Wongia gen. nov. (Papulosaceae, Sordariomycetes), a new generic name for two root-infecting fungi from Australia

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**Abstract:** The classification of two root-infecting fungi, Magnaporthe garrettii and M. griffinii, was examined by phylogenetic analysis of multiple gene sequences. This analysis demonstrated that M. garrettii and M. griffinii were sister species that formed a well-supported separate clade in Papulosaceae (Diaporthomycetidae, Sordariomycetes), which clusters outside of the Magnaporthales. Wongia gen. nov, is established to accommodate these two species which are not closely related to other species classified in Magnaporthe nor to other genera, including Nakataea, Magnaporthiopsis and Pyricularia, which all now contain other species once classified in Magnaporthe.

**Key words:** Ascomycota Cynodon Diaporthomycetidae multigene analysis one fungus-one name molecular phylogenetics root pathogens

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**INTRODUCTION**

The taxonomic and nomenclatural problems that surround generic names in the Magnaporthales (Sordariomycetes, Ascomycota), together with recommendations for the suppression and protection of some of these names, were explained by the Pyricularia/Magnaporthe Working Group established under the auspices of the International Commission on the Taxonomy of Fungi (ICTF; Zhang et al. 2016). One of these generic names, Magnaporthe, was proposed for suppression by Zhang et al. (2016) because Magnaporthe is congeneric with Nakataea (Hara 1939) as the types of both genera, Magnaporthe salvinii (syn. Leptosphaeria salvinii) and Nakataea sigmoidea (syn. Helminthosporium sigmoideum) are conspecific (Krause & Webster 1972, Luo & Zhang 2013).

Magnaporthe was morphologically characterised by having dark perithecia with long necks immersed in host tissue, unitunicate asci, and 4-celled fusiform hyaline to pale brown ascospores (Krause & Webster 1972). Subsequently, seven species were assigned to Magnaporthe based on morphology, namely, M. salvinii (Krause & Webster 1972), M. grisea (Barr 1977), M. rhizophila (Scott & Deacon 1983), M. poae (Landschoot & Jackson 1989), M. oryzae (Couch & Kohn 2002), and M. garrettii and M. griffinii (Wong et al. 2012). Most of these species belong to other genera, specifically Magnaporthiopsis, Nakataea, and Pyricularia (Luo & Zhang 2013). The two exceptions are the Australian ectotrophic species, M. garrettii and M. griffinii, which infect roots of some turf grasses (Wong et al. 2012). One of these species, M. griffinii, was found by Klaubauf et al. (2014) to be distant from Sordariomycetes based on ITS sequences (GenBank JQ390311, JQ390312).

This study aims to resolve the classification of M. garrettii and M. griffinii using molecular sequence data from the type specimens. Four loci from the nuclear genome namely, ITS and the large subunit (LSU) of rDNA, translation elongation factor 1-alpha (TEF1), and the largest subunit of RNA polymerase II (RPB1) were selected for analysis.

**MATERIALS AND METHODS**

**Fungal cultures and DNA extraction**

Dried specimens of the holotypes of Magnaporthe garrettii (DAR 76937) and M. griffinii (DAR 80512) were borrowed from the Plant Pathology Herbarium, New South Wales Agriculture (DAR). Dried perithecia were excised with a needle and soaked in extraction buffer overnight at 65 °C before extraction of DNA with an UltraClean® Microbial DNA Isolation Kit (MoBIO Laboratories) as per the manufacturer’s instructions. An additional culture of M. griffinii (BRIP 60377) was grown on PDA for 6 wk before enough mycelium was produced for DNA extraction.

