Molecular phylogenetics of Pleosporales: Melanommataceae and Lophiostomataceae re-circumscribed (Pleosporomycetidae, Dothideomycetes, Ascomycota)

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Abstract: The classification of Pleosporales has posed major challenges due to the lack of clear understanding of the importance of the morphological characters used to distinguish between different groups in the order. This has resulted in varied taxonomic treatments of many families in the group including Melanommataceae and Lophiostomataceae. In this study we employ two nuclear DNA gene markers, nuclear ribosomal large subunit DNA and translation elongation factor 1-alpha in order to examine the molecular phylogenetics of Pleosporales with strong emphasis on the families Melanommataceae and Lophiostomataceae. Phylogenetic analyses recovered Melanommataceae, Lophiostomataceae, Hypsostromataceae, and a few others as strongly supported clades within the Pleosporales. Melanommataceae as currently circumscribed was found to be polyphyletic. The genera Byssosphaeria, Melanoma, and Pseudotrichia were recovered within the family, while others such as Ostropella and Xenolipodium nested outside in a weakly supported group along with Platystomum compressum and Pseudotrichia guatopoensis that may correspond to the family Platystomataceae. The genus Byssosphaeria was recovered as a strongly supported group within the Melanommataceae while Melanoma was weakly supported with unclear relationships among the species. The genera Herpotrichia and Bertiella were also found to belong in the Melanommataceae. Lophiostomataceae occurs as a strongly supported group but its concept is here expanded to include a new genus Misturatosphaeria that bears morphology traditionally not known to occur in the family. The strongly supported clade of Misturatosphaeria contains nine species that have gregarious, papillate ascomata with lighter coloured apices and plugged ostioles and that vary in ascospore morphology from 1- to 3-septate to muriform. Along with a strongly supported Lophistoma clade, also within the family are Thyridaria macrostomoides based on new sequences from Kenyan collections and Massariosphaeria frisipata, M. grandispora, Westerdykella cylindrica and Preussia tennicola based on GenBank sequences. The family Hypsostromataceae was recovered as a strongly supported monophyletic group nested within the Pleosporales.

Key words: Eumycota, evolution, fungi, Hypsostromataceae, phylogeny, taxonomy.

Taxonomic novelties: Misturatosphaeria Mugambi & Huhndorf, gen. nov., M. aurantotata Mugambi & Huhndorf, sp. nov., M. claviformis Mugambi & Huhndorf, sp. nov., M. cruciformis Mugambi & Huhndorf, sp. nov., M. kenyensis Mugambi & Huhndorf, sp. nov., M. minima Mugambi, A.N. Mill. & Huhndorf, sp. nov., M. tennesseensis Mugambi, A.N. Mill. & Huhndorf, sp. nov., M. unisepulta Mugambi, A.N. Mill. & Huhndorf, sp. nov., M. uniseriata Mugambi, A.N. Mill. & Huhndorf, sp. nov.

INTRODUCTION

Pleosporales is one of the largest orders of loculoascomycetous fungi and includes a complex array of organisms (Schoch et al. 2009, Zhang et al. 2009). Consequently, Barr (1987) considered arrangement of the genera and families to be far from satisfactory and work continues to this day to try to clarify the relationships. Luttrell (1955) included seven families and Barr (1987) recognised 18 families in her revised concept of the group. Presently it contains 20 families encompassing roughly 167 genera (Lumbsch & Huhndorf 2007). In synonymy with it contains 20 families encompassing roughly 167 genera, while Kirk (1970, Holm & Holm 1988, Barr 1984, 1987, 1990a, b, Lumbsch & Huhndorf 2007, Kirk et al. 2008). In Melanommataceae, Barr (1990a) accepted five genera, Kirk et al. (2008) accepted 21 genera, while Lumbsch & Huhndorf (2007) accept 18 genera with six of questionable placement. The taxonomy of Lophiostomataceae, another family in Pleosporales, has followed a similar path with Barr (1987) recognising six genera, Holm & Holm (1988) five genera, while Kirk et al., (2008) treated 15 genera and Lumbsch & Huhndorf (2007) 12 genera in the family.

Barr’s (1990a) treatment of Melanommataceae included the following genera: Ostropella, Keissleriella, Strickeria, Byssosphaeria and Melanoma united on the basis of similar erumpent to superficial ascomata with walls composed of small, thick-walled cells. Byssosphaeria was re-instated by Barr (1984) for species that are separable from Herpotrichia, where it had been in synonymy for many years. The classification of Byssosphaeria, Herpotrichia and Pseudotrichia has posed major challenges to many authors because the morphological characters used to distinguish between the genera are not necessarily obvious (Samuels & Müller 1978). This has resulted in varied taxonomic treatments of the groups (e.g. Bose 1961, Samuels 1973, Samuels et al. 2008, Zhang et al. 2008), many authors have differed on the circumscription of the families therein (e.g. Chesters & Bell 1970, Holm & Holm 1988, Barr 1984, 1987, 1990a, b, Lumbsch & Huhndorf 2007, Kirk et al. 2008). In Melanommataceae, Barr (1990a) accepted five genera, Kirk et al. (2008) accepted 21 genera, while Lumbsch & Huhndorf (2007) accept 18 genera with six of questionable placement. The taxonomy of Lophiostomataceae, another family in Pleosporales, has followed a similar path with Barr (1987) recognising six genera, Holm & Holm (1988) five genera, while Kirk et al., (2008) treated 15 genera and Lumbsch & Huhndorf (2007) 12 genera in the family.
& Müller 1978, Barr 1984). Detailed taxonomic revision on the genera is offered by Bose (1961) and Barr (1984). The two studies provide detailed morphological characters distinguishing the genera. Pseudotrichia differs from Herpotrichia in its rather large ascomata, often with compressed apices, while in Herpotrichia smaller ascomata are often covered with long flexuous hyphae and with subiculum that sometimes overgrows the fruiting bodies (Bose 1961, Barr 1984). Byssosphaeria on the other hand possesses superficial ascomata that are turbinated and sometimes overgrows the fruiting bodies (Bose & Müller 1978, Barr 1984).

MugaMbi and Huhndorf study on transferred it to initially segregated apical area that is usually light colored (Barr, 1984). Barr (1984) superficia ascomata that are turbinate with a rounded pore and with subiculum that sometimes overgrows the fruiting bodies (Bose 1961, Barr 1984).

Holm & Holm (1988) accepted it in the strict sense for the group comprising the type species. Massariosphaeria was revised by Crivelli (1983) and Leuchtmann (1984) and its principal characteristics are the gelatinous ascospore sheath and tendency towards formation of red pigment, especially in mycelial cultures (Holm & Holm 1988). Leuchtmann (1984) transferred Lophiotrema microthecum to the group as M. grandispora. Recent molecular study of Massariosphaeria by Wang et al. (2007) indicated that the genus is highly polyphyletic with only M. grandispora among the species included in the analyses grouping in Lophiotremataceae.

Lophiotremataceae (Huhndorf 1994) was described for two tropical genera, Hypsostroma and Manglicola. In setting up the family Huhndorf (1994) noted its affinities to taxa in the Melanommatiales (= Pleosporales) where she suggested it belonged but appeared unrelated to any known families. Characters that united the two genera in the family included superficial, large, elongate ascomata, soft-textured pseudoparenchymatous wall, trabeculate pseudoparaphyses, asci with an apical chamber and fluorescing ring and stipe, basally arranged and fusiform, septate ascospores (Huhndorf 1994). Hypsostroma was erected by Huhndorf (1992) for two tropical wood-inhabiting species H. saxicola and H. caimitensis. The two species bear close morphological resemblance, only slightly differing in their ascomatol and ascospore characters, with H. caimitensis bearing long papillate ascomata and ascospores constricted at septum. Currently the family resides in the Dothideomycetes, family incertae sedis (Lumbsch & Huhndorf 2007).

Many of the recent molecular phylogenetic studies involving the Pleosporales seem to reject the monophyly of the families in the order (e.g. Liew et al. 2000, Lumbsch & Lindemuth 2001, Kryus et al. 2006, Schoch et al. 2006, Wang et al. 2007, Zhang et al. 2008). This polyphylly witnessed in major lineages within Pleosporales indicates that the order is in urgent need for revision. This study contributes to this endeavor and it employs two nuclear gene markers, nuclear ribosomal large subunit DNA (LSU) and translation elongation factor 1-alpha (TEF) in order to: 1) assess the generic constitution and relationships within Melanommataceae and Lophiotremataceae, 2) verify the phylogenetic placement of the genus Hypsostroma, 3) discuss phylogenetic findings with respect to morphological-based classification schemes.
MATERIALS AND METHODS

Taxon sampling and morphological analyses

The taxa used in this study are listed in Table 1 - see online Supplementary Information. Those newly sequenced together with their collection information are indicated in bold while the others were obtained from GenBank. Representative species covering 10 families in the Dothideomycetes were targeted for analyses. A total of 149 taxa were included in the analyses with 75 taxa newly sequenced during this study (Table 1). The microscopy and image capture follow methods outlined in Huhndorf & Fernández (1998). The ascomata were squash-mounted in water and images of anatomical structures captured with a Dage DC-330 video system (Dage-MTI®, U.S.A.) mounted on a Zeiss Axioskop microscope (Carl Zeiss®, U.S.A.). Format of the individual figures for most of the species follow those produced for the pyrenomycete website (Pyrenomycetes of the World: www-s.life.illinois.edu/pyrenos/).

