Hypogeous fungi and *Phytophthora* survey, Nungatta Feral Predator-Free Area

Technical report to New South Wales national Parks and Wildlife Service by the Royal Botanic Gardens Victoria – Melbourne

Photo: Hypogeal fungal sporocarp found at site for Nungatta Feral Predator Free Area, November 2021, NSW NPWS (Rachel Butterworth)

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The present report summarises survey results of hypogeous fungal sporocarps in the Nungatta feral predator free area (FPFA) within South East Forests National Park in New South Wales. Results from soil testing for *Phytophthora* are also discussed.

Methods

Collection of hypogeous fungal sporocarps

Surveys were conducted over two periods: most recently from June 17th to June 27th 2022 (autumn/winter survey), and previously from October 30th to November 7th 2021 (spring survey). Sporocarps of hypogeous fungi were collected from 24 plots established by National Parks & Wildlife Service (NPWS) staff (12 inside and 12 outside the proposed FPFA) (Figure 1).

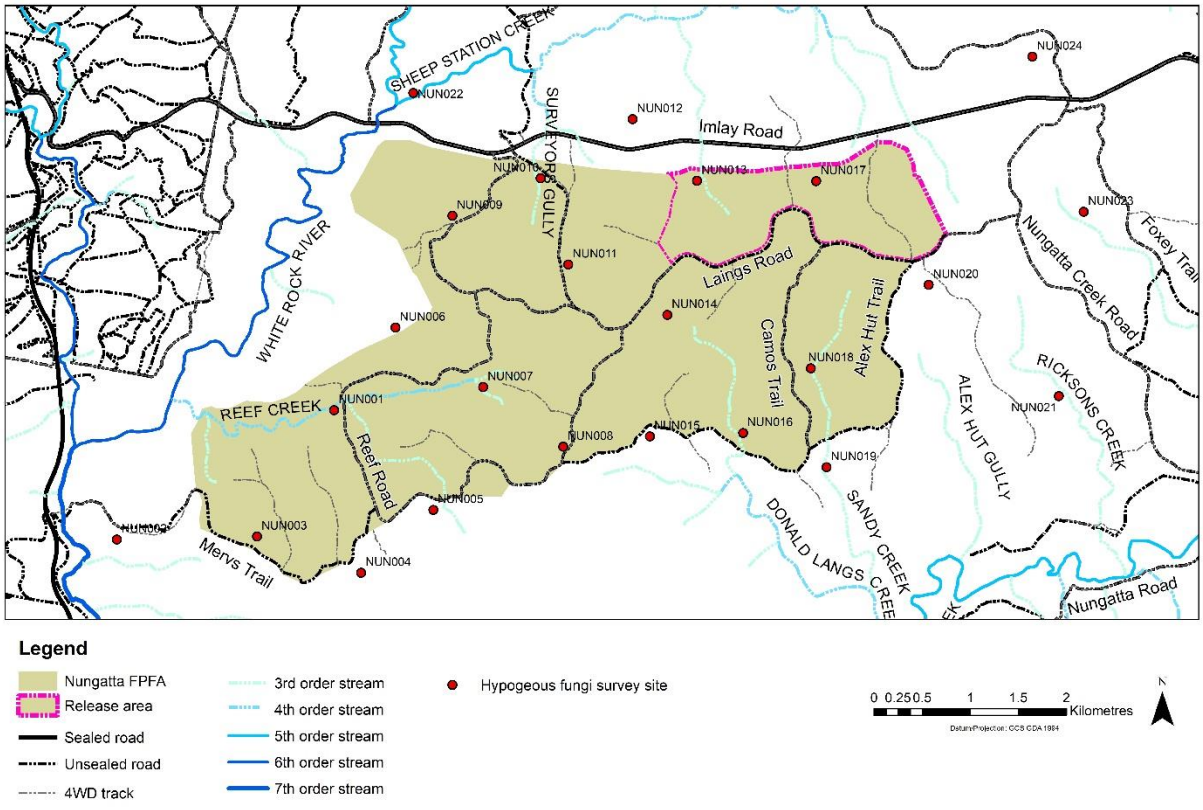

Figure 1 Location of autumn survey sites

For the autumn/winter survey, following methodology in Claridge et al. (2000), plots of 50 x 20 meters in area were searched for 100 person-minutes, entailing raking away litter, topsoil, and subsoil (up to 15 cm) with a four-pronged rake in suitable microhabitats to reveal sporocarps.

