Woylie Conservation Research Project

Preliminary Survey of Hypogeous Fungi in the Upper Warren Region

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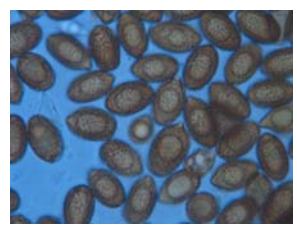

Department of Environment and Conservation

SUMMARY

As part of an integrated study on woylie decline in the Upper Warren region, a survey on hypogeous fungi was undertaken to test survey methods for a more detailed study on food resources for woylies. Surveys were undertaken in June, August and September 2006, at five separate locations in the Upper Warren region. Thirty-four species of hypogeous fungi were recorded with the highest diversity occurring in August. All species except one, *Royoungia* sp., have previously been recorded as being consumed by native mammals in Australia. *Royoungia* sp. is a new record for the genus in Western Australia.

Acknowledgements

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Cover photos: Top left, *Endogone* sp.; top centre, *Scleroderma* sp.; top right, spores of *Descomyces angustisporus*; bottom left, *Descomyces angustisporus*; bottom right, woylie (*Bettongia penicillata*).

Introduction

The woylie (*Bettongia penicillata*) was once widespread throughout southern Australia, but has undergone significant reductions in both numbers and range since European settlement. By the 1960s only three small isolated populations remained in southwest Western Australia (Start *et al.* 1995). Woylie numbers have increased since the 1970s following the introduction of fox-baiting programs, such as Western Shield, and reintroductions into the area. Detailed ecological studies of woylies commenced in the Perup Nature Reserve in the 1970s, and provide baseline population data for the species (Christensen 1980, Burrows and Christensen 2002). The woylie recovery was sufficient to de-list the species from State and Commonwealth endangered/threatened species lists in 1996. However, woylie numbers have declined by as much as 90% in the years between 2000 and 2003 (Wayne *et al.* 2006), despite on-going fox-baiting programs. The Department of Environment and Conservation has been investigating the possible reasons behind the decline (Wayne *et al*. 2006), and as part of the project an assessment of woylie food resources, of which hypogeous fungi is a component, is being conducted.

In Australia, six species of bettong, including the woylie, depend on hypogeous fungi as a substantial part of their diet. Approximately 2000 species of native hypogeous fungi are estimated to occur in Australia, with about 90 genera and 300 species currently known (Bougher and Lebel 2001). Analyses of stomach contents of woylie revealed seasonal variation in spore types and numbers, with peak spore diversity occurring in spring, and a smaller peak in autumn (Christensen 1980), indicating that hypogeous fungi are more abundant or available in the spring. During times of peak fungi availability, body condition and fecundity of the Tasmanian bettong (*Bettongia gaimardi*) increased when fungi comprised up to 90% of their diet (Johnson 1994). Variations in stable isotope ratios were used to trace the sources of nutrition in northern bettongs (*Bettongia tropica*) and showed that the majority of dietary carbon and almost all the nitrogen were derived from hypogeous fungi (McIlwee and Johnson 1998).

Given the prevalence of mycophagy within *Bettongia* spp., a survey and pilot study was undertaken to aid in the development of more detailed research of woylie food resources. The principle aims of this pilot study were to;

- Conduct a preliminary assessment of whether there are any relationships between species and abundance of hypogeus fungi and woylie abundances and population trends.
- Assess the feasibility and sampling requirements required to more rigorously test whether there are any significant differences in hypogeous fungi available as food resources in relation to woylie abundances and population trends

Methods

As an integrated component of the woylie population comparison study (PCS) (Wayne *et al*. 2006), surveys for hypogeous fungi were undertaken in June, August and September 2006 at five locations, Warrup, Winnejup, Balban, Keninup and Boyicup, northeast of Manjimup (Fig. 1). Having undergone recent decline, Boyicup and Winnejup represent sites with very low-density Woylie populations. Warrup, Keninup and Balban represent the last remaining sites with moderate-density populations. Each fungal survey location was established within five kilometres of existing PCS trapping grids. For these preliminary surveys, time since fire was not considered when selecting locations, except that no fire had occurred in the previous 6 months.

Because topography has been shown to have an effect on the distribution and abundance of certain hypogeous fungi (Claridge *et al.* 1996), three plots each were established in valley, mid-slope and ridge areas of each location. A total of 45 plots, each 20 x 50 m with the long edge running along the slope, were established. Plot size was determined following advice from T. Lebel (Royal Botanic Gardens, Melbourne) and a review of resources available to undertake the survey. Hypogeous fungal surveys result in disturbance of litter and soil to a depth of about 10-15 cm, therefore each plot was utilised only once. One set of plots (comprising a valley, mid-slope and ridge plot) was monitored at each location in June, August and September 2006.

