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Pesotum australi sp. nov. and *Ophiostoma quercus* associated with *Acacia mearnsii* trees in Australia and Uganda, respectively

G. Kamgan Nkuekam[A,C], *K. Jacobs*[B], *Z. W. de Beer*[A], *M. J. Wingfield*[A] *and J. Roux*[A]

[A]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.
[B]Department of Microbiology, University of Stellenbosch, Stellenbosch, South Africa.
[C]Corresponding author. Email: Gilbert.kamgan@fabi.up.ac.za

Abstract. *Pesotum* accommodates synnematal anamorphs of *Ophiostoma* spp. with sympodially proliferating conidiogenous cells. These fungi are usually closely associated with wounds on trees and the insects that visit them. During tree disease surveys in Uganda, as well as studies of fungi infecting wounds on *Acacia mearnsii* trees in Uganda and Australia, many isolates resembling species of *Pesotum* were collected. The aim of this study was to identify these fungi using both morphological and DNA sequence comparisons. The *Pesotum*, anamorph of *O. quercus* was the only species collected from multiple collections in Uganda. Collections from Australia represent a new species of *Pesotum* described here as *P. australi* sp. nov.

Additional keywords: forestry, hardwood, ophiostomatoid, sapstain.

Introduction

The genus *Ophiostoma* Syd. & P. Syd. accommodates virulent pathogens such as *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier, which result in tree death (Brasier 1990; Wingfield *et al*. 1993). It also includes many species that result in sapstain of lumber, which can lead to great losses in revenue (Münch 1907; Lagerberg *et al*. 1927; Seifert 1993; Uzunovic and Webber 1998). *Ophiostoma* spp. require wounds for infection and most species are closely associated with insects such as bark beetles (Curculionidae: Scolytinae) that act as wounding agents (Grosmann 1931, 1932; Six 2003; Kirisits 2004; Harrington 2005).

Ophiostoma sensu lato is a polyphyletic taxon, including at least three genera. These include *O. sensu stricto* with *Pesotum* J.L. Crane & Schokn. and *Sporothrix* Hektoen & C.F. Perkins anamorphs, *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. with *Hyalorhinocladiella* H.P. Upadhyay & W.B. Kendr. anamorphs and *Grosmannia* Goid. with *Leptographium* Lagerb. & Melin anamorphs (Upadhyay 1981; Zipfel *et al*. 2006). Sexual forms of these fungi commonly produce ascomata with long, erect necks giving rise to sticky spore drops that facilitate dispersal by insects (Malloch and Blackwell 1993). Asexual structures are typically erect conidiophores with sticky spores at their apices (*Pesotum*, *Hyalorhinocladiella* and *Leptographium*) or dry spores (*Sporothrix*) that can be wind dispersed (Ingold 1971; Crane and Schoknecht 1973; Malloch and Blackwell 1993).

The anamorph genus *Pesotum* was established to accommodate species that produce both synnematous and mononematous conidiophores, with sympodially proliferating conidiogenous cells (Crane and Schoknecht 1973). However, its taxonomic placement in *Ophiostoma* has been the source of considerable debate. Okada *et al*. (1998) treated *Pesotum* to include all synnematal anamorphs with affinities to *Ophiostoma*. In a more recent treatment based on DNA sequence comparison, Harrington *et al*. (2001) recommended that *Pesotum* should be restricted only to anamorphs of the *O. piceae* (Münch) Syd. & P. Syd. complex.

Acacia mearnsii de Wild is a woody legume of the family Mimosaceae (Orchard and Wilson 2001). It is endemic to Australia and has been introduced into many countries for tannins that can be extracted from its bark and for its high value, short fibre wood used in pulp and fuel production (Acland 1971; Sherry 1971; Gibson 1975). In many developing countries, such as Uganda, *A. mearnsii* trees are utilised extensively for fuel wood, often growing in dense clumps of naturally regenerating trees.

Very little is known regarding the occurrence of *Ophiostoma* spp. in parts of the world other than Europe and North America. In the southern hemisphere, reports of *Ophiostoma* spp. are restricted to a few countries and from a limited number of studies. Other than those from South Africa, there are no reports of *Ophiostoma* spp. from Africa. Reports of *Ophiostoma* spp. from Australia are also relatively limited with a few species known to be associated with introduced pine-infesting bark beetles (Vaartaja 1967; Stone and Simpson 1987, 1991) and *O. quercus* has been recorded from *Pinus radiata* D. Don (Harrington *et al*. 2001). The aim of this study was to identify *Pesotum* spp. collected from artificially induced wounds on *A. mearnsii* trees in Australia, where this tree is native, and those collected from non-native *A. mearnsii* in Uganda. For this purpose both phenotypic and DNA sequence comparisons were used.

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Materials and methods

Cultures

Seventeen isolates from *A. mearnsii* in Uganda were obtained from stumps shortly after harvesting, in the Kabale area of south-western Uganda and from stem cankers on these trees in the same area. Four isolates from Australia were obtained from artificially induced wounds made on the stems of *A. mearnsii* trees near Cann River in the state of Victoria, Australia, collected as part of a previous study (Barnes *et al*. 2003). All cultures used in this study have been preserved in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and representative cultures have also been deposited with the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS).

Morphology

Isolates for morphological characterisation were grown on 2% malt extract agar (MEA, 20 g/L malt extract and 15 g/L agar) (Biolab, Midrand, South Africa) containing the antibiotic streptomycin sulfate (0.05 g/L) (Sigma-Aldrich, Steinheim, Germany) at 24°C for 7 days. Single drops of conidia or segments of mycelium were transferred from pure cultures to oatmeal agar medium (OMA, 30 g/L oats and 20 g/L Biolab agar) to promote sporulation and for comparisons with previously published descriptions. Cultures were incubated at 24°C until sporulation and then assembled in morphologically similar groups based on differences in colony colour (Rayner 1970), arrangement of fruiting bodies and morphology. Fruiting structures (synnemata and conidia) were mounted in 80% lactic acid on microscope slides and measured using a Zeiss AxioCam light microscope (Carl Zeiss, Hallbergmoos, Germany). Fifty measurements were made for each structure from each isolate chosen as the type and 10 measurements were made for additional isolates. The means were computed for relevant morphological structures and measurements were noted as (minimum−) mean minus s.d.–mean plus s.d. (−maximum). To induce the production of sexual fruiting structures, cultures were grown on 1.5% water agar (15 g/L Biolab agar) supplemented with sterile pieces of *A. mearnsii* wood. Plates were incubated at room temperature and inspected weekly for the appearance of ascomata and ascospore production.

