

# 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*, *Melanomma*, and *Pseudotrichia* were recovered within the family, while others such as *Ostropella* and *Xenolophium* 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 *Melanomma* 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 *Lophiostoma* clade, also within the family are *Thyridaria macrostomoides* based on new sequences from Kenyan collections and *Massariosphaeria triseptata*, *M. grandispora*, *Westerdykella cylindrica* and *Preussia terricola* 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. aurantonotata* 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. uniseptata* 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 *Pleosporales* is the order *Melanommatales*, created by Barr (1983) for taxa that had a combination of centrum (peripherally occurring asci) and hamathecium (trabeculate pseudoparaphyses) characters she believed were important at the ordinal level. Recent molecular phylogenetic studies (*e.g.*, Berbee 1996, Liew *et al.* 2000, Winka 2000, Lumbsch & Lindemuth 2001, del Prado *et al.* 2005, Schoch *et al.* 2006, Krays *et al.* 2006, Wang *et al.* 2007) have not supported the separation of *Melanommatales* from *Pleosporales*.

Although the concept of *Pleosporales* has recently attained some consensus (*e.g.* Winka 2000, Lumbsch & Lindemuth 2001, Krays *et al.* 2006, Schoch *et al.* 2006, Lumbsch & Huhndorf 2007,

Kirk *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.

Barr's (1990a) treatment of *Melanommataceae* included the following genera: *Ostropella*, *Keissleriella*, *Strickeria*, *Byssosphaeria* and *Melanomma* 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

& 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. *Pseudotrachia* 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 turbinate with a rounded pore and apical area that is usually light coloured (Barr, 1984). Barr (1984) initially segregated *Herpotrichia* into *Massariaceae* but later transferred it to *Lophiostomataceae* (Barr 1987). Bose's (1961) study on *Massarina* and related genera concluded that *Massarina*, *Herpotrichia* and *Keissleriella* are distinct but closely related, and he placed them in the *Pleosporaceae* citing lack of striking characters to justify the creation of a new family. *Pseudotrachia* was described for ascomycete fungi with immersed-erumpent to superficial ascomata with rounded or laterally compressed apex. On the basis of the shape of the apex, Petrak (1940) placed it in *Lophiostomataceae*. However, Barr (1990a) accepted the genus in *Platystomaceae* and noted substantial variability in ascomatal apical morphology. She observed that even in a single collection it could be rounded or compressed to somewhat triangular.

The genus *Ostropella* was originally named as a subgenus of *Ostropa* for *O. albocincta* and was raised to generic status and redescribed by von Höhnel (1918). Müller & von Arx (1962) reduced *Schizostoma*, *Xenolophium* and *Ostreionella* all to synonymy under *Ostropella*. Barr (1990a) provided the history of *Ostropella* and its relationship to these other taxa. Holm & Yue (1988) presented work on *Schizostoma* in which they tried to clarify some misconceptions that have surrounded the genus since its establishment and to resolve the placement of some species. Chesters & Bell (1970) reduced *Xenolophium* into synonymy under *Lophiostoma* and treated it in their "*L. pachythele* group". Barr (1990a) pointed to the close relationship of *Xenolophium* to *Ostropella* and proposed it be accommodated under *Ostropella*. She also noted the differences between the low apical crest observed in *Ostropella* (that appears almost ornamental) from the compressed papilla in *Lophiostoma* and thus placed the genus in *Melanommataceae*. *Xenolophium* was established for two species of fungi from Hawaii by Sydow (in Stevens 1925). However, the genus was in synonymy for a long time until resurrected by Huhndorf (1993) who also added two new species to the group. She treated *Ostropella* and *Xenolophium* in *Melanommataceae* and distinguished the two genera based on their ascomatal surface structure, morphology of the ascomatal wall in longitudinal section and ascospore morphology.

*Lophiostomataceae* was first erected by Nitschke (1869) and in its long existence the group has greatly increased in size. The overall character that has historically been used to distinguish the family is the slit-like ostiolar opening on a laterally compressed papilla (Chesters & Bell 1970, Holm & Holm 1988). Chesters & Bell (1970) considered the family to comprise taxa with laterally compressed apices, hence they included *Lophiostoma*, and *Platystomum* in the group. However, the variability in the papillate form in the lophiostomataceous fungi had been noted for quite some time with certain species exhibiting mixed morphologies even within a single collection (Holm 1957, Eriksson 1981, Holm & Holm 1988, Barr 1990a). Holm & Holm (1988) took a broad concept of the family including taxa with laterally compressed and rounded apices in the group. They accepted five genera in the family, *Lophiostoma*, *Lophiotrema*, *Massariosphaeria*, *Navicella* and *Trematosphaeria*, while Barr (1987) accepted *Dangeardiella*, *Herpotrichia*, *Massarina*,

*Lophiostoma*, *Lophidiopsis*, *Trichometasphaeria*, and *Cilioplea*. Recent molecular studies seem to support the view that the family is not exclusively composed of taxa with compressed papillae and that some taxa traditionally placed in this group belong elsewhere (e.g. Wang *et al.* 2007, Zhang *et al.* 2008).

*Lophiostoma* was circumscribed by Holm & Holm (1988) to include taxa that have immersed-erumpent ascomata with a distinctly flattened neck and opening by a slit-like ostiole. Asci are mostly clavate and ascospores are 1-septate, multiseptate or muriform, hyaline to dark brown. Recent phylogenetic work carried out on *Lophiostoma* species bearing these typical characters including the type species, *L. macrostomum*, indicated that the genus formed a monophyletic group (Tanaka & Hosoya 2008). *Lophiotrema* on the other hand was erected by Saccardo for "*Hyalophragmia*" and has been used in this sense for a long time. Its circumscription is thus highly heterogeneous (Holm & Holm 1988). Chester & Bell (1970) did not recognise *Lophiotrema* but 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 *Lophiostomataceae*.

*Hypsostromataceae* (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 *Melanommatales* (= *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 stipitate, basally arranged and fusiform, septate ascospores (Huhndorf 1994). *Hypsostroma* was erected by Huhndorf (1992) for two tropical wood-inhabiting species *H. saxicola* and *H. caimitalensis*. The two species bear close morphological resemblance, only slightly differing in their ascomatal and ascospore characters, with *H. caimitalensis* 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, Krus *et al.* 2006, Schoch *et al.* 2006, Wang *et al.* 2007, Zhang *et al.* 2008). This polyphyly 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 *Lophiostomataceae*, 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-MT<sup>®</sup>, U.S.A.) mounted on a Zeiss Axioskop microscope (Carl Zeiss<sup>®</sup>, 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/](http://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](http://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 *Taq* polymerase (Roche<sup>®</sup>, 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 °C for LSU, and initial 58 °C for TEF and then reduced by 1 °C during each of the first eight cycles and maintained at 50 °C for the remaining cycles. The fragments were sequenced using the Big Dye<sup>®</sup> Terminator Cycle Sequencing kit v. 3.1 (ABI PRISM, Applied Biosystems, Forster City, U.S.A.). Sequencing was performed using the same set of primers as the PCR. The other sequences used in the analyses were obtained from GenBank. Sequences were aligned using multiple sequence program Muscle<sup>®</sup> v. 3.6 (Edger 2004). The alignments were further manipulated manually and those regions, which could not be aligned with confidence were excluded from analyses. The final data matrices comprised of LSU data set with 140 taxa with 1179 unambiguously aligned characters, and a TEF data set with 57 taxa and 750 unambiguously aligned characters while the combined data set comprised of 49 taxa for which both genes were available with 1929 unambiguously aligned characters. Voucher specimens are deposited in the Field Museum Herbarium (F), while Kenyan specimens are deposited at the East Africa Herbarium (EA).

