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* (LE*tef1*) 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 eucorticioides* 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, *tef1* 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 acremonium- or verticillium-like conidiophores with hyaline conidia, and are assignable to *Trichoderma* sect. *Hypocreanum* Bissett.

Hypocrea sulphurea (Schw.) Sacc. is a common, yellow, effused fungicolous 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 stromata. The relationship between *H. egmontensis* and *H. sulphurea* has not been established. Doi (1972) described *Hypocrea sulphurea* f. *macrospora* Yoshim. Doi. We compared morphologically type material (NY) of this forma 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 *Stilbocrea 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.

Kullnig-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 acremonium- 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 (LE*tef1*) 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 paraphyletic. The seven species included are compared to selected species treated by Overton *et al.* (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 *Protocrea farinosa*; (5) to investigate the phylogenetic boundaries of *Hypocrea* 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 (LE*tef1*), 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 LE*tef1* regions which differs from the *tef1*region amplified in Overton *et al*. (2006): for LE*tef1*, EF1-983F (5'-GC(C/T)CC(C/T)GG(A/C/T)CA(C/T)GGTGA(C/T)TT(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 LE*tef1* were evaluated in single and combined analyses to establish phylogenetic species limits. These analyses excluded the taxa *H. avellanea*, *H. parmastoi*, *H. cinereoflava* 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 *Hypocrea* isolates with *Trichoderma* sect. *Hypocreanum* anamorphs in phylogenetic context with other *Hypocrea*/*Trichoderma* species. For the second objective, *Sphaerostilbella* cf. *aureonitens, Arachnocrea scabrida* Yoshim. Doi., and *Hypomyces stephanomatis* Rogerson & Samuels were used as outgroup taxa for the combined LE*tef1* 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 LE*tef1* 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 LE*tef1* 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 LE*tef1*, *rpb2*, and combined alignments using MODELTEST 3.06 (Posada & Crandall 1998) as implemented in PAUP*. For the LE*tef1* data, the likelihood ratio test (LRT) implemented in MODELTEST, 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 1.0000 (A–C), 3.1252 (A–G), 1.6847 (A–T), 1.6847 (C–G), 10.5209 (C–T), and 1.0000 (G–T). For the *rpb2* data, the LRT implemented in MODELTEST, 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 1.0000 (A–C), 6.5499 (A–G), 1.0000 (A–T), 1.0000 (C–G); 9.0762 (C–T), and 1.0000 (G–T). For the combined LE*tef1* and *rpb2* data set, the LRT implemented in MODELTEST, selected GTR G+I 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 LE*tef1* 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 LE*tef1* 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 LE*tef1* (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.

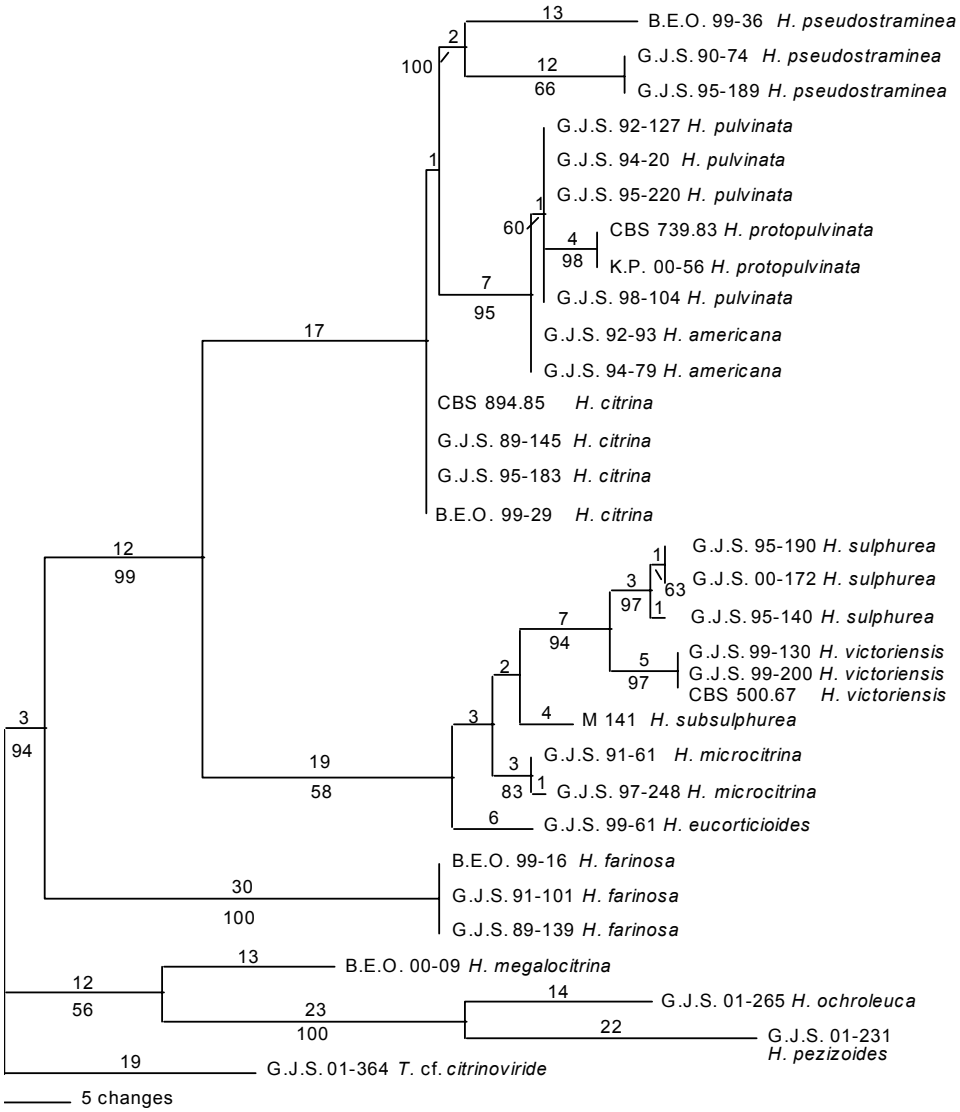


Fig. 1. Parsimony analysis of ITS. One phylogram of 3193 most parsimonious trees; 217 steps; consistency index 0.779; retention index 0.855; homoplasy index 0.221; numerical values of branch lengths are given above and bootstrap values (500 replicates with 10 random addition replications) are indicated 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	LE*tef1*	*rpb2*
H. pulvinata Fuckel	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-Hundsrück, 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.) *Fr.*	G.J.S. 95-183	Daniel Boone National Forest, Kentucky, U.S.A.	DQ835413	DQ835469	DQ835458
	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. 96-275	Ascutung, Vermont, U.S.A.	DQ835418	–	–
	CBS 708.73	Baarn, Zandheuvelweg, Netherlands	DQ835415	–	–
	CBS 853.70	Pelmer Wald near Gerolstein, Germany	DQ835416	–	–
	CBS 894.85*	Hestreux near Eupen, Belgium	DQ835417	DQ835481	AF545561
H. pseudostraminea Yoshim. Doi	G.J.S. 91-135	Prince Georges County, Maryland, U.S.A.	DQ835419	–	–
	G.J.S. 90-74	Dutches County, New York, U.S.A.	DQ835420	DQ835470	DQ835454
	G.J.S. 95-169	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. megalocitrina Yoshim. Doi	B.E.O. 00-09	North Carolina, U.S.A.	DQ835511	AY225855	AF545563
H. microcitrina	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 500.67	New Zealand	DQ835466	–	–
H. eucorticioides Overton	G.J.S. 99-61	Limon, Costa Rica	DQ835467	DQ835474	DQ835518
H. subsulphurea Kalchbr. & Cooke	M 141	Kurokami Kumamoto, Japan	DQ835509	DQ835492	DQ835522
H. farinosa Berk. &	G.J.S. 91-101	Maryland, U.S.A.	DQ835507	DQ835476	DQ835520
Broome	G.J.S. 89-139	Unknown mushroom farm, U.S.A.	DQ835508	DQ835477	DQ835521
H. alcalifuscescens	B.E.O. 99-16	Maryland, U.S.A.	DQ835506	DQ835475	DQ835519
Overton	TFC 181548*	Estonia	–	DQ834455	DQ834462
H. parmastoi Overton	TFC 97-143*	Voru Commune, Estonia	–	DQ834456	DQ834463
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		
			ITS	LE*tef1*	*rpb2*
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 E. 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 *rpb2* 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 LE*tef1* 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*tef1* 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

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 subclade 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 LE*tef1* and *rpb2* regions distinguished between North American and European isolates of *H. sulphurea*, whereas ITS did not. The LE*tef1* gene region was less variable than *tef1* sequences generated by Overton *et al*. (2006), using the primer pair ef-1/2, for *H. citrina* and allies. Sequences were generated from the *tef1* gene region for selected species of *H. sulphurea* and allies included in this study and deposited in GenBank (Table 3). The introns of *tef1* were highly variable making alignments between species such as *H. citrina* and *H. sulphurea* problematic. Consequently, the *tef1* region was excluded from this study.

