

## *Hypocrea flaviconidia*, a new species from Costa Rica with yellow conidia

Irina S. Druzhinina<sup>1</sup>, Priscila Chaverri<sup>2,4</sup>, Payam Fallah<sup>2,5</sup>, Christian P. Kubicek<sup>1</sup> and Gary J. Samuels<sup>3\*</sup>

<sup>1</sup>Research Area Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, TU Wien, Getreidemarkt 9-1665, A-1060 Wien, Austria; <sup>2</sup>Department of Plant Pathology, The Pennsylvania State University, 301 Buckhout Laboratory, University Park, PA 16802, U.S.A.; <sup>3</sup>United States Department of Agriculture-ARS, Systematic Botany and Mycology Laboratory, Rm. 304, B-011A, Beltsville, MD 20705 U.S.A.; <sup>4</sup>Current address: Department of Plant Pathology, Cornell University, 334 Plant Science Building, Ithaca, New York 14853, U.S.A.; <sup>5</sup>Current address: Environmental Microbiology Laboratory, Inc., 10636 Scripps Summit Court Suite 103, San Diego, California 92131, U.S.A.

\*Correspondence: Gary J. Samuels, gary@nt.ars-grin.gov

**Abstract:** The new species *Hypocrea flaviconidia* and its *Pachybasium*-like *Trichoderma* anamorph are described based on collections from the Southern region of Costa Rica. This species is distinguished by its yellow conidia, a character that is rare and unusual in *Hypocrea/Trichoderma*. The phylogenetic relationship of *H. flaviconidia* to other species of *Hypocrea/Trichoderma* is explored based on ITS1 and 2 regions of rDNA, *tef1* and *ech42* gene genealogies. Phylogenetic analyses show that *H. flaviconidia* belongs in *Trichoderma* sect. *Trichoderma*, where it forms a sister group to a clade that includes *T. hamatum* and *T. pubescens*. A key to species of *Hypocrea* with known anamorphs with yellow conidia is presented.

**Taxonomic novelty:** *Hypocrea flaviconidia* Chaverri, Druzhinina & Samuels sp. nov.

**Key words:** *Hypocreales*, *Hypocreaceae*, *Trichoderma*, systematics, trees.

### INTRODUCTION

Three specimens of a species of *Hypocrea* that produced an anamorph with yellow conidia were collected by Chaverri and Samuels in the southern region of Costa Rica (Puntarenas Prov.). This specimen was encountered during a National Biodiversity Institute (INBio) inventory of the fungi of Costa Rica. Only two species of *Trichoderma* are described as having yellow conidia, viz. *T. flavofuscum* (J. Miller *et al.*) Bissett and *T. croceum* Bissett. *Trichoderma flavofuscum* is a synonym of *T. virens* (J. Miller *et al.*) Arx, the anamorph of *H. virens* Chaverri *et al.* (Chaverri & Samuels 2003), and *T. croceum* is a synonym of *T. polysporum* (Link : Fr.) Rifai, the anamorph of *H. pachybasioides* Yoshim. Doi (Lu *et al.* 2004). These species are not closely related to each other nor to the new species. In the present paper we describe a new species of *Hypocrea* and its *Trichoderma* anamorph, show its phylogenetic relationships to other species of *Hypocrea/Trichoderma* based on multiple gene genealogies and provide a key to species of *Hypocrea/Trichoderma* with yellow conidia.

### MATERIALS AND METHODS

#### Isolates

Single ascospores were isolated from fresh collections of *Hypocrea* with the use of a micromanipulator and

placed on cornmeal-dextrose agar (CMD), consisting of Difco cornmeal agar (Difco Laboratories, Detroit, MI, U.S.A.), 2 % dextrose; 1 % antibiotic solution (0.2 % Sigma [Sigma-Aldrich Corp., St. Louis, MO, U.S.A.] Streptomycin Sulfate + 0.2 % Sigma Neomycin Sulfate + distilled water) was added after autoclaving. The cultures obtained are maintained at the U.S. National Fungus Collection (BPI) on Difco cornmeal agar (CMA) slants at 8 °C and in liquid nitrogen in cryovials with 10 % glycerol. Representative isolates have been deposited Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS). Herbarium specimens have been deposited in BPI and in the Department of Botany Herbarium of INBio, the National Biodiversity Institute of Costa Rica (INB).

