Identification and pathogenicity of *Graphium* and *Pesotum* species from machete wounds on *Schizolobium parahybum* in Ecuador

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Schizolobium parahybum is native to Ecuador and is widely distributed throughout South America. This tree has ideal timber properties and is a favored species for plantation development. *Schizolobium parahybum* trees, however, suffer from a serious disease that causes substantial losses to plantations in Ecuador. Most diseased trees have been regularly wounded with machetes and it has been suggested that these wounds might provide entry portals for pathogens. To determine the possible role that fungi associated with machete wounds might play in disease development, wood samples were taken from these wounds and screened for possible pathogens. A number of potential pathogens were identified, including *Ceratocystis fimbriata*, *C. moniliformis*, *Graphium* spp. and *Pesotum* spp. The objective of this study was to identify the twenty-one synnematous *Hyphomycetes*, from wounds on *S. parahybum*, using small subunit (SSU) and internal transcribed spacer (ITS) sequence data from the ribosomal RNA operon. We also investigated the possible role of these species in disease development in a greenhouse inoculation trail. Results showed that fifteen isolates reside in the *Graphium penicillioides* complex (Order: *Microascales*). Four isolates resided in the *Ophiostomatales* and represent the *Pesotum* anamorph of *Ophiostoma quercus*. The remaining two isolates were unidentified *Pesotum* anamorphs of *Ophiostoma*. None of the three species produced significant lesions in a greenhouse inoculation trail and we do not consider them pathogens of *S. parahybum*.

Key words: *Ceratocystis*, ITS, *Microascales*, *Ophiostoma quercus*, *Ophiostomatales*, SSU

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Introduction

Schizolobium parahybum (Vell.) Blake is a native tree species in South America, where it occurs in Ecuador and the Amazon Basin. Its timber is highly prized for production of a clear light-coloured veneer. *Schizolobium parahybum* has thus been used to establish plantations in Ecuador, where the tree is commonly known as "pachaco". In 1982, plantations of *S. parahybum* were established in the Los Sachas area of the Ecuadorian Amazon. However, trees soon developed a serious die-back disease known as pachaco die-back.

Observations of diseased trees in Ecuador have indicated that pathogens are probably introduced into the trees through mechanical damage caused by machetes. Preliminary isolations from machete wounds led to the isolation of the well-known tree pathogen, *Ceratocystis fimbriata* Ellis & Halsted, as well as several other fungi. These included *C. moniliformis* (Hedgcock) Moreau and a collection of isolates of synnematous *Hyphomycetes* tentatively identified as belonging to the *Graphium sensu lato* complex (Roux *et al*., 2000; Geldenhuis *et al*., 2001).

Species of *Graphium sensu lato*, including *Graphium*, *Pesotum* and *Phialographium*, are typified by well-developed, generally dark synnemata, producing single-celled conidia in slimy masses at their apices. The genera in this group were treated collectively for many years but were later separated based on their respective modes of conidial development. *Graphium* was first described in 1837 for five species, including *G. penicillioides* Corda (Corda, 1837), later named the lectotype species for the genus, which was defined as having percurrent conidial development (Hughes, 1958). Münch (1907) assigned the synnematous anamorphs of *O. piceae* (Münch) H. & P. Sydow and *O. canum* (Münch) H. & P. Sydow to *Graphium*. Crane and Schoknecht (1973), however, showed that conidiogenesis in *G. penicillioides* differs from that of the synnematous anamorphs of *O. piceae* and *O. ulmi* (Buisman) Nannfeldt. They transferred the anamorph of the latter species, *Graphium ulmi* Schwarz, to the new genus, *Pesotum*, with *Pesotum ulmi* (Schwarz) Crane & Schoknecht as the type species. Subsequently a number of other genera were described based on patterns of conidiogenesis, including *Phialographium* (Upadhyay and Kendrick, 1974) with the anamorph of *O. sagmatospora* (Wright & Cain) Solheim as the type species.