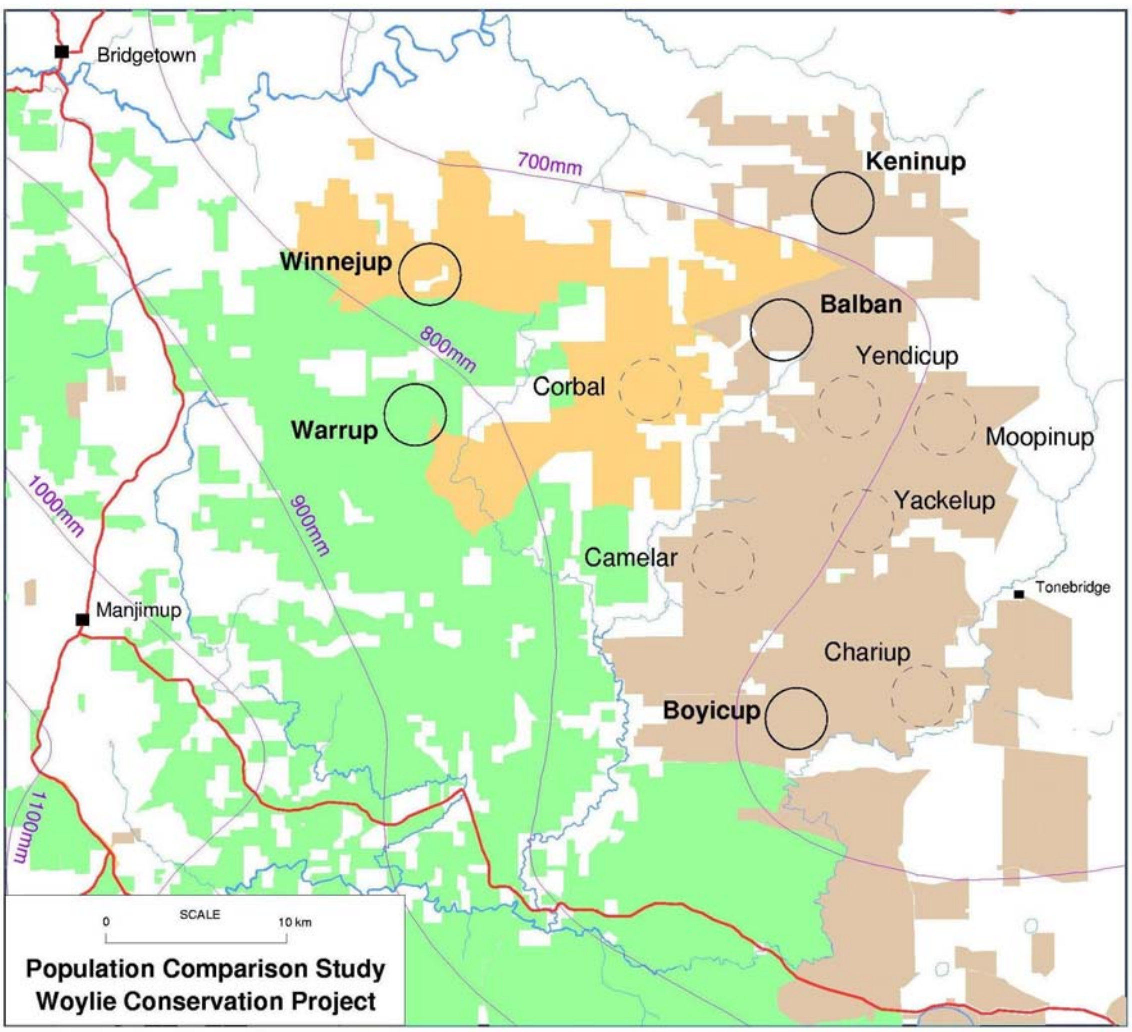

Figure 1. Location of hypogeous fungi survey sites in the Upper Warren region (showing isohyets).

Hypogeous sporocarps were collected by raking the litter and organic soil layer with a four-pronged Canterbury hoe to a depth of 10-15 cm. Soil was then replaced to minimise disturbance. Hypogeous sporocarps are generally distributed non-randomly in the landscape as discrete clusters (Trappe *et al.* 1996) in mycorrhizal association with roots, often near the base of host trees and shrubs (Claridge *et al* .1993). Therefore, raking was concentrated at the base of trees and understorey shrubs and around logs where moisture can be retained in the soil. Each plot was raked for the equivalent of 100 person minutes, with survey time varying depending on the number of participants. Generally five personnel raked plots for 20 minutes. The aim was to cover approximately 25-40% of the total plot area, with effort made to distribute the search evenly across each plot.

Each fungal collection was assigned a collection number on site and taken back to the laboratory for preliminary macroscopic identification. In the laboratory, each collection was briefly described, assigned a species number and photographed. Collections were then air dried and weighed (grams) on an analytical precision balance to three decimal places. It is intended that each collection will be lodged at the Western Australian Herbarium (PERTH). At the conclusion of the field survey each species “reference type collection” was microscopically examined, photographed and where possible identified.

Soil and litter moisture also effects fruiting of fungi. Rainfall (and SDI) data from the automatic weather stations at Manjimup, Bridgetown and Rocky Gully was collected from the Australian Government Bureau of Meteorology (2007) website in order to retrospectively examine climatic conditions prior to and at the time of survey.

Additional data collected at each site, but not utilised in this preliminary analysis

Vegetation structure and floristics, including tree basal area, overstorey, midstorey and lowerstorey species cover and strata heights, were recorded in each plot. All plots were assigned a vegetation health score and *Phytopthora cinnamomi* presence score. Soil colour and type were also noted along with the amount of litter in each plot. To aid in determining relationships between hypogeous fungal diversity and abundance, leaf litter and soil moisture data was collected during the September fungal survey.

Signs of woylie nests, remains or scats were also noted, along with signs of digging activity. Digging activity was scored by establishing circular plots, 7 m radius at each of the four corners of fungi survey plots. The number and age of woylie diggings was recorded in each quadrant of each circular plot along with its location in proximity to trees, understorey shrubs, coarse woody debris or open ground.

Results and Discussion

During the three surveys, 388 collections comprising 826 sporocarps of hypogeous fungi were made. Thirty-four species of fungi were recognized. Thirty-one species were Basidiomycetes; two were Glomeromycetes (*Endogone* spp.) and one was an Ascomycete (*Hydnoplicata convoluta*) (Table 1, Appendix 1). Seven were identified to species level, 15 were identified to genus only.

Table 1. Species of hypogeous fungi recorded in surveys (WDP = woylie decline project).

WDP Species reference number	WDP reference collection number	Species name	Number of Collections	Number of sporocarps collected	Total dry wt of each species (gms)
1	001	*Hysterogaster* sp.	0	0	0
2	002	*Alpova*-like	4	12	4.945
3	008	Unknown	5	92	6.849
4	052	*Descomyces angustisporus*	9	61	2.955
5	141	*Cystangium* sp.	12	114	8.326
6	247	*Octaviania* sp.	6	13	2.543
7	013	*Hysterangium* sp.	9	116	12.012
8	030	*Pseudohysterangium* sp.	3	14	4.485
9/8a/9a	010	*Scleroderma* sp. A	8	48	6.808
10	082	*Royoungia* sp.	3	5	0.364
11	121	Unknown	2	3	0.907
12	113	*Pogiesperma* sp.	5	33	3.845
13	117	*Dermocybe globuliformis*	9	148	10.295
14	116	Unknown	1	4	1.123
15	147	*Hydnoplicata convoluta*	3	3	0.586
16	152	Unknown	2	7	0.735
17	094	*Endogone* sp.	4	6	1.118
18	108	Unknown	1	5	3.020
19	190	*Hysterangium* sp.	2	6	0.763
20	221	*Endogone* sp. *?*	2	3	0.245
21	234	*Gymnomyces* sp.	3	12	0.837
22	241	Unknown – sterile	6	10	0.446
23/28/33	197/259 /389	*Hysterangium aggregatum*	4	40	3.995
24	198	*Pseudohysterangium* sp.	2	4	0.761
25	210	*Mesophellia* sp.	2	2	0.611
26	213	*Hysterangium* sp.	1	2	0.084
27	219	Unknown	1	2	0.033
29	277	*Protoglossum luteum*	1	1	0.615
30		Unknown	4	6	1.215
31	274	*Scleroderma* sp. (immature)	1	3	1.195
32/17a	454/186	*Cystangium sessile*	5	41	3.139
32a	455	*Gymnomyces* sp.	4		
34	364	*Descomyces* sp.	1	1	0.019
35	400	*Rhizopogon* sp.	1	2	0.075
36	401	*Mesophellia angustispora*	1	3	0.560
37	403	Unknown	1	2	0.420
38	385	*Phallobata/Protubera/Gelopellis*	1	1	0.086
		TOTAL	124	826	84.539