DNA extraction and PCR amplification

A selection of isolates, representing each of the different groups identified based on culture and morphological characteristics were selected for DNA sequence comparisons. Single spore drops from synnemata in pure cultures were grown on 2% MEA for 7–10 days. Mycelium was then transferred to 1.5-mL Eppendorf tubes using a sterile scalpel. DNA was extracted using the protocol described by Möller *et al*. (1992), except that 10 µL of RnaseA were added at the final step and incubated overnight at room temperature to digest RNA. The presence of DNA was verified by separating an aliquot of 5 µL on 1% agarose gels containing ethidium bromide and visualised under UV light. The internal transcribed spacer regions (ITS1 and ITS2) and 5.8S gene of the rRNA operon were amplified using an Eppendorf Mastercycler (Merck, Hamburg, Germany) and primers ITS1 and ITS4 (White *et al*. 1990). Parts of two other gene regions comprising the nuclear large subunit (LSU) rDNA and the β-tubulin gene were also amplified, using primers LROR (5′-ACCCGCTGAACTTAAGC-3′) and LR5 (5′-TCCTGAG GGAAACTTCG-3′) (http://www.biology.duke.edu/fungi/mycolab/primers.htm, verified 17 March 2008) for the LSU, and primers T10 (5′-ACGATAGGTTCA CCTCCAGAGAC-3′) (O'Donnell and Cigelnik 1997) and Bt2b (5′-GGTAACCAA ATCGGTGCTGCTTTC-3′) (Glass and Donaldson 1995) for the β-tubulin regions.

DNA template (60 ng) was used to prepare a 25-µL PCR, that also contained 2.5 µL of 10× reaction buffer with $MgCl_2$ (25 mM) (Roche Diagnostics, Mannheim, Germany), 2.5 µL $MgCl_2$ (25 mM) (Roche Diagnostics), 1U of Taq polymerase (Roche Diagnostics), 2.5 µL of dNTP (10 mM) and 0.5 µL of each primer (10 mM). The conditions used for the thermal cycling were as follows: an initial denaturation of the DNA at 96°C for 2 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, primer extension at 72°C for 1 min and a final extension at 72°C for 10 min. An aliquot of 5 µL of the PCR product was separated on a 1% agarose gel and visualised under UV light after staining with ethidium bromide. For a few isolates, multiple bands were obtained. In each of these cases, the annealing temperatures were adjusted until a single band was obtained.

DNA sequencing

PCR products were purified using Sephadex G-50 Gel (Sigma-Aldrich), as recommended by the manufacturer. Purified products (1 µL) were separated by electrophoresis in a 1% agarose gel to estimate the concentration of DNA. Subsequently, an accurate concentration of the purified PCR product was determined using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Rockland, DE, USA). Sequencing reactions were performed using the Big Dye cycle sequencing kit with Amplitaq DNA polymerase, FS (Perkin-Elmer, Warrington, UK), following the manufacturer's protocols on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). Between 60–100 ng PCR product was used to prepare 10 µL sequencing reactions that also contained 2 µL of ready reaction mixture (Big Dye), 2 µL of 5× reaction buffer, 1 µL of primer (10 mM) and enough water to complete the volume of 10 µL. The same primers were used as those used for the PCR amplifications. Both DNA strands were sequenced.

Phylogenetic analyses

A preliminary identity for isolates from Uganda and Australia was obtained by performing a similarity search (standard nucleotide BLAST) against the GenBank database (http://www.ncbi.nlm.nih.gov, verified 17 March 2008) using ITS sequence data. Thereafter, sequences for both strands for each isolate were checked visually and combined using the program Sequence Navigator version 1.01 (ABI PRISM, Perkin-Elmer), by comparing the nucleotides and their corresponding peaks. Additional sequences of related *Pesotum* spp. were obtained from the GenBank database (Table 1). Sequences were then aligned online (http://www.imtech.res.in/raghava/mafft/, verified 7 April 2008) with those from GenBank using

Table 1. *Ophiostoma* spp. included in DNA sequence comparison studies
NA, not available