Wingfield *et al*. (1991) reassessed *Graphium*, *Pesotum* and *Phialographium*, and found that there is more than one mode of conidiogenesis present in both *Pesotum* and *Phialographium*. This suggested that differences in conidiogenesis do not provide a reliable characteristic to distinguish between these genera. They, therefore, transferred species of *Pesotum* and

Phialographium back to *Graphium*. Seifert and Okada (1993) concluded that *Graphium* should be restricted to anamorphs of the *Ophiostomataceae*. However, small subunit (18S) sequences showed that *G. penicillioides*, the lectotype species of *Graphium*, is not phylogenetically related to anamorphs of *Ophiostoma* (Okada *et al*., 1998). Results of their study, furthermore, showed that *G. penicillioides* includes several different taxa and it should be considered a species aggregate. The *G. penicillioides* aggregate forms a monophyletic group within the order *Microascales*, while the synnematous anamorphs of *O. piceae* and *O. ulmi* group with the *Ophiostomatales*. This classification was supported by Okada *et al*. (2000) who, based on 18S rDNA sequence data, showed that *Graphium*-like synnematous fungi currently occur in the *Microascales* (*Graphium*), *Ophiostomatales* (*Pesotum*) and *Chaetothyriales* (*Exophiala*). They also introduced a fourth phylogenetic group for an undescribed genus (or genera) belonging to the *Erysiphales*. Most recently, Harrington *et al*. (2001) recommended that the name *Pesotum* should be restricted to anamorphs related to the *O. piceae* complex, within the *Ophiostomatales*.

One means to distinguish between species of *Graphium* and *Pesotum* is to assess their ability to grow on media containing cycloheximide. *Ophiostoma* species are well known to be able to tolerate high levels of this antibiotic substance and it is commonly used in selective media for these fungi (Jacobs and Wingfield, 2001; Zhou *et al*., 2001). However, cycloheximide tolerance is not sufficiently robust to group *Graphium* and *Pesotum* (Harrington *et al*., 2001). Currently the only definitive method to determine whether fungi loosely resembling species of *Graphium* reside in this or other genera is to compare DNA sequence data for them (Okada *et al*., 1998).

Several *Pesotum* species are important plant pathogens. Most notable of these is *Pesotum ulmi*, the anamorph of *Ophiostoma ulmi*. This fungus together with *O. novo-ulmi* Brasier are the causal agents of Dutch elm disease (Brasier, 1990). The genus *Graphium* does not include important pathogens, but it commonly occurs in niches together with *Pesotum* spp. such as bark beetle galleries in trees killed by Dutch elm disease (Webber and Brasier, 1984).

The objective of this study was to identify isolates loosely assigned to *Graphium* that had been collected from wounds on diseased *S. parahybum* trees in Ecuador. This was accomplished by sequencing the small subunit (SSU) and internal transcribed spacer (ITS) regions (including the 5.8S gene) of the ribosomal RNA operon. The possible role of these fungi in die-back of *S. parahybum* was also considered by conducting preliminary pathogenicity tests under greenhouse conditions.

Materials and methods

Isolates

One hundred wood samples associated with machete wounds on different *S. parahybum* trees in Ecuador were collected. These samples were placed in moist chambers at 25ºC to induce fungal growth and sporulation. Synnematal anamorphs loosely assigned to the genus *Graphium* were commonly found sporulating on the wood surface after 8-10 days. These fungi were isolated by transferring masses of conidia from individual synnemata to 2% malt extract agar (MEA: $20gL^{-1}$ malt extract and $20gL^{-1}$ agar, Biolab). The single spore drops were allowed to grow for 10 days at 25ºC before they were grouped into morphotypes according to differences in colony colour (Rayner, 1970), fruiting structures and hyphal growth characteristics on MEA (Lacap *et al*., 2003). All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

DNA isolation

Two isolates loosely identified as *Graphium* spp., were randomly selected from each morphological group (Table 1) and grown in 2% malt extract broth ($20gL^{-1}$ malt extract, Biolab) at 25ºC. After 10 days, mycelium was harvested by filtration through sterile filter paper. DNA was extracted using a modification of the DNA extraction procedure described by Raeder and Broda (1985). The mycelium was ground to a fine powder using liquid nitrogen and a mortar and pestle, transferred to Eppendorf tubes and suspended in 800µL DNA extraction buffer (200mM Tris-HCl pH 8, 25mM EDTA pH 8, 150mM NaCl, 0.5% SDS). After addition of phenol:chloroform (5:3, v/v), the mixture was centrifuged (10 000 rpm, 60 minutes) to remove cell debris. This was followed by a series of phenol:chloroform (1:1, v/v) extractions on the upper aqueous layer until the interphase was clean. Excess phenol was removed by a final chloroform extraction. Nucleic acids were allowed to precipitate overnight at -20ºC in cold 100% ethanol (2:1, v/v). The DNA was collected by centrifugation (10 000 rpm, 30 minutes), washed in 70% ethanol and resuspended in 50µL sterile water. RNA was removed through addition of Rnase-A (Roche Molecular Biochemicals, Germany) and incubation at 37ºC for 60 minutes. The DNA solution was stored at -20ºC.