There was not a strong relationship between rainfall recorded at Bridgetown, Manjimup or Rocky Gully (Fig. 2) during the period of this study and the diversity and abundance of hypogeous fungi recorded at each site (Figs 3 & 4). The average rainfall pattern shown in Figure 1 suggests that Bridgetown data may be applicable to the Warrup site, but neither Manjimup nor Rocky Gully stations correlate with the isohytes associated with the other sites. Rainfall patterns in the Upper Warren region appear to be variable and highly localized. Future hypogeous fungal surveys will need to include the installation of portable weather stations at each site in order to analyse, in appropriate detail, relationships between climatic conditions and fungal species diversity and richness.

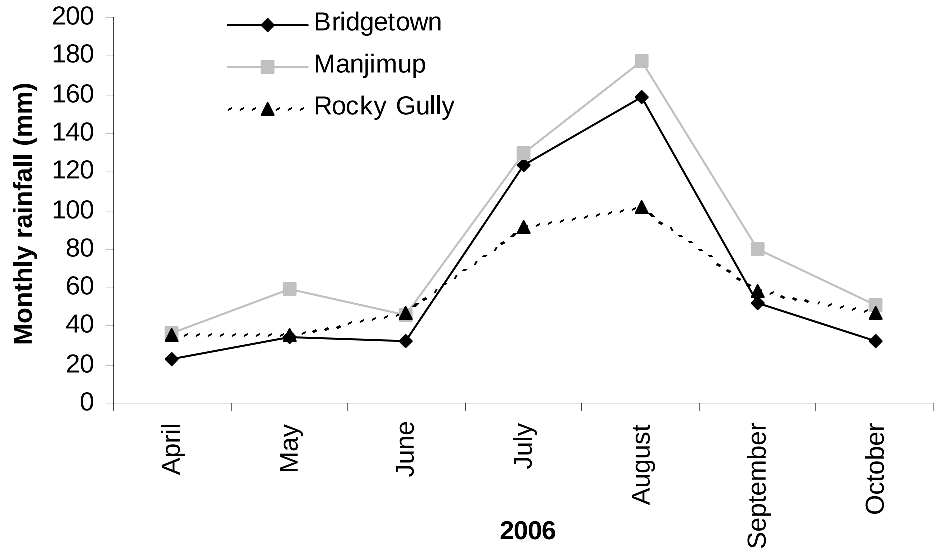

Figure 2. Total monthly rainfall recorded at Bridgetown, Manjimup and Rocky Gully from April to September 2006 (Australian Bureau of Meteorology, 2007).

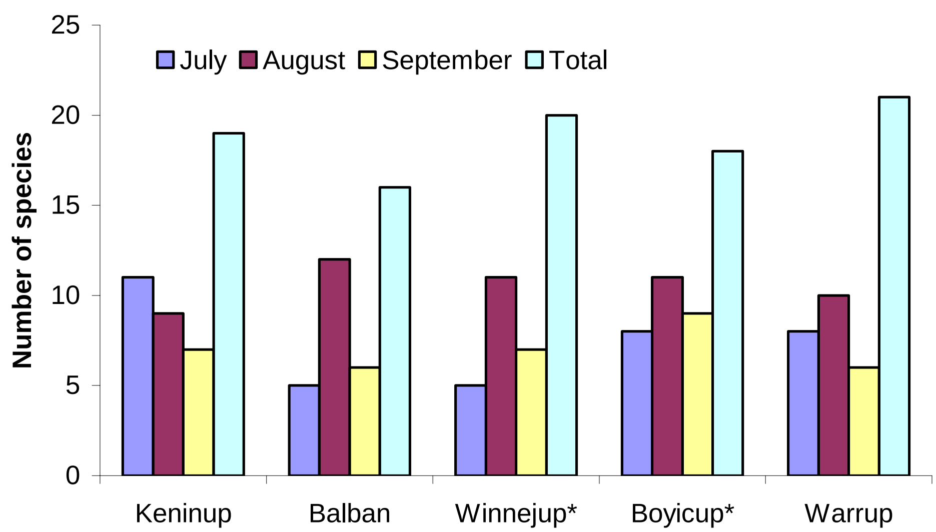

Figure 3. The total number of species of hypogeous fungi recorded at each location on each survey. * = sites with low woylie populations.

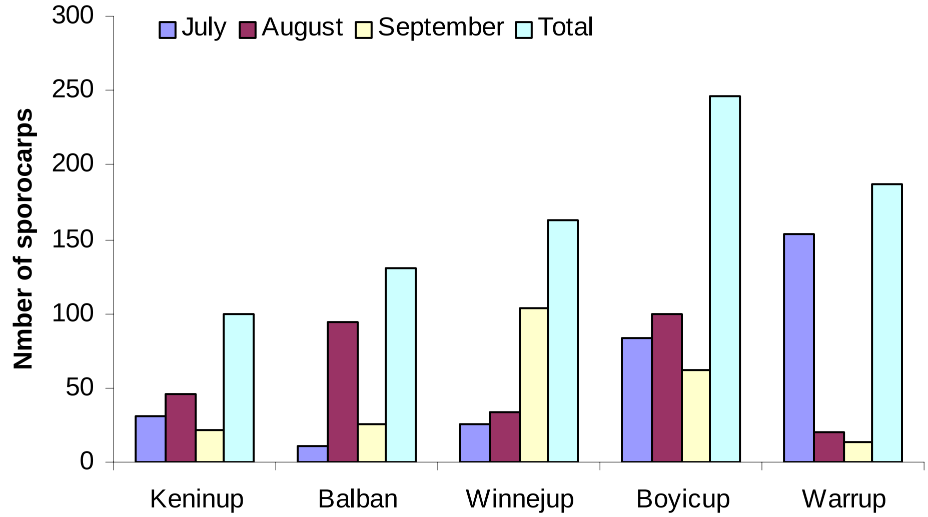

Figure 4. The number of hypogeous fungal sporocarps recorded at each location on each survey.

