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Taxonomy and phylogeny of *Heterobasidion* in South Korea

Yeongseon Jang[1], Seokyoon Jang[1], Young Woon Lim[2], Changmu Kim[3] & Jae-Jin Kim[1]*

[1]*Division of Environmental Science & Ecological Engineering, College of Life Sciences & Biotechnology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul, 136-701, Korea*

[2]*School of Biological Sciences, Seoul National University, Seoul, 151-747, Korea*

[3]*National Institute of Biological Resources, Environmental Research Complex, Incheon, 404-708, Korea*

*Correspondence to: *jae-jinkim@korea.ac.kr*

Abstract — ITS and TEF gene sequences from eleven basidiome collections from South Korea and one mycelial culture were analyzed to infer the phylogeny of Korean *Heterobasidion* species. Comparison of morphological characters with phylogenetic analyses revealed that specimens previously recorded as '*H. annosum*' and '*H. araucariae*' in fact represent *H. ecrustosum*, while those recorded as '*H. insulare*' represent *H. orientale*. The two species currently recognized in South Korea are described and illustrated.

Key words — *Basidiomycota, Bondarzewiaceae*, polypore

Introduction

Heterobasidion Bref. is a well-known polypore genus that includes the destructive tree pathogen, *H. annosum* (Fr.) Bref. The genus is characterized by resupinate to pileate basidiocarps, a dimitic hyphal system with mostly simple septate generative hyphae and dextrinoid skeletal hyphae, and finely asperulate inamyloid basidiospores (Gilbertson & Ryvarden 1986). In addition, its skeletal hyphae are cyanophilous in Cotton Blue (Dai & Korhonen 2009). Until recently, five species — *H. annosum*, *H. araucariae* P.K. Buchanan, *H. insulare* (Murrill) Ryvarden, *H. pahangense* Corner, and *H. rutilantiforme* (Murrill) Stalpers — were accepted, but both *H. annosum* and *H. insulare* have been found to represent species complexes (Korhonen 1978, Dai et al. 2002), so that now eleven *Heterobasidion* species are recognized (Dai & Korhonen 1999,

Tokuda et al. 2009, Dai & Korhonen 2009, Otrosina & Garbelotto 2010). Dai et al. (2003), Tokuda et al. (2009), and Dai & Korhonen (2009) accept *H. australe* Y.C. Dai & Korhonen, *H. ecrustosum*, *H. linzhiense* Y.C. Dai & Korhonen, *H. orientale*, and *H. parviporum* Niemelä & Korhonen in northeast Asia (China and Japan).

In Korea, Lee (1988), Lee & Jung (2006), and Paul et al. (2012) have reported three *Heterobasidion* species: *H. annosum*, *H. araucariae,* and *H. insulare. Heterobasidion insulare* was reported (as *Fomitopsis insularis*) from a conifer trunk (Lee 1988), *H. annosum* was found at the base of *Pinus rigida* (Lee & Jung 2006), and *H. araucariae* was found as an endophyte from roots of red pepper, *Capsicum annuum* (Paul et al. 2012). However, as none of these three species had previously been reported for northeast Asia, re-examination of those materials was required.

In this study, we list the *Heterobasidion* species in South Korea and describe the morphological characteristics for specimens collected in the country and, when possible, for voucher specimens from previous studies. In addition, we analyzed sequences from the internal transcribed spacer rDNA (ITS) and translation elongation factor 1-alpha (TEF). The ITS was selected because it is used for barcoding fungal species (Schoch et al. 2012), and the TEF has been used to infer species relationships within *Heterobasidion* (Ota et al. 2006, Paul et al. 2012). We describe and illustrate the two accepted species and compare their key characters with the *Heterobasidion* species in northeast Asia.

Materials & methods

Collection and morphological examination

The basidiocarps of *Heterobasidion* spp. identified by Lee & Jung (2006) and materials deposited in Seoul National University Fungal Collection (SFC) and the Korea University Culture Collection (KUC) were used in this study (Table 1). The macro- and microscopic characteristics of the specimens were examined according to Jang et al. (2013). Color codes in the descriptions follow Munsell (2000).

