Mycol Progress (2010) 9:131–143
DOI 10.1007/s11557-009-0612-6

ORIGINAL ARTICLE

Comparative taxonomy of desert truffles of the Australian outback and the African Kalahari

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Received: 19 March 2009 / Revised: 23 June 2009 / Accepted: 24 July 2009 / Published online: 26 August 2009

Abstract Seven truffle species are reported from the Australian Outback-six Ascomycota (*Elderia arenivaga, Mattirolomyces mulpu* sp. nov., *Mycoclelandia arenacea, M. bulundari, Reddelomyces westraliensis, Ulurua non-paraphysata* gen. & sp. nov.) and one Basidiomycota (*Horakiella watarrkana* sp. nov.) Three Ascomycota species are redescribed from the African Kalahari (*Eremiomyces echinulatus, Kalaharituber pfeilii* and *Mattirolomyces australiafricanus* comb. nov.). The phylogenetic analyses of nrDNA of the Australian Ascomycota provided strong support for placement of all but one in the Pezizaceae (*Reddellomyces* belongs in the Tuberaceae), as is true of most, if not all, other ascomycetous desert truffles, These genetic results also highlight that the genus *Mattirolomyces* is more taxonomically, ecologically, and geographically diverse than previously realized.

Keywords Ascomycota Basidiomycota . Pezizaceae . Sclerodermataceae . Tuberaceae

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Introduction

The central Australian Outback and the African Kalahari, great desert regions of the southern hemisphere, have broadly comparable geographic, botanical, and cultural attributes. Among these are low and variable average rainfall, extremely high temperatures in summer, hypogeous fungi (truffles), and indigenous peoples, the Aborigines and San, respectively, that have hunted and harvested these fungi for food from prehistoric times. Trappe et al. (2008a, b) have reviewed the literature and other sources of information on the ecology and ethnomycology of these truffles and briefly outlined their taxonomy. Here, we present the formal taxonomy and molecular phylogenetics of these taxa together with the resulting nomenclatural modifications.

Materials and methods

Macroscopic descriptions of the fungi were taken from a combination of the literature and notes with individual collections, or in the case of *Reddellomyces westraliensis,* from fresh specimens. Razor-blade sections mounted in water, 3% KOH, Melzer's reagent, and cotton blue in lactic acid, respectively, were used for microscopy. Spore dimensions were measured in water mounts of mature

spores; the other mounting media can cause ascospores or their ornamentation to shrink or swell. When two-dimensional measurements are given, length is first, followed by width. Herbarium abbreviations are according to the Index Herbariorum (2009).

Methods for DNA extraction, PCR amplification and sequencing were described in detail by Kovacs et al. (2008). Total DNA was extracted from small pieces of dried herbarium specimens. The SSU (NS 1-NS8), the ITS, and the LSU (LROR-LR5) regions of rDNA were amplified and sequenced. The sequences were compiled from electrophoregrams by Pregap4 and Gap4 (Staden et al. 2000). Amplifications of nrDNA succeeded for eight specimens of six species from the Australian Outback plus one species from the African Kalahari; sequences obtained for each specimen have been deposited in GenBank (Table 1); the ITS (GQ231754) and LSU (GQ231755) regions of a *Mattirolomyces terfezioides* collected in Europe (France, OSC Trappe 4548) were also sequenced.

With a previously set datamatrix of Pezizales (Kovacs et al. 2008), we established that sequences of all but one ascomycetous taxon unambiguously grouped within the Pezizaceae (Fig. 1). The LSU region is a reliable marker for intrafamily phylogenetic analyses of Pezizaceae (Hansen et al. 2001, 2005); it amplifies relatively well from dried ascomata (Hansen et al 2005). For the final analyses, we used a reduced LSU dataset of previously published phylogenetic analyses of Pezizaceae "lineage A" (Leesae and Hansen 2007) together with sequences in our present study. The sequences were aligned by use of ClustalX (Thompson et al. 1997). As *Kalaharituber pfeilii* and *Eremiomyces echinulatus* are represented by LSU sequences approximately 600 bp long in GenBank (while the sequencing of the LROR-LR5 segment results were more than 900 bp), an alignment 671 characters long was used in the final analyses. *Ascobolus crenulatus* served as outgroup. Alignments were checked and adjusted manually with ProSeq 2.9 (Filatov 2002). The best-fit nucleotide substitution model was selected with the program Modeltest 3.06 (Posada and Crandall 1998), considering the selection of Akaike Information Criterion (AIC). The best-fit model was used to calculate distances for NJ analysis with the PAUP*4.0bl0 software package (Swofford 2003). The supports of the branches were tested by NJ bootstrap (NJB) with 1,000 replicates. Phylogenies were also inferred by parsimony analyses by the PAUP heuristic search for the most parsimonious (MP) trees. Gaps were handled as missing characters, "MulTrees" was in effect, and TBR was used as branch-swapping algorithm. Supports of the branches were tested by parsimony bootstrap (PB) with a fast-heuristic search with 1,000 replicates. ML phylogenetic analyses were conducted with the program PHYML (Guindon and Gascuel 2003). The GTR nucleotide substitution model was used with ML estimation of base frequencies. The proportion of invariable sites was estimated and optimized. Four substitution rate categories were set and the gamma distribution parameter estimated and optimized. ML bootstrap (MLB) analysis with 1,000 replicates was used to test support of the branches. The same substitution model was used in Bayesian analyses performed with program MrBayes 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The Markov chain was run over 4,000,000 generations, sampling at every 100 steps and with a burn in at 7,500 sampled trees. The phylogenetic trees were visualized and edited by Tree Explorer of the MEGA 4 program (Tamura et al. 2007) and a text editor.

Results

Six truffle genera encompassing seven species have been found in the deserts of the Australian outback. One genus, *Ulurua,* is proposed as new, while another, *Mattirolomyces,* is reported from Australia for the first time. Three of the seven species are described as new: *Horakiella watarrkana, Mattirolomyces mulpu,* and *Ulurua nonparaphysata.* The

Table 1 Desert truffle Ascomycota from the Australian Outback and African Kalahari and the GenBank accession numbers of their SSU, ITS, and LSU nrDNA

Source	Taxon	OSC[a] No.	GenBank No.		
			SSU	ITS	LSU
Australia	*Elderia arenivaga* (isotype)	111751	GQ231732	GQ231733	GQ231734
	Elderia arenivaga (isoepitype)	111641	GQ231735	GQ231736	GQ231737
	Mattirolomyces mulpu (isotype)	131319	GQ231738	GQ231739	GQ231740
	Mycoclelandia arenacea	131125	GQ231741	GQ231742	GQ231743
	Mycoclelandia arenacea	131132	GQ231744	GQ231745	GQ231746
	Mycoclelandia bulundari	130648			GQ231747
	Reddellomyces westraliensis	111640			GQ231748
	Ulurua nonparaphysata (isotype)	131126		GQ231749	GQ231750
Africa	*Mattirolomyces austroafricanus* (isotype)	58845	GQ231751	GQ231752	GQ231753

