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There it is! *Fusarium pseudograminearum* did not lose the fusaristatin gene cluster after all

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ABSTRACT

Fusarium pseudograminearum is a significant pathogen of cereals in arid regions worldwide and has the ability to produce numerous bioactive secondary metabolites. The genome sequences of seven *F. pseudograminearum* strains have been published and in one of these strains, C5834, we identified an intact gene cluster responsible for biosynthesis of the cyclic lipopeptide fusaristatin A. The high level of sequence identity of the fusaristatin cluster remnant in strains that do not produce fusaristatin suggests that the absence of the cluster evolved once, and subsequently the resulting locus with the cluster fragments became widely dispersed among strains of *F. pseudograminearum* in Australia. We examined a selection of 99 Australian *F. pseudograminearum* isolates to determine how widespread the ability to produce fusaristatin A is in *F. pseudograminearum*. We identified 15 fusaristatin producing strains, all originating from Western Australia. Phylogenetic analyses could not support a division of *F. pseudograminearum* into fusaristatin producing and nonproducing populations, which could indicate the loss has occurred relatively recent.

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1. Introduction

Fusarium pseudograminearum is the primary cause of Fusarium crown rot (FCR) of wheat and barley in the arid cereal growing regions of the world including Australia (Burgess et al., 2001), Southern Europe (Balmas, 1994), Northern Africa (Gargouri et al., 2011), South Africa (Lamprecht et al., 2006), China (Ji et al., 2016; Li et al., 2012; Xu et al., 2017) and the United Stated of America (Smiley et al., 2005). The disease is one of the most severe in cereals in Australia with yearly economic losses of approximately 100 million Australian dollars (Murray and Brennan, 2009, 2010). *F. pseudograminearum* is heterothallic (Aoki and O'Donnell, 1999b; Summerell et al., 2001) and was initially recognized as a population within the *Fusarium graminearum* species group (Group 1) based on cultivation and its inability to form homothallic perithecia (Burgess et al., 1975; Francis and Burgess, 1977). Later, the two species were formally segregated by molecular analyses (Aoki and O'Donnell, 1999a) and further sequence analyses suggested that *F. pseudograminearum* is a single globally occurring species (Scott and Chakraborty, 2006), while *F. graminearum* can be divided into more than 16 phylogenetically distinct species (Aoki et al., 2012; O'Donnell et al., 2000). *F. graminearum* is involved in Fusarium head blight (FHB) in cereals, a disease which *F. pseudograminearum* has only been observed to cause in Australia (Backhouse et al., 2004) and China (Ji et al., 2016). Both species are known producers of the trichothecene mycotoxin deoxynivalenol (and derivatives) and of the mycoestrogen zearalenone (Sydenham et al., 1991).

Comparative analyses of the first genome sequenced strains of *F. graminearum* (NRRL 31084) and *F. pseudograminearum* (CS3096) revealed only minor differences in the composition of polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs) (Hansen et al., 2015). The two strains differ, however, in their ability to produce polyketide lipopeptides: in their ability to produce two polyketide lipopeptides: *F. graminearum* NRRL 31084 produces

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fusaristatin A but not W493, while *F. pseudograminearum* CS3096 produces W493 but not fusaristatin A (Fig. 1 (Sørensen et al., 2014a)).

Biosynthesis of W493 and fusaristatin A are suggested to follow similar routes starting with production of a partially reduced polyketide which serves as a substrate for a NRPS that catalyzes the condensation of the polyketide and amino acids before the compounds are released by cyclization (Sørensen et al., 2014a). The key enzymes involved in biosynthesis of W493 are PKS32, which produces a reduced polyketide (C_{14}) chain and NRPS40, which catalyzes condensation of six amino acids (threonine, alanine, alanine, glutamine, tyrosine and valine/isoleucine (W493-A/W493-B)). Fusaristatin biosynthesis is initiated by production of a reduced polyketide (C_{24}) by PKS6 prior to incorporation of three amino acids (dehydroalanine, β-aminoisobutyric acid and glutamine) by NRPS7.

The fusaristatin gene cluster has also been identified in the more distantly related *Botrytis fuckeliana, Cochliobolus heterostrophus* and *Pyrenophora teres* (Sieber et al., 2014). Following the first genome release of a *F. pseudograminearum* strain, six additional strains were published (Gardiner et al., 2017; Moolhuijzen et al., 2013). In one of these strains, CS5834, we identified the intact fusaristatin gene cluster and the aim of the current study was to determine how common this cluster is in *F. pseudograminearum* and whether its presence or absence arose from a gain or loss event.