Phylogenetic analysis

Genomic DNAs were extracted according to Jang et al. (2013) from the materials in Table 1. PCR reactions for ITS regions were performed using Accupower PCR Premix Kit (Bioneer, Korea) with the primers ITS1F/ITS4 according to Jang et al. (2013). TEF region was amplified using the same kit with the primers, EF1-728F (5´-catcgagaagttcgagaagg-3´) /EF1-1567R (5´-achgtrccratacca-ccratctt-3´) and the PCR conditions: an initial denaturation step of 95°C for 7 min, followed by 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 1 min, with an elongation step of 72°C for 7 min at the end. The DNA was sequenced by the Macrogen sequencing service (Seoul, Korea). Sequences obtained in this study were deposited in GenBank, NCBI; the accession numbers are shown in Table 1. The ITS sequences

Table 1. The Korean *Heterobasidion* specimens and their ITS and TEF sequences used in this study.

Species	Original determination	Specimen no.[a]	GenBank ITS No.	GenBank TEF No.
H. ecrustosum	*H. annosum*	KUC20080904-43	—	—
	H. annosum	KUC20110916-44	KF218829	—
	H. annosum	KUC20111001-14	KF218830	KF154267
	H. annosum	KUC20120810-11	KF218831	KF154268
	H. annosum	KUC20120810-14	KF218832	KF154269
	H. araucariae	CNU081069	JQ691621[b]	JQ691622[b]
	H. annosum	SFC 20020927-11	—	—
	H. annosum	SFC 20120820-01	KF218834	KF154270
H. orientale	*H. insulare*	KUC20081030C-04	KF218828	—
	H. insulare	KUC20121019-01	—	KF154271
	H. insulare	KUC20121019-30	KF218833	KF154272
	H. insulare	KUC20121123-14	—	KF154273

[a] Examined basidiocarps now deposited in National Institute of Biological Resources (KB); *H. ecrustosum* CNU081069 is a mycelial isolate from Paul et al (2012).

[b] Sequences retrieved from GenBank, NCBI.

were aligned with the *Heterobasidion* sequences from Dalman et al. (2010). TEF sequences were aligned with the sequences from Ota et al. (2006) and Paul et al. (2012). The sequence alignment, model selection, and Bayesian phylogenetic analyses were performed according to Jang et al. (2013).

Results & discussion

Morphological comparisons

Although the voucher specimens of '*Heterobasidion insulare*' in Lee (1988) were not available, we feel that his fungus represents *H. orientale* based on his description and colored illustration of the basidiocarps. He described the basidiocarps as sessile and 2.5–5 × 4–8 × 1–1.5 cm; the pileus had a brick-red surface and white to cream margin. The 1.5–3 mm context was white to cream, the tubes were 1 cm with round to labyrinthiform pores, and the spores were subglobose and 4–5 µm long. In contrast, *H. insulare* has a mostly light brown pileus surface and round to angular (not labyrinthiform) pores (Tokuda et al. 2009). In addition, other specimens formerly known as '*H. insulare*' (Table 1) are actually *H. orientale:* Tokuda et al. (2009) note that specimens previously recognized as *H. insulare* in China and Japan represent *H. orientale* and that *H. insulare* is known only from the type specimen found in the Philippines.

Re-examination '*Heterobasidion annosum*' specimen SFC 20020927-11 from Lee & Jung (2006) revealed that it represents instead *H. ecrustosum,*

and the species description in Lee & Jung (2006) is quite similar to that of *H. ecrustosum* by Tokuda et al. (2009). Furthermore, all specimens previously identified as '*H. annosum*' have been since identified as *H. ecrustosum* (Table 1). Dai et al. (2003) showed that *H. annosum* has not been found from eastern Asia and Altai region and that Russia represents its easternmost distribution.

