CSIRO PUBLISHING

RESEARCH NOTE
https://doi.org/10.1071/PC24085

PACIFIC CONSERVATION BIOLOGY

Fungal consumption by marsupials in southern Tasmania

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Handling Editor:
Tim Doherty

Received: 4 November 2024
Accepted: 19 January 2025
Published: 10 February 2025

Cite this: Elliott K *et al.* (2025) Fungal consumption by marsupials in southern Tasmania. *Pacific Conservation Biology* **31**, PC24085. doi:10.1071/PC24085

ABSTRACT

In Australia, many mycophagous (fungus feeding) mammals that disperse fungal spores are extinct or threatened throughout much of their historic range. Using live-trapping, we collected scats from eastern bettongs (*Bettongia gaimardi*), long-nosed potoroos (*Potorous tridactylus*), brush-tailed possums (*Trichosurus vulpecula*), eastern barred bandicoots (*Perameles gunnii*) and southern brown bandicoots (*Isoodon obesulus*) at two sites in southern Tasmania. Microscopic analysis of scats revealed that all species in this study consumed fungi (over 24 fungal taxa), and the composition varied between some species and sites. This study highlights the need for additional research to gain insight into the ecological implications of spore dispersal by native marsupials.

Keywords: critical weight range mammals, diets, fungal ecology, fungivory, mammal ecology, mycophagy, mycorrhizal fungi, Potoroidae, spore dispersal, truffle ecology.

Introduction

Globally, over 600 vertebrates contribute to fungal spore dispersal (Elliott *et al.* 2019*a*, 2019*b*, 2022*a*; Nest *et al.* 2023) by eating fungi that form mycorrhizal associations key to plant nutrient uptake (Claridge and May 1994; Johnson 1996). Many fungal species ingested by animals form sequestrate (enclosed) fruiting bodies that are hypogeous (fruit underground). These habits have likely developed in response to animal feeding behaviours, as strong aromas released by the fungi are attractive to animals (Donaldson and Stoddart 1994; Stephens *et al.* 2020). In the absence of the aid of animal dispersal, most fungi disperse spores by wind (Geml *et al.* 2008) or move vegetatively via mycelial spread (Jonsson *et al.* 1999). However, only a small percentage of spores disperse beyond a few metres of the parent fruiting body (Li 2005; Galante *et al.* 2011) and a variety of factors can impede mycelial spread. The excavation, consumption and subsequent dispersal of fungi provided by vertebrate mycophagists is therefore a key factor in the health of mycorrhizal forest systems.

There has been significant research focus on these animal–fungal interactions within Australia (Claridge and May 1994; Nuske *et al.* 2017) but these associations also are widespread beyond this region. Rodents have been shown to be the most diverse group of mycophagists globally, including many Australian taxa (Vernes *et al.* 2015; Elliott *et al.* 2022*a*). In Australia, potoroid marsupials are the most thoroughly studied obligate mycophagists. All eight extant members of this Australian endemic family eat a diversity of fungi (Taylor 1992; Claridge and Trappe 2005; Nuske *et al.* 2017; Elliott *et al.* 2022*a*).

The fungal foraging activities of potoroids are important to soil disturbance and organic matter decomposition (Garkaklis *et al.* 2004; Newell 2009; Fleming *et al.* 2014; Davies *et al.* 2019; Palmer *et al.* 2020). This ecosystem service is vital to soil hydration and organic matter decomposition (Fleming *et al.* 2014; Davies *et al.* 2019; Palmer *et al.* 2020). While estimating soil disturbance was beyond the scope of this study, Tasmanian potoroids and other mycophagists would be expected to play a similar role in bioturbation as has been shown for related species on mainland Australia. Since colonial invasion, Australia's potoroid mammals have suffered serious population declines and extinctions (Short and Smith 1994; Short 1998; Vernes *et al.* 2021). This loss has led to a reduction in spore dispersal that in turn has impacted plant and fungal communities. Given the ecological significance of services provided by members of the Potoroidae, our aim was to explore the fungal diets of these mammals in an ecosystem in which the animals remain in healthy numbers.

