



Original Article

First record of *Ceriporia inflata* and *Ceriporia lacerata* (*Phanerochaetaceae*, Basidiomycota) from Indonesian tropical forestRetno Wulandari,^{a, b} Pongtharin Lotrakul,^b Rudianto Amirta,^d Seung Wook Kim,^e Hunsu Punnapayak,^{b, c} Sehanat Prasongsuk^{b, *}^a Biological Sciences Program, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand^b Plant Biomass Utilization Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand^c Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia^d Faculty of Forestry, Mulawarman University, Samarinda, 75199, Indonesia^e Department of Chemical and Biological Engineering, Korea University, Seoul, 136-701, South Korea

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ABSTRACT

Resupinate fungi (Basidiomycota) are wood-inhabiting fungi found abundantly in the tropical rainforest of Indonesia. A survey in East Kalimantan discovered two new recorded species for Indonesia: *Ceriporia inflata* and *Ceriporia lacerata*. The morphological characteristics of these two resupinate fungi were similar to those of the holotype specimens found in sub-tropical forest in China and Japan. Based on maximum parsimony and Bayesian phylogenetic analysis of internal transcribed spacers and large subunit nuclear ribosomal RNA sequences, these two new records were placed within the *Ceriporia* group.

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Introduction

Resupinate fungi are mostly polypores that have adopted a resupinate habit and can occupy similar ecological niches on wood as the corticioid fungi that look similar in anatomical, physiological characteristics (Gates, 2009). They are well known for their ability as wood decomposers in the forest (Permpornsakul et al., 2016). Resupinate fungi are distributed among all major clades of Homobasidiomycetes (Hibbet and Binder, 2002; Larsson et al., 2004). Taxonomy and molecular tools have been used to identify 50 putative families for corticioid fungi (Larsson, 2007) with up to 1853 species of resupinate polypores and 766 species reported from Asia (Hjortstam and Ryvarden, 2007). The collection of resupinate fungi conducted in a Southeastern China tropical forest resulted in many new species being described (Cui et al., 2006; Dai, 2012). In contrast, there have been only a few reports about taxonomy and sequence data of resupinate fungi from Indonesia. Therefore, intensive studies must be conducted regarding resupinate fungi from Indonesian tropical forests.

Ceriporia is a genus of wood-inhabiting polypores that cause white rot. This genus is characterized by a resupinate basidiocarp with white, red, purple, orange, pink or green pore surfaces, a monomitic hyphal system, simple septate or rarely clamped generative hyphae, lack of cystidia or other sterile hymenial elements, and subglobose to cylindric or allantoid basidiospores (Gilbertson and Ryvarden, 1986). The genus is cosmopolitan, with its species being recorded worldwide (Donk, 1965). More than 30 species of the *Ceriporia* genus have been described; however, only a few species were referred to using molecular phylogeny (Jia et al., 2014). The genus was at first considered as monophyletic (Kim and Jung, 1999), but it was recently shown that it is polyphyletic and the presence or absence of a few morphological characteristics were not considered as a phylogenetic character in delimiting species (Jia et al., 2014).

In the current study, two isolates of resupinate fungi belonging to *Ceriporia* species were found. *Ceriporia inflata* (*C. inflata*) and *Ceriporia lacerata* (*C. lacerata*) were identified based on their morphological characters and sequence analyses of internal transcribed spacer (ITS) and nuclear ribosomal large subunit (nLSU) data. Phylogenetic analyses of these two new records were also carried out.

