Are *Melanomma pulvis-pyrius* and *Trematosphaeria pertusa* congeneric?

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The neotypes of *Trematosphaeria pertusa* and *Melanomma pulvis-pyrius* have been examined and are fully described. New collections of *M. pulvis-pyrius* and *T. pertusa* with living cultures have been obtained and these are assigned as epitypes with ex-epitypes. Cellular pseudoparaphyses as compared to trabeculae were observed in the neotypes of *M. pulvis-pyrius* and *T. pertusa*. The traditional concept of *Melanommataceae* as based on *Melanomma* as having trabeculate pseudoparaphyses is therefore imprecise. This study confirms that *Melanomma* and *Trematosphaeria* clearly belong in *Pleosporales*, however inclusion at the family level is partially resolved. The type species of *Melanomma*—*M. pulvis-pyrius* and *Trematosphaeria*—*T. pertusa* fall into two separate well-supported groups. Both morphology and molecular data support the fact that they are separate genera. We present a new colour coding scheme to indicate robustness of phylogenetic trees with bold with light blue background representing type strains (e.g. holotypes, epitypes, isotypes), bold with yellow background representing fungi with verified vouchered specimens, red background representing doubtful strains and lack of a coloured background representing unverified GenBank accessions. We illustrate how this scheme can increase confidence in conclusions drawn from phylogenetics trees (e.g. in *Pleosporaceae* and *Botryosphaeriales*) and suggest that the fungal community use type, authentic or verified strains or deposit voucher specimens in public collections for sequences deposited in GenBank whenever possible. We recommend that the colour coding scheme for phylogenetic trees be adopted, with possible modification in future publications as it will improve understanding and reliability of the phylogenetic trees.

Key words: epitype, *Melanommataceae*, phylogeny, *Pleosporales*, trabeculae

Introduction

Melanomma pulvis-pyrius (Pers.) Fuckel (1870) is the type species of *Melanomma* Nitschke ex Fuckel (1870) and *Trematosphaeria pertusa* (Pers.) Fuckel (1870) is the type species of *Trematosphaeria* Fuckel (1870) (Winter, 1887; Clements and Shear, 1931; Boise, 1985). These genera were introduced by Fuckel (1870), and there has been much written comparing them. They share similar morphological characters in having brittle and heavily carbonized ascomata, and brown, phragmosporous, elliptical to fusiform ascospores (Samuels and Müller, 1979). The distinction between these genera is, however, not always considered obvious (Samuels and Müller, 1979).

Winter (1887) placed *Melanomma* in *Melanommataceae* and *Trematosphaeria* in *Amphisphaeriaceae*, however, these genera have usually been included in *Melanommataceae* by later authors (e.g. Barr 1979, 1990; Eriksson, 2005). Munk (1953, 1957) compared these genera and noted from Chesters' (1938) drawings that *Melanomma* and *Trematosphaeria* could be distinguished as *M. pulvis-pyrius* has a peridium of distinct *textura angularis*, while in *T. pertusa* it is indistinct. Holm (1957) distinguished *Mela-*

nomma from *Trematosphaeria* using three characters: 1) *Melanomma* has superficial to immersed, and subglobose to collabent ascomata, whereas in *Trematosphaeria* the ascomata are slightly to deeply immersed and pyriform to conical; 2) the peridium in *Melanomma* comprises pigmented, small-celled *textura angularis*, while in *Tremato-sphaeria* it is composed of several layers of irregular pigmented, thin-walled cells which are variable in size; and 3) ascospores of *Melanomma* are uniformly coloured, whereas in *Trematosphaeria* ascospores are lighter at their extremities. Luttrell (1973) considered that the degree of immersion of ascomata and shape and pigmentation of the ascospores could distinguish between these genera: *Melanomma* has rarely immersed ascomata, and brown, ellipsoidal to cuneiform or almost clavate concolorous ascospores, while *Tremato-sphaeria* has partially to wholly immersed ascomata, and brown, fusoid ascospores which are usually paler at the ends. Arx and Müller (1975) separated *Melanomma* from *Tremato-sphaeria* by ascomatal size and thickness of the ascomatal wall; *Trematosphaeria* having larger ascomata, up to 1 mm in diam., with "thick" walls.

Information concerning the anamorphs has been not helpful in distinguishing these genera. *Melanomma pulvis-pyrius*, *M. fusci-dulum* Sacc., *M. radicans* Samuels & E. Müll. and *Trematosphaeria heterospora* (De Not.) G. Winter have *Phoma*-like anamorphs (Chesters, 1938; Samuels and Müller, 1979), however the anamorphic stage of *T. pertusa* is unknown.

In an attempt to distinguish between these species, Samuels and Müller (1979) randomly studied one specimen of *M. pulvis-pyrius* and one of *T. pertusa* from ZT and made sections to reveal the peridial structure. They found similarities in peridial structure; the only major difference being in the width of the ascomatal base relative to that of the lateral wall. The specimen of *T. pertusa* had a much thinner ascomatal base than that of the lateral wall, while in *M. pulvis-pyrius* the base and lateral walls were about the same width and the base was flattened. Chesters (1938) however had described *M. pulvis-pyrius* as having a round and superficial base. Based on the above characters, Samuels and Müller (1979) were

convinced that *Melanomma* and *Tremato-sphaeria* were congeneric. Unfortunately, their conclusion was drawn from studies of collections other than the type material. Some important characters such as asci and pseudoparaphyses were also neglected; the significance of which have been re-evaluated and emphasized by other mycologists (Groenhart, 1965; Barr, 1976, 1979, 1987; Liew *et al.*, 2000).

This study deals with the types of *M. pulvis-pyrius* and *T. pertusa*, genera traditionally placed in the *Melanommataceae* (*sensu* Barr, 1990). Considering the confusion surrounding these genera we have examined the type specimens of *M. pulvis-pyrius* and *T. pertusa*. We also collected and isolated fresh material from France. These were confirmed to be morphologically identical with the type material of *M. pulvis-pyrius* and *T. pertusa* and thus assigned as epitypes. Since isolates of the type species of *Trematosphaeria* and *Mela-nomma* are newly available, we obtained DNA sequences to evaluate the phylogenic status of these two taxa.

Materials and methods
Sample collection and specimen examination
Fresh specimens of *Melanomma pulvis-pyrius* and *Trematosphaeria pertusa* were collected in Belgium and France in 2008 and 2004 respectively. In all cases ascomata were collected directly from natural wood without incubation. The samples were processed and examined following the method described by Tsui *et al.* (2000). Specimens were deposited in IFRD (epitypes). Type material of *M. pulvis-pyrius* and *T. pertusa* was also obtained from Uppsala University (UPS) and the National Herbarium Nederland, Leiden University Branch (L) respectively. Observations, mea-surements and photographs were prepared from squash mounts or sections in water or in 10% lactic acid. The terminology utilized here for types of pseudoparaphyses, trabeculate pseudoparaphyses and cellular pseudopara-physes follows Barr (1987), Eriksson (1981) and Hyde *et al.* (2000).

Fungal isolates and DNA extraction
Isolates were grown on potato dextrose agar (PDA) and malt extract agar (MEA) for

two to four weeks, and total genomic DNA was extracted from mycelia following the protocols as outlined by Cai *et al*. (2006) and Shenoy *et al*. (2007).

