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RESEARCH ARTICLE

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Zymoseptoria gen. nov.: a new genus to accommodate *Septoria*-like species occurring on graminicolous hosts

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Key words

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Abstract The *Mycosphaerella* complex is both poly- and paraphyletic, containing several different families and genera. The genus *Mycosphaerella* is restricted to species with *Ramularia* anamorphs, while *Septoria* is restricted to taxa that cluster with the type species of *Septoria*, *S. cytisi*, being closely related to *Cercospora* in the *Mycosphaerellaceae*. Species that occur on graminicolous hosts represent an as yet undescribed genus, for which the name *Zymoseptoria* is proposed. Based on the 28S nrDNA phylogeny derived in this study, *Zymoseptoria* is shown to cluster apart from *Septoria*. Morphologically species of *Zymoseptoria* can also be distinguished by their yeast-like growth in culture, and the formation of different conidial types that are absent in *Septoria* s.str. Other than the well-known pathogens such as *Z. tritici*, the causal agent of septoria tritici blotch on wheat, and *Z. passerinii*, the causal agent of septoria speckled leaf blotch of barley, both for which epitypes are designated, two leaf blotch pathogens are also described on graminicolous hosts from Iran. *Zymoseptoria brevis* sp. nov. is described from *Phalaris minor*, and *Z. halophila* comb. nov. from leaves of *Hordeum glaucum*. Further collections are now required to elucidate the relative importance, host range and distribution of these species.

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INTRODUCTION

More than 10 000 names have been described in the genus *Mycosphaerella* (*Capnodiales*, *Dothideomycetes*) and its associated anamorph genera (*Cercospora*, *Pseudocercospora*, *Septoria*, *Ramularia*, etc.) (Crous et al. 2009a), making it one of the largest genera of plant pathogenic Ascomycetes known to date (Crous 2009). However, in contrast to earlier phylogenetic studies based on the ITS region (Stewart et al. 1999, Crous et al. 1999, 2000, 2001, Goodwin et al. 2001), more robust multi-gene phylogenies have revealed *Mycosphaerella* to be polyphyletic (Crous et al. 2007, 2009b, Schoch et al. 2009a, b), suggesting that *Mycosphaerella* s.l. should be subdivided to reflect natural groups (genera) as defined by their anamorphs.

The genus *Mycosphaerella* is typified by *M. punctiformis*, which has a *Ramularia* anamorph, *R. endophylla* (Verkley et al. 2004a). Ever since it was established, the name *Mycosphaerella* has been used to describe related and unrelated, small loculoascomycetes (in some cases even asexual coelomycetes) (Aptroot 2006), prompting Crous et al. (2009b), to suggest that the older generic name *Ramularia* (1833), rather than the confused name *Mycosphaerella* (1884) should be used for this well-defined morphologic (Braun 1998) and phylogenetic clade of fungi (Crous et al. 2009b, Kirschner 2009).

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The genus *Septoria* Sacc. (1884) currently contains almost 3 000 species (Verkley & Priest 2000, Verkley et al. 2004b), several of which have *Mycosphaerella*-like teleomorphs. The type species is *Septoria cytisi* (Fig. 1), a pathogen of *Cytisus laburnum* (= *Laburnum anagyroides*). *Septoria* represents a polyphyletic assembly of anamorph genera that cluster mostly in the *Mycosphaerellaceae* (a family incorporating many plant pathogenic coelomycetes), although *Septoria*-like anamorphs have also evolved outside this family (Crous et al. 2009b). In this regard some *Septoria* species on graminicolous hosts (e.g. *S. passerinii* and *S. tritici*) have a distinct dimorphic lifestyle. Besides their mycelial state, they can exhibit a yeast-like growth in culture via microcyclic conidiation, distinguishing them from *Septoria* s.str. Furthermore, phylogenetically the *Septoria*-like species occurring on graminicolous hosts have also been found to cluster apart from *Septoria* species occurring on other hosts (Crous et al. 2001, Verkley et al. 2004b). This clear phylogenetic separation, together with the unique yeast-like growth for *S. tritici* and *S. passerinii*, led to the hypothesis that the *S. tritici* clade did not belong to *Septoria* s.str., but should be classified as a separate genus. In order to prove this hypothesis, the phylogenetic relationship of the type species of the genus *Septoria* (*S. cytisi*) needs to be determined. However these data are not currently available, as other than herbarium material, we have not been able to recollect or locate any living strains of *S. cytisi*.

The aims of this study were thus to isolate and sequence part of the nuclear ribosomal DNA operon from *S. cytisi* herbarium material, and to test the hypothesis whether the *S. tritici* clade can represent a new genus of fungi. A further aim was to resolve the identity of *Septoria*-like species occurring on graminicolous hosts. To this end partial gene sequences of five loci viz. actin (ACT), calmodulin (CAL), β-tubulin (TUB), RNA polymerase II second largest subunit (RPB2) and 28S nuclear ribosomal RNA gene (LSU) were generated and analysed.

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Table 1 Details of cultures subjected to DNA sequencing.

Species	Isolate no[1]	Host	Location	Collected by	GenBank Accession no[2]					
					ACT	CAL	ITS	TUB	RPB2	LSU
Cercospora apii	CBS 118712	–	Fiji	P. Tyler	–	–	–	–	–	GQ852583
C. ariminensis	CBS 137.56	*Hedysarum coronarium*	Italy	M. Ribaldi	–	–	–	–	–	JF700933
C. beticola	CBS 124.31	*Beta vulgaris*	Romania	–	–	–	–	–	–	JF700934
Cladosporium bruhnei	CBS 188.54	–	–	–	–	–	–	–	–	JF700935
	CBS 115683	Douglas-fir pole	USA	–	–	–	–	–	–	JF700936
Dissoconium australiensis	CBS 120729	*Eucalyptus platyphylla*	Australia	P.W. Crous	–	–	–	–	–	GQ852588
D. commune	CPC 12397	*Eucalyptus globulus*	Australia	I.W. Smith	–	–	–	–	–	GQ852591
D. dekkeri	CPC 13479	*Eucalyptus camaldulensis*	Thailand	W. Himaman	–	–	–	–	–	GQ852595
Dothistroma pini	CBS 116484	*Pinus nigra*	USA	G. Adams	–	–	–	–	–	JF700937
D. septosporum	CPC 16799	*Pinus mugo uncinata*	The Netherlands	W. Quaedvlieg	–	–	–	–	–	JF700938
	CPC 3779 (= 112498)	*Pinus radiata*	Ecuador	–	–	–	–	–	–	JF700939
Lecanosticta acicola	CBS 871.95	*Pinus radiata*	France	M. Morelet	–	–	–	–	–	GU214663
	CPC 17940	*Pinus* sp.	Mexico	M. de Jesus Yanez Morales	–	–	–	–	–	JF700940
	IMI 281598	*Pinus oocarpa*	Guatemala	H.C. Evans	–	–	–	–	–	JF700941
Mycosphaerella ellipsoidea	CBS 111167	*Eucalyptus cladocalyx*	South Africa	A.R. Wood	–	–	–	–	–	GU214450
M. elongata	CBS 120735	*Eucalyptus camaldulensis*	Venezuela	M.J. Wingfield	–	–	–	–	–	JF700942
M. marksii	CBS 110981	*Eucalyptus* sp.	Tanzania	M.J. Wingfield	–	–	–	–	–	JF700943
Mycosphaerella sp.	CBS 110843	*Eucalyptus cladocalyx*	South Africa	P.W. Crous	–	–	–	–	–	GQ852602
M. vietnamensis	CBS 119974	*Eucalyptus grandis*	Vietnam	T.I. Burgess	–	–	–	–	–	JF700944
Passalora eucalypti	CBS 111318	*Eucalyptus saligna*	Brazil	P.W. Crous	–	–	–	–	–	GU214458
Phaeophleospora eugeniae	CPC 15143	*Eugenia uniflora*	Brazil	A.C. Alfenas	–	–	–	–	–	FJ493206
P. eugeniicola	CPC 2557	*Eugenia* sp.	Brazil	A.C. Alfenas	–	–	–	–	–	JF700945
Pseudocercospora gracilis	CPC 11144	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	–	–	–	–	–	JF700946
P. heimii	CPC 11716	–	Brazil	A.C. Alfenas	–	–	–	–	–	JF700947
P. heimioides	CBS 111190	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	–	–	–	–	–	GU214439
P. irregulariramosa	CBS 111211	*Eucalyptus saligna*	South Africa	M.J. Wingfield	–	–	–	–	–	GQ852609
P. pseudoeucalyptorum	CPC 13769	*Eucalyptus punctata*	South Africa	P.W. Crous	–	–	–	–	–	GQ852642
P. robusta	CBS 111175	*Eucalyptus robur*	Malaysia	M.J. Wingfield	–	–	–	–	–	JF700948
P. stromatosa	CBS 101953	*Protea* sp.	South Africa	S. Denman	–	–	–	–	–	EU167598
Ramularia endophylla	CBS 113265	*Quercus robur*	The Netherlands	G. Verkley	–	–	–	–	–	DQ470968
R. eucalypti	CBS 120726	*Corymbia grandifolia*	Italy	W. Gams	–	–	–	–	–	JF700949
R. lamii	CPC 11312	*Leonurus sibiricus*	Korea	H.D. Shin	–	–	–	–	–	JF700950
Ramulispora sorghi	CBS 110578	*Sorghum* sp.	South Africa	D. Nowell	–	–	–	–	–	JF700951
	CBS 110579	*Sorghum* sp.	South Africa	D. Nowell	–	–	–	–	–	GQ852654
Septoria azaleae	CBS 352.49	*Rhododendron* sp.	Belgium	J. van Holder	–	–	–	–	–	JF700952
S. betulae	CBS 116724	*Betula pubescens*	Scotland	S. Green	–	–	–	–	–	JF700953
S. cytisi	USO 378994 (Herbarium specimen)	*Laburnum anagyroides*	'Czechoslovakia'	J. A. Baumler	–	–	JF700932	–	–	JF700954
S. gerberae	CBS 410.61	*Gerbera jamesonii*	Italy	W. Gerlach	–	–	–	–	–	JF700955
S. menthae	CBS 404.34	–	Japan	T. Hemmi	–	–	–	–	–	JF700956
S. rosae	CBS 355.58	*Rosa* sp.	–	–	–	–	–	–	–	JF700957
S. rubi	CBS 102327	*Rubus* sp.	The Netherlands	G. Verkley	–	–	–	–	–	JF700958
S. verbenae	CBS 113481	*Septoria* sp.	New Zealand	G. Verkley	–	–	–	–	–	JF700959
Teratosphaeria fibrillosa	CBS 121707	*Protea* sp.	South Africa	P.W. Crous & L. Mostert	–	–	–	–	–	GU323213
T. molleriana	CBS 117926	*Eucalyptus globulus*	Australia	–	–	–	–	–	–	JF700960
T. nubilosa	CPC 12830	*Eucalyptus globulus*	Portugal	A. Philips	–	–	–	–	–	GQ852697
T. pseudocryptica	CPC 11264	*Eucalyptus* sp.	New Zealand	J. Stalpers	–	–	–	–	–	JF700961
T. secundaria	CBS 115608	*Eucalyptus grandis*	Brazil	A.C. Alfenas	–	–	–	–	–	JF700962
T. suberosa	CPC 13090	*Eucalyptus agglomerata*	Australia	A.J. Cargenie	–	–	–	–	–	JF700963
Verrucisporota daviesiae	CBS 116002	*Daviesia latifolia*	Australia	V. beilhartz	–	–	–	–	–	GQ852730
V. proteacearum	CBS 116003	*Grevillea* sp.	Australia	J.L. Alcorn	–	–	–	–	–	GQ852731
Zasmidium anthuriicola	CBS 118742	*Anthurium* sp.	Thailand	C.F. Hill	–	–	–	–	–	GQ852732
Z. citri-grisea	CPC 13467	*Eucalyptus* sp.	Thailand	W. Himaman	–	–	–	–	–	GQ852733
Z. nabiacense	CBS 125010	*Eucalyptus* sp.	Australia	A.J. Cargenie	–	–	–	–	–	JF700964
Z. pseudoparkii	CBS 110999	*Eucalyptus grandis*	Colombia	M.J. Wingfield	–	–	–	–	–	JF700965
Z. xenoparkii	CBS 111185	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	–	–	–	–	–	JF700966
Zymoseptoria brevis	IRAN1485C (= CPC 18102)	*Phalaris paradoxa*	Iran	–	JF701035	JF701103	JF700866	JF700967	JF700798	–
	CPC 18106 (= 8S) = CBS 128853	*Phalaris minor*	Iran	–	JF701036	JF701104	JF700867	JF700968	JF700799	–
	IRAN1486C (= CPC 18107)	*Phalaris minor*	Iran	–	JF701037	JF701105	JF700868	JF700969	JF700800	–
	CPC 18109 (= 81)	*Phalaris paradoxa*	Iran	–	JF701038	JF701106	JF700869	JF700970	JF700801	–
	CPC 18110 (= 83)	*Phalaris paradoxa*	Iran	–	JF701039	JF701107	JF700870	JF700971	JF700802	–

