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Review

What is in a name? *Terfezia* classification revisited

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ABSTRACT

Desert truffles (mycorrhizal hypogeous *Ascomycota*) are found in arid and semi-arid areas of the globe and have great ecological and economic importance. *Terfezia* is undoubtedly the most diversified of all desert truffle genera, but its taxonomy is far from resolved. Specifically, the large number of newly described species plus the high intraspecific morphological variability observed within some *Terfezia* lineages as rendered the use of molecular techniques mandatory for specimen's discrimination. But the subsequent increasing amount of sequence data produced also a huge number of undescribed *taxa* that required determination. We compiled and used the public available ITS data on *Terfezia* spp. on the custom-curated UNITE database to reconstruct the genus phylogeny. We found at least 17 distinct lineages within the genus and successfully resolved some of the more pressing taxonomic issues, namely the *T. leptoderma/olbiensis* complex and some misapplied synonymy. Based on this resolved phylogeny, and motivated by the recent new described species, we proposed an identification key to *Terfezia* genus highlighting the importance of morphological and ecological characterization.

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1. Introduction

Desert truffles are hypogeous *Ascomycota* that have evolved in several lineages within the *Pezizaceae*, and are typically found in arid and semi-arid areas throughout the world (Moreno et al., 2014; Morte et al., 2009; Navarro-Ródenas et al., 2011). They represent a key component of the mycological flora around the Mediterranean basin, establishing important mycorrhizal symbioses with diverse host plants, most often members of the Cistaceae (Díez et al., 2002; Kagan-Zur and Roth-Bejerano, 2008). Many of them are endemic and overall play an essential role in soil conservation - preventing erosion and desertification - of Mediterranean shrublands and xerophytic grasslands (Honrubia et al., 1992).

Terfezia Tul. & Tul. is undoubtedly the most diversified of all desert truffle genus (Kovács and Trappe, 2014). The first *Terfezia* species was described by Moris, as *Tuber arenarium* Moris, from Sardinia in 1829. Soon after, Tulasne and Tulasne, described two more *Terfezia* species which they named *Choiromyces olbiensis* and *C. leptodermus*. Only in 1851, the same authors proposed the creation of the genus *Terfezia*. At the time, they included five species within the genus: *T. arenaria*, *T. leptoderma*, *Terfezia olbiensis* - previously described - plus *T. berberidiodora* and *T. oligosperma* (Alsheikh, 1994; Kovács and Trappe, 2014). Meanwhile several other species were described and later, in 1869, summarized in the book "La Truffe" by Chatin. The first identification keys for the African, Asian, European and North American species were provided by Fischer, Bataille, Mattirolo and Gilkey but it was Alsheikh who, in 1994, first monographed the genus worldwide (Alsheikh, 1994).

Despite the aforementioned contributions, the nomenclatural and taxonomic history of the genus is filled with several old species names, many of them synonyms of earlier described species (Alsheikh, 1994), some lacking useful diagnostic features and most of them rarely cited after the first time (Zitouni-Haouar et al., 2018). The situation lingered in the pre-molecular era because the criteria for separating and/or identifying groups of species were limited largely to morphological, anatomic and chemical features (Bordallo and Rodríguez, 2014) which were not always unambiguous. As pointed out by Díez et al. (2002), the use of this type of features alone for classifying desert truffles is challenging, due to the reduced set of morphological characters and their homoplasy. The observed morphological convergence is likely to be environmental conditioned, but the possibility that in some cases speciation has occurred with hardly detectable morphological changes should also be acknowledged (Bordallo and Rodríguez, 2014; Díez et al.,

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2002). Intra-specific plasticity of phenotypes also contributes to the challenge of using some of these morphological characters for taxonomic purposes, and one has to consider that this ability of a genotype to produce different phenotypes might also be induced by the plant host or by other interacting microorganisms.

