Mycosphere 7 (9): 1346–1356 (2016) www.mycosphere.org ISSN 2077 7019

Article – special issue
Doi 10.5943/mycosphere/7/9/8

Taxonomy and phylogenetic appraisal of *Montagnula jonesii* sp. nov. (Didymosphaeriaceae, Pleosporales)

Tennakoon DS[1,2,3,4], Hyde KD[1,2,3,4], Wanasinghe DN[1,2,3,4], Bahkali AH[5], Camporesi E[6,7], Khan S[1,8] and Phookamsak R[1,2,3]

[1] *World Agro Forestry Centre, East and Central Asia, 132 Lanhei Road, Kunming 650201, Yunnan China*
[2] *Key Laboratory for Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan China*
[3] *Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand*
[4] *School of Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand*
[5] *Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia*
[6] *A.M.B. Gruppo Micologico Forlivese "Antonio Cicognani", Via Roma 18, Forlì, Italy; A.M.B. Circolo Micologico "Giovanni Carini", C.P. 314, Brescia, Italy*
[7] *Società per gli Studi Naturalistici della Romagna, C.P. 144, Bagnacavallo (RA), Italy*
[8] *Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences; Kunming 650201, Yunnan China.*

Tennakoon DS, Hyde KD, Wanasinghe DN, Bahkali AH, Camporesi E, Khan S, Phookamsak R 2016 – Taxonomy and phylogenetic appraisal of *Montagnula jonesii* sp. nov. (Didymosphaeriaceae, Pleosporales). Mycosphere 7 (9), 1346–1356, Doi 10.5943/mycosphere/7/9/8

Abstract

A saprobic member of Dothideomycetes was collected from dead branches of *Fagus sylvatica* in Italy. Morphology coupled with combined gene analysis of LSU, SSU, ITS and tef1-α sequence data, showed it to be a novel *Montagnula* species, which is introduced in this paper. *Montagnula jonesii* sp. nov. differs from other *Montagnula* species in having immersed, brown ascomata and ellipsoidal to fusiform, 3-septate ascospores with rounded ends and prominent guttules in each cell and is enlarged at the second cell from the apex. The new species is compared with other *Montagnula* species and a comprehensive description and micrographs are provided.

Key words – Dothideomycetes – Morphology – New species

Introduction

Didymosphaeriaceae is an important family in Pleosporales, Dothideomycetes (Aptroot 1995, Hyde et al. 2013, Ariyawansa et al. 2014a, b, Liu et al. 2015, Wanasinghe et al. 2016). Munk (1953) introduced Didymosphaeriaceae and typified the family by *Didymosphaeria* Fuckel with *D. epidermidis* as the type species. Didymosphaeriaceae is characterized by brown, thick-walled, 1-septate ascospores and trabeculate pseudoparaphyses, which anastomose above the asci in a gelatinous matrix (Aptroot 1995, Hyde et al. 2013, Ariyawansa et al. 2014a, b). The members of Didymosphaeriaceae play a vital role as saprobes, endophytes and pathogens of plant substrates (Aptroot 1995, Ariyawansa et al. 2014a, Liu et al. 2015, Wanasinghe et al. 2016). Ariyawansa et al. (2014a) discussed the confusion surrounding genera of Didymosphaeriaceae and mentioned that the family appears to be a distinct family of Pleosporales based on the morphological considering, but the molecular data could not be resolved its phylogenetic placement as the distinct family from

Submitted 1 November 2016, Accepted 29 November 2016, Published online 28 December 2016
Corresponding Author: Rungtiwa Phookamsak – E-mail – jomjam.rp2@gmail.com

Montagnulaceae. The representative species *Didymosphaeria rubi-ulmifolii* which was introduced by Ariyawansa et al. (2014a), clustered within the Montagnulaceae as a separate genus. Hence, Ariyawansa et al. (2014a) showed that Montagnulaceae and Didymosphaeriaceae are synonyms and thus, Ariyawansa et al. (2014b) synonymized Montagnulaceae under Didymosphaeriaceae based on priority of the oldest name. Ariyawansa et al. (2014b) re-circumscribed genera in Didymosphaeriaceae and accepted 16 genera in this family. Wijayawardene et al. (2014a, b) introduced another two asexual genera in family viz. *Paracamarosporium* and *Pseudocamarosporium*. Furthermore, Crous et al. (2015a, b) introduced *Verrucoconiothyrium* and *Xenocamarosporium* and Ariyawansa et al. (2015) referred *Austropleospora* and *Pseudopithomyces* to Didymosphaeriaceae. Wanasinghe et al. (2016) introduced *Laburnicola* and *Paramassariosphaeria* to the family and thus 24 genera are presently accepted in Didymosphaeriaceae.

