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Magnicamarosporium diospyricola sp. nov. (*Sulcatisporaceae*) from Thailand

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Abstract

A new species of *Magnicamarosporium*, *M. diospyricola* was found on dead or dying twigs of a dicotyledonous plant in southern Thailand. The new species is distinct from other species in *Sulcatisporaceae*, as it has dematiaceous dictyosporous conidia. It differs from *Magnicamarosporium iriomotense* in its smaller conidiomata and conidia. Bayesian inference and maximum likelihood analysis of combined LSU, SSU, ITS, and TEF1-α sequence data indicate that *M. diospyricola* is a well-resolved species, sister to *M. iriomotense*, in the family *Sulcatisporaceae*. The morphology and phylogenetic placement of the new species are discussed in this paper.

Keywords – asexual morph – coelomycetes – Massarineae – Pleosporales – saprobes

Introduction

Camarosporium-like species account for a well-defined coelomycetous group characterized by dictyosporous conidia (Schulzer 1870, Sutton 1980). Based on molecular data, the type species of *Camarosporium*, *C. quaternatum*, is placed in the suborder Pleosporinae (Pleosporales) (Saccardo 1883, Crous et al. 2006, Wijayawardene et al. 2014a, b, Liu et al. 2015). Wijayawardene et al. (2016) illustrated brown-spored coelomycetes, including various *Camarosporium*-like taxa. Several authors have attempted to establish the phylogenic placement of *Camarosporium*-like taxa (Wijayawardene et al. 2014b, Liu et al. 2015). The *Camarosporium*-like taxa are presently shown to be polyphyletic.

Tanaka et al. (2015) illustrated the suborder Massarineae and described *Magnicamarosporium* based on *Diplospora dubia* (Rubiaceae). The genus is typified by *M. iriomotense* Tanaka & Hirayama and shares a similar morphology with *Camarosporium* in its muriform, brown conidia. Nevertheless, molecular data shows that it belongs to *Sulcatisporaceae*. The genus is characterized by pycnidial conidiomata, with ellipsoid, subglobose, and muriform conidia (Crous et al. 2014, Tanaka et al. 2015).

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In this study, analysis of concatenated rDNA and TEF1-α sequence data using maximum-likelihood and Bayesian posterior probabilities, clearly showed that our strain clusters with *Magnicamarosporium.* Therefore, we introduce *Magnicamarosporium diospyricola* sp. nov. isolated from *Diospyros malabarica* in Thailand, based on both morphology and phylogenic analysis.

Material & Methods

Sample collection, morphological study and isolation

Fresh specimens were collected from fallen twigs of *Diospyros malabarica* (Ebenaceae) in Krabi, Thailand, during 2015 and brought to the laboratory in plastic ziplock bags. Pure cultures were established from single ascospores on malt extract agar following the method of Chomnunti et al. (2014). Cultures were incubated at 25°C for up to 8 weeks. Type specimens were deposited in Mae Fah Luang University (MFLU) herbarium. Ex-type living cultures were deposited at the Mae Fah Luang Culture Collection (MFLUCC), and also deposited at the International Collection of Microorganisms from Plants (ICMP). Fungal slides were examined under a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 600D digital camera fitted to the microscope. Measurements were made using Tarosoft (R) Image Frame Work program and photo-plates using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, United States). Faces of fungi numbers and Index Fungorum numbers are provided (Jayasiri et al. 2015, Index Fungorum 2016).

DNA extraction, amplification and sequencing

DNA was extracted from mycelium with Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) (Hangzhou, P. R. China), following the manufacturer's protocol. Primer sequences are available at the WASABI database at the AFTOL website (aftol.org). Amplification reactions for LSU, SSU and ITS were performed according to Phukhamsakda et al. (2015). The PCR thermal cycle program for EF1-983F and EF1-2218R (Carbone & Kohn 1999) for translation elongation factor 1-alpha (TEF1-α) was set for denaturation at 96°C for 2 minutes, followed by 40 cycles of denaturation at 96°C for 45 seconds, annealing at 52°C for 30 seconds and extension at 72°C for 1.30 minutes, with a final extension step at 72°C for 5 minutes. DNA extracted and PCR proliferation products were checked on 1% Agarose gel, the purified PCR products and the sequencing were performed by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China).

