**Hypocrea virens** sp. nov., the teleomorph of *Trichoderma virens*

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**Abstract:** The new species *Hypocrea virens*, has been found to be the teleomorph of *Trichoderma virens*, a species commonly used in biological control applications. This conclusion is based on the comparison of morphological and molecular data from four isolates of *T. virens* and a single collection and isolate of *H. virens*. Data for several morphological characters, including colony and growth characteristics, were collected. In addition, sequence data from ITS1, 5.8S, ITS2 rDNA and translation elongation factor (tef-1α) were analyzed for the five isolates. Analysis of phenotypic characters show that cultures and microscopic characters of the anamorph of *H. virens* are indistinguishable from those of *T. virens*. This is consistent with sequence data from ITS1, 5.8S, ITS2 rDNA which show that the sequence of *H. virens* is identical to that of the ex-type isolate of *T. virens*. Despite minor variation in tef-1α sequences, *T. virens* isolates and *H. virens* form a monophyletic group that is different from other examined species of *Hypocrea*; this clade is supported by a 100% bootstrap value. Molecular and morphological data confirm the connection between *H. virens* and *T. virens*.

**Key Words:** Ascomycetes, biological control, Hypocreales, systematics, teleomorph-anamorph connection

**INTRODUCTION**

*Trichoderma virens* (Miller et al) Arx is an important species used in biological control of several soil-borne plant pathogens (Papavizas 1985). It has been proven effective against pathogens such as *Rhizoctonia solani* Kuhn., *Pythium ultimum* Trow, *Colletotrichum* spp., *Sclerotinia* spp., among others (Lfs hitz et al 1985, Ghisalberti and Sivasithamparam 1991, Lumsden et al 1996, Harris and Lumsden 1997). In addition, *T. virens* is the active ingredient in the commercial product SoilGard® (formerly GlioGard®), which is used in biocontrol applications (Ricard 1981, Lumsden et al 1993).

*Trichoderma virens* was first placed in Glciocladium Corda based on the gliocladium-like branching pattern. Bisby (1939) suggested that the anamorph of *Hypocrea gelatinosa* (Tode : Fr.) Fr., which is very similar to *T. virens*, is typical of *Trichoderma* Pers. and erroneously concluded that *Hypocrea gelatinosa* is a synonym of *Hypocrea rufa* (Pers.) Fr. Later, Webster (1964) and Rifai (1969) determined that *T. virens* is morphologically closer to *Gliocladium* than to *T. viride* Pers., the type species of *Trichoderma*. On the other hand, Seifert (1985), in his monograph of *Stilbella* Lindau and relatives, stated that the gliocladium-type anamorphs of *Hypocrea* Fr. do not fit into the concept of true *Gliocladium* based on *G. penicilliooides* Corda, the type species. Molecular data based on 28S and ITS rDNA (Rehner and Samuels 1994, 1995, Chaverri et al 2000) suggest that all anamorphs of *Hypocrea* are *Trichoderma*, and that *T. virens* is clearly nested among other *Hypocrea* species and phylogenetically distinct from *G. penicilliooides*. The placement of *T. virens* in *Trichoderma* (Arx 1987) is now generally accepted (Bissett 1991a, b, Samuels 1996, Gams and Bissett 1998).

*Trichoderma virens* has not previously been connected to a teleomorph and has been thought to be a strictly asexual and clonal hyphomycete. Recent evidence from diverse fungi, e.g., *Coccidioides immitis* (Siles (Burt et al 1996) and *Aspergillus flavus* Link : Fr. (Geiser et al 1998), have demonstrated genetic diversity in fungi that were previously thought to be clonal, a finding that is best explained by sexual or asexual recombination. In addition, teleomorphs have been found for species that were thought to be only asexual, e.g., *Trichoderma reesei* Simmons-*Hypocrea jecorina* Berk. & Broome (Kuhls et al 1996) and *Tolypocladium inflatum* Gams-Cordyceps subsessilis Petch (Hodge et al 1996). Numerous examples suggest that strictly clonal fungi are probably uncommon.

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(Taylor et al. 1999). The connection of T. virens to a teleomorph, H. virens, suggests possible sexual recombination in this common widespread species.

