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Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains

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Abstract

Trichoderma harzianum is known as a cosmopolitan, ubiquitous species associated with a wide variety of substrates. It is possibly the most commonly used name in agricultural applications involving *Trichoderma*, including biological control of plant diseases. While various studies have suggested that *T. harzianum* is a species complex, only a few cryptic species are named. In the present study the taxonomy of the *T. harzianum* species complex is revised to include at least 14 species. Previously named species included in the complex are *T. guizhouense, T. harzianum*, and *T. inhamatum*. Two new combinations are proposed, *T. lentiforme* and *T. lixii*. Nine species are described as new, *T. afarasin, T. afroharzianum, T. atrobrunneum, T. camerunense, T. endophyticum, T. neotropicale, T. pyramidale, T. rifaii* and *T. simmonsii*. We isolated *Trichoderma*

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cultures from four commercial biocontrol products reported to contain *T. harzianum*. None of the biocontrol strains were identified as *T. harzianum* s. str. In addition, the widely applied culture '*T. harzianum* T22' was determined to be *T. afroharzianum*. Some species in the *T. harzianum* complex appear to be exclusively endophytic, while others were only isolated from soil. Sexual states are rare. Descriptions and illustrations are provided. A secondary barcode, nuc translation elongation factor 1-α (*TEF1*) is needed to identify species in this complex.

Keywords

cryptic speciation; endophytes; fungal barcode; *Hypocrea*; phylogenetics; species concepts; taxonomy

Introduction

Trichoderma harzianum Rifai (Sordariomycetes, Hypocreales, Hypocreaceae) is known as a cosmopolitan and ubiquitous species found on a wide variety of substrates. It has been isolated from soil, rotting plant material, other fungi, and recently as one of the species most commonly isolated as endophytes in the sapwood of tropical trees (Chaverri and Samuels 2003, Evans et al. 2003, Samuels 2006, Hoyos-Carvajal et al. 2009, Jaklitsch 2009, Gazis and Chaverri 2010, Druzhinina et al. 2011, Chaverri and Samuels 2013). Although there are occasional reports of *T. harzianum* being a plant saprobe (Ahmad and Baker 1987a,b; Savoie et al. 2001), recent studies suggest that species in this complex are mycoparasites or fungicoles (Kubicek et al. 2011, Chaverri and Samuels 2013).

In connection with its antifungal properties, *T. harzianum* has a long history in agricultural applications (see reviews in Harman and Kubicek 1998, Paulitz and Belanger 2001, Harman et al. 2004, Sharma et al. 2009, Chowdappa et al. 2013). It is effective in the control of soil-borne diseases (Harman and Kubicek 1998, Paulitz and Belanger 2001, Sharma et al. 2009), and is the active ingredient for several commercially available biological control and plant growth enhancing products (see Fravel 2005, Funck-Jensen and Lumsden 1999, Kaewchai et al. 2009, Samuels and Hebbar 2015).

Trichoderma harzianum was one of the nine 'aggregate species' recognized by Rifai (1969). Rifai considered that each aggregate species potentially included two or more morphologically cryptic but biologically distinct species. Several morphologically similar species are now known to be phylogenetically related to *T. harzianum* (Veerkamp and Gams 1983, Samuels et al. 2002, Chaverri and Samuels 2003, Park et al. 2006, Jaklitsch 2009, Chaverri et al. 2011, Li et al. 2012), and they join that species in the Harzianum Clade of *Trichoderma* (Jaklitsch 2009), earlier known as the 'Catoptron/LixiiClade' (Chaverri and Samuels 2003). However, *T. harzianum* in the broad sense of Rifai has resisted taxonomic revision, this despite the phylogenetic diversity of the *T. harzianum* morphological construct shown by multilocus phylogenetic studies (Chaverri et al. 2003, Druzhinina et al 2010b).

Because of the ecological and economic importance of the name *'T. harzianum,'* it is essential that taxonomy reflects phylogenetically defined lineages, and that names are associated with the true biology and evolutionary history of those lineages. These

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phylogenetically defined lineages may have distinct features of practical relevance, such as production of secondary metabolites, growth requirements, pathogenic potential, host ranges, and geographical distributions, among others. Misidentification of a cryptic taxon by the use of a collective name may have far-reaching negative consequences for strategic matters in industry, plant quarantine and other fields, such as human and animal health (Sandoval-Denis et al. 2014).

The present study aims at (i) revising the taxonomy of species in the *T. harzianum* complex, including their sexual and asexual stages, (ii) naming and describing the species in this complex, and (iii) reassessing the identity of some commercial *T. harzianum*-like biocontrol strains. We will use a combination of morphological, ecological, biogeographical and phylogenetic data. Because the limitations of the nuc internal transcribed spacers rDNA regions (ITS 1 – 5.8S – ITS2) in delimiting species are well known in *Trichoderma* in general and the *T. harzianum* complex specifically, we also propose a more reliable secondary barcode nuc translation elongation factor 1-α (*TEF1*) for species identification in this complex.

Materials and methods

Cultures from nature and specimens.—Specimens and living cultures were isolated from soil, decaying plant material and living stem tissues of the vascular cambium and phloem (= sapwood), other fungi and from sexual stromata. Isolations from stromata followed methods described in Chaverri and Samuels (2003) or Jaklitsch (2009). Cultures derived from conidia and ascospores were grown on Difco™ cornmeal agar + 2% dextrose (CMD; Difco, Detroit, Michigan) plus 1% antibiotic (Sigma-Aldrich streptomycin-neomycin-penicillin) to suppress bacterial growth. Endophytic strains were isolated from living sapwood tissue, mostly from the tropical trees *Cola, Hevea* and *Theobroma*, using techniques described in Kowalski and Kehr (1996), Evans et al. (2003) and Gazis and Chaverri (2010). Pure cultures were obtained by dilution plating on PDA. Cultures are stored at the University of Maryland and the Systematic Mycology and Microbiology Laboratory (USDA-ARS-SMML, including cultures designated as “G.J.S.” and “Dis”) in 20% glycerol in cryovials at −80 C or in public culture collections including Agriculture & Agri-Food Canada National Mycological Culture Collection, Ottawa, Canada (DAOM); the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS); and the CABI Microbial Services culture collection, Egham, UK (IMI).

For each species we have listed type specimens and extype cultures with the diagnoses; additional specimens and cultures examined are provided (Supplementary table V).

Commercial biocontrol agents included in this study.—We attempted to confirm the identity of the *T. harzianum* strains reported to be active in four biological control products. Canna AkTRIvator® was purchased from Canna International BV (Breda, the Netherlands). Trichosan® (Vitalin Pflanzengesundheit GmbH, Ober-Ramstadt, Germany) was obtained from Sauter and Stepper GmbH (Ammerbuch, Germany). Vitalin® was provided by the manufacturer, Vitalin Pflanzengesundheit GmbH (Ober-Ramstadt, Germany). Promot® WP (JH Biotech Inc., Ventura, California) was purchased from Ernst Mack Fellbach GmbH &

Co. KG (Fellbach, Germany). To isolate the bioactive principle from these products, every container was thoroughly surface-sterilized with Fermicidal D2 spray from IC Products (Minusio, Switzerland). Containers were opened and sampled aseptically in a laminar flow cabinet. Pure cultures of the *Trichoderma* species were obtained by dilution plating on Difco™ potato dextrose agar (PDA; Difco, Detroit, Michigan).

Morphological characterizations.—Morphological observations of colonies were based on isolates grown on SNA (Synthetischer Nährstoffarmer Agar, Nirenberg 1976, without filter paper) and PDA (Difco or Merck potato-dextrose agar) for up to 3 wk in an incubator at 25 C with alternating 12 h/12 h fluorescent light/darkness. Characters of the conidium-bearing structures and conidia were assessed from cultures grown on SNA. Sexual state characters also were observed and described, including reaction of the stroma to 3% KOH. To observe internal microscopic characteristics of perithecia and stromata, stromata were rehydrated in 3% KOH and sectioned with a freezing microtome at a thickness of ca. 15 mm. Characteristics of asci and ascospores were observed by rehydrating stromata in KOH, removing part of the centrum with a fine glass needle and placing it on a glass slide with 3% KOH. Measurements of continuous characters such as length and width were made with the Scion Image beta 4.0.2 software (Scion Corp., Frederick, Maryland). Continuous measurements are based on 10–30 or more measured units for each selected specimen, unless otherwise indicated. Extremes (maximum and minimum in brackets), 95% confidence interval and mean values are reported only for morphological traits that are informative (e.g. phialide length and width, conidium length and width, ascospore length and width). For some traits only 95% confidence intervals are provided.

Growth-rate trials were done in 9 cm diam Petri dishes with 20 mL PDA and SNA at 15, 20, 25, 30 and 35 C. Cultures were incubated in darkness up to 1 wk or until the colony covered the agar surface. Measurements of colony radius, the greatest distance from the edge of the plug of inoculum to the edge of the colony, were taken daily. Trials were replicated three times. The ranges of growth rates (radius after 72 h) are provided. In the descriptions below colony characters were recorded after growth in 9 cm diam Petri dishes for 72 h under a 12 h photoperiod at 25 C on SNA and PDA unless otherwise noted.

DNA extraction, PCR and sequencing.—Strains (SUPPLEMENTARYTABLE I) were grown in 6 cm diam Petri dishes containing Difco™ potato-dextrose broth. Cultures were incubated at 25 C for ca. 1 wk. DNA was extracted from the mycelial mat harvested from the surface of the broth with the Power-Plant™ DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, California). DNA sequences of a-actin (*ACT*) (ca. 700 bp); calmodulin (*CAL*) (ca. 500 bp); nuc internal transcribed spacers rDNA regions (ITS 1 – 5.8S – ITS2 = ITS), (ca. 600 bp); RNA polymerase II subunit 2 (*RPB2*) (ca. 900 bp); and nuc translation elongation factor 1-α (*TEF1*) (ca. 600 bp) were used in the phylogenetic analyses. The primers used were: for *ACT*, TRI-ACT1, TRI-ACT2, act500, act511 (Carbone and Kohn 1999, Samuels and Ismaiel 2009); for *CAL*, cal-228, cal-737 (Carbone and Kohn 1999); for ITS, ITS5 and ITS4 (White et al. 1990); for *RPB2*, rpb2-5f1 and *frpb2-7cr* (Liu et al. 1999); and for *TEF1*, ef-728M, ef2, ef700f, tef1r (Carbone and Kohn 1999, Rehner 2001). PCR reactions used GoTaq® Green Master Mix (Promega Corp., Madison, Wisconsin) and were run in an Eppendorf Master-cycler ep. Detailed protocols are described in various publications

(Chaverri and Samuels 2003, Chaverri et al. 2011, Chaverri and Samuels 2013). PCR products were cleaned with ExoSAP-IT® (USB Corp., Cleveland, Ohio). Sequencing was done at the DNA sequencing facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland) or at the Systematic Mycology and Microbiology Laboratory (USDA-ARS, Beltsville, Maryland).

Phylogenetic analyses.—Sequences were aligned with SATe (simultaneous alignment and tree estimation, Liu et al. 2009) with MAFFT (Katoh et al. 2005) as the external sequence alignment tool and RAxML (Stamatakis 2006) as the tree estimator. SATé is an automated method that quickly and accurately estimates both DNA alignments and trees with the maximum likelihood criterion. Where needed, the alignment was improved by hand with MESQUITE 2.72 (Maddison and Maddison 2009). Ambiguously aligned and poly-A/T regions were manually excluded from all analyses. Alignments are deposited in TreeBASE under submission number SN 16519. A reciprocal 70% bootstrap threshold (Mason-Gamer and Kellogg 1996, Reeb et al. 2004) was used to determine whether partitions could be combined into a single phylogeny.

Maximum likelihood (ML) and Bayesian (BI) analyses were performed with all sequences, first with each gene/locus separately and then with the concatenated datasets. For both analyses, the data were partitioned by gene and by codon position. JModeltest (Posada 2008) was used to select the models of nucleotide substitution for the ML and BI analyses. Once the likelihood scores were calculated, the models were selected according to the Akaike information criterion (AIC). After jModeltest the models for all loci were set to GTR + I + G.

GARLI-PART 0.96 (Zwickl 2006) was used for the ML and bootstrap analyses through the Grid computing and the Lattice Project, which includes several clusters and desktops in one distributed system (Cummings and Huskamp 2005, Bazinet and Cummings 2008, Myers et al. 2008). GARLI-PART allows for ML analysis with partitioned data. The starting tree was obtained by stepwise addition and the number of runs or search replicates was set to 50. Bootstrap (BP) analyses were replicated 2000 times. BI analysis was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001, Ronquist 2004), also with the partitioned data. Two independent analyses of two parallel runs and four chains were carried out for 25 000 000 generations. Analyses were initiated from a random tree and trees were sampled every 1000th generation. Convergence of the log likelihoods was analyzed with TRACER 1.4.1 (Rambaut and Drummond 2007). The first 20% of the resulting trees were eliminated as burn in. Both runs were pooled and a consensus tree (SUMT option) and posterior probabilities (PP) were calculated in MrBayes.

Phylogenetic analyses with *ACT, CAL*, ITS, *RPB2* and *TEF1* were used to illustrate the placement of the *T. harzianum* species complex in the Harzianum clade (Fig. 1 overview tree) and to define species limits in the *T. harzianum* complex (Fig. 2). To construct the overview multilocus phylogeny, several species were used as well as 10 representatives of the *T. harzianum* complex. *Trichoderma crassum* and *T. virens* were used as outgroup taxa (Supplementary table I).

To reconstruct the multilocus phylogeny of species in the *T. harzianum* complex, 192 isolates were used in the analyses (SUPPLEMENTARY TABLE I) and species were defined with genealogical concordance criteria (Avise and Ball 1990, Dettman et al. 2003). Previously named and described species such as *T. guizhouense*, *T. harzianum*, *T. inhamatum* and *T. lixii* were included in the analyses because they fit in the core of the *T. harzianum* complex (Chaverri et al. 2003, Li et al. 2012). Two *T. aggressivum* isolates were used as outgroup taxa. For this analysis, *ACT*, *CAL*, ITS and *TEF1* were used because *RPB2* did not provide sufficient variability within the species complex (results not shown).

ITS vs, TEF1 *as barcode for the* T. harzianum *complex*.—ITS nrDNA is the official barcode region for fungi (Schoch et al. 2012), but several studies demonstrate that ITS is imprecise in species identification, especially in the Hypocreales (Chaverri et al. 2003, Hirooka et al. 2011, Salgado et al. 2012). Therefore the value of *TEF1* as a secondary barcode for species in the *T. harzianum* complex is compared to ITS. Intra- and interspecific variation in ITS and *TEF1* sequences were calculated with MEGA (molecular evolutionary genetics analysis) 5.2.2 (Tamura et al. 2011). Gaps and missing data were not included in the analyses. *P* distance was used to estimate within- and between-groups divergence or base-pair differences per site. Standard error also was calculated. Other loci used in this study (*ACT* and *CAL*) were not evaluated because PCR amplification success is much lower than for ITS and *TEF1*.

Results

Phylogenetic analyses.—An overview phylogeny that includes the core *T. harzianum* complex and demonstrates its relationship with other species in the Harzianum clade sensu Chaverri and Samuels (2003) is provided (Fig. 1). Most nodes are well supported by both ML and BI analyses (BP ≥ 70% and PP ≥ 0.9). The phylogenetic analyses show that the *T. harzianum* species complex is most closely related to the clade that contains *T. amazonicum*, *T. pleuroti* and *T. pleuroticola* and the clade with *T. aggressivum, T. alni, T. compactum, T. epimyces, T. parepimyces* and *T. tawa*.

A multilocus phylogeny of only taxa and isolates within the *T. harzianum* complex is presented herein (Fig. 2). The results of the phylogenetic analyses and relative importance of the loci used is summarized (SUPPLEMENTARY TABLE II). *TEF1* has the most phylogenetically informative characters, followed by *CAL, ACT* and ITS. In general ML and BI analyses supported many clades, but the backbone remains largely unresolved (Fig. 2). The phylogenetic analyses based on the concatenated loci matrix and the genealogical concordance species concept suggest that 14 lineages represent different species in the *T. harzianum* complex. Both ML and BI analyses support all the putative species. Nine species are new (*T. afarasin, T. afroharzianum, T. atrobrunneum, T. camerunense, T. endophyticum, T. neotropicale, T. pyramidale, T. rifaii, T. simmonsii*), and these names will be used hereafter. The other five lineages represent previously named species: *T. guizhouense*, *T. harzianum* s. str., *T. inhamatum, T. lentiforme* comb. nov. and *T. lixii* comb. nov.

Phylogeographic structure.—Phylogenetic analyses reveal some segregation according to geography, especially at continental and latitudinal levels (Asia vs. Africa vs. America vs.

Europe; tropical vs. temperate) (Fig. 2). The only major exceptions are *T. afroharzianum* and *T. guizhouense*, which are distributed worldwide. *Trichoderma harzianum* and *T. simmonsii* both are found in temperate North America and Europe, with the exception of one Colombian soil isolate CIB T100 (Hoyos-Carvajal et al. 2009), which is *T. harzianum* s. str. *Trichoderma lixii* is known only from southeastern Asia; *T. afarasin* and *T. camerunense* are found in Africa; *T. atrobrunneum* and *T. pyramidale* are known only from Europe; and the remaining species thus far are known only from tropical South America (*T. endophyticum, T. inhamatum, T. lentiforme, T. rifaii*).

