



Macrodiplodiopsis in *Lophiostomataceae*, *Pleosporales*

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Abstract

Macrodiplodiopsis desmazieri, the type species of *Macrodiplodiopsis*, was collected from Italy, and morpho-molecular studies were carried out. Phylogenetic analyses (maximum-likelihood, maximum parsimony and Bayesian) using combined data set of LSU, SSU and EF1- α sequences showed our strain to group in *Lophiostomataceae* (*Pleosporales*) with *Misturatosphaeria uniseriata* and other *Misturatosphaeria* spp. *Floricola striata*, the type species of *Floricola* also groups in the same clade with a close relationship with *Misturatosphaeria cruciformis*. The *Macrodiplodiopsis* clade is well supported with high bootstrap and posterior probability. *Floricola striata* and nine species of *Misturatosphaeria* are transferred to the older name *Macrodiplodiopsis* as new combinations.

Key words: coelomycetes, molecular phylogeny, morphology, multi-gene analyses, taxonomy

Introduction

The linking of asexual and sexual ascomycetous genera with a single name (Dai *et al.* 2012, Liu *et al.* 2012) has become a hot topic in fungal taxonomic studies. Recent studies on coelomycetous and hyphomycetous taxa have established several links with their sexual states (Crous *et al.* 2007, 2009, Boonmee *et al.* 2011, Chomnunti *et al.* 2011, Wijayawardene *et al.* 2013). Molecular techniques, such as PCR (White *et al.* 1990) and computer based phylogenetic and taxonomic studies, have allowed integration of previously unrelated groups of fungi (Shenoy *et al.* 2007, Taylor 2011, Dai *et al.* 2012, Liu *et al.* 2012, Manamgoda *et al.* 2012, Wijayawardene *et al.* 2012b, Hyde *et al.* 2013a,b).

Macrodiplodiopsis Petr. (1922: 343) is a coelomycetous ascomycete (Morgan-Jones *et al.* 1972, Sutton 1980), and Shear & Davidson (1936) mentioned that *M. desmazieri* (Mont.) Petr. (1922: 343) (as *Hendersonia desmazieri* Mont. (1849: 310)), the type species of *Macrodiplodiopsis*, is the asexual state of *Massaria platani* Ces. in Rabenhorst (1861: 323) [current name *Splanchnonema platani* (Ces.) M.E. Barr (1982: 364)]. However, Glawe (1985) rejected this link and to date *Macrodiplodiopsis* has not been placed in a natural classification system (Wijayawardene *et al.* 2012a), and sequence data for *Macrodiplodiopsis* is not available in GenBank.

We collected *Macrodiplodiopsis desmazieri* from Italy and carried out morpho-molecular studies. Combined gene (LSU, SSU rDNA and EF1- α) analyses using maximum-likelihood (ML), maximum-parsimony (MP) and MrBayes clearly showed this species groups along with *Floricola striata* Kohlm. & Volkm.-Kohlm. (2000: 385) and *Misturatosphaeria* species in *Lophiostomataceae*.

Materials and methods

Collection

Decaying plant litter was collected in Montebello (Ibola Valley), Italy. Specimens were placed in paper bags, taken to the laboratory and observed under a stereoscope to reveal the fungal taxa. Materials lacking fruiting bodies were incubated in a moist chamber to promote their development.

Morphological studies and isolation

Conidiomata were removed, placed in a droplet of distilled water on a clean slide, neatly squashed and examined under a compound microscope (Nikon Eclipse E600 DIC microscope and a Nikon DS-U2 camera or a Nikon Eclipse 80i compound microscope fitted with a Canon 450D digital camera) to observe the conidial characters. Single conidial isolation was carried out following the method described in Chomnunti *et al.* (2011). Germinating conidia were transferred aseptically to potato dextrose agar (PDA) plates and grown at 18°C. Colony colour and other characters were assessed after 5 days and 1 week. The specimens were deposited in the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Living cultures were also deposited in the Culture Collection at Mae Fah Luang University (MFLUCC), Landcare Research, New Zealand (ICMP) and Department of Plant Pathology, Agriculture College, Guizhou University, China (HGUP).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh fungal mycelia by using A BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416) (Wijayawardene *et al.* 2013). The amplification of rDNA regions of internal transcribed spacers (ITS), small subunit rDNA (SSU) and large subunit (LSU) was carried out by using ITS5 and ITS4, NS1 and NS4 (White *et al.* 1990) and LROR and LR5 (Vilgalys & Hester 1990) primers. We tried to get PCR products for EF1- α by using EF1-688F (Alves *et al.* 2004), EF1-986R (Carbone & Kohn 1999), EF1-526F, EF1-983F and EF1-1567R (Mugambi & Huhndorf 2009), however, these attempts were not successful. The amplification conditions for ITS, LSU and SSU were carried out according to Liu *et al.* (2012) and amplified PCR fragments were then sent to SinoGenoMax Co., Beijing, China for DNA sequencing. The nucleotide sequence data obtained were deposited in GenBank (Table 1).

