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ORIGINAL ARTICLE

Characterization of natural habitats and diversity of Libyan desert truffles

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Abstract Desert truffles have traditionally been used as food in Libya. Desert truffle grows and gives fruit sporadically when adequate and properly distributed rainfall occurs with existence of suitable soil and mycorrhizal host plant. The present study aimed to identify and characterize two kinds of wild desert truffles from ecological and nutritional points that were collected from the studied area. The truffle samples were identified as *Terfezia* (known as red or black truffle) and *Tirmania* (known as white truffle). The nutritional values (protein, lipid and carbohydrate) of both Libyan wild truffle (*Terfezia* and *Tirmania)* were determined on a dry weight basis and result showed that *Tirmania* and *Terfezia* contained 16.3 and 18.5% protein, 6.2 and 5.9% lipid, 67.2 and 65% carbohydrate, respectively, in ascocarp biomass. The soil pH of the upper and lower regions of the Hamada Al-Hamra ranged between 8.2 and 8.5 giving suitable conditions for fructification. The plants, *Helianthemum kahiricum* and *Helianthemum lippii* were the dominant plants in Hamada Al-Hamra region found to form a mycorrhiza with desert truffles. The phylogenetic analysis of the genomic rDNA ITS region showed that, out of five collections three represented *Tirmania pinoyi* (Maire) Malencon, one *Tirmania nivea* (Desf.) Trappe, and one *Terfezia boudieri* Chatin.

Keywords Libya · Desert truffle · *Terfezia* · *Tirmania* · *Helianthemum* · Phylogeny

Introduction

Desert truffles from genera *Terfezia* and *Tirmania* (hypogeous ascomycetes) grow naturally in North Africa, Middle East, North America (Bokhary and Parvez 1993; Trappe

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et al. 2007) and in substantial areas of Mediterranean South Europe (Montecchi and Sarasini 2000), while other genera belonging to ecological term "desert truffles" were recorded also in Africa (e.g., *Kalaharituber*, *Eremiomyces* and *Mattirolomyces*) (Trappe et al. 2008a) and Australia (*Elderia*, *Horakiella*, *Mycoclelandia*, *Reddellomyces*, *Ulurua* and *Mattirolomyces*) (Trappe et al. 2008b). The members of mycorrhizal fungus genera *Terfezia* and *Tirmania* are mostly endemic to arid and semi-arid areas. The phylogenetic analysis of ITS/5.8S rDNA sequences revealed hypogeous representatives of the genera as monophyletic groups with morphological species well corresponding to the obtained phylogenetic species (Dìez et al. 2002) while the LSU phylogeny indicate the scattered position of *Terfezia*, *Tirmania* and other hypogeous "desert truffles" among several clades in Pezizaceae (Læssøe and Hansen 2007). The species from *Terfezia* and *Tirmania* are mycorrhizal with members of the Cistaceae family, mainly from the genus *Helianthemum* (Fortas and Chevalier 1992) and may form either or both types of associations, ectomycorrhiza [mainly under in vitro conditions (Gutiérrez et al. 2003)] or endomycorrhiza in natural conditions (Kagan-Zur et al. 1999). A new *Terfezia* species were identified and tested in the mycological collection of the Herbarium of Real Jardin Botanico, Madrid (Gabor et al. 2011).

Ascomycetes from genera *Terfezia* and *Tirmania* grow naturally in many parts of Libya, especially in Hamada Al-Hamra region where truffles are highly appreciated as delicious food. Locally truffles are known as Terfasa and Al-Kamaa or Al-Faga in Qatar and Kuwait. Truffle fruiting depends on several parameters such as soil type and properties, climate conditions, timing and quantity of the rainy season (Bokhary and Parvez 1988). The development of desert truffle is linked to amount of rainfall during the winter and fall (Bradai et al. 2014). In Spain (Murcia region with rainfall of between 350 and 400 mm), the estimated production of desert truffle varied between 50 and 170 kg/ha in natural areas (Honrubia et al. 2003). An irrigation system in the truffle plantation is not necessary when the rainfall is available because the mycorrhizal association is well adapted to arid and semi-arid climates (Morte et al. 2001).

The purpose of this study was to explore knowledge about the wild growing Libyan truffles (desert truffles from genera *Terfezia* and *Tirmania*), their occurrence and identity in the Hamada Al-Hamra region. Data on total production and consumption of Libyan truffles are not available since several species, including *Terfezia boudieri* (Chatin, La Truffe: 72, 1892), are sold on local markets in Libya without quantity and quality control. They grow wild and are sold on day-to-day basis by truffle collectors without control over the market. The prices recently reached 120 Libyan dinars/kg (about 70€/kg), whereas in the 80s the price was less than 10 dinars/kg (about 5€/kg). Gastronomic and nutritional values for species from genera *Terfezia* and *Tirmania* have been proven years ago (Ahmed et al. 1981). Despite the vivid local markets, the molecular tools for the identification of fungus from local origin have not been applied yet. The purposes of the work was to identify five unknown samples (including samples with unripe spores) from genera *Terfezia* and *Tirmania* found growing wildly in (semi) desert areas of Libya, reveal their soil and habitat properties, potential host plant species, and chemical composition (nutrition value/protein content). In addition to the identification of ascocarps, a phylogenetic position of collections in relation to the available sequences, mainly focusing on the genus *Terfezia* and *Tirmania* was done.

