Metals X Limited

Wingellina Nickel Project

Supplementary Report: Wingellina Borefields Subterranean fauna Assessment

May 2013

FINAL REPORT

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Wingellina Borefields Subterranean Fauna Assessment

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Executive Summary

Metals X Limited (Metals X) commissioned Outback Ecology to undertake an assessment of subterranean fauna (stygofauna and troglofauna) in three potential borefield areas, Cobb Depression, Nyikukura and Officer Basin, for the proposed Wingellina Nickel Project (the Project). The Project is located around 1,400 kilometres (km) north-east of Perth. Approximately 12 gigalitres (GL) per year of groundwater will be required for the Project's construction and mining operations over the estimated 40 year lifespan of the mine.

The subterranean fauna assessment of the three potential borefield areas encompassed a habitat characterisation and Level 1 reconnaissance survey, to clarify if suitable habitat was present for stygofauna (groundwater fauna) or troglofauna (terrestrial subterranean fauna), as well as a low-intensity level of sampling for stygofauna. The assessment complements previously completed subterranean fauna surveys undertaken for several other Project areas.

Survey Effort

The stygofauna survey effort (ES Table 1) involved 18 net haul samples collected from 18 sites over two survey periods.

ES Table 1: Stygofauna net haul sample effort

Study Area	October 2011	January 2013
Cobb Depression		4
Nyikukura		2
Officer Basin	7	5

Results

The Wingellina region has previously been demonstrated to have limited potential for hosting stygofauna and troglofauna species with only a single obligate stygofauna species and one troglofauna species recorded from what were considered optimal habitat conditions that existed within the Pipalyatjara calcrete. The assessment undertaken of the three potential borefield areas are consistent with these earlier findings. No subterranean fauna species were recorded from the Nyikukura and Officer Basin borefield areas. Only one putative stygofauna species, Enchytraeidae OES 18, was collected at Cobb Depression from a single bore (CWRC2) located within the maximum modelled groundwater drawdowns. Enchytraeidae OES 18, is an oligochaete worm, and these are commonly recorded in subterranean fauna surveys. They occur in both surface and subterranean aquatic systems (freshwater and marine), or semi-aquatic and terrestrial habitats.

Assessment

The survey results are consistent with the habitat characterisation of both Nyikukura and Officer Basin borefield areas, which indicated that neither area was likely to host stygofauna and troglofauna. Therefore the development of the Nyikukura and Officer Basin groundwater supply borefields would not be considered likely to pose a long term conservation risk to species of stygofauna or troglofauna.

At Cobb Depression, the proposed lowering of the groundwater table through development of the borefield is not considered likely to pose a long term conservation risk to Enchytraeidae OES18, when considering the:

- extent of saturated habitat that would persist in the immediate locality from where the putative stygofauna species was collected, as well as within the neighbouring area, during the operational life of the borefield;
- greater extent of habitat present outside the proposed groundwater drawdown zones; and
- broader distribution patterns of other members of the oligochaete enchytraeid family.

Given the findings that the development of the potential borefields would represent little or no conservation risk to stygofauna or troglofauna, further assessment of the Cobb Depression, Nyikukura and Officer Basin borefield areas, via a Level 2 (Baseline) survey, is not considered necessary .

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1. INTRODUCTION

1.1. Project Background

Metals X Limited (Metals X) commissioned Outback Ecology to undertake an assessment of subterranean fauna (stygofauna and troglofauna) in the proposed Wingellina Nickel Project (the Project), located within Ngaanyatjarra lands on Aboriginal Reserve 17614. The Project is situated along the Gunbarrel Highway approximately 1,400 kilometres (km) north-east of Perth and 8 km south-west of the Surveyor General's Corner that marks the intersection of the Northern Territory, South Australian and Western Australian borders. The Wingellina nickel deposit is located between the North and South Hinkley Ranges and within the Wingellina Hills that are part of the Musgrave Ranges region. It is estimated that 180 million tonnes (Mt) of ore at 0.98% nickel and 0.08% cobalt is present providing a mine life of over 40 years at the expected average mining rate of 4.3 Mt per annum (pa). Approximately 12 gigalitres (GL) per year of groundwater will be required for the Project's construction and operation over the life of the mine.

A subterranean fauna assessment was previously completed for several Project areas (Wingellina Pit, Wingellina Tailings Storage Facility (TSF), Pipalyatjara Calcrete, and including exploration areas Area 3, the Wedge and Yapan) (**Table 1**). This included comprehensive sampling within the Pipalyatjara calcrete system that represented the most optimal subterranean fauna habitat within the Project area (Outback Ecology 2011b). This earlier work identified limited occurrence of subterranean fauna in the region, with only one stygofauna species and one troglofauna species collected, both from within the Pipalyatjara calcrete.

At the time when the field surveys were undertaken for the previous subterranean fauna assessment, potential aquifers for the supply of water for the Project had not been identified. Three potential aquifers have now been identified at Cobb Depression, Nyikukura and Officer Basin (**Table** 1). Cobb Depression has been recognised as having the most potential to satisfy the Project's groundwater demand (Rockwater 2013).

1.2. Scope and Objectives

The objective of the assessment presented in this report was to assess the occurrence of subterranean fauna in each of the potential borefield areas and to establish if habitat modification associated with the proposed groundwater drawdown during development and operation of the Project would pose a conservation risk to any subterranean fauna species.

The subterranean fauna assessment presented in this report is supplementary to the earlier, more extensive assessments undertaken for the Project (Outback Ecology 2011b). The scope of the this assessment encompassed a reconnaissance survey, to clarify if suitable habitat was present for stygofauna (groundwater fauna) or troglofauna (terrestrial subterranean fauna), and low-intensity sampling for stygofauna.

Table 1: Components of the Wingellina Nickel Project that had been previously assessed for subterranean fauna (Outback Ecology 2011b) and the three areas included within this Wingellina Borefields subterranean assessment (shaded light orange).

Study Area		Tenement	State	Activity	Potential Impact on Subterranean Fauna
Previous surveys	Wingellina Pit	E69/535	WA	Open pit nickel mining	Physical removal of habitat, groundwater drawdown
	Wingellina TSF	E69/535	WA	Tailings storage facility	Surface disturbance, alteration of resource flow, contamination
	Pipalyatjara Calcrete	E3555	SA	Potential source of limestone	Physical removal of habitat above groundwater level
	Area 3, The Wedge and Yapan	E3555	SA	Additional resource exploration	N/A
Borefield Assessment	Cobb Depression	L69/19	WA	Potential water supply borefield	Groundwater drawdown
	Nyikukura	E3555	SA		
	Officer Basin	L69/12	WA		

2. METHODS

2.1. Survey Levels

Previous stygofauna and troglofauna sampling undertaken for the Project (**Table 2**) demonstrated an absence of subterranean fauna in all areas, with the exception of a single stygal copepod species and troglobitic pseudoscorpion that were both recorded from the large Pipalyatjara calcrete system (Outback Ecology 2011b). In light of previous desktop and survey results, a Level 1 reconnaissance survey coupled with the low-intensity sampling of stygofauna was considered sufficient for assessing the likelihood of stygofauna and troglofauna occurring within the potential borefield areas. This level of survey is in accordance with new draft guidelines released in March 2013 (Environmental Protection Authority 2013) that supersede Guidance Statement 54 (Environmental Protection Authority 2003).