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Materials and methods

Morphological study

Fungal fruiting bodies were collected from Samarinda Botanical Garden (KRUS), East Kalimantan, Indonesia (0°25'10" N and 117°14'4" S). KRUS was characterized as a tropical rainforest. The forest was divided into several zones: primeval reserve forest (117 ha), natural and artificial forest for research (60 ha), indigenous species arboretum (7 ha), coniferous forest (16 ha) and a recreation zone (60 ha). All fungal specimens were identified to the species level using identification keys (Nunez and Ryvarden, 2001). Isolation of pure culture was conducted in plastic Petri dishes containing potato dextrose agar supplemented with 0.015% benomyl. The morphological study was conducted following the methodology from Jia et al. (2014). Macro-morphological descriptions were compared to the morphological characters from previous reports (Suhara et al., 2003; Jia and Cui, 2012; Jia et al., 2014). Micro-morphological identification was conducted using 10% potassium hydroxide (KOH), cotton blue, Melzer's reagent and phloxine. Sections were carefully made by hand to observe the basidia, cystidia and basidiospore (Q; length/width ratio). The sections were studied at magnifications up to $\times 1000$ using a microscope (model CH30; Olympus Corp.; Tokyo, Japan) and phase contrast illumination. *Ceriporia inflata* PBURU R1 and *C. lacerata* PBURU 141 were submitted into the fungal section of the Professor Kasin Suva-tabhandhu Herbarium with herbarium numbers 0071 BCU and 0072 BCU, respectively.

DNA extraction, amplification and sequencing

Mycelia of each fungal sample grown on 2% (weight per volume) malt extract agar were harvested 7 d after inoculation. Genomic DNA was extracted using the standard phenol-chloroform method (Sambrook and Russell, 2001). The ITS and LSU regions were amplified using ITS4 (TCCTCCGCTTATTGATATGC), ITS5 (TCCGTAGGTGAACCTGCGG), and LROR (ACCGCTGAACCTAAGC) LR7 (TACTACCACCAAGATCT) as primer pairs (White et al., 1990). Polymerase chain reaction (PCR) was performed using i-Startaq™ DNA polymerase (iNtRON Biotechnology; Kyung-ki Do, South Korea) in a final volume of 25 μ L. The PCR reaction conditions (for both ITS and LSU) were thermocycled at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, with the annealing point at 48 °C for 30 s, 72 °C of 2.5 min and 3 min of final extension at 72 °C. The PCR products were purified using a MEGA-spin™ agarose gel DNA extraction kit (iNtRON Biotechnology; Kyung-ki Do, South Korea). DNA sequencing was performed using the dideoxy termination method at Macrogen Korea Corp. (Seoul, Korea). The new sequences were submitted to GenBank and the nBLAST tools (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) were used to compare the identity with the NCBI database. The ITS and nLSU accession numbers were listed for *Ceriporia inflata* PBURU R1 (KY234234, KY234236) and *C. lacerata* PBURU 141 (KY234235, KY234232).

Phylogenetic analysis

The *Ceriporia* species included in the phylogenetic analysis were downloaded from GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Table 1). The voucher species from this analysis either had ITS or nLSU sequences, which is why it was not combined for the analysis. Each set of sequences (ITS and nLSU) were aligned using Clustal Omega (Sievers et al., 2011) using default parameters, then manually edited in Mega 6 version 6.0 (Tamura et al., 2013). Gaps in the alignments were treated as missing data. The analysis was conducted using the software PAUP* version 4.0b10 (Sinauer

Associates; Inc.; Sunderland; MA; USA). Bootstrap analysis involved 1000 replicates, using the same settings. Maximum parsimony (MP) analysis was used in the phylogeny construction using the software PAUP* version 4.0b10 (Sinauer Associates; Inc.; Sunderland; MA; USA). The tree was generated through a heuristic search using 1000 bootstrapping replications with 10 random sequence additions using tree bisection reconnection (TBR) branch swapping. Max-trees were set to automatically increase by 100, branches of zero length were collapsed and all parsimonious trees were saved. Clade stability was assessed by performing 1000 bootstrap (BS) replicates. Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), homoplasy index (HI) were calculated for each tree generated. The ITS and nLSU sequences from other species in this clade were downloaded from GenBank. *Antrodia xantha* (ITS = DQ491424; nLSU = AJ583430) was selected as an outgroup (Jia et al., 2014). For MP phylogenetic analyses, gaps were treated as missing characters and trees were generated by heuristic searches, with the random addition of sequences (1000 replicates), tree bisection reconnection (TBR) branch swapping and MULTREES effective. Characters were weighted to reduce the effect of homoplasy by including all phylogenetically informative characters and applying successive weighting according to the mean consistency index of each character. Bayesian analysis used the Mr. Bayes version 3.2.2 software package (Huelsenbeck and Ronquist, 2001) and Bayesian posterior probabilities for each clade were presented.

