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Pyrenophora

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Abstract

This is the first in a series of papers in which we revisit genera of fungi to provide baseline data for future study. In this article we examine the genus *Pyrenophora* and provide details of morphology, phylogeny and the current status of species. *Pyrenophora* is a genus of saprobic and plant pathogenic fungi with a worldwide distribution, commonly associated with leaves, wood, cereals and other grasses. A phylogeny for *Pyrenophora* (sexual state of *Drechslera*) and allied genera is presented based on analysis of ITS, GPDH, RPB2, nrSSU and nrLSU DNA sequence datasets. *Pyrenophora* is a monophyletic genus in *Pleosporaceae*. *Pyrenophora* sexual states cluster with their expected *Drechslera* asexual states. As a genus can now only have one name we synonymise *Drechslera* under *Pyrenophora*.

Key words – GPDH **–** RPB2 **–** Epitypification **–** Nomenclature

Introduction

Pyrenophora (sexual state = *Drechslera*) causes disease on many graminicolous hosts (Zhang & Berbee 2001) where they are commonly observed in their asexual state (Zhang & Berbee 2001). Several recent studies using multigene analysis and some coupled with morphology have provided the groundwork for classification in *Pyrenophora* (Berbee 1996, Zhang & Berbee 2001, Zhang et al. 2012, Hyde et al. 2013). We have been working on the genera of *Pleosporales* in order to provide a natural classification via morphological and phylogenetic characterization (Zhang et al. 2012, Ariyawansa et al. 2013a, b, c, Hyde et al. 2013, Ariyawansa et al. 2014). In this paper we bring together data on the genus *Pyrenophora.*

History

The type species of *Pyrenophora*, *P. phaeocomes* (Rebent.) Fr., was described as *Sphaeria phaeocomes* by Rebentisch (1804) and placed in *Xylariaceae*. Later, Fries (1849) reclassified the genus as *Pyrenophora* and placed in Pleosporales. Wehmeyer (1961) placed *Pyrenophora* in the family *Pleosporaceae*. Barr (1987) redefined *Pleosporaceae* to comprise *Clathrospora*, *Kirschsteiniothelia*, *Lewia* and *Pleospora* and grouped *Cochliobolus*, *Pyrenophora* and

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Setosphaeria in the family *Pyrenophoraceae*. Berbee (1996) disagreed, suggesting these genera belong to *Pleosporaceae* and this has been followed by later researchers (Zhang et al. 2012, Hyde et al. 2013). *Pyrenophora* is characterized by immersed to semi immersed ascomata and neck covered with brown to reddish-brown setae, lack of pseudoparaphyses, clavate to saccate asci, usually with a large apical ring, and muriform terete (cylindrical, frequently circular in section but narrowing to one end) ascospores. Morphologically, the terete ascospores of *Pyrenophora* can be readily distinguished from *Clathrospora* and *Platyspora*. The lack of pseudoparaphyses and smaller ascospores of *Pyrenophora* can easily be differentiated from those of *Pleospora* (Sivanesan 1984). *Pyrenophora* has usually clustered in *Pleosporaceae* with *Bipolaris* and *Setosphaeria* (Zhang & Berbee 2001). *Pyrenophora* species can easy be distinguished from species in *Bipolaris* as ascospores are filiform and *Setosphaeria* as ascospores are phragmosporous and hyaline (Wehmeyer 1953, Zhang & Berbee 2001).

Sexual and asexual states

Pyrenophora has been linked to asexual morphs in *Drechslera*. *Drechslera* species were initially categorized in *Helminthosporium* on the basis of their dark colour, transversely septate conidia and a graminicolous habitat (Drechsler 1923, Shoemaker 1959, 1961). Consequently, graminicolous *Helminthosporium* species were segregated into three genera, *Bipolaris*, *Drechslera*, and *Exserohilum*, defined based on their association with their sexual states *Cochliobolus*, *Pyrenophora* and *Setosphaeria* respectively (Zhang & Berbee 2001).

Importance and role

Pyrenophora species are phytopathogens or as saprobes are involved in nutrient cycling. Many species cause disease on their graminicolous hosts and are usually present in their asexual state (*Drechslera*) (Zhang & Berbee 2001). Some species of *Pyrenophora* are serious plant pathogens (Zhang & Berbee 2001). *Pyrenophora teres* (= *Drechslera teres*) is a necrotrophic pathogen of economically important crops, such as barley (Kingsland 1991, Gupta & Loughman 2001). *Pyrenophora graminea* (= *Drechslera graminea*) causes barley stripe resulting in significant yield losses (Tekauz 1983). *Pyrenophora graminea* lives within barley kernels as mycelium, and when seeds germinate, hyphae enter the seedling through the coleorrhiza, causing a systemic infection (Pecchia et al. 1998, Leisova 2005). *Pyrenophora tritici-repentis* causes tan spot of wheat (Lamari & Bernier 1989, Balance et al. 1996) which occurs in all the major wheat growing areas of the world and causes 3 to 50% yield losses (Lamari & Bernier 1989) and its prevalence has increased recently.

Number of species

Currently 198 species of *Pyrenophora* and 135 species of *Drechslera* are listed in Index Fungorum (2014).