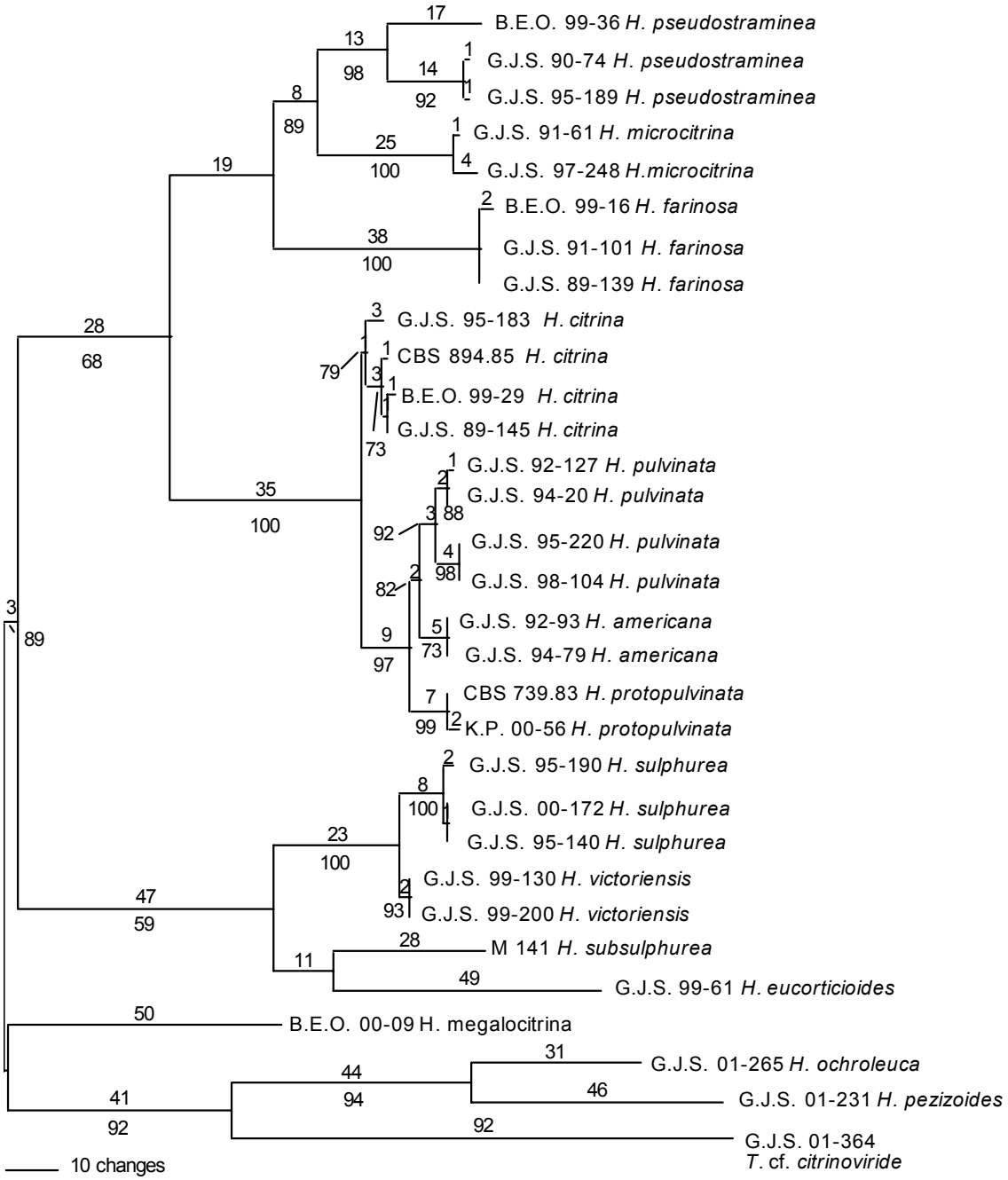


Fig. 2. Parsimony analysis of partial sequences of *rpb2*. Single most parsimonious tree; 650 steps; consistency index 0.628; retention index 0.771; homoplasy index 0.372, rest as Fig. 1.

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 Hyphal	1	15156.58	<0.0001*

[1]Probability 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.

[2]The best –ln likelihood tree from the maximum parsimony analysis.

Species of *Hypocrea* with anamorphs assignable to *Trichoderma* sect. *Hypocreanum* did not form a monophyletic group. The K-H test on the combined LE*tef1* 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 megalocitrina* 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 LE*tef1* 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: HI = 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).

DISCUSSION

Species recognition

The combined phylogenetic analyses using ITS and partial sequences of LE*tef1* and *rpb2* show that *Hypocrea sulphurea*, *H. subsulphurea*, *H. victoriensis*, *H. farinosa*, and *H. eucorticioides* 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 megalocitrina* 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

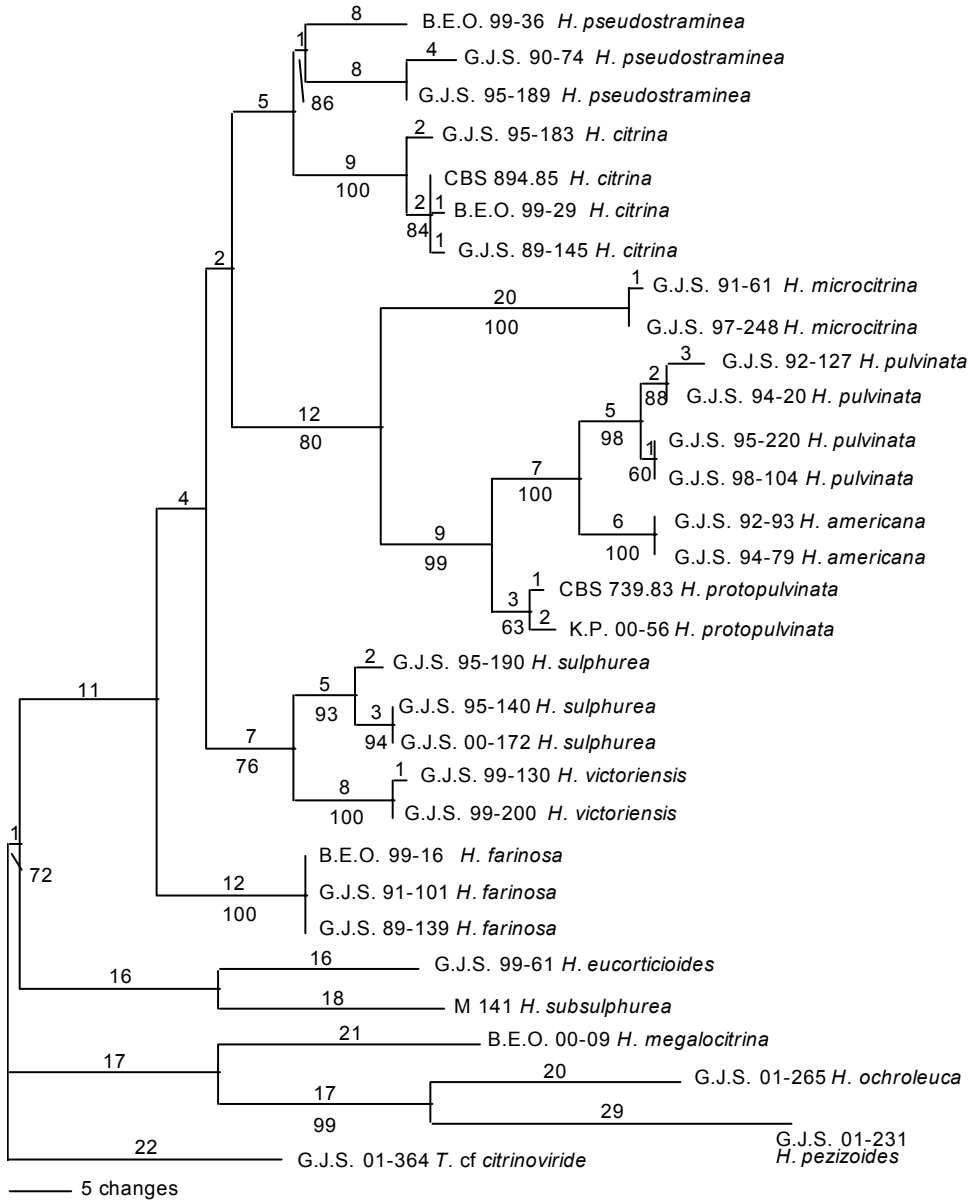


Fig. 3. Parsimony analysis of partial sequences of LE*tef1*. One phylogram of 64 most parsimonious trees; 314 steps; consistency index: 0.567; retention index: 0.744; homoplasy index: 0.433, rest as Fig. 1.

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 teleomorphs 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*.

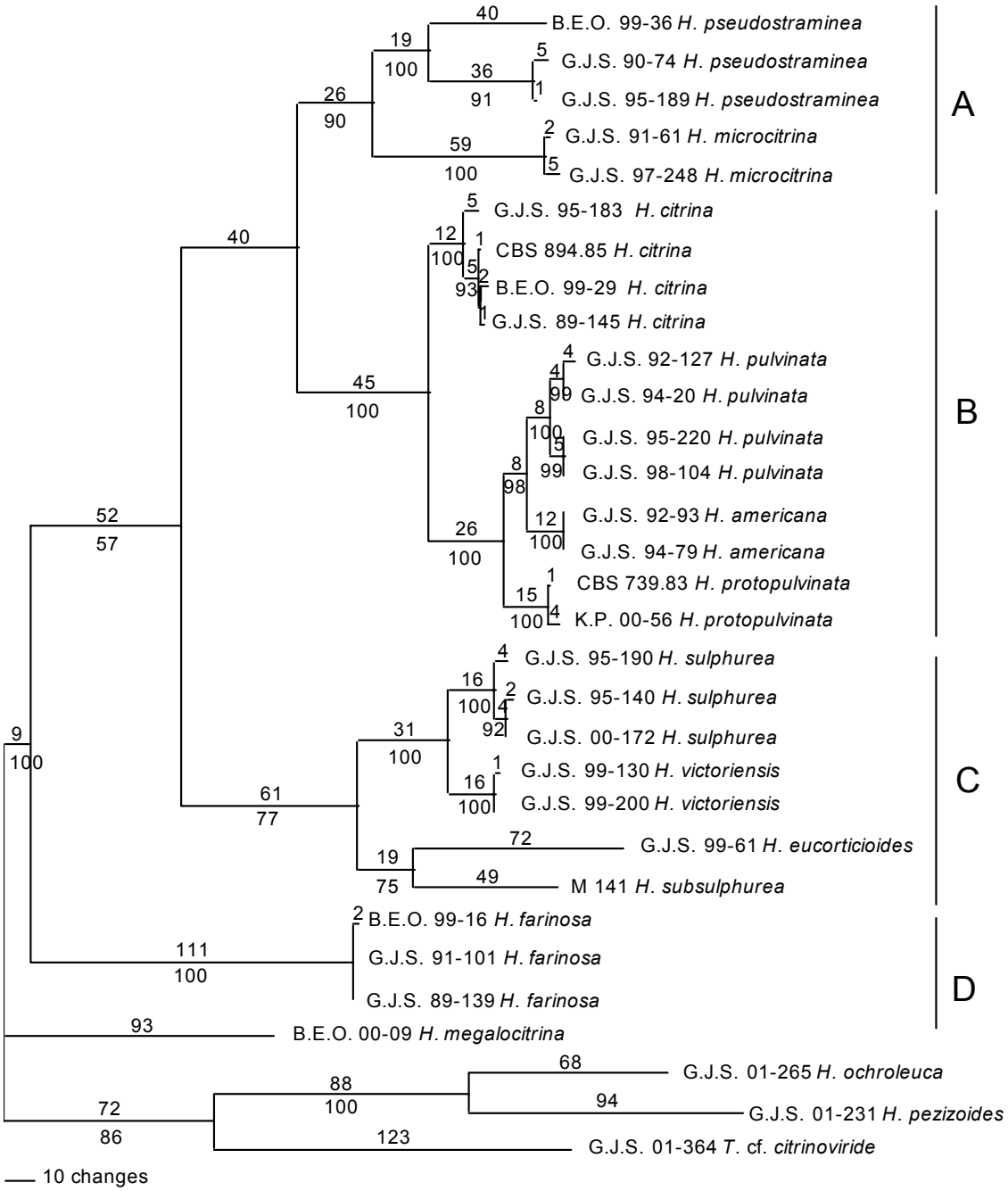


Fig. 4. Combined parsimony analysis of ITS, LE*tef1*, *rpb2*. Phylogram of one of three most parsimonious trees; 428 steps; consistency index: 0.626; retention index: 0.766; homoplasy index: 0.374, rest as Fig. 1.