For the spring survey, a central coordinate and its surrounding area (within 70 m), was searched using the same method for 60 person-minutes. For the spring survey, in addition to searching by coordinates/plots determined by NPWS, additional sites inside and outside the proposed FPFA were also searched (Figure 2).

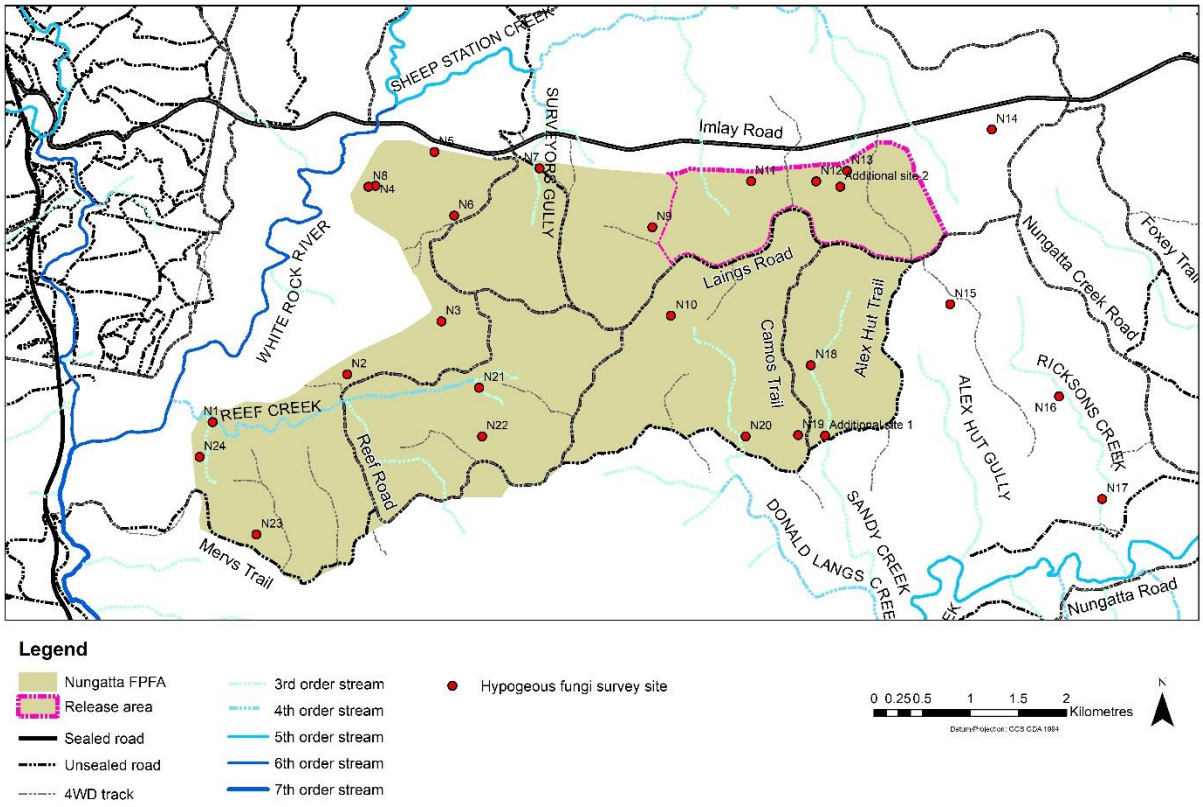

Figure 2 Location of spring survey sites

Identification of fungal taxa

Species diversity was estimated using hypothesised morphotaxa: determinations were made based on external macromorphology, then identifications were further refined based on dissection of sporocarps to reveal internal macromorphology and brief study where needed of micromorphology using compound microscopes at the Royal Botanic Gardens Victoria. Given the highly reduced morphology of hypogeous fungi and the state of flux of their taxonomy (e.g. Davoodian et al. 2021), accurate species identification must be done with molecular approaches, implementation of which is ongoing: results from molecular study of the collected specimens will be made available when complete.

