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Two novel species of *Parastagonospora* (Phaeosphaeriaceae, Pleosporales) on grasses from Italy and Russia

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Abstract

Phaeosphaeriaceae comprises many fungal species occuring mainly on grasses and cereal crops as endophytes, saprobes and especially pathogens. *Parastagonospora* is an important genus in Phaeosphaeriaceae that includes pathogens causing leaf and glume blotch on cereal crops. In this study, a sexual morph species and an asexual morph species, occuring as saprobes on Poaceae are introduced based on morphology and a combined molecular analysis of the LSU, ITS and RPB2 gene sequence data. The sexual morph of a new *Parastagonospora* species, *P. elymi* was isolated from dead stems of *Elymus repens* in Russia. *Parastagonospora elymi* is similar to the sexual morph of *P. avenae* in having cylindrical asci, bearing eight, overlapping biseriate, fusiform ascospores but can be distinguished by its subglobose to conical-shaped, wider ascomata. In addition, no sheath was observed surrounding the ascospores. An asexual morph of *Parastagonospora* was isolated from dead stems of *Dactylis glomerata* in Italy and is introduced as *P. macrouniseptata*. *Parastagonospora macrouniseptata* is a coelomycete and bears a close resemblance to *P. allouniseptata* and *P. uniseptata* in having globose to subglobose, pycnidia and hyaline, cylindrical, 1-septate conidia. However, the new species is morphologically distinct in its conidiomata characteristics and phylogenetic affinity.

Key words – 2 new species – Dothideomycetes – multi-gene analysis – Poaceae – saprobes – taxonomy

Introduction

Phaeosphaeriaceae M.E. Barr is an important family of the order Pleosporales and was introduced by Barr (1979) with *Phaeospharia* I. Miyake as the generic type (Zhang et al. 2009, 2012, Hyde et al. 2013, Phookamsak et al. 2014, 2017). It consists of species that are mostly

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phytopathogenic on economically important crops, saprobic or endophytic on plants, and some associated with lichens or humans infections (Shoemaker & Babcock 1989, Kirk et al. 2008, Zhang et al. 2009, Lawrey et al. 2012, Phookamsak et al. 2014, Wijayawardene et al. 2014, Jayasiri et al. 2015b, Li et al. 2015, Tennakoon et al. 2016, Ahmed et al. 2017, Senanayake et al. 2018). Phookamsak et al. (2014) comprehensively reviewed the family Phaeosphaeriaceae, accepting 30 genera (including 17 asexual morph genera) based on morphological data, with the addition of multi-gene (ITS, LSU, SSU, RPB2 and TEF1) analyses. Several more genera have been added to Phaeosphaeriaceae following recent studies (Liu et al. 2015, Ariyawansa et al. 2015, Crous et al. 2015, Li et al. 2015, Phukhamsakda et al. 2015, Tennakoon et al. 2016, Hyde et al. 2017, Thambugala et al. 2017, Karunarathna et al. 2017, Phookamsak et al. 2017, 2019, Wanasinghe et al. 2018, Bakhshi et al. 2019, Yang et al. 2019, Maharachchikumbura et al. 2019). The majority of asexual morphs in this family are coelomycetous (Li et al. 2015). The family currently comprises more than 400 species from over 60 genera (Phookamsak et al. 2017, 2019, Wijayawardene et al. 2018, Bakhshi et al. 2019).

Among the pathogenic microfungi occuring on grasses, asexual morph genera in the Phaeosphaeriaceae are commonly reported and are of great importance (Lamprecht et al. 2011, Phookamsak et al 2014, 2017, Bakhshi et al. 2015). *Parastagonospora* Quaedvl., Verkley & Crous is one such genus. *Parastagonospora avenae* (A.B. Frank) Quaedvl., Verkley & Crous and *P. nodorum* (Berk.) Quaedvl., Verkley & Crous (the generic type), are both pathogens that cause leaf and glume blotch on cereal crops such as barley, wheat and rye, leading to heavy losses of yield (Cunfer 2000, Stukenbrock et al. 2006, Vergnes et al. 2006, Quaedvlieg et al. 2013, Phookamsak et al. 2014).

