Trichoderma asperellum, a new species with warted conidia, and redescription of *T. viride*

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The new species *Trichoderma asperellum* is distinguished from *T. viride* by finer conidial ornamentation, slightly ovoidal conidia, a faster growth rate, mostly paired branches, ampulliform phialides, and consistent presence of chlamydospores. *Trichoderma asperellum* cannot be unequivocally linked to a teleomorph. The teleomorph of *T. viride* is *Hypocrea rufa*. A key to *Trichoderma* and *Hypocrea* species with warted or roughened conidia is presented.

Keywords: biological control, *Hypocrea*, Hypocreales, systematics.

Trichoderma viride Pers., the type species of the genus, is characterized by globose to subglobose, warted conidia (Rifai 1969, Domsch & al., 1980; Bissett 1991a). Rifai (1969) and Bissett (1991a) recognized that there was considerable variation in conidial shape and in the appearance of the conidiophores among strains of *T. viride*, but they did not draw any formal taxonomic conclusions from the variation. When Rifai (1969) revised *Trichoderma*, he accepted the *T. viride*-aggregate as one of the nine 'aggregate' species that comprised the genus. Bisset (1991a) and Gams & Bissett (1998) included *T. viride* in *Trichoderma* sect. *Trichoderma*, along with *T. atroviride* Karsten, *T. koningii* Oud. and *T. aureoviride* Rifai. Meyer & Plaskowitz (1989) concluded conidia of some collections were more conspicuously warted than conidia of other collections. These different types of warts corresponded to mtDNA sequences (Meyer, 1991), leading Meyer (1991) to suggest that more than one species was involved within what has traditionally been called *T. viride*. He also did not propose a formal taxonomy to account for the variation.

Following Meyer's (1991) suggestion that all *Trichoderma* strains that have subglobose to globose, warted conidia are possibly not conspecific, Lieckfeldt & al. (1999) undertook a multifaceted study of the question. Sequences of the ITS-1 and ITS-2 regions and the first part of the 28S region of the rRNA gene along with RFLP analysis of the endochitinase gene and PCR fingerprinting, morphometrics, and physiological characters were utilized in a study of seventy-one strains that could be classified as *T. viride*. This study also included strains of species included in *Trichoderma* sect. *Trichoderma* (Bissett, 1991a, Gams & Bissett 1998), viz. *T. atroviride* and *T. koningii*, as well as strains derived from ascospores of *Hypocrea* collections that could be identified as *H. rufa* (Pers.) Fr., the teleomorph of *T. viride*. The mixed-type data sets were combined in correspondence analyses to demonstrate whether there was any correlation between the types of conidial ornamentation, termed groups I and II by Meyer & Plaskowitz (1989), to several genetic and phenotypic characters. Conidia of the lectotype specimen of *T. viride* (910 263 877, L!; Bisby, 1939) were strongly warted and correspond to group I of Meyer & Plaskowitz (1989), which is thus *T. viride s. str.* The results of Lieckfeldt & al. (1999) supported Meyer's (1991) contention that a new species was indicated for group II strains. In the present paper we describe the new species, *T. asperellum*, and compare it to *T. viride*.

Materials and methods

All measurements of anamorph characters were taken from cultures grown on CMD (cornmeal dextrose agar: Difco cornmeal agar + 2% dextrose) and SNA (low nutrient medium, Nirenberg, 1976) for about one week at 20–22 C. Conidiophores and conidia were measured from 3% KOH or from water; KOH was used first in all cases to aid in wetting the conidia. Measurements of teleomorph characters were taken from herbarium material that was rehydrated briefly in 3% KOH. Where it was possible, thirty measurements were made for each parameter in each collection. Measurements are reported as the maximum and minimum means of the collections included. A complete list of characters studied is given in Lieckfeldt & al. (1999).

Growth rates and colony characters at 20, 25, 30, and 35 C were taken from potato dextrose agar (PDA, Difco) and from SNA (Nirenberg, 1976).

Reference to colors is taken from Kornerup and Wanscher (1978), and is indicated as "K&W".

Material for SEM studies was obtained from cultures that were grown on PDA for 2 weeks at 20 C. Agar blocks with abundant conidia were prepared for SEM. Specimens were examined with a

JEOL T300 scanning electron microscope. With permission of the director of Persoon's herbarium (Leiden) we studied conidial ornamentation in the 200-yr-old lectotype collection of *T. viride* (910 263 877) using SEM and light microscopy. All SEM procedures followed the protocol of Meyer & Plaskowitz (1989).