Molecular data

Rapid identification of diseases cause by *Pyrenophora* has been determined via different DNA markers. Identification of molecular genetic markers in *Pyrenophora teres* f. *teres* associated with low virulence on ‘Harbin’ barley was assessed by random amplified polymorphic DNA (RAPD) (Weiland et al. 1999) and five RAPD markers were obtained that were associated in coupling with low virulence. The data suggested that the RAPD technique can be used to tag genetic determinants for virulence in *P. teres* f. *teres* (Weiland et al. 1999). Specific polymerase chain reaction (PCR) primers were developed from amplified fragment length polymorphism (AFLP) fragments of *Pyrenophora teres*, the causal agent of net blotch on barley leaves (Leisova et al. 2005). The primers were designed to amplify DNA from *P. teres* f. *teres* (net form) and allow its differentiation from *P. teres* f. *maculata* (spot form), which is morphologically very similar *to P. teres* f. *teres* in culture (Leisova et al. 2005). The PCR assay was certified with 60 samples of *Pyrenophora* species. The amplification with four designed PCR primer pairs provided *P. teres*

form-specific products. No cross-reaction was observed with DNA of several other species, such as *P. tritici-repentis* and *P. graminea* (Leisova et al. 2005). *Pyrenophora graminea* is the causal agent of barley leaf stripe disease (Lubna et al. 2012, Mokrani et al. 2012). Two leaf stripe isolates PgSy3 (exhibiting high virulence on the barley cultivar 'Arabi Abiad') and PgSy1 (exhibiting low virulence on Arabi Abiad), were mated and 63 progeny were isolated and phenotyped for the reaction on Arabi Abiad (Lubna et al. 2012). From 96 AFLP markers, three AFLP markers, E37M50-400, E35M59-100 and E38M47-800 were linked to the virulence locus VHv1 in isolate PgSy3. Lubna et al. 2012 suggested that the three markers are closely linked to VHv1 and are unique to isolates carrying the virulence locus. Pecchia et al. (1998) developed an efficient PCR protocol for amplification of the IGS region in *P. graminea* and to characterize this region by restriction fragment analysis. During the study based on the length of the IGS-PCR product, ca. 3.8 or 4.4 kb, two groups of isolates were identified from six cultures *i.e* I3/88 (Italy; CBS 100862), I7/88 (Italy; CBS100861), 60/93 (Austria; CBS 100866), I10/95 (Tunisia; CBS 100863), I28/95 (Tunisia; CBS 100864), I33/95 (Tunisia; CBS 100865). The RFLP patterns of isolates obtained with the 6-base cutting enzymes *ApaI, BglII, DraI, EcoRV, HindIII* and *SacI* were similar within each group and different between the two groups (Pecchia et al. 1998). Restriction patterns of IGS-PCR products digested with the 4-base cutting enzyme *AluI* were polymorphic among isolates in spite of their IGS-PCR product length (Pecchia et al. 1998).

DNA sequence-based phylogenetics has dramatically influenced both the taxonomy and systematic of *Pyrenophora* (Zhang & Berbee 2001, Zhang et al. 2012). In phylogenetic analysis based on 18s rRNA *Pyrenophora* clustered within *Pleosporaceae* (Zhang & Berbee 2001) and thus, excluded from *Pyrenophoraceae* (Zhang & Berbee 2001). Later, phylogenetic analysis of the ITS and *gdp* data showed that *Pyrenophora* is monophyletic (Zhang & Berbee 2001). In the same study Zhang & Berbee 2001 has shown that the asexual states of the *Pyrenophora*, *Drechslera* clustered with their predicted sexual relatives.

Aim of study

The genera of ascomycetes are relatively confused as most 20th century classifications were based on morphological characters and thus personal opinions. Molecular data has changed this approach and we can now use morphological and molecular characters to develop more natural classifications. This is the first in a series of papers in which we detail provide data on a genus, including molecular data and the morphology of type material with illustrations.

Materials and Methods

Specimen examination

The basic methodology used in this study was the same as Ariyawansa et al. (2013c, d). Type specimens were loaned from the Museum of Evolution, Uppsala University, Sweden (UPS). Ascomata were rehydrated in 5% KOH prior for examination and sectioning. Hand sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. The fungus was examined in a Nikon ECLIPSE 80i compound microscope and photographed by a Cannon 450D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems Inc., The United States).

Phylogenetic analyses

Multiple sequence alignments were generated with MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html). The alignments were checked visually and improved manually where necessary. Two different datasets were used to estimate two phylogenies; a Pleosporineae family tree and a *Pyrenophora* phylogeny. The first tree focuses on phylogenetic placement of *Pyrenophora* in *Pleosporaceae* and Pleosporineae, the second one was

Table 1 Taxa used in the phylogenetic analysis and their corresponding GenBank numbers

