Taxonomy and phylogenetic relationships of nine species of *Hypocrea* with anamorphs assignable to *Trichoderma* section *Hypocreanum*

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Abstract: Morphological studies and phylogenetic analyses of DNA sequences from the internal transcribed spacer (ITS) regions of the nuclear ribosomal gene repeat, a partial sequence of RNA polymerase II subunit (rpb2), and a partial sequence of the large exon of tef1 (LEtef1) were used to investigate the taxonomy and systematics of nine *Hypocrea* species with anamorphs assignable to *Trichoderma* sect. *Hypocreanum*. *Hypocrea corticioides* and *H. sulphurea* are reevaluated. Their *Trichoderma* anamorphs are described and the phylogenetic positions of these species are determined. *Hypocrea sulphurea* and *H. subcitrina* are distinct species based on studies of the type specimens. *Hypocrea egmontensis* is a facultative synonym of the older name *H. subcitrina*. *Hypocrea* with anamorphs assignable to *Trichoderma* sect. *Hypocreanum* formed a well-supported clade. Five species with anamorphs morphologically similar to sect. *Hypocreanum*, *H. avellanea*, *H. parmastoi*, *H. megalocitrina*, *H. alcalifuscescens*, and *H. pezizoides*, are not located in this clade. *Protocrea* farinosa belongs to *Hypocrea* s.s.

**Taxonomic novelties:** *Hypocrea euorticoides* Overton, nom. nov., *Hypocrea victoriensis* Overton, sp. nov., *Hypocrea parmastoi* Overton, sp. nov., *Hypocrea alcalifuscescens* Overton, sp. nov.

**Key words:** Ascomycetes, *Hypocreales*, *Hypocreanum*. *Hypocrea corticioides*, *H. egmontensis*, *H. parmastoi*, *H. alcalifuscescens*, *H. subsulphurea*, *H. farinosa*, *H. subcitrina*, *H. sulphurea*, *H. victoriensis*, ITS rDNA, *Lentinula* edodes, systematics, rpb2 gene sequences, *Trichoderma*.

INTRODUCTION

Nine species of *Hypocrea* Fr. (*Ascomycetes, Hypocreales, Hypocreaceae*) with effused stromata from Japan, Australia, New Zealand, North America, Europe, and Central America, are newly described or redescribed. Anamorphs of these species are morphologically similar, having acrümion- or verticillium-like conidiophores with hyaline conidia, and are assignable to *Trichoderma* sect. *Hypocreanum* Bissett.

*Hypocrea sulphurea* (Schw.) Sacc. is a common, yellow, effused fungicidal species recorded from North America and Europe that occurs on *Exidia* spp. Dingley (1956) considered *H. subcitrina* Kalchbr. & Cooke, recorded from Africa, as a synonym of the older *H. sulphurea*, but this synonymy has never been critically examined. Dingley (1956) published the new name *H. egmontensis* from New Zealand based on a fungus with yellow effused stroma. The relationship between *H. egmontensis* and *H. sulphurea* has not been established. Doi (1972) described *Hypocrea sulphurea* f. *macrospora* Yoshim. Doi. We compared morphologically typical material (NY) of this form with collections of *H. sulphurea* from North America and Europe. A specimen identified as *Hypocrea subsulphurea* Syd. in De Wild. was recently collected and cultured in Japan and redescribed. *Hypocrea corticioides* Speg. is similar in appearance to *H. sulphurea*, but *H. corticioides* occurs on decorticated wood and has a tropical distribution. *Hypocrea corticioides* Speg. is a later homonym of *H. corticioides* Berk. & Broome.

The type material of *H. corticioides* Berk. & Broome is indistinguishable from and therefore synonymous with *Stilbocorea macrostoma* (Berk. & M.A. Curtis) Höhn., a member of the *Bionectriaceae* (Rossman et al. 1999). A new name is proposed for *H. corticioides* Speg.

Two apparently new species of *Hypocrea* with hyphal stromata were studied. Their relationship to *Hypocrea* spp. with pseudoparenchymatous tissue was unclear. In addition, the relationship of these hyphal species to *Protocrea* farinosa (Berk. & Broome) Petch, which also has a hyphal stroma, had to be examined.

Kulling-Gradinger et al. (2002) showed that some *Trichoderma* species with anamorphs in *Trichoderma* sect. *Hypocreanum* form a highly supported subclade of sect. *Pachybasium sensu lato* and suggested that sections *Hypocreanum* and *Pachybasium* are phylogenetically indistinguishable. Their analysis included a limited number of taxa with acrümion- or verticillium-like anamorphs. More recently, Chaverri et al. (2003) used partial sequences of the RNA polymerase II subunit (rpb2) and the large exon of tef-1α (LEtef1) and found that anamorphs referable to sect. *Hypocreanum* do not form a monophyletic group, as *H. pezizoides* Berk. & Broome and *H. avellanea* S.T. Carey & Rogerson were situated in the *H. rufa* clade. Chaverri et al. (2003) showed that *H. citrina* (Pers. : Fr.) Fr. and *H. pulvinata* Fuckel form a highly supported clade, the limits of which were not established. Dodd et al. (2002) showed, using the ITS1-5.8S-ITS2 rDNA (ITS) region, that *H. pulvinata* and *H. sulphurea* form two distinct subclades of a strongly supported but phylogenetically unresolved clade. These authors
did not conclude that sect. Hypocreanum and sect. Pachybasium were phylogenetically indistinguishable. The results of Dodd et al. (2002) and Chaverri et al. (2003) support the conclusion of Kullnig-Gradinger et al. (2002) that section Pachybasium is paraplectic. The seven species included are compared to developed by Taylor species delineated in this study according to criteria selected species treated by Overton (2006) to establish the phylogenetic limits of Trichoderma sect. Hypocreanum.

The objectives of this study are: (1) to determine whether H. sulphurea, H. subcitrina, and H. egmontensis are distinct species; (2) to verify the phylogenetic relationship between H. subsulphurea and H. sulphurea; (3) to verify the relationship between H. corticioides and H. sulphurea; (4) to determine the relationships of two new hyphal species to Proteocrea farinosa; (5) to investigate the phylogenetic boundaries of Hypocreanum with anamorphs in Trichoderma sect. Hypocreanum; and (6) to describe the phylogenetic species delineated in this study according to criteria developed by Taylor et al. (2000).

**MATERIALS AND METHODS**

**Collections and isolates**

Doi’s illustrations and descriptions (1971, 1972, 1975) were used in making initial species determinations. Table 1 lists the accession numbers used in this study. Frequently cited collectors are abbreviated: B.E. Overton (B.E.O.), G. J. Samuels (G.J.S.), and K. Põldmaa (K.P.). All isolates with G.J.S. designations were obtained by isolating single ascospores on CMD with the aid of a micromanipulator. All isolates with B.E.O. designations were obtained from plating the entire contents of individual perithecia. Unless otherwise noted, host and substratum data are taken from herbarium labels. The presentation of measurements is the same as in Overton et al. (2006).

**Molecular phylogenetic analyses**

DNA sequence analysis was conducted using three gene sequences: ITS 1-5.8S-ITS2 (ITS), a partial sequence of the large exon of translation elongation factor (LEtef1), and a partial sequence of the RNA polymerase II subunit (rpb2). ITS and rpb2 sequences were generated following the protocol and primers described in Overton et al. (2006). The following primers were employed for amplifying the LEtef1 regions which differs from the tef1 region amplified in Overton et al. (2006): for LEtef1, EF1-983F (5’-GC(G/C/T)CC(C/T)GG(A/C/T)CA(C/T)GGTGAC(T/C)T(C/T)AT-3’) (Carbone & Kohn 1999), EF1-2218R (5’-ATGAC(A/G)TG(A/G)GC(A/G)AC(A/G)GT(C/T)TG-3’) (S.A. Rehner, pers. comm.). Two percent dimethyl sulfoxide (DMSO) from AMRESCO® was added to each 50 µL PCR reaction. PCR products were purified and sequenced following the protocol in Overton et al. (2006). Sequences were assembled using SeqMan® II option and aligned using Clustal W in DNA Star (DNA Star Inc., Madison, Wisconsin), and a phylogenetic analysis was performed using PAUP* v. 4.0 b4 (Swofford 1999). Alignments were manually adjusted in PAUP*. Outgroup taxa varied depending on the phylogenetic analysis to meet two different objectives in this study. For the first objective, ITS, rpb2, and LEtef1 were evaluated in single and combined analyses to establish phylogenetic species limits. These analyses excluded the taxa H. avellanae, H. parmastoi, H. cineoreflava Samuels & Seifert, and H. alcalifuscescens, with isolates of T. cf. citrinoviride, H. megalocitrina, H. pezizoides, and H. cf. ochroleuca used as outgroup taxa. The second objective was to place Hypocreanum isolates with Trichoderma sect. Hypocreanum anamorphs in phylogenetic context with other Hypocreanum species. For the second objective, Sphaerostilbella cf. aureonitens, Arachnocrea scabrida Yoshim. Doi, and Hypomyces stephanomannatis Rogerson & Samuels were used as outgroup taxa for the combined LEtef1 and rpb2 analysis with representative isolates from the different sections of Trichoderma included in the analysis. Maximum parsimony (MP) analyses were done using the heuristic search option under the following conditions: TBR branch swapping, 10 random addition sequences, and gaps (insertions/deletions) treated as missing. Bootstrap analysis was performed in 500 replicates with random sequence addition (10 replicates). For the combined LEtef1 and rpb2 analysis, sequences were trimmed to the same starting position because some GenBank sequences not generated in this study were significantly shorter. All sequences and alignments were deposited in GenBank (Table 1).

