In Press at Mycologia, preliminary version published on June 6, 2012 as doi:10.3852/11-374

Short title: *Z. tritici progenitors*

Zymoseptoria ardabiliae and *Z. pseudotritici*, two progenitor species of the septoria tritici leaf blotch fungus *Z. tritici* (synonym: *Mycosphaerella graminicola*)

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Abstract: *Zymoseptoria* is a newly described genus that includes the prominent wheat pathogen *Zymoseptoria tritici* (synonyms *Mycosphaerella graminicola* and *Septoria*

tritici). Studies indicated that the center of origin of *Z. tritici* is in the Middle East where this important pathogen emerged during the domestication of wheat. Several *Zymoseptoria* species have been found on uncultivated grasses in the Middle East, and in this article we describe two new *Zymoseptoria* species from Iran. These species, isolated from *Elymus repens*, *Dactylis glomerata* and *Lolium perenne*, are named *Z. ardabiliae* and *Z. pseudotritici*. Both species were identified by means of morphological characteristics and phylogenetic analyses of a seven-gene DNA dataset. These taxa comprise some of the closest known relatives of the wheat pathogen *Z. tritici*, confirming the reported close phylogenetic relationship between *Z. tritici* and *Z. pseudotritici*.

Key words: *Dactylis* sp., ITS, *Lolium* sp., LSU, multilocus sequence typing, *Mycosphaerella*, *Septoria*, systematics, *Triticum aestivum*

INTRODUCTION

The genus *Mycosphaerella* in recent years has been revealed to be polyphyletic, not only incorporating numerous genera within *Mycosphaerellaceae* but also within *Davidiellaceae*, *Dissoconiaceae*, *Schizothyriaceae* and *Teratosphaeriaceae* (Crous et al. 2007, 2009a, b). In this regard, *Zymoseptoria* recently was proposed as a novel genus within *Mycosphaerellaceae* to accommodate *Septoria*-like species occurring on *Poaceae* (Quaedvlieg et al. 2011). *Zymoseptoria* also includes the prominent wheat pathogen *Z. tritici* (synonyms *Mycosphaerella graminicola* and *Septoria tritici*), which is the causal agent of septoria tritici leaf blotch on wheat. The pathogen can be found on wheat worldwide. Population genetics and evolutionary studies indicated that the center of origin of *Z. tritici* was in the Middle East, overlapping the center of origin of its wheat host in the Fertile Crescent (Banke and McDonald 2005, Stukenbrock et al. 2007).

To further investigate the evolutionary history of *Z. tritici* material from uncultivated graminicolous species showing septoria-like leaf symptoms was collected from five locations along a transect of approximately 600 km in the northwestern province, Ardabil, in Iran. Two distinct fungal populations were isolated and identified as close relatives of *Z. tritici* based on sequencing of seven loci (Stukenbrock et al. 2007). One of the two new *Zymoseptoria* populations called S1 was recognized as the closest known relative of *Z. tritici*. The two species share the same sequence for 500 base pairs of the ribosomal internal transcribed spacer region (rDNA ITS) that often is used to distinguish ascomycete species (White et al. 1990, Seifert 2009). Coalescent analyses of a multilocus dataset including more than 100 isolates resolved *Z. tritici* and the two new species into distinct lineages and suggested that the divergence of *Z. tritici* from the closest of the new *Zymoseptoria* species took place ~ 11 000 y ago (Stukenbrock et al. 2007). Subsequent genome sequencing of the three *Zymoseptoria* lineages revealed a genome-wide divergence of 6% and 10% between *Z. tritici* and the two Iranian lineages (Stukenbrock et al. 2010, 2011), supporting the separation of these lineages as distinct species.

The *Zymoseptoria* species most closely related to *Z. tritici* (S1) was isolated from the grass hosts *Elymus repens* and *Dactylis glomerata*, and the other species (named S2) was isolated from *Dactylis glomerata* and *Lolium perenne*. Both species were found along a transect of approximately 600 km, indicating they are widely distributed within this region. Of note, the two new species were not found on wheat hosts in adjacent fields. Only *Z. tritici* was isolated from leaves sampled in wheat fields. Similarly *Z. tritici* was not found on the undomesticated grass hosts, consistent with the evolution of host specificity among the *Zymoseptoria* pathogens. Experimental infection on detached leaves let us demonstrate that, although all three

species can infect the same hosts, *T. aestivum*, *E. repens*, *D. glomerata* and *L. perenne*, they differ in their degree of virulence on them (Stukenbrock et al. 2011). Given this overlapping host range, this complex of *Zymoseptoria* pathogen species provides an excellent model system to study host specialization and speciation processes.

Here we present descriptions for the two Iranian *Zymoseptoria* species based on phylogenetic analyses of seven loci sequenced in multiple, closely related *Zymoseptoria*. We also present a detailed description of morphological and culture characteristics. Our multilocus sequence analyses place the two new Iranian *Zymoseptoria* species within the boundaries of the *Zymoseptoria* clade and confirm the reported close phylogenetic relationship with *Z. tritici*.

MATERIALS AND METHODS

Isolates.—Leaves with visible asexual fruiting bodies, pycnidia, were 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 procedure of Crous et al. (2009c). Cultures were plated on fresh MEA, 2% tap water agar supplemented with green, sterile barley leaves (WAB), synthetic nutrient-poor agar (SNA), 2% potato-dextrose agar (PDA) and oatmeal agar (OA) (Crous et al. 2009c) and 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 (TABLE I). Descriptions, nomenclature and illustrations were deposited in MycoBank (www.mycobank.org, Crous et al. 2004).

DNA extraction, amplification and sequencing.—Genomic DNA was extracted from mycelium on MEA with the UltraClean® Microbial DNA Isolation Kit (MoBio Laboratories Inc., Solana Beach, California). These strains were screened for seven loci, namely ITS, LSU, actin (ACT), calmodulin (CAL), RNA polymerase II second largest subunit (RPB2), translation elongation factor 1-alpha (EF1) and β-tubulin (TUB) (TABLE I). DNA amplification and sequencing reactions were performed as described by Cheewangkoon et al. (2008). (See TABLE II for detailed primer descriptions.)

Phylogenetic analysis.—A basic alignment of the obtained sequence data was completed with MAFFT 6 (Katoh et al. 2002) and consequently checked manually in BioEdit 7.0.5.2 (Hall 1999). A Bayesian analysis (critical value for the topological convergence diagnostic set to 0.01) was performed for both the generic RPB2/LSU tree and the ACT, CAL, EF1, TUB, RPB2, ITS and LSU multilocus tree with MrBayes 3.1.1 (Huelsenbeck and Ronquist 2001). Parallel to this, a neighbor joining distance tree (1000 repeats) was generated for the RPB2/LSU dataset with the Kimura 2-parameter substitution model to augment the Bayesian posterior probability values with bootstrap support values. These datasets consisted of approximately 1028 nucleotides for the RPB2/LSU tree (293 nucleotides for RPB2 and 735 nucleotides for LSU) and approximately 2991 nucleotides for the multilocus tree (293 nucleotides for RPB2, 735 nucleotides for LSU, 224 nucleotides for ACT, 269 nucleotides for CAL, 334 nucleotides for EF1, 392 nucleotides for TUB and 478 nucleotides for ITS). *Dissoconium musae* (CBS 122453) was used as outgroup for the RPB2/LSU analyses while *Mycosphaerella punctiformis* (CBS 113265) was used as outgroup for the multigene analyses (see TABLE II for detailed primer descriptions). All novel sequences derived from this study were deposited in GenBank (TABLE I).

