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Six novel species of *Fusarium* from natural ecosystems in Australia

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Abstract Six new species of *Fusarium* associated with soil and plant hosts from ecosystems of minimal anthropogenic disturbance in Australia are described. *Fusarium coicis* from *Coix gasteenii*, *F. goolgardi* from *Xanthorrhoea glauca*, *F. mundagurra* from soil and *Mangifera indica*, *F. newnesense* from soil, *F. tjaetaba* from *Sorghum interjectum* and *F. tjaynera* from soil, *Triodia microstachya*, *Sorghum interjectum* and *Sorghum intrans*. Morphology and phylogenetic analysis of *EF-1α*, *RPB1* and *RPB2* sequence data were used to delineate species boundaries. The new species were phylogenetically distributed in the *Fusarium sambucinum*, *F. fujikuroi*, and *F. chlamydosporum* species complexes, and two novel species complexes. These six new species have particular phylogeographic significance as not only do they provide further insight into the geographic patterns of *Fusarium* evolution but also challenge current phylogeographic hypotheses.

Keywords Species diversity · Endophyte · Phylogenetics · Mycoflora · Phylogeography

Introduction

Fusarium is considered a ubiquitous fungal genus commonly isolated from the majority of bioclimatic regions and ecosystems (Summerell et al. 2010; Backhouse et al. 2001). Representatives cause devastating plant diseases (Nelson et al. 1983) and are major contributors to mycotoxin contamination of human and animal food supplies (Marasas et al. 1984; Desjardins 2006; Bryden 2012). Some species also cause diseases of humans and other animals (Boutati and Anaissie 1997; Mitchell and Attleber 1973). Consequently, *Fusarium* is one of the most studied fungal genera in ecosystems associated with anthropogenic activities.

Fusarium species also occur widely in natural ecosystems with considerable ecological plasticity, occurring as endophytes or latent plant pathogens and soil saprobes (Gordon and Martyn 1997; Burgess 1981; Walsh 2007). Despite the human and ecological importance of this genus, surveys in natural ecosystems of minimal anthropogenic disturbance have largely been undertaken in Australia with relatively few such surveys in other geographic regions of the world (Summerell et al. 2010).

A series of continental scale surveys of *Fusarium* in natural ecosystems of Australia have been conducted over the past 40 years, encompassing a wide range of bioclimatic regions, including tropical, arid, temperate and alpine bioregions (Summerell et al. 1993, 2010; Burgess and Summerell 1992; Sangalang et al. 1995b; Walsh et al. 2010). Biogeographical surveys in natural ecosystems of Australia have resulted in the discovery of novel species including *F. nygamai* (Burgess and Trimboli 1986), *F. beomiforme* (Nelson et al. 1987), *F. babinda* (Summerell et al. 1995), *F. aywerte*, *F. nurragi* (Benyon et al. 2000), *F. gaditjirri* (Phan et al. 2004), *F. lyarnte*, *F. werrikimbe* (Walsh et al. 2010) and *F. burgessii* (Laurence et al. 2011); as well as novel species complexes (Laurence et al. 2011); in addition to high levels of intraspecific diversity in plant pathogenic *Fusarium* species (Laurence et al. 2012, 2014). The fact that novel species have been discovered in each of these surveys suggests that the species diversity in Australia and indeed globally is yet to be fully determined.

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Fusarium is a complex and diverse genus with a controversial taxonomic history (Summerell et al. 2010). The number of recognised species has fluctuated wildly from over 1, 000 to as few as nine (Summerell and Leslie 2011), with current estimations ranging from 100 to 500 (Kirk et al. 2008; Leslie and Summerell 2006), depending on the species concept applied. Details of the historical taxonomic controversies have been comprehensively covered elsewhere (Leslie et al. 2001; Nelson 1991; Leslie and Summerell 2006) but many have resulted from the paucity and considerable plasticity of morphological characters. The key morphological markers for the genus are the shape of the macroconidia and microconidia, the mode of formation of microconidia and the presence or absence of chlamydospores in older cultures. The utility of morphology to describe the inherent diversity in *Fusarium,* however, is questionable as there is considerable plasticity of the characters between isolates within a species and the expression of these characters is also affected by environmental conditions (Leslie et al. 2001). DNA sequencing technology has enabled the taxonomic resolution of *Fusarium* based on multi-gene genealogies (Laurence et al. 2014; O'Donnell et al. 2000; O'Donnell et al. 1998b, 2004). Surveys of natural populations of *Fusarium* are helping to ensure that the intraspecific diversity is represented, improving not only the phylogenetic accuracy (Zwickl and Hillis 2002; Pollock et al. 2002; Hillis et al. 2003) but also taxonomic stability.

This study describes six novel morphospecies of *Fusarium* recovered from natural ecosystems of minimal anthropogenic disturbance throughout Australia. A bi-phasic approach of morphological description and DNA sequence analysis is adopted, consisting of a two-step phylogenetic analysis of deep and shallow node resolutions. An initial deep node phylogenetic analysis of the novel taxa is conducted using the largest and second largest subunits of RNA polymerase II (*RPB1* and *RPB2*). These loci have been used previously to determine deep level *Fusarium* phylogeny (Laurence et al. 2011; O'Donnell et al. 2007; Gräfenhan et al. 2011). Phylogenetic resolution within species complexes is determined using the translation elongation factor 1-alpha (*EF-1α*) locus. The description and investigation of these species adds to existing knowledge on the phylogeny, taxonomy, phylogeography, ecology, and species evolution in the genus *Fusarium*.

Materials and methods

Origin of isolates examined

Six novel *Fusarium* morphospecies were recovered from three independent series of surveys conducted between 2003 and 2010 in natural ecosystems of minimal anthropogenic disturbance throughout Australia. Three novel *Fusarium* morphospecies species were recovered from *Coix gasteenii, Triodia microstachya* and *Sorghum* species in Litchfield National Park, Northern Territory in the first survey series (2003–2006). Two novel *Fusarium* morphospecies were recovered from soil samples obtained from Carnarvon Gorge National Park, Queensland and Rocky Cape National Park, Tasmania in the second survey series (2006–2007); and one novel *Fusarium* morphospecies associated with *Xanthorrhoea glauca* decline in Bungonia National Park, New South Wales in the third survey series (2010). The isolation, culture purification techniques and incubation conditions used were described by Burgess et al. (1994b).

Accession of types

The ex-type cultures were deposited in the Agricultural Research Service Culture Collection, Peoria, Illinois USA (NRRL number) with replicates maintained in the culture collection at the Royal Botanic Gardens Trust, Sydney, New South Wales, Australia (RBG and FRL numbers). The holotype for each species was deposited in the culture collection at the Royal Botanic Gardens Trust, Sydney, New South Wales, Australia.

Morphological characterisation

Morphological characteristics were examined after 12 days growth of cultures initiated from a single germinated macroconidium. Cultures were grown under 12 h light–dark (l/d) cycles with UV and daylight colour fluorescent lights at 24 °C. Morphological characters examined included the shape and size of macroconidia produced in sporodochia on Carnation Leaf Agar (CLA) (Fisher et al. 1982), the shape and mode of formation of microconidia on CLA and Spezieller Nährstoffarmer Agar (SNA) (Nirenberg 1976), the production of chlamydospores on CLA, and pigmentation of the agar on Potato Dextrose Agar (PDA). Descriptions of pigmentation colour were based on the Methuen Handbook of Colour (Kornerup 1978). The dimensions of the conidia were measured using a minimum of 30 conidia of each spore type.

DNA sequencing and phylogenetic analysis

Isolates were grown on PDA for 5 days under dark incubation at 25 °C, after which the mycelium was harvested and the genomic DNA extracted using the FastDNA® Kit (Q-biogene Inc., Irvine, California, USA) according to the manufacturer's instructions.

Portions of the DNA loci were amplified using primer sets and PCR conditions described in Carbone and Kohn (1999) for *EF-1α*, O'Donnell et al. (2010) for *RPB1* and Reeb et al. (2004) for *RPB2*. All PCR were amplified in 25 μl reaction volumes containing PCR buffer (Promega Corporation,

Madison, Wisconsin, USA), 2.5 mM of $MgCl_2$ (Sigma-Aldrich Corporation, Louis, Missouri, USA), 1.25 units of GoTaq™ (Promega Corporation, Madison, Wisconsin, USA) and 0.25 mM each of dATP, dCTP, dGTP and dTTP (Promega).

The resulting PCR products were purified using ExoSAP-IT (USB Corporation Cleveland, Ohio, USA) following the manufacturer's instructions and then electrophoresed to assess product integrity and estimate concentration. The PCR amplicons were sent to the Ramaciotti Centre for Gene Function Analysis at the University of New South Wales (Randwick, NSW, Australia) for DNA sequence determination by an ABI PRISM® 3700 DNA Analyser (Applied Biosystems Inc., Foster City, California, USA), using the same primers as for the PCR amplifications for each gene region, respectively. Both the forward and reverse strands were sequenced to minimise the presence of ambiguous nucleotides. All sequences from this study have been deposited in GenBank (Table 1).

Sequences from the current study were aligned with reference sequences obtained from GenBank using the multiple alignment program ClustalW (Version 1.83) plug-in (Thompson et al. 1997) in the software Geneious (Version 5.3.6) (Drummond et al. 2011). The alignment was edited manually within the sequence alignment editing program Geneious (Version 5.3.6) (Drummond et al. 2011) and all polymorphisms were confirmed by reexamining the electropherograms. Reference sequences for each set of analyses were selected on the basis of previously published phylogenetic relationships within the genus (O'Donnell et al. 1998a, 2007; O'Donnell 2000; Schroers et al. 2004; Baayen et al. 2001). Table 1 lists all the *Fusarium* strains used in the phylogenetic analyses.