[a] Oregon State University Mycological Collections

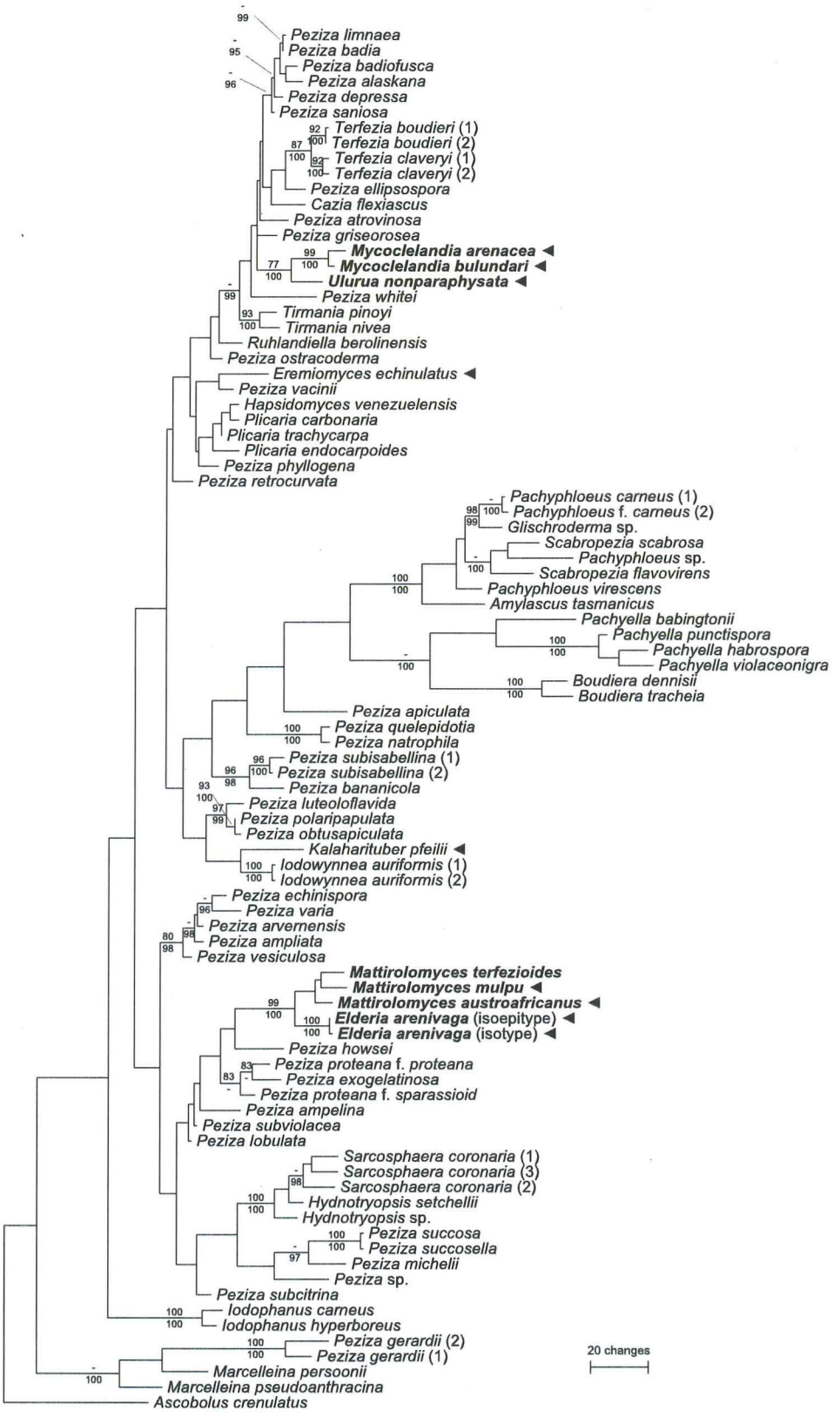


Fig. 1 One of the 3,362 most parsimonious phylogenetic trees (score 1,562, CI: 0.312, RI: 0.665) showing the positions of desert truffles within the Pezizaceae (lineage A) as inferred by parsimony analysis of nrDNA LSU sequences using the PAUP* program (Swofford 2003). *Ascobolus crenulatus* served as an outgroup. Sequences obtained in this study are shown in *bold*. Other sequences were obtained from earlier studies as given in Læsøe and Hansen (2007). Parsimony bootstrap (PB) values are above the branches, posterior probabilities calculated by Bayesian analysis (PP) are below. Both values are presented as percentages; bootstrap values below 75% and the posterior probabilities below 95% are not shown. *Bar* 20 changes

other four species are redescribed: *Elderia arenivaga, Mycoclelandia arenacea, M bulundari,* and *Reddellomyces westraliensis. R. westraliens is* is not strictly a desert species; it also occurs in dry sclerophyll woodlands of Western Australia, South Australia, Victoria, and New South Wales (Trappe et al. 1992). It has been collected only once in the desert country, and then in a riparian swale under *Eucalyptus* sp. in the Northern Territory. The other six species are restricted to genuinely arid habitats.

Three genera have been found in the Kalahari, each represented by one species: *Eremioniyces echinulatus, Kalaharituber pfeilii,* and *Mattirolomyces austroafricanus,* the last named being a new combination and the first report of the genus in southern Africa.

In our phylogenetic analyses, *Redellomyces westraliensis* grouped unambiguously in the Tuberaceae and branched together with *Redellomyces donkii* (data not shown). The other Ascomycota grouped within the Pezizaceae. Several fine-scale lineages were resolved within Pezizaceae (Fig. 1), overall corresponding to those found in previous studies (Hansen et al. 2005). The lineages differed in their supports, but as in previous analyses based on LSU sequences (Hansen et al. 2001; Lesee and Hansen 2007), their relative positions were not resolved.

Mattirolomyces mulpu and *Mattirolomyces austroafricanus* always grouped with the European *Mattirolomyces terfezioides* (Fig. 1), although this monophyletic *Mattirolomyces* clade had not received strong support in any previous analyses The *Mattirolomyces* taxa formed a well-supported (PB:99% NJB,MLB,PP: 100%) lineage with *Elderia arenivaga* (Fig. 1). The *Mattirolomyces-Elderia* clade branched from a polytomy during the NJ analyses from where the *"Peziza* s. str." lineage (Hansen et al. 2005) also branched. In the ML and parsimony analyses, the *Mattirolomyces-Elderia* clade grouped with the *"Peziza* s. str. b" lineage (Hansen et al. 2005) but without good support. In both the ML and parsimony analyses, the "a" and "b" group *of "Peziza s*. str." (Hansen et al. 2005) grouped separately. None of these positions of the *Mattirolomyces-Elderia* clade received strong support. In the strict consensus tree of the 3,362 equally MP trees, the *Mattirolomyces-Elderia* clade nested within the *"Peziza* s. str. b" similarly to the nesting of *Mattirolomyces* in *"Peziza* s. str." in strict consensus of MP trees in the analysis of Leesee and Hansen (2007). In the Bayesian analyses, the *Peziza* s. str. grouped together and the *Mauirolomyces-Elderia* clade plus *Iodophanus* nested into this group, which received relatively strong support (PP:97%).

The phylogenetic position of *Kalaharituber pfeilii* was the same as described previously (Lessee and Hansen 2007); the species branched with *Iodowynnea* in all analyses. The *Kalaharituber - Iodowynnea* lineage had an ambiguous position and received only moderate support (pB:65% NJB:85% MLB:78% PP: 85%). *Eremiomyces* formed a distinct lineage with no unambiguous affinity to any of the known Pezizaceae lineages, but in all analyses grouped in the "inclusive group A" of Pezizaceae (Hansen et al. 2005) as suggested by Leesee and Hansen (2007).

Mycoclelandia arenacea and *M bulundari* formed a strongly supported clade (PB,NJB:99% MLB,PP:100%) in a monophyletic well-supported group (PB:77% NJB:91 % MLB:87% PP:100) with *Ulurua nonparaphysata.* This *Mycoclelandia-Ulurua* clade nested into the *"Peziza depressa-Ruhlandiella"* lineage in all analyses.

Keys **and descriptions**

Spore and ascus morphology and presence or absence of paraphyses form the basis for discriminating each of the species described below. Juvenal spores of all species are smooth, including those that become ornamented by rnaturity. Ifmost asci are empty or have clearly young spores, one must search diligently to find relatively thick-walled spores to determine whether or not mature spores are ornamented. Staining mounts in cotton blue facilitates finding scattered mature spores in that case. The asci only of *Mycoclelandia* and *Ulurua* species turn blue in Melzer's reagent.

