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.

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1 AFTOL: Assembling the Fungal Tree of Life; ANM: A.N. Miller; BCC: BIOTEC Culture Collection, Bangkok, Thailand; CBS: Centraalbureau voor Schimmecultures, 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, Halothiaceae, Lindgomycetaceae, Lophiostomataceae, Melanommataceae, Pleomassariaceae, Roussoellaceae, Sporormiaceae, Tetraplosphaeriaceae, 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 (BMCMMC) 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.

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)


*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)


*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, annellicidic, 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 (x̄ = 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. Forlì-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.

Other accepted species


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


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


**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).


**Key to the species of Macrodiplodiopsis**

1. Asexual state with cylindrical, oblong or ellipsoidal conidia ................................................................. 2
2. Sexual state with phragmosporous or dictyosporous ascospores ............................................................. 3
2. Conidia 44–52 × 18–22 µm ........................................................................................................... *M. desmazieri*
3. Ascospores with gelatinous sheath ........................................................................................................ 4
3. Ascospores lacking a gelatinous sheath ................................................................................................. 6
4. Gelatinous sheath only on immature ascospores ................................................................................... *M. aurantiacinotata*
5. Gelatinous sheath on mature ascospores ............................................................................................... 5
5. Ascospores 15–24 × 4–6 µm .............................................................................................................. *M. kenensis*
6. Ascospores 18–22 × 3–4 µm ............................................................................................................. *M. minima*
7. Ascospores with 1 septum ................................................................................................................... *M. uniseriata*
8. Ascospores with 3 septa ...................................................................................................................... *M. uniseriata*
9. Ascospores dark brown .................................................................................................................... *M. tennesseensis*
10. Ascospores pale brown ................................................................................................................... *M. tennesseensis*
9. Pseudoparaphyses covered by a gelatinous matrix ........................................................................... *M. mariæ*
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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**References**


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