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Two new records of the resupinate polypore fungi, *Ceriporia cystidiata* and *Macrohyporia dictyopora*, in Thailand

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ABSTRACT: Wood-inhabiting resupinate polypore fungi are abundant and widely distributed in tropical forests of Thailand. In our survey, *Ceriporia cystidiata* and *Macrohyporia dictyopora* were found in Thailand for the first time. Identification was based on morphological characteristics and DNA sequences of internal transcribed spacers and large subunit (LSU) nuclear ribosomal RNA genes. The initial phylogenetic analyses showed that *C. cystidiata* clustered as a sister clade to *C. lacerata*, and *M. dictyopora* was placed firmly in the phlebioid clade.

KEYWORDS: molecular phylogeny, taxonomy, tropics

INTRODUCTION

Resupinate fungi refer to basidiomycetes that have flattened fruiting bodies developed on or under wood surfaces[1,2]. They resemble each other in gross morphology, but possess diverse anatomical, physiological, and molecular characteristics[3,4]. These fungi are widely represented in tropical forests as efficient wood decomposers. To date, there are up to 1853 resupinate polypore species in 282 genera within 50 putative families: 766 species are reported in Asia[5–11]. Recently, comprehensive studies on wood-rotting fungi in tropical areas of southern China have been completed, and many new species have been described[5–8]. Taxonomic and systematic studies of the resupinate fungi have been performed mostly based upon their morphology since their molecular data, especially the information from tropic regions is limited[3,9,10].

The genus *Ceriporia* Donk (Phanerochaetaceae, Polyporales) of wood-inhabiting Polyporales was characterized by producing resupinate basidiomata with various colours, causing a white rot, and having cylindrical to oblong-ellipsoid basidiospores[12–15]. About 36 species of *Ceriporia* have been morphologically identified but only a few of them were described with molecular support[13]. Although a phylogeny study could place this genus in phlebioid clade of polypore group, it is not monophyletic[13].

Macrohyporia Johan. & Ryv. (Polyporaceae, Polyporales) is recorded in Africa, Argentina, Australia, and New Zealand[16–19]. The morphological markers of this genus are very broad ($> 6\,\mu$m diameter) generative hyphae, refractive thickened walls of binding hyphae, and thin-walled subglobose basidiospore[16]. There are currently only two known species namely *M. pileata* and *M. dictyopora*[17]. Molecular biological data do not appear to have been reported[9]. *M. dictyopora* is the type species of the genus *Macrohyporia*. However, it differs from *M. pileata* in having resupinate basidiomata[17–20].

Wood-inhabiting Homobasidiomycetes in Thailand have been extensively studied. About 2000

species have been described[21,22], about 190 of which are resupinate polypore fungi which includes 6 new species for Asia and 9 species have only been reported in SE Asia[11,23]. During a survey of resupinate fungi in Thailand, two previously unknown species were found. Hence this study contributes further information of new resupinate polypore species for Thailand. Their morphological description, sequences of internal transcribed spacer (ITS) and nuclear ribosomal large subunit (nLSU) are outlined and the evolutionary relationships of this interesting group of wood-inhabiting fungi are discussed.

MATERIALS AND METHODS

Morphological observation

Basidiomata of resupinate fungi were collected from tropical forests around Thailand. The specimens were dried and deposited in the herbarium of the Plant Biomass Utilization Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, Thailand, and specimen codes were assigned. Species identification of the resupinate fungi samples was performed using the standard morphological criteria[12,24,25]. Macromorphological characteristics of the hymenial surface were observed using fresh specimens. Micromorphological characterization and drawings were made from slide preparations by free-hand section of dried specimens mounted with 5% KOH and stained with phloxine, cotton blue, and Melzer's solutions under light microscopy. Microscopic structures, including the basidiospores (Q; length/width ratio), hyphae, and cystidia, were measured using an ocular micrometre under light microscope.