In the dawn of the molecular era, early phylogenies revealed various misidentifications at the genus and species level. Also, re-examination of some herbarium specimens and personal collections around the world, using molecular methods, exposed their inaccurate generic assignments and removed the ambiguity around their taxonomic status (Zitouni-Haouar et al., 2018). These early molecular approaches decreased considerably *Terfezia* richness and increased the geographic uniformity of the genus to encompass only those species regularly collected, at the time, from the Mediterranean region and the Middle East: *T. arenaria* (Moris) Trappe, *Terfezia boudieri* Chatin, *T. claveryi* Chatin, *T. leptoderma* Tul. and *T. olbiensis* Tul. & C. Tul (Kovács and Trappe, 2014). Later, molecular studies also revealed the intraspecific diversity of some *Terfezia* species and the existence of diverse species complex (including cryptic species) (Aviram et al., 2004; Bordallo et al., 2013; Díez et al., 2002; Ferdman et al., 2009; Kovács et al., 2011). Specifically, a high intraspecific and/or intrasporocarpic rDNA internal transcribed spacer (ITS) variability was detected among collections of *T. leptoderma* (for some authors synonym of *T. fanfani*) and *T. olbiensis*, revealing at least four well supported lineages of *Terfezia* with spiny spores in addition to *T. leptoderma* (TLO-1a) (Kovács et al., 2011). Also, considerable genetic variation was reported in *T. boudieri* (Aviram et al., 2004; Ferdman et al., 2009; Sbissi et al., 2011), and *T. claveryi* (Sbissi et al., 2011). In relation to the synonymy between *T. leptoderma* and *T. fanfani* considered as separated species in the pre-molecular era, recent phylogenetic studies do not show a clear distinction between the sequences assigned to each of those species names. Instead, most sequences previously identified either as *T. leptoderma* or *T. fanfani* are phylogenetically always nesting together in a well-supported monophyletic group - see for instance in Bordallo et al. (2013) and Bordallo et al. (2015) - or placed together with other spiny spored *Terfezia* (e.g. *T. leptoderma/T. fanfani/T. cistophila)* - see the work of Zitouni-Haouar et al. (2018). A similar problem is poised between *T. olbiensis* and *T. lepdoderma*, since the former was considered by many authors as synonym or an immature form of *T. leptoderma* based on their morphological similarities (Díez et al., 2002). However, newly molecular and morphological studies seem nowadays to support that *T. olbiensis* is in fact a distinct and valid species (Bordallo et al., 2013). Recently, 12 new *Terfezia* species were described from the Iberian Peninsula, Canary Island, Greece and Algeria: (1) *T. alsheikhii* Kovács, M.P. Martín & Calonge (Kovács et al., 2011); (2) *Terfezia canariensis* Bordallo & Ant. Rodr (Bordallo et al., 2012); (3) *T. albida* Ant. Rodr., Mohedano & Bordallo; (4) *T. eliocrocae* Bordallo, A. Morte & Honrubia; (5) *T. extremadurensis* Mohedano, Ant. Rodr. & Bordallo; (6) *Terfezia pini* Bordallo, Ant. Rodr. & Mohedano; (7) *T. pseudoleptoderma* Bordallo, Ant. Rodr. & Mohedano (Bordallo et al., 2013); (8) *T. grisea* Bordallo, Kaounas & Ant. Rodr.; (9) *T. cistophila* Ant. Rodr., Bordallo, Kaounas & A. Morte (Bordallo et al., 2015); (10) *T. trappei* (R. Galán & G. Moreno) A. Paz & Lavoise (Paz et al., 2017); (11) *T.* crassiverrucosa Zitouni-Haouar, G. Moreno, Manjón, Fortas, & Carlavilla (Zitouni-Haouar et al., 2018); and (12) *Terfezia lusitanica* Bordallo, Ant. Rodr., Louro, Santos-Silva & Mohedano (Bordallo et al., 2018). This granted *Terfezia* the title of the most speciated desert truffle genus, totalling 17 species.

What is in a name? Accurate *Terfezia* species determination is important for our understanding of the ecological functioning of the system (e.g. essential role in soil conservation), and is crucial if we consider their economic significance for the rural populations on the Mediterranean basin. Desert truffles fruit bodies are a potentially important food source for animals and humans, rich in proteins and poor in carbohydrates and lipids (Chevalier, 2014; Kovács et al., 2011). Plus, given the considerable prices they may reach in local markets, their cultivation has the potential to enhance the socio-economic development of rural and/or local populations around the Mediterranean basin.

