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Irpex hacksungii sp. nov. (*Polyporaceae*) from Korea

Jin Sung Lee[1], Changmu Kim[2] & Young Woon Lim[3]

[1]*jinsung@me.go.kr*
[2]*snubull@me.go.kr*
[3]*youngwlim@me.go.kr*
National Institute of Biological Resources
Incheon 404-708, Korea

Abstract — *Irpex* is one of the most common polypore genera and characterized by its poroid to hydnaceous hymenophores, encrusted cystidia, and simple-septate hyphae. An *Irpex* specimen with conspicuous morphological differences from *I. lacteus* and *I. hydnoides* was collected in Korea and this taxon is proposed as a new species, *Irpex hacksungii*.

Key words — *Basidiomycota*, taxonomy, phylogeny, ITS

Introduction

The genus *Irpex* Fr. (*Polyporaceae, Basidiomycetes*) is characterized by its poroid, irpicoid, hydnaceous hymenophores, encrusted cystidia, and simple-septate hyphae (Cejp 1931, Gilbertson & Ryvarden 1986, Ryvarden & Gilbertson 1993). Because of a broad generic concept, the genus *Irpex* included numerous species. However, Maas Geesteranus (1974) treated many *Irpex* species as synonyms of *I. lacteus* (Fr.) Fr. and moved all other species from the genus *Irpex* to other genera. The monotytic genus *Irpex* remained stable then for thirty years (Ryvarden & Gilbertson 1993). Recently, Lim & Jung (2003) included a second species, *Irpex hydnoides* Y.W. Lim & H.S. Jung, in this monotypic genus. *I. hydnoides* is distinguished from *I. lacteus* based on hymenophoral configuration and cultural and molecular characters.

The national biological inventory organized by the National Institute of Biological Resources (NIBR, www.nibr.go.kr) is currently underway and has yielded numerous collections of fungi from Korea. During observations of dry specimens at NIBR, a previously unknown species of *Irpex* was encountered. This fungus is distinct from other *Irpex* species based on morphological and molecular characters. Therefore, it is described in this paper as a new species.

Materials and methods

Specimens and observations

Macroscopic and microscopic characters were based on voucher specimens deposited at Seoul National University Fungi Herbarium (SFC) with duplicates at NIBR. The measurements and drawings were made on slide preparations mounted with 3% (w/v) potassium hydroxide and stained with 1% (w/v) phloxine in water (Largent et al. 1977) using a Nikon microscope and drawing tube.

Molecular analyses

Specimens that used on molecular approaches are listed in Table 1. Total DNA was extracted from dried specimens using a Genomic DNA Extraction Kit (Bioneer, Korea). Extracted DNA was stored at –20°C. The internal transcribed spacer (ITS) region was amplified using primers ITS5 and ITS4 (White et al. 1990). PCR amplification was performed as described by Lee et al. (2000). Amplified products were visualized by electrophoresis on a 1% agarose gel, and purified with the PCR purification kit (Bioneer, Korea). Sequencing was performed using the primers described above on an ABI 3730XL automated sequencer (Applied Biosystems, USA). The sequence was proofread, edited, and aligned with other *Irpex* species ITS sequences retrieved from the GenBank using the jPHYDIT program (Jeon et al. 2005). Phylogenetic trees were inferred from sequence alignment using neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) methods implemented in PAUP 4.0b10 (Swofford 2002). Likelihood settings were parameterized using the best-fit model (TrN+I) selected by AIC in Modeltest 3.7 (Posada & Crandall 1998).

Table 1. *Irpex* specimens used in this study.

Species	Voucher No.	Locality	GenBank Acc. No. (ITS)
I. hacksungii	SNU m-990326-16[a]	Korea	EU301650
I. hydnoides	SNU m-971215-19	Korea	AF479667
I. hydnoides	SNU m-971011-12	Korea	AF479668
I. lacteus	SNU m-951007-39	Korea	AF479666
I. lacteus	SNU m-971006-12	Korea	AF163046

[a] SNU, Seoul National University Herbarium, Seoul, Korea, Holotype.