Sequence alignment and phylogenetic analysis

SeqMan v. 7.0.0 (DNASTAR, Madison, WI) was used to assemble consensus sequences. Sequences of closely related strains were retrieved using BLAST searches against GenBank (Benson et al. 2013). We also followed the strains from Tanaka et al. (2015) and these are listed in Table 1. Sequences were aligned with MUSCLE in MEGA 7 (Tamura et al. 2013) and MAFFT online tool version 7 (Katoh & Standley 2013). The alignments were checked visually and improved manually wherever obligate nucleotides are necessary. Leading or trailing gaps exceeded from primer binding site were trimmed prior to tree building. Phylogenetic analyses were performed with the CIPRES webportal for maximum likelihood (ML) analysis (Miller et al. 2010) comparing with RAxML (O'meara et al. 2006) maximum likelihood analyses (ML), including 1,000 bootstrap replicates, as implemented in raxmlGUI version v.1.3.1 (Silvestro & Michalak 2011). MrBayes v. 3.2.2 was performed for Bayesian analysis (Huelsenbeck & Ronquist 2001). The search strategy was set to rapid bootstrapping. Analysis was carried out with the general time reversible (GTR) model for nucleotide substitution and a discrete gamma-distributed with invariable sites (GTRGAMMA+I) (Stamatakis et al. 2008, Guindon et al. 2010). The bootstrap replicates were summarized on to the best scoring tree. Maximum likelihood bootstrap values equal or greater than 50% are given in black below or above each node (Fig. 1).

Table 1 Culture collection code and accession numbers used in this study.