#### Morphological characterization

Morphological observations of the anamorph were based on cultures grown on 15–20 mL CMD in vented plastic, 9 cm diam Petri dishes in an incubator at 20–21 °C, with 12 h cool white fluorescent light and 12 h darkness. Morphological characters of the teleomorph and anamorph are as described in Chaverri & Samuels (2004). Observations of the anamorph within approximately 1 wk or when the first mature conidia were formed. The presence of chlamydo-spores was recorded by examining the reverse of a colony grown on CMD for *ca.* 1 wk with the 40 × objective of a compound microscope.

**Table 1.** Sequenced strains of *Hypocrea* (H) and *Trichoderma* (T) species, their provenance and GenBank numbers of their DNA sequences

Species	Strain	GenBank no. ITS1 & ITS2			Provenance
			<i>tef1</i>	<i>ech42</i>	
<i>H. flaviconidia</i>	G.J.S. 99-49	AY665696/AY665700	AY665710	AY665691	Costa Rica
<i>H. flaviconidia</i>	G.J.S. 99-57	AY665697/AY665701	AY665711	AY665692	Costa Rica
<i>T. asperellum</i>	CBS 433.97	AJ230668	AF456907	AY665688	U.S.A., Maryland
<i>T. atroviride</i>	DAOM 165779	Z48817	AF348113	AF276650	U.S.A., North Carolina
<i>T. hamatum</i>	DAOM 167057	Z48816	AY665702	AY665690	Canada, Ontario
<i>T. koningii</i>	CBS 979.70	Z79628	AY665703	AF188918	Germany
<i>T. pubescens</i>	CBS 345.93	AF011979/AF398496	AY665704	AY665712	U.S.A., North Carolina
<i>T. strigosum</i>	CBS 348.93	AF011982/AF398497	AY665705	-	U.S.A., North Carolina
<i>T. viride</i> B	W.J. 2450*	AY665593	AY665595	AY665591	Sweden
<i>T. viride</i> D	C.P.K. 998	AY665698	AY665706	AY665693	Russia, South Taiga
<i>T. viride</i> D	C.P.K. 999	AY665699	AY665707	AY665694	Russia, South Taiga
<i>T. viride</i> D	CBS 111094	AJ507084	AY665708	AY665689	Austria
<i>T. viride</i> D	CBS 111096	AJ507138	AY665709	AY665695	Austria
<i>T. viride</i> D	W.J. 2374*	AY665592	AY665594	AY665590	Austria

\*Isolated from the teleomorph by Walter Jaklitsch.

Measurements of continuous characters were made using the beta 4.0.2 version of Scion Image software (Scion Corporation, Frederick, MD, U.S.A.). Confidence intervals ( $\alpha = 0.05$ ), minimum and maximum values for the anamorph and teleomorph morphological characters measured were calculated using Systat 8.0 (SPSS, Inc., Chicago, IL, U.S.A.). Colony appearance was described from CMD at 20 °C and potato-dextrose-agar (PDA, Difco) at 25 °C, and included observations on the formation, distribution and shape of tufts or pustules. Colour terminology is from Kornerup & Wanscher (1978).

#### DNA extraction, PCR amplification and sequencing

Mycelia were harvested after 2–4 d growth on MEA at 25 °C and genomic DNA was isolated using QIAGEN DNeasy® Plant Maxi Kit (Qiagen, Inc., Valencia, CA, U.S.A.) following the manufacturer's protocol. Amplification of nuclear rDNA, containing the ITS1 and 2 and the 5.8S rRNA gene, and of a 0.4 kb fragment of *ech42* was done as described previously (Kullnig-Gradinger *et al.* 2002). A 0.3 kb fragment of *tef1*, containing the large intron, was amplified by the primer pair EF1-728F (5'-CATCGAGAAGTTCGAG AAGG-3') and EF1-986R (5'-TACTTGAAG GAACCCTTACC-3') (Druzhinina *et al.* unpubl. data). Amplicon purification and sequencing was also done as described in detail previously (Kullnig-Gradinger *et al.* 2002). All sequences obtained in this study have been submitted to NCBI GenBank, their accession numbers are indicated in Table 1. Previously published sequences used for phylogenetic analyses in this study are given by accession numbers as they were retrieved from GenBank.

