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Phylogenetic Studies of *Terfezia pfeilii* and *Choiromyces echinulatus* (*Pezizales*) support new genera for southern African truffles: *Kalaharituber* and *Eremiomyces*

Yael FERDMAN[1], Sharon AVIRAM[1], Nurit ROTH-BEJERANO[1], James M. TRAPPE[2] and Varda KAGAN-ZUR[3]*

[1] *Department of Life Sciences, Ben-Gurion University, POB 653, Beer-Sheva, 84105, Israel.*
[2] *Department of Forest Science, Oregon State University, Corvallis, Oregon, USA 97331-5752.*
[3] *Institutes for Applied Research, Ben-Gurion University, POB 653, Beer-Sheva, 84105, Israel.*
E-mail: zur@bgumail.bgu.ac.il

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The ITS region including the 5.8S rRNA gene as well as the 5′ end of the 28S rRNA gene of hypogeous *Pezizaceae* and *Tuberaceae* were studied to clarify the generic placement of two southern African desert truffles, *Terfezia pfeilii* and *Choiromyces echinulatus*. The results show that neither species belongs in the genus to which it has been assigned on the basis of morphological characters. As expected, two *Choiromyces* spp. grouped close to the representative of the *Tuberaceae* (*Tuber melanosporum*). However, *C. echinulatus* diverged from the other *Choiromyces* species and emerged near members of the genus *Terfezia*, being even closer to that genus than *T. pfeilii*. Two new genera and new species combinations, *Kalaharituber* gen. nov. *with K. pfeilii* (syn. *T. pfeilii*) comb. nov. and *Eremiomyces* gen. nov. with *E. echinulatus* (syn. *C. echinulatus*) comb. nov. are therefore introduced to accomodate these taxa. Both genera are closely related to *Terfezia*, and thus are placed in the *Pezizaceae*.

INTRODUCTION

Truffles are the hypogeous ascomata of fungi belonging to the *Pezizales* (Trappe 1979, 1990). The assignment of truffle genera to a particular family and species to a particular genus was traditionally founded on the morphology of ascomata, asci and spores (e.g. Trappe 1979). Thus, *Terfezia pfeilii* was described from Damaraland, Namibia, by Hennings (1897) and *Choiromyces echinulatus* from Cape Province, South Africa, by Marasas & Trappe (1973).

In recent years, however, molecular phylogenetic research on sequestrate fungi has repeatedly demonstrated that morphology of hypogeous fungi can be misleading. Evolution of hypogeous species typically involves a convergent reduction in macromorphological characters, often entailing loss of features otherwise useful to distinguish related epigeous taxa. More specifically, molecular analyses of the *Pezizales* and the phylogenetic relations among epi- and hypogeous species have been conducted by O'Donnell *et al.* (1997), Norman & Egger (1999), Percudani *et al.* (1999), Roux *et al.* (1999), Hansen, Læssøe & Pfister (2001), and Díez, Manjón & Martin, (2002). Some of these investigations confirm earlier morphological findings. O'Donnell *et al.* (1997) and Hansen *et al.* (2001) demonstrated that certain hypogeous members of the *Pezizales* show greater affinity to epigeous members than to other hypogeous ones. Similarly, the monophyletic origin of some members of *Terfezia* and *Tirmania* was confirmed by Díez *et al.* (2002). However, other results challenge some of the earlier placements, for example along with *Tirmania*, *Terfezia* belongs to the *Pezizaceae* rather than the *Terfeziaceae* (Norman & Egger 1999; Percudani *et al.* 1999). Further, *Choiromyces* was moved from the *Terfeziaceae* to the *Tuberaceae* (O'Donnell *et al.* 1997, Percudani *et al.* 1999), and *Terfezia terfezioides* was removed from *Terfezia* and reinstated as *Mattirolomyces terfezioides* (Percudani *et al.* 1999, Díez *et al.* 2002).

A preliminary molecular study of *Terfezia pfeilii* and *Choiromyces echinulatus* suggested that these two Kalahari desert truffles are molecularly close, though currently placed in different families. This apparent affinity inspired us to study the phylogenetic relationships between these two species in relation to other *Terfezia* and *Choiromyces* species. To this end, we analyzed DNA sequences from the internal transcribed

* Corresponding author.

Table 1. Species analyzed for this work.