TABLE 1. Sequence data used in this study.

Taxon	Culture collection number ¹	GenBank accession number		
		LSU	SSU	EF1- α
<i>Aigialus parvus</i>	BCC 32558	GU479779	GU479743	GU479843
<i>Amniculicola immersa</i>	CBS 123083	FJ795498	GU456295	GU456273
<i>Amniculicola parva</i>	CBS 123092	GU301797	GU296134	GU349065
<i>Anteaglonium abbreviatum</i>	ANM925a	GQ221877		
<i>Anteaglonium globosum</i>	SMH5283	GQ221911		GQ221919
<i>Floricola striata</i>	JK 5678I	GU301813	GU296149	GU479852
<i>Halothia posidoniae</i>	BBH 22481	GU479786		GU479752
<i>Lindgomyces breviappendiculatus</i>	KT 1215	AB521748	AB521733	
<i>Lindgomyces rotundatus</i>	KT 1096	AB521740	AB521723	
<i>Lophiostoma caulium</i>	CBS 624.86	GU301832		GU349007
<i>Lophiostoma compressum</i>	IFRD 2014	GU301834	GU296164	
<i>Lophiostoma crenatum</i>	AFTOL-ID 1581	DQ678069	DQ678017	DQ677912
<i>Lophiostoma quadrinucleatum</i>	GKM 1233	GU385184		GU327760
<i>Lophiotrema nucula</i>	CBS 627.86	GU301837	GU296167	GU349073
<i>Lophiotrema vagabundum</i>	KT664	AB619024	AB618706	
<i>Macrodiplodiopsis desmazieri</i>	MFLUCC 12-0088	KF531928	KF531927	
<i>Massaria anomia</i>	CBS 591.78	GU301839	GU296169	
<i>Massariosphaeria phaeospora</i>	CBS 611.86	GU301843	GU296173	
<i>Melanomma pulvis-pyrius</i>	CBS 124080	GU456323	GU456302	GU456265

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TABLE 1. (Continued)

Taxon	Culture collection number ¹	GenBank accession number		
		LSU	SSU	EF1- α
<i>Melanomma pulvis-pyrius</i>	CBS 371.75	GU301845	FJ201989	GU349019
<i>Misturatosphaeria aurantonotata</i>	GKM 1280	GU385174		GU327762
<i>Misturatosphaeria claviformis</i>	GKM 1210	GU385212		GU327763
<i>Misturatosphaeria cruciformis</i>	SMH 515	GU385211		
<i>Misturatosphaeria kenyensis</i>	GKM 1195	GU385194		GU327767
<i>Misturatosphaeria mariae</i>	YZ-2012	JN851819		
<i>Misturatosphaeria minima</i>	GKM 169N	GU385165		GU327768
<i>Misturatosphaeria tennesseensis</i>	ANM 911	GU385207		GU327769
<i>Misturatosphaeria uniseptata</i>	SMH 4330	GU385167		GU327770
<i>Misturatosphaeria uniseriata</i>	ANM 909	GU385206		
<i>Monotosporella tuberculata</i>	CBS 256.84	GU30185		GU349006
<i>Phaeomyco-centrospora cantuariensis</i>	CPC 10157	GU253712		GU384381
<i>Platystomum scabridisporum</i>	BCC 22835	GQ925844	GQ925831	GU479857
<i>Pontoporeia biturbinata</i>	BBH 23338	GU479796	GU479763	
<i>Preussia funiculata</i>	CBS 659.74	GU301864	GU296187	GU349032
<i>Preussia terricola</i>	DAOM 230091	NG_027612	DQ471063	
<i>Prosthemium betulinum</i>	VM20070116R	AB553757		
<i>Prosthemium betulinum</i>	CBS 279.74	AB553759		
<i>Prosthemium canba</i>	MK30	AB553770		
<i>Roussoella hysterioides</i>	HH 26988	AB524622	AB524481	AB539115
<i>Roussoellopsis tosaensis</i>	KT 1659	AB524625	AB524484	AB539117
<i>Tetraploa aristata</i>	CBS 996.70	AB524627	AB524486	AB524836
<i>Tetraplospora nagasakiensis</i>	KT 1682	AB524630	AB524489	AB524837
<i>Thyridaria rubronotata</i>	CBS 419.85	GU301875		GU349002
<i>Ulospora bilgramii</i>	CBS 110020	DQ384108	DQ384083	
<i>Verruculina enalia</i>	BCC 18401	GU479802	GU479770	GU479863
<i>Westerdykella dispersa</i>	CBS 508.75	DQ384099		
<i>Westerdykella ornata</i>	CBS 379.55	GU301880	GU296208	GU349021