### 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 ran 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 100<sup>th</sup> 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.* 1990) 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 *Melanommataceae*, *Pleosporaceae*, *Lophiostomataceae*, *Delitschiaceae*, *Arthopyreniaceae* and *Hypsostromataceae* in at least one of the trees (Figs 1–3). The LSU tree showed clades with stronger BS support than the TEF tree. *Melanommataceae* 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 *Melanommataceae*, 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



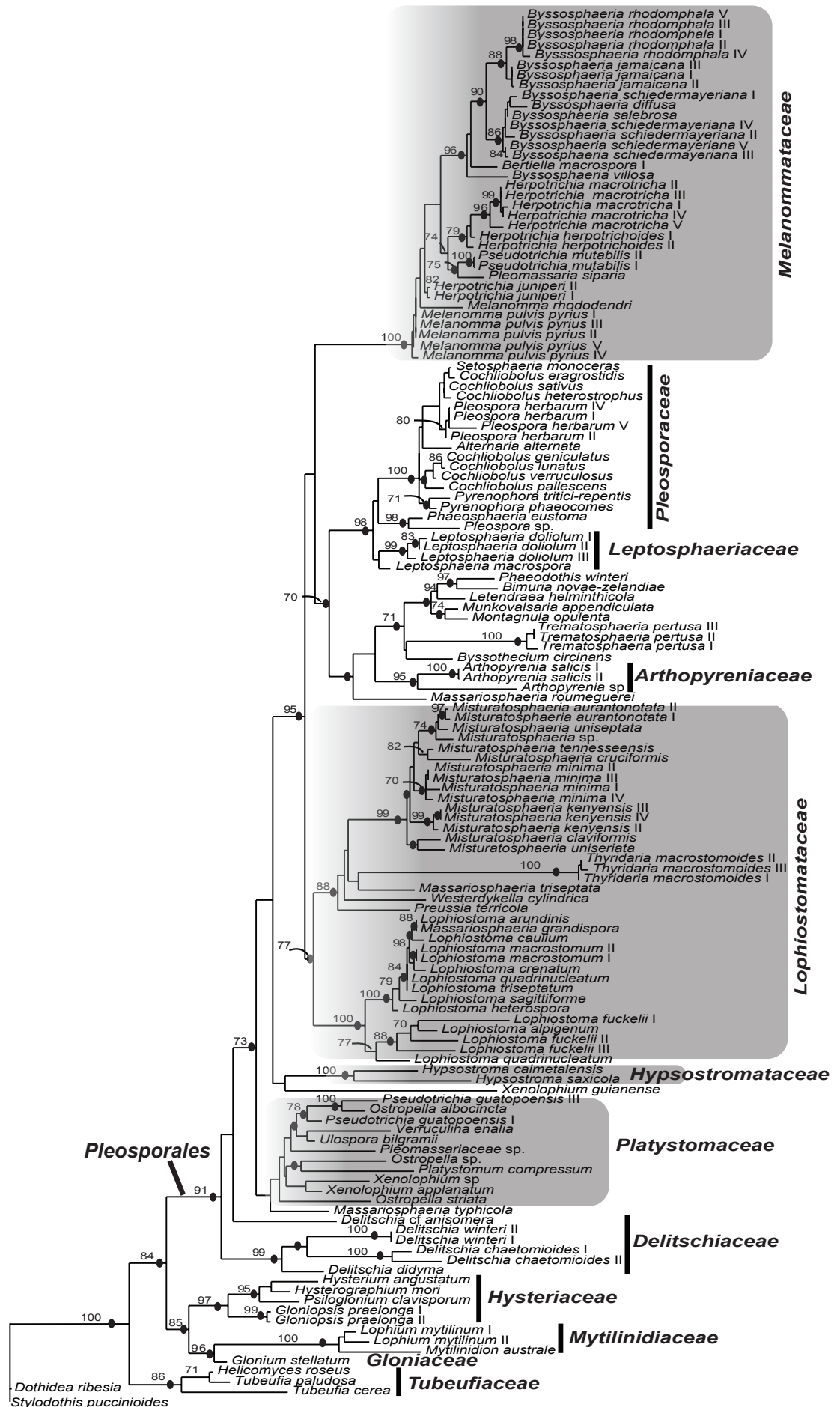
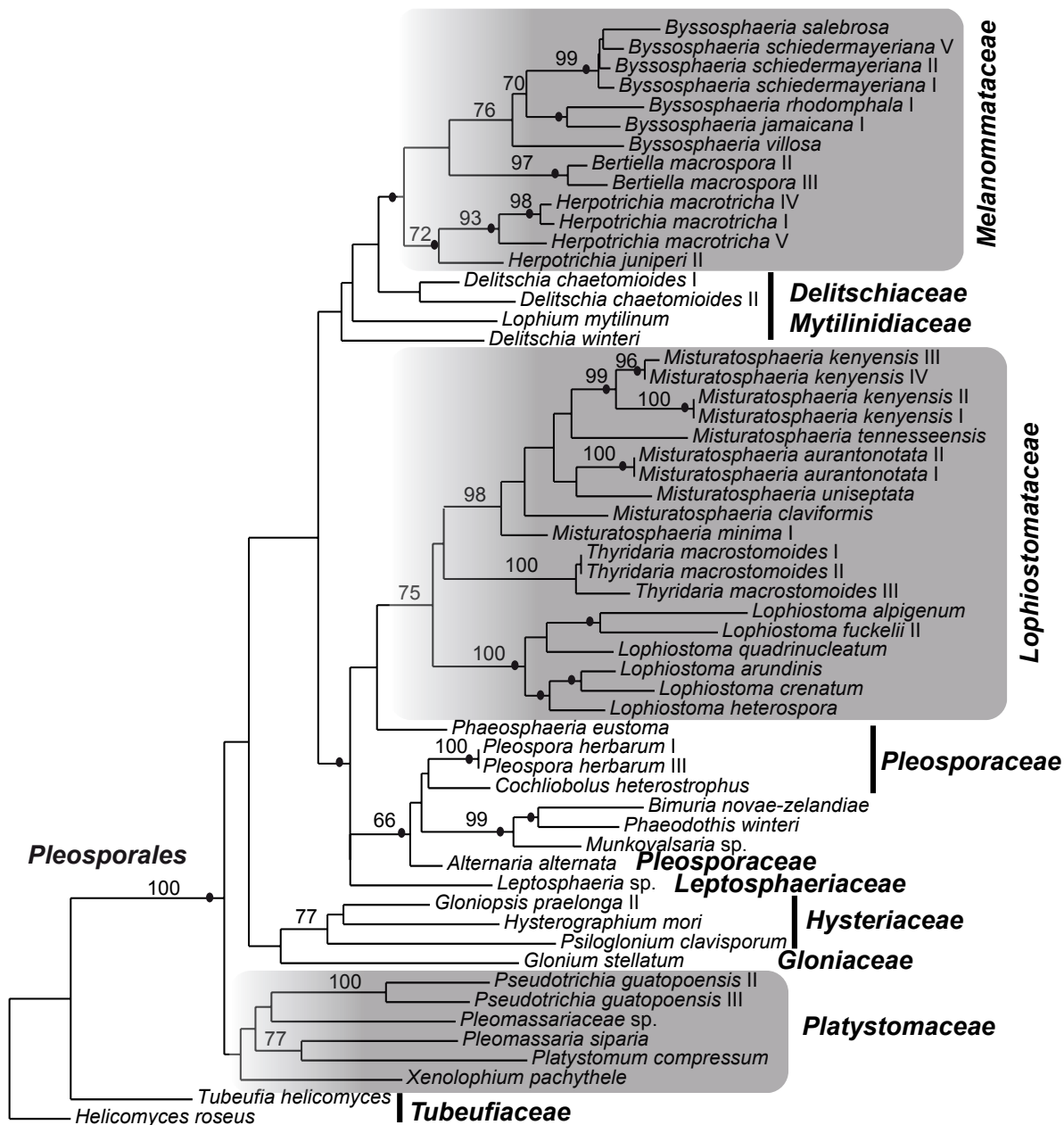


Fig. 1. Phylogram of the maximum likelihood analyses generated from LSU sequences. Bootstrap support values  $\geq 70\%$  are shown above or below the branches. Black circles indicate branches with Bayesian posterior probabilities  $\geq 95\%$ . The families treated in this study are indicated (shaded).