DNA amplification

The polymerase chain reaction (PCR) was used to amplify specific regions of the ribosomal RNA operon. The SSU gene of all representative isolates (Table 2) was amplified using the primer pair 2F (5`-ATCTGGTTGAT CCTGCCAGTAG-3`) and 1794R (5`- GATCCTTCCGCAGGTTCACC-3`) (Okada *et al*., 1997). Small subunit sequences revealed the identity of only two of the isolates. Thus, the internal transcribed spacer regions (ITS1 and ITS2)

Table 1. The three morphological groups of the *Graphium* and *Pesotum* species isolated from *S. parahybum*. Total number of isolates in each group are presented in parentheses, and numbers of isolates sequenced in this study are printed in bold type.

Morpho-logical group	Isolate numbers[a]	Colony colour		Fruiting structures	Characteristics of hyphal growth on MEA
		Reverse	Top		
Group 1	**CMW5564; CMW5573** (2)	white, center olivaceous buff (21’’’d)	white, center olivaceous buff (21’’’d)	dominant	suppressed growth, colony margin smooth
Group 2	**CMW5565;** CMW5567; **CMW5568;** CMW5575 (4)	buff (19’’f)	white, center olivaceous buff (21’’’d)	dominant	aerial mycelium present, colony margin smooth
Group 3	CMW5551; **CMW5554**-5556; CMW5558-5563; **CMW5566**; CMW5570-5572; CMW5574 (15)	buff (19’’f)	greenish glaucous (33’’’f), margin buff	sparse	suppressed growth, colony margin smooth

[a] CMW refers to the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

and the 5.8S gene of the other four isolates (CMW5565, CMW5568, CMW5564, CMW5573) were amplified using primers ITS1 (5'-TCCGTAGGT GAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3`) (White *et al*., 1990). PCR reactions were carried out in a total volume of 50µL and included 1ng DNA template, Expand HF buffer containing 1.5mM $MgCl_2$ (supplied with the enzyme), 0.2µM of each primer, 200µM of each dNTP and Expand^{TM} High Fidelity Taq polymerase mixture (1.75 U) (Roche Pharmaceuticals, Germany). PCR conditions described by Okada *et al*. (1997) were followed to amplify the SSU and ITS regions. PCR products were

visualized under UV illumination after electrophoreses in a 1% agarose (Sigma) gel, stained with ethidium bromide to determine the success of the reactions.

DNA sequencing

PCR fragments were purified using a High Pure™ PCR Product Purification Kit (Boehringer Mannheim) and sequenced using a ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq®DNA Polymerase, FS (Perkin-Elmer, Warrington, U.K.) following the manufacturers protocol on a ABI 377 Autosequencer (Applied Biosystems). The purified amplification products were sequenced in both directions with the same primers used for PCR. Additional internal primers 404F (5`-GCTACCACATC CAAGGAAGG-3`) and 581R (5`-ATTACCGCGGCTGCTGGC-3`) (Okada *et al*., 1997) were used for sequencing of the SSU.

Sequence analysis

Sequence data were manually aligned by inserting gaps that were treated as missing data. Ambiguously aligned sequences were not included in the analysis. DNA sequences were analyzed using PAUP 4.0* (Phylogenetic Analysis Using Parsimony* and other Methods version 4) (Swofford, 1998). Heuristic searches were done with stepwise addition (random sequence addition), branch swapping (tree bisection reconnection) and MULPAR effective with MaxTrees set to auto-increase. The tree length distributions over 100 randomly generated trees were evaluated to access the phylogenetic signal in the data sets (g1) (Hilles and Haulsenbeck, 1992). Statistical support for internal branches of the most parsimonious SSU and ITS trees were estimated by bootstrap analysis of a 1000 replicates. Sequences for other species included in the SSU and ITS analysis were obtained from GenBank (Table 2). *Neurospora crassa* Shear & B.O. Dodge was used as monophyletic outgroup for the SSU data and *O. ulmi* for ITS sequence analysis. A BLAST search was done with ITS sequences that could not be included in the ITS analysis, due to unambiguous alignment.

