

QUAMBALARIA, A NEW GENUS OF EUCALYPT PATHOGENS

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Abstract

The plant pathogenic species of *Ramularia* and *Sporothrix* previously described from species of eucalypts are transferred to the new genus *Quambalaria*. A relationship with the Ustomycota (possibly Exobasidiales or Ustilaginales) is postulated for these pathogens.

Introduction

Ramularia pitereka J. Walker & Bertus was described from a species of *Corymbia* K.D. Hill & L.A.S. Johnson in Australia causing a leaf spot and shoot blight of young plants growing in nurseries and glasshouses. In artificial inoculation tests, similar symptoms were produced on young plants of *Angophora costata* (Gaertn.) Britten. With the expansion of eucalypt plantations in New South Wales and Queensland in the 1990s, large areas were planted to spotted gums, *Corymbia* series *Maculatae*. A high incidence of shoot death in young spotted gum plantations caused by *R. pitereka* has caused a move away from these species for commercial plantations in subtropical eastern Australia.

Walker & Bertus (1971) noted that species of *Ramularia* occur mostly on herbaceous hosts and that *R. pitereka* was the only species known from Myrtaceae. Braun (1998) accepted 325 species in the genus. The teleomorphs of species of *Ramularia* Unger (*nom. cons.*) are, so far as is known, species of *Mycosphaerella* Johanson (Dothideales: Mycosphaerellaceae) (von Arx 1983, Braun 1998). Crous (1998) excluded *R. pitereka* from Mycosphaerellaceae when he revised the species of *Mycosphaerella* that occur on eucalypts. Braun (1998) concurred with that opinion and decided that *R. pitereka* and two similar species from eucalypts growing in South Africa and Thailand would be placed better in *Sporothrix* Hektoen & C.F. Perkins ex Nicot & Mariat. The characters used in arriving at this decision were 1) conidial scars and hila are neither darkened nor thickened though sometimes slightly refractive, 2) acropetal conidial chains are not formed, 3) secondary conidia are sometimes produced from primary conidia directly or on a short, minutely denticulate conidiophore.

Sporothrix as presently accepted is a diverse assemblage of anamorphs of species of Cerinomycetaceae Jülich, Clavicipitaceae (Lindau) O.E. Erikss., Dacrymycetaceae J. Schröt., Endomycetaceae J. Schröt., Ophiostomataceae Nannf., and Tilletiaceae J. Schröt. (de Hoog 1974, 1993, de Hoog *et al.* 1985). Weijman & de Hoog (1985) acknowledged the disparate taxonomic groups in *Sporothrix* and proposed a number of sections to accommodate them. Moore (1987) transferred some of the basidiomycetous (Dacrymycetales) species of *Sporothrix* to the new genus *Cerinosterus* R.T. Moore, type species *C. luteoalba* (de Hoog) R.T. Moore (= *S. luteoalba* de Hoog). The type species of *Sporothrix* is *S. schenckii* Hektoen & C.F. Perkins with attributes of an anamorph of *Ophiostoma* Syd. & P. Syd. (Summerbell *et al.* 1993, Takeda *et al.* 1991). The *Sporothrix* states of species of *Cordyceps* (Fr.) Link (Clavicipitaceae) are entomogenous (de Hoog 1974, 1993). *Blastobotrys* Klopotek in an emended sense may be an appropriate genus for yeast-like *Sporothrix* species (Endomycetales).

Traquair *et al.* (1988) described two new species of *Stephanoascus* M.T. Sm., Van der Walt & Johannsen with *Sporothrix* anamorphs. However, de Hoog (1993) considered that these were smut fungi. Species of *Tilletiopsis* Derx resemble species of *Sporothrix* (Boekhout 1991). In the past conidial forms of Tilletiales have often been confused with anamorphs of *Mycosphaerella* in the genera *Cercospora* Sacc. and *Ramularia* (Boekhout 1991, Braun 1995). Smut fungi produce two kinds of conidia: ballistoconidia and passively liberated blastoconidia. Ballistoconidia are bilaterally symmetrical, cylindrical, allantoid or falcate, colourless, usually aseptate, with a truncate base, and are formed on short denticles (Boekhout 1991). Blastoconidia are narrow, fusiform or filiform, straight or slightly curved, aseptate or sparsely septate, hyaline, with ends attenuated (Boekhout 1991). Ballisto- and blastoconidia may be produced together or separately. Conidiophores are usually filiform, narrow, evanescent, formed in fascicles, and erumpent through stomata or through the cuticle. Unlike the anamorphs of Mycosphaerellaceae, anamorphs of smuts do not have conspicuously thickened or darkened conidial scars and hila (Braun 1995), nor do they usually have conspicuous sympodially elongating conidiogenous cells.