The genus *Montagnula* was introduced by Berlese (1896) to accommodate *M. infernalis* (Niessl) Berl. and *M. gigantean* (Mont.) Berl. based on the morphology and phylogeny, Ariyawansa (2014b) placed *Montagnula* in Didymosphaeriaceae. The genus is characterized by globose or sphaerical, immersed ascomata with a clypeus, claviform asci, fusoid or ellipsoid ascospores with transverse septa and one or more longitudinal septa (Barr 1990, Ariyawansa et al. 2014b). There have been several recent studies on the taxonomy of *Montagnula* with introducing novel species (Table 1). Presently, there are 32 epithets for *Montagnula* (Index Fungorum 2016).

The aim of this study is to introduce a new species, *Montagnula jonesii*. Maximum-likelihood (ML), maximum-parsimony (MP) and Bayesian analyses (BI) of combined LSU, SSU, ITS and tef1- α sequence data clearly showed this species grouped in *Montagnula* (99% ML, 70% MP and 0.99 PP support, Fig. 1). The new species is described, illustrated and compared with similar taxa.

Table 1. Recorded *Montagnula* species in recent studies (2015–2016)

***Montagnula* species**	**Authority**	**Reference**
M. bellevaliae	Wanasinghe, Camporesi, E.B.G. Jones &K.D. Hyde	Hongsanan et al. (2015)
M. scabiosae	Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde	Hongsanan et al. (2015)
M. graminicola	Chethana, Thambugala, Camporesi & K.D. Hyde	Liu et al. (2015)
M. saikhuensis	Wanasinghe, E.B.G. Jones & K.D. Hyde	Wanasinghe et al. (2016)
M. cirsii	Qing Tian, Camporesi & K.D. Hyde	Hyde et al. (2016)

Materials & methods

Sample collection, morphological studies and isolation

Fresh specimens were collected from Arezzo (AR) Province in Italy. Specimens were taken to the laboratory in zip lock bags and observed with a JNOEC JSZ4 stereomicroscope. Ascomata and ascospores were examined with an OLYMPUS SZ61 compound microscope. Sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. Images were taken using a Nikon ECLIPSE 80i compound microscope with a Canon EOS 600D digital camera. Permanent slides were prepared by mounting fungal material in lactoglycerol and sealed by applying nail-polish around the margins of cover slips. All measurements were calculated using Tarosoft Image Frame work program (IFW) and images used for figures processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

The specimens were deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand and the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS), Yunnan, China. Living cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection

(KUMCC). Faces of Fungi and Index Fungorum numbers are registered as described in Jayasiri et al. (2015) and Index Fungorum (2016).

DNA extraction and PCR amplification

Genomic DNA was extracted from mycelium using Biospin fungus Genomic DNA extraction kit (BioFlux®, Hangzhou, P. R. China) following the manufacturer's protocol. The DNA product was kept at 4 ℃ for the DNA amplification and maintained at -20 ℃ for long term storage. The DNA amplification was carried out by polymerase chain reaction (PCR) using four genes, the large subunit (28S, LSU), small subunit (18S, SSU), internal transcribed spacers (ITS1, 5.8S, ITS2) and translation elongation factor 1-alpha gene (tef1- α). The LSU gene was amplified by using the primers LROR and LR5 (Vilgalys & Hester 1990, Liu et al. 1999, Sung et al. 2007), SSU gene was amplified using the primers NS1 and NS4 (White et al. 1990), nuclear ITS was amplified by using the primers ITS5 and ITS4 (White et al. 1990) and tef1- α gene was amplified using the primers EF1-983F and EF1-2218R (Rehner et al. 2001). The amplification reactions were performed in 25µl of total reaction which contained 9.5 µl of sterilized water, 12.5 µl of 2× Power Taq PCR MasterMix (Bioteke Co., China), 1 µl of each forward and reverse primers and 1 µl of DNA template. The polymerase chain reaction (PCR) thermal cycle program for LSU, SSU, ITS and tef1- α genes amplification were provided as: initially 95 ℃ for 3 mins, followed by 35 cycles of denaturation at 95 ℃ for 30 sec, annealing at 55 ℃ for 50 sec, elongation at 72 ℃ for 30 sec and final extension at 72 ℃ for 10 mins. The quality of PCR products were checked on 1% agarose gel electrophoresis stained with ethidium bromide. PCR products were purified and sequenced by Sangon Biotech (Shanghai) Co., Ltd, China. Nucleotide sequences were deposited in GenBank (Table 2).

Sequencing and alignment

Phylogenetic analysis used combined LSU, SSU, ITS and tef1- α sequence data and other related sequences used in the analyses (Table 2) were obtained from GenBank (http://www.ncbi.nlm.nih.gov/) based on recently published data (Hyde et al. 2016, Wanasinghe et al. 2016) and BLAST searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The combined dataset consists of 72 sequences including our newly generated sequences. *Pleospora herbarum* (CBS 191.86, IT 956) and *Pleospora tarda* (CBS 714.68) were selected as the outgroup taxa. The multiple alignments were automatically aligned by MAFFT v. 7 at the web server (http://mafft.cbrc.jp/alignment/server; 2016). Alignments were refined where necessary and combined sequence alignments were obtained by using BioEdit v. 7.0.5.2 (Hall 1999).

