economically important forest insects. They include many primary pest species, which can attack healthy living trees and have caused significant economic losses to the global forestry industry (Wood & Bright 1992). In South Africa, three non-native bark beetle species, *Hylastes angustatus*, *Hylurgus ligniperda*, and *Orthotomicus erosus* infest various *Pinus* spp. (Tribe 1992). They are generally considered as secondary pests, although *H. angustatus* may undergo maturation feeding on healthy living seedlings causing significant losses during plantation establishment (Tribe 1992).

Bark beetles are well-known vectors of fungi, especially *Ophiostoma* species (Six 2003, Kirisits 2004, Harrington 2005). The ophiostomatoid fungi are a polyphyletic group of morphologically similar fungi, adapted for insect dispersal. Several ophiostomatoid fungi are important pathogens of conifers (Harrington & Cobb 1988, Wingfield *et al*. 1993b, Jacobs & Wingfield 2001), while many others can cause sapstain on logs and freshly cut wood (Wingfield *et al*. 1993b). The group includes the genera *Ceratocystis* Ellis & Halst., *Gondwanamyces* G.J. Marais & M.J. Wingf., *Sphaeronaemella* P. Karst. and *Cornuvesica* C.D. Viljoen, M.J. Wingf. & K. Jacobs and their anamorphs in the *Microascales* (Spatafora & Blackwell 1994, Hausner *et al.* 2000), and *Ophiostoma* Syd. & P. Syd., *Grosmannia* Goid. and *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr., with their *Pesotum* J.L. Crane & Schokn., *Leptographium* Lagerb. & Melin, *Sporothrix* Hektoen & C.F. Perkins and *Hyalorhinocladiella* H.P. Upadhyay & W.B. Kendr. anamorphs in the *Ophiostomatales* (Zipfel *et al.* 2006).

More than 30 ophiostomatoid fungi have been reported from South Africa (Table 1), of which at least 12 are associated with the three exotic pine-infesting bark beetle species in the country (Zhou *et al.* 2001). These fungi have been isolated from the insects or their galleries and identified based on their morphological characteristics (Zhou *et al*. 2001). Eight of these species belong to the genus *Ophiostoma* (*sensu* Zipfel *et al*. 2006) or its anamorphs. However especially those of which only the anamorphs were observed remained to be identified to species level (Zhou *et al*. 2001). The aim of this study was to use DNA sequence comparisons to confirm the identities of the *Ophiostoma* spp. (Zipfel *et al.* 2006) from South African pine bark beetles, previously identified based only on morphology (Zhou *et al*. 2001).

MATERIALS AND METHODS

Fungal isolates

Twelve isolates (Table 2) used in this study originated from a previous investigation of ophiostomatoid fungi associated with the three pine-infesting bark beetle species in South Africa (Zhou *et al*. 2001). All cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. A relevant sub-set of cultures has been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands.

DNA sequencing and phylogenetic analyses

Single hyphal-tip cultures from the 12 isolates were grown on 2 % MEA (20 g Biolab malt extract, 20 g Biolab agar, and 1000 mL deionised water). DNA was extracted using PrepMan Ultra Sample reagent (Applied Biosystems) as described by Aghayeva *et al*. (2004). The ITS (internal transcribed spacer) region of

the ribosomal RNA operon was amplified using primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al*. 1990). PCR products were sequenced with the same primers. Conditions for PCR amplification and sequencing reactions were as described by Zhou *et al*. (2004b). For comparisons, ITS sequences of closely related taxa (Table 3) were obtained from GenBank.

All sequences were aligned using MAFFT v. 5.667 (Katoh *et al*. 2002). Phylogenetic relationships among the isolates were determined using distance analyses in MEGA3 (http://www.megasoftware.net/). Trees were constructed using the Neighbour-joining tree-building algorithm (Saitou & Nei 1987) and rooted using GenBank sequences of *Leptographium guttulatum* M. J. Wingf. & K. Jacobs (AY649782 and AY649783). Bootstrap analyses (1000 replicates) were run to determine confidence levels of the branching points (Felsenstein 1985).

Three of the 12 isolates (CMW 19362, CMW 19363, and CMW 19364) grouped in a clade separate from the other isolates, all of which grouped with known taxa. For these three isolates, part of the β-tubulin gene was amplified using primers Bt2a and Bt2b (Glass & Donaldson 1995). For each of the two regions, phylogenetic analyses were done separately, followed by a distance analysis of the combined data set. A partition homogeneity test was performed in PAUP v. 4.0b8 (Phylogenetic Analyses Using Parsimony) (Swofford 2002) to determine the congruence of the two data sets.

