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The *Trichoderma koningii* aggregate species

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Abstract: The morphological concept of *Trichoderma koningii* is found to include several species that differ from each other in details of phenotype (including conidium morphology, growth rate) and biogeography. Phylogenetic analysis utilizing partial sequences of the translation-elongation factor 1 alpha (*tef*1), as well as fragments of actin and calmodulin genes, indicate that phenotypic characters typical of *T. koningii* evolved independently in three well-separated main lineages. Combined molecular and phenotype data lead to the development of a taxonomy with the recognition of twelve taxonomic species and one variety within the three lineages. These lineages include: (1) *T. koningii* and *T. ovalisporum* and the new species *T. caribbaeum* var. *caribbaeum*, *T. caribbaeum* var. *aequatoriale*, *T. dorotheae*, *T. dingleyae*, *T. intricatum*, *T. koningiopsis*, *T. petersenii* and *T. taiwanense*; (2) the new species *T. rogersonii* and *T. austrokoningii*, and (3) the new anamorph *T. stilbohypoxyli*.

Trichoderma koningii s. str. is an uncommon species restricted to Europe and eastern North America; *T. caribbaeum* var. *aequatoriale, T. koningiopsis*, and *T. ovalisporum* were isolated as endophytes of trunks of *Theobroma* species in tropical America, and *T. ovalisporum* from the woody liana *Banisteropsis caapi* in Ecuador; *T. koningiopsis* is common in tropical America but was isolated also from natural substrata in East Africa, Europe and Canada, and from ascospores in eastern North America, and as an endophyte in *Theobroma* species; *T. stilbohypoxyli*, originally described as a parasite of *Stilbohypoxylon* species in Puerto Rico, is found to be more common in the tropics, besides an endophytic isolate from *Fagus* in U.K. The additional new species are known almost exclusively from their teleomorphs. Isolates of *T. ovalisporum* and *T. koningiopsis* may have biological control potential. A morphophenetic key and a set of tools for molecular species identification were developed.

Taxonomic novelties: *Trichoderma austrokoningii/Hypocrea austrokoningii* Samuels & Druzhinina sp.nov.*, T. caribbaeum* var *caribbaeum/H. caribbaea* Samuels & Schroers sp.nov.*, T. caribbaeum* var. *aequatoriale* Samuels & H.C. Evans var.nov.*, T. dingleyae/H. dingleyae* Samuels & Dodd sp.nov., *T. dorotheae/H. dorotheae* Samuels & Dodd sp.nov.*, T. intricatum/H. intricata* Samuels & Dodd sp.nov.*, T. koningiopsis/H. koningiopsis* Samuels, C. Suarez & H.C. Evans sp.nov.*, T. petersenii/H. petersenii* Samuels, Dodd & Schroers sp.nov.*, T. rogersonii/H. rogersonii* Samuels sp.nov.*, T. stilbohypoxyli* Samuels & Schroers sp.nov.*, T. taiwanense/H. taiwanensis* Samuels & M.L. Wu sp.nov.

Key words: Actin, barcode, Bayesian phylogeny, local BLAST, biogeography, biological control, cacao, calmodulin, endophytes, GCPSR, *Hypocrea*, *Hypocreales*, *Hypocreaceae*, ISTH, ITS1 and 2, molecular identification, morphological key, nomenclature, rDNA, RNA polymerase, sequence similarity search, species identification, systematics, translation elongation factor 1-alpha.

INTRODUCTION

Trichoderma koningii Oudem. is one of the most commonly cited species of *Trichoderma* Pers., the anamorph genus of *Hypocrea* Fr. (*Hypocreales*, *Hypocreaceae*). Literally hundreds of publications report the involvement of this species in the biological control of plant diseases caused by other fungi. Among these, *T. koningii* is reported to produce 6-pentyl alpha pyrone, a spore germination inhibitor (Worasatit *et al.* 1994). Song *et al.* (2006) characterized trichokonins, which are peptaibols that have antimicrobial activity, in *T. koningii*. A search of the literature reveals a role of *T. koningii* in many activities in addition to biological control of fungus-induced plant disease. For example, culture filtrates of *T. koningii* and *T. harzianum* killed 100 % of root-knot nematodes in Sri Lanka (Sankaranarayanan *et al.* 1997). *Trichoderma koningii* also benefits plant health and nutrient uptake when it was determined to be highly active in biomineralizing calcium oxalate crystals in soil (Oyarbide *et al.* 2001), the first reference to indicate this species as a biomineral-producing agent.

What is *T. koningii*? Despite the fact that the genus *Trichoderma* Pers. was proposed late in the 18th century, prior to 1984 only 35 species were included in the genus, and before 1969 very few of these were reported in the literature subsequent to their original description. *Trichoderma koningii*, described in 1902 (Oudemans & Koning 1902), was included by Rifai (1969) as one of the nine "aggregate" or "morphological" species that he recognized. Bissett (1991a) included it in *Trichoderma* sect. *Trichoderma*, which includes the type of the genus, *T. viride* Pers., on the basis of the morphology of the conidiophore. Lieckfeldt *et al.* (1998) confirmed membership of *T. koningii* in sect. *Trichoderma* using ITS1 and 2 sequences of the rDNA gene cluster, and PCR fingerprinting, a result that has been affirmed in additional publications with other genes (e.g. Kullnig-Gradinger *et al.* 2002). Lübeck *et al.* (2004) showed that infra-species variation was greater

than inter-species in ITS in the *T. koningii* aggregate species. Essentially, in that study ITS1 and 2 were not helpful in separating closely related species of sect. *Trichoderma*, but the authors found that UP-PCR fingerprinting could distinguish *T. koningii* from *T. viride* and other members of *Trichoderma* sect. *Trichoderma.* The first version of an oligonucleotide barcode based on ITS1 and 2 implemented in *TrichO*KEY program (Druzhinina *et al.* 2005) is able to identify the *T. koningii/ T. ovalisporum/H. muroiana* species triplet and attribute it to the "*Pachybasium* A" clade after Kullnig-Gradinger *et al.* (2002).

Bissett (1991a) divided *Trichoderma* species among several sections. Among them was sect. *Trichoderma*, which included *T. viride*. Chaverri & Samuels (2004) proposed a move towards the classification based on phylogenetic clades rather than dividing the genus into sections. They referred to the "Rufa Clade," named for *Hypocrea rufa,* the type species of the genus, which included members of sections *Trichoderma* and species from the "Pachybasium A" Clade. The latter group includes *T. hamatum* (Bonord.) Bainier, the type species of *Pachybasium* Sacc., and other species. It was refered to as the "Hamatum clade" by Jaklitsch *et al.* (2006a). In the present work we refer to the combined "Rufa Clade" and the "Pachybasium A" Clade as the "Viride Clade." *Trichoderma koningii* and the species discussed in the current paper belong to that clade.

Lieckfeldt *et al.* (1998) narrowly defined the morphology of *T. koningii* and linked it to a teleomorph, *Hypocrea koningii* Lieckfeldt *et al*. Lieckfeldt *et al*. (1998) and Lübeck *et al.* (2004) demonstrated genetic diversity within the *T. koningii* aggregate species. Lieckfeldt *et al*. (1998) noted four additional, morphologically similar and phylogenetically closely related species that they identified as *H.* cf. *muroiana* or *Hypocrea* sp. One of the strains identified by Lieckfeldt *et al.* (1998) as *H.* cf. *muroiana* has since been described as *H. stilbohypoxyli* B.S. Lu & Samuels (Lu & Samuels 2003). Later, in a revision of *T. viride*, Lieckfeldt *et al.* (1999) found nine ITS haplotypes among isolates that conformed to the broadly defined morphospecies *T. koningii*, of which one was true *T. koningii* in the narrow sense of Lieckfeldt *et al.* (1998). Holmes *et al.* (2004) distinguished *T. ovalisporum* Samuels & Schroers from *T. koningii s. str*. and other members of the *T. koningii* morphological aggregate on the basis of sequences of the protein-encoding gene translation-elongation factor 1-α (*tef1)* and conidium morphology. In addition to these *T. koningii*-like species, Holmes *et al*. (2004) designated four clades of *Trichoderma* collections that have the *T. koningii* morphology as "Tkon 20," "Tkon 21," "Tkon 22," and "Tkon 3."

Since the study of Lieckfeldt *et al*. (1999) we have received many additional collections from geographically and biologically diverse sources that can be assigned generally to sect. *Trichoderma* and specifically to the *T. koningii* aggregate species. In the present work we examine the phenotypic and phylogenetic diversity found within the *T. koningii* aggregate species, and develop a taxonomy for those fungi by combining results of morphological, cultural, and molecular-phylogenetic analyses.

MATERIALS AND METHODS

COLLECTIONS AND ANALYSIS OF PHENOTYPE

The isolates originated from three natural sources: isolations from ascospores of *Hypocrea* specimens, direct isolations by a variety of means from soil or dead herbaceous tissue, and as isolations as endophytes from living stems of *Theobroma* and related tree species as reported by Evans *et al.* (2003). A smaller number of isolates was obtained from the American Type Culture Collection (ATCC), Centraalbureau voor Schimmelcultures (CBS) and colleagues. Cultures derived from single part-ascospores that were germinated on cornmeal agar with 2 % dextrose (CMD, Difco cornmeal agar + 2 % dextrose w/v) and isolated using a micromanipulator; usually two or more single-spore cultures were combined in a single stock culture and polyspore cultures were used in all subsequent analyses. Representative cultures are deposited in ATCC and CBS. Kornerup & Wanscher (1978) was used as the colour standard. Isolates and their GenBank numbers are listed in Table 1. The name of the most commonly cited collectors are abbreviated as G.J.S. (G.J. Samuels) and C.T.R. (C.T. Rogerson).

Cultures used for study of anamorph micro-morphology were grown on CMD or, less frequently SNA (without filter paper, Nirenberg 1976), at 20 or 25 ºC for 7–10 d under alternating 12 h cool white fluorescent light and 12 h darkness; in the descriptions that follow, these alternating light conditions are referred to when the word "light" is used. Approximately 20 mL of agar was poured into Petri dishes.

We did not observe any difference in anamorph morphology between CMD and SNA but there was a tendency for more reliable conidial production on SNA than on CMD. Conidial pustules of *Trichoderma* isolates grown on these two media appeared to be more similar to how they appear in nature than conidia formed on other commonly-used media, including potato-dextrose agar, malt agar and oatmeal agar (Gams *et al.* 1998).

Morphological analysis of microscopic characters was undertaken from material that was first hydrated in the case of herbarium material, or wetted in the case of living cultures, in 3 % KOH. The KOH was subsequently replaced by distilled water. Measurements were made from KOH or water; we did not observe any differences between the two reagents. Where possible, 30 units of each parameter were measured for each collection. Ninety-five percent confidence intervals of the means (CI) are provided; this figure represents the interval within which 95 % of the individuals of the parameter will be found. The parameters used for analysis are listed in Table 3. Chlamydospores were measured by inverting a 7–10 d old CMD culture on the stage of a compound microscope and observing with a 40 × objective. Data were gathered using a Nikon DXM1200 digital camera and Nikon ACT 1 software and measured using Scion Image (release Beta 4.0.2; Scioncorp, Frederick, MD).

Five types of microscopy were used, viz. stereo microscopy (stereo), bright field (BF), phase contrast (PC), Nomarski differential interference contrast (DIC) and epifluorescence (FL). The fluorescent brightener

Table 1. Strains used in phylogenetic analysis, their origin and GenBank numbers.

Strain[1]	Species	Geography	GenBank			
			ITS 1+2	*act*	*cal*	*tef1*
G.J.S. 02-134	*T. atroviride*	Sri Lanka	DQ315466			DQ307547
G.J.S. 02-135	*T. atroviride*	Sri Lanka	DQ315467			DQ307548
G.J.S. 02-139	*T. atroviride*	Sri Lanka	DQ315463			DQ307546
G.J.S. 02-96	*T. atroviride*	Sri Lanka	DQ315469			DQ307550
G.J.S. 98-8	*T. atroviride*	France	DQ315462			DQ307545
C.T.R. 85-57	*T. austrokoningii*	U.S.A. (FL)	DQ315469	DQ333551	DQ379003	DQ307564
CBS 247.63	*T. austrokoningii*	New Zealand	DQ315470	DQ341183	DQ381953	DQ307568
G.J.S. 00-73	*T. austrokoningii*	Russia	DQ315465	DQ341177	DQ379006	DQ307569
G.J.S. 96-163	*T. austrokoningii*	Taiwan	DQ323408	DQ367709	DQ378997	DQ307565
G.J.S. 99-146	*T. austrokoningii* (T)	Australia (QL)	DQ323423	DQ367716	DQ379001	DQ307561
G.J.S. 99-147	*T. austrokoningii*	Australia (QL)	DQ323422	DQ333569	DQ379000	DQ307562
G.J.S. 99-116	*T. austrokoningii*	New Zealand	DQ333546	DQ379011	DQ379004	
G.J.S. 97-3	*T. caribbaeum* (T)	Guadeloupe	DQ313131	DQ328607	DQ367688	DQ284977
G.J.S. 98-43	*T. caribbaeum*	Puerto Rico	DQ313139	DQ328606	DQ367673	DQ284976
DIS 320c	*T. caribbaeum var. aeqatoriale* (T)	Ecuador	DQ323436	DQ328610	DQ367704	DQ289010
G.J.S. 02-87	PS E	Sri Lanka	DQ315461			DQ307544
G.J.S. 02-50	*T. dingleyae* (T)	New Zealand	DQ333548	DQ367718	DQ370461	DQ284978
G.J.S. 99-105	*T. dingleyae*	New Zealand	DQ313151		DQ378998	AF348117
						DQ289008
G.J.S. 99-203	*T. dingleyae*	New Zealand	AY380899	AY376674	DQ367666	AF348111, AY376040
G.J.S. 99-194	*T. dorotheae*	Australia (VI)	DQ313136	DQ328602	DQ367674	DQ284974
G.J.S. 99-202	*T. dorotheae* (T)	New Zealand	DQ313144	DQ323454	DQ367706	DQ307536
G.J.S. 99-97	*T. dorotheae*	New Zealand	DQ333547	DQ379009		DQ288990
DIS 7	*T. erinaceus*	Peru	DQ109534	DQ111968	DQ122162	DQ109547
DIS 8	*T. erinaceus*	Peru	DQ313147	DQ323450	DQ384930	DQ284970
G.J.S. 96-13	*T. intricatum*	Puerto Rico	DQ381955	AY376683	DQ367679	AY376059
G.J.S. 97-88	*T. intricatum* (T)	Thailand	AY380913	AY376684	DQ367680	AY376060
ATCC 64262	*T. koningii*	Hungary	AY380903	AY376679	DQ367671	AY376046
DAOM 167645 = CBS 979.70	*T. koningii*	Netherlands	DQ323410	DQ379007	DQ367682	DQ288994
G.J.S. 00-156	*T. koningii*	U.S.A. (PA)	DQ323433	DQ379010		DQ288991
G.J.S. 00-168	*T. koningii*	U.S.A. (?MD)	DQ323427	DQ333562		DQ307571
G.J.S. 89-122 = CBS 989.97 = IMI 378801	*T. koningii* (T of *H. koningii)*	U.S.A. (MD)	AY380902	AY376678	DQ367693	AY376045
G.J.S. 90-18 = CBS 988.97	*T. koningii*	U.S.A. (WI)	DQ323409	DQ341181	DQ370463	DQ289007
G.J.S. 92-18 = CBS 987.97	*T. koningii*	Canada	DQ323435	DQ367712	DQ370458	DQ289005
G.J.S. 96-118	*T. koningii*	Netherlands	DQ323444	DQ341178	DQ367683	DQ288996
CBS 458.96						
G.J.S. 96-119	*T. koningii*	Netherlands	DQ323424	DQ341179	DQ367681	DQ289003
CBS 460.96						
G.J.S. 96-120	*T. koningii*	Netherlands	DQ109536	DQ111972	DQ122168	DQ109548
CBS 459.96						
G.J.S. 96-117	*T. koningii* (neotype)	Netherlands	Z79628	DQ341180	DQ367686	AF456909
CBS 457.96						
DAOM 222105	*T. koningiopsis*	Canada (ON)	DQ313146	DQ379012	DQ367668	AY376042
DIS 172ai = CBS 119067 = IMI 385811	*T. koningiopsis*	Brazil	DQ313138	DQ323453	DQ367675	DQ284972
DIS 205f = CBS 119068 = IMI 385805	*T. koningiopsis*	Ecuador	DQ323419	DQ328609	DQ367698	DQ288993
DIS 229d = CBS 119069 = IMI 391590	*T. koningiopsis*	Ecuador	DQ313143	DQ323452	DQ367676	DQ284971
DIS 326h = CBS 119070 = IMI 393639	*T. koningiopsis*	Ecuador	DQ379015	DQ379008	DQ367695	DQ288997

Table 1. (Continued).

Strain[1]	Species	Geography	GenBank			
			ITS 1+2	*act*	*cal*	*tef1*
DIS 339c = CBS 119065 = IMI 391591	*T. koningiopsis*	Ecuador	DQ313142	DQ323447	DQ367677	DQ284968
DIS 94c = CBS 119066 = IMI 391592	*T. koningiopsis*	Peru	DQ313130	DQ379014	DQ367678	DQ284967
G.J.S. 01-07	*T. koningiopsis*	Ecuador	DQ313132	AY376676	DQ367669	AY376043
G.J.S. 01-08	*T. koningiopsis*	Ecuador	–	–	–	–
G.J.S. 01-09	*T. koningiopsis*	Ecuador	–	–	–	–
G.J.S. 01-10	*T. koningiopsis*	Ecuador	–	–	–	–
G.J.S. 01-11	*T. koningiopsis*	Ecuador	–	–	–	–
G.J.S. 01-12	*T. koningiopsis*	Ecuador	–	–	–	–
G.J.S. 04-10	*T. koningiopsis*	Texas	DQ323413	DQ341184	DQ367692	DQ284981
G.J.S. 04-11	*T. koningiopsis*	Texas	DQ323421	DQ341182	DQ367687	DQ289009
G.J.S. 04-373	*T. koningiopsis*	Brazil	DQ323437	DQ367713	DQ367703	DQ289006
G.J.S. 04-379	*T. koningiopsis*	Brazil	DQ323439	DQ367719	DQ367702	DQ289001
G.J.S. 04-378	*T. koningiopsis*	Brazil	DQ323432	DQ367714	DQ367699	DQ289011
G.J.S. 04-375	*T. koningiopsis*	Brazil	DQ323411	DQ367711	DQ367697	DQ288989
G.J.S. 04-376	*T. koningiopsis*	Brazil	DQ323425	DQ341176	DQ367696	DQ288992
G.J.S. 04-377	*T. koningiopsis*	Brazil	DQ323416	DQ333552	DQ367701	DQ289002
G.J.S. 04-374	*T. koningiopsis*	Brazil	DQ323412	DQ367709	DQ367700	DQ288995
G.J.S. 91-6	*T. koningiopsis*	Brazil	DQ313135	DQ323446	DQ367705	DQ307539
G.J.S. 91-7	*T. koningiopsis*	Brazil	DQ313137	DQ323449	DQ367689	DQ284969
G.J.S. 95-175	*T. koningiopsis*	U.S.A. (KY)	AF456923	DQ323448	DQ367707	AF456910
G.J.S. 96-47	*T. koningiopsis*	Puerto Rico	DQ313133	AY376677	DQ367670	AY376044
G.J.S. 93-20	*T. koningiopsis* (T)	Cuba	DQ313140	DQ381954	DQ367694	DQ284966
G.J.S. 97-273 = BBA 65450	*T. koningiopsis*	Germany	DQ109537	DQ111973	DQ122169	DQ109549
DIS 172h	*T. ovalisporum*	Brazil	AY380896	AY376670	DQ122167	AY387660
DIS 172i	*T. ovalisporum*	Brazil	DQ323438	DQ328611	–	DQ288999
DIS 203c	*T. ovalisporum*	Brazil	DQ315458	DQ328608	DQ378996	DQ307540
DIS 70a	*T. ovalisporum* (T)	Ecuador	AY380897	AY376671	DQ379005	AY376037
DAOM 165782	*T. petersenii*	U.S.A. (NC)	DQ313149	DQ328604	DQ367690	DQ289000
G.J.S. 04-164	*T. petersenii*	U.S.A. (TN)	DQ323442	DQ333566	DQ367684	DQ289004
G.J.S. 04-351	*T. petersenii*	U.S.A. (TN)	DQ323440	DQ333568	–	DQ284979
G.J.S. 04-355	*T. petersenii* (T)	U.S.A. (TN)	DQ323441	DQ333570	DQ367691	DQ284980
G.J.S. 90-86	*T. petersenii*	U.S.A. (NC)	DQ313153	DQ328605	DQ367708	DQ284975
G.J.S. 91-99	*T. petersenii*	U.S.A. (VA)	DQ313134	AY376673	–	AY376039
G.J.S. 98-139	*T. petersenii*	France	DQ323426	DQ379013	DQ378999	DQ288998
G.J.S. 98-140	*T. petersenii*	France	DQ313148	AY376675	DQ367667	AY376041
G.J.S. 99-48	*T. petersenii*	Costa Rica	AY380898	AY376672	DQ367665	AY376038
G.J.S. 04-157	*T. rogersonii*	U.S.A. (TN)	DQ323415	DQ333565	DQ370454	DQ307558
G.J.S. 04-158	*T. rogersonii* (T)	U.S.A. (TN)	DQ323434	DQ333567	DQ370460	DQ307563
G.J.S. 90-108	*T. rogersonii*	U.S.A. (MD)	DQ333550	DQ333561	DQ370448	DQ307570
G.J.S. 90-125	*T. rogersonii*	U.S.A. (NC)	DQ323417	DQ341175	DQ370453	DQ307567
G.J.S. 90-78	*T. rogersonii*	U.S.A. (NC)	DQ333549	DQ333558	DQ370457	DQ307556
G.J.S. 90-79	*T. rogersonii*	U.S.A. (NC)	DQ381956	DQ333563	DQ370451	DQ381959
G.J.S. 90-93	*T. rogersonii*	U.S.A. (NC)	DQ313154	DQ333560	DQ370450	DQ307559
G.J.S. 92-116	*T. rogersonii*	U.S.A. (MD)	DQ313150	DQ333554	DQ370455	DQ307557
G.J.S. 94-115	*T. rogersonii*	U.S.A. (MO)	Z95927	DQ333555	DQ370452	AF456906
G.J.S. 95-217	*T. rogersonii*	Austria	DQ323445	DQ333553	DQ370462	DQ307566
G.J.S. 98-75	*T. rogersonii*	U.S.A. (NJ)	DQ323443	DQ333556	DQ370449	DQ307560
G.J.S. 98-77	*T. rogersonii*	U.S.A. (NJ)	DQ323414	DQ333559		DQ307572
G.J.S. 98-82	*T. rogersonii*	U.S.A. (NJ)	AY380910	AY376682	DQ367672	AY376055
G.J.S. 96-32	*T. stilbohypoxyli*	Puerto Rico	AY380915	AY376686	DQ122160	AY376062
G.J.S. 96-30 = CBS 992.97	*T. stilbohypoxyli* (T)	Puerto Rico	DQ109533	DQ111967	DQ122161	DQ109546

Table 1. (Continued).

Strain[1]	Species	Geography	GenBank			
			ITS 1+2	*act*	*cal*	*tef1*
G.J.S. 96-30A	*T. stilbohypoxyli*	Puerto Rico	AY380916	AY376687	–	AY376063
G.J.S. 96-42A	*T. stilbohypoxyli*	Puerto Rico	AY380914	AY376685	–	AY376061
G.J.S. 96-43	*T. stilbohypoxyli*	Puerto Rico	–	–	–	–
G.J.S. 95-93	*T. taiwanense* (T)	Taiwan	DQ313141	DQ323455	DQ367685	DQ284973
C.P.K. 984[2]	*T. viride* VB	Sweden	–	–	–	–
C.P.K. 965[2]	*T. viride* VB	Czech Republic	–	–	–	–
ATCC 28020	*T. viride* VB	U.S.A. (WA)	DQ109535	DQ111970	DQ122164	AY937449
CBS 101526	*T. viride* VB	Netherlands	X93979	–	–	AY376053
C.P.K. 1001	*T. viride* VB	Russia	–	–	–	–
C.P.K. 1002	*T. viride* VB	Russia	–	–	–	–
C.P.K. 1006	*T. viride* VB	Russia	–	–	–	–
C.P.K. 1007	*T. viride* VB	Russia	–	–	–	–
C.P.K. 1008	*T. viride* VB	Russia	–	–	–	–
C.P.K. 1009	*T. viride* VB	Russia	–	–	–	–
G.J.S. 04-369	*T. viride* VB	Austria	DQ323430	–	–	DQ307553
G.J.S. 04-370	*T. viride* VB	Austria	DQ323429	–	–	DQ307554
G.J.S. 04-371	*T. viride* VB	France	DQ323431	–	–	DQ307552
G.J.S. 04-372	*T. viride* VB	Czech Rep.	DQ323428	–	–	DQ307555
G.J.S. 90-95 = CBS 120066	*T. viride* VB	U.S.A. (NC)	DQ315455	–	–	DQ307535
G.J.S. 92-14	*T. viride* VB	New Zealand	DQ313155	–	–	DQ288988
G.J.S. 92-15	*T. viride* VB	Canada	DQ315452	–	–	DQ307537
G.J.S. 97-271 = BBA 70239 = ITB 8212	*T. viride* VB	Denmark	DQ315456	–	–	AF348116
G.J.S. 99-16 = FP 5563	*T. viride* VB	Japan?	DQ315460	–	–	DQ307543
TR 2 = ATCC 18652 = CBS 240.63	*T. viride* VB	U.S.A. (WA)	DQ315457	–	–	DQ307538
TR 21 = ATCC 28038	*T. viride* VB	U.S.A. (VA)	AY380909	AY376681	DQ122165	AY376054
G.J.S. 04-40	*T. viride* VB?	Brazil	DQ315454	–	–	DQ307534
301rufa[2] = C.P.K. 1999	*T. viride* VB	Austria	–	–	–	–
19d[2] = G.J.S. 89-127	*T. viride* VB	U.S.A. (NC)	–	–	–	–
292rufa[2] = C.P.K. 1997	*T. viride* VB	Czech Republic	–	–	–	–
6138i[2] = C.P.K. 947	*T. viride* VD	Austria	–	–	–	–
712341[2] = C.P.K. 2341	*T. viride* VD	Italy (Sardinia)	–	–	–	–
ATCC 20898	*T. viride* VD	U.S.A. (NY)	DQ315434	–	–	DQ307518
ATCC 32630	*T. viride* VD	Sweden	DQ315445	–	–	DQ307526
CBS 274.79	*T. viride* VD	Austria	DQ315428	–	–	DQ307513
CBS 333.72	*T. viride* VD	Netherlands	DQ315441	–	–	DQ307523
CBS 438.95	*T. viride* VD	U.K.	DQ315438	–	–	DQ307522
CBS 439.95	*T. viride* VD	Northern Ireland	DQ315439	–	–	AY937413
273[2]	*T. viride* VD	U.K.		–	–	–
C.P.K. 998	*T. viride* VD	Russia		–	–	–
C.P.K. 999	*T. viride* VD	Russia		–	–	–
246[2]	*T. viride* VD	U.K.		–	–	–
234[2]	*T. viride* VD	Germany	–	–	–	–
G.J.S. 00-67	PS F *T. viride* VD	U.S.A. (WV)	DQ315418	–	–	DQ307502
G.J.S. 89-142 = CBS 120065	*T. viride* VD	U.S.A. (NC)	DQ109532	DQ111965	DQ122157	AY376049
G.J.S. 92-11	*T. viride* VD	New Zealand	DQ315422	–	–	DQ307524
G.J.S. 94-10	PS F *T. viride* VD	Taiwan	DQ315420	–	–	DQ307506
G.J.S. 94-11	PS F *T. viride* VD	Taiwan	DQ313156	–	–	DQ307508
G.J.S. 94-118	*T. viride* VD	France	DQ315424	–	–	DQ307510
G.J.S. 94-9	PS F *T. viride* VD	Taiwan	DQ315421	–	–	DQ307507
G.J.S. 97-243	PS F *T. viride* VD	U.S.A. (GA)	DQ315419	–	–	DQ307503

Table 1. (Continued).

Strain[1]	Species	Geography	GenBank			
			ITS 1+2	*act*	*cal*	*tef1*
G.J.S. 97-272 = BBA 66069	*T. viride* VD	Germany	DQ315429	–	–	DQ307504
G.J.S. 97-274 = BBA 68432	*T. viride* VD	Russia	DQ315440	–	–	DQ307505
G.J.S. 98-129	*T. viride* VD	France	AY737773	–	–	DQ307542
G.J.S. 98-182 = W.J. 1223 = CBS 120067	*T. viride* VD	Austria	DQ315425	–	–	DQ307511
G.J.S. 98-86	*T. viride* VD	Mexico	DQ315423	–	–	DQ307509
G.J.S. 99-10 = NR 5510	*T. viride* VD	Czech Rep.	DQ315430	–	–	DQ307514
G.J.S. 99-128	*T. viride* VD	Australia (VI)	DQ315431	–	–	DQ307515
G.J.S. 99-142	*T. viride* VD	Australia (VI)	DQ315427	–	–	DQ307512
G.J.S. 99-175	*T. viride* VD	Australia (VI)	DQ315437	–	–	DQ307521
G.J.S. 99-8 = NR 5541	*T. viride* VD	Japan	DQ315433	–	–	DQ307517
G.J.S. 99-18 = FP 5564	*T. viride* VD	Japan?	DQ315435	–	–	DQ307519
TR 4	*T. viride* VD	U.S.A. (OR)	DQ315436	–	–	DQ307520
TR 5	*T. viride* VD	U.S.A. (OR)	DQ315443	–	–	DQ307525
TR 6	*T. viride* VD	U.S.A. (OR)	DQ315444	–	–	AY376050
CBS 433.34	*T. viride* VD, T of *Eidamia viridescens*	U.K.	AF456922	–	–	AF456905, AY376048
G.J.S. 04-09	PS C	U.S.A. (TX)	DQ315459	–	–	DQ307541
G.J.S. 92-60	PS C	Australia	DQ315448	–	–	DQ307529
C.P.K. 1011	PS C	Central Russia	–	–	–	DQ641674
C.P.K. 1010	PS C	Central Russia	–	–	–	DQ641673
G.J.S. 02-54	*H. vinosa*	New Zealand	DQ315447	–	–	DQ307528
G.J.S. 99-156	*H. vinosa*	U.S.A. (TX)	DQ315446	–	–	DQ307527
G.J.S. 99-158	*H. vinosa*	New Zealand	AY380904	AY376680	DQ122158	AY376047
DIS 217i	PS B	Ecuador	DQ323420	–	–	DQ307549
G.J.S. 04-353	PS B	U.S.A. (TN)	DQ323418	–	–	DQ307551
G.J.S. 90-97	PS B	U.S.A. (NC)	DQ315449	–	–	DQ307530
G.J.S. 99-127	PS A	Australia (VI)	DQ315453	–	–	DQ307533
G.J.S. 99-83	PS A	Australia (VI)	AF456921	DQ111966, DQ333557	DQ122159	AF348118
G.J.S. 99-191	PS A	Australia (VI)	DQ315451	–	–	DQ307532
G.J.S. 99-86	PS A	Australia (VI)	DQ315432	–	–	DQ307516
G.J.S. 99-204	PS A	New Zealand	DQ315450	–	–	DQ307531
371Tsp27Bnov = J. B. ID424	PS A	–	–	–	–	–
230T.spnov.278 = J. B. ID422	PS A	–	–	–	–	–
G.J.S. 98-170	*T. hamatum*	New Zealand	DQ109530	DQ111963	DQ122155	DQ109544
DAOM 167057	*T. hamatum* (ex-neotype)	Canada	Z48816	DQ111962	DQ122154	AF456911, AY750893
DAOM 166162	*T. hamatum*	U.S.A. (NC)	DQ083016	DQ111961	DQ122153	AY750887
G.J.S. 04-217	*T. asperellum*	Peru	DQ381957	DQ333564	DQ379002	DQ381958
Tr 3 = CBS 433.97	*T. asperellum*	U.S.A. (MD)	X93981	DQ111974	DQ122170	AY376058

[1]Abbreviations of culture collections and collectors: ATCC = American Type Culture Collection, Manassas, VA, U.S.A., BBA = Biologisches Bundesanstalt, Berlin, Germany; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; C.P.K. = Collection of C.P. Kubicek, Technische Universität, Abteilung für Mikrobielle Biochemie, Vienna; C.T.R., G.J.S., TR = Culture collection of the United States Department of Agriculture, Systematic Botany and Mycology Lab, Beltsville, MD U.S.A. (BPI); DAOM = Canadian Collection of Fungal Cultures, Ottawa, Canada; DIS refers to CABI-Bioscience, Ascot, cultures held by G.J.S; FP = unknown provenance, received from Toru Okuda, Nippon Roche Corp., Tokyo, Japan; IBT = Culture Collection of Fungi, Technical University of Denmark, Lyngby, Denmark; IMI = CABI-Bioscience, Egham, U.K.; J.B. = John Bissett, Agriculture and Agri-Food, Eastern Cereal and Oilseed Research Centre, Ottawa, Canada; W.J. = Culture collection of Walter Jaklitsch, Technische Universität, Abteilung für Mikrobielle Biochemie, Vienna; NR = Nippon Roche Corp. Tokyo, Japan.

[2]Collection of C.P. Kubicek, Technische Universität, Abteilung für Mikrobielle Biochemie, Vienna.

calcofluor (Sigma Fluorescent Brightener 28 C.I. 40622 Calcofluor white M2R in 2 molar phosphate buffer at pH 8.00) was used for FL.

Sections of *Hypocrea* stromata were prepared by rehydrating small blocks of substratum supporting stromata in 3 % KOH. The blocks were supported by Tissue Tek O.C.T. embedding medium 4583 (Miles, Inc., Elkhart, IN) and sectioned at about 15 µm on a Microtome-Cryostat (International Equipment Co., Needham Heights, MA). The sections were first floated in water and then placed on slides to make semi-permanent preparations following Volkmann-Kohlmeyer & Kohlmeyer (1996). Slides are deposited with the specimens.

Growth rate trials were performed in darkness on potato-dextrose agar (PDA, Difco or Sigma) and SNA following the procedure described by Samuels *et al.* (2002) with the addition that cultures were also grown at 25 ºC under 12 h darkness/12 h cool white fluorescent light for 96–120 h. Each growth-rate trial was repeated three times and the results of the three were averaged.

The slope of the growth curve, which reflects rate of growth per hour, is determined by linear regression. Regression is used to characterize the manner in which the colony radius changes (x's) with the time (y's) when measurements of colony radius are made. By revealing how the mean of the y measures changes as the various x measures change, the regression line is understood to describe the regression of y (colony radius) over x (time of measurement). This regression line is the slope of the growth curve; it is the predicted value of each colony radius for each time of measurement and essentially reports growth per hour (see http://www.animatedsoftware.com/statglos/sgregres.htm).

Principal Components Analysis (PCA), a multivariate analysis (Multivariate Statistical Package, version 1.131; Kovach Computing Services, U.K.), was utilized to determine patterns of variation of phenotype within phylogenetically defined groups. The eigenanalysis is shown in Table 2 and graphical output is shown in Fig. 4. The standardized data used in PCA, and other data analyses, were obtained using Systat version 10 (SPSS Inc., Chicago, IL, U.S.A.).

Dry cultures of *Trichoderma* species were prepared by placing all or part of a culture growing in 9-cm-diam Petri dish in a cardboard two-slide micro-slide holder (e.g. VWR Scientific, West Chester, PA, U.S.A.) and drying them for *ca.* 2 h over low heat of a fruit dryer. Dry cultures were prepared so as to preserve essential characters of conidiophore branching and phialides.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

The extraction of genomic DNA was performed as reported previously (Dodd *et al.* 2002).

The PCR for amplification of the internal transcribed spacers 1 and 2 of the rDNA gene cluster (ITS1 and 2 including the 5.8S RNA gene) was performed in a 50 µL reaction volume using 5 µL of 10 × PCR buffer (Applied Biosystems), 200 µM dNTPs, 25 pmole of each primer (ITS1 and ITS4), 1.25 units AmpliTaq Gold (Applied Biosystems), and about 10–50 ng of template DNA. The reaction mixture was placed in a 0.2 mL PCR tube. The PCR was carried out on a PT-200 PCR system (MJ Research, Waltham, MA, U.S.A.) according to the following protocol: initial activation of AmpliTaq Gold at 95 °C for 10 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min; and a final extension period at 72 °C for 10 min. Five µL of the PCR product was analyzed on 1 % agarose gel in TAE buffer. The positive PCR reactions were purified using the Qiagen QIAquick PCR purification kit (Qiagen, California, U.S.A.) following the manufacturer's instructions. The concentration of the PCR products in ng/µL was determined on 1 % agarose gel electrophoresis in TAE buffer with Lambda Hind III DNA as a marker.

Similarly, a portion of translation elongation factor 1 alpha (*tef1*) was amplified using the primers EF1-728F (Carbone & Kohn 1999) and TEF1 rev (Samuels *et al.* 2002), which resulted in a PCR product of approximately 600 bp, and was sequenced in both directions. The primers for amplification of the calmodulin-encoding gene (*cal*) were CAL-228F and CAL-737R (Carbone & Kohn 1999). Initially a fragment of actin gene (*act*) was amplified using the primers Fung.ACT.F1 and Fung.ACT.R1 and the conditions described by Wirsel *et al.* (2002). Based on the sequences obtained, two *Trichoderma*-specific act primers were designed T*act*1 (5'- TGGCACCACACCTTCTACAATGA) and T*act*2 (5'-TCTCCTTCTGCATACGGTCGGA). These two primers were used for amplification of *act* for all the isolates in this study. Additionally two sequencing primers for *act* were designed called T*act*500F (5'-ATTCCGTGCTCCTGAG) and T*act*511R (5'- CTCAGGAGCACGGAAT) and were used for sequencing reactions.

The portion of the RNA polymerase subunit B 2 (*rpb2*) gene was amplified and sequenced as described by Chaverri & Samuels (2004) using fRPB2-5F and fRPB2-7cR (Liu *et al.* 1999) as forward and reverse primers, respectively.

DNA sequences were obtained using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, California). Products were analyzed directly on a 3100 DNA sequencer (Applied Biosystems). Both strands were sequenced for each gene.

ANALYSIS OF SEQUENCE DATA

Sequences were edited and assembled using Sequencher 4.1 (Gene Codes, WI). Clustal X (Thompson *et al.* 1997) was used to align the sequences; the alignment of each locus was manually edited using MacClade and GeneDoc 2.6 (Nicholas & Nicholas 1997). The sequences were deposited in GenBank (Table 1) and alignments were deposited in TreeBase (http://herbaria.harvard.edu/treebase/), submission number SN 1008). The multiple sequence alignment file for the *tef1* locus is also available at http://www.isth.info/phylogeny/koningii.php.

The interleaved NEXUS file was formatted using PAUP* v. 4.0b10 (Sinauer Associates, Sunderland, MA) and manually formatted for the MrBayes v3.0B4 program. The Bayesian approach to phylogenetic

reconstructions (Rannala & Yang 2005) was implemented using MrBayes 3.0B4 (Huelsenbeck & Ronquist 2001). The MODELTEST3-06 package (http://bioag.byu.edu/zoology/crandall_lab/modeltest.htm) was used to compare the likelihood of different nested models of DNA substitution and select the best-fit model for the investigated data set. The modelblock3.nex which is compatible with the current version of PAUP* v. 4.0b10 was downloaded from http://workshop.molecularevolution.org/software/modeltest/files/modelblock3. Both hierarchical LRT and AIC output strategies were considered, although the preference was given to the last one. The unconstrained GTR + I + G substitution model was selected for all tree loci.

Metropolis-coupled Markov chain Monte Carlo (MCMCMC) sampling was performed with four incrementally heated chains with the default heating coefficient λ = 0.2, heats for cold chains 1 and heated chains 2, 3 and 4 are 1, 0.83, 0.71 and 0.63, respectively) that were simultaneously run for 5 million generations for the *tef1* alignment, which comprised more than 200 sequences. Alignments of the other two loci (*cal* and *act*), neither of which exceeded 100 sequences, were analysed using 3 million generations. To check for potentially poor mixing of MCMCMC, each analysis was repeated at least three times. The convergence of MCMCMC was monitored by examining the value of the marginal likelihood through generations. Convergence of substitution rate and rate heterogeneity model parameters were also checked. Bayesian posterior probabilities (PP) were obtained from the 50 % majority rule consensus of trees sampled every 100 generations after removing the first 2000 trees for *tef1* and the first 500 for *cal* and *act* using the "burn" command. According to the protocol of Leache & Reeder (2002), PP values lower then 0.95 were not considered significant, while values below 0.9 are not shown on phylograms and radial trees. Model parameter summaries after MCMC run and burning first samples were collected. For *tef1* mean substitution values were estimated as G↔T =1, C↔T = 3.33, C↔G = 1.14, A↔T = 1.32, A↔G = 5.98, A↔C = 1.43; nucleotide frequencies were estimated as 0.19(A), 0.28(C), 0.17(G), 0.36(T); alpha parameter of gamma distribution shape was 0.23. For *cal* mean substitution values were estimated as G↔T =1, C↔T = 4.43, C↔G = 0.83, A↔T = 1.15, A↔G = 3.55, A↔C = 1; nucleotide frequencies were estimated as 0.26(A), 0.26(C), 0.24(G), 0.24(T); alpha parameter of gamma distribution shape was 0.1. For *act* mean substitution values were estimated with a high affinity to pyrimidine transitions (C↔T = 81.9); other transitions were G↔T =1, C↔G = 0.3, A↔T = 0.85, A↔G = 0.83, A↔C = 0.61; nucleotide frequencies were estimated as 0.2(A), 0.3(C), 0.24(G), 0.26(T); alpha parameter of gamma distribution shape was 0.09. The genetic distance was computed in PAUP* v. 4.0b10 under the GTR + I model.

RESULTS

Our work leads us to recognize several species, many undescribed. In the following we have anticipated the formal taxonomy by adopting those names in order to facilitate the presentation of the results.

PHYLOGENETIC ANALYSES OF SEQUENCE DATA

The position of *T. koningii*-like species on the *Hypocrea/Trichoderma* genus phylogeny is shown on the radial tree obtained after the analysis of partial *rpb2* sequences (Fig. 1). This complex species takes the terminal position on the sect. *Trichoderma* branch, which consists of "Pachybasium A" and "Viride Clades" ("Rufa Clade" in Chaverri & Samuels 2004, and Druzhinina *et al.* 2005). It is interesting to note the relatively short genetic distances within clades and species on this branch. The neighbouring *H. voglmayrii*, which was recently described from the Austrian Alps (Jaklitsch *et al.* 2006a), or species from "Hypocreanum" and "Lutea Clades" are separated by longer evolutionary distances.

Visual inspection of ITS1 and 2 sequences of strains of the *T. koningii* complex show a very low degree of variability (max. 6 % of variable sites), corresponding to findings in other studies (Lübeck *et al*. 2004, Druzhinina *et al.* 2005). Therefore, this locus was not used in phylogenetic reconstructions. However, we were able to develop a species-specific oligonucleotide barcode from ITS sequences for some of these species. It was integrated into the upgraded version of *Trich*OKEY previously published by Druzhinina *et al.* (2005). The program allows the identification of four individual species with *T. koningii*-like morphology and one group of seven species (for details see below).