The highest species diversity and abundance was generally recorded in August, during the peak winter rainfall period (Fig. 2), at all locations except Keninup where the highest diversity was recorded during the July survey (Fig. 3) and at Warrup and Winnejup where the highest abundance was recorded in July and September respectively (Fig. 4). Yet overall species diversity was similar at all locations (Fig 3) and overall abundance appeared to be related to the rainfall gradient with the lowest at Keninup and Balban and the highest at Boyicup and Warup (Fig. 4). Sporocarps were also most abundant in valleys and on midslopes at all locations except Boyicup (Fig. 5), which also suggests that soil and litter moisture may influence abundance. Sixteen species were recorded at Balban, 18 at Boyicup, 19 at Keninup, 20 at Winnejup and 21 at Warrup (Fig. 3).

At each location species composition varied and only four species, *Cystanguin* sp. (WDP sp.5), *Dermocybe globuliformis*, *Descomyces angustisporus* and *Hysterangium* sp. (WDP sp.7) were common to all locations.

The most abundant species collected were *Dermocybe globuliformis* (WDP sp.13), *Hysterangium* sp. (WDP sp.7), *Cystangium* sp. (WDP sp.5), Unidentified (WDP sp.3), and *Descomyces angustisporus* (WDP sp.4) (See Table 1). When air-dried weight was considered, the most productive species were *Hysterangium sp.* (WDP sp.7) followed by *Dermocybe globuliformis* (WDP sp.13), *Cystangium sp.* (WDP sp.5), unidentified (WDP sp3) and *Scleroderma* sp. (WDP sp.9) (Table 1).

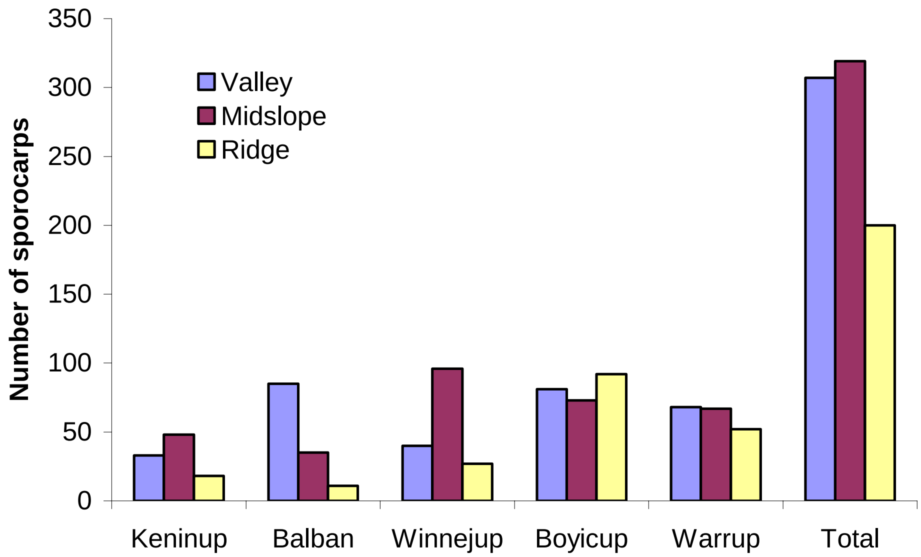

Figure 5. Sporophore abundance recorded in valley, midslope and ridge plots at each location.

The total combined weight of all sporocarps was 84.539 grams (Table 1). Differences in the size of sporocarps generally makes comparisons of air-dried weight and abundance difficult for most species of fungi, however, in this study the relationship between dry weight and abundance appeared to be consistent between treatments (Fig. 6) and between locations (Fig 7).

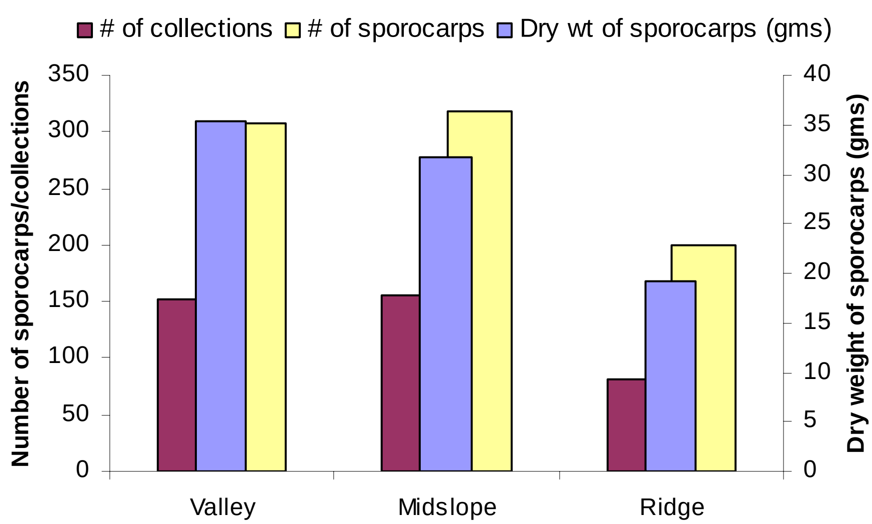

Figure 6. The number and total weight of sporophores, and number of collections from valley, midslope and ridge plots from all survey locations.