To determine whether the multilocus DNA sequence datasets were congruent a partition homogeneity test (Farris et al. 1994) of all possible combinations was performed in PAUP 4.0b10 (Swofford 2003) with 1000 replications. Parallel to this, a 70% neighbor joining (NJ) reciprocal bootstrap method with maximum likelihood distance (Mason-Gamer and Kellogg 1996, Lombard et al. 2010) also was employed to check congruency.

Morphology.—Descriptions were based on fungal cultures sporulating in vitro on WAB, incubated under continuous near-ultraviolet light 2–4 wk. Wherever possible, 30 measurements (1000× magnification) were made of structures mounted in lactic acid, with the extremes of spore measurements in parentheses. Colony colors (surface and reverse) were assessed after 2 wk on MEA, PDA and OA at 25 C in the dark with the color charts of Rayner (1970).

RESULTS

Phylogenetic analyses.—In the *Zymoseptoria* dataset, multilocus sequence data of four *Z. ardabiliae* and four *Z. pseudotritici* isolates were combined partially with the existing dataset used in Quaedvlieg et al. (2011). The adjusted sequence alignment for

each locus consisted of 46 ingroup taxa with *Dissoconium musae* (CBS 122453) acting as outgroup.

Congruency testing.—The strict consensus tree (unpubl), based on the multilocus maximum parsimony analysis, had an identical topology to those of the strict consensus trees obtained for the individual loci. Partition homogeneity tests for all the possible multigene combinations of all seven loci consistently yielded a *P* value of 0.001 and thus were incongruent. However, the 70% reciprocal bootstrap trees of the individual gene regions showed no conflicting tree topologies between the individual loci. Based on these results, the DNA sequences of the seven loci were concatenated and used for phylogenetic analyses (Mason-Gamer and Kellogg 1996, Cunningham 1997).

The RPB2/LSU dataset contains 47 taxa (including the outgroup taxon). The multilocus dataset contains 31 taxa (including the outgroup taxon). Well supported clades for both *Z. ardabiliae* and *Z. pseudotritici* emerged alongside the four previously described *Zymoseptoria* species at both the generic- (Rpb2/LSU) (FIG. 1) and species-level (multigene) trees (FIG. 2). The RPB2/LSU tree (FIG. 1) provides strong posterior probability support for the separation of *Ramularia* from *Zymoseptoria* (0.98). This support is enhanced by the neighbor joining bootstrap support values (calculated with the K2P substitution model), depicted on the right side of the posterior probability values. Bootstrap values also strongly support the split between *Ramularia* and *Zymoseptoria* because these clades only cluster together randomly (51%) in the generated NJ trees.

TAXONOMY

Based on the multigene dataset (FIG. 2), and differences observed in morphology, two new species are introduced herewith for taxa occurring on *Poaceae*.

Zymoseptoria ardabiliae B. McDonald, Stukenbrock & Crous, sp. nov. FIG. 3

MycoBank MB560629

Zymoseptoriae triticii similis, sed conidiis minoribus, (15–)20–25(–30) × 2(–3) µm.

On sterile barley leaves on WA. Conidiomata pycnidial, semi-immersed to erumpent, dark brown to black, subglobose, up to 250 µm diam, with central ostiole, up to 20 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, 5–12 × 3–4 µm, with 1–2 inconspicuous, percurrent proliferations at apex, 1–1.5 µm diam. Type I conidia rarely observed, solitary, hyaline, smooth, guttulate, narrowly cylindrical to subulate, tapering toward acutely rounded apex, with tapered, subtruncate to truncate base, (0–)1-septate, (15–)20–25(–30) × 2(–3) µm. Type II conidia (phragmospores) rarely observed, subcylindrical, guttulate, aseptate, 8–15 × 2–3 µm. Type III conidia abundant on all media studied; hyaline, smooth, guttulate, subcylindrical, 8–12(–15) × 2(–3) µm.

Culture characteristics. Colonies on OA erumpent, spreading, with moderate, dirty white aerial mycelium and lobate, feathery margins; olivaceous gray to fuscous black; on PDA spreading, erumpent, with sparse to moderate aerial mycelium and feathery, lobate margins, olivaceous gray (surface and reverse); reaching 15 mm diam after 2 wk at 25 C; fertile.

Etymology. Named after the location where it was first collected, Ardabil Province, Iran.

Specimen examined. IRAN, ARDABIL PROVINCE: *Lolium* sp. (presumably *L. perenne*), Sep 2004, coll. *M. Javan-Nikkhah*, (holotype CBS H-20732, culture ex-type CBS 130977 = S2).

Notes. *Zymoseptoria ardabiliae* is phylogenetically closest related to *Z. brevis*

(FIGS. 1, 2). Morphologically, however, it is most similar to *Z. passerinii* (conidia 1–3-septate, 21–52 × 1.5–2.2 μm), although its conidia are shorter and wider (conidia 0–1-septate, 15–30 × 2–3 μm) (Quaedvlieg et al. 2011).

Zymoseptoria pseudotritici B. McDonald, Stukenbrock & Crous, sp. nov. FIG. 4

MycoBank MB560628

Zymoseptoriae triticii similis, sed conidiis minoribus, (7–)10–12(–22) × 2.5(–3) μm.

On sterile barley leaves on WA. *Conidiomata* pycnidial, semi-immersed to erumpent, dark brown to black, subglobose, up to 200 μm diam, with central ostiole, up to 20 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, 6–10 × 3–6 μm, with 1–3 inconspicuous, percurrent proliferations at apex, 1–2 μm diam. Type I conidia rarely observed, solitary, hyaline, smooth, guttulate, narrowly cylindrical to subulate, tapering toward acutely rounded apex, with bluntly rounded to truncate base, 0(–1)-septate, (7–)10–12(–22) × 2.5(–3) μm. Forming Type II conidia (phragmospores), subcylindrical, 8–20 × 1.5–3 μm, and Type III conidia (microcyclic conidiation) on SNA. Type III conidia abundant on all media studied, hyaline, smooth, guttulate, subcylindrical, apex obtuse, base truncate, aseptate, (5–)10–12(–15) × 1.5–2(–3) μm.

Culture characteristics. Colonies on OA somewhat erumpent, spreading, with sparse aerial mycelium and smooth, even margins; olivaceous black with patches of pale violet; on PDA surface more erumpent than on OA, with moderate aerial mycelium and feathery, lobate margins, pale olivaceous gray with patches of olivaceous gray; reverse olivaceous gray; reaching 25–30 mm diam after 2 wk at 25 C; fertile.