Morphology

Isolates (CMW 19362, CMW 19363, and CMW 19364) that resided in a defined phylogenetic clade of unknown identity were grown on 2 % WA (20 g Biolab agar and 1000 mL deionised water) with sterilised pine twigs,

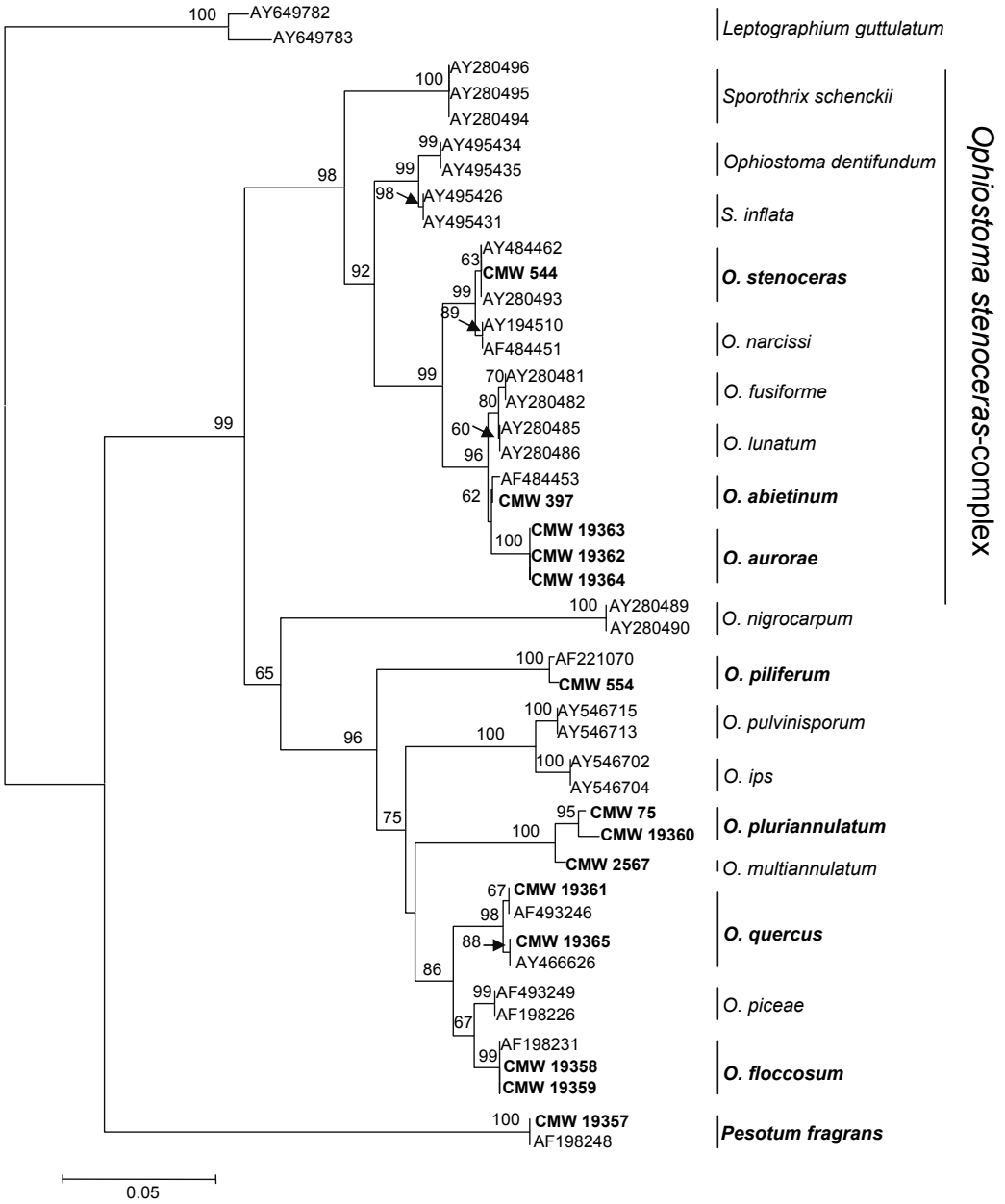

Fig. 1. Neighbour-joining tree of *Ophiostoma* species associated with bark beetles in South Africa based on ITS sequences (ITS1 and ITS2 regions, as well as 5.8S rRNA gene). Isolates sequenced in this study are printed in bold. Bar = total nucleotide differences between taxa. Bootstrap values (1000 replicates) are indicated above the branches.

and on 1.5 % oatmeal agar (15 g oats powder, 20 g Biolab agar and 1000 mL deionised water) to induce production of perithecia. Perithecia with ascospores were formed in two isolates (CMW 19362 and CMW 19363) on oatmeal agar. Thirty measurements were made for each structure, and the ranges and averages were computed. Anamorph structures were observed on 7-d-old slide cultures (Riddell 1950), mounted in lactophenol.

