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Terfezia disappears from the American truffle mycota as two new genera and *Mattirolomyces* species emerge

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Abstract: Reexamination and molecular phylogenetic analyses of American *Terfezia* species and *Mattirolomyces tiffanyae* revealed that their generic assignments were wrong. Therefore we here propose these combinations: *Mattirolomyces spinosus* comb. nov. (≡ *Terfezia spinosa*), *Stouffera longii* gen. & comb. nov. (≡ *Terfezia longii*) and *Temperantia tiffanyae* gen. & comb. nov. (≡ *Mattirolomyces tiffanyae*). In addition we describe a new species, *Mattirolomyces mexicanus* spec. nov. All species belong to the Pezizaceae. Based on these results *Terfezia* is not known from North America, *Mattirolomyces* is represented by two species and two new monotypic genera are present.

Key words: Ascomycota, ascospore, biogeography, Pezizaceae, Pezizales, scanning electron microscopy, *Stouffera*, *Temperantia*, truffle

INTRODUCTION

The truffle genus *Terfezia* (Pezizaceae, lineage A in Læssøe and Hansen 2007) is represented by the well known desert truffles of the Mediterranean region, Middle-east and southwestern Asia. Recent studies on *Terfezia* species from the Kalahari Desert, South Africa, revealed that these belong to different genera, *Kalaharituber pfeilii* (Henn.) Trappe and Kagan-Zur (≡ *Terfezia pfeilii* Henn.) (Ferdman et al. 2005) and *Mattirolomyces austroafricanus* (Trappe & Marasas) Kovács, Trappe & Claridge (≡ *Terfezia austroafricana* Trappe & Marasas) (Trappe et al. 2010a, b). Three *Terfezia* species, *T. longii* Gilkey, *T. spinosa* Harkn. and *T. gigantea* Imai, have been described from North America with additional provisional species proposed to exist on the continent (Harkness 1899, Gilkey 1947, Alsheikh 1994, Kovács et al. 2008). *Terfezia gigantea*, collected in northeastern North America and Japan, was shown to represent a new truffle genus *Imaia* belonging to the Morchellaceae (Kovács et al. 2008). *Terfezia spinosa* Harkn. was described from a collection in Louisiana (Harkness 1899). Trappe (1971) reduced *Mattirolomyces* to a subgenus under *Terfezia* and placed *T. spinosa* in that subgenus. Molecular phylogenetic studies confirmed that *Mattirolomyces* merited a separate genus (Percudani et al. 1999, Díez et al. 2002) and it was suggested that the generic placement of *T. spinosa* needed revision (Læssøe and Hansen 2007). The type specimen of *Terfezia longii* Gilkey was collected in New Mexico (Gilkey 1947). The species also has a South American record; Gilkey (Alsheikh 1994) determined *Tuber argentinum* var. *pampeanum* Speg. (Spegazzini 1909) to be a taxonomic synonym of *T. longii*.

During a reevaluation of American *Terfezia* species molecular phylogenetic analyses revealed that neither *T. longii* nor *T. spinosa* belong to genus *Terfezia*. We included *Mattirolomyces tiffanyae* Healy, described from Iowa (Healy 2003), in the molecular phylogenetic analyses to complete the phylogeny of American members of the genus. Here we present a taxonomic revision of these species consistent with their morphologies and phylogenetic relationships.

MATERIALS AND METHODS

Examination of specimens and microscopy.—Macroscopic descriptions of the fungi were taken from the literature and notes accompanying individual collections. Hand sections mounted in water, 3% KOH, Melzer's reagent and cotton blue in lactic acid were used for microscopy. Spore dimensions were measured in water mounts of mature spores. Light microscopy with Nomarski interference contrast optics also was used. For microscopy (SEM)

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spores of dry herbarium samples were affixed on double-sided tape, gold coated, and examined in a Hitachi 2360N SEM.

DNA extraction, PCR amplification and sequencing.—DNA was extracted from holo- or isotypes of the taxa in this study as well as from supplementary collections when available (see *Collections examined* under each taxon). Methods for DNA extraction, PCR amplification and sequencing were described in detail by Kovács et al. (2008). DNA was extracted from small pieces of dried herbarium specimens. SSU, ITS and LSU (LR0R–LR5) regions of nrDNA were amplified and sequenced. Sequences were compiled from electrophoregrams by Pregap4 and Gap4 (Staden et al. 2000). Amplification of regions of nrDNA was successful in the different specimens; sequences were deposited in GenBank (HQ660377–HQ660390, SUPPLEMENTARY TABLE I). The LSU region had been shown to be phylogenetically informative within Pezizaceae (e.g. Hansen et al. 2001, 2005); moreover it can be amplified relatively easily from DNA extracted from dried ascomata (Hansen et al. 2005).

Phylogenetic analyses.—For the final analyses we used a reduced, family-level LSU dataset of the phylogenetic analyses of Pezizaceae (Læssøe and Hansen 2007) together with sequences from Trappe et al. (2010a) and the present study. *Ascobolus crenulatus* was used as outgroup. We also analyzed a combined dataset of LSU and ITS sequences of *Mattirolomyces* species with *Elderia arenivaga* as outgroup. *Elderia* formed a sister group of *Mattirolomyces* in Trappe et al. (2010a). The alignments were deposited in TreeBASE (S11065).