Trichoderma sect. *Hypocreanum* and classification

The phylogeny of the major clades in *Trichoderma*/ *Hypocrea* is essentially unresolved based on the genes used in this study. However, *Hypocrea* 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. *Pachybasium* 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. *Pachybasium* could not be resolved in the combined LE*tef1* and *rpb2* dataset. The anamorphs of *H. megalocitrina*, *H. parmastoi*, 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. *Pachybasium*.

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

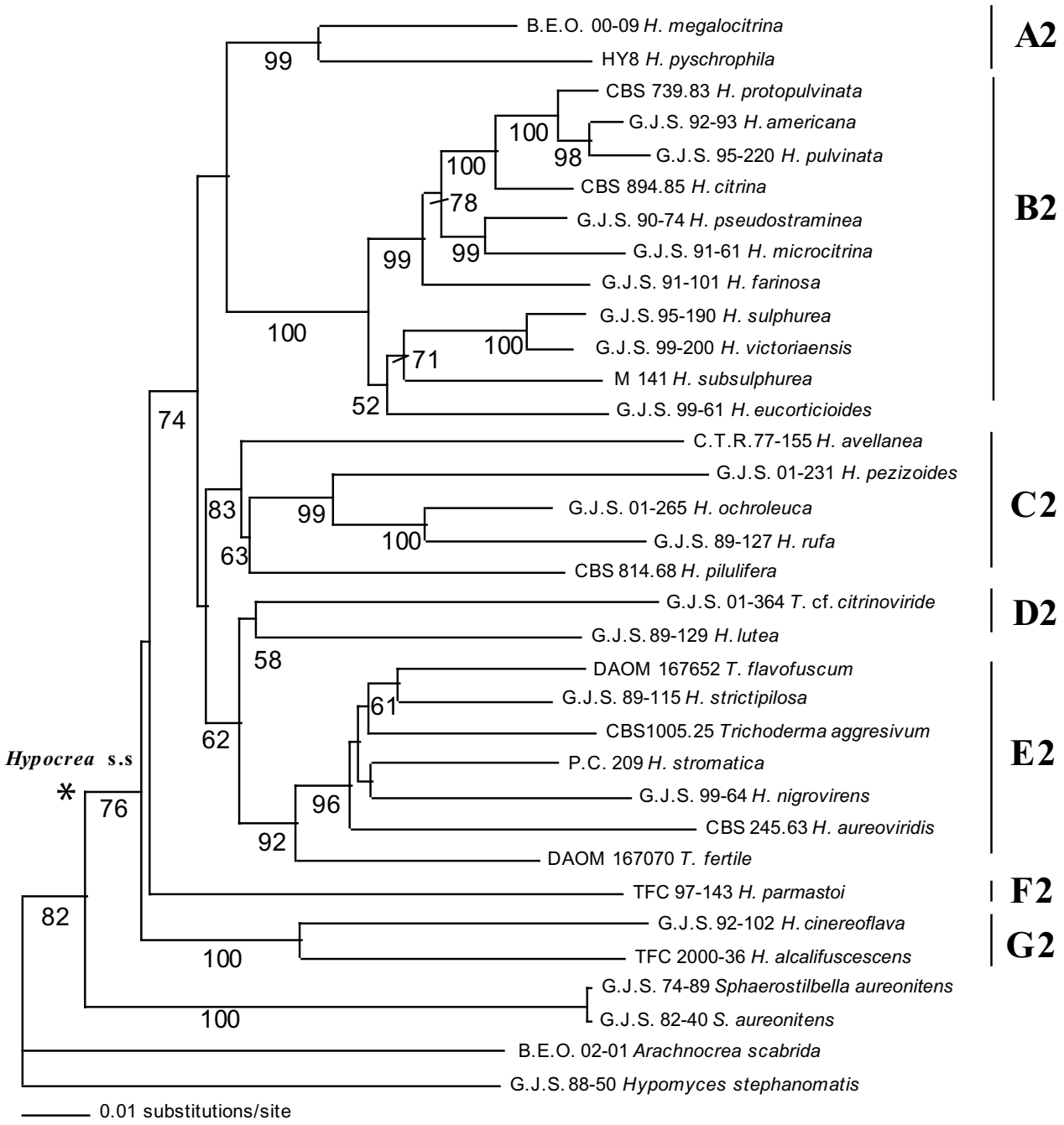


Fig. 5. Parsimony analysis of the combined LE*tef1* and *rpb2* data set. Phylogram of one of 3 most parsimonious trees; 2469 steps; consistency index: 0.354; retention index: 0.515; homoplasy index: 0.646, rest as Fig. 1. Outgroup taxa: *Hypomyces stephanomatis*; *Acrachnocrea scabrida*; *Sphaerostibella* cf. *aureonitens*.

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 (LE*tef1*) were generated for the seven species, including several of those treated by Overton *et al.* (2006). The LE*tef1* 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 LE*tef1* 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 *Hypocrea* have been sequenced to date, with published accounts placing an over-reliance on ITS rDNA sequence data. The LE*tef1* 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 *Hypocrea/Trichoderma*. Additional taxa from other genera such as *Sarawakus* Lloyd, with *Trichoderma* anamorphs, need to be sequenced before a complete phylogeny of *Hypocrea/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 *Hypocrea* with acremonium- or verticillium-like anamorphs with hyaline conidia have lost the ability to produce a primary trichoderma-like anamorph, with pyramidally 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 *Aphysiostroma* 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). *Hypocrea 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 LE*tef1* gene sequences and based on ITS sequence data, a finding consistent with Kullnig-Gradinger *et al*. (2002). *Hypocrea cinereoflava* produces a primary synnematous anamorph and a verticillium-like synanamorph. This species of *Hypocrea* 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. *Hypocrea cinereoflava* is located in an unresolved basal clade of *Hypocrea s.s.* (Fig. 5) and, based on the molecular results of this study, it could not be excluded from the genus *Hypocrea*. The phylogenetic placement of this species basal to all other *Hypocrea* 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. *Hypocrea sulphurea* (Schw.) Sacc., Syll. Fung. 2: 535. 1883. Figs 6–8.

≡ *Sphaeria sulphurea* Schw., Trans. Amer. Philos. Soc. 2: 193. 1832.

= *Hypocrea 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. Stroma surface 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 ellipsoidal, (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. Asci 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

obovate, sometimes subellipsoidal, (4.2–)5.2–6.6 (–7.6) × (4.2–)5.3–7.1(–8.3) µm, L/W ratio, (0.8–)1.1–1.4 (–1.7) (n = 294); proximal part ellipsoidal, sometimes subcylindrical, (4.4–)5.5–6.9(–8.5) × (2.7–)3.9–5.1 (–6.6) µm, L/W ratio (0.9–)1.2–1.6(–2.2) (n = 294).

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 conidiophores; conidiophores irregularly branched, on long hyphal elements, usually verticillium-like; phialides in whorls of 2–4, solitary or alternating in pairs on long hyphal elements; phialides subulate, (8–)17–32(–45) × (2.4–)3–4(–4.8) µm (n = 92); 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.

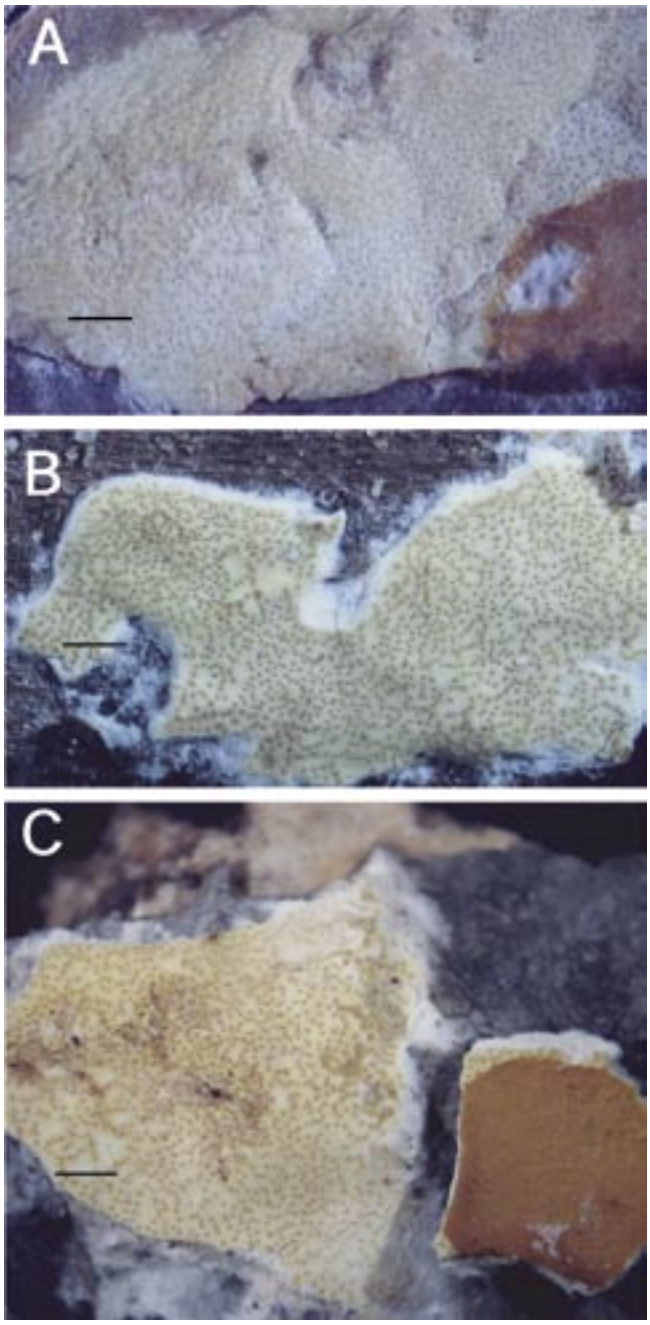


Fig. 6. A–C. *H. sulphurea*. A. Stroma with KOH reaction, BPI 737764; bar = 1 mm. B. Stroma with byssoid margin, BPI 737752; bar = 1 mm. C. Stroma with KOH reaction, TNS-F-190169; bar = 1 mm.

