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Blue pigment in *Hypocrea caerulescens* sp. nov. and two additional new species in sect. *Trichoderma*

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Abstract

Three new species of *Hypocrea*/*Trichoderma* sect. *Trichoderma* (Hypocreaceae, Hypocreales, Ascomycota, Fungi) are described from recent collections in southern Europe and the Canary Islands. They have been characterized by morphological and molecular methods, including microscopic examination of the teleomorph in thin sections, the anamorph, growth rate experiments and phylogenetic analyses based on a part of the translation elongation factor 1- α encoding gene (*tef1*) containing the two last introns and a part of the *rpb2* gene, encoding the second largest RNA polymerase subunit. Analyses involving *tef1* did not unequivocally resolve the sister clade relationship of *Hypocrea caerulescens* relative to the *Koningii* and *Viride* clades, while analyses based on *rpb2* clearly suggest a close relationship with the former, although the phenotype of *H. caerulescens* is similar to *H. viridescens*, particularly by its warted conidia and a coconut-like odor in CMD culture. *Hypocrea hispanica* and *T. samuelsii* however are clearly related to the *Viride* clade by both phylogenetic markers, despite their morphological similarity to *H. koningii* and its relatives. An apparently specific blue pigment is formed in CMD cultures by *Hypocrea caerulescens* but could not be obtained by extraction with organic solvents.

Keywords

Ascomycetes; DNA barcode; Hypocreales; morphology; phylogenetic analysis; pigments; *rpb2*; *tef1*; *Trichoderma*

Introduction

Hypocrea (Hypocreaceae, Hypocreales), often addressed in the non-taxonomic literature under the anamorph name *Trichoderma*, is a well known genus due to its importance for humans (Harman and Kubicek 1998). Many physiological or biochemical activities have been ascribed to the type species *H. rufa* and its anamorph *T. viride* (Jaklitsch et al. 2006). The antagonistic action of many *Trichoderma* species mainly against asco- and

basidiomycetous fungi and certain oomycetous *Chromista* has made them powerful biocontrol agents to fight plant diseases, thereby also acting as opportunistic plant symbionts enhancing systemic resistance of plants (Harman et al. 2004, Harman 2006), for example as endophytes (Samuels et al. 2006b, Hanada et al. 2008, Chaverri et al. 2011).

Antimicrobial action is driven by mechanisms that comprise both enzymatic and antibiotic activities. Various industrial applications in food and textile processing involve *Trichoderma* enzymes such as β -glucanases, pectinases, xylanases, but industrially most lucrative or promising is mass production of cellulases and hemicellulases, on the one hand for the detergent industry, on the other for production of second generation biofuels from cellulosic waste (Schuster and Schmoll 2010). More than 430 entries on different secondary metabolites are listed in the current version of the natural product database, Antibase (Laatsch 2010), and 30 are known from *Hypocrea*; hence these fungi undoubtedly belong to the most prolific metabolite producers among the eukaryotes. Important secondary metabolites comprise antibiotics such as peptaibols and a broad array of volatile organic compounds including 6-pentyl- α -pyrone, which causes the coconut-like aroma of several species of sect. *Trichoderma*, but also trichothecene mycotoxins (Ghisalberti and Sivasithamparam 1991; Degenkolb et al. 2006; 2008a, b). Some species have gained particular attention due to their destructive action: Those that are able to grow at 37 C, mostly belonging to the *Longibrachiatum* clade (e.g. *Hypocrea orientalis*), are opportunistic human pathogens of immunocompromised patients (Kredics et al. 2003; Druzhinina et al. 2008a, b). Other species causing green mold disease are responsible for serious losses in commercial mushroom cultivation (Park et al. 2006, Hatvani et al. 2007). Species of *Trichoderma* are susceptible to strain improvement and genetic engineering via transformation, opening a wide field of applications in addition to their wide array of intrinsic potential (Schuster and Schmoll 2010).

Pigments of Hypocreales so far have not been reported to have potential importance for industrial applications. In contrast to many members of the Xylariaceae, brightly colored stromata of *Hypocrea* are devoid of pigments extractable with KOH. In culture *Hypocrea* species, particularly in sect. *Hypocreanum* and *Longibrachiatum*, often form yellow to orange, less commonly reddish pigments on PDA. On CMD however pigments are only rarely formed and mostly after prolonged storage, for example yellow in *H. atroviridis* (at 30 C), *H. aureoviridis*, *H. petersenii*, *H. psychrophila* or *H. voglmayrii*, brown in *H. crystalligena* and green in *H. aeruginea* (Jaklitsch 2009, 2011).

Blue pigments from fungi however are produced extremely rarely. Even though numerous lichenized as well as nonlichenized ascomycetes show blue, these colors cannot be extracted with organic solvents and might be due to the presence of metal ion complexes of certain secondary metabolites that do not constitute pigments themselves. For instance the characteristic blue of certain *Penicillium* species is probably due to the presence of dimeric zinc complexes of phenaleones (Robinson et al. 1992). Likewise the blue and green pigments of certain hypoxyloid Xylariaceae coincide with the presence of “lichen compounds” of the lepralic acid type, which are major extractable stromatal constituents (Læssøe et al. 2010), but are uncolored in their pure state.

Jaklitsch (2009, 2011) gave a comprehensive account of European taxa of *Hypocrea*. However southern Europe could be studied only after that work. Conidial colonies were collected from plant and fungal material in addition to *Hypocrea* teleomorphs in that region including the Canary Islands. As a preliminary result the most commonly encountered species belong to the section *Trichoderma*. This is the most complex and morphologically most conserved group of species within the genus. Three new species in the section

Trichoderma are described below, of which *Hypocrea caerulescens* is the most common and most remarkable due to the formation of a blue pigment in CMD cultures.

Materials and methods

Isolates and specimens

Ascospore and conidial isolates were prepared as described by Jaklitsch (2009). Specimen information is given for each species after its description; the letter S stands for southern Europe and is used for both specimens and cultures. Representative isolates have been deposited at the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands (CBS). Specimens have been deposited in the Herbarium of the University of Vienna (WU).

Growth, morphology, DNA extraction, PCR and sequencing

Growth rate experiments were carried out and teleomorph and anamorph morphology was studied as described by Jaklitsch (2009). Genomic DNA extraction, PCR and sequencing were performed as described in that work, except that before DNA extraction by the DNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) mycelium was grown in 2% liquid malt extract culture, harvested by filtration, freeze dried and ground according to Voglmayr and Jaklitsch (2008). GenBank accession numbers of sequences generated in this study are provided (Table I). Accession numbers of sequences retrieved from GenBank are given on the phylogenetic trees (Figs. 1, 2).

Analysis of sequence data

ITS, *tef1* and *rpb2* sequences of all available species of section *Trichoderma* were downloaded from GenBank and combined with those used by Jaklitsch (2009, 2011). For ITS only a sequence alignment was produced with MUSCLE 3.6 (Edgar 2004) to enable sequence comparisons, but no further phylogenetic analyses were performed due to insufficient phylogenetic information. *tef1* and *rpb2* sequences were aligned for a first rough phylogenetic analysis to select representative sequences for the final analyses. A single representative sequence subsequently was selected for each species, preferably from the types. Because several type sequences were short these were replaced by identical or highly similar sequences from other authentic sources where available or by those determined for newly isolated strains. For the new species several representative sequences were included in the matrix. Sequence alignments were produced with MUSCLE3.6 (Edgar 2004). The resulting alignments were checked and refined with BioEdit 7.0.9.0 (Hall 1999). After exclusion of excessive leading/trailing gap regions, the *tef1/rpb2* matrices contained 1353/1067 characters from 85/44 sequences respectively.

Maximum parsimony (MP) analyses of the data matrices were performed with PAUP* 4.0 b10 (Swofford 2002), using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, COLLAPSE = MAXBRLEN, steepest descent option not in effect). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data. BS analysis with 1000 replicates was performed in the same way, but using five rounds of random sequence addition and subsequent branch swapping during each BS replicate; the number of rearrangements per replicate was limited to 10 000 000 in the *tef1* analyses.

For ML analyses 500 bootstrap replicates were computed with RAxML 7.0.4 (Stamatakis 2006) using the GTRCAT algorithm. For Bayesian analyses the computer program MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) was used, implementing the well known general time reversible model and additionally assuming a proportion of invariant sites with gamma-distributed substitution rates of the remaining sites (GTR + I + G). Three parallel runs of

four incrementally heated simultaneous Markov chains were performed over 5 000 000 generations from which every 500th tree was sampled in each run. The first 1000 trees were discarded. A 90% majority rule consensus of the remaining trees was computed to obtain estimates for the probabilities that groups are monophyletic given the sequence data (posterior probabilities).

Analytical studies on the pigments of *H. caeruleus*

Studies were carried out with a well established methodology that had been used in the past to detect and identify secondary metabolites of Xylariaceae (Stadler and Fournier 2006, Stadler et al. 2008) and relying on one of the most comprehensive spectral libraries of natural products (Bitzer et al. 2007). Well grown mycelia from cultures of *H. caeruleus* were retrieved from the agar plates, transferred into glass vials and extracted with methanol or acetone for 1 h. The extracts were concentrated in vacuo, redissolved in methanol and subjected to HPLC-DAD/MS profiling. Because the results for the regular organic extracts appeared to be inconclusive the procedure was repeated by a modified extraction procedure. The methanol was adjusted to pH 2 or pH 11 by adding respectively concentrated trifluoroacetic acid or KOH solution, and the extraction was repeated in a similar manner as described above for the preparation of conventional methanol extracts.

Results

Molecular phylogenetic analyses

The final alignments and the trees obtained were deposited in TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S11946>). ITS sequences of *H. hispanica* and *T. samuelsii* were almost or completely identical with those of *H. koningii*, *viridescens* and *H. petersenii*, demonstrating the unsuitability of ITS as barcoding marker within this group, as already shown by other authors (Druzhinina et al. 2005). On the contrary, the ITS of *H. caeruleus* was characteristically distinct from all other species of sect. *Trichoderma*, with sequence differences (including gaps) of at least 19 bp.