Australian outback species

I. Basidiospores; gleba with minute chambers filled with brown, globose, reticulate spores at maturity. *Horakiella watarrkana*

I. Ascospores; gleba solid or with large chambers or veins; spores hyaline or pale brown, globose or ellipsoid, smooth, warty or reticulate. 2

2. Mature asci blue in Melzer's reagent; spores ellipsoid to subglobose. 3

2. Mature asci not blue in Melzer's reagent; spores globose. 5

3. Spores ornamented with minute warts; hymenium lacking paraphyses. *Ulurua nonparaphysata*

3. Spores smooth; hymenium with paraphyses. 4

4. Spores 10-12 x 8-10 um, length/width ratio 1-1.25. *Mycoclelandia arenacea*

4. Spores 12-16 x 8-10 um, length/width ratio 1.4-*1.75. Mycoclelandia bulundari*

5 (2). Spores 27-42 um broad, at maturity ornamented with rounded warts 1-4 um broad. *Reddellomyces westraliensis*

5. Spores:<26 um broad, minutely warty or reticulate. 6

6. Mature spores 8.5-13.5 um broad, minutely warty. *Elderia arenivaga*

6. Mature spores nearly all 19-21 um broad, reticulate. *Mattirolomyces mulpu*

Elderia arenivaga (Cooke & Massee) McLennan, Proc R Soc Victoria 74:113–114. 1960

≡ *Stephensia arenivaga* Cooke & Massee in Cooke, Grevillea 21:38. 1892
≡ *Choiromyces aboriginum* Trappe, Mycotaxon 9:334–335, 1979

Ascomata (Fig. 2) 4-8 cm broad, subglobose to turbinate, furrowed and lobed. *Peridium* pubescent in youth, by maturity glabrous on exposed surfaces, white to pale cream color. *Gleba* white to pale cream color, with narrow, labyrinthine chambers partly empty to filled by opposing hymenia or stuffed with hyphae. *Taste* and *odor* not recorded.

Ascospores (Fig. 3a) in water hyaline, subglobose to globose, 9.5-12.5 um broad, when thin-walled and immature swelling up to 14 um in KOH mounts, smooth in youth, by maturity ornamented with minute, crowded warts<0.2 x 0.2 Um, mostly with a de Bary bubble, nonguttulate; spore walls 1.5-2 um thick. *Asci* in a hymenium or occasionally embedded in the glebal trama, hyaline, cylindric to clavate or saccate with a long, tapered stem, 90-150 x 15-30 um, nonamyloid, thin-walled, with 8 uni- or biseriate spores, disintegrating at maturity. *Paraphyses* hyaline, 3-5 (-10) um broad, sinuous, ranging from half the length of the asci to longer than the asci.

Peridium of interwoven, hyaline, thin-walled hyphae 3-14 um broad, with many cells inflated to 15-20 um and scattered to abundant sphaerocyst-like cells 40 - 120 um broad; suprapellis in youth a tangle of hyphae 2-4 um broad, these often collapsing as the ascoma matures. *Glebal* tissue similar to peridium except with few inflated cells.

Etymology: Elderia, in recognition of the Elder Scientific Exploring Expedition of 1891-1892 to central and western Australia, during which the type collection was found, and Latin, *aren-* (sandy) and *-vaga* (wandering). The intent of Cooke and Massee (Cooke 1892) in coining the species name is uncertain; it may refer to the "wandering" of the expedition in the sandy desert.

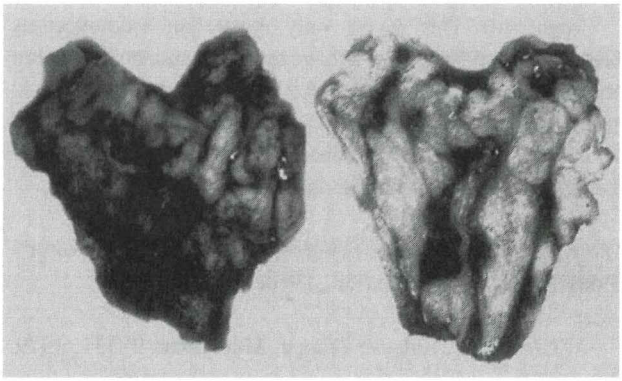


Fig. 2 Ascoma of *Elderia arenivaga* preserved in formalin, ×1; surface view (*left*) and cross-section (*right*)

Family: Pezizaceae.

Distribution, habitat and season: Eastern Great Sandy Desert of Western Australia to the Tanami Desert of the Northern Territory and the Simpson and Great Victoria Deserts of South Australia. April, June and August.

Collections examined: HOLOTYPE: AUSTRALIA: *South Australia*—Great Victoria Desert, 27°20′S, 130°53′E, R. Helms, 25 Jun 1891 (K, isotypes ADW 2431, MEL 40052 and OSC 111751). EPITYPE here designated: *Northern Territory*—Alice Springs Desert Park, 23°42′S, 133°50′E, David Albrecht, Jun 2000 (NT n.s., isoepitype OSC 111641) OTHER COLLECTIONS: *South Australia*—Simpson Desert, J. B. Cleland, 23 Apr 1940 (AD 9768, ADW 5995, OSC 131133). *Western Australia*—near Lake Hazlett, 21°26′S, 128°26′E, D. Thompson, Aug 1958 (MELU, holotype of *Choiromyces aboriginum*, isotype OSC 39478).

Comments: The holotype was collected near Camp 9 of the Elder Scientific Exploring Expedition. It has smooth spores, whereas the later collection described by McLennan (1961) has minutely but distinctly warty spores. Trappe (1979) regarded the latter collection as different from the holotype and described it as *Choiromyces aboriginum.* In re-examining the holo- and isotypes, however, we noted that both are quite immature, as indicated by many empty asci and thin-walled spores. Moreover, the faint beginnings of a warty ornamentation could be discerned on infrequent spores. Because *E. arenivaga* and C. *aboriginum* do not differ in any other respects, and because the holo- and isotypes are immature and in poor condition, we here designate the excellent, mature collection by David Albrecht as isoepitype for *E. arenivaga.* The molecular taxonomic results also support this isoepitype designation. nrDNA regions of both the 108-year-old holotype and the 8-year-old isoepitype could be sequenced and only minimal differences were found between them: 0,057%, 0,806%, 0.115% of the SSU, ITS, and LSU, respectively.

Horakiella watarrkana Trappe & Claridge, sp. nov.

MycoBank MB 513025

A Horakiella clelandii sporis reticulatioribus et parvioribus (11-15 um longis) differt.

Basidiomata hypogeous, as dried up to 25 x 35 mm, dark brown, glabrous, with a very few, concolorous rhizomorphs adpressed at the base. *Peridium* in cross section 0.5 x 1 mm thick, concolorous with the surface. *Gleba* with irregular locules 0.2-0.5 mm broad, at maturity packed with coherent, brown masses of spores separated by narrow, brown tramal veins. *Taste* and *odor* not recorded.

Basidiospores (Fig. 3b) in water pale yellow singly, orange-brown in mass, globose to subglobose, 11-15 um broad; spore wall with 2 layers: outer wall 0.2-0.5 um thick with an obscure to prominent, partial to complete reticulum 0.2-0.5 (-0.5) um tall; inner wall 2-3 um thick at maturity; nurse hyphae not detected, but debris clinging to spore surfaces may be remnants; in Melzer's reagent, spores hyaline to light orange singly, light orange to brownish orange in mass (weakly dextrinoid); in cotton blue, light to moderate blue. *Basidia* early evanescent, not seen.