Phylogenetic structure according to ecology.—Species concepts in the *T. harzianum* complex appear to correlate with ecology, specifically to the substrates and habitats. *Trichoderma endophyticum, T. neotropicale* and *T. rifaii* are known only as endophytes in leaves and stems of tropical trees. One subclade within *T. guizhouense*, strongly supported by BP and PP values, contains only endophytes from Africa isolated from wild *Cola* trees. *Trichoderma afarasin* and *T. lentiforme* contain mostly endophytes, with a few soil isolates; and one *T. lentiforme* sexual state (G.J.S. 98-6 = CBS 100542) was found on another ascomycetous fungus on decaying wood. *Trichoderma atrobrunneum, T. lixii, T. pyramidale* and *T. simmonsii* were found in soil or on decaying wood, clearly or cryptically parasitizing other fungi. *Trichoderma afroharzianum*, *T. camerunense*, *T. guizhouense*, *T. harzianum* and *T. inhamatum* are known mostly from soil. *Trichoderma rifaii* is known only as an endophyte of *Theobroma cacao* and *Th. gileri*. Within *T. endophyticum*, three subclades segregated according to host (*Hevea brasiliensis, H. guianensis, Theobroma gileri*), which are highly supported by BP and PP values (Fig. 2).

Identification of some biocontrol strains.—We included *T.* cf. *harzianum* isolates that are contained in commercial biological control preparations. The active ingredient of Canna AkTRIvator® (G.J.S. 08-137) and the strain T22 (= G. Harman 129522 = G.J.S. 09-1563) (Ahmad and Baker 1987a, b; Stasz et al. 1988; Lo et al. 1997; Stasz et al. 2000), Kullnig et al. 2001) are *T. afroharzianum;* the active ingredient of Promot® WP (G.J.S. 08-135), is *T. guizhouense*; and in WP Trichosan® (G.J.S. 08-134) and Vitalin® (G.J.S. 08-136) it is *T. simmonsii*. These strains belong in three different clades/species; none are *T. harzianum* s. str. (Fig. 2).

Inter- and intraspecific variation in TEF1 *and ITS*.—The number of base differences per site, calculated by averaging all sequence pairs within each group, is provided (Supplementary tables III and IV). The analyses involved 123 and 189 nucleotide sequences for *TEF1* and ITS, respectively. For ITS and *TEF1* there were a total of 337 and 96 positions, respectively, in the final dataset after ambiguously aligned poly-T/A regions were excluded. In ITS the average within-group distance was 0.002 ± 0.001 (or 0.17%); in *TEF1* the average was 0.007 ± 0.004 (or 0.70%). The average between-groups distance was 0.003 ± 0.002 (0.29%) and 0.057 ± 0.021 (5.7%) in ITS and *TEF1*, respectively. In *TEF1* the 95% confidence interval (CI) was 5.1–6.2% (minimum 0.5%, maximum 11.9%). In ITS the 95% CI was 0.2–0.3 % (min. 0%, max. 0.6%). ITS sequences of *T. afroharzianum* and *T. lixii* are identical. *Trichoderma endophyticum*, *T. lentiforme*, *T. neotropicale* and *T. rifaii* also have identical ITS sequences. In contrast, *TEF1* of *T. lixii* can be distinguished from *T. afroharzianum* by 7.5% of the total nucleotides analyzed. *Trichoderma endophyticum, T. lentiforme, T.*

neotropicale and *T. rifaii* can be distinguished by an average of 3.4 ± 1.5 % (minimum 1.6%, maximum 5.9%). With ITS the maximum difference in % distance was observed between *T. pyramidale* and *T. guizhouense* (0.6%). With *TEF1* the maximum was between *T. atrobrunneum* and *T. inhamatum* (11.9%) and the minimum between *T. camerunense* and *T. rifaii* (0.5%).

Morphology and in vitro colony characters.—The morphology of the asexual and sexual states in the *T. harzianum* species complex is highly conserved (Table I). While conidium and conidiophore morphology of members of this complex are diagnostic in the genus as a whole, variation in characters of visible phenotype within the group is extremely small. This, combined with the small number of continuous characters (conidia, phialides etc.) available for analysis, suggests that for most cases the only reliable way to identify a species of this complex is with *TEF1* sequences. The dimensions of continuous characters overlap in their means +/– standard deviation and therefore do not allow discrimination of species. The 95% confidence intervals of these measurements indicate trends within clades and, although not useful by themselves for species identification because they tend to overlap, they lend support to our species hypotheses.

We have observed sexual states for five of the 14 species that we recognize in the *T. harzianum* species complex. Sexual states are rarely found, but they are common in *T. atrobrunneum* and *T. simmonsii*. The sexual state of *T. lixii* is known only from the type locations in southeastern Asia (holotype: Papua New Guinea; epitype: Thailand). The sexual state of *T. lentiforme* also is known only from the type location in Brazil and from one specimen from French Guiana, and the sexual state of *T. guizhouense* was collected once in Indonesia. When formed, stromata are very dark, almost black. Stromata of *T. lixii* and *T. simmonsii* are dark brown becoming black (*T. simmonsii* stromata green when immature), and stromata of *T. lentiforme* and *T. atrobrunneum* are green, becoming black. *Trichoderma lentiforme* and *T. lixii* have stromata ca. 1 mm diam, while those of *T. atrobrunneum* and *T. simmonsii* are larger, averaging > 1.5 mm diam. The part-ascospores of all species are green, warted and similar in size. *Trichoderma atrobrunneum* and *T. lixii* have slightly smaller distal part ascospores than *T. lentiforme* and *T. simmonsii*. *Trichoderma atrobrunneum* and *T. lentiforme* have smaller proximal part ascospores than *T. lixii* and *T. simmonsii*.

The asexual states have the typical *T. harzianum*-like morphology (see Rifai 1969, Bissett 1991, Gams and Meyer 1998, Samuels et al. 2002), which is described more precisely as follows: Conidial pustules usually are not well formed, conidiophores loosely joined and often covering extensive areas of a colony. Conidiophores comprise a well developed and often long main axis from which pairs or verticils of a few fertile branches often arise at right angles in regular and closely or widely spaced intervals. Fertile branches increase in length with distance from the tip of the main axis and rebranch, and the secondary branches decrease in length with distance from the branching point. Conidiophores do not extend into hairs, and there are no sterile hairs apart from conidiophores. Phialides arise in whorls at the tips of secondary branches and from the tip of the main axis. The whorls of phialides usually comprise fewer than five phialides and are typically divergent or “cruciate”, the phialides arising at right angles to the cell that produced them, although phialides also may arise singly or in pairs. Phialides are typically ampulli-form to lageniform with length/width ratios

greater than 2 and are greater than 2× the width of the cell that produced them, but there is considerable variation in length of phialides depending upon the species. The ampulliform phialides are typically constricted below the tip, forming a narrow neck. The harzianum-type branching pattern intergrades with the pachybasium-type in species such as *T. harzianum* and *T. inhamatum*, where the spacing between fertile branches can be short. The harzianum-type conidiophore has a more or less pyramidal aspect because of the long or, at least, distinct main axis, and the regularly and closely spaced, frequently paired primary and secondary fertile branches (sometimes unpaired or in whorls) that arise at right angles from the main axis and that increase in length with distance from the tip of the conidiophore. We refer to the branching pattern described here as “pyramidal”. Pyramidal branching also is found in unrelated species of *Trichoderma* such as *T. atroviride* (Viride Clade, Samuels 2006) in addition to *T. harzianum*. The average dimensions of phialides of the members of the harzianum complex are 6.0 ± 1.7 × 3.5 ± 0.4 μm (Table I). The longest phialides are found in *T. afroharzianum* and *T. pyramidale* (average 9.0 and 8.5 μm, respectively), while the shortest phialides are found in *T. inhamatum*, which average < 5 μm long. The narrowest phialides are in *T. camerunense* and *T. inhamatum*, and the widest in *T. lixii* and *T. neotropicale*. Conidia do not vary in shape and most are globose to subglobose or broadly ovoidal. The average conidium dimensions of all members are 2.9 ± 0.4 × 2.6 ± 0.3 μm, L/W 1.1 ± 0.1. The width and length of conidia increase linearly from smallest to largest with few breaks to distinguish species. As can be seen (Table I), the differences in conidial dimensions of species at the extremes are substantial but adjacent species in the progression cannot be distinguished from each other. The shortest are those of *T. inhamatum, T. lentiforme* and *T. rifaii* (2.7 × 2.5 μm), and the largest are in *T. afroharzianum, T. harzianum, T. lixii* and *T. pyramidale* (3.2−3.6 × 2.8−3.3 μm).

Chlamydospores are not a conspicuous feature of members of the *T. harzianum* complex. Although they are produced by all species treated herein, they are never numerous. They are subglobose, 5−10 μm diam, and terminate hyphae or form within cells of hyphae.

The growth curves on PDA and SNA for almost all species recognized in this study are identical. The optimum temperature for growth is 30 C, and both PDA and SNA colonies fill, or nearly fill, a 9 cm diam Petri dish within 72 h. In general growth on PDA is only slightly faster than on SNA at 30 C. At 35 C, where most *Trichoderma* species do not grow, or grow very poorly, the average colony radius for members of the *T. harzianum* complex is 35 mm on PDA and 30 mm on SNA after 72 h. Two notable exceptions are *T. atrobrunneum* and *T. pyramidale*, both of which grow slowly. The optimum temperature for *T. pyramidale* is 25 C and it does not grow at 35 C; the colony radius for *T. atrobrunneum* is only ca. 20 mm after 72 h at 30 C.

Discussion

Species delimitation in Trichoderma *and in the* T. harzianum *complex*.—We provide evidence based on a combination of genealogical concordance, morphology and ecology that the morphologically defined species *T. harzianum* is a species complex and that *T. harzianum* s. str. may be an uncommon species found primarily in the northern hemisphere. The fact that *T. harzianum* in a wide sense comprises a multiplicity of species is supported

by earlier phylogenetic studies (Grondona et al. 1997, Chaverri et al. 2003, Druzhinina et al. 2010b) and recombination (Druzhinina et al. 2010b) studies. Based on our sampling the most common species isolated from environmental samples are *T. guizhouense* (cosmopolitan), *T. simmonsii* (northern hemisphere) and *T. lentiforme* (Neotropics).

In some species clades, highly supported subclades are observed. *Trichoderma guizhouense* includes one such subclade with strains from Europe and another subclade only with endophytes of wild trees of *Cola altissima* in Africa. However, these subclades were not formally named because they might represent populations in the process of speciation. The endophyte subclade may represent a case of allopatric speciation in which the new population developed a key adaptation (i.e. internal colonization of living tissues), for example. If these endophytic and European subclades were defined as separate species, many of the other sister strains would be left as single-isolate lineages. For this reason a more conservative species concept was adopted. This same phenomenon applies to *T. endophyticum* and *T. lentiforme*, which also contain several highly supported subclades that correlate to either host or geographic origin.

The many relict or singleton lineages (i.e. a branch with just one representative) in the complex shown by Druzhinina et al. (2010b) are not included in the analyses. While they possibly represent distinct taxa, these lineages have unresolved phylogenetic positions and thus it is unclear whether they should be placed in the closest clade, whether they represent hybrids, whether they have retained the ability to recombine with other species in the complex or whether their solitary position has resulted from some other phenomenon (Brasier 2000, Sang and Zhong 2000, Wagner et al. 2013). Further increase in taxon sampling may reveal a different phylogenetic structure. Seifert and Rossman (2010) discuss this issue and new species ideally should be described based on more than one specimen or culture.

Ecology.—Species in the *T. harzianum* complex are commonly fungicolous, living in different types of habitats (Samuels 1996, Chaverri and Samuels 2013). They are most commonly isolated from soil or found on decomposing plant material where they cryptically or obviously parasitize other fungi (Chaverri and Samuels 2003, 2013; Hoyos-Carvajal et al. 2009; Jaklitsch 2009; Li et al. 2012). In addition, species in the *T. harzianum* complex are possibly the most common endophytic “species” in wild tropical trees (Evans et al. 2003, Gazis and Chaverri 2010, Samuels unpubl). The present study indicates a tendency for specialization of species for habitat. Thus species commonly isolated from soil tend not to be endophytes and species isolated as endophytes tend not to be isolated from soils. *T. afroharzianum, T. camerunense, T. guizhouense*, and *T. simmonsii* are common in the soil, for example. *Trichoderma afarasin, T. endophyticum, T. lentiforme, T. neotropicale* and *T. rifaii* are found most commonly as endophytes (*T. afarasin* and *T. lentiforme* also sometimes are found in soil). Hoyos-Carvajal et al. (2009) described a great diversity of *Trichoderma* in soils in Colombia and some adjacent countries using ITS and *TEF1* sequences. Many of the isolates they found were placed in the *T. harzianum* complex and labeled as three clades, A, B and C. In the present study their *TEF1* sequences were compared and placed in a phylogenetic context with the sequences produced (results not shown). Most of their isolates are *T. afroharzianum* or *T. lentiforme*. Their clade A corresponds to *T. lentiforme*, clade B to

the *T. afroharzianum/T. atrobrunneum/T. guizhouense/T. pyramidale* clade and clade C to the *T. lixii/T. camerunense/T. simmonsii/T. harzianum/T. rifaii/T. afarasin/T. endophyticum/T. neotropicale* clade. In the same manner *TEF1* sequences produced in Druzhinina et al. (2010b) were compared to those produced here. *Trichoderma afroharzianum* corresponds to their "afroharzianum" subclade (II), *T. atrobrunneum* to subclade III, *T. guizhouense* to subclade IV and *T. lentiforme* to subclade V. *Trichoderma simmonsii* corresponds to one of their hypothetical phylogenetic species within the Lixii subclade (in Druzhinina et al. 2010b, green circle in Fig. 1).

The identity of some biocontrol strains.—Advances in the science of systematic mycology and the adoption of molecular phylogenetics has rendered the classical morphological paradigm all but obsolete, with the result that names change. Unfortunate victims of this new approach to classification of *T. harzianum* are the names of strains that are used in patented products. Despite their product labels, the reportedly active ingredients of the four biocontrol products that we sampled (Canna AkTRIvator®, Promot® WP, Trichosan®, Vitalin®) are not *T. harzianum* s. str. as the species is defined here but instead they are other members of the *T. harzianum* species complex. Moreover, the most widely reported strain of *T. harzianum*, T22, which is the active ingredient in several biocontrol and plant growth-stimulating products (Samuels and Hebbar 2015), is not *T. harzianum* but *T. afroharzianum*.

If they are so closely related, does it matter that these fungi are not actually *T harzianum* s. str.? We know that in *Trichoderma*, morphologically identical, phylogenetic sister species express different biological properties. As example, we note the significant physiological, ecological and reproductive differences separating the morphologically identical sympatric, cosmopolitan, tropical species *T. reesei* E.G. Simmons and *T. parareesei* Atanasova et al. (Druzhinina et al. 2010a). In this case differences in carbon utilization, light sensitivity and ecological niche correlated closely with the phylogenetic differences between the apparently clonal *T. parareesei* and the sexually reproducing *T. reesei*. The members of the *Trichoderma harzianum* species complex similarly show little or no morphological differentiation and also are closely related. Small but consistent differences in morphology combine with geographic distribution and ecological (e.g. endophyte vs. soilborne) and reproductive modes (possibly clonal vs. sexual and presumably outcrossing) to characterize individual species (Table I). These observations indicate differentiation in the genome, where additional biochemical differences no doubt will be found. The taxonomy for the *T. harzianum* complex that we propose here formalizes phylogenetic lineages and opens new prospects for discovery of biologically utility. For example, representatives of the newly recognized species *T. neotropicale* (Dis 219f) and *T. lentiforme* (Dis 110a) were isolated as endophytes of wild *Theobroma* spp. and both had strong in vitro antagonistic effects against *Moniliophthora roreri*, the causal agent of frosty pod rot of *Theobroma cacao* (Bailey et al. 2008). This suggests the endophytic niche as a promising souce of novel beneficial species and strains of *Trichoderma*.

It should be emphasized here that thorough taxonomic revision is of major practical relevance for the manufacturers and users of *"T. harzianum"*-based biocontrol agents. One culture (CGMCC 1780), identified as *T. harzianum*, for example, is cited in three Chinese patents (CN101037656A, CN101275152B, CN101979620B) for a proposed antifungal

product. However, examination of the sequences of the peptaibiotics that were published after the patent application (Pan et al. 2012) reveals that culture CGMCC 1780 produces peptaibol sequences that are typical of two groups of species. One group resembles the 20-residue alamethicins that are known exclusively from the *T. brevicompactum* clade (Degenkolb et al. 2008). The other group resembles 18-residue peptaibols of the trichokindin-type known from *T. harzianum* s lat. (Iida et al. 1994, Degenkolb et al. 2015). There are no reports of an individual species producing the synthetases for peptaibiotics of both types and chain lengths. Thus it seems likely that culture GCMCC 1780 is mixed and that one of its components produces large amounts of the potent mycotoxin trichodermin that is typical of some members of the *T. brevicompactum* clade. A mycotox-in-producing *Trichoderma* species that has been misidentified as *T. harzianum* clearly must not be introduced into any biological control or integrated pest management scheme for bacterial or fungal pathogens in the field or greenhouse.

Limitations of the nuclear transcribed spacers in species delimitation.—Limitations of the nuclear internal transcribed spacers rDNA regions (ITS 1 – 5.8S – ITS2) in delimiting species are well known in *Trichoderma* in general and the *T. harzianum* complex specifically. In this study we propose a more reliable secondary barcode, nuc translation elongation factor 1-α (*TEF1*), for species identification in this complex. *TEF1* is confirmed as a substantially more useful marker than ITS for the identification of *Trichoderma* species, especially those in the *T. harzianum* complex. With *TEF1*, there is a 90% probability of identifying the correct species with a 2% similarity threshold and a 95% probability with a 1.2% similarity threshold. In contrast, some species have identical ITS sequences. The utility of *TEF1*, often many times in combination with other loci such as *CAL*, for delimiting species in *Trichoderma* and other species complexes has been highlighted (e.g. Jaklitsch 2009, 2011; Rojas et al. 2010, Hirooka et al. 2011). In addition *TEF1* can clearly separate clades of taxa associated with specific hosts or ecological traits, suggesting possible host preferences and biogeographic structure (for the *Colletotrichum gloeosporioides* species complex; Rojas et al. 2010, Gazis et al. 2011), as was also observed in the present study

Taxonomy

Additional material examined is listed for all species (Supplementary table V).