Of the 1353 characters of the *tef1* alignment included in the analyses, 284 were parsimony informative. MP analyses revealed 224 MP trees 1191 bp long, one of which was selected and included herein (Fig. 1). Topologies of all MP trees were identical except for minor differences within the *caeruleus*, *hispanica* and *samuelsii* subclades, within the subclade containing *H. rufa*, *T. scalesiae* and *T. martiale*, and a slightly different position of the *atroviridis-erinaceus* subclade (Fig. 1). The three Bayesian runs revealed almost identical posterior probabilities (PP) and were fully compatible with the MP strict consensus tree.

Of the 1067 characters of the *rpb2* alignment included in the analyses, 227 were parsimony informative. MP analyses revealed two MP trees 847 bp long, one of which was selected and illustrated (Fig. 2). Topologies of the two MP trees differed only slightly in the phylogenetic position of *H. rufa* and *H. valdunensis*. The three Bayesian runs revealed almost identical posterior probabilities (PP) and were fully compatible with the MP strict consensus tree. Topologies of *tef1* and *rpb2* trees differed especially in the deeper nodes (Figs. 1, 2); however most of these topological differences did not receive significant support in the *tef1* tree (Fig. 1). The *caeruleus*, *hispanica* and *samuelsii* subclades were highly supported in both analyses, as was the sister group relationship of the latter two taxa. In the *rpb2* analyses *H. caeruleus* is a closest relative to a core *Koningii* clade with high support, whereas its phylogenetic position remains unclear in the *tef1* trees due to poor backbone support. In both phylogenetic trees *H. hispanica* and *T. samuelsii* are members of the Viride clade with medium to high support, but sister group relationship to *H. junci* lacks significant support (Figs. 1, 2).

Characterization of the pigment formed in culture

The native methanol and acetone extracts of mycelia of *H. caerulescens* grown in CMA as well as extracts prepared with acidified methanol remained largely uncolored, and the remaining mycelia remained grayish blue. Accordingly the HPLC analyses were inconclusive and no compounds featuring UV-visible maxima in the range of blue light were detected. A discoloration of the mycelia was observed only upon extraction with alkaline methanol. However in this case the extracts immediately turned yellowish, suggesting that the pigments of the mycelia had been dissolved. Treatments of these yellowish solutions by careful titration with trifluoroacetic acid or HCl to neutralize or acidify them in the hope of recovering blue pigment failed however, and the yellowish tint persisted. In the resulting HPLC profiles of such samples no distinct peaks with absorption maxima in the range of yellow light, but only a substantial tailing was observed, indicating that an original constituent of the mycelia might have decayed. The pigment of *H. caerulescens* is either an unstable compound that changed its chemical composition under the drastic extraction conditions or it could as well be an ion complex that is destroyed by high pH and cannot be reinstated by neutralization, according to these preliminary results.

Taxonomy

Hypocrea caerulescens Jaklitsch & Voglmayr sp. nov. Figs. 3, 4

Mycobank MB563315

Etymology: *Caerulescens* for the blue CMD agar discoloration upon storage at 15 C.

Stromata pulvinata, in vivo aurantiobrunnea, sicca fusce rubrobrunnea, tomentosa, 0.5–4 mm lata. Asci cylindrici, (72–)82–96(–102) × (5.0–)5.5–6.0(–6.5) μm. Ascospores bicellulares, hyalinae, verruculosae, ad septum disarticulatae, pars distalis (sub)globosa, vel ellipsoidea, (4.0–)4.2–4.7(–5.0) × (3.0–)3.5–4.0(–4.5) μm, pars proxima oblonga vel ellipsoidea, (4.0–)4.8–5.7(–6.5) × (2.7–)3.0–3.7(–4.2) μm. Agarum CMD caerulescens in cultura ad 15 C. Conidiophora anamorphosis effuse disposita in agaris CMD at SNA, verticillio vel trichodermati similia. Phialides divergentes, anguste lageniformes, (4.5–)6.5–11(–16) × (2.0–)2.7–3.7 (–4.5) μm. Conidia ellipsoidea vel subglobosa, viridia, verruculosa, (3.0–)3.5–4.3(–5.5) × (2.7–)3.0–3.5(–3.8) μm.

Stromata when fresh 0.5–3(–4) mm, 0.5–1 mm thick, scattered or aggregated in small groups, first subeffuse, whitish to yellow, velutinous, later pulvinate with variable outline, pale orange, orange–brown or light brown. Surface becoming smooth and slightly tubercular; margin free, undulate. Ostiolar dots invisible, rarely appearing as diffuse minute dark spots. Spore deposits white. Stromata when dry (0.7–)1–2 mm diam, up to 4 mm long when young and subeffuse, 0.2–0.7 mm thick (n = 35), first appearing as white hairy tufts, turning yellow from the center, subeffuse or discoid when young, often wrinkled, with evanescent rust surface hairs and sometimes radiating white marginal mycelium; later flat pulvinate or discoid; outline variable, often angular or oblong; margin mostly free, sometimes lobed; surface smooth, uneven to tubercular; ostiolar dots invisible, only rarely visible under high magnification, 15–31(–55) μm diam, reddish to subhyaline. Stromata mostly dark reddish brown, or orange–brown, violet–brown to nearly black, 8–10F5–8, 8E5–8. Rehydrated stromata dark reddish or orange–brown, turning slowly dark red in 3% KOH.

Stroma anatomy: Cortical layer (17–)19–28(–35) μm thick (n = 30), orange–brown, paler and more yellow downward, comprising a dense t. angularis of indistinct, thin– to thick–walled cells (3.5–)4–8(–12) × (2.5–)3.0–6.0(–7.3) μm (n = 30) in section. Hairs, (7.5–)9–23(–37) × (2.5–)3.3–4.5(–5.0) μm (n = 30), scant on the surface, more frequent in lateral

regions, cylindrical with narrowly rounded ends, 1–3-celled, light brown, thick-walled, warted. Subcortical tissue a t. intricata of hyaline, thin-walled hyphae (3–)4–6(–8) μm wide (n = 30), mixed with some angular cells. Subperithecial tissue a t. angularis–epidermoidea comprising hyaline, thick-walled cells (7.5–)9–21(–26) $\times$ (5–)6–13(–19) μm (n = 30). Stroma base mostly of parallel, often compressed, thick-walled yellow–brown hyphae (2–)3–5(–8) μm wide (n = 30). Perithecia (174–)195–235(–250) μm high, (127–)150–206(–230) μm wide (n = 30). Peridium (12–)15–21(–25) wide at the base, (10–)12–18(–20) μm at the sides (n = 30). Ostioles not projecting, (63–)70–84(–90) μm long, at the apex (19–)24–39(–47) μm wide inside, (40–)45–60(–70) μm wide outside (n = 30), periphysate. Asci (72–)82–96(–102) $\times$ (5.0–)5.5–6.0(–6.5) μm ; apex slightly thickened, with a minute ring; stipe (4–)5–11 (–15) μm long (n = 40). Ascospores hyaline, verruculose; cells dimorphic, distal cells (sub)globose or ellipsoid, (4.0–)4.2–4.7(–5.0) $\times$ (3.0–)3.5–4.0(–4.5) μm , l/w (1.0–)1.1–1.3(–1.5) (n = 95), proximal cells oblong or ellipsoid, (4.0–)4.8–5.7(–6.5) $\times$ (2.7–)3.0–3.7(–4.2) μm , l/w (1.2–)1.4–1.7(–2.1) (n = 95).

Anamorph on natural substrates: Colonies effuse or pulvinate, first white, turning light to dark green with or without white margin, often with bluish tone; from few millimeters to 9 cm long, granulose, farinose, hairy or fluffy, sometimes associated with yellow mycelium. Conidia and phialides, determined for the specimen of the holomorph, as given below under CMD cultures.

Cultures and anamorph: Optimal growth at 25 C on all media, slow and restricted growth at 30 C, no growth at 35 C. On CMD after 72 h 24–28 mm at 15 C, 30–41 mm at 25 C, 0.5–3.5 mm at 30 C; mycelium covering the plate after 5–6 d at 25 C. Colony hyaline, dense, circular or with ill-defined margin; mycelium not zonate, with conspicuous difference in width between primary and secondary hyphae, the latter short, thin and curly. Aerial hyphae only at the distal margin conspicuous and long, becoming fertile. Autolytic excretions absent, coilings inconspicuous. Chlamydospores detectable after 6–7 d, infrequent, (8–)9–13(–14) $\times$ (6–)8–12(–13) μm , l/w 0.9–1.3(–1.9) (n = 30). Within 2 wk no diffusing pigment detectable, but after 1–2 mo at 15 or 25 C a peculiar grayish blue pigment, 22–23B1–3, appearing in the agar in patches or on the entire plate. Often also mycelial spots or tufts or brown spots formed by pigmented hyphal segments. Coconut-like odor formed after more than 2 wk, rarely earlier (e.g. after 10 d). Conidiation appearing after 2 d, effuse, dry, short, in loose shrubs or on aerial hyphae, spreading over the entire plate, concentrated in one or several, broad, ill-defined, scarcely separated, loose, downy to floccose, later farinose zones including distal margin, with radial arrangement of shrubs within, after 4–6 d turning faintly greenish or dark bluish green 25–26EF4–6. Conidiation structure (four isolates, after 6–16 d on CMD and SNA at 25 C): Stipe or aerial hypha and primary branches of shrubs thick-walled, to 6–7 μm wide (with some thickenings and globose branching points to 8 μm), asymmetrically terminating in long and often broad, loosely branched, regularly tree-like conidiophores. Conidiophores verticillium-like with little rebranching, comprising loosely arranged side branches or phialides mostly in whorls of 2–4(–5), or singly, around a distinct main axis. Side branches 1.5–4 μm wide, consisting of 1–3 long cells, with some 1–2-celled terminal side branches up to ca. 130 μm long; side branches usually perpendicular to the main axis or aerial hypha, terminal branches also in steep angles upward. Thickenings inconspicuous, except for phialide origins that are slightly thickened terminally (e.g. 3.5 μm vs. 2.5 μm of the residual cell). Phialides solitary or in whorls of 2–4, (4.5–)6.2–10.8(–16) $\times$ (2.0–)2.7–3.7(–4.5) μm , l/w (1.2–)1.6–3.9(–7.1), (1.2–)1.5–2.3(–3.2) μm wide at the base (n = 145), narrowly lageniform, less commonly ampulliform and then often with long neck, straight or curved or sometimes sigmoid, non-repetitive. Conidia (3.0–)3.7–4.5(–5.5) $\times$ (2.7–)3.0–3.5(–3.8) μm , l/w (1.0–)1.1–1.4(–1.9) (n = 120), ellipsoid, oval or subglobose,

green, distinctly warted when young; warts less conspicuous when mature or old (e.g. after 12 d), scar inconspicuous.