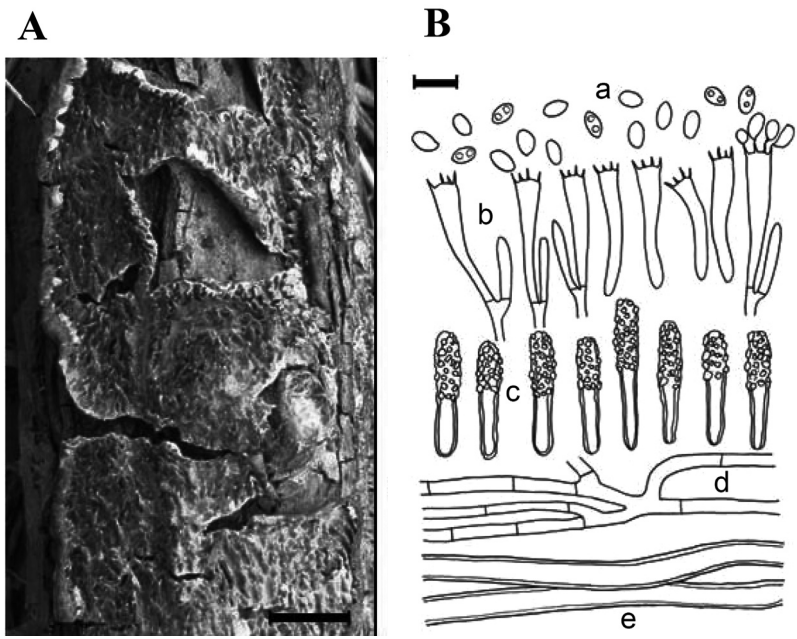

Figure 1. Basidiocarps (A) and microscopic features (B) of *Irpex hacksungii* (holotype). a, basidiospores. b, basidia. c, lamprocystidia. d, generative hyphae. e, skeletal hyphae.
Scale bars: A = 5 mm, B = 10 μm.

Results

Taxonomy

Irpex hacksungii J.S. Lee & Y.W. Lim, **sp. nov.** Figure 1

MycoBank MB 512279

Basidiocarpus annuus, resupinatus vel effuso-reflexus; superficies canus, tomentoso-hirsuto; hymenium cremeo-lutea vel ochraceo, hydnoidea, 2 mm longis; systema hypharum dimiticum; hyphae generatoriae tenuitunicatae, 2–3.5 μm; lamprocystidia incrustata, 24–33 × 5–6.5 μm; basidia clavata, 4-sterigmatibus, 26–32 × 4.5–5.7 μm; basidiosporae ellipsoideae, hyalinae, laeves, non-amyloideae, 5.0–6.2 × 3.0–3.5 μm.

***Holotypus** in herbarium SNU; numero accessiono m-990326-16.*

Etymology: "hacksungii", named in honour of Dr. Hack Sung Jung, a pioneering Korean mycologist. Among his many notable contributions to mycological research in Korea were excellent descriptions and keys for identifying polyporoid genera and species.

Basidiocarps annual, usually resupinate or effused-reflexed; pilei protrude up to 3 mm, upper surface light gray; hymenophore cream, ocher to gray, tooth up to 2 mm long; margin loose and often somewhat upcurved. Hyphal system dimitic; generative hyphae thin-walled with frequent branching, frequently

septate without clamp connection, 2–3.5 µm wide; skeletal hyphae hyaline, thick-walled, rarely simple septate, 5–6 µm wide. Lamprocystidia abundant, thick-walled, heavily incrusted at apex, 24–33 × 5–6.5 µm, incrusted part 10–14 × 5–6.5 µm. Basidia clavate, 4-sterigmata, 26–32 × 4.5–5.7 µm, simple-septate at the base. Basidiospores elliptical, hyaline, smooth, negative in Melzer's reagent, 5.0–6.2 × 3.0–3.5 µm.