Trichoderma afarasin P. Chaverri & F.B. Rocha, sp. nov. Figs. 3A, 3O, 4A–E

MycoBank MB809944

Etymology: "afarasin," from the Yoruba word "afarasin", which means one who hides, with reference to the cryptic occurrence of this species as an endophyte and sometimes in soil.

Typification: CAMEROON. Central Province: location unknown, isolated from soil, 1999 (**holotype BPI** 88109). (Ex-type culture CBS 130755 = IMI 393967 = G.J.S. 99-227).

Teleomorph: Unknown.

Characters in culture: Colony radius after 72 h at 25 C on PDA 55–67 mm, on SNA 60–65 mm; at 35 C on PDA and SNA 32–42 mm. On PDA after 96 h at 25 C under 12 h photoperiod aerial mycelium abundant, cottony, conidia developing within 72 h beginning at the inoculation point and progressing in distinct concentric rings with the youngest and newly formed conidia at the colony margin; a brown diffusing pigment developing after 48 h at 35 C; sometimes with a sweet odor. On SNA after 1 wk at 25 C conidia forming abundantly within 72 h in more or less distinct, broad concentric rings, first in the aerial mycelium and later in compact, flat, gray-green, 1–3 mm diam pustules beginning around the inoculum, the degree of pustule formation variable and dependent on the culture, sometimes no pustules observed. Conidiophores pyramidal, with closely spaced, opposing branches, each branch terminating in a cruciate whorl of up to five phialides. Phialides ampulliform, sharply constricted to form a narrow neck, obpyriform or obclavate and sometimes conical, (4.2−)4.5−5.5 (−8.0) × (2.2−)2.7−3.2 µm (mean 5.5 × 3.2 µm), base 1.0–2.7 µm (mean 1.9 µm), supporting cells (2.0−)2.5−3.0(−3.5) µm wide (mean 2.7 µm), phialide length/width ratio (1.3−)1.4−2.2(−3.0) (mean 1.9); ratio of phialide length to width of supporting cell (1.4−)1.7−2.5(−2.9); ratio of width of phialide to width of supporting cell (1.0−)1.1−1.3 (−1.4). Conidia globose to subglobose, (2.2−)2.5−3.2(−3.7) × (2.2−)2.5−3.0(−3.2) µm (mean 2.9 × 2.7 µm), length/width ratio 0.9–1.1 (mean 1.1), hyaline when young becoming green to dark green, rarely yellowish green with age, smooth. Chlamydospores infrequent.

Habitat: Endophytic in stems of *Cola* sp. and isolated from soil; possibly fungicolous (Chaverri and Samuels 2013).

Geographic distribution: Africa (Cameroon).

Notes: Trichoderma afarasin can be distinguished from other African or widespread species (*T. afroharzianum, T. camerunense, T. guizhouense*) by phialide length and growth rate at 30 and 35 C. Phialides of *T. afarasin* are shorter phialides than those of *T. afroharzianum* and longer than those of *T. camerunense*. *Trichoderma afarasin* grows faster at 30 and 35 C than *T. guizhouense*.

Trichoderma afroharzianum P. Chaverri, F.B. Rocha, Degenkolb & I. Druzhinina, sp. nov. FIGS. 3B, 3P, 4F–I

MycoBank MB809945

Etymology: A *T. harzianum-like* fungus originally found in Africa.

Typification: PERU. JUNÍN: on *Moniliophthora roreri* on *Theobroma cacao*, date unknown, *W. Soberanis 47* (**holotype** BPI 881096). Ex-type culture CBS 124620 = G.J.S. 04-186.

Teleomorph: unknown.

Characters in culture: Colony radius after 72 h at 25 C on PDA 50–65 mm, on SNA 55–60 mm; at 35 C on PDA 35–45 mm, on SNA 42–50 mm. On PDA after 96 h at 25 C aerial mycelium abundant, cottony, radiating; conidia appearing within 48–72 h, typically

abundant and disposed in two or three concentric rings around the point of inoculation; yellow pigment sometimes diffused in the medium, especially at 35 C and in old cultures; a sweet odor sometimes detected at 30 C. On SNA aerial mycelium sparse, conidia forming abundantly within 72–96 h from aerial mycelium in broad concentric bands or flat, often coalescing pustules to form a continuous lawn covering extensive areas. Conidiophores pyramidal, with opposing, somewhat widely spaced branches, the main axis and each branch terminating in a cruciate whorl or verticil of up to five phialides. Phialides lageniform to ampulliform, (3.5−)5.2−10.2(−17.5) × (2.0−)2.5−3.5(−4.2) µm (mean 7.8 × 3.1 µm), base (1.0−)1.5−2.2(−3.5) µm wide (mean 1.9 µm), supporting cell (2.0−)2.2−3.2(−5.0) µm wide (mean 2.7 µm), phialide length/width ratio (1.0−)1.6−3.8(−7.0) (mean 2.7); ratio of phialide length to width of supporting cell (1.0−)1.8−4.4(−7.0); ratio of phialide width to width of supporting cell (0.6−)1.0−1.4(−1.9). Conidia subglobose to ovoid, (2.0−)2.7−3.5(−4.5) × (2.0−)2.5−3.2(−4.0) µm (mean 3.1 × 2.8 µm), length/width ratio (0.9−)1.0−1.2 (−1.5), smooth, green to dark green with age, infrequently yellow. Chlamydospores rare.

Habitat: Isolated from soil, roots and other fungi; possibly fungicolous (Chaverri and Samuels 2013).

Geographic distribution: Widespread.

Notes: The name, *"Trichoderma afroharzianum"*, was introduced in a phylogenetic study of *T. harzianum* (Druzhinina et al. 2010b) without a description, thus was nomen nudum and not validly published. We adopted the name *T. afroharzianum* to retain continuity with that work. *Trichoderma afroharzianum* is distinctive for its widely spaced, often verticillate, conidiophores. *Trichoderma afroharzianum* has the longest phialides in the complex, and the supporting cell for the phialides is the narrowest. The phialides of *T. pyramidale* are somewhat shorter than those of *T. afroharzianum*, but the cell supporting the phialide of *T. pyramidale* is on average wider. These two species can be distinguished by the ability of *T. afroharzianum* to grow well at 35 C. *Trichoderma pyramidale* does not grow at all at 35 C. *Trichoderma afroharzianum* is a widespread species, while *T. pyramidale* is known only from Europe. Strain *Sharon 248* (as *T. harzianum*) was cited by Sharon et al. (2007) in their study of antagonism of the root knot nematode *Meloidogyne javanica* and was unable to attach to or parasitize eggs of the nematode.

Trichoderma atrobrunneum F.B. Rocha, P. Chaverri & W. Jaklitsch, sp. nov. FIGS. 3C, 3Q, 5, 12A

MycoBank MB809946

Etymology: "atrobrunneum" means dark brown, which is the color of the stromata.

Typification: FRANCE. PYRÉNÉES ATLANTIQUES: 64 Oloron, on decorticated wood of *Fagus sylvatica*, possibly growing on another fungus, 13 Sep 1992, *F. Candoussau* (**holotype** BPI 802854). Ex-type culture CBS 548.92 = G.J.S. 92-110.

Teleomorph: Unknown.

Characters in culture: Colony radius after 72 h at 25 C on PDA and SNA (35–)40–55(–61) mm; at 35 C on PDA and SNA (4–)8–30(–35) mm, slightly slower on SNA than on PDA, on SNA at 30 C 40–65 mm, at 35 C 5–20 mm. On PDA after 96 h at 25 C aerial mycelium abundant, cottony or wooly, conidia forming abundantly within 48–72 h in broad concentric rings; diffusing pigment rarely noted after 96 h at 25 C; a sweet odor often detected at 30 C. On SNA conidia forming within 96 h at 25 C, at first abundant in the aerial mycelium throughout the colony, later in cottony pustules; conidiation sometimes sparse or absent. Conidiophores pyramidal, with often opposing, often somewhat widely spaced branches, the main axis and each branch terminating in a cruciate, sometimes verticillate, whorl of up to four phialides. Phialides ampulliform to lageniform, (4.5−)5.5−8.0 (−13.0) × (2.2−)3.0−3.7(−4.5) µm (mean 7.0 × 3.4 µm), base 1.0−2.7 µm wide (mean 2.0 µm), supporting cell (2.0−)2.2−3.2(−4.0) µm wide (mean 2.9 µm), phialide length/width ratio (1.2−)1.6−2.6(−4.4) (mean 2.1); ratio of phialide length to width of supporting cell (1.4−)1.8–3.0(−5.1); ratio of phialide width to width of supporting cell (0.8−)1.0−1.4(−1.8). Conidia subglobose to ovoid, (2.0−)2.7−3.8(−4.0) × (2.0−)2.5−3.0 (−3.5) µm (mean 3.0 × 2.7 µm), length/width ratio (0.9−)1.0−1.1 (−1.5) (mean 1.1), smooth, hyaline when young becoming green to dark green with age.

Stromata solitary or aggregated, pulvinate, 0.5–1.7 mm high, 0.5–6.0 mm diam, glabrous, light green and sometimes with reddish or brownish tones when young, dark green to black when dry, formed of tissue of *textura angularis*, in 3% KOH and lactic acid cells at the surface pigmented brown, angular when viewed from the top, and internal cells hyaline to pale brown. Perithecia immersed in the stroma, closely aggregated, sometimes scattered, distorted when crowded, and globose to subglobose when scattered, 145−320 × 80−230 µm, wall formed of compact pseudoparenchymatous tissue, 6.5−20.0 µm thick, ostiole central. Asci cylindrical, 53−117 × 3.0−6.8 µm, ascospores uniseriate. Ascospores bicellular, warted, green, disarticulating at the septum to form 16 part spores per ascus; part spores dimorphic; distal part spores subglobose, ovoid or subovoid with truncate base and rounded apex, (3.0−)4.0−4.3 (−6.0) × (2.8−)3.7−4.0(−5.0) µm (mean 4.2 × 3.8 µm); proximal part spores subcylindrical, sometimes with rounded base and truncate apex, (3.3−) 4.2− 4.5(−7.0) × (2.5−)3.0−3.7(−4.4) µm (mean 4.3 × 3.4 µm).

Habitat: Isolated from soil, decaying wood and fungi; possibly all fungicolous (Chaverri and Samuels 2013).

Geographic distribution: Europe and North America.

Notes: Trichoderma atrobrunneum is one of the few species in this complex for which we have found stromata in nature. These can be distinguished from those of other species in the group (i.e. *T. lentiforme, T. lixii*, *T. simmonsii*) by the color and size of the stroma and the size of the ascospores. *Trichoderma atrobrunneum* and *T. lentiforme* have light green stromata that become dark green to black with age, but ascospores are slightly smaller in *T. atrobrunneum. Trichoderma lixii* and *T. simmonsii* have dark brown to black stromata at maturity, but the stroma diameter and part-ascospores of *T. lixii* are slightly smaller. *Trichoderma atrobrunneum* grows more slowly at 30 and 35 C than *T. simmonsii. Trichoderma atrobrunneum* and *T. simmonsii* are only known from temperate regions,

whereas *T. lentiforme* and *T. lixii* are only known from the tropics. The asexual state of *T. atrobrunneum* can be distinguished easily because it has the slowest growth at 35 C of all species in the complex, except for *T. pyramidale*, which does not grow at 35 C.

Trichoderma camerunense P. Chaverri & Samuels, sp. nov. FIGS. 3D, 3R, 6E–H

MycoBank MB809997

Etymology: "camerunense" reflects the type locality, Cameroon.

Typification: CAMEROON. CENTRAL PROVINCE: specific location unknown, isolated from soil, 1999, *P.R. Tondje* (**holotype** CBS 137272, a permanently preserved, metabolically inactive culture). Ex-type culture G.J.S. 99-230.

Teleomorph: Unknown.

Culture characters: Colony radius after 72 h at 25 C on PDA ca. 65 mm, on SNA ca. 55 mm; at 35 C on PDA ca. 30 mm, on SNA ca. 25 mm. On PDA after 96 h at 25 C aerial mycelium abundant, cottony, conidia forming abundantly within 48–72 h in the center of the colony and in a single broad band; no diffusing pigment noted; a sweet odor detected at 30 C. On SNA conidia forming within 96 h at 25 C, abundant in the aerial mycelium and ill-formed pustules in two concentric rings. Conidiophores pyramidal with opposing closely spaced branches; the main axis and each branch terminating in a cruciate whorl of up to four phialides. Phialides ampulliform, often lageniform, at the tip of the main axis obpyriform or obclavate, sharply constricted, below the tip, (3.7−) 3.2− 5.7(−7.2) × (2.0−)2.5−3.2(−3.5) µm (mean 5.0 × 3.0 µm), phialide length/width ratio (1.2−)1.4−1.9 (−2.5) (mean 1.7), base 1.5−2.0 µm wide (mean 1.7 µm), supporting cell (2.0−)2.5−3.2(−3.5) wide (mean 2.9 µm); ratio of phialide length to width of supporting cell (1.2−)1.4−2.0(−2.5); ratio of width of phialide to width of supporting cell (0.7−)0.8−1.2 (−1.5). Conidia subglobose to ovoid, (2.5−)2.5−3.2 (−4.0) × (2.2−)2.5−3.0(−3.2) µm (mean 2.9 × 2.7 µm), length/width ratio (0.9−)1.0−1.1(−1.5) (mean 1.1), smooth, hyaline when young becoming green to dark green with age.

Habitat: Isolated from soil.

Geographic distribution: Cameroon.

Notes: We know this species from two cultures that were isolated in Cameroon. After we were able to extract and sequence DNA from G.J.S. 99-231, the culture was lost. *Trichoderma camerunense* is difficult to distinguish morphologically from other species in the complex. However, only one species (*T. inhamatum*) has a slower growth rate than *T. camerunense* at 15 C on PDA, and it has smaller phialides than any of the other African members of the *T. harzianum* complex, which include *T. afarasin*, *T. guizhouense* and *T. afroharzianum*.

Trichoderma endophyticum F.B. Rocha, Samuels & P. Chaverri, sp. nov. FIGS. 3E, 3S, 6I–L

MycoBank MB809989

Etymology: "endophyticum" refers to the endophytic habit.

Typification: ECUADOR. ESMERALDAS PROVINCE: Lita, isolated as an endophyte from lower stems of *Theobroma gileri*, 5 May 2000, *H.C. Evans & K.A. Holmes Dis 217a* (**holotype** CBS 130729, a permanently preserved metabolically inactive culture). Ex-type cultures Dis 217a = IMI 395208.

Teleomorph: Unknown.

Culture characters: Colony radius after 72 h at 25 C on PDA and SNA 55–65 mm, growing more slowly on SNA than on PDA. On PDA after 96 h at 25 C under 12 h photoperiod aerial mycelium cottony, radiating; conidia typically forming abundantly within 48 h in pronounced, continuous concentric rings; colonies rarely sterile; diffusing yellow pigment rarely noted; no distinctive odor detected. On SNA conidia forming 48–72 h; after 96 h conidial production typically abundant in a few concentric rings; conidia forming in the aerial mycelium and in cottony pustules; pustules becoming confluent and sometimes the confluent pustules covering extensive areas, conidia gray green to dark green in mass. Conidiophores pyramidal with opposing branches, the distance between branches typically relatively short, the main axis and each branch terminating in a cruciate or botryose whorl of up to five phialides; phialides often solitary on lateral branches. Phialides ampulliform, (3.5−)4.0−6.0(−9.5) × (2.0−)3.0−3.7(−4.2) µm (mean 5.2 × 3.3 µm), phialide length/width ratio (1.0−)1.3−1.9(−2.9) (mean 1.6), base (1.0−)1.7−2.5(−3.5) µm wide (mean 2.0 µm), supporting cell (2.0−)2.5−3.7(5.0) µm wide (mean 3.2 µm); ratio of phialide length to width of supporting cell (0.7−)1.3−2.1(−3.2); ratio of phialide mid to width of supporting cell (0.6−)0.8−1.2(−1.7). Conidia globose, subglobose to ovoid, (2.2−)2.5−3.0(−3.5) × (1.7−)2.2−2.7(−3.2) µm (mean 2.7 × 2.5 µm), length/width ratio (0.8−)1.0−1.2(−1.5), smooth, hyaline when young becoming green to dark green with age. Chlamydospores not observed.

Habitat: Endophytic in stems of *Theobroma* spp. and *Hevea* spp.

Geographic distribution: Ecuador and Peru.

Notes: Trichoderma endophyticum is known only as an endophyte in Neotropical trees. It is one of the most common *Trichoderma* endophytes of sapwood in wild *Theobroma* spp. and *Hevea* spp. In general the phialides of *T. endophyticum* tend to be among the shortest. The endophytic members of this group from tropical America, *T. endophyticum, T. lentiforme, T. neotropicale* and *T. rifaii*, are morphologically indistinguishable. All tend to have short, ampulliform phialides that arise from supporting cells that are as wide as the phialides are long. *Trichoderma rifaii* stands out for having the smallest conidia in the *T. harzianum* complex and in its inability to grow at 35 C.

Trichoderma guizhouense Q.R. Li, E.H.C McKenzie & Yong Wang, Mycol. Prog. 12:170 (2012). FIGS. 3F, 3T, 7A–C

MycoBank MB563664

Typification: CHINA. Guizhou Province: Duyun City, Niujiaotang, isolated from soil, Dec 2009, *Q. R. Li* (**holotype** a dried culture deposited in the herbarium of the Department of Plant Pathology, Guizhou University, China as HGUPd0038). (ex-type culture = CBS 131803).

Teleomorph unknown.