DNA extraction, PCR amplification, sequencing and sequence alignment

Total fungal DNA was extracted from whole fruiting bodies using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer. Phylogenetic analyses were conducted using partial sequences of two genes, the Translation Elongation Factor 1-Alpha (TEF) and nuclear ribosomal large subunit (LSU) DNA. Nuclear LSU was amplified using the primers LR0R, LR6 and LR3 (Vilgalys & Hester 1990) and TEF was amplified using primers EF1-526F, EF1-983F, EF1-1567R, Ef-df and EF-gr obtained from D. Hibbett's website (www.clarku.edu/faculty/dhibbett/Protocols_Folder/Primers/Primers.htm).

Polymerase chain reaction (PCR) was carried out using the following protocol: The final volume of the PCR reaction was 25 µL and contained 2.5 µL buffer, 2.5 µL dNTP mix, 1 µL of each primer (10 µM), 5 µL of Bovine Serum Albumin (BSA), 1.5 µL Tag polymerase (Roche®, U.S.A.), 2 µL genomic DNA extract and 9.5 µL deionised water. The reaction was then allowed to run for 34 cycles. The annealing temperature was 50 ºC for LSU, and initial 58 ºC for TEF and then reduced by 1 ºC during each of the first eight cycles and maintained at 50 ºC for the remaining cycles. The fragments were sequenced using the Big Dye® Terminator Cycle Sequencing kit v. 3.1 (ABI PRISM, Applied Biosystems, Forster City, U.S.A.). Sequencing was performed using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer. Phylogenetic analyses were conducted using partial sequences of two genes, the Translation Elongation Factor 1-Alpha (TEF) and nuclear ribosomal large subunit (LSU) DNA. Nuclear LSU was amplified using the primers LR0R, LR6 and LR3 (Vilgalys & Hester 1990) and TEF was amplified using primers EF1-526F, EF1-983F, EF1-1567R, Ef-df and EF-gr obtained from D. Hibbett's website (www.clarku.edu/faculty/dhibbett/Protocols_Folder/Primers/Primers.htm).

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Phylogenetic analyses

MODELTEST v. 3.7 (Posada & Crandall, 1998) following Akaike Information Criterion was used to determine the best-fit model of evolution for each data set for Bayesian and Maximum Likelihood analyses. Bayesian analyses employing Markov Chain Monte Carlo (MCMC) were carried out using MrBayes v. 3.1 (Huelsenbeck & Ronquist, 2001). Four MCMC chains were run simultaneously for 5–7 million generations for single-gene and combined gene analyses, the temperature of the heated chains was set at 0.05 for LSU and at 0.2 for TEF and combined gene analyses. Trees were sampled every 100th generation. The TEF gene matrix was partitioned into three parts to take into account the codon positions, while combined gene matrix had four partitions. Independent models of evolution were applied on the partitions for Bayesian analyses. AWTY was used to check the stationarity of the Bayesian tree sampling procedure (Nylander et al. 2007). All the trees obtained before the MCMC chains attained stationarity in each analysis were discarded and posterior clade probabilities were determined from the consensus tree generated from the rest. The majority rule consensus tree was obtained by executing the MrBayes sumt command. Maximum likelihood (ML) analyses were carried out for each of the three data sets using RAxML (Stamatakis et al. 2008) employing mixed models of evolution settings of the program and Bootstrap support obtained by running 1000 pseudo replicates. Five independent ML tree searches were done in RAxML (Stamatakis et al. 2008) each one starting from randomised tree.

Test of conflict was based on single gene analyses and doing comparison based on Bootstrap and Bayesian posterior probabilities support. Clades with greater than or equal to 70 % bootstrap support (BS) and 95 % posterior probabilities (PP) were considered strongly supported. There were no major conflicts in the phylogenies obtained from single-gene analyses. The differences observed were mainly the family relationships in the Pleosporales, which nonetheless received low support. As a result the data sets were combined for Maximum Likelihood (ML) and Bayesian analyses.

RESULTS

The best-fit model of evolution for LSU, TEF and combined gene data sets was GTR+I+G (Rodriguez et al. 1999) following Akaike Information Criterion implemented by ModelTest v. 3.7 (Posada & Crandall, 1998). Analyses using Maximum Likelihood and Bayesian methods resulted in phylogenies with similar topologies. Consequently only results of ML phylograms of single-gene analyses (Figs 1–2) and combined gene matrix (Fig. 3) are presented. The gene genealogies recovered a strongly supported Pleosporales that is composed of strongly supported clades for Melanomataceae, Pleosporaceae, Lophidiaceae, Delitschiaceae, Arthopyreniaceae and Hypostomataceae in at least one of the trees (Figs 1–3). The LSU tree showed clades with stronger BS support than the TEF tree. Melanomataceae includes Melanomma, Byssosphaeria, Herpotrichia, and Pseudotrichia. Nested in the family are collections representing Bertiella macrospora and Pleomassaria siparia. Taxa in Ostropella and Xenolophium that were previously placed in Melanomataceae, group in an unsupported clade distant from the family (Figs 1–3). The genus Herpotrichia was recovered as polyphyletic using LSU, with H. juniperi grouping separate from a well-supported clade that includes H. macrotricha and H. herpotrichoides (Fig. 1), but the genus resolves as monophyletic in the TEF and combined gene trees (Figs 2–3). A well-supported clade for Byssosphaeria was recovered in all three trees. Only LSU sequences were obtained for taxa in Melanomma and they did not resolve in a monophyletic
Fig. 1. Phylogram of the maximum likelihood analyses generated from LSU sequences. Bootstrap support values ≥ 70 % are shown above or below the branches. Black circles indicate branches with Bayesian posterior probabilities ≥ 95 %. The families treated in this study are indicated (shaded).
clade (Fig. 1). Pseudotrichia is polyphyletic with the type species, P. mutabilis occurring in the Melanommataceae and P. guatopoensis grouping with Ostropella spp. (Figs 1–2). A strong BS and PP supported clade composed of a new genus with nine species was recovered nested within Lophiostomataceae (Figs 1, 3) and the taxa are described below. The clade was obtained in TEF analyses but was not strongly supported (Fig. 2). Sister to the new genus are three collections of Thyridaria macrostomoides (Figs 1–3). A strongly supported clade for Lophiostoma was recovered (Figs 1–3) including two collections of Lophiostoma macrostomum. These grouped together in a strongly supported clade that included nine other species in the genus (Fig. 1). Massariosphaeria grandispora, a sequence obtained from GenBank, was found nested within Lophiostoma (Fig. 1). Single collections of Preussia terricola and Westerdykella cylindrica whose sequences were obtained from GenBank were found nested within Lophiostomataceae (Fig. 1).

Hypsostromataceae was recovered as a well-supported clade within Pleosporales comprising two species accepted in the family (Fig. 1). Species of Ostropella, Xenolophium (except Xenolophium pachythele that groups separate in TEF tree) and other taxa assemble in a mostly unsupported clade together with a collection of Platystomum compressum, possibly representing the Platystomaceae. The taxa group together in all three trees but only obtain PP support in the combined gene tree (Fig. 3). A monophyletic clade for Delitschiaceae was recovered within Pleosporaceae; Hysteriaceae, Mytilinidiaceae, Gloniaceae and Tubeufiaceae were recovered as strongly supported monophyletic groups outside the Pleosporales (Figs 1–3).

Fig. 2. Phylogram of the maximum likelihood analyses generated from TEF sequences. Bootstrap support values ≥ 70 % are shown above or below the branches. Black circles indicate branches with Bayesian posterior probabilities ≥ 95 %. The families treated in this study are indicated (shaded).
Images of sequenced taxa are included and grouped together to facilitate comparison of morphological characteristics. The species of *Byssosphaeria* and *Bertiella* are arranged together in the first plate: *Byssosphaeria jamaiaca* (Figs 4–5), *B. rhodomphala* (Figs 6, 8), *Bertiella macrospora* (Fig. 7), and *Byssosphaeria villosa* (Fig. 9). The second plate contains species of *Byssosphaeria* and *Bertiella* (Figs 10–13, 15), *Melanomma* (Figs 10–13, 15), *cf. H. herpotrichoides* (Figs 18). The third plate contains *Tubefusaria* (Fig. 18). The third plate contains *Tubefusaria* (Fig. 18).

**Misturatospaerha** Mugambi & Huhndorf, gen. nov. MycoBank MB515583.

*Etymology:* *Misturatus* (L.) = mixed, refers to the mixed ascospore morphology in the group.

*Ascomata* erumpent to superficial, occurring singly or aggregated in clusters, subiculum present or lacking, apex rounded, with or without a gelatinous sheath covering. Asci cylindrical or clavate, short stipitate, 8-spored, held in gelatinous matrix, pseudoparaphyses numerous, septate, branching and anastomosing between and above the asci. Ascospores brown or hyaline, phragmosporous or dictyosporous, external walls roughened or smooth, with or without a gelatinous sheath covering.