Phylogenetic analysis

Maximum parsimony analysis (MP) was performed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002), with parameters as described in Wanasinghe et al. (2014). Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI]) were calculated for trees generated under different optimality criteria. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were significantly different.

Maximum likelihood analysis was performed by RAxML v.7.2.8 (Stamatakis 2010) implemented in RaxmlGUI 1.3 (Silvestro & Michalak 2012). Bootstrap support for the branches was generated with 1000 replicates.

The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). A Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001) to evaluate Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 3,000,000 generations and trees were sampled every 100th generations. The first 3000 trees representing the burn-in phase of the analyses were discarded and the remaining 27,000 (Post

burning) trees used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai et al. 2006, Liu et al. 2012). Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2012) and annotated in Microsoft Power Point (2010). The finalized alignment and tree were deposited in TreeBASE, submission ID: 20319 (http://www.treebase.org/).

Table 2 GenBank and culture collection accession numbers of species included in the phylogenetic study. The newly generated sequence is shown in red bold. The ex-type strains are in black bold.

Species name	**Strains no.**	**GenBank accession number**			
		LSU	**SSU**	**ITS**	***tef1- α***
Alloconiothyrium aptrootii	**CBS 980.95^{T}**	**JX496234**	**-**	**JX496121**	**-**
Alloconiothyrium aptrootii	**CBS 981.95^{T}**	**JX496235**	**-**	**JX496122**	**-**
Austropleospora archidendri	**CBS 168.77^{T}**	**JX496162**	**-**	**JX496049**	**-**
Austropleospora osteospermi	**LM 2009a^{T}**	**-**	**-**	**FJ481946**	**-**
Bimuria novae-zelandiae	**CBS 107.79^{T}**	**AY016356**	**AY016338**	**-**	**-**
Deniquelata barringtoniae	**MFLUCC 11-0422^{T}**	**JX254655**	**JX254656**	**JX254654**	**-**
Deniquelata barringtoniae	**MFLUCC 11-0257^{T}**	**KM213997**	**KM214000**	**KM214003**	**-**
Didymocrea sadasivanii	**CBS 438.65^{T}**	**DQ384103**	**DQ384066**	**-**	**-**
Didymosphaeria rubi-ulmifolii	**MFLUCC 14-0023^{T}**	**KJ436586**	**KJ436588**	**KJ436586**	**-**
Didymosphaeria rubi ulmifolii	CBS 100299	JX496124	AY642523	JX496011	-
Didymosphaeria sp.	CBS 587 84	JX496212		JX496099	-
Kalmusia ebuli	**CBS 123120^{T}**	**JN644073**	**JN851818**	**-**	**-**
Kalmusia italica	**MFLUCC 14-0560^{T}**	**KP744487**	**KP753953**	**KP744441**	**-**
Kalmusia spartii	**MFLUCC 13-0352^{T}**	**KM658315**	**KM658316**	**KM658314**	**-**
Kalmusia variisporum	**CBS 121517^{T}**	**JX496143**	**-**	**JX496030**	**-**
Karstenula rhodostoma	CBS 690.94	GU301821	GU296154	-	-
Karstenula rhodostoma	CBS 691.94	AB807531	AB797241	-	AB808506
Laburnicola hawksworthii	**MFLUCC 13-0602^{T}**	**KU743195**	**KU743196**	**KU743194**	**-**
Laburnicola muriformis	**MFLUCC 16-0290^{T}**	**KU743198**	**KU743199**	**KU743197**	**KU743213**
Laburnicola muriformis	**MFLUCC 14-0921^{T}**	**KU743201**	**KU743202**	**KU743200**	**-**
Letendraea cordylinicola	MFLUCC 11-0148	KM213995	KM213998	KM214001	-
Letendraea cordylinicola	**MFLUCC 11-0150^{T}**	**KM213996**	**KM213999**	**KM214002**	**-**
Letendraea helminthicola	CBS 884.85	AY016362	AY016345	-	-
Letendraea padouk	CBS 485.70	AY849951	-	-	-
Montagnula aloes	**CPC 19671^{T}**	**JX069847**	**-**	**JX069863**	**-**
Montagnula appendiculata	**CBS 109027^{T}**	**AY772016**	**-**	**DQ435529**	**-**
Montagnula bellevaliae	**MFLUCC 14-0924^{T}**	**KT443902**	**KT443904**	**KT443906**	**-**
Montagnula cirsii	**MFLUCC 13-0680**	**KX274249**	**KX274255**	**KX274242**	**KX284707**
Montagnula donacina	HVVV01	KJ628377	KJ628376	KJ628375	-
Montagnula graminicola	**MFLUCC 13-0352^{T}**	**KM658315**	**KM658316**	**KM658314**	**-**
Montagnula jonesii	**MFLUCC 16-1448**	**KY273276**	**KY313618**	**KY313619**	**KY313620**
Montagnula opulenta	CBS 168.34	NG027581	NG 013127	AF383966	-
Montagnula saikhuensis	**MFLUCC 16-0315^{T}**	**KU743210**	**KU743211**	**KU743209**	**-**
Montagnula scabiosae	**MFLUCC 14-0954^{T}**	**KT443903**	**KT443905**	**KT443907**	**-**
Neokalmusia brevispora	**KT 2313^{T}**	**AB524601**	**AB524460**	**-**	**AB539113**
Neokalmusia didymospora	**MFLUCC 11-0613^{T}**	**KP091434**	**KP091435**	**KP091433**	**-**
Neokalmusia scabrispora	KT 2202	AB524594	AB524453	-	AB539107
Paracamarosporium fagi	CPC 24892	KR611905	-	KR611887	-
Paracamarosporium fagi	CPC 24890	KR611904	-	KR611886	-
Paracamarosporium hawaiiense	**CBS 120025^{T}**	**JX496140**	**EU295655**	**JX496027**	**-**
Paracamarosporium psoraleae	**CPC 21632^{T}**	**KF777199**	**-**	**KF777143**	**-**
Paraconiothyrium cyclothyrioides	**CBS 972.95^{T}**	**JX496232**	**AY642524**	**JX496119**	**-**
Paraconiothyrium estuarinum	**CBS 109850^{T}**	**JX496129**	**AY642522**	**JX496016**	**-**
Paraconiothyrium fungicola	**CBS 113269^{T}**	**JX496133**	**AY642527**	**JX496020**	**-**
Paramassariosphaeria clematidicola	**MFLU 16-0172^{T}**	**KU743207**	**KU743208**	**KU743206**	**-**
Paramassariosphaeria anthostomoides	CBS 615.86	GU205223	GU205246	-	-
Paraphaeosphaeria angularis	CBS 167.70^{T}	JX496160	-	JX496047	-
Paraphaeosphaeria michotii	MFLUCC 13-0349^{T}	KJ939282	KJ939285	KJ939279	-
Paraphaeosphaeria minitans	CBS 111750	JX496130	-	JX496017	-
Paraphaeosphaeria minitans	CBS 859.71	JX496229	-	JX496116	-