	CPC 18111 (= 84)	*Phalaris paradoxa*	Iran	–	JF701040	JF701108	JF700871	JF700972	JF700803	–
	CPC 18112 (= 85)	*Phalaris paradoxa*	Iran	–	JF701041	JF701109	JF700872	JF700973	JF700804	–
	CPC 18113 (= 86)	*Phalaris paradoxa*	Iran	–	JF701042	JF701110	JF700873	JF700974	JF700805	–
	CPC 18114 (= 87)	*Phalaris paradoxa*	Iran	–	JF701043	JF701111	JF700874	JF700975	JF700806	–
	CPC 18115 (= 88)	*Phalaris paradoxa*	Iran	–	JF701044	JF701112	JF700875	JF700976	JF700807	–
Zymoseptoria halophila	IRAN1483C (= CPC 18105) = CBS 128854	*Hordeum glaucum*	Iran	–	JF701045	JF701113	JF700876	JF700977	JF700808	–
	CBS 120382	*Hordeum vulgare*	USA	S. Goodwin	JF701046	JF701114	JF700877	JF700978	JF700809	–
Z. passerinii	CBS 120384	*Hordeum vulgare*	P71 × P83A, USA	S. Ware	JF701047	JF701115	JF700878	JF700979	JF700810	–
	CBS 120385	*Hordeum vulgare*	P71 × P83B, USA	S. Ware	JF701048	JF701116	CJF700879	JF700980	JF700811	–
	IRAN1489C (= CPC 18099)	*Aegilops tauschii*	Iran	–	JF701049	JF701117	JF700880	JF700981	JF700812	–
	CPC 18100	*Aegilops tauschii*	Iran	–	JF701050	JF701118	JF700881	JF700982	JF700813	–
	CPC 18101	*Aegilops tauschii*	Iran	–	JF701051	JF701119	JF700882	JF700983	JF700814	–
	IRAN1484C (= CPC 18103)	*Calamagrostis* sp.	Iran	–	JF701052	JF701120	JF700883	JF700984	JF700815	–
	CPC 18116	*Avena* sp.	Iran	–	JF701053	JF701121	JF700884	JF700985	JF700816	–
	CPC 18117	*Avena* sp.	Iran	–	JF701054	JF701122	JF700885	JF700986	JF700817	–
Z. tritici	CBS 392.59	*Triticum aestivum*	–	E. Becker	JF701055	JF701123	AY152603	JF700987	JF700818	–
	CBS 398.52	*Triticum aestivum*	Switzerland	E. Muller	JF701056	JF701124	JF700886	JF700988	JF700819	–
	IPO 01001	*Triticum aestivum*	New Zeeland	–	JF701057	JF701125	JF700887	JF700989	JF700820	–
	IPO 02158	*Triticum aestivum*	Iran	–	JF701058	JF701126	JF700888	JF700990	JF700821	–
	IPO 03008	*Triticum aestivum*	Germany	–	JF701059	JF701127	JF700889	JF700991	JF700822	–
	IPO 320	*Triticum aestivum*	Romania	–	JF701060	JF701128	JF700890	JF700992	JF700823	–
	IPO 323	*Triticum aestivum*	The Netherlands	–	JF701061	JF701129	AF181692	JF700993	JF700824	–
	IPO 86013	*Triticum aestivum*	Turkey	–	JF701062	JF701130	JF700891	JF700994	JF700825	–
	IPO 86015	*Triticum aestivum*	Morocco	–	JF701063	JF701131	JF700892	JF700995	JF700826	–
	IPO 86036	*Triticum aestivum*	Israel	–	JF701064	JF701132	JF700893	JF700996	JF700827	–
	IPO 87016	*Triticum aestivum*	Uruguay	–	JF701065	JF701133	JF700894	JF700997	JF700828	–
	IPO 88004	*Triticum aestivum*	Ethiopia	–	JF701066	JF701134	JF700895	JF700998	JF700829	–
	IPO 90012	*Triticum aestivum*	Mexico	–	JF701067	JF701135	JF700896	JF700999	JF700830	–
	IPO 90015	*Triticum aestivum*	Peru	–	JF701068	JF701136	JF700897	JF701000	JF700831	–
	IPO 91009	*Triticum durum*	Tunisia	–	JF701069	JF701137	JF700898	JF701001	JF700832	–
	IPO 91010	*Triticum aestivum*	Tunisia	–	JF701070	JF701138	JF700899	JF701002	JF700833	–
	IPO 91012	*Triticum durum*	Tunisia	–	JF701071	JF701139	JF700900	JF701003	JF700834	–
	IPO 91014	*Triticum durum*	Tunisia	–	JF701072	JF701140	JF700901	JF701004	JF700835	–
	IPO 91016	*Triticum durum*	Tunisia	–	JF701073	JF701141	JF700902	JF701005	JF700836	–
	IPO 91020	*Triticum durum*	Morocco	–	JF701074	JF701142	JF700903	JF701006	JF700837	–
	IPO 92002	*Triticum aestivum*	Portugal	–	JF701075	JF701143	JF700904	JF701007	JF700838	–
	IPO 92003	*Triticum aestivum*	Portugal	–	JF701076	JF701144	JF700905	JF701008	JF700839	–
	IPO 92005	*Triticale* sp.	Portugal	–	JF701077	JF701145	JF700906	JF701009	JF700840	–
	IPO 92032	*Triticum aestivum*	Algeria	–	JF701078	JF701146	JF700907	JF701010	JF700841	–
	IPO 92050	*Triticum aestivum*	Kenya	–	JF701079	JF701147	JF700908	JF701011	JF700842	–
	IPO 94231	*Triticum aestivum*	USA	–	JF701080	JF701148	JF700909	JF701012	JF700843	–
	IPO 94236	*Triticum aestivum*	USA	–	JF701081	JF701149	JF700910	JF701013	JF700844	–
	IPO 95001	*Triticum aestivum*	Switzerland	–	JF701082	JF701150	JF700911	JF701014	JF700845	–
	IPO 95006	*Triticum durum*	Syria	–	JF701083	JF701151	JF700912	JF701015	JF700846	–
	IPO 95013	*Triticum aestivum*	Syria	–	JF701084	JF701152	JF700913	JF701016	JF700847	–
	IPO 95025	*Triticum durum*	Syria	–	JF701085	JF701153	JF700914	JF701017	JF700848	–
	IPO 95026	*Triticum durum*	Syria	–	JF701086	JF701154	JF700915	JF701018	JF700849	–
	IPO 95027	*Triticum durum*	Syria	–	JF701087	JF701155	JF700916	JF701019	JF700850	–
	IPO 95028	*Triticum aestivum*	Syria	–	JF701088	JF701156	JF700917	JF701020	JF700851	–
	IPO 95031	*Triticum durum*	Syria	–	JF701089	JF701157	JF700918	JF701021	JF700852	–
	IPO 95046	*Triticum durum*	Syria	–	JF701090	JF701158	JF700919	JF701022	JF700853	–
	IPO 95047	*Triticum aestivum*	Algeria	–	JF701091	JF701159	JF700920	JF701023	JF700854	–
	IPO 95050	*Triticum aestivum*	Algeria	–	JF701092	JF701160	JF700921	JF701024	JF700855	–
	IPO 95052	*Triticum aestivum*	Algeria	–	JF701093	JF701161	JF700922	JF701025	JF700856	–
	IPO 95054	*Triticum aestivum*	Algeria	–	JF701094	JF701162	JF700923	JF701026	JF700857	–
	IPO 95062	*Triticum aestivum*	Algeria	–	JF701095	JF701163	JF700924	JF701027	JF700858	–
	IPO 95071	*Triticum aestivum*	Algeria	–	JF701096	JF701164	JF700925	JF701028	JF700859	–
	IPO 95072	*Triticum aestivum*	Algeria	–	JF701097	JF701165	JF700926	JF701029	JF700860	–
	IPO 95073	*Triticum aestivum*	Algeria	–	JF701098	JF701166	JF700927	JF701030	JF700861	–
	IPO 95074	*Triticum aestivum*	Algeria	–	JF701099	JF701167	JF700928	JF701031	JF700862	–
	IPO 97016	*Triticum aestivum*	Italy	–	JF701100	JF701168	JF700929	JF701032	JF700863	–
	IPO 98115	*Triticum aestivum*	Hungary	–	JF701101	JF701169	JF700930	JF701033	JF700864	–
	IPO 99048	*Triticum aestivum*	France	–	JF701102	JF701170	JF700931	JF701034	JF700865	–