The new species of Hypocreopsis described in this paper was collected in Indiana, USA. The specimen was first identified as H. gelatinosa but it differed from typical H. gelatinosa in the production of abundant chlamydospores, a characteristic of T. virens. Comparison of ITS sequences showed that the new Hypocreopsis was distinct from H. gelatinosa (data from Chaverri et al. 2000 and additional unpublished data) but identical to four isolates of T. virens, including the ex-type. A portion of the tef-1α gene was sequenced in order to obtain more support for the hypothesis that the unknown species of Hypocreopsis was the teleomorph of T. virens.

In this paper we demonstrate the phylogenetic and phenotypic link between T. virens and its teleomorph and describe that teleomorph as a new species of Hypocreopsis, thereby elucidating the life cycle of this important species.

MATERIALS AND METHODS

Collections and isolates.—Molecular and morphological data were analyzed to compare the unidentified Hypocreopsis to isolates of T. virens, including the ex-type culture and isolates used in biological control. Table I lists the isolates and collections used in this study. These were lyophilized for preservation and for DNA extraction. The lyophilization protocol is described in Stewart et al. (1999). Lyophilized samples were stored at −20°C.

Determination of optimum temperature for growth.—A growth trial for the five isolates studied was carried out using two media, PDA (Difco) and CMD (Difco, +2% Dextrose); each plate contained 20 mL of medium. Inoculum was prepared by growing isolates on CMD until there was visible growth. A 1-cm-diam disk from the actively growing colony was placed 1 cm from the edge of the plate. One plate of each medium for each culture was incubated at five temperatures, 15, 20, 25, 30, and 35°C, at 12 h fluorescent light and 12 h darkness. The radius of the colony was measured from the edge of the plug of inoculum at 24 hourly intervals for 4 d. The experiment was repeated for a total of three times in subsequent weeks.

Morphological analysis.—Single ascospores of the unidentified Hypocreopsis were isolated with the aid of a micromanipulator on CMD. Cultures of T. virens and of the unidentified Hypocreopsis were grown on CMD in 9-cm-diam petri plates in an incubator at 20°C, with 12 h fluorescent light and 12 h darkness, to observe and measure the microscopic details of the anamorph. The observations were made after ca one wk of growth. Colony characteristics were observed from cultures on PDA after ca one week, under the same conditions as with CMD. The anamorph characters measured are the following: conidiophore length and width, width of phialide at the base, phialide width at its widest, phialide length, conidium length and width, metulae (conidiophore branch from which phialides arise) length and width, chlamydospore length and width, and colony characteristics. The herbarium specimen of the unidentified Hypocreopsis was rehydrated 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. Twenty-seven teleomorphic characteristics were evaluated and measured only for descriptive purposes. Measurements of continuous characters were collected using the image capturing software Scion Image beta 4.0.2 (Scion Corporation, Frederick, Maryland). Basic statistics of micromorphology were made based on 30 measurements except where indicated.

A one-way analysis of variance using Tukey’s comparison (p-value = 0.05) was carried out using Minitab 10.5 Xtra (Minitab Inc., State College, Pennsylvania). Each mean for each anamorph variable was compared to the other four isolates. A Principal Coordinates analysis (PCoA) was performed using MVSP Plus 3.1 (Kovach Computing Services, Wales, U.K.). PCoA analysis was used to test the phenotypic coherence of the monophyletic groups and/or to identify morphological characters that are phylogenetically informative. The Gower General Similarity Coefficient, a similarity measure, was used to perform the PCoA since the data matrix includes continuous and binary anamorph morphological data. For this analysis, morphological data for 10 and 3 additional isolates of Trichoderma harzianum and H. flavovirens, respectively (Table I), were used to show the different groups of taxa.

Molecular phylogenetic analysis.—The molecular analysis of the five isolates was done using two gene sequences: translation elongation factor (tef-1α), and internal transcribed spacers (ITS1, 5.8S, ITS2 rDNA). ITS sequences for T. virens isolates Gli 3, Gli 20, Gli 21 and Gli 39 were obtained from GenBank (NCBI, EMBL).

