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Re-evaluation of *Hypocrea pseudogelatinosa* and *H. pseudostraminea* isolated from shiitake mushroom (*Lentinula edodes*) cultivation in Korea and Japan

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(Received on May 23, 2012; Revised on July 20, 2012; Accepted on July 23, 2012)

Shiitake (*Lentinula edodes*) is the most economically important cultivated mushroom, but yields are impacted by its competitor, *Trichoderma* spp. We previously found two unidentified *Trichoderma* species growing in bedlogs and sawdust shiitake media in Korea. Here, we identify and re-describe those two species based on molecular sequence data, morphology, and culture characteristics. Well-supported clades based on phylogenetic analyses of internal transcribed spacer, translation elongation factor 1- α , and RNA polymerase subunit II sequences grouped one of the unidentified *Trichoderma* spp. with *Hypocrea pseudogelatinosa* and the other with *Hypocrea pseudostraminea*, and their morphologies matched well with the original descriptions of the two *Hypocrea* species. This study reports the first phylogenetic analyses of *H. pseudogelatinosa* and Japanese strains of *H. pseudostraminea*. Based on the phylogenetic results, we re-described these two species using modern taxonomic concepts in *Hypocrea/Trichoderma*.

Keywords : morphology, phylogeny, *Trichoderma pseudogelatinosum*, *Trichoderma pseudostramineum*

Shiitake (*Lentinula edodes*) is the most economically important edible mushroom and is widely cultivated in China, Japan and Korea (Chen, 2005). The first credible written record of shiitake cultivation can be traced to Wu Sang Kwuang – born during the Sung Dynasty (AD 960–1127) – in China (Wasser, 2005).

Trichoderma (teleomorph: *Hypocrea*) is the most important competitor/pathogen in shiitake cultivation, and they can cause severe damage to production. Recently Kim et al. (2012a) isolated six species of *Trichoderma* from bedlogs and sawdust media of shiitake mushroom in Korea. All six

Trichoderma could potentially cause large economic losses in shiitake crops, as determined by competition tests against *L. edodes* on PDA and sawdust media. Two of those *Trichoderma* species were unidentified.

Here, we clearly identify the two unidentified *Trichoderma* species of Kim et al. (2012a) using modern taxonomic concepts for *Hypocrea/Trichoderma* and combined phylogenetic and phenotypic data, including teleomorph and anamorph morphology, growth rates, colony characteristics, and DNA sequence data (Chaverri and Samuels, 2003; Jaklitsch, 2009; Kim et al. 2012b). We re-describe these species and propose new combinations based on new nomenclatural rules that became part of the International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) in 2011.

Materials and Methods

Strains and specimens. Cultured strains of *Hypocrea/Trichoderma* were obtained from the culture collection and herbarium of the Fungus/Mushroom Resource and Research Center, Tottori University, Japan, and the culture collection of Chungnam National University, Korea (Table 1). The specimens, including holotypes, were loaned by the Tottori Mycological Institute and the National Science Museum, Tokyo, Japan. Fourteen strains of representative cultures of the two species were maintained in potato dextrose agar (Becton, Dickson and Co. Sparks, MD, USA) slants at 15°C.

PCR amplification and sequencing. Fungal strains were grown in potato dextrose broth for 3–4 days at 25°C in a shaking incubator. Mycelia were collected from the cultures by filtration and then transferred to 1.5 mL tubes. DNA was extracted following Cubero et al. (1999).

For the amplification of rRNA internal transcribed spacer (ITS) regions, translation elongation factor 1- α (*tef1*), and

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RNA polymerase subunit II (*rpb2*) genes, three different primer sets were used; ITS5 and ITS4 (White et al., 1990), EF1-728F (Carbone and Kohn 1999) and *tefl*-rev (Samuels et al., 2002), and fRPB2-5F and fRPB2-7cR (Liu et al., 1999), respectively. The PCR mixtures contained 0.5 pmol of each primer, 0.25 mM dNTPs, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 2.5 U of Taq DNA polymerase, and 15 ng of template DNA. The PCR cycling conditions for ITS and *tefl* were as follows: an initial denaturation step at 94°C for 10 min; followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s; with a final elongation step at 72°C for 10 min. For *rpb2* gene amplification, the number of cycles was modified to 40 and the annealing temperature to 50°C. PCR products were purified using an ExoSAP kit (USB, Cleveland, OH, USA). The purified double-stranded PCR fragments were directly sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), following the manufacturer's instructions. The same primer sets used to amplify ITS and *tefl* were employed for sequencing. For *rpb2*, two internal primers, RPB-432F and RPB-450R (Degenkolb et al., 2008), were used for the sequencing reactions. Capillary electrophoresis and data collection were performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Sequence data were submitted to GenBank (Table 1).