2. Materials and methods

2.1. Fungal strains

Ninety-nine strains of *F. pseudograminearum* were obtained from the CSIRO collection in Brisbane Australia. These strains were isolated from four different Australian states; New South Wales (42 strains), Queensland (18 strains), South Australia (4) and Western Australia (35).

2.2. Fusaristatin gene cluster analyses

The available genome sequences of seven *F. pseudograminearum* strains (CS3096, CS3220, CS3270, CS3427, CS3487, CS5834 and RBG5266) were screened for presence of the fusaristatin gene cluster using the published gene cluster from *F. graminearum* (Sørensen et al., 2014a). Remnant fragments of the fusaristatin gene cluster were identified though BlastN analyses (Altschul et al., 1990) using the fusaristatin gene cluster from *F. pseudograminearum* CS5834 against the whole-genome sequence (WGS) database of the six other *F. pseudograminearum* strains.

2.3. Analyses of W493-B and fusaristatin A production

For secondary metabolite analyses the 99 *F. pseudograminearum* strains were cultivated on solid yeast extract sucrose (YES) agar medium (Sørensen and Sondergaard, 2014) and corn meal agar (CM; corn meal 60 g/L, $ZnSO_4 \times 7H_2O$ 10 mg/L, $CuSO_4 \times 5H_2O$ 5 mg/L, agar 20 g/L) medium for two weeks in the dark at 25 °C. The extraction of secondary metabolites were performed as previously described (Smedsgaard, 1997). The resulting extracts were analyzed on a Hitachi Elite LaChrom HPLC system equipped with a 150 × 4.6 mm Ascentis Xpress 2.7 μm phenyl-hexyl column (Sigma–Aldrich, USA) and coupled to a high resolution mass spectrometer (compact qTOF, Bruker, Germany) with an electrospray source using a 3:97 flowsplitter. 40 μL extract was separated using a flow of 1 mL/min with a linear water–acetonitrile gradient, with both eluents buffered with 0.1 % formic acid. The gradient started at 10 % acetonitrile and reached 100 % in 20 min, which was held for 5 min.

2.4. Determination of presence or absence of the fusaristatin gene cluster

The fungal strains were cultivated in 30 mL liquid Czapek dox (Sigma–Aldrich) medium prior to DNA extraction. The cultivated fungi were filtered through sterile MiraCloth (Calbiochem®) and ground in liquid nitrogen before genomic DNA was extracted with the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany) (Droce et al., 2013). The isolated genomic DNA served as template in a polymerase chain reaction (PCR) targeting *PKS6* with primers PKS6conFw (5′-3′: CTG TTG TTG GCA TGA GTT GC) and PKS6conRv (5′-3′: TGG CCC ATG CGA GGA TAC TG), which amplify a 1751 bp product in strains with intact *PKS6* and 1564 bp product in strains with *PKS6* remnants. The PCR reactions were performed in 50 μL volume using the Phusion Hot Start II DNA Polymerase (Thermo Fisher Scientific) according to manufactures protocol. The resulting PCR products were run on 1 % agarose gels with 1 kbp plus DNA ladder (Thermo Fisher Scientific).

2.5. Phylogenetic analyses of F. pseudograminearum strains

For phylogenetic analyses the primers PHO1 (5′-3′: ATC TTC TGG CGT GTT ATC ATG) and PHO6 (5′-3′: GAT GTG GTT GTA AGC AAA GCC C) were used to amplify a fragment of the Phosphate permease gene (FPSE_11047 in *F. pseudograminearum* CS3096) (Scott and Chakraborty, 2006) by PCR. The PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced at Eurofins Genomics (Ebersberg, Germany) using the

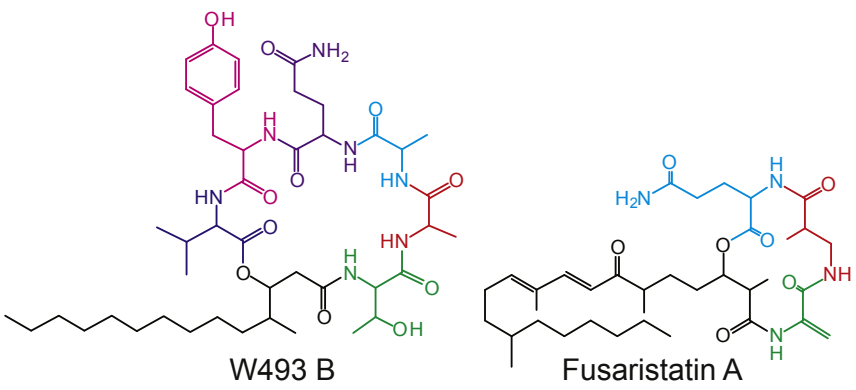

Fig. 1. Structures of W493-B and fusaristatin A highlighting the reduced polyketide (black) and peptide (colored) parts.