Peridium poorly rehydrating, with a patchy suprapellis of tangled, hyaline hyphae 2-5 um broad; subpellis of tightly interwoven, adpressed, hyaline, thin-walled hyphae 3-6 um broad at the septa, the cells often inflated up to 10 um or more, especially towards the gleba; clamp connections scattered. *Rhizomorph* surface a tangle of looping hyphae 2-5 um broad overlying parallel hyphae 3-5 um broad; clamp connections abundant. *Glebal trama* of parallel to interwoven, hyaline, thin-walled hyphae 3-6 um broad at the septa, most cells inflated up to 8-20 um; clamp connections scattered.

Etymology: Horakiella, in honor of Austrian mycologist Dr. Egon Horak, who has contributed much to knowledge of Australasian fungi, and *watarrka-* with suffix *-na* (Watarrkanan), Latinized aboriginal name of the only place where it has been found.

Famity: Sclerodermataceae.

Distribution and season: Australia, known only from the type collection from Northern Territory. May.

Collection examined: HOLOTYPE: AUSTRALIA: *Northern Territory-* Watarrka National Park, *R. Southgate* (MEL 263949, OSC 80820).

Comments: Many *Horakiella watarrkana* spores have a fairly well developed reticulum. The only other species so far described for the genus, *H. clelandii* (Rodway) Castellano & Trappe, has some spores with patches of reticulum, but it is known only from forests in Tasmania, and its spores are 25-35 (-40) um long, much larger than those of *H. watarrkana* (Castellano and Trappe 1992).

Mattirolomyces mulpu Kovacs, Trappe & Claridge, sp. nov.

Mycobank MB 513071

Ascomata 3-7 cm lata, turbinata, rugosa. Peridium caespitibus hypharum agglutinarum. Gleba venis irregularibus. Sporae hyalinae, globosae, (l3-) 19-21 (-26) um, reticulo irregulari 2-4 um alto. Asci in hymenio inordinato, nonamyloidei, 8-spori. Paraphyses hyalinae, 2-4 um latae. Peridium glebaque cellulis multo inflatis.

Ascomata 3-7 cm broad, turbinate, rugose. *Peridium* white, with tufts of agglutinated hyphae that incorporate sand grains. *Gleba* with irregular veins, color not recorded. *Taste* and *odor* not recorded.

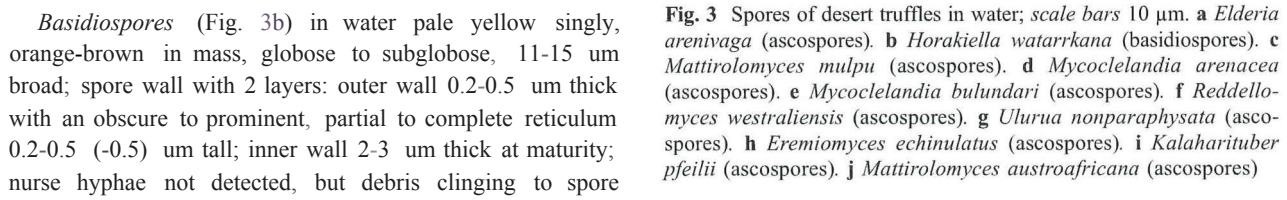


Fig. 3 Spores of desert truffles in water; *scale bars* 10 µm. **a** *Elderia arenivaga* (ascospores). **b** *Horakiella watarrkana* (basidiospores). **c** *Mattirolomyces mulpu* (ascospores). **d** *Mycoclelandia arenacea* (ascospores). **e** *Mycoclelandia bulundari* (ascospores). **f** *Reddellomyces westraliensis* (ascospores). **g** *Ulurua nonparaphysata* (ascospores). **h** *Eremiomyces echinulatus* (ascospores). **i** *Kalaharituber pfeilii* (ascospores). **j** *Mattirolomyces austroafricana* (ascospores)

Ascospores (Fig. 3c) in water hyaline to pale yellow, globose, (13-) 19-21 (-26) um broad excluding the ornamentation of blunt spines connected in an irregular reticulum 2-4 um tall that is sometimes obscured by amorphous deposits, mostly with a de Bary bubble and uniguttulate; walls 1 - 1.5 um thick. *Asci* in a tangled, disorderly hymenium, hyaline, cylindric to clavate, ellipsoid or saccate, difficult to separate from tissues, ±140-170x25-75 um, nonamyloid, the walls 1-2 um thick, with 8 uni- or biseriate spores. *Paraphyses* hyaline, 2-4 um broad, the tips generally enclosing the asci.

Pertdium 400-500 um thick, of hyaline, thin-walled, interwoven hyphae 4-7 um broad at the septa, most cells inflated to 10-40 um, with a suprapellis of hyaline, tangled hyphae 2-4 (-10) um broad and mostly agglutinated into tufts with incorporated sand grains. *Gleba* of hyphae similar to those of the peridium.

Etymology: Mattirolomyces, in honor of Italian mycologist Oreste Mattirolo (l856 - 1947), who described the first species of the genus, and *-myces* (fungus), hence "the Mattirolo fungus;" *mulpu,* the Ngaatjatjarra name for this or similar truffles (Kalotas 1996).

Family: Pezizaceae.

Distribution, habitat and season: Australia, southern part of Northern Territory, close to a sand dune under *Cassia* and *Eragrostis* spp, May

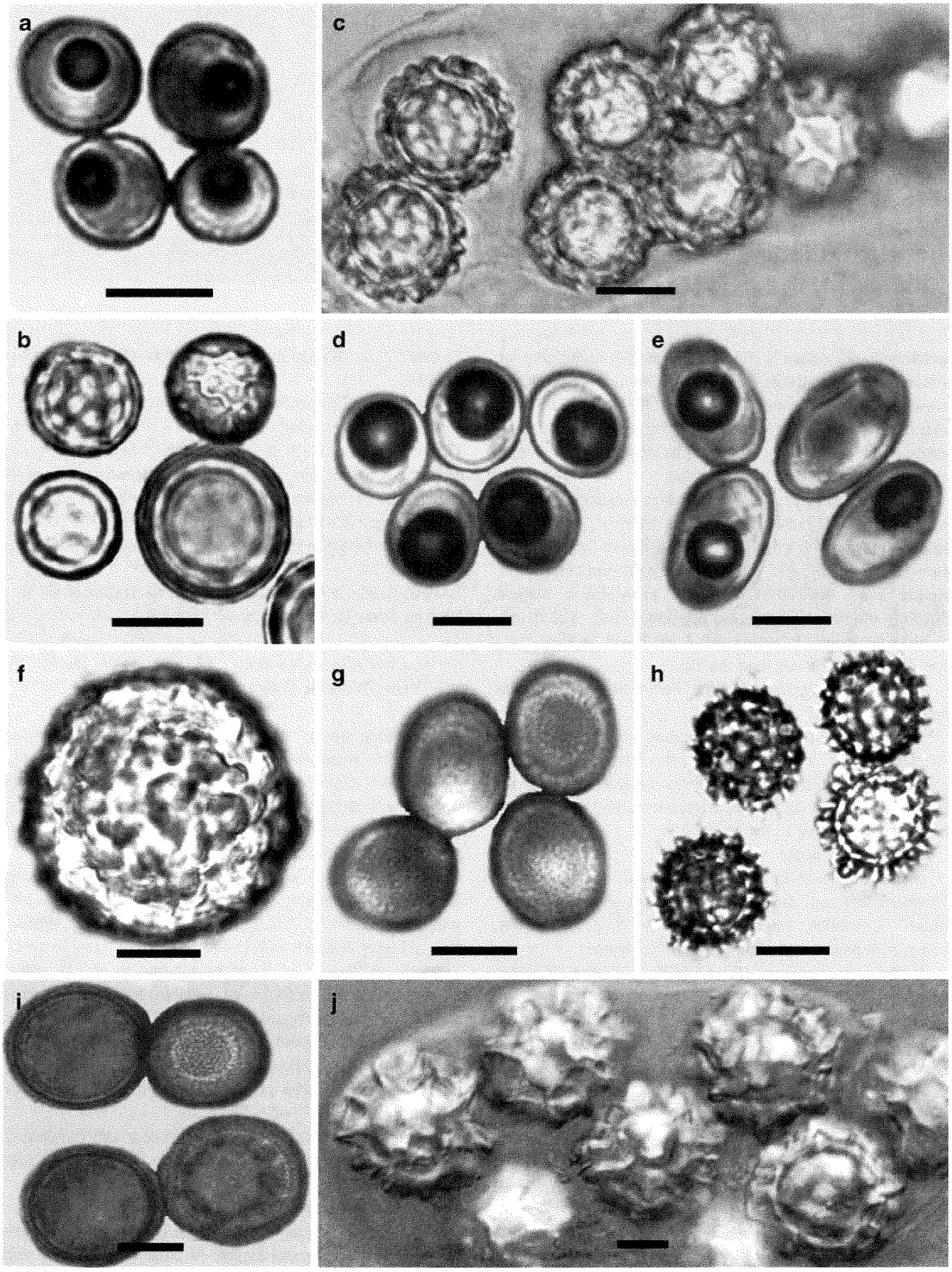
Collection examined: HOLOTYPE: AUSTRALIA: *Northern Territory*—2 km S of Roe Cr., 23°51′S, 133°50′ E, Mantatjara, 26 May 1983 (NT461 as *Choiromyces aboriginum*, isotype OSC 131319). PARATYPE: *Northern Territory*—Tanami Desert, 20°35′S, 131°31′E, D. Gibson & J. Cole, 15 Jun 1983 (NT 506 M, OSC 130644).