Species name	Strains no.	GenBank accession number			
		LSU	SSU	ITS	*tef1- α*
Phaeodothis winteri	AFTOL-ID 1590	DQ678073	DQ678021	-	DQ677917
Phaeodothis winteri	CBS 182.58	GU301857	GU296183	-	-
Pleospora herbarum	**CBS 191.86T**	**GU238160**	**GU238232**	**NR111243**	**KC584731**
Pleospora herbarum	IT 956	KP334709	KP334729	KP334719	-
Pleospora trada	**CBS 714.68T**	**KC584345**	**KC584603**	**KC584238**	**KC584729**
Pseudocamarosporium corni	**MFLUCC 13-0541^{T}**	**KJ813279**	**KJ819946**	**KJ747048**	**-**
Pseudocamarosporium cotinae	**MFLUCC 14-0624^{T}**	**KP744505**	**KP753964**	**KP744460**	**-**
Pseudocamarosporium lonicerae	**MFLUCC 13-0532^{T}**	**KJ813278**	**KJ819947**	**KJ747047**	**-**
Pseudocamarosporium propinquum	**MFLUCC 13-0544^{T}**	**KJ813280**	**KJ819949**	**KJ747049**	**-**
Pseudopithomyces chartarum	UTHSC 04-678	HG518065	-	HG518060	-
Pseudopithomyces chartarum	UTHSC 03-2472	HG518064	-	HG518059	-
Pseudopithomyces sp.	MUCL 15905	LK936383	-	LK936375	-
Pseudopithomyces sp.	MUCL 4329	LK936382	-	LK936374	-
Spegazzinia deightonii	yone 212	AB807582	AB797292	-	AB808558
Spegazzinia sp.	yone 279	AB807583	AB797293	-	AB808559
Spegazzinia tessarthra	SH 287	AB807584	AB797294	JQ673429	AB808560
Trematеia arundicola	**MFLU 16-1275^{T}**	**KX274248**	**KX274254**	**KX274241**	**KX284706**
Trematеia guiyangensis	**GZAAS01^{T}**	**KX274247**	**KX274253**	**KX274240**	**KX284705**
Trematеia halophila	JK 5517J	-	GU296201	-	-
Verrucoconiothyrium nitidae	CBS 119209	EU552112	-	EU552112	-
Xenocamarosporium acaciae	**CPC 24755^{T}**	**KR476759**	**-**	**KR476724**	**-**

Abbreviations of culture collections: **AFTOL-ID**: Assembling the Fungal Tree of Life, **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, **CPC**: Working collection of Pedro Crous housed at CBS, **GZAAS**: Guizhou Academy of Agricultural Sciences herbarium, China, **JK**: J. Kohlmeyer, **KT**: K. Tanaka, **LM**: Secçáo de Botânica e Ecologia, Mozambique. MAPUTO, **MFLU**: Mae Fah Luang University, Chiang Rai, Thailand, **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, **MUCL**: Université Catholique de Louvain, Belgium, **SH**: Academia Sinica People's Republic of China. Shanghai, **UTHSC**: Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA, **Yone**: H. Yonezawa.