'*Heterobasidion araucariae*' from Paul et al. (2012) was found as an endophyte. The cultural characteristics of *H. araucariae* and *H. ecrustosum* are similar. The data of Paul et al. (2012) for their '*H. araucariae*' (conidiophores <300 × 5.5–10.5 µm; conidia 4–11.5 × 2–7 µm) lie within the data range of Tokuda et al. (2009) for *H. ecrustosum* (conidiophores <300 × 4–10 µm; conidia 3.2–17.1 × 2.9–12 µm), but only partially overlap the data range of Buchanan (1988) for authentic *H. araucariae* (conidiophores <400 × 5.5–13.5 µm; conidia 5–16 × 3–13.5 µm). Authentic *H. araucariae* has been found only in Australia, New Zealand, and Fiji (Buchanan 1988; Ota et al. 2006; Tokuda et al. 2009), and Tokuda et al. (2009) consider it a species restricted to the southern hemisphere.

Phylogeny

Among the 11 specimens examined in this study, ITS gene region sequences were obtained from seven specimens (594–627 bp) and TEF gene region sequences from seven specimens (718–774 bp) (Table 1). No sequence was amplified from KUC20080904-43 and SFC 20020927-11. The phylogenetic analyses supported our morphological conclusions that the examined '*H. annosum*' and '*H. araucariae*' specimens represent *H. ecrustosum* and that the examined '*H. insulare*' specimens represent *H. orientale*.

Bayesian analysis of ITS sequences (not presented) was congruent with the tree from Dalman et al. (2010). No intraspecific ITS sequence variation was found in Korean *H. ecrustosum* and *H. orientale*. *Heterobasidion ecrustosum* was monophyletic with *H. araucariae* and our *H. ecrustosum* ITS sequences were 99% similar (5 positions different) to *H. araucariae* from Australia and New Zealand (GenBank FJ627521, FJ627522, FJ627526, and FJ627527). *Heterobasidion orientale* was monophyletic and sister to the *H. araucariae*/*H. ecrustosum* clade.

The TEF gene region tree (Fig. 1) showed a topology similar to that of Ota et al. (2006) except that Japanese isolates of *H. parviporum* did not form a separate clade but were positioned on the basal part of the clade comprising *H. occidentale*, *H. abietinum*, and *H. parviporum*. Korean *H. orientale* collections produced two different TEF sequences although they were collected from the same location; their basal position within the *H. orientale* clade was supported with high posterior probability (1.0 p.p.). *Heterobasidion orientale*