Pathogenicity

A preliminary greenhouse inoculation trial was conducted with one isolate of each of the three species identified in this study (Table 2). The isolates were transferred to fresh MEA and incubated at 25ºC. After 8 days,

each isolate was inoculated into 20 trees (1-2cm diam.) and an equal number of trees served as controls. The inoculations were carried out using the technique described by Zhou *et al*. (2002). After six weeks, the outer bark was removed with a scalpel and the length of the inner lesions measured. Pieces of wood (2mm × 2mm) were cut several distances from the lesions and placed on MEA to determine whether the inoculated fungi were present in the wounds.

Differences in lesion lengths were analysed using one-way analysis of variance (ANOVA) with SAS (version 8, SAS Institute Inc., Cary, NC). Data were log transformed where they did not meet the assumptions of ANOVA.

Results

Isolates

A total of 21 *Graphium*-like isolates were obtained from the wood samples. Each of these isolates was specifically from a different tree. The isolates could be placed in three morphotypes based on colony colour, production of fruiting bodies and the characteristics of hyphal growth on MEA (Table 1).

Sequence analysis

Both strands of the SSU rDNA of the six representative isolates were sequenced to determine whether these isolates represented *Pesotum* or *Graphium* species. After manual alignment, a total of 1032 base pairs were obtained. Uninformative characters were excluded from the data set, resulting in a total of 908 excluded characters and 124 parsimony informative characters. The heuristic search option generated 20 most parsimonious trees with similar topologies but with different branch lengths. Only one tree was selected for presentation (Fig. 1). The phylogram generated from the SSU sequence data indicated that two of the isolates reside in the *G. penicillioides* aggregate, Order *Microascales*. The other four isolates grouped with *Pesotum* (*Ophiostomatales*). Two of the latter isolates grouped closely with *O. piceae* and *O. ulmi*, while the other two resided in a separate clade.

The ITS rDNA regions of the four *Pesotum* spp. were sequenced in both directions to compare them with *O. quercus* (Georgevitch) Nannfeldt and *O. piceae*. Only sequences for two of the isolates could be unambiguously aligned. The other two isolates were thus excluded from the analysis. The total number of characters after alignment was 579, including 196 bp of ITS1, 158 bp of the 5.8S gene and 225 bp of ITS2. Uninformative characters were

Table 2. Fungal isolates used in DNA sequence comparisons. Numbers of isolates sequenced in this study are printed in bold type and those used in the pathogenicity trial are underlined.

Species	Culture no.	Host	Origin	GenBank no.	
				ITS	SSU
Ceratocystis fimbriata	[a]C89	-	-	-	U32418
Chaetomium elatum	[b]UCB81-063	-	-	-	M83257
Graphium penicillioides	[c]**CMW5566**	*S. parahybum*	Ecuador	-	AY351895
	CMW5554	*S. parahybum*	Ecuador	-	AY351894
	[d]CBS506.86	*Ulmus procera*	UK	-	AB007652
	CBS320.72	forest soil	Solomon Islands	-	AB007653
	CBS470.71	*Fagus sylvatica*	Germany	-	AB007681
	CBS781.85	*Orthotomicus erosus*	South Africa	-	AB007682
	[e]JCM9301	*Salix* sp.	Netherlands	-	AB007654
G. putredinis	JCM7866	grass	Japan	-	AB007683
Microascus cirrosus	[f]UAMH963	-	-	-	M89994
Neurospora crassa	Unknown	-	-	-	X4971
Ophiostoma ainoae	JCM9356	*Ips typographus*	Japan	-	AB007665
O. bicolor	JCM9358	*I. typographus*	Japan	-	AB007666
O. cucullatum	JCM8815	*I. typographus*	Japan	-	AB007664
O. europhioides	JCM9360	*I. typographus*	Japan	-	AB007667
O. penicillatum	JCM9362	*Picea jezoensis*	Japan	-	AB007668
O. piceae	CMW2468	*P. abies*	France	AF493240	-
	CMW7644	*P. abies*	Austria	AF493245	-
	CMW7646	*P. abies*	Poland	AF493247	-
	CMW7648	*P. sitchensis*	UK	AF493249	-
	JCM6016	*Betula platyphylla*	Japan	-	AB007663
O. quercus	CMW2463	*Fagus sylvatica*	France	AF493239	-
	CMW7645	*Quercus robur*	Austria	AF493246	-

