Multi-locus phylogeny of *Pleosporales*: a taxonomic, ecological and evolutionary re-evaluation

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Abstract: Five newly introduced families: *Spatafora*⁷ and K.D. Hyde⁸, ⁹*

Recent phylogenetic studies indicated that *Pleosporales* has undergone great changes in the last half century. The name *Pleosporales* was first proposed in 1955 by Luttrell to accommodate members of *Dothideomycetes* having ascomata with pseudoparaphyses amongst the asci, and seven families, i.e. *Botryosphaeriaceae*, *Didymosphaeriaceae*, *Herpotrichiellaceae*, *Lophiostomataceae*, *Mesnieraceae*, *Pleosporaceae* and *Venturaceae* were included. Luttrell (1973) redefined the concept of *Pleosporales* based on ascomatal morphology, ascal arrangement in locules, presence or absence of hamathecal tissue, shape of papilla or ostioles, ascospore features and type of habitats, and added three more families, i.e. *Dimeriaceae*, *Mycoporaceae* and *Sporormiaceae*. The morphology of the pseudoparaphyses was given much importance at the ordinal level classification when Barr (1987) introduced *Melanommales* to accommodate pleosporalean taxa with trabeculate pseudoparaphyses (Sporormia-type centrum development) as compared to cellular pseudoparaphyses (*Pleospora*-type centrum development) possessed by other members of *Pleosporales*. Due to the lack of a Latin description in the original publication, *Pleosporales* was formally established in 1987 (Barr 1987b), and was characterised by perithecioid ascomata with conspicuous ocular chambers and apical rings. Nutritional shifts in *Pleosporales* likely occurred from saprotrophic to hemibiotrophic or biotrophic.

INTRODUCTION

*Pleosporales* is the largest order in the class *Dothideomycetes*, with a reported 23 families, 332 genera and more than 4 700 species (Kirk et al. 2008), or 19 families and 174 genera in Lumbsch & Huhndorf (2007)*. Members of *Pleosporales* can be endophytes or epiphytes (Huang et al. 2008, Sánchez Márquez et al. 2008, Tao et al. 2008), parasitic on green leaves or stems (Wetzel et al. 1999, Solomon et al. 2006), lichenicolous (Calatayud et al. 2001), saprobic on dead leaves or stems in terrestrial or aquatic environments (Cámara et al. 2002, Ramesh et al. 2003, Kadse et al. 2008, Zhang et al. 2008b, 2009a), or occur on animal dung (Kruys et al. 2006, Kruys & Weedin 2009).

The circumscription of *Pleosporales* has undergone great changes in the last half century. The name *Pleosporales* was first proposed in 1955 by Luttrell to accommodate members of *Dothideomycetes* having ascomata with...
Coccoideaceae, Cucurbitariaceae, Dacampiaceae, Hysteriaceae, Leptosphaeriaceae, Micropelidaceae, Parodiellaceae, Phaeosphaeriaceae, Phaeotrichaceae, Pleomassariaceae, Polystomellaceae, Pyrenophoraceae, Tuberiaceae, Vizellaceae. Recent phylogenetic analysis based on DNA sequence data however, have indicated that the Pleospora-type and Sporormia-type of centrum development (cellular versus trabeculate pseudoparaphyses) are not natural groupings, as taxa with these centrum types are dispersed in phylogenetic trees (Liew et al., 2000, Lumbsch & Lindemuth 2001). Thus members of Melanomatales were assigned to Pleosporales, and consequently, Melanomatales was treated as a synonym of Pleosporales (Eriksson 2006). Nineteen families have been assigned to Pleosporales in Kirk et al. (2001), 13 in Eriksson (2006), and 19 in Lumbsch & Huhndorf (2007).

One important reason for the unstable circumscriptions in the traditional classification of the Pleosporales is that the value given to the various morpho-characters, even those used at high-level classification, has proven to be overstated. For instance, fruiting-body shapes, i.e. cleistotheciod, perithecioid and apothecioid, previously considered sanctum at class level classification, were found to have undergone convergent evolution (Hawksworth & Lagreca 2007), as can be seen across Ascomycota (Schoch et al. 2009a). Another important distinguishing character, ascus type, has been reported to be phylogenetically misleading in numerous natural groups (Schmitt & Lumbsch 2004, Wedin et al. 2005, Lumbsch et al. 2007). Indeed, several DNA sequence based phylogenetic reconstructions have shown that ascospore morphology has little phylogenetic significance at familial or generic level classification (Cous et al. 2003, Schmitt & Lumbsch 2004, Kodueba et al. 2006, Wang et al. 2007, Zhang et al. 2009b). Consequently, an increasing number of taxa designated only by morphological characterisations in Pleosporales have been reported to be polyphyletic, such as the families Pleosporaceae (Kodueba et al. 2006), Melanomataaceae (Liew et al. 2000, Wang et al. 2007) and genera Massariosphaeria (Wang et al. 2007), Melanomma (Wang et al. 2007), Massarina and Lophiostoma (Liew et al. 2002, Zhang et al. 2009b).

Various anamorph genera have been recorded in Pleosporales and include both hyphomycetes and coelomycetes. Anamorph genera are often associated with multiple teleomorph genera, and in many cases anamorph relationships described in older literature have not yet been tested with DNA sequence data (Farr et al. 1989, de Gruyter et al. 2009). In the few cases where this was done, anamorph genera such as Ampelomyces, Ascochyta, Coniothyrium and Phoma proved to be polyphyletic and associated with multiple teleomorph genera (Aveskamp et al. 2008, de Gruyter et al. 2009).

Besides the morphological characters used in traditional taxonomy, several other biological characters have been used to define families. For instance, metabolite production and substrate staining reactions have been shown to be phylogenetically informative in xylariaceous and pleosporalean taxa (Stadler et al. 2001, 2004, 2007, Stadler & Fournier 2006, Bitzer et al. 2008, Zhang et al. 2009a). Host spectrum has been used to distinguish between Phaeosphaeria and Leptosphaeria (Holm 1957, Shoemaker & Babcock 1989), and anamorphic stages have been used to distinguish Pleospora and Lewia (Simmons 1986, 2007).

Since the first attempts at a classification of the order Pleosporales it has been a challenge to address the enormous diversity in biology, morphology and ecology within a stable classification. Thus, in molecular studies comprehensive taxon sampling is essential in order to avoid biased conclusions. To counteract this, a large number of taxa from various families and habitats, in particular generic types were included in the present phylogenetic analysis. The aims of the present investigation are: 1) to build up an overall molecular phylogenetic framework based on a multi-gene analysis showing the interfamilial relationships in the Pleosporales; 2) to re-evaluate the significance of morphological or ecological characters used in phylogeny and taxonomy of the order; and 3) to redefine hypotheses for evolutionary trends in the Pleosporales.

**MATERIALS AND METHODS**

**Collection and examination of specimens**

Twenty-eight fresh specimens were collected in Europe (the majority from France) during 2004 to 2008 by J. Fournier, and returned to the laboratory for examination. In most cases ascomata were collected directly on natural wood without incubation. The samples were processed and examined following the method described in Tsui et al. (2000). Colonies were sub-cultured onto 2 % potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), 2 % malt extract agar (MEA), and oatmeal agar (OA) (Crous et al. 2009b), and incubated under continuous near-UV light at 25 °C to promote sporulation. Observations and photographs were prepared from material mounted in water, congo red, cotton blue, chlorazol black, aqueous nigrosin, lactic acid or Indian ink. Additional cultures used in this study were obtained from the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands. Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004).

**Fungal isolates and DNA extraction**

Total genomic DNA was extracted from mycelia following the protocols as outlined by Cai et al. (2006) and Shenoy et al. (2007). A second set of DNA samples were obtained following DNA extraction protocols outlined in Schoch et al. (2007). In cases where no cultures could be obtained, a Forensic Kit (UltraClean™ Forensic Kit, Cambio) was used to extract DNA from specimens directly.

**DNA amplification and sequencing**

DNA amplification was performed by PCR. For partial large subunit (28S, LSU) nuclear rDNA amplification (nu-rDNA), LROR and LR5 primers (Vilgalys & Hester 1990) were used. Primer pairs NS1 (28S, LSU) nuclear rDNA amplification (nu-rDNA), LROR and LR5 were used for partial large subunit DNA amplification and sequencing. DNA amplification was performed by PCR. For partial large subunit (28S, LSU) nuclear rDNA amplification (nu-rDNA), LROR and LR5 primers (Vilgalys & Hester 1990) were used. Primer pairs NS1 and NS4 were used to amplify a region from the small subunit (18S, SSU) of the nu-rDNA (White et al. 1990). The fRPB2-5F and fRPB2-7Cr primers were used for the amplification of the partial RNA polymerase second largest subunit (RPB2) (Liu et al. 1999). The EF1-α-F and EF1-α-R primers were used to amplify a region from the translation elongation factor 1-alpha gene (TEF1) (Schoch et al. 2006) and the RPB1-Ac and RPB1-Cr primers were used for RPB1 region (Schoch et al. 2009; this volume). The amplification reaction for partial LSU, SSU and TEF1 nu-rDNA genes was performed in a 50 μL reaction volume as outlined by Jeewon et al. (2004) and Shenoy et al. (2007): 1 × PCR buffer, 0.2 mM dNTPs, 0.3 μM of each primer; 1.5 mM MgCl₂, 0.8 units Taq polymerase and 5–10 ng gDNA. The PCR thermal cycle programme for partial LSU nu-rDNA amplification was as follows: 95 °C for 3 min, followed by 34 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 30 s and elongation at 72 °C for 1 min.
with a final extension step of 72 °C for 10 min (Vigalys & Hester 1990). The PCR thermal cycle programme for the partial RPB2 gene amplification consisted of 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 2 min and elongation at 72 °C for 90 s, with a final extension step of 72 °C for 10 min (Liu et al. 1999). The PCR products, spanning approximately 700 bp (TEF1), 900 bp (partial LSU) and 1200 bp (partial SSU and RPB2), were checked on 1 % agarose electrophoresis gels stained with ethidium bromide. The PCR products were then purified using minicolumns, purification resin and buffer according to the manufacturer’s protocols (GFX PCR DNA and Gel Band Purification Kit, Amersham Biosciences, Buckinghamshire, U.K.). DNA sequencing was performed using the above-mentioned primers in an Applied Biosystem 3730 DNA analyser at the Genome Research Centre, the University of Hong Kong.

**Sequence alignment and phylogenetic analyses**

Sequences were obtained from WASABI (Kauff et al. 2007) as well as from previous publications (e.g. Lutzoni et al. 2004, Schoch et al. 2009b). Taxa was aligned by using default options for a simultaneous method of estimating alignments and tree phylogenies, SATé (Liu 2009b). Taxa was aligned by using default options for a simultaneous method of estimating alignments and tree phylogenies, SATé (Liu et al. 2009). Protein coding fragments were translated in BioEdit v. 7.0.1 (Hall 2004) and aligned within SATé as amino acids. These were aligned with their respective DNA sequences using the RevTrans 1.4 Server (Wernersson & Pedersen 2003). Subsequently, newly generated sequences were added to this initial alignment with MAFFT v. 6.713 (Katoh et al. 2009).

A supermatrix of five genes (LSU, SSU, TEF1, RPB1, RPB2) consisting of 47 % gaps and undetermined characters across 171 taxa was obtained. Most taxa had at least two genes present – except for a set of nine taxa with closely related species needed to confirm their identity (Table 1 - see online Supplementary Information).

**Conflict tests**

Conflict tests were conducted by selecting single gene data sets and doing comparisons on a gene-by-gene basis applying the bootstrapping criterion in RAxML v. 7.0.4 (Stamatakis et al. 2008), using the CIPRES 2.1 webportal (Miller et al. 2009) to produce trees of comparative gene sets where all taxa have the gene present. Comparisons between two sets of gene trees were done using a script (compat.py; Kauff & Lutzoni 2002) obtained through the Lutzoni lab website (www.lutzonilab.net/downloads/index.shtml) to detect taxa within clades with a cut-off value of 70 %. This is also performed as in Schoch et al. (2009).