Alternate phylogenetic hypotheses reflecting different species relationships were compared by the Kishino-Hasegawa (K-H) test (Table 2) in PAUP* for the combined LEtef1 and rpb2 data set. The most parsimonious trees recovered with and without constraints were compared by likelihood scores (Table 2). The likelihood model implemented in the K-H test assumed equal rates of substitution and empirical base frequencies. Models of sequence evolution were tested and model parameters obtained for the LEtef1, rpb2, and combined alignments using MODELEST 3.06 (Posada & Crandall 1998) as implemented in PAUP*. For the LEtef1 data, the likelihood ratio test (LRT) implemented in MODELEST, selected the TIM+I+G model with unequal base frequencies; nucleotide frequencies were set to A: 0.2133, C: 0.3337, G: 0.2211, T: 0.2320; a gamma-shape parameter of 0.5234; and substitution rates set to A: 0.2413, C: 0.2787, G: 0.2551, T: 0.2320; a gamma-shape parameter of 0.5234; and substitution rates set to 1.0000 (A–C), 3.1252 (A–G), 1.0000 (A–T), 1.6847 (C–G), 1.6847 (C–T), 10.5209 (C–T), and 10.5209 (C–T), and 1.0000 (G–T). For the rpb2 data, the LRT implemented in MODELEST, selected the Trn+I+G model with unequal base frequencies; nucleotide frequencies were set to A: 0.2413, C: 0.2787, G: 0.2551, T: 0.2248; a gamma-shape parameter of 1.1736; and substitution rates set to A: 0.2413, C: 0.2787, G: 0.2551, T: 0.2248; a gamma-shape parameter of 1.1736; and substitution rates set to 1.0000 (A–C), 6.5499 (A–G), 1.0000 (A–T), 10.5209 (C–T), and 9.0762 (C–T), and 1.0000 (G–T). For the combined LEtef1 and rpb2 data set, the LRT implemented in MODELEST, selected GTR +I+G model with unequal base frequencies; nucleotide frequencies were set to A: 0.22590, C: 0.30330, G: 0.24090, T: 0.22990; a gamma-shape parameter of 0.87796; and
substitution rates set to 1.0000 (A–C), 5.2773 (A–G), 1.0000 (A–T), 1.0000 (C–G), 8.4309 (C–T), and 1.0000 (G–T). A maximum likelihood (ML) tree was then obtained in PAUP* using 10 random sequence addition replicates and the substitution model suggested by MODELTEST. Bootstrap analysis was performed with 500 replicates and fast stepwise addition.

**Morphology**
Anamorph and teleomorph characteristics were measured from isolates and specimens representative of each phylogenetic species. Cultures of Hypocrea were grown on PDA, CMD and SNA at 20°C, with 12 h fluorescent light and 12 h darkness. Observations of anamorphs were made at ca. 7–10 d post inoculation. Anamorph and teleomorph characters were measured following Overton et al. (2006) with the exception that optimal growth temperatures were not determined. Colour terminology was obtained from Kornerup & Wanscher (1981). Important morphological characters used in species recognition are discussed in the comments section immediately following each species description.

**RESULTS**

**Phylogeny**
Except for minor differences, the gene trees are concordant (Figs 1–3). The gene tree generated from ITS is slightly different from those obtained from LEfet1 and rpb2. Hypocrea sulphurea isolate G.J.S 00-172 from Russia grouped with North American isolates in the ITS tree (Fig. 1) but grouped with G.J.S. 95-140 from Europe in rpb2 and LEfet1 gene trees (Figs 2–3). This point of discordance between the gene trees establishes a phylogenetic species limit for isolates of H. sulphurea. In all three gene trees, isolates of H. victoriensis from Australia are phylogenetically distinct from isolates of H. sulphurea. The phylogenetic position of Protocrea farinosa varies between the gene trees. In ITS (Fig. 1) and LEfet1 (Fig. 3) gene trees, P. farinosa is basal to other species in Trichoderma sect. Hypocreanum. In the rpb2 gene tree, P. farinosa resides in the H. pseudostraminea clade (Fig. 2) with no bootstrap support. Consequently, the exact phylogenetic position P. farinosa in relation to Hypocrea spp. with anamorphs referable to sect. Hypocreanum, is unresolved.

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**Fig. 1.** Parsimony analysis of ITS. One phylogram of 3193 most parsimonious trees; 217 steps; consistency index 0.779; retention index 0.855; homoplasy index 0.221; numerical values of branch lengths are given above and bootstrap values (500 replicates with 10 random addition replications) are indicated below branches. Outgroup taxa: H. megalocitrina; H. ochroleuca; H. pezizoides; T. cf. citrinoviride.
Table 1. Isolates used in molecular phylogenetic analyses. (* = ex-type strain).

| Name                              | Accession number | Origin                     | GenBank accession number |
|-----------------------------------|------------------|----------------------------|--------------------------|
|                                   |                  |                            | ITS                      |
| H. pulvinata Fockel               | G.J.S. 94-20     | Tushar Mountains, Utah, U.S.A. | DQ835409 DQ835485 DQ835451 |
|                                   | G.J.S. 92-127    | Olympia National Park, Washington, U.S.A. | AF487666 DQ835484 DQ835461 |
|                                   | G.J.S. 98-104    | Naturpark Saar-Hunsrück, Germany | AF487665 DQ835490 AF545559 |
|                                   | G.J.S. 95-220    | Waldviertel, Lower Austria, Austria | DQ835407 DQ835486 DQ835452 |
| H. americana                      | G.J.S. 92-93     | New Mexico, U.S.A.          | DQ835410 DQ835489 DQ835455 |
| (Canham) Overton                  | G.J.S. 94-79     | White Mountains, Arizona, U.S.A. | DQ835408 DQ835491 DQ835456 |
| H. protopulvinata                 | K.P. 00-56       | Unknown, U.S.A.             | DQ835406 DQ835488 DQ835453 |
| Yoshim. Doi                       | CBS 739.83*      | Chiba Pref., Kiyosumi, Fudagou, Japan | DQ835405 DQ835487 DQ835463 |
| H. citrina (Pers. : Fr.)          | G.J.S. 95-183    | Daniel Boone National Forest, Kentucky, U.S.A. | DQ835413 DQ835469 DQ835458 |
| Fr.                               | B.E.O. 99-29     | Oswego County, New York, U.S.A. | DQ835412 DQ835482 DQ835464 |
|                                   | G.J.S. 89-145    | Devon, Budleigh, Saltaton, U.K. | DQ835414 DQ835483 DQ835457 |
|                                   | G.J.S. 96-257    | Ascotung, Vermont, U.S.A.   | DQ835418 – – |
|                                   | CBS 708.73       | Baarn, Zandheuvelweg, Netherlands | DQ835415 – – |
|                                   | CBS 853.70       | Palmer Wald near Gerolstein, Germany | DQ835416 – – |
|                                   | CBS 894.85*      | Hestreux near Eupen, Belgium | DQ835417 DQ835481 AF545561 |
| H. pseudostreminata               | G.J.S. 91-135    | Prince Georges County, Maryland, U.S.A. | DQ835419 – – |
| Yoshim. Doi                       | G.J.S. 90-74     | Dutches County, New York, U.S.A. | DQ835420 DQ835470 DQ835454 |
|                                   | G.J.S. 95-189    | Daniel Boon National Forest, Kentucky, U.S.A. | DQ835421 – – |
|                                   | G.J.S. 95-189    | Brown County, Indiana, U.S.A. | DQ835422 DQ835480 DQ835459 |
|                                   | B.E.O. 99-36     | Patapsco State Park, Maryland, U.S.A. | DQ835423 DQ835468 DQ835465 |
| H. megacitrina                     | B.E.O. 00-09     | North Carolina, U.S.A.      | DQ835511 AY225855 AF545563 |
| Yoshim. Doi                       | G.J.S. 97-248    | Georgia, U.S.A.             | DQ835424 DQ835479 DQ835462 |
| Yoshim. Doi                       | G.J.S. 91-61     | Virginia, U.S.A.            | DQ835426 DQ835478 DQ835460 |
| H. sulphurea (Schw.)              | G.J.S. 95-190    | Indiana, U.S.A.             | DQ835425 AY225858 AF545560 |
| Sacc.                             | G.J.S. 95-140    | Styria, Austria             | AF487664 DQ835471 DQ835515 |
|                                   | G.J.S. 00-172    | Moscow, Russia              | DQ835510 DQ835493 DQ835523 |
| H. victoriensis                    | G.J.S. 99-130    | Victoria, Australia         | DQ835504 DQ835472 DQ835516 |
| Overton                           | G.J.S. 99-200*   | Victoria, Australia         | DQ835505 DQ835473 DQ835517 |
|                                   | CBS 500.87       | New Zealand                | DQ835466 – – |
| H. eucticioides                    | G.J.S. 99-61     | Limon, Costa Rica           | DQ835467 DQ835474 DQ835518 |
| Overton                           | M 141            | Kurokami Kumamoto, Japan    | DQ835509 DQ835492 DQ835522 |
| Kalchbr. & Cooke                  |                  |                            |                          |
| H. farinosa Berk. & Broome        | G.J.S. 91-101    | Maryland, U.S.A.            | DQ835507 DQ835476 DQ835520 |
|                                   | G.J.S. 89-139    | Unknown mushroom farm, U.S.A. | DQ835508 DQ835477 DQ835521 |
| H. alcalifusescens                | B.E.O. 99-10     | Maryland, U.S.A.            | DQ835506 DQ835475 DQ835519 |
| Overton                           | TFC 181548*      | Estonia                     | – DQ834455 DQ834462    |
| H. parvastoi                      | TFC 97-143*      | Voru Commune, Estonia       | – DQ834456 DQ834463    |
| Overton                           |                  |                            |                          |
| H. avellanea Carey & Rogerson     | C.T.R. 77-155*   | Type locality, Massachusetts, U.S.A. | – AY225857 AF545562 |
| H. cf. ochroleuca                  | G.J.S. 01-265    | Thailand                    | DQ835512 DQ835494 DQ835524 |
Table 1. (Continued).

| Name | Accession number | Origin | GenBank accession number |
|------|------------------|--------|-------------------------|
| *H. pezizoides* Berk. & Broome | G.J.S. 01-231 | Unknown | DQ835513 AY225859 AF545564 |
| *H. cf. cinereoflava* | G.J.S. 92-102 | Unknown | – DQ834454 DQ834461 |
| *H. psychrophila* Müll., Aebi & J. Webster | HY8 (CBS 262.71) | Switzerland | – AF534584 AF545520 |
| *H. rufa* (Pers.) Fr. | G.J.S. 89-127 | North Carolina, U.S.A. | – AF534585 AF545521 |
| *H. pilulifera* J. Webster & Rifai | CBS 814.68 | Yorkshire, U.K. | – AF534583 AF545519 |
| *H. lutea* (Tode) Petch | G.J.S. 89-129 | New York, U.S.A | – AF534581 AF545517 |
| *H. strictipilosa* Chaverri & Samuels | G.J.S. 89-115 | Maryland, U.S.A. | – AF534596 AF545528 |
| *H. aureoviridis* Plowr. & Cooke | CBS 245.63 | England, U.S.A. | – AF534575 AF545504 |
| *H. nigrovirens* Chaverri & Samuels | G.J.S. 99-64 | Limón, Costa Rica | – AF534582 AF545518 |
| *Trichoderma* cf. *citrinoviride* G.J.S. 01-364 | Unknown | – DQ835514 AY225860 AF545565 |
| *T. fertile* Bissett | DAOM 167070 | Canada | – AF534617 AF545545 |
| *T. aggressivum* Samuels & W. Gams | CBS 100525 | United Kingdom | – AF534614 AF545541 |
| *T. flavofuscum* (J.M. Mill., Giddens, A.A. Foster) Bissett | DAOM 167652 | Georgia, U.S.A. | – AF534619 AF545547 |
| *T. stromaticum* Samuels & Pardo-Schultheiss | P.C. 209 | Brazil | – AF534613 AF545539 |
| *Arachnocrea scabrida* Yoshim. Doi | B.E.O. 02-01 | New York, U.S.A. | – DQ834457 DQ834458 |
| *Sphaerostilbella* cf. *aureonitens* | G.J.S. 74-87 | New Zealand | – DQ834452 DQ834460 |
| *S. cf. aureonitens* | G.J.S. 82-40 | New Zealand | – DQ834453 DQ834459 |

Nevertheless, *P. farinosa* is clearly situated in *Hypocrea* s.s. (Fig. 5, clades A2+B2), with a bootstrap score of 100 uniting the clades, and it will be referred to as *Hypocrea farinosa* in the remaining sections of this text (see Taxonomy).

