Phylogeny of the *Quambalariaceae* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia

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Abstract: The genus *Quambalaria* consists of plant-pathogenic fungi causing disease on leaves and shoots of species of *Eucalyptus* and its close relative, *Corymbia*. The phylogenetic relationship of *Quambalaria* spp., previously classified in genera such as *Sporothrix* and *Ramularia*, has never been addressed. It has, however, been suggested that they belong to the basidiomycete orders *Exobasidiales* or *Ustilaginales*. The aim of this study was thus to consider the ordinal relationships of *Q. eucalypti* and *Q. pitereka* using ribosomal LSU sequences. Sequence data from the ITS nrDNA were used to determine the phylogenetic relationship of the two *Quambalaria* species together with *Fugomyces* (= *Cerinosterus*) *cyanescens.* In addition to sequence data, the ultrastructure of the septal pores of the species in question was compared. From the LSU sequence data it was concluded that *Quambalaria* spp. and *F. cyanescens* form a monophyletic clade in the *Microstromatales,* an order of the *Ustilaginomycetes*. Sequences from the ITS region confirmed that *Q. pitereka* and *Q. eucalypti* are distinct species. The ex-type isolate of *F. cyanescens*, together with another isolate from *Eucalyptus* in Australia, constitute a third species of *Quambalaria*, *Q. cyanescens* (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer comb. nov. Transmission electron-microscopic studies of the septal pores confirm that all three *Quambalaria* spp. have dolipores with swollen lips, which differ from other members of the *Microstromatales* (i.e. the *Microstromataceae* and *Volvocisporiaceae*) that have simple pores with more or less rounded pore lips. Based on their unique ultrastructural features and the monophyly of the three *Quambalaria* spp. in the *Microstromatales*, a new family, *Quambalariaceae* Z.W. de Beer, Begerow & R. Bauer fam. nov., is described.

Taxonomic novelties: *Quambalariaceae* Z.W. de Beer, Begerow & R. Bauer fam. nov., *Quambalaria cyanescens* (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer comb. nov.
Key words: *Cerinosterus, Fugomyces,* ITS, LSU, *Microstromatales, Sporothrix, Ramularia,* ultrastructure, *Ustilaginomycetes*.

INTRODUCTION

During the 1950's, a shoot disease was observed on *Corymbia maculata* (then *Eucalyptus maculata*) seedlings in New South Wales, Australia. The causal fungus was later described as *Ramularia pitereka* J. Walker & Bertus (Walker & Bertus 1971). In 1987, a similar disease was noted on a *Eucalyptus grandis* clone in South Africa. Wingfield *et al.* (1993) described the South African fungus as a new species, *Sporothrix eucalypti* M.J. Wingf., Crous & Swart. In his monograph of *Ramularia* Unger, Braun (1998) transferred *R. pitereka* to *Sporothrix* Hektoen & C.F. Perkins. In the same volume, a third *Sporothrix* species, *S. pusilla* U. Braun & Crous, isolated from leaf spots on *Eucalyptus camaldulensis* in Thailand, was described. Braun (1998) distinguished the three species based on morphology and host specificity. The treatment of the three species in *Sporothrix* (*Ophiostomataceae, Ophiostomatales*), and not *Ramularia* (*Mycosphaerellaceae, Mycosphaerellales*), was based largely on conidial scar morphology (Braun 1998).

Studies prior to Braun's (1998) treatment of the *Eucalyptus* pathogens as species of *Sporothrix* had shown that this genus accommodates superficially similar species with diverse phylogenetic relationships (Weijman & De Hoog 1985, De Hoog 1993). The type species for the genus *Sporothrix*, *S. schenckii* Hekt. & C.F. Perkins, was placed in the teleomorph genus *Ophiostoma* Syd. & P. Syd., based on 18S rDNA sequences (Berbee & Taylor 1992). More recently, Simpson (2000) showed that isolates of *R. pitereka* are not cycloheximide-tolerant, as is almost always the case with *Sporothrix* isolates with affinities to *Ophiostoma* (Harrington 1981). Based on the cycloheximide intolerance, pathogenicity to species of *Eucalyptus* and *Corymbia*, the dense growth of white conidiophores on agar media and the host, and the absence of distinct denticles on the conidiogenous cells, Simpson (2000) concluded that the affinities of *R. pitereka* and the two related species, *S. eucalypti* and *S. pusilla*, are not with the *Ophiostomataceae*. He consequently erected the new genus, *Quambalaria* J.A. Simpson, to accommodate the three species. Simpson (2000), like Braun (1998), distinguished the species based on conidial morphology and specificity to their respective *Eucalyptus* or *Corymbia* hosts. Furthermore, based on the apparent absence of dolipore septa in their hyphae observed by light microscopy, he suggested that these fungi probably reside in either one of the basidiomycete orders *Exobasidiales* Henn., emend. R. Bauer & Oberw., or *Ustilaginales* G. Winter, emend. R. Bauer & Oberw. (Simpson 2000).

There had been one other *Sporothrix*-like fungus isolated from *Eucalyptus pauciflora* in Australia by V.F. Brown. This isolate was sent to CBS in 1973 and was identified as *Sporothrix cyanescens* de Hoog & G.A. de Vries, earlier described

from human skin (De Hoog & De Vries 1973). Smith & Batenburg-Van der Vegte (1985) confirmed that *S. cyanescens,* and also *S. luteoalba* de Hoog, have dolipores in their septa and are thus the anamorphs of basidiomycetes. Based on this fact and the presence of the basidiomycetous coenzyme Q-10 system (Suzuki & Nakase 1986), Moore (1987) erected a new genus, *Cerinosterus* R.T. Moore, for the two *Sporothrix* spp., with *C. luteoalbus* (de Hoog) R.T. Moore as generic type species. The first phylogenetic study that included the two *Cerinosterus* spp. showed that *C. luteoalbus* groups within the *Dacrymycetales* Henn. based on LSU sequences (Middelhoven *et al.* 2000). However, *C. cyanescens* (de Hoog & G.A. de Vries) R.T. Moore grouped in the *Microstromatales* R. Bauer & Oberw*.,* and it was suggested that it could not be accommodated in *Cerinosterus*. Sigler & Verweij (2003) thus described a new genus, *Fugomyces* Sigler, with *F. cyanescens* (de Hoog & G.A. de Vries) Sigler as type species.

The aim of this study was to determine whether *Quambalaria* spp. are monophyletic and what their relationship was to *F. cyanescens,* using ITS sequences. Furthermore, ribosomal LSU sequences and ultrastructural characters were used to determine an appropriate order in which species of *Quambalaria* should reside.