Phylogenetic analyses. Raw sequences were proofread, edited, and merged into contigs using the PHYDIT program version 3.2 (Chun, 1995; available at <http://plaza.sun.ac.kr/>

~jchun/phydit). DNA sequences were aligned using Clustal X 1.81 (Thompson et al., 1997), and then visually corrected using PHYDIT. Ambiguously aligned regions were excluded from subsequent analyses.

To determine the phylogenetic positions of these fungi, datasets were analyzed using maximum parsimony (MP) and neighbor-joining (NJ) in PAUP version 4.0b10 (Swofford, 2002) and using Bayesian analyses in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Parsimony analysis was conducted using a heuristic search with 1000 random addition replicates and TBR branch-swapping. Neighbor-joining analysis used the Kimura two-parameter model. Bootstrap support values for nodes were computed from 1000 replicates for both MP (MPBS) and NJ (NJBS) analyses. The best model of nucleotide substitution for the Bayesian approach was determined from among 88 models using the Akaike information criterion in jModeltest (Posada, 2008) with 11 substitution schemes, equal or unequal base frequencies, a proportion of invariant sites, and rate variation among sites. The base tree for likelihood calculations was ML optimized. The general time-reversible model, under the assumption of a discrete gamma-shape rate variation with a proportion of invariable sites (GTR+I+G), was estimated as the best-fit likelihood model for the combined sequences dataset (*tefl* and *rpb2*). Posterior probabilities (PP) were approximated using the metropolis-coupled Markov Chain Monte Carlo method. Two parallel runs were conducted with one cold and three heated chains for 10 million generations, starting with a random tree. The three chains were heated at 0.2 for the combined-sequences

Table 1. Strains and GenBank accession numbers of *Hypocrea pseudogelatinosa*/Trichoderma *pseudogelatinosum* comb. nov. and *H. pseudostraminea*/T. *pseudostramineum* comb. nov. obtained in this study

Species	Strain	Origin	GenBank number		
			ITS	<i>tefl</i>	<i>rpb2</i>
<i>H. pseudogelatinosa</i> /T. <i>pseudogelatinosum</i> comb. nov.	CNU N309	Korea	HM769754	HM920202	HM920173
	CNU N349	Korea	HM769755	HM920203	HM920174
	CNU N417B	Korea	HM769756	HM920204	HM920175
	TUFC 60183	Japan	JQ797388	JQ797396	JQ797404
	TUFC 60186 T	Japan	JQ797389	JQ797397	JQ797405
	TUFC 60223	Japan	JQ797390	JQ797398	JQ797406
	TUFC 60933	Japan	JQ797391	JQ797399	JQ797407
	CNU N109	Korea	HM769757	HM920205	HM920176
<i>H. pseudostraminea</i> /T. <i>pseudostramineum</i> comb. nov.	CNU N334	Korea	HM769758	HM920206	HM920177
	TUFC 60104	Japan	JQ797392	JQ797400	JQ797408
	TUFC 60440	Japan	JQ797393	JQ797401	JQ797409
	TUFC 60753	Japan	JQ797394	JQ797402	JQ797410
	TUFC 60848	Japan	JQ797412	—	—
	TUFC 60854	Japan	JQ797395	JQ797403	JQ797411

dataset (the heats for cold chain 1 and heated chains 2, 3, and 4 were 1.00, 0.83, 0.71, and 0.63, respectively). The trees were sampled every 100th generation. We deemed that the two runs had converged when the average standard deviation of the split frequencies dropped below 0.01. The trees obtained before convergence was reached were discarded with the burn-in command, and the remaining trees were used to calculate a 50% majority consensus topology and PP. PP values below 0.95 were not considered significant while values below 0.9 are not shown on the resulting phylogram.

Phenotypic analyses. Dry stromata briefly rehydrated in 3% KOH and fresh stromata formed on agar were embedded in Tissue-Tek O.C.T. Compound 4583 (Sakura Fine-technical, Tokyo, Japan) and sectioned at a thickness of *ca.* 20 μ m using a freezing microtome (REM-710, Yamato Kohki Industrial, Saitama, Japan). The following teleomorph characteristics were evaluated: diameter, color, and shape of stromata; texture of subcortical and subperithecial tissue; perithecial shape and reaction to 3% KOH; ostiolar canal length; ascus length and width; and lengths and widths of distal and proximal ascospores. Whenever possible, 30 representatives were measured for each character.

The cultures used to study anamorph micromorphology were grown on cornmeal agar + 2% dextrose (CMD; Becton Dickson), synthetic low-nutrient agar (SNA; Nirenberg 1976), or potato dextrose agar (PDA; Becton Dickson). All measurements for the morphological analyses were made in 3% KOH or water. Where possible, 30–50 representatives were measured for each morphological parameter in each collection. Growth rate and optimum temperature were determined based on an examination of cultures on PDA and SNA (Samuels et al., 2002). After several days, when colony growth was visible on PDA and SNA, but before conidial production, a plug (5 mm diameter) was taken from the actively growing edge of the colony and inoculated onto freshly prepared medium. The inoculum plug was placed mycelial side down, approximately 1 cm from the edge of a vented Petri dish (9 cm diameter) containing 20 mL of freshly made medium. Petri dishes were incubated in the dark at 15–35°C (in increments of 5°C), and colony radius was measured after 72 h. Growth tests were conducted three times at roughly weekly intervals, and the average radius was calculated from the three independent measurements.