Taxonomy

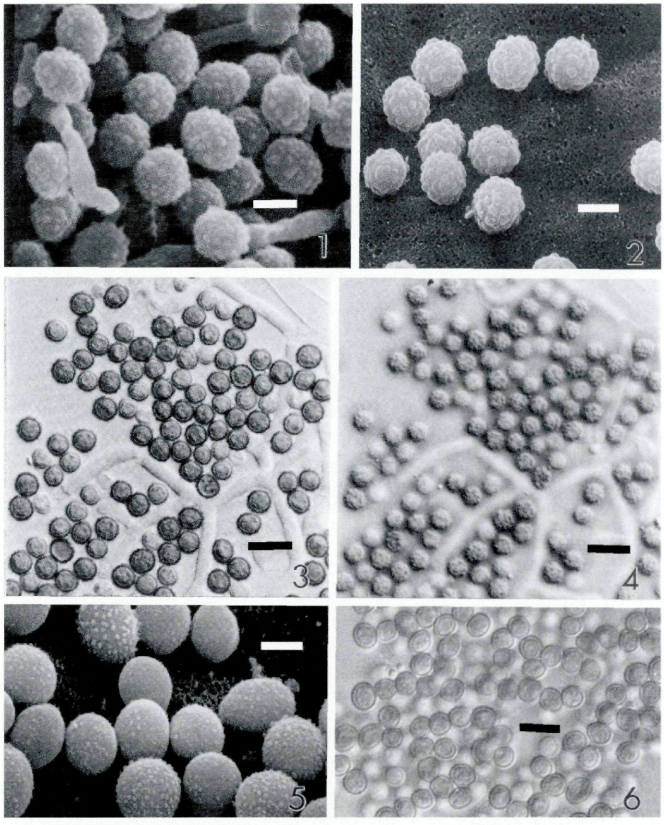
Lieckfeldt & al. (1999) detailed the molecular and phenotypic characters that separate *T. asperellum* from *T. viride*. The most important molecular characters distinguishing *T. asperellum* from *T. viride* are divergent ITS-1 and 28S sequences, RFLP's of the endochitinase gene and the RAPD pattern M13. Phenotypic characters that distinguish the species are given here in Tab. 1, the most important of which are the conidial ornamentation (Figs. 1–6), arrangement and branching of the conidiophores, the rather wider phialides and narrower conidiophores in *T. viride* and the relatively slow growth of *T. viride* on PDA and SNA coupled with later formation of conidia in *T. viride* (Figs. 7–10). The phenotypic differences between the two species are discussed as follows.

Because readily definable conidiophores are typically not formed in *Trichoderma* species, for the purposes of the present discussion we refer to the conidiophore as the phialides and the branch from which they arise. In practice, this is the terminal branch of often complexly intertwined hyphae within a more or less dense cushion or aggregate (Figs. 11, 20). In *T. viride* and *T. asperellum* conidiophores formed on CMD and SNA arise within aggregates that measure 1–1.5 mm diam. Conidiophores protrude from the conidial aggregates of both species. Conidiophores that protrude from those of *T. asperellum* are fertile along their length and often appear as plumes (Fig. 11, 12, 19 B). Individual conidiophores (Figs. 13–17, 19 A, B) of *T. asperellum* tend to be regularly branched, with lateral branches being more or less uniformly spaced and paired, the longest branches occurring the farthest from the tip. Phialides form at the tips of branches in verticillate or 'cruciate whorls,' wherein each of the 3–4 phialides are held at approximately 100° with respect to the other phialides and to the terminal branch from which the whorl arose (Figs. 15–17; 19 A, B). Phialides are straight and tend to be slightly wider in the middle than at the base (Figs. 15–17; 19 A, B).

Conidiophores that protrude from the aggregates of *T. viride* tend to be fertile only at the tip, or phialides are only sparingly produced along the main axis (Figs. 20, 21). Within the aggregate, conidiophores mostly branch irregularly, with lateral branches formed at regular intervals along the main axis or infrequently paired (Figs. 22–26; 28A–D). Lateral branches tend to be shorter than

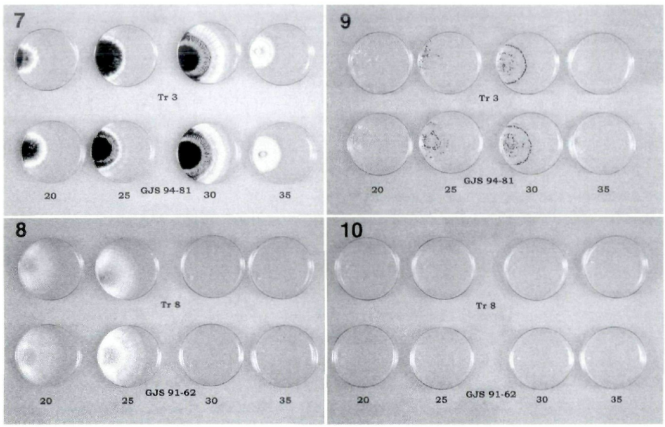
Tab. 1. – Salient phenotypic characters of *T. viride* and *T. asperellum*

	conidial ornamentation	**conidiophore**	**phialide**	**chlamydospores**	**temperature optimum (PDA)**	**colony radius (PDA) at optimum temperature (mm) after 48 h**
T. viride	conspicuous	irregularly branched, branches often not paired	often sigmoidal or hooked; l/w = 3.3	typically absent	25	11–33
T. asperellum	inconspicuous	regularly branched, branches typically paired	straight, l/w = 2.4	typically present	30	31–47



Figs. 1–6. Conidia of *Trichoderma viride* (1–4) and *T. asperellum* (5, 6). – 1. From the lectotype specimen of *T. viride*. – 2. From GJS 92-14. – 3, 4. From BBA 70239. – 5. From GJS 91-1. – 6. From Tr 3. – 1, 2, 5 SEM. 3, 6 Conidia in optical section using interference contrast microscopy. 4 Conidia in surface view using interference contrast microscopy. All from CMD except Fig. 1, which is a from herbarium specimen. – Scale bars: 1, 2, 5 = 2.5 µm; 3, 4, 6 = 10 µm.

in *T. asperellum*. Phialides form singly or in cruciate whorls. Phialides are straight, markedly sinuous or hooked (Figs. 24–26; 19 A, C). They are almost cylindrical rather than swollen in the middle, in contrast to those of *T. asperellum*.

