Hypocrea stilbohypoxyli and its *Trichoderma koningii*-like anamorph: a new species from Puerto Rico on *Stilbohypoxylon moelleri*

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The new species *Hypocrea stilbohypoxyli* and its *Trichoderma* anamorph are described. The species is known only from Puerto Rico where it grows on stromata of *Stilbohypoxylon moelleri* (Xylariales, Xylariaceae). *Hypocrea stilbohypoxyli* is readily distinguished from the morphologically most similar *H. koningii/T. koningii* by its substratum and slower growth rate, which is especially evident on SNA. Stromata are morphologically very similar to those of *H. koningii* and *H. rufa*. The anamorph is morphologically close to *T. koningii*, differing from *T. koningii* in having somewhat shorter and wider conidia. *Hypocrea stilbohypoxyli* differs from *H. koningii/T. koningii* in 3 bp in ITS-1 and 2 bp in ITS-2 sequences. Both are members of *Trichoderma* sect. *Trichoderma*.

Keywords: anamorph, Ascomycetes, Hypocreales, Hypocreaceae, systematics, teleomorph.

This work is part of an ongoing study of *Hypocrea* and *Trichoderma* sect. *Trichoderma* (Ascomycetes, Hypocreales, Hypocreaceae) as delineated by Bissett (1991; see also (Bissett 1991, Lieckfeldt & al., 1998, Lieckfeldt & al., 1999, Dodd & al., 2002, Dodd & al., 2003). A *Hypocrea* was found repeatedly on the stromata of *Stilbohypoxylon moelleri* Rogers & Y.-M. Ju (Xylariales, Xylariaceae; see Rogers & Ju, 1997) in Puerto Rico. Lieckfeldt & al. (Lieckfeldt & al., 1998) noted the morphological similarities between the anamorph of the Puerto Rican collections and that of *H. koningii/T. koningii* s. str. The authors found that the two taxa differed in 3 bp (1.6%) in the ITS-1 and 2 bp (1.1%) in the ITS-2 regions of rDNA.

Within *Hypocrea/Trichoderma* there is typically no intraspecies variation in ITS-1 and ITS-2 sequences (up to a maximum of 2 bp in

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ITS-1 (0.9%) in *Trichoderma* sect. *Longibrachiatum* (Kuhls & al., 1997), and interspecies differences in that section ranged from 5 to 11 bp in the ITS–1 and 0-18 bp in ITS–2 (Kuhls & al., 1997). Differences between the Puerto Rican collections and *T. koningii* s. str. of 3 and 2 bp in the ITS-1 and ITS-2 regions of rDNA, respectively, are of intermediate magnitude, suggesting to us that the Puerto Rican collections represent a distinct taxon. Here we examine the Puerto Rican collections found on *Stilbohypoxylon moelleri* for phenotypic characters that could distinguish them from *H. koningii/T. koningii* at some taxonomic level.

Materials and methods

Ascospores were isolated from fresh stromata onto cornmeal dextrose agar (CMD, Difco) with the aid of a micromanipulator. Conidia adjacent to stromata on the substratum were isolated by touching the conidial mass with the sterile tip of a scalpel and transferring the conidia to CMD. Measurements of morphological parameters and growth rate and colony characters were all made as was described in recent publications (Samuels & al., 2002, Dodd & al., 2003) The media used for growth rate measurements were potato dextrose agar (PDA, Difco) and 'synthetic nutrient-poor agar' (SNA, (Nirenberg 1976). Morphological parameters of the anamorph were taken from cultures grown on CMD at 20–21 C, 12 h dark/12 h cool white fluorescent light. Cultures are deposited in ATCC, CBS and DAOM. Measurements are given as maximum and minimum values in parentheses and mean values ± standard deviation.

Results

Following are results of observations of the Puerto Rican collections. Morphological, biological and biogeographic characters are summarized in Tab. 1.

Morphological characters of the *Hypocrea* teleomorph (Figs. 1–13)