1 AFTOL: Assembling the Fungal Tree of Life; ANM: A.N. Miller; BCC: BIOTEC Culture Collection, Bangkok, Thailand; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Collection of Pedro Crous housed at CBS; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; GKM; G.K. Mugambi; IFRD: Culture Collection, International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China; KT: K. Tanaka; MFLUCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MK: M. Kamiyama; SMH: S.M. Huhndorf; VM: Vadim A. Mel'nik.

Phylogenetic analyses

Blast searches of LSU and SSU sequences were carried out to reveal the closest taxa to our strain. Combined analyses of LSU, SSU rDNA and EF1- α sequences of closest relatives in *Aigialaceae*, *Amniculicolaceae*, *Anteagloniaceae*, *Halotthiaceae*, *Lindgomycetaceae*, *Lophiostomataceae*, *Lophiotremataceae*, *Melanommataceae*, *Pleomassariaceae*, *Roussoellaceae*, *Sporormiaceae*, *Tetraplosporaaceae*, *Testudinaceae* and *Thyridariaceae* were used to confirm the phylogenetic placement in *Pleosporales*. These sequences were downloaded from GenBank and aligned separately using Bioedit (Hall 2004) and ClustalX (Kohli & Bachhawat 2003). Alignments were checked and manual adjustments made where necessary and individual datasets concatenated into a combined dataset. Maximum-likelihood (ML) analysis was performed in RAXML (Stamatakis 2006) implemented in raxmlGUI v.0.9b2 (Silvestro & Michalak 2010). Maximum-parsimony (MP) analysis was carried out using PAUP v. 4.0b10 (Swofford 2003). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were valued by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Maximum trees were visualized with Tree View (Page 1996).

Results and discussion

Phylogenetic analyses

The combined LSU, SSU and EF1- α data set comprised 47 sequences from 45 taxa with *Massaria anomia* (CBS 591.78) as the outgroup taxon. The dataset consists of 4,016 characters after alignment, of which 1,859 are conserved, 1,177 are variable and 690 are parsimony informative in the ML and MP analyses. A best scoring RAxML tree is shown (Fig. 1) and bootstrap support (BS) values of ML and MP (equal to or above 50% based on 1,000 replicates) are shown on the upper branches. Values of the bayesian posterior probabilities (PP) from MCMC analyses are shown under the branches.