Taxon	voucher/culture	ITS	LSU	SSU	RPB2	GPDH
Alternaria alternata	CBS 916.96		DQ678082	KC584507	KC584375	
Alternariaster helianthi	CBS 119672		KC584368	KC584626	KC584493	
Alternariaster helianthi	CBS 327.69		KC584369	KC584627	KC584494	
Bipolaris maydis	ACCC 38152		KC445317	KC445317		
Clathrospora diplospora	IMI 68086		U43481_	U43464		
Clathrospora elynae	CBS 196.54		GU323214	GU296142	KC584496	
Clathrospora elynae	CBS 161.51		KC584370	KC584628	KC584495	
Clathrospora heterospora	CBS 175.52		KC584320	KC584577	KC584445	
Cochliobolus heterostrophus	CBS134.39		AY544645	AY544727	DQ247790	
Cochliobolus sativus	DAOM226212		DQ678045	DQ677995	DQ677939	
Comoclathris compressa	CBS 156.53		KC584372	KC584630	KC584497	
Comoclathris compressa	CBS 157.53		KC584373	KC584631	KC584498	
Comoclathris magna	CBS 174.52		DQ678068	KC584578	DQ677964	
Coniothyrium palmarum	CBS:400.71		EU754153	EU754054	DQ677956	
Coniothyrium palmarum	CBS 758.73		EU754154	EU754055		
Cucurbitaria berberidis	CBS 394.84		GQ387605	GQ387544		
Cucurbitaria berberidis	CBS 363.93		GQ387606	GQ387545		
Dendryphiella salina	CBS 142.60		KC793339	KC584583	KC793340	
Didymella exigua	CBS 183.55		EU754155	EU754056		
Dothidotthia aspera	CPC 12933		EU673276	EU673228		
Dothidotthia symphoricarpi	CPC 12929		EU673273	EU673224		
Drechslera andersenii	CBS 258.80	AY004804				AY004835
Drechslera andersenii	CBS 967.87	AY004805				
Drechslera andersenii	DAOM 229292	JN943646	JN940084	JN940958		
Drechslera avenae	CBS 189.29	AY004795				AY004827
Drechslera avenae	CBS 279.31	AY004796				AY004828
Drechslera biseptata	DAOM 208987	AY004786				AY004817
Drechslera biseptata	CBS 308.69	JN712464	JN712530			AY004819
Drechslera biseptata	CBS 599.7	AY004787				AY004818
Drechslera biseptata	CBS 108940	AY004788				
Drechslera campanulata	BRIP15927	AF163058				
Drechslera catenaria	DAOM 63665A	AY004802				AY004833
Drechslera catenaria	CBS 191.29	AY004803				AY004834
Drechslera dactylidis	DAOM 92161	AY004781				AY004812
Drechslera dematioidea	CBS 108963	AY004789	JN712532			AY004820
Drechslera dematioidea	DAOM 229295	JN943648	JN940094			
Drechslera dematioidea	CBS 108962	JN712465	JN712531			
Drechslera dematioidea	CBS 108962	AY004790	JN712531			AY004821
Drechslera dictyoides	DAOM 63666	AY004806	JN940080			AY004836
Drechslera erythrospila	CBS 108941	AY004782				AY004813
Drechslera erythrospila	DAOM 55122	AY004783				AY004814
Drechslera fugax	CBS 509.77	AY004791				AY004822
Drechslera nobleae	CBS 259.80	AY004792				AY004823
Drechslera nobleae	DAOM 229296	JN943647	JN940095			
Drechslera nobleae	CBS 966.87	AY004793				AY004824
Drechslera nobleae	CBS 316.69	AY004794				AY004825
Drechslera phlei	CBS 315.69	AY004807				AY004837
Drechslera phlei	DAOM 225627	JN943656	JN940077	JN940964	JN993627	
Drechslera poae	DAOM 145373	AY004801	JN940082	JN940961	JN988321	AY004832
Drechslera poae	DAOM 169240	JN943651				
Drechslera siccans	DAOM 115701	AY004797	JN940078	JN940963	JN993626	
Drechslera siccans	DAOM 115702	AY004799				
Drechslera sp.	DAOM126766	AY004800				AY004831
Drechslera sp.	DAOM126772	AY004784				AY004815
Drechslera sp	CBS313.69	AY004785				AY004816
Drechslera triseptata	NZ6120	AF163059				