The oldest valid name for the anamorph of a smut is *Fusidium ranunculi* Bonord., described in 1851, followed by *Ascomyces trientalis* Berk. 1860 (Ciferri 1959). *Paepalopsis* J.G. Kühn, type species *P. irmischiae* J.G.

Kühn, the anamorph of *Urocystis primicola* Magnus, was the first genus to be described specifically for a smut anamorph. Species of *Paepalopsis* are anamorphs of species of *Urocystis* Rabenh. ex Fuckel (including *Ginanniella* Cif.). The conidia of these anamorphs are subglobose, obovoid or obpyriform and may germinate to form a germ tube or secondary conidia (Nagler & Oberwinkler 1984, Vánky 1994). *Ascomyces trientalis* is now *Paepalopsis trientalis* (Berk.) Cif., the anamorph of *Urocystis trientalis* (Berk. & Broome) B. Lindeb.

Entylomella Höhn. was described for conidial forms of *Entyloma* de Bary and *Doassansia* Cornu (von Höhnel 1924). *Tilletiopsis* (anamorphs of *Entyloma* and *Melanotaenium* de Bary) is very similar to *Entylomella*. Boekhout (1991) proposed that these anamorphs all be placed in the genus *Tilletiopsis*. Braun (1995) has argued that *Entylomella* be used for host specific pathogenic anamorphs and *Tilletiopsis* for saprophytic and phylloplane inhabiting taxa. *Fusidium ranunculi* is now regarded as a synonym of the *Tilletiopsis* anamorph of *Entyloma ficariae* (Boekhout 1991). Braun (1995) considers *Doassansiella* Zambett. *nom. inval.* and *Savulescuella* Cif., anamorphs of *Doassansia* and *Tracya* Syd. & P. Syd., to be synonyms of *Entylomella*.

Some species of *Exobasidium* Woronin have anamorphs that resemble species of *Entylomella*, *Tilletiopsis*, or *Ramularia* (Boekhout 1991, Braun 1995). Species of Exobasidiales are associated mainly with species of Commelinaceae and Ericaceae. *Ceraceosorus bombacis* (B.K. Bakshi) B.K. Bakshi (Brachybasidiaceae) that causes a serious disease of *Bombax ceiba* L. in India produces a blastoconidial anamorph in culture (Cunningham *et al.* 1976). Braun (1995) thought the *Entylomella*-like *Exobasidium* anamorphs should be placed in a separate genus.

In Western Australia a fungus very similar to *R. piterika* causes a shoot dieback of *Corymbia ficifolia* (F. Muell.) K.D. Hill & L.A.S. Johnson (Cass Smith 1970). The Western Australian fungus has been known as *Sporotrichum destructor* H.A. Pittman (*nom. nud.*). The genus *Sporotrichum* Link is now restricted to anamorphs of lignicolous basidiomycetes (species of *Laetiporus* Murrill, *Phanerochaete* P. Karst. and *Pycnoporellus* Murrill) with stalked, broadly attached terminal blastoconidia borne on randomly to racemosely branched conidiophores, clamps absent or limited to advancing hyphae (Stalpers 1984). *Sporothrix schenckii*, the type species of *Sporothrix*, has been described repeatedly and incorrectly as species of *Sporotrichum* (Stalpers 1984).