Etymology. Named after its close phylogenetic affinity to *Zymoseptoria tritici*.

Specimen examined. IRAN, ARDABIL PROVINCE: *Dactylis* sp. (presumably *D. glomerata*), Sep 2004, coll. *M. Javan-Nikkhah*, (holotype CBS H-20731, culture ex-type CBS 130976 = S1).

Notes. *Zymoseptoria pseudotritici* is phylogenetically closely related to *Z. tritici*, although it has much shorter and wider pycnidial conidia (one-septate, 7–22 × 2.5–3 µm) than *Z. tritici* (three-septate, 28–85 × 1.5–2.2 µm), and is more similar to *Z. brevis* (one-septate, 12–17 × 2–2.5 µm) (Quaedvlieg et al. 2011).

Genome data of *Z. pseudotritici* are deposited in the NCBI fungal genome database under accession numbers STIR04_5.9.1: AFIT00000000 and of *Z. ardabiliae* STIR04_1.1.1: AFIU00000000.

DISCUSSION

Although the genus *Mycosphaerella* had been regarded as the largest genus of Ascomycetous fungi (Crous 2009), it recently was shown to be polyphyletic (Crous et al. 2007, 2009b). To complicate matters further, the recent trend to delineate genera as natural monophyletic lineages and to link these to single names in contrast to dual nomenclature (Hawksworth et al. 2011, Wingfield et al. 2012) has resulted in many anamorph names being used to name the former *Mycosphaerella*-like clades. Because many of the type species of these anamorph genera currently are being recollected, it has become possible to better delineate the application of different generic names (Frank et al. 2010, Minnis et al. 2011). With the characterization of *Septoria cytisi*, the type of the genus *Septoria*, and the subsequent introduction of *Zymoseptoria* to accommodate species occurring on *Poaceae*, a new niche was discovered representing many *Zymoseptoria* species on grasses. Although the bootstrap support for the separation of *Zymoseptoria* from *Ramularia* was inconclusive (82% in the parsimony analysis of Quaedvlieg et al. 2011), this value has been significantly improved by adding more genes to the analyses in the present study (0.98 posterior probability,

51% bootstrap support, Rpb2/LSU, FIG. 1). The separation of *Ramularia* (*Mycosphaerella* s.str.) from *Zymoseptoria* on morphological grounds also is defendable because the former genus consists of more than 1000 names, representing fasciculate hyphomycetes with hyaline, septate, catenulate conidia having darkened, thickened scars (Braun 1998), none of which have ever been observed to have coelomycete synanamorphs or to exhibit a yeast-like state in culture.

Many fungal pathogens within the newly described genus *Zymoseptoria* in recent years have been isolated and characterized from uncultivated grasses collected in the Middle East (Stukenbrock et al. 2007, Seifbarghi et al. 2009, Quaedvlieg et al. 2011). Among these are the two new species, *Z. pseudotritici* and *Z. ardabiliae*, described here. *Z. pseudotritici* (formerly called *Mycosphaerella* S1) and *Z. ardabiliae* (formerly called *Mycosphaerella* S2) were isolated from wild grasses in Iran to study their genealogical relationship with the prominent wheat pathogen *Z. tritici*. The evolutionary history of *Z. tritici* was inferred by coalescent analyses using DNA sequence information from the three species. It was shown that *Z. tritici* emerged in parallel with the domestication and cultivation of wheat in the Fertile Crescent 10 000–11 000 y ago from a common ancestor of *Z. tritici* and *Z. pseudotritici* (Stukenbrock et al. 2007, 2011). *Z. ardabiliae* represents another closely related species of both *Z. tritici* and *Z. pseudotritici* that diverged about 20 000–22 000 y ago (Stukenbrock et al. 2007, 2011).

Here we describe the phylogenetic and morphological characters of the two pathogens, *Z. pseudotritici* and *Z. ardabiliae*. *Z. pseudotritici* shares the same ITS sequence as *Z. tritici* but can be resolved as a distinct species based on multilocus phylogenetic analyses, comparative genomics and morphological characters. This close relative of *Z. tritici* also was able to infect *T. aestivum* in a detached leaf assay

but to our knowledge has never been sampled from wheat fields in the Middle East. The pathogen however was isolated from grass hosts of different genera (*Dactylis* sp. and *Elymus* sp.), suggesting that the species can infect a variety of grass hosts.

Between *Z. tritici* and *Z. pseudotritici* the overall genome identity at the nucleotide level is 94%, supporting the distinction of the pathogens as different species. This high genome-wide nucleotide similarity let us compare > 9000 predicted genes (80% of the total number of predicted genes; Goodwin et al. 2011) and to identify a small set of positively selected genes (Stukenbrock et al. 2010). These genes likely played a role in either host specialization or speciation, and their functional roles currently are being investigated.

In addition to *Z. pseudotritici* we also provide here a detailed description of the other close relative of *Z. tritici*, *Z. ardabiliae*. Like *Z. pseudotritici*, *Z. ardabiliae* produces lesions on grass hosts similar to the septoria tritici leaf blotch symptoms caused by *Z. tritici* on wheat. *Zymoseptoria ardabiliae* was isolated from *Elymus* sp. and *Lolium* sp. and has a broad host range similar to *Z. pseudotritici*. Our comparative genome analyses of *Z. ardabiliae* and *Z. tritici* have an average nucleotide identity of 90%, supporting the multilocus phylogenetic analyses reported here that place *Z. ardabiliae* more distant to *Z. tritici* than *Z. pseudotritici*. In *Z. ardabiliae* we also have identified a set of positively selected genes, which likely are involved in host specialization or speciation processes.

The detailed species characterization of *Z. pseudotritici* and *Z. ardabiliae* contributes to our understanding of *Zymoseptoria* species. *Zymoseptoria tritici* has co-evolved with a cultivated host and thereby became specifically adapted to the wheat agro-ecosystem. On the other hand *Z. pseudotritici* and *Z. ardabiliae* both evolved in natural grassland. The host ranges of the three species likely reflect the host

environments where the fungi exist and where they have evolved. This group of closely related dothideomycete species provides an excellent model system to investigate processes of host specialization in plant pathogenic fungi and to increase our understanding of speciation processes in fungi.

LITERATURE CITED

Banke S, McDonald BA. 2005. Migration patterns among global populations of the pathogenic fungus *Mycosphaerella graminicola*. Mol Ecol 14:1881–1896.

Braun U. 1998. A monograph of *Cercosporella*, *Ramularia* and allied genera (phytopathogenic hyphomycetes). Vol. 2. Eching, Germany: IHW-Verlag.

Cheewangkoon R, Crous PW, Hyde KD, Groenewald JZ, To-anan C. 2008. Species of *Mycosphaerella* and related anamorphs on *Eucalyptus* leaves from Thailand. Persoonia 21:77–91.

Crous PW. 2009. Taxonomy and phylogeny of the genus *Mycosphaerella* and its anamorphs. Fungal Divers 38:1–24.