Phylogenetic analyses were performed using PAUP 4.0b10 (Swofford 2002) on the combined *RPB1* and *RPB2* data set and individual *EF-1α* data set. Unweighted Maximum Parsimony (MP), Neighbour Joining (NJ) and Bayesian Likelihood (BL) analyses were performed on all data sets. The heuristic search option with 1,000 random addition sequences and tree bisection reconnection branch swapping was used to infer maximum parsimony. Gaps were treated as missing data. The Consistency Index (CI) and Retention Index (RI) were calculated to indicate the amount of homoplasy present. NJ analyses were also conducted on individual and combined datasets with trees generated using the HKY85 model (Hasegawa et al. 1985) with ties broken randomly. Base frequencies were estimated with among-site ratios assumed to be equal and the gamma distribution was also estimated. Clade stability was assessed in PAUP 4.0b10 (Swofford 2002) using 1,000 heuristic search bootstrap replications with random sequence addition. Trees were rooted using the outgroup method. Bayesian inference was used to estimate posterior probabilities for consensus nodes using MrBayes 3.1 (Huelsenbeck 2001) run with a 4,000,000-generation Monte Carlo Markov chain method with a burnin of 10,000 trees. JModeltest (Posada and Crandall 1998) was used to determine the most appropriate model for Bayesian analysis of the combined *RPB1* and *RPB2* and individual *EF-1α* data sets. Trees were visualised using FigTree v1.4 (Rambaut 2013). Appropriate outgroups for the intra-species complex *EF-1α* analyses were determined using the results from the *RPB1* and *RPB2* phylogenetic analyses.

Results

Phylogenetic analyses

Phylogenetic analyses of the *RPB1* and *RPB2* resolved the phylogenetic positions of the six novel morphospecies in relation to the 15 currently recognised monophyletic species complexes in the genus *Fusarium* (Fig. 1). No major topological variations were detected between trees derived from MP, NJ and BL phylogenetic inferences (data not shown). The *RPB1* and *RPB2* data set consisted of 3074 nucleotides, of which 1230 were parsimony-informative (PIC). The MP analysis yielded 7 equally most-parsimonious trees (CI=0.28, RI=0.74) (Fig. 1).

The *RPB1* and *RPB2* phylogenetic analyses placed five of the novel *Fusarium* morphospecies in three *Fusarium* species complexes: the F. fujikuroi Species Complex (FFSC), F. chlamydosporum Species Complex (FCSC) and F. sambucinum Species Complex (FSAMSC) (Fig. 1). *Fusarium newnesense* sp. nov. did not cluster within any of the currently recognised complexes but formed an independent lineage closely related to the F. oxysporum Species Complex (FOSC), FFSC and F. nisikadoi Species Complex (FNSC).

The *RPB1* and *RPB2* phylogeny was used to select appropriate outgroup taxa for species level resolution using the *EF-1α* locus. It was determined that *F. equiseti* (NRRL20697), *F. lacertarum* (NRRL20423) and *Fusarium* sp. (NRRL5537) were appropriate outgroup taxa for resolving the phylogeny of *F. goolgardi* and *F. tjaynera* (Fig. 2). Reference taxa for separating these two novel species from close relatives were selected from the FCSC and FSAMSC. The *EF-1α* analyses did not result in any major topological variations between trees derived from MP, NJ and BL phylogenetic inferences (data not shown). The FCSC/FSAMSC *EF-1α* data set consisted of 557 nucleotides, of which 160 were PICs. The MP analysis yielded six equally most-parsimonious trees (CI=0.64, RI=0.84) (Fig. 2).

Fusarium goolgardi clustered in the FSAMSC and was closely related to the described species *F. langsethiae*, *F. palustre*, *F. sporotrichoides* and the undescribed *Fusarium* species NRRL36351. *Fusarium tjaynera* formed a sister relationship to *F. aywerte* (Figs. 1 and 2). Although the closest

Table 1 *Fusarium* species used in the current study

Species	Isolate	GenBank accession numbers		
		EF-1α	*RPB1*	*RPB2*
Fusarium acutatum	NRRL 13308	AF160276	N/A	N/A
F. ambrosium	NRRL20438	N/A	JX171470	JX171584
F. anguioides	NRRL25385	N/A	JX171511	JX171624
F. anthophilum	NRRL13602	AF160292	N/A	N/A
F. armeniacum	NRRL6227	N/A	JX171446	JX171560
F. asiaticum	NRRL13818	N/A	JX171459	JX171573
F. aywerte	NRRL25410	N/A	JX171513	JX171626
F. aywerte	RBG5743	KP083250	KP083273	KP083278
F. aywerte	RBG5736	KP083248	N/A	N/A
F. aywerte	RBG5741	KP083249	N/A	N/A
F. babinda	NRRL25539	N/A	JX171519	JX171632
F. bactridioides	NRRL20476	AF160290	N/A	N/A
F. begoniae	NRRL25300	AF160293	N/A	N/A
F. beomiforme	NRRL25174	N/A	JX171506	JX171619
F. brevicatenulatum	NRRL25446	AF160265	N/A	N/A
F. buharicum	NRRL13371	N/A	JX171449	JX171563
F. bulbicola	NRRL13618	KF466415	N/A	N/A
F. burgessii	RBG5319	N/A	XXXXX	HQ646392
F. cerealis	TUR057[a]	JN541063	N/A	N/A
F. circinatum	NRRL25331	N/A	JX171510	JX171623
F. coicis sp. nov.	RBG5368/NRRL66233	KP083251	KP083269	KP083274
F. coicis sp. nov.	RBG5369/NRRL66234	KP083252	N/A	N/A
F. commune	NRRL22903	AF008513	N/A	N/A
F. commune	NRRL28387	AF246832	JX171525	JX171638
F. concentricum	NRRL25181	AF160282	N/A	N/A
F. concolor	NRRL13459	N/A	JX171455	JX171569
F. culmorum	NRRL25475	N/A	JX171515	JX171628
F. culmorum	VI01002	AJ543541	N/A	N/A
F. denticulatum	NRRL25302	AF160569	N/A	N/A
F. dimerum	NRRL20691	N/A	JX171478	JX171592
F. dlaminii	NRRL13164	AF160277	N/A	N/A
F. equiseti	NRRL13402	N/A	JX171452	JX171566
F. equiseti	NRRL20697	N/A	JX171481	JX171595
F. falciforme	NRRL43529	N/A	JX171541	JX171653
F. flocciferum	NRRL25473	N/A	JX171514	JX171627
F. foetens	NRRL38302	N/A	JX171540	JX171652
F. foetens	NRRL31852	AY320087	N/A	N/A
F. foetens	NRRL52749	JF740825	N/A	N/A
F. fujikuroi	NRRL13566	N/A	JX171456	JX171570
F. gaditjirri	NRRL45417	N/A	JX171542	JX171654
F. globosum	NRRL26131	AF160285	N/A	N/A
F. goolgardi sp. nov.	RBG5412/NRRL66248	KP083253	N/A	N/A
F. goolgardi sp. nov.	RBG5418/NRRL66249	KP083254	N/A	N/A
F. goolgardi sp. nov.	RBG5411/NRRL66250	KP101123	KP083270	KP083280
F. graminearum	NRRL31084	N/A	JX171531	JX171644
F. guttiforme	NRRL22945	N/A	JX171505	JX171618
F. heterosporum	NRRL20693	N/A	JX171480	JX171594

Table 1 (continued)

Species	Isolate	GenBank accession numbers		
		EF-1α	*RPB1*	*RPB2*
F. hostae	NRRL29889	N/A	JX171527	JX171640
F. hostae	FRCO-2071	AF331817	N/A	N/A
F. oxysporum	NRRL20433	N/A	JX171469	JX171583
F. kyushuense	VI01325	AJ427274	N/A	N/A
F. lacertarum	NRRL20423	N/A	JX171567	JX171581
F. langsethiae	NRRL54940	N/A	JX171550	JX171662
F. langsethiae	VI01280	AJ427272	N/A	N/A
F. lateritium	NRRL13622	N/A	JX171457	JX171571
F. longipes	NRRL13368	N/A	JX171448	JX171562
F. longipes	NRRL13374	N/A	JX171450	JX171564
F. longipes	NRRL20723	N/A	JX171483	JX171596
F. lunatum	NRRL36168	N/A	JX171536	JX171648
F. lyarnte	NRRL54252	N/A	JX171549	JX171661
F. mangiferae	NRRL25226	N/A	JX171509	JX171622
F. miscanthi	NRRL26231	N/A	JX171521	JX171634
F. mundagurra sp. nov.	RBG5717/NRRL66235	KP083256	KP083272	KP083276
F. mundagurra sp. nov.	RBG5599/NRRL66236	KP083255	N/A	N/A
F. napiforme	NRRL13604	AF160266	N/A	N/A
F. nelsonii	NRRL13338	GQ505402	JX171447	GQ505466
F. newnesense sp. nov.	RBG5443/NRRL66237	KJ397074	KP083271	KP083277
F. newnesense sp. nov.	RBG5444/NRRL66238	KP083257	N/A	N/A
F. newnesense sp. nov.	RBG5445/NRRL66239	KP083258	N/A	N/A
F. newnesense sp. nov.	RBG5446/NRRL66240	KP083259	N/A	N/A
F. newnesense sp. nov.	RBG610/NRRL66241	KP083261	N/A	N/A
F. newnesense sp. nov.	RBG6847	KP083262	N/A	N/A
F. newnesense sp. nov.	RBG609/NRRL66242	KP083260	N/A	N/A
F. nisikadoi	NRRL25179	N/A	JX171507	JX171620
F. nurragi	NRRL36452	N/A	JX171538	JX171650
F. nygamai	NRRL13488	AF160273	N/A	N/A
F. oxysporum	NRRL25387	N/A	JX171512	JX171625
F. oxysporum	NRRL34936	N/A	JX171533	JX171646
F. oxysporum	NRRL22902	AF160312	N/A	N/A
F. palustre	NRRL54054	GQ856949	N/A	N/A
F. phaseoli	NRRL22276	N/A	JX171495	JX171608
F. phyllophilum	NRRL13617	AF160274	N/A	N/A
F. poae	NRRL13714	N/A	JX171458	JX171572
F. poae	VI01265	AJ420839	N/A	N/A
F. proliferatum	NRRL22944	N/A	JX171504	JX171617
F. pseudocircinatum	NRRL22946	AF160271	N/A	N/A
F. pseudograminearum	NRRL28062	N/A	JX171524	JX171637
F. pseudograminearum	CS5791[a]	JN541056	N/A	N/A
F. pseudograminearum	RBG3580	HQ667168	N/A	N/A
F. pseudonygamai	NRRL13592	AF160263	N/A	N/A
F. ramigenum	NRRL25208	KF466423	N/A	N/A
F. redolens	NRRL22901	N/A	JX171503	JX171616
F. redolens	FRCO-681	AF331816	N/A	N/A
F. sacchari	NRRL13999	N/A	JX171466	JX171580