DNA amplification and sequencing

DNA amplification was performed by PCR. For partial large subunit (28S) nu-rDNA amplification, LROR and LR5 primers (Vilgalys and Hester, 1990) were used. Primer pairs NS1 and NS4 were used to amplify a region from the small subunit (18S) of the rDNA (White *et al.*, 1990). The amplification reaction for rDNA (18S and 28S) was performed in a 50 μl reaction volume as outlined by Jeewon *et al*. (2004) and Shenoy *et al*. (2007) respectively. The purified PCR products were sequenced 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

Multiple alignment was carried out in BioEdit (Hall, 2005) and Clustal X (Thompson *et al*. 1997) and analyses were performed in PAUP* 4.0b10 (Swofford, 2002). Maximum Parsimony (MP) was conducted using heuristic searches as implemented in PAUP, with the default options method. Clade stability was assessed in a bootstrap analysis with 1000 replicates, random sequence additions with maxtrees set to 1000 and other default parameters as implemented in PAUP*. Independent Bayesian phylogenetic analysis was performed in MrBayes 3.0 using a uniform GRT+I+G model, as selected by hLRT in Mrmodeltest 2.2. The Metropolis-coupled Markov chain Monte Carlo (MCMC) approach were used to calculate posterior probabilities. Chains were analyzed with random starting trees for 1,000,000 generations. Trees collected before the stable likelihood value point were discarded as "burn-in" (Kodsueb *et al*., 2006). Trees were viewed in Treeview (Page, 1996). The nucleotide sequences reported in this paper have been deposited in GenBank (Table 1). The dataset of a combined 18S rDNA and 28S rDNA was analysed in this study.

Colour coding of phylogenetic trees.

In this study we colour code the branches of the dengrograms to indicate better the robustness of trees as follows: taxa in bold with a light blue background represents the type strain (holotype / isotype / epitype), taxa in bold with a yellow background means the collection was confirmed by comparison with type material, taxa with red background representing doubtful strain (not used in this study) and lack of colour represent unverified GenBank accessions.

Results

Taxonomy
Melanomma pulvis-pyrius (Pers.) Fuckel, Jahrbücher des Nassauischen Vereins für Naturkunde 23-24: 160 (1870). (Fig. 1)

≡ *Sphaeria pulvis-pyrius* Pers., Synopsis Methodica Fungorum (Göttingen) 1: 86 (1801).
For other synonyms see:
http://www.indexfungorum.org (18 July 2008).
Description from neotype.

Ascomata 215-471 μm high and 260-440 μm diam., gregarious, superficial, globose, sub-globose, broadly or narrowly conical, often laterally flattened, wall black, roughened and irregular, often bearing remnants of wood fibres; apex short papillate, often somewhat puckered or sulcate (Figs 1A, B). *Peridium* 70-90 μm thick, to 180 μm thick at the base, coriaceous, two-layered, outer layer composed of small heavily pigmented thick-walled cells of *textura angularis*, apical cells smaller and walls thicker, individual cell walls to 6 μm thick, inner layer composed of lightly pigmented to hyaline thin-walled cells of *textura angularis*, 5-8 μm diam., individual cell wall to 1.5-2 μm thick, in places with columns of *textura prismatica*, and larger, paler cells of *textura prismatica* towards the interior and at the base (Fig. 1B). *Hamathecium* dense, filamentous, 1-2 (-2.5) μm broad, branching, rarely anastomosing, septate (Figs 4A-C). *Asci* 98-123 × 6.5-7.5 (-9) μm ($\bar{x} = 109 \times 7.5$ μm), 8-spored, with a short, furcate pedicel, to 25 μm long, bitunicate, dehiscence fissitunicate, cylindrical to fusiform with an ocular chamber (Figs 1C-G). *Ascospores* 14-17.5 (-19) × 4.5-6.5 μm ($\bar{x} = 15.8 \times 5.2$ μm), obliquely

Table 1. Species and sequences database accession numbers used in this study (newly generated sequences are indicated in bold).

Taxon	Source	Genbank Accession numbers	
		LSU	SSU
Alternaria alternate	CBS 916.96	DQ678082	DQ678031
Ascochyta pisi	CBS 126.54	DQ678070	DQ678018
Bimuria novae-zelandiae	CBS 107.79	AY016356	AY016338
Botryosphaeria dothidea	CBS 115476	DQ678051	DQ677998
Botryosphaeria ribis	CBS 115475	DQ678053	DQ678000
Botryosphaeria stevensii	CBS 431.82	DQ678064	DQ678012
Botryosphaeria tsugae	CBS 418.64	DQ767655	AF271127
Botryosphaeria viticola	CBS 117009	DQ678087	DQ678036
Byssothecium circinans	CBS 675.92	AY016357	AY016339
Clathrospora diplospora	IMI 68086	U43481	U43464
Cochliobolus heterostrophus	CBS 134.39	AY544645	AY544727
Cochliobolus sativus	DAOM 226212	DQ678045	DQ677995
Coniothyrium obiones	CBS 453.68	DQ678054	DQ678001
Coniothyrium palmarum	CBS 400.71	DQ767653	DQ678008
Cucurbitaria elongata	CBS 171.55	DQ678061	DQ678009
Delitschia didyma	UME 31411	DQ384090	AY853318
Delitschia winteri	CBS 225.62	DQ678077	DQ678026
Delphinella strobiligena	CBS 735.71	DQ470977	DQ471029
Dendryphiella arenaria	CBS 181.58	DQ470971	DQ471022
Diaporthe phaseolorum	FAU458	AY346279	AY779326
Didymella cucurbitacearum	IMI 373225	AY293792	AY293779
Dothidea insculpta	CBS 189.58	DQ247802	DQ247810
Dothidea ribesia	CBS 195.58	AY016360	AY016343
Dothidea sambuci	DAOM 231303	AY544681	AY544722
Dothiora cannabinae	CBS 737.71	DQ470984	DQ479933
Guignardia bidwellii	CBS 237.48	DQ678085	DQ678034
Guignardia gaultheriae	CBS 447.70	DQ678089	NS
Herpotrichia diffusa	CBS 250.62	DQ678071	DQ678019
Herpotrichia juniperi	CBS 200.31	DQ678080	DQ678029
Leptosphaeria doliolum	ATCC 32813	U43473	U43455
Leptosphaeria maculans	DAOM 229267	DQ470946	DQ470993
Lewia eureka	DAOM 195275	DQ678044	DQ677994
Lewia infectoria	IMI 303186	U43482	U43465
Lophiostoma arundinis	CBS 269.34	DQ782384	DQ782383
Lophiostoma caulium	CBS 623.86	DQ528763	U42485
Lophiostoma crenatum	CBS 629.86	DQ678069	DQ678017
Macrophomina phaseolina	CBS 227.33	DQ678088	DQ678037
Massarina eburnea	CBS 473.64	**FJ201983**	AF164367
Massariosphaeria grandispora	CBS 613.86	EF165034	EF165038
*Melanomma pulvis-pyrius**	IFRDCC 2044	**FJ201984**	**FJ201985**
Melanomma pulvis-pyrius	CBS 109.77	**FJ201986**	**FJ201987**
Melanomma pulvis-pyrius	CBS 371.75	**FJ201988**	**FJ201989**
Montagnula opulenta	CBS 168.34	DQ678086	AF164370
Ophiobolus fulgidus	ATCC 9556	U43472	U43454
Ophiosphaerella herpotricha	CBS 620.86	DQ678062	DQ678010
Ophiosphaerella herpotricha	CBS 240.31	DQ767656	DQ767656
Phaeodothis winteri	CBS 182.58	DQ678073	DQ678021
Phaeosphaeria avenaria	DAOM 226215	AY544684	AY544725
Phaeosphaeria eustoma	CBS 573.86	DQ678063	DQ678011
Platychora ulmi	CBS 361.52	EF114702	EF114726
Pleomassaria siparia	CBS 279.74	DQ678078	DQ678027
Pleospora herbarum	CBS 714.68	DQ678049	DQ767648

*Ex-epitypes designated in this study.
NS: no sequence available in GenBank.

