Available online at www.sciencedirect.com

MYCOSCIENCE

ISSN 1340-3540 (print), 1618-2545 (online)

journal homepage: www.elsevier.com/locate/myc

Short communication

Polyporus thailandensis, a new species of group Polyporellus in *Polyporus* (Polyporales, Agaricomycota) from Northeastern Thailand

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ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form
27 July 2015
Accepted 29 July 2015
Available online 18 November 2015

Keywords:
Morphology
Phylogenetic relationship
Polypore
Taxonomy
Wood decay fungi

ABSTRACT

Polyporus thailandensis is described and illustrated as a new species from Thailand based on morphological and phylogenetic investigations. This species is characterized by centrally stipitate basidiocarps with umber to sienna glabrous pileus having minute cilia along the margin, round to angular pores, a dimitic hyphal system with often inflating generative hyphae, and cylindrical to ellipsoid basidiospores measuring 7–10.5 × 3–4.5 μm. Phylogenetic analyses based on the combined large subunit (LSU) and internal transcribed spacer (ITS) regions showed that *P. thailandensis* and *P. tricholoma* are closely related, but they differ in color of the pore surface and size of the basidiospores.

Polyporus Fr. (Polyporales, Basidiomycota) is characterized by stipitate basidiocarps; a dimitic hyphal system with arboriform skeletal-binding hyphae; cylindrical, smooth basidiospores; and white rot in their woody substrate (Gilbertson and Ryvarden 1987; Núñez and Ryvarden 1995). A phylogenetic study based on the large subunit (LSU) of the nuclear ribosomal RNA gene (nrDNA), RNA polymerase II second largest subunit (*RPB2*), and mitochondrial ATPase subunit 6 (*ATP6*) regions revealed that *Polyporus* and its allied genera sharing similar microscopic characters and white rotting type included six major clades (Sotome et al. 2008). Recently, two of the six major clades were segregated from the polyphyletic

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http://dx.doi.org/10.1016/j.myc.2015.07.006

genus *Polyporus* as distinct genera, *Favolus* Fr. and *Neofavolus* Sotome & T. Hatt., (Sotome et al. 2013).

Historically, the genus *Polyporus* has accommodated morphologically and phylogenetically various species. Núñez and Ryvarden (1995) divided *Polyporus* into six infrageneric groups based on macro-morphological characters; group *Polyporus*, group Admirabilis, group Dendropolyporus, group Melanopus, group Polyporellus and group Favolus. They included *Polyporus* species with medium-sized basidiocarps (up to 10 cm high), a central stipe lacking black cuticles, and inflated skeletal-binding hyphae in the 'group Polyporellus'. Phylogenetic analyses showed that members of 'group Polyporellus' form a monophyletic group together with an allied genus *Lentinus* Fr. (Hibbett and Donoghue 1995; Krüger and Gargas 2004; Sotome et al. 2008, 2009). Altogether, ten species of 'group Polyporellus' are accepted in the world (Núñez and Ryvarden 1995; Boulet 2003; Silveira and Wright 2005; Sotome et al. 2009), and six of them have been reported in Southeast and East Asia: *P. arcularius* (Batsch) Fr., *P. brumalis* (Pers.) Fr., *P. ciliatus* Fr., *P. longiporus* Audet, Boulet & Sirard, *P. tricholoma* Mont., and *P. rhizophilus* (Pat.) Sacc. (Núñez and Ryvarden 1995; Zhao 1998; Sotome et al. 2009; Dai 2012).

Recently, two newly sampled specimens from Northeastern Thailand obviously belonging to 'group Polyporellus' was collected from a mixed deciduous forest in Demonstration Center 6, which is part of the Phu Kao-Phu Phan Kham National Park, Khon Kaen Province, Northeastern of Thailand. These two specimens could not be identified based on the keys provided by Núñez and Ryvarden (1995) and Sotome et al. (2009). In this study, we examined the phylogenetic position of these two specimens in relation to 'group Polyporellus' of *Polyporus* and *Lentinus* based on the LSU and internal transcribed spacer (ITS) nrDNA regions. After detailed morphological examinations, we describe them as a new species.