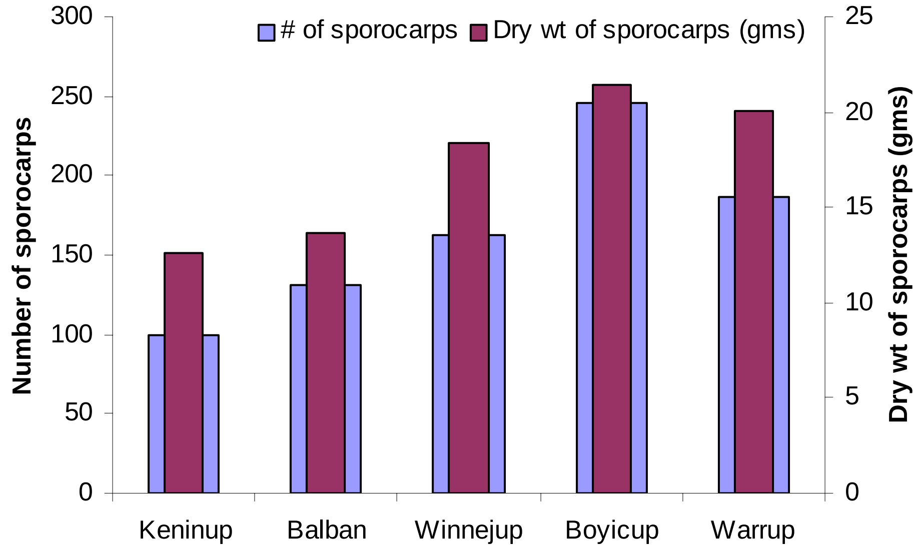

Figure 7. The number of sporocarps and the dry weight (gms) of sporocarps collected at each survey location.

The Boyicup plots yielded the greatest biomass and abundance of sporocarps. The Boyicup location is also known to have the lowest woylie trapping success rates of the locations surveyed. Keninup sites have the highest Woylie trapping success rate and yielded the least individual sporocarps and biomass of the locations (Fig. 7). This result may be related to woylie foraging activities, but further survey and study is required before conclusions can be made. However it does indicate that hypogeous fungi, and therefore presumably food resources, are available at sites where woylies have declined.

Royoungia sp. (WDP sp.10) is the first record of this genus in Western Australia. All species identified except *Royoungia* sp. have been documented as being consumed by mammals (Claridge *et al*.1996), and are likely to contribute to woylie food resources in the Upper Warren region and elsewhere in the southwest of WA. *Rhizopogon* sp. (WDP sp.35) is not a native species, but is a common mycorrhizal species associated with pines. *Rhizopogon* species are also used in the captive diet of the Gilbert's potoroo (*Potorous gilbertii*) (Friend 2004). *Rhizopogon* sp. was recorded and collected from the Warrup valley site where a number of *Pinus radiata* wildlings were present due to its close proximity to a mature pine plantation. Future analysis of spore diversity in woylie scats will provide information on woylie feeding preferences and seasonal availability of sporocarps for species collected in this study.

In a single survey at Karakamia Wildlife Sanctuary (the location of a high non-declining woylie population 50 km east of Perth) in October 2006 only five species of hypogeous fungi were recorded (Bougher 2006). Of these, two species, *Cystangium sessile* and *Dermocybe globuliformis* were also recorded in the Upper Warren region. The species diversity and abundance at Karakamia, was likely hampered by low autumn/winter rainfall prior to the survey (Bougher 2006).

Fire affects both woylie habitat and fungal communities (Lamont 1995, Robinson and Bougher 2003), but site selection for this survey did not take into account fire history. The effect of fire on the majority of mycorrhizal fungi that develop hypogeous sporocarps is poorly known (Claridge *et al*. 1996). Those species that fruit in the litter and organic layer are initially lost during fire (Robinson and Bougher 2003) but those that fruit deeper in mineral soil can survive and form a substantial part of the post fire diet for a number of Australian mammals (Christensen 1980, Taylor 1991, Johnson 1995, Vernes *et al*. 2001). For jarrah forest, it was shown that Basidiomycete ectomycorrhiza were 10 times more abundant on a site unburnt for 50 years compared to a site burnt 2 years previously (Reddell & Malajczuk 1984). In a recent study in eastern Australia, *Dermocybe globuliformis* (the most abundant fungus in this study) dominated non-burnt sites and was absent in burnt sites (Trappe *et al*. 2006). That study did not support an earlier hypothesis (Taylor 1991, Johnson 1995) that suggested burning enhanced fruiting of truffle fungi as food for mycophagous mammals. The reliance of the woylie on hypogeous fungi as a food resource, and the response of hypogeous fungi to fire require further investigation. Fire management for woylie habitat also needs to consider possible effects on hypogeous fungi communities.

Woylie trapping in the Upper Warren region and data analysis is ongoing. The survey methods used to record hypogeous fungi in this study were very successful. Survey and collection of hypogeous sporocarps will continue in 2007 and be combined with analyses of woylie scats and stomach contents to provide a more complete understanding of the woylie diet and its reliance on hypogeous fungi. Diversity and abundance of hypogeous fungi will also be compared within and between the five PCS study locations in order to investigate whether there is any relationship between the availability of hypogeous fungi and declines in woylie populations.

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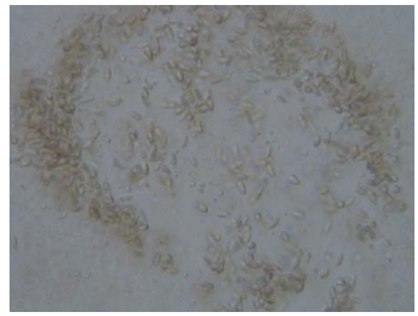
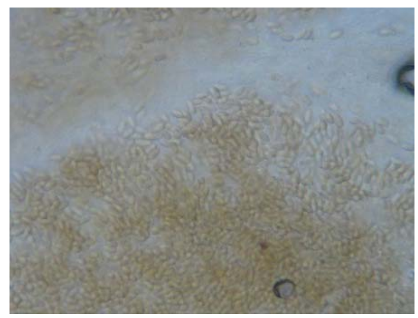
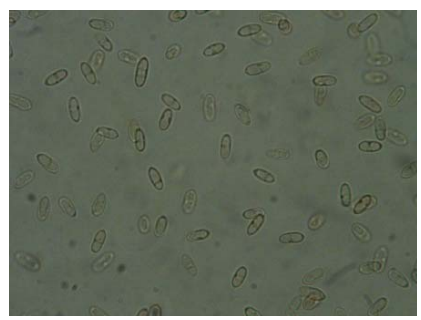
Appendix 1

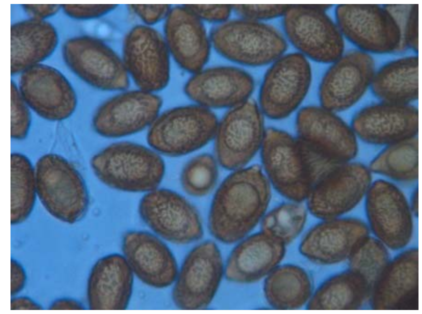
Species of hypogeous fungi recorded in surveys

Species number, name & voucher reference number	Section through fruit body showing gleba (internal tissues)	Micrograph of spores (not to same scale)
 Species 1: *Hysterogaster* (WDP 001)		
 Species 2: *Alpova*-like (WDP 002)		
 Species 3: Unknown (WDP 008)		

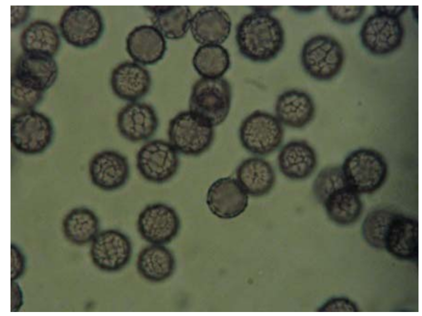
Appendix 1 continued. Species of hypogeous fungi recorded in surveys.

