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 ºC 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

Table 1. Collection details and GenBank accession numbers of isolates included in this study.

Species	Voucher[1]	Substrate	Locality	GenBank accession no.[2]			
				ITS	LSU	RPB1	TEF1
Annulusmagnus triseptatus	CBS 128831	Decayed wood	France		GQ996540		
Bambusicularia brunnea	CBS 133599[T]	*Sasa* sp.	Japan	KM484830	KM484948	KM485043	
Barretomyces calatheae	CBMAI 1060[T]	*Calathea longifolia*	Brazil	GU294490			
Brunneosporella aquatica	HKUCC 3708	Submerged wood	Hong Kong		AF132326		
Budhanggurabania cynodonticola	BRIP 59305[T]	*Cynodon dactylon*	Australia	KP162134	KP162140	KP162143	KP162138
Buergenerula spartinae	ATCC 22848[T]	*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	-		AY198388		
Cordana pauciseptata	CBS 121804	-	Spain		HE672160		
Cryphonectria havanensis	CBS 505.63	*Eucalyptus saligna*	Russia		AF408339		
C. parasitica	ATCC 38755	*Castanea dentata*	USA	Genome[3]	Genome[a]	Genome[3]	Genome[3]
Diaporthe eres	CBS 109767	*Acer campestre*	Austria		AF408350		
Diaporthe phaseolorum	ATCC 64802	-	-		AY346279		
Fluminicola coronata	HKUCC 3717	-	Hong Kong		AF132332		
Gaeumannomyces oryzinus	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 59300[T]	*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-CM12m9[T]	*Eragrostis* sp.	USA	KF689648	KF689638	KF689618	KF689628
Pseudopyricularia kyllingae	CBS 133597[T]	*Kyllinga brevifolia*	Japan	KM484876	KM484992	KM485096	
Pyricularia grisea	M 83	*Digitaria* sp.	USA	JX134671	JX134683	JX134725	JX134697
P. oryzae	70-15	-	USA	Genome[4]	Genome[4]	Genome[4]	Genome[4]
Togniniella acerosa	CBS 113648	Decayed wood	New Zealand		AY761076		

Table 1. (Continued).

Species	Voucher[1]	Substrate	Locality	GenBank accession no.[2]			
				ITS	LSU	RPB1	TEF1
Wongia garrettii	DAR 76937[T]	*Cynodon dactylon*	Australia	**KU850474**		**KU850469**	**KU850467**
W. griffinii	DAR 80512[T]	*Cynodon dactylon* × *transvaalensis*	Australia	**KU850473**	**KU850471**		
W. griffinii	BRIP 60377	*Cynodon dactylon* × *transvaalensis*	Australia	**KU850472**	**KU850470**	**KU850468**	**KU850466**

[1]AFTOL: Assembling the Fungal Tree of Life; ATCC: American Type Culture Collection, Manassas, VA; BRIP: Plant Pathology Herbarium, Department of Agriculture and Forestry, Queensland, Australia; CBMAI: Coleção Brasileira de Microrganismos para Ambiente e Indústria, Paulinia, Brazil ; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; DAR: Plant Pathology Herbarium, Orange Agriculture Institute, NSW, Australia; F: Field Museum Mycology Herbarium, Chicago, IL; G: Culture Collection of the Wood Science Department, University of British Columbia, Vancouver, BC, Canada; HKUCC: Hong Kong University Culture Collection; MUCL: Mycothèque de l´Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NRRL: American Research Service (ARS) culture collection, Beltsville, MD; RUTPP = Rutgers Mycological Herbarium, New Brunswick, NJ; YMF: Yunnan Microbiological Fermentation Culture Collection Center, Kunming, Yunnan, China.

[2] GenBank accession numbers of sequences newly generated in this study are in bold.

[3] Joint Genome Institute, Walnut Creek, CA.

[4] Broad Institute, Cambridge, MAA.