———, Braun U, Groenewald JZ. 2007. *Mycosphaerella* is polyphyletic. Stud Mycol 58:1–32.

———, Gams W, Stalpers JA, Robert V, Stegehuis G. 2004. MycoBank: an online initiative to launch mycology into the 21st century. Stud Mycol 50:19–22.

———, Schoch CL, Hyde KD, Wood AR, Gueidan C, Hoog GS de, Groenewald JZ. 2009a. Phylogenetic lineages in the *Capnodiales*. Stud Mycol 64:17–47.

———, Summerell BA, Carnegie AJ, Wingfield MJ, Hunter GC, Burgess TI, Andjic V, Barber PA, Groenewald JZ. 2009b. Unraveling *Mycosphaerella*: Do you believe in genera? Persoonia 23:99–118.

———, Verkley GJM, Groenewald JZ, Samson RA, eds. 2009c. Fungal biodiversity. CBS Laboratory Manual Series 1:1–269. Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Cunningham CW. 1997. Can three incongruence tests predict when data should be combined? Mol Biol Evol 14:733–740.

Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Testing significance of incongruence. Cladistics 10:315–319.

Frank J, Crous PW, Groenewald JZ, Oertel B, Hyde KD, Phengsintham P, Schroers HJ. 2010. *Microcyclospora* and *Microcyclosporella*: novel genera accommodating epiphytic fungi causing sooty blotch on apple. Persoonia 24:93–105.

Goodwin SB, Ben M'barek S, Dhillon B, Wittenberg A, Crane CF et al. 2011. Finished genome of *Mycosphaerella graminicola* reveals stealth pathogenesis and extreme plasticity. PLoS Genet 7: e1002070. doi:10.1371/journal.pgen.1002070.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield, MJ et al. 2011. The Amsterdam declaration on fungal nomenclature. IMA Fungus 2:105–112.

Lombard L, Crous PW, Wingfield BD, Wingfield MJ. 2010. Multigene phylogeny and mating tests reveal three cryptic species related to *Calonectria pauciramosa*. Stud Mycol 66:15–30.

Mason-Gamer RJ, Kellogg EA. 1996. Testing for phylogenetic conflict among molecular datasets in the tribe *Triticeae* (*Gramineae*). Syst Biol 45:524–545.

Minnis AM, Kennedy AH, Grenier DB, Rehner SA, Bischoff JF. 2011. *Asperisporium* and *Pantospora* (Mycosphaerellaceae): epitypifications and phylogenetic placement. Persoonia 27:1–8.

Page RDM. 1996. TreeVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

Quaedvlieg W, Kema GHJ, Groenewald JZ, Verkley GJM, Seifbarghi S, Razavi M, Mirzadi Gohari A, Mehrabi R, Crous PW. 2011. *Zymoseptoria* gen. nov.: a new genus to accommodate *Septoria*-like species occurring on graminicolous hosts. Persoonia 26:57–69.

Rayner RW. 1970. A mycological color chart. Kew, Surrey, UK: CMI and British Mycological Society.

Seifbarghi S, Razavi M, Aminian H, Zare R, Etebarian H. 2009. Studies on the host range of *Septoria* species on cereals and some wild grasses in Iran. Phytopathol Mediterr 48:422–429.

Seifert KA. 2009. Progress toward DNA barcoding of fungi. Mol Ecol Resour 9:83–89.

Stukenbrock EH, Banke S, Javan-Nikkhah M, McDonald BA. 2007. Origin and domestication of the fungal wheat pathogen *Mycosphaerella graminicola* via sympatric speciation. Mol Biol Evol 24:398–411.

———, Jørgensen FG, Zala M, Hansen TT, McDonald BA, Schierup MH. 2010. Whole genome and chromosome evolution associated with host adaptation and speciation of the wheat pathogen *Mycosphaerella graminicola*. PLoS Genet 6: e1001189.

———, Bataillon T, Dutheil JY, Hansen TT, Li R, Zala M, McDonald BA, Wang J, Schierup MH. 2011. The making of a new pathogen: insights from comparative population genomics of the domesticated wheat pathogen *Mycosphaerella graminicola* and its wild sister species. Genome Res doi: 10.1101/gr.118851.110

Swofford DL. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

White TJ, Bruns TD, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. New York: Academic Press. p 315–322.

Wingfield MJ, de Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, Crous PW. 2011. One fungus, one name promotes progressive plant pathology. Mol Plant Path DOI: 10.1111/J.1364-3703.2011.00768.X

LEGENDS

FIG. 1. A Bayesian 50% majority rule RPB2/LSU consensus tree, containing all known *Zymoseptoria* species, and representative taxa from four closely related genera. Bayesian posterior probabilities and neighbor joining K2M bootstrap support values for the genus nodes. A stop rule (set to 0.01) for the critical value for the topological convergence diagnostic was used for the Bayesian analysis. The tree was rooted to *Dissoconium musae* (CBS 122453). Bar indicates 0.1 expected changes per site.

FIG. 2. A Bayesian 50% majority rule ACT, CAL, EF1, TUB, RPB2, ITS and LSU consensus tree, containing all known *Zymoseptoria* species, including the newly described *Z. ardabiliae* and *Z. pseudotritici*. Bayesian posterior probabilities and neighbor joining K2M bootstrap support values are at the nodes. A stop rule (set to 0.01) for the critical value for the topological convergence diagnostic was used for the Bayesian analysis. The tree was rooted to *Mycosphaerella punctiformis* (CBS 113265). Bar indicates 0.1 expected changes per site.

FIG. 3. *Zymoseptoria ardabiliae* (CBS 130977). A. Colony sporulating on oatmeal agar. B. Yeast-like growth on synthetic nutrient-poor agar. C–G. Type I conidia undergoing microcyclic conidiation and Type III conidia (yeast-like). H. Type II conidia (phragmospores). Bars = 10 µm.

FIG. 4. *Zymoseptoria pseudotritici* (CBS 130976). A. Colony sporulating on oatmeal agar. B. Yeast-like growth on synthetic nutrient-poor agar. C, D. Type III conidia (yeast-like) on synthetic nutrient-

poor agar. E. Type I conidia undergoing microcyclic conidiation. Type III conidia (yeast-like) formed on potato dextrose agar. Bars = 10 µm.

FOOTNOTES

Submitted 12 Nov 2011; accepted 21 Mar 2012.