RESULTS

DNA Sequence analyses

PCR of the ITS regions delivered products ranging from about 530 to 610 bp in size. Comparison of the ITS sequences with GenBank sequences confirmed the identities of seven *Ophiostoma* spp. (Fig. 1). These included *O. stenoceras* (Robak) Nannf., *O. abietinum* Marm. & Butin, *O. piliferum* (Fr.) Syd. & P. Syd., *O. pluriannulatum* (Hedgc.) Syd. & P. Syd., *O. quercus* (Georgev.) Nannf., *O. floccosum* Math.-Käärik , and *Pesotum fragrans* (Math.-Käärik) G. Okada & Seifert. The identity of *O. ips* (Rumbold) Nannf. (also included in the study) had previously been confirmed based on DNA sequence comparisons (Zhou *et al*. 2004a).

Fragments 541 bp in size from the ITS region, and 345 from the partial β-tubulin gene were amplified for the three unidentified isolates (CMW 19362, CMW 19363, and CMW 19364). The β-tubulin region included intron 5, but no intron 4 was present. This corresponds with species in the *O. stenoceras* -complex (Zipfel *et al*. 2006). Sequences of isolates representing the majority of species in this complex were thus selected for further phylogenetic analyses, with *O. nigrocarpum* as outgroup. *Ophiostoma* spp. from outside the *O. stenoceras* -complex were not included in these analyses because of the presence of intron 4, but no intron 5 (Zipfel *et al*. 2006). The partition homogeneity test (P = 0.003) confirmed that the ITS and β-tubulin data sets were congruent. Distance analyses for the combined data set showed that the three unidentified isolates grouped together with a bootstrap support of 100 % (Fig. 2), and that they either represented an undescribed species or a species for which no sequence data are available.

Morphology

The three isolates (CMW 19362, CMW 19363, and CMW 19364) are morphologically similar to each other and different from any other described *Ophiostoma* species. They produced a typical *Sporothrix* anamorph in culture with swollen clavate conidia. Two of the isolates produced ascomata with allantoid rounded ascospores.

TAXONOMY

Based on combined sequence comparisons of the ITS regions and partial β-tubulin gene, as well as morphology, we conclude the three isolates from *H. angustatus* infesting pines in South Africa represent an undescribed taxon. This is described as follows:

Ophiostoma aurorae X.D. Zhou & M.J. Wingf., **sp. nov.** MycoBank MB500888. Figs 3A–3F.
Anamorph: *Sporothrix* (Fig. 3D–F).

Etymology: The type locality of this species is in Mpumalanga Province, South Africa. In siSwati, the name of the province means “the place where the sun rises”. Aurora was the Roman (Latin) goddess of dawn, so the specific epithet is an oblique reference to the type locality.

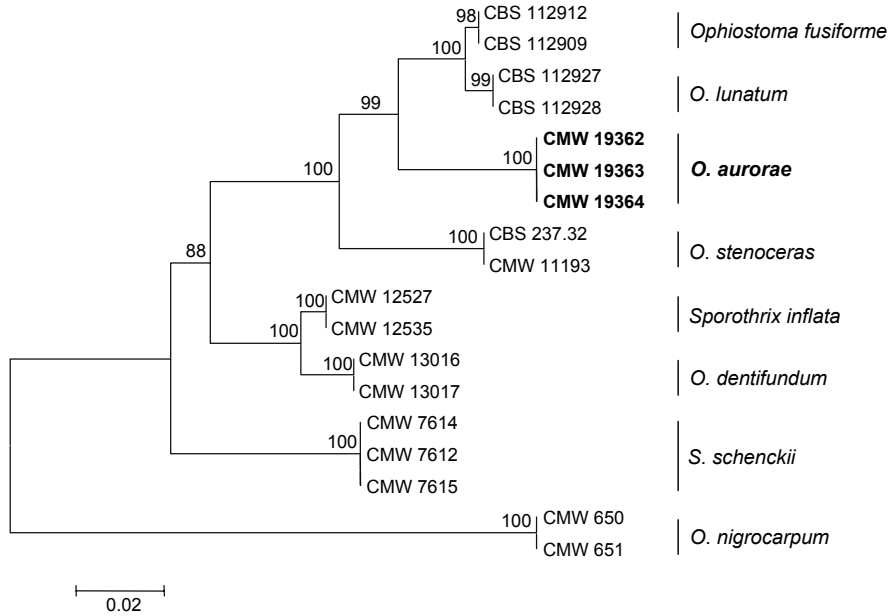

Fig. 2. Neighbour-joining tree of the *Ophiostoma stenoceras - Sporothrix schenckii* complex of species, including *O. aurorae* based on the combined ITS and β-tubulin sequences. Isolates sequenced in this study are printed in bold. Bar = total nucleotide differences between taxa. Bootstrap values (1000 replicates) are indicated above the branches.