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Pedro Crous working collection housed at CBS; IMI: International Mycological Institute; USO: United States Department of Agriculture, National Fungus Collections (BPI); IPO: Research Institute for Plant Protection, Wageningen (IRAN); Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection. [2] ACT = Actin, TUB = β-tubulin, CAL = Calmodulin, LSU = 28S large subunit of the nrRNA gene, RPB2= RNA polymerase II second largest subunit.

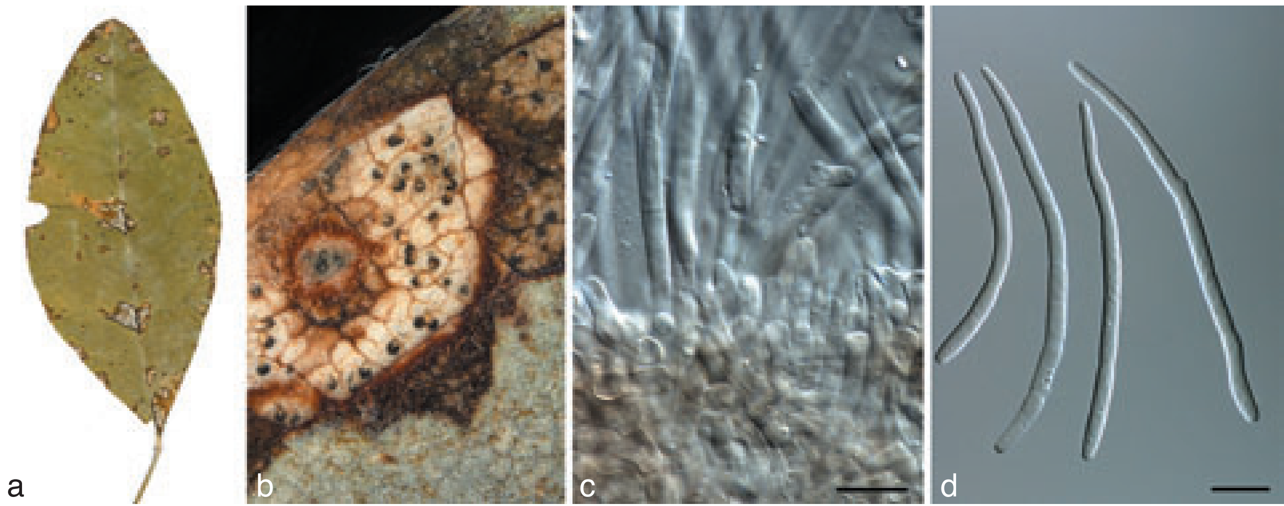

Fig. 1 *Septoria cytisi* (BPI 378994). a. Leaf with leaf spots; b. lesion with pycnidia oozing conidial cirrhi; c. conidiogenous cells showing sympodial and percurrent proliferation; d. conidia. — Scale bars = 10 µm.

MATERIALS AND METHODS

Isolates

Symptomatic leaves were collected from several localities (Table 1), and leaves with visible asexual fruiting bodies were immediately subjected to direct fungal isolation, or alternatively were first incubated in moist chambers to stimulate sporulation. Single-conidial isolates were established on malt extract agar (MEA; 20 g/L Biolab malt extract, 15 g/L Biolab agar) using the previously described procedure (Crous et al. 2009c). Cultures were later plated on fresh MEA, 2 % tap water agar supplemented with green, sterile barley leaves (WAB), 2 % potato-dextrose agar (PDA), and oatmeal agar (OA) (Crous et al. 2009c), and subsequently incubated at 25 °C under near-ultraviolet light to promote sporulation. Reference strains are maintained in the culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands, the Plant Research Institute, Wageningen, the Netherlands, and the Iranian Research Institute of Plant Protection, Tehran, Iran (Table 1), and supplemented with other relevant isolates present in the CBS collection. Descriptions, nomenclature, and illustrations were deposited in MycoBank (www.mycobank.org, Crous et al. 2004).

DNA extraction, amplification and sequencing

Herbarium specimens

Ten *S. cytisi* herbarium specimens occurring on *Cytisus laburnum* (= *Laburnum anagyroides*), were obtained from the U.S. National Fungus Collections (BPI) in Beltsville, Maryland, USA (Table 2). After microscopic inspection, the five specimens with the least amount of surface contamination (yeast and saprobes) where selected for DNA extraction (Table 2). Using a stereo microscope, ± 25 pycnidia, including their dried conidial cirrhi, where excised from each respective herbarium specimen, and suspended in tubes with 20 µL STL buffer from an E.Z.N.A. ® Forensic DNA Kit (Omegabiotek, Norcross). Special care was taken to keep the amount of contaminant leaf material, excised together with the fungal tissue, as low as possible. The fungal material was kept in STL buffer to rehydrate for 24 h at 4 °C, after which the fungal cell walls were degraded by two cycles of freezing with liquid nitrogen and immediate re-heating to 99 °C.

Table 2 Herbarium specimens of *Laburnum anagyroides* infected with *Septoria cytisi*, obtained from the U.S. National Fungus Collections (BPI), Maryland, USA. Specimens marked with an asterisk were selected for DNA extraction.

BPI accession number	Host	Year collected	Location
0378986	*Laburnum anagyroides*	1913	France
0378987	*Laburnum anagyroides*	1933	Romania
0378988	*Laburnum anagyroides*	1893	Italy
0378989*	*Laburnum anagyroides*	1929	‘Czechoslovakia’
0378990*	*Laburnum anagyroides*	1874	Italy
0378991*	*Laburnum anagyroides*	1885	‘Czechoslovakia’
0378992	*Laburnum anagyroides*	1903	Italy
0378993*	*Laburnum anagyroides*	1929	Austria
0378994*	*Laburnum anagyroides*	1884	‘Czechoslovakia’
0378995	*Laburnum anagyroides*	1876	Italy

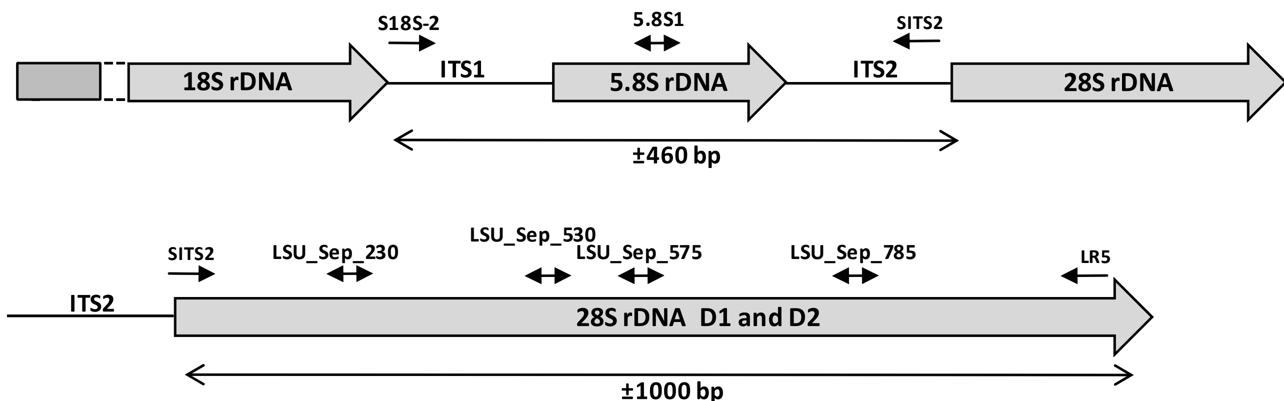

Fig. 2 A diagrammatic representation of part of the nrDNA operon indicating the positions of the *Septoria*-specific primers used to generate ITS and LSU sequences of *S. cytisi*.