Sequences were aligned with Clustal X (Thompson et al. 1997) and checked and adjusted manually with ProSeq 2.9 (Filatov 2002). The best-fit nucleotide substitution model was selected with the program jModeltest (Posada 2008) considering the selection of Akaike information criterion (AIC). The best-fit model was used to calculate distances for NJ analysis with PAUP* 4.0b10 (Swofford 2003). Support of the branches was tested by NJ bootstrap (NJB) with 1000 replicates. Phylogenies also were inferred by parsimony analyses (MP) by heuristic search. Gaps were treated as missing characters, MULTREES option was in effect and TBR was used as the branch-swapping algorithm. Support of the branches was tested by parsimony bootstrap (PB) with a fast-heuristic search with 1000 replicates. A maximum likelihood (ML) phylogenetic analysis was carried out with the online version of PHYML 3.0 (Guindon and Gascuel 2003). The GTR nucleotide substitution model was used with ML estimation of base frequencies. The proportion of invariable sites was estimated and optimized. Four substitution rate categories were set, and the gamma distribution parameter estimated and optimized. ML bootstrap (MLB) analysis with 1000 replicates was used to test support of branches. The same substitution model was used in Bayesian analyses performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) running at the Computational Biology Service Unit at Cornell University (http://cbsuapps.tc.cornell.edu/index.aspx). Four incrementally heated Markov chains were run for 5 000 000 generations, sampled every 100 generations. The burn-in was set to 7500 sampled trees, and Bayesian posterior probabilities (PP) were obtained from the trees retained. A 50% majority rule consensus phylogram of the trees kept was computed. The phylogenetic trees were viewed and edited by Tree Explorer in the MEGA 4 program (Tamura et al. 2007) and a text editor.

RESULTS

Molecular phylogenetic analyses.—The phylogenetic analyses all resolved similar topologies, in accordance with previous analyses of the Pezizaceae (Hansen et al. 2005, Læssøe and Hansen 2007). *Terfezia longii* and *T. spinosa* separated from genus *Terfezia* represented by *T. claveryi* and *T. boudieri* in our analyses (FIG. 1). The type species of *Terfezia*, *T. arenaria*, was not included in the analyses, but based on previous results with ITS (Díez et al. 2002) and β-tubulin sequences (Hansen et al. 2005) it is closely related to *T. claveryi* and *T. boudieri*, and we regarded these species as *Terfezia* s. str. *Terfezia spinosa* and a new species from Mexico were nested within *Mattirolomyces*. Similar to Trappe et al. (2010a) *Mattirolomyces* formed a strongly supported monophyletic group with *Elderia* (PB 97, PP 100, NJB 95). *Terfezia spinosa* was represented by two collections, one from Louisiana, USA, the other from Ladhar Sheikhupura, western Pakistan, which had a high degree of sequence similarity. The two collections grouped together and showed almost no sequence differences; their partial LSU sequences were identical, the partial SSU sequences differed in one character while the ITS sequences differed in two substitutions and one indel. The new *Mattirolomyces* species from Mexico was identified as a sister taxon of *M. spinosus* (FIGS. 1, 2). This species, described below as *M. mexicanus*, is distinguished from other *Mattirolomyces* species by its coarse spore ornamentation (FIG. 3). Although *Mattirolomyces* was only weakly supported in the analyses of the LSU (FIG. 1), it received full support (NJB, PB, MLB, PP: 100%) in the analyses of the combined ITS and LSU sequences (FIG. 2). *Mattirolomyces spinosus* formed a strongly supported group with *M. mexicanus* (NJB, PB, MLB, PP: 100%). *Mattirolomyces mulpu*, *M. austroafricanus* and *M. terfezioides* were resolved as successive sister taxa to the *M. spinosus-mexicanus* clade.

Mattirolomyces tiffanyae was found to represent a separate lineage within Pezizaceae (FIG. 1) distinct from *Mattirolomyces*. *Terfezia longii* similarly appeared to be distinct from species of *Terfezia* (FIG. 1). Although *T. longii* and *M. tiffanyae* were grouped together, this group lacked support and could be a long-branch attraction (LBA) artifact. *Mattirolomyces tiffanyae* and *T. longii* formed a well supported group

