Fungi in scats of Gilbert's Potoroo (*Potorous gilbertii*) - Australia's most critically endangered mammal

A consultancy report for Edith Cowan University (ECU) and the WA Department of Conservation and Land Management (CALM) - 1998

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Summary of major findings

1. Fungal spores present and abundant in all Gilbert's Potoroo scats (from 4 sites, and variously dated March to December 1994 - 1998).
2. Little or no identifiable plant material in scat portions.
3. All scat portions predominantly (about 90% or more) fungal, remainder mainly unidentifiable detritus.
4. Diverse range of fungi represented in all scats - about 25 spore types, up to 9 spore types per scat.
5. Some spore types in scats matched to spores in fruit body herbarium specimens from Gilbert's Potoroo habitat.
6. Origin of spores: most diversity and frequency from basidiomycete truffle-like fruit bodies; less diversity from mushrooms and puffballs, or from Ascomycetes.

Background and aims

Two Peoples Bay Nature Reserve is situated along the south coast of Western Australia to the east of Albany. The reserve has an average rainfall of about 800 mm, various vegetation types including coastal heath and woodland, and was gazetted in the 1960's to protect habitat for a long unsighted rare bird - the Noisy Scrub bird. In similarity with this bird, Gilbert's Potoroo (*Potorous gilbertii*) had not been sighted anywhere since last century (1870's) and was thought to be extinct until it's recent rediscovery at Two Peoples Bay in 1994. Soon after its rediscovery, a captive colony of Gilbert's Potoroo was established to alleviate the precarious situation of only one known wild population. Management options currently underway with Gilbert's Potoroo such as breeding programs to provide animals for translocation to new areas, require fundamental understanding of the animal's biology. Important aspects of potoroo biology determining breeding and translocation success are likely to include diet and interaction with other organisms. Fungi are known to be a significant food source for many small mammals including potoroos in Australia (Claridge *et al* 1996). In particular, truffle-like fungi are likely to be a major component of the Gilbert's potoroo diet, and interact with major potoroo habitat plants via their mycorrhiza (see below). Australia has a high diversity of truffle-like fungi probably exceeding that of other continents, and many species are endemic (Castellano and Bougher 1994).

It has been estimated that there may be 250 000 species of fungi in Australia, including about 5 000 larger fungi (Pascoe 1991). However, only 5–10% of Australian fungi may have been named so far. Larger fungi (also referred to as macrofungi) include many of the best known fungi such as the mushrooms and toadstools, puffballs, coral fungi, earthstars and other forms growing on the ground, bracket fungi growing on tree trunks, and truffle-like fungi which fruit below the ground. These are the fruit bodies or spore-bearing organs of the fungi, akin to the fruits of plants. In Western Australia, about 500 species of larger fungi have been recorded, most from the South-West (Hilton 1982, 1988). A survey of the larger fungi at Two Peoples Bay (Syme 1992) collected a total of 441 species (mainly unidentified) over two consecutive years, with an estimated 365 of these probably not yet known to science. These included some truffle-like fungi. Subsequent surveys (CSIRO unpublished data) have revealed that a large diversity of truffle-like fungi occur in Two Peoples Bay Nature Reserve, including fungi with putative mycorrhizal associations with plants such as *Gastrolobium* within main potoroo habitats and *Eucalyptus* plants outside main potoroo habitats.

Fungi have crucial ecosystem roles, e.g. in decomposition of organic matter and engineering major soil nutrient cycling processes. In addition to having such functions, many fungi including the truffle-like fungi are symbionts or mutualists - fungi that form mutually beneficial associations with other organisms. All partners benefit in some way, but each on its own may be unable or less likely to complete its life cycle. More specifically truffle-like fungi form co-evolved mycorrhizal relationships with plants via two-way exchange that occurs in modified roots known as mycorrhiza. Photosynthates (sugars) from the plant are transferred to the fungus in one direction, while soil nutrients such as phosphorus are transported from the fungus to the plant in the other direction. Mycorrhizal fungi not only enhance plant uptake of nutrients from poor soils but also may buffer plants against environmental stresses such as disease. About 660 Australian species of ectomycorrhizal fungi (most of which produce large fruit bodies) have been named so far (Bougher 1995).