Results and discussion

Over 30 *Ceriporia* species have been reported from Europe, Japan, Korea and China (Jang et al., 2012; Jia et al., 2014). This previous study suggests that most of these species have overlapping characters such as presence or absence of hymenial cystidia which cannot be used to delimit species relationship. Consequently, there have been many species complexes leading to misplacement. Jia et al. (2014) studied 203 specimens of *Ceriporia* morphologically and constructed a phylogenetic relationship among these genera, emphasizing the China collection and they suggested that *Ceriporia* is not monophyletic. Among 30 species of *Ceriporia*, *C. inflata* and *C. lacerata* were only reported from China, Korea and Japan, with no report from Southeast Asia.

Taxonomy

Ceriporia jiangxiensis/*Ceriporia inflata* (Jia and Cui)

Mycotaxon, 121:306 (2012), MycoBank MB 800809.

Specimens examined: Indonesia, East Kalimantan, Samarinda Botanical Garden, on fallen angiosperm wood, collector: R. Wulandari, June 2012. PBURU R1.

Distribution: Asia; China and South East Asia; Indonesia.

This species was characterized by resupinate basidiocarps, soft corky when fresh and hard when dry. They are firmly attached to the substrate, and are a type of white rot. The pore surface is hymenophore poroid, white to cream when fresh and buff to clay-buff when dry. The pores are angular to irregular, 0.8–2 mm, and dissepiments thin, lacerate. Sometimes pores extend to the very edge, flocculent. The tubes are concolorous with the pore surface and up to 1–2 mm thick. Hyphal system is monomitic; with generative hyphae and simple septa. The tissue was unchanged after treatment with KOH. Generative hyphae are hyaline and thin-walled with frequent simple septa, frequently branched, interwoven with cystidia and cystidioles present. Tramal hyphae are hyaline, thin to slightly thick-walled, frequently branched, with frequent simple septa, sometimes constricted at septa, subparallel

Table 1

Taxa used for phylogenetic analysis in this study.