Species	Isolate numbers	GenBank accession number			Hosts	Collectors	Origin
		ITS	β-Tubulin	LSU			
O. catonianum	C1084 (=CBS263.35)	AF198243	NA	NA	*Pyrus communis*	G. Goidanich	Italy
O. floccosum	C1086 (=CBS799.73)	AF198231	NA	NA	NA	A. Käärik	Sweden
	CMW7661	AF493253	NA	NA	*Pinus elliottii*	Z. W. de Beer	South Africa
	KAS708	NA	AY305691	NA	NA	NA	NA
	NZFS637	NA	AY789141	NA	NA	NA	New Zealand
	CMW1713	NA	NA	DQ294367	NA	NA	USA
O. himal-ulmi	C1183 (=CBS374.67) (=ATCC36176) (=ATCC36204)	AF198233	NA	NA	*Ulmus* sp.	H. M. Heybroek	India
	C1306 (=HP27)	AF198234	NA	NA	*Ulmus* sp.	C. M. Brasier	India
O. kryptum	DAOM229702 (=IFFFBW/1)	AY304434	NA	NA	*Larix decidua*	T. Kirisits & M. J. Wingfield	Austria
	IFFFHasd/1	AY304437	NA	NA	*Larix deciduas*	T. Kirisits & M. J. Wingfield	Austria
	DAOM229702	NA	AY305686	NA	*L. decidua*	M. J. Wingfield & T. Kirisits	Austria
	DAOM229701	NA	AY305685	NA	*L. decidua*	T. Kirisits	Austria
O. multiannulatum	CBS124.39	AY934512	NA	NA	NA	NA	NA
O. novo-ulmi	C510	AF198236	NA	NA	*Ulmus* sp.	NA	Iowa, USA
	C1185 (=CBS298.87) (=WCS637)	AF198235	NA	NA	*Ulmus* sp.	H. M. Heybroek	Russia
	CMW10573	NA	DQ296095	NA	NA	NA	Austria
	CMW10373	NA	NA	DQ294375	NA	NA	Austria
O. perfectum	C1104 (=CBS636.66)	DQ062970	NA	NA	NA	NA	NA
O. piceae	C1087 (=CBS108.21)	AF198226	NA	NA	NA	E. Munch	Germany
	CMW7648 (=C967) H2181	AF493249	NA	NA	*Picea sitchensis*	D. B. Redfern & J. F. Webber	United Kingdom
	CMW7648	NA	AY789152	NA	NA	NA	United Kingdom
	NZFS332.01	NA	AY789151	NA	NA	NA	New Zealand
	CMW8093	NA	DQ296091	DQ294371	NA	NA	Canada
O. piliferum	CBS129.32	AF221070	NA	NA	*Pinus sylvestris*	H. Diddens	United Kingdom
	NA	AF221071	NA	NA	NA	NA	NA
	CMW7877	NA	DQ296098	DQ294378	NA	NA	NA
	CMW7879	NA	DQ296097	NA	NA	NA	NA
	CBS12932	NA	NA	DQ294377	NA	NA	NA
O. pluriannulatum	MUCL18372	AY934517	NA	NA	NA	NA	USA
	C1033, NZ-150	DQ062971	NA	NA	*P. radiata*	R. Farrell	New Zealand
	C1567 (=UAMH9559) (=WIN(M)869)	DQ062972	NA	NA	*Podocarpus* sp.	J. Reid	New Zealand
O. quercus	C970 (=CBS102353) (=H1039)	AF198239	NA	NA	*Quercus* sp.	P. T. Scard & J. F. Webber	United Kingdom
	CMW7656	AF493250	NA	NA	*Q. robur*	M. J. Wingfield	South Africa
	CMW2463 (=0.96)	AF493239	NA	NA	*Fagus sylvatica*	M. Morelet	France
	CMW7650 (=C969) CBS102352 (=H1042)	AF198238	NA	NA	*Quercus* sp.	P. T. Scard & J. F. Webber	United Kingdom
	CMW7645 (=W3) (=HA367)	AF493246	NA	NA	*Q. robur*	T. Kirisits & E. Halmschlager	Austria

(*continued next page*)

Table 1. (*continued*)

Species	Isolate numbers	GenBank accession number			Hosts	Collectors	Origin
		ITS	β-Tubulin	LSU			
	C970	NA	AY789157	NA	NA	NA	UK
	KUC2210	NA	AY789155	NA	NA	NA	NZ
	NZFS3182	NA	AY789156	NA	NA	NA	NZ
	CMW3110	NA	DQ296096	NA	NA	NA	USA
	CBS118713	NA	NA	DQ294376	NA	NA	USA
	CMW5826[A]	NA	NA	NA	*A. mearnsii*	J. Roux	Uganda
	CMW5928[A]	EF408598	NA	NA	*A. mearnsii*	J. Roux	Uganda
	CMW5932[A]	NA	NA	NA	*A. mearnsii*	J. Roux	Uganda
	CMW5952[A]	NA	NA	NA	*A. mearnsii*	J. Roux	Uganda
	CMW5948[A]	EF408600	NA	NA	*A. mearnsii*	J. Roux	Uganda
	CMW5679[A]	NA	NA	NA	*A. mearnsii*	J. Roux	Uganda
	CMW5955[A]	NA	NA	NA	*A. mearnsii*	J. Roux	Uganda
	CMW5943[A]	EF408599	NA	NA	*A. mearnsii*	J. Roux	Uganda
O. setosum	AU16053	AF128927	NA	NA	NA	NA	Canada
	AU16038	AF128929	NA	NA	NA	NA	Canada
	NZFS3652	NA	AY789159	NA	NA	NA	NA
	AU160–53	NA	AY305703	NA	NA	NA	Canada
O. subannulatum	CBS188.86	AY934522	NA	NA	NA	NA	NA
O. tetropii	CBS428.94	AY194507	NA	NA	*Picea abies*	T. Kirisits	Austria
	DAOM229566 (=C01-015)	AY194493	NA	NA	*P. glauca*	G. Alexander	McNabs Island, Canada
	CBS428.94	NA	AY305702	NA	NA	NA	Austria
	C00-003	NA	AY305701	NA	NA	NA	Canada
O. ulmi	C1182 (=CBS102.63) (=IMI101223) (=JCM9303)	AF198232	NA	NA	*Ulmus* sp.	W. F. Holmes & H. M. Heybroek	Netherlands
	CMW1462	NA	DQ296094	DQ294373	NA	NA	USA
P. australi	CMW6590[A]	EF408601	NA	NA	*A. mearnsii*	M. J. Wingfield	Australia
	CMW6588[A]	EF408604	NA	NA	*A. mearnsii*	M. J. Wingfield	Australia
	CMW6606[A]	EF408603	EF408606	EF408608	*A. mearnsii*	M. J. Wingfield	Australia
	CMW6589[A]	EF408602	EF408605	EF408607	*A. mearnsii*	M. J. Wingfield	Australia

[A]Isolates sequenced in this study.

Mafft version 5.851 (Katoh *et al*. 2002). Phylogenetic analyses were performed using PAUP*4.0b10 (Swofford 1998). For isolates from Australia, the nuclear LSU and the β-tubulin genes were also included in the study and their phylogenetic analyses were performed independently of each other in PAUP*4.0b10. For parsimony analyses, heuristic searches with 10 random addition sequence replicates, branch swapping and tree bisection reconstruction were performed. Trees were rooted using *O. piliferum* (Fr.) Syd. & P. Syd. as an outgroup taxon. Confidence levels of the branching points in the phylogenetic trees were estimated with the bootstrap method (1000 replications) (Felsenstein 1985). A distance tree for each dataset was obtained by performing neighbour-joining analyses using the Kimura 2-parameter model. Trees were rooted using *O. piliferum* as outgroup. Confidence levels of the phylogenies were estimated with the bootstrap method (1000 replications) (Felsenstein 1985).