Aiming the establishment of a consensual *Terfezia* classification, we revised the public available data on this genus and constructed an identification key to the known *Terfezia* species. Based on data deposited at the custom-curated UNITE database (https://unite.ut.ee/), we have reconstructed the genus phylogeny and we confronted the results with putative plant host and soil parameters associated with the different specimens, whenever available. We discuss the results integrating them with meaningful morphologic and ecologic characters towards a simple to use identification key of the several *Terfezia* species.

2. Methods

2.1. Data collection

Sequence data was obtained from the Unified system for the DNA based fungal species linked to the classification (UNITE, https://unite.ut.ee/). UNITE is the product of a consortium of fungal ecologists, taxonomists, and bioinformaticians. The custom-curated UNITE database includes many sequences from specimens that were collected and deposited by taxonomic specialists. A total of 220 *Terfezia* spp. genomic DNA sequences - containing a full (or partial) region comprising 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene - were retrieved [by February 2018, considering only sequences of 500 bp or more, plus the available sequences of the recently described species - *T. crassiverrucosa* (Zitouni-Haouar et al., 2018) and *T. lusitanica* (Bordallo et al., 2018)] together with information on ectomycorrhizal lineage, UNITE taxon name, geographic location, putative host, source of DNA. Whenever specimen's ecological and geographical information was missing, the European Nucleotide Archive was consulted for additional information (Supplemental Table 1).

2.2. Phylogenetic analysis

Three known non-*Terfezia* sequences were added to the dataset as putative outgroups (*Tirmania* JF908769.1, *Cazia* AY830852.1 and *Peziza* JX414200.1). Sequences were aligned with online MAFFT version 7 using the E-INS-i strategy (Katoh et al., 2017). The phylogenetic reconstruction analysis based on the above ITS sequences was performed in BEAST v.4.2.8 software (Drummond and Rambaut, 2007), allowing the software to estimate the evolutionary model. All other settings were left as default. The output of BEAST was analysed in the software Tracer v.1.6 to determine chain convergence and burnin. The single tree that best represents the posterior distribution was summarized using the program TreeAnnotator v.2.4.8, considering a burn-in of 10 % (first 1000 trees were removed).

First we tested the hypothesis that all the database retrieved samples belong to *Terfezia* genus. This implied a comparison between a first analysis of the full data set with no priors and one with all the samples putatively belonging to *Terfezia* species constraint to monophyly. Bayes factors were used to test if the topological constraints were significantly different than the optimal topology, and were measured using twice the difference of −ln likelihood (2lnBF) with 2lnBF = 0–2 meaning not worth a mention, 2lnBF = 2–6 meaning positive support, 2lnBF = 6–10 meaning strong support, and 2lnBF > 10 meaning decisive support (Grummer et al., 2014). The non-*Terfezia* samples were removed.

With the final dataset of 202 *Terfezia* samples (after removal of non-*Terfezia* sequences) the same approach was used to estimate *Terfezia* phylogeny. Additionally, and for comparison purposes, phylogenetic relationships were also estimated using two methods: approximate maximum-likelihood (ML) and Neighbour-joining (NJ) using the software MEGA7 (Kumar et al., 2016). Branch support in the ML and NJ trees was tested by means of 1000 bootstrap replicates. This data is shown as supplementary material (Supplemental Fig. S1 and S2).

3. Results

The reconstructed optimal phylogeny of all our dataset identified 18 sequences falling outside the clade of the *Terfezia* genus (Fig. 1). This model is significantly better ($2 \cdot \ln BF = 287.85$; with BI ln [optimal model] = −6856.27 and BI ln [alternative model] = −7000.19) than when monophyly of all putative *Terfezia* samples is constrained. The final alignment consists of 783 bp including gaps, of which 382 have full coverage by all 202 *Terfezia* sequences. Considering the complete alignment, 314 positions are variable of which 236 are parsimonious informative. Given the available *Terfezia* sequences, the reconstructed phylogeny ample supports the existence of 17 distinct clades representing well supported monophyletic groups (Fig. 2). Only one sequence (GenBank accession no. AF396864), did not cluster in any of the 17 identified clades.