The genomic DNA extraction was performed using the 'Isolation of DNA from dried blood' protocol available in the E.Z.N.A. ® Forensic DNA Kit with one modification: in order to increase the final DNA concentration, only 50 µL of preheated (70 °C) elution buffer was used to elude the DNA from the column.

Genus-specific primers had to be designed because the use of generic fungal ITS and LSU primers only generated sequences of contaminants (mostly yeasts). For the amplification reactions concerning the herbarium specimens, the Verbatim High Fidelity DNA Polymerase Kit (Thermo Scientific) was used in combination with the *Septoria*-specific S18S-2 forward primer (annealing to the nuclear rDNA operon at the 3'-end of the 18S nrRNA gene (SSU); Table 2), together with the *Septoria*-specific SITS2_Fd reverse primer (annealing to the nuclear rDNA operon at the 5'-end of the 28S nrRNA gene (LSU); Table 2), in order to amplify a region spanning the 5.8S nrRNA gene and the first and second internal transcribed spacer regions (Fig. 2). This amplification reaction was set up in a volume of 12.5 µL using 5× High Fidelity buffer (with $MgCl_2$), 0.8 µM of each primer, 2 µL of gDNA, 150 µM dNTP mix and 0.1 unit of Verbatim polymerase using a MyCycler thermal cycler (Bio-Rad). PCR amplification conditions were set as follows: an initial denaturation temperature at 98 °C for 2 min, followed by 50 cycles of denaturation temperature at 98 °C for 30 s, primer annealing at 52 °C for 30 s, primer extension at 72 °C for 30 s and final extension at 72 °C for 2 min. The resulting PCR products were then size-fractionated on a 3 % (w/v) agarose gel stained with ethidium bromide, excised from the gel and subsequently sequenced as described by Cheewangkoon et al. (2008).

Degradation and shearing of the *S. cytisi* herbarium gDNA made it impossible to directly amplify and sequence the approximate 1 300 bp needed to cover both the ITS and D1–D3 domains of the 28S nrDNA in a single reaction. Therefore, specific primers were developed from the obtained *S. cytisi* ITS1 sequence, spaced about 300 bp apart (Table 2, Fig. 2), which made it possible to sequentially amplify and sequence the entire regions of both the ITS, and the D1–D3 domains of the LSU of *S. cytisi* sequentially, and later to sequence it as described by Cheewangkoon et al. (2008).

Fungal cultures

Genomic DNA was extracted from mycelium growing on MEA (Table 1), using the UltraClean® Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, USA). These strains were screened for five loci, namely ITS, Actin (ACT), calmodulin (CAL), RNA polymerase II second largest subunit (RPB2) and β-tubulin (TUB) (Table 3). DNA amplification and sequencing reactions were performed as described by Cheewangkoon et al. (2008).

Phylogenetic analysis

To determine whether the multi-locus DNA sequence datasets were congruent, a partition homogeneity test (Farris et al. 1994) of all possible combinations was performed in PAUP v4.0b10 (Swofford 2003) with 1 000 replications. Parallel to this, a 70 % Neighbour-Joining (NJ) reciprocal bootstrap method with Maximum Likelihood distance (Mason-Gamer & Kellogg 1996, Lombard et al. 2010) was also employed to check congruency. The models of evolution for the NJ tree were estimated with Modeltest v3.7 (Posada & Crandall 1998) and bootstrap analyses (10 000 replicates) were performed in PAUP. Resulting NJ tree topologies were visually compared for conflicts between the individual gene regions. Maximum-parsimony genealogies for individual datasets and the combined dataset were estimated in PAUP using heuristic searches based on 1 000 random taxon addition sequences and the best trees were saved. All characters were weighted equally and alignment gaps were treated as missing data. Branches of zero length were collapsed and all multiple, equally most parsimonious trees were saved. Tree length (TL), consistency index (CI), retention index (RI) and the rescaled consistency index (RC) were calculated in PAUP for the equally most parsimonious trees and the resulting trees were printed with TreeView (Page 1996) and the alignments and phylogenetic trees were lodged in TreeBASE (www.treebase.org). All novel sequences derived from this study were deposited in GenBank (Table 1). Trees were either rooted to *Cladosporium bruhnei* for the LSU tree, or to *Mycosphaerella punctiformis* for the multigene tree.

Morphology

Descriptions were based on fungal cultures sporulating in vitro on WAB, incubated under continuous near-ultraviolet light for 2–4 wk. Wherever possible, 30 measurements (×1 000 magnification) were made of structures mounted in lactic acid, with the extremes of spore measurements given in parentheses. Colony colours (surface and reverse) were assessed after 1 mo on MEA, PDA and OA at 25 °C in the dark, using the colour charts of Rayner (1970).

RESULTS

ITS and LSU amplification and sequencing of S. cytisi

The gDNA extractions from the *S. cytisi* herbarium samples were performed on the herbarium specimens indicated in Table 2, and both the ITS and a partial LSU regions where targeted for these isolates using *Septoria*-specific primers (Table 4). An ITS amplicon length of 486 bp was achieved from herbarium sample US0378993 while the other samples yielded only partial ITS amplicons varying in length from 440 bp in sample US0378994 to ± 200 bp in sample US0378990; amplicons of sample US0378991 only yielded contamination sequences with general primers and did not amplify with either *Septoria*- or *S. cytisi*-specific primers.

A comparison between the full-length *S. cytisi* ITS sequence and 287 other *Septoria* ITS sequences that were generated as part of a larger unpublished study, broadly linked *S. cytisi* to a distinct ITS clade containing *S. astralagi* and *S. hippocastani*, basal to a clade consisting of the majority of sequenced *Septoria*

Table 3 Primer combinations used during this study for generic amplification and sequencing.

Locus	Primer	Primer sequence 5' to 3'	Orientation	Reference
Actin	ACT-512F	ATGTGCAAGGCCGGTTTCGC	Forward	Carbone & Kohn (1999)
Actin	ACT2Rd	ARRTCRCGDCCRGCCATGTC	Reverse	Groenewald, unpubl. data
Calmodulin	CAL-228F	GAGTTCAAGGAGGCCTTCTCCC	Forward	Carbone & Kohn (1999)
Calmodulin	CAL2Rd	TGRTCNGCCTCDCGGATCATCTC	Reverse	Groenewald, unpubl. data
β-tubulin	TUB2Fd	GTBCACCTYCARACCGGYCARTG	Forward	Aveskamp et al. (2009)
β-tubulin	TUB4Rd	CCRGAYTGRCCRAARACRAAGTTGTC	Reverse	Aveskamp et al. (2009)
RPB2	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	Forward	Liu et al. (1999)
RPB2	fRPB2-5F+414R	ACMANNCCCCARTGNGWRTTRTG	Reverse	Present study
LSU	LSU1Fd	GRATCAGGTAGGRATACCCG	Forward	Crous et al. (2009a)
LSU	LR5	TCCTGAGGGAAACTTCG	Reverse	Vilgalys & Hester (1990)

Table 4 *Septoria cytisi*-specific ITS and LSU primers used for amplification and sequencing. Nucleotide positions were determined relative to the ITS/LSU sequence of *Zymoseptoria tritici* (GenBank accession FN428877).

Primer name	Primer sequence 5' to 3'	Orientation	Relative position
S18S-2	CGTAGGTGAACYTGCGRAGGGATCATTACYGAGTGA	Forward	7
5.8S1Fd	CTCTTGGTTCBVGCATCG	Forward	240
SITS2_Fwd	CCGCCCGCACTCCGAAGCGATTAATGAAATC	Forward	459
SITS2_Rev	GATTTCATTAATCGCTTCGGAGTGCGGGCGG	Reverse	459
LSU_Sep_230_Fwd	TATGTGACCGGCCCGCACCCTTTAC	Forward	710
LSU_Sep_230_Rev	GTAAAGGGTGCGGGCCGGTCACATA	Reverse	710
LSU_Sep_530_Fwd	AAGACCTTAGGAATGTAGCTCACCT	Forward	999
LSU_Sep_530_Rev	AGGTGAGCTACATTCCTAAGGTCTT	Reverse	999
LSU_Sep_575_Fwd	CTTGGGCGAGGTCCGCGCT	Forward	1059
LSU_Sep_575_Rev	AGCGCGGACCTCGCCCAAG	Reverse	1059
LSU_Sep_785_Rev	AGGACATCAGGATCGGTCGAT	Reverse	1225

Annotation: ITS1 = 1–172 bp, 5.8S = 173–330 bp, ITS2 = 331–525 bp, LSU D1 & D2 domain = 525–1110 bp.

Table 5 Polymorphisms found in the ITS and LSU sequence between the *S. cytisi* herbarium specimens. Data marked with – are not available.

BPI specimen	Collection year	ITS position (bp)			LSU position (bp)					
		93	219	411	176	377	446	536	561	563
USO 378989	1929	A	–	–	T	T	G	T	T	C
USO 378993	1929	A	C	C	C	C	C	A	G	–
USO 378994	1884	C	G	T	C	C	G	A	G	G
USO 378990	1874	A	G	C	–	–	–	–	–	–

species (data not shown). Interspecific variation in the *S. cytisi* ITS sequences were present; however, it was limited to a few nucleotides per isolate sequenced (Table 5).