A phylogenetic analysis was performed using RAxML v. 7.2.2 (Stamatakis 2006) applying unique model parameters for each gene and codon. The data set was thus partitioned in 11 partitions as previously done in Schoch et al. (2009b). In addition a general time reversible model (GTR) was applied with a discrete gamma distribution and four rate classes. One hundred successive most likely tree searches were done in RAxML under the same model, each one starting from a randomised tree with joint branch length optimisation and a rapid hill climbing option. Bootstrap pseudoreplicates were performed 145 times using the fast bootstrapping option and a frequency-based bootstrapping criterion (Stamatakis et al. 2008). These were plotted above the nodes in the most likely tree obtained earlier. The values below the nodes are percentages of 500 jacknife resamplings performed in TNT for MS windows with a new technology search set to 20 (Goloboff et al. 2008).

**RESULTS AND DISCUSSION**

**DNA phylogeny**

The tree presented in Fig. 1 represents the most complete phylogeny of Pleosporales produced to date. In addition it contains the members of other potential orders in Pleosporomycetidae and Dothideomycetes for outgroup comparisons. The tree was rooted with two Anthonomycetes as outgroups, Opegrapha varia and O. dolomitica (not shown). The supermatrix analysed in this study produced 4 290 distinct alignment patterns distributed as follows across the various partitions: SSU – 563, LSU – 807, RPB1 codon1 – 232, RPB2 codon2 – 198, RPB1 codon3 – 333, RPB2 codon1 – 467, RPB2 codon2 – 404, RPB2 codon3 – 614, TEF1 codon1 – 185, TEF1 codon2 – 176 and TEF1 codon3 – 311. The highest scoring likely tree had a log likelihood of -107754.307532.

**Families of Pleosporales**

In total, 151 taxa (171 strains) of Ascomycota (including the outgroups Opegrapha dolomitica and O. varia) were included in the analysis. It comprises 149 taxa (169 strains) of Dothideomycetes, of which 129 taxa (148 strains) were Pleosporales. The Pleosporales formed a well-supported clade (Fig. 1). The pleosporalean taxa comprised of representatives from 59 pleosporalean genera out of about 200 known genera (ca. 30 %), with 39 generic types of Pleosporales included in the analysis. As shown in Fig. 1, Pleosporales can be subdivided into 17 clades with more than 70 % ML bootstrap (MBL) or 65 % Jackknife (JK); 15 representing familial ranks, i.e. Agilaceae, Delitschiaeae, Didymellaceae, Lepotosphaeriaceae, Lophiotomataceae s. str., Massarinaceae, Melanommataceae, Montagnulaceae, Phaeosphaeriaceae, Pleosporaceae, Sporormiaceae, Trematosphaeriaceae and Massariaceae (Lumbsch & Huhndorf 2007, Kirk et al. 2008), as well as Amniculicolaceae and Lentitheciaceae, which are newly introduced in this paper. Based on the multi-gene phylogenetic data generated here, a new circumscription of Pleosporales is given as follows:

**Pleosporales** Lutr. ex M.E. Barr, *Prodromus to class Loculoascomycetes*: 67. 1987. emend.

Hemibiotrophic, saprobic, hypersaprobic, or lichenised. Habitats in freshwater, marine or terrestrial environment. Ascomata perithecioid, rarely cleistothecoid, immersed, erumpent to superficial, globose to subglobose, or lenticular to irregular, with or without conspicuous papilla or ostioles. Ostioles with or without paraphyses. Peridium usually composed of a few layers of cells with various shapes and structures. Hamathecium persistent, filamentous, very rarely decomposing. Asci bitunicate, fissitunicate, cylindrical, clavate to obclavate, with or without pedicel.

Anamorphs: Acroconidiellina, Alternaria, Aposphaeria, Ascochyta, Ascochyttella, Bipolaris, Ceratophoma, Coniothyrium, Corynespora, Curvularia, Cytoptea, Drechslera, Exserohilum, Hendersonia, Leptophoma, Metabolotryon, Microsphaeropsis, Myxococcus, Nigroentilocus, Nimbya, Phoma, Pilimmyces, Pleurophomopsis, Prosthemium, Pseudospiroplas, Pyrenochaeta, Scolocosporiella, Scolicosporium, Shearia, Sphaerellopsis, Stagonospora, Steganosporium, Stemphylium and Tiarospora (www.cbs.knaw.org).
### Pleosporaceae

| Species | Ascus | Ascosporae | SZ | SO | SP | ST | CL |
|---------|-------|------------|----|----|----|----|----|
| Phaeosphaeria herpotricha | M | P | Fi | >3 | H | Y |
| Phaeosphaeria nigra | M | M | P | Fi | >3 | H | Y |
| Phaeosphaeria elongata | M | M | P | Fi | >3 | H | Y |
| Phaeosphaeria typharam | S | S | P | NF | >3 | H/Y |
| Phaeosphaeria sp. | M | M | P | Fi | >3 | H | Y |
| Phaeosphaeria nodorum | M | M | P | Fi | >3 | H | Y |

### Phaeosphaeriaceae

| Species | Ascus | Ascosporae | SZ | SO | SP | ST | CL |
|---------|-------|------------|----|----|----|----|----|
| Phaeosphaeria nodorum | M | P | Fi | >3 | H | Y |
| Phaeosphaeria truncata | M | M | P | Fi | >3 | H | Y |
| Phaeosphaeria aphidiformis | S | S | P | NF | >3 | H/Y |
| Phaeosphaeria avencaria | M | M | P | Fi | >3 | H | Y |
| Phaeosphaeria sp. | M | M | P | Fi | >3 | H | Y |

### Didymellaceae

| Species | Ascus | Ascosporae | SZ | SO | SP | ST | CL |
|---------|-------|------------|----|----|----|----|----|
| Didymella bryoniae | M | P | Fi | >3 | H | Y |
| Didymella sphaericus | M | M | P | Fi | >3 | H | Y |
| Didymella sp. | S | S | P | NF | >3 | H/Y |

### Coniothyrium
genera

| Species | Ascus | Ascosporae | SZ | SO | SP | ST | CL |
|---------|-------|------------|----|----|----|----|----|
| Coniothyrium palmarum | M | M | P | Fi | >3 | H | Y |
| Coniothyrium sp. | S | S | P | NF | >3 | H/Y |
| Coniothyrium sp. | M | M | P | Fi | >3 | H | Y |
| Coniothyrium sp. | S | S | P | NF | >3 | H/Y |

### Phaeosphaeria
genera

| Species | Ascus | Ascosporae | SZ | SO | SP | ST | CL |
|---------|-------|------------|----|----|----|----|----|
| Phaeosphaeria sp. | M | M | P | Fi | >3 | H | Y |
| Phaeosphaeria sp. | S | S | P | NF | >3 | H/Y |
| Phaeosphaeria sp. | M | M | P | Fi | >3 | H | Y |
| Phaeosphaeria sp. | S | S | P | NF | >3 | H/Y |

### Ophiostomataceae

| Species | Ascus | Ascosporae | SZ | SO | SP | ST | CL |
|---------|-------|------------|----|----|----|----|----|
| Ophiostoma herpotricha | M | P | Fi | >3 | H | Y |
| Ophiostoma nigra | M | M | P | Fi | >3 | H | Y |
| Ophiostoma elongata | M | M | P | Fi | >3 | H | Y |
| Ophiostoma typharam | S | S | P | NF | >3 | H/Y |
| Ophiostoma sp. | M | M | P | Fi | >3 | H | Y |
| Ophiostoma nodorum | M | M | P | Fi | >3 | H | Y |
| Ophiostoma sp. | S | S | P | NF | >3 | H/Y |

### Montagulaceae

| Species | Ascus | Ascosporae | SZ | SO | SP | ST | CL |
|---------|-------|------------|----|----|----|----|----|
| Montagula arundinearum | M | P | Fi | >3 | H | Y |
| Montagula sp. | S | S | P | NF | >3 | H/Y |
| Montagula sp. | M | M | P | Fi | >3 | H | Y |
| Montagula sp. | S | S | P | NF | >3 | H/Y |

### Fungi
genera

| Species | Ascus | Ascosporae | SZ | SO | SP | ST | CL |
|---------|-------|------------|----|----|----|----|----|
| Didymella bryoniae | M | P | Fi | >3 | H | Y |
| Didymella sphaericus | M | M | P | Fi | >3 | H | Y |
| Didymella sp. | S | S | P | NF | >3 | H/Y |
| Didymella sp. | M | M | P | Fi | >3 | H | Y |
| Didymella sp. | S | S | P | NF | >3 | H/Y |

### Phylogenetic relationships

Fig 1. RAAML tree with bootstrap values after 1000 pseudo replications on the nodes. The values below the nodes are percentages of 500 jackknife resamplings. Pleospora-like leaves highlighted in red and bold are marine or maritime taxa, in blue and bold are freshwater taxa, and others are terrestrial ones. Relevant biological or morphological characters plotted on the leaves are abbreviated as follows: Biology: Mono – monocotyledons; Dico – dicotyledons; Gy – Gymnosperm; SF – Stream foam; ? – unknown; X – no information. Morphology: SZ – size, OS – ostiole, SP – shape, ST – septum, CL – colour: Ascusoma size: S – small (<300 µm), M – medium (300 µm < diam < 600 µm), L – large (diam > 600 µm); ostiole: P – pore-like ostiole, Sl – slit-like ostiole, Nil – no opening. Ascospore shape: Fi – filiform, Fu – fusiform, NF – narrowly fusiform, BF – broadly fusiform, Cy – cylindrical; ascospore septum: 1 – one transverse septum, 2 – two transverse septae, 3 – three transverse septa, 3+ – more than three transverse septa, M – muriform, Ap – apiosporous; ascospore colour: H – hyaline, B – brown, PB – pale brown, RB – reddish brown, DB – dark brown, Y – yellow, PY – pale yellow, ? – characters unknown. -- -- anamorph strain.
Pleosporineae

Pleosporineae contains many notorious plant pathogens, most belonging to one of four families, viz. Didymellaceae, Leptosphaeriaceae, Phaeosphaeriaceae and Pleosporaceae. These four families cluster together with high support (MLB = 99 %, JK = 92 %) (Fig. 1). Most taxa in these families are associated with living plants and many are serious plant pathogens (Shoemaker & Babcock 1989, Ueng et al. 2003, Rouxel & Balesdent 2005). Examples of important plant pathogens representing the different families are Cochliobolus heterostrophus (Pleosporaceae), the cause of southern corn leaf blight on maize (White 1999), Phaeosphaeria nodorum (anamorph Stagonospora nodorum) the cause of wheat glume blotch (Vergnes et al. 2006, Rouxel & Balesdent 2005). Because of their economic importance, members of Pleosporineae have already been subject to extensive molecular phylogenetic and pathogenic investigations over several decades (Wehmeyer 1961, Shoemaker 1976, 1984a, Shoemaker & Babcock 1985, Simmons 1986, Barr 1992). This includes studies on taxonomy, fungus-host interactions, biochemistry and genomics. Recently, the production of full genome data sets have spurred renewed interest in species such as Stagonospora nodorum (Solomon et al. 2006, Hane et al. 2007), Leptosphaeria maculans (Rouxel & Balesdent 2005), and Alternaria brassicicola (Pedras et al. 2009). The designation of Pleosporineae was first proposed by Barr (1979) to accommodate fungi having “globose, depressed, conic or vertically elongated ascomata, with a peridium equal in thickness or thickened at the lower sides”. Six families were included, viz. Mesnieraceae, Phaeosphaeriaceae, Pleosporaceae, Pyrenophoraceae, Tubulifloraceae and Venturiaceae (Barr 1979). The findings here support previous phylogenetic studies in concluding that the ordinal type, Pleosporaceae, and the families Phaeosphaeriaceae, Leptosphaeriaceae and Didymellaceae form a robust clade, and consistently occupy the terminal branches of pleosporalean dendrograms (Liew et al. 2000, Kodseue et al. 2006, Krusy et al. 2006, Schoch et al. 2006, de Gruyter et al. 2006). Thus Pleosporineae is emended here to accommodate these four families. Many anamorphic stages of the Pleosporineae are coelomyceteous genera, which includes Ascochyta, Chalospororhiza, Ditylenchus, Microsphaerops, Pleurophoma, Phoma, and Stagonospora (de Gruyter et al. 2009). However, hyphomyceteous anamorphs such as Bipolaris, Alternaria or Stemphylium are also included (Simmons 1986).