On PDA after 72 h 19–24 mm at 15 C, 27–36 mm at 25 C, 0.5–2 mm at 30 C; mycelium covering the plate after 6–7 d at 25 C. Colony circular, dense. Aerial hyphae abundant, forming strands and a whitish hairy or floccose mat; plug and surroundings turning green. Autolytic excretions and coilings inconspicuous. Conidiation appearing after 1–2 d, remaining colorless or turning green after 3–5 d, effuse, inconspicuous, with low conidial yield, often only around the plug. Sometimes grayish bluish patches appearing from the center, also at 15 C. Odor indistinct.

On SNA after 72 h 17–22 mm at 15 C, 24–30 mm at 25 C, 0.7–3.5 mm at 30 C; mycelium covering the plate after 6–7 d at 25 C. Colony hyaline, dense, more or less circular, of hyphae as on CMD, colony not zoned. Aerial hyphae inconspicuous, common and long toward margin, becoming fertile. Autolytic excretions absent or rare; coilings frequent; Chlamydospores detected after 5–7 d, uncommon but more frequent than on CMD. No pigment produced; odor indistinct. Conidiation appearing after 2 d, abundant, effuse, spreading from the center over the entire colony, becoming concentrated in up to 5 indistinctly separated concentric zones, after 3–4 d successively turning green 26–27DE3–6 from the center outward, farinose and with radial arrangement within each zone; delicate, in loose but densely disposed shrubs and on aerial hyphae; conidia formed in mostly dry heads less than 20 µm diam; more abundant and faster than on CMD.

Distribution: Southern Europe (Croatia, Italy, Portugal (Madeira), Spain, including the Canaries).

Habitat: On wood and bark of various shrubs and trees and on fungi growing on them.

Holotype: SPAIN, CANARIAS, LA PALMA, Montaña Tagoja, 28°43′18″N, 17°47′07″W, 1040 m, on medium decayed branches 1–5 cm thick of *Erica arborea*, on wood, holomorph, soc. *H. cf. minutispora*, 14 Dec 2009, *W. Jaklitsch* (WU 31600; culture S195 = CBS 130011).

Other material examined (generally on branches or twigs, except where noted): *Teleo-, holomorphs:* ITALY, APULIA, Andria, Parco Nazionale dell'Alta Murgia, Castel del Monte, between SP234 and Masseria Savignano, 41°02′33″N, 16°18′24″E, 380 m, on medium to well decayed *Olea europaea* twigs, 0.5–1 cm diam, lying in moist grass and moss, on wood and a *Hymenochaete* sp. soc. effete pyrenomycete in wood, a Hysteriaceae, a coelomycete, *Mollisia* sp. and ample anamorph, 19 Nov 2009, *W. Jaklitsch & H. Voglmayr* (WU 31599; culture S71). Foggia, Gargano, NE from Mattinata, 41°44′48″N, 16°08′00″E, 80 m, on medium to well decayed twigs, 1–5 cm thick, of *Olea europaea*, on wood, teleomorph immature, 20 Nov 2009, *H. Voglmayr & W. Jaklitsch* (culture S76). PORTUGAL, MADEIRA, Portela, PR5, close to its start, 32°44′48″N, 16°49′16″W, 560 m, on twigs 0.7–2 cm thick of *Clethra arborea*, on medium decayed wood, soc. *Biscogniauxia cf. capnodes*, *Stictis* sp., white mold and algae, 21 Feb 2010, *W. Jaklitsch & O. Sükösd* (WU 31602; culture S206 = CBS 130012). Ribeiro Frio, Levada to Portela, 32°44′11″N, 16°53′11″W, 880 m, on well decayed, decorticated branches, 3–4 cm thick, of *Persea indica*, on bark, 16 Feb 2010, *W. Jaklitsch & O. Sükösd* (WU 31601; culture S196). SPAIN, CANARIAS, LA PALMA, Cumbre Nueva, 28°38′27″N, 17°49′50″W, 1040 m, on *Erica arborea*, 2 Dec 2010, *W. Jaklitsch* (WU 31603; culture S439); branching off LP-301 at Area Recreativa del Pilár, 28°37′24″N, 17°49′53″W, 1460 m, on branch, 4 cm thick, of *Myrica faya*, teleomorph scant and immature, 11 Dec 2009, *W. Jaklitsch* (culture S173); Mazo, LP2062, Pista Cabrita, 28°35′48″N, 17°48′04″W, 950 m, on *Erica*

arborea, 5 Dec 2010, *W. Jaklitsch* (WU 31605; culture S460); El Paso, *Castanea* plantation at LP 301, close to crossing with LP 3, on *Chamaecytisus proliferus*, 2 Dec 2010, *W. Jaklitsch* (WU 31604; culture S441).

Anamorphs: CROATIA, ISTRIA, forest N of Barbariga, ca. 20 m, on *Quercus ilex*, 14 May 2010, *W. Jaklitsch* & *H. Voglmayr* (cultures S259, S261). Fazana, forest at Valbandon, on *Phoenix canariensis*, 26 Sep 2010, *H. Voglmayr* & *W. Jaklitsch* (culture S277); same place, on *Cornus mas*, soc. *Stictis* sp., 17 Oct 2010, *W. Jaklitsch* (culture S304); close to St Golas, on *Diatrype stigma/Quercus pubescens*, 26 Sep 2010, *W. Jaklitsch* (culture S285). Vrsar, beach forest at Petalon Resort, 20 m, on *Ulmus minor*, *Phillyrea angustifolia* and *Quercus ilex*, 13 May 2010, *H. Voglmayr* & *W. Jaklitsch* (cultures S252-S255). Losinj, hiking trail from Veli Losinj to Javorna Uvala, on *Laurus nobilis*, 16 Oct 2010, *W. Jaklitsch* (culture S296); Jamna Uvala, on *Arbutus unedo*, 16 Oct 2010, *W. Jaklitsch* (culture S299). ITALY, APULIA, Foggia, Gargano, NE from Mattinata, 41°44'49"N, 16°07'51"E, 120 m, on 1 cm thick, medium decayed twigs of *Phillyrea latifolia*, on bark and wood, 20 Nov 2009, *W. Jaklitsch* & *H. Voglmayr* (culture S78). CALABRIA, Cosenza, Parco Nazionale del Pollino, above Morano Calabro, 39°50'48"N, 16°06'47"E, 650 m, on *Ostrya carpinifolia*, 3 cm diam, on wood, soc. *Echinospaeria* aff. *canescens*, 18 Nov 2009, *H. Voglmayr* & *W. Jaklitsch* (culture S57). Lazio, close to Magagnano, at the Strada Magagnano-Roccalvecce, left shortly before reaching the brook, to ½ m up, on corticated, standing branch, 2–4 cm thick, of *Quercus virgiliana*, soc. *Peniophora* sp., *Vuilleminia*, *Steccherinum ochraceum*, 25 Nov 2009, *H. Voglmayr* & *W. Jaklitsch* (culture S126). SARDINIA, roadside of SP 38 at junction to Isalle Orrule, 40°21'49"N, 09°31'00"E, 230 m, on *Cistus monspeliensis*, on bark, soc. old pyrenomycete, 2 Nov 2009, *W. Jaklitsch* (culture S2); at Su Gologone, 40°17'23"N, 09°29'38"E, 215 m, on *Alnus glutinosa*, 3 Nov 2009, *W. Jaklitsch* (culture S8) and 40°17'19"N, 09°29'36"E, 230 m, on *Pistacia lentiscus*, soc. *Xylaria* sp., 3 Nov 2009, *W. Jaklitsch* (culture S9); S of Oliena along SP22 to Orgosolo, 40°14'24"N, 09°23'59"E, 600 m, on *Stereum hirsutum/Quercus ilex*, 4 Nov 2009, *W. Jaklitsch* (culture S14); N of Sarule, 40°14'21"N, 09°09'57"E, 650 m, on *Quercus virgiliana*, on bark, 5 Nov 2009, *W. Jaklitsch* (culture S17). PORTUGAL, MADEIRA, Funchal, Botanical Garden, on ?*Callistemon* sp., 19 Feb 2010, *W. Jaklitsch* & *O. Sükösd* (culture S203). Portela, route PR5, close to its start, on ?*Acacia melanoxylon*, soc. *Mollisia* sp., 21 Feb 2010, *W. Jaklitsch* & *O. Sükösd* (culture S205). SPAIN, BALEARES, MALLORCA, Es Capdella, along Torrent de Galatzó, on a *Diatrypaceae*, 21 Nov 2010, *W. Jaklitsch* (culture S421); same place, same date, on *Calicotome spinosa*, soc. *Hypocrea* sp., *W. Jaklitsch* (culture S424a). CANARIAS, LA GOMERA, TF713, shortly after junction to Hermigua, on *Myrica faya*, 9 Dec 2010, *W. Jaklitsch* (culture S464). La Palma, El Paso, *Castanea* plantation at LP 301, close to crossing with LP 3, on *Castanea sativa*, 2 Dec 2010, *W. Jaklitsch* (culture S432); same place, same date, on *Erica arborea*, *W. Jaklitsch* (culture S434). Mazo, LP2062, beginning of the walking path Camino La Banda, on *Erica arborea*, 5 Dec 2010, *W. Jaklitsch* (culture S459). Montaña Tagoja, on *Erica arborea*, 7 Dec 2010, *W. Jaklitsch* (culture S462). TENERIFE, Macizo de Anaga, Pico del Ingles, on *Erica platycodon*, 11 Apr 2010, *W. Jaklitsch* (culture S214); El Pijaral, Chinobre, on moss on *Erica platycodon*, 15 Apr 2010, *W. Jaklitsch* (culture S232); Montaña Chamuscada, on *Laurus novocanariensis*, 16 Dec 2010, *W. Jaklitsch* (culture S479); at the road to Pedro Álvarez shortly before the crossing Los Batanes, on stromata of a *Nemania* sp. on *Persea indica*, 15 Apr 2010, *W. Jaklitsch* (culture S237). Orotava, above Aguamansa/La Orotava, path at Mirador de Matanzos, on a *Corticaceae* on *Myrica faya*, 12 Apr 2010, *W. Jaklitsch* (culture S221); Pista de Benijos, on a corticiaceous fungus on *Myrica faya*, 12 Apr 2010, *W. Jaklitsch* (culture S218); on *Erica arborea*, *W. Jaklitsch* (culture S219); slightly above Pista de Benijos/camping place La Caldera, on resupinate polypore on *Eucalyptus globulus*, 16 Apr 2010, *W. Jaklitsch* (culture