forward primer PHO1. The sequences were aligned with by multiple alignment using fast fourier transform (MAFFT) at the T-REX web server (Boc et al., 2012). The alignments were analysed with CLC main workbench (CLC Bio, Qiagen, Germany) using maximum likelihood with 1000 bootstraps and visualized with EvolView (http://evolgenius.info/evolview) (Zhang et al., 2012).

2.6. Whole-genome sequencing

With minor modifications, genomic DNA was extracted from strains CS3894, CS3900, CS5541, CS7093, CS7108, CS7081, CS7088, CS7065 and CS7060 using the FastDNA™ SPIN kit for Soil (MP Biomedicals, USA). Following clean-up with Agencourt AMPure XP beads (Beckman Coulter, USA), 2 μg DNA was used as input for the SQK-LSK8 ligation sequencing kit protocol (NBE_9006_v103_revQ_21Dec2016). The protocol was modified to allow for barcoding with the Native Barcoding Kit (EXP-NBD103, Oxford Nanopore Technologies, UK) directly following the end-prep step and for downstream compatibility with sequencing on the PromethION alfa/beta sequencer (Oxford Nanopore Technologies, UK). Briefly, 10 μL Native barcode (NB01-NB9) was mixed with 30 μL end-prepped DNA mix (2 μg DNA), 10 μL nuclease-free water, 40 μL Ultra II ligation master mix (New-England Biolabs, USA), 1 μL ligation enhancer (New-England Biolabs, USA) and incubated at room temperature for 10 min before being further processed according to the PromethION SQK-LSK9 protocol (GDLE_9056_v109_revE_02-Feb2018). Approximately 600 ng of pooled DNA was loaded onto a primed FLO-PRO001 flow-cell (Oxford Nanopore Technologies, UK) and sequenced on the PromethION alfa/beta sequencer with live base-calling enabled. Approximately 60 Gbp reads were demultiplexed and trimmed in Porechop version 0.2.3 and subsequently mapped to the reference genome of *F. pseudograminearum* CS3096 (Gardiner et al., 2017) in CLC Genomics Workbench version 9.5.5 (CLC Bio, Qiagen, Germany). Consensus sequences from the complete genes of beta-tubulin (FPSE_03337), translation elongation factor 1-alfa (FPSE_11980), trichothecene 3-O-acetyltransferase (FPSE_11049), ammonia-ligase (FPSE_11050) and phosphate permease (FPSE_11047) were finally extracted for phylogenetic analysis (O'Donnell et al., 2000). The alignment was executed with MUSCLE (Edgar, 2004). A few nucleotides (1–3 pr. sequence) resulting in non-sense mutation were excluded from the final alignments to eliminate Nanopore sequencing-biases (in some homopolymeric nucleotide-region). The alignments were fused and analysed using the same approach as for the phosphate permease gene. CANU version 1.7 was used to assemble the genome of CS3894 with default settings (genome size set at 36 gbp) (Koren et al., 2017).

3. Results and discussion

3.1. The fusaristatin cluster is conserved in F. pseudograminearum CS5834

The predicted fusaristatin cluster in *F. pseudograminearum* CS5834 was initially compared to the published clusters in *F. graminearum* and *Fusarium avenaceum* (Sørensen et al. 2014a, 2014b). The comparison showed that the hypothetical proteins are of comparable length and identity (Table 1) suggesting that the gene cluster is also functional in *F. pseudograminearum* CS5834. Based on their phylogenetic relationship (Kristensen et al., 2005; O'Donnell et al., 2013) it was not surprising that a higher identity was observed to *F. graminearum* (94–98 %) than to *F. avenaceum* (73–86 %).