Truffle	Collection site	Date	Provided by	DNA fragment	GenBank accession no.
Choiromyces alveolatus (Trappe 22830)	Oregon, USA	1998	*J. Trappe*	ITS	AF501258
				28S 5′ region	AF435826
C. echinulatus (voucher no. 12)	Ghanzi, Botswana	1999	*V. Kagan-Zur*	ITS	AF435829
				28S 5′ region	AF435825
C. echinulatus (44894)	South West Africa (Namibia)	1974	*J. Trappe*	ITS	AF435823
				28S 5′ region	AY232725
C. venosus (536)	Germany	1972	*J. Trappe*	28S 5′ region	AF435827
Mattirolomyces terfezioides	Hungary	2001	*Z. Bratek*	28S 5′ region	AY649792
Terfezia boudieri form 1	Zeelim, Israel	1996	*Bedouins*	ITS	AF092096
				28S 5′ region	AF435820
T. boudieri form 2	Zeelim, Israel	1996	*Bedouins*	ITS	AF092097
				28S 5′ region	AF435822
T. boudieri form 3	Zeelim, Israel	1996	*Bedouins*	ITS	AF092098
				28S 5′ region	AF435828
T. boudieri 42a	Zeelim, Israel	1997	*Bedouins*	ITS	AF301418
T. claveryi	Jarada, Morocco	1994	*M. Achouri*	ITS	AF301421
				28S 5′ region	AF435824
T. pfeilii a	Kalahari, Botswana	1996		ITS	AF301420
T. pfeilii b	Kalahari, Botswana	1996	*F. Taylor*	ITS	AF301422
				28S 5′ region	AY232726
Tuber melanosporum Mycelium, isolate 'asco'			*G. Chevalier*	ITS	AF167097
				28S 5′ region	AF435821

spacer (ITS) of the rRNA gene cluster, that includes ITS1, 2 and the 5.8S gene, as well as the variable D1 and D2 5′ regions (van Tuinen, Zhao & Gianinazzi-Pearson 1998*b*) of the large subunit (LSU) of the rRNA gene cluster.

Much of the molecular research aimed at identifying fungi in general (e.g. Gardes *et al.* 1991, Henrion, Le Tacon & Martin 1992, Buscot *et al.* 1996, Mello *et al.* 1996, Kårén *et al.* 1997, Chillali *et al.* 1998) and truffles in particular (e.g. Henrion, Chevalier & Martin 1994, Paolocci *et al.* 1995, 1999) relies on comparison of sequences and RFLP patterns of the ITS region. Several phylogenetic studies of truffles were also based on this DNA region (Roux *et al.* 1999, Díez *et al.* 2002). Other molecular phylogenetic studies have been based on sequences from the 18S and the 28S (LSU) rRNA genes with or without the ITS region (e.g. O'Donnell *et al.* 1997, van Tuinen *et al.* 1998a, Norman & Egger 1999, Percudani *et al.* 1999, Hansen *et al.* 2001). The rRNA genes present different levels of conservation which can be exploited to analyze any desired phylogenetic level (Hillis & Dixon 1991).

Molecular data now available for the genera *Terfezia* and *Tirmania* place them, as mentioned above, in the *Pezizaceae* (Norman & Egger 1999, Percudani *et al.* 1999). Such molecular data are not yet available for *Mycoclelandia* (an Australian desert genus with two species), but it was assigned to the *Pezizaceae* because of its strongly amyloid asci (Trappe 1979, Trappe & Beaton 1984). Morphology alone has been the basis for putting two other desert species, *Choiromyces echinulatus* and *C. aboriginum*, in the *Tuberaceae* (Marasas & Trappe 1973, Trappe 1979) and a third, *Phaeangium lefebvrei*, in the *Pyronemataceae* (Alsheikh & Trappe 1983).

In this study we present molecular data that supports removal of *Terfezia pfeilii* from *Terfezia* and *Choiromyces echinulatus* from *Choiromyces*. We describe two new genera to accommodate these truffles, still within the *Pezizaceae*.

MATERIALS AND METHODS

The fungi

The origin of fruit bodies obtained for the analysis presented in this work is summarized in Table 1. Voucher collections of the specimens studied are deposited in the Department of Botany Mycological Herbarium, Oregon State University (OSC). Previously published sequences used in this work are presented in Table 2.

DNA extraction and amplification

The method described by Grube *et al.* (1995) was employed to obtain small amounts of amplifiable DNA. DNA was amplified with primers ITS 1 and ITS 4 (White *et al.* 1990) for ITS or LR 1 and U2 (van Tuinen *et al.* 1998b and Sandhu *et al.* 1995, respectively) for the large subunit (LSU). Primers were synthesized by Rhenium, Jerusalem. Both ITS and LSU amplifications were performed as described for ITS by Kagan-Zur *et al.* (1999).

Cloning

PCR amplified ITS or the 5′ end of the LSU fragments were cloned into InsT/Aclone™ PCR Product Cloning kit No. K1214 (MBI Fermentas, Lithuania).

Table 2. Sequences obtained from the National Center for Biotechnology Information (NCBI).