All three datasets have similar homoplasy indices. The heuristic search of the most parsimonious tree for the ITS dataset yielded 3193 trees with 217 steps. The minimal possible tree length is 169; the homoplasy index (HI) is 0.221 (Fig. 1). From 550 total characters, 421 characters are constant: 41 variable characters are parsimony-uninformative and 88 characters are parsimony-informative. The heuristic search of the most parsimonious trees for the LE*tef* dataset resulted in a tree with 650 steps with the minimum possible tree length of 408: HI = 0.372 (Fig. 2). From 956 total characters, 651 characters are constant: 88 variable characters are parsimony-uninformative, and 217 characters are parsimony-informative. The heuristic search of the most parsimonious trees for the rpb2 data set yielded 64 trees with 314 steps with the minimum possible tree length of 178: HI = 0.433 (Fig. 3). From 863 total characters, 705 characters are constant, 35 variable characters are parsimony-uninformative, and 123 characters are parsimony-informative.

The combined phylogenetic analysis using ITS, partial sequences of LE*tef* and rpb2 showed that *H. sulphurea*, *H. subsulphurea*, *H. victoriensis*, *H. farinosa*, and *H. corticioides* represent phylogenetically distinct species. *Hypocrea sulphurea*, *H. victoriensis*, *H. subsulphurea*, and *H. corticioides* formed a monophyletic clade C, supported by a bootstrap
Fig. 2. Parsimony analysis of partial sequences of rp2. Single most parsimonious tree; 650 steps; consistency index 0.628; retention index 0.771; homoplasy index 0.372, rest as Fig. 1.

score of 77 %, with *H. sulphurea* distinguished from *H. victoriensis* by a bootstrap score of 100 % (Fig. 4). European and North American isolates of *H. sulphurea* formed a distinct clade supported by bootstrap scores of 92 % in the combined analysis (Fig. 4), but more European isolates must be sequenced before determining whether European isolates represent a distinct phylogenetic species. *Hypocrea citrina*, *H. americana*, *H. pulvinata*, and *H. protopulvinata* formed a strongly supported monophyletic clade B with a bootstrap score of 100 % (Fig. 4). *Hypocrea microcitrina* and *H. pseudostraminea* are located in an unresolved clade A, sister to *H. citrina*, supported by a bootstrap score of 90 %. The heuristic search of the most parsimonious trees yielded three trees with 1202 steps, with the minimum possible tree length of 753: $HI = 0.374$ (Fig. 4). From 2358 total characters, 1767 characters are constant; 163 variable characters are parsimony-uninformative, and 428 characters are parsimony-informative.

The LF1 and rp2 regions distinguished between North American and European isolates of *H. sulphurea*, whereas ITS did not. The LF1 region was less variable than rf2 sequences generated by Overton et al. (2006), using the primer pair ef-1/2, for *H. citrina* and allies. Sequences were generated from the rf1 gene region for selected species of *H. sulphurea* and allies included in this study and deposited in GenBank (Table 3). The introns of rf1 were highly variable making alignments between species such as *H. citrina* and *H. sulphurea* problematic. Consequently, the rf1 region was excluded from this study.
Species of Hypocrea with anamorphs assignable to Trichoderma sect. Hypocreanum did not form a monophyletic group. The K-H test on the combined LEteff and rpb2 dataset indicated a significantly worse tree (p < 0.0001) when all Hypocrea with anamorphs in Trichoderma sect. Hypocreanum were constrained to monophyly (Monophyletic Hypocreanum, Table 2). When taxa with hyphal stromata were constrained to monophyly (Monophyletic Hyphal, Table 2) the –log likelihood was significantly worse (P < 0.0001) than that of the unconstrained tree.

The phylogenetic relationship of Trichoderma sect. Pachybasium s.l. to clades A2, B2, and C2 could not be established. Hypocrea megacorticita is situated in clade A2, which was supported by a bootstrap score of 93 %. Hypocrea avellanea and H. pezizoides, both of which have a verticillium-like anamorph, reside in the H. rufa clade C2 (Fig. 5) supported by a bootstrap score of 75 %. Hypocrea parmastoi and H. alcalifuscescens are located in the unresolved clades F2 and G2, basal to all species of Hypocrea included in this analysis (Fig. 5), but have verticillium-like anamorphs referable to Trichoderma sect. Hypocreanum. Based on this dataset, it is unclear whether Hypocrea cinereoflava, H. parmastoi, and H. alcalifuscescens should be maintained within Hypocrea, as all three species were basal to other members of the genus (Fig. 5). For the combined LEteff and rpb2 dataset, the heuristic search of the most parsimonious trees yielded three trees with 2469 steps with the minimal possible tree length of 875: H1 = 0.646 (Fig. 5). From 1588 total characters, 1009 characters were constant, 127 variable characters were parsimony-uninformative, and 452 characters were parsimony-informative. ML analysis of the combined data resulted in two trees with log Likelihood scores of −12767.00537 (not shown). These trees did not significantly differ from the tree generated in the MP analysis (Fig. 5).

**Table 2.** Results of the Kishino-Hasegawa likelihood test

| Topology          | Trees | −ln likelihood | P^1 |
|-------------------|-------|----------------|-----|
| Unconstrained     | 1^2   | 14817.41       | Best|
| Monophyletic      |       |                |     |
| Hypocreanum       | 5     | 15048.56−15048.67 | <0.0001*|
| Monophyletic      | 1     | 15156.58       | <0.0001*|

^1Probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (two-tailed test); indicates significance at P < 0.05.

^2The best −ln likelihood tree from the maximum parsimony analysis.

DISCUSSION

Species recognition

The combined phylogenetic analyses using ITS and partial sequences of LEteff and rpb2 show that Hypocrea sulphurea, H. subsulphurea, H. victoriensis, H. farinosa, and H. euctriciooides represent phylogenetically distinct species that are members of a strongly supported clade C (Figs 4, 5). Dingley (1956) suggested that morphology could not be used to distinguish between H. subcitrina and H. sulphurea and considered these species synonymous. Based on type studies, we found the ascospores of H. subcitrina to be consistently shorter and narrower than those of H. sulphurea. In contrast, Hypocrea egmontensis is considered a facultative synonym of the older H. subcitrina.

Dingley deposited a culture of H. sulphurea (CBS 500.67) from New Zealand in CBS. Specimens recently collected from Australia had the same ITS sequence as CBS 500.67 and represent Dingley’s concept of H. sulphurea. Molecular phylogenetic results indicate that the Australian specimens and the New Zealand culture (CBS 500.67) represent a new phylogenetic species, described here as H. victoriensis, that differs morphologically from H. subcitrina and H. sulphurea. The morphological similarities between Australian specimens of H. victoriensis and North American specimens of H. sulphurea are striking, but the part-ascospores of the Australian species are more strongly spinulose than the part-ascospores found in H. sulphurea. In addition, none of the Australian specimens occurred on Exidia spp., which is a common substrate in North America. This suggests that ascospore ornamentation and substratum are informative species characters for members of the H. sulphurea subclade (clade C, Fig. 4).

Hyphal versus pseudoparenchymatous stromata

Hypocrea species with hyphal stromata and anamorphs assignable to Trichoderma sect. Hypocreanum are situated in different clades. Hypocrea megacorticita resides in clade A2 (Fig. 5) with H. psychrophila. Hypocrea avellanea has a hyphal stroma and a verticillium-like anamorph with conidia that are uniform in size and shape. Anamorphs in Trichoderma sect. Hypocreanum typically produce conidia that are variable in size and shape. Hypocrea avellanea resides in the H. rufa clade (Fig. 5) with species having pseudoparenchymatous stromata. Anamorphs in the Hypocrea rufa clade generally produce conidia that are typically more uniform in size and shape than those found in Trichoderma sect. Hypocreanum. Hypocrea alcalifuscescens and H. parmastoi have hyphal stromata and verticillium-like anamorphs and are basal to other major clades of Hypocrea/Trichoderma. Species found in clade B2 (Fig. 5), have effused, extensive
### Table 3. Additional tef1 sequences deposited in GenBank.

| Name                  | Strain Number | Origin                  | GenBank accession number (tef1, primers ef-1, ef-2) |
|-----------------------|---------------|-------------------------|--------------------------------------------------|
| *H. sulphurea* (Schw.) Sacc | G.J.S. 95-190 | Indiana, U.S.A.         | DQ835448                                         |
|                       | G.J.S. 95-140 | Styria, Austria          | DQ835499                                         |
|                       | G.J.S. 00-172 | Moscow, Russia           | DQ835495                                         |
|                       | G.J.S. 95-176 | Kentucky, U.S.A.         | DQ835498                                         |
|                       | B.E.O. 98-44 | Pennsylvania, U.S.A.     | DQ835496                                         |
|                       | B.E.O. 98-45 | Pennsylvania, U.S.A.     | DQ835497                                         |
| *H. victoriensis* Overton | G.J.S. 99-200 | Victoria, Australia      | DQ835500                                         |
|                       | G.J.S. 99-201 | Victoria, Australia      | DQ835501                                         |
| *H. eucorticioides* Overton | G.J.S. 99-61 | Limon, Costa Rica        | DQ835502                                         |
| *H. farinosa* Berk. & Broome | G.J.S. 91-101 | Maryland, U.S.A.         | DQ835503                                         |

![Parsimony analysis of partial sequences of LEtef1. One phylogram of 64 most parsimonious trees; 314 steps; consistency index: 0.567; retention index: 0.744; homoplasy index: 0.433, rest as Fig. 1.](image)

Fig. 3. Parsimony analysis of partial sequences of LEtef1. One phylogram of 64 most parsimonious trees; 314 steps; consistency index: 0.567; retention index: 0.744; homoplasy index: 0.433, rest as Fig. 1.
stromata, with pseudoparenchymatous tissue, except one, *H. subsulphurea*, which is hyphal. Anamorphs in clade B2 produce conidia variable in size and shape, typical of *Trichoderma* sect. *Hypocreanum*. *Hypocrea pezizoides*, known to have a pseudoparenchymatous stroma and a verticillium-like anamorph also resides in the *H. rufa* clade (C2, Fig. 5), a finding consistent with Chaverri et al. (2003). The anamorph of *H. pezizoides* produces conidia that initially are light green, but become hyaline after repeated transfers. Species with pseudoparenchymatous stroma and anamorphs that produce hyaline conidia variable in size and shape are located in clade B2 (Fig. 5). Species with hyphal stromata and anamorphs that produce uniform conidia (of similar size and shape) are polyphyletic.