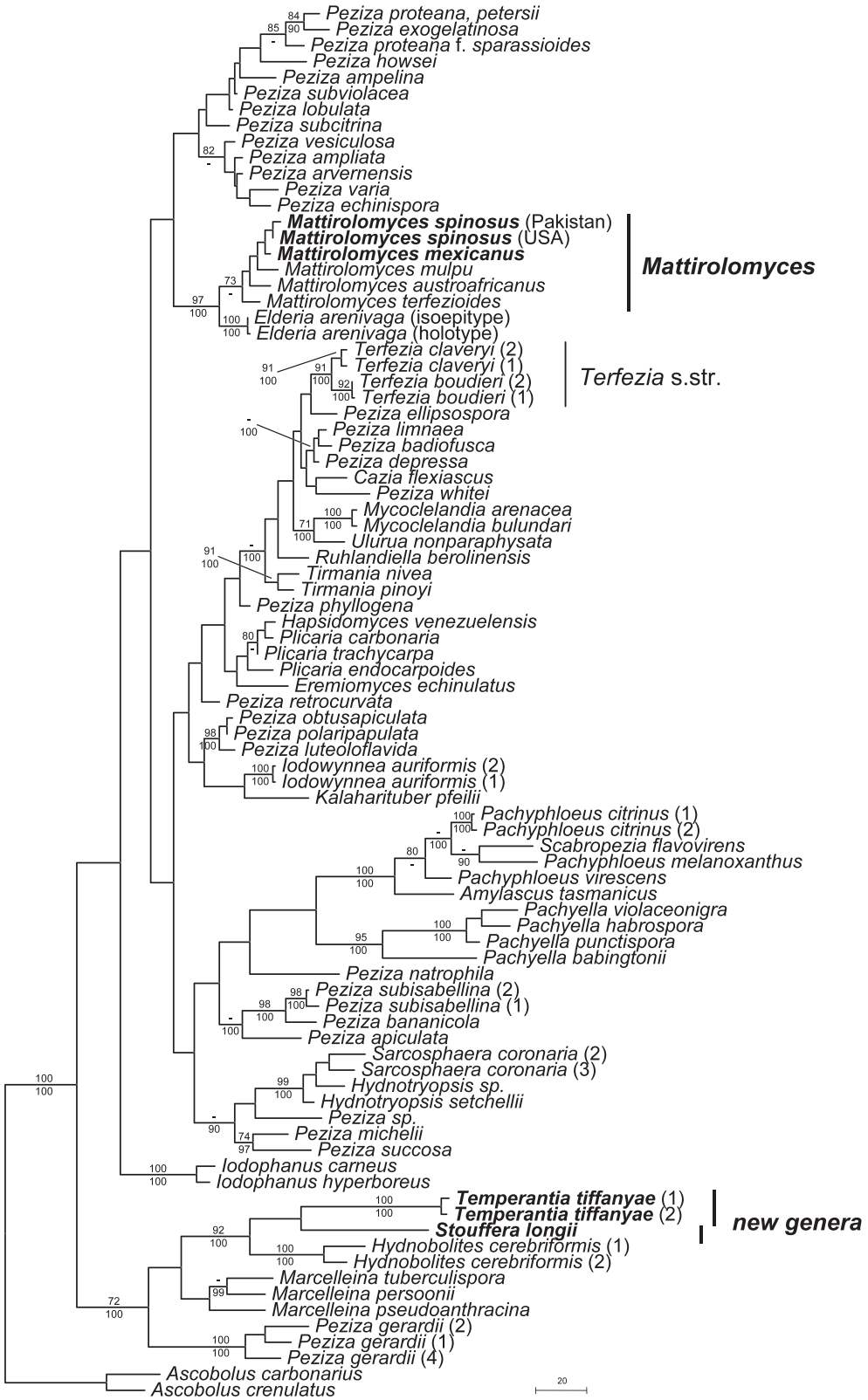

FIG. 1. One of 375 most parsimonious phylogenetic trees inferred from a dataset of partial LSU sequences showing positions of the studied desert truffles within the Pezizaceae. *Ascobolus crenulatus* served as outgroup. Sequences obtained in this study are shown in boldface. Other sequences were obtained from Læssøe and Hansen (2007) and Trappe et al. (2010b). Parsimony bootstrap (PB) values are shown above the branches, while the Bayesian posterior probabilities (PP) are below. Bootstrap values below 70% and PP below 95% are not shown.

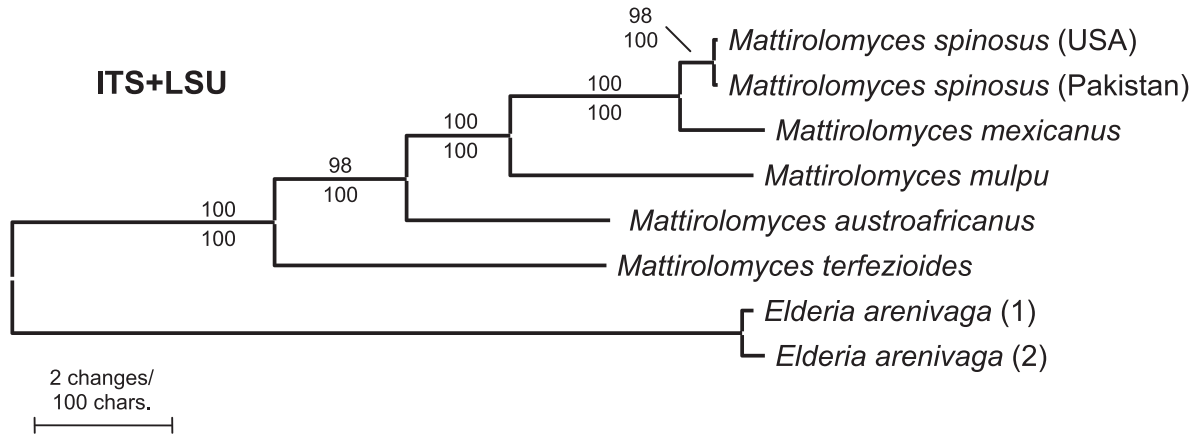

FIG. 2. The maximum likelihood (ML) tree of the combined ITS and LSU sequences of *Mattirolomyces species* with *Elderia arenivaga* as outgroup. The ML bootstrap (MLB) values are above the branches, while the Bayesian posterior probabilities (PP) are below.

with *Hydnobolites cerebriformis* (MP 92%, PP 100%, MLB 97%, NJB 99%) (FIG. 1). This well supported group was nested within the *Marcelleina-P. gerardii* lineage (MP: 72%, PP: 100%).

The phylogenetic analyses showed that the American ''*Terfezia*'' species and ''*Mattirolomyces tiffanyae*'' require generic reassignment. We here describe the new genera *Stouffera* and *Temperantia* for *T. longii*