Results

Phylogenetic analyses. The ITS dataset included 60 taxa and 555 characters, of which 108 were parsimony-informative. The MP tree was 414 steps long with a consistency

index (CI) of 0.6280, a retention index (RI) of 0.8435, and a homoplasy index (HI) of 0.3720. The Bayesian analysis used a GTR+I+G model. The first 25,000 trees were discarded as burn-in. One of 838 most-parsimonious ITS tree is shown in Figure 1. The seven strains of *Hypocrea pseudogelatinosa*/*Trichoderma pseudogelatinosum* (CNU N309, CNU N349, CNU N417B, TUFC 60183, TUFC 60186, TUFC 60223, and TUFC 60933) formed a clade with strong support (MPBS and NJBS > 70%; PP > 0.95). They appeared closely related to *Hypocrea straminea* CBS 114248 (ex-type), with sequence identities of *ca.* 99%. The seven strains of *H. pseudostraminea*/*T. pseudostramineum* (CNU N109, CNU N334, TUFC 60104, TUFC 60440, TUFC 60753, TUFC 60848 and TUFC 60854) also formed a strongly-supported clade (MPBS and NJBS > 70%; PP > 0.95). They were closely related to *H. decipiens*, with sequence identities of *ca.* 97%.

The *tef1* dataset included 68 taxa and 271 characters, of which 127 were parsimony-informative. The MP tree was 1,099 steps long, with a CI of 0.4004, an RI of 0.6491, and an HI of 0.5996. The Bayesian analysis used a TrN+I+G model. The first 40,000 trees were discarded as burn-in. Figure 2 shows one of 21 most-parsimonious *tef1* trees. All seven strains of *H. pseudogelatinosa*/*T. pseudogelatinosum* form a group with strong support (MPBS and NJBS > 70%; PP > 0.95). They were closely related to *H. catoptron* CBS 114232, with sequence identities of *ca.* 95%. The six *H. pseudogelatinosa*/*T. pseudostramineum* strains (strain TUFC 60848 was not sequenced for *tef1*) also formed a strongly-supported clade (MPBS and NJBS > 70%; PP > 0.95) that was closely related to *H. decipiens*, with sequence identities of *ca.* 92–93%.

The *rpb2* dataset included 84 taxa and 316 characters, of which 123 were parsimony-informative. The MP tree was 802 steps long with a CI of 0.3167, an RI of 0.7493, and an HI of 0.6833. The Bayesian analysis used a TPM1uf+I+G model. The first 40,000 trees were discarded as burn-in. One of 126 most-parsimonious *rpb2* trees is depicted in Fig. 3. The seven strains of *H. pseudogelatinosa*/*T. pseudogelatinosum* formed a clade with strong support (MPBS and NJBS > 70%; PP > 0.95). They were closely related to *H. catoptron* CBS 114232, with sequence identities of *ca.* 99%. The six strains of *H. pseudostraminea*/*T. pseudostramineum* (strain TUFC 60848 was not sequenced for *rpb2*) also formed a strongly-supported clade (MPBS and NJBS > 70%; PP > 0.95) that was closely related to *H. decipiens*, with sequence identities of *ca.* 96–97%.

The combined sequences dataset (*tef1* and *rpb2*) included 60 taxa and 590 characters, of which 293 were parsimony-informative. The MP tree was 1780 steps long with a CI of 0.3742, an RI of 0.6537, and an HI of 0.6258. The Bayesian analysis used a GTR+I+G model. The first 45,000 trees

Fig. 1. One of 838 most parsimonious trees from a heuristic analysis of the ITS region. Broad black branches indicate maximum parsimony (MPBS) and neighbor-joining (NJBS) bootstrap support values > 70% and Bayesian posterior probabilities (PP) > 0.95. Broad gray branches indicate MPBS, NJBS > 50% and 0.89 < PP < 0.95. Nodes with asterisks (*) indicate MPBS, NJBS > 50% and PP < 0.90. The strains of *Hypocrea pseudostraminea sensu* Overton et al. (2006a) are named in bold and underlined. *Nectria cinnabarina* CBS 279.48 was used as the outgroup.

were discarded as burn-in. One of 15 most-parsimonious tree of combined sequences dataset is depicted in Figure 4. The *H. pseudogelatinosa/T. pseudogelatinosum* and *H. pseudostraminea/T. pseudostramineum* formed a clade with

strong support (MPBS and NJBS > 70%; PP > 0.95), and they were closely related to *H. catoptron* and *H. decipiens*, respectively. In each of the three gene trees and combined dataset, *H. pseudogelatinosa/T. pseudogelatinosum* belonged

