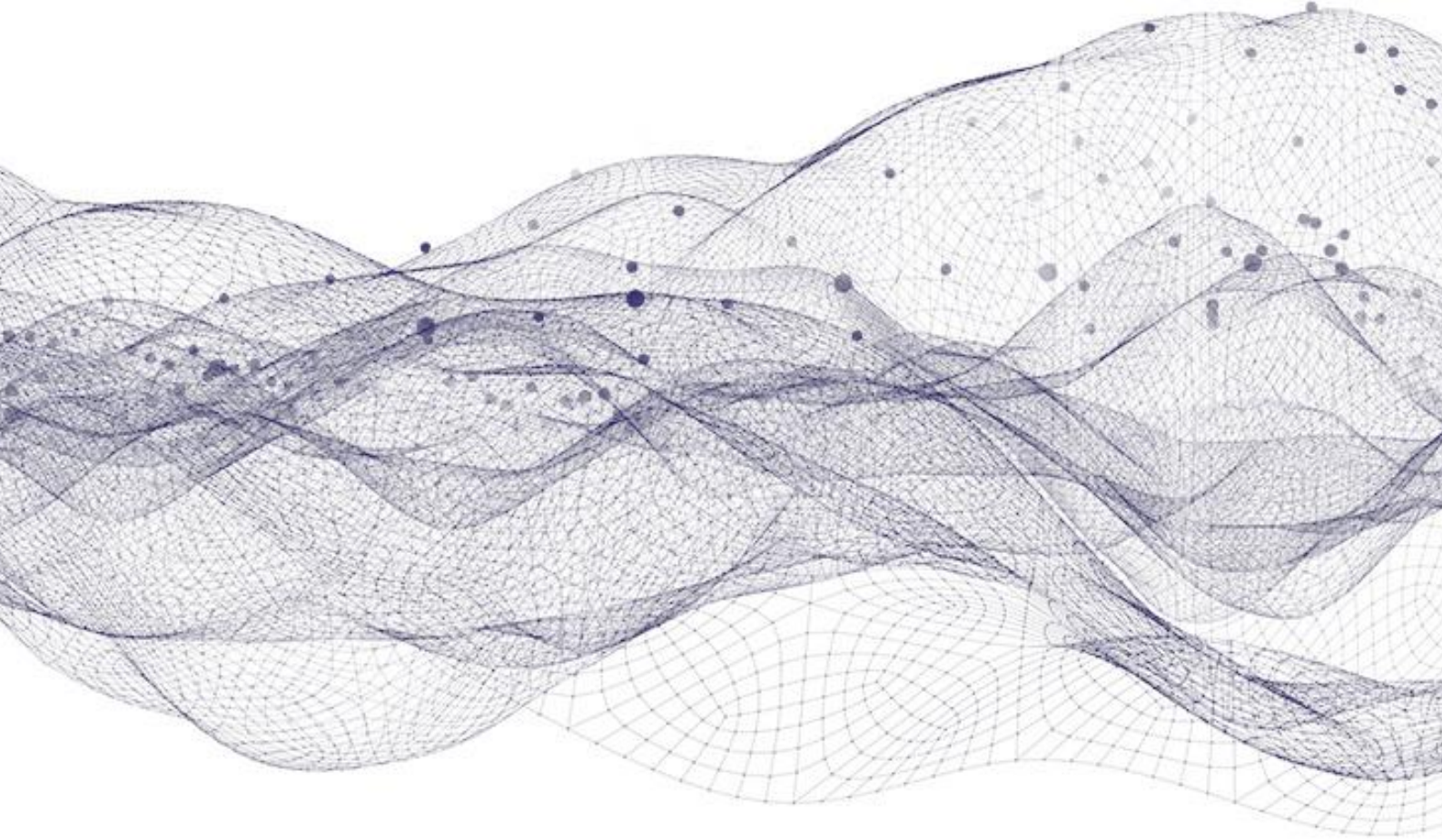


Yinnetharra Lithium Project Subterranean Fauna Study: Malinda Prospect

Prepared For Delta Lithium Limited
November 2024



Bestiolas Consulting

Document Schedule

Revision #	Date	Description	Prepared by	Reviewed by	Approved by
1	12/08/2024	Draft Report	N. Stevens	N. Stevens	N. Stevens
2	14/11/2024	Final	N. Stevens	Lisa Chandler (Delta) Claire McGuire (Delta)	N. Stevens

This document has been prepared for the benefit of Delta Lithium Limited to inform of subterranean fauna values via a detailed survey study in the Malinda Prospect study area of the Yinnetharra Lithium Project. No liability is accepted by Bestiolas Consulting with respect to its use by any other person for anything other than its purpose. This disclaimer shall apply notwithstanding that the report may be made available to other persons for an application for permission or approval to fulfil a legal requirement.

COPYRIGHT: This document has been prepared to the requirements of the client identified above, and no representation is made to any third party. Copyright and any other Intellectual Property associated with the document belongs to Bestiolas Consulting and may not be reproduced without written permission of the Client or Bestiolas Consulting. It may be cited for the purposes of scientific research or other fair use, but it may not be reproduced or distributed to any third party by any physical or electronic means without the express permission of the client for whom it was prepared or Bestiolas Consulting.

© Copyright 2024 Bestiolas Consulting. All rights reserved.

Cover page graphic attribution: Free Lines Png vectors by Lovepik.com

Executive Summary

Electrostate Malinda Pty Ltd (Electrostate), a wholly owned subsidiary of Delta Lithium Ltd (Delta), owns the Yinnetharra Lithium Project (Yinnetharra), which is located within the Gascoyne region of Western Australia (WA), approximately 120 km northeast of Gascoyne Junction and 825 km north of Perth. The current tenement package for Yinnetharra covers more than 520 km² encompassing a highly prospective Lithium-caesium-tantalum (LCT) bearing metasedimentary belt within a regional scale granite unit that trends in a north-westerly orientation for approximately 80 km. Current exploration drilling activities are focused on a small area (5 km²) of the Malinda Prospect (Tenement E0902169) where lithium bearing pegmatites extending from the surface to depths greater than 350 metres below ground level (m bgl) have been confirmed.

Delta engaged Bestiolas Consulting (Bestiolas) to undertake a subterranean fauna (stygo fauna and troglo fauna) study at the Malinda Prospect to investigate if subterranean fauna will represent a significant environmental factor that may be impacted by the development of Yinnetharra's Malinda Prospect. The study findings will inform future environmental impact assessment (EIA) and applications for regulatory approval of the Project. This report presents the findings of the Yinnetharra stygo fauna and troglo fauna study (the Project) of the Malinda Prospect study area (Malinda). This report does not provide a formal environmental impact assessment (EIA) because the full extent of the proposed footprint has not yet been finalised, plus additional exploration drilling of neighbouring prospects that is currently ongoing have not yet been assessed for subterranean fauna.

Survey Effort

The subterranean fauna study involved three survey phases each more than three months apart: Phase 1— June 2023; Phase 2— November 2023; and Phase 3— March 2024.

Stygo fauna — The total stygo fauna sampling involved the collection of 94 haul net samples from 53 sites, consisting of 49 uncased exploration drill holes, three cased pastoral bores, and one cased production bore.

Troglo fauna — The total troglo fauna survey involved the collection of 88 scrape samples from 48 uncased exploration drill holes, and 101 litter traps deployed in 56 uncased exploration drill holes.

ES Table 1: Subterranean Fauna survey effort.

Area	Stygo fauna		Troglo fauna	
	Haul Net	Litter Trap	Scrape	
Malinda Study Area	89	101	88	
Regional	5	0	0	
Total	94	101	88	

Findings

Stygo fauna

A total of 823 stygo fauna specimens, representing six species from four higher level taxonomic groups (Amphipoda, Cyclopoida, Harpacticoida, and Isopoda), were collected from 18 of the 53 sites sampled across the three survey phases. Four stygo fauna species were collected in 89 haul net samples from 49 sites in the Malinda study area, compared to five species collected from four samples from three neighbouring regional sites. Of the four species recorded from the Malinda study area, three species (Paramelitidae sp. 'Biologic-AMPH095', Halicyclops sp. 'Biologic-CYCL099', and Harpacticoida sp. 'Biologic-HARP087') were also collected from regional reference sites. Only one species, Robustura sp. YIN01, was not collected from beyond the Malinda study area. The regional site YIN01, nearly 5 km north of Malinda, was the most species rich site with four of the six recorded species found to occur there, including three of the four species recorded from within the Malinda study area.

Troglo fauna

Only three troglo fauna specimens, representing two species from two higher level taxonomic groups (Diplura and Polyxenida), were collected from two of the 56 sites sampled across the three survey Phases. Both species were each recorded from a single sample only, collected from different sites within or near to the same proposed pit area. The badly damaged dipluran specimen is cautiously regarded as a potential troglo fauna, because although it could likely be a soil dwelling species, this cannot be

conclusively determined. The distribution range of the indeterminate dipluran species is considered to be of a wider extent beyond the Malinda study area within the broader expanse of contiguous subterranean habitat present. The polyxenid species, *Lophoturus madecassus*, is a widespread species with a global circum-tropical distribution so is not confined to the Malinda study area.

Discussion

Key factors influencing subterranean fauna diversity and distribution are the presence of extensive interconnected porosity within suitable geological and hydrogeological units that are in connection with adequate hydrological regimes to ensure pathways for the infiltration (vertical and/ or lateral) of resources such as oxygen and nutrients. Within the Gascoyne bioregion, the palaeodrainage channel calcrete habitats, as well as associated alluvial and colluvial aquifer systems, are considered to host diverse stygofauna and troglifauna assemblages, however, these have not yet been as extensively studied as many Pilbara and Yilgarn subterranean fauna assemblages.

Studies of the Gifford Creek stygofauna PEC found that all of the recorded stygofauna diversity occurred within the calcrete and associated surficial alluvial/ colluvial aquifer habitats. The occurrence of stygofauna from such environments is not unexpected as such habitats are known to provide optimal conditions that host diverse assemblages. The associated fractured rock groundwater environments hosted a much lower diversity with only three of the recorded 62 stygofauna species collected from within granite fractured rock aquifer habitats, each of which were also present in the calcrete and associated alluvial/ colluvial aquifer systems.

The Project findings show that the more prospective, higher value stygofauna habitat in the region occurs along the main drainage channels within calcrete aquifers and associated alluvial/ colluvial aquifer environments. The target ore resource of the Malinda Prospect occurs in an elevated fractured rock aquifer system that is surrounded by more extensive surficial alluvial/ colluvial aquifers lower in the landscape that are associated with the larger drainage systems, which in areas host calcrete aquifer systems. The elevated fractured rock aquifer environment would provide less optimal habitat conditions for stygofauna, fringing the more optimal alluvial/ colluvial and calcrete aquifer habitats lower in the landscape.

It is considered unlikely that any stygofauna species are restricted to the fractured rock aquifer system present in the Malinda study area. Three of the four stygofauna species recorded from the fractured rock aquifer system within the Malinda study area were found to have distributions that extended to neighbouring regional sites that intercepted or were closer to the higher value alluvial/ colluvial aquifer habitats. These regional sites recorded a higher stygofauna species richness compared to Malinda from a much lower sampling intensity.

It is considered highly unlikely that any troglifauna species are restricted to the Malinda study area. The troglifauna values of the weathered and fractured rock habitat within Malinda were found to be very low, to potentially zero, considering the single potential troglifauna species collected could more likely be edaphofauna and not troglobitic. Unfortunately, there were no regional sites available that were suitable for troglifauna sampling so no comparison of diversity could be made.

Conclusion

The total sample effort completed, primarily from within the focussed exploration drilling area of the Malinda Prospect, along with the habitat assessment and subterranean fauna records from the broader region, is considered more than sufficient to provide a reliable characterisation of the subterranean fauna values present in the Malinda study area. The Project findings showed that the fractured rock habitat present in Malinda hosts low stygofauna values and very low, to potentially zero, troglifauna values. Higher valued stygofauna habitats are considered to occur in surficial alluvial/ colluvial aquifers associated with the main drainage channels in the neighbouring area as well as the broader region, particularly where larger calcrete bodies have formed.

