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RESEARCH ARTICLE

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Pyricularia graminis-tritici, a new *Pyricularia* species causing wheat blast

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Key words

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Abstract *Pyricularia oryzae* is a species complex that causes blast disease on more than 50 species of poaceous plants. *Pyricularia oryzae* has a worldwide distribution as a rice pathogen and in the last 30 years emerged as an important wheat pathogen in southern Brazil. We conducted phylogenetic analyses using 10 housekeeping loci for 128 isolates of *P. oryzae* sampled from sympatric populations of wheat, rice, and grasses growing in or near wheat fields. Phylogenetic analyses grouped the isolates into three major clades. Clade 1 comprised isolates associated only with rice and corresponds to the previously described rice blast pathogen *P. oryzae* pathotype *Oryza* (PoO). Clade 2 comprised isolates associated almost exclusively with wheat and corresponds to the previously described wheat blast pathogen *P. oryzae* pathotype *Triticum* (PoT). Clade 3 contained isolates obtained from wheat as well as other *Poaceae* hosts. We found that Clade 3 is distinct from *P. oryzae* and represents a new species, *Pyricularia graminis-tritici* (Pgt). No morphological differences were observed among these species, but a distinctive pathogenicity spectrum was observed. Pgt and PoT were pathogenic and highly aggressive on *Triticum aestivum* (wheat), *Hordeum vulgare* (barley), *Urochloa brizantha* (signal grass), and *Avena sativa* (oats). PoO was highly virulent on the original rice host (*Oryza sativa*), and also on wheat, barley, and oats, but not on signal grass. We conclude that blast disease on wheat and its associated *Poaceae* hosts in Brazil is caused by multiple *Pyricularia* species. *Pyricularia graminis-tritici* was recently found causing wheat blast in Bangladesh. This indicates that *P. graminis-tritici* represents a serious threat to wheat cultivation globally.

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INTRODUCTION

Pyricularia oryzae is a species complex (Couch & Kohn 2002) that causes blast disease on more than 50 species of poaceous plants, including important crops such as rice, wheat, barley, millet, and oats (Urashima & Kato 1998, Couch & Kohn 2002, Takabayashi et al. 2002, Murakami et al. 2003, Couch et al. 2005). On the basis of host specificity, mating ability, and genetic relatedness, *P. oryzae* isolates were classified into several subgroups with restricted host ranges, including: the *Oryza* pathotype, pathogenic on rice (*Oryza sativa*); the *Setaria* pathotype, pathogenic on foxtail millet (*Setaria italica*); the *Panicum* pathotype, pathogenic on common millet (*Panicum miliaceum*); the *Eleusine* pathotype, pathogenic on finger millet (*Eleusine coracana*); the *Triticum* pathotype, pathogenic on wheat (*Triticum aestivum*); the *Avena* pathotype, pathogenic on oats (*Avena sativa*); and the *Lolium* pathotype, pathogenic on perennial ryegrass (*Lolium perenne*) (Urashima et al. 1993, Kato et al. 2000, Tosa et al. 2004, Tosa & Chuma 2014). Kato and collaborators (Kato et al. 2000) reported that isolates of *P. oryzae* recovered from *Eleusine*, *Panicum*, *Oryza, Setaria,* and *Triticum* spp. form a highly related group that is partially inter-fertile with the *Oryza* subgroup (i.e. the rice blast pathogen). In addition, the *Oryza* and *Setaria* pathotypes contain physiological races that show distinct patterns of virulence on cultivars within their host species (Tosa & Chuma 2014). Both host species-specificity and cultivar-specificity can be governed by gene-for-gene interactions (Silue et al. 1992, Takabayashi et al. 2002, Tosa et al. 2006, Valent & Khang 2010).

The *P. oryzae* pathotype *Triticum* is considered the causal agent of wheat blast in South America and has also been associated with blast disease on barley, rye, triticale, and signal grass (*Urochloa* sp., ex *Brachiaria* sp.) in central-western and southern Brazil (Lima & Minella 2003, Verzignassi et al. 2012). Wheat blast was first reported in Paraná State, Brazil in 1985 (Igarashi et al. 1986, Anjos et al. 1996). Due to the lack of resistant cultivars and effective fungicides for disease management, wheat blast is widely distributed across all the wheat-cropping areas in Brazil, causing crop losses from 40–100 % (Silva et al. 2009, Maciel 2011, Castroagudín et al. 2015). Wheat blast also occurs in Bolivia, Argentina, and Paraguay (Duveiller et al. 2010). The disease was not found outside South America (Maciel 2011) until a recent outbreak reported in Bangladesh (Callaway 2016), though wheat blast is considered a major quarantine disease and a threat to wheat crops in the United States (Duveiller et al. 2007, Kohli et al. 2011).

As wheat blast emerged in an area of southern Brazil where rice blast is prevalent, it was originally proposed that the rice

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pathogen had evolved to parasitize wheat (Igarashi et al. 1986). Urashima et al. (1993) provided evidence based on pathogenicity, reproductive isolation, and genetic data that indicated the existence of two distinct groups of *P. oryzae* causing wheat blast in Brazil: one that infects rice and wheat, and one that infects only wheat. In that study, wheat-derived isolates were reported to infect grass plants from six different tribes within *Poaceae*. In addition, crosses of wheat-derived isolates with strains from *Eleusine coracana*, *Urochloa plantaginea* (ex *Brachiaria plantaginea*), and *Setaria italica* produced mature perithecia with viable ascospores, i.e. evidence of fertile crosses (Urashima et al. 1993). On the contrary, progeny from the crosses between wheat- and rice-derived isolates were infertile (Urashima et al. 1993). In the same study, crosses between wheat-derived isolates and isolates obtained from *Cenchrus echinatus*, *Setaria geniculata*, and *Echinocloa colonum* produced no perithecia (Urashima et al. 1993). The work of Urashima and his collaborators indicated that two distinct pyricularia-like pathogens cause wheat blast disease in Brazil. However, it is not clear whether a population of *P. oryzae* able to infect both rice and wheat coexists with a population that infects only wheat.

Several studies suggested that the wheat-adapted *P. oryzae* population was derived *de novo* from a non-rice host. DNA fingerprinting with the repetitive DNA probes MGR563 and MGR586 found a high level of differentiation between *P. oryzae* pathotype *Oryza* (PoO) and *P. oryzae* pathotype *Triticum* (PoT) from Brazil (Farman 2002). In fact, the fingerprints from wheat-derived isolates resembled those from isolates non-pathogenic to rice (Hamer 1991, Valent & Chumley 1991, Urashima et al. 1999, Farman 2002). Maciel et al. (2014) showed that the Brazilian wheat-adapted population of *P. oryzae* was highly differentiated (F_{CT} = 0.896, $P \leq 0.001$) from the local rice-adapted population. Analyses of the current pathotype diversity of *P. oryzae* showed that none of the 69 wheat-derived isolates were able to infect rice (Maciel et al. 2014).

Phylogenetic analyses demonstrated that *Pyricularia* is a species-rich genus in which different species evolved through repeated radiation events from a common ancestor (Hirata et al. 2007, Choi et al. 2013, Klaubauf et al. 2014). Multi-locus phylogenetic analyses revealed that *P. oryzae* and *P. grisea* are independent phylogenetic species (Taylor et al. 2000, Couch & Kohn 2002) and showed that the contemporary rice-infecting pathogen (*P. oryzae* pathotype *Oryza*) originated via a host shift from millet onto rice ~7 000 years ago during rice domestication in China (Couch et al. 2005). More recent phylogenetic analyses combined pre-existing biological and morphological data to re-examine the relationships among pyricularia-like species. These comprehensive studies favoured the classification of new cryptic species that were recently identified within *Pyricularia* and other relevant changes within the order *Magnaporthales* (Hirata et al. 2007, Choi et al. 2013, Luo & Zhang 2013, Klaubauf et al. 2014, Murata et al. 2014). Most relevant for agricultural scientists is that despite the extensively reported differentiation between *P. oryzae* pathotypes *Oryzae* and *Triticum*, these two pathotypes have been kept under the same species name *P. oryzae*. Therefore, we sought to determine whether the pathotypes *Oryza* and *Triticum* of *P. oryzae* are distinct species that should be given different names. We conducted phylogenetic analyses based on 10 housekeeping genes using sympatric populations of *Pyricularia* sampled from rice, wheat, and other poaceous hosts in Brazil. We also conducted cultural, morphological, and pathogenic characterisation of the *Pyricularia* isolates to provide a complete description for each species. Our phylogenetic analyses revealed a new *Pyricularia* species causing blast on wheat and other poaceous hosts in Brazil. We name and describe *Pyricularia graminis-tritici* in this report.

MATERIALS AND METHODS

Fungal isolates and DNA extraction

A unique collection of 128 monoconidial isolates of *Pyricularia* spp. obtained in sympatry from the Brazilian wheat agro-ecosystem was analysed in this study (Table 1). *Pyricularia* spp. isolates were obtained from *Triticum aestivum* (*N* = 79), *Oryza sativa* (*N* = 23), *Avena sativa* (*N* = 5), *Cenchrus echinatus* (*N* = 3), *Cynodon* sp. (*N* = 1), *Digitaria sanguinalis* (*N* = 4), *Elionurus candidus* (*N* = 2), *Echinochloa crusgalli* (*N* = 1), *Eleusine indica* (*N* = 1), *Rhynchelytrum repens* (*N* = 3), and *Urochloa brizantha* (ex *Bracharia brizanta*) (*N* = 6). Isolates recovered from wheat and other poaceous hosts located within or adjacent to sampled wheat plots were obtained from symptomatic head and leaf tissue in commercial wheat fields located in seven states in Brazil during 2012. A detailed description of wheat field sampling strategies was provided earlier (Castroagudín et al. 2015). The rice-derived isolates of *P. oryzae* were recovered from rice leaves, necks and panicles exhibiting typical rice blast symptoms, comprising a representative group including all races of *P. oryzae* pathotype *Oryza* prevalent in the major Brazilian rice growing areas (Maciel et al. 2014). The rice-derived isolates were provided by EMBRAPA-Rice and Beans, Santo Antônio de Goiás, Goiás, Brazil. The isolate collection is maintained at the Laboratory of Phytopathology, UNESP-DEFERS Campus Ilha Solteira, São Paulo, Brazil. A duplicate of the collection is hosted at the Laboratory of Phytopathology, EMBRAPA-Wheat, Passo Fundo, Brazil. Specimens were deposited at Culture Collection Mycobank Prof. Maria Auxiliadora Cavalcanti, Federal University of Pernambuco, Recife, Brazil, and at the Coleção de Culturas da Microbiologia Agrícola (Agriculture Microbiology Culture Collection) of the Federal University of Lavras, Lavras, Minas Gerais, Brazil. Holotype specimen was deposited at INCT-HISA Herbário Virtual da Flora e dos Fungos at UNESP – Campus Ilha Solteira (Virtual Herbarium of Flora and Fungi, University of São Paulo State – Campus Ilha Solteira, Ilha Solteira, São Paulo, Brazil).

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from freeze-dried mycelia with the GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA), according to the specifications of the manufacturer. Partial sequences of 10 nuclear housekeeping loci previously used to characterise *Pyricularia* species (Carbone & Kohn 1999, Couch & Kohn 2002, Couch et al. 2005, Zhang et al. 2011) were included in the analyses. The loci amplified were: *ACT* (actin), *BAC6* (putative vacuolar import and degradation protein), *βT-1* (beta-tubulin), *CAL* (calmodulin), *CH7-BAC7* (hypothetical protein), *CH7-BAC9* (anonymous sequence), *CHS1* (chitin synthase 1), *EF-1α* (translation elongation factor 1-alpha), *MPG1* (hydrophobin), and *NUT1* (nitrogen regulatory protein 1). The loci were amplified using PCR cycling conditions described previously (Carbone & Kohn 1999, Couch et al. 2005). The PCR primers and the annealing temperatures used to amplify each locus are described in Table 2. The PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea) using the ABI Prism BigDye Terminator v.3.1 Cycle Sequencing Ready Reaction Kit in an ABI 3730xl automated sequencer (Applied Biosystems, Foster City, CA). Newly generated DNA sequences were deposited in NCBIs GenBank nucleotide database (Table 1).

Phylogenetic analyses

The complete set of sequence data was obtained from 125 isolates of *Pyricularia* spp., including two identified as *P. pennisetigena* (URM7372 = CML3524, isolate 12.0.100) and *P. grisea* (URM7371 = CML3525, isolate 12.0.082) from Brazil, which

Table 1 Details of isolates of *Pyricularia* spp. used in this study and NCBI accession numbers.