Materials and methods

Collection of truffle sample

For the purpose of the study, a representative number of unknown hypogeous specimens presumably from the genus *Tirmania* or *Terfezia* growing in upper (*Tirmania* collections) and lower (*Terfezia* collections) Hamada Al-Hamra regions of Libya were collected. The specimens were washed free of adhering soil and were kept in alcohol until the microscopic and molecular analyses. From the pool of samples, five samples with distinct macroscopic characteristics were selected. The samples (ascocarps) were deposited in Herbarium of Juva Truffle Centre, Finland and parallelly in the Herbarium and Mycotheca at the Slovenian Forestry Institute (ascocarps and extracted DNA).

Determination of nutritional value of Libyan desert truffles

From 1 year sampling, triplicate samples of desert truffle from each collection were used for the determination of nutritional value. The protein, lipid and carbohydrate contents were determined and calculated on a dry weight basis. The measurement of total crude protein share in ascocarps (N × 6.25) was determined by Kjeldahl method (AOAC 2016) and represented as a % of total protein in ascocarps dry biomass. The % of protein in total ascocarps biomass serves as an orientate/guidance nutrition value in supporting the importance of genera in diet and to support their market price. Total carbohydrate content was determined by phenol sulphuric acid method (Nielsen 2009). Lipid/fat content was determined by AOAC (2016) method. Water content (moisture content) was determined

by heating the truffle samples to 75 °C in a vacuum oven (45–50 mm Hg) to a constant weight. Ash content was determined by placing sample into porcelain crucible and place in temperature-controlled furnace preheated at 200 °C and hold at this temperature for 2 h. Further crucible was transfer directly to the desiccator, cool and weighed immediately, and ash content was reported in percent (AOAC 2016).

Microscopic characterization

Specimens with fully developed ascomata and spores were used for the examination of macro, microscopic and morphological characters while unripe specimens were used for molecular identification approach. Microscopic slides of hymenium, including spores were prepared in tap water or lactic acid. Slides made with lactic acid were not used for any further analyses due to the immediate dissolving of oil droplets in spores. Water immerse slides were immediately used for daylight microscopy using Olympus BX51 microscope with attached digital camera and image analysis software. For unripe ascocarps with no mature spores only molecular identification was performed.

Analysis of soil and host plants

Representative volume of top soil layers were collected from locations directly next to ascocarps of *Tirmania* or *Terfezia* (from the lower and upper regions of Hamada Al-Hamra). The detailed soil analysis was carried out by the soil and water group of Biotechnology Research Center, Libya.

All potential mycorrhizal host plants from the vicinity of collected sporocarps were mapped and kept in herbarium for identification. All collected plants were identified at the Botany Department, Faculty of Science, Tripoli University, Libya.

DNA sequence analysis

DNA was extracted from 20 mg of the hymenium from alcohol-stored material using Plant DNeasy Mini Kit (Promega). Extracted DNA was resuspended in pre-warmed, sterile milli-Q water to the approximate final concentration of 100 ng/µl and kept at −80 °C. Primer pair ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) was used for PCR amplification of the ITS region, including 5.8S rDNA. Amplification reactions were performed as described in Kraigher et al. (1995) in a PE 9700 DNA thermocycler, with an annealing temperature of 55 °C. Negative controls, lacking fungal DNA, were run for each experiment to check for any contamination of the reagents. Amplified DNA was separated and analyzed as described in Grebenc et al. (2000). Potential desert truffle host plants were not subjected to molecular identification approach.

Amplified fragments were first separated and purified from the agarose gel using the Wizard SV Gel and PCR Clean-Up System (Promega) or send directly to the selected sequencing service (Macrogen) for sequencing. Sequencher 4.8 (GeneCodes) was used to identify the consensus sequence from the two strands of each isolate. The sequences were submitted to EMBL nucleotide sequence database with the accession numbers indicated in Table 1.

Additional sequences from genera *Terfezia, Tirmania, Cazia, Mattirolomyces, Pachyphloeus, Kalaharituber* and *Peziza*, as variously distinct relatives (Dìez et al. 2002; Læssøe and Hansen 2007) to analyze ascocarps, were obtained from GenBank for phylogenetic analysis of unknown desert truffle samples. Sequences were aligned using l-INS-I algorithm in stand-alone version of MAFFT (Katoh et al. 2005). A simple NJ phylogenetic tree was constructed using Jukes–Cantor substitution model.