Truffle-like fungi in south-western and other parts of Australia also have co-evolved symbiotic links with some mycophagous (fungus-eating) small mammals which depend upon the fungi as a food source (Christensen 1980; Claridge and May 1994; Claridge *et al.* 1996, Bougher and Tommerup 1996). Indigenous mammals such as woylies and potoroos are lured by irresistible aromas to dig up and eat the spore-bearing fruit bodies of the fungi. The truffle spores pass unharmed through the mammalian gut, and are deposited in dung potentially far from the original site of consumption. The fungus benefits by dispersal of its spores. The mammal benefits from the nutrition value of the fruit body. Hence truffle-like fungi are involved in a highly specialised three-way interaction that is vital to each of the participant organisms - fungus, plant, and mammal.

This preliminary investigation based on a limited number of scats was commissioned to determine whether fungi are likely to be a major component of the Gilbert's Potoroo diet at Two Peoples Bay Nature Reserve. To assess the extent of mycophagy, this consultancy focussed on analysing faecal scats from wild animal populations of Gilbert's Potoroo for fungal material to:

1. Determine the presence of fungal material (spores, hyphae) in potoroo scats.
2. Characterise/identify the diversity of fungi in scat samples.
3. Assess the fungal fruiting type from which the fungal material originated.
4. Compare fungal material in scats with fruit bodies of fungi collected so far in mycorrhizal association with potoroo-habitat plants.

The findings were expected to raise some key questions/issues for further investigation about the relationship between the Gilbert's Potoroo and fungi (see also Bougher *et al.*, 1998).

Methods

Scats from wild populations of Gilbert's Potoroo were obtained from Two Peoples Bay over a period of about 3 years ranging from 1994 to 1998, including samples from March, May, June, and December (Table 1). The scats were air-dried and then stored in sealed plastic bags until examination. Five random subsamples of approximately 0.5 x 0.5 mm were extracted from each individual scat used for analysis. Subsamples were rehydrated in 3% KOH then split into two portions - one directly mounted onto a microscope slide, and the other rinsed in water and mounted in Melzer's Reagent. The latter reagent is an iodine solution which induces an amyloid (blue colour change) or dextrinoid (brown colour change) in some types of fungal spores, hyphae and other structures. Subsamples were examined under a compound high powered (100 X oil immersion objective) Olympus microscope and an interference contrast Leitz microscope. General abundance and major characteristics of fungal material were recorded for each subsample. Microscopic measurements are rounded to the nearest 0.5 µ. Measurements of spores are given as a range, e.g. (10) 10.5–12 (12.5) x (7) 7.5–9 (9.5) µ, where the values in brackets represent the uncommon extremes. Major fungal types encountered were allocated a code. Microscopic line drawings of major fungal types were created with an Olympus drawing tube. Spores are presented at 2000x.

Where possible in the short time available for this brief consultancy, about 30 collections of truffle-like fruit bodies of fungi collected so far in mycorrhizal association with potoroo-habitat plants (and lodged at CSIRO see below) were examined microscopically and compared to fungal material in scats to aid in the identification of the material. Above-ground fruiting fungi collected so far in Gilbert's Potoroo habitats were not examined microscopically during this consultancy. Reference samples of fungal fruit bodies of fungi collected from potoroo habitats at Two Peoples Bay used in this report are lodged as air-dried voucher specimens at CSIRO Forestry and Forest Products Mycology Herbarium, Perth WA. The details of these specimens are not made available for this report.

Results

Fungal spores were present and abundant in all scat samples examined. A total of 15 major spore types were observed (Table 1, Figures 1-10). A further 10 other spore types in very low numbers were estimated to be present among the scat samples. Immature and mature spores were present, as is consistent with ingestion of fruit bodies containing spores of various stages of development.

A female potoroo from site 1bd 236 in December 1994 had the largest number of spore types (9) and a male from site 3 in March 1995 had the least (4).

Some spore types were rare and restricted to one sample, e.g. NB10 *Quadrispora* 'WA2' sp. nov., whereas others were present and abundant in all scat samples, e.g. NB1 *Elaphomyces*? and NB2 *Hysterangium/Mesophellia* (Table 1). The spore types originated from three major fruit body forms - truffle-like, puffball (e.g. NB15), and epigeous (mushroom-like, e.g. NB11). Truffle-like fungi were the most frequent and diverse type. Most of the spore types appear to be Basidiomycetes, e.g. spores with a hilar appendage. However spore type NB1 and large reticulate, non-amyloid spores such as type NB13 may represent Ascomycete truffle-like fungi.