Pleosporineae Barr, Mycologia 71: 947. 1979. emend.

Mostly hemibiotrophic or saprobic, rarely symbiotic. Ascomata perithecioid, immersed, erumpent to superficial; globose to subglobose, ovoid or obpyriform. Hamathecium broadly to narrowly trabeculate or cellular pseudoparaphyses, rarely deliquescent at maturity. Asci bitunicate, fissitunicate, usually basal, rarely extending laterally, cylindrical, clavate to oblong. Ascospores mostly pigmented, rarely hyaline, one- to multi-septate or multinucleate, symmetrical or rarely assymetrical. Anamorphs: Acroconidiellina, Alternaria, Ascochyta, Ascochyttella, Bipolaris, Coniothyrium, Curvularia, Drechslera, Exserohilum, Leptosphaeria, Metaboltron, Nimbya, Phoma, Pithomyces, Scolecosporiella, Stagonospora, Stemphylium and Tiarospora (www.cbs.knaw.nl/databases/anateleo.htm 04-2009, www.indexfungorum.org/ 12-2009, www.mycobank.org/DefaultPage.aspx 12-2009).

Clade I Phaeosphaeriaceae

The clade of Phaeosphaeriaceae (MLB = 92 %, JK = 83 %) comprises 19 taxa including the generic types of Amarenomyces (A. ammophilae), Entodesmium (E. rude) and Setomelanomma (S. holmi), as well as the species Leptosphaeria derasa, Ophiopsphaerella herpotricha and some other Phaeosphaeria species, such as P. avenaria, P. eustoma and P. nodorum (Fig. 1). This clade could be further subdivided into four subclades, i.e. I-A–D. Of these, I-A comprises species of Ophiopsphaerella and Phaeosphaeria; and I-B–D Phaeosphaeria species.

Phaeosphaeriaceae is an important family in the Pleosporales, comprising 19 genera and 394 species (Kirk et al. 2008), with many plant pathogens or forming associations with plants (Shoemaker & Babcock 1989, Carson 2005, Stukenbrock et al. 2006). Phaeosphaeriaceae was introduced by Barr (1979) based on a pseudoparenchymatous peridium almost equal in thickness, and narrowly fusiform or filiform, hyaline, pale brown or rarely dark brown ascospores, and was assigned under Pleosporales sensu Barr. The anamorphs are coelomycetes. Fourteen genera were included, viz. Comocladithra, Didymella, Euderluca, Heptameria, Leptosphaeria, Loculohypoxylon, Metameris, Microthelia, Nodulosphaeria, Ophiobolus, Paraphaeosphaeria, Rhopograps, Scirhodothids and Teichospora (Barr 1979). Subsequent phylogenetic studies indicated that the Phaeosphaeriaceae is heterogeneous, and Leptosphaeriaceae was introduced to accommodate species related to Leptosphaeria (Barr 1987a), which is supported by subsequent phylogenetic results (Fig. 1; Khashnobish & Shearer 1996, Câmara et al. 2002, de Gruyter et al. 2009).

Phaeosphaeria, as the familial type of Phaeosphaeriaceae, was first introduced by Miyake (1909), but was regarded as a synonym of Leptosphaeria for a long time. Holm (1957) noticed the presence of pseudoparaphyses in the generic type of Phaeosphaeria (P. oryzae), reinstated Phaeosphaeria, assigned some Leptosphaeria (s. 1) species with relatively small ascomata which occurred on monocotyledons to Phaeosphaeria, and treated 17 species. Subsequently, more species and information were added (Hedjaroude 1968, Leuchtmann 1984, Shoemaker & Babcock 1989). In a world monograph, 114 species of Phaeosphaeria were described, and they were further divided into 6 subgenera, viz. Ovispora, Fusisor, Phaeosphaeria, Spathispora, Vigaspisora and Sicispora, based on differences in ascospore shape and the number of septa (Shoemaker & Babcock 1989). Many species of Phaeosphaeria have characteristic gelatinous sheaths on spores, and some are dicyosporous (Eriksson 1967). Currently, ca. 80 species are accepted under Phaeosphaeria, and many of them have Stagonospora anamorphs (Kirk et al. 2008).

Two of the three strains in subclade I-B are isolated from maritime environments; e.g. P. ammophilae from beach grass Ammophila arenaria and Phaeosphaeria spartiinae from stems of Spartina alterniflora in estuarine salt marshes. A strain of Phaeosphaeria caricis (CBS 120249) used here was isolated from Typha latifolia occurring in or near freshwater. All species in the other three subclades (I-A, C–D, Fig. 1) are associated
with terrestrial or near freshwater grasses such as *P. elongata* with *Miscanthus sinensis*, *P. juncofila* with *Juncus articulatus* and *Ophiopsphaerella herpotricha* with *Bromus erectus*. The only exception is *Phaeosphaeria spartincola*, which was isolated from salt marsh grass (*Spartina alterniflora*).

Amarenomyces was separated from *Phaeosphaeria* (as Amarenomyces *ammonilae*) based on its multilayered endotunica and large and thick-walled, sheathed ascospores (Eriksson 1981). However, its relationship with other *Phaeosphaeria* species is supported in this study. Thus *Amarenomyces* is treated as a synonym of *Phaeosphaeria*. *Entodesmium* is exclusively associated with legumes, and is traditionally assigned to *Lophiostomataceae* based on its periphysate papilla (Eriksson & Hawksworth 1990, Barr 1992). But its immersed ascomata, non-compressed papilla and thin peridium, plus the multiseptate, lightly pigmented ascospores, which break up into part-spores support its inclusion in *Phaeosphaeriaceae*. In particular, *Entodesmium multiseptatum* and *E. nieslemanum* were originally described as a *Leptosphaeria* species (Shoemaker 1984b), indicating their similarity with *Phaeosphaeria* which is commonly confused with *Lentosphaeria* (Shoemaker 1984a, Shoemaker & Babcock 1989).

**Notes:** Although members of the *Phaeosphaeriaceae* are usually known as saprobes or parasites of plants or other fungi, the strain of *Phaeosphaeria luctuosa* (CBS 308.79) in this clade is recorded as an endophyte in *Zea mays*. In addition, the inclusion of *Entodesmium rude* in this clade indicates the ascospores of this family can be filiform.

Currently accepted genera: *Ophiopsphaerella*, *Phaeosphaeria*, *Entodesmium* and *Setomelanomma*.

**Anamorphs:** *Ampelomyces*, *Chaetosphaeronema*, *Coniothyrium*, *Phoma*, *Plenodomus*, *Stagonospora* and *Wojnowicia* (Leuchtmann 1984, de Gruyter et al. 2009).

The genera *Ampelomyces*, *Coniothyrium*, *Phoma* and *Plenodomus* are polyphyletic (de Gruyter et al. 2009). The generic type species *Ampelomyces quisqualis* clustered in the *Phaeosphaeriaceae*, whereas *A. quericus* grouped in the *Didymellaceae*. The type species of the genera *Phoma*, *Coniothyrium* and *Plenodomus* clustered in the *Didymellaceae* and *Leptosphaeriaceae* respectively. Although *Chaetosphaeronema* was associated with *Ophiobolus* (Petrak 1944), this teleomorph-anamorph relation has not been confirmed. An isolate preserved as *Trematophoma* sp. was found in the *Phaeosphaeriaceae* (de Gruyter et al. 2009); however, its identity needs to be studied in more detail.

**Clade II Pleosporaceae**

Pleosporaceae (Clade II), including the generic type of *Pleospora* — *P. herbarum*, forms a robust clade (MLB = 100 %, JK = 100 %), and comprises four subclades as well, i.e. II-A–D. Clade II-A, including the generic type – *Cochliobolus heterostrophus* represents *Cochliobolus*, II-B comprises two taxa, i.e. *Pleospora herbarum* and the anamorphic *Dendryphiella arenaria* (*Scolesterolosidium arenaria*), which represents *Pleospora*, II-C represents anamorphic fungi – *Alternaria*, and II-D contains the generic type – *Pyrenophora phaeocomes*, represents *Pyrenophora*.

Pleosporaceae comprises 36 genera and 769 species (Kirk et al. 2008) and is the largest family in *Pleosporales*. Members have been reported as plant parasites or saprobes occurring on herbaceous or woody plant leaves or stems (Sivasenans 1984). *Pleosporaceae* was introduced by Nitschke (1869), which had been assigned to *Sphaeriales* based on the immersed ascomata and presence of pseudoparaphyses, then to *Pseudospherales* (Theissen & Sydow 1917, Wehmeyer 1975), and the name of *Pseudospherales* subsequently was replaced by *Pleosporales* (Luttrell 1955). Morphology of ascospores, i.e. shape, colour, septation and presence or absence of sheaths has been emphasised in defining the circumscriptions of genera under *Pleosporaceae* (Luttrell 1955, 1973, Wehmeyer 1961, 1975, von Arx & Müller 1975, Sivasenans 1984, Barr 1987b, Aberl 2003). The polyphyletic nature of *Pleosporaceae* has been indicated in previous investigations, and some genera have been assigned to other families, such as *Leptosphaerulina* to *Leptosphaeriaceae*, and *Macroventuria* to *Phaeosphaeriaceae* (Kodsueb et al. 2006). In this study however, the generic types of both *Macroventuria* (*M. anomochaeta*) and *Lephtosphaerulina* (*L. australis*) cluster within the *Didymellaceae*, as previously recorded (de Gruyter et al. 2009).

The current clade of *Pleosporaceae*, comprising the generic types of *Cochliobolus* (*C. heterostrophus*), *Pleospora* (*P. herbarum*) and *Pyrenophora* (*P. phaeocomes*), represents the core members of *Pleosporaceae*, and are mostly plant pathogens (Fig. 1). Species in subclades II-A and II-D are exclusively associated with monocotyledons, such as *Pyrenophora tritici-repentis* with wheat and *P. phaeocomes* with *Festuca rubra*. *Pleospora herbarum* (Clade II-B) has been recorded as associates of numerous monocotyledons and dicotyledons, while the strain of *Dendryphiella arenaria* is from the root zone soil of beachgrass (*Amphophila arenaria*). Subclade II-C comprises two *Alternaria* species and one *Alllewia* species, of which *Alternaria maritima* was isolated from submerged wood in seawater, *A. alternata* is generally occurring on all kinds of substrates, and *Alllewia eureka* is associated with terrestrial dicotyledons.

**Notes:** Members of this clade mostly have middle-sized ascomata, and the hyaline and filiform ascospores possessed by *Setosphaeria monoceras* expanded the familial concept from “brown” by Cannon & Kirk (2007) to “hyaline or brown”.

Currently accepted genera: *Alllewia*, *Lewia*, *Cochliobolus*, *Pleospora*, *Pyrenophora* and *Setosphaeria*.

**Anamorphs:** *Alternaria*, *Ascochyta*, *Bipolaris*, *Curvularia*, *Drechslera*, *Embellisia*, *Exserohilum*, *Phoma* and *Stemphylium* (Simmons 1986, 1989, 1990, Cannon & Kirk 2007, Aveskamp et al. 2008, de Gruyter et al. 2009).

Most of the anamorphs in the *Pleosporaceae* are hyphomycetes. Both *Ascochyta* and *Phoma* species have been described in the *Pleosporaceae*. However, the generic type species, *Ascochyta pisi* and *Phoma herbarum*, belong to the *Didymellaceae* (de Gruyter et al. 2009).
Clade III Leptosphaeriaceae

The clade containing members of Leptosphaeriaceae is sister to the Pleosporaceae, but receives poor statistical support (Fig. 1), indicating the need for more thorough analysis. It comprises the generic types of Leptosphaeria (D. exigua) and Neophaeosphaeria (N. filamentosa), as well as other taxa from numerous groups, such as Coniothyrium palmarum, L. maculans (Leptosphaeriaceae) and Pyrenochaeta nobilis (Herpotrichia, Melanommataceae).