Results

Phylogenetic analysis

The combined LSU, SSU, ITS and tef1- α sequence data were analyzed with *Pleospora herbarum* (CBS 191.86, IT 956) and *Pleospora tarda* (CBS 714.68) as the outgroup taxa. The data set comprised 72 taxa including *Montagnula jonesii*. The maximum parsimony dataset comprised 3261 characters, including 2475 constant characters, 184 variable parsimony-uninformative characters and 602 parsimony-informative characters. The most parsimonious tree is shown where TL = 2023, CI = 0.519, RI = 0.724, RC = 0.375, HI = 0.481. Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. Maximum likelihood (ML), maximum parsimony (MP) and Bayesian posterior probability analyses (PP) resulted in trees with similar topologies that did not differ significantly from one another (data not shown). The final RAxML tree is shown in Fig. 1, with the final ML optimization likelihood value of -15278.83918 (ln). The phylogeny showed that *Montagnula jonesii* grouped in *Montagnula* with strong support (99% ML, 70% MP and 0.99 PP), sister to *M. saikhuensis* (MFLUCC 16-0315), *M. donacina* (HVVV01) and *M. graminicola* (MFLUCC 13-0352). All analyses (ML, MP and PP) gave similar results of the generic placements in agreement with previous studies based on multi-gene analyses (Hyde et al. 2016, Li et al. 2016, Wanasinghe et al. 2016).

Taxonomy

Montagnula jonesii Tennakoon, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF552577; Facesoffungi number: FoF02719, Fig. 2