Extraction of genomic DNA was carried out using phenol and chloroform (protocol described in Stewart et al. 1999). A polymerase chain reaction (PCR) was performed using the following protocol: 5 μL buffer, 5 μL dNTPs, 1 μL of each primer, 0.1 μL of Taq Polymerase, and 2 μL of template PCR for a total reaction volume of 50 μL. Afterwards, PCR was performed using the following parameters: a 5 min step at 94°C, followed by 35 steps of 1 min at 94°C, 1 min at the annealing temperature (53°C), and 1 min at 72°C, and then a final step of 5 min at 72°C. For ITS, the primers utilized were ITS 1 (5’TCCGTAGGTAACCTGCGG-3’) and ITS 4 (5’CTCGTCGGCAGGTAATGC-3’) and for tef-1α the primers were ef1 (5’-ATGGGTAAGGA(A/C)GGAACAAGAC-3’) and ef2 (5’-GGAGA(A/G)GTACCAGT(G/C)ATCATGTTC-3’) (O’Donnell et al. 1998). A PCR product purification was carried out using Promega Wizard® PCR Preps purification system. After PCR products were cleaned, sequencing reactions and precipitations were done for the forward and reverse primers separately using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase FS, by PE Applied Biosystems. The sequencing was done at the Nucleic Acid Facility (Life Science Consortium, The Pennsylvania State University).
<table>
<thead>
<tr>
<th>Name</th>
<th>Strain number</th>
<th>Origin</th>
<th>GenBank accession number (ITS1, 5.8S, ITS2 rDNA)</th>
<th>GenBank accession number (tef1α)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hypocrea flavovirens</em> Berk.</td>
<td>PC 4</td>
<td>Pennsylvania, USA</td>
<td>AF275322</td>
<td>AF328561</td>
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<tr>
<td><em>H. flavovirens</em></td>
<td>PC 8</td>
<td>New York, USA</td>
<td></td>
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<tr>
<td><em>H. flavovirens</em></td>
<td>PC 14</td>
<td>New York, USA</td>
<td></td>
<td></td>
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<tr>
<td><em>H. cf. flavovirens</em></td>
<td>G.J.S. 97-61</td>
<td>Nakorn Nayok, Thailand</td>
<td>AF328552</td>
<td>AF328555</td>
</tr>
<tr>
<td><em>H. virens</em></td>
<td>G.J.S. 95-194</td>
<td>Indiana, USA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. nigricans</em> (Imai) Doi</td>
<td>G.J.S. 98-183</td>
<td>Vienna, Austria</td>
<td>AF275330</td>
<td>AF328560</td>
</tr>
<tr>
<td><em>T. harzianum</em> Rifai</td>
<td>G.J.S. 94-26</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>G.J.S. 94-27</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>G.J.S. 95-14</td>
<td>New Zealand</td>
<td></td>
<td></td>
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<tr>
<td><em>T. harzianum</em></td>
<td>G.J.S. 95-40</td>
<td>England</td>
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<tr>
<td><em>T. harzianum</em></td>
<td>G.J.S. 95-69</td>
<td>Jilin Province, People’s Republic of China</td>
<td>AF099006</td>
<td>AF328558</td>
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<tr>
<td><em>T. harzianum</em></td>
<td>G.J.S. 95-70</td>
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<tr>
<td><em>T. harzianum</em></td>
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<td>Kanagawa, Japan</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>G.J.S. 97-264</td>
<td>Okinawa, Japan</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>G.J.S. 97-266</td>
<td>Okinawa, Japan</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>G.J.S. 97-267</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma virens</em> (Miller et al.) Arx</td>
<td>Gli 3 (E. Nelson #27)</td>
<td>Oregon, USA</td>
<td>AF099006</td>
<td>AF328558</td>
</tr>
<tr>
<td><em>T. virens</em></td>
<td>Gli 20</td>
<td>Unknown (active ingredient in SoilGard™)</td>
<td>AF099007</td>
<td>AF328557</td>
</tr>
<tr>
<td><em>T. virens</em> (ex-type)</td>
<td>Gli 39 (ATCC 13213, CBS 249.59, J.C. Giddens 167)</td>
<td>Georgia, USA</td>
<td>AF099005</td>
<td>AF328559</td>
</tr>
<tr>
<td><em>T. virens</em></td>
<td>Gli 21</td>
<td>Unknown (derived from GL 20)</td>
<td>AF099008</td>
<td>AF328556</td>
</tr>
</tbody>
</table>
The assembly of contiguous sequences was done using SeqMan® II option in DNA Star (DNA Star Inc., Madison, Wisconsin). Clustal × 1.81 (Thompson et al 1997) was used to align the consensus sequence, then the alignment was refined by hand.