DNA extraction, amplification, and sequencing

Mycelia of each fungal sample growing on the top of cellophane membranes placed on 2% (w/v) malt extract agar were harvested after 7 d of inoculation. Their genomic DNA was isolated by standard phenol-chloroform extraction[26]. The ITS and LSU regions were amplified using the ITS4/ITS5 and LROR/LR7 primer pairs, respectively[27]. The PCR reactions were performed using Chroma *Taq* DNA polymerase (Denville Scientific, Metuchen, NJ, USA) in a final volume of 25 µl. The PCR was thermocycled at 94 °C for 2 min, followed by 25 cycles of 94 °C for 10 s, 55 °C for 30 s, and 72 °C for 1 min, and subsequently a final 72 °C for 10 min. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and

Table 1 Taxa used for phylogenetic analysis in this study with and GenBank accession numbers.

Species name	Sample no.	GenBank accession no.	
		ITS	nLSU
Abortiporus biennis	TFRI 274	EU232187	EU232277
Antrodia albida	CBS 308.82	DQ491414	AY515348
Ceriporia alachuana	Li 1011	JX623898	JX644047
C. alachuana	Li 1115	JX623900	JX644050
C. aurantiocarnescens	Yuan 2066	JX623902	JX644042
C. aurantiocarnescens	Dai 6055	JX623904	JX644043
C. bubalinomarginata	Dai 11 327	JX623953	JX644045
C. bubalinomarginata	Dai 12 499	JX623954	JX644043
C. camaresiana	Cui 3238	JX623931	JX644060
C. crassitunicata	Dai 10 833	JX623935	JX644064
C. crassitunicata	Dai9995	JX623905	
C. cystidiata	PBU 0048	KC570339	KU760725
C. excels	Dai 3204	KF856503	
C. excels	LE247365		JX644056
C. inflata	Dai 10 376	JX623929	JX644062
C. inflata	Cui 7712	JX623930	JX644063
C. lacerata	Dai 9501	JX623908	JX644069
C. lacerata	Dai 10 734	JX623916	JX644068
C. lacerata	Cui 7229	JX623919	
C. lacerata	Dai 10 522	JX623915	
C. mellea	Dai 9453	JX623932	JX644059
C. mellea	Dai 9667	JX623933	JX644058
C. nanlingensis	Li 1670	JX623939	JX644055
C. nanlingensis	Dai 8107	JX623938	JX644052
C. pseudocystidiata	Cui 6878	JX623943	JX644057
C. purpurea	Dai 6205	JX623951	JX644046
C. purpurea	Dai 6366	JX623952	JX644047
C. reticulata	Li 1045	JX623946	
C. reticulata	KHL 11981		EU118614
C. spissa	Dai 10 477	KC182769	KC182781
C. spissa	Yuan 5862	KC182771	KC182782
C. sulphuricolor	Dai 6090	JX623934	JX644066
C. tarda	Dai 10 226	JX623945	
C. variegata	Li 1780	JX623936	JX644065
C. viridans	Yuan 2744	KC182773	
C. viridans	Cui 8012	KC182774	
Ceriporiopsis alboaurantia	Cui 2877	KF845954	KF845947
C. aneirina	Dai 12 657	KF845952	KF845945
Dacryobolus karstenii	KHL 11162	EU118624	EU118624
Gelatoporia subvermispora	BRNU 592909	FJ496694	FJ496706
Gloeoporus taxicola	GL52	AM231907	
G. taxicola	O 146 364	AM231903	
H. annosum	PFC 5252	DQ384592	DQ384592
Junghuhnia nitida	KHL 11903	EU118638	EU118638
L. persicinus	DA-41	EU402566	EU402533
L. sulphureus	GR-12	EU402561	EU402534
M. dictyopora	PBU 0051	KC570331	KU760726
Phanerochaete chrysosporium	BKM-F-1767	HQ188436	GQ470643
P. magnoliae	HHB 9701	KP135089	
P. magnoliae	HHB 9829	KP135090	
P. sordida	CY180	HQ608013	
P. sordida	T8	JN253600	
Phlebia livida	FCUG 2189	AF141624	AF141624
P. livida	MG104	HQ153416	
P. livida	MG103	HQ153415	
P. radiata	ATCC 64658	FJ746663	
P. tremellosa	BRFM 968	JX082340	
P. tremellosa	GU062266	GU062266	
Polyporus tuberaster	CulTENN 8976	AF516598	AJ488116
Steccherinum fimbriatum	KHL 11905	EU118668	EU118668
Trametes pubescens	PRM 900586	AY684173	AY855906
Trametopsis cervina	LEBIN2974	JX463662	JX463662
Wolfiporia dilatohypha	FP72162	KC585401	KC585236

