Hypocrea phyllostachydis and its *Trichoderma* anamorph, a new bambusicolous species from France

Priscila CHAVERRI[1,*], Françoise CANDOUSSAU[2], and Gary J. SAMUELS[3]

Hypocrea phyllostachydis was collected from the bamboo species *Phyllostachys bambusoides* in southwestern France (Dept. Pyréneés Atlantiques). It can be distinguished from other morphologically similar species by the small subglobose or broadly ellipsoidal conidia and small ascospores. Conidiophores of the *Trichoderma* state of *H. phyllostachydis* do not branch in a pyramidal fashion, as is typical of most species of *Trichoderma*. Rather, it has an irregular branching pattern, with a long central axis and relatively short lateral secondary branches. A key to species of *Hypocrea* with green ascospores from France is presented.

Taxonomy novelty: *Hypocrea phyllostachydis* Chaverri & Candoussau

Hypocrea Fr. and its anamorph *Trichoderma* Pers. (*Hypocreaceae, Hypocreales, Ascomycota*) are fungi commonly encountered in the environment. They can be found in soil, on decaying wood and other fungi, but rarely on leaves or monocotyledonous substrata. The teleomorph is typically a cushion-shaped fleshy stroma, brightly or lightly colored, sometimes almost black, that is no more than 5 mm in diameter, although stromata of some species may be several centimeters in extent and may even be club-shaped. Perithecia are completely immersed in the stroma. The asci are cylindrical and the ascospores are uniseptate, disarticulating early in their development to yield 16 part-ascospores that are hyaline or green, generally warted or spinulose. Anamorphs of *Hypocrea* have a highly variable morphology, but in general they are species of *Trichoderma*. They have hyaline phialides formed on exposed fertile conidiophores. The conidia are generally smooth, rarely ornamented, typically ellipsoidal to nearly oblong, rarely globose, mostly green or hyaline, rarely yellow. If chlamydospores are formed, they are typically globose to subglobose and formed within or at the tips of hyphae.

[1] The Pennsylvania State University, Department of Plant Pathology, Buckhout Laboratory, University Park, Pennsylvania 16802, USA. Current address: United States Department of Agriculture, Agricultural Research Service, Systematic Botany and Mycology Laboratory, Rm. 304, B-011A, 10300 Baltimore Ave, Beltsville, Maryland 20705, USA.

[2] 53, rue Aristide Briand, F–64000 Pau, France.

[3] United States Department of Agriculture, Agricultural Research Service, Systematic Botany and Mycology Laboratory, Rm. 304, B-011A, 10300 Baltimore Ave, Beltsville, Maryland 20705, USA.

* Corresponding author: Fax: +1 (301) 504-5810; e-mail: priscila@nt.ars-grin.gov

The present paper deals with a species of *Hypocrea* with green ascospores and brownish stromata that was found on decaying culms of the bamboo *Phyllostachys bambusoides* Siebold & Zucc. (*Poaceae*, *Gramineae*) in southwestern France (Dept. Pyrénées Atlantiques). Most species of *Hypocrea*/*Trichoderma* occur on decaying woody substrata; species on monocotyledonous substrata are rare. Among the species of *Hypocrea* described from grasses and monocotyledonous plants are *H. bambusella* Höhnel, *H. muroiana* Hino & Katumoto, *H. placentula* W.B. Grove, *H. pilulifera* J. Webster & Rifai, *H. spinulosa* Fuckel, *H. tuberculata* Pat., *H. saccharalis* Racib., and *H. virescentiflava* Speg. Of these species, only *H. spinulosa*, which is found on grasses in alpine and boreal regions, and *H. virescentiflava* have green ascospores. The yellowish tuberculate stromata of *H. spinulosa* readily distinguish it from the new species on *Phyllostachys*. *Hypocrea virescentiflava* was described from Brazil and has a pale yellow stroma. *Hypocrea bambusella*, *H. muroiana, H. placentula, H. pilulifera, H. tuberculata*, and *H. saccharalis* have hyaline ascospores.

Most recent studies of *Hypocrea*/*Trichoderma* include phenotypic and DNA-based characters to distinguish species. The use of molecular tools have aided in the confirmation of taxonomic proposals based on morphology, such as some Bissett's *Trichoderma* sections (BISSETT 1984, 1991a, b, c, 1992) or the uncovering of new cryptic species (CHAVERRI et al. 2003, DODD et al. 2002, KUHLS et al. 1997, LIECKFELDT et al. 1998, 1999, 2001, SAMUELS et al. 1998, 2002). Comparison of the morphology of the *Hypocrea* on *Phyllostachys* with other species found on monocotyledonous substrata and phylogenetically closely related species indicates that it is undescribed. On the basis of phenotypic characters, including

teleomorph, anamorph and growth rates, and molecular phylogenetic data, the new species of *Hypocrea* on *Phyllostachys* is described in this paper as *Hypocrea phyllostachydis*. A key to species of *Hypocrea* with green ascospores from France is presented and a larger work that includes all species of *Hypocrea* with green ascospores is in preparation.