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TABLE I. Cultures subjected to DNA sequencing

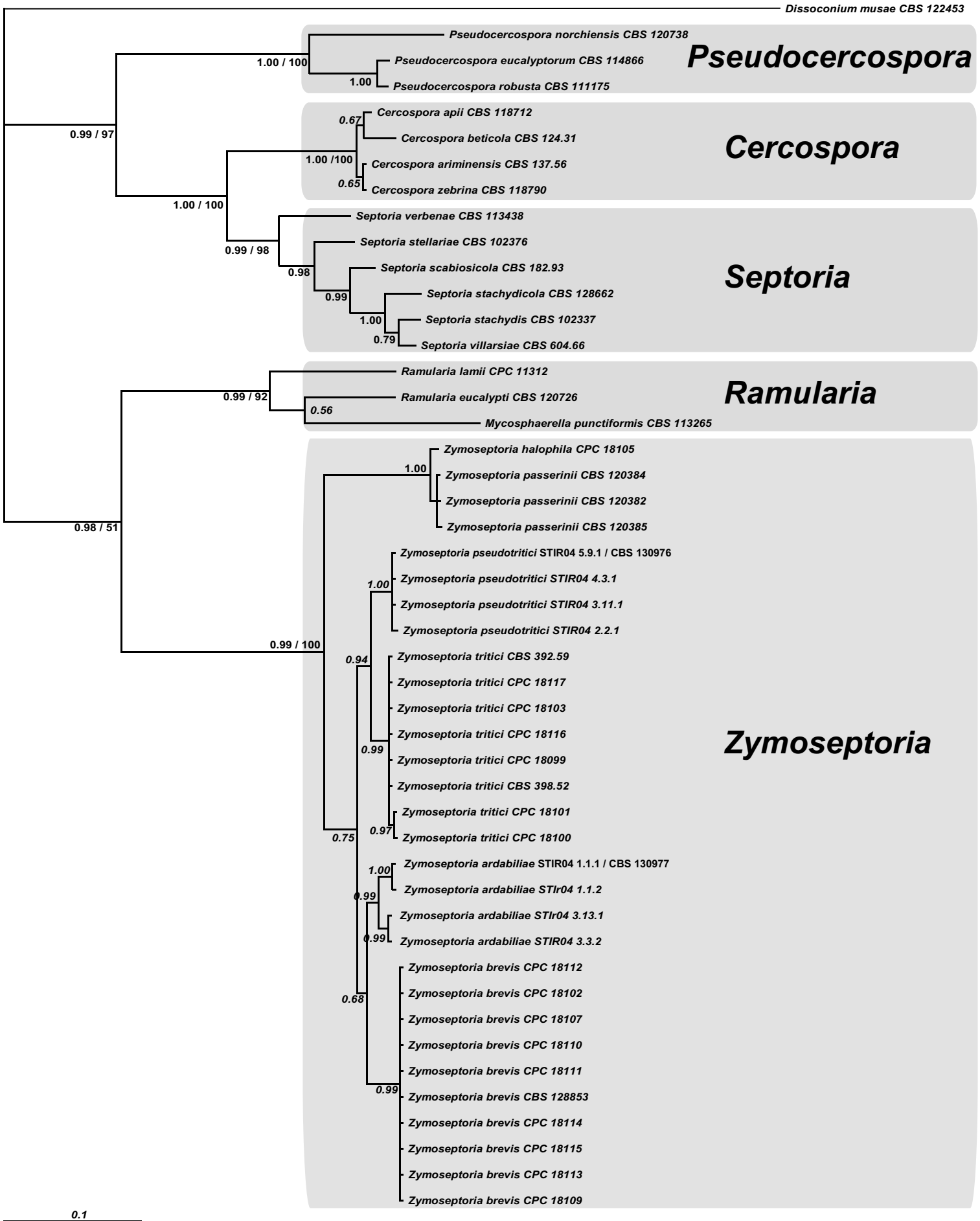
Species	Isolate no[a]	Host	Location	Collected by	ACT	CAL	ITS	TUB	RPB2	LSU	EF1
Cercospora apii	CBS 118712	—	Fiji	P. Tyler	—	—	—	—	JQ739858	GQ852583	—
Cercospora ariminensis	CBS 137.56	*Hedysarum coronarium*	Italy	M. Ribaldi	—	—	—	—	JQ739859	JF700933	—
Cercospora beticola	CBS 124.31	*Beta vulgaris*	Romania	—	—	—	—	—	JQ739860	JF700934	—
Cercospora zebrina	CBS 118790	*Trifolium subterraneum*	Australia	M.J. Barbetti	—	—	—	—	JQ739861	JQ739815	—
Dissoconium musae	CBS 122453	*Musa acuminata*	India	I. Buddenhagen	—	—	—	—	JQ739862	JQ739816	—
Mycosphaerella punctiformis	CBS 113265	*Quercus robur*	the Netherlands	G. Verkley	JQ739739	JQ739759	JQ739802	JQ739752	JQ739863	DQ470968	JQ739771
Pseudocercospora eucalyptorum	CBS 114866	*Eucalyptus nitens*	South Africa	P.W. Crous	—	—	—	—	JQ739864	JQ739817	—
Pseudocercospora norchiensis	CBS 120738	*Eucalyptus* sp.	Italy	W. Gams	—	—	—	—	JQ739865	JQ739818	—
Pseudocercospora robusta	CBS 111175	*Eucalyptus robus*	South Africa	P.W. Crous	—	—	—	—	JQ739866	JQ739819	—
Ramularia eucalypti	CBS 120726	*Eucalyptus grandiflora*	Italy	W. Gams	—	—	—	—	JQ739867	JQ739820	—
Ramularia lamii	CPC 11312	*Vicia amurens*	Korea	H.D. Shin	—	—	—	—	JQ739868	JQ739821	—
Septoria scabiosicola	CBS 182.93	*Succissa pratensis*	France	H.A. van de Aa	—	—	—	—	JQ739869	JQ739822	—
Septoria stachydicola	CBS 128662	*Stachydis riederi*	Republic of Korea	S.B. Hong	—	—	—	—	JQ739870	JQ739823	—
Septoria stachydis	CBS 102337	*Stachys sylvatica*	Netherlands	G. Verkley	—	—	—	—	JQ739871	JQ739824	—
Septoria stellariae	CBS 102376	*Stellaria media*	Netherlands	G. Verkley	—	—	—	—	JQ739872	JQ739825	—
Septoria verbenae	CBS 113438	*Verbena officinalis*	New Zealand	G. Verkley	—	—	—	—	JQ739873	JQ739826	—
Septoria villarsiae	CBS 604.66	*Nymphoides peltata*	Netherlands	L. Marvanová	—	—	—	—	JQ739874	JQ739827	—
Zymoseptoria pseudotritici	STIR04 5.9.1 (= CBS 130976)	*Dactylis glomerata*	Iran	M. Javan-Nikkhah	JN982476	JN982478	JN982480	JN982484	JN982482	JQ739828	JQ739772
Zymoseptoria pseudotritici	STIR04 2.2.1	*Dactylis* sp.	Iran	M. Javan-Nikkhah	JQ739740	JQ739760	JQ739803	JQ739753	JQ739875	JQ739829	JQ739773
Zymoseptoria pseudotritici	STIR04 3.11.1	*Agropyron* sp.	Iran	M. Javan-Nikkhah	JQ739741	JQ739761	JQ739804	JQ739754	JQ739876	JQ739830	JQ739774
Zymoseptoria pseudotritici	STIR04 4.3.1	*Agropyron* sp.	Iran	M. Javan-Nikkhah	JQ739742	JQ739762	JQ739805	JQ739755	JQ739877	JQ739831	JQ739775
Zymoseptoria brevis	IRAN1485C (= CPC 18102)	*Phalaris paradoxa*	Iran	—	JF701035	JF701103	JF700866	JF700967	JQ739878	JQ739832	JQ739776
Zymoseptoria brevis	CPC 18106 (=8S) = CBS 128853	*Phalaris minor*	Iran	—	JF701036	JF701104	JF700867	JF700968	JF700798	JQ739833	JQ739777
Zymoseptoria brevis	IRAN1486C (= CPC 18107)	*Phalaris minor*	Iran	—	JF701037	JF701105	JF700868	JF700969	JF700799	JQ739834	JQ739778
Zymoseptoria brevis	CPC 18109 (= 81)	*Phalaris paradoxa*	Iran	—	JF701038	JF701106	JF700869	JF700970	JF700800	JQ739835	JQ739779
Zymoseptoria brevis	CPC 18110 (=83)	*Phalaris paradoxa*	Iran	—	JF701039	JF701107	JF700870	JF700971	JF700801	JQ739836	JQ739780
Zymoseptoria brevis	CPC 18111 (=84)	*Phalaris paradoxa*	Iran	—	JF701040	JF701108	JF700871	JF700972	JF700802	JQ739837	JQ739781