Comments: This is the only Australian ascomycetous desert truffle so far known to have reticulate spores. Collection notes by A. C. Kalotas state, "Revealed by cracks and rise in ground. Cooked in hot sand, ashes, coals, and eaten." It and *M. austroafricanus*, the Kalahari species reported below, are the first reports of the genus in the southern hemisphere.

Mycoclelandia arenacea (Trappe) Trappe & G.W. Beaton, Trans Br Mycol Soc 83:536. 1984.

≡ *Clelandia arenacea* Trappe, Mycotaxon 9: 331. 1979.

Ascomata (Fig. 4a, b) 2-4 (-9) cm broad, even to deeply furrowed, globose to turbinate and narrowed to a basal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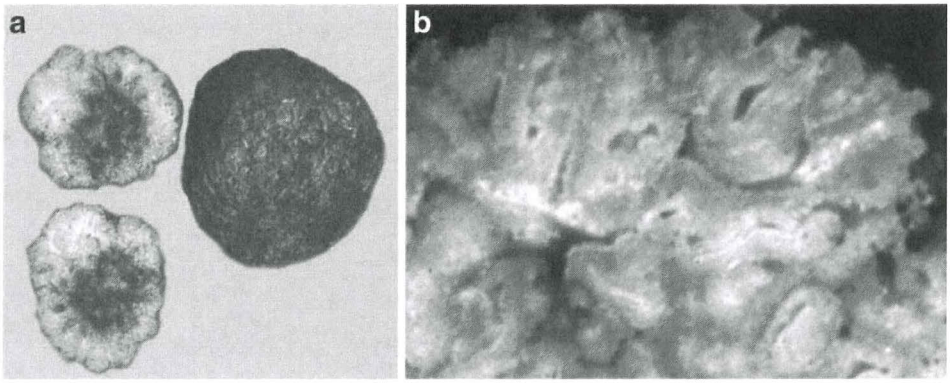

Fig. 4 Fresh ascomata of *Mycoclelandia arenaceae*: **a** surface and cross-sectional views, ×0.5; **b** closeup of gleba, ×4. Photograph by David Albrecht

attachment. *Peridium* glabrous to patchily pubescent or tomentose, especially in the furrows, pale orange-brown to brown but often appearing dark reddish brown from adherent sand. *Gleba* whitish, with labyrinthine chambers partly empty to filled by opposing hymenia or stuffed with hyphae. *Taste* and *odor* not recorded.

Ascospores (Fig. 3d) in water hyaline, broadly ellipsoid to subglobose, 10-12 x 8-11 um, length/width ratio 1.0-1.25, smooth, mostly with a de Bary bubble, uniguttulate, the walls 1 um thick. *Asci* hyaline, cylindric to saccate and tapered to a simple base, 90-120 x 12-25 um, in Melzer's reagent diffusely blue but bluest at the tip, thin-walled, with 8 uni- or biseriate spores. *Paraphyses* 5-8 um broad, hyaline, thin-walled, septate, shorter than the asci.

Peridium and *gleba* of hyaline, thin-walled hyphae with cells inflated to 20-40 um broad.

Etymology: Greek *myco-* (fungus) + *Clelandia,* the "Cleland fungus" in honor of Prof. J.B. Cleland (1878-1971), eminent mycologist at the University of Adelaide until his retirement in 1948, and Latin, *arenacea* (sandy), in reference to the sand adhering to the peridium.

Family: Pezizaceae.

Distribution, habitat and season: Australia, southeastern Northern Territory, sandy soil, spinifex/shrubland plains, in dunefield swales, or near groves of *Acacia aneura,* sometimes near dunes; June through September.

Collections examined: HOLOTYPE (of *Clelandia arenacea):* AUSTRALIA: *Northern Territory-* Karrinyarra (Mt. Wedge Station), 22°39'S, 131°52'E, J. B. Cleland, 25 Aug. 1957 (ADW 15885, isotype OSC 39481). PARATYPE: *Northern Territory-Haast's* Bluff Aboriginal Land Trust, 23°29'S, 130°34'E, J.B. Cleland, 3 Sep 1957 (ADW 8615, OSC 130645). OTHER COLLECTIONS: *Northern Territory-Road* to Wingkilina 10 km from Docker River Road, 25°14'S, 128°34'E, A. C. Kalotas AK455M, July 1980's but year not recorded in the collection notes (NT M464, OSC 131125). Uluru Kata Tjuta National Park, 25°17' 25"S, 130°54'30"E, I. Richards & S. McAlpin, 26 Aug 1998 (NT M588, OSC 131132) and 25°17'665"S, *130°* 52′899″E, S. McAlpin, 14 Jun 2000 (NT s.n., OSC 131124).

Comments: This species is the type of the genus *Mycoclelandia,* originally described by Trappe (1979) as *Clelandia.* That name proved to be invalid, because it had already been used for a monotypic genus in the Violaceae. Trappe and Beaton (1984) corrected the error for both this and the following species. Cleland annotated his Haast's Bluff collection, "Subterranean in sandy soil. Dug up and eaten by natives."

Mycoclelandia bulundari (G. W. Beaton) Trappe & G. W. Beaton, Trans Br Mycol Soc 83:536. 1984.

≡ *Clelandia bulundari* G. W. Beaton in Beaton & Weste, Trans Br Mycol Soc 79:459–460.

Ascomata up to 8 x 6 cm, subglobose-cerebriform. *Peridium* as preserved in formalin glabrous to roughened, dark brown, with adhering sand. *Gleba* light brownish orange, with elongated, labyrinthine chambers partly empty to filled by opposing hymenia or stuffed with hyphae. *Taste* and *odor* not recorded.