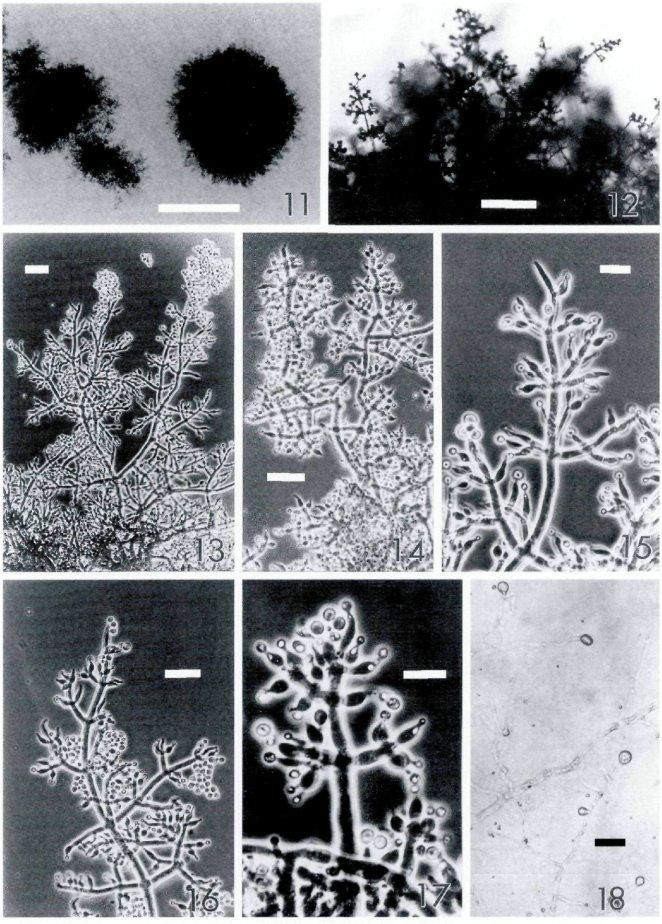


Figs. 7–10. Colony characters of two strains each of *Trichoderma asperellum* (7, 8) and *T. viride* (9, 10) from PDA (left) and SNA (right) when grown in darkness at 20, 25, 30 and 35 C for 72 h. – Note that there is growth on SNA for *T. viride*, but colonies are barely visible against the background of the medium.

Conidia of both species ultimately become dark green (28D8; K&W). Conidia of both are globose to subglobose, but in *T. asperellum* (Figs. 5, 6) they are slightly more ovoidal than in *T. viride* (Figs. 1–4) (median length/width measured from CMD = 1.3, 1.2 respectively).

The most conspicuous morphological difference between the species is seen in the different types of conidial ornamentation (Figs. 1–6). As seen with the light microscope, conidial warts in *T. asperellum* are small and inconspicuous whereas warts in *T. viride* are easily seen (compare Figs. 3 and 6). In SEM, warts of *T. viride* are broadly rounded (Figs. 1, 2; group I of Meyer & Plaskowitz, 1989 = *T. viride*) and warts of *T. asperellum* are slightly more irregular and pyramidal (Fig. 5, group II = *T. asperellum*). In *T. asperellum* the warts are unevenly dispersed and parts of individual conidia are smooth (Fig. 5). Warts on conidia of *T. viride* are uniformly dispersed (Figs. 1, 2). Conidia of the Persoon lectotype specimen of *T. viride* (Fig. 1) have conspicuous, broadly rounded, uniformly dispersed warts of group I; the conidia average 4.1×3.4 µm, l/w = 1.2 (n=38).

Strains of *T. asperellum* have a much faster growth rate than *T. viride* (Figs. 7–10). This is especially evident at 30 C or higher. At 30 C, *T. viride* had a colony radius of less than 10 mm within 40 h, whereas *T. asperellum* reached nearly 30 mm in the same time. Only



Figs. 11–18. *Trichoderma asperellum*. – 11. Conidial aggregate with projecting conidiophores. – 12. Projecting conidiophores from a conidial aggregate. – 13–17. Conidiophores and phialides showing regular branching and phialides that are arranged in 'cruciate' whorls and that are slightly swollen in the middle. – 18. Chlamydospores. – Fig. 11 from GJS 91-160; 12 from BBA 68646; 13, 15, 16 from Tr 3; 14 from BBA 68543; 17 from GJS 91-7; 18 from Tr 31. – Scale bars: 11 = 1 mm; 12 = 100 µm; 13, 14, 16, 18 = 25 µm; 15, 17 = 10 µm.