Table 1 (continued)

Species	Isolate	GenBank accession numbers		
		EF-1α	*RPB1*	*RPB2*
F. sambucinum	NRRL22187	N/A	JX171493	JX171606
F. sarcochroum	NRRL20472	N/A	JX171472	JX171586
F. sibiricum	NRRL53429	HM744683	N/A	HQ154471
F. solani f.sp. pisi	NRRL45880	N/A	JX171543	JX171655
F. sporotrichioides	NRRL3299	N/A	JX171444	JX171558
F. sporotrichioides	VI1313	AJ420818	N/A	N/A
F. stilbioides	NRRL20429	N/A	JX171468	JX171582
F. subglutinans	NRRL22016	N/A	JX171486	JX171599
F. subglutinans	NRRL22016	AF160289	N/A	N/A
F. sublunatum	NRRL13384	N/A	JX171451	JX171565
F. succisae	NRRL13613	AF160291	N/A	N/A
F. thapsinum	NRRL22045	N/A	JX171487	JX171600
F. tjaetaba sp. nov.	RBG5361/NRRL66243	KP083263	KP083267	KP083275
F. tjaetaba sp. nov.	RBG5363/NRRL66244	KP083264	N/A	N/A
F. tjaetaba sp. nov.	RBG5364/NRRL66245	KP083265	XXXX	XXXX
F. tjaynera sp. nov.	RBG5367/NRRL66246	EF107152	KP083268	KP083279
F. tjaynera sp. nov.	FRL19318	EF107150	N/A	N/A
F. tjaynera sp. nov.	RBG5366/NRRL66247	KP083266	N/A	N/A
F. tjaynera sp. nov.	FRL19315	EF107151	N/A	N/A
F. tjaynera sp. nov.	FRL11240	EF107155	N/A	N/A
F. torulosum	NRRL22748	N/A	JX171502	JX171615
F. tricinctum	NRRL25481	N/A	JX171516	JX171629
F. udum	NRRL22949	AF160275	N/A	N/A
F. venenatum	NRRL22196	N/A	JX171494	JX171607
F. verticillioides	NRRL20956	N/A	JX171485	JX171598
F. virguliforme	NRRL31041	N/A	JX171530	JX171643
F. werrikimbe	FRL19361	EF107132	N/A	N/A
F. xylarioides	NRRL25486	N/A	JX171517	JX171630
F.sibiricum	NRRL53430	HM744684	N/A	HQ154472
Fusarium cf. compactum	NRRL13829	N/A	JX171460	JX171574
Fusarium lactis	NRRL25200	AF160272	N/A	N/A
Fusarium sp.	NRRL22436	N/A	JX171497	JX171610
Fusarium sp.	NRRL22632	N/A	JX171501	JX171614
Fusarium sp.	NRRL13444	N/A	JX171454	JX171568
Fusarium sp.	NRRL28578	N/A	JX171526	JX171639
Fusarium sp.	NRRL13338	N/A	JX171447	JX171561
Fusarium sp.	NRRL31008	N/A	JX171529	JX171642
Fusarium sp.	NRRL26417	N/A	JX171522	JX171635
Fusarium sp.	NRRL32175	N/A	JX171532	JX171645
Fusarium sp.	NRRL52700	N/A	JX171544	JX171656
Fusarium sp.	NRRL25184	AF008514	JX171508	JX171621
Fusarium sp.	RBG5116	N/A	XXXXX	HQ646395
Fusarium sp.	NRRL25533	N/A	JX171518	JX171631
Fusarium sp.	NRRL22566	N/A	JX171500	JX171613
Fusarium sp.	NRRL54149	N/A	JX171548	JX171660
Fusarium sp.	NRRL36351	GQ915500	N/A	N/A
Fusarium sp.	NRRL46670	GU250579	N/A	GU250693

Table 1 (continued)

Species	Isolate	GenBank accession numbers		
		EF-1α	*RPB1*	*RPB2*
Fusarium sp.	NRRL5537	GQ505588	N/A	EF470111
Fusarium sp.	CML 908	EU574681	N/A	N/A
Fusarium sp.	NRRL25204	AF160299	N/A	N/A
Fusarium sp.	CML 914	EU574682	N/A	N/A
Fusarium sp.	NRRL29123	AF160310	N/A	N/A
Fusarium sp.	NRRL29124	AF160311	N/A	N/A
Fusarium sp.	NRRL25195	AF160298	N/A	N/A
Fusarium sp.	NRRL25623	AF160300	N/A	N/A
Fusarium sp.	NRRL25807	AF160305	N/A	N/A
Fusarium sp.	NRRL26756	AF160307	N/A	N/A
Fusarium sp.	NRRL26757	AF160308	N/A	N/A
Fusarium sp.	NRRL25346	AF160296	N/A	N/A
Fusarium sp.	NRRL25622	AF160301	N/A	N/A
Fusarium sp.	NRRL25615	AF160304	N/A	N/A
Fusarium sp.	NRRL26793	AF160309	N/A	N/A
Fusarium sp.	NRRL25303	AF160283	N/A	N/A
Fusarium sp.	NRRL25309	AF160284	N/A	N/A
Fusarium sp.	NRRL26427	AF160286	N/A	N/A
Fusarium sp.	NRRL26061	AF160303	N/A	N/A
Fusarium sp.	NRRL26152	AF160306	N/A	N/A
Fusarium sp.	NRRL26794	AF160287	N/A	N/A
Fusarium sp.	NRRL28852	AF160288	N/A	N/A
Fusarium sp.	NRRL25221	AF160268	N/A	N/A
Fusarium sp.	NRRL26064	AF160302	N/A	N/A

NRRL Agricultural Research Service Culture Collection, Peoria, Illinois USA, *FRL* University of Sydney, Sydney, New South Wales, Australia, *RBG* Royal Botanic Gardens Trust, Sydney, New South Wales, Australia, *VI* National Veterinary Institute, Norway, *CML* Coleção Micológica de Lavras, Brasil, *FRC* Fusarium Research Center, Penn State University, Pennsylvania, USA, *N/A* The sequence is either not available or not applicable to current study

[a] Unknown culture collection

relatives to *F. aywerte* and *F. tjaynera* were in the FCSC there were high levels of divergence (170 PICs from the combined RPB1 and RPP2 data set) suggesting that *F. aywerte* and *F. tjaynera* should be considered a separate monophyletic species complex/lineage, hereby designated Fusarium aywerte Species Complex (FASC).

Taxa in the Fusarium redolens Species Complex (FRSC) were determined to be appropriate outgroups for resolving the phylogeny of *F. coicis*, *F. mundagurra*, *F. newnesense* and *F. tjaetaba* (Fig. 3). Reference taxa for separating *F. coicis*, *F. mundagurra*, *F. newnesense* and *F. tjaetaba* were selected from the FFSC and FOSC. The FOSC/FFSC *EF-1α* data set consisted of 585 nucleotides, of which 142 were PICs. The MP analysis yielded over 5,000 equally most-parsimonious trees (CI=0.64, RI=0.85) (Fig. 1).

Fusarium coicis, *F. mundagurra* and *F. tjaetaba* clustered within the African Clade of the FFSC (O'Donnell et al. 1998a) (Fig. 1). *Fusarium coicis* was closely related to the undescribed species NRRL25615 (origin: Nigeria) and NRRL26793 (origin: Sudan), differing by 12 and 18 *EF-1α* PICs, respectively. *Fusarium mundagurra* was closely related to the undescribed species NRRL25221 (origin: Zimbabwe) and *F. sacchari*, differing by 6 and 29 *EF-1α* PICs, respectively. *Fusarium tjaetaba* was closely related to *F. brevicatenulatum* NRRL25446 (origin: Madagascar) differing by 19 *EF-1α* PICs.

Fusarium newnesense did not cluster within the established *Fusarium* species complexes and formed an independent lineage that was basal to the FOSC, FFSC and near *F. commune*. The *F. newnesense* lineage resolved into two phylogenetic species separated by 3 *EF-1α* mutations and