Sporocarp specimen processing

Gathered sporocarps were gently air-dried with low heat after dissection and preliminary identification, and mass in grams recorded. Specimens are assigned voucher numbers and housed at the National Herbarium of Victoria (MEL).

Phytophthora *testing*

From October 30th to November 7th 2021, visual search for signs of *Phytophthora* infection in plants was conducted, which resulted in detection of possible *Phytophthora* infection at three localities (approximate coordinates of center of locality, in decimal degrees: -37.136900, 149.390458; -37.152047, 149.390825; -37.143991, 149.389087). On April 27th 2022, soil samples were taken at these three locations and submitted to Royal Botanic Garden Sydney's PlantClinic for molecular diagnosis of *Phytophthora* and *Pythium* (rbgsyd.nsw.gov.au/science/plant-id-disease-diagnostic-services). From June 17th to June 27th 2022, 19 further soil samples for *Phytophthora* and *Pythium* testing were taken by NPWS across the proposed FPFA.

Results

Hypogeous fungi

From June 17th to June 27th 2022, 208 collections (defined as sporocarps from a single hypothesised species taken at a single raking spot) were retrieved. Taxonomic diversity, biomass per site, and number of sporocarps per site are tabulated in Table 1. The average biomass of sporocarps per site was 1.65g. However, it should be noted that sporocarp biomass is difficult to accurately measure given the tendency for tissue to grow around and embed soil, roots, and small stones. Any comparisons with biomass reported in previous studies may therefore be misleading.

Across the 24 surveyed sites, the average number of sporocarps found per site during the autumn survey was approximately 19.8, with a standard error of 3.82. The minimum number of sporocarps found per site during the autumn survey was 0 and the maximum number was 68. Across the 24 sites surveyed, 37 species/form taxa were recovered in the autumn survey, with an average of approximately 4.6 per site (standard deviation approximately 2.7); the minimum number of species recovered per site was 0 and the maximum was 10.

From October 30th to November 7th 2021, 22 collections were retrieved. Taxonomic diversity, biomass per site, and number of sporocarps per site are tabulated in Table 2. Across all the surveyed sites, the average number of sporocarps found per site during the spring survey was approximately 2.5, with a standard error of 0.96; the minimum number of sporocarps found per site during the spring survey was 0 and the maximum number was 20. Across all surveyed sites, 9 species/form taxa were recovered in the spring survey, with an average of approximately 0.85 per site (standard deviation approximately 1); the minimum number of species recovered per site was 0 and the maximum was 3.

Phytophthora *and* Pythium *testing*

Phytophthora cinnamomi was detected at all three sites surveyed in April 2022 (-37.136900, 149.390458; -37.152047, 149.390825; -37.143991, 149.389087). Results from further testing have indicated *Phytophthora* and similar pathogens to be widespread across the site.

Discussion

Based on the surveys outlined above, diversity and biomass/sporocarp production of hypogeous fungi at Nungatta are substantial, especially in the autumn/winter. Though recorded sporocarp production per site at Nungatta is significantly less than the average (43.66 with standard error 2.32) and maximum (120) sporocarp numbers reported by Claridge et al. (2000) for autumn surveys, it is important to note the reported numbers from Claridge et al. (2000) are based on 136 sites distributed at landscape scale. Based on recent fieldwork by Davoodian (including within long-footed potoroo habitat), the proposed Nungatta FPFA appears to be a promising location for reintroduction of native mycophagous mammals. Recent surveys undertaken in known long-footed potoroo habitat in East Gippsland, Victoria, generally yielded lower diversity and abundance of hypogeous fungi in the autumn/winter period than recorded at Nungatta in the autumn/winter (Davoodian, unpublished data). While these surveys were undertaken using a somewhat different methodology to that employed at Nungatta, in the opinion of the author the results nevertheless appear to indicate more substantial hypogeous fungal resources at Nungatta during the survey period.