Species, isolate	Race	Host	Origin	Sampling year	NCBI GenBank accession number									
					ACT	*BAC6*	*βT-1*	*CAL*	*CH7-BAC7*	*CH7-BAC9*	*CHS*	*EF-1α*	*MPG1*	*NUT1*
Pyricularia graminis-tritici														
12.0.038i	–[d]	*Urochloa brizantha*	Paraná	2012	KU952115	KU952241	KU952995	KU952869	KU952367	KU952492	KU953120	KU953245	KU952618	KU952744
12.0.051i	–	*Rhynchelytrum repens*	Paraná	2012	KU952116	KU952242	KU952996	KU952870	KU952368	KU952493	KU953121	KU953246	KU952619	KU952745
12.0.073	–	*Avena sativa*	Mato Grosso do Sul	2012	KU952117	KU952243	KU952997	KU952871	KU952369	KU952494	KU953122	KU953247	KU952620	KU952746
12.0.194[a,c]	–	*Elionorus candidus*	Mato Grosso do Sul	2012	KU952118	KU952244	KU952998	KU952872	KU952370	KU952495	KU953123	KU953248	KU952621	KU952747
12.0.321	–	*Avena sativa*	Mato Grosso do Sul	2012	KU952119	KU952245	KU952999	KU952873	KU952371	KU952496	KU953124	KU953249	KU952622	KU952748
12.0.326[a,b,c]	–	*Echinochloa crusgalli*	Mato Grosso do Sul	2012	KU952120	KU952246	KU953000	KU952874	KU952372	KU952497	KU953125	KU953250	KU952623	KU952749
12.0.345[a,b,c]	–	*Avena sativa*	Mato Grosso do Sul	2012	KU952121	KU952247	KU953001	KU952875	KU952373	KU952498	KU953126	KU953251	KU952624	KU952750
12.0.346	–	*Avena sativa*	Mato Grosso do Sul	2012	KU952122	KU952248	KU953002	KU952876	KU952374	KU952499	KU953127	KU953252	KU952625	KU952751
12.0.347	–	*Avena sativa*	Mato Grosso do Sul	2012	KU952123	KU952249	KU953003	KU952877	KU952375	KU952500	KU953128	KU953253	KU952626	KU952752
12.0.366[a,b,c]	–	*Urochloa brizantha*	Mato Grosso do Sul	2012	KU952124	KU952250	KU953004	KU952878	KU952376	KU952501	KU953129	KU953254	KU952627	KU952753
12.0.368[a,b]	–	*Urochloa brizantha*	Mato Grosso do Sul	2012	KU952125	KU952251	KU953005	KU952879	KU952377	KU952502	KU953130	KU953255	KU952628	KU952754
12.0.534i[a,b,c]	–	*Eleusine indica*	Paraná	2012	KU952126	KU952252	KU953006	KU952880	KU952378	KU952503	KU953131	KU953256	KU952629	KU952755
12.0.535i	–	*Cenchrus echinatus*	Paraná	2012	KU952127	KU952253	KU953007	KU952881	KU952379	KU952504	KU953132	KU953257	KU952630	KU952756
12.0.543i[a]	–	*Elionorus candidus*	Paraná	2012	KU952128	KU952254	KU953008	KU952882	KU952380	KU952505	KU953133	KU953258	KU952631	KU952757
12.0.555i[a,c]	–	*Digitaria sanguinalis*	Paraná	2012	KU952129	KU952255	KU953009	KU952883	KU952381	KU952506	KU953134	KU953259	KU952632	KU952758
12.0.578i[c]	–	*Cynodon sp.*	Paraná	2012	KU952130	KU952256	KU953010	KU952884	KU952382	KU952507	KU953135	KU953260	KU952633	KU952759
12.0.607i[a,b,c]	–	*Rhynchelytrum repens*	Paraná	2012	KU952131	KU952257	KU953011	KU952885	KU952383	KU952508	KU953136	KU953261	KU952634	KU952760
12.0.613i	–	*Rhynchelytrum repens*	Paraná	2012	KU952132	KU952258	KU953012	KU952886	KU952384	KU952509	KU953137	KU953262	KU952635	KU952761
12.0.625i	–	*Digitaria sanguinalis*	Paraná	2012	KU952133	KU952259	KU953013	KU952887	KU952385	KU952510	KU953138	KU953263	KU952636	KU952762
12.0.642i[a,b,c]	–	*Cenchrus echinatus*	Paraná	2012	KU952240	KU952366	–	KU952994	–	KU952617	–	–	KU952743	–
12.0.655i[a]	–	*Digitaria sanguinalis*	Paraná	2012	KU952134	KU952260	KU953014	KU952888	KU952386	KU952511	KU953139	KU953264	KU952637	KU952763
12.1.002	–	*Triticum aestivum*	Minas Gerais	2012	KU952135	KU952261	KU953015	KU952889	KU952387	KU952512	KU953140	KU953265	KU952638	KU952764
12.1.002i	–	*Triticum aestivum*	Paraná	2012	KU952136	KU952262	KU953016	KU952890	KU952388	KU952513	KU953141	KU953266	KU952639	KU952765
12.1.019i	–	*Triticum aestivum*	Paraná	2012	KU952137	KU952263	KU953017	KU952891	KU952389	KU952514	KU953142	KU953267	KU952640	KU952766
12.1.037[a,c]	–	*Triticum aestivum*	Goiás	2012	KU952138	KU952264	KU953018	KU952892	KU952390	KU952515	KU953143	KU953268	KU952641	KU952767
12.1.048i	–	*Triticum aestivum*	São Paulo	2012	KU952139	KU952265	KU953019	KU952893	KU952391	KU952516	KU953144	KU953269	KU952642	KU952768
12.1.049i	–	*Triticum aestivum*	São Paulo	2012	KU952140	KU952266	KU953020	KU952894	KU952392	KU952517	KU953145	KU953270	KU952643	KU952769
12.1.050i	–	*Triticum aestivum*	São Paulo	2012	KU952141	KU952267	KU953021	KU952895	KU952393	KU952518	KU953146	KU953271	KU952644	KU952770
12.1.051i	–	*Triticum aestivum*	São Paulo	2012	KU952142	KU952268	KU953022	KU952896	KU952394	KU952519	KU953147	KU953272	KU952645	KU952771
12.1.052i	–	*Triticum aestivum*	São Paulo	2012	KU952143	KU952269	KU953023	KU952897	KU952395	KU952520	KU953148	KU953273	KU952646	KU952772
12.1.053i[a]	–	*Triticum aestivum*	São Paulo	2012	KU952144	KU952270	KU953024	KU952898	KU952396	KU952521	KU953149	KU953274	KU952647	KU952773
12.1.061	–	*Triticum aestivum*	Goiás	2012	KU952145	KU952271	KU953025	KU952899	KU952397	KU952522	KU953150	KU953275	KU952648	KU952774
12.1.075	–	*Triticum aestivum*	Goiás	2012	KU952146	KU952272	KU953026	KU952900	KU952398	KU952523	KU953151	KU953276	KU952649	KU952775
12.1.109	–	*Triticum aestivum*	Federal District	2012	KU952147	KU952273	KU953027	KU952901	KU952399	KU952524	KU953152	KU953277	KU952650	KU952776
12.1.112	–	*Triticum aestivum*	Federal District	2012	KU952148	KU952274	KU953028	KU952902	KU952400	KU952525	KU953153	KU953278	KU952651	KU952777
12.1.117[a]	–	*Triticum aestivum*	Federal District	2012	KU952149	KU952275	KU953029	KU952903	KU952401	KU952526	KU953154	KU953279	KU952652	KU952778
12.1.149	–	*Triticum aestivum*	Federal District	2012	KU952150	KU952276	KU953030	KU952904	KU952402	KU952527	KU953155	KU953280	KU952653	KU952779
12.1.153	–	*Triticum aestivum*	Federal District	2012	KU952151	KU952277	KU953031	KU952905	KU952403	KU952528	KU953156	KU953281	KU952654	KU952780
12.1.191[c]	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952152	KU952278	KU953032	KU952906	KU952404	KU952529	KU953157	KU953282	KU952655	KU952781
P. oryzae pathotype *Triticum*														
12.0.007i[a]	–	*Urochloa brizantha*	Paraná	2012	KU952238	KU952364	–	KU952992	–	KU952615	–	–	KU952741	–
12.0.009i[a,b,c]	–	*Urochloa brizantha*	Paraná	2012	KU952176	KU952302	KU953056	KU952930	KU952428	KU952553	KU953181	KU953306	KU952679	KU952805
12.0.012i[a,b]	–	*Urochloa brizantha*	Paraná	2012	KU952239	KU952365	–	KU952993	–	KU952616	–	–	KU952742	–
12.1.001	–	*Triticum aestivum*	Minas Gerais	2012	KU952177	KU952303	KU953057	KU952931	KU952429	KU952554	KU953182	KU953307	KU952680	KU952806
12.1.005i	–	*Triticum aestivum*	Paraná	2012	KU952178	KU952304	KU953058	KU952932	KU952430	KU952555	KU953183	KU953308	KU952681	KU952807
12.1.007	–	*Triticum aestivum*	Minas Gerais	2012	KU952179	KU952305	KU953059	KU952933	KU952431	KU952556	KU953184	KU953309	KU952682	KU952808
12.1.009	–	*Triticum aestivum*	Minas Gerais	2012	KU952180	KU952306	KU953060	KU952934	KU952432	KU952557	KU953185	KU953310	KU952683	KU952809
12.1.010i	–	*Triticum aestivum*	Paraná	2012	KU952181	KU952307	KU953061	KU952935	KU952433	KU952558	KU953186	KU953311	KU952684	KU952810
12.1.014	–	*Triticum aestivum*	Minas Gerais	2012	KU952182	KU952308	KU953062	KU952936	KU952434	KU952559	KU953187	KU953312	KU952685	KU952811
12.1.014i	–	*Triticum aestivum*	Paraná	2012	KU952183	KU952309	KU953063	KU952937	KU952435	KU952560	KU953188	KU953313	KU952686	KU952812
12.1.015	–	*Triticum aestivum*	Minas Gerais	2012	KU952184	KU952310	KU953064	KU952938	KU952436	KU952561	KU953189	KU953314	KU952687	KU952813
12.1.020i	–	*Triticum aestivum*	Paraná	2012	KU952185	KU952311	KU953065	KU952939	KU952437	KU952562	KU953190	KU953315	KU952688	KU952814
12.1.021i	–	*Triticum aestivum*	Paraná	2012	KU952186	KU952312	KU953066	KU952940	KU952438	KU952563	KU953191	KU953316	KU952689	KU952815

Table 1 (cont.)

Species, isolate	Race	Host	Origin	Sampling year	NCBI GenBank accession number									
					ACT	*BAC6*	*βT-1*	*CAL*	*CH7-BAC7*	*CH7-BAC9*	*CHS*	*EF-1α*	*MPG1*	*NUT1*
12.1.032i[b]	–	*Triticum aestivum*	São Paulo	2012	KU952187	KU952313	KU953067	KU952941	KU952439	KU952564	KU953192	KU953317	KU952690	KU952816
12.1.034i	–	*Triticum aestivum*	São Paulo	2012	KU952188	KU952314	KU953068	KU952942	KU952440	KU952565	KU953193	KU953318	KU952691	KU952817
12.1.035	–	*Triticum aestivum*	Minas Gerais	2012	KU952189	KU952315	KU953069	KU952943	KU952441	KU952566	KU953194	KU953319	KU952692	KU952818
12.1.045i	–	*Triticum aestivum*	São Paulo	2012	KU952190	KU952316	KU953070	KU952944	KU952442	KU952567	KU953195	KU953320	KU952693	KU952819
12.1.058	–	*Triticum aestivum*	Goiás	2012	KU952191	KU952317	KU953071	KU952945	KU952443	KU952568	KU953196	KU953321	KU952694	KU952820
12.1.078	–	*Triticum aestivum*	Goiás	2012	KU952192	KU952318	KU953072	KU952946	KU952444	KU952569	KU953197	KU953322	KU952695	KU952821
12.1.085	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952193	KU952319	KU953073	KU952947	KU952445	KU952570	KU953198	KU953323	KU952696	KU952822
12.1.087	–	*Triticum aestivum*	Minas Gerais	2012	KU952194	KU952320	KU953074	KU952948	KU952446	KU952571	KU953199	KU953324	KU952697	KU952823
12.1.089	–	*Triticum aestivum*	Minas Gerais	2012	KU952195	KU952321	KU953075	KU952949	KU952447	KU952572	KU953200	KU953325	KU952698	KU952824
12.1.097	–	*Triticum aestivum*	Minas Gerais	2012	KU952196	KU952322	KU953076	KU952950	KU952448	KU952573	KU953201	KU953326	KU952699	KU952825
12.1.100	–	*Triticum aestivum*	Minas Gerais	2012	KU952197	KU952323	KU953077	KU952951	KU952449	KU952574	KU953202	KU953327	KU952700	KU952826
12.1.107	–	*Triticum aestivum*	Goiás	2012	KU952198	KU952324	KU953078	KU952952	KU952450	KU952575	KU953203	KU953328	KU952701	KU952827
12.1.116	–	*Triticum aestivum*	Federal District	2012	KU952199	KU952325	KU953079	KU952953	KU952451	KU952576	KU953204	KU953329	KU952702	KU952828
12.1.119[b]	–	*Triticum aestivum*	Federal District	2012	KU952200	KU952326	KU953080	KU952954	KU952452	KU952577	KU953205	KU953330	KU952703	KU952829
12.1.127[a]	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952201	KU952327	KU953081	KU952955	KU952453	KU952578	KU953206	KU953331	KU952704	KU952830
12.1.132[a,c]	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952202	KU952328	KU953082	KU952956	KU952454	KU952579	KU953207	KU953332	KU952705	KU952831
12.1.135	–	*Triticum aestivum*	Minas Gerais	2012	KU952203	KU952329	KU953083	KU952957	KU952455	KU952580	KU953208	KU953333	KU952706	KU952832
12.1.139	–	*Triticum aestivum*	Minas Gerais	2012	KU952204	KU952330	KU953084	KU952958	KU952456	KU952581	KU953209	KU953334	KU952707	KU952833
12.1.146	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952205	KU952331	KU953085	KU952959	KU952457	KU952582	KU953210	KU953335	KU952708	KU952834
12.1.147	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952206	KU952332	KU953086	KU952960	KU952458	KU952583	KU953211	KU953336	KU952709	KU952835
12.1.148	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952207	KU952333	KU953087	KU952961	KU952459	KU952584	KU953212	KU953337	KU952710	KU952836
12.1.158[a,b,c]	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952208	KU952334	KU953088	KU952962	KU952460	KU952585	KU953213	KU953338	KU952711	KU952837
12.1.169[a,b,c]	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952209	KU952335	KU953089	KU952963	KU952461	KU952586	KU953214	KU953339	KU952712	KU952838
12.1.174	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952210	KU952336	KU953090	KU952964	KU952462	KU952587	KU953215	KU953340	KU952713	KU952839
12.1.179[a]	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952211	KU952337	KU953091	KU952965	KU952463	KU952588	KU953216	KU953341	KU952714	KU952840
12.1.180	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952212	KU952338	KU953092	KU952966	KU952464	KU952589	KU953217	KU953342	KU952715	KU952841
12.1.181	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952213	KU952339	KU953093	KU952967	KU952465	KU952590	KU953218	KU953343	KU952716	KU952842
12.1.182	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952214	KU952340	KU953094	KU952968	KU952466	KU952591	KU953219	KU953344	KU952717	KU952843
12.1.183	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952215	KU952341	KU953095	KU952969	KU952467	KU952592	KU953220	KU953345	KU952718	KU952844
12.1.186	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952216	KU952342	KU953096	KU952970	KU952468	KU952593	KU953221	KU953346	KU952719	KU952845
12.1.187	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952217	KU952343	KU953097	KU952971	KU952469	KU952594	KU953222	KU953347	KU952720	KU952846
12.1.193	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952218	KU952344	KU953098	KU952972	KU952470	KU952595	KU953223	KU953348	KU952721	KU952847
12.1.194	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952219	KU952345	KU953099	KU952973	KU952471	KU952596	KU953224	KU953349	KU952722	KU952848
12.1.197	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952220	KU952346	KU953100	KU952974	KU952472	KU952597	KU953225	KU953350	KU952723	KU952849
12.1.204[a]	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952221	KU952347	KU953101	KU952975	KU952473	KU952598	KU953226	KU953351	KU952724	KU952850
12.1.205[a,c]	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952222	KU952348	KU953102	KU952976	KU952474	KU952599	KU953227	KU953352	KU952725	KU952851
12.1.207	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952223	KU952349	KU953103	KU952977	KU952475	KU952600	KU953228	KU953353	KU952726	KU952852
12.1.209	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952224	KU952350	KU953104	KU952978	KU952476	KU952601	KU953229	KU953354	KU952727	KU952853
12.1.213	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952225	KU952351	KU953105	KU952979	KU952477	KU952602	KU953230	KU953355	KU952728	KU952854
12.1.217	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952226	KU952352	KU953106	KU952980	KU952478	KU952603	KU953231	KU953356	KU952729	KU952855
12.1.219	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952227	KU952353	KU953107	KU952981	KU952479	KU952604	KU953232	KU953357	KU952730	KU952856
12.1.225	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952228	KU952354	KU953108	KU952982	KU952480	KU952605	KU953233	KU953358	KU952731	KU952857
12.1.228	–	*Triticum aestivum*	Rio Grande do Sul	2012	KU952229	KU952355	KU953109	KU952983	KU952481	KU952606	KU953234	KU953359	KU952732	KU952858
12.1.234	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952230	KU952356	KU953110	KU952984	KU952482	KU952607	KU953235	KU953360	KU952733	KU952859
12.1.236	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952231	KU952357	KU953111	KU952985	KU952483	KU952608	KU953236	KU953361	KU952734	KU952860
12.1.241	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952232	KU952358	KU953112	KU952986	KU952484	KU952609	KU953237	KU953362	KU952735	KU952861
12.1.243[a]	–	*Triticum aestivum*	Mato Grosso do Sul	2012	KU952233	KU952359	KU953113	KU952987	KU952485	KU952610	KU953238	KU953363	KU952736	KU952862
12.1.288	–	*Triticum aestivum*	Paraná	2012	KU952234	KU952360	KU953114	KU952988	KU952486	KU952611	KU953239	KU953364	KU952737	KU952863
12.1.291[a,b,c]	–	*Triticum aestivum*	Paraná	2012	KU952235	KU952361	KU953115	KU952989	KU952487	KU952612	KU953240	KU953365	KU952738	KU952864
12.1.311	–	*Triticum aestivum*	Paraná	2012	KU952236	KU952362	KU953116	KU952990	KU952488	KU952613	KU953241	KU953366	KU952739	KU952865
12.1.315	–	*Triticum aestivum*	Paraná	2012	KU952237	KU952363	KU953117	KU952991	KU952489	KU952614	KU953242	KU953367	KU952740	KU952866
P. oryzae pathotype *Oryza*														
97	ID-1	*Oryza sativa*	Tocantins	2007	KU952175	KU952301	KU953055	KU952929	KU952427	KU952552	KU953180	KU953305	KU952678	KU952804
284	IB-34	*Oryza sativa*	Tocantins	2007	KU952158	KU952284	KU953038	KU952912	KU952410	KU952535	KU953163	KU953288	KU952661	KU952787
323	IC-1	*Oryza sativa*	Tocantins	2006	KU952159	KU952285	KU953039	KU952913	KU952411	KU952536	KU953164	KU953289	KU952662	KU952788