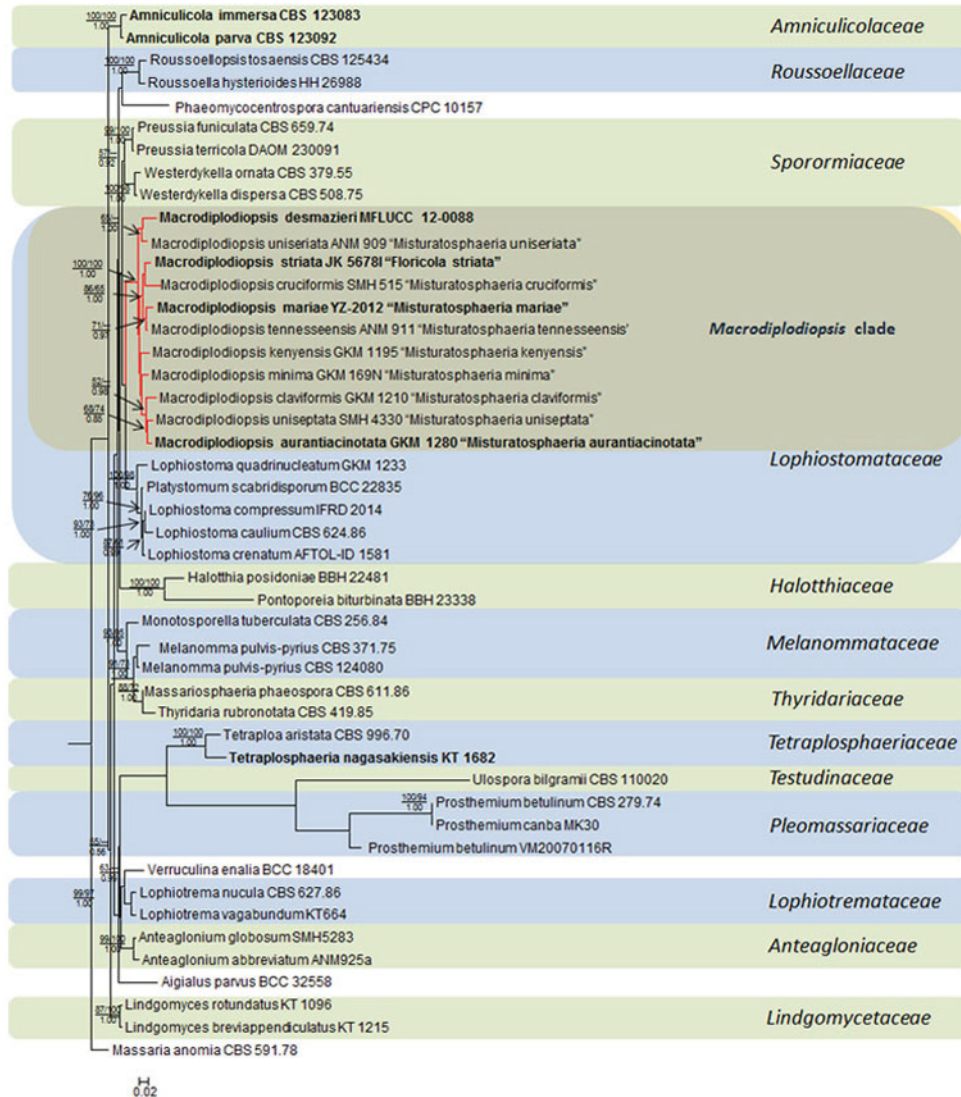


FIGURE 1. RAxML tree based on a combined dataset of LSU, SSU and EF1- α sequences. Bootstrap support values for maximum likelihood (ML) and maximum parsimony (MP) greater than 50% are given above the nodes; Bayesian posterior probabilities are given below the nodes. The original culture numbers are given after the species names. The tree is rooted to *Massaria anomia* (CBS 591.78). All sequences from type strains are in bold.

The *Macrodiplodiopsis* clade is a sister clade to the *Lophiostoma* clade and represents a well resolved genus in *Lophiostomataceae*. Our strain *Macrodiplodiopsis desmazieri* (MFLUCC 12-0088) grouped with *Misturatosphaeria uniseriata* (bootstrap value 65% in ML analysis and 1.00 in bayesian analysis) in a well-supported clade (100% in ML and 100% in MP analyses and 1.00 in PP analysis) with other *Macrodiplodiopsis* species and *Floricola striata*. *Floricola striata* grouped with *Misturatosphaeria cruciformis* within this clade, but with low bootstrap and low PP values. The grouping of *Floricola*, *Macrodiplodiopsis* and *Misturatosphaeria* as a single clade indicates that this is a monophyletic genus and should be represented by *Macrodiplodiopsis*, which is

the oldest name. We therefore combine all species under *Macrodiplodiopsis* below. The *Macrodiplodiopsis* clade is well supported and is morphologically distinct from *Lophiostomataceae* as shown in Hyde *et al.* (2013b). Although it would be premature to introduce a new family for this group, this may become necessary as further members of the group are sequenced.

Taxonomy

In this section, we synonymize *Floricola* and *Misturatosphaeria* under *Macrodiplodiopsis*.