A second analysis was done using a Bayesian approach (Larget and Simon 1999). Posterior probabilities were approximated by sampling trees using a Markov chain Monte Carlo (MCMC) method. The posterior probabilities of each branch were calculated by frequency of trees that were visited during the course of the MCMC analysis. The analysis was performed assuming the general time reverse model including estimation of invariant sites and assuming a discrete gamma distribution with six categories (GTG + I + G). No molecular clock was assumed. A run with 2,000,000 generations starting with a random tree and employing four simultaneous chains was executed. Every 100th tree was saved. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ac.uk/software.html?i=tracer) and determined that stationary state was achieved when the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck and Ronquist 2001). The initial 2000 trees were discarded as burn-in before stationary state was reached. Using the "sumt" command of MrBAYES, majority-rule consensus trees were obtained from 15,000 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes. DNA sequence phylogenetic trees were drawn in MEGA 4 and edited in PhotoShop.

Results and discussion

Truffle sample morphology and phylogeny

All truffles collected in this study from the lower and upper Hamada Al-Hamra regions were morphologically identified as *Tirmania* and *Terfezia*. Comparative morphology of

Table 1 List of analyzed samples with basic information, herbarium voucher numbers, identification and GenBank accession number

Herbarium voucher at the Herbarium of Juva Truffle Centre	DNA extracts voucher deposited at the Slovenian Forestry Institute	Collection place	EMBL nucleotide sequence database accession numbers
Tirmania 1-2008/HHU	E09_4_13	Lower region of Hamada Al-Hamra, North West of Libya	FN395012
Tirmania 2-2008/HHU	E09_4_14	Lower region of Hamada Al-Hamra, North West of Libya	FN395013
Tirmania 3-2008/HHU	E09_4_15	Lower region of Hamada Al-Hamra, North West of Libya	FN395014
Tirmania 4-2008/HHU	E09_4_23	Lower region of Hamada Al-Hamra, North West of Libya	FN395015
Terfezia 5-2008/HHL	E09_4_22	Upper region Al-Hamra, North West of Libya	FN395016

collected *Tirmania* and *Terfezia* was represented in Fig. 1. Five representative fresh or alcohol-stored ascocarps were examined for spore characteristics. Samples *Tirmania* 1, 2 and 3-2008/HHU exhibited mature spores (Fig. 2a–c). All three samples with identical subglobose to globose spores, smooth or nearly reticulate, correspond best with spores of *Tirmania pinoyi* (Maire) (Malençon, Persoonia 7(2): 277. 1973). In the sample *Tirmania* 4-2008/HHU, we found only unripe spores (Fig. 2d), whereas no spores were observed in *Terfezia* 5-2008/HHL; thus, the morphological identification was not possible.

DNA sequence analysis

DNA extraction and amplification of genomic ITS region was successful from all five samples. Both, the simple NJ phylogenetic approach tree (Fig. 3) and the phylogenetic tree generated with Bayesian approach with MCMC method (Fig. 4), revealed identical position of unknown samples in terminal clades, well corresponding to morphologic species. Collections *Tirmania* 1, 2 and 3-2008/HHU form a single cluster with *Tirmania pinoyi* (AF276669), which supports the morphological identification. *Tirmania* 4-2008/HH sequence with unripe spores is identical to several sequences of *Tirmania nivea* (Desf.) (Trappe, Trans. Br. mycol. Soc. 57(1): 88. 1971), while the *Terfezia* 5-2008/HHL collection belongs to one of the two obtained phylogenetic clades of *Terfezia boudieri.*

The phylogenetic analysis based on the ITS sequences revealed similar phylogenetic distribution of *Terfezia* and *Tirmania* samples after the simple NJ phylogenetic approach as previously published by Dìez et al. (2002) (Fig. 3). The relative position of hypogeous genera within Pezizaceae also corresponds well with the general Pezizaceae clade in, Læssøe and Hansen (2007). A preliminary intent to include several representatives from the genus Peziza, an epigeous genera showed low resolution of the tree and uneven distribution among clades (data not shown). Hence, any further analyses were omitted and *Peziza badia* was the only included from the genus. The resolution of the phylogenetic tree based on the rDNA ITS sequences was high enough at the phylogenetic species for reliable identification of unknown samples while some parts of phylogenetic tree remain unsolved or does not correspond well to the morphological species as described by authors of available sequences. The information used (ITS) in this case does not give enough information for a reliably phylogenetic study of the family yet it indicates that the position of analyzed genera corresponds well to the distribution of genera based on the rDNA SSU ML tree within the Pezizaceae (Percudani et al. 1999; Kovács et al. 2008) with close relation of *Cazia* and *Terfezia*, while *Mattirolomyces* appears to be less distant using ITS rDNA. Our results also support close relation of the genus *Tirmania* with *Terfezia olbiensis/leptoderma* (Tul., G. bot. ital. 2(1): 60. 1844/Tul., Annls Sci. Nat., Bot., sér. 3 2: 350. 1844) clade observed by Ferdman et al. (2005), with an exception of genus *Cazia*, which was not analyzed in the mentioned study of Ferdman et al. (2005). According to the rDNA, ITS phylogeny (Fig. 3) forms a well-supported clade between *Tirmania* and *Terfezia*. Phylogenetic approach based on the Bayesian analysis with MCMC method for sampling trees (Fig. 4) did not give satisfactory resolution of more basal clades in the consensus tree based on 15,000 trees to support distances. For a clear phylogenetic position among analyzed genera the LSU phylogenetic relationships published in Læssøe and Hansen (2007) should be referred.