Fig. 2. One of 21 most parsimonious trees from a heuristic analysis of the *tef1* gene. Broad black branches indicate maximum parsimony (MPBS) and neighbor-joining (NJBS) bootstrap support values > 70% and Bayesian posterior probabilities (PP) > 0.95. Broad gray branches indicate MPBS, NJBS > 50% and 0.89 < PP < 0.95. Nodes with asterisks (*) indicate MPBS, NJBS > 50% and PP < 0.90. The strains of *Hypocrea pseudostraminea sensu* Overton et al. (2006a) are named in bold and underlined. *Nectria cinnabarina* HMAS 91782 was used as the outgroup.

to the Harzianum clade and *H. pseudostraminea*/*T. pseudostramineum* to Hypocreanum clade. The section and clades of *Hypocrea*/*Trichoderma* generally corresponded to those reported by Samuels (2006), ISTH (<http://www.isth.info/>

biodiversity/index.php), and Jaklitsch (2009).

Taxonomy. We re-describe the two species *Hypocrea pseudo gelatinosa* and *H. pseudostraminea*. However, accord-

Fig. 3. One of 126 most parsimonious trees from a heuristic analysis of the *rpb2* gene. Broad black branches indicate maximum parsimony (MPBS) and neighbor-joining (NJBS) bootstrap support values > 70% and Bayesian posterior probabilities (PP) > 0.95. Broad gray branches indicate MPBS, NJBS > 50% and 0.89 < PP < 0.95. Nodes with asterisks (*) indicate MPBS, NJBS > 50% and PP < 0.90. The strains of *Hypocrea pseudostraminea sensu* Overton et al. (2006a) are named in bold and underlined. *Nectria cinnabarina* GJS 91-111 was used as the outgroup.

Fig. 4. One of 15 most parsimonious trees from a heuristic analysis of the combined sequences dataset. Broad black branches indicate maximum parsimony (MPBS) and neighbor-joining (NJBS) bootstrap support values > 70% and Bayesian posterior probabilities (PP) > 0.95. Broad gray branches indicate MPBS, NJBS > 50% and 0.89 < PP < 0.95. Nodes with asterisks (*) indicate MPBS, NJBS > 50% and PP < 0.90. The *Hypocrea pseudostraminea* sensu Overton et al. (2006a) is named in bold and underlined. *Nectria cinnabarina* was used as the outgroup.

ing to the new rules of nomenclature that became part of the International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) in 2011, the genus *Hypocrea*/*Trichoderma* will be changed to have one name after 1 Jan. 2013 (Hawksworth, 2012). Because *Trichoderma* Per. (1794) is older than *Hypocrea* Fr. (1825), it probably has

priority and will be the accepted holomorphic name. Therefore, we established new combinations of these species within the genus *Trichoderma*.

Hypocrea pseudogelatinosa M. Komatsu and Yoshim. Doi, *Rep. Tottori Mycol. Inst.* 10: 425. 1973.

Fig. 5. *Hypocrea pseudogelatinosa*. A-F. Dry stromata (A-D, holotype; E-F, TMI 8165). G. Longitudinal section of stroma. H. Subperithecium in section. I. Cortical and subcortical tissue in section. J. Stroma base in section. K-N. Asci with ascospores (M, N, in 3% KOH). Scale bars: B = 1 cm. C, E, F = 1 mm. D = 500 μ m. G = 200 μ m. H-J, M = 20 μ m. K, L = 10 μ m. N = 100 μ m.

Anamorph: *Trichoderma pseudogelatinosum* (M. Komatsu and Yoshim. Doi) C.S. Kim comb. nov. [Harzianum clade] (Figs. 5 and 6)

Stromata 0.8–1.3 mm diam., 0.3–0.8 mm thick (n=10), solitary, scattered, gregarious or aggregated; pulvinate. Out-

line roughened with slight perithecial protuberances. Yellow, yellow-brown to brown. Ostiolar openings obvious due to green ascospores. Cortical layer composed of thick-walled angular cells, not changing color in 3% KOH. Subcortical tissue of *textura angularis*, hyaline, not changing color in

Fig. 6. *Trichoderma pseudogelatinosum* comb. nov. (TUFC 60186; ex-type). A-D, H, K. Conidiophores. F, G. Chlamydospores. I-K. Conidia. L-N. Cultures after 10 d at 25 °C (L, on PDA; M, on SNA; N, on CMD). Scale bars: A-F, H-K = 20 μ m. G = 10 μ m. L-N = 15 mm.

3% KOH. Perithecia immersed in the stroma, generally closely aggregated or slightly separated, subglobose to ellipsoidal, 134.7–168.6 $\times$ 105.3–143.0 μ m (n=15), wall composed of compacted cells, not changing color in 3% KOH. Subperithecial tissue of *textura angularis* to *textura epidermoidea*, hyaline, not changing color in 3% KOH.