Materials and methods

We established two trapping sites in the South East Tasmanian bioregion: The Peter Murrell Conservation Area and State Reserve near Huntingfield, Tasmania (43°00′S, 147°18′E) and on private property near The Lea, Tasmania (42°56′08.2″S, 147°18′55.9″E). These sites are distinct and ~7 km apart. The Peter Murrell Reserves are dominated by *Eucalyptus amygdalina* coastal woodland with a heathy understory on sandy soils and include stands of *Acacia* spp. and *Allocasuarina* sp. The Reserves also include heathlands and some wetland areas. The site near The Lea is dominated by *Eucalyptus pulchella* forest and woodland, pasture (~9 ha) and some *Eucalyptus globulus* dry forest and woodland.

We used standard cage trapping protocols approved by the University of New England Animal Ethics Committee (Approval Number AEC18-065) and the Department of Primary Industries, Parks, Water and Environment's *Standard Operating Procedures for Live-trapping and Handling of Wild Tasmanian Mammals 2013*. Traps were baited with peanut butter and vanilla on bread, and set from dusk to dawn. Captured animals were transferred to clean cloth bags to be identified, weighed and sexed. To ensure identification of recaptures, we clipped a small patch of fur from the flank of each animal for temporary identification or used subcutaneous microchips for more permanent identification. Scats were collected from each trap and placed in 70% alcohol. Traps were cleaned after each capture to prevent contamination between captures. We had 120 trap nights in the Peter Murrell Conservation Area between 26 and 29 August 2019 and 160 trap nights at The Lea site between 29 August 2019 and 1 September 2019.

Scat samples were collected from each capture and multiple pellets (from the same individual) were collected when possible to ensure accurate assessment of which fungal taxa had been eaten. Samples were wet-mounted on glass slides and analysed at 400× magnification to determine presence and species richness of fungal spores (Fig. 1). To avoid reporting incidental consumption of fungi from spores that happened to be present in the environment, only spores found to occur frequently (a minimum of five times) in each sample were considered. Spores were identified based on standard microscopic characters. Ambiguous identities were confirmed by comparison with spores of identified fungal fruiting body collections made by the authors in the same or similar habitats. We generally did not attempt to refine fungal identification beyond family or genus level. We focused on the diversity of fungi consumed and therefore apply some older nomenclatural concepts that help to outline the diversity of taxa. For example, we mention the genera *Thaxterogaster* and *Quadrispora* and our use refers to sequestrate taxa allied with *Cortinarius*, a speciose genus that contains both epigeous and sequestrate hypogeous species. Many recent taxonomic studies of sequestrate fungi have shown that members of historically sequestrate genera are often synonymous with epigeous genera (Gasparini 2014; Elliott and Trappe 2018; Pastor *et al.* 2019; Elliott *et al.* 2020*a;* Nouhra *et al.* 2021). We agree with these taxonomic revisions but in the context of this study we mention *Thaxterogaster* and *Quadrispora* in reference to spores of the Cortinariaceae but have characters indicating that these likely belong to sequestrate taxa. The taxa we list as *Cortinarius* could also be sequestrate but we have no evidence to indicate the fruiting habits.