Culture characters: Colony radius after 72 h at 25 C on PDA and SNA 45–70 mm, somewhat slower on SNA than on PDA. On PDA after 96 h at 25 C under 12 h photoperiod aerial mycelium abundant, cottony, radiating or not, conidia forming within 24 h in broad concentric bands, in the aerial hyphae, sometimes in dense mats, after 48 h becoming yellowish then green, typically forming a yellowish diffusing pigment; at 30 C reverse turning partly yellow-orange or dull orange-brown; at 35 C reverse dull orange to brown; no distinctive odor noted. On SNA conidial production abundant, conidia forming from aerial hyphae in broad concentric rings, typically numerous large (to 3 mm), flat, irregularly shaped pustules forming around the inoculum and in a few concentric rings, pustules often becoming confluent. Conidiophores pyramidal with opposing branches, the distance between branches short or sometimes more open, the main axis and each branch terminating in a cruciate whorl of 2–4 phialides, sometimes phialides solitary; rarely conidiophores nodose and phialides disposed in more or less botryose clusters (DIS 386ai). Phialides ampulliform, typically strongly constricted below the tip, less frequently lageniform and then usually terminal and inequilateral to strongly curved, (4.0−)4.7−7.5(−12.2) × (2.3−)3.0−3.7(−4.0) µm (mean 6 × 3.3 µm), phialide length/width ratio (1.2−)1.4−2.4(−4.2), base (1.0−)1.5−2.4(−3.0) µm wide; supporting cell (1.7−)2.5−3.2(−4.2) µm wide (mean 2.8 µm); ratio of phialide length to width of supporting cell (1.3−)1.7−2.9(−4.6); ratio of phialide width to width of supporting cell (0.8−)1.0−1.4(−1.9). Conidia globose to subglobose, (2.0−)2.5−3.2 (−4.0) × (2.0−)2.5−3.0(−3.5) µm (mean 2.9 × 2.8 µm), length/width ratio (0.8-)1.0−1.2(−1.5), smooth, green. Chlamydospores not observed.

Habitat: On decaying wood, bark and fungi. Also isolated from soil and as an endophyte in stems of *Ancistrocladus korupensis* and *Cola* spp. Possibly fungicolous (Chaverri and Samuels 2013).

Geographic distribution: Africa (Cameroon, Ghana). Europe (Croatia, Greece, Italy, Spain). Asia/SE Asia (Indonesia, Japan). Possibly cosmopolitan.

Notes: Trichoderma guizhouense is monophyletic and has high statistical support. However, the strains that we include fall into two highly supported internal clades (see Fig. 2). One of the subclades comprises seven cultures isolated in Cameroon as endophytes from sapwood of two *Cola* trees and one isolated from the liana *Ancistrocladus korupensis*. The remaining strains come from sources that range from Europe, Ghana, Indonesia and China (type), and a diversity of substrates (wood of various tree species, fungi, soil). Growth rates, temperature optima and colony morphology are identical in both subclades. However, there are small but statistically significant differences in size and shape of phialides and in the conidial dimensions. Phialides of the endophytes are slightly longer and narrower than are those of

the nonendophytes (95% CIs: 6.2–6.4 and 5.4–5.9, respectively), and the conidia of the endophytes are slightly shorter and narrower than are those of the nonendophytes (95% CIs: 2.8–2.9 µm, n = 894; 2.9–3.0 µm, *n* = 120, respectively). We do not consider these two clades distinct species. The phylogenetic and phenotypic differences are small but when combined with the endophytic vs. non-endophytic habit may point to an ongoing speciation process. One of our cultures (G.J.S. 85-119) is derived from ascospores of a collection made in Indonesia; this collection was reported as *Hypocrea nigricans* by Samuels et al. (1990) and by Chaverri and Samuels (2003) as *H. lixii*. An immature stroma was found associated with a European collection of *T. guizhouense*. In general, *T. guizhouense* is among the slowest growing species in the complex, especially on PDA at 15 C, where only *T. inhamatum* is slower. In contrast to *T. pyramidale*, *T. guizhouense* grows much faster at all temperatures, including 35 C.

Trichoderma harzianum Rifai, Mycol. Pap. 116:38 (1969) emend P. Chaverri, G.J. Samuels & F.B. Rocha Figs. 3G, 3U, 7D–G.

MycoBank MB198225

Typification: ENGLAND. YORKSHIRE: South Yorkshire, Sheffield, isolated from botanical garden soil, Jan 1994, *J.L. Kinderlerer* (**ex-neotype** CBS 226.95, designated by Gams and Meyer 1998).

Teleomorph: Unknown.

Culture characters: Colony radius after 72 h at 25 C on PDA and SNA 50–65 mm, somewhat slower on SNA than on PDA. On PDA after 96 h at 25 C under 12 h photoperiod aerial mycelium cottony, radiating; conidia forming abundantly within 48 h in a dense, nearly crustose central disk and in broad concentric rings, conidia gray green when young, becoming dark green; sometimes a pale yellow diffusing pigment after 48 h at 35 C; a sweet odor sometimes detected at 30 C. On SNA conidia forming within 72 h; after 96 h conidia forming abundantly in a few obscure, broad concentric rings or uniformly throughout the colony; at best loosely aggregated with few minute (< 1 mm) pustules. Conidiophores pyramidal with opposing branches, the distance between branches relatively large, the main axis and each branch terminating in a whorl of 2–5 phialides or phialides solitary from the main axis; whorls typically cruciate but often nearly verticillate. Adjacent phialides ampulliform to lageniform, typically constricted below the tip to form a narrow neck, terminal phialides in a whorl often lageniform, (4.7−)5.2−8.5(−16.0) × (2.7−)3.0−4.0 (−4.7) µm (mean 6.9 × 3.5 µm), phialide length/width ratio (1.2−)1.4−2.5(−5.0) (mean 2.0), base 1.2−3.0 µm wide (mean 2.0 µm), supporting cell (2.0−)2.5−3.5 (−4.0) µm wide (mean 3.0 µm); ratio of phialide length to width of supporting cell (1.3−)1.7−3.4 (−5.2); ratio of phialide width to width of supporting cell (0.7−)1.0−1.3(−1.9). Conidia subglobose to ovoid, (2.2−)2.7−3.5(−4.2) × (2.0−)2.5−3.0 (−3.7) µm (mean 3.2 × 2.8 µm), length/width ratio (0.9−)1.0−1.2(−1.6), smooth, hyaline when young becoming green to dark green with age. Chlamy-dospores rare.

Habitat: Isolated mostly from soil; also mushroom compost and sometimes as an endophyte in stems; possibly fungicolous (Chaverri and Samuels 2013).

Geographic distribution: Europe and North America.

Notes: Although the name is widely used, *T. harzianum* s. str. is an uncommon species known only from Europe and North America. Tendencies in some morphological characters may be helpful in recognizing this species. For example, *T. harzianum* is among the fastest growing species at 35 C with an average colony radius after 72 h of > 35 mm on SNA and > 40 mm on PDA. Conidia of *T. afroharzianum, T. atrobrunneum, T. harzianum* and *T. lixii* are the largest in the complex, averaging ≥ 3.0 × 2.7 μm; and phialides of *T. harzianum* are the largest, averaging ≥ 6.5 × 3.5 μm.

Trichoderma inhamatum Veerkamp & W. Gams, Caldasia 13:710 (1983). Figs. 3H, 3V, 7H.

Typification: COLOMBIA. Departamento Meta: Municipio de Villavicencio, 25 km from Villavicencio to Acacías, isolated from soil under maize, 1978, *O. Rangel isolated by W. Gams* (**isotype** a dry culture BPI 748209). ex-type culture CBS 273.78 = IMI 287526.

Teleomorph: Unknown.

Culture characters: Colony radius after 96 h at 25 C on PDA ca. 60 mm, on SNA ca. 50 mm; at 35 C on PDA ca. 35 mm, on SNA ca. 20 mm. On PDA after 1 wk at 25 C aerial mycelium cottony, abundant, conidia developing abundantly in two or three distinct concentric rings; conidia forming at the edges of the rings; a pale yellow pigment diffusing into the agar; a sweet odor sometimes noted at 30 C; conidial masses dark yellowish green. On SNA within 1 wk at 25 C, conidia forming abundantly in small (< 1 mm diam), poorly formed pustules disposed in more or less distinct concentric rings, conidia first appearing within 72 h. Conidiophores pyramidal, with closely spaced, opposing branches, each branch terminating in a cruciate whorl of up to five phialides. Phialides ampulliform but terminal phialides in a whorl tending to be lageniform, (3.5−)4.0−6.0(−9.2) × (2.2−) 2.7−3.5(−4.2) μm (mean 5.0 × 3.2 μm), base (1.2−) 1.5−2.2(−3.0) μm wide (mean 2.0 μm), supporting cell (1.7−)2.5−3.5(−4.5) μm wide (mean 3.0 μm), phialide length/width ratio (1.1−)1.2−2.0(−3.3) (mean 1.6); ratio of phialide length to width of supporting cell 1.1− 2.3(−5.3); ratio of the width of the phialide to the width of the supporting cell (0.7−)0.8−1.2(−1.7). Conidia subglobose to ovoid, (2.2−)2.5−3.0(−3.5) × (2.0−)2.2−2.7(−3.0) μm, length/width ratio (0.8−)0.9− 1.3(−1.4), smooth, becoming green to dark green with age, smooth. Chlamydospores subglobose, rare.

Additional descriptions and illustrations: Veerkamp and Gams (1983).

Habitat: Soil.

Geographic distribution: Colombia, Peru.

Notes: Phylogenetically *T. inhamatum* is most closely related to *T. lentiforme*. Although some could consider them conspecific, the long branch that separates them and the low bootstrap and posterior probability values strongly suggest that they are distinct. Because *T. inhamatum* is known only from only two cultures, it is impossible to evaluate variation within the species. Based on our study of the type culture, *T. inhamatum* is relatively easy to

distinguish from other species in the complex because of its small conidia, short and narrow phialides and its growth at 15 C on PDA and SNA, and at 35 C on SNA it is among the slowest of the group.

Trichoderma lentiforme (Rehm) P. Chaverri, Samuels & F.B. Rocha, comb. nov. FIGS. 3I, 3W, 8, 12B

≡ *Hypocrea lentiformis* Rehm, Hedwigia 37:193 (1898). Basionym.

Typification: BRAZIL. SANTA CATARINA STATE: on decaying leaves of *Euterpe*, soc. dematiaceous fungi, Aug. 1888, *Ule* (**isotype** HBG #812!).

Epitype, here designated: FRENCH GUIANA. SAUL: Commune de Saul, Mont. Galbao, base camp on NE side, near headwater of Mara River, 50–150 m, 09°10′0″N 79°50′0″W, on decaying bark possibly growing on another fungus, 11 Nov. 1997, *S.M. Huhndorf 3758* (BPI 744709, ex-epitype culture G.J.S. 98-6 = CBS 100542.

Culture characters: Colony radius after 96 h at 25 C on PDA (57–)60–70(–75) mm, on SNA (25–)32–43 (–47) mm. On PDA after 96 h at 25 C under 12 h photoperiod aerial mycelium abundant cottony to granular, conidia forming within 48 h, typically abundant, disposed in a central disk and one or two broad concentric rings; no pigment noted at 25 C but diffusing pale yellow, reddish or brownish pigment developing at 35 C; in some cultures a sweet odor detected at 30 C. On SNA after 96 h at 25 C under 12 h photoperiod conidia forming abundantly in the aerial mycelium in one or two broad centric rings; frequently flat, irregularly shaped, 1–3 mm diam pustules forming beginning around the inoculum; pustules becoming confluent and sometimes forming a continuous ring; pigmentation absent on SNA. Conidiophores pyramidal with opposing, closely spaced branches, each branch and the main axis terminating in a cruciate whorl of phialides. Phialides ampulliform, sharply constricted below the tip to form a narrow neck, phialides terminating the main axis frequently lageniform, obpyriform or obclavate, some strains developing conical phialides mainly at the end of the conidiophores, up to five at the end of each conidiophore, (3.0−)4.2−6.5(−12.2) × (2.2−) 3.0− 4.0(−5.0) µm (mean 5.5 × 3.5 µm), phialide length/width ratio (0.9−)1.3−1.9(−3.7) (mean 1.6), base 1.0−3.5 µm wide (mean 2.0 µm), supporting cell (1.7−)2.7−3.7(−5.0) µm wide (mean 3.2 µm); ratio of phialide length to width of supporting cell (0.9−)1.2− 2.2(−5.3); ratio of phialide width to width of supporting cell (0.6−)0.9−1.3(−2.1). Conidia globose to subglobose, or subglobose to ovoid, (2.0−)2.5−3.2 (−3.7) × (1.7−)2.5−2.7(−3.2) µm (mean 2.8 × 2.6 µm), length/width ratio (0.7−)1.0−1.2(−1.7) (mean 1.1), smooth, hyaline when young becoming green to dark green with age, some strains developing yellowish green conidia. Chlamydospores not observed.

Stromata solitary or aggregated, pulvinate, 1.0–1.3 mm diam (mean 1.0 mm), 0.5–1.0 mm high (n = 9), glabrous, dark green appearing black, tissue of the stroma of textura angularis, cells at surface pigmented greenish and brownish in KOH, angular when viewed from the top, internal cells of the stroma hyaline to slightly pigmented pale brown in KOH. Perithecia immersed in the stroma, closely aggregated, sometimes scattered, distorted when crowded, globose to subglobose, 180−220 × 150−170 µm, wall formed of compact pseudoparenchyma, 7.5−8.5 µm thick. Asci cylindrical, 85−90 × 4.0−4.5 µm, ascospores

uniseriate. Ascospores bicellular, warted, green, disarticulating at the septum to form 16 partspores, dimorphic, distal part-spores globose to subglobose (3.5−)4.3−4.6(−5.5) × (3.0−)3.7−4.0 (−4.5) µm (mean 4.4 × 3.9 µm); proximal part spores subcylindrical, (3.5−)4.3−4.6(−5.5) × (2.5−)3.3−3.5 (−4.0) µm (mean 4.5 × 3.4 µm).

Habitat: Isolated as endophytes in stems of tropical trees, and from soil. The sexual state has been found on decaying leaves and bark. Possibly fungicolous (Chaverri and Samuels 2013).

Geographic distribution: Neotropical.

Notes: Trichoderma lentiforme is possibly the most commonly found member of the *T. harzianum* complex in the Neotropics. This species is notable for the broad cells that support the phialides, which are as wide as the phialides are long; in this respect *T. lentiforme* is similar to the apparently rare Neotropical species *T. inhamatum*. In its sexual state *T. lentiforme* resembles the temperate species *T. atrobrunneum*, both having dark green to black stromata; the stromata of the other species in the complex are brown.

Trichoderma lixii (Pat.) P. Chaverri, comb. nov. FIGS. 3J, 3X, 9, 12C

≡ *Hypocrea lixii* Pat., Rev. Mycol. Toulouse 13:138 (1891). Basionym.

= *Chromocrea nigricans* Imai, Trans. Sapporo Nat. Hist. Soc. 14:102 (1935).

≡ *Hypocrea nigricans* (Imai) Yoshim. Doi, Bull. Natl. Sci. Mus. Tokyo 15:732 (1972).

= *Hypocrea nigricans* f. *octospora* Yoshim. Doi, Bull. Natl. Sci. Mus. Tokyo 15:734 (1972).

MycoBank: MB 809999

Typification: PAPUA NEW GUINEA. On hymenium of *Ganoderma pourii*, Jul 1891, *Lix* (**holotype** FH).

Epitype (vide Chaverri and Samuels 2003): THAILAND. SARABURI PROVINCE: Khao Yai National Park, Wang Jumpee Trail, on hymenium of *Ganoderma* sp., 31 Jul. 1997, *K. Põldmaa, P. Chaverri, G.J. Samuels 8233* (BPI 745654, ex-epitype culture CBS 110080 = G.J.S. 97-96 = ATCC MYA-2478).

Culture characters: Colony radius after 72 h at 25 C on PDA ca. 60 mm, on SNA ca. 55 mm; at 35 C on PDA ca. 40 mm, on SNA ca. 30 mm. On PDA after 96 h at 25 C under 12 h photoperiod aerial mycelium cottony, radiating; conidia developing within 48 h, after 96 h abundant and green to dark green around the inoculum, beginning to turn green in one or two concentric rings; no pigment or odor noted. On SNA conidia appearing in the aerial mycelium throughout the colony within 72 h at 25 C under 12 h photoperiod, after 96 h conidia forming abundantly in loosely formed, confluent, pale green pustules in a single ring around the inoculum. Conidiophores pyramidal, typically with opposing pairs of branches, less frequently solitary, closely spaced branches, each branch and the main axis terminating in 2–4, cruciately to nearly verticillately disposed phialides. Phialides ampulliform to lageniform, (4.0−)5.2−8.2 (−12.2) × (2.7−)3.5−4.0(−4.5) µm, (mean 6.7 × 3.7 µm), phialide

length/width ratio (1.0−)1.3−2.1(−2.7) (mean 1.7), base 1.5−2.7 µm wide (mean 2.0 µm), supporting cell (2.2−)2.7−3.7(−4.0) µm wide (mean 3.2 µm); ratio of length of phialide to width of supporting cell (1.4−)1.5−2.5(−3.0); ratio of width of phialide to width of supporting cell (0.9−)1.0−1.4 (−1.6). Conidia globose to subglobose, (2.5−)3.0−3.5 (−3.7) × (2.2−)2.5−3.2(−3.5) µm (mean 3.2 × 3.0 µm), length/width ratio (0.9−)1.0−1.2 (−1.3) (mean 1.1), smooth, hyaline when young becoming green to dark green with age. Chlamydospores not observed.

Stromata solitary or aggregated, pulvinate, 0.8–1.2 mm diam (mean 1.0 mm), 0.5–0.8 mm high (*n* = 8), glabrous, dark brown appearing black, tissue of the stroma of textura angularis, cells at surface pigmented greenish, becoming brownish in KOH, angular when viewed from the top, internal cells of the stroma hyaline to slightly pigmented pale brown in KOH. Perithecia immersed in the stroma, closely aggregated, sometimes scattered, distorted when crowded, globose to subglobose, 230−250 × 140−150 µm, wall formed of compact pseudoparenchyma, 12.0−13.5 µm thick. Asci cylindrical, 73−78 × 4.5−4.7 µm, ascospores uniseriate. Ascospores bicellular, warted, green, disarticulating at the septum to form 16 part spores, dimorphic, distal part spores globose to subglobose (3.0−)4.1−4.5(−5.6) × (3.0−)3.8−4.1(−5.0) (mean 4.3 × 3.9 µm); proximal part-spores wedge-shaped to subcylindrical, (3.0−)4.3−4.8(−5.5) × (2.5−)3.4−3.7 (−5.0) µm (mean 4.6 × 3.5 µm).