Asci cylindrical, 73–81 $\times$ 4.6–5.6 μ m (n=30). Part-ascospores pale green to green, warted, dimorphic, sometimes upper-part spores of asci bigger than lower-part spores, distal part subglobose or sometimes obovate, 4.1–5.4 $\times$ 3.7–4.4 μ m, L/W 1.1–1.3 (n=30), proximal part obovate to oblong, 4.6–5.8 $\times$ 3.3–4.2 μ m, L/W 1.2–1.6 (n=30).

Colonies on CMD at 25°C after *ca.* 10 d flat, with discrete small pustules *ca.* 0.3–0.8 mm diam. (n=10) forming concentric rings, mycelium loose; no distinctive odor; agar not pigmented. Conidiophores gliocladium- to verticillium-like; phialides slender or flask-shaped, sometimes hooked, 6.7–10.1 × 2.2–2.7 µm, L/W 2.7–4.3 (n=30). Conidia green, smooth, mostly subglobose to ellipsoidal, rarely oblong, 2.8–6.2 × 2.3–3.3 µm, L/W 1.2–1.9 (n=30). Chlamydospores observed, globose to subglobose, terminal or intercalary in hyphae, 6.7–8.3 × 5.5–7.0, L/W 1.0–1.4 (n=30).

Colonies on PDA at 25°C after *ca.* 10 d flat, with abundant conidia in aggregated concentric rings; conidia formed after *ca.* 7 day or less; no distinctive odor; agar not pigmented; mycelium dense. Colonies on SNA at 25°C after *ca.* 10 d flat, conidia produced generally in pustules, forming concentric rings, mycelium loose; no distinctive odor; agar not pigmented; mycelium loose.

Colony radius on PDA after 72 h at 15°C 10.3–17.1 mm, at 20°C 21.7–29.7 mm, at 25°C 20.8–32.8 mm, at 30°C 0–0.8 mm, and at 35°C 0 mm (n=7). Colony radius on SNA 72 h at 15°C 14.2–16.8 mm, at 20°C 18.7–27.8 mm, at 25°C 21.2–28.5 mm, and at 30 and 35°C 0 mm (n=7).

Habitat: On bedlogs (*Quercus* sp.) and sawdust media of shiitake mushroom

Known distribution: Korea and Japan

Holotype: Japan, Tottori Pref., 13 Oct. 1969. M. Komatsu (specimen: Tottori 209=D-1963=TNS-F-192712; ex-type culture: TMIC 60186=UFC 60186)

Other specimens and/or cultures examined: Korea, Gyeonggi Pref. 2003, 2004 (cultures: CNU N309, CNU 417B). Jeonbuk Pref. 2004 (culture: CNU N349). Japan, Hiroshima Pref. 1 Apr. 1983 (culture: TMIC 60933=UFC 60933). Tottori Pref. (specimen: TMI 8165, 2 Jul. 1975) (cultures: TMIC 60183=UFC 60183, 2 Jul. 1969; TMIC 60223=UFC 60223, 27 Jul. 1974)

Notes: Phylogenetically, this species is related to *Hypocrea straminea*/*Trichoderma stramineum* in the ITS tree and to *H. catoptron*/*T. catoptron* in the *tef1* and *rpb2* trees. Morphologically, this species is similar to *H. cinnamomea*/*T. cinnamomeum*. However, this species can be distinguished from *H. straminea*/*T. stramineum*, *H. catoptron*/*T. catoptron* and *H. cinnamomea*/*T. cinnamomeum* by the size of the phialides and conidia and the growth rates, especially at 30°C on PDA and SNA media (Table 2).

Hypocrea pseudostraminea Yoshim. Doi, *Bull. Natl. Sci. Mus.* 15: 676. 1972.

Anamorph: *Trichoderma pseudostramineum* (Yoshim. Doi) C.S. Kim comb. nov. [Hypocreanum clade] (Figs. 7 and 8)

Stromata *ca.* 0.4–14 mm diam., *ca.* 0.1–0.4 mm thick (n=10), solitary or largest continuous stroma broadly attach-

ed with irregular margins, thin, pale yellow, brownish orange to light brown. Cortical layer composed of thick-walled angular cells, reddish-brown in 3% KOH. Subcortical tissue of *textura angularis*, hyaline, not changing color in 3% KOH. Perithecia immersed in the stroma, generally closely aggregated or slightly separated, subglobose to ellipsoidal, 197–237 × 139–177 µm (n=30), wall composed of compacted cells, reddish-brown in 3% KOH, ostiolar canal 28–40 µm long (n=21), 24–31 µm diam. (n=21), subperithecial tissue of *textura globulosa* to *textural anularis*, hyaline, not changing color in 3% KOH. Asci cylindrical, 62–70 × 3.5–4.1 µm (n=30). Part-ascospores hyaline, minutely warted, dimorphic, distal part subglobose 3.3–3.8 × 2.8–3.3 µm, L/W 1.1–1.3 (n=30), proximal part obovate to ellipsoidal 3.7–4.4 × 2.7–3.3 µm, L/W 1.2–1.5 (n=30).