Material and methods

Isolates and specimens

The isolates of the new species, *H. phyllostachydis*, used in this study are G.J.S. 92-123 (ex holotype specimen BPI 802617) and G.J.S. 92-81 (ex specimen BPI 802861). Both isolates have been deposited in the American Type Culture Collection (ATCC), Eastern Cereals and Oilseeds Research Centre, Ottawa, Canada (DAOM), and Centraalbureau voor Schimmelcultures (CBS). Single-ascospore isolations from fresh collections of *Hypocrea* were made on CMD (Difco cornmeal agar + 2 % dextrose + distilled water + 1 % antibiotic solution (0.2 % Sigma Streptomycin Sulfate + 0.2 % Sigma Neomycin Sulfate + distilled water) with the aid of a micromanipulator. These cultures are also maintained at BPI in CMA (Difco cornmeal agar) slant tubes at 4 °C and in liquid nitrogen in vials with 10 % glycerol.

Growth and colony characterization

Growth trials were performed to determine the growth rate and optimum temperature for growth following the protocol of SAMUELS et al. (2002) on PDA (Difco potato-dextrose agar) and synthetic low-nutrient agar (SNA, NIRENBERG 1976). Colony radius was measured at 24, 48, and 72 h at 15, 20, 25, 30, and 35 °C. Each growth-rate experiment was repeated three times and the results averaged for each isolate. The time of first appearance of green conidia, the presence of yellow pigmentation of young conidia, the presence of diffusing pigment in the agar, odor and colony appearance were also noted.

Morphological observations

Morphological observations of the anamorph were taken from cultures grown on CMD in 9-cm-diam vented plastic Petri plates in an incubator at 20 °C, with 12 h fluorescent light and 12 h darkness within approximately one wk. The following characters were measured: width of phialide base, phialide width at the widest point, phialide length, and length/width ratio (L/W), conidium length, width and length/width ratio (L/W), width of cell from which phialides arise (= metulae = subtending hypha), presence of chlamydospores, and chlamydospore width. Measurements of continuous characters were taken from images using the beta 4.0.2 version of Scion Image (Scion Corporation, Frederick, Maryland). Colony appearance was described from CMD at 20 °C and PDA at 25 °C, including formation and shape of tufts or pustules. Color terminology was obtained from KORNERUP & WANSCHER (1978).

Herbarium specimens of the *Hypocrea* were rehydrated briefly in 3% KOH. Rehydrated stromata were supported by Tissue-Tek O.C.T. Compound 4583 (Miles Inc., Elkhart, Indiana) and sectioned at a thickness of ca 15 µm with a freezing microtome. The following teleomorph characters were evaluated: diameter, height, color and shape of the stroma; texture of surface of the stroma; perithecium shape, length and width; reaction to 3% KOH, color and width of perithecium wall; length of the ostiolar canal; color reaction of the outer region of the stroma to 3% KOH; shape, length and wall thickness of cells of the outer, middle (immediately below the outer region) and inner region (below perithecia) of the stroma; ascus length and width; distal and proximal part-ascospore length and width. Measurements of continuous characters were also gathered using the image-capturing software Scion Image beta 4.0.2. Confidence intervals (a=0.05), minimum and maximum values for the anamorph and teleomorph morphological characters measured were calculated using Systat 8.0 (SPSS, Inc., Illinois).

Molecular phylogenetic analysis

This study used sequences produced in CHAVERRI et al. (2003), including G.J.S. 92-123, in addition to another isolate of the new species, G.J.S. 92-81, to show the phylogenetic relationship to other species of *Hypocrea/Trichoderma*. All other sequences were obtained from GenBank. The techniques for isolating DNA, primers, PCR and sequencing are described in CHAVERRI et al. (2003). The extraction of genomic DNA was done using Puregene™ Genomic DNA Isolation Kit (Gentra Systems, Minneapolis, Minnesota). Two partial coding gene regions were amplified and sequenced, viz. RNA Polymerase II Subunit (RPB2) and Translation Elongation Factor 1-alpha (EF- 1a). The primers used for PCR and sequencing were for RPB2: fRPB2-5F (5'- GA(T/C)GA(T/C)(A/C)G (A/T)GATCA(T/C)TT(T/C)GG-3') and fRPB2-7cR (5'-CCCAT(A/G)GCTTG(T/C)TT(A/G)CCCAT-3') (LIU et al. 1999); and for EF- 1a: EF1-983F (5'-GC(C/T)CC(C/T)GG (A/C/T)CA(C/T)GGTGA(C/T)TT(C/T)AT-3') (CARBONE & KOHN 1999) and EF1-2218R (5'-ATGAC(A/G)TG(A/G)GC (A/G)AC(A/G)GT(C/T)TG-3') (S. A. Rehner, pers. comm.). The purification of PCR products was done using QIAquick® PCR Purification Kit (Qiagen, Inc., Valencia, California) and QIAquick® Gel Extraction Kit, when more than one band was amplified. The sequencing was done by the DNA Sequencing Facility (Center for Agricultural Biotechonology, University of Maryland, College Park, Maryland) using Perkin-Elmer big dye terminators with dITP (Applied Biosystems) and an Applied Biosystems DNA sequencer model 3100. Sequences were edited and assembled using Sequencher 4.1 (Gene Codes, Wisconsin). Clustal X 1.81 (THOMPSON et al. 1997) was used to align the sequences, and then the alignment was refined by hand. The sequences were deposited in GenBank as AY39198, AY391927, for isolate G.J.S. 92-81. Additional *Hypocrea/Trichoderma* reference isolates, viz. *H.* cf. *chlorospora* G.J.S. 95-203, *T. polysporum* G.J.S. 90-4, and *T. minustisporum*

CBS 901.72, were sequenced; their sequences were deposited in GenBank under accession numbers: AY391993, 391936; 392010, 481589; and 392209, 48158, respectively.