Additional illustrations: Chaverri and Samuels (2002).

Habitat: Fungicolous, on basidiomata of *Ganoderma* and on other fungi growing on palm leaves.

Geographic distribution: Southeastern Asia (Papua New Guinea, Thailand).

Notes: Trichoderma lixii is based originally on its sexual form and is represented by only a single culture that was collected many years later. Thus it is difficult to evaluate the range of variation of the species. The dry stromata of the type collection are dark green appearing black. Only *T. simmonsii*, which is mostly known from temperate regions, has stromata similar to *T. lixii*. The other species form dark green to black stromata. Based on the single culture of *T. lixii* examined, *T. lixii* and *T. pyramidale* have relatively large conidia, the largest in the complex. The phialides of *T. lixii* are long and especially wide in the group.

Trichoderma neotropicale P. Chaverri & F.B. Rocha, sp. nov. FIGS. 3K, 3Y, 10A–D

MycoBank MB810000

Etymology: "neotropicale" because it is known only from the Neotropics.

Typification: PERU. MADRE DE DIOS: Manu, Los Amigos Biological Station, endophytic in stems of *Hevea guianensis*, May 2008, *R. Gazis LA11* (**holotype** CBS 130633, a permanently preserved, metabolically inactive culture). Ex-type culture = G.J.S. 11-185).

Teleomorph: Unknown.

Culture characters: Colony radius after 72 h at 25 C on PDA 55–60 mm, on SNA ca. 55 mm; at 35 C on PDA 30–35 mm, on SNA ca. 30 mm. On PDA after 96 h at 25 C under 12 h photoperiod aerial mycelium abundant, cottony, radiating or not; conidia forming within 48 h, at 96 h conidial production abundant, conidia forming in a dense disk around the inoculum and in two narrow or broad concentric rings; no pigment noted at 25 C, a pale yellow diffusing pigment within 48 h at 35 C; no odor detected at 30 C. On SNA at 25 C under 12 h photoperiod conidia forming within 72 h, after 96 h conidia forming abundantly in the aerial mycelium and in loose or flat, compact pustules disposed in two concentric rings; pustules becoming confluent. Conidiophores pyramidal with opposing closely spaced branches, the main axis and each branch terminating in a cruciate to slightly verticillate whorl of up to five phialides. Phialides ampulliform, obpyriform or obclavate, sharply constricted below the tip to form a narrow neck; (4.2−)4.7−7.5(−12.2) × (2.5−)3.2−4.2(−4.7) µm (mean 6.0 × 3.7 µm), phialide length/width ratio (1.1−)1.2−2.2(−4.1) (mean 1.7), base 1.5−3.0 µm wide (mean 2.2 µm), supporting cell (2.5)2.7−4.0(−4.2) µm wide (mean 3.2 µm); ratio of length of phialide to width of supporting cell (1.3−)1.6−2.4(−4.0); ratio of width of phialide to width of supporting cell (0.8−) 1.0− 1.4(−1.7). Conidia globose to subglobose, sometimes ovoid, (2.5−)2.7−3.2(−3.5) × (2.0−)2.5−3.0 (−3.5) µm (mean 3.0 × 2.7 µm), length/width ratio (0.9−)1.0−1.2(−1.3), smooth, hyaline when young becoming green to dark green with age. Chlamydospores not observed.

Habitat: Known only as endophytes of *Hevea* spp. and *Theobroma* spp.

Geographic distribution: Ecuador, Peru.

Notes: Trichoderma neotropicale is morphologically a typical member of the *T. harzianum* complex and cannot be reliably distinguished from other species by phenotype characters.

Trichoderma pyramidale W. Jaklitsch & P. Chaverri, sp. nov. FIGS. 3L, 3Z, 10E–H

MycoBank MB809990

Etymology: “pyramidale” describes the terminal cluster of phialides, which assumes a pyramidal aspect.

Typification: ITALY. APULIA: Foggia, Gargano, Mattinata, 41°44′48″N, 16°08′01″E, 120 m, on branch of *Olea europaea*, asexual state, 20 Nov. 2009, *H. Voglmayr & W. Jaklitsch* (**holotype** CBS 135574, permanently preserved in a metabolically inactive state). Other culture number S73.

Teleomorph: Unknown.

Culture characters: Optimal growth at 25–30 C on all media, no growth at 35 C. On PDA colony radius after 72 h 43–47 mm at 25 C and 39–42 mm at 30 C; on SNA colony radius 46–51 mm at 25 C and 43–50 mm at 30 C after 72 h. On PDA mycelium covering the plate after 5–6 d at 25 C. Colony circular, dense, marginal surface hyphae conspicuously wide, surface soon becoming covered by a whitish, zonate mat of aerial hyphae; center flat,

turning yellowish, scarcely separated concentric zones turning yellow-green by effuse conidiation; central reverse yellow, odor indistinct. Conidiation starting after 48 h, turning green after ca. 1 wk. At 30 C yellow-green conidiation zones well defined. On SNA mycelium covering the plate after 4–5 d at 25 C. Colony circular, hyaline, thin, loose, with more or less conspicuous differences in hyphal width, marginal surface hyphae particularly wide. Margin or distant half of the colony becoming floccose and covered by a loose whitish mat of abundant aerial hyphae. Autolytic excretions from frequent coils. No diffusing pigment formed, odor indistinct. Conidiation starting after 24 d; first effuse on simple verticillium-like conidiophores, soon followed by the formation of numerous loose, cottony, roundish or amorphous tufts or pustules up to 1–2 mm diam in a broad distant or marginal zone, partly confluent. Pustules first white, turning (yellowish) green after 4–5 d from within the pustule, eventually dark green. Pustules arising on a narrow stipe, branching asymmetrically into a loose reticulum with joints mostly at right angles. Terminal conidiophores departing at the reticulum, often distinctly vertically projecting up to ca. 0.4 mm from the pustule surface, first as sterile elongations but becoming fertile and eventually integrated into the pustule. Conidiophores regularly tree-like, 2−4.5(−5) µm wide, with enlargements at nodes to 6−7 µm, straight, curved or slightly sigmoid, comprising a distinct main axis with side branches unpaired, paired or often in whorls of 3−4 perpendicular to the main axis, branches often replaced by phialides toward the conidiophore apex. Phialides (4.0−)5.5−11.5(−17.5) × (2.5−)2.8−3.7(−4.5) µm (mean 8.5 × 3.3 µm), phialide length/width ratio (1.3−)1.6−3.9(−5.8), base (1.2−)1.5−2.5(−3.2) µm wide, arising from a cell ca. 3.0 µm wide; solitary or in whorls of 2−4(−5); variable, mostly lageniform, acute, inequilateral, straight, curved or sigmoid, sometimes with cylindrical bent neck, or ampulliform; terminal whorls typically comprising a long, acute curved lageniform central phialide surrounded by 2–3 ampulliform phialides nearly perpendicular to it; also minute aphanophialides present, ca. 2−4 × 1−1.5 µm, situated laterally on phialides or directly below the phialide septum. Conidia globose, subglobose or ovoid, (2.8−) 3.2− 4.0(−4.7) × (2.5−) 3.0−3.5(−4.0) µm (mean 3.6 × 3.3 µm), length/width ratio (1.0−) 1.1−1.2(−1.4), green, smooth, scar indistinct. Chlamydospores uncommon.

Habitat: On decaying wood and bark, possibly growing on other fungi.

Geographic distribution: Southern Europe (Italy, Spain).

Notes: This species is easily distinguished from other members of the *T. harzianum* complex by its slow growth on PDA and SNA and no growth at 35 C.

Trichoderma rifaii F.B. Rocha, P. Chaverri & Samuels, sp. nov. FIGS. 3M, 3AA, 10I–L

MycoBank MB809991

Etymology: In honor of M.A. Rifai for his pioneering contributions to the taxonomy of *Trichoderma*.

Typification: ECUADOR. PICHINCHA PROVINCE: specific location unknown, isolated as endophyte of stems of *Theobroma gileri*, collecting date unknown, *H.C. Evans Dis 355b*

(**holotype** CBS 130746, a permanently preserved, metabolically inactive culture). Extype culture Dis 355b.

Teleomorph: Unknown.

Culture characters: Colony radius after 72 h at 25 C on PDA 62–67 mm, on SNA 55–65 mm; at 35 C on PDA and SNA 0–42 mm. On PDA after 96 h at 25 C under 12 h photoperiod aerial mycelium abundant, cottony, radiating, sporulating abundantly in broad concentric rings, conidia just beginning to turn green; yellow brown around the inoculum and some yellow pigmentation in the aerial hyphae; a sweet odor at 30 C. On SNA conidia appearing within 72 h at 25 C; after 96 h under 12 h photoperiod conidia forming in the aerial mycelium throughout the colony with no pustules. Conidiophores pyramidal with closely spaced, opposing branches; each branch terminating in a cruciate whorl of up to five phialides. Phialides ampulliform to lageniform, often sharply constricted below the tip to form a narrow neck (4.2−)5.0−7.2(−9.0) × (2.7−)3.0− 4.0(−4.2) µm (mean 6.1 × 3.5 µm), phialide length/width ratio (1.1−)1.4−2.2 (−2.8) (mean 1.8), base 1.5− 2.5(−3.0) µm wide (mean 2.0 µm), supporting cell (2.0−)2.5−3.2(−4.2) µm wide (mean 2.9 µm); ratio of phialide length to width of supporting cell (1.3−)1.5− 2.6(−3.4); ratio of width of phialide to width of supporting cell (0.8−)1.0−1.4 (−1.7). Conidia globose, subglobose to ovoid, (2.0−)2.2−3.0 × 2.0−2.7(−3.0) µm (mean 2.6 × 2.4 µm), length/width ratio (0.8−)1.0−1. 2(−1.6) (mean 1.1), smooth, hyaline when young becoming green, sometimes appearing yellowish. Chlamydospores not observed.

Habitat: Known only as endophyte in stems of *Theobroma* spp.

Geographic distribution: Ecuador, Panama.

Notes: This species is known only from two collections. *Trichoderma rifaii* tends to have the fastest growth in the *T. harzianum* complex on SNA, especially at 25 C, and conidia of *T. rifaii* are among the smallest in the group.

Trichoderma simmonsii P. Chaverri, F.B. Rocha, Samuels, Degenkolb & W. Jaklitsch, sp. nov. FIGS. 3N, 3AB, 11, 12D

MycoBank MB809947

Etymology: To the memory of Emory G. Simmons, who described the famous *Trichoderma reesei*, and for his many other contributions to Mycology.

Typification: UNITED STATES. MARYLAND: Prince Georges County, on decaying bark, 11 Oct 1991, *G.J. Samuels, S.E. Rehner, A.Y. Rossman & F.A Uecker* (holotype BPI 1112907). Ex-type cultures: CBS 130431 = G.J.S. 91-138.

Culture characters: Colony radius after 72 h at 25 C on PDA 55–65 mm, on SNA 45–60 mm; at 35 C on PDA 25–55 mm (mean 33 mm), on SNA 10–35 mm (mean 22 mm). On PDA after 96 h at 25 C under 12 h photoperiod aerial mycelium abundant, radiating, conidia forming abundantly in a large dark green disk around the inoculum and in concentric rings; no diffusing pigment noted at 25 C, a pale yellow diffusing pigment sometimes formed

within 48 h at 35 C; no odor detected. On SNA conidia forming within 72 h at 25 C under 12 h photoperiod within 72 h, after 96 h conidia forming abundantly throughout the colony, first in aerial mycelium, later in loosely formed ≤ 1 mm diam pustules; pustules becoming confluent and sometimes covering an extensive area. Conidiophores pyramidal with opposing branches, each branch terminating in a cruciate whorl of up to five phialides; phialides frequently solitary or in a whorl of two or three. Phialides ampulliform to lageniform, often constricted below the tip to form a narrow neck (4.2−)5.2−6.5 (−9.0) × (2.5−)3.0−3.7 (−4.0) µm (mean 6.2 × 3.3 µm), phialide length/width ratio (1.2−)1.5−2.4(−3.3) (mean 1.9), base 1.7−2.2 µm (mean 2.0 µm), supporting cells (2.0−)2.5−3.2(−4.0) µm wide (mean 2.7 µm); ratio of length of phialide to width of supporting cell (1.4−)1.8−2.8 (−4.0); ratio of width of phialide to width of supporting cell (0.7−) 1.0−1.4(−1.7). Conidia subglobose to ovoid, (2.5−) 2.7−3.2(−3.7) × (2.2−)2.5−3.0 (−3.5) µm (mean 3.0 × 2.7 µm), length/width ratio 1.0−1.1 (−1.4), smooth, hyaline when young becoming green to dark green, rarely yellowish green with age. Chlamydospores sometimes present.

Stromata solitary, pulvinate when seen from above, 0.5–1.0 mm high, 1.0–2.0 mm diam, glabrous, dark brown to black, formed of textura angularis tissue, cells at the surface dark brown or black, angular when viewed from the top, internal cells ranging from hyaline to pale brown. Perithecia immersed in the stroma, forming dense amorphous aggregates, sometimes scattered, distorted when densely packed, globose to subglobose when scattered, 150−275 × 93−182 µm, wall formed of compact pseudoparenchyma, 10−15 µm thick, ostiole central. Asci cylindrical, 85−90 × 4.5−5.0 µm, ascospores uniseriate. Ascospores green, warted, bicellular, disarticulating at the septum to form 16 part spores per asci, dimorphic, distal part spores subglobose, ovoid or subovoid with truncate base and rounded apex, (3.0−)4.4−4.5(−5.5) × (3.0−) 4.0− 4.2(−5.0) µm (mean 4.4 × 4.1 µm); proximal spores subcylindrical sometimes with rounded base and truncate apex, (3.5−)4.5−4.7(−6.0) × (2.7−)3.5− 3.7(−4.7) µm (mean 4.6 × 3.6 µm).

Habitat: Mostly found on decomposing bark and decorticated wood, and on other fungi.

Geographic distribution: Europe (Austria, Croatia, France, Germany, Greece, Italy, Spain). United States (Alabama, Illinois, North Carolina, Wisconsin).

Notes: The asexual state of *T. simmonsii* is difficult to distinguish morphologically from other members of the *T. harzianum* complex. As in *T. lixii*, stromata of *T. simmonsii* are dark brown to black but the ascospores of *T. simmonsii* are larger than those of *T. lixii*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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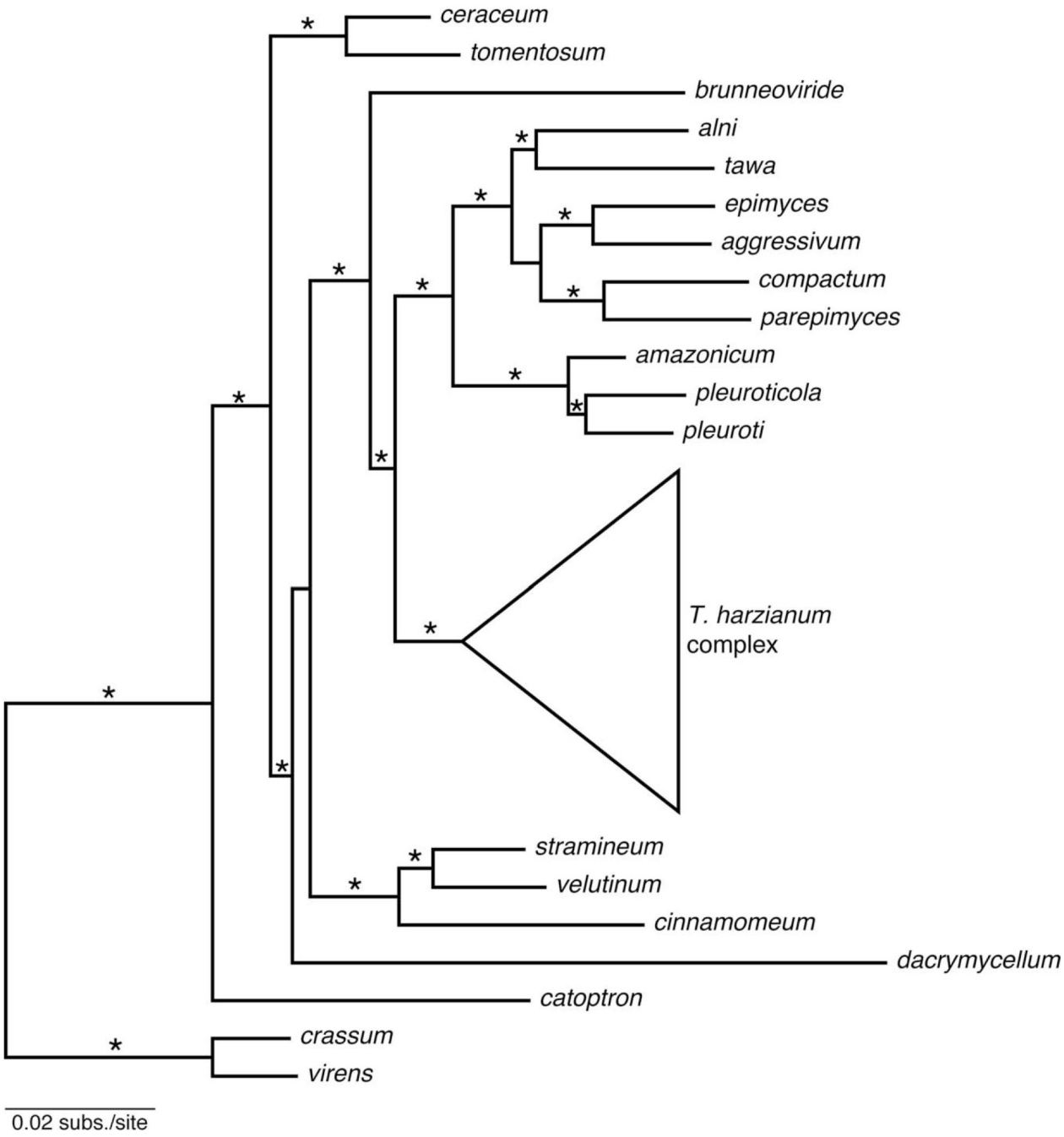

Fig. 1.