Amplification of the D1–D3 domains of the LSU region was attempted on the same *S. cytisi* gDNA extracts as mentioned before. A full-length sequence read of the *S. cytisi* D1–D3 domains (the first ± 900 bp of the 28S nrRNA gene) was only obtained from a single sample (US0378994). The four remaining herbarium specimens only yielded LSU sequences varying in length from 500–800 bp. Interspecific variation in the LSU nucleotide sequences was limited to a few nucleotides per sequenced isolate (Table 5).

Phylogenetic analyses

LSU dataset

During phylogenetic analyses, the *S. cytisi* LSU sequence was aligned with LSU sequence data of 64 *Capnodiales* taxa, including 19 representative *Septoria* taxa, in order to determine which of these *Septoria* isolates belonged to *Septoria* s.str. (i.e. high association with *S. cytisi*) and to establish how this clade is related to other well-established genera within the *Capnodiales*. For the LSU tree, ± 759 characters were determined for 64 *Capnodiales* taxa, including 19 *Septoria* taxa as well as the two *Cladosporium bruhnei* isolates that were used as outgroups (CPC 5101 and CBS 188.54). The phylogenetic analysis showed that 164 characters were parsimony-informative, 38 were variable and parsimony-uninformative and 557 were constant. Thirty-two equally most parsimonious trees were obtained from the heuristic search, the first of which is shown in Fig. 3 (TL = 574, CI = 0.495, RI = 0.848, RC = 0.419). The phylogenetic analysis of the *Capnodiales* LSU dataset, including *S. cytisi*, showed this species clustering in a well-defined clade incorporating the majority of the *Septoria* spp. used in this analysis, clearly delineating this clade as *Septoria* s.str. These results also show a distinct monophyletic clade that are referred to as *Zymoseptoria* gen. nov. below, which contains *S. tritici* and *S. passerinii* together with two other species in this genus.

Multi-locus dataset

For the multi-locus phylogenetic analyses of the graminicolous isolates, ± 220 nucleotides where determined for ACT, 345 for CAL, 513 for ITS, 350 for TUB, and 305 for RPB2 (see Table 3 for detailed primer description). The adjusted sequence alignment for each locus consisted of 69 ingroup taxa with *Ramularia endophylla* (*Mycosphaerella punctiformis*; strain CBS 113265) as outgroup.

The strict consensus tree (Fig. 4) based on the multi-locus maximum-parsimony analysis had an identical topology to those of the strict consensus trees obtained for the individual loci. The partition homogeneity tests for all of the possible combinations of the five gene regions consistently yielded a P-value of 0.001, and were therefore incongruent. However, the 70 % reciprocal bootstrap trees of the individual gene regions showed no conflicting tree topologies between the separate datasets. Based on the result of the 70 % reciprocal bootstrap trees (Mason-Gamer & Kellogg 1996, Cunningham 1997), the DNA sequences of the five gene regions (ACT, CAL, RPB2, TUB and ITS) were concatenated for the phylogenetic analyses.

The concatenated and manually aligned multi-locus alignment contained 70 taxa (including the outgroup sequence) and, out of the 1 723 characters used in the phylogenetic analysis, 233 were parsimony-informative, 291 were variable and parsimony-uninformative and 1 199 were constant. 810 equally parsimonious trees were obtained from the heuristic search, the first of which is shown in Fig. 4 (TL = 768, CI = 0.815, RI = 0.922, RC = 0.751). Phylogenetic results showed two well-supported new species emerging besides the conserved *S. tritici* and *S. passerinii* clades, with a significant amount of genetic variation within the *S. tritici* clade as previously found by Goodwin et al. (2007). This intraspecific variation is most likely the cause of the partition homogeneity test failure.

The overall genetic diversity of *S. tritici*, examined over five loci, was found to be quite significant within the 54 global isolates of *S. tritici* used for this study. Most of the existing phylogenetic variation observed between the *S. tritici* isolates used in the combined tree (Fig. 4) was caused by single insertion and deletion events of triplets within tandem repeats inside the ACT and RPB2 intron sequences of these isolates. The most significant

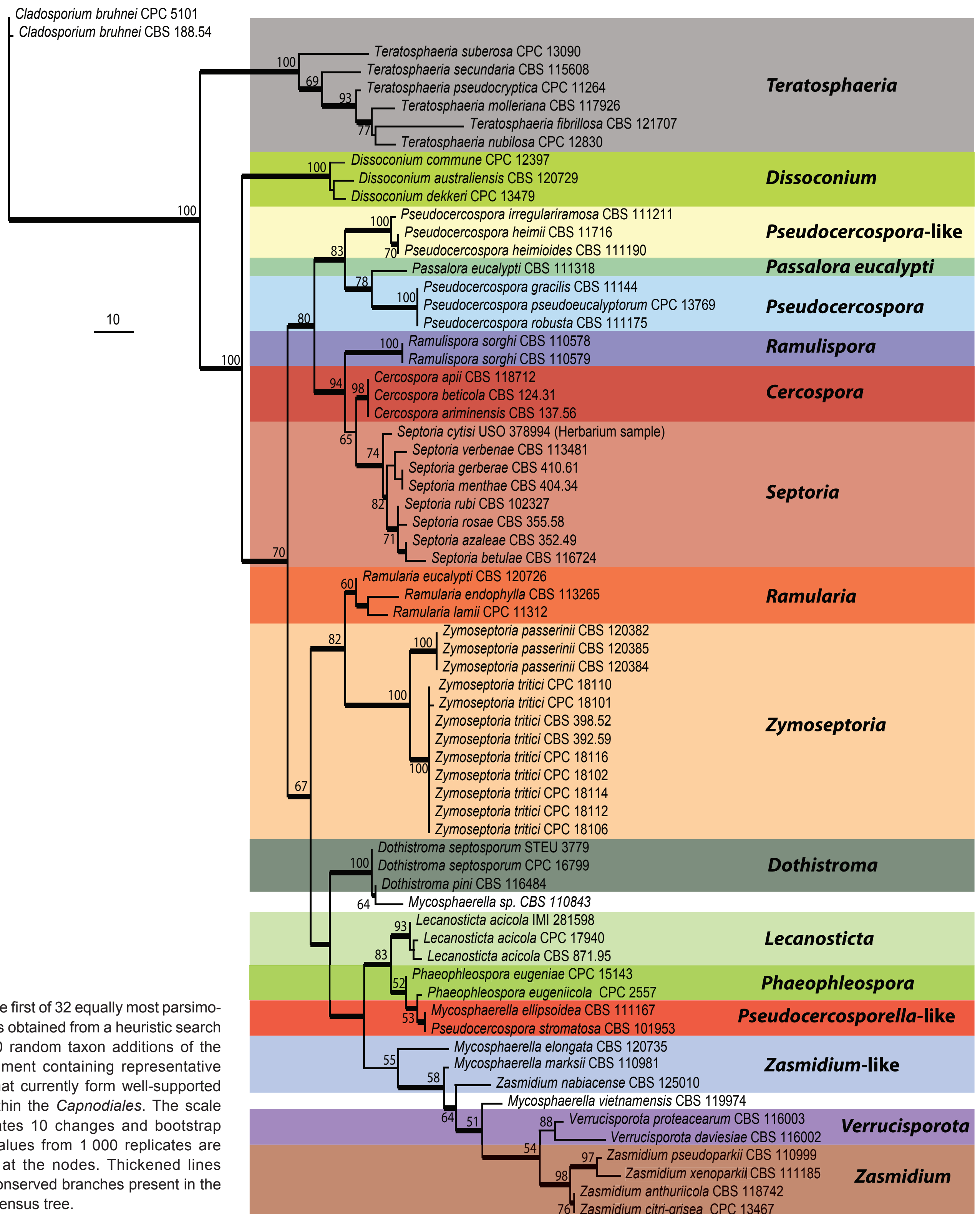

Fig. 3 The first of 32 equally most parsimonious trees obtained from a heuristic search with 1 000 random taxon additions of the LSU alignment containing representative species that currently form well-supported clades within the *Capnodiales*. The scale bar indicates 10 changes and bootstrap support values from 1 000 replicates are indicated at the nodes. Thickened lines indicate conserved branches present in the strict consensus tree.

impact of these indel events can be seen in the phylogenetic cluster containing CPC 18099–18101 (on *Aegilops tauschii*, Iran), that arises in the *S. tritici* clade of the combined tree (Fig. 4). This small clade has a bootstrap support value of 94 %, suggesting that it could represent a cryptic or ancestral lineage of what is currently considered to be *S. tritici*. Further study using more isolates would be required to address this issue.

Taxonomy

Based on the LSU dataset (Fig. 3), *S. cytisi* was shown to cluster within the major *Septoria* clade, while the taxa occurring on graminicolous hosts clustered in a separate clade, distinct from *Septoria* (*S. cytisi*) and *Mycosphaerella* (*M. punctiformis*, represented by *R. endophylla*), suggesting that they represented a distinct genus in the *Mycosphaerellaceae*. Morphologically these phylogenetic differences were supported by the distinct yeast-like growth exhibited in culture by the graminicolous species, as well as their mode of conidiogenesis, e.g. phialidic, with periclinal thickening and occasional inconspicuous percurrent proliferation(s), but lacking blastic sympodial proliferation which occurs in many species of *Septoria* s.str. Based on these differences in culture, morphology and phylogeny, a new genus is hereby introduced for the taxa occurring on graminicolous hosts.