Species name	Sample no.	Locality	GenBank accession number	
			ITS	nLSU
<i>Antrodia xantha</i> (Fr.) Ryvarden	CBS 155.79	South Korea	DQ491424	—
<i>Antrodia xantha</i>	25S	USA	—	AF287845
<i>Ceriporia alachuana</i>	Li1115	China	JX623900	JX644050
<i>C. alachuana</i>	Li1011	China	JX623898	JX644049
<i>C. alachuana</i>	Li1001	China	JX623897	—
<i>C. aurantiocarnescens</i>	Yuan2066	China	JX623902	JX644042
<i>C. aurantiocarnescens</i>	Yuan975	China	JX623901	—
<i>C. aurantiocarnescens</i>	Dai6055	China	JX623904	JX644043
<i>C. bubalinomarginata</i>	Dai11327	China	JX623953	JX644045
<i>C. bubalinomarginata</i>	Dai12499	China	JX623954	JX644044
<i>C. bubalinomarginata</i>	KUC20130725-09	South Korea	KJ668567	—
<i>C. camaresiana</i>	Cui3238	China	JX623931	JX644060
<i>C. crassitunicata</i>	Dai10833	China	JX623935	JX644064
<i>C. crassitunicata</i>	Dai9995	China	JX623905	—
<i>C. cystidiata</i>	PBU 0048	Thailand	KC570339	—
<i>C. excelsa</i>	LE247365	Russia	KF856503	—
<i>C. excelsa</i>	Dai3204	China	—	JX644056
<i>C. inflata</i>	Dai10376	China	JX623929	JX644062
<i>C. inflata</i>	Cui7712	China	JX623930	JX644063
<i>C. inflata</i>	Dai9587	China	—	JX644061
<i>C. inflata</i>	PBURU R1	Indonesia	KY234234	KY234236
<i>C. lacerata</i>	Dai10734	China	JX623916	JX644068
<i>C. lacerata</i>	Cui7229	China	JX623919	—
<i>C. lacerata</i>	Dai9501	China	JX623908	JX644069
<i>C. lacerata</i>	KUC3108	South Korea	DQ912694	—
<i>C. lacerata</i>	ZJYS	China	KP677607	—
<i>C. lacerata</i>	PBURU 141	Indonesia	KY234235	KY234232
<i>C. mellea</i>	Dai9453	China	JX623932	JX644059
<i>C. mellea</i>	Dai9667	China	JX623933	JX644058
<i>C. nanlingensis</i>	Yuan5749	China	JX623939	JX644054
<i>C. nanlingensis</i>	Dai8107	China	JX623938	—
<i>C. nanlingensis</i>	Dai8173	China	—	JX644053
<i>C. pseudocystidiata</i>	Cui6878	China	JX623943	JX644057
<i>C. purpurea</i>	Dai6205	China	JX623951	JX644046
<i>C. purpurea</i>	Dai6366	China	JX623952	JX644047
<i>C. reticulata</i>	Li1045	China	JX623946	—
<i>C. reticulata</i>	L-7837	USA	KP135040	—
<i>C. reticulata</i>	Li1316	China	JX623947	—
<i>C. spissa</i>	Dai10477	China	KC182769	KC182781
<i>C. spissa</i>	Dai8110	China	KC182767	KC182784
<i>C. spissa</i>	Dai8168	China	KC182768	KC182785
<i>C. spissa</i>	Yuan5862	China	KC182771	KC182782
<i>C. spissa</i>	Yuan5965	China	KC182772	KC182783
<i>C. spissa</i>	PRM915964	USA	GU594154	—
<i>C. spissa</i>	PRM915965	USA	GU594155	—
<i>C. sulphuricolor</i>	Dai6090	China	—	JX644066
<i>C. tarda</i>	Dai10226	China	JX623945	—
<i>C. tarda</i>	Wu9506–64	China	—	GQ470632
<i>C. variegata</i>	Li1780	China	JX623936	JX644065
<i>C. viridans</i>	Yuan2744	China	KC182773	—
<i>C. viridans</i>	Cui8097	China	KC182780	—
<i>C. viridans</i>	KUC20131022-11	South Korea	KJ668563	—
<i>C. viridans</i>	Dai5183	China	—	KC182786

along the tubes, 2.1–3 µm in diameter, basidia clavate, with four sterigmata and a basal simple septum, 10.1–23.5 × 3.3–6.2 µm. Basidiospores are allantoid to ellipsoid, hyaline, thin-walled, smooth, some slightly curved, 3–5.5 × 1.2–3.4 µm ($Q = 1.6–2.5$; $n = 15/1$). The general characters of Indonesian *C. inflata* (Fig. 1) matched the holotype from China.

Notes: The resupinate fruiting-body of *C. inflata* is similar to *C. lacerata* in having a whitish to ochreous pore surface. However, it differs in pore size, the presence of cystidia and having broadly ellipsoid spores. *C. inflata* also differs from other *Ceriporia* species by its variably shaped basidiospores and the presence of cystidia.

Ceriporia lacerata (Maek, Suhara and Kondo)
Mycotaxon, 86:342 (2003), MycoBank MB 372339.

Specimen examined: Indonesia, East Kalimantan, Samarinda Botanical Garden, on fallen angiosperm tree, collector: R. Wulandari, June 2012. PBURU 141.

Distribution: Asia; China, Japan, Korea and South East Asia; Indonesia.

This species is characterized by resupinate basidiocarps, effused, confluent, soft when fresh, then fragile. They are firmly attached to the substrate and are a type white rot. The hymenophore is poroid, white, buff to ochreous, dissepiments entire to lacerate, white to cream when fresh and buff to clay-buff when dry, firmly attached to the substrate with pores angular to irregular, 2.5–5 mm. Hyphal system is monomitic with generative hyphae having simple septa. Tissue is unchanged in KOH.