**Fig. 2.** Phylogram of the maximum likelihood analyses generated from TEF sequences. Bootstrap support values  $\geq 70\%$  are shown above or below the branches. Black circles indicate branches with Bayesian posterior probabilities  $\geq 95\%$ . The families treated in this study are indicated (shaded).

clade (Fig. 1). *Pseudotrachia* 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 Pleosporales; Hysteriaceae, Mytiliniaceae, Gloniaceae and Tubeufiaceae were recovered as strongly supported monophyletic groups outside the Pleosporales (Figs 1–3).

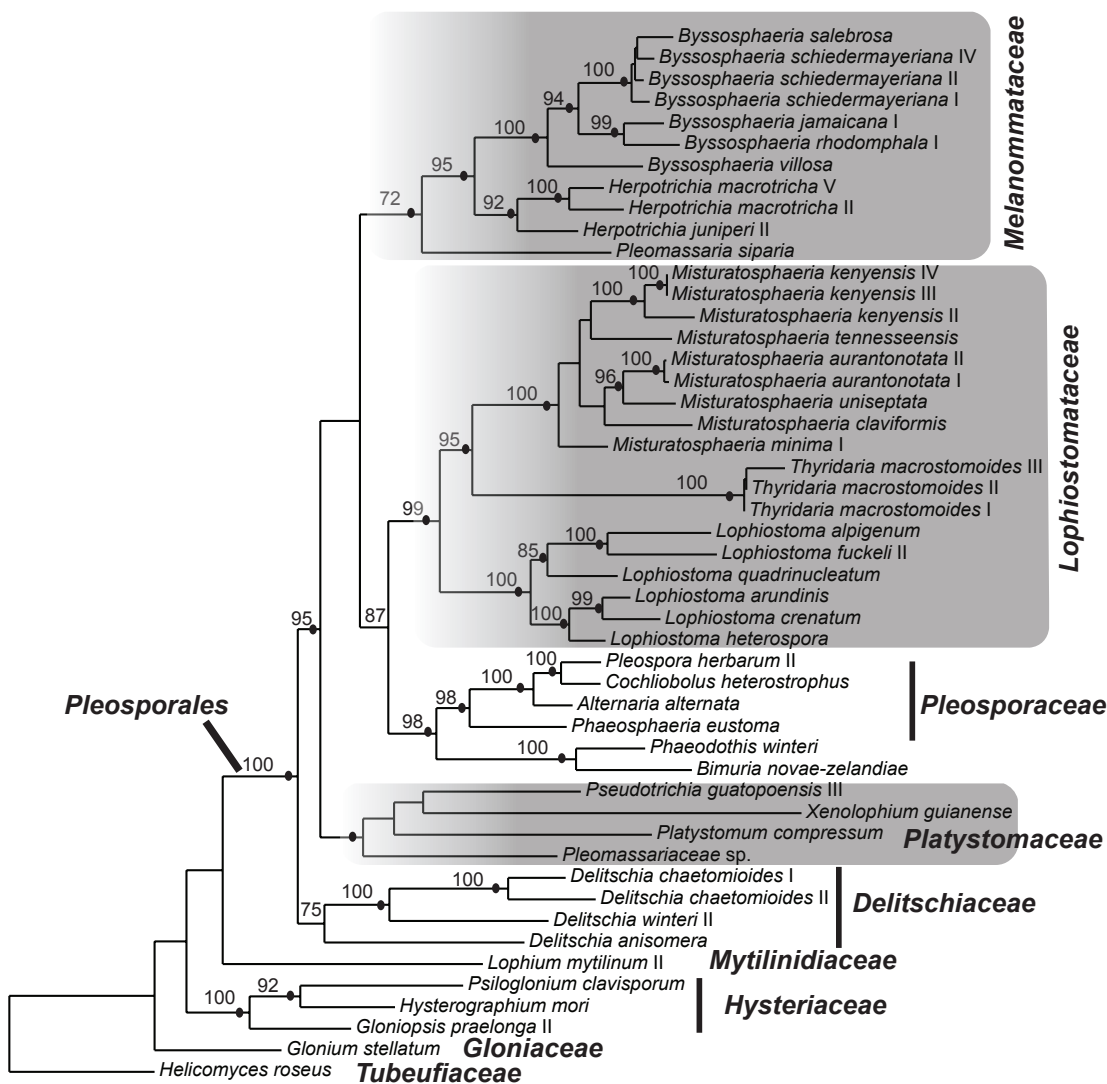


Fig. 3. Phylogram of the maximum likelihood analyses generated from the combined genes (LSU and TEF). Bootstrap support values  $\geq 70\%$  are shown above or below the branches. Black circles indicate branches with Bayesian posterior probabilities  $\geq 95\%$ . The families treated in this study are indicated (shaded).

## TAXONOMY

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 jamaicana* (Figs 4–5), *B. rhodomphala* (Figs 6, 8), *Bertiella macrospora* (Fig. 7), and *Byssosphaeria villosa* (Fig. 9). The second plate contains species of *Byssosphaeria*, *Melanomma* and *Pseudotrachia*: *Byssosphaeria schiedermayeriana* (Figs 10–13, 15), *B. salebrosa* (Fig. 14), *Melanomma pulvis-pyrius* (Fig. 16), *M. rhododendri* (Fig. 17), and *Pseudotrachia mutabilis* (Fig. 18). The third plate contains *Herpotrichia macrotricha* (Figs 19–22) and *H. cf. herpotrichoides* (Figs 23–24).

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

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

Ascomata erumpentia ad superficialia, solitaria vel aggregata, cum subiculum vel sine subicolo, apicibus rotundatis pallide coloratis vel incoloratis. Asci claviti vel cylindrici, breve stipitati, octospori, pseudoparaphysibus numerosis, hyalinis et septatis, in matrice mucosa. Ascospores hyalinae vel brunneae, septatae, cum vagina mucosa vel sine vagina.

**Typus:** *Misturatosphaeria aurantonotata* Mugambi & Huhndorf, sp. nov.

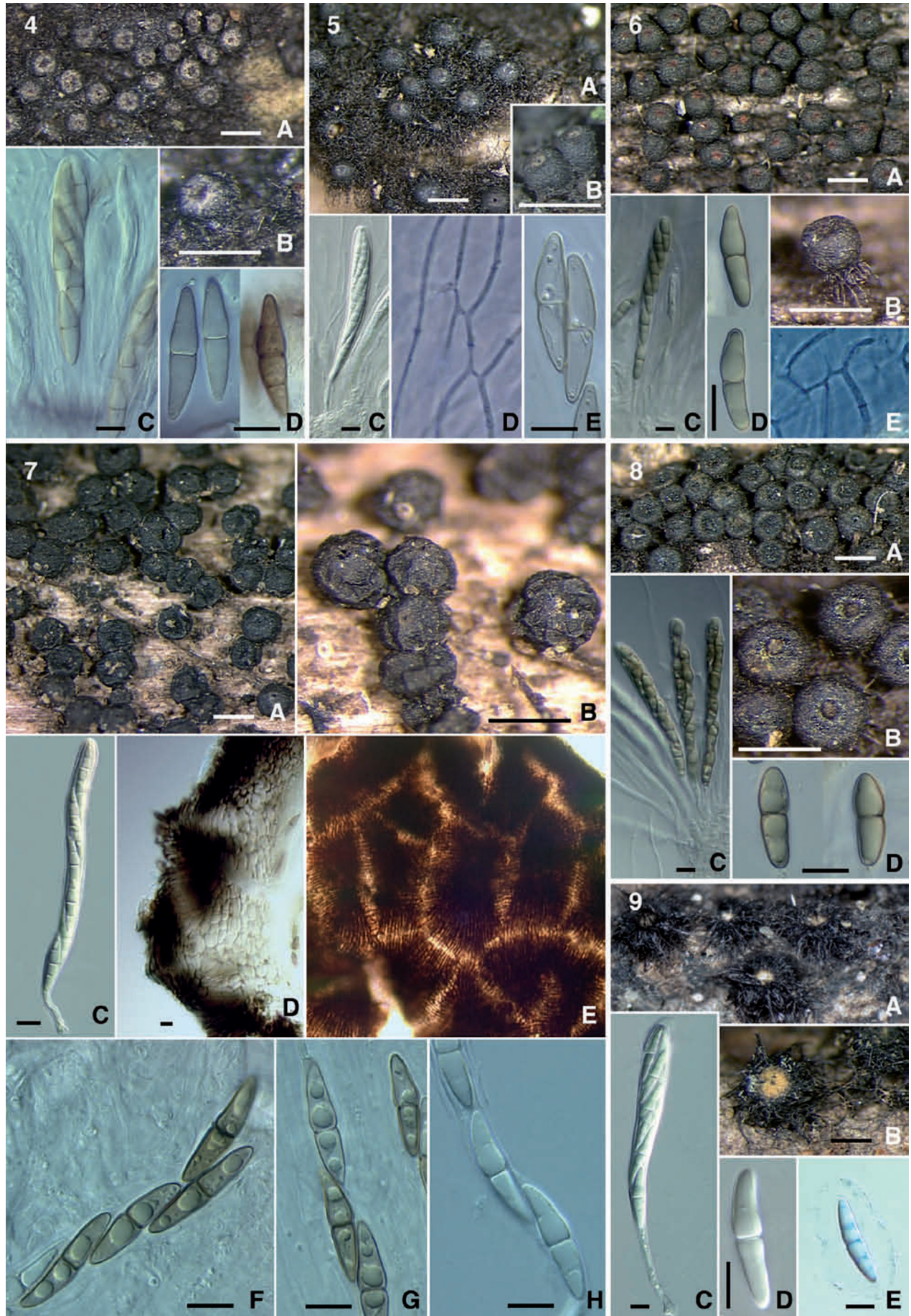
Ascomata erumpent to superficial, occurring singly or aggregated in clusters, subiculum present or lacking, apex rounded, with raised papilla or not, ostiole area light coloured or not and ostiole opening appearing plugged by gelatinous tissue. Asci cylindrical or clavate, short stipitate, 8-spored, 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.

