Hypocrea lixii, the teleomorph of *Trichoderma harzianum*

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Cultures derived from ascospores of *Hypocrea lixii* (= *H. nigricans*, *H. lentiformis*) produced the morphological species *Trichoderma harzianum* in pure culture. *Trichoderma harzianum,* the most commonly found species of the genus, is also one of the most species frequently used in biocontrol of plant pathogens. It has not been connected previously to a teleomorph. The connection was confirmed by DNA sequence analysis. Similar to the anamorph, the teleomorph collections have a wide geographic distribution. Described in the 19th century, *Hypocrea lixii* is epitypified by a collection from Thailand.

Trichoderma harzianum Rifai (*Ascomycota, Hypocreales, Hypocreaceae*) is a cosmopolitan species that is found in a variety of substrata. This species is known for its effectiveness in biocontrol of plant-pathogenic fungi, specially soil-borne diseases (WELLS et al. 1972, AL-HEETI & SINCLAIR 1988, YANG et al. 1995, BAILEY & LUMSDEN 1998, MATHEW & GUPTA 1998). In addition, *T. harzianum* has been reported to have potential in the enhancement of plant growth and resistance to plant pathogens (BAILEY & LUMSDEN 1998, GROMOVICH et al. 1998).

Cultures derived from ascospores of fresh collections of *Hypocrea lixii* Pat. produced the morphological species *T. harzianum* in pure culture. In an unpublished study, *H. lixii* isolates were shown to group with isolates of *T. harzianum* based on phylogenies of four genes, translation elongation factor-1α, calmodulin, actin and ITS rDNA, and morphological and cultural data (CHAVERRI et al. 2002). Despite the fact that *T. harzianum* is genetically diverse, several phylogenetic lineages can be found that include cultures from specimens of *H. lixii*. Thus, there is no doubt of the genetic link between the two morphs.

Hypocrea lixii was originally described on the basis of a single collection from a rotting *Ganoderma* basidioma in Papua New Guinea (PATOUILLARD 1891). It is morphologically indistinguishable from the type collection of *H. lentiformis* Rehm, described from leaves of a palm (*Euterpe* sp.) in southern Brazil (REHM 1898) and from a paratype collection of *H. nigricans* f. *octospora* Doi (1972), which was collected in the western Pacific island of New Britain, also on decaying palm leaves. Although we have not been able to study type material of *H. nigricans* f. *nigricans* (Imai) Doi, the description of the anamorph and teleomorph of this species provided by DOI (1972) and DOI & DOI (1980) is morphologically consistent with *T. harzianum* and *H. lixii*. We have collected many specimens from New World and Asian tropical regions as well as from North America and Europe that agree with type material of *H. lixii*.

The purpose of the present paper is to redescribe and epitypify *H. lixii*.

Material and methods

The majority of the specimens examined in this study were collected and deposited in the BPI culture collection. In addition, herbarium specimens were studied from BPI, FH, HBG, NY. Cultures were obtained from single-ascospore isolations from fresh specimens of *Hypocrea lixii*. The herbarium specimens of *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 approx. 15 μm with a freezing microtome. Thirty-four teleomorph characteristics were evaluated, including: substratum; 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; ostiolar canal length; thickness, color and 3% KOH reaction of stroma outer region; 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

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were gathered using the image-capturing software Scion Image beta 4.0.2. Confidence intervals (a = 0.05), minimum and maximum values for the morphological characters measured were calculated using Systat 8.0 (SPSS, Inc., Illinois).

Results

Analysis of morphological data of type specimens of *H. lentiformis*, *H. lixii*, and *H. nigricans* f. *octospora*, and additional specimens of *H. lixii* show that these species are indistinguishable from each other and therefore represent one species. Because *Hypocrea lixii* Pat. is the oldest name, it is the correct name for this species.

Several cultures derived from specimens of *Hypocrea* identified by Y. Doi as *H. nigricans* (IFO 31294, 31289, 30611, 31285, 31286) were examined and were morphologically identical to *T. harzianum*. Unfortunately we were not able to study the *Hypocrea* specimens from which those cultures were derived. The type specimen of *H. nigricans* f. *nigricans* was not available from TNS. Specimens deposited by Doi in NY as *H. nigricans* are indistinguishable from the type collection of *H. lixii*.