Ascospores (Fig. 3e) in water hyaline singly but faintly brown in mass, broadly ellipsoid, 12-18 x 8-12 um, length/width ratio 1.4-1.75, smooth, some with a de Bary bubble, uni- to biguttulate, the walls up to 1 um thick. *Asci* hyaline, cylindric to clavate or irregular, tapered to an obscurely forked base, 130-165 x 15-24 um; in Melzer's reagent, diffusely blue, 8-spored, thin-walled, with 8 uni- or biseriate spores. *Paraphyses* hyaline, ±8 um broad, as long as or somewhat shorter than the asci

Peridium and gleba of ellipsoid to globose cells up to 45 um broad.

Etymology: Genus as per *M. arenacea,* and *bulundari,* the word for this truffle in the Gugadja language (Beaton and Weste 1982).

Family: Pezizaceae.

Distribution, habitat and season: northeastern Western Australia to the Tanami Desert of Northern Territory, red

sandy soil in spinifex country, sometimes in dunefield swales. May through July, September.

Collections examined: HOLOTYPE (of *Clelandia bulundari):* AUSTRALIA: *Western Australia-* Near Lake Gregory, Halls Creek, ca 20°12'S, 127°27'E, A. Peile 20B, July 1979 (PERTH-UWA 2427; isotypes MELU, OSC 131128). OTHER COLLECTIONS: *Northern Territory-* Sangsters Bore, Tanami Desert, 20°53'S, 130°24'E, D. Jampijinpa, 2 Sep 1986 (NT M577, OSC 131127). Tanami Desert, 20°26'S, 131°45'E, D. Gibson & J. Cole, 15 Jun 1983 (NT M505, OSC 131129). Uluru Kata Tjuta National Park, 20 km E of Mutijulu along Old Curtin Springs Road, 25°30.314'S, 131°14.045'E, S. McAlpin, 16 June 2000 (NT n.s., OSC 130648). Yulara, 25°14'S, 131°00'30"E, P. K. Latz 16218,31 May 2000 (NT n.s., OSC 131130).

Comments: According to Beaton and Weste (1982), the species "is apparently plentiful in favourable seasons in the area around Halls Creek, and when available in sufficient quantity is used as food by the Aboriginal Gugadja." They quote the collector, Fr A. Peile: "The term *bulundari,* I believe, applies to a single fungus. All the old people here know about it, so it would be widely distributed in spinifex sand country ...When you dig it out of the ground you have to break it open before eating it as that is the only way you can differentiate it from another fungus having the same external appearance (which) ... cannot be eaten." Notes with the collection by Steve McAlpin state, "Detected by crack in the sand with raised piece of earth. The upper surface of this witita was ca 10 cm below the surface of the ground."

Reddellomyces westraliensis (G.W. Beaton & Malajczuk) Trappe, Castellano & Malajczuk, Aust Syst Bot 5:609–610. 1992.

≡ *Labyrinthomyces westraliensis* G.W. Beaton & Malajczuk. Trans Br Mycol Soc. 86:505. 1986.

Ascomata (Fig. 5) up to 18 x 28 mm, irregularly subglobose to ellipsoid or lobed and furrowed. *Peridium* white and pubescent in youth, later becoming cream color to brownish white and glabrous. *Glebal* trama white, with gray, ellipsoid to labyrinthine chambers up to 5 x 1.5 mm and partly empty to filled by opposing hymenia. *Taste* and *odor* mild.

Ascospores (Fig. 3f) in water hyaline in youth, pale brown at maturity, globose, smooth in youth, at maturity 27-42 um broad excluding the ornamentation of hyaline, rounded warts 1-4 (-6) x 1-4 um, the warts swelling in KOH, multiguttulate, the walls up to 5 um thick, with two equal layers. *Asci* hyaline, borne in a hymenium, cylindric to clavate, ventricose or reniform-saccate, 180-285 x 40-60 (-70) um, tapering to a narrow, obscurely forked base, nonamyloid, the walls in KOH and Melzer's reagent

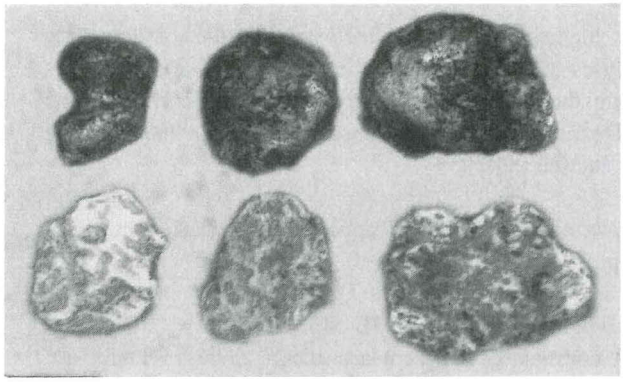

Fig. 5 Fresh ascomata of *Reddellomyces westraliensis,* ×1, surface view in *upper row,* cross-section in *lower row*

swelling up to 5 um thick, by maturity generally <2 um thick with (1-) 3-5 (-6) irregularly disposed spores. *Paraphyses* up to 4.5 um broad, hyaline, the apices rounded, equal to or longer than the asci.

Peridium 300--400 um thick, in youth with a suprapellis of tangled, emergent hyphae 2-4 um broad but these soon collapsing, the pellis and subpellis of interwoven or occasionally somewhat inflated hyphae 2-7 (-10) um broad. *Gleba* a tissue of hyphae similar to the subpellis.

Etymology: In honor of Australian biologist Dr. Paul Reddell + Greek *myces* (fungus), "the Reddell fungus," and a contraction of "Western Australia" with the Latin suffix *"-ensis"* (place of occurrence) (Beaton and Malajzcuk 1981).

Family: Tuberaceae.

Distribution, habitat and season: Southern part of Northern Territory, under *Eucalyptus microtheca* F. Muell. in a swale along Roe Creek southwest of Alice Springs. July. Also occurring in dry habitats from Western Australia east to South Australia and New South Wales.

Collection examined: AUSTRALIA: *Northern Territory*— along Highway 87, 3.9 km SW of Roe Cr. crossing, 23°51′S, 133°49′E, J. Trappe 25433, 9 Jul 2000 (CANB, OSC 111640).

Comments: This is not a desert species in the strict sense, as it occurred in a moist swale, Nonetheless, it occurs in an otherwise arid landscape and is the first record of the species for the Northern Territory. It was originally described from forests and woodlands of southwestern Western Australia and South Australia (Trappe et al. 1992). Subsequently, it has been discovered in box-ironbark woodlands of the South West Slopes of New South Wales and under introduced *Eucalyptus* spp. planted in Spain (Trappe, unpublished data). As it is too small to hump up the soil, it is difficult to find and thus may not have been important as a traditional Aboriginal food. The collection site was not far from the type locality of *Mattirolomyces mulpu.* In the phylogenetic analyses, *Reddellomyces westraliensis* branched together with *R. donkii* (data not shown).

The inclusion of *R. westraliensis* in the phylogenetic analyses did not change the position of *Redellomyces* within the Tuberaceae, thereby confirming earlier reports (O'Donnell et al. 1997; Hansen and Pfister 2006; Leesae and Hansen 2007).

Ulurua nonparaphysata Trappe, Claridge & Kovacs, gen. & sp. nov.

MycoBank MB 513072, MB 513073

Ascomata usque ad 4 cm lata, sulcata, globosa vel turbinata, tomentosa, brunnea. Gleba venis labyrinthinis, vacuis vel hymeniis opponentibus repletis. Sporae hyalinae, late ellipsoideae vel subglobosae, 12-15 x 10-12 um, venucis minutis ornatae. Asci in hymenio innati, amyloidei, sporis octo. Paraphyses destitutae. Peridium glebaque cellulis multo inflatis.

Ascomata as dried ±4 cm broad, furrowed, globose to turbinate. *Peridium* tomentose, especiaIly in the furrows, brown, often appearing dark reddish brown from adherent sand. *Gleba* with labyrinthine veins partly empty to filled by opposing hymenia. *Taste* and *odor* not recorded.