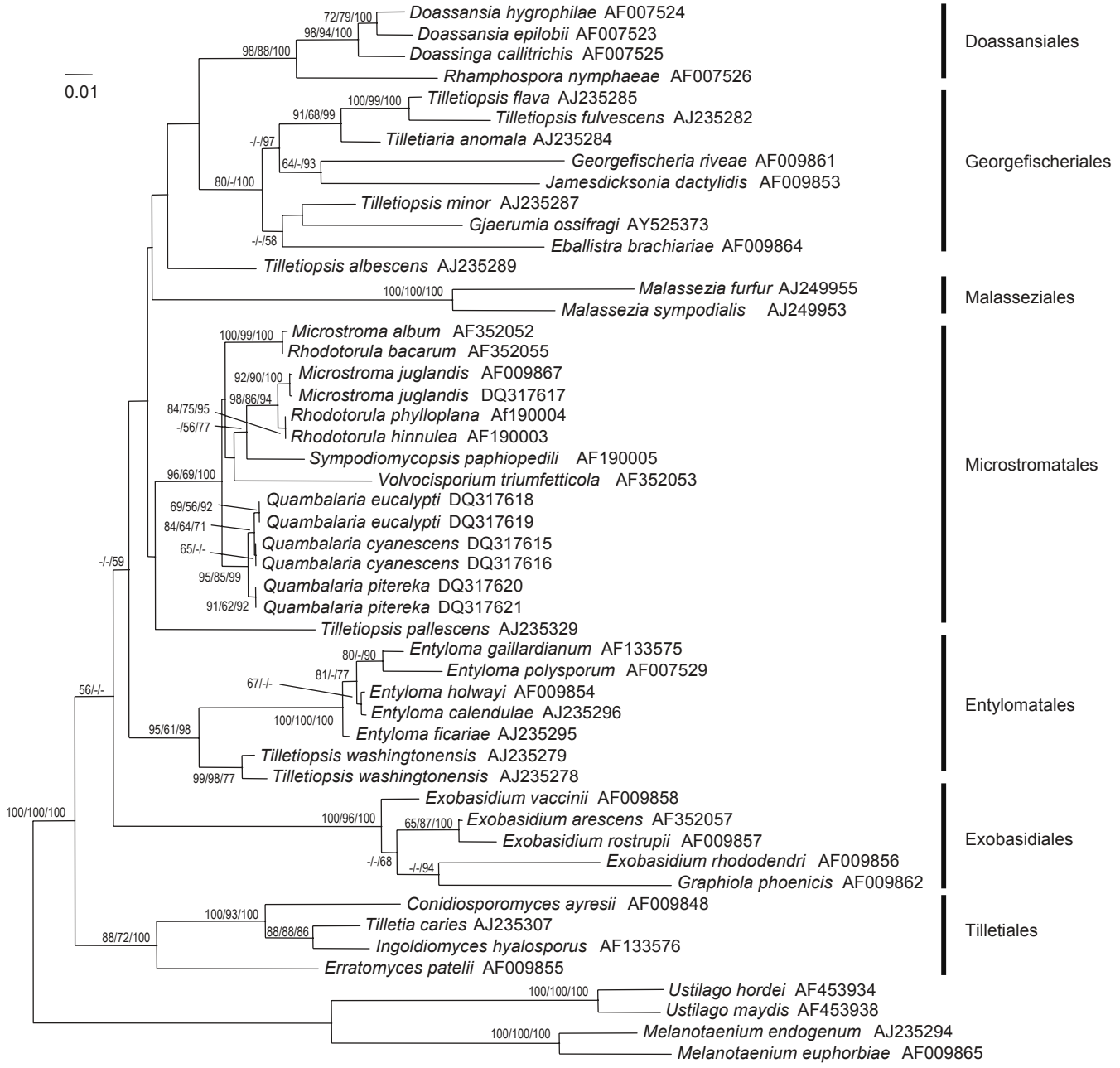


Fig. 1. Phylogram obtained by neighbour-joining analysis using GTR+I+G substitution model of the nuclear LSU region sequences of species in the *Microstromatales*. The topology was rooted with four members of the *Ustilaginomycetidae*. The numbers from left to right refer to percentage bootstrap values of 1000 replicates of neighbour-joining, maximum parsimony, and to *a posteriori* probabilities of Bayesian Markov chain Monte Carlo analysis. Values smaller than 50 % are not shown. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site.

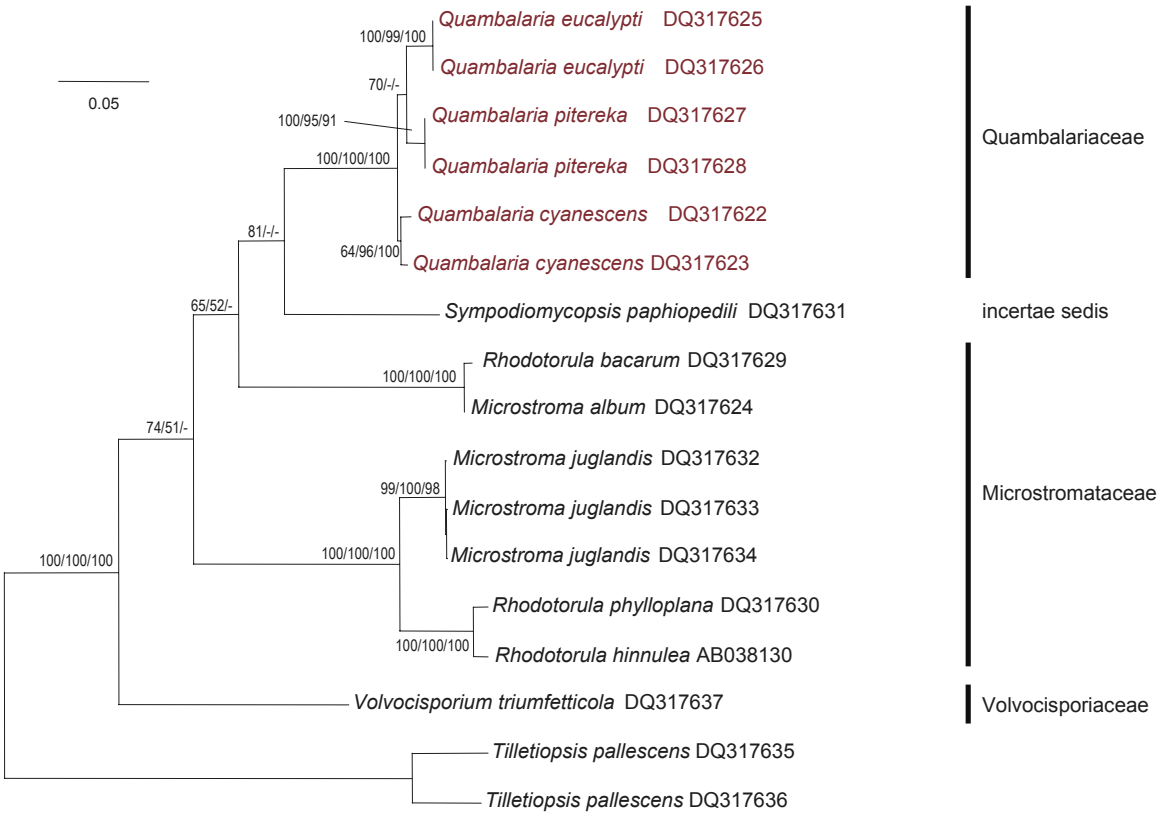


Fig. 2. Phylogram obtained by neighbour-joining analysis of DNA sequences of the nuclear ITS region of species in the *Microstromatales,* using the TVM+I+G substitution model. The topology was rooted with two isolates of *Tilletiopsis pallescens*. The numbers refer to percentage bootstrap values of 1000 replicates of neighbour-joining and maximum parsimony, and to *a posteriori* probabilities of Bayesian Markov chain Monte Carlo analysis. Values smaller than 50 % are not shown. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site.

