

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

Xudong Zhou<sup>1\*</sup>, Z. Wilhelm de Beer<sup>2</sup> and Michael J. Wingfield<sup>1</sup>

<sup>1</sup>Tree Protection Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI); <sup>2</sup>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 *Hyalorhinochlaeniella* 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

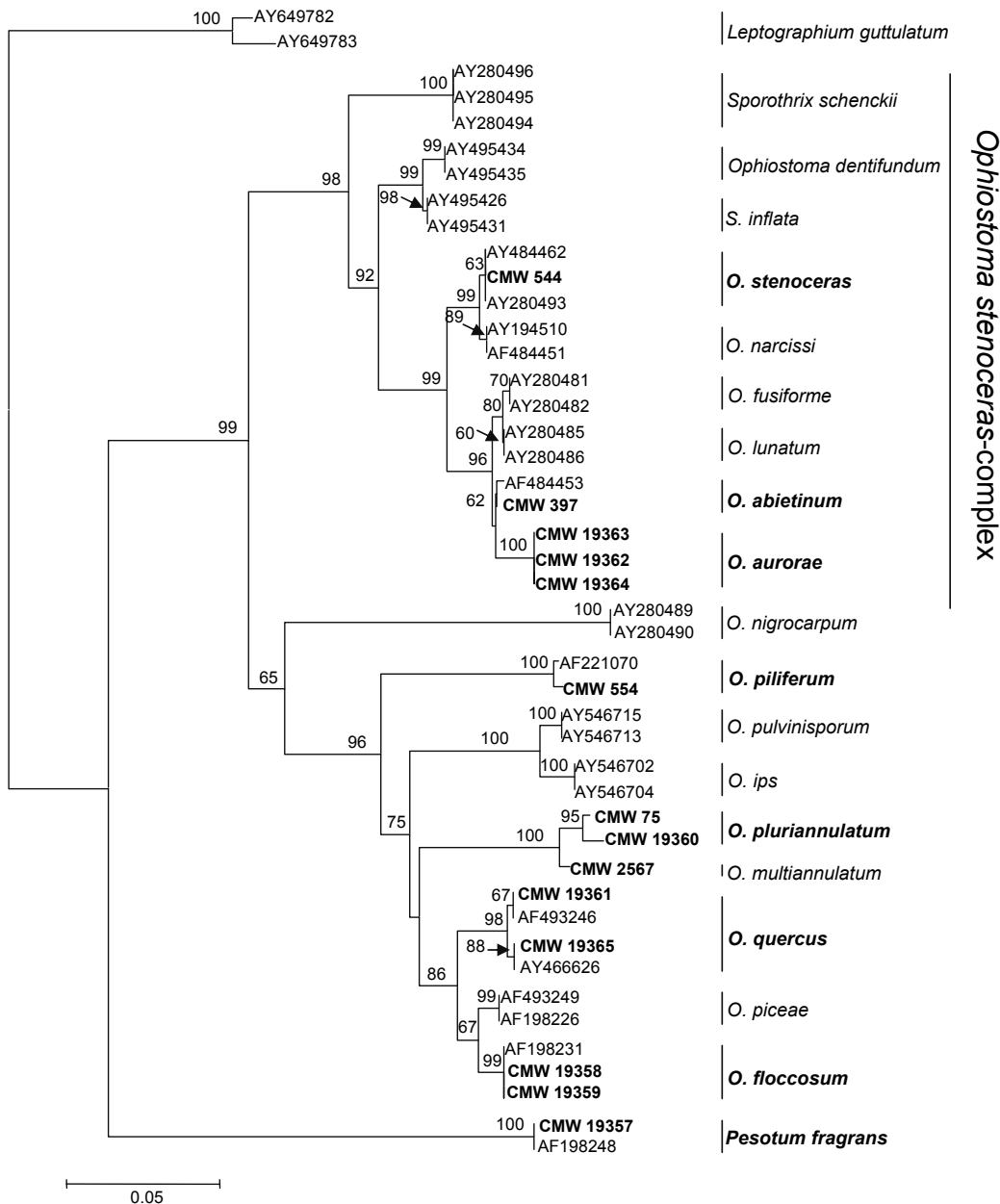
the ribosomal RNA operon was amplified using primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). PCR products were sequenced with the same primers. Conditions for PCR amplification and sequencing reactions were as described by Zhou *et al.* (2004b). For comparisons, ITS sequences of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

All sequences were aligned using MAFFT v. 5.667 (Kato *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  $\beta$ -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.



