Phytotaxa 203 (1): 037–046
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.203.1.3

Molecular phylogenetic analysis reveals two new species of *Discosia* from Italy

WEN-JING LI[1,2,3,4], JIAN-KUI LIU[3,4], D. JAYARAMA BHAT[3,5], ERIO CAMPORESI[6], DONG-QING DAI[3,4], PETER E MORTIMER[1,2], JIAN-CHU XU[1,2], KEVIN D. HYDE [1,2,3,4,7] & PUTARAK CHOMNUNTI[3,4]

[1]*World Agroforestry Centre, East and Central Asia, 132 Lanhei Road, Kunming 650201, China*
[2]*Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Lanhei Road No 132, Panlong District, Kunming, Yunnan Province, 650201, PR China*
[3]*Institute 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]*Formerly, Department of Botany, Goa University, Goa 403206, India*
[6]*A.M.B. Gruppo Micologico Forlivese "Antonio Cicognani", Via Roma 18, Forlì, Italy*
[7]*Botany and Microbiology Department, College of Science, King Saud University, Riyadh, KSA 11442, Saudi Arabia*
Corresponding author: Putarak Chomnunti, email address: putarak.cho@mfu.ac.th

Abstract

Two fresh collections of *Discosia* were made from dead leaves of *Fagus sylvatica* in Italy. As these collections could not be cultured, the fruiting bodies were directly used for sequencing using a Forensic DNA Extraction Kit. Based on analyses of the concatenated internal transcribed spacer regions of the nrDNA operon (ITS) and large subunit rDNA (LSU) gene sequences, as well as morphological characters, the fresh collections are introduced as two new species, namely *D. italica* and *D. fagi*. Phylogenetically, these two species are distinct from all other *Discosia* species. Morphologically, *D. italica* is somewhat similar with *D. fagi*, but can be distinguished using dimension of conidiomata and conidiogenous cells. Descriptions and illustrations of the new taxa are provided herein.

Key words: Asexual morphs, Amphisphaeriaceae, Phylogeny, Taxonomy

Introduction

Discosia (Amphisphaeriaceae) is a widely distributed genus of coelomycetes (Sutton 1980, Vanev 1992b, Nag Raj 1993, Wołczańska *et al.* 2004). Most species are endophytes or saprobes on various vascular plants in the tropical and temperate regions (Subramanian & Reddy 1974, Sutton 1980, Vanev 1992b, 1996, Nag Raj 1993, Okane *et al.* 1998). Some species in this genus are pathogens, such as *Adisciso yakushimense* Kaz. Tanaka, Okane & Hosoya on *Symplocos prunifolia* (Tanaka *et al.* 2011), as well as *A. kaki* Kaz. Tanaka, J. Yamam. & Toy. Sato on *Diospyros kaki* (Yamamoto *et al.* 2012), forming leaf spots on terrestrial plants from June to September.

The genus *Discosia* is characterized by uni- to multilocular conidiomata with multi-layered walls, occurring singly or in clusters. Conidiogenous cells are monoblastic, phialidic to annellidic. Conidia are almost hyaline to pale brown, and with polar or subpolar appendages inserted in the median part of the end cells (Sutton 1980, Nag Raj 1993).

Traditionally, morphological characters and host-association have been used to classify *Discosia* to species (Subramanian & Reddy 1974, Sutton 1980, Vanev 1991, 1992a, 1996, Nag Raj 1993). However, most species in this genus have overlapping characters, such as the location of the conidial septa and appendages, varying proportional lengths of conidial cells, and overall conidium size (Sutton 1977, 1980, Nag Raj 1993, Jeewon *et al*. 2002, Barber *et al.* 2011, Tanaka *et al.* 2011). The classification, validity, and delimitation of this genus have been problematic (Subramanian & Reddy 1974, Vanev 1991, 1992b, Sotton 1980, Nag Raj 1993, Jeewon *et al*. 2002). Subramanian & Reddy (1974) divided the taxa under *Discosia* into four sections based on the conidial morphology (size, septation and pigmentation). Vanev (1991) expanded this concept and grouped the species of *Discosia* into six sections, viz., *D*. sect. *Discosia*, *D*. sect. *Laurina* Vanev, *D*. sect. *Clypeata* Vanev, *D*. sect. *Libertia* Vanev, *D*. sect. *Strobilina* Vanev and *D*. sect. *Poikilo-*

Accepted by Sajeewa Maharachchikumbura: 19 Jan. 2015; published: 19 Mar. 2015

mera Vanev (Vanev 1991, 1992a, 1996). However, the concept of *Discosia* has been significantly amended based on molecular sequence data. Phylogenetic analyses generated from 18 strains of *Discosia,* based on β-tubulin sequence data by Tanaka *et al*. (2011), showed that species in section *Libertia* are distributed among the two *Discosia* clades, and species in section *Discosia* and section *Strobilina* did not constitute a natural grouping. Therefore, the subdivision proposed based on conidial characters by Vanev (1991) is not phylogenetically significant in the three above sections (Tanaka *et al.* 2011). Whether the conidial septa and appendages and varying proportional lengths of conidial cells used to distinguish taxa of *Discosia* have phylogenic significance, requires further assessment using molecular data.

The aim of this paper is to introduce two new species in *Discosia*, *D. italica* and *D. fagi*, based on combination of morphological data and results of phylogenetic analyses. Descriptions and illustrations of the two new species are provided.