The high degree of similarity of teleomorphs and anamorphs within the *T. koningii* species complex led us to anticipate the higher level of sequence similarity of protein-encoding DNA sequences. Therefore we chose phylogenetic markers with relatively big introns such as (i) the partial sequence of the translation elongation factor 1-alpha (*tef1*) covering the fourth (large) and fifth (short) introns (Kopchinskiy *et al.* 2005), (ii) the partial actin (*act*) and (iii) the partial calmodulin (*cal*) genes with two introns each. Since *tef1* is the most variable locus (>50 % of variable sites) it was selected as a reference phylogenetic marker and, consequently, sequenced for all investigated strains. The *cal* and *act* genes were used to apply the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept of Taylor *et al.* (2000) to representatives of the main groups detected by the phylogenetic analysis of *tef1*.

In order to examine the phylogeny of *T. koningii*-like strains with respect to their position to the "Viride Clade" we aligned a portion of the *tef1* gene for a large number of isolates. First, we attempted to analyse *T. koningii*-like strains against a background of few representatives of the nearest clades such as *T. viride* VD and *T. viride* VB (data not shown). However, the log probability plotted against the number of up to 5 million generations did not reach a stationarity. This indicated a low reliability of

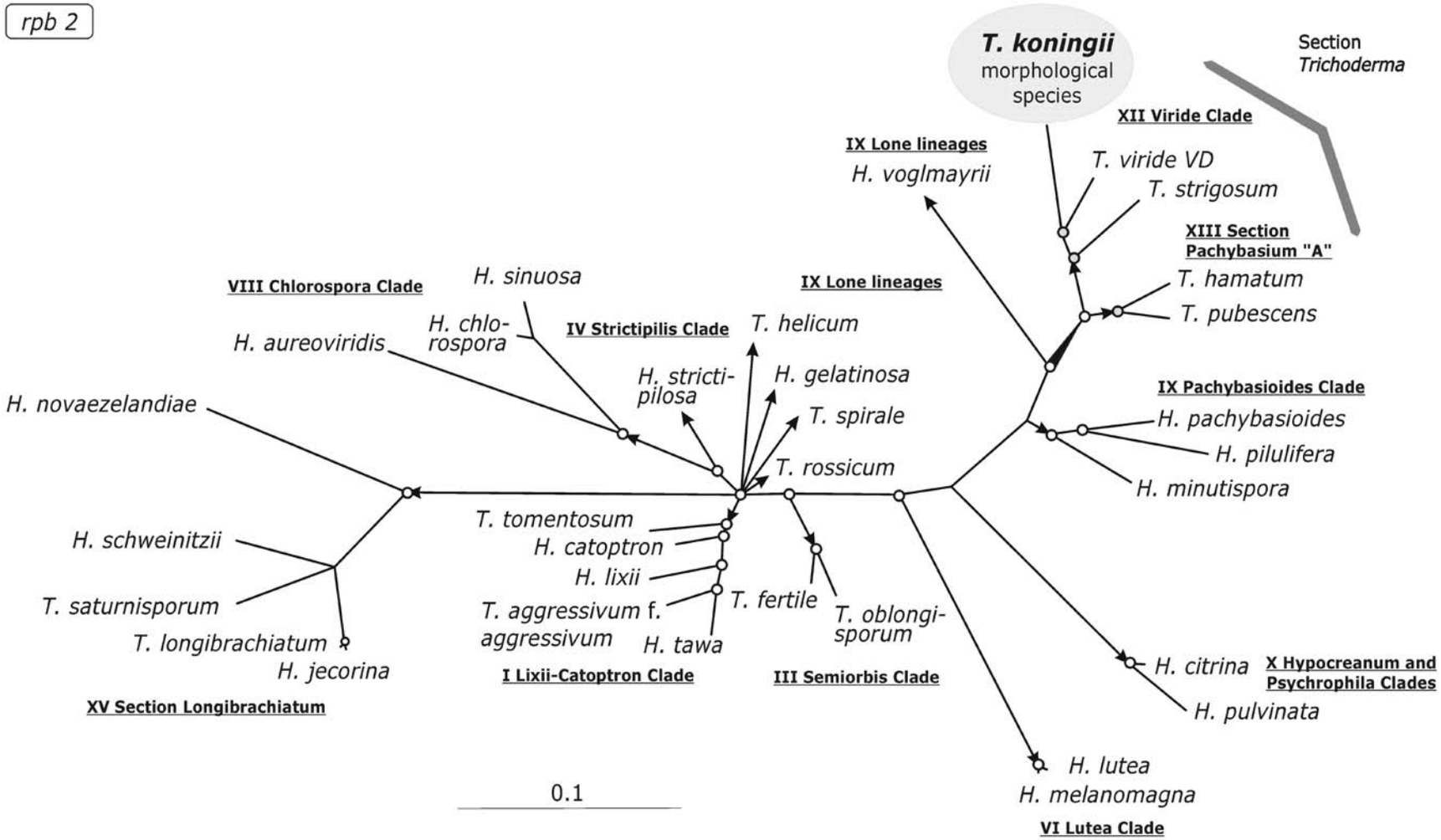


Fig. 1. Bayesian radial tree showing position of *T. koningii* aggregate species on *Hypocrea/Trichoderma* phylogeny based on partial *rpb2* sequences. Arrows indicate branches leading to currently recognized clades within the genus in sense of Chaverri & Samuels (2003), Druzhinina *et al*. (2005) and the present work. Circles at nodes indicate the posterior probability coefficients higher 0.95 as they were obtained after 3 million generations. All sequences except *T. koningii* CBS 979.70 DQ641671 and *H. novaezelandiae* G.J.S. 81-264 DQ 641672 were retrieved from NCBI GenBank as follows: *H. voglmayrii* CBS 117710 DQ086151; *T. viride* VD G.J.S. 89-127 AF545521; *T. pubescens* CBS 345.93 AF545552; *T. hamatum* CBS 102160 AF545548; *T. strigosum* CBS 348.93 AF545556; *H. minutispora* CBS 901.72 AY481588; *H. pachybasioides* CBS 820.68 DQ087238; *H. pilulifera* CBS 814.68 AF545519; *H. citrina* CBS 894.85 AF545561; *H. pulvinata* G.J.S. 98-104 AF545559; *H. melanomagna* CBS 114236 AY391926; *H. lutea* G.J.S. 89-129 AF545517; *T. oblongisporum* CBS 344.98 AF545551; *T. fertile* CBS 339.93 AF545546; *H. chlorospora* CBS 114231 AY391903; *H. sinuosa* CBS 114247 AY391942; *H. aureoviridis* CBS 245.63 AF545509; *H. strictipilosa* G.J.S 98-91 AF545538; *T. spirale* CBS 346.93 AF545553; *T. aggressivum* CBS 100525 AF545541; *H. tawa* CBS 114233 AY391956; *H. lixii* CBS 226.95 AF545549; *H. catoptron* CBS 114232 AY391900; *T. tomentosum* DAOM 178713a AF545557; *H. gelatinosa* CBS 114246 AY391924; *T. helicum* DAOM 230021 DQ087239; *T. rossicum* TUB F-718 DQ087240; *H. jecorina* TUB F-430 DQ087241; *T. longibrachiatum* CBS 816.68 DQ087242; *T. saturnisporum* CBS 330.70 DQ087243; *H. schweinitzii* G.J.S. 01-364 AF545565.

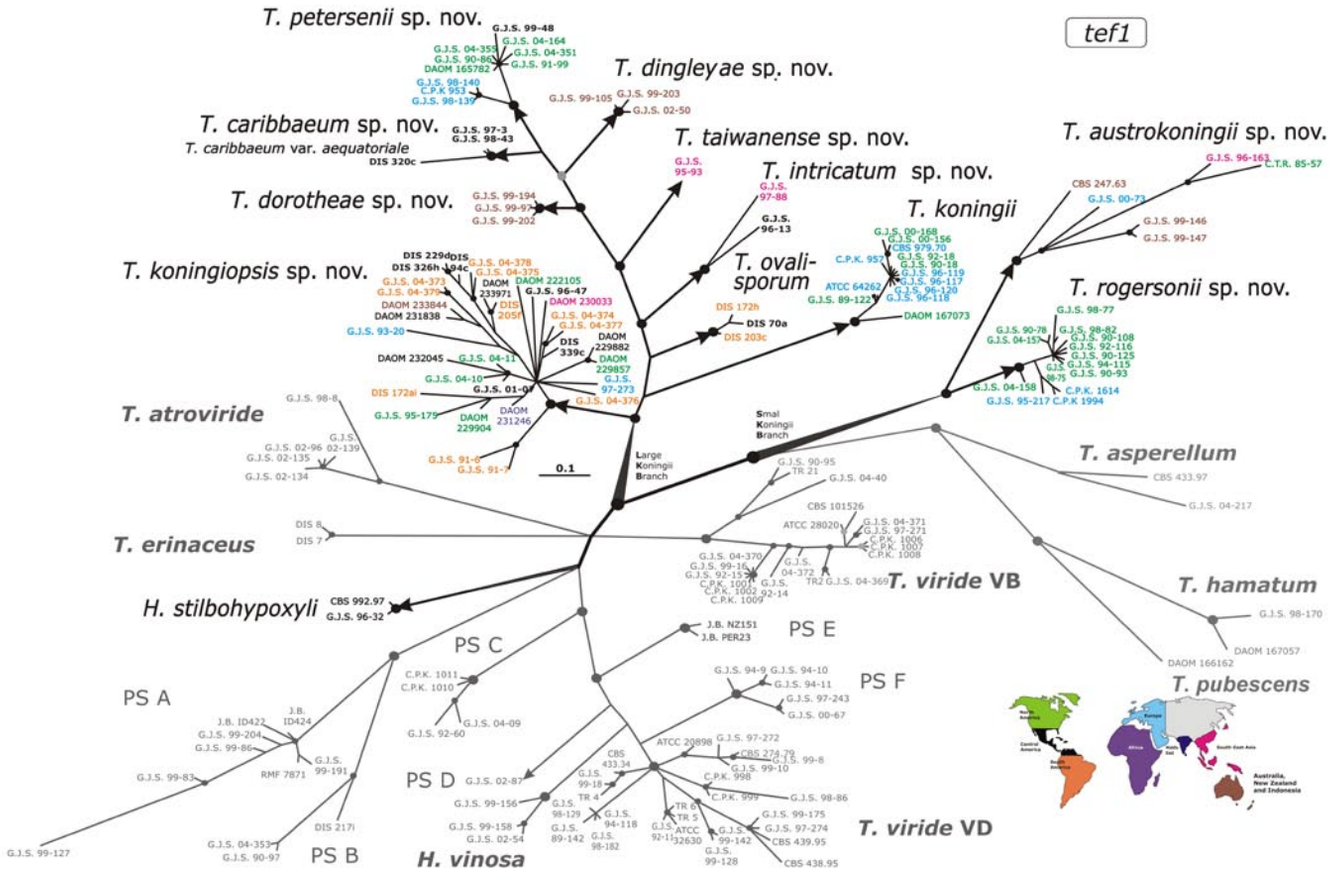


Fig. 2. Bayesian radial phylogram showing the structure of the *'Viride'* Clade as it was inferred based on sequences of two introns of *tef1*. Grey colour is used to separate specimens which are not discussed in this study but whose sequences were used to produce the multiple sequence alignment. Arrows indicate branches leading to species recognized within *T. koningii* aggregate species. In the highlighted part of the tree, grey filled circles at nodes indicate posterior probability coefficients higher than 0.90 as they were obtained after 5 million generations; black filled circles at nodes show support higher than 0.95. Font colours correspond to regions of sampling on the schematic map. Clades identified as "PS A–F" in the lower half of the tree represent undescribed phylogenetic species (see Table 1).

the resulting tree. Moreover, trees obtained in different runs with equal priors showed inconsistent topologies and were poorly resolved. Our strategy to solve this obstacle was based on the inclusion of the maximum known variability within the "Viride Clade" in the multiple sequence alignment, including *T. viride* VB, *T. viride* VD, *H. stilbohypoxyli*, *T. erinaceus*, *T. atroviride* and several potentially new taxa. The repeated consecutive use of intermediate phylogenetic analyses, rearrangements of sequences in MSA and realignments, particularly of the highly variable forth (large) intron of *tef1*, made it possible to produce the most correct final MSA file (available at www.isth.info/phylogeny/koningii). In this file, sequences of the *T. koningii* and *T. viride* complexes were aligned to representatives of *T. asperellum* and *T. hamatum* as members of the next neighbouring phylogenetic clade. As expected, likelihood estimations reached stationarity over generations, indicating reproducibility of the MCMC analyses. Fig. 2 represents a radial Bayesian phylogenetic tree obtained after 5 million chain generations.

Analyses of *cal* and *act* sequences did not produce problems during repeated MCMC runs; results are shown in Fig. 3.

All three trees show clear separation with high statistical support between fungi of the "Viride Clade" and species from "Pachybasium A" (Kullnig-Gradinger *et al.* 2002), of which the latter was represented by *T. asperellum* and *T. hamatum*. The monophyletic origin of the entire "Viride Clade" was confirmed by phylogenetic analyses of all three loci. As may be seen in the *tef1* radial tree (Fig. 2), the majority of strains with a *T. koningii* morphology appear on a single proliferating branch, which is well-separated from other large species aggregates such as *T. viride* VB, *T. viride* VD, *T. atroviride*, *H. stilbohypoxyli* and *T. erinaceus*. The same tree topology is supported by both *cal* and *act* trees. This lineage was named "Large Koningii Branch"(**LKB**) (Figs 2–3).

In the *tef1* tree, the most basal position of the LKB is occupied by the highly supported multifurcating clade of *T. koningiopsis*. *Trichoderma koningiopsis* presents a genetically variable species because only few internal nodes within that species are well-supported and are of numerous long intraspecific genetic distances. Although strains of *T. koningiopsis* have the same position on the LKB on the trees of Fig. 3, its identity as a distinct monophyletic clade is not statistically supported in

Fig. 3. (Page 77). The concordance between two Bayesian phylograms as inferred based on partial *act* and *cal* gene sequences. Black circles at nodes indicate the posterior probability coefficients higher than 0.95 as they were obtained after 3 million generations. Grey circles in the cal tree indicate differences in topology when compared to the same isolates in the act tree. Taxon "PS A" indicates an undescribed phylogenetic species (see Table 1).


act
cal
T. petersenii (1)
T. dorotheae (2)
T. caribbaeum (3)
T. taiwanense (4)
T. dingleyae (5)
T. ovalisporum (6)
T. intricatum (7)
T. koningii (8)
T. koningiopsis (9)
T. rogersonii (10)
T. austrokoningii (11)
Large Koningii Branch
Small Koningii Branch
T. viride VB
T. erinaceus
H. stilbohypoxyli
H. vinosa
PS A
T. asperellum
T. hamatum
0.1

analyses of the *cal* and *act* genes. These findings could indicate the presence of a relatively intensive recombination process due to sexual reproduction, despite the fact that the vast majority of the several strains of *T. koningiopsis* studied were derived directly from substrata with no known teleomorph.

In the LKB of the *tef1* tree, *T. ovalisporum* and *T. koningii s. str*. possess two equally supported clades. The insignificant support of nodes where both of these species diverge from the main stem suggests approximately simultaneous speciation even for both taxa. However, the divergence was clearly allopatric, because *T. koningii* is common in North America and Europe, while *T. ovalisporum* is an endophyte from South America. The phylogenetic position of these two species is not contradicted by *cal* or *act* trees, although these genes did not always provide significant support. Compared to other species such as *T. koningiopsis*, *T. koningii s. str.* appears to be a relatively homogeneous taxon represented by strains from North America and Europe. *Trichoderma koningii* strains have almost identical sequences in the hypervariable large intron of *tef1*. Only one strain (DAOM 167073 from Québec) appeared distinct from numerous other strains, which had very similar or identical sequences, irrespective of their broad geographic distribution.

The upper part of the LKB on all three trees has a stepped structure with well-supported internal nodes (except the *cal* tree). Based on the concordance between the three loci trees, it consists of at least six phylogenetic species. In general, their phylogeny may be attributed to allopatric speciation because *T. taiwanense* is Asian, both *T. dorotheae* and *T. dingleyae* are isolated, known only from Australia and New Zealand, while strains of *T. caribbaeum* var. *aequatoriale* have a South American origin. The terminal position is occupied by *T. petersenii*. It consists of strictly North American and European clades. *Trichoderma intricatum,* which is located basal to the species listed above, may be an exception because it is represented by one Asian and one Caribbean strain.

Results of phylogenetic analysis show that additional species having *T. koningii*-like morphology have evolved independently from the taxa of the LKB. The majority of these species appear on the “Small Koningii Branch” (**SKB**), which is segregated from the LKB by taxa of the “Viride Clade” (Figs 2–3). The first species on the SKB is *T. rogersonii,* which is represented by mainly North American and a few European strains. The terminal part of the SKB (*tef1* tree) consists of a number of long lineages that lead to geographically separated strains. The divergence among these strains may be explained by allopatric speciation. Three of these six strains originated from Australia and New Zealand, one from Europe, one from the U.S.A., and one from Taiwan. Based on both *tef1* and *cal* loci, Taiwan and North American strains form the most terminal well-supported clade, although on the *act* tree this clade also includes a European strain. Thus, there is no concordance between topologies of the *act* tree and trees inferred from sequences of the other two loci. This finding makes it difficult to draw conclusions about phylogenetic species on the terminal part of the SKB.

The third lineage that is characterized by the *koningii* morphology is represented by the single species *T. stilbohypoxyli.*

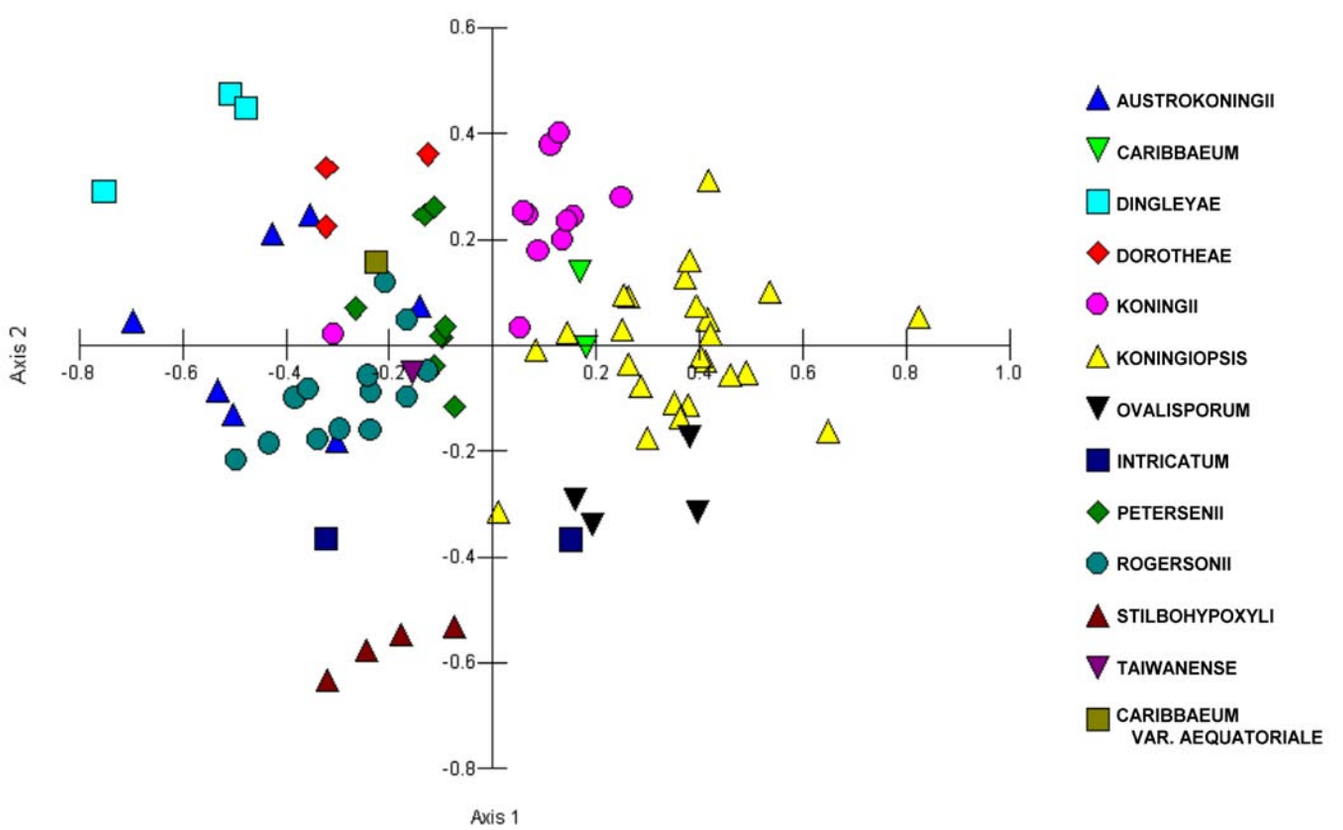


Fig. 4. Principal Components Analysis, scatter plot of Eigen vectors. See Table 2 for statistics.

PHENOTYPE: ANAMORPH

For summary of continuous characters see Table 3.

A total of eighty-six strains were studied. Typical of *Trichoderma*, very little aerial mycelium forms on CMD or SNA, and mycelial production on PDA is typically lush. There is variation among the strains of individual clades as to whether conidiophores form in aerial mycelium or in complex cottony pustules on CMD as well as in relative amounts of conidial production. Conidial production on CMD and SNA tends to occur at the margin of the colony. Discrete conidial pustules sometimes form on CMD and SNA, but on PDA pustules are not formed, rather, conidia form in dense, effused areas. On CMD and SNA pustules are at most 1.5 mm diam and usually smaller, hemispherical, uniformly cottony. Entirely fertile, somewhat plumose conidiophores can often be seen within pustules (e.g. Figs 176, 197). Usually projecting sterile hairs or conidiophores that are only fertile at the apex are absent, but occasionally long, apically fertile conidiophores are seen in *T. austrokoningii* (Figs 55, 97), *T. dingleyae* (Fig. 138), and *T. koningiopsis* (Fig. 212). Conidiophores also form in the aerial mycelium. One isolate of *Trichoderma dorotheae* (G.J.S. 99-202) formed hemispherical pustules in addition to conidiophores in the aerial mycelium. Conidiophores in the pustules were not easily discerned (Figs 156, 161). In older cultures of this species phialides appeared to proliferate percurrently to form a second phialide (Fig. 158). The newly formed phialides were often abruptly swollen in the middle. This aspect is also seen in *Eidamia viridescens* A.S. Horne & H.S. Williamson (1923), which is *T. viride* VD of recent publications (e.g. Lieckfeldt *et al.* 1999, Dodd *et al.* 2003, Holmes *et al.* 2004) and distinct from "true"*T. viride* (VB in Figs 2–3). Pustules generally are compact, formed of intertwined hyphae that tend to branch dichotomously near the surface and to produce short branches that sometimes act as phialides, or for the cells near the surface of the pustules to swell and produce two or more short cells. In addition, verticillium-like conidiophores arise from near the surface of the pustules. Conidia are dark green (27E–F7–8). There is variation in the time and temperature at which conidia appear. Most of the isolates of *T. dingleyae* and *T. dorotheae* lost their ability to produce conidia after storage on cornmeal agar slants at *ca.* 8 °C.

None of the isolates produces sterile hairs, although, as noted above, occasionally long conidiophores that are fertile only at the tip form in some cultures of some strains, as is described for *Trichoderma* sect. *Pachybasium* (Bissett 1991b). Often individual branched conidiophores can be seen within pustules when viewed with the stereo microscope. Conidiophores reaching the surface of pustules in members of all clades form a discernable major axis, from which primary lateral branches arise. Primary branches arise at or near 90° with respect to the main axis often singly but also often they arise in pairs or three at a node, with the members at a single node equal in length and progressively longer with distance from the tip of the main axis. Primary (*1°*) branches rebranch to form secondary (*2°*) branches. *2°* branches follow the same pattern of branching as the *1°* branches with longer side branches closer to the main axis and short branches more distal. Phialides arise singly, directly from the main axis near its tip and the *1°* branches; they also terminate *1°* and *2°* branches in whorls of 3 or 4. *1°* and *2°* branches, respectively, of conidiophores reaching the surface of the pustule tend to be widely spaced from each other. Branches arising from conidiophores found in the interior of the pustule tend to be crowded, with short internodal distances, and phialides tend to be held in dense heads of several.

Conidiophores of *T. taiwanense* were unusual in often being conspicuously enlarged and verrucose at the base (Figs 290–293).

Phialides were nearly cylindrical, only slightly swollen in the middle, when formed on widely spaced branches and shorter and conspicuously swollen in the middle when crowded. Often phialides were densely clustered with a very short internode between phialides (e.g. *T. koningii* Fig. 201, *T. koningiopsis* Fig. 214), we have termed these dense clusters pseudowhorls. Within any culture there can be considerable variation in the size and shape of phialides but there was no difference among the clades in the degree of variation in any of the continuous attributes of the phialides, the mean variation of phialide length for all collections being 6.1 ± 1.8 µm. The mean continuous measurements for phialides in the 86 strains studied was 7.9 ± 1.0 µm long, 3.0 ± 0.3 µm at the widest point, 2.0 ± 0.2 µm at the base, L/W = 2.7 ± 0.6. The cell from which phialides arise was 2.8 ± 0.4 µm and the ratio of the phialide length to the cell from which it arose was 2.8 ± 0.6. Mean phialide length in most collections ranged ≤7.5–8.5 µm. In two species, *T. dingleyae* and *T. caribbaeum* var. *aequatoriale*, mean phialide length was 9.5–10 µm and in *T. intricatum* and *T. stilbohypoxyli* mean phialide length was *ca.* 7.0 µm.

Conidia of all collections included in this study were oblong to ellipsoidal or ovoidal and smooth; the mean length was 4.0 µm, the mean width was 2.7 µm, and the mean L/W was 1.4. The mean length of conidia in most collections ranged 3.8–4.0 µm; conidia in collections of *T. dingleyae* and *T. koningii* were longest, 4.1–4.3 µm, while the mean length of conidia of *T. austrokoningii*, *T. ovalisporum*, *T. intricatum* and *T. stilbohypoxyli* ranged 3.4–3.5 µm. The mean width of conidia of most collections ranged 2.7–3.0; conidia of *T. dingleyae*, *T. dorotheae*, *T. intricatum* and *T. ovalisporum* were somewhat wider, the mean ranging 3.0–3.2 µm, while conidia of collections of *T. koningii*, *T. koningiopsis* and *T. stilbohypoxyli* were somewhat narrower, ranging 2.6–2.7 µm. The mean length/width ratio of most collections ranged 1.4–1.5; the mean L/W ratio of conidia in collections of *T. koningii* was 1.6 and in *T. ovalisporum* and *T. intricatum*, which have broadly ellipsoidal to ovoidal conidia, the mean L/W was *ca.* 1.2.

Chlamydospores are produced sporadically by members of the various species and of the clades, only the two collections of *T. intricatum* failed to produce chlamydospores. Chlamydospores are typical of *Trichoderma*, being terminal or intercalary within hyphae, and globose to subglobose.

PHENOTYPE: TELEOMORPH

Most of the strains that we studied were derived from ascospores of *Hypocrea* specimens. The most notable exception was *T. koningiopsis*, a common tropical species that was most often encountered as direct isolations from substrata, including as an endophyte from trunks of trees of *Theobroma cacao* and *Th. gileri*, and only a few isolates were derived from ascospores. *Trichoderma koningii s. str*. was most often directly isolated from substrata but three cultures were derived from ascospores of *Hypocrea* collections made in the United States and one from the Netherlands. *Trichoderma ovalisporum* is known only from four isolations, all from natural substrata. *Trichoderma caribbaeum* and its variety *aequatoriale* (DIS 320c) are represented by three strains; of these, two were isolated from ascospores of *Hypocrea* specimens collected in, respectively, Guadeloupe (G.J.S. 97-3) and Puerto Rico (G.J.S. 98-43), where they were growing on fructifications of black ascomycetes; the variety (DIS 320c) was isolated from the trunk of a live tree of *Theobroma gileri* in Ecuador and may be an endophyte. Despite strong phylogenetic similarity between DIS 320c, on one hand, and G.J.S. 97-3/ G.J.S. 98-43 on the other, DIS 320c is phenotypically quite different from the other two and we regard it as a variety of *T. caribbaeum*.

Stromata (when dry, Figs 24–50) were typically 6C–D8, brownish orange to light brown, but in *T. petersenii* stromata are darker, 7–8E–F8, reddish brown; stromata were typically pulvinate and broadly attached or at most slightly free at the margins. Perithecial elevations, or mounds, were not visible; the stroma surface was plane or wrinkled. Ostiolar openings were not visible, at least in dry specimens, or were barely visible as viscid circular areolae or dots on the stroma surface. There was no reaction to KOH in any tissue. When young, stromata were semi-effused, light brown or tan and villose; the developing stroma retained the villose aspect, which eventually was lost. The villose aspect is the result of short hairs that arise from the cells of the surface of the stroma; these are 5–10 µm long, 2.5–3.5 µm wide, septate, unbranched, often spinulose. The stroma surface, seen in face view, appeared mottled with unevenly deposited brown pigment in the cell walls. The cells at the surface of the stroma, when seen in face view, were angular, 3–7 µm diam, with walls slightly thickened. The stroma comprised three anatomically distinct regions. The surface region was 15–25(–35) µm thick and pigmented, in section appearing yellow when mounted in lactic acid. Cells of the surface region were angular, 2.5–5 µm diam, with slightly thickened walls. The tissue immediately below the stroma surface consisted of compact to loosely disposed hyphae. The tissue below the perithecia was pseudoparenchymatous, the cells measured 5–10(–15) × 3–7(–10) µm; their walls were slightly thickened or not visibly thickened; cells were oriented perpedicular to the surface of the substratum. The stromata of *T. taiwanense* (G.J.S. 95-93) are atypical in the group because they are luteous, lack hairs and have conspicuous ostiola; however this specimen is old and possibly has lost the traits that are typical of this group.

Perithecia were elliptic in section, 160–280 µm tall, 100–185 µm wide, ostiolar canal 53–90 µm long, cells of the perithecial apex not sharply differentiated from the cells of the surrounding stroma.

Asci were cylindrical, 60–70 × 4–5.7 µm, completely filled with ascospores; there was a thickening at the tip of each ascus. Ascospores were bicellular; they disarticulated at the septum into two part-ascospores early in development.

There was little variation in ascospore morphology or measurements among the 46 teleomorph collections that were studied. Differences are noted as follows: Part-ascospores were hyaline, spinulose, dimorphic; distal parts ranging (3.0–)3.5–4.0(–4.5) × (2.0–)3.1–3.8(–4.0) µm; proximal parts ranging (2.7–)3.7–4.5(–5.0) × (2.2–)2.7–3.2(–3.7) µm. The means of distal part-ascospores of most collections ranged 3.6–4.0 × 3.2–3.7 µm and of proximal part-ascospores 4.0–4.6 × 2.8–3.2 µm. The ascospores of *H. intricata* were somewhat smaller overall than in the other species. The distal part-ascospores of *H. intricata* were somewhat shorter and narrower than in the other species, *ca.* 3.3 × 3.1 µm. The proximal part-ascospores of *H. intricata* were also smaller, falling in the lower end of the range of spore dimensions overall. The distal part-ascospores of *H. koningii* were somewhat longer than most species (mean 4.1 µm) while those of *H. petersenii* were somewhat wider than in most species (mean = *ca.* 3.3 µm).

PHENOTYPE: COLONY MORPHOLOGY AND GROWTH RATE

Colony morphology is described from PDA at 25 and 30 °C in light or darkness after 72–96 h. Colony morphology is more or less consistent within a species. The cultures illustrated in Figs 6–23 are representative of the respective species. There is a tendency for conidia to form in concentric rings that are more or less pronounced; this is especially clear in *T. petersenii*. With the exception of *T. dingleyae* and *T. dorotheae*, conidia tended to form in abundance and to be dark green; conidial production in these two species is poor.

A summary of growth rate curves is shown in Fig. 5. In general, these are rather slow-growing species of *Trichoderma*.The colony radius is typically less than 50 mm and none reaching a colony radius of 70 mm on PDA, and most less than 40 mm on SNA, when grown for 72 h at optimum temperature of 25–30 °C in darkness (Fig. 5). The temperature optimum for most species is 25–30 °C; the temperature optimum for DIS 203c (*T. ovalisporum)* and *T. dingleyae* is lower, 20–25 °C. There is little (radius typically < 5 mm) or no growth at 35 °C for any of the species. On SNA, only *T. caribbaeum*, *T. koningiopsis* and *T. ovalisporum* reach a radius of 40 mm at the optimum temperature; the rest of the species reach a radius of 20–30 mm. On PDA after 72 h darkness, the mean colony radius of most species was < 50 mm; the radius of *T. caribbaeum* var. *caribbaeum* and *T. ovalisporum* was 55–60 mm and the mean radius of *T. koningiopsis* was 60–65 mm. Most

species grow faster at 30 °C than at 20 °C. However, *T. dingleyae* grew very poorly at 30 °C on both PDA and SNA (radius < 5 mm after 72 h in darkness), whereas at 20 °C colony radius was *ca.* 20–25 mm, and *T. caribbaeum* var. *aequatoriale* grew considerably more slowly at 30 °C (10 mm) than at 20 °C (35 mm).

Green conidia were first observed in PDA cultures of most species within 48–72 h on PDA at 25–30 °C, although individual isolates of a species varied in this regard. In *T. caribbaeum* var. *caribbaeum, T. ovalisporum* and *T. dorotheae* conidia of most isolates were first observed at 20 °C within 48–72 h. All but a few isolates of all species, except *T. caribbaeum* var. *aequatoriale* and *T. dingleyae*, formed conidia within 96 h. There was a correlation between species and temperature and time of the first appearance of green conidia, with the exception of *T. austrokoningii,* in which conidia overall appeared after 72 h; but there was considerable variation among the isolates as to the time of first appearance of conidia, which ranged from 48–96 h. In *T. dorotheae*, *T. ovalisporum,* and *T. petersenii* first green conidia were seen beginning after 48 h at 20 °C.

No distinctive odour was detected in any cultures, or rarely a coconut odour in *T. koningii*.

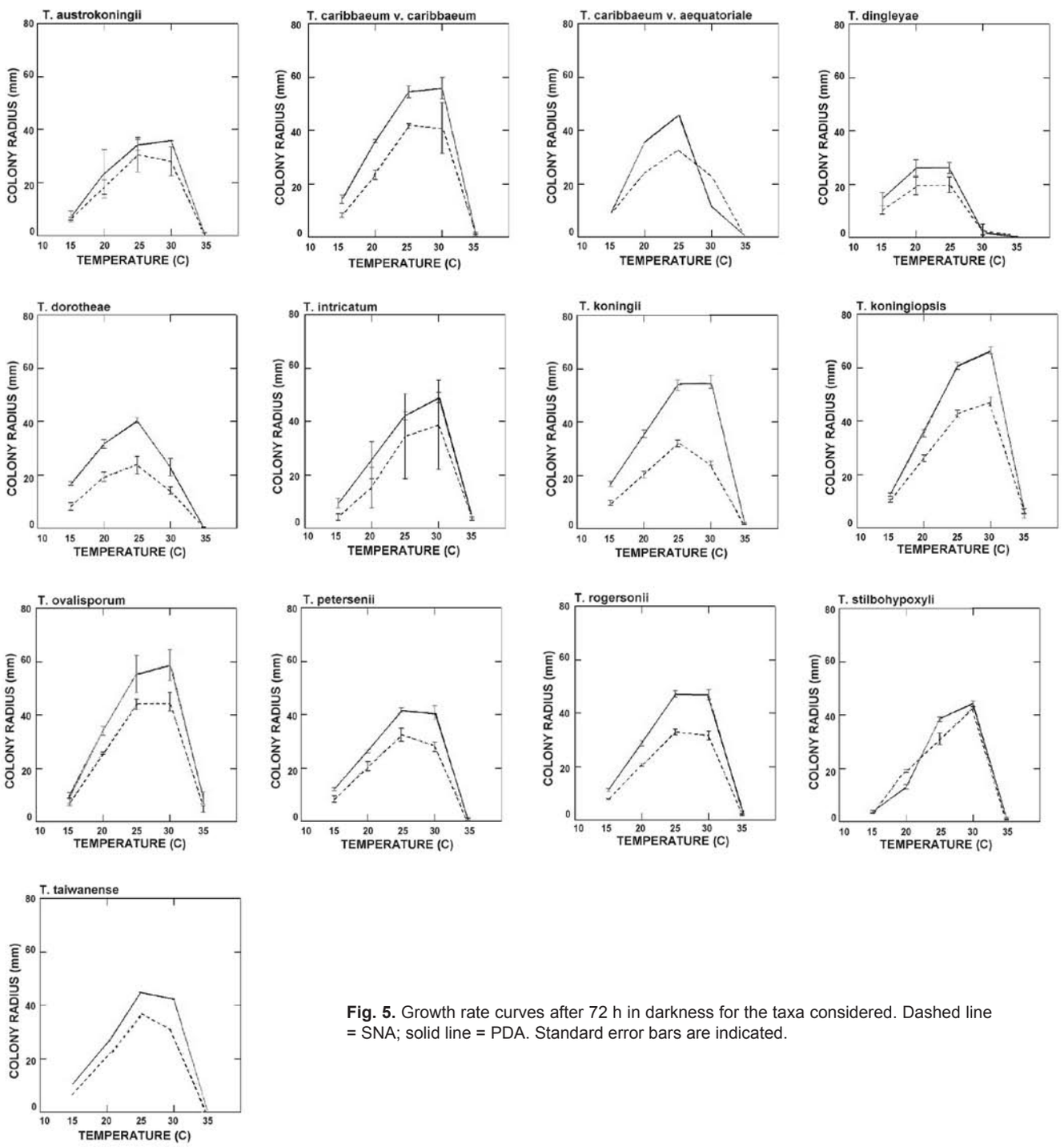


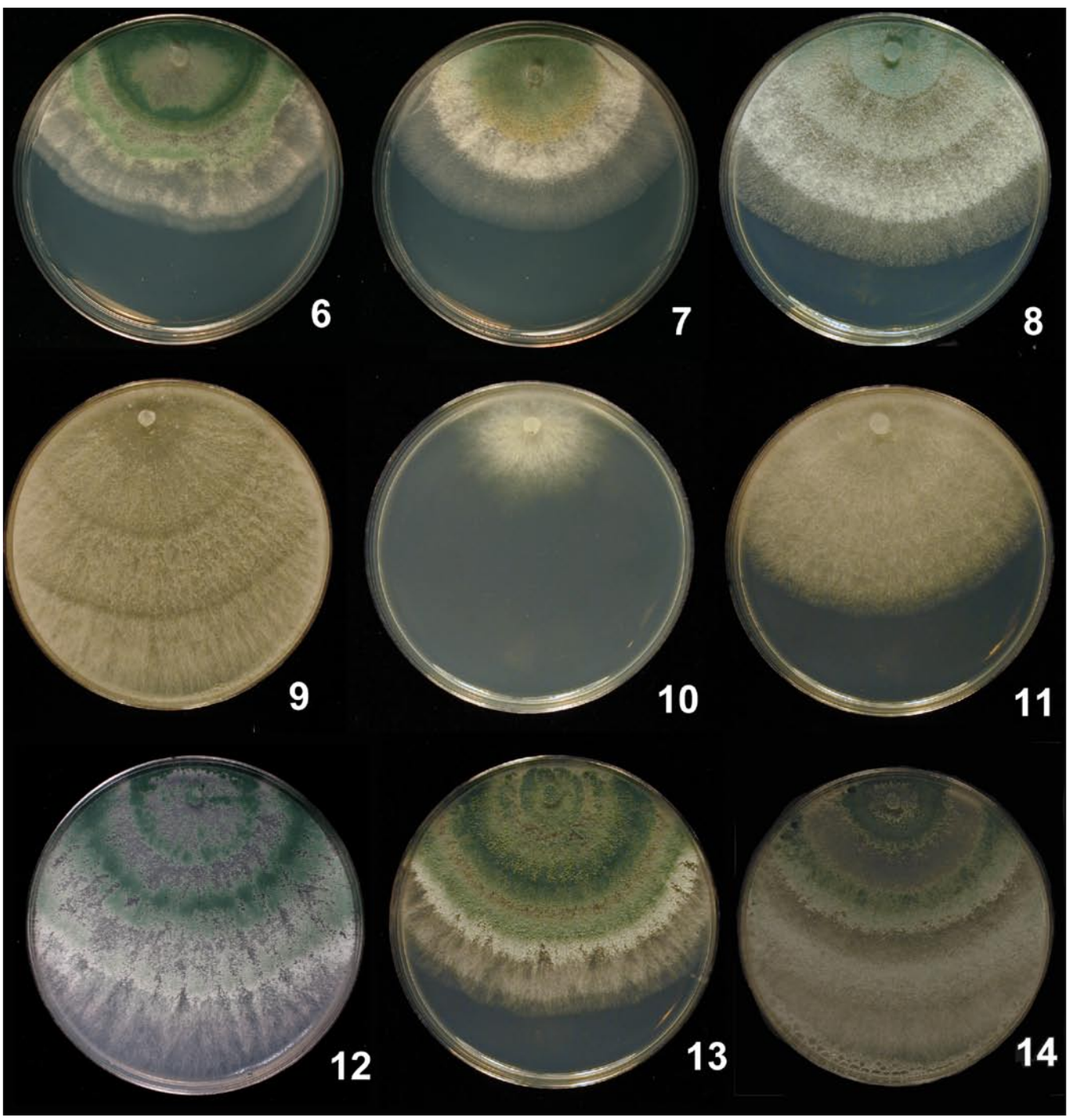
Fig. 5. Growth rate curves after 72 h in darkness for the taxa considered. Dashed line = SNA; solid line = PDA. Standard error bars are indicated.

BIOGEOGRAPHY

With few exceptions, a biogeographic bias was seen in the respective clades (Fig. 2, Table 3). The many reports in the literature of wide distribution not withstanding, distribution of *T. koningii* is limited to eastern North America and Europe. *Trichoderma koningiopsis* is a common and cosmopolitan species, but it is more common at tropical than at temperate latitudes.

Most of our isolates originated in the American tropics, but the species occurs in Canada (Ontario) and Germany, and its teleomorph has been found in the U.S.A. (Kentucky). It was also found in the rhizosphere of *Coffea arabica* from the main coffee-growing area in Ethiopia, where sampling was done from elevations of 1300–2000 m (T. Belayneh, pers. comm.). *Trichoderma stilbohypoxyli* was also revealed in this work to be a common tropical species, being widespread in tropical America and found in one location in Ghana, but it also occurs in the U.K.