Elymus repens (L.) Gould, commonly known as couch grass, is important for grazing animals, used in traditional Austrian medicine and is considered an invasive weed in some parts of the world (Werner & Rioux 1977, Klein 2011, Vogl et al. 2013). *Dactylis glomerata* L. (cocksfoot) is a commonly found grass distributed in temperate regions throughout Asia, Europe and North Africa (Sánchez Márquez et al. 2007). It is widely used as fodder or for pastures (Hackney & Dear 2007). Five fungal species have been recorded from *Elymus* spp. in Russia (Farr & Rossman 2019) while more than ten species have been recorded on *D. glomerata* from Italy (Farr & Rossman 2019). In this study two novel species, one on *E. repens* from Russia and the other on *D. glomerata* from Italy are introduced based on phylogenetic analyses of ITS, LSU and RPB2 sequence data complete with descriptions and illustrations.

Materials & Methods

Sample collection and specimen examination

Samples were collected in Italy and Russia, and brought back to the laboratory in paper bags. Morphological features of the fungi were examined using a Motic SMZ 168 dissecting microscope. Free-hand sections of the fungal fruiting structures were obtained and mounted in water on a slide to observe their microscopic features. Micro-morphologies were examined using a Nikon ECLIPSE 80i compound microscope and photographed using a Canon EOS 600D digital camera fitted to the microscope. The images were processed using Adobe Photoshop CS5 Extended version 12.0 software (Adobe Systems, USA). The Tarosoft (R) Image Frame Work program v. 0.9.7 was used for taking measurements. Isolates were obtained by single spore isolation as detailed in Chomnunti et al. (2014) in a petri dish containing 2% water agar and incubated overnight at 16–18°C. Germinating spores were then transferred aseptically onto potato dextrose agar (PDA) and further incubated at 16–18°C to obtain pure colonies. Herbarium material is deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU) and Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS). Living cultures are deposited at Kunming Culture Collection (KUMCC) and in Mae Fah Luang University Culture Collection (MFLUCC). Facesoffungi and Index Fungorum numbers were obtained as described in Jayasiri et al. (2015a) and Index Fungorum (2019).

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from fresh mycelia grown on PDA, following the manufacturer's standard protocol described in the DNA extraction kit (Biospin Fungus Genomic DNA Extraction Kit). Polymerase chain reactions (PCR) were carried out using the primer pairs of ITS5 and ITS4 to amplify the internal transcribed spacers (ITS) (White et al. 1990), and LROR and LR5 for large subunit rDNA (LSU) (Vilgalys & Hester 1990) and fRPB2-5F and fRPB2-7CR to amplify the partial RNA polymerase II second largest subunit (RPB2) (Liu et al. 1999). The amplification reaction was performed in a 25 µl reaction volume containing 1µl DNA, 12.5 µl Taq polymerase and PCR buffer mix, 9.5 µl double distilled water and 1 µl of each primer. The PCR thermal cycling program for ITS and LSU gene regions were as follows: an initial denaturing step of 94°C for 3 min, followed by 35 amplification cycles of 94°C for 30 s, annealation at 57°C for 45 s, elongation at 72°C for 60 s and a final extension step of 72°C for 10 min. For the RPB2 gene region an initial step of 95°C for 5 min, followed by 40 cycles of amplification at 95°C for 1 min, annealation at 52°C for 2 min, elongation at 72°C for 90 s, and final extension at 72°C for 10 min was followed. PCR products were verified using 1% agarose gel electrophoresis, stained with 4S Green Stain. Purification and sequencing of PCR products were carried out at Shanghai Sangon Biological Engineering Technology and Services Co., China. Sequences derived from this study are deposited in GenBank. Small subunit rDNA (SSU) sequence data for both *Parastagonospora elymi* and *P. macrouniseptata*, and TEF for *P. elymi* were obtained but not used in the phylogenetic tree and can be obtained from the corresponding author on request.

Phylogenetic analyses

Sequences generated from this study were subjected to BLAST (NCBI) searches to obtain the closest matches in GenBank (Table 1), from recently published sequences (Li et al. 2015, Tennakoon et al. 2016, Thambugala et al. 2017, Karunarathna et al. 2017) The single gene sequences alignments were initially aligned using BioEdit v. 7.0.9.0 (Hall 1999) and MEGA version 6 (Tamura et al. 2013). Multiple sequence alignments were generated with MAFFT v. 7 (http://mafft.cbrc.jp/ alignment/server /index.html) and manually improved using MEGA v.6 for maximum alignment and minimum gaps. Evolutionary models for phylogenetic analyses were independently selected for each gene region following the Akaike Information Criterion (AIC) of the MrModeltest v. 3.7 (Nylander 2004), implemented using both PAUP v. 4.0b10 and MrBayes v. 3. Phylogenetic reconstructions of combined gene trees were performed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) criteria.