Holotype – MFLU 16-1363

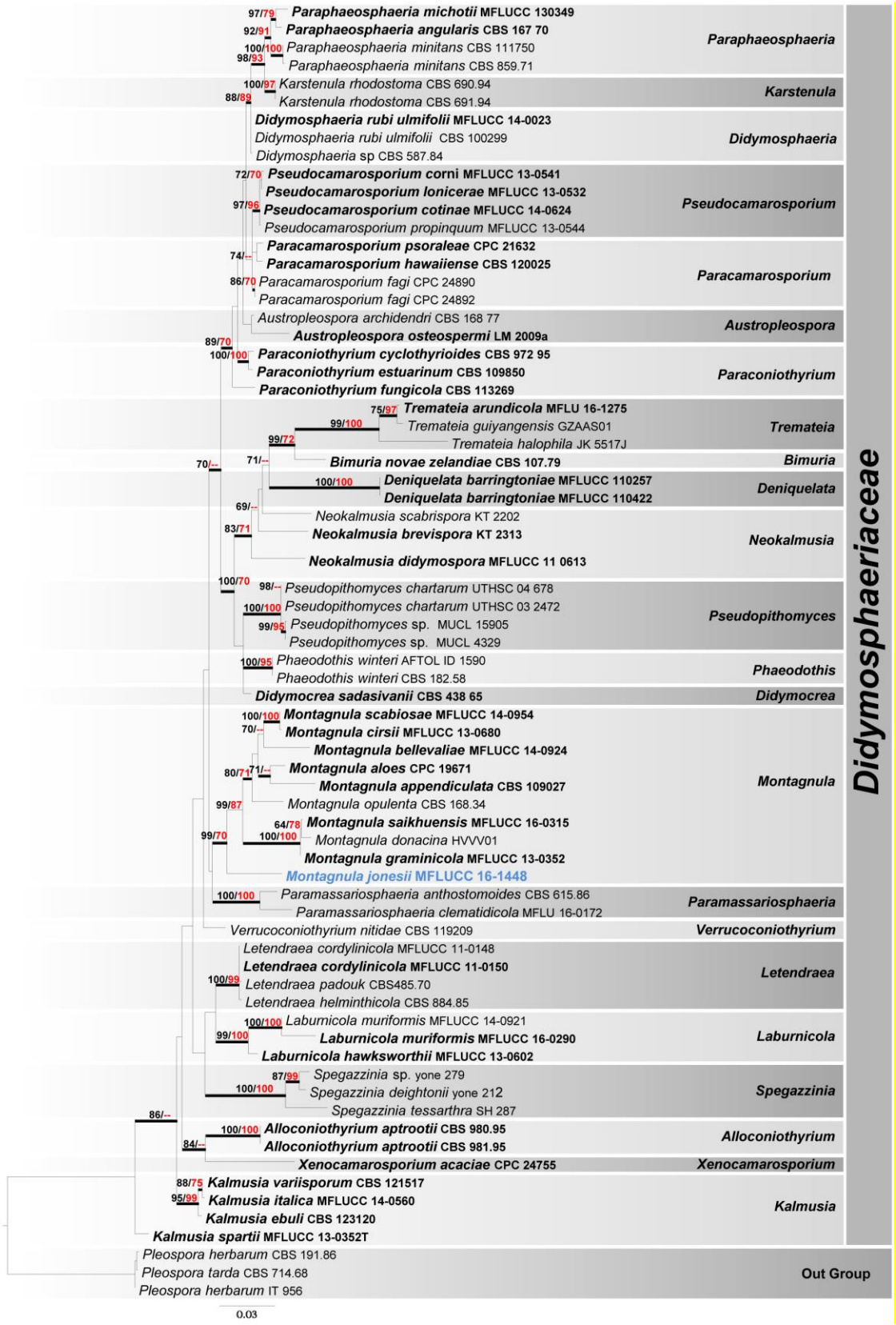

Fig. 1 – RAxML tree based on analysis of a combined LSU, SSU, ITS and tef1- α partial sequences. Bootstrap support values for maximum parsimony (MP, red) and maximum likelihood (ML, black) greater than 70 % are defined above the nodes. Bayesian posterior probabilities (PP) greater than 0.90 are shown as bold branches. The tree is rooted to *Pleospora herbarum* (CBS 191.86, IT 956) and *Pleospora tarda* (CBS 714.68). The new strain is shown in blue. Ex-type strains are shown in bold.

Etymology – In honour of Professor E.B. Gareth Jones for his immense contribution to mycology

Saprobic on dead branches of *Fagus sylvatica* L. Sexual morph: Ascomata 140–210 µm diam., solitary, scattered to clustered, immersed or erumpent through host surface, visible as slightly raised, brown spots on host surface, globose to subglobose, glabrous, uniloculate, ostiole central with minute papilla. *Peridium* 10–25 µm wide, thin-walled with equal thickness, slightly thin at the base, composed of two layers of pseudoparenchymatous cells, inner layer comprising several cell layers of flattened, hyaline cells, arranged in a *textura prismatica*, outer layer comprising several layers of dark brown to black cells, arranged in a *textura angularis*. *Hamathecium* composed of 2–2.5 µm wide, dense, broad, filamentous pseudoparaphyses, distinctly septate, not constricted at the septum, anastomosing at the apex, embedded in a hyaline, gelatinous matrix. *Asci* (53–)60–70(–83) × (7.6–)9–10(–11.5) µm ($\bar{x}$ = 66.8 × 9.5 µm, n = 35), 8-spored, bitunicate, fissitunicate, clavate, long pedicellate, apically rounded, with well-developed ocular chamber. *Ascospores* 14–16(–17) × 4–5.5 µm ($\bar{x}$ = 15.5 × 5 µm, n = 35), overlapping 1–2-seriate, initially hyaline to pale brown, becoming brown to reddish-brown at maturity, ellipsoidal to fusiform with rounded ends, 1-septate when young, becoming 3-septate when mature, constricted at the septa, straight to curved, enlarge at the second cell from apex, smooth-walled, with guttules. Asexual morph: Undetermined.

Culture characteristics – Colonies on PDA fast growing, reaching 7–8 cm diam. after two weeks at 20–25 °C, colonies medium sparse, circular, flat, surface slightly rough with edge entire, margin well-defined, cottony to fairly fluffy with sparse aspects, colony from above, white to cream at the margin, light brown at the centre; from below, white brown to yellowish brown at the margin, mycelium green to grey with tufting; not producing pigmentation in PDA.

Material examined – ITALY, Arezzo Province (AR), near Croce di Pratomagno, on aerial and dead branches of *Fagus sylvatica* (Fagaceae), 21 June 2015, E. Camporesi, IT 2545 (MFLU 16-1363, holotype; HKAS93702, isotype**)**, ex-type living cultures, MFLUCC 16-1448, KUMCC 15-0556.

Notes: *Montagnula jonesii* resembles to *M. aloes* Crous and *M. scabiosae* in having reddish-brown, 3-septate ascospores and immersed ostiolate ascomata. *Montagnula jonesii* has an unique character that can be used to distinguish it from *M. aloes* and *M. scabiosae* as ascospores have an enlarged second cell from the apex. Additionally, the size of asci and ascospores are different in each species (Table 3). *Montagnula jonesii* has ellipsoidal to fusiform ascospores, while they are ellipsoidal to ovoid in *M. aloes*. *Montagnula jonesii* is deeply constricted at septa, whereas *M. scabiosae* is slightly constricted (Table 3). Furthermore, each species is associated with a different host species (Table 3). A synopsis of the characters of species of *Montagnula* are provided in Table 3.