Collections Examined: **KOREA**: Mungyeongsaejae, Mungyeong-si, Gyeongsangbuk-do, on a fallen branch of an unknown deciduous tree, 26 March 1999, Y. W. Lim, deposited in Seoul National University Herbarium (SNU), Specimen No. SNU m-990326-16 (**HOLOTYPE**), and National Institute of Biologial Resources (NIBR), Specimen No. NIBRFG0000001610 (**ISOTYPE**).

Notes – Although this fungus is macroscopically similar to *Irpex hydnoides*, the teeth are shorter and similar to those of *Steccherinum* Gray species. Microscopic differences of *I. hacksungii* include: smaller lamprocystidia than those in *I. hydnoides* and *I. lacteus* (*I. hacksungii*: 24–33 × 5–6.5 µm, *I. hydnoides*: 40–57 × 8–10 µm, *I. lacteus*: 65–80 × 6.8–11.5 µm); smaller basidiospores than *I. hydnoides* (*I. hacksungii*: 5–6.2 × 3–3.5 µm, *I. hydnoides*: 5.9–6.7 × 3.5–4 µm); and smaller basidia than *I. hydnoides* (*I. hacksungii*: 26–32 × 4.5–5.7 µm, *I. hydnoides*: 30–32 × 5.5–6.5 µm) (Table 2).

Table 2. Morphological characters of *Irpex* species.

Species	*Irpex hacksungii*	*Irpex hydnoides*	*Irpex lacteus*
Basidiocarp	Resupinate to effuse-reflexed	Resupinate to effuse-reflexed	Resupinate to effuse-reflexed
Hymenophore	hydnoid, yellow to gray	hydnoid, deep yellow	poroid, irpicoid, hydnoid, milky white to cream
Hyphal system	dimitic	dimitic	dimitic
Lamprocystidia (µm) (encrusted part)	24–33 × 5–6.5 [a] (10–14 × 5–6.5)	40–57 × 8–10 (23–25 × 8–10)	65–80 × 6.8–11.5 (25–31 × 6.8–11.5)
Basidia (µm)	26–32 × 4.5–5.7	30–32 × 5.5–6.5	24–25 × 4–5
Basidiospores (µm)	5–6.2 × 3–3.5	5.9–6.7 × 3.5–4	5.5–6 × 2.2–2.8

[a] Twenty (20) cystidia, basidia and spores of each specimen were measured.

Sequence analyses

The amplified ITS1-5.8S rDNA-ITS2 region of the new *Irpex* species was 594 bp long. There was little intraspecific variation in the ITS region of *I. lacteus* (mean value=0.40%, 0~3/436; 0~3 out of 436 positions showed nucleotide difference) and in the ITS region of *I. hydnoides* (0.46%, 1~5/434). Average sequence variation between *I. hacksungii* and *I. lacteus* or *I. hydnoides* was 3.05% (12~15/433) and 2.88% (12~13/434) respectively. These differences