**Misturatospaerha aurantonotata** Mugambi & Huhndorf, sp. nov.

*Ascomata* erumpent to superficial, occurring singly or aggregated in clusters, subiculum present or lacking, apex rounded, with raised papilla or not, ostiole area light coloured or not and ostiole opening appearing plugged by gelatinous tissue. Ascii cylindrical or clavate, short stipitate, 8-spored, held in gelatinous matrix, pseudoparaphyses numerous, septate, branching and anastomosing between and above the ascii. Ascospores brown or hyaline, phragmosporous or dictyosporous, external walls roughened or smooth, with or without a gelatinous sheath covering.

**Misturatospaerha aurantonotata** Mugambi & Huhndorf, sp. nov. MycoBank MB516007. Fig. 25.

*Etymology:* *Aurantiacum* (L.) = orange, *notatus* (L.) = marked, refers to the orange colour markings at the ascomata apices.

*Ascomata* superficialia, solitaria vel aggregata, cum subiculum vel sine subiculo, apicibus rotundatis pallide coloratis vel incoloratis. Ascii cylindrici, breve stipitati, octospori, pseudoparaphyses numerosis, hyalinis et septatis, in matrice mucosa. Ascosporeae hyalinae vel bruneae, septatae, cum vagina mucosa vel sine vagina.

**Fig. 3.** Phylogram of the maximum likelihood analyses generated from the combined genes (LSU and TEF). Bootstrap support values ≥ 70 % are shown above or below the branches. Black circles indicate branches with Bayesian posterior probabilities ≥ 95 %. The families treated in this study are indicated (shaded).
Figs 4–9. *Byssosphaeria jamaicana* (SMH1403) A–B. Ascomata. C. Ascus. D. Ascospores. 5. *B. jamaicana* (SMH3646/3085) A–B. Ascomata. C. Ascus. D. Pseudoparaphyses. E. Ascospores. 6. *B. rhodomphala* (ANM942) A, B. Ascomata. C. Ascus. D. Ascospores. E. Pseudoparaphyses. 7. *Bertiella macrospora* (IL5005) A–B. Ascomata. C. Ascus. D. Ascomatal wall section. E. Ascomatal wall surface. F–H. Ascospores. 8. *B. rhodomphala* (SMH3402) A. Ascomata. B. Asci. C. Ascospores. 9. *B. villosa* (GKM204N) A–B. Ascomata. C. Ascs. D–E. Ascospores. Scale bars: Ascomata = 500 μm. Wall = 10 μm. Ascus = 10 μm. Ascospore = 10 μm.
Figs. 10–18. 10. Byssosphaeria schiedermayeriana (SMH1296) A. Ascomata. B. Pseudoparaphyses. C. Ascus. D. Ascospores. 11. B. schiedermayeriana (SMH3157) A. Ascomata. B. Ascus. C. Ascospores. 12. B. schiedermayeriana (SMH1816) A. Ascomata. B. Ascus. C. Ascospores. 13. B. schiedermayeriana (GKM1197) A–B. Ascomata. C. Ascus. D. E. Ascospores. 14. B. salebrosa (SMH2387) A, C. Ascomata. B. Ascomatal wall surface. D. Ascus. E. Ascospore. 15. B. schiedermayeriana (GKM152N) A. Ascomata. B–C. Asci. D. Ascospores. 16. Melanoma pulvis-pyrius (SMH3291) A–B. Ascomata. C. Ascus. D. Ascospores. 17. M. rhododendri (ANM73) A–B. Ascomata. C. Ascii. D. Ascospores. 18. Pseudotrichia mutabilis (SMH5288) A. Ascomata. B. Ascus. C. Ascospores. Scale bars: Ascomata = 500 µm. Ascus = 10 µm. Ascospore = 10 µm.
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Figs 19–24. *Herpotrichia macrotricha* (SMH269) A–B. Ascomata. C. Ascomatal section. D. Ascus. E. Ascospore. 20. *H. macrotricha* (GKM196N) A. Ascoma. B. Ascomatal section. C. Ascus. D. Ascospores. 21. *H. macrotricha* (GKM1128) A–B. Ascomata. C. Ascus. D. Ascospores. 22. *H. macrotricha* (SMH913) A–B. Ascomata. C. Ascomatal neck section. D. Ascomatal wall section. E. Ascomatal section. F. Ascus. G. H. Ascospores. 23. *H. cf. herpotrichoides* (GKM212N) A–B. Ascomata. C. Ascus. D. Ascospores. 24. *H. cf. herpotrichoides* (SMHS167) A–B. Ascomata. C. Ascus. D. Ascospores. Scale bars: Ascomata = 500 µm. Section = 100 µm. Wall = 10 µm. Ascus = 10 µm. Ascospore = 10 µm.
Figs 25–27. 25. Misturatosphaeria aurantonotata (GKM1238) A–C. Ascomata. D. pseudoparaphyses. E. Asci. F–G. Ascospores. 26. Misturatosphaeria claviformis (GKM1210) A–C. Ascomata. D. Pseudoparaphyses. E. Asci. F–G. Ascospores. 27. Misturatosphaeria cruciformis (SMHS151) A–B. Ascomata. C. E. Asci. D. G. Ascospores. F. Pseudoparaphyses. Scale bars: Ascomata = 500 µm. Ascus = 10 µm. Ascospore = 10 µm.
Ascomata superficial, occurring singly or aggregated into large clusters, occasionally even growing on old ascomatal tissue, often sited on sparse brown subiculum, pyriform in shape, dark brown, ascomatal wall smooth, 441–710 µm high, 461–573 µm wide. Apices rounded, usually with raised papillae, ostiole area orange in colour or the colouring lacking altogether. Asci are cylindrical-clavate with short stipes, 8-spored partially biseriate in arrangement, 103–122 x 8–12 µm. *Pseudoparaphyses* are numerous, septate, branching and anastomosing between and above the asci, held in gelatinous matrix, 1–2 µm diam. Ascospores fusiform, often slightly curved, first hyaline later becoming brown to dark brown, the outer walls are thick and roughened, 3-septate at maturity, one of the middle cells is often larger than the rest, only slightly constricted at the middle septum. Mucilaginous sheath present when the spores are young and falls off upon attaining maturity, 17–22 x 5–6 µm.

**Substratum:** Found on decorticated woody branches on the ground.

**Anamorph:** Unknown.

**Distribution:** Presently only known from two tropical forests in the central highlands of Kenya.

Specimens examined: *Kenya*. Rift Valley Province, Kajiando District, Ngong hills forest res., 1°23'34''S, 36°38'28''E, 12 July 2006, on woody branch, G.K. Mugambi, GKM1238, *holotype* EA, *isotype* F; Koibatek District, Lembus forest along Eldoret-Eldama Ravine Rd, 0°04'N, 35°35'E, 19 Jan. 2007, on branch on the ground, G.K. Mugambi, GKM1280, EA, F.

**Misturatosphaeria claviformis** Mugambi & Huhndorf, sp. nov. MycoBank MB516008. Fig. 26.

**Etymology:** *Clavatus* (L) = club, refers to the club-shaped ascomata usually witnessed in the species.

Ascomata erumpentia ad superficialia, atrobrunnea, solitaria vel sparse aggregata, sine subiculo, pyriformia ad obclavata, 235–520 µm alta, 195–322 µm diam, apicibus rotundatis, ostiolata. Asci cylindrici-clavati, brevi stipitati, octospori, 85–134 x 12–18 µm. *Pseudoparaphyses* numerosae, septatae, in matrice mucosa, 1–2 µm diam. Ascosporeae fusiformes, ovoideoe ad atrobrunneae, muniformes, sine vagina mucosa, 12–20 x 7–9 µm.

Ascomata erumpent to superficial, occurring singly or aggregated into small groups, subiculum absent, pyriform to obclavate in shape, dark brown, ascomatal wall smooth, 500–535 µm high and 545–649 µm wide. Apices are rounded with papillae that are usually raised, ostiole opening rounded. *Asci* cylindric-clavate in shape and bearing short stipes, 8-spored partially biseriate to sometimes overlapping uniseriate, 127–154 x 14–17 µm. *Pseudoparaphyses* are numerous, septate, branching and anastomosing between and above the asci, held in a gelatinous matrix, 1–2 µm diam. Ascospores oblong to elliptical, brown becoming dark brown with age, outer wall smooth, dictyosporous, usually constricted at the middle transverse septum, possess no mucilaginuous sheath, 19–26 x 8–13 µm.

**Substratum:** Found on decorticated woody branches on the ground.

**Anamorph:** Unknown.

**Distribution:** Currently only known from a forest reserve in Illinois, U.S.A.

Specimens examined: *U.S.A.* Illinois, Cook Co., Swallow Cliff Woods Forest Preserve, 21 May 2004, on woody branch, G.K. Mugambi, SMH5151, *holotype* F.

**Misturatosphaeria kenensis** Mugambi & Huhndorf, sp. nov. MycoBank MB516010. Figs 28–29.

**Etymology:** Refers to the country the species was collected, Kenya.

Ascomata erumpentia ad superficialia, solitaria vel dense aggregata, atrobrunnea, pyriformia ad obclavata, 185–305 µm alta, 245–334 µm diam, apicibus rotundatis pallide coloratis, ostiolo rotundato. Asci cylindrici-clavati, brevi stipitati, octospori, 71–79 x 9–9 µm. *Pseudoparaphyses* numerosae, septatae, in matrice mucosa, 1–2 µm diam. Ascosporeae fusiformes, hyalineae, 1–3-septatae vulgo 1–septatae, cum vagina mucosa parva, 15–24 x 4–6 µm.