Contents

1	Introduction.....	7
1.1	Scope and Objectives.....	7
2	Subterranean Fauna.....	9
2.1	Stygofauna.....	9
2.2	Troglofauna.....	9
2.3	Habitat.....	10
2.4	Short Range Endemics.....	10
2.5	Relevant Legislation & Guidelines.....	10
2.6	Impacts & Associated Risks.....	11
2.6.1	Direct & Indirect Impacts.....	11
3	Methods.....	12
3.1	Database Searches & Literature Review.....	12
3.2	Stygofauna Survey.....	12
3.2.1	Stygofauna Sample Effort.....	12
3.2.2	Haul Net Sampling.....	13
3.2.3	Groundwater Properties.....	13
3.3	Troglofauna Survey.....	16
3.3.1	Troglofauna Sample Effort.....	16
3.3.2	Litter Trap Sampling.....	16
3.3.3	Scrape Sampling.....	16
3.4	Field Personnel, Licence, & Limitations.....	17
3.5	Laboratory Processes.....	17
3.6	Genetic Analysis.....	17
4	Environmental Context.....	18
4.1	Biogeographic Region.....	18
4.2	Environmental Significant Areas.....	18
4.3	Land Use and Tenure.....	18
4.4	Climate.....	20
4.5	Geology.....	22
4.6	Hydrology.....	30
4.7	Hydrogeology.....	30
5	Results.....	31
5.1	Database Searches.....	31
5.2	Literature Review.....	31
5.2.1	Stygofauna.....	31
5.2.2	Troglofauna.....	31
5.3	Groundwater Properties.....	37
5.3.1	Salinity.....	37
5.3.2	pH.....	37
5.3.3	Dissolved Oxygen.....	37
5.3.4	SWL.....	38

5.3.5	Groundwater Assessment	38
5.4	Survey Results	42
5.4.1	Stygofauna	42
5.4.2	Troglofauna	48
6	Discussion.....	53
7	Conclusion.....	53
8	Reference.....	54
Appendices.....		59
Appendix A:	Yinnetharra Subterranean Fauna Site Details, Sample Effort, & Groundwater Details. ..	59
Appendix B:	Subterranean Fauna Sample Results	70
Appendix C:	Genetic Analysis.....	75

List Of Tables

Table 3-1:	Defined database and internet sourced search parameters.....	12
Table 3-2:	Stygofauna survey effort.	13
Table 3-3:	Troglofauna survey effort.....	16
Table 4-1:	Bedrock geology code descriptions most relevant to the Project area as shown in Figure 4-5 (source Geological Survey of Western Australia (2020)).....	25
Table 4-2:	Surface geology code descriptions most relevant to the Project area as shown in Figure 4-7 (source Raymond et al. (2012) and Sheppard et al. (2010)).....	27
Table 5-1:	Stygofauna studies of relevance to the Project.	35
Table 5-2:	Troglofauna studies of relevance to the Project.....	36
Table 5-3:	Stygofauna diversity recorded.	44
Table 5-4:	Troglofauna diversity recorded.....	48

List Of Figures

Figure 1-1:	Regional setting and tenements of the Yinnetharra Lithium Project.	8
Figure 3-1:	Stygofauna and troglofauna regional sample sites beyond Malinda Prospect study area....	14
Figure 3-2:	Stygofauna and troglofauna sample sites within the Malinda Prospect study area.	15
Figure 4-1:	Location of the Yinnetharra Lithium Project in the western part of the Augustus (GAS03) subregion of the Gascoyne IBRA bioregion.....	19
Figure 4-2:	Monthly mean, minimum and maximum rainfall compared to 2023 and 2024 monthly totals recorded from Burringurrah Airstrip weather station (# 007210; 2012 to 2024). Mean minimum and maximum temperatures recorded from the Gascoyne Junction weather station (# 006022; 1940 to 2024) (Bureau of Meteorology 2024).	21
Figure 4-3:	Daily rainfall recorded from Burringurrah Airstrip weather station (# 007210; 2023 and 2024). 21	
Figure 4-4:	Yinnetharra Lithium Project location in relation to tectonic units of Western Australia (adapted from Geological Survey of Western Australia (2022)).	22
Figure 4-5:	Regional bedrock geology (source Geological Survey of Western Australia (2020)). Refer Table 4-1 for relevant geology code descriptions.	23
Figure 4-6:	Diagrammatic bedrock stratigraphy for Sections A—B and C—D in Figure 4-5 and Figure 4-7 (adapted from Johnson et al. (2012) and Sheppard et al. (2008)). Pink asterix highlights occurrence of Leake Springs Metamorphics (P_MR_mlst) that host prospective spodumene bearing pegmatites in Malinda Prospect.....	24
Figure 4-7:	Surface geology of the Yinnetharra region (source Raymond et al. (2012)). Refer Table 4-2 for relevant geology code descriptions.....	26
Figure 4-8:	Diamond drill core images (0 to 68.8 mbgl) of YNRD009, located within proposed pit area, 30 m east of YRRD036 (recorded SWL ranged from 27.4 to 28.8 mbgl), from which stygofauna and troglofauna were collected.	28
Figure 4-9:	Diamond drill core images (0–70.1 mbgl) of YNEX001, located within proposed pit area, 150 m west of YRRD025 (recorded SWL ranged from 17.4 to 18.8 mbgl), from which stygofauna were recorded.	29
Figure 5-1:	Groundwater assemblage PEC and subterranean fauna collection records from database and literature searches in relation to Yinnetharra.	32
Figure 5-2:	Gifford Creek PEC and subterranean fauna records in relation to bedrock geology. Refer Table 4-1 for relevant geology code descriptions.	33

Figure 5-3: Gifford Creek PEC and subterranean fauna records in relation to surface geology. Refer Table 4-2 for relevant geology code descriptions.	34
Figure 5-4: Minimum, maximum, and mean of recorded groundwater parameters: A) specific electrical conductivity (EC); B) pH; C) dissolved oxygen (DO).	39
Figure 5-5: Groundwater standing water levels (SWL) recorded from Malinda sites relative to site surface elevation, as metres above sea level (Australian Height Datum (AHD)): A) SWL expressed as metres below ground level (mbgl); B) SWL expressed as mAHD.	40
Figure 5-6: Minimum, maximum, and mean of recorded groundwater standing water levels (SWL) and site surface elevation, as metres above sea level (Australian Height Datum (AHD)): A) SWL expressed as metres below ground level (mbgl); B) SWL expressed as mAHD; C) site ground surface elevation.	41
Figure 5-7: Distribution of stygofauna species recorded.	45
Figure 5-8: Distribution of stygofauna species recorded in relation to bedrock geology. Refer Table 4-1 for relevant geology code descriptions.	46
Figure 5-9: Distribution of stygofauna species recorded in relation to surface geology. Refer Table 4-2 for relevant geology code descriptions.	47
Figure 5-10: Distribution of troglofauna species recorded.	50
Figure 5-11: Distribution of troglofauna species recorded in relation to bedrock geology. Refer Table 4-1 for relevant geology code descriptions.	51
Figure 5-12: Distribution of troglofauna species recorded in relation to surface geology. Refer Table 4-2 for relevant geology code descriptions.	52

1 Introduction

Electrostate Malinda Pty Ltd (Electrostate), a wholly owned subsidiary of Delta Lithium Ltd (Delta), owns the Yinnetharra Lithium Project (Yinnetharra) that is located within the Gascoyne Lithium Province of Western Australia (WA), approximately 120 km northeast of Gascoyne Junction and 825 km north of Perth (**Figure 1-1**). The current tenement package for Yinnetharra covers more than 520 km² encompassing a highly prospective Lithium-caesium-tantalum (LCT) bearing metasedimentary belt within a regional scale granite unit that trends in a north-westerly orientation for approximately 80 km. Current exploration drilling activities are focused on a small area, just over 5 km², of the Malinda Prospect (Tenement E 0902169) where lithium bearing pegmatites extending from the surface to depths greater than 350 metres below ground level (m bgl) have been confirmed.

Delta engaged Bestiolas Consulting (Bestiolas) to undertake a subterranean fauna (stygo fauna and troglo fauna) study of the Malinda Prospect area, starting with a desktop study and a basic survey (previously referred to as Level 1 or pilot survey). Initial desktop findings identified in the region the Priority 1 Gifford Creek, Mangaroon, Wanna calcrete groundwater invertebrate (stygo fauna) Priority Ecological Community (PEC), hosted within the Lyons Palaeodrainage channel in the upper catchment area of the Lyons River, the buffer boundary of which extends to within 32 km to the north of the Malinda Prospect (**Figure 1-1**). The basic survey, consisting of 21 stygo fauna haul net samples, 20 troglo fauna scrape samples, and 20 troglo fauna litter trap samples, recorded the presence of at least two stygo fauna species and two troglo fauna species. The desktop and basic survey findings triggered the requirement for a detailed subterranean fauna survey (previously referred to as a Level 2 or comprehensive/ baseline survey) to be completed, involving an additional two survey phases, as per Western Australian Environmental Protection Authority (WA EPA) 2021 *Technical Guidance — Subterranean fauna surveys for environmental impact assessment*.

The development of resource projects can potentially directly impact subterranean fauna (stygo fauna and troglo fauna) with the physical removal of habitat from mining excavation and associated groundwater drawdown. This report presents the findings of the Yinnetharra stygo fauna and troglo fauna assessment (the Project) within the Malinda Prospect study area (Malinda) in relation to sampled regional (reference) sites. This report does not provide a formal environmental impact assessment (EIA) because the full extent of the proposed footprint has not yet been finalised, plus additional exploration drilling of neighbouring prospects (e.g., Jamesons) that is currently ongoing have not yet been assessed for subterranean fauna.

1.1 Scope and Objectives

The aim of the Project is to investigate if subterranean fauna will represent a significant key environmental factor that may be impacted by the development of Yinnetharra's Malinda Prospect. The assessment will inform future environmental impact assessment (EIA) and applications for regulatory approval of the Project. The objectives of the Project are to:

- Evaluate the likelihood of subterranean fauna (stygo fauna and troglo fauna) species existing within the Project's study area through stygo fauna and troglo fauna sampling and desktop assessment; and
- Determine if subterranean fauna may represent a significant key environmental factor.

The approach of the subterranean fauna study are aligned with the principles and objectives of relevant regulatory guidelines that include, but are not limited to:

- EPA (2021b) *Technical Guidance — Subterranean fauna surveys for environmental impact assessment*.
- EPA (2016) *Environmental Factor Guideline — Subterranean Fauna*
- EPA (2021a) *Statement of Environmental Principles, factors, and objectives*.

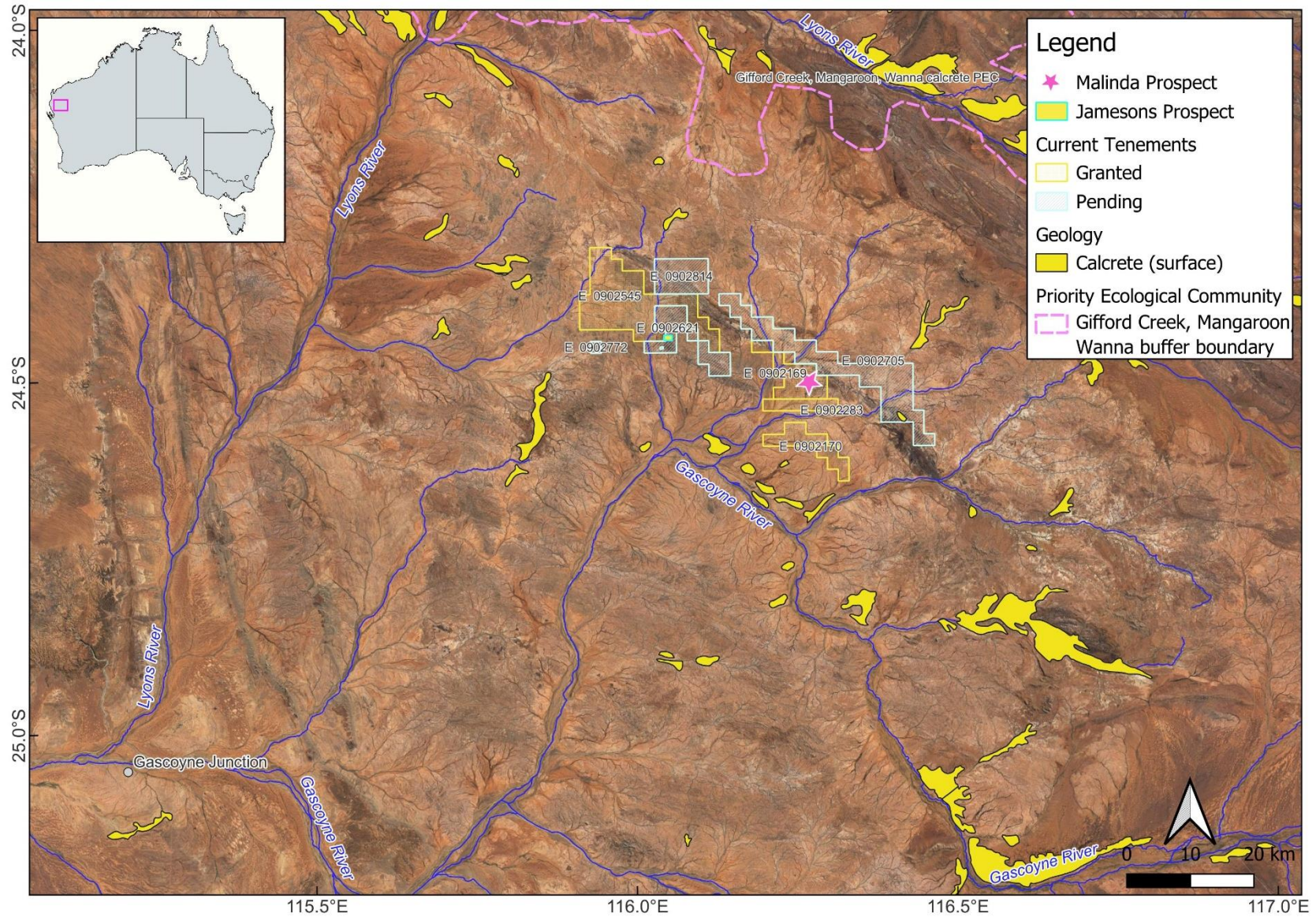


Figure 1-1: Regional setting and tenements of the Yinnetharra Lithium Project.

2 Subterranean Fauna

Subterranean fauna may be distinguished from surface dwelling (epigean) species by morphological characteristics typical of a subterranean existence, such as a reduction or absence of pigmentation, absence or reduction of eyes, and the presence of extended locomotory and sensory appendages (Gorički *et al.* 2019, Humphreys 2008, Humphreys 2019). They may also be defined by ecological parameters such as longer life history stages, and lower rates of metabolism and fecundity (Cooper *et al.* 2002, Danielopol and Pospisil 2000, Gorički *et al.* 2019, Humphreys 2017, 2019).

2.1 Stygofauna

Stygofauna are predominantly comprised of invertebrate species, particularly crustaceans, that inhabit suitable groundwater systems across the globe, but can include vertebrate species, with the blind cave gudgeons (*Milyeringa justitia* and *M. veritas* (Larson *et al.* 2013, Page *et al.* 2018)) and the blind cave eel (*Ophisternon candidum* (Moore *et al.* 2018, White *et al.* 2020)) the only notable examples to occur in Australia. Non-crustacean invertebrate stygofauna taxa can include gastropods, insects, water mites and worms (Humphreys 2019). Stygofauna are considered to play an important role in influencing groundwater ecosystem dynamics and health mainly via enhancement of the diversity and dispersal potential of prokaryotic microbes involved in maintaining groundwater purity (Smith *et al.* 2016).