Further analyses of the available *Fusarium* genome sequences revealed that the fusaristatin gene cluster is present with conserved synteny in *F. pseudograminearum* CS5834, *F. graminearum*, *Fusarium culmorum, Fusarium meridionale, Fusarium asiaticum, Fusarium langsethiae, Fusarium acuminatum* and *F. avenaceum* (Fig. 2). The flanking genes were, however, different in *F. avenaceum* and *F. acuminatum* compared to the other *Fusarium* species, indicating that the cluster is present in a different genomic location these two species. The identical location of the fusaristatin cluster in *F. pseudograminearum* CS5834 and the majority of other *Fusarium* species suggests that CS5834 did not acquire the cluster through horizontal gene transfer. This in turn suggests that the fusaristatin cluster was present in *F. pseudograminearum* after it diverged from other fusaria but was subsequently lost. To further investigate the nature of the loss, we examined the genomic region between the flanking genes of the fusaristatin gene cluster by which five conserved remnant fragments (88–95 % sequence identity) of the cluster could be found in all six *F. pseudograminearum* strains (Fig. 2A). One of the fragments (R1; 897 bp) originates from a predicted aminotransferase gene (BN849_0052070), three other fragments (R2-R4; 120, 446 and 273 bp, respectively) originate from *PKS6* (BN849_0052040) while a fifth fragment (R5; 407 bp) originates from *NRPS7* (BN849_0052030). To illustrate that the fragments originate from PKS6 the three remnant fragments of PKS6 in *F. pseudograminearum* CS3096 were translated into amino acid sequences and aligned against the functional *PKS6* of *F. pseudograminearum* CS5834 (Fig. 2B). In these alignments, a high sequence identity was observed for the three fragments as R2 had 90 % (60 amino acids), R3 had 89 % (148 amino acids) and R4 had 82 % identity (91 amino acids). The presence of conserved remnant fragments suggests that the missing fusaristatin gene cluster is a result of a deletion event in a common ancestor.

3.2. Fusaristatin-producing F. pseudograminearum strains are geographically co-localized

The distribution of the fusaristatin-producing ability in Australian *F. pseudograminearum* strains was further investigated through chemical analyses of the 99 strains, which originated from five different states (New South Wales, Queensland, South Australia and Western Australia). The analyses showed that while nearly all strains (except CS3002 and CS5897) were able to produce W493-B only 15 strains produced fusaristatin when cultivated on solid YES or CM medium (Table 2). The ability to produce fusaristatin seemed to be geographically confined, because all 15 fusaristatin A-producing strains were isolated from Western

Table 1
Description of genes in the fusaristatin cluster in *F. pseudograminearum* CS5834 and comparison (% identity on amino acid level) to *F. graminearum* NRRL 31084 and *F. avenaceum* Fa05001.

F. pseudograminearum	Length	Function	*F. graminearum*	*F. avenaceum*
BN849_0052030	4355 aa	Non-ribosomal peptide synthetase	FGSG_08209 (94 %)	FAVG1_08708 (73 %)
BN849_0052040	2554 aa	Polyketide synthase	FGSG_08208 (98 %)	FAVG1_08709 (84 %)
BN849_0052050	520 aa	Cytochrome P450 monooxygenase	FGSG_08207 (98 %)	FAVG1_08710 (86 %)
BN849_0052060	138 aa	Hypothetic protein	FGSG_08206 (96 %)	FAVG1_08711 (79 %)
BN849_0052070	511 aa	Aminotransferase	FGSG_08205 (96 %)	FAVG1_08712 (78 %)