Mating studies

To produce a tester strain that could be used for isolate identification, 15 single ascospore cultures were prepared from ascomata produced by an isolate of *O. quercus* (CMW5826) from Uganda on sterilised *A. mearnsii* wood. The single ascospore cultures were crossed in every possible combination on MEA supplemented with *A. mearnsii* wood pieces. To induce the production of ascomata, these cultures were first incubated at 24°C for 2 weeks, and then at 20°C for 3 weeks and checked weekly using a dissection microscope. Some crosses gave rise to ascomata, and it was then possible to select tester strains of opposite mating type. The tester strains were used in crosses with three single conidium cultures prepared from each of 14 other isolates from Uganda, which did not produce ascomata on the wood. The strains were not subjected to DNA sequence comparisons. Thus, the aim was to determine their identity based on mating compatibility alone. A few isolates from Uganda that had been compared based on DNA sequences were also included in the mating tests to serve as controls. Isolates from Australia were not used in the mating tests as there were only four isolates and DNA sequence comparisons were made for all of them. The tester strains [CMW17256, CMW17257 (+) and CMW17258, CMW14307 (−)] are maintained in the CMW of FABI.

Growth in culture

Discs of agar (9 mm in diameter) bearing mycelium of selected isolates (CMW6606, CMW6589) of two Australian

isolates were transferred from the actively growing margins of 7-day-old cultures and placed upside down at the centres of 90-mm Petri dishes containing 2% MEA. Plates were incubated in the dark for 10 days at temperatures ranging from 5 to 35°C at intervals of 5°. Five replicates of each isolate were used at each temperature. Growth of cultures after 10 days was measured using two diameter measurements perpendicular to each other for each plate at each temperature tested. The averages of the 10 measurements were then computed.

Results

Morphology

A total of 21 isolates (17 isolates from Uganda and 4 isolates from Australia) resembling *Pesotum* spp. were obtained from *A. mearnsii* and examined. These isolates could be assigned to one of four different morphotypes, one from Australia and three from Uganda, based on colony colour and the production of fruiting structures on OMA. Morphotype A included isolates with brown colonies and synnemata scattered over the plates. Morphotype B comprised isolates with white or lightly coloured mycelium and synnemata organised in a circular pattern. Morphotype C included isolates with light-brown colonies, with synnemata scattered at the edges of the plates, but forming circular rings towards the middle of the plates and Morphotype D included only isolates from Australia, which could be distinguished from Ugandan isolates on OMA by their cream-coloured colonies and synnemata with slimy heads, arranged in concentric rings. On water agar supplemented with wood chips, only isolate CMW5826 from Uganda produced sexual fruiting structures. These were characteristic of an *Ophiostoma* sp. and this isolate was used to produce tester strains for the mating studies.

Phylogenetic analyses

All isolates selected for DNA sequencing produced PCR products of ~650 bp, using the primers ITS1 and ITS4. BLAST searches suggested that the *A. mearnsii* isolates from Uganda and Australia represent *O. quercus*. Comparisons of Ugandan and Australian isolates with those from GenBank and analysis in PAUP resulted in a total of 666 characters including gaps, with 399 constant characters, 25 parsimony-uninformative and 242 parsimony informative characters. Similar values for the β-tubulin dataset were 293 characters including gaps, with 198 constant characters, 6 parsimony-uninformative characters and 89 parsimony informative characters, while for the nuclear LSU dataset, there were a total of 703 characters including gaps, with 586 constant characters, 20 parsimony uninformative characters and 97 parsimony informative characters. PAUP and the heuristic search option resulted in 463 trees for ITS, 237 trees for β-tubulin and 210 trees for LSU. For the respective datasets, the consistency index values were 0.83, 0.67 and 0.64, while the retention index values were 0.94, 0.83 and 0.85. Neighbour-joining analyses of the three datasets resulted in phylograms presented in Figs 1–3. All eight isolates from Uganda clustered with *O. quercus*, supported by a bootstrap value of 60% (Fig. 1). Isolates from Australia did not group with any of the representative *Ophiostoma* reference strains (Fig. 1), suggesting that they represent a previously undescribed species, most closely related to *O. quercus*. Sequence comparisons using Australian isolates, the β-tubulin (Fig. 2) and the LSU (Fig. 3) gene regions produced trees of similar topology to those of the ITS, confirming that it represents an undescribed taxon.

Mating studies

Nine isolates from Uganda that did not produce sexual fruiting structures, and that were not sequenced, were crossed with two tester strains of opposite mating type (Table 2). These had been identified as *O. quercus* based on DNA sequence comparisons. Five other isolates from Uganda that had been identified based on DNA sequences were also subjected to mating compatibility tests. Ten isolates gave positive results with the (−) tester strain (CMW14307) while four isolates gave positive results with the (+) tester strain (CMW14257), confirming that all 14 isolates from Uganda represented *O. quercus*.

Taxonomy

Pesotum australi Kamgan-Nkuekam, Jacobs & Wingfield, **sp. nov.** (Fig. 4)

Etymology: refers to the country where the fungus was first collected.

Coloniae umbrinae, capitula cremea mucosa in annulis concentricis disposita formantes. Conidiophorae synnematae, erectae, basin atrobrunneae, apicem *v.* pallescentes, (202−) 224.5–275.5 (−324.5) µm altae, basin (16.5−) 20–37.5 (−60) µm latae. Rhizoidea adsunt. Capitulum conidiogenum maxime (47−) 63–96 (−122) µm diametro, laete brunneum apicem *v.* hyalinescens. Cellulae conidiogenae (17.5−) 25.3–65.4 (−133.7) µm longae, (1.6−) 1.8–2.6 (−3.5) µm latae, apicem *v.* angustatae. Conidia aseptata, hyalina, oblonga vel cylindrica, 1.5–2 (−2.5) × 0.5–1 µm.