In Western Australia, the Pilbara and the Yilgarn regions are global hotspots for stygofauna diversity (Guzik *et al.* 2010, Halse *et al.* 2014, Humphreys 2008). The karstic limestone anchialine and freshwater aquifer systems of Cape Range and Barrow Island (Bishop *et al.* 2020) host species rich, ecologically diverse, and globally significant stygofauna assemblages, which include the stygobitic blind cave gudgeons, blind cave eel, blind shrimps (*Stygiocaris lancifera* and *S. stylifera* (Page *et al.* 2008)), and the only southern hemisphere records of thermobanaceans (*Halosbaena tulki*) and remipedes (*Lasioneectes exleyi*) that are both considered relicts from the ancient Tethys Ocean (Humphreys 2019, Jurado-Rivera *et al.* 2017, Poore and Humphreys 1992, Yager and Humphreys 1996). The calcrete and alluvial aquifer systems associated with palaeodrainage channels of the arid and semi-arid zones of Western Australia can contain rich stygofauna communities, with more than 70 species recorded from the Yeelirrie calcrete system (Bennelongia 2015, Subterranean Ecology 2011).

Stygofauna are classified according to their ecological dependency on the subterranean environment:

- stygoxenes — epigean species that enter groundwaters passively or by accident.
- stygophiles — have incipient adaptations to survive in groundwater habitats, so can exist in epigean and hypogean aquatic environments.
- stygobites — obligate groundwater inhabitants that cannot persist in surface water environments and are the focus of the stygofauna component of this assessment.

2.2 Troglifauna

Troglifauna are air-breathing subterranean fauna inhabitants of suitable humid underground environments, often considered relictual forms that evolved from epigean ancestors to avoid increasingly harsh arid surface conditions (Humphreys 2000b). Troglifauna are predominantly invertebrate taxa, well represented by species of arachnids, myriapods, and insects, but rarely can include vertebrate species such as troglobitic salamanders (Gorički *et al.* 2019) and snake species. The blind snake, *Ramphotyphlops longissimus*, from the Barrow Island karst has apparent troglomorphies (Aplin 1998) and is the only known troglobitic reptile globally (Humphreys 2019). Troglifauna occur worldwide and historically were classified as cave organisms (Culver and Sket 2000). The discovery of diverse troglifauna communities inhabiting sub-surface rock fractures in non-karstic environments in Europe in the 1980's led to a broader consideration of potential habitat (Juberthie 2000).

In Western Australia, the well-researched karstic cave systems of Cape Range and Barrow Island first recorded the occurrence of relatively species rich troglifauna assemblages (Hamilton-Smith and Eberhard 2000, Humphreys *et al.* 2013, Humphreys 1991, 2000a). Further sampling in the Pilbara region identified diverse troglifauna assemblages from non-karstic geologies such as vuggy pisolite ore beds (Biota 2006, Humphreys 2008). Diverse troglifauna assemblages are commonly collected from groundwater associated calcrete (i.e. non-pedogenic calcrete) and alluvial/colluvial geologies within palaeodrainage channels of the arid and semi-arid zones, particularly in the Pilbara and Yilgarn regions (Harrison *et al.* 2014, MWH 2015, Outback Ecology 2011a, 2012a, c, Platnick 2008), but less so in the more arid interior of Australia (Outback Ecology 2011b). Less diverse troglifauna assemblages have also been recorded from weathered fractured rock (Outback Ecology 2014, Subterranean Ecology 2011) and metamorphic mafic rock systems (Bennelongia 2009). The most common environments in which

troglofauna occur are those that support suitably sized and extensively connected crevices, small cavities or vugs associated with secondary porosity from erosion, fractures and shears zones, that remain relatively humid, an important condition considered to be a key requirement for troglofauna existence (EPA 2021b).

Troglofauna are classified according to their ecological dependency on the subterranean environment:

- Troglonexes — use subsurface habitats passively or incidentally.
- Troglaphiles — undergo most of their early lifecycle stages underground, with latter lifestage/s able to / needing to survive in epigeal habitats.
- Troglobites — are obligate or permanent subterranean inhabitants and are the focus of the troglofauna component of this assessment.

2.3 Habitat

An important abiotic factor influencing the prospectiveness of habitat for stygofauna and troglofauna is the presence of vugs and voids of suitable size and connectivity to satisfy biological requirements. For this reason, subterranean fauna were believed to be mostly restricted to karst landscapes that provide a relatively high degree of secondary porosity, including large cave systems. In more recent times various types of non-karstic geologies and aquifer systems have been found to provide suitable voids to host diverse faunal assemblages (Humphreys 2008). Stygofauna are now known to occur in non-karstic aquifers in coarse alluvial sediments, fractured rock, pisolites and thin, rocky regolith (Eberhard *et al.* 2005, Guzik *et al.* 2010, Halse *et al.* 2014, Humphreys 2006, 2008, MWH 2016a, Outback Ecology 2014). Likewise, recent surveys have identified troglofauna from non-karstic geologies such as vuggy pisolite ore beds and fractured and weathered rock formations in the Pilbara and Yilgarn regions (Barranco and Harvey 2008, Bennelongia 2009, Halse *et al.* 2002, MWH 2015, Outback Ecology 2011a, Subterranean Ecology 2011).

Ecologically, there are many factors that influence the extent of suitable subterranean fauna habitat and the persistence and distribution of species at a range of habitat and temporal scales (Boulton 2000). At the microhabitat (sediment) scale some of the more influential factors include sediment size and interstitial pore size (i.e. provision of connected network of habitable cavities), hydrological exchange inflow rates of resources (e.g. dissolved oxygen, organic carbon, biofilm growth, prey), and water quality parameters such as temperature, pH, dissolved oxygen and organic carbon levels (Humphreys 1991, Korbel and Hose 2015, Korbel *et al.* 2019, Sacco *et al.* 2019, Saccò *et al.* 2022, Schmidt *et al.* 2007). At the mesohabitat (catchment) scale, factors include surface water flow patterns influencing infiltration zones and hydrological exchange influx rates into the groundwater systems of energy resources or dissolved oxygen according to geomorphological features, as well as interactions with vegetation and parafluvial sediments that can shape the hydrochemical groundwater characteristics (Boulton *et al.* 1998, Framenau *et al.* 2021, Humphreys 2009, Saccò *et al.* 2022, Schmidt *et al.* 2007, Strayer 1994).

2.4 Short Range Endemics

Subterranean fauna, particularly stygobites and troglobites, are considered to be mostly short-range endemic (SRE) species because they have limited distributions due to been restricted to their subterranean environment, resulting in high rates of endemism. SRE species (subterranean and epigeal) are classified as having a geographically restricted range of less than 10,000 km², therefore, are considered more vulnerable to extinction due of their limited distribution range (Harvey *et al.* 2011, Harvey 2002).

2.5 Relevant Legislation & Guidelines

Subterranean fauna are considered under State and Federal legislation, governed by three Acts:

- *Biodiversity Conservation Act 2016* (WA) (BC Act);
- *Environmental Protection Act 1986* (WA) (EP Act); and
- *Environment Protection and Biodiversity Conservation Act 1999* (Commonwealth) (EPBC Act).

In Western Australia, subterranean fauna environmental impact assessments (EIA) are required to be aligned with the principles and objectives of the Western Australian Environmental Protection Authority (EPA) guidelines:

- EPA (2016) *Environmental Factor Guideline — Subterranean Fauna*;
- EPA (2021a) *Statement of Environmental Principles, factors, and objectives*; and
- EPA (2021b) *Technical Guidance — Subterranean fauna surveys for environmental impact assessment*.

The EPA's environmental objective for subterranean fauna is:

- “To protect subterranean fauna so that biological diversity and ecological integrity are maintained”; whereby,
- Ecological integrity is defined as “the composition, structure, function and processes of ecosystems, and the natural range of variation of these elements” (EPA 2016).

2.6 Impacts & Associated Risks

2.6.1 Direct & Indirect Impacts

The development and operation of resource projects in Western Australia can pose a number of risks to subterranean fauna and their habitat. Impacts may be either direct or indirect (EPA 2016, 2021b).

Direct impacts include the removal of prospective subterranean fauna habitats through:

- Excavation — e.g., mining, construction earthworks. Impacting both stygofauna and troglofauna.
- Groundwater abstraction — lowering of the groundwater table. Stygofauna are considered the more sensitive factor, but large-scale drawdowns would also impact troglofauna.
- Inundation — groundwater reinjection of waste or excess water can flood troglofauna habitat.
- Groundwater quality — alterations to parameter levels (e.g., salinity, pH) that exceed stygofauna species tolerance limits.

Indirect impacts include alterations to prospective subterranean fauna habitats through:

- Clearing of surface vegetation — causing increased siltation rates and decreased resource inputs (e.g., oxygen, nutrients). Impacting both stygofauna and troglofauna.
- Changes to surface topography — leading to altered hydrology regimes, reduced surface water infiltration and aquifer recharge, changes to groundwater flow paths and rates. Impacting both stygofauna and troglofauna.
- Contamination — including operational spills or leaks, leaching from tailings and wastewater resulting in alterations to ground water chemistry and quality, and introduction of toxins or radiation. Stygofauna are considered the more sensitive factor, but large-scale contamination would also impact troglofauna.
- Salinisation — intrusion of saline aquifers into freshwater aquifers, or the leaching of saline water from pit void lakes. Stygofauna are considered the more sensitive factor.

In general, direct impacts are considered to pose the greater risk to subterranean fauna values as they are often of a larger scale and result in the total removal of a species' population/s. Indirect impacts are considered to usually be of a smaller scale that may result in the decline of the abundance of a species' population/s. However, if the scale of indirect impacts is sufficiently widespread in relation to the distribution of a species of a subterranean fauna assemblage, then the risks to maintaining the ecological integrity of a habitat can be as high as for direct impacts.

3 Methods

3.1 Database Searches & Literature Review

The desktop review included searches of both Federal and State Government databases to reveal if any stygofauna or troglofauna taxa had been previously recorded from within or near the Project area, and to identify if any subterranean fauna threatened or priority ecological communities (TECs and PECs) were in the vicinity. The database and internet information sources included:

- Department of Biodiversity, Conservation and Attractions (DBCAs) threatened and priority ecological communities database was searched for TECs and PECs occurring within a 100 km radius of the Project.
- Atlas of Living Australia (ALA) including Western Australian Museum's (WAM) subterranean fauna records.
- Department of Water and Environmental Regulation's (DMER) Index of Biodiversity Surveys for Assessments (IBSA).

Federal and State government lists were checked against search results to identify if any threatened or priority subterranean fauna species occurred within the search area:

- WC Act Schedule Species List;
- EPBC Act Threatened Ecological Community (TEC) List; and
- EPBC Act Threatened Fauna List.

Defined search areas were either from a central point in the Project area or of a designated rectangular search area (**Table 3-1**).

A literature review was conducted to update the information that may exist on subterranean fauna within the Project vicinity or of relevance to the Project. The review included environmental consultant technical reports, scientific journal articles and government publications.

Table 3-1: Defined database and internet sourced search parameters.

Data Source	Search Area	Co-ordinates
TEC's & PEC's	100 km radius	Central point @ 24.54210 S, 116.23521 E
ALA / WAM records	38,775 km ²	NE corner: 23.72166 S, 117.35294°E SW corner: 25.22362°S 115.00712°E

3.2 Stygofauna Survey

3.2.1 Stygofauna Sample Effort

The detailed stygofauna survey completed for the Yinnetharra subterranean fauna assessment involved three survey phases, each more than three months apart, and the collection of 94 haul net samples (refer Section 3.2.2) from 53 sites consisting of 49 uncased exploration drill holes, three cased pastoral bores, and one cased production bore (**Appendix A, Table 3-2, Figure 3-1, Figure 3-2**). Most of the stygofauna sampling occurred within the focussed exploration drilling area of the Malinda Prospect with only four regional (i.e., reference) sites found that were suitable for stygofauna sampling. The Phase 1 survey (June 9-11, 2023) was a basic survey to investigate the prospectivity of the Malinda prospect for stygofauna. The collection of stygofauna from the 21 haul net samples, prompted the requirement for a detailed survey to be completed involving two additional phases of survey: Phase 2 (November 9-14, 2023) and Phase 3 (March 7-9, 2024).

Table 3-2: Stygofauna survey effort.

Phase	Area	Haul Net
Phase 1 June 2023	Malinda	20
	Regional	1
Phase 2 November 2023	Malinda	37
	Regional	4
Phase 3 March 2024	Malinda	32
	Regional	0
Total		94

3.2.2 Haul Net Sampling

Haul net samples are generally collected from a mixture of exploration holes, water bores, and monitoring bores (collectively referred to as sites) using haul nets, which have been found to be the most efficient stygofauna collection method (Allford et al. 2008). The haul net sampling method outlined below is consistent with the procedures outlined in the EPA (2021) technical guidance. The sampling method is as follows:

- The 6 net hauls collected per site (= 1 sample) used weighted haul nets with a mesh size of 65 µm. Each haul net was fitted with a 30 ml polyethylene vial.
- The first net haul was lowered to the bottom of the site where it was gently raised up and down several times to agitate the sediments with the intention of resuspending any stygofauna specimens, or parts thereof, that may be present.
- On retrieval, the net was raised slowly to filter the stygofauna from the water column on ascent. It is important to haul the net up slowly, to minimise the 'bow wave' effect that may prevent specimens from entering the net if raised too quickly.
- The retrieved haul net and sample vial were processed (washed down) into a larger 'sample bucket'.
- This process was repeated an additional five times until all six net hauls had been washed down into the sample bucket.
- The sample in the sample bucket was then elutriated by pouring through a 100 mm diameter net with mesh size of 65 µm and 30 ml vial attached.
- The elutriation step concentrates the sample, reducing the sediment/debris in the sample and getting rid of as much excess water as possible.
- Absolute ethanol (100 %) is used to flush the elutriation net so all remaining components of the sample that may be lining the net are washed into the 30 ml collection vial prior to removal and subsequent preservation of the sample.
- If there was a considerable amount of sediment/debris in a sample making it difficult/impossible to fit in 30 ml vial then the elutriation net contents were flushed into a 250 ml jar instead.
- The processed and contained sample was allocated a unique Sample Number (SN).
- To prevent any cross-contamination among sample sites, all sampling equipment was decontaminated after each site.
- In the field, all samples were placed into eskies with ice bricks prior to being transferred into a refrigerated environment onsite at the end of each survey day.