#### Phylogenetic analysis

DNA sequences were visually aligned using Genedoc 2.6 (Nicholas & Nicholas 1997). The interleaved

NEXUS file was formatted using PAUP v. 4.0b10 and was manually edited in order for it to be recognized by MrBayes v. 3.0B4 programme. The Bayesian approach to phylogenetic reconstructions (Rannala & Yang 1996, Yang & Rannala 1997) was implemented using MrBayes 3.0B4 (Huelsenbeck & Ronquist 2001). The model of evolution and prior settings for individual loci and the combined dataset were used as has been estimated by Druzhinina *et al.* (unpublished) for different taxa of *Hypocrea/Trichoderma*. Metropolis-coupled Markov chain Monte Carlo (MCMCMC) sampling was performed with four incrementally heated chains that were simultaneously run for 10<sup>6</sup> generations. To check for potentially poor mixing of MCMCMC, each analysis was repeated four to six times. The convergence of MCMCMC was monitored by examining the value of the marginal likelihood through generations. Convergence of substitution rate and rate heterogeneity model parameters was also checked. Bayesian posterior probabilities (PP) were obtained from the 50 % majority rule consensus of 80 000 trees sampled every 100 generations after removing the 2 000 first trees as the "burn-in" stage. According to the protocol of Leache & Reeder (2002), PP values lower than 0.95 were not considered significant while values below 0.9 were not shown on phylograms.

The MSA file and phylogenetic trees have been deposited in the Treebase <http://www.treebase.org/treebase/submit.html> database under the submission code SN1926.