Materials & Methods

Specimen examination

Fresh specimens were collected from Forlì-Cesena in Italy on *Fagus sylvatica*, dried and sent to Thailand for examination. Samples were processed and examined, following the method described in Li *et al.* (2014). The specimens were observed and examined under a Nikon ECLIPSE 80i and 90i compound microscopes and photographed using a Canon 550D and 600D digital camera fitted to the microscope. Measurements were made using Tarosoft v. 0.9.7. (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

DNA extraction, PCR amplification and sequencing

Despite several attempts we could not isolate *D. italica* and *D. fagi* into culture. Therefore, DNA was extracted directly from the conidiomata on dried specimens. The materials were surface disinfected with 75% alcohol for 1 min and subsequently rinsed with sterile water for 1 min. Target conidiomata (5–10) were removed directly from the sterilized material using sterile fine forceps and collected in a 1.5 ml micro-centrifuge tube. Genomic DNA was extracted from conidiomata and infusion of conidiomata using the E.Z.N.A. ™ Forensic DNA Extraction Kit (OMEGA Bio-Tek, D3591-01, Norcross, GA, U.S.A.).

DNA amplification was performed by polymerase chain reaction (PCR). The primer pairs LROR and LR5 as defined by Vilgalys & Hester (1990) were used to amplify a segment of the large subunit rDNA (LSU). Primer pairs ITS4 and ITS5 as defined by White *et al.* (1990) were used to amplify the internal transcribed spacers (ITS). The amplifications were performed in a 50 µl reaction volume as follows: 3 µg DNA template, 1.5 µl of each forward and reverse primers, 25 µl of 2 x Taq PCR SuperMix (Mixture of 0.1 U Taq Polymerase/µl, 500 µm Dntp each, 20 mM Tris-HCL PH8.3, 100 Mm KCl, 3 mM MgCl2 and optimized buffer) (TIANGEN BIOTECH Co., Ltd., Chaoyang District, Beijing, PR China) and 19 µl sterilized distilled water. The amplification conditions were as follows: initially 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30–50 seconds, annealing at 55 °C for 1 minute, elongation at 72 °C for 90 seconds, and final extension at 72 °C for 5–10 minutes. The PCR products were checked on 1% agarose electrophoresis gel stained with ethidium bromide, and sent to Beijing Bai Mai Hui Kang Biological Engineering Technology Co. Ltd (Beijing, P. R. China) for molecular sequencing.

DNA sequence data analysis

The sequence data generated from different primers were compared by Blast search with reference sequences in GenBank (Altschul *et al.* 1990). The sequence alignments were generated with MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html). Bioedit v. 7.2 (Hall 1999) and Clustal X v. 1.83 (Thompson *et al.* 1997) are used to align the sequence data. The alignments were checked visually and improved manually wherever necessary. Phylogenetic analyses of sequence data were carried out using PAUP v.4.0b 10 (Swofford 2002) for Maximum-Parsimony (MP) analyses, MrBAYES v. 3.0b4 (Ronquist & Huelsenbeck 2003) for Bayesian analyses, and RAxMLGUI v. 1.3 (Silvestro & Michalak 2011) for maximum likelihood (ML) analysis.

In order to perform Maximum Likelihood analysis, alignments in PHYLIP format were loaded and automatically checked their compatibility in raxmlGUI. The optimal ML tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run (Maharachchikumbura *et al.* 2014). The

final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTRGAMMA substitution model.

Maximum-parsimony analyses were performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1000 bootstrap replications (Hillis & Bull 1993). Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated and the resulting trees were printed with TreeView v. 1.6.6 (Page 1996).

Bayesian analysis was performed on a MP starting tree automatically generated by using software MrBAYES v.3.0b4 (Ronquist & Huelsenbeck 2003), MrModeltest2.3 (Nylander 2004). The performed procedure followed as described previously (Liu *et al.* 2012). Trees were rooted using *Pestalotiopsis versicolor* (BRIP 14534) as the outgroup taxon and visualized with TreeView (Page 1996).

Results

Phylogenetic analyses

Two gene regions (ITS and LSU) were determined for the two fresh collections from Italy. The other sequences data used in the analyses were downloaded from GenBank (Table 1). The combined ITS-LSU gene datasets comprised of 43 taxa. The dataset consists of 1475 characters (including alignment gaps) after alignment, of which 1269 were constant, 54 variable characters parsimony-uninformative and 152 characters parsimony-informative. Kishino-Hasegawa (KH) test showed length=349 steps, CI=0.679, RI=0.868, RC=0.589 and HI=0.321. Bayesian, Maximum parsimony (MP) and RAxML analyses of the combined dataset resulted in phylogenetic reconstructions with similar topologies and not significantly different (data not shown). The best scoring RAxML tree was chosen as the backbone tree and shown in Figure 1.

The phylogenetic reconstruction based on ITS and LSU combined sequence data resulted in four groups corresponding to five genera, namely *Discosia*, *Seimatosporium*, *Immersidiscosia*, *Bartalinia* and *Truncatella*. Twenty-seven taxa of *Discosia* clustered together and were close to the *Seimatosporium* clade. The genus *Immersidiscosia* was represented by the type species, and the fourth group represents *Bartalinia* and *Truncatella* represented by their type species. Within the *Discosia* clade, there were three distinct sub-clades, in which the first represents the type species of *Discosia* (*D. artocreas*), with *D. pini*, *D.* aff. *brasiliensis*, *D. aquatica*, *D. pseudoartocreas* and one strain of *D.* aff. *artocreas* (KT2118), along with four *Discosia* isolates which could not be identified to species level, the second polytomy cladogram includes the two new species *D. italica* and *D. fagi* clustering with *Adisciso tricellulare* (Okane, Nakagiri & Tad. Ito) Kaz. Tanaka, Okane & Hosoya, *A. yakushimense* Kaz. Tanaka, Okane & Hosoya, as well as two strains of *D.* aff. *pleurochaeta*, *D.* aff. *artocreas* (MAFF 242785, MAFF 238070), and the third sub-clade includes two *Discosia* species isolates. *D. italica* is morphologically somewhat similar with *D. fagi*, and it is difficult to recognize these two species only by morphology. However, *D. italica* and *D. fagi* did not cluster together with any other strains within *Discosia* and formed two separate branches. Therefore, we treat them as two different new species which we introduce in this study.