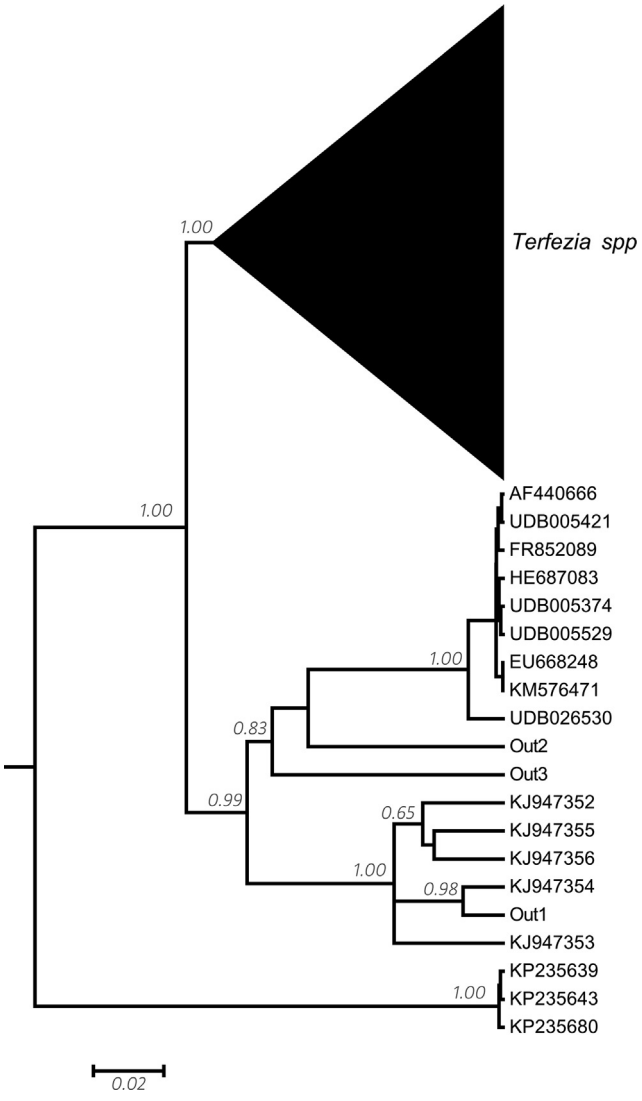

Fig. 1. Phylogenetic relationship between putative *Terfezia* species retrieved from the databases (see methods). The phylogeny corresponds to the majority rule consensus tree of trees sampled in a Bayesian analysis, and the posterior probability values are shown for main nodes. Three known non-*Terfezia* sequences were added to the dataset as putative outgroups (Out1: *Tirmania* JF908769; Out2: *Cazia* AY830852 and Out3: *Peziza* JX414200). Qatar samples (KJ947352; KJ947353; KJ947354; KJ947355; KJ947356); Soil isolates unspecified geographical region (KP235639; KP235643; KP235680); Australia (DQ061109); all other sequences are from several different sources.

In line with our reassembled phylogenetic tree, all sequences previously determined by UNITE database as *T. alsheikhii*, and only these, clustered together producing a strongly supported homogenous group. The same was also true for the following clades: *T. arenaria*, *T. boudieri*, *T. claveryi*, *T. eliocrocae* and *T. grisea*. These species seem robust in terms of identification, as all sequence-s identified by UNITE database are correct and no other available sequences (e.g. *Terfezia* sp.) match the aforementioned species.

As to Clade A, we verified that it was fundamentally composed by numerous sequences identified at UNITE database only to genus level, namely, 1 isolate retrieved from a mycorrhizal root tip and 34 sequences formerly classified by Kovács et al. (2011) as *Terfezia* aff. *olbiensis* belonging to the TLO-3 group. However, the only 2 confirmed *T. pini* sequences also nested within this clade. From the above, it seems fairly likely that the well supported Clade A, might indeed represent the species *T. pini*. This claim seems to be further corroborated by the ecology of the specimens within the group, which as far as we could retrieve the information, share the same putative plant hosts (*Pinus* spp. and *Quercus* spp.) (see Supplemental Table 1).

The clade named *T. albida* (Fig. 2) included the only 2 samples formerly determined as such, plus 2 more sequences identified as *T. olbiensis* in the UNITE database. These later sequences had been suggested to represent *Terfezia* aff. *olbiensis* by Kovács et al. (2011), which at the time included them in their proposed group TLO-2. The remaining *T. olbiensis* recognized in the UNITE database (10 sequences) clustered together in our phylogenetic analysis forming the well supported clade named *T. olbiensis*. Thus, our results corroborate the notion that *T. olbiensis* represents a true valid species.

