Identity and Taxonomy of Truffle Fungi from an initial Survey at Karakamia Wildlife Sanctuary

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The Department of Environment and Conservation (DEC) Woylie Decline Project aims to determine the factors contributing to recent declines in woylie (*Bettongia penicillata*) populations in south west Australia (Wayne *et al.* 2006). One of the potential factors is the diversity and abundance of fungal food resources available for woylies, particularly truffle fungi. As part of a program to assess this factor, survey work was initiated to compare the truffle fungi at Karakamia sanctuary where woylie densities are remaining high, with fungi in areas where woylie populations are declining. The current report provides details about the fungi obtained from the initial survey at Karakamia approximately 50 km east of Perth, on 4th October 2006.

Five species were represented among 11 collections of truffle fungi from Karakamia during the survey - three Basidiomycetes and two Zygomycetes. Identifications, descriptive details, and images of the specimens are provided below (Tables 1, 2). Example collections of each of the species have been preserved and permanently lodged at the WA Herbarium (PERTH). Some of the truffle fungi from this survey remain identified only to genus pending further collections or more detailed comparative analyses.

Only one of the species - *Dermocybe globuliformis* – could be matched positively to any of the approximately 40 "reference species types" of truffles from the DEC Manjimup-based Woylie Decline Project surveys. A species of *Hysterangium* superficially resembling, but microscopically different to, species 7 of the "reference species types" was collected once on the mid-slope plot and twice on the ridge plot. This fungus closely matches an un-named species of *Hysterangium* associated with *Eucalyptus platyphylla* in Queensland (H4684 in PERTH, collected near Gunawarra in May 1991).

Table 1: Summary of fungi obtained from the initial survey at Karakamia sanctuary
[Protocol: three 50 x 20 m plots – valley, mid-slope, ridge; 100 person minutes per plot]

Identity of Fungus	KAR Code	Herbarium Code	Woylie Decline Project Ref. Fungi Type	Comments
Cystangium sessile	1	E8383	?	Many genera and species with similar appearance
Cystangium sessile	2	none	?	Many genera and species with similar appearance
Cystangium sessile	3	E8384	?	Many genera and species with similar appearance
***Hysterangium* sp. nov.**	4	E8385	?	Many similar species of this genus (including 'type 7')
Cystangium sessile	5	E8386	?	Many genera and species with similar appearance
Dermocybe globuliformis	6	E8387	13	Distinctive fruit bodies occur near or at surface amid extensive yellow mycelial mats
***Hysterangium* sp. nov.**	7	none	?	Many similar species of this genus (including 'type 7')
***Hysterangium* sp. nov.**	8	none	?	Many similar species of this genus (including 'type 7')
***Glomus* sp.**	9	E8388	?	Zygomycete genus reported in Australian mammal mycophagy. This particular species may be epigeous?
***Hysterangium* sp. nov.**	10	none	?	Many similar species of this genus (including 'type 7')
Glomus caledonium	11	E8389	?	Zygomycete genus reported in Australian mammal mycophagy
Lactarius eucalypti	-	none	-	Epigeous
***Inocybe* sp.**	-	none	-	Epigeous
Laccaria lateritia	-	none	-	Epigeous

Many of the truffle fungi obtained in the survey at Karakamia are endemic to Australia but known to be widespread throughout the continent. *Cystangium sessile* and *Dermocybe globuliformis* have been recorded in many parts of south-west and south-east Australia. In the Perth region *C. sessile* occurs on the Swan coastal Plain and the Darling Scarp, whereas *D. globuliformis* favours the lateritic soils of the Scarp and has not been confirmed on the Swan Coastal Plain. Unlike the Basidiomycete truffles mentioned above, and more than 95% of other known Australian truffle species (Bougher & Lebel 2001), the sporocarpic (fruit body producing) Zygomycete - *Glomus caledonium* - is not endemic. It occurs in tropical and temperate regions of Australia and is also known from many other parts of the world (McGee & Trappe 2002).

Only three species of epigeous fungi (mushrooms, corals, puffballs etc…), all Basidiomycetes, were observed on the survey plots (Table 1). Never the less, a high diversity of epigeous macro fungi is likely to occur at Karakamia sanctuary, as this is the case for eucalypt forests/woodlands in general. There is evidence that mammals at Karakamia have been feeding on epigeous fungi as well as truffle fungi (Karakamia staff, pers. comm.). It is recommended that both categories of fungi be considered in future surveys. The distinction between the two categories of fungi is often blurred, e.g. *Dermocybe globuliformis* fruits in mycelial mats often exposed above the soil/litter (Bougher & Trappe 2002), and some sporocarpic Zygomycetes such as some *Glomus* species are truffle-like in form but fruit above the ground.

