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

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**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. plurianulatum*, *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-infecting 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-infecting 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* Gold. and *Ceratocystis* 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-infecting 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-infecting 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 Bi2a and Bi2b (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.
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.

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**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 | '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 | '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 albimanensis D.B. Scott & J.W. du Toit | Platypus extremedentatus ex Ficus sycomorus | Scott & Du Toit (1970) |
| 1970 | R. hennebertii D.B. Scott & J.W. du Toit | P. extremedentatus ex F. sycomorus | 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 | '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. [= Ceratocystisips proteae M.J. Wingf., P.S. van Wyk & Marasas] | Protea repens | Wingfield et al. (1988a) |
| 1993 | '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 | '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 radicola (Bliss) C. Moreau | Phoenix dactylifera | Linde & Smit (1999) |
| 2000 | Ophiostoma galeiforme (B.K. Bakshi) Math.-Käärik | P. pinaster, Hyurgus 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 gagueudi | Marais & Wingfield (2001) |
| 2001 | Ceratocystisips minuta (Siemaszko) H.P. Upadhay & W.B. Kendr. | O. erosus, Hylastes angustatus, H. ligniperda ex Pinus spp. | Zhou et al. (2001) |
| 2001 | Ophiostoma piceae (Münch) Syd. & P. Syd. | H. ligniperda | Zhou et al. (2001) |
| 2001 | Ophiostoma pluriannullatum (Hedgec.) Syd. & P. Syd. | O. erosus, H. ligniperda, H. angustatus ex Pinus spp. | Zhou et al. (2001) |
| 2001 | 'Leptographium lundbergii Lagerb. & Melin | Roots of Pinus taeda | Zhou et al. (2001) |
| 2001 | Pesotum spp. | 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 moniliiformis* (Hedgc.) | Erythrina sp.         | Barnes et al. (2003)             |
| 2004 | *Chalara Hughesii* Nag Raj & W.B. Kendr. | Elogia capensis      | Lee et al. (2004)                |
| 2004 | *Graphium calicioides* (Fr.) Cooke & Massee | Leucadendron sp.     | Lee et al. (2004)                |
| 2004 | *Ceratocystis pirilliiformis* I. Barnes & M.J. Wingf. | Eucalyptus grandis   | Roux et al. (2004)               |

*Colonies in agar 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 agar 1.5 % avenae. Bases peritheciorum globosa, obscuresae, 130–220(–350) µm diam, hyphis laete griseis 65–150(–280) µm longis, 1.5–2.0 (–2.5) µm latis omatae. 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 ostiolaris adsunt. Ascopores 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 incassatum denticulos arcus perferentes; conidia hyalina, unicellularia, clavata vel guttuliformia, 3–4.5(–8) x 1–1.5(–2.5) µm.

*Ascomata with glosbe 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). Ascopores 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, hyalinae, 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.*

*Species 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. ligniperta*, 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. aurora* 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). *Pestotum 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 aurora* 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          |
|--------------------------|----------------|-------------|--------------------|---------------|---------------|
| Pesotum fragrans         | ^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. plurianulatum         | 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           | H. elliottii  | Mpumalanga    |
|                          | CMW 19363      | DQ396797    | DQ396801           | H. elliottii  | Mpumalanga    |
|                          | CMW 19364      | DQ396798    | DQ396802           | H. elliottii  | Mpumalanga    |

*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 |
|--------------------------|------------|-------------|----------------------|--------|---------------|
| Leptographium guttulatum | ^CMW 1310  | AY649782    | J. N. Gibbs          | England| Pinus / Tomicus piniperda |
|                          | ^CMW 742   | AY649783    | M. Morelet           | France | P. sylvestris / Tomicus sp. |
| Pesotum fragrans         | ^CBS 279.54 | AF196248    | A. Mathiesen-Kälärk | Sweden | P. sylvestris / Ips sexdentatus |
| Ophiostoma abietinum     | ^CBS 125.89 | AF484453    | J. G. Marmolejo      | Mexico | Abies vejari / Pseudohylesinus sp. |
| O. dentifundum           | ^CBS 115790 | AY495434   | C. Delatour          | Hungary| Quercus wood |
|                          | CBS 115865 | AY495435   | T. Kowalski          | Poland | Quercus robur |
| O. floccosum             | ^CBS 799.73 | AF196231    | A. Kälärk           | Sweden | Picea or Pinus |
| O. fusiforme             | ^CBS 112912 | AY280481   | D. N. Aghayeva       | Azerbaijan| Populus nigra |
|                          | CBS 112909 | AY280482   | D. N. Aghayeva       | Azerbaijan| Castanea sativa |
| O. ips                   | ^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         | CBS 112927 | AY280485    | AY280466             | T. Kirisits | Austria Carpinus betulus       |
|                    | CBS 112928 | AY280486    | AY280467             | T. Kirisits | Austria Larix decidua          |
| O. multiannulatum  | CBS 357.77 | AY280467    | R.W. Davidson        | U.S.A.      | Pinus sp.                      |
| O. narcissi        | CBS 1648   | AF484451    | –                    | U.K.        | Narcissus sp. Narcissus sp.    |
|                    | CBS 138.50 | AY194510    | D.P. Limber          | Netherlands | Narcissus sp. Narcissus sp.    |
| O. nigrocarpum     | 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          | CBS 108.21 | AF198226    | E. Münch             | Germany     | Abies or Picea                 |
| O. piliferum       | CMW 7648   | AF493249    | D.B. Redfern, J.F. Webber | U.K.       | Picea sitchensis               |
| O. plurianulatum   | CBS 129.32 | AF221070    | H. Diddens           | –           | Pinus sylvestris               |
| O. pulvinisporum   | CMW 75     | R.W. Davidson | U.S.A.              | –           |                                |
| O. quercus         | CMW 2467   | AY466626    | M. Morelet           | France      | Quercus sp.                    |
| O. stenoceras      | CMW 7645   | AF493246    | H. Kirisits, E. Halmschlager | Austria  | Q. robur                       |
| Sporothrix inflata | CMW 237.32 | AY484462    | AY280471             | H. Robak    | Norway Pinus pulp              |
|                    | CMW 11193  | AY280493    | AY280475             | R. Farrell | New Zealand wood               |
|                    | CBS 239.68 | AY495426    | AY495437             | W. Gams     | Germany wheatfield soil        |
| S. schenckii       | CBS 841.73 | AY495431    | AY495442             | J. Grinbergs | Chile soil                     |
|                    | 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 |

aCMW = Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Ex-type culture or authentic strain.

bCBS = Culture collection of the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.

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

ACKNOWLEDGEMENTS

We thank the National Research Foundation (NRF), members of Tree Protection Co-operative Programme (TPCP) and the THRIP initiative of the Department of Trade and Industry, South Africa for financial support. We also acknowledge Sappi for a fellowship awarded to the first author and Dr Hugh F. Glen for providing the Latin diagnosis.

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