Overview phylogenetic tree with position of the *T. harzianum* complex in the Harzianum clade. This is the best maximum likelihood tree (Ln –15471.5174) (Bayesian inference Ln –15951.3457), using five genes. Values at nodes represent ML bootstrap/BI posterior probability.

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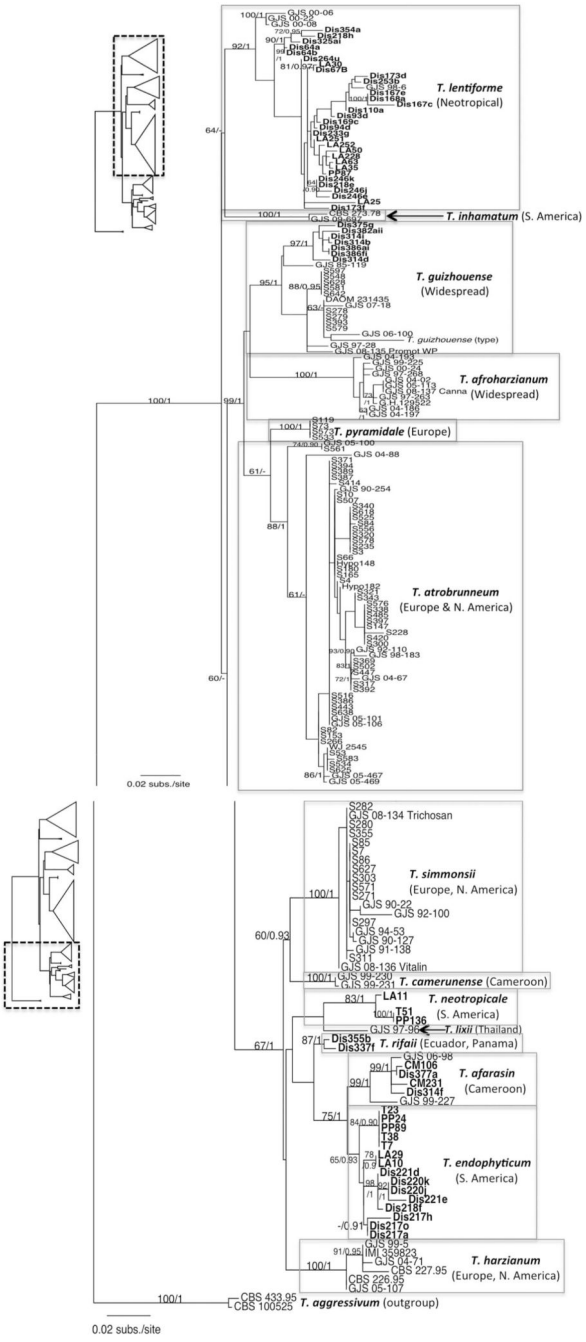

Fig. 2.

Maximum Likelihood tree of *ACT, TEF1, CAL* and ITS (Ln –8211.5679) (Bayesian Inference Ln –9717.5440), which includes only species in the *T. harzianum* complex. The figure is divided in two subtrees (A, B) according to the small cartoon inset tree (top left corner). Values at nodes represent ML bootstrap/BI posterior probability. Boldface numbers represent endophytic strains.

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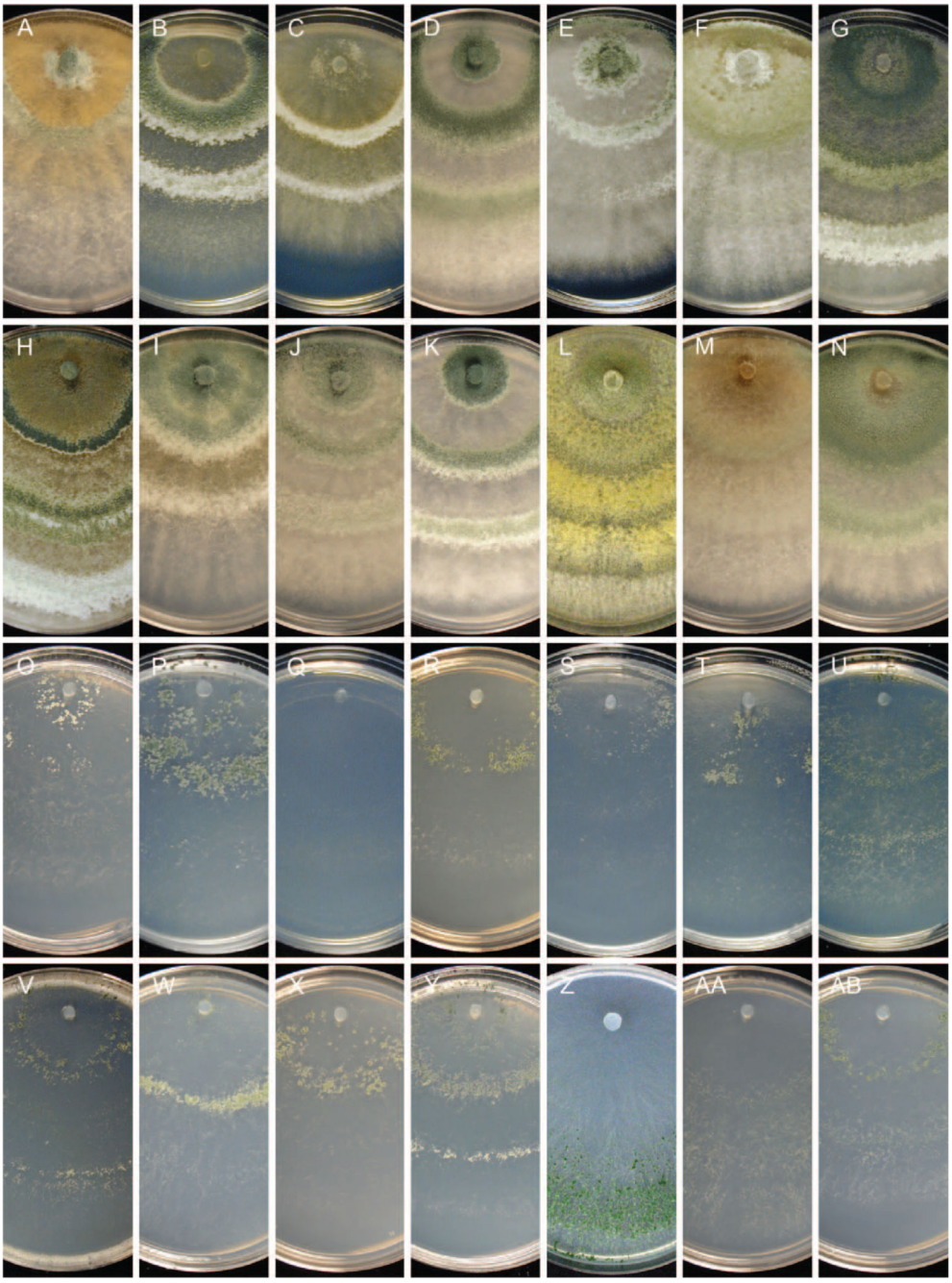

Fig. 3.

Cultures of members of the *Trichoderma harzianum* complex species on PDA (A–N) and SNA (O–AB) at 25 C after 96 h under 12 h light regime. A, O. *T. afarasin* (G.J.S. 99-227). B, P. *T. afroharzianum* (G.J.S. 04-186). C, Q. *T. atrobrunneum* (G.J.S. 05-106). D, R. *T. camerunense* (G.J.S. 99-230). E, S. *T. endophyticum* (Dis 220j). F, T. *T. guizhouense* (Dis 375g). G, U. *T. harzianum* s. str. (CBS 226.95). H, V. *T. inhamatum* (CBS 273.78). I, W. *T. lentiforme* (Dis 168a). J, X. *T. lixii* (G.J.S. 97-96). K, Y. *T. neotropicale* (LA11). L, Z. *T. pyramidale* (S73). M, AA. *T. rifaii* (Dis 337f). N, AB. *T. simmonsii* (G.J.S. 91-138).

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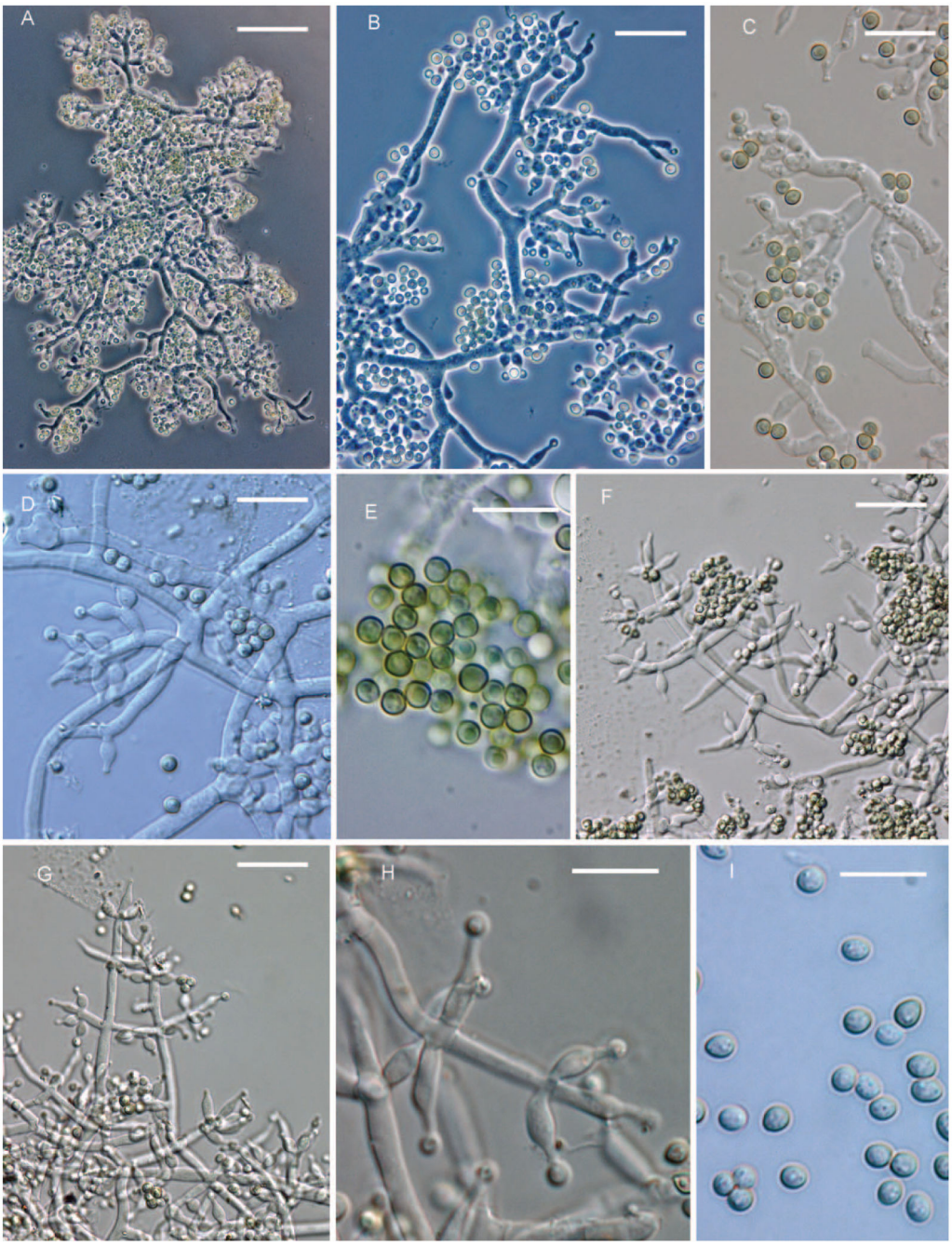

Fig. 4.

Trichoderma afarasin (A–E) and *T. afroharzianum* (F–I). A-D, F, G. Conidiophores. H. Phialides. E, I. Conidia. A-C from Dis 377a, D from G.J.S. 06-98, E from Dis 355a; F, G from G.J.S. 04-186; H, I from G.J.S. 04-197. Bars: A-D, F, G = 20 µm; E, H, I = 10 µm.

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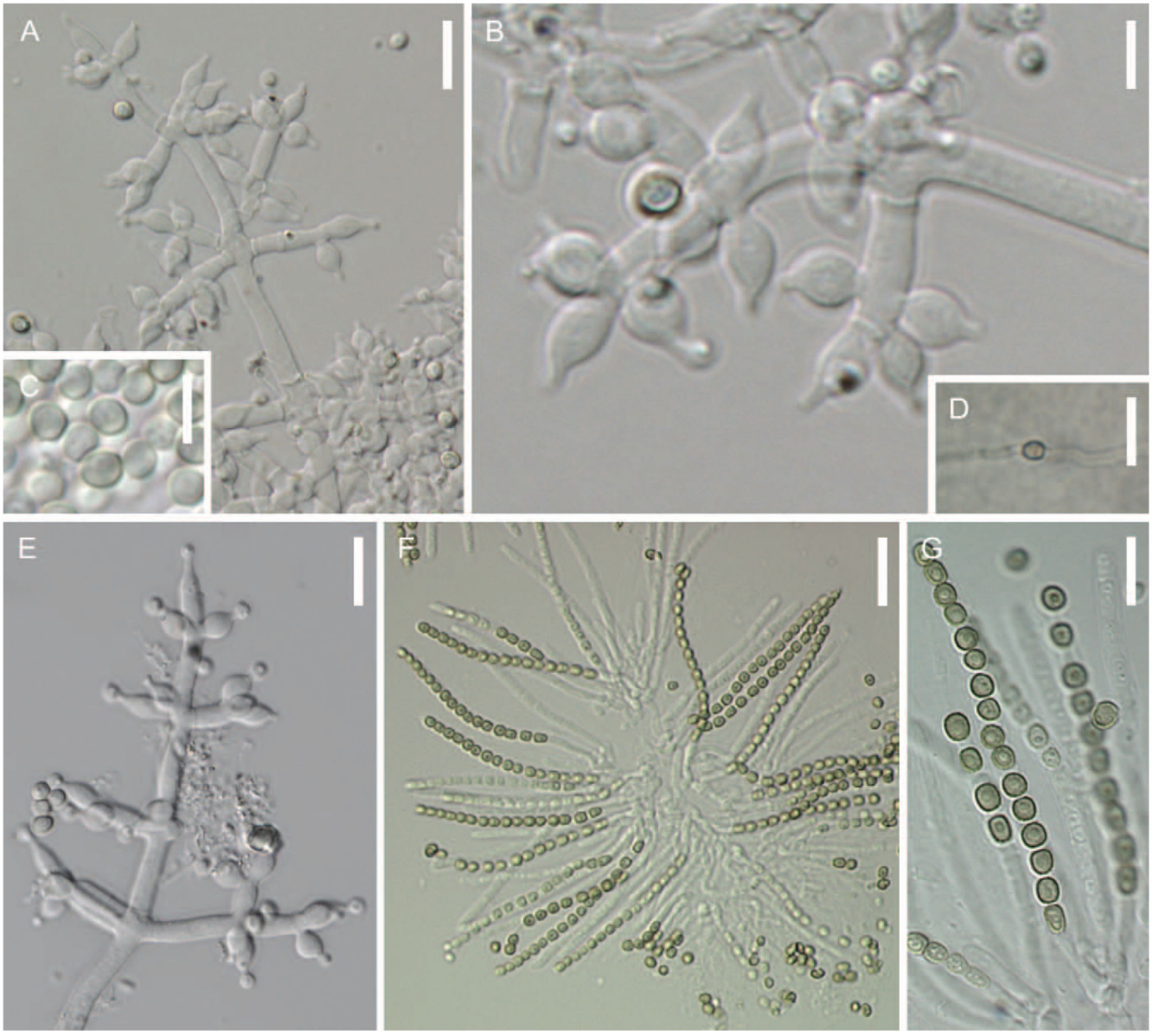

Fig. 5.

Trichoderma atrobrunneum. A, E. Conidiophores. B. Phialides. C. Conidia. D. Chlamydospore. F, G. Asci and ascospores. A, B from G.J.S. 90-254, C from G.J.S. 04-67, D from G.J.S. 05-106; E from G.J.S. 05-100, F, G from G.J.S. 98-183. Bars: A, D, E, G = 10 µm; B, C = 5 µm; F = 25 µm.