Accession no.	Species	Origin
U42688	*Choiromyces venosus*, LSU partial sequence[a]	Oregon USA
AF003910	*C. venosus* (as *meandriformis*), ITS[b]	Italy
AF276681	*Mattirolomyces terfezioides* 1, ITS[c]	Hungary
AF276680	*M. terfezioides* 2, ITS[c]	Hungary
AF276679	*Terfezia leptoderma* 1, ITS[c]	Spain
AF276678	*T. leptoderma* 2, ITS[c]	Spain
AF276675	*T. arenaria* 1, ITS[c]	Spain
AF276674	*T. arenaria* 2, ITS[c]	Spain
AF387657	*T. olbiensis* 1, ITS[d]	Not specified
AF387656	*T. olbiensisI* 2, ITS[d]	Not specified
AF387648	*T. claveryi* 3, ITS[d]	Not specified
AF276669	*Tirmania pinoyi*, ITS[a]	Algeria
AF276668	*T. nivea*, ITS[a]	Kuwait
AF335178	*T. pinoyi*, LSU partial sequence[e]	Algeria
AF335177	*T. nivea*, LSU partial sequence[e]	Kuwait

[a] O'Donnell *et al.* (1997).
[b] Amicucci, Stocchi & Martin (Direct submission).
[c] Díez, Manjón & Martin (2002).
[d] Gutierrez, Honrubia & Morte (Direct Submission).
[e] Hansen *et al.* (2001).

The plasmid mixture was transformed into *Escherichia coli* XL1-blue in accordance with the manufacturer's recommendations. Transformants were plated on agar solidified Luria Broth (LB) plates (Miller 1972) containing 50 μg ml^{-1} ampicillin.

Inserts sequencing

Single colonies were picked up and grown in LB overnight. Plasmids were extracted from these cultures by the alkaline method (Birnboim & Doly 1979). Plasmids containing inserts were sent to the sequencing unit of the Hebrew University of Jerusalem, where the Big Dye Terminator kit (Perkin-Elmer, Branchburg, NJ) was used in conjunction with the primer pair M13 (Forward) and pUC (Reverse) (Sambrook & Russell 2001) for bi-directionally sequencing of inserted fragments directly from each plasmid. Primers were synthesized at the Molecular Biology Laboratory, Hadassa Hospital, Jerusalem. The sequence was determined with an ABI prism 377 DNA sequencer (Applied Biosystems, Foster City, CA). Sequences were deposited in the National Center for Biotechnology Information (NCBI) databases (Table 1).

Alignments and phylogenetic analysis

Several sequences were obtained for phylogenetic analysis from the National Center for Biotechnology Information (Table 2). Sequence alignments were submitted to TreeBASE PIN no. 12889. Sequences were aligned with ClustalX version 1.81, downloaded for the MacIntosh from http://www.embl.de/~chenna/clustal/darwin/. The results were manually checked and adjusted.

Phylogenetic evolutionary trees based on these data were constructed along with 1000 bootstrapping repeats by MEGA version 2.1 (Kumar *et al.* 2001). Several reshuffled lists of sequences were presented to both the ClustalX and Mega programs. The Mega program's 'Subtree Swapping' option was used to adjust outputs. Trees were built with and without deleting gaps. Trees presented here were constructed using the gap delete options. Phylogenetic analysis of the ITS and the large subunit (LSU) sequences were carried out by the neighbour-joining (NJ) and maximal parsimony (MP) methods.

Morphological analyses

Dried specimens were hand-sectioned with a razor blade and mounted in 5% KOH, Melzer's reagent and cotton blue in lactic acid, respectively. Spore dimensions are based on at least 30 randomly selected spores plus the smallest and largest seen, excluding the ornamentation. Spores were measured in cotton blue mounts, because the walls or ornamentation of many ascomycetes tend to swell in KOH. Other tissues were measured in KOH.

RESULTS AND DISCUSSION

Phylogenetic analyses

ITS analyses

The ITS phylogenetic tree presented as Fig. 1 was the output result of both NJ and MP methods of analysis, although there are differences in percent bootstrapping. In this tree some of the *Terfezia* group nodes, notably separation of *T. claveryi* and *T. arenaria* (**40**/30) as a distinct clade and grouping of the latter with the *T. boudieri* clade (**47**/36), were only weakly supported by either method. Including sequences leading to extensive gap formation resulted in an NJ tree very similar to Fig. 1. The only placing changes observed involved the weakly supported branches (data not shown) within the *Terfezia* clade. Reshuffling the input data did not change the grouping presented in Fig. 1 but in one or two cases changed the placing order (e.g. putting *T. boudieri* first, or separating *T. claveryi* from *T. arenaria*). These are easily achieved or corrected by 'Subtree Swapping' at will. However, examining the best bootstrapping tree (data not shown) revealed several other differences: *Tuber melanosporum*, chosen as an out group, which forms a *Tuber-Choiromyces* clade together with *C. venosus* (submitted to the GenBank as *C. meandriformis*) and *C. alveolatus* in Fig. 1, emerges as a true out-group, though close to the above mentioned *Choiromyces* spp.