Taxon	Strain number	GenBank accession numbers			
		LSU	SSU	ITS	TEF1-α
Bambusicola bambusae	MFLUCC 11-0614	JX442035	JX442039	NR_121546	–
Bambusicola massarinia	MFLUCC 11-0389^{T}	JX442037	JX442041	NR_121548	–
Bambusicola pustulata	MFLUCC 15-0190	KU863107	KU872112	KU940118	KU940190
Bambusicola splendida	MFLUCC 11-0439	JX442038	JX442042	NR_121549	–
Bambusicola triseptatispora	MFLUCC 11-0166	KU863109	–	KU940120	–
Bambusistroma didymosporum	MFLUCC 13-0862^{T}	KP761730	KP761737	KP761734	KP761727
Camarographium koreanum	CBS 117159^{T}	JQ044451	–	JQ044432	–
Camarosporium aloes	CPC 21572	KF777198	–	KF777142	–
Camarosporium quaternatum	CBS 483.95	GU301806	GU296141	–	GU349044
Deniquelata barringtoniae	MFLUCC 11-0422^{T}	NG_042696	JX254656	NR_111779	–
Dictyosporium digitatum	JCM 19404	AB807515	–	LC014545	AB808491
Dictyosporium elegans	NBRC 32502^{T}	DQ018100	DQ018079	DQ018087	–
Dictyosporium thailandicum	MFLUCC 13-0773	KP716707	–	KP716706	–
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0024	KJ436585	KJ436587	–	–
Keissleriella cladophila	CBS 104.55	GU301822	GU296155	–	GU349043
Latorua caligans	CBS 576.65^{T}	KR873266	–	NR_132923	–
Latorua grootfonteinensis	CBS 369.72	KR873267	–	–	–
Lentithecium fluviatile	CBS 122367^{T}	GU301825	GU296158	–	GU349074
Lentithecium lineare	IFRD 2008	FJ795435	FJ795478	–	–
Macrodiplodiopsis desmazieri	CBS 221.37	JX681100	–	KR873236	–
Macrodiplodiopsis desmazieri	CPC 24971	KR873272	–	KR873240	–
Magnicamarosporium iriomotense	KT 2822^{T}	AB807509	AB797219	AB809640	AB808485
Magnicamarosporium diospyricola	**MFLUCC 16-0419**	**KY554212**	**KY554211**	**KY554210**	**KY554209**
Massarina eburnea	CBS 473.64	GU301840	GU296170	–	GU349040
Montagnula aloes	CPC 19671	JX069847	–	JX069863	–
Murilentithecium clematidis	MFLUCC 14-0561^{T}	KM408758	KM408760	KM408756	KM454444
Neobambusicola strelitziae	CBS 138869^{T}	KP004495	–	KP004467	–
Neokalmusia brevispora	CBS 120248^{T}	AB524600	AB524459	–	AB539112
Neottiosporina paspali	CBS 331.37	EU754172	EU754073	KP170653	GU349079
Palmiascoma gregariascomum	MFLUCC 11-0175	KP744495	KP753958	KP744452	–
Paracamarosporium psoraleae	CPC 21632^{T}	KF777199	–	KF777143	–
Periconia homothallica	CBS 139698	AB807565	AB797275	AB809645	AB808541
Periconia pseudodigitata	CBS 139699	AB807564	AB797274	LC014591	AB808540
Phragmocamarosporium hederae	MFLUCC 13-0552	KP842916	KP842919	–	–
Phragmocamarosporium platani	MFLUCC 14-1191^{T}	KP842915	KP842918	–	–
Pseudocamarosporium lonicerae	MFLUCC 13-0532	KJ813278	KJ819947	KJ747047	–
Pseudocamarosporium propinquum	MFLUCC 13-0544^{T}	KJ813280	KJ819949	KJ747049	–
Pseudocamarosporium tilicola	MFLUCC 14-0093	KJ813281	KJ819950	KJ747050	–
Pseudochaetosphaeronema larense	CBS 639.94	KF015610	KF015651	KF015655	KF015683
Pseudochaetosphaeronema larense	CBS 640.73^{T}	KF015611	KF015652	NR_132038	KF015684
Stagonospora pseudocaricis	CBS 135132	KF251762	–	KF251259	KF253209
Sulcatispora acerina	KT 2982^{T}	LC014610	LC014605	LC014597	LC014615
Sulcatispora berchemiae	KT 1607	AB807534	AB797244	AB809635	AB808509
Suttonomyces clematidis	MFLUCC 14-0240^{T}	KP842917	KP842920	–	–
Xenocamarosporium acaciae	CPC 24755^{T}	KR476759	–	KR476724	–

Type species from ex-type of each genus indicated with (T), new generated sequences in this study indicated in bold.

The model of evolution for the Bayesian inference analysis was determined with MrModeltest 2.3 (Nylander 2004) and the GTR+I+G nucleotide substitution model was used for each partition based on the results from MrModeltest. Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.2 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation. 10,000 trees were obtained. The suitable burn-in phase was determined by traces inspected in Tracer version 1.6 (Rambaut et al. 2014). Based on the tracer analysis, the first 1,000 trees representing 10% of burn-in phase of the analyses were discarded. The remaining trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence

diagnostic set to ≤0.01). Bayesian Posterior Probabilities (PP) equal or greater than 0.90 are given above each node (Fig. 1).

Phylogenetic trees and data files were visualized in FigTree v. 1.4 (Rambaut & Drummond 2008). The phylograms with bootstrap values and posterior probabilities on the branches are presented in Fig. 1, using graphical options available in Adobe Illustrator CS v. 6. All sequences generated in this study were submitted to GenBank. The finalized alignment and tree were deposited in TreeBASE, submission ID: 20569 (Piel et al. 1999).

Results

Topology of phylogenetic analysis

Partial nucleotides of LSU, SSU, ITS and TEF1-α dataset comprising 43 strains from the suborder Massarineae were used to determine the placement of *Magnicamarosporium diospyricola*. *Camarosporium quaternatum* (CBS 483.95) and *C. aloes* (CPC 21572) were used as the outgroup taxon (Fig. 1). The individual datasets were initially performed and compares the similarity of the placement topology. Overall topology was consistent (data not shown), therefore the alignments were combined and the results from phylogenetic analyses are given in Fig. 1.