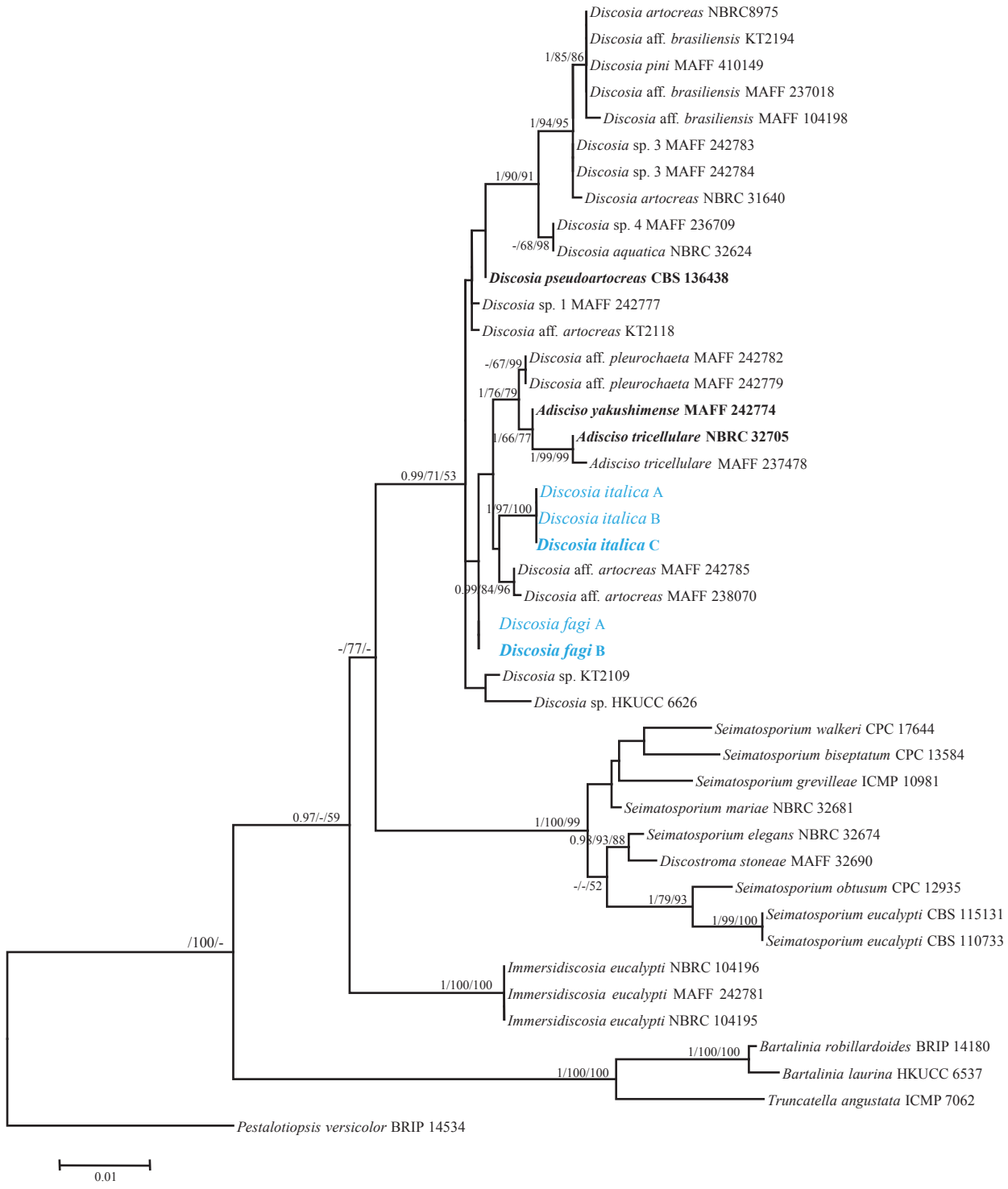

FIGURE 1. Best scoring RAxML tree of *Discosia* strains obtained from combined dataset of LSU and ITS sequence alignment. Bootstrap support (BS) values of maximum parsimony (MP) and maximum likelihood (ML) (equal to or greater than 50% based on 1.000 replicates) and Bayesian posterior probabilities (PP) (equal to or above 0.95) are shown at the nodes. New species strains are in blue and bold the ex-types (type). The tree is rooted to *Pestalotiopsis versicolor* (BRIP 14534).

TABLE 1. GenBank and culture collection accession numbers of species treated in the phylogenies. The newly generated sequences are in dicated in bold.