Ecology of Libyan desert truffles

Vegetation in Libyan deserts is generally scarce and mainly limited to areas with sufficient annual precipitation. In our study, *Helianthemum kahiricum* (Delile, Flora Égypte: 237. 1813) was the dominant plant found in upper part of Hamada Al-Hamra region while *Helianthemum lippii* (Syn.

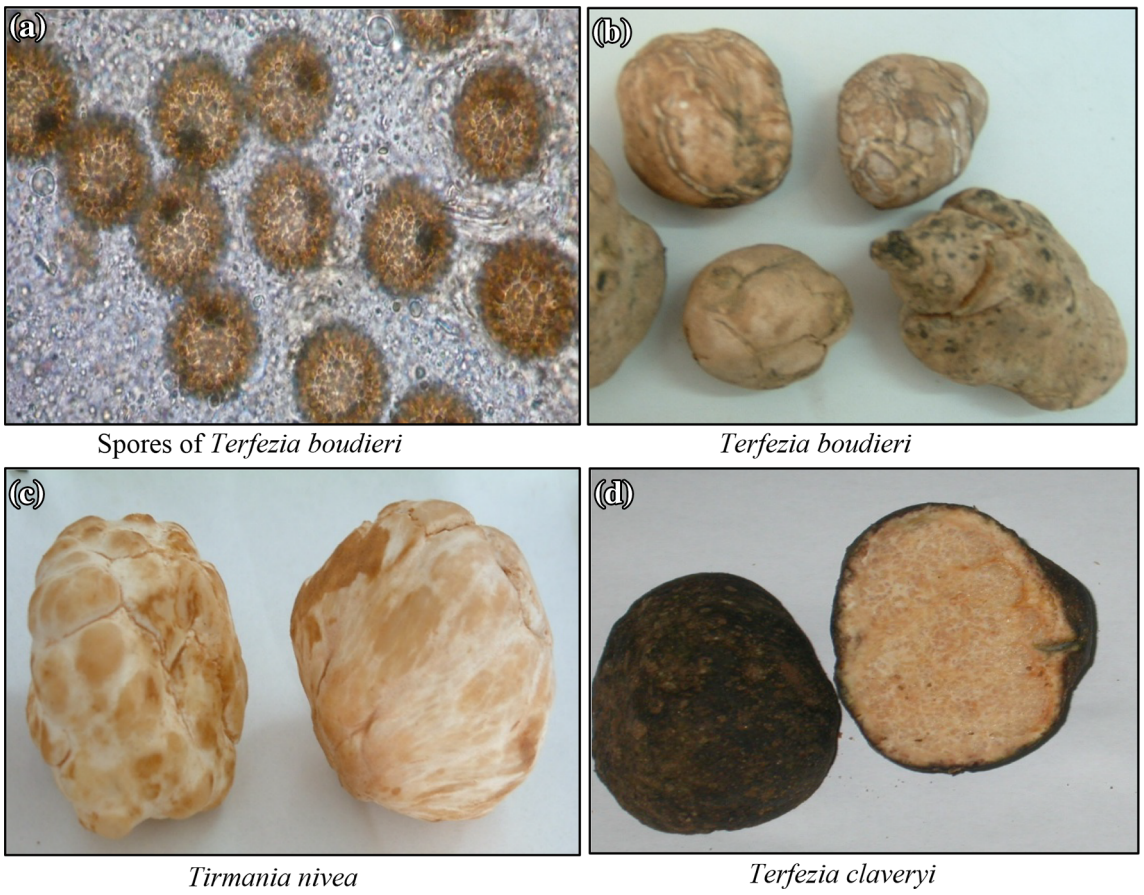

Fig. 1 Comparative morphology of collected *Tirmania and Terfezia* species. **a** Spores of *Terfezia boudieri*, **b** *Terfezia boudieri*, **c** *Tirmania nivea*, **d** *Terfezia claveryi*