Table 1. Ophiostomatoid fungi, including species with affinities to both the *Microascales* and *Ophiostomatales*, reported from South Africa. Currently accepted species names are listed first, with the name used in the original report in square brackets. Species reported as associates of bark or ambrosia beetles are printed in blue.

Year	Species	Host	References
1927	*Sporothrix schenckii* Hektoen & C.F. Perkins [= *Sporotrichum beurmanii* Matr. & Ramond]	Human	Doidge (1950)
1931	*Thielaviopsis basicola* (Berk. & Broome) Ferraris	*Nicotiana tabacum*	Gorter (1977)
1937	[a]*Ophiostoma piliferum* (Fr.) Syd. & P. Syd. [= *Ceratostomella pilifera* (Fr.) G. Winter]	Logs of *Pinus radiata*	Laughton (1937)
1937	*Thielaviopsis paradoxa* (De Seynes) Höhn.	*Saccharum officinarum*	Doidge (1950)
1947	[b]*Graphium* sp. associated with *Sporotrichum* sp., producing perithecia	Timber and air	Brown *et al.* (1947)
1956	*Ceratocystis adiposa* (E.J. Butler) C. Moreau	Shoots of *Pinus* sp.	Talbot (1956)
1965	*Chalara terrestris* Agnihothr. & K.C. Barna	*Eucalyptus saligna*	Marasas *et al.* (1966)
1970	*Raffaelea albimanens* D.B. Scott & J.W. du Toit	*Platypus externedentatus ex Ficus sycamorus*	Scott & Du Toit (1970)
1970	*R. hennebertii* D.B. Scott & J.W. du Toit	*P. externedentatus ex F. sycamorus*	Scott & Du Toit (1970)
1970	*R. arxii* D.B. Scott & J.W. du Toit	*Xyleborus torquatus ex Cussonia umbellifera*	Scott & Du Toit (1970)
1974	*Graphium putredinis* (Corda) S. Hughes	Soil	Eicker (1974)
1977	*Ceratocystis fimbriata* Ellis & Halst.	*Protea gigantea*	Gorter (1977)
1978	*Leptographium reconditum* Jooste	*Triticum* rhizosphere	Jooste (1978)
1980	*Ophiostoma ips* (Rumbold) Nannf. [= *Ceratocystis ips* (Rumbold) C. Moreau]	*Orthotomicus erosus ex Pinus* spp.	Wingfield & Marasas (1980a), Zhou *et al.* (2001, 2004a)
1980	[c]*Grosmannia serpens* Goid. [= *Verticicladiella alacris* M.J. Wingf. & Marasas]	Roots of *Pinus pinaster* and *P. radiata*	Wingfield & Marasas (1980b), Zhou *et al.* (2001)
1983	*Leptographium truncatum* (M.J. Wingf. & Marasas) M.J. Wingf. [= *Verticicladiella truncata* M.J. Wingf. & Marasas]	Roots of *Pinus taeda*	Wingfield & Marasas (1983)
1988	*Gondwanamyces proteae* (M.J. Wingf., P.S. van Wyk & Marasas) Marais & M.J. Wingf. [= *Ceratocystiopsis proteae* M.J. Wingf., P.S. van Wyk & Marasas]	*Protea repens*	Wingfield *et al.* (1988a)
1993	[d]*Quambalaria eucalypti* (M.J. Wingf., Crous & W.J. Swart) J.A. Simpson [= *Sporothrix eucalypti* M.J. Wingf., Crous & W.J. Swart]	*Eucalyptus grandis*	Wingfield *et al.* (1993a)
1993	*Gondwanamyces capensis* (M.J. Wingf. & P.S. van Wyk) Marais & M.J. Wingf. [= *Ophiostoma capense* M.J. Wingf. & P.S. van Wyk]	*Protea* spp.	Wingfield & Van Wyk (1993)
1994	*Ophiostoma splendens* G.J. Marais & M.J. Wingf.	*Protea* spp.	Marais & Wingfield (1994)
1994	[e]*Graphium pseudormiticum* M. Mouton & M.J. Wingf.	*Orthotomicus erosus*	Wingfield *et al.* (1988b), Mouton *et al.* (1994b)
1995	*Ophiostoma quercus* (Georgev.) Nannf.	*Olinia* sp., *Eucalyptus grandis, Quercus robur*	De Beer *et al.* (1995, 2003b)
1996	*Ceratocystis albifundus* M.J. Wingf., De Beer & M.J. Morris	*Protea* sp.	Wingfield *et al.* (1996)
1997	*Ophiostoma protearum* G.J. Marais & M.J. Wingf.	*Protea* sp.	Marais & Wingfield (1997)
1999	*Ophiostoma stenoceras* (Robak) Nannf.	*Eucalyptus* spp., *Acacia mearnsii, Malus* sp.	De Beer *et al.* (1999, 2003a), Zhou *et al.* (2001)
1999	*Ceratocystis radicicola* (Bliss) C. Moreau	*Phoenix dactylifera*	Linde & Smit (1999)
2000	*Ophiostoma galeiforme* (B.K. Bakshi) Math.-Käärik	*P. pinaster, Hylurgus ligniperda ex Pinus elliottii*	Zhou *et al.* (2001, 2004b)
2000	*Leptographium procerum* (W.B. Kendrick) M.J. Wingf.	*Hylastes angustatus ex Pinus* sp.	Zhou *et al.* (2001)
2001	*Ophiostoma africanum* G.J. Marais & M.J. Wingf.	*Protea gaguedi*	Marais & Wingfield (2001)
2001	*Ceratocystiopsis minuta* (Siemaszko) H.P. Upadhyay & W.B. Kendr.	*O. erosus, Hylastes angustatus, H. ligniperda ex Pinus* spp.	Zhou *et al.* (2001)
2001	[f]*Ophiostoma piceae* (Münch) Syd. & P. Syd.	*H. ligniperda*	Zhou *et al.* (2001)
2001	*Ophiostoma pluriannulatum* (Hedgc.) Syd. & P. Syd.	*O. erosus, H. ligniperda, H. angustatus ex Pinus* spp.	Zhou *et al.* (2001)
2001	[g]*Leptographium lundbergii* Lagerb. & Melin	Roots of *Pinus taeda*	Zhou *et al.* (2001)
2001	*Pesotum* spp. *Sporothrix* sp. *Hyalorhinocladiella* sp.	*O. erosus, Hylastes angustatus, H. ligniperda ex Pinus* spp.	Zhou *et al.* (2001)