Taxon	Original code	Culture Accession	Host	GenBank Accession	
				ITS	LSU
Adisciso tricellulare	-	MAFF 237478	*Rhododendron indicum*	AB594798	AB593730
Adisciso tricellulare	-	NBRC 32705 (ex-type)	*Rhododendron indicum*	AB594796	AB593728
Adisciso yakushimense	KT 1907	MAFF 242774 (ex-type)	*Symplocos prunifolia*	AB594789	AB593721
Bartalinia robillardoides		BRIP 14180	*Macrotyloma daltonii*	AF405301	AF382366
Bartalinia laurina	-	HKUCC 6537	Unidentified dead leaf	AF405302	AF382369
Discosia aff. *artocreas*	-	MAFF 238070	*Fallopia japonica*	AB594788	AB593720
Discosia aff. *artocreas*	SH 290	MAFF 242785	*Hamamelis japonica*	AB594779	AB593711
Discosia aff. *artocreas*	KT 2118	MAFF 242776	*Betula ermanii*	AB594772	AB593704
Discosia aff. *brasiliensis*	KT 2193	MAFF 104198	Decayed leaves	AB594774	AB593706
Discosia aff. *brasiliensis*	-	MAFF 237018	*Rosa rugosa*	AB594787	AB593719
Discosia aff. *brasiliensis*	-	KT 2194	Decayed leaves	AB594775	AB593707
Discosia aff. *pleurochaeta*	KT 2192	MAFF 242782	Decayed leaves	AB594782	AB593714
Discosia aff. *pleurochaeta*	KT 2188	MAFF 242779	Decayed leaves	AB594781	AB593713
Discosia aquatica	-	NBRC 32624	*Quercus fusiformis*	3262401	-
Discosia artocreas	-	NBRC 8975	*Poa pratensis*	AB594773	AB593705
Discosia artocreas	-	NBRC 31640	Decayed leaves	3164001	-
Discosia fagi	**IT-722 A**	**-**	***Fagus sylvatica***	**KM678040**	**KM678048**
Discosia fagi	**IT-722 B**	**-**	***Fagus sylvatica***	**KM678039**	**KM678047**
Discosia italica	**IT-712 A**	**-**	***Fagus sylvatica***	**KM678042**	**KM678045**
Discosia italica	**IT-712 B**	**-**	***Fagus sylvatica***	**KM678043**	**KM678046**
Discosia italica	**IT-712 C**	**-**	***Fagus sylvatica***	**KM678041**	**KM678044**
Discosia pini	-	MAFF 410149	*Pinus densiflora*	AB594776	AB593708
Discosia pseudoartocreas	CPC 21117	CBS 136438 (ex-type)	*Tilia* sp.	KF777161	KF777214
Discosia sp.	-	HKUCC 6626	Unidentified dead leaf	AF405303	AF382381
Discosia sp. 1	KT 2131	MAFF 242777	*Betula platyphylla* var. *japonica*	AB594778	AB593710
Discosia sp. 3	SH 288	MAFF 242784	*Machilus thunbergii*	AB594784	AB593716
Discosia sp. 3	SH 125	MAFF 242783	*Castanea crenata*	AB594783	AB593715
Discosia sp. 4	-	MAFF 236709	*Parthenocissus tricuspidata*	AB594786	AB593718
Discosia sp.	-	KT 2109	Decayed leaves	AB594780	AB593712
Discostroma stoneae	-	MAFF 32690	-	AB594797	AB593729
Immersidiscosia eucalypti	KT 2091	NBRC 104195	*Quercus myrsinifolia*	AB594790	AB593722
Immersidiscosia eucalypti	KT 2115	NBRC 104196	*Quercus myrsinifolia*	AB594791	AB593723
Immersidiscosia eucalypti	KT 2191	MAFF 242781	Decayed leaves	AB594793	AB593725
Pestalotiopsis versicolor	-	BRIP 14534	Psidium guajava	AF405298	AF382357
Seimatosporium biseptatum	-	CPC 13584	*Eucalyptus oresbia*	JN871199	JN871208
Seimatosporium obtusum	-	CPC 12935	*Corymbia henryi*	JN871206	JN871215
Seimatosporium elegans	-	NBRC 32674	*Melaleuca ericifolia*	AB594801	AB593733
Seimatosporium eucalypti	-	CBS 115131	*Eucalyptus smithii*	JN871200	JN871209
Seimatosporium eucalypti	-	CBS 110733	*Eucalyptus smithii*	JN871201	JN871210
Seimatosporium walkeri	-	CPC 17644	*Eucalyptus* sp.	JN871207	JN871216
Seimatosporium mariae	-	NBRC 32681	*Correa reflexa*	AB594807	AB593740
Seimatosporium grevilleae	-	ICMP 10981	*Protea* sp.	AF405304	AF382372
Truncatella angustata	-	ICMP 7062	*Malus X domestica*	AF405306	AF382383

Sequence ID of NBRC strains with blue highlights were obtained from NBRC web site: (http://www.nbrc.nite.go.jp/NBRC2/NBRCDispSearchServlet?lang=jp)

Taxonomy

Discosia italica W.J. Li, J.K Liu & K.D. Hyde *sp. nov.* **FIGURE 2.**

Index Fungorum number: IF550758
Faces of Fungi number: FoF00330
Etymology: Named after the country from where the fungus was collected, Italy.
Holotype: MFLU 14–0298