In all scats examined fungal material was dominant to the exclusion of any other types of ingested organic material, and only in one case (male potoroo 22 site 6) was plant material observed (then as a minor component). 90% or more of the material observed in all scat portions under the microscope was fungal, with the remainder as mainly unidentifiable detritus.

Spore types could be matched to some, but not all, of the 30 reference fruit bodies of truffle-like fungi from Two Peoples Bay, e.g. spores of fruit bodies of a collection of *Castoreum tasmanicum* cf. match spore type NB8 found in two of the scats examined (Figure 8), and spores from fruit bodies of *Quadrispora* 'WA2' sp. nov. matched those of NB10 in scats (Figure 10).

In addition to spores, fungal hyphae and tissue were present in two of the scat samples (types NB16, NB17 Table 1). Type NB17 has an elastic 'chewing gum' consistency characteristic of the fungal genus *Gummiglobus* (Family Mesophelliaceae).

Brief notes about ten of the main spore types observed in the scats follow:

NB1 (Figure 1)
Main features: Brown, spherical, minutely ornamented, thick walled.
Notes on identity: Complex thick spore wall suggestive of sporocarpic VA mycorrhizal fungi such as *Endogone* or *Acaulospora*, but these generally have larger spores. More likely an ascomycete, and has similarity to some of the *Elaphomyces* species recorded in Australia.
Microscopic features: golden then dark brown in KOH, darker brown in Melzer's reagent, (11)11.5–14.5 (15) µ diam., globose, verruculose with few interconnections, in profile pegs embedded in thick (up to 2.5 µ) outer layer, hilar appendage not seen.

NB2 (Figure 2)
Main features: Fusoid, hyaline, smooth, thin-walled, not dextrinoid.
Notes on identity: Hysterangium or *Mesophellia* likely genera.
Microscopic features: hyaline in KOH and in Melzer's reagent (some dextrinoidy when immature), (10)10.5–13(13.5) x 4–6 (6.5) µ, fusoid, smooth and thin-walled, hilar appendage absent or with a truncate pad.

NB3 (Figure 3)
Main features: Small, rod-like, hyaline, smooth, thin-walled, not dextrinoid.
Notes on identity: Unknown as could match a range of fungi.
Microscopic features: hyaline in KOH and in Melzer's reagent (some dextrinoidy when immature), 6–10 x 2.5–4 µ, bacilloid, smooth and thin-walled, hilar appendage not usually visible.

NB4 (Figure 4)
Main features: Fusoid, hyaline, thin-walled with wrinkled perisporium, not dextrinoid.
Notes on identity: Hysterangium likely genus.
Microscopic features: hyaline in KOH and in Melzer's reagent (some dextrinoidy when immature), 10–13.5 (14) x 4.5–6 µ, broad fusoid, smooth and thin-walled, hilar appendage tapered.

NB5 (Figure 5)
Main features: Hyaline or greenish, faintly longitudinally ridged, with wrinkled perisporium.
Notes on identity: Austrogautieria manjimupana or *A. chlorospora.*
Microscopic features: hyaline in KOH and in Melzer's reagent, 12–15.5 x 5–8.5 (9) µ, fusoid, ellipsoidal, sometimes attenuate at apex, faintly ornamented with 5–8 longitudinal or slightly oblique ridges extending along entire spore length, perisporium flaring, hilar appendage large.

NB6 (Figure 6)
Main features: similar to those of NB5 except brown.
Notes on identity: Austrogautieria sp.
Microscopic features: as for NB5 except brown in KOH and in Melzer's reagent.

NB7 (Figure 7)
Main features: Brown, citriniform with attenuated apex, conspicuous perisporium.
Notes on identity: Descomyces sp.
Microscopic features: bright brown in KOH darker brown in Melzer's reagent, (14) 14.5–19 x 7.5–10 (10.5) µ, citriniform, broadly fusiform, attenuate at apex, faintly verruculose or appearing smooth, unwrinkled perisporium brown conspicuous but not covering spore apex, hilar appendage large.

NB8 (Figure 8)
Main features: Hyaline or greenish, faintly longitudinally ridged, with wrinkled perisporium.
Notes on identity: Castoreum tasmanicum cf. (matched to fruit body lodged at CSIRO.)
Microscopic features: hyaline in KOH, pale brown (dextrinoid) in Melzer's reagent, 15–17 (17.5) x 7–9 µ, ellipsoid, asymmetric, smooth with heavily wrinkled outer spore wall layer, hilar appendage large oblique.