quantified. A 20 ng sample of each PCR product was sequenced by the dideoxy chain termination method (GENEWIZ DNA Sequencing Service, NJ). The sequences were assembled and analysed using the DNASTAR sequence analysis software (Lasergene, Madison, WI), and manually edited and assembled into contigs using Seqman (DNASTAR, Inc., Madison, WI). All new sequences were submitted to GenBank (Table 1) and screened for homologues against the GenBank database using the BLAST tool (www.ncbi.nlm.nih.gov/blast).

Phylogenetic analysis

The newly generated ITS and LSU sequences of each species in this study were combined as dataset (ITS+LSU). Each dataset was aligned with the sequences of related taxa downloaded from GenBank (Table 1) using the Muscle algorithm[28] with default parameters and manually edited in MEGA6[29]. All characters were equally weighted and gaps were treated as missing data. Maximum parsimony phylogenetic analysis was conducted using PAUP* version 4.0b10[30]. The sequence of *Antrodia albida* from *Antrodia* clade was selected as an outgroup for the analysis of *C. cystidiata*[13] and *Heterobasidion annosum* from Russuloid clade was used as an outgroup for the analysis of *Macrohyporia dictyopora*[3] (Table 1). Trees were then generated through a heuristic search with TBR branch swapping and 10 random sequence additions. Max-trees were set to 100 branches. Topology stability was assessed by performing 1000 bootstrap replicates. Descriptive tree statistics tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RCI), were calculated for each tree. Bayesian analysis was analysed by MrBayes 3.2.2 and Bayesian posterior probabilities for each clade were presented[13,31].

RESULTS AND DISCUSSION

Taxonomic

C. cystidiata Ryvarden & Iturr. 2003[14] (Fig. 1)

Specimen examined: Thailand, Botanical garden of Roi-Et College of Agriculture and Technology, Roi-Et Province, on dead wood, coll. P. Permpornsakul, June 2008, PBU 0048.

Distribution: Latin America; Venezuela[14] and Southeast Asia; Thailand.

Resupinate basidioma, brittle when dry, 1 mm thick, pore surface white to pale cream, pores thin-walled, round to angular, 6–8 per mm, tube layer concolourous to pore surface, up to 1 mm deep.