MATERIALS & METHODS

Isolates and herbarium specimens

For phylogenetic studies, two South African isolates of *Q. eucalypti* (M.J. Wingf., Crous & W.J. Swart) J.A. Simpson, including the ex-type culture (CMW 1101 = CBS 118844), were compared with two isolates representing *Q. pitereka* (J. Walker & Bertus) J.A. Simpson from recent disease outbreaks in Queensland, Australia (Table 1). Two isolates representing *F. cyanescens,* including the ex-type culture (CBS 357.73), were also included. Other isolates for which DNA sequences were obtained in this study, are listed in Table 1. GenBank accession numbers of sequences obtained in previous studies, are indicated in Figs 1–2.

For ultrastructural examinations of *Q. pitereka* and *Q. eucalypti*, herbarium specimens of naturally infected leaves and stems were used (Table 1). These specimens had been deposited in the National Collection of Fungal Specimens, Pretoria, South Africa (PREM). The holotype of *Q. eucalypti* (PREM 51089) consists of a dried culture on 2 % MEA. However, some important morphological and ultrastructural characters are only expressed on host tissue. The *Q. eucalypti* specimen we used for ultrastructural work (PREM 58939), consists of symptomatic leaf tissue, collected from the same host in the same location as the holotype (Table 1). This material is designated here as epitype for *Q. eucalypti*. The culture associated with the epitype (CBS 119680 = CMW 11678), was also included in the phylogenetic analyses. Specimen or isolate numbers of other species in the *Microstromatales* used for ultrastructural work, are underlined in Table 1.

The ex-type culture of *Q. pusilla* (U. Braun & Crous) J.A. Simpson (CMW 8279) was found to be contaminated with a *Verticillium* species and could not be purified. Attempts to extract DNA from the holotype specimen (HAL) were not successful. This species was therefore not included in the study.

DNA extraction and PCR

For the phylogenetic analyses, isolates were grown for 7 d on 2 % malt extract agar. DNA extraction, PCR conditions, visualization and purification of PCR products, as well as DNA sequencing, were done as described by Aghayeva *et al.* (2004). The internal transcribed spacer region (ITS1, the 5.8S rRNA gene and ITS2), was amplified using PCR with the primers ITS1 and ITS4 (White *et al.* 1990). The 5' end of the ribosomal large subunit (LSU) was amplified using primers NL1 and NL4 (O'Donnell 1993).

Phylogenetic analyses

Both alignments were assembled with MAFFT 3.85 (Katoh *et al.* 2002) using the accurate and iterative refinement method (FFT-NS-i settings). After trimming of both ends, the LSU alignment consisted of 572 bp and the ITS alignment of 726 bp. Phylogenetic analyses were carried out using PAUP v. 4.0b10 (Swofford 2001).

Table 1. Isolates and herbarium specimens used in this study.

Species	CBS numbers	Isolate number	Herbarium number	Host	Origin	Collector	GenBank	
							ITS	LSU
Microstroma album			R.B. 2072	*Quercus robur*	Germany	R. Bauer	DQ317624	AF352052
M. juglandis		F3381		*Juglans regia*	Germany	M. Göker	DQ317632	–
			R.B. 2054	*J. regia*	Germany	R. Bauer	DQ317633	–
			R.B. 2042	*J. regia*	Germany	R. Bauer	DQ317634	DQ317617
Quambalaria cyanescens = *Fugomyces cyanescens*	CBS 357.73[1T]	CMW 5583		skin of man	Netherlands	T.F. Visser	DQ317622	DQ317615
	CBS 876.73	CMW 5584		*Eucalyptus pauciflora*	New South Wales, Australia	V.F. Brown	DQ317623	DQ317616
Q. eucalypti	CBS 118844[T]	CMW 1101	PREM 51089[T]	*E. grandis*	Kwambonambi, South Africa	M.J. Wingfield	DQ317625	DQ317618
	CBS 119680	CMW 11678	PREM 58939[E]	*E. grandis* clone NH58	Kwambonambi, South Africa	L. Lombard	DQ317626	DQ317619
Q. pitereka		CMW 6707		*Corymbia maculata*	New South Wales, Australia	M.J. Wingfield	DQ317627	DQ317620
	CBS 118828	CMW 5318		*C. citriodora* subsp. *variegata*	Queensland, Australia	M. Ivory	DQ317628	DQ317621
			PREM 58940	*C. citriodora* subsp. *variegata*	Queensland, Australia	G.S. Pegg	–	–
Rhodutorula bacarum	CBS 6526[T]	IGC4391		*Ribes nigrum*	United Kingdom	R.W.M. Buhagiar	DQ317629	AF352055
R. hinnulea	CBS 8079[T]	IGC4849		*Banksia collina*	Australia	R.G. Shivas	AB038130	AF190003
R. phylloplana	CBS 8073[T]	IGC4246		*B. collina*	Australia	R.G. Shivas	DQ317630	AF190004
Sympodiomycopsis paphiopedili	CBS 7429[T]	IGC5543		nectar of *Paphiopedilum primurinum*	Japan	K. Tokuoka	DQ317631	AF190005
Tilletiopsis pallescens		F3370		fern leaf	Germany	J.P. Sampaio	DQ317635	–
	CBS 606.83[T]	ATCC24345		basidiome of *Sirobasidium* sp.	Japan	R.J. Bandoni	DQ317636	–
Volvocisporium triumfetticola			R.B. 2070[T]	*Triumfetta rhomboidea*	India	M.S. Patil	DQ317637	AF352053

[1]Underlined culture collection or herbarium numbers indicate isolates or specimens used in TEM studies.

[T]Holotype specimens or ex-type isolates.

[E]Epitype; CBS = Centraalburaeu voor Schimmelcultures, Utrecht, The Netherlands; CMW = Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; R.B. = Herbarium R. Bauer, Tübingen, Germany; F = Culture Collection, Tübingen, Germany; PREM = National Collection of Fungal Specimens, Pretoria, South Africa; IGC = Portugese Yeast Culture Collection, Portugal; ATCC = American Type Culture Collection, Manassas, Virginia, U.S.A.

Table 2. Higher classification and definitions of families in the *Microstromatales*. Extracted from Bauer *et al*. (1997), Begerow *et al*. (2001), and the results of this study.