Colonies on CMD at 25°C after *ca.* 10 d flat with a thin white layer of mycelium, not forming concentric rings; no distinctive odor; agar not pigmented. Conidiophores visible near the edge of the plate; conidiophores acremonium- to verticillium-like; phialides solitary, slender, subulate, 10.8–26.7 × 1.9–2.5 µm, L/W 4.3–13.5 (n=30). Conidia hyaline, smooth, subglobose to ellipsoidal, rarely oblong, 3.7–6.2 × 2.1–3.3 µm, L/W 1.4–2.3 (n=30). Chlamydospores observed, subglobose to ellipsoidal, 4.8–6.7 × 3.8–5.1 µm, L/W 1.1–1.5 (n=30). Some strains do not form conidiophores or conidia on media.

Colonies on PDA at 25°C after *ca.* 10 d flat, with a dense white or slightly yellowish layer of mycelia close to the agar surface and a layer of cotton-like aerial mycelia, not forming concentric rings; no distinctive odor; reverse plate weakly yellowish brown to pink colored. Colonies on SNA at 25°C after *ca.* 10 d flat, thin layer of mycelia close to the agar surface, forming cotton-like aerial mycelia especially near the edge of the plate, not forming concentric rings; no distinctive odor; agar not pigmented.

Colony radius on PDA after 72 h at 15°C 17.3–21.9 mm, at 20°C 38.9–50.8 mm, at 25°C 57.5–67.2 mm, at 30°C 54.0–66.3 mm, and at 35°C 0 mm (n=5). Colony radius on SNA 72 h at 15°C 4.9–15.7 mm, at 20°C 15.9–34.2 mm, at 25°C 25.9–51.9 mm, at 30°C 29.4–58.2 mm, and at 35°C 0 mm (n=5).

Habitat: On bedlogs (*Quercus* sp.) of shiitake mushroom and on bark

Known distribution: Korea and Japan

Holotype: Japan, Kochi Pref., Tosayama-cho, Mt. Kuishi, 15 Oct. 1967. Y. Doi (specimen: D-366=TNS-F-223432)

Paratype: Japan, Tottori Pref., Kokoge, 3 Oct. 1969. Y. Doi (specimen: D-1085 = TNS-F-190524), Hokkaido Pref., Hidaka, Mt. Poroshiri-Dake, 25 Dec. 1971. (specimen: D-1124=TNS-F-190525)

Other specimens and/or cultures examined: Korea, Jeonbuk Pref., Jangsu-gun, 2002 (culture: CNU N109). Gyeonggi

Table 2. Comparison of *Hypocrea pseudogelatinosa*/Trichoderma *pseudogelatinosum* comb. nov. to related *Hypocrea*/Trichoderma species

Character	Species			
	<i>H. pseudogelatinosa</i> /T. <i>pseudogelatinosum</i> comb. nov.	<i>H. straminea</i> /T. <i>stramineum</i> *	<i>H. catoptron</i> /T. <i>catoptron</i> *	<i>H. cinnamomea</i> /T. <i>cinnamomeum</i> *
Origin	Korea and Japan	Sri Lanka	Sri Lanka	Taiwan and U.S.A.
Conidiophore type	verticillium- to gliocladium-like	pachybasium- and verticillium-like	pachybasium- and verticillium-like	pachybasium-like
Phialides:				
shape	ampulliform or lageniform	in whorls of 1–3, short, ampulliform	ampulliform	ampulliform
size (µm)	6.7–10.1 × 2.2–2.7	2.7–5.0 × 3.0–5.0	5.5–7.2 × 3.2–4.2	9.0–10.3 × 3.7–4.0
L/W	2.7–4.3	1.5–4.6	1.4–2.2	2.4–2.8
Conidia:				
shape	ellipsoidal to rarely oblong	broad ellipsoidal, sometimes oblong	ellipsoidal to oblong	ellipsoidal, rarely oblong
size (µm)	2.8–6.2 × 2.3–3.3	3.0–3.2 × 2.0–2.2	3.5–4.0 × 2.3–2.7	4.2–4.5 × 3.3–3.5
L/W	1.2–1.9	1.4–1.5	1.4–1.6	1.2–1.3
Chlamydospores:				
formation	+	–	–	–
size (µm)	6.7–8.3 × 5.5–7.0	–	–	–
Colony radius (mm) on PDA after 72 h:				
15 °C	10.3–17.1	13–18	3–9	0–2
20 °C	21.7–29.7	37–41	17–29	1–5
25 °C	20.8–32.8	55–67	36–49	2–6
30 °C	0–0.8	66–75	22–27	3–8
35 °C	0	2–6	0	0–1
Colony radius (mm) on SNA after 72 h:				
15 °C	14.9–16.8	10–15	2–6	0–3
20 °C	18.7–27.8	25–31	15–20	1–5
25 °C	21.2–28.5	42–48	22–26	1–9
30 °C	0	52–56	12–18	3–11
35 °C	0	2–5	0	0–2
Stromata color	yellow, yellow-brown to brown	pale yellow to grayish yellow	yellow	brown to light brown
Subperithecial tissue	<i>t. angularis</i>	<i>t. angularis</i>	<i>t. angularis</i> to <i>t. epidermoidea</i>	<i>t. angularis</i> to <i>t. epidermoidea</i>
Asci (µm)	73–81 × 4.6–5.6	82–93 × 4.7–5.3	91–109 × 5.0–5.5	83–87 × 4.2–4.7
Part ascospores				
distal (µm)	4.1–5.4 × 3.7–4.4	4.5–5.0 × 3.7–4.0	4.8–5.3 × 4.4–4.8	4.0–4.5 × 4.0–4.2
proximal (µm)	4.6–5.8 × 3.3–4.2	4.5–5.2 × 3.2–3.5	5.2–5.5 × 4.0–4.3	4.0–4.5 × 3.5–3.8