Maximum likelihood analysis was performed using RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) with GTR+I+G as the model of evolution and bootstrap support obtained by running 1000 pseudo replicates. The MP analysis was performed using PAUP v. 4.0b10 (Phylogenetic Analysis Using Parsimony) (Swofford 2002). Ambiguously aligned regions were excluded and gaps were treated as missing data. The trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were set up to 1000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony tree length [TL], consistency index [CI], retention index [RI], relative consistency index [RC] and homoplasy index [HI] were calculated for the maximum parsimonious tree. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications resulting from MP analysis, each with ten replicates of random stepwise addition of taxa (Felsenstein 1985). The Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed to determine whether the trees were significantly different. The BI analysis was conducted using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) to evaluate posterior probabilities (BYPP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation. The distribution of log-likelihood scores were examined to determine the stationary phase for each search and to decide if extra data runs were required to achieve convergence, using Tracer v. 1.6 program (Rambaut et al. 2014). First 10% of generated trees representing the burn-in phase were discarded and the remaining trees were used to calculate prosterior probabilities of the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01).

Phylograms were visualized using FigTree v1.4.0 program (Rambaut 2012) and re-edited and formatted using Microsoft Power Point (2013) and Adobe Photoshop CS6 extended version 13.1.2 software. The final tree alignment was submitted to TreeBASE (Submission ID: 24486, http://www.treebase.org/).

Table 1 GenBank accession numbers of the strains used for phylogenetic analysis. Sequences generated in this study are in blue.

Taxon*	**Strain numbers**	**GenBank accession numbers**		
		ITS	**LSU**	**RPB2**
Camarosporioides phragmitis	**MFLUCC 13-0365**	KX572340	KX572345	-
Didymocyrtis cladoniicola	CBS 128025	KP170641	MH877975	-
D. consimilis	CBS 129140	MH865190	MH876627	-
D. foliaceiphila	CBS 129141	KP170648	MH876628	-
Juncaceicola alpina	CBS 456.84	KF251181	KF251684	KF252188
J. luzulae	**MFLUCC 16-0780**	KX449529	KX449530	-
Melnikia anthoxanthii	**MFLUCC 14-1011**	-	KU848204	-
Neosetophoma samarorum	**CBS 138.96**	KF251160	KF251664	KF252168
Neostagonospora caricis	**CBS 135092**	KF251163	KF251667	KF252171
N. phragmitis	MFLUCC 16-0493	KX926416	KX910090	KX880498
Ophiosphaerella agrostidis	MFLUCC 16-0895	MF351996	MF197563	MF351995
O. agrostidis	MFLUCC 12-0007	KM434272	KM434282	KM434308
Paraphoma radicina	**CBS 111.79**	KF251172	KF251676	KF252180
Parastagonospora allouniseptata	**MFLUCC 13-0386**	KU058711	KU058721	-
Pa. avenae	CBS 289.69	KF251174	KF251678	KF252182
Pa. avenae	CBS 290.69	KF251175	KF251679	KF252183
Pa. caricis	**CBS 135671/S615**	KF251176	KF251680	KF252184
Pa. cumpignensis	**MFLU 15-1480**	KU842388	KU842389	-
Pa. dactylidis	**MFLUCC 13-0375**	KU058712	KU058722	-
Pa. elymi	**KUMCC 16-0125**	MN002867	MN002870	-
Pa. forlicesenica	**MFLUCC 13-0557**	KY769660	KY769661	-
Pa. fusiformis	**MFLUCC 13-0215**	KX926418	KX910088	KX863711
Pa. italica	**MFLUCC 13-0377**	KU058714	KU058724	-
Pa. macrouniseptata	**KUMCC 16-0111**	MN002869	MN002868	MN019669
Pa. minima	**MFLUCC 13-0376**	KU058713	KU058723	-
Pa. nigrans	CBS 307.79	KF251184	KF251687	KF252191
Pa. nodorum	CBS 110109	KF251177	KF251681	KF252185
Pa. nodorum	CBS 287.52	-	JX681113	-
Pa. poaceicola	**MFLUCC 15-0471**	-	KX910092	KX880499
Pa. poae	**CBS 135089**	KF251178	KF251682	KF252186
Pa. poagena	**CBS 136776**	KJ869116	KJ869174	-
Pa. uniseptata	**MFLUCC 13-0387**	KU058715	KU058725	-
Phaeosphaeria chiangraina	**MFLUCC 13-0231**	KM434270	KM434280	KM434307
Ph. thysanolaenicola	**MFLUCC 10-0563**	KM434266	KM434276	KM434303