**PCR amplification**

The primer pairs ITS1/ITS4 (White et al. 1990), RPB-Ac/ RPB-Cr (Castlebury et al. 2004, Matheny et al. 2002), LR5/ LROR and EF1983F/2218R (Schoch et al. 2009) were...
| Species                              | Voucher          | Substrate          | Locality | GenBank accession no.  |
|-------------------------------------|------------------|--------------------|----------|------------------------|
|                                     |                  |                    |          | ITS  | LSU  | RPB1 | TEF1 |
| **Annulusmagnus triseptatus**       | CBS 128831       | Decayed wood       | France   | GQ996540               |
| **Bambusicularia brunnea**          | CBS 133599¹      | Sasa sp.           | Japan    | KM484830 KM484948 KM485043 |
| **Barretomyces calatheae**          | CBMAI 1060¹      | Calathea longifolia| Brazil   | GU294490               |
| **Brunneosporella aquatica**        | HKUCC 3708       | Submerged wood     | Hong Kong| AF132326               |
| **Budhanggurabania cynodonticola**  | BRIP 59305¹      | Cyordon dactylon   | Australia| KP162134 KP162140 KP162143 KP162138 |
| **Buergenerula spartinae**          | ATCC 22848¹      | Spartina alterniflora|          | JX134666 DQ341492 JX134720 JX134692 |
| **Calosphaeria pulchella**          | CBS 115999       | Prunus avium       | France   | AY761075               |
| **Camarops ustulinoides**           | AFTOL-ID 72      | -                  | -        | DQ470941 DQ471121 DQ471050 |
| **Coniochaeta ligniaria**           | NRRL 30616       | Soil               | Hong Kong| AF132332               |
| **Cordana pauciseptata**            | CBS 121804       | -                  | Spain    | HE672160               |
| **Cryphonectria havanensis**        | CBS 505.63       | Eucalyptus saligna | Russia   | AF408339               |
| **C. parasitica**                   | ATCC 38755       | Castanea dentata   | USA      | Genome³ Genome⁴ Genome⁵ Genome⁶ |
| **Diaporthe eres**                  | CBS 109767       | Acer campestre     | Austria  | AF408350               |
| **Diaporthe phaseolorum**           | ATCC 64802       | -                  | -        | AY346279               |
| **Fluminicola coronata**            | HKUCC 3717       | -                  | Hong Kong| AF132332               |
| **Gaemunnomyces oryzainus**         | CBS 235.32       | Oryza sativa       | USA      | JX134669 JX134681 JX134723 JX134695 |
| **Harknessia eucalypti**            | CBS 342.97       | Eucalyptus regnans | Australia| AF408363               |
| **Lecythophora luteoviridis**       | CBS 206.38       | -                  | Switzerland| FR691987               |
| **Magnaporthiopsis agrostidis**     | BRIP 59300¹      | Agrostis stolonifera| Australia| KT364753 KT364754 KT364755 KT689623 |
| **M. poae**                         | ATCC 64411       | Triticum aestivum  | USA      | JF414836 JF414885 JF710433 JF710415 |
| **Nakataea oryzae**                 | ATCC 44754       | Oryza sativa       | Japan    | JF414838 JF414887 JF710441 JF701406 |
| **Neurospora crassa**               | MUCL 19026       | -                  | -        | AF286411               |
| **Ophioceras leptosporum**          | CBS 894.70       | Dead stem          | UK       | JX134678 JX134690 JX134732 JX134704 |
| **O. dolichostomum**                | CBS 114926       | Rotten wood        | China    | JX134677 JX134689 JX134731 JX134703 |
| **O. commune**                      | YMF1.00980       | Rotten wood        | China    | JX134675 JX134687 JX134729 JX134701 |
| **Ophiostoma floccosum**            | AU55-6 in G      | Pinus sp.          | Canada   | AF234836               |
| **O. stenoceras**                   | AFTOL-ID 1038    | -                  | -        | DQ836904               |
| **Papulosa amerospora**             | AFTOL-ID 748     | -                  | -        | DQ470950 DQ471143 DQ471069 |
| **Pseudophialophora eragrostis**    | RUTTP-CM12m²     | Eragrostis sp.     | USA      | KF689648 KF689638 KF689618 KF689628 |
| **Pseudopyricularia kyllingae**     | CBS 133597¹      | Kylilnga brevifolia| Japan    | KM484876 KM484992 KM485096 |
| **Pyricularia grisea**              | M 83             | Digitaria sp.      | USA      | JX134671 JX134683 JX134725 JX134697 |
| **P. oryzae**                       | 70-15            | -                  | USA      | Genome⁴ Genome⁵ Genome⁶ Genome⁷ |
| **Togniniella acerosa**             | CBS 113648       | Decayed wood       | New Zealand| AY761076               |
used to amplify ITS, RPBI, LSU, and TEF1 sequences, respectively. PCR amplifications were conducted in a 20 µl reaction volume containing 1 µl of 5-10 ng DNA, 10 µl of high fidelity Phusion DNA Polymerase (New England Biolabs), 1 µl of primers (10 µM) and 7 µl of sterile water with the thermal cycling program as follows: 98 ºC for 30s, 30 cycles of 98 ºC for 10 s, 58–62 ºC for 30 s and 72 ºC for 1 min, and a final extension of 72 ºC for 10 min. PCR products were sent to Macrogen (Korea) for direct sequencing using the amplification primers.