The Leptosphaeriaceae is likely paraphyletic (Schoch et al. 2009a; this volume). This taxon was separated from the Pleosporaceae and formally introduced by Barr (1987a) based on its “coelomycetous anamorphs” and “narrower and thinner-walled ascii” (Barr 1987b), and supported by phylogenetic data (Dong et al. 1998). Initially, five genera, i.e. Curreya, Didymolepta, Heptameria, Leptosphaeria and Ophiobolus, were accepted under Leptosphaeriaceae (Barr 1987b), while Eriksson & Hawksworth (1990) only accepted Leptosphaeria and Ophiobolus under this family. The Leptosphaeriaceae only comprises some species of Leptosphaeria and Neophaeosphaeria filamentosa, as well as the anamorph Coniothyrium palmarum. Pyrenochaeta nobilis also clustered in the Leptosphaeriaceae. However, this species probably represents a closely related subclade (de Gruyter et al. 2009).

Morphologically, Leptosphaeriaceae is mostly comparable with Phaeosphaeriaceae, and numerous characters have been used to distinguish them at generic or family level. For instance, anamorphic states (Câmara et al. 2002), peridium structure (Khoshnabish & Shearer 1996, Câmara et al. 2002) and host spectrum (Câmara et al. 2002) have all been proposed in distinguishing Leptosphaeria s. str. and Phaeosphaer. Of these characters, the host preference of Leptosphaeria on dicotyledons in contrast to Phaeosphaeria on monocotyledons has been widely reported (Eriksson 1967, Hedjaroude 1968, Eriksson 1981, Shoemaker & Babcock 1989). Currently, six of the eight species included in Leptosphaeriaceae (Fig. 1) have dicotyledonous hosts, while Coniothyrium palmarum is associated with palms. Thus present results further support the fact that the host spectrum has phylogenetic significance to some degree (Câmara et al. 2002, Voigt et al. 2005).

Currently accepted genera: Leptosphaeria and Neophaeosphaeria.

Anamorphs: Chaetodiplodia, Coniothyrium, Phoma, Plectophomella and Pyrenochaeta (Wehmeyer 1975, de Gruyter et al. 2009).

The genus Chaetodiplodia has been recorded as an anamorph of Leptosphaeria (Wehmeyer 1975), but not confirmed. A Chaetodiplodia sp. isolate clustered in the Leptosphaeriaceae (de Gruyter et al. 2009); however the identity of this strain is uncertain.

Clade IV Didymellaceae

The Didymellaceae (Clade IV) receives high bootstrap support, and includes the generic types of Didymella (D. exigua), Macroventuria (M. innumerosum), Monascostroma (M. innumerosum), Leptosphaerulina (L. australis) and Platychora (P. ulmi), as well as some species of Phoma and Ascochyta (Fig. 1).

This family was introduced to accommodate some species of Phoma and their phylogenetically closely related anamorphic taxa, as well as telemorphs such as Didymella and Leptosphaerulina (de Gruyter et al. 2009, Woudenberg et al. 2009). The generic types of Platychora, Monascostroma and Macroventuria are also located in Clade IV. In particular, both Platychora ulmi and Monascostroma innumerosum have immersed ascomata and clavate asci with lightly pigmented, 1-septate ascospores, and they form a robust subclade (Fig. 1), which most likely represents a single genus. When compared with M. innumerosum, the apiosporous ascospores are the most striking character of Platychora ulmi. Thus the symmetry of ascospores might have no phylogenetic significance at the generic level.

What is most interesting is that Leptosphaerulina argentinensis forms a robust clade with two strains of L. australis. Although L. argentinensis can be distinguished from L. australis by its larger ascospores, their morphological similarity can not be ignored (Graham & Luttrell 1961). Thus this subclade most likely represents a species complex for L. australis.

Most species in this clade are associated with dicotyledons, such as Macroventuria anomochaeta with Medicago sativa, Phoma cucurbitacearum with Cucurbita spp., Didymella exigua with Rumex arifolius, Leptosphaerulina argentinensis with Lonicera periclymenum and Ascochyta pisii with Pisum sativum, while Leptosphaerulina australis and Phoma herbarum are associated with a wide range of hosts including dicotyledons and monocotyledons.

Notes: Besides the characters described by de Gruyter et al. (2009), members of Didymellaceae are also mostly hemibiotrophic or saprobic, and have sometimes setose ascomata, persistent or deliquescing pseudoparaphyses and fusiform, symmetric or apiosporous ascospores.

Currently accepted genera: Didymella, Leptosphaerulina, Macroventuria, Monascostroma and Platychora.

Anamorphs: Chaetosbolisia, Diplodina, Microsphaeropsis and Phoma (Aveskamp et al. 2008, de Gruyter et al. 2009).

The genus Phoma is subdivided in nine sections with telemorphs in the genera Didymella, Leptosphaeria, Mycosphaerella and Pleospora (Boerema 1997). Molecular studies confirmed the polyphylectic character of Phoma in the Pleosporineae (de Gruyter et al. 2009). The generic type, Phoma herbarum, grouped in the Didymellaceae, and therefore, Phoma species in the Didymellaceae are considered as Phoma s. str. (de Gruyter et al. 2009). The taxonomy of Phoma species in the Leptosphaeriaceae, Phaeosphaeriaceae and Pleosporaceae needs further study.

Clade V Lentitheciaceae

The clade of Lentitheciaceae comprises the generic type Lentithecium fluitatile, as well as L. arundinaceum, Stagonospora macrocynidia, Wettsteinia lacinosa, Keisslerella cladophila, and the bambusicolous species Katumotoa bambusicola and Ophiophaerella sasicola, which receives high bootstrap support (MLB = 100 %, JK = 100 %). The telemorphs have lenticular ascomata, trabeculate to broadly cellular pseudoparaphyses, cylindrical to clavate asci with short pedicles, uni-, 3- to multiseptate, fusiform or filiform ascospores. Based on morphological characters and current molecular phylogenetic results, a new family — Lentitheciaceae is introduced to accommodate them.

This clade is further subdivided into two groups. One subclade comprises Lentithecium arundinaceum, Katumotoa bambusicola, W. lacinus, Ophiophaerella sasicola and Stagonospora macrocynidia (Clade V-A), while the other subclade (Clade
V-B) comprises *L. fluviatil* and *L. aquaticum* with Keissleriella cladophila basal to both. Species of Clade V-A exclusively occur on monocotyledons, such as Lentithecium arundinaceum and Stagonospora macroycnidia which are isolated from Phragmites sp. and Wettsteinina lacustris which is record on Schoenoplectus sp. The strain of *W. lacustris* (CBS 618.86) used here was isolated from Schoenoplectus lacustris, and both Ophiophaerea sasicala (from Sasa senanensis) and Katumotoa bambusica (from Sasa kuniensis) are bambusicolous. In contrast, species of Clade V-B seem to be exclusively associated with dicotyledonous woody substrates in freshwater environments, i.e. *L. aquaticum* and *L. fluviatil* are from submerged wood of Fraxinus sp. and Populus sp. from France, respectively. The habit details of the Keissleriella cladophila strain (CBS 104.55) used here are unknown, but it was isolated from dicotyledonous woody plants (*Smilax parvifolia*) in Pakistan.

The relatively larger ascomata (500–600 vs. 300–400 µm) and the sheathed ascospore of Ophiophaerea sasicala make it readily distinguishable from *O. herpotricha*, and the latter is morphologically similar to the generic type of Ophiophaerea (*O. graminicola*).

The identification of the strain of Wettsteinina lacustris (CBS 618.86) used here could not be verified. According to Shoemaker & Babcock (1989, p. 1596) however, the collections studied by Leuchtmann (collector of CBS 618.86) under this name, represent “a good Massarina”, which is “not conspecific with Wettsteinina”. Thus the strain of CBS 618.86 most likely is of Massarina s. l., which is closely related to Lentithecium. Both Ophiophaerea sasilica and Katumotoa bambusica are bambusicolous, and they have lenticular ascomata with a simple peridium structure, as well as numerous persistent pseudoparaphyses. All of these characters fit in the traditional concept of Lentithecium. However, their ascospores are asymmetrical (*K. bambusicola*) or filiform (*Ophiophaerea sasicala*), which differs from the symmetrical and cylindrical to fusiform ascospores possessed by other species of Lentithecium (Nagasawa & Otani 1997, Tanaka & Harada 2005a).

**Lentithecium** Yin. Zhang, C.L. Schoch, J. Fourn., Crous & K.D. Hyde, fam. nov. MycoBank MB515470.

Aquaticus vel terristrius. Saprophytus. Ascomata immensa, lenticulare, solitaria vel disseminata, nigra. Asci bitunicati, fissitunicati, clavati vel oblongato- cylindricali, pedicellati. Ascosporae cylindrica vel fusiforme vel filiforme, uniseptatae vel aliunquando 3-septatae cum supra-maturae, parce multiseptatum, hyalinae vel fulvum.

Terrestrial habitat. Saprobic. Ascomata immersed, lenticular, solitary or scattered. *Peridium* comprising a few layers of thin-walled cells. *Asci* bitunicate, fissitunicate, cylindro-clavate to cylindro-oblong, short pedicellate. *Ascosporae* fusiform or filiform, hyaline to pale yellow, 1-septate, constricted at the septum, sometimes becoming 3-septate when mature, rarely multiseptate.

**Type genus**: Lentithecium K.D. Hyde, J. Fourn. & Yin. Zhang.

**Notes**: Lentithecium was introduced to accommodate some freshwater taxa as lenticular ascomata and hyaline, 1-septate ascospores (Zhang et al. 2009b). Wettsteinina lacustris, Ophiophaerea sasicala, and the anamorphic Stagonospora macroycnidia, as well as Keissleriella cladophila and Katumotoa bambusica are also included in this clade. The strain of Wettsteinina lacustris used here may be misidentified (see comments above). However, they all have immersed and lenticular ascomata, with thin peridium usually almost equal in thickness, short pedicellate asci and fusiform or filiform, hyaline or rarely lightly pigmented, 1- to multi-septate ascospores. Phylogenetically, they form a robust clade separating them from all other pleosporalean families. Thus a new family, Lentithecaceae, is introduced to accommodate these species of Massarina s. l., a “genus” which should contain species from numerous genera.

**Currently accepted genera**: Lentithecium, Katumotoa and ? Keissleriella.

**Anamorph**: ? Stagonospora macroycnidia.

The genus Stagonospora is polyphyletic and considered as the anamorph of Phaeosphaeria (Leuchtmann 1984), while a strain of Stagonospora macroycnidia used here clusters in Lentithecaceae in this study.

**Clade VI Massarinaceae**

The Massarinaceae clade comprises the generic types of Massarina (*M. eburnea* and *Byssothecium* (*B. cirincans*), as well as *M. cisti* and *M. igniaria*, and receives high bootstrap support (MLB = 100 %, JK = 97 %). Massarinaceae was introduced to accommodate species having immersed, flattened or sphaerical ascomata with or without clypeus, trabeculate or cellular pseudoparaphyses, clavate to cylindro-clavate asci, hyaline, fusiform to narrowly fusiform, 1- to 3-septate ascospores with or without sheath. Five genera were accepted, i.e. *Keissleriella*, *Massarina*, *Metasphaeria*, *Pseudotrichia* and *Trichometasphaeria* (Munk 1956). This family name has not been commonly used and the familial type — Massarina has usually been placed under the Lophiostomataceae (Bose 1961, Eriksson & Yue 1986, Barr 1987b, 1990). The polyplethic nature of *Massarina* has been noted (Liew et al. 2002, Zhang et al. 2009b), and a narrow concept of *Massarina* was accepted, which comprises the generic type (*M. eburnea*) and morphologically similar species (e.g. *M. cisti*) (Zhang et al. 2009b). The strain of *Byssothecium cirincans* (CBS 675.92) in this clade is unverified, thus its status remains unresolved (see comments by Zhang et al. 2009b). *Massarina s. str.* comprising *M. cisti*, *M. eburnea* and *M. igniaria* is confirmed based on these five nuclear loci, which represents a separate branch in Pleosporales.

**Massarinaceae** Munk, Friesia 5: 305. 1956. emend.

Terrestrial habitat. Saprobic. Ascomata immersed, erumpent to superficial with small to wide papila, solitary or scattered. Pseudoparaphyses cellular to narrowly cellular. Asci clavate to cylindrical, with short pedicels. Ascosporae fusiform to broadly fusiform, hyaline or brown, 1- to 3-septate, with or without sheaths.