Past collections of hypogeous fungi within the area also suggest it is likely suitable for reintroduction of native mammals, and this has been corroborated by the 2021/2022 surveys. Hypogeous genera such as *Cortinarius*, *Hysterangium*, and *Mesophellia* have been collected in the general area in past years (e.g. herbarium specimens MEL2238585A, MEL2329244A, and MEL2238580A, respectively) and over the course of these surveys (Tables 1 & 2); these taxa are known constituents of the diets of various Australian mammals that inhabit New South Wales and Victoria (Davoodian et al. unpublished data, Green et al. 1999, Nuske et al. 2017), including the long-footed potoroo (*Potorous longipes*). Based on supplementary data to Nuske *et al.* (2017), 11 of the 37 hypogeous fungi genera recorded during the 2021/2022 surveys are known to comprise part of the diet of the long-footed potoroo and 10 are known to comprise part of the diet of the eastern bettong (Table 4). These include two of the most widespread genera recorded during the surveys, which are components of the diet of both species: *Hydnangium* (recorded at 12 sites) and *Hysterangium* (recorded at 11 sites).

It should be noted that, in the opinion of the author, the long-footed potoroo, eastern bettong and other mycophagous species are likely to consume a much broader range of hypogeous fungi than has been documented to date, since many taxa may not have been readily identifiable using the techniques available at the time past dietary studies were undertaken, over 20 years ago. Current eDNA techniques would likely reveal a more complete suite of the taxa consumed by these species, which may include more of the taxa present at Nungatta.

Discussions on *Phytophthora* management are underway; details of a *Phytophthora* management plan will be dependent on results from further soil testing.

The aim of these surveys was to explore potential hypogeous fungi abundance and diversity across the proposed Nungatta FPFA. The results from these surveys indicate that sufficient hypogeous fungal diversity and abundance exists at the site to justify proceeding with further planning, including determining contingencies related to *Phytophthora* management.

Arrangements are being made to return to Nungatta to conduct eDNA sampling in the proposed FPFA in late 2022 or 2023, and this will provide further data on the hypogeous fungi species at the site.

Based on the survey results to date and previously available data outlined in this report, it can be stated that the proposed FPFA is likely suitable for the reintroduction of native mycophagous mammals, dependent on further monitoring, investigation, and planning.

References

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Nuske, S.J., Vernes, K., May, T.W., Claridge, A.W., Congdon, B.C., Krockenberger, A. and Abell, S.E., 2017. Data on the fungal species consumed by mammal species in Australia. *Data in Brief* 12: 251–260.

Table 1. Results from hypogeous fungi survey of Nungatta sites (June 17th to June 27th 2022) where 100 person-minutes of searching was conducted. Table lists sites, species, mass (sum of all collections from a site; dry biomass; rounded to nearest 0.05 g), number of sporocarps found at each site, additional notes, and approximate central coordinates of where collections were made (latitude and longitude in decimal degrees). Plots that were surveyed but yielded no sporocarps are left blank.

Site	Species	Mass	Sporocarps	Notes	Latitude	Longitude
NUN001	*Hydnangium* sp.; *Descomyces* sp.; *Hysterangium* sp.; *Russula* sp.; *Cortinarius* sp.	1 g	21		-37.161938	149.352079
NUN002	*Descomyces* sp. 1; *Hydnangium* sp.	0.15 g	4		-37.174935	149.326438
NUN003					-37.173777	149.342888
NUN004	Basidiomycota sp.; Boletaceae sp.; *Lactarius* "Red B" sp.; *Descomyces* sp.; *Cystangium* sp.; *Hydnangium* sp.	1 g	25	"Red B" is likely a new species	-37.177269	149.355360
NUN005	*Hysterangium* sp.; *Hydnangium* sp.; *Descomyces* sp.; Russulaceae sp.: Basidiomycota sp.	1 g	22		-37.171381	149.364241
NUN006					-37.153964	149.359073
NUN007	*Cystangium* sp.; *Lactarius* "Red B" sp.; Agaricales sp.	2.75 g	10		-37.159903	149.370459
NUN008	*Cystangium* sp.; *Descomyces* sp.; Basidiomycota sp.; unknown	1.4 g	11		-37.164864	149.379172
NUN009	*Hysterangium* sp.; *Zelleromyces* sp.; Phallales "Purple" sp.; Russulales "Peach" sp.	1 g	22	"Purple" is likely a new species	-37.142970	149.365872
NUN010	*Lactarius* "Red B" sp.; *Hydnangium* sp.; Basidiomycota sp. 1	0.8 g	7		-37.139486	149.375847
NUN011	*Descomyces* sp.; *Rossbeevera* sp.; *Cystangium* sp.; *Gymnomyces* sp.;	3.95 g	53		-37.147298	149.379290