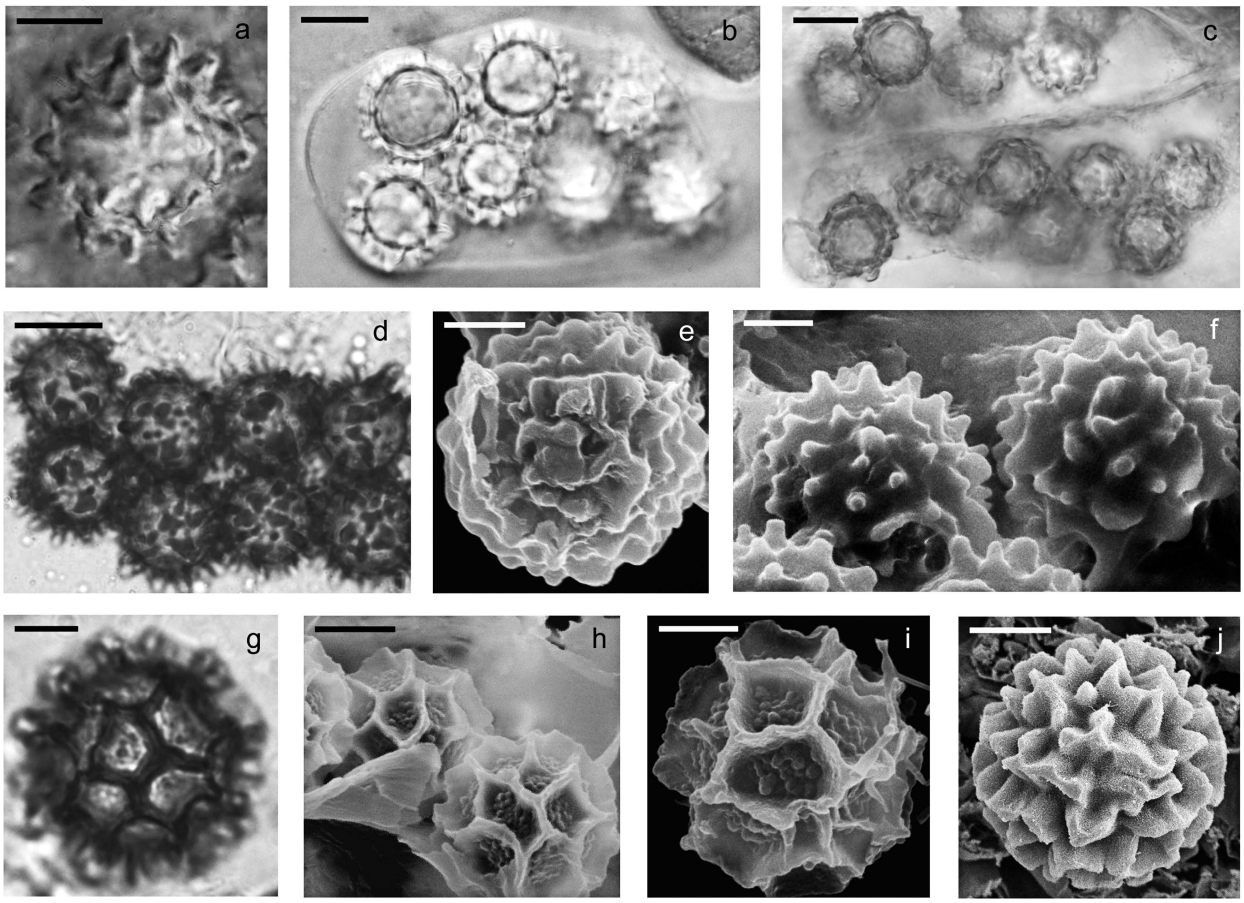

FIG. 3. Micrographs of the ascospores of the truffles studied. a–b. *Mattirolomyces spinosus*; c–f. *Mattirolomyces mexicanus*; g–i. *Stouffera longii* (g–h = New Mexico, i = Argentina); j. *Temperantia tiffanyae*. (a–d, g = light micrographs, e, f, i, j = scanning electron micrographs). Bars: a, b, h, j = 10 µm; c, d = 20 µm; e–g, i = 5 µm.

and *M. tiffanyae* respectively, transfer *T. spinosa* to *Mattirolomyces* as *M. spinosus* comb. nov. and describe the new species, *M. mexicanus* sp. nov.

TAXONOMY

Mattirolomyces spinosus (Harkn.) Kovács, Trappe and Alsheikh comb. nov. FIGS. 1, 2, 3a–b

≡ *Terfezia spinosa* Harkn., Proc. Calif. Acad. Sci., Ser. 3, 1:277. 1899.

MycoBank MB519282

Ascomata hypogeous, globose to subglobose or turbinate, the top rounded to depressed, lobed, occasionally faintly pubescent, substipitate, with a short basal attachment, 1.5–3.1 × 1.5–5 cm, fragile. Excipulum smooth to wrinkled, radiate-rugose from the base, unpolished, often with deep to shallow crevices or grooves 0.1–3 mm broad and 0.1–0.2 mm deep in cross section when dry or up to 1.5 mm when rehydrated, near white to light greenish yellow or light yellow brown in youth, when mature with patches of darker brown, pale reddish brown or dark brown. Gleba fleshy, solid, whitish marbled when fresh, light pale yellowish brown to pale brown or dull light brown overall when dry, gelatinous-moist in youth, later moist but not gelatinous, consistency fragile when dry, with more or less isodiametric or elongate, light brown to grayish brown pockets of fertile tissue 0.2–2 × 0.1–2 mm separated by meandering, pale cream-colored, sterile veins, 0.01–2 mm broad, and a pallid, sterile base. Odor lightly mushroomy or strongly fishy when fresh. Flavor not distinctive.

Spores globose or rarely subglobose, 14–20(–25) µm broad excluding ornamentation, 16–25(–29) µm with ornamentation, hyaline, the walls 0.5–2 µm thick, smooth in youth, at maturity with tapered, blunt to pointed, often curved rods and spines 2–3(–4) µm tall, 1–3 µm broad at the base and ± 0.5 µm broad at tips, irregularly joined by low walls 0.5–1 µm broad to form a partial, irregularly alveolate reticulum, the alveolae 2–5 µm broad, in Melzer's reagent deep yellow to yellowish brown, in cotton blue light blue. Asci randomly arranged in gleba, eight-spored, hyaline, reniform to saccate, ellipsoid, pyriform, clavate, obovoid, or globose, (40–)65–115(–140) × (25–)45–90 µm, sessile or occasionally substipitate with a base or short stalk 10–25 µm broad, disintegrating with age, the walls ± 0.5–1 µm thick, in youth the spores clustered in the tip of the ascus, later migrating to the middle, biseriate or irregularly arranged, nonamyloid.