Ascomata erumpent to superficial, occurring singly or aggregated into large clusters, without subiculum, pyriform to globose in shape, dark brown, ascomatal wall smooth, 185–305 µm high and 245–334 µm wide. Apices rounded, usually with raised papillae, opening rounded and the ostiole area often of lighter colour. *Asci* cylindric-clavate in shape, short stipitate, 8-spored, partially biseriate in arrangement, 71–79 x 8–9 µm. *Pseudoparaphyses* numeroses and septate, branching and anastomosing between and above the asci, held in gelatinous matrix, 1–2 µm diam. Ascospores fusiform in shape, hyaline, outer wall smooth, usually 1–3-septate but commonly 1-septate, occasionally one of the middle cells broader than others, possess small mucilaginuous sheaths that extends at the tip of spores, 15–24 x 4–6 µm.
**Misturatosphaeria minima** Mugambi, A.N. Mill. & Huhndorf, *sp. nov.* MycoBank MB516011. Figgs 30–32.

**Etymology**. *M. minima* is an diminutive from *minima*, Latin for smallest, referring to the smallest size of the ascospores in the species.

**Anamorph**: Unknown.

**Distribution**: Presently only known from a tropical cloud forest in Kenya.

Specimen examined. **Kenya**, Coast Province, Taita Taveta District, Taita hills, Ngangao forest res., 3° 22’ 30” S, 38° 20’ 45” E, 1800 m elev., 13 May 1996, on woody branch, S.M. Huhndorf, F.; 35° 30’ 19” S, 83° 12’ 32” W, 868 m elev., 23 May 2006, on woody branch, A.N. Miller, G.K. Mugambi, ANM911, **holotype** EA, **isotype** F.

**Misturatosphaeria uniseptata** Mugambi, A.N. Mill. & Huhndorf, *sp. nov.* MycoBank MB516013. Fig. 34.

**Etymology**. *Unicus* (L.) = one, referring to one septate nature of the ascospores in the species.

**Anamorph**: Unknown.

**Distribution**: Presently only known from a forested area in Tennessee, U.S.A.

Specimen examined. **U.S.A.**, Tennessee, Cocke Co., Great Smoky Mountains National Park, Lower Mount Cammerer Trail, 35° 45’ 256” N, 83°12’ 329” W, 686 m elev., 19 May 2006, on woody branch, A.N. Miller, G.K. Mugambi, ANM911, **holotype** F, **isotype** ILLS.

**Misturatosphaeria tennesseensis** Mugambi, A.N. Mill. & Huhndorf, *sp. nov.* MycoBank MB516012. Fig. 33.

**Etymology**. Refers to the locality where the species was collected.

**Anamorph**: Unknown.

**Distribution**: Currently only known from a tropical forest in Ecuador.
Melanomataceae and Lophostomataceae

Figs 28–33.

28. Misturatosphaeria kenynsis (GKM1195) A–B. Ascomata. C–D. Asci. E, F. Ascospores.
29. M. kenynsis (GKM L100Na) A. Ascomata. B. Ascospores. C. Pseudoparaphyses.
30. Misturatosphaeria minima (SMH2448) A. B. Ascomata. C. Ascus. D. Ascospores.
31. M. minima (GKM169N) A. Ascomata. B. Ascus. C. Pseudoparaphyses.
32. M. minima (ANM60) A, B. Ascomata. C. Ascus. D. Pseudoparaphyses. E–F. Ascospores.
33. Misturatosphaeria tennesseensis (ANM911) A–B. Ascomata. C–D. Asci. E. Pseudoparaphyses. F. Ascospores.

Scale bars: Ascomata = 500 µm. Ascus = 10 µm. Ascospore = 10 µm.
**Misturatosphaeria uniseriata** Mugambi, A.N. Mill. & Huhndorf, *sp. nov*. MycoBank MB516014. Fig. 35.

**Etymology**: *Unicus* (L.) = one, *Serialis* (L.) = row, refers to single-row arrangement of the ascospores in the ascii.

Ascomata erumpent, solitary vel dense aggregata, abruto brunnnea, pyriformia ad globosa, 332–343 µm alta, 309–379 µm diam, sine subiculo, apicibus rotundatis, pallide coloratis. Acsi cylindrici ad cylindric-clavati, brevi stipitati, octospori, 100–130 x 8–10 µm. Pseudoparaphyses numerosae, septatae in matrice mucosa, 1–2 µm diam. Ascospore fusiformes ad ellipiticae, brunnnea ad atrobrunnea, 1–3-septatae, vulgo 3-septatae, constrictae ad septa omnia, sine vagina mucosa, 14–19 x 4–7 µm.

Ascomata erumpent, occurring aggregated into large clusters, subiculum lacking, pyriform to subglobose in shape, dark brown, ascomatal wall smooth, 332–343 µm high and 309–379 µm wide. Apices rounded, may possess slightly raised papillae, the ostiole area usually of lighter colour, possess prominent pore opening that appears plugged by central tissue. Acsi cylindrici ad cylindric-clavati, bearing short stipes, 8-spored, overlapping uniseriate in arrangement, 100–130 x 8–10 µm. Pseudoparaphyses are numerous and septate, branching and anastomosing between and above the asci, held in gelatinous matrix, 1–2 µm diam. Ascospores fusiform to elliptical, brown to dark brown in colour, outer wall smooth, 1–3-ascospore but commonly 3-ascospore, occasionally constricted at all three septa, with no mucilaginous sheath, 14–19 x 4–7 µm.

**Substratum**: Found on decorticated woody branches on the ground.

**Anamorph**: Unknown.

**Distribution**: Currently only known from forested area in the Great Smoky Mountains National Park in Tennessee, U.S.A.

Specimen examined: **U.S.A.**, Tennessee, Cocke Co., Great Smoky Mountains National Park, Lower Mount Cammerer Trail, 35° 45’ 256” N, 83° 12’ 329” W, 686 m elev., 19 May 2006, on woody branch, A.N. Miller, G.K. Mugambi, ANM909, holotype F.

**Notes**: The nine species newly described in *Misturatosphaeria* (Figs 25–35) were collected from wide geographic localities from Africa, North, Central and South America. An additional collection was included in the analyses (*Misturatosphaeria* sp., Fig. 1) that probably represents another species but asci were not seen so the specimen is not described at this time (Fig. 36). *Misturatosphaeria aurantionotata* bears some similarity with *M. tennesseensis* and *M. uniseriata* but differs from both in its much larger superficial ascomata and quite verrucose ascospores. It also differs in its phylogenetic placement (Figs 1–3). *Misturatosphaeria tennesseensis* differs from *M. uniseriata* in the shape and loose aggregation of the ascomata, asci that are predominantly partially biseriate in arrangement and ascospores that are paler brown and strongly constricted at the mid-septum. In contrast *M. uniseriata* ascomata tend to occur in large clusters, asci have predominantly overlapping uniseriate ascospore arrangement, ascospores are dark brown at maturity and are only rarely slightly constricted. Molecular data also support the separation of these two species (Figs 1–3). *Misturatosphaeria uniseriata* differs from all the other species in the group in its pale brown, 1-septate ascospores. *Misturatosphaeria minima* and *M. tennesseensis* are quite similar in their morphologies but the former differs in its ascomata that are solitary or in small groups usually less than 5 individuals. *Misturatosphaeria kenynensis* ascomata are aggregated in larger clusters and ascospores are slightly broader. *Misturatosphaeria cruciformis* shares ascospore morphology with *M. claviformis*, but the former differs by having much larger ascomata that are predominantly pyriform and ascospores that are often constricted at the middle septum. *Misturatosphaeria claviformis* has smaller pyriform to obclavate ascocoma, and ascospores that are very rarely slightly constricted. Molecular data also indicate the two species are distantely related (Figs 1–3). In addition to species of *Misturatosphaeria*, the sixth plate of figures contains two taxa illustrated for morphological comparison: *Platystomum compressum* (Fig. 37), and *Thyridaria macrostomoides* (Fig. 38). Both species share features with members of the Lophiotistomataceae but only *T. macrostomoides* finds placement in the family based on molecular data (Figs 1–3).

**DISCUSSION**

**Melanommataceae**

Phylogenetic analyses recovered *Melanommataceae*, *Lophistomataceae*, *Hypsostromataceae*, and a few others as strongly supported clades within the Pleosporales (Figs 1–3). Although some genera currently accepted in the *Melanommataceae*, such as *Melanomma*, *Pseudotrichia* and *Byssosphaeria*, were recovered within the family, others such as *Ostropella* and *Xenolophium* are nested outside the family (Figs 1–3). *Herpotrichia* and *Bertiella* currently reside in other families (Kirk et al. 2008) but they, along with a single representative of *Pleomassaria sippia*, find their placement within the *Melanommataceae* based on our data. *Byssosphaeria*, *Herpotrichia*, and *Pseudotrichia* are taxa that have been united in the past, mostly under *Herpotrichia* and all share the distinctive characteristic that at least some species bear subiculate ascomata (Bose 1961, Sivanesan 1971, Barr 1984). Previous analyses of the Pleosporales have included the GenBank sequences *Lophistoma macrostomum* (DQ384094) and *O. compressum* (DQ678020) that nested within *Melanommataceae*. Although voucher specimens were not obtained to verify their identity, we strongly believe that these collections were misidentified. *Lophistoma macrostomum*, the type species of the Lophistomata, has already been confirmed to reside outside *Melanommataceae* (Tanaka & Hosoya 2008), which was corroborated by the results of this study (Figs 1–3). Recently, Zhang et al. (2008) using the epitype strain of *Trematosphaeria pertusa* have also demonstrated that this species is not closely related to *Melanomma* and belongs outside *Melanommataceae s. str*. This is corroborated here by our own collection of *T. pertusa* (Fig. 1).