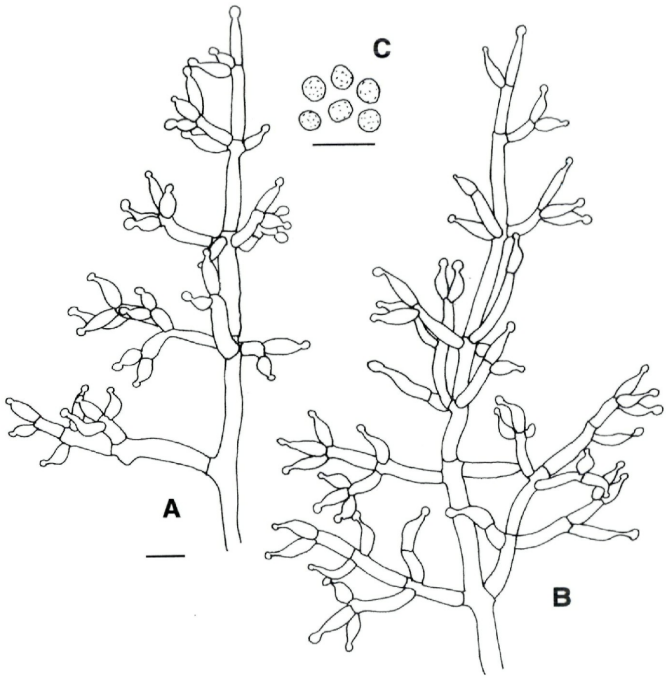
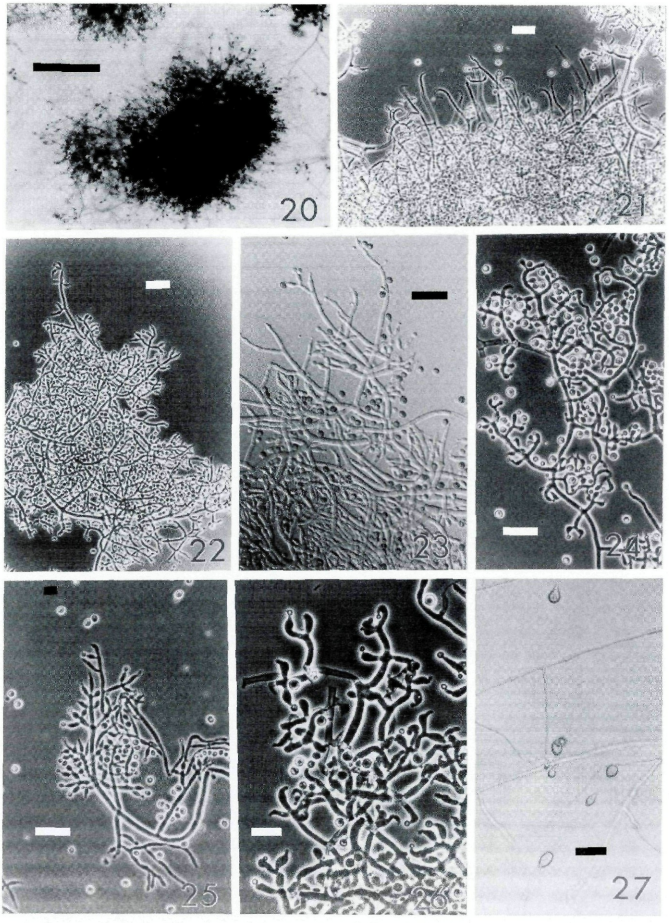


Fig. 19. *Trichoderma asperellum*, conidiophores and conidia. – A. TR 32, from CMD. – B, C. Tr 3, from SNA. – Scale bars = 10 µm.

strains of *T. asperellum* were able to grow at 35 C. In cultures of *T. asperellum* grown on PDA in darkness at 25 C conidia formed on average about 24 h earlier (average of 15 strains = 40 h) than in cultures of *T. viride* (average of 18 strains = 64 h). Of the eighteen strains of *T. viride* that we studied, only two produced conidia on SNA at 25 C in darkness, whereas conidia formed on SNA after an average of 40 h in all fifteen strains of *T. asperellum*.

Chlamydospores were formed by individual strains of both *T. asperellum* (Fig. 18) and *T. viride* (Fig. 27). However, of the strains of *T. asperellum* that we examined, only one failed to produce chlamydospores, whereas only six strains of *T. viride* produced chlamydospores. In *T. asperellum* chlamydospores were immediately obvious in the colony reverse and the thirty measurements were made



Figs. 20–27. *Trichoderma viride*. – 20. Conidial aggregate with projecting conidiophores. – 21. Projecting conidiophores from a conidial aggregate. – 22–26. Conidiophores and phialides showing irregular branching and phialides that are often solitary and often hooked or sinuous and that tend to be cylindrical. – 27. Chlamydospores. – Fig. 20 from Tr 6; 21 from GJS 92-17; 22 from GJS 92-11; 23 from GJS 89-127; 24 from Tr 6; 25, 27 from GJS 94-118; 26 from Tr 8. – Scale bars: 20 = 250 µm; 21–25 = 20 µm; 26 = 10 µm; 27 = 25 µm.

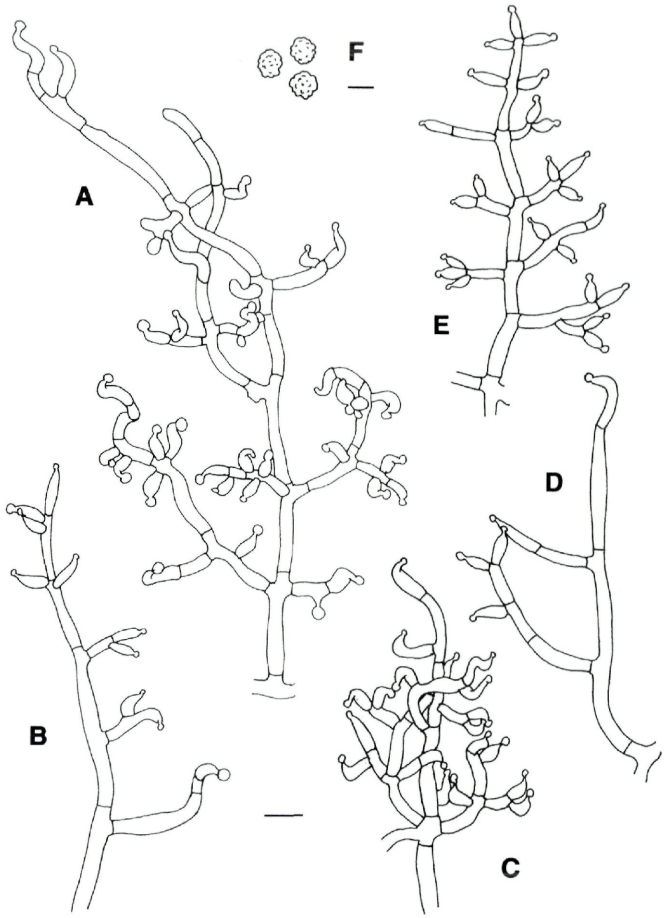


Fig. 28. *Trichoderma viride*, conidiophores and conidia. – A, B, F from GJS 91-62. – C, D from GJS 92-14. – E from Tr 26. – Scale bars = 10 µm.

well before half of the colony was scanned. On the other hand, no chlamydospores were found when the whole colony reverse was scanned; of the six strains in which chlamydospores were found, twenty or fewer were found in three. In two of the remaining three,

chlamydospores were as abundant as in *T. asperellum* whereas in the third chlamydospores were widely scattered and thirty were found only after the whole colony reverse had been scanned.