Macrodiplodiopsis Petr., *Annls mycol.* 20(5/6): 343 (1922)

= *Floricola* Kohlm. & Volk.-Kohlm., *Bot. Mar.* 43(4): 385 (2000)

= *Misturatosphaeria* Mugambi & Huhndorf, *Stud. Mycol.* 64: 108 (2009)

Saprobic on decorticated branches, bark of *Platanus* and senescent leaves and inflorescences of *Juncus roemerianus*. Sexual state: *Ascomata* single or gregarious, erumpent to superficial, with or without a subiculum, rounded at the apex, with or without a raised papilla. *Ostiole* light or dark, opening appearing plugged by gelatinous tissue. *Hamathecium* with numerous branched pseudoparaphyses, held in a gelatinous matrix, septate. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short-pedicellate. *Ascospores* fusiform or oblong to elliptical, phragmosporous or dictyosporous, with roughened or smooth external walls, brown or hyaline, with or without a gelatinous sheath covering. Asexual state: *Conidiomata* separate or gregarious, immersed, globose to collabent, papillate, dark brown to black, unilocular, thick-walled, with wall cells of *textura porrecta* except at the base where they are *textura angularis*. *Ostiole* single, circular, papillate. *Conidiophores* absent. *Conidiogenous cells* annellidic, discrete, indeterminate, cylindrical, hyaline, formed from the inner cells of the pycnidial wall. *Conidia* ellipsoid to obovoid, or clavate, 3-distoseptate, occasionally with a longitudinal septum in the middle cell, pale brown, with lumina very much reduced and often surrounded by dark brown wall deposits, continuous, thick-walled, with truncate base and with an abscission scar, with obtuse apex, surrounded by a large gelatinous sheath.

Notes:—We are unaware of any *Misturatosphaeria* species having been linked to *Macrodiplodiopsis*, *Floricola* or any other asexual states (Mugambi & Huhndorf 2009, Zhang *et al.* 2012). In our collection of *Macrodiplodiopsis* we found no evidence of a “*Misturatosphaeria*” sexual state on the host substrate, nor did one form in culture. In this study we linked these genera through molecular analyses indicating the power of such techniques.

Type species:—*Macrodiplodiopsis desmazieri* (Mont.) Petr., *Annls mycol.* 20: 343 (1922) (Figs 2, 3)

≡ *Hendersonia desmazieri* Mont., *Annls Sci. Nat., Bot., sér. 3*, 12: 310 (1849) [1848–49]

Saprobic on dead branches of *Platanus*. Sexual state: unknown. *Conidiomata* separate or gregarious, immersed, globose, papillate, dark brown to black, unilocular, thick-walled, with wall cells *textura porrecta* except at the base, where they are *textura angularis*. *Ostiole* central, single, circular, papillate. *Conidiophores* absent. *Conidiogenous cells* obclavate to cylindrical, wider at the base, annellidic, discrete, indeterminate, cylindrical, hyaline, arising from the inner cells of the pycnidial wall, 12–25 × 3–5 μm. *Conidia* ellipsoid to obovoid, or clavate, 3-distoseptate, with granular cytoplasm and much reduced lumina and often surrounded by dark brown wall deposits, continuous, pale brown, thick-walled, with truncate base and obtuse apex, surrounded by a thick gelatinous sheath, 43–49 × 17–19 μm (\bar{x} = 45.25 × 18.22 μm, n = 20).

Colonies on PDA, brown on surface and white at margin, slow growing, attaining 2 cm diam. after 2 weeks at 18°C, with thin mycelium, circular. Reverse of the colony brown after 2 weeks.

Material examined:—ITALY. Forli-Cesena Province: Modigliana, Montebello (Ibola Valley), on branches of *Platanus acerifolia*, 15 April 2013, Erio Camporesi NNW-IT39 (MFLU 13-0090), ex-type cultures = MFLUCC 12-0088 = HGUP T69 = ICMP 19882.

Notes:—In our phylogenetic analyses (Fig. 1), *Macrodiplodiopsis uniseriata* (≡ *Misturatosphaeria uniseriata*) (ANM 909) groups with *Macrodiplodiopsis desmazieri* (MFLUCC 12-0088) with relatively high bootstrap values in maximum-likelihood analysis (65%) and 1.00 in PP analysis. However, in maximum parsimony analysis the bootstrap value is less than 50%. These two strains may be sexual and asexual states of the same species, but because of the low maximum parsimony support values we maintain both species until further molecular data is available for more strains.