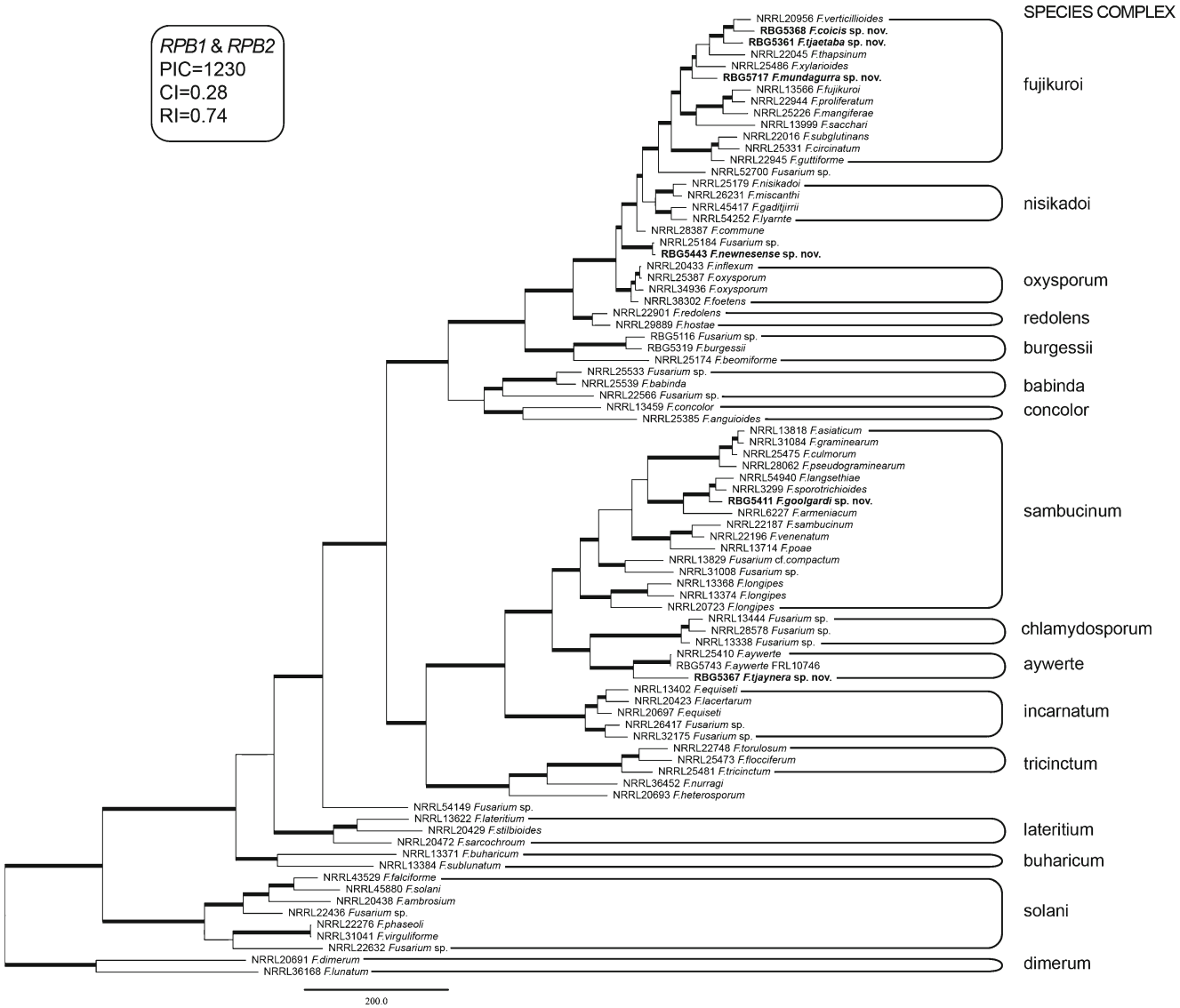


Fig. 1 One of 7 most-parsimonious trees inferred from the combined *RPB1* and *RPB2* data set indicating the phylogenetic relationship between the novel species *Fusarium coicis*, *F. goolgardi*, *F. mundagurra*, *F. newnesense*, *F. tjaetaba* and *F. tjaynera* and the major species complexes in the Fusarium genus. Branches with most parsimonious bootstrap partitions >70 % and Bayesian posterior probabilities >0.95 are indicated in bold. The number of Parsimony-Informative Characters (PIC), Retention Index (RI) and Consistency Index (CI) are indicated

correlated to geographic origin. The closest relative was the undescribed species NRRL25184 (origin: Germany) and was separated by 3 *EF-1α* PICs.

Taxonomy

Fusarium coicis R.M. Johanssen, J.L. Walsh, M.H. Laurence, L.W. Burgess, E.C.Y. Liew & T. Petrovic**, sp. nov. -**Figs. 4–8 MB812304

Etymology: Refers to the host, *Coix gasteenii,* from which this species was isolated.

Description Colonies on PDA with powdery to floccose aerial mycelium, white to pale pink in colour, darkening with age. Pigment on reverse side is greyish-rose to dark violet. *Sporodochia* small, cream in colour, forming on carnation leaf pieces on CLA, difficult to observe in some isolates. *Macroconidia* very long, slender, parallel dorso-ventral sides, tapering apical cells and barely notched basal cells, 4–10 septate, usually 7 septate, 55–123 μm ($\bar{x}$ = 85 μm) long×4–6 μm ($\bar{x}$ =5 μm) wide. *Microconidia* forming in false heads and short to long chains, arising from monophialides and occasionally polyphialides, oval to clavate in shape, 8–23 μm ($\bar{x}$ =14 μm) long, 0–3 septate. *Chlamydospores* absent.

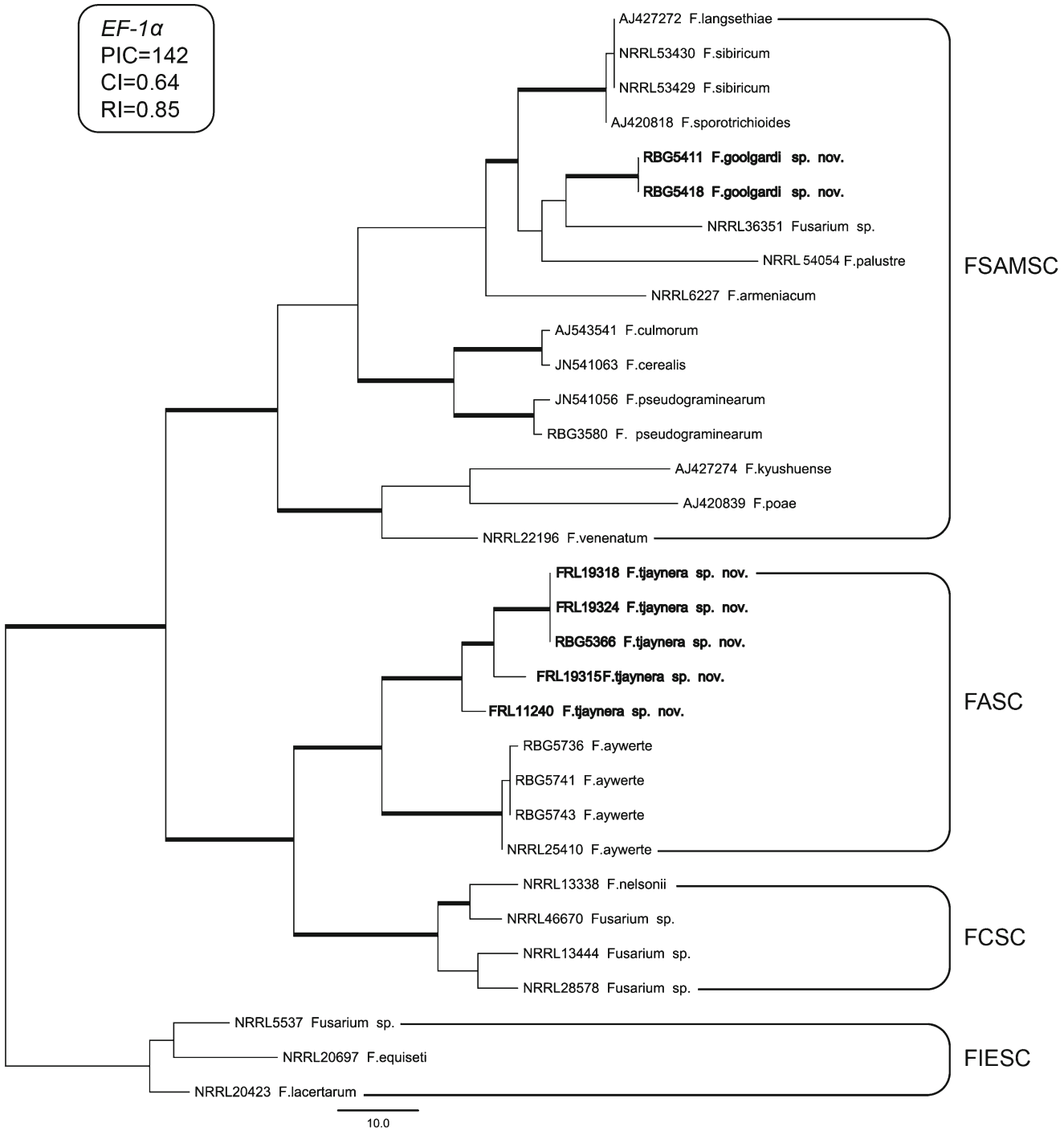


Fig. 2 One of six most-parsimonious trees inferred from the *EF-1α* data set indicating species resolution of *Fusarium goolgardi* sp. nov. and *F. tjaynera* sp. nov. Branches with most parsimonious bootstrap partitions >70% and Bayesian posterior probabilities >0.95 are indicated in bold. The number of Parsimony-Informative Characters (PIC), Retention Index (RI) and Consistency Index (CI) are indicated.

Specimen examined Type Strain (living and dried) NRRL66233, RBG5368, FRL19329 and additional strains FRL19328, FRL19333, FRL19335, FRL19336 isolated from *Coix gasteenii*, Mareeba, Queensland; 16°59′S, 145°19′E 10/06/2005. Additional strains FRL19330, FRL19331, FRL19332, FRL19334, FRL19337 isolated from *Coix gasteenii* Lakefield National Park, Queensland; 14°36′S, 143°56′E.