Ectal excipulum (10–)25–150 µm thick, interwoven hyphae 2–6 µm broad at septa, the cells inflated up to 25 µm, the walls ± 0.5–1 µm thick, hyaline to pale yellowish brown. Ental excipulum 300–800 µm thick, gradually differentiated from ectal excipulum as an inner layer of circumferentially aligned to interwoven, hyaline, thin-walled hyphae 4–8 µm broad at septa, with many cells inflated up to 35 µm broad, the walls 0.2–0.5 µm thick. Glebal hyphae hyaline, interwoven, the walls 0.2–0.5 µm thick, 3–10 µm broad at septa, the cells inflated 5–35 µm broad, in sterile veins often more or less spherical, 25–30 µm broad. Fertile tissues less compact than ectal and ental excipula, of hyphae 3–10 µm broad at septa, the cells generally not inflated.

Etymology. Latin, *spinosus* (spiny), referring to the spiny-reticulate spore ornamentation.

Illustrations. Harkness (1899), plate 45, fig. 24a, b; Mattirolo (1922), fig. 35; Gilkey (1939), plate 2, fig. 37; Alsheikh (1994), plates 2–19, p 145.

Distribution, habitat and season. USA, Arizona and Louisiana south into Mexico, in desert (Arizona), in sandy ground along banks of Red River (Louisiana) and Cachain de Echeverria River (Michoacan, Mexico), variously under *Ficus* spp., *Enterolobium cyclocarpum* Griseb. *Acacia* sp., and *Quercus* spp. in matorral and cercano, a shrubby vegetation type; July to November.

Collections examined. HOLOTYPE: USA: LOUISIANA: Natchitoches County [sic], Red River Valley near Natchitoches, *E. Forges*, Nov 1886, Ellis & Everhart North American Fungi Ser. 2, #1782, as *Terfezia leonis*, comm. Rev. A.B. Langlois, Harkness 108A (BPI; isotypes BPI, CUP, FH, M, MICH, MPPD, NY, OSC, TO, UPS). OTHER COLLECTIONS: MEXICO: MICHOACAN: Aquila, 6 km north from Cachain de Echeverria River bridge, *Horalia B. Diaz, Trappe 11265*, Oct–Nov 1983 (ITCV 872, OSC). USA: ARIZONA: *Chester Leathers, Trappe 5279*, undated (OSC). LOUISIANA: Natchitoches County [sic], banks of Red River, *C.F. Meschutt, J.B. Ellis 6970*, Nov 1886, as *Terfezia leonis* (CUP); *A.B. Langlois 704*, 25 Sep 1886, as *Terfezia leonis* (NY, PRC). PAKISTAN: LADHAR: Sheikhupura (near Lahore): *S. Ahmed*, Aug 1949, as *Terfezia* sp. (CUP).

Commentary. Microscopically *M. spinosus* so closely resembles the type species of the genus, *M. terfezioides* (Mattir.) E. Fischer, that Alsheikh (1994) only tentatively regarded the two as distinct. Phylogenetically however the two clearly differ (FIG. 1). The description of *M. spinosus* is based on poorly annotated, dried, herbarium specimens. When fresh collections, meticulously described, become available more morphological differences might emerge. The startling disjunction between the North American and Pakistani collections of *M. spinosus* cannot be explained on the basis of present information. We include it here as a new record for this species otherwise presently known only from North America.

Mattirolomyces mexicanus Kovács, Trappe and Alsheikh sp. nov. FIGS. 1, 2, 3c–f

MycoBank MB519283

A Mattirolomycetibus ceteris ornamento sporarum grosse verrucoso–reticulato verrucis conicis interdum latis quam

altis, 1–4(–5) × (0.5)1.5–3(–4) μm, saepe lineis vel cristis usque ad 3 μm altis connexis et ascis sporis 4–7(–8) differt.

Ascomata as dried, hypogeous, globose to subglobose, turbinate or napiform, the top rounded to depressed, often lobed, substipitate with short basal attachment, faintly pubescent, when dry fragile to hard, 3 × 2.5 cm. Excipulum smooth to more or less wrinkled, often radiate-rugose from the base, 0.1–0.8 mm thick when dry, light brownish gray to light brown mottled with brown. Gleba solid, light brown to moderate brown, fragile to hard, gelatinous-moist in youth, later moist but not gelatinous; fertile pockets moderate brown to dark brown, 0.3–0.8 × 0.2–0.4 mm, globose to subglobose, elongate or polygonal, separated by meandering sterile veins 0.1–0.3 mm broad, generally paler than the fertile pockets, pale orange yellow to light yellowish brown when dry. Odor and flavor not recorded.

Spores globose at maturity, 14–19(–30) μm broad excluding ornamentation, 20–28(–33) μm including ornamentation, at maturity hyaline to yellow, deep olive brown or light brown, the walls 1–2 μm thick, in youth smooth, becoming warty-reticulate, of conical warts with rounded tips, sometimes as broad as tall, 1–4(–5) × (0.5–)1.5–3(–5) μm, the tips 0.5–2(–4) μm broad, frequently connected by fine lines ± 0.25 μm broad or ridges up to 2 μm broad rising above spore surface to form an incomplete reticulum 1–3 μm tall; in Melzer's reagent orange yellow, in cotton blue lightly cyanophilic. Asci (4–7)–8-spored, hyaline, often globose to subglobose when young but later ovoid, saccate, elongate, reniform, ellipsoid, or globose, 80–135(–145) × 50–90 μm, astipitate to substipitate with a base ± 5 × 6 μm, walls in youth 2 μm thick, at maturity 0.1–0.5 μm thick, disintegrating with age, compactly and randomly arranged in fertile pockets, readily separable from glebal hyphae; spores formed at ascus tips, uni-, bi- or at times triseriate in elongate asci or irregularly arranged in broader asci; in Melzer's reagent young asci orangish yellow to pale yellowish brown, in cotton blue light blue.