2.2. Study Areas and Stygofauna Survey Sites

All suitable bores available for stygofauna sampling were accessed within each of the three potential borefield areas. In total, 18 samples from 18 bores were sampled across the three areas (**Table 2**, **Figure 1**), as follows:

- *Cobb Depression* — Four bores were sampled in the Cobb Depression (approximately 80 km northeast of the Wingellina deposit), intersecting an alternating sequence of predominantly clays and gravels, underlain by sandstone at approximately 125 m (Rockwater 2013);
- *Nyikukura* — Two bores were sampled near Nyikukura in South Australia, approximately 10 km east of the Wingellina deposit. The bores extend approximately 130 m and intersect palaeochannel sediments composed largely of clay and gravel (Rockwater 2012a).
- *Officer Basin* — Twelve bores in the central Officer Basin, were sampled approximately 95 km southwest of the Wingellina deposit. Within the water exploration area the three main lithostratigraphic units intersected, in order of deposition were the Wahlgu Formation, the Lungkarta Formation, and younger Vines Formation (Rockwater 2012b).

Table 2: Subterranean fauna sampling effort for the Wingellina Nickel Project, including the previous survey areas (Outback Ecology 2011b) and the three areas included within this Wingellina Borefields assessment (shaded light orange). Stygofauna net haul sample effort non-italcised; Troglofauna litter trap sample effort italcised and placed to right of reverse solidus symbol (\ ).

	Study Area	Apr 2008	Sep 2010	Nov 2010	Jan 2011	Oct 2011	Jan 2013	Total
Previous surveys	Wingellina Pit	11	2	10	9			**32**
	Wingellina TSF	2		4	4			**10**
	Pipalyatjara Calcrete		8	28	26			**62**
	Area 3, The Wedge and Yapan		4	13	11			**28**
Borefield Assessment	Cobb Depression						4	**4**
	Nyikukura						2	**2**
	Officer Basin					7	5	**12**
							Total number of samples	**150**

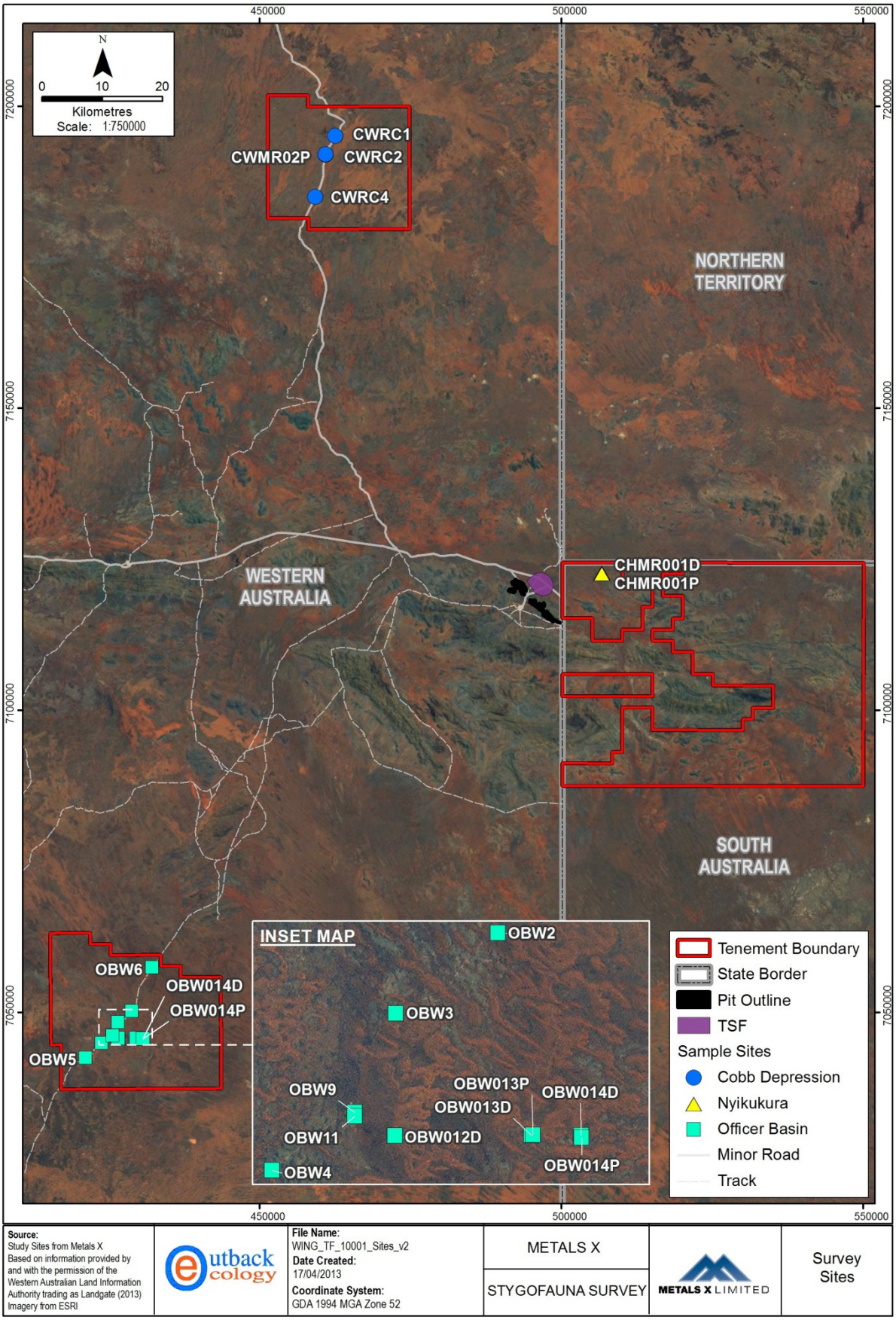

Figure 1: Location of bores sampled for the Wingellina Borefields stygofauna assessment.

2.3. Stygofauna Sampling

Reconnaissance and field surveys were undertaken over two periods:

- on the 4th of October, 2011, by Adrian Rakimov and Mike Young (both Outback Ecology); and
- from the 24th to the 26th of January, 2013, by Jason Coughran (Outback Ecology) and Zian Minhinnick (Metals X), under DEC permit SF009031.

Stygofauna were sampled using haul nets, which have been found to be the most efficient retrieval method (Allford *et al.* 2008). Sampling was consistent with the procedures outlined in the EPA Draft Guidance Statement No. 54a (EPA 2007). The sampling method was as follows:

- samples were collected using two weighted haul nets with mesh sizes of 150 µm and 50 µm. Each net was fitted with a collection vial with a base mesh of 50 µm;
- the 150 µm net was lowered first, near to the bottom of the hole;
- once at the bottom, the net was gently raised up and down to agitate sediments;
- the net was then raised slowly to minimise the 'bow wave' effect that may limit collection of specimens, filtering the stygofauna from the water column on retrieval;
- once retrieved, the collection vial was removed, the contents emptied into a 250 ml polycarbonate vial, and preserved with 100% undenatured ethanol;
- this process was repeated three times with the 150 µm net and a further three times using the 50 µm net;
- to prevent cross-contamination, all sampling equipment was washed thoroughly with Decon 90 (2 to 5% concentration) and rinsed with potable water after each bore;
- in the field, samples were placed into eskies with ice bricks prior to being transferred into a refrigerated environment on-site at the end of each survey day; and
- samples were couriered back to the Outback Ecology laboratory in Perth, where they were stored at approximately minus 20°C in 100% ethanol.

2.4. Groundwater Quality

Basic physicochemical data was collected during the stygofauna surveys. The approximate standing water level (SWL), given as metres below ground level (mbgl), was measured using a Heron Dipper T100 water level meter. A calibrated YSI ProPlus multi-parameter field instrument was used to measure pH, water temperature, dissolved oxygen (DO), electrical conductivity (EC), salinity and reduction-oxidation potential (Redox) of the groundwater. The end of hole (EoH) was estimated using the number of rotations of the stygofauna sampling winch reel required to retrieve stygofauna nets.

2.5. Sorting and Identification of Specimens

Preserved samples were sorted manually under Leica MZ6, MZ7.5, M80 and M205C stereo microscopes, by Lina Ramlee and Syngeon Rodman. Stygofauna specimens were identified to the lowest possible taxonomic rank and morpho-typed by Dr Jason Coughran and Dr Erin Thomas of Outback Ecology. However, Enchytraeidae specimens could not be identified below family level due to the current limitations in taxonomy for the group in Australia. Terrestrial invertebrates that were inadvertently collected as by-catch in samples were identified to Order level.