Discussion

Montagnula species play a vital role as saprobes growing on dead plants, especially dead wood and bark, sometimes on dead leaves (Ariyawansa et al. 2014b). Host-specificity of the taxa in this group have not yet been clarified according to they have been recorded from various plant families (i.e. Agavaceae, Arecaceae, Asparagaceae, Caprifoliaceae, Fagaceae, Poaceae, Xanthorrhoeaceae) (Table 3). Species of *Montagnula* seem to be cosmopolitan in distribution since they have been recorded from both temperate and tropical countries (i.e. Algeria, Australia, Italy, South Africa, Thailand) (Aptroot 1995, Wanasinghe et al. 2016). At the present, a well-resolved revision of the genus *Montagnula* is difficult since it lacks molecular data. From the 32 epithets present in Index Fungorum, there have been only 45 sequences from 12 species available in GenBank. The type species, *M. infernalis* (Niessl) Berl. does not have molecular data to verify its generic status and some sequences are not represented from the ex-type cultures, such as *M. anthostomoides* (Rehm) Leuchtm. (CBS 615.86), *M. opulenta* (De Not.) Aptroot (CBS 168.34). The connectively of sexual and as asexual morphs is not proven yet, as nobody has obtained any asexual morph for these new species from an ex-type culture which has molecular data. Also there

is no molecular support to link possible asexual taxa. Therefore, representative species of these *Montagnula* species are essentially needed to be recollected and obtained molecular data for clarifying its phylogenetic affinity (especially from *M. infernalis*).

Table 3 Synopsis of recorded *Montagnula* species discussed in this study

***Montagnula* species**	**Size (µm)**			**Septa in ascospores**	**Host**	**References**
	Ascomata (diam.)	**Asci**	**Ascospores**			
M. aloes	450	110–250 × 20–30	33–36 × 13–14	3	*Aloe* sp.	Crous et al. 2012
M. appendiculata	100–200	-	12–15 ×4–5	1	*Zea mays*	Aptroot 2004
M. *bellevaliae*	100–120 × 150–175	70–100 × 9–12	15–18 × 5–6	2	*Bellevalia romana*	Hongsanan et al. 2015
M. cirsii	385–415 × 510–525	84.5–119.5 × 10.5–13.5	18–23.5 ×6.5–9.5	3	*Cirsium* sp.	Hyde et al. 2016
M. donacina	-	-	12–17 ×4–6.5	1	*Arundo donax*	Aptroot 1995
M. graminicola	37–117.22	50–132 × 8–13	9.8–13 × 3.8–5.5	1	Grass	Liu et al. 2015
M. jonesii	325–350 × 300–325	72–95 × 9–13	14–16 ×5–6	3	*Fagus sylvatica*	This study
M. opulenta	400–1200	-	19–25 ×9–13	1	*Opuntia* sp.	Aptroot 1995
M. saikhuensis	400–450 × 400–500	70–100 × 10–12	12–16 × 4–6	1	*Citrus* sp.	Wanasinghe et al. 2016
M. scabiosae	300–320 × 300–360	110–130 × 14–20	20–23 × 7–9	3	*Scabiosa* sp.	Hongsanan et al. 2015

Acknowledgments

The authors extend their sincere appreciations to the Deanship of Scientific Research at King Saud University for its funding this Prolific Research Group (PRG-1436-09). K.D Hyde thanks the Chinese Academy of Sciences, (project number 2013T2S003), for the award of visiting Professorship for Senior International Scientists at Kunming Institute of Botany. CGIAR Research Program 6: Forest, Trees and Agroforestry and Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences are thanked to the help of DNA molecular experiments. MFLU grant number 56101020032 is thanked for supporting studies on Dothideomycetes. R. Phookamsak thanks the Royal Golden Jubilee Ph.D Program (PHD/0090/2551) under the Thailand Research Fund for financial support. S. Khan thankful to National Natural Science Foundation China for the support of this work (Grant No. 31550110215). D. Tennakoon is grateful to K. Thambugala, QJ Shang, M. Samarakoon and N. de Silva for their valuable suggestions.

References

Aptroot A. 1995 – Redisposition of some species excluded from Didymosphaen'a (Ascomycotina). Nova Hedwigia 60, 325–379.

Aptroot A. 2004 – Two new ascomycetes with long gelatinous appendages collected from monocots in the tropics. Studies in Mycology 50, 307–312.

Ariyawansa HA, Camporesi E, Thambugala KM, Mapook A et al. 2014a – Confusion surrounding *Didymosphaeria* phylogenetic and morphological evidence suggest Didymosphaeriaceae is not a distinct family. Phytotaxa 176, 102–119.

Ariyawansa HA, Tanaka K, Thambugala KM, Phookamsak R et al. 2014b – A molecular phylogenetic reappraisal of the Didymosphaeriaceae (= Montagnulaceae). Fungal Diversity 68, 69–104.

Ariyawansa HA, Hyde KD, Jayasiri SC, Buyck B et al. 2015 – Fungal Diversity Notes 111–252—taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75, 27–274.

Barr ME. 1990 – Some dictyosporous genera and species of Pleosporales in North America. Memoirs of the New York Botanical Garden 62, 1–92.

Fig. 2 – *Montagnula jonesii* (MFLU 16-1363, holotype). a Ascomata visible as black dots on the host surface. b Vertical section of ascoma. c Section through peridium. d Pseudoparaphyses. e–h Asci i–k Ascospores. l Germinated ascospore. m Colony from above. n Colony from below. Scale bars: b = 50 µm, c = 10 µm, d–l = 5 µm.