Petch (1937) established the genus *Protocrea* Petch for species that have simple ascomata immersed or seated upon a byssoid stroma with ascospores that disarticulate into part-ascospores. Rossman et al. (1999) described the anamorph of *Protocrea* as acremonium- or verticillium-like. *Protocrea farinosa* resides in clade B2 (Fig. 5) with other species with acremonium- and verticillium-like anamorphs. A well-defined layer of pseudoparenchymatous tissue was observed below the perithecia in specimens of *P. farinosa*. Although the telemorphs of specimens examined varied in the degree of pseudoparenchymatous tissue present, the part-ascospore measurements obtained are identical to those published for *P. farinosa* by Rossman et al. (1999) and the anamorph characteristics are identical to those described by Doi (1972) for *P. farinosa*.

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**Fig. 4.** Combined parsimony analysis of ITS, LEf1, rpb2. Phylogram of one of three most parsimonious trees; 428 steps; consistency index: 0.626; retention index: 0.766; homoplasy index: 0.374, rest as Fig. 1.
Trichoderma sect. Hypocreanum and classification

The phylogeny of the major clades in Trichoderma Hypocreanum is essentially unresolvable based on the genes used in this study. However, Hypocreanum spp. with well-defined pseudoparenchymatous stroma tissue, and acremonium- or verticillium-like conidiophores (hypocreanum-like), that produce hyaline conidia variable in size and shape, can be accommodated in a large monophyletic assemblage of species B2 (Fig. 5). Kullnig-Gradinger et al. (2002) suggested that Trichoderma sect. Hypocreanum and sect. Pachybasiom should be merged as they are phylogenetically indistinguishable. The present study, which included 17 taxa morphologically belonging to sect. Hypocreanum, shows that the phylogenetic relationship of Trichoderma sect. Hypocreanum to sect. Pachybasiom could not be resolved in the combined LEf1 and rpb2 dataset. The anamorphs of H. megaloclitina, H. parmsgustoi, and H. alcalifuscescens are morphologically similar to anamorphs typical of Trichoderma sect. Hypocreanum; nevertheless, these fungi do not belong to the major Hypocreanum clade B2 (Fig. 5), nor are they phylogenetically related to members of Trichoderma sect. Pachybasiom.

The multigene phylogeny of Kullnig-Gradinger et al. (2002) should serve as an example for future phylogenetic analyses to determine sectional relationships, but future studies should include a larger number of taxa and exclude ITS rDNA sequences. The

Fig. 5. Parsimony analysis of the combined LEf1 and rpb2 data set. Phylogram of one of 3 most parsimonious trees; 2469 steps; consistency index: 0.354; retention index: 0.515; homoplasy index: 0.646, rest as Fig. 1. Outgroup taxa: Hypomyces stephanomatis, Arachnocrea scabrida, Sphaerostibella cf. aureonitens.

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**Fig. 5**

A2

B2

C2

D2

E2

F2

G2

0.01 substitutions/site

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ITS region proved useful in distinguishing between closely related species (Overton et al. 2006) and has been used for the revision of sections Longibrachiatum and Trichoderma (Kuhls et al. 1996, 1997; Kinderman et al. 1998; Samuels et al. 1998, 1999).

Overton et al. (2006) demonstrated that ITS rDNA, rpb2, and the tef1 region could establish phylogenetic species limits, but the introns found in the tef1 region, delimited by the primers ef-1 and ef-2, were highly divergent among morphologically similar species. In this study partial sequences of the large exon (LEtef1) were generated for the seven species, including several of those treated by Overton et al. (2006). The LEtef1 region also resolved all major clades established by these authors using the tef1 gene region and distinguished between North American and European isolates of H. pulvinata; therefore LEtef1 is better suited for phylogenetic studies than the tef1 region previously sequenced by Overton et al. (2006).

Comparatively few of the approximately 200 named species of Hypocreap have been sequenced to date, with published accounts placing an over-reliance on ITS rDNA sequence data. The LEtef1 and rpb2 sequences generated in this study, work by Chaverri et al. (2003), and data from other gene regions published by Kullnig-Gradinger et al. (2002), have helped to clarify our understanding of the sectional relationships of Hypocreap/Trichoderma. Additional taxa from other genera such as Sarawakus Lloyd, with Trichoderma anamorphs, need to be sequenced before a complete phylogeny of Hypocreap/Trichoderma can be established.

The evolution of anamorphs referable to Trichoderma sect. Hypocreanum

There has been considerable speculation published on the evolution of the anamorphs in Trichoderma sect. Hypocreanum. Samuels (1996) hypothesized that the anamorphs referable to Trichoderma sect. Hypocreanum may be synanamorphs or spermatial states, suggesting that Hypocreap with acremonium- or verticillium-like anamorphs with hyaline conidia have lost the ability to produce a primary trichoderma-like anamorph, with pyramidal branched conidiophores and green conidia. Kullnig-Gradinger et al. (2002) presented molecular data suggesting that the more typical trichoderma-like anamorph with green conidia may have evolved from genera having verticillium-like anamorphs, in particular Aphysiosstroma Barrasa and Arachnocrea Z. Moravec.

Results based on molecular data have not conclusively established the evolution of the Trichoderma anamorph, including those referable to sect. Hypocreanum. Two species are of particular interest when considering the hypotheses promulgated by Kullnig-Gradinger et al. (2002) and Samuels (1996). Hypocreap pezizoides has light green conidia that become completely hyaline in subsequent transfers, suggesting an incomplete reversal to the primitive verticillium-like form with hyaline conidia. This species resides in the H. rufa clade C2 (Fig. 5) based on combined rpb2 and LEtef1 gene sequences and based on ITS sequence data, a finding consistent with Kullnig-Gradinger et al. (2002). Hypocreap cinereoflava produces a primary synnematous anamorph and a verticillium-like synanamorph. This species of Hypocreap is important when considering the hypothesis of Samuels (1996) that the verticillium-like anamorphs found in Trichoderma sect. Hypocreanum represent spermatial states, in which the primary trichoderma-like anamorph was lost. Hypocreap cinereoflava is located in an unresolved basal clade of Hypocreap s.s. (Fig. 5) and, based on the molecular results of this study, it could not be excluded from the genus Hypocreap. The phylogenetic placement of this species basal to all other Hypocreap species sequenced in this analysis could suggest that the ability to produce a synnematous primary anamorph has subsequently been lost. The data obtained in this study provide some support for the hypotheses of Samuels (1996) and Kullnig-Gradinger et al. (2002), leaving room for speculation. Additional taxa need to be sequenced before the evolution of Trichoderma anamorphs can be more accurately determined.

TAXONOMY

1. Hypocreap sulphurea (Schw.) Sacc., Syll. Fung. 2: 535. 1883. Figs 6–8. ≡ Sphaeriap sulphurea Schw., Trans. Amer. Philos. Soc. 2: 193. 1832. ≡ Hypocreap sulphurea f. macrospora Yoshim. Doi, Bull. Natl. Sci. Mus. 15: 699. 1972.

Anamorph: Trichoderma sp. [sect. Hypocreanum].

Teleomorph: Stromata effuse, extensive, largest continuous stroma 70 × 30 mm, smallest continuous stroma 1 × 1 mm, many stromata not larger than 25 × 10 mm, varying in colour, sometimes vivid yellow, usually light yellow to greyish yellow (3A8; 3A5–3A6; 4A5–4A6), KOH⁺, reaction variable, usually very weak, the stroma becoming light orange (6A4); ostiolar canals visible at the stroma surface, appearing light orange (6A4), giving rise to the greyish yellow overall appearance of the stroma. Stromata smooth; tissue immediately below the stroma surface formed of compact to loose pseudoparenchymatous cells of textura globulosa to t. angularis. Perithecia completely immersed, generally widely spaced, compact in some regions, sometimes completely absent near the margins or regions of extensive stroma growth. Perithecia ellipsoid, (128–)190–250(–277) μm long (including the length of the ostiolar canal, n = 26); width of perithecia near the base (measured from 3/4 total length of the perithecium), (87–)100–142(–175) μm (n = 26); length of ostiolar canal (42–)52–74(–85) μm; width of ostiolar canal from outer perithecial wall to the opposite internal perithecial wall (22–)30–50(–64) μm (n = 26); wall KOH⁺, reaction variable, weak. Ascis cylindrical, (80–)94–116(–150) × (4.2–)5.3–7.1(–8.3) μm (n = 196); tip slightly thickened. Part-ascospores hyaline, thick-walled, spinulose, dimorphic; distal part...
Anamorph: Colonies covering a 100 mm diam Petri plate with PDA in 10 d, not producing concentric rings or radial rays of mycelium; a layer of aerial mycelium covering the entire Petri plate, aerial mycelium consisting of visible hyphal elements; phialides subulate, (8–)17–32(–45) µm long hyphal elements, usually verticillium-like; phialides on conidiophores; conidiophores irregularly branched, on entire Petri plate, aerial mycelium consisting of visible rays of mycelium; a layer of aerial mycelium covering the Petri plate with PDA in 10 d, not producing concentric rings or radial rays of mycelium; a layer of aerial mycelium consisting of visible hyphal elements; phialides subulate, (8–)17–32(–45) × (2.4–)3–4(–4.8) µm (n = 294); conidia variable in size, obovate to subcylindrical, often ellipsoidal, (3.9–5.6–9.0(–12.6) × (3.0–)3.3–4.3(–6.6) µm (n = 112), with some conidia asymmetric, having a flat edge; no distinctive odour; yellowish orange pigment (4A6–4A8) produced near the inoculation point. After 10 d conidia beginning to swell and more variable in size. Colonies on SNA or CMD did not produce conidiophores in 10 d.