The genus *Melanomma* formed the weakest structure in the clade. While the family was strongly supported, the genus did not unite in a strong clade. All five collections of *Melanomma pulvispyrus* clustered at the base of the family clade in the LSU tree, separated by very short branches (two collections appeared to be identical). The other represented species, *M. rhododendri* was on a long branch near *M. pulvispyrus*. Morphologically our collections fit within the genus, both species having the clustered, superficial ascomata and small 3-septate brown ascospores in an uniseriate arrangement in the ascii (Figs 16–17). It appears that additional collections and other genes might be necessary to understand how the specimens and species relate to each other.
Figs 34–38. 34. Misturatosphaeria uniseptata (SMH4330) A–B. Ascomata. C, G. Ascospores. D–F Asci. 35. Misturatosphaeria uniseriata (ANM909) A–C. Ascomata. D. Ascomatal section. E. Ascomatal wall section. F. Asci. G. Ascospores. 36. Misturatosphaeria sp. (SMH3747) A–B. Ascomata. C. Ascomatal wall surface. D. Ascospores. 37. Platystomum compressum (GKM1048) A–B. Ascomata. C. Ascospores. 38. Thyridaria macrostomoides (GKM1033) A. Ascomata. B. Ascus. C. Ascospores. Scale bars: Ascomata = 500 µm. Section = 100 µm. Wall = 10 µm. Ascus = 10 µm. Ascospore = 10 µm.
In contrast, a strongly supported, monophasyic Byssosphaeria was recovered, which in this study was represented by six species, three of them with multiple collections (Figs 1-3). Byssosphaeria was described for taxa bearing superficial ascomata, separate or usually gregarious, turbinate, globose to ovoid and with rounded or minute papilla and opening by rounded pore. The pore and surrounding cells are pallid, whitish, or grey, or bright yellow, orange or red pigmented and the pore region appearing sulcate or plicate at times. Ascomatal wall surfaces are often irregular or slightly roughened, with protruding cells and often bear hyphal hairs (described as appendages in Barr 1984). Asci are clavate to cylindric, peripherally arranged and 8-spored. Ascospores are at first hyaline becoming light reddish brown or clear brown, ellipsoid or fusoid (Barr, 1990a). Byssosphaeria schiedermayeriana, the type species of the genus, is represented by five collections that form a strongly supported clade. The collections are neither morphologically nor molecularly identical. Morphologically the main differences seen are in the ascomatal structure. The collections differ in their amount of subiculum or hyphal hairs, amount of vertical collapsing (tendency toward becoming collabent, see Fig. 11A) and in the amount of colouration around the pore area (Figs 10A, 11A, 12A, 13A, 14A, 15A). The ascospores of these collections have measurements within the size range for the species given by Barr (1984), 25–42 x 5–9 µm and a sheath is seen in some of the collections (Figs 10D, 13D). Nusted within the clade is the sequence of B. diffusa from GenBank (as Herpotrichia in GenBank) and one collection representing B. salebrosa. Byssosphaeria diffusa is reported to differ from B. schiedermayeriana in having a pallid or whitish pore area and smaller ascospores (Sivanesan 1971, Barr 1984). Byssosphaeria salebrosa is distinguished by non-subiculate ascomata with a surface that is roughened by projecting masses of cells, a feature present in our collection (Fig. 14B, C). Additional collections with the characteristics of these two species should be added to see if these morphological characters are phylogenetically informative.

Byssosphaeria rhodomphala and B. jamaicana, each represented by several collections, occur in a strongly supported sister relationship (Fig. 1). The collections of Byssosphaeria jamaicana fit well within the description of the species (Figs 4–5). The subiculate, clustered ascomata, varying in the amount of hyphal hairs, have a pale-coloured pore area, the ascospores appear to lack appendages and in at least one of our collections, the ascospores turn somewhat darker brown and become 8-spored (Fig. 4D) (as was illustrated for the species by Sivanesan 1971). Five collections represent R. rhodomphala and morphologically and molecularly they are remarkably consistent given that they are geographically distant (Figs 1, 6, 8). Among the collections, the pore region of the ascomata can show variation from red to orange to yellowish granular deposits and can macroscopically appear quite dark. The most distinct characteristic of this species and the most useful aid in identification is the oblong-ellipsoid ascospore with obtuse ends. Byssosphaeria rhodomphala differs from B. jamaicana by the colouration of the ascomatal pore region. Hyphal appendages are lacking in B. rhodomphala and the ascospores are smaller than in B. jamaicana (Barr 1994).

On a basal branch in the Byssosphaeria clade is a single collection of B. villosa. The species is distinguished by ascomata bearing a dense covering of outwardly-projecting, long villose hairs and a pale coloured apex (Samuels & Müller 1978). Its general appearance was described as being “similar to Lasiosphaeria phyllophila” (= lodosphaeria) (Samuels & Müller 1978), an observation that aided us in correctly identifying our own collection. Our collection differs from the description by having a distinctly yellow pore area (Fig. 9). However, Samuels & Müller’s (1978) description of a bright yellow colour in the colonies they obtained in pure culture allowed us to conclude that the colour of the pore area might vary among collections of the species.

Herpotrichia also found its placement in the Melanommataceae in contrast to the Teliosporaceae where it was previously placed (Lumbsch & Huhndorf 2007). Herpotrichia macrospora is represented by three collections (Costa Rica, Kenya, U.S.A.) and slightly different placements in the LSU and TEF trees. It was unfortunate that we were not able to sequence one of the same collections for both genes. The collections show the distinctive arrangement of thick-walled and highly melanised ascomatal cells bearing bands of less pigmented, thin-walled cells (Fig. 7) that was described by Eriksson & Yue (1986) as “resembling cephalaecithoci.” As reported for the type specimen (Eriksson & Yue 1986), all three recent collections have ascomata that are superficial, gregarious and non-papillate (Fig. 7) with a roughened surface and lacking appendages or subiculum. The main difference occurs in the size of the ascospores. Whereas Eriksson & Yue (1986) describe the type specimen as having ascospores in the size range of 37–43 x 8–9 µm, our collections have smaller ascospores, ranging 22–30 x 5–7 µm in size. Based on the description given by Barr (1984), another taxon that may be similar to these collections is Byssosphaeria semen. This species differs from other Byssosphaerias in having a roughened ascomatal surface that lacks hairs or subiculum and has ascospores in the same range as our collections. The main difference is that no cephalothecioid cell arrangement is reported for this species and this is quite a prominent feature of our collections.

Barr (1984) revised the genus Herpotrichia and in the process re-established several genera, including Byssosphaeria and Pseudotrichia that had been in synonymy. She suggested that Herpotrichia belonged in Massarinaceae, a family later synonymised with Lophioistomataceae (Barr 1987). Herpotrichia is characterised by immersed, erumpent or superficial ascomata, with tomentum, rounded apex opening by broad pore. The asci are clavate or cylindrical, basal and pseudoparaphyses are narrowly cellular. The ascospores are fusoid, ellipsoid or oblong, usually surrounded by a mucilaginous sheath, and are hyaline becoming light yellow to reddish brown, mostly 1-septate but developing more septa with age. Herpotrichia is represented in our analyses by H. herpotrichioïdes, H. juniperi and H. macrotrocha, and these taxa collectively do not form a monophasyic group in the LSU analyses. Subsets of these taxa group together in the TEF and combined gene trees. Two collections are tentatively identified as H. herpotrichioïdes, the type species of the genus. Morphologically the collections match this species and they do not differ significantly from each other (Figs 23–24); however in the LSU tree they occur in the same clade but separate from each other. The collections are geographically distant (one from the U.S.A. and one from Kenya) so a sequenced European collection would be useful to aid in the placement of the species. Herpotrichia juniperi, represented by two strains obtained from GenBank grouped consistently separate from the other two species. They also did not appear to closely align with the other taxa in the clade but occupied a position in Melanommataceae (Fig. 1). Assuming these collections were correctly identified, the results presented suggested that H. juniperi may belong outside the genus and thus may bear affinities to Melanommataceae. However, this might only become clear with broader taxon sampling in the groups. The described morphologies of Melanommata and Herpotrichia overlap in many ways, with both sharing immersed, erumpent to superficial ascomata that are tomentose and usually...
sit on an ample subiculum. Ascii and ascospore morphologies are also similar, although in *Herpotrichia* ascospores tend to initially start hyaline and 1-septate later become brown and more septate. Since the *Melanomma* clade did not receive strong support the true placement of *H. juniperi* or its relationships to the taxa in the genus remain unclear. *Herpotrichia macrotricha* is reported to have a wide distribution (Barr 1984) and the five collections of *H. macrotricha* (representatives from Costa Rica, Kenya and Puerto Rico) form a strongly supported clade (Fig. 1). The species is easily recognised by its distinctive broad cap-like ascomatal apex with a thick inner layer of hyaline pseudoparenchymatous cells (Figs 19–22). One collection stands out from the rest both in sequence data and morphology. The Costa Rican collection (SMH4913, V on Fig. 1) differs from the others in having an upper, ascomal wall with a central hyaline layer that gives the ascomata a wrinkled, collapsed appearance in the dried collections (Fig. 22). It was thought perhaps to be a separate species but other known species should be checked before making that decision.