Ascopores (Fig. 3g) in water hyaline, broadly ellipsoid to subglobose, 12-15 x 10-12 um, length/width ratio 1.2-1.75, at maturity ornamented with warts <0.5 um broad and tall, mostly with a de Bary bubble, uniguttulate, the walls *1-1.5* um thick. *Asci* borne in a hymenium, hyaline, cylindric to ventricose and often sinuous or strongly curved towards the base, tapered to an obscurely forked base, (115-) 150-200 x 20-30 um, in Melzer's reagent diffusely blue, the walls thin or up to 1 um thick, with 8 biseriate or clustered spores. *Paraphyses* lacking.

Peridium and *gleba* of hyaline, thin-walled, inflated hyphae 3-5 (-8) um broad at the septa, most cells inflated to 10-20 um together with clusters of sphaerocyst-like cells up to 40 um broad.

Etymology: Latinized *Uluru,* the area where the type collection was found, and Latin *non-* (not-) and *-paraphysata* (having paraphyses), "not having paraphyses" for that character distinctive among truffles that form hymenial palisades.

Family: Pezizaceae.

Distribution, habit and season: Northern Territory, known only from the type collection "from the ring of habitat classified as 'sandplain' that encircles Uluru" (collection notes by Steve McAlpin). June.

Collection examined: HOLOTYPE here designated: AUSTRALIA: *Northern Territory-Uluru* Kata-Tjuta National Park, ca 2 km E of Sunset Viewing Area, 25° 19.578'S, 131°00.61l'E, Steve McAlpin, 15 June 2000 (NT n.s., isotype OSC 131126).

Comments: The lack of paraphyses in the hymenium of *U. nonparaphysata* is unique for hymenium-forming truffle species. The molecular data indicate a close relationship between *Ulurua* and *Mycoclelandia* but the lack of paraphyses in combination with the ornamented spores suggest a new genus in the Pezizaceae. All three of the desert truffles with amyloid asci *(Mycoclelandia arenacea, M. bulundari, Ulurua nonparaphysata)* occur in Uluru Kata Tjuka National Park and surrounds.

African Kalahari species

1. Spores 10–14 µm broad excluding the ornamentation of somewhat distant, blunt rods and cones 1–2×0.5–1 (–1.5) µm. *Eremiomyces echinulatus*
1. Spores 16–22 µm broad excluding the ornamentation of crowded spines or a reticulum.
   2. Spores ornamented with densely crowded, acute spines 0.5–1.5 (–2)×≤ 0.5 µm. *Kalaharituber pfeilii*
   2. Spores ornamented with an alveolar reticulum (2–) 3–5 (–6) µm tall. *Mattirolomyces austroafricanus*

Eremiomyces echinulatus (Trappe & Marasas) Trappe & Kagan-Zur in Ferdman et al, Mycol Res 109:244. 2005.

≡ *Choiromyces echinulatus* Trappe & Marasas in Marasas & Trappe, Bothalia 11: 139-–140. 1973.

Ascomata subglobose. *Peridium* pale cream colored. *Gleba* marbled with veins enclosing hymenial palisades.

Ascospores (Fig. 3h) in water globose, 10-14 um broad excluding the ornamentation of straight, somewhat distant, obtuse rods and cones 1-2 x 0.5-1 (-1.5) um. *Asci* in a hymenium, hyaline, cylindric to ellipsoid or saccate, non-amyloid, thin-walled, mostly with 8 uni- to more often biseriate or clustered spores. *Paraphyses* hyaline, ±4 um broad, septate, thin-walled.

Peridial and *glebal* tissues of thin-walled hyphae with many cells inflated to 15-50 um broad.

Etymology: Greek, *eremio-* (desert) + *-myces* (fungus), the "desert fungus," and Latin, *echinulatus* (spiny) in reference to the spore ornamentation.

Family: Pezizaceae.

Distribution and season: South Africa, Botswana and Namibia. June.

Collections examined: HOLOTYPE: SOUTH AFRICA: *North Cape-Gordonia* near Upington, O. A. Leistner 2612, 1 Jun 1961 (PRE 42202). OTHER COLLECTION: BOTSWANA- Kgalagadi District, V. Kagan-Zur, Trappe 30593, 2000 (OSC 111368). NAMIBIA-Stampriet District, A. du Plessis, 7 May 1974 (PRE 44894).

Comments: This rare southern hemisphere desert truffle mimics the northern hemisphere forest truffle genus *Choiromyces* in macromorphology, but DNA sequences indicate that *Eremiomyces* belongs in the *Pezizaceae,*

whereas *Choiromyces* is in the *Tuberaceae* (Ferdman et al. 2005). These two genera exemplify morphologically convergent but genetically divergent evolution.

Kalahari pfeilii (Henn.) Trappe & Kagan-Zur in Ferdman et al., Mycol. Res. 109:242–243. 2005.

≡ *Terfezia pfeilii* Henn., Bot. Jahrb. 22:75. 1897.

Ascomata (Fig. 6) turbinate to obpyriform or subglobose, up to 7 (-9) x 7 cm, with a basal tangle of hyphae, roots and soil dense enough to form a stalk-like structure. *Peridium* minutely pubescent but often becoming glabrous on exposed areas, rugose, yellowish brown to dark brown with yellowish wrinkles or cracks. *Gleba* solid, marbled with white veins and yellowish white to brown fertile pockets. *Taste* mild, *odor* strongly fungoid.

Ascospores (Fig. 3i) in water globose, 16-22 (-26) um broad excluding the ornamention of densely crowded, acute spines 0.5-1.5 (-2)x < 0.5 um. *Asci* randomly clustered in fertile pockets, globose to ellipsoid or obovoid, 70-90x65-75 um,

Peridial and *glebal* tissues with many cells thin-walled and greatly inflated.

Etymology: Kalahari + Latin *tuber* (truffle), the "Kalahari truffle," and *pfeilii,* in honor of Count Joachim von Pfeil (1857-1924), German colonial politician, traveller and author who obtained the type collection.

Family: Pezizaceae.

Distribution, habitat and season: Botswana, Namibia and South Africa, Kalahari Desert and adjacent arid areas.

Collections examined: see Ferdman et al (2005) for extensive listing.

Comments: Yet another case of morphological similarity but genetic divergence, the southern hemisphere *Terfezia pfeilii* has been shown by DNA analysis to merit a separate genus from the northern hemisphere desert truffle genus *Terfezia* (Ferdman et al. 2005). *Kalaharituber* and *Terfezia* both belong to the Pezizaceae (Lessee and Hansen 2007). *K. pfeilii* is the common truffle of the Kalahari, traditionally sought by indigenous people and now an item of commerce. *K. pfeilii* has been variously misidentified in times past as *Terfezia boudieri* Chatin (Pole-Evans 1918; Ceruti 1960), *T claveryi* Chatin (Doidge 1950; Marloth 1913; Pole-Evans 1918), or *Tirmania pinoyi* (Maire) Malencon (Mattirolo 1922).

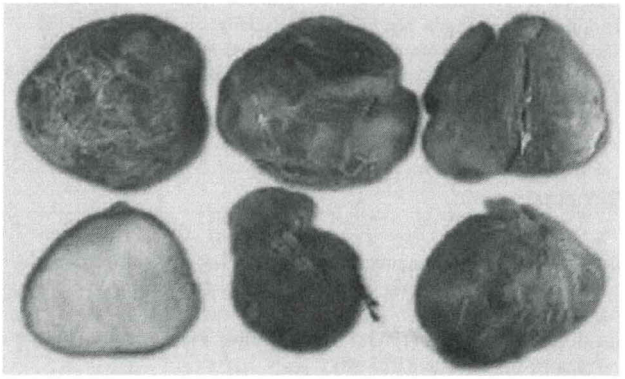

Fig. 6 Fresh ascomata of *Kalaharituber pfeilii,* ×0.5, cross-section *lower left*, others are surface views. Photograph by by G.C.A van der Westhuizen and Albert Eicker

Mattirolomyces westraliensis (Trappe & Marasas) Kovács, Trappe & Claridge, comb. nov.