Ten strains of *T. viride* were derived from ascospores of *Hypocrea rufa*. Only one strain of *T. asperellum* was reported to have originated from ascospores (GJS 94–81, ICMP 5411 as *H. vinosa*), but we were not able to link that culture to a specimen (PDD) and thus cannot confirm that connection.

The *H. rufa* collections agreed well with the description published by Webster (1964), which was based on collections made in the United Kingdom, except that ascospores of the specimens that we studied were on average smaller.

Stromata mostly formed on decorticated wood, less frequently on bark of hardwood trees and on other fungi. They were pulvinate to discoidal, 0.5–1.5 mm diam, light brown and, when young, slightly effused and surrounded by a lighter ring. The surface was velvety as the result of short hairs, and ostiolar openings were not visible except when water or 3% KOH was added. None of our strains of either *Hypocrea* or apparently asexual *T. viride* produced stromata in agar culture.

Descriptions of the species

Trichoderma asperellum Samuels, Lieckfeldt & Nirenberg, sp. nov. – FIGS. 5–8, 11–19.

Trichodermati viridi species similis sed conidiorum verrucis minutioribus, phialidibus rectis, conidiophoris rectis regulariterque ramosis distincta. Coloniae celeriter crescentes. Phialides rectae, ampulliformes, 7.0–11.5 µm longae, 3.0–5.5 µm in medio, 2.0–3.5 µm ad basim crassae. Conidia globosa vel subglobosa ad leviter ovoidea, 3.7–6.0 × 3.0–5.0 µm. Coloniae optime in agaro dicto "PDA" temperatura 30 C crescunt. Teleomorphosis ignotus.

Holotype. – UNITED STATES. Maryland: Prince George County, Beltsville, isolated from sclerotia of *Sclerotinia minor* buried in soil of sesame plot, 1986, *M. T. Dunn TS 1 (c)* (Tr3, BBA 70684, CBS 433.97, AJ230668; dry culture BPI; live cultures deposited at ATCC and CBS).

Optimum temperature for growth: ca. 30 C. – Radius of colonies grown on PDA for 48 h at 30 C 31–47 mm (average of 18 = 35 ± 9 mm). – Colonies grown on PDA for 72 h at 30 C in darkness (with brief exposure to ambient fluorescent light at roughly 8-hr intervals) forming up to 5 concentric rings of dense conidial production, with conidia toward the center dark green (K&W 27F8) and conidia toward the margin just beginning to form, aerial mycelium lacking. – Diffusing pigment and odor lacking. – Colony reverse cream colored and often folded or convoluted.

Colonies grown on CMD 5 d at 20 C alternating equal periods of darkness and cool white fluorescent light >9 cm diam; colony characters and conidial formation nearly identical on CMD and SNA: conidia forming in abundance on pulvinate to hemispherical, 0.5–2 mm diam, discrete to confluent aggregates that are scattered throughout the colony or arranged in 2 or 3 concentric rings; conidia not forming or sparsely formed on conidiophores apart from the aggregates; conidial masses 'dark green' (27F8). Aggregates cottony with individual branches that are fertile to the tip; long, projecting conidiophores that are fertile only at the tip are not formed. – Conidiophores within each aggregate have a symmetric aspect, 2.8–5.4 µm wide (median of 16 collections = 3.3 µm), terminating in two or more phialides, and primary branches arising below the tip frequently paired and projecting at nearly 90° to the main axis. Primary branches progressively longer as the distance from the tip increases, members of a pair tending to have the same length, producing secondary branches that are longest close to the main axis; secondary branches producing tertiary branches that do not rebranch. – Phialides typically produced at the tips of primary, secondary and tertiary branches, rarely directly along the length of primary or secondary branches, typically held in whorls of 2–4 phialides. Phialides straight, ampulliform, only slightly enlarged in the middle, 7.0–11.5 µm (median of 16 collections = 8.4 µm) long, 3.0–5.5 µm (median = 3.4 µm) in the middle, 2.0–3.5 µm (median = 2.2 µm) at the base, l/w = 2.3–4.3 (median = 3.3), l/w = 2.0–3.5 (median = 2.5), slightly constricted at the base, held at an angle of ca. 100° with respect to the cell from which they arose. – Conidia globose to subglobose or ovoidal, 3.7–6.0 × 3.0–5.0 µm (median of 16 collections 3.8 × 3.2 µm, l/w = 1.2), lacking a visible basal abscission scar, finely spinulose, ornamentation sometimes difficult to observe. – Chlamydospores forming abundantly within one wk in cultures of all strains grown on CMD; terminal, or infrequently intercalary, on immersed hyphae, subglobose to ovoidal, smooth, pale green, 6.2–11.5 µm diam.

Habitat. – Soil.

Cultures examined. – See Tab. 2.

***Trichoderma* viride** Persoon in Roemer, Neu Mag. Bot. 1: 92. 1794 : Fries, Syst. mycol. 3: 215. 1832. Figs. 1–4, 9, 10, 20–28.