Phylogenetic analyses were performed using PAUP* 4.0 b8 (SWOFFORD 1999) using *Hypomyces stephanomatis* C.T. Rogerson & Samuels and *Nectria cinnabarinna* (Tode : Fr.) Fr. sequences as outgroup (GenBank accession numbers AF534633, AF545567, AF534632, AF545566). Neighbor-Joining analyses (NJ) were performed using the Kimura-2-parameter model. Bootstrap values were calculated from 1000 replicates. Maximum parsimony (MP) was also performed using a heuristic search, with a starting tree obtained via step-wise addition, with 1000 random addition sequences, tree-bi-section-reconnection as the branch-swapping algorithm, and MULTREES off. Bootstrap values from 1000 replicates were calculated using a fast step-wise addition search. A consensus tree was calculated using 50 % majority rule. Because the purpose of this paper is to describe a new species and show its relationship to other species of *Hypocrea/Trichoderma*, only a tree with the combined RPB2 and EF-1α data is presented.

Results

A total of 1061 bp were analyzed, 698 bp of EF-1α and 903 bp of RPB2. Separate phylogenetic analyses of both genes (RPB2 and EF- 1α) were done and yielded similar topologies. Therefore, for the purposes of this paper, only a combined analysis will be shown. Parsimony analyses of the combined EF-1α and RPB2 produced three equally most parsimonious trees, with 2494 steps, 1013 constant characters, 147 parsimony-uninformative and 441 parsimony informative characters. The consistency index (CI) is 0.35, the retention index (RI) is 0.54, and the homoplasy index (HI) is 0.65. The NJ analysis gave better bootstrap values for internal branches, compared to MP, which had lower bootstrap values. NJ and MP analyses of RPB2 and EF-1α show that the relationship of *H. phyllostachydis* to other species of *Hypocrea/Trichoderma* is not supported by bootstrap values (Fig. 1). *Hypocrea phyllostachydis* is in a clade (Clade B in CHAVERRI et al. 2003 and KINDERMANN et al. 1998) that includes other species with pachybasium-like anamorphs and the majority of the species of *Hypocrea* with green ascospores. Clade B is supported by 96% bootstrap in the NJ tree but is not supported by bootstrap in the MP tree; however it is present in the 50% majority rule consensus. The analyses of multiple phenotypic characters, including anamorph, teleomorph and growth data, and DNA sequence data of two genes demonstrate that *H. phyllostachydis* is distinct from other described species.

Discussion

CHAVERRI et al. (2003) presented a phylogeny of several species of *Hypocrea/Trichoderma* with conidiophore elongations and green conidia, based on RPB2 and EF-1α genealogies. One of the reference sequences used in that study was a *H. phyllostachydis* isolate G.J.S. 92-123, identified as *H.* cf. *dichromospora* Doi (see Table 1 and Figures 2–7 in CHAVERRI et al. 2003). *Hypocrea dichromospora* was described from Japan as having patellate, pale yellow-brown stromata, green ascospores, and a pachybasium-like anamorph (DOI 1968). Although specimens and cultures of *H. dichromospora* are not available for comparison, the description of the species given by DOI (1968) suggests a similarity between *H. dichromospora* and the species on *Phyllostachys*. The main differences between them are the color of the stroma and size of conidia and ascospores. CHAVERRI et al. (2003) obtained weak evidence of a close phylogenetic relationship between isolate G.J.S. 92-123 and *T. spirale* Bissett; however, these species are morphologically distinct.

The combination of multiple morphological and molecular characters is useful in the recognition of species within *Hypocrea/Trichoderma*. The examination of *Hypocrea/Trichoderma* specimens and the analyses of phenotype and genotype show that even though *H. phyllostachydis* is similar to *H. lixii/T. harzianum, T. aggressivum*, and *H. dichromospora*, it is nonetheless distinct. The morphology of the stroma of *H. phyllostachydis* resembles that of *Hypocrea lixii* Pat./*T. harzianum* Rifai and of the undescribed *Hypocrea* teleomorph of *T. aggressivum* Samuels & W. Gams. The teleomorphs of *H. lixii/T. harzianum*, the teleomorph of *T. aggressivum*, and the new species of *Hypocrea* differ in ascospore size and appearance of the stroma. The teleomorphs of *H. lixii/T. harzianum* and *T. aggressivum* have larger ascospores, darker and duller stromata than those of *H. phyllostachydis*. The anamorph of the new *Hypocrea* species is also similar to *T. aggressivum* and *H. lixii/T. harzianum*. The anamorphs of *H. lixii/T. harzianum* and *T. aggressivum* are almost indistinguishable phenotypically and are phylogenetically closely related (SAMUELS et al. 2002). *Hypocrea lixii/T. harzianum* and *T. aggressivum* have conidiophores that branch in a pyramidal pattern, with a relatively short main axis and crowded, often paired lateral branches. The phialides are typically in whorls of 2–4 and held at right angles with respect to the hyphae from which they arise (metulae, subtending hyphae). The conidia of these two species are subglobose to ovoidal, smooth, green, 2.7–3.5 x 2.5–3.2 µm, and with a length/width ratio of 1.0–1.5. In addition, at 25 °C after 3 d on PDA the colony radius is approximately 50–60 mm. *Hypocrea phyllostachydis* has a more irregular branching pattern, with a long central axis with relatively short lateral branches, and slower growth rates than are found in *T. harzianum* and *T. aggressivum*. The teleomorph and anamorph of *H. lixii/T. harzianum* were described and re-described in CHAVERRI & SAMUELS (2002) and SAMUELS et al. (2002), respectively. *Trichoderma aggressivum* is described in SAMUELS et al. (2002), however its undescribed teleomorph was found on decaying wood and other fungi only recently. The morphological description of *H. phyllostachydis* is detailed in the following section.