With respect to the *T. extremadurensis* clade, besides 6 sequences previously identified as such, 7 other sequences nested within the clade: one sequence previously named *T. leptoderma* and six labelled *Terfezia* sp., these latter having previously been considered as *Terfezia* aff. *olbiensis* (TLO-4) (Kovács et al., 2011).

As for the clade *T. cistophila*, the majority of the sequences (9) were correctly assigned to *T. cistophila* by UNITE. Still, 2 more sequences named *Terfezia* sp. also nested inside this clade, one being an isolate from a mycorrhizal root tip and another previously identified by Kovács et al. (2011) as *Terfezia* aff. *olbiensis* (TLO-1). The clade *T. pseudoleptoderma* clustered together 3 sequences previously identified as such, plus 1 sequence coming from a strain isolated from a mycorrhizal root tip, which was, to date, determined only to genus level (see Supplemental Fig. S1 and Fig. S2).

The clade *T. fanfani* grouped 5 sequences identified as such, and 12 *T. leptoderma* sequences. These latter were identified as belonging to TLO-1a and named as *T. leptoderma* by Kovács et al. (2011). In light of this result, our analysis supports the viewpoint advocated by Venturella et al. (2004) and Chevalier (2014) that the two names are in fact synonyms, denominating specimens belonging to a same species; therefore, the correct name should be assigned according to the rules of the International Code of Nomenclature for algae, fungi, and plants. To the only sequence identified in UNITE as *T. trappei*, our analyses suggest the inclusion of 4 more sequences, one previously identified as *T. fanfani* and

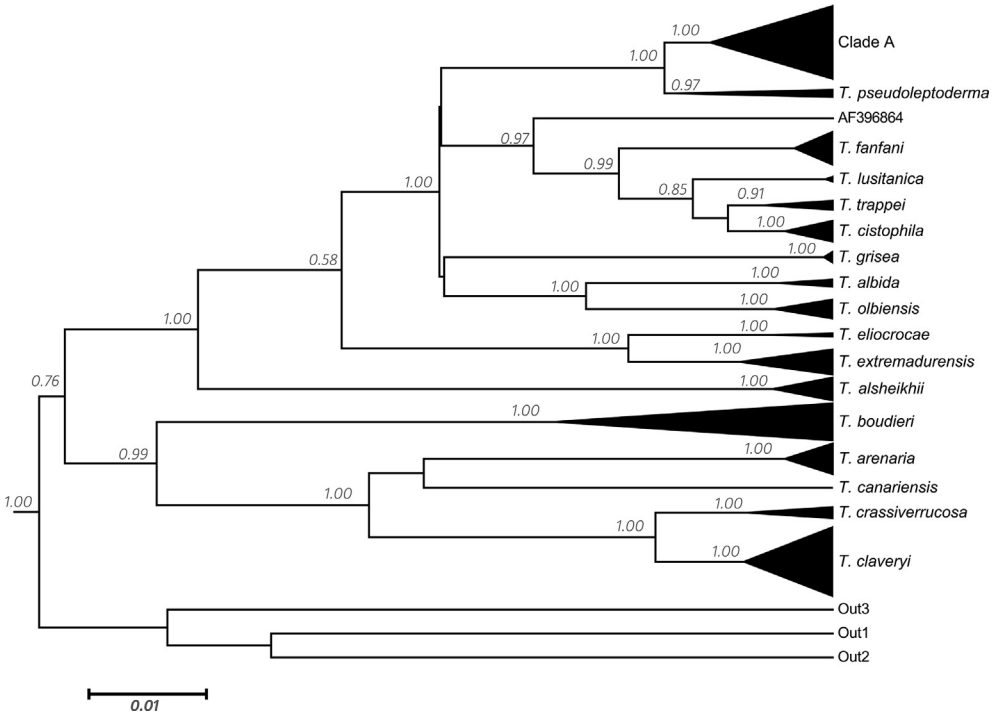

Fig. 2. Phylogenetic relationship between *Terfezia* species. The phylogeny corresponds to the majority rule consensus tree of trees sampled in a Bayesian analysis, and the posterior probability values are shown for main nodes. (ML and NJ analyses presented in Supplemental Fig. S1 and Fig. S2 respectively).

three as *Terfezia* sp., reported by Kovács et al. (2011) as *Terfezia* aff. *olbiensis* (TLO-1). Yet, the name *Terfezia trappei* was only considered taxonomically valid in 2017, after the publication of Paz and co-workers (Paz et al., 2017) which, in their monograph of the genus *Elaphomyces* in Europe, suggest that the isotype of *Elaphomyces trappei*, described by Galán and Moreno (1991), is indeed *T. trappei*.