S245). San Juan de la Rambla, Barranco Rambla de Ruíz, on *Erica arborea*, 12 Apr 2010, W. Jaklitsch (cultures S216, S217).

Notes: *Hypocrea caerulescens* is a common species; 50 specimens were collected within 17 mo. Most frequently this species is found as anamorph or in association with immature stromata (e.g. S71, S76) in the Mediterranean region. It is unknown whether the species also occurs farther north because no anamorphs have been collected earlier. Blaszczyk et al. (2011) did not report this species from Poland.

The most conspicuous trait of *Hypocrea caerulescens* is the grayish blue discoloration of CMD agar upon storage 1–2 mo at 15 C. This has been observed in 46 of 50 isolates of this species and in none of any other species of the genus. *Hypocrea caerulescens* shares verruculose conidia with *H. viridescens* but differs from that species by more delicate and effuse conidiation, in non-repetitive phialides, a retarded formation of the coconut-like odor, poor growth at 30 C on all media, and particularly in the peculiar grayish blue pigment on CMD. Young conidia of *H. caerulescens* sometimes show wing-like projections as known from *Trichoderma saturnisporum*.

***Hypocrea hispanica* Jaklitsch & Voglmayr sp. nov. Figs. 3, 4**

MycoBank MB563316

Etymology: for its currently exclusive occurrence in Spain including the Canaries.

Stromata subeffusa vel pulvinata, fusce brunnea, tomentosa, 0.5–3 mm lata. Asci cylindrici, (72–)75–87(–96) × (5.5–)5.8–6.8(–7.2) µm. Ascospores bicellulares, hyalinae, verruculosae, ad septum disarticulae, pars distalis (sub)globosa, (3.8–)4.0–4.7(–5.0) × (3.5–)3.8–4.2(–4.5) µm, pars proxima subglobosa vel oblonga, (3.7–)4.2–5.3(–6.0) × (3.0–)3.3–3.8(–4.0) µm. Conidiophora in pustulis disposita in agar CMD et SNA. Phialides divergentes, lageniformes, (4.5–)6–11(–17) × (2.0–)2.5–3.2(–4.0) µm, interdum repetitae, saepe arcuatae. Conidia ellipsoidea vel oblonga, viridia, glabra, (3.5–)4.3–5.0(–6.0) × (2.8–)3.0–3.5(–4.0) µm.

Stromata solitary, gregarious or densely aggregated into amorphous masses of ca. 6 mm diam, when fresh ca. 0.5–3 mm diam, subeffuse or flat pulvinate with irregular outline, velutinous, orange–brown. Stromata when dry (0.5–)1–2.4(–2.9) × (0.5–)0.8–1.7(–2.1) mm, (0.2–)0.3–0.6(–0.9) mm thick (n = 17), 20–25% larger after rehydration, pulvinate or forming thin crusts with stromata of irregular shapes; outline round, angular, oblong or irregular; margin free, often for a large part, sterile sides often covered by rust mycelium; surface tubercular, stroma tubercular, projecting ostioles involving overlying cortex; ostiolar openings hyaline, only visible after rehydration under strong magnification; dark brown or violaceous brown, turning dark red in 3% KOH, clothed with yellow–brown to rust hairs. Spore deposits white.

Stroma anatomy: Cortical layer (14–)17–30(–35) µm thick (n = 30), reddish brown, around entire stroma where unattached, comprising a t. angularis of indistinct small cells (2.5–)3.5–7.5(–9.0) × (2.0–)2.7–4.5(–5.5) µm in section (n = 30), lighter and more thin-walled downward. Hairs on stroma surface (8–)10–18(–22) × (2.5–)3.0–4.5(–5.7) µm (n = 30), numerous, cylindrical with rounded ends, smooth or warted, yellow– or light brown, narrow, thick-walled, sometimes basally widened. Subcortical tissue a hyaline, dense t. intricata of thin-walled hyphae (2.5–)3–5(–6) µm wide (n = 30). Subperithecial tissue a hyaline t. angularis–epidermoidea of thin-walled cells, (6–)8–20(–29) × (4–)6.5–12 (–15) µm (n = 30). Stroma base of thick-walled cells and (2.2–)2.5–5.5(–8.5) µm wide (n = 30), reddish brown hyphae; attached areas comprising a hyaline cellular tissue deeply emerging from

bark. Perithecia ellipsoid to globose, $(190\text{--}210\text{--}250\text{--}265) \times (104\text{--}120\text{--}192\text{--}246) \mu\text{m}$ ($n = 20$); peridium $(14\text{--})16\text{--}23\text{--}(29) \mu\text{m}$ wide at the base ($n = 20$), $(9\text{--})12\text{--}21\text{--}(24) \mu\text{m}$ at sides ($n = 20$), sometimes slightly yellowish at the base. Ostioles cylindrical, $(59\text{--})76\text{--}108\text{--}(120) \mu\text{m}$ long, projecting to ca. $40 \mu\text{m}$ including cortex, $(13\text{--})16\text{--}27\text{--}(35) \mu\text{m}$ wide at the apex inside. Asci $(72\text{--})75\text{--}87\text{--}(96) \times (5.5\text{--})5.8\text{--}6.8\text{--}(7.2) \mu\text{m}$, stipe $(6\text{--})8\text{--}15\text{--}(17) \mu\text{m}$ long ($n = 20$). Ascospores hyaline, verruculose, cells dimorphic, distal cells globose or subglobose, $(3.8\text{--})4.0\text{--}4.7\text{--}(5.0) \times (3.5\text{--})3.8\text{--}4.2\text{--}(4.5) \mu\text{m}$, l/w $1.0\text{--}1.2\text{--}(1.3)$ ($n = 30$), proximal cells oblong, subglobose or wedge-shaped, $(3.7\text{--})4.2\text{--}5.3\text{--}(6.0) \times (3.0\text{--})3.3\text{--}3.8\text{--}(4.0) \mu\text{m}$, l/w $(1.1\text{--})1.2\text{--}1.5\text{--}(1.7)$ ($n = 30$), often subglobose and often smaller than the distal cell.

Cultures and anamorph: Optimal growth at 25 C on all media, no growth at 35 C. On CMD after 72 h 27–28 mm at 15 C, 50–51 mm at 25 C, 23–24 mm at 30 C; mycelium covering the plate after 4–5 d at 25 C. Colony hyaline, dense, not zonate, without distinct radial arrangement, margin ill defined. Hyphae with conspicuous difference in width, marginal primary surface hyphae wide, surface hyphae soon degenerating, hyper branching causing small white spots within the agar particularly in a broad marginal zone. Aerial hyphae inconspicuous. Autolytic excretions and coilings absent or infrequent. Agar within 3 wk turning at most slightly yellowish but after storage at 15 C for 6 mo or longer agar distinctly yellow (except S423). Odor strongly coconut-like. Chlamydospores infrequent, terminal. Conidiation after 6–7 d in loosely disposed, small shrubs and pustules on the entire surface, concentrated and larger, 1–3 mm diam, along the plate margin, turning first yellow then dark green after 7–9 d; phialides sometimes repetitive.

On PDA after 72 h 20–22 mm at 15 C, 38–42 mm at 25 C, 20–22 mm at 30 C; mycelium covering the plate after 5–6 d at 25 C. Colony circular, dense. Aerial hyphae abundant, forming a thick white floccose mat except for narrow plug surroundings, which turn yellowish. Autolytic excretions abundant, large; coilings inconspicuous. Odor unpleasant, with a coconut-like component. Conidiation starting after 2–3 d, scant, mostly in the center, also spreading on aerial hyphae, not turning green.