*data from Chaverri & Samuels (2003)

Pref., Yeosu-gun, 2003 (culture: CNU N334). Japan, Miyazaki Pref., Ayaminami National Forest, 20 Oct. 1966. Y. Doi (specimen: D-242=TNS-F-190520). Shimane Pref., Oda-shi, Kurada-cho, Tsurumura, 7 Jul. 1968. Taniguchi (specimen: TMI 8154; culture: TMIC 60440=TUFC 60440). Tochigi Pref., Otawara-shi, 19 Aug. 1977. (culture: TMIC

60753=TUFC 60753). Tottori Pref., Iwami-gun, Kokufu-cho, Aug. 1980 (culture: TMIC 60854=TUFC 60854), Kokoge, 20 Oct. 1979. M. Komatsu (specimen: TMI 8160; culture: TMIC 60841=TUFC 60841), 19 Aug. 1980. M. Komatsu (specimen: TMI 8161; culture: TMIC 60848=TUFC 60848), Tottori-shi, Tsunoi, Sugizaki, 10 Jul. 1987.

Fig. 7. *Hypocrea pseudostraminea*. A-D. Dry stromata (A-B, holotype; C, paratype; D-E, TMI 8154). F-G. Perithecium in section (F, in water; G, in 3% KOH). H. Cortical and subcortical tissue in section. I. Ostiole in section. J-K. Subperithecial tissue in section. L. Stroma base in section. M-N. Asci with ascospores. Scale bars: A, B, D = 1 mm. C = 2 cm. E = 500 μ m. F, G = 100 μ m. H-N = 20 μ m.

M. Komatsu (specimen: TMI 8495; culture: TMIC 61407= TUFC 61407), Tottori, 1965 (culture: TMIC 60104= TUFC 60104)

Notes: *Hypocrea pseudostraminea*/*Trichoderma pseudostramineum* is almost indistinguishable from *H. pseudostraminea sensu* Overton et al. (2006a) and *H. decipiens* in teleomorphic characteristics. However, the length of phialides and conidia of this species are slightly different from those of *H. pseudostraminea sensu* Overton et al. (2006a) and *H. decipiens* in anamorphic characteristics (Table 3). In

addition, they could be distinguished by their ITS, *tef1* and *rpb2* sequences.

Discussion

Chaverri and Samuels (2003) suggested that *Hypocrea pseudogelatinosa*/*Trichoderma pseudogelatinosum* may be a synonym of *H. cinnamomea*/*T. cinnamomeum* based on the morphological description of Doi (1973), despite differences in the size of the conidia. We found that *H.*

Fig. 8. *Trichoderma pseudostramineum* comb. nov. (TUFC 60440). A-D, I-K. Conidiophores. E, F. Chlamydospores. G, H. Conidia. L-N. Cultures after 10 d at 25 °C (L, on PDA; M, on SNA; N, on CMD). O. Reverse of PDA after 10 d at 25 °C. Scale bars: A = 50 µm. B-G, I-K = 20 µm. H = 10 µm. L-O = 15 mm.

pseudogelatinosa/T. *pseudogelatinosum* produced abundant chlamydospores, but *H. cinnamomea*/T. *cinnamomeum* did not. These species, including the ex-type TUFC 60186 (specimen: Tottori 209=D-1963=TNS-F-192712), were clearly distinguished for the first time in our molecularly phylogenetic analyses on the basis of three genes. *Hypocrea pseudogelatinosa*/T. *pseudogelatinosum* was related to *H. straminea*/T. *stramineum* in the ITS tree and to *H. catoptron*/T. *catoptron* in the *tef1* and *rpb2* trees. These phylo-

genetic placements suggest that this species is different from both *H. straminea*/T. *stramineum* and *H. catoptron*/T. *catoptron*, although they are teleomorphically indistinguishable due to their similar morphological characteristics. However, *H. pseudogelatinosa*/T. *pseudogelatinosum* was easily distinguished from other *Hypocrea*/*Trichoderma* species by the size of phialides and conidia and also in growth rates, especially at 30 °C on PDA and SNA media in the anamorphic state.