The best scoring tree presented in Fig. 1, with a final likelihood value of In: -17532.41. *Magnicamarosporium diospyricola* clustered in the *Sulcatisporaceae*. The strains cluster with strong support with *M. iriomotense* (95%ML/1.00PP) and separated from other members in the family. *Magnicamarosporium* formed a sister clade and resided with S*ulcatispora berchemiae* (KT 1607), *S. acerina* (KT 2982), and *Neobambusicola strelitziae* (CBS 138869) and another genus in *Sulcatisporaceae* with significant support (100%ML/1.00 PP).

Taxonomy

Magnicamarosporium diospyricola Phukhams, sp. nov. Fig. 2

Index Fungorum number: IF552777; Facesoffungi number: FoF 02897

Etymology – The species habitat in reference of host

Holotype – MFLU 17-0001

Saprobic on dead twigs of *Diospyros malabarica* (Desr.) Kostel. **Sexual morph** Undetermined. **Asexual morph** *Conidiomata* 277–301 µm high × 289–337 µm diam. protruding, partly immersed in the host, subglobose to depressed globose, uniloculate, dark brown to black, with a centrally located ostiole. *Ostioles* 129–145 µm high × 74–116 µm diam. ($\bar{x}$ = 135 × 96 µm, n = 5), central, oblong, thick-walled, periphysate, dark brown. *Pycnidial wall* 10–29 µm (–36 µm at base corner) wide, composed of 7–9 layers of brown to dark brown cells; outer layers with *textura globose* to *textura angularis* cells; inner layers with hyaline cells bearing conidiogenous cells. *Paraphyses* 29–63 µm high × 2–4 µm diam. ($\bar{x}$ = 43 × 3 µm, n = 30), branched, regularly 1−2-septate, hyaline. *Conidiophores* reduced to conidiogenous cells with one supporting cell. *Conidiogenous cells* 5–10 × 3–5 µm, ($\bar{x}$ = 8 × 4 µm, n = 20), holoblastic to annelidic, indeterminate, integrated, cylindrical, hyaline, smooth, with 1–2 prominent annellations. *Conidia* 24–35 × 14–21 µm ($\bar{x}$ = 30 × 17 µm, n = 50), obovoid to broadly oblong, sometimes pyriform, obtuse at apex, slightly tapered at base, with a circular basal scars, euseptate, with 5–7 transverse and 1–2 vertical septa, slightly constricted at median septa, hyaline when young, dark brown at maturity, smooth without a gelatinous sheath.

Culture characteristics – Colonies on MEA, reaching 40 mm diam. after 4 weeks at 25°C, colonies dark-brown to black, dense, irregular, umbonate, with rough surface, strongly irregular at margin covering with white mycelium; reverse white at edges, dark brown to black at the center, radiating, irregular, margin rough, with orange pigment diffused to the agar.