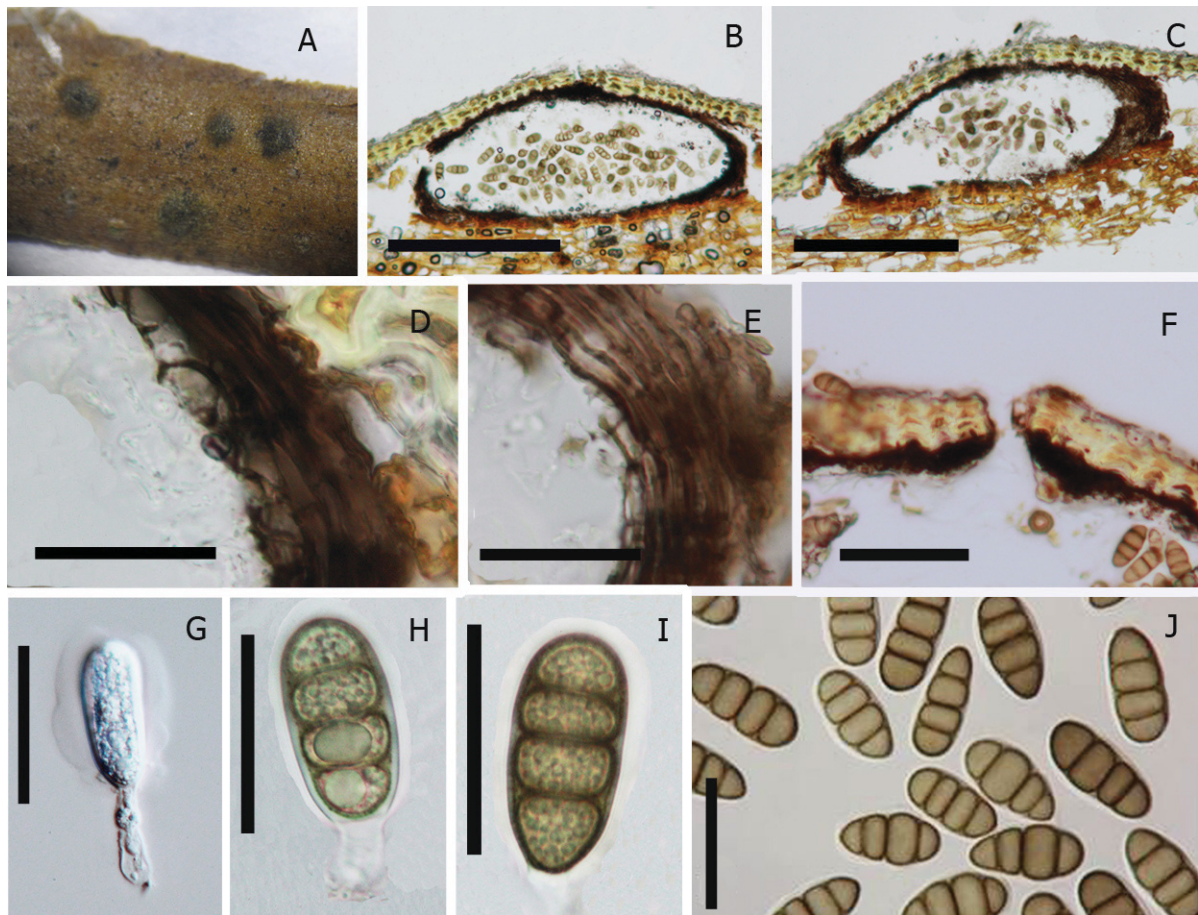


FIGURE 2. *Macrodiplodiopsis desmazieri* (MFLU 13-0090) A. Conidiomata on the host. B–C. Cross section of conidiomata. D–E. Conidiomata wall. F. Short neck of the conidiomata. G–I. Different stages of conidium development. J. Conidia. Scale bars: B, C=200 μ m, D, E, I, J = 40 μ m, F = 100 μ m, G, H = 30 μ m.