**Currently accepted genera**: ? Byssothecium and *Massarina*.

**Anamorph**: Periconia.

The hyphomycete genus *Periconia* is polyplethic, and in the *Massarinaceae* associated with Didymosphaeria (Booth 1968). The coelomycete genus *Neottiosporina* has not been associated with a teleomorph. In this study however, a strain of *N. paspali* grouped in the *Massarinaceae*. 
Clade VII Montagnulaceae

The well-supported clade of Montagnulaceae (MLB = 100 %, JK = 100 %) comprises the generic types of Bimuria (B. novae-zelandiae), Didymocrea (D. saddasivani), Karstenula (K. rhodostoma) and Paraphaeosphaeria (P. michotii), as well as some species of Kalmusia, Paraconiothyrium, Letendraea and Montagnula. Based on the morphological and ecological similarities, Phaeosphaeria brevispora was assigned to Kalmusia (see comments below). Species in this clade can be saprobic (Kalmusia scabrispora, Phaeosphaeria brevispora and Bimuria novae-zelandiae), plant pathogenic (Paraphaeosphaeria michotii) or mycoparasitic (Paraconiothyrium minutans) (Fukuhara 2002, Verkley et al. 2004). Montagnulaceae was introduced by promoting the heterogeneric Montagnula to familial level, which contains species with three types of ascospores, i.e. muriiform (Montagnula), phagmosporous (Kalmusia) and didymosporous (Didymosphaerella) (Barr 2001).

Paraphaeosphaeria has been treated as a segregate of Leptosphaeria based on its swollen cell above the A1 septum and a longer more highly septate upper part and Coniothyrium s. l. anamorphs (Eriksson 1967). By analysing the ITS and 18S rDNA sequences, Paraphaeosphaeria was shown to be polyphylectic, and a narrow generic concept accepted (Câmara et al. 2001). The familial placement of Paraphaeosphaeria under Montagnulaceae is verified in this study.

Remarkably, our phylogenetic results indicated that the generic type of Bimuria, B. novae-zelandiae is included in this group. Bimuria novae-zelandiae was initially isolated from soil in a barley field in New Zealand, and is characterised by a very thin peridium, mostly 2-spored, fissitunicate asci and muriiform, dark brown, verrucose ascospores, which is considered somewhat comparable with Montagnula (Hawksworth et al. 1979). The thick carbonaceous peridium, however, distinguishes Montagnula from Bimuria. In addition, the ascospores of Montagnula are discharged forcibly through the ostiole instead of simply deliquescing and gathering at the apex of the ascomata as happens in Bimuria (Hawksworth et al. 1979). Because of its unique morphological characters, the familial placement of this genus has been debatable and it has been placed in Pleosporaceae by Hawksworth et al. (1979), in Phaeosphaeriaceae by Barr (1987b) and in Melanommataceae by Lumbsch & Huhndorf (2007). In agreement with previous phylogenetic studies (Schoch et al. 2006), its affinity to other members of Montagnulaceae is noted here.

The generic type of Karstenula (K. rhodostoma) clusters in this group, which is characterised by immersed ascomata, usually with a wide ostiolar opening, narrowly cellular pseudoparaphyses, cylindrical asci with short pedicels, and reddish-brown, muriiform ascospores (information obtained from type material). Traditionally, Karstenula has been assigned to Melanommataceae, but the immersed ascomata, narrowly cellular pseudoparaphyses and reddish-brown, muriiform ascospores fit the definition of Montagnulaceae (Barr 2001), and this placement is confirmed by the present phylogenetic data (Fig. 1). The clade also contains sequences of Didymocrea saddasivani (Zopfiaceae) obtained from GenBank, confirming the polyphyly of Zopfiaceae, and its placement in relation to Bimuria, as noted before (Kruys et al. 2006).

The fact that this species produces ostensibly unisuniculate asci within ascostromatic ascomata makes it especially interesting (Rogerson 1970, Parguey-Leduc & Janex-Favre 1981).

Notes: The 2- or 3-spored ascus possessed by Bimuria novae-zelandiae is another unique character in Montagnulaceae.

Clade VIII Trematosphaeriaceae

The generic type of Trematosphaeria (T. pertusa) and the marine fungus, Halomassarina thalassiae, form a well supported clade (MLB = 100 %, JK = 100 %), and represent a pleosporalean family, Trematosphaeriaceae. Details of this family are addressed by Suetrong et al. 2009; this volume).

Clade IX Melanommataceae (syn. Pleomassariaceae)

Currently accepted genera: Bimuria, Didymocrea, ? Kalmusia, Karstenula, ? Letendraea, ? Montagnula and Phaeosphaeria. Anamorph: Paraconiothyrium (Verkley et al. 2004).

Kalmusia brevispora (Nagas. & Y. Otani) Yin. Zhang, Kaz. Tanaka, C.L. Schoch, comb. nov. MycoBank MB515474. Basionym: Phaeosphaeria arundinacea var. brevispora Nagas. & Y. Otani, Rep. Tottori Mycol. Inst. 15: 38. 1977.

Notes: Morphological characters of Phaeosphaeria brevispora, such as the immersed ascomata with clypei, thin peridium, clavate asci with relatively long pedicels, and the reddish-brown, verrucose ascospores constitute at the primary septum, fit Kalmusia well. Phylogenetically, P. brevispora and K. scabrispora form a robust clade. In particular, both of these two species occur on Sasa sp. (Tanaka & Harada 2004, Tanaka et al. 2005b).

Differing from other terrestrial members of this clade, both Beverwykella pulmonaria and Monotosporella tuberculosis are from freshwater. A Phoma-like anamorph (Aposphaeria ?) has been reported for Melanomma pulvis-pyrius (Chesters 1938, Sivanesan 1994). Both Beverwykella pulmonaria and Monotosporella tuberculosis are aquatic hyphomycetous fungi isolated from Europe (Netherlands and U.K., respectively), which indicates that the anamorphs of Melanommataceae should include hyphomycetes as well.

Genera currently accepted: ? Herpotrichia, Melanomma and Pleomassaria.
Anamorphs: Aposphaeria (or Phoma-like according to Chesters 1938), Beverwykella pulmonaria, Monotospora tuberculata, Prosthennium and ? Pyrenocheaeta (Sivanesan 1984, Paavolainen et al. 2000).

The genus Pyrenocheaeta is polyphyletic (de Gruyter et al. 2009), and the generic type species P. nobilis grouped in the Leptosphaeriaceae in this study.

Clade X Sporormiaceae

The Sporormiaceae including the generic types of Preussia (P. funiculata) and Westerdykella (W. ornata), and some other species such as Sporormiella minima, Preussia lignicola, P. tenticola and Westerdykella cylindrica form a well-supported clade (MLB = 98 %, JK = 82 %). The Sporormiaceae is the largest coprophilous family of Pleosporales, which contains 10 genera and 143 species (Kirk et al. 2008). The absence of periphyses and well-developed apical rings together with ascomata with or without ostioles, ascospores with or without germ slits have been used to distinguish the Sporormiaceae from other coprophilous families, such as the Deltitciaceae and the Phaeotrichiaceae (Barr 2000, Krüys et al. 2006). Phylogenetic analysis based on ITS-nLSU rDNA, mtSSU rDNA and ß-tubulin sequences indicated that compared to the genes or phylogenetic analyses are needed to separate those genera. All three species were collected in Europe, and stain the woody substrate purple, which could be indicative of metabolite activity (Zhang et al. 2009a). Metabolites have rarely been used in the phylogeny and taxonomy of Pleosporales, but it is widely used in the taxonomy of xylariaceous taxa (Stadler et al. 2004, Bitzer et al. 2008). In addition, all species in this clade are from freshwater environments, which may indicate this as a unique ecological habit for the Amniculicolaceae.

Amniculicolaceae Yin. Zhang, C.L. Schoch, J. Fourn., Crous & K.D. Hyde, fam. nov. MycoBank MB515469.

Aquaticus. Saprobius. Ascomata globosa vel subglobosa vel lenticular, nigra, solitaria, immersa vel partim immersa vel superficialia. Apex productum. Peridium exilis. Trabeeculae, hyalinae, gelatinae circumdatae. Asci, 8-spori, cylindrical vel clavati, fissitunicati, brevi pedicellati. Ascosporeae, fusiforme vel peranguste fusiforme, unisepatae vel multisepatae vel muriforme, hyalinae vel pallide brunneae vel nufrobrunneae, tunica gelatinosa praeditae. Substratum malvaceo purpureus.

Freshwater habitat. Saprobius. Ascomata solitary, scattered, or in small groups, immersed, erumpent, or nearly superficial, globose, subglobose to lenticular; surface black, roughened; apex elongated. Peridium thin. Pseudoparaphyses trabeicate, embedded in mucilage. Asci 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short pedicellate, with an ocellar chamber. Ascospores fusiform or narrowly fusiform, hyaline, pale or reddish-brown, one to multi-septate or muriform, constricted at the median septum, usually surrounded by an irregular, hyaline gelatinous sheath. Ascomata usually stain the woody substrate in shades of purple.

Type genus: Amniculicola Yin. Zhang & K.D. Hyde.

Currently accepted genera: Amniculicola, Murispora and Neomassariosphaeria.

Anamorphs: ? Anguillulospora longissima, Spirosphaera cupreorufescens and Repetophragma ontariense (Zhang et al. 2009a).

Murispora Yin. Zhang, J. Fourn. & K.D. Hyde, gen. nov. MycoBank MB515472.

Etymology: Named after its muriform ascospores.

Aquaticus. Saprobius. Ascomata immersa vel partim immersa vel superficialia. Peridium exilis. Trabeeculae, hyalinae, gelatinae circumdatae. Asci, 8-spori, clavati vel late clavati, fissitunicati; brevi pedicellati. Ascosporeae, fusiforme, muriforme, brunneae, tunica gelatinosa praeditae. Substratum malvaceo purpureus.

Freshwater habitat. Saprobius. Ascomata scattered, or in small groups, immersed, erumpent, or nearly superficial, globose to subglobose, wall black, roughened; apex weakly papillate, conical to laterally flattened. Peridium thin. Pseudoparaphyses trabeicate, embedded in mucilage. Asci 8-spored, bitunicate, fissitunicate, oblong to clavate, short pedicellate, with an ocellar chamber. Ascospores fusiform, pale or reddish brown, muriform, constricted at the median septum, usually surrounded by an irregular, hyaline, gelatinous sheath. Ascomata stain the woody substrate purple.
Type species: *Murispora rubicunda* (Niessl) Yin. Zhang, J. Fourn. & K.D. Hyde.

Note: The studied specimens from which the cultures were obtained are identified in the sense used by Webster (1957), who studied the type specimens, while they might be referred to *Pleospora rubelloloides* sensu Crivelli (1983).

**Murispora rubicunda** (Niessl) Yin. Zhang, J. Fourn. & K.D. Hyde, comb. nov. MycoBank MB515477.

**Basionym:** *Pleospora rubicunda* Niessl, Notiz. Pyr.: 31. 1876.
≡ *Massariosphaeria rubicunda* (Niessl) Crivelli, Über die Heterogene AscomycetenGattung Pleospora Rabh.: 144. 1983.
≡ *Karstenella rubicunda* (Niessl) M.E. Barr, N. Amer. Fl., Ser. 2 (New York): 52. 1990.

**Neomassariosphaeria** Yin. Zhang, J. Fourn. & K.D. Hyde, gen. nov. MycoBank MB515473.

**Etymology:** “Neo-” meaning “new”, named after its similarity with *Massariosphaeria*.

Aquatic. Saprophyticus. Ascomata dispergere vel gregariculus, immersa vel parim immersa. Apex productum. Peridium exilis. Trabeulae, hyalinae vel gelatinosa praeditae. Substratum plerumque purpureus.

Aquatic. Saprobic. Ascomata scattered or in small groups, immersed to erumpent, subglobose to lenticular; wall black, apex elongated. *Peridium* thin. *Pseudoparaphyses* trabeculate, embedded in mucilage. *Asci* 8-spored, bitunicate, fissitunicate, breve pedicellati. *Ascosporae*, peranguste fusiforme, multiseptatae, hyalinae vel rufobrunneus, tunica circumdatae. *Asci*, 8-spori, clavati vel late clavati, fissitunicati, breve pedicellati.