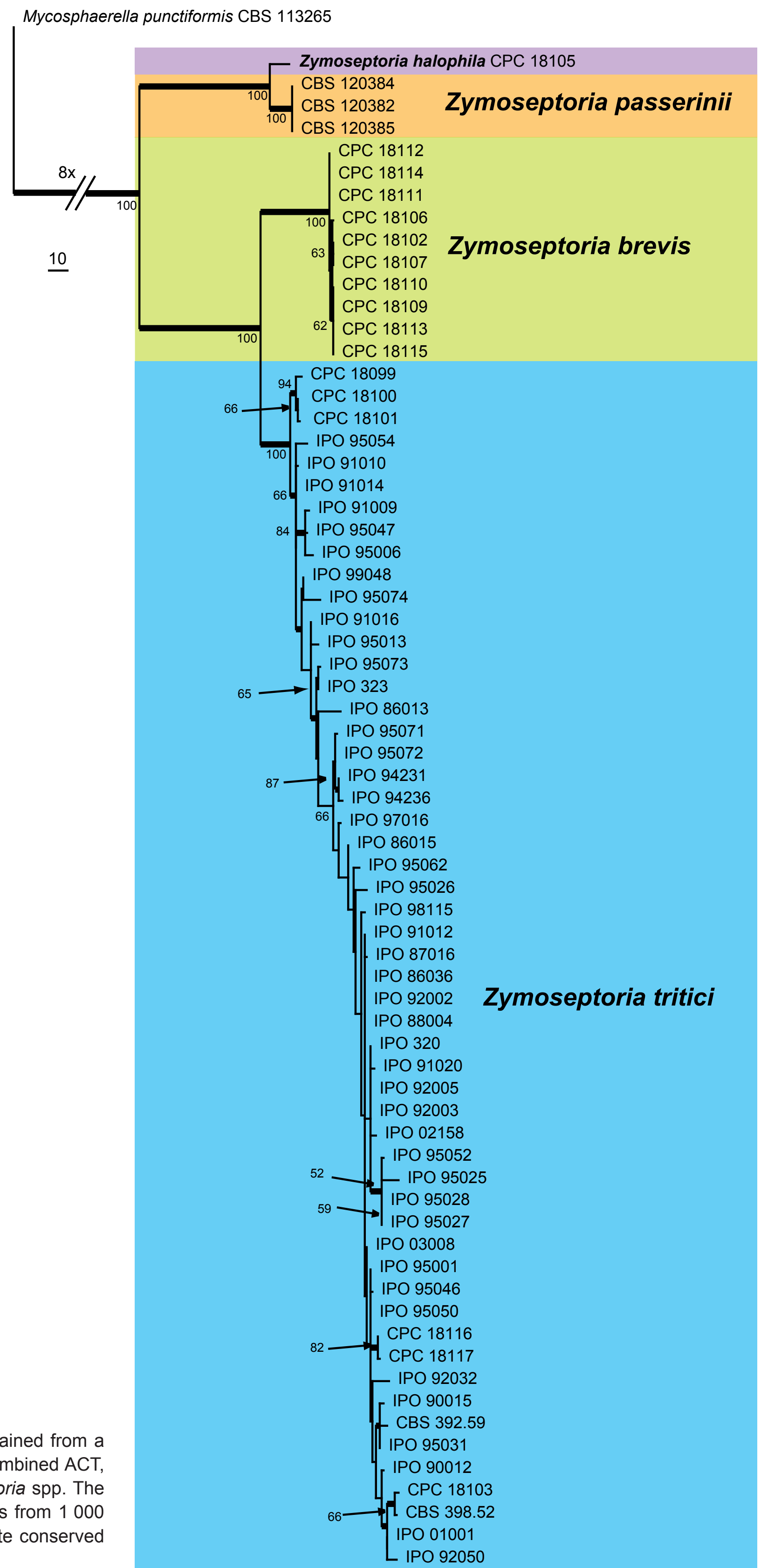

Fig. 4 The first of 810 equally most parsimonious trees obtained from a heuristic search with 1 000 random taxon additions of the combined ACT, CAL, TUB, RPB2 and ITS sequence alignment of *Zymoseptoria* spp. The scale bar indicates 10 changes and bootstrap support values from 1 000 replicates are indicated at the nodes. Thickened lines indicate conserved branches present in the strict consensus tree.

Zymoseptoria Quaedvlieg & Crous, *gen. nov.* — MycoBank MB517922

Septoriae similis, sed adaucto fermentoide, sine formatione blastica-sympodiali conidiorum, in cultura typis conidiorum usque ad 3.

Type species. *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous.

Etymology. *Zymo* = yeast-like growth; *Septoria* = *Septoria*-like in morphology.

Conidiomata pycnidial, semi-immersed to erumpent, dark brown to black, subglobose, with central ostiole; wall of 3–4 layers of brown *textura angularis*. *Conidiophores* hyaline, smooth, 1–2-septate, or reduced to conidiogenous cells, lining the inner cavity. *Conidiogenous cells* tightly aggregated, ampulliform to doliiform or subcylindrical, phialidic with periclinal thickening, or with 2–3 inconspicuous, percurrent proliferations at apex. *Type I conidia* solitary, hyaline, smooth, guttulate, narrowly

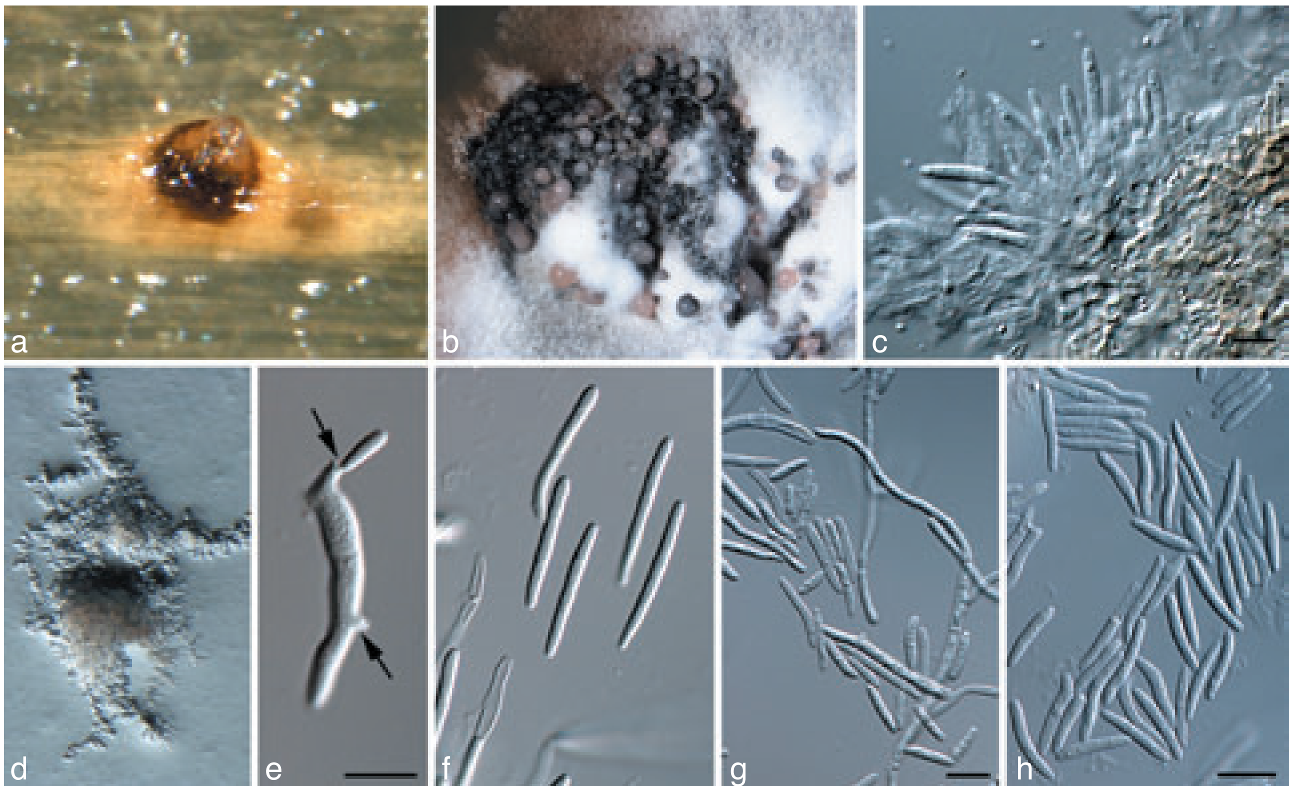

Fig. 5 *Zymoseptoria brevis* (CPC 18106) a. Pycnidium forming on barley leaves in vitro; b. colony sporulation on potato-dextrose agar; c. conidiogenous cells; d. colony on synthetic nutrient-poor agar, showing yeast-like growth; e. conidium undergoing microcyclic conidiation (arrows; Type III); f–h. pycnidiospores (Type I). — Scale bars = 10 μm.

cylindrical to subulate, tapering towards acutely rounded apex, with bluntly rounded to truncate base, transversely euseptate; hila not thickened nor darkened. On OA and PDA aerial hyphae disarticulate into phragmospores (*Type II conidia*), that again give rise to *Type I conidia* via microcyclic conidiation; yeast-like growth and microcyclic conidiation (*Type III conidia*) common on agar media.

Zymoseptoria brevis M. Razavi, Quaedvlieg & Crous, *sp. nov.* — MycoBank MB517923; Fig. 5

Zymoseptoriae passerinii similis, sed conidiis minoribus, (12–)13–16(–17) × 2(–2.5) μm.

Etymology. Named after its conidia, which are shorter (*brevis*) than those of the other species.

On sterile barley leaves on WA: *Conidiomata* pycnidial, substomatal, immersed to erumpent, globose, dark brown, up to 200 μm diam, with central ostiole, 5–10 μm diam; wall of 3–4 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells, or with one supporting cell, lining the inner cavity. *Conidiogenous cells* hyaline, smooth, tightly aggregated, subcylindrical to ampulliform, straight to curved, 7–15 × 2–4 μm, with 1–2 inconspicuous, percurrent proliferations at apex, 1–1.5 μm diam. *Type I conidia* solitary, hyaline, smooth, guttulate, subcylindrical to subulate, tapering towards bluntly rounded apex, with truncate base, 0–1-septate, (12–)13–16(–17) × 2(–2.5) μm; on PDA, 9–21 × 2–3.5 μm; hila not thickened nor darkened, 1–2 μm. On OA and PDA yeast-like growth and microcyclic conidiation (*Type III conidia*) common, also forming on aerial hyphae via solitary conidiogenous loci.