Table 1. (Continued).

Year	Species	Host	References
2003	*Ophiostoma floccosum* Math.-Käärik	*Pinus elliottii*	De Beer *et al.* (2003b)
2003	*Ceratocystis moniliformis* (Hedgc.) C. Moreau	*Erythrina* sp.	Barnes *et al.* (2003)
2004	*Chalara hughesii* Nag Raj & W.B. Kendr.	*Elegia capensis*	Lee *et al*. (2004)
2004	[h]*Graphium calicioides* (Fr.) Cooke & Massee	*Leucadendron* sp.	Lee *et al*. (2004)
2004	*Ceratocystis pirilliformis* I. Barnes & M.J. Wingf.	*Eucalyptus grandis*	Roux *et al.* (2004)

[a]Doubtful report based on perithecia without ascospores (Laughton 1937). The present study provides the first substantiated report of *O. piliferum* in South Africa.

[b]Probably a *Pesotum* sp. with *Sporothrix* synanamorph.

[c]Only the *Leptographium* anamorph of this species has been observed in South Africa.

[d]Initially reported as a *Sporothrix* sp., *Q. eucalypti* has now been shown to be a smut in the *Exobasidiomycetes* order *Microstromatales* (De Beer *et al*. 2006).

[e]In 1988 reported as a *Graphium* sp. (Wingfield *et al*. 1998b).

[f]This identification of *O. piceae* was based on morphology and host specificity (Zhou *et al*. 2001). Sequence data in the present study show that this identification was incorrect and that the fungus was *O. quercus*.

[g]*Leptographium truncatum* was treated a synonym of *L. lundbergii* (Jacobs & Wingfield 2001); however, Jacobs *et al*. (2005) showed that South African isolates identified as *L. lundbergii* by Zhou *et al*. (2001), have sequences distinct from *L. lundbergii*, and should be treated as *L. truncatum*.

[h]*Graphium calicioides* has been placed in the *Chaetothyriales* (black yeasts) (Okada *et al.* 1998).