Table 1 Continued.

Taxon*	Strain numbers	GenBank accession numbers		
		ITS	**LSU**	**RPB2**
Sclerostagonospora lathyri	MFLUCC 14-0958	MG828955	MG829066	-
S. opuntiae	CBS 118224	DQ286768	DQ286772	-
Sclerostagonospora sp.	CBS 118152	JX517283	JX517292	-
Scolicosporium minkeviciusii	**MFLUCC 12-0089**	-	KF366382	-
Wojnowicia dactylidis	**MFLUCC 13-0735**	KP744470	KP684149	-
Wojnowicia spartii	MFLUCC 13-0402	KU058719	KU058729	-
Wojnowiciella eucalypti	**CPC 25024**	KR476741	KR476774	-
Wojnowiciella viburni	MFLUCC 12-0733	KC594286	KC594287	-
Xenoseptoria neosaccardoi	**CBS 128665**	KF251281	KF251784	KF252286
Yunnanensis phragmitis	MFLUCC 17-0315	MF684862	MF684863	-
Yunnanensis phragmitis	**MFLUCC 17-1361**	MF684869	MF684865	-

*Ex-type strains are in bold

Results

As a result of the current study, a fungal sexual morph from *Elymus repens* and an asexual morph from *Dactylis glomerata* were isolated. Based on morphological characters and molecular analyses presented below, two new species of *Parastagonospora* are described.

Phylogenetic analyses

A combined analysis of the ITS, LSU and RPB2 sequence data of 45 strains consisting of *Parastagonospora* and other representative genera in Phaeosphaeriaceae were used to confirm the phylogenetic placement of our strains, with *Neosetophoma samarorum* (CBS 138.96) as the outgroup taxon (Fig. 1). This combination of sequence data was performed after confirming that the topologies of the trees obtained from each gene were overall congruent. Bootstrap values obtained for MP and ML analyses and PP values resulted in BI analysis are given at each node (Fig. 1).

The Bayesian analysis resulted in 10,001 trees after 1,000,000 generations. The first 1000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 9001 trees were used for calculating posterior probabilities in the majority rule consensus tree presented in Fig. 1. The average standard deviation of split frequencies was 0.010067. The RAxML analysis of the combined dataset yielded a best scoring tree with a ML optimization likelihood value of -11145.503449. The matrix had 796 distinct alignment patterns, with 37.7% of undetermined characters or gaps. Parameters for the GTR + I + G model of the combined ITS, LSU and RPB2 were as follows: estimated base frequencies; A = 0.249573, C = 0.222647, G = 0.273922, T = 0.253857; substitution rates AC = 1.771863, AG = 3.436302, AT = 1.824852, CG = 0.770295, CT = 7.693174, GT = 1.000000; proportion of invariable sites I = 0.001000; gamma distribution shape parameter α = 0.146118. The maximum parsimony dataset consisted of 2584 characters, of which 1906 characters were constant, 505 were parsimony-informative and 173 were parsimony-uninformative. The most parsimonious tree showed values as follows: CI = 0.620, RI = 0.707, RC = 0.438, HI = 0.380.

Both KUMCC 16–0125 and KUMCC 16–0111 strains clustered within *Parastagonospora* with KUMCC 16–0111 forming a sister clade to *P. allouniseptata* with strong statistical support (96% ML/ 88% MP/ 1.00 PP). KUMCC 16–0125 grouped closely with *P. avenae*, *P. caricis*, *P. forlicesenica* and *P. italica* with moderate support (61% ML/ 49% MP/ 0.93 PP).