***Misturatosphaeria 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.

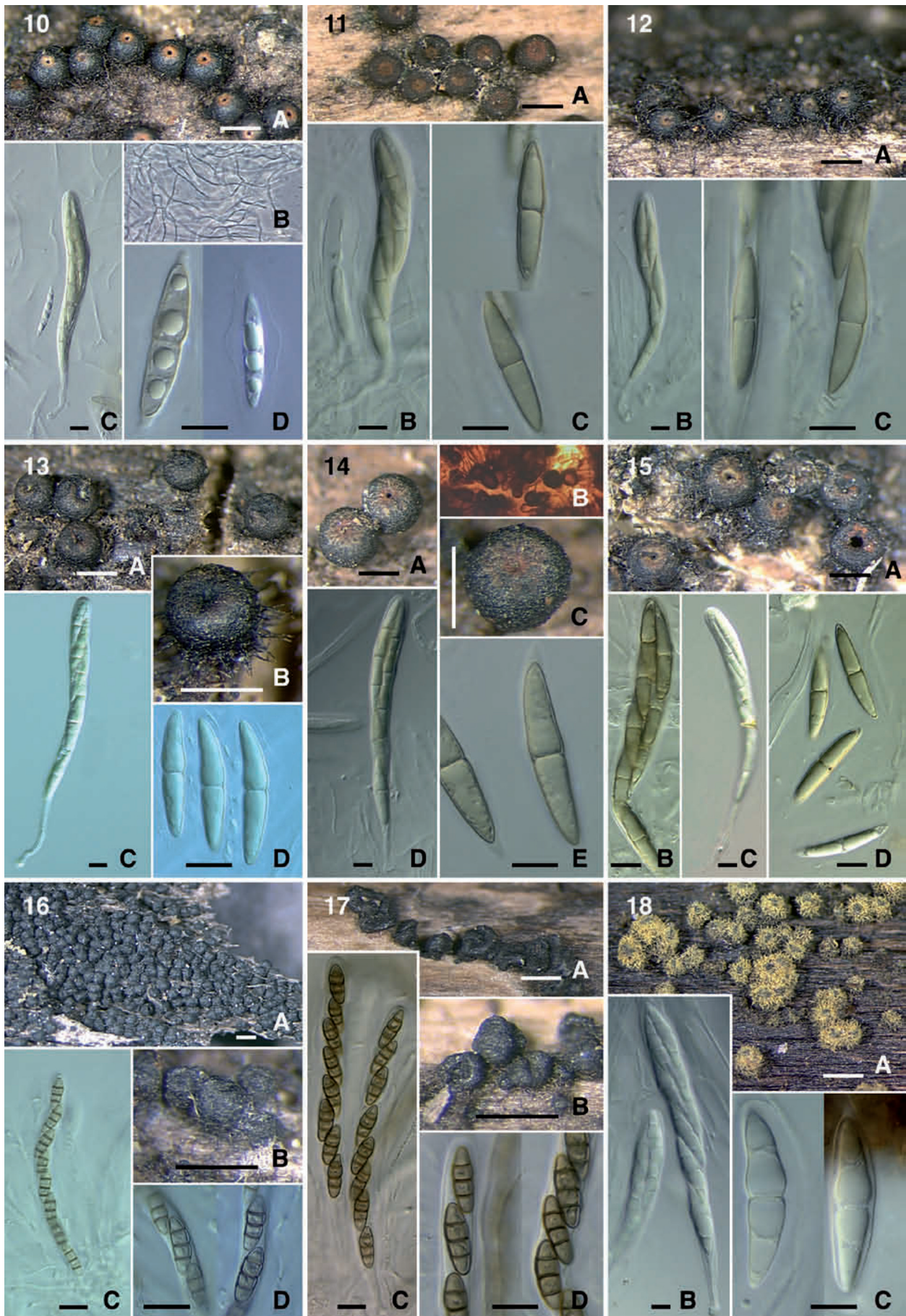
Ascomata superficialia, atrobrunnea, solitaria vel dense aggregata, in subiculo sparso ex hyphis brunneis, pyriformia, 441–710  $\mu\text{m}$  alta, 461–573  $\mu\text{m}$  diam, apicibus rotundatis, saepe aurantiacis. Asci cylindrici-clavati, breve stipitati, octospori, 103–122  $\times$  8–12  $\mu\text{m}$ . Pseudoparaphyses numerosae, septatae, in matrice mucosa, 1–2  $\mu\text{m}$ . Ascospores fusoides, primo hyalinae, deinde atrobrunnae, 3-septatae, cum vagina mucosa, 17–22  $\times$  5–6  $\mu\text{m}$ .