Ectal excipulum 350–500 μm thick, of more or less thin-walled, tightly interwoven, pale brown to light brown hyphae, 3–6 μm broad at septa, the cells globose to subglobose or elongate and 8–17 μm broad, some inflated up to 30 μm, the walls ± 0.5 μm thick, in Melzer's reagent dark orange yellow to strong yellow brown. Ental excipulum 200–1100 μm thick, differentiated from ectal excipulum as an inner layer of more or less loosely interwoven, orange yellow to pale brown hyphae, 7–11 μm broad at septa; cells elongate to spherical, 10–25 μm broad, paler than those in ectal excipulum, walls 0.1–0.2 μm thick. Gradation of cells from ectal to ental excipulum gradual, with less compact, less pigmented and smaller cells toward the gleba, in Melzer's reagent orange yellow to pale brown or light brown, in cotton blue light blue. Glebal hyphae hyaline to light yellow or pale brown when mature, the cells more loosely arranged than in ectal and ental excipula, 4–10(–13) μm broad at septa; cells elongate to spherical, 10–20 μm broad, some inflated up to 45 μm broad, the walls 0.1–0.2 μm thick; fertile tissue of similar hyphae but with smaller and narrower cells, in Melzer's reagent orange yellow to pale brown, in cotton blue light blue.

Etymology. Latin, *mexicanus*, Mexican.

Illustrations. Alsheikh (1994), plates 2–16, p 134.

Distribution, habitat and season. Known only from type locality, Mexico, Nuevo Leon, Guadalupe, in sandy soil near a municipal garbage dumpsite under *Acacia* sp. and *Quercus* sp. in matorral and cercano, a shrubby vegetation type; July.

Holotype here designated. MEXICO: NUEVO LEON, Guadalupe. Colonia Carmen Romano. J. Muñoz, *Chacón 49*, *Trappe 11399*, 8 Jul 1980, as *Choiromyces* sp. (ENCB, isotype OSC).

Stouffera Kovács and Trappe gen. nov.
MycoBank MB519284

A *Terfezia* ac *Mattirolomyes* excipulo verrucoso, papillulis rotundatis in pagi sporarum inter parietes reticuli et ordinibus DNA differt.

Differs from *Terfezia*, *Mattirolomyces* and *Temperantia* by the warty excipulum, the small, rounded hemispheres on the spore surface between the walls of the reticulum, and the DNA sequences.

Type species. Stouffera longii (Gilkey) Kovács and Trappe, comb. nov.

Etymology. Named after mycologist David J. Stouffer who along with W.H. Long collected the type species.

Stouffera longii (Gilkey) Kovács and Trappe comb. nov. FIGS. 1, 3g–i

≡ *Terfezia longii* Gilkey, Mycologia 39:448. 1947.

= *Tuber argentinum* var. *pamparum* Speg., Anal. Mus. Nac. B Aires Ser. 3, 12:423. 1909.

MycoBank MB519285

Ascomata hypogeous, pale brown to dark brown, subglobose, up to 3.5 cm broad, with a basal mycelial tuft or occasionally a sterile basal projection. Excipulum a thin, pale yellow to pale brown layer overlying a thicker white to pale yellow inner layer 0.2–1 mm thick, with prominent, rounded to subpolygonal warts (Gilkey described it as whitish and smooth); excipulum thickest near the base. Gleba solid, fleshy, white in youth, at maturity becoming pale yellow, with pockets of fertile tissue separated by sterile tramal veins concolorous with the ental excipulum at maturity. Odor and flavor not recorded.

Spores globose, 14–22 µm broad excluding ornamentation, (14–)20–24 µm with ornamentation, hyaline in youth, at maturity pale yellow to light ochraceous, the walls ± 1 µm thick, smooth in youth, at maturity with distant rods, cones or spines 2–4 × 1–3(–4) µm tall and ± 1 µm thick at the tip, joined by walls 1–2(–3) µm tall to form a complete alveolar reticulum of quadrangular to pentagonal alveolae, the spore surface within the alveolae covered with even, crowded, hemispheres ≤ 1 µm broad; in Melzer's reagent deep yellow to golden yellow, lightly cyanophilic in cotton blue. Asci eight-spored, hyaline, globose to subglobose, subpyriform to obovoid, 62–108(–135) × 43–64(–85) µm, generally astipitate but often with a basal projection ± 20 × 12 µm, the walls up to 4 µm thick in youth but ± 1 µm thick at maturity, disintegrating with age, randomly arranged in fertile pockets in the gleba, the spores randomly placed within the asci. In Melzer's reagent golden yellow to deep yellow; light blue in cotton blue.

Ectal excipulum 60–100 µm thick, at maturity of hyaline to pale yellow, subglobose to polyhedral or irregular cells overlying a layer 300–500 µm thick of cells 8–23(–30) µm broad, the walls in all layers ± 1 µm thick. Ental excipulum 20–120 µm thick, at maturity of hyaline to pale yellow cells larger and with thinner walls than those of the ectal excipulum grading to loosely interwoven narrow hyphae with some cells inflated up to 25–30 µm broad near the gleba, the walls ± 0.2 µm thick. Glebal hyphae hyaline, loosely interwoven, 4–13 µm broad at septa, cells often inflated up to 40 µm, walls ± 0.2 µm thick; sterile tramal veins of hyphae similar to those of fertile pockets. In Melzer's reagent tissues golden yellow or orange to yellowish, with many hyphae having highly refractive, yellow-orange content.

Etymology. Named after mycologist W.H. Long, who along with D.J. Stouffer collected the species holotype.

Illustrations. Gilkey (1947), fig. 17, p 443; Trappe (1979), fig. 27c, p 310, Alsheikh (1994), plates 2–14, p 123.

Distribution, habitat and season. Argentina, found under a humus layer, and USA in New Mexico on sand hills and juniper dunes in arid areas with *Artemisia* and *Juniperus* spp.; September and January.