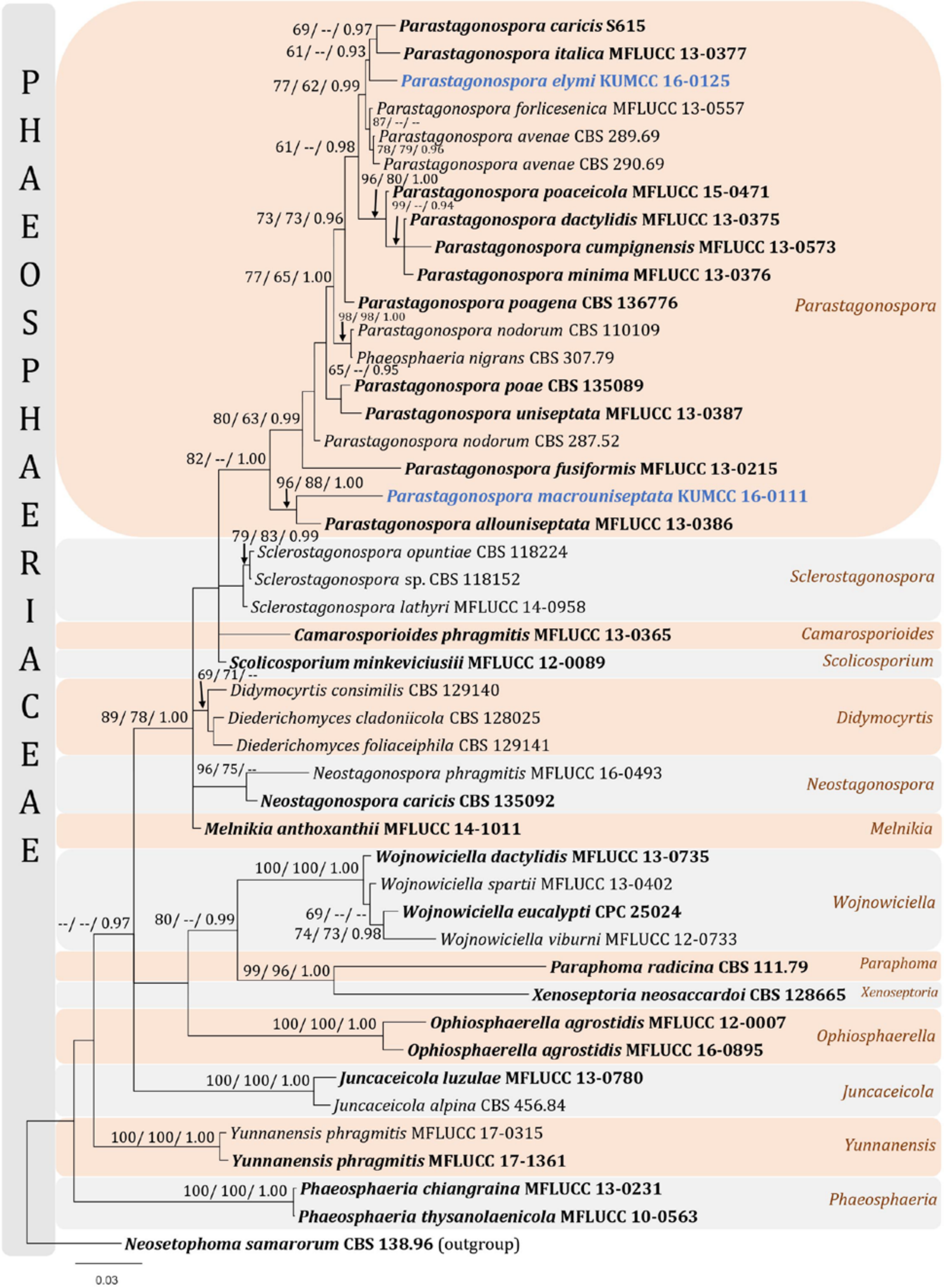

Fig. 1 – Bayesian majority consensus tree based on the combined ITS, LSU and RPB2 sequence dataset. Maximum likelihood bootstrap values (ML) and maximum parsimony values (MP) equal or greater than 60% are given at the nodes. Bayesian posterior probabilities (PP) of more than 0.90 are given at the nodes. Ex-type strains are in bold; the new isolates are in blue. The tree is rooted to *Neosetophoma samarorum* CBS 138.96.