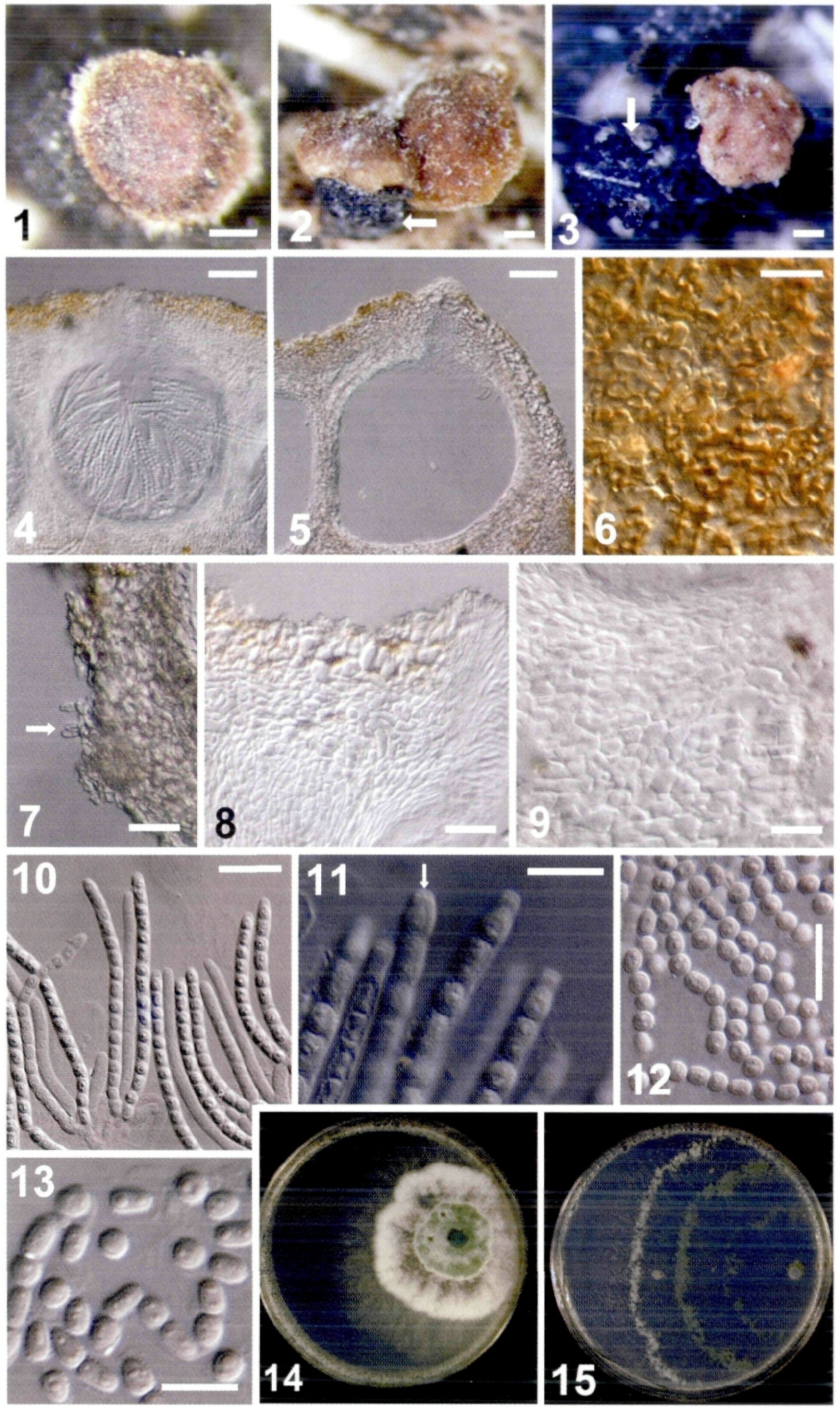
Stromata mostly solitary, sometimes gregarious, occasionally in pairs, forming on the ostiolar region of stromata of *Stilbohypoxylon moelleri* and adjacent palm leaf midrib tissue, mostly rounded or elongate, sometimes irregular in outline, (0.2–)0.5–0.9(–1.5) × 0.2–0.7(–1.3) mm (mean = 0.7 ± = 0.229 × 0.5, ± 0.189, n = 72), pulvinate, mostly constricted around the margin but sometimes broadly attached to the substratum, brown, somewhat reddish, when young a

Tab. 1. – Salient characters of *Hypocrea stilbohypoxli* and *H. koningii/T. koningii*