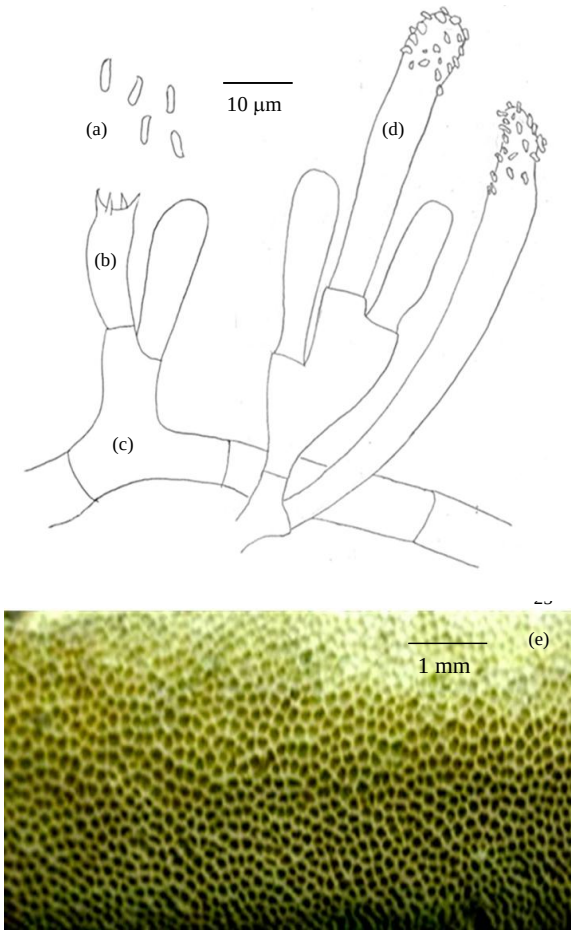

Fig. 1 *Ceriporia cystidiata*; microscopic features: (a) allantoid basidiospores, (b) basidia, (c) generative hyphae, and (d) tubular apical encrusted cystidia; (e) basidioma.

Subiculum very thin, cottony and whitish. Hyphal system monomitic; generative hyphae hyaline, thin to slightly thick-walled, simple-septate, with sparse branching, negative in Melzer's reagent, 3–8 µm in diameter. Basidia clavate, 4-sterigmate, 10–12 × 3.5–4.5 µm, simple-septate at the base. Cystidia present in the hymenium, tubular, thin-walled, with a slight apical encrustation, up to 90 µm long, 5–12 µm wide. Basidiospore allantoid ($Q = 2.5$–2.7; $n = 15/1$), thin-walled, smooth, 4–4.5 × 1 µm, hyaline, and inamyloid.

Notes: The occurrence of *C. cystidiata* was previously only known from the type locality in Venezuela[14]. The *C. cystidiata* differs from another species within *Ceriporia* in presenting an encrusted cystidia[14,15]. Basidioma of *C. cystidiata* is similar to *C. alachuana*, *C. ferruginicincta*, *C. lacerata*, and *C. pseudocystidiata* by having a whitish to ochreous

pore surface. However, it differs from *C. alachuana*, and *C. ferruginicincta* by pore size and presence of cystidia, also from *C. pseudocystidiata* by the presence of an apical encrusted cystidia[13,14]. Even the basidiomata of *C. lacerata*, and *C. cystidiata* are not different, *C. lacerata* can be distinguished from *C. cystidiata* by its pore size and presence of cystidia since the pore size of *C. lacerata* is larger than that of *C. cystidiata*, and *C. lacerata* has some cystidia[13,14].

M. dictyophora (Cooke) I. Johans. & Ryvarden, 1979[18] (Fig. 2)

Specimen examined: One specimen from Thailand, Erawan National Park, Kanchanaburi Province, on dead wood, coll. P. Permpornsakul, June 2008, PBU 0051.