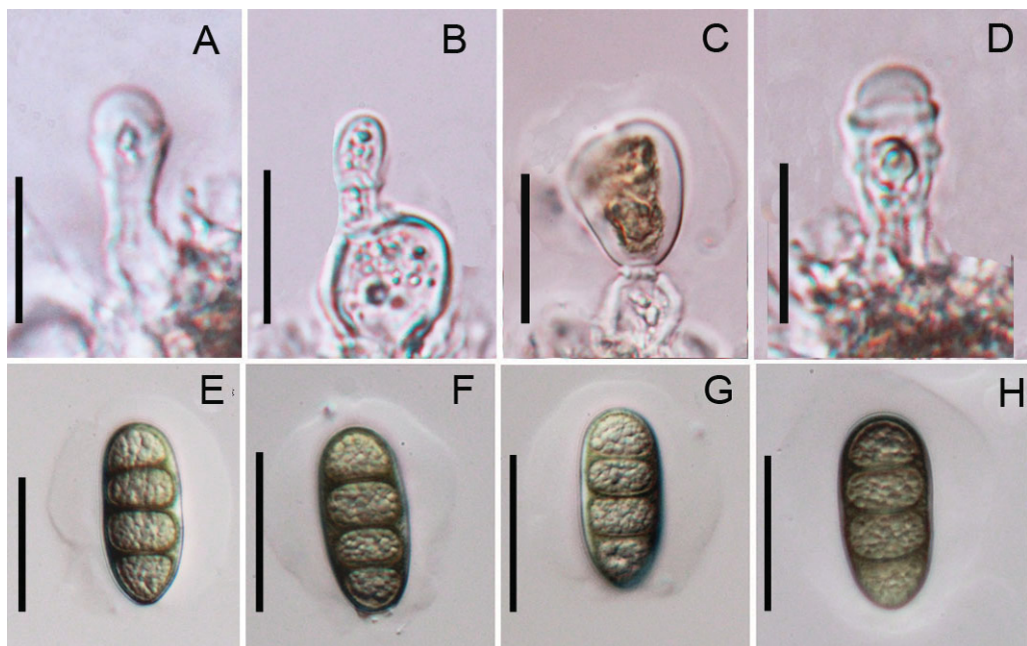


FIGURE 3. *Macrodiplodiopsis desmazieri* (MFLU 13-0090) A–D. Different stages of conidiogenesis. E–H. Mature conidia with mucilaginous sheath. Scale bars: A, B, D = 15 μ m, C, E–H = 30 μ m.

Other accepted species

Macrodiplodiopsis aurantiacinotata (Mugambi & Huhndorf) Wijayawardene, Camporesi, D.J. Bhat & K.D. Hyde, *comb. nov.* MycoBank: MB805549

≡ *Misturatosphaeria aurantonotata* Mugambi & Huhndorf, *Stud. Mycol.* 64: 108 (2009)

Notes:—This species was listed as *Macrodiplodiopsis aurantonotata* Mugambi & Huhndorf in Mugambi & Huhndorf (2009). However Index Fungorum (2013) listed it as *Misturatosphaeria aurantiacinotata* and we follow the name as it is in Index Fungorum.

Macrodiplodiopsis claviformis (Mugambi & Huhndorf) Wijayawardene, Camporesi, D.J. Bhat & K.D. Hyde, *comb. nov.* MycoBank: MB805550

≡ *Misturatosphaeria claviformis* Mugambi & Huhndorf, *Stud. Mycol.* 64: 113 (2009)

Macrodiplodiopsis cruciformis (Mugambi & Huhndorf) Wijayawardene, Camporesi, D.J. Bhat & K.D. Hyde, *comb. nov.* MycoBank: MB805551

≡ *Misturatosphaeria cruciformis* Mugambi & Huhndorf, *Stud. Mycol.* 64: 113 (2009)

Macrodiplodiopsis kenyensis (Mugambi & Huhndorf) Wijayawardene, Camporesi, D.J. Bhat & K.D. Hyde, *comb. nov.* MycoBank: MB805552

≡ *Misturatosphaeria kenyensis* Mugambi & Huhndorf, *Stud. Mycol.* 64: 113 (2009)

Macrodiplodiopsis mariae (Ying Zhang, J. Fourn. & K.D. Hyde) Wijayawardene, Camporesi, D.J. Bhat & K.D. Hyde, *comb. nov.* Index Fungorum: IF550236

≡ *Misturatosphaeria mariae* Ying Zhang, J. Fourn. & K.D. Hyde, *Mycol. Progr.* 54(2): 2 (2012)

Notes:—We were unable to get MycoBank number for this species hence included Index Fungorum number.