Parameter	Species	
	Hypocrea koningii/T. koningii	*Hypocrea stilbohypoxyli*
Phialides:		
Length[1]	(4.2–)8.0–8.2(–15.5) µm	(4.2–)5.5–9.0(–12.5) µm
95% CI	7.9–8.3 µm	6.9–7.5 µm
Widest point[1]	(2.0–)2.7–3.5(–4.2) µm	(1.0–)2.7–3.7(–4.5) µm
95% CI	3.0–3.1 µm	3.2–3.3 µm
L/W[1]	(1.2–)2.0–3.7(–6.6)	(1.2–)1.2–3.4(–11.5)
95% CI	2.6–2.9	2.1–2.5
Base[1]	(1.1–)1.5–2.2(–3.0) µm	(1.5–)1.7–2.7(–3.5) µm
95% CI	1.9–2.0 µm	2.1–2.3 µm
Cells supporting phialides[1]	(1.5–)2.2–3.0(–3.7) µm	(1.6–)2.5–3.5(–5.0) µm
95% CI	2.6–2.7 µm	2.1–2.3 µm
Conidia:		
Length[1]	(3.1–)3.7–4.5(–6.0) µm	(2.6–)3.2–4.2(–5.0) µm
95% CI	4.1–4.2 µm	3.7–3.9 µm
Width[1]	(2.0–)2.2–3.0(–3.3) µm	(2.0–)2.5–3.2(–3.5) µm
95% CI	2.5–2.6 µm	2.7–2.8 µm
L/W[1]	(1.2–)1.5–1.7(–2.3)	(1.0–)1.2–1.6(–1.9)
95% CI	1.6–1.7	1.35–1.42
Colony radius		
PDA 25 C, 72 h[1]	(42–) 54 ± 6 (–58) mm	(42–) 39 ± 2 (–58) mm
PDA 30 C, 72 h[1]	(41–) 55 ± 7 (–61) mm	(36–) 42 ± 5 (–48) mm
SNA 25 C, 72 h	(24–) 32 ± 4 (–35) mm	(34–) 35 ± 2 (–37) mm
SNA 30 C, 72 h[1]	(22–) 25 ± 3 (–29) mm	(42–) 43 ± 2 (–46) mm

[1] Indicates a statistically significant difference between the two taxa at the $p < 0.001$ level

white or light-colored margin visible. Surface velvety when young from short projecting hyphae, velvety aspect diminishing with age, all parts remaining unchanged in 3% KOH, plane. Ostiolar openings at most barely visible as darker dots (Figs. 1–3). Cells of the stroma in face view elongate, angular or irregular in outline, (4.0–)5.5–7.5(–10.0) × (3.0–)4.0–5.5(–7.5) µm (mean = 6.6 ± 1.765 × 1.020 ± 4.8, n = 30), reddish-brown, cell-walls 0.5–1 µm thick (n = 10) (Fig. 6), stromatal surface in vertical section 30–60(–88) µm thick, brown, cells compressed, angular or sometimes round, (2.4–)3.5–5.5(–10.0) × (2.0–)2.5–4.0(–6.5) µm (mean = 4.0 ±= 1.393 × 3.1 ± 0.923, n = 30), cell-walls 0.5–1 µm thick (Fig. 8). Hyphal hairs arising from stroma surface conspicuous, scattered, to 10 µm long, 3 µm wide at the base, hyaline to pale-brown (Fig. 7). Cells immediately below the stroma surface hyphal, thin-walled, hyaline (Figs. 4, 5, 8). Tissue below perithecia tending to comprise compact intertwined hyphae, less frequently of pseudoparenchymatous cells, (5–)7–10(–15) × (3.5–)4.5–7.0(–9.0) µm (mean = 8.1 × 5.7, SD 2.432, 1.326, n = 30), thin-walled, hyaline, not changing color in 3% KOH (Fig. 9). – Perithecia immersed in stroma, closely spaced, mostly globose to subglobose, laterally compressed, pyriform to clavate when densely disposed, (216–)250–320(–339) µm high, 149–200(–220) µm wide, ostiolar canal (46.5–)65–95(–103) µm long, cells of perithecial wall brown or red-



dish-brown to hyaline; ostiolar region not sharply delimited from surrounding tissue of the stromatal surface (Figs. 4, 5). – Asci cylindrical, (70–)75–90(–102.8) × (3.9–)4.5–5.5(–6.4) m, with a thickened tip and a pore. – Ascospores hyaline, bicellular, finely spinulose, uniseriate in asci, disarticulating at the septum early in development into 16 part-ascospores (Figs. 10–13). Part-ascospores dimorphic. Distal part-ascospores globose to subglobose or conical, proximal part-ascospores subglobose or elongate to wedge-shaped, tending to be more elongate toward the base of the ascus.

Colony characters (Figs. 14, 15)

Optimum temperature for growth on PDA and SNA 30 C. Colonies grown on PDA and SNA at 25 C and 30 C for 120 h in darkness nearly covering the agar surface. – Colonies on PDA dense, dark green in the center fading to gray-green toward the margin (Fig. 14). First green conidia observed on PDA and SNA at 25 or 30 C within 48–72 h in darkness. – On SNA conidia forming in conspicuous, narrow concentric rings separated by wider bands of less conidial production (Fig. 15). – On CMD at 25 C after 4 d under 12 h darkness/12 h cool white fluorescent light aerial mycelium scant, conidial pustules minute, at first scattered discrete and then more abundant and confluent toward the margin of the colony; at first light-green toward the center and white-gray to white near the margin, becoming uniformly green within 4 d. – No distinct odor or pigment noted.