On SNA after 72 h 19–20 mm at 15 C, 34–37 mm at 25 C, 16–18 mm at 30 C; mycelium covering the plate after 6 d at 25 C. Colony hyaline, dense, margin ill defined, secondary hyphae fine, short, curly; surface hyphae scarcely degenerating. Aerial hyphae inconspicuous, more frequent and long at the distal margin. Autolytic excretions absent, coilings abundant. Chlamydospores infrequent. Conidiation starting after 3–5 d, short effuse and predominantly in small pustules 1–2 mm diam formed mostly at the lateral and distal plate margin, becoming densely aggregated or confluent, turning dark green 27–28F3–8 after 6–7 d. Pustules compact, velutinous, pulvinate to globose, with a smooth surface; consisting of a stipe ca. $6\text{--}8\text{--}(11) \mu\text{m}$ wide with irregularly or radially emergent primary branches up to $6.5 \mu\text{m}$ wide with often constricted septa, forming a reticulum. Main axes of conidiophores mostly embedded within the reticulum, difficult to separate, mostly short, resulting conidiophores therefore variable, irregular or tree-like, loosely or densely branched, often broad and with conspicuous curvatures particularly in terminal branches and phialides; branches mostly unpaired, often in right angles or inclined upward, $2\text{--}5 \mu\text{m}$ wide, thickening to $6.5 \mu\text{m}$. Phialides arising singly or in whorls of 2–4, sometimes repetitive (i.e. supporting cell of similar shape). Phialides $(4.5\text{--})6\text{--}11\text{--}(17) \times (2.0\text{--})2.5\text{--}3.2\text{--}(4.0) \mu\text{m}$, l/w $(1.4\text{--})2\text{--}4.1\text{--}(7.2)$, $(1.1\text{--})1.5\text{--}2.2\text{--}(2.7) \mu\text{m}$ wide at the base ($n = 160$), lageniform or ampulliform, inequilateral when long and narrow, often distinctly curved. Conidia $(3.5\text{--})4.3\text{--}5.0\text{--}(6.0) \times (2.8\text{--})3.0\text{--}3.5\text{--}(4.0) \mu\text{m}$, l/w $(1.1\text{--})1.3\text{--}1.5\text{--}(2.0)$ ($n = 200$), green, smooth, oblong or mostly ellipsoid, thick-walled.

Distribution: Spain, including the Balearic and Canary Islands.

Habitat: On twigs and branches of shrubs and trees and stalks of herbaceous plants.

Holotype: SPAIN, CANARIAS, LA PALMA, Garafía, at LP1 close to the junction to El Tablado, 28°47′48″N, 17°53′40″W, 1110 m, on branch, 6 cm thick, of *Chamaecytisus proliferus*, stromata on bark and an effuse Diatrypaceae, holomorph, 4 Dec 2010, W. Jaklitsch (WU 31606; culture S453 = CBS 130540).

Other material examined: SPAIN, ANDALUCIA, Alcalá de los Gazules, Via de Servicio south of the exit at km 54 off the A7 (A381), 36°22′09″N, 5°38′41″E, 75 m, on *Olea europaea* subsp. *sylvestris*, 17 Mar 2011, W. Jaklitsch & H. Voglmayr (S491). El Colmenar, roadside of MA9300 at km 8, 36°31′39″N, 5°22′39″E, 330 m, on branches of *Calicotome spinosa*, soc. *Hypocrea* sp., 22 Mar 2011, H. Voglmayr & W. Jaklitsch (S536a). Jerez de la Frontera, pasture near the hotel La Cueva Park, 36°42′43″N, 6°01′23″E, 70 m, on *Cynara* sp., 16 Mar 2011, W. Jaklitsch & H. Voglmayr (S486). CANARIAS, LA PALMA, Cumbre Nueva, branching off LP-301 at Area Recreativa del Pilar, 28°37′24″N, 17°49′53″W, 1460 m, on *Cistus symphytifolius*, 11 Dec 2009, W. Jaklitsch & R.M. Dähncke (culture S172 = CBS 130538); Pista Cabrito, 28°35′47″N, 17°49′02″W, 1355 m, on *Cistus symphytifolius*, 13 Dec 2009, W. Jaklitsch & R.M. Dähncke (S183); Garafía, at LP1 close to the junction to El Tablado, 28°47′48″N, 17°53′39″W, 1110 m, on *Chamaecytisus proliferus*, 4 Dec 2010, W. Jaklitsch (S450). TENERIFE, Teno Alto, 28°20′28″N, 16°52′26″W, 775 m, on *Teline canariensis*, 10 Dec 2010, W. Jaklitsch (S465). ISLAS BALEARES, MALLORCA, W Santa Eugenia at Ma-3011, 39°36′00″N, 2°49′00″W, 115 m, on *Pistacia lentiscus*, 21 Nov 2010, W. Jaklitsch (S419); Es Capdella, along Torrent de Galatzó, 39°35′03″N, 2°29′01″W, 115 m, on *Ceratonía siliqua*, 21 Nov 2010, W. Jaklitsch (S423).

Notes: *Hypocrea hispanica* phylogenetically is closely related to *H. junci* and *Trichoderma samuelsii*. *Hypocrea junci* so far is known only from *Juncus* in Denmark and differs from *H. hispanica* also by its long, straight, radial conidiophores, larger conidiation pustules formed in a broad marginal zone on CMD and SNA, lack of a coconut-like odor on CMD, and by slower growth particularly on CMD and at 30 C on all media. Also in comparison with *T. samuelsii*, the anamorph of *H. hispanica* tends to have shorter conidiophores often with conspicuous curvatures in terminal branches and phialides and the conidia tend to be more ellipsoid. On SNA *T. samuelsii* grows faster and on CMD it sporulates much earlier (after 2 d vs. 6–7 d), on SNA in a conspicuous distal down and in pustules that are widely disposed on the agar surface, which is consistent in all examined strains. In *H. hispanica* pustules on SNA are generally formed only at the lateral and distal margin of the Petri dish. However the only ascospore isolate S453 deviates from all conidial isolates in the formation of some pustules within the colony, coinciding with a slightly different *rpb2* sequence. Conidiation pustules are more compact than in *T. samuelsii*, with fewer projecting elements. *Hypocrea hispanica* produces a yellow pigment on PDA only after storage for several months at 15 C, while in *T. samuelsii* the reverse of PDA cultures already turns distinctly yellow within a week at 25 C.

Trichoderma samuelsii Jaklitsch & Voglmayr sp. nov. Fig. 4

MycoBank MB563317

Etymology: In honor of Gary J. Samuels for his excellent and comprehensive work on *Hypocrea/Trichoderma*, particularly on the section *Trichoderma*.

Conidiophora effusa et in pustulis disposita in agaris CMD et SNA, verticillio vel trichodermati similia. Phialides divergentes, lageniformes, (5–)7–11(–16) × (2.2–)2.5–3.0 (–

3.5) μm . Conidia oblonga, interdum ellipsoidea, viridia, glabra, (3.7–)4.2–5.0(–5.7) $\times$ (2.4–)2.7–3.2(–3.7) μm .

Cultures and anamorph: Optimal growth at 25 C on all media, no growth at 35 C. On CMD after 72 h 25–27 mm at 15 C, 51–52 mm at 25 C, 44–48 mm at 30 C; mycelium covering the plate after 4 d at 25 C. Colony hyaline, dense, radial, not zonate; hyphae with conspicuous difference in width, marginal primary surface hyphae wide, surface hyphae soon degenerating from the center. Broad marginal zone becoming downy to floccose by numerous long aerial hyphae. Autolytic excretions and coilings nearly absent. Chlamydospores infrequent, terminal and intercalary. Diffusing pigment absent, odor coconut-like. After longer storage (e.g. 1 y) at 15 C on CMD agar evenly dull yellow. Conidiation starting after 2 d, first effuse in numerous delicate shrubs and on aerial hyphae in the broad distal zone, later also in some pustules to ca. 1.5 mm diam mostly laterally and at the distal margin of the same zone or pustules not formed, after 5–6 d turning bluish green, 25EF3–6, 26E3–5.

On PDA after 72 h 20–23 mm at 15 C, 50–51 mm at 25 C, 35–40 mm at 30 C; mycelium covering the plate after 4 d at 25 C. Colony circular, dense. Aerial hyphae abundant, forming a thick whitish mat except for the flat center, the latter turning light yellow to green. Autolytic excretions abundant and large, coilings absent. Reverse turning yellow, 3–4A3–5. Odor distinctly unpleasant, chemical, more or less pyridine-like, as in *T. bavaricum*, pronounced at 30 C. Conidiation starting after 2 d, effuse on flat delicate shrubs in the granulose center, turning greenish after 5–6 d; also slightly spreading on aerial hyphae.

On SNA after 72 h 21–22 mm at 15 C, 49–51 mm at 25 C, 34–39 mm at 30 C; mycelium covering the plate after 4–5 d at 25 C. Colony hyaline, circular, dense; marginal primary surface hyphae conspicuously wide, surface hyphae within the colony degenerating. Aerial hyphae abundant in a major distal part of the colony excluding the plug area or a broad marginal zone or several ill-separated zones becoming hairy or floccose after few days, turning greenish. Autolytic activity moderate, coilings frequent. Chlamydospores noticeable after 5–6 d, infrequent, terminal and intercalary. Diffusing pigment and distinct odor absent. Conidiation more abundant than on CMD, starting after 2 d, first effuse, mostly on aerial hyphae, later (after 5–6 d) in pustules ca. 0.5–2 mm diam first formed from proximal ends of a floccose central concentric zone, eventually in several ill separated zones, turning dark green 25–26EF3–6, 27F4–8, partly confluent to several mm. Pustule periphery long remaining white, surface loose, hairy, with projecting conidiophores. Conidiophores with the same structure on aerial hyphae, in shrubs and pustules, originating on 4–5 μm wide hyphae, loose, long and straight, regularly tree-like but often also broad due to long upper branches, typically comprising a distinct main axis and mostly unpaired side branches loosely arranged in right angles or inclined upward that give rise to phialides or short terminal branches; all branches narrow, 1.5–3.5 μm wide. Phialides non-repetitive, solitary or in whorls of 2–4, (5–)7–11(–16) $\times$ (2.2–)2.5–3.0(–3.5) μm , l/w (1.7–)2.5–4.6(–7.1), (1.2–)1.5–2.2(–2.7) μm wide at the base (n = 115), lageniform, straight, thickening median or above the middle, neck short, often inequilateral, infrequently curved. Conidia (3.7–) 4.2–5.0(–5.7) $\times$ (2.4–)2.7–3.2(–3.7) μm , l/w (1.2–)1.4–1.7(–2.1) (n = 130), green, typically oblong with distinctly parallel sides, but with a fraction of ellipsoid ones varying among strains, smooth, sometimes pinched at sides, with two or more small guttules; abscission scar indistinct.