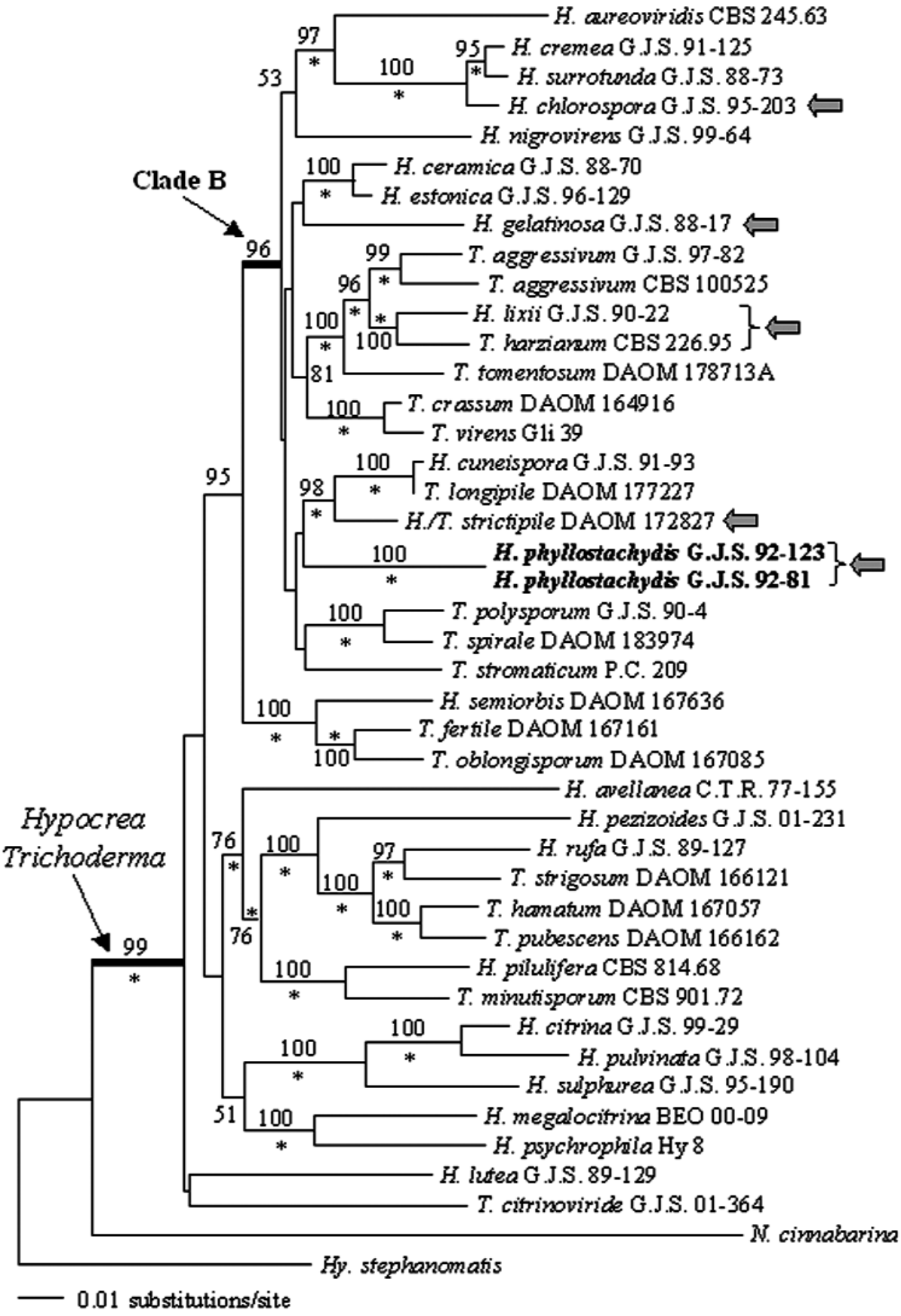