Fusarium goolgardi D.M. Robinson, M.H. Laurence, E.C.Y. Liew & B.A. Summerell **sp. nov.**-Figs. 9–18, MB812305


EF-1α
PIC=160
CI=0.64
RI=0.84
EU574681 Fusarium sp. strain CML908
NRRL25204 Fusarium sp.
NRRL25300 F.begoniae
EU574682 Fusarium sp. strain CML914
NRRL29123 Fusarium sp.
NRRL29124 Fusarium sp.
NRRL25331 F.circinatum
NRRL22945 F.guttiforme
FRL19361 F.werrikimbe
NRRL13618 F.bulbicola
NRRL20476 F.bactridioides
NRRL13602 F.anthophilum
NRRL13613 F.succisae
NRRL25195 Fusarium sp.
NRRL25623 Fusarium sp.
NRRL25807 Fusarium sp.
NRRL22016 F.subglutinans
NRRL26756 Fusarium sp.
NRRL26757 Fusarium sp.
NRRL25346 Fusarium sp.
NRRL25622 Fusarium sp.
NRRL25302 F.denticulatum
RBG5361 F.tjaetaba sp. nov.
RBG5363 F.tjaetaba sp. nov.
RBG5364 F.tjaetaba sp. nov.
NRRL25446 F.brevicatenulatum
NRRL13592 F.pseudonygamai
NRRL13604 F.napiforme
NRRL25208 F.ramigenum
RBG5368 F.coicis sp. nov.
RBG5369 F.coicis sp. nov.
NRRL25615 Fusarium sp.
NRRL26793 Fusarium sp.
NRRL25200 F.lactis
NRRL22946 F.pseudocircinatum
NRRL13488 F.nygamai
NRRL22045 F.thapsinum
NRRL25303 Fusarium sp.
NRRL25309 Fusarium sp.
NRRL25181 F.concentricum
NRRL25226 F. mangiferae
NRRL26427 Fusarium sp.
NRRL26061 Fusarium sp.
NRRL26152 Fusarium sp.
NRRL13164 F.dlaminii
NRRL22944 F.proliferatum
NRRL26131 F.globosum
NRRL13566 F.fujikuroi
NRRL26794 Fusarium sp.
NRRL28852 Fusarium sp.
RBG5599 F.mundagurra sp. nov.
RBG5717 F.mundagurra sp. nov.
NRRL25221 Fusarium sp.
NRRL13999 F.sacchari
NRRL13617 F.phyllophilum
NRRL22949 F.udum
NRRL26064 Fusarium sp.
NRRL13308 F.acutatum
NRRL31852 F.foetens
NRRL52749 F.foetens
NRRL22902 F.oxysporum
RBG5444 F.newnesense sp. nov.
RBG5445 F.newnesense sp. nov.
RBG5446 F.newnesense sp. nov.
RBG610 F.newnesense sp. nov.
RBG6847 F.newnesense sp. nov.
RBG609 F.newnesense sp. nov.
NRRL25184 Fusarium sp.
NRRL22903 F.commune
NRRL28387 F.commune
AF331816 F.redolens
AF331817 F.hostae
FFSC
FOSC
FRSC
7.0

◀ **Fig. 3** One of ≥5,000 most-parsimonious trees inferred from the *EF-1α* data set indicating species resolution of *Fusarium coicis* sp. nov., *Fusarium mundagurra* sp. nov., *F. newnesense* sp. nov., and *F. tjaetaba* sp. nov.. Branches with most parsimonious bootstrap partitions >70 % and Bayesian posterior probabilities >0.95 are indicated in bold. The number of Parsimony-Informative Characters (PIC), Retention Index (RI) and Consistency Index (CI) are indicated

Etymology: Goolgardi is the Australian Aboriginal Dharug name for *Xanthorrhoea*, the genus from which this fungus was isolated.

Description Colonies on PDA powdery, velvety to floccose with abundant dense aerial mycelium, orange white, pale orange, greyish orange, brownish orange in colour. Pigment on reverse side brown, brownish-orange, reddish-orange, greyish-orange, yellowish-brown. *Sporodochia* pale orange to orange white forming on carnation leaf pieces in CLA and on agar surface. *Macroconidia* long, slender, slightly curved, parallel dorso-ventral sides, hooked apical cells, distinctly notched basal cells, 3 to 5 septate, mostly 3 septate, 23–66 μm ($\bar{x}$ =40 μm) long×3–11 μm ($\bar{x}$ = 5 μm) wide. *Microconidia* produced on monophialides and polyphialides proliferating sympodially creating a zig-zag or spiral appearance. Microconidia straight or curved, oval 0–3 septate, mainly 1 septate, 10-31 μm ($\bar{x}$ =17 μm) long×3–5 μm ($\bar{x}$ =4 μm) wide. *Chlamydospores* produced sporadically in most cultures after 2 weeks in the dark at 23 °C on CLA, hyaline, smooth walled, formed in chains, terminal and intercalary, globose, subglobose, cylindrical to subcylindrical, 3–18 μm ($\bar{x}$ = 8 μm) long×4–10 μm ($\bar{x}$ =7 μm) wide.

Specimen examined Type Strain (living and dried) NRRL66250, RBG5411 and additional strains RBG5412 and RBG5418 isolated from *Xanthorrhoea glauca* Bungonia State Conservation Area, NSW, Australia, 34°48′S 150° 0′E on January 2010 by D.M Robinson and M.H. Laurence.

Fusarium mundagurra M.H. Laurence, E.C.Y. Liew, L.A. Shuttleworth & L.W. Burgess **sp. nov.-**

Figs 19–26, MB812306

Etymology: Mundagurra is named for the rainbow serpent that created Carnarvon Gorge in the Australian Aboriginal Dreamtime. Carnarvon Gorge is the geographic origin of the isolate first recognised as belonging to this species.

Description Colonies on PDA incubated in the dark for 2 weeks at 23 °C. Mycelia abundant, floccose, velvety to powdery, sometimes touching lid of agar plate, powdery pale violet, greyish-violet, greyish-blue, dull blue, greyish magenta and white. Reverse greyish blue, dark blue, blackish blue and greyish yellow. *Sporodochia* white, formed extensively on carnation leaf pieces of CLA. *Macroconidia* long, straight to falcate, dorso-ventral sides, blunt apical cells, foot-shaped basal cells, hyaline, 3 to 5 septate, mostly 3 septate, 20.5-51.5 μm ($\bar{x}$ =31.2 μm) long×3.2-5.2 μm ($\bar{x}$ =4 μm) wide. *Microconidia* formed abundantly on long and short monophialides and polyphialides, hyaline, 4.2-68.7 μm ($\bar{x}$ =23 μm) long, microconidia formed abundantly in sliding chains 2–20 ($\bar{x}$ =10) in number, and less abundantly in false heads. Microconidia oval to obovate, 0 to 1 septate, 6.2-18.9 μm ($\bar{x}$ =11 μm) long×2.7-4 μm ($\bar{x}$ =3.2 μm) wide. Terminal microconidium on sliding chains often larger than the sub-terminal microconidia in the chain. *Chlamydospores* formed abundantly under 12 h l/d cycles within 2 weeks at 23 °C, hyaline, smooth walled, formed in chains, terminal and intercalary, globose, subglobose, cylindrical to subcylindrical, 3.7-17.2 μm ($\bar{x}$ =9.3 μm) long×4.9-16.6 μm ($\bar{x}$ =8.7 μm) wide.

Specimen examined Type Strain (living and dried) NRRL66235, RBG5717 isolated from soil Carnarvon Gorge National Park, Queensland, Australia, 25° 02'S 148° 11'E on October 2008 by S.M. Dunstan and M.H. Laurence. Additional strains RBG5599 from

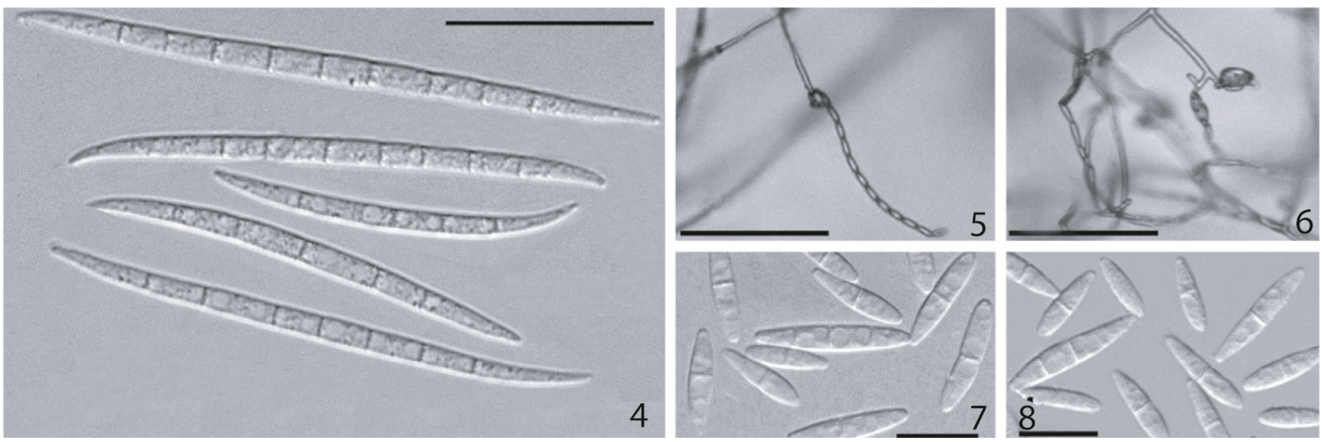
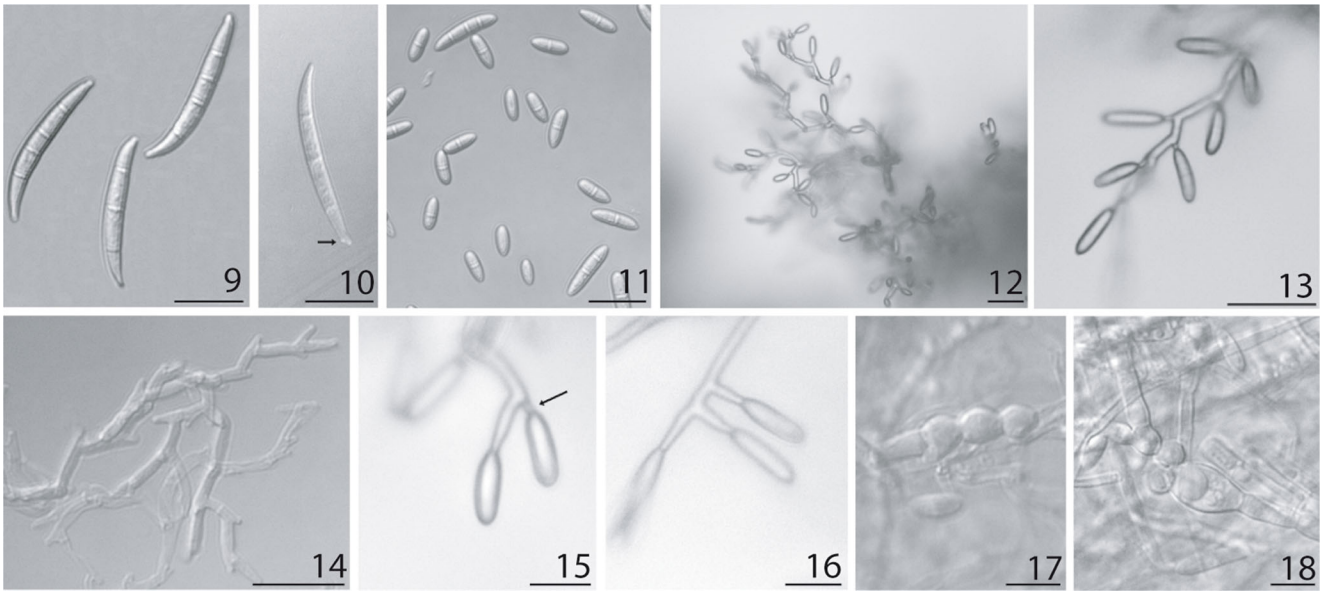