[T] Type specimen or ex-type culture.

used to amplify ITS, RPB1, 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.

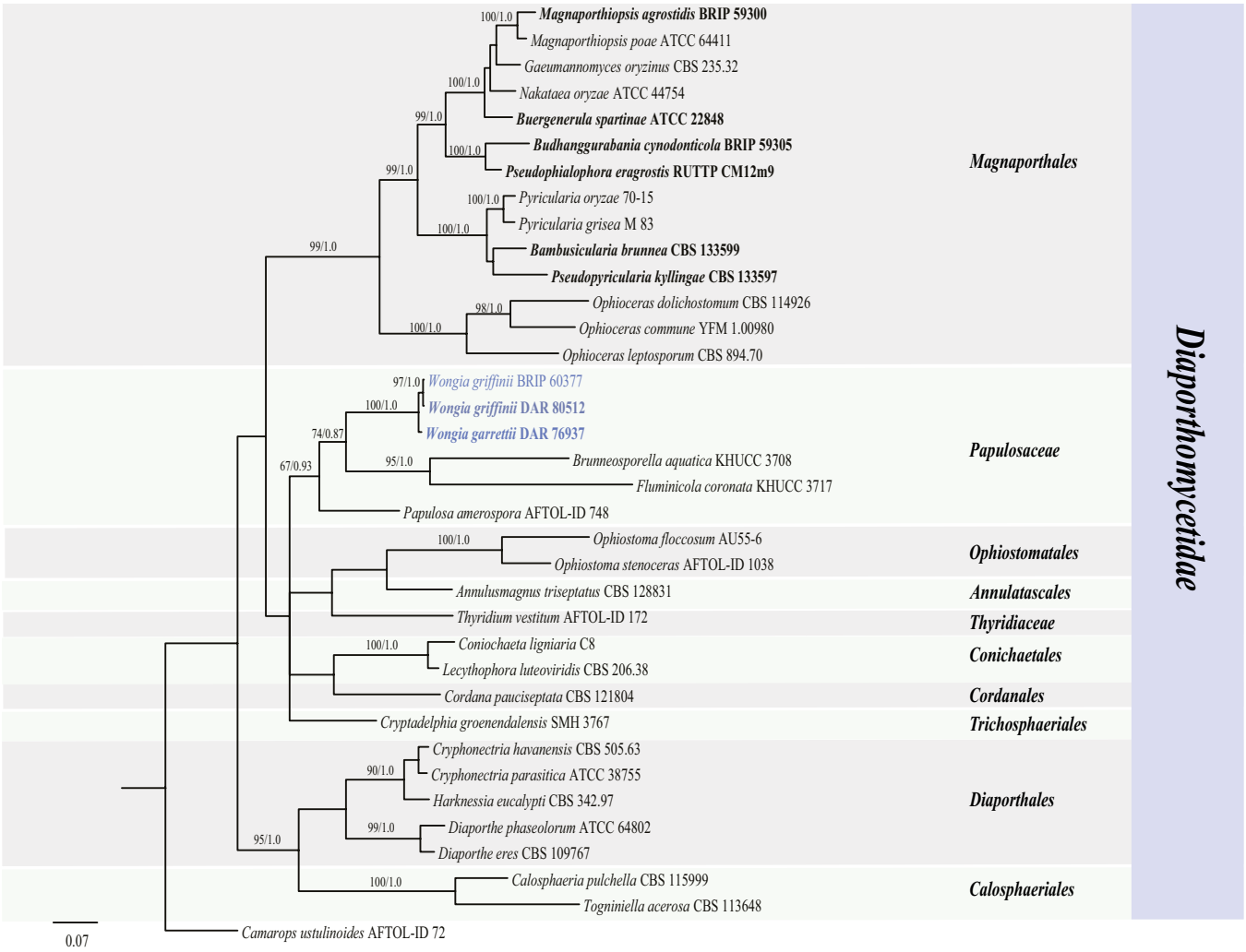

Fig. 1. Phylogenetic tree obtained from a maximum likelihood analysis of the combined ITS/LSU/RPB1/TEF1 alignment. The bootstrap support values from 1 000 replicates and posterior probabilities obtained in Bayesian analysis are indicated at the nodes. The scale bar indicates the expected changes per site. Ex-type cultures of species are indicated in **bold**.