364	IC-17	*Oryza sativa*	Tocantins	2007	KU952160	KU952286	KU953040	KU952914	KU952412	KU952537	KU953165	KU953290	KU952663	KU952789
421	ID-2	*Oryza sativa*	Tocantins	2007	KU952161	KU952287	KU953041	KU952915	KU952413	KU952538	KU953166	KU953291	KU952664	KU952790
611	IA-65	*Oryza sativa*	Tocantins	2007	KU952162	KU952288	KU953042	KU952916	KU952414	KU952539	KU953167	KU953292	KU952665	KU952791
641	IB-41	*Oryza sativa*	Goiás	2007	KU952163	KU952289	KU953043	KU952917	KU952415	KU952540	KU953168	KU953293	KU952666	KU952792
658	IB-9	*Oryza sativa*	Goiás	2006	KU952164	KU952290	KU953044	KU952918	KU952416	KU952541	KU953169	KU953294	KU952667	KU952793
674	IB-33	*Oryza sativa*	Goiás	2007	KU952165	KU952291	KU953045	KU952919	KU952417	KU952542	KU953170	KU953295	KU952668	KU952794
678[a,b,c]	IA-33	*Oryza sativa*	Goiás	2006	KU952166	KU952292	KU953046	KU952920	KU952418	KU952543	KU953171	KU953296	KU952669	KU952795
695	IA-41	*Oryza sativa*	Tocantins	2007	KU952167	KU952293	KU953047	KU952921	KU952419	KU952544	KU953172	KU953297	KU952670	KU952796
704[a,c]	IA-1	*Oryza sativa*	Tocantins	2007	KU952168	KU952294	KU953048	KU952922	KU952420	KU952545	KU953173	KU953298	KU952671	KU952797
706	IA-25	*Oryza sativa*	Tocantins	2007	KU952169	KU952295	KU953049	KU952923	KU952421	KU952546	KU953174	KU953299	KU952672	KU952798
8762[a,b,c]	–	*Oryza sativa*	Central Brazil	2013	KU952170	KU952296	KU953050	KU952924	KU952422	KU952547	KU953175	KU953300	KU952673	KU952799
8763	–	*Oryza sativa*	Central Brazil	2013	KU952171	KU952297	KU953051	KU952925	KU952423	KU952548	KU953176	KU953301	KU952674	KU952800
8772	–	*Oryza sativa*	Central Brazil	2013	KU952172	KU952298	KU953052	KU952926	KU952424	KU952549	KU953177	KU953302	KU952675	KU952801
8844	–	*Oryza sativa*	Central Brazil	2013	KU952173	KU952299	KU953053	KU952927	KU952425	KU952550	KU953178	KU953303	KU952676	KU952802
8847	–	*Oryza sativa*	Central Brazil	2013	KU952174	KU952300	KU953054	KU952928	KU952426	KU952551	KU953179	KU953304	KU952677	KU952803
10659[b]	–	*Oryza sativa*	Central Brazil	2013	KU952153	KU952279	KU953033	KU952907	KU952405	KU952530	KU953158	KU953283	KU952656	KU952782
10783	–	*Oryza sativa*	Central Brazil	2013	KU952154	KU952280	KU953034	KU952908	KU952406	KU952531	KU953159	KU953284	KU952657	KU952783
10877	–	*Oryza sativa*	Central Brazil	2013	KU952155	KU952281	KU953035	KU952909	KU952407	KU952532	KU953160	KU953285	KU952658	KU952784
10879	–	*Oryza sativa*	Central Brazil	2013	KU952156	KU952282	KU953036	KU952910	KU952408	KU952533	KU953161	KU953286	KU952659	KU952785
10880[a,b,c]	–	*Oryza sativa*	Central Brazil	2013	KU952157	KU952283	KU953037	KU952911	KU952409	KU952534	KU953162	KU953287	KU952660	KU952786
Outgroup isolates														
P. pennisetigena, 12.0.100		*Cenchrus echinatus*	Mato Grosso do Sul	2012	KU963214	KU963216	KU953118	KU963218	KU952490	KU963220	KU953243	KU953368	KU963222	KU952867
P. grisea, 12.0.082		*Digitaria sanguinalis*	Mato Grosso do Sul	2012	KU963215	KU963217	KU953119	KU963219	KU952491	KU963221	KU953244	KU953369	KU963223	KU952868

[a] Isolates included in the cultural and morphological characterization assays.
[b] Isolates included in the pathogenicity spectra assays.
[c] Isolates listed in the Taxonomy section as specimens examined.
[d] '–' indicates no data available.

were used as outgroups. Sequence data from the 10 loci were assembled, aligned, and concatenated using Geneious R v. 9.0.5 (Biomatters, Auckland, New Zealand) for further phylogenetic analyses.

The phylogeny for the *Pyricularia* species was reconstructed through Bayesian inference using BEAST v. 1.8.2 and in-files created with the help of BEAUti (Drummond et al. 2012). The 10-locus dataset was partitioned and the best substitution model for each locus was determined using JModelTest2 (Darriba et al. 2012). Exploratory BEAST runs were conducted to determine the optimal clock- and tree-models. Model comparisons were based on the likelihoods using the Akaike information criterion (AICM) as implemented in the program Tracer v. 1.6 (Rambaut et al. 2014). The selected nucleotide substitution model was GTR for all loci, the strict clock model and the birth-death speciation process as the tree model.

Four independent final runs were conducted with MCMC length set to 10^8 generations with sampling intervals every 1 000 generations. Runs were assessed for convergence and combined using LogCombiner v. 1.8.0, which is part of the BEAST package. Posterior sampled trees were extracted using TreeAnnotator v. 1.8.2. (Drummond et al. 2012) with the following parameters: burn-in 10 %, 0.50 posterior probability limit, maximum clade credibility target tree type, and mean node height. The final tree was visualised with FigTree v. 1.4.2 (Institute of Evolutionary Biology, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree). A phylogenetic tree was reconstructed for *MPG1* using the same settings as described for the combined tree. The resulting trees and respective alignments were deposited into TreeBASE (submission 19365). Based on the phylogenetic results, non-fixed and fixed nucleotide differences across all loci among the major clades were calculated using DnaSP (Librado & Rozas 2009).

Cultural characterisation

To examine macroscopic features, a representative subgroup of 30 isolates (Table 1) were grown on Corn Meal Agar (CMA), Malt Extract Agar (MEA), Oatmeal Agar (OA), Potato Dextrose Agar (PDA), and Synthetic Nutrient-poor Agar (SNA). All media were prepared as previously described (Crous et al. 2009) and amended with streptomycin sulphate (INLAB, São Paulo, Brazil) 0.05 g/L, and chloramphenicol (INLAB, São Paulo, Brazil) 0.05 g/L.

Stored isolates were re-activated on PDA. For this assay, a 6-mm-diam disk of colonized PDA from a 7-d-old re-activated culture was transferred to the centre of a Petri plate containing one of the media described above. Colony diameter and cultural features were assessed after 7 d of incubation at 25 °C under a 12 h dark/12 h fluorescent light regime, following the procedures described by Klaubauf et al. (2014). Three replicates were made for each isolate and the assay was conducted twice. For colony descriptions, isolates were grouped according to their clustering in the phylogenetic analyses. A general description representing the colony morphology of each group of isolates was recorded. In addition, one isolate from each group was chosen as representative of the group.

Morphological characterisation

The same subgroup of 30 isolates selected for the description of colony morphology was examined using bright field and electron microscopy to characterise fungal structures. Isolates were re-activated on CMA and incubated for 7 d at 25 °C in darkness. They were subsequently transferred to SNA with sterile barley seeds to induce sporulation and incubated for 3 wk at 25 °C under a 12 h dark/12 h fluorescent light regime. Samples were prepared following methods described previously (Bozzola & Russell 1999).

Table 2 Primers used in this study.

Locus	Forward primer (5' - 3')	Reverse primer (5' - 3')	AT (°C)[a]	Expected PCR product (bp)	Reference
ACT	ACT-34F: CGTCTTCCGTAAGTGCCC	ACT-322R: GCCCATACCAATCATGATAC	58	279	This study
BAC6	BAC6-F: ACATCATTGTCCTCCTCGTC	BAC6-R: GTTCCTGTCATTCATTTTCAA	54	283	Couch et al. 2005
βT-1	BT-26F: CCAGCTCAACTCTGATCTCC	BT-630R: GGTACTCGGAAACAAGATCG	56–58[b]	604	This study
CAL	CAL-35F: CTTACCGAAGAGCAAGTTTCCG	CAL-607R: TYTTCCTGGCCATCATGGTS	55	648	This study
CH7-BAC7	CH7-BAC7-F: AAGACACGAGAGCAAAGAAAGAAG	CH7-BAC7-R: CGATACATTACAGTGCCTACGAA	55	313	Couch et al. 2005
CH7-BAC9	CH7-BAC9-F: TGTAAGAAGCTCGGTGACTGAT	CH7-BAC7-R: AGTGTTGCTTGAACGGCTAA	59	296	Couch et al. 2005
CHS1	CHS-79F: TGGGGCAAGGATGCTTGGAAGAAG	CHS-354R: TGGAAGAACCATCTGTGAGAGTTG	55	300	Carbone & Kohn 1999
EF-1α	EF-98F: CTYGGTGTTAGGCAGCTCA	EF-820R: GAAMTTGCAGGCRATGTGGG	55	722	This study
MPG1	MPG1-F: AGATCCCCATCGACGTTCTC	MPG1-R: TCCCTCACAGAAACTCCAAAC	55	368	Couch et al. 2005
NUT1	NUT1-F: AAGTATGGCGCTTCTTCAGC	NUT1-R: GCGCATTGGTCTTTAGTGGT	55	268	Couch et al. 2005

[a] AT: Annealing temperature.
[b] AT of 56 °C was used with DNA from isolates obtained from wheat and rice, and annealing temperature of 58°C was used with DNA of isolates obtained from other poaceous hosts.

Observations were made with a Nikon SMZ25 stereo-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and a Nikon DS-Ri2 camera and software. The bright field images were taken with a Nikon SMZ1500 stereoscope microscope using NIS Elements D 3.2 software. Scanning electron microscope (SEM) images and measurements were acquired on a Zeiss LEOEVO 40 microscope using SmartSem Zeiss software (Oberkochen, Germany) operating at 10 kV and 10 to 30 mm work distance. When possible, biometric data were obtained from 30 observations per fungal structure per isolate. The photo plates were created on Corel Draw X7 software (Corel Corporation, Ottawa, Canada).