Morphological characters of the anamorph (Figs. 16–29)

Conidiophores (Figs. 17–21, 24–29) comprising a more or less distinct central axis, phialides and/or fertile branches arising along

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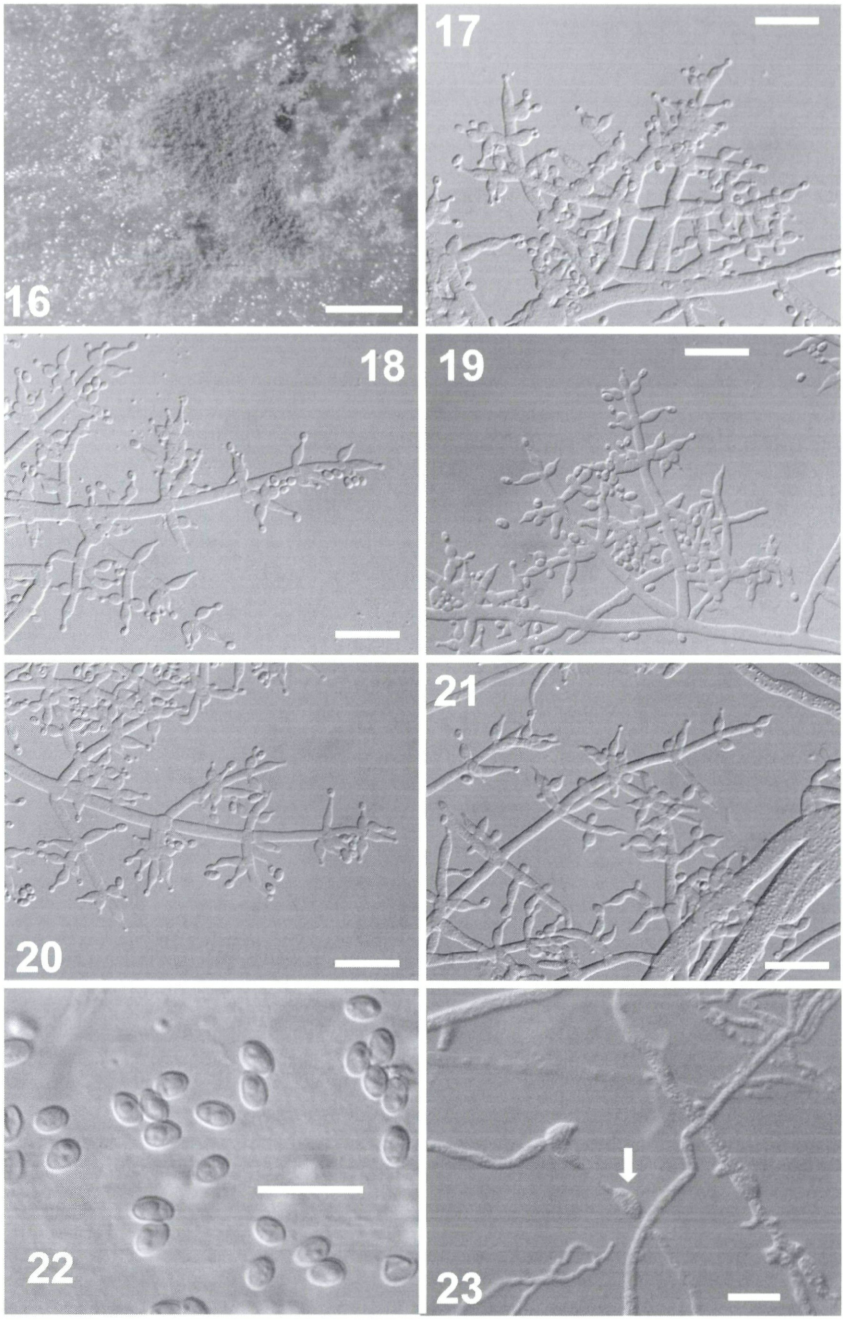
Figs. 1–15. *Hypocrea stilbohypoxyli* (teleomorph). – 1–3. Stromata formed on stroma of *Stilbohypoxylon moelleri* (arrows in 2, 3); note also the apparent absence of ostiolar openings in the *Hypocrea* stroma. – 4, 5. Median longitudinal section of perithecia immersed in a stroma. – 6. Cells of the stroma surface in face view. – 7. Hairs arising from the *Hypocrea* stroma surface (arrow). – 8. Ostiolar region of a perithecium and the stroma surface. – 9. Hyphal cells of the interior of the stroma below a perithecium; perithecial base seen on the top. – 10, 11. Asci, note thick apex with a pore (arrow in 11). – 12, 13. Part-ascospores, note distal part-ascospores globose to subglobose or conical, proximal part-ascospores subglobose or elongate to wedge-shaped. – 14, 15. Colony on PDA (14) after 96 h at 25 C and SNA (15) in darkness (with intermittent light) after 4 d at 25 C. – Figs. 2, 4, 8–11, 13–15 from G. J. S. 96–30; 3, 12 from G. J. S. 96–32; 1, 5, 7 from G. J. S. 96–42a, 6 from G. J. S. 96–43. Scale bars: 1–3 = 0.2 mm; 4, 5 = 50 µm; 6–10 = 20 µm; 11–13 = 10 µm.