<table>
<tr><td colspan="4">Ustilaginomycetes</td></tr>
<tr><td colspan="4">phytoparasitic members of the Basidiomycota lacking dolipores with multilayered pore caps
interactions with deposits of specific fungal vesicles</td></tr>
<tr><td colspan="4">Exobasidiomycetidae</td></tr>
<tr><td colspan="4">local interaction zones
septa having pores with membranous caps or septa poreless at maturity</td></tr>
<tr><td colspan="4">Microstromatales</td></tr>
<tr><td colspan="4">no interaction apparatus
no teliospores</td></tr>
<tr><td>Microstromataceae</td><td>Volvocisporiaceae</td><td>Quambalariaceae</td><td>incertae sedis</td></tr>
<tr><td>aseptate basidiospores</td><td>septate basidiospores</td><td>sexual state unclear</td><td>sexual state not observed</td></tr>
<tr><td colspan="2">simple pores with more or less rounded pore lips</td><td>dolipores with swollen pore lips</td><td rowspan="2">no pores, septa occasionally with median swellings</td></tr>
<tr><td colspan="3">septal pores enclosed on both sides by membrane caps</td></tr>
<tr><td colspan="4">Teleomorphic genera & species</td></tr>
<tr><td>Microstroma</td><td>Volvocisporium</td><td colspan="2"></td></tr>
<tr><td>basidia protrude through stomata & sporulate in gasteroid mode of spore release on leaf surface</td><td>septate basidiospores with peripheral layer of cells</td><td colspan="2">teleomorph unclear or not observed</td></tr>
<tr><td>> 37 species</td><td>V. triumfetticola</td><td colspan="2"></td></tr>
<tr><td colspan="4">Anamorphic genera & species</td></tr>
<tr><td>Rhodotorula</td><td rowspan="4"></td><td>Quambalaria</td><td>Sympodiomycopsis</td></tr>
<tr><td>R. bacarum
R. phylloplana
R. hinnulea</td><td>Q. pitereka
Q. eucalypti
Q. cyanescens
Q. pusilla?</td><td>S. paphiopedili</td></tr>
</table>

Modeltest 3.0 (Posada & Crandall 1998) was applied to determine a model of DNA substitution that fits the data set. GTR+I+G was selected from the Akaike information criterion for the LSU alignment (base frequencies: π_A = 0.2563, π_C = 0.1950, π_G = 0.2911, π_T = 0.2576; substitution rates: A/C = 0.7670, A/G = 2.6760, A/T = 0.7823, C/G = 0.3153, C/T = 5.9744, G/T = 1.0000; gamma shape parameter = 0.7950; percentage of invariant sites = 0.3790). TVM+I+G was selected from the Akaike information criterion for the ITS alignment (base frequencies: π_A = 0.2535, π_C = 0.2188, π_G = 0.2157, π_T = 0.3120; substitution rates: A/C = 0.14911, A/G C/T = 5.2884, A/T = 2.1848, C/G = 0.8252, G/T = 1.0000; gamma shape parameter = 1.6440; percentage of invariant sites = 0.3892). Neighbour-joining analysis was done determining genetic distances according to the specified substitution model.

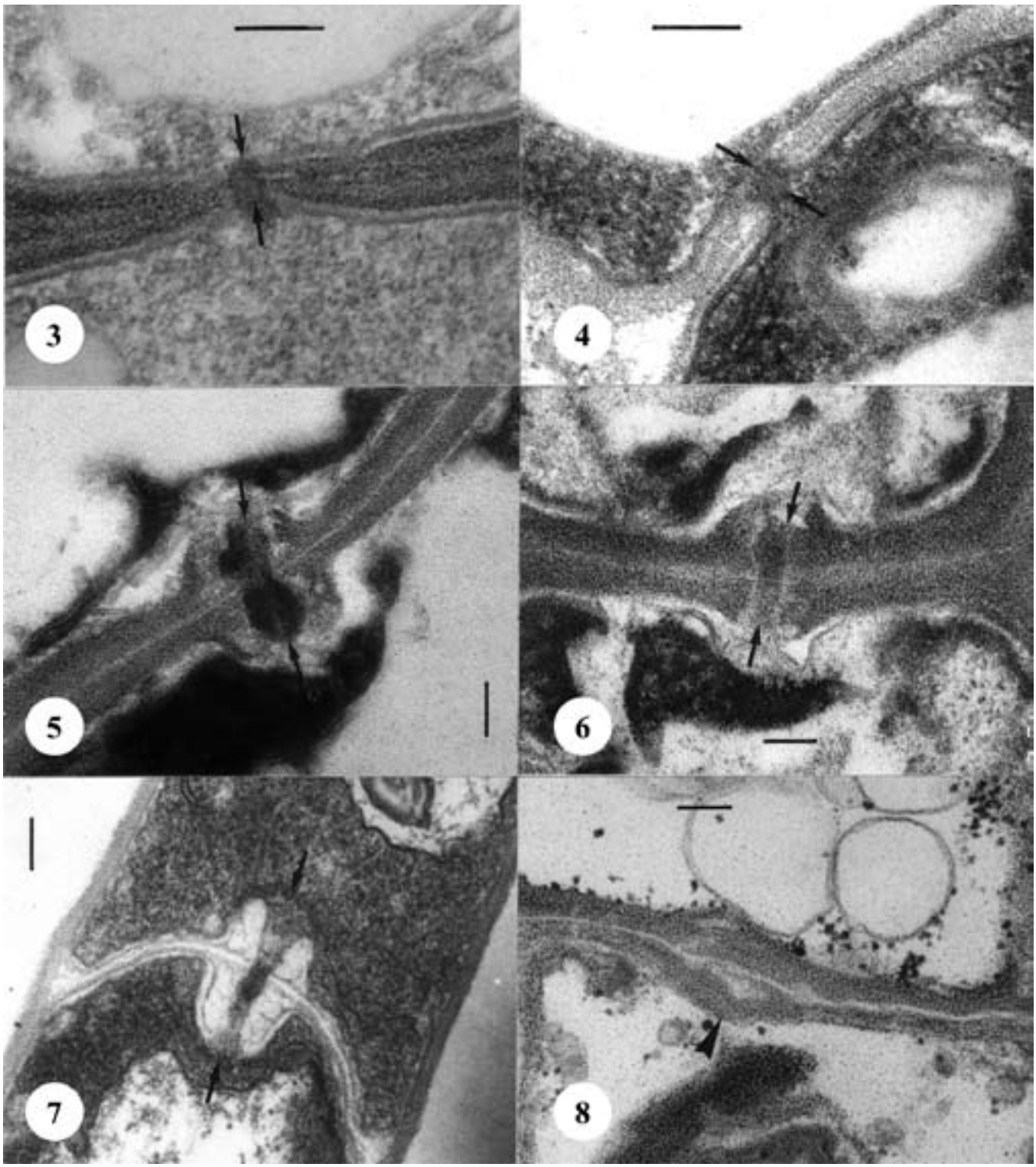
Parsimony analysis was conducted in two steps where the first with 10.000 random additions without branch swapping resulted in two islands for the LSU alignment and six for the ITS alignment. Subsequent TBR swapping over the best trees of these islands resulted in four most parsimonious trees for the LSU alignment with 1025 steps (CI = 0.404; RI = 0.665; RC = 0.269), and six trees for the ITS alignment with 507 steps (CI = 0.789; RI = 0.857; RC = 0.676), using 1000 replicates for bootstrap analyses.