The separation of *T. boudieri* 2 (and 42a2) from *T. boudieri* 1 and 3 is well supported in Fig. 1, but all 3 *T. boudieri* ITS forms (Holdengraeber *et al.* 2001) stem from the same root in the bootstrapping tree

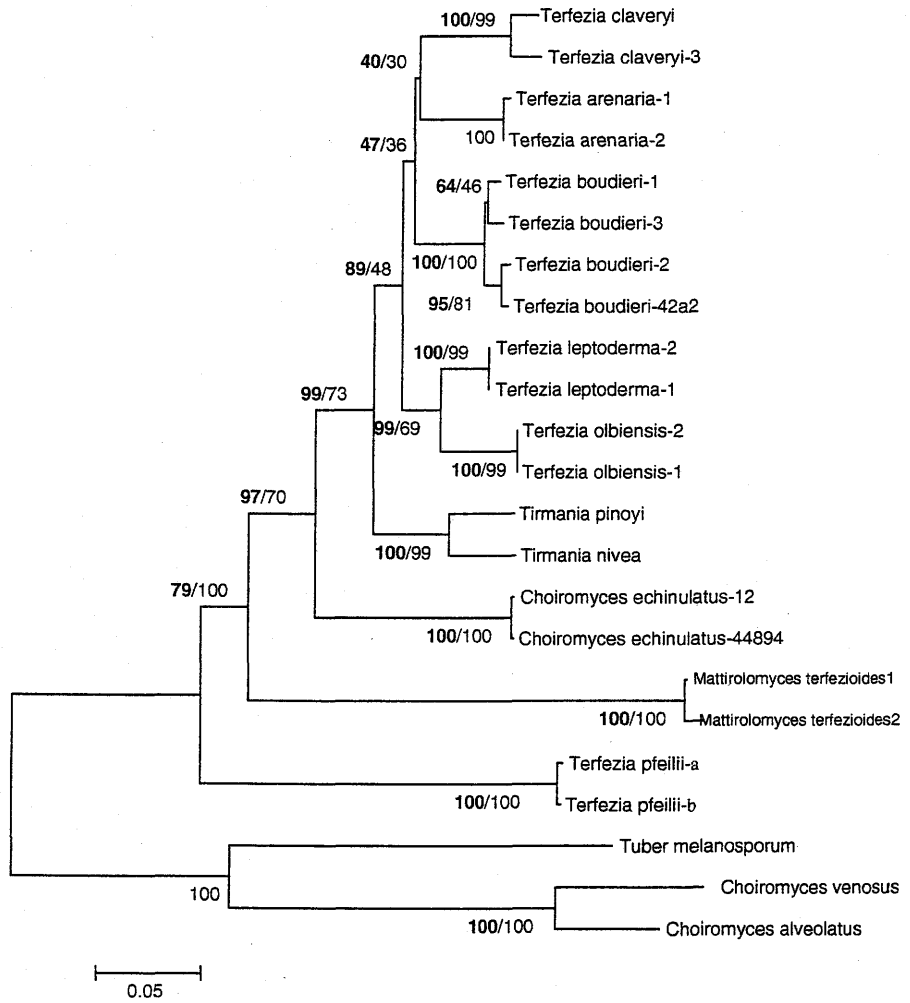


Fig. 1. Neighbour-joining consensus phylogenetic tree of ITS sequences. Bold numbers are % of 1000 bootstrapping replicates supporting the NJ tree presented here. Non-bold numbers are % of 1000 bootstrapping replicates supporting the same node by the MP method.

(no hierarchy). Some of the less supported nodes were also eliminated from the latter, resulting in the loss of the less supported information. Both, however, essentially support removal of *C. echinulatus* and *Terfezia pfeilii* from the genera to which they were assigned solely on morphological grounds.

C. echinulatus, emerges closer to the *Tirmania* clade than to the other *Choiromyces* species, being placed within the in-group. Most of the *Terfezia* spp. group together to constitute a monophyletic *Terfezia* clade, the closest neighbour of which is the *Tirmania* lineage. *T. leptoderma* and *T. olbiensis* form a distinct group as sister to *T. boudieri*, *T. arenaria* and *T. claveryi*. However, *T. pfeilii*, the Kalahari *Terfezia*, is distant from other *Terfezia* species and forms a separate lineage. *Mattirolomyces terfezioides*, *T. pfeilii* and *C. echinulatus*, belonging to separate clades, are placed between the *Tirmania* and *Tuber-Choiromyces* groups. The data support reinstatement of *Mattirolomyces terfezioides* (Fischer 1938) as a replacement for *Terfezia terfezioides* and representative of a separate genus distinct from *Terfezia*, as suggested by Percudani *et al.* (1999) and supported by Díez *et al.* (2002).