Colonies umber (13 m) on OMA with conidiophores forming cream-coloured slimy heads arranged in concentric rings, reverse dark mouse grey (13’’’’’k) to almost black. On MEA colonies avellaneous (17’’’b) with conidiophores forming cream-coloured slimy heads arranged in concentric rings, reverse colonies tawny olive (17’’i). Colony diameter reaching 14 mm in 10 days on MEA at 25°C. Optimal growth temperature 20°C, no growth at 5°C or above 30°C. *Conidiophores* synnematal, erect, dark brown at the bases, becoming lighter towards the apex, (202−) 224.5–275.5 (−324.5) µm long, (19−) 17–42 (−31) µm wide in the middle, (16.5−) 20–37.5 (−60) µm wide at the base. *Rhizoids* present. Conidiogenous heads (47−) 63–96 (−122) µm across the widest part, light brown becoming hyaline towards the apex. Conidiogenous cells, hyaline (17.5−) 25.3–65.4 (−133.7) µm long, (1.6−) 1.8–2.6(−3.5) µm wide tapering towards the apex. *Conidia* produced through holoblastic, annellidic development. Conidia aseptate, hyaline, oblong to cylindrical, accumulating in slimy heads on the apices of the synnemata, 1.5–2 (−2.5) µm × 0.5–1 µm.

Specimens examined: Australia, isolated from wounds on *A. mearnsii*. Cann River, New South Wales, November

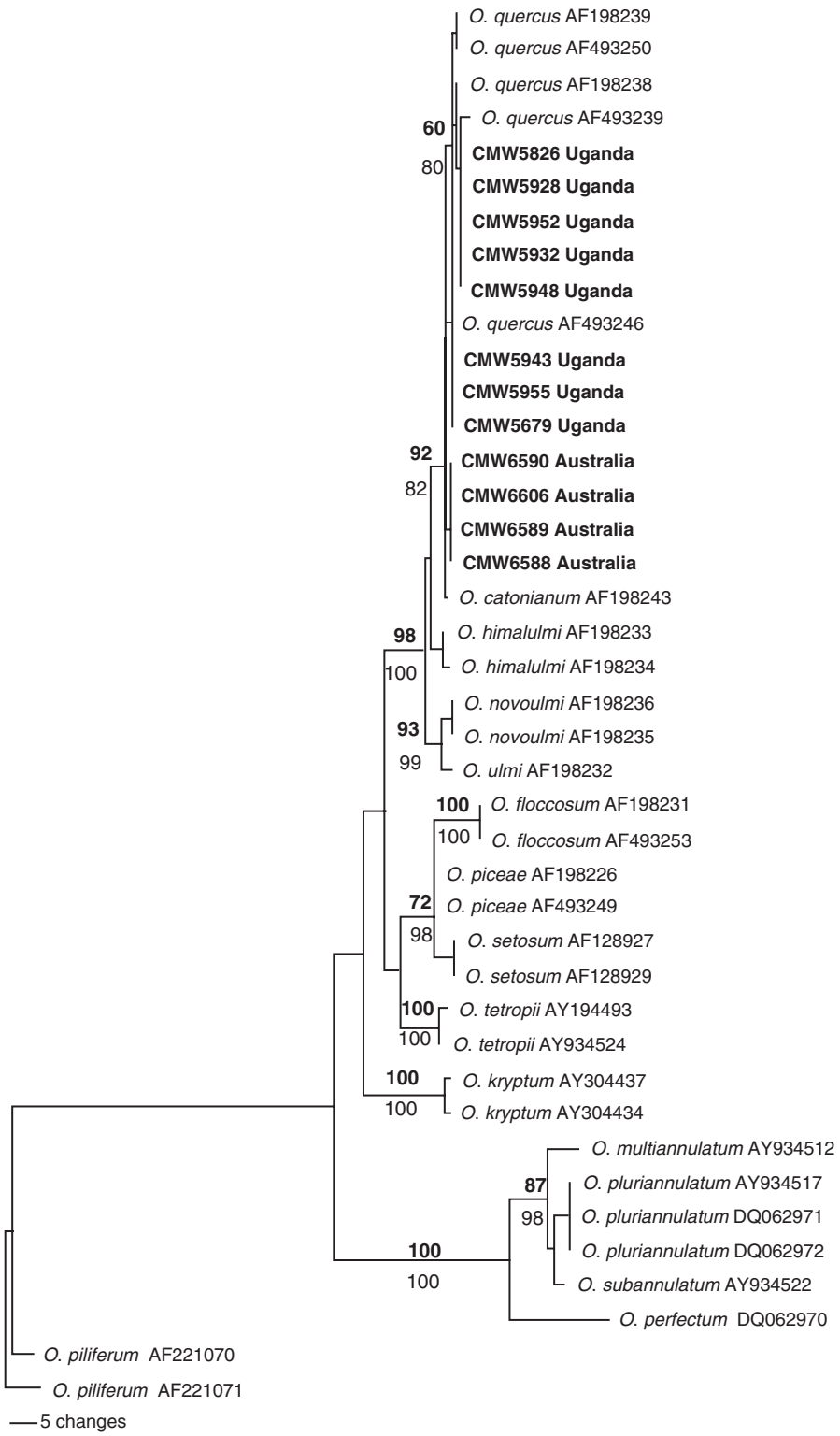

Fig. 1. Phylogenetic tree produced by a neighbour-joining analysis of the internal transcribed spacer sequence data. *Ophiostoma piliferum* was used as outgroup taxon. Bootstrap values for both parsimony analysis (indicated above each branch in bold font) and neighbour-joining (indicated below each branch) were derived from 1000 replicates.