3.2.3 Groundwater Properties

Basic groundwater physicochemical parameters (specific electrical conductivity (EC), pH, dissolved oxygen (DO), reduction-oxidation potential (Redox), and water temperature) were recorded in the field using a calibrated YSI water quality meter. The groundwater sample was collected by bailer from the upper few metres of the holes water column. The standing water levels (SWL) were measured using a Solinst 101 water level meter and corrected for hole inclination.

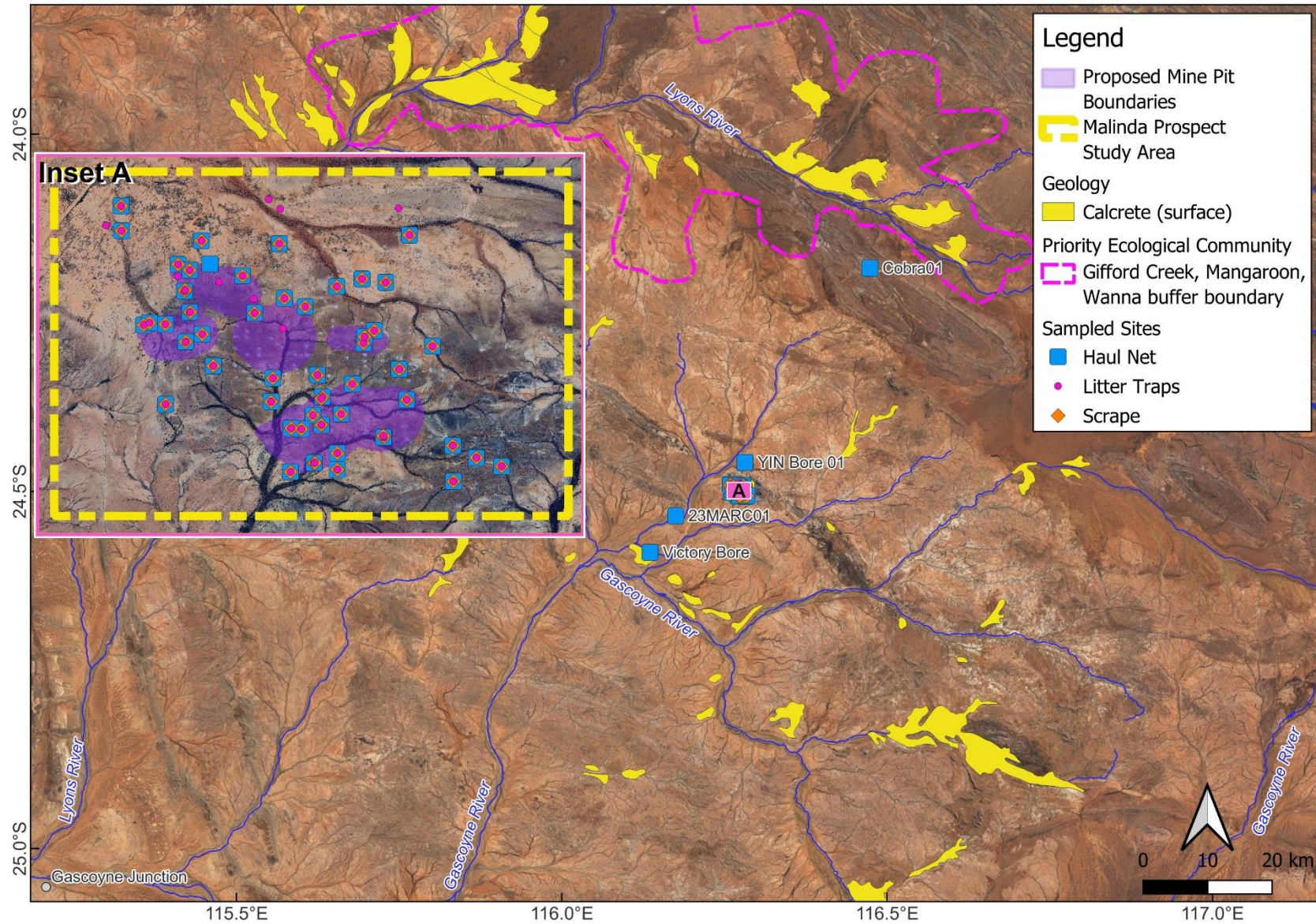


Figure 3-1: Stygofauna and troglofauna regional sample sites beyond Malinda Prospect study area.

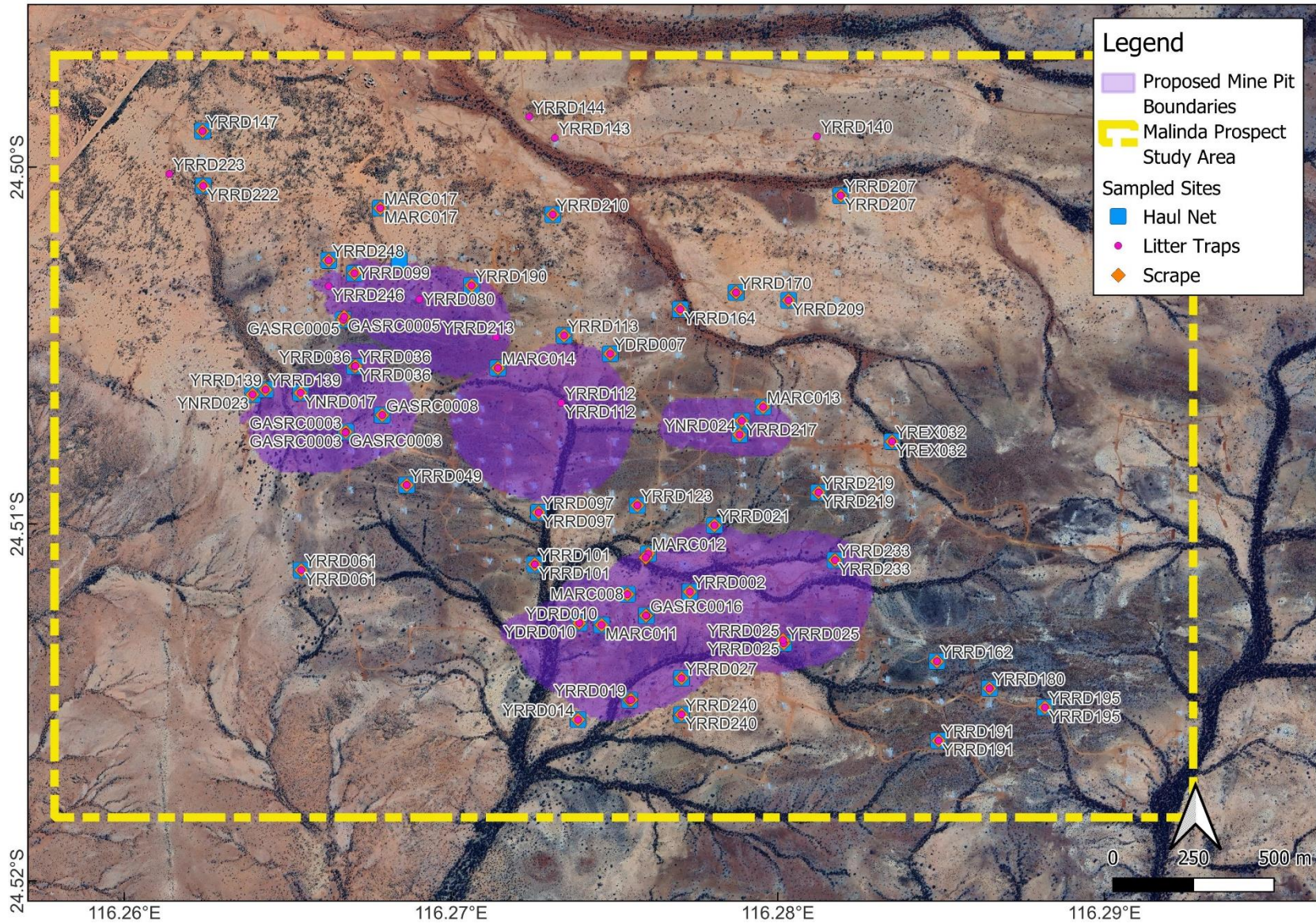


Figure 3-2: Stygofauna and troglofauna sample sites within the Malinda Prospect study area.

3.3 Troglifauna Survey

3.3.1 Troglifauna Sample Effort

The detailed troglifauna survey completed for the Yinnetharra subterranean fauna assessment involved the collection of 88 scrape samples (refer Section 3.3.3) from 48 uncased exploration drill holes, and 101 litter traps (refer Section 3.3.2) deployed in 56 uncased exploration drill holes over three survey phases (**Appendix A, Table 3-3, Figure 3-1, Figure 3-2**).

All the troglifauna sampling occurred within the focussed exploration drilling area of the Malinda Prospect. No suitable regional (i.e., reference) sites were available for troglifauna sampling as the few regional sites found for stygofauna were all fully cased above the groundwater table. The troglifauna component of the Phase 1 survey involved the collection of 20 scrape samples (June 9-11, 2023) and 20 litter traps that were deployed *in situ* for nearly nine weeks (61 days) from June 9 to August 8, 2023. The troglifauna component of the Phase 2 survey involved the collection of 36 scrape samples (November 9-14, 2023) and 45 litter traps that were deployed *in situ* for nine weeks (63 days) from November 11 2023, to January 11, 2024. The Phase 3 survey included the collection of 32 scrape samples (March 7-9, 2024) and 36 litter traps that were deployed *in situ* for nearly nine weeks (61 days) from March 7 to May 6, 2024.

Table 3-3: Troglifauna survey effort.

Phase	Area	Litter Trap	Scrape	Total
Phase 1 June 2023	Malinda	20	20	40
Phase 2 November 2023	Malinda	45	36	81
Phase 3 March 2024	Malinda	36	32	68
Total		101	88	189

3.3.2 Litter Trap Sampling

Litter traps were used to sample for troglifauna in accordance with EPA (2021) technical guidance. The litter traps consist of 250 mm long x 50 mm diameter PVC tubing with several ingress points cut into the side to allow troglifauna taxa from the surrounding unsaturated substrate to colonise the organic litter (e.g., pea straw) packed into the trap. The troglifauna litter traps were prepared, deployed, and collected in the following way:

- Litter traps were packed with prepared organic litter (pea straw soaked in warm water for several hours prior to draining to removing excess water) then sealed to maintain moist, sterile conditions prior to deployment.
- Traps may be moistened with water prior to deployment to ensure they are not too dry. Important to ensure the contents are not dripping wet, just moist.
- Traps were installed by lowering on cord to suitable depth, dependent on geology, hole depth and standing water level. Traps were left *in situ* for more than eight weeks to allow adequate time for colonisation by troglifauna.
- Retrieved traps were sealed in sturdy zip lock bags, labelled, and couriered to Bestiolas for processing, sorting and identification.

Processing of litter traps involved using Tullgren funnels to extract troglifauna specimens from the litter. The trap litter contents are placed into funnels, and light and low heat is applied from overhead lamps to encourage the migration of troglifauna, which are light sensitive and prefer humid conditions, downwards through the litter as it dries and ultimately into the collection vials at the base of the funnels, containing 100 % ethanol. The trap contents took approximately three to four days to run on the funnels before the litter was dry. The dried out litter was then removed from the funnels and manually searched under magnification for any troglifauna specimens that might be remaining.

3.3.3 Scrape Sampling

Haul net sampling of uncased holes or cased holes slotted above the groundwater table, has been found to be an effective method, referred to as scrape sampling, to collect troglifauna that complements litter trap sampling (Halse and Pearson 2014, Outback Ecology 2011a, Subterranean Ecology 2008). The haul net/scrape sampling methods outlined below is consistent with the procedures outlined in the EPA (2021b) technical guidance. The sampling method is the same as for stygofauna haul net sampling but may have the following variations:

- lowering a stygofauna net to the bottom of a dry site or at least 1 m below the standing water level if groundwater is present.
- scraping the net up along the uncased wall surface of the site on retrieval with the aim of dislodging and collecting any invertebrates that may be present.
- this process is repeated four times per site with each scrape sampling a different side of the wall surface of the site.

Scraping for troglofauna can also be conducted simultaneously when sampling uncased bores with water present for stygofauna so that the stygofauna sample also counts as a troglofauna scrape sample. The only difference is the sample effort is greater with six net hauls taken per sample rather than four. Stygofauna sampling of fully-cased bores are not regarded as net haul scrape samples, regardless of whether potential troglofauna taxa may have been collected.

3.4 Field Personnel, Licence, & Limitations

Field personnel involved in the haul net and scrape sampling, and litter trap deployment were Dr Nicholas Stevens and Jake Daviot. The retrieval of litter traps was completed by Delta Lithium representatives Claire McGuire (Phase 1 traps) and David Berman (Phase 2 & 3 traps). Sampling was completed under the Regulation 27 Fauna Taking (Biological Assessment) Licence (#BA27000832), Biodiversity Conservation Regulations 2018. The Phase 3 survey round was cut short by three days due to acute health concerns of a relative in Perth of the field team. However, most sampling was completed in the Malinda Study area, but unfortunately no sampling from regional sites was able to be achieved due to the shortened trip.

3.5 Laboratory Processes

In Perth at Bestiolas, Dr Nicholas Stevens processed all the preserved stygofauna haul net and troglofauna net scrape and litter trap samples and identified all collected invertebrate material using Leica M80 and M205C stereomicroscopes. All samples were stored at minus 20°C before being processed. Any potential subterranean fauna specimens that were found were preserved in 100 % ethanol and stored at minus 20°C to optimise tissue viability for DNA analysis (if required). Lower taxonomic level identifications of taxa collected used published and unpublished keys and taxon descriptions. Any material that may be present of juvenile or badly damaged specimens that are too difficult to reliably identify morphologically but which may have viable tissue present for DNA sequencing were recommended for genetic analysis.