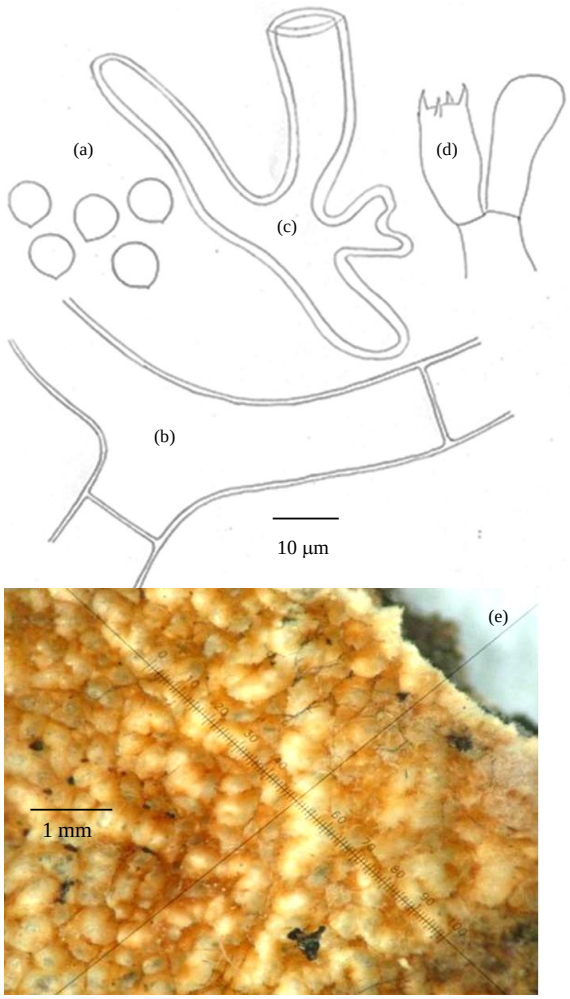

Fig. 2 *Macrohyporia dictyopora*; microscopic features: (a) subglobose basidiospores, (b) generative hyphae, (c) basidia, and (d) binding hyphae; (e) basidioma odontoid.

Distribution: Australia[16,17,32], East Africa[18,19], and Southeast Asia; Thailand.

Resupinate-adnate basidioma, covering large areas, 2–3 mm thick. Pore surface white to pale brown, margin thin, white, odontoid; 3–4 per mm. Hyphal system dimitic: generative hyphae generally 2–3 thick, up to 10 µm in diameter, colourless, simple septate and without clamp-connections; binding hyphae much branched outer wall curved, nearly solid, 1.5–2 µm thick, up to 6 µm in diameter, colourless. Cystidia absent. Basidia clavate, 12–14 × 3.5–4 µm, four-spored. Basidiospore ($Q = 1.1$–1.3; $n = 13/1$) subglobose, thick-walled, smooth, 4.5–6 µm in diameter, hyaline, and inamyloid.

Note: The hyphal system of *Macrohyporia* resembles to that of *Laetiporus sulphureus* and *Poria cocos*. It is separated from *Laetiporus* by the basidioma and binding hyphae, also differs from *Poria* by the spore characteristics[33]. However, the specimen of *M. dictyopora* in this study differs from the previous specimen reported from New Zealand by producing narrower binding hyphae[16].

Molecular and phylogenetic analysis

The newly generated ITS and LSU sequences of *C. cystidiata* and *M. dictyopora* were BLAST searched in GenBank and Mycobank databases and these molecular information appeared to be the first sequences to both species. The evolutionary relationship among them could therefore be established for the first time in this study. So far, almost 40 species of the genus *Ceriporia* have been described based on morphology, but molecular biological data (ITS and/or LSU sequences) from only 16 species are reported[12–14]. The ITS+LSU dataset including 42 sequences from 28 *Ceriporia* and 14 other related species comprised 2369 characters alignment with 1601 constant, 212 variable, and 556 parsimony informative sites; tree length was 2561 with CI = 0.454, RI = 0.616, and RCI = 0.280 (Fig. 3). Bayesian analysis based GTR+G model resulted in an average standard deviation of split frequencies = 0.0094 established identical tree topology as the MP analysis (Fig. 3). The tree topology showed that most of *Ceriporia* species formed a clade, except *C. inflata*, *C. mellea*, and *C. camaresiana* which clustered with *Phanerochaete* species. All morphologically identified *Ceriporia* species were clustered well within the species level. Although *C. cystidiata* was clustered as a sister clade with *C. lacerata* (Fig. 3), the morphological differences between them clearly support their identities.