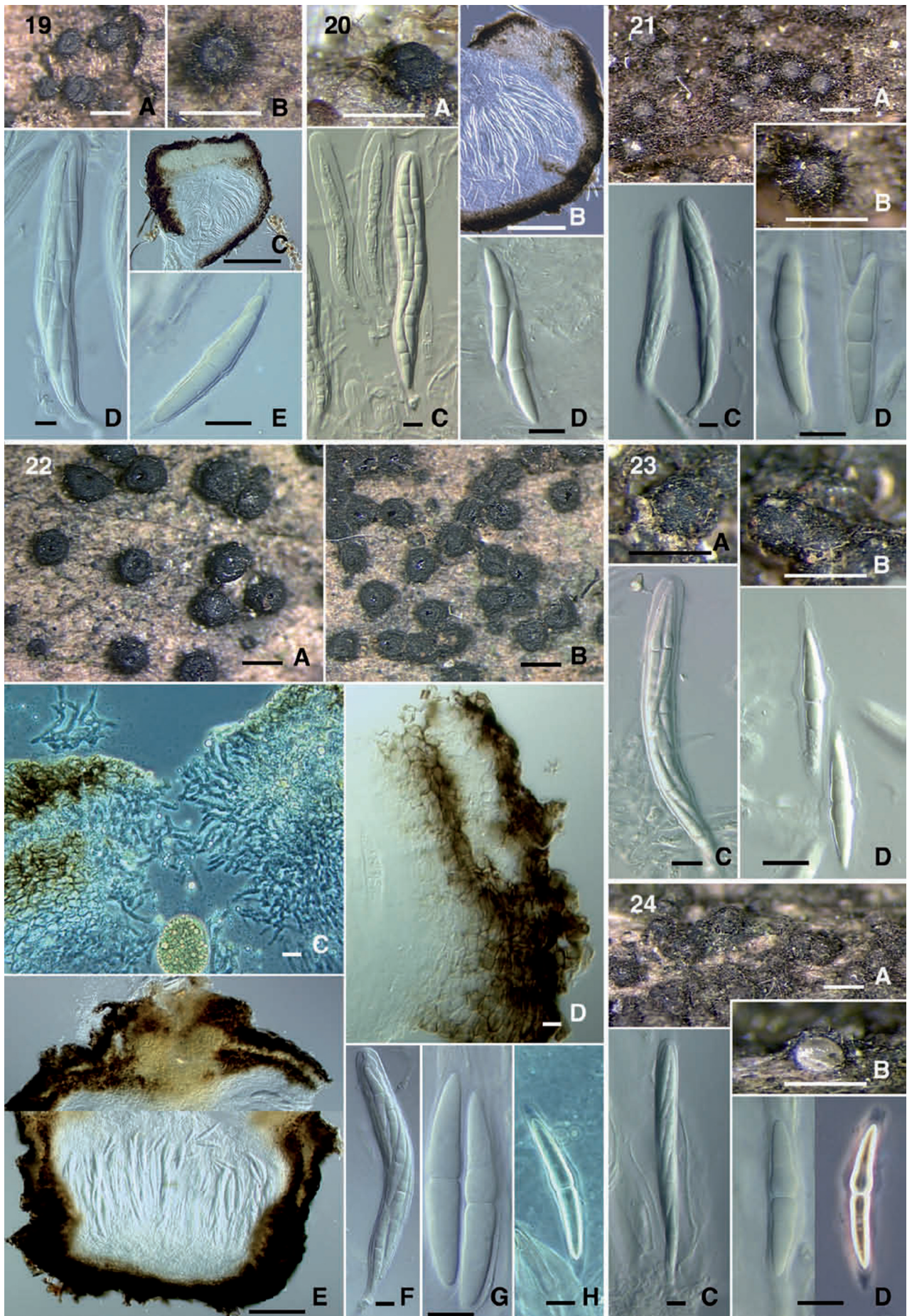
**Figs 4–9.** 4. *Byssosphaeria jamaicana* (SMH1403) A–B. Ascomata. C. Ascus. D. Ascospores. 5. *B. jamaicana* (SMH3464/3085) A–B. Ascomata. C. Ascus. D. Pseudoparaphyses. E. Ascospores. 6. *B. rhodomphala* (ANM942) A, B. Ascomata. C. Ascus. D. Ascospores. E. Pseudoparaphyses. 7. *Bertiella macrospora* (IL5005) A–B. Ascomata. C. Ascus. D. Ascospores. E. Ascomatal wall surface. F–H. Ascospores. 8. *B. rhodomphala* (SMH3402) A. Ascomata. B. Ascus. C. Ascospores. 9. *B. villosa* (GKM204N) A–B. Ascomata. C. Ascus. D–E. Ascospores. Scale bars: Ascomata = 500 µm. Wall = 10 µm. Ascus = 10 µm. Ascospore = 10 µm.





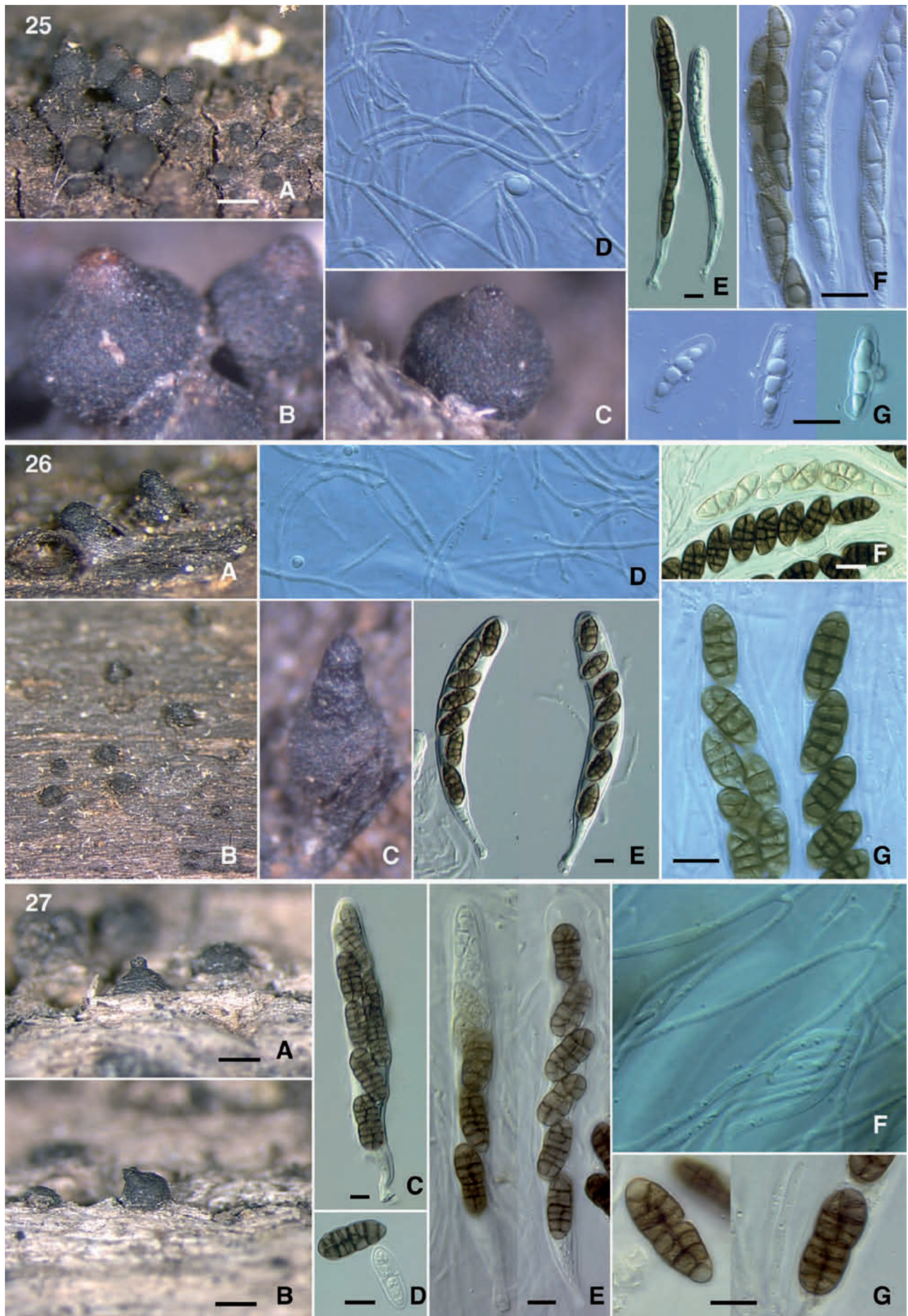
**Figs 10–18.** 10. *Byssosphaeria schiedermayeriana* (SMH1296) A. Ascomata. B. Pseudoparaphyses. C. Ascus. D. Ascospores. 11. *B. schiedermayeriana* (SMH3157) A. Ascomata. B. Ascus. C. Ascospores. 12. *B. schiedermayeriana* (SMH1816) A. Ascomata. B. Ascus. C. Ascospores. 13. *B. schiedermayeriana* (GKM1197) 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. Ascus. D. Ascospores. 16. *Melanomma pulvis-pyrius* (SMH3291) A–B. Ascomata. C. Ascus. D. Ascospores. 17. *M. rhododendri* (ANM73) A–B. Ascomata. C. Ascus. D. Ascospores. 18. *Pseudotrachia mutabilis* (SMH5288) A. Ascomata. B. Ascus. C. Ascospores. Scale bars: Ascomata = 500  $\mu\text{m}$ . Ascus = 10  $\mu\text{m}$ . Ascospore = 10  $\mu\text{m}$ .