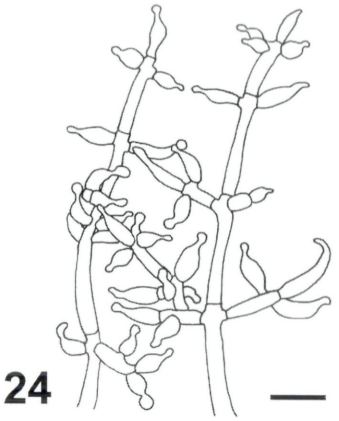


Figs. 16–23. *Hypocrea stilbohypoxyli* (anamorph). – 16. Conidial pustule on CMD. – 17–21. Conidiophores and phialides. – 22. Conidia. – 23. Chlamydospores (arrow). – Figs. 17, 18 from G.J.S. 96–30; 16, 19, 20, from G. J. S. 96-32; 21 from G. J. S. 96–42; 22, 23 from G. J. S. 96–43. – Scale bars: 16 = 0.5 mm, 17–21, 23 = 20 µm, 22 = 10 µm.

the entire length. 1° branches arising at or near 90° with respect to the main axis, paired or solitary, progressively longer and more profusely branched with distance from the tip, producing phialides directly or producing 2° branches. 2° branches mostly arising at or near 90° with respect to the 1° branches, paired or solitary, producing phialides directly or producing 3° branches. 3° branches typically unicellular, producing phialides directly, singly or in a terminal whorl of 2–4. – Phialides flask-shaped to pyriform (Figs. 17–21, 24–29). – Conidia ellipsoidal, green, smooth (Figs. 22, 25–27). – Chlamydospores scattered, not abundant, terminal and intercalary in hyphae, globose or subglobose, (2.3–)5.5–8(-14.0) × (1.6–) 4.0–7.5(–10.8) µm (mean = 6.3 × 5.4, SD = 2.437, 1.884, n = 89), wall smooth or somewhat roughened (Fig. 23).

Comparison of the Puerto Rican collections to *Hypocrea koningii/Trichoderma koningii* (Tab. 1)

Hypocrea koningii/T. koningii are illustrated in (Lieckfeldt & al., 1998) and Figs. 30–35. The teleomorph of *Hypocrea koningii/T. koningii* is uncommon, confirmed by us only from a single collection from eastern North America. The culture CBS 979.70 (cited in Lieckfeldt & al., 1998), from Germany, is reported to be a mass ascospore isolate from *H. muroiana* but that specimen cannot be located. The anamorph of *H. koningii/T. koningii* s. str. is also uncommon. Most of the few cultures that we have examined originated in eastern North America and the Netherlands but we have studied one culture from Brazil (Lieckfeldt & al., 1998). The isolates of *T. koningii* all originated from soil while the *Hypocrea* stromata formed on decaying wood of a dicotyledonous tree. There are no morphological or anatomical differences between the stromata of *H. koningii* and the Puerto Rican collections. The asci of the Puerto Rican collections are longer than those of *H. koningii*. The proximal part-ascospores of the Puerto Rican collections are shorter and wider than in *H. koningii*. Both *H. koningii/T. koningii* and the Puerto Rican collections have an anamorph that could be called the '*T. koningii* morphospecies.' The differences between the two are subtle but constant. Conidia of *H. koningii/T. koningii* (Fig. 34) are longer and narrower than in the Puerto Rican collections, and the L/W of conidia of *H. koningii* is greater than in the Puerto Rican collections. The phialides of *H. koningii/T. koningii* (Figs. 30–33) are slightly longer and narrower than those of the Puerto Rican collections. Finally, *H. koningii/T. koningii* (Fig. 35) grows faster than the Puerto Rican collections on PDA at 25 and 30 C and on SNA at 30 C, although surprisingly, not at 25 C.