Zymoseptoria brevis	CPC 18112 (=85)	*Phalaris paradoxa*	Iran	—	JF701041	JF701109	JF700872	JF700973	JF700803	JQ739838	JQ739782
Zymoseptoria brevis	CPC 18113 (=86)	*Phalaris paradoxa*	Iran	—	JF701042	JF701110	JF700873	JF700974	JF700804	JQ739839	JQ739783
Zymoseptoria brevis	CPC 18114 (=87)	*Phalaris paradoxa*	Iran	—	JF701043	JF701111	JF700874	JF700975	JF700805	JQ739840	JQ739784
Zymoseptoria brevis	CPC 18115 (=88)	*Phalaris paradoxa*	Iran	—	JF701044	JF701112	JF700875	JF700976	JF700806	JQ739841	JQ739785
Zymoseptoria halophila	IRAN1483C (= CPC 18105) = CBS 128854	*Hordeum glaucum*	Iran	—	JF701045	JF701113	JF700876	JF700977	JF700808	JQ739842	JQ739786
Zymoseptoria passerinii	CBS 120382	*Hordeum vulgare*	USA	S. Goodwin	JF701046	JF701114	JF700877	JF700978	JF700809	JQ739843	JQ739787
Zymoseptoria passerinii	CBS 120384	*Hordeum vulgare*	USA	S. Ware	JF701047	JF701115	JF700878	JF700979	JF700810	JQ739844	JQ739788
Zymoseptoria passerinii	CBS 120385	*Hordeum vulgare*	USA	S. Ware	JF701048	JF701116	CJF700879	JF700980	JF700811	JQ739845	JQ739789
Zymoseptoria ardabiliae	STIR04 1.1.1 (= CBS 130977)	*Lolium perenne*	Iran	M. Javan-Nikkhah	JN982477	JN982479	JQ739806	JN982485	JN982483	JQ739846	JQ739790
Zymoseptoria ardabiliae	STIR04 3.13.1	*Agropyron* sp.	Iran	M. Javan-Nikkhah	JQ739743	JQ739763	JQ739807	JQ739756	JQ739878	JQ739847	JQ739791
Zymoseptoria ardabiliae	STIR04 3.3.2	*Agropyron* sp.	Iran	M. Javan-Nikkhah	JQ739744	JQ739764	JQ739808	JQ739757	JQ739879	JQ739848	JQ739792
Zymoseptoria ardabiliae	STIR04 1.1.2	*Lolium* sp.	Iran	M. Javan-Nikkhah	JQ739745	JQ739765	JQ739809	JQ739758	JQ739880	JQ739849	JQ739793
Zymoseptoria tritici	CBS 392.59	*Triticum aestivum*	—	E. Becker	JF701055	JF701123	AY152603	JF700987	JF700818	JQ739850	JQ739794
Zymoseptoria tritici	CBS 398.52	*Triticum aestivum*	Switzerland	E. Muller	JF701056	JF701124	JF700886	JF700988	JF700819	JQ739851	JQ739795
Zymoseptoria tritici	CPC 18099	*Aegilops tauschii*	Iran	M. Razavi	JQ739746	JQ739766	JF700880	JF700981	JQ739881	JQ739852	JQ739796
Zymoseptoria tritici	CPC 18116	*Avena* sp.	Iran	A.M. Gohari	JQ739747	JQ739767	JQ739810	JF700985	JQ739882	JQ739853	JQ739797
Zymoseptoria tritici	CPC 18103	*Calamagrostis* sp.	Iran	M. Razavi	JQ739748	JQ739768	JQ739811	JF700984	JQ739883	JQ739854	JQ739798
Zymoseptoria tritici	CPC 18101	*Aegilops tauschii*	Iran	M. Razavi	JQ739749	JQ739769	JQ739812	JF700983	JQ739884	JQ739855	JQ739799
Zymoseptoria tritici	CPC 18100	*Aegilops tauschii*	Iran	M. Razavi	JQ739750	JQ739770	JQ739813	JF700982	JQ739885	JQ739856	JQ739800
Zymoseptoria tritici	CPC 18117	*Avena* sp.	Iran	A.M. Gohari	JQ739751	JQ739771	JQ739814	JF700986	JQ739886	JQ739857	JQ739801

[a]CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; STIR04: Culture collection of Bruce McDonald, Institute of Integrative Biology, ETH Zurich, Switzerland.

TABLE II. Primers used in this study for generic amplification and sequencing

Locus	**Primer**	**Primer sequence 5'–3':**	**Orientation**	**Reference**
Actin	ACT-512F	ATGTGCAAGGCCGGTTTCGC	Forward	Carbone and Kohn (1999)
Actin	ACT2Rd	ARRTCRCGDCCRGCCATGTC	Reverse	Quaedvlieg et al. (2011)
Calmodulin	CAL-235F	TTCAAGGAGGCCTTCTCCCTCTT	Forward	Present study
Calmodulin	CAL-228F	GAGTTCAAGGAGGCCTTCTCCC	Forward	Carbone and Kohn (1999)
Calmodulin	CAL2Rd	TGRTCNGCCTCDCGGATCATCTC	Reverse	Quaedvlieg et al. (2011)
Elongation factor-1α	EF1-728F	CAT CGA GAA GTT CGA GAA GG	Forward	Carbone and Kohn (1999)
Elongation factor-1α	EF-2	GGA RGT ACC AGT SAT CAT GTT	Reverse	O'Donnell et al. (1998)
β-tubulin	T1	AACATGCGTGAGATTGTAAGT	Forward	O'Donnell and Cigelnik (1997)
β-tubulin	β-Sandy-R	GCRCGNGGVACRTACTTGTT	Reverse	Present study
RPB2	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	Forward	Liu et al. (1999)
RPB2	fRPB2-414R	ACMANNCCCCARTGNGWRTTRTG	Reverse	Quaedvlieg et al. (2011)
LSU	LSU1Fd	GRATCAGGTAGGRATACCCG	Forward	Crous et al. (2009a)
LSU	LR5	TCCTGAGGGAAACTTCG	Reverse	Vilgalys and Hester (1990)
ITS	ITS1	GAAGTAAAAGTCGTAACAAGG	Forward	White et al. (1990)
ITS	ITS4	TCC TCC GCT TAT TGA TAT GC	Reverse	White et al. (1990)