T. crassiverrucosa well supported clade comprises the sequence from the holotype and 5 more sequences previously identified as *Terfezia* sp. in the UNITE database. *T. crassiverrucosa* is one of the most recently described *Terfezia* species and is a striking example of a cryptic species hidden under the name *T. claveryi* throughout the pre-molecular era: their morphologic similarity and sharing of known ecology, made them non-distinguishable. Finally, *T. lusitanica*, a recently described *Terfezia* of which only the recently published sequences are known (Bordallo et al., 2018), also formed a distinct well-supported clade with none of the remaining *Terfezia* sequences analysed being included.

4. Discussion

Traditional *Terfezia* classification has largely relied on morphological, chemical and organoleptic characters, and later, also on host plant and soil features. However, the large number of newly described species (Bordallo et al., 2012, 2013; 2015, 2018; Kovács et al., 2011; Zitouni-Haouar et al., 2018), plus the high intraspecific morphological variability observed within some *Terfezia* lineages (Aviram et al., 2004; Bordallo et al., 2013; Díez et al., 2002; Ferdman et al., 2009; Kovács et al., 2011) as rendered the use of molecular techniques mandatory for specimen's discrimination. Subsequently, the increasing amount of sequence data, flowing from fungal molecular ecology studies, using either classic Sanger sequencing or high-throughput sequencing technologies, produced also a huge number of undescribed *taxa* that needed determination. Yet, *Terfezia* species identification has not undergone the needed adjustment and updating towards minimization of data base errors.

Regardless of how the sequences are obtained, *taxa* determination is mainly inferred by homology, which means that the outcome of the inference is never better than the reference(s) itself. The most popular current nucleotide search tool, the National Centre for Biological Information (NCBI) Basic Local Alignment Search Tool, or BLAST, has numerous errors, poor-quality sequences, and many deposited sequences with little or no associated taxonomic nor ecological information (Nilsson et al., 2006). The downstream impact of it goes beyond naming, as it affects evolutionary studies and the biological understanding of an organism and its ecology, pathways analyses, systems, and metabolic processes as well (Klimke et al., 2011). Researcher's awareness on the shortcomings of the databases references can certainly minimize error propagation but one needs to keep in mind that this is a dynamic process that needs to be revised and updated in frame of the continuous new flow of data (Nobre et al., 2016).

Through our reconstructed phylogenetic analysis, based exclusively on the custom-curated UNITE database, which includes many sequences from specimens that were collected and deposited by taxonomic specialists, we have reconstructed the genus phylogeny and were able to assign a name to almost all *Terfezia* sequences therein, that were identified only to genus level (52 sequences) or that were misinterpreted (17 sequences).

A relevant topic that also required resolution is *T. trappei*, which was described for the first time as *E. trappei*, in a period where molecular biology was not the widely disseminated taxonomical tool it is today, but that recently has undergone a reclassification as *T. trappei*, comb. nov. by Paz et al. (2017). The former authors also suggested that *T. cistophila* was a later synonym of *T. trappei* (Paz et al., 2017). However, we strongly disagree with the applied synonymy and propose that these are two independent *taxa*. To support this claim we highlight that despite both species share the same ecology (acid soils and *Cistus* spp. as putative plant host) and some morphological similarities, specifically in sporocarp size and colour, there are also marked differences between them, namely, in

the *peridium* thickness (thicker in *T. trappei*), in spore dimension and ornamentation size (bigger in *T. trappei*). Our phylogenetic analysis further corroborates the existence of two distinct well-supported clades for *T. trappei* and for *T. cistophila*.