*Pseudotrichia* was considered to be in the family *Platystomataceae* by Barr (1990b), but is correctly placed in the *Melanomnataceae* as in the most recent outline of the Ascomycota in *Myconet* (Lumbsch & Huhndorf 2007). The type species, *P. mutabilis*, is represented by two U.S.A. collections and in our LSU tree occurs as a sister taxon monophyletic as our other included species, *forests*. As currently circumscribed however, the genus is not tomentose ascomata and its hyaline, fusiform, septate ascospores distinctive and easily identified with its gregarious, yellow-green by Barr (1990b), but is correctly placed in the *macrotricha* should be checked before making that decision.

In the LSU tree, *Pleomassaria* is representative of the *Melanomnataceae* in the clade with *Pseudotrichia mutabilis*. According to Barr (1982: 370) *Pleomassaria siparia* is characterised by immersed, depressed globose ascomata containing oblong asci with simple muriform ascospores. The ascospores are dark brown with a verruculose surface, with 5–7 transverse septa and one longitudinal septum in several cells. The characteristics this species shares with others in the clade are not obvious. Our other collection identified as belonging in the family (*Pleomassariaceae*; SMH5232) does not cluster with *P. siparia* but rather finds a placement among the taxa in the unsupported *Platystomataceae* clade. Additionally, our other unpublished phylogenetic analyses using sequences from putative *Asteromassaria* and *Splanchnonema* species (other taxa that are arranged in the morphologically defined *Pleomassariaceae*) found that these species also do not cluster with *P. siparia*. It will be necessary to have additional sequences from other collections of the species to confirm its placement.

In the LSU tree, *Ostropella* and *Xenolophium* occur distantly related to *Melanomnataceae* in a clade lacking significant support that also includes *Pseudotrichia guatopoensis*, *Platystomum compressum* (Fig. 37) and an unnamed *Pleomassariaceae* (Fig. 1). The clade, however, received significant PP support in the combined-gene analysis (Fig. 3). *Ostropella* and *Xenolophium* share a combination of morphological characters that appears distinct from that observed in *Melanomnataceae s. str.* They possess relatively large ascomata, bearing raised apices with silt-like ostiolar openings. The asci in these two genera are clavate with quite long stipes, which is unlike taxa that are here treated in *Melanomnataceae*. The mostly tropical collections of *Ostropella*, *Xenolophium*, and *P. guatopoensis* all share the distinctive, extensive network of trabeculate pseudoparaphyses that Barr (1983, 1990a) emphasised as an important diagnostic character that separates *Melanomnatales* from *Pleosporales*. Subsequent studies using DNA sequence data have established that pseudoparaphysis type is not a phylogenetically informative character at the ordinal level and hence separation of *Melanomnatales* from *Pleosporales* based on this character is not supported (Liew et al. 2000, Lumbsch & Lindemuth 2001, Kruys et al. 2006, Schoch et al. 2006, Wang et al. 2007). The results of the phylogenetic analyses presented in this study corroborate these findings. Additional genera in this clade are *Ulospora bilgramii* and *Verruculina enalia* whose sequences were obtained from GenBank. Since the clade for the most part did not receive significant support, further studies involving more collections and more markers are needed to confirm the observed relationships. Furthermore, the occurrence of *P. guatopoensis* and *P. compressum* in this clade, which were previously not thought to be related to *Ostropella* and *Xenolophium*, underscores the need for more work to establish with confidence the nature of the relationships.

**Lophiostomataceae**

Our analyses recovered a strongly supported *Lophiostomataceae* comprised of *Lophiostoma* and some species currently placed in *Thyridaria*. Sister to the *Lophiostoma* clade is the strongly supported clade of the genus *Misturatosphaeria* comprising nine new species (Figs 1–3). The genus *Lophiostoma* was established by Fries (1849) to accommodate taxa that possess mostly erumpent ascomata, bearing apices that are raised and laterally compressed with phragmosporous hyaline or brown ascospores. Since its inception, many more species have been added to the genus but the taxonomy of the group remains uncertain, requiring urgent revision. Chesters & Bell (1970) and Holm & Holm (1988) provide comprehensive information on the taxonomic history and morphology of the genus. Barr (1990a) transferred several species of *Lophiostoma* into *Thyridaria*, which she placed in the *Platystomataceae* that included *T. macrostomoides*. We had among our specimens, collections that bear the morphology of the species and in analyses *T. macrostomoides* groups separate from taxa in *Lophiostoma* (Figs 1–3). *Thyridaria macrostomoides* (Fig. 38) collections occur in an unsupported sister relationship with *Misturatosphaeria* in single-gene trees but receives significant PP in the combined-gene tree (Figs 1–3). Barr (1990a) described *Thyridaria* to include taxa with the following morphology: ascomata that are immersed or erumpent, in volsid groups or separate or gregarious; ascomata globose with a well developed papilla or short beak that is rounded or compressed; ostioles that are rounded or silt-like and periphysate or filled with pallid or brightly pigmented hyphal ends. Ascii were described as clavate or cylindrical with trabeculate pseudoparaphyses held in gelatinous matrix. Ascospores are brown, symmetric or asymmetric, phragmosporous, three or more septate, smooth, verruculose or striate. The results presented here demonstrate that at least one of the species of *Thyridaria* resides in *Lophiostomataceae*. However, since we did not include the type species of *Thyridaria* in our analyses the generic placement remains unclear.

*Misturatosphaeria* differs from other genera in *Lophiostomataceae* by possessing ascomata that are erumpent to superficial, with rounded apices that are often raised. Ostiolar openings are rounded and plugged by gelatinous tissue and
occasionally lighter coloured. Asci are cylindrical to clavate with phragmosporous or dictyosporous ascospores (Figs 25–36). Despite morphological differences of Misturatospaeria from other lophiostomataceous fungi, we feel justified in placing it in Lophiostomataceae at this point due to the strong support the clade received in our analyses (Figs 1–3). The only other genus with dictyosporous ascospores accepted by Barr (1980b) in this family is Ciliopoea. This genus was not included in our analyses but it differs markedly from the dictyosporous members of Misturatospaeria and others in the group in general. It possesses ascomata with thick walled brown or dark brown setae around the apex and a narrow peridium of few internal rows of compressed pallid cells, surrounded by brown hyphae into the substrate. It also differs in the ascospore shape and septation, with the ascospore longitudinal septum in this genus primarily limited to the central cells (Barr 1990b).

**Hypsostromataceae**

Huhndorf (1992) described the genus Hypsostroma for two tropical species, *H. saxicola* and *H. caimitalenesis*. She did not prefer familial placement at the time but suggested they bear affinities in the Melanommataceae (= Pleosporales). Later, Huhndorf (1994) erected family Hypsostromataceae for the genera Hypsostroma and Mangicola. The two species of Hypsostroma were included in our analyses and they grouped together in a strongly supported clade within Pleosporales distinct from other families in the order (Fig 1). This study is only the second time the two specimens used for sequencing have been collected and this expands their range outside of the Caribbean and South America. Hypsostroma is distinctive in having asci with extremely long stipes, similar to those found in Ostropella and Xenophomum but much longer (Huhndorf 1992, 1994). Although the clade shows no supported relationship with the Platystomaceae taxa, *X. guianense* tentatively joins the clade in the LSU tree and the morphological similarities of these long-stipitate taxa suggest that they may in some way be related.

**CONCLUSION**

The Melanommataceae is found to contain taxa that have gregarious, superficial ascomata and the ascomata may be smooth (Byssosphaeria *p.p.*), roughened (Melanomma, Bertiella macrospora), or clothed in hyphal hairs (Herpotrichia, Byssosphaeria *p.p.*) or coloured tomentum (*F. mutabilis*). Subicular hyphae may be present in some taxa in the family (Herpotrichia, Byssosphaeria or absent (Melanomma, Bertiella). Cephalothecoid-like ascomatal wall structure (Bertiella macrospora), versicolourous-layered walls (*H. macrotricha p.p.*) or uniformly brown-pigmented walls (Byssosphaeria, Melanomma) occur in the family. The ascospores that occur in the group are hyaline or brown, mostly fusiform in shape and 1–3-septate. Anomalous in the family is the presence of Pleomassaria siparia, differing from the other sequenced taxa by having immersed ascomata and muriform ascospores. Former family members, Ostropella and Xenophomum are found to occur outside the Melanommataceae in a weakly supported group along with Platystomum compressum and Pseudotrichia guatopoensis, that may correspond to the family Platystomaceae.

