A NEW *TERFEZIA* FROM SPAIN

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Summary. MORENO G., J.L. MANJÓN & P. ALVARADO (2019). A new *Terfezia* from Spain. *Bol. Soc. Micol. Madrid* 43: 55–60.
A new mycorrhizal species of the genus *Terfezia* from *Tuberaria guttata* in acidic soil is described. DNA-based phylogenetic analysis, as well as macro/microscopic photographs of its main morphological characteristics are provided.

Key words: *Ascomycetes*, desert truffles, *Pezizales*, taxonomy, SEM, *Terfeziaceae*.

Resumen. MORENO G., J.L. MANJÓN & P. ALVARADO (2019). Una nueva *Terfezia* de España. *Bol. Soc. Micol. Madrid* 43: 55–60.
Se describe una nueva especie de *Terfezia* micorrícica de *Tuberaria guttata* en suelo ácido. Se aportan análisis filogenéticos y fotografías macro/microscópicas de sus principales características morfológicas.

Palabras clave: *Ascomycetes*, MEB, *Pezizales*, taxonomía, *Terfeziaceae*, Trufas de desierto.

INTRODUCTION

DNA-based molecular techniques are helpful to solve taxonomic problems between similar macro/microscopically species of the genus *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul. This was the case of the type specimen of *Terfezia trappei* (R. Galán & G. Moreno) A. Paz & Lavoise, which was shown to represent a synonym of *T. fanfanii* Mattir. (RODRÍGUEZ & al., 2019). Another sample identified as *Terfezia trappei* (GenBank AF276676) was, however, genetically different from the type. It is here, together with new collections, accommodated into a new species.

MATERIALS AND METHODS

All the studied material is preserved in the herbarium of the Department of Plant Biology of Alcalá de Henares (AH). Spores were measured under an oil immersion lens of a Nikon microscope (Nikon Eclipse 80i) equipped with an automatic photographic system (Nikon DS–5M). The measurements provided herein included the surface structures of the spores, such as spines or warts.

Scanning electron microscopy (SEM) micrographs were obtained at the University of Alcalá de Henares using a Zeiss DSM–950 device.

DNA extraction, amplification and sequen-

cing: Total DNA was extracted from dry specimens employing a modified protocol based on MURRAY & THOMPSON (1980). PCR reactions (MULLIS & FALOONA, 1987) included 35 cycles with an annealing temperature of 54 ºC. Primers ITS1F and ITS4 (WHITE & *al*., 1990, GARDES & BRUNS 1993) were employed to amplify the ITS rDNA region, while LR0R and LR5 (VILGALYS & HESTER, 1990; CUBETA & *al*., 1991) were used for the 28S rDNA region. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected.

Phylogenetic analyses: BLAST (ALTSCHUL & *al*., 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration (INSDC) public databases. Sequences came mainly from BORDALLO & *al*. (2012, 2013, 2015, 2018), RODRÍGUEZ & *al*. (2019) and VIZZINI & *al*. (2019). Sequences first were aligned in MEGA 5.0 (TAMURA & *al*., 2011) software with its Clustal W application and then corrected manually. The final alignment included 44 ITS rDNA sequence with 177/575 variable sites, and 20 28S rDNA sequences with 51/555 variable sites. Aligned loci were loaded in MrBayes 3.2.6 (RONQUIST & HUELSENBECK, 2003), where a Bayesian analysis was performed (partitions: ITS-28S, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 0.18 M generations, standard deviation having fell below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML 8.2.12 (Stamatakis 2014) using the standard search algorithm (partitions: ITS-28S, GTRCAT model, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

TAXONOMY

Terfezia honrubiae G. Moreno, Manjón, A. Morte & P. Alvarado, *sp. nov.* (Figs. 1–11).