Taxon	voucher/culture	ITS	LSU	SSU	RPB2	GPDH
Halojulella avicenniae	BCC 20173		GU371822	GU371831	GU371786	
Halojulella avicenniae	BCC 18422		GU371823	GU371830	GU371787	
Leptosphaeria maculans	DAOM 229267		DQ470946	DQ470993	DQ470894	
Leptosphaerulina australis	CBS 317.83		GU301830	GU296160	GU371790	
Neophaeosphaeria filamentosa	CBS 102202		GQ387577	GQ387516	GU371773	
Ophiosphaerella herpotricha	CBS 620.86		DQ678062	DQ678010	DQ677958	
Paraphoma radicina	CBS 111.79		EU754191	EU754092	KF252180	
Phaeosphaeria eustoma	CBS 573.86		DQ678063	DQ678011	DQ677959	
Phoma betae	CBS 109410		EU754178	EU754079	GU357804	
Phoma exigua	CBS 431.74		EU754183	EU754084	GU371780	
Pleospora calvescens	CBS 246.79		EU754131	EU754032	KC584500	
Pleospora chenopodii	CBS 206.80		JF740266	JF740095	KC584501	
Pleospora halimiones	CBS 432.77		JF740267	JF740096	KC584503	
Pleospora herbarum	CBS 191.86	DQ491516	DQ247804	DQ247812	DQ247794	AY316969
Pleospora incompta	CBS 467.76		GU238087	GU238220	KC584504	
Pleospora typhicola	CBS 132.69		JF740325	JF740105	KC584505	
Pyrenochaeta corni	CBS 248.79		GQ387608	GQ387547		
Pyrenophora bromi	DAOM 127414	AY004809	JN940074	JN940954		AY004839
Pyrenophora chaetomioides	DAOM 208989	AF081445	JN940091			AF081371
Pyrenophora dictyoides	DAOM 75616	JN943654	JN940079	JN940962	JN988322	
Pyrenophora japonica	DAOM 169286	AF071347				AF081369
Pyrenophora lolii	CBS 318.69	AY004798				AY004829
Pyrenophora phaeocomes	DAOM 222769	JN943649	DQ499596		DQ497614	
Pyrenophora semeniperda	DAOM 213153	AF081446	JN940089		JN993630	AY004826
Pyrenophora tetrarrhenae	DAOM 171966	JN943663	JN940090		JN993620	
Pyrenophora tritici-repentis	DAOM 226213	JN943670	AY544672			AF081370
Pyrenophora tritici-repentis	DAOM 208990	AF071348	JN940071			AY004838
Pyrenophora tritici-repentis	DAOM 107224	AY004808	DQ384097			
Pyrenopora graminea	11	Y10748				
Pyrenopora teres	PM2	Y08746				AY004830
Setosphaeria monoceras	CBS 154.26		AY016368	AY016352		
Setosphaeria turcica	ATCC 64835		KF278475	KF278475		
Trematosphaeria pertusa	CBS 122371		GU301876	GU348999	GU371801	

generated to show the placement of *Pyrenophora* and its asexual state *Drechslera*. All sequences obtained from GenBank were previously used in Zhang & Berbee (2001), Schoch et al. (2009) and Woudenberg et al. (2013) and are listed in Table 1.

Maximum-parsimony analysis was performed by using PAUP v. 4.0b10 (Swofford 2002) to obtain the most parsimonious tree. Trees were inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were setup to 5000 and 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 trees generated under different optimality criteria. Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. Maximum parsimony bootstrap values (MP) equal or greater than 50 % are given below or above each node in blue (Fig. 1 and 2).

Maximum likelihood analyses including 1000 bootstrap replicates were run using RAxML v. 7.2.6 (Stamatakis 2006, Stamatakis et al. 2008, Stamatakis & Alachiotis 2010). The online tool Findmodel (http:// www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) was used to find out the best nucleotide substitution model for each partition. For both SSU (Pleosporineae family