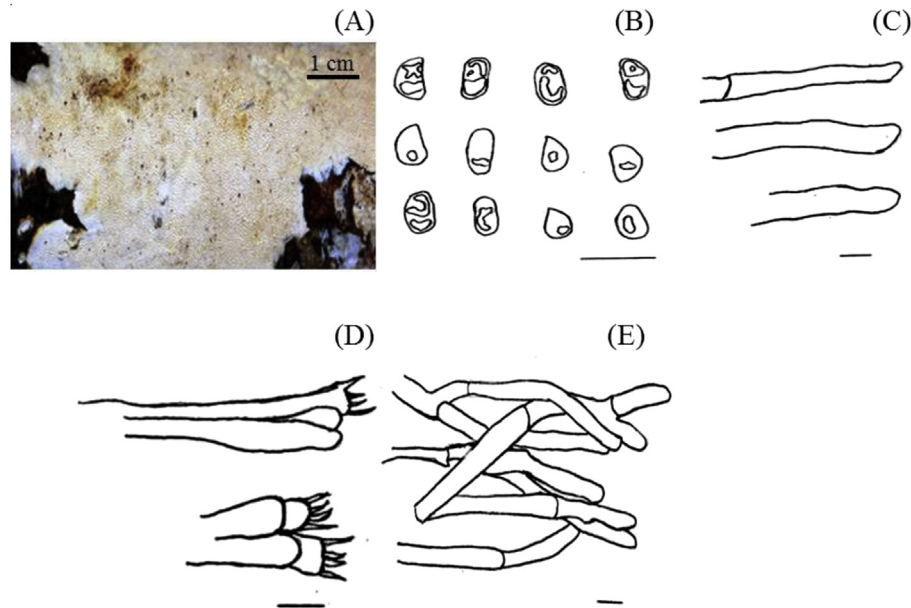


Fig. 1. *Ceriporia inflata* PBURU R1 (A) Basidioma (scale bar = 1 cm); (B) Basidiospore; (C) Cystidia; (D) Basidia and Basidiole; (E) Generative hyphae (scale bar = 10 μ m).

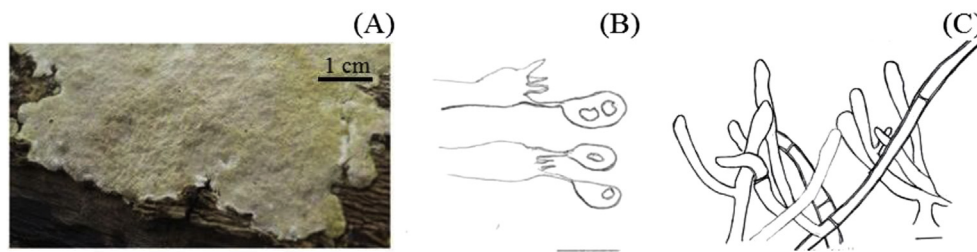


Fig. 2. *Ceriporia lacerata* PBURU 141 (A) Basidioma (scale bar = 1 cm); (B) Basidia and Basidiospore; (C) Generative hyphae (scale bar = 10 μ m).

Generative hyphae are hyaline, thin-walled, frequently branched, interwoven, lack of cystidia, hyaline, thin-walled. Tramal hyphae are hyaline, thin to slightly thick-walled, frequently branched, with frequent simple septa, sometimes constricted at septa, subparallel along the tubes, 3–3.7 μ m in diameter. Basidia are clavate, with four sterigmata and a basal simple septum, 9–18.2 $\times$ 3.7–5.5 μ m. Basidioles are similar in shape to basidia, but smaller. Basidiospores are oblong-ellipsoid to ellipsoid, hyaline, thin-walled, smooth, some slightly curved, 3.5–5 $\times$ 1.9–3.7 μ m ($Q = 1.4$ –1.8; $n = 15/1$) (Fig. 2).

Notes: Basidiocarp of *Ceriporia lacerata* is similar to *C. cystidiata*, *C. alachuana*, *C. pseudocystidiata* and *C. inflata* in having a whitish to ochreous pore surfaces.

Both *C. inflata* PBURU R1 and *C. lacerata* PBURU 141 species share similar morphological characters with those of holotypes from China and Japan (Table 2). Variations in size and dimensions of macroscopic and microscopic characters such as basidiospores, basidia and pores are not characteristically unique to both species.