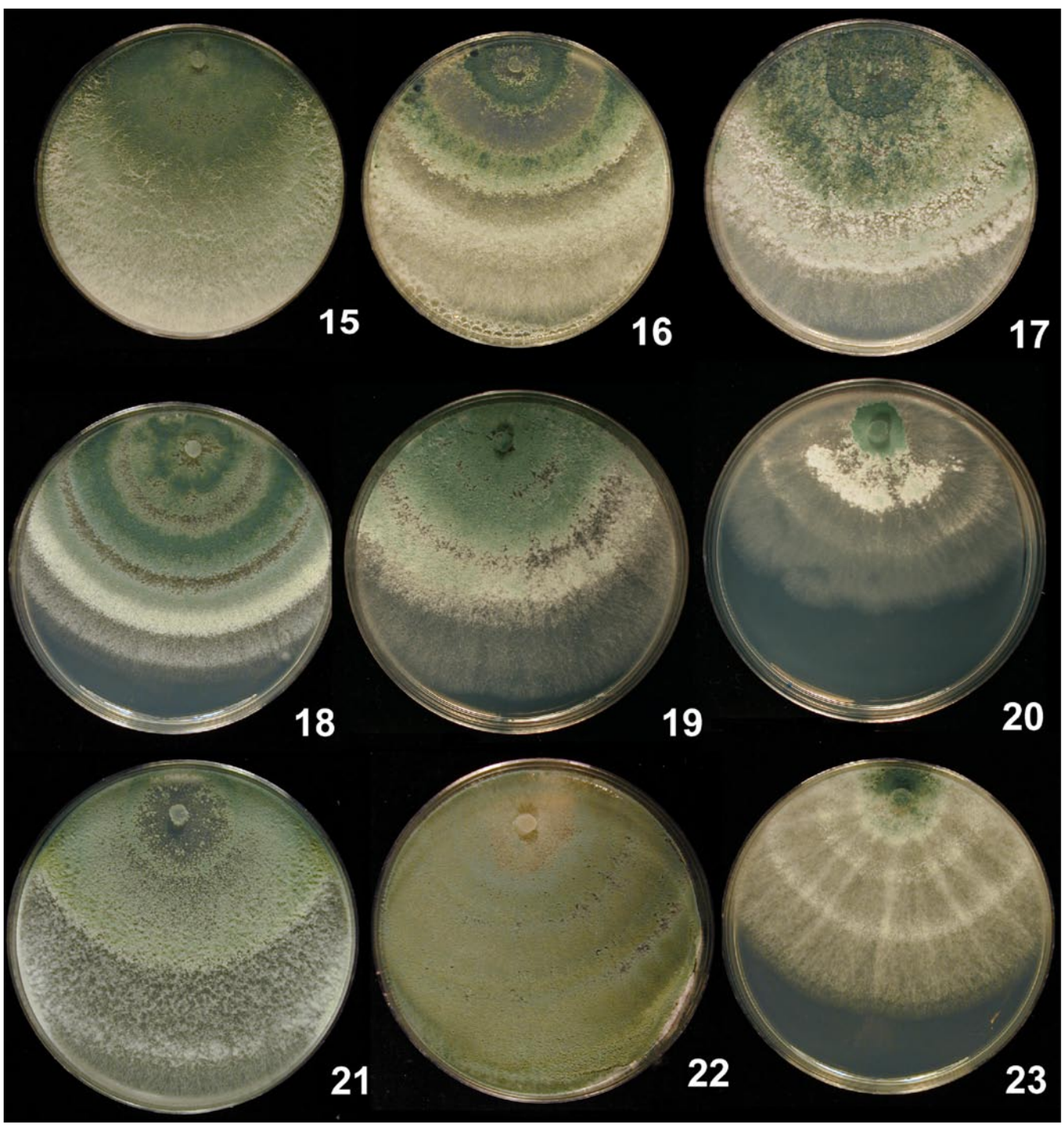
Trichoderma petersenii and *T. rogersonii* are common and sympatric in eastern North America and central Europe; we have only seen *T. rogersonii* as isolations from ascospores but we have a single



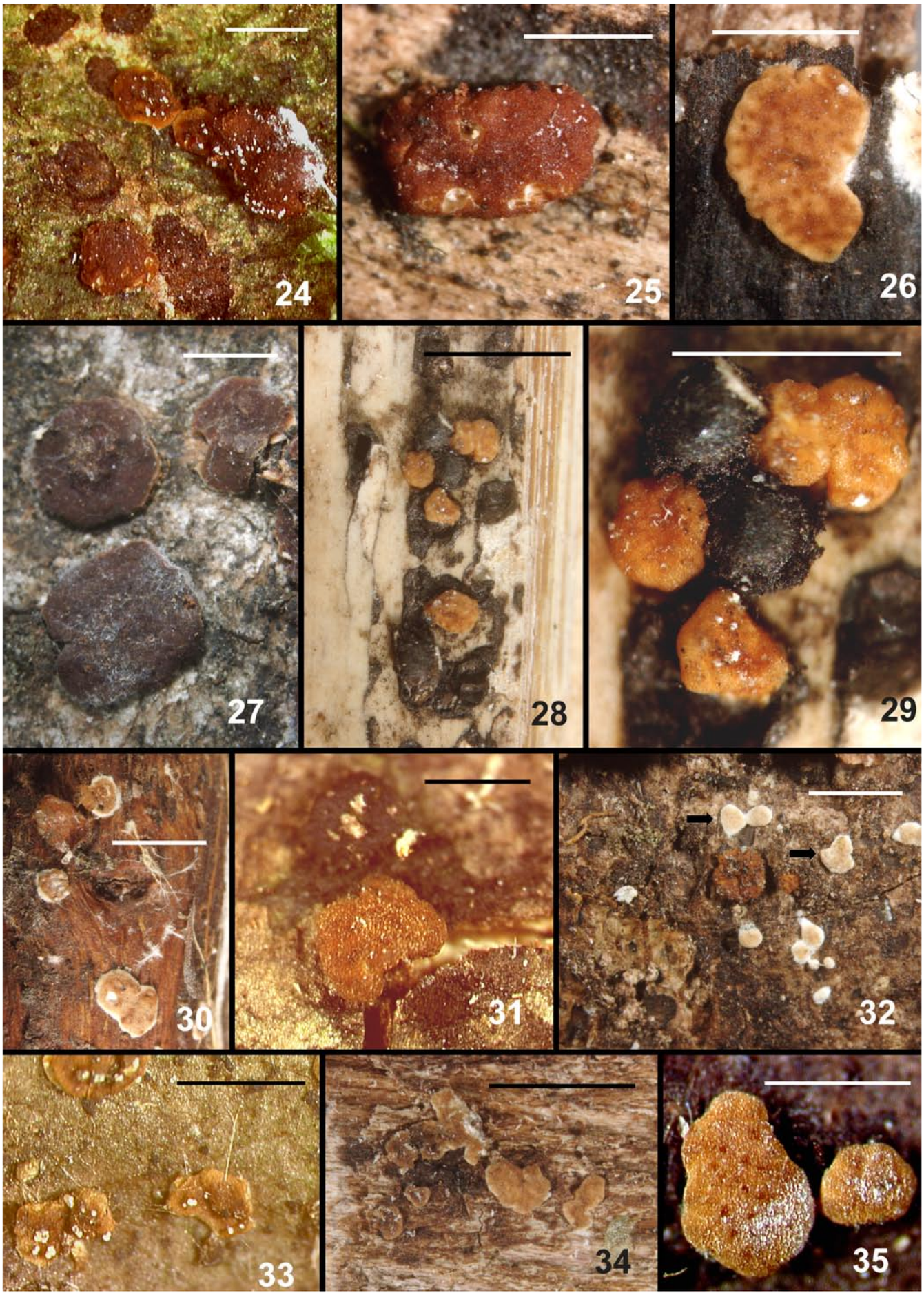
Figs 6–14. *Trichoderma cultures* grown on PDA in 9-cm-diam Petri dishes for 96 h at 25 °C under 12 h darkness/12 h cool white fluorescent light. 6: *T. austrokoningii* G.J.S. 99-146. 7. *T. austrokoningii* (New Zealand) G.J.S. 99-116. 8. *T. austrokoningii* (Russia) G.J.S. 00-73. 9. *T. caribbaeum* var. *caribbaeum* G.J.S. 97-3. 10. *T. dingleyae* G.J.S. 99-105. 11. *T. dorotheae* G.J.S. 99-97. 12–13. *T. intricatum* (12: G.J.S. 96-13, 13: G.J.S. 97-88). 14. *T. koningii* G.J.S. 96-119.

soil isolate of *T. petersenii*; stromata of *T. petersenii* have been collected also in Costa Rica. *Trichoderma intricatum* is known only from two ascospore-derived cultures that originate, respectively, in Puerto Rico and Thailand.

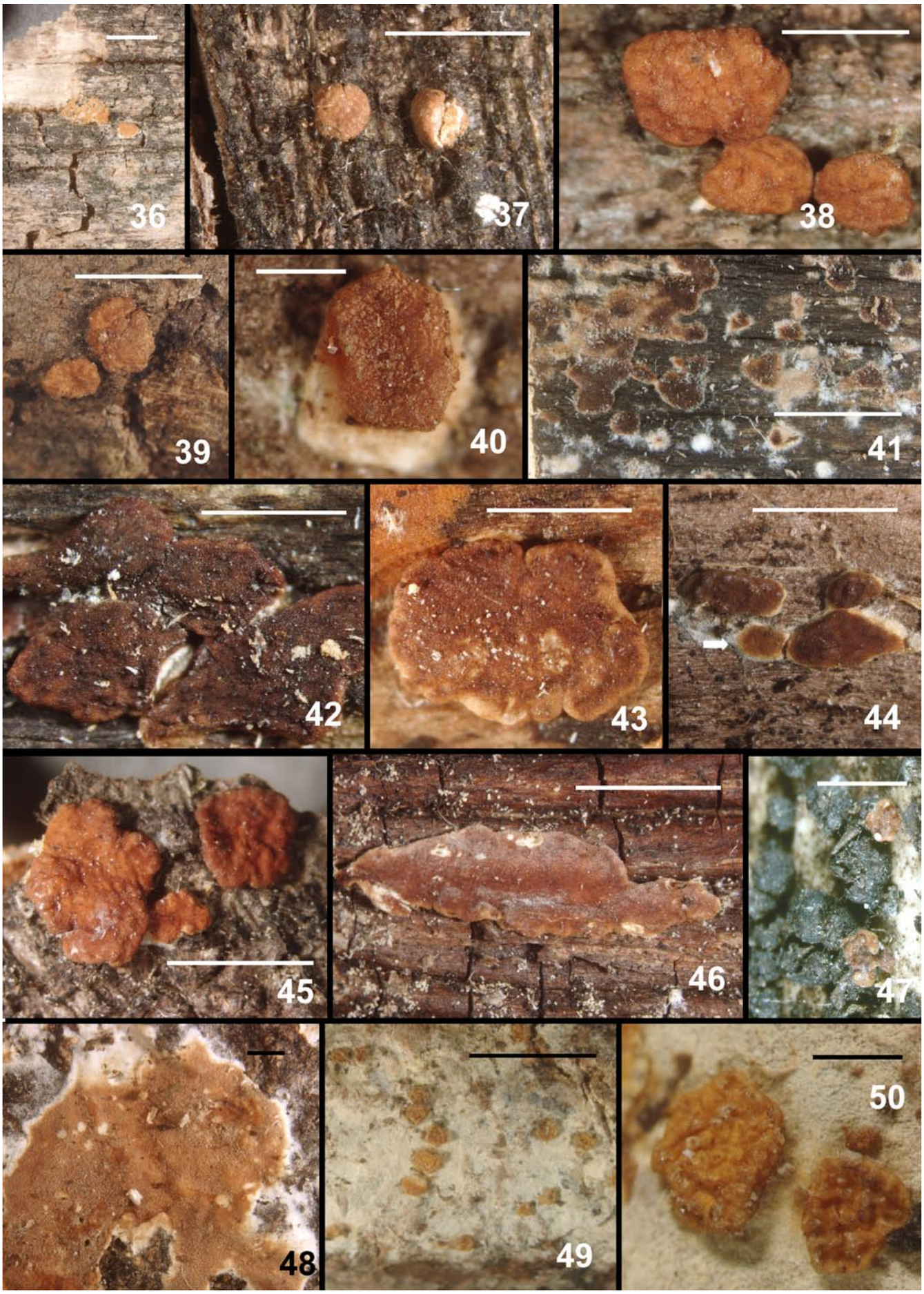
The most problematic clade from the point of biogeography is the clade comprising strains identified here as *T. austrokoningii.* This clade includes six isolates with unclear phylogenetic position because topologies of corresponding branches are nonconcordant among three loci. Most divergent are two strains, respectively, from Florida and Taiwan. The basal lineage on the *tef1* tree comprises two strains (only one shown in Fig. 2) from the South Island of New Zealand. There is a single lineage/isolate from Russia and two closely related collections from tropical Australia. The sequence divergence in this clade suggests that additional sampling would resolve it into two or more species. Although collections in the two Australasian clades are physically relatively close to each other, their actual locations are climatically very different, tropical in the case of the two Australian cultures and south-temperate in the case of the collections from New Zealand.



Figs 15–23. *Trichoderma* cultures grown on PDA in 9-cm-diam Petri dishes for 96 h at 25 °C under 12 h darkness/12 h cool white fluorescent light. 15–16. *T. koningiopsis* (15: G.J.S. 01-07, 16: G.J.S. 91-6). 17. *T. ovalisporum* DIS 172h. 18. *T. petersenii* DAOM 165782. 19–20. *T. rogersonii* (19: G.J.S. 90-125; 20: G.J.S. 92-116). 21–22. *T. stilbohypoxyli* (21: G.J.S. 02-143, in darkness; 22: G.J.S. 03-103, 7 d). 23. *T. taiwanense* G.J.S. 95-93.



Figs 24–35. *Hypocrea* teleomorphs of *Trichoderma* species. 24–27. *T. austrokoningii*. 24–25 from type, 26 from Florida (C.T.R. 85-57), 27 from Russia (G.J.S. 00-73). 28–29. *T. caribbaeum* var. *caribbaeum* on stroma of ?*Penzigia* sp. (G.J.S. 97-3). 30–31. *T. dingleyae* immature (30: G.J.S. 02-50) and mature (31: G.J.S. 99-105) stromata. 32–33. *T. dorotheae* mature and immature (arrow) stromata (32: G.J.S. 99-97, 33: G.J.S. 99-194). 34–35. *T. intricatum* immature (34: G.J.S. 96-13, Puerto Rico) and mature (35: G.J.S. 97-88, Thailand) stromata. Microscopy: all stereo. Bars: 24–25, 27–28, 30–34 = 1 mm; 26, 29, 35 = 0.5 mm.



Figs 36–50. *Hypocrea* teleomorphs of *Trichoderma* species. 36–38. *T. koningii* (36, 38: G.J.S. 89-122, 37: G.J.S. 00-156). 39–40. *T. koningiopsis* (G.J.S. 93-20, type). 41–43. *T. petersenii* (41–42: G.J.S. 98-139; 43 G.J.S. 04-355 France), immature stromata shown in 41. 44–46. *T. rogersonii*. Immature stroma (arrow) in 44 (44: G.J.S. 95-217; 45: G.J.S. 90-79; 46: G.J.S. 90-125). 47–48. *T. stilbohypoxyli* (47: G.J.S. 96-43, 48: G.J.S. 03-103, immature). 49–50. *T. taiwanense*, overmature stromata (G.J.S. 95-93, type). Microscopy: all stereo. Bars: 36 = 2 mm; 37–39, 41, 44, 46, 47–49 = 1 mm; 40, 42–43, 45, 50 = 0.5 mm.

We cannot say that any geographic region is more diverse than any other as regards the genetic diversity represented in Fig. 2. As was noted above, *T. petersenii* and *T. rogersonii, T. koningii* and *T. koningiopsis* are sympatric in eastern North America. Three species are found in Australia and New Zealand, viz. *T. dingleyae*, *T. dorotheae* and *T. austrokoningii*, although the latter species was found on the northern, tropical Queensland coast of Australia, whereas the other two were collected in south-temperate *Nothofagus* forests of Australia (Victoria) and New Zealand (S. Island).

SUBSTRATUM

The *Hypocrea* specimens from which most of the cultures were derived were found either directly on ascomata of, often, members of the *Xylariaceae* or on indeterminate black fungi on rotting decorticated wood or bark of rotting trees. In only a few cases was a fungal substratum not seen. The isolates taken directly from the substratum were taken from soil, less frequently from fallen leaves and mushroom casing. Several isolates were recovered as endophytes from the sapwood of stems of *Theobroma* species or, in one case (*T. ovalisporum*, DIS 70a), a liana. The *Trichoderma* endophytes of *Theobroma gileri* were reported by Evans *et al.* (2003). Five isolates of *T. koningiopsis* from Ecuador, represented in the cladogram by G.J.S. 01-07, were isolated directly from pods of *Theobroma cacao* infected with *Moniliophthora roreri* that had been placed on cacao leaf litter in a search for parasites of the *Moniliophthora*. Perhaps most interesting of the *Trichoderma* endophytes of woody plants was *T. stilbohypoxyli*. We have found (Samuels, unpubl.) that *Trichoderma* stem endophytes tend strongly to be specific to host genus and to biogeography, but *T. stilbohypoxyli* was isolated as an endophyte from trunks of ancient *Fagus sylvatica* in the United Kingdom and *Theobroma* species in Ecuador and Brazil.

RESULTS OF PRINCIPAL COMPONENTS ANALYSIS (PCA)

PCA was performed to determine the correlation between phenotype traits and clades; the phylogenetic clades were used as the grouping factor. Only characters of the anamorph and of colony morphology and growth rates and geographic distribution were used in the analysis because they were common to all isolates. Characters of the teleomorph were not utilized in PCA because those characters were not common to all strains. Analysis of the teleomorph characters did not resolve groups. In the PCA of the geographic and phenotype characters listed in Table 3, 51 % of the variation is accounted for by the first three axes. While the results of the Eigen analysis (Table 2) do not indicate a strong fit of the data to the model, a scatter plot of the Eigenvalues reveals that isolates of the same clade/species tend to group together (Fig. 4). The two geographically distinct isolates of *T. intricatum* were separated because of the slow growth of G.J.S. 97-88. Slow growth also pulled DIS 94c from the rest of the isolates of *T. koningiopsis*, and CBS 979.70 from the rest of *T. koningii*. The four Puerto Rican gatherings of *T. stilbohypoxyli* clustered together and distant from the other 8 cultures; this may be because the Puerto Rican strains have, on average, slightly longer and wider conidia than the others. The phylogenetic diversity of *T. koningiopsis* and, especially, *T. austrokoningii* is reflected in their wide dispersion on the scatter plot of eigenvalues.

IDENTIFICATION OF SPECIES

Three methods of *Hypocrea/Trichoderma* species identification based on the analysis of DNA sequences have been developed. Most recently, an automated identification system using oligonucleotide DNA barcodes of ITS1 and 2 sequences was developed. If it is already available for the group under investigation, a barcode is the easiest method to obtain an absolute result. The second possibility is to perform a similarity search (BLAST) against a pool of voucher sequences. This method is very useful because a search can be made using multiple loci; however, results from this technique are unavoidably uncertain, because the user must subjectively weigh every mismatch in the resulting sequence alignment. Moreover, since gene evolution does not always reflect the speciation process, it is highly recommended to obtain a concordant result of several unlinked loci. The third method of molecular species identification is the most reliable one but, at the same time, the most laborious because it implies phylogenetic analyses and the application of the Gene Concordance Phylogenetic Species Recognition (GCPSR) concept of Taylor *et al.* (2000). A detailed description of the application of each of these methods to the *T. koningii* aggregate species is given below. Molecular identification is available via a dedicated on-line "*T. koningii* morphological species project", which is located at www.isth.info/phylogeny/koningii.

Table 2. Principal Components Analysis Eigenvalues

	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6
Eigenvalues	8.041	4.125	3.038	1.854	1.814	1.536
Percentage	26.803	13.750	10.128	6.179	6.048	5.120
Cumulative Percentage	26.803	40.554	50.682	56.861	62.909	68.028

Using ITS1 and 2 and the oligonucleotide barcode program TrichOKEY v. 1.1

The first version of DNA oligonucleotide barcode integrated in *Trich*OKEY v. 1.0 (www.isth.info, Druzhinina *et al*. 2005) is able to recognize *Trichoderma* sect. *Trichoderma* and all species from the "Viride Clade" that were known prior to this study. Thus, the barcode distinguished the *T. koningii* aggregate species as a triplet of *T. koningii/T. ovalisporum/H. muroiana.* We have investigated the inter- and intraspecific variability of ITS1 and 2 sequences from the complex based on the present larger sample size. Unique species-specific oligonucleotide hallmarks for *T. petersenii* and *T. rogersonii* and *T. koningii s. str*. have been discovered. Because all three species are known from many specimens, all of which were considered in the development of the barcode, the resulting identification is reliable ("standard" in *Trich*OKEY v.1.1). In addition, a characteristic DNA signature that is common to both isolates of *T. intricatum* is incorporated in the program. Although, due to the low number of available isolates, the barcode identification is of low reliability and needs to be confirmed by other methods of sequence analysis. Other species from the *T. koningii* aggregate species such as *T. koningiopsis, T. caribbaeum, T. ovalisporum, T. dingleyae, T. dorotheae, T. taiwanense* and *T. austrokoningii* are not distinguishable based on ITS1 and 2 sequences, at least based on the observed diversity. Therefore, they will be identified as "*T. koningiopsis* or 6 rare species with *T. koningii* morphology" because *T. koningiopsis* is the most abundant and cosmopolitan species, known from more isolates than the total number of specimens from other species with ITS1 and 2 haplotype identical to it. Help involving biogeography is provided for distinguishing these species. Thus, the updated version of the ITS1 and 2 barcode (*Trich*OKEY v. 1.1) is able to distinguish all sympatric species from the complex of the *T. koningii* aggregate species.

Using *tef1* and sequence similarity search program TrichoBLAST

The *Tricho*BLAST tool for *Hypocrea/Trichoderma* sequence identification installed on www.isth.info (Kopchinskiy *et al.* 2005) determines the sequence from the database to which the query sequence is most similar. The main *Tricho*BLAST database consists of sequences of five phylogenetic markers (two introns and one exon of *tef1*, partial exon of *rpb2* and ITS1 and 2). With respect to the present group, the first version of this database included only two sequences of each *tef1* intron from two strains of *T. koningii s. str.* and one strain of *T. ovalisporum*. The remaining biodiversity was not considered. In order to facilitate the identification of species from the *T. koningii* morphological species, we have extracted both most variable *tef1* phylogenetic markers (forth large and fifth short introns) from the type sequences of each new species and inserted them in the main database, which is named "Nucleotide DB of Phylogenetic Markers" (http://isth.info/tools/blast/blast.php). Because of the high degree of intraspecific variability, we assume some difficulty in species identification based on *tef1* introns and similarity search, because the user would need to charge the weight of multiple mismatches on the pairwise alignment. Therefore, in order to minimize the possible dissimilarity between the query and subject sequences in a blast result, we have composed a separate database of both *tef1* introns for all available sequences from the *T. koningii* morphological species. This database is named "koningii *tef1*" and it is directly available from the *T. koningii* project page (www.isth.info/phylogeny/koningii) or as a selectable database in the main *Tricho*BLAST. Thus, the user has a possibility to perform the primary round of species identification using the default, main, database. A positive result ("*koningii*-positive") leads to the possibility of searching for the most similar haplotype among highly homologous sequences and making allowance in the final identification according to it. The conclusion about species identification may be drawn if the query sequence is significantly more similar (significantly higher bit score) to sequence(s) of one species compared to the similarity to others and if all precautions explained in Kopchinskiy *et al.* (2005) are taken into account.

Using multilocus phylogenetic analysis

As has been shown in this study, the "Viride Clade" is an extremely species-rich group. Therefore, we anticipate the further discovery of new taxa with the *T. koningii* morphology. In this case, both methods of molecular species identification would provide uncertain results, e.g. the ITS1 and 2 based barcode will lead to an identification at the level of clade and/or "*T. koningiopsis* and six rare species" (see above), while the result of similarity search will show the same relation to the group of species instead of only a single taxon. Such a situation may be resolved only by the use of phylogenetic analysis based on several unlinked phylogenetic markers. In order to facilitate the task, we have included type sequences of newly recognized species in the multiloci database of phylogenetic markers (http://www.isth.info/tools/blast/show_all_seq.php). This database is especially designed to assist in retrieving of type sequences for the subsequent phylogenetic analyses. In addition, as has been mentioned above, the *T. koningii* project page also contains a table listing all the ITS1 and 2, and both *tef1* sequences of species from the *T. koningii* aggregate species, and the geographic origin of the corresponding strain is given. Thus, the final species identification or the detection of the new species may be done based on the phylogenetic analysis, with phylogenetic markers retrieved from www.isth.info/phylogeny/koningii.

Using phenotypic characters

Geography and reproductive isolation have played a large part in our species concept. PCA revealed phenotype-based groups that combine with GCPSR in species delimitation. While there is significant homoplasy in the phenotypic characters, the species that we recognize in most cases are not sympatric. Although the species that we recognize in this work are characterized to some extent by phenotypic characters,

we recognize that often the characters are at best subtle and difficult to observe. It will be difficult to recognize a species if it is out of the currently known geographic range. Nevertheless we have provided a key for species identification based on phenetic characters, which should resolve doubts that may derive from sequence similarities.

KEY TO SPECIES OF THE *TRICHODERMA KONINGII* AGGREGATE SPECIES BASED ON THE PHENOTYPE

1. 95 % CI L/W of conidia 1.1–1.3 2
1. 95 % CI L/W of conidia > 1.3 3

2. 95 % CI L/W of conidia 1.2–1.3; known only from ascospore-derived cultures ..... 6. ***T. intricatum***
2. 95 % CI L/W of conidia 1.1–1.2; known only as an endophyte of *Theobroma* species and woody tissue infected with *Moniliophthora perniciosa* ..... 9. ***T. ovalisporum***

3. 95 % CI L/W of conidia 1.6–1.7; colony radius on PDA after 72 h at 25 ºC in darkness 50–60 mm; primarily Europe and North America ..... 7. ***T. koningii***
3. 95 % CI L/W of conidia L/W ≤ 1.5 4

4. Colony radius on PDA after 72 h at 25 ºC in darkness ≤ 50 mm 5
4. Colony radius on PDA after 72 h at 25 ºC in darkness > 50 mm 11

5. Europe and North and Central America, Caribbean Region, Thailand; sporulating well on PDA; known from teleomorph and isolated directly from substratum 6
5. Australia and New Zealand; often sporulating poorly on PDA; known only from teleomorph 9

6. Colony radius on PDA after 72 h at 25 ºC in darkness 33–40 mm; North and Central America, Caribbean Region, West Africa, Europe 7
6. Colony radius on PDA after 72 h at 25 ºC in darkness > 40 mm; North America, Europe, Taiwan 8

7. North and Central America and Europe; conidia lacking yellow colour at any time, cultures on PDA with several conspicuous concentric rings ..... 10. ***T. petersenii***
7. Central and South America, Caribbean region, Africa (Ghana), United Kingdom; on stromata of pyrenomycetes, found as an endophyte in woody tissue; PDA cultures often with a diffusing yellow pigment and conidia yellow-green at least at first ..... 12. ***T. stilbohypoxyli***

8. Taiwan; distal part-ascospores 3–4 × (2.5–)3.0–3.5(–4.0) µm; proximal part-ascospores (3.0–)3.5–4.5(–5.2) × 2.5–3.0(–3.2) µm ..... 13. ***T. taiwanense***
8. Eastern North America and Europe; distal part-ascospores (3.0–)3.5–4.5(–5.2) × (2.5–)3.2–4.0(–5.0) µm; proximal part-ascospores (3.2–)4.2–5.2(–5.9) × (2.5–)2.7–3.0(–3.2) µm ..... 11. ***T. rogersonii***

9. Colony radius on PDA after 72 h at 25 ºC in darkness *ca.* 40 mm; 95 % CI of conidia 4.0–4.1 × 2.8–3.0 µm, L/W = 1.2–1.3 ..... 5. ***T. dorotheae***
9. L/W of conidia 1.3–1.4; slower growing 10

10. Colony radius on PDA after 72 h at 25 ºC in darkness 33–35 mm, 95 % CI of conidia 3.7–3.9 × 2.5–2.6 µm; cosmopolitan ..... 1. ***T. austrokoningii***
10. Colony radius on PDA after 72 h at 25 ºC in darkness 25–30 mm; 95 % CI of conidia 4.1–4.3 × 3.1–3.2 µm; New Zealand ..... 4. ***T. dingleyae***

11. Colony radius on PDA and SNA after 72 h at 30 °C in darkness < 10 mm; known only as an endophyte of cacao .....3. ***T. caribbaeum* var. *aequatoriale***
11. Colony radius on PDA after 72 h at 30 ºC ≥ 30 mm 12

12. Colony radius on PDA after 72 h at 25 and 30 °C in darkness 50–60 mm; known only from ascospore isolations made in Puerto Rico and Guadeloupe .....2. ***T. caribbaeum* var. *caribbaeum***
12. Faster growing, colony radius on PDA after 72 h at 25 ºC in darkness 55–70 mm; known primarily from direct isolations from substrata, rarely from ascospore isolations ..... 8. ***T. koningiopsis***

DISCUSSION AND TAXONOMIC CONCLUSIONS

We studied 86 strains of *Trichoderma*, any of which could have been identified as *T. koningii* following the schemes of Rifai (1969) or Gams & Bissett (1998). In the absence of reproductive isolation and clear morphological differentiation for detecting species boundaries, the GCPSR concept (Taylor *et al.* 2000) remains the only currently applicable choice. It requires the concordant phylogenetic position of a taxon among closely related other taxa based on at least three unlinked loci. The concordant phylogenetic position should also not be contradicted by analyses of other loci. In this study we found few phylogenetic markers that could reliably resolve groups of closely related, apparently recently evolved *Hypocrea*/*Trichoderma* species. The choice of phylogenetic markers for a particular group of fungi is a delicate task. Druzhinina & Kubicek (2005) have listed eleven phylogenetic markers attributed to eight DNA loci used in phylogenetic analyses of *Hypocrea*/*Trichoderma* species. The internal transcribed spacers 1 and 2 (ITS1 and 2), which provide considerable diagnostic properties in *Hypocrea*/*Trichoderma* (Druzhinina *et al.* 2005), are insufficient for phylogenetic modelling even at the intercladal level. Therefore, for the analysis of *T. koningii*-like species, we have selected intron-rich fragments of the protein-encoding genes *tef1*, *act*, and *cal* that deliver higher levels of variation. However, sufficiently high resolution was obtained only from both *tef1* introns, while the phylogenetic signal of *act* and *cal* was moderate. Tree topologies based on *act* and *cal* loci are concordant with the selected tree based on *tef1*, however, overall statistical support of species nodes was relatively low. Therefore, an integrated approach for the identification of *Trichoderma* species was developed that adopts multivariate analyses of phenotypic characters and patterns of geographical distributions as well as phylogenetic inferences and oligonucleotide barcoding (see also Kraus *et al*. 2004, Harrington & Rizzo 1999).

The molecular phylogenetic analysis based on three protein-encoding genes revealed several weakly or well supported clades representing taxa that could be characterized and therefore recognized at least partly by phenotypic characters and patterns of geographic distributions. With the help of this integrated approach, a taxonomy was developed that currently accepts twelve species and one variety.

Trichoderma, like many other fungi, suffers from homoplasy of morphological characters, which have been the basis of species descriptions since the genus was described more than 200 years ago. In the absence of characters derived from DNA, all of the isolates that we studied could have been identified as *T. koningii* in the sense of Rifai (1969). In the present work, geographic distribution and rate of growth in agar culture were among the most significant characters separating species. PCA that included only the few available characters of the anamorph *per se* (i.e. measurements of conidia and phialides) did not result in a clustering of strains that was consistent with the results of phylogenetic analysis. Certain characters of the anamorph are variable within a phylogenetic species; others suggest homoplasy in different phylogenetic species. There are usually no finite conidiophores; instead, conidiophores are aggregated within more or less well-developed pustules and individual elements cannot be measured and are difficult to characterize. The formation and extent of pustules, or degree of aggregation of “conidiophores”, in most species is highly variable and the ability to form pustules may decline with length of time of strain preservation or after successive transfers. Phialides in most groups are longer or shorter, wider or narrower, depending upon whether they are formed near the surface of the pustule, where they are less crowded, or at the interior of the pustule where they are more crowded. However, crowded and less crowded phialides can hardly be analyzed separately from each other. Conidia present the most consistent morphological character because they can be measured and because their morphology remains constant over successive transfers. What compounds the difficulty in finding phenotypic characters is the general lack of pigments in cultures, a character that has been used in taxonomy of *Trichoderma* sect. *Longibrachiatum* (Samuels *et al.* 1998) or other species-rich genera such as *Fusarium*.

The teleomorph is not helpful in species recognition and is only of limited use in recognizing phylogenetically distinct clades. In general, individual species within a clade cannot be distinguished on the basis of the morphological characters of the teleomorph. However, clades may or may not have a distinctive teleomorph morphology. Chaverri & Samuels (2004) found that among species with green ascospores, the same anatomy of the *Hypocrea* stroma was found in phylogenetically distant groups, however, all of those teleomorphs were very different from the teleomorphs reported here and from those formed in *T.* sect. *Longibrachiatum* (Samuels *et al*. 1998). In gross morphology, most of the *Hypocrea* collections studied here cannot be distinguished from *H. rufa* (Pers. : Fr.) Fr., the teleomorph of the closely related *T. viride*, and the type species of *Hypocrea.* Doi (1972) subdivided *Hypocrea* of Japan on the basis of ascospore colour and stroma anatomy, but phylogenetic analysis has not upheld those subdivisions.

With the adoption of molecular phylogenetics, it has become obvious that a species delineated by morphological characters is likely to comprise multiple phylogenetic species. If we had limited our study of phenotype characters to those of the conidia, conidiophores and ascospores, we might have concluded (as did Rifai in 1969) that there were potentially more than one “cryptic species” within the morphological species *T. koningii*. We would not have been able, however, to identify most of them unambiguously. This phenomenon is common in the ascomycetes and led Hawksworth (2001) to revise upward the estimated number of species of fungi. Minute or subtle characters that would have, in an earlier time, been disregarded as insignificant, are now accepted and sought for

characterization of clades. For example, the common species *T. harzianum* was distinguished from the cause of green mould of mushrooms, *T. aggressivum* Samuels & W. Gams, by the inability of the latter species to grow at 35 °C (Samuels *et al.* 2002).

The paucity of phenotypic characters to distinguish clades is not unique to *Trichoderma* but rather is likely to become increasingly found in the ascomycetes in general as genera are subjected to phylogenetic analyses. In the example of *Coccidioides,* Fisher *et al.* (2005) found that salt tolerance was the only phenotypic character to distinguish *Coccidioides posadasii* from the closely related *C. immitis. Botryosphaeria* has received much attention recently. Differences in yellow pigment and host distinguished between the phylogenetically closely related *Botryosphaeria lutea* and *B. acaciae* (Slippers *et al.* 2004). The *Fusarium solani* complex remains an important challenge to taxonomic revision (O'Donnell 2000).

As increasing numbers of members of species-rich genera such as *Trichoderma*, *Fusarium* or *Botryosphaeria* are included in phylogenetic analyses, increasing numbers of morphologically defined species will be found to be paraphyletic or comprise numerous cryptic species. As much as we would wish for a taxonomy that would permit accurate species identification using only the microscope, we must face the reality that there may not be enough characters in morphology and growth to reflect the differences revealed in diverging DNA sequences. Homoplasy of morphological characters may well be the result if genetically distinct lineages occupy the same niche for a long time. This is also confirmed by many cases where data for the overall carbon utilization profiles (Biolog) did not correspond to the phylogenetic analysis, while the analysis of the utilization of certain single carbohydrates did (Kubicek *et al.* 2003). A similar situation was observed in marine species of the unrelated genus *Dendryphiella,* collected from different marine sites (Dela Cruz & Druzhinina, unpublished).

One could argue that our species concept in *Trichoderma* is too narrow, too strongly influenced by phylogeny, but an example from the present study argues against that. *Trichoderma rogersonii* and *T. petersenii* are well separated in the phylogenetic analysis (Figs 2–3). They are common and sympatric in the Eastern U.S.A. However, as can be seen from the PCA (Fig. 4), the two species are incompletely separated by a suite of phenotype characters. The characters separating the two species (Table 3) are the L/W and length of conidia and the length of the proximal part-ascospores. In addition, growth rates on SNA are also characteristic of the respective species. While these differences are statistically significant, there will be many cases that cannot be identified on the basis of their phenotype.

In *Trichoderma*, at least, traditional characters may be too few to provide practical identification of the apparent large number of species. Carbon utilization estimated using a Phenotype MicroArray technique may provide additional characters (Kubicek *et al.* 2003, Kraus *et al.* 2004). In *Trichoderma* we do not yet have the ability to perform *in vitro* mating experiments, which would help immensely in determining whether strains belong to the same or different biological species.

The species that we have studied in the current work represent a small part of the diversity of the species-rich "Viride Clade." Work is continuing on taxonomy of this group.

The refined definition of *T. koningii* given by Lieckfeldt *et al.* (1998) was reinforced in this study; the species is distinguished by its longer and narrower conidia and slow rate of growth on SNA. Although *T. koningii* is among the most commonly cited species in the genus, our results suggest that it is an uncommon species of Europe and North America. The far more common species isolated directly from natural substrata and the species often used in biological control applications is the closely related *T. koningiopsis*.

Trichoderma koningiopsis is essentially a tropical species, known from South America and Africa (Ethiopia), but its *Hypocrea* teleomorph has been found as far north as New York State. *Trichoderma koningiopsis* is the most commonly encountered species having a *T. koningii*-like morphology. This species was reported earlier as "*Hypocrea* sp. (8)" in part (Lieckfeldt *et al*. 1998), "*T. koningii* II" (Dodd *et al.* 2003) and "*T. koningii* Tkon 21" (Holmes *et al.* 2004).

Webster (1964) described *"Hypocrea* sp. 1" from the United Kingdom. The *T. koningii*-like anamorph described by Webster (1964) strongly resembles *T. petersenii* in the morphology of conidia, conidiophores and in the formation of concentric rings in agar culture. We have obtained the cultures cited by Prof. Webster from CBS. Based on anamorph morphology as observed in these cultures and sequences of *tef1*, we can see that *Hypocrea* sp. 1 was based on a mixture of two species. Webster 2534 = CBS 257.62 = *T. harzianum*, the anamorph of *H. lixii* (Chaverri & Samuels 2004); the other cultures (Webster 2545 = CBS 258.62, 2617 = CBS 259.62 and 2644 = CBS 260.62) are all *T. minutisporum* Bissett, the anamorph of *H. minutispora* (Lu *et al.* 2004). Neither of these species is closely related to members of the "Viride Clade" (Samuels 2006).

Doi (1974) reported a *T. koningii*-like anamorph for Japanese collections of *H. muroiana* Hino & Katumoto. However, the range of conidial types – including those with surface ornamentation – described by him lead us to suspect that more than one species was involved. Moreover, none of the collections cultured by Doi was taken from bamboo, which is the substratum of the type collection of *H. muroiana*. In our experience, bamboo is an unusual substratum supporting fungi that are not usually found on other substrata, at least not on woody substrata, which was the source of specimens reported by Doi. We have examined the type collection of *H. muroiana* (YAM) and conclude on the basis of its morphology that it is a member of the "Viride Clade," but there is no material with a living culture available. We are not able to identify to species the *T. koningii*-like anamorph(s) reported by Doi (1974) for *H. muroiana.* Two cultures isolated from rhizomorphs of, respectively,

Armillaria mellea (IFO 31288) and *Lentinula edodes* (IFO 31293) and identified by Y. Doi as *H. muroiana* are *T. atroviride*, the anamorph of *H. atroviridis.*

Several isolates of *T. koningiopsis*, represented by G.J.S. 01-10 and G.J.S. 01-11 but not included in the phylogenetic analysis, were isolated in Ecuador from pods of *Theobroma cacao* that were infected by the destructive parasite *Moniliophthora roreri*. These isolates are currently in field trials to protect cacao from the *Moniliophthora* (C. Suarez, pers. comm.). *Trichoderma koningiopsis* isolates G.J.S. 04-10 and 04-11 are effective in protecting cotton plants from infection by *Thielaviopsis basicola* in Texas (C.R. Howell, pers. comm.), and isolate G.J.S. 05-462 (received too late to be included in the present study) is showing potential for control of *Fusarium verticillioides* in maize (I. Yates, pers. comm.). A single isolate of this species, G.J.S. 97-273 (= BBA 65450), was isolated from soil in Germany. We tested several isolates of *T. koningiopsis* for their ability to parasitize the cacao pathogen *Moniliophthora roreri in vitro* (results not shown) following the "preinoculated plate test" described by Evans *et al.* (2003) and found that several were able to parasitize the mycelium of *M. roreri*, with the German isolate being especially effective.

Trichoderma koningiopsis is probably cosmopolitan but perhaps more common in tropical regions. In Figs 2–3 the species can be seen to comprise several well-supported internal branches; however, we were not able to detect any geographic or phenotypic bias to any of the clades. Six strains (designated as "DIS" in Fig. 2) of *T. koningiopsis* were isolated as endophytes from freshly exposed, living sap-wood of trunks of species of *Theobroma* in Brazil, Ecuador and Peru. Following the protocol described in Holmes *et al.* (2004), strains DIS 172ai (from *Theobroma grandiflorum*) and DIS 229d (from *Th. gileri*) could be introduced into seedlings of *Theobroma cacao* and were reisolated from woody tissue but not from the apical meristem. The isolate DIS 339c (from *Th. gileri*) could be reisolated from all stem sections of *Th. cacao* seedlings, including the apical meristems, and it could be reisolated from inoculated pods of *Th. cacao* after 12 weeks, indicating a potential for protecting pods against infection by *M. roreri* (K. Holmes, pers. comm.). Ecuadorian strains (G.J.S. 01-07–G.J.S. 01-12, Table 1) were isolated from pods of *Th. cacao* that were naturally infected with the parasite *M. roreri*, the cause of frosty pod rot, and have been included in a field trial in Ecuador against that pathogen (C. Suarez, pers. comm.).

Trichoderma koningiopsis occupies the most basal position of the Large Koningii Branch (LKB) in the *tef1* tree (Fig. 2), although the statistical support of this species on both *act* and *cal* trees is particularly low. This finding in combination with confirmed wide distribution of the species in tropical countries may indicate a relatively intensive recombination process due to sexual reproduction. However, the majority of *T. koningiopsis* strains were isolated as anamorphs from natural substrata. Teleomorph specimens are only known from the Caribbean region and from the U.S.A. Alternately, the paraphyly of *T. koningiopsis* could be explained if the species were relatively old. Partially sympatric old, clonal lineages could occur sympatrically and, over evolutionary time, accumulated mutations in the introns and other parts of the DNA could explain the variation in the species.

Trichoderma petersenii and *T. rogersonii* are common and sympatric in eastern North America. *Trichoderma petersenii* was reported earlier as "*Hypocrea* sp. (8)" in part (Lieckfeldt *et al.* 1998), "*T. koningii* Tkon 3" and "Tkon 22" in part (Holmes *et al.* 2004). *Trichoderma rogersonii* was reported as "*Hypocrea* sp. (4) and (5)" (Lieckfeldt *et al.* 1998). The similarity between these two species was noted above. The most obvious difference between these very similar but phylogenetically relatively distantly related species is that *T. rogersonii* grows more slowly on SNA than does *T. petersenii*; moreover, conidia of *T. petersenii* are slightly shorter and broader than those of *T. rogersonii* (95 % CI of L/W respectively 1.35–1.39, 1.40–1.46). Their *Hypocrea* morphs are indistinguishable from each other and, at least in gross morphology, they are indistinguishable from *H. rufa* (anamorph: *T. viride*). However, *H. rufa* is an uncommon species, albeit sympatric with the other two, despite the many reports of its occurrence. It differs from *T. petersenii* and *T. rogersonii* in having slightly larger ascospores (distal part-ascospores approx. 4.5–5 × 4–4.5 µm; proximal part-ascospores approx. 5–6 × 3–4 µm). *Trichoderma viride* is readily distinguished from *T. petersenii* and *T. rogersonii* by its subglobose, warted conidia.

Trichoderma koningii is also sympatric with *T. petersenii* and *T. rogersonii*. Because these highly similar species are common, despite their phylogenetic distance from each other, it is important that they may be reliably distinguished by the ITS1 and 2 oligonucleotide barcodes.