Table 3. Comparison of *Hypocrea pseudostraminea*/Trichoderma *pseudostramineum* comb. nov. to related *Hypocrea* species

Character	Species		
	<i>H. pseudostraminea</i> /T. <i>pseudostramineum</i> comb. nov.	<i>H. pseudostraminea</i> <i>sensu</i> Overton et al. (2006a)	<i>H. decipiens</i> *
Origin	Korea and Japan	U.S.A.	France and U.S.A.
Conidiophore type	verticillium- to acremonium-like	irregularly verticillate	verticillium- to acremonium-like
Phialides:			
shape	solitary or subulate, variable in length	solitary and alternating, in whorls of 3–5, subulate	in whorls of 3, subulate
size (µm)	10.8–26.7 × 1.9–2.5	14–21 × 2.3–3.2	13–25 × 2.4–3.0
L/W	4.3–13.5	ND	ND
Conidia:			
shape	cylindrical to obovate-ellipsoid	subellipsoidal to elongate ellipsoidal	subglobose or obovate to subellipsoidal
size (µm)	2.8–6.2 × 2.3–3.3	2.5–3.5 × 1.5–2.2	4.4–6.7 × 2.5–3.5
L/W	1.2–1.9	1.3–1.8	ND
Chlamydospores:			
formation	+	+	ND
size (µm)	4.8–6.7 × 3.8–5.1	ND	ND
Colony radius (mm) on PDA after 72 h:			
15 °C	17.3–21.9	ND	ND
20 °C	38.9–50.8	ND	ND
25 °C	57.5–67.2	ND	ND
30 °C	54.0–66.3	ND	ND
35 °C	0	ND	ND
Colony radius (mm) on SNA after 72 h:			
15 °C	4.9–15.7	ND	ND
20 °C	15.9–34.2	ND	ND
25 °C	25.9–51.9	ND	ND
30 °C	29.4–58.2	ND	ND
35 °C	0	ND	ND
Asci (µm)	62–70 × 3.5–4.1	58–79 × 4.1–5.7	65–73 × 3.5–4.5
Part ascospores			
distal (µm)	3.3–3.8 × 2.8–3.3	3.0–4.0 × 2.8–3.6	3.3–3.7 × 3.0–3.2
proximal (µm)	3.7–4.4 × 2.7–3.3	3.5–4.5 × 2.6–3.3	3.5–4.5 × 2.3–2.7

*data from Overton et al. (2006 a, b) and Jaklitsch (2011); ND, not described

Hypocrea pseudostraminea was originally described from Japan by Doi (1972). Recently Overton et al. (2006a) re-described the species based on teleomorph morphology and phylogenetic analyses, but they did not sequence DNA from any Japanese strains. Jaklitsch (2011) suggested that *H. pseudostraminea sensu* Overton et al. (2006a) was misidentified based on Jaklitsch's re-examination of European specimens, including specimens used by Overton et al. (2006a). According to Jaklitsch (2011), European specimens of *H. pseudostraminea sensu* Overton et al. (2006a) produced green conidia, but in the original description of *H.*

pseudostraminea, it produced hyaline conidia.

We re-examined specimens of this species, including the holotype and cultures of Japanese *Hypocrea pseudostraminea*. Our specimens showed nearly identical morphological characters to *H. pseudostraminea sensu* Overton et al. (2006a). Because morphology alone was insufficient to identify this fungus, we also conducted detailed phylogenetic analyses. Ex-type cultures do not exist, and we failed to directly amplify ITS, *tefl*, and *rpb2* from the holotype specimen. However, we could sequence these genes from the Japanese strains. In all three of our phylogenetic

gene trees, our strains were clearly distinguished from *H. pseudostraminea* sensu Overton et al. (2006a). Based on these results, we concluded that our strains were true *H. pseudostraminea*, but *H. pseudostraminea* sensu Overton et al. (2006a) may be a new species. *H. pseudostraminea*/T. *pseudostramineum* is closely related to *H. decipiens* based not only on morphological characters, but also on phylogeny and habitat. These two species were suspected to be synonyms, however, they were clearly distinguished in our phylogenetic analyses and slightly different in anamorphic characteristics. Therefore, *H. pseudostraminea*/T. *pseudostramineum* and *H. decipiens* should be considered to be different species.

Acknowledgments

We are grateful to Dr. Tsuyoshi Hosoya, fungi curator the mycological herbarium of the National Museum of Nature and Science, Tsukuba, Japan (TNS), for providing the holotype and other specimens. This work was supported by a grant from the Regional Subgenebank Support Program of the Rural Development Administration, Republic of Korea, and by the Global Center of Excellence (COE) Program, “Advanced Utilization of Fungus/Mushroom Resources for Sustainable Society in Harmony with Nature”, from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

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