We were not able to obtain the holotype specimen of *H. nigricans* f. *octospora* for study, but a paratype of this form, deposited in NY, was studied. *Hypocrea nigricans* f. *octospora* was distinguished from f. *nigricans* because it has eight green part-ascospores and eight smaller colorless part-ascospores. Within a perithecium of *H. nigricans* f. *octospora*, ascospore pigmentation and size segregated in various patterns, viz. 8:0, 4:4, 2:4:2, 2:2:2:2, or 1:6:1 suggesting that the loss of pigment and diminution in size of ascospores is the result of a mutation or a rare allele. The illustration provided by Doi (1972, Fig 41) suggests that the smaller, colorless part-ascospores are in fact malformed and probably not viable, further suggesting a 'spore killer' mutant (Van Der Gaaga et al. 2000, Raju & Perkins 1991). Doi (1972) noted that collections of f. *octospora* often only had 8 normal part-ascospores (as opposed to the expected 16), which is additional evidence for a 'killer' mutant. In our experience, in older specimens of *Hypocrea* it is not unusual to find some asci with aborted ascospores. Based on the study of available herbarium specimens of f. *octospora* and literature, we conclude that this *forma* falls within the normal circumscription of *H. lixii*.

The holotype of *H. lixii* is a collection made in Papua New Guinea, and no anamorph is associated with this specimen. Because stroma morphology in *Hypocrea* can be shared by more than one species, anamorph morphology can be critical to species recognition. Therefore, in the interest of nomenclatural stability we designate a specimen from Thailand (BPI 745654, culture G.J.S. 97-96, CBS 110080, ATCC MYA-2478), which we have cultured, and deposited in culture collections, to be the epitype of *H. lixii*. The designated epitype is on the same substratum (*Ganoderma*) as the holotype.

Taxonomy

Hypocrea lixii Pat., Rev. Mycol. Toulouse 13: 138. 1891 **Figs 1–9**

= *Hypocrea lentiformis* Rehm, Hedwigia 37: 193. 1898.

= *Chromocrea nigricans* Imai, Trans. Sapporo Nat. Hist. Soc. 14: 102. 1935.

≡ *Hypocrea nigricans* (Imai) Doi, Bull. Natl. Sci. Mus. Tokyo 15: 732. 1972.

= *Hypocrea nigricans* f. *octospora* Doi, Bull. Natl. Sci. Mus. 15: 734. 1972.

Anamorph: *Trichoderma harzianum* Rifai, Mycol. Pap. 116: 38. 1969 (for illustrations see Bissett 1991, Figs 56–63; Samuels et al. 2002, Figs 6–16).

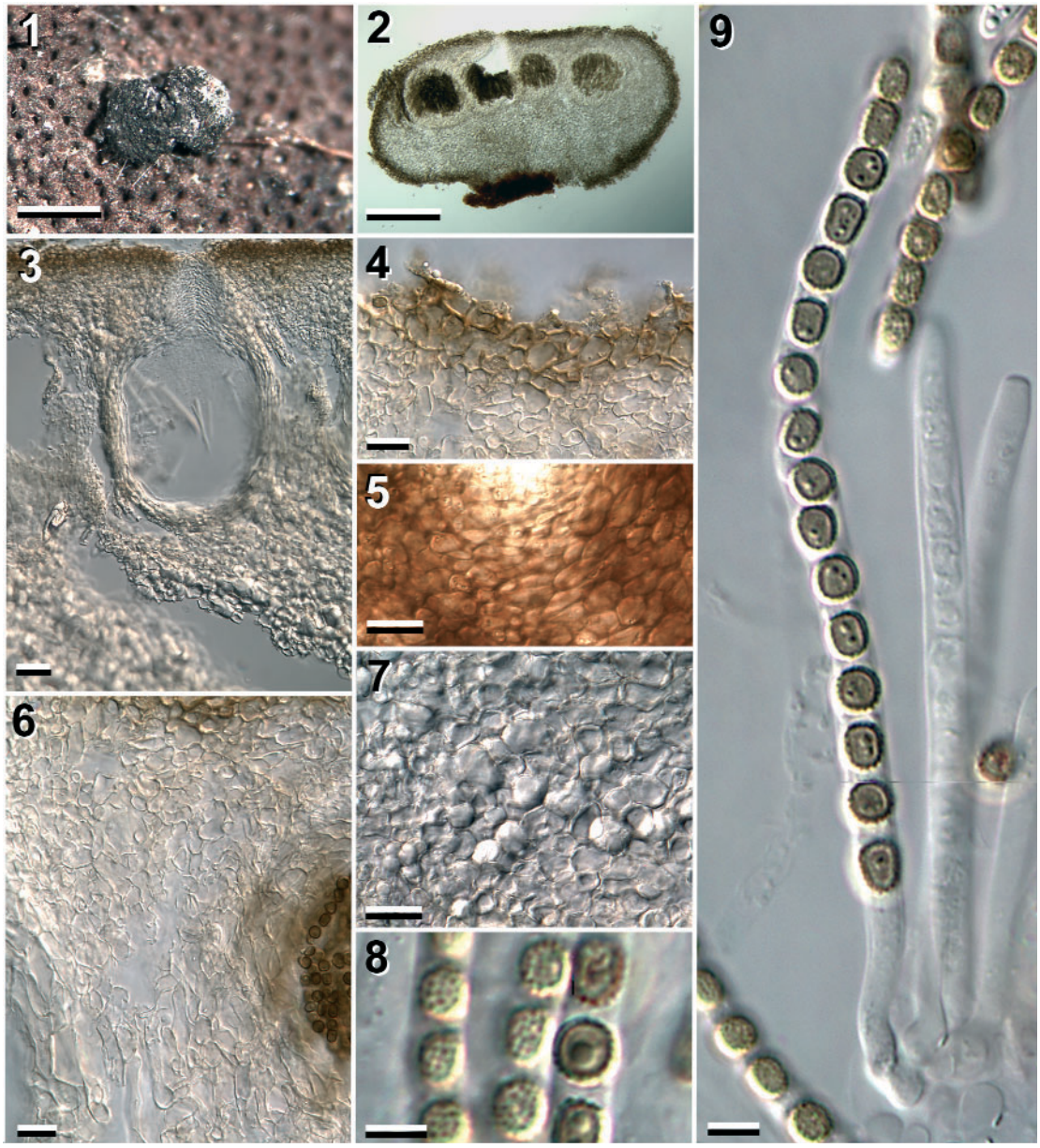
= *Trichoderma inhamatum* Veerkamp & W. Gams, Caldasia 13: 710. 1983.