Trichoderma ovalisporum is distinguished by its subglobose to more ovoidal conidia (Figs 235–236). It was found as an endophyte of *Theobroma* species and was also isolated from a woody stem of the liana *Banisteropsis caapi* that was infected by *Moniliophthora* (*Crinipellis*) *perniciosa,* the cause of Witches' Broom disease of cacao in tropical America (Holmes *et al*. 2004). The fifth isolate was isolated from soil in Panama, where cacao is grown; it was not included in the phylogenetic analysis. The liana isolate (DIS 70a) reinfected and was reisolated from meristematic tissue of *Th. cacao*, and inhibited radial growth of the frosty pod rot pathogen (*Moniliophthora roreri*) *in vitro*. It also persisted on the surface, and within tissues, of cocoa pods in the field for at least 10 weeks. Initial field trials in Costa Rica, where conidia were applied as a spray, indicated an ability to protect pods against infection by *M. roreri* (Holmes *et al.* 2004).

With the exception of the one isolate of *T. ovalisporum* (DIS 70a), all of the DIS isolates studied for this work (Table 1) were isolated as endophytes from woody stems of South American *Th. cacao*, *Th. gileri* and other *Theobroma* species. These and other *Trichoderma* isolates were reported previously by Evans *et al*. (2003) and Holmes *et al*. (2004) to

be endophytes of *Th. gileri*. In the current work we identify several additional endophytes as members of the "Viride Clade" of *Trichoderma* sect. *Trichoderma*. *Trichoderma koningiopsis* was especially well represented in the endophyte isolations but they did not fall into an endophyte-specific lineage in this species. In contrast to *T. ovalisporum* and *T. caribbaeum* var. *aequatoriale*, which are known only as endophytes of cacao and cacao relatives but which are not common even in that niche, it is not surprising that a species that is as common as *T. koningiopsis* should be found as an endophyte of a common tropical tree. Judging by the large number of isolates of *T. koningiopsis* that we received from many sources, it would be surprising not to find it as an endophyte of stems of other tropical trees. Equally, it would not be surprising to find additional isolates that have a biological control potential for fungus-induced plant diseases. Interesting is that Arnold & Herre (2003) did not report *Trichoderma* species as leaf endophytes in Panama. Outside of the *T. koningii* aggregate species, *T. erinaceus* (DIS 7, DIS 8 in Fig. 2) was extended to Peru. This species was previously known only from Southeast Asia (Thailand, Cambodia, Malaysia). *Trichoderma stilbohypoxyli* was originally described as a parasite of the xylariaceous fungus *Stilbohypoxylon muelleri* in Puerto Rico in 1996. Since then several isolates considerably expand the biological and geographic distribution of this species by discovery of its teleomorph in Costa Rica and Ghana on bark and perithecia of *Neonectria jungneri*, and endophyte isolations from woody tissue of *Theobroma* species in Ecuador and Brazil and from *Fagus sylvatica* in the United Kingdom. The diffusing yellow pigment, especially seen in colony reverse on PDA, and fast growth rate characterize this species.

Trichoderma caribbaeum var. *caribbaeum* is represented by two collections (G.J.S. 97-3, G.J.S. 98-43); both are derived from ascospores of *Hypocrea* specimens collected in, respectively, Guadeloupe and Puerto Rico. As can be seen from the PCA (Fig. 4), these two strains are closely similar in phenotype and also genotype (Fig. 2). The isolate DIS 320c, *T. caribbaeum* var. *aequatoriale,* forms a highly supported clade with the other two isolates but is phenotypically and apparently biologically distinct. It was isolated as an endophyte from stems of *Th. gileri* in Ecuador. The considerable differences in phenotype, biogeography and habit, despite its phylogenetic proximity to the ascospore isolates, lead us to recognize the endophyte as a variety, var. *aequatoriale*. The apparent close relationship between the two varieties is possibly an artifact of sampling; additional sampling could support their separation at the species rank.

At least three species occur in New Zealand and Australia, viz. *T. dorotheae, T. dingleyae* and *T. austrokoningii*. *Trichoderma dingleyae* is the slowest-growing species in the present study; its temperature optimum is 20–25 °C and the colony radius is < 5 mm after 72 h at 30 °C. The first two species were collected in *Nothofagus* forests whereas the third, *T. austrokoningii,* was found in the tropical Queensland coast and in *Nothofagus* forests of New Zealand. *Hypocrea vinosa* Cooke was described from New Zealand (Cooke 1879) and is reported often in the literature, or on the World Wide Web, from diverse geographic regions (e.g. Brazil, Bresadola 1896; Japan, Komatsu & Hashioka 1966; New Guinea, Doi 1971). Ascospores in the type specimen of *H. vinosa* (K!) are unusually large (distal part-ascospores 5.1–6.7 × 5.0–5.5 µm; proximal part-ascospores 5.7–7.2 × 4.6–5.3 µm), suggesting that most or all of the reports of this species outside of New Zealand are based on misidentifications. CBS 247.63, *H. austrokoningii,* is derived from a *Hypocrea* specimen received from New Zealand (J.M. Dingley No. 3, Auckland, Te Aroha). However, we cannot locate that specimen in CBS or PDD to confirm its identity. We have collected specimens in New Zealand that conform to the type collection of *H. vinosa* and redescribe the species in another publication (Jaklitsch *et al*. 2006b).

Unlike most clades, the one that includes *T. austrokoningii* is geographically diverse, including lineages (Figs 2, 3) from tropical Australia (Queensland, Figs 51–59), temperate New Zealand (Figs 80–88), Russia (Figs 71–79), and a single lineage that includes one collection from the United States (Florida) and one from Taiwan (Figs 89–101). Subtle phenotypic differences characterize each clade (e.g. growth rates, Fig. 102, and ascospore measurements). The phylogenetic and phenotypic diversity of the isolates in this "*austrokoningii"* clade, which occupies the terminal position of the SKB clade (Fig. 2), suggests that more than one taxon could be involved and that additional sampling would resolve this clade.

Some of the species are represented by one or two strains. We would not normally describe a species based on such a small amount of material because there is no way to estimate intraspecific variability. Nonetheless, *T. taiwanense*, based on a single collection from Taiwan (G.J.S. 95-93), and *T. intricatum*, based on two collections (G.J.S. 97-88 from Thailand and G.J.S. 96-13 from Puerto Rico), are phylogentically distinct from all other species that we have included. The two strains of *T. intricatum* are phenotypically distinct as shown by PCA (Fig. 4). *Trichoderma intricatum* was formerly reported as "*H.* cf. *muroiana*/*Hypocrea* sp. (6)" in Lieckfeldt *et al.* (1998).

THE SPECIES OF THE *TRICHODERMA KONINGII* AGGREGATE

(continuous characters used in the PCA are presented in Table 3)

1. *Trichoderma austrokoningii* Samuels & Druzhinina**, sp. nov.** MycoBank MB501032. Figs 6–8, 51–59, 71–101.

Teleomorph: ***Hypocrea austrokoningii*** Samuels & Druzhinina**, sp. nov**. MycoBank MB501033. Figs 24–27, 60–70.

Etymology: Refers to a similarity to *T. koningii* and to Australia, the locality of the type collection.

Stromata rufobrunnea, *H. rufae* (Fr.) Fr. similia. Ascosporae hyalinae, spinulosae. Pars distalis ascosporarum (3.2–)3.5–4.2(–5.0) × (2.7–)3.2–3.7(–4.2) µm, pars proxima (3.2–)2.5–4.5(–5.0) × 2.7–)3.0–3.5(–4.2) µm. Anamorphosis *T. koningii* Oudem. similis, conidia viridia, late ellipsoidea, (3.2–)3.5–4.2(–4.7) × (2.0–)2.2–2.7(–3.2) µm, ratio longitudinis:latitudinis (0.9–)1.2–1.6(–1.7). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate 33–35 mm.

Holotypus *H. austrokoningii* BPI 870962; holotypus anamorphosis *T. austrokoningii* cultura sicca ex ascospora oriens BPI 870962B.

Stromata scattered, semi-effused and lenticular to irregular in outline, to pulvinate or tuberculate, 8D–E8 (English-red to reddish brown), not reacting to KOH, 1–2 mm diam, broadly attached with edges slightly free, plane, appearing velvety or smooth, perithecial elevations not evident, ostiolar openings not visible. Cells of stroma surface in face view circular, 3.5–9 × 2.5–5.5 µm, often in chains of 2–4 cells, walls *ca.* 1.5 µm thick, unevenly pigmented. Surface region of stroma 10–25 µm thick, composed of pigmented, pseudoparenchymatous cells 1.5–5.5 × 2.0–4.5 µm, walls *ca.* 1.5 µm thick. Tissue below the stroma surface region of intertwined hyhae. Perithecia elliptic in section, 175–300 µm high, 120–150 µm wide; ostiolar canal 55–75 µm long; perithecial apex protruding slightly through the stroma surface, formed of narrow hyphal elements. Tissue below the perithecia comprising vertically elongated, thin-walled cells, (6–)10–20(–30) × (4.5–)5.2–10(–14) µm. Asci cylindrical, apex thickened, with a pore. Part-ascospores hyaline, finely spinulose, dimorphic; distal part subglobose, proximal part wedge-shaped to oblong or slightly ellipsoidal.

Characteristics in culture: Optimum temperature for growth on PDA and SNA 25–30 °C. Colonies grown on PDA in intermittent light forming conidia within 48 h at 25 °C; after 96 h in light, conidial production in 2 concentric rings. Conidia on PDA and CMD 26E–F8 (deep green to dark green). No pigment diffusing through the agar; no distinctive odour. Colonies grown on CMD at 20–25 °C under light filling the Petri plate within 1 wk, conidia abundant, continuously dispersed around the colony margin and also forming in few 1–2 mm diam cottony pustules; individual conidiophores visible within the pustules, completely fertile to the tip. Conidiophores more ore less symmetrical, comprising a recognizable main axis, 2–3 µm wide, fertile branches arising along the length of the main axis, often paired, with longer or shorter internodes. Branches arising at an angle of slightly less than 90° with respect to the main axis, longer branches near the base and short branches or solitary phialides arising near the tip; *1°* branches rebranching or producing phialides directly; *2°* branches producing phialides at the tip and often along the length. Phialides typically straight, lageniform, cylindrical or slightly swollen in the middle, held in whorls of 3 or 4, sometimes crowded when formed within a pustule; intercalary phialides formed but uncommon. Conidia broadly ellipsoidal, smooth. Chlamydospores not observed.

Habitat: Bark of hardwood trees.

Known distribution: Australia (Queensland), possibly also New Zealand, Republic of China (Taiwan), Russia, United States (Florida).

Holotype: **Australia**, Queensland, Wongakill State Forest, *ca.* 10 km S of Atherton, on bark of decaying log, 30 Aug. 1999, K. Põldmaa 238 (BPI 870962A; holotype of anamorph = BPI 870962B; ex-type culture G.J.S. 99-146 = CBS 119092).

Paratype: Data as for the holotype, on wood, K. Põldmaa 240 (BPI 870963A; dry culture = culture BPI 870963B; live culture: G.J.S. 99-147 = CBS 119079 = ICMP 16282).

Additional specimens examined: **New Zealand**, North Island, Auckland, Te Aroha, substratum not known, Feb. 1963, J. M. Dingley No. 3 (specimen not located, culture CBS 247.63, as *H. vinosa*); South Island, Westland, Buller River Gorge, "Sinclair's Castle", the point where the Ohikanui River joins the Buller River, 41°51' S, 171°43' E, elev. 50 m, along flood plain of the Ohikani River, on *Nothofagus menziesii*, 6 Sep. 1999, G.J.S. 8698 & S. Dodd (PDD 83836; culture G.J.S. 99-116 = CBS 119080 = ICMP 16280). **Republic of China,** Taiwan, Fushan Botanical Garden, on decorticated wood, 13 July 1996, M.-L. Wu 960713T8 (BPI 744491, culture G.J.S. 96-163 = CBS 119078). **Russia**, Kostroma Region, Manturovo, forest near the river, on rotting wood of *Alnus glutinosa,* 21 Aug. 1999, A. Alexandrova 434 (BPI 842331; culture G.J.S. 00-73 = CBS 119077). **U.S.A.,** Florida, Alachua County, San Felasco Hammock State Preserve, on rotten log, 10 Aug. 1985, C.T. Rogerson (NY: culture C.T.R. 85-57 = CBS 119076).

Notes: The description given above is based on the two cited Australian collections. We specifically did not designate specimens not found in Queensland as paratypes because there is reason to doubt that they are truly collections of *T. austrokoningii.*

Trichoderma austrokoningii s. lat., *T. dingleyae* and *T. dorotheae* occur in Australia and New Zealand. In Australasia, *T. austrokoningii* is known from a tropical region of Australia, the Queensland Coast, and from subtropical (CBS 247.63) to temperate (G.J.S. 99-116) parts of New Zealand (Figs 80–88). The teleomorphs of these species are indistinguishable. Conidia of *T. dingleyae* and *T. dorotheae* are longer and, especially, wider than are those of *T. austrokoningii*. Conidia in the Australian collections of *T. austrokoningii* (Figs 51–59) are shorter [(2.5–)3.0–3.5(–4.2) µm, CI = 3.3–3.4 µm] than are those of the other collections of this species. Growth (Fig. 102) of *T. austrokoningii* at 30 °C on PDA is the same as at 25 °C (radius = *ca.* 30 mm after 72 h), whereas growth of the two New Zealand isolates is considerably slower at 30 °C (radius = < 15 mm) than at 25 °C (radius = *ca.* 30 mm). Australian collections of *T. austrokoningii* grow faster on SNA than either *T. dingleyae* or *T. dorotheae* or any of the other cited collections of *T. austrokoningii* (colony radius for Australian *T. austrokoningii* at 25 and 30 °C = 25–35 mm as compared to a radius of < 25 mm at 25 °C and < 15 mm for *T. dingleyae, T. dorotheae* and the other collections of *T. austrokoningii*).

In Taiwan the occurrence of *T. austrokoningii* overlaps with that of *T. taiwanense*. *Trichoderma taiwanense* has larger conidia and somewhat smaller part-ascospores than does the Taiwanese collection of *T. austrokoningii*.

Table 3. Continuous characters, geographic distribution and colony phenotype of the *Trichoderma* species discussed.

	Species												
	T. austrokoningii 2[2]	*T. caribbaeum* var. *caribbaeum* 2[2]	*T. caribbaeum* var. *aequatoriale* 1[2, 4]	*T. dingleyeae* 3[2]	*T. dorotheae* 3[2]	*T. intricatum* 2[2]	*T. koningii* 11[2]	*T. koningiopsis* 23[2]	T. *ovalisporum* 4[2]	*T. petersenii* 8[2]	*T. rogersonii* 13[2]	*T. stilbohypoxyli* 11[2]	*T. taiwanense* 1[2]
Geography[1]													
	Australia (Queensland), also New Zealand, Taiwan, U.S.A. (Florida), Russia	Puerto Rico, Guadeloupe	Ecuador	New Zealand	Australia (Victoria), New Zealand	Puerto Rico, Thailand	Eastern N. America, Europe	Probably pantropical, also Eastern North America and Europe	Brazil (Amazonia), Ecuador	Europe, Eastern North America and Central America	Eastern North America, Europe	Central & South America, West Africa, U.K.	Taiwan
Conidium characters													
Conidium L[1] (µm)	(2.5–)3.0–3.7 (–4.2)	(3.5–)3.7–4.5 (–4.7)	(3.0–)3.5–3.7 (–4.0) [7]	(3.2–)3.7–4.5 (–6.2)	(3.0–)3.5–4.2 (–5.0)	(3.0–)3.5–4.0(–4.5)	(3.0–)3.7–4.5 (–4.7)	(3.0–)3.5–4.5 (–6.2)	(2.7–)3.2–4.0 (–4.2)	(2.5–)3.5–4.5(–5.5)	(2.7–)3.5–4.5(–5.5)	(2.5–)3.0–4.0 (–5.0)	(3.5–)3.7–4.2 (–4.7)
95% CI	3.3–3.4	4.0–4.1	3.5–3.7	4.2–4.3	3.8–3.9	3.6–3.7	4.1–4.2	3.9–4.0 x	3.5–3.6	3.9–4.0	4.0–4.1	3.5–3.6	3.9–4.1
Conidium W[1] (µm)	(2.0–)2.2–2.7 (–3.2)	(2.2–)2.5–3.2 (–3.5)	(2.0–)2.2–2.7 (–3.0)[7]	(2.5–)3.0–3.5 (–3.7)	(2.5–)2.7–3.2 (–3.7)	(2.5–)2.7–3.2(–3.5)	(2.0–)2.5–3.0 (–3.5)	(2.0–)2.5–3.0 (–3.5)	(2.5–)3.0–3.2 (–3.7)	(2.2–)2.7–3.0(–3.5)	(2.2–)2.5–3.2(–4.2)	(1.7–)2.5–3.0 (–3.5)	(2.5–)2.7–3.2 (–3.5)
95% CI	2.5–2.6	2.8–3.0	2.4–2.6	3.1–3.2	3.0–3.1	3.0–3.1	2.6-2.7	2.6–2.7	3.0–3.1	2.8–2.9	2.8–2.9	2.6–2.7	2.8–3.1
Conidium L/W[1]	(0.9–)1.2–1.6 (–1.7)	(1.0–)1.2–1.6(–1.9)	(1.2–)1.3–1.5 (–1.9)[7]	(1.1–)1.2–1.6 (–2.1)	(1.1–)1.2–1.4(–1.6)	(0.9–)1.1–1.3(–1.5)	(1.3–)1.5–1.7 (–2.0)	(1.0–)1.3–1.8 (–2.5)	1.1–1.3(–1.6)	(1.0–)1.3–1.5(–1.8)	(0.8–)1.3–1.7(–2.2)	(0.9–)1.1–1.5 (–1.9)	(1.0–)1.2–1.6 (–1.8)
95% CI	1.3–1.4	1.4–1.5	1.4–1.5	1.3–1.4	1.2–1.3	1.2–1.3	1.5-1.6	1.49–1.52	1.15–1.19	1.35–1.39	1.4–1.5	1.3–1.4	1.3–1.5
Phialide characters													
Phialide length[1]	(5.2–)7.0–10.5 (–13)	3.5–9.0(–15.0)	(5.5–)6.0–10.2 (–14.0)[7]	(4.2–)6.7–11.2(–15.0)	(4.7–)6.5–10.0(–13.7)	(5.0–)5.7–8.2(–11.5)	(4.2–)6.2–10.0(-15.5)	(3.5–)5.5–9.2 (–16.0)	(4.0–)5.7–9.2 (–13.5)	(4.0–)6.2–10.2(–16.5)	(3.5–)6.0–10.0(–17.5)	(4.2–)5.5–9.0 (–12.5)	(5.2–)7.0–10.2(–13.2)
95% CI	8.3–9.2	5.2–6.2	7.4–9.0	8.5–9.5	7.9–8.7	6.6–7.2	7.9–8.3	7.2–7.5	7.2–7.8	8.1–8.6	7.8–8.3	7.1–7.5	8.0–9.2
Phialide at widest point[1]	(2.0–)2.5–3.0 (–3.5)	(2.0–)2.5–3.2 (–4.0)	(2.2–)2.5–3.2 (–3.7)[7]	(1.7–)2.2–3.2 (–4.0)	(1.7–)2.2–3.0(–3.5)	(2.2–)3.0–3.7(–4.5)	(2.0-)2.7–3.5 (–4.2)	(2.0–)2.7–3.5 (–4.5)	(2.0–)2.5–3.5 (–4.2)	(2.0–)2.5–3.5(–4.0)	(2.0–)2.5–3.5(–4.0)	(2.0–)2.7–3.5 (–4.5)	(2.2–)2.5–3.2(–3.5)
95% CI	2.6–2.8	2.8–3.0	2.8–3.1	2.7–2.9	2.6–2.8	3.2–3.4	3.0–3.1	3.1–3.2	3.0–3.2	2.9–3.0	3.0–3.1	3.1–3.2	2.6–2.9
Phialide base[1]	(1.5–)1.5–2.2 (–3.2)	(1.2–)1.5–2.2 (–2.5)	1.5–2.2(–2.5)	(1.2–)1.7–2.5 (–4.0)[7]	(1.2–)1.5–1.5 (–2.7)	(1.2–)1.5–2.5(–3.0)	(1.0–)1.5–2.2 (–3.0)	(1.0–)1.5–2.2 (–3.7)	(1.0–)1.5–2.5 (–3.2)	(1.0–)1.5–2.5(–3.5)	(1.2–)1.7–2.5(–3.2)	(1.2–)1.7–2.5 (–3.5)	(1.5–)1.8–2.5(–2.5)
95% CI	1.8–2.0	2.8–3.0	1.8–2.0	2.0–2.2	1.9–2.0	1.9–2.2	1.9-2.0	1.9–2.0	1.9–2.1	2.9–3.0	2.05–2.13	2.0–2.1	2.0–2.7
L/W of Phialide[1]	(1.8–)2.4–3.7 (–5.4)	1.2–3.1(–6.6)	(1.6–)1.9–3.9 (–5.7)[7]	(1.8–)2.5–4.5(–6.3)	(1.5–)2.3–4.1 (–6.8)	(1.4–)1.6–2.8(–4.0)	(1.2–)1.8–3.6 (–6.6)	(1.0–)1.7–3.3 (–6.0)	(1.2–)1.7–3.3 (–6.8)	(1.3–)2.1–3.9(–6.9)	(1.0–)2.7–3.7(–8.3)	(1.2–)1.4–3.1 (–5.6) µm	(2.1–)2.7–4.0(–5.1)
95% CI	3.1–3.6	1.8–2.4	2.5–3.3	3.3–3.7	3.0–3.3	2.0–2.3	2.6-2.8	2.4–2.5	1.6–3.4	2.8–3.0	2.6–2.8	2.3–2.4 µm	2.9–3.5

Phialide supporting cell[1]	(1.5–)2.0–3.0 (–3.5)	(1.5–)2.0–3.0(–3.7)	(1.7–)2.0–2.7 (–3.0)[7]	(1.7–)2.2–4.2(–6.5)	(1.5–)1.7–5.0(–9.5)	(2.0–)2.5–3.5(–4.0)	(1.5–)2.2–3.0 (–4.0)	(1.7–)2.2–3.5 (–7.7)	(1.7–)2.2–4.0 (–6.2)	(1.5–)1.7–2.2 (–8.0)	(1.5–)2.5–3.2(–4.0)	(1.6–)2.2–3.2 (–5.0) µm	(2.0–)2.0–3.0(–3.5)
95% CI	2.4–2.6	2.4–2.6	2.2–2.5	3.1–3.5	3.0–3.7	2.9–3.1	2.6-2.7	2.8–2.9	3.0–3.3	2.9–3.1	2.7–2.8	2.7–2.8 µm	2.4–2.7
Ascus characters													
Ascus length	(65–)75–90 (–100)	65–75(–85)		(45–)66–95 (–105)	*ca.* 70[3]	(56–)65–77 (–84)	(56-)62-72 (-80)	(60–)65–75 (–80)		(55–)67–85 (–105)	(51–)69–89 (–128)	(65–)72–88 (–98) µm	(60–)63–73 (–78)
95% CI	81–85	70–75		76–85		70–73	66–70	69–72		75–78	78–80	79–82 µm	63–72
Ascus width	(3.2–)4.2–5.7 (–6.5)	(3.5–)4.5–6.0 (–7.0)		(3.0–)4.0–6.0 (–7.7)	4.5–5.5[3]	(2.7–)4.0–5.2(–6.2)	(4.2-)4.5-6.2 (-7.0)	(3.5–)4.0–6.0 (–7.5)		(3.0–)4.5–6.2 (–8.0)	(3.5–)4.2–6.2(–8.2)	(4.0–)5.0–6.5 (–7.7) µm	(3.2–)3.7–5.0 (–5.2)
95% CI	4.8–5.2	4.9–5.4		4.8–5.4		4.4–4.8	5.2–5.8	4.7–5.3		5.2–5.4	5.3–5.5	5.5–5.8 µm	3.7–4.8
Ascospore characters													
Distal part-ascospore length	(3.2–)3.5–4.2 (–5.0)	(2.7–)3.2–4.2 (–4.7)		(2.0–)3.0–4.2 (–5.0)	3.0-4.5(–6.5)	(2.5–)3.0–3.7(–4.0)	(2.7-)3.5–4.5(-4.6)	(2.7–)3.2–4.0 (–4.5)		(2.2–)3.5–4.5 (–5.5)	(3.0–)3.5–4.5(–5.2)	(3.2–)3.5–4.5 (–5.0) µm	(2.7–)3.0–4.0 (–4.7)
95% CI	3.8–3.9	3.7–3.8		3.6–3.9	3.6–4.0	3.2–3.4	3.9–4.2	3.7–3.8		3.8–4.0	3.9–4.0	3.9–4.1 µm	3.2–3.7
Distal part-ascospore width	(2.7–)3.2–3.7 (–4.2)	(2.5–)3.0–3.5 (–4.0)		(1.7–)2.5–4.0 (–4.5)	(2.2–)2.7–3.7 (–5.2)	(2.2–)2.7–3.5(–3.7)	(2.5-)3.0–3.5 (-4.0)	(2.0–)2.7–3.5 (–4.0)		(2.0–)3.2–4.2 (–5.0)	(2.5–)3.2–4.0(–5.0)	(2.7–)3.2–3.7 (–4.2) µm	(2.5–)3.0–3.5 (–4.0)
95% CI	3.5–3.6	3.2–3.4		3.1–3.4	2.8–3.8	3.0–3.2	3.2–3.4	3.1–3.3		3.7–3.8	3.6–3.7	3.4–3.5 µm	3.1–3.3
Proximal part-ascospore length	(3.2–)2.5–4.5 (–5.0)	(2.7–)3.5–4.7 (–5.2)		(2.5–)3.0–4.7 (–6.5)	(3.5–)3.7–5.0 (–6.5)	(2.5–)2.5–4.2(–4.5)	(3.2-)3.7-4.5 (-5.2)	(3.0–)3.7–4.7 (–6.0)		(2.7–)3.7–5.0 (–6.5)	(3–)4–5(–6)	(3.2–)4.0–5.0 (–6.0) µm	(3.0–)3.5–4.5 (–5.2)
95% CI	3.9–4.1	4.1–4.4		3.8–4.1	4.2–4.6	3.6–3.9	4.0–4.3	4.1–4.4		4.4–4.6	4.4–4.5	4.5–4.7 µm	3.7–4.1
Proximal-part width	(2.7–)3.0–3.5 (–4.2)	(2.5–)2.7–3.2 (–3.7)		(1.7–)2.5–3.5 (–4.2)	2.5–3.5 (–4.2)	(2.2–)2.5–3.0(–3.2)	(2.2-)2.5-3.0 (-3.7)	(1.7–)2.2–3.0 (–3.5)		(2.2–)2.7–3.7 (–5.2)	(2.0–)2.7–3.5(–4.0)	(2.2–)2.7–3.2 (–4.0) µm	(2.2–)2.5–3.2
95% CI	3.1–3.2	3.0–3.1		2.9–3.2	2.9–3.1	2.7–2.8	2.6–2.9	2.6–2.8		3.2–3.3	3.1–3.2	3.0–3.1 µm	2.7–2.9
Colony radius and slope of growth curve on PDA after 72h (mm)													
Radius 15 °C[1]	**65**	**13–15[5]**	7–10[4]	11–17[5]	16–17[5]	**8**–10[5]	(12–)15–19	(8–)9–17(–30)	(5–)6–13	(10–)11–13 (–14)	(8–)8.5–11 (–13)	(2–)3–13(–17)	10–12[5]
Radius 20 °C[1]	**18–20[5]**	365	25–42[4]	22–37[5]	31–42[5]	25–42[5]	(26–)31–38	(23–)30–40 (–42)	(31–)31–37	(19–)23–29	(23–)24–28(–31)	(11–)15–39 (–43)	21–29[5]
Radius 25 °C[1]	33–35[5]	53–56[5]	41–50[4]	24–29[5]	39–41[5]	40–50[6]	(41–)49–58	(45–)51–63 (–67)	(38–)43–66	(26–)33–45	(38–)40–48(–50)	(36–)38–54 (–64)	43–46[5]
Radius 30 °C[1]	(36–37[5]	54–58[5]	9–16[4]	1–3[5]	20–28[5]	9–16[5]	(35–)45–61	(20–)52–72	48–70	(25–)32–48	(40–)40–52 (–60)	36–54(–69)	42–43[5]
Radius 35 °C[1]	0.0–1.0[5]	1–2[5]	0–1[4]	15	1–2[5]	0–1[5]	(0–)1–2(–3)	(0–)2–10(–12)	5–14	0–4	(0–)0.7–4 (–5)	0–3	0
slope PDA 15 °C[1]	0.113–0.135[5]	0.204–0.222[5]	0.2[4]	0.209–0.263[5]	0.272–0.318[5]	0.125–0.169[5]	(0.225–) 0.255–0.311 (–0.314)	(0.053–)0.163–0.281(–0.310)	0.125–0.185[5]	(0.178–) 0.187–0.223(–0.232)	(0.138–) 0.151–0.191 (–0.210)	(0.049–)0.092–0.222(–0.242)	0.163–0.216[5]

Table 3. (Continued).

	Species												
	T. austrokoningii [2]	*T. caribbaeum* var. *caribbaeum* [2]	*T. caribbaeum* var. *aequatoriale* [1, 4]	*T. dingleyeae* [3]	*T. dorotheae* [3]	*T. intricatum* [2]	*T. koningii* [11]	*T. koningiopsis* [23]	T. *ovalisporum* [4]	*T. petersenii* [8]	*T. rogersonii* [13]	*T. stilbohypoxyli* [11]	*T. taiwanense* [1]
slope PDA 20 °C[1]	0.329–0.336[5]	0.580–0.599[5]	0.475–0.625[4]	0.353–0.428[5]	0.493–0.554[5]	0.329–0.492[5]	(0.492–) 0.540–0.610	(0.272–)0.503–0.676	0.522–0.654[5]	(0.340–)0.396–0.475 (–0.468)	(0.371–) 0.414–0.504 (–0.540)	(0.271)0.340–0.683	0.395–0.495[5]
slope PDA 25 °C[1]	0.522–0.540[5]	0.603–0.608[5]	0.695–0.721[4]	0.332–0.389[5]	0.610–0.617[5]	0.608–0.624[5]	(0.547–) 0.544–0.706 (–0.829)	(0.558–)0.550–0.742(–0.815)	0.551–0.856[5]	(0.562–) 0.586–0.622 (–0.629)	(0.585–) 0.602–0.674 (–0.724)	(0.631–)0.673–0.823(–0.848)	0.625–0.633[5]
slope PDA 30 °C[1]	0.055–0.539[5]	0.593–0.601[5]	0.108–0.150[4]	5	0.261–0.486[5]	0.547–0.631[5]	(0.540–) 0.556–0.606 (–0.618)	(0.269–)0455–0.735(–0.807)	0.519–0.793[5]	(0.214–) 0.362–0.648	(0.550–) 0.587–0.629 (–0.641)	(0.650–)0.659–0.749(–0.782)	0.592–0.629[5]
slope PDA 35 °C[1]	0	0	0	0	0	0	0	(0.003–0.063 (–0.111)	0.007–0.333	0	0	0	**0**
Colony radius and slope of growth curve on SNA after 72h													
Colony radius SNA 15 °C[1]	6.0–7.0[5]	8–9[5]	8–15[4]	8–17[5]	7–10[5]	8–15[5]	(5–)7–13	(5.3–)8–12(–14)	(5.0–)5.0–10.0	(6–)7–12(–13)	(1.5–)3.0–8.0(–10)	(2–)3–11(–12)	6–8[5]
Colony radius SNA 20 °C[1]	17–20[5]	22–24[5]	20–27[4]	14–22[5]	17–21[5]	20–27[5]	(14–)18–23	(16–)22–31 (–43)	(25–)19–27	(19–)20–24	(10–)13–19(–21)	(13–)17–27 (–30)	16–25[5]
Colony radius SNA 25 °C[1]	27–34[5]	41–42[5]	31–33[4]	22–30[5]	19–28[5]	31–33[5]	(25–)29–35	(32–)37–47 (–51)	40–48	(27–)31–35	(15–)21–31(–33)	(22–)28–49 (–41)	27–48[5]
Colony radius SNA 30 °C[1]	25–31[5]	36–46[5]	224	1–2[5]	12–15[5]	65–70[5]	(20–)21–27 (–28)	(15–)32–54 (–64)	39–41(–51)	(22–)26–31 (–34)	(12–)14–28(–35)	(25–)30–43 (–45)	30–50[5]
Colony radius SNA 35 °C[1]	0–1[5]	0.5–1.0	0	0–1	0–1	0	(0.5–)1.0–1.8 (–2.0)	(0–)1.5–6.2 (–14)	4–7	(0–)0.2–1.5 (–2)	0–3.2	0–1	0–2[5]
Colony slope SNA 15 °C[1]	0.119–0.146[5]	0.156–0.182[5]	0.167–0.183[4]	0.156–0.189[5]	0.134–0.186[5]	0.057–0.085[5]	(0.057–) 0.110–0.202 (–0.226)	(0.085–)0.121–0.201(–0.232)	0.164–0.236[5]	(0.115–) 0.124–0.214 (–0.232)	(0.054–) 0.064–0.148 (–0.190)	0.087–0.199	0.104–0.114[5]
Colony slope SNA 20 °C[1]	0.236–0.340[5]	0.397–0.406[5]	0.341–0.413[4]	0.385–0.442[5]	0.253–0.396[5]	0.200–0.367[5]	(0.200–) 0.272–0.444 (–0.482)	(0.161–)0.290–0.462(–0.518)	0.401–0.554[5]	(0.311–) 0.313–0.419 (–0.461)	(0.213–) 0.248–0.318 (–0.321)	0.227–0.381	0.220–0.383[5]

Colony slope SNA 25 °C[1]	0.460–0.557[5]	0.451–0.594[5]	0.495–0.550[4]	0.603–0.633[5]	0.275–0.614[5]	0.424–0.640[5]	(0.275–) 0.328–0.618 (–0.712)	(0.215–)0.453–0.675(–0.779)	0.504–0.789[5]	(0.465–) 0.486–0.554 (–0.520)	(0.354–) 0.392–0.482 (–0.485)	0.264–0.574	0.371–0.650[5]
Colony slope SNA 30 °C[1]	0.371–0.451[5]	0.160–0.403[5]	0.325[4]	0.321–0.619[5]	0.075–0.619[5]	0.457–0.653[5]	(0.014–) 0.100–0.558 (–0.654)	(0.004–)0.296–0.676(–0.782)	0.297–0.769[5]	(0.206–) 0.290–0.450 (–0.483)	(0.200–) 0.229–0.391 (–0.461)	0.038–0.537	0.354–0.567[5]
Colony slope SNA 35 °C[1]	0	0	0	0	0	0.168	0	0	0	0	0	0	0–0.004[5]
Colony morphology on PDA													
Colony type after 96 h in light	growth slow; conidia formed abundantly in two concentric rings	fast; faint concentric rings; few conidia	growth slow; abundant conidia in concentric rings	growth slow; mycelium uniform or abundant in faint concentric rings; sterile	growth slow; mycelium uniform or abundant in faint concentric rings; sterile or not	fast; faint concentric rings; few conidia	growth fast; faint to pronounced concentric rings, conidia typically abundant	growth fast; faint to pronounced concentric rings, conidia typically abundant	growth fast; faint to pronounced concentric rings, conidia typically abundant	growth slow; conspicuous concentric rings; conidia abundant, lacking any yellow color	growth slow; few faint concentric rings; conidia formed abundantly in the center of the colony	growth slow, abundant conidial production in 3 or 4 broad concentric rings. Some yellow pigment in developing conidia and PDA reverse	growth slow, few conidia in concentric rings
Time of appearance of green conidia on PDA in darkness[1] hours	48	72–96	48	>96	72–96	>96	72	48	48	48–72	24(–48)	48(–72)	48
Temperature of appearance of green conidia on PDA in darkness[1] °C	25	20	25–30	25	20–25	25	25–30	(20–)25–30	20	20–25	25–30	25–30	25

[1] Characters used in PCO analysis.

[2] Number of collections.

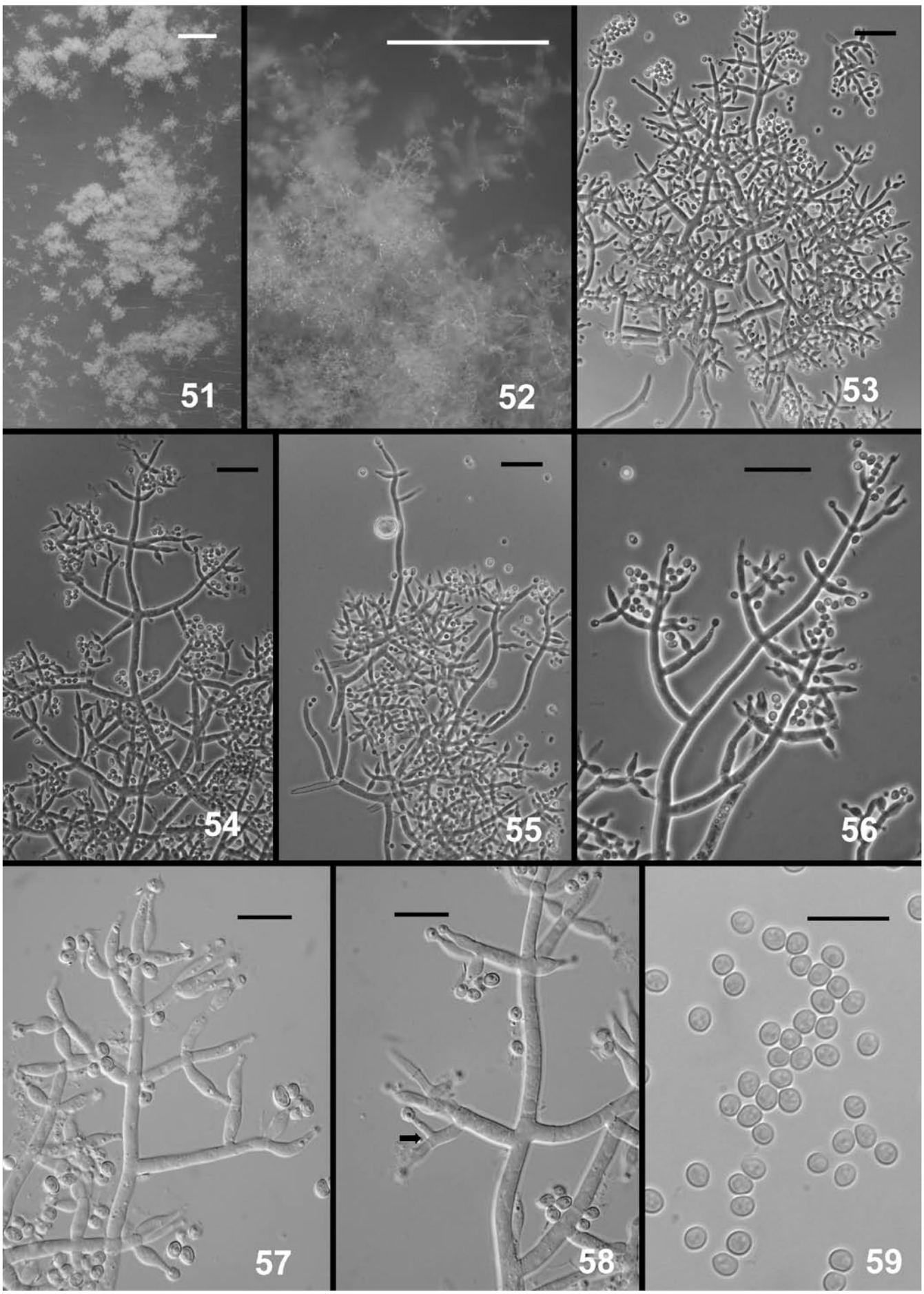
[3] Only two asci could be measured from three collections.

[4] Continuous measurements from SNA. All measurements pertaining to growth rate report the range observed in three, independent trials.

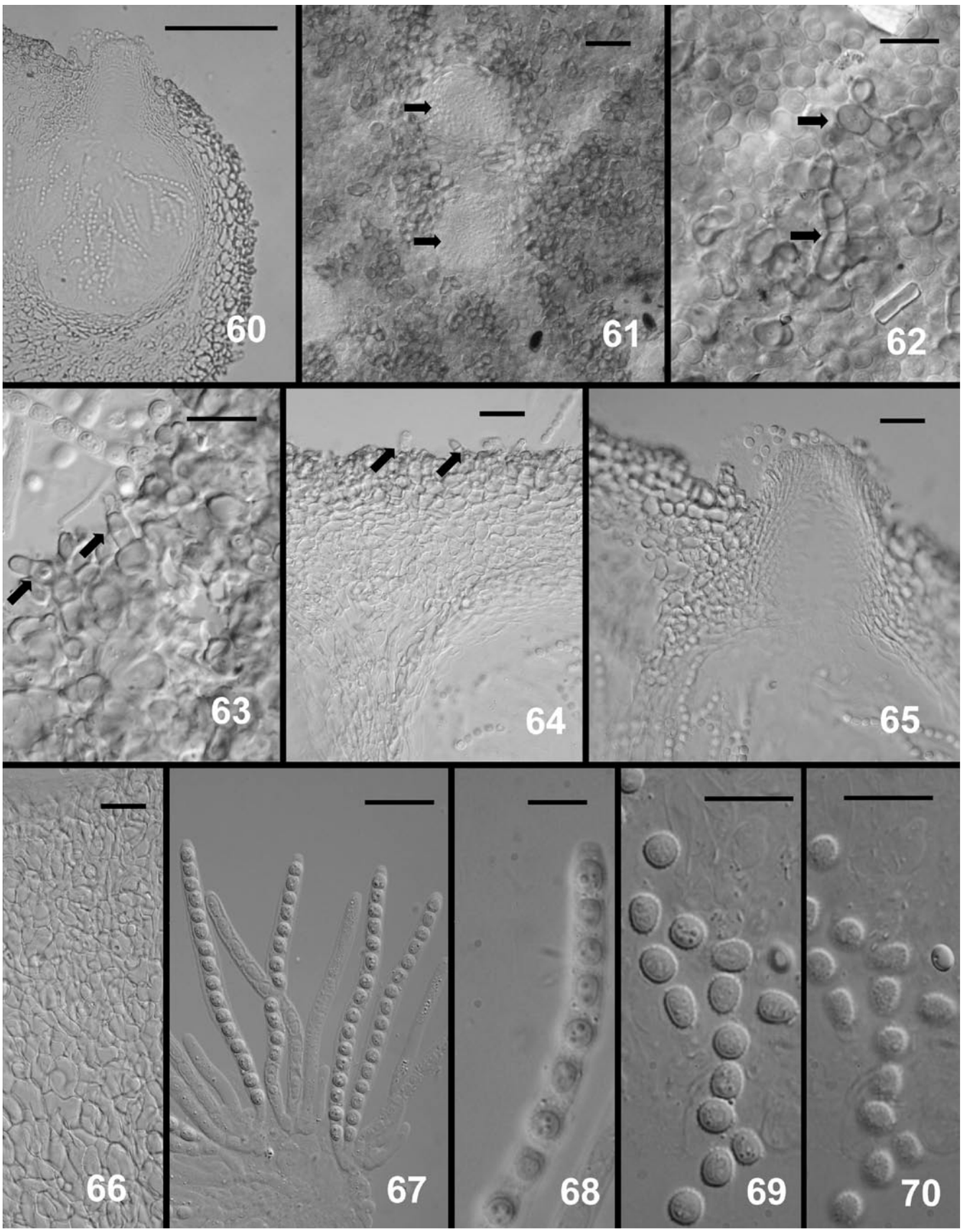
[5] maximum and minimum of all cultures studied.