Species number, name & voucher reference number	**Section through fruit body showing gleba (internal tissues)**	**Micrograph of spores (not to same scale)**

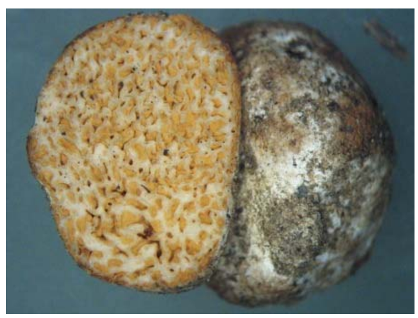
Species 4: *Descomyces angustisporus* (WDP 052)

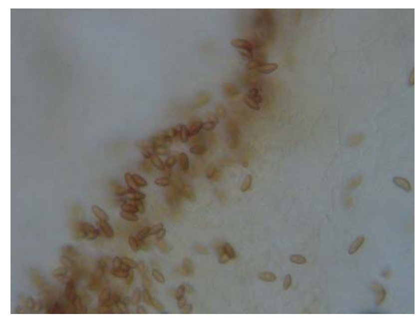


Species 5: *Cystangium* sp. (WDP 024)

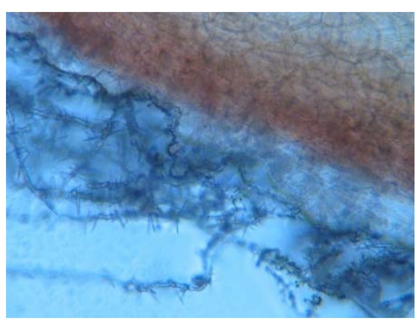


Species 6: *Octaviana* sp. (WDP 026)

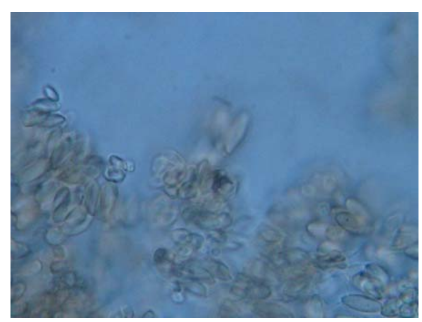




Species 7: *Hysterangium* sp. (WDP 013)



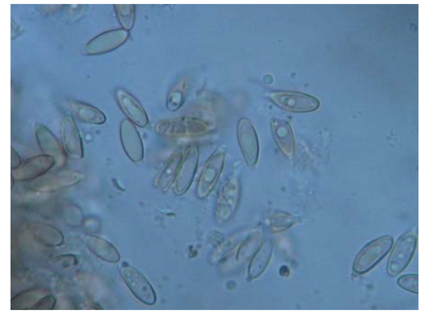
Setal hyphae on surface



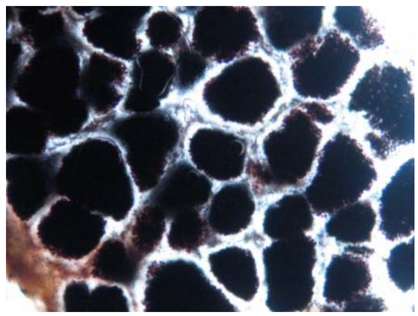
Appendix 1 continued. Species of hypogeous fungi recorded in surveys.

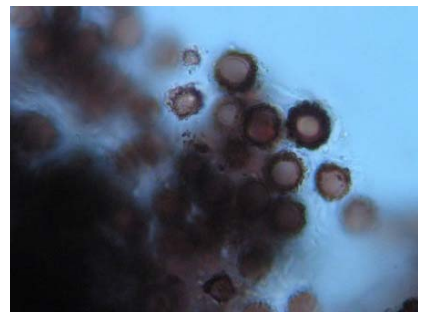
Species number, name & voucher reference number	**Section through fruit body showing gleba (internal tissues)**	**Micrograph of spores (not to same scale)**

Species 8: *Pseudohysterangium* sp. (WDP 030)

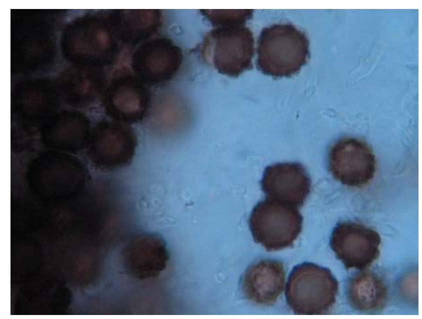



Species 9: *Scleroderma* sp. A (WDP 010)

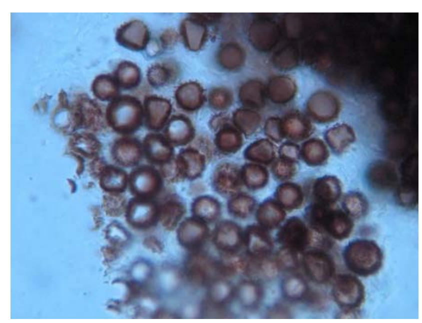

Locules in gleba



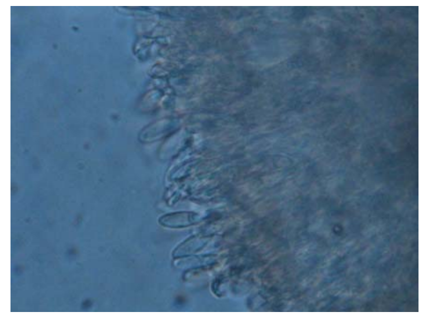
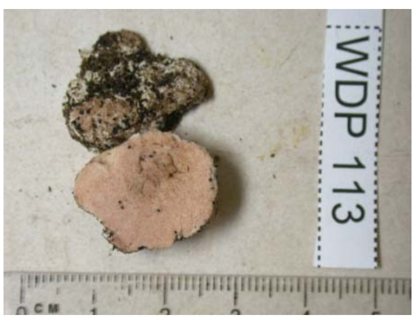
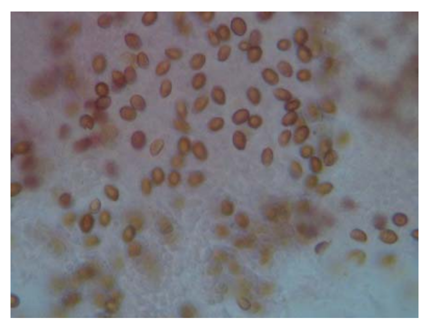
Species 9a: *Scleroderma* sp. A (WDP 224)