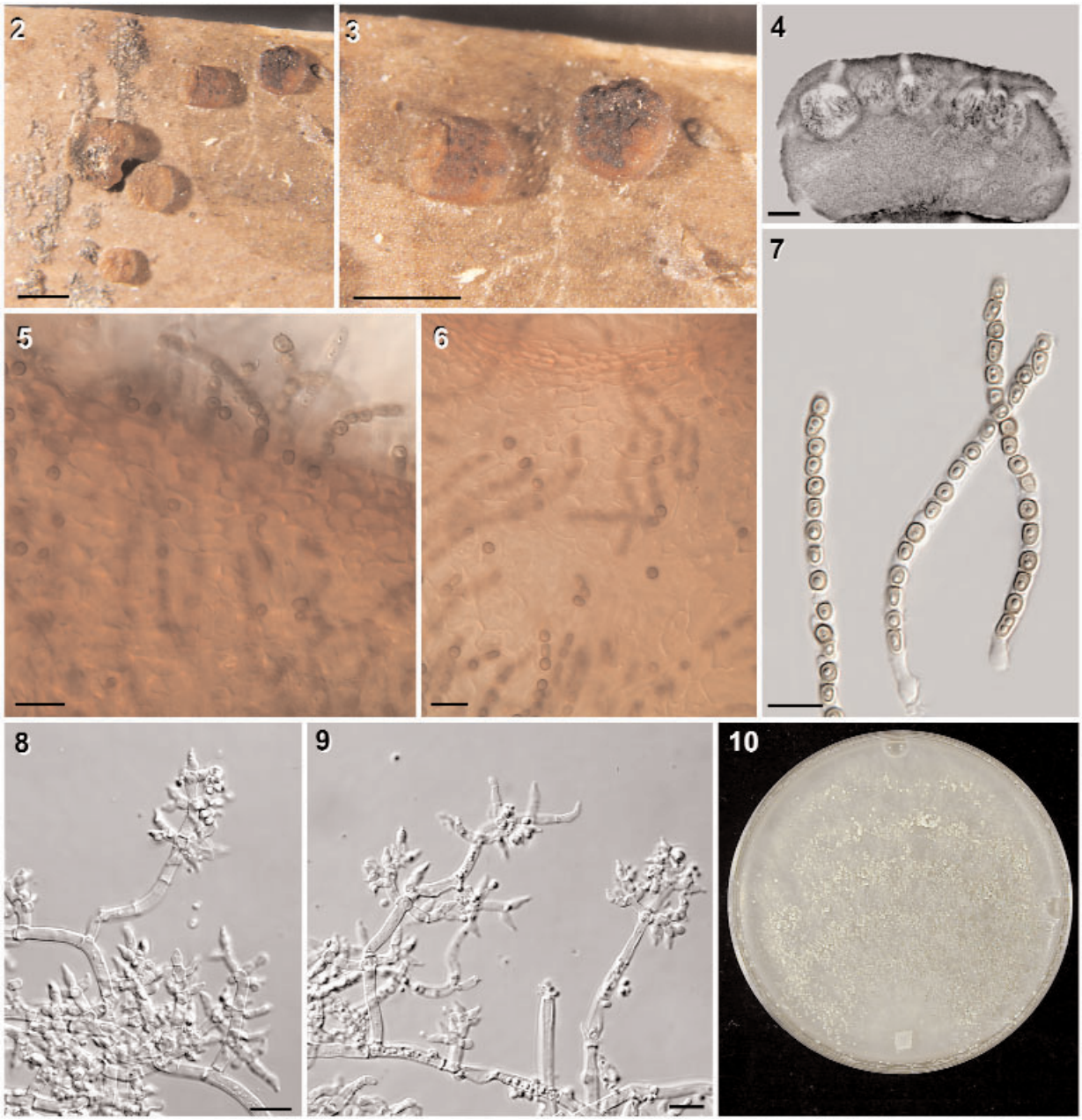