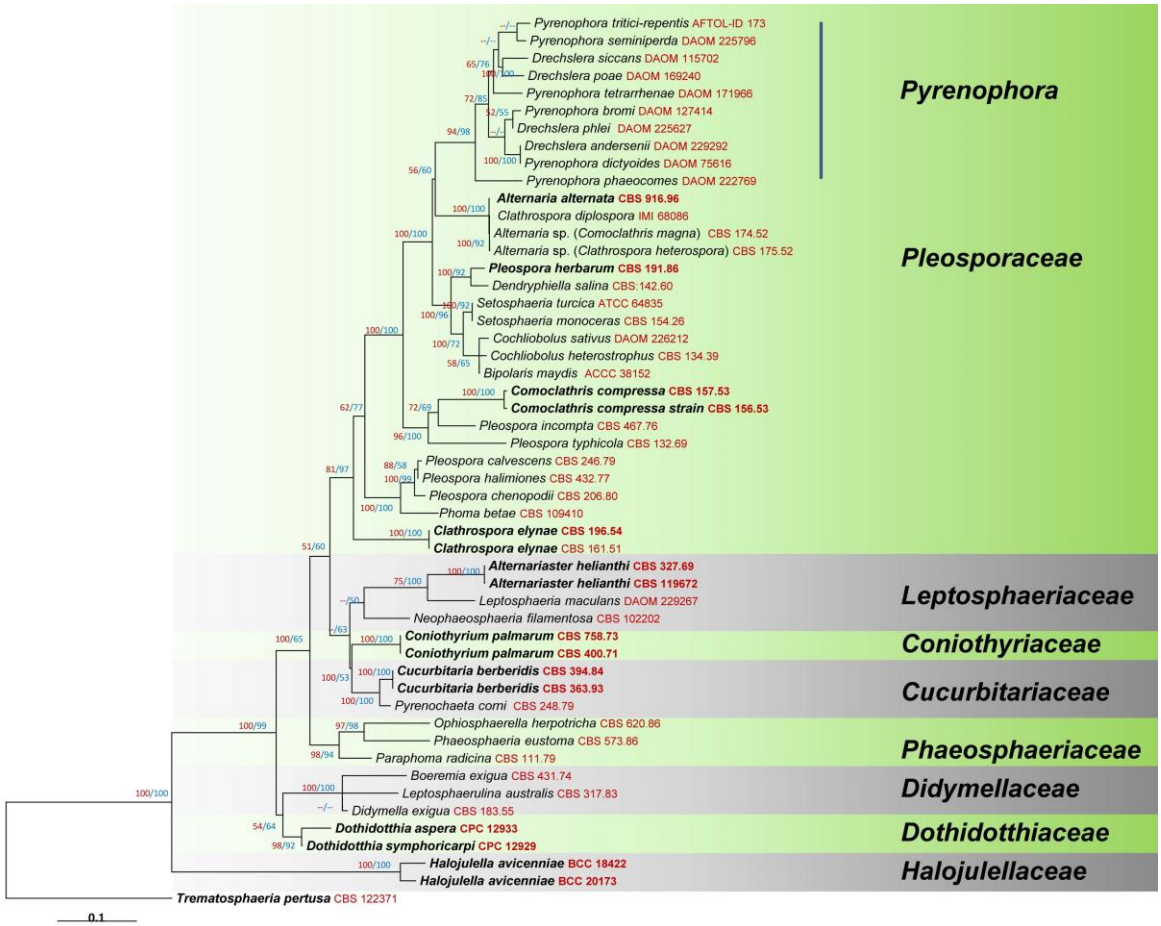

Fig. 1 – RAxML tree based on based on the LSU, SSU and RPB2 sequences of 51 strains representing the Pleosporineae. Bootstrap support values >50% are shown above or below the branch. The tree is rooted to *Trematosphaeria pertusa*. The original isolate numbers are noted after the species names. Bold indicates ex-type strains.

122371) in the Pleosporineae phylogeny. The resulting trees were printed with TreeView v. 1.6.6 (Page 1996). The resulting replicates were plotted on to the best scoring trees obtained. Maximum Likelihood bootstrap values (ML) equal or greater than 50 % are given below or above each node in red (Fig.1 and Fig.2).

Results and discussion

Phylogeny

The final Pleosporineae alignment included 51 strains, representing eight families, and consisted of 2795 characters, of which 2008 characters were constant, 143 variable characters were parsimony-uninformative and 644 characters were parsimony-informative. Kishino-Hasegawa (KH) test showed length= 3145 steps, CI= 0.391, RI= 0.649, RC= 0.254 and HI= 0.609. All trees were similar in topology and not significantly different (data not shown). In the SSU alignment a large insertion at position 480 in the isolate *Ophiosphaerella herpotrichia* (CBS 620.86) was excluded from the phylogenetic analyses. A best scoring RAxML tree is shown in Fig. 1 and 2 with the value of -6929.59087and -8276.09706 respectively. Phylogenetic trees obtained from maximum likelihood and maximum parsimony analyses yielded trees with similar overall topology at species relationship in agreement with previous work based on maximum likelihood (Zhang & Berbee 2001, Schoch et al. 2009, Hyde et al. 2013, Woudenberg et al. 2013). The support values for the different phylogenetic methods vary, with the RAxML bootstrap being higher than the maximum parsimony bootstrap support values in most cases.