**Neomassariosphaeria** Yin. Zhang, J. Fourn. & K.D. Hyde, comb. nov. MycoBank MB515478.

**Basionym:** *Leptosphaeria typhicola* P. Karst., Bidrag Kännedom Finlands Natur Folk. 23: 100. 1873.
≡ *Phaeosphaeria typhicola* (P. Karst.) Hedg., Sydowia 22: 86. 1969.
≡ *Massariosphaeria typhicola* (P. Karst.) Leuchtm., Sydowia 37: 168. 1984.
≡ *Chaetomastia typhicola* (P. Karst.) M.E. Barr, Mycotaxon 34: 514. 1989.

**Neomassariosphaeria grandispora** (Sacc.) Yin. Zhang, J. Fourn. & K.D. Hyde, comb. nov. MycoBank MB515478.

**Basionym:** *Leptosphaeria grandispora* Sacc., Michelia 1: 341. 1878.
≡ *Metasphaeria grandispora* (Sacc.) Sacc., Syll. Fung. 2: 181. 1883.
≡ *Massariosphaeria grandispora* (Sacc.) Leuchtm., Sydowia 37: 172. 1984.
≡ *Lophiotrema grandispora* (Sacc.) Shoemaker & C.E. Babc., Sydowia 37: 172. 1989.

Notes: Although the living habit of *Neomassariosphaeria grandispora* (CBS 613.86) can not be clarified, the fresh water habit of species under this clade seems characteristic (see comments by Zhang et al. 2009a). In addition, the ascomata of telemorphs usually stain the woody substrate purple. Their morphological characters, however, vary greatly. For instance, *Amniculicola* species have cylindrical asci, while *N. grandispora*, *N. typhicola* and *Murispora rubicunda* have clavate asci. *Amniculicola* species have hyaline, fusiform 1- or rarely 3-septate ascospores, while the ascospores of *N. typhicola* and *N. grandispora* are narrowly fusiform and multisepate, but ascospores of *N. typhicola* are brown and *N. grandispora* are hyaline. The ascospores of *M. rubicunda* are brown and muriiform. Based on their phylogenetic affinity and morphological distinctions, two new genera, i.e. *Murispora* (based on *Pleospora rubicunda*) and *Neomassariosphaeria* (based on *Massariosphaeria typhicola*) and a new family, *Amniculicolaceae*, are introduced.

**Clade XII Lophiostomataceae (uncertain)**

The *Lophiostomataceae* comprises some *Lophiostoma* species, such as *L. caullum*, *L. semiliberrum*, *L. arundinis*, *L. compressum*, *L. viridarium* and *L. macrostomoides* (MLB = 100 %, JK = 89 %) while *L. fuckelii* is basal (MLB = 94 %, JK = 77 %), as previously reported (Tanaka & Hosoya 2008, Zhang et al. 2009b).

Traditionally, *Lophiostomataceae* comprised some other genera with various morphological characters, such as *Entodesmium* and *Lophionema* with filiform ascospores, and *Herpoticinia* and *Lophiotrema* with fusiform, brown or hyaline, 1-septate ascospores are usually multisepate when senescent (Sivanesan 1984, Holm & Holm 1988). The present phylogeny does not support their placement in *Lophiostomataceae*. The paragynetic nature of *Lophiostomataceae* has been previously noted (Schoch et al. 2006), and Clade XII is likely to represent the narrow concept of *Lophiostomataceae*, although it is still too early to draw this conclusion until verified sequences of the generic type of *Lophiostoma* (L. *macrostomum*) are obtained (see comments by Zhang et al. 2009b).

Geographically, most species used in this study are from European locations such as Switzerland (*Lophiostoma caullum*, *L. arundinis* and *L. compressum*), Sweden (*L. semiliberrum*) and France (*L. viridarium*, *L. compressum* and *L. macrostomoides*). *Lophiostoma fuckelii*, the only strain from South Africa, diverged earlier than all other members (Fig. 1).

**Lophiostomataceae** s. str. Sacc., Syll. Fung. 2: 672. 1883. emend.

Terrestrial or aquatic habitat. Saprobic. Ascomata perithecoid, medium to large-sized, solitary or scattered, immersed to erumpent or rarely superficial with protruding, compressed papilla and slit-like ostioles. *Pseudoparaphyses* numerous, narrowly cellular. Asci cylindrical to cylindro-clavate, with short pedicels. Ascosporae fusiform to narrowly fusiform, and mostly multisepate and heavily pigmented, sometimes with longitudinal septa in one or two cells, rarely 1-septate and hyaline, with or without sheath.

Currently accepted genus: *Lophiostoma* s. str.

Anamorphs: Reported as *Pleurophomopsis*-like (Leuchtmann 1985).

**Clade XIII Massariaceae**

The well-supported clade of the *Massariaceae* comprises the generic type of *Massaria* (*M. inquinans*) as well as species of *Roussosella* and *Arthopyrenia* that form a robust clade. The phylogeny in Fig. 1 includes the generic type of *Massaria — M. inquinans*. Morphologically, all of them have immersed ascomata,
pseudoparaphyses from abundant to rare, asci from cylindrical to clavate, ascospores from hyaline to reddish-brown, 1- or 3-septate.

Traditionally, Massariaceae (Melanommatales) is defined as having large ascomata, a peridium comprising compact, small cells, trabeculate pseudoparaphyses, large, and symmetric distoseptate ascospores usually surrounded with a sheath (Barr 1979). Based on these characters, six genera were included, i.e. Aglaospora, Caryospora, Dothivalaria, Massaria, Titanella and Zopfia (Barr 1979). Massaria inquinans and Aglaospora profusa are the generic types of Massariaceae and Aglaospora respectively, and they share numerous morphological characters, such as the large, immersed ascomata, trabeculate pseudoparaphyses, cylindrical asci with large and conspicuous apical rings and large, reddish-brown, 3-distoseptate ascospores (Shoemaker & Leclair 1975). The phylogenies here exclude the placement of Aglaospora under Massariaceae, and the placement of other four traditional genera under Massariaceae, i.e. Caryospora, Dothivalaria, Titanella and Zopfia can not be verified here either.

**Massariaceae** Nitschke, Verh. Naturhist. Vereines Preuss. Rheinl. 26: 73. 1869.

*Note*: Members of this clade are mostly saprobic.

**Currently accepted genera**: ? Arthopyrenia, Massaria, ? Roussoeilla.

**Anamorph**: ? *Torula herbarum*.

**Clade XIV**

The current phylogenetic data show that *Lophiotrema* as well as the generic types of *Lophiotrema* (*L. nucula*), *Verruculina* (*V. enalii*), *Ulospora* (*U. bilgramii*), *Lepidosphaeria* (*L. nicotiae*) and *Xenolophium* (*X. applanatum*) cluster apart from the clade of *Lophiotomataceae s. str*. Members of this clade are all saprobos, but have diverse morphological characters. *Lophiotrema* was introduced as a genus closely related to *Lophiostoma*, but having hyaline ascospores, and was assigned to *Lophiotomataceae* (Saccardo 1878, Holm & Holm 1988). The relatively smaller ascomata, peridium of almost equal in thickness, and the hyaline, 1-septate ascospores have been used to distinguish *Lophiotrema* from *Lophiostoma* (Holm & Holm 1988, Yuan & Zhao 1994, Kirk *et al*. 2001). The peridium concept, however, is not supported by the lectotype specimen, which has a flattened, thin-walled base (Zhang *et al*. 2009b). Species with brown ascospores are found in *Lophiotrema* based on molecular phylogenetic results (Zhang *et al*. 2009b).

*Lepidosphaeria*, *Ulospora* and *Verruculina* are all genera of the *Testudinaceae*, which is characterised by the cleistothecioid ascomata, 1-septate, brown, glabrous or ornamented ascospores (von Arx 1971). The size, shape and ornamentation of the ascospores serve as the distinguishing character between different genera (von Arx 1971, von Arx & Müller 1975, Hawksworth 1979). Based on the present phylogenetic result, these three genera of *Testudinaceae* are closely related. In addition, the non-ostiolate ascomata of the *Testudinaceae* provides evidence that taxa with cleistothecioid fruiting bodies have evolved from taxa with perithecioid ones in the *Pleosporales*.

The diverse morphological characters possessed by members of clade XIV might indicate that they are from more than one family. A more firmly stated hypothesis can only be obtained by further phylogenetic study which should include more genera and related species.

**Lophiotrema** Sacc., Michelia 1: 338, 1878. *emend*.

*Saprobic*. *Ascomata* perithecioid, mostly immersed, rarely erumpent; globose, subglobose or ovoid. *Hamathecium* of broadly to narrowly trabeculate or cellular pseudoparaphyses, persistent. *Asci* bitunicate, fissitunicate, cylindrical to clavate. Ascospores mostly hyaline, rarely brown, 1-septate, smooth.

**Anamorph**: unknown.

**Lophiotrema neoarundinaria** (Ellis & Everh.) Yin. Zhang, Kaz. Tanaka & K.D. Hyde, *comb. nov*. MycoBank MB515475. Basionym: *Didymosphaeria arundinariae* Ellis & Everh., N. Amer. Pyren. (Newfield): 732. 1892.

≡ *Microthelia arundinariae* (Ellis & Everh.) Kuntze, Revis. gen. pl. (Leipzig) 3: 498. 1896.
≡ *Massarina arundinariae* (Ellis & Everh.) M.E. Barr, Mycotaxon 45: 211. 1992.
≡ *Lophiostoma arundinariae* (Ellis & Everh.) Aptroot & K.D. Hyde, in Hyde, Wong & Aptroot, Fungal Divers. Res. Ser. 7: 107. 2002.

*Note*: To avoid the duplication with *Lophiotrema arundinariae* Rehm, a new name – *Lophiotrema neoarundinaria* is proposed here.

**Lophiotrema rubi** (Fuckel) Ying. Zhang, C.L. Schoch & K.D. Hyde, *comb. nov*. MycoBank MB515476. Basionym: *Massaria rubi* Fuckel, Jahrb. Nassauischen Vereins Naturk. 25–26: 303. 1871.

≡ *Massarina rubi* (Fuckel) Sacc., Syll. Fung. (Abellini) 2: 155. 1883.
≡ *Didymelina rhaphithamni* Keissl., Nat. Hist. Juan. Fernandez Easter Lsl. 2: 480. 1927.
≡ *Mycosphaerella rhaphithamni* (Keissl.) Petr., Ann. Mycol. 38: 221. 1940.
≡ *Massarina emergens* (P. Kunt.) L. Holm, Les Pleosporaceae: 149. 1957.
≡ *Lophiostoma rubi* (Fuckel) E.C.Y. Liew, Aptroot & K.D. Hyde, Mycologia 94: 812. 2002.

**Clade XV Aigialaceae**

The generic type of *Aigialus* (*A. grandis*) and *Lophiostoma mangrovei* form a well-supported cluster, which represents a marine pleosporalean family, *Aigialaceae*. This new family is addressed by Suetrong *et al*. (2009; this volume).

**Clade XVI Delitschiaceae**

The generic type of *Delitschia* (*D. didyma*) and *D. winteri*, represent *Delitschiaceae* and form a robust clade that diverges before all other members of *Pleosporales*. The *Delitschiaceae* is a small group of coprophilous fungi, which comprises three genera (i.e. *Delitschia*, *Ohleriella* and *Semidelitschia*) and 54 species (Barr *et al*. 2008). This family was introduced to accommodate coprophilous pleosporalean species with periphysate ostiole, wide ascus endotunica, conspicuous apical ring and heavily pigmented 1- to multisepitate ascospore with germ slits in each cell (Barr 2000).