2.6. DNA sequencing

Representative specimens of Enchytraeidae from the Cobb Depression were sent to Dr Remko Leijs (South Australian Museum) for DNA analysis, together with specimens of Enchytraeidae OES19 collected in stygofauna net haul and troglofauna litter trap samples during previous surveys at Pipalyatjara calcrete (Outback Ecology 2011b).

3. RESULTS AND DISCUSSION

3.1. Regional Context

The subterranean fauna of the central arid zone is poorly known and the area is considered to have limited potential for supporting species rich subterranean fauna communities (Environmental Protection Authority 2007, 2013). In other semi-arid to arid areas of Australia, particularly the northern Goldfields region of Western Australia, valley calcrete deposits, that form in association with groundwater aquifers, are recognized as providing optimal habitat conditions for both stygofauna and troglofauna, with many diverse and abundant assemblages recorded (Allford *et al.* 2008, Cooper *et al.* 2008, Environmental Protection Authority 2007, Humphreys 2008, Outback Ecology 2011a, 2012). Calcretes such as these are formed in association with groundwater, rather than through soil development (pedogenic calcrete), and their prevalent void spaces provide favourable habitat for subterranean fauna (Humphreys 2001, Humphreys *et al.* 2009). The valley calcretes occur extensively in the northern Goldfields, but less frequently below the Menzies line (latitude 29 degrees South). Pedogenic calcretes, which dominate to the south of the Menzies line (Anand and Paine 2002, Morgan 1993), form within the soil above the groundwater and have not been found to provide suitable habitat for subterranean fauna species.

For the Wingellina Project, the Pipalyatjara groundwater calcrete system was considered to have a high likelihood of supporting subterranean fauna (Environmental Protection Authority 2013). However, the comprehensive stygofauna and troglofauna survey undertaken collected only a single obligate stygofauna species, the copepod *Dussartcyclops* sp. TK1, and only one troglofauna species, *Tyrannochthonius* sp. OES6, from within the Pipalyatjara calcrete (Outback Ecology 2011b). In other habitats sampled in other Project areas, no stygofauna or troglofauna species were recorded (Outback Ecology 2011b). The previously reported study, represents the only comprehensive subterranean fauna study of the Wingellina region. There are no documented records in the database of the Western Australian Museum of subterranean fauna occurring within 400 km of the Project (Outback Ecology 2011b). Therefore, based on current knowledge, the central arid zone, particularly the Wingellina region, appears to have limited potential for hosting subterranean fauna species.

3.2. Subterranean Fauna Habitat

Cobb Depression

The Cobb Depression is a trough, approximately 20 km wide, that has filled with fluvial (possibly also aeolian) sediments extending up to 140 mbgl (Rockwater 2013). The sediments within the Cobb depression are composed of red sand overlying successive layers of clayey to slightly clayey, fine to coarse grained quartz sand interlaid with gravel layers (Rockwater 2013). The test pump results indicate that the aquifer is confined with most of the groundwater stored within the gravel and coarse grained sand strata that are confined by intervening, less-permeable, massive layers of finer grained material (Rockwater 2013). The aquifer thickness within the vicinity of bores CWMR02P and CWRC2

was 98 m with the standing water level occurring at 34 mbgl, one metre below a confining massive clay layer (Rockwater 2013). Confined aquifers are not regarded as providing suitable conditions for stygofauna as by there very nature, resources (e.g., food, dissolved oxygen) can be limited, particularly in parts of the aquifer that are distant from recharge or discharge zones (Strayer 1994).

Given that the substrate in the Cobb Depression is dominated by sand and clay, it would be expected to lack cavities or voids that might provide potential habitat for subterranean fauna. The bore completion log for CWMR02P indicated a layer of calcrete, intermixed with fine and coarse grained sand, from 3 to 9 mbgl, well above the groundwater table (Rockwater 2013). This calcrete is considered to be of pedogenic origin and is not extensive or likely to provide suitable habitat for subterranean fauna. In summary, the likelihood of the substrate within the Cobb Depression supporting stygofauna and troglofauna is considered low.

Nyikukura

The aquifer investigated at Nyikukura occurs within palaeovalley sediments and has been demonstrated to be a potable source of water and potentially has sufficient permeability, and storage capacity to meet the Project's water demands for the construction phase of the operation (Metals X pers, comm.). The sediments intersected below the overlying sand layer are largely composed of clay and gravel with a dominant band of sandy clay present approximately 20 m below the standing water level that is present at around 23 mbgl (Rockwater 2012a). The strata intersected below the groundwater table are not considered to represent prospective stygofauna habitat due to the lack of pore space available for colonisation, evidenced by the mainly fine sediments present (Rockwater 2012a). At or above the standing water level, sediments may contain pebble sizes that are generally larger, but these are hosted within fine grained sand, siltstone or clay, or cemented within pedogenic calcrete (Rockwater 2012a) that would infill interstitial spaces that might otherwise have provided some pore space for potential colonisation. The likelihood of the habitat present at Nyikukura supporting stygofauna and troglofauna is considered low.

Officer Basin Borefield

Within the surveyed area of the Officer Basin Borefield three main lithostratigraphic units are present (Rockwater 2012b). The older Wahlgu Formation is generally overlain by the younger Vines and Lungkarta Formations, except in the northern part of the survey area where the unit subcrops beneath a thin layer of fine grained sand (Rockwater 2012b). The unit dips to the south, to greater than 175 to 220 mbgl. This formation was deposited during fluvio-glacial environmental conditions and is mostly composed of sandy diamictite with bands of mudstone and sandstone also present (Rockwater 2012b). The clay content was found to be relatively high and increased with depth (Rockwater 2012b). The permeability of this unit is much lower than the Vine and Lungkarta Formations (Rockwater 2012b) and indicates that there is insufficient interstitial space present for potential

subterranean fauna colonisation. Therefore, the likelihood of the Wahlgu Formation within the central Officer Basin supporting stygofauna or troglofauna is considered low.

In the central part of the survey area the Vines Formation ranges in extent from around 8 to 140 mbgl, and extends from 16 to greater than 290 mbgl towards the south (Rockwater 2012b). This formation is believed to have been deposited in a fluvial environment over the older Lungkarta and Wahlgu Formations (Rockwater 2012b). The Vines Formation is generally comprised of well consolidated sandstone and conglomerate with minor occurrences of claystone and siltstone interbedded (Rockwater 2012b). Bore completion logs of several bores indicated minor amounts of pedogenic calcrete that generally occurred as clasts or interbedded within sands or mudstone layers close to the surface and well above the groundwater level (Rockwater 2012b). The porosity and permeability of the Vines Formation was low (Rockwater 2012b) and indicates that there is limited interstitial space present for potential subterranean fauna colonisation. Therefore, the likelihood of the habitat present within the geological strata of the Vines Formation supporting stygofauna or troglofauna is considered low.

The Lungkarta Formation was considered to be the most prospective geological unit for providing a source of groundwater for the proposed Project (Rockwater 2012b). The formation is mostly comprised of well consolidated fine to medium grained sandstone that was deposited within an aeolian environment (Rockwater 2012b). The unit subcrops in the northern part of the survey area, extending from 12 to 34 mbgl, with some outcrops present that extend to 120 mbgl (Rockwater 2012b). Centrally, the upper strata of the Lungkarta Formation ranged from around 40 to 170 mbgl with lower strata extending to depths as great as 200 or 300 mbgl (Rockwater 2012b). Analyses indicated that the aquifer is confined by the overlying fine grained clay sediment at the base of the Vines Formation (Rockwater 2012b). As mentioned above, confined aquifers are not regarded as providing suitable conditions for stygofauna. The porosity and permeability of the Lungkarta Formation was low (Rockwater 2012b) indicating that there is limited interstitial space present for potential subterranean fauna colonisation. Therefore, the likelihood of the saturated or unsaturated habitat present within the geological strata of the Lungkarta Formation supporting stygofauna or troglofauna is considered low.