Collections examined. HOLOTYPE: USA: NEW MEXICO: Lincoln County, Corona. W.H. *Long & D.J. Stouffer, W.H. Long 9740, Gilkey 283*, 17 Sep 1941 (holotype OSC, isotypes BPI, NY). OTHER COLLECTIONS: ARGENTINA: COL. RESISKACIA: C. Spegazzini, Jan 1887, as type of *Tuber argentinum* var. *pampeanum* Speg., (OSC, TO).

Commentary. *Stouffera longii* differs principally from *Terfezia* and *Mattirolomyces* in its unusual, double-spore ornamentation. The minute hemispheres on the spore surface and enclosed by the reticular walls are easily overlooked under 1000× magnification unless stained with cotton blue and were neither mentioned in the original description (Gilkey 1947) nor shown in the line drawings in Gilkey (1947) or Trappe (1979) or Spegazzini's drawing enclosed with his collection. They are clearly evident with SEM (FIG. 3h–i), the spore surface within each alveola is reminiscent of looking down on a bucket of balls.

Temperantia K. Hansen, Healy and Kovács gen. nov. MycoBank MB519286

Peridium constans ex textura intricata. Gleba a peridio non separabilis, solida, marsupiis fertilibus et venis in sicco cremicoloribus. Asci sporis 1–4, paraphyses carentes. Sporae hyalinae, uniguttulatae, globosae, e verrucato porcatae. A Hydnobolite ac Mattirolomycete ordinibus DNA differt.

Ascoma a stereothecium without a basal mycelial tuft, not changing color with handling or damage, darkening slightly to cream-colored when dry. Excipulum thin, of slightly inflated, interwoven hyphae similar to those of the sterile glebal veins. Gleba solid, lacking paraphyses or channels. Asci 1–4-spored, irregularly arranged in fertile pockets. Spores globose, with one guttule, hyaline and ornamented with blunt-tipped warts and irregularly thickened ridges (FIG. 3j).

Type species. *Temperantia tiffanyae* (Healy) K. Hansen, Healy and Kovács, comb. nov.

Etymology. In honor of Dr Lois H. Tiffany, the epithet namesake of the type species, referring to her self-discipline and modesty.

Temperantia tiffanyae (Healy) K. Hansen, Healy and Kovács comb. nov. FIGS. 1, 3j

≡ *Mattirolomyces tiffanyae* Healy, Mycologia 95:766. 2003.

MycoBank MB519308

Description and illustrations. Healy (2003).

Distribution, habitat and season. Hypogeous to emergent in temperate deciduous oak-hickory woodland. So far collected only in Iowa but expected to occur in other North American deciduous woodlands with calcareous soil.

Collections examined. HOLOTYPE: USA: IOWA: Story County, Hickory Grove Park. *R.A. Healy*, 18 Aug 1998, Healy, R. 231, as *Mattirolomyces tiffanyae* (holotype ISC, isotypes BPI, OSC). OTHER COLLECTIONS: (all as *Mattirolomyces tiffanyae* at ISC) USA: IOWA: Story County, McFarland Park (42°6′00″N, 93°34′30″W), near Ames, *R.A. Healy*, 23 Aug 1998, *RH237*; Story County, Hickory Grove Park (41°59′30″N, 93°21′30″W), near Nevada, *R.A. Healy*, 1 Sep 1998 *RH251*; *R.A. Healy* 7 Sep 1998 *RH257*; *R.A. Healy* 17 Sep 1998 *RH274*; *R.A. Healy* 6 Aug 1999 *RH520*; *R.A. Healy* 16 Sept 2007 *RH869*; Reactor Woods (42°02′30″N, 93°39′20″W), Ames, *C. Notis* 28 Aug 1999 *RH556*; *R.A.*

Healy 3 Oct 1999 *RH601*, Webster County, Woodman Hollow State Preserve, *R.A. Healy* 4 Oct 2007 *RH884*; all as *Mattirolomyces tiffanyae* (ISC).

Commentary. The LSU rDNA sequence of *Temperantia tiffanyae* diverges significantly from *Mattirolomyces* and the rest of the ingroup. Morphologically it is distinguished by the lack of a mycelial tuft; unchanging, white throughout the ascomata; low ascus spore number; and spore ornamentation of blunt-tipped warts and irregularly thickened ridges (FIG. 3j). *Temperantia tiffanyae* forms a monophyletic group with *Stouffera longii* and *Hydnobolites cerebriformis* in our molecular phylogenetic analyses and, although we take this grouping with caution, *Temperantia* shares several morphological characters with *Hydnobolites* (i.e. a thin excipulum, lack of paraphyses, apparent lack of basal mycelial tuft, disorganized arrangement of asci in pockets of glebal tissue, and globose, hyaline spores). *Hydnobolites* differs by usually having glebal channels, spores ornamented with a complete reticulum of relatively thin walls, 4–8 spores per ascus, and a tendency to turn orange with handling and as it dries.

Stouffera differs from *Temperantia* by having a basal mycelial tuft, brown ascomata, warted excipulum, and spores ornamented with a reticulum that encloses minute hemispheres on the spore surface. Of those Pezizaceae that have been studied by TEM we know of no others with stacked parallel microtubule-like structures observed in the early secondary wall development of *Temperantia* (Healy 2003, Fig. 21).

DISCUSSION

Genus *Terfezia* has been represented in America by two described species, *T. spinosa* and *T. longii*. Our molecular phylogenetic study of these, along with *Mattirolomyces tiffanyae* (Healy 2003) and an undescribed collection labeled *Choiromyces*, revealed that none had good generic placement (FIG. 1). *Terfezia longii* represents a new genus, and *T. spinosa* belongs to *Mattirolomyces*. Thus genus *Terfezia* disappears from North America. The undescribed ''*Choiromyces*'' collection also belongs in *Mattirolomyces*. *Mattirolomyces tiffanyae* was discovered to be a distinct lineage, here described as a new genus of Pezizaceae.