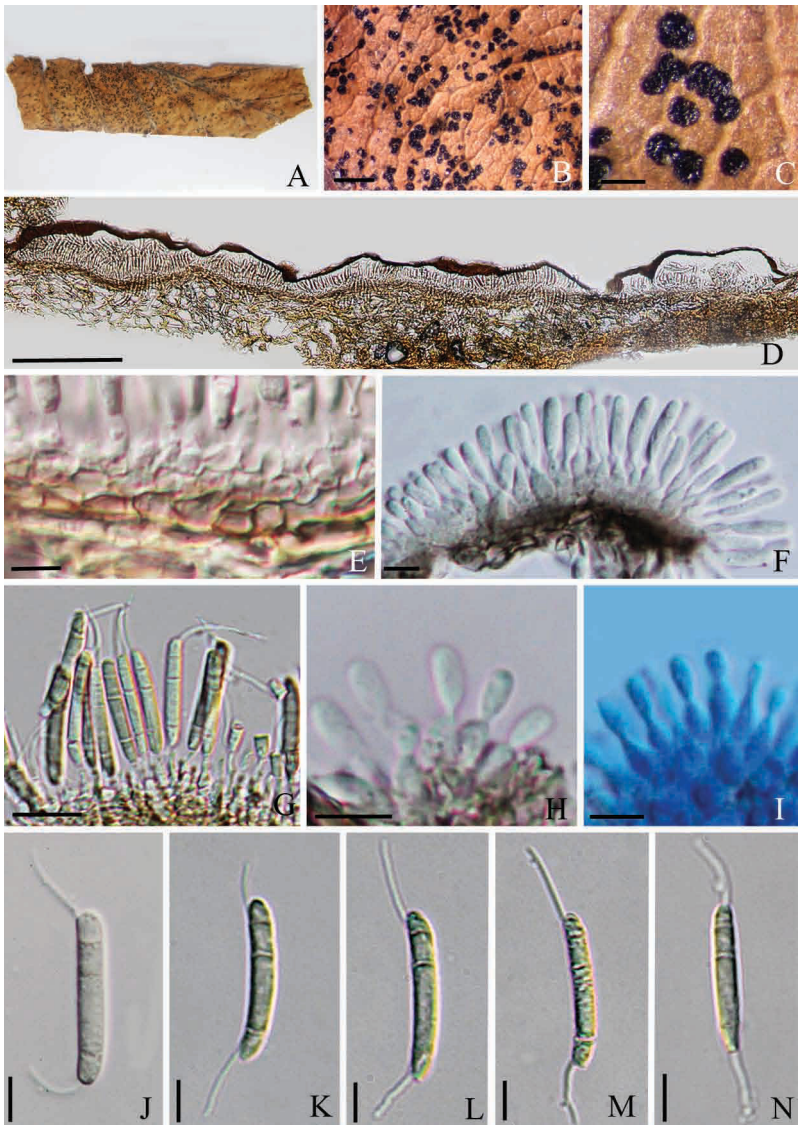

FIGURE 2. *Discosia italica* (MFLU 14–0298, holotype). A**.** Specimen. B, C**.** Black conidiomata on host surface. D. Vertical section of conidioma. E. Section of peridium. F–I. Conidiogenous cells and developing conidia. J–N. Conidia. Scale bars: B = 500 µm. C = 200 µm. D = 100 µm. E, F = 5 µm. G = 10 µm. H–N = 5 µm.

Saprobic on dead leaves of *Fagus sylvatica*. **Sexual state:** Unknown. **Asexual state**: coelomycetous. *Conidiomata* 100–250 ($\bar{x}$ = 180) µm diam., 20–45 ($\bar{x}$ = 30) µm high, conspicuous, pycnidial, stromatic, amphigenous, scattered or aggregated and confluent, flattened or concave at the centre with a convex margin and a relatively thin stromatic base, rounded, black, glabrous, epidermal, unilocular or multilocular, with locules separated by cells of *textura porrecta*, ostiolate. *Peridium* 6–17 µm wide, composed of 3–4-layers, with outer 1–2-layers brown and inner 1–2-layers hyaline, composed of thin-walled cells of *textura angularis*. *Ostiole* circular, papillate. *Conidiophores* absent. *Conidiogenous cells* 2–5 × 1–3 ($\bar{x}$ = 3.5 × 2) µm, holoblastic to phialidic, ampulliform, integrated, hyaline, smooth-walled. *Conidia* 10–19 × 1.5–4 µm ($\bar{x}$ = 15 × 2.5, n = 50), cylindrical to allantoid, initially hyaline, becoming pigmented to pale brown

at maturity, smooth-walled, guttulate, 3-euseptate, slightly constricted at septa, thin-walled; with basal cell obconic, slightly truncate at the base and appendaged; 2 median cells sucylindrical, with second cell from the base 4–7 µm ($\bar{x}$ = 5.5 µm) long and third cell 2.5–4 µm ($\bar{x}$ = 3 µm) long; apical cell subconical with a obtuse apex; apical and basal cells each with a single, simple, unbranched, filamentous appendage at the ends, apical appendage 4–10 µm ($\bar{x}$ = 8 µm) and basal appendage 6–9 µm ($\bar{x}$ = 8 µm).

Material examined:—ITALY, Forlì-Cesena [FC], Campigna, Santa Sofia, on dead leaves of *Fagus sylvatica* L. (*Fagaceae*), 1 September 2012, Erio Camporesi, IT-712 (MFLU 14–0298 **holotype**). *ibid*. (KUN! HKAS 83869, **isotype**)

Notes:—The fresh collections (MFLU 14–0298) could not be cultured. Therefore, the fruiting bodies were directly used for sequencing and three sequences of *D. italica* were obtained and marked as *D. italica* A, *D. italica* B, *D. italica* C (Figure 1). Similarly, two sequences of *D. fagi* were made from another collection (MFLU 14–0299), viz. *D. fagi* A and *D. fagi* B.

Discosia fagi W.J. Li, J. K. Liu & K.D. Hyde *sp. nov.* **FIGURE 3.**

Index Fungorum number: IF550759

Faces of Fungi number: FoF00331

Etymology: Referring to the host genus, *Fagus*, from which the species was isolated.