Fig. 1: Neighbor-Joining tree of the combined EF-1α and RPB2 sequence data. Asterisks (*) represent nodes that were also present in the Maximum Parsimony analysis and supported by bootstrap values > 50 %. All clades were present in the 50 % majority rule consensus tree. Bootstrap values are from 1000 replicates. The gray block arrows indicate species of *Hypocrea/Trichoderma* with green ascospores that have been found in France. The new species is indicated in bold



Figs. 2-10: *Hypocrea phyllostachydis* teleomorph and anamorph. **2, 3**. Stromata. **4**. Longitudinal section of stroma. **5**. Tissue of surface of stroma. **6**. Tissue of stroma below perithecia. **7**. Asci and ascospores. **8, 9**. Conidiophores on CMD at 20 °C. **10**. Colony on PDA at 25 °C after 2 wk. Figs. 2-4, 8-10. Holotype, BPI 802617 (isolate G.J.S. 92-123). Figs. 5-7. BPI 802861 (isolate G.J.S. 98-21). Bars: 2, 3 = 1 mm; 4 = 100 µm; 5-9 = 10 µm

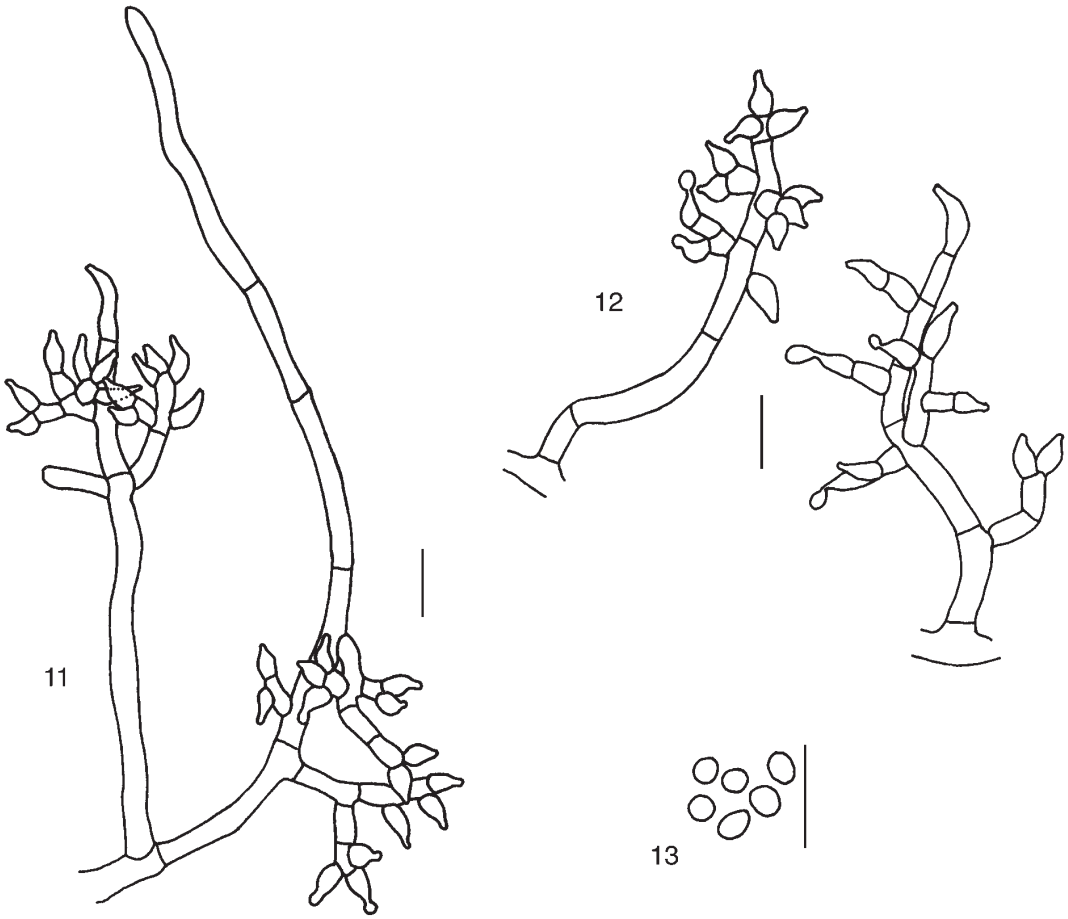
Taxonomy

Hypocrea phyllostachydis Chaverri et Candoussau, sp. nov. Figs. 2-13

Stromata rufobrunnea, (0.5) 0.9–1.0 (1.5) mm. Ascosporae bicellulares, verruculosae, ad septum disarticulatae, atrovirentes, parte distali globosa vel subglobosa, (3.0) 3.5–4.0 (4.5) x (3.0) 3.5–4.0 (4.5) µm, parte proximali oblonga vel cuneiformi, (3.0) 4.0 (5.0) x (2.5) 3.0–3.5 (4.0) µm. Anamorphe *Trichoderma* sp. Phialide (4.5) 6.5–7.0 (12.0) x (2.5) 3.0–3.5 (4.0) µm, longitudo/crassitudo (1.5) 2.0–2.2 (3.4). Conidia subglobosa vel ellipsoidea, viridia, glabra, (2.3) 3.0 (3.5) x (2.0) 2.3–2.5 (3.0) µm, longitudo/crassitudo (0.9) 1.2–1.3 (1.8). Incrementum radii in agaro dicto 'PDA' post 72 h 15 °C = 10–16 mm, 20 °C = 22–25 mm, 25 °C = 28–34 mm, 30 °C = 12–21 mm, 35 °C = 0–1 mm. Holotypus: BPI 802617.

Anamorph: *Trichoderma* sp.

Stromata scattered, solitary, pulvinate, circular to irregular in outline, (0.5) 0.9–1.0 (1.5) mm diam (n = 27), 0.5–0.7 (0.8) mm high (n = 15), broadly attached, surface smooth, somewhat gelatinous, waxy and translucent, with small perithecial pro-



Figs. 11-13: *Hypocrea phyllostachydis* anamorph. **11, 12**. Conidiophores. **13**. Conidia. Fig. 11. Isolate G.J.S. 92-81. Figs. 12, 13. Isolate G.J.S. 92-123 (holotype). Bars = 10 µm