Distribution: Southern Europe (Italy, Spain).

Habitat: On twigs and branches of shrubs and deciduous trees and on fungi growing on them.

Holotype: ITALY, SARDINIA, roadside of SP 38 at junction to Isalle Orrule, 40°21'49"N, 9°31'00"E, 190 m, on *Hymenochaete* sp./*Cistus monspeliensis*, 2 Nov 2009, W. Jaklitsch (dry culture WU 31607; living culture S5 = CBS 130537).

Other material examined: ITALY, APULIA, Andria, Parco Nazionale dell'Alta Murgia, Castel del Monte, between SP234 and Masseria Savignano, 41°02'34" N, 16°18'02" E, 510 m, on *Crataegus laciniata*, 19 Nov 2009, W. Jaklitsch & H. Voglmayr (S67). CAMPANIA, Roscigno, 40°24'26"N, 15°21'51"E, 745 m, on *Quercus pubescens*, 16 Nov 2009, H. Voglmayr & W. Jaklitsch (S42). SPAIN, MALLORCA, east of Calvià, Ma-1016 roadside, 39°34'54"N, 2°32'37"E, 190 m, on *Stereum* sp./*Quercus ilex*, 17 Nov 2010, W. Jaklitsch (S398).

Notes: *Trichoderma samuelsii* is a fungus so far known from four gatherings. This species is closely related phylogenetically to *Hypocrea junci* and *H. hispanica*. Morphological and other differences from the latter see under that species. Conidial morphology, conidiophores and cultures resemble that of *H. junci*, but that species so far has only been recorded from *Juncus* in Denmark, differs from *T. samuelsii* by lack of a coconut-like odor on CMD and by slower growth particularly on CMD and at 30 C. In the latter the distinctly oblong conidia are conspicuous, although this varies considerably among the studied strains. *T. koningii* has also oblong conidia of similar size (see Jaklitsch 2011, Samuels et al. 2006a). Characteristic and so far unique in sect. *Trichoderma* is the distinctly unpleasant odor produced in PDA cultures, which is much reminiscent of PDA cultures of *H. bavarica* and *H. leucopus*.

Discussion

The taxonomy of the section *Trichoderma* was treated in detail by Samuels et al. (2006a), Jaklitsch et al. (2006) and Jaklitsch (2011), and some species were added (Hanada et al. 2008; Samuels and Ismaiel 2009; Samuels et al. 2006b, 2010), but this phylogenetically complex and morphologically highly conserved section is still far from monographed.

Including the three newly described species, the section *Trichoderma* comprises 43 species, for which some DNA data are available. ITS sequences are identical or nearly identical for several species of the section (e.g. those of *H. hispanica*, *H. koningii*, *H. viridescens* and *T. samuelsii*), therefore this marker is not useful for phylogenetic reconstruction or for bar-coding (Druzhinina et al. 2005, www.isth.info). Samuels et al. (2006a), using *tef1* introns, *cal1* and *act*, included up to 20 named and some unnamed clades in their phylogenetic analyses. Jaklitsch et al. (2006) used *tef1* introns to reconstruct the phylogeny of 29 named and five unnamed species, and Jaklitsch (2009, 2011), using *rpb2* plus *tef1* exon, included 27 species. *tef1* introns have been shown in most analyses to permit a resolution that is superior to other phylogenetic markers. They are available for all species of the section, although they are short or of low quality in a few species. On the other hand *rpb2* sequences are lacking for six species while *cal*, *act* and *tef1* exon are unavailable for many species. In the current analysis 37 (*rpb2* analysis) or 39 (*tef1* analysis) species are included. The resolution of the *tef1* tree has been considerably improved since earlier analyses, but backbone support is still not optimal for all nodes. The three new species are statistically highly supported in both phylogenetic analyses. However it has not been possible to determine the evolutionary (sister clade) relationships of *H. caerulescens* because the *tef1* tree failed to show whether the species is closer related to the *Koningii* (containing *H. koningii*) or the *Viride* (containing *H. rufa*, anamorph *T. viride*) clade, which together with the comparatively long branches in the phylograms indicates an isolated position within sect. *Trichoderma*. This is also supported by distinct ITS sequences of *H. caerulescens*. On the other hand both analyses show that *H. hispanica* and *T. samuelsii* belong to the *Viride* clade.

Recognition based on phenotype is seriously impaired in *Hypocrea hispanica* and *Trichoderma samuelsii*, where *tef1* sequences have proven to be basically the only reliable source of species recognition. Particularly notable is the distinctly unpleasant *H. bavarica*-like odor produced in PDA cultures of *T. samuelsii*, which is new to the sect. *Trichoderma*. Some other useful differences in anamorphs and cultures are given under the notes of the respective species. However conidiophore and conidial morphology, particularly in *T. samuelsii*, are much like those of *T. koningii* and some of its relatives (see Samuels et al. 2006a), although these species are phylogenetically not closely related with that group.

The most remarkable of the three new species described above, *H. caerulescens*, is extremely common in southern Europe, but despite several studies (Migheli et al. 2008, Zachow et al. 2008) it has not yet been reported. Morphological differentiation and recognition based on stromata has its limits because the light orange-brown fresh stromata drying dark reddish brown are shared with other species, particularly *H. viridescens*, which is even more common in the same habitats and regions, but also other species (e.g. *H. hispanica* or *H. petersenii*) may be similar. Most diagnostic of *H. caerulescens* is the blue pigment formed on CMD, but this occurs late, often only after storage for a month. This may be the reason why the species has not received its due attention. Another diagnostic set of traits is the combination of effuse conidiation, warted conidia, non-repetitive phialides on delicate, narrowly verticillate conidiophores and late emergence of a coconut-like odor.

The specific blue pigments of *H. caerulescens* were not identified in this study. It could be feasible to isolate the yellowish pigments that were extracted from the mycelia under alkaline conditions, elucidate their chemical structures and then study the compounds in the presence of metal ions.

Fungal pigment chemistry certainly should not be considered trivial. For instance the intense but highly polar and extremely unstable dark violet pigments of *Cortinarius violaceus* turned out to be conjugates of ferric ions with beta dopamine, following several decades of intensive work aimed at their identification (von Nussbaum et al. 1998), and many other pigments of these organisms still have not been identified. The few true blue fungal pigments known to science include scleroderis blue from *Gremmeniella abietina* (Ayer et al. 1986, Ayer and Pedras 1987), asperopterin A from *Aspergillus oryzae* (Kaneko and Sanada 1969) and 1-(3,8-Dimethyl-azulen-5-yl)-ethanone from sporocarps of *Entoloma hochstetteri* (Gill 1999). Several additional species of *Entoloma* also feature blue, and their pigments are likely azulene terpenoids as well. Basidiomata of the cobalt crust fungus *Terana caerulea* (syn. *Pulcherricium caeruleum*, *Corticium caeruleum*) are sky-blue owing to the presence of benzobisbenzofurans named corticins (Briggs et al. 1976). Compounds that also are known from other biological sources such as indigo also were reported from fungi and can be produced using biotechnological systems (e.g. Falanghe and Bobbio 1962); an indigo-like pigment is also known from cultures of *Morchella* (Eyal et al. 1991). Finally Gill and Steglich (1987) provided an overview on the manifold compounds from basidiomata of various *Boletales*, such as variegatic acid, which are converted enzymatically to blue anions upon injury of the fruiting bodies. A similar reaction also occurs in injured basidiomata of *Lactarius indigo*, yielding azulene pigments (Harmon et al. 1980). Most of these compounds are unfortunately either not accessible in large quantities or they are highly unstable or insoluble, which has precluded their commercialization. In view of the increasing interest of both the food and cosmetics industries in natural pigments (see Mapari et al. 2005, 2009 and references therein), rapidly growing organisms such as species of *Hypocrea/Trichoderma*, which already have found wide biotechnological application, certainly would be better candidates for industrial production of natural pigments than the above mentioned mycorrhizal mushrooms. Therefore further studies on the pigments of *H. caerulescens* might be warranted.