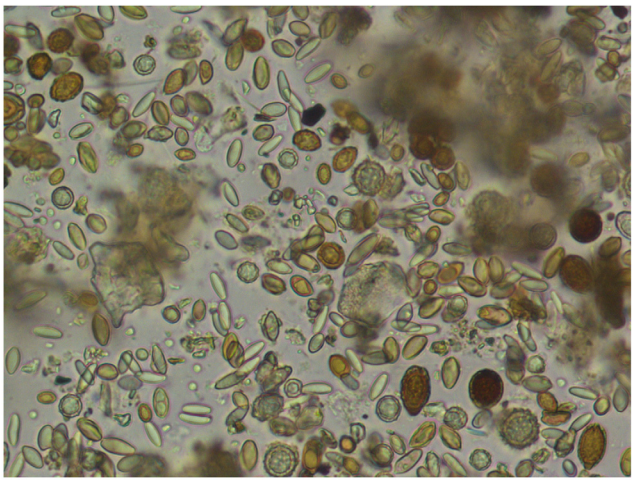

Fig. 1. A micrograph showing a typical view of a diversity of fungal spores in the scat of a long-nosed potoroo.

To compare fungal consumption between mammal species and sites, we conducted analysis of similarities (ANOSIM) in Primer 7 using presence/absence of fungal species in scat samples to generate Bray–Curtis similarity measures. ANOSIM returns an R-statistic that yields a measure of similarity between categories. *R*-values most commonly range from zero to one; the closer the *R*-value is to one, the more the categories differ; a value close to zero indicates that assemblages can barely be separated (Clarke and Warwick 2001). ANOSIM tests are robust to differences in the number of samples in factor groups (Clarke and Warwick 2001). To visualise multivariate patterns in vertebrate assemblage structure, multidimensional scaling was performed. Scats without fungi were excluded from the analysis, as was the single eastern barred bandicoot sample (see Table 1).

Results

Fungi were a major component of the diets of the five mammal species trapped during this study. A total of 24 fungal spore types was recognised in the scats (Table 1 and Fig. 1). We had eight captures of brush-tailed possums (*Trichosurus vulpecula*), one of eastern barred bandicoot (*Perameles gunnii*) and three of southern brown bandicoots (*Isoodon obesulus*) (Table 1). We

Table 1. Percentage occurrence of fungal taxa in mammal diets from the Peter Murrell Reserves and Lea Conservation Area, Tasmania (calculated from scats that contained fungus).

Fungal taxa	Mammal species				
	Bettongia gaimardi (6)	*Potorous tridactylus* (31)	*Trichosurus vulpecula* (8)	*Perameles gunnii* (1)	*Isoodon obesulus* (3)
Amylascus	50.0	38.7	–	–	–
Mesophelliaceae	100.0	80.6	62.5	–	–
Aroramyces 1	–	16.1	–	–	–
Ascomycete (Dicina-like)	16.7	–	–	–	–
Austrogautieria	66.7	35.5	12.5	–	–
Boletoid	100.0	58.1	–	–	33.3
Cortinarius	100.0	83.9	25.0	–	–
Descomyces	–	–	–	100.0	–
Descomyces 2	50.0	–	–	–	–
Descomyces 5	33.3	100.0	37.5	–	–
Elaphomyces	–	22.6	–	–	–
Glomeromycota	100.0	74.2	–	–	–
Hydnangium	66.7	19.4	–	–	–
Hymenogaster	16.7	9.7	–	–	–
Hysterangium	50.0	77.4	12.5	–	–
Hymenogaster 2	–	–	–	–	–
Labyrinthomyes	100.0	96.8	–	–	–
Octaviania	–	3.2	–	–	–
Quadrispora	83.3	83.9	–	–	–
Rossbeevera	16.7	–	–	–	–
Russulaceae	100.0	87.1	50.0	–	–
Scleroderma	–	6.5	–	–	–
Thaxterogaster	66.7	96.8	12.5	–	66.7
Unknown spore taxon	–	–	–	100.0	–
Number of scats examined	6	31	8	1	3
Number of fungal taxa	17	18	7	2	2
% occurrence of fungus	100	100	75		

Total taxa in diets and percentage occurrence of fungus in all scats (where sufficient samples were gathered) are also shown.

had six captures of eastern bettongs (*Bettongia gaimardi*) and 31 captures of long-nosed potoroos (*Potorous tridactylus*), and every scat from these two species contained fungal spores (Table 1 and Fig. 2).