All truffle fungi, including the fungi obtained in this survey, are presumed to be ectomycorrhizal, i.e. they have and depend upon mutually beneficial partnerships with plants. In order to gain a better understanding of the ecology of woylie habitats it is recommended that data about the identity, abundance, and health of putative ectomycorrhizal host plants at Karakamia be assessed in relation to fungi survey data.

Unusually low rainfall in the autumn and winter period prior to this survey probably greatly affected the abundance and diversity of fungi observed in the survey. This is evident by the few collections made, and the few fruit bodies - often only 1, maximum of 3 - per collection. Far more truffle fungi species are likely to occur at Karakamia sanctuary than the five species captured in this pilot survey. The current survey may have been undertaken towards or beyond the end of the likely main fruiting season for fungi in the region. It is recommended that multiple surveys be undertaken at Karakamia to adequately assess the diversity and abundance of fungal food resources available to mycophagous animals at the sanctuary. Multiple surveys are also required to make accurate comparisons between the fungi at Karakamia and fungi of areas where woylie populations have declined.

Table 2: Descriptive details: truffle fungi specimens from survey at Karakamia sanctuary

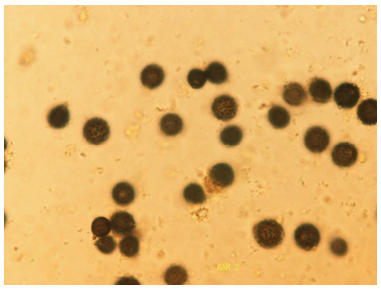
Morphological Details	Images
Cystangium sessile KAR 1, E8383 A single specimen only. Dry weight: 0.056 g. *Fruit body* 10 x 7 mm, broadly ellipsoid. *Peridium* cream, not bruising, dry, smooth, thin, single-layered in TS. *Gleba* white, of radially oriented empty minute locules, no latex evident. *Sterile tissue* abrupt indentation at base of fruit body, with a small (1x 1 mm) sterile, non-emergent base. *Spores* 8.3-9.0 µm diam., globose, densely spinose, spines tubular up to 0.6 µm tall, not interconnected at base, strongly amyloid. *Peridium* cellular elements present, some projecting hyphae.	 
Cystangium sessile KAR 2 A single specimen only. Dry weight: 0.029 g.	

Table 2 (cont.): Descriptive details: truffle fungi specimens from survey at Karakamia sanctuary