Table 2 continued. Fungal isolates used in DNA sequence comparisons. Numbers of isolates sequenced in this study are printed in bold type and those used in the pathogenicity trial are underlined.

	CMW7647	*Q. robur*	Poland	AF493248	-
	CMW7650	*Quercus* sp.	UK	AF198238	-
	CMW5565	*S. parahybum*	Ecuador	AY351899	AY351901
	CMW5568	*S. parahybum*	Ecuador	AY351900	AY351896
O. stenoceras	UCB57-013	-	-	-	M85054
O. ulmi	[g]MH75	-	-	U23424	-
	[h]ATCC32437	*U. americana*	USA	-	M83261
Pesotum fragrans	CBS 219.83	*P. abies*	Norway	-	AB007656
Pesotum sp.	**CMW5573**	*S. parahybum*	Ecuador	-	AY351897
	CMW5564	*S. parahybum*	Ecuador	-	AY351898
Podospora anserina	Unknown	-	-	-	X54864
Pseudallescheria boydii	Unknown	-	-	-	U43915
Sporothrix schenckii	ATCC14284	human	USA	-	M85053

[a] C = Culture Collection of T.C. Harrington, Department of Plant Pathology, Iowa State University, Iowa, USA. [b] UCB = Culture Collection of University of California Berkeley, CA, USA. [c] CMW = Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. [d] CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands. [e] JCM = Japanese Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Saitama, Japan. [f] UAMH = University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada. [g] MH = Culture Collection of M. Hubbes, Faculty of Forestry, University of Toronto, Ontario, Canada. [h] ATCC = American Type Culture Collection, Manassas, VA, USA.

excluded from the data analysis resulting in a total of 557 characters excluded from the data set. The remaining 22 characters were all parsimony informative. Heuristic searches generated three most parsimonious trees with the same topologies but different branch lengths. One of these trees was selected for presentation (Fig. 2). The ITS tree included sequences of four *O. quercus* isolates, four *O. piceae* isolates and the two *Pesotum* isolates from Ecuador. The two *Pesotum* isolates clearly reside with *O. quercus*. The BLAST search that was done with ITS sequences of the two *Pesotum* isolates that could not be included in the ITS analysis, did not result in a positive identification of these isolates.

Pathogenicity

Inoculations with *G. penicillioides*, *O. quercus* and the *Pesotum* sp. resulted in very small lesions. Control inoculations did not give rise to any lesions. The inoculated fungi could be re-isolated from only slightly coloured wood, up to 10 mm from the lesions. The average lesion length for *G. penicillioides*, *O. quercus*, the *Pesotum* sp. and the control were 12.1 mm, 16.7 mm, 12.9 mm and 12 mm respectively. Lesions associated with *O. quercus* were significantly longer than the others, including the control ($P = 0.0031$). Neither *G. penicillioides* nor the *Pesotum* sp. differed from the control inoculations in terms of lesion length (Fig. 3).

Discussion

In this study SSU and ITS sequence data were used to identify *Graphium*-like isolates collected from diseased *S. parahybum* trees in Ecuador. Three different species, one within the *Microascales* and two in the *Ophiostomatales* were isolated from machete wounds. This is in addition to *C. fimbriata* and *C. moniliformis* that were isolated from these wounds in previous studies (Roux *et al*., 2000; Geldenhuis *et al*., 2001). The *Graphium* sp. identified is most likely related to *G. penicillioides* and the one *Pesotum* sp. is *O. quercus*. The other *Pesotum* sp. appears to represent an undescribed taxon that we have chosen not to name, due to the low number of isolates available. This study represents the first reports of *G. penicillioides* and *O. quercus* from Ecuador and from *S. parahybum*.