Molecular and phylogenetic analysis

The ITS and nLSU sequences of newly record *C. inflata* PBURU R1 and *C. lacerata* PBURU 141 from Indonesia were compared using the BLASTn search in the GenBank NCBI and MycoBank databases. Based on nucleotides comparison using BLASTn search of the GenBank NCBI, the best hits using ITS as markers for two strains of

Table 2

Morphological comparison of *Ceriporia inflata* PBURU R1 and *C. lacerata* PBURU 141 with the type specimens.

Characteristic	<i>Ceriporia inflata</i> (current study)	<i>Ceriporia inflata</i> Jia and Cui (2012)	<i>Ceriporia lacerata</i> (current study)	<i>Ceriporia lacerata</i> Maek Suhara et al (2003)
Basidiocarp	Resupinate, soft corky when fresh and hard when dry	Annual, resupinate corky to brittle when dry	Resupinate, effused, confluent, soft when fresh	Resupinate, effused, confluent, soft when fresh
Pore surface	White to cream when fresh, buff to clay-buff when dry	White to cream when fresh, buff to clay-buff when dry	White, buff to ochraceous	White, buff to ochraceous
Pores (mm)	0.8–2	2–3	2.5–5	2–5
Hypha structure	Monomitic	Monomitic	Monomitic	Monomitic
Basidia (μ m)	10.1–23.5 $\times$ 3.3–6.2	12.9–19 $\times$ 3.8–4.2	9–18.2 $\times$ 3.7–5.5	11–16.5 $\times$ 3.5–5.5
Basidiospores (μ m)	3–5.5 $\times$ 1.2–3.4	4.93 $\times$ 2.15	3.5–5 $\times$ 1.9–3.7	3.5–5 $\times$ 2–3

Ceriporia from Indonesia resulted in isolate PBURU R1 being 99% similar to *C. inflata* Dai10376 (GenBank JX623929), while isolate PBURU 141 was 96% similar to *C. lacerata* Cui7229 (GenBank JX623919). The BLASTn result of nLSU showed that the best hit for isolate PBURU 141 was *C. lacerata* Dai10734 (GenBank JX644068) with 96% similarity while isolate PBURU R1 shared 97% similarity with *C. inflata* Dai10376 (GenBank JX644062).

Antrodia xantha (Fr.) Ryvarden CBS 155.79 (GenBank DQ491424) was used as an outgroup for ITS, while *A. xantha* 25S

was used for nLSU analyses. The ITS rDNA dataset of 46 sequences comprised 813 characters with 333 constants, 94 variables and 386 parsimony-informative characters. The tree length was 1611 steps with consistency index (CI) = 0.4922 and retention index (RI) = 0.7417. Bayesian analysis based on the standard model resulted in an average standard deviation of split frequencies of 0.00955 and established an identical tree topology as the MP analysis (Fig. 3), while the nLSU dataset of 33 (including out-group) sequences comprised 1367 characters with 1082 constant,