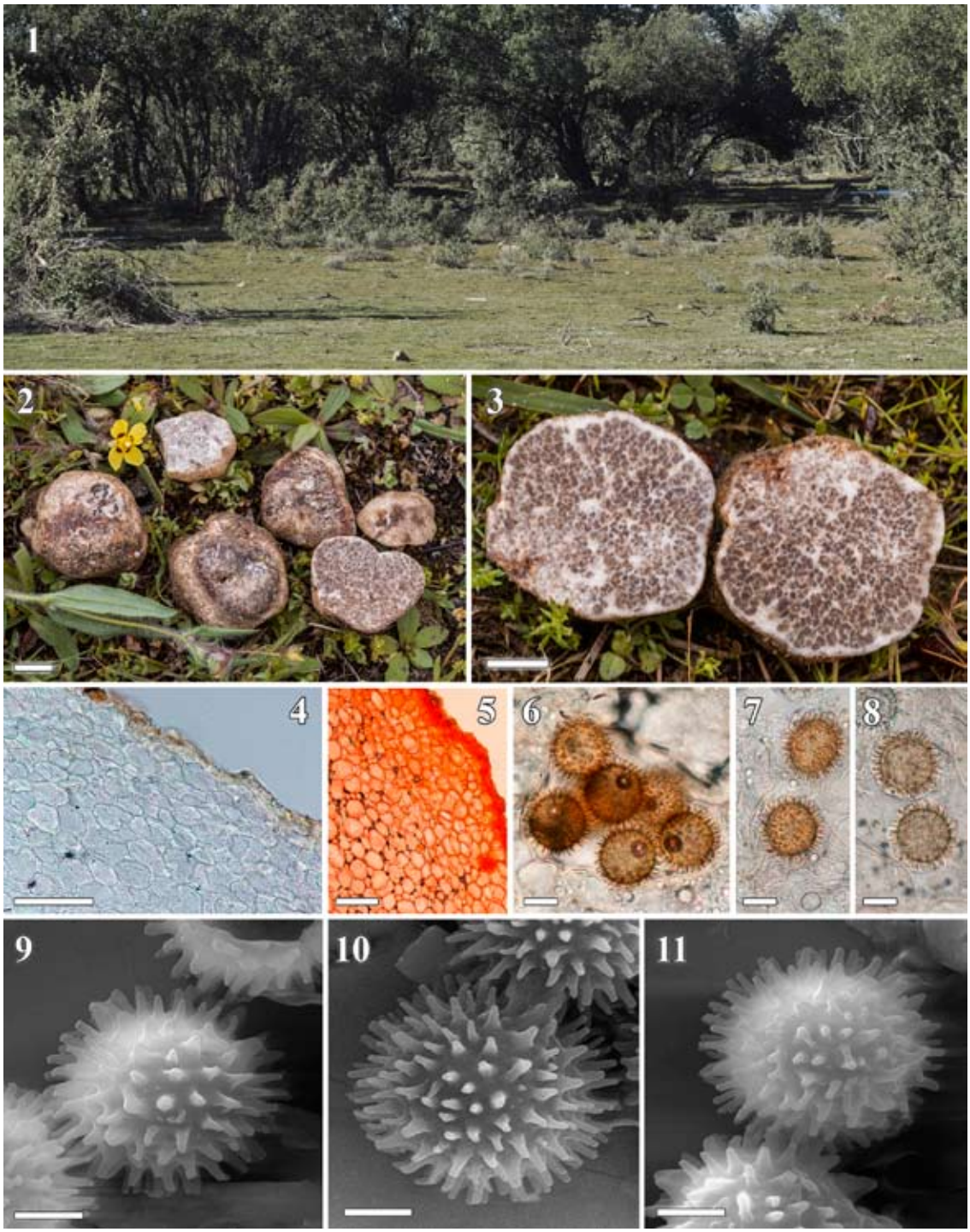
Material examined: *Holotypus.* SPAIN, Segovia, Revenga, under *Tuberaria guttata*, 20 April 2016, *M. Jeppson, E. Larsson, F. Esteve-Raventós, M. Heykoop, A. Sánchez & G. Moreno* (holotype AH 45976, ITS, LSU sequences GenBank, MN512332, MN512336, MycoBank MB 832758).

Additional specimens examined. *Terfezia honrubiae*: SPAIN, Salamanca, Guijuelo, under *Tuberaria guttata*, 26 April 1996, *G. Moreno, M. Lizárraga, E. Pérez-Silva & T. Herrera*, paratype AH 19619, (ITS sequence GenBank, MN512331). Córdoba, Cardeña, 31 April 2001, *Soc. Micol. Madrid*, paratype AH 46030, (ITS, sequence GenBank, MN512333). Ávila, Medinilla, granitic sandy acid soil under *Tuberaria guttata*, 1 May 2016, *Soc. Micol. Madrid*, paratype AH 46458 (ITS, LSU sequences GenBank, MN512334, MN512337).

Etymology: Named after Mario Honrubia, Spanish mycologist and specialist in hypogeous fungi and mycorrhizae, who passed away in June 2015.

Diagnosis: *Terfezia honrubiae* is morphologically characterized by its brown gleba at maturity, peridium pseudoparenchymatous, spiny spores with well-marked spines, by fruiting isolated to gregarious on acid soil under *Tuberaria guttata*, and its ITS nrDNA sequence from all other ITS sequenced *Terfezia* species.