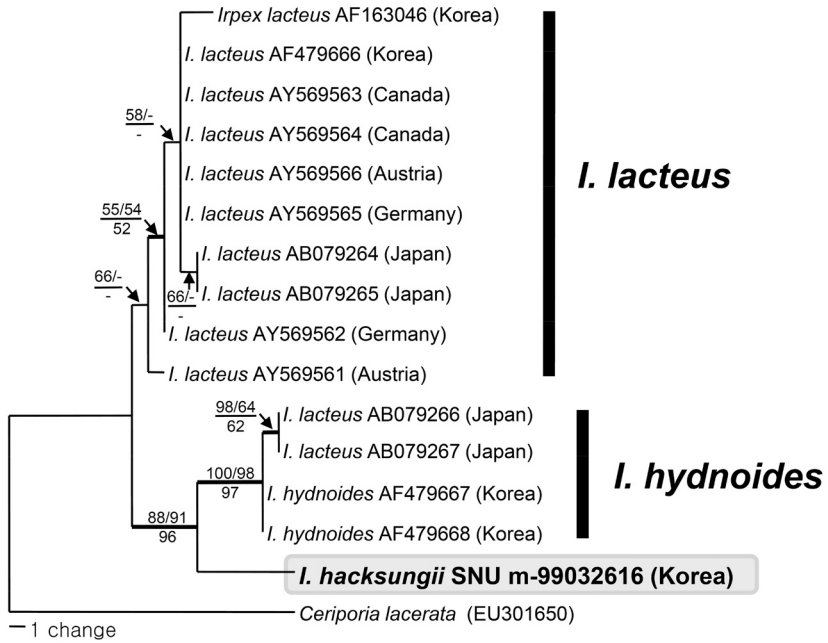

Figure 2. The NJ tree of *Irpex hacksungii, I. lacteus* and *I. hydnoides* based on sequence data of the ITS regions. Branches maintained in three different analyses (MP, ML and NJ analysis) were presented by bold lines. Numbers above branches that are before the slash are NJ bootstrap proportions and those that are after the slash are MP bootstrap proportions. Values below branches are ML bootstrap proportions. The ITS sequence of *Ceriporia lacerata* (EU301650) was used as an outgroup.

are greater than the variation observed between *I. lacteus* and *I. hydnoides* (2.87%, 11~14/433). The aligned sequences of 15 *Irpex* species and *Ceriporia lacerata* N. Maek., Suhara & R. Kondo (outgroup) formed a matrix with 604 nucleotide positions in length. Among these, 554 sites were constant, 38 sites were variable but parsimony-uninformative, and 12 sites were parsimony-informative. NJ, MP, and ML analyses yielded the same phylogenetic trees. The NJ tree is presented in Figure 2. *Ceriporia lacerata* (DQ912694) was selected as an outgroup, as justified in previous phylogenetic study (Lim & Jung 2003). The tree showed that the new *Irpex* species was included in a monophyletic clade with the two other *Irpex* species, *I. lacteus* and *I. hydnoides*. However, its phylogenetic position was distinct within the *Irpex* clade (Fig. 2). Two Japanese *I. lacteus* were grouped into *I. hydnoides*. They might be *I. hydnoides* because i) *I. lacteus* had been treated as a monotypic genus for a long time and ii) *I. hydnoides* was reported only recently (Lim & Jung 2003).

Discussion

Ryvarden (1991) classified the *Polyporaceae* into eleven groups and included *Irpex* in the *Junghuhnia* group along with ten other related genera. However, recent phylogenetic studies using rDNA sequence suggested that the genus *Irpex* showed more relationship with the genera, *Ceraceomyces* Jülich, *Ceriporia* Donk, *Ceriporiopsis* Domański, *Cystidiodontia* Hjortstam, *Hexagonia* Fr. and *Oxyporus* (Bourdot & Galzin) Donk (Ko et al. 2001, Lee 2006). *I. lacteus* and species from the genus *Ceriporia* have received greater attention as they are important white rot fungi due to their production of endo- and exotype cellulases (Hamada et al. 1999, Hoshino et al. 1994), antibiotics and irpexans (Silberborth et al. 2000). They are also capable of decolorizing chemically complex dyes and bioremediation of hazardous organic compounds (Kim & Song 2000, Novotny et al. 2000).

Since Maas Geesteranus's (1974) revision, two species have become accepted in the genus *Irpex*: *I. lacteus* and *I. hydnoides*. We include one more species, *I. hacksungii*, in this genus. Morphological characteristics and phylogenetic analysis of the ITS region show that *I. hacksungii* is closer to *I. hydnoides* than to *I. lacteus*. However, the new species is quite distinct from *I. hydnoides*, because it possesses short teeth, and small lamprocystidia and basidiospores (Table 2). In the molecular phylogenetic analysis, the three *Irpex* species formed a monophyletic clade, but were separated into independent lineages. Additionally *I. lacteus* is distributed worldwide as a cosmopolitan species whereas *I. hydnoides* and *I. hacksungii* appear to be distributed in Far East Asia. *I. hydnoides* was found in China recently (Dai et al. 2008) and Japan (Fig. 2). *I. hydnoides* and *I. hacksungii* might be derived from *I. lacteus* and may have a potential for biotechnological applications, similar to or higher that of *I. lacteus*.

Acknowledgments

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