Pl. (Persoon) 2(1): 78. 1806) was the common host plant in lower part of Hamada Al-Hamra. Both dominant plant species in the truffle habitat areas in Libya could have a symbiotic association with the Libyan wild truffles were collected and identified (Fig. 5). Other mapped plants (namely *Artemisia campestris*, *Scorzonera undulata*, *Medicago laciniate*, *Marrubium deserti*, *Astragalus tribulricks* and several *Atractylis* spp.) occur on both sites and were reported in previous surveys by Shamekh (1986). The roots of *Helianthemum* spp. plants were previously reported by Moubasher (1993). To support the growth of the *Terfezia* and *Tirmania* mycorrhizas, we did not follow the fungal mycelium from ascocarps to plant roots to confirm the mycorrhiza partner's identity (Agerer 1991). Based on the observed vegetation, only *Helianthemum* spp. has a potential to form endomycorrhiza in natural field conditions. Previous reports showed ecto- and endomycorrhiza without a sheath in pot cultures, while an ectomycorrhiza with a characteristic sheath and Hartig net in in vitro cultures (Gutiérrez et al. 2003).

Spanish desert truffles are collected in the spring from the roots of annual and perennial plants, often *Helianthemum* and *Xolantha* spp. (Moreno et al. 1986), while the fruiting period in upper and lower areas of Hamada Al-Hamra mainly corresponds to flowering season of particular genus, from November until April, respectively. Moreno et al. (2000) pointed out that these mycorrhizal fungi play an important role in the maintenance of arid Mediterranean shrub-lands by preventing erosion and desertification which is the case in Libya as well.

Nutritional value of Libyan desert truffles

Representative amount of morphologically identified sporocarps was pooled for measuring the nutritional value of fungal material. Nutritional value of Libyan desert truffle is shown in Table 2. The results showed that pooled *Tirmania* and *Terfezia* contained 16.3 and 18.5% protein, 6.2 and 5.9% lipid, 67.2 and 65% carbohydrate, respectively, in ascocarp biomass on dry weight basis. *Tirmania* and *Terfezia* contained 77.6 and 73.5% of moisture and 10.2 and 10.6% of ash, respectively. The protein content of the Saudi desert truffle, *Terfezia*