= *Trichoderma lignorum* Harz, Einige neue Hyphomyceten p. 29. 1871. (*fide* Domsch & al., 1980)

= *Trichoderma glaucum* E. V. Abbott, Iowa State Coll. J. Sci. 1: 27. Oct. 1926. (*fide* Domsch & al., 1980)

Tab. 2. – Cultures of *T. asperellum*

Strain	Geographic location/substratum	Genbank accession number
Tr 31	USA, lab strain	AJ230669
Tr 32	Korea, soil	AJ230669
Tr 50	USA/North Carolina	AJ230669
GJS 90-14	Vietnam	AJ230669
GJS 91-1	South Africa	AJ230669
BBA 68646R	Germany, compost	AJ230680
Tr 3 (TYPE) = CBS 433.97 = BBA 70684	USA/Maryland, *Sclerotinia*	AJ230668
Tr 7 = CBS 983.97	USA, lab strain	AJ230668
Tr 13 = CBS 984.97	USA, mutant of Tr 3	AJ230668
Tr 44	USA/Georgia, soil	AJ230668
GJS 94-81	New Zealand, *Hoheria*	AJ230668
BBA 68543	Germany, *Dieffenbachia*	AJ230668
GJS 91-160	Brazil	AJ230668
GJS 90-7	Vietnam	AJ230668
GJS 91-162	Brazil, soil	AJ230668
GJS 91-24	?	AJ230668

Optimum temperature for growth: ca. 25 C. – Radius of colonies grown on PDA for 48 h at 25 C 8–33 mm (average of 22 = 20.5 ± 5.6 mm). Radius of colonies grown on PDA for 48 h at 30 C 18–32 mm (average of 18 = 26 ± 8.0 mm). – Colonies typically not growing on PDA after 72 h at 30 C in darkness. Colonies grown on PDA for 72 h at 25 C in darkness forming only white mycelium on the surface of the agar and no conidial production. – Diffusing pigment lacking. – Colony reverse gray-green, not cream colored, folded or convoluted.

Colonies grown on CMD 5 d at 20 C alternating equal periods of darkness and cool white fluorescent light >9 cm diam; a more or less strong coconut odor detected in approximately half of the strains studied. Colony characters and conidial formation identical on CMD and SNA; morphologically distinctive conidiophores lacking, conidia forming in abundance on pulvinate to hemispherical, 0.5–3 µm diam, discrete to confluent aggregates that are typically located in a broad band around the margin of the colony or arranged in 2 or 3 concentric rings; conidial masses 'dark green' (27–28F8). Aggregates cottony to compact, sometimes conidia appearing to form only at the surface of individual aggregates; individual branches usually not visible within the aggregate; long, projecting conidiophores that are sparingly branched or are fertile only at the tip typically extending beyond the aggregate. – Conidiophores within each aggregate typically having a linear and often asymmetric aspect wherein primary branches of the conidiophore tend to

be short (typically <25 µm long), 2.5–3.5 µm (median of 22 collections = 2.8 µm) wide, unpaired and unbranched and terminating in a whorl of phialides; tip of conidiophore terminating in one or two phialides or sterile. – Phialides frequently arising directly from the conidiophore, especially near the tip, typically solitary or paired at the tip of the conidiophore or at the tip of secondary branches. Less frequently, within the same cultures, conidiophores symmetrically branched and phialides held in cruciate whorls. Phialides straight, tending to be sigmoidal or hooked, cylindrical or slightly wider in the middle than at the base, 7.2–11.5 µm (median of 22 collections = 9.2 µm) long, 3.0–5.5 µm in the middle, 2.0–3.5 µm at the base, slightly constricted at the base, forming an angle of ca. 100° with respect to the conidiophore axis below. – Conidia globose to subglobose, 3.5–4.5 × 2.7–4.0 µm (median of 22 collections = 4.1 × 3.5 µm, l/w = 1.1), lacking a visible basal abscission scar, conspicuously warted. – Chlamydospores forming in cultures of six of fifteen strains within one wk on CMD, terminal on immersed hyphae, subglobose to ovoidal, smooth, pale green, 8.5–10.5 µm diam.

Habitat. – Typically on decaying wood but also isolated from many different kinds of soils (Domsch et al., 1980).

Known distribution. – Reportedly cosmopolitan.

Cultures/specimens examined. – See Tab. 3.

Discussion

Trichoderma asperellum and *T. viride* are readily distinguished when examined using light microscopy because of the differences in the size of the warts on the conidia. Warts of *T. viride* are easily seen (Figs. 1–4) whereas those of *T. asperellum* are difficult to see (Figs. 5, 6). In addition to differences in the conidial warts, other phenotypic characters distinguish these species (Tab. 1). Sequencing of the internal transcribed spacers (ITS-1 and ITS-2) of the ribosomal gene complex revealed base pair differences totaling 13–17 bp, which is consistent with interspecies differences in *Trichoderma* (Kuhls & al., 1997; Lieckfeldt & al., 1998).

Trichoderma viride has been recognized to be the anamorph of *H. rufa* since the middle of the 19th Century (Tulasne & Tulasne, 1865). Several of the strains that are included in this and a previous study (Lieckfeldt & al., 1999) were derived from collections made primarily in the United States. *Hypocrea rufa, H. koningii* Lieckfeldt & Samuels (anamorph = *T. koningii*, Lieckfeldt & al., 1998), *H. muroiana* Hino & Katsumoto and an unidentified *Hypocrea*

Tab. 3. Cultures and specimens of *T. viride* and *H. rufa*.