NB9 (Figure 9)
Main features: Brown, verrucose, asymmetric, without conspicuous perisporium.
Notes on identity: Thaxterogaster likely genus.
Microscopic features: brown in KOH and in Melzer's reagent, 9–12 x 5–7 µ, ellipsoid, asymmetric, verrucose, without conspicuous perisporium, hilar appendage large oblique.

NB10 (Figure 10)
Main features: Brown ornamented spores in clusters of four.
Notes on identity: Quadrispora 'WA2' *sp. nov.* (matched to fruit body lodged at CSIRO).
Microscopic features: bright brown in KOH, yellowish in Melzer's reagent, 12-15.5 (16.5) x 7-8.5 (9) µ, ellipsoid, elongate-ellipsoid, asymmetric with large, irregular/angular verrucae of uneven, perisporium gelatinised, fragmenting, conspicuous, continuous between adjacent spores of a tetrad, loosening/fragmenting, hilar appendage large oblique.

Table 1: Fungal spore types, and two fruit body tissue types observed in scats of Gilbert's Potoroo

Scat sample **Spore code & type**	**Fruit body type**	**Female 236 site 1bd 20.12.94**	**Female 10 site 3 17.5.95**	**Male 9 site 3 3.6.95**	**Male 34 site 7 26.3.98**	**Male 22 site 6 24.3.98**
NB1 *Elaphomyces?* spherical, dark brown	truffle-like	++	++	++	++	++
NB2 *Hysterangium, or Mesophellia*	truffle-like	++	++	++	++	++
NB3 small, rod-like	truffle-like	+	+	-	-	-
NB4 *Hysterangium* wrinkled	truffle-like	+	++	++	+	+
NB5 *Austrogautieria manjimupana*	truffle-like	(+)	-	-	-	(+)
NB6 *Austrogautieria* brown	truffle-like	-	-	-	(+)	-
NB7 *Descomyces* (mucronate)	truffle-like	-	-	-	+	-
NB8 *Castoreum tasmanicum* cf.	truffle-like	(+)	-	-	-	++
NB9 *Thaxterogaster* or *Cortinarius*	truffle-like?	(+)	-	(+)	-	-
NB10 *Quadrispora* 'WA2' sp. nov.	truffle-like	-	(+)	-	-	-
NB11 Stropharioid (with germ pore)	epigeous	(+)	(+)	-	-	-
NB12 *Stephanospora flava*	truffle-like	(+)	-	-	-	-
NB13 Large pale reticulate, spherical	truffle-like	-	-	-	(+)	(+)
NB14 Very large globose brown spore	truffle-like	-	-	-	-	(+)
NB15 Gasteromycete (globose, apiculate)	puffball	-	-	-	+	-
NB16 Intact fungal fruit body tissue	undetermined	+	-	+	+	+
NB17 *Gummiglobus*-like mycelium	truffle-like	-	-	-	-	+
10 other spore types in very low numbers observed overall in scat samples	truffle-like puffball epigeous	+	+	+	+	+

Figures 1-10: Ten spore types in scats of Gilbert's Potoroo (all shown at X 2000).

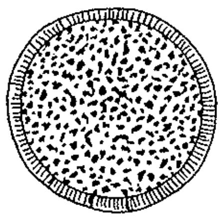

Fig. 1: **NB1**

Fig. 2: **NB2**

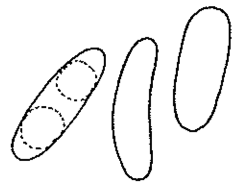

Fig. 3: **NB3**

Fig. 4: **NB4**

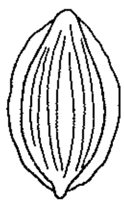

Fig 5: **NB5**

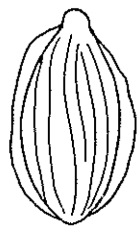

Fig. 6: **NB6**

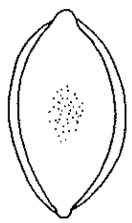

Fig. 7: **NB7**

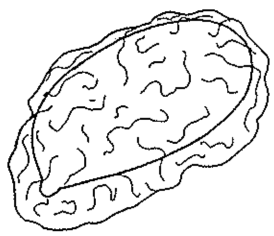

Fig. 8: **NB8**

Fig. 9: **NB9**

Fig. 10: **NB10**

Discussion and conclusion

This investigation demonstrates that a wide diversity of fungi comprised a major part of the Gilbert's Potoroos' diet at the time of sampling. Evidence from scat composition suggests that little or no plant material was eaten by the potoroos at this time. If the potoroos had eaten plant material it probably would have appeared in the scat samples, as intact fungal material was observed in some of the scats perhaps indicating a rapid transit of some incompletely digested food through the gut.