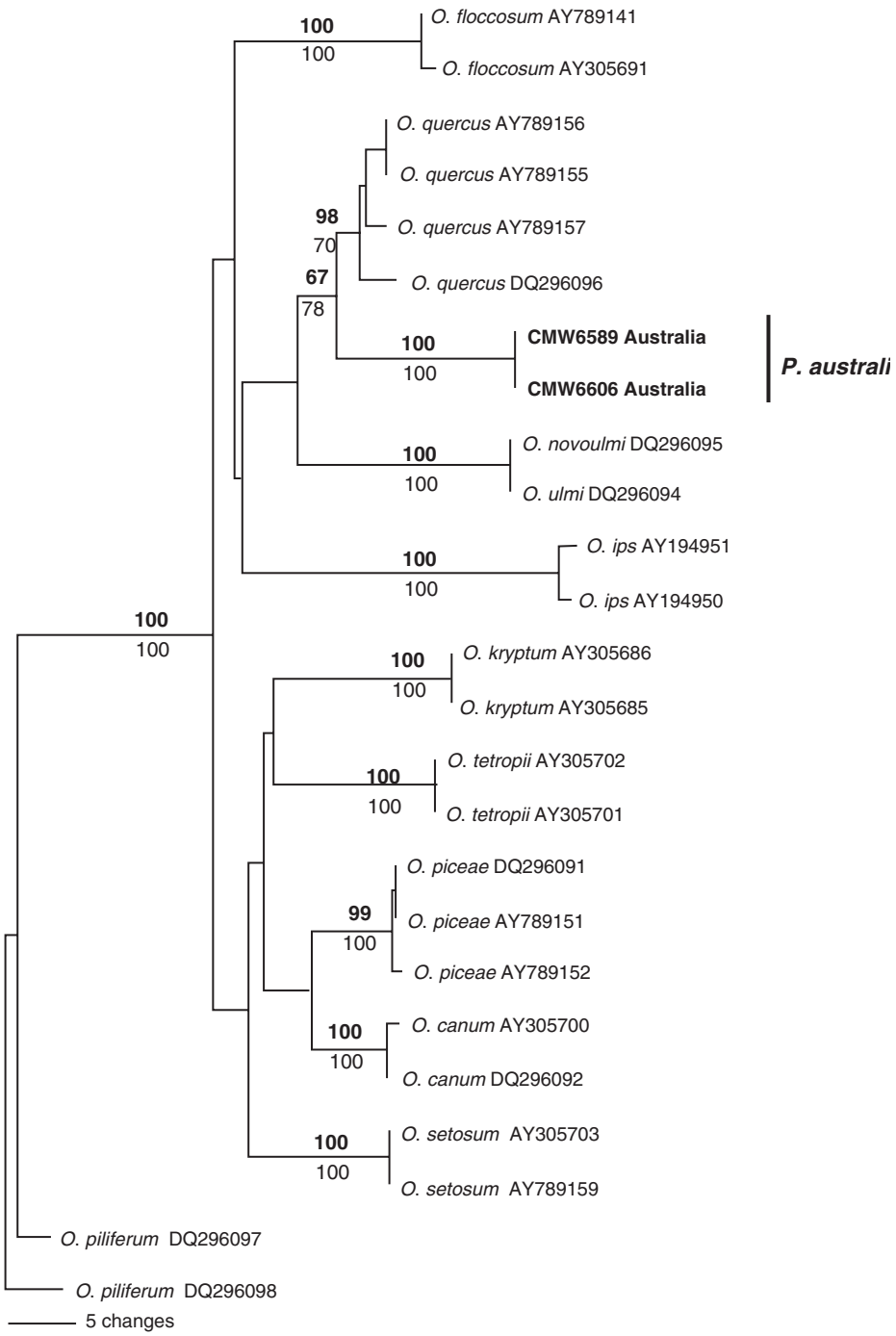

Fig. 2. Phylogenetic tree produced by a neighbour-joining analysis of the β-tubulin sequence data. *Ophiostoma piliferum* was used as outgroup taxon. Bootstrap values for both parsimony analysis (indicated above each branch in bold font) and neighbour-joining (indicated below each branch) were derived from 1000 replicates.

2000, M.J. Wingfield, **holotype** PREM 59426, dried specimen of living culture CMW6606/CBS121025.

Additional specimens: Australia, isolated from wounds on *A. mearnsii.* Cann River, New South Wales, November 2000, M.J. Wingfield, **paratype**, PREM 59741, dried specimen of living culture, CMW6589/CBS121026.

Discussion

In this study, we expand the host and geographic range of *O. quercus* and the new species, *P. australi* is described. These two fungi were isolated from *A. mearnsii* trees in Uganda and in Australia, respectively, where few studies on *Ophiostoma* spp. have been conducted in the past. Both