Habitat: Found on decorticated wood with *Exidia* spp., sometimes occurring on decorticated wood without visible evidence of *Exidia* spp.

**Known distribution**: Europe, Japan and North America.

**Isotype**: U.S.A., Pennsylvania, Salem & Bethlehem, on *Exidia* sp., *H. sulphurea* (K, herb. Schweinitz).

**Other specimens examined**: Austria, Styria, Leibnitz, St. Nikolai, alt. 310 m, on decorticated wood, *Exidia* sp. not visible, 26 Aug. 1995, H. Voglmayr (BPI 737705; culture G.J.S. 95-140). Japan, Amori Prefecture, near Tsuta-Onsen, Towanda National Park, Towanda-Cho, Kami-kita-Gun, on *Exidia* sp., 10 Sep. 1971, Y. Doi, (NY, TNS. D-1169 = TNS-F-190169), **paratype** of *H. sulphurea* f. macrospora. Russia, 10 km northeast of Moscow, mixed deciduous forest, 17 Oct. 2000, A. Alexandrova (BPI 748252; culture G.J.S. 00-172). U.S.A., Indiana, Brown County, vic. Pikes Peak, Happy Hollow Camp, alt. 250 m, 39°09′ N, 86°06′ W, on bark with unidentified fungus, 29 Sep. 1995, G. J. Samuels (BPI 737764; culture G.J.S. 95-190); Brown County, Yellow Wood State Forest, Jackson Creek Management Trail, alt. 200 m, 39°09′ N, 86°06′ W, on *Exidia* sp., 30 Sep. 1995, G.J. Samuels (BPI 737772; culture G.J.S. 95-198); Illinois, Carbondale, Giant City State Park, on *Exidia* sp., 9 Aug. 1999, B.E. Overton, B.E.O. 99-02 (BPI); Union County, Carbondale, Giant City State Park, on leaf litter and decorticated wood, 19 Sep. 1994, G. J. Samuels (BPI 749353; culture G.J.S. 94-58); Kentucky, Rowan County, Daniel Boone National Forest, Cave Run Lake, Sheltowee Trail, on *Exidia* sp., 26 Sep. 1995, G. J. Samuels (BPI 737752; culture G.J.S. 95-176); Maryland, Prince Georges County, Laurel, Patuxent Refuge, on *Exidia* sp., 2 July 2000, Kadri Põldmaa, K.P. 00-14 (BPI; TFC 2000-52); Tacoma Park, on *Exidia* sp., Dec. 1906, C.L. Shear (BPI 631489); New York, Green County, on bark, no *Exidia* sp. visible, 27 Sep. 1998, B.E. Overton, B.E.O. 98-50 (BPI); North Carolina, Durham County, Hill Forest, on Carya glabra var. glabra with *Exidia* sp., 18 May 2002, L. Grand (NCSU Mycological Herbarium); North Dakota, Fargo, on branches of *Tilia americana* with *Exidia* sp., 1907–1908, G. W. Wilson & F. J. Seaver (BPI 631488); Pennsylvania, Centre County, Rock Springs Agricultural Research Center, on *Exidia* sp., 19 Sep. 1998, B.E. Overton, B.E.O. 98-44 (BPI); same origin B.E.O. 98-45 (BPI); Vermont, Burlington, Indian Brook Conservation Area, Aug. 2000, B.E. Overton, B.E.O. 00-07 (BPI; culture G.J.S. 00-76).

**Comments**: The paratype specimen of *H. sulphurea* f. macrospora and the specimens from Russia and Austria had part-ascospores that were on average 1 µm larger than specimens of *H. sulphurea* from North America; *H. sulphurea* f. macrospora and European specimens (Russia and Austria) had distal part-ascospores, (5.6–)6.0–7.1(–7.6) × (4–)4.8–5.9(–6.5) µm, and proximal part-ascospores, (5.6–)6.4–7.6(–8.5) × (3.5–)4.3–5.7(–6.6) µm; *H. sulphurea* specimens from North America had distal part-ascospores (4.2–)5.3–6.4(–7.1) × (3.6–)4.2–5(–5.8) µm, and proximal part-ascospores (4.4–)5.3–6.4(–8.2) × (2.7–)3.9–4.7(–5.7) µm.

European and North American isolates were slightly different in LEdf1 and rpb2 gene trees, but in the ITS tree one European isolate grouped with isolates of *H. sulphurea* from North America. We use this point of discordance to establish the phylogenetic species limit for *H. sulphurea*. *Hypocrea sulphurea* f. macrospora is not considered sufficiently distinct from *H. sulphurea*.

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**Fig. 6.** A–C. *H. sulphurea*. A. Stroma with KOH reaction, BPI 737764; bar = 1 mm. B. Stroma with byssoid margin, BPI 737752; bar = 1 mm. C. Stroma with KOH reaction, TNS-F-190169; bar = 1 mm.
Fig. 7. A–F. *H. sulphurea*. A. Section of stroma showing ostiolar papilla; bar = 20 µm. B. Section of stroma showing textura globulosa to t. angularis with t. intricata below perithecium; bar = 20 µm. C. Asci with ascospores; bar = 20 µm; A–C. BPI 737752. D. Section showing t. angularis near surface, B.E.O. 98-44. E. F. TNS-F-190169, *H. sulphurea* f. macrospora; E. Section through ostiole; F. asci; bars = 20 µm. G. Smooth stroma surface, BPI 747764, *H. sulphurea* f. sulphurea; bar = 1 mm.
The teleomorph description provided above for *H. sulphurea* consists of combined measurements from all specimens examined for this species. Even with variable part-ascospores, the ascospores of *H. sulphurea* are significantly larger than those of *H. subcitrina*, even at the lower extremes; therefore the synonymy proposed by Dingley 1956 is rejected.

Doi (1972) described an additional species *H. megalosulphurea* Yoshim. Doi in which proximal part-ascospores can be as large as 10 µm diam. Type material or cultures were not available for study, but it is doubtful that *H. megalosulphurea* is a synonym of *H. sulphurea* because, even though part-ascospores can vary in size, variation of this magnitude was never observed in the specimens of *H. sulphurea* examined.

2. *Hypocrea subcitrina* Kalchbr. & Cooke, Grevillea 9: 26. 1880) = *H. egmontensis* Dingley, Trans. Roy. Soc. New Zealand 83: 647. 1956.

*Anamorph:* Unknown.

*Notes:* G.J. Samuels (pers. comm.) provided measurements of part-ascospores that allow a comparison of type specimens as follows: *Hypocrea subcitrina*, distal part-ascospores subglobose to obovate conical, (4.5–)4.7–5.1(–5.4) × (3.6–)3.8–4.2(–4.3) µm; proximal part tending to be oblong and narrow, (4.2–)4.7–5.6(–6.0) × (3.6–)3.5–3.7(–3.8) µm; *H. egmontensis*, distal part-ascospores subglobose, conical, (4.1–)4.5–5.3(–5.7) × (3.8–)4.4–4.6(–5.2) µm; proximal part-ascospores (4.3–)5.0–5.6(–6.7) ×
Fig. 9. A–F. H. victoriensis.
A. Stroma with discharged ascospores, BPI 747361; bar = 1 mm. B. Stroma showing KOH reaction, BPI 747363; bar = 1 mm. C. Section of Stroma, showing ostiolar papillae; bar = 40 µm. D, F. Section of stroma showing t. globulosa to t. angularis; bar = 20 µm. E. Asci with spinulose ascospores; bar = 20 µm; C–E. BPI 747362.
Fig. 10. H. victoriensis. A–C. Irregular verticillium-like conidiophore branching pattern; bars = 20 µm. D. Conidiophore, G. J. S. 99-201; bar = 20 µm. E. Conidia; bar = 20 µm; A–C, E. G.J.S. 99-200.
(2.7–)3.5–4.3(–4.1) µm. In this respect H. subcitrina and H. egmontensis are identical, differing from the larger ascospores of H. sulphurea and H. victoriensis. Based on ascospore measurements, H. egmontensis is considered a facultative synonym of the older name H. subcitrina. Doi (1971) already suggested that H. egmontensis and H. subcitrina were similar species. Doi (1971) described H. subcitrina var. dimorphospora Yoshim. Doi, based on the presence of part-ascospores of different size classes in specimens collected in New Guinea. Type material was not available for study and the relationship of this variety to H. subcitrina cannot be formally evaluated.

Specimens examined: South Africa, Port Natal, H. subcitrina, Wood 184 (K; isotype). New Zealand, Taranaki, Mt Egmont, Apr. 1946, J.M. Dingley 6272, H. egmontensis (PPD 6272; holotype).

3. H. victoriensis Overton, sp. nov. MycoBank MB501055. Figs 9–10.

Anamorph: Trichoderma sp. [sect. Hypocreanum].

Etymology: Named after the location where it was collected, Victoria, Australia.

Teleomorph: Stromata effusa, extensa, rubido-lutea vel griseo-lutea, KOH⁺. Ascosporeae hyalinae, crassitunicatae, spinulosae, dimorphicae; pars distalis plus minus ellipsipoidea, (4.8–)5.6–6.8(–7.4) × (3.9–)4.5–5.9(–5.9) µm, pars proxima (4.7–)5.8–7.4(–8.6) × (3.6–)4.1–5.1(–6.1) µm, L/W ratio (1–)1.1–1.3(–1.6) (n = 72); conidia variable in size, obovate to elongate-ovobate or subellipsoidal, (4.5–)5.9–9.3(–12.0) × (2.8–)3.2–4.2(–5.2) µm (n = 40); no distinctive odour; yellowish orange pigment (4A8) produced near the inoculation point. After 10 d conidia beginning to swell and more variable in size. Colonies on SNA or CMD did not produce conidiophores within 10 d.

Habitat: Typically found on decorticated wood or bark, species of Exidia not observed, possibly fungicolous.

Known distribution: Australia, New Zealand.