Ascomata 0.8–5.5 cm broad, hypogeous to semihypogeous in maturity, tuberiform, globose to subglobose, sometimes broadly lobed, pale creamy to slightly brownish, but with dark brown tones on the epigeal surface; surface dry, smooth, the epicutis cracking in age with brown tonalities in the cracks on the upper part. Peridium pseudoparenchymatous, consisting of globoses to subgloboses cells, hyalines, up to 65 µm diam., 1–2 µm thick walled and without pigments. Gleba solid, fleshy, whitish to brown at maturity without greenish tones, fertile tissue separated by light whitish sterile veins. Odor and taste not distinctive. Spores 16.7–21.8(–22) × 16.7–21.8(–22) µm, av 19.25 × 19.25 µm (*holotypus*), Qav = 1 (n = 25) in diam. including ornamentation, globose to subglobose, with spines, hyaline to brown-ochraceous at maturity, not amyloid, nor dextri-



Figs. 1–6.- ***Terfezia honrubiae*** G. Moreno, Manjón, A. Morte & P. Alvarado, *holotypus*. AH 45976. Segovia, Revenga, prairie under *Tuberaria gutatta* in *Quercus ilex* forest, where the holotype was collected. 2. Ascomata. 3. Fruit body section. 4–5. Detail of the outermost layer of the peridium with pseudoparenchymatic structure. 6. Spored ascus. 7–8. Spines spores. 9–11. Spores under SEM.
Scale bars = 2–3 cm, 4–5 = 100 µm, 6–8 = 10 µm, 9–11 = 5 µm.