A phylogenetic analysis was performed using PAUP* 4.0 b4 (Swofford 1999). Trichoderma virens isolates and the unknown Hypocreæ were used as the ingroup and Hypocreæ flavovirens Berk. and H. nigricans (Imai) Doi were used as outgroup species. Chaverri et al (2000) showed that H. nigricans is closely related to T. virens and that H. flavovirens has a more distant relationship with T. virens. A parsimony analysis was done using exhaustive search. Gaps (insertions/deletions) were treated as missing. Bootstrap values (500 replicates) were also calculated. PAUP* was also used to determine the base pair differences. The sequences and alignment were deposited in GenBank and TreeBase (SN649; http://herbaria.harvard.edu/treecase/), respectively.

RESULTS

Morphological analysis.—The ANOVA (Table II) revealed statistically significant differences (at 0.05) in anamorph variables among Gli 3, Gli 39 (ex-type), Gli 20, Gli 21, and G.J.S. 95–194 (teleomorph), however these differences are not large enough to be taxonomically significant. The PCoA analysis (Fig. 1) shows that the new species of Hypocreæ, G.J.S. 95–194, forms a compact group together with the T. virens isolates, while the other isolates are grouped in two additional groups. Hypocreæ flavovirens isolates group together, as well as the isolates in the H. nigricans/T. harzianum group. The cumulative percentage of eigenvalues in axis 1, 2, and 3 are 36.84%, 52.39%, and 63.50%, respectively, indicating that most of the variance is explained in those 3 first axes, thus satisfactorily supporting the grouping of the different taxa. Although PCoA does not plot variables (characters), progressive elimination of all but the formation of compact tufts or pustules in culture and the shape of the phialide resulted in the three different groups (i.e., T. virens/H. virens, T. harzianum/H. nigricans and H. flavovirens), and the cumulative percentage of eigenvalues on Axis 1 and 2 increases to 80% and 100%, respectively. When all variables except these two are included in the PCoA analysis, the cumulative percentage of eigenvalues decreases and the isolates do not cluster into well-defined groups. Therefore, the variables that distinguish the T. virens/H. virens group from the outgroup species are the straight phialides and the absence of compact tufts in culture. These results are consistent with molecular sequence data.

The optimum temperatures for growth were between 25 and 30 C for all isolates. In addition, the colony characteristics were almost indistinguishable. There are no significant differences in growth rate. On CMD, all five isolates had a flat colony with sparse aerial mycelium. The conidia were produced concentrically or near the margin of the plate. Some differences can be seen in the amount of conidia formed. On PDA, the colonies are floccose with effuse conidiation most often covering the entire surface of the plate. A light yellow pigmentation of the agar (CMD) was present in Gli 20, Gli 39 (ex-type), and G. J. S. 95–194 (teleomorph). A yellow pigmentation of agar on PDA was observed on Gli 21 and Gli 39.

Molecular analysis.—Phylogenetic analysis using ITS1, 5.8S, ITS2 rDNA, and tef-1α sequences show that the T. virens isolates studied, including the unknown Hypocreæ, form a strongly supported monophyletic group (Figs. 2 and 3). Parsimony analysis of ITS sequences revealed that the five T. virens isolates (including the new species of Hypocreæ) are identical and form a clade supported by a 100% bootstrap value (Fig. 2). The exhaustive search of the most parsimonious trees found 2 trees with 57 steps. The minimal possible tree length was also 57, showing no homoplasy (parallelism, reversal, convergence, or recombination), thus the homoplasy index (HI) is 0. From a total of 658 characters, 603 characters were constant, 42 were parsimony-uninformative and 13 were informative. The sequence of the unknown Hypocreæ, G.J.S 95–194, was identical to the ex-type isolate Gli 39.

Parsimony analysis of tef-1α produced a similar result to ITS rDNA (Fig. 3), revealing small differences among the T. virens isolates. Even though there are differences among T. virens isolates, the group containing these species is monophyletic and is supported by a 100% bootstrap value. The exhaustive search found 5 most parsimonious trees with 481 steps. The minimal tree length was 470, 11 steps shorter than the most parsimonious tree obtained. This is reflected in a homoplasy index (HI) of 0.03. Of the 908 characters, 489 were constant, 370 were parsimony-uninformative, and 49 were informative. The consensus (50% majority rule) of the five most parsimonious trees shows two clades with frequencies of 100%, and two clades with frequencies of 60%. The unknown Hypocreæ groups with the ex-type Gli 39 and forms a clade present in the consensus (frequency of 60%) but with a bootstrap value less than 50%. The clade containing G.J.S. 95–194, Gli 39, Gli 21, and Gli 20 was supported by a bootstrap value of 100%. In addition, the clade that shows Gli 3 as a basal isolate in the clade is supported by a bootstrap value of 99%. In the analysis of the aligned sequences, 14 transitions and 4 transversions were detected be-
TABLE II. Anamorphic morphological characters of the isolates and taxa analyzed. Values represent 95% confidence intervals.