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).

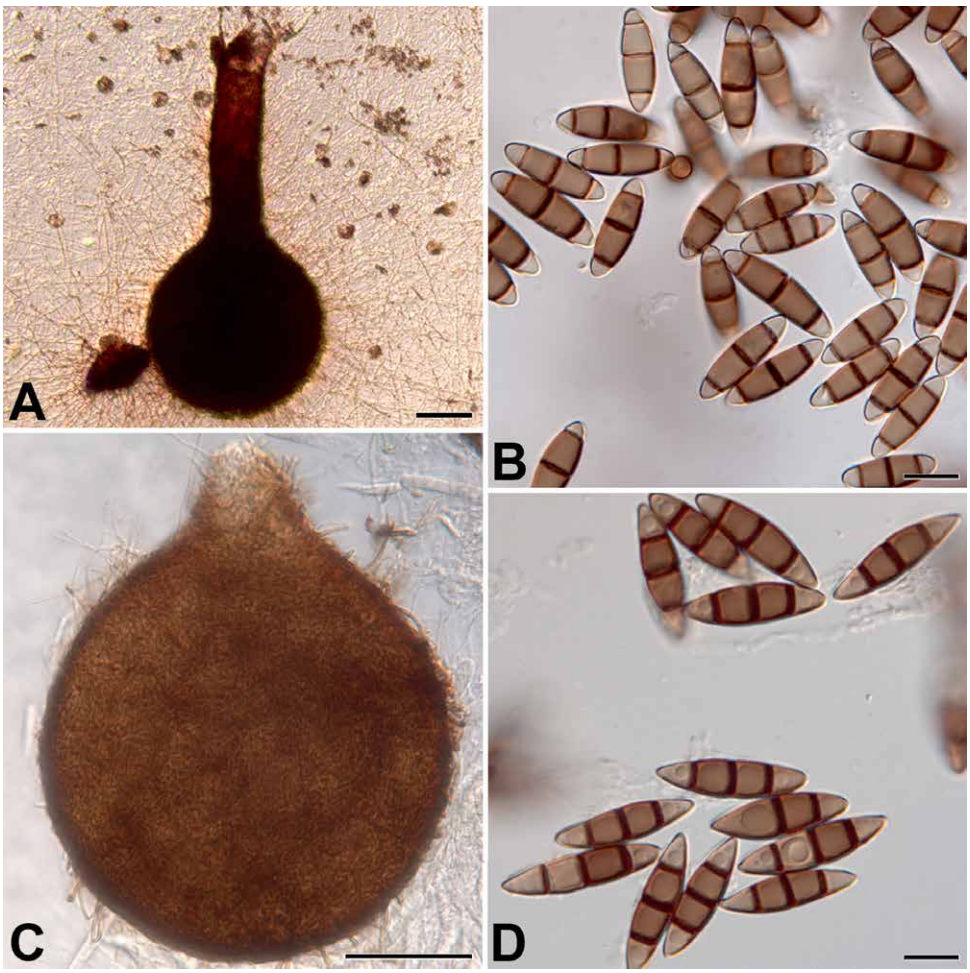

Fig. 2. Morphological features of *Wongia* species. **A–B**. *W. garrettii* (DAR 76937 – holotype). **A**. Perithecium. **B**. Ascospores. **C–D**. *W. griffinii* (BRIP 60378). **C**. Perithecium. **D**. Ascospores. Bars: A, D = 100 µm; B, D = 10 µm.

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* (Ranghoo & 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 (Ranghoo & 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 & Eriksson (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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