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Table 1 (continued). Species and sequences database accession numbers used in this study (newly generated sequences are indicated in bold).

Taxon	Source	Genbank Accession numbers	
		LSU	SSU
Pleospora herbarum	CBS 191.86	DQ247804	AF382386
Preussia terricola	DAOM 230091	AY544686	AY544726
Pyrenophora phaeocomes	DAOM 222769	DQ499596	DQ499595
Pyrenophora tritici-repentis	OSC 100066	AY544672	AY544716
Setosphaeria monoceras	CBS 154.26	AY016368	AY016352
Sporormiella minima	CBS 524.50	DQ678056	DQ678003
Stylodothis puccinioides	CBS 193.58	AY004342	AY016353
Sydowia polyspora	CBS 116.29	DQ678058	DQ678005
Trematosphaeria heterospora	CBS 644.86	AY016369	AY016354
*Trematosphaeria pertusa**	CBS 122368	**FJ201990**	**FJ201991**
Trematosphaeria pertusa	CBS 122371	**FJ201992**	**FJ201993**
Westerdykella dispersa	CBS 50875	DQ468050	U42488

*Ex-epitypes designated in this study.
NS: no sequence available in GenBank.

uniseriate and partially overlapping, broadly fusiform to fusiform with broad rounded ends, straight or slightly curved, smooth, lightly pigmented, four-celled, slightly constricted at the septa, the second cell from the top slightly wider than the others, no sheath (Figs 1H-L).

Colonies (of epitype) reaching 4 cm diam after 20 days growth on PDA at 25°C, depressed to raised, cottony to woolly, with rhizoidal margin, grey, reverse darkened. *Phoma*-like anamorph has been reported by Chesters (1938) and Sivanesan (1984), but no anamorphic stage was observed in the cultures of IFRDCC 2044, CBS 109.77 and CBS 371.75 after culturing 3 months on PDA.

Specimens examined: **Neotype** (as *Sphaeria pulvis-pyrius* Pers.) Scler. suec. n. 120, UPS, on decaying wood, designated by Holm (1957), Barr (1990). **Epitype designated here** (IFRD 2001): FRANCE, Ariège, Rimont, Saurine, on bark of *Salix caprea*, 10 April 2008, Jacques Fournier, ex-epitype living culture deposited in the IFRD culture collection (IFRDCC 2044).

Notes: Persoon originally described this taxa as *Sphaeria pulvis* in 1794 (p. 27), and as *S. pulvis-pyrius* in 1801 (p. 86). Fries treated this species as early as 1817 (p. 259), and sanctioned it in 1823 (p. 458). According to Art. 9.1 of Vienna Code (2006, http://www.ibot.sav.sk/icbn/main.htm), the holotype of *S. pulvis-pyrius* would be a single collection designated by Persoon (1794, p. 27);no collection was designated by Persoon (1794, 1797, 1801), and relevant Persoon collections cannot be located. Fries (1823, p. 458) cited Scler. suec. n. 312 in his treatment of *S. pulvis-pyrius*. but Scler. suec. n. 312 had not been issued in 1823 (and was possibly never issued), and was subsequently assigned by Fries as a different taxon, *Sphaeria aurantia* (see comments by Holm, 1957; Holm and Nannfeldt, 1962), whereas Scler. suec. n. 120 is extant at UPS and is confirmed as *Sphaeria pulvis-pyrius*. Holm (1957) stated that Scler. suec. n. 120 should be chosen as the type material of *M. pulvis-pyrius*. In discussing the genus *Melanomma*, Barr (1990, p. 18) cited n. 120 as the "holotype" [an error for neotype] of *M. pulvis-pyrius* (Pers.: Fr.) Fuckel (UPS!).

We designate an epitype based on its similarity to the neotype. The only difference between the neotype and epitype specimens is the peridium, which is thicker in the neotype (70-90 vs. 35-60 µm) with a thickened base. However as Samuels and Müller (1979) point out, the "wall structure of *M. pulvis-pyrius* is sufficiently variable to present a different aspect from collection to collection". We corroborate this finding.

Trematosphaeria pertusa (Pers.) Fuckel, Jahrbücher des Nassauischen Vereins für Naturkunde 23-24: 161 (1870). (Fig. 2)

≡ *Sphaeria pertusa* Pers., Synopsis Methodica Fungorum (Göttingen) 1: 83 (1801).
For other synonyms see Chesters (1938).
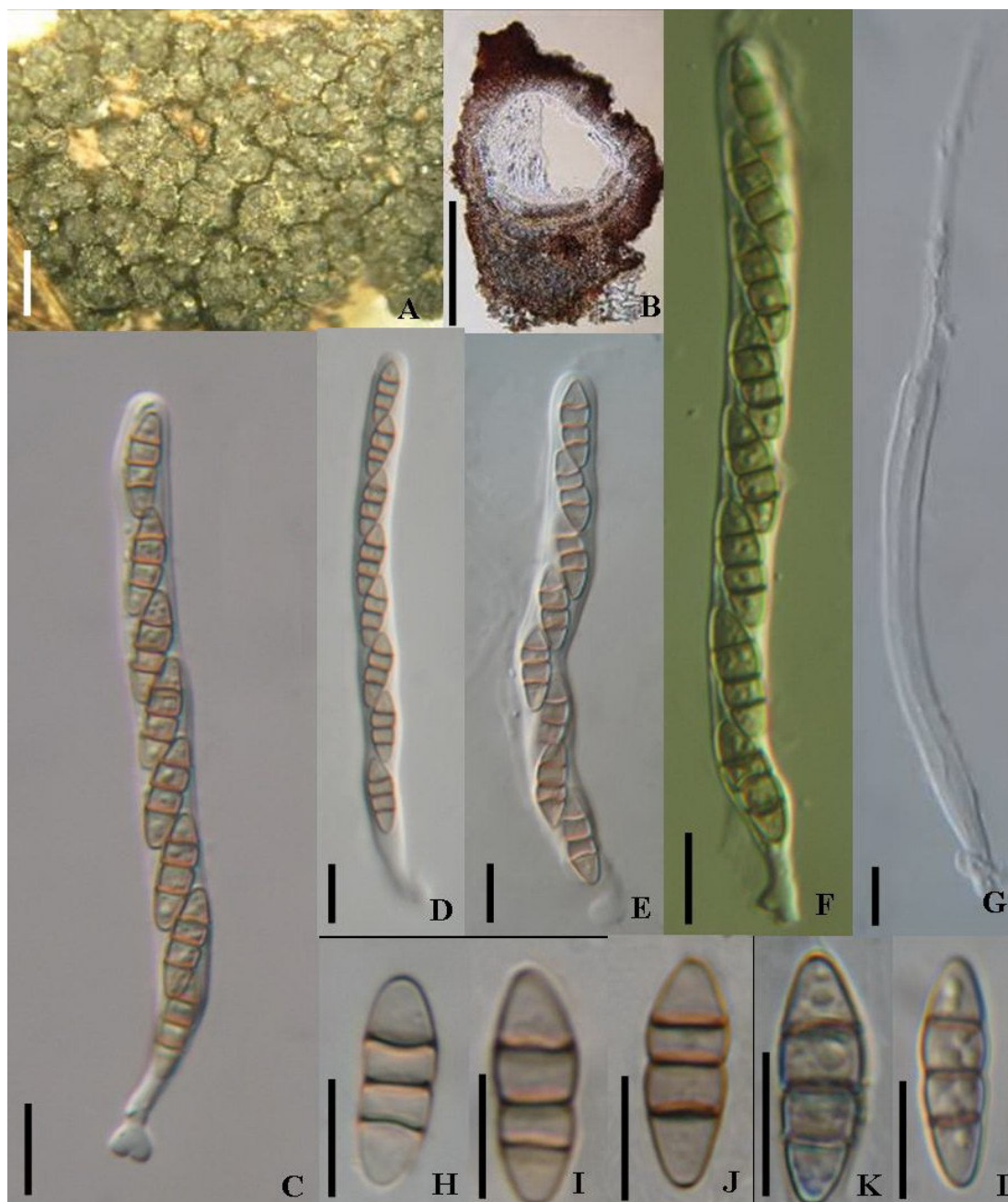Description from neotype.