Pathogenicity spectrum

A subgroup of 18 isolates was tested for pathogenicity spectra in greenhouse assays on barley (*Hordeum vulgare*) cvs. BRS Korbel, signal grass (*Urochloa brizantha*, ex *Brachiaria brizantha*) cvs. Piatã and Marandú, oats (*Avena sativa*) cvs. EMBRAPA 29 and IAPAR 61, rice (*Oryza sativa*) cv. IRGA 409, and wheat cv. Anahuac 75. Seeds of the different hosts were planted in 10-cm-diam plastic pots filled with Tropstrato HT potting mix (Vida Verde, Mogi Mirim, São Paulo, Brazil). Fifteen seeds were planted per pot. Fifteen d after seedling emergence, pots were thinned to eight seedlings per pot for barley, signal grass, oats, and rice; and to five seedlings per pot for wheat. Pots were kept in the greenhouse under natural conditions until inoculation and watered daily from the top. Plants were fertilised with NPK 10 : 10 : 10 granular fertiliser (N : P_2O_5 : K_2O, Vida Verde, Mogi Mirim, São Paulo, Brazil). A forty gram dose of NPK granular fertiliser was sprinkled across every 100 pots 1 d after emergence. Fertilisation was repeated every 15 d until inoculation. In addition, rice plants were fertilised with a solution of 4 g/L $FeSO_4 \cdot 7H_2O$ (Dinâmica, Diadema, São Paulo, Brazil) once after emergence, with 1 L of solution applied to every 100 pots.

Isolates were recovered from long-term storage and re-activated on PDA plates and then transferred either to OA plates (rice-derived isolates) or PDA plates (wheat and other isolates originating from poaceous hosts). Fifteen plates were prepared for each isolate. Plates were incubated for 15 d at 25 °C under a 12 h dark/12 h fluorescent light regime. Mycelium was gently scraped and washed with 3–5 mL of sterile distilled water amended with Tween 80 (two drops/L) to release the spores. Conidia concentration was quantified using a Neubauer counting chamber and adjusted to 1×10^5 spores/mL for inoculation.

Pathogenicity assays were conducted on seedlings, 1-mo-old plants at growth stage 14 (Zadocks et al. 1974) on all hosts, and on immature heads of 2-mo-old wheat plants at the beginning of anthesis in growth stage 60 (Zadocks et al. 1974). Spore suspensions (1×10^5 spores/mL) were uniformly applied either onto the adaxial leaf surfaces or onto wheat heads until runoff. Fifty millilitres of spore suspension was used for every 20 inoculated pots.

Inoculated pots were placed onto plastic trays and incubated in a plant growth chamber for 7 d at 26 °C (barley, oats, rice, and wheat) or 30 °C (signal grass). Plants were kept in the dark for the first 24 h, followed by a 12 h dark/12 h fluorescent light regime. Plants were watered every other day from the bottom to avoid cross-contamination. Humidifiers were used to insure that relative humidity would stay above 85 % within the chamber during the entire experiment. Temperature and relative humidity were recorded in the chamber using an ITLOG80 Datalogger (Instrutemp, Belenzinho, São Paulo, Brazil). As negative controls, five pots of each host were mock-inoculated with sterile deionised water amended with Tween 80 (two drops/L) in each experimental replication.

Plants were examined for lesions 7 d after inoculation. For the seedling inoculation tests, the disease severity index was calculated using an ordinal scale from 0 to 5 as previously described (Urashima et al. 2005). The disease severity index (DI) was scored as follows: lesion type 0 = no visible reaction; 1 = minute, pinhead-sized spots; 2 = small brown to dark brown lesions with no distinguishable centres; 3 = small eyespot shaped lesions with grey centres; 4 = typical elliptical blast lesions with grey centres; 5 = completely dead plant. Index values 0, 1, and 2 were considered non-compatible and index values 3, 4 and 5 were considered compatible. When different types of lesions were found on a single leaf, the most abundant lesions were considered.

Disease severity on wheat heads was assessed following the procedure described by Maciel et al. (2014), calculating the percentage of each wheat head affected by blast using Assess v. 2.0 image analysis software (APS, St. Paul, Minnesota). Wheat head tissue was considered affected by blast when it was chlorotic and/or it was covered with pathogen spores. For each head, a picture from each side of the head was taken, and the percentage of affected area in the two pictures was averaged.

Seedling and head inoculation experiments were conducted using a one-factor completely randomized unbalanced design. Five pots containing five (wheat) or eight (barley, signal grass, oats, and rice) plants in the seedling tests, or five non-detached heads in the wheat-head tests were inoculated with each of the 18 isolates. The seedling inoculation experiments were conducted twice. The head inoculation experiment was conducted six times, but only two randomly chosen replicates were used for further statistical analyses. For statistical analyses,

isolates were grouped according to their phylogenetic clustering (i.e. based on the species clades identified using the 10 loci sequences).

Analyses of variance (ANOVA) were performed to evaluate the effects of experiment's replicates, *Pyricularia* species, and their interactions in the different inoculation tests. Analyses were performed independently for each host species. For non-parametric data (seedlings inoculation tests) ANOVAs were conducted using the PROC NPAR1WAY procedure computed with the Wilcoxon rank-sum test and by using Monte Carlo estimations for the exact *p*-values (*P*) with the EXACT/MC statement, at α = 0.01. A Dunn all Pairs for Joint Ranks test was used for non-parametric means comparisons. In the seedlings inoculation experiment, replicates were not significantly different (exact $P \geq 0.05$), thus the two replicates were combined for these analyses. For parametric data (wheat heads inoculation tests) ANOVAs were conducted with the PROC GLM procedure, considering species as fixed factors and isolates as random factors nested inside species factors. Fisher's protected Least Significant Difference (LSD) test was used for comparison of disease severity means for species, at α = 0.05. Since the experiment was unbalanced, the harmonic cell size was used to calculate the average LSD. The experiment effect was statistically significant ($P = 0.02$), therefore the two replicates of the experiment were analysed independently. All statistical analyses were performed with Statistical Analysis System program, v. 9.4 (SAS Institute, Cary, North Carolina)

RESULTS

Phylogenetic analyses

The final alignment for partial sequences of the 10 genes had a total length of 3 381 bases (3 301 un-gapped bases) from 125 isolates, including sequences retrieved from Brazilian isolates of *P. grisea* and *P. pennisetigena* used as outgroups. A total of 471 polymorphic sites were found, equivalent to 14.3 % of the un-gapped alignment total length, and 168 of these sites (5.1 %) were phylogenetically informative (Table 3). This resulted in 109 multilocus haplotypes, i.e. 87.2 % of isolates had a unique multilocus haplotype.

The Bayesian analyses grouped the isolates into three major phylogenetic clades (Fig. 1, 2). In the 10-locus phylogeny, Clade 1 (Bayesian posterior probability, BPP = 1) comprised isolates exclusively associated with rice and corresponds to the previously described *P. oryzae* pathotype *Oryza* (PoO). Clade 2 (BPP = 0.99) comprised isolates almost exclusively associated with wheat. A single isolate (12.0.009i) collected from signal grass plants invading a wheat field in Paraná state also clustered within this clade. This clade corresponds to the previously described *P. oryzae* pathotype *Triticum* (PoT). Clade 3 (BPP = 0.99) contained isolates obtained from wheat as well as other *Poaceae* hosts. Based on the combined evidence presented in this study, we propose that this clade is distinct from *P. oryzae* and represents a new species, *Pyricularia graminis-tritici* (Pgt).

Non-fixed and fixed nucleotide differences among the three identified phylogenetic clades were examined for each locus, excluding the outgroups (Table 3, 4). A total of 242 polymorphic sites were found, corresponding to 7.3 % of the un-gapped alignment total length. Of those sites, 120 (3.6 %) were phylogenetically informative. Four of the 10 loci (*βT-1, CH7-BAC9, EF-1α*, and *MPG1*) showed a total of 18 (0.6 %) fixed differences across the three clades (Table 4, 5). *Pyricularia graminis-tritici* could be distinguished from PoT by 14 differences at *MPG1*. These fixed differences were at the following positions: 10 (C), 13–14 (TC), 20 (A), 22–25 (CCAG), 27 (C), 33–34 (CA), 41–42 (AG), and 87 (C). Likewise, Pgt could be distinguished from PoO by 18 fixed differences. These mutations are: one fixed difference at *βT-1*: 338 (A), one at *CH7-BAC9*: 20 (C), one at *EF-1α*: 325 (T), and 15 fixed differences at *MPG1*, as follows: 4 (T), 10 (C), 13–14 (TC), 20 (A), 22–25 (CCAG), 27 (C), 33–34 (CA), 41–42 (AG), and 87 (C). PoT was differentiated from PoO only by fixed differences: one difference at *CH7-BAC9*: 20 (C) and one at *EF-1α*: 325 (T) (Table 4, 5).

Sequences for only six genes were obtained for three isolates; therefore these isolates were not included in the phylogenetic analyses. However, by analysing variation in the diagnostic genes *CH7-BAC9* and *MPG1*, we were able to assign isolate 12.0.642i to Pgt, and isolates 12.0.007i and 12.0.012i to PoT.

Table 3 Number of polymorphic sites in ten loci across *Pyricularia* species examined in this study.

Locus	Alignment length (bp)	Un-gapped sequence mean length (bp)	Polymorphic sites[a]	
			including outgroups[b]	excluding outgroups[c]
ACT	184	179	16 (2)[d]	0 (0)
BAC6	254	253	18 (0)	0 (0)
βT-1	501	500	28 (9)	19 (9)
CAL	524	520	92 (33)	12 (5)
CH7-BAC7	285	285	54 (34)	54 (34)
CH7-BAC9	293	268	40 (20)	38 (20)
CHS	229	224	78 (8)	26 (2)
EF-1α	658	643	83 (31)	66 (30)
MPG1	229	205	55 (26)	22 (16)
NUT1	224	224	7 (5)	5 (4)
Total	3381	3301	471 (168)	242 (120)

[a] Sequences of isolates 12.0.100 (*P. pennisetigena*, URM7372) and 12.0.082 (*P. grisea*, URM7371) were used as outgroups.
[b] *N* = 125.
[c] *N* = 123.
[d] The number of phylogenetically informative sites is indicated between parenthesis.

Cultural and morphological characterisation

For description of cultural and morphological characteristics, *Pyricularia* isolates were grouped according to their phylogenetic placement, following the assignments *P. graminis-tritici* (Pgt), *P. oryzae* pathotype *Triticum* (PoT) and *P. oryzae* pathotype *Oryza* (PoO).

In general, similar colony morphologies were observed for isolates of Pgt, PoT, and PoO on the five media tested. No morphological differences were observed among the *Pyricularia* species. Cultural and morphological characteristics observed for *Pyricularia graminis-tritici* and *Pyricularia oryzae* pathotypes *Triticum* and *Oryza* (Fig. 6–8, a–j) are described in the Taxonomy section.

Pathogenicity spectrum of Pyricularia spp. on wheat, barley, signal grass, oats, and rice

The replicates of the seedlings inoculation tests were combined due to the lack of experiment effect (Table 6). *Pyricularia* species caused symptoms ranging from hypersensitive response lesions composed of diminutive, 1-mm-diam brown spots (mean disease index (DI) = 1), to typical elliptical blast lesions with grey centres (> 5 mm diam), usually coalescing and causing plant death on all hosts (DI ≥ 3) (Kato et al. 2000, Cruz et al. 2016) (Fig. 3–5). This virulence variation was observed even among isolates of the same *Pyricularia* species and pathotypes, indicating the presence of host-physiological race interactions. For all tests, host seedlings or wheat heads used as negative controls showed no blast lesions on their leaves (DI = 0.00).

Table 4 Number of fixed polymorphic sites in ten loci across *Pyricularia* species.

Species, clade	Locus	*ACT*	*BAC6*	*βT-1*	*CAL*	*CH7-BAC7*	*CH7-BAC9*	*CHS*	*EF-1α*	*MPG1*	*NUT1*	Total	%[a]
	Alignment length (bp)	184	254	501	524	285	293	229	658	229	224	3381	
	Ungapped sequence mean length (bp)	179	253	500	520	285	268	224	643	205	224	3301	
P. graminis-tritici vs. *P. oryzae* pathotype *Triticum*		0	0	0	0	0	0	0	0	14	0	14	0.42
P. graminis-tritici vs. *P. oryzae* pathotype *Oryza*		0	0	1	0	0	1	0	1	15	0	18	0.55
P. oryzae pathotype *Triticum* vs. *P. oryzae* pathotype *Oryza*		0	0	0	0	0	1	0	1	0	0	2	0.06
	Total	0	0	1	0	0	1	0	1	15	0	18	0.55

[a] Percentage of fixed mutation with reference to the total number of 3301 nucleotides in the ungapped alignment.