Site	Species	Mass	Sporocarps	Notes	Latitude	Longitude
	Hydnangium sp.; *Cortinarius globuliformis*; *Hysterangium* sp.					
NUN012	*Cortinarius globuliformis*; *Rossbeevera* sp.; *Hysterangium* sp. 6	4.45 g	24		-37.133657	149.386623
NUN013	*Descomyces* sp.; Basidiomycota sp.; *Russula* sp.; *Hydnangium* sp.; *Lactarius* "Red B" sp.	1 g	41		-37.139337	149.394684
NUN014	*Glomus* sp.; *Descomyces* sp. 1; Basidiomycota sp. 2	0.5 g	6		-37.151938	149.391725
NUN015	*Lactarius* sp.; *Glomus* sp.; Basidiomycota sp.; *Hydnangium* sp.; *Descomyces* sp.; Hysterangiales sp.; *Mesophellia* sp.; *Lactarius* "Red B" sp.; *Russula* sp.; *Hysterangium* sp.	2.6 g	68		-37.163760	149.389804
NUN016	*Descomyces* sp. 2; Hysterangiales sp.; *Hysterangium* sp. 5; unknown	3.15 g	25		-37.163169	149.400620
NUN017	*Mycoamaranthus* sp.; *Cortinarius* sp.; *Scleroderma* sp.	1.6 g	6		-37.139101	149.408418
NUN018	*Hysterangium* sp. 5; Boletaceae sp.; *Descomyces* sp. 1; *Glomeromycota* sp.	3.15 g	21		-37.156844	149.408507
NUN019	*Hysterangium* sp. 6; *Hydnangium* sp.; *Hysterangium* sp.; *Russula* sp.; *Descomyces* sp.	2.3 g	26		-37.166282	149.410846
NUN020	*Chondrogaster* sp.; *Russula* sp.; *Ammarendia* sp.; *Hydnangium* sp.;	2.8 g	23		-37.148365	149.422192

Site	Species	Mass	Sporocarps	Notes	Latitude	Longitude
	Ascomycota sp.; *Zelleromyces* sp.; *Lactarius* "Red B" sp.; unknown; Basidiomycota sp.; *Stephanospora* sp.					
NUN021	*Russula* sp.; *Descomyces* sp.; *Hydnangium* sp.	0.1 g	6		-37.159041	149.438211
NUN022	*Russula* sp.; *Nothocastoreum cretaceum*; *Cystangium* sp.; Basidiomycota sp.	1 g	7		-37.131370	149.360169
NUN023	*Thaxterogaster* sp.; *Hydnangium* sp.; *Hysterangium* sp.; *Descomyces* sp.; *Hydnangium* sp.; *Lactarius* sp.; *Russula* sp.; Russulaceae sp.; *Ammarendia* sp.	1 g	40		-37.141374	149.439595
NUN024	*Hysterangium* sp.; *Descomyces* sp.; *Rossbeevera* sp.; *Cystangium* sp.; *Zelleromyces* sp.; *Mesophellia* sp.; *Russula* sp.; *Cortinarius globuliformis*	2.95 g	26		-37.126647	149.433679

Table 2. Results from hypogeous fungi survey of Nungatta sites (October 30th to November 7th 2021) where one person-hour of searching was conducted. Table lists sites, species, mass (sum of all collections from a site; dry biomass; rounded to nearest 0.05 g), number of sporocarps found at each site, additional notes, and approximate central coordinates of where collections were made (latitude and longitude in decimal degrees). Plots that were surveyed but yielded no sporocarps are left blank. Plots that were not surveyed are indicated with "n/a (not surveyed)."