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, Center 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 LE*tef1* 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*.

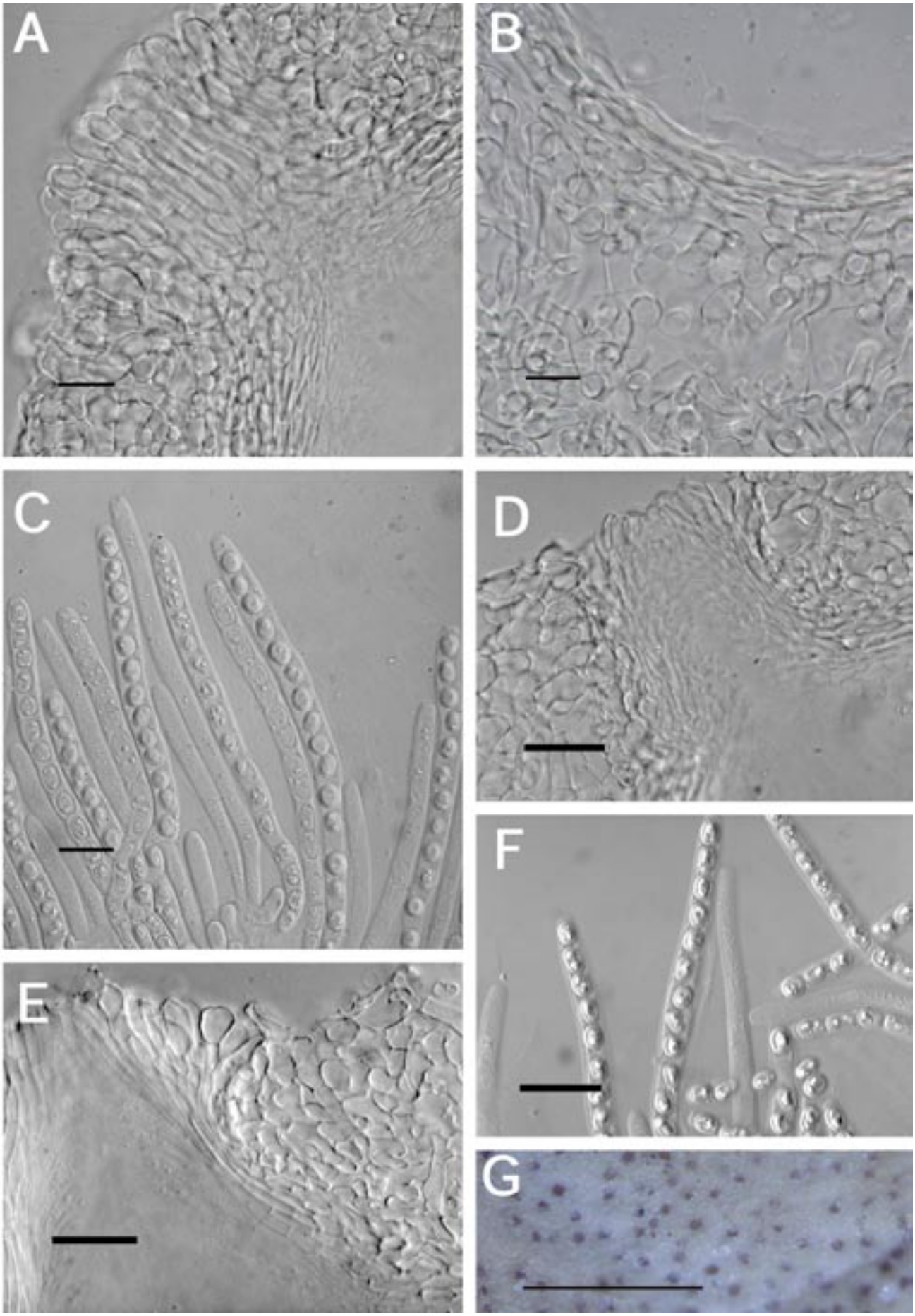


Fig. 7. A–F. *H. sulphurea*. A. Section of stroma showing ostiolar papilla; bar = 20 µm. B. Section of stroma showing *textura globulosa* to *t. angularis* with *t. intricata* below perithecium; bar = 20 µm. C. Asci with ascospores; bar = 20 µm; A–C. BPI 737752. D. Section showing *t. angularis* near 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.

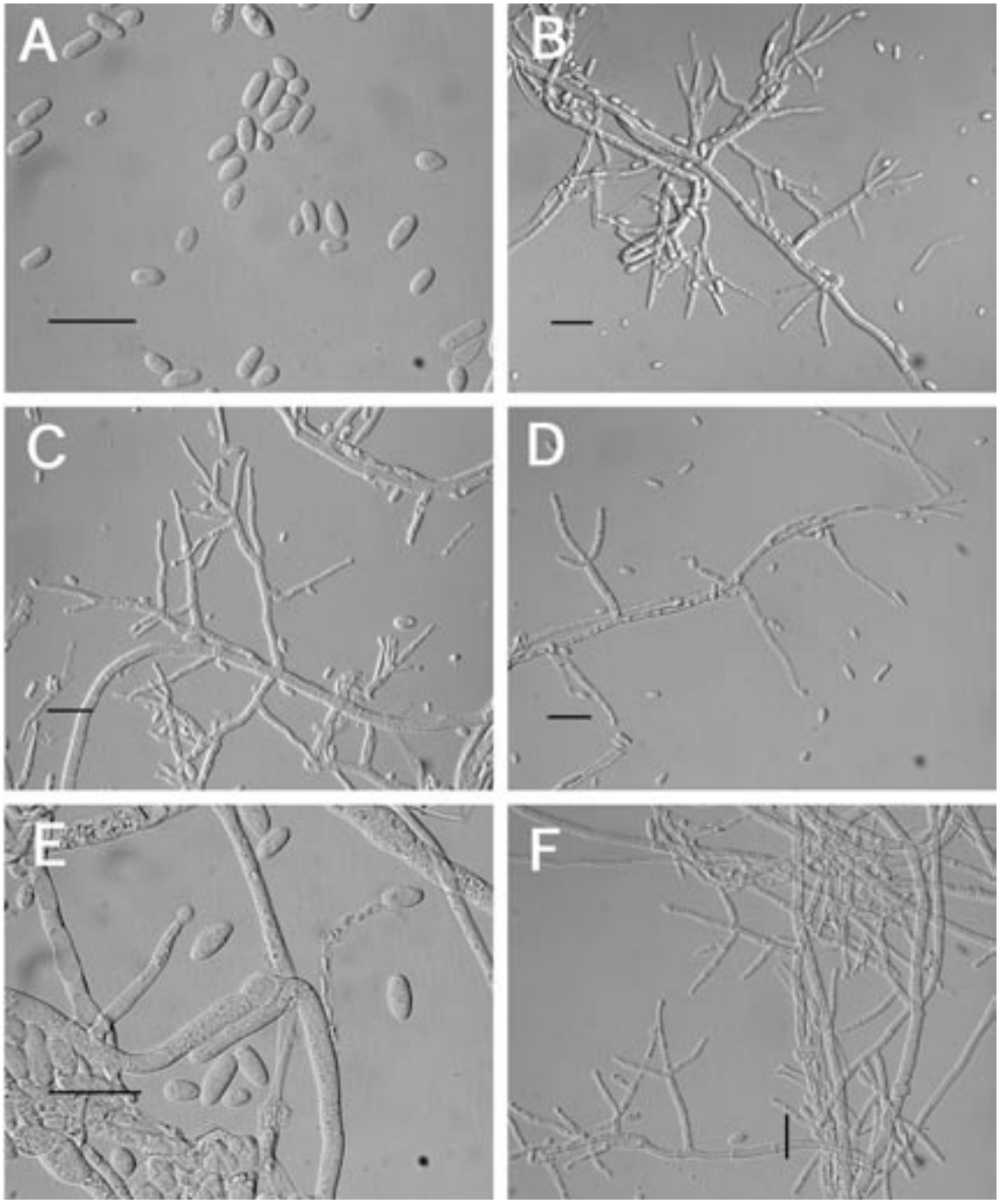


Fig. 8. A–F. *H. sulphurea*. A. Conidia of variable size, on PDA; bar = 20 µm. B–C. Conidiophores, on PDA; bars = 20 µm; A–C. B.E.O. 98-44. D-F. Conidiophores and conidia from G.J.S. 95-140 on PDA; bars = 20 µm.

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–)4–4.6(–5.2) µm; proximal part-ascospores (4.3–)5.0–5.6(–6.7) ×