We found significant differences in fungal diet among species (ANOSIM $R = 0.698$, $P = 0.001$, Fig. 2). Composition of fungi in the long-nosed potoroo samples was well separated from that in southern brown bandicoots ($R = 1.00$) and brush-tailed possums ($R = 0.97$) but not well separated from eastern bettongs ($R = 0.29$). Fungi composition in eastern bettongs was well separated from that in southern brown bandicoots ($R = 1.00$) and moderately separated from that in brush-tailed possums ($R = 0.59$). Fungi composition in brush-tailed possums was moderately separated from that in southern brown bandicoots ($R = 0.47$).

The single brush-tailed possum sample from the Peter Murrell Reserves is clustered with that of the southern brown bandicoots from the same site (Fig. 2). The other brush-tailed possum samples are from The Lea. Eastern bettong samples are all from The Lea. The long-nosed potoroos are the only mammal for which we had sufficient data to compare between the two study sites. There was no significant difference in fungal consumption between The Lea and the Peter Murrell Reserves ($R = 0.08$, $P = 0.23$, Fig. 3).

Discussion

This study builds on existing knowledge about mycophagy in Australia and highlights the ecological importance of

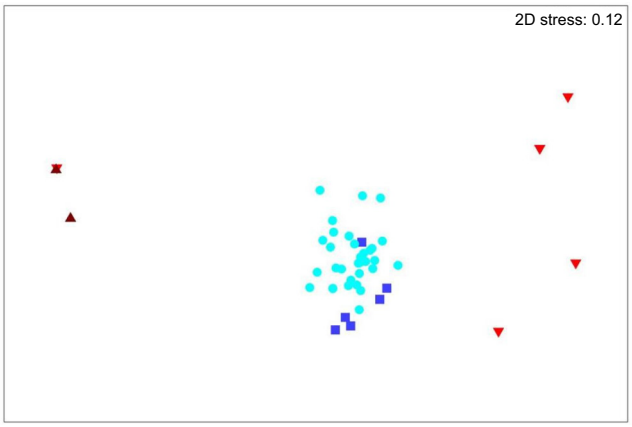

Fig. 2. Ordination of individual mammal diets based on presence/absence of fungal taxa. Brown triangles = southern brown bandicoot, light blue circles = long-nosed potoroo, dark blue squares = eastern bettong and red inverted triangles = brush-tailed possum.

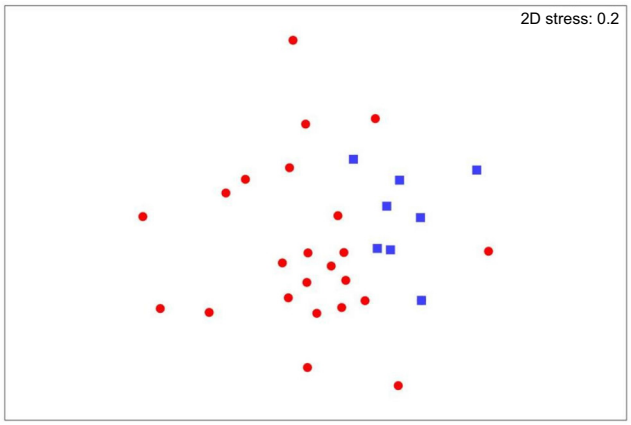

Fig. 3. Ordination of individual long-nosed potoroo diets based on presence/absence of fungal taxa. Red circles = Peter Murrell Reserves, blue squares = The Lea.

conservation of potoroids and other critical weight range mammals. Mycophagy levels among potoroids have been shown to vary between seasons and with the availability of fungi (Taylor 1992; Johnson 1994; Johnson and McIlwee 1997; Tory *et al.* 1997; Vernes 1999; Robley *et al.* 2001; Vernes *et al.* 2015). Our study is a temporal snapshot and does not represent consumption throughout the entire year but many studies of this group have shown these to be regular fungal consumers (Nuske *et al.* 2017; Elliott *et al.* 2022*a*). Our study supports the idea that obligate mycophagists such as our two potoroids share similar fungal dietary components. Our observation of mycophagy among brush-tailed possums, eastern barred bandicoots and southern brown bandicoots supports more robust mycophagy data sets about the diets of these marsupials (see: Nuske *et al.* 2019; Elliott *et al.* 2022*a*, 2023).