Materials and Methods

Isolates of *R. piterika* were cultured on 1.5% malt extract (Difco) agar (MEA), 2% malt extract plus 0.3% yeast extract (Difco) agar (MEYA) at 22°C in darkness or on the laboratory bench (Traquair *et al.* 1988), carnation leaf agar (Fisher *et al.* 1982) or *Corymbia* leaf agar. Light microscope observations were made on fresh field collections, herbarium collections and on cultures up to 28 days old. Mycelium and conidiophores were mounted in water, aqueous Congo Red, or 0.5% acid fuchsin in lactic acid. Cycloheximide tolerance was determined on 1.5% MEA amended with 10, 100 or 1000 µg/mL of the antibiotic (Siegfried *et al.* 1992). Growth on media containing cycloheximide has been used as a diagnostic character for species of *Ophiostoma* (Seifert *et al.* 1993, Siegfried *et al.* 1992, Vujanovic *et al.* 2000).

Young shoots from apparently healthy trees in two to four year old *Corymbia* plantations in north-eastern New South Wales were removed with secateurs, placed in plastic bags in a cool box, and returned to the laboratory to determine if *R. piterika* was present as an endophyte. After surface sterilisation in 2.0% sodium hypochlorite solution for 5 min. shoots were cut into lengths of about 10 mm and plated on water agar. Plates were examined after seven days. Shoots from young trees, from plantations or natural regeneration, with small lesions exuding kino were treated in a similar way.

Results

Ramularia piterika did not grow on 1.5% MEA amended with 10, 100 or 1000 µg/mL of cyclohexamide, indicating it is not an anamorph of a species of Ophiostomataceae. No teleomorph was found on cultures, herbarium specimens, or on living or dead plant material collected in the field despite extensive searches. Conidia and conidiogenous cells were confirmed as hyaline and thin-walled. Hyphae stained with Congo Red revealed only simple septa and no dolipore septa. Sporulating colonies of *R. piterika* on agar plates inverted over fresh plates showed no evidence of production of ballistoconidia.

Isolations from young woody shoots from living apparently healthy *C. henryi* (S.T. Blake) K.D. Hill & L.A.S. Johnson, *C. maculata* (Hook.) K.D. Hill & L.A.S. Johnson, and *C. variegata* (F. Muell.) K.D. Hill & L.A.S. Johnson consistently yielded *R. piterika* as did shoots with kino lesions. Isolates from woody tissue were

identical to those from living leaves. An isolate from living leaves of *C. ficifolia* from Western Australia was similar in all regards to *R. pitereka* isolates from New South Wales and Queensland. *Ramularia pitereka* is occasionally found on large *C. maculata* trees and is common on young spotted gum natural regeneration in eastern New South Wales.

Over the years several collections of species of ghost gums from Central Australia being cultivated in gardens have been seen with shoot dieback. In each instance the fungus has been *R. pitereka*. Ghost gums are members of *Eucalyptus* subgenus *Blakella* (paper fruited bloodwoods), series *Scutiformis* Maiden (Brooker 2000). *Blakella* was included in *Corymbia* by Hill & Johnson (1995) although there is dispute whether or not it should be recognised as a separate genus (Brooker 2000, Ladiges & Udovicic 2000, Ladiges *et al.* 1995, Sale *et al.* 1993, Steane *et al.* 1999, Udovic *et al.* 1995). It is noteworthy that *R. pitereka* infects species of *Angophora*, *Blakella* and *Corymbia* as these taxa form a monophyletic clade in molecular classifications of eucalypts (Ladiges & Udovicic 2000). *Eucalyptus grandis* and *E. camaldulensis*, the hosts of *S. eucalypti* and *S. pusilla*, are members of *Eucalyptus* subgenus *Symphomyrtus* (Schauer) Brooker (Braun 1998, Wingfield *et al.* 1993).

Discussion

Isolations from apparently healthy shoots of species of *Corymbia* show *R. pitereka* can be an endophyte. The kino oozing lesions in bark of young shoots of living spotted gums are similar to lesions reported on *C. ficifolia* in Western Australia (Cass Smith 1970). The lesions resemble wounds made by hailstones.

Conidial scars on conidiophores may be conspicuous by thickened walls, dark colouration, or being refractive. Tooth-like projections supporting the conidia are called denticles. Denticles are more or less subcylindric to tapered, continuous with the conidiogenous cell and usually formed sympodially. Conidial scars located on small shoulders on the conidiogenous cell have been termed geniculations (Chesters & Greenhalgh 1964) and appear as minute protruberances. Braun (1995) redefined the genus *Ramularia* based on peculiarities of the conidial scars. The characteristics of the conidiophores, conidiogenous cells and conidia of *Sporothrix*, *Ramularia* and *R. pitereka* and its relatives are shown in Table 1.