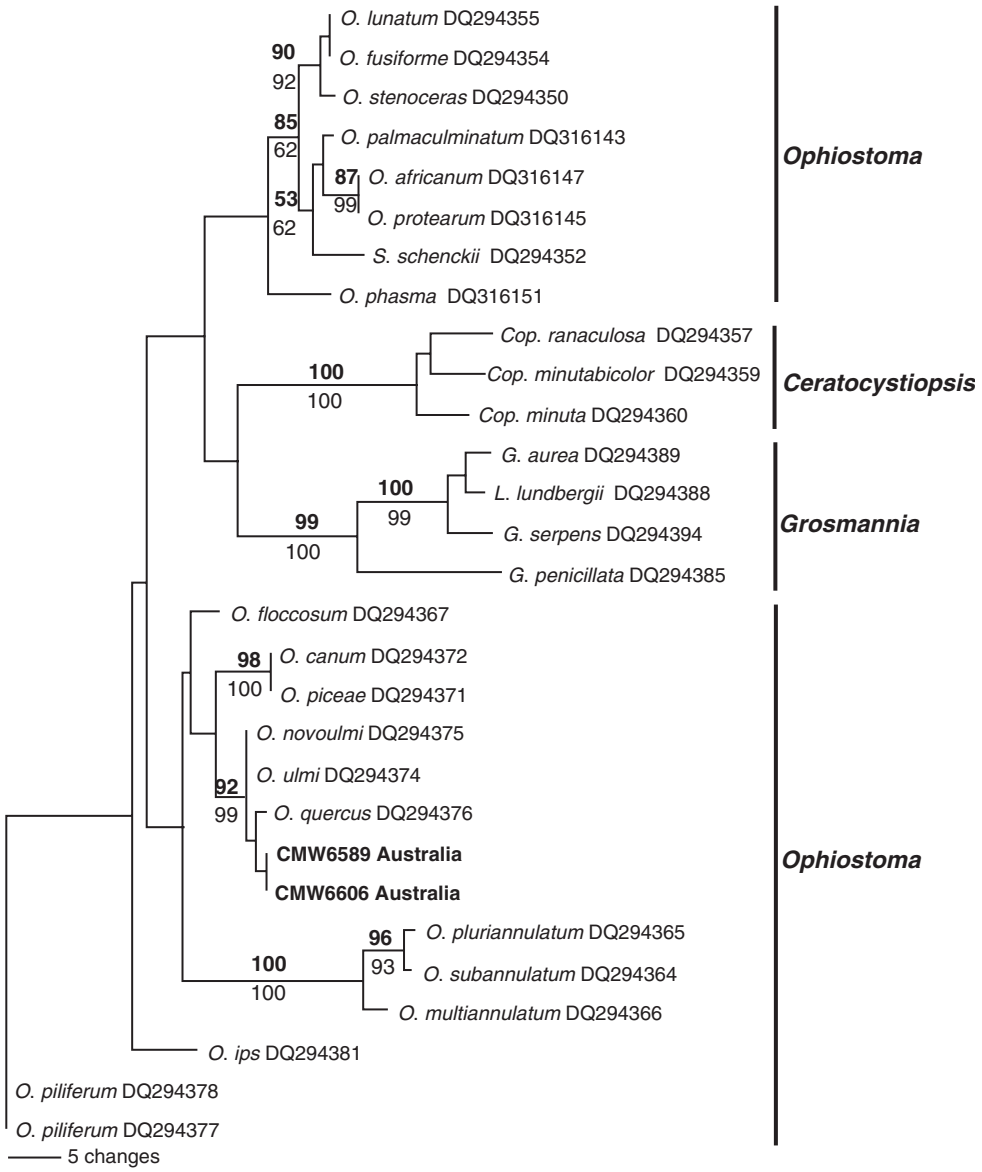

Fig. 3. Phylogenetic tree produced by a neighbour-joining analysis of the large subunit sequence data. *Ophiostoma piliferum* was used as outgroup taxon. Bootstrap values for both parsimony analysis (indicated above each branch in bold font) and neighbour-joining (indicated below each branch) were derived from 1000 replicates.

Table 2. Results of mating compatibility tests using tester strains and isolates from Uganda

Isolates where the identity was confirmed with internal transcribed spacer sequences are underlined. CMW, culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa

Tester strains	Isolates from Uganda crossed													
	CMW5679	CMW5910	CMW5948	CMW5900	CMW5825	CMW5933	CMW5917	CMW5902	CMW5651	CMW5955	CMW5930	CMW5952	CMW5922	CMW5943
CMW14307 (–)	+	+	–	–	+	+	+	+	+	+	+	–	+	–
CMW17257 (+)	–	–	+	+	–	–	–	–	–	–	–	+	–	+

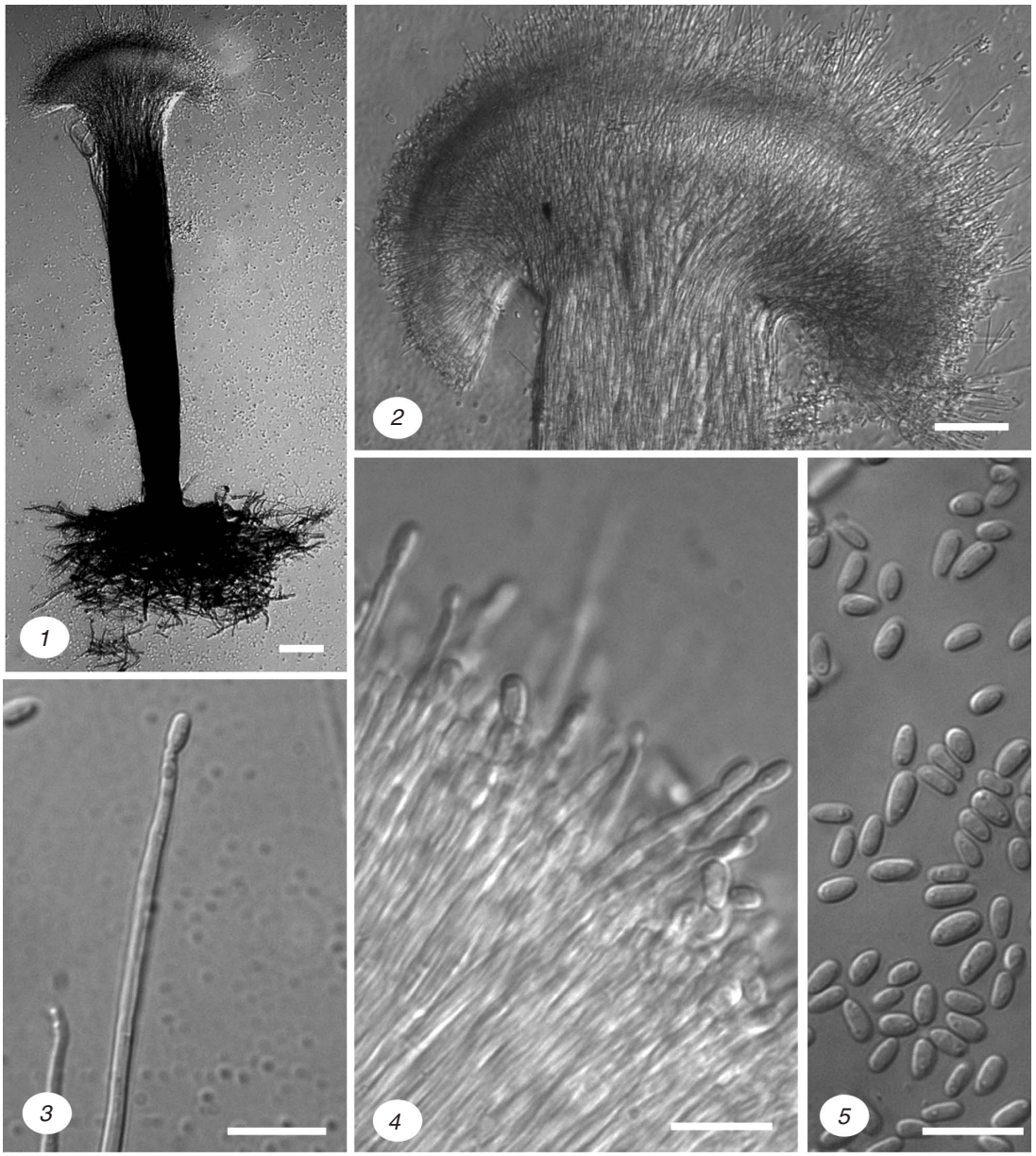

Fig. 4. Morphological characteristics of *Pesotum australi* sp. nov. (CMW6606). (*1*) Synnema (scale bar = 100 µm) showing rhizoids at base, (*2*) head of synnema (scale bar = 20 µm), (*3–4*) conidiogenous cells with conidia at the tips of percurrently proliferating conidiogenous cells (scale bar = 5 µm), (*5*) oblong to cylindrical conidia (scale bar = 5 µm).