3.3. Groundwater Quality

The groundwater in all three potential borefield areas was of a suitable quality for stygofauna (**Table** 3). The pH was generally circum-neutral (6.5 to 7.5), only slightly lower than the typical pH range of calcrete aquifers (7.2 to 8.2) where diverse stygal communities can occur (Humphreys 2008). Groundwater was classified as fresh at Cobb Depression and Nyikukura, and slightly hyposaline (*sensu* Hammer 1986) at Officer Basin. Fresh to mesosaline conditions suit stygofauna, and although diversity generally decreases in hypersaline conditions, some species can tolerate salinities exceeding 100,000 ppm (Outback Ecology 2012). All of the salinities recorded in the Wingellina Borefield areas were sufficiently low for stygofauna. Dissolved oxygen (DO) levels were also generally suitable in each area, with only two bores, OBW4, OBW13P, in the Officer Basin recording

less than 1.0 ppm. Although stygofauna taxa have been recorded over a wide range of dissolved oxygen concentrations and have evolved to tolerate oxygen deficient groundwaters (Malard and Hervant 1999), species diversity and abundance decline strongly at levels below 1.0 ppm, with levels less than 0.5 ppm representing a critical threshold for long term persistence (Hahn 2006).

Table 3: Summary of groundwater properties for the Wingellina Borefields stygofauna assessment.

Study Area		pH (units)	Salinity (ppm)	Temp (°C)	DO (ppm)	Redox (mV)
Cobb Depression	min	7.18	480	24.4	4.17	66.4
	max	7.31	900	28.4	5.42	79.4
	mean	7.24	690	26.3	5.07	71.2
	n	4	4	4	4	4
Nyikukura	min	6.96	480	28.0	-	95.8
	max	7.10	500	28.9	3.12	121.2
	mean	7.03	490	28.5	3.12	108.5
	n	2	2	2	2	2
Officer Basin	min	7.01	1,031	25.1	0.40	-240.0
	max	8.00	15,900	28.9	4.40	193.0
	mean	7.29	4,439	26.6	1.97	46.2
	n	12	12	12	12	12

3.4. Stygofauna Assessment

No stygofauna species were collected from the Nyikukura and Officer Basin borefields (**Table 4**). At the Cobb Depression borefield, four specimens of a putative stygofauna species, Enchytraeidae OES18 were collected (**Figure 2**). All four specimens were collected from a single bore, CWRC2 (**Appendix B**), located within the maximium modelled groundwater drawdown contours (Rockwater 2013).

Table 4: Putative stygofauna species diversity, abundance and distribution.

Taxa	Cobb Depression	Nyikukura	Officer Basin
Oligochaeta			
Enchytraeidae OES18	4		

Figure 2. Enchytraeidae OES 18 collected from Cobb Depression during the Wingellina Borefields stygofauna assessment.

A genetic analysis was undertaken to determine if Enchytraeidae OES18 was the same species as Enchytraeidae OES19, recorded from Pipalyatjara calcrete in 2011 (Outback Ecology 2011b). The respective specimens were indistinguishable morphologically from each other based on external features. Enchytraeidae OES19 had been collected in stygofauna net haul samples as well as troglofauna litter trap samples. Therefore, it was not considered to be a stygofauna species as was not confined to the groundwater, and is likely to be semi-aquatic or terrestrial. Genetic analysis, using the DNA species barcoding fragment of the CO1 gene (Hebert *et al.* 2003), confirmed that

Enchytraeidae OES18 and Enchytraeidae OES19 are separate, but closely related species, with an inter-species genetic pairwise sequence divergence of 9.4 - 9.5% (**Appendix D**)(Leijs 2013).

The taxonomy and ecology of the Enchytraeidae is poorly known, with no stygal species described to date (Pinder 2009, Pinder 2007). These worms are commonly recorded in subterranean fauna surveys, and occur in both surface and subterranean aquatic systems (freshwater and marine), or semi-aquatic and terrestrial habitats (Outback Ecology 2011a, Rota *et al.* 2007, van Vliet *et al.* 1997). It is not known whether Enchytraeidae OES18 is an aquatic (inhabiting the aquifer), semi-aquatic or terrestrial oligochaete species. Terrestrial fauna are often collected in stygofauna nets having either being dislodged from the soil profile during retrieval of the nets or collected from the water column into which they may have fallen (Outback Ecology 2011a, Outback Ecology unpublished data, Subterranean Ecology 2008a, b). This could have occurred in this instance as bore CWRC2 is slotted for approximately 15 m above the standing water level (**Appendix A**). At the location from which Enchytraeidae OES18 was collected, the aquifer was considered to be a confined and therefore generally not considered to provide suitable conditions for stygofauna, particularly in parts of the aquifer that are distant from recharge or discharge zones. The close evolutionary relationship demonstrated by the CO1 analysis to exist between Enchytraeidae OES18 and the semi-aquatic or terrestrial Enchytraeidae OES19 may indicate that Enchytraeidae OES18 is also not an obligate groundwater inhabitant. However, this cannot be determined conclusively from this survey. It is for this reason that Enchytraeidae OES18 is considered a putative stygofauna species.

The two Enchytraeidae species, Enchytraeidae OES 18 and Enchytraeidae OES 19, recorded from the Wingellina region may be more widespread than has currently been demonstrated. In other areas, distribution ranges of individual Enchytraeidae species, as well as other better known oligochaete groups, such as phreodrillids, have been found to be widespread, occurring across disjunct aquifers and drainage catchments (Biota 2010, Outback Ecology unpublished data, Pinder 2008, Pinder *et al.* 2010).

3.5. Troglofauna Assessment

Troglofauna were not actively sampled for in this assessment of the potential borefield areas because previous desktop and survey results demonstrated that the Wingellina area has limited potential for hosting troglofauna species (Outback Ecology 2011b). A Level 1 reconnaissance survey, as well as geological characterisation as part of groundwater investigations (Rockwater 2012a, b, 2013) (refer 3.2 above), of each of the borefield areas indicated that there was a low likelihood of the habitat supporting troglofauna. Further to this, the extents of the unsaturated subsurface geologies present within each borefield area appear to be relatively widespread and contiguous with the surrounding region. This means that the distributions of troglomorphic species, if any were present, are unlikely to be restricted to a small area. In addition to this, the likely degree of impact to any troglofauna species, if any were present, posed by the drawdown of groundwater would be considered low. Therefore, the proposed development of the Project's groundwater supply borefields is not considered to pose a risk to the long-term survival of any species of troglofauna.

3.6. Risk Assessment

The direct potential impact of the proposed development of the Project's borefields on stygofauna and troglofauna is the removal of habitat by the lowering of the groundwater table through groundwater abstraction. Groundwater drawdowns are considered to have greater impacts on stygofauna compared to troglofauna because lowering of the water table can directly reduce the extent of habitat available. In the case of troglofauna, the lowering of the water table could mean portions of any saturated vuggy geology that may be present, may become unsaturated and potentially available for colonisation. Therefore the risk assessment outlined below focuses on stygofauna.

Cobb Depression Borefield

The habitat present within the Cobb Depression area, together with the broader characterisation of the surrounding Wingellina region as having low prospectivity for stygofauna species (based on habitat assessments and results of comprehensive sampling from various Project areas), indicates that it is likely that there are limited stygofauna values present. Low intensity sampling confirmed this, with only one putative stygofauna species collected. The species Enchytraeidae OES18, was found from a single bore located within the maximum drawdown contours of a number of modelled groundwater abstraction scenarios (Rockwater 2013). The distribution ranges of species that are only known from a single location are difficult to reliably assess in relation to proposed impacts.