We also performed multiple alignment of the 5.8S. Table 3 shows that out of the 155 bp analyzed for the 5.8S gene, 23 sites exhibited variability, representing 22 independent events. Of these, ten were transitions (5 C-T), eight were transversions, two were inversions of dinucleotides, one was an insertion and one deletion of two nucleotides.

The above sequences fall into two main groups, one including *T. melanosporum*, *C. alveolatus*, *C. venosus* (as *meandriformis*) and *C. venosus*, and the other *Terfezia* spp., *C. echinulatus*, *Tirmania* spp., and

Table 3. All deviations from the consensus sequence (deduced from this work) found in the 5.8S rRNA gene.

Site no.	*Tuber melanosporum*	*Choiromyces mend. & C. alveolatus*	*Terfezia arenaria*	*T. claveryi*	*T. boudieri* 2	*Mattirolomyces terfezioides* 2	*M. terfezioides* 1	*Tirmania nivea*	*T. pinoyi*	*Terfezia olbiensis*	*T. leptoderma*	*T. pfeilii*	*Choiromyces echinulatus* 44894	*C. echinulatus* 12
17	G to A													
24	A to T	A to T												
27		A to T												
31	T to C	T to C												
35-36									gap					
41						G to A								
51	T to C	T to C												
55												A to T		
63	A to G					A to C	A to C							
79						G to A	G to A							
83			ins-T											
84-85	inv-TC	inv-TC												
86	C to A	C to A												
96			C to T		C to T		C to T							
120			C to G											
123	A to T	A to T	A to T	A to T										
127			T to C											
133	G to T	G to T	G to A	G to A	G to A									
148-149	inv-TC	inv-TC												
153						C to T	C to T							

M. terfezioides. Both show relatively small differences within and relatively large differences between the groups. Detailed analysis of the 5.8S gene revealed three variable sites within the *Tuber-Choiromyces* group (three sequences), and eight sites variable solely within the *Terfezia-Tirmania* group (ten sequences). An additional seven sites were identical within each group but varied between them. *T. pfeilii* and *C. echinulatus* displayed greater conformity to the 5.8S gene consensus sequence (emerging from this study) than *T. arenaria* and *T. claveryi*.

Analysis of the 5′ end of the LSU

To further test these results, the divergent domains D1 and D2 of the LSU rRNA gene were also analyzed. This DNA region is better conserved than the ITS but still variable enough to show differences between species (Hillis & Dixon 1991, van Tuinen *et al.* 1998b). The LSU NJ tree (Fig. 2), like the ITS tree, shows *C. alveolatus* and *C. venosus* branching together with *T. melanosporum* to form a *Tuber-Choiromyces* clade, an outgroup; this tree is supported by the bootstrapping. As in the ITS tree, the genus *Tirmania* is placed closer to the *Terfezia* clade, while *C. echinulatus*, *M. terfezioides*, and *T. pfeilii*, form separate clades where *C. echinulatus* is closer to *Terfezia* spp. than to other *Choiromyces* spp. However, the MP trees were somewhat different. One of the better supported trees (Fig. 3) shows a non-hierarchical relationship of *M. terfezioides*, *C. echinulatus*, *Tirmania* spp. and the *Terfezia* clade, while *T. pfeilii* emerges in a hierarchically once removed clade. The non-hierarchical branching is rather poorly supported (59% bootstrap value), but the separation of *T. pfeilii* is well supported. All MP trees, however, placed *T. melanosporum* on its own, as an outgroup, while *C. alveolatus* and *C. venosus* formed a separate clade. Essentially, the two rRNA gene cluster regions examined in our phylogenetic analysis of *Terfezia* and *Choiromyces* species, namely the ITS region including ITS1, 2 and the 5.8S rRNA gene, as well as the 5′ end of the LSU gene, generated similar results. However, as GenBank contains rather few 28S sequences, the range of the 28S (LSU) gene tree was necessarily limited as compared with the ITS tree. The LSU result concerning *Terfezia boudieri* was somewhat unexpected, as this region is rather better conserved than the ITS, we had expected a resolution along species lines rather than within the species. While *T. pfeilii* a and b had identical 28S sequences and *T. boudieri* ITS forms 1 and 3 were also almost identical to each other in their 28S sequences, *T. boudieri* ITS 2 deviated from the latter. The hypothesis that these forms, though they may seem morphologically indistinguishable, actually represent two different species, deserves further investigation.