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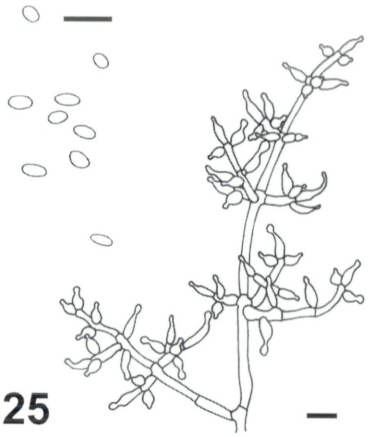

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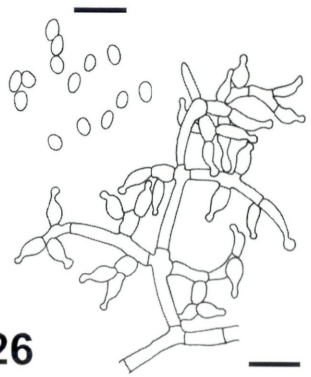

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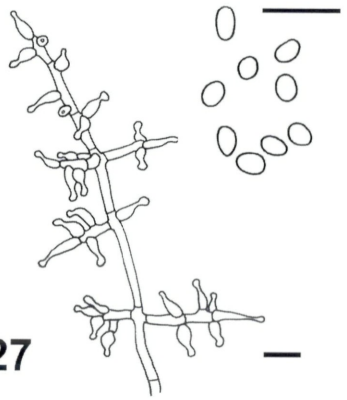

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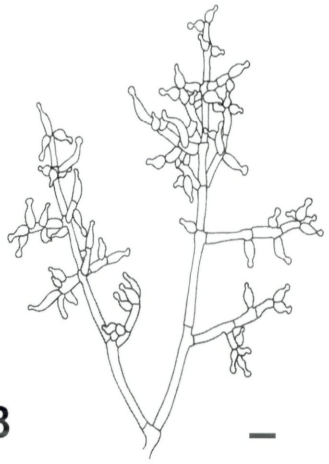

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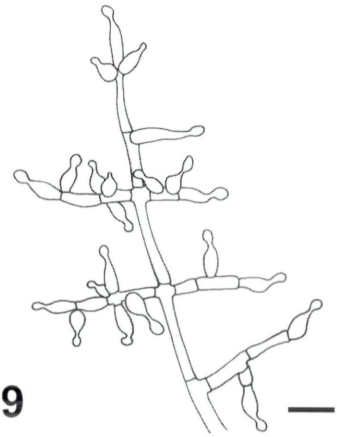

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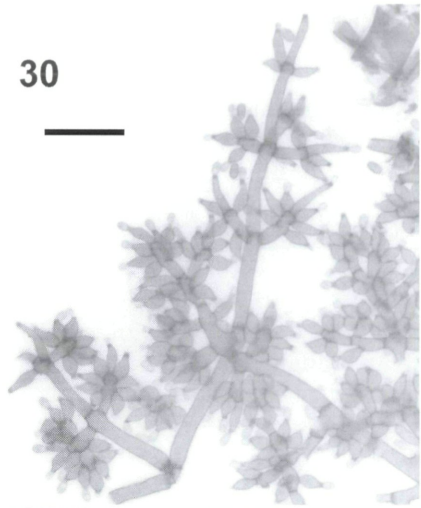
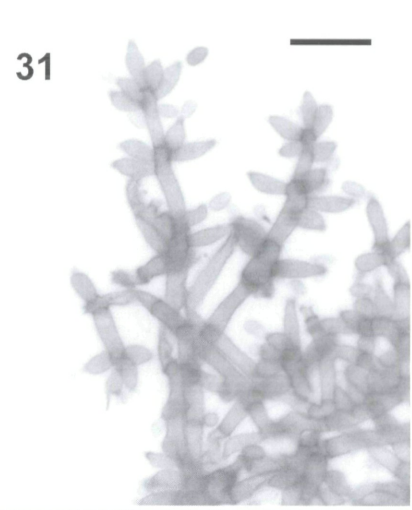
Discussion

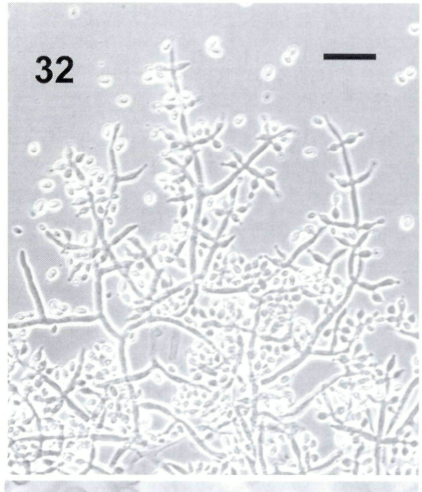
The initial results of ITS sequencing suggested a taxonomic distinction between *H. koningii/T. koningii* and the Puerto Rican collections from *Stilbohypoxylon* (Lieckfeldt & al., 1998). The Puerto Rican collections were identified by Lieckfeldt & al. (1998) as *H.* cf. *muroiana*.