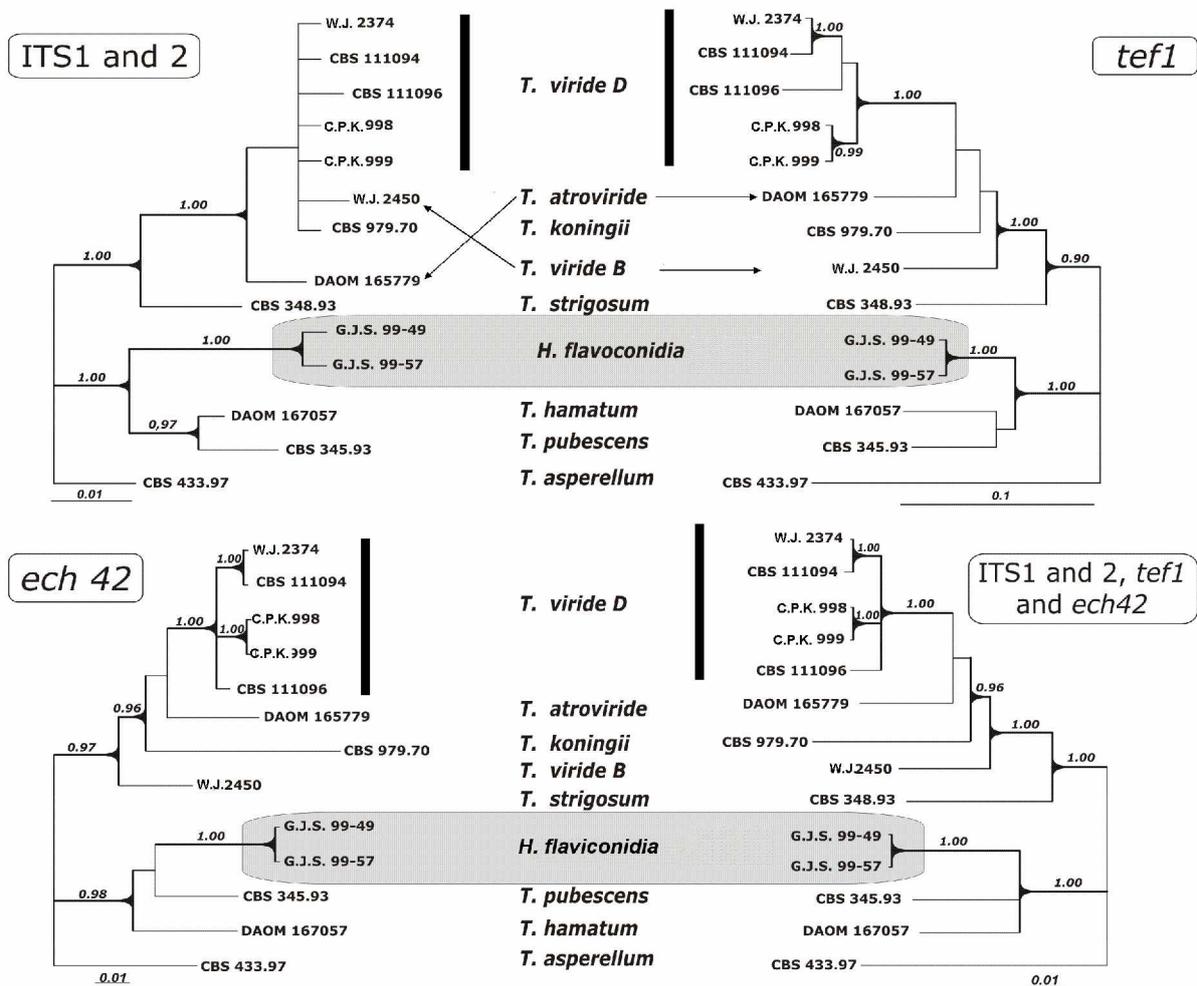
## RESULTS

#### Phylogenetic analysis

Preliminary analysis of the ITS1 and 2 sequence of two isolates of the unknown *Hypocrea* indicated that it is likely a new taxon belonging to section *Tricho-*

*derma*. In order to prove this by the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) approach (Taylor *et al.* 2000), we amplified and sequenced fragments from three genomic loci from the two isolates and other taxa of section *Trichoderma*, i.e. the internal transcribed spacers 1 and 2 (ITS1 and 2) of the ribosomal rDNA; the large intron of the translation elongation factor 1- $\alpha$  gene (*tef1*); and a portion of the last exon of the endochitinase 42-encoding gene (*ech42*). The corresponding sequences were subjected to Bayesian phylogenetic analysis, using the General Time Reversible model (GTR, Rodriguez *et al.* 1990). As shown in Fig. 1, when the trees were rooted with *T. asperellum* as an outgroup, the remaining taxa of section *Trichoderma* split into two major clades: one containing the species of the “*H. rufa*” species complex (*H. rufa*, *T. viride*, *T. atroviride*, *T. koningii*); and a second clade containing *T. hamatum*, *T. pubescens* and the two isolates of the

unknown *Hypocrea* species. As no *ech42* sequence was available for *T. strigosum*, it was analyzed only in the ITS1 and 2, *tef1* and combined trees. While it clustered in the first with high posterior probability basal to the species of the *H. rufa* clade, this position received no PP support in the *tef1* tree and its phylogenetic position remains therefore unclear. The unknown *Hypocrea* consistently formed a sister clade to *T. hamatum* and *T. pubescens* in all three single-gene trees and the combined tree, which was characterized by high posterior probabilities in the ITS1 and 2 and the *tef1* tree. Although the clade of the unknown *Hypocrea* received low PPs in the *ech42* tree, this does not reject its phylogenetic position. These analyses therefore confirm that the two isolates of the unknown *Hypocrea* fulfill the criteria of GCPSR to be considered as a separate phylogenetic taxon.



**Fig. 1.** Phylogenetic position of *H. flaviconidia* by Bayesian analysis of ITS1 and 2, *tef1* large intron, *ech42* large exon and a combined dataset of all three loci. Posterior probabilities are given in italic numbers over the branches, and values below 0.9 are not shown. Arrows indicate position of taxa which were incongruent between different trees.

### Phenotype analysis

The teleomorph of the unknown *Hypocrea* is almost nondescript. The stroma is small, pulvinate, light brown; the ostiolar openings are barely visible as paler colored dots. The surface of the stroma is plane and cells of the surface are almost angular. The stroma surface region is 60–70 µm wide and composed of angular cells *ca.* 10 µm diam. Ascospores are hyaline.