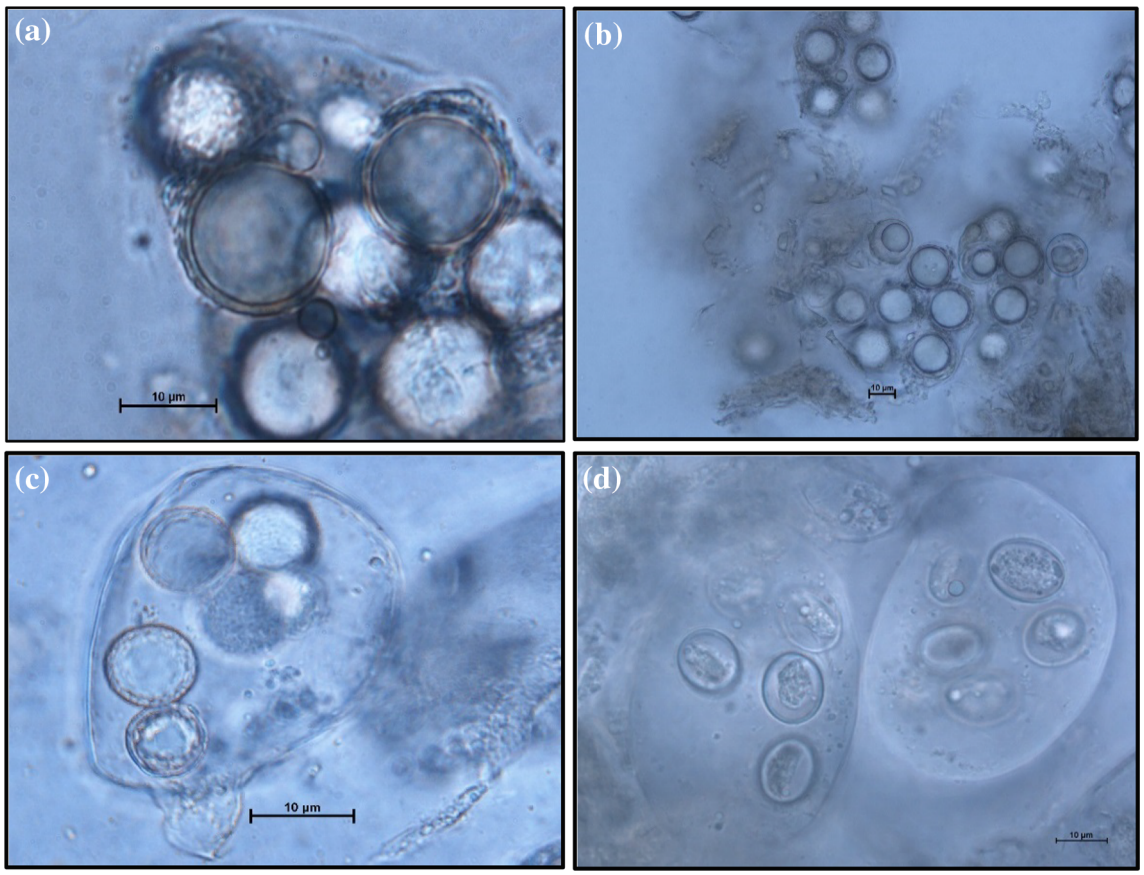

Fig. 2 **a** Spores of sample 1. Globose spores are shown with oil droplets in the center, confusedly arranged in ascus, smooth to nearly reticulate. Light microscopy, bar 10 µm. **b** Spores of sample 2. Globose–subglobose spores are shown with oil droplets, confusedly arranged in ascus, lightly reticulate. Light microscopy, bar 10 µm. **c** Spores of sample 3. Globose–subglobose spores are shown, confusedly arranged in ascus, lightly reticulate. Light microscopy, bar 10 µm. **d** Spores of sample 4. Spores subglobose are shown, irregularly arranged in ascus, the surface smooth probably due to unripe stage of spores. Light microscopy, bar 10 µm

claveryi (Chatin, La Truffe: 74. 1892), reported by Bokhary and Parvez (1993) was lower than Libyan *Terfezia* but the same as Libyan *Tirmania* (16%). Whereas the results of Sawaya et al. (1985) showed that the brown species (Gibaah) of Saudi truffles (*Terfezia*) contained 25% protein while the white truffle, *T. nivea* (Zubaidi), contained 27% protein, which are much higher than the values of both Libyan truffles. Nutritional value of analyzed *Terfezia* collections is comparable to *Tuber texense* (Heimsch, Mycol. 50: 657. 1958) (Beuchat et al. 1993) while *Terfezia* samples appeared more nutritious in terms of protein content. The differences in protein content for the phylogenetically close representatives of truffles might be due to the small change in environmental conditions and habitats of the truffle. Hypogeous fungi often contain high concentrations of nitrogenous compounds, vitamins, and minerals and are major dietary items for many rodents and other small mammals (Cork and Kenagy 1989). Therefore, the *Terfezia* and *Tirmania* sporocarps can be considered as an important protein source in the diet of local inhabitants. The water content of Libyan truffles was 73–78% of fresh weight and that is close to values (78%) reported for Saudi truffles (Bokhary et al. 1987; Bokhary and Parvez 1993).

Physical properties soil

Particle size distribution

The soil analysis revealed that the upper and lower Hamada Al-Hamra bear similar soil conditions. The average amount of sand, silt and clay in Hamada soil samples was found to be 75.05, 10.00 and 14.46%, respectively. Therefore, the textural class of soil was identified as 'sandy loam' using textural triangle (Fitzpatric 1974).

True and bulk density

The true and bulk densities of the soil sample were found to be 2.65 and 1.40 gm/cm^3, respectively. These values were comparable with earlier report by Fitzpatric (1974) who indicated that true density varies from about 2.65 for

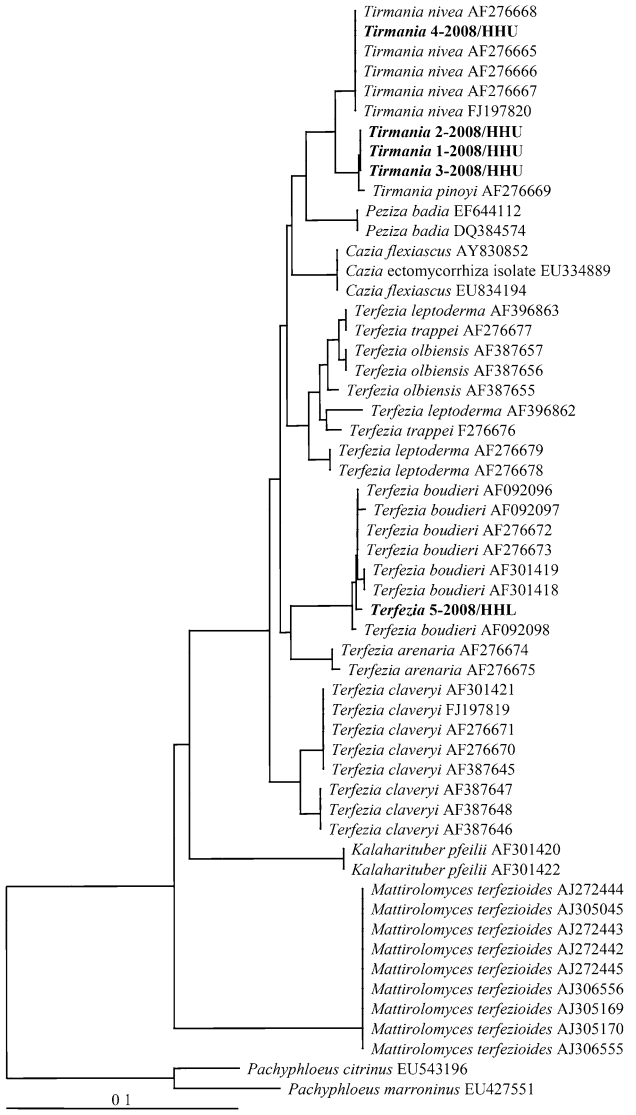

Fig. 3 NJ phylogenetic tree for selected desert truffle sequences and unknown ascocarps. The tree was constructed using Jukes–Cantor substitution model, based on all conserved sites in the alignment of the genomic rDNA ITS region