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Phialide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>length</td>
<td>8.7-9.5 µm</td>
<td>8.6-9.6 µm</td>
<td>7.9-8.9 µm</td>
<td>8.6-9.6 µm</td>
<td>8.7-9.9 µm</td>
<td>7.6 µm</td>
<td>8.3 µm</td>
</tr>
<tr>
<td>width at the base</td>
<td>2.4-2.8 µm</td>
<td>2.1-2.3 µm</td>
<td>2.3-2.5 µm</td>
<td>2.1-2.3 µm</td>
<td>2.5-2.7 µm</td>
<td>2.0 µm</td>
<td>2.5 µm</td>
</tr>
<tr>
<td>width at the widest</td>
<td>4.1-4.5 µm</td>
<td>4.1-4.3 µm</td>
<td>3.8-4.0 µm</td>
<td>3.4-3.8 µm</td>
<td>3.9-4.3 µm</td>
<td>2.8 µm</td>
<td>3.8 µm</td>
</tr>
<tr>
<td>shape</td>
<td>straight</td>
<td>straight</td>
<td>straight</td>
<td>straight</td>
<td>straight</td>
<td>straight</td>
<td>curved</td>
</tr>
<tr>
<td>Conidium</td>
<td>4.5-4.9 × 3.6-3.8 µm</td>
<td>4.3-4.7 × 3.8-4.2 µm</td>
<td>4.2-4.6 × 3.6-3.8 µm</td>
<td>4.4-4.6 × 3.9-4.1 µm</td>
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<td>3.2 × 2.8 µm</td>
<td>3.9 × 3.4 µm</td>
</tr>
<tr>
<td>Conidiophore</td>
<td>34.6-60.0 × 4.4-5.8 µm</td>
<td>21.7-55.3 × 4.2-5.6 µm</td>
<td>20.2-33.6 × 4.7-5.7 µm</td>
<td>107.8-133.0 × 4.2-4.8 µm</td>
<td>124-59.2 × 5.4-6.0 µm</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Metulae (subtending hyphae)</td>
<td>10.1-12.7 × 4.2-4.8 µm</td>
<td>9.8-11.9 × 4.1-4.6 µm</td>
<td>8.6-11 × 3.8-4.2 µm</td>
<td>8.5-11.7 × 4.2-4.8 µm</td>
<td>11.0-13.8 × 3.9-4.5 µm</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chlamydospore</td>
<td>8.2-10.0 × 7.8-8.8 µm</td>
<td>7.8-11.0 × 7.3-9.9 µm</td>
<td>6.3-8.1 × 6.1-7.5 µm</td>
<td>9.8-12.4 × 7.9-10.1 µm</td>
<td>9.0-10.4 × 7.9-9.7 µm</td>
<td>7.8 × 9.0 µm</td>
<td>9.8 × 10.0</td>
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<td>Optimum growth temperature</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pigment of agar</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>on CMD</td>
<td>absent</td>
<td>light yellow</td>
<td>absent</td>
<td>light yellow</td>
<td>light yellow</td>
<td>—</td>
<td>absent</td>
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<tr>
<td>on PDA</td>
<td>absent</td>
<td>absent</td>
<td>yellow</td>
<td>yellow</td>
<td>absent</td>
<td>yellow, when present</td>
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<tr>
<td>Formation of pustules/tufts in culture</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
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<td>yes</td>
<td>yes</td>
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</table>

*Average of 11 isolates of T. harzianum/H. nigricens and 4 isolates of H. flavovirens.
between the unknown *Hypocrea* and the ex-type Gli 39. However, these substitutions were synonymous. The single non-synonymous substitution was present between Gli 20 and the rest of the isolates.