Strain/Collection	Geographic location/substratum	Genbank accession number
Tr 8 = WSF 2023 = CBS 994.97	USA/Wisconsin, soil	X93986
Tr 2 = ATCC 18652	Colombia, soil	X93978
Tr 22 = ATCC 28020	USA/Washington	AJ230678
GJS 91-62	USA/Virginia	AJ230678
BBA 70238	Germany, turf	AJ230678
GJS 92-14	New Zealand, pine	AJ230678
GJS 92-15	Canada, peat	AJ230678
Tr 21 = ATCC 28038	USA/Virginia, soil	AJ230675
GJS 90-95	USA/North Carolina	–
BBA 68432	Russia/St. Petersburg, wheat	AJ230686
BBA 70470	Germany, wet building	AJ230682
GJS 92-11	New Zealand, pine	AJ230682
Tr 4	USA/Oregon, roots of Douglas fir infected with *Phellinus werii*	AJ230682
Tr 5	USA/Oregon, see Tr4	AJ230682
Tr 6	USA/Oregon, see Tr4	AJ230682
Tr 26 = ATCC 32630	Sweden, beach wood	AJ230682
BBA 66069R	Germany, soil	AJ230681
BBA 65450	Germany, soil	AJ230673
BBA 70471		AJ230682
Hy 70	New Zealand	AJ230678
GJS 89-127	USA/North Carolina	X93980
GJS 89-142	USA/North Carolina	X93987
GJS 94-118	France, bark	AJ230679

teleomorph of *T. atroviride* (Lieckfeldt & al., 1999) cannot be distinguished by their teleomorphs. The species are separable only through their anamorphs. Among *T. viride*, *T. atroviride* and *T. koningii* the small sequence variation (ITS-1: 1–2 bp; ITS-2: 2–5 bp) and RFLP analysis of the endochitinase gene and PCR fingerprinting indicate that these species are closely related. These differences included two ITS-subgroups of *T. viride s. str.* (Vb and Vd in Lieckfeldt & al., 1999). These two subgroups could not be distinguished reliably on the basis of their morphology, although conidia of Vd tended to be slightly larger and more ovoidal than conidia of Vb (the differences in size and l/w ratio were not statistically significant). Moreover, Samuels & al. (1994) included one strain of each of the groups Vb (GJS 89-127) and Vd (GJS 89-142) in a study of the *H. schweinitzii* complex. These strains, which were derived from ascospores of collections of *H. rufa* gathered in the same area of the Smoky Mountains of North Carolina, showed a greater difference in isozyme characters than the 'within species' variation of the other *Hypocrea* and *Trichoderma* species that were included in that study. Further,

we observed that most strains of *T. atroviride* produced a pronounced coconut odor, as did several strains of *T. viride*. Within *T. viride*, however, only one of the strains of group Vb and several strains of group Vd exhibited the odor. How to taxonomically evaluate the differences between these groups is the subject of continuing study.

Within sect. *Trichoderma* (Bissett, 1991a; Gams & Bissett, 1998), *T. asperellum* and *T. viride* are the only two described species that have subglobose, warted conidia. In addition, Doi (1974) and Doi & al. (1989) observed that conidia of *H. muroiana* could be either ellipsoidal and smooth, or subglobose to ovoidal and finely punctate. In this section, conidial morphology is closely correlated with small differences (ITS-1: 1–2 bp, ITS-2: 2–5 bp) in DNA sequence types (Lieckfeldt & al., 1999). Strains having smooth, ellipsoidal conidia correspond to *H. koningii* while strains having smooth, subglobose to ovoidal conidia were derived from *Hypocrea* stromata that are readily identified as *H. rufa*. Given that teleomorphs of *T. viride*, *T. koningii* and *T. atroviride* are indistinguishable from each other and from *H. muroiana*, we consider it possible that Doi (1974: Fig. 2) illustrated conidia of all of these ascomycetes under the name *H. muroiana*. The SEM illustrations provided by Doi & al. (1989; Fig. 2, A, B) for the ornamented conidia of *H. muroiana* agree well with what has been illustrated for *T. viride*. Doi & al. (1984) distinguished *H. rufa* var. *formosana* Yoshim. Doi and *H. albomedullosa* Yoshim. Doi (Doi, 1972; see also Doi & al., 1989) from *H. rufa* var. *rufa* on the basis of color of the stroma and on size and shape of conidia and phialides. Although material of the newly described taxa has not been made available to us for study, the illustrations of the anamorphs and teleomorphs strongly suggest that only one taxon is involved.

Although *T. viride* is the most frequently reported species of *Trichoderma* with warted or verrucose conidia (including anamorphs of *Hypocrea* species, see Domsch & al., 1980), additional species have roughened to tuberculate conidia. Conidial ornamentation in *T. flavofuscum* (Miller et al.) Bissett, *T. spirale* Bissett and *T. virens* (Miller et al.) Arx is visible only with scanning electron microscopy (Bissett, 1991b). Light microscopy reveals warts or tubercules on conidia of *T. saturnisporum* Hammill (Hammill, 1970; Samuels & al., 1998), *T. ghanense* Yoshim. Doi & al. (Doi & al., 1987; Samuels & al., 1998) and the anamorphs of *H. andinensis* Samuels & O. Petrini (Samuels et al., 1998) and *H. pachybasioides* Yoshim. Doi (reported by Doi, 1972). The species of *Trichoderma* that are known to have conidia and roughened or tuberculate conidia when viewed using light microscopy are distinguished in the following key.