**Figs 19–24.** 19. *Herpotrichia macrotricha* (SMH269) A–B. Ascomata. C. Ascumatal section. D. Ascus. E. Ascospore. 20. *H. macrotricha* (GKM196N) A. Ascoma. B. Ascumatal section. C. Ascus. D. Ascospores. 21. *H. macrotricha* (GKM1128) A–B. Ascomata. C. Ascus. D. Ascospores. 22. *H. macrotricha* (SMH4913) A–B. Ascomata. C. Ascumatal neck section. D. Ascumatal wall section. E. Ascumatal section. F. Ascus. G, H. Ascospores. 23. *H. cf. herpotrichoides* (GKM212N) A–B. Ascomata. C. Ascus. D. Ascospores. 24. *H. cf. herpotrichoides* (SMH5167) 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* (SMH5151) 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 all together. *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°23'934"S, 36°38'287"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, breve stipitati, octospori, 85–134 x 12–18 µm. *Pseudoparaphyses* numerosae, septatae, in matrice mucosa, 1–2 µm diam. *Ascosporae* ellipticae, brunneae ad atrobrunnae, muriformes, 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, 235–520 µm high and 195–322 µm wide. Apices rounded, usually possess raised, broad rounded papillae also with rounded openings. *Asci* cylindrical-clavate, with short stipes, 8-spored partially biseriate or uniseriate, oblique or sometimes irregularly arranged in an oblique fashion or irregular, 85–134 x 12–18 µm. *Pseudoparaphyses* numerous, septate, branching and anastomosing between and above the *asci*, held in gelatinous matrix, 1–2 µm diam. *Ascospores* elliptical, straight or inequilateral, brown to dark brown in colour, outer wall smooth, dictyosporous, with no mucilaginous sheath, 12–20 x 7–9 µm.

*Substratum*: Found on decorticated woody branches on the ground in forested areas.

*Anamorph*: Unknown.

*Distribution*: Currently known only from a tropical highland forest in central Kenya.

*Specimen examined*: Kenya, Central Province, Nyeri District, Mt Kenya forest, behind Bantu lodge, 0°6'907"S, 37°2'699"E, 30 Nov. 2006, on woody branch, G.K. Mugambi, GKM1210, **holotype** EA, **isotype** F.

***Misturatosphaeria cruciformis* Mugambi & Huhndorf, sp. nov.** MycoBank MB516009. Fig. 27.

*Etymology*: *Cruciatus* (L.) = cross-wise, refers to the *ascospore* septation, transverse and longitudinal septa.

*Ascomata* erumpentia, atrobrunnea, solitaria vel sparse aggregata, pyriformia, globosa, 500–535 µm alta, 545–649 µm diam, apicibus rotundatis, ostiolata, sine subiculo. *Asci* cylindrici-clavati, breve stipitati, octospori, 127–154 x 14–17 µm. *Pseudoparaphyses* numerosae, septatae, in matrice mucosa, 1–2 µm diam. *Ascosporae* oblongae ad ellipticae, brunneae ad atrobrunnae, muriformes, saepe constrictae ad septum medium, sine vagina mucosa, 19–26 x 8–13 µm.

*Ascomata* erumpent, usually occurring singly rarely clustered into small groups, subiculum lacking, pyriform to globose 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* cylindrical-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 mucilaginous 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.

*Specimen 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 kenyensis* 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 globosa, 185–305 µm alta, 245–334 µm diam, apicibus rotundatis pallide coloratis, ostiolo rotundato. *Asci* cylindrici-clavati, breve stipitati, octospori, 71–79 x 8–9 µm. *Pseudoparaphyses* numerosae, septatae, in matrice mucosa, 1–2 µm diam. *Ascosporae* fusoides, hyalinae, 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* cylindrical-clavate in shape, short stipitate, 8-spored, partially biseriate in arrangement, 71–79 x 8–9 µm. *Pseudoparaphyses* numerous 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 mucilaginous sheaths that extends at the tip of spores, 15–24 x 4–6 µm.

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

**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, 30 Oct. 2006, on woody branch, G.K. Mugambi, GKM1195, **holotype** EA, **isotype** F.

**Misturatosphaeria minima** Mugambi, A.N. Mill. & Huhndorf, **sp. nov.** MycoBank MB516011. Figs 30–32.

**Etymology:** *Minus* (L.) = less, refers to the relatively smaller size ascumata observed in this species.

Ascomata erumpentia ad superficialia, solitaria vel sparse aggregata, atrobrunnea, pyriformia ad globosa, 194–389 µm alta, 244–355 µm diam, sine subiculo, apicibus rotundatis saepe aurantiacis, ostiolo rotundato. Asci cylindrici-clavati, breve stipitati, octospori, 72–112 x 8–11 µm. Pseudoparaphyses numerosae, septatae, in matrice mucosae, 1–2 µm diam. Ascospores fusoides, hyalinae, 1–3-septatae, constrictae ad septum medium, cum vagina mucosa parva, 18–22 x 3–4 µm.

*Ascomata* erumpent through the bark or sometimes appearing superficial after the breakdown of the surrounding plant tissue, occurring singly or aggregated into small clusters usually less than five individuals, possesses no subiculum, pyriform to subglobose in shape, dark brown, ascomatal wall smooth, 194–389 µm high and 244–355 µm wide. Apices rounded, raised with rounded openings, occasionally the pore area appear orange in colour or the colouring is lacking. *Asci* cylindrical-clavate in shape bearing short stipes, 8-spored partially biseriate in arrangement, 72–112 x 8–11 µm. *Pseudoparaphyses* are numerous, septate, branching and anastomosing between and above the asci, held in a gelatinous matrix, 1–2 µm diam. *Ascospores* are fusiform, hyaline, the outer wall smooth, 1–3-septate and constricted at the middle septum, one of the middle cells is broader than the rest, possesses a small mucilaginous sheath that extends at the tips of the spore, 18–22 x 3–4 µm.

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

**Anamorph:** Unknown.

**Distribution:** Currently known from forested areas in Kenya, Costa Rica and U.S.A.

**Specimens examined:** Kenya, Coast Province, Taita Taveta District, Taita hills, Ngangao forest res., 3° 22' 30" S, 38° 20' 45" E, 1800 m elev., 13 Apr. 2005, on woody branch, G.K. Mugambi, GKM169N, **holotype** EA, **isotype** F. Costa Rica, San Jose, Albergue de Montagne, Savegre, Sendero la Cataracta, 9° 32' 38" N, 83° 48' 51" W, 13 May 1996, on woody branch on the ground, S.M. Huhndorf, F. Fernández, SMH2448, F. U.S.A., North Carolina, Smoky Mountains National Park, Cataloochee, Rough Fork Trail, 35° 61' N, 83° 12' W, 868 m elev., 23 May 2006, 1 cm diam woody branch on ground, A.N. Miller, A.Y. Rossman, M. Sogonov, L. Vasilyeva, G.K. Mugambi, ANM933, ILLS. Tennessee: Smoky Mountains National Park, vic. of Gatlinburg, Grotto Falls Trailhead, 35° 68' N, 83° 46' W, 944 m elev., 12 July 2004, 1 cm diam woody branch on ground, A.N. Miller, S.M. Huhndorf, G.K. Mugambi, L. Ruiz-Sanchez, ANM60, ILLS.

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

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

Ascomata erumpentia, solitaria vel dense aggregata, atrobrunnea, pyriformia, 265–398 µm alta, 201–401 µm diam, apicibus rotundatis. Asci cylindrici-clavati, breve

stipitati, octospori, 84–118 x 10–11 µm. Pseudoparaphyses numerosae, septatae, in matrice mucosa, 1–2 µm diam. Ascospores fusoides, brunneae, 3-septatae, constrictae ad septum medium, sine vagina mucosae vagina, 14–19 x 5–6 µm.

*Ascomata* erumpent through the bark, usually occurring singly or aggregated into small to large clusters, subiculum lacking, pyriform in shape, dark brown, ascomatal wall smooth, 265–398 µm high and 201–401 µm wide. Apices rounded, papillae are usually raised and often slightly sulcate with rounded opening. *Asci* cylindrical-clavate in shape, bearing short stipes, 8-spored partially biseriate, 84–118 x 10–11 µm. *Pseudoparaphyses* are numerous, septate, branching and anastomosing between and above the asci, held in gelatinous matrix, 1–2 µm diam. *Ascospores* fusiform, sometimes slightly curved, pale brown in colour, outer wall smooth, 3-septate with the septal area often of darker colour than rest of the spore, slightly constricted at the middle septum, one half of the spore composed cells that are slightly broader, possess no mucilaginous sheath, 14–19 x 5–6 µm.