Taxonomy

Parastagonospora elymi Goonas., Bulgakov & McKenzie, sp. nov. Fig. 2

Index Fungorum number: IF556608, Facesoffungi number: FoF06260

Etymology – Named after the host genus *Elymus* from which it was collected.

Holotype – MFLU 15–2243

Saprobic on dead stems of *Elymus repens*, appearing as black, raised, shiny, oval lesions on host surface. Sexual morph: *Ascomata* perithecial, solitary, scattered to gregarious, glabrous, semi-immersed to superficial, 100–150 µm high, 145–200 µm diam. (n = 10), subglobose to conical, dark brown, with central ostiole. *Peridium* 15–20 µm wide, composed of several layers, outer layers; brown, thick-walled cells of *textura prismatica* to *textura angularis* and inner layers, hyaline to lightly pigmented, thin-walled cells of *textura angularis*. *Hamathecium* composed of many filamentous, septate, 1.5–2 µm wide, cellular pseudoparaphyses, periphyses present. *Asci* 55–95 × 7–13 µm ($\overline{x}$ = 80 × 10.5 µm, n = 20), cylindrical, 6–8-spored, bitunicate, fissitunicate, rounded at the apex, with bi-lobed pedicel. *Ascopores* 21.5–25.5 × 3.5–5 µm ($\overline{x}$ = 22.5 × 4.5 µm, n = 20), uniseriate to overlapping bi-seriate, hyaline, fusiform, with acute ends, straight or slightly curved, 3-septate at maturity, cells near the mid septum, especially second cell from apex slightly swollen, constricted at the middle septum, smooth-walled. Asexual morph: Undetermined.

Material examined – Russia, Rostov region, Krasnosulinsky district, Donskoye forestry, artificial forest (47.85472° N, 40.2318907° E), on dead stems of *Elymus repens* (L.) Gould [=*Elytrigia repens* (L.) Nevski] (Poaceae), 28 June 2015, Timur S. Bulgakov T-539 (MFLU 15–2243, holotype; HKAS, isotype) – ex-type living culture KUMCC 16–0125 = MFLUCC 16–0887.

Culture characteristics – Colonies reaching 15–20 mm diam. in 4 weeks at 16–18°C on PDA, woolly and raised, margins irregular, from above grey, reverse grey to white.

Notes – In the phylogenetic analyses, *Parastagonospora elymi* groups closely with the two strains of *P. avenae* (CBS 289.69 and CBS 290.69). Morphologically, *P. elymi* is similar to the sexual morph of *P. avenae* (= *Phaeosphaeria avenaria*) in the characteristics of their asci and ascospores (cylindrical asci, with 8, overlapping biseriate, fusiform ascospores), however their dimensions of the ascomata vary. *P. elymi* ascomata are subglobose or conical, 145–200 µm wide and 100–150 µm high, while *P. avenae* has smaller, globose ascomata that are 120–150 µm wide, 120–150 µm high (Shoemaker & Babcock 1989, this study). The ascospores of *P. avenae* are guttulate and surrounded by a sheath, while those of *P. elymi* have neither guttules nor a sheath (Shoemaker & Babcock 1989, this study).

Parastagonospora macrouniseptata Goonas., Camporesi & McKenzie, sp. nov. Fig. 3

Index Fungorum number: IF556607, Facesoffungi number: FoF06261

Etymology – "uniseptata" referring to its similarity to *P. uniseptata*; and "macro" to its larger conidiomata.

Holotype – MFLU 15–0774

Saprobic on dead stem of *Dactylis glomerata* L. (Poaceae) appearing as black dots on the host surface. Sexual morph: Undetermined. Asexual morph: Coelomycetous. *Conidiomata* 150–180(–190) µm wide, (120–)130–160 µm high ($\overline{x}$ = 167 × 145 µm, n = 10), dark brown to black, pycnidial, solitary to gregarious, immersed, globose to subglobose, unilocular, glabrous, ostiolate. *Ostiole* central, short papilla. *Conidiomatal wall* 8–18 µm wide ($\overline{x}$ = 13.7 µm), composed of thin, dark brown, pseudoparenchymatous cells, gradually merging with hyaline cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 4.2 × 3 µm, hyaline, phialidic, ampulliform to lageniform, discrete, determinate, aseptate, smooth, arising from the inner layers of conidioma. *Conidia* (14–)15–18(–20) × (1–)2–2.5 µm ($\overline{x}$ = 17.3 × 2 µm, n = 30), hyaline, cylindrical to subcylindrical, rounded at apex, slightly truncate at base, 1-septate, slightly constricted at the septum, smooth-walled, guttulate.