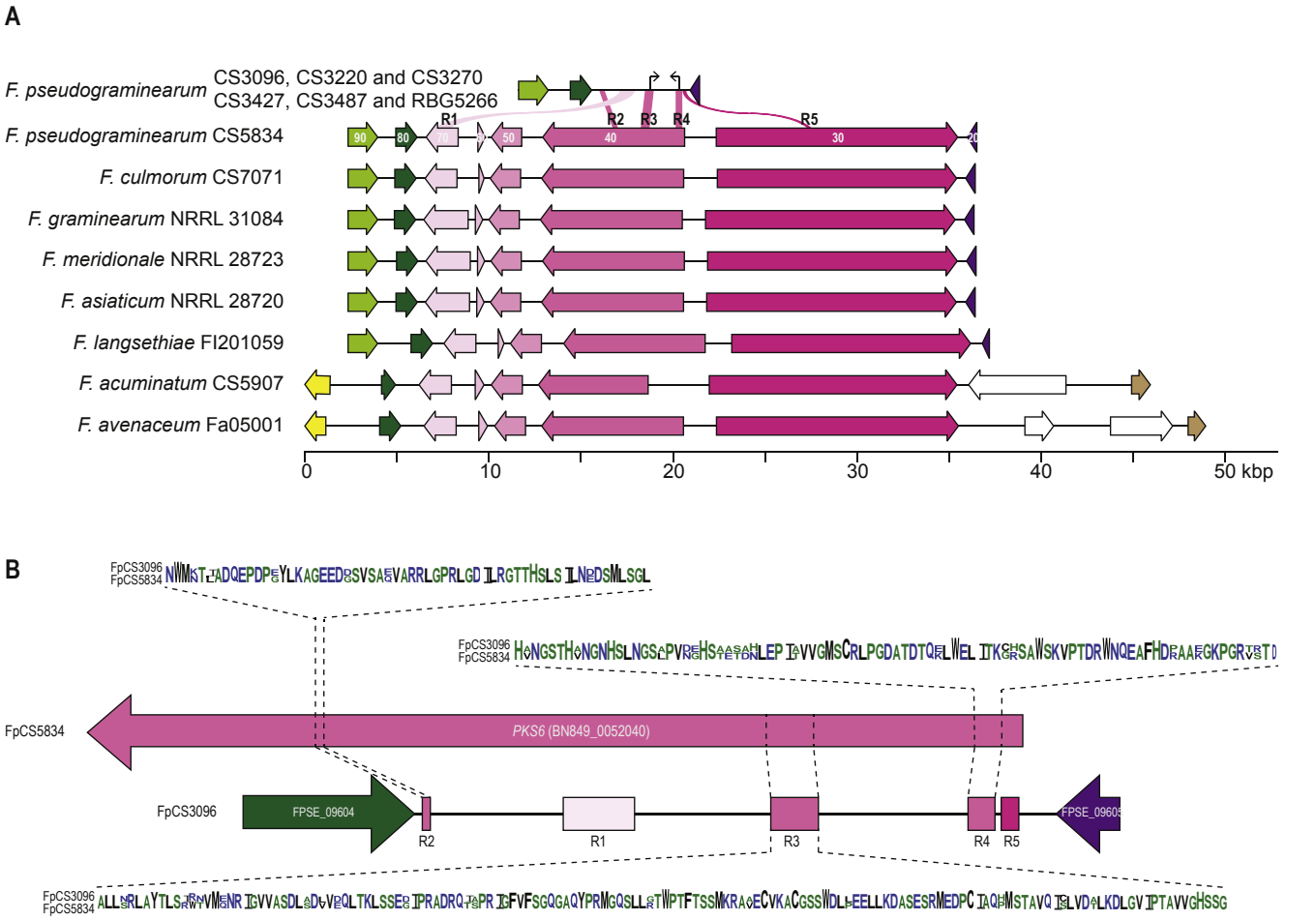

Fig. 2. Comparative analysis of the fusaristatin gene cluster and remnant fragments in *Fusarium*. **A**. Illustration of the intact cluster in *F. pseudograminearum* CS5834 (BN849_0052030 - BN849_0052070) and seven other *Fusarium* species. Only five remnant fragments (R1-R5) are present in *F. pseudograminearum* CS3096, CS3220, CS3487, CS3270, CS3427 and RBG5266. **B**. Predicted amino acid sequence of regions corresponding to PKS6 fragments R2 - R4 in *F. pseudograminearum* strains CS3096 (lacks intact cluster) and CS5834 (has intact cluster). Amino acids are represented by standard single-letter abbreviations, and two letters stacked one on top of the other indicate a difference in the sequence of the two strains.

Australia. Although a slight decrease in W493-B levels was observed in the fusaristatin A producers, this difference was not significant ($P > 0.05$; Supplementary Figure 1).

3.3. *Fusaristatin producing isolates do not form a unique lineage*

The phosphate permease gene was partially sequenced (807 of 1851 bp) to investigate whether fusaristatin-producing and nonproducing strains constitute phylogenetically distinct lineages of *F. pseudograminearum*. Assumedly, this locus is inherited independently of the fusaristatin gene cluster, as they are located on two different chromosomes. The phosphate permease gene is located near the middle of chromosome IV, while the fusaristatin gene cluster is located near and end of chromosome II. Phylogenetic analyses of the resulting sequences resulted in a tree with two major clades (Fig. 3A), separated by 26 variable sites (3 %). The first clade contained the majority of the strains isolated from New South Wales (40/42) and Queensland (16/18). Three nonproducers of fusaristatin A from Western Australia were also present in clade I, while the remaining thirty-two strains were located in the second clade. This second clade consisted of two different sequence types, sharing 805 of 807 nucleotides and contained both fusaristatin producers and nonproducers without any signs of segregation.