Table 1. Attributes of *Quambalaria*, *Ramularia* and *Sporothrix*.

Character	<i>Quambalaria</i>	<i>Ramularia s. str.</i>	<i>Sporothrix s. str.</i>
Stromatic tissue	Present, hyaline	Sometimes present, hyaline or faintly pigmented	Absent
Conidiophores	Solitary to caespitose	Solitary to fasciculate	Solitary
Conidiogenous cells hyaline	Yes	Usually, or with basal pigment	Yes
Growth sympodial	Yes	Yes	Yes
Conidia	Solitary	Solitary or catenate	Solitary
Thickened spore scars	No	Slightly to strongly thickened	Inconspicuous
Conidial scars darkened	No	Always; usually strongly	No
Refractive spore scars	Slightly to moderately refractive	Always strongly refractive	No
Spore scars protruding on small geniculations on conidiogenous cells	Yes	Yes	No
Denticles	Minute	No	Usually prominent
Secondary conidia	Present but not on all primary conidia	None	Formed in some species
Teleomorph	Unknown	Mycosphaerellaceae	Ophiostomataceae

No teleomorph is known for *R. pitereka*, *S. eucalypti* or *S. pusilla*. The opinion of Braun (1998) that *R. pitereka* is not an anamorph of a species of Mycosphaerellaceae is accepted for the reasons listed above. The absence of growth on media containing cycloheximide, the pathogenicity to eucalypts, the dense growth of white conidiophores on both host and agar media, and the absence of distinct denticles indicate the affinities of *R. pitereka* are not with Ophiostomataceae. Hosts of species of Entylomellaceae Cif. (Braun 1995, Ciferri 1959) are herbaceous. Furthermore *Entylomella* conidiophores and conidiogenous cells are relatively inconspicuous unlike the profuse growth from stroma-like tissue of the eucalypt pathogens. The apparent absence of dolipore septa in the hyphae indicates *R. pitereka* and related taxa are not anamorphs of Holobasidiomycetes or Tilletiales (Vánky & Bauer 1992). Rather the affinities appear to lie with the Exobasidiales or Ustilaginales (Ustomycota *sensu* J. Walker 1996).

There are three reports of fungi described as smuts on Myrtaceae but probably none is valid. *Ustilago vriesiana* Vuill. was described from young plants of *Eucalyptus* spp. raised from seed in the Amsterdam Botanic Garden. The type or other specimens have not been seen and its true identity is not known. *Entyloma eugeniarum* Cooke & Masee apud Cooke was described from *Eugenia* sp. (?*Syzygium* sp.) in Queensland (Cooke 1891). Examination of the type has shown it is a species of *Phyllachora* (J. Walker pers. comm.). *Entyloma paradoxa* Syd. & P. Syd. from Java on leaves of *Syzygium aqueum* (Burm.f.) Alston was excluded from the Ustilaginales by Ciferri (1963).

Under these circumstances it is thought appropriate to describe a new genus for *Ramularia pitereka* and related taxa.

Taxonomy

Quambalaria J.A. Simpson *gen. nov.*

Pustulae strato stromatico exorientes, subcuticulariae vel in cavitate stromatum, albae. *Conidiophora* erumpentia, singularia vel caespitosa, hyalina, simplicia, raro ramosa, parce septata, proliferatione sympodiali et cicatricibus parvis leviter refractivis non incrassatis pallidis planis vel in geniculis parvis vel in denticulis parvis. *Conidia* holoblastica, hyalina, unicellularia, levia, in forma variabilia, clavata vel elongata-clavata, cylindracea, fusiformia vel anguste pyriformia, vel obovoidea, singularia, cicatrice leviter refractiva non incrassata ad unum vel ambo extrema, germinatio a hyphis una vel duabus. *Conidia secundaria* conidiis primariis exorientia, vel gemmationibus vel ex cellulis conidiogenis brevissimis cum cicatricibus et proliferatione sympodiali, catenas breves simplices vel ramosas formantia. Differt a *Ramularia* in cicatricibus hyalinis non incrassatis et in conidiis secundariis; differt a *Sporotriche* in cicatricibus refractivis et geniculis parvis. *Teleomorphus* ignotus.