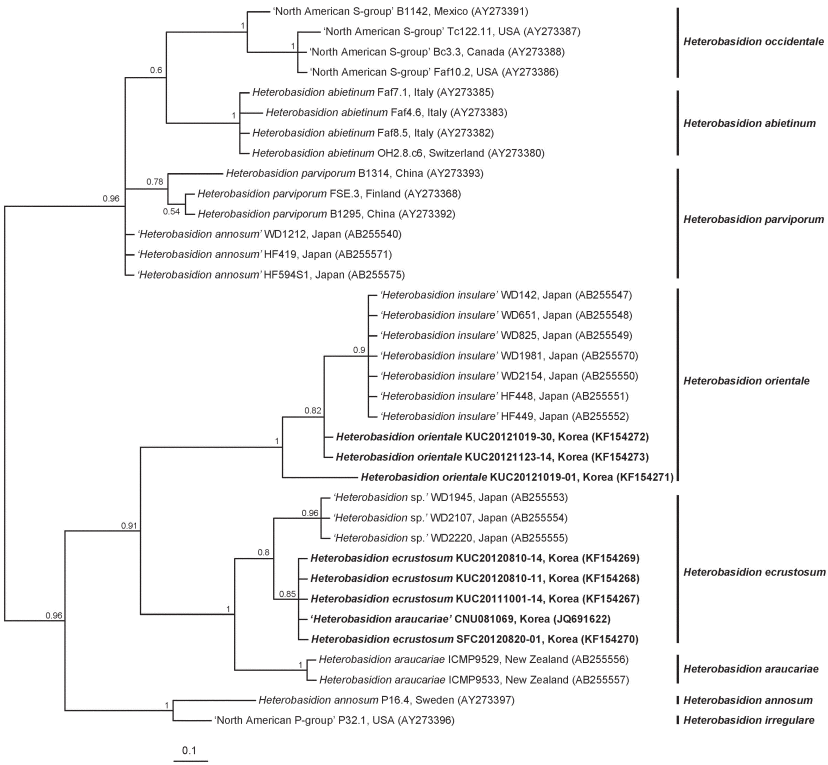

Fig. 1. 50% major-rule consensus tree of Korean *Heterobasidion* spp. and allies using TEF region sequences. The tree, containing 36 taxa and 258 characters, was constructed from 15,000 trees produced by Bayesian analysis and was mid-point rooted. Posterior probabilities ≥50% are given. Korean specimens are shown in bold type. For each node, the former names, isolate numbers, and localities are given, and the species names accepted by recent studies (Tokuda et al. 2009, Otrosina & Garbelotto 2010) are presented on the right side of the tree. GenBank accession numbers are provided in parentheses.

KUC20121019-30 and KUC20121123-14 were 98% similar to Japanese isolates (254 out of 258 sequences matched), and KUC20121019-01 was 96% similar (247 out of 258 sequences matched). Korean *H. ecrustosum* collections (including '*H. araucariae*' CNU081069 from Paul et al. 2012) had 100% identical TEF sequences and were sister to Japanese *H. ecrustosum* isolates with moderate support (0.8 p.p.). The *H. ecrustosum* clade again clustered with authentic *H. araucariae* with high support (1.0 p.p.). Korean *H. ecrustosum* collections were 98% similar to Japanese *H. ecrustosum* (245 out of 251 sequences matched) and 97% similar to *H. araucariae* (242 out of 249 sequences matched).

Taxonomy

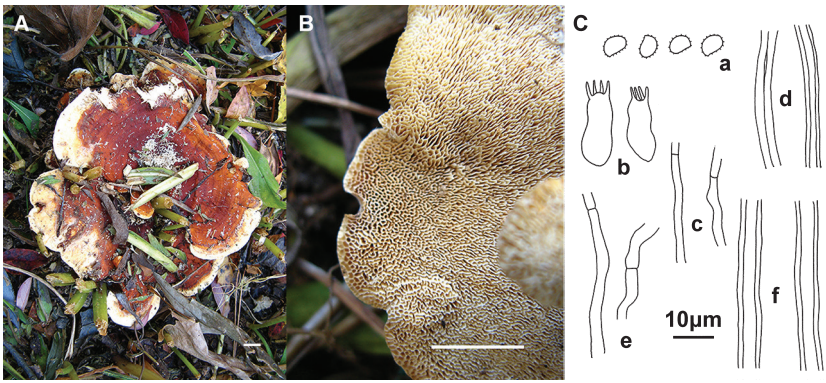

Fig. 2. *Heterobasidion orientale* (KUC20121019-01). A, B. Basidiocarp. Scale bar = 1 cm. C. Microscopic features. a, basidiospores; b, basidia; c, generative hyphae from trama; d, skeletal hyphae from trama; e, generative hyphae from subiculum; f, skeletal hyphae from subiculum.

Heterobasidion orientale Tokuda, T. Hatt. & Y.C. Dai,
Mycoscience 50: 193. 2009. Fig. 2

Basidiocarps annual, sessile, solitary to imbricate, ≤7 × 5 × 2 cm. Pileus broadly attached to the substrate, dimidiate, applanate to elongated. Pileus surface glabrous, sometimes radially rugose when dry, zonate with dusky red (10R3/3) to dark red (7.5R3/6) in the middle, yellow (10YR7/6) to very pale brown (10YR8/2-3) near the margin. Pileus margin acute. Pore surface very pale brown (10YR8/4) to yellow (10YR7/6-8, 10YR8/6-8). Pores round to angular or labyrinthiform, 2–4/mm, dissepiments eroded. Sterile margin 0.5 mm wide or absent. Context corky, very pale brown (10YR8/2), 1–2 mm thick. Tubes 3–6 mm deep, concolorous with context.

Hyphal system dimitic; generative hyphae without clamp connection and skeletal hyphae.