Lophiostomataceae occurs as a strongly supported monophyletic group but its concept is here expanded to include a new genus Misturatospaeria that bears morphology traditionally not known to occur in the family. The gregarious ascomata of Misturatospaeria, especially *M. minima*, suggests a resemblance and potential relationship to Byssosphaeria species or other taxa in the Melanommataceae. The molecular data shows this not to be the case. The ascomata differ with papillate apices present in Misturatospaeria versus plane to collapsed asci in Byssosphaeria, along with the lack of hyphal hairs or subiculum in the former. Distinctive characteristics of Misturatospaeria are the tendencies towards lighter coloured apices (*M. aurantonotata*) and plugged ostioles (*M. minima, M. uniseriata*). Additionally the ascomata in the group are actually mostly erumpent from the substrate and not superficial as in Byssosphaeria. The mixture of didymosporous, phragmosporous to dictyosporous ascospore morphologies that gives the genus its name was problematic and for awhile we thought that more than one generic clade would resolve but the molecular data did not provide that satisfaction. Some of the phragmosporous species have ascospores that resemble those in Lophiostoma s. l.

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### Table 1. Taxa used in the study with those newly sequenced for LSU and TEF genes shown in bold, the rest obtained from GenBank.

| Taxon                                | Collection locality                        | Collector and accession number                | LSU       | TEF       |
|--------------------------------------|--------------------------------------------|----------------------------------------------|-----------|-----------|
| Alternaria alternata                 | Costa Rica, Puntarenas, Monteverde         | S.M. Huhndorf, SMH4000                      | DQ678082  | DQ677927  |
| Arthopyrenia salicis I              | Costa Rica, Puntarenas, Monteverde         | I. Lopez, IL5005                            | AY538339  |           |
| Arthopyrenia salicis II             | Costa Rica, Puntarenas, Monteverde         | I. Lopez, IL5005                            | AY607730  |           |
| Arthopyrenia sp.                    | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.K. Mugambi, GKM L122N                      | GU385149  |           |
| Bertiella macrospora I              | Costa Rica, Puntarenas, Monteverde         | S.M. Huhndorf, SMH3953                      | GU327744  |           |
| Bertiella macrospora II             | U.S.A., Michuon, Huron Mt. Club            | S.M. Huhndorf, SMH3953                      | GU327744  |           |
| Byssosphaeria jamaicana I           | U.S.A., Puerto Rico, Luquillo Mts.         | S.M. Huhndorf, SMH1403                      | GU385152  | GU327746  |
| Byssosphaeria jamaicana II          | U.S.A., Puerto Rico, Luquillo Mts.         | S.M. Huhndorf, SMH3085                      | GU385154  |           |
| Byssosphaeria rhodomphala I         | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.K. Mugambi, GKM L153N                      | GU385157  | GU327747  |
| Byssosphaeria rhodomphala II        | U.S.A., North Carolina, Smoky Mts.         | A.N. Miller, ANM942                         | GU385160  |           |
| Byssosphaeria rhodomphala III       | U.S.A., Puerto Rico, Luquillo Mts.         | S.M. Huhndorf, SMH3086                      | GU385155  |           |
| Byssosphaeria rhodomphala IV        | Panama, Barro Colorado Island              | S.M. Huhndorf, SMH3402                      | GU385170  |           |
| Byssosphaeria salebrosa             | Costa Rica, San Jose, San Gerardo de Dota  | S.M. Huhndorf, SMH2387                      | GU385162  | GU327748  |
| Byssosphaeria schiedermayeriana I   | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.K. Mugambi, GKM152N                      | GU385168  | GU327749  |
| Byssosphaeria schiedermayeriana II  | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.K. Mugambi, GKM1197                      | GU385161  | GU327750  |
| Byssosphaeria schiedermayeriana III | U.S.A. Puerto Rico, Luquillo Mts.          | S.M. Huhndorf, SMH1296                      | GU385158  |           |
| Byssosphaeria schiedermayeriana IV  | U.S.A. Puerto Rico, Luquillo Mts.          | S.M. Huhndorf, SMH1816                      | GU385159  |           |
| Byssosphaeria schiedermayeriana V   | U.S.A. Puerto Rico, Luquillo Mts.          | S.M. Huhndorf, SMH3157                      | GU385163  | GU327745  |
| Byssosphaeria villosa                | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.M. Mugambi, GKM204N                      | GU385151  | GU327751  |
| Byssothecium cinctum                | Kenya, Mt. Kenya Forest, along the track past Sirimon entrance | G.K. Mugambi, GKM1205                      | GU385171  |           |
| Cochliobolus eragrostidis           |                                           |                                              | AB288215  |           |
| Cochliobolus geniculatus            |                                           |                                              | AB444670  |           |
| Cochliobolus heterostrophus         |                                           |                                              | AY544645  | DQ497603  |
| Cochliobolus lunatus                |                                           |                                              | AB444681  |           |
| Cochliobolus pallescens             |                                           |                                              | AB288225  |           |
| Cochliobolus sativus                |                                           |                                              | DQ678045  |           |
| Cochliobolus verruculosus           |                                           |                                              | AB444680  |           |
| Delitschia cf. anisomera            | Kenya, Mt. Kenya Forest, along the track past Sirimon entrance | G.K. Mugambi, GKM1205                      | GU385171  |           |
| Delitschia chaetomoides I           | Kenya, Rift Valley Province, Lembus forest along Eldore – Elderma Ravine road | G.K. Mugambi, GKM1283                      | GU385172  | GU327752  |
| Delitschia chaetomoides II          | Costa Rica, Guanacaste, Bosque Encantado   | S.M. Huhndorf, SMH3253.2                    | GU390656  | GU327753  |
| Delitschia didyma                   |                                            |                                              | DQ384090  |           |
| Delitschia winteri                  |                                            |                                              | DQ384091  |           |
| Taxon | Collection locality | Collector and accession number | LSU | TEF |
|-------|---------------------|--------------------------------|-----|-----|
| Delitschia winteri II | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.K. Mugambi, GKM212N | DQ678077 | DQ677922 |
| Dothidea ribesia | | | AY016360 | |
| Gloiopsis praelonga I | | | FJ61193 | |
| Gloiopsis praelonga II | | | FJ61195 | FJ61103 |
| Glonium stellatum | | | FJ61179 | FJ61095 |
| Helicomyces roseus | | | DQ678083 | DQ677928 |
| **Herpotrichia cf. herpotrichoides I** | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.K. Mugambi, GKM196N | GU385176 | GU327755 |
| **Herpotrichia cf. herpotrichoides II** | U.S.A., Wisconsin, Upham Woods | S.M. Huhndorf, SMH5167 | GU385175 | |
| *Herpotrichia diffusa (= Byssosphaeria)* | | | DQ678071 | |
| **Herpotrichia juniperi I** | Kenyan Province, Kajiando District, Ngorng hills forest | G.K. Mugambi, GKM1128 | GU385178 | |
| **Herpotrichia juniperi II** | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.K. Mugambi, GKM1193 | GU385179 | |
| **Herpotrichia macrotricha I** | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.K. Mugambi, GKM196N | GU385176 | GU327755 |
| **Herpotrichia macrotricha II** | Kenya, Rift Valley Province, Kajiando District, Ngorng hills forest | G.K. Mugambi, GKM1128 | GU385178 | |
| **Herpotrichia macrotricha III** | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest | G.K. Mugambi, GKM1193 | GU385179 | |
| **Herpotrichia macrotricha IV** | U.S.A., Puerto Rico, Toru Negro Forest | S.M. Huhndorf, SMH269 | GU385177 | GU327756 |
| **Herpotrichia macrotricha V** | Costa Rica, Puntarenas, Monteverde | S.M. Huhndorf, SMH4913 | GU385164 | GU327754 |
| **Hypsostroma caimetalensis** | Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park | G.K. Mugambi, GKM1165 | GU385180 | |
| **Hypsostroma saxicola** | Costa Rica, San Jose, INBio Parque | S.M. Huhndorf, SMH5005 | GU385181 | |
| *Hysterium angustatum* | | | FJ611194 | |
| *Hysterographium mori* | | | FJ611196 | FJ611104 |
| *Leptosphaeria doliolum I* | | | U43473 | |
| *Leptosphaeria doliolum II* | | | U43474 | |
| *Leptosphaeria doliolum III* | | | U43475 | |
| *Leptosphaeria macrospora* | | | DQ94092 | |
| *Letendraea helminthicola* | | | AY016362 | |
| **Lophiostoma alpigenum** | Kenya, Rift Valley Province, Kajiando District, Ololua forest | G.K. Mugambi, GKM1091b | GU385193 | GU327758 |
| *Lophiostoma anundinis* | | | DQ782384 | DQ782387 |
| *Lophiostoma caullium* | | | DQ528763 | |
| *Lophiostoma crenatum* | | | DQ678069 | DQ677912 |
| **Lophiostoma fuckelii I** | U.S.A., Puerto Rico, Luquillo Mts. | S.M. Huhndorf SMH1371 | GU385186 | |
| **Lophiostoma fuckelii II** | Kenya, Nairobi Province, Nairobi Museum Botanic Garden grounds | G.K. Mugambi, GKM1063 | GU385192 | GU327759 |
| *Lophiostoma fuckelii III* | | | DQ399531 | |
| *Lophiostoma heterospora* | | | AY016369 | DQ497609 |
| *Lophiostoma macrostomum I* | | | AB433273 | |
| *Lophiostoma macrostomum II* | | | AB433274 | |
| **Lophiostoma quadrinucleatum** | Kenya, Central Province, Nyeri district, Mt. Kenya forest, behind Bantu lodge | G.K. Mugambi, GKM1233 | GU385184 | GU327760 |
| *Lophiostoma sagittiforme* | | | AB369267 | |
| **Lophiostoma triseptatum I** | U.S.A., Michigan, Huron Mt. Club | S.M. Huhndorf, SMH2591 | GU385183 | |
| **Lophiostoma triseptatum II** | U.S.A., Michigan, Headland Park | S.M. Huhndorf, SMH5287 | GU385187 | |
| Taxon                              | Collection locality                                                                 | Collector and accession number | LSU               | TEF               |
|-----------------------------------|-------------------------------------------------------------------------------------|--------------------------------|-------------------|------------------|
| Lophium mytilinum I               | U.S.A., North Carolina, Highlands Biological Station                               | S.M. Huhndorf, SMH3291         | GU385197          |
| Lophium mytilinum II              | U.S.A., Tennessee, Smoky Mts                                                       | A.N. Miller, ANM73             | GU385198          |