tuberances, pale to dark reddish brown, becoming darker in KOH, ostiolar openings obvious due to the green ascospores. Outermost layer of stroma composed of slightly thick-walled angular cells, (4.0) 7.0–7.5 (11.5) µm diam (n = 60), walls (0.5) 1.0–1.5 (2.0) µm thick (n = 50). Tissue between perithecia and below the outermost layer composed of hyaline cells, becoming light reddish brown in KOH, of *textura angularis*, cells (3.5) 6.0–6.5 (10.0) µm diam (n = 65), walls (0.3) 0.5–0.7 (1.0) µm thick (n = 55). Internal tissue below the perithecia of *textura angularis*, hyaline, becoming light reddish brown in KOH, (4.5) 10.5–12.5 (23.5) µm diam (n = 62), walls (0.4) 0.5–0.6 (0.9) µm thick (n = 56). Perithecia completely immersed in stroma, generally closely aggregated or slightly separated, subglobose, 101–121 (127) x (60) 61–84 (99) µm (n = 10), wall composed of compacted cells, slightly changing color in KOH, (8.5) 13.5–16.5 (21.0) µm thick (n = 20), ostiolar canal (25) 28–37 (40) µm long (n = 10). Asci cylindrical, uniseriate, (57.0) 69–73.0 (90.0) x (3.0) 4.0–4.5 (6.0) µm (n = 50). Part-ascospores green, warted, dimorphic, distal part globose to subglobose (3.0) 3.5–4.0 (4.5) x (3.0) 3.5–4.0 (4.5) µm, proximal part generally oblong to wedge-shaped, (3.0) 4.0 (5.0) x (2.5) 3.0–3.5 (4.0) µm (n = 60).

Colonies on CMD at 20 °C after ca one wk, flat, with few discrete, pulvinate compact tufts 1–2 mm diam forming at the edges or the sides of the plate, conidia produced after ca 2 wk, conidial masses grayish-green; no distinctive odor; no pigmentation of the agar. Branching pattern of the conidiophore irregular, with short secondary branches, generally not paired, and with phialides arising from subtending hyphae, rarely solitary; intercalary phialides not observed; subtending hyphae (metulae) cylindrical; sterile elongations of the conidiophore rarely present. Phialides short, flask-shaped, formed in whorls of 2–4, (4.5) 6.5–7.0 (12.0) µm long, (2.5) 3.0–3.5 (4.0) µm at the widest point, (1.7) 2.3–2.5 (3.3) µm at the base, L/W (1.5) 2.0–2.2 (3.4) (n = 60). Conidia green, smooth, subglobose to broadly ellipsoidal, (2.3) 3.0 (3.5) x (2.0) 2.3–2.5 (3.0) µm, L/W (0.9) 1.2–1.3 (1.8) (n = 65). No chlamydospores observed.

Colonies on PDA at 25 °C after ca one wk with abundant aerial mycelium, with loose to compact tufts scattered throughout the plate, but typically more abundant towards the edge of the plate; conidia not formed before ca two wk, conidial masses grayish-green; no pigmentation of agar; no distinctive odor. Colony radius on PDA after 3 d at 15 °C: 10–16 mm, 20 °C: 22–25 mm, 25 °C: 28–34 mm, 30 °C: 12–21 mm, and

Key to species of *Hypocrea* with green ascospores occurring in France

1 Stromata light-colored, from pale yellow to pale brown; on wood or other fungi **2**
- **2** Stromata bright yellow, later orange to pale brown, gelatinous; anamorph gliocladium-like with conidia held in drops of liquid; known from Europe; on wood . . . . . *H. gelatinosa* (Tode: Fr.) Fr.
- **2** Stromata pale yellow to darker yellow; gelatinous or not; anamorph typical *Trichoderma* **3**
  - **3** Stromata pale yellow, somewhat waxy or transparent; part-ascospores monomorphic globose to subglobose; conidiophores frequently and irregularly branched and forming a reticulum; conidia 4.5–4.8 x 4.0 µm, L/W 1.2; on decorticated wood; cosmopolitan . . . . . *H. chlorospora* Berk. & Curtis
  - **3** Stromata pale yellow to grayish yellow, opaque; part-ascospores dimorphic, distal part globose to subglobose, proximal part wedge-shaped; conidiophores straight and branching uniformly, branches often paired, typically with sterile or fertile elongations; conidia 4.5–4.7 x 3.5–3.7 µm, L/W 1.3; on wood and other fungi; known from North America and Europe (anamorph *T. strictipile* Bissett) . . . . . *H. strictipilosa* Chaverri & Samuels

1 Stromata dark-colored, dark brown to nearly black; on wood, bamboo or other fungi **4**
- **4** Stromata very dark brown or green, nearly black; conidiophores branching in a regular pyramidal fashion; conidia globose to subglobose, 3.0–3.2 x 2.7–3.0 µm, L/W 1.0–1.2; colony radius on PDA after 3 d at 25 °C 50–60 mm; on wood and other fungi; cosmopolitan (anamorph *T. harzianum* Rifai) . . . . . *H. lixii* Pat.
- **4** Stromata pale to dark reddish brown; anamorph with conidiophores branching irregularly; conidia subglobose to broadly ellipsoidal, ca 3.0 x 2.3–2.5 µm, L/W 1.2–1.3; colony radius on PDA after 3 d at 25 °C 28–34 mm; on culms of the bamboo species *Phyllostachys bambusoides*; known from southwestern France . . . . . *H. phyllostachydis*

35 °C: 0–1 mm (n = 6). Colony radius on SNA after 3 d at 15 °C: 4–13 mm, 20 °C: 15–21 mm, 25 °C: 34–37 mm, 30 °C: 12–23 mm, and 35 °C: 0 mm (n = 6).
Habitat: Decaying culms of *Phyllostachys bambusoides*.
Known distribution: France.