The BLASTn search results of ITS and LSU

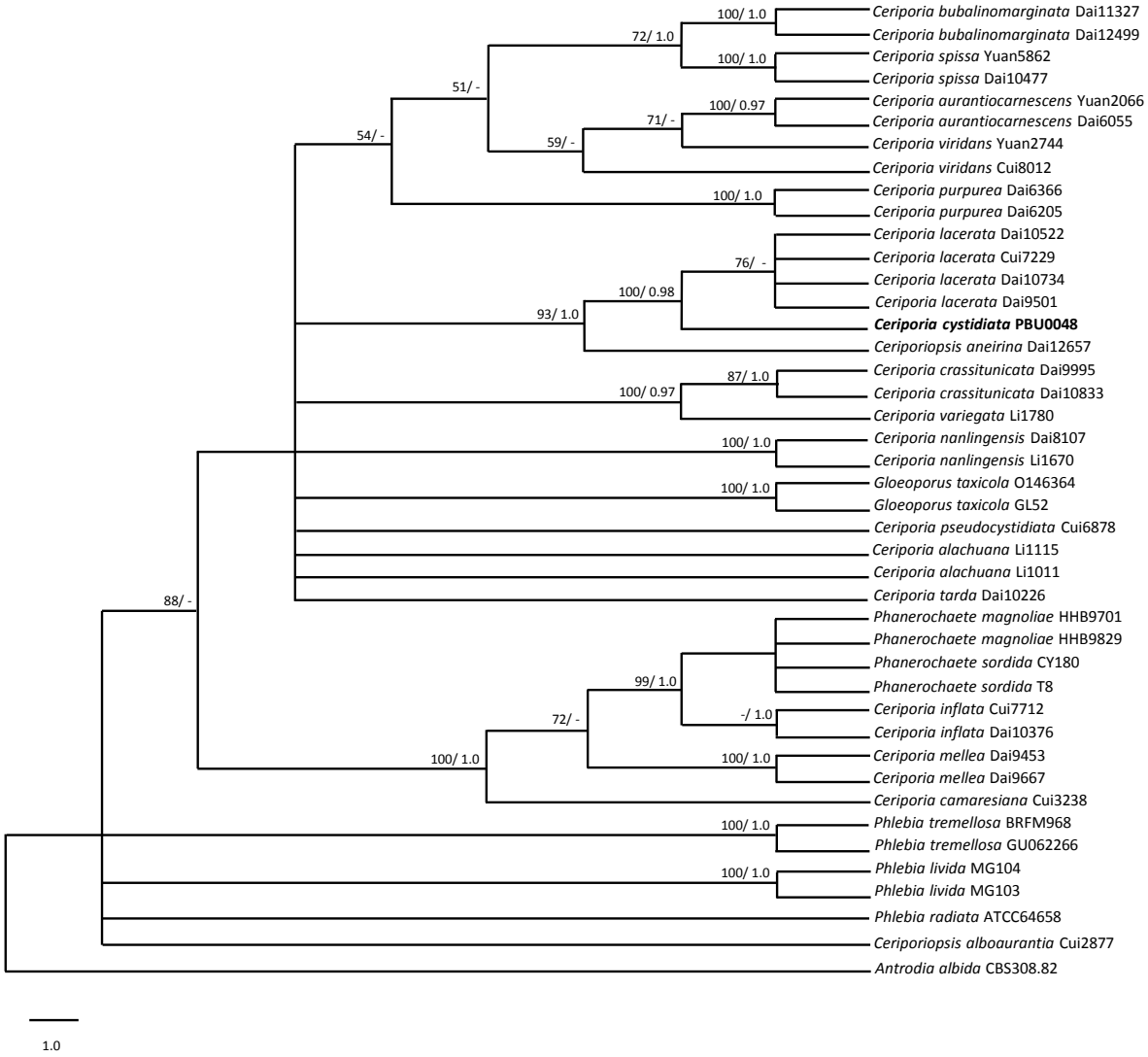

Fig. 3 Phylogenetic tree of the newly isolated *C. cystidiata* and its related species based on ITS + LSU sequences from GenBank generated by maximum parsimony method. *Antrodia albida* is the outgroup. Values labelled above the branches are bootstrap proportions (greater than 50%) / Bayesian probabilities (greater than 0.95).