Fig. 1. *Melanomma pulvis-pyrius* (in water). **A-B, D-E, H-J** from **neotype, C, G, K, L** from **epitype**. **A.** Ascoma on the host surface. **B.** Section of an ascoma. **C-F.** Ascus with pedicle. **G.** Dehiscent ascus. **H-L.** Ascospores. Scale bars: **A =** 500 µm**, B =** 200 µm**, C-L =** 10 µm.

Ascomata 350-550 µm high and 320-480 µm diam., solitary, scattered, or in groups, initially immersed, becoming erumpent to semi-immersed, subglobose, wall black; apex with a short ostiole usually slightly conical and widely porate, to 100 µm high (Figs 2A, B). *Peridium* 48-55 µm thick laterally, to 80 µm thick at the apex, thinner at the base, 30-40 µm thick, coriaceous, a single layer, composed of small heavily pigmented thick-walled cells of *textura angularis*, cells 4-8 µm diam, cell wall 1.5-3 µm thick in places with columns of *textura prismatica* orientated perpendicular to the ascomatal surface, apex cells smaller and walls thicker, forming thick-walled cells of *textura pseudoparenchymata*, and larger, paler cells of mixture of *textura epidermoidea* and *textura angularis* at the base, 10-25 µm (Figs 2B, C, H). *Hamathecium* dense, filamentous, 1.5-2.5 µm broad embedded in mucilage
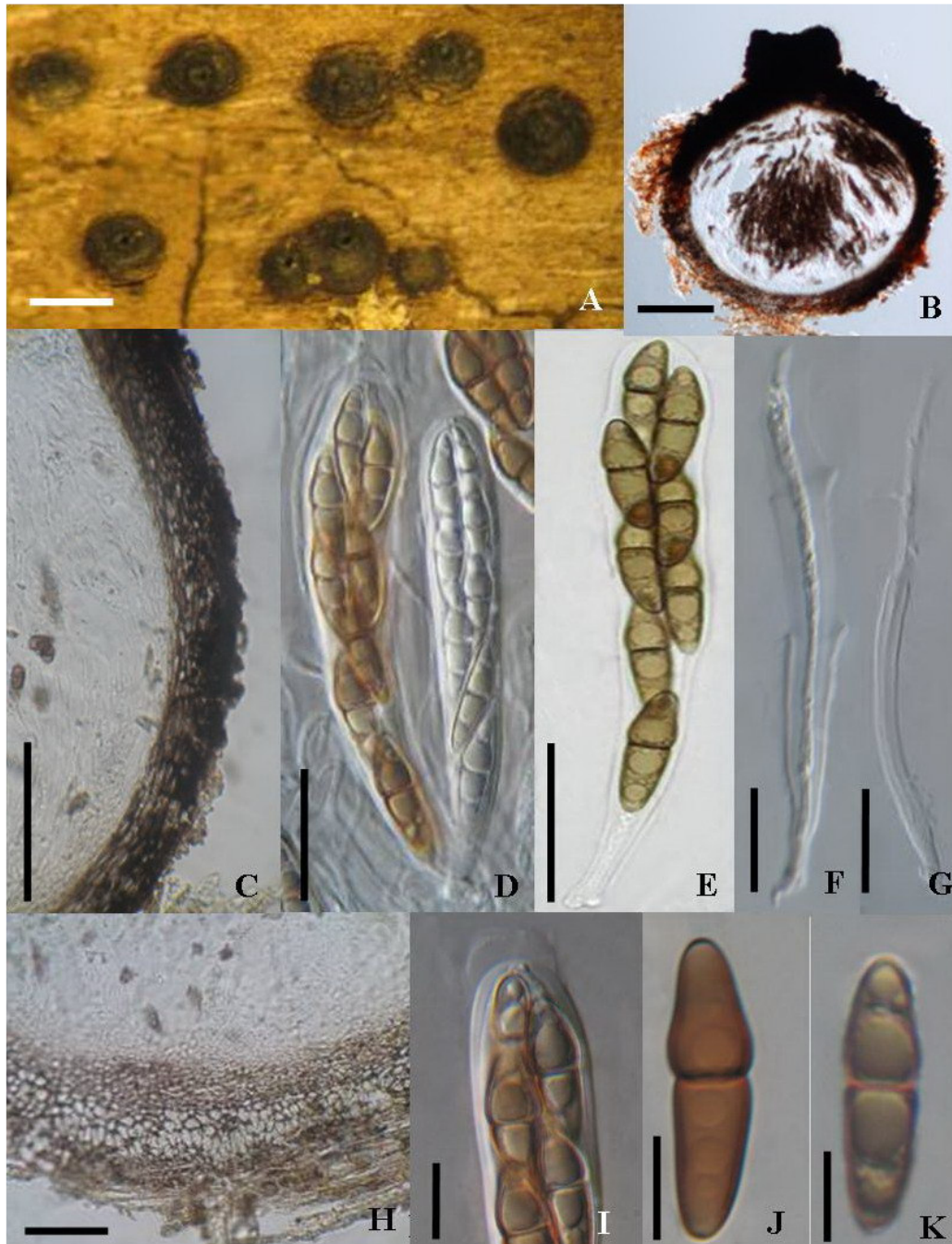
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Fig. 2. *Trematosphaeria pertusa* (in water). **A, D, F-I** from **epitype**, **B, C, E, J** from **neotype**. **A.** Ascoma on the host surface. **B.** Section of an ascoma. **C, H.** Section of the peridium. **D.** Asci in the pseudoparaphyses. **E.** Ascus with pedicle. **F, G.** Dehiscent ascus. **I.** Upper part of the ascus, showing the ocular chamber and the mucilage covering the apex. **J, K.** Ascospore. Scale bars: **A** = 0.5 mm, **B, C** = 100 µm, **D-H** = 20 µm, **I-K** = 10 µm.

branching and anastomosing between and above the asci, septate (Figs 4D-F). *Asci* 100-145 × 15-17 µm ($\bar{x}$ = 118 × 15.5 µm), 8-spored, bitunicate, dehiscence fissitunicate, clavate, with a short, thick, furcate pedicel which is 12-30 µm long, with a truncate ocular chamber (Figs 2D-G, I). *Ascospores* 27.5-32.5 × 7.5-8.5 µm ($\bar{x}$ = 29.5 × 8 µm), biseriate to uniseriate near the base, fusiform with broadly to narrowly rounded ends, dark brown, 1-3-septate, secondary septum forming late or often absent, deeply constricted at the median septum, the upper cell often shorter and broader than the lower one, smooth to finely

Fig. 3. Hyphopodia like structure of *Trematosphaeria pertusa* (in water). **A-D** from **ex-type**-CBS 122368. **A, B, D.** Nearly hyaline lobed hyphopodia like structures produced on side of hyphae. **C.** Light brown lobed hyphopodia like structures on side or tip of hypha. Scale bars = 10 µm.

verruculose, containing refractive globules (Figs 2J-K).