All trees presented here provide strong support for a close relationship between *T. melanosporum* and the *Choiromyces* clade, while equally strongly supported is

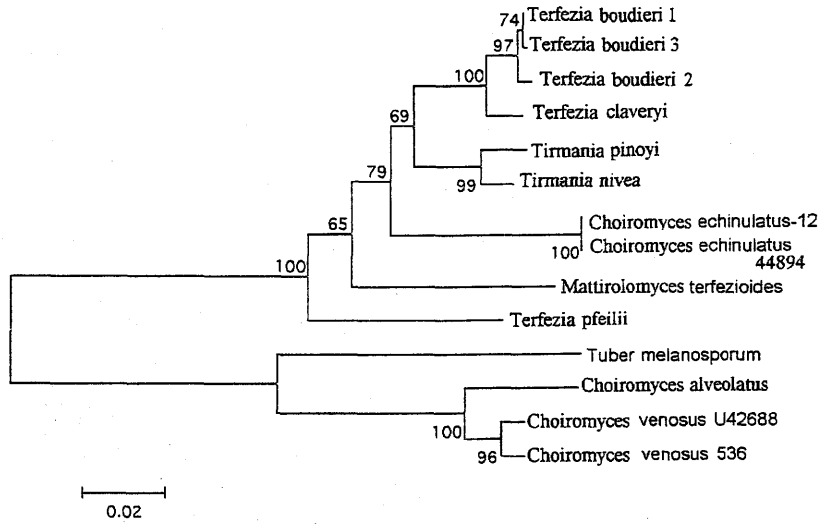


Fig. 2. Neighbour-joining consensus phylogenetic tree of the D1–D2 5′ end of the 28S (LSU) rRNA gene sequences. Numbers are % of 1000 bootstrapping replicates supporting the tree presented here.

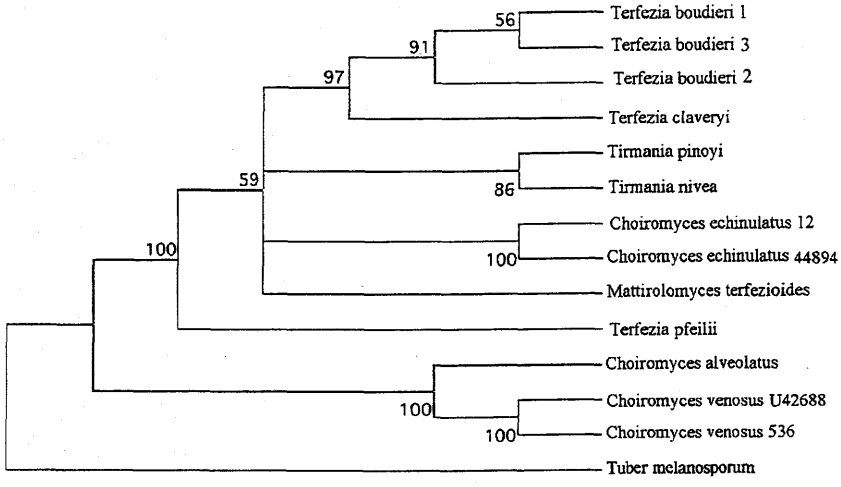


Fig. 3. A representative Maximal Parsimony (MP) phylogenetic tree of the D1–D2 5′ end of the 28S (LSU) rRNA gene sequences. Numbers are % of 1000 bootstrapping replicates supporting the tree presented here.

the placing of *C. echinulatus* outside this clade within the ingroup. *T. pfeilii*, although located within the ingroup is phylogenetically farthest from the *Terfezia* clade.

To conclude, our findings evidence that the assignment of *C. echinulatus* to the genus *Choiromyces* and therefore to the *Tuberaceae* is incorrect. It is also clear that *T. pfeilii* does not belong to the *Terfezia* clade and its classification needs to be revised. In spite of the molecular resemblance between *T. pfeilii* and *C. echinulatus*, which initiated this study, the finding that the two species occupy distinct clades in all types of analyses precludes their placement in a single genus. We therefore propose two new genera, described below, to accommodate the above findings.

TAXONOMY

Kalaharituber Trappe & Kagan-Zur, **gen. nov.**

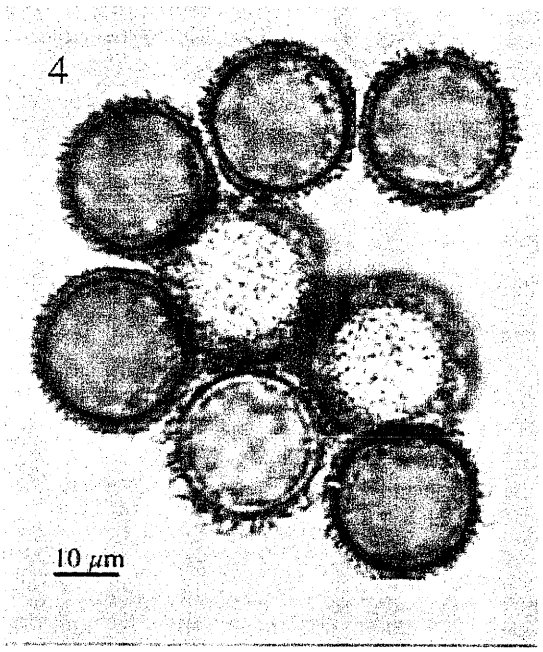
Etym.: Kalahari-+-tuber (Latin, 'truffle'), 'truffle of the Kalahari.'