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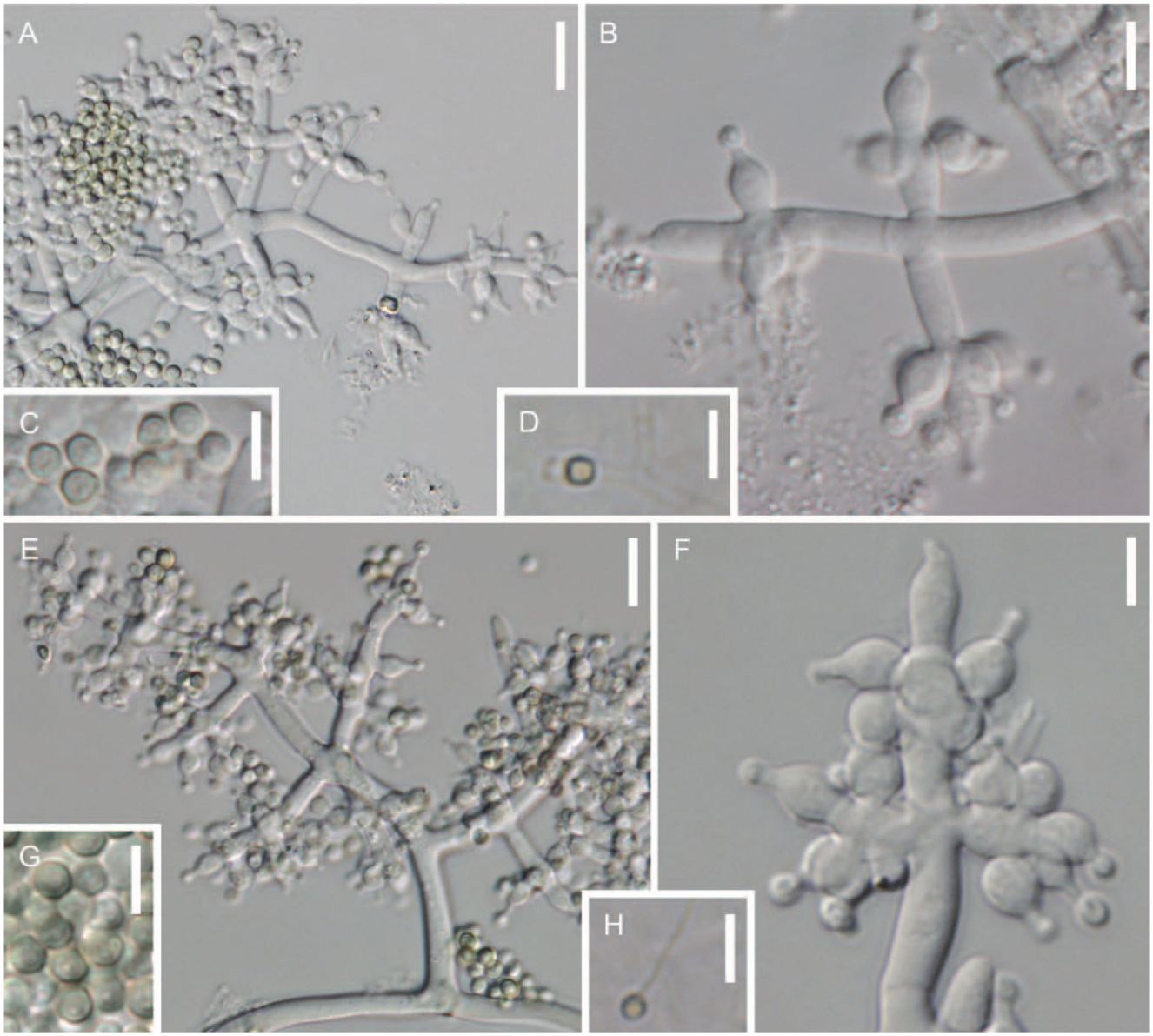

Fig. 6.

Trichoderma camerunense (E–H) and *T. endophyticum* (I–L). A, E. Conidiophores. B, F. Phialides. C, G. Conidia. D, H. Chlamydospore. A-D from G.J. S. 99-230; E from Dis 220k, F from Dis 218f, G from T 38 H from LA 10. Bars: A, E = 10 μm; B, C, D, F, G, H = 5 μm.

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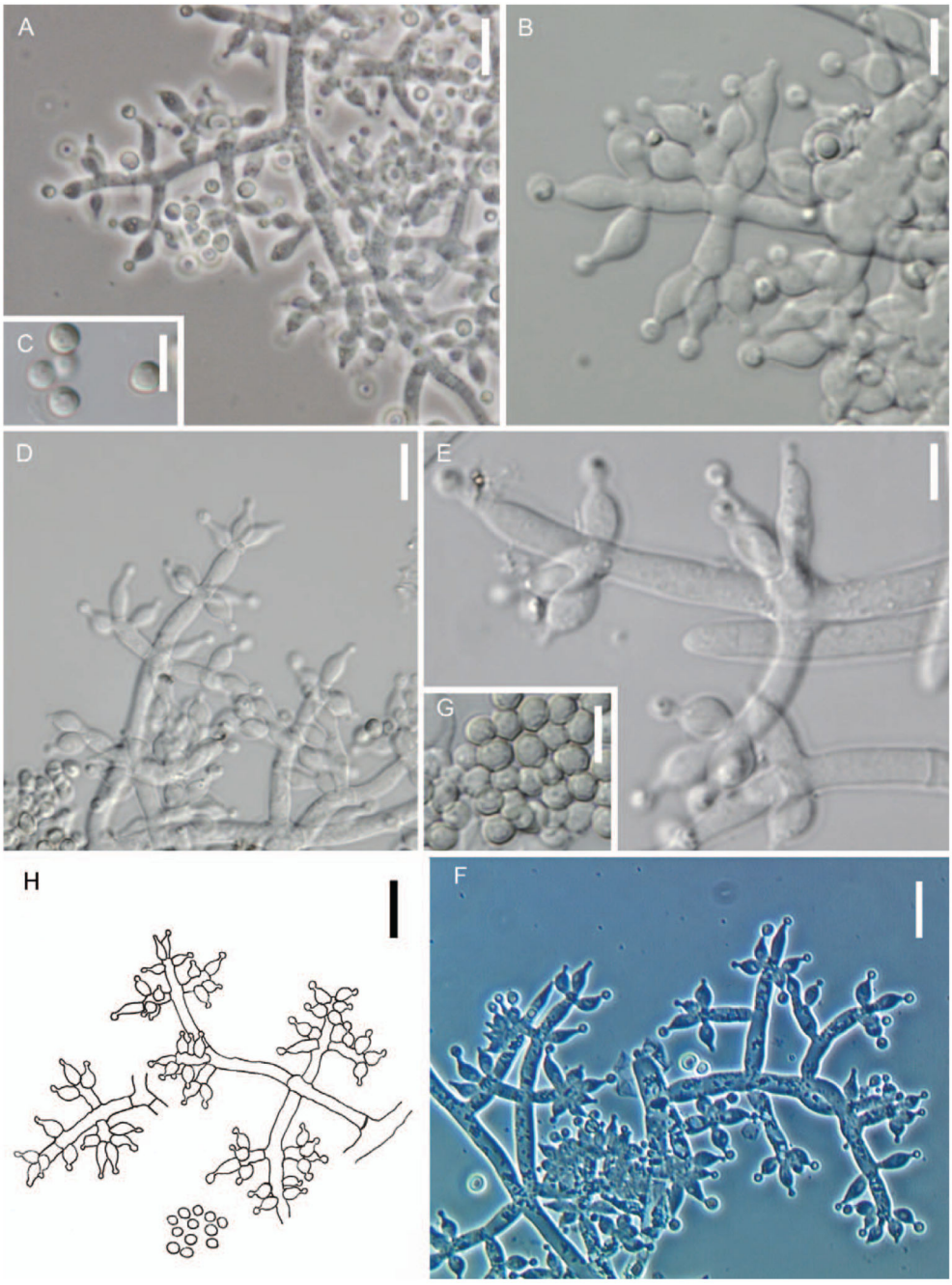

Fig. 7.

Trichoderma guizhouense (A–C), *T. harzianum* s. str. (D–G) and *T. inhamatum* (H). A, D, F. Conidiophores. B, E. Phialides. C, G. Conidia.. H. Conidiophores and conidia. A from Dis 314i, B from CBS 227.95, C from Dis 314d, E from CBS 226.95, F from IMI 359823, G from G.J.S. 04-71. H redrawn from Veerkamp and Gams (1983), original drawing courtesy of W. Gams. Bars: A, D, F, H = 10 µm; B, C, E, G = 5 µm.

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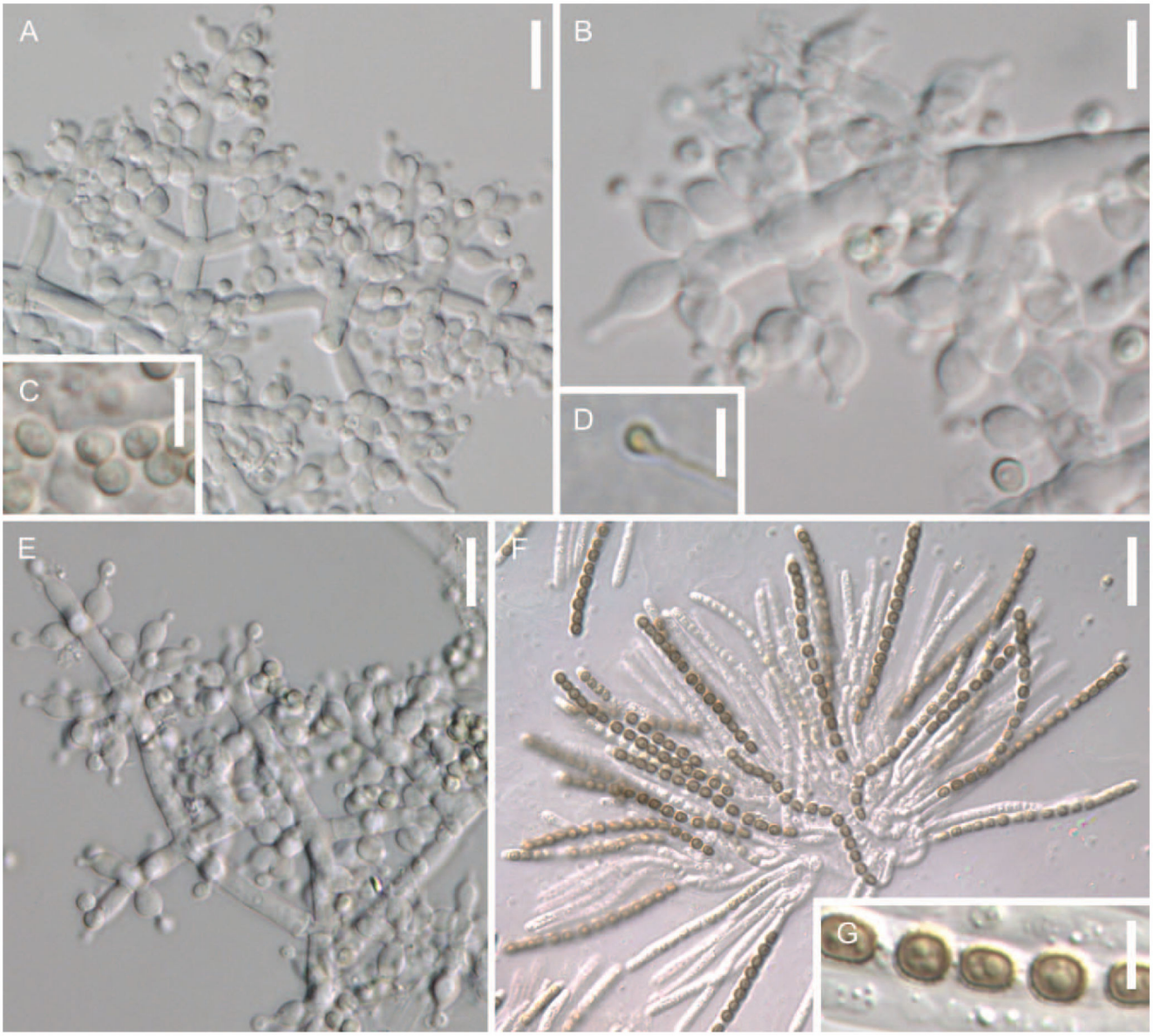

Fig. 8.

Trichoderma lentiforme. A, E. Conidiophores. B. Phialides. C. Conidia. D. Chlamydospore. F. Asci and ascospores. G. Ascospores. A, C from Dis 55f, B from Dis 167c, E from Dis 64b, F, G from G.J.S. 98-6. Bars: A, E = 10 µm; B, C, D, G = 5 µm; F = 25 µm.

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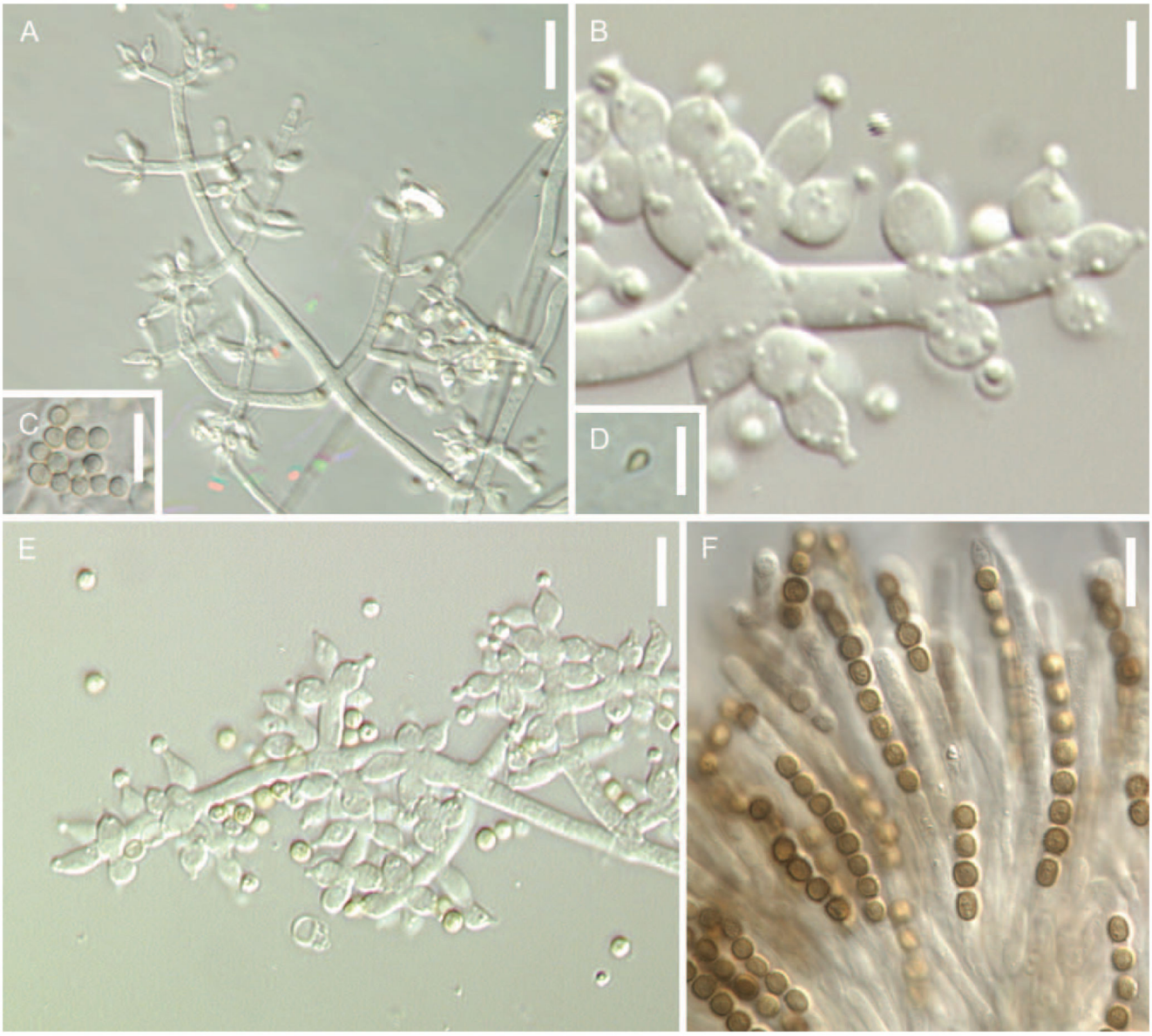

Fig. 9.

Trichoderma lixii. A, E. Conidiophores. B. Phialides. C. Conidia. D. Chlamydospores. F. Asci and ascospores. All from G.J.S. 97-96. Bars: A = 20 µm; B, F = 5 µm; C, D, E = 10 µm.

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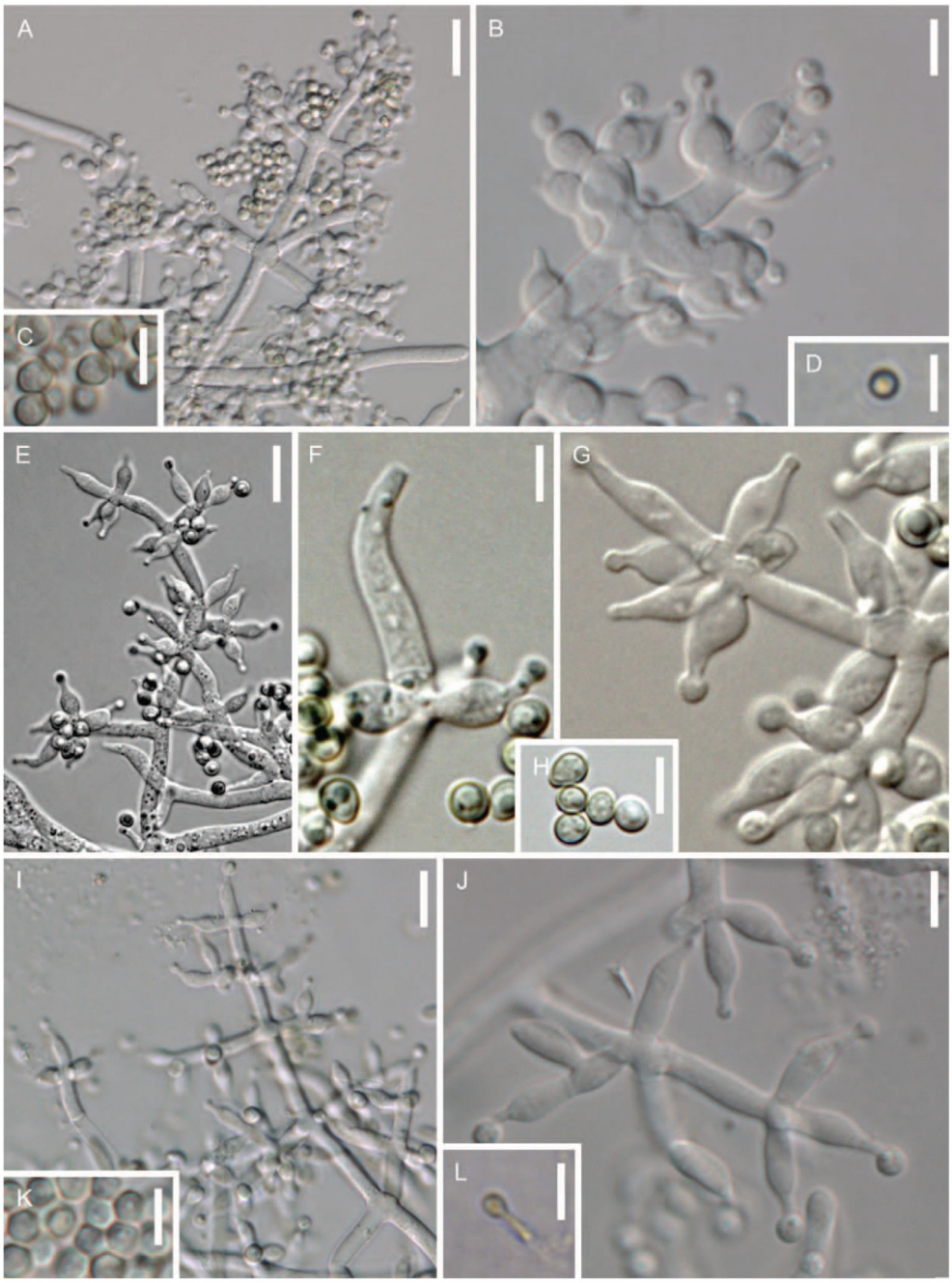

Fig. 10.