In fresh isolates on CMD conidia formed in sharply delimited yellow pustules. After six years storage at 5–9 °C on cornmeal agar (no dextrose) in culture tubes conidia no longer form on CMD. On SNA yellow-green conidia form slowly in pustules, after more than 1 wk under ambient laboratory conditions; on oatmeal agar (Gams *et al.* 1998) large, flat, pale yellow pustules form after 2 wk under ambient laboratory conditions, although in one oatmeal plate incubated at 25 °C with 12 h dark/12 h cool white fluorescent a green pustule formed.

Conidiophores from SNA are variable in morphology. In part they are somewhat *Verticillium*-like (Figs 11, 14), with a discernible main axis from which single phialides arise at an angle < 90 ° with respect to the main axis, or phialides terminate short lateral branches and are then held in a verticil; these phialides tend to be long, narrow and to taper uniformly from base to tip. In part there is no discernible main axis; conidiophores are irregularly and frequently branched and the septa were conspicuous (Figs 12, 13); phialides are *Pachybasium*-like, being relatively short and broad and are often crowded at the tips of short branches or along the length of branches. Intercalary phialides are common.

Conidia are light yellow by transmitted light in fresh cultures; green cultures conidia formed derived from old stock cultures grown on SNA. Conidia in G.J.S. 99-49 are ellipsoidal to oblong or subcylindrical, while in G.J.S. 99-51 and G.J.S. 99-57 they are ellipsoidal with at most few oblong conidia.

Chlamydospores were observed in one (G.J.S. 99-49) of three cultures grown on CMD after 2 wk.

### TAXONOMY

*Hypocrea flaviconidia* Chaverri, Druzhinina & Samuels, **sp. nov.** MycoBank MB500100.

*Anamorph:* *Trichoderma* sp.

Stromata pulvinata, brunnea ad subbrunnea, 1–1.7(–2) mm diam. Ascospores bicellulares, verruculosae, ad septum disarticulatae, hyalina, parte distali globosa ad subglobosa, (4–)4.5–4.7(–45.5) × (3.7–)4.2–4.3(–5) µm, parte proximali cuneiformi ad subcylindrica, (4.2–)5.2–5.5(–6.7) × (3.2–)3.7–4(–4.5) µm. Anamorphosis *Trichoderma* sp. Phialides (4–)6.2–7(–12.7) × (2.2–)2.7–3(–4) µm, longitudo/latitudo (1.4–)2.2–2.5(–5.6). Conidia oblonga ad ellipsoidea, flava,