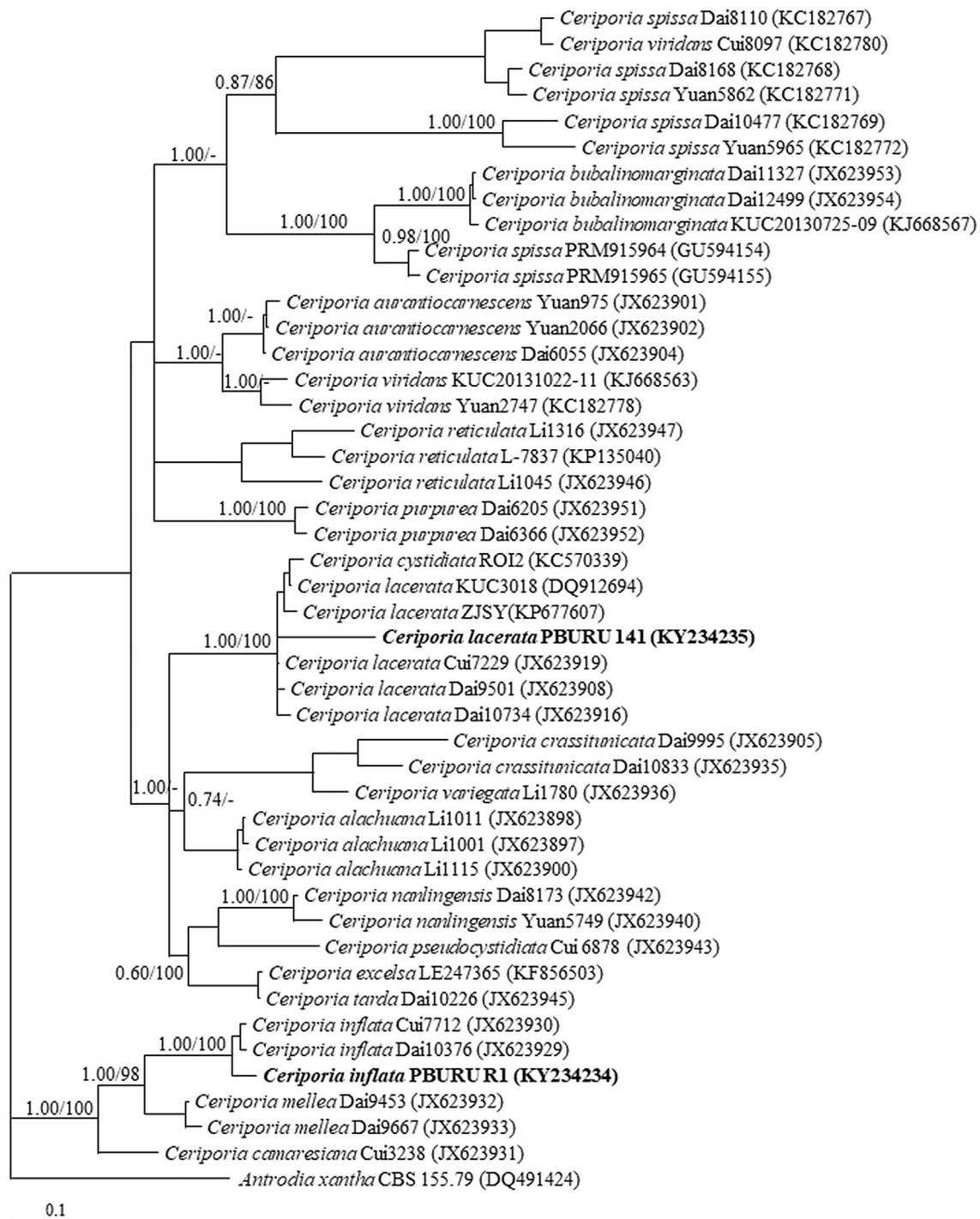


Fig. 3. Bayesian analysis and maximum parsimony of the ITS rDNA region of *Ceriporia inflata* PBURU R1 and *C. lacerata* PBURU 141 among *Ceriporia* group, where the tree was generated from Bayesian analysis; bootstrap values $\geq 50\%$ are shown above branches; specimens found in the current study are in bold.