Spore types in the scat samples are representative of a wide taxonomic diversity of Basidiomycete and to a lesser extent Ascomycete fungi, e.g representing Basidiomycetes families such as Cortinariaceae, Hysterangiaceae, and Mesophelliaceae. Represented are a wide range of major fruit body forms - mostly truffle-like forms, with less puffball and epigeous (mushroom-like) forms. Surprisingly, a major truffle-like group in Australia - the Russulales - were not detected in any of the scat samples. These fungi have been collected as fruit bodies from Two Peoples Bay, e.g. *Martellia* species, but perhaps they were not fruiting at the time when the animals from which the examined scats originated were foraging. Above-ground fruiting fungi collected so far from Two Peoples Bay were not examined microscopically during this consultancy, and it is suggested that these need to be examined, as some of the spore types observed in scats relate to those of epigeous fungi. However puffballs and mushroom-like fungi are probably less favoured by Gilbert's Potoroo than truffle-like fungi, as is the case with other mycophagous mammals (Claridge *et al.,* 1996)

A further indication that fungi are a significant part of the potoroo diet is that fungi are eaten by Gilbert's Potoroo throughout the annual cycle - at least in this study from March to December within the years 1994 to 1998. Indeed scats from a female potoroo at site 1bd 236 in December 1994 had the largest number of spore types, emphasising that truffle-like fungi are fruiting in potoroo habitats at a time of the year outside the main fungal fruiting season - generally thought to range from about April to August/September. That some major groups of fungi such as the Russulales were absent from the examined scats is perhaps indicative of variability in fungal fruiting phenology, and there is a need to frequently sample scats from all year-round to assess seasonal fluctuation of fungi in the potoroo diet.

That only some of the spore types match any of the 30 fruit body collections examined, suggests that a more extensive collection survey of truffle-like fruit bodies in potoroo habitats is required to match spores in the scats. It is also possible that the appearance of some spores may be altered during passage through the gut of the potoroos, e.g. swelling or erosion of outer perisporial spore wall layers, or changes in colour. Therefore some interpretation may be needed when comparing spores from scats with those of uneaten fruit bodies.

Due to the brief time available in this consultancy, less than adequate time was given to important aspects of this work which are recommended for further attention, namely: (1) the specific identity of fungi in potoroo scats, (2) the frequency and timing of scat samples analysed, and (3) matching fungi in scats to fungal fruit bodies. Further studies should aim to match spores from scats to fruit bodies of fungi occurring in the area so that the nutritional contribution of fruit bodies to the diet of Gilbert's Potoroo can be assessed, and also so fungal spores may be more accurately identified. Significant to the proposed translocation of potoroos to new areas is evidence that many of the fungi eaten by Gilbert's Potoroo are truffle-like fungi having mycorrhizal associations particularly with N-fixing *Gastrolobium bilobum* shrubs in main potoroo habitats.

Hence a number of questions and issues concerning the relationship between fungi and Gilbert's Potoroo remain to be investigated (some of which are listed below).

- To what degree are truffle-like fungi nutritionally important to the diet of potoroos?
- What is the phenology of the fungi fruiting and relationship of this to their contribution to the diet of the potoroos over annual cycles?
- What is the biodiversity, species identity, and systematic relationships of the fungi eaten by potoroos?
- Do potoroos restrict themselves to feeding on fungi mycorrhizal with plants in thickets with *Gastrolobium* shrubs or do they venture out and forage on fungi associated with a wide range of plants and habitats?
- Which plant species are the mycorrhizal associates of truffle-like fungi in potoroo habitats?
- What is the role of epigeous fungal fruiting bodies as food for the potoroos?
- Do target areas for translocation of potoroos have sufficient abundance and diversity of truffle-like fungi to assist their survival?
- Are certain plants that have truffle-like associates such as *Gastrolobium* present in translocation target areas, and if not do the plants need to be re-introduced also?

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Dr Jackie Courtenay (Edith Cowan University/CALM) arranged this consultancy, and provided the scats of Gilbert's Potoroo examined in this study. Thanks to Alan Danks (CALM) for cooperation during field collecting of fungal fruit bodies at Two Peoples Bay for which CALM provided collecting permits to CSIRO.

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