For Bayesian analysis, four incrementally heated simultaneous MCMC Markov chains were run over 1 000 000 generations using the general time-reversible model (six rate classes) including a proportion of invariant sites and gamma-distributed substitution rates of the remaining sites (GTR+I+G) (for description of models see Swofford *et al.* 1996). Trees were sampled every 100th generation, resulting in an overall sampling of 10 000 trees. From these, the first 3000 trees were discarded (as burn-in). MrBayes 3.0b3 (Huelsenbeck & Ronquist 2001) was used to compute a 50 % majority rule consensus of the remaining trees to obtain estimates for the posterior probabilities.

Transmission Electron Microscopy

Species representing the major groups in the *Microstromatales*, were selected for ultrastructural

studies (Table 1). For transmission electron microscopy (TEM), samples were fixed overnight with 2 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 20 °C. Following six transfers in 0.1M sodium cacodylate buffer, samples were postfixed in 1 % osmium tetroxide in the same buffer for 1 h in the dark, washed in bidistilled water, and stained with 1 % aquaeous uranyl acetate for 1 h in the dark. After five consecutive washes in bidistilled water, samples were dehydrated in acetone, using 10 min transfers at 10, 25, 50, 70, 95, and three times in 100 % acetone. Samples were embedded afterwards in Spurr´s plastic and sectioned with a diamond knife. Ultra-thin serial sections were mounted on formvar-coated, single-slot copper grids, stained with lead citrate at room temperature for 5 min, and finally washed with bidistilled water. The samples were studied using a Zeiss EM 109 transmission electron microscope operating at 80 kV.



Figs 3–8. Septation in the *Microstromatales*. 3. Simple pore with two membrane caps (arrows) of *Microstroma juglandis*. 4. Simple pore with two membrane caps (arrows) of *Volvocisporium triumfetticola*. 5. Dolipore of *Quambalaria eucalypti* with two membrane caps (arrows) from herbarium material. 6. Dolipore with two membrane caps (arrows) of *Quambalaria pitereka* from herbarium material. 7. Dolipore with two membrane caps (arrows) of *Fugomyces cyanescens* (CBS 357.73). 8. Pore equivalent in *Sympodiomycopsis paphiopedili* (CBS 7429). Septum with median swelling (arrowhead), but without cytoplasmic continuim between adjacent cells. Scale bars = 0.1 µm.

RESULTS

Phylogenetic analyses

The different phylogenetic analyses of the LSU dataset resulted in similar topologies resolving all known orders of *Exobasidiomycetidae* Jülich, emend. R. Bauer & Oberw. (Fig. 1). The *Tilletiales* H. Kreisel *ex* R. Bauer & Oberw. were weakly supported as sistergroup to the other orders. Although the backbone was not resolved in all parts, the specimens of *Quambalaria* and *Fugomyces* considered in this study clustered within the *Microstromatales* as a highly supported monophylum in both datasets. *Tilletiopsis pallescens* Gokhale clustered together with members of the *Microstromatales* and it was, therefore, used as outgroup for the ITS dataset of the *Microstromatales*.

The ITS regions were used to elucidate the inner phylogeny of the *Microstromatales* (Fig. 2). *Volvocisporium triumfetticola* (Patil) Begerow, R. Bauer & Oberw., the only known member of the *Volvocisporiaceae* Begerow, R. Bauer & Oberw., was sister to the other members of the *Microstromatales*. *Microstroma* Niessl appeared paraphyletic in the LSU and ITS analyses, and the relationship between the two *Microstroma* clusters was weakly supported. This could have resulted from the unclear positions of *Sympodiomycopsis paphiopedili* Sugiy., Tokuoka & Komag. and *V. triumfetticola*. All studied specimens of *Quambalaria* and *Fugomyces* appeared to form a monophylum. The monophyly of *Quambalaria eucalypti* and *Q. pitereka* was supported only in the ITS neighbour-joining analysis and was rejected by maximum parsimony and Bayesian inference and by the LSU data. *Quambalaria eucalypti*, *Q. pitereka* and the *Fugomyces* isolates formed three separate, well-supported clusters. Sequences of the two *Q. eucalypti* isolates (ex-type and ex-epitype cultures) were identical, and also those of the two *Q. pitereka* isolates. The ITS sequences of two *F. cyanescens* isolates differed from each other by 4 bp.

Transmission Electron Microscopy

Septal pore apparatuses in the studied species of *Microstroma* and *Volvocisporium* Begerow, R. Bauer & Oberw. were simple with more or less rounded pore lips, which were enclosed on both sides by membrane caps (Figs 3–4). In *Quambalaria pitereka*, *Q. eucalypti* and *Fugomyces cyanescens,* the pores were also enclosed by membrane caps, but the septal pore apparatus consisted of dolipores with swollen pore lips (Figs 5–7). In the anamorphic yeast, *Sympodiomycopsis paphiopedili* we found no septal pores. Occasionally, the septa possess median swellings resembling septal pores, but there was no cytoplasmic continuum between adjacent cells (Fig. 8).

TAXONOMY

Phylogenetic analyses of the LSU data obtained in this study showed that the genus *Quambalaria* resides in the *Microstromatales*. However, the ultrastructure of the septal pores of *Quambalaria* spp. differ substantially from those of species in the *Microstromataceae* Jülich and *Volvocisporiaceae*. We, therefore, describe a new family, *Quambalariaceae,* to accommodate the species with dolipores. Thus, the *Microstromatales* now include not only taxa having septa with simple pores, but also taxa with dolipores or septa without pores. Ultrastructural characteristics, together with LSU and ITS data, show that *Fugomyces cyanescens* is clearly monophyletic with the two sampled *Quambalaria* spp. *Fugomyces* is therefore synonymised here with *Quambalaria* and the necessary new combination is established.

Quambalariaceae Z.W. de Beer, Begerow & R. Bauer, **fam. nov.** MycoBank MB500889.

Socii Microstromatalium doliporos cum labiis pororum tumidis facientes.

Members of the *Microstromatales* having dolipores with swollen pore lips.

Quambalaria J.A. Simpson, Australas. Mycol. 19: 60–61. 2000.

= *Fugomyces* Sigler, *Manual of clinical microbiology, Vol. 2*: 1753. 2003.

(1) Type species: ***Quambalaria pitereka*** (J. Walker & Bertus) J.A. Simpson, Australas. Mycol. 19: 60. 2000.

Basionym: *Ramularia pitereka* J. Walker & Bertus, Proc. Linn. Soc. New South Wales 96(2): 108. 1971.