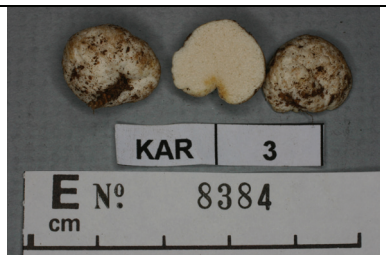
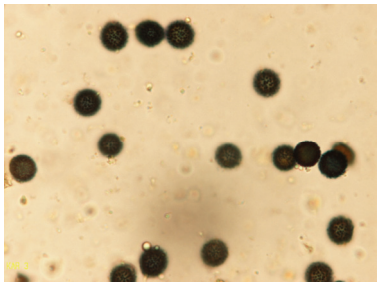
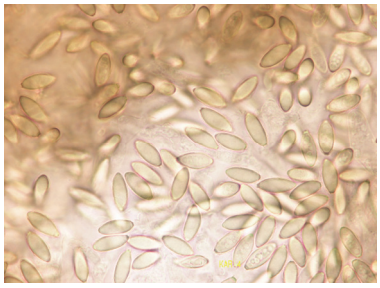
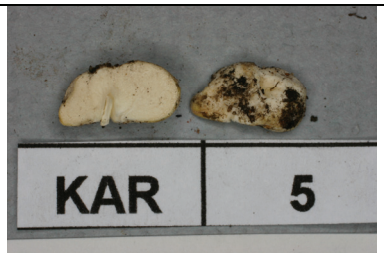
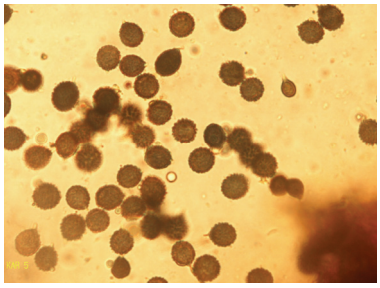
Cystangium sessile KAR 3, E8384 Two fruit bodies. Dry weight: 0.186 g. *Fruit bodies* up to 15 x 12 mm, broadly ellipsoid. *Peridium* thin, single layered in TS, surface dry, smooth, white. *Gleba* white, empty loculate, slight radial pattern evident. *Sterile tissue* again (like in E8383) there is an abrupt indentation at the base of the fruit body but no development of a columella – only a small plug of tissue at the indentation but not emerging. *Spores* globose, strongly amyloid, densely spinose, isolated spines, tubular or gently tapering towards a blunt apex. *Peridium* cellular elements and some projecting hyphae.	 
***Hysterangium* sp. nov.** KAR 4, E8385 Two fruit bodies. Dry weight: 0.363 g. *Fruit bodies* up to 15 x 10mm, sub-globose/sub-ellipsoid. *Peridium* thick (up to 1mm), two-layered: inner layer thick whitish-watery, abruptly delineated from the gleba; outer layer very thin, deep rose-pink in section and on surface (near 8E7), shiny, smooth, dry. Surface is cream at least in part when first un-earthed but rapidly bruises and changes to the deep reddish colouration which then after persists. *Gleba* loculate, empty locules but with incomplete gelatinization evident in some parts, dull olive greenish (near 30F4 but more olive), no strong radial pattern evident. Sterile tissue absent or as a truncate, dull gelatinized/watery columella. *Spores* 11.7-12.9 x 4.0-5.1 µm, fusoid with truncate collar-like apiculus, hyaline to pale grayish-greenish in KOH, smooth-walled, thin-walled, perisporium adhering and sometimes wrinkling but never flanging.	 
Cystangium sessile KAR 5, E8386 A single specimen only. Dry weight: 0.048 g. Note in this collection: the presence of a narrow emergent white stipe 2 x 0.5 mm, emergent but not projecting beyond the basal indentation, and not intruding into the gleba.	 

Table 2 (cont.): Descriptive details: truffle fungi specimens from survey at Karakamia sanctuary

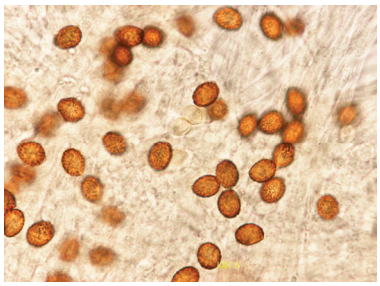
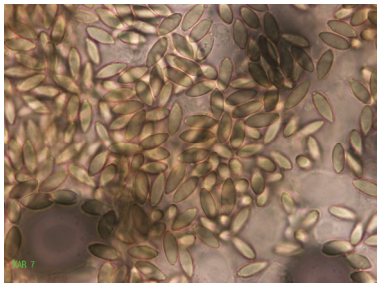
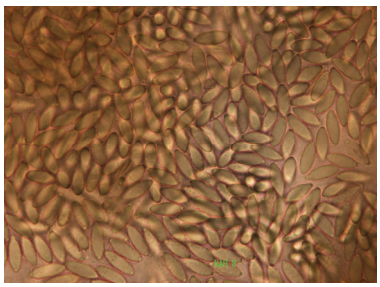
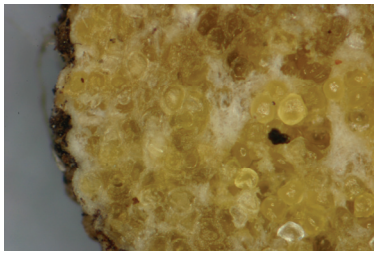
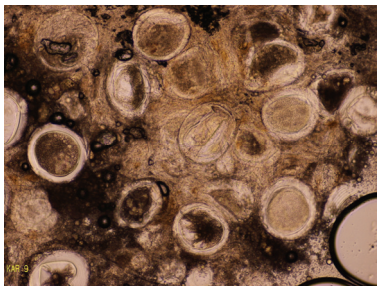
Dermocybe globuliformis KAR 6, E8387 Two fruit bodies. Dry weight: 0.071 g. Characterised by: (i) golden yellow fruit bodies amid same colour mycelial mats; (ii) squat stature with short stipe; (iii) persistent thick silky yellow partial veil; (iv) spores e.g. 9.9 x 7.4 µm, 9.5 x 6.7 µm, broad ellipsoid, verrucose.	 
***Hysterangium* sp. nov.** KAR 7 A single specimen only. Dry weight: 0.125 g.	 
***Hysterangium* sp. nov.** KAR 8 A single specimen only. Dry weight: 0.125 g.	 