There are many factors that influence the distribution of stygofauna at a range of habitat and temporal scales (Boulton 2000). Some of the more influential factors at the microhabitat (sediment) scale include interstitial pore size, inflow rates of energy resources (e.g. organic carbon, biofilm growth, prey), and water quality parameters such as water temperature, pH, dissolved oxygen and organic carbon levels. At the mesohabitat (catchment) scale, factors include geomorphological features influencing zones of upwelling and downwelling of energy resources or dissolved oxygen, as well as interactions with riparian and parafluvial sediments (Boulton *et al.* 1998). The seemingly restricted distribution of a taxon to a single bore is likely to be an artefact of that species occurring at low population densities with an irregular distribution pattern within the aquifer in response to micro and mesohabitat factors, rather than the actual distribution being confined to one limited area that happened to be intercepted by a single bore.

The oligochaete family, Enchytraeidae, is known to occur in many regions in Western Australia, with many enchytreaid species, as well as species from other better known oligochaete groups, such as phreodrilids, having widespread distributions that can occur across disjunct aquifers and drainage catchments (Biota 2010, Outback Ecology unpublished data, Pinder 2008, Pinder *et al.* 2010). Although a wider distribution range for Enchytraeidae sp. OES18 was not demonstrated in this study, it is considered likely that the distribution range of this species would extend to other areas of the Cobb Depression and would not be confined to the immediate vicinity of the bore from which it was collected.

If Enchytraeidae OES18 was confirmed to be a stygofauna species and was restricted to within the various modelled groundwater drawdown impact areas, with drawdowns ranging from 25 to 40 m, its long term persistence would be considered to be a low risk because sufficient saturated habitat, ranging from 58 to 73 m deep is expected to persist within the immediate vicinity of the bore. In addition, the modelled steep and narrow drawdown contours around each production bore mean that greater vertical and lateral extents of habitat will remain within the nearby surrounding area during the proposed operational life of the borefield.

Lowering of the groundwater table through the proposed development of the Cobb Depression Borefield is not considered likely to pose a long term conservation risk to Enchytraeidae OES18, when taking into consideration the:

- extent of saturated habitat that would persist in the immediate locality from where the putative stygofauna species was collected, as well as within the neighbouring area, during the operational life of the borefield;
- greater extent of habitat present outside the proposed groundwater drawdown zones; and
- broader distribution patterns of other members of the oligochaete enchytraeid family.

Nyikukura Borefield

For the Nyikukura borefield, no stygofauna were recorded. Although sampling within this study area was limited to two sites only, the habitat characterisation indicated that it was unlikely that this area would support stygofauna. Therefore, the potential development of the Nyikukura groundwater supply borefield is not considered likely to pose a long term conservation risk to stygofauna.

Officer Basin Borefield

For the Officer Basin borefield, no stygofauna were recorded. The level of sampling undertaken for this study area was more intensive and therefore, can be considered to provide a reliable demonstration that the habitat characterisation for the Officer Basin Borefield as unlikely to support any stygofauna species was correct. Therefore, the potential development of the Officer Basin groundwater supply borefield is not considered likely to pose a long term conservation risk to stygofauna.

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5. GLOSSARY

alluvium – sediment deposited by a stream or river

aquatic – relating to water

aquifer – a body of permeable rock or sediment capable of storing groundwater

arid – a region characterised by a severe lack of available water, to the extent that the growth and development of biota is hindered or prevented

bedrock – consolidated rock attached to the earth's crust

biodiversity – the diversity of biota in a particular environment or region

calcrete – carbonate deposits that form in arid environments, as a result of groundwater evaporation

cave – a subsurface cavity of sufficient size that a human could enter

dissolved oxygen – a measure of the amount of gaseous oxygen dissolved in a solution; oxic = > 3 mg/L; dysoxic = 0.3 to 3.0 mg/L; suboxic = < 0.3 mg/L levels

distribution range – the overall geographic area that a species is known to occur in

divergence – degree of separation from a common ancestor

diversity – species richness

drawdown – the lowering of the adjacent water table or piezometric surface as a result of groundwater extraction

ecotone – zone of transition among different ecosystems

electrical conductivity – an estimate of the total dissolved salts in a solution, or salinity

endemic – having a distribution restricted to a particular geographic region

epigean – pertaining to the surface zone

fractured rock – a rock formation characterized by separation or discontinuity, usually as a result of geological stress (e.g. faulting)

geological ages (e.g. Cainozoic) – distinct time periods within the geological history of the earth

groundwater – water occurring below the ground surface

habitat – an ecological or environmental area that is inhabited by a particular animal or plant species

hypogean – pertaining to the subterranean zone

hyporheic zone – spatially fluctuating ecotone within the bed of a river or stream between surface and groundwater. Considered important component of groundwater ecosystems and involved in the 'interstitial highway', forming hyporheic corridor linking associated aquifers.

invertebrates – animals lacking vertebrae

karst – a region of limestone or other soluble rock, characterized by distinctive features such as caves, caverns, sinkholes, underground streams and springs

lineage – a group of organisms related by descent from a common ancestor

molecular – pertaining to the genetic characteristics of an organism or group

morphology – the specific form and structure of an organism or taxon

morphospecies – a general grouping of organisms that share similar morphological traits, but is not necessarily defined by a formal taxonomic rank

palaeoriver, palaeochannel, palaeodrainage – a remnant of a stream or river channel cut in older rock and filled by the sediments of younger overlying rock

pH – a measure of the hydrogen ion concentration of a soil or solution (values below pH of 6.5 are 'acidic', and those above pH 7.5 are 'alkaline')

relictual – having survived as a remnant

salinity – the concentration of all dissolved salts in a solution; freshwater = salinity less than 5,000 µS/cm (3,000 mg/L); hyposaline = salinity ranging from 5–30 mS/cm (3–20 ppt); mesosaline = salinity ranging from 30–70 mS/cm (20–50 ppt); hypersaline = salinity equal to or greater than 70 mS/cm (50 ppt)

semi-arid – a climatic region that receives low annual rainfall (250 – 500 mm)

species – a formal taxonomic unit defining a group or population of organisms that share distinctive characters or traits, are reproductively viable and/or are otherwise identifiable as a related group

species diversity – the number of species present in a particular habitat, ecosystem or region

species accumulation curve – a model used to estimate species diversity or richness

standing water level (SWL) – the depth to groundwater from a particular reference point (e.g. in a monitoring bore)

stygal, stygo – pertaining to groundwater biota

stygobite – an obligate aquatic species of groundwater habitats

stygobiont – another term used to describe obligate inhabitants of groundwater systems

stygofauna – a general term for aquatic groundwater fauna

stygophile – an aquatic species that temporarily or permanently inhabits groundwater habitats

stygoxene – an aquatic species that has no fixed affinity with groundwater habitats, but may nonetheless occur in groundwater habitats

sympatry / **sympatric** – two or more species that are considered to exist in the same or overlapping geographic area and may regularly interact with, or encounter, each another (without interbreeding)

taxon – an identifiable group of organisms, usually based on a known or inferred relationship or a shared set of distinctive characteristics

troglobite – an obligate terrestrial species of subterranean habitats

troglofauna – a general term for terrestrial subterranean fauna

troglomorphic features – morphological characteristics resulting from an adaptation to subterranean habitats (e.g. a reduction in pigment)

troglophile – a terrestrial species that temporarily or permanently inhabits subterranean habitats

trogloxene – a terrestrial species that has no fixed affinity with subterranean habitats, but may nonetheless occur in subterranean habitats

void – a pore space in the rock or stratum

Yilgarn – pertaining to the Yilgarn Craton, a 65,000 km2 body of the earth's crust in south-western Australia that dates back to the Archaean period, 2.6 to 3.7 million years ago

Appendix A

Bore Data

Bore Data: Map Zone is 52 J; EoH = End of Hole; SWL = standing water level; mbgl = metres below ground level.

