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, Ophiostomatiales), 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 conidigenous 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

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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.

![Phylogram](image-url)

**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.
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.

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).
| Species                  | CBS numbers | Isolate number | Herbarium number | Host              | Origin          | Collector | GenBank     |
|-------------------------|-------------|----------------|------------------|-------------------|-----------------|-----------|-------------|
| Microstoma album        |             | R.B. 2072      | Quercus robur    | Germany           | R. Bauer        | DQ317624  | AF352052    |
| M. juglandis            | F3381       | R.B. 2054      | Juglanthes regia | Germany           | M. Göker        | DQ317632  | –           |
|                         |             | R.B. 2042      | J. regia         | Germany           | R. Bauer        | DQ317633  | –           |
| Quambalaria cyanescens  | CBS 357.73TT| CMW 5583       | skin of man      | Netherlands       | T.F. Visser     | DQ317622  | DQ317615    |
| Fugomyces cyanescens    | CBS 876.73  | CMW 5584       | Eucalyptus pauciflora | New South Wales, Australia | V.F. Brown | DQ317623  | DQ317616    |
| Q. eucalypti            | CBS 118844T | CMW 1101       | E. grandis       | Kwambonambi, South Africa | M.J. Wingfield | DQ317625  | DQ317618    |
|                         |             | PREM 51089F    | E. grandis clone NH58 | Kwambonambi, South Africa | L. Lombard | DQ317626  | DQ317619    |
| Q. piteika              | CMW 6707    | C. citriodora subsp. variegata | Queensland, Australia | M. Ivory | DQ317628  | DQ317621    |
|                         | CMS 118828  | CMW 5318       | E. grandis       | kwambonambi, South Africa | M.J. Wingfield | DQ317627  | DQ317620    |
|                         | PREM 58940F | C. citriodora subsp. variegata | Queensland, Australia | G.S. Pegg | – | –           |
| Rhodotorula bacarum     | CBS 6526T   | IGC4391        | Ribes nigrum     | United Kingdom    | R.W.M. Buhagiar | DQ317629  | AF352055    |
| R. hinnulea             | CBS 8079T   | IGC4849        | Banksia collina  | Australia         | R.G. Shivas     | AB038130  | AF190003    |
| R. phylloplana          | CBS 8073T   | IGC4246        | B. collina       | Australia         | R.G. Shivas     | DQ317630  | AF190004    |
| Sympodiomyces paphiopedil | CBS 7429T  | IGC5543        | nectar of Paphiopedilum primulinum | Japan | K. Tokuoka | DQ317631  | AF190005    |
| Tilletiopsis pallescens | F3370       | fern leaf      | Germany           | J.P. Sampaio      | DQ317635  | –           |
| Volvocisporium triumfettlicola | CBS 606.83T | ATCC24345     | basidiome of Sirobasidium sp. | Japan | R.J. Bandoni | DQ317636  | –           |

1Underlined culture collection or herbarium numbers indicate isolates or specimens used in TEM studies.
2Holotype specimens or ex-type isolates.

*Note: CBS = Centraalbureau 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 = Portuguese Yeast Culture Collection, Portugal; ATCC = American Type Culture Collection, Manassas, Virginia, U.S.A.*
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: \( \pi_A = 0.2563, \pi_C = 0.1950, \pi_G = 0.2911, \pi_T = 0.2576 \); 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: \( \pi_A = 0.2535, \pi_C = 0.2188, \pi_G = 0.2157, \pi_T = 0.3120 \); substitution rates: A/C = 0.14911, A/G = 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
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 % aqueous 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 paphiopedilli (CBS 7429). Septum with median swelling (arrowhead), but without cytoplasmic continuum 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 *Exobasidionymycetidae* Jülich, emend. R. Bauer & Oberw. (Fig. 1). The *Tilletiades* 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 palescens* 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. *Microstoma* Niessl appeared paraphyletic in the LSU and ITS analyses, and the relationship between the two *Microstoma* clusters was weakly supported. This could have resulted from the unclear positions of *Sympodiumycopsis paphiopedilli* 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 *Microstoma* 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, *Sympodiumycopsis paphiopedilli* 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 Microstromatales Jülich and Volvocisporiaceae. We, therefore, describe a new family, *Quambalariaeae*, 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.

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

Socii Microstromatium dolipororum 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 hypomycescae): 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, Oroningen, skin of man, 18 Oct 1959, T.F. Visser, holotype culture ex-type CBS 357.73 = CMW 5593.

(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) *Sporothrix* destructor H.A. Pittman, In Cass
DISSCUSSION

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, Quambalaricaceae, 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 Exobasidiales is Graphiola Poit., which includes more than 12 species, occurring exclusively on Arecales (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, Fabaceae and Juglandaceae (http://nt.arg-grin.gov/fungaldatabases/fungushost/FungusHost.cfm and http://www.indexfungorum.org). Only two Microstremma 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 Microstremma spp. have been reported from Australia: again M. album from Quercus and, additionally, M. juglandis (Berenger) Sacc. from Juglans (Sampson & Walker 1982, Shivas 1989). Microstremma album (Fig. 2) is known only from Quercus and has been reported widely from the Northern hemisphere. Microstremma juglandis (Fig. 2) has been found on different genera belonging to the Juglandaceae, with a global distribution. Microstremma 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 Microstremma 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. hinnulae (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 colina (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 Sporidiiales 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 opportunistic, 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 telemorph 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 Symphyomyces paphiopedilli. 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. paphiopedilli, but the respective micrograph is insufficient. The pore structure of the hyphal phase of S. paphiopedilli 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). Cospication of groups of species in the Entylomatales R. Bauer & Oberw. with their hosts, has also been shown (Begerow et al. 2002b). To test cospication 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 Quambalariaeae 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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