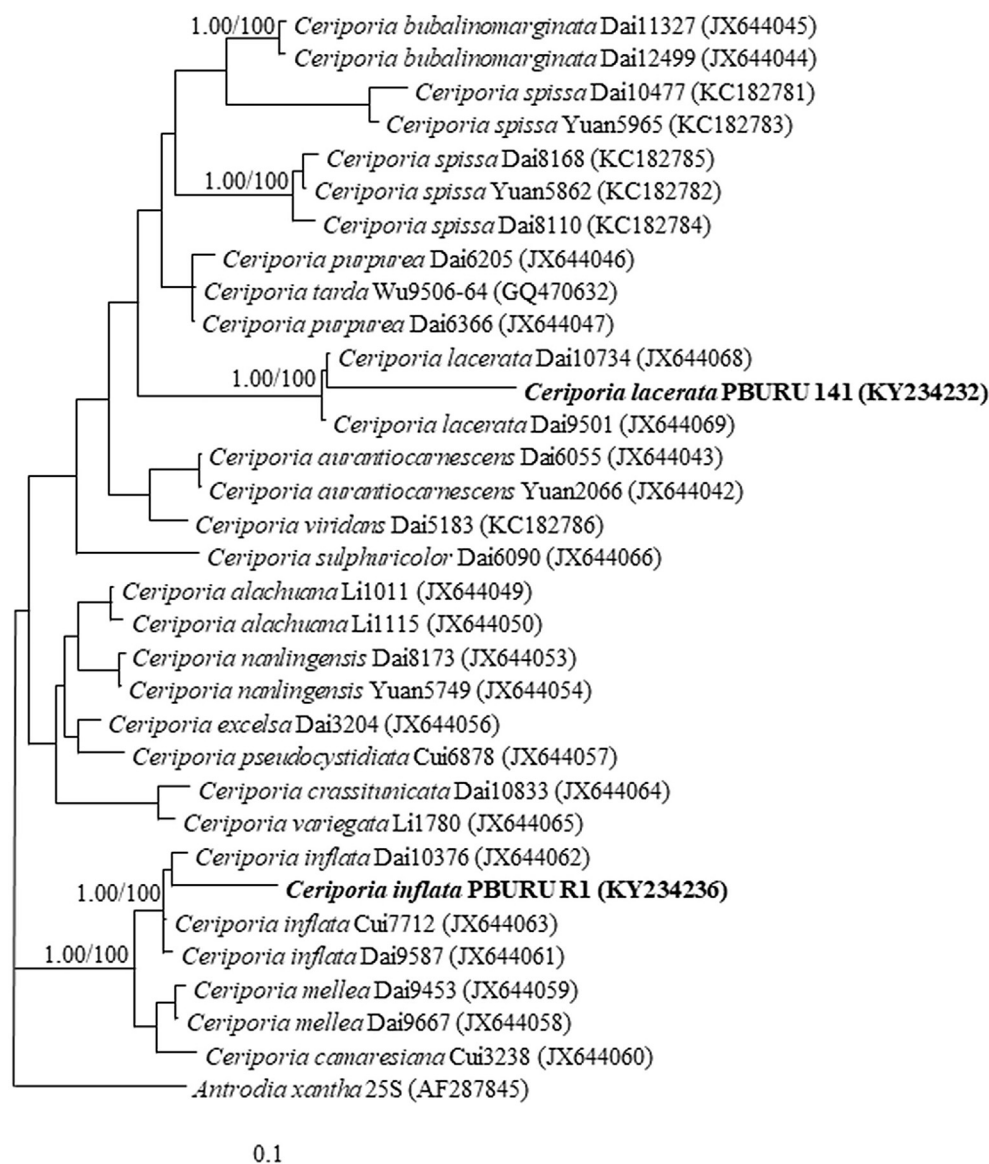


Fig. 4. nLSU region of Bayesian analysis and maximum parsimony of *Ceriporia inflata* PBURU R1 and *C. lacerata* PBURU 141 among *Ceriporia* group, where the tree was generated from Bayesian analysis; bootstrap values $\geq 50\%$ are shown above branches; specimens found in the current study are in bold.

84 variable and 201 parsimony-informative characters. The tree length was 221 steps with CI = 0.5515 and RI = 0.7505 while the Bayesian analysis based on the standard model resulted in an average standard deviation of split frequencies of 0.028069 (Fig. 4).

In the phylogenetic analyses, *C. inflata* PBURU R1 and *C. lacerata* PBURU 141 were highly supported as two distinct taxa with both ITS and nLSU datasets (Figs. 3 and 4), which corresponds with the morphological results. Furthermore, the analysis indicated that *C. inflata* PBURU R1 is closely related to China species, whereas *C. lacerata* PBURU 141 is closely related to Asian species.

In conclusion, the first morphological and molecular evidence (ITS and nLSU) for *C. inflata* and *C. lacerata* from Indonesian tropical forest were obtained, showing the relationships within *Ceriporia* spp. Moreover, the current study and data will contribute previously unknown information on this resupinate fungal diversity group from Indonesia.

Conflict of interest

The authors declare no conflict of interest.

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