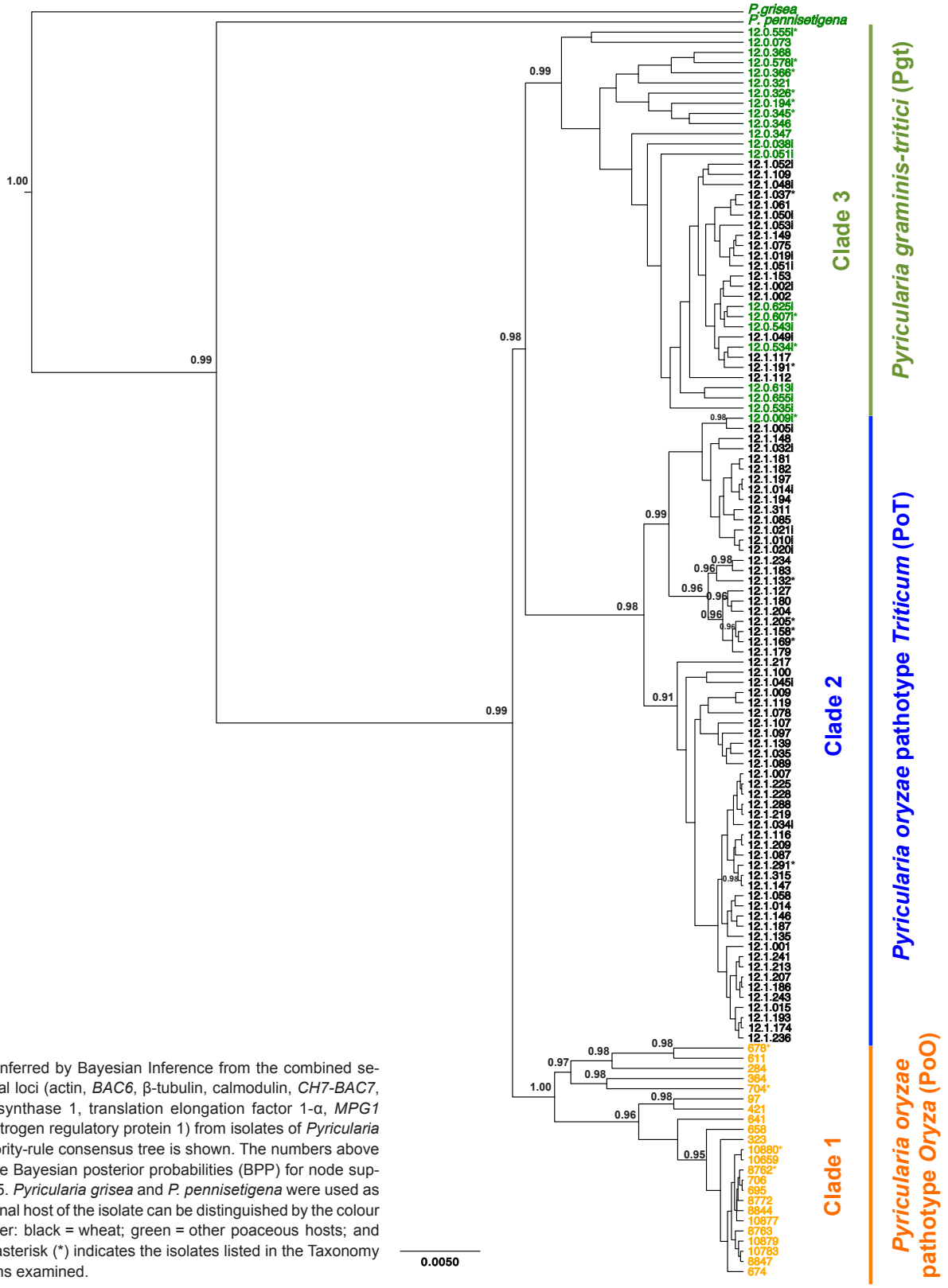

Fig. 1 Phylogeny inferred by Bayesian Inference from the combined sequences of 10 partial loci (actin, *BAC6*, β-tubulin, calmodulin, *CH7-BAC7*, *CH7-BAC9*, chitin synthase 1, translation elongation factor 1-α, *MPG1* hydrophobin, and nitrogen regulatory protein 1) from isolates of *Pyricularia* spp. The 50 % majority-rule consensus tree is shown. The numbers above the branches are the Bayesian posterior probabilities (BPP) for node support with BPP > 0.95. *Pyricularia grisea* and *P. pennisetigena* were used as outgroups. The original host of the isolate can be distinguished by the colour of the isolate number: black = wheat; green = other poaceous hosts; and orange = rice. The asterisk (*) indicates the isolates listed in the Taxonomy section as specimens examined.

Table 5 Fixed polymorphic sites in four loci across *Pyricularia* spp.

Species, clade	Locus	*βT-1*	*CH7-BAC9*	*EF-1α*	*MPG1*														
	Aligment position	776	1771	2597	2934	2940	2943	2944	2950	2952	2953	2954	2955	2957	2964	2965	2973	2974	3019
	Locus position	338	20	325	4	10	13	14	20	22	23	24	25	27	33	34	41	42	87
Pyricularia graminis-tritici		A	C	T	T	C	T	C	A	C	C	A	G	C	C	A	A	G	C
P. oryzae pathotype *Triticum*		A/C	C	T	T/C	T	C	G	C	T	T	C	–	T	T	C	–	–	A
P. oryzae pathotype *Oryza*		C	A	C	C	T	C	G	C	T	T	C	–	T	T	C	–	–	A
P. pennisetigena		A	C	C	T	A	A	T	T	A	T	C	A	T	T	C	–	G	A
P. grisea		C	C	C	A	T	T	T	C	A	T	G	G	C	C	G	A	–	A

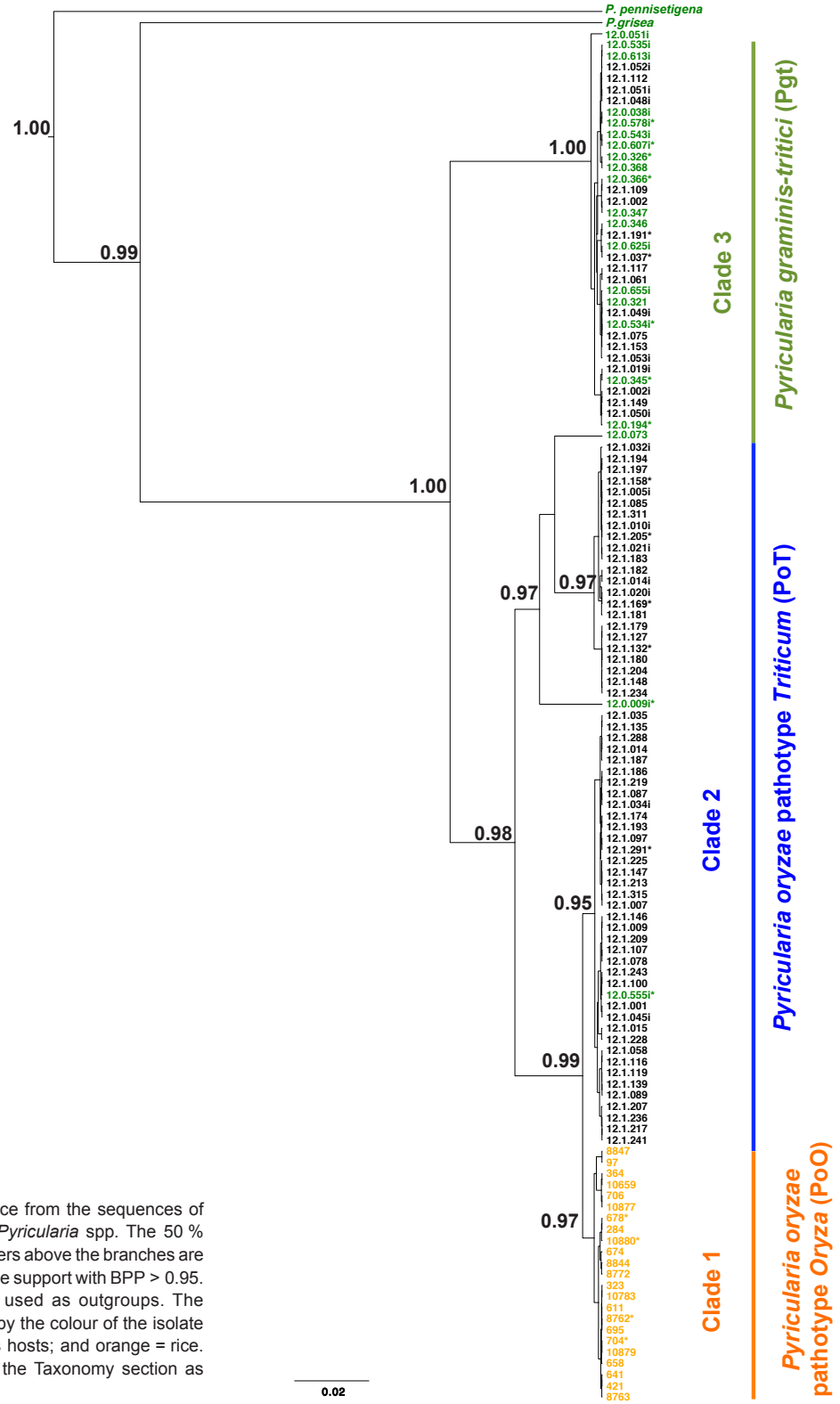

Fig. 2 Phylogeny inferred by Bayesian Inference from the sequences of the *MPG1* hydrophobin locus from isolates of *Pyricularia* spp. The 50 % majority-rule consensus tree is shown. The numbers above the branches are the Bayesian posterior probabilities (BPP) for node support with BPP > 0.95. *Pyricularia grisea* and *P. pennisetigena* were used as outgroups. The original host of the isolate can be distinguished by the colour of the isolate number: black = wheat; green = other poaceous hosts; and orange = rice. The asterisk (*) indicates the isolates listed in the Taxonomy section as specimens examined.

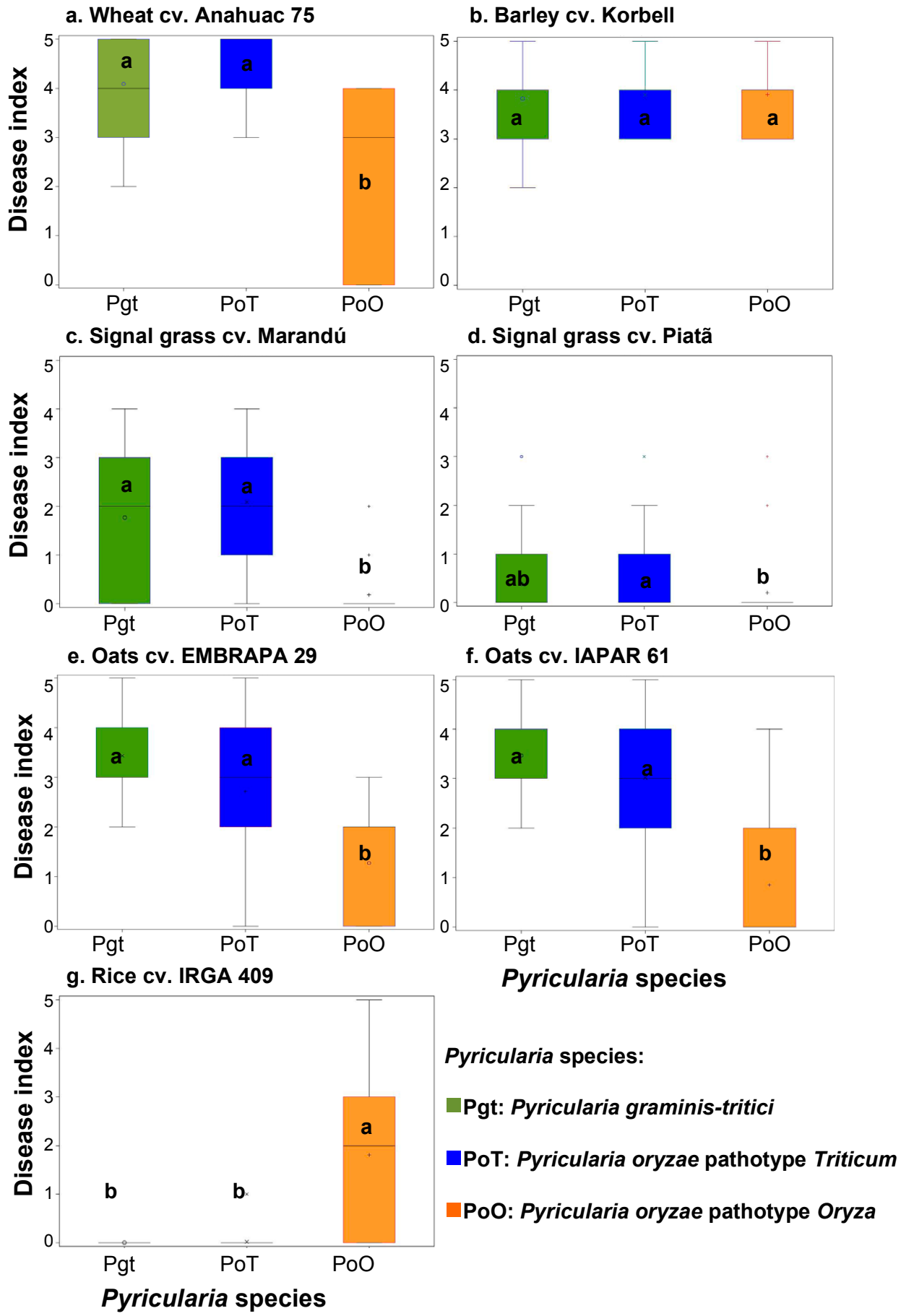

Fig. 3 Boxplot distribution of leaf blast severity of seedlings of five poaceous hosts in response to inoculations with isolates of *P. graminis-tritici* (Pgt, *N* = 7), *P. oryzae* pathotype *Triticum* (PoT, *N* = 7), and *P. oryzae* pathotype *Oryza* (PoO, *N* = 4). Boxplots represent blast severity as mean disease index assessed 7 d after inoculation using an ordinal scale from 0 to 5, and based on lesion type (Urashima et al. 2005). Disease index means with the same letter are not significantly different according to Dunn's All Pairs for Joint Ranks non-parametric test ($P > \chi 2 \leq 0.05$). a. Inoculation tests on seedlings of wheat (*Triticum aestivum*); b. barley (*Hordeum vulgare*) cv. BRS Korbell; c. signal grass (*Urochloa brizantha,* ex *Brachiaria brizanta*) cv. Marandú; d. signal grass cv. Piatã; e. oats (*Avena sativa*) cv. EMBRAPA 29; f. oats cv. IAPAR 61; g. rice (*Oryza sativa*) cv. IRGA 409.

Table 6 Pathogenicity of isolates of *Pyricularia* spp. on seedlings of five poaceous hosts.

Species	Host	Mean scores for disease index[a]						
		Wheat	Barley	Signal grass		Oat		Rice
	Cultivar	Anahuac 75	BRS Korbell	Marandú	Piatã	EMBRAPA 29	IAPAR 61	IRGA 409
Pyricularia graminis-tritici (*N* = 7)		4.0882 a	3.8286 a	1.7612 a	0.3857 ab	3.4328 a	3.4627 a	0.0000 b
P. oryzae pathotype *Triticum* (*N* = 7)		4.4857 a	3.8986 a	2.0882 a	0.4714 a	2.7121 a	3.0145 a	0.0143 b
P. oryzae pathotype *Oryza* (*N* = 4)		2.0000 b	3.9143 a	0.1750 b	0.2051 b	1.2750 b	0.8500 b	1.8000 a
Species effect								
χ^2		80.6093	0.5303	48.8753	2.9844	56.0390	81.2610	92.7152
$P > \chi^2$		< 0.0001	0.7671	< 0.0001	0.2249	< 0.0001	< 0.0001	< 0.0001
Experiment effect								
χ^2		1.8216	3.9535	0.5244	2.9081	2.3851	0.3639	0.7286
$P > \chi^2$		0.1771	0.0500	0.4690	0.0881	0.1225	0.5463	0.3934

[a] Mean disease index was averaged over five repetitions per test, and two test replicates were conducted. Each repetition (pot) had five seedlings for wheat, and eight seedlings for the other hosts. Disease index was assessed 7 d after inoculation using an ordinal scale from 0 to 5, and based on lesion type (Urashima et al. 2005). In this scale, 0 = no visible reaction; 1 = minute, pinhead-sized spots; 2 = small brown to dark brown lesions with no distinguishable centers; 3 = small eyespot shaped lesions; with grey centers; 4 = typical elliptical blast lesions with grey centers; 5 = complete dead plant. Disease index means with the same letter are not significantly different according to Dunn's All Pairs for Joint Ranks non-parametric test ($P > \chi^2 \leq 0.05$).

