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

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**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...
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 *Verrucocammarosporium* and *Xenocamarosporium* and Ariyawansa et al. (2015) referred *Austroleospora* 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.

| 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
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 °C for the DNA amplification and maintained at -20 °C 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 °C for 3 mins, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 50 sec, elongation at 72 °C for 30 sec and final extension at 72 °C 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 & Ronquist 2001) to evaluate Posterior probabilities (PP) (Rannala & Yang 1996, Zhaoybayeva & 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 aptrooii** | CBS 980.95⁹ | JX496234                 | -             | JX496121     |
| **Alloconiothyrium aptrooii** | CBS 981.95⁹ | JX496235                 | -             | JX496122     |
| **Austropleospora archidendri** | CBS 168.77⁹ | JX496162                 | -             | JX496049     |
| **Austropleospora osteospermi** | LM 2009a⁹ | -                        | -             | FJ481946     |
| **Bimuria nova-zelandiae**    | CBS 107.79⁹ | AY016356                 | AY016338      | -            |
| **Deniquelata baringtoniae**  | MFLUCC 11-0422⁹ | JX254655               | JX254656      | JX254654     |
| **Deniquelata baringtoniae**  | MFLUCC 11-0425⁹ | KM213997               | KM214000      | KM214003     |
| **Didymocrea sasavini**       | CBS 438.65⁹ | DQ384103                 | DQ384066      | -            |
| **Didymosphaeria rubi-ulinfolii** | MFLUCC 14-0023⁹ | KJ436586               | KJ436588      | KJ436586     |
| **Didymosphaeria rubi ulinfolii** | CBS 100299 | JX496124                 | AY642523      | JX496011     |
| **Didymosphaeria sp.**        | CBS 587 84 | JX496212                 | JX496099      | -            |
| **Kalmusia ebuli**            | CBS 123120⁹ | JN644073                 | JN851818      | -            |
| **Kalmusia italic**           | MFLUCC 14-0560⁹ | KP744487               | KP753953      | KP744441     |
| **Kalmusia varriisporum**     | MFLUCC 13-0352⁹ | KM658315               | KM658316      | KM658314     |
| **Kalmusia varriisporum**     | CBS 121517⁹ | JX496143                 | JX496030      | -            |
| **Karstenula rhodostoma**     | CBS 690.94 | GU301821                 | GU296154      | -            |
| **Karstenula rhodostoma**     | CBS 691.94 | AB807531                 | AB797241      | AB80506     |
| **Laburnicola hawskworthii**  | MFLUCC 13-0602⁹ | KU743195               | KU743196      | KU743194     |
| **Laburnicola muriformis**    | MFLUCC 16-0290⁹ | KU743198               | KU743199      | KU743197     | KU743213 |
| **Laburnicola muriformis**    | MFLUCC 14-0921⁹ | KU743201               | KU743202      | KU743200     |
| **Lettredraea cordylinicola**  | MFLUCC 11-0148 | KM213995               | KM213998      | KM214001     |
| **Lettredraea cordylinicola**  | MFLUCC 11-0150⁹ | KM213996               | KM213999      | KM214002     |
| **Lettredraea helminthicola**  | CBS 884.85  | AY016362                 | AY016345      | -            |
| **Lettredraea padouk**         | CBS 485.70 | AY849951                 | -             | -            |
| **Montagnula aloe**           | CPC 1967¹   | JX69894                  | -             | JX69863      |
| **Montagnula appendiculata**  | CBS 109027⁹ | AY772016                 | -             | DQ435529     |
| **Montagnula bellevaiae**     | MFLUCC 14-0924⁹ | KT443902               | KT443904      | KT443906     |
| **Montagnula cirri**          | MFLUCC 13-0680 | KX274249               | KX274255      | KX274242     | KX284707 |
| **Montagnula donacina**       | HVVV01      | KJ628377                 | KJ628376      | KJ628375     |
| **Montagnula graminicola**    | MFLUCC 13-0352⁹ | KM658315               | KM658316      | KM658314     |
| **Montagnula jonesii**        | MFLUCC 16-1448 | KY273276               | KY313618      | KY313619     | KY313620 |
| **Montagnula opulenta**       | CBS 168.34 | NG027581                 | NG013127      | AF383966     |
| **Montagnula saikhuensis**    | MFLUCC 16-0315⁹ | KU743210               | KU743211      | KU743209     |
| **Montagnula scabiosa**       | MFLUCC 14-0954⁹ | KT443903               | KT443905      | KT443907     |
| **Neokalmusia brevispora**    | KT 2313¹ | AB524601                 | AB524460      | -            | AB539113 |
| **Neokalmusia didymospora**   | MFLUCC 11-0613⁹ | KP091434               | KP091435      | KP091433     |
| **Neokalmusia scabrispora**   | KT 2202    | AB524594                 | AB524543      | -            | AB539107 |
| **Paracamarosporium fagi**    | CPC 24890 | KR611990                 | KR611887      | -            |
| **Paracamarosporium fagi**    | CPC 24890 | KR611990                 | KR611886      | -            |
| **Paracamarosporium hawaiense** | CBS 120025⁹ | JX496140               | EU295655      | JX496027     |
| **Paracamarosporium psoraleae** | CPC 21632⁹ | KF771199                 | -             | KF771143     |
| **Paraconiothyrium cyclothyroides** | CBS 972.95⁹ | JX496232               | AY642524      | JX496119     |
| **Paraconiothyrium estuarinar** | CBS 10985⁹ | JX496129               | AY642522      | JX496016     |
| **Paraconiothyrium junigcola** | CBS 11326⁹ | JX496133               | AY642527      | JX496020     |
| **Paramassariosphaeria clematidicola** | MFLU 16-0172⁹ | KU743207               | KU743208      | KU743206     |
| **Paramassariosphaeria aestuariomades** | CBS 615.86 | GU205223               | GU205246      | -            |
| **Paraphaeosphaeria angularis** | CBS 167.70⁹ | JX496160                 | -             | JX496047     |
| **Paraphaeosphaeria michotii** | MFLUCC 13-0349⁹ | KJ392822               | KJ392828      | KJ39279     |
| **Paraphaeosphaeria muntians** | CBS 111750 | JX496130                 | -             | JX496017     |
| **Paraphaeosphaeria muntians** | CBS 859.71 | JX496229                 | JX496116      | -            |
### 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 characters and 602 parsimony-uninformative 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).