Key to *Trichoderma* with roughened, warted conidia

1. Conidia hyaline (white in mass), roughened when young; sterile, sinuous, spinulose conidiophore extensions projecting from conidial tufts ..... *H. pachybasioides*
1. Conidia in mass green 2

2. Conidia conspicuously tuberculate, appearing as blisters; many phialides arising singly from the main axis ..... *T. saturnisporum*
2. Warts on conidia smaller or inconspicuous; many or most conidia appearing smooth 3

3. Length/width ratio of conidia measured from CMD >1.8; conidia obscurely tuberculate or smooth 4
3. Length/width ratio of conidia measured from CMD <1.5; conidia conspicuously or obscurely tuberculate 5

4. Most conidia measured from CMD 4–5 × 1.7–2.5 µm .. *H. andinensis*
4. Most conidia measured from CMD 4.5–6.2 × 2.2–3 µm ... *T. ghanense*

5. Most conidia conspicuously roughened or warted; conidia typically globose to subglobose (l/w = 1.1) but ellipsoidal in some collections; phialides cylindrical, often sinuous or hooked ..... *T. viride*
5. Conidia finely warted, many appearing smooth, globose, subglobose or ovoidal, l/w averaging 1.2; conidiophores regularly branched with branches conspicuously paired; phialides ampulliform, arranged in cruciate whorls of 3 or more, rarely solitary ..... *T. asperellum*

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References

Bisby, G. R. (1939). *Trichoderma viride* Pers. ex Fries, and notes on *Hypocrea*. – Trans. Brit. Mycol. Soc. 23: 149–168.

Bissett, J. (1991a). A revision of the genus *Trichoderma*. II. Infrageneric classification. – Canad. J. Bot. 69: 2357–2372.

—— (1991b). A revision of the genus *Trichoderma*. III. Section *Pachybasium*. – Canad. J. Bot. 69: 2373–2417.

Doi, N., K. Aoki & Y. Doi (1989). Conidia ornamentation studies in the genus *Trichoderma* 1. – Bull. Nat. Sci. Mus. B, 15: 27–33.

Doi, Y. (1972). Revision of the Hypocreales with cultural observations IV. The genus *Hypocrea* and its allies in Japan (2) Enumeration of the species. – Bull. Nat. Sci. Mus. 15: 649–751.

—— (1974). *Hypocrea rufa* (Pers. ex Fr.) Fr., *Hypomyces aurantius* (Pers. per S. F. Gray) Tul. and their allies in Japan. – Jap. J. Bot. 20: 403–412.

——, Y. Abe & J. Sugiyama (1987). *Trichoderma* sect. *Saturnisporum*, sect. nov. and *Trichoderma ghanense*, sp. nov. – Bull. Nat. Sci. Mus. Ser. B (Botany) 13: 1–9.

——, N. Doi & K. Toyozawa (1984). Notes on *Trichoderma* and its allies 3. Comparison of three strains of *Trichoderma viride* aggr. and their teleomorphs. – Bull. Nat. Sci. Mus. B, 10: 73—85.

Domsch, K. H., W. Gams & T.-H. Anderson (1980). Compendium of soil fungi. Vol. 1. – IHW Verlag, Berlin. 859 pp. (reprint 1993).

Gams, W. & J. Bissett (1998). Morphology and identification of *Trichoderma*. pp. 3–34. – In C. P. Kubicek and G. E. Harman (eds.), *Trichoderma* and *Gliocladium*. Taylor & Francis Ltd., London, U.K.

Hammill, T. M. (1970). *Paecilomyces clavisporus* sp. nov., *Trichoderma saturnisporum* sp. nov., and other noteworthy soil fungi from Georgia. – Mycologia 62: 107-122.

Kornerup, A. & J. H. Wanscher (1978). Methuen Handbook of Colour. – Methuen, London, UK.

Kuhls, K., E. Lieckfeldt, G. J. Samuels, W. Meyer, C. P. Kubicek, and T. Börner (1997). Revision of *Trichoderma* sect. *Longibrachiatum* including related teleomorphs based on analysis of ribosomal DNA internal transcribed spacer sequences. – Mycologia 89: 442–460.

Lieckfeldt, E., G. J. Samuels, T. Börner, & W. Gams (1998). *Trichoderma koningii*: Neotypification and *Hypocrea* teleomorph. – Canad. J. Bot. 76: 1507–1522.

——, G. J. Samuels & H. I Nirenberg (1999). What is *Trichoderma viride*? - A morphological and molecular perspective. – Appl. Environ. Microbiol. (submitted)

Meyer, R. (1991). Mitochondrial DNAs and plasmids as taxonomic characteristics in *Trichoderma viride*. – Appl. Environ. Microbiol. 57: 2269–2276.

—— & J. S. Plaskowitz (1989). Scanning electron microscopy of conidia and conidial matrix of *Trichoderma*. – Mycologia 81: 312–317.

Nirenberg, H. I. (1976). Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. – Mitt. Biol. Bundesanst. Land- Forstw. Berlin-Dahlem 169: 1–117.

Rifai, M. A. (1969). A revision of the genus *Trichoderma*. – Mycol. Pap. 116: 1–56.

Samuels, G. J., O., Petrini & S. Manguin (1994). Morphological and macromolecular characterization of *Hypocrea schweinitzii* and its *Trichoderma* anamorph. – Mycologia 86: 421-435.

——, O. Petrini, K. Kuhls, E. Lieckfeldt & C. P. Kubicek (1998). The *Hypocrea schweinitzii* complex and *Trichoderma* sect. *Longibrachiatum*. – Stud. Mycol. 41, 1–54.

Tulasne, L.-R & C. Tulasne (1865). Selecta fungorum carpologia. Tomus Tertius. Nectrei.-Phacidiei.-Pezizei. – Paris. – 221 pp. + 22 plates.

Webster, J. (1964). Culture studies on *Hypocrea* and *Trichoderma*. I. Comparison of perfect and imperfect states of *H. gelatinosa*, *H. rufa*, and *Hypocrea* sp. 1. – Trans. Brit. Mycol. Soc. 47: 75–96.

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