≡ *Sporothrix pitereka* (J. Walker & Bertus) U. Braun & Crous, In Braun, *A monograph of Ramularia, Cercosporella and allied genera (phytopathogenic hyphomycetes)*: 416. 1998.

Specimens examined: **Australia**, Queensland, *Corymbia citriodora* subsp. *variegata* leaves, 09 June 1999, M. Ivory, CBS 118828 = CMW 5318; *C. citriodora* subsp. *variegata* leaves, 2002, G.S. Pegg, PREM 58940; New South Wales, Grafton, *C. maculata* leaves, Dec. 2000, M.J. Wingfield, CMW 6707.

(2) ***Quambalaria cyanescens*** (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer, **comb. nov.** MycoBank MB500890.

Basionym: *Sporothrix cyanescens* de Hoog & G.A. de Vries, Antonie van Leeuwenhoek 39: 515. 1973.

≡ *Cerinosterus cyanescens* (de Hoog & G.A. de Vries) R.T. Moore, Stud. Mycol. 30: 216. 1987.

≡ *Fugomyces cyanescens* (de Hoog & G.A. de Vries) Sigler, In Murray, *Manual of clinical microbiology, Vol. 2*: 1753. 2003.

Specimens examined: **Australia**, New South Wales, Armidale, *Eucalyptus pauciflora*, 1973, V.F. Brown, CBS 876.73 = CMW 5584. **Netherlands**, Groningen, skin of man, 18 Oct 1959, T.F. Visser, **holotype** culture ex-type CBS 357.73 = CMW 5583.

(3) ***Quambalaria eucalypti*** (M.J. Wingf., Crous & W.J. Swart) J.A. Simpson, Australas. Mycol. 19: 61. 2000.

Basionym: *Sporothrix eucalypti* M.J. Wingf., Crous & W.J. Swart, Mycopathologia 123: 160. 1993.

Specimens examined: **South Africa**, KwaZulu-Natal, Kwambonambi, *Eucalyptus grandis* leaves, 19 May 1987, M.J. Wingfield, holotype PREM 51089; KwaZulu-Natal, Kwambonambi, *E. grandis* leaves, 2001, L. Lombard, PREM 58939, **epitype designated here**, culture ex-epitype CBS 119680 = CMW 11678.

Species of uncertain status

(a) ***Sporotrichum destructor*** H.A. Pittman, In Cass

Smith, J. Agric. W. Austral. 11 (2): 34. 1970. (*nom. nud.*)

Note: This fungus, resembling other *Quambalaria* spp., was isolated by H.A.J. Pittman in 1935 from diseased *Corymbia ficifolia* in Western Australia. Cultures were sent to Kew where it was identified as a new species named *Sporotrichum destructor* H.A. Pittman (Cass Smith 1970). However, a Latin diagnosis was never published and material of this species was not available for this study.

(b) ***Quambalaria pusilla*** (U. Braun & Crous) J.A. Simpson, Australas. Mycol. 19: 61. 2000.
Basionym: *Sporothrix pusilla* U. Braun & Crous, In Braun, *A monograph of Ramularia, Cercosporella and allied genera (phytopathogenic hyphomycetes)*: 418. 1998.

Note: The ex-type culture of this species (CMW 8279) was contaminated and DNA could not be extracted from the holotype specimen (HAL). The phylogenetic status of this species shall only become clear if fresh material can be obtained.

DISCUSSION

In this study we have produced phylogenetic evidence showing that *Q. pitereka* infecting *Corymbia* spp. in Australia and *Q. eucalypti,* the fungal pathogen on *Eucalyptus grandis* in South Africa, indeed represent two distinct species. Both LSU and ITS sequence data sets revealed that the two *Quambalaria* spp. and *F. cyanescens* (now *Q. cyanescens*) form a monophyletic lineage in the basidiomycete order *Microstromatales*. The monophyly of *Quambalaria* is supported by ultrastructural features. *Quambalaria* differs from other genera in the *Microstromatales* because it has dolipores with swollen pore lips in the septa, and not simple pores with more or less rounded pore lips, which are characteristic of the *Microstromataceae* and *Volvocisporiaceae*. We have thus described a new family, *Quambalariaceae*, in the *Microstromatales* to accommodate *Quambalaria* spp.

Taxa in the *Microstromatales* are classified in the subclass *Exobasidiomycetidae* of the *Ustilaginomycetes* (Table 2). With few exceptions, the *Ustilaginomycetes* are restricted to angiosperms, and most are parasites of monocots (Bauer *et al.* 1997). Of the at least seven orders in the *Ustilaginomycetes* (Fig. 1), members of only two, the *Exobasidiales* and the *Microstromatales,* do not form teliospores and occur on woody bushes or trees (Begerow *et al.* 2001). The *Exobasidiales* differ from the *Microstromatales* by the formation of complex interaction apparatuses including interaction rings (Bauer *et al.* 1997). The *Exobasidiales* represent a large order including at least nine genera in four families (Begerow *et al.* 2002a). The largest of these is *Exobasidium* Woronin with over 100 species occurring world-wide on flowering plants such as the *Ericaceae*. Another well-known genus of the *Exobasiales* is *Graphiola* Poit., which includes more than 12 species, occurring exclusively on *Arecaceae* (palms), also with a global distribution (http://nt.arg-grin.gov/fungaldatabases/fungushost/FungusHost.cfm and http://www.indexfungorum.org). A third genus of this order is *Muribasidiospora* O. Kamat & Rajendren (Begerow *et al*. 2001). *Muribasidiospora indica* O. Kamat & Rajendren was recently reported from South Africa for the first time, causing a prominent leaf spot on native *Rhus lancea* (Crous *et al.* 2003).

The *Microstromatales* are characterised by the lack of teliospores and interaction apparatus (Bauer *et al.* 1997). Only two teleomorphic genera are known in the *Microstromatales* (Table 2). One of these is *Volvocisporium* (Table 2 and Fig. 2) which is monotypic. This fungus has such a unique morphology that it was placed in a family of its own (Begerow *et al.* 2001). The dominant genus in the *Microstromatales* is *Microstroma* including about 35 species occurring world-wide, primarily on *Leguminosae*, *Fagaceae* and *Juglandaceae* (http://nt.arg-grin.gov/fungaldatabases/fungushost/FungusHost.cfm and http://www.indexfungorum.org). Only two *Microstroma* spp. have been reported from South Africa: *M. album* (Desm.) Sacc. from *Quercus*, both exotic, and *M. albiziae* Syd. & P. Syd. from three native *Albizia* spp. (Doidge 1950). Similarly, two exotic *Microstroma* spp. have been reported from Australia: again *M. album* from *Quercus* and, additionally, *M. juglandis* (Berenger) Sacc. from *Juglans* (Sampson & Walker 1982, Shivas 1989). *Microstroma album* (Fig. 2) is known only from *Quercus* and has been reported widely from the Northern hemisphere. *Microstroma juglandis* (Fig. 2) has been found on different genera belonging to the *Juglandaceae*, with a global distribution. *Microstroma albiziae* has only been reported from *Albizia* spp. in South Africa (Doidge 1950) and India (Mathur 1979). Material of these species was not available for study.