3.6 Genetic Analysis

Forty-eight stygofauna specimens were submitted to Biologic Environmental Survey Pty Ltd (Biologic) for DNA sequencing of the barcoding mitochondrial gene Cytochrome Oxidase subunit 1 (COI; Hebert et al., 2003) and molecular systematics analysis. The main objectives of the genetic analysis were to test the interspecific and intraspecific relationships of designated morphospecies and investigate relationships of sequenced taxa collected as part of this Project to previously sequenced material from the wider region. Methods used to address the objectives are clearly stated in Biologic (2024) (**Appendix D**).

4 Environmental Context

4.1 Biogeographic Region

Yinnetharra is located in the western portion of the Augustus subregion (GAS03) within the Gascoyne Interim Biogeographic Regionalisation (IBRA) bioregion (**Figure 4-1**). The Augustus subregion is 96,695 km² in size and classified as a Desert and Xeric Shrubland ecoregion, characterised by rugged low Proterozoic sedimentary and granite ranges separated by wide flat valleys with extensive areas of alluvial valley fills (DCCEEW 2024, Desmond *et al.* 2001). Vegetation consists mainly of mulga woodland over *Triodia* species on shallow stony loams and rises, and mulga parkland on shallow earthy loams over hardpan on plains (Desmond *et al.* 2001).

The Gascoyne River is the main drainage system in the Augustus subregion, draining the entire southern portion of the subregion (**Figure 4-1**). The headwaters of both the Ashburton and Fortescue River systems originate in the northeastern portion of the subregion. In Western Australia, many groundwater calcrete bodies have formed within the palaeodrainage channels associated with the main current drainage systems. These calcrete systems are known to host diverse and largely endemic subterranean fauna (stygo fauna and troglo fauna) assemblages (Cooper *et al.* 2002, Humphreys 2008). Stygo fauna assemblages hosted in calcrete aquifer ecosystems are listed as vulnerable rare features for the Augustus subregion, but incorrectly as in the Carnegie drainage (Desmond *et al.* 2001) that occurs in the Carnegie subregion (GAS02) (Cowan 2001). Groundwater calcrete systems occur in all three of the Gascoyne bioregion's subregions (Ashburton (GAS01), Augustus (GAS03), and Carnegie (GAS02)), with 10 calcrete stygo fauna assemblages listed as Priority Ecological Communities (PECs) for the Gascoyne and Lyons palaeodrainage systems: Curbur, Dalgety and Landor, Doolgunna, Gifford Creek Mangaroon Wanna, Milgun Central, Milgun South, Mingah Springs, Mount Clere, Three Rivers, and Three Rivers Plutonic (DBCA 2023b).

4.2 Environmental Significant Areas

The Gascoyne River is a wetland listed as of subregional significance, the main channel of which runs approximately 18 km south of the Malinda prospect area (**Figure 4-1**) (Desmond *et al.* 2001). Additional wetlands within 75 km of the Project area that are listed as of subregional significance include Edithana Pool (47 km² north-northeast of the Project) and Cattle Pool (60 km northeast). Both listed wetlands are high quality river pools on the Lyons River. Located within 150 km of the Malinda Prospect area are the Mount Augustus National Park (92 km², 55 km east), the Kennedy Range National Park (1,423 km², 110 km west), and the Class "A" Barlee Range Nature Reserve (1,050 km², 125 km north). In addition to the currently gazetted conservation reserves mentioned above, there are large tracts of ex-pastoral leases in the region that have been approved to be added to Western Australia's conservation estate. This includes an additional 4,421 km² to the Mount Augustus (Burringarruh) National Park and 1,774 km² to the Kennedy Range National Park.

4.3 Land Use and Tenure

The majority of land within the Gascoyne region is used for pastoralism involving mostly grazing of native pastures, with leases covering 75 % of the area (GDC 2023). Smaller areas serve horticultural and mining purposes (GDC 2023). Land within the Augustus subregion is mainly used for native pasture grazing (84 %), with smaller areas classified as Unallocated Crown Land (UCL) and Crown Reserves (10 %), and Aboriginal reserves (3.4 %) (Desmond *et al.* 2001). The current Yinnetharra mining leases stretch across three pastoral leases, Yinnetharra Station, Mount Phillip Station and Eudamullah Station. The Malinda prospect occurs entirely on the Yinnetharra Station pastoral lease.

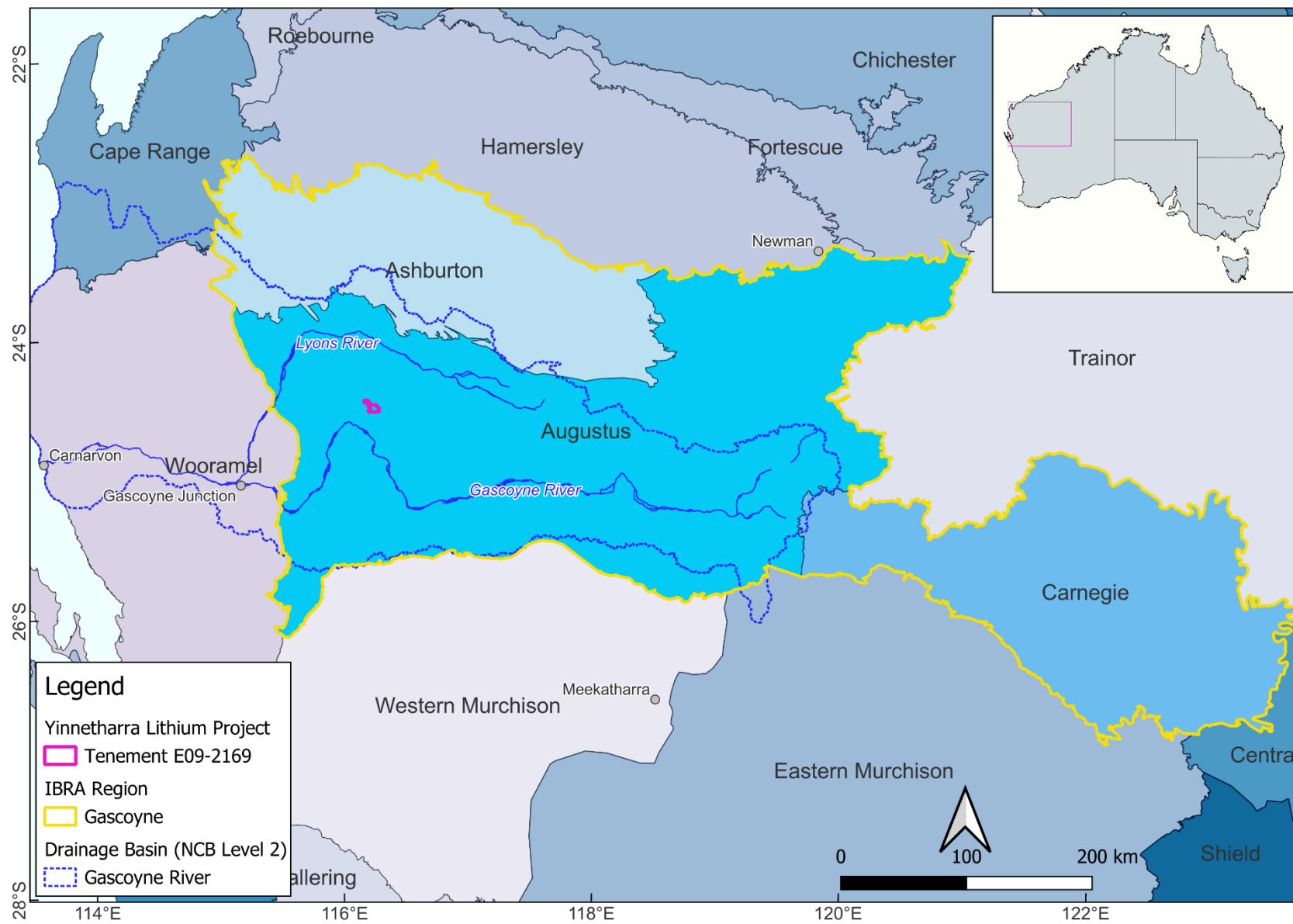


Figure 4-1: Location of the Yinnetharra Lithium Project in the western part of the Augustus (GAS03) subregion of the Gascoyne IBRA bioregion.

4.4 Climate

The Gascoyne region typically receives low amounts of variable rainfall that can often be influenced by northern tropical systems including cyclonic events (GDC 2023). The Augustus subregion is a desert area that is generally characterised by a bimodal rainfall pattern, meaning that the region receives rainfall in the warmer months, associated with tropical systems, as well as receiving winter rainfall from southerly cold fronts (Desmond *et al.* 2001, GDC 2023).

There were three Bureau of Meteorology (BOM) weather stations in close proximity to the Project area: Mount Phillip (#007058, 12 km northeast), Yinnetharra (#007094, 18 km southwest) and Burringurrah Airstrip (#007210, 67 km east-southeast). Of these, the Burringurrah Airstrip was the only weather station that had complete rainfall data for 2023 and 2024. The Mount Phillip weather station records ceased in 2020 and for the Yinnetharra weather station ceased in early 2021. The Gascoyne Junction weather station (#006022, 120 km southwest) was the closest weather station to the Project area with reliable long-term and recent temperature data, with the exception that there was no data for 2023.

The annual rainfalls (2012 to 2024) recorded by the Burringurrah Airstrip weather station are highly variable, ranging from 79 mm (recorded in 2019) to 370 mm (2015)) with a mean of 200 mm (Bureau of Meteorology 2024) (**Figure 4-2**). The warmer months (October to March, with mean maximum temps ranging from 35.1°C to 41.2°C) generally receive higher rainfalls (mean 125 mm (63 % of mean annual total) compared to the cooler period (April to September, with mean maximum temps ranging from 26°C to 34°C) with mean falls of 67 mm (34 %) (Bureau of Meteorology 2024).

In 2023, the annual rainfall received (123 mm) was lower than the mean annual (200 mm) and followed two years of above average rainfalls of 267 mm in 2021 and 279 mm in 2022. The rainfall received in 2023 prior to the Phase 1 survey round (June 9 to August 8, 2023) was 113 mm compared to the mean January to May rainfall of 136 mm (**Figure 4-2, Figure 4-3**). The rainfall received in late 2023 (9 mm) prior to the Phase 2 survey round (November 11 2023, to January 11, 2024) was much lower compared to the mean June to October rainfall of 39 mm. For the Phase 3 survey round (March 7 to May 6, 2024) conditions were much drier than average in the five months prior with only 6 mm received compared to the mean of 79 mm (Bureau of Meteorology 2024). During the Phase 3 trap deployment period more than 40 mm of rain was received (**Figure 4-3**). The climate over each of the sample phases would have provided favourable environmental conditions for subterranean fauna, if present.

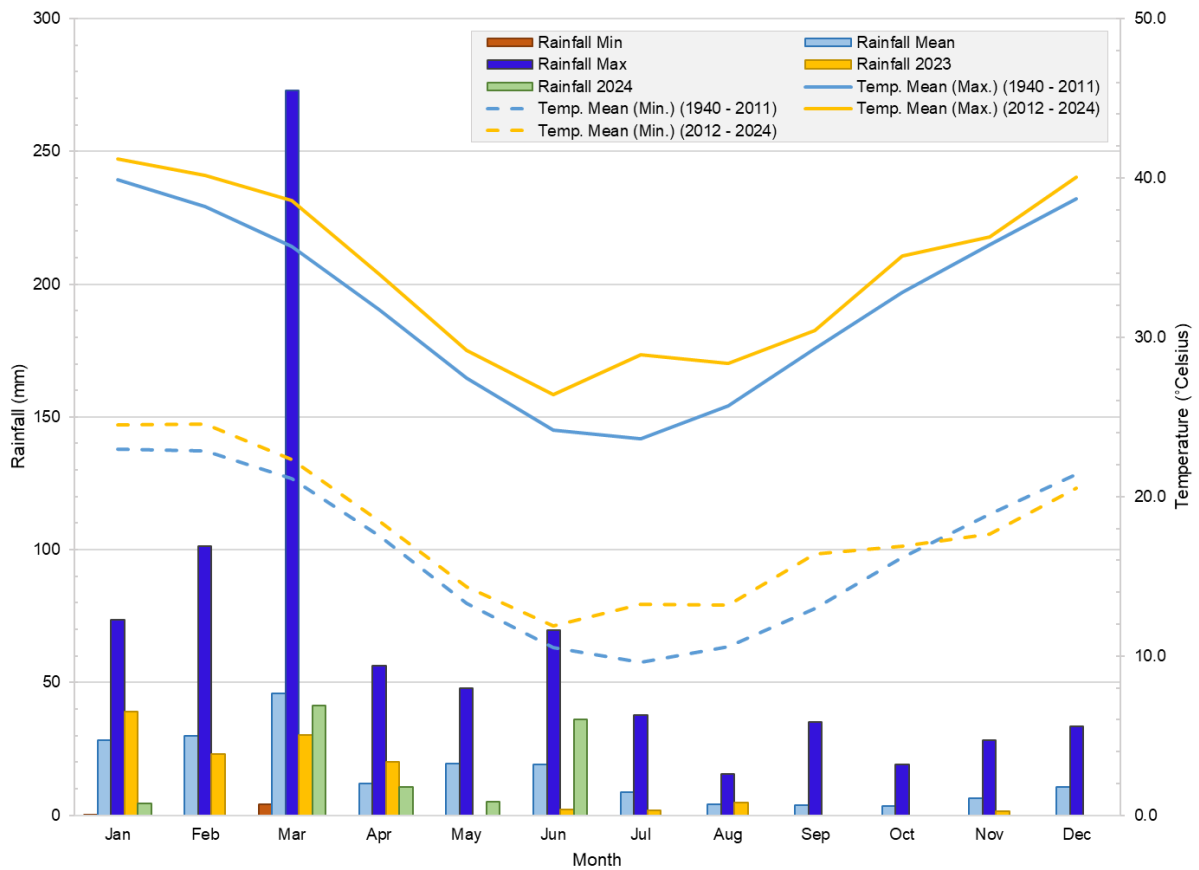


Figure 4-2: Monthly mean, minimum and maximum rainfall compared to 2023 and 2024 monthly totals recorded from Burringurrah Airstrip weather station (# 007210; 2012 to 2024). Mean minimum and maximum temperatures recorded from the Gascoyne Junction weather station (# 006022; 1940 to 2024) (Bureau of Meteorology 2024).