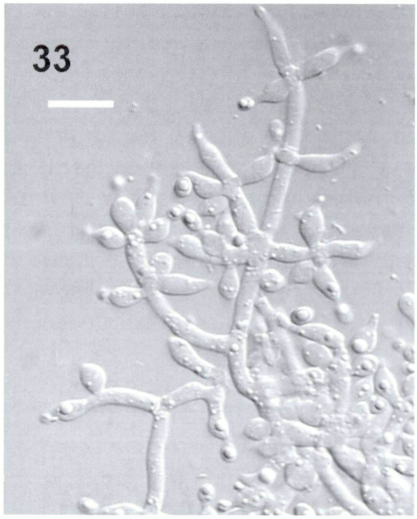
Hypocrea muroiana (Hino & Katumoto, 1958) was originally described from bamboo in Japan; the original authors did not report an anamorph for it. Doi (1974) reported several Japanese collections of *H. muroiana* on wood and bamboo, and described a *Trichoderma* anamorph for the species. The illustrated anamorph (Doi, 1974) has a branching pattern that is consistent with members of *Trichoderma* sect. *Trichoderma*, including *T. koningii* (Lieckfeldt & al., 1998), *T. viride* (Lieckfeldt & al., 1999; Samuels & al., 1999) and *T. atroviride* (Dodd & al., 2003). Conidia of three different collections were illustrated by Doi (1974); depending on the collection the conidia were globose and smooth, globose and warted, or ellipsoidal to ovoidal and smooth. Based on our experience with members of this group, a single *Hypocrea* morphotype can produce different anamorphs, the species differences being manifested in the anamorph and DNA sequences (see Dodd & al., 2002). The three conidial types illustrated by Doi (1974) strongly indicate three distinct species of *Hypocrea*, viz., *H. rufa* (Pers. : Fr.) Fr./ *T. viride* Pers.(subglobose, warted conidia), *H. atroviridis* Dodd & Samuels/*T. atroviride* P. Karst. (conidia subglobose, smooth) and one of several *Hypocrea* species (conidia ellipsoidal to ovoidal, smooth; Samuels, unpubl.). The teleomorphs of these lignicolous fungi are essentially indistinguishable. From the publication (Doi, 1974) it is not possible to know which, if any, of the cultured specimens came from bamboo and the identity of *H. muroiana* is in doubt. Of these, only the last could possibly be the same as the Puerto Rican collections that are the object of the present work. A portion of that collection (TNS-F-223405) is preserved at NY (!). Despite the fact that no measurements were given for those smooth and ellipsoidal to ovoidal conidia illustrated by Doi, the fungus illustrated is not the same species as the Puerto Rican collections. Ascospores of the Japanese collection are slightly larger than in *H. koningii*; it is corticolous and not fungicolous, and it was found in Japan. We do not know why this new *Hypocrea* is apparently restricted to *Stilbohypoxylon moelleri*. Species of other genera of the Xylariaceae in the area were not affected.

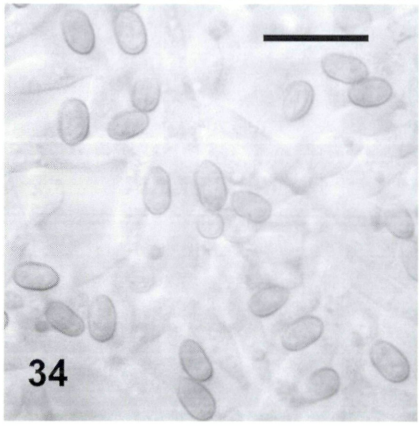
Figs. 24–29. *Hypocrea stilbohypoxyli* (anamorph). – 24–29. Phialides and conidia. – Figs. 24, 25 from G. J. S. 96–30; 26 from G. J. S. 96–42; 27, 28 from G. J. S. 96–32. – Scale bars = 10 μm.