Begerow *et al*. (2001) showed with LSU sequence analyses that two anamorphic yeasts, *Rhodotorula bacarum* (Buhagiar) Rodr. Mir. & Weijman and *R. phylloplana* (R.G. Shivas & Rodr. Mir.) Rodr. Mir. & Weijman are phylogenetically closely related to *Microstroma album* and *M. juglandis*, respectively. Our ITS data (Fig. 2), support their results and show that *R. bacarum* might be the same species as *M. album*. We included a third species, *R. hinnulea* (R.G. Shivas & Rodr. Mir.) Rodr. Mir. & Weijman, and it differs from *R. phylloplana* in only 2 bp. (Fig. 2). Both these species were isolated from the leaves of *Banksia collina* (*Proteaceae*) in Australia, and were described then as new *Cryptococcus* species (Shivas & Rodrigues de Miranda 1983). However, the biochemical and morphological differences (Shivas & Rodrigues de Miranda 1983) between the two species are small and they might represent individuals of the same species. The three *Rhodotorula* spp. should not be accommodated in the genus *Rhodotorula*, because the type species for *Rhodotorula*, *R. glutinis* (Fresen.) F.C. Harrison, is phylogenetically (based on sequence data) placed in the *Sporidiales* R.T. Moore in the *Urediniomycetes* (Swann & Taylor 1995). We have chosen not to erect a new anamorph genus for these fungi at the present time, since they might be linked to

teleomorphs (probably *Microstroma* spp.) and could be more appropriately treated at a time when additional material is available for study.

The monophyly (Fig. 2) and ultrastructural similarities (Figs 5–7) between the three *Quambalaria* spp. recognised in this study, is supported by the ecology of these species. The fact that all three species, as well as *Q. pusilla* (not included), occur on tree species native to Australia, suggests that Australia is the centre of origin of these species. Although *Q. cyanescens* has been isolated from human tissues on several occasions, the fungus has not been associated with specific disease symptoms of humans (Middelhoven *et al.* 2000, Sigler & Verweij 2003). Inoculation trials on mice failed to demonstrate virulence of the fungus on mammals (Sigler *et al.* 1990). The fungus is, therefore, rather regarded as an opportunist, and potentially can be implicated in disease in immunocompromised patients (Tambini *et al.* 1996).

The recognition of *Quambalaria* spp. as basidiomycetes has not been widely considered because the teleomorph has never been observed. When the teleomorph morphology of the closely related fungus *M. juglandis* is considered (Begerow *et al.* 2001), it might be found that the teleomorph of *Quambalaria* is masquerading as an anamorph. This is entirely possible as the anamorph and teleomorph states would be difficult to distinguish from each other.

One of the species for which the position in the *Microstromatales* remains uncertain (Table 2 and Fig. 2), is the anamorphic yeast *Sympodiomycopsis paphiopedili*. This fungus was described from the nectar of an orchid in Japan (Sugiyama *et al.* 1991). Although the conidiogenous cells in culture (Sugiyama *et al.* 1991) resemble those of *Quambalaria*, its phylogenetic position (Fig. 2) sets it apart from all the other members of the *Microstromatales*. Because this yeast forms pseudomycelia, occasionally with retraction septa, it is not surprising that we did not observe pores (Bauer *et al.* 2001), but septa with median swellings (Fig. 8). Suh *et al.* (1993) reported simple pores in *S. paphiopedili*, but the respective micrograph is insufficient. The pore structure of the hyphal phase of *S. paphiopedili* is thus unknown.

Recognition of three families in the *Microstromatales* and emerging lineages that correspond with host families, follows a trend that has been observed in other orders in the *Ustilaginomycetes* (Begerow *et al.* 2004). The four families in the *Exobasidiales*, for example, can be distinguished based on basidial morphology and host range, but these characteristics also match phylogenetic lineages based on LSU rDNA sequences (Begerow *et al.* 2002a). Cospeciation of groups of species in the *Entylomatales* R. Bauer & Oberw. with their hosts, has also been shown (Begerow *et al.* 2002b). To test cospeciation processes in the *Microstromatales*, additional fungal isolates from a wider variety of hosts would need to be included in phylogenetic studies together with their host species. However, there is good evidence that *Q. pitereka* infects only *Corymbia* and *Q. eucalypti* is restricted to hosts in the genus *Eucalyptus*. These two tree genera are phylogenetically distinct (Hill & Johnson 1995, Wilson *et al.* 2001) and it appears that the pathogens have specifically evolved to infect them.

Studies on members of the *Microstromatales* have been limited, most likely because they have not been considered an economically important group of fungi. This perception is changing rapidly with the reported spread of disease caused by members of the *Quambalariaceae* in commercial *Eucalyptus* plantations in South Africa (Wingfield *et al.* 1993), Brazil and Uruguay (Alfenas *et al.* 2001, Zauza *et al.* 2003), and in *Corymbia* plantations in Australia (Simpson 2000, Pegg *et al.* 2005). That we have only touched the "tip of the iceberg" of the *Microstromatales* (Begerow *et al.* 2001) should be regarded as a challenge, since so many questions surrounding the biology and distribution of this intriguing group of fungi remain unanswered.

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REFERENCES

Aghayeva DN, Wingfield MJ, De Beer ZW, Kirisits T (2004). Two new *Ophiostoma* species with *Sporothrix* anamorphs from Austria and Azerbaijan. *Mycologia* **96**: 866–878.

Alfenas AC, Zauza EAV, Rosa OPP, Assis TF (2001). *Sporothrix eucalypti*, um novo patógeno do eucalipto no Brazil. *Fitopatologia Brasileira* **26**: 221.

Bauer R, Begerow D, Oberwinkler F, Piepenbring M, Berbee ML (2001). *Ustilaginomycetes*. In: *Mycota VII, Part B. Systematics and evolution* (McLaughlin DJ, McLaughlin EG, Lemke PA, eds). Springer Verlag, Heidelberg: 57–83.

Bauer R, Oberwinkler F, Vánky K (1997). Ultrastructural markers and systematics in smut fungi and allied taxa. *Canadian Journal of Botany* **75**: 1273–1314.

Begerow D, Bauer R, Oberwinkler F (1997). Phylogenetic studies on nuclear large subunit ribosomal DNA sequences of smut fungi and related taxa. *Canadian Journal of Botany* **75**: 2045–2056.

Begerow D, Bauer R, Oberwinkler F (2001). *Muribasidiospora*: *Microstromatales* or *Exobasidiales*? *Mycological Research* **105**: 798–810.

Begerow D, Bauer R, Oberwinkler F (2002a). The *Exobasidiales*: an evolutionary hypothesis. *Mycological Progress* **1**: 187–199.

Begerow D, Göker M, Lutz M, Stoll M (2004). On the evolution of smuts and their hosts. In: *Frontiers in Basidiomycete Mycology* (Agerer, Piepenbring, Blanz, eds). IHW-Verlag, Eching: 81–98.