Context generative hyphae without clamp connections, hyaline, thin to slightly thick-walled, IKI–, 2.0–3.5 µm in diameter; skeletal hyphae abundant, hyaline, thick-walled, dextrinoid, 4.5–6.5 µm in diameter.

Tubes generative hyphae without clamp connections, hyaline, thin-walled, IKI–, 2–2.5 µm in diameter; skeletal hyphae abundant, hyaline, thick-walled, weakly dextrinoid, 3–5 µm in diameter; cystidia none; basidia barrel-shaped, 4-sterigmate, slightly constricted in the middle, 17–29 × 5–7 µm.

Basidiospores globose to subglobose, hyaline, finely asperulate, IKI–, (4.6–)4.8–6.7 × 3.9–5.4(–5.8) µm, L = 5.6 µm, W = 4.5 µm (n = 88/3).

Distribution — China, Japan, and Korea.

Specimens examined: **KOREA**, **Kyeonggi-do**, Mt. Bori, 37°39′06″N 127°32′02″E, on *Pinus densiflora* Siebold & Zucc., 30 October 2008, Yeongseon Jang (KB, KUC20081030C-04). **Gangwon-do**, Mt. Odae, 37°43′59″N 128°35′23″E, on unknown wood, 19 October 2012, Yeongseon Jang (KB, KUC20121019-01); 37°44′13″N 128°35′16″E, *Abies holophylla* Maxim., 19 October 2012, Yeongseon Jang (KB, KUC20121019-30); 37°44′30″N 128°35′03″E, on unknown wood, 23 November 2012, Yeongseon Jang (KB, KUC20121123-14).

Remarks — The basidia of Korean *H. orientale* specimens are larger than those given by Tokuda et al. (2009).

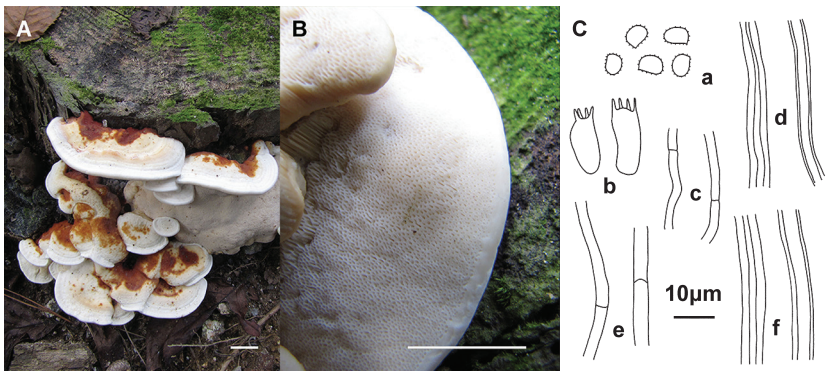

Fig. 3. *Heterobasidion ecrustosum* (KUC20110916-44). A, B. Basidiocarp. Scale bar = 1 cm. C. Microscopic features. a, basidiospores; b, basidia; c, generative hyphae from trama; d, skeletal hyphae from trama; e, generative hyphae from subiculum; f, skeletal hyphae from subiculum.

Heterobasidion ecrustosum Tokuda, T. Hatt. & Y.C. Dai, Mycoscience 50: 196. 2009. Fig. 3

Basidiocarps annual, sessile, solitary to imbricate, ≤6 × 3.5 × 2 cm. Pileus broadly attached to the substrate, dimidiate, applanate to convex, somewhat inrolled when dry. Pileus surface glabrous, usually rough, subzonate to azonate, radially sulcate in one specimen (KUC20110916-44), very pale brown (10YR8/3-4) to yellow (10YR8/6) or reddish yellow (5YR6/8), with crust at the base or without crust, crust dark yellowish brown (10YR3/4) to very dark brown (10YR2/2) or dark reddish brown (5YR3/4). Pileus margin rounded. Pore surface very pale brown (10YR8/2-4) or pale to light yellowish brown (10YR6/4-6/6), pores round to angular, sometimes elongated, 2–3/mm, dissepiments entire. Sterile margin narrow, concolorous with the pore surface, 0.5–1.5 mm wide, or absent. Context corky, very pale brown (5YR8/2-3), 2–5 mm thick. Tubes concolorous with the context, 1–7 mm deep.