Phylogenetic analysis
All sequences were assembled with Sequencher v. 5.1 (Gene Codes, Ann Arbor, MI). Alignments were generated for individual loci using MAFFT v. 6.611 (Katoh & Toh 2008), and then the alignments concatenated for the phylogenetic analyses. DNA sequences were deposited in GenBank with the accession numbers listed in Table 1 and the final curated alignment deposited in TreeBASE under accession no. ID 19968. Phylogenetic trees were reconstructed with two phylogenetic criteria, Maximum likelihood (ML) and Bayesian Inference (BI). ML was carried out with RAxML v. 7.2.6 using GTRGAMMA as the model of evolution (Stamatakis 2006), choosing the rapid bootstrap analysis (command –f a) with a random starting tree and 1000 maximum likelihood bootstrap replications. BI was done with MrBayes v. 3.1.2 (Ronquist et al. 2012), utilizing four parallel MCMC chains, which were allowed to run for 10 million generations, with sampling every 1000 generations and saving trees every 5 000 generations. The cold chain was heated at a temperature of 0.25. All phylogenetic trees were visualized using FigTree (Morariu et al. 2009).

RESULTS
Molecular phylogeny
The phylogenetic trees recovered from the ML and BI analyses had identical topologies and were well-supported by bootstrap and posterior probabilities (Fig. 1). The analyses comprised 36 taxa belonging to eight orders and two families in the subclass Diaporthomycetidae (Sordariomycetes). Camarops ustulinoides (Boniliales, Sordariomycetes) was used as the outgroup (Table 1). The phylogenetic analysis revealed Magnaporthe garrettii (DAR 76937) and M. griffinii (DAR 80512) as sister species that formed a distinct well-supported (100/1.0) monophyletic clade in Papulosaceae that sat outside Magnaporthales. The analysis provided moderate support (67/0.93) for placement of M. garrettii and M. griffinii in Papulosaceae, which has not yet been assigned to any order of Diaporthomycetidae. Based on this analysis, a new generic name is established here to accommodate M. garrettii and M. griffinii.

TAXONOMY
Wongia Khemmuk, Geering & R.G. Shivas, gen. nov.
MycoBank MB817529

Etymology: Named after the eminent Australian mycologist and plant pathologist, Percy T.W. Wong (University of Sydney), who first studied and classified these fungi.

Diagnosis: Differs from all other genera in the subclass Diaporthomycetidae in having non-amyloid apical rings in the asci with 3-septate ascospores that have dark brown middle cells and pale brown to subhyaline shorter distal cells.
Type species: Wongia garrettii (P. Wong & M.L. Dickinson) Khemmuk et al. 2016

**Classification:** Ascomycota, Sordariomycetes, Diaporthomycetidae.

**Description:** Mycelium comprised of brown, straight or flexuous hyphae, with simple hyphopodia. Ascomata perithecial, superficial and immersed, mostly solitary or sometimes aggregated in small groups, globose, black, ostiolate, with a long or short neck, perithecial wall composed of textura epidermoidea, external cell much darker. Paraphyses thin-walled, hyaline, filiform, septate.