The presence of a large ocellar chamber with an apical ring in the ascus is the most striking character of most members of *Delitschiaceae* as well as species in clade XVII, *Aglaospora profusa*. These two clades are consistently the earliest diverging lineage in *Pleosporales* as in several other phylogenies (Kruys *et al*. 2006, Schoch *et al*. 2006).
Fig. 2. A. Xenolophium applanatum. Ascomata on the host surface. Note the slit-like ostiole. B. Trematosphaeria pertusa. Ascomata on the host surface. Note the pore-like ostiole. C, E, H. Murispora rubicunda. C. Ascomata on the host surface. Note the purple woody substrate. E. Clavate 8-spored asci with short pedicels in pseudoparaphyses. H. Muriform ascospore with wide mucilaginous sheath. D. I. Trematosphaeria sp. D. Fusiform mature or immature 8-spored asci with pseudoparaphyses. I. Multiseptate dark brown ascospore. F. Neomassariosphaeria grandispora. Ascosporas with sheath. G. Aglospora profusa. Apical apparatus. Note the conspicuous apical ring. J. Amniculicola immersa. Hyaline fusiform ascospores in ascus. Scale bars; A–C = 100 μm, D–J = 20 μm.
SUMMARY

Phylogeny

The results presented here indicate that nutritional modes and environmental habits may have phylogenetic significance in Pleosporales, although more extensive statistical analyses remain to be done. Host spectrum (monocotyledon/dicotyledon) appears closely related to the phylogeny of plant associated fungi or plant pathogens (e.g. in Pleosporineae). Of the morphological characters, the size, shape and immersion degree of ascomata, ostiole characters and ascule shape can be of phylogenetic significance to varying degrees. The purple staining nature of the substrate found in some Amniculicolaceae might indicate that secondary metabolites have phylogenetic significance for this group.

However, even closely related species can exhibit diverse morphologies. Ascospores can vary from 1- to multiseptate to even muriform, hyaline to pigmented in many families, such as Amniculicolaceae (given as an example in Fig. 2), Lophiotomaceae s. str., Melanommataceae and Didymellaceae. From an evolutionary perspective, the “bipolar symmetrical ascospore tends to be correlated to passive dispersal”, and “the colour, size, shape and texture of spores should be viewed as probable functional adaptations modified in evolution by requirements of liberation, of flotation in fluids, and ultimately of deposition and survival” (Ingold 1971, Gregory 1973, Hawksworth 1987). Thus ascospore shape should be viewed as a highly adaptive character that can obscure underlying relationships.

Evolutionary trends

Most plant pathogens in Pleosporales belong to Pleosporineae, which tends to occupy the terminal branches on the Pleosporales tree (Fig. 1). On the other hand, a clade of coprophilous fungi — Delitschiaceae — consistently occurs as an early-diverged lineage compared to all other pleosporalean members, with numerous other saprotrophic members interspersed. Parasitic fungi are usually considered as “highly specialised”, and may require nutritional shifts from several other modes (Cain 1972, Heath 1987, Berbee 2001, Sung et al. 2008). This may indicate that Pleosporales originated from saprotrophic fungi, and that the transition from saprotrophic to necrotrophic and hemibiotrophic (or biotrophic) is likely, in agreement with earlier ideas (Lewis 1974, Cooke 1977, Cooke & Whipps 1986), also mirroring what is seen in the Capnodiales phylogeny (Crous et al. 2009a, Schoch et al. 2009a; this volume).

It is remarkable that as with the Delitschiaceae, Agleaspora profusa is also an early diverging lineage. Members of both Delitschiaceae and Agleaspora have a striking morphological character in having a large apical apparatus, which is rare in Pleosporales. According to the hypothesis of Hawksworth (1987), “……foremost of these trends is the loss of apical apparatus associated with a change from active to passive discharge of the ascospores…….”. Thus this striking apical apparatus might further indicate the plesiomorphic status of both Delitschiaceae and Agleaspora, supporting the premise that the ancestor of Pleosporales was saprobic with a well-developed apical ring.

Shortcomings and further work

Attempts to write a familial dichotomous key based on the present phylogenetic data has proven to be unsuccessful. The traditional keys rely on single morpho-characters, which are polyphyletic. Thus it appears to be impossible to find any single criterion which can be used to key out a family in such a way as to include all genera or species belonging to it, without incorporating the genus or species in several places in the key, as have been mentioned by Cain (1972).

Compared with the ca. 3 000 reported species in Pleosporales, the 130 species (< 5 %) used in present investigation are far from sufficient to obtain a comprehensive phylogenetic survey for the genetic diversity in the order, but will hopefully provide a framework for directing further work. Members of some families, such as Curvibatiriaecae and Diademaceae, are absent from our analysis, thus their status remains unresolved. In particular, erroneous strains or names in databases and culture collections necessitate verification, and circumscriptions of families within the clades currently remain preliminary. Importantly, this data set is geographically biased as most strains originated from temperate areas in the Northern Hemisphere, mainly Europe. Obtaining correctly identified fungal strains from various locations is crucial for further molecular phylogenetic investigations, necessitating the consistent analysis and interpretation of large taxon datasets.

It seems clear that most morphological criteria used by traditional taxonomy for Pleosporales at various taxonomic levels (such as genus or family) do not strictly correlate with distinct evolutionary groups. We will therefore have to rely on expanding our base of knowledge in ecology, biochemistry and other biological fields, to supplement the genetic information. The expected expansion in pleosporalean genome sequences makes this especially important.

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### SUPPLEMENTARY INFORMATION

Table 1. Isolates used in this study and their GenBank accession numbers. Name changes from their originals are indicated in brackets and newly generated sequences are indicated in bold.

| Classification | Species name                  | Culture/voucher | SSU    | LSU    | RPB1     | RPB2     | TEF1     |
|----------------|--------------------------------|-----------------|--------|--------|----------|----------|----------|
| Agialaceae     | Aigialus grandis               | JK 5244A        | GU296131 | GU301793 | GU371762 |          |          |
|                | Astrosphaeriella aggregata     | MAFF 239486     | AB524450 | AB524591 | AF242264 | AB539092 | AB539105 |
|                | Rimora mangrovei (as Lophiostoma mangrovei) | JK 5246A | GU296193 | GU301868 | GU371759 |          |          |
| Amniculicolaceae | Amniculicola immersa           | CBS 123083      | GU456295 | FJ795498 | GU456358 | GU456273 |          |
|                | Amniculicola lignicola         | CBS 123092      | EF493861 | EF493863 | EF493862 | EF456128 |          |
|                | Amniculicola parva             |                 | GU296134 | FJ795497 | GU349065 |          |          |
|                | Neomassariosphaeria grandispora | CBS 613.86   | GU296172 | GU301842 | GU371725 | GU371725 | GU349036 |
|                | Neomassariosphaeria tephicola  | CBS 123126      | GU296174 | FJ795004 | GU371795 |          |          |
|                | Muriispora rubicunda           | IFRD 2017       | GU456308 | FJ795507 | GU456289 |          |          |
| Delitschiaceae | Delitschia didyma 1            | UME 31411       | DQ384090 |          |          |          |          |
|                | Delitschia didyma 2 (duplicate) | UME 31411      | AF242264 | DQ384090 | DQ677975 | DQ677922 |          |
|                | Delitschia winteri             | CBS 225.62      | DQ678026 | DQ678077 | DQ677975 | DQ677922 |          |
| Didymellaceae  | Ascochyta pisi                 | CBS 126.54      | DQ678018 | DQ678070 | DQ677967 | DQ677913 |          |
|                | Didymella exigua               | CBS 183.55      | GU296147 | GU357800 | GU371764 |          |          |
|                | Didymella bryoniae             | CBS 133.96      | GU456335 | GU371767 |          |          |          |
|                | Leptosphaerulina argentinensis | CBS 569.94      | AY849947 | GU357859 | GU349008 |          |          |
|                | Leptosphaerulina australis 1   | CBS 311.51-T    | FJ795500 | GU345357 | GU456272 |          |          |
|                | Leptosphaerulina australis 2   | CBS 317.83      | GU296160 | GU301830 | GU371790 | GU349070 |          |
|                | Macroventuria arnomochea       | CBS 525.71      | AY878736 | GU456315 | GU456346 | GU456262 |          |
|                | Monasostroma innumerosum       | CBS 345.50      | GU296179 | GU301850 | GU349033 |          |          |
|                | Phoma complanata               | CBS 268.92      | EUT54081 | EUT54180 | GU371778 | GU349078 |          |
|                | Phoma exigua                   | CBS 431.74      | EUT54084 | EUT54183 | GU371789 | GU349080 |          |
|                | Phoma glomerata                | CBS 528.66      | EUT54184 | GU371781 | GU349081 |          |          |
|                | Phoma herbarum                 | CBS 276.37      | DQ678014 | DQ678066 | GU357792 | DQ677962 | DQ677909 |
|                | Phoma zeal-maydis              | CBS 588.69      | EUT54093 | EUT54192 | GU371782 | GU349082 |          |
|                | Platychora ulmi                | CBS 361.52      | EF114726 | EF114702 |          |          |          |
| Lentitheciaceae | Katumotoa bambusicolor         | JCM 13131, MAFF | AB524454 | AB524595 | AB539095 | AB539108 |          |
|                | Keissleriella cladophila        | CBS 104.55      | GU296155 | GU301822 | GU371735 | GU349043 |          |
|                | Lentithecium aquaticum         | CBS 123099      | FJ795477 | FJ795434 | FJ795455 | GU349068 |          |
|                | Lentithecium arundinaceum 1    | CBS 123131      | GU456298 | GU456320 | GU456281 |          |          |
|                | Lentithecium arundinaceum 2    | CBS 619.86      | DQ813513 | DQ813599 | FJ795473 |          |          |
|                | Lentithecium fluviatile(as Massarina fluviatile) | CBS 122367  | FJ795493 | GU456290 |          |          |          |
|                | Ophiopsphaerella sasicala      | JCM 13134, MAFF | AB524458 | AB524599 | AB539098 | AB539111 |          |
|                | Stagonospora macropycnidia     | OSC 100965      | GU296198 | GU301873 | GU349026 |          |          |
|                | Wettsteinin acidus             | CBS 618.86      | DQ678023 | DQ677972 | DQ677919 |          |          |
| Leptosphaeriaceae | Coniothyrium palmarum         | CBS 400.71      | DQ678008 | DQ677653 | DQ677956 | DQ677930 |          |
|                | Leptosphaeria biglobosa        | CBS 303.51      | GU301826 | GU349010 |          |          |          |
|                | Leptosphaeria dolichum         | CBS 505.75      | GU296159 | GU349096 |          |          |          |
|                | Leptosphaeria drysia           | CBS 643.86      | GU301828 | GU349009 |          |          |          |
|                | Leptosphaeria maculans         | DAOM 229267     | DQ470993 | DQ470946 | DQ471136 | DQ471062 |          |
|                | Neophaeosphaeria filamentososa | CBS 102202      | GU387516 | GU387577 | GU371773 | GU349084 |          |
| Classification               | Species name       | Culture/voucher | SSU            | LSU            | RPB1      | RPB2      | TEF1      |
|-----------------------------|--------------------|----------------|----------------|----------------|-----------|-----------|-----------|
|                            | Phoma heteromorphospora | CBS 115.96  | EU754089       | EU754188       | GU371775  | GU349077  |
|                            | Pyrenochaeta nobilis  | CBS 407.76   | DQ678096       | DQ677991       | DQ677936  |
| Lophiostomataceae s. str.  | Lophiostoma arundinis  | CBS 621.86   | DQ782383       | DQ782384       | DQ782386  | DQ782387  |
|                            | Lophiostoma caulum 1  | CBS 623.86   | FJ795479       | FJ795436       | FJ795456  |
|                            | Lophiostoma caulum 2  | CBS 624.86   | GU301832       |               |           |           |           |
|                            | Lophiostoma compressum 1  | IFRD 2014   | FJ795486       | FJ795437       | FJ795457  |
|                            | Lophiostoma compressum 2  | IFRDCC2081  | GU456321       |               |           |           |           |
|                            | Lophiostoma crenatum  | CBS 629.86   | DQ678017       | DQ678069       | DQ677965  | DQ677912  |
|                            | Lophiostoma fuckelii  | CBS 101952   | FJ795496       | DQ399531       | FJ795472  |
|                            | Lophiostoma macrostomoides  | CBS 123097  | FJ795482       | FJ795439       | FJ795458  |
|                            | Lophiostoma semiliberum  | CBS 626.86   | FJ795484       | FJ795441       | FJ795460  |
|                            | Lophiostoma viridarium  | IFRDCC2090  | FJ795486       | FJ795443       | FJ795468  |
| Massariaceae                | Arthopyrenia salicis 1  | CBS 368.94   | AY538333       | AY538339       | GU371814  |
|                            | Arthopyrenia salicis 2  | 1994Coppins  | AY607730       |               |           |           |           |
|                            | Massaria inquinans  | CBS 122369   | GU456300       | GU456322       | GU456282  |
|                            | Pleosporales sp. 1 (as Thelenella luridella)  | CBS 101277  | GU456309       |               |           |           |           |
|                            | Roussella hystericoides 1  | JCM 13126, MAFF 239636 | AB524480 | AB524621 | AB539101 | AB539114 |
|                            | Roussella hystericoides 2  | CBS 125434  | AB524481       | AB524622       | AB539102 | AB539115 |
|                            | Roussella postulans  | JCM 13127, MAFF 239637 | AB524482 | AB524623 | AB539103 | AB539116 |
|                            | Roussellopsis tosaensis  | NBRC 106245  | AB524625       |               | AB539104 | AB539117 |
|                            | Torula herbarum  | CBS 379.58   | GU456362       |               |           |           |           |
| Massarinae                  | Byssothecium ciricnans  | CBS 675.92   | AY016339       | AY016357       | DQ767646  |
|                            | Massarina cisti  | CBS 266.62   | FJ795490       | FJ795447       | FJ795464  |
|                            | Massarina elburnea  | CBS 473.64   | AF164367       | FJ795449       | FJ795466  |
|                            | Massarina igniaria  | CBS 845.96   | FJ795494       | FJ795452       | FJ795469  |
|                            | Neotiosporina papall  | CBS 331.37   | EU754073       | EU754172       | GU371779  | GU349079  |
| Melanomataceae              | Beveneykella pulmonaria  | CBS 283.53   |                  | GU301804       | GU371768  |
|                            | Herpotrichia diffusa  | CBS 250.62   | DQ678019       | DQ678071       | DQ677968  | DQ677915  |
|                            | Herpotrichia juniperi  | CBS 202.31   | DQ678029       | DQ678080       | DQ677978  | DQ677925  |
|                            | Melanomma pulvis-pyrius 1  | CBS 109.77  | FJ201987       | FJ201986       | GU456359  | GU456274  |
|                            | Melanomma pulvis-pyrius 2  | CBS 124080  | GU456302       | GU456323       | GU456350  | GU456265  |
|                            | Monotosporella tuberculata  | CBS 256.84  | GU301851       |               | GU349006  |
|                            | Pleomassaria siparia  | CBS 279.74   | DQ678027       | DQ678078       | DQ677976  | AY544726  |
| Sporomiaceae                | Preussia funiculata  | CBS 659.74   | GU296187       | GU301864       | GU371799  | GU349032  |
|                            | Preussia lignicola (as Sporormia lignicola)  | CBS 264.69  | GU296197       | GU301872       | GU371765  | GU349027  |
|                            | Preussia terricola  | DAOM 230091  | AY544726       | AY544686       | DQ471137  | DQ470895  | DQ471063  |
|                            | Sporormiella minima  | CBS 524.50   | DQ678003       | DQ678056       | DQ677950  | DQ677897  |
|                            | Westerdykella cylindrica  | CBS 454.72  | AY016355       | AY004343       | DQ471168  | DQ470925  | DQ497610  |
|                            | Westerdykella ornata  | CBS 379.55   | GU296208       | GU301880       | GU371803  | GU349021  |
| Montagnulaceae              | Bimunia novae-zelandiae  | CBS 107.79   | AY016338       | AY016356       | DQ471159  | DQ470917  | DQ471087  |
|                            | Didymocrea sadasivani  | CBS 438.65   | DQ384066       | DQ384103       |           |           |           |
|                            | Kalmusia brevispora 1  | NBRC 106240  | AB524459       | AB524600       | AB539100  | AB539113  |
|                            | Kalmusia brevispora 2  | MAFF 239276  | AB524460       | AB524601       | AB539099  | AB539112  |
|                            | Kalmusia scabrispora 1  | NBRC 106237  | AB524453       | AB524594       | AB539094  | AB539107  |
Table 1. (Continued).