sequences from *M. dictyopora* PBU 0051 revealed less than 92% maximum sequence similarity with the sequences in GenBank and Mycobank databases. The length of aligned dataset is 2288 characters with 1482 constant, 291 variable, and 515 parsimony informative sites; tree length was 1449 steps, CI = 0.508, RI = 0.333, and RCI = 0.169. Bayesian analysis based GTR+I+G model with an average standard deviation of split frequencies = 0.0086 established similar tree topology as the MP analysis. The phylogeny could establish five major clades of including Antrodia, Core polyporoid, phlebioid, Gelatoporia, and Residual polyporoid clades which is similar to the previous studies[3]. Previous reports described *M. dictyopora* based on morphology with the supposed genus position related to *Wolfiporia* and *Laetiporus* groups which were located in *Antrodia* clade[34]. However, the present phylogenetic analysis revealed that this species is clustered well within phlebioid clade and closely related to *C. lacerata* and *Trametopsis cervina* (Fig. 4).

CONCLUSIONS

Information of tropical resupinate fungi continues to unfold. In this study, the first molecular data for *C. cystidiata* and the genus *Macrohyporia* were

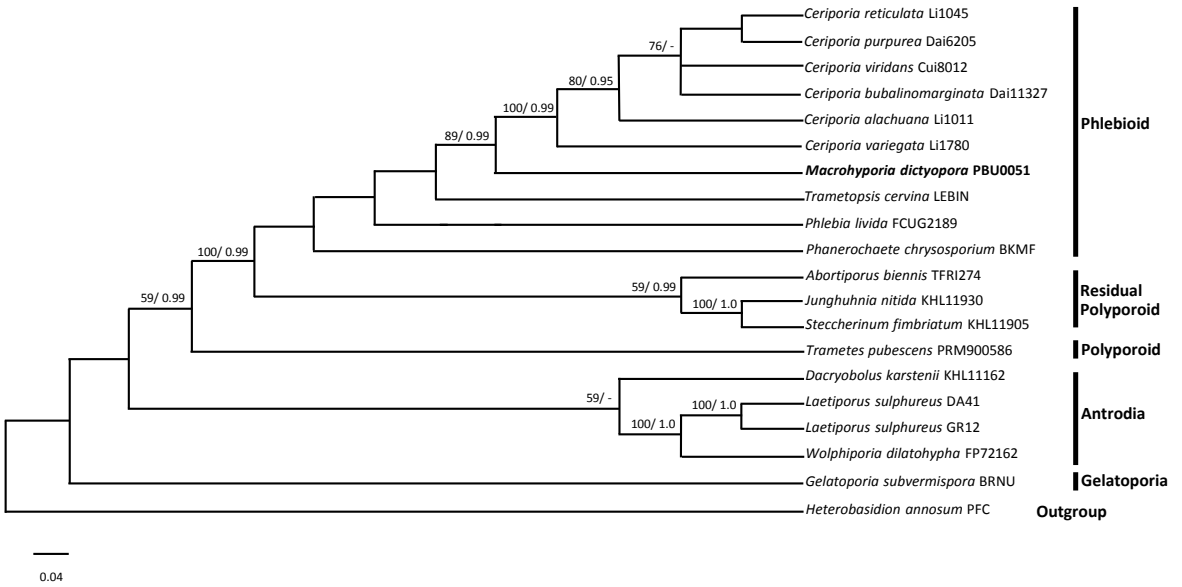

Fig. 4 Phylogenetic tree of the newly isolated *M. dictyopora* and its closely related taxa based on ITS + LSU sequences from GenBank generated by maximum parsimony method. *Heterobasidion annosum* is the outgroup. Values labelled above the branches are bootstrap proportions (greater than 50%) / Bayesian probabilities (greater than 0.95).

obtained, showing the relationships of *C. cystidiata* within *Ceriporia* spp. and *Macrohyporia* genus within polyporoid group, respectively. Further studies are needed to address the taxonomic and systematic status of this complex Polyporales group.

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