Hyphal system dimitic; generative hyphae without clamp connections and skeletal hyphae.

Context generative hyphae without clamp connections, hyaline, thin to thick-walled, IKI–, 2.5–5 µm in diameter; skeletal hyphae abundant, hyaline, dextrinoid, thick-walled, 3.5–5.5 µm in diameter.

Tubes generative hyphae without clamp connections, hyaline, thin walled, IKI–, 2.0–4.5 µm in diameter; skeletal hyphae abundant, hyaline, dextrinoid, thick-walled, 3–5.5 µm in diameter; cystidia none; basidia barrel-shaped, 4-sterigmate, 17–26 × 4.5–7 µm.

Basidiospores globose to subglobose, hyaline, finely asperulate, IKI–, 4.9–6.9(–7.7) × (3.7–)4.1–5.9(–6.5) µm, L = 5.9 µm, W = 5.0 µm (n = 99/3).

Distribution — China, Japan, and Korea.

Specimen examined: **KOREA**, **Seoul**, Mt. Cheonggye, 37°25′47″N 127°02′51″E, on the branch of wood, 4 September 2008, Yeongseon Jang (KB, KUC20080904-43). Heonilleung, 37°28′03″N 127°05′00″E, on the stump of *Pinus densiflora*, 16 September 2011, Yeongseon Jang (KB, KUC20110916-44). **Jeollabuk-do**, Gochang, 35°27′35″N 126°30′51″E, on unknown wood, 1 October 2011, Yeongseon Jang (KB, KUC20111001-14). **Jeollanam-do**, Wando arboretum, 34°23′05″N 126°39′07″E, on unknown wood, 10 August 2012, Yeongseon Jang (KB, KUC20120810-11); on unknown wood, 10 August 2012, Yeongseon Jang (KB, KUC20120810-14). **Chungcheongbuk-do**, Mt. Songni, on *Pinus rigida* Mill., 27 September 2002, Jin Sung Lee (KB, SFC 20020927-11). **Chungcheongnam-do**, Mt. Gaya, on *Pinus densiflora*, 20 August 2012, Young Woon Lim (KB, SFC 20120820-01).

Remarks — The ranges of basidia and basidiospore sizes of Korean *H. ecrustosum* specimens overlap with those given by Tokuda et al. (2009), but Korean specimens tend to have larger basidia and basidiospores.

We confirm that there are only two *Heterobasidion* species — *H. ecrustosum* and *H. orientale* — in South Korea and compare their characteristics with other northeastern Asian *Heterobasidion* species in Table 2. Because we analyzed

Table 2. Key characteristics of *Heterobasidion* species in northeast Asia.

Species	Basidiocarps	Pores	Basidiospores (µm)	Substrates
H. australe (Dai & Korhonen 2009)	Perennial	Mostly round, occasionally angular, 4–5/mm	4.1–6.2 × 3.3–5.5	*Keteleeria, Picea, Pinus, Pseudolarix, Tsuga*
H. ecrustosum (this study)	Annual	Angular to round, 2–3/mm	4.9–7.7 × 3.7–6.5	*Pinus*
H. linzhiense (Dai et al. 2007)	Annual	Angular, 2–4/mm	5–10 × 4–10	*Abies, Picea*
H. orientale (this study)	Annual	Angular to round or labyrinthiform, 2–4/mm	4.6–6.7 × 3.9–5.8	*Abies, Pinus*
H. parviporum (Tokuda et al. 2009)	Mostly perennial	Round, regular, 4–7/mm	3.3–5.6 × 2.9–4.8	*Abies, Larix, Picea, Pinus, Populus, Tsuga*

only a limited number of specimens, there might be other species present in this region. Thus, further studies are needed to determine the complete diversity of *Heterobasidion* in South Korea.

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