Bore		Eastings	Northings	Sample Date	Casing description	EoH (mbgl)	SWL (mbgl)
Cobb Depression	CWRC4 (CW05)	459266	7184952	26/01/2013	0 to 13m unslotted PVC 13 to 37m slotted PVC 37 to 127m alternating unslotted and slotted PVC 127 to 133m unslotted PVC	133	24.2
	CWRC2 (CW07)	460976	7191945	26/01/2013	0 to 19m unslotted PVC 19 to 43m slotted PVC 43 to 133m alternating unslotted and slotted PVC 133 to 139m slotted PVC	139	33.4
	CWMR02P	460953	7191988	26/01/2013	0.20 to 30.25m unslotted PVC 30.25 to 132.25m slotted PVC	137	33.2
	CWRC1 (CW08)	462595	7195045	26/01/2013	0 to 25m unslotted PVC 25 to 49m slotted PVC 49 to 127m alternating unslotted and slotted 127 to 133m slotted PVC	133	39.5
Nyikukura	CHMR001D	506763	7122730	24/01/2013	0.40 to 16.6m unslotted PVC 16.6 to 130.6m slotted PVC	129	21.8
	CHMR001P	506764	7122648	24/01/2013	0.51 to 17.8m, 29.8 to 35.8m & 45.8 to 70.8m unslotted PVC 17.8 to 29.8m, 35.8 to 45.8m & 70.8 to 123.0m slotted PVC	129	22.4
Officer Basin	OBW2	428871	7050189	4/10/2011	4 to 10m & 100 to 250m slotted PVC	250	8.9
	OBW3	426611	7048406	4/10/2011	16 to 22m & 100 to 250m slotted PVC	250	17.8
	OBW4	423878	7044949	4/10/2011	0 to 66m slotted PVC	294	9.6
	OBW5	421222	7042473	4/10/2011	16 to 22m & 100 to 250m slotted PVC	250	12.7
	OBW6	432231	7057440	4/10/2011	96 to 216m slotted PVC	216	25.6
	OBW9	425701	7046223	4/10/2011	123 to 153m slotted PVC	187	15.6
	OBW11	425706	7046137	4/10/2011	14 to 20m & 74 to 224 slotted PVC	225	15.8
	OBW12D	426594	7045706	25/01/2013	0.44 to 167.6m unslotted PVC 167.6 to 299.6m slotted PVC	300	25.5
	OBW13D	429610	7045713	25/01/2013	0.73 to 47.6m unslotted PVC 47.6 to 215.6m slotted PVC 215.6 to 233.6m unslotted PVC	235	12.3
	OBW13P	429641	7045721	25/01/2013	0.52 to 5.83m unslotted PVC 58.3 to 219.2m slotted PVC	222	12.6
	OBW14D	430708	7045715	25/01/2013	0.65 to 39.9m unslotted PVC 39.9 to 171.9m slotted PVC 171.9 to 183.9m unslotted PVC	185	9.6
	OBW14P	430716	7045669	25/01/2013	0.52 to 55.2m unslotted PVC 55.2 to 175.2m slotted PVC	176	9.7

Appendix B

Representative Survey Site Photographs

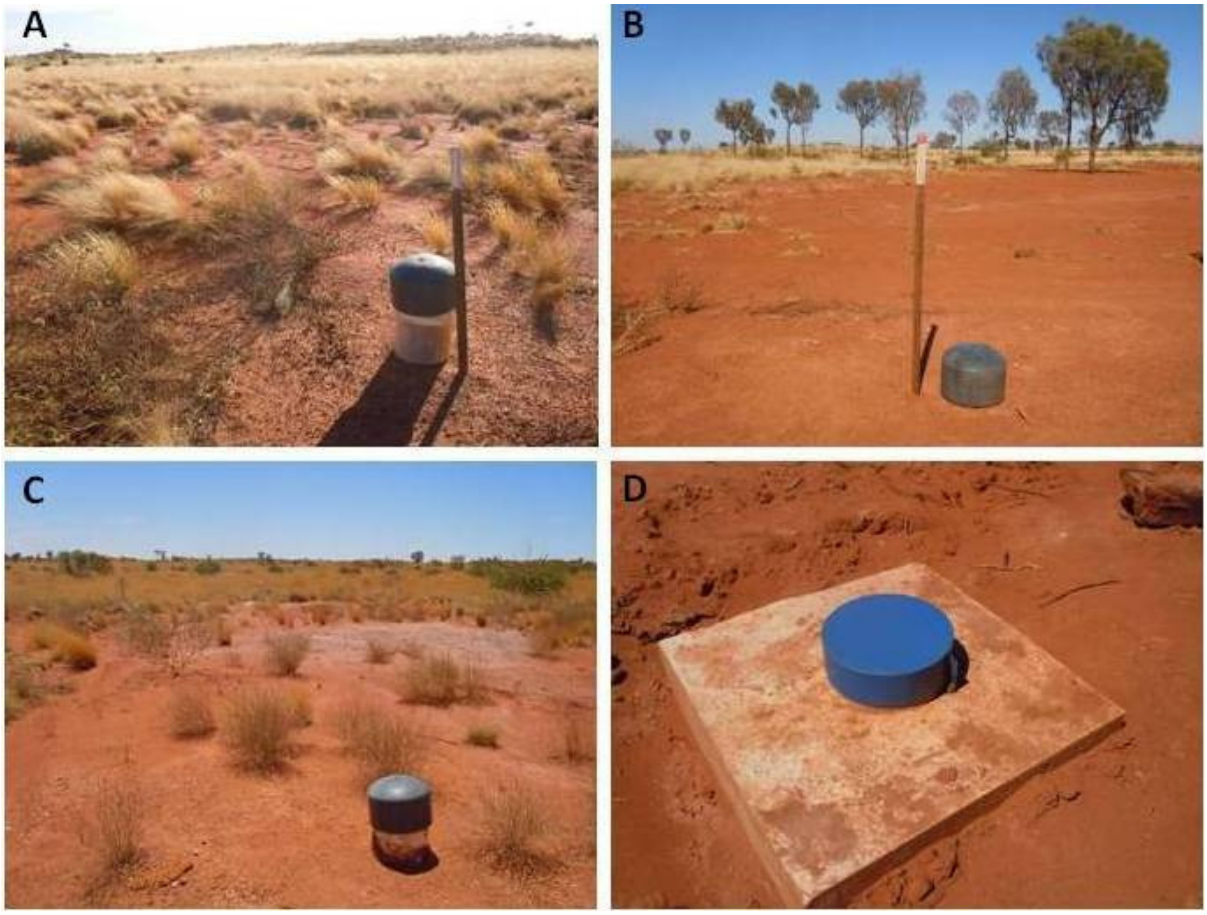

Plate 1. Representation of Cobb Depression survey bores: A) CWRC4; B) CWRC2; C) CWRC1; and D) CWMR02P.