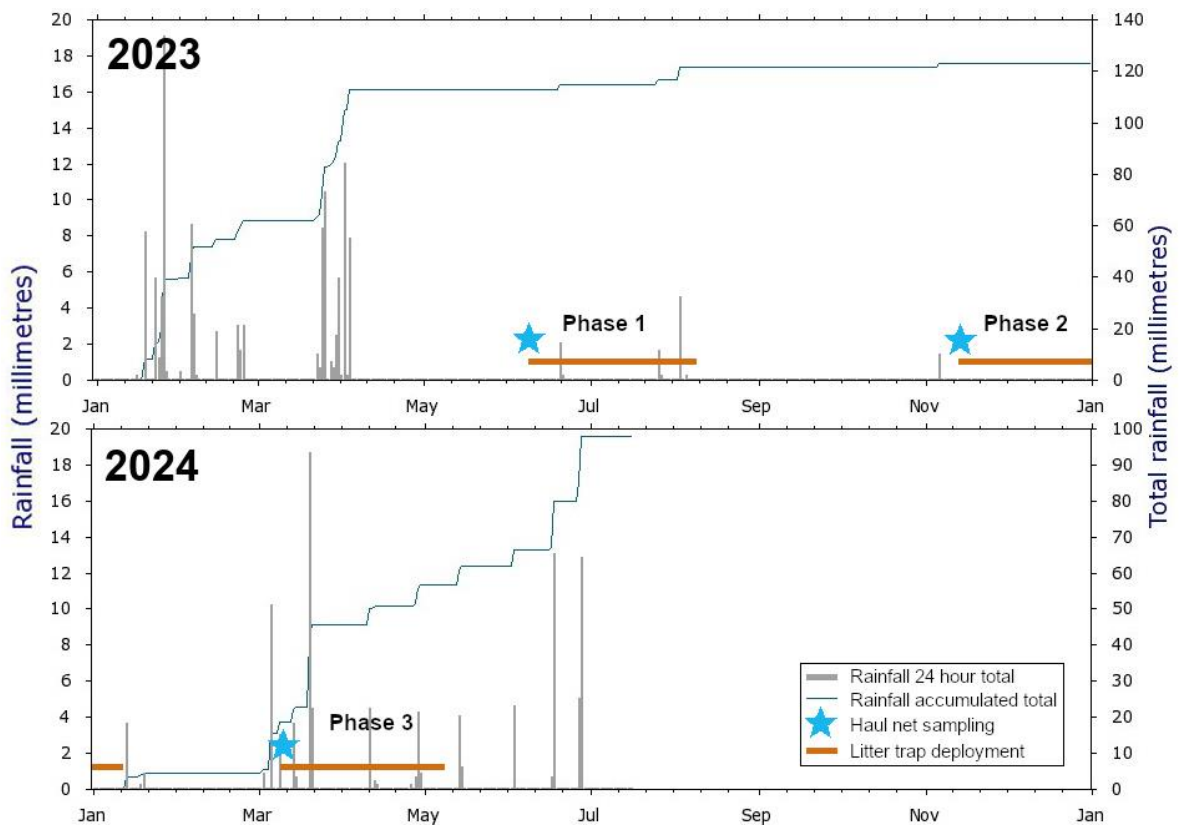


Figure 4-3: Daily rainfall recorded from Burringurrah Airstrip weather station (# 007210; 2023 and 2024).

4.5 Geology

The Yinnetharra Lithium Project (Yinnetharra) is located within the Gascoyne Province of the Capricorn Orogen, which is marked by widespread deformation through various phases of metamorphism, largely considered to have resulted from distant continental-edge interactions, and not from the collision of the Pilbara and Yilgarn cratons as was first thought (**Figure 4-4**) (Johnson *et al.* 2011, Sheppard *et al.* 2010).

Yinnetharra tenements encompass a highly prospective Lithium-caesium-tantalum (LCT) spodumene bearing pegmatite belt within a regional scale granite unit that trends in a north-westerly orientation for approximately 80 km, more recently referred to as the 'Volta corridor' (Delta Lithium 2024, Voltaic Strategic Resources 2024). Pegmatites are a coarse grained and fractionated granitic rock known to often host spodumene (lithium) mineralisation in the Gascoyne region and elsewhere in the world. LCT bearing pegmatites are structurally controlled, largely by shear zones or faults that play a critical role in the location of pegmatite intrusions into overlying geological units from source molten granite upwellings and intrusions (Phelps-Barber *et al.* 2022). Pegmatite swarms in the Pilbara were shown to be related to major lineaments on a province scale and to shear zones on a district to orebody scale (Phelps-Barber *et al.* 2022). The Ti Tree shear zone appears to be the main structure that facilitated and controlled the pegmatite intrusions of the Volta corridor into the overlying metasedimentary geological units such as the Leake Springs Metamorphics (**Figure 4-5**, **Figure 4-6**). The more prospective pegmatite intrusions of the Volta corridor occur within 5 km from the edge of the source granite extensions of the Thirty Three Supersuite (Voltaic Strategic Resources 2024).

The surface geology of the Malinda Prospect area generally consists of outcropping of weathered rocks and quartz veins of the Leake Springs Metamorphics that form the low ridgelines, largely surrounded by shallow red-brown and red earthy loam and sand colluvials, and intersected by channel and floodplain alluvials (**Figure 4-7**). The secondary porosity present is principally associated with fractures within the less weathered saprolite and saprock beneath a shallow soil profile (**Figure 4-8**, **Figure 4-9**).

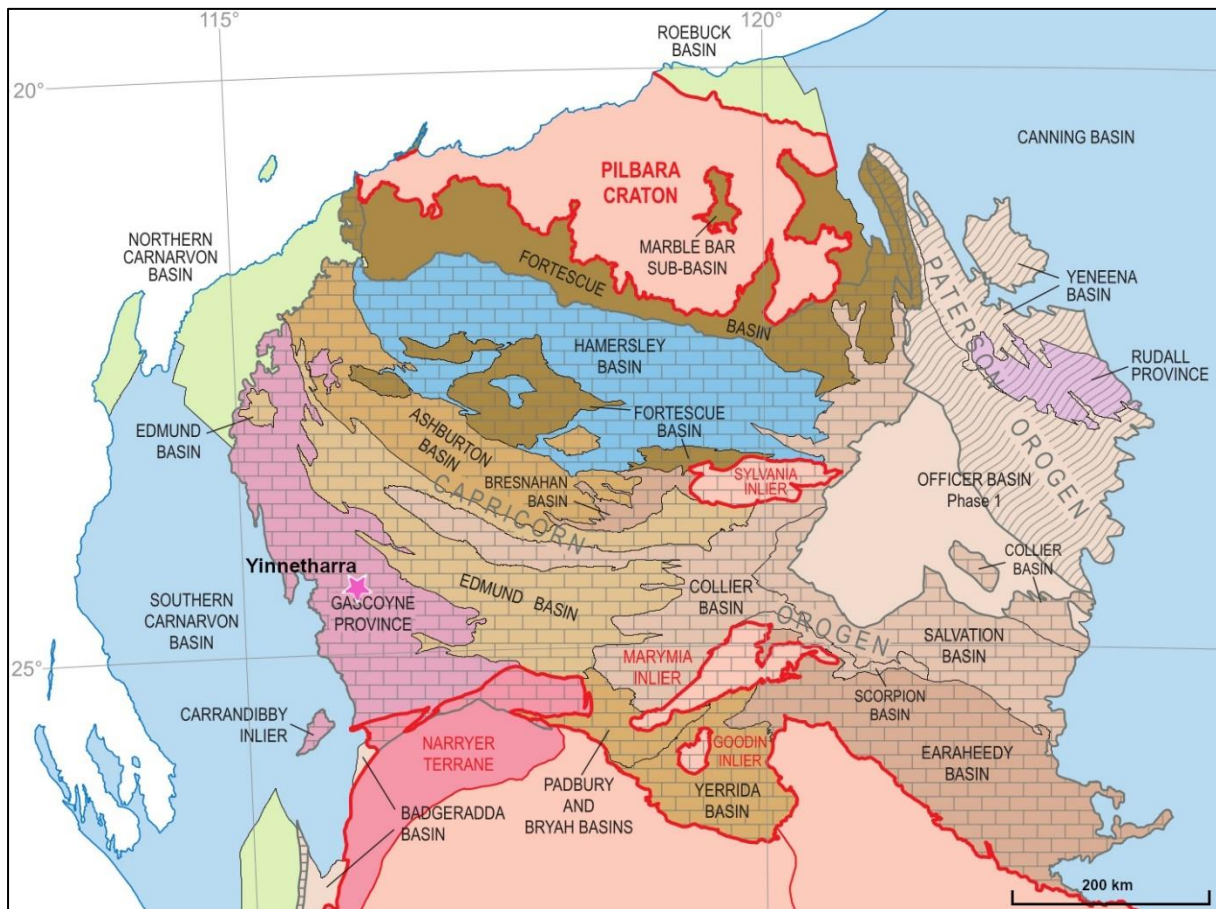


Figure 4-4: Yinnetharra Lithium Project location in relation to tectonic units of Western Australia (adapted from Geological Survey of Western Australia (2022)).

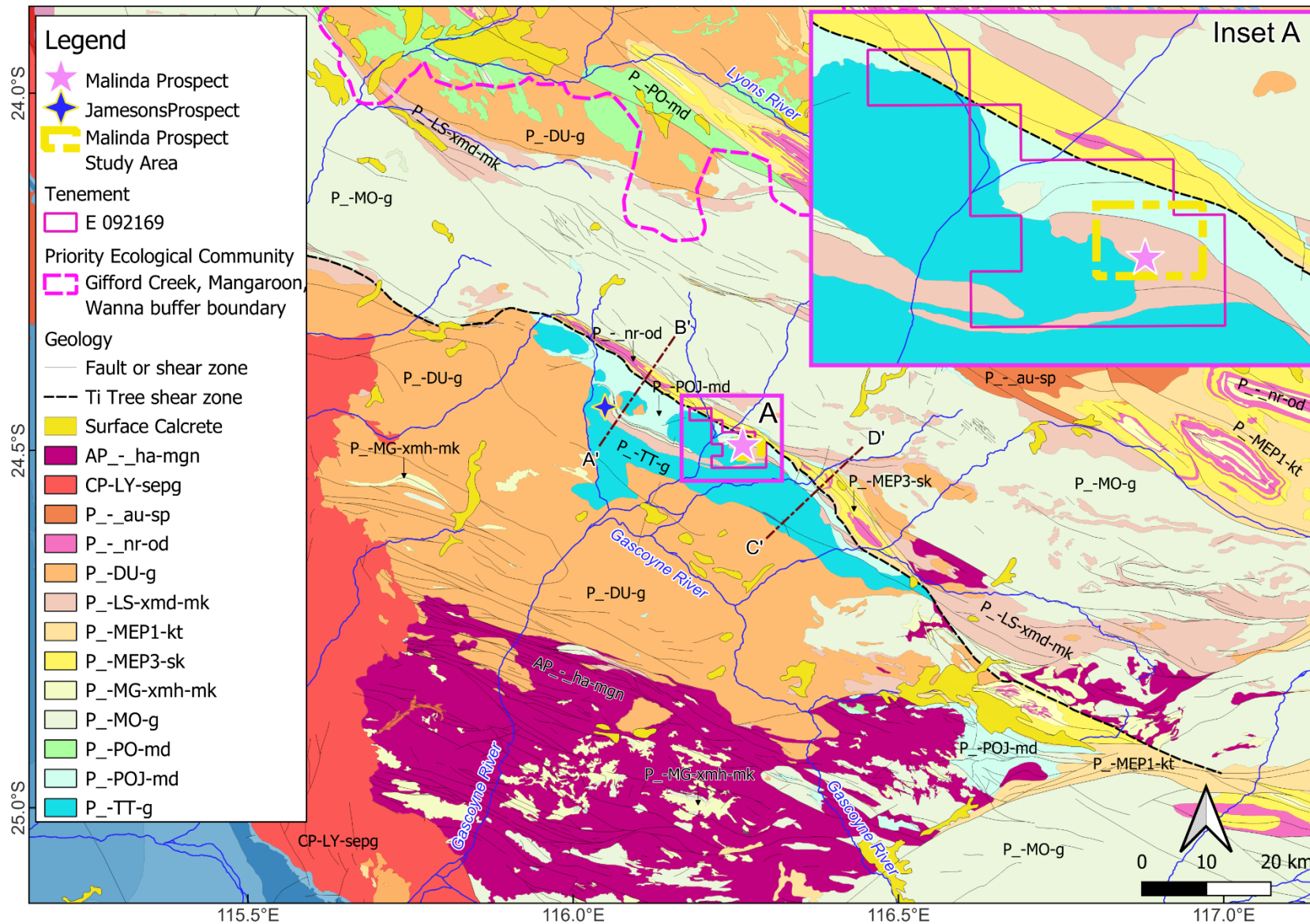


Figure 4-5: Regional bedrock geology (source Geological Survey of Western Australia (2020)). Refer Table 4-1 for relevant geology code descriptions.