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

**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 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.

Ascomata superficialia, solitaria vel sparse ad dense aggregata, atrobrunnea, pyriformia ad globosa, 230–293 µm alta, 318–385 µm diam, sine subiculo, apicibus rotundatis pallide coloratis. Asci cylindrici-clavati, breve stipitati, octospori, 70–82 x 5–6 µm. Pseudoparaphyses numerosae, septatae, in matrice mucosa, 1–2 µm diam. Ascospores fusoides, brunneae, 1-septatae, constrictae ad septum medium, sine vagina mucosa, 12–14 x 3–4 µm.

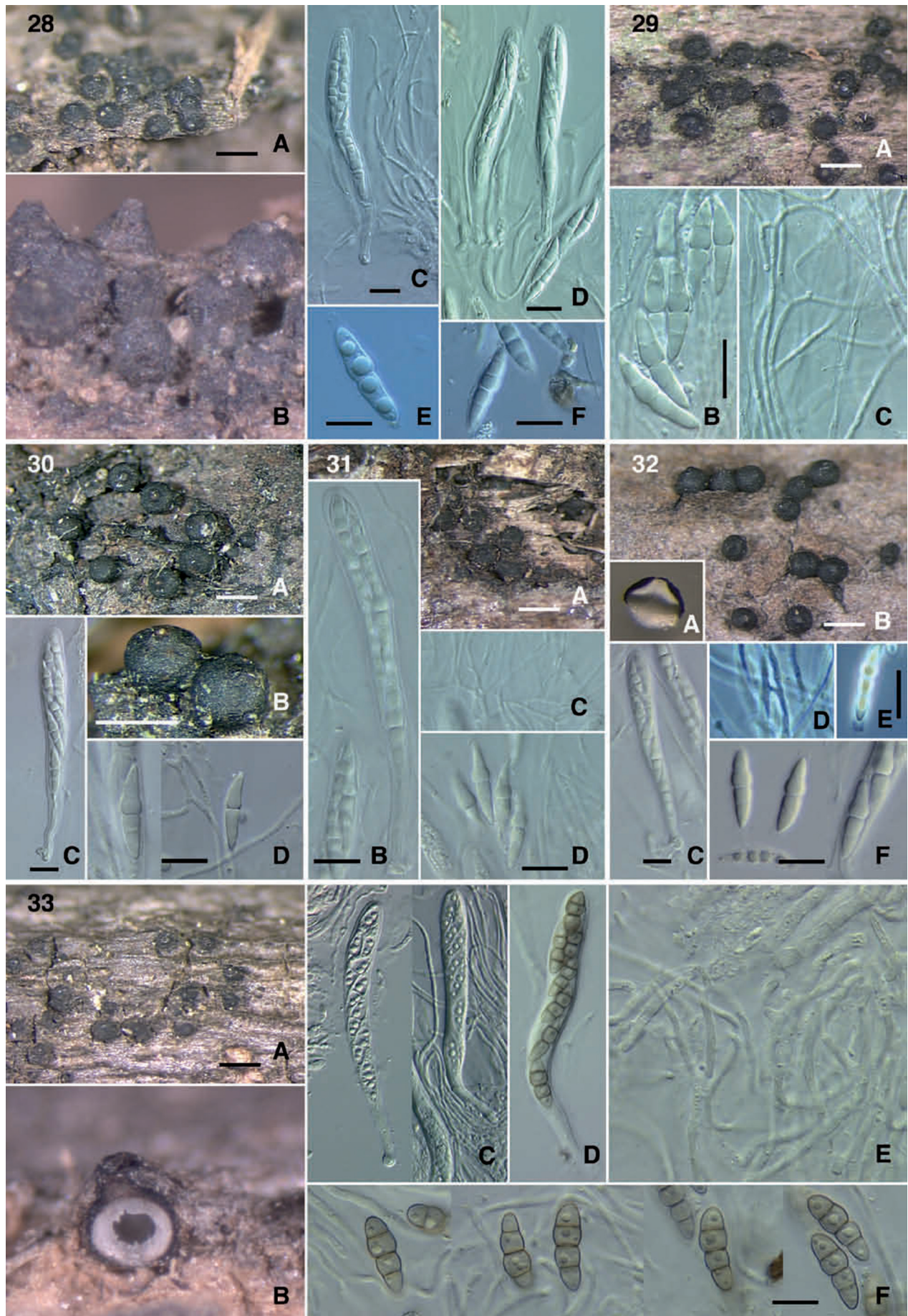
*Ascomata* superficial, occurring singly or aggregated into small to large clusters, subiculum absent, pyriform to globose in shape, dark brown, ascomatal wall smooth, 230–293 µm high and 318–385 µm wide. Apices rounded, often with slightly raised papillae, ostiole area often of lighter colour, ostiole openings often quite prominent but appear plugged by centrum tissue. *Asci* cylindrical-clavate in shape and bearing short stipes, 8-spored partially biseriate or sometimes overlapping uniseriate in arrangement, 70–82 x 5–6 µm. *Pseudoparaphyses* numerous, septate, branching and anastomosing between and above asci, held in gelatinous matrix, 1–2 µm diam. *Ascospores* fusiform, sometimes slightly curved or straight, brown, outer wall smooth, 1-septate with the septal area of darker colour, upper cell usually shorter and broader than the basal cell, slightly constricted at septum, possess no mucilaginous sheath, 12–14 x 3–4 µm.

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

**Anamorph:** Unknown.

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





**Figs 28–33.** 28. *Misturatosphaeria kenyensis* (GKM1195) A–B. Ascomata. C–D. Asci. E, F. Ascospores. 29. *M. kenyensis* (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. D. Ascospores. 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.



*Specimen examined:* Ecuador, Orellana Province, Yasuni National Park, Botanico trail, 5 Mar. 2001, on woody branch, F.A. Fernández, A.N. Miller, R. Briones, SMH4330, **holotype** F.

***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 asci.

Ascomata erumpentia, solitaria vel dense aggregata, atrobrunnea, pyriformia ad globosa, 332–343 µm alta, 309–379 µm diam, sine subiculo, apicibus rotundatis, pallide coloratis. Asci cylindrici ad cylindrici-clavati, breve stipitati, octospori, 100–130 x 8–10 µm. Pseudoparaphyses numerosae, septatae in matrice mucosa, 1–2 µm diam. Ascospores fusoides ad ellipticae, brunneae ad atrobrunnae, 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 centrum tissue. *Asci* cylindrical to cylindrical-clavate, 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-septate but commonly 3-septate, 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 aurantonotata* bears some similarity with *M. tennesseensis* and *M. uniseriata* but differs from both in its much larger superficial ascomata and quite verruculose 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 biserial 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 uniseptata* differs from all the other species in the group in its pale brown, 1-septate ascospores. *Misturatosphaeria minima* and *M. kenyensis* 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 kenyensis* 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 ascomata, and ascospores that are very rarely slightly constricted. Molecular data also indicate the two species are distantly 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 Lophiostomataceae but only *T. macrostomoides* finds placement in the family based on molecular data (Figs 1–3).