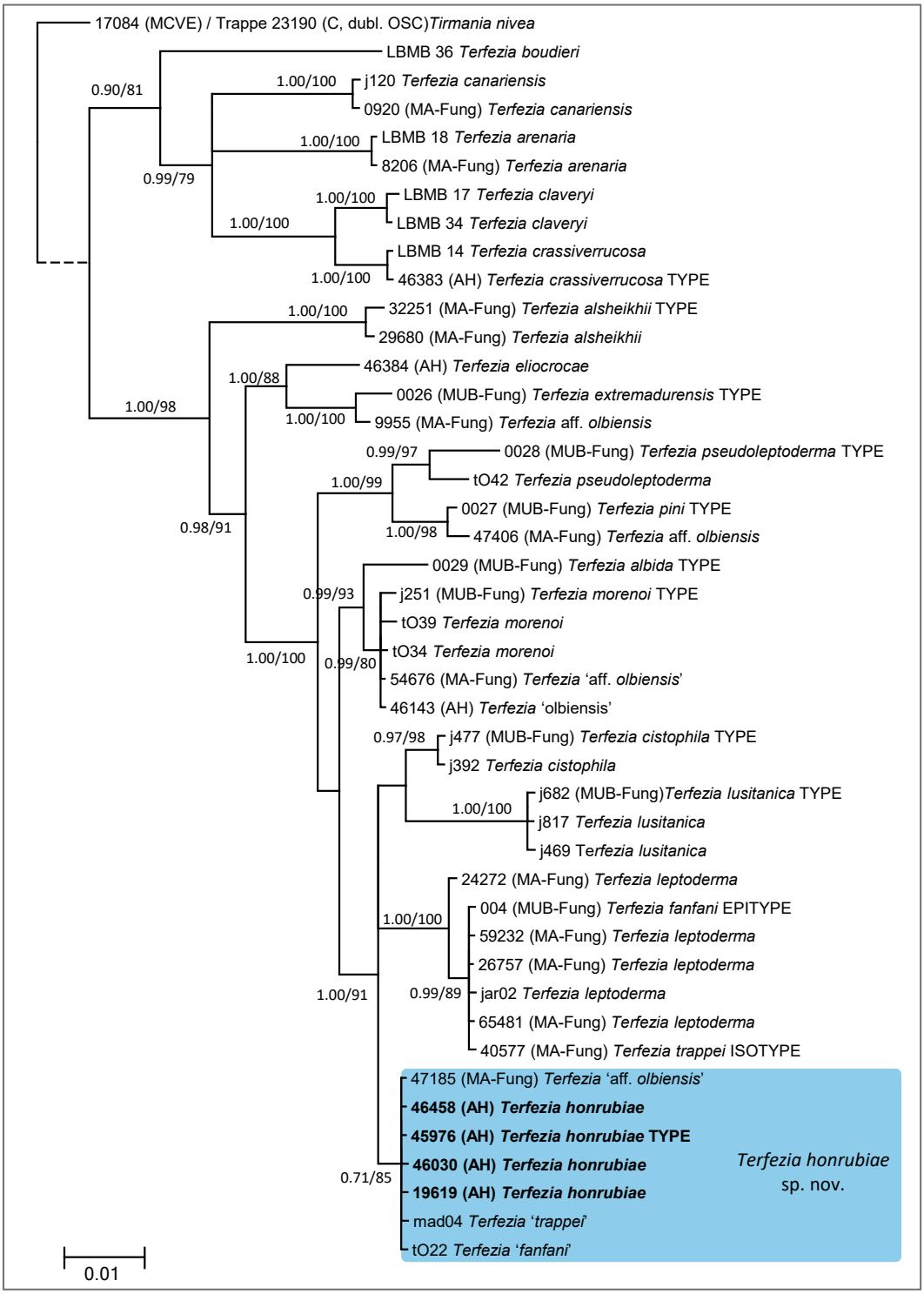


Fig. 12.- 50% majority rule ITS-28S rDNA consensus phylogram of genus *Terfezia* with *Tirmania nivea* as outgroup. It was obtained in MrBayes from 1350 sampled trees. Values next to nodes represent Bayesian PP and Maximum Likelihood BP. Only nodes supported by >0.95 PP or >70% BP were annotated. Rooting branch was reduced for publishing. Bold names represent samples sequenced in the present work.

noid. Spines de 2.5–3(–4) µm. Asci (6–)8 spored, hyaline, ellipsoid to ovoid or at times subglobose, 60–75 × 50–70 µm, nonstalked.

Habitat and distribution ─ Growing solitary to gregarious on sandy acid soil under *Tuberaria guttata*. Abundant in the studied area.

Observations**:** In our combined ITS-28S rDNA phylogeny (MycoBank supplementary data) *Terfezia honrubiae* is significantly related to *T. cistophila* (Bordallo & *al*., 2015), *T. lusitanica* (Bordallo & *al*., 2018), and *T. fanfani* (Vizzini & *al*., 2019).

Other mycorrhizal *Terfezia* species under *Tuberaria guttata* (*Cistaceae*) with spiny spores are:

Terfezia extremadurensis Muñoz-Mohedano, Ant. Rodr. & Bordallo differs from other spiny-spored *Terfezia* species in its *Tuber*-like gleba, with meandering veins of the gleba not completely surrounding the fertile tissue and not forming pockets as is typical for all other *Terfezia* species (BORDALLO & *al*., 2013).

Terfezia fanfanii Mattir. presents larger ascocarp dimensions 2–5(–7) cm diam. and the gleba takes greenish tints at maturity. This species has been confused with *T. leptoderma* (Tul. & C. Tul.) Tul. & C. Tul. (VIZZINI & *al*., 2019).

Terfezia lusitanica Bordallo, Ant. Rodriguez, Louro & Muñoz-Mohedano differs by ochraceous peridium, with blackish gray gleba at maturity and its ITS nrDNA sequence from all other ITS sequenced *Terfezia* species (BORDALLO & *al*., 2018).

Terfezia trappei (R. Galán & G. Moreno) A. Paz & Lavoise must be considered a *T. fanfanii* synonym (RODRÍGUEZ & *al*., 2019).

Other species de *Terfezia* con spiny spores not associated with *Tuberaria* spp. are:

Terfezia pini Bordallo, Ant. Rodr. & Muñoz-Mohedano, is diagnosed by its occurrence on burnt areas under pine and oak and lack of association with cistaceous plants and its spore ornamentation comprising long spines joined at the bases to form a pseudo-reticulum (BORDALLO & *al*., 2013).

Terfezia pseudoleptoderma Bordallo, Ant. Rodr. & Muñoz-Mohedano, is associated with cistaceous plants near pine and oak forests, the ascocarps are similar in size to *T. leptoderma*, which differs in the symmetric bases of the spore spicules (BORDALLO & *al*., 2013).

Terfezia albida Ant. Rodr., Muñoz-Mohedano & Bordallo, differs from other spiny-spored *Terfezia* species in its larger average size, white peridial colour, and spermatic odour. It is the only spiny-spored *Terfezia* species associated with *Helianthemum* spp. in alkaline soils (BORDALLO & *al*., 2013).

Terfezia grisea Bordallo, V. Kaounas & Ant. Rodr., se diferencia is a spiny-spored *Terfezia* species characterized by its ochraceous brownish, almost black peridium, blackish gray gleba and growing in alkaline sandy soils associated with *Helianthemum* spp. (BORDALLO & *al*., 2015).

Finally, *Terfezia cistophila* Ant. Rodr., Bordallo, V. Kaounas, & Morte is characterized by its intense blackening of peridium, light ochre gleba, spermatic odour and growing in acid soils associated with *Cistus* spp. (BORDALLO & *al*., 2015).

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