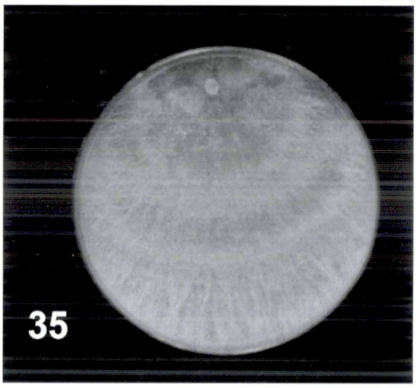












Although in *Hypocrea/Trichoderma* differences in ITS sequences alone are not necessarily indicative of taxonomic distinction, the coincidence of these base pair divergences with several subtle but consistent phenotype characters, biology and biogeography lead us to propose a new species for the Puerto Rican collections on *Stilbohypoxylon*, *H. stilbohypoxyli*. *Hypocrea stilbohypoxyli* is most easily distinguished from *H. koningii/T. koningii* by substratum, conidium sizes and growth rates.

Like other members of *Trichoderma* sect. *Trichoderma* (Lieckfeldt & al., 1998, Dodd & al., 2002, Dodd & al., 2003), the *Hypocrea* stroma is velvety when young and pale brown; perithecial openings are never conspicuous and may not be visible at all. The overall aspect of the *Trichoderma* anamorph is consistent with the pattern seen in *Trichoderma* sect. *Trichoderma* (see Samuels & al., 1999; Lieckfeldt & al., 1998; Samuels & al., 1999, 2002).

Species description

Hypocrea stilbohypoxyli B. S. Lu & Samuels, **sp. nov.** — Figs. 1–29.

Anamorph: *Trichoderma* sp. – Figs. 14–29.

Stromata solitaria vel gregaria, pulvinata, rotundata vel oblonga, (0.2–)0.5–0.9(–1.5) × 0.2-0.7(–1.3) mm, ostiolis invisibilibus, in vel inter stromata *Stilbohypoxyli* crescentia. Asci cylindrici, (65–)70–83(–90) × (65–)70–83(–90) µm, apice incrassato, poro apicali praediti. Ascosporae hyalinae, bicellulares, ad septum disarticulatae; parte distali globosa vel subglobosa, (3.2–)3.5–4.2(–4.7) × (2.7–)3.2–3.7(–4.0) µm, parte proximali ellipsoidea vel oblonga, (3.2–)4.2–5.2(–5.9) × (2.2–)2.7–3.2(–3.7) µm, hyalinae, verrucosae. Forma anamorphosis *Trichodermati koningii* similis sed differt propter phialides (4.2–)5.5–9.0(–12.5) µm longas, (1.0–)2.7–3.7(–4.5) µm latas. Conidia viridia, ellipsoidea, laevia, (2.6–)2.5–3.2(–5.0) × (2.0–)2.5–3.2(–3.5) µm, ratione longitudinis et latitudinis = (1.0–)1.2–1.6(–1.9).

Holotype. – PUERTO RICO: Caribbean National Forest, El Yunque Recreation Area, El Yunque, Trail from Palo Colorado, elev. 700–800 m, on *Stilbohypoxylon muelleri* on midribs of *Praestoea* palm leaves, 22 Feb 1996, G. J. Samuels 8076 (BPI 744463; ex-type culture G.J.S. 96-30 = CBS 992.97).

Etymology. – referring to the substratum, *Stilbohypoxylon moelleri* (Xylariaceae).

Known distribution. – Puerto Rico.

Known host. – *Stilbohypoxylon moelleri*.

Figs. 30–35. *Hypocrea koningii/T. koningii*, anamorph. – 30–33. Conidiophores and phialides. – 34. Conidia. – 35. Colony grown on PDA in darkness (with intermittent light) for 96 h at 25 C. – Fig. 30 from G. J. S. 96–117; 31 from G. J. S. 90–18; 32, 33, 34 from ATCC 64262; 35 from G. J. S. 92–18. – Scale bars: 30–32 = 20 µm; 33, 34 = 10 µm. For provenance of isolates see Lieckfeldt & al., 1998.

Additional specimens examined (all on *Stilbohypoxylon moelleri* on *Praestoea* palm leaves). – PUERTO RICO: Caribbean, National Forest, Big Tree Trail to Lamina, North end, off Rte. 191, elev. 500 m, 23 Feb 23, G. J. Samuels 8071a & H.-J. Schroers (BPI 744467; culture G.J.S. 96-32 = CBS 112888); Caribbean National Forest, Luquillo Mountains, Interpretive Trail, Serra Palm, off Rte 191, elev. 650 m, 23 Feb 1996, G. J. Samuels 8079a & H.-J. Schroers (BPI 744471; conidial culture G. J. S. 96-42a = CBS 112886); Serra Palm, off Rte. 191, elev. 700–800m, 23 Feb 1996, G. J. Samuels 8076a & H.-J. Schroers (BPI 744756; culture G. J. S. 96-43 = CBS 450.96).

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