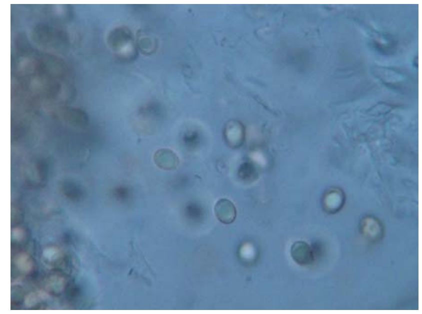
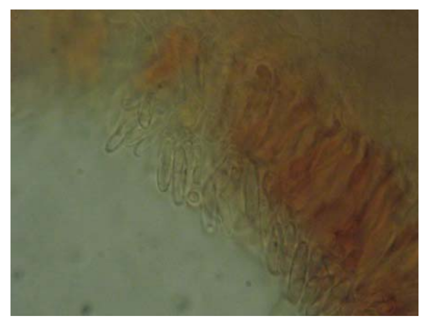
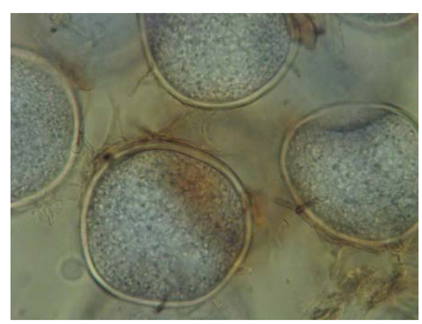
Species 8a: *Scleroderma* sp. A (WDP 223)



Appendix 1 continued. Species of hypogeous fungi recorded in surveys.

Species number, name & voucher reference number	**Section through fruit body showing gleba (internal tissues)**	**Micrograph of spores (not to same scale)**
 Species 10: *Royoungia* sp. (WDP 082)		
 Species 11: Unknown (WDP 121)		
 Species 12: *Pogiesperma* sp. (WDP 113)		
 Species 13: *Dermocybe globuliformis* (WDP 117)	 section through fruit body	

Appendix 1 continued. Species of hypogeous fungi recorded in surveys.

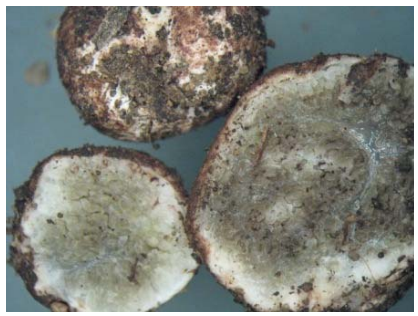
Species number, name & voucher reference number	**Section through fruit body showing gleba (internal tissues)**	**Micrograph of spores (not to same scale)**
 Species 14: Unknown (WDP 116)		
Species 15: *Hydnoplicata convolute* (WDP 147)		
Species 16: Unknown (WDP 152)		
Species 17: *Endogone* sp. (WDP 094)		

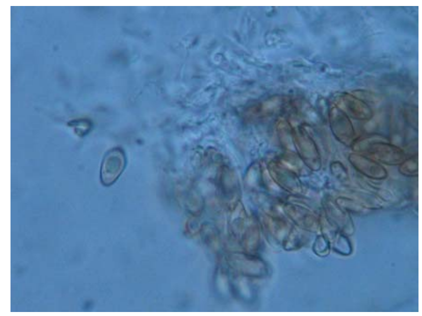
Appendix 1 continued. Species of hypogeous fungi recorded in surveys.

Species number, name & voucher reference number	Section through fruit body showing gleba (internal tissues)	Micrograph of spores (not to same scale)

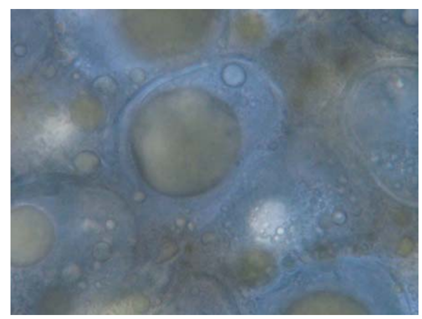
Species 18: Unknown (WDP 108)

Species 19: *Hysterangium* sp.
(WDP 190)

