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

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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*

*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
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 tef1-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 tef1 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 tef1 were employed for sequencing. For rpb2, two internal primers, RBP-432F and RBP-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 invariant sites (GTR+I+G), was estimated as the best-fit likelihood model for the combined sequences dataset (tef1 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 | ITS GenBank number | tef1 GenBank number | rpb2 GenBank number |
|---------|--------|--------|--------------------|---------------------|--------------------|
| *H. pseudogelatinosa* / *T. pseudogelatinosum* comb. nov. | CNU N309 | Korea | HM769754 | HM920202 | HM920173 |
| | CNU N349 | Korea | HM679755 | 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 |
| *H. pseudostraminea* / *T. pseudostramineum* comb. nov. | CNU N109 | Korea | HM769757 | HM920205 | HM920176 |
| | 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 |
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 trees 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. pseudogelatinosina/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. pseudogelatinosina/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. pseudogelatinosina/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
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. pseudostaminea*/*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

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**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 pseudostaminea sensu* Overton et al. (2006a) are named in bold and underlined. *Nectria cinnabarina* CBS 279.48 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).

**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.

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

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**Biology**

- **68 taxa**
- **271 characters**
- **127 parsimony-informative**
- **21 trees**
- **1099 steps**
- **CI = 0.4004**
- **RI = 0.6491**
- **HI = 0.5996**

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**Clade**

- **tef1**
- **true H. pseudostraminea/T. pseudostramineum comb. nov.**
- **H. pseudo gelatinosa/T. pseudo gelatinosum comb. nov.**

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**H. pseudogelatinosa**

- **H. pseudogelatinosa**
- **H. pseudogelatinosa GUS 91-135 USA (DQ835443)**
- **H. pseudogelatinosa BEG 99-36 USA (DQ835447)**
- **H. pseudogelatinosa**
- **H. citrina**
- **H. citrina GUS 89-65 T (DQ835444)**
- **H. citrina**
- **H. citrina GUS 99-130 (EU328333)**
- **H. sulphurea**
- **H. sulphurea GUS 99-190 (DQ835448)**
- **H. pulvinata**
- **H. pulvinata GUS 94-20 (DQ835433)**
- **H. protopulvinata**
- **H. protopulvinata CBS 739.63 T (DQ835427)**
- **H. americana**
- **H. americana GUS 92-93 (DQ835434)**
- **H. microscirtina**
- **H. microscirtina GUS 97-248 (DQ835449)**
- **H. decipiens**
- **H. decipiens GUS 97-207 (EF550995)**
- **H. decipiens**
- **H. decipiens GUS 91-101 (FJ660635)**
- **T. brevicompactum**
- **T. brevicompactum CBS 109720 T (EU338299)**
- **H. lutea**
- **H. lutea GUS 86-129 (AY737731)**
- **H. melanomagnata**
- **H. melanomagnata GUS 99-153 (AY737751)**
- **T. asperellum**
- **T. asperellum GUS 90-7 (EU338333)**
- **H. neohemibroccoli**
- **H. neohemibroccoli CBS 119120 (EU866321)**
- **T. haromatum**
- **T. haromatum DAOM 167057 (AY750893)**
- **T. lieckfeldiae**
- **T. lieckfeldiae CBS 123050 T (EU865325)**
- **H. stibbyhoojisi**
- **H. stibbyhoojisi CBS 112888 (AY376062)**
- **H. rufa**
- **H. rufa CBS 101526 (AY378053)**
- **H. korvigi**
- **H. korvigi CBS 988.97 (DQ289007)**
- **T. kongiognopus**
- **T. kongiognopus GUS 93-20 T (DQ289496)**
- **T. atroviride**
- **T. atroviride CBS 142.95 (AF456991)**
- **T. viridescens**
- **T. viridescens CBS 333.72 (DQ975223)**
- **H. rodmani**
- **H. rodmani GUS 89-120 (EU338285)**
- **T. aggressivus**
- **T. aggressivus DAOM 100525 (AF348095)**
- **H. cinnamomea**
- **H. cinnamomea GUS 97-237 (AY737732)**
- **H. straynia**
- **H. straynia GUS 114248 (AY737746)**
- **T. pleurotus**
- **T. pleurotus CBS 124387 T (HM142362)**
- **T. pleurotociola**
- **T. pleurotociola CBS 124393 T (HM142381)**
- **T. harizianum**
- **T. harizianum CBS 226.95 T (AF348101)**
- **H. lixi**
- **H. lixi IMI 38996 (AF443933)**
- **H. cataptrion**
- **H. cataptrion CBS 114232 (AY737726)**
- **CNU N309 KOR**
- **TUF C 60933 JPN**
- **TUF C 60233 JPN**
- **TUF C 60183 JPN**
- **TUF C 60186 Holotype JPN**
- **CNU N4170 KOR**
- **CNU N349 KOR**
- **H. sulawesiensis**
- **H. sulawesiensis GUS 85-228 (AY737730)**
- **H. irlandica**
- **H. irlandica DAOM 114234 (AY737748)**
- **H. virescensflava**
- **H. virescensflava PC 278 (AY737749)**
- **H. nigrovires**
- **H. nigrovires DAOM 114330 (AY737744)**
- **T. flavofuscum**
- **T. flavofuscum DAOM 167652 (AY750891)**
- **T. vires DAOM 01-207 (AY750894)**
- **H. pachybasiosides**
- **H. pachybasiosides CBS 820.60 (AY605810)**
- **T. oblongisporum**
- **T. oblongisporum DAOM 167085 (AY750884)**
- **T. fertile**
- **T. fertile DAOM 167161 T (AY750881)**
- **H. semiorbis**
- **H. semiorbis DAOM 167636 (AY737750)**
- **T. spirale**
- **T. spirale DAOM 183714 (AY750890)**
- **H. gelatinosa**
- **H. gelatinosa CPK 1618 (FJ179669)**
- **H. chlorospora**
- **H. chlorospora GJS 98-1 (AY737737)**
- **H. candida**
- **H. candida DAOM 114249 (AY737742)**
- **H. aureovires**
- **H. aureovires CBS 245.63 T (AY865322)**
- **H. pseudokorvigi**
- **H. pseudokorvigi GJS 81-300 (AY937429)**
- **H. schwetsinitzii**
- **H. schwetsinitzii CBS 258.85 T (AY865637)**
- **T. longibrachiatum**
- **T. longibrachiatum DAOM 166989 (EU338335)**
- **T. saturnispore**
- **T. saturnispore IMI 146852 (AY865542)
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.