The exhaustive search of the combined sequence data from the two genes produced 5 most parsimonious trees with 538 steps (Fig. 4). From a total of 1566 characters, 1092 were constant, 412 were variable uninformative, and 62 were parsimony informative.

**DISCUSSION**

Despite minor variation in tef1α sequences and in some morphological characters among isolates, we conclude that the unknown *Hypocrea* is the teleomorph of *T. virens*. From the results of the phylogenetic analysis of two gene sequences, the data suggest that the unknown *Hypocrea* is nested within the *T. virens* clade, and that this clade is monophyletic and supported by bootstrap values of 100%. Some variation among isolates can be observed in tef1α sequences; ITS sequences show no divergence among *T. virens* isolates and the unknown *Hypocrea*. It is noted that tef1α might be more useful than ITS in multilocus analyses to detect boundaries because in the present study tef1α had 49 phylogenetically informative characters compared to 13 in ITS rDNA. Most of the variable sites in tef1α sequences were found in the introns.

The discovery of a teleomorph of *T. virens* suggests the possibility of sexual recombination. Taylor et al (1999) mention that different genes have different evolutionary histories (topologies) when recombination has occurred, but are inherited as a unit under clonality. In the present paper it is shown that both ITS and tef1α phylogenies are not in conflict, thus revealing similar evolutionary histories. To support a hypothesis of clonality or recombination in the *T. virens* clade, a phylogenetic analysis of other genes, besides tef1α and ITS, is desirable. A teleomorph has never been observed in cultures of *T. virens* and seems to be rare in nature. Our ascospore-derived culture did not form a teleomorph so we cannot know whether *H. virens* is homothallic or heterothallic.

Even though there are some statistically significant differences among the morphological characters, the measurements of the different variables are in the ranges noted by Miller et al (1957) and Bissett (1991b) for *T. virens*. Moreover, the differences albeit statistically different—are not biologically significant. The PCoA analysis of the morphological data showed that the isolate of the new species of *Hypocrea* groups with the *T. virens* isolates and is different from the outgroup taxa, *H. flavovirens* and *H. nigricans/T. harzianum*. The PCoA analysis also showed that the morphological characters that separate *T. virens* and its teleomorph from *T. harzianum/H. nigricans* and *H. flavovirens* are the shape of the phialides and the formation of compact tufts or pustules in culture.

Despite the authors’ collecting and culturing *Hypocrea* specimens in several temperate and tropical countries over many years, only the new species described in this paper, with one specimen, has been found to produce an anamorph that is indistinguishable from *T. virens*. *Hypocrea gelatinosa* and the unknown *Hypocrea* are similar in morphology and anatomy of their teleomorphs and anamorphs, however, there are differences that separate them. *Hypocrea virens* has globose to subglobose, at most slightly dimorphic part-ascospores, whereas *H. gelatinosa* has distinctly dimorphic part-ascospores. In addition, the part-ascospores of *H. gelatinosa* are smaller than are those of *H. virens*. The anamorphs of the two species can be distinguished based on morphology, Bissett
(1991b) distinguished between the two species on the basis of more profusely branched conidiophores in *H. gelatinosa*. Other distinguishing features include the colony characteristics and the formation of chlamydospores. *Hypocrea gelatinosa* grows more slowly than *H. virens*. In addition, the conidiation in *H. gelatinosa* is more sparse and generally in the form of small irregular fascicles. In *T. virens* the formation of fascicles was not observed. Formation of chlamydospores in *H. gelatinosa* is rare, restricted to submerged older mycelium, while in *T. virens* chlamydospores are abundant in young and old colonies. These differences were supported by DNA sequence analysis (Chaverri et al 2000). The *Hypocrea* teleomorph of *T. virens* has green ascospores, as do *H. gelatinosa* and many other *Hypocrea* species.