Fig. 4–8 Morphological characters of *F. coicis*. **4.** Macroconidia. **5-6.** Microconidia produced in false heads and chains on SNA. **7-8.** Microconidia. Bars: 20 μm



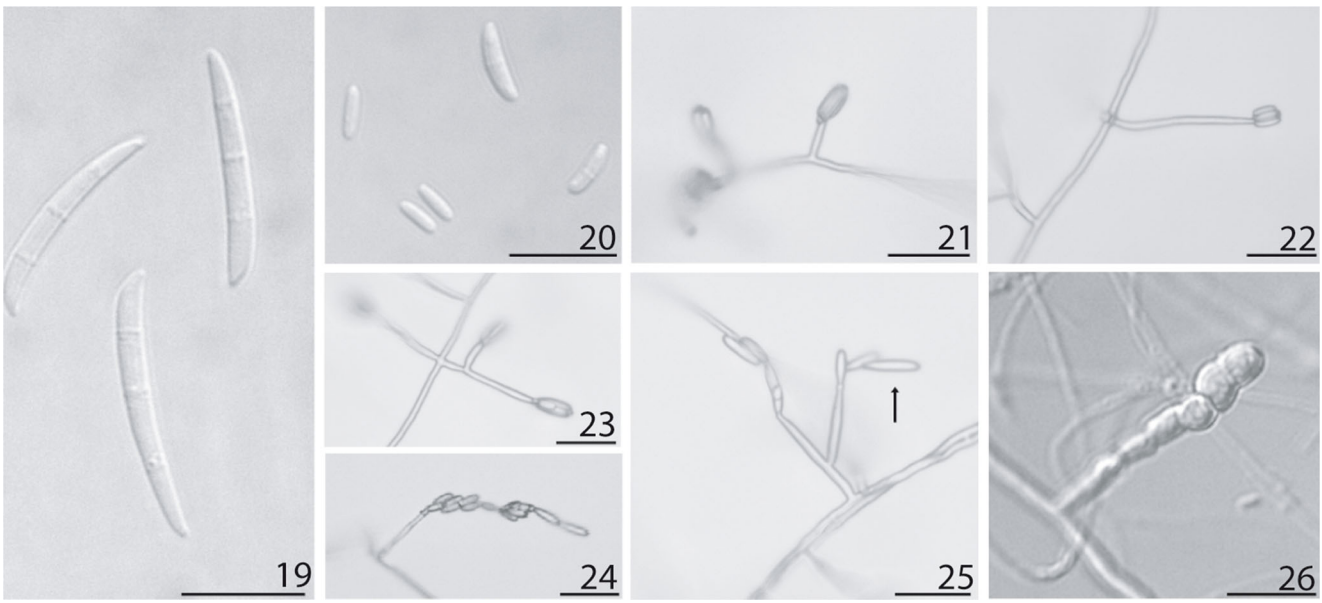
Figs. 9–18 Morphological characters of *F. goolgardi*. **9-10.** Macroconidia (arrow in Fig. 10 indicates basal notch). **11.** Microconidia, aseptate and with 1, 2 and 3 septa. **12-14.** Sympodial hyphal branching (Figs. 12-13 with attached microconidia, Fig. 14 without microconidia). **15.** Monophialide with attached microconidium (arrow indicates monophialide). **16.** Polyphialide with attached microconidia. **17-18.** Chlamydospores. Bars: Figs. 9-12, 14= 20 μm, Figs. 13, 15-18 = 10 μm

Northern Territory, Australia and RBG94 Queensland, Australia.

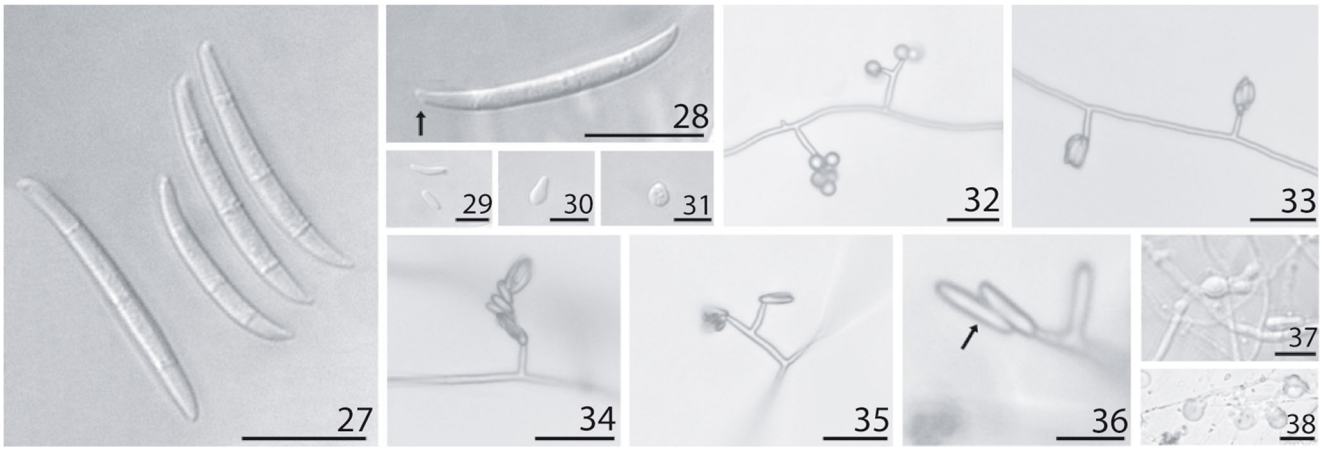
Fusarium newnesense M.H. Laurence & B.A. Summerell **sp. nov. -**Figs. 27–38, MB812307

Etymology: Refers to Newnes Plateau State Forest, the location where this species was first collected.

Description Colonies on PDA in dark for 2 weeks, 23 °C. Abundant velvety, floccose to powdery aerial mycelium, top surface violet white, yellowish white to white in colour, reverse beaver, blonde, coffee, dull yellow and olive brown. *Macroconidia*, slender, straight to falcate, slightly hooked apical cells and foot shaped basal cells, 2–4 septate, mostly 3 septate, 24.9-54 μm ($\bar{x}$ =41.2 μm) long×3.1-5.5 μm ($\bar{x}$ = 4.5 μm) wide. *Sporodochia* when produced are orange. *Microconidia*, two forms, pyriform to napiform and oval. Pyriform to napiform aseptate, 5.2-12.2 μm ($\bar{x}$ =7.6 μm) long×



Figs. 19–26 Morphological characters of *Fusarium mundagurra*. **19.** Macroconidia. **20-25.** Microconidia. **21.** Produced in false heads on short monophialide. **22.** Produced in false heads on long monophialide. **23.** Produced in false heads on polyphialide. **24.** Produced in sliding chains on monophialide. **25.** Produced in sliding chain on polyphialide (arrow indicates the larger terminal microconidium). **26.** Chlamydospores. All bars: 20 μm



Figs. 27–38 Morphological characters of *F. newnesense*. **27-28.** Macroconidia (arrow in Fig. 28 shows basal notch). **29-31.** Microconidia. **29.** Oval to obovate, straight and curved. **30.** Pyriform. **31.** Napiform. **32.** Pyriform and napiform microconidia on monophialide and polyphialide. **33.** Oval to obovoid microconidia in false heads. **34.** Oval to obovoid microconidia in disordered chains and false heads. **33.** On short monophialide. **35.** On long polyphialide. **36.** Microconidia on sliding/disordered chain. **37-38.** Chlamydospores. Bars: Figs. 27-28, 32-35 =20 μm, Figs. 29-31, 37-38 =10 μm

3.2-8.7 μm wide ($\bar{x}$ =5.5 μm) arising terminally on long and short monophialides and polyphialides, oval straight or curved 2.6-24.7 μm ($\bar{x}$ =12.2 μm) long×2.7-14.7 μm wide ($\bar{x}$ = 4 μm), aseptate to 3 septate, mostly aseptate, arising in disordered chains 1–17 in number ($\bar{x}$ =6) or in false heads, on the end of long and short monophialides and polyphialides 1.7-46.4 μm ($\bar{x}$ =16.5 μm) long. *Chlamydospores* formed abundantly in isolates RBG610 and seldom in RBG614 and RBG5444 within 2 weeks at 23 °C under 12 h l/d cycles, hyaline, smooth walled, single, in pairs and in short chains, mostly single, terminal and intercalary, globose, subglobose, cylindrical to subcylindrical, 4.4-18.1 μm ($\bar{x}$ =9.9 μm) long× 4.5-15.2 μm ($\bar{x}$ =8.4 μm) wide.

Specimen examined Type Strain (living and dried) NRRL66241, RBG610 and additional strains RBG614 from soil Newnes State Forest, New South Wales, Australia 33°20′ S, 150°15′E, March 1992 by B.A. Summerell. Additional strains RBG5444 from soil Rocky Cape National Park, Tasmania, Australia 40° 52′S 145° 31′E on March 2009 by B.H. Laurence and M.H. Laurence and RBG6847 from soil Grampians National Park, Victoria, Australia 37° 28′S 142° 25′E on May 2007 by E.L. Laurence and M.H. Laurence.