Holotype: MFLU 14–0299

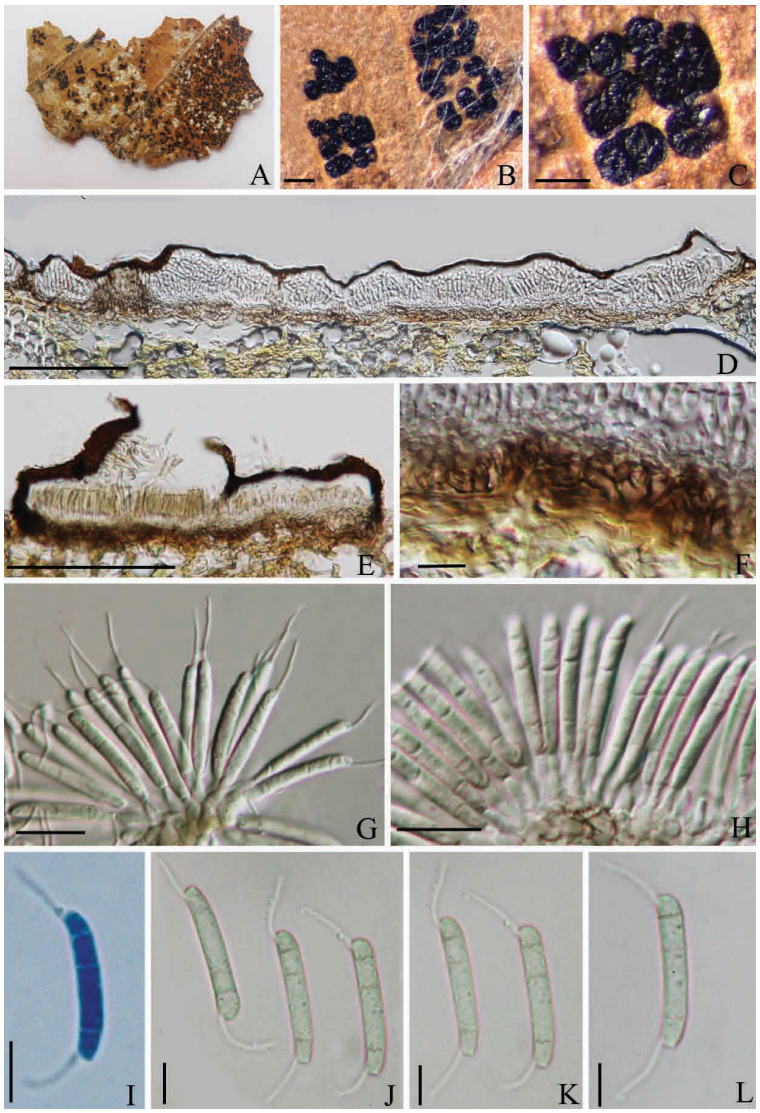

FIGURE 3. *Discosia fagi* (MFLU 14–0299, holotype). A. Specimen. B, C. Black conidiomata on the host surface. D, E. Vertical section of conidioma. F. Section of peridium. G–H. Conidiogenous cells and developing conidia. I–L. Conidia. Scale bars: B = 500 µm. C = 200 µm. D, E = 100 µm. F–L = 5 µm.

Saprobic on dead leaves of *Fagus sylvatica*. **Sexual state:** Unknown. **Asexual state:** coelomycetous. *Conidiomata* 200–500 ($\bar{x}$ = 300) µm diam., 30–75 µm high, pycnidial, stromatic, epiphyllous or hypophyllous, solitary, scattered to gregarious or confluent, applanate to disc-like, partly immersed or superficial, rounded to irregular in outline, glabrous, unilocular or divided into several locules by tissue cells of *textura porrecta,* ostiolate. *Peridium* 10–15 µm thick, composed of 5–6 layers of cells, with outer 2–3 layers dark brown, inner 1–2 layers colourless, comprising thin-walled cells of *textura angularis*. *Ostiole* circular, papillate. *Conidiophores* reduced to conidiogenous cells, arising from the upper cells of the basal stroma. *Conidiogenous cells* 4–8 × 1–3.5 ($\bar{x}$ = 6 × 2) µm, subcylindrical to narrowly flask-shaped, developing directly on the inner-most layer of peridium-wall, phialidic, each producing a single conidium, integrated, hyaline, smooth. *Conidia* 13–20 × 2.5–3 µm ($\bar{x}$ = 17 × 2.8 µm, n = 50), cylindrical to subcylindrical, straight or slightly curved, 3-septate, slightly constricted at the septa, with cells of equal width and colour (yellowish to colourless); basal cell obconic, with a truncate base; 2 median cells subcylindrical, second cell adjacent to the base 5–9 µm ($\bar{x}$ = 7 µm) long, the third cell adjacent to the apex 2–4 µm ($\bar{x}$ = 3 µm) long; apical cell subconical with a rounded apex; apical and basal cells each with a subapical and suprabasal, unbranched, filiform, flexuous or straight appendage; apical appendage 6–10 µm ($\bar{x}$ = 8 µm), basal appendage 6–10 µm ($\bar{x}$ = 8 µm).

Material examined:—ITALY, Forlì-Cesena [FC], Montefalco, Santa Sofia, on dead leaves of *Fagus sylvatica*, 2 September 2012, Erio Camporesi, IT-722 (MFLU 14–0299 **holotype**). *ibid.* (KUN! HKAS 83870, **isotype**)

Notes:—There are six species so far described from *Fagus sylvatica*, namely, *D. artocreas*, *D. faginea*, *D. maculiformis* (Nag Raj 1993), *D. minuta* (Farr & Rossman 2011), as well as the new species *D. italica* and *D. fagi*. However, the original specimen of *D. maculiformis* Syd. & P. Syd. has not been examined, thus the morphological characters of this species are not clear. Comparative morphological characters of the *D. fagi* and *D. artocreas* show that *D. fagi* share similar characters with *D. artocreas* in the form of conidiomata and conidia, but differ in conidiogenous cells (phialidic in *D. fagi* and annellidic in *D. artocreas*). Moreover, the differences between *D. fagi* and *D. faginea* can to be observed in the shape of the conidiomata (unilocular to muti-locular, applanate to disc-like in *D. fagi* and monoloculate, disc-shaped in *D. faginea*), and the pigmentation of conidia, with *D. fagi* conidia cells having equal width and colour, while in *D. fagi* the two middle cells are wider and darker than the two end cells. Furthermore, *D. fagi* can be distinguished from *D. minuta* in dimensions of conidiomata (200–500 ($\bar{x}$ = 300) µm diam. in *D. fagi* versus 120–170 µm diam. in *D. minuta*), and appendage (6–10 µm in *D. fagi*, whereas up to 10 µm in *D. minuta*). The differences between *D. italica* and *D. fagi* are further explained in the discussion section.

Discussion

The genus *Discosia* was introduced by Libert in 1837. Subramanian & Reddy (1974) reviewed the genus based on morphological characters, and designated *D. strobilina* Lib. ex Sacc. as the lectotype for *Discosia* (Nag Raj 1993, Tanaka *et al.* 2011). However, Vanev (1992a) re-examined the original specimen of *D. artocreas* (Tode) Fr. and designated this species as the lectotype. This concept was followed by Crous *et al.* (2013), as well as Index Fungorum (http://www.indexfungorum.org). *D. artocreas* is cosmopolitan in distribution, occurring on more than 100 plant species in 67 genera (Sutton 1980, Nag Raj 1993, Vanev 1996, Farr & Rossman 2011, Tanaka *et al.* 2011). In a recent phylogenetic study on *Discosia* and related genera, Tanaka *et al.* (2011) showed that *D. artocreas* is paraphyletic (Tanaka *et al.* 2011, Crous *et al.* 2013).