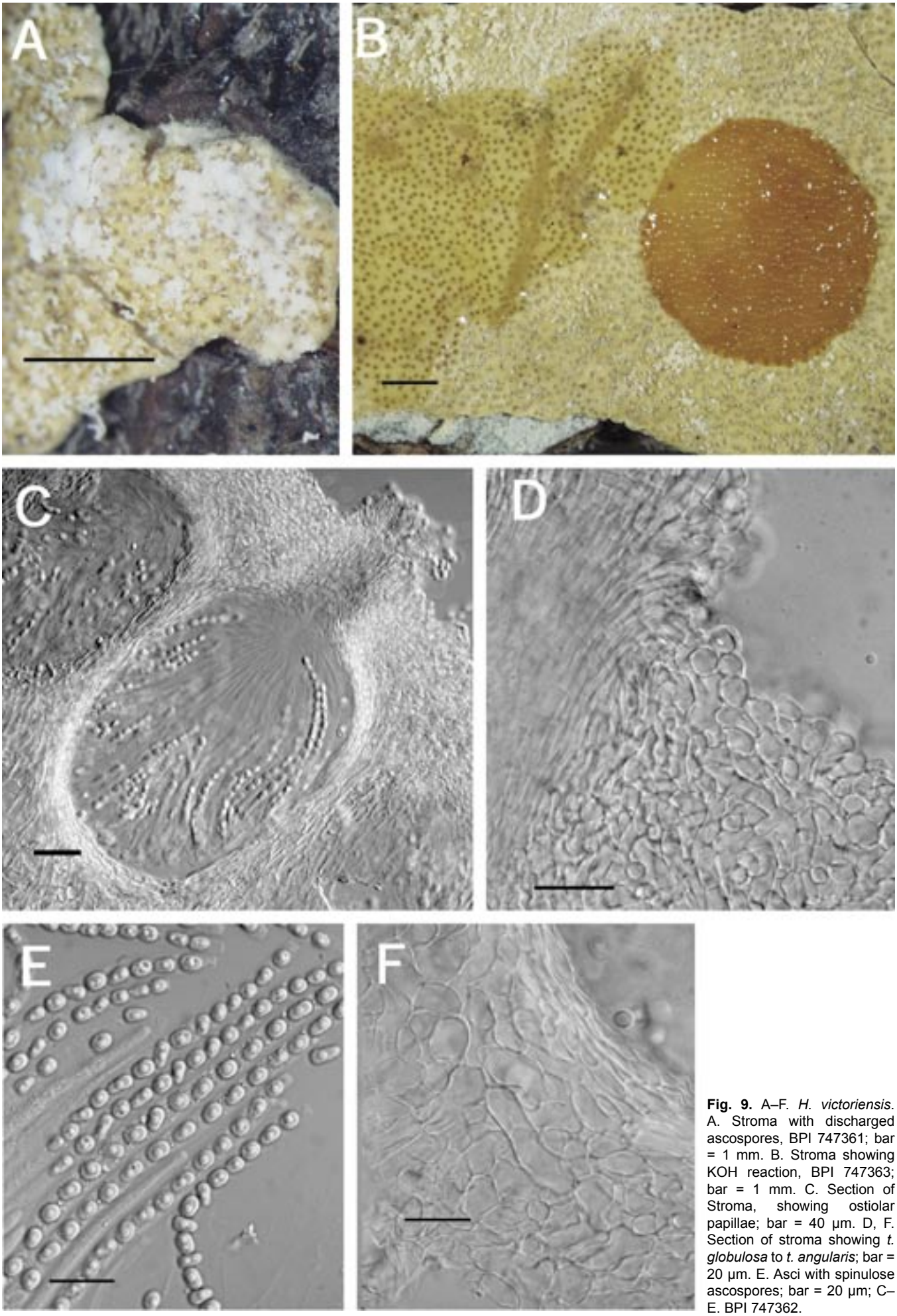


Fig. 9. A–F. *H. victoriensis*. A. Stroma with discharged ascospores, BPI 747361; bar = 1 mm. B. Stroma showing KOH reaction, BPI 747363; bar = 1 mm. C. Section of Stroma, showing ostiolar papillae; bar = 40 µm. D, F. Section of stroma showing *t. globulosa* to *t. angularis*; bar = 20 µm. E. Asci with spinulose ascospores; bar = 20 µm; C–E. BPI 747362.

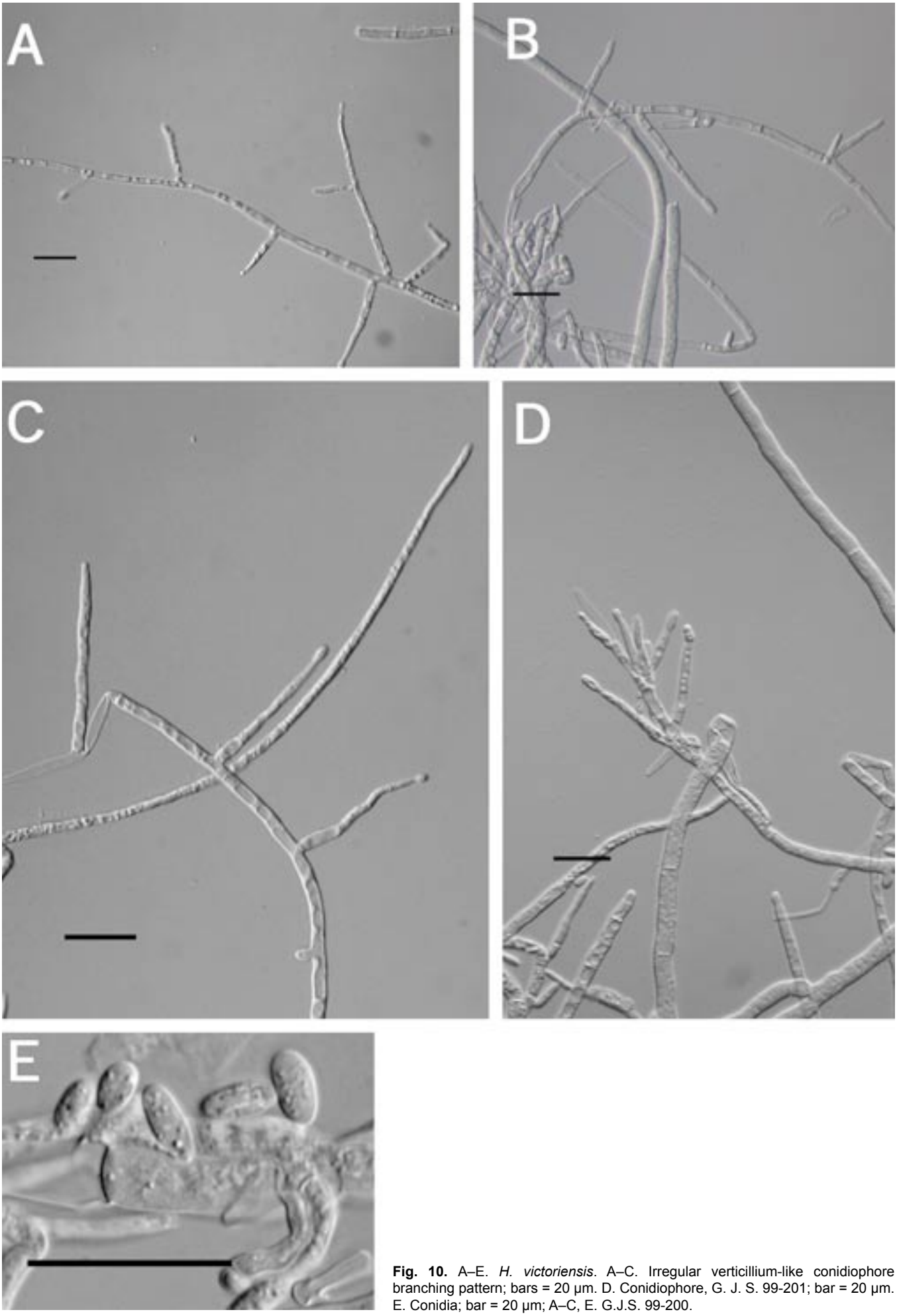


Fig. 10. A–E. *H. victoriensis*. A–C. Irregular verticillium-like conidiophore branching pattern; bars = 20 µm. D. Conidiophore, G. J. S. 99-201; bar = 20 µm. E. Conidia; bar = 20 µm; A–C, E. G.J.S. 99-200.

(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* (PDD 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.

Stromata effusa, extensa, rubido-lutea vel griseo-lutea, KOH$^{+/-}$. Ascosporae hyalinae, crassitunicatae, spinulosae, dimorphicae; pars distalis plus minusve ellipsoidea, (4.8–)5.6–6.8(–7.4) × (3.9–)4.5–5.5(–5.9) µm, pars proxima (4.7–)5.8–7.4(–8.6) × (3.6–)4.1–5.1(–6.1) µm. Anamorphosis *Trichoderma* sectionis *Hypocreanum*. Conidia hyalina, obovata vel subellipsoidea, (4.5–)5.9–9.3(–12.0) × (2.8–)3.2–4.2(–5.2) µm.
Typus: BPI 747361.

Teleomorph: Stromata effuse, 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), giving rise to the greyish yellow overall appearance of the stroma. Stroma surface smooth, tissue immediately below the stromatal surface formed of compact to loose pseudoparenchymatous cells, *textura globulosa* to *t. angularis*. Perithecia completely immersed, with ostiolar canals projecting from the stroma surface, (35–)36–51(–61) µm (n = 10); perithecia generally widely spaced, compact in some regions, sometimes completely absent near the margins or regions of extensive stroma growth. Perithecia ellipsoidal, (335–)345–405(–435) µm high (including the length of the ostiolar canal, n = 10); width of perithecia near the base (measured from 3/4 total length of the perithecium), (152–)160–205(–225) µm (n = 10); length of ostiolar canal (100–)105–140(–155) µm; width of ostiolar canal, from outer perithecial wall to opposite internal perithecial wall, (38–)41–53(–54) µm (n =10); wall KOH$^{+/-}$, reaction variable, weak. Asci cylindrical, (92–)112–142(–152) × (5.5–)6–7(–9) µm (n = 49); tip slightly thickened. Part-ascospores hyaline, thick-walled, distinctly spinulose, dimorphic; distal part subellipsoidal, sometimes obovate, (4.8–)5.6–6.8 (–7.4) × (3.9–)4.5–5.5(–5.9) µm, L/W ratio (1–)1.1–1.3(–1.6) (n = 72); proximal part, ellipsoidal, sometimes subellipsoidal, (4.7–)5.8–7.4(–8.6) × (3.6–)4.1–5.1 (–6.1) µm, L/W ratio (1–)1.2–1.7(–2) (n = 72).

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 agar surface; conidiophore and conidium production limited; conidiophores irregularly branched, on long hyphal elements, usually verticillium-like; phialides not in whorls, solitary or alternating in pairs on long hyphal elements, subulate, (3.8–)18–36(–57) × (2.6–)3.1–3.9(–4.4) µm (n = 62); conidia variable in size, obovate to elongate-obovate 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 "*Hypocrea sulfurea"*, 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. *Hypocrea eucorticioides* Overton, **nom. nov.** MycoBank MB501056. Figs 11–12.
≡ *Hypocrea corticioides* 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 effuse, extensive, largest continuous stroma 15 × 10 mm, smallest continuous stroma 7 × 1 mm, varying in colour, typically greyish yellow (4A5–4B5), sometimes pastel-yellow to light yellow (2A4–2A5), KOH$^{+/-}$, reaction variable, usually very weak with stroma becoming orange (6A8); ostiolar canals visible at stroma surface, appearing light orange (6A4), giving rise to the greyish yellow overall appearance of the stroma. Stroma surface smooth, tissue immediately below the stromatal surface formed of compact to loose pseudoparenchymatous cells,