Macrodiplodiopsis minima (Mugambi, A.N. Mill. & Huhndorf) Wijayawardene, Camporesi, D.J. Bhat & K.D. Hyde, *comb. nov.* MycoBank: MB805553

≡ *Misturatosphaeria minima* Mugambi, A.N. Mill. & Huhndorf, *Stud. Mycol.* 64: 114 (2009)

Macrodiplodiopsis striata (Kohlm. & Volkm.-Kohlm.) Wijayawardene, Camporesi, Bhat & K.D. Hyde, *comb. nov.* MycoBank: MB805554

≡ *Floricola striata* Kohlm. & Volkm.-Kohlm., *Bot. Mar.* 43(4): 385 (2000)

Notes:—The genus *Floricola* Kohlm. & Volkm.-Kohlm. (2000: 385) was introduced by Kohlmeyer & Volkmann-Kohlmeyer (2000) to accommodate a coelomycetous fungus isolated from a marine habitat, with cylindrical to ellipsoidal, brown conidia with 3-distosepta and a thick gelatinous sheath. Kohlmeyer & Volkmann-Kohlmeyer (2000) compared this monotypic genus (type species *Floricola striata* Kohlm. & Volkm.-Kohlm. (2000: 385) with *Sclerostagonospora heraclei* (Sacc.) Höhn. (1917: 252), the type species of *Sclerostagonospora* Höhn. (1917: 252). However, they did not compare *Floricola striata* with *Macrodiplodiopsis*, which is characterised by ‘ellipsoid to obovoid, or clavate, 3-distoseptate, occasionally with a longitudinal septum, lumina very much reduced and often surrounded by dark brown wall deposits, continuous, pale brown, thick-walled, base truncate, apex obtuse, surrounded by a large gelatinous sheath’, according to Sutton (1980). This morphological similarity is supported in our phylogenetic analyses (Fig. 1), which groups *Floricola striata* with *Macrodiplodiopsis*. Hence, we conclude that *Floricola striata* is a species of *Macrodiplodiopsis* and is thus transferred. *Macrodiplodiopsis desmazieri* (43–49 × 17–19 µm) has larger conidia than *M. striata* (13–17 × 5–7 µm).

Macrodiplodiopsis tennesseensis (Mugambi, A.N. Mill. & Huhndorf) Wijayawardene, Camporesi, D.J. Bhat & K.D. Hyde, *comb. nov.* MycoBank: MB805555

≡ *Misturatosphaeria tennesseensis* Mugambi, A.N. Mill. & Huhndorf, *Stud. Mycol.* 64: 114 (2009)

Macrodiplodiopsis uniseptata (Mugambi, A.N. Mill. & Huhndorf) Wijayawardene, Camporesi, D.J. Bhat & K.D. Hyde, *comb. nov.* MycoBank: MB805556

≡ *Misturatosphaeria uniseptata* Mugambi, A.N. Mill. & Huhndorf, in Mugambi & Huhndorf, *Stud. Mycol.* 64: 114 (2009)

Macrodiplodiopsis uniseriata (Mugambi, A.N. Mill. & Huhndorf) Wijayawardene, Camporesi, D.J. Bhat & K.D. Hyde, *comb. nov.* MycoBank: MB805557

≡ *Misturatosphaeria uniseriata* Mugambi, A.N. Mill. & Huhndorf, in Mugambi & Huhndorf, *Stud. Mycol.* 64: 116 (2009)

Key to the species of *Macrodiplodiopsis*

1. Asexual state with cylindrical, oblong or ellipsoidal conidia 2
1. Sexual state with phragmosporous or dictyosporous ascospores 3
2. Conidia 44–52 × 18–22 µm *M. desmazieri*
2. Conidia 13–17 × 5–7 µm *M. striata*
3. Ascospores with gelatinous sheath 4
3. Ascospores lacking a gelatinous sheath 6
4. Gelatinous sheath only on immature ascospores *M. aurantiacinctata*
4. Gelatinous sheath on mature ascospores 5
5. Ascospores 15–24 × 4–6 µm *M. kenyensis*
5. Ascospores 18–22 × 3–4 µm *M. minima*
6. Ascospores with 1 or more septa in one plane 7
6. Ascospores dictyosporous 9
7. Ascospores with 1 septum *M. unisepta*
7. Ascospores with 3 septa 8
8. Ascospores dark brown *M. uniseriata*
8. Ascospores pale brown *M. tennesseensis*
9. Pseudoparaphyses not covered by a gelatinous matrix *M. mariae*
9. Pseudoparaphyses covered by a gelatinous matrix 10
10. Ascospores oblong to elliptical, 19–26 × 8–13 µm *M. cruciformis*
10. Ascospores elliptical, straight or inequilateral, 12–20 × 7–9 µm *M. claviformis*

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