Colonies (of epitype) reaching 5 cm diam after 20 dyas growth on MEA at 25°C, raised, woolly, deep grey, with irregular to rhizoidal margin, reverse darkened. Hyphopodia-like structures (or conidia) produced after 6 months, hyaline to light-brown, lobed, 4-4.5 (-5) µm long and 3-3.5µm diam (Figs 3A-D).

Specimens examined**:** *Neotype* (as *Sphaeria pertusa* Pers.): EUROPE, Upsala, on decaying wood, designated by Boise (1985), L-Pers 910269-172; *Epitype designated here* (IFRD 2002): FRANCE, Deux Sèvres, Sansais, Le Vanneau, Les Grandes Mottines, swamp, on bark of a dead stump of *Fraxinus excelsior*, 25 April 2004, collected by Jacques Fournier, ex-epitype living culture deposited in CBS (CBS 122368).

Notes: The neotype (L-Pers 910269-172) and the newly designated epitype are indistinguishable. Boise (1985) provided a detailed description of the neotype, in which the asci and ascospores are consistent with those shown here. However, the diagram of the ascoma by Boise (Fig. 3) show them as having an almost flattened base and lacking a papillate ostiole, which is inconsistent with our observation of the neotype and the original description of this species (Persoon, 1801).

Molecular data

Phylogenetic analysis was carried out on sequence data comprising 2,264 bp from nrDNA (18S and 28S rDNA). There were 344 parsimony-informative characters. The out-group taxon was *Diaporthe phaseolorum*. The heuristic search with random addition of taxa (1000 replicates) and treating gaps as missing performed by PAUP* generated a single most parsimonious tree of length 1377 (CI = 0.509, RI = 0.828, RC = 0.421, HI = 0.491). Bayesian analyses resulted in a tree with similar topologies obtained from Maximum Parsimony (MP). We selected the MP tree to explain systematic relationships pertaining to *Melanomma pulvis-pyrius* and *Trematosphaeria pertusa*.

The maximum parsimony tree generated from our sequence analysis of the combined 18S and 28S rDNA dataset clustered into ten monophyletic clades (Fig. 5). Clade containing *Pleosporaceae*, *Phaeosphaeriaceae* and *Delitschiaceae* form a well supported monophyletic group with 97% MP bootstrap support and 100% Bayesian posterior probabilities (PP) support. Clade I with 96% MP bootstrap support and 100% Bayesin PP support, includes *Bimuria novae-zelandiae*, *Montagnula opulenta*, *Massarina eburnea*, *Phaeodothis winteri*, and *Trematosphaeria pertusa*. This group is basal to members of *Pleosporaceae*, *Phaeosphaeriaceae* and *Delitschiaceae*, and the node supporting them received a moderate Maximum Parsimony bootstrap value (77%) but a high Bayesian PP value (100%). The node supporting *Lophiostomataceae*, *Sporomiaceae* and group II receives no bootstrap
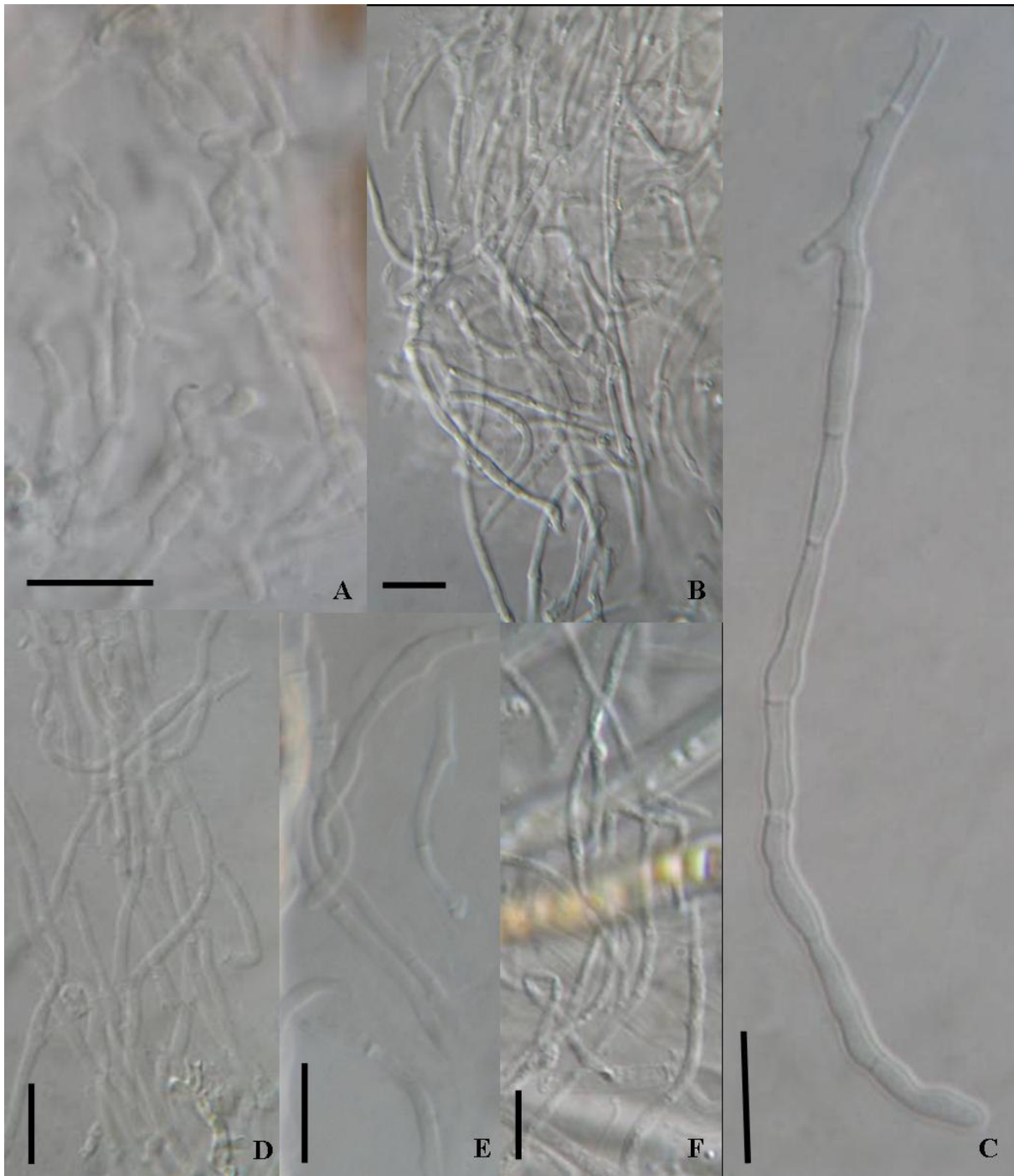
Fig. 4. Pseudoparaphyses (in 10% lactic acid). **A-C** *Melanomma pulvis-pyrius*, **D-F** *Trematosphaeria pertusa*. **A** from **neotype** of *M. pulvis-pyrius*, **B, C** from **epitype** of *M. pulvis-pyrius*, **D, E** from **neotype** of *T. pertusa*, **F.** from **epitype** of *T. pertusa*. Scale bars = 10 µm.

support; whereas they form three well supported monophyletic clusters respectively. Group II comprising *Melanomma pulvis-pyrius*, *Herpotrichia diffusa* (Schwein.) Ellis & Everh., *Herpotrichia juniperi* (Duby) Petr. and

Pleomassaria siparia (Berk. & Broome) Sacc. is well supported in both analyses (MP bootstrap = 100%, Bayesian PP = 100%). *Delitschiaceae* containing *Delitschia didyma* Auersw. and *Delitschia winteri* (W. Phillips &

Plowr.) Sacc. is monophyletic and strongly supported. The pleosporalean taxa above form a well supported monophyletic group (MP bootstrap value = 96%, Bayesian PP = 98%) (Fig. 5). Members of *Bortryosphaeriales* and *Dothideales* are also monophyletic and strongly supported in our study.