Holotype: Australia, Victoria, Otway National Park, along Great Ocean Road, Cannan's Track, alt. 350 m, on bark, 27 Aug. 1999, G. J. Samuels (BPI 747361; culture G.J.S. 99-200).

Other specimens examined: Australia, same origin as holotype (BPI 747362; culture G.J.S. 99-201); Otway Ranges, Melba Gully State Park, Madsen Track along the Johanna River, alt. 350 m, on bark, 27 Aug. 1999, G. J. Samuels (BPI 747363; culture G.J.S. 99-130). New Zealand, Culture CBS 500.67, as "Hypocreopsis squalida", PDD 6332, specimen not located.

Comments: Isolate CBS 500.67 had the same ITS sequence as isolates from Australia included here under the name H. victoriensis. The part-ascospore measurements of H. victoriensis and H. sulphurea are substantially larger than those found in the type of H. subcitrina. Molecular phylogenetic data indicate that H. sulphurea and H. victoriensis are phylogenetically close, but distinct species. H. victoriensis has part-ascospores that are distinctly more spinulose than those of H. sulphurea. In addition, the ostioles project conspicuously from the surface in H. victoriensis whilst in H. sulphurea the ostioles protrude only slightly. Furthermore, H. victoriensis does not appear to grow on species of Exidia.

4. Hypocreopsis eucorticioides Overton, nom. nov. MycoBank MB501056. Figs 11–12.

≡ Hypocreopsis eucorticioides Speg., An. Mus. Nac. Hist. Nat. Buenos Aires 23: 75. 1912 [non Berk. & Broome, J. Linn. Soc. Bot. 14: 111. 1873].

Anamorph: Trichoderma sp. [sect. Hypocreanum].

Teleomorph: Stromata effusa, extensive, largest continuous stroma 30 × 10 mm, smallest continuous stroma 3 × 2 mm, varying in colour, usually reddish yellow to greyish yellow (4A7–4B7), KOH⁻; reaction variable, usually very weak with stroma becoming light orange (6A4); ostiolar openings visible at the stroma surface, appearing light orange (6A4); ostiolar canal, Mela: Gulf State Park, Madsen Track along the Johanna River, alt. 350 m, on bark, 27 Aug. 1999, G. J. Samuels (BPI 747363; culture G.J.S. 99-130). New Zealand, Culture CBS 500.67, as "Hypocreopsis squalida", PDD 6332, specimen not located.

Comments: Isolate CBS 500.67 had the same ITS sequence as isolates from Australia included here under the name H. victoriensis. The part-ascospore measurements of H. victoriensis and H. sulphurea are substantially larger than those found in the type of H. subcitrina. Molecular phylogenetic data indicate that H. sulphurea and H. victoriensis are phylogenetically close, but distinct species. H. victoriensis has part-ascospores that are distinctly more spinulose than those of H. sulphurea. In addition, the ostioles project conspicuously from the surface in H. victoriensis whilst in H. sulphurea the ostioles protrude only slightly. Furthermore, H. victoriensis does not appear to grow on species of Exidia.
Fig. 11. A–F. *H. eucorticoides*. A. Effuse stromata with irregular margins, NY 29223; bar = 1 mm. B. Effuse stromata with irregular margins showing reaction in KOH, BPI 747358; bar = 1 mm. C. Asci with subglobose part-ascospores, BPI 747358; bar = 20 µm. D. Asci with subglobose part-ascospores, NY 29223; bar = 20 µm. E–F. Section of stroma showing *t. globulosa* to *t. angularis* tissue, BPI 747358; bar = 20 µm.

Fig. 12. A–D. *H. eucorticoides*, G.J.S. 99-61. A. Irregular verticillium-like branching pattern; bar = 40 µm. B–D. Phialides with developing conidia; bar = 20 µm.
textura globulosa to t. angularis. Perithecia completely immersed, generally widely spaced, compact in some regions, sometimes completely absent near the margins or regions of extensive stroma growth. Perithecia subglobose to subellipsoidal, (130–)147–193(–200) μm high (including the length of the ostiolar canal, n = 14); width of perithecia near the base (measured from 3/4 total length of the perithecium) (79–)89–125(–135) μm (n = 14); length of ostiolar canal (25–)40–57(–60) μm; width of ostiolar canal from outer perithecial wall to opposite internal perithecial wall (23–)37–42(–46) μm (n =14); wall KOH+ to opposite internal perithecial wall (3.5–)5.1–6.0(–7.3) μm (n = 60); tip slightly thickened. Part-ascospores hyaline, thin-walled, spinulose, monomorphic; generally subglobose, (2.6–)2.8–3.5(–3.9) × (2.4–)2.6–3.2(–3.7) μm, L/W ratio (0.8–)0.9–1.2(–1.3) (n = 60).

Anamorph: Colonies covering a 100 mm diam Petri plate with PDA in 10 d, not producing concentric rings or radial rays of mycelium; a layer of aerial mycelium covering the entire plate; conidiophores irregularly branched, on long hyphal elements, usually verticillium-like; phialides in whors of 2–3, solitary or alternating on long hyphal elements; phialides subulate, (4.1–)8–16(–23.2) × (1.5–)2.3–3.1(–5.6) μm (n = 34); conidia variable in size, typically subglobose, but sometimes subellipsoidal, (2.4–)3.3–4.2(–10) × (2.0–)2.5–3.1(–6) μm (n = 19); no distinctive odour or pigment. Colonies on SNA or CMD did not produce conidiophores within 10 d.

Habitat: Typically found on bark of decaying wood.

Known distribution: Central and South America.

Holotype: Argentina, Entre Rios, Ibiguary, 28 June 1911, C. Spegazzini no. 911 (LPS 1719).

Other specimens examined: Costa Rica, Limón, Puerto Viejo, Refugio Nacional Mendoça-Manzanilla, on decorticated wood, July 1999, G.J. Samuels & P. Chaverri (BPI 747358; culture G.J.S. 99–Refugio Nacional Mendaca-Manzanilla, on decorticated wood, July NYBG from the Venezuelan Expedition 1950–1951, on bark, 19 Oct. Other specimens examined no. 911 (LPS 1719).

Comments: The new name Hypocrea eucorticoides is proposed because H. corticioides Speg. is a later homonym of H. corticioides Berk. & Broome. The condition of the type of H. corticioides Speg. has degraded over time, but ascospore measurements were obtained: part-ascospores monomorphic, subglobose, (2.0–)2.6–2.8(–4.0) × (1.9–)2.3–2.9(–3.0) μm (G.J. Samuels, pers. comm.). The specimens of H. eucorticoides examined from Costa Rica and Venezuela were identical to the holotype from Argentina.

Doi (1975) included specimens in NY previously described as H. flava (from Costa Rica) under the name H. corticioides Speg. However, the part-ascospores of the holotype (LPS 1719 bis) illustrated by Doi (1975) in fig. 4 R and confirmed by our study of the holotype, are monomorphic and subglobose, differing substantially from the dimorphic part-ascospores illustrated by Doi for specimens of H. flava, fig. 4, M, O (Doi 1975). Specimens of H. flava were not examined in this study, but it is clear that H. flava should be retained as a distinct species.

5. Hypocrea subsulphurea Syd. in De Wildeman, Flores Bas et Moyen-Congo: 15. 1909. Figs 13–14. Anamorph: Trichoderma sp. [sect. Hypocreanum].

Teleomorph: Stromata effuse, extensive, surface hyphal, largest continuous stroma 10 × 10 mm, smallest continuous stroma 2 × 2 mm, varying in colour, usually light yellow to greyish yellow (4A5–4B5), KOH–, reaction weak, slightly darkening; ostiolar canals visible at the stroma surface, appearing golden-yellow or light orange (4A8–6D6). Stroma surface rough, tissue immediately below the stroma surface formed of compact to loose hyphal elements, textura intricata. Perithecia subglobose to ellipsoidal; wall KOH–, reaction variable, weak. Ascii cylindrical, often wider near the tip, (52–)59–72(–80) × (3.9–)4.1–5.6(–6.4) μm (n = 21); tip slightly thickened. Part-ascospores hyaline, thick-walled, spinulose, slightly dimorphic; distal part subglobose, (3.0–)3.4–4.0(–4.7) × (2.7–)2.9–3.5(–4.2) μm, L/W ratio (0.87–)1.0–1.2(–1.4) (n = 57); proximal part subglobose to subellipsoidal, (2.8–)3.6–4.4(–5.4) × (2.3–)2.9–3.6(–4.1) μm, L/W ratio (0.90–)1.0–1.4 (–2.4) (n = 58).

Anamorph: Colonies covering a 100 mm diam Petri plate with PDA in 10 d, not producing concentric rings or radial rays of mycelium; a layer of aerial mycelium covering the entire plate; conidiophores irregularly branched, on long hyphal elements, usually verticillium-like; phialides in whors of 2–4, solitary, or alternating in pairs on long hyphal elements, phialides subulate, (15.7–)22–30(–46) × (2.9–)3.3–3.7(–4.2) μm (n = 41); conidia variable in size, ellipsoidal to subcylindrical, (5.9–)6.4–10.4(–13.5) × (2.7–)3.2–4.3(–4.7) μm (n = 41), truncate base not prevalent, or when present not pronounced; no distinctive odour or pigment. Colonies on SNA or CMD did not produce conidiophores within 10 d.

Habitat: Typically found on bark with Exidia spp., also producing new stromata over remnants of previous stroma fructifications.

Known distribution: Africa and Japan.

Specimen examined: Japan, Kurokami Kumamoto, Tathuta Mt., Research Forest at Kyushu Research Center, Forestry and Forest Products Research Institute, old Hypocrea subsulphurea fruitbody on Exidia sp., 14 Feb. 2002, K. Miyazaki and B.E. Overton, M141 (BPI).

Comments: The type could not be located (in S) and is probably lost. The specimen examined in this study is in poor condition and from a different geographic location and cannot serve as neotype material. The size of the subglobose part-ascospores and smooth-walled conidia distinguish this species from H. microsulfurea. It is likely that H. microsulfurea is a phylogenetic sister species of H. subsulphurea and that globose part-ascospores and yellow extensive stromata represent apomorphic characters. The specimen of Hypocrea subsulphurea collected in Japan was severely

57
Fig. 13. A–D. *H. subsulphurea*, M 141. A–B. Perithecia forming on old stromata; bar = 1 mm. C. Asci and ascospores from old stroma; bar = 20 µm. D. Asci and ascospores from perithecia developing on old stroma surface; bar = 20 µm. Note: repeated attempts to section perithecia in old and developing stromata have failed.