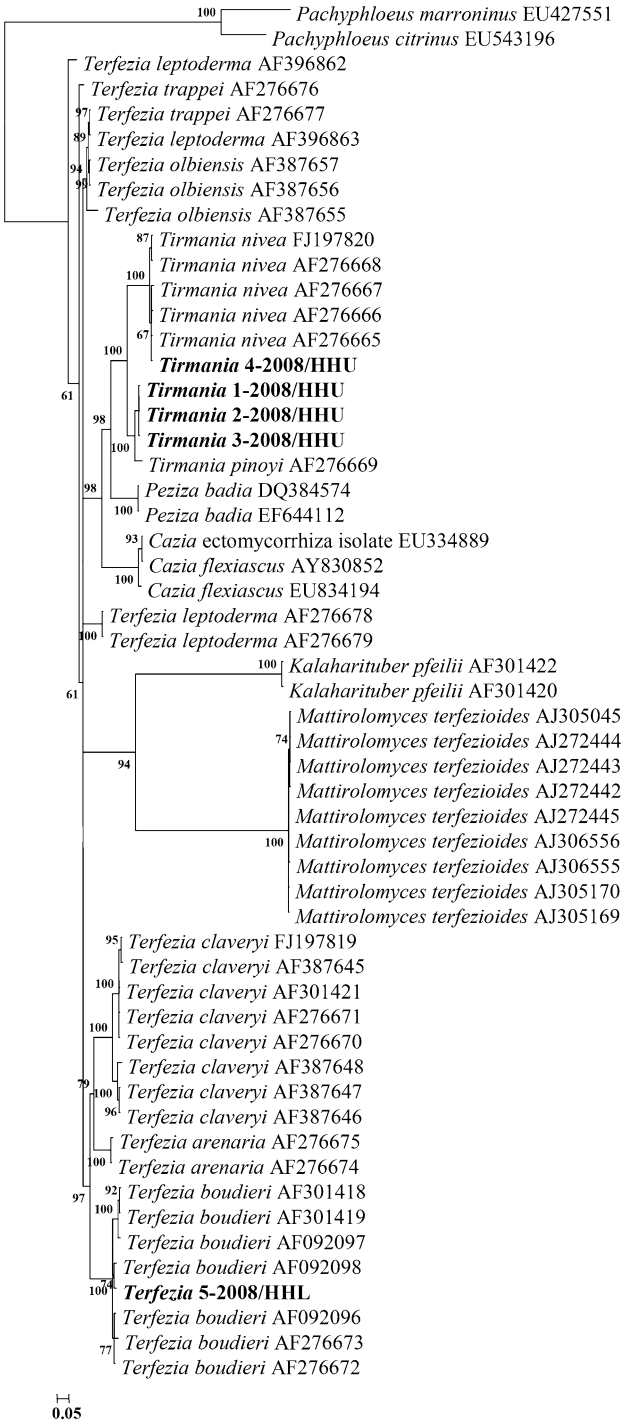

Fig. 4 Consensus tree for selected desert truffles and unknown ascocarps based on the genomic rDNA ITS region sequences. The tree is based on the Bayesian analysis with MCMC method for sampling trees

mineral particles to about 0.2 gm/cm^3 for the organic matter.

Porosity, field capacity and electrical conductivity

High value of porosity (45.28%) for soil sample was found. The field capacity value was found to be considerably low (22.23%). Both results were as expected due to the textural class (sandy loam) of soil sample. The high macroporosity of value seems to be a characteristic of great importance of the soil from the standpoint of providing sufficient air for good growth and reproduction of truffles. The average value of electrical conductivity of soil was 0.86 mm hos/cm at 25 °C. This value is generally considered to be lower than that for good crop production on most soil (Arar 1973).