≡ *Terfezia austroafricana* Trappe & Marasas in Marasas & Trappe, Bothalia 11:140–141. 1973.

MycoBank MB 513074

Ascomata subglobose. *Peridium* as dried orange brown. *Gleba* as dried ochraceous, marbled with pallid veins and pockets. *Taste* and *odor* not recorded.

Ascospores (Fig. 3j) in water globose, 25-30 um broad excluding the ornamentation of straight, truncate to obtuse spines (2-) 3-5 (-6) x 1-3 um connected by walls to form a partial to complete, alveolate reticulum. *Asci* randomly clustered in fertile pockets, hyaline, ellipsoid to obovoid, subcylindric, reniform or asymmetric, nonamyloid, thin-walled, with (4-) 8 clustered spores. *Paraphyses* lacking.

Etymology: Mattirolo- (renowned Italian mycologist who discovered the first species of the genus) + Greek, *-myces* (fungus), the "Mattirolo fungus," and Latin, *austro-* (southern) + Latin, *-african us,* (African), "southern African."

Family: Pezizaceae.

Distribution, habitat and season: South Africa, Cape Province, habitat not recorded; April.

Collections examined: HOLOTYPE: South Africa, *Cape-*Griqualand West, Barkly West, E. L. Stephens (PRE 35577, isotype OSC 58845). OTHER COLLECTION: South Africa, *Cape-Kimberley,* Wilman, 10 Apr 1918 (PRE 11542, OSC 58846).

Comments: Marasas and Trappe (1973) placed this species in the subgenus *Mattirolomyces* of the genus *Terfezia,* because its morphology resembles other species of that group in most respects. However, molecular analysis by Percudani et al. (1999) and Diez et al. (2002) clearly support restoration of *Mattirolomyces* to the independent generic status it originally had. Our phylogenetic analysis shows that the species belongs in *Mallirolomyces.*

Discussion

The results of the phylogenetic analyses suggest that Australian and southern African desert truffles represent at

least four distinct lineages within the Pezizaceae. The Australian taxa form two distinct groups, *Mycoclelandia-Ulurua* and *Mattirolomyces-Elderia,* the latter also including one African species, *M austroafricanus,* and the European *M terfezioides.* In some analyses, the hypogeous *Mattirolomyces-Elderia* clade grouped with *"Peziza* s. str. b." but with no strong support, this clade might represent a 15th fine-scale lineage additionally to the 14 already identified within the Pezizaceae by Hansen et al. (2005). The *Mycoclelandia-Ulurua* clade nested into the *Peziza depressa-Ruhlandiella* lineage of Hansen et al. (2005) but formed a separate, well-supported clade within this major lineage (Fig. 1). Although the diffuse bluing in iodine solutions by asci of *Mycoclelandia* and *Ulurua* differs from the intense apical bluing of the *Peziza* species in this lineage (Hansen et al 2005), it resembles that of the hypogeous genus *Tirmania* also in the lineage. Like *Tirmania* spp. (Alsheikh and Trappe 1983a), *Mycoclelandia* and *Ulurua* have smooth to finely ornamented spores. *Mycoclelandia* and *Ulurua* thus join the other, hypogeous, desert genera, *Tirmania* and *Terfezia,* in this major lineage.

Several features of the desert truffles are striking. All are in the Ascomycota except *Horakiella watarrkana,* the only hypogeous Basidiomycota so far reported from deserts. Of the Ascomycota, our data show that the Pezizaceae strongly predominate the desert truffle mycota in the southern hemisphere as is true of the northern hemisphere (Trappe 1979; Percudani et al 1999; Diez et al 2002). Only *Reddelomyces westraliensis* in the Tuberaceae departed from this trend: it was found in a swale along a streambank. The genus *Tuber* is notably absent from deserts, as are hypogeous members of the Pyronemataceae.

Our morphological studies show that the peridium or gleba or both of southern hemisphere desert truffles are characteristically composed of thin-walled hyphae with inflated cells, in contrast to most hypogeous Ascomycota in nonarid habitats. The same is true of nearly all northern hemisphere desert truffles (Alsheikh 1994; Alsheikh and Trappe 1983a; Trappe and Weber 2001). This would not seem a useful anatomy to conserve water in habitats that may dry out quickly after soil-wetting rain, but it could be an adaptation to enhance water and nutrient uptake and rapid transfer from the ascoma surface to the interior; that, in turn, might promote rapid maturation and spore formation. Desert rain is often intense but of relatively short duration. If followed by clear, sunny weather, the soil can dry out quickly. Truffles under those conditions need to form and mature in a short time. This was strikingly demonstrated to the senior author, who visited a known truffle producing area in central Australia 3 weeks after it had experienced drenching rains that filled dry lakes and stream beds. Fresh native truffles had, indeed, been brought to the Northern Territory Museum at Alice Springs not long after the rain, but by the time he arrived, the soil was dust dry. The only truffles he found were the *Reddellomyces westraliensis* in a moist swale along a still running creek.

The thin-walled glebal cells also appear to be an adaptation for spore dispersal. Animal mycophagy plays a role in spore dispersal in some conditions (Alsheikh and Trappe 1983b; Zak and Whitford 1986; Trappe et al. 2008a, b), but sporocarps not eaten may dry in situ. The thin-walled glebal cells and asci disintegrate into powdery fragments upon drying. Specimens that are near the surface are then exposed to wind-blown sand, which abrades away the peridium and gleba, so that the spores become airborne (Alsheikh and Trappe 1983a), or entire sporocarps may be blown about, breaking up as they go to release the powdery spores (Trappe and Weber 2001).

In conclusion, we have established that all but a few desert truffles of the African Kalahari and Australian Outback are members of the Pezizaceae. The same is true of the northern hemisphere desert truffles. The Kalahari and Outback genera are endemic to their known distributions with one notable exception, *Mattirolomyces.* Originally described from southern Europe, it is now known to occur in Asia and America (Kovacs and Trappe, unpublished data) as well as Africa and Australia.

Acknowledgements These studies were supported in part by C.S.I.R.O. Sustainable Ecosystems, Canberra, Australian Commonwealth Territory, Australia, the U.S. Forest Service, Pacific Northwest Research Station, Forestry Science Laboratory, Corvallis, Oregon, and the Hungarian Research Fund (OTKA K72776). Dr. David Albrecht, Northern Tenitory Museum, Alice Springs, Northern Territory, Australia, provided specimens of Central Australian truffles and the photograph of *Mycoclelandia.* Dr. Karen Hansen of the Swedish Museum of Natural History, Stockholm, offered many helpful suggestions on drafts of the manuscript. We also much appreciate access to collections afforded by: the Australian National Herbarium, Canberra; the Mycological Collections of Oregon State University, Corvallis; the National Botanical Institute, Pretoria; the National Herbarium of Victoria, South Yarra; the Northern Tenitory Museum, Alice Springs; the Royal Botanic Gardens, Kew; the State Herbarium of South Australia, Adelaide; the University of Melbourne Herbarium, Parkville; and the Western Australian Herbarium, Perth. Magda Nel of the University of Pretoria granted permission to use the photo of *Kalaharituber pfeilii* by G.C.A van der Westhuizen and Albert Eicker. Dr. Michael Castellano provided the photograph of *Horakiella watarrkana* spores.

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