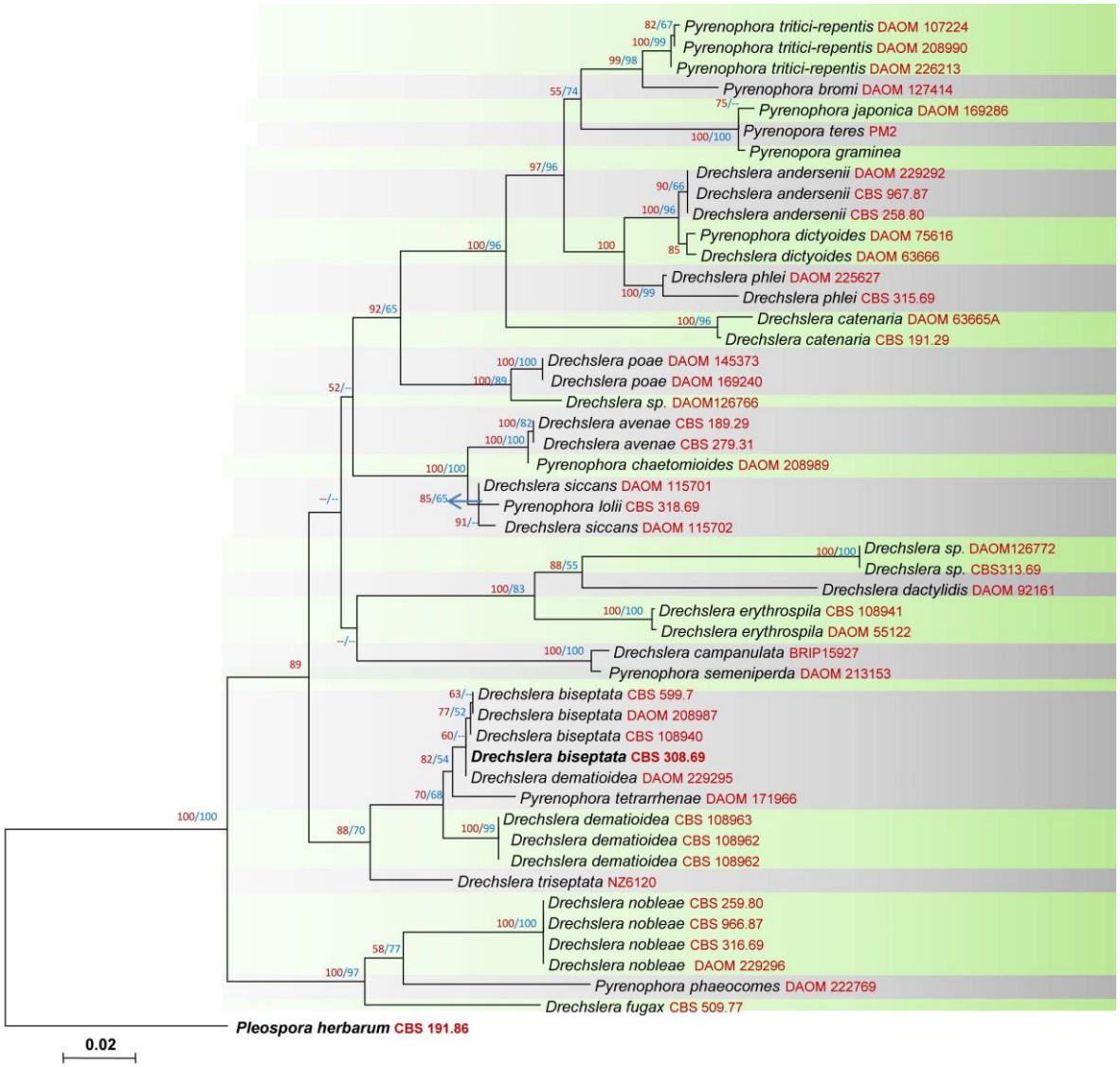

Fig. 2 – RAxML tree based on a combined dataset of ITS, LSU and GPDH. Bootstrap support values >50% are shown above or below the branch. The tree is rooted with *Pleospora herbarum*. The original isolate numbers are noted after the species names. Bold indicates ex-type strains.

For defining the taxonomy of *Pyrenophora* and its asexual state *Drechslera*, 49 strains were included in the alignment. The maximum parsimony dataset consists of 2194 characters; of which 1781 characters were constant, 95 variable characters were parsimony-uninformative and 318 characters were parsimony-informative. Kishino-Hasegawa (KH) test showed length= 5108 steps, CI=0.399, RI=0.508, RC= 0.203 and HI=0.601. All trees were similar in topology and not significantly different (data not shown). Phylogenetic trees obtained from maximum likelihood and maximum parsimony analyses yielded trees with similar overall topology at species relationship in agreement with previous work based on maximum likelihood (Zhang & Berbee 2001, Schoch et al. 2009, Hyde et al. 2013).

Taxonomy

Pyrenophora Fr., Summa veg. Scand., Section Post. (Stockholm): 397 (1849). MycoBank: MB 4596

= *Drechslera* S. Ito, Proc. Imp. Acad. Japan 6: 355 (1930)

Sexual state – *Ascomata* immersed, becoming erumpent to near superficial, solitary or scattered, globose to subglobose, broadly or narrowly conical, smooth-walled, ostiolate. *Ostiole* papillate, covered with brown to reddish-brown setae, which are darkened at the base. *Peridium* comprising 2–4 layers of brown, thick-walled cells of *textura angularis*. *Pseudoparaphyses* not observed. *Asci* 8-spored, bitunicate, fissitunicate, clavate to sub-cylindrical, with a short, broad pedicel, with a distinct ocular chamber surrounded by a large apical ring. *Ascospores* 2–3-seriate, muriform, constricted at the septum, smooth-walled, surrounded by a mucilaginous sheath. Asexual state: hyphomycetous, *Conidiophore*s macronematous, mononematous, sometimes caespitose, straight or flexuous, often geniculate, unbranched or in a few species loosely branched, brown, smooth in most species. *Conidiogenous cells* polytretic, integrated, terminal, frequently becoming intercalary, sympodial, cylindrical, cicatrized. *Conidia* solitary, in certain species also sometimes catenate or forming secondary conidiophores which bear conidia, acropleurogenous, simple, straight or curved, clavate, cylindrical rounded at the ends, ellipsoidal, fusiform or obclavate, straw-coloured or pale to dark brown or olivaceous brown, sometimes with cells unequally coloured, the end cells then being paler than intermediate ones, mostly smooth, rarely verruculose, pseudoseptate (description of asexual state from Ellis 1971).

Type species – *Pyrenophora phaeocomes* (Rebent.) Fr., Summa veg. Scand., Section Post. (Stockholm): 397 (1849) MycoBank: MB 222199

= *Sphaeria phaeocomes* Rebent., Prodr. fl. neomarch. (Berolini): 338 (1804)

Notes – The genus *Pyrenophora* clusters in the suborder Pleosporineae of the family *Pleosporaceae* with a relatively high bootstrap support (Fig 1, 60%). Phylogenetic analysis (Fig. 2) shows that sexual *Pyrenophora* states cluster with asexual *Drechslera* states, *i.e. Pyrenophora dictyoides* (DAOM 75616) clusters with *Drechslera dictyoides* (DAOM 63666). The putative strain of *Pyrenophora phaeocomes* (DAOM 222769), which is the type species of the genus clusters with other *Pyrenophora* species and forms a sister clade with *Drechslera biseptata*. As a genus can now only have one name *Drechslera* is synonymized under *Pyrenophora*.