Trichoderma neotropicale (A–D), *Trichoderma pyramidale* (E–H) and *Trichoderma rifaii* (I–L). A, E, I. Conidiophores (T 51, S73, Dis 337f). B, F, G, J. Phialides (T51, S73, S73, Dis 337f). C, H, K. Conidia (T 51, S73, Dis 355b). D, L. Chlamydospores (T51, Dis 355b). A-D from T 51, E-H from S73; I, J from Dis 337f; K. L from Dis 355b. Bars: A, E, I = 10 μm; B, C, D, F, G, H, J, K, L = 5 μm.

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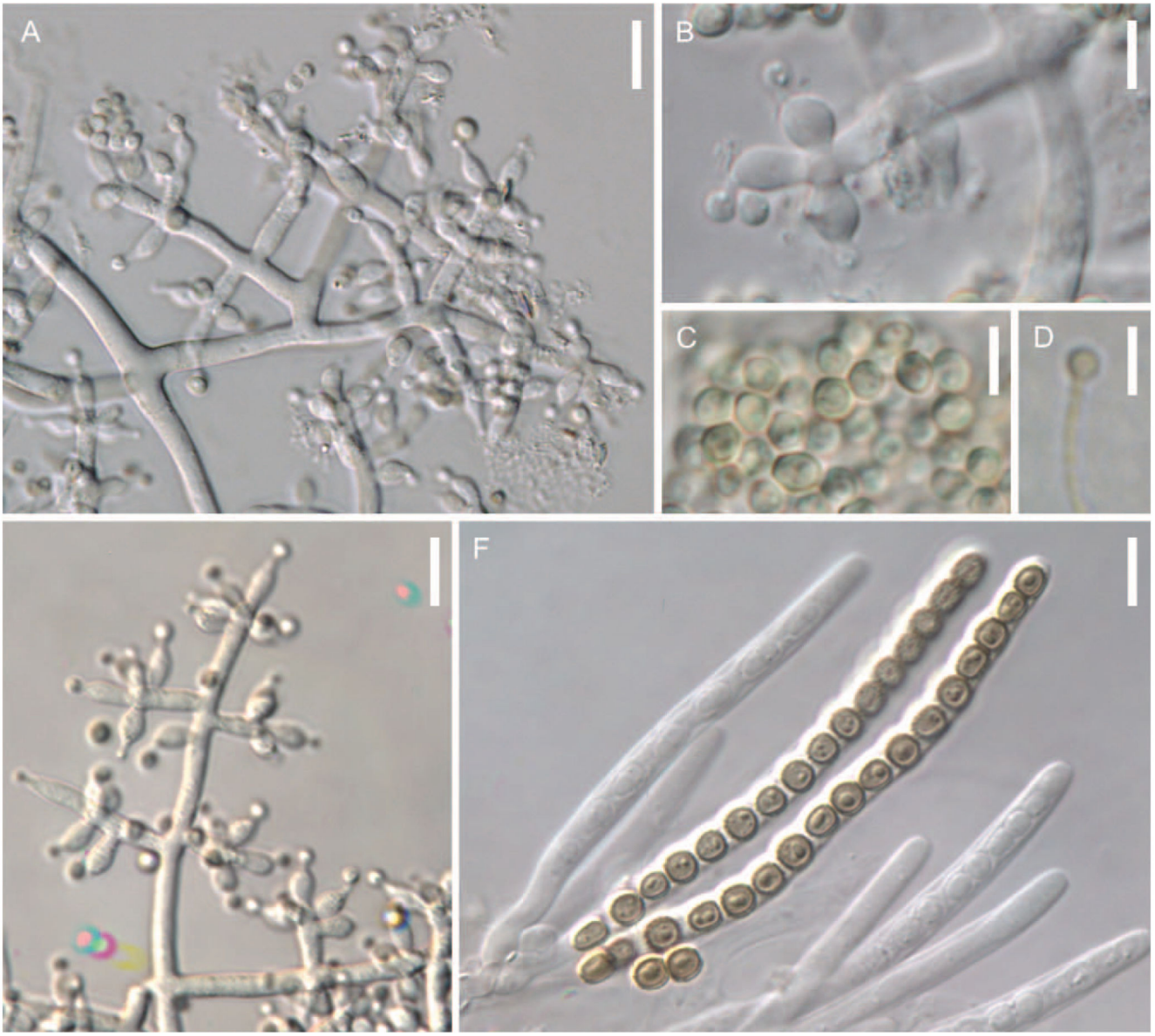

Fig. 11.

Trichoderma simmonsii. A, E. Conidiophores (G.J.S. 90-22, G.J.S. 91-138). B. Phialides (G.J.S. 91-138). C. Conidia (G.J.S. 91-138). D. Chlamydospores (G.J.S. 90-22). F. Asci and ascospores (G.J.S. 90-22). A, D, F from G.J.S. 90-22; B, C, D from G.J.S 91-138. Bars: A, E, F = 10 μm; B, C, D = 5 μm.

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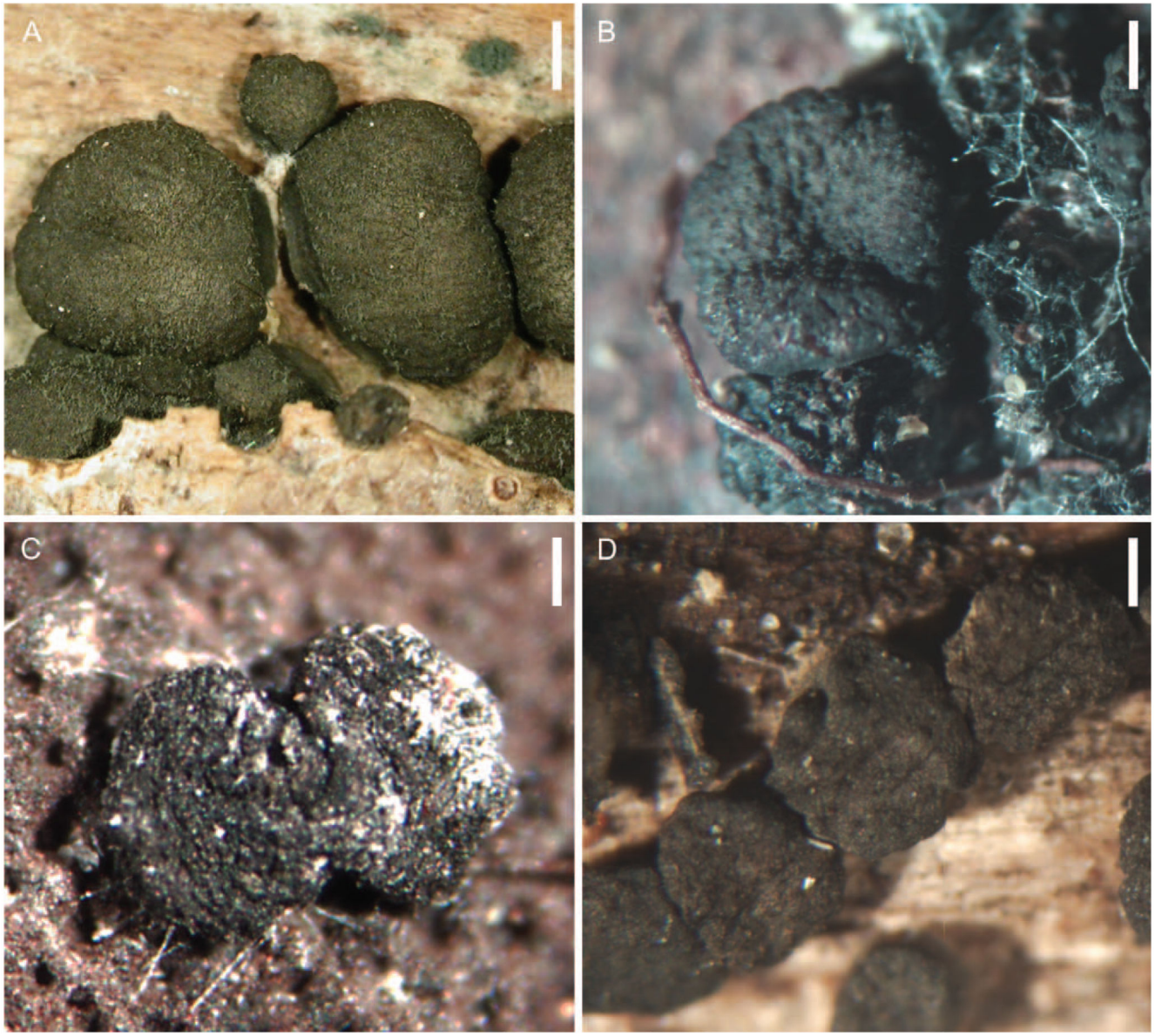

Fig. 12.

Stromata of members of the *T. harzianum* complex. A. *T. atrobrunneum* (WU 29099 = Hypo 348). B. *T. lentiforme* (G.J.S. 98-6). C. *T. lixii* (G.J.S. 97-96). D. *T. simmonsii* (G.J.S. 92-100). Bars = 250 μm.

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Table I

Comparison of morphology of species in the *T. harzianum* complex

Character/species	afarasin	afroharzianum	atrobrunneum	camerunense	endophyticum	guizhouense
Geographic distribution	West Africa	Cosmopolitan	Europe, North America	West Africa	Ecuador, Peru	Cosmopolitan
Known habitat	Endophyte, soil	Diverse	Soil, decaying wood and fungi	Soil	Endophyte	Diverse; soil, endophyte
Conidium length (µm)	2.8	3.2	3.0	2.9	2.9	2.8
	(2.7−2.9)	(3.1−3.3)	(3.0−3.1)	(2.9−3.0)	(2.9−3.0)	(2.8−2.9)
Conidium width (µm)	2.6	2.8	2.8	2.7	2.5	2.7
	(2.5−2.6)	(2.8−2.9)	(2.7−2.8)	(2.7−2.8)	(2.5−2.6)	(2.6−3.0)
Conidium L/W ratio	1.1	1.1	1.1	1.1	1.1	1.1
	(1.12−1.06)	(1.12−1.09)	(1.10−1.03)	(1.06−1.10)	(1.10−1.14)	(1.07−1.08)
Phialide length (µm)	5.6	9.1	7.0	5.0	5.4	6.4
	(5.3−6.0)	(8.6−9.5)	(6.6−7.3)	(4.8−5.2)	(5.2−5.6)	(6.2−6.3)
Phialide width (µm)	3.2	3.0	3.4	2.9	3.5	3.3
	(3.1−3.4)	(3.0−3.1)	(3.3−3.5)	(2.8−3.0)	(3.4−3.6)	(3.2−3.3)
Growth after 72 h[a] PDA 25 C (mm)	55−62	49−65	37−55	66	54−66	45−69
	(59)	(59)	(48)	n = 1	(60)	(59)
	n = 4	n = 4	n = 7		n = 6	n = 10
Growth after 72 h[a] PDA 30 C (mm)	65−70	55−70	35−59	70	61−73	54−70
	(65)	(66)	(52)	n = 1	(68)	(65)
	n = 4	n = 4	n = 7		n = 6	n = 10
Growth after 72 h[a] PDA 35 C (mm)	0−44	14−45	4−35	32	37−48	45−69
	(31)	(35)	(19)	n = 1	(42)	(57)
	n = 4	n = 4	n = 7		n = 6	n = 10
Growth after 72 h[a] SNA 25 C[a](mm)	50−58	53−59	35−53	55	46−65	45−69
	(56)	(56)	(48)	n = 1	(55)	(57)
	n = 4	n = 4	n = 7		n = 6	n = 10
Growth after 72 h[a] SNA 30 C (mm)	60−65	58−65	32−57	59	50−70	52−69
	(62)	(62)	(49)	n = 1	(59)	(60)
	n = 4	n = 4	n = 7		n = 6	n = 10

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Character/species	afarasin	afroharzianum	atrobrunneum	camerunense	endophyticum	guizhouense
Growth after 72 h[a] SNA 35 C (mm)	3–46	14–50	7–30	24	33–43	4–43
	(31)	(37)	(57)	n = 1	(36)	(26)
	n = 4	n = 4	n = 7		n = 6	n = 10)
Stroma color	—	—	Dark green to black	—	—	—
Stroma diam (mm)	—	—	1.5 (0.9–2.5)	—	—	—
Distal part ascospore (µm)	—	—	4.2 × 3.8	—	—	—
			(4.0–4.3 × 3.7–3.9)			
Proximal part ascospore (µm)	—	—	4.3 × 3.4	—	—	—
			(4.2–4.4 × 3.3–3.5)			

Character/species	harzianum	inhamatum	lentiforme	lixii	neotropicale	pyramidale	rifaii	simmonsii
Geographic distribution	Europe, North America	Neotropical	Neotropical	Southeast Asia	Ecuador, Peru	Southern Europe	Neotropical	Europe, North America
Known habitat	Soil, sometimes endophyte	Soil	Endophyte; sexual stage on leaves a nd bark	Aphyllophorales	Endophyte	Wood and bark (?fungi)	Endophyte	Decomposing wood (?fungi)
Conidium length (µm)	3.2	2.7	2.8	3.2	3.0	3.5	2.6	3.0
	(3.1–3.2)	(2.6–2.8)	(2.8–3.0)	(3.1–3.2)	(2.1–3.0)	(3.5–3.6)	(2.5–2.7)	(3.0–3.1)
Conidium width (µm)	2.8	2.5	2.6	3.2	23.0	3.5	2.6	3.0
	(2.7–2.8)	(2.4–2.6)	(2.5–2.7)	(3.1–3.2)	(2.1–3.0)	(3.4–3.6)	(2.5–2.7)	(3.0–3.1)
Conidium L/W ratio	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
	(1.1–1.2)	(1.0–1.2)	(1.07–1.08)	(1.07–1.11)	(1.075–1.11)	(1.12–1.15)	(1.06–1.12)	(1.1–1.2)
Phialide length (µm)	6.9	4.8	5.6	6.7	6.1	8.4	6.1	6.3
	(6.7–7.2)	(4.2–5.3)	(5.3–5.5)	(6.3–7.0)	(5.8–6.5)	(7.7–9.1)	(5.8–6.2)	(6.1–6.5)
Phialide width (µm)	3.5	2.9	5.4	3.7	3.7	3.2	3.5	3.3
	(3.5–3.6)	(2.8–3.0)	(3.50–3.52)	(3.6–3.8)	(3.6–3.8)	(3.1–3.3)	(3.4–3.6)	(3.3–3.4)
Growth after 72 h[a] PDA 25 C (mm)	48–60	60	n = 28	62	56–59	43–47	55–67	58–65
	(55)	n = 1		n = 1	n = 2	n = 2	n = 2	(61)
	n = 5							n = 5
Growth after 72 h[a] PDA 30 C (mm)	56–72	70	n = 28	70	62–68	39–42	60–70	68–70
	(68)	n = 1		n = 1	n = 2	n = 2	n = 2	(70)
	n = 5)							n = 5
Growth after 72 h[a] PDA 35 C (mm)	20–49	33	57–74	40	31–35	0	32–43	10–52
	(39)	n = 1	(66)	n = 1	n = 2	n = 2	n = 2	(33)

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Character/species	harzianum	inhamatum	lentiformе	lixii	neotropicale	pyramidale	rifaii	simmonsii
	n = 5)		n = 28					n = 5
Growth after 72 h[a] SNA 25 C[a](mm)	29−60	50	44−72	55	53−54	46−51	62−65	46−58
	(49)	n = 1	(60)	n = 1	n = 2	n = 2	n = 2	(53)
	n = 5)		n = 28					n = 5
Growth after 72 h[a] SNA 30 C (mm)	36−70	50	45−72	63	55−58	43−50	64−69	50−67
	(57)	n = 1	(65)	n = 1	n = 2	n = 2	n = 2	(58)
	n = 5		n = 28					n = 5
Growth after 72 h[a] SNA 35 C (mm)	17−44	19	26−47	30	30	0	33−43	3−34
	(35)	n = 1	(38)	n = 1	n = 2	n = 2	n = 2	(22)
	n = 5		n = 28					n = 5
Stroma color	—	—	Dark green to black	Dark brown to black	—	—	—	Dark brown to black
Stroma diam (mm)	—	—	1.0 (0.9−1.3)	1.0 (0.8−1.2)	—	—	—	2.0 (1.0−3.0)
Distal part ascospore (µm)	—	—	4.4 × 3.9	4.3 × 3.9	—	—	—	4.4 × 4.1
			(4.3−4.6 × 3.7−4.0)	(4.1−4.5 × 3.8−4.1)				(4.4−4.5 × 4.0−4.2)
Proximal part ascospore (µm)	—	—	4.5 × 3.4	4.6 × 3.5	—	—	—	4.6 × 3.6
			(4.3−4.6 × 3.3−3.5)	(4.3−4.8 × 3.4−3.7)				(4.5−4.7 × 3.6−3.7)

[a]Range of n cultures, average with 95% confidence intervals in parentheses.