Testing viability of spores after transit through the digestive systems was beyond the scope of our study. However, among the more than 40 mammal species that have been studied – including members of Peramelidae, Phalangeridae and Potoroidae – all have been shown to pass viable fungal spores and some have been shown to increase spore germination rates (Elliott *et al.* 2022*a*). Although we did not test for evidence of detrimental degradation of spores, no evidence of this was observed.

Accurately estimating spore dispersal potential requires data on digestive transit time and telemetry data on movement. These data were lacking, therefore we could not model dispersal potential in this study. However, for comparison, modeling of swamp wallabies (*Wallabia bicolor*) has shown that these animals frequently disperse mycorrhizal fungal spores hundreds of metres and possibly as far as 1265 m from the point of consumption (Danks *et al.* 2020). Dispersal estimates based on home range size indicate that some murid rodents could also move spores hundreds and possibly over 1000 m (Elliott *et al.* 2020*b*, 2022*b*), and bandicoots likely hundreds of metres (Elliott *et al.* 2022*c*).

Spores often linger longer than other dietary items in digestive systems and therefore have a longer period of potential dispersal (Danks 2012). Ultimately, spore dispersal potential is dependent upon movement patterns, home range size and gut passage rate. No studies have assessed passage rate of fungal spores through potoroid digestive systems but based on what is known of passage rates of other animals discussed in the aforementioned studies, maximum retention times are likely to be 2 days. Mean home range sizes vary significantly between the two potoroids in this study. Mean home range size is 61 ha for the eastern bettong (Taylor 1993), and 4 ha among males and 1.9 ha among females for long-nosed potoroos (Long 2001). The differences in home range size suggest that bettongs may contribute to longer distance dispersal than potoroos. Another variable not considered in this study is secondary dispersal that occurs when these prey species are ingested by a predator or scavenger. In mainland Australia, dingoes (*Canis familiaris*) contribute to spore dispersal by eating primary mycophagists (including long-nosed potoroos), with modelling suggesting that dingoes potentially disperse fungal spores over more than 10 km (Elliott *et al.* 2024). Avian raptors, Tasmanian devils (*Sarcophilus harrisii*) and quolls (*Dasyurus* spp.) likely provide – and at one point the Tasmanian tiger (*Thylacinus cynocephalus*) provided – a similar function of long-distance spore dispersal.

Our data show that possums, bandicoots, bettongs and potoroos consume a diversity of mycorrhizal fungi in Tasmania. We hope this study will stimulate further research into viability, passage rates and dispersal estimation of fungal spores, and provide an additional ecological rationale for the value of conserving Australian mammal communities.

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Data availability. All relevant data are presented in the paper but clarification or notes are available upon request.

Conflicts of interest. The authors declare that they have no conflicts of interest.

Declaration of funding. This project was made possible by funding provided to the corresponding author by two Holsworth Wildlife Research Endowment Grants from the Ecological Society of Australia and an *In Situ* Science Research Excellence Award. The trapping program at Peter Murrell Reserves by the second author was funded by the Tasmanian Government.

Acknowledgements. We are grateful to Carly Lambert for assisting with trapping at The Lea site and the Walch family for allowing us to trap on their private property. Peter J. Jarman also provided assistance with trapping at the Peter Murrell sites. The School of Environmental and Rural Science at the University of New England provided facilities and an International Postgraduate Research Scholarship to the corresponding author.

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