[6] Total range of two isolates.

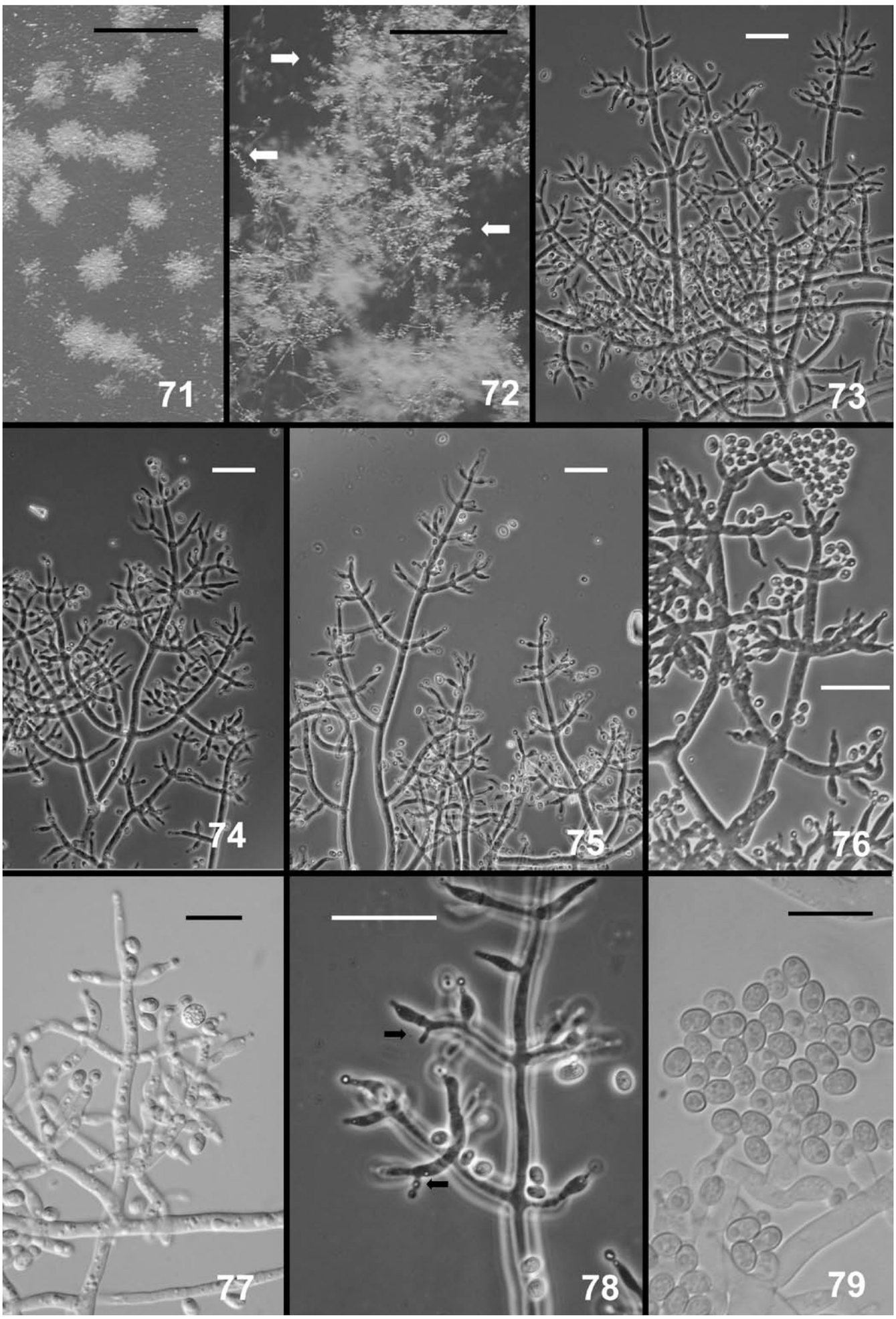
[7] Measured from pustules.



Figs 51–59. *T. austrokoningii*, anamorph (Queensland, including type; all from CMD). 51–52. Conidial pustules. 53–58. Conidiophores and phialides. Intercalary phialide shown in 58 (arrow). 59. Conidia. Figs 51, 53–55, 59 from G.J.S. 99-147; 52, 56–58 from G.J.S. 99-146. Microscopy: 51–52 = stereo; 53–56 = PC; 57–59 = DIC. Bars: 51 = 1 mm; 52 = 0.5 mm; 53–58 = 20 μm; 59 = 10 μm.



Figs 60–70. *T. austrokoningii*, *Hypocrea* teleomorph (Queensland, including type). 60. Median longitudinal section of a mature perithecium. 61. Surface view of stroma showing two ostiolar openings (arrow) and small cells that form hairs. 62–63. Hairs arising from stroma surface (arrows). 64. Section of stroma surface showing hairs (arrows) and pigmented outer region. 65. Median longitudinal section through an ostiolar canal. 66. Cells of stroma interior below perithecia. 67–68. Asci. Apical ring visible in 68–70. Discharged ascospores. Spores shown in optical section in 69, surface view showing ornamentation in 70. Figs 60, 63–64, 66–67, 69–70 from G.J.S. 99-147; 61–62, 65, 68 from G.J.S. 99-146. Microscopy: 60 = BF; all others DIC. Bars: 60 = 100 µm; 61–68 = 20 µm; 69–70 = 10 µm.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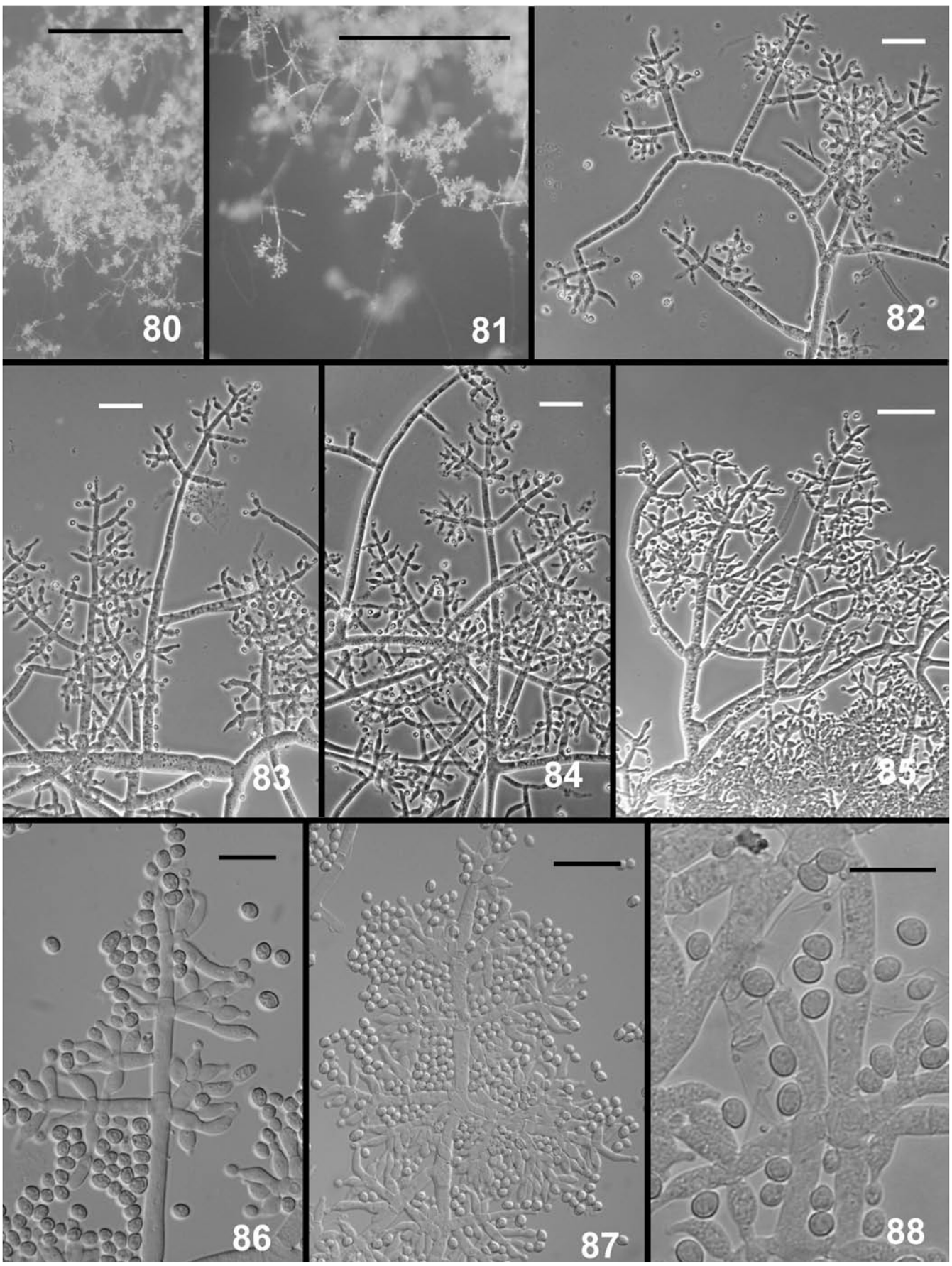
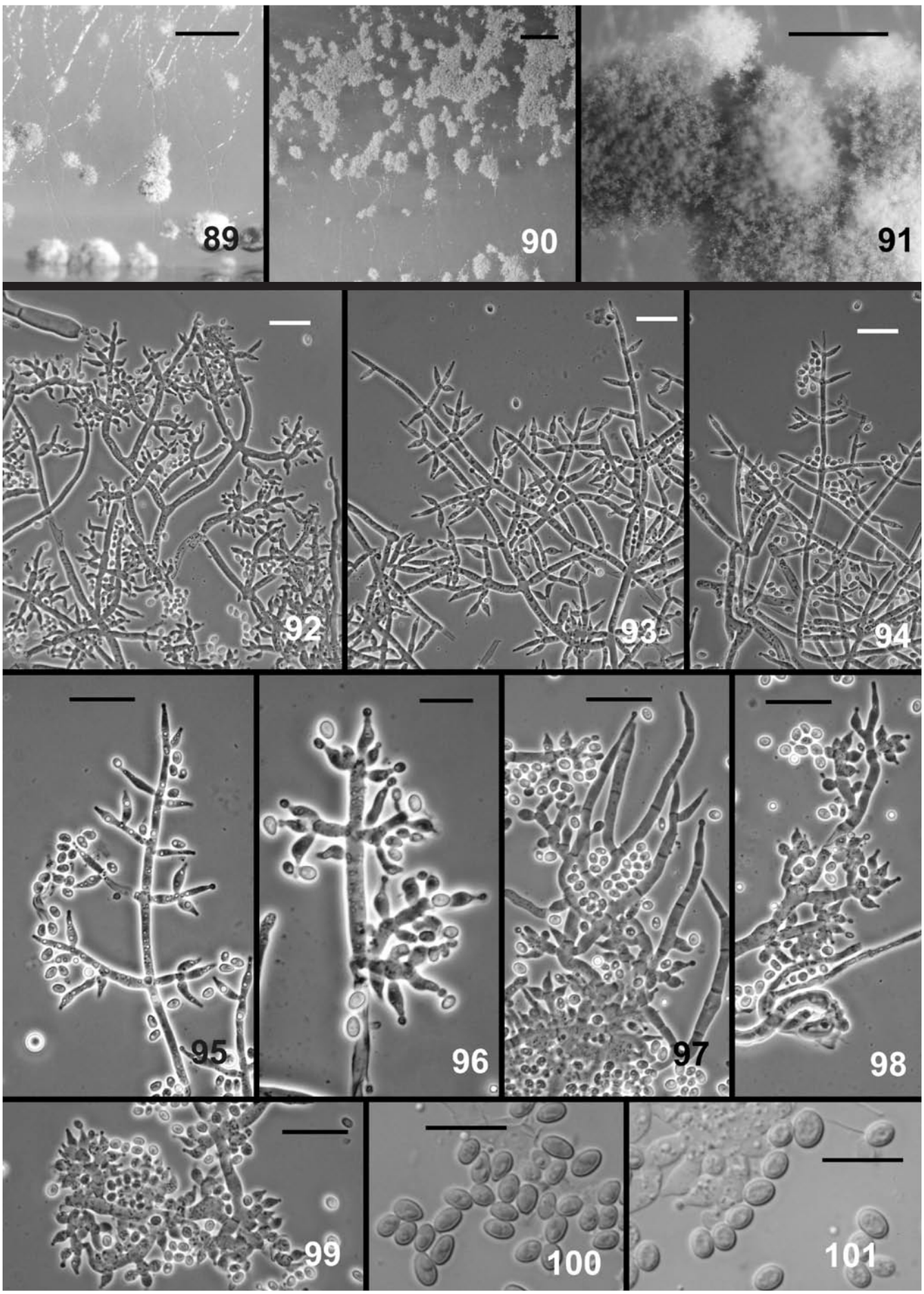
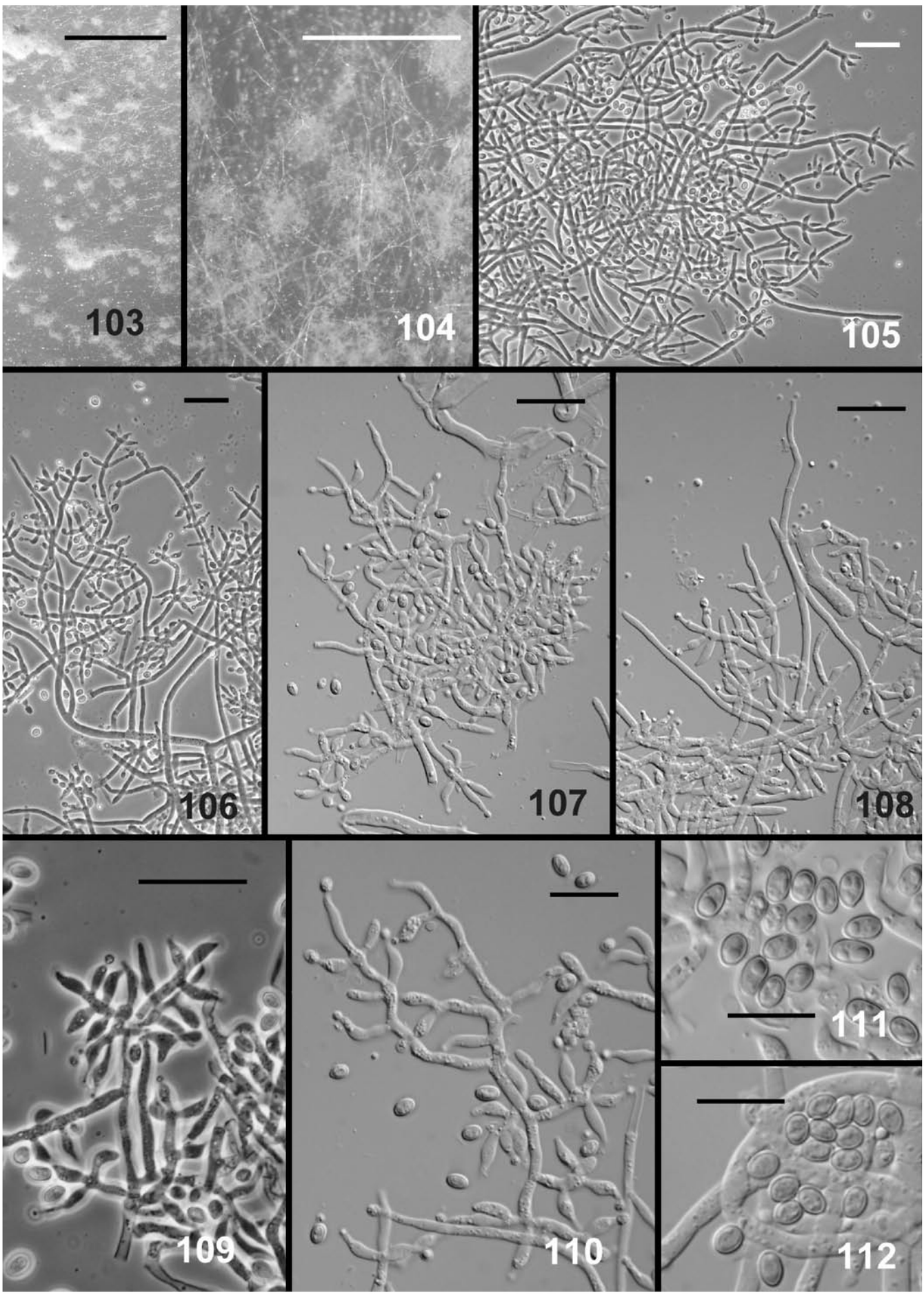


Figs 80–88. *Trichoderma austrokoningii* from New Zealand on CMD. 80. Aggregated conidiophores. 81–87. Conidiophores. 88. Conidia. Figs 80–84, 86–87 from G.J.S. 99-116; 85, 88 from CBS 243.63. Microscopy: 80–81 = stereo; 82–85 = PC; 86–88 = DIC. Bars: 80 = 1 mm; 81 = 0.5 mm; 82–87 = 20 µm; 88 = 10 µm.

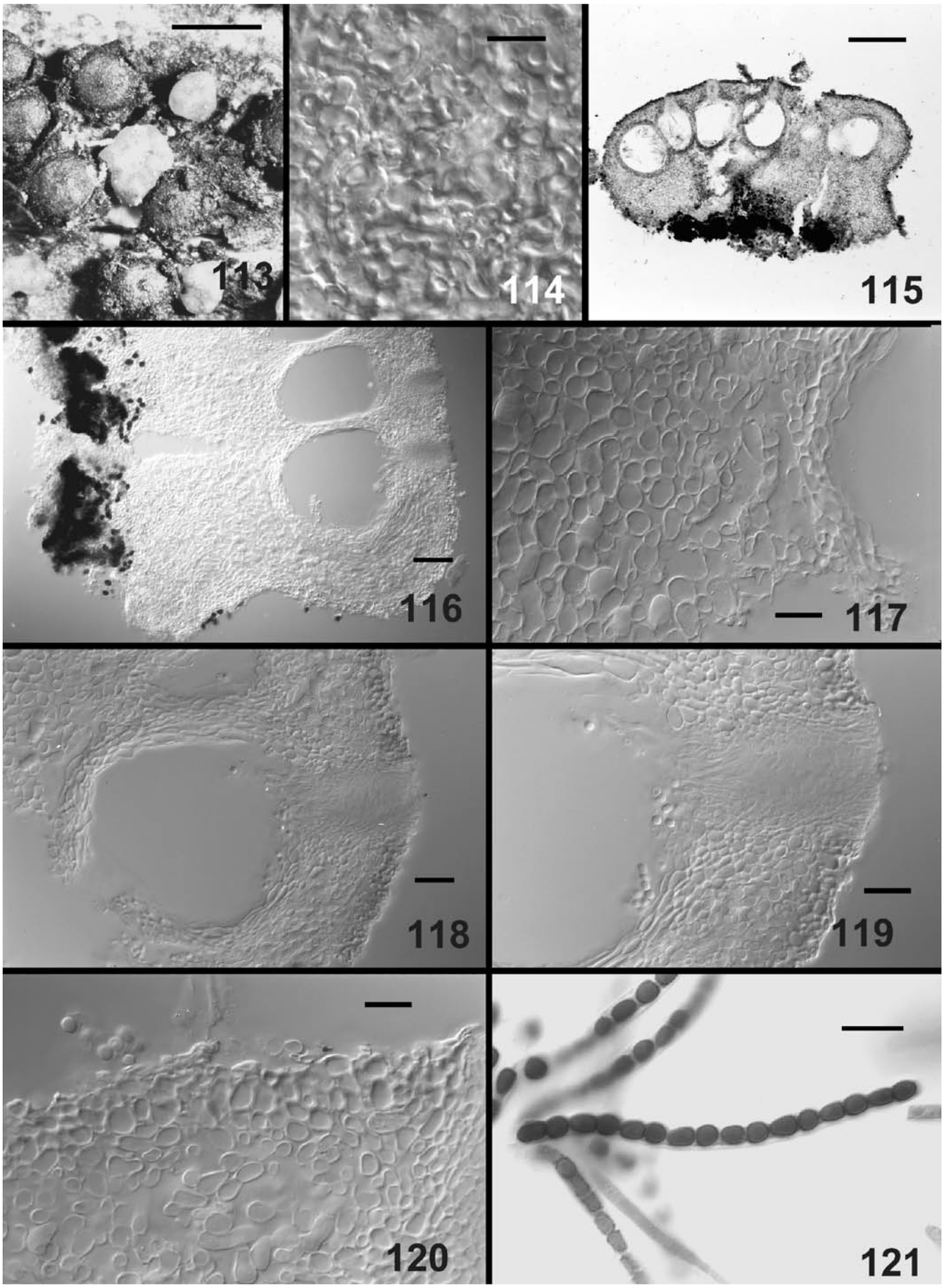
Figs 71–79. (Page 100). *Trichoderma austrokoningii* from Russia (G.J.S. 00-73) on CMD. 71–72. Conidial pustules. Note long, entirely fertile conidiophores in 72 (examples marked with arrows). 73–78. Conidiophores and phialides. Intercalary phialides indicated by arrows in 78. 79. Conidia. Microscopy: 71–72 = stereo; 73–76, 78 = PC; 77, 79 = DIC. Bars: 71 = 1 mm; 72 = 0.5 mm; 73–78 = 20 µm; 79 = 10 µm.



Figs 89–101. *Trichoderma austrokoningii* from Florida and Taiwan on CMD. 89–91. Conidial pustules. 92–99. Conidiophores. 100–101. Conidia. Figs 89, 91, 97–99, 101 from G.J.S. 96-163; 90, 92-96, 100 from C.T.R. 85-57. Microscopy: 89–91 = stereo; 92–99 = PC; 100–101 = DIC. Bars: 89–90 = 1 mm; 91 = 0.5 mm; 92–99 = 20 µm; 100–101 = 10 µm.



Figs 103–112. *Trichoderma caribbaeum* var. *caribbaeum*, anamorph from CMD. 103–104. Conidial pustules. 105–110. Conidiophores. 111–112. Conidia. Figs 103, 107, 109–111 from G.J.S. 98-43; 104–106, 108, 112 from G.J.S. 97-3. Microscopy: 103–104 = stereo; 105–106, 109 = PC; 107–112 = DIC. Bars: 103 = 1 mm; 104 = 0.5 mm; 105–110 = 20 µm; 111–112 = 10 µm.



Figs 113–121. *Trichoderma caribbaeum* var. *caribbaeum*, *Hypocrea* teleomorph. 113. Stromata of *Hypocrea* (light coloured) growing on xylariaceous host. 114. Face view of stroma surface. 115. Section through a single stroma. 116, 118. Median longitudinal sections through mature perithecia. 117. Internal tissue of the stroma below perithecia; the lower wall of a perithecium seen on the right. 119. Section through ostiolar canal. 120. Stroma surface. 121. Ascus with ascospores (in 1 % aq. phloxine). Figs 113, 115–121 from G.J.S. 97-3; 114 from G.J.S. 98-43. Microscopy: 113 = stereo, 114, 116–120 DIC; 115, 121 = BF. Bars: 113 = 1 mm; 114, 121 = 10 µm; 115 = 200 µm; 116–120 = 20 µm.

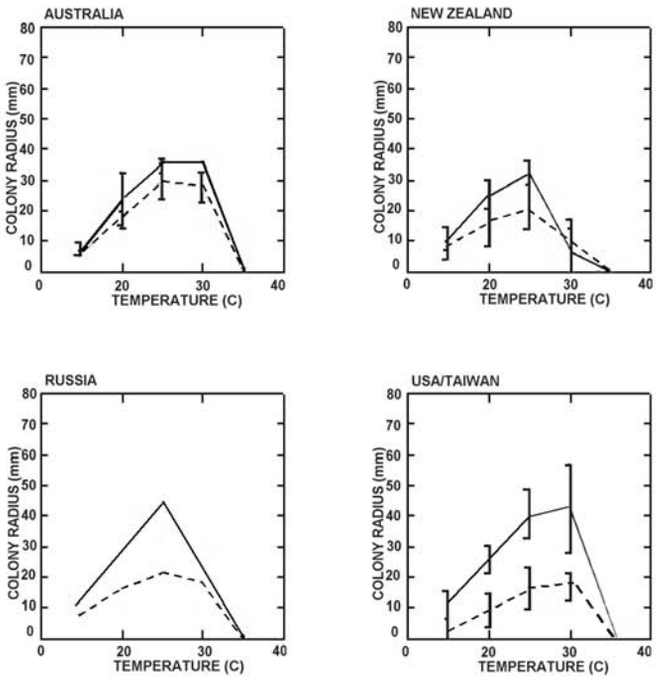


Fig. 102. Growth curves of variants of *T. austrokoningii*. Solid line = PDA, broken line = SNA. The following isolates were included: Australia (G.J.S. 99-146, G.J.S. 99-174), New Zealand (G.J.S. 99-116, CBS 243.63), Russia (G.J.S. 00-73), U.S.A./Taiwan (C.T.R. 85-57, G.J.S. 96-163). Standard error bars are shown.

2. *Trichoderma caribbaeum* Samuels & Schroers var**. *caribbaeum*, sp. nov.** MycoBank MB501034. Figs 9, 103–112.

Teleomorph: ***Hypocrea caribbaea*** Samuels & Schroers**, sp**. **nov**. MycoBank MB501035. Figs 28–29, 113–121.

Etymology: In reference to the Caribbean Ocean region where the species has been collected.

Stromata pallide ad aurantio-brunnea, *H. rufae* (Fr.) Fr. similia**.** Ascosporae hyalinae, spinulosae. Pars distalis ascosporarum (2.7–)3.2–4.2(–4.7) × (2.5–)3.0–3.5(–4.0) µm; pars proxima (2.7–) 3.5–4.7(–5.2) × (2.5–)2.7–3.2(–3.7) µm. Anamorphosis *T. koningii* Oudem. similis, conidia viridia, ellipsoidea, (3.5–)3.7–4.5(–4.7) × (2.2–)2.5–3.2(–3.5) µm; ratio longitudinis:latitudinis (1.0–)1.2–1.6 (–1.9). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate 53–56 mm.

Holotypus *H. caribbaea* BPI 746700; holotypus anamorphosis *T. caribbaeum* cultura sicca ex ascospora oriens BPI 746700B.

Stroma scattered, light brown to brownish orange (6C–6D), not reacting to KOH, discrete, 0.5–1.0 mm diam, irregular or nearly circular in outline, more or less pulvinate, broadly attached; plane, appearing smooth, perithecial elevations not evident or appearing as low tubercules, ostiolar openings barely visible as slightly dark areolae, young stroma appearing velvety. Hyphal hairs arising from the stroma surface 7–10 µm long, septate, 2–3 µm wide, absent from mature stromata. Cells of the stroma surface in face view pseudoparenchymatous, 4–10 µm diam, walls thickened, unevenly pigmented. Surface region of the stroma 20–35 µm thick, composed of pigmented, compact, pseudoparenchymatous cells with thickened walls. Tissue below the stroma surface of intertwined hyphae or more compact and then more or less pseudoparenchymatous. Tissue below the perithecia pseudoparenchymatous, cells 8–12 × 5–7 µm, thin-walled. Perithecia subglobose, 150–210 µm high, 80–150 µm wide; ostiolar canal 60–80 µm long; perithecial apex around the ostiolar opening not anatomically distinct from the cells of the stroma surface. Asci cylindrical, apex thickened, with a minute pore. Part-ascospores hyaline, finely spinulose, dimorphic; distal part subglobose to slightly conical.

Characteristics in culture: Optimum temperature for growth on PDA and SNA 25–30 °C. Colonies grown on PDA with faint concentric rings and poor conidial production after 96 h at 25–30 °C. Colonies grown on SNA producing abundant aerial mycelium, sterile after 96 h. Conidia green (27E7–8), without yellow coloration. No pigment diffusing through the agar, no distinctive odour. Conidia forming slowly on PDA, after 72–96 h at 20 °C, later at higher temperatures. Colonies grown on CMD at 20 °C under light filling the Petri plate within 1 wk; conidia forming in scattered pustules in concentric rings; pustules to 1 mm diam, tending to coalesce, dense; conidia also forming well apart from the pustules in the scant aerial mycelium. Conidiophores projecting from the pustules, entirely fertile or sparingly branched along the length, sometimes bearing only one or a few phialides at the tip but otherwise sterile. Conidiophores highly intricated within the pustule, a strongly developed main axis not discernable, lateral branches tending to be solitary, paired branches uncommon. Phialides held in cruciate to verticillate whorls of 3 or 4 or arising singly, especially near the tip of the main axis and along the length of *1°* branches, straight, lageniform, somewhat swollen in the middle; intercalary phialides present but not common. Conidia ellipsoidal to nearly oblong, smooth. Chlamydospores produced sparingly on CMD, terminal on hyphae, subglobose, (n = 30) (5.5–)6.7–10(–11) µm diam.

Habitat: On pyrenomycetes (incl. *Xylariaceae*) and decorticated wood.

Known distribution: Guadeloupe, Puerto Rico.

Holotype: **Guadeloupe**, Rain forest St. Claude, Basse Terre, on ?*Penzigia* on *Bambusa vulgaris*, 11 Jan. 1997, J. Vivant Guad 97-03, comm. F. Candoussau (teleomorph: BPI 746700; holotype of *T. caribbaeum* var. *caribbaeum* a dry culture BPI 746700B; ex-type culture G.J.S. 97-3 = CBS 119093, culture derived from ascospore isolates of the *Hypocrea* sp. teleomorph).

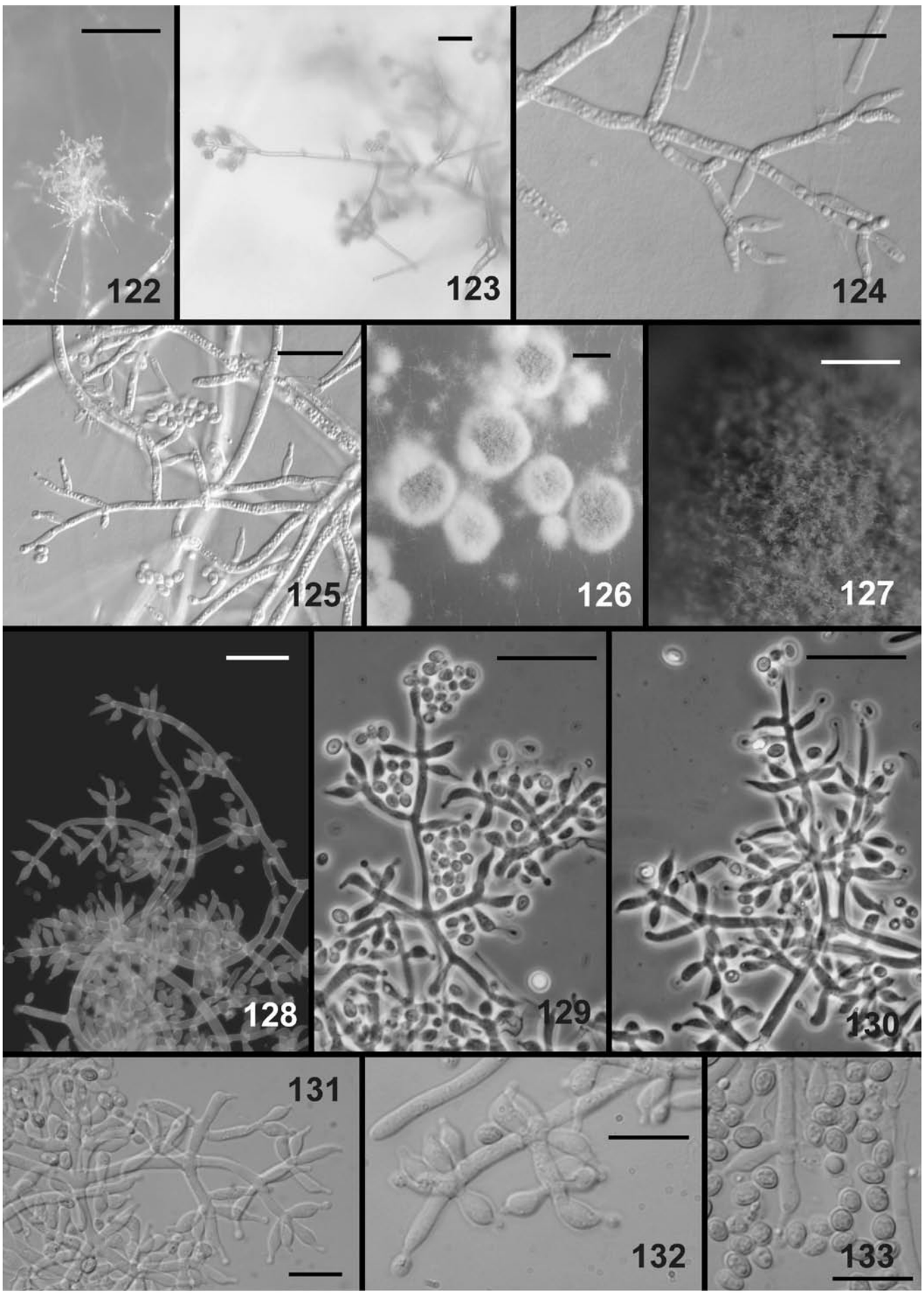
Additional specimen examined: **Puerto Rico**, Río Grande, Caribbean National Forest, Luquillo Mts., Trade Winds Trail, elev. 950 m, on fungus on decorticated wood, 11 June 1998, G.J.S. (BPI 748388, culture G.J.S. 98-43 = CBS 119054).

Notes: *Trichoderma caribbaeaum* var. *caribbaeum* is sympatric in Puerto Rico with *T. stilbohypoxyli* and *T. intricatum.* For comparison of these species see the discussion under *T. intricatum.*

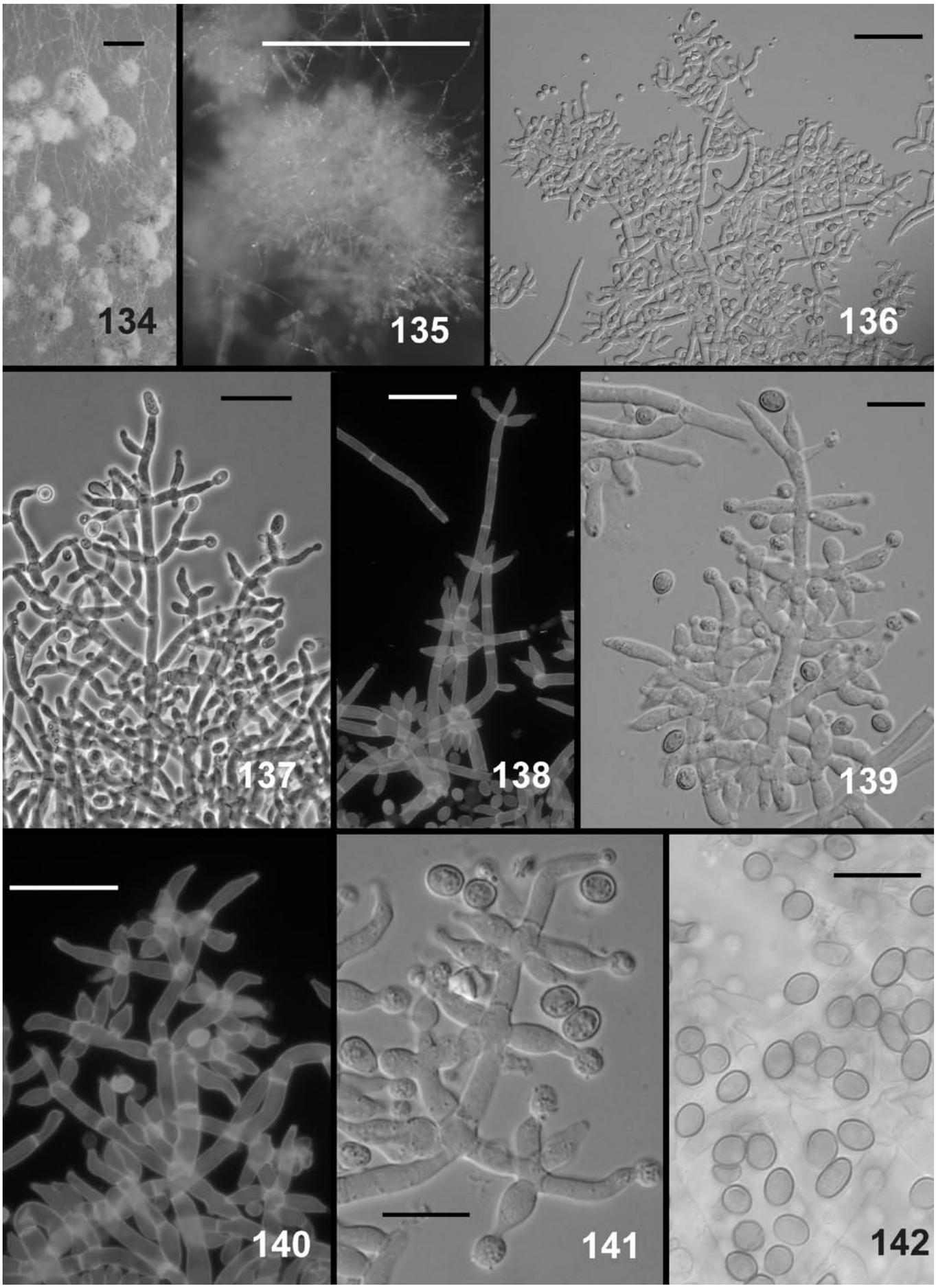
3. *Trichoderma caribbaeum* var. *aequatoriale* Samuels & H.C. Evans, **var. nov**. MycoBank MB500561. Figs 122–133.

Etymology: "*Aequatoriale"* in reference to Ecuador, where the species has been found.

Conidiophora mononemata, verticillata vel in pustulis orientia, *T. koningii* Oudem. similia. Conidia viridia, late ellipsoidea, (2.5–)3.0–



Figs 122–133. *Trichoderma caribbaeum* var. *aequatoriale* from SNA (DIS 320c). 122–125. Conidiophores formed in the aerial mycelium. 126–127. Conidial pustules. 128–132. Conidiophores produced from pustules. 133. Conidia. Microscopy: 122, 126–127 = stereo, 123 = BF; 124–125, 131–133 = DIC; 128 = FL; 129–130 = PC. Bars: 122, 127 = ¼ mm; 126 = 1 mm; 123, 125, 128–130 = 20 µm; 124, 131–133 = 10 µm.



Figs 134–142. *Trichoderma dingleyae*, anamorph from CMD. 134–135. Conidial pustules. 136–141. Conidiophores. 142. Conidia. Figs 134–135 from G.J.S. 02-50; 136, 139, 141 from G.J.S. 99-203; 137–138, 140 from G.J.S. 02-50; 142 from G.J.S. 99-203. Microscopy: 134–135 = Stereo, 136, 139, 141–142 = DIC, 137 = PC; 138, 140 = FL. Bars: 134–135 = 1 mm; 136–138, 140 = 20 µm; 139, 141–142 = 10 µm.

3.5(–3.7) × (1.1–)1.2–1.6(–2.1) µm; ratio longitudinis:latitudinis (1.2–)1.3–1.5(–1.9). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate *ca.* 45 mm.

Holotypus cultura sicca, BPI 870965.

Teleomorph: none known.

Optimum temperature for growth on PDA and SNA 25 °C. Not growing at 35 °C. Colonies grown on PDA and SNA in darkness sterile within 96 h; conidia forming on PDA, CMD and SNA at 20–25 °C within 10 d when grown under light. On PDA and CMD mononematous conidiophores obscure, conidia held in pale green drops of watery liquid. Conidiophores on CMD mononematous and produced in pustules; mononematous conidiophores 75–85 µm long, 3.5–5.5 µm wide at the base, branching verticillium- or gliocladium-like with convergent phialides terminating each branch. Phialides tapering uniformly from base to tip. Conidia from mononematous conidiophores broadly ellipsoidal, (3.5–)3.7–4.2(–4.5) × (2.5–)2.7(–3.0) µm. On SNA conspicuous pustules scattered throughout the colony; pustules hemispherical, 1–2 mm diam, dense, without projecting sterile hairs, slowly producing greyish green conidia (27D5). Conidiophores entirely integrated into pustules, not projecting from pustules; conidiophores near the surface of the pustules with a barely discernable, short main axis producing short *1°* branches at 90° and solitary phialides; branches typically unicellular, terminating in 3–5 phialides in a whorl; phialides often arising directly, separated by short internodes and then forming a dense "pseudowhorl". Phialides more or less cylindrical or slightly swollen in the middle, straight or slightly hooked. Conidia ellipsoidal, smooth. Mononematous conidiophores similar to those found on CMD forming on SNA. Chlamydospores not observed on CMD.

Holotype: **Ecuador**, Pichincha, vic. Vicente Maldonado, Arasha Resort forest, km 120, Rio Caoni, isolated from stem of *Theobroma gileri*, 3 Nov. 2001, H.C. Evans DIS 320C (BPI 870965, a dry culture; ex-type culture DIS 320c = IMI 393638 = CBS 119055).

Notes: *Trichoderma caribbaeum* var. *aequatoriale* was originally isolated as an endophyte from the trunk of a *Theobroma gileri* tree. When inoculated onto leaves of *Th. cacao*, it could be re-isolated from the lower parts of the plant. The variety is unusual in growing faster at 20 °C than at 30 °C.

4. *Trichoderma dingleyae* Samuels & Dodd, **sp. nov.** MycoBank MB501036. Figs 10, 134–142.
Teleomorph: ***Hypocrea dingleyae*** Samuels & Dodd, **sp. nov**. MycoBank MB501037. Figs 30–31, 143–152.

Etymology: Named in honour of Joan M. Dingley in recognition of her pioneering studies of hypocrealean fungi, especially those found in New Zealand.

Stromata aurantio-brunnea, *H. rufae* (Fr.) Fr. similia. Ascosporae hyalinae, spinulosae. Pars distalis ascosporarum (2.0–)3.0–4.2 (–5.0) × (1.7–)2.5–4.0(–4.5) µm, pars proxima (2.5–)3.0–4.7 (–6.5) × 2.5–3.5(–4.2) µm. Anamorphosis *T. koningii* Oudem. similis, conidia viridia, ellipsoidea vel late ellipsoidea, (3.2–)3.7–4.5(–6.2) × (2.5–)3.0–3.5(–3.7) µm; ratio longitudinis:latitudinis (1.1–)1.2–1.6 (–2.1). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate 24–29 mm.

Holotypus *H. dingleya*e PDD 83838; holotypus anamorphosis *T. dingleyae* cultura sicca ex ascospora oriens PDD 83838.

Stromata at first pulvinate, tan with a white margin, velutinous, solitary or crowded; becoming discoidal, darker (*ca.* 6–7C–D8: brownish orange, burnt sienna), with or without a velutinous surface, not reacting to KOH, circular to irregular in outline, 1–2 mm diam, broadly attached or with margins slightly free; surface plane to wrinked; perithecial elevations not evident, ostiolar openings barely visible as slightly darker areolae or not visible. Cells of stroma surface in face view pseudoparenchymatous, with unevenly pigmented walls, (2.5–)3.2–6.0(–8.5) µm diam. Surface region of stroma 15–30 µm thick, composed of pseudoparenchymatous cells, (2–)3–6(–11) × (1.5–) 2.7–3.7(–8.0) µm, walls slightly thickened. Hyphal hairs arising from stroma surface, 5–10(–20) µm long, 2–3 µm wide, septate, unbranched, thin-walled. Tissue immediately below the stroma surface compact, of *textura epidermoidea* or pseudoparenchyma but hyphal below. Tissue below the perithecia pseudoparenchymatous, lacking hyphal elements, cells 3–10(–14) µm diam, thin-walled. Perithecia elliptical in section, 175–280(–350) µm high, (80–)120–200(–215) µm diam; ostiolar canal (50–)60–90(–100) µm long; perithecial apex around the ostiolar opening not anatomically distinct from the stroma surface. Asci cylindrical, apex thickened, with a pore. Part-ascospores hyaline, finely spinulose, dimorphic; distal part subglobose, proximal part wedge-shaped to oblong or slightly ellipsoidal.