In a further attempt to achieve a phylogenetic separation of fusaristatin producers and nonproducers, we performed a multiplexed genome sequencing of four producers (CS5541, CS7108, CS7081, and CS7060) and five nonproducers (CS3894, CS3900, CS7065 CS7088, CS7093). In addition to the phosphate permease gene, sequences of five genes were extracted (β-tubulin, translation elongation factor 1α, trichothecene 3-O-acetyltransferase and ammonia-ligase) and used to generate an additional phylogenetic tree. The resulting tree failed to separate fusaristatin producers and nonproducers, although this combination of genes has previously been used to separate *F. graminearum* into different phylogenetic species (Fig. 3C). Due to the inadequacy of this multigene approach, future studies could focus on full genome analyses in order to determine whether producers and nonproducers of fusaristatin can be separated into two groups.

The lack of fusaristatin production in a strain does not necessarily mean that the strain does not have a functional fusaristatin gene cluster, because lack of production can also be caused by too low production levels or repression under the tested conditions. A PCR based strategy was used to determine the presence or absence of a functional *PKS6* yielding predicted products of 1751 bp in strains with an intact *PKS6* and 1564 bp in strains with *PKS6* remnant fragments. Thus, the two fragments are markers for the two alternative alleles of the locus (i.e., an intact and a deleted gene

Table 2
Production[a] of W493-B and Fusaristatin A (Fst A) by *F. pseudograminearum* strains collected from New South Wales (NSW), Queensland (QLD), South Australia (SA) and Western Australia (WA).

Strain[a]	W493-B[b]	Fst A[b]	Location	State	Strain	W493-B	Fst A	Location	State
CS3096	●		Moree	NSW	CS7114	●		Bowenville	QLD
CS3164	●		Qurindi	NSW	CS7118	●		Marmaduaz	QLD
CS3166	●		Qurindi	NSW	CS7124	●		Hannaford	QLD
CS3173	●		Qurindi	NSW	CS7126	●		Hannaford	QLD
CS3184	●		Bladeville	NSW	CS7133	●		Toobeak	QLD
CS3220	●		Liverpool Plains	NSW	CS7139	●		Toobeak	QLD
CS3270	●		Liverpool Plains B	NSW	CS7145	●		Wyaga	QLD
CS3293	●		Boggabri	NSW	CS7147	●		Wyaga	QLD
CS3319	●		Boggabri	NSW	CS7149	●		Warra	QLD
CS3361	●		Bellata	NSW	CS7153	●		Warra	QLD
CS3768	●		North Stat	NSW					
CS3784	●		North Stat	NSW	CS3891	●		Foolunga Street	SA
CS3941	●		Cooper Creek K	NSW	CS3894	●		Foolunga Street	SA
CS3950	●		Cooper Creek K	NSW	CS3900	●		Angus Valley	SA
CS3965	●		9 Miles Road	NSW	CS3907	●		Angus Valley	SA
CS3967	●		9 Miles Road	NSW					
CS3983	●		Livingstone Farm	NSW	CS5541	●	●	Stockdale	WA
CS3986	●		Livingstone Farm	NSW	CS5573	●	●	Stockdale	WA
CS7291	●		Nombi 1	NSW	CS5588	●	●	Tammin	WA
CS7302	●		Spring Ridge 1	NSW	CS5703	●	●	Tammin	WA
CS7305	●		Spring Ridge 1	NSW	CS5834	●	●	Tammin	WA
CS7311	●		Nombi 1	NSW	CS5877	●		Farm 3	WA
CS7313	●		Nowbi 1	NSW	CS5894	●	●	Jerramungub	WA
CS7319	●		Spring Ridge 2	NSW	CS5897		●	Jerramungub	WA
CS7344	●		Nowbi 2	NSW	CS7054	●	●	Lake Grace	WA
CS7350	●		Nowbi 2	NSW	CS7055	●		Boxwood Hill	WA
CS7358	●		Tambar Springs	NSW	CS7056	●	●	Boxwood Hill	WA
CS7374	●		Tambar Springs	NSW	CS7060	●	●	Lake Grace	WA
CS7385	●		Spring Ridge 3	NSW	CS7062	●	●	Lake Grace	WA
CS7391	●		Spring Ridge 3	NSW	CS7065	●		Mettler	WA
CS7405	●		Spring Ridge 4	NSW	CS7066	●	●	Wellstead	WA
CS7407	●		Bladeville	NSW	CS7069	●		Wellstead	WA
CS7420	●		Spring Ridge 5	NSW	CS7078	●		Lake Grace	WA
CS7427	●		Spring Ridge 5	NSW	CS7080	●		Lake Grace	WA
CS7436	●		Spring Ridge 2	NSW	CS7081	●	●	Carnamagh	WA
CS7453	●		Spring Ridge 6	NSW	CS7082	●		Lake King	WA
CS7460	●		Werris Creek	NSW	CS7084	●		Lake King	WA
CS7461	●		Werris Creek	NSW	CS7085	●	●	Lake King	WA
CS7463	●		Kelvin	NSW	CS7088	●		Lake King	WA
CS7464	●		Kelvin	NSW	CS7089	●		Grasspatch	WA
CS7465	●		Caroona 4	NSW	CS7090	●		Grasspatch	WA
CS7467	●		Caroona 4	NSW	CS7091	●		Grasspatch	WA
					CS7093	●		Grasspatch	WA
CS3002				QLD	CS7094	●		Grasspatch	WA
CS3427	●		Wilga Downs	QLD	CS7098	●		Grasspatch	WA
CS3438	●		Wilga Downs	QLD	CS7099	●		Salmon Gums	WA
CS3442	●		Coondiwindi	QLD	CS7100	●		Salmon Gums	WA
CS3744	●		Kentare Condamine	QLD	CS7104	●		Salmon Gums	WA
CS3752	●		Kentare Condamine	QLD	CS7105	●		Lake Grace	WA
CS3910	●		Westfield Condamine	QLD	CS7108	●	●	Lake Grace	WA
CS7113	●		Bowenville	QLD					