Dissoconium musae CBS 122453
Pseudocercospora norchiensis CBS 120738
Pseudocercospora eucalyptorum CBS 114866
Pseudocercospora robusta CBS 111175
Pseudocercospora
Cercospora apii CBS 118712
Cercospora beticola CBS 124.31
Cercospora ariminensis CBS 137.56
Cercospora zebrina CBS 118790
Cercospora
Septoria verbenae CBS 113438
Septoria stellariae CBS 102376
Septoria scabiosicola CBS 182.93
Septoria stachydicola CBS 128662
Septoria stachydis CBS 102337
Septoria villarsiae CBS 604.66
Septoria
Ramularia lamii CPC 11312
Ramularia eucalypti CBS 120726
Mycosphaerella punctiformis CBS 113265
Ramularia
Zymoseptoria halophila CPC 18105
Zymoseptoria passerinii CBS 120384
Zymoseptoria passerinii CBS 120382
Zymoseptoria passerinii CBS 120385
Zymoseptoria pseudotritici STIR04 5.9.1 / CBS 130976
Zymoseptoria pseudotritici STIR04 4.3.1
Zymoseptoria pseudotritici STIR04 3.11.1
Zymoseptoria pseudotritici STIR04 2.2.1
Zymoseptoria tritici CBS 392.59
Zymoseptoria tritici CPC 18117
Zymoseptoria tritici CPC 18103
Zymoseptoria tritici CPC 18116
Zymoseptoria tritici CPC 18099
Zymoseptoria tritici CBS 398.52
Zymoseptoria tritici CPC 18101
Zymoseptoria tritici CPC 18100
Zymoseptoria ardabiliae STIR04 1.1.1 / CBS 130977
Zymoseptoria ardabiliae STIr04 1.1.2
Zymoseptoria ardabiliae STIr04 3.13.1
Zymoseptoria ardabiliae STIR04 3.3.2
Zymoseptoria brevis CPC 18112
Zymoseptoria brevis CPC 18102
Zymoseptoria brevis CPC 18107
Zymoseptoria brevis CPC 18110
Zymoseptoria brevis CPC 18111
Zymoseptoria brevis CBS 128853
Zymoseptoria brevis CPC 18114
Zymoseptoria brevis CPC 18115
Zymoseptoria brevis CPC 18113
Zymoseptoria brevis CPC 18109
Zymoseptoria
1.00 / 100
1.00
0.99 / 97
0.67
1.00 /100
0.65
1.00 / 100
0.99 / 98
0.98
0.99
1.00
0.79
0.99 / 92
0.56
0.98 / 51
1.00