Table 7 Pathogenicity of isolates of *Pyricularia* spp. on non-detached heads of wheat (*Triticum aestivum*) cv. Anahuac 75.

Species, clade	Disease index (% head affected area)[a]			
	Experiment 1		Experiment 2	
	Least Mean Square	Standard Error	Least Mean Square	Standard Error
Pyricularia graminis-tritici (*N* = 7)	57.0364 a	1.6566	47.9202 a	2.3065
P. oryzae pathotype *Triticum* (*N* = 7)	39.7740 b	1.6996	43.6509 a	2.3065
P. oryzae pathotype *Oryza* (*N* = 4)	2.1330 c	2.1241	8.3485 b	2.8691
Species effect				
F	209.0400		65.2000	
P	< 0.0001		< 0.0001	
LSD	5.123		7.016	

[a] Disease index was calculated as the percentage of the wheat head affected by blast using Assess v. 2.0 Image Analysis software. Head tissue was considered diseased when it was chlorotic and/or covered in pathogen spores. Disease was assessed 7 d after inoculation. Mean disease index was averaged over five repetitions (wheat heads) for each test replicate. The inoculation experiment was conducted twice, and replicates were analyzed independently due to significant experiment effect ($P = 0.0170$). Disease index means with the same letter are not significantly different according to Fisher's protected Least Significant Difference (LSD) test at $P \leq 0.05$.

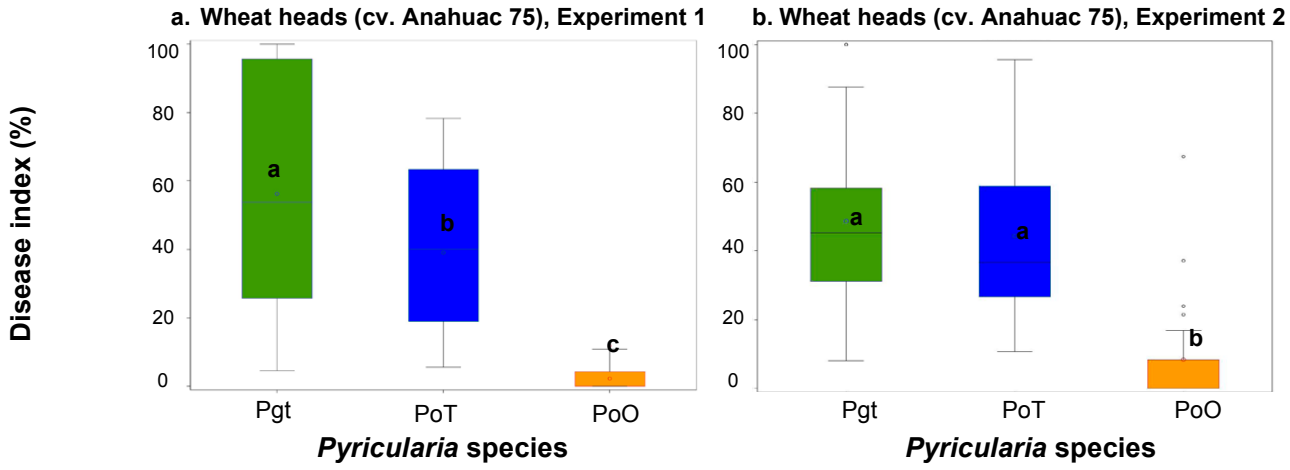

Fig. 4 Boxplot distribution of blast severity observed on heads of wheat (*Triticum aestivum*) cv. Anahuac after inoculations with isolates of *P. graminis-tritici* (Pgt, *N* = 7), *P. oryzae* pathotype *Triticum* (PoT, *N* = 7), and *P. oryzae* pathotype *Oryza* (PoO, *N* = 4). Heads were not detached from the plant. Boxplots represent blast severity as mean disease index assessed 7 d after inoculation as percentage wheat head affected by blast using Assess v. 2.0 Image Analysis software. Head tissue was considered diseased when it was chlorotic and/or covered in pathogen spores. The test was conducted twice, and replicates (experiment 1 and 2) were analysed independently (a, b). Disease index means with the same letter are not significantly different according to Fisher's protected Least Significant Difference test at $P \leq 0.05$.

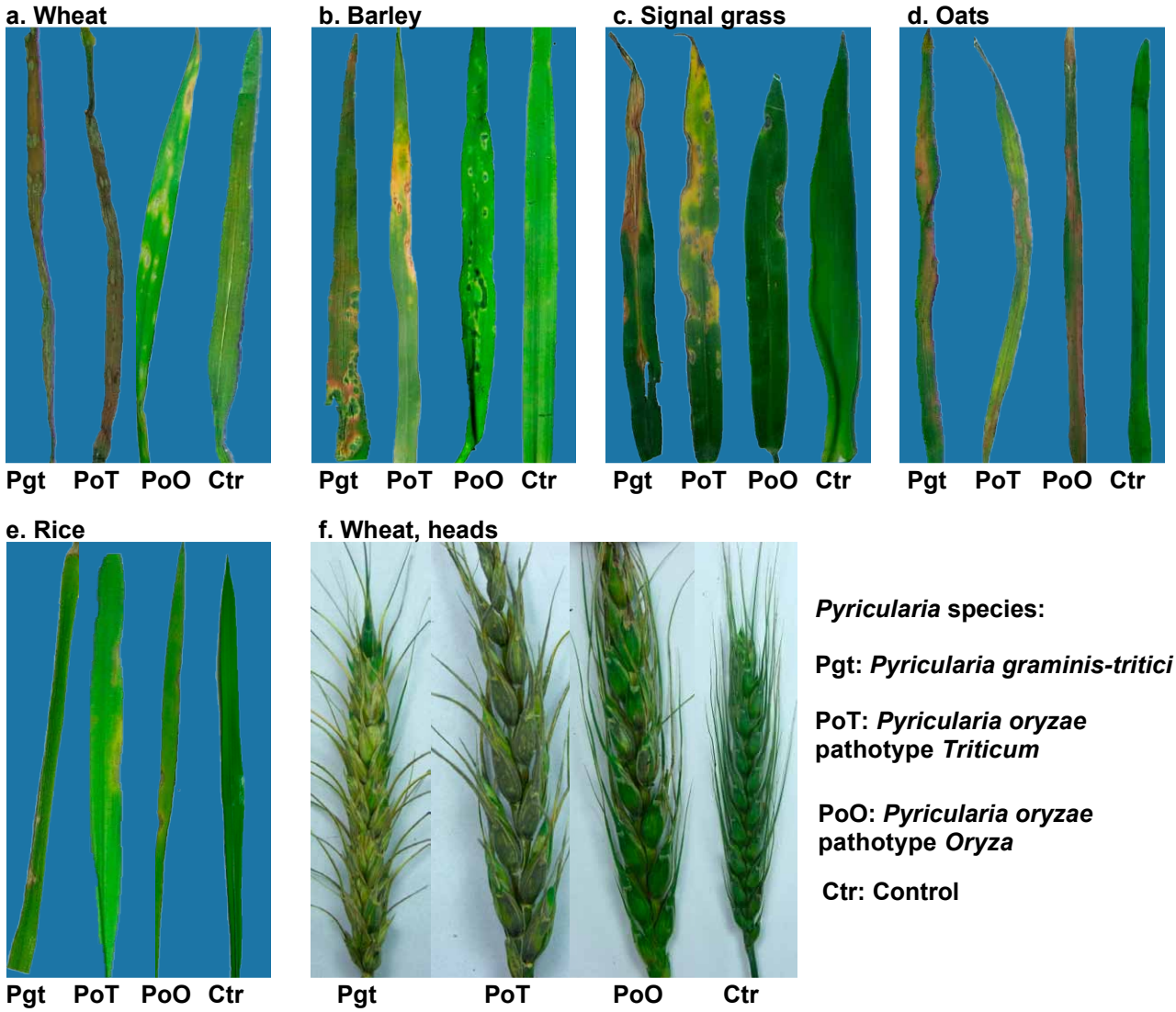

Fig. 5 Blast symptoms on leaves and heads of poaceous host after inoculation with *Pyricularia* species. Inoculated hosts: a and f. wheat (*Triticum aestivum*); b. barley (*Hordeum vulgare*); c. signal grass (*Urochloa brizantha,* ex *Brachiaria brizantha*); d. oats (*Avena sativa*); e. rice (*Oryza sativa*). *Pyricularia* species: *Pyricularia graminis-tritici* (Pgt), *P. oryzae* pathotype *Triticum* (PoT), and *P. oryzae* pathotype *Oryza* (PoO). Control plants (Ctr) were inoculated with sterile deionized water amended with Tween 80 (2 drops/L). Plants were assessed for disease symptoms 7 d after inoculation.

Inoculation tests on seedlings of wheat cv. Anahuac 75 showed significant differences among *Pyricularia* species in pathogenicity ($P > \chi^2 < 0.0001$). Seedlings were highly susceptible to isolates of PoT and Pgt (DIs of 4.48 and 4.09, respectively). In addition, isolates of PoO caused lesions on wheat seedlings (DI = 2.00); however, conspicuous differences were observed in the levels of virulence of isolates of this group. Isolates 8762 and 10659 sporadically produced lesions that ranged from minute, pinhead-sized spots (type 1 lesion) to small eyespot shaped lesions with grey centres (type 3 lesions). On the other hand, isolates 678 and 10880 consistently produced typical elliptical blast lesions with grey centres (type 4 lesions) (Fig. 3a, 5a).

Seedlings of barley cv. BRS Korbell did not show significant differences in their susceptible response to the inoculated *Pyricularia* species ($P > \chi^2 = 0.7671$). All species were highly virulent on this host (DIs ≥ 3.82), showing that barley is very susceptible to both wheat and rice blast pathogens (Fig. 3b, 5b).

Inoculations on signal grass seedlings showed that cv. Marandú was more susceptible to *Pyricularia* species than cv. Piatã. On cv. Marandú, PoT (DI = 2.08) showed the highest level of virulence, but it was not significantly different from Pgt (DI = 1.76). PoO was not pathogenic on this cultivar (DI = 0.18). None of the species were pathogenic on signal grass cv. Piatã (DIs ranged from 0.21 to 0.47, and were not significantly different at $P > \chi^2 = 0.2249$) (Fig. 3c, d, 5c).

Inoculation tests on oats showed similar seedling reactions for cvs. EMBRAPA 29 and IAPAR 61. Both Pgt and PoT had similar, high average levels of aggressiveness with DIs > 2.71 for cv. EMBRAPA 29 and DI > 3.01 for cv. IAPAR 61. Furthermore, significant differences in the level of aggressiveness of individual isolates of these species were observed. The most aggressive isolates on oats cv. EMBRAPA 29 were 12.0.534i (Pgt), 12.1.169 and 12.1.119 (both PoT), and the least aggressive isolates were 12.0.607i (Pgt), 12.1.032i and 12.1.291 (both PoT). Likewise, on cv. IAPAR 61 the most aggressive isolates were 12.0.607i (Pgt), 12.1.158 and 12.1.119 (both PoT), and the least aggressive isolates were 12.0.642i (Pgt), 12.0.009i and 12.1.291 (both PoT). Isolates of PoO showed the lowest level of aggressiveness on oats (DI = 1.28 on cv. EMBRAPA 29, and 0.85 on cv. IAPAR 61), significantly lower ($P > \chi^2 < 0.0001$) compared to PoT and Pgt. Differences in virulence among isolates of PoO were significant only on cv. IAPAR 61, on which isolate 10659 was the most aggressive while isolate 8762 was not pathogenic (Fig. 3e, f, 5d).

Inoculation tests on rice seedlings showed generally low levels of disease severity. On cultivar IRGA 409, PoO was pathogenic

with a mean DI = 1.80 which was significantly different from the DI of the other two species ($P > \chi^2 < 0.0001$). Pgt and PoT were not pathogenic on rice (DI = 0.00 and DI = 0.01, respectively). PoO isolates showed a wide range of aggressiveness. Whereas isolates 8762 and 10880 consistently produced small eyespot-shaped lesions with grey centres (type 3 lesions) and sporadically typical elliptical blast lesions (type 4 lesions), isolate 678 produced small dark brown lesions with no distinguishable centres (type 2 lesions) and isolate 10659 sporadically produced type 2 lesions or no lesions at all on cv. IRGA 409 (Fig. 3h, 5e). This variation in virulence among the isolates is consistent with race-cultivar interactions.

A significant experiment effect was observed in the wheat head inoculation tests ($P = 0.02$). Therefore, statistical analyses of the two test replicates were conducted independently (Table 7, Fig. 4, 5f). The mean disease indexes obtained for PoT and PoO were higher in the second experiment; nevertheless, results from both experiments were congruent. All species tested were pathogenic on heads of wheat cv. Anahuac 75 and significant differences were found in their levels of aggressiveness ($P < 0.0001$ for both experiment 1 and experiment 2). Pgt was the most aggressive species, followed by PoT (Table 7). Isolates of PoO were able to infect wheat heads, but the disease did not progress to more than 10 % of the head of cv. Anahuac 75. However, similar to the seedling inoculation tests, PoO isolate 10880 was very aggressive on wheat heads, infecting 20–60 % of the inoculated heads (mean DI = 33.39 %; Fig. 4, 5f).

TAXONOMY

Pyricularia graminis-tritici V.L. Castroagudín, S.I. Moreira, J.L.N. Maciel, B.A. McDonald, Crous & P.C. Ceresini, *sp. nov*. — MycoBank MB816086; Fig. 6

Etymology. Referring to the major association of this fungal species with multiple grasses, and to the most common cultivated species this fungal species infects causing blast, *Triticum aestivum*.