cluster) based on available genome sequence data. The results showed that the PCR of the 15 fusaristatin producing strains resulted in amplified fragments of the expected size for the intact and functional *PKS6* (Fig. 3B). The PCR fragments for all the nonproducing strains, except CS3894, were smaller, which corresponds to the presence of the *PKS6* remnant region. The slightly larger PCR fragment in CS3894 was investigated further using the full genome sequence of CS3894, which showed that overall the sequence was very similar to the nonproducing CS3096 remnant region with the exception of an additional 100 bp (Supplementary Figure 2) which accounts for the intermediate size of the band observed for this isolate (Fig. 3B).

Together the molecular analyses suggests that the presence of the fusaristatin gene cluster is reflected to some extend in the phylogenetic analyses of genes used in the present study. However, the genes do not contain sufficient variation to segregate the strains into clades reflecting the ability to produce fusaristatin A. A phylogenetic analysis of *F. pseudograminearum* based on the phosphate permease, reductase, translation elongation factor-1α and β-tubulin genes concluded that *F. pseudograminearum* is a single monophyletic species (Scott and Chakraborty, 2006). The high sequence conservation within *F. pseudograminearum* is also reflected in the RNA polymerase II largest (RPB1) and second largest subunit (RPB2) genes, which have been successfully used for separating closely related *Fusarium* species (O'Donnell et al., 2013). In these genes CS3096 and CS5834 share high sequence identity (1604/1606 and 901/902).

The loss of the fusaristatin gene cluster in *F. pseudograminearum* could represent an evolutionary development where the compound is not needed for spread and survival. Biosynthesis of huge proteins, like PKS6 and NRPS7, represent a significant energy cost for the fungus; thus, losing the redundant gene cluster can result in an improved fitness.

One of the reasons for losing the fusaristatin gene cluster could be due to an overlapping mode of action for W493 and