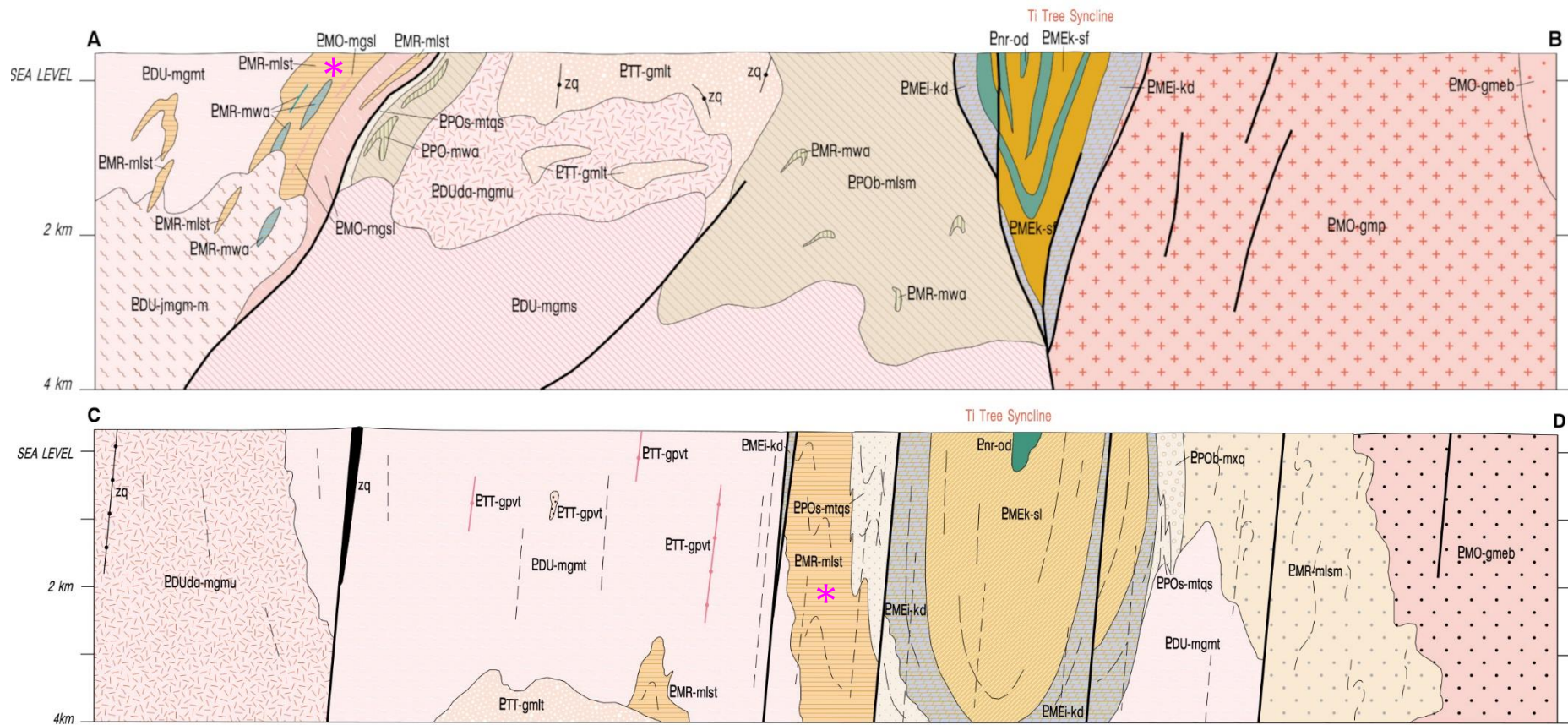


Figure 4-6: Diagrammatic bedrock stratigraphy for Sections A—B and C—D in Figure 4-5 and Figure 4-7 (adapted from Johnson *et al.* (2012) and Sheppard *et al.* (2008)). Pink asterisk highlights occurrence of Leake Springs Metamorphics (P_MR_mlst) that host prospective spodumene bearing pegmatites in Malinda Prospect.

Table 4-1: Bedrock geology code descriptions most relevant to the Project area as shown in Figure 4-5 (source Geological Survey of Western Australia (2020)).

Geological Code	Unit Name	Description
AP_-_ha-mgn	Halfway Gneiss	Interlayered leucocratic and mesocratic granitic gneiss, pale-grey granitic gneiss and foliated metagranite, and gneissic to foliated porphyritic metagranodiorite.
CP-LY-sepg	Lyons Group	Diamictite, sandstone and siltstone (locally calcareous), shale, and boulder beds and lags; glaciogene.
P_-_au-sp	Mount Augustus Sandstone	Sandstone, pebbly sandstone, and conglomerate; minor siltstone.
P_-_nr-od	Narimbunna Dolerite	Dolerite and gabbro sills intruded into Edmund Group.
P_-DU-g	Durlacher Supersuite	Granite and minor gabbro, and metamorphosed equivalents.
P_-LS-xmd-mk	Leake Spring Metamorphics	Pelitic and psammitic schist; calc-silicate rock; minor amphibolite. Previously referred to as Morrissey Metamorphics.
P_-MEP1-kt	Edmund Group, Depositional package 1	Stromatolitic and non-stromatolitic dolostone, dolomitic siltstone, sandstone, siltstone, and conglomerate.
P_-MEP3-sk	Edmund Group, Depositional package 3	Siltstone, mudstone, sandstone, and dolostone; minor conglomerate.
P_-MG-xmh-mk	Moogie Metamorphics	Psammitic and pelitic schist; calc-silicate rock; minor quartzite, marble, amphibolites, and ultramafic schist.
P_-MO-g	Moorarie Supersuite	Granite and minor gabbro, and metamorphosed equivalents.
P_-PO-md	Pooranoo Metamorphics	Pelitic and psammitic schist, quartz metasandstone, feldspathic metasandstone and metaconglomerate, and phyllite.
P_-POJ-md	Mount James Subgroup	Pelitic and psammitic schist, metamorphosed quartz sandstone, feldspathic metasandstone and metaconglomerate.
P_-TT-g	Thirty Three Supersuite	Leucocratic granite and metagranite, and pegmatite.

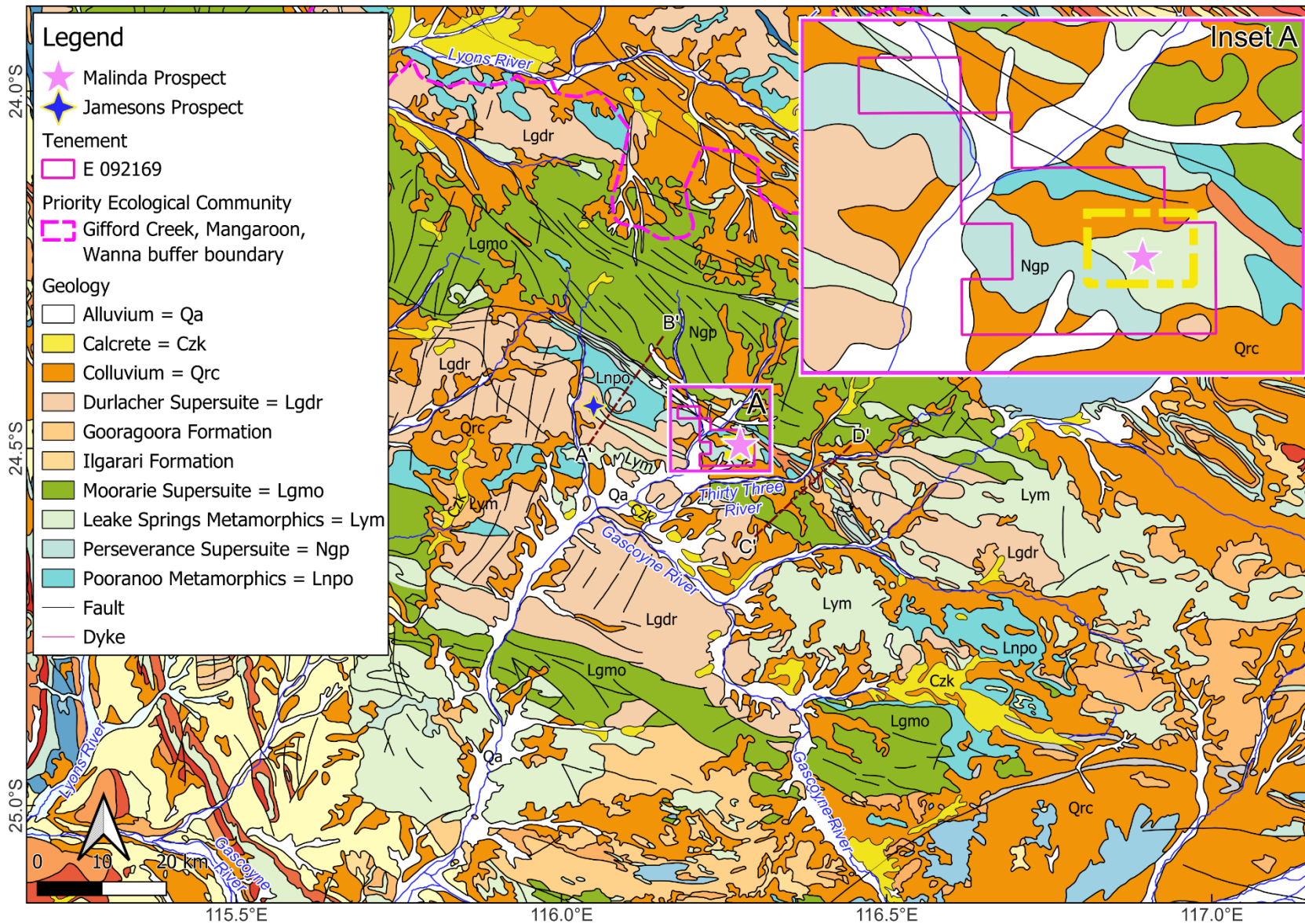


Figure 4-7: Surface geology of the Yinnetharra region (source Raymond *et al.* (2012)). Refer Table 4-2 for relevant geology code descriptions.

Table 4-2: Surface geology code descriptions most relevant to the Project area as shown in Figure 4-7 (source Raymond *et al.* (2012) and Sheppard *et al.* (2010)).

Unit Name	Geology Code	Description
Alluvium	Qa	Channel and flood plain alluvium; gravel, sand, silt, clay; can be locally calcreted.
Calcrete	Czk	Calcrete, travertine; calcareous cementing of bedrock and transported materials; pisolitic to nodular or massive; as low mounds, in playa lakes, valley calcrete, or in subsurface; may contain intercalated chalcedony; locally dissected and karstified.
Colluvium	Qrc	Colluvium and/or residual deposits, sheetwash, talus, scree; boulder, gravel, sand; may include minor alluvial or sand plain deposits, local calcrete and reworked laterite.
Durlacher Supersuite	Lgdr	Monzogranite and granodiorite, minor tonalite, syenogranite, gabbro.
Moorarie Supersuite	Lgmo	Monzogranite, granodiorite and tonalite, diorite, gneissic to schistose granodiorite to monzogranite; local schist, amphibolite, calc-silicate and quartzite.
Leake Spring Metamorphics	Lym	Comprise pelitic schist with numerous thin layers and lenses of amphibolite and psammitic schists (after feldspathic sandstone) interlayered with calc-silicate rock. Previously considered to be Morrisey Metamorphics.
Muntharra Formation, Kiangi Creek Formation	Msd3	Siltstone and mudstone; sandstone, dolostone, minor conglomerate.
Perseverance Supersuite	Ngp	Leucocratic medium-grained muscovite-tourmaline (-biotite) monzogranite; equigranular to porphyritic.
Pooranoo Metamorphics	Lnp0	Pelitic schist, gneiss and granofels, and metamorphosed feldspathic sandstone and conglomerate.

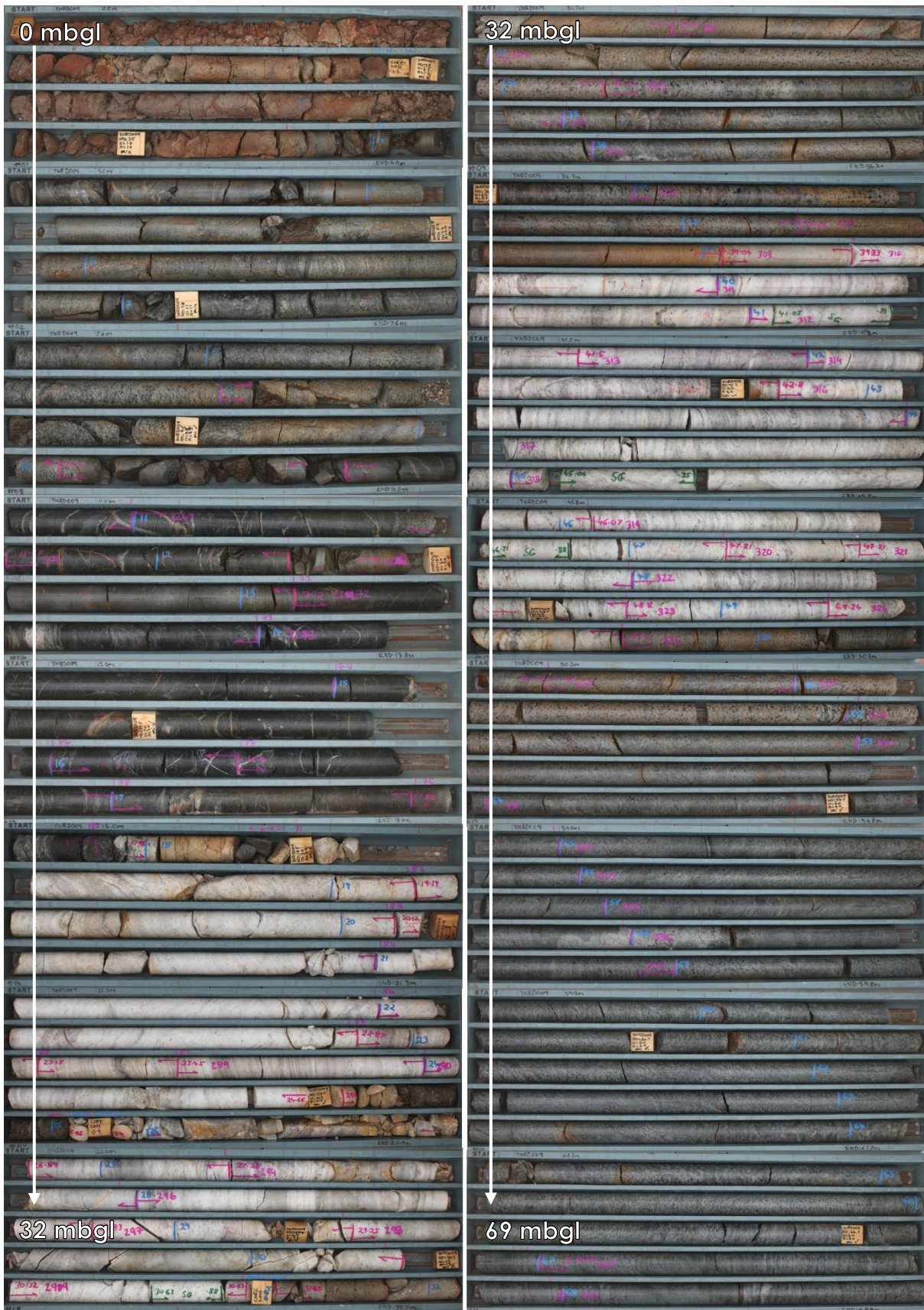


Figure 4-8: Diamond drill core images (0 to 68.8 mbgl) of YNRD009, located within proposed pit area, 30 m east of YRRD036 (recorded SWL ranged from 27.4 to 28.8 mbgl), from which stygofauna and troglfauna were collected.