Coloniae in agaro 1.5 % avenae in medio 45 mm diam aetate duarum hebdomadum in 25 °C, laete hyalinae vel albae. Mycelium aerium adest. Ascomata superficialia vel subimmersa in agaro 1.5 % avenae. Bases perithiciorum globosae, obscurae, 130–220(–350) µm diam, hyphis laete griseis 65–150(–280) µm longis, 1.5–2.0 (–2.5) µm latis ornatae. Colla peritheciorum brunnea vel nigra, laevia, 340–800 (1415) µm longa, ad basim 35–42(–58) µm, ad apicem 12–15(–27) µm lata. Hyphae ostiolares adsunt. Ascosporae hyalinae, non septatae, allantoideae, in sectione transversali rotundae, 2–3(–3.5) x 1–1.5(–2) µm.

Cellulae conidiogenae micronematae, mononematae, hyalinae, 12–60(–85) x 1.5–2(–2.5) µm, ad apicem incrassatum denticulos acres perferentes; conidia hyalina, unicellularia, clavata vel guttuliformia, 3–4.5(–8) x 1–1.5(–2.5) µm.

Ascomata with globose bases, dark, 130–220(–350) µm diam (Fig. 3A), ornamented with light grey hyphae, 65–150(–280) µm long, 1.5–2(–2.5) µm wide. *Perithecial necks* brown to black, smooth, 340–800 (1415) µm long, 35–42(–58) µm wide at the base, 12–15(–27) µm at the apex (Fig. 3A, B). *Ostiolar hyphae* present (Fig. 3B). *Ascospores* hyaline, aseptate, allantoid, round in side view, 2–3(–3.5) x 1–1.5(–2) µm (Fig. 3C).

Conidiogenous cells (Fig. 3D–E), micronematous, mononematous, hyaline, 12–60(–85) x 1.5–2(–2.5) µm, sharp denticles present in the swollen apical part. *Conidia* (Fig. 3F) hyaline, single 1–celled, clavate to guttuliform, 3–4.5(–8) x 1–1.5(–2.5) µm.

Cultural characteristics: Colonies on 1.5 % oatmeal agar reaching on average 45 mm diam in two weeks at 25 °C. Colonies light hyaline to cotton–white. Aerial mycelium present. Perithecia produced superficially on or partially immersed in 1.5 % oatmeal agar.

Substrates: *Hylastes angustatus* and infested bark of *Pinus patula*.

Distribution: Mpumalanga Province, South Africa.

Specimens examined: **South Africa**, Mpumalanga Province, *Hylastes angustatus*, Sep. 1999, X.D. Zhou, **holotype** PREM 58886, culture ex-type CBS 118837 = CMW 19362; paratype PREM 58887, culture ex-paratype = CMW 19363; paratype PREM 58888, culture ex-paratype CBS 118827 = CMW 19364.

DISCUSSION

Results of this study have confirmed the identities of five *Ophiostoma* spp. associated with the non-native pine-infesting bark beetles *H. angustatus*, *H. ligniperda*, and *O. erosus* in South Africa. These fungi are *O. ips*, *O. floccosum*, *O. pluriannulatum, O. quercus* and *O. stenoceras*. In addition, *O. abietinum*, *O. piliferum* and *P. fragrans* are recognised for the first time from South Africa. One of the fungi associated with these bark beetles represents an undescribed taxon, for which the name *O. aurorae* has been provided.

The three fungal species *O. abietinum*, *O. piliferum* and *P. fragrans* reported from South Africa for the first time, are well-known associates of conifer timber. *Ophiostoma abietinum* was first described from *Abies vejari* attacked by a *Pseudohylesinus* sp. in Mexico (Marmolejo & Butin 1990), and was considered as an intermediate between *O. stenoceras* and *O. nigrocarpum* (R. Davidson) De Hoog (De Beer *et al*. 2003a). *Ophiostoma piliferum* is considered economically important to the forestry industry, and a colourless mutant of this species has been marketed as biocontrol agent against sapstaining fungi (Farrell *et al*. 1993). *Pesotum fragrans* was described from galleries of *Ips sexdentatus* infesting *Pinus sylvestris* in Sweden (Mathiesen-Käärik 1953), and the species has been reported from Australia, California, Canada, and New Zealand (Harrington *et* al. 2001, Jacobs *et al*. 2003).

Ophiostoma aurorae described in this study is morphologically similar to species in the *O. stenoceras*-complex (De Beer *et al*. 2003a, Aghayeva *et al*. 2004, 2005). Species in the complex have typical orange-

Table 2. Fungal isolates from pine bark beetles in South Africa used in this study.