Our results have shown that lesions caused by *O. quercus* after inoculation on young *S. parahybum* trees in the greenhouse, differed significantly from the control. These lesions however, did not differ more than 5 mm from the control and although the differences are statistically significant they are not pathogenetically important. *Graphium penicillioides* and the *Pesotum* sp. were unable to cause any signs of disease. These three fungi were most probably transferred to machete wounds by casual insects and they clearly appear to be saprobic. It was interesting that we were able to re-isolate them from apparently healthy wood in advance of the inoculation wounds. This indicates that they are able to grow inside the tree without causing disease. Although we do not consider them primary pathogens, it is possible that their presence may contribute to sapstain in response to the decline of the tree.

Graphium penicillioides was the most commonly isolated synnematous fungus from the machete wounds on *S. parahybum*. Identification of *Graphium* and *Pesotum* species is difficult because these genera are morphologically very

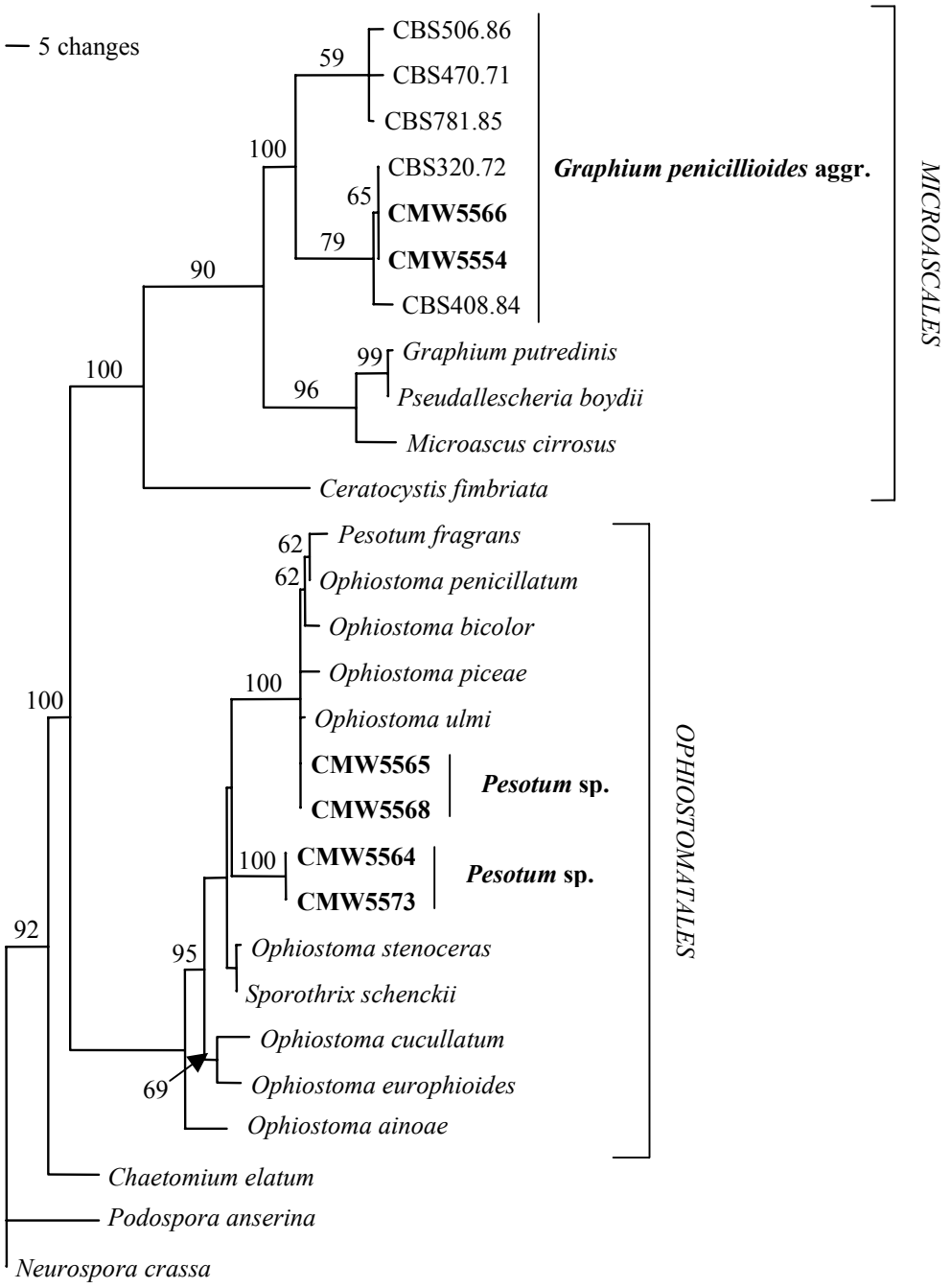