**Table 1.** Ophiostomatooid 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 | <i>Sporothrix schenckii</i> Hektoen & C.F. Perkins [= <i>Sporotrichum beurmanii</i> Matr. & Ramond]   | Human   | Doidge (1950)  |
| 1931 | <i>Thielaviopsis basicola</i> (Berk. & Broome) Ferraris   | <i>Nicotiana tabacum</i>  | Gorter (1977)  |
| 1937 | <sup>a</sup> <i>Ophiostoma piliferum</i> (Fr.) Syd. & P. Syd. [= <i>Ceratostomella pilifera</i> (Fr.) G. Winter]  | Logs of <i>Pinus radiata</i>  | Laughton (1937)  |
| 1937 | <i>Thielaviopsis paradoxa</i> (De Seynes) Höhn.   | <i>Saccharum officinarum</i>  | Doidge (1950)  |
| 1947 | <sup>b</sup> <i>Graphium</i> sp. associated with <i>Sporotrichum</i> sp., producing perithecia  | Timber and air  | Brown <i>et al.</i> (1947)                                     |
| 1956 | <i>Ceratocystis adiposa</i> (E.J. Butler) C. Moreau   | Shoots of <i>Pinus</i> sp.  | Talbot (1956)  |
| 1965 | <i>Chalara terrestris</i> Agnihothr. & K.C. Barna   | <i>Eucalyptus saligna</i>   | Marasas <i>et al.</i> (1966)                                   |
| 1970 | <i>Raffaelea albimanens</i> D.B. Scott & J.W. du Toit   | <i>Platypus externedentatus</i> ex <i>Ficus sycamorus</i>                                 | Scott & Du Toit (1970)   |
| 1970 | <i>R. hennebertii</i> D.B. Scott & J.W. du Toit   | <i>P. externedentatus</i> ex <i>F. sycamorus</i>  | Scott & Du Toit (1970)   |
| 1970 | <i>R. arxii</i> D.B. Scott & J.W. du Toit   | <i>Xyleborus torquatus</i> ex <i>Cussonia umbellifera</i>                                 | Scott & Du Toit (1970)   |
| 1974 | <i>Graphium putredinis</i> (Corda) S. Hughes  | Soil  | Eicker (1974)  |
| 1977 | <i>Ceratocystis fimbriata</i> Ellis & Halst.  | <i>Protea gigantea</i>  | Gorter (1977)  |
| 1978 | <i>Leptographium reconditum</i> Jooste  | <i>Triticum</i> rhizosphere   | Jooste (1978)  |
| 1980 | <i>Ophiostoma ips</i> (Rumbold) Nannf. [= <i>Ceratocystis ips</i> (Rumbold) C. Moreau]  | <i>Orthotomicus erosus</i> ex <i>Pinus</i> spp.   | Wingfield & Marasas (1980a), Zhou <i>et al.</i> (2001, 2004a)  |
| 1980 | <sup>c</sup> <i>Grosmannia serpens</i> Goid. [= <i>Verticicladiella alacris</i> M.J. Wingf. & Marasas]  | Roots of <i>Pinus pinaster</i> and <i>P. radiata</i>                                      | Wingfield & Marasas (1980b), Zhou <i>et al.</i> (2001)         |
| 1983 | <i>Leptographium truncatum</i> (M.J. Wingf. & Marasas) M.J. Wingf. [= <i>Verticicladiella truncata</i> M.J. Wingf. & Marasas]                                   | Roots of <i>Pinus taeda</i>   | Wingfield & Marasas (1983)                                     |
| 1988 | <i>Gondwanamyces proteae</i> (M.J. Wingf., P.S. van Wyk & Marasas) Marais & M.J. Wingf. [= <i>Ceratocystiopsis proteae</i> M.J. Wingf., P.S. van Wyk & Marasas] | <i>Protea repens</i>  | Wingfield <i>et al.</i> (1988a)                                |
| 1993 | <sup>d</sup> <i>Quambalaria eucalypti</i> (M.J. Wingf., Crous & W.J. Swart) J.A. Simpson [= <i>Sporothrix eucalypti</i> M.J. Wingf., Crous & W.J. Swart]        | <i>Eucalyptus grandis</i>   | Wingfield <i>et al.</i> (1993a)                                |