Pesotum spp. reported in this study, group within the larger *O. piceae* complex, which is a group of morphologically similar species difficult to identify and that have been the subject of considerable taxonomic confusion (Przybyl and De Hoog 1989; Okada *et al*. 1998; Harrington *et al*. 2001).

P. australi is phylogenetically most closely related to *O. quercus*. However, DNA sequence data for several gene regions, including the ITS1 and ITS2, 5.8S, β-tubulin and the LSU of the rDNA operon have shown that this fungus is distinct from other *Pesotum* spp. In these analyses, it forms a well resolved clade, supported by a bootstrap value of 92% on the parsimony tree and 82% on the neighbour-joining tree for ITS data. *P. australi* is most closely related to members of the *O. piceae* complex that had previously been recognised to include nine species (Harrington *et al*. 2001). Species in the *O. piceae* complex are morphologically similar to each other (Przybyl and De Hoog 1989; Harrington *et al*. 2001), and recognition of *O. quercus* as distinct from *O. piceae* only became clear in the early 1990s (Brasier 1993; Halmschlager *et al*. 1994; Pipe *et al*. 1995). Thus, many species identified as either *O. piceae* or *O. quercus* before the advent of DNA sequence comparisons may represent other species in the complex.

P. australi can be distinguished from *O. quercus* and from other members in the *O. piceae* complex, by the fact that it produces only a *Pesotum* anamorph in culture. All other members of the *O. piceae* complex form *Sporothrix* synanamorphs in addition to the *Pesotum* state, and this separates the complex from other species of *Ophiostoma* (Harrington *et al*. 2001). Additionally, *O. quercus* grows at 32°C (Brasier and Stephens 1993; Harrington *et al*. 2001) while *P. australi* does not grow at 30°C or higher on MEA. The optimum growth temperature and the maximum temperature of growth of *P. australi* were 20 and 25°C, respectively. Most isolates of *O. quercus* form concentric rings of aerial mycelium on MEA (Halmschlager *et al*. 1994; Harrington *et al*. 2001) with synnemata bearing viscous drops of ellipsoid to ovoid conidia. *P. australi* has a similar culture morphology to *O. quercus* on OMA. However, its synnemata terminate in creamy masses of oblong to cylindrical conidia that are also shorter than those of *O. quercus*.

In this study, we were able to develop positive and negative mating tester strains from one *O. quercus* isolate from Uganda. Crosses between the tester strains and 14 other isolates from Uganda produced ascomata confirming that these all represent *O. quercus*. These mating tester strains will be useful in future studies aimed at identifying collections of *Ophiostoma* spp. from hardwoods that include *O. quercus*.

O. quercus was a common inhabitant of wounds on *A. mearnsii* in Uganda. This is interesting, given the fact that this fungus was not isolated from *A. mearnsii* in Australia. Neither was *P. australi* found on this tree in Uganda. *O. quercus* has, however, been recorded from *P. radiata* in Australia (Harrington *et al*. 2001). In the present study, sampling was undertaken from a very limited area thus sampling from different countries and from a wider range of areas in Australia where *A. mearnsii* is native are needed to better understand the host specificity of *P. australi*.

This study represents the first record of *O. quercus* from Uganda. Its occurrence in this country is not surprising as the fungus occurs worldwide, predominantly on hardwoods, but also on conifers in the northern hemisphere (Morelet 1992; Brasier and Kirk 1993; Halmschlager *et al*. 1994; Pipe *et al*. 1995; Kim *et al*. 1999; Harrington *et al*. 2001). It has also been reported in many countries of the southern hemisphere, from both native and non-native trees (De Beer *et al*. 2003). The only previous reports of the fungus from Africa are from South Africa, where it has been found on native *Olinia* sp. (De Beer *et al*. 1995), non-native *Eucalyptus grandis* (Hill) Maiden and *Quercus robur* L. (De Beer *et al*. 1995) and from three bark beetle species infesting *Pinus* spp. (Zhou *et al*. 2001).

The origin of *O. quercus* in the southern hemisphere has been a matter of controversy. It has been suggested that the fungus was introduced from the northern hemisphere, where it is probably native (Brasier and Kirk 1993; Harrington *et al*. 2001). However, the fact that *O. quercus* is common on various native trees in the southern hemisphere might equally suggest that it is also native to this part of the world (De Beer *et al*. 2003). Furthermore, *O. quercus* grows at high temperature ranges up to 32°C (Brasier and Stephens 1993), which suggests that it is well adapted to warmer climates (De Beer *et al*. 2003). Recent reports of the fungus on native *Schizolobium parahybum* in Ecuador (Geldenhuis *et al*. 2004) support the notion that it has a wide natural distribution, beyond the boreal region.

The taxonomy of *O. quercus* appears not to be fully resolved. The differences in branch lengths for isolates treated as *O. quercus* in the phylogenetic component of this study and the low bootstrap values for these branches lead us to hypothesise that *O. quercus* represents a complex of species with a wide geographic distribution. There are likely different strains or subspecies among those currently treated as *O. quercus* occurring on different hosts and under different geographical and climatic conditions. This hypothesis deserves further study, particularly at the population level where gene diversity among *O. quercus* strains collected from different parts of the world and from different substrates can be considered.

This study has extended the host and geographic range of *O. quercus* and it has identified a new closely related species. Native hardwood species in Australia represent an excellent plant material where new *Ophiostoma* spp. is likely to be found. Thus, surveys of fungi occurring, particularly on wounds on native Australian tree species, will probably result in the description of many new Ophiostomatoid fungi.

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