Stouffera longii originally was described from New Mexico by Gilkey (1947) as *Terfezia longii*. Although she discussed some resemblance of that species with *Terfezia spinosa* described from Louisiana (Harkness 1899), she found several consistent differences in for example spore ornamentation and ascus size and accordingly described a new species, naming it after Dr W.H. Long who co-collected the species. Gilkey's (1947) single illustration of the new species is a line drawing of its ascospores. Our results support those that declare spore ornamentation is an important character for *Stouffera longii*. The ornamentation as described by Gilkey is ''coarsely alveolate, with generally slender spines at angles'' (Gilkey 1947, p 448), whereas the drawing shows a spore with alveolar ornamentation more slender than coarse but spines more long than slender. We found the ascospores ornamented with a complete alveolar reticulum of quadrangular to pentagonal alveolae (FIG. 3g–i). The SEM study of the ascospores revealed a double ornamentation of the spores; the surface of the spores in the area of the alveolae is crowded with minute hemispheres. This ornamentation is detectable by light microscopy only when spores are stained with cotton blue and observed at 1000× magnification (FIG. 3g). Our results support Gilkey's decision to resist the ''intriguing possibility of linking the New Mexican material with this … long-lost *Terfezia*'' (i.e. *T. spinosa*) (Gilkey 1947, p 449). Moreover the molecular phylogenetic analyses show that the New Mexican material is not a different species but a different genus, which belongs to a completely different lineage of Pezizaceae than genus *Terfezia*. An Argentinian collection, type of *Tuber argentinum* var. *pampeanum*, is also *Stouffera longii*. Gilkey's notes on its herbarium vial indicate she determined it to be conspecific with *Terfezia (Stouffera) longii*. Unfortunately we could not amplify nrDNA from the Argentinian material, but the conspecificity could not be falsified, according to the morphological similarities and SEM of the spore ornamentation (FIG. 3i).

Trappe (1971) reduced the rank of *Mattirolomyces* to a subgenus of *Terfezia* and placed both *Terfezia spinosa* and the newly recombined *T. terfezioides* in that subgenus on the basis of their morphological similarities to *Terfezia* spp. Molecular phylogenetic analyses subsequently supported *Mattirolomyces* as a separate genus (Percudani et al. 1999, Díez et al. 2002); Læssøe and Hansen (2007) suggested generic revision of *T. spinosa* but did not formally transfer it to *Mattirolomyces*. Our analyses clearly showed that *T. spinosa* belongs to *Mattirolomyces*. Gilkey was correct to call it ''long-lost'' because apart from additional collections from the type locality the same year as the type was found only two later records are known from America—one from Arizona and another from Mexico. Although ornamentation of the mostly immature spores of the Arizonan material is not quite the same as the type, their LSU and SSU sequences are identical while the ITS rDNA sequences differ slightly. Unfortunately the Mexican collection was checked only by microscopy and no DNA analysis could be done. The collection from Pakistan showing high similarities to *M. spinosus* was included

in the molecular phylogenetic study and results supported its conspecificity with *M. spinosus*. We have no details about its locality, it being simply recorded as ''on the ground''. Its geographic disjunction with the American distribution might be interpreted as biologically meaningless, especially because the localities bear no striking biogeographic or habitat similarities. Nevertheless we cannot rule out the possibility that further genetic analyses could reveal more differences between the Pakistani and American materials than detected in the nrDNA sequences.

The new species *M. mexicanus* is separated from *M. spinosus* and all other *Mattirolomyces* species in the phylogenetic analyses and morphologically by its spore ornamentation. Although *M. mexicanus* has some spores with the ''*Mattirolomyces*-type'' ornamentation, most show other characteristics such as robust, thick, spine-like and/or warty ornamentation that do not occur elsewhere in the genus.

Mattirolomyces was represented by one species, *M. terfezioides*, for almost a century. Recent studies revealed that the genus is broadly distributed and includes several species (Trappe et al. 2010a, b). With the two new American species it now contains five—*M. spinosus* from USA, *M. mexicanus* from Mexico, *M. terfezioides* from Europe, *M. mulpu* from Australia and *M. austroafricanus* from South Africa.

Another species, *Mattirolomyces tiffanyae*, was described from the United States (Healy 2003), but its placement in that genus proved to be wrong. Molecular phylogenetic analyses showed that *Temperantia tiffanyae* is distinct from species of *Mattirolomyces* and other genera of Pezizaceae. It forms a monophyletic group with *Stouffera longii* and *Hydnobolites cerebriformis*. However this grouping could result from long-branch attraction. Still *Temperantia* shares several morphological characters with *Hydnobolites*. Although *Temperantia* separated from *Mattirolomyces*, the ultrastructure of the type species of both genera (Healy 2003, Healy and Kovács 2010) clearly showed characteristics of the Pezizaceae.

New species or even genera of hypogeous fungi are still being discovered, not only from less studied regions such as Australia (Trappe et al. 2010a, b) but from the US Pacific Northwest (Bonito et al. 2010, Trappe et al. 2010), where truffles and their biology have been studied intensively for the past 90 y. Trappe (in Mueller et al. 2007, Table 6) estimated that 4500–5500 hypogeous fungal species exist worldwide, and only about 700 have been described. As truffle diversity continues to be uncovered the new data will widen our understanding of their relationships and biogeography. Such is the case here, where molecular data from newly discovered taxa caused us to reexamine and revise described species and collections. Our revision has taxonomic consequences and gives new insight into the biogeography of these truffles. In addition to finding two new genera the range of *Terfezia* decreased, disappearing from America, while the presence of genus *Mattirolomyces* on the continent was established.

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