Typus. Brazil, Goiás, isolated from head of *Triticum aestivum*, 2012, *J.L.N. Maciel* (holotype HISA 10298, culture ex-type URM7380 = CML 3547 = isolate 12.1.037).

On SNA on sterile barley seeds — *Mycelium* consisting of smooth, hyaline, branched, septate hyphae, 2–3 µm diam. *Conidiophores* solitary, erect, straight or curved, unbranched, 1–5-septate, medium brown, smooth, (14–)125(–255) × (1–)3.5(–6) µm. Abundant conidiogenesis observed on the top half of the conidiophore. *Conidiogenous cells* 50–80(–170) × 3–5 µm, terminal and intercalary, pale brown, smooth, forming a rachis with sympodial proliferation, with several protruding denticles, 1–2 µm long, 1.5–2 µm diam. *Conidia* solitary, pyriform to obclavate, pale brown, finely verruculose, granular to guttulate, 2-septate, (23–)25–29(–32) × (8–)9(–10) µm; apical cell 10–13 µm height, basal cell 6–9 µm long; frill hilum, protruding, 1–1.5 µm long, 1.5–2 µm diam, unthickened, not darkened; central cell turning dark brown with age. *Chlamydospores* and *microconidia* not observed.

Culture characteristics — Colonies on CMA with moderate dark grey aerial mycelium, irregular margins, reaching up to 6.5 cm diam after 1 wk; reverse dark grey. Colonies on MEA with abundant white aerial mycelium, and pale grey sporulation at the centre; reaching up to 7.6 cm diam after 1 wk; reverse dark grey; sometimes, fewer colonies (5.1 cm diam) with dark grey sporulation at centre and abundant white aerial mycelium at margins. Colonies on OA with dark grey sporulation in concentric circles, with sparse margins, up to 5.8 cm; reverse pale grey; sometimes, larger growth with abundant white aerial mycelium, pale grey at the centre. Colonies on PDA with abundant white aerial mycelium, olivaceous at centre, growth in concentric circles, up to 6.5 cm diam; reverse black in centre with white margins. Colonies on SNA with sparse olivaceous mycelium irregular margins, up to 5.2 cm diam; reverse sparse olivaceous.

Specimens examined. Brazil, Goiás, isolated from head of *Triticum aestivum*, 2012, *J.L.N. Maciel* (URM7380, isolate 12.1.037); Mato Grosso do Sul, isolated from leaves of *Avena sativa*, 2012, *J.L.N. Maciel* (URM7366 = CML3516, isolate 12.0.345); Mato Grosso do Sul, isolated from leaves of *Echinochloa crusgalli*, 2012, *J.L.N. Maciel* (URM7381, isolate 12.0.326); Mato Grosso do Sul, isolated from leaves of *Elionorus candidus*, 2012, *J.L.N. Maciel* (URM7377, isolate 12.0.194); Mato Grosso do Sul, isolated from leaves of *Urochloa brizantha*, 2012, *J.L.N. Maciel* (URM7367 = CML3517, isolate 12.0.366); Paraná, isolated from leaves of *Cenchrus equinatus*, 2012, *J.L.N. Maciel* (URM7378, isolate 12.0.642i); Paraná, isolated from leaves of *Cynodon* spp., 2012, *J.L.N. Maciel* (URM7375, isolate 12.0.578i); Paraná, isolated from leaves of *Digitaria sanguinalis*, 2012, *J.L.N. Maciel* (URM7376, isolate 12.0.555i); Paraná, isolated from leaves of *Eleusine indica*, 2012, *J.L.N. Maciel* (URM7365 = CML3518, isolate 12.0.534i); Paraná, isolated from leaves of *Rhynchelytrum repens,* 2012, *J.L.N. Maciel* (URM7384, isolate 12.0.607i); Rio Grande do Sul, isolated from head of *T. aestivum*, 2012, *J.L.N. Maciel* (URM7387, isolate 12.1.191).

Notes — *Pyricularia graminis-tritici* causes blast disease on *Triticum aestivum*, *Avena sativa*, *Hordeum vulgare,* and *Urochloa brizantha* but not on *Oryza sativa*.

Based on morphological and cultural comparisons, isolates of *P. graminis-tritici* are indistinguishable from those of *P. oryzae* pathotypes *Oryza* and *Triticum.* However, these taxa are readily distinguished based on their DNA phylogeny, host range and pathogenicity spectra. Sequencing of the *MPG1* gene is a diagnostic tool to distinguish *P. graminis-tritici* from *P. oryzae*.

Pyricularia oryzae Cavara, Fungi Longobard. Exsicc. 1: no. 49. 1891

= *Magnaporthe oryzae* B.C. Couch, Mycologia 94: 692. 2002.

Pyricularia oryzae* pathotype *Triticum (Kato et al. 2000) — Fig. 7

On SNA on sterile barley seeds — *Mycelium* consisting of smooth, hyaline, branched, septate hyphae, 1.5–2 µm diam. *Conidiophores* solitary, erect, straight or curved, unbranched, medium brown, smooth, 60–150 × 4–6 µm, 2–3-septate; base arising from hyphae, not swollen, lacking rhizoids. *Conidiogenous cells* 40–95 × 3–5 µm, integrated, terminal and intercalary, pale brown, smooth, forming a rachis with several protruding denticles, 0.5–1 µm long, 1.5–2 µm diam. *Conidia* solitary, pyriform to obclavate, pale brown, smooth, granular to guttulate, 2-septate, (25–)27–29(–32) × (8–)9(–10) µm; apical cell 10–13 µm long, basal cell 6–9 µm long; hilum truncate, protruding, 1–1.5 µm long, 1.5–2 µm diam, unthickened, not darkened. *Chlamydospores* and *microconidia* not observed (based on isolate CPC 26580 = 12.1.132).

Culture characteristics — On CMA colonies with moderate dark grey aerial mycelium with irregular margins, sometimes with black aerial mycelium with sporulation in concentric circles, or sparse white mycelial colonies, reaching up to 5.9 cm diam after 1 wk; reverse dark grey with brown margins. On MEA, colonies presented different forms: cottony white aerial mycelia within concentric growth rings, sometimes with a grey sporulation at the centre, reaching up to 6.9 cm diam after 1 wk; reverse dark grey. Colonies on OA with grey aerial mycelium and sporulation in concentric circles; sometimes surface mycelia were white or cream, showing concentric growth, up to 7.9 cm diam; reverse dark grey; sometimes, larger growth with abundant white aerial mycelium, pale grey at the centre. PDA colonies exhibited many variations in culture, often with concentric growth: abundant white aerial mycelia and pale grey sporulation at centre; abundant white aerial mycelia; or

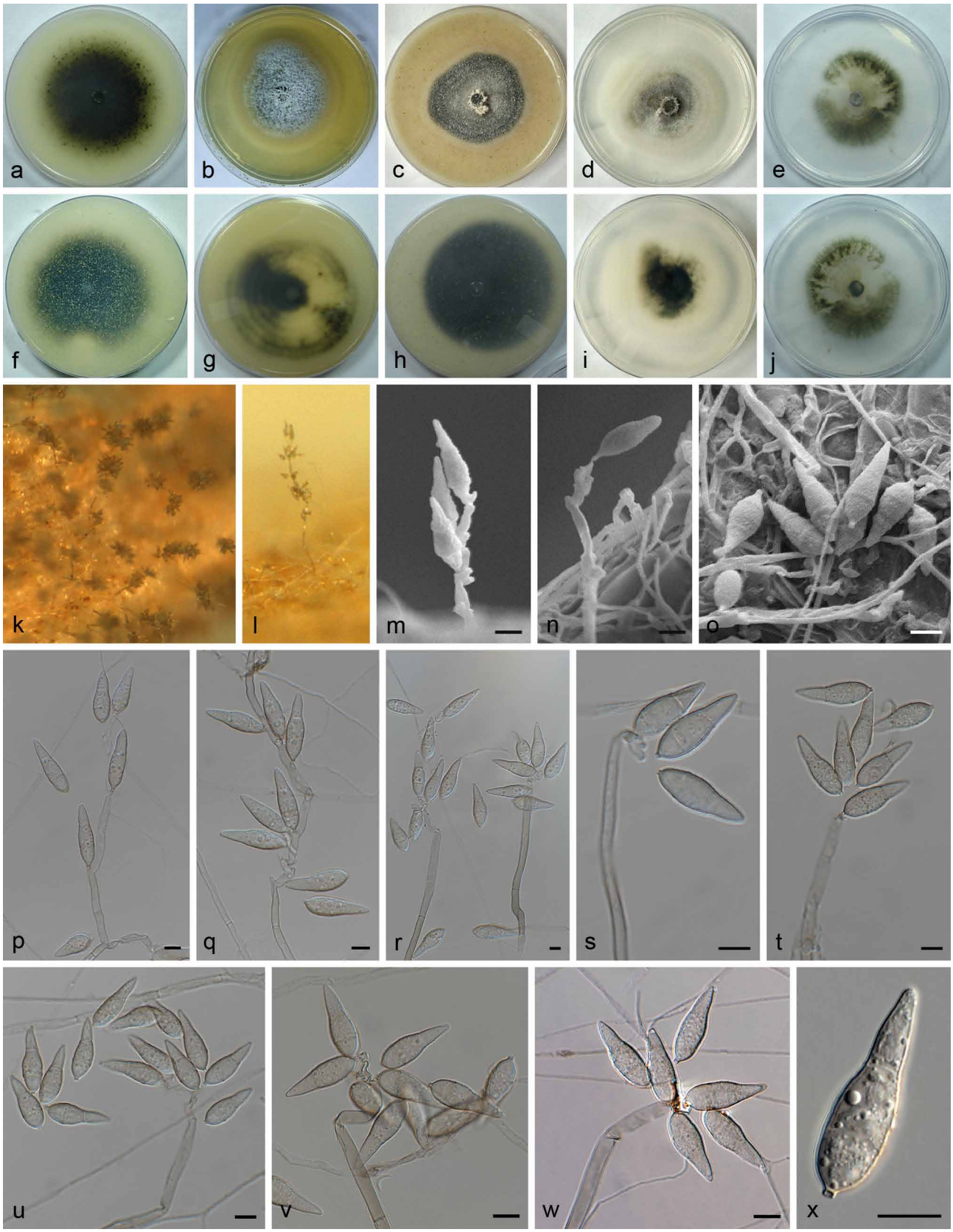

Fig. 6 *Pyricularia graminis-tritici.* a–j. Cultures of isolate 12.1.037 grown for 7 d at 12 h photoperiod and 25 °C in CMA (a, f), MEA (b, g), OA (c, h), PDA (d, i), and SNA (e, j) media; k–l. sporulation on SNA on sterile barley seeds; m–o. scanning electron micrographs of conidiophores and conidia; p–x. bright field microscopy images of conidiophores and conidia. — Scale bars = 10 µm.

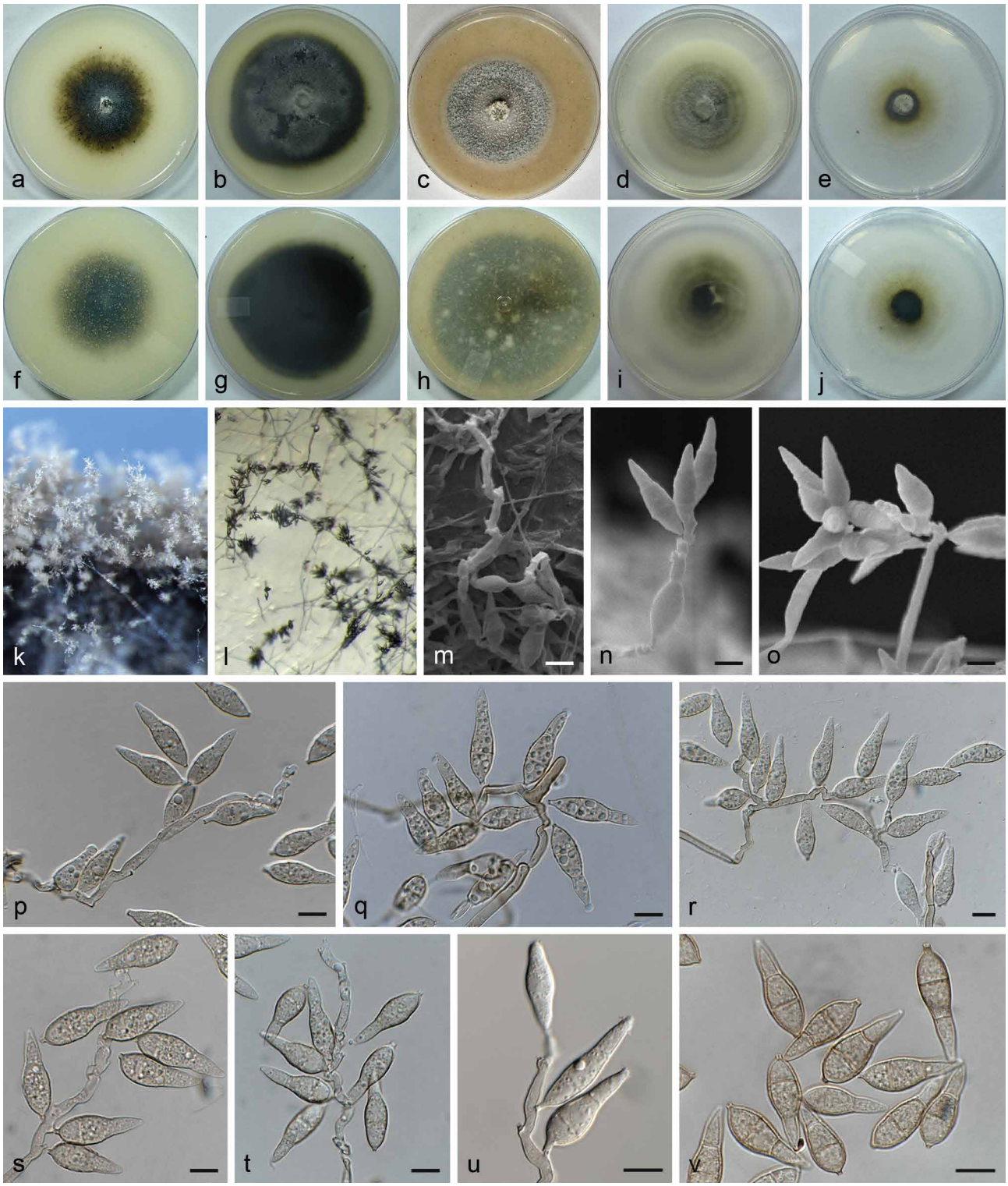

Fig. 7 *Pyricularia oryzae* pathotype *Triticum*. a–j. Cultures of isolate 12.1.291 grown for 7 d at 12 h photoperiod and 25 °C in CMA (a, f), MEA (b, g), OA (c, h), PDA (d, i), and SNA (e, j) media; k–l. sporulation on SNA on sterile barley seeds; m–o. scanning electron micrographs of conidiophores and conidia; p–v. bright field microscopy images of conidiophores and conidia. — Scale bars = 10 µm.

dark grey mycelia at the bottom, with white aerial mycelia up to 7 cm diam; reverse, concentric growth, black in centre with olivaceous margins. On SNA the colonies with dark green centres with sparse pale brown margins; or pale grey at the centre and sparse pale brown margins; reverse dark green to black at the centre and with pale brown margins.