| Classification | Species name | Culture/voucher | SSU            | LSU            | RPB1         | RPB2         | TEF1         |
|----------------|--------------|----------------|----------------|----------------|--------------|--------------|--------------|
| **Pleosporaceae** | Aleuca eureka | DAOM 192275    | DQ677984       | DQ678044       | DQ677838     | DQ677883     |
|                | Alternaria alternata | CBS 916.96     | DQ678031       | DQ678082       | DQ677980     | DQ677927     |
|                | Alternaria maritima | CBS 126.60     | GU456294       | GU456317       | GU456347     |
|                | Cochliobolus heterostrophus | CBS 134.39    | AY544727       | AY546465       | DQ477990     | DQ477603     |
|                | Cochliobolus sativus | DAOM 226212    | DQ677985       | DQ678045       | DQ677939     |
|                | Phoma betae | CBS 109410     | EU754079       | EU754178       | GU371774     | GU349075     |
|                | Pyenophora herbarum | CBS 714.68     | DQ767648       | DQ768049       | DQ71163      | DQ677888     |
|                | Pyenophora phaeocomes | DAOM 222769    | DQ499565       | DQ499596       | DQ497614     | DQ497607     |
|                | Pyenophora tritici-repentis 1 (as Pyenophora trichostoma) | OSC 100066 | AY544672       |               |              | DQ677882     |
|                | Pyenophora tritici-repentis 2 (as Pyenophora trichostoma) | CBS 392.54    |               |               |              | GU349017     |
|                | Pyenophora tritici-repentis 3 | CBS 328.53    |               |               |              | GU349029     |
|                | Scolobasidium arenarium (as Dendryphiella arenaria) | CBS 181.58    | DQ471022       | DQ470971       | GU349071     | DQ470924     | DQ677890     |
|                | Setosphaeria monoceras | CBS 154.26    | AY016352       | AY016368       |              |              |              |
|                | Scolecobasidium arenarium | CBS 110217    | GU296196       | GU301871       | GU371800     | GU349028     |              |

**Phaeosphaeriaceae**
| Classification | Species name | Culture/voucher | SSU     | LSU     | RPB1    | RPB2    | TEF1    |
|----------------|--------------|----------------|---------|---------|---------|---------|---------|
| Trematosphaeriaceae | *Asteromassaria pulchra* | CBS 124082 | GU296137 | GU301800 | GU371772 | GU349066 |
|                 | *Halomassarina thalassiae (as Massarina thalassiae)* | JK 5262D | GU301816 |         |         |         | GU349011 |
|                 | *Trematosphaeria pertusa 1* | CBS 122368 | FJ201991 | FJ201990 | FJ795476 | GU456276 |
|                 | *Trematosphaeria pertusa 2* | CBS 122371 | FJ201992 | FJ201993 | GU371801 | GU349085 |
| Pleosporales Incertae sedis | *Aglaospora profusa 1* | CBS 123109 | GU296130 | GU301792 |         |         | GU349062 |
|                 | *Aglaospora profusa 2* | CBS 123129 | GU456293 | GU456316 |         |         | GU456280 |
|                 | *Byssolophis sphaerioides* | IFRDCC 2053 | GU296140 |         |         |         |         |
|                 | *Lepidosphaeria nicotiae* | CBS 101341 | DQ678067 | DQ677963 | DQ677910 |         |         |
|                 | *Lophiotrema brunneosorum* | CBS 123095 | FJ795487 | FJ795444 |         |         | GU349071 |
|                 | *Lophiotrema lignicola* | CBS 123094 | FJ795489 | FJ795445 |         |         | GU349072 |
|                 | *Lophiotrema neoarundinaria 1* | NBRC 106238 | AB524455 | AB524596 |         |         | GU339097 |
|                 | *Lophiotrema neoarundinaria 2* | MAFF 239461 | AB524456 | AB524597 |         |         | GU339096 |
|                 | *Massaria anomia* | CBS 591.78 | GU296169 | GU301839 | GU349062 |         | GU349073 |
|                 | *Massarina rubi* | CBS 691.95 | GU456301 |         |         |         |         |
|                 | *Massariosphaeria phaseospora* | CBS 611.86 | GU296173 | GU301843 | GU349074 |         |         |
|                 | *Munkvalssaria rubra* | CBS 109605 | GU456303 | GU456324 | GU456339 | GU456344 | GU456260 |
|                 | *Thyristaria rubronotata* | CBS 419.85 | GU301875 |         |         |         | GU349002 |
|                 | *Ulospora bilgramii* | CBS 110021 | DQ678025 | DQ677974 | DQ677921 |         |         |
|                 | *Valaria insitiva* | CBS 123098 | GU456310 | GU460204 |         |         | GU456264 |
|                 | *Valaria insitiva* | CBS 123125 | GU456311 | GU460205 | GU456353 | GU456268 |         |
|                 | *Verruculina enalia* | JK 5235A | DQ678028 | DQ677977 | DQ677924 |         |         |
|                 | *Xenolophium applanatum* | CBS 123123 | GU456312 | GU456329 | GU456354 | GU456269 |         |
|                 | *Xenolophium applanatum* | CBS 123127 | GU456313 | GU456330 | GU456355 | GU456270 |         |
| Botryosphaeriales (outgroup) | *Botryosphaeria dothidea* | CBS 115476 | DQ677998 | DQ678051 | GU357802 | GU371774 | DQ67637 |
|                 | *Botryosphaeria tsuage* | CBS 171.55 | DQ678009 | DQ678061 | GU357796 | GU371774 | DQ677904 |
|                 | *Guignardia gaultheriae* | CBS 447.70 | DQ678089 | DQ677987 |         |         | GU349071 |
|                 | *Guignardia bidwellii* | CBS 237.48 | DQ678034 | DQ678085 | GU357794 |         |         |
| Dothideales (outgroup) | *Dothidea hippochaës* | CBS 188.58 | U42475 | DQ678048 | GU357801 | GU371774 | DQ677887 |
|                 | *Phaeosclera dematicides* | CBS 157.81 | GU296184 | GU301855 | GU357764 | GU349047 |         |
|                 | *Dothidea samtuci* | DAO 123130 | AY544722 | AY544681 |         |         | GU349066 |
| Hysteriales (outgroup) | *Psilotogonium clavisporum* | CBS 123339 | FJ161157 | FJ167526 | FJ161124 | FJ161105 |         |
|                 | *Hysteriales sp. 1* | CBS 243.34 | GU456297 | GU456319 | GU456338 | GU456343 | GU456259 |
|                 | *Hysterinum angustatum* | CBS 236.34 | GU397359 | GU161180 | GU456341 |         |         |
| Jahnulales (outgroup) | *Jahnula seychellensis* | SS2113.1 | EF175644 | EF175665 |         |         |         |
|                 | *Jahnula aquatica* | R89-1 | EF175633 | EF175655 |         |         |         |
|                 | *Aliquandostipite khaoiyaiensis* | CBS 118232 | AF201453 | GU301796 |         |         | GU349048 |
| Mytilinidiales (outgroup) | *Mytilinidion andinense* | CBS 123562 | FJ161159 | FJ161199 | FJ161125 | FJ161107 |         |
|                 | *Lophium mytilinum* | CBS 269.34 | DQ678030 | DQ678081 | GU456342 |         | DQ677926 |
### Table 1. (Continued).

| Classification         | Species name         | Culture/voucher | SSU      | LSU      | RPB1     | RPB2     | TEF1     |
|------------------------|----------------------|-----------------|----------|----------|----------|----------|----------|
| Venturiaceae           | Venturia pyrina      | ATCC 38995      |          |          | EF114714 |          |          |
|                        | Venturia inaequalis  | CBS 476.61      |          | GU456336 |          | GU456288 |          |
|                        | Metacoleroa diciei   | medipc          |          |          |          |          |          |
| Arthoniomycetes        | Opegrapha dolomitica | DUKE 0047528    | DQ883706 | DQ883717 | DQ883714 | DQ883732 |          |
| (outgroup)             | Opegrapha varia      | DUKE 0047526    |          |          |          |          |          |
|                        |                      |                 |          | FJ772242 | FJ772243 | FJ772244 |          |

*Public culture collections and herbaria*: ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; DUKE: Duke University Herbarium Durham, North Carolina, U.S.A.; IFRD: International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunmin, People’s Republic of China; JCM: Japan Collection of Microorganisms, RIKEN BioResource Center, Japan; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; OSC: Oregon State University Herbarium, Corvallis, Oregon, U.S.A.; NBRC: National Institute of Technology and Evaluation, Chiba, Japan; UME: Umeå University Herbarium, Umeå, Sweden.