Asci unitunicate in structure, cylindrical, mostly straight, short stalked, tapered towards a rounded apex, with a light refractive, non-amyloid apical ring, 8-spored. Ascospores uniseriate, cylindrical to fusiform, straight or slightly curved with rounded ends, 3-septate, middle cells dark brown and distal cells pale brown to subhyaline and shorter.

**Wongia garrettii** (P. Wong & M.L. Dickinson) Khemmuk, Geering & R.G. Shivas, **comb. nov.** (Fig. 2A–B)

MycoBank MB817530
Basionym: Magnaporthe garrettii P. Wong & M.L. Dickinson, Australasian Plant Pathology 41: 326 (2012).

**Type:** Australia: South Australia: Adelaide, Colonel Light Gardens Bowling Club, on Cynodon dactylon, 30 Oct. 2004, M.L. Dickinson (DAR 76937 – holotype).

**Description and Illustration:** Wong et al. (2012).

**Wongia griffinii** (P.Wong & A.M. Stirling) Khemmuk, Geering & R.G. Shivas, **comb. nov.** (Fig. 2C–D)

MycoBank MB817531
Basionym: Magnaporthe griffinii P. Wong & A.M. Stirling, Australasian Plant Pathology 41: 327 (2012).
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Type: Australia: Queensland: Coolum, Hyatt Coolum Golf Club, on Cynodon dactylon × transvaalensis, 13 Mar. 2008, M. Whatman (DAR 80512 – holotype).

Description and illustration: Wong et al. (2012)

Other specimens examined: Australia: New South Wales: Cobbitty, on Cynodon dactylon, 19 Apr. 2013, G. Beehag, (BRIP 60378). Queensland: Brisbane, on on Cynodon dactylon × transvaalensis, Jan. 2000, A.M. Stirling (BRIP 60377).

DISCUSSION

Magnaporthe is a synonym of Nakataea as their respective type species, Magnaporthe salvinii and Nakataea sigmoidea, refer to the same species (Krause & Webster 1972, Luo & Zhang 2013, Klaubauf et al. 2014, Zhang et al. 2016). This led us to re-examine two Australian species, M. garrettii and M. griffinii, pathogenic on roots of couch (Cynodon dactylon) and hybrid couch (C. dactylon × transvaalensis) (Wong et al. 2012). We establish Wongia here to accommodate these two species, based on molecular and morphological analysis.

Multigene analyses placed W. garrettii and W. griffinii in Papulosaceae (Diaporthomycetidae, Sordariomycetes; Maharachchikumbura et al. 2015) with moderate bootstrap support (Fig. 1). The Papulosaceae has not yet been placed in an order within Sordariomycetes (Winka & Erikson 2000). Wongia is the fourth genus to be placed in Papulosaceae, along with Brunneosporella (Rancho & Hyde 2001), Fluminicola (Wong et al. 1999), and Papulosa (Kohlmeyer & Volkmann-Kohlmeyer 1993). Most members in this family are found on submerged wood in freshwater habitats and grow slowly in culture on potato dextrose agar (Rancho & Hyde 2001). Wongia garrettii and W. griffinii are morphologically different from other genera of Papulosaceae in having non-amyloid apical rings in the asci using Melzer’s reagent, while others have amyloid apical rings (Winka & Erikson 2000). The long perithecial necks of W. garrettii differentiate it from W. griffinii (Wong et al. 2012), which also has larger ascospores (24–35 x 6–9 µm) than W. garrettii (19–25 x 5–7 µm) (Wong et al. 2012). Asexual morphs have not been found in either W. garrettii or W. griffinii in nature or in cultures grown on artificial media under laboratory conditions (Wong et al. 2012).

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