| 1993 | <i>Gondwanamyces capensis</i> (M.J. Wingf. & P.S. van Wyk) Marais & M.J. Wingf. [= <i>Ophiostoma capense</i> M.J. Wingf. & P.S. van Wyk]                        | <i>Protea</i> spp.  | Wingfield & Van Wyk (1993)                                     |
| 1994 | <i>Ophiostoma splendens</i> G.J. Marais & M.J. Wingf.   | <i>Protea</i> spp.  | Marais & Wingfield (1994)                                      |
| 1994 | <sup>e</sup> <i>Graphium pseudormiticum</i> M. Mouton & M.J. Wingf.   | <i>Orthotomicus erosus</i>  | Wingfield <i>et al.</i> (1988b), Mouton <i>et al.</i> (1994b)  |
| 1995 | <i>Ophiostoma quercus</i> (Georgev.) Nannf.   | <i>Olinia</i> sp., <i>Eucalyptus grandis</i> , <i>Quercus robur</i>                       | De Beer <i>et al.</i> (1995, 2003b)                            |
| 1996 | <i>Ceratocystis albifundus</i> M.J. Wingf., De Beer & M.J. Morris   | <i>Protea</i> sp.   | Wingfield <i>et al.</i> (1996)                                 |
| 1997 | <i>Ophiostoma protearum</i> G.J. Marais & M.J. Wingf.   | <i>Protea</i> sp.   | Marais & Wingfield (1997)                                      |
| 1999 | <i>Ophiostoma stenoceras</i> (Robak) Nannf.   | <i>Eucalyptus</i> spp., <i>Acacia mearnsii</i> , <i>Malus</i> sp.                         | De Beer <i>et al.</i> (1999, 2003a), Zhou <i>et al.</i> (2001) |
| 1999 | <i>Ceratocystis radicola</i> (Bliss) C. Moreau  | <i>Phoenix dactylifera</i>  | Linde & Smit (1999)  |
| 2000 | <i>Ophiostoma galeiforme</i> (B.K. Bakshi) Math.-Käärik   | <i>P. pinaster</i> , <i>Hylurgus ligniperda</i> ex <i>Pinus eliottii</i>                  | Zhou <i>et al.</i> (2001, 2004b)                               |
| 2000 | <i>Leptographium procerum</i> (W.B. Kendrick) M.J. Wingf.   | <i>Hylastes angustatus</i> ex <i>Pinus</i> sp.  | Zhou <i>et al.</i> (2001)                                      |
| 2001 | <i>Ophiostoma africanum</i> G.J. Marais & M.J. Wingf.   | <i>Protea gaguedi</i>   | Marais & Wingfield (2001)                                      |
| 2001 | <i>Ceratocystiopsis minuta</i> (Siemaszko) H.P. Upadhyay & W.B. Kendr.  | <i>O. erosus</i> , <i>Hylastes angustatus</i> , <i>H. ligniperda</i> ex <i>Pinus</i> spp. | Zhou <i>et al.</i> (2001)                                      |
| 2001 | <sup>f</sup> <i>Ophiostoma piceae</i> (Münch) Syd. & P. Syd.  | <i>H. ligniperda</i>  | Zhou <i>et al.</i> (2001)                                      |
| 2001 | <i>Ophiostoma pluriannulatum</i> (Hedgc.) Syd. & P. Syd.  | <i>O. erosus</i> , <i>H. ligniperda</i> , <i>H. angustatus</i> ex <i>Pinus</i> spp.       | Zhou <i>et al.</i> (2001)                                      |
| 2001 | <sup>g</sup> <i>Leptographium lundbergii</i> Lagerb. & Melin  | Roots of <i>Pinus taeda</i>   | Zhou <i>et al.</i> (2001)                                      |
| 2001 | <i>Pesotum</i> spp.<br><i>Sporothrix</i> sp.<br><i>Hyalorhinocladia</i> sp.   | <i>O. erosus</i> , <i>Hylastes angustatus</i> , <i>H. ligniperda</i> ex <i>Pinus</i> spp. | Zhou <i>et al.</i> (2001)                                      |