Fig. 1. One of the most parsimonious phylogenetic trees produced by a heuristic search of the SSU sequence data. *Neurospora crassa* was used as outgroup taxon. Bootstrap values were derived from 1000 replicates and are indicated above the branches of the tree. Length of tree = 238 steps, CI = 0.664, RI = 0.891, RC = 0.592 and g1 = –0.57.

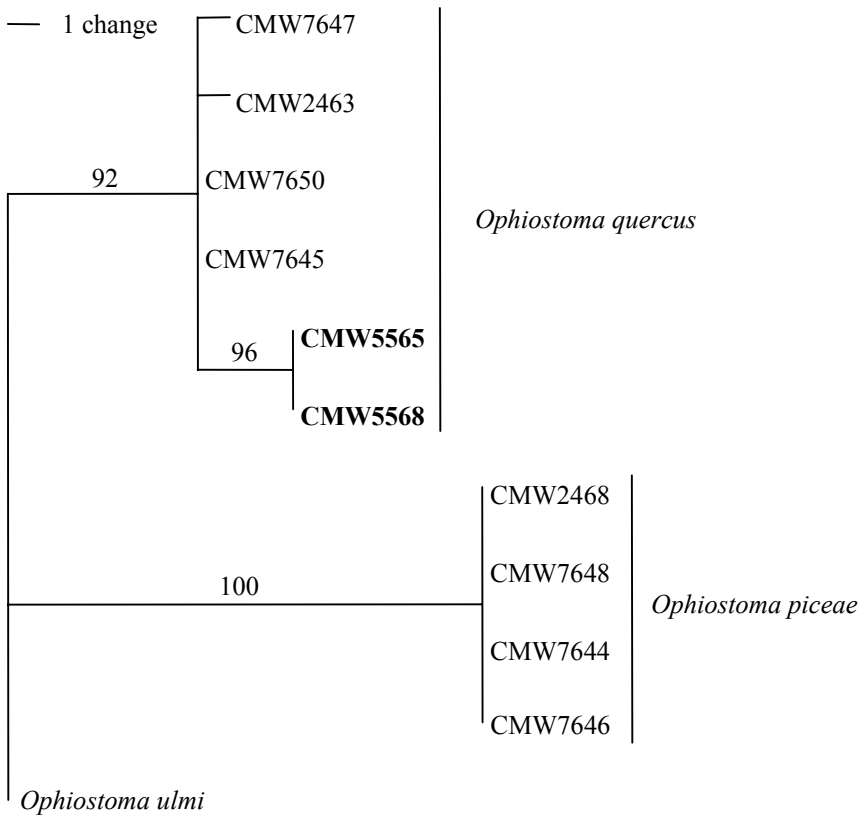

Fig. 2. One of the most parsimonious phylogenetic trees constructed from ITS sequence data. A heuristic search was done with *Ophiostoma ulmi* as outgroup taxon. Bootstrap values (1000 replicates) are indicated above the tree branches. Length of tree = 24 steps, CI = 0.958, RI = 0.984, RC = 0.943 and g1 = –0.934.

similar. *Graphium penicillioides* isolates were thus distinguished from the other isolates based on cultural characteristics and their identity established using DNA sequence data. Little is known regarding the biology of *G. penicillioides*. The fungus was originally described from *Populus italica* Moench. in Prague (Corda, 1837) and there are no reports of it being pathogenic on the trees from which it has been isolated. It clearly has a wide host and distribution range on wounds of deciduous trees.