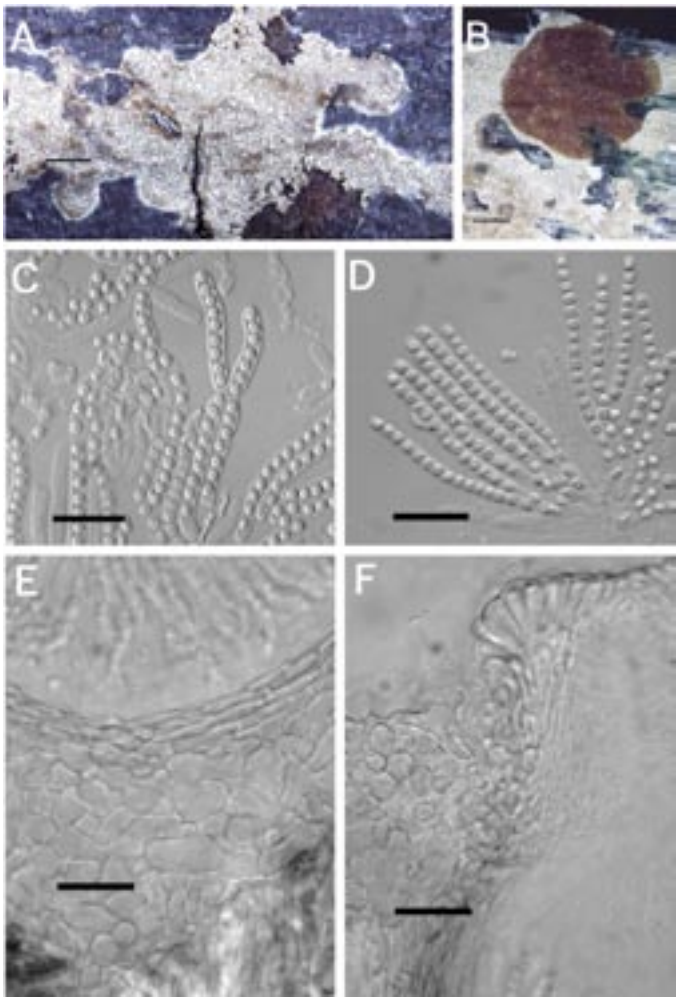


Fig. 11. A–F. *H. eucorticioides*. A. Effuse stromata with irregular margins, NY 29223; bar = 1 mm. B. Effuse stromata with irregular margins showing reaction in KOH, BPI 747358; bar = 1 mm. C. Asci with subglobose part-ascospores, BPI 747358; bar = 20 µm. D. Asci with subglobose part-ascospores, NY 29223; bar = 20 µm. E–F. Section of stroma showing *t. globulosa* to *t. angularis* tissue, BPI 747358; bar = 20 µm.

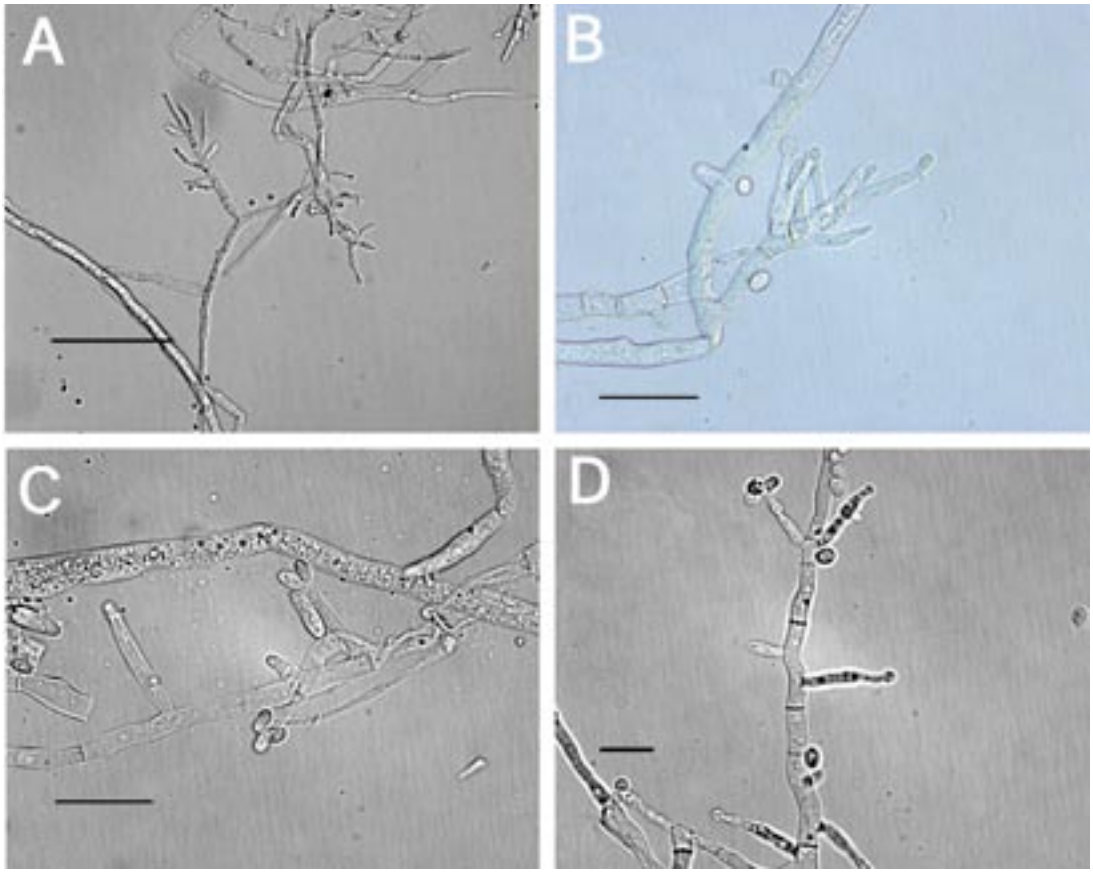


Fig. 12. A–D. *H. eucorticioides*, G.J.S. 99-61. A. Irregular verticillium-like branching pattern; bar = 40 µm. B–D. Phialides with developing conidia; bar = 20 µm.

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$^{+/-}$, reaction variable, weak. Asci cylindrical, (56–)62–72(–80) × (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 whorls 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, Ibicuy, 28 June 1911, C. Spegazzini no. 911 (LPS 1719).

Other specimens examined: **Costa Rica,** Limón, Puerto Viejo, Refugio Nacional Mendaca-Manzanilla, on decorticated wood, July 1999, G.J. Samuels & P. Chaverri (BPI 747358; culture G.J.S. 99-61). **Venezuela,** Bolívar, La Urbana, Orinoco River, Plants of the NYBG from the Venezuelan Expedition 1950–1951, on bark, 19 Oct. 1950, Bassett Maquire, R.S. Cowan & J. J. Wurdack 29223 (NY).

Comments: The new name *Hypocrea eucorticioides* 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. eucorticioides* 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, *Flore 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. Asci 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 whorls of 2–4, solitary, or alternating in pairs on long hyphal elements, phialides subulate, (15.7–)22–30(–46) × (2.9–)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

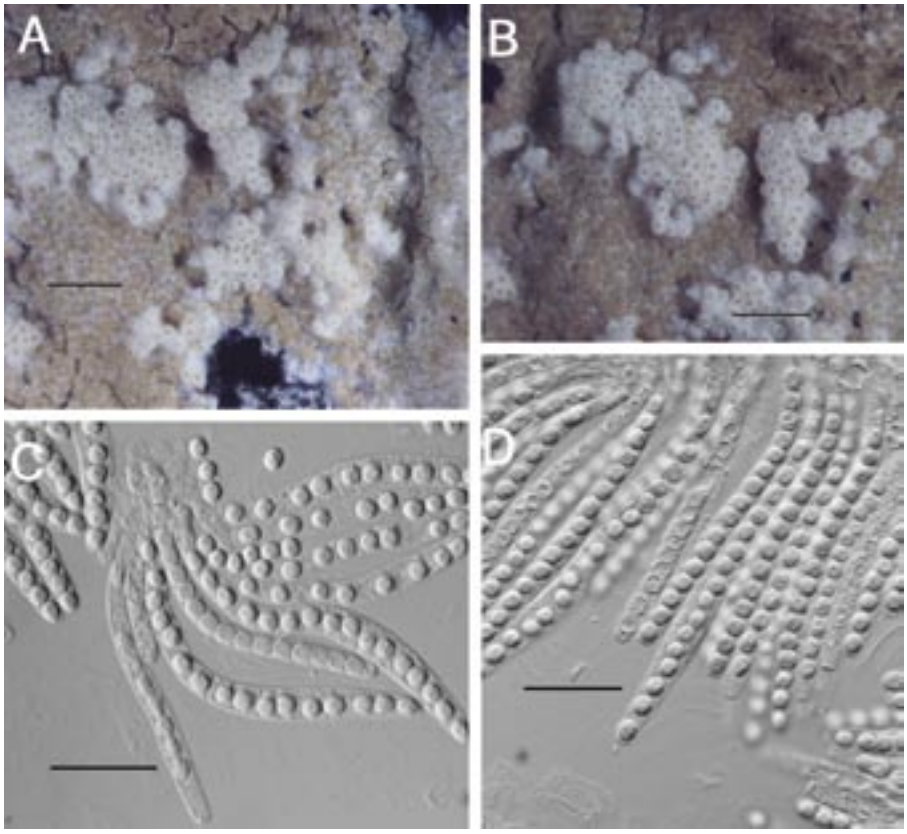


Fig. 13. A–D. *H. subsulphurea*, M 141. A–B. Perithecia forming on old stromata; bar = 1 mm. C. Asci and ascospores from old stroma; bar = 20 µm. D. Asci and ascospores from perithecia developing on old stroma surface; bar = 20 µm. Note: repeated attempts to section perithecia in old and developing stromata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