Of the 63 fungal strains used in this molecular work, 10 were type strains, and the correct identification of 14 (22 %) strains were verified by comparing with type specimens and voucher specimens deposited in CBS.

Discussion

In this study we have examined the morphological characters of the type specimens of *Melanomma pulvis-pyrius* and *Tremato-sphaeria pertusa* and analysed 18S and 28S rDNA sequence data from fresh specimens. Both morphological and molecular data indicate that *M. pulvis-pyrius* and *T. pertusa* belong to different genera, thus they are not congeneric.

Morphological data

There are several morphological differences between *T. pertusa* and *M. pulvis-pyrius* both in the types and subsequent collections. In *T. pertusa* ascomata are usually scattered, while those of *M. pulvis-pyrius* are gregarious. The ostiole of *T. pertusa* is distinctive and papilla-like with a wide opening, while ostioles of *M. pulvis-pyrius* are short and obscure and no wide opening occurs. The ascoma wall surface in *T. pertusa* is relatively smooth, while in *M. pulvis-pyrius* the peridium is roughened. The ascus is cylindrical in *M. pulvis-pyrius* and clavate in *T. pertusa*. Ascospores of *T. pertusa* are dark brown and one to three-septate, while those of *M. pulvis-pyrius* are lightly pigmented and three-septate.

Trabeculate pseudoparaphyses have been regarded as an important diagnostic character of the *Melanommataceae* (Sivanesan, 1984; Barr, 1990; Liew *et al.*, 2000; Kirk *et al.*, 2001). This family is represented by *Mela-nomma* and the type species is *M. pulvis-pyrius* (Chesters, 1938; Cannon and Kirk, 2008). Trabeculate pseudoparaphyses are defined as very narrow, remotely septate, branched

anastomosing filaments (Hyde *et al.*, 2000). However, no typical trabeculae were observed in the neotypes of *M. pulvis-pyrius* or *T. pertusa* and therefore the status of the *Melanommataceae* is doubtful. The phylogenic significance of pseudoparaphyses at the family level has been considered inconclusive (Silva-Hanlin and Hanlin, 1999; Liew *et al.*, 2000; Lumbsch and Lindemuth, 2001). However, whether they have taxonomic significance at the genus level is undetermined.

Phylogenetic analysis

Melanomma pulvis-pyrius and *Trematosphaeria pertusa* nested within the *Pleosporales* (Fig. 5) with strong support, but grouped in two separate well-supported sub-clades. *Trematosphaeria pertusa* forms a robust cluster with *Bimuria novae-zelandiae*, *Phaeodothis winteri*, *Montagnula opulenta* and *Massarina eburnea*, which form a sister group with *Pleosporaceae*, *Phaeosphaeriaceae* and *Delitschiaceae* with moderate bootstrap support. This cluster may represent a distinct family (e.g. *Massarinaceae*) but more taxa should be included to verify this. *Bimuria novae-zelandiae*, *Montagnula opulenta* and *Phaeodothis winteri* form a subclaster with higher bootstrap support (MP = 84%, PP = 100%). Initially, both *Montagnula opulenta* and *Phaeodothis winteri* were accommodated in *Didymosphaeria* which is characterized by the brown 1-septate ascospores, but were transferred to other genera by Aptroot (1995). *Bimuria novae-zelandiae* (CBS 107.79, type strain) produced ascomata in culture medium, in which cellular pseudoparaphyses were detected. The strain of *Massarina eburnea* (CBS 473.64) is correctly identified as it clustered with 100% bootstrap support with a new strain that we isolated and compared with the type (data not shown). Strains of *Montagnula opulenta* (CBS 168.34) and *Phaeodothis winteri* (CBS 182.58) could not be verified as no voucher specimens examined. *Trematosphaeria pertusa* is comparable with *Kirschsteiniothelia aethiops*, which served as the type species of *Kirschsteiniothelia*. Unfortunately, the only strain of this taxon sequenced is CBS 109.53 and this clustered
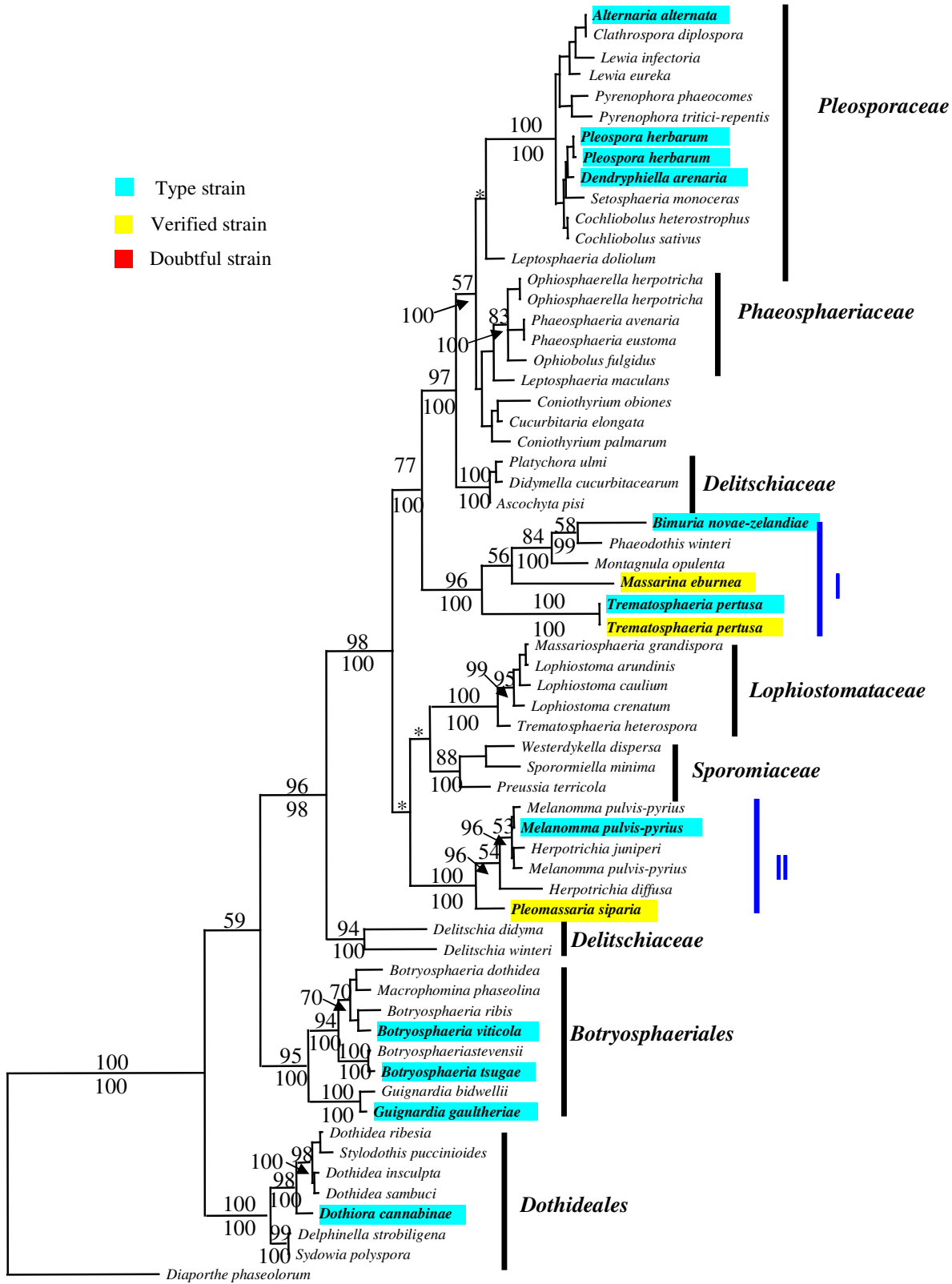
Legend:
- **Type strain** (cyan)
- **Verified strain** (yellow)
- **Doubtful strain** (red)