Begerow D, Lutz M, Oberwinkler F (2002b). Implications of molecular characters for the phylogeny of the genus *Entyloma*. *Mycological Research* **106**: 1392–1399.

Berbee ML, Taylor JW (1992). 18S Ribosomal RNA gene sequence characters place the human pathogen *Sporothrix schenckii* in the genus *Ophiostoma*. *Experimental Mycology* **16**: 87–91.

Braun U (1998). *A monograph of Ramularia, Cercosporella and allied genera (phytopathogenic hyphomycetes)*. IHW-Verlag, Eching.

Cass Smith WP (1970). Stem canker disease of red flowering gums. *Journal of the Department of Agriculture, Western Australia* **11**: 33–39.

Crous PW, Groenewald JZ, Carroll G (2003). *Muribasidiospora indica* causing a prominent leaf spot disease on *Rhus lancea* in South Africa. *Australasian Plant Pathology* **32**: 313–316.

De Hoog GS (1993). *Sporothrix*-like anamorphs of *Ophiostoma*

species and other fungi. In: *Ceratocystis and Ophiostoma*: *Taxonomy, Ecology and Pathogenicity* (Wingfield MJ, Seifert KA, Webber J, eds). American Phytopathological Society, St. Paul, Minnesota: 53–60.

De Hoog GS, De Vries GA (1973). Two new species of *Sporothrix* and their relation to *Blastobotrys nivea*. *Antonie van Leeuwenhoek* **39**: 515–520.

Doidge EM (1950). The South African fungi and lichens to the end of 1945. *Bothalia* **5**: 1–1094.

Harrington TC (1981). Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. *Mycologia* **73**: 1123–1129.

Hill KD, Johnson LAS (1995). Systematic studies in the eucalypts – 7. A revision of the bloodwoods, genus *Corymbia* (*Myrtaceae*). *Telopea* **6**: 173–505.

Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Katoh K, Misawa K, Kuma K-I, Miyata T (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.

Mathur RS (1979). *The Coelomycetes of India*. Bishen Singh Mahendra Pal Singh, Delhi, India.

Middelhoven WJ, Guého E, De Hoog GS (2000). Phylogenetic position and physiology of *Cerinosterus cyanescens*. *Antonie van Leeuwenhoek* **77**: 313–320.

Moore RT (1987). Micromorphology of yeasts and yeast-like fungi and its taxonomic implications. *Studies in Mycology* **30**: 203–226.

Pegg GS, Drenth A, Wingfield MJ (2005). *Quambalaria pitereka* on spotted gum plantations in Queensland and northern New South Wales, Australia. Abstracts of XII IUFRO World Congress, 8–13 August 2005, Brisbane, Australia. *International Forestry Review* **7**: 337.

Posada D, Crandall KA (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Sampson PJ, Walker J (1982). An Annotated List of Plant Diseases in Tasmania. Department of Agriculture Tasmania.

Shivas RG (1989). Fungal and bacterial diseases of plants in Western Australia. *Journal of the Royal Society of Western Australia* **72**: 1–62.

Shivas RG, Rodrigues de Miranda L (1983). *Cryptococcus phylloplanus* and *Cryptococcus hinnuleus*, two new yeast species. *Antonie van Leeuwenhoek* **49**: 153–158.

Sigler L, Harris JL, Dixon DM (1990). Microbiology and potential virulence of *Sporothrix cyanescens*, a fungus rarely isolated from blood and skin. *Journal of Clinical Microbiology* **29**: 1009–1115.

Sigler L, Verweij PE (2003). *Aspergillus*, *Fusarium*, and other opportunistic moniliaceous fungi. In: *Manual of Clinical Microbiology* (Murray PR, ed). ASM Press, Washington, D.C.: 1726–1760.

Simpson JA (2000). *Quambalaria*, a new genus of eucalypt pathogens. *Australasian Mycologist* **19**: 57–62.

Smith MT, Batenburg–Van der Vegte WH (1985). Ultrastructure of septa in *Blastobotrys* and *Sporothrix*. *Antonie van Leeuwenhoek* **51**: 121–128.

Sugiyama J, Tokuoka K, Suh S–O, Hirata A, Komagata K (1991). *Sympodiomycopsis*: a new yeast-like anamorph genus with basidiomycetous nature from orchid nectar. *Antonie van Leeuwenhoek* **59**: 95–108.

Suh S–O, Hirata A, Sugiyama J, Komagata K (1993). Septal ultrastructure of basidiomycetous yeasts and their taxonomic implications with observations on the ultrastructure of *Erythrobasidium hasegawianum* and *Sympodiomycopsis paphiopedili*. *Mycologia* **85**: 30–37.

Suzuki M, Nakase T (1986). Heterogeneity of ubiquinone systems in the genus *Sporothrix*. *Journal of General and Applied Microbiology* **32**: 165–168.

Swann EC, Taylor JW (1995). Phylogenetic diversity of yeast-producing basidiomycetes. *Mycological Research* **99**: 1205–1210.

Swofford DL (2001). PAUP*. Phylogenetic analysis using parsimony (*and other methods) version 4.0b1. Sinauer Associates, Sunderland, Massachusetts.

Swofford DL, Olsen GJ, Waddell PJ, Hillis DM (1996). Phylogenetic Inference. In: *Molecular Systematics* (Hillis DM, Moritz C, Mable BK, eds). Sinauer Associates Inc., Sunderland, Massachusetts, U.S.A.: 407–514.

Tambini R, Farina C, Fiocchi R, Dupont B, Guého E, Delvecchio G, Mamprin F, Gavazzeni G (1996). Possible pathogenic role for *Sporothrix cyanescens* isolated from a lung lesion in a heart transplant patient. *Medical Mycology* **34**: 195–198.

Walker J, Bertus AL (1971). Shoot blight of *Eucalyptus* spp. caused by an undescribed species of *Ramularia*. *Proceedings of the Linnean Society of New South Wales* **96**: 108–115.

Weijman ACM, De Hoog GS (1985). Carbohydrate patterns and taxonomy of *Sporothrix* and *Blastobotrys*. *Antonie van Leeuwenhoek* **51**: 111–120.

White TJ, Bruns TD, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols*: *A guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, Inc., San Diego, California: 315–322.

Wilson PG, O'Brien MM, Gadek PA, Quinn CJ (2001). *Myrtaceae* revisited: a reassessment of infrafamilial groups. *American Journal of Botany* **88**: 2013–2025.

Wingfield MJ, Crous PW, Swart WJ (1993). *Sporothrix eucalypti* (sp. nov.), a shoot and leaf pathogen of *Eucalyptus* in South Africa. *Mycopathologia* **123**: 159–164.

Zauza EAV, Alfenas AC, Langrell SRH, Tommerup IC (2003). Detection and identification of *Quambalaria* species in *Eucalyptus* nurseries and plantations. 8th International Congress of Plant Pathology, Christchurch, New Zealand.