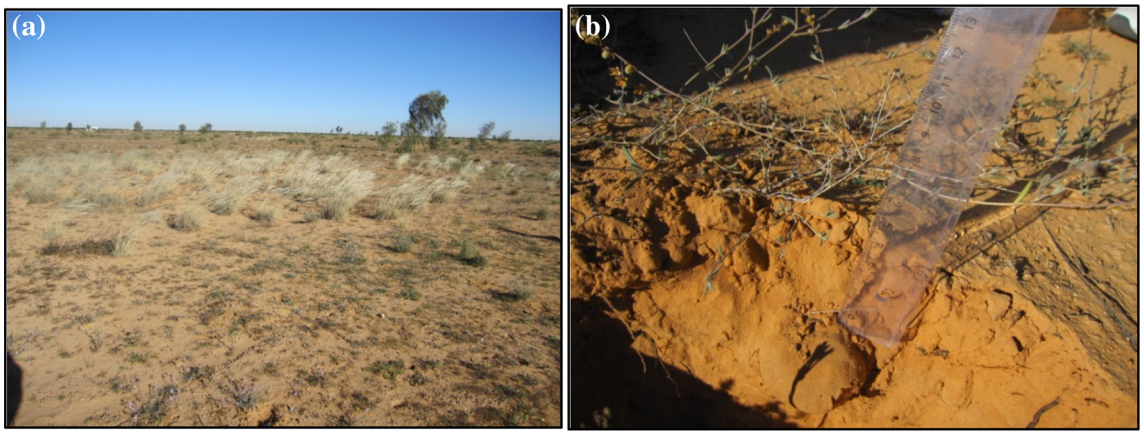

Fig. 5 **a** Habitat areas of the collected *Tirmania and Terfezia* species and the potential host plant species. **b** *Terfezia boudieri* under their hot plant *H. sessiliflorum* (*H. lippii* var. sessiliflorum)

Table 2 Nutritional value of Libyan desert truffles

Truffle species	Protein (%)	Lipid (%)	Carbohydrate (%)	Moisture (%)	Ash (%)
Tirmania	16.3 ± 0.5[a]	6.2 ± 0.35[a]	67.2 ± 0.45[a]	77.6 ± 0.35	10.2 ± 0.44
Terfezia	18.5 ± 0.4[a]	5.9 ± 0.43[a]	65 ± 0.35[a]	73.5 ± 0.45	10.6 ± 0.34

[a] Contents were calculated on a dry weight basis. Data are result of triplicate analyses and all the standard deviations were less than ±5%)

Chemical properties of soil

pH value of soil

Both areas appear to be suitable as truffle habitats with pH ranging from 8.1 to 8.5. Relatively high pH, generally above 7 or 7.5 (depending on the species) and similar soil texture as observed in Hamada Al-Hamra is also required by several cultivated truffles (*Tuber* spp.) as well (Chevalier and Frochot 1997; Weden et al. 2004; Hall et al. 2007). In general, the soils where the desert truffles occur naturally can be and in small plots already are used for desert truffle plantations. Areas with suitable soil conditions (e.g., clay-loamy texture and basic pH around 8.5) can in future be more intensively propagated and managed for controlled cultivation, after the consideration of other environmental properties for their suitability for truffle growth and fructification.

Mineral elemental analysis of soil

Data in Table 3 showed mineral content of the soil sample. The concentration of the calcium was found to be the highest followed by sodium and magnesium. The high calcium content of the soil sample (160 ppm) was probably due to the presence of calcite and seems to be important in providing suitable habitat for good growth and

Table 3 Physical properties of the soil from the habitats of the truffle

Properties	Quantity
Mechanical analysis	
Sand	75.04%
Clay	14.96%
Silt	10.00%
Porosity	45.28%
Field capacity	22.23%
True density	2.65 gm/cm^3
Bulk density	1.40 gm/cm^3

Table 4 Mineral elemental analysis of soil

Mineral element	Concentration (ppm)
Calcium	160 ± 0.35
Sodium	89.3 ± 0.30
Potassium	10.9 ± 0.18
Magnesium	36 ± 0.15
Nitrogen	7.5 ± 0.18
Phosphorus	0.25 ± 0.02
Chloride	0.25 ± 0.0015

Data are result of triplicate analyses and all the standard deviations were less than ±5%

reproduction of truffle. Earlier report showed that the calcium content of natural truffles habitat ranges from 21.3 to 292.5 ppm (Singer 1961). Calcium ion plays very important role in maintaining the pH value of soil. Nitrogen and phosphorus contents of the soil were found to be very low (7.5 and 0.25 ppm, respectively) (Table 4).

Conclusion

This study showed representatives of both genera, *Terfezia* and *Tirmania*, collected in the area of Hamada Al-Hamra were successfully identified, representing already known species and genotypes. Their natural distribution, the presence of potential mycorrhizal hosts from the genus *Helianthemum* and brief soil analyses indicated suitable conditions for mycelium growth and fruiting of hypogeous fungi. Their high nutritional value should encourage even more intensive exploration and exploitation of natural resources both, in upper and lower areas of Hamada Al-Hamra. Suitable natural conditions in the region and at the same time unfavorable conditions for majority of other ectomycorrhizal fungi should encourage an initiation of plantations with species-targeted plant hosts for an easy cultivation of those precious and nutritious hypogeous fungi.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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