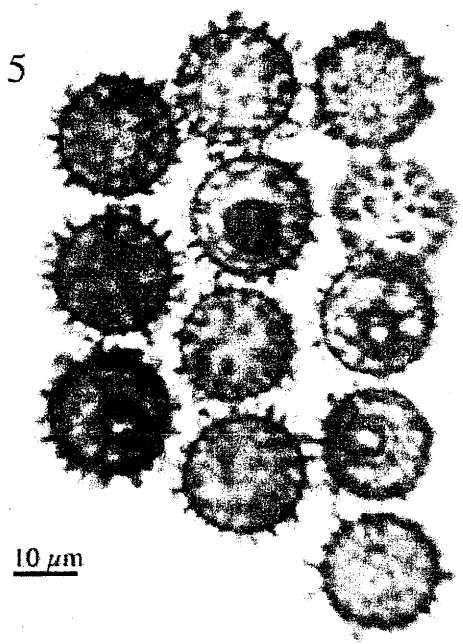
A *Terfezia* sporis spinis minutissimis dense congestis, ordiniibus DNA abhorrentibus, distributione in Hemisphaerio Meridionali differt.

Typus: *Terfezia pfeilii* Henn.

Kaliharituber pfeilii (Henn.) Trappe & Kagan-Zur, **comb. nov.** (Fig. 4)

Basionym: *Tuber pfeilii* Henn., *Bot. Jahrb.* **22**: 75 (1897).





Figs 4–5. Ascospores. **Fig. 4.** *Kalaharituber pfeilii* in KOH. **Fig. 5.** *Eremiomyces echinulatus* in Cotton blue.

Etym.: in honor of Count Pfeil, the original collector of the type species.

Ascomata hypogeous, smooth, unpolished, minutely pubescent, turbinate to obpyriform or subglobose, to 6.5 × 6 cm. *Peridium ca* 1 mm thick, wrinkled (especially on the upper ascoma surface), dark brown, the wrinkles yellowish. *Gleba* solid, fleshy, marbled with sterile, white veins and yellowish white to brown, fertile pockets, *Odour* strongly fungoid. *Spores* globose, 16–22 (–26) μm diam, at first hyaline, in age light brown, the walls ± 1.5 μm thick, 2-layered, light yellow to light orange in Melzer's reagent; ornamentation appearing in KOH as a minutely papillose or granulose, mucilaginous epispore but in cotton blue clearly seen as densely crowded, acute spines 0.5–1.5 (–2) × ≤ 0.5 μm. *Asci* nonamyloid, with (4–) 5–8, loosely arranged spores, globose to ellipsoid or obovoid, 70–100 × 50–80 μm, sessile to substipitate, the walls ± 1 μm thick at maturity, randomly arranged in fertile pockets. *Ectal excipulum* 180–220 μm thick, of brown hyphae 4–12 μm broad at septa but the cells inflated to 15–30 μm. *Ental excipulum* of generally circumferentially aligned, hyaline to pale yellow hyphae 4–10 μm broad at the septa, the cells often slightly inflated. *Glebal hyphae* of sterile veins and fertile pockets undifferentiated, hyaline, thin-walled, 5–12 μm broad at septa but the cells generally inflated to 20 μm.

Distribution, Habitat and Season: Kalahari Desert of southern Africa: Namibia, Botswana, and adjacent areas of South Africa, In compact to fairly compact, calcareous, white and pink sands (Leistner 1967), associated with *Acacia spp.* (Marloth 1913, as *Terfezia claveryi*, Storey 1958, Taylor *et al.* 1995) and various other plants, including *Citrullus vulgaris* (Kagan-Zur *et al.* 1999), April through June.

Illustrations (as *Terfezia claveryi* or *T. pfeilii*): Pole-Evans (1918: pl. 7), Mattirolo (1922: fig. 28), Leistner (1967: pl. 50), Marasas & Trappe (1973: fig. 2), Alsheikh (1994: pl. 2–18).

Comments: The spore ornamentation of minute, densely crowded spines of *K. pfeilii* was recognized as different from that of any other *Terfezia* spp. by Mattirolo (1922), Marasas & Trappe (1973), and Alsheikh (1994). Nonetheless, it has been variously misidentified as *Tefezia boudieri* or *T. claveryi* (Marloth 1913, Pole-Evans 1918, Ceruti 1960). Marasas & Trappe (1973) ascribe the early confusion of *K. pfeilii* with other *Terfezia* spp. to the paucity of good specimens for study and the nature of its spore ornamentation: spines so minute and crowded that they can be discerned only when stained, even with the oil immersion objective. Use of *K. pfeilii* for food by natives of the Kalahari was reviewed by Trappe (1990).