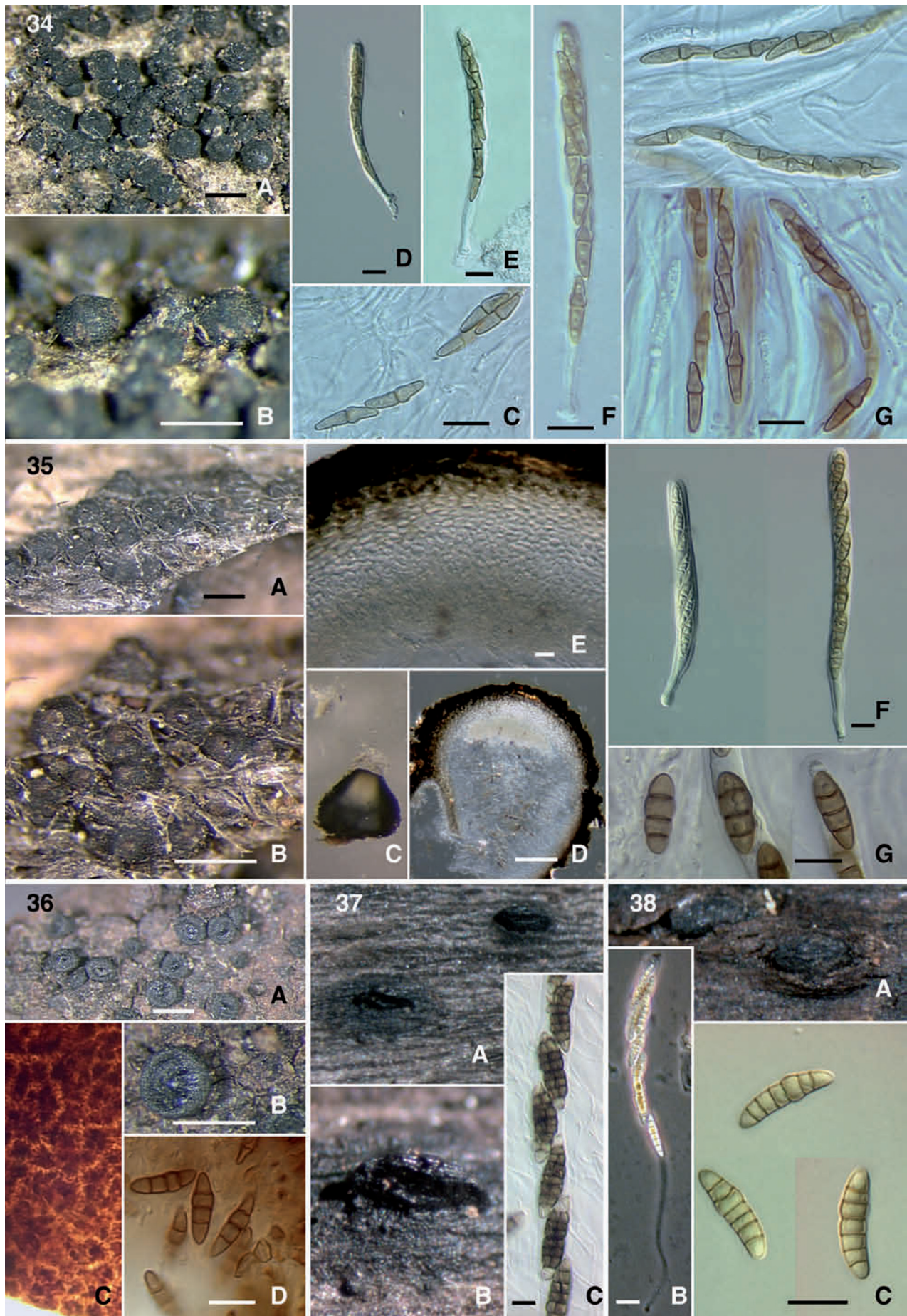
## DISCUSSION

### *Melanommataceae*

Phylogenetic analyses recovered *Melanommataceae*, *Lophiostomataceae*, *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*, *Pseudotrachia* and *Byssosphaeria*, were recovered within the family, others such as *Ostropella* and *Xenophium* 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 siparia*, find their placement within the *Melanommataceae* based on our data. *Byssosphaeria*, *Herpotrichia*, and *Pseudotrachia* 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 *Lophiostoma macrostomum* (DQ384094) and / or *Trematosphaeria pertusa* (DQ678020) that nested within *Melanommataceae*. Although voucher specimens were not obtained to verify their identity, we strongly believe that these collections were misidentified. *Lophiostoma macrostomum*, the type species of the *Lophiostoma*, 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 pulvis-pyrius* 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. pulvis-pyrius*. Morphologically our collections fit within the genus, both species having the clustered, superficial ascomata and small 3-septate brown ascospores in a uniseriate arrangement in the asci (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  $\mu$ m. Section = 100  $\mu$ m. Wall = 10  $\mu$ m. Ascus = 10  $\mu$ m. Ascospore = 10  $\mu$ m.



In contrast, a strongly supported, monophyletic *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 cylindrical, 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  $\mu\text{m}$  and a sheath is seen in some of the collections (Figs 10D, 13D). Nested 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 3-septate (Fig. 4D) (as was illustrated for the species by Sivanesan 1971). Five collections represent *B. 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 1984).

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* (= *Iodosphaeria*)” (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.

*Bertiella* also found its placement in the *Melanommataceae* in contrast to the *Teichosporaceae* where it was previously placed (Lumbsch & Huhndorf 2007). *Bertiella 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 wall cells bearing bands of less pigmented, thin-walled cells (Fig. 7) that was described by Eriksson & Yue (1986) as “resembling cephalothecoid”. 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  $\mu\text{m}$ , our collections have smaller ascospores, ranging 22–30 x 5–7  $\mu\text{m}$  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 cephalothecoid 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 *Lophiostomataceae* (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. herpotrichoides*, *H. juniperi* and *H. macrotricha*, and these taxa collectively do not form a monophyletic 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. herpotrichoides*, 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 near *Melanomma* (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 *Melanomma*. However, this will only become clear with broader taxon sampling in the groups. The described morphologies of *Melanomma* and *Herpotrichia* overlap in many ways, with both sharing immersed, erumpent to superficial ascomata that are tomentose and usually



sit on an ample subiculum. Asci 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 ascumatal 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, ascumatal wall with a central hyaline layer that gives the ascumata 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 *Platystomaceae* by Barr (1990b), but is correctly placed in the *Melanommataceae* 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 to the single collection of *Pleomassaria*. *Pseudotrichia mutabilis* is distinctive and easily identified with its gregarious, yellow-green tomentose ascumata and its hyaline, fusiform, septate ascospores (Fig. 18). It is a common entity on decaying wood in temperate forests. As currently circumscribed however, the genus is not monophyletic as our other included species, *P. guatopoensis*, finds a placement among the taxa in the unsupported *Platystomaceae* clade.

*Pleomassaria siparia* (lectotype species of the genus) is represented by two LSU sequences in GenBank (one used here), both coming from the same CBS 279.74 culture. In our analyses the species is nested within the *Melanommataceae* in the clade with *Pseudotrichia mutabilis*. According to Barr (1982: 370) *Pleomassaria siparia* is characterised by immersed, depressed globose ascumata 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 *Platystomaceae* 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 *Melanommataceae* 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 *Melanommataceae* s. str. They possess relatively large ascumata, bearing raised apices with slit-like ostiolar openings. The asci in these two genera are clavate with quite long stipes, which is unlike taxa that are here treated in

*Melanommataceae*. 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 *Melanommatales* 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 *Melanommatales* from *Pleosporales* based on this character is not supported (Liew *et al.* 2000, Lumbsch & Lindemuth 2001, Krays *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 ascumata, 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 *Platystomaceae* 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: ascumata that are immersed or erumpent, in valsoid groups or separate or gregarious; ascumata globose with a well developed papilla or short beak that is rounded or compressed; ostioles that are rounded or slit-like and periphysate or filled with pallid or brightly pigmented hyphal ends. Asci 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 ascumata 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 *Misturatosphaeria* 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 dictyospores accepted by Barr (1990b) in this family is *Cilioplea*. This genus was not included in our analyses but it differs markedly from the dictyosporous members of *Misturatosphaeria* 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. caimitalensis*. She did not prefer familial placement at the time but suggested they bear affinities in the *Melanommatales* (= *Pleosporales*). Later, Huhndorf (1994) erected family *Hypsostromataceae* for the genera *Hypsostroma* and *Manglicola*. 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 *Xenolophium* 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 suggests 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 (*P. 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 *Xenolophium* are found to occur outside the *Melanommataceae* in a weakly supported group along with *Platystomum compressum* and *Pseudotrachia 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 *Misturatosphaeria* that bears morphology traditionally not known to occur in the family. The gregarious ascomata of *Misturatosphaeria*, 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 *Misturatosphaeria* versus plane to collapsed apices in *Byssosphaeria*, along with the lack of hyphal hairs or subiculum in the former. Distinctive characteristics of *Misturatosphaeria* 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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