Culture Characteristics – Colonies reaching 15–20 mm diam. in 4 weeks at 18–20°C on

PDA, dense, margins undulate to irregular, from above white, slightly raised and umbonate in the center, becoming grey at the margins, reverse grey to white.

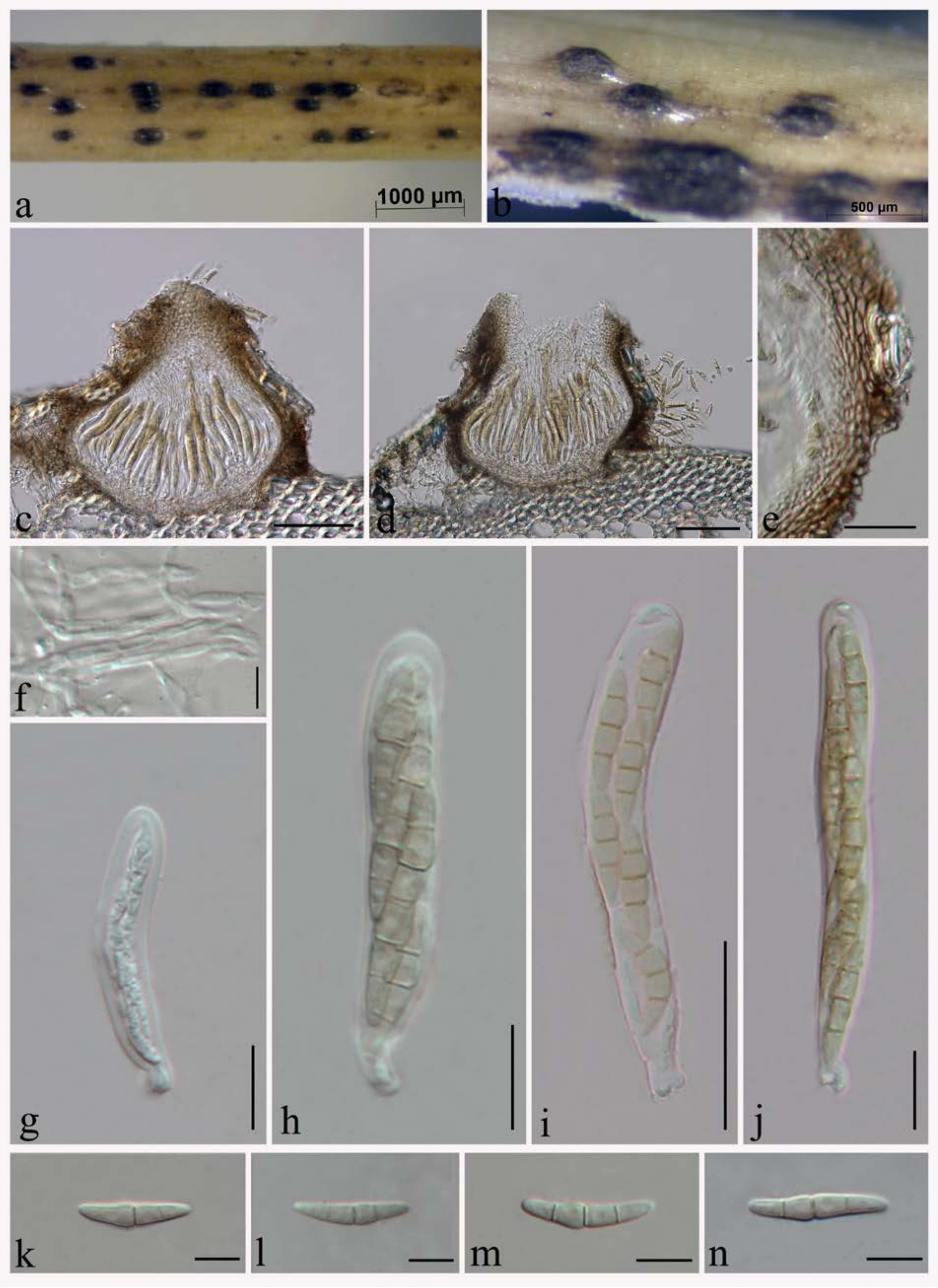

Fig. 2 – *Parastagonospora elymi* (MFLU 15–2243, holotype) a Appearance of ascomata on host. b Close up of ascomata. c, d Vertical section of ascoma. e Peridium. f Pseudoparaphyses. g–j Asci. k–n Ascospores. Scale bars: c, d = 100 µm, e = 50 µm, f = 10 µm, g, h, j–n = 15 µm, i = 20 µm.

Material examined – ITALY, Province of Forlì-Cesena, near Predappio Alta, on dead aerial stem of *Dactylis glomerata* L. (Poaceae), 15 January 2012, Erio Camporesi IT 109 (MFLU 15–0774, holotype; HKAS 96288, isotype) – ex-type living culture, KUMCC 16–0111 = MFLUCC 16–0885.

Notes – *Parastagonospora macrouniseptata* is morphologically similar to *P. allouniseptata* in having globose to subglobose, pycnidia and hyaline, cylindrical, 1-septate conidia. Both species are described from the same host. However, *P. macrouniseptata* has larger conidiomata ($\bar{x}$ = 167 × 145 µm) than *P. allouniseptata* (60–90 µm high, 70–90 µm diam.) (Li et al. 2015). Phylogenetically, *P. macrouniseptata* forms a sister clade with *P. allouniseptata*, within the *Parastagonospora* clade with high bootstrap support. The differences in base pairs between the two species were 14 for the ITS region, six for LSU and 20 for the TEF gene region.

Fig. 3 – *Parastagonospora macrouniseptata* (MFLU 15–0774, holotype) a Specimen from above. b Conidiomata on host. c Squash mount of conidioma. d Section through conidioma. e Wall of conidioma. f, g Conidiogenous cells. h–k Conidia. l Colony on PDA. Scale bars: c, d = 50 µm, e = 20 µm, f, g = 10 µm, h–k = 5 µm.

Discussion