Culture characteristics — Colonies on PDA flat, spreading, with moderate aerial mycelium and feathery, lobate margins; surface olivaceous-grey, outer region dirty white, reverse iron-grey; on MEA more erumpent, with less aerial mycelium; surface iron-grey with patches of white, reverse greenish black; on OA somewhat fluffy with dirty white to pale olivaceous aerial mycelium, and submerged, olivaceous-grey margin; reaching 15 mm diam after 1 mo at 25 °C; fertile.

Specimen examined. Iran, Ilam province, Dehloran, on living leaves of *Phalaris minor*, *M. Razavi*, holotype CBS H-20542, cultures ex-type No 8S = CPC 18106 = CBS 128853.

Notes — *Zymoseptoria brevis* can easily be distinguished from the other taxa presently known within the genus based on its shorter conidia.

Zymoseptoria halophila (Speg.) M. Razavi, Quaedvlieg & Crous, *comb. nov.* — MycoBank MB517924; Fig. 6

Basionym: *Septoria halophila* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires, Ser. 3, 13: 382. 1910.

Initial symptoms of the disease were dark-brown lesions which soon became pale buff in the centre. The leaves were heavily mottled later, and the solitary, sometimes aggregated pycnidia formed on the lesions. The disease was more severe on the lower leaves. *Pycnidia* were observed on adaxial surface of the infected leaves, and were dark-brown, globose, measuring 90–150 μm, with an ostiole ± 10 μm diam. *On sterile barley leaves on WA*: *Conidiomata* pycnidial, semi-immersed to erumpent, dark brown to black, subglobose, up to 300 μm diam, with central ostiole, up to 30 μm diam; wall of 3–4 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells, lining the inner cavity. *Conidiogenous cells* hyaline, smooth, tightly aggregated, ampulliform to doliiform, 10–15 × 4–7 μm, with 2–3 inconspicuous, percurrent proliferations at apex, 1–2 μm diam. *Type I conidia* solitary, hyaline, smooth, guttulate, narrowly cylindrical to subulate, tapering towards acutely rounded apex, with bluntly rounded to truncate base; basal cell long obconically truncate, 1(–3)-septate, (30–)33–38(–50) × 2(–3) μm; conidia in vivo 1–2-septate, 36–45 × 1.5–2 μm; hila not thickened nor darkened, 1–2 μm. On OA and PDA conidia can be up to 62 μm long, and aerial hyphae disarticulate into

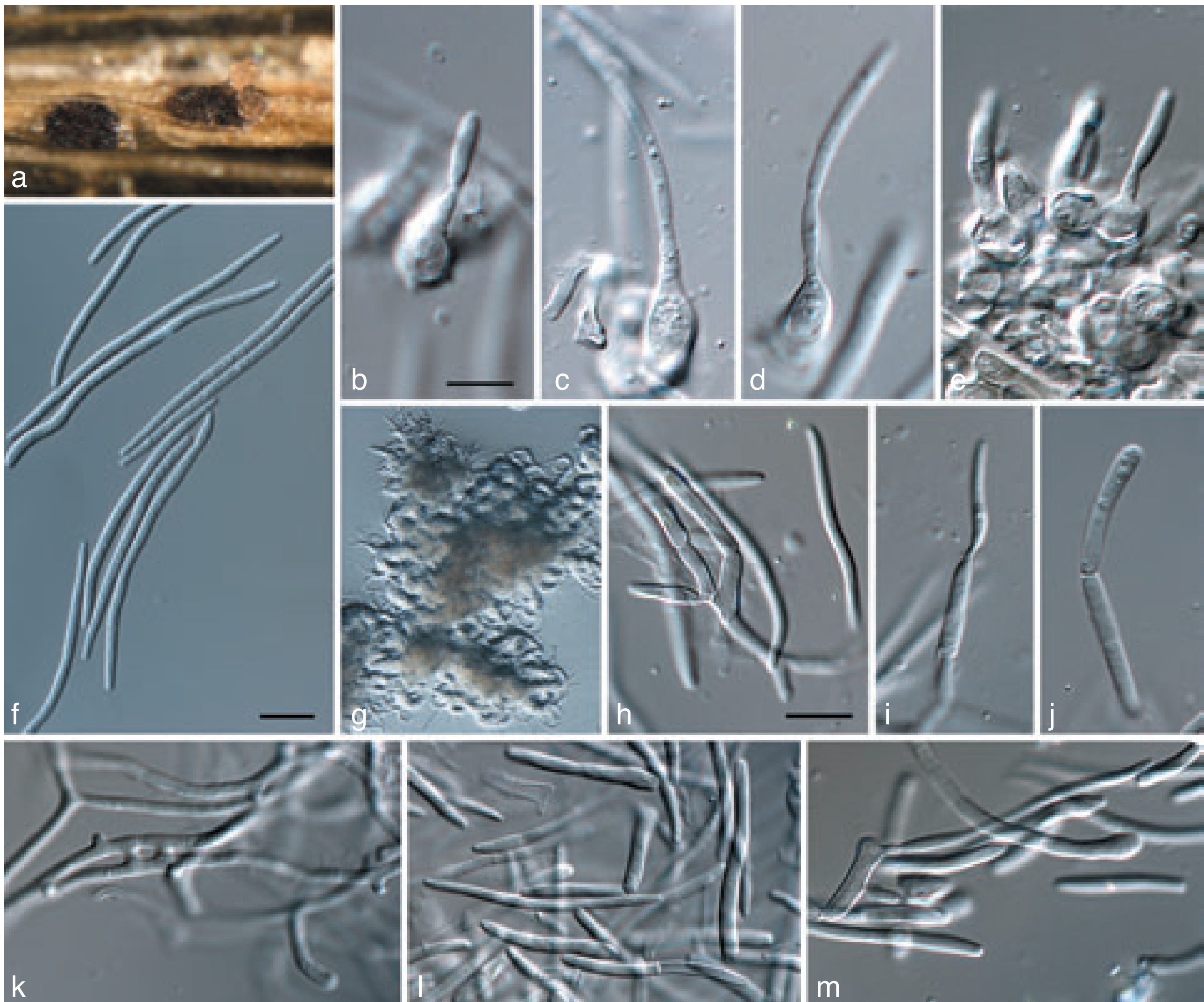

Fig. 6 *Zymoseptoria halophila* (CPC 18105). a. Pycnidia forming on barley leaves in vitro, with oozing conidia cirrhus; b–e. conidiogenous cells formed in pycnidia; f. conidia (Type I); g. colony with yeast-like growth on synthetic nutrient-poor agar; h, j–l. conidia formed as phragmospores in aerial hyphae (Type II); i, m. conidia formed via microcyclic conidiation (Type III). — Scale bars = 10 µm.

phragmospores (*Type II conidia*), that again give rise to *type I conidia* via microcyclic conidiation; yeast-like growth and microcyclic conidiation (*Type III conidia*) common on agar media.

Culture characteristics — Colonies on PDA flat, spreading, with sparse aerial mycelium and feathery, lobate margins; centre olivaceous-grey, outer region iron-grey; reverse iron-grey; on MEA surface and reverse greenish black; on OA iron-grey, reaching 20 mm diam after 1 mo at 25 °C; fertile.

Specimen examined. Iran, Ilam province, Dehloran, on living leaves of *Hordeum glaucum*, 25 Apr. 2007, *M. Razavi*, specimens IRAN12892F, CBS H-20543, cultures ex-type GLS1 = IRAN1483C = CPC 18105 = CBS 128854.

Notes — The present collection of *Z. halophila* was initially reported from Iran as *S. halophila* by Seifbarghi et al. (2009) (GenBank HM100267, HM100266), based on the description of *S. halophila* provided by Priest (2006). *Zymoseptoria halophila* was originally described from *Hordeum halophilum* collected in Argentina, with conidia being (0–)1(–2)-septate, 36–58 × 1.5(–2) µm, and conidiogenous cells being 8–10 × 2.5–3.5 µm. It is likely that the various collections on *Hordeum* and *Poa* spp. from Australia listed by Priest (2006) could represent different species, but this can only be resolved once additional collections and cultures have been obtained to facilitate further molecular comparisons.

Zymoseptoria halophila is closely related to *Z. passerinii*, which is also reflected in its conidial size, which overlaps in length, but can only be distinguished based on their difference in width. It is possible that some published records of *Z. passerinii* could in fact represent *Z. halophila*, but more collections would be required to resolve its host range and geographic distribution.

Zymoseptoria passerinii (Sacc.) Quaedvlieg & Crous, *comb. nov.* — MycoBank MB517925; Fig. 7

Basionym: *Septoria passerinii* Sacc., Syll. Fung. (Abellini) 3: 560. 1884.

Specimens examined. Italy, Vigheffio, near Parma, on *Hordeum murinum*, June 1879 (F. von Thümen, Mycotheca Univ. No. 1997, isotype in MEL, see Priest 2006, f. 107). – USA, North Dakota, Foster county, on *Hordeum vulgare*, coll. *S. Goodwin*, isol. *D. Long*, epitype designated here CBS H-20544, culture ex-epitype P83 = CBS 120382.