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| Species name                        | Strains no. | 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 13-0541f | KJ813278 | KJ819946 | KJ747048 | -      |
| *Pseudocamarosporium cotiniae*      | MFLUCC 14-0624f | KP744505 | KP753964 | KP744460 | -      |
| *Pseudocamarosporium lonicerae*     | MFLUCC 13-0352f | KJ813278 | KJ819947 | KJ747047 | -      |
| *Pseudocamarosporium propinquum*    | MFLUCC 13-044f | KJ813280 | KJ819949 | KJ747049 | -      |
| *Pseudopithomyces chartarum*        | UTHSC 03-678 | HG518065 | HG518064 | HG518059 | -      |
| *Pseudopithomyces chartarum*        | UTHSC 03-2472 | HG518064 | HG518059 | HG518059 | -      |
| *Spegazzinia deightonii*             | MUCL 15905  | LK936383 | LK936375 | -       | -      |
| *Spegazzinia tesserilhara*           | MUCL 4329   | LK936382 | LK936347 | -       | -      |
| *Tremateia arundicola*               | CPC 125469f | AB807582 | AB797292 | -       | AB808558 |
| *Tremateia gruyangensis*             | CPC 125465f | AB807582 | AB797293 | -       | AB808559 |
| *Tremateia halophilica*              | SH 241737f  | AB807584 | AB797294 | JQ673429 | AB808560 |
| *Verrucostoconiothyrium nitidae*     | CBS 119209  | EU552112 | EU552112 | -       | -      |

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**: Secção 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.

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**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 (χ = 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 (χ = 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 (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 ascii 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 assexual 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                  |
|----------------------------------|-----------|---------------------|------------|-----------------------------|
| M. aloeis                        | 450       | 3                   | Aloe sp.   | Crous et al. 2012           |
| M. appendiculata                 | 100–200   | 1                   | Zea mays   | Aptroot 2004                |
| M. bellevaleiae                  | 100–120 × 150–175 | 2              | Belleviala romana | Hongsanan et al. 2015     |
| M. cirsi                        | 385–415 × 510–525 | 3              | Cirsium sp. | Hyde et al. 2016           |
| M. donacina                      | -         | 1                   | Arundo donax | Aptroot 1995               |
| M. graminicola                  | 37–117.22 | 1                   | Grass      | Liu et al. 2015             |
| M. jonesii                      | 325–350 × 300–325 | 3              | Fagus sylvatica | This study               |
| M. opalenta                     | 400–1200  | 1                   | Opuntia sp. | Aptroot 1995               |
| M. saikhuensis                   | 400–450 × 400–500 | 1            | Citrus sp. | Wanasinghe et al. 2016     |
| M. scabiosae                    | 300–320 × 300–360 | 3           | Scabiosa sp. | Hongsanan et al. 2015     |

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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.

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