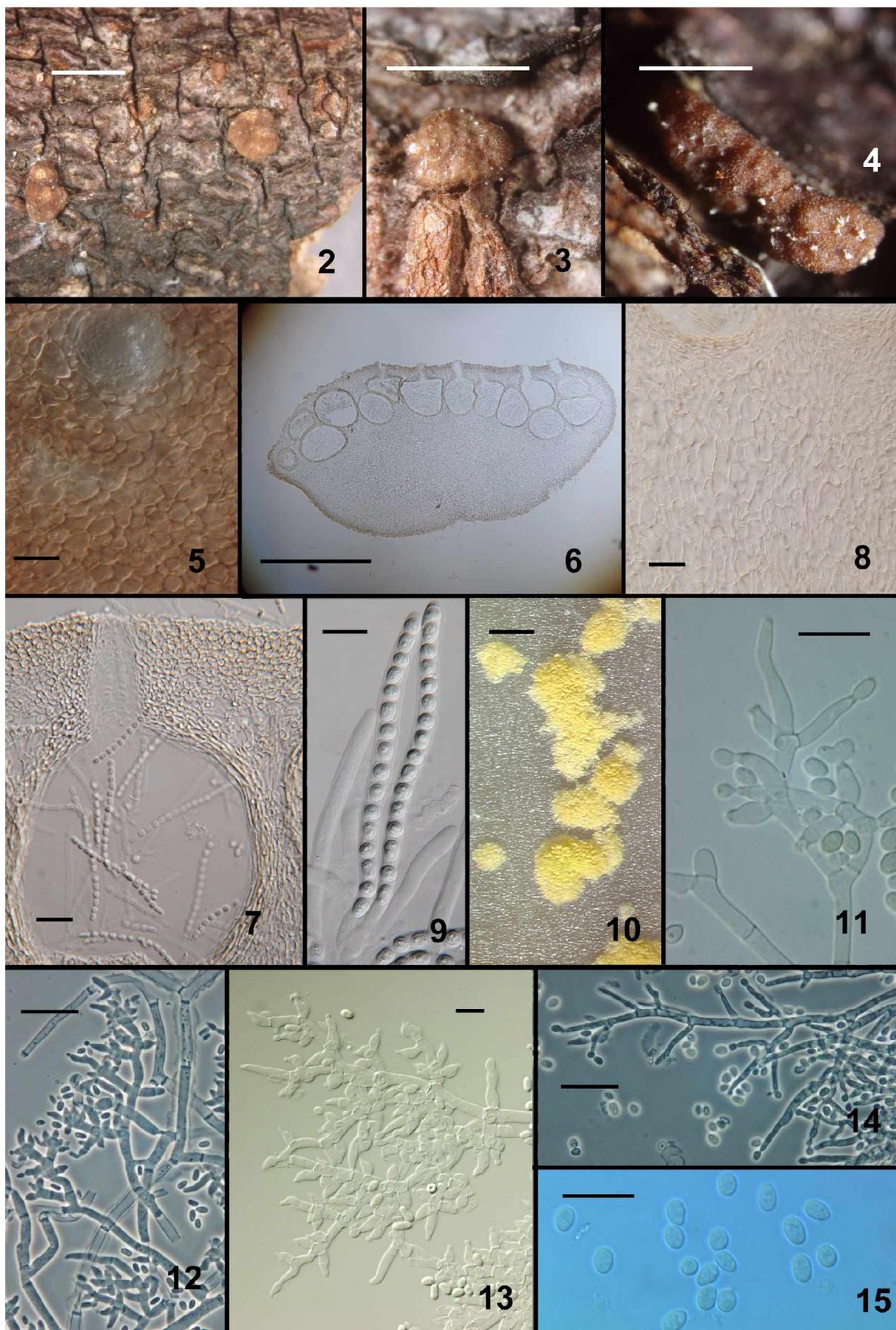
glabra, (3–)3.7–4(–4.7) × (2–)2.2–2.5(–3) µm, longitudo/latitudo (1.2–)1.5–1.6(–2) µm.

*Holotypus:* INB 3862698, isotypus BPI 746538.

*Stromata* solitary, pulvinate, circular in outline, 1–1.7(–2) mm diam, (0.5–)0.7–0.9(–1) mm high (n = 10), somewhat constricted at the base, smooth, opaque, with small perithecial protuberances, pale brown to brown, becoming darker brown in KOH; ostiolar openings visible. Cells of the *stroma* surface nearly angular in outline, (3.5–)4.5–7(–10) µm, walls at most slightly thickened. Surface region of stroma 60–70 µm thick, comprising thin-walled angular cells, hyaline, (5.7–)7.5–8.5(–14.5) µm diam. Tissue between the perithecia and below the outermost layer consisting of intertwined hyphae. Internal tissue below the perithecia of *textura angularis*, cells hyaline, thin-walled, (7–)12.5–14.5(–21) µm diam. *Perithecia* completely immersed in the stroma, generally closely aggregated, subglobose in section, (216–)243–275(–330) × (147–)161–184(–215) µm, wall composed of compacted cells, KOH–, ostiolar canal (54–)66–85(–109) µm long. Cells of the *ostiolar region* not sharply differentiated from the surrounding cells of the stroma surface. *Asci* cylindrical, (72–)86–91(–102) × (4.7–)5.2–5.5(–6.2) µm (n = 50); apex slightly thickened and with a pore. *Part-ascospores* hyaline, warted, dimorphic, distal part globose to subglobose, (4–)4.5–4.7(–45.5) × (3.7–)4.2–4.3(–5) µm, proximal part wedge-shaped to subcylindrical, (4.2–)5.2–5.5(–6.7) × (3.2–)3.7–4(–4.5) µm (n = 70).