Characteristics in culture: Optimum temperature for growth on PDA and SNA 20–25 °C. Colonies grown on PDA in darkness or in light for 96 h with abundant white aerial mycelium, sterile. No pigment diffusing through the agar; no distinctive odour. Colonies grown on CMD at 20 °C under light producing scant aerial mycelium; conidia forming in scattered, hemispherical, grey-green pustules 0.5–1.0 mm diam. Pustules very compact; long, terminally fertile conidiophores barely protruding beyond the surface; many small, easily detached pustules forming in the aerial mycelium. Conidiophores within the pustules irregularly branched, typically without a discernable main axis; phialides often held in dense divergent clusters, often arising from swollen nodes. Protruding conidiophores with a long stipe and terminating in 2–4 phialides in a single verticil. Phialides formed within pustules cylindrical to broadly flask-shaped, straight or slightly hooked; those forming at the tips of long conidiophores often narrowly cylindrical or tapering uniformly from base to tip, or slightly swollen in the middle. Conidia ellipsoidal to broadly ellipsoidal, smooth. Chlamydospores few, terminal, subglobose, (3.2–)4.0–6.7(–8.7) µm diam.

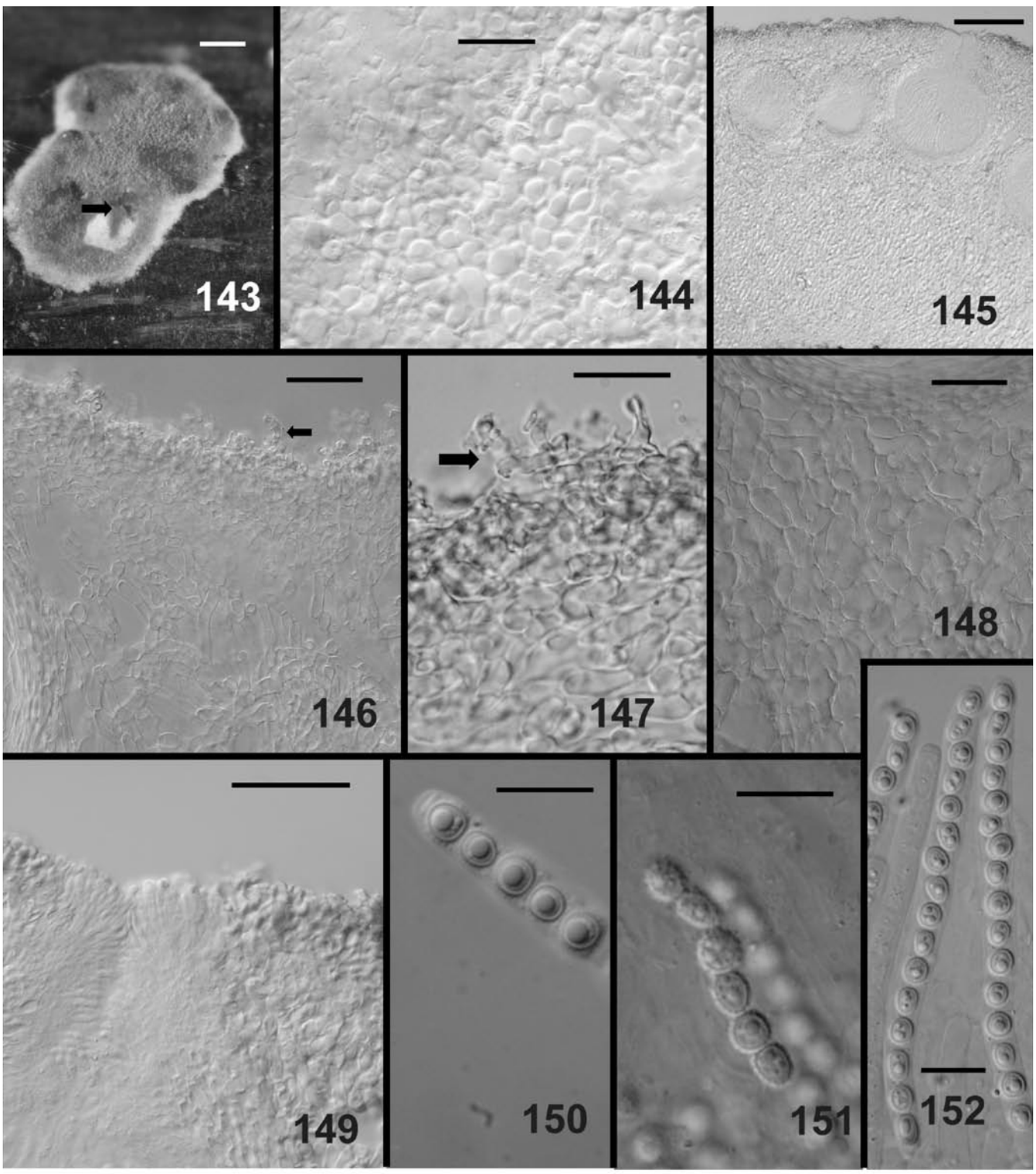
Habitat: Bark and wood of *Nothofagus* spp.

Known distribution: New Zealand.

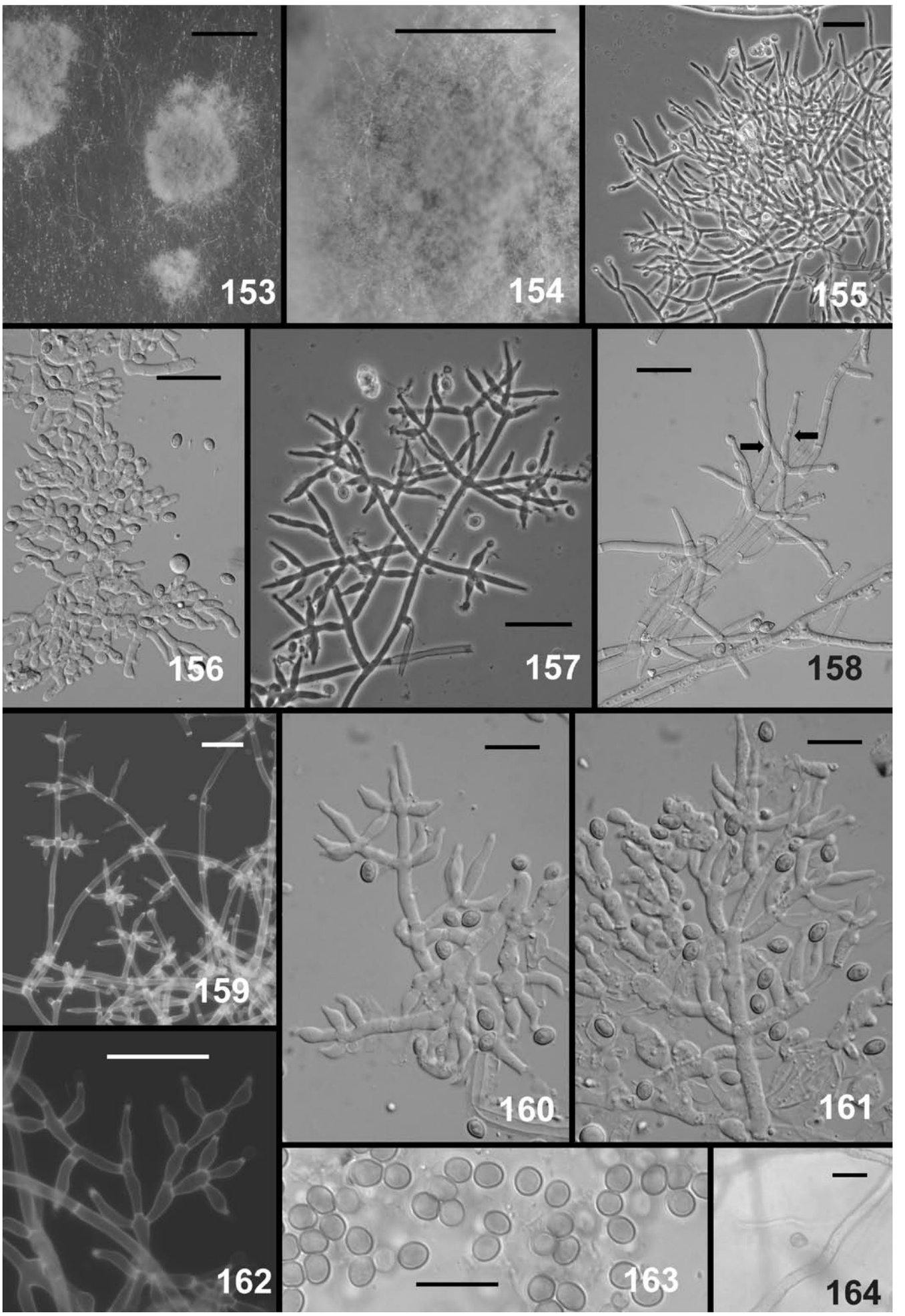
Holotype: **New Zealand,** South Westland, Haast River, Pleasant Flat, 44°07' S, 169°23' E, on wood of *Nothofagus solandri* var. *cliffortioides*, 10 May 2002, J.A. Cooper, comm. S.R. Pennycook (PDD 83837, isotype BPI 842438, ex-type culture G.J.S. 02-50 = ICMP 16285 = CBS 119056).

Additional specimens examined: **New Zealand,** Westland, Lower Buller Gorge, road to Berlins Bluff, under *Nothofagus* and *Dacrydium*, 41°53' S, 171°50' E, on an ascomycete on *Nothofagus* sp., 6 Sep. 1999, G.J.S. & S. Dodd 8706 (PDD 83834, culture G.J.S. 99-105 = ICMP 16283 = CBS 119053); Paparoa National Park, vic. Punakaikai, end of Bullock Creek Rd., S on Inland Pack Track, 42°06' S, 171°24' E, elev. 75–100 m, under *Nothofagus* and mixed podocarp secondary forest, on bark and decorticated wood of *Nothofagus* sp., 1 Sep. 1999, G.J.S. & S. Dodd 8659 (PDD 83841, culture G.J.S. 99-203 = ICMP 16284 = CBS 119235).

Notes: *Trichoderma dingleyae* and *T. dorotheae* were found in the same secondary forest in New Zealand. *Trichoderma dingleyae* has slightly broader conidia than *T. dorotheae*. The growth rate of *T. dingleyae* is much slower than that of *T. dorotheae*; the difference is especially strong at 30 °C after 72 h on PDA or SNA.



Figs 143–152. *Trichoderma dingleyae*, *Hypocrea* teleomorph. 143. Young stroma, note velutinous surface and poorly visible ostiolar openings (one shown at arrow with exuded ascospores). 144. Face view of a stroma. 145. Section through stroma with immature perithecia. 146–147. Section through stroma surface; note loosely hyphal nature of stroma surface and hairs (arrows) that give a velutinous aspect. 148. Cells of interior of stroma below perithecia. 149. Section through ostiolar canal. 150–152. Asci and ascospores; note ornamented ascospores in 151. Figs 143, 146–148, 150, 152 from G.J.S. 02-05; 144–145, 149 from G.J.S. 99-105; 151 from G.J.S. 99-194. Microscopy: 143 = stereo; 144–152 = DIC. Bars: 143 = 1 mm; 144, 146, 148–149 = 20 µm; 145 = 100 µm; 147 = 20 µm; 150–152 = 10 µm.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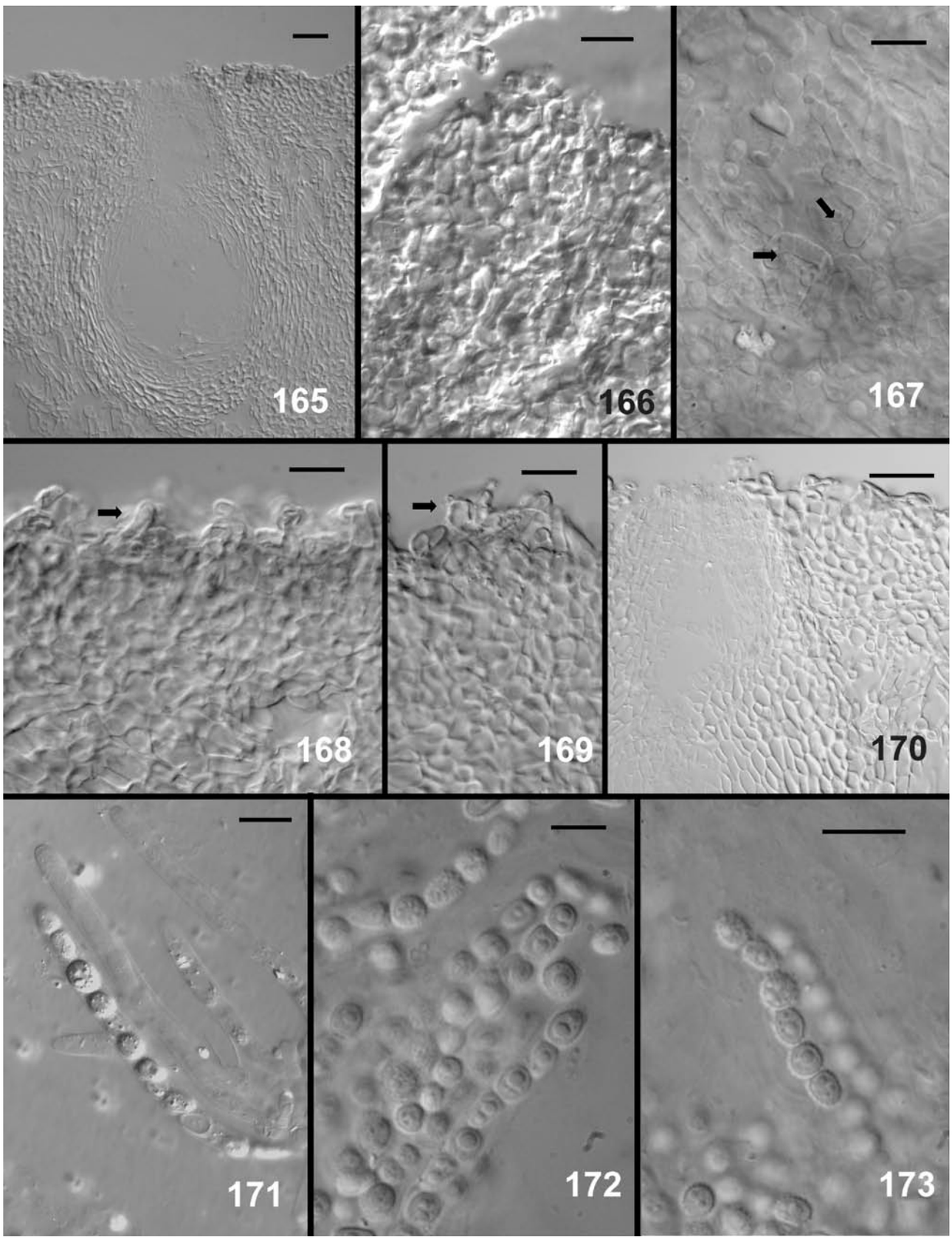


Figs 165–173. *Trichoderma dorotheae*, *Hypocrea* teleomorph. 165. Section through an immature perithecium. 166. Face view of stroma. 167–169. Hairs (arrows) formed on stroma surface in face view (167) and in section (168–169). 168–170. Section through stroma surface and ostiolar region (170). 171–173. Asci and ascospores. Note ornamented ascospores in 173. Figs 165–166, 168–170 from G.J.S. 99-202, 167, 172–173 from G.J.S. 99-194, 171 from G.J.S. 99-97. Microscopy: all from DIC. Bars: 165, 170 = 20 µm; 166–169, 171–173 = 10 µm.

Figs 153–164. (Page 110). *Trichoderma dorotheae*, anamorph from CMD. 153–154. Conidial pustules. Note in Fig. 154 that individual conidiophores are not visible in the pustule. 155–162. Conidiophores and phialides; arrows in Fig. 158 indicate percurrently proliferated phialides. 163. Conidia. 164. Chlamydospore. Figs 153–154, 159, 162, 164 from G.J.S. 99-97; 155–158, 160–161 from G.J.S. 99-202; 163 from G.J.S. 99-194. Microscopy: 153–154 = stereo; 155, 157 = PC; 156, 158, 160–161, 163 = DIC; 159, 162 = FL; 164 = BF. Bars: 153 = 1 mm; 154 = 0.5 mm; 155–157, 159, 162, 164 = 20 µm; 158, 160–161, 163 = 10 µm.

5. *Trichoderma dorotheae* Samuels & Dodd, **sp. nov**. MycoBank MB501038. Figs 11, 153–164.
Teleomorph: ***Hypocrea dorotheae*** Samuels & Dodd, **sp. nov**. MycoBank MB501039. Figs 32–33, 165–173.

Etymology: Named in honour of Dorothy Gale, who went from Kansas to the Land of Oz with her dog Toto (Frank Baum, "Wizard of Oz").

Stromata flavobrunnea aetate provecta. Ascosporae hyalinae, spinulosae. Pars distalis ascosporarum 3.0–4.5(–5.2) × (2.2–)2.7–3.7(–5.2) µm; pars proxima (3.5–)3.7–5.0(–6.5) × 2.5–3.5(–4.2) µm. Conidiophora irregulariter ramosa, internodia inter ramos saepe curta. Conidia viridia, late ellipsoidea, (3.0–)3.5–4.2(–5.0) × (2.5–)2.7–3.2(–3.7) µm; ratio longitudinis:latitudinis (1.1–)1.2–1.4 (–1.6). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate 39–41 mm.

Holotypus *H. dorotheae* PDD 83839; holotypus anamorphosis *T. dorotheae* cultura sicca ex ascospora oriens PDD 83839.

Stromata at first pinkish white (*ca.* 8A2) with white margin, flat and pulvinate, becoming brownish yellow, light brown, yellowish brown (5B–E8) and more raised and discoidal to tuberculate, not reacting to KOH, scattered, circular to irregular in outline, 0.5–1.5 mm diam, broadly attached or with margins slightly free, plane, appearing smooth; perithecial elevations not evident; ostiolar openings barely visible as slightly darker aerolae or not visible. Cells of stroma surface in face view more or less pseudoparenchymatous with unevenly pigmented walls, 7–10 µm diam. Surface region of stroma *ca.* 20 µm thick, composed of pigmented, pseudoparenchymatous cells, (1.7–)2.5–4.5(–7.0) × (1.5–)2.0–3.5(–5.2) µm, walls slightly thickened. Hyphal hairs arising from stroma surface, 5–10(–20) µm long, 2–3 µm wide, septate, unbranched, thin-walled. Tissue below the stroma surface compact, of *textura epidermoidea* or pseudoparenchyma but hyphal below. Tissue below the perithecia pseudoparenchymatous, lacking hyphal elements, cells 5–15(–25) µm diam. Perithecia 169–250 µm high, 65–160 µm wide, ostiolar canal 45–95 µm long; perithecial apex around the ostiolar opening not anatomically distinct from the surrounding stroma surface. Asci cylindrical, apex thickened, with a pore. Part-ascospores hyaline, finely spinulose, dimorphic; distal part subglobose, proximal part wedge-shaped to oblong or slightly ellipsoidal.

Characteristics in culture: Optimum temperature for growth on PDA and SNA 25 °C. Colonies grown on PDA in darkness or in light forming conidia only after 96 h or remaining sterile. Colonies grown on CMD at 20 and 25 ºC under light filling the Petri plate within 1 wk, conidia forming in 1–3 mm diam pustules formed around the periphery of the colony or pustules not evident and conidiophores arising in the aerial mycelium. Often long, entirely fertile conidiophores visible in the pustules. Conidiophores lacking a discernable main axis and branches not obviously paired or sometimes arising in whorls of 3, or unilaterally branched; conidiophores arising from pustules often fasciculate (Figs 155, 156), internodes between the branches often short, branches increasing in length with distance from the tip of the main axis, rebranching to form 2° branches, which terminate in slightly divergent whorls of 3–4 phialides. Phialides lageniform, only slightly swollen in the middle. With age of culture, phialides tending to proliferate percurrently to form new phialides, the newly formed phialide often abruptly swollen in the middle (Fig. 158). After storage, cultures sterile or forming dense, subglobose, grey-green pustules on the surface of the agar and in the aerial mycelium. Conidia broadly ellipsoidal, smooth. Chlamydospores few, terminal, 2.7–5.7(–8.5) µm diam.

Habitat: On bark and wood of *Nothofagus* and *Eucalyptus* species.

Known distribution: New Zealand, Australia (Victoria).

Holotype: **New Zealand,** Westland, Paparoa National Park, vic. Punakaikai, end of Bullock Creek Rd., S on Inland Pack Track, 42°06' S, 171°24' E, elev. 75–100 m, in *Nothofagus* and mixed podocarp forest, on decorticated wood of *Nothofagus* sp., 1 Sep. 1999, G.J.S. & S. Dodd 8657 (PDD 83839; ex-type culture G.J.S. 99-202 = CBS 119089 = ICMP 16288).

Additional specimens examined: **Australia**. Victoria, Otway Ranges, Otway State Forest, Aire Valley, Hopetoun Falls, elev. 300 m, on bark of *Eucalyptus* sp., 27 Aug. 1999, G.J.S. 8651 (BPI 746863, culture G.J.S. 99-194 = CBS 119071 = ICMP 16287). **New Zealand.** Westland, Kahurangi National Park, N of Karamea, between Nenya Creek and Vilya Creek, *ca.* 41°12' S, 172°12' E, elev. 150 m in *Nothofagus fusca* and *N. menziesii* forest, on rotting bark of *Nothofagus* sp., 5 Sep. 1999, G.J.S. & S. Dodd 8689 (PDD 83842, BPI 746628, culture G.J.S. 99-97 = CBS 119057 = ICMP 16286).

Notes: *Trichoderma dorotheae* is distinguished from the sympatric *T. dingleyae* by its faster growth rate and slightly larger conidia. The three teleomorph collections that are linked to this species are in poor condition, mainly overmature.

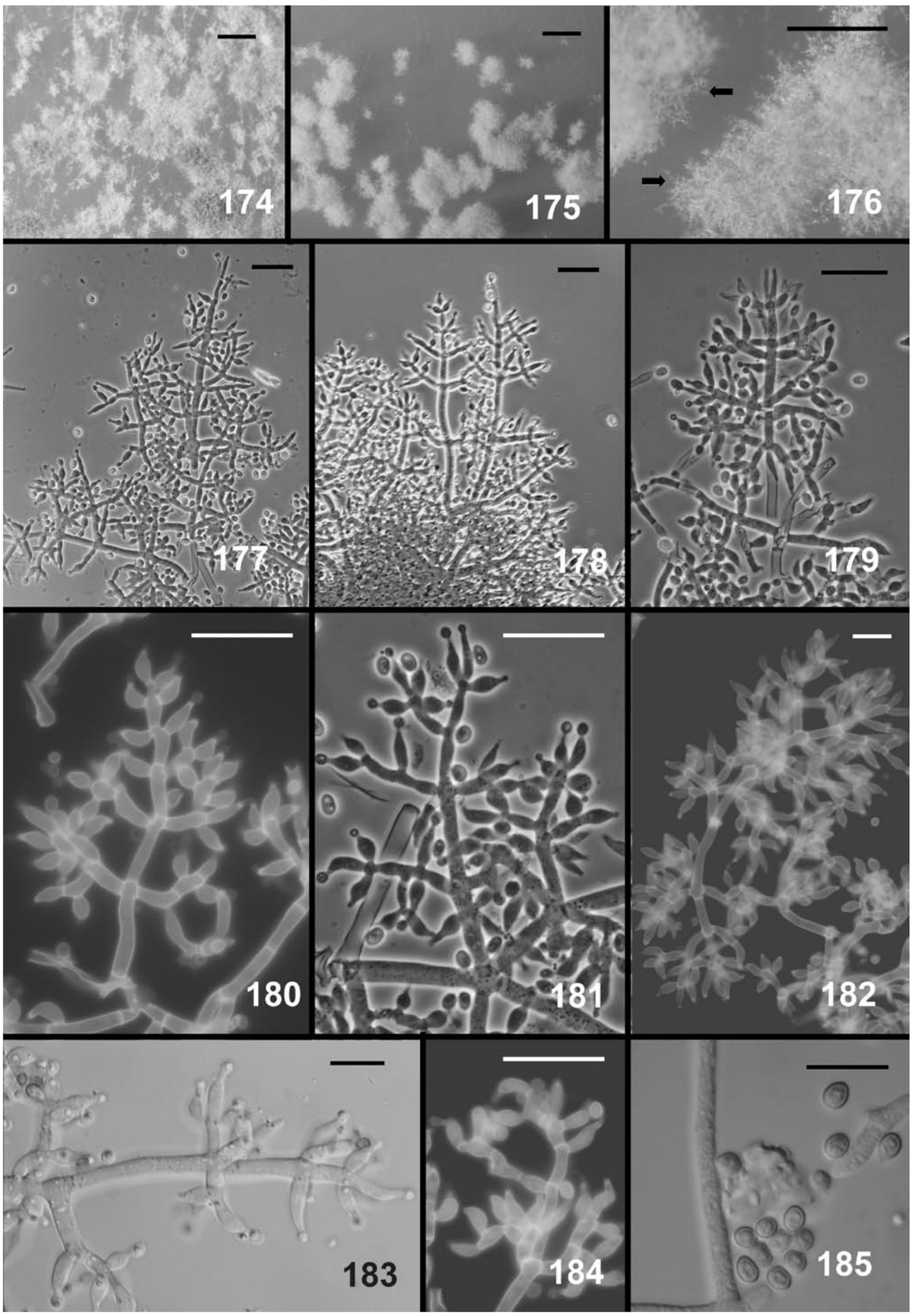
6. *Trichoderma intricatum* Samuels & Dodd, **sp. nov**. MycoBank MB501040. Figs 12–13, 174–185.
Teleomorph: ***Hypocrea intricata*** Samuels et Dodd, **sp. nov**. MycoBank MB501041. Figs 34–35, 186–195.

Etymology: Refers to the intricately arranged conidiophores in the conidial pustules.

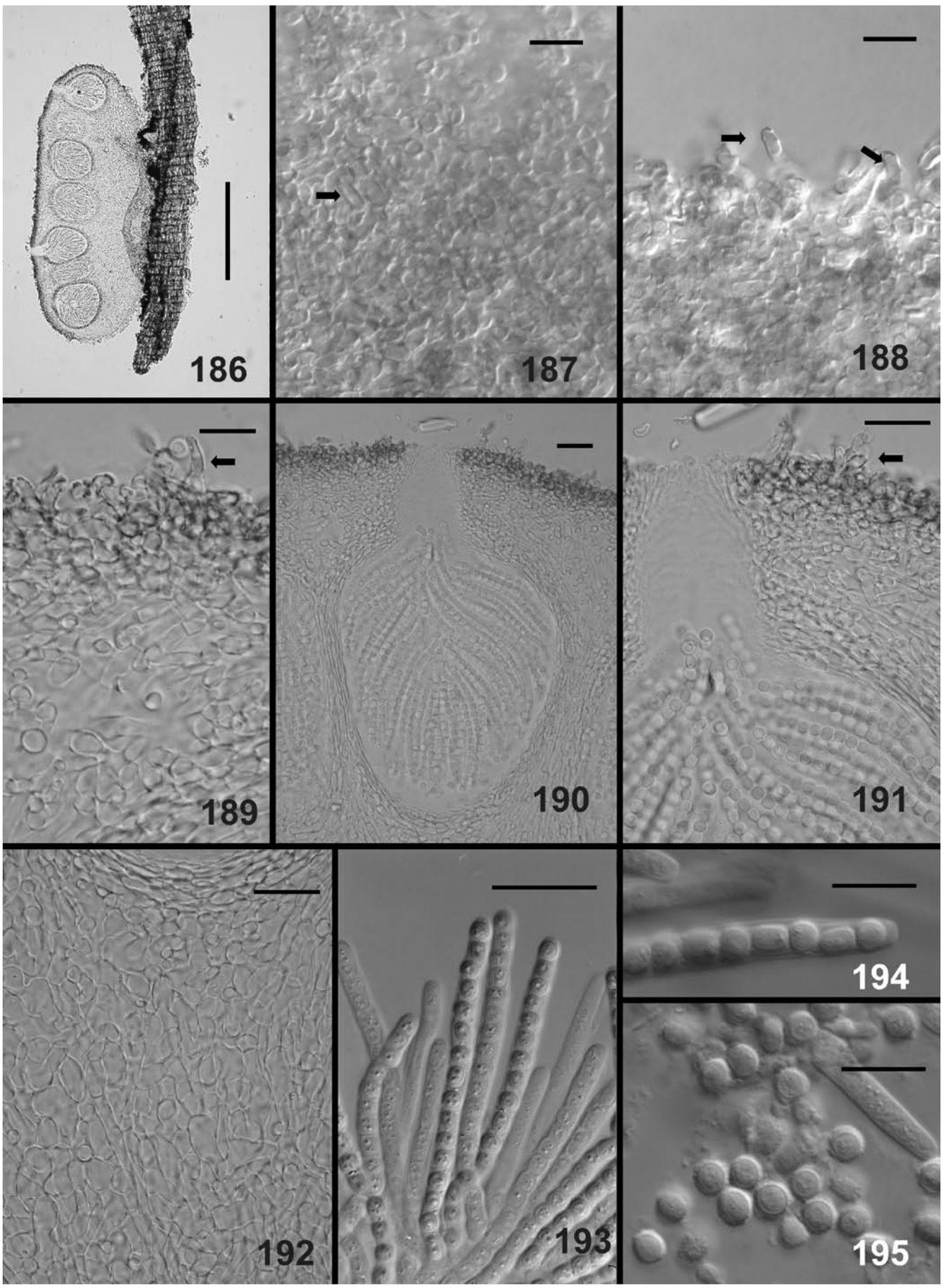
Stromata aurantio-brunnea aetate provecta. Ascosporae hyalinae, spinulosae. Pars distalis ascosporarum (2.5–)3.0–3.7(–4.0) × (2.2–)2.7–3.5(–3.7) µm; pars proxima (2.5–)2.5–4.2(–4.5) × (2.2–)2.5–3.0(–3.2) µm. Anamorphosis *T. koningii* Oudem. similis, conidia late ellipsoidea vel ovoidea, (3.0–)3.5–4.0(–4.5) × (2.5–)2.7–3.2(–3.5) µm; ratio longitudinis:latitudinis (0.9–)1.1–1.3(–1.5). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate 40–50 mm.

Holotypus *H. intricata* BPI 745751; holotypus anamorphosis *T. intricatum* cultura sicca ex ascospora oriens BPI 745751B.

Stromata at first semi-effused, brownish orange to light brown (*ca.* 6C–D8) with a white margin, velvety, becoming tuberculate to discoidal, circular to irregular in outline, 0.5–10 mm diam, greyish orange to brownish orange (*ca.* 5B–D6–7), with or without a slightly scaly or velvety surface, not reacting to KOH, broadly attached with margins slightly free; surface plane; perithecial elevations not evident; ostiolar openings not visible, barely visible as slightly darker points. Cells of stroma surface in face view pseudoparenchymatous, with unevenly pigmented walls, (1.5–)2.2–5.5(–9.5) × (1.2–)1.7–3.0(–4.0) µm, walls slightly thickened. Pigmented surface region of stroma 10–20 µm thick, composed



Figs 174–185. *Trichoderma intricatum*, anamorph from CMD. 174–176. Conidial pustules; individual plumose conidiophores can be seen in the pustule (176, arrows). 177–184. Conidiophores and phialides. 185. Conidia. Figs 174, 177–179, 181, 183 from G.J.S. 97-88; 175–176, 180, 182, 184–185 from G.J.S. 96-13. Microscopy: 174–176 = stereo; 177–179, 181 = PC, 180, 182, 184 = FL; 183, 185 = DIC. Bars: 174–175 = 1 mm; 176 = 0.5 mm; 177–182, 184 = 20 µm; 183, 185 = 10 µm.



Figs 186–195. *Trichoderma intricatum*, *Hypocrea* teleomorph. 186. Section through a stroma with mature perithecia. 187–189. Stroma surface in face view (187) and in section (188–189); hairs shown at arrows; note the loose nature of the stroma surface that gives the stroma a velutinous aspect (188–189, 191). 190. Median longitudinal section through a mature perithecium. 191. Section through the ostiolar canal. 192. Cells of the stroma interior below perithecia. 193–194. Asci and ascospores. 195. Discharged part-ascospores. Figs 186, 189–192 from G.J.S. 97-88; 187–188, 193–195 from G.J.S. 96-13. Microscopy: 186 = BF, all others DIC. Bars: 186 = 200 ìm; 187–188, 194–195 = 10 µm; 189–193 = 20 µm.

of pseudoparenchymatous cells, (1.5–)2.0–4.0(–6.5) × (1.2–)1.7–2.7(–4.0) µm, walls slightly thickened. Hyphal hairs arising from stroma surface, 6.5–10 µm long, 3–4 µm wide, septate, unbranched, thin-walled. Tissue below the stroma surface compact, of *textura epidermoidea* but hyphal below. Tissue below the perithecia pseudoparenchymatous but with many long hyphal elements, cells (2–)4–9(–13) × (2.5–)3.0–5.0(–6.5) µm, thin-walled. Perithecia elliptical in section, (150–)160–270(–360) µm high, 65–220(–350) µm wide; ostiolar canal 50–100 µm long; perithecial apex around the ostiolar opening not anatomically distinct from the stroma surface. Asci cylindrical, apex thickened, with a pore. Part-ascospores hyaline, finely spinulose, dimorphic; distal part subglobose, proximal part wedge-shaped to oblong or slightly ellipsoidal.

Characteristics in culture: Optimum temperature for growth on PDA and SNA 30 °C; less than 5 mm at 35 °C. Colonies grown on PDA in darkness or under light at 25 °C producing conidia within 8 h; within 96 h conidia abundant in the aerial mycelium in marked concentric rings; on SNA conidia beginning to form in small pustules in a ring around the original inoculum. No diffusing pigment or distinctive odour detected on any medium. Colonies on CMD filling the Petri plate within 1 wk at 20 °C; conidiophores forming around the margin of the colony in a more or less continuous, *ca.* 1 cm broad band of confluent, poorly-defined, cottony pustules, within which entirely fertile conidiophores can be seen (Fig. 176); conidia dark green (27F8). On SNA conidia forming in more or less discrete, hemispherical pustules. Pustules lacking projecting terminally fertile conidiophores or sterile hairs. Conidiophores with a discernable main axis, more or less symmetrical, often with 2 branches arising on either side of a single node, *1°* branches arising at or near 90° with respect to the main axis, progressively longer with distance from the tip, rebranching to form unicellular *2°* branches. Phialides arising directly from the main axis, *1°* branches and terminating the *2°* branches, in whorls of 3–5; dense whorls at the tips of branches not noted; phialides lageniform and somewhat swollen in the middle to cylindrical, straight, rarely slightly hooked or sinuous; supporting cell not significantly different in width from the widest part of the phialide. Conidia broadly ellipsoidal to ovoidal, smooth. Chlamydospores not observed.

Habitat: Decorticated wood inhabited by other ascomycetous fungi including *Rosellinia* sp.

Known distribution: Puerto Rico, Thailand.

Holotype: **Thailand**. Saraburi Prov., Khao Yai Natl. Park, Haew Narok, elev. 350 m, bark of very rotten tree, 11 Aug. 1997, G.J.S. & P. Chaverri 8422 (teleomorph: BPI 745751; holotype of *T. intricatum* BPI 745751B; ex-type culture G.J.S. 97-88 = CBS 119059).

Additional specimen examined: **Puerto Rico**, Caribbean Natl. Forest, Luquillo Mts., El Verde Research Area, on decorticated wood, 9 Feb. 1996, G.J.S. & H.-J. Schroers 8038 (BPI 744458, culture G.J.S. 96-13 = CBS 986.97).

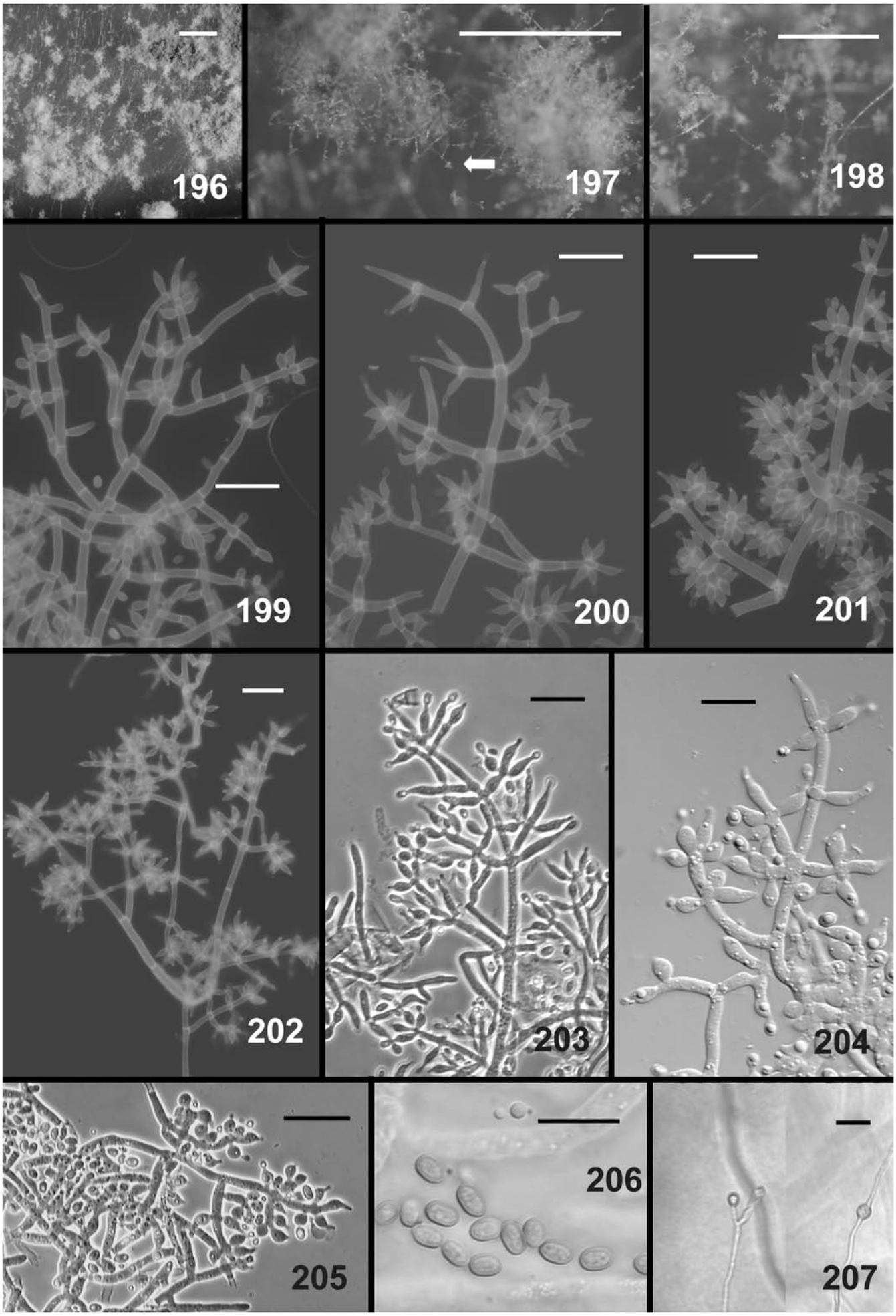
Notes: Despite the fact that the two known collections of *T. intricatum* were found in widely separated geographic locations, they were joined together with high bootstrap support in analyses of *tef*, *act* and *cal*. The apparent geographic separation may account for the small differences between the two collections, viz. conidia of G.J.S. 97-88 [3.7 ± 0.2 (3.5–4.5) × 2.5 ± 0.2 (2.5–3.5) µm] are statistically longer than those of G.J.S. 96-13 [3.5 ± 0.3 (3.0–4.0) × 3.1 ± 0.3 (2.5–3.5) µm]; the distal and proximal part-ascospores of G.J.S. 96-13 [distal: 3.5 ± 0.3 (3.0–4.0) × 3.5 ± 0.2 (3.0–3.7) µm; proximal: 3.2 ± 0.3 (3.2–4.5) × 2.7 ± 0.2 (2.5–3.2) µm] are statistically longer than those of G.J.S. 97-88 [distal: 2.7 ± 0.2 (2.5–3.5) × 2.2 ± 0.3 (2.2–3.5); proximal: 2.5 ± 3.5 (2.5–3.7) × 2.2 ± 0.2 (2.7–3.2) µm] and the distal part-ascospores of G.J.S. 96-13 are wider than those of 97-88. Finally, although the two collections have virtually identical growth curves on PDA, 97-88 grows considerably more slowly on SNA, reaching a colony radius of only 30 mm on PDA after 72 h as opposed to *ca.* 50 mm for 96-13. In the absence of addional collections, we do not recognize these differences in our taxonomy.

Trichoderma intricatum is sympatric with *T. koningiopsis*, *T. caribbaeum* var. *caribbaeum* and *T. stilbohypoxyli* in Puerto Rico. The species can be distinguished with some difficulty. *Trichoderma koningiopsis* and *T. caribbaeum* have a faster growth rate on PDA than either *T. intricatum* or *T. stilbohypoxyli*, the radius of *T. koningiopsis* reaching 70 mm at 30 °C and that of *T. intricatum* 50–60 mm at 30 °C, whereas the colony radii of *T. intricatum* and *T. stilbohypoxyli* reach a maximum of only *ca.* 50 mm. Conidia form slowly, after 96 h at 25°C, in PDA and SNA cultures of *T. caribbaeum*, but conidia form in abundance within 96 h on these media in the other species. The distal and proximal part-ascospores of *T. intricatum* (Table 3) are shorter than those of either *T. stilbohypoxyli* [distal: (3.2–)3.7–4.2(–4.7) µm, 95 % CI = 3.9–4.0 µm; proximal: (3.2–)4.2–5.2(–5.7) µm, 95 % CI = 4.6–4.8 µm] or *T. koningiopsis* (Table 3). Conidia of *T. intricatum* are wider and the L/W of conidia is smaller than in *T. caribbaeum*, *T. koningiopsis* and *T. stilbohypoxyli* (1.3–1.5). PDA cultures of *T. stilbohypoxyli* typically produce a diffusing yellow pigment.

7. *Trichoderma koningii* Oudem. in Oudemans & Koning, Arch. Néerl. Sci. Exactes Nat., Sér. 2, 7: 291. 1902. Figs 14, 196–207.
Teleomorph: ***Hypocrea koningii*** Lieckfeldt, Samuels & W. Gams, Canad. J. Bot. 76: 1519. 1998. MycoBank MB446367. Figs 36–38.

See Lieckfeldt *et al*. (1998) for descriptions and illustrations of stromata. Measurements of the teleomorph are given in Table 3.