Fusarium tjaetaba T.T.H. Vu, J.L. Walsh, M.H. Laurence, L.W. Burgess, E.C.Y. Liew, & B.A. Summerell**, sp. nov. --** Figs. 39–43, MB812308

Etymology: Refers to Tjaetaba Falls, Litchfield National Park, the geographic origin of this species.

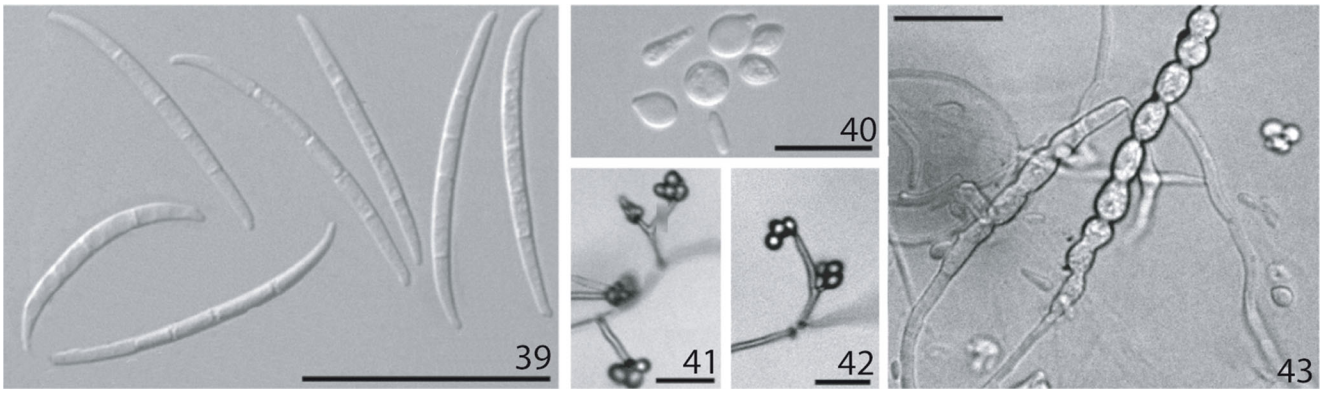
Description Colonies on PDA with powdery aerial mycelium, pale pink to orange in colour. Pigment on reverse side pale orange to violet or dark blue. *Sporodochia* orange in colour, forming on CLA within 10 d on carnation leaf pieces and occasionally on the agar surface. *Macroconidia* long, slender, falcate, parallel dorso-ventral sides, hooked apical cells and distinctly notched basal cells, 3–7 septate, mostly 4 septate, 40–60 μm ($\bar{x}$ =50 μm) long×3–4 μm ($\bar{x}$ =3 μm) wide. *Microconidia* forming abundantly in false heads and sometimes in short chains, arising from monophialides and polyphialides, pyriform 5–19 μm ($\bar{x}$ =9 μm) long, 0–1 septate or oval 5–25 μm ($\bar{x}$ =10 μm) long, 0–1 septate in shape. *Chlamydospores* formed within 11 d on SNA in some isolates, varying in abundance in different isolates, produced in chains on aerial hyphae, terminal or intercalary, round 4–7.9 μm ($\bar{x}$ = 6.2 μm) diameter to cylindrical 6.8 –12.9 μm ($\bar{x}$ =9.5 μm)× 4.2-7.4 μm ($\bar{x}$ =4.8 μm), smooth walled, and hyaline.

Specimens examined Type Strain (living and dried) NRRL66243, RBG5361 and FRL14350, additional strains FRL14348, FRL 14349, FRL14351, FRL14352, FRL14354, FRL14355, FRL14356, FRL14357 and FRL14358 isolated from *Sorghum interjectum*; Litchfield National Park, Northern Territory, Australia; 13°14′S, 130°42′E in July 2009 by J.L. Walsh.

Fusarium tjaynera J.L. Walsh, M.H. Laurence, L.W. Burgess, E.C.Y. Liew, & B.A. Summerell**, sp. nov. -**Figs. 44–48, MB812309

Etymology: Refers to Tjaynera Falls, Litchfield National Park, the geographic origin of isolates first recognised as belonging to this species.

Description Colonies on PDA with abundant dense aerial mycelium, white to greyish-rose in colour becoming darker as the culture ages. Pigment on reverse side is red to burgundy, sometimes concentric. Orange sporodochial masses are produced in most isolates. *Sporodochia* large, orange in colour, forming on CLA within 10 d on carnation leaf pieces and on the agar surface. *Macroconidia* very long, slender, falcate, parallel dorso-ventral sides, tapering apical



Figs. 39–43 Morphological characters of *F. tjaetaba*. **39.** Macroconidia. **40.** Microconidia. **41-42**. Microconidia produced in false heads on monophialides and polyphialides. **43.** Chlamydospores. Bars: Fig. 39 = 50 μm. Figs. 40-42 = 20 μm. Fig. 43 = 25 μm

cells and distinctly notched basal cells, 4–7 septate, usually 5 septate, 60–95 μm ($\bar{x}$ =78 μm) long×4–5 μm ($\bar{x}$ =4 μm) wide. *Microconidia* forming singly or in false heads, arising from monophialides and polyphialides, oval in shape, 8–20 μm ($\bar{x}$ =13 μm) long, 0–1 septate, may be absent or very sparse in some isolates, but more abundant on SNA than CLA. *Chlamydospores* absent.

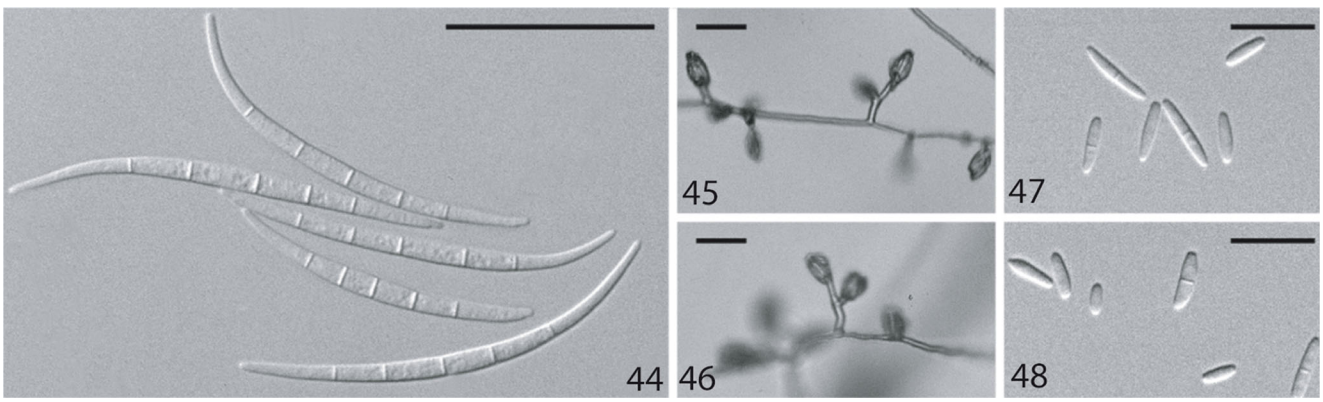
Specimens examined Type Strain (living and dried) NRRL66246, RBG5367 and additional strains FRL14482, FRL14483, FRL14484, FRL14485, FRL19308, FRL19309, FRL19310, FRL19311, FRL19312, FRL19313, FRL19314, FRL19315, FRL19316, FRL19318, FRL19319, FRL19320, FRL19321, FRL19322, FRL19323 FRL19325, FRL19326 isolated from *Triodia microstachya* Litchfield National Park, Northern Territory, Australia; 13°13′S, 130°44′E in 2009 by J.L. Walsh. Additional strains FRL14486, FRL14487 isolated from *Sorghum intrans* Litchfield National Park, Northern Territory; 13°15′S, 130°45′E, FRL19317 isolated from *Sorghum interjectum*; Litchfield National Park, Northern Territory; 13°14′S, 130°42′E and FRL11240, FRL11241 isolated from soil Kununurra, Western Australia; 15°46′S, 128°44′E in 2009 by J.L. Walsh.

Discussion

The current study identified and described six novel *Fusarium* species from Australian natural ecosystems of minimal anthropogenic disturbance. These novel species were phylogenetically distributed throughout the *Fusarium* genus in the FSAMSC, FFSC, FCSC and at least one novel lineage (FASC).

Morphological affinities

Morphologically *F. coicis* is similar to *Fusarium* species producing microconidia in medium to long chains, including *F. andiyazi, F. fractiflexum, F. fujikuroi, F. gaditjirri, F. globosum, F. miscanthi, F. napiforme, F. nisikadoi, F. phyllophilum, F. proliferatum, F. pseudonygamai, F. thapsinum*, and *F. verticillioides*. *Fusarium coicis* can be differentiated from the majority of these species by its very long macroconidia and the presence of long chains of large oval-clavate microconidia, which may be up to 3 septate. The macroconidia of *F. nisikadoi* are within the range reported for *F. coicis*; however, on average *F. coicis* produces longer macroconidia than *F. niskadoi* (Nirenberg and Aoki 1997).



Figs. 44–48 Morphological characters of *F. tjaynera*. **44.** Macroconidia. **45, 46.** Microconidia produced in false heads on SNA. **47, 48.** Microconidia. Bars: Fig. 44 = 50 μm; Figs. 45-48 = 20 μm

Fusarium coicis can be differentiated from *F. nisikadoi* and *F. miscanthi* morphologically by the colour of the sporodochia that are cream in *F. coicis* and orange in *F. niskadoi* and *F. miscanthi*. These latter species are also reported to produce pyriform microconidia, which have not been observed in *F. coicis* (Gams et al. 1999; Nirenberg and Aoki 1997).

Fusarium goolgardi has morphological affinities with *F. acuminatum*, *F. armeniacum*, *F.avenaceum*, *F. langsethiae*, *F. sambucinum*, *F. sporotrichoides* and *F. venenatum*, sharing the shape of the macroconidia, typical of species in the FSAM SC. However, *F. goolgardi* can be differentiated from these and all other *Fusarium* species by the sympodially branching monophialides that produce the microconidia.