Another pressing issue that needs to be addressed is that little or no associated taxonomic, geographic and ecological information is available for many of the deposited sequences in most popular nucleotide databases. And even when that information exists, there is always the possibility of being incorrect. This seems to be the case of *T. cistophila* sequence (GenBank accession no. FJ013087) referred as associated with *Pinus pinaster* Aiton (see Supplemental Table 1); in our reconstructed phylogenetic tree it nests inside the clade of *T. cistophila*. If plant host is really an important feature in *Terfezia* specimen's determination (Díez et al., 2002), as we believe it is, then the information regarding the putative plant host must be given with care. As far as we know, *Terfezia cistophila* lives exclusively with *Cistus* spp (Bordallo et al., 2015).

Still on misidentifications, two sequences (GenBank accession no. HQ698145 and HQ698146) previously identified as *T. olbiensis* in the UNITE, in our analysis clustered inside *T. albida* clade. Given these specimens ecology, found associated with *Helianthemum* (see Supplemental Table 1), it is fairly clear that they are not *T. olbiensis*, which by all accounts lives in association with *Pinus* spp. and *Quercus* spp (Bordallo et al., 2013).

T. pini has only two UNITE identified sequences which clustered inside Clade A (Fig. 2) of our reconstructed phylogeny. Considering that most other sequences in this well supported clade are identified only to the genus level (all the *Terfezia* aff. *olbiensis* belonging to TLO-3) and given that the majority of them seem to be associated with *Pinus* and *Quercus*, it is most likely that this clade represents *T. pini*.

One of the most discussed issues over *Terfezia* classification, and one which persist to date despite the efforts to resolve it, is the taxonomic placement of *T. leptoderma* and *T. olbiensis*. While some authors tend to agree that *T. olbiensis* represent an immature form and a synonym of *T. leptoderma* (Bordallo et al., 2013; Díez et al., 2002; Moreno et al., 1986), others have chosen to consider these two as separated species (Montecchi and Sarasini, 2000) and more recently Kovács et al. (2011) proposed it to represent a species complex hiding several lineages within (TLO-1, TLO-2, TLO-3 and TLO-4). We believe that our reconstructed phylogeny has for the first time successfully disentangled this species complex and allowed the assignment of a species name linked to each former TLO designations. Accordingly, TLO-3 sequences belong to *T. pini*, TLO-4 to *T. extremadurensis*, TLO-2 mainly to *T. olbiensis* and 2 sequences to the newly described *T. albida*, a very close species to *T. olbiensis*. And finally, TLO-1 mainly *T. fanfani* (former *T. leptoderma*), *T. cistophila*, *T. trappei*, *T. lusitanica* and seq. AF396864 (which remains a puzzle). Regarding the only sequence which did not clustered in any of the above described clades, Díez et al. (2002) determined that the specimen which originated the sequence was morphologically similar to *T. leptoderma* - though they also noticed that it had slightly smaller spores with shorter spines - and it was associated with *Pinus halepensis* in acid soils. The combination of all the above mentioned features and taxonomic placement of this sequence in our phylogenetic reconstruction makes us hypothesize the possibility that this might indeed be a new *taxon*. Nevertheless, more specimens and sequences are still needed in order to confirm this hypothesis.

Direct sampling of sequences from environment or even from fungal tissue, with no further characterization, does facilitates large-scale mechanical production of *Terfezia taxa* names, based on minor sequence divergence, without taking in account the direct observation and characterization of individual organisms. Although molecular techniques are valuable tools to discriminate species, they should be always complemented with specimen's morphological and ecological description. Despite the information available on different websites, to the best of our knowledge, no complete identification key for *Terfezia* genus was produced so far. In this context, we propose a dichotomous identification key to aid in the identification of mature *Terfezia* specimens, based on morphological and ecological characters.

Key to *Terfezia* species

(Ascospore measurements do not include ornamentations)

1 Spiny spores 2
Warty or warty reticulated spores 11

2(1) In alkaline soils 3
In acid soils 5

3(2) With *Cistaceae*; spores ≥14 µm with blunt and/or truncated spines 4
With *Pinus* and/or *Quercus*; spores <14 µm with pointed spines ..... ***olbiensis***

4(3) Peridium light colour; gleba with green colours… ..... ***albida***
Peridium dark colour; gleba without green colours…… ..... ***grisea***