Typus generis hic designatus: *Quambalaria pitereka* (J. Walker & Bertus) J.A. Simpson *comb. nov.*

Basionym: *Ramularia pitereka* J. Walker & Bertus, *Proc. Linn. Soc. New South Wales*, ser. 2, **96**, 108 (1971)
 = *Sporothrix pitereka* (J. Walker & Bertus) U. Braun & Crous, in Braun, *Monograph on Cercosporella, Ramularia and Allied Genera*, Vol. 2, p. 416 (1998)
 = *Sporotrichum destructor* H.A. Pittman, in Cass Smith, *J. Agric. W. Australia*, ser. 4, **11** (2), 34 (1970), *nom. nud.*

Pustules arising from stromatic layer, subcuticular or in the stomatal cavity, white. *Conidiophores* erumpent, single or caespitose, hyaline, simple, rarely branched, sparsely septate, with sympodial proliferation and small slightly refractive non-thickened pale scars, flat or on small geniculations or on small denticles. *Conidia* holoblastic, hyaline, unicellular, smooth, variable in shape, clavate to elongated-clavate, cylindrical, fusiform or narrowly pyriform, or obovoid, single, with a slightly refractive non-thickened scar at one or both ends, germination by one or two hyphae. *Secondary conidia* arising from primary conidia, either by budding or from very short conidiogenous cells with scars and sympodial proliferation, forming short simple or branched chains. Differs from *Ramularia* in hyaline, non-thickened scars and in secondary conidia; differs from *Sporothrix* in refractive scars and in small geniculations. *Teleomorph* unknown.

Etymology: quambaloo, Australian aboriginal word for gum tree (eucalypt).

Selected specimens examined

New South Wales: West Pennant Hills, Forestry Nursery, *C. eximia* (Schauer) K.D. Hill & L.A.S. Johnson, 3.iv. 1970, A.L. Bertus & J. Walker (DAR 19773, holotype); Grafton, *C. variegata*, Zeull's plantation, Sept. 1998, J.A. Simpson (DAR 75022); Grafton, *C. henryi*, natural regeneration at Zeull's plantation, Sept. 1998, J.A. Simpson (DAR 75023); Jackadgery, *C. maculata*, Oct. 1999, J.A. Simpson (DAR 75024); Jackadgery, *C. henryi*,

Oct. 1999, J.A. Simpson (DAR 75025). Queensland: Beerburrum, Forestry Nursery, *C. citriodora* (Hook.) K.D. Hill & L.A.S. Johnson, Nov. 1998, J.A. Simpson (DAR 75027); Beerburrum, Forestry Nursery, *C. variegata*, Nov. 1998, J.A. Simpson (DAR 75026). Western Australia: Denmark, *C. ficifolia*, Dec. 1996, K. Syme (DAR 75028).

Quambalaria eucalypti (M.J. Wingf., Crous & W.J. Swart) J.A. Simpson *comb. nov.*

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

Known only from the holotype collection: South Africa, Natal, Kwambonambi, *Eucalyptus grandis* W. Hill ex Maiden, 19.v.1987, M.J. Wingfield (PREM 51089).

Quambalaria pusilla (U. Braun & Crous) J.A. Simpson *comb. nov.*

Basionym: *Sporothrix pusilla* U. Braun & Crous, in Braun, *Monograph on Cercosporiella, Ramularia and Allied Genera*, Vol. 2, p. 418 (1998).

Known only from the holotype collection: Thailand, *Eucalyptus camaldulensis* Dehnh., Oct. 1995, M.J. Wingfield (HAL).

Key to described species of *Quambalaria*

1. Secondary conidia similar to primary conidia; on species of *Angophora* or *Corymbia* *Q. pitereka*
 1: Secondary conidia about half the size of the primary conidia; on species of *Eucalyptus* subg. *Symphomyrtus* ..
 2.
 2. Primary conidia 3.0–12.0 × 1.0–2.0 µm, secondary conidia 1.5–3.5 × 0.5–1.5 µm *Q. pusilla*
 2: Primary conidia 6.0–12.0 × 2.5–4.0 µm, secondary conidia 3.0–5.0 × 1.5–2.5 µm *Q. eucalypti*

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