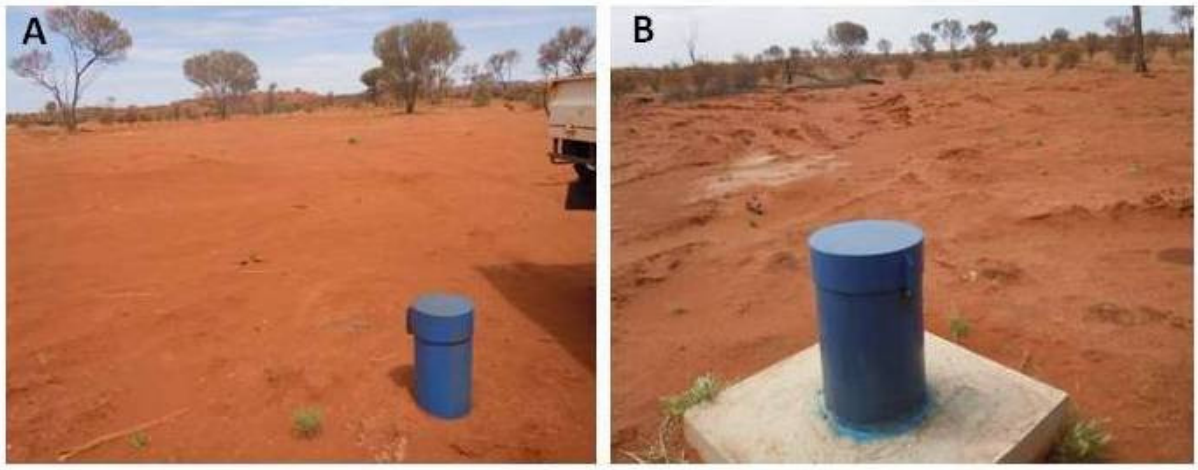

Plate 2. Representation of Nyikukura survey bores: A) CHMR001D; and B) CHMR001P.

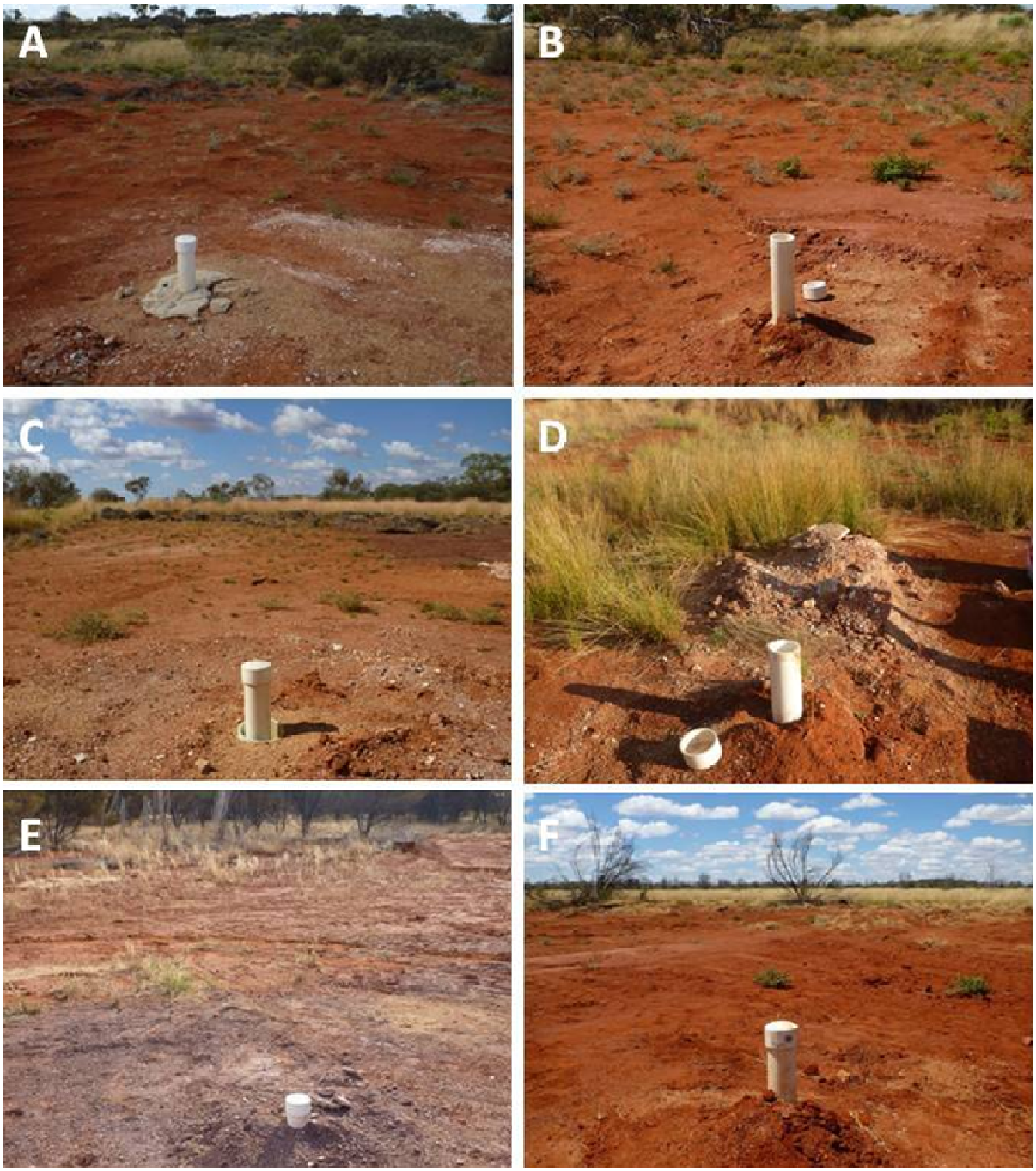

Plate 3. Representation of Officer Basin survey bores: A) OBW2; B) OBW3; C) OBW4; D) OBW5; E) OBW6 and F) OBW9.