Specimens examined: **Botswana**: Ghanzi, *G. Scholtz* (PRE 41869). **Namibia**: Damaraland, *Graf Pfeil* (S – holotype of *Terfezia pfeilii*); Gibeon, *Burger* (PRE 42082, 42203); Gobabis, *Verbüchlein* (PRE 4390); Klein Karas, 20 Apr. 1921, *A. E. Hill* (OSC, TO); *loc. cit.*, 8 June 1923 (BPI 38083, PRE 17799, OSC, TO); *Windhoek*, *Gies* (PRE 42076). **South Africa**: *North Cape*: Kakamas, June 1931, *M. J. Oosthuizen* (PRE 26335, OSC); Kalahari Gemsbok National Park, May 1956, *R. Story 5616* (PRE 41602); Postmasburg, May 1911, *F. L. Hunter* (PRE 11293, OSC); Prieska, *MacCleod* (PRE 11619); Upington, *O. A. Leistner* 2610 (PRE 42201); Vryburg, *Stephens* 527 (PRE 36103); unspecified locality,

Le Riche (PRE 41870) – **Kalahari Desert:** unspecified locality, *Bottomley* (PRE 44310); herb. *G. Bresadola* (BPI, as *Terfezia boudieri*); 1913, *R. Marloth* (K, as *Terfezia claveryi*); *Nash* (PRE 44254); *Weintraub* (PRE 32394).

Eremiomyces Trappe & Kagan-Zur, **gen. nov.**

Etym.: Eremi- (Greek, 'desert') + -myces (Greek, 'fungus'), 'desert fungus.'

A *Choiromyces* sporis virgis conicis rectis obtusis, cellulis magnopere inflatis, ordiniibus DNA abhorrentibus (ad Pezizaceas, non Tuberaceas, affinis) et distributione in desertis Hemisphaerio Meridionali differt.

Typus: *Choiromyces echinulatus* Trappe & Marasas.

Eremiomyces echinulatus (Trappe & Marasas) Trappe & Kagan-Zur, **comb. nov.** (Fig. 5)

Basiosym: *Choirmyces echinulatus* Trappe & Marasas, *Bothalia* **11**: 139 (1973).

Etym: '*echinulatus*' (Latin, 'having small spines').

Ascomata pale cream colored and subglobose when fresh, as dried with a black peridium and solid *gleba* marbled by dark brown veins embedding hymenial palisades; opposing palisades deformed from pressing against each other.

Spores globose, 10–14 µm diam, light yellow to light brownish yellow, the walls 1 µm thick, light blue in cotton blue; ornamentation of straight, obtuse rods and cones 1–2 × 0.5–1 (–1.5) µm, pale yellow in KOH and light blue in cotton blue, *ca* 20–25 around the spore circumference, unconnected except for some spores on which barely perceptible lines on the spore surface join occasional ornaments. *Asci* hyaline, thin-walled, mostly 8-spored, in youth cylindric to ellipsoid or saccate, by maturity mostly cylindric, 140 × 12–17 µm, astipitate or with a short basal projection, the spore arrangement occasionally uniseriate but mostly incompletely biseriate to irregular. *Paraphyses* thin-walled, hyaline, ±4 µm broad. *Ectal excipulum* of light yellow, thin-walled hyphae 4–8 µm broad at the septa; *ental excipulum* similar but the hyphal cells often inflated to 15–50 µm broad. *Glebal veins* of subparallel-interwoven hyphae 3–6 µm broad at the septa, the cells mostly inflated.

Distribution, Habitat and Season: North Cape, South Africa, and Botswana. June, in sand dunes.

Illustrations (as *Choiromyces echinulatus*): Marasas & Trappe (1973: fig. 1), Trappe (1979: fig. 24a).

Comments: This rarely collected fungus mimics *Choiromyces* of the *Tuberaceae* in its macromorphology. However, its spore ornamentation of straight, obtuse, rods and cones differs from the sinuous rods with apical depressions of *Choiromyces venosus*, the type species of that genus. The other *Choiromyces spp.* have pitted spore surfaces (Trappe 1979). *Eremiomyces echinulatus* is a desert dweller, and like most other desert truffles has tissues with greatly inflated cells, a character not found in *Choiromyces* species of temperate forests. Of course, the DNA sequences place *E. echinulatus* in the *Pezizaceae*, whereas *Choiromyces sensu stricto* is in the *Tuberaceae* (O'Donnell *et al.* 1997, this study).

Specimens examined: **South Africa**: *North Cape*: Gordonia near Upington, 1 June 1961, *O. A. Leistner* 2612 (PRE 42202 – holotype of *Choiromyces echinulatus*).

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