Alternaria alternata
Clathrospora diplospora
Lewia infectoria
Lewia eureka
Pyrenophora phaeocomes
Pyrenophora tritici-repentis
Pleospora herbarum
Pleospora herbarum
Dendryphiella arenaria
Setosphaeria monoceras
Cochliobolus heterostrophus
Cochliobolus sativus
Leptosphaeria doliolum

Pleosporaceae

Ophiosphaerella herpotricha
Ophiosphaerella herpotricha
Phaeosphaeria avenaria
Phaeosphaeria eustoma
Ophiobolus fulgidus
Leptosphaeria maculans
Coniothyrium obiones
Cucurbitaria elongata
Coniothyrium palmarum
Platychora ulmi
Didymella cucurbitacearum
Ascochyta pisi

Phaeosphaeriaceae

Delitschiaceae

Bimuria novae-zelandiae
Phaeodothis winteri
Montagnula opulenta
Massarina eburnea
Trematosphaeria pertusa
Trematosphaeria pertusa

I

Massariosphaeria grandispora
Lophiostoma arundinis
Lophiostoma caulium
Lophiostoma crenatum
Trematosphaeria heterospora

Lophiostomataceae

Westerdykella dispersa
Sporormiella minima
Preussia terricola

Sporomiaceae

Melanomma pulvis-pyrius
Melanomma pulvis-pyrius
Herpotrichia juniperi
Melanomma pulvis-pyrius
Herpotrichia diffusa
Pleomassaria siparia

II

Delitschia didyma
Delitschia winteri

Delitschiaceae

Botryosphaeria dothidea
Macrophomina phaseolina
Botryosphaeria ribis
Botryosphaeria viticola
Botryosphaeriastevensii
Botryosphaeria tsugae
Guignardia bidwellii
Guignardia gaultheriae

Botryosphaeriales

Dothidea ribesia
Stylodothis puccinioides
Dothidea insculpta
Dothidea sambuci
Dothiora cannabinae
Delphinella strobiligena
Sydowia polyspora

Dothideales

Diaporthe phaseolorum

10

Fig. 5. Phylogeny of the *Melanomma pulvis-pyrius* and *Trematosphaeria pertusa* within *Pleosporales* estimated under Maximum Parsimony (MP) (Total length = 1377, CI = 0.509, RI = 0.828, RC = 0.421, HI = 0.491). Outgroup is *Diaporthe phaseolorum*. Bold with light blue background represents type strains (e.g. holotypes, epitypes, isotypes), bold with yellow background represents fungi with verified vouchered specimens, red background represents doubtful strains (not shown) and lack of a coloured background representing unverified GenBank accessions.

with *Dendryphiopsis atra* outside the *Pleosporales* (Schoch *et al*., 2006). Thus this strain is probably wrongly identified and needs verification. Sequence data from this strain were therefore excluded in the present analysis.

Melanomma pulvis-pyrius formed a well supported clade (MP bootstrap = 100%, Bayesian PP=100%) with *Herpotrichia juniperi* and *H. diffusa* and *Pleomassaria siparia* sequences obtained from GenBank. This may therefore represent a family *Melanommataceae*, however more taxa should be incorporated before conclusions can be made.

Colour coding phylogenetic trees

There have been several recent molecular papers dealing with the *Pleosporales* and families therein (Kodsueb *et al*., 2006; Kruys *et al*., 2006; Schoch *et al*., 2006; Vijaykrishna *et al*., 2006). Some previous papers have illustrated types or material studied with bold text or asterisks (see Cai *et al*., 2008; Damm *et al*., 2008). Here we have used colour coding to indicate the robustness of the sequences used in our analysis. For instance, *Bimuria novae-zelandiae* is an orginal isolate from the type material (CBS 107.79, ex-holotype strain), while *Trematosphaeria pertusa* is a strain (CBS 122371) linked to a voucher specimen (IFRD 2003) which we checked against the type material (L-Pers 910269-172). We therefore use bold with light blue highlighting to indicate *Bimuria novae-zelandiae* and a bold with yellow highlighting for *Trematosphaeria pertusa* to show that these sequences are from type or verified strains. In Fig. 5 the *Pleosporaceae* comprise 12 sequenced strains of which four (*Alternaria alternata*, *Dendryphiella arenaria*, *Leptosphaeria doliolum*, *Pleospora herbarum*) are derived from type or verified taxa. This grouping also has strong bootstrap support (MP bootstrap = 100%, Bayesian PP=100%) and thus we can be highly confidence of the status of the *Pleosporaceae*.

A similar case can be made for the *Botryosphaeriales* (Kruys *et al*., 2006; Schoch *et al*., 2006; Phillips *et al*., 2008). Clade I also has strong bootstrap support as sequences from four out of the six strains are types or have been verified. We can therefore be highly confident in this grouping which may represent the *Massarinaceae*. However more taxa should be included to confirm this.

The *Lophiostomataceae* and *Phaeosphaeriaceae* contain no sequences from type or verified taxa. These sequences are not linked to preserved reference specimens and therefore the morphological characters of these taxa could not be verified, and therefore we can have less confidence in this grouping of taxa. Nilsson *et al*. (2006) and Hyde and Soytong (2007) have pointed out that many sequences in GenBank are possibly derived from wrongly identified taxa. We therefore reiterate that in the future sequences deposited in GenBank should be linked to preserved reference specimens in particularly as recommended by Agerer *et al*. (2000). This has the advantage that the voucher specimens can be re-examined and various characters discerned and identifications verified. For example, in Fig. 5, *Pleomassaria siparia* clustered with *M. pulvis-pyrius*. We were able to examine the voucher specimen (CBS H-258) of *P. siparia* and thus confirm that the GenBank accession is correctly named. This is particularly important as *P. siparia* is the type species of *Pleomassaria*, and thus serves as a representative strain for the *Pleomassariaceae*.

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References

Agerer, R., Ammirati, J., Blanz, P., Courtecuisse, R., Desjardin, D.E., Gams, W., Hallenberg, N., Halling, R., Hawksworth, D.L., Horak, E., Korf, R.P., Mueller, G.M., Oberwinkler, F., Rambold, G., Summerbell, R.C., Triebel, D. and Watling, R. (2000). Always deposit vouchers. Mycological Research 104: 641-644.

Aptroot, A. (1995). A monograph of *Didymosphaeria*. Studies in Mycology 37: 1-160.

Arx von, J.A. and Müller, E. (1975). A re-evaluation of the bitunicate ascomycetes with keys to families and genera. Studies in Mycology 9: 1-159.

Barr, M.E. (1976). Perspectives in the Ascomycotina. Memoirs of the New York Botanical Garden 28: 1-8.

Barr, M.E. (1979). A classification of Loculoasco-mycetes. Mycologia 71: 935-957.

Barr, M.E. (1987). *Prodromus to Class Loculoasco-mycetes*, Bigelow, Amherst, MA.

Barr, M.E. (1990). North American flora, Melanommatales (Loculoascomycetes). Series II, part 13. 1-129.

Boise, J.R. (1985). An amended description of *Trematosphaeria*. Mycologia 77: 230-237.

Cai, L., Jeewon, R. and Hyde, K.D. (2006). Phylogenetic investigations of *Sordariaceae* based on multiple gene sequences and morphology. Mycological Research 110: 137-150.