Fig. 14. A–D. *H. subsulphurea*, M 141. A–B. Irregular verticillium-like branching pattern, on PDA; bar = 40 µm. C. Phialides with developing conidia, on PDA; bar = 20 µm. D. Conidia, variable in size, on PDA; bar = 20 µm.
degraded but discharging ascospores. The ascospores germinated on the surface of the old specimen and began producing additional perithecia in a thin byssoid layer. The hyphal stromata in this specimen may be an aberration caused by in-vitro production of perithecia.

6. *Hypocrea farinosa* Berk. & Broome, Ann. Mag. Nat. Hist., Ser. 2, 7: 186. 1851. Figs 15–16. ≡ *Protocrea farinosa* (Berk. & Broome) Petch, J. Bot. 75: 219. 1937.  

**Anamorph**: *Trichoderma* sp. [sect. *Hypocreanum*].

**Teleomorph**: Stromata effuse, extensive, surface hyphal, largest continuous stroma 40 × 25 mm, smallest continuous stroma 5 × 3 mm, varying in colour, usually light yellow to light brown (4A6–6D6), KOH+, reaction strong, with stroma becoming dark brown (6E6); ostiolar openings visible at the stroma surface, appearing orange or light brown (5A7–6D4). Stroma surface rough, formed of compact to loose hyphal elements, *textura intricata*; below the hyphal layer is a well-defined layer of loosely compacted pseudoparenchymatous tissue, *textura globulosa*. Perithecia surrounded by a loose layer of hyphae; perithecia generally widely spaced, compact in some regions, sometimes completely absent near the margins or regions of extensive stroma growth. Perithecia subglobose to subellipsoidal, (128–)140–200(–230) µm high (including the length of the ostiolar canal, n = 16); width of perithecia near the base (measured from 3/4 total length of the perithecium) (99–)110–155(–171) µm (n = 16); length of ostiolar canal (35–)40–60(–72) µm; width of ostiolar canal from the outer perithecial wall to the opposite internal perithecial wall (23–)27–40(–46) µm (n = 16); wall KOH+, reaction variable. Asci cylindrical, (43–)60–90(–113) × (2.8–)4.0–5.6(–6.8) µm (n = 96); tip slightly thickened. Part-ascospores hyaline, thick-walled, spinulose, dimorphic; distal part subglobose, (2.7–)3.3–4.0(–4.8) × (2.3–)3–3.6(–4.7) µm, L/W ratio (0.80–)0.98–1.2(–1.4) (n = 189); proximal part subellipsoidal, sometimes wedge-shaped, (2.7–)3.4–4.5(–5.6) × (2.3–)2.7–3.3(–3.9) µm, L/W ratio (0.90–)1.1–1.5(–1.9) (n = 189).

**Anamorph**: Colonies covering a 100 mm diam Petri plate with PDA in 10 d, not producing concentric rings or radial rays of mycelium; a layer of aerial mycelium covering the entire plate; brown pigment near the

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**Fig. 15.** A–D. *H. farinosa*. A. Effuse extensive stroma, BPI 802598. B. Effuse extensive stroma, BPI 747356. C. Colour variation in stroma, BPI 737771. D. KOH reaction, BPI 802598. bars = 1 mm.
point of inoculation (7D7–7E7). Colonies covering a 100 mm diam Petri plate with CMD in 10 d, producing a thin layer of effuse mycelium across the agar, with a thin cottony layer of aerial mycelium near the agar plug and the edge of the Petri plate; light brownish orange pigment (6C3) diffusing into the agar. Colonies covering a 100 mm diam Petri plate with SNA in 10 d, a thin layer of mycelium covering the agar surface, with a very thin cottony layer of aerial mycelium near the edge of the Petri plate; pinkish white pigment (10A2) produced near the point of inoculation. Isolates variably produce conidiophores and conidia on all three media. A combined description of the anamorph from all three media follows: Conidiophores produced on long a very thin cottony layer of aerial mycelium near the agar surface; conidia produced terminally on phialides, some conidia with basal abscission scar; conidia subglobose or obovate to subellipsoidal, sometimes elongate ellipsoidal, (3–)4.4–6.7(–11.6) × (1.9–)2.5–3.5(–5.0) µm (n = 79).

Habitat: Fungicolous, found on lichen-covered bark, Stereum spp., on logs inoculated with Lentinula edodes, and in bag cultures of L. edodes.

Known distribution: Japan, Europe, North America.

Epitype (designated here): France, Pyrénées Atlantiques, Forêt Domaniale d’Oloron 64, on bark, 30 Aug. 1997, F. Candoussau, # G.J.S. 513 (BPI 747356; culture G.J.S. 97-207).

Other specimens examined: Canada, Ontario, Bear Island, on Hymenochea sp., 28 Aug. 1937, H. S. Jackson, det. R. F. Cain (BPI 631473). Japan, Morotomuka Mura, anonymous mushroom farm, on log inoculated with Lentinula edodes, 29 July 1999, K. Miyazaki, M107 (BPI). U.S.A., Indiana, Brown County, Yellow Wood State Forest, alt. 200 m, Jackson Creek Management Trail, 39°09’ N, 86°06’ W, on bark, 30 Sep. 1995, G. J. Samuels (BPI 737771; culture G. J. S. 95-197); Louisiana, St. Martinville, on decayed wood, 24 Aug. 1890, A.B. Langlois, Flora Ludovicana # 2294 (BPI 631450); Maryland, Ellicot City, Papatasco Valley State Park, on Stereum cf. ostrea, 8 Sep. 1999, B.E. Overton, B.E.O. 99-16 (BPI; culture B.E.O. 99-16); Prince Georges County, Greenbelt Forest, on bark, fall 1991, G. J. Samuels (BPI 1112870; culture G.J.S. 91-101); same origin, 8 Nov. 1991, S.E. Rehner (BPI 1112896); unknown mushroom farm, on wood and grain inoculated with Lentinula edodes, 1989, sent to D. Farr at USDA/SBML (BPI 802598; culture G.J.S. 89-139).

Comments: The ascospore measurements given for Protocrea farinosa by Rossman et al. (1999), based on a reexamination of the type, are nearly identical to those of H. farinosa recorded in this study: distal part subglobose, (3–)3.4–3.7(–4.6) × (2–)2.5–3(–3.3) µm; proximal part wedge-shaped to ellipsoid, (3.2–)3.5–4.5 × 2–2.7(–3) µm. Rossman et al. (1999) agreed with Doi (1972) and suggested that P. farinosa has an acremonium-like anamorph, but noted that the anamorph of P. farinosa described by Doi (1972) may be questionable. The anamorph characteristics observed in this study are identical to what Doi (1972) described. The only anomalous character is the well-defined layer of pseudoparenchymatous tissue near the base of the perithecium, which is not consistent with the completely hyphal stromata described in the type description of H. farinosa. Teleomorph anatomy is variable, with some specimens of H. farinosa being more hyphal than others. In older specimens it is difficult to recognize the pseudoparenchymatous layer near the base of the stroma in H. farinosa. The significance of a purely hyphal stroma versus a stroma composed of two distinct layers is unclear but it is likely that this character has been misinterpreted because of the original condition of specimens used to delineate H. farinosa.

Hypocreia farinosa has been observed in the United States and Japan associated with the cultivation of Lentinula edodes. Japanese isolates of this species have been shown to be aggressive against commercial isolates of Lentinula (Kazuhiro Miyazaki, unpublished). In the United States, collections were made on a species of Stereum and lichen-covered bark. *Hypocreia farinosa* was collected once from *Lentinula* bag culture.

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Fig. 16. A–H. *H. farinosa*. A. Section of stroma; note the layer of *T. intricata* near the stroma surface and loose layer of pseudoparenchymatous tissue near the base of the perithecium; bar = 40 µm. B. asci and part-ascospores, both of BPI 802598, bar = 20 µm. C. Section of stroma; bar = 40 µm. D–E. *Textura intricata* near stroma surface and *T. globulosa* to *T. angularis* below perithecium, notably different from that shown in A; bar = 20 µm. C–E. BPI 112870. F–G. Conidiophores and developing conidia, on PDA; bars = 20 µm. H. Conidia on PDA; bar = 40 µm; F–H. G.J.S. 89-139.
HYPOCREA spp. WITH ANAMORPHS IN TRICHODERMA sect. HYPOCREANUM

Fig. 17. A–F. *H. alcalifuscescens*, TAA 181584. A. Stroma showing reaction in KOH; bar = 1 mm. B. Section of stroma showing compact *t. intricata* and KOH-positive layer of stroma tissue; bar = 20 µm. C. Asci and ascospores; bar = 20 µm. D. Conidia; bar = 20 µm. E–F. Conidiophore branching pattern; bars = 40 µm, and 20 µm, respectively; D–F. TFC 181584. Note: in spite of several attempts, no illustrative sections of perithecia were obtained.
It is likely that this species is fungicolous in nature and is present on other fungi on logs used for production of *L. edodes*; thus it can easily switch from natural substrates to the commercial strains of *L. edodes*. Consequently, *H. farinosa* poses a greater concern to log cultivation of *L. edodes* than to bag culture. An additional *Hypocrea* species, similar in overall appearance to *H. lactea sensu* Doi, was observed associated with log cultivation of *L. edodes* in Japan (Kazuhiro Miyazaki, unpublished). Specimens of *H. lactea sensu* Doi were not available for direct comparison. The epitype specimen designated here for *H. farinosa* from Europe does not appear to be associated with an identifiable fungus. Nevertheless, ITS data from this specimen are identical to those of specimens obtained from *Stereum* sp., lichen-covered bark, and cultivated *L. edodes*.

7. *Hypocrea alcalifuscescens* Overton, sp. nov. MycoBank MB501057. Fig. 17.

**Anamorph**: *Trichoderma* sp. [sect. *Hypocreanum*].

**Etymology**: *fuscescens* (L.), turning dark in alkali; the yellow-brown stroma of this species becomes dark reddish brown when a drop of 3 % KOH is placed on the surface.

Stromata effusa, extensa, luto-brunnea vel olivaceo-brunnea, KOH ope brunnescentia. Ascosporae hyalinae, crassitunicatae, spinulosae, dimorficae; pars distalis (3.5–)4.0–5.3(–6.7) × (3.2–)3.7–4.5(–5.6) µm, pars proxima (3.2–)4.5–5.8(–6.6) × (2.8–)3.2–4.0(–5.2) µm. Anamorphosis *Trichoderma* sectionis *Hypocreanum*. Conidia hyalina, subglobosa vel subellipsoida, (2.6–)4.0–7.3(–11) × (2.2–)2.7–4.7(–6.3) µm.