To establish the phylogenetic placement of *Pyrenophora* species at the higher level, combined analysis of LSU (LROR/LR5), SSU (NS1/NS4) and RPB2 (fRPB2-SF/fRPB2-7cR) sequence datasets are recommended. For resolving species we recommend combined of ITS (ITS1/ITS4), LSU (LROR/LR5) and GPDH (*gpd*1/*gpd*2) datasets. Phylogenetic inferences from sequence data of parts of the 18S nrDNA (SSU), 28S nrDNA (LSU), the internal transcribed spacer regions 1 and 2 and intervening 5.8S nrDNA (ITS) and glyceraldehyde-3-phosphate dehydrogenase(GAPDH) genes has shown that GAPDH and ITS regions provide more resolution for species of *Pyrenophora* as compared to LSU.

Pyrenophora phaeocomes (Rebent.) Fr., Summa veg. Scand., Section Post. (Stockholm): 397 (1849) MycoBank: MB 222199

= *Sphaeria phaeocomes* Rebent., Prodr. fl. neomarch. (Berolini): 338 (1804

Sexual state – *Ascomata* 380–450 × 370–430 µm ($\bar{x}$ = 395 × 380 µm, n = 10), solitary or scattered, initially immersed, becoming erumpent to near superficial, globose to subglobose, broadly or narrowly conical, coriaceous, smooth-walled, ostiolate. *Ostiole* usually broadly papillate, central ostiolar canal filled with periphyses and covered with setae. *Setae* brown to reddish-brown, darkened at the base, septate and tapered towards the apex. *Peridium* 40–70 µm ($\bar{x}$ = 45 µm, n = 20) wide, comprising two types of cells, outer cells of 1–2 layers of heavily pigmented cells of *textura angularis*, inner layer composed of small, light brown to hyaline cells of *textura angularis*. *Pseudoparaphyses* not observed. *Asci* 300–400 × 130–160 µm ($\bar{x}$ = 345 × 140 µm, n = 20), 8-spored, bitunicate, fissitunicate, clavate to sub-cylindrical, with a short, broad pedicel, thickened and rounded at apex with a distinct ocular chamber surrounded by a large, distinct, apical ring. *Ascospores* 78–96 × 27–34 µm ($\bar{x}$ = 88 × 30 µm, n = 40), biseriate to overlapping triseriate, ellipsoidal with broadly rounded ends, hyaline to light brown when immature, becoming brown to chestnut brown when mature, muriform with 5–6 transverse septa

and single longitudinal septa in one or all cells, constricted at the septa, smooth-walled, relatively thick-walled, with a 5–9 μm thick mucilaginous sheath. Asexual state – not observed, but see notes.

Material examined – SWEDEN, on leaves of *Anthoxanthum* (*Poaceae*), 7 August 1951, J. Ax. Nannfeldt (UPS 170980, neotype).

Distribution – Putative collections of *Pyrenophora phaeocomes* have reported from Belgium, Czech Republic, Denmark, Norway, Portugal, Sweden (GBIF, 2014), but these identifications have not been confirmed by molecular data.

Type specimen **–** UPS (neotype), Putative collections of *Pyrenophora phaeocomes* are available in BG, BPI, C and O

Sequence data **–** There is no extype sequence data.

Molecular data is available in GenBank for a putative strain of *Pyrenophora phaeocomes* (DAOM 222769). However it is not clear if this species was correctly identified. DAOM 222769 was initially used by the Assembling the Fungal Tree of Life (AFTOL) project in 2007.
AFTOL ID: AFTOL: 283
ITS: JN943649.1 (ITS1/ITS4)
LSU: JN940093.1 (LROR/LR5)
SSU: JN940960.1 (NS1/NS4)
EF1a: DQ497607.1 (983/2218R)
RPB2: DQ497614.1 (fRPB2-SF/fRPB2-7cR)

Notes – *Pyrenophora phaeocomes* is the type species of *Pyrenophora*. Sivanesan (1987) stated that *P. phaeocomes* has a *Drechslera* asexual state, but the species was not identified. In our study we did not observe the asexual state of *Pyrenophora phaeocomes* on the neotype.

Industrial relevance

Some *Pyrenophora* species have been used as biocontrol agents. *Bromus tectorum* is a dominant winter annual weed in cold deserts of the western United States. (Meyer et al. 2007). *Bromus tectorum* and other annual brome grasses have invaded many ecosystems of the western United States, and because of an annual-grass influenced alteration of the natural fire cycle on arid western range lands near monocultures are created and conditions in which the native vegetation cannot compete have been established (Meyer et al. 2007).

Biosecurity

Some species of *Pyrenophora* are considered as economically important plant pathogens. *i.e Pyrenophora avenae* causes seedling blight of oats in different climatic zones (Motovilin, 2000). Because of the destruction of leaf tissue, photosynthesis is reduced in diseased plants, resulting in light or shriveled grains. Direct attack of kernels by the fungus also results in light or shriveled kernels. Severe disease attacks have caused yield losses as high as 30-40 percent (Motovilin & Strigekozin, 2000). *Drechslera cactivora* (stem rot and fruit rot on *Cactus* species), *Drechslera curvispora*, *Drechslera gigantean, Drechslera longirostrata* (seed rot), *Drechslera maydis* (Southern corn blight), *Drechslera musae-sapientium* (leaf spot), *Drechslera nodulosa* (seed rot), *Drechslera patereae, Drechslera pedicellata* (root rot), *Drechslera sorghicola* (grain mould), *Drechslera stenospila* (leaf spot), *Pyrenophora cerastii*, *Pyrenophora chrysospora* and *Pyrenophora tetramera* (net blotch) are listed in New Zealand Ministry for Primary Industries as unwanted organisms(http://www.biosecurity.govt.nz/ Accession Date – 2 April 2014).