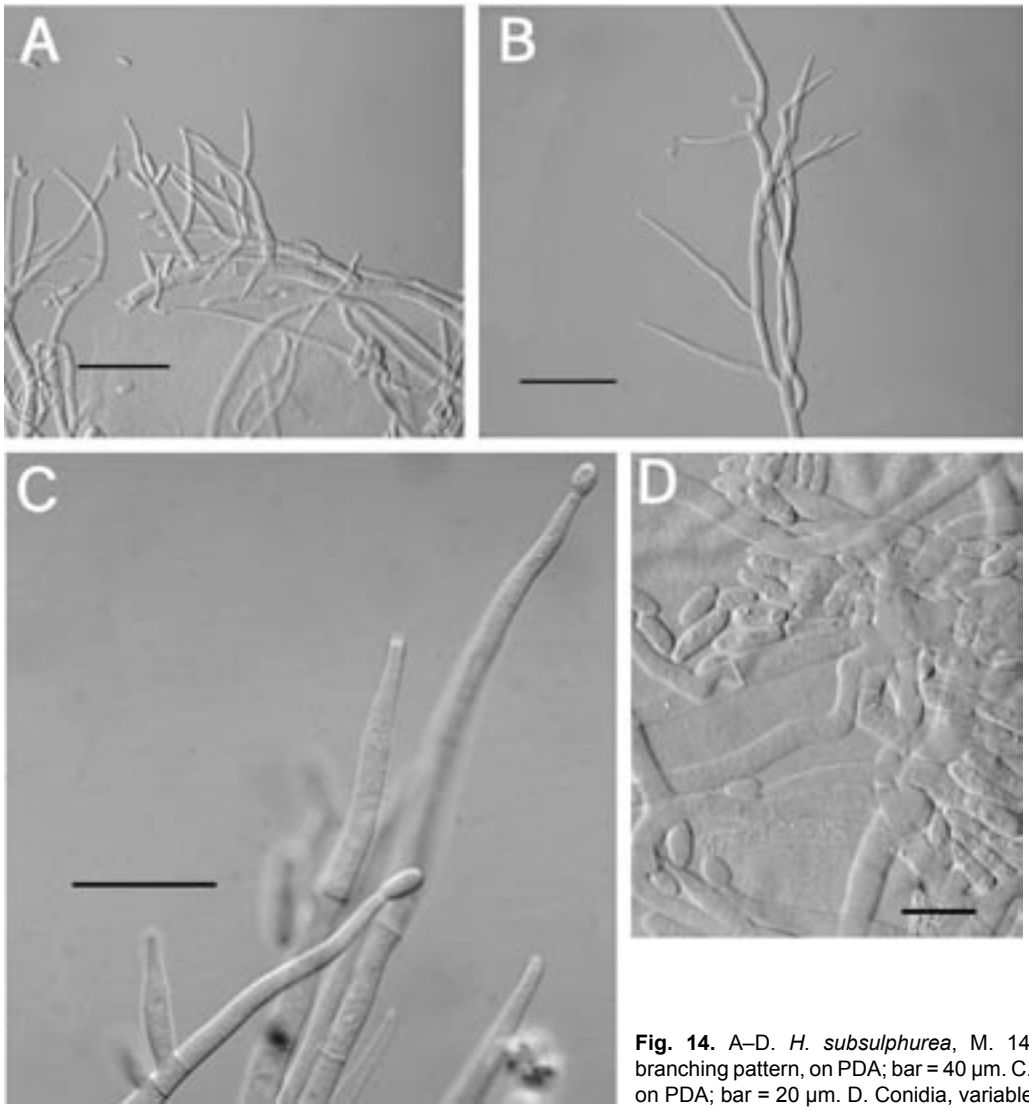


Fig. 14. A–D. *H. subsulphurea*, M. 141. A–B. Irregular verticillium-like branching pattern, on PDA; bar = 40 µm. C. Phialides with developing conidia, on PDA; bar = 20 µm. D. Conidia, variable in size, on PDA; bar = 20 µm.

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

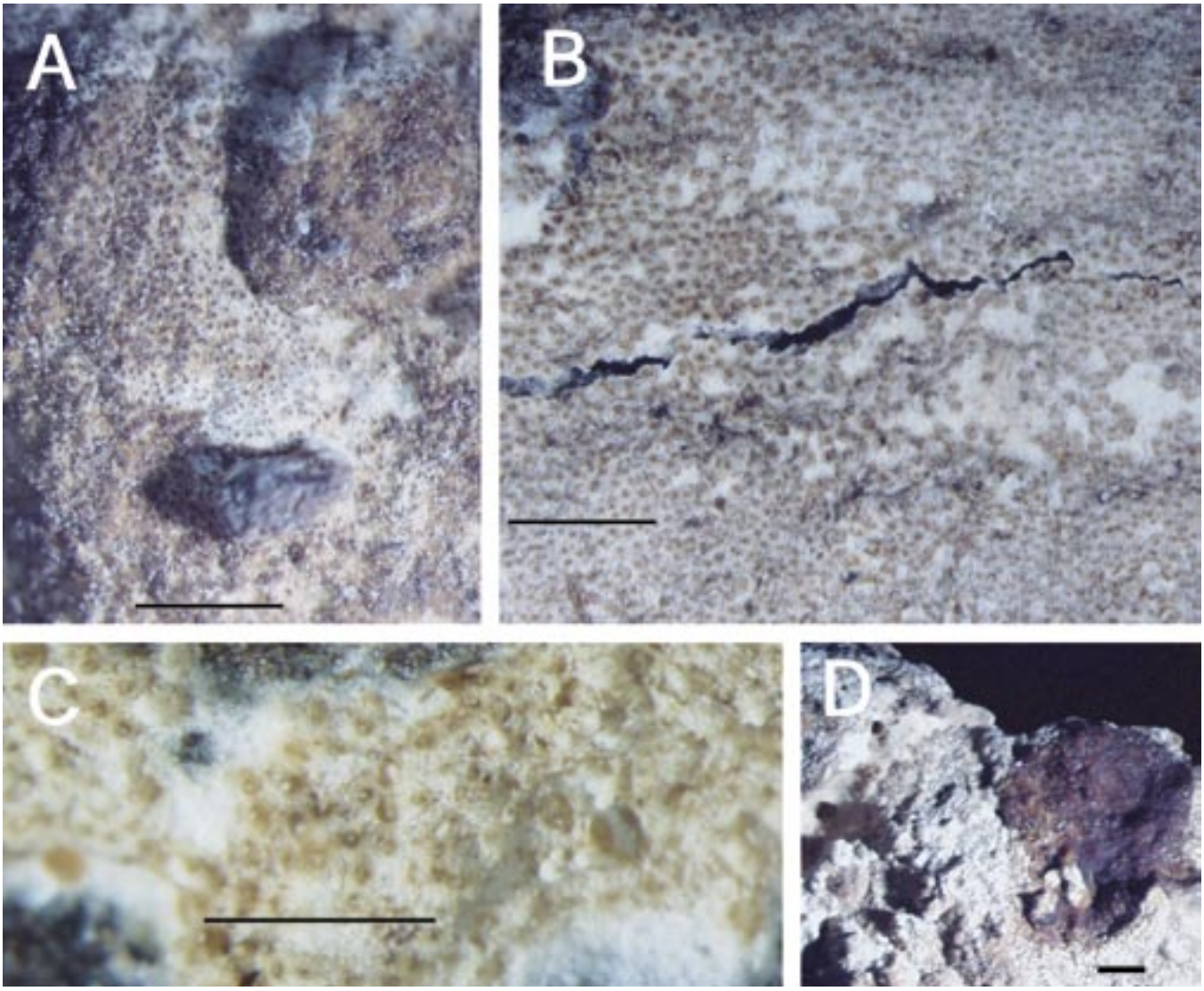


Fig. 15. A–D. *H. farinosa*. A. Effuse extensive stroma, BPI 802598. B. Effuse extensive stroma, BPI 747356. C. Colour variation in stroma, BPI 737771. D. KOH reaction, BPI 802598. bars = 1 mm.

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 hyphal elements near the agar surface or in the aerial mycelium, irregularly branched; phialides in whorls of (2–)3(–4); phialides subulate, (9.5–)13–25(–36) × (1.5–)2.4–3.0(–3.4) µm (n = 64); 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).

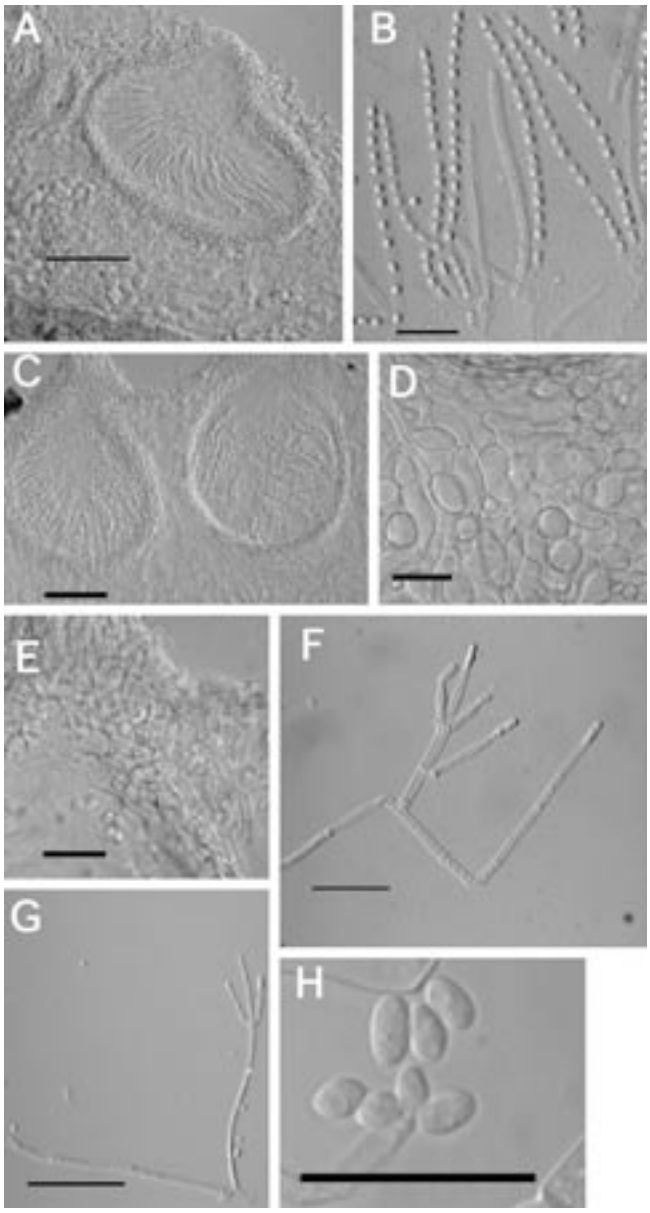


Fig. 16. A–H. *H. farinosa*. A. Section of stroma; note the layer of *t. intricata* near the stroma surface and loose layer of pseudoparenchymatous tissue near the base of the perithecium; bar = 40 µm. B. asci and part-ascospores, both of BPI 802598, bar = 20 µm. C. Section of stroma; bar = 40 µm. D–E. *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.

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, # 513 (BPI 747356; culture G.J.S. 97-207).

Other specimens examined: **Canada,** Ontario, Bear Island, on *Hymenochaete* sp., 28 Aug. 1937, H. S. Jackson, det. R. F. Cain (BPI 631473). **Japan,** Morothuka 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. Martinsville, on decayed wood, 24 Aug. 1890, A.B. Langlois, Flora Ludoviciana # 2294 (BPI 631450); Maryland, Ellicot City, Paptapsco 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 perithecia, 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*.

Hypocrea 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. *Hypocrea farinosa* was collected once from *Lentinula* bag culture.