Macroscopic characteristics were described based on fresh and dried specimens. Color descriptions were derived according to the Munsell System. Microscopic characters were based on dried specimens, examining free-hand sections mounted in 3% (w/v) KOH solution after staining with 1% (w/v) phloxine solution and in Melzer's reagent. Measurements and line-drawing of microscopic elements were made using Nikon 80i microscope with Microscope Zoom Drawing arm (Nikon Co., Tokyo, Japan). Basidiospore measurements were made from material mounted in Melzer's reagent. The following abbreviations were used for basidiospore measurements: L = mean basidiospore length, W = mean basidiospore width, R = the ratio of length/width of a basidiospore, r = arithmetic mean of R; (n = x/y) means x measurement of basidiospores from y specimens. The examined specimens were deposited in the Natural Medicinal Mushroom Museum, Faculty of Science, Mahasarakham University (MSUT), Thailand.

DNA was extracted from specimens or isolates using EZNA Fungal DNA Kit (Omega Bio-Tek, Norcross, GA, USA) or following Hosaka and Castellano (2008). PCR reactions for the LSU and ITS regions were performed as described by Sotome et al. (2014). PCR products were purified using ExoSAP-IT (GE Healthcare, Tokyo, Japan) or MonoFas DNA Purification Kit (GL Sciences, Tokyo, Japan). Amplified fragments were cloned in the pGEM-T Easy cloning vector (Promega, Fitchburg, WI, USA) and transformed into competent cells (*Escherichia coli* JM109). The DNA of five transformed clones from each sample was amplified as described by Sotome et al. (2014). DNA sequences were determined using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with an ABI 3130 DNA sequencer (Applied Biosystems). The generated sequences were deposited in the DDBJ/EMBL/GenBank databases shown in Table 1 (Accession nos. LC052215–LC052222).

The LSU and ITS sequences were aligned using MAFFT v. 7 (Katoh and Standley 2013) and corrected manually in BioEdit (Hall 1999). Phylogenetic analyses of the aligned sequences were performed with maximum parsimony (MP) and maximum likelihood (ML) in MEGA v. 6.06 (Tamura et al. 2013). The positions where gaps were present were deleted. The strength of the internal branches of the resulting MP and ML trees was tested statistically by bootstrap (BT) analysis (Felsenstein 1985) from 1000 bootstrap replications. The K2+G model was used for the combined LSU and ITS dataset. Best-fit substitution model was estimated using MEGA v. 6.06. The combined LSU and ITS dataset was 1129 sites with 86 parsimony informative characters. The alignment dataset and resulting trees were deposited in TreeBase (http://www.treebase.org/) under the accession number 17512.

The MP and ML trees showed no inconsistency in any supported clades (both bootstrap supported values ≥65%) (Fig. 1). In the MP analysis, we obtained two equally most parsimonious trees (length = 198, CI = 0.67, RI = 0.73). The ML analysis resulted in a best scoring tree with a likelihood of InL = −2599.0465. Two newly sampled specimens formed a distinct clade with strong bootstrap support (MPBT/MLBT = 99/99) and were most closely related to *Polyporus tricholoma*, forming a strongly supported clade (MPBT/MLBT = 99/99).

Polyporus thailandensis Sotome, sp. nov. Fig. 2

MycoBank no.: MB 813755.

Holotype: THAILAND, Khon Kaen Prov., Demonstration Center 6 in a part of Phu Kao-Phu Phan Kham National Park, on hardwood, Sophon Boonlue, T. Aimi, K. Sotome, T. Matozaki, 29 Aug 2014 (MSUT_6734).

Gene sequences ex holotype: LC052219 (LSU); LC052221 (ITS).

Etymology: *thailandensis* (Lat.), referring to Thailand, the country of origin.

Basidiocarps annual, centrally stipitate, solitary. Pileus circular in outline, flat to convex, depressed at the center, 1.1–2.1 cm in diam, up to 4.5 mm thick; surface smooth, glabrous, umber to sienna (10YR5–6/6–8), azonate; margin incurved to flat, with minute cilia. Stipe cylindrical, equal, 0.8–2.8 cm long, up to 2 mm in diam; surface smooth, isabelline (10YR5–6/4). Context fleshy-tough to leathery in fresh condition, corky in dried condition, pale yellow (10YR9/4) in fresh condition, up to 2 mm thick. Pore surface pale yellow (10YR8–9/4–8), round to angular, (4–)5–7 pores/mm, occasionally irregular and elongated up to 1.5 × 0.3 mm, dissepiments thin, entire. Tubes concolorous with pore surface, up to 3.5 mm deep. Hyphal system dimitic with generative hyphae