Characters of cultures: Optimum temperature for growth on PDA 25–30 °C; on SNA significantly faster at 25 °C than at 30 °C. Colonies grown on PDA at 25 °C for 96 h under light tending to produce abundant white mycelium; conidial production beginning in the centre of the colony in dense green patches; production spreading outward in broad, faint concentric rings. First green conidia appearing at *ca.* 72 h in PDA cultures grown at 25 or 30 °C in darkness.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Characters of anamorph: Colonies grown on CMD filling the Petri plate within one wk; no diffusing pigment, no distinctive odour. Conidia forming in a narrow band around the colony margin in confluent, compact to cottony pustules up to 1 mm diam and in the sparingly produced aerial mycelium, sometimes with only a slight tendency to form pustules; individual conidiophores often visible within the pustule (Fig. 197). Conidiophores with a discernable main axis, more or less symmetrical, often with 2 branches arising on either side of a single node, *1°* branches arising at or near 90° with respect to the main axis, progressively longer with distance from the tip, rebranching to form unicellular *2°* branches. Phialides arising directly from the main axis, the *1°* and *2°* branches, in whorls of 3 or 4, often dense whorls at the tips of branches and in intercalary positions, lageniform, somewhat swollen in the middle, straight; phialides often densely clustered in "pseudowhorls" (Figs 201, 202). Conidia oblong, smooth, green. Chlamydospores sparingly produced, subglobose, (6.5–)7.5–11.5(–14.5) µm, terminal on hyphae.

Habitat: Isolated from soil, perithecia forming on bark.

Known distribution: U.S.A., Canada, Europe

Neotype of *Trichoderma koningii*: **The Netherlands**. Spanderswoud near Bussum, under *Pinus sylvestris*, 1997, W. Gams (BPI 744883; ex-neotype culture CBS 457.96 = IMI 374798 = G.J.S. 96-117).

Holotype of *Hypocrea koningii*: **U.S.A.,** Maryland, Garrett County, approx. 10 mi SSE of Grantsville, near Bittinger, western Maryland 4-H, High Bog, on decorticated wood, 23 Sep. 1989*,* G.J.S. (89-122), C.T. Rogerson, W.R. Buck & R.C. Harris *(*BPI 745885, ex-type culture G.J.S. 89-122 = IMI 378801 = CBS 989.97).

Additional specimens and cultures examined: **Canada**, isolated from mushroom compost, C. Fordyce D8129-6 (conidial culture G.J.S. 92-18 = CBS 987.97). **Hungary**, locality not known, conidial isolate from soil (ATCC 64262; dry culture = BPI 870956). **The Netherlands**, collecting data as the neotype of *T. koningii,* conidial isolates (CBS 458.96, under *Pseudotsuga menziesii;* CBS 459.96, under *Larix Leptolepsis;* CBS 460.96, under *Fagus sylvatica*); Baarn, Groeneveld, on decaying angiosperm wood, Oct. 1970, W. Gams (ascospore culture CBS 979.70, as *H. muroiana,* specimen not located). **U.S.A.,** ?Maryland, on bark, Nov.–Dec. 2000, G. Arnold 00-155 (BPI 842351, culture G.J.S. 00-168 = CBS 119060, ascospore isolate); Pennsylvania, Westmoreland County, Laurel Summit Picnic Area, on blackened, decorticated wood, 16 Sep. 2000, K. Põldmaa 00-143 (BPI 842346; culture G.J.S. 00-156 = CBS 119061, ascospore isolate); Wisconsin, Sand County, Aldo Leopold Reserve, on burned wood, 23 June 1990, G.J.S. (conidial culture 90-18 = CBS 988.97).

8. ***Trichoderma koningiopsis*** Samuels, C. Suarez & H.C. Evans, **sp. nov**. MycoBank MB487454. Figs 15–16, 208–216.
Teleomorph: ***Hypocrea koningiopsis*** Samuels, **sp. nov**. MycoBank MB501042. Figs 39–40, 217–225.

Etymology: "*Koningiopsis"* in reference to the similarity to *T. koningii* Oudem.

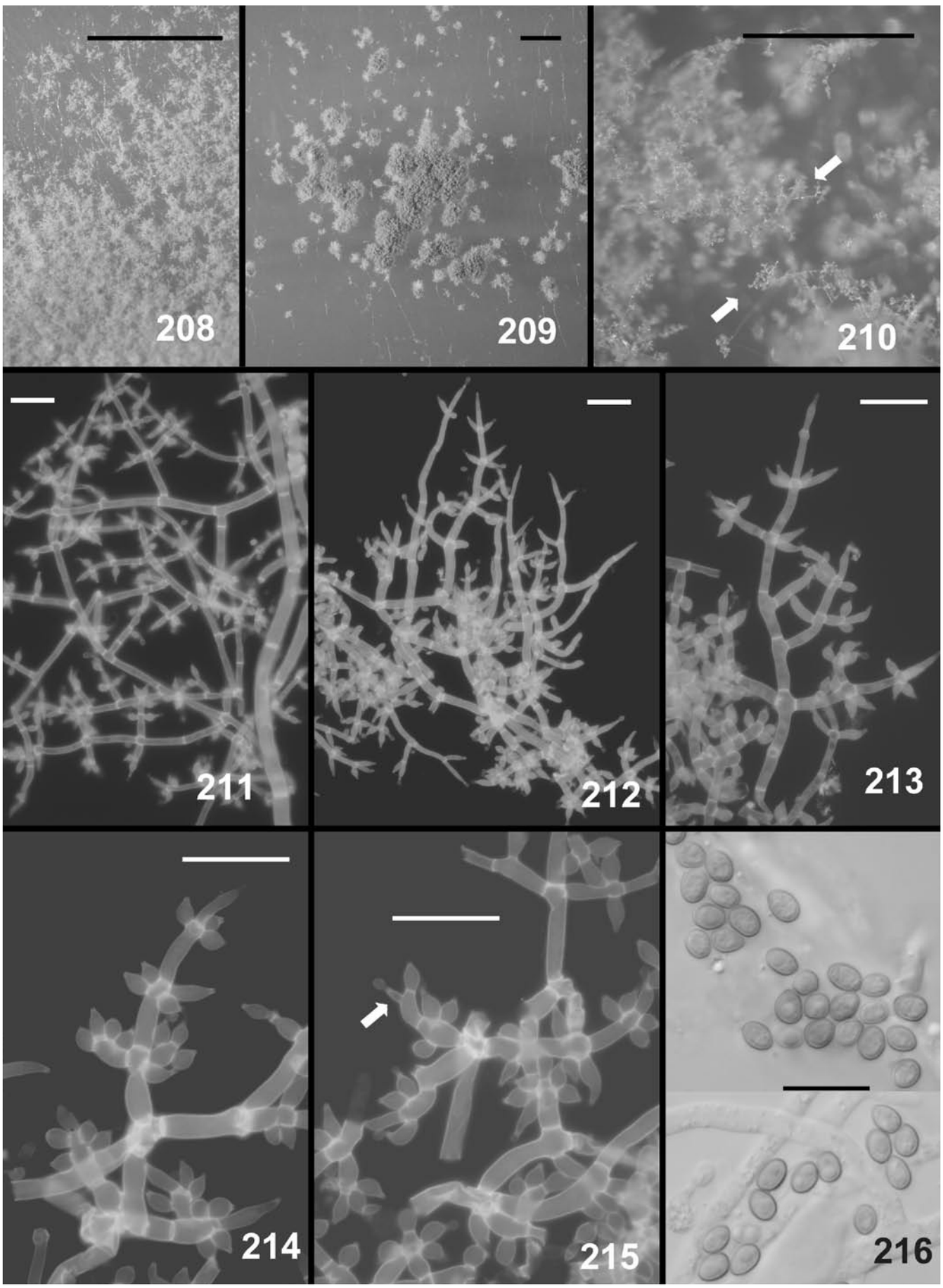
Stromata brunnea, ostiola plerumque occulta. Ascosporae hyalinae, spinulosae. Pars distalis ascosporarum (2.7–)3.0-3.5 (–4.5) × (2.0–)2.5–3.5 (–4.0) µm; pars proxima (3.0–)3.7–4.7 (–6.0) × (1.7–)2.2–3.0 (–3.5) µm. Conidiophora regulariter ramosa, interdum longa internodia inter ramos apicem versus praebentia. Conidia ellipsoidea, (3.0–)3.5–4.5 (–6.2) × (2.0–)2.2–3.0 (–3.5) µm; ratio longitudinis : latitudinis (1.0–)1.3–1.8 (–2.5). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate (45–)51–63(–67) mm.

Holotypus *H. koningiopsis*: BPI 802571; holotypus anamorphosis *T. koningiopsis* cultura sicca ex ascospora oriens BPI 802571B.

Stromata brown (6E8), not reacting to KOH, scattered, nearly circular in outline, 1.5–2.5 mm diam, pulvinate, broadly attached, margins sometimes free, convex to plane, appearing smooth, perithecial elevations not evident or appearing as low tubercules, ostiolar openings barely visible as slightly dark areolae. Hyphal hairs arising from stroma surface, 8–10 µm long, septate, 2–3 µm wide, absent from mature stromata. Cells of the stroma surface in face view pseudoparenchymatous, 10–20 × 5–15 µm, walls slightly thickened, unevenly pigmented. Surface region of stroma *ca.* 20 µm thick, composed of pigmented, compact pseudoparenchymatous cells 5–12 × 3–10 µm, walls slightly thickened. Tissue below the stroma surface of loosely disposed, thin-walled hyphae. Tissue below the perithecia vertically oriented, long-celled pseudoparenchyma; cells 5–15 × 5–10 µm, with some short hyphal elements. Perithecia subglobose, (130–)150–250(–275) µm high, (60–)90–150 µm wide; ostiolar canal 50–100 µm long; perithecial apex around the ostiolar opening not anatomically distinct from cells of the surrounding stroma surface. Asci cylindrical, apex slightly thickened, a pore not visible; ascospores uniseriate. Part-ascospores hyaline, finely spinulose, dimorphic; distal part globose to subglobose; proximal part oblong to wedge-shaped or slightly ellipsoidal.

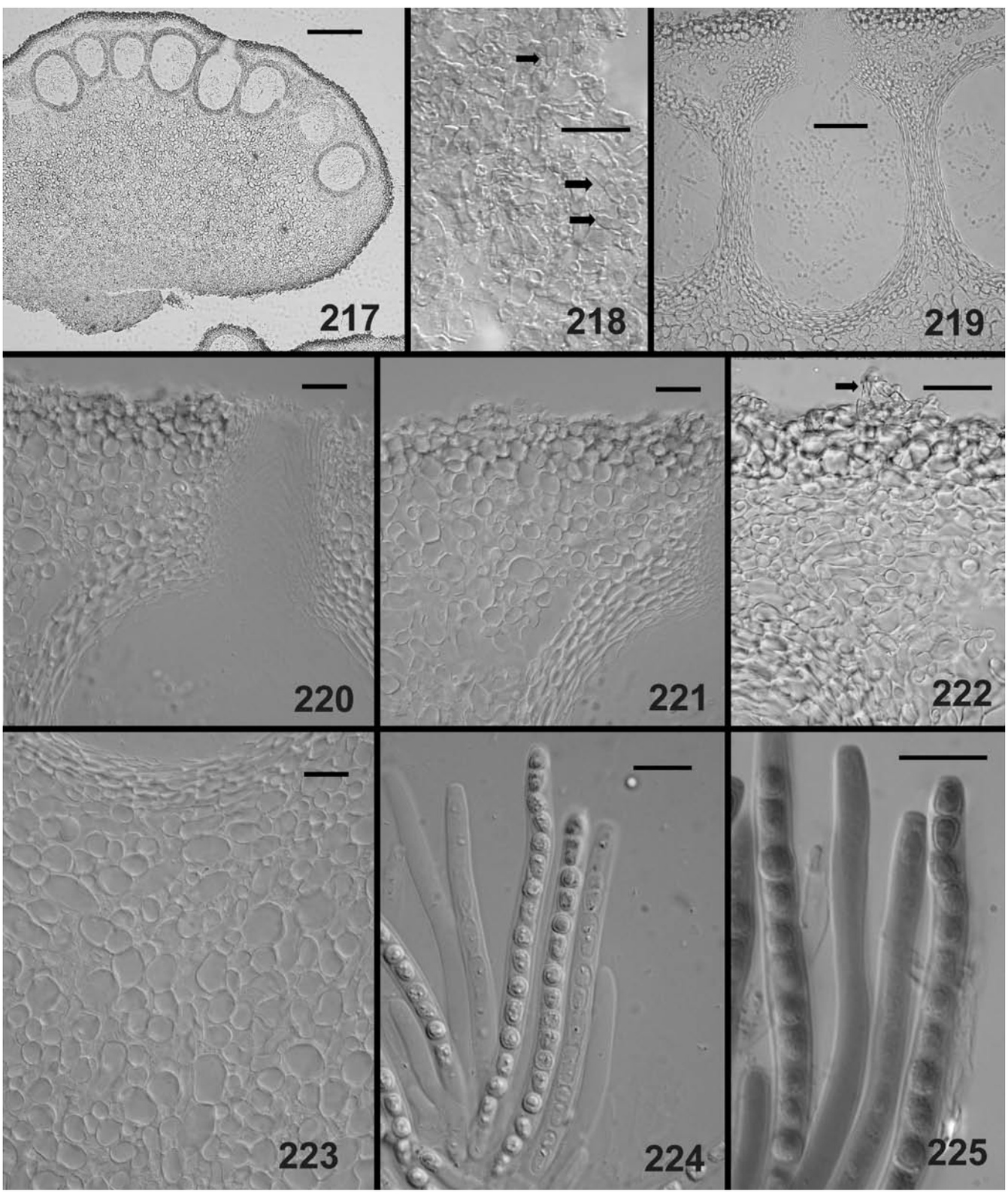
Characteristics of cultures: Optimum temperature for growth on PDA and SNA 30 °C. Colonies grown on PDA in darkness or in light for 96 h often forming conidia after 48 h at 25 and 30 °C in a dense lawn or the centre remaining sterile; conidia also forming in 2 or 3 concentric rings; conidia abundant in light-grown colonies, less abundant and sometimes lacking from colonies grown in darkness. Colonies grown on SNA in darkness or in light for 96 h producing abundant aerial mycelium; conidia tending to be uniformly dispersed in the aerial mycelium in broad concentric rings, sometimes forming cottony pustules. Conidial masses 25E–F8 (deep green to dark green), seldom with yellow coloration (26–27E–F). No pigment diffusing through the agar; no distinctive odour (rarely a faint coconut odour detected). Colonies grown on CMD at 20 °C under light filling the Petri plate within 1 wk, conidial production nearly continuous with a tendency to form highly compact to cottony, 1–2 mm diam pustules; conidial production sometimes

Figs 196–207. (Page 116). *Trichoderma koningii*, anamorph from CMD. 196–197. Conidial pustules; individual conidiophores can be seen in 197 (e.g. arrow). 198–205. Conidiophores and phialides; in 198 conidiophores as viewed with the stereo microscope at the periphery of a pustule can be seen; note the densely clustered phialides in 201–202 and 205. 206. Conidia. 207. Chlamydospores. Fig. 196 from G.J.S. 96-119; 197 from G.J.S. 90-18; 198, 202 from CBS 979.70; 199 from G.J.S. 92-18; 200–201 from G.J.S. 97-117; 203 from G.J.S. 00-156; 204–207 from ATCC 64262. Microscopy: 196–198 = stereo; 199–202 = FL; 203, 205 = PC; 204, 206 = DIC, 207 = BF. Bars: 196 = 1 mm; 197–198 = 0.5 mm; 199–202, 205, 207 = 20 µm; 203–204, 206 = 10 µm.



Figs 208–216. *Trichoderma koningiopsis*, anamorph from CMD. 208–210. Conidial pustules; note individual conidiophores at the periphery of a pustule in 210 (e.g. arrow). 211–215. Conidiophores and phialides; note intercalary phialide in 215 (arrow); note "pachybasium"-like arrangement of phialides in 214. 216. Conidia. Figs 208, 210–211 from G.J.S. 91-6; 209, 212–215 from G.J.S. 01-09; 216 above from G.J.S. 97-273, 216 below from G.J.S. 91-7. Microscopy: 208–210 = stereo; 211–215 = FL, 216 = DIC. Bars: 208–209 = 1 mm; 210 = 0.5 mm; 211–215 = 20 µm; 216 = 10 µm.

restricted to the margin of the colony. Often long, entirely fertile branches visible in the pustules (Fig. 210). Conidiophores comprising a recognizable main axis, *ca.* 3 µm wide; fertile branches arising along the length of the main axis, more or less paired with longer or shorter internodes; terminal part of conidiophore often sparingly branched and with long internodes between branches (Figs 212–213); branches formed from the interior of pustules, sometimes pachybasium-like, with short, crowded phialides (Figs 214–215). Branches arising at an angle of slightly less than 90º with respect to the main axis, longer branches near the base and short branches or solitary phialides arising near the tip; *1°* branches rebranching or



Figs 217–225. *Trichoderma koningiopsis*, *Hypocrea* teleomorph. 217. Section through a mature stroma. 218. Stroma surface in face view with hairs visible (e.g. arrows). 219. Median longitudinal section through a mature perithecium. 220–222. Section through stroma surface; the remains of the loose surface can be seen in 221 and a hair is visible in 222 (arrow). 223. Cells of the stroma interior below perithecia. 224–225. Asci and ascospores; 225 stained with 1 % aq. phloxine; the thickened ascus apex can be seen in 225. All from G.J.S. 93-20. Microscopy: 217 = BF, all others from DIC. Bars: 217 = 200 ìm; 218, 220–223 = 20 µm; 219 = 50 µm; 224–225 = 10 µm.

producing phialides directly, *2°* branches tending to be paired; all fertile branches terminating in a whorl of phialides. Phialides typically straight, sometimes hooked or sinuous, narrowly lageniform or sometimes conspicuously swollen in the middle (especially when crowded at the interior of a pustule), held in whorls of 2–5, sometimes several phialides arising from the same point and crowded (Fig. 214); intercalary phialides forming but not common (Fig. 215). Conidia ellipsoidal, smooth. Chlamydospores abundant to sparse or lacking, terminal to intercalary, globose to subglobose, (n = 168) (3.0–)9–9.5 (–16) µm diam.

Habitat: Isolated from pods of *Theobroma cacao* and from trunks of *Th. grandiflorum, Th. gileri, Theobroma* sp. ("cacao de monte"), mushroom compost, soil, twigs and decaying leaves. Teleomorph found on wood, possibly associated with ascomycetes.

Known distribution: Teleomorph: Cuba, Puerto Rico, U.S.A. (Kentucky). Anamorph: Brazil, Canada, Ethiopia, Ecuador, Germany, Ghana, Peru.

Holotype: **Cuba**, Sanctu Spiritus, Moyote Mi Ritiro, elev. 700–750 m, 21º52' N, 80º01' W, on branch, 2 July 1993, S.M. Huhndorf 572 (BPI 802571, isotype NY; holotype of *T. intricatum* BPI 802571B; ex-type culture G.J.S. 93-20 = CBS 119075).

Additional specimens examined, teleomorph: **Puerto Rico**, Cordillera Central, Chario Azul, off Rte.184, elev. 550 m, on decorticated wood, G.J.S. 8111, H.-J. Schroers & D.J. Lodge (BPI 744473, culture G.J.S. 96-47 = CBS 991.97). **U.S.A.,** Kentucky, Rowan County, Cave Run Lake, on decorticated wood, 26 Sep. 1995, G.J.S. (BPI 737751, culture G.J.S. 95-175 = CBS 991.97).

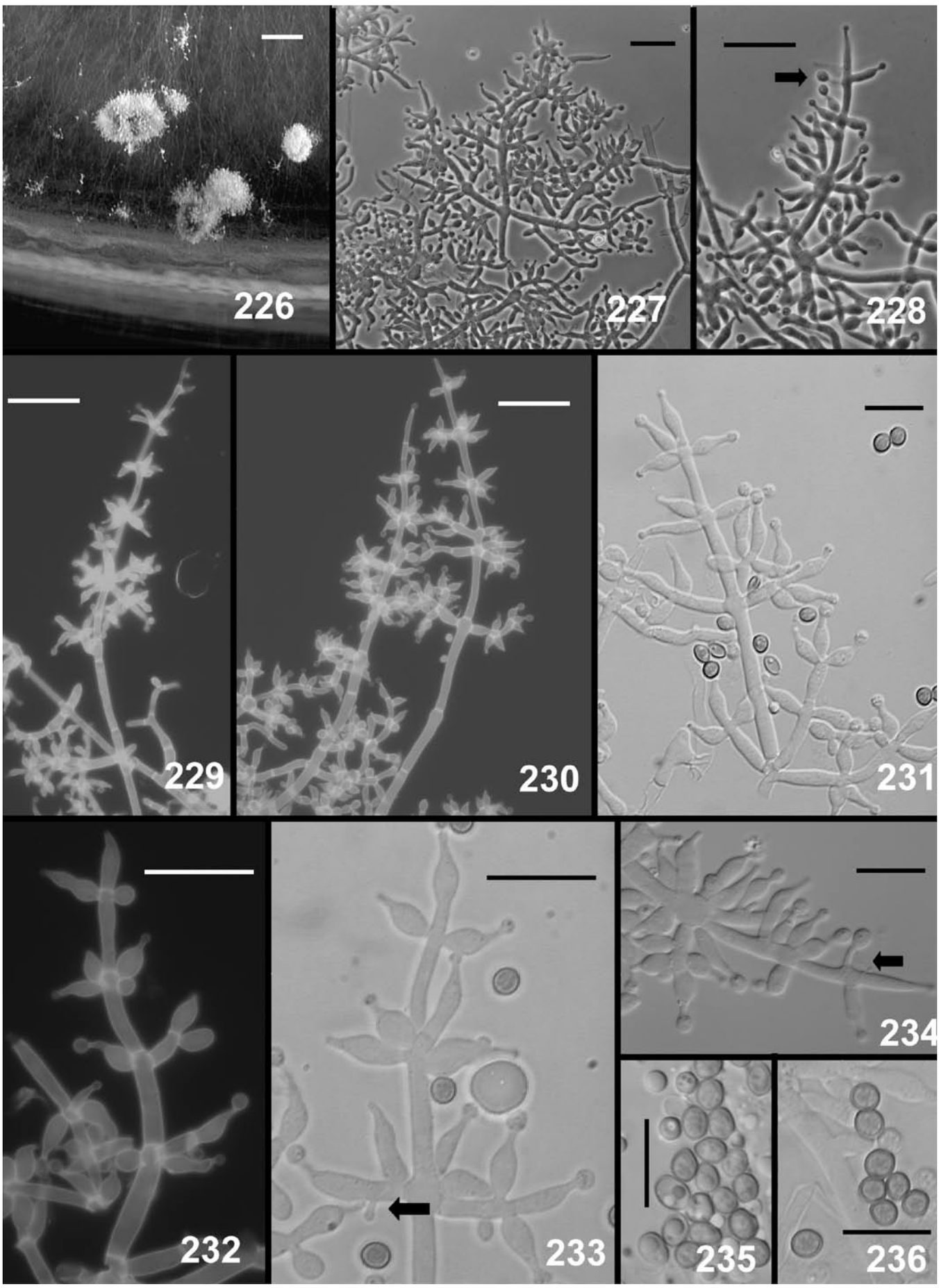
Additional specimens examined, anamorph: **Brazil**, Rio Xingu, Bella Vista Farm, isolated from trunk of 20 m tall *Theobroma grandiflorum*, 5 Mar. 2000, H.C. Evans & K. A. Holmes DIS 205f (BPI 870959; culture DIS 205f = IMI 385805 = CBS 119068); vic. Iguasu Falls, in soil in rain forest, 6 Sep. 2004, I. Druzhinina (two cultures: TUB F 1134 = G.J.S. 04-378 = CBS 119073; TUB F 1145 = G.J.S. 04-375); Pará, Belém, EMBRAPA, isolated from stem of 50–60-year-old *Theobroma grandiflorum*, 29 Feb. 2000, H.C. Evans & K. A. Holmes DIS 172ai (CBS 119067 = IMI 385811); Rio de Janeiro, location and substratum unknown, 27 Oct. 2004, I. Druzhinina (TUB F 824); Sugarloaf Mt., in soil under bamboo, 6 Sep. 2004, I. Druzhinina (two cultures: TUB F 727 = G.J.S. 04-377 = CBS 119074; TUB F 1079 = G.J.S. 04-374); Botanical Garden, in soil, 19 Sep. 2004, I. Druzhinina (two cultures: TUB F 682 and 687); São Paulo, Atlantic Forest, vic. Paranapicaba, *ca.* 60 km from São Paulo, on leaves of *Alchornea triplinervia* in stream, 1989–1990, I. Schoenlein-Crusius (G.J.S. 91-6); same location data, isolated from soil (G.J.S. 91-7). **Canada**, Ontario, Leamington, mushroom farm, isolated from mushroom casing, 18 Aug. 1994, collector unknown (DAOM 222105). **Ecuador**, Esmeraldas Prov., El Rocio, Guadual, isolated from stem of *Theobroma gileri*, 5 Nov. 2001, H. C. Evans & R. Reeder DIS 326h (BPI 870961; live culture DIS 326h = IMI 393639 = CBS 119070); Los Rios Prov., Pichilingue, vic. Quevedo, isolated from pods of *Theobroma cacao* infected with *Moniliophthora roreri,* 1999, C. Suarez & K. Solis [cultures 1.09 = G.J.S. 01-07; 2.09 = G.J.S. 01-09; 4.09 = G.J.S. 01-10 = CBS 119063; 5.09 = G.J.S. 01-11, 6.09 = G.J.S. 01-12 = CBS 119064 (dry culture BPI 870957)]; Pichincha Prov., Vicente Maldonado, Arasha Resort forest, km 120, Río Caoni, isolated from stem of *Th. gileri,* 5 Aug. 2000, H.C. Evans & K.A. Holmes DIS 229d (BPI 870960; live culture CBS 119069 = IMI 391590); Vicente Maldonado, Rancho Marionita, km 122, on old, damaged *Th. gileri,* 14 Apr. 2001, H.C. Evans & K.A. Holmes DIS 339c (CBS 119065 = IMI 391591). **Germany**, Müncheberg, isolated from arable sandy soil, 1990, H. Nirenberg (BBA 65450). **Ghana,** Western Region, Wiaswo Distr., Bia National Park, from forest headquarters to Rock Pool, 06°37' N, 03°04' W, elev. 506 m, to 06°36' N, 03°05' W, elev. 300 m, disturbed primary forest, on twig, 22 Oct. 2003, G.J.S. 9417 & H.C. Evans (BPI 872112, live culture G.J.S. 03-160). **Peru**, Quebrada Payarote, Río Marañon, isolated from stem of 15–20 m tall *Theobroma* sp. ("cacao de monte"), 3 May 1999, H.C. Evans & D.H. Dejeddour DIS 94c (BPI 870958; live culture CBS 119066 = IMI 391592). **U.S.A.,** Texas, Karnes County, vic. Kennedy, from farm soil, date unknown, C. Howell T.K. 3 (G.J.S. 04-10 = CBS 119063).

Notes: *Trichoderma koningiopsis* has been isolated directly from substrata much more often than it has been encountered as its teleomorph. It is a common species in the Americas, especially in tropical regions, but has also been found in soil under coffee in East Africa (Ethiopia, T. Belayneh, pers. comm.) and on a twig in forest in Ghana. In the United States its range overlaps with *T. koningii*, *T. petersenii*, and *T. rogersonii*. Early evidence suggests that it is effective in protecting cacao pods against *Moniliophthora roreri* in Ecuador (C. Suarez, pers. comm.), cotton from *Thielaviopsis basicola* (C. Howell, pers. comm.), and maize from *Fusarium verticillioides* (I. Yates, pers. comm.).

9. *Trichoderma ovalisporum* Samuels & Schroers, Mycol. Prog. 3: 204. 2004. MycoBank MB493497. Figs 17, 226–236.
Teleomorph: none known.

Characters of cultures: Optimum temperature on PDA and SNA 25–30 °C. Colonies grown on PDA and SNA in intermittent light forming green conidia on PDA and SNA at 25 and 30 °C after 48 h. PDA mycelium nearly or completely filling the Petri dish with dense, confluent green pustules forming in the centre of the colony and subsequent conidia developing toward the margin; no diffusing pigment or distinctive odour noted. Colonies grown on CMD filling the Petri plate within 4 d at 20–21 °C, conidia forming abundantly around the margin of the colony in cottony, grey-green pustules. Each pustule comprising intertwined, *ca.* 3.0–4.5 µm wide hyphae; phialides and conidia arising from the terminal 40–200 µm of the hyphae at or near the surface of the pustules. Conidiophores with conspicuous, distinctly linear central axes with relatively short lateral branches. *1º* branches tending to be paired and evenly spaced, 10–20 µm apart (more closely spaced toward the tip of the axis), arising at or near 90º with respect to the main axis, producing phialides directly, or *1º* branches less frequently producing *2º* branches, which form phialides directly. In DIS 172h often up to six phialides or *2º* branches arise from enlarged nodes on the main axis (pseudowhorls), sometimes fertile *1º* branches clustered at the top of the main axis with a long, sterile part below. Phialides paired or arising in whorls of 2–5 directly from the main axis and *1º* and *2º* branches; phialides typically arising at 90º with respect to the cell below, flask-shaped and more or less swollen below the tip to (less frequently) cylindrical. Intercalary phialides forming (Figs 233–234). Conidia ovoidal to broadly ellipsoidal or subglobose, green, smooth. Chlamydospores scattered, subglobose, terminal in submerged hyphae, (6–)7–10(–12) × (4.0–)6.2–8.7 (–10.5) µm (n = 30).

Habitat: Endophytic within woody tissues of *Banisteriopsis caapi* (*Malpighiaceae*), *Theobroma grandiflorum* and *Th. speciosum* (*Malvaceae*).



Figs 226–236. *Trichoderma ovalisporum*, anamorph from CMD. 226. Conidial pustules. 227–234. Conidiophores and phialides; intercalary phialides visible in 228, 233–234 (arrows). 235–236. Conidia. Figs 226–230, 234–235 from DIS 172h; 231, 233, 236 from DIS 203c; 232 from DIS 70a. Microscopy: 226 = stereo, 227–228 = PC, 229–230, 232 = FL, 231, 233–236 = DIC. Bars: 226 = 1 mm; 227–230, 232–234 = 20 µm; 231, 235–236 = 10 µm.

Known distribution: Amazonian Ecuador (Sucumbios Prov.) and Brazil (Pará state).

Holotype: **Ecuador**. Sucumbios Prov., Napo River, Panaco-cha-Río Yanayacu, isolated from the liana *Banisteropsis caapi*, Mar. 1999, H.C. Evans DIS 70a (BPI 843692, ex-type culture DIS 70a = CBS 113299 = DAOM 232077 = IMI 390990).

Additional cultures examined (all conidial isolates): **Brazil**, Pará, Belém, EMBRAPA Research Station, isolated from trunk of 50–60-yr-old *Th. grandiflorum*, Feb. 2000, H.C. Evans & K.A. Holmes DIS 172h (BPI 843691; cultures DIS 172h = CBS 113300 = DAOM 232078 = IMI 385808); second isolate from the same tree (DIS 172i = IMI 385811); Rio Xingu, Bella Vista Farm, isolated from 20-m-tall *Theobroma speciosum*, 5 Mar. 2000, H.C. Evans & K.A. Holmes DIS 203c (culture DIS 203c = IMI 385934).

Notes: This species was originally described and illustrated based on two endophytic isolates, DIS 70a and DIS 172i (Holmes *et al.* 2004). Since that time, two additional isolates (DIS 172h, DIS 203c) have been identified as this species. The isolates DIS 172h and DIS 172i were isolated from the same tree and are likely to be clones.

10. *Trichoderma petersenii* Samuels, Dodd & Schroers, **sp. nov**. MycoBank MB501043. Figs 18, 237–247.
Teleomorph: ***Hypocrea petersenii*** Samuels, Dodd & Schroers, **sp. nov.** MycoBank MB501044. Figs 41–43, 248–259.

Etymology: Named to honour Ronald H. Petersen, University of Tennessee, in recognition of his love for, and knowledge of, the Smoky Mountains.

Stromata rufobrunnea, velutina, ostiola plerumque occulta, *H. rufae* (Fr.) Fr. similia. Ascosporae hyalinae, spinulosae. Pars distalis ascosporarum (2.2–)3.5–4.5(–5.5) × (2.0–)3.2–4.2(–5.0) µm; pars proxima (2.7–)3.7–5.0(–6.5) × (2.2–)2.7–3.5(–5.2) µm. Anamorphosis *T. koningii* Oudem. similis. Conidia viridia, laevia, ellipsoidea ad late ellipsoidea, (2.5–)3.5–4.5(–5.5) × (2.2–)2.7–3.0(–3.5) µm; ratio longitudinis : latitudinis (1.0–)1.3–1.5(–1.8). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate (26–)33–45 mm.

Holotypus *H. petersenii*: BPI 864092A; holotypus anamorphosis *T. petersenii*: cultura sicca ex ascospora oriens BPI 864092B.

Stromata scattered to gregarious, at first thin, semi-effused, tan with a lighter-coloured margin, velvety, gradually becoming thicker, pulvinate to discoidal and reddish brown (8E–F8), not reacting to KOH, circular to elliptic in outline, 0.5–1.0(–1.7) mm diam (n = 109), broadly attached or with edges slightly free; plane, appearing velvety or smooth; perithecial elevations not evident; ostiolar openings barely visible as slightly dark areolae. Cells of stroma surface in face view more or less pseudoparenchymatous with unevenly thickened and pigmented walls, 3–7 µm diam. Surface region of the stroma *ca.* 20 µm thick, composed of pigmented, pseudoparenchymatous cells, (2.0–)3.0–6.5(–11) × (1.5–)2.2–5(–10) µm, walls slightly thickened. Tissue below the stroma surface compact, pseudoparenchyma or hyphal, thin-walled. Tissue below the perithecia pseudoparenchymatous, lacking hyphal elements. Perithecia subglobose, (166–)190–375(–485) µm high, (90–)125–265(–370) µm diam; ostiolar canal (50–)60–100(–150) µm long; perithecial apex around the ostiolar opening not anatomically distinct from the surrounding stroma surface. Asci cylindrical, apex thickened, with a pore. Part-ascospores hyaline, finely spinulose, dimorphic; distal part subglobose, proximal part wedge-shaped to oblong or slightly ellipsoidal.

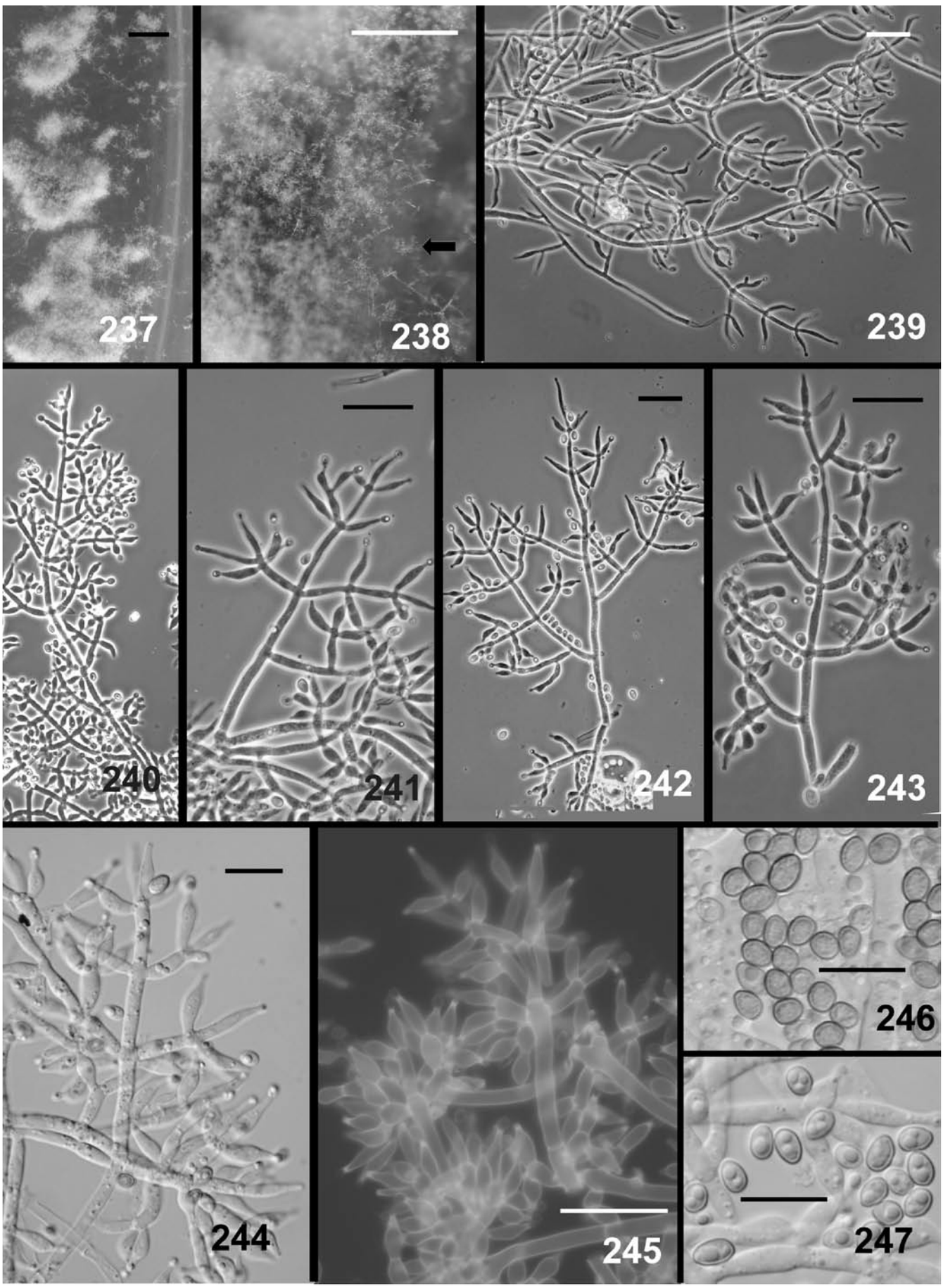
Characteristics of cultures: Optimum temperature for growth on PDA 25–30 °C, on SNA 25 °C. Colonies grown on PDA in darkness or in light for 96 h forming conidia in conspicuous concentric rings with a barraging aspect. Conidia typically forming abundantly on SNA in marked concentric rings in the aerial mycelium and/or in pustules. Conidia formed on CMD dark green, 27E–F8. No pigment diffusing through the agar; no distinctive odour. Colonies grown on CMD at 20 °C under light filling the Petri plate within 1 wk, conidial production often in one or more concentric rings near the margin, often in minute, confluent or discrete, cottony pustules. Conidiophores often visible in pustules, entirely fertile and “plumose” (Fig. 238). Conidiophores symmetrical, comprising a recognizable main axis, *ca.* 3 µm wide, fertile branches arising along the length of the main axis, more or less paired, with longer or shorter internodes; lateral branches and phialides on conidiophores formed at the interior of pustules tending to be densely clustered in pseudowhorls (Fig. 245). Branches arising at an angle of slightly less than 90° with respect to the main axis, longer branches near the base and short branches or solitary phialides arising near the tip; *1°* branches rebranching or producing phialides directly, *2°* branches producing phialides at the tip and often along the length. Phialides typically straight, lageniform, cylindrical or slightly swollen in the middle, held in whorls of 3 or 4, sometimes crowded when formed within a pustule; intercalary phialides not seen. Conidia ellipsoidal to broadly ellipsoidal, smooth. Chlamydospores abundant to sparse or lacking, terminal or intercalary, globose to subglobose, (3.5–)6.5–12(–28) µm diam (n = 109).

Habitat: Stromata developing on stromata of pyrenomycetous fungi and on decorticated wood; known also from one soil isolation in North Carolina.

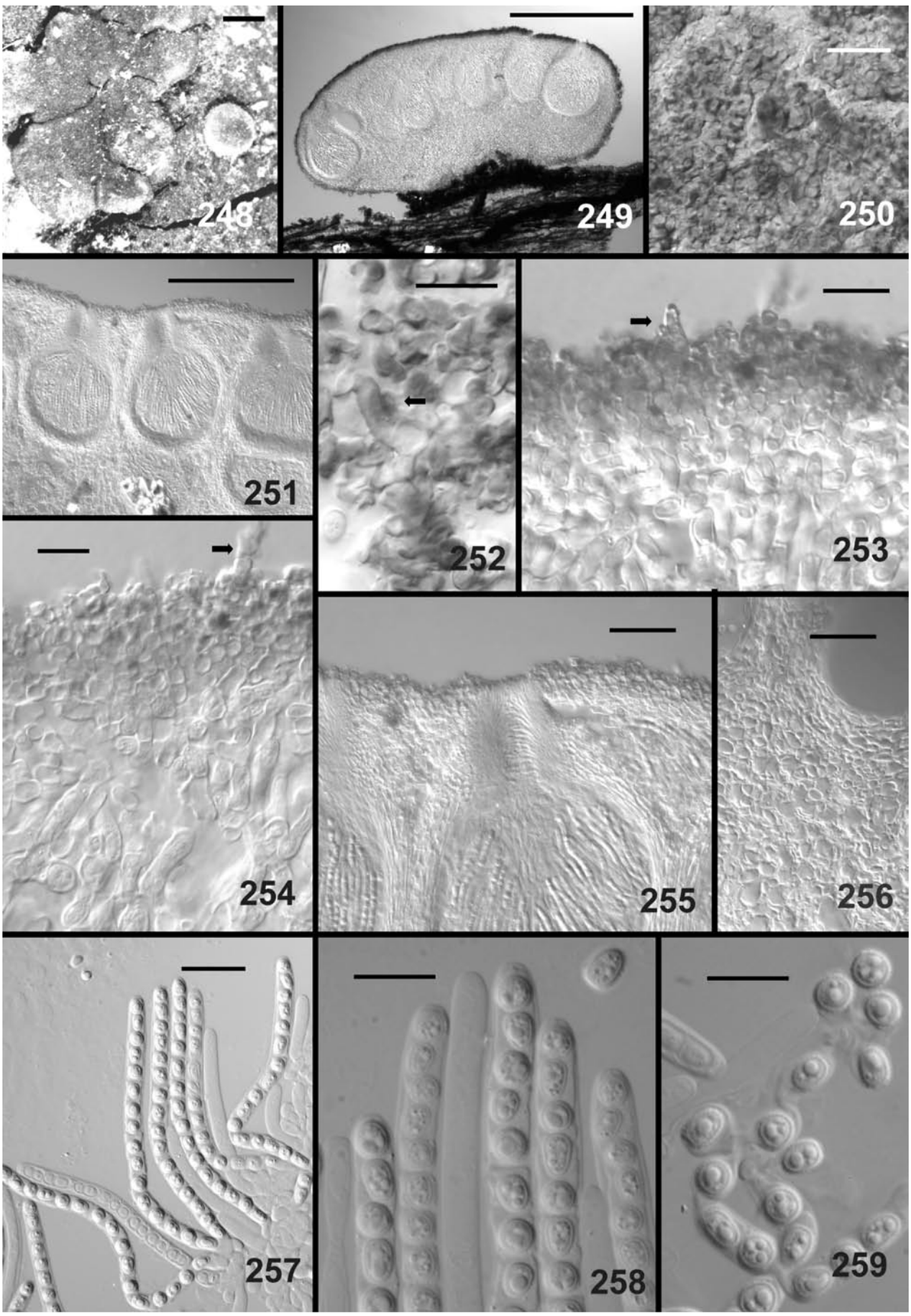
Known distribution: Europe (France), U.S.A. (TN, NC, VA), Costa Rica; common in the eastern U.S.A.