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LITERATURE CITED

- Ayer WA, Hoyano Y, Pedras MSC, van Altena I. Metabolites produced by the scleroderris canker fungus *Gremmeniella abietina* 1. Can J Chem. 1986; 64:1585–1589. doi:10.1139/v86-262.
- Ayer WA, Pedras MSC. Metabolites produced by the scleroderris canker fungus *Gremmeniella abietina* 3. Some further metabolites. Can J Chem. 1987; 65:754–759. doi:10.1139/v87-128.
- Bitzer J, Köpcke B, Stadler M, Hellwig V, Ju YM, Seip S, Henkel T. Accelerated dereplication of natural products, supported by reference libraries. Chimia. 2007; 51:332–338. doi:10.2533/chimia.2007.332.
- Blaszczak L, Popiel D, Chelkowski J, Koczyk G, Samuels GJ, Sobieralski K, Siwulski M. Species diversity of *Trichoderma* in Poland. J Appl Genetics. 2011; 52:233–243. doi:10.1007/s13353-011-0039-z. [PubMed: 21465156]
- Briggs LH, Cambie RC, Dean IC, Hodges R, Ingram WB, Rutledge PS. Chemistry of fungi XI. Corticins A, B and C, benzobisbenzofurans from *Corticium caeruleum*. Austr J Chem. 1976; 29:179. doi:10.1071/CH9760179.
- Chaverri P, Gazis RO, Samuels GJ. *Trichoderma amazonicum*, a new endophytic species on *Hevea brasiliensis* and *H. guianensis* from the Amazon basin. Mycologia. 2011; 103:139–151. doi:10.3852/10-078. [PubMed: 20943534]
- Degenkolb T, Dieckmann R, Nielsen KF, Gräfenhan T, Theis C, Zafari D, Chaverri P, Ismael A, Brückner H, von Döhren H, Thrane U, Petrini O, Samuels GJ. The *Trichoderma brevicompactum* clade: a separate lineage with new species, new peptaibiotics and mycotoxins. Mycol Prog. 2008a; 7:177–219. doi:10.1007/s11557-008-0563-3.
- Degenkolb T, Gräfenhan T, Berg A, Nirenberg HI, Gams W, Brückner H. Peptaibiotics: screening for polypeptide antibiotics (peptaibiotics) from plant-protective *Trichoderma* species. Chem Biodivers. 2006; 3:593–610. doi:10.1002/cbdv.200690063. [PubMed: 17193294]
- Degenkolb T, von Döhren H, Nielsen KF, Samuels GJ, Brückner H. Recent advances and future prospects in peptaibiotics, hydrophobin and mycotoxin research and their importance for chemotaxonomy of *Trichoderma* and *Hypocrea*. Chem Biodivers. 2008b; 5:671–680. doi:10.1002/cbdv.200890064. [PubMed: 18493954]
- Druzhinina IS, Komon-Zelazowska M, Kredics L, Hatvani L, Antal Z, Belayneh T, Kubicek CP. Alternative reproductive strategies of *Hypocrea orientalis* and genetically close but clonal *Trichoderma longibrachiatum*, both capable of causing invasive mycoses of humans. Microbiology. 2008a; 154:3447–3459. doi:10.1099/mic.0.2008/021196-0. [PubMed: 18957598]
- Druzhinina IS, Kopchinskiy AG, Komon M, Bissett J, Szakacs G, Kubicek CP. An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. Fungal Genet Biol. 2005; 42:813–828. doi:10.1016/j.fgb.2005.06.007. [PubMed: 16154784]
- Druzhinina IS, LaFe K, Willinger B, Komon-Zelazowska M, Ammirati J, Kubicek CP, Rogers JD. An unknown species from *Hypocreaceae* isolated from human lung tissue of a patient with non-fatal pulmonary fibrosis. Clin Microbiol Newslett. 2008b; 29:180–184. doi:10.1016/j.clinmicnews.2007.11.002.
- Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004; 32:1792–1797. doi:10.1093/nar/gkh340. [PubMed: 15034147]
- Eyal J, Mabud MdA, Walter JF. Production of indigotin in submerged culture using *Morchella* nov. ES-1. Appl Biochem Biotechnol. 1991; 30:303–312. doi:10.1007/BF02922034.

- Falanghe H, Bobbio PA. Identification of indigo produced in submerged culture of *Agaricus campestris* mutant culture. Arch Biochem Biophys. 1962; 96:430–433. doi: 10.1016/0003-9861(62)90431-9. [PubMed: 13891397]
- Ghisalberti EL, Sivasithamparam K. Antifungal antibiotics produced by *Trichoderma* spp. Soil Biol Biochem. 1991; 23:1011–1020. doi:10.1016/0038-0717(91)90036-J.
- Gill M. Pigments of fungi (macromycetes). Nat Prod Rep. 1999; 16:301–317. doi:10.1039/a705730j.
- Gill M, Steglich W. Pigments of Fungi (macromycetes). Prog Ch Org Nat Prod. 1987; 51:1–317.
- Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999; 41:95–98.
- Hanada RE, Jorge Souza de T, Pomella AWV, Prakash Hebbar K, Pereira JO, Ismaiel A, Samuels GJ. *Trichoderma martiale* sp. nov., a new endophyte from sapwood of *Theobroma cacao* with a potential for biological control. Mycol Res. 2008; 112:1335–1343. doi:10.1016/j.mycres.2008.06.022. [PubMed: 18672059]
- Harman GE. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology. 2006; 96:190–194. doi:10.1094/PHYTO-96-0190. [PubMed: 18943924]
- Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species—opportunistic, avirulent plant symbionts. Nat Rev Microbiol. 2004; 2:43–56. doi:10.1038/nrmicro797. [PubMed: 15035008]
- Harman, GE.; Kubicek, CP. *Trichoderma & Gliocladium*. Vol. 2. Enzymes, biological control and commercial applications. Taylor & Francis Ltd.; London: 1998. p. 393
- Harmon AD, Weisgraber KH, Weiss U. Preformed azulene pigments of *Lactarius indigo* (Schw.) Fries (*Russulaceae*, *Basidiomycetes*). Experientia. 1980; 36:54–56. doi:10.1007/BF02003967.
- Hatvani L, Antal Z, Manczinger L, Szekeres A, Druzhinina IS, Kubicek CP, Nagy A, Nagy E, Vagvolgyi C, Kredics L. Green mold diseases of *Agaricus* and *Pleurotus* spp. are caused by related but phylogenetically different *Trichoderma* species. Phytopathology. 2007; 97:532–537. doi: 10.1094/PHYTO-97-4-0532. [PubMed: 18943294]
- Huelsenbeck JP, Ronquist F. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics. 2001; 17:754–755. doi:10.1093/bioinformatics/17.8.754. [PubMed: 11524383]
- Jaklitsch WM. European species of *Hypocrea* I. The green-spored species. Stud Mycol. 2009; 63:1–91. doi:10.3114/sim.2009.63.01. [PubMed: 19826500]
- Jaklitsch WM. European species of *Hypocrea* II: species with hyaline ascospores. Fungal Divers. 2011; 48:1–250. doi:10.1007/s13225-011-0088-y. [PubMed: 21994484]
- Jaklitsch WM, Samuels GJ, Dodd SL, Lu B-S, Druzhinina IS. *Hypocrea rufa*/ *Trichoderma viride*: a reassessment and description of five closely related species with and without warted conidia. Stud Mycol. 2006; 56:135–177. doi:10.3114/sim.2006.56.04. [PubMed: 18490991]
- Kaneko Y, Sanada M. Studies on the fluorescent substances produced by *Aspergillus* fungi VII. Purification and isolation of asperopterin B and chemical properties of asperopterin B and A. J Ferment Technol. 1969; 47:8–19.
- Kredics L, Antal Z, Dóczy I, Manczinger L, Kevei F, Nagy E. Clinical importance of the genus *Trichoderma*. A review. Acta Microbiol Immunol Hung. 2003; 50:105–117. doi:10.1556/AMicr.50.2003.2-3.1. [PubMed: 12894482]
- Laatsch, H. AntiBase—the natural compound identifier. Database, Wiley-VCH; Weinheim, Germany: 2010.
- Læssøe T, Srikitikulchai P, Fournier J, Köpcke B, Stadler M. Lepralic acid derivatives as chemotaxonomic markers in *Hypoxyton aeruginosum*, *Chlorostroma subcubisporum* and *C. cyaninum* sp. nov. Fungal Biol. 2010; 114:481–489. doi:10.1016/j.funbio.2010.03.010. [PubMed: 20943159]
- Mapari SAS, Meyer AS, Thrane U, Frisvad JC. Identification of potentially safe promising fungal cell factories for the production of polyketide natural food colorants using chemotaxonomic rationale. Microb Cell Fact. 2009; 8:24. doi:10.1186/1475-2859-8-24. [PubMed: 19397825]
- Mapari SAS, Nielsen KF, Larsen TO, Frisvad JC, Meyer AS, Thrane U. Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. Curr Opin Biotechnol. 2005; 16:231–238. doi:10.1016/j.copbio.2005.03.004. [PubMed: 15831392]