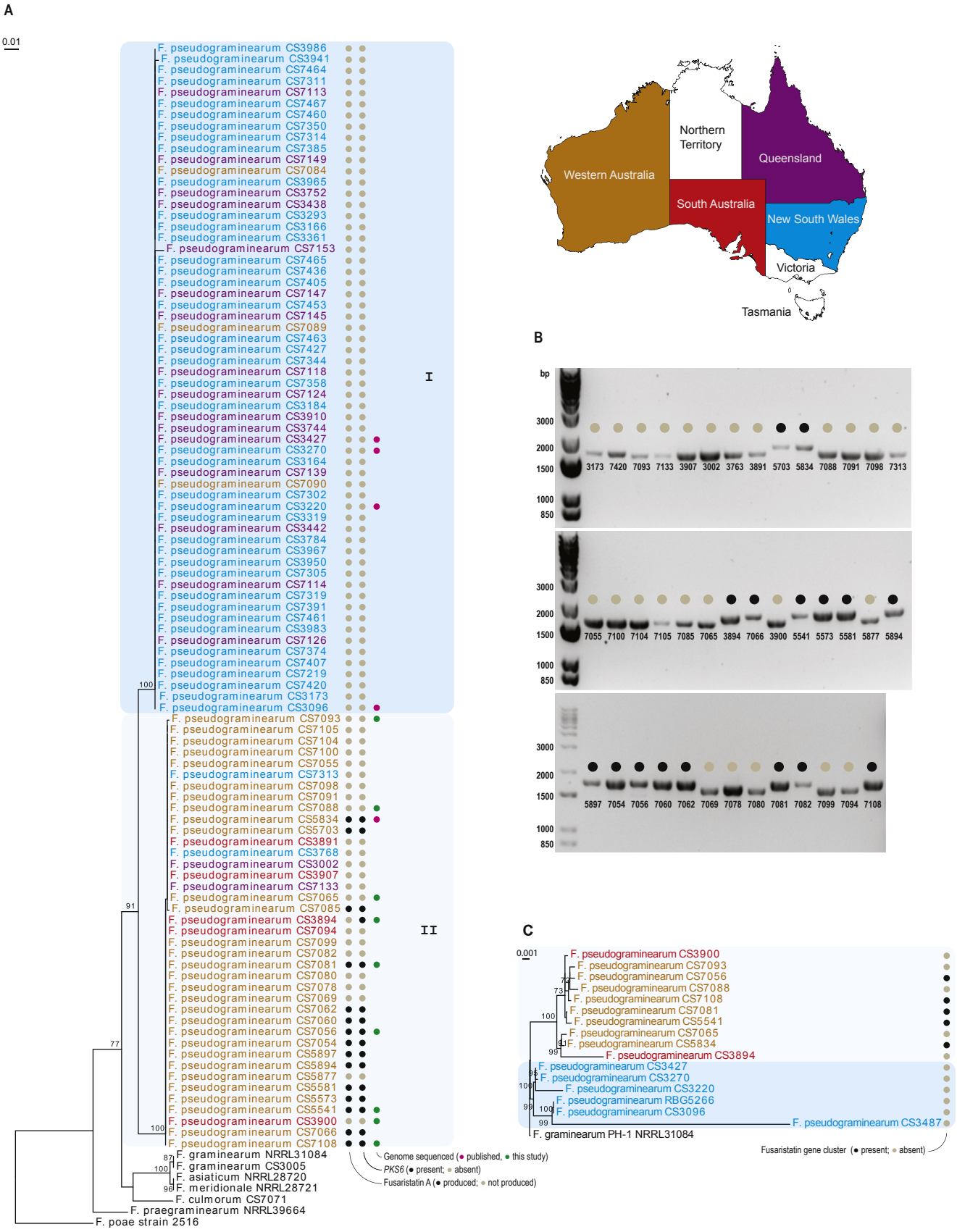

Fig. 3. Molecular analyses of the *F. pseudograminearum* strains. **A**. Phylogenetic analyses of the 99 *F. pseudograminearum* strains (orange: Western Australia; red: South Australia; blue: New South Wales; purple: Queensland) and of selected genome sequenced *Fusarium* strains with *F. poae* strain 2516 as outgroup. Numbers indicate bootstrap values from 1000 replications. **B**. 1 % agarose gels visualizing the PCR products for determining the presence (●) and absence (●) of *PKS6* of strains located in clade II. **C**. Multi-locus phylogeny of 16 *F. pseudograminearum* isolates with and without the fusaristatin gene cluster. Numbers indicate bootstrap values from 1000 replications. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

fusaristatin A, which is not an unlikely scenario given their similar biosynthetic background and structural similarities. The high level of identity of the sequence of the remnant fusaristatin cluster in strains CS3096, CS3220, CS3270, CS3427, CS3487 and RBG5266 suggests that presence a deletion event occurred in one strain or lineage of the fungus rather than multiple times in multiple strains or lineages. However, the presence of the additional region in CS3894 suggests that some modifications has occurred locus where the fusaristatin gene cluster was lost. Understanding when this loss event occurred may provide some indication of the evolutionary reason for the absence of the cluster in most strains. The climatic conditions (and native grass populations) in WA can be drastically different to the eastern states of Australia. The restricted geographic location of isolates containing the fusaristatin cluster may suggest different evolutionary pressures exist in WA but the widespread (and overlapping) presence of isolates carrying the cluster loss in the same location and the absence of obvious lineages are contrary to this scenario.

Although Fusarium crown rot has likely been present in WA for a long time, it has only recently emerged as a significant economic impediment to wheat production in this area (Murray and Brennan, 2009). Further complicating our understanding of the evolutionary pressures that have shaped the *F. pseudograminearum* genome is the likelihood that *F. pseudograminearum*, like *F. graminearum*, has not co-evolved with wheat (Lofgren et al., 2018) and can be considered an opportunistic pathogen of wheat. Thus, it will be extremely challenging to pinpoint the reason for loss of the cluster or even whether maintaining the clusters provides some advantage in the WA environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2018.10.004.

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