In this study, *Discosia italica* shares morphological similarity with *D. pseudoartocreas*, *D. artocreas* and *Adisciso yakushimense* in having conidial cells of equal width and colour, with the second cell adjacent to the base is always twice or more than two times longer than the third cell adjacent to the apex. However, *D. italica* closely resembles *D. artocreas* in shape of conidiomata, conidiophores and conidia. Yet, *D. italica* remains distinct from *D. artocreas* in the dimension of conidiomata (100–250 µm diam., 25–45 µm deep in *D. italica* whereas 150–500 µm diam. up to 60 µm deep in *D. artocreas*), with conidia being slightly shorter than *D. artocreas* (12.5–) 14–22), as well as nature of conidiogenous cells (phialidic in *D. italica* and annellidic in *D. artocreas*). The phylogenetic analysis based on ITS sequence data showed that *D. italica* may have phylogenetic affinity with *A. kaki*, however, *D. italica* is morphologically quite distinct from *A. kaki*. While the latter is homothallic species without discosia-like conidial state (Yamamoto *et al*. 2012). Therefore, *D. italica* is described as new based on morphological characters as well as molecular data (ITS and LSU).

A mega Blast search of NCBLS Genbank nucleotide based on LSU sequence shows that *D. fagi* (GenBank, KM678039) sequences are simialr *to D.* aff. *artocreas* (GenBank, AB593711, Identities = 807/808 (99%), no gaps),

D. aff. *pleurochaeta* (GenBank, AB593713, Identities = 806/808 (99%), no gaps) and *Discosia* sp. 1' (GenBank, AB593710, Identities = 806/808 (99%), no gaps). The closest hits using the ITS sequence had similarity to *Discosia* sp. (GenBank, AF405303, Identities = 549/560 (98%), Gaps = 2/560 (0%)), *D.* aff. *artocreas* (GenBank, AB594772, Identities = 525/536 (98%), Gaps = 3/536 (0%)) and *D. pseudoartocreas* (GenBank, KF777161, Identities = 557/569 (98%), Gaps = 4/569 (0%)). However, phylogenetic analyses derived from combined ITS and LSU sequences show that *D. fagi* is clearly distinct from all other *Discosia* species (Figure 1). Morphologically, *D. fagi* resembles *D. italica*, but can be distinguished using dimension of conidiomata, being slightly larger than *D. italica* (100–250 µm diam., and 20–45 µm high). Furthermore, the conidiogenous cells of *D. fagi* also longer than *D. italica* 2–5 ($\bar{x}$ = 3) µm. Based on the substantive evidence provided above, *D. fagi* is introduced as novel species of *Discosia*.

In this paper, it has not been possible to apply LSU alone in resolving the species within *Discosia*, especially for species where very few morphological differences exist. Therefore, analyses of the combined sequences of ITS and LSU genes are used to delimit the species within *Discosia*. They also provide a significant evidence to justify that *D. italica* and *D. fagi* are distinct from each other. Nevertheless, the identification of interspecific relationship is problematic within the genus *Discosia*. *D. pini*, *D.* aff. *brasiliensis* and the type species *D. artocreas* clustered together with high bootstrap support, and this is in congruence with the observations of Tanaka *et al*. (2011). Thus, the interspecific relationship within *Discosia* should be studied further using multi-gene data. Moreover, on the bases of molecular data (ITS, LSU), the dimension of conidiomata and morphology of the conidiogenous cells and conidia, interspecies relationship can be distinguished. It would be premature to discuss the phylogenetic significance of various species, given that sequence data is available for only a small number of *Discosia* species. Future studies should focus on larger sampling of *Discosia* species and evaluation by molecular sequence data.

Acknowledgments

We would like to thank Humidtropics, a CGIAR Research Program that aims to develop new opportunities for improved livelihoods in a sustainable environment, for partially funding this work. KD Hyde thanks The Chinese Academy of Sciences, project number 2013T2S0030, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
http://dx.doi.org/10.1016/S0022-2836(05)80360-2

Barber, P.A., Crous, P.W., Groenewald, J.Z., Pascoe, I.G. & Keane, P. (2011) Reassessing *Vermisporium* (Amphisphaeriaceae), a genus of foliar pathogens of eucalypts. *Persoonia* 27: 90–118.
http://dx.doi.org/10.3767/003158511X617381

Cai, L., Hyde, K.D., Taylor, P.W.J., Weir, B., Waller, J., Abang, M.M., Zhang, J.Z., Yang, Y.L., Phoulivong, S., Liu, Z.Y., Prihastuti, H., Shivas, R.G., Mckenzie, E.H.C. & Johnston, P.R. (2009) A polyphasic approach for studying *Colletotrichum*. *Fungal Diversity* 39: 183–204.

Crous, P.W., Wingfield, M.J., Guarro, J., Cheewangkoon, R., van der Bank, M., Swart, W.J., Stchigel, A.M., Cano-Lira, J.F., Roux, J., Madrid, H., Damm, U., Wood, A.R., Shuttleworth , L.A., Hodges, C.S., Munster, M., de Jesús Yáñez-Morales, M., Zúñiga-Estrada, L., Cruywagen, E.M., de Hoog, G.S., Silvera, C., Najafzadeh, J., Davison, E.M., Davison, P.J.N., Barrett, M.D., Barrett, R.L., Manamgoda, D.S., Minnis, A.M., Kleczewski, N.M., Flory, S.L., Castlebury, L.A., Clay, K., Hyde, K.D., Maússe-Sitoe, S.N.D., Chen, Shuaifei, Lechat, C., Hairaud, M., Lesage-Meessen, L., Pawłowska, J., Wilk, M., Śliwińska-Wyrzychowska, A., Mętrak, M., Wrzosek, M., Pavlic-Zupanc, D., Maleme, H.M., Slippers, B., Mac Cormack, W.P., Archuby, D.I., Grünwald, N.J., Tellería, M.T., Dueñas, M., Martín, M.P., Marincowitz, S., de Beer, Z.W., Perez, C.A., Gené, J., Marin-Felix, Y. & Groenewald, J.Z. (2013) Fungal Planet description sheets: 154–213. *Persoonia* 31: 188–296.
http://dx.doi.org/10.3767/003158513X675925

Farr, D.F. & Rossman, A.Y. (2011) Fungal databases, systematic mycology and microbiology laboratory, ARS, USDA. Retrieved February 15, 2011, from /fungal databases/.