Six of the 21 *Graphium*-like isolates obtained from *S. parahybum* in this study occur within the genus *Pesotum*. Comparisons of sequence data showed that four of these isolates represent the *Pesotum* anamorph of *O. quercus*. Okada *et al*. (1998) assigned *P. pirinum* (Goid.) Okada & Seifert as the anamorph of *O. quercus*, but Harrington *et al*. (2001) considered *P. pirinum* to be distinct from *O. quercus* and proposed that *G. roboris* Georgescu & Teodoru might be an appropriate anamorph. Resolving this issue did not form part of the scope of this study and until this has been clarified, we will refer to

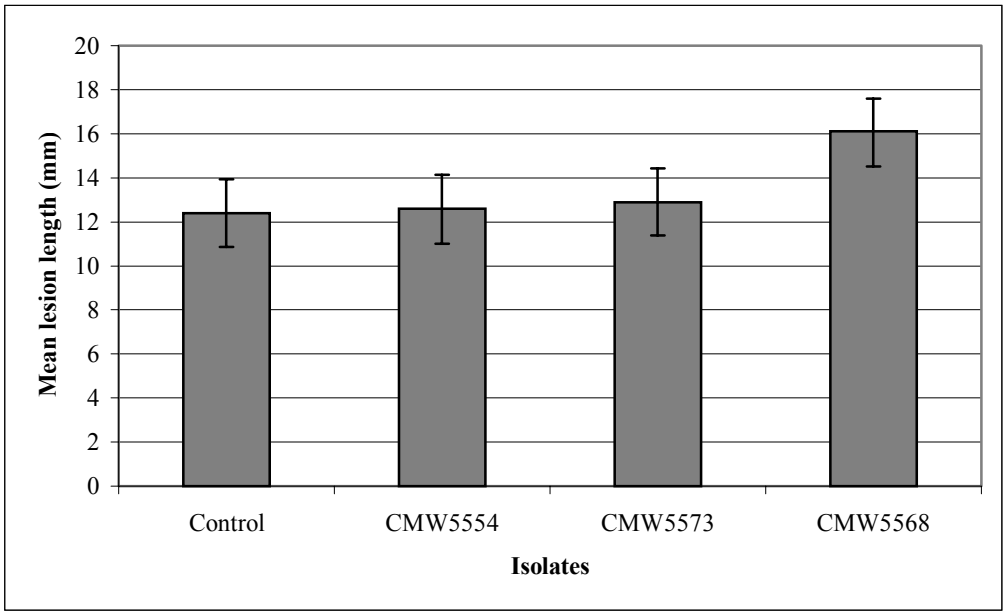

Fig. 3. Mean lesion length after inoculation of *Schizolobium parahybum* with *Graphium penicillioides* (CMW5554), the *Pesotum* sp. (CMW5573) and *Ophiostoma quercus* (CMW5568). Bars represent mean (Least Squares Means) lesion lengths for each isolate. Lesion length caused by *O. quercus* differed significantly from the control (P = 0.0031), while *G. penicillioides* and the *Pesotum* sp. showed no significant differences.

the isolates from Ecuador as *O. quercus*. *Ophiostoma quercus* is a common and widespread fungus, occurring predominantly on hardwoods, but also on conifers (Harrington *et al*., 2001). The fungus was originally described from oak in Yugoslavia (Georgevitch, 1926), but for many years, was treated as a synonym of *O. piceae* (Hunt, 1956; Upadhyay, 1981; Przybyl and de Hoog, 1989), with the consequence that many reports of *O. piceae* from hardwoods could have represented *O. quercus*. Its occurrence on native hardwood trees in Ecuador, confirms previous suggestions that it might be native to the southern continents (De Beer *et al*., 2003). Although *O. quercus* has been linked to vascular wilt on oak in Europe (Anonymous, 1990), it appears to be only a weak pathogen or a saprobe on *S. parahybum* in Ecuador.

Two isolates from *S. parahybum* could only be identified as representing a *Pesotum* sp. This fungus appears to be an undescribed species.

This study has extended both the host and geographic range of *O. quercus* and *G. penicillioides*. These fungi, and the unnamed *Pesotum* sp., were also shown not to contribute to the death of *S. parahybum* in Ecuador. Although they are related to plant pathogenic fungi and were associated with

dying trees, we do not believe that they justify inclusion in future pathogenicity tests that are planned for trees in Ecuador plantations.

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