In the absence of a comprehensive monograph of *Hypocrea*, the most complete work is that of Doi (1966, 1969, 1971, 1972, 1975, 1976, 1978), for Japan and elsewhere, who described both anamorphs and teleomorphs for many *Hypocrea* species. Prior to the work of Doi, taxonomy of *Hypocrea* largely emphasized the teleomorph (e.g., Seaver 1909a, b, 1910a, b, 1911) or was too narrow in scope of species studied (e.g., Dingley 1951, 1952, 1957, Rifai 1969). Our unknown *Hypocrea*—which is defined by the combined teleomorph and anamorph—is not described in Doi's works or others. Differences among closely

**Fig. 2.** Parsimony analysis of ITS1, 5.8S, ITS2 rDNA. One of two most parsimonious trees; 57 steps; consistency index: 1.0; retention index: 1.0; homoplasy index: 0.0; bootstrap values (500 repetitions) indicated above branches. The branches present in the consensus (50% majority rule) are indicated by the thicker branches. Outgroup taxa: *H. flavovirens* and *H. nigricans*. 
related *Hypocrea* species are manifested in their anamorphs, the respective teleomorphs being indistinguishable (e.g., Samuels et al. 1998: *Hypocrea schweinitzii* complex, Lieckfeldt et al. 1999: *Trichoderma viride*). Thus we propose it as a new species.

**TAXONOMY**

**Hypocrea virens** Chaverri, Samuels et E.L. Stewart, sp. nov.  

Figs. 5–20

Stromata solitaria, abolutea, (0.7–)0.8–1.0(–1.4) mm. Asci cylindrici, (85–)95–103(–114) × (5.0–)5.5–6.0(–6.5) μm, apice incrassato praediti. Ascosporae bicellulares, ad septum disarticulatae; globosae ad subglobosae, parte distali (4.0–)5.0–5.5(–6.5) × (4.0–)5.0–5.5(–6.5) μm, parte proximali (4.0–)5.0–5.5(–6.5) × (4.5–)5.0–5.5 μm, atrovirentes, verrucosae. HOLOTYPUS: Herb BPI 737768.


= *Gliocladium virens* Miller, Giddens & Foster, Mycologia 49:792. 1957.

Stromata solitary and scattered, pulvinate, light yellow, KOH+, nearly circular in outline, (0.7–)0.8–1.0(–1.4) mm diam (n = 14), (0.7–)0.8 mm (n = 10) high, with a wide base, surface smooth with slight perithecial protuberations, (13–)25–50(–80) perithe-
cia per stroma (n = 12), ostiolar openings narrow, visible due to green contents of centrum. Stroma surface (25–)26–33 μm thick (n = 10), formed of angular cells, slightly pigmented almost hyaline, KOH+, (7.0–)10.0–11.5 (–14.0) μm diam, densely compacted with walls 0.5–1.0 μm thick. Tissue immediately below the stromatal surface formed of compact to loose, pseudoparenchymatous cells, textura angularis to t. epidermoidea, colorless, KOH-, (4.5–)8.5–10.0 (–14.5) μm diam, walls 0.5–1.0 μm thick. Internal tissue below perithecia formed of angular cells, colorless, KOH-, (7.0–)11.0–13.0 (–17.5) μm diam, walls (0.2–)0.5–0.7 (–1.0) μm thick. Perithecia generally widely spaced, subglobose, (175–)180–218 (–241) μm high (n = 8), (95–)127–174 (–184) μm wide (n = 8), ostiolar canal (49–)56–80 μm long (n = 8), wall KOH+. Asci cylindrical, (85–)95–103 (–114) × (5.0–)5.5–6.0 (–6.5) μm (n = 20), with a slightly thickened tip; part-ascospores uniseriate. Part-ascospores slightly dimorphic, globose to subglobose, distal part globose to subglobose (4.0–)5.0–5.5 (–6.5) × (4.0–)5.0–5.5 (–6.5) μm, proximal part globose to subglobose, sometimes slightly tapered, (4.0–)5.0–5.5 (–6.5) × (4.5–)5.0–5.5 μm, dark green, warty.

For the description of the anamorph and colonies refer to Miller et al (1957) and Bissett (1991b). Anamorph of H. virens and the ex-type isolate of T. virens clade

![Diagram](image-url)

**Habitat.**—The teleomorph was found on decorticated wood, probably growing on black mycelium of another fungus. *Trichoderma vires* has been isolated from soil, other fungi, and decaying woody substrata.

**Known distribution.**—The teleomorph is known only from the type locality in Indiana, USA. The anamorph is cosmopolitan.

**Holotype.**—UNITES STATES. INDIANA: Brown County, vic. Pike's Peak, Happy Hollow Camp, 39°09'N, 86°06'W, elev. 250 m, 29 Sep 1995, G.J. Samuels 95–194 (BPI 737768; CBS 109339; ATCC MYA-1298).

**Other specimens examined.**—*Trichoderma vires* isolates:

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LITERATURE CITED