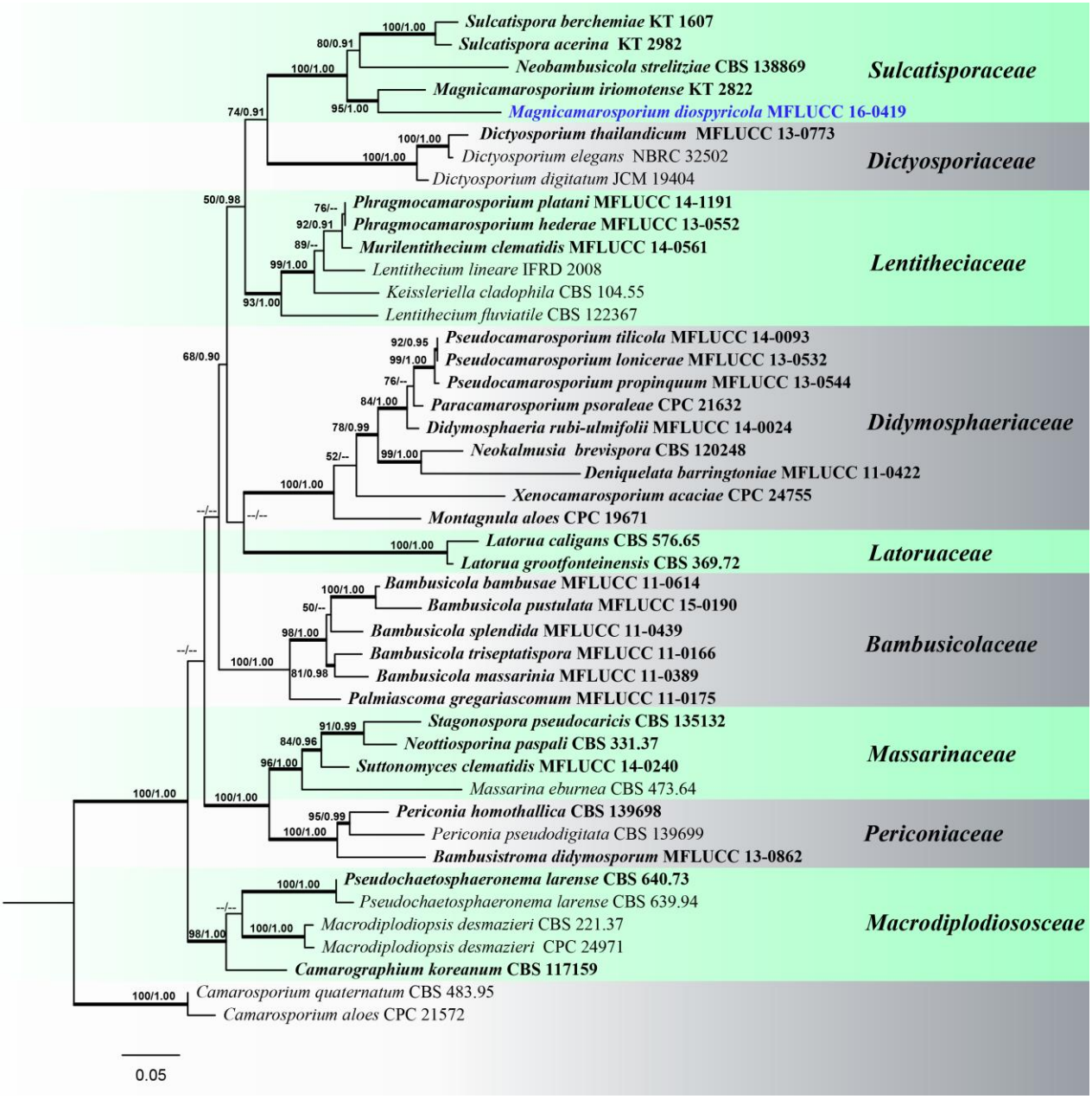

Figure 1 – The best scoring RAxML tree based on a combined partial LSU, SSU, ITS and TEF1-α gene datasets. Bootstrap values ≥50% from the maximum likelihood (ML) analysis are followed by Bayesian posterior probabilities (PP) values ≥ 0.90. The tree is rooted with *Camarosporium sensu stricto.* The species determine in this study indicated in blue. The ex-type and references strains are indicated in black bold. Hyphen (-) represents support values ≤ 50%/0.90. Bold lines represent significant support values from both analyses (BS ≥ 70%/PP ≥ 0.95).

Material examined – THAILAND, Krabi Province, Muang City, on dead and twigs of *Diospyros malabarica* (Ebenaceae), 15 December 2015, C. Phukhamsakda, Kr009 (MFLU 17-0001, **holotype**), ex-type living culture, MFLUCC 16-0419, ICMP 21581.