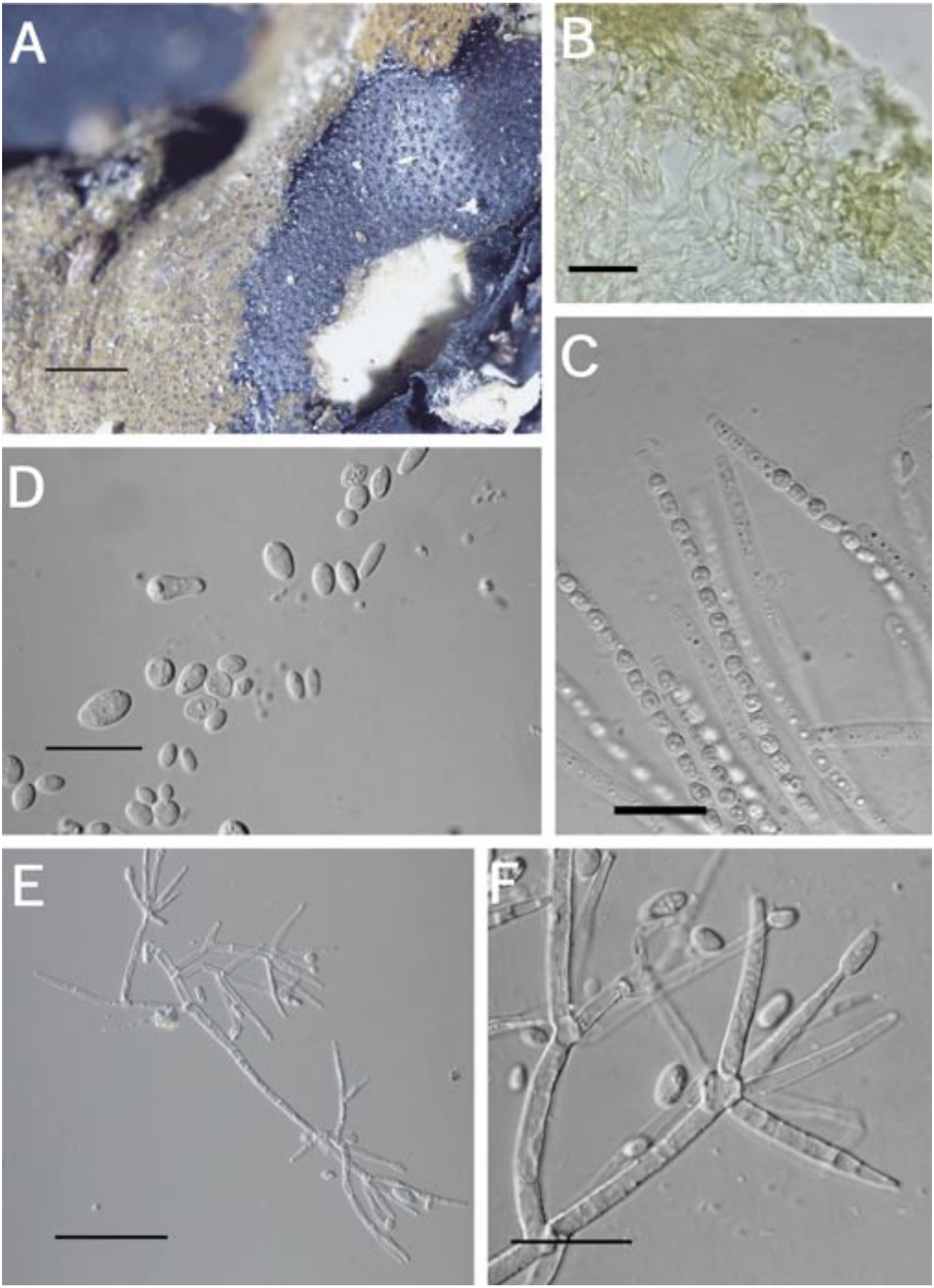


Fig. 17. A–F. *H. alcalifuscescens*, TAA 181584. A. Stroma showing reaction in KOH; bar = 1 mm. B. Section of stroma showing compact *t. intricata* and KOH-positive layer of stroma tissue; bar = 20 µm. C. Asci and ascospores; bar = 20 µm. D. Conidia; bar = 20 µm. E–F. Conidiophore branching pattern; 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, luteo-brunnea vel olivaceo-brunnea, KOH ope brunnescentia. Ascosporae hyalinae, crassitunicatae, spinulosae, dimorphicae; 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 subellipsoidea, (2.6–)4.0–7.3(–11) × (2.2–)2.7–4.7(–6.3) µm.

Typus: TAA(M) 181548 in BPI.

Teleomorph: Stromata effuse, extensive, surface hyphal; largest continuous stroma 25 × 10 mm, varying in colour, usually yellow-brown to olive-brown (5E8–4E7), KOH^{+}, darkening, reddish brown (8E8); 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) × (4.0–)4.4-5.7(–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.

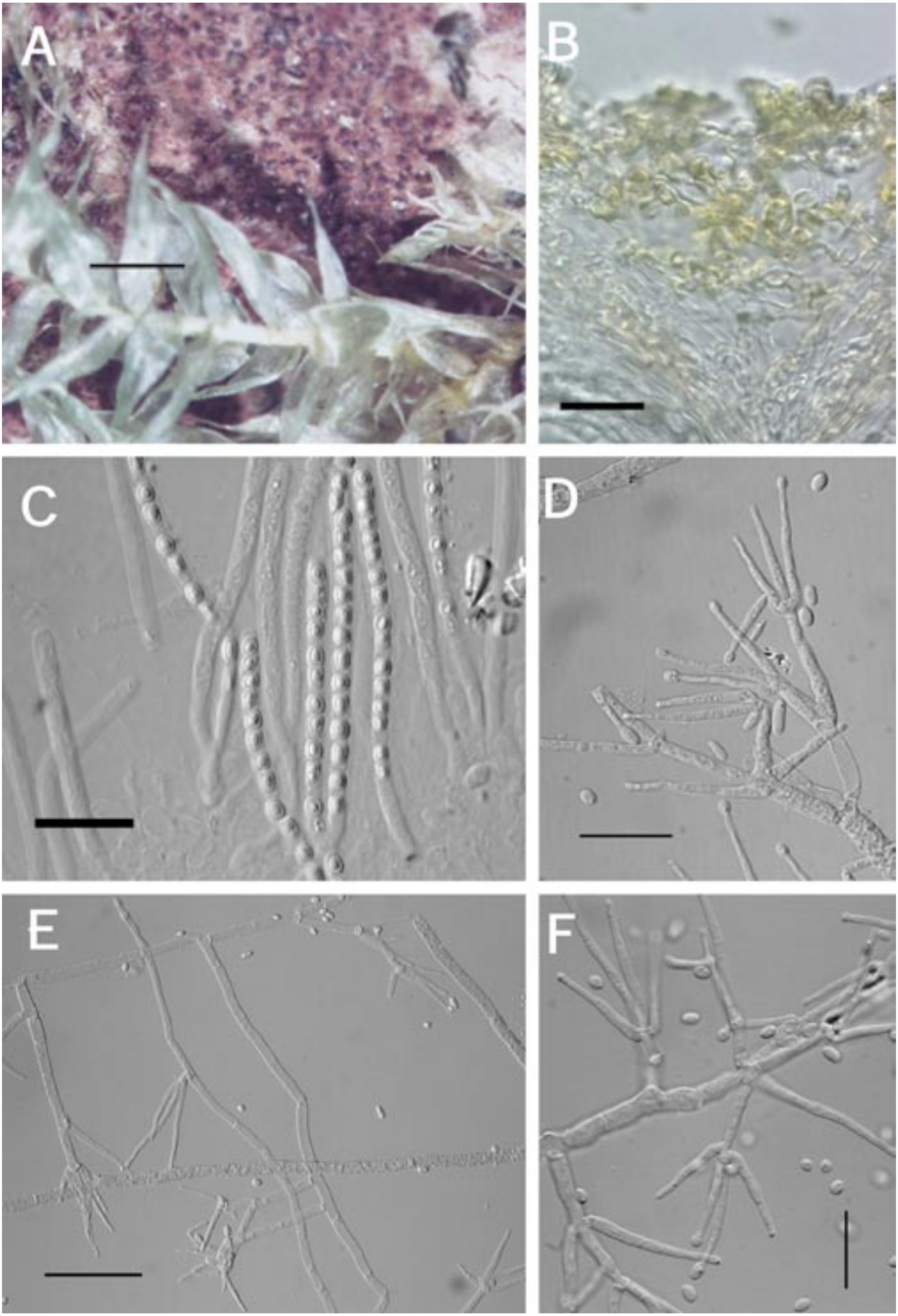
Stromata effusa, extensa, violaceo-brunnea vel griseo-purpurea,. Ascosporae hyalinae, crassitunicatae, spinulosae, dimorphicae; pars distalis ascosporarum subglobosa vel obovata, (2.7–)3.8–5.2(–6.7) × (2.7–)3.5–4.5(–5.6) µm, pars proxima sublgoobsa vel ellipsoidea, (3.2–)4.2–5.7(–6.6) × (2.4–)3.0–4.0(–5.2) µm. Anamorphosis *Trichoderma* sectionis *Hypocreanum.* Conidia hyalina, subglobosa vel subcylindrica, (3.0–)3.4–5.0(–7.8) × (2.2–)2.5–3.1(–3.6) µm.

Teleomorph: Stromata effuse, 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(–5.6) 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

Fig. 18. (Page 63). A–F. *H. parmastoi*. A–B. Stroma; bars = 1 mm and 20 µm, respectively. C. Asci and ascospores; bar = 20 µm; A–C. TAA 169055. D, F. Conidiophores on PDA; bar = 20 µm. E. Conidiophores on CMD; bar = 40 µm; D–F. TFC 97-143. Note: in spite of several attempts, no illustrative sections of perithecia were obtained.


A
B
C
D
E
F

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 whorls 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 whorls 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
1. 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(–7.6) × (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***
2. Part-ascospores monomorphic, hyaline, thick-walled, spinulose, subglobose to sub-ellipsoidal, (2.8–)3.6–4.4(–5.4) × (2.3–)2.9–3.6(–4.1) µm ..... 5. ***H. subsulphurea***

3. Stromata hyphal or sometimes with a thin layer of pseudoparenchymatous tissue below the perithecia 4
3. Stroma tissue pseudoparenchymatous, no hyphal layer; stromata yellow to yellow-brown, effuse or subpulvinate 6

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 ..... 8. ***H. parmastoi***
4. Stromata either composed of compact hyphae, or with both a hyphal layer near the surface and a pseudoparenchymatous layer below the perithecia 5

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 ..... 7. ***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–)3–3.6(–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. eucorticioides***
6. 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***
7. 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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