Parastagonospora was introduced by Quaedvlieg et al. (2013) to accommodate several species previously classified as *Leptosphaeria*, *Phaeosphaeria*, *Septoria* and *Stagonospora*. This genus is characterized by a sexual morph having immersed ascomata with slightly papillate ostiole, bitunicate, shortly stipitate asci, fusoid, subhyaline to pale brown, septate ascospores and a coelomycetous asexual morph with hyaline, cylindrical, granular to multi-guttulate, transversely euseptate conidia (Quaedvlieg et al. 2013, Li et al. 2015, Thambugala et al. 2017). *Parastagonospora* species are widespread occuring mostly on wheat, grasses such as *Dactylis* sp. and *Poa* sp. as well as other cereal crops (Quaedvlieg et al. 2013, Li et al. 2015, Ghaderi et al. 2017). Apart from *Parastagonospora*, the genera *Neosetophoma*, *Phaeosphaeria*, *Phaeosphaeriopsis*, *Setophoma*, *Wojnowicia* and *Xenoseptoria* in Phaeosphaeriaceae have been associated with leaf spots from various hosts (Carson 2005, Arzanlou & Crous 2006, Quaedvlieg et al. 2013, Phookamsak et al. 2014).

As most sexual morphs in Phaeosphaeriaceae are morphologically similar, it is their asexual morph characteristics, coupled with molecular data, that are essential in classification (Phookamsak et al. 2017). In most cases, the addition of protein coding genes is necessary to clearly distinguish species in Phaeosphaeriaceae (Phookamsak et al. 2014). Based on the phylogenetic results of this study, the combined use of ITS, LSU and RPB2 sequence data satisfactorily segregated the species of *Parastagonospora*, although this may not be sufficient for all genera in the family. There is a need for broader collections, and more importantly, the re-collection of type spcies, in order to firmly establish the phylogenetic placements of certain genera, especially those with poor species representation (Phookamsak et al. 2014, 2017, Tennakoon et al. 2016). It is doubtful whether most species of this family are host specific (Phookamsak et al. 2014) as initially thought, hence more fresh collections are necessary.

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