Holotype: **U.S.A.**, Tennessee, Great Smoky Mts National Park, vic. Cosby, Maddron Bald Track, 35°46’ N, 83°16’ W, elev. 500 m, 12 July 2004, on decorticated wood (?*Tsuga*), G.J.S. (BPI 864092A, holotype of *T. petersenii* is a dry culture BPI 864092B; ex-type culture G.J.S. 04-355 = CBS 119051).

Additional specimens examined, teleomorph: **Costa Rica**, Punta Arenas, Sabalito, Coto Brus, elev. 1500 m, on decorticated wood, 30 June 1999, G.J.S. *et al.* 8488 (BPI 746550, InBio; culture G.J.S. 99-48 = CBS 119090). **France**, Pyrénées Atlantiques, 64 Oloron, Forêt Bugangue, on decorticated hardwood, G.J.S. *et al.* (BPI 748317, culture G.J.S. 98-139); same collecting data, on *Hypoxylon* (BPI 748318, culture G.J.S. 98-140 = CBS 119091). **U.S.A.,** North Carolina, Clay County, Standing Indian Campground off highway 64, on decorticated wood (?*Fagus*), 15 Oct. 1990, Y. Doi, A.Y. Rossman & G.J.S. (BPI 1109378, TNS; culture G.J.S. 90-86 = CBS 119052); Tennessee, Great Smoky Mts National Park, vic. Cosby, Snake Den Rock Trail, 35°45’ N, 83°13’ W, elev. 940 m, on *Hypoxylon* on hardwood, 14 July 2004, G.J.S. (BPI 871100, culture G.J.S. 04-351 = CBS 119050); Virginia, Giles County, Cascades Recreation Site, 4



Figs 237–247. *Trichoderma petersenii*, anamorph from CMD. 237–238. Conidial pustules; individual conidiophores can be seen in 238 (e.g. arrow). 239–245. Conidophores and phialides; note densely clustered phialides in 245. 246–247. Conidia. Figs 237–239, 241, 243 from G.J.S. 05-351; 240 from G.J.S. 90-86; 242, 244, 247 from G.J.S. 04-355; 245 from G.J.S. 99-48; 246 from DAOM 165782. Microscopy: 237–238 = stereo; 239–243 = PC; 245 = FL; 244, 246–247 = DIC. Bars: 237 = 1 mm; 238 = 0.5 mm; 239–243 = 20 µm; 244, 246–247 = 10 µm; 245 = 20 µm.



Figs 248–259. *Trichoderma petersenii*, *Hypocrea* teleomorph. 248. Stromata formed on bark. 249. Longitudinal section through a mature stroma. 250, 252. Face view of a stroma; hairs visible in 252 (arrow). 251, 255. Median longitudinal sections through perithecia. 253–254. Sections through stroma surface; hairs marked by arrows. 256. Cells of stroma interior below a perithecium. 257–258. Asci. 259. Discharged part-ascospores. Figs 248, 252–254, 256–259 from G.J.S. 91-99; 249, 255 from G.J.S. 99-48; 250 from G.J.S. 04-355. Microscopy: 248 = stereo; all others = DIC. Bars 248–249 = 0.5 mm; 250, 253, 255–257 = 20 µm; 251 = 250 µm; 252, 254, 258–259 = 10 µm.

mi N of Pembroke, Little Stony Creek, 37°02' N, 80°35' W, elev. 840 m, on *Diatrype* sp., 18 Sep. 1991, G.J.S. *et al.* (BPI 1112869, culture G.J.S. 91-99).

Notes: With the exception of the soil isolate DAOM 165782 (North Carolina), we only know *T. petersenii* from ascospore isolations. It is common in the eastern United States together with *T. rogersonii, T. koningii* and *T. koningiopsis*. *Trichoderma petersenii* is characterized by a moderate growth rate, PDA colonies that are distinctive because of the conspicuous concentric rings and abundant conidia (Fig. 18), and stromata that are darker in colour, tending to be red-brown, than stromata of the other species (Figs 41–43).

11. *Trichoderma rogersonii* Samuels, **sp. nov**. MycoBank MB501045. Figs 19, 260–268.
Teleomorph: ***Hypocrea rogersonii*** Samuels, **sp. nov**. MycoBank MB501046. Figs 44–46, 269–280.

Etymology: Named in honour of Clark T. Rogerson who introduced a younger generation of mycologists to the Great Smoky Mts. National Park.

Stromata aurantio-brunnea ad rufo-brunnea, velutina, ostiola plerumque occulta, *H. rufa* (Fr.) Fr. similia. Ascosporae hyalinae, spinulosae. Pars distalis ascosporarum (3.0–)3.5–4.5(–5.2) × (2.5–) 3.2–4.0(–5.0) µm, pars proxima (3–)4–5(–6) × (2.0–)2.7–3.5 (–4.0) µm. Anamorphosis *T. koningii* Oudem. similis. Conidia viridia, laevia, ellipsoidea, (2.7–)3.5–4.5(–5.5) × (2.2–)2.5–3.2(–4.2) µm; ratio longitudinis:latitudinis (0.8–)1.3–1.7(–2.2). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate (38–)40–48 (–50) mm.

Holotypus *H. rogersonii*: BPI 870964A; holotypus anamorphosis *T. rogersonii*: cultura sicca ex ascospora oriens BPI 870964.

Stromata scattered to gregarious, at first thin, semi-effused, tan with a lighter-coloured margin, velvety, gradually becoming thicker, pulvinate to discoidal and reddish brown (8D–F8), not reacting to KOH, circular to elliptic in outline when mature, (0.5–)0.7–2.0(–4.0) mm diam (n = 58), broadly attached or with edges slightly free, plane, appearing velvety or smooth, perithecial elevations not evident, ostiolar openings not visible or barely visible as slightly dark areolae. Cells of stroma surface in face view more or less pseudoparenchymatous with unevenly thickened and pigmented walls or tending to be hyphal, 5–7 µm diam; hairs to 15 µm long, 3–4 µm wide, 1–3-septate, arising directly from cells of the stroma surface. Surface region of the stroma 20–25 µm thick, composed of pigmented, pseudoparenchymatous cells, (1.5–)2–4(–6) × (1.5–) 2–3(–4) µm, walls slightly thickened. Tissue below the stroma surface hyphal; cells *ca.* 5 µm wide. Perithecia elliptic to subglobose in section, (150–)200–260(–350) µm high, (55–)120–180(–215) µm diam; ostiolar canal (40–)55–75(–100) µm long; perithecial apex around the ostiolar opening not anatomically distinct from the surrounding stroma surface. Asci cylindrical, apex thickened, with a pore. Part-ascospores hyaline, finely spinulose, dimorphic; distal part subglobose, proximal part wedge-shaped to oblong or slightly ellipsoidal.
Characters of cultures: Optimum temperature for growth on PDA 25–30 °C, on SNA 25 °C. Colonies grown on PDA in intermittent light forming conidia within 48 h at 25 °C; after 96 h in light conidial production most abundant in a central disk with 1 or 2 widely spaced concentric rings beginning to form. Conidia on CMD deep green (26–27D–F8). No pigment diffusing through the agar; no distinctive odour.

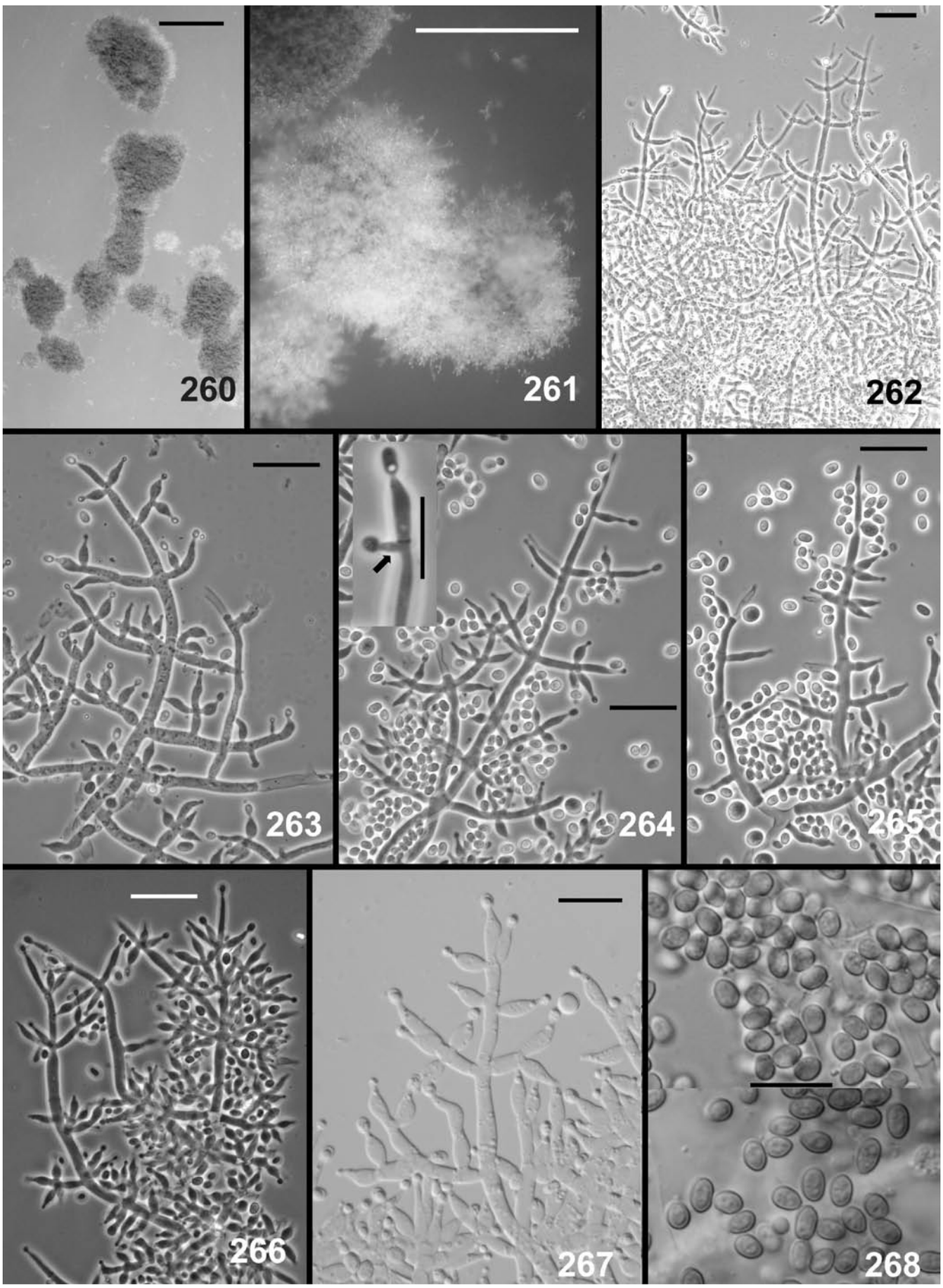
Colonies grown on CMD at 20–25 °C under light filling the Petri plate within 1 wk, conidia forming abundantly behind the colony margin in 1–2 concentric rings of confluent, minute cottony pustules or conidial production continuous; individual conidiophores visible within the pustules, completely fertile to the tip (Fig. 261). Conidiophores symmetrical, comprising a recognizable main axis, *ca.* 3 µm wide, fertile branches arising along the length of the main axis, often paired, with longer or shorter internodes. Branches arising at an angle of slightly less than 90° with respect to the main axis, longer branches near the base and short branches or solitary phialides arising near the tip; *1°* branches rebranching or producing phialides directly, *2°* branches producing phialides at the tip and often along the length; conidiophores produced at the interior of pustules becoming much-branched with short internodes between the branches (Fig. 266). Phialides typically straight, lageniform, cylindrical or slightly swollen in the middle, held in whorls of 3 or 4, sometimes crowded; intercalary phialides observed. Conidia narrowly ellipsoidal, smooth. Chlamydospores typically not produced but abundant in some collections, terminal on hyphae and forming within hyphal cells, subglobose when terminal, (7–)9–15(–26) µm diam.

Habitat: Bark of hardwood trees.

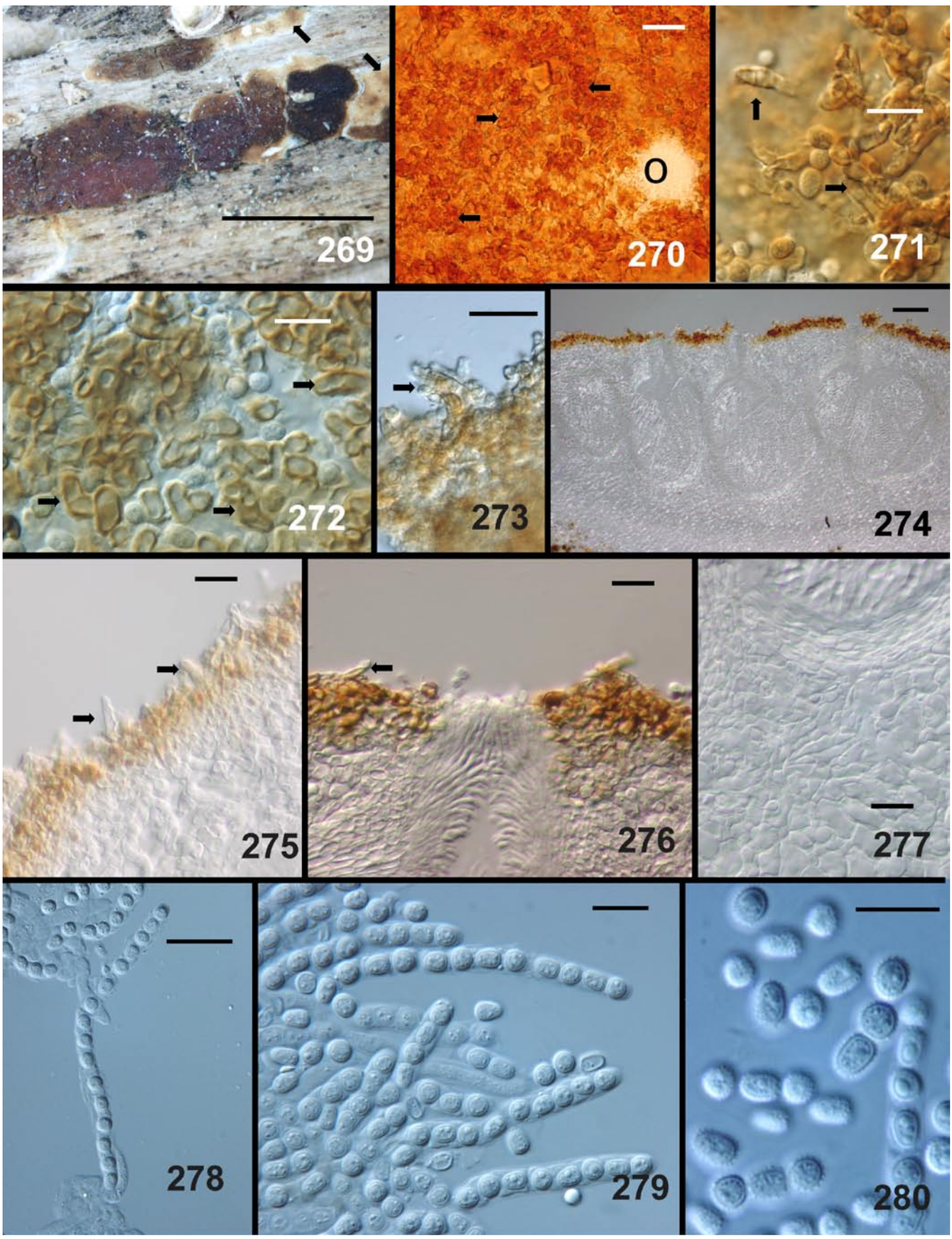
Known distribution: U.S.A. (MD, MO, NC, NJ, TN), Europe (Austria).

Holotype: **U.S.A.,** Tennessee, Great Smoky Mts. National Park, vic. Cosby, Albright Trail, on decorticated wood, July 2005, B.E. Overton 04-04 (BPI 870964A, [GSMNP 77359]; holotype of anamorph BPI 870964B; ex-type culture G.J.S. 04-158 = CBS 119233).

Additional specimens examined: **Austria**, Lower Austria, Waldviertel, elev. 420–500 m, virgin forest at Dobra Kamp, on *Fagus sylvatica*, fallen log, 10 Oct. 1995, H. Voglmayr (951004/2) (BPI 737800; culture G.J.S. 95-217 = CBS 119085). **U.S.A.,** Maryland, Prince George County; Greenbelt, on bark, 11 Oct. 1992, S.A. Rehner (BPI 802859; culture G.J.S. 92-116 = CBS 119083); Missouri, Mingo Wilderness Area, Lake Wappen Wilderness Area, on decorticated wood, 18 Sep. 1994, G.J.S. (BPI 737858, culture G.J.S. 94-115 = CBS 119081); New Jersey, Cumberland County, S. of Newfield, *ca.* 1 mi off Rte 40, elev. 250 m, on decorticated hardwood, 15 Aug. 1998, G.J.S., H. C. Chamberlain & B.E. Overton (BPI 748406; culture G.J.S. 98-77); second collection, on decorticated wood (BPI 748404; culture G.J.S. 98-75), third collection (BPI 748411; culture G.J.S. 98-82 = CBS 119084); North Carolina, Macon County, Ammons Branch Campground, off Bull Pen Rd., 35°01' N, 83°08' W, elev. 3000 ft, on well-rotted wood of *Quercus* sp., 14 Oct. 1990, Y. Doi (50), A.Y. Rossman & G.J.S. (BPI 1107174, TNS; culture G.J.S. 90-108); Macon County, Blue Valley off Clear Creek Rd., along Overflow Creek, 35°03' N, 83°15' W, on *Diaporthales* on *Betula* sp., 16 Oct. 1995, G.J.S., A. Y. Rossman & Y. Doi (95) (BPI 1109384, TNS, culture G.J.S. 90-93); second collection, on bark, 14 Oct. 1990, Y. Doi, A.Y. Rossman & G.J.S. (BPI 1109371; culture G.J.S. 90-79); third collection, on decorticated wood of *Quercus* sp., 16 Oct. 1990, Y. Doi (35) *et al.* (BPI 1107185, TNS; culture G.J.S. 90-125 = CBS 119082); Macon County, Ellicott Rock Trail, off Bull Pen Rd., 31°01' N, 83°08' W, elev. 3000 ft., on decorticated log, 14 Oct. 1990, Y. Doi (4) *et al.* (BPI 1109370, TNS; culture G.J.S. 90-78 = CBS 985.97); Tennessee, Great Smoky Mts. National Park, vic. Cosby, Albright Trail, on decorticated wood, July 2005, B.E. Overton 04-05 (BPI 864093, GSMNP 77395; culture G.J.S. 04-157).



Figs 260–268. *Trichoderma rogersonii*, anamorph from CMD. 260–261. Conidial pustules; individual conidiophores can be seen protruding from the periphery of the immature pustule in 261. 262–267. Conidiophores and phialides; an intercalary phialide can be seen in 264 inset (arrow). 268. Conidia. Figs 260–261 from G.J.S. 90-78; 262 from G.J.S. 04-158; 263, 267 from G.J.S. 04-157; 264–265 from G.J.S. 90-108; 266, 268 above from G.J.S. 90-79; 268 below from G.J.S. 98-82. Microscopy: 260–261 = stereo; 262–266 = PC; 267–268 = DIC. Bars: 260 = 1 mm; 261 = 0.5 mm; 262–267 = 20 µm; 264 inset, 268 = 10 µm.



Figs 269–280. *Trichoderma rogersonii*, *Hypocrea* teleomorph. 269. Stromata; immature stromata indicated by arrows. 270. Stroma surface in face view; ostiolar opening of a perithecium indicatd by "O"; note the mottled appearance resulting from small piles of cells and short hairs (e.g. arrows). 271–273. Cells and hairs (arrows) at stroma surface in face view (271–272) and in section (273). 274. Median longitudinal section through mature perithecia. 275. Section through stroma surface; note hairs (e.g. arrows); note the loose stroma surface in 274–275. 276. Section through the ostiolar canal and surrounding stroma tissue; hair indicated by arrow. 277. Tissue of stroma interior below a perithecium. 278–279. Asci and ascospores. 280. Discharged part-ascospores. Fig. 269 from G.J.S. 95-217; 270–271, 274 from G.J.S. 98-75; 272, 278–279 from G.J.S. 04-157; 273, 275–276 from G.J.S. 90-108; 277 from G.J.S. 92-116; 280 from G.J.S. 04-158. Microscopy: 269 = stereo, all others DIC. Bars: Fig. 269 = 1 mm; 270–278 = 20 µm; 279–280 = 10 µm.

Notes: The geographic range of *T. rogersonii* overlaps with that of *T. petersenii*, *T. koningii* and, to a lesser extent, *T. koningiopsis*. Both *T. rogersonii* and *T. petersenii* are common in forests of the eastern United States. Despite their phylogenetic differences, very few phenotypic characters separate them. See notes with *T. petersenii.*

12. *Trichoderma stilbohypoxyli* Samuels & Schroers, **sp. nov**. MycoBank MB501047. Figs 21–22, 281–289. *Teleomorph*: ***Hypocrea stilbohypoxyli*** Samuels & B.-S. Lu, Sydowia 55: 265 (2003). MycoBank MB488313 Figs 47, 48.

Anamorphosis *T. koningii* Oudem. similis. Conidia viridia, laevia, ellipsoidea, (2.6–3.2–4.2(–5.0) × (2.0–)2.5–3.2(–3.5) µm; ratio longitudinis:latitudinis (1.0–)1.2–1.6(–1.9). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate 36–41 mm. Anamorphosis *Hypocreae stilbohypoxyli* Samuels & B.-S. Lu.
Holotypus: BPI 744463B, cultus in agaro siccus.

Stromata mostly solitary, sometimes gregarious, occasionally in pairs, forming on ascomata of host fungi and adjacent plant tissue, semi-effused, irregular in outline and covering extensive areas of plant tissue, sometimes slightly constricted at the margin, (0.2–)0.5–0.9(–1.5) × 0.2–0.7(–1.3) mm, plane to conspicuously tuberculate due to perithecial apices, *ca*. 5C–D8 or more reddish, not changing colour in 3 % KOH; margin light tan to white; young surface of stroma velvety due to short ends of projecting hyphae; velvety aspect diminishing with age. Ostiolar openings at most barely visible as darker dots. Cells of the stroma in face view elongate, angular or irregular in outline, (4.0–)5.5–7.5 (–10) × (3.0–)4.0–5.5(–7.5) µm, reddish brown, cell-walls 0.5-1.0 µm thick (n = 10); stromatal surface in vertical section 30–60(–88) µm thick (n = 5), brown, KOH^-, cells compressed, angular or sometimes rounded, (2.4–)3.5–5.5(–10) × (2.0–)2.5–4.0(–6.5) µm, cell-walls 0.5–1.0 µm thick (n = 10). Hyphal hairs arising from the stroma surface conspicuous, scattered, *ca.* 10 µm long, 3 µm wide at the base, hyaline to light brown. Cells immediately below the stroma surface hyphal, thin-walled, hyaline, not changing colour in 3 % KOH. Tissue below the perithecia of more or less compact intertwined hyphae, less frequently pseudoparenchymatous, cells (5–)7–10(–15) × (3.5–)4.5–7(–9) µm, thin-walled, hyaline, not changing colour in 3 % KOH. Perithecia immersed in stroma, closely spaced, mostly globose to subglobose, laterally compressed, pyriform to clavate when densely disposed, (216–)250–320(–340) µm high (n = 11), 150–200(–220) µm wide (n = 11), ostiolar canal (46–)65–95(–103) µm long (n = 11), cells of the perithecial wall brown or reddish brown to hyaline; ostiolar region not sharply delimited from the surrouding tissue of the stroma surface. Asci cylindrical, with a thickened tip and a pore. Ascospores hyaline, finely spinulose. Part-ascospores dimorphic; distal part globose to subglobose or conical, proximal part subglobose or elongate to wedge-shaped, tending to be more elongate toward the base of the ascus.

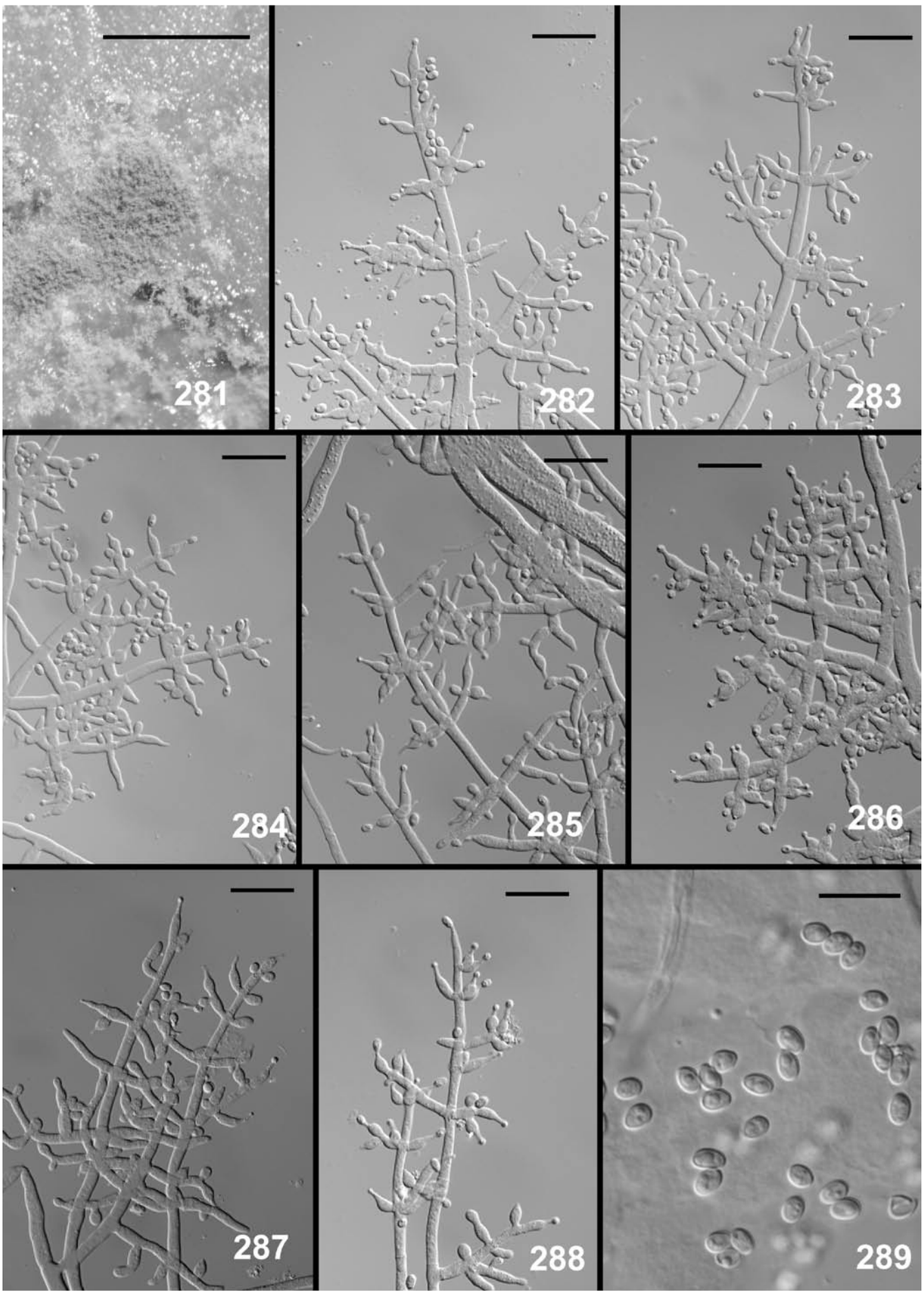
Characteristics in culture: Optimum for growth on PDA and SNA 30 °C. Colonies grown on PDA and SNA at 25 °C and 30 °C for 72 h reaching a radius of 20–25 mm. After 1 wk on PDA at 25 °C under light or in darkness typically forming a lawn of conidia in up to three broad concentric rings of green to yellow-green conidia, usually a diffusing yellow pigment visible in colony reverse. On SNA conidia forming in conspicuous narrow concentric rings separated by wider bands of less conidial production. On CMD at 25 °C after 4 d under light, aerial mycelium scant, conidial pustules minute, at first scattered discrete and then more abundant and confluent toward the margin of the colony; at first light green toward the centre and white-grey to white near the margin, becoming uniformly green within 4 d. No odour or pigment noted. Conidiophores comprising a more or less distinct central axis; phialides and/or fertile branches arising along the entire length. *1º* branches arising at 90º with respect to the main axis, paired or solitary, progressively longer and more profusely branched with distance from the tip, producing phialides directly or producing *2º* branches, which mostly arise at 90º with respect to the *1º* branches, paired or solitary, producing phialides directly or producing *3°* branches; *3°* branches typically unicellular, producing phialides directly, singly or in a terminal whorl of 2–4. Phialides flask-shaped to pyriform. Conidia ellipsoidal, green, smooth. Chlamydospores scattered, not abundant, terminal and intercalary in hyphae, globose or subglobose, (2.3–)5.5–8(–14.0) × (1.6–)4.0–7.5(–10.8) µm, wall smooth or somewhat roughened.

Habitat: On diverse lignicolous ascomycetes including perithecia of *Neonectria jungneri* and stromata of *Stilbohypoxylon muelleri* (*Xylariaceae*); endophytic in sapwood of *Theobroma grandiflorum*, *Th. cacao* and *Fagus sylvatica*.

Known distribution: Brazil, Costa Rica, Ecuador, Puerto Rico, Ghana, United Kingdom

Holotype: **Puerto Rico**, Caribbean National Forest, El Yunque Recreation Area, Trail from Palo Colorado, elev. 700–800 m, on palm leaf midribs with *Stilbohypoxylon muelleri*, 22 Feb. 1996, G.J.S. 8076 (teleomorph BPI 744463; holotype of *T. stilbohypoxyli* BPI 744463B; ex-type culture G.J.S. 96-30 = ATCC MYA 2970 = CBS 992.97 = DAOM 231834).

Additional specimens and cultures examined: **Brazil,** Rio Xingu, Bella Vista, isolated from trunk of 20 m tall *Theobroma* grandiflorum, 5 Mar. 2000, H.C. Evans & K.A. Holmes (culture DIS 205bi = IMI 385821). **Costa Rica,** Pta Arenas, Coto Brus, Sabalito, Sitio Coton Cito, sendero Sutuba, elev. 1400 m, *Quercus* forest, on bark of recently cut tree, 28 Sep. 2000, L. Umaña (G.J.S. 8850) (INBio 13274; culture G.J.S. 02-143). **Ghana**, Eastern Region, Mkawkaw Distr., Mpraeso Scarp, vic. Mpraeso, near telecom station, 06°34' N, 00°46' W, elev. 785 m, in disturbed forest, on bark, 20 Oct. 2003, G.J.S. 9391 & H.C. Evans (BPI 872115, culture G.J.S. 03-103); same collecting data, on twig, G.J.S. 9390 & H.C. Evans (BPI 872116; conidial isolate G.J.S. 03-156); same collecting data, on *Neonectria jungneri*, G.J.S. 9397 & H.C. Evans (BPI 872117; conidial isolate G.J.S. 03-157). **Puerto Rico**, Caribbean National Forest, Big Tree Trail to Lamina, North end, off Rte 191, elev. 500 m, on *Stilbohypoxylon muelleri*, 23 Feb. 1996, G.J.S. 8071a & H.-J. Schroers (BPI 744467, culture G.J.S. 96-32 = CBS 112888, DAOM 231835); Caribbean National Forest, Luquillo Mountains, Interpretive Trail, Serra Palm, off Rte 191, elev. 650 m, on palm leaf midribs with *Stilbohypoxylon muelleri*, 23 Feb. 1996, G.J.S. 8079a & H.-J. Schroers (BPI 744471, conidial isolate G.J.S. 96-42a = CBS 112886 = DAOM 231836); Serra Palm, off Rte 191, elev. 700–800 m, 23 Feb. 1996, G.J.S. 8076a & H.-J. Schroers (BPI 744756, culture G.J.S. 96-43 = CBS 450.96 = DAOM 231837).



Figs 281–289. *Trichoderma stilbohypoxyli*, anamorph from CMD. 281. Conidial pustule. 282–288. Conidiophores and phialides. 289. Conidia. Figs 281, 283–284, 289 from G.J.S. 96-32; 282, 286 from G.J.S. 96-30; 285, 287–288 from G.J.S. 96-42a. Microscopy: 281 = stereo, all others DIC. Bars: 281 = 0.5 mm; 282–288 = 20 µm; 289 = 10 µm.

United Kingdom, Wiltshire, Savernake Forest, isolated from trunk of *Fagus sylvatica*, S.E. Thomas B69-6A (culture G.J.S. 05-475); same locality, second collection, S.E. Thomas B69-1A (culture G.J.S. 05-474).

Notes: As is evident from the several specimens and cultures cited here, *T. stilbohypoxyli* is a common tropical species, the teleomorph of which we have seen in Ghana and Puerto Rico. Additional *tef* analysis (not shown) has shown *T. stilbohypoxyli* to be phylogenetically diverse, the diversity corresponding to some extent with the geographic diversity that is indicated in the specimens examined cited above. However, we lack consistent morphological information to use in recognizing species within the phylogenetic diversity. Ascospores of the single Ghanaian collection were somewhat larger than they were in the Puerto Rican collections (respectively: distal part-ascospores: 4.2 ± 0.4 × 3.7 ± 0.2 µm vs 4.0 ± 0.3 × 3.5 ± 0.3 µm; proximal part-ascospores 4.5 ± 0.5 × 3.5 ± 0.2 µm vs 4.7 ± 0.5 × 3.0 ± 0.2 µm). The Puerto Rican cultures grew more slowly than the others on PDA, reaching a maximum radius of 45 mm after 72 h at the optimum temperature of 30 °C; colony radius in the other isolates was 55–70 mm. This difference was not seen on SNA. We were surprised to find *T. stilbohypoxyli* as an endophyte of trunks of ancient beech trees in the United Kingdom and *Theobroma* species in South America. Interestingly, the *Fagus* endophytes (G.J.S. 05-474, G.J.S. 05-475) clustered independently of the *Theobroma* endophyte (DIS 205b) from Brazil.

In the Caribbean region, *T. stilbohypoxyli* is found with *T. caribbaeum* var. *caribbaeum, T. intricatum* and *T. koningiopsis*. *Trichoderma caribbaeum* and *T. koningiopsis* have the longest conidia and, with *T. stilbohypoxyli*, they have the longest ascospores and highest L/W for their conidia. *Trichoderma stilbohypoxyli* is distinguished by having shorter and narrower conidia than *T. caribbaeum* and *T. koningiopsis* and by having the slowest growth rate of all these species when grown on PDA, a difference that is especially noticeable at 20 °C; this difference is not seen on SNA. On SNA at 20 °C *T. caribbaeum* and *T. koningiopsis* grow considerably faster than *T. stilbohypoxyli* and *T. intricatum,* a difference that is not seen at higher temperatures.

The two *Fagus* endophytes from the U.K. grew much more slowly than the other isolates of *T. stilbohypoxyli* on SNA; the difference was marked at 25–30 °C. No difference was seen in growth rate on PDA, but the *Fagus* endophytes remained sterile and produced a distinctive, diffusing orange pigment when grown one week in intermittent light at 20–30 °C.

13. *Trichoderma taiwanense* Samuels & M.L. Wu, **sp. nov**. MycoBank MB501048. Figs 23, 290–295
Teleomorph: ***Hypocrea*** sp. Figs 49–50, 296–299.

Etymology: Named for the type locality, Taiwan.

Stromata discoidea, lutea ad rufa, glabra, papillata, ostiola plerumque occulta. Ascosporae hyalinae, spinulosae. Pars distalis ascosporarum (2.7–)3.0–4.0(–4.7) × (2.5–)3.0–3.5(–4.0) µm, pars proxima (3.0–)3.5–4.5(–5.2) × (2.2–)2.5–3.2 µm. Conidiophora *Trichodermati koningii* Oudem. similia, regulariter ramosa, ad basim incrassata, verrucosa. Conidia viridia, laevia, ellipsoidea, (3.5–)3.7–4.2(–4.7) × (2.5–)2.7–3.2(–3.5) µm; ratio longitudinis:latitudinis (1.0–)1.2–1.6(–1.8). Radius coloniae in substrato PDA dicto post 72 horas 25 °C obscuritate 43–46 mm.

Holotypus cultura sicca ex ascospora oriens BPI 737694.

Stromata more or less circular in outline, more or less discoidal, luteous when dry, rufous in 3 % KOH, 0.5–1 mm diam, margins free, smooth; surface tuberculate due to the rounded perithecial elevations, ostiolar openings not visible. Cells of the stroma surface distinctly pseudoparenchymatous, (2–)6–10(–12) µm diam, walls slightly thickened, hairs not observed at the stroma surface. Surface region of the stroma 12–20 µm thick, composed of pigmented, pseudoparenchymatous cells, 3.5–10 µm diam, walls slightly thickened. Tissue immediately below the surface region of intertwined hyphae. Perithecia elliptic in section, 160–200 µm high, 90–120 µm diam, ostiolar canal 50–70 µm long; perithecial apex protruding slightly through the stroma surface, formed of narrow hyphal elements. Asci cylindrical, apex thickened, with a pore. Part-ascospores hyaline, finely spinulose, dimorphic; distal part ellipsoidal to subglobose, proximal part wedge-shaped to oblong.

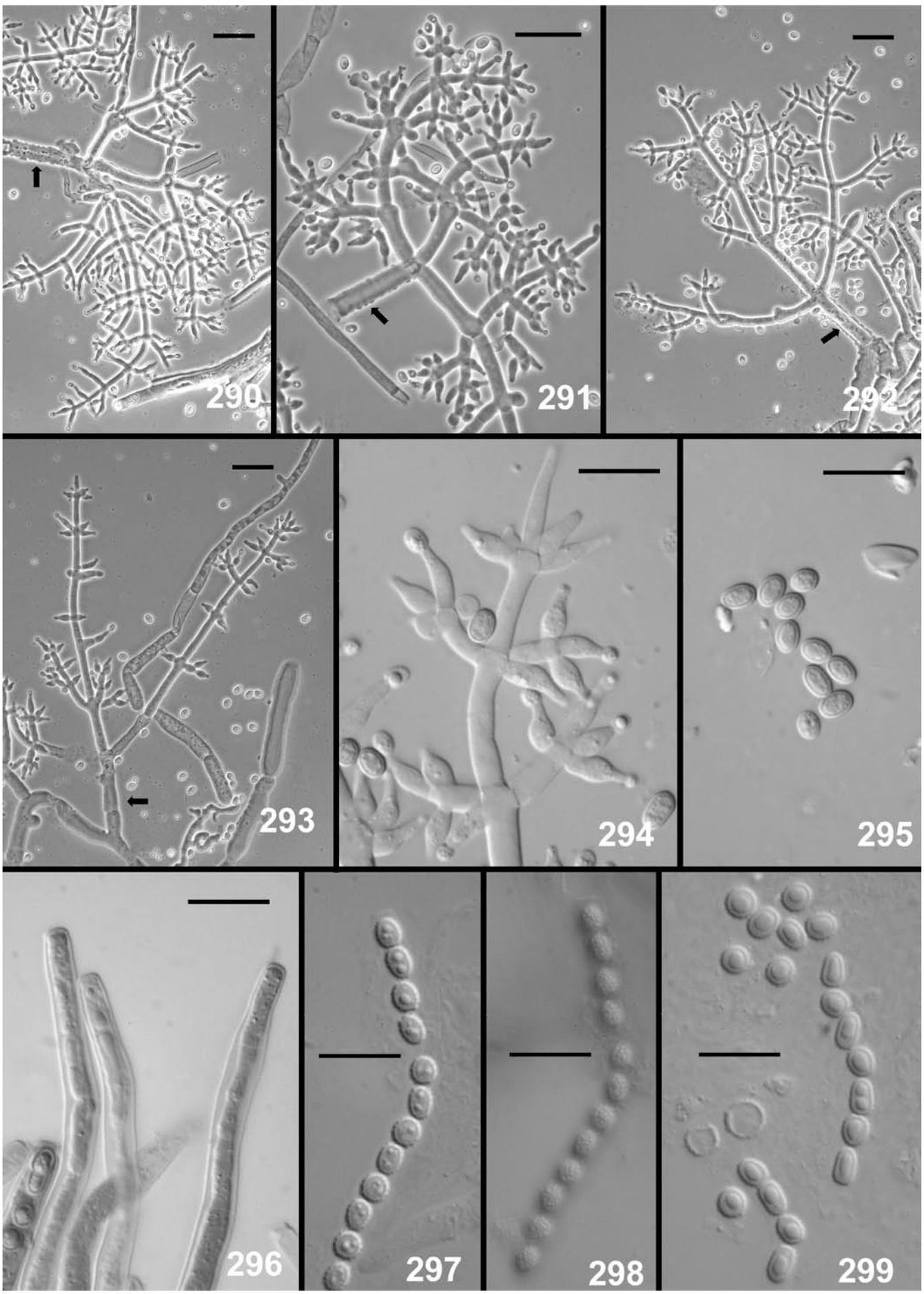
Characteristics in culture: Optimum temperature for growth on PDA 25–30 °C, on SNA 25 °C. Colonies grown on PDA in darkness or in light forming conidia in the centre of the colony with conidial production gradually spreading outwardly in faint concentric rings. Conidia dark green on CMD. No pigment diffusing through the agar; no distinctive odour. Colonies grown on CMD at 20 °C under light filling the Petri plate within 1 wk, conidia forming in minute, ill-defined tufts and in the aerial mycelium. Conidiophores symmetrical, comprising a recognizable main axis, 2–3 µm wide, often arising from an enlarged, verrucose base (Figs 290–293), fertile branches arising along the length of the main axis, more or less paired, with longer or shorter internodes. Branches arising at an angle of slightly less than 90° with respect to the main axis, longer branches near the base and short branches or solitary phialides arising near the tip; *1°* branches rebranching to a limited extent or producing phialides directly, *2°* branches producing phialides at the tip and often along the length. Phialides typically straight, lageniform, cylindrical or slightly swollen in the middle, held in whorls of 3 or 4, sometimes crowded and penicillate when formed within a pustule; intercalary phialides not seen. Conidia ellipsoidal, smooth. Chlamydospores not observed.

Habitat: On bark.

Known distribution: Republic of China (Taiwan).

Holotype: **Republic of China**, Taiwan, Fushan Botanical Garden, on bark, 23 Jan. 1995, M.-L. Wu 95-F1-11-T3 (BPI 737694; ex-type culture G.J.S. 95-93 = CBS 119058).

Notes: The *Hypocrea* specimen from which *T. taiwanense* is derived is overmature. Few asci remain and the stroma is collapsed. The stroma does not appear to be typical of members of this group in its gross morphology, but this could be the result of overmaturity. We have not described the *Hypocrea* teleomorph of



Figs 290–299. *Trichoderma taiwanense*. 290–295. Anamorph from CMD. 290–294. Conidiophores and phialides; note enlarged and roughened conidiophore base (arrows). 295. Conidia. 296–299. Asci and ascospores. Figs 296, 298 stained in 1 % aq. phloxine. All from G.J.S. 95-93. Microscopy: 290–293 = PC; 294–299 = DIC. Bars: 290–293 = 20 µm; 294–299 = 10 µm.

this species as new pending the discovery of material in better condition.

Two species having the *T. koningii* morphology and included in the present work are found in Taiwan, *T. taiwanense* and *T. austrokoningii*. For distinctions between these two phylogenetically distinct but phenotypically similar species see commentary under *T. austrokoningii* and Table 3.

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