| Massariosphaeria grandispora      | Kenya, Rift Valley Province, Kajando District, Ngong hills forest                   | G.K. Mugambi, GKM1238          | GU385173          | GU327761         |
| Massariosphaeria roumeguerei       | Kenya, Rift Valley Province, Lembus forest, along Eldoret – Elderma Ravine road     | G.K. Mugambi, GKM1280          | GU385174          | GU327762         |
| Massariosphaeria triseptata        | Kenya, Central Province, Nyeri District, Mt. Kenya forest, behind Bantu lodge       | G.K. Mugambi, GKM1210          | GU385212          | GU327763         |
| Massariosphaeria cruziformis       | U.S.A., Illinois, Swallow Cliff Woods                                              | S.M. Huhndorf, SMH5151         | GU385211          |
| Massariosphaeria kenyensis I       | Kenya, Coast Province, Taita District, Taifa hills, Ngango forest                   | G.K. Mugambi, GKM194N          | GU385188          | GU327765         |
| Massariosphaeria kenyensis II      | Kenya, Coast Province, Taita District, Taifa hills, Ngango forest                   | G.K. Mugambi, GKM234N          | GU385189          | GU327766         |
| Massariosphaeria kenyensis III     | Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park               | G.K. Mugambi, GKM L100Na       | GU385194          | GU327767         |
| Massariosphaeria minima I          | Kenya, Coast Province, Taita District, Taifa hills, Ngango forest                   | G.K. Mugambi, GKM169N          | GU385165          | GU327768         |
| Massariosphaeria minima II         | U.S.A., North Carolina, Smoky Mts                                                   | A.N. Miller, ANM60             | GU385182          |
| Massariosphaeria minima III        | U.S.A., North Carolina, Smoky Mts                                                   | A.N. Miller, ANM933            | GU385195          |
| Massariosphaeria minima IV         | Costa Rica, San Jose, San Gerardo de Dota                                            | S.M. Huhndorf, SMH2448         | GU385166          |
| Misturatosphaeria sp.              | French Guiana, Saül                                                                  | S.M. Huhndorf, SMH3747         | GU385196          |
| Misturatosphaeria tennesseensis    | U.S.A., Tennessee, Smoky Mts                                                        | A.N. Miller, ANM911            | GU385207          | GU327769         |
| Misturatosphaeria uniseptata       | Ecuador, Yasuni                                                                     | F.A. Fernández, A.N. Miller, SMH4330 | GU385167 | GU327770         |
| Misturatosphaeria uniseriata       | U.S.A., Tennessee, Smoky Mts                                                        | A.N. Miller, ANM909            | GU385206          |
| Montagnula opulenta                | U.S.A., Wisconsin, Upham Woods                                                      | S.M. Huhndorf, SMH5174         | GU385199          |
| Munkovalsaria appendiculata        | U.S.A., North Carolina, Highlands Biological Station                               | S.M. Huhndorf, SMH3291         | GU385197          |
| Mytilinidion australe              | Panama, Barro Colorado Island                                                       | S.M. Huhndorf, SMH3536         | GU385200          |
| Ostropella albocincta I            | Costa Rica, Arenal                                                                  | SMH1854                        | GU385203          |
| Ostropella striata                 | Costa Rica, Arenal                                                                  | SMH1854                        | GU385203          |
| Phaeodothis winteri                | U.S.A., Tennessee, Smoky Mts                                                        | A.N. Miller, ANM933            | GU385195          |
| Phaeosphaeria eustoma              | Costa Rica, Arenal                                                                  | SMH1854                        | GU385203          |
| Platystomum sp.                    | U.S.A., Wisconsin, Upham Woods                                                      | S.M. Huhndorf, SMH5174         | GU385199          |
| Taxon                              | Collection locality                                                                 | Collector and accession number | LSU       | TEF        |
|-----------------------------------|-------------------------------------------------------------------------------------|--------------------------------|-----------|------------|
| *Platystomum compressum*          | Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park               | G.K. Mugambi, GKM1048          | GU385204  | GU327772   |
| *Pleomassaria siparia*            |                                                                                     |                                | DQ678078  | DQ677923   |
| *Pleomassariaceae*                | New Zealand, Auckland, Wenderholm Regional Park                                     | S.M. Huhndorf, SMH5232         | GU385205  | GU327773   |
| *Pleospora herbarum* I            |                                                                                     |                                | DQ247804  | DQ471090   |
| *Pleospora herbarum II*           |                                                                                     |                                | DQ678049  |            |
| *Pleospora herbarum III*          |                                                                                     |                                | DQ677888  |            |
| *Pleospora herbarum IV*           |                                                                                     |                                | AF382386  |            |
| *Pleospora herbarum V*            |                                                                                     |                                | U43476    |            |
| *Pleospora* sp.                   |                                                                                     |                                | EF177848  |            |
| *Preussia tericola*               |                                                                                     |                                | DQ471137  |            |
| *Pseudotrichia guatopoensis* I    | U.S.A., Puerto Rico, Luquillo Mts                                                   | S.M. Huhndorf, SMH1288         | GU385208  |            |
| *Pseudotrichia guatopoensis II    | Costa Rica, San Jose, San Gerardo de Dota                                            | S.M. Huhndorf, SMH2383         | GU327775  |            |
| *Pseudotrichia guatopoensis III   | Costa Rica, Alajuela, Volcan Arenal                                                 | S.M. Huhndorf, SMH4535         | GU385202  | GU327774   |
| *Pseudotrichia mutabilis I*       | U.S.A., Wisconsin, New Glarus State Park                                            | S.M. Huhndorf, SMH1541         | GU385209  |            |
| *Pseudotrichia mutabilis II*      | U.S.A., Michigan, Headlands Park                                                    | S.M. Huhndorf, SMH5288         | GU385210  |            |
| *Psiloglonium clavisporum* II      |                                                                                     |                                | FJ167526  | FJ161105   |
| *Pyrenophora phaeocomes*          |                                                                                     |                                | DQ499996  |            |
| *Pyrenophora tritici repentinis*   |                                                                                     |                                | AY544672  |            |
| *Setosphaeria monoceras*          |                                                                                     |                                | AY016368  |            |
| *Stylophoris puccinioides*        |                                                                                     |                                | AY004342  |            |
| *Thyridaria macrostomoides* I     | Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park               | G.K. Mugambi, GKM1033          | GU385190  | GU327776   |
| *Thyridaria macrostomoides II     | Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest                  | G.K. Mugambi, GKM224N          | GU385191  | GU327777   |
| *Thyridaria macrostomoides III    | Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park               | G.K. Mugambi, GKM1159          | GU385185  | GU327778   |
| *Trematosphaeria pertusa I*       | U.S.A., Wisconsin, Madison, Picnic Point Park                                      | S.M. Huhndorf, SMH1448         | GU385213  |            |
| *Trematosphaeria pertusa II*      |                                                                                     |                                | FJ201990  |            |
| *Trematosphaeria pertusa III*     |                                                                                     |                                | FJ201992  |            |
| *Tubefugia cerea*                 |                                                                                     |                                | DQ70982   |            |
| *Tubefugia helicomyces*           |                                                                                     |                                | DQ767638  |            |
| *Tubefugia paludosa*              |                                                                                     |                                | AY849966  |            |
| *Ulospora bilgramii*              |                                                                                     |                                | DQ678076  |            |
| *Verruculina enalia*              |                                                                                     |                                | DQ678079  |            |
| *Westerdykella cylindrica*        |                                                                                     |                                | AY004343  |            |
| *Xenolophium sp.*                 | Panama, Barro Colorado Island                                                       | S.M. Huhndorf, SMH3537         | GU385201  |            |
| *Xenolophium applanatum*          | U.S.A., Puerto Rico, Luquillo Mts                                                   | S.M. Huhndorf, SMH2055         | GU385214  |            |
| *Xenolophium guianense*           | Ecuador, Yasuni                                                                      | F.A. Fernández, A.N. Miller, SMH4711 | GU385215 |            |
| *Xenolophium pachythele*          | French Guiana, Saúl                                                                 | S.M. Huhndorf, SMH996          | GU327779  |            |