Stromata solitary or aggregated, pulvinate, nearly circular in outline, (0.3)1.0–1.1(3.0) mm diam (n = 154), (440)730–785 (1400) µm high (n = 139), broadly attached, surface smooth, sometimes with slight perithecial protuberations, dark brown or green, almost black, changing color from dark green to brown in KOH, if stromata dark brown then KOH–, ostiolar openings not obvious due to the dark color of the stromata. Stroma surface (8)17–20(45) µm thick (n = 176), formed of angular cells, compacted, pigmented brown (KOH-) or dark green (KOH+), (2.7)7.8–8.5(17) µm diam (n = 398), walls (0.5)1.0 (2.0) µm thick (n = 294). Tissue immediately below the stromatal surface of compact to loose *textura angularis* to *t. epidermoidea*, colorless or slightly pigmented, (2.0)7.0–7.5 (22.0) µm diam (n = 366), walls (0.2)0.5–0.6(1.0) µm thick (n = 321). Internal tissue below the perithecia formed of angular cells, colorless, KOH–, (5.0)13–14(31) µm diam, walls (0.4)0.7–0.8(1.5) µm thick (n = 365). Perithecia completely immersed in stroma, generally closely aggregated, sometimes widely spaced, subglobose, (153)231–244(336) µm high, (75) 142–152(259) µm wide (n = 142), wall composed of compacted cells, KOH+ or –, (6.0)12.5–13.2(25.0) µm thick (n = 154), ostiolar canal (44)63–68(101) µm long (n = 125). Asci cylindrical, (44)73–78(138) x (3.0)4.5–4.7(6.5) µm (n = 372), ascospores uniseriate. Part-ascospores green, warted, dimorphic, distal part globose to subglobose, (3.0)4.3–4.4(5.6) x (2.8)3.9–4.0(5.2) µm, proximal part wedge-shaped to cylindrical, (3.4)4.5–4.6(6.5) µm (n = 472).

Habitat: Fungicolous, corticolous, lignicolous; infrequently on rotting leaves of palms.

Known distribution: Cosmopolitan.

Holotype: PAPUA NEW GUINEA, on hymenium of *Ganoderma pourii*, Jul. 1891, *Lix* (FH!).



Figs 1-9: *Hypocrea lixii* (Epitype BPI 745654). Fig 1. Stroma. Fig 2. Section of stroma. Fig 3. Perithecium. Fig 4. Outer tissue of stroma. Fig 5. Top view of stroma surface cells. Fig 6. Tissue immediately below the stromatal surface. Fig 7. Inner tissue below perithecia. Fig 8. Ascus and ascospores. Fig 9. Ascospore (notice the warted ornamentation). Bars: Fig 1 = 1 mm, Fig 2 = 250 μm, Fig 3 = 25 μm, Figs 4–7 = 15 μm, Figs 8, 9 = 5 μm.