Species	Isolate Number	GenBank no.		Host	Insect vector	Area
		ITS	β-tubulin			
Pesotum fragrans	[a]CMW 19357	DQ396790		*Pinus patula*	*Hylastes angustatus*	Mpumalanga
Ophiostoma abietinum	CMW 397	DQ396788		–	*Orthotomicus erosus*	Western Cape
O. floccosum	CMW 19358	DQ396791		*P. elliottii*	*Hylurgus ligniperda*	Kwazulu-Natal
	CMW 19359	DQ396792		*P. patula*	*O. erosus*	Mpumalanga
O. pluriannulatum	CMW 19360	DQ396793		*P. elliottii*	*H. ligniperda*	Kwazulu-Natal
O. piliferum	CMW 554	DQ396789		–	*O. erosus*	Western Cape
O. quercus	CMW 19361	DQ396794		*P. patula*	*H. angustatus*	Mpumalanga
	CMW 19365	DQ396795		*P. elliottii*	*H. ligniperda*	Kwazulu-Natal
O. stenoceras	CMW 544	DQ396799		–	*H. angustatus*	Western Cape
O. aurorae	CMW 19362	DQ396796	DQ396800	*P. elliottii*	*H. angustatus*	Mpumalanga
	CMW 19363	DQ396797	DQ396801	*P. elliottii*	*H. angustatus*	Mpumalanga
	CMW 19364	DQ396798	DQ396802	*P. elliottii*	*H. angustatus*	Mpumalanga

[a]Culture Collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

Table 3. Isolates of selected species of *Ophiostoma* used for comparative purpose in this study.

Species	Strain No.	GenBank no.		Collector / supplier	Origin	Host / insect
		ITS	β-tubulin			
Leptographium guttulatum	[a]CMW 1310	AY649782		J.N. Gibbs	England	*Pinus / Tomicus piniperda*
	[b]CMW 742	AY649783		M. Morelet	France	*P. sylvestris / Tomicus* sp.
Pesotum fragrans	[b,c]CBS 279.54	AF198248		A. Mathiesen-Käärik	Sweden	*P. sylvestris / Ips sexdentatus*
Ophiostoma abietinum	[b]CBS 125.89	AF484453		J.G. Marmolejo	Mexico	*Abies vejari / Pseudohylesinus* sp.
O. dentifundum	[b]CBS 115790	AY495434	AY495445	C. Delatour	Hungary	*Quercus* wood
	CBS 115865	AY495435	AY495446	T. Kowalski	Poland	*Quercus* robur
O. floccosum	[b]CBS 799.73	AF198231		A. Käärik	Sweden	*Picea* or *Pinus*
O. fusiforme	[b]CBS 112912	AY280481	AY280461	D.N. Aghayeva	Azerbaijan	*Populus nigra*
	CBS 112909	AY280482	AY280462	D.N. Aghayeva	Azerbaijan	*Castanea sativa*
O. ips	[b]CBS 137.36	AY546704		C.T. Rumbold	U.S.A.	*Ips integer*
	CMW 6418	AY546702		X.D. Zhou	South Africa	*Pinus elliottii / Orthotomicus erosus*

Table 3. (Continued).

Species	Strain No.	GenBank no.		Collector / supplier	Origin	Host / insect
		ITS	β-tubulin			
O. lunatum	[b]CBS 112927	AY280485	AY280466	T. Kirisits	Austria	*Carpinus betulus*
	CBS 112928	AY280486	AY280467	T. Kirisits	Austria	*Larix decidua*
O. multiannulatum	CBS 357.77			R.W. Davidson	U.S.A.	*Pinus* sp.
O. narcissi	[d]C 1648	AF484451		–	U.K.	*Narcissus* sp.
	[b]CBS 138.50	AY194510		D.P. Limber	Netherlands	*Narcissus* sp.
O. nigrocarpum	[b]CBS 637.66	AY280489	AY280479	R.W. Davidson	U.S.A.	*Abies* sp.
	CBS 638.66	AY280490	AY280480	R.W. Davidson	U.S.A.	*Pseudotsuga menziesii*
O. piceae	[b]CBS 108.21	AF198226		E. Münch	Germany	*Abies* or *Picea*
	CMW 7648	AF493249		D.B. Redfern, J.F. Webber	U.K.	*Picea sitchensis*
O. piliferum	CBS 129.32	AF221070		H. Diddens	–	*Pinus sylvestris*
O. pluriannulatum	CMW 75			R.W. Davidson	U.S.A.	–
O. pulvinisporum	[b]CMW 9020	AY546713		X.D. Zhou	Mexico	*P. pseudostrobus / Dendroctonus mexicanus*
	CMW 9026	AY546715		X.D. Zhou	Mexico	*Pinus maximinoi / Ips calligraphus*
O. quercus	[b]CMW 2467	AY466626		M. Morelet	France	*Quercus* sp.
	CMW 7645	AF493246		T. Kirisits, E. Halmschlager	Austria	*Q. robur*
O. stenoceras	[b]CBS 237.32	AY484462	AY280471	H. Robak	Norway	*Pinus* pulp
	CMW 11193	AY280493	AY280475	R. Farrell	New Zealand	wood
Sporothrix inflata	[b]CBS 239.68	AY495426	AY495437	W. Gams	Germany	wheatfield soil
	CBS 841.73	AY495431	AY495442	J. Grinbergs	Chile	soil
S. schenckii	CMW 7612	AY280494	AY280476	H.F. Vismer	South Africa	human sporotrichosis
	CMW 7614	AY280495	AY280477	H.F. Vismer	South Africa	human sporotrichosis
	CMW 7615	AY280496	AY280478	H.F. Vismer	South Africa	human sporotrichosis