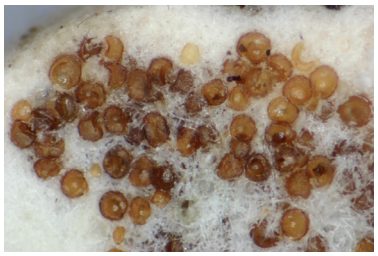
Table 2 (cont.): Descriptive details: truffle fungi specimens from survey at Karakamia sanctuary

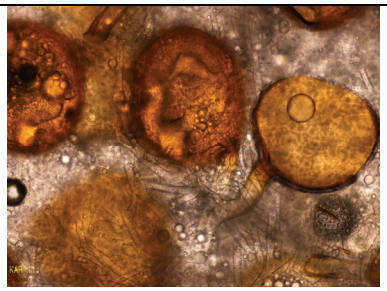
***Glomus* sp.** KAR 9, E8388
A single specimen only. Dry weight: 0.033 g.
Fruit body 6 x 3 mm, ellipsoid. *Peridium* thin, smooth, dry, dirty whitish, lacking rhizomorphs. *Gleba* mottled cream and dull yellow to the eye; under hand lens globose, shiny spores embedded in gleba visible. *Sterile tissue* no discernable columella or stipe tissue. *Spores* globose to subglobose, 150-200 µm wide, pale (not coloured) in KOH, walls double-layered: inner wall glassy 4-5 µm wide; outer layer multi-laminated 18-25 µm wide also glassy. Attachment by single hypha 9 µm wide, unswollen, slightly occluded, hyaline, thin- and smooth-walled. Trama hyaline, densely compacted hyphae, mostly gelatinized with most walls dissolved, hyphae (when intact and visible) mostly less than 3 µm wide.

***Hysterangium* sp. nov.** KAR 10
A single specimen only. Dry weight: 0.021 g.

Glomus caledonium KAR 11, E8389
A single specimen only. Dry weight: 0.039 g.
Fruit body 7 x 5 mm, ellipsoid, with shallowly depressed base. *Peridium* thick (up to 1 mm), white in TS and surface, TS composed of dense fibrous to cottony white hyphae (which extend into the gleba and are more loosely intertwined there); surface white to cream with some tan brown regions, smooth to the eye but with surface white fibrils evident under hand lens, and some adhering and embedded soil and organic debris. *Gleba* tough, dry, dark tan brown, granular to the eye, under hand lens abundant loose cottony white hyphae (some almost crystalline-like due to right angle branching of them). Embedded in these hyphae are shiny, globose smooth spores, brown, each with a dark "rind" and paler interior, not in clusters but separated from each other. *Spores* bright golden brown in KOH, 150-220 µm wide, globose, with a double wall: inner wall dark honey brown, 5-8 µm wide, finely laminated; outer layer of mucilaginous, hyaline material, 1 – 9 µm wide. Attachment by single aseptate (but with yellowish plug of material in some) hypha, thick walled (up to



15 µm thick) especially near attachment point tapering down to about 80 µm from attachment point. Two or more spores may arise from a branched pedicel hypha but no centrum observed, nor any massive clusters. *Matrix hyphae* hyaline to glassy, 7-20 µm wide, smooth-walled, thick-walled (up to 1.5 µm), right-angle or near so branching predominant. *Peridium* a loose trichodermium of hyaline, smooth, non-septate hyphae 5 - 9 µm wide, thin-walled, apices not swollen (but cytoplasm easily streams and bubbles out), many of the terminal hyphae sinuous or knobbly/gnarled.	

Field Survey Team: initial fungi survey at Karakamia sanctuary

Marika Maxwell and Neale Bougher (DEC), and volunteers/staff/students associated with Karakamia sanctuary including: Berdina Ballast, Simon Cherriman, Andrew Hide, Alison McGilvray, Joy McGilvray, Mac McGilvray, and Jacqui Purvis.

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