Morphologically *F. mundagurra* is similar to *Fusarium* species that produce microconidia in short to medium length chains, including *F. brevicatenulatum, F. fractiflexum, F. globosum*, *F. lactis*, *F. napiforme*, *F. nygamai*, *F. phyllophilum*, *F. proliferatum*, *F. pseudocircinatum*, and *F. pseudonygamai. Fusarium mundagurra*; however, is differentiated from *F. fractiflexum, F. globosum*, *F. lactis, F. phyllophilum, F. proliferatum, F. pseudocircinatum* and *F. pseudonygamai* in the production of chlamydospores. In addition, *F. mundagurra* is differentiated from *F. brevicatenulatum* in that it does not produce pyriform microconidia. *Fusarium mundagurra* is morphologically difficult to distinguish from *F. nygamai* and can only be reliably separated on the basis of DNA sequence comparison.

Fusarium newnesense has morphological affinities with species in both the FOSC and FFSC. *Fusarium newnesense* resembles *F. oxysporum* (FOSC) and *F. foetens* (FOSC) in the production of oval microconidia in false heads on short monophialides. However, *F. newnesense* is differentiated from *F. oxysporum* and *F. foetens* by the production of pyriform microconidia and short disordered chains of oval microconidia. *Fusarium newnesense* is also morphologically similar to species in the FFSC that produce pyriform microconidia, including *F. anthophilum*, *F. brevicatenulatum*, *F. dlaminii, F. globosum*, *F. konzum*, *F. miscanthi*, *F. nisikadoi* and *F. proliferatum*. However, it is differentiated from these species by the production of chlamydospores. Furthermore, *F. newnesense* only produces short disordered chains of microconidia compared to the long chains of microconidia formed by *F. globosum, F. miscanthi*, *F. nisikadoi* and *F. proliferatum. Fusarium newnesense* is also differentiated from the two species outside of the FFSC that produce pyriform microconidia, *F. sporotrichioides* and *F. tricinctum,* by the macroconidial shape as *F. newnesense* produces macroconidia typical of species in the FFSC and FOSC, being mostly slender, thin-walled and slightly falcate. In contrast, *F. sporotrichioides* and *F. tricinctum* produce macroconidia that are falcate to almost lunate.

Morphologically *F. tjaetaba* is most similar to *F. anthophilum*, *F. brevicatenulatum*, and *F. konzum*, but differs from them by the production of pyriform microconidia in short chains and false heads from both monophialides and polyphialides, as well as the presence of chlamydospores. It should be cautioned, however, that chlamydospores are not formed by all isolates of *F. tjaetaba* and the ability to produce chlamydospores may be lost with repeated sub-culturing of isolates within this species.

Fusarium tjaynera morphologically resembles *F. aywerte*, a species first described by Sangalang et al. (1995a) as a subspecies of *F. avenaceum. Fusarium aywerte* was later promoted to species rank by Benyon et al. (2000). Isolates of *F. tjaynera* are morphologically similar to *F. aywerte*, but are distinguished by the production of microconidia and red pigmentation on PDA. *Fusarium aywerte* does not produce microconidia and produces peach or pale orange to orange-white pigmentation on PDA (Sangalang 1992).The two species are distinguished on basis of the shape of the basal and apical cells of the macroconidium. In *F. tjaynera* the basal cell is distinctly notched, whereas *F. longipes* has a very distinctive exaggerated elongate foot shape (Leslie and Summerell 2006). Both species have tapering apical cells; however the apical cell of *F. tjaynera* is not as exaggerated as the elongate whip-like apical cell of *F. longipes* (Burgess et al. 1994a; Leslie and Summerell 2006).

Phylogeography

Surveys of natural ecosystems are not only important for uncovering *Fusarium* species diversity but also for detecting phylogeographical signals which are likely to be influenced by anthropogenic disturbance (Summerell et al. 2010). In the current study we recovered species in the FSAMSC, FFSC, FCSC and at least one novel lineage (FASC). The phylogeographical relationships within each species complex raise interesting hypotheses on the distribution and evolution of this genus.

Multi-gene genealogies of the FFSC have generated phylogeographical hypotheses and debates within the *Fusarium* community. A landmark phylogenetic study of the FFSC uncovered three major clades (O'Donnell et al. 1998a). The authors presented a phylogeographical hypothesis to explain the origin of these clearly demarcated clades, correlating them to three major geographical regions; 'American', 'African' and 'Asian'. Although a number of exceptions have been noted (Kvas et al. 2009; Walsh et al. 2010), the distribution of FFSC species generally fit the 'American', 'African' and 'Asian' hypothesis, while allowing for the anthropogenic movement of species. Adding to the exceptions, the three novel FFSC species recovered from natural ecosystems in the current study all belong to the 'African' clade. *Fusarium coicis* has a restricted geographic and host distribution in Australia and has been thus far only recovered in association with the extremely rare grass, *Coix gasteenii*. This grass is

known to occur naturally only in small populations occurring in Lakefield National Park, Queensland, Australia (Simon 1989). The closest phylogenetic relatives to *F. coicis* are the undescribed *Fusarium* species NRRL25615 (host: *Oryza sativa* seed) and NRRL26793 (host: *Striga hermonthica*) that were both isolated from Africa. Significant *EF-1α* sequence divergence was detected between these relatives, suggesting a long evolutionary separation from *F. coicis*. The pattern for *F. tjaetaba* is similar with high levels of sequence divergence from its closest relative in the 'African' clade, *F. brevicatenulatum* NRRL25446 (host: *Striga asiatica*), isolated from Madagascar. *Fusarium tjaetaba* also has a very narrow known host range, isolated only from *Sorghum interjectum*, in spite of extensive sampling of other grass species from the same locality, including *Heteropogon triticeus*, *Pseudopogonatherum irritans*, *Sehima nervosum*, *Sorghum intrans* and *Triodia microstachya*. The closest relative of *F. mundagurra* is the undescribed *Fusarium* sp. NRRL25221 (host: *Zea mays*) that was isolated from Zimbabwe, Africa. Once again there were high levels of *EF-1α* sequence divergence suggesting a long period of separation. Although *F. mundagurra* was recovered from both soil and mango, a cultivated species not endemic to Australia, the isolation frequency from mango was low. The recovery of *F. mundagurra* from mango may reflect an opportunistic rather than a close host association. The close phylogenetic relationships between the novel Australian species and African species in the FFSC supports the post Gondwanaland radiation of the FFSC in the late Miocene as proposed by O'Donnell et al. (2013), perhaps in association with hosts endemic to Australia, as in the case of *F. coicis* and *F. tjaetaba*. However, we believe that further survey work that compares *Fusarium* communities in natural ecosystems between Africa, Australia and South America is required to shed further light on the phylogeography of species in the FFSC.

Fusarium goolgardi belongs to the FSAMSC, a trichothecene producing clade, with its closest relative being *Fusarium* sp. NRRL36351 that was recovered from stored peanuts (*Arachis hypogaea*) in Lisboa, Portugal. The closest described species are *F. langsethiae* (origin: Europe), *F. palustre* (origin: USA) and *F. sibiricum* (origin: Russia). It is interesting that *F. goolgardi* was associated with *Xanthorrhoea* decline that was symptomatically similar to the dieback associated with *F. palustre* in the USA (Elmer and Marra 2011; Elmer et al. 2012). Trichothecenes are involved in plant pathogenesis (Proctor et al. 2002; Desjardins and Hohn 1997) and given that *F. goolgardi* clusters in a trichothecene producing clade it would be valuable to determine if it produces trichochenes, which may have a role in *Xanthorrhoea* decline at the Bungonia National Park site.

Fusarium newnesense is an interesting species morphologically, sharing characters with both the FOSC and FFSC, suggesting close ancestry. However, phylogenetically the deep nodes are conflicted, with *RPB1* placing *F. newnesense* as basal to the FOSC and *RPB2* as basal to the FFSC (data not shown). The combined *RPB1/RPB2* phylogeny places this lineage as an intermediate, within the FOSC and basal to the FFSC. These observations may indicate that this species is the ancestor to these important *Fusarium* species complexes. Further characterisation of this species may provide interesting insights into the evolution of pathogenicity and mycotoxin production in *Fusarium*.

Biogeographically *F. newnesense* was recovered from three locations in Australia: Tasmania, Victoria and New South Wales. Phylogenetic analyses of the *EF-1α* region showed clustering that corresponded to geography, suggesting allopatric divergence. The broad geographic range in natural ecosystems and polymorphisms suggest that *F. newnesense* may be endemic to Australia. The closest relative is the undescribed *Fusarium* sp. NRRL25184 that was isolated as an endophyte of grape vines in Europe. As discussed, it is difficult to draw phylogeographic conclusions based on isolates from heavily modified ecosystems, especially in a crop, such as grapes, which has experienced significant global movement of germplasm.

Fusarium tjaynera clusters with *F. aywerte* in a well-supported and differentiated clade basal to the FCSC. *Fusarium aywerte* has only been recovered from endemic spinifex grasses in central Australia. Similarly, *F. tjaynera* is primarily associated with the spinifex species *Triodia microstachya*. No species in the FASC have been recovered from international surveys, suggesting that the FASC is endemic to Australia, perhaps evolving with endemic flora.

The current study highlights the importance of mycogeographic surveys in natural ecosystems of minimal anthropogenic disturbance for providing base line information for rigorous studies on the phylogeny, taxonomy, phylogeography, pathology and ecology of *Fusarium* (Burgess 2014). This base line research can be used to infer the origin and evolutionary relationships in this genus. Further continental scale surveys are justified in Australian natural ecosystems as novel species continue to be recovered from biogeographic areas and plant species that have not previously been studied. Furthermore substantive progress in understanding the phylogeography of *Fusarium* will depend on rigorous phylogeographic surveys of *Fusarium* in soil and plants in natural ecosystems in other regions of the world, particularly South America, which is largely unsurveyed.

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