5(2) Ascocarp diameter >4.5 cm 6
Ascocarp diameter <4.5 cm 7

6(5) Ascocarp brown-reddish; spores with straight pointed spines ..... ***fanfani***
Ascocarp brown; spores with conical (≈3-4 µm at base) blunt and truncated spines ..... ***extremadurensis***

7(5) Gleba without green colours; with *Cistaceae* 8
Gleba with green colours; with *Cistaceae*, *Pinus* or *Quercus* 9

8(7) Spores ≥15 µm with flexuous blunt spines; with *Cistaceae* ..... ***pseudoleptoderma***
Spores <15 µm with straight spines; with *Cistus* exclusively ..... ***cistophila***

9(7) Peridium <1 mm 10
Peridium ≈1 mm ..... ***trappei***

10(9) With *Pinus* and/or *Quercus*; spores with spines >4 µm long ..... ***pini***
With *Tuberaria guttata*; spores with spines <4 µm long ..... ***lusitanica***

11(1) In alkaline 12
In acid soils 16

12(11) Spores with warts making a complete and clear reticulum 13
Spores with warts sometimes forming an incomplete reticulum 14

13(12) Gleba strong pink; spores ≥18 µm ..... ***canariensis***
Gleba whitish; spores <18 µm ..... ***eliocrocae***

14(12) Spores <22 µm 15
Spores ≥22 µm ..... ***boudieri***

15(14) Asci with 6-8 spores; spores with spines >1.5 µm long ..... ***claveryi***
Asci with 4-6 spores; spores with spines <1.5 µm long ..... ***crassiverrucosa***

16(11) Ascocarps >2 cm; spores warty without reticulum ..... ***arenaria***
Ascocarps <2 cm; spores warty with a complete reticulum ..... ***alsheikhii***

5. Additional note

The increasing amount of sequence data, and the time lapse between manuscript preparation, submission and acceptance (and sometimes even later availability of the data) makes this type of work never complete. Already in the final stages of manuscript publication, we were aware of a new *Terfezia* species description (Crous et al., 2018) - *Terfezia morenoi*. We have re-run the main analysis (Supplemental file 1) and the newly described *T. morenoi* nests inside the monophyletic group by us designated (based on UNITE curated taxonomy and the majority rule) *T. olbiensis*. This implies that, the inclusion of these sequences does not alter the phylogenetic relationships observed and that all well supported clades remain unchanged. The morphological description of *T. morenoi* coincides on the diagnostic characters with the one of *T. olbiensis* Tul. & C. Tul., G. Bot. et al. (1845) as transcribed in Bordallo et al. (2013) (Supplemental file 1). Hence the above identification key to *Terfezia* genus remains valid. The name of the clade in question (in our manuscript, as *T. olbiensis* species) should be assigned - as stated before for the *T. leptoderma/T. fanfani* issue - according to the rules of the International Code of Nomenclature for algae, fungi, and plants.

6. Conclusions

The present analysis on the *Terfezia* ITS sequences accessible through the custom-curated UNITE database revealed 17 well supported independent lineages within the genus. Overall, the ITS region performed well in discriminating almost all analysed sequences, with the exception of seq. (GenBank accession no. AF396864), which we hypothesize that may represent an undescribed *taxa* given its unique set of morphological characters and its placement in our phylogeny. Further sampling is necessary to test this hypothesis. Our results show beyond doubt that the applied synonymy between *T. trappei* and *T. cistophila* is incorrect and we propose that they should be considered as two independent *taxa*. Regarding the sizzling debate around some lineages of *Terfezia* with spiny spores, our results highlighted several lineages hidden within the *T. leptoderma*/*olbiensis* complex (first proposed by Kovács et al., 2011). Furthermore, our reconstructed phylogeny allowed the assignment of a species name linked to each former TLO designations (TLO-3 sequences are *T. pini*; TLO-4 are *T. extremadurensis*; TLO-2 are mainly *T. olbiensis* and 2 sequences are the newly described *T. albida*; TLO-1 comprises species of *T. fanfani*, *T. cistophila*, *T. trappei*, *T. lusitanica* and seq. AF396864). The next step is to search the remaining sequences deposited in databases as *Terfezia* and access the extent of misidentifications and whenever possible, confirm or assign a species name based on the established taxonomy.

Conflicts of interest

The authors declare they have no conflict of interest in this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2019.01.003.

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