Typus: TAA(M) 181548 in BPI.

**Teleomorph**: Stromata effusa, extensive, surface hyphal; largest continuous stroma 25 × 10 mm, varying in colour, usually yellow-brown to olive-brown (5E8–4E7), KOH: darkening, reddish brown (6E8); ostiolar canals visible at the stroma surface, appearing brown (6E8), stroma surface with furrows; tissue immediately below the stroma surface formed of compact hyphal elements, textura intricata. Perithecia subglobose to ellipsoidal; wall KOH++, reaction variable, weak. Asci cylindrical, often wider near the tip, (78–)90–112(–121) × (3.9–)4.4–5.8(–6.4) µm (n = 30); tip slightly thickened. Part-ascospores hyaline, thick-walled, spinulose, dimorphic; distal part subglobose, sometimes obovate, (3.5–)4.0–5.3(–6.7) × (3.2–)3.7–4.5(–5.6) µm, L/W ratio (0.87–)1.0–1.3(–1.5) (n = 65); proximal part subglobose to oblong ellipsoidal, sometimes wedge-shaped, (3.2–)4.5–5.8(–6.6) × (2.8–)3.2–4.0(–5.2) µm, L/W ratio (0.90–)1.2–1.6(–2.1) (n = 65).

**Anamorph**: Colonies covering a 100 mm diam Petri plate with PDA in 10 d, not producing concentric rings or radial rays of mycelium; a layer of aerial mycelium covering the entire plate; conidiophores irregularly branched, on long hyphal elements, usually verticillium-like; phialides in whorls of 4–6(–8), rarely solitary, or alternating in pairs; phialides subulate, (6.7–)13–24(–30.3) × (1.6–)2.3–3.0(–3.5) µm (n = 38); conidia variable in size, subglobose to obovate, subellipsoidal, rarely subcylindrical, (2.6–)4.0–7.3(–11) × (2.2–)2.7–4.7(–6.3) µm (n = 63), infrequently with an indistinct flat edge; no distinctive odour, brownish orange pigment (6C4) produced near the point of inoculation. Colonies on SNA or CMD did not produce conidiophores within 10 d.

**Habitat**: Possibly fungicolous, found on leaf litter with *Piloderma/Amauroderma*, also known from bark of *Liriodendron tulipifera* without visible evidence of another fungus present.

**Known distribution**: Eastern Europe and North America, apparently not common.

**Holotype**: Estonia, on leaf litter and *Piloderma/Amauroderma*, 13 Sep. 2000, U. Kõljalg, TAA(M) 181548 (BPI, ex-type culture TFC 2000-36).

**Other specimen examined**: U.S.A., Delaware, Rockland, on bark of *Liriodendron tulipifera*, 7 July 1890 (BPI 631474; herb. J. B. Ellis).

8. *Hypocrea parmastoi* Overton, sp. nov. MycoBank MB501058. Fig. 18.

**Anamorph**: *Trichoderma* sp. [sect. *Hypocreanum*].

**Etymology**: This species is named after Dr E. Parmasto in recognition of his significant mycological contributions, and in appreciation of his assistance in identifying polypores in this study.

Stromata effusa, extensa, violaceo-brunnea vel griseo-purpurea. Ascosporae hyalinae, crassitunicatae, spinulosae, dimorficae; pars distalis ascosporarum subglobosa vel obovata, (2.7–)3.8–5.2(–6.7) × (2.7–)3.5–4.5(–6.6) µm, pars proxima subgloboesa vel ellipsoida, (3.2–)4.2–5.7(–6.6) × (2.4–3.0) × (2.0–2.5) × (2–5.2) µm. Anamorphosis *Trichoderma* sectionis *Hypocreanum*. Conidia hyalina, subglobosa vel subcylindrica, (3–)4.3–5.0(–7.8) × (2.2–)2.5–3.1(–3.6) µm.

**Teleomorph**: Stromata effusa, extensive, surface hyphal, largest continuous stroma 20 × 10 mm, varying in colour, usually violet-brown to greyish ruby (10E7–12DE7), KOH: ; ostiolar canals visible at the stroma surface, greyish brown (10F3); stroma surface rugulose, tissue immediately below the stroma surface formed of compact to loose hyphal elements, textura intricata. Perithecia subglobose to ellipsoidal; wall KOH++, reaction variable, weak. Asci cylindrical, often wider near the tip, (78–)89–112(–121) × (3.9–)4.4–5.6(–6.4) µm (n = 30); tip slightly thickened.

Part-ascospores hyaline, thick-walled, spinulose, dimorphic; distal part subglobose, sometimes obovate, (2.7–)3.8–5.2(–6.7) × (2.7–)3.5–4.5(–6.6) µm, L/W ratio (0.75–)1.0–1.3(–1.4) (n = 77); proximal part, subglobose to ellipsoidal, (3.2–)4.2–5.7(–6.6) × (2.4–3.0) × 4.0(–5.2) µm, L/W ratio (0.90–)1.2–1.6(–2.1) (n = 77).

**Anamorph**: Colonies covering a 100 mm diam Petri plate with PDA in 10 d, producing radial rays of mycelium.
HYPOCREA spp. with anamorphs in Trichoderma sect. Hypocreanum
mycelium and a layer of aerial mycelium near the agar plug and the edge of the plate; conidiophores irregularly branched, on long hyphal elements, usually verticillium-like; phialides in whors of 2–4(–10), sometimes solitary or in pairs forming a fork shape; phialides subulate, (12–)16–29(–33.3) × (1.6–)2.1–3.0(–3.4) µm (n = 34); conidia variable in shape, subglobose to obovate, or subellipsoidal to subcylindrical, (3.0–)3.4–5.0(–7.8) × (2.2–)2.5–3.1(–3.6) µm (n = 38), infrequently with an flat base, which is then not conspicuous; no distinctive odour; dark rose pigment (11A3) produced near the point of inoculation. Colonies covering a 100 mm diam Petri plate with CMD in 10 d, producing a layer of aerial mycelium near the agar plug and the edge of the plate; conidiophores irregularly branched, on long hyphal elements, usually verticillium-like; phialides in whors of 2–4(–10), sometimes solitary or in pairs; phialides subulate, with the same measurements as on PDA; no distinctive odour; reddish white pigment (11A2) produced near the point of inoculation. Colonies on SNA did not produce any conidiophores within 10 d.

**Habitat:** Lignicolous, on *Alnus incana* and *Castanea* sp.; possibly *Quercus* spp.

**Known distribution:** Eastern Europe and France, apparently not common.

**Holotype:** Estonia, Võru Commune, Võrumaa County, 57°47’ N, 27°9’ E, on *Alnus incana* (fallen trunk), Kütiorg, 3 Oct. 1997, I. Parmasto, TAA(M) 169055 (BPI; ex-type culture TFC 97-143).

**Other specimen examined:** France, St. Gaudens 31, Haute Garonne, Arboretum De Cudeilhac, on *Castanea* sp., possibly *Quercus* sp., 1 Nov. 1994, Françoise Candoussau (BPI 737853).

## KEY TO THE SPECIES TREATED

1. Occurring on *Exidia* spp.; stromata yellow, effuse, extensive ................................................................. 2
2. Not occurring on *Exidia* spp.; stromata variable in colour, discrete to extensive ........................................ 3

### 2. Part-ascospores dimorphic, hyaline, thick-walled, spinulose; distal part obovate, sometimes subellipsoidal, (4.2–)5.2–6.6(–7.8) × (4.2–)5.3–7.1(–8.3) µm, proximal part ellipsoidal, sometimes subcylindrical, (4.4–)5.5–6.9(–8.5) × (2.7–)3.9–5.1(–6.6) µm .................................................. 1. *H. sulphurea*

### 5. Stromata composed of tightly packed hyphae, olive-brown at the surface; part-ascospores dimorphic, hyaline, thick-walled, spinulose; distal part subglobose, sometimes obovate, (3.5–)4.0–5.3(–6.7) × (3.2–)3.7–4.5(–5.6) µm, proximal part subglobose to subellipsoidal, (3.2–)4.5–5.8(–6.6) × (2.8–)3.2–4.0(–5.2) µm .................................................. 8. *H. par mastoi*

### 4. Stromata composed of a loose layer of hyphae, violet-brown; part-ascospores dimorphic, hyaline, thick-walled, spinulose; distal part subglobose, sometimes obovate, (3.5–)4.0–5.3(–6.7) × (3.2–)3.7–4.5(–5.6) µm, proximal part subglobose to subellipsoidal, (3.2–)4.5–5.8(–6.6) × (2.8–)3.2–4.0(–5.2) µm .................................................. 3. *H. alcalifuscescens*

### 5. Stromata composed of two layers, a hyphal layer near the surface, and a pseudo-parenchymatous layer near the base of the perithecia, light yellow to light brown; part-ascospores dimorphic, hyaline, thick-walled, spinulose, distal part subglobose, (2.7–)3.3–4.0(–4.8) × (2.3–)3.3–3.6(–4.7) µm, proximal part subellipsoidal, sometimes wedge-shaped, (2.7–)3.4–4.5(–5.6) × (2.3–)2.7–3.3 (–3.9) µm .................................................. 6. *H. farinosa*

### 6. Part-ascospores monomorphic, subglobose, spinulose, (2.6–)2.8–3.5(–3.9) × (2.4–)2.6–3.2(–3.7) µm; stromata yellow, effuse, extensive .................................................. 4. *H. eucorticoides*

### 7. Part-ascospores dimorphic; stromata effuse to subpulvinate, yellow to yellowish brown ................................................................. 7

### 7. Part-ascospores dimorphic, hyaline, thick-walled, spinulose; distal part subellipsoidal, sometimes obovate, (4.8–)5.6–6.8(–7.4) × (3.9–)4.5–5.5(–5.9) µm, proximal part ellipsoidal, sometimes wedge-shaped, (4.7–)5.8–7.4(–8.6) × (3.6–)4.1–5.1(–6.1) µm .................................................. 3. *H. victoriensis*

### 8. Part-ascospores dimorphic, distal part subglobose to obovate conical, (4.5–)4.7–5.1 (–5.4) × (3.6–)3.8–4.2(–4.3) µm; proximal part tending to be oblong or ellipsoidal, narrow, (4.2–)4.7–5.6(–6.0) × (3.6–)3.5–3.7(–3.8) µm; known from Africa and New Zealand ........................................ 2. *H. subcitrina*
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