Notes – *Magnicamarosporium diospyricola* is somewhat similar to *M. iriomotense* in its morphology. Both species occur as saprobic on twigs of dicotyledonous plants. However, *M. diospyricola* differs from *M. iriomotense* in having smaller, thick-walled, rather more hemisphaerical conidiomata, holoblastic to annelidic conidiogenous cells and oblong to pyriform conidia. Whereas *M. iriomotense* differs from the new species by lager conidiomata with cylindrical ostiole, thinner peridium wall, doliiform, holoblastic conidiogenous cells and large and oval conidia (Table. 2). The distinctness of both species is supported in phylogenetic analysis with high supported values.

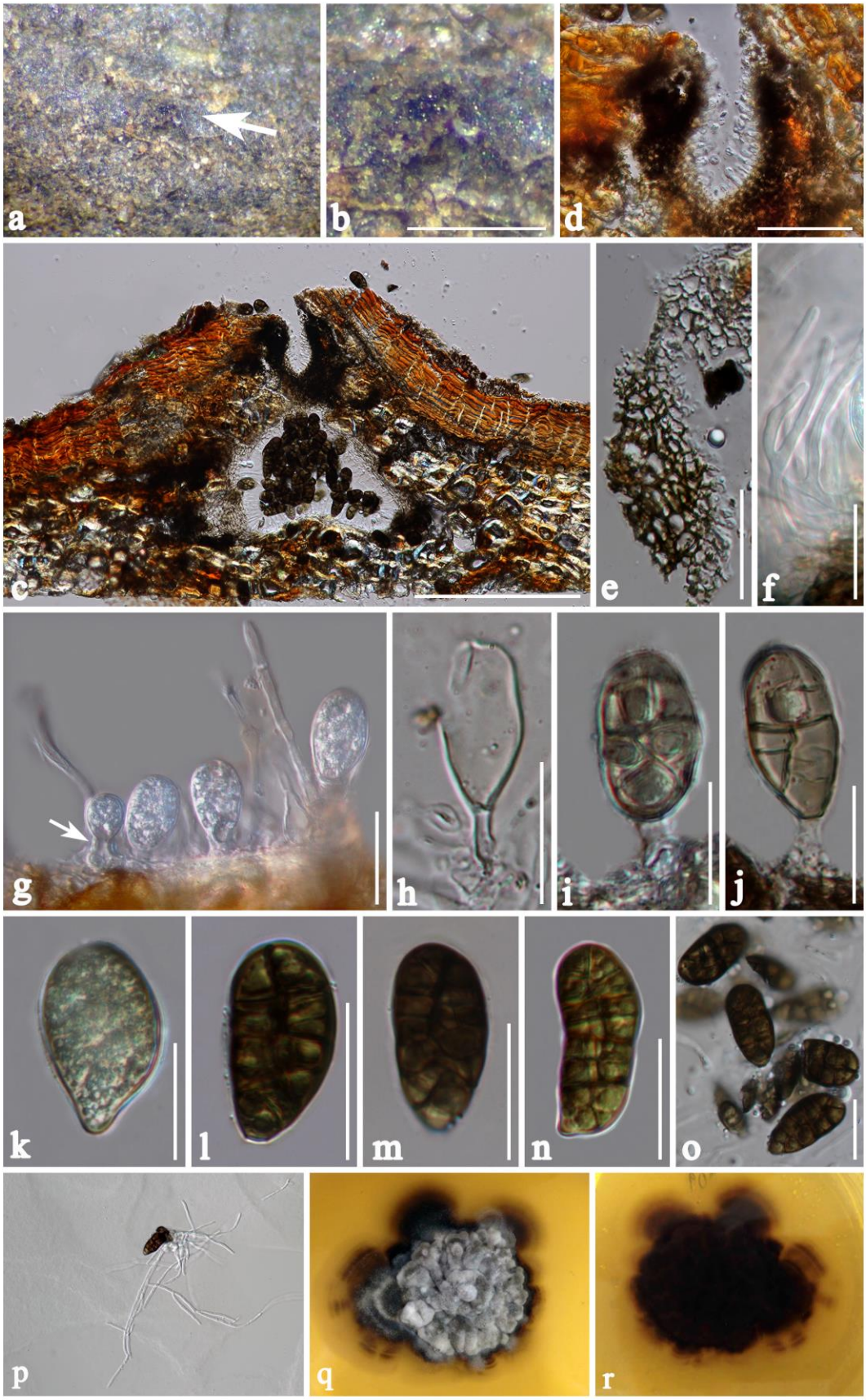

Figure 2 – *Magnicamarosporium diospyricola* (MFLU 17-0001, **holotype**). **a** Appearance of conidiomata on host surface. **b** Close-up of conidioma on host surface. **c** Vertical section of conidioma. **d** Ostiole part. **e** Pycnidial walls. **f** Paraphyses. **g**–**j** Developing stages of conidia from conidiogenous cells. **k**–**o** Developing stages of conidia. **p** Germinated conidia. **q**–**r** Culture characters on MEA. Scale bars: b = 500 µm, c = 200 µm, d = 100 µm, e = 50 µm, f–o = 20 µm.

Table 2 Synopsis of characters of *Magnicamarosporium iriomotense* and *M. diospyricola*.

Name	*M. diospyricola* (This study)	*M. iriomotense* (Tanaka et al. 2015)
Conidiomata	277–301 × 289–337 µm under clypeus, pycnidial, subglobose to depressed globose	330–440 × 700–760 µm, pycnidial, depressed globose
Ostiole	129–145 × 74–116 µm, oblong, canal filled with periphyses	120–150 × 80–100 µm diam., cylindrical, papillate, canal filled with periphyses
Peridium	10–29 µm (–36 µm at base corner), 7–9 layers, brown to dark brown-walled	10–20 µm wide, 2–3 layers, brown-walled
Paraphyses	29–63 × 2–4 µm, regularly 1–2-septate	20–50 (–80) × 1.5–2.5 µm, septate
Conidiophores	Absent	Absent