[a]CMW = Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

[b]Ex-type culture or authentic strain.

[c]CBS = Culture collection of the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.

[d]C = Culture collection of T.C. Harrington, Department of Plant Pathology, Iowa State University, U.S.A.

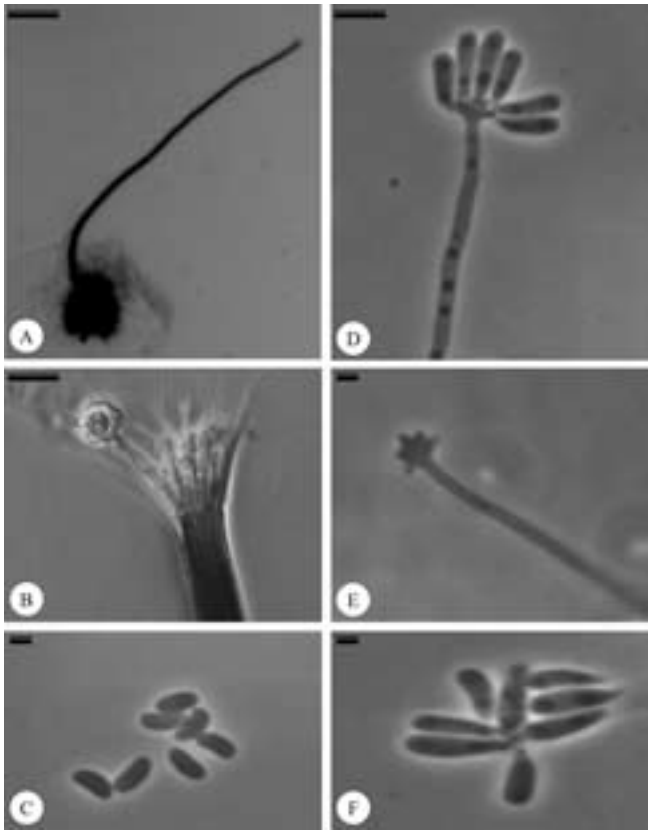

Fig. 3. *Ophiostoma aurorae* (CMW 19362) on 1.5 % oatmeal agar. A. Perithecium with long neck. (Scale bar = 190 µm). B. Apex of the neck with ostiolar hyphae. (Scale bar = 15 µm). C. Allantoid round ascospores. (Scale bar = 1.5 µm). D. Conidiophore. (Scale bar = 3.5 µm). E. Conidiogenous cell. (Scale bar = 1.5 µm). F. Clavate conidia. (Scale bar = 1.5 µm).

section-shaped ascospores and *Sporothrix* anamorphs. *Ophiostoma aurorae* can be distinguished from other species in the complex by its very obviously rounded ascospores and swollen clavate conidia. Its association with the root feeding scolytid bark beetle *H. angustatus* also appears to be a useful characteristic that might be applied in identification. In addition to its morphologically unique nature, analyses of ITS and partial β-tubulin gene sequences confirmed that *O. aurorae* resides in a phylogenetic clade, distinct from all morphologically similar *Ophiostoma* spp. for which sequence data are available.

Results of this study emphasise that a surprisingly large number of *Ophiostoma* spp. are associated with the three non-native conifer-infesting bark beetles accidentally introduced into South Africa. They also highlight the fact that the introduction of what might initially appear to be a single organism (plant, insect, fungus) is often considerably more complex. It seems likely that most of the fungi treated in this study are specifically associated with the insects in their areas of origin and like their insect vectors, they are also introduced exotics.

Species such as *O. quercus* that have a wide distribution on many woody substrates in South Africa could have invaded the bark beetle niche. It would be interesting to understand the long-term changes in such vector/fungus relationships, as has recently been found with *Tomicus piniperda* (Linnaeus) and *Leptographium wingfieldii* M. Morelet in the United States (Jacobs *et al*. 2004). Clearly, the bark beetle/ fungal association represents a complex and dynamic environment that deserves further study.

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