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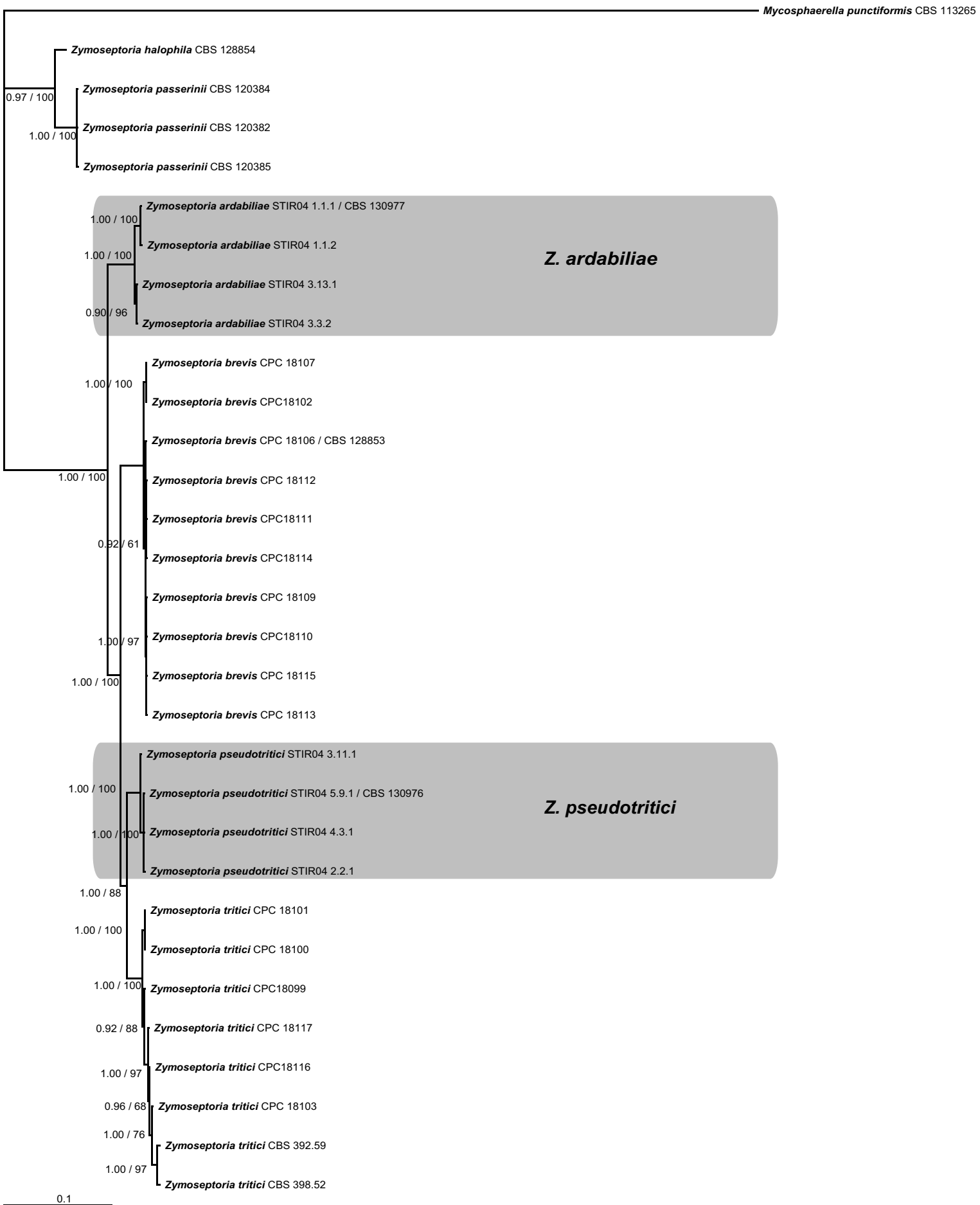
Mycosphaerella punctiformis CBS 113265
Zymoseptoria halophila CBS 128854
Zymoseptoria passerinii CBS 120384
Zymoseptoria passerinii CBS 120382
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0.97 / 100
1.00 / 100
Zymoseptoria ardabiliae STIR04 1.1.1 / CBS 130977
Zymoseptoria ardabiliae STIR04 1.1.2
Zymoseptoria ardabiliae STIR04 3.13.1
Zymoseptoria ardabiliae STIR04 3.3.2
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1.00 / 100
0.90 / 96
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Zymoseptoria brevis CPC 18107
Zymoseptoria brevis CPC18102
Zymoseptoria brevis CPC 18106 / CBS 128853
Zymoseptoria brevis CPC 18112
Zymoseptoria brevis CPC18111
Zymoseptoria brevis CPC18114
Zymoseptoria brevis CPC 18109
Zymoseptoria brevis CPC18110
Zymoseptoria brevis CPC 18115
Zymoseptoria brevis CPC 18113
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1.00 / 97
1.00 / 100
Zymoseptoria pseudotritici STIR04 3.11.1
Zymoseptoria pseudotritici STIR04 5.9.1 / CBS 130976
Zymoseptoria pseudotritici STIR04 4.3.1
Zymoseptoria pseudotritici STIR04 2.2.1
1.00 / 100
1.00 / 100
Z. pseudotritici
1.00 / 88
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Zymoseptoria tritici CPC 18100
Zymoseptoria tritici CPC18099
Zymoseptoria tritici CPC 18117
Zymoseptoria tritici CPC18116
Zymoseptoria tritici CPC 18103
Zymoseptoria tritici CBS 392.59
Zymoseptoria tritici CBS 398.52
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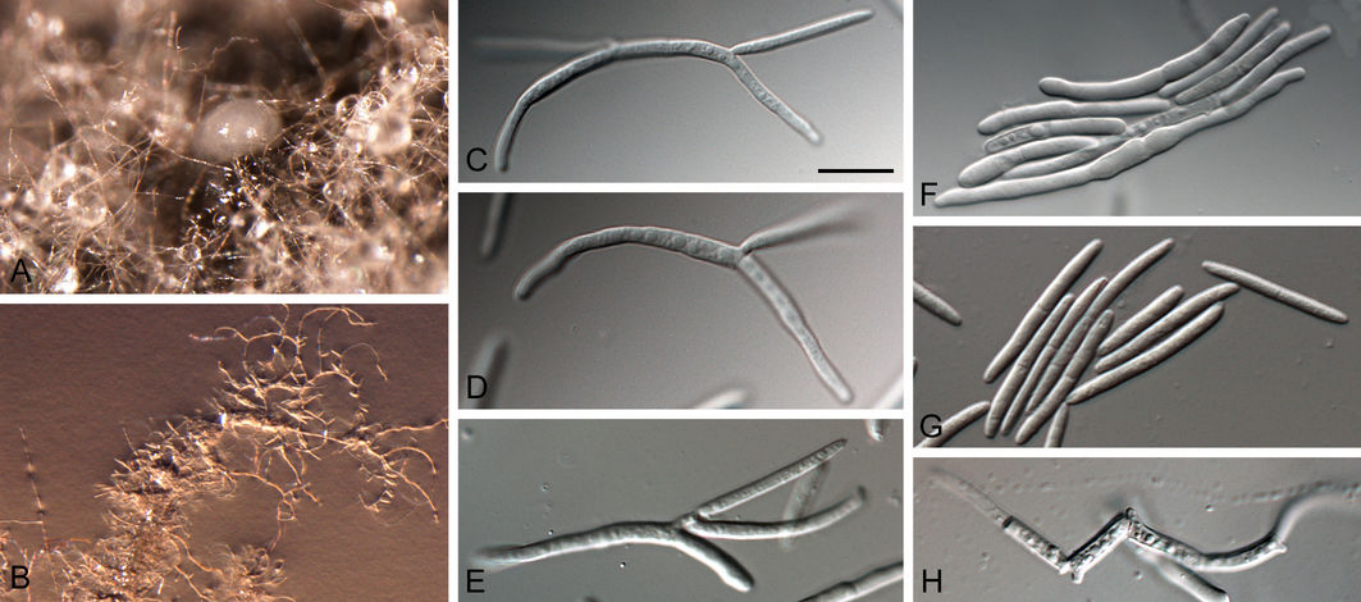
1.00 / 97
0.96 / 68
1.00 / 76
1.00 / 97
0.1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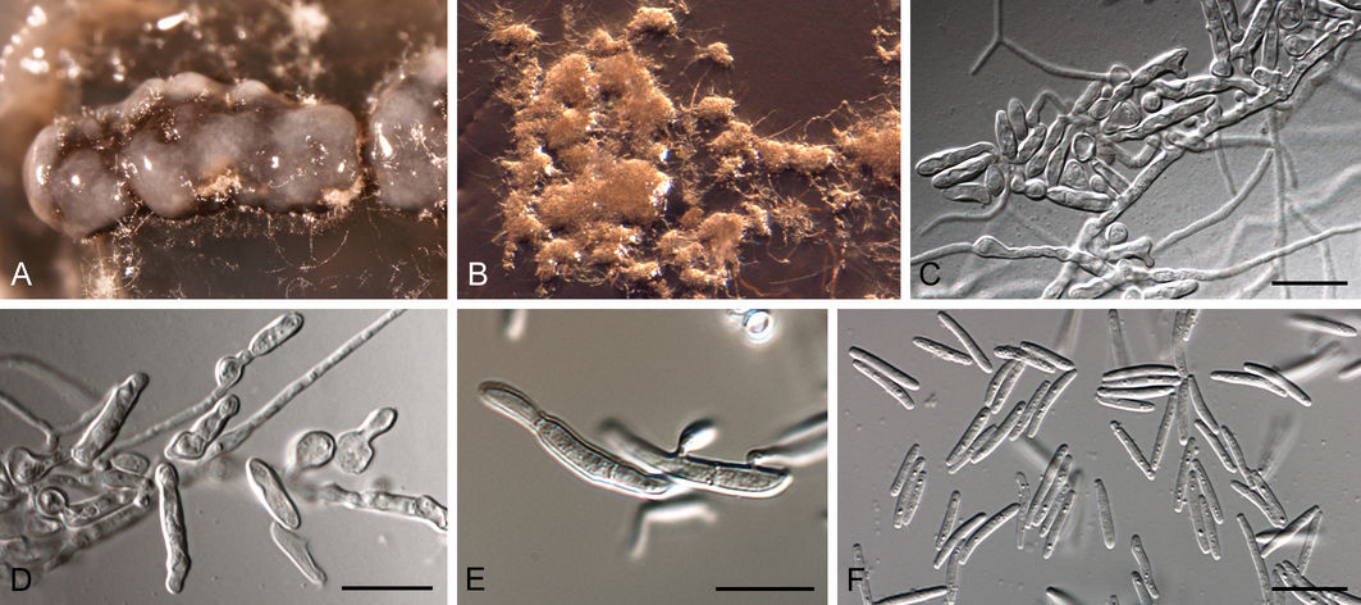
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