Notes — Priest (2006) reported *Z. passerinii* from several *Hordeum* species collected in Western Australia and deposited them at IMI (now in Kew), and found them to be identical to type material examined, suggesting that this pathogen is widely distributed along with its host. Ware et al. (2007) reported a *Mycosphaerella*-like teleomorph from a heterothallic mating of isolates of *Z. passerinii*. Single ascospore isolates have been deposited as CBS 120384 (P71 × P83A) and CBS 120385 (P71 × P83B). Isolate P63, which is genetically similar to P83 on the loci sequenced in this study, has been used for whole genome analysis of *Z. passerinii* (E.H. Stukenbrock, pers. comm.).

Fig. 7 *Zymoseptoria passerinii* (CBS 120382). a. Colony sporulating on potato-dextrose agar; b. colony sporulating on synthetic nutrient-poor agar; c. conidiogenous cells formed inside pycnidia; e, f. conidia from pycnidia (Type I). — Scale bars = 10 µm.

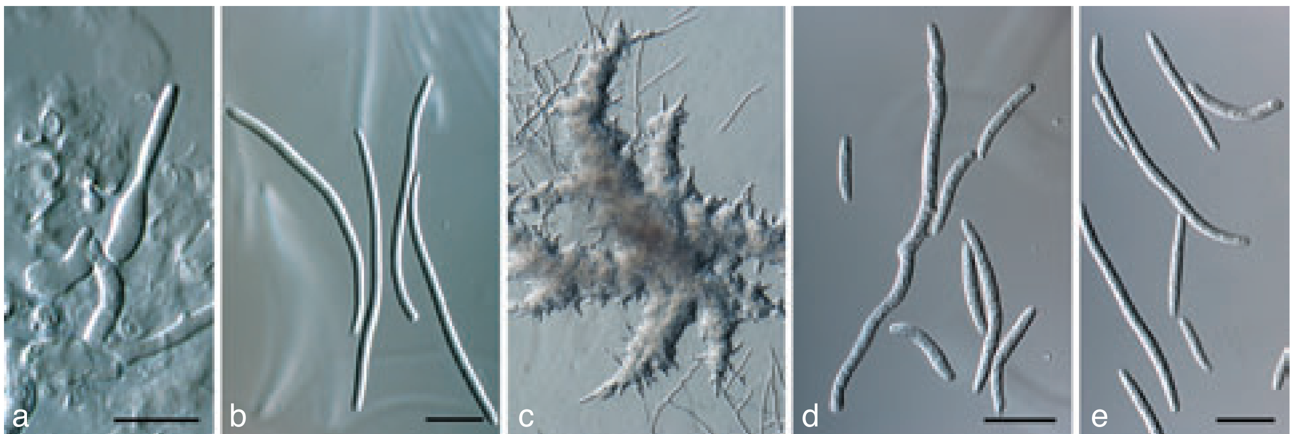

Fig. 8 *Zymoseptoria tritici* (CBS 115943). a. Conidiogenous cells formed inside pycnidia; b. conidia from pycnidia (Type I); colony sporulating on synthetic nutrient-poor agar, showing yeast-like growth; d, e. conidia formed via microcyclic conidiation (Type III). — Scale bars = 10 µm.

Zymoseptoria tritici (Desm.) Quaedvlieg & Crous, *comb. nov.* — MycoBank MB517926; Fig. 8

Basionym: *Septoria tritici* Desm., Ann. Sci. Nat., Bot., sér. 2, 17: 107 (1842).

Teleomorph: '*Mycosphaerella*' *graminicola* (Fuckel) J. Schröt., in Cohn, Krypt.-Fl. Schlesien 3, 2: 340. 1894 ('1893').

Basionym: *Sphaeria graminicola* Fuckel, Fungi Rhenani Exsicc.: no. 1578. 1865.

≡ *Sphaerella graminicola* (Fuckel) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 101. 1870.

Specimens examined. France, on *Triticum* sp. (holotype of *Septoria tritici*; PC). – Germany, Oestrich, on *Triticum repens*, Fuckel, Fungi Rhenani Exsiccati no. 1578 (L, isotype of *Mycosphaerella graminicola*). – Netherlands, Brabant West, on *Triticum aestivum*, coll. *R. Daamen*, 6 May 1981, isol. as single conidium, *W. Veenbaas*, 810507/1, 7 May 1981, epitype designated here CBS H-20545, including teleomorph material on *Triticum* leaf of heterothallic mating IPO 323 (MAT 1-1) × IPO 94269 (MAT 1-2), culture ex-epitype IPO 323 = CBS 115943.

Notes — The isolate designated here as ex-epitype (IPO 323 = CBS 115943) is also the strain used in the whole genome amplification and sequencing of this species (http://genome.jgi-psf.org/Mycgr3/Mycgr3.download.html).

DISCUSSION

For many years the genus *Mycosphaerella* has been treated as a wide general concept to accommodate a range of related and unrelated species and genera that have small ascomata, and hyaline, 1-septate ascospores, without pseudoparaphyses (Aptroot 2006). The observation that *Mycosphaerella*-like teleomorphs were linked to more than 40 different anamorphs (Crous 2009) was thus seen as rather odd, though acceptable within this wider concept used to accommodate these thousands of mostly phytopathogenic fungi. It was only in recent years when the higher order phylogenetic relationships of *Mycosphaerella* was addressed as part of the Assembling the Fungal Tree of Life initiative (Schoch et al. 2006), that *Mycosphaerella* was shown to be polyphyletic (Crous et al. 2007), even containing different families within the *Dothideomycetes* (Crous et al. 2009a, b, Schoch et al. 2009a, b).

The fact that *Septoria* also contains significant morphological variation was commented on by Sutton (1980), who stated that the genus is heterogeneous, and should be revised, containing conidiomata that ranged from acervuli to pycnidia, and conidiogenesis that ranged from blastic sympodial to annellidic (percurrent proliferation) or phialidic (with periclinal thickening). As can be seen with the taxa treated to date, however, these characters alone are also insufficient to delineate all natural

genera, as several modes of conidiogenesis or conidiomatal types occur within the same genus in the *Septoria*-like complex. Part of the reason for the confusion surrounding the genus *Septoria* is based on the fact that until now no DNA sequence data were available for the type species, *S. cytisi*. Due to the lack of cultures of this species, DNA was subsequently extracted from several herbarium specimens. Using this technique, however, some intraspecific variation was observed in both the LSU and ITS sequences of *S. cytisi.* This could possibly be explained by geographical and temporal spread in the sampling sites, spanning 54 years from a region encompassing South and Central Europe, making some sequence variation within these specimens probable. Even if one or two nucleotides might actually be scored wrong in the US0378994-derived LSU sequence for *S. cytisi*, this would not have any impact on the phylogenetic position of *S. cytisi* within the *Septoria* s.str. clade, its nearest sister genus being *Cercospora* in the *Mycosphaerellaceae* (Groenewald et al. 2006).

As shown in the present study (Fig. 2), the genus *Mycosphaerella* is unavailable to accommodate the taxa occurring on graminicolous hosts, as *Mycosphaerella* is restricted to species with *Ramularia* anamorphs (Verkley et al. 2004a, Crous et al. 2009b). Furthermore, *Septoria* s.str. also clusters apart from the species on cereals (Fig. 3), making the name *Septoria* unavailable for these pathogens.

In the present study we introduce a novel genus *Zymoseptoria* to accommodate the *Septoria*-like species occurring on graminicolous hosts. Although species of *Zymoseptoria* tend to have phialides with apical periclinal thickening, this mode of conidiogenesis has also evolved in *Septoria* s.str. (e.g. *S. apiicola*), and is not restricted to *Zymoseptoria*. More importantly, species of *Zymoseptoria* exhibit a yeast-like growth in culture, and have up to three different conidial types that can be observed, namely Type I (pycnidial conidia), Type II (phragmospores on aerial hyphae), and Type III (yeast-like growth proliferating via microcyclic conidiation). Introducing a novel genus for this group of important plant pathogens was not taken lightly, as *Z. passerinii* causes septoria speckled leaf blotch (SSLB) on barley (*Hordeum vulgare*), and has been reported around the globe on this crop (Mathre 1997, Cunfer & Ueng 1999, Goodwin & Zismann 2001, Ware et al. 2007). Septoria tritici blotch (STB) is caused by *Z. tritici* (teleomorph '*Mycosphaerella' graminicola*), and is currently present in all major wheat growing areas. This disease is consistently ranked amongst the most damaging wheat diseases in Australia, Europe, North and South America, and in Europe more than 70 % of all the fungicides applied to wheat are to control STB (Eyal et al. 1987). Wheat, together with maize and rice directly contribute 47 % to global human consumption (Tweeten & Thompson 2009). Since 1961, wheat production has increased globally with almost 300 % on a virtually stable cultivation area of 200 M ha. This progress was largely achieved by increased average yields (FAO 2010). However, the annual growth rate of global wheat production cannot meet the global market requirements in the coming four decades (Fischer et al. 2009, Fischer & Edmeades 2010).

Although *Z. passerinii* and *Z. tritici* share many similarities (Goodwin et al. 2001) (Fig. 3, 4), both pathogens having a dimorphic lifestyle (Mehrabi et al. 2006); one major difference between them is that *Z. tritici* has a year-round and very active sexual cycle (Shaw & Royle 1993, Kema et al. 1996, Zhan et al. 2003), whereas there have been no reports of a sexual cycle for *S. passerinii* observed in nature, despite isolates of *S. passerinii* having opposite mating types being commonly found in natural populations, even on the same leaf (Goodwin et al. 2003), suggesting cryptic sex does exist for *Z. passerinii* (Ware et al. 2007). With respect to the two additional species treated in the present study, *Z. brevis* and *Z. halophila*, almost nothing is known about their relative importance, geographical distribution, host range or sexual behaviour. Given the importance of their known host crops, however, this complex is in dire need of further study.

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