Biochemistry

A new phytotoxic sesquiterpenoid penta-2,4-dienoic acid, named pyrenophoric acid, was isolated from solid wheat seed culture of *Pyrenophora semeniperda*, which is a fungal pathogen proposed as a mycoherbicide for bio-control of cheat grass (*Bromus tectorum*) and other annual bromes (Masi et al. 2014). This genus should be assessed for its chemical diversity and novel compounds.

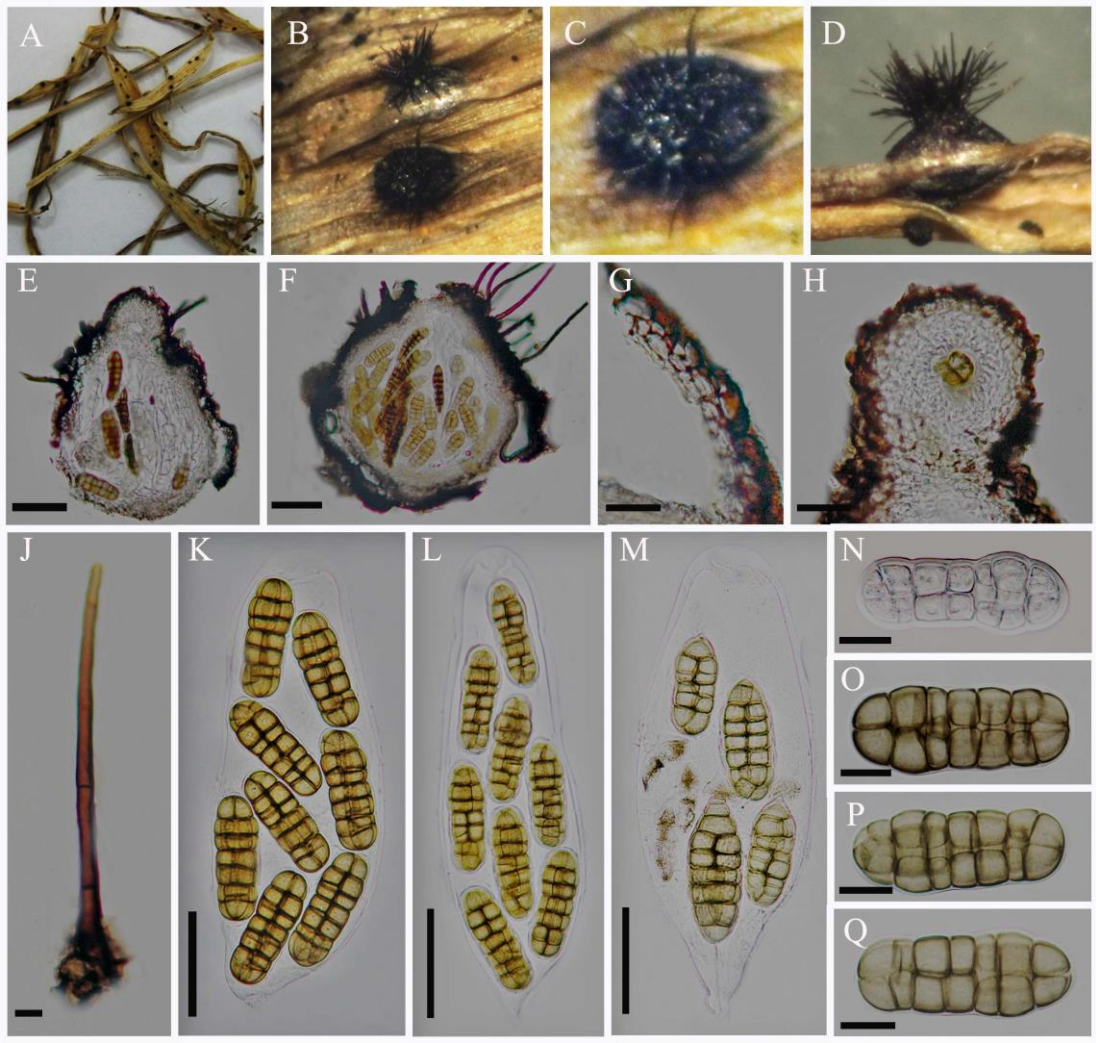

Fig. 3 – ***Pyrenophora phaeocomes*** (neotype) A, B. Ascomata on host specimen. C. Close up of ascoma. D. Side view of ascoma with neck covered with setae. E, F. Sections of ascomata. G. Section of peridium. H. Ostiole, with central periphyses. J. Light brown seta. K-M. Asci with 8 ascospores, distinct ocular chamber and apical ring. N-Q. Mature and immature muriform ascospores. Scale bars: E-F=200 µm, G=30 µm, H=80 µm, J=50 µm, K-M=50 µm, N-Q=15 µm.

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