Specimens examined. Brazil, Mato Grosso do Sul, isolated from head of *Triticum aestivum*, 2012, *J.L.N. Maciel* (URM7388, isolate 12.1.132); Mato Grosso do Sul, isolated from head of *T. aestivum*, 2012, *J.L.N. Maciel* (URM7368 = CML3521, isolate 12.1.158); Mato Grosso do Sul, isolated from head of *T. aestivum*, 2012, *J.L.N. Maciel* (URM7386, isolate 12.1.169); Paraná, isolated from head of *T. aestivum*, 2012, *J.L.N. Maciel* (URM7369 = CML3522, isolate 12.1.291); Paraná, isolated from leaves of *Urochloa brizantha*, 2012, *J.L.N. Maciel* (URM7385, isolate 12.0.009i); Rio Grande do Sul, isolated from head of *T. aestivum*, 2012, *J.L.N. Maciel* (URM7389, isolate 12.1.205).

Pyricularia oryzae **pathotype *Oryza*** (Kato et al. 2000) — Fig. 8

On SNA on sterile barley seeds — *Mycelium* consisting of smooth, hyaline, branched, septate hyphae, 2–3 µm diam. *Conidiophores* were (70.5–)146.5(–247) × (3.5–)4.5(–5.5) µm, solitary, erect, straight or curved, septate, hyaline, sometimes light brown. Sometimes, the conidiophores branched. Conidio-

genous cells apical and intercalary, sporulating frequently at the apical part, with protruding denticles 0.9–1.1 µm long. *Conidia* pyriform to obclavate, narrowed towards the tip, rounded at the base, 2-septate, hyaline to pale olivaceous, (18–)24–28(–32) × (8–)9(–10) µm; apical cell 7–14 µm long, basal cell 7–12 µm long; hilum 1.5–2 µm diam. *Chlamydospores* and *microconidia* not observed.

Culture characteristics — On CMA the predominant colony morphology was the moderate pale grey aerial mycelium with irregular margins reaching up to 5.6 cm diam after 1 wk; reverse dark grey centre and grey edges; fewer colonies with regular margin formed by sparse white aerial mycelia; sometimes, moderate dark grey aerial mycelium with irregular margins; or white aerial mycelium. Colonies on MEA were often pale grey, sporulation in concentric circles, with dark grey margins; sometimes dark grey at the bottom with sparse white aerial mycelia; or white colonies with regular margins, dark grey at the centre, reaching up to 7.6 cm diam after 1 wk; reverse dark grey. On OA colonies with dark grey sporulation at centre and regular margins of white aerial mycelia up to 7.3 cm. PDA colonies were variable, with grey growth in concentric circles, sometimes pale grey or olivaceous; in some cases, with regular

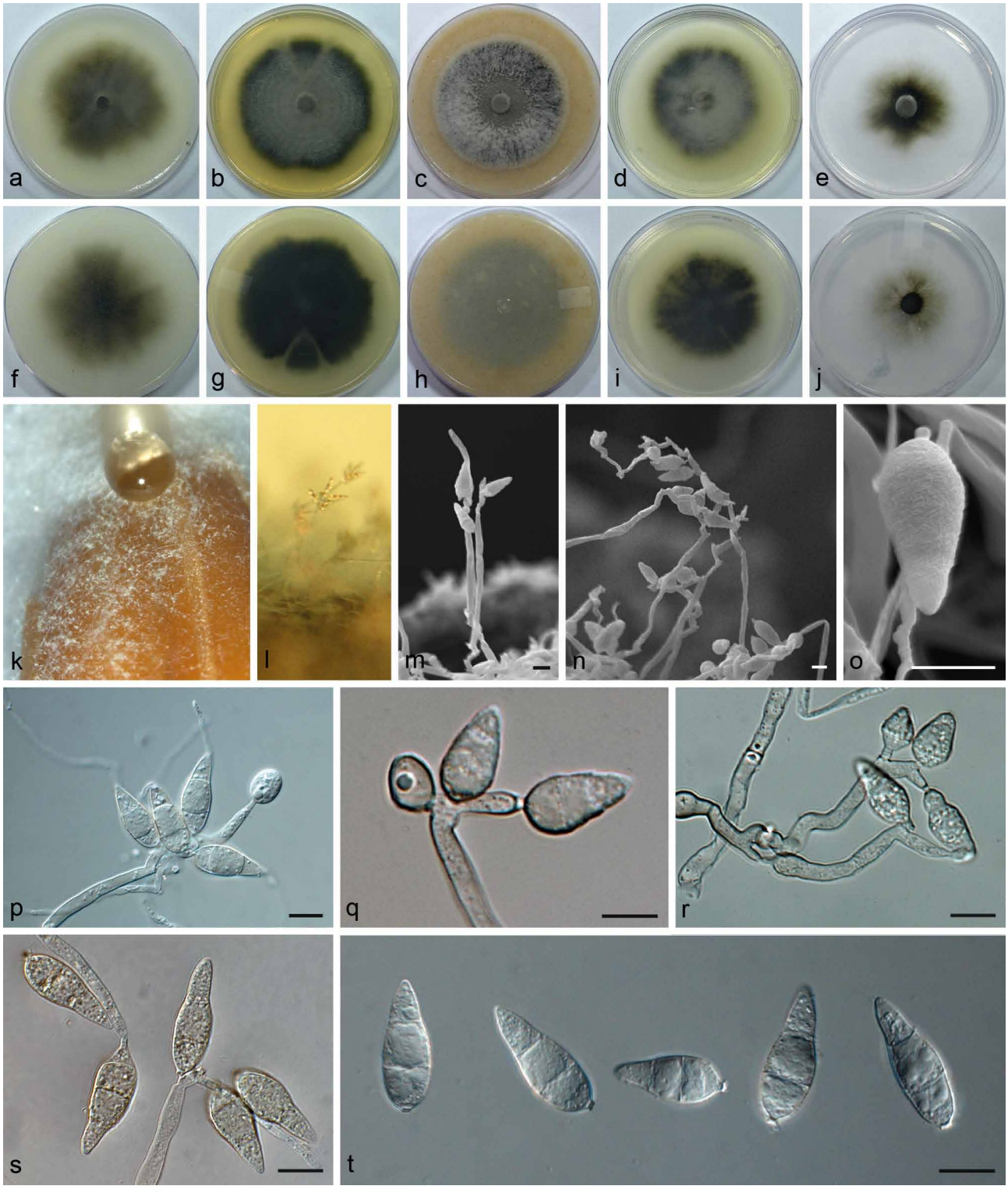

Fig. 8 *Pyricularia oryzae* pathotype *Oryza*. a–j. Cultures of isolate 10880 grown for 7 d at 12 h photoperiod and 25 °C in CMA (a, f), MEA (b, g), OA (c, h), PDA (d, i), and SNA (e, j) media; k–l. sporulation on SNA on sterile barley seeds; m–o. scanning electron micrographs of conidiophores and conidia; p–t. bright field microscopy images of conidiophores and conidia. — Scale bars = 10 µm.

margins of white mycelia, reaching up to 6.4 cm; reverse dark grey. On SNA colonies with pale green or dark green mycelia, with sparse margins; in rare cases with abundant pale grey aerial mycelia at centre and white mycelia in regular margins, up to 3.1 cm; reverse dark green in centre and olivaceous at the borders.

Specimens examined. Brazil, Central Brazil, isolated from leaves of *Oryza sativa*, 2013, *Unknown* (URM7382, isolate 8762); Central Brazil, isolated from leaves of *O. sativa*, 2013, *Unknown* (URM7370 = CML3523, isolate 10880); Goiás, isolated from leaves of *O. sativa*, 2006, *Unknown* (URM7379, isolate 678); Tocantins, isolated from leaves of *O. sativa*, 2007, *Unknown* (URM7383, isolate 704).

DISCUSSION

We conducted comprehensive phylogenetic, morphological, and pathogenicity analyses to characterise *Pyricularia* isolates associated with the blast disease on rice, wheat and other poaceous hosts from the Brazilian agro-ecosystem. Urashima, Igarashi & Kato (1993) demonstrated that the blast pathogens infecting wheat and rice were distinct. These authors also reported that isolates recovered from wheat did not infect rice and that most isolates recovered from rice did not infect wheat, except for a few isolates capable of producing small leaf lesions. Although Urashima & Kato (1998), and several follow-up studies demonstrated that the wheat and rice pathogens were phenotypically and genetically different, they have been treated as subgroups of the same species: *Pyricularia oryzae* (Urashima & Kato 1998, Kato et al. 2000, Murakami et al. 2000, Couch & Kohn 2002, Farman 2002, Klaubauf et al. 2014, Chiapello et al. 2015).

The results of our phylogenetic analyses indicate that wheat blast is caused by *Pyricularia* strains assigned to Clade 2, previously described as *P. oryzae* pathotype *Triticum,* and to Clade 3 (Fig. 1, Table 5). Here, we propose that Clade 3 is distinct from *P. oryzae* and represents a new species, *Pyricularia graminis-tritici* (Pgt).

We confirmed that the two host-associated clades *P. oryzae* pathotype *Triticum* and *P. oryzae* pathotype *Oryza* correspond to different pathotypes. This distinction is supported by the combined phylogenetic reconstruction that clearly separates the two taxa. Interestingly, the combined tree (Fig. 2) does not suggest that PoO and PoT are sister taxa. Instead, PoT forms a sister group with Pgt that includes all isolates collected from wheat and other poaceous hosts. This combined group is the sister group to the rice-associated PoO. However, we postulate that this pattern should be interpreted with caution as explained below.

Among the *Pyricularia* species examined in this study, non-fixed polymorphic sites and phylogenetically informative sites were found in nine of the ten loci examined (locus *BAC6* was monomorphic). Fixed nucleotide differences that are diagnostic for the three taxa were located in four loci: *βT-1, CH7-BAC9, EF-1α*, and *MPG1*. Among these, *MPG1* was the most diagnostic locus with 15 fixed differences. Hence, sequencing the *MPG1* locus could provide a simple and informative tool to establish the identity of *Pyricularia* isolates at the species level.

Fig. 2 shows the phylogenetic tree reconstructed for *MPG1* using the same settings as described for the combined tree. Significant differences in tree topology are visible compared to the combined tree. Variation at the *MPG1* locus can distinguish Pgt and PoO with high confidence. However, this analysis splits PoT into two sub-clades. Furthermore, PoO and PoT now join together to form the sister-group, as opposed to Pgt. The observation that single loci can produce different phylogenetic patterns has been referred to as 'phylogenetic incongruence'. The concept of genealogical concordance of different sequence loci (genealogical concordance phylogenetic species recognition, GCPSR) was proposed as a possible solution for phylogenetic species recognition (Taylor et al. 2000, Dettman et al. 2003). In the GCPSR approach, concordant grouping of species based on several sequences is regarded as evidence for restricted exchange of genetic material and, thus, for the reproductive isolation of taxonomic units, indicating speciation. However, in an extensive analysis Grünig et al. (2007) showed that this combined phylogenetic approach also has its limits. The authors concluded that in ambiguous cases (such as cryptic species complexes) phylogenetic approaches should be complemented with population genetic analyses that more easily detect reproductive isolation between taxa. Until additional evidence emerges, likely based on comparative population genomics analyses that include entire genome sequences, we suggest a conservative interpretation and propose to maintain the pathotype-based denomination system of *P. oryzae* pathotype *Oryza and P. oryzae Triticum* (Kato et al. 2000), recognizing that PoT and Pgt may eventually be fused into a single, highly diverse species.

Under our experimental conditions, *P. graminis-tritici* and *P. oryzae* pathotypes *Oryza* and *Triticum* did not present consistent cultural or morphological differences. However, distinctive pathogenicity spectra were observed. *Pyricularia graminis-tritici* and *P. oryzae* pathotypes *Triticum* and *Oryza* caused blast symptoms on wheat, barley, and oats with different levels of aggressiveness. These findings agree with Urashima's pioneering observation that two different pyricularia-like pathogens caused wheat blast disease in Brazil (Urashima et al. 2005). Furthermore, our results confirmed that isolates of *P. oryzae* pathotype *Oryza* can cause blast on seedlings and heads of wheat under greenhouse conditions that favour infection, as previously reported (Urashima et al. 1993, Urashima & Kato 1998). An important question that remains to be answered is whether compatible interactions also occur under natural field conditions. Our observation that none of the wheat-derived isolates was genetically assigned to PoO suggests that PoO infections on wheat are very rare or absent under natural field conditions.

In conclusion, our study suggests that blast disease on wheat and other *Poaceae* in Brazil represents a disease complex caused by more than one species of *Pyricularia*. A recent population genomics analysis performed by D. Croll showed that the Bangladeshi wheat blast strains responsible for the 2016 outbreak were closely related to strains of *Pyricularia graminis-tritici* collected in Brazilian wheat fields (Callaway 2016). Given these findings, recognising and properly naming the causal agents of wheat blast will not only increase our understanding of the biology and epidemiology of the disease, but will also enable the establishment of proper quarantine regulations to limit the spread of these pathogens into disease-free areas that grow susceptible wheat cultivars, including Asia, Europe, and North America (McTaggart et al. 2016).

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