- Migheli Q, Balmas V, Komon-Zelazowska M, Scherm B, Fiori S, Kopchinskiy AG, Kubicek CP, Druzhinina IS. Soils of a Mediterranean hot spot of biodiversity and endemism (Sardinia, Tyrrhenian Islands) are inhabited by pan-European, invasive species of *Hypocrea/Trichoderma*. *Environ Microbiol*. 2008; 11:35–46. doi:10.1111/j.1462-2920.2008.01736.x. [PubMed: 18764873]
- Park MS, Bae KS, Yu SH. Two new species of *Trichoderma* associated with green mold of oyster mushroom cultivation in Korea. *Mycobiology*. 2006; 34:111–113. doi:10.4489/MYCO.2006.34.3.111.
- Robinson N, Wood K, Hylands PJ, Gibson TM. Blue pigments of *Penicillium herquei*. *J Nat Prod*. 1992; 55:814–817. doi:10.1021/np50084a019.
- Samuels GJ, Dodd S, Lu B-S, Petrini O, Schroers H-J, Druzhinina IS. The *Trichoderma koningii* aggregate species. *Stud Mycol*. 2006a; 56:67–133. doi:10.3114/sim.2006.56.03. [PubMed: 18490990]
- Samuels GJ, Ismaiel A. *Trichoderma evansii* and *T. lieckfeldtia*: two new *T. hamatum*-like species. *Mycologia*. 2009; 101:142–156. doi:10.3852/08-161. [PubMed: 19271677]
- Samuels GJ, Ismaiel A, Bon M-C, de Respinis S, Petrini O. *Trichoderma asperellum* sensu lato consists of two cryptic species. *Mycologia*. 2010; 102:944–966. doi:10.3852/09-243. [PubMed: 20648760]
- Samuels GJ, Suarez C, Solis K, Holmes KA, Thomas SE, Ismaiel A, Evans HC. *Trichoderma theobromicola* and *T. paucisporum*: two new species isolated from cacao in South America. *Mycol Res*. 2006b; 110:381–392. doi:10.1016/j.mycres.2006.01.009. [PubMed: 16621496]
- Schuster A, Schmoll M. Biology and biotechnology of *Trichoderma*. *Appl Microbiol Biotechnol*. 2010; 87:787–799. doi:10.1007/s00253-010-2632-1. [PubMed: 20461510]
- Stadler M, Fournier J. Pigment chemistry, taxonomy and phylogeny of the *Hypoxyloideae* (*Xylariaceae*). *Rev Iberoamericana Micol*. 2006; 23:160–170. doi:10.1016/S1130-1406(06)70037-7.
- Stadler M, Fournier J, Beltrán-Tejera E, Granmo A, Glawe DA, Ammirati JF. The “red Hypoxylons” of the temperate and subtropical northern hemisphere. *North Am Fungi*. 2008; 3:73–125. A Festschrift in honor of Professor Jack D. Rogers.
- Stamatakis E. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*. 2006; 22:2688–2690. doi:10.1093/bioinformatics/btl446. [PubMed: 16928733]
- Swofford, DL. PAUP*4.0b10: phylogenetic analysis using parsimony (*and other methods). Sinauer Associates; Sunderland, Massachusetts: 2002.
- Voglmayr H, Jaklitsch WM. *Prosthecium* species with *Stegonsporium* anamorphs on Acer. *Mycol Res*. 2008; 112:885–905. doi:10.1016/j.mycres.2008.01.020. [PubMed: 18554889]
- von Nussbaum F, Spiteller P, Rütth M, Steglich W, Wanner G, Gamblin B, Stievano L, Wagner FE. Ein Eisen(III)-Catechol-Komplex als Pilzfarbstoff. *Angew Chem*. 1998; 110:3483–3485. doi:10.1002/(SICI)1521-3757(19981204)110:23,3483::AID-ANGE3483.3.0.CO;2-5.
- Zachow C, Berg C, Müller H, Meincke R, Komon-Zelazowska M, Druzhinina IS, Kubicek CP, Berg G. Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): relationship to vegetation zones and environmental factors. *ISME J*. 2008; 3:79–92. doi:10.1038/ismej.2008.87. [PubMed: 18830279]

Fig. 1.

Phylogram of one of 224 MP trees of length 1191 revealed by PAUP from an analysis of the *tef1* matrix of sect. *Trichoderma*, showing the phylogenetic position of *H. caerulea*, *H. hispanica* and *T. samuelsii* (formatted in boldface). Thin branches represent nodes collapsing in the strict consensus tree of all MP trees. MP and ML BS above 60% and Bayesian PP above 90% are given respectively at first, second and third position, above or below the branches; GenBank accession or isolate numbers follow the taxon labels.

Fig. 2. Phylogram of one of two MP trees of length 847 revealed by PAUP from an analysis of the *rpb2* matrix of sect. *Trichoderma*, showing the phylogenetic position of *H. caeruleus*, *H. hispanica* and *T. samuelsii* (formatted in boldface). Thin branches represent nodes collapsing in the strict consensus tree of the two MP trees. MP and ML BS above 60% and Bayesian PP above 90% are given respectively at first, second and third position, above or below the branches; GenBank accession or isolate numbers follow the taxon labels.

Fig. 3.

a–s. Teleomorph of *Hypocrea caerulea*. a–c. Fresh stromata. d–g. Dry stromata (d. with spore deposits on the surface; e. immature). h. Rehydrated stroma part in 3% KOH. i. Stroma surface with hairs. j. Perithecium in section. k. Cortex in section. l. Subcortical tissue in section. m. Subperithecial tissue in section. n, o. Stroma base in section (n. substrate-attached; o. free). p–s. Asci (r, s. in cotton blue/lactic acid). t–x. Teleomorph of *Hypocrea hispanica*. t. Ascus. u. Fresh stromata. v. Dry stroma. w. Rehydrated stroma part in 3% KOH. x. Perithecium in section. y–aa. Cultures of *Hypocrea caerulea* after 10 d at 25 C (y. on CMD; z. on PDA; aa. on SNA). a–c, g–p, r. WU 31600. d. WU 31601. e, f, q, s. WU 31602. t–x. WU 31606. y, z. S8. aa. S252. Bars: a–c = 1 mm. d, e, w = 0.2 mm. f–h = 0.5 mm. i, l, m = 20 μ m. j = 30 μ m. k, n, o = 15 μ m. p–t = 10 μ m. u = 1.5 mm. v = 0.3 mm. x = 60 μ m. y–aa = 15 mm.

Fig. 4.

a–i. *Hypocrea caerulescens*. a. Reverse of CMD culture after 64 d at 15 C. b, c, f. Conidiophores and phialides (b, c. from SNA after 7 d; b. at 15 C, c. at 25 C; f. from CMD after 16 d at 25 C). e. Anamorph on natural substrate. d, g–i. Conidia from SNA (d, g. after 6–7 d at 15 C; h, i. after 7–12 d at 25 C). j–s. *Trichoderma samuelsii*. Cultures and anamorph at 25 C on SNA except k. j, k. Cultures (j. after 10 d; k. on PDA, yellow reverse after 7 d). l. Conidiation pustule (after 7 d). m–q. Conidiophores and phialides (m, o. after 4 d; n, p, q. after 5–7 d). r, s. Conidia after 4–6 d). t–z. *Hypocrea hispanica*. Cultures and anamorph on SNA at 25 C. t. Culture after 9 d. u–x. Conidiophores and phialides after 6 d (x. repetitive curved phialides). y, z. Conidia after 6 d. a, b, f. S8. c, g, h. S206. d, i. S252. e. S439. j–m, o, r. S5. n, p, q. S398. s. S67. t. S172. u, x, y. S450. v. S486. w, z. S423. Bars: a, j, k, t = 15 mm. b, c, f, n, u = 15 μ m. d, g–i, r, z = 5 μ m. e, l = 0.5 mm. m, o, p = 30 μ m. q, w = 20 μ m. s, v, x, y = 10 μ m.

Table 1

GenBank accessions of sequences obtained in this study for the listed strains

Name	Origin	Strain	GenBank accession number		
			<i>tefl</i>	<i>rpb2</i>	ITS
<i>Hypocrea caenulescens</i>	Italy (Sardinia)	S2	JN715615	JN715602	JN715591
	Italy (Sardinia)	S8	JN715616		
	Italy (Sardinia)	S9	JN715617		
	Italy (Sardinia)	S14	JN715618		
	Italy (Sardinia)	S17	JN715619		
	Italy (Apulia)	S71		JN715603	
	Italy (Apulia)	S78	JN715620		
	Spain (La Palma)	S195 = CBS 130011	JN715621	JN715604	JN715589
	Portugal (Madeira)	S196	JN715622		
	Portugal (Madeira)	S203	JN715623		
	Portugal (Madeira)	S206 = CBS 130012	JN715624	JN715605	JN715590
	Spain (Tenerife)	S214	JN715625		
	Spain (Tenerife)	S216	JN715626		
	Spain (Tenerife)	S217	JN715627		
	Spain (Tenerife)	S218	JN715628		
	Spain (Tenerife)	S219	JN715629		
	Spain (Tenerife)	S221	JN715630		
	Spain (Tenerife)	S232	JN715631	JN715606	
	Croatia (Istria)	S255	JN715632		
	Croatia (Istria)	S261	JN715633		
	Croatia (Istria)	S277	JN715634		
	Croatia (Losinj)	S296	JN715635		
	Croatia (Losinj)	S299	JN715636		
	Croatia (Istria)	S304	JN715637		
	Spain (Mallorca)	S421	JN715638		
	Spain (Mallorca)	S424a	JN715639	JN715607	
	Spain (La Palma)	S432	JN715640		
	Spain (La Palma)	S434	JN715641		

Name	Origin	Strain	GenBank accession number	
			<i>tefl</i>	<i>rpb2</i> ITS
<i>H. hispanica</i>	Spain (La Palma)	S439	JN715642	
	Spain (La Palma)	S441	JN715643	
	Spain (La Palma)	S459	JN715644	
	Spain (La Palma)	S460	JN715645	
	Spain (La Palma)	S462	JN715646	
	Spain (La Gomera)	S464	JN715647	
	Spain (Tenerife)	S479	JN715648	
	Spain (Andalucia)	S519	JN715649	
	Spain (Andalucia)	S539	JN715650	
	Spain (La Palma)	S172 = CBS 130538	JN715655	JN715597
	Spain (La Palma)	S183	JN715656	JN715594
	Spain (Mallorca)	S419	JN715657	
	Spain (Mallorca)	S423	JN715658	
	Spain (La Palma)	S453 = CBS 130540	JN715659	JN715600
	Spain (Tenerife)	S465	JN715660	JN715595
	Spain (Andalucia)	S486	JN715661	
<i>H. koningii</i>	Spain (Andalucia)	S491	JN715662	
	Spain (Andalucia)	S536a	JN715663	
	Spain (Tenerife)	S227		JN715609
	France (Meurthe-et-Moselle)	e) C.P.K. 1937	JN715611	
	Spain (La Palma)	S170	JN715612	
<i>H. petersenii</i>	Thailand	C.P.K. 775 = G.J.S. 97-83		JN715610
<i>H. pezizoides</i>	Italy (Sardinia)	S5 = CBS 130537	JN715655	JN715599
<i>Trichoderma samuelsii</i>	Italy (Campania)	S42	JN715652	JN715598
	Italy (Apulia)	S67	JN715653	JN715592
<i>T. taiwanense</i>	Spain (Mallorca)	S398	JN715654	
	Singapore	C.P.K. 416 = TUB F-597		JN715608