Site	Species	Mass	Sporocarps	Notes	Latitude	Longitude
N1	*Russula* sp.; Boletales sp.	0.1 g	2	Both species recorded near two large *E. viminalis*	-37.163322	149.337687
N24					-37.166648	149.336250
N21	*Hysterangium* sp. 3	6 g	20	Near *E. viminalis*	-37.159438	149.369210
N9					-37.143824	149.389308
N4					-37.140555	149.356335
N3					-37.153248	149.364540
N18	*Hysterangium* sp. 1	0.4 g	5	Near *E. cypellocarpa*	-37.156510	149.408531
N20	*Hysterangium* sp. 1	1 g	5		-37.163420	149.400997
N17	n/a (not surveyed)	n/a		n/a (not surveyed)	-37.168483	149.443496
N16					-37.158868	149.438078
N15	*Mesophellia* sp.	0.1 g	1	The *Mesophellia* is a burned sporocarp from last wildfire	-37.150404	149.424873
N22					-37.164052	149.369721
N19					-37.163171	149.407221
N14	Phallales sp.	0.05 g	1		-37.133741	149.429271
N6					-37.143196	149.365754
N7	*Hysterangium* sp. 1	3 g	15		-37.138512	149.375747
N13					-37.138012	149.412226
N12					-37.139087	149.408600
N11	*Hysterangium* sp. 1; *Hysterangium* sp. 2; Boletaceae sp. 1	1 g	6		-37.139225	149.400868
N8					-37.140660	149.355481
N5					-37.137204	149.363222
N10	*Mesophellia* sp.;	0.2 g	2	The *Mesophellia* is a burned	-37.152149	149.391767

Site	Species	Mass	Sporocarps	Notes	Latitude	Longitude
	Hysterangium sp. 1			sporocarp from last wildfire		
N2	Hysterangiales sp.; Boletales sp.; Boletaceae sp. 1	0.3 g	4		-37.158488	149.353524
N23	*Hysterangium* sp. 1	0.1 g	1	Near a few *E. cypellocarpa*	-37.173881	149.343215
Additional site 1	*Hysterangium* sp.	0.1 g	1		-37.163153	149.410422
Additional site 2	*Hysterangium* sp. 3/4	0.1 g	1	Found approx. between plots 92 and 93	-37.139530	149.411474

Table 3. Complete list of all hypogeous fungi recorded during both spring and autumn surveys at Nungatta

Taxa name
Agaricales sp.
Ammarendia sp.
Ascomycota sp
Basidiomycota sp.
Basidiomycota sp. 1
Basidiomycota sp. 2
Boletaceae sp.
Boletaceae sp. 1
Boletales sp.
Chondrogaster sp.
Cortinarius globuliformis
Cortinarius sp.
Cystangium sp.
Descomyces sp. 1
Descomyces sp. 2
Descomyces sp.
Glomeromycota sp.
Glomus sp.
Gymnomyces sp.
Hydnangium sp.
Hysterangiales sp.
Hysterangium sp. 1
Hysterangium sp. 2
Hysterangium sp. 3/4
Hysterangium sp. 5
Hysterangium sp. 6
Lactarius "Red B" sp.
Lactarius sp.
Mesophellia sp.
Mycoamaranthus sp.
Nothocastoreum cretaceum
Phallales sp.
Phallales "Purple" sp.
Rossbeevera sp.
Russula sp.
Russulaceae sp.

Taxa name
Russulales "Peach" sp.
Scleroderma sp.
Stephanospora sp.
Thaxterogaster sp.
Zelleromyces sp.
Unknown (treated as 1 taxon)

Table 4. Hypogeous fungi genera recorded at Nungatta that are known components of the long-footed potoroo (LFP) and eastern bettong (EB) diet, and number of sites they were recorded in during each survey period

Genus	**Confirmed component of LFP diet**	**Confirmed component of EB diet**	**No sites recorded, autumn**	**No sites recorded, spring**
Descomyces sp.		*	15	-
Hydnangium sp.	*	*	12	-
Hysterangium spp.	*	*	11	9
Russula sp.		*	9	1
Cystangium sp.	*	*	6	-
Cortinarius spp.	*		5	-
Rossbeevera sp.	*	*	3	-
Zelleromyces sp.	*	*	3	-
Ammarendia sp.	*		2	-
Mesophellia sp.	*	*	2	2
Gymnomyces sp.	*	*	1	-
Scleroderma sp.	*	*	1	-
Stephanospora sp.	*		1	-

Supplement 1. *Descomyces* sp. 2 from site NUN016, collection from 2022; 0.05 AUD coin for scale.

Supplement 2. Phallales “Purple” sp. from site NUN009, collection from 2022; 0.05 AUD coin for scale.