Berlese, A.N. 1896. Icones Fungorum. Pyrenomycetes. 2, 1–216.

Cai L, Jeewon R, Hyde KD. 2006 – Molecular systematics of *Zopfiella* and allied genera: evidence from multi-gene sequence analyses. Mycological Research 110, 359–368.

Crous PW, Schumacher RK, Wingfield MJ, Lombard L et al. 2015a – Fungal systematics and evolution: FUSE 1. Sydowia 67, 81–118.

Crous PW, Summerell BA, Shivas RG, Burgess TI et al. 2012 – Fungal Planet description sheets: 107–127. Persoonia 28, 138–182.

Crous PW, Wingfield MJ, Guarro J, Hernandez-Restrepo M et al. 2015b – Fungal Planet description sheets: 320–370. Persoonia 34, 167–266.

Hall TA. 1999 – BioEdit: a user- friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

Hongsanan S, Hyde KD, Bahkali AH, Camporesi E et al. 2015 – Fungal Biodiversity Profiles 11–20, Cryptogamie, Mycologie 36, 355–380.
Huelsenbeck JP, Ronquist F. 2001 – MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Hyde KD, Jones EBG, Liu JK, Ariyawansa H et al. 2013 – Families of Dothideomycetes. Fungal Diversity 63, 1–313.
Hyde KD, Jones EBG, Camporesi E, McKenzie EHC et al. 2016 – Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 80, 1–270.
Index Fungorum 2016 – http://www.indexfungorum.org/Names/Names.asp.
Jayasiri CS, Hyde KD, Ariyawansa HA, Bhat DJ et al. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18.
Kishino H, Hasegawa M. 1989 – Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA – sequence data, and the branching order in Hominoidea. Journal of Molecular Evolution 29, 170–179.
Li GJ, Hyde KD, Zhao RL, Hongsanan S et al. 2016 – Fungal diversity notes 253–366: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 78, 1–237.
Liu JK, Hyde KD, Jones EBG, Ariyawansa HA et al. 2015 – Fungal diversity notes 1–110: taxonomic and phylogenetic contributions to fungal species. Fungal Diversity 72, 1–197.
Liu JK, Phookamsak R, Doilom M, Wikee S et al. 2012 – Towards a natural classification of Botryosphaeriales. Fungal Diversity 57, 149–210.
Liu YJ, Whelen S, Hall BD. 1999 – Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16, 1799–1808.
Munk A. 1953 – The system of the pyrenomycetes. A contribution to a natural classification of the group Sphaeriales sensu Lindau. Dansk Botanisk Arkiv 15, 1–163.
Nylander JAA. 2004 – MrModeltest v2. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University.
Rambaut A. 2012 – FigTree version 1.4.0 Available at http://tree.bio.ed.ac.uk/software/figtree. Accessed November 2016.
Rannala B, Yang Z. 1996 – Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. Journal of Molecular Evolution 43, 304–311.
Rehner S. 2001 – Primers for elongation factor 1-α (EF1-α). http://www.aftol.org/pdfs/EF1primer.pdf
Silvestro D, Michalak I. 2012 – raxmlGUI: a graphical front-end for RAxML. Organisms Diversity and Evolution 12, 335–337.
Stamatakis A, Alachiotis N. 2010 – Time and memory efficient likelihood-based tree searches on phylogenomic alignments with missing data. Bioinformatics 26, 132–139.
Sung GH, Hywel-Jones NL, Sung JM, Luangsa-ard JJ et al. 2007 – Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Studies in Mycology 57, 5–59.
Swofford DL. 2002 – PAUP: Phylogenetic analysis using parsimony. Version 4.0 b10, Sinauer Associates, Sunderland.
Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172, 4238–4246.
Wanasinghe DN, Jones EBG, Camporesi E, Boonmee S et al. 2014 – An exciting novel member of Lentitheciaceae in Italy from *Clematis vitalba*. Cryptogamie Mycologie 35, 323–337.
Wanasinghe DN, Jones EBG, Camporesi E, Dissanayake AJ et al. 2016 – Taxonomy and phylogeny of *Laburnicola* gen. nov. and *Paramassariosphaeria* gen. nov. (Didymosphaeriaceae, Massarineae, Pleosporales). Fungal Biology 120, 1354–1373.
White T, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications 18, 315–322.

Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL et al. 2014a – Naming and outline of Dothideomycetes – 2014 including proposals for the protection or suppression of generic names. Fungal Diversity 69, 1–55.

Wijayawardene NN, Hyde KD, Bhat DJ, Camporesi E et al. 2014b – Camarosporium-like species are polyphyletic in Pleosporales; introducing *Paracamarosporium* and *Pseudocamarosporium* gen. nov. in Montagnulaceae. Cryptogamie Mycologie 35, 177–198.

Zhaxybayeva O, Gogarten JP. 2002 – Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC genomics 3, 4.