Table 1. (Continued).

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

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

<sup>b</sup>Probably a *Pesotum* sp. with *Sporothrix* synanamorph.

<sup>c</sup>Only the *Leptographium* anamorph of this species has been observed in South Africa.

<sup>d</sup>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).

<sup>e</sup>In 1988 reported as a *Graphium* sp. (Wingfield *et al.* 1998b).

<sup>f</sup>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*.

<sup>g</sup>*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*.

<sup>h</sup>*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 hebdomadam in 25 °C, laete hyalinae vel albae. Mycelium aerium adest. Ascumata 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 perithiciorum 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. Ascospores hyalinae, non septatae, allantoidae, 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.

*Ascumata* 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. *Perithicial 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         | $\beta$ -tubulin |                     |                            |               |
| <i>Pesotum fragrans</i>    | <sup>a</sup> CMW 19357 | DQ396790    |                  | <i>Pinus patula</i> | <i>Hylastes angustatus</i> | Mpumalanga    |
| <i>Ophiotoma abietinum</i> | CMW 397                | DQ396788    |                  | –                   | <i>Orthotomicus erosus</i> | Western Cape  |
| <i>O. floccosum</i>        | CMW 19358              | DQ396791    |                  | <i>P. elliotii</i>  | <i>Hylurgus ligniperda</i> | Kwazulu-Natal |
|                            | CMW 19359              | DQ396792    |                  | <i>P. patula</i>    | <i>O. erosus</i>           | Mpumalanga    |
| <i>O. pluriannulatum</i>   | CMW 19360              | DQ396793    |                  | <i>P. elliotii</i>  | <i>H. ligniperda</i>       | Kwazulu-Natal |
| <i>O. piliferum</i>        | CMW 554                | DQ396789    |                  | –                   | <i>O. erosus</i>           | Western Cape  |
| <i>O. quercus</i>          | CMW 19361              | DQ396794    |                  | <i>P. patula</i>    | <i>H. angustatus</i>       | Mpumalanga    |
|                            | CMW 19365              | DQ396795    |                  | <i>P. elliotii</i>  | <i>H. ligniperda</i>       | Kwazulu-Natal |
| <i>O. stenoceras</i>       | CMW 544                | DQ396799    |                  | –                   | <i>H. angustatus</i>       | Western Cape  |
| <i>O. aurorae</i>          | CMW 19362              | DQ396796    | DQ396800         | <i>P. elliotii</i>  | <i>H. angustatus</i>       | Mpumalanga    |
|                            | CMW 19363              | DQ396797    | DQ396801         | <i>P. elliotii</i>  | <i>H. angustatus</i>       | Mpumalanga    |
|                            | CMW 19364              | DQ396798    | DQ396802         | <i>P. elliotii</i>  | <i>H. angustatus</i>       | Mpumalanga    |

<sup>a</sup>Culture Collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

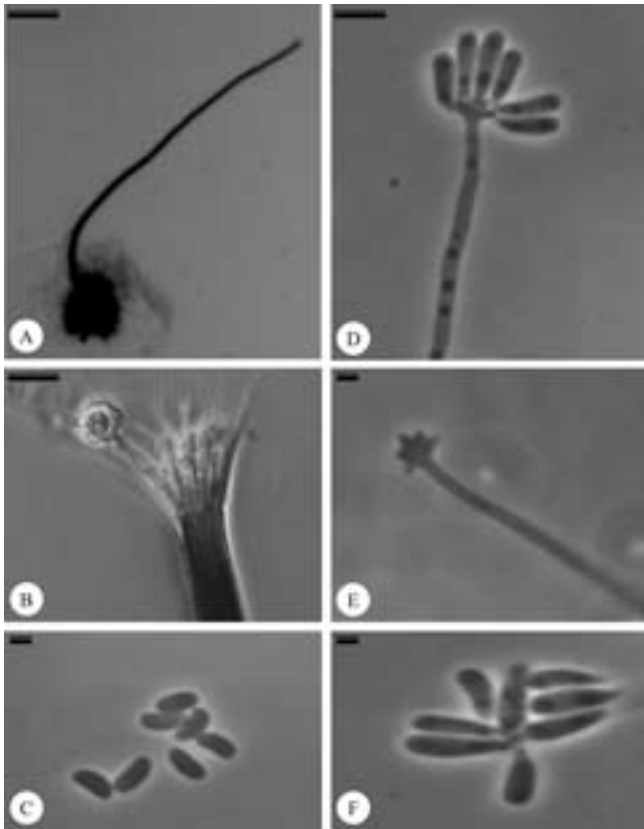
**Table 3.** Isolates of selected species of *Ophiotoma* used for comparative purpose in this study.

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

Table 3. (Continued).

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

<sup>a</sup>CMW = Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.<sup>b</sup>Ex-type culture or authentic strain.<sup>c</sup>CBS = Culture collection of the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.<sup>d</sup>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  $\mu$ m). B. Apex of the neck with ostiolar hyphae. (Scale bar = 15  $\mu$ m). C. Allantoid round ascospores. (Scale bar = 1.5  $\mu$ m). D. Conidiophore. (Scale bar = 3.5  $\mu$ m). E. Conidiogenous cell. (Scale bar = 1.5  $\mu$ m). F. Clavate conidia. (Scale bar = 1.5  $\mu$ m).

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  $\beta$ -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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