Conidiogenous cells	5–10 × 3–5 µm, integrated, cylindrical. holoblastic, annellidic, with 1–2 prominent annellations	7–11 × 5–6 µm, holoblastic, cylindrical to doliiform
Conidia	24–35 × 14–21 µm, with 5–7 transverse, obovoid to broad oblong	29–43 × 24–27 µm, with 4–6 transverse, oval to ellipsoid, smooth
Habitat/Host	*Diospyros malabarica* (Ebenaceae)	*Diplospora dubia* (Rubiaceae)

Discussion

Sulcatisporaceae comprises *Magnicamarosporium*, *Neobambusicola* and *Sulcatispora* (Tanaka et al. 2015). The asexual morph of *Neobambusicola* and *Sulcatispora* are distinct from *Magnicamarosporium.* The genus *Neobambusicola* has erumpent, globose conidiomata and hyaline, smooth, 1-septate, fusoid, lipsoid conidia, with hyaline and aseptate microconidia produced in cultures (Crous et al. 2014). The genus *Sulcatispora* produces its asexual morph in culture which is characterized by pycnidial, globose conidiomata, annellidic conidiogenous cells and pale-brown to brown, phragmosporous conidia (Tanaka et al. 2015). Whereas *Magnicamarosporium* has immersed pycnidial conidiomata, holoblastic or annellidic, discrete or integrated conidiogenous cells and muriform conidia.

The genus *Magnicamarosporium* also shares some characters with *Paracamarosporium* Wijayaw. & K.D. Hyde (*Didymosphaeriaceae*) in having paraphyses among the conidia (Sutton 1980, Nag Raj 1993, Wijayawardene et al. 2014a). However, *Magnicamarosporium* is unique by its immersed, large-sized, depressed, globose conidiomata and dark-brown conidia. Phylogeny analysis (Fig. 1) shows that *Magnicamarosporium* resides within the *Sulcatisporaceae* instead of *Didymosphaeriaceae* (Tanaka et al. 2015, this study). Our new species *Magnicamarosporium diospyricola,* found on twigs *Diospyros malabarica* is placed in *Sulcatisporaceae* with other strains of *Magnicamarosporium*, but as a distinct species.

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