*Colonies* on CMD at 20 °C after *ca.* 2 wks 9 cm diam, flat, with no aerial mycelium, no distinctive odour; agar not pigmented; conidia produced in pustules mainly at the margins of the colony; pustules compact, dry, yellow (3A–B8), 1–3 mm diam. Conidiophores *Trichoderma*-like, branching irregularly, generally terminating in short branches, branches arising singly or in pairs from the main axis; phialides arising in whorls of 2–3, rarely singly, intercalary phialides common. *Sterile elongations* of conidiophores and long protruding conidiophores lacking. *Phialides* cylindrical to flask-shaped, tapering towards the tip, sometimes slightly hooked, (4–)6.2–7(–12.7) µm long, (2.2–)2.7–3(–4) µm wide at the widest point, (1.5–)2.2–2.5(–3) µm at the base, L/W (1.4–)2.2–2.5(–5.6) (n = 90), arising from a cell 2.5–3.5(–4.5) µm wide. *Conidia* pale yellow, smooth, ellipsoidal to oblong, (3–)3.7–4(–4.7) × (2–)2.2–2.5(–3) µm, L/W (1.2–)1.5–1.6(–2) (n = 90). *Chlamydospores* sometimes observed after 1 wk; globose to subglobose, (7–)9.5–11.5(–20.5) × (5.2–)7.5–9.5(–15.2) µm (n = 30).

*Habitat:* On bark.



**Figs 2–15.** *Hypocrea flaviconidia*. 2–4. Stromata. 5. Cells at the stroma surface. 6. Section through a stroma showing perithecia. 7. Median longitudinal section through a perithecium showing the surface and subsurface regions of the stroma. 8. Stroma tissue below perithecia. 9. Two asci with ascospores. 10. Conidial pustules on CMD. 11–14. Conidiophores, from SNA. 15. Conidia, from CMD. Figs 2–5, 8, 9, 15 from G.J.S. 99-51; 6, 9, 10, 12, 13 from G.J.S. 99-49; 7, 11, 14 from G.J.S. 99-57. Scale bars: Figs 1–3 = 2 mm, 4, 10 = 1 mm, 5, 9, 11, 13, 15 = 10  $\mu$ m, 6 = 500  $\mu$ m, 7, 8, 12, 14 = 20  $\mu$ m.

*Known distribution:* Costa Rica.

*Specimens examined:* **Costa Rica**, Puntarenas. Coto Brus, Sabalito, Sitio Las Tablas, Sendero Siénege, elevation 1500 m, on bark, 29 Jun. 1999, G.J. Samuels (8477), P. Chaverri, H.L. Chamberlain (INB 3862187, BPI 746540, culture G.J.S. 99-49); Sendero Siénege, elevation 1500 m, on bark; 29 Jun. 1999, G.J. Samuels (8475), P. Chaverri, H.L. Chamberlain (**holotype** INB 3862698, **isotype** BPI 746538); Sendero Siénege, elevation 1350 m, on bark, 1 Jul. 1999, G.J. Samuels (8498), P. Chaverri, H.L. Chamberlain (BPI 746561, INB 3862702; culture: G.J.S. 99-57, CBS 116238).

*Notes:* We know this species from three collections, all made in the same area of Costa Rica. Ascospores isolated from all three gave identical cultures; only G.J.S. 99-49 and G.J.S. 99-57 are now viable, and these were sequenced. Unfortunately the only specimen suitable to be the type specimen is BPI 746538 from which the no longer viable culture G.J.S. 99-51 was derived. There is no doubt in our minds of the identity of this collection with the other two paratype specimens, and their cultures, cited above.

Because of its morphology, the anamorph would be assigned to *Trichoderma* sect. *Pachybasium* (Sacc.) Bissett *sensu* Bissett (1991), a section that is now known to be paraphyletic (Kullnig-Gradinger *et al.* 2002). The combined phenotype and genotype data lead us to conclude that this unknown *Hypocrea* is not one of the species for which the whole life cycle is

known and, accordingly, we describe it as a new species.

Using cultures stored for 6 years as described above, the optimum temperature for growth in darkness on PDA and SNA was 25 °C. After 72 h the radius of cultures grown on PDA after 72 h was 27–30 mm; on SNA < 5 mm; there was no growth at 15 or 30 °C. On SNA the colony was transparent and the margin deeply dissected or lobed; on PDA the mycelium was more or less cottony and formed in concentric rings.