H. pseudogelatinosa
/T. pseudogelatinosum comb. nov.

true H. pseudostraminea
/T. pseudostramineum comb. nov.
Re-evaluation of *Hypocrea pseudogelatinosa* and *H. pseudostraminea* from shiitake mushroom

According 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. 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.
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. Outline 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

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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 µm. G = 200 µm. H-I, M = 20 µm. K, L = 10 µm. N = 100 µm.
3% KOH. Perithecia immersed in the stroma, generally closely aggregated or slightly separated, subglobose to ellipsoidal, 134.7–168.6 × 105.3–143.0 μm (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 × 4.6–5.6 μm (n=30). Part-ascospores pale green to green, warty, dimorphic, sometimes upper-part spores of asci bigger than lower-part spores, distal part subglobose or sometimes obovate, 4.1–5.4 × 3.7–4.4 μm, L/W 1.1–1.3 (n=30), proximal part obovate to oblong, 4.6–5.8 × 3.3–4.2 μm, L/W 1.2–1.6 (n=30).

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 μm. G = 10 μm. L-N = 15 mm.
Colony radius on CMD 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=TNF-F-192712; ex-type culture: TMIC 60186=TUFC 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=TUFC 60933). Tottori Pref. (specimen: TMI 8165, 2 Jul. 1975) (cultures: TMIC 60183=TUFC 60183, 2 Jul. 1969; TMIC 60223=TUFC 60223, 27 Jul. 1974)

Notes: Phylogenetically, this species is related to Hypocreanum 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).

Hypocreanum straminea 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 attached 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 textula angularis, 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).

Colony 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 acermonium- 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.

Colony 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.) and sawdust media 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=TNF-F-223432)

Paratype: Japan, Tottori Pref., Kokoge, 3 Oct. 1969. Y. Doi (specimen: D-1085 = TNS-F-190524), Hokkaido Pref., Hakodate, 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 | Hypocrea pseudogelatinosa / Trichoderma pseudogelatinosum comb. nov. | Hypocrea straminea / Trichoderma stramineum* | Hypocrea catoptron / Trichoderma catoptron* | Hypocrea cinnamomea / Trichoderma cinnamomeum* |
|-----------|--------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|
| 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 | t. angularis | t. angularis | t. angularis to t. epidermoidea | t. angularis to t. epidermoidea |
| 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 |

*a data from Chaverri & Samuels (2003)*
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, ref1 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.*
pseudogelatinosa/T. pseudogelatinosum produced abundant chlamydospores, but \textit{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. \textit{Hypocrea pseudogelatinosa}/T. pseudogelatinosum was related to \textit{H. straminea}/T. stramineum in the ITS tree and to \textit{H. catoptron}/T. catoptron in the \textit{tef1} and \textit{rpb2} trees. These phylogenetic placements suggest that this species is different from both \textit{H. straminea}/T. stramineum and \textit{H. catoptron}/T. catoptron, although they are teleomorphically indistinguishable due to their similar morphological characteristics. However, \textit{H. pseudogelatinosa}/T. pseudogelatinosum was easily distinguished from other \textit{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.
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 mis-identified 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, tef1, and rpb2 from the holotype specimen. However, we could sequence these genes from the Japanese strains. In all three of our phylogenetic

| Character | H. pseudostraminea / T. pseudostramineum comb. nov. | H. pseudostraminea sensu Overton et al. (2006a) | H. decipiens* |
|-----------|-----------------------------------------------|-----------------------------------------------|----------------|
| 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             |

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

Table 3. Comparison of Hypocrea pseudostraminea/Trichoderma pseudostramineum comb. nov. to related Hypocrea species
gene trees, our strains were clearly distinguish-
ed from \textit{H. pseudostraminea sensu} Overton et al. (2006a). Based on
these results, we concluded that our strains were true \textit{H. pseudostraminea}, but \textit{H. pseudostraminea sensu} Overton et al.
(2006a) may be a new species. \textit{H. pseudostraminea/T. pseudostramineum} is closely related to \textit{H. decipiens} based
not only on morphological characters, but also on phylo-
genom 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, \textit{H. pseudostraminea/T. pseudo-
stramineum} and \textit{H. decipiens} should be considered to be
different species.

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