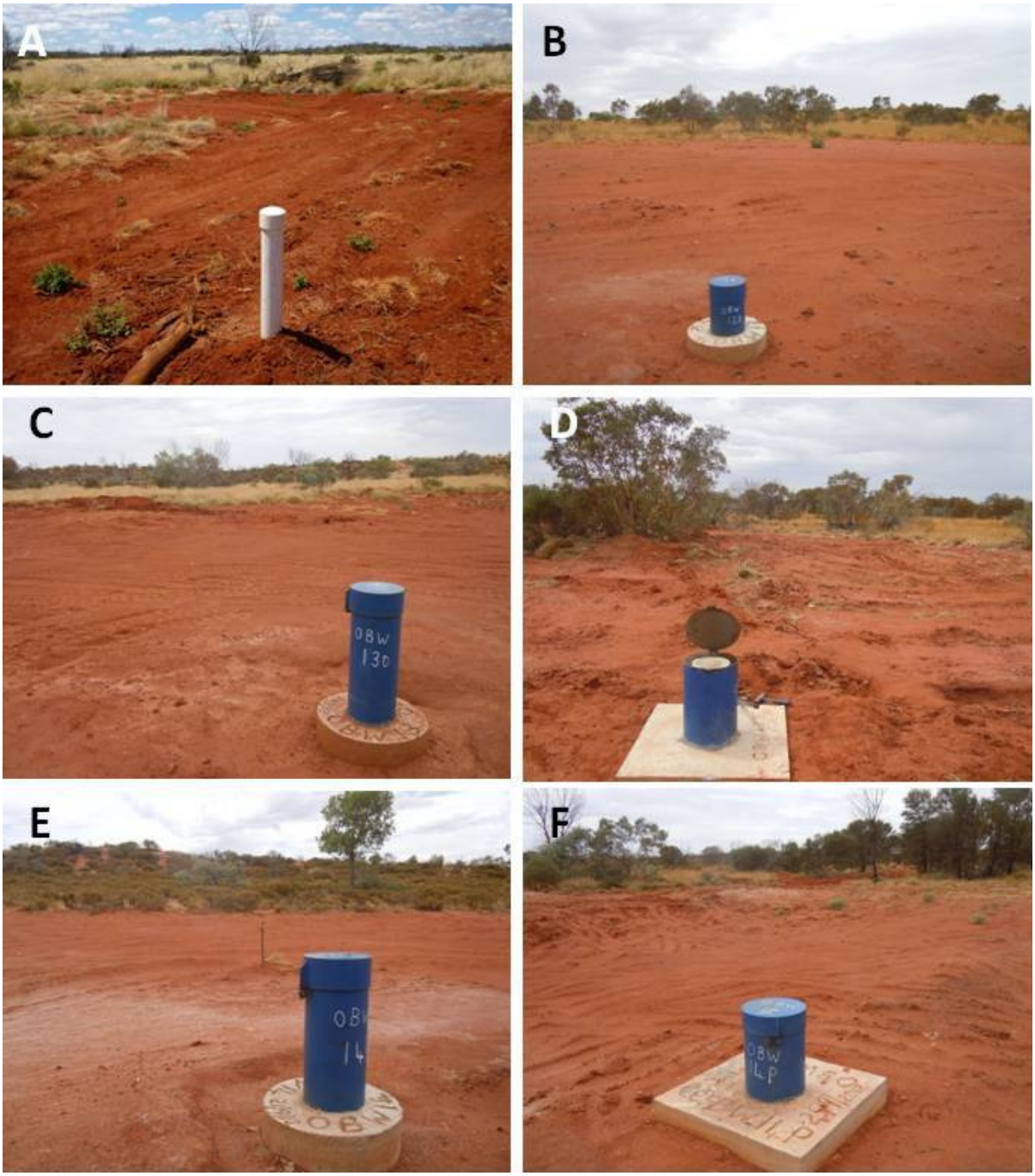

Plate 3 (continued): Representation of Officer Basin survey bores: A) OBW11; B) OBW12D; C) OBW13D; D) OBW13P; E) OBW14D; and F) OBW14P.

Appendix C

Groundwater Physicochemical Data

Groundwater Physicochemical Data: EoH = End of Hole; SWL = standing water level; mbgl = metres below ground level.

Bore ID		Sample Date	SWL (mbgl)	EoH (mbgl)	pH	Salinity (ppm)	EC (µS/cm)	Temp (°C)	DO (ppm)	Redox (mV)
Cobb Depression	CWRC4 (CW05)	26/01/2013	24.2	133	7.31	900	1748	24.4	5.4	66.4
	CWRC1 (CW08)	26/01/2013	39.5	133	7.23	480	1017	28.4	5.4	79.4
	CWMR02P	26/01/2013	33.2	137	7.24	680	1379	26.1	4.2	69.9
	CWRC2 (CW07)	26/01/2013	33.4	139	7.18	700	1442	26.2	5.3	68.9
Nyikukura	CHMR001D	24/01/2013	21.8	129	6.96	480	1038	28.0	-	95.8
	CHMR001P	24/01/2013	22.4	129	7.10	500	1107	28.9	3.1	121.2
Officer Basin	OBW2	4/10/2011	8.9	207	7.04	2,110	3820	27.9	1.1	-12.0
	OBW3	4/10/2011	17.8	207	7.01	2,880	5280	28.9	4.4	155.0
	OBW4	4/10/2011	9.6	51	7.17	15,900	25900	26.3	0.7	188.0
	OBW5	4/10/2011	12.7	207	7.22	3,470	6240	25.9	2.5	193.0
	OBW6	4/10/2011	25.6	-	8.00	1,031	1923	26.8	1.1	-186.0
	OBW9	4/10/2011	15.6	154	7.53	3,190	5700	27.5	1.2	-240.0
	OBW11	4/10/2011	15.8	207	7.24	2,650	4850	28.2	2.3	131.0
	OBW12D	25/01/2013	25.5	300	7.11	3,200	5904	25.1	2.5	44.0
	OBW13D	25/01/2013	12.3	235	7.71	3,870	7127	25.4	3.7	52.8
	OBW13P	25/01/2013	12.6	222	7.41	3,590	6833	26.2	0.4	75.3
	OBW14D	25/01/2013	9.6	185	7.05	5,420	9770	25.5	2.4	73.0
	OBW14P	25/01/2013	9.7	176	7.02	5,960	10695	25.7	1.3	80.7

Appendix D

DNA Assessment of Oligochaetes

Prepared for Outback Ecology, March 2013
by Dr Remko Leijs, South Australian Museum.

Biodiversity assessment of the Oligochaeta of the Wingellina area using molecular methods

Summary

- In the Wingellina area 2 enchytraeid oligochaete species are identified by molecular biodiversity assessment: these specimens have not been found in earlier studies, and should be considered as new species.

Methods

Biodiversity assessment of the collected fauna (Table 1) included morphological assessment and PCR amplification and sequencing of a 677 bp fragment of CO1, commonly used for DNA barcoding (Hebert et al. 2003). Morphological assessment is indicated in Table 1 in the column "SAM identification". To increase sequencing success rate, PCR's for all specimens were set up with two different sets of primers. The sequences were added to large datasets that consists of related taxa from the region complemented with data from Genbank and unpublished sequence data at the South Australian Museum.
Phylogenetic analyses using neighbour joining of uncorrected sequence distances in PAUP* (Swofford 1998) were used to estimate the number of species among the received specimens from each of the areas, as well as for checking whether these species were found at other localities in the region. Results of phylogenetic analyses are presented as partial phylogenetic trees showing the target species with some closest related species as well as a matrix of uncorrected ("p") pairwise distances between target species and relevant taxa in the phylogenetic trees. The target species are highlighted in yellow in the phylogenetic trees. In the distance matrices *intra*-specific distances are highlighted in yellow, *inter*-specific distances are highlighted in orange.

Extraction	Code	OE identification	SAM identification	Extr.date	Coll.Date	locality	Site	CO1
ST1884	LN4633	Enchytraeidae	sp. WING 1	4-Mar-13	26-Jan-13	Cobb	CWRC02D/CW07	good seq
ST1885	LN4633	Enchytraeidae	sp. WING 1	4-Mar-13	26-Jan-13	Cobb	CWRC02D/CW07	good seq
ST1886	LN4631	Enchytraeidae		4-Mar-13	15-Jan-11	Pipalyatjara calcrete	CHRC0215	no PCR
ST1887	LN4631	Enchytraeidae	sp. WING 2	4-Mar-13	15-Jan-11	Pipalyatjara calcrete	CHRC0215	good seq

Table 1. Overview of the analysed specimens. The first column gives the DNA extraction number, the last column indicates whether the DNA sequencing was successful.

Oligochaeta – Enchytraeidae

Three out of the four five specimens submitted for biodiversity assessment produced good sequences (Table 1). These specimens represent two different species that belong to species not found earlier in any of the identified in previous analyses subterranean fauna. Specimens ST1884 and ST1885 are conspecific, pairwise sequence divergence 0.46% (Table 2, yellow highlighted value). Specimen ST1887 belongs to a different species and is found as sister species of the latter species (Figure 1). The pairwise sequence divergences among these specimens of 9.4-9.5% (Table 2, orange highlighted values) indicate that they

belong to different species. The species are distantly related to a species (specimens ST1689 and ST1693) from Ethel Creek in the Pilbarra.

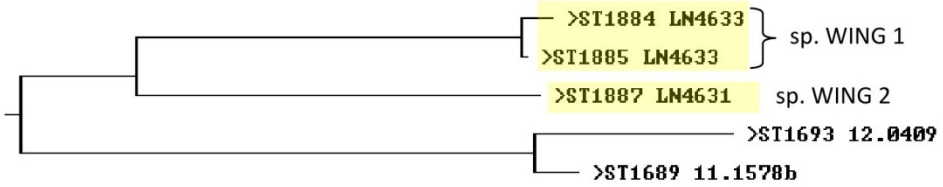

Figure 1. Partial neighbour joining cladogram of enchytraeid oligochaetes.

Uncorrected ("p") distance matrix

		277	278	279	316	318
277	>ST1884 LN4633	-				
278	>ST1885 LN4633	0.00456	-			
279	>ST1887 LN4631	0.09531	0.09389	-		
316	>ST1693 12.0409	0.14460	0.14055	0.15132	-	
318	>ST1689 11.1578b	0.12933	0.12266	0.12749	0.02747	-

Table 2. Pairwise uncorrected sequence divergences enchytraeid oligochaetes.

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