Additional specimens examined

AUSTRIA, Niederösterreich, 23rd district of Vienna, Mauer Wald, on decorticated wood and black pyrenomycete, 3 Oct. 1998, *W. Jaklitsch* (G.J.S. 98-183, BPI 841387). – BRAZIL, Sta. Catharina State, on leaves of *Euterpe* sp., Aug. 1888, *Ule* (HBG #812, ISOTYPE of *H. lentiformis*). – FRANCE, Pyrénées Atlantiques, 64 Oloron, Forêt de Josbaig, on decorticated wood of *Fagus* sp., 13 Sep. 1992, *F. Candoussau* (G.J.S. 92-110, BPI 802854). – GERMANY, Thuringia, Weimar, Belvedere, on decaying wood of *Pinus sylvestris*, 8 Oct. 1990, *G. Arnold* (G.J.S. 90-254, BPI 1109306). – INDONESIA, North Sulawesi, Dumoga-Bone National Park, between Madison's Camp and '1440' Camp, on decaying wood, 5 Oct. 1985, *G.J. Samuels 2161* (G.J.S. 85-119, NY). – PAPUA NEW GUINEA, New Britain Island, Rabaul, on decaying palm leaves, 2 Jan. 1970, *Y. Doi* (NY, PARATYPE of *H. nigricans* f. *octospora* NS-1323, TNS.D-723, TNS-F-191628). – SWITZERLAND, on artificially inoculated *Pinus* sp. blocks, *O. Petrini* (G.J.S. 92-135, BPI 802883). – THAILAND, Saraburi Province, Khao Yai National Park, Wang Jumpee Trail, on hymenium of *Ganoderma* sp., 31 Jul. 1997, *K. Põldmaa, P. Chaverri, G.J. Samuels 8233* (BPI, EPITYPE of *H. lixii,* BPI 745654, G.J.S. 97-96, ATCC MYA-2478, CBS 110080); vicinity of park headquarters, on bark and corticioid basidiomycete, 30 Jul. 1997, *K. Põldmaa, P. Chaverri, S. Sivichai, G.J. Samuels 8201* (G.J.S. 97-106, BPI 745629). – UNITED STATES OF AMERICA, Alabama: Winston County, W.B. Bankhead National Forest, Sipsey Wilderness Area, Sipsey Recreation River along trail, on decorticated wood, 25 Sep. 1992, *C.T. Rogerson, S.M. Huhndorf, G.J. Samuels* (G.J.S. 92-

100, BPI 802845). Illinois: Shawnee National Forest, vicinity of Pomona, Pomona Natural Bridge, on decorticated wood and basidiomycete, 30 Sep. 1994, *W. Sundberg, G.J. Samuels* (G.J.S. 94-53, BPI 749348). Maryland: Prince George County, E of Largo, in old growth forest at Church Rd., on bark of decaying wood, 11 Oct. 1991, *A.Y. Rossman, S.E. Rehner, F.A. Uecker, G.J. Samuels* (G.J.S. 91-138, BPI 1112907). North Carolina: Macon County, Blue Valley, off Clear Creek Rd., along overflow Creek, on bark of decaying wood (probably on dematiaceous mycelia and lichen), 16 Oct. 1990, *Y. Doi, A.Y. Rossman, G.J. Samuels* (G.J.S. 90-127, BPI 1109390). Wisconsin: Sand County, Aldo Leopold Reserve, on decorticated wood (probably growing on dematiaceous mycelia), 23 Jun. 1990, *G. J. Samuels* (G. J. S. 90-22, BPI 802600).

Acknowledgments

This research was supported by NSF-PEET grant 9712308 to the Pennsylvania State University, Dept. of Plant Pathology, "Monographic Studies of Hypocrealean Fungi: *Hypocrea* and *Hypomyces*". Also a grant from the National Geographic Society to G.J.S. provided support for fieldwork in Indonesia, in collaboration with Royal Entomological Society 'Project Wallace.' We appreciate the assistance given by Dr. Nigel Hywel-Jones (Bangkok) in support of our fieldwork in Thailand. We thank our colleagues Günter Arnold, Françoise Candoussau and Walter Jaklitsch for providing us with European collections of *Hypocrea lixii*. Revisions and comments given by Drs. Amy Y. Rossman and Elwin L. Stewart were very helpful.

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Accepted: 17.5.2002