Cai, L., Guo, X.Y., Hyde, K.D. (2008). Morphological and molecular characterisation of a new anamorphic genus *Cheirosporium*, from freshwater in China. Persoonia 20: 53-58.

Cannon, P.F. and Kirk, P.M. (2008). *Fungal Families of the World*. Wallingford, UK: CAB International.

Chesters, C.G.C. (1938). Studies on British pyreno-mycetes II. A comparative study of *Melanomma pulvis-pyrius* (Pers.) Fuckel, *Melanomma fuscidulum* Sacc. and *Thyridaria rubro-notata* (B. & Br.) Sacc. Transactions of the British Mycological Society 22: 116-150.

Clements, F.E. and Shear, C.L. (1931). *The Genera of Fungi*. H.W. Wilson, New York, USA.

Damm, U., Crous, P.W., and Fourie, P.H. (2008). A fissitunicate ascus mechanism in the Calosphaeriaceae, and novel species of *Jattaea* and *Calosphaeria* on *Prunus* wood. Persoonia 20: 39-52.

Eriksson, O.E. (1981). The families of bitunicate ascomycetes. Opera Botanica 60: 1-209.

Eriksson, O.E. (2005). Outline of Ascomycota—2005. Myconet 11: 1-113.

Fries, E.M. (1817). Uppställning af de i Sverige funne Vårtsvampar (Scleromyci). Kongliga Vetenskaps Academiens Handlingar 38: 245–271.

Fries, E.M. (1823). Systema Mycologicum. 2: 275-621.

Fries, E.M. (1849). *Summa Vegetabilium Scandinaviae. Sectio posterior*. Sweden, Stockholm, Uppsala & Germany, Leipzig; Typographia Academica: 259-572..

Fuckel, K.W.G.L. (1870). Symbolae mycologicae. Beiträge zur Kenntniss der Rheinischen Pilze. Jahrbücher des Nassauischen Vereins für Naturkunde 23-24: 1-459.

Groenhart, P. (1965). Studies in ascostromatic lichen-fungi II. Types of ascostromata. Persoonia 1: 9-13.

Hall, T. (2005). Bioedit version 7.0.4. Department of Microbiology, North Carolina State University.

Holm, L. (1957). Etudes taxonomiques sur les pléosporacées. Symbolae Botanicae Upsaliensis 14: 1-188.

Holm, L. and Nannfeldt, J.A. (1962). Fries's Sclero-myceti Sueciae. A study on its editorial history with an annotated check list. Friesia 7: 10-59.

Hyde, K.D., Taylor, J.E. and Fröhlich, J. (2000). Genera of Ascomycetes from Palms. Fungal Diversity Research Series 2: 1-247.

Hyde, K.D. and Soytong, K. (2007). Understanding microfungal diversity: a critique. Cryptogamie Mycologie 28: 281-289.

Jeewon, R., Liew, E.C.Y. and Hyde, K.D. (2004). Phylogenetic evaluation of species nomenclature of *Pestalatiopsis* in relation to host association. Fungal Diversity 17: 39-55.

Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A.(eds) (2001). *Ainsworth & Bisby's Dictionary of the Fungi. 9th edn*. Oxon: CAB International.

Kodsueb, R., Jeewon, R., Vijaykrishna, D., McKenzie, E.H.C., Lumyong, P., Lumyong, S. and Hyde, K.D. (2006). Systematic revision of *Tubeufiaceae* based on morphological and molecular data. Fungal Diversity 21: 105-130.

Kruys, A., Eriksson, O.E. and Wedin, M. (2006). Phylogenetic relationships of coprophilous Pleosporales (Dothideomycetes, Ascomycota), and the classification of some bitunicate taxa of unknown position. Mycological Research 110: 527-536.

Liew, E.C., Aptroot, A. and Hyde, K.D. (2000). Phylogenetic significance of the pseudo-paraphyses in loculoascomycete taxonomy. Molecular Phylogenetics and Evolution 16: 392-402.

Liu, Y.J., Whelen, S. and Hall, B.D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA Polymerase II subunit. Molecular Biology and Evolution 16: 1799-1808.

Lumbsch, H.T. and Lindemuth, R. (2001). Major lineages of Dothideomycetes (Ascomycota)

inferred from SSU and LSU rDNA sequences. Mycological Research 105: 901-908.

Luttrell, E.S. (1973). Loculoascomycetes. In: *The Fungi, an Advanced Treatise, Vol. 4 A. A Taxonomic Review with Keys*: *Ascomycetes and Fungi Imperfecti* (eds G.C. Ainsworth, F.K. Sparrow and A.S. Sussman). Academic Press. New York & London.

Munk, A. (1953). The system of the Pyrenomycetes. A contribution to a natural classification of the group Sphaeriales *sensu* Lindau. Dansk Botanisk Arkiv 15:1-163.

Munk, A. (1957). Danish Pyrenomycetes: a prelimi-nary flora. Dansk Botanisk Arkiv 17: 1-491.

Nilsson, R.H., Ryberg, M., Kristiansson, E., Abarenkov, K., Larsson, K.H. and Kolgalg, U. (2006). Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLoS One 1: e59.

Persoon, C.H. (1794). Nähere Bestimmung und Beschreibungen einiger sich nahe verwandter Pflanzen. Annalen der Botanik (Usteri) 11: 1–32.

Persoon, C.H. (1797). *Tentamen Dispositionis Methodicae Fungorum.* Lipsiae.

Persoon, C.H. (1801). Synopsis Methodica Fungorum. 1: 1-240.

Phillips, A.J.L., Alves, A., Pennycook, S.R., Johnston, P.R., Ramaley, A., Akulov, A. and Crous, P.W. (2008). Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the *Botryosphaeriaceae*. Persoonia 21: 29-55.

Samuels, G.J. and Müller, E. (1979). Life-history studies of Brasilian ascomycetes 3. Sydowia 31: 142-156.

Schoch, C.L., Shoemaker, R.A., Seifert, K.A., Hambleton, S., Spatafora, J.W. and Crous, P.W. (2006). A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98: 1041-1052.

Shenoy, B.D., Jeewon, R., Lam, W.H., Bhat, D.J., Than, P.P., Taylor, P.W.J. and Hyde, K.D. (2007). Morpho-molecular characterisation and epityfication of *Collectotrichum capsici* (*Glomerellaceae*, *Sordariomycetes*), the causative agent of anthracnose in chilli. Fungal Diversity 27: 197-211.

Silva-Hanlin, D.M.W. and Hanlin, R.T. (1999). Small subunit RNA gene phylogeny of several loculoascomycetes and its taxonomic implications. Mycological Research 103: 153-160.

Sivanesan, A. (1984). *The Bitunicate Ascomycetes and their Anamorphs*. J. Cramer, Vaduz Lichtenstein, 701 p

Swofford, D.L. (2002). PAUP*: Phylogenetic Analysis Using Parsimony (and other methods). Version 4b10. Sinauer Associates, Sunderland, USA.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876-4882.

Tsui, C.K.M., Hyde, K.D. and Hodgkiss, I.J. (2000). Biodiversity of fungi on submerged wood in Hong Kong streams. Aquatic Microbial Ecology 21: 289-298.

Vijaykrishna, D., Jeewon, R. and Hyde, K.D. (2006). Molecular taxonomy, origins and evolution of freshwater ascomycetes. Fungal Diversity 23: 351-390.

Vilgalys, R. and Hester, M. (1990). Rapid genetic identification and mapping enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238-4246.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *Protocol: a guide to methods and applications. San Diego* (eds M.A. Innis, D.H. Gelfand, J.S. Sninsky and T.J. White). Academic Press: 315-322.

Winter, G. (1887). Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz 1 (2): 1-928.