Jeewon, R., Liew, E.C.Y. & Hyde, K.D. (2002) Phylogenetic relationships of *Pestalotiopsis* and allied genera inferred from ribosomal DNA sequences and morphological characters. *Molecular Phylogenetics and Evolution* 25: 378–92.

http://dx.doi.org/10.1016/S1055-7903(02)00422-0
Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
Hillis, D.M. & Bull, J.J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.
http://dx.doi.org/10.2307/2992540
Li, W.J., Liu, J.K., Bhat, D.J., Camporesi, E., Chukeatirote, E., Xu, J.C. & Hyde, K.D. (2014) Introducing the novel species, *Dothiorella symphoricarposicola*, from snowberry in Italy. *Cryptogamie*, *Mycologie* 35(3): 257–270.
http://dx.doi.org/10.7872/crym.v35.iss3.2014.257
Liu, J.K., Phookamsak, R., Doilom, M., Wikee, S., Li, Y.M., Ariyawansha, H., Boonmee, S., Chomnunti, P., Dai, D.Q., Bhat, J.D., Romero, A.I., Zhuang, W.Y., Monka, J., Jones, E.B.G., Chukeatirote, E., Ko-Ko, T.W., Zhao, Y.C. & Wang, Y. (2012) Towards a natural classification of Botryosphaeriales. *Fungal Diversity* 57: 149–210.
http://dx.doi.org/10.1007/s13225-012-0207-4.
Maharachchikumbura, S.S.N., Hyde, K.D., Groenewald, J.Z., Xu, J.C, & Crous, P.W. (2014) *Pestalotiopsis* revisited. *Studies in Mycology* 79: 121–186.
http://dx.doi.org/10.1016/j.simyco.2014.09.005
Nag Raj, T.R. (1993) *Coelomycetous anamorphs with appendage-bearing conidia*. Mycologue Publications, Waterloo, Canada, 1101 pp.
Nylander, J.A.A. (2004) MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
Okane, I., Nakagiri, A. & Ito, T. (1998) Endophytic fungi in leaves of ericaceous plants. *Canadian Journal of Botany* 76: 657–663.
http://dx.doi.org/10.1139/b98-032.
Page, R.D.M. (1996) TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357–358.
http://dx.doi.org/10.1093/bioinformatics/12.4.357.
Phookamsak, R., Liu, J.K., Chukeatirote, E., Mckenzie, E.H.C. & Hyde K.D. (2013) Phylogeny and morphology of *Leptosphaerulina saccharicola sp. nov.* and *Pleosphaerulina oryzae* and relationships with *Pithomyces*. *Cryptogamie, Mycologie* 34: 303–319.
http://dx.doi.org/10.7872/crym.v34.iss4.2013.303.
Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
http://dx.doi.org/10.1093/bioinformatics/btg180.
Silvestro, D. & Michalak, I. (2011) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* 12: 335–337.
http://dx.doi.org/10.1007/s13127-011-0056-0
Subramanian, C.V. & Reddy, K.R.C. (1974) The genus *Discosia*. I, Taxonomy. *Kavaka* 2: 57–89.
Sutton, B.C. (1977) Coelomycetes. VI. Nomenclature of generic names proposed for coelomycetes. *Mycological Papers* 141: 1–253.
Sutton, B.C. (1980) *The Coelomycetes-Fungi imperfecti with pycnidia, acervuli and stromata*. Commonwealth Mycological Institute, Kew, UK, 496 pp.
Swofford, D.L. (2002) PAUP: phylogenetic analysis using parsimony. version 4.0 b10. Sinauer Associates, Sunderland MA.
Tanaka, K., Endo, M., Hirayama, K., Okane, I., Hosoya, T. & Sato, T. (2011) Phylogeny of *Discosia* and *Seimatosporium*, and introduction of *Adisciso* and *Immersidiscosia* genera nova. *Persoonia* 26: 85–98.
http://dx.doi.org/10.3767/003158511X576666.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.
http://dx.doi.org/10.1093/nar/25.24.4876.
Vanev, S.G. (1991) Species conception and sections delimitation of genus *Discosia*. *Mycotaxon* 41: 387–396.
Vanev, S.G. (1992a) Comparative morphological studies of *Discosia artocras* and *Discosia faginea*. *Mycotaxon* XLIV: 461–470.
Vanev, S.G. (1992b) On the typification and nomenclature of some *Discosia* species. *International Journal of Mycology and Lichenology* 5: 293–300.
Vanev, S.G. (1996) Fungi of the genus *Discosia* (Deuteromycotina) in the Mediterranean area. *Bocconea* 5: 351–357.
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.
White, T., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In: PCR protocols: a guide to methods and applications.* pp. 315–322.
Wołczańska, A.G.W., Ozłowska, M.O.K., Iątek, M.A.P. & Ułenko, W.I.M. (2004) Survey of the genus *Discosia* (anamorphic fungi) in Poland. *Polish Botanical Journal* 49: 55–61.
Yamamoto, J., Tanaka, K., Ohtaka, N. & Sato, T. (2012) Black leaf spot of Japanese persimmon (Diospyros kaki), a new disease caused by Adisciso kaki sp. nov. *Journal of General Plant Pathology* 78: 99–105.
http://dx.doi.org/10.1007/s10327-012-0367-9