Material examined: FRANCE. PYRÉNÉES ATLANTIQUES: Parc du Château D'Uhart-Mixe, 20 km S of Sauveterre, vicinity of Spanish frontier; on *Phyllostachys bambusoides*; 26 Sep 1992; F. Candoussau (FC 250) & J.P. Chaumeton (BPI 802617 holotype; culture: G.J.S. 92-123 = ATCC MYA-3066 = CBS 814071 = DAOM 232101). – Osserain, Sauveterre, Bambous d'Osserain; on *Phyllostachys bambusoides*; 23 Aug 1992; J.P. Chaumeton (FC 249) (BPI 802861; culture: G.J.S. 92-81).

Notes: This species is recognized by the waxy, somewhat transparent stroma that becomes darker and more reddish in KOH, and relatively small ascospores. The anamorph is distinguished from other similar anamorphs (*e.g., T. harzianum* and *T. aggressivum*) by the irregular conidiophore branching, small subglobose conidia, and slower growth on PDA and SNA. In addition, few species with green ascospores grow on monocotyledonous hosts, such as *Phyllostachys*.

Acknowledgments

This study was supported in part by the NSF-PEET grant 9712308 to the Pennsylvania State University, Dept. of Plant Pathology "Monographic Studies of Hypocrealean Fungi: *Hypocrea* and *Hypomyces*". We thank Dr. Amy Y. Rossman for her comments on the paper.

References

Bissett J (1984) A revision of the genus *Trichoderma*. I. Section *Longibrachiatum* sect. nov. – Canadian Journal of Botany **62**: 924-931.

Bissett J (1991a) A revision of the genus *Trichoderma*. II. Infrageneric classification. – Canadian Journal of Botany **69**: 2357-2372.

Bissett J (1991b) A revision of the genus *Trichoderma*. III. Section *Pachybasium*. – Canadian Journal of Botany **69**: 2373-2417.

Bissett J (1991c) A revision of the genus *Trichoderma*. IV. Additional notes on section *Longibrachiatum*. – Canadian Journal of Botany **69**: 2418-2420.

Bissett J (1992) *Trichoderma atroviride*. – Canadian Journal of Botany **70**: 639-641.

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. – Mycologia **91**: 553-556.

Chaverri P, Castlebury LA, Overton BE, Samuels GJ (2003) *Hypocrea/Trichoderma*: species with conidiophore elongations and green conidia. – Mycologia **95**: in press.

Chaverri P, Samuels GJ (2002) *Hypocrea lixii* Pat., the teleomorph of *Trichoderma harzianum* Rifai. Mycological Progress **1**: 283-286.

Dodd SL, Lieckfeldt E, Chaverri P, Overton BE, Samuels GJ (2002) Taxonomy and phylogenetic relationships of two species of *Hypocrea* with *Trichoderma* anamorphs. – Mycological Progress **1**: 409-428.

Doi Y (1968) Revision of the Hypocreales with cultural observations. II. *Hypocrea dichromospora*, sp. nov. and its *Trichoderma* state. – Bulletin of the National Science Museum **11**: 185-189.

Kindermann J, El-Ayouti Y, Samuels GJ, Kubicek CP (1998) Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA cluster. – Fungal Genetics and Biology **24**: 298-309.

KORNERUP A, WANSCHER JH (1978) Methuen handbook of colour. Methuen London Ltd., London.

KUHLS K, LIECKFELDT E, SAMUELS GJ, BÖRNER T, MEYER W, KUBICEK CP (1997) Revision of *Trichoderma* sect. *Longibrachiatum* including related teleomorphs based on analysis of ribosomal DNA internal transcribed spacer sequences. – Mycologia **89**: 442-460.

LIECKFELDT E, KULLNIG C, KUBICEK CP, SAMUELS GJ, BÖRNER T (2001) *Trichoderma aureoviride*: phylogenetic position and characterization. – Mycological Research **105**: 313-322.

LIECKFELDT E, SAMUELS GJ, BÖRNER T, GAMS W (1998) *Trichoderma koningii*: Neotypification and *Hypocrea* teleomorph. – Canadian Journal of Botany **76**: 1507-1522.

LIECKFELDT E, SAMUELS GJ, NIRENBERG HI, PETRINI O (1999) A morphological and molecular perspective of *Trichoderma viride*: Is it one or two species? – Applied environmental microbiology **65**: 2418-2428.

LIU YJ, WHELEN S, HALL BD (1999) Phylogenetic relationships among Ascomycetes: Evidence from an RNA Polymerase II Subunit. – Molecular Biology and Evolution **16**: 1799-1808.

NIRENBERG H (1976) Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. – Mitt Biol Bundesanstalt für Land-Forstw Berlin-Dahlem **169**: 1-117.

SAMUELS GJ, DODD SL, GAMS W, CASTLEBURY LA, PETRINI O (2002) *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. – Mycologia **94**: 146-168.

SAMUELS GJ, PETRINI O, KUHLS K, LIECKFELDT E, KUBICEK CP (1998) The *Hypocrea schweinitzii* complex and *Trichoderma* sect. *Longibrachiatum*. – Studies in Mycology **41**: 1-54.

SWOFFORD D (1999) PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, Massachusetts.

THOMPSON JD, GIBSON TJ, PLEWNIAK F, JEANMOUGIN F, HIGGINS DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. – Nucleic Acids Research **24**: 4876-4882.

Accepted: 17.10.2003