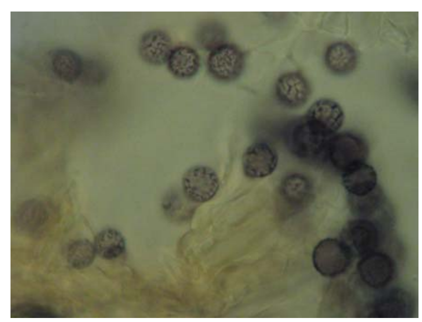




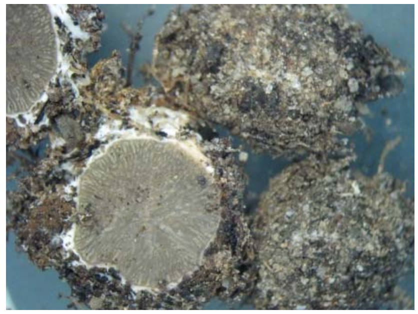
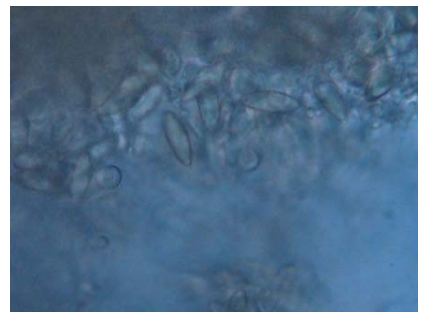
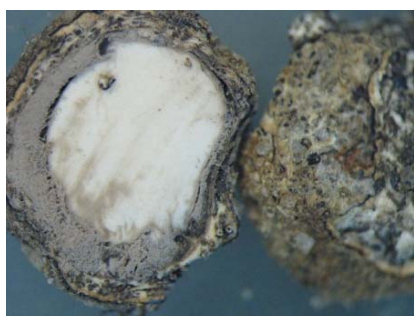
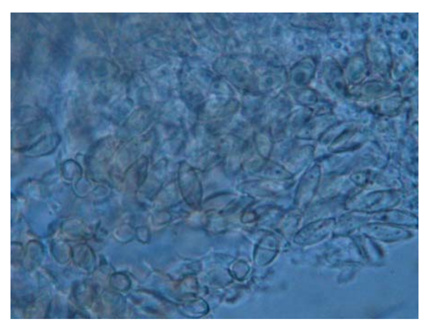
Species 20: *Endogone* sp.? (WDP 221)



Species 21: *Gymnomyces* sp.
(WDP 234)

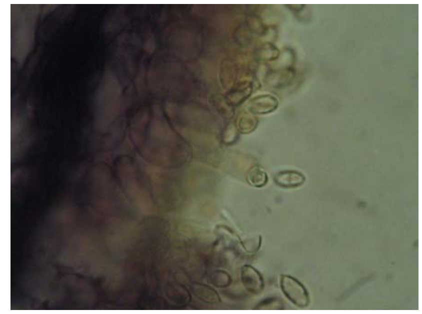


Appendix 1 continued. Species of hypogeous fungi recorded in surveys.

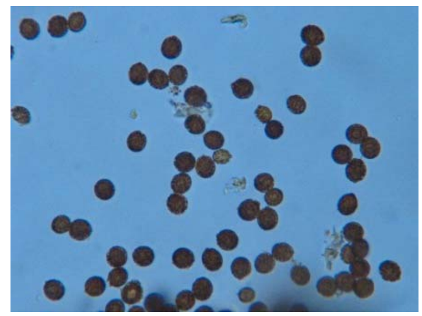
Species number, name & voucher reference number	**Section through fruit body showing gleba (internal tissues)**	**Micrograph of spores (not to same scale)**
 Species 23/28/33 *Hysterangium* sp. (WDP 197)		
 Species 24: *Pseudohysterangium* sp. (WDP 198)		
 Species 25: *Mesophellia* sp. (WDP 210)		
 Species 26: *Hysterangium* sp. (WDP 213)		

Species number, name & voucher reference number	Section through fruit body showing gleba (internal tissues)	Micrograph of spores (not to same scale)

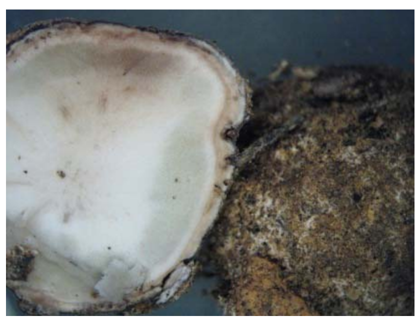
Species 27: Unknown (WDP 219)



Species 29: *Protoglossum luteum* (WDP 227)



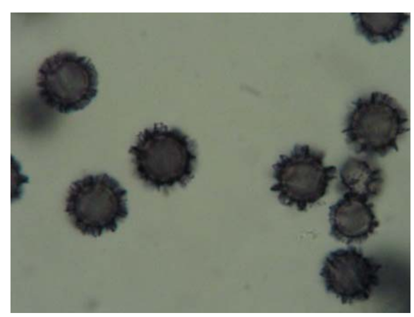
Species 31: *Scleroderma* sp. (WDP 274)



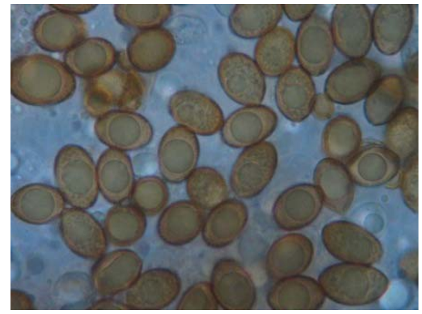
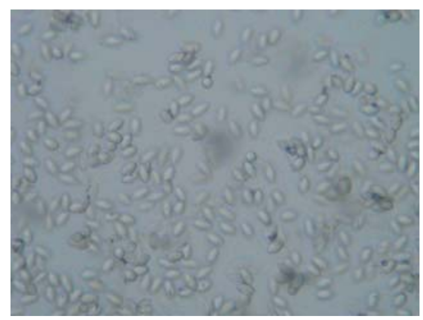
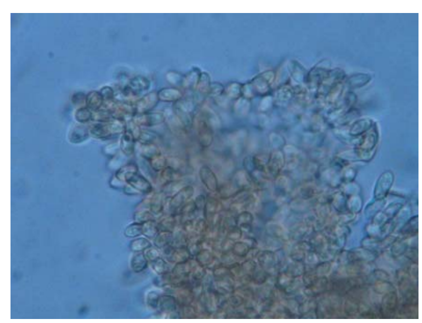
Immature gleba

No spores observed - immature

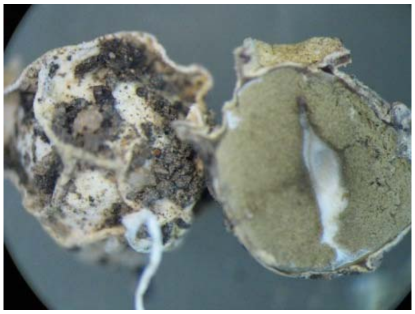
Species 32/17a: *Cystangium sessile* (WDP 285)



Appendix 1 continued. Species of hypogeous fungi recorded in surveys.

Species number, name & voucher reference number	**Section through fruit body showing gleba (internal tissues)**	**Micrograph of spores (not to same scale)**
Species 34: *Descomyces* sp. (WDP 364)		
Species 35: *Rhizopogon* sp. (WDP 400)		
Species 36: *Mesophellia angustispora* (WDP 401)		
Species 37: Unknown (WDP 403)		

Appendix 1 continued. Species of hypogeous fungi recorded in surveys.

Species number, name & voucher reference number	Section through fruit body showing gleba (internal tissues)	Micrograph of spores (not to same scale)
 Species 38: *Phallobata/Protubera/Gelopellis* group (WDP 385)