Although yellow conidia sometimes form in some cultures of some species, very few isolates have permanently yellow isolates. As was said in the introduction, *T. flavofuscum* and *T. croceum* were described on the basis of yellow conidia, but these have been shown to be synonyms of *T. virens* and *T. polysporum*, respectively. Conidia of *T. virens* are typically green while conidia of *T. polysporum* are typically white to cream-colored. Even were it not for the peculiar conidium pigmentation, this new species is morphologically different from other described members of the morphological *T.* sect. *Pachybasium*. Conidia of *H. flaviconidia* were unmistakably yellow on CMD when first isolated. Conidium pigmentation was equivocal after storage, yellow-green on SNA but white on OA.

### Key to species of *Hypocrea/Trichoderma* with sometimes yellow conidia

1. Ascospores green; anamorph *Gliocladium*-like, producing mostly green, rarely yellow-brown conidia ..... *H. virens/T. virens*
1. Ascospores hyaline; anamorph not *Gliocladium*-like ..... 2
2. Distal part-ascospores 3.2–4.5 × 3–4 µm, proximal part-ascospores 3.7–5.2 × 2.5–3.5 µm; anamorph *Pachybasium*-like; conidia hyaline (white in mass) in most isolates, rarely yellow, 2.5–3.5 × 1.5–2.5 µm; known only from temperate regions ..... *H. pachybasioides/T. polysporum*
- 2'. Distal part-ascospores 4.5–4.7 × 4.2–4.3 µm, proximal part-ascospores 5.2–5.5 × 3.7–4 µm; anamorph *Trichoderma*- to *Pachybasium*-like; conidia light yellow, 3.7–4 × 2.2–2.5 µm; known only from Costa Rica ..... *H. flaviconidia*

### ACKNOWLEDGMENTS

P.C., P.F. and G.J.S. were supported part by INBio and by National Science Foundation Grant 9712308, 'Monographic studies of the Hypocrealean fungi: *Hypocrea* and *Hypomyces*,' to the Department of Plant Pathology, The Pennsylvania State University. C.P.K. and I.D. were supported in part by the Austrian Science Fund grants P-12748-MOB and FWF P-16601 to C.P.K. C.P.K. and I.D. thank Monika Komon (first author's laboratory) for her help in PCR amplifications. G.J.S. and P.C. thank Lutorri Ashley for technical assistance.

### REFERENCES

- Bissett J (1991). A revision of the genus *Trichoderma*. III. Section *Pachybasium*. *Canadian Journal of Botany* **69**: 2372–2417.
- Chaverri P, Samuels GJ (2003). *Hypocrea/Trichoderma* (Ascomycota, Hypocreales, Hypocreaceae): Species with green ascospores. *Studies in Mycology* **48**: 1–116.
- Gams W, Hoekstra ES, Aptroot A (1998). *CBS course of mycology*. 4<sup>th</sup> ed. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

- Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Kornerup A, Wanscher JH (1978). *Handbook of colour*. 3<sup>rd</sup> ed. Methuen London Ltd., London.
- Kullnig-Gradinger CM, Szakacs G, Kubicek CP (2002). Phylogeny and evolution of the fungal genus *Trichoderma* – a multigene approach. *Mycological Research* **106**: 757–767.
- Leache AD, Reeder TW (2002). Molecular systematics of the Eastern Fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood and Bayesian approaches. *Systematic Biology* **51**: 44–68.
- Lu BS, Druzhinina IS, Fallah P, Chaverri P, Gradinger C, Kubicek CP, Samuels GJ (2004). *Hypocrea/Trichoderma* species with pachybasium-like conidiophores: teleomorphs for *T. minutisporum* and *T. polysporum*, and their newly discovered relatives. *Mycologia* **96**: 310–342.
- Nicholas KB, Nicholas HB Jr (1997). *Genedoc: a tool for editing and annotating multiple sequence alignments*. <http://www.psc.edu/biomed/genedoc>.
- Nirenberg HI (1976). Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* **169**: 1–117.
- Rannala B, Yang Z (1996). Probability distribution of molecular evolutionary trees: a new method of phylogenetic interference. *Journal of Molecular Evolution* **43**: 304–311.
- Rodriguez F, Oliver FJ, Marin A, Medina JR (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142**: 485–501.
- Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* **31**: 21–32.
- Yang Z, Rannala B (1997). Bayesian phylogenetic interference using DNA sequences: a Markov chain Monte Carlo method. *Molecular Biology and Evolution* **14**: 717–724.