Figure 4-9: Diamond drill core images (0–70.1 mbgl) of YNEX001, located within proposed pit area, 150 m west of YRRD025 (recorded SWL ranged from 17.4 to 18.8 mbgl), from which stygofauna were recorded.

4.6 Hydrology

The Yinnetharra area extends across numerous minor incised ephemeral tributaries and broader alluvial valleys that would flow southwards during periods of high rainfall into the Gascoyne River, 20 km south of the Malinda Prospect (**Figure 4-7**). Following heavy rain, surface drainage would occur along small watercourses or as sheet flow. Surface water flows in the northern drainage area of the Malinda study area initially flow westwards before reaching a broader, braided drainage system flowing southwards. The drainage pathways are broader and less distinct in the northern drainage, occurring more as sheet flow across hardpan, compared to the narrower, more incised, ephemeral creek lines in the southern drainage area that flow southwards into the Thirty Three River (4.5 km south), a larger tributary of the Gascoyne River (**Figure 3-1, Figure 3-2**).

4.7 Hydrogeology

Groundwater in the Yinnetharra region occurs in three main aquifer types that are each associated with different geology:

1. *Fractured Rock Aquifers*— Groundwater storage occurs principally in fractures within weathered and unweathered basement rocks comprised of greenstones, granitoids and minor intrusives. The more substantial groundwater storage generally occurs within the boundary zone between the unweathered and weathered bedrock, as well as within fault and shear zones in, and between greenstones and granitoid units.
2. *Alluvial / Colluvial Aquifers*— The surficial alluvial and colluvial deposits may be of lower permeability due to higher silt and clay content. These deposits support surficial unconfined to semi confined aquifer systems.
3. *Calcrete Aquifers*— The formation of calcrete by the precipitation of calcium and magnesium carbonates from the groundwater (i.e. non-pedogenic formation) has displaced or replaced the co-alluvial materials generally in low lying areas with shallow water table (~ 5 mbgl). Calcrete formations can provide important local aquifers due to the high degree of secondary porosity from chemical dissolution. The groundwater can be highly stratified with freshwater lenses overlying increasing salinity with depth. Calcrete systems are renowned for providing optimal environments for stygofauna and troglifauna (refer Sections 2.1 and 2.2).

Groundwater in the Malinda study area occurs principally within a fractured rock aquifer system (Rockwater 2023). Salinity levels range from freshwater (<5 mS/cm) to mesosaline conditions (>30–70 mS/cm) (refer section 5.3.1 for further details). Aquifer recharge would occur following reasonable rainfall events by direct rainfall infiltration and stream flow. The recorded SWLs within the Malinda study area indicate a low hydraulic gradient from areas located higher in the landscape, towards the surrounding aquifer systems lower in the adjoining landscape (refer section 5.3.4 for further details). At the local scale the anisotropic permeability of the weathered and fractured rock aquifer system would result in a limited and complex pattern of groundwater movement.

5 Results

5.1 Database Searches

The Gifford Creek, Mangaroon, Wanna calcrete groundwater assemblage type (commonly referred to as the Gifford Creek PEC) is the only stygofauna PEC present within a 100 km radius of the Malinda prospect, having formed within the Lyons River palaeodrainage channel on the Gifford Creek, Lyons and Wanna pastoral stations (**Figure 5-1, Figure 5-2, Figure 5-3**) (DBCA 2023c). The Gifford Creek PEC is categorised as Priority 1 due to it being considered a 'poorly known ecological community' that is 'known from very few occurrences with a very restricted distribution' (DBCA 2023a).

Other database search results are as follows:

- No subterranean fauna TECs occur within a 100 km radius of the Project (DBCA 2023c).
- No threatened or priority subterranean fauna species are listed in the DBCA's threatened and priority fauna database for the East Murchison subregion (DBCA 2022).
- No subterranean fauna ecological communities in the search area are listed under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act).
- There were no IBSA listed subterranean fauna studies in the Augustus subregion relevant to the Project.
- There were two stygofauna and no troglifauna collection records in the combined ALA and WAM database within the search area, both of which were from Stone Tank Well located within the Gifford Creek calcrete PEC (**Table 5-1, Figure 5-1, Figure 5-2, Figure 5-3**).

5.2 Literature Review

5.2.1 Stygofauna

Published and unpublished records of stygofauna occurring in the region surrounding Yinnetharra (≤ 100 km) are all from within, or near to, the Gifford Creek stygofauna PEC, the closest record of which is approximately 40 km to the north to the Malinda Prospect (**Table 5-1, Figure 5-1, Figure 5-2, Figure 5-3**). Habitats sampled that hosted most of the stygofauna diversity (abundance and species richness) included calcrete and associated surficial alluvial/ colluvial aquifer system/s. The occurrence of stygofauna from such environments is not unexpected as such habitats are known to provide optimal conditions that host diverse assemblages (refer Section 2.1). The associated fractured rock groundwater environment hosted a much lower diversity with only three of the recorded 62 stygofauna species collected from within the granite fractured rock aquifer system, each of which were also present in the calcrete and associated alluvial/ colluvial aquifer systems.

5.2.2 Troglifauna

There were no published records of troglifauna of relevance to the Project. Unpublished records (consultant reports) of troglifauna collections were all from the unsaturated, weathered and fractured granites of the Durlacher Supersuite, the only habitat sampled for troglifauna, which is associated with the Gifford Creek calcrete PEC (**Table 5-2, Figure 5-1, Figure 5-2, Figure 5-3**). The closest troglifauna records to the Malinda Prospect are approximately 60 km to the north.

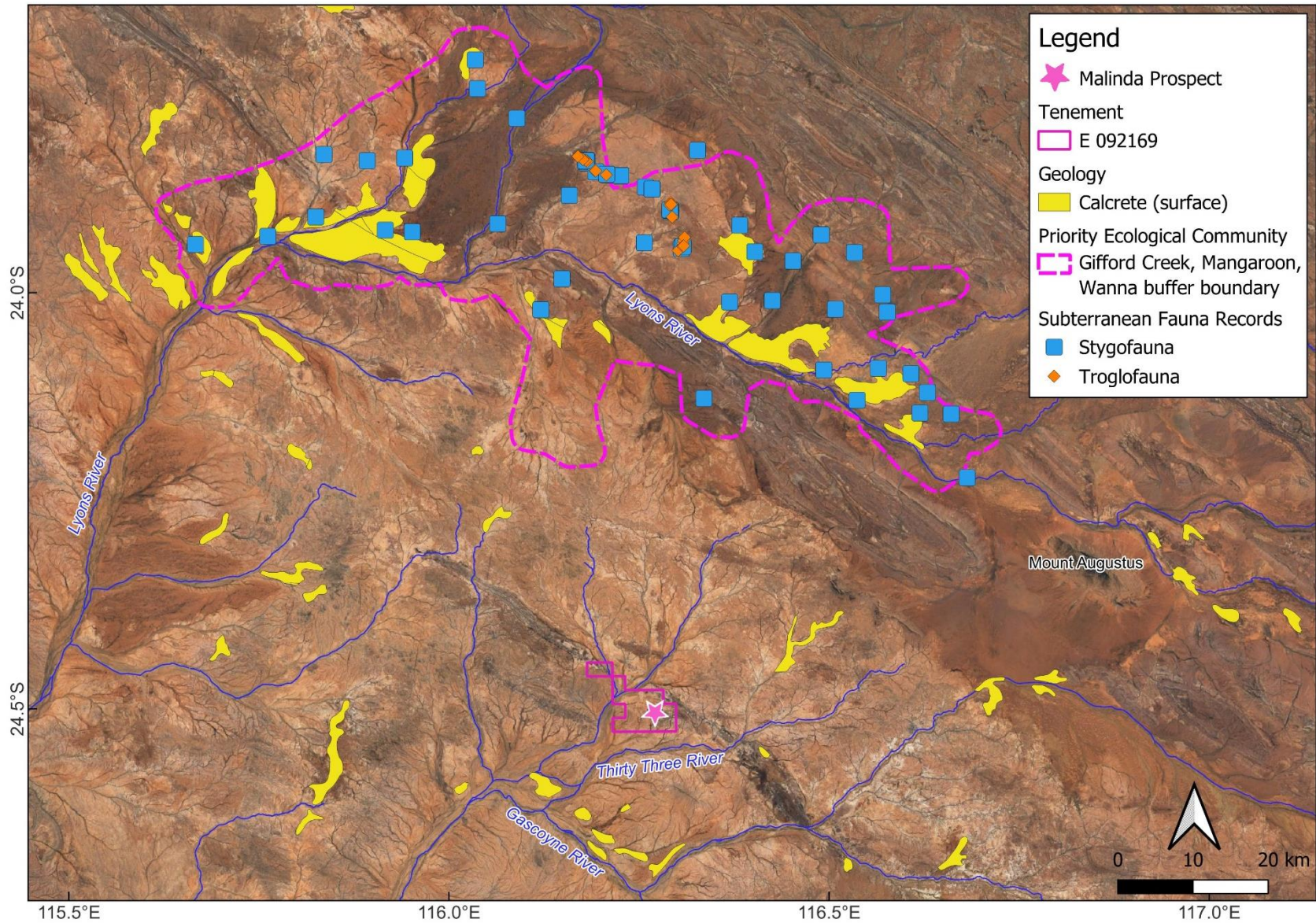


Figure 5-1: Groundwater assemblage PEC and subterranean fauna collection records from database and literature searches in relation to Yinnetharra.

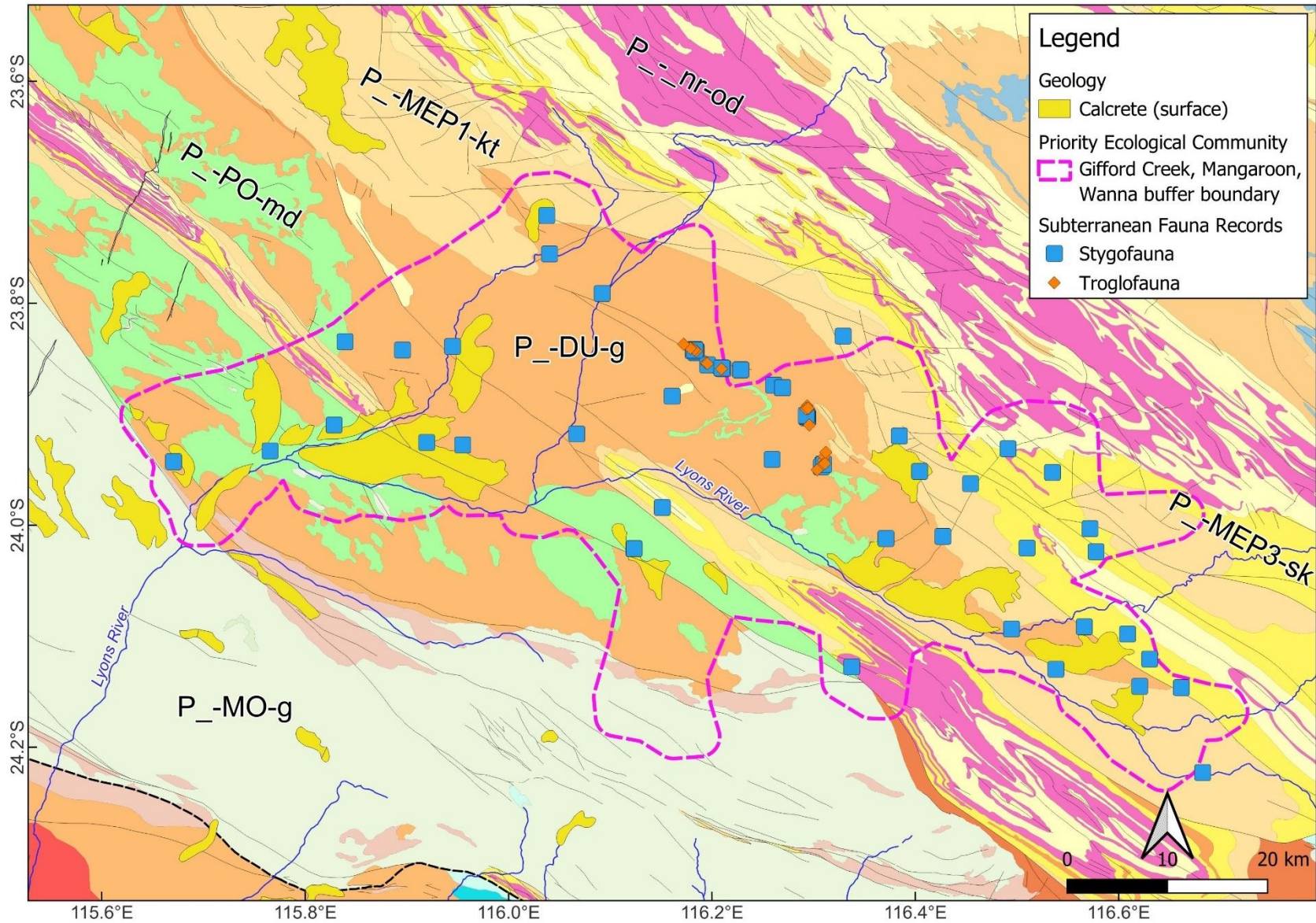


Figure 5-2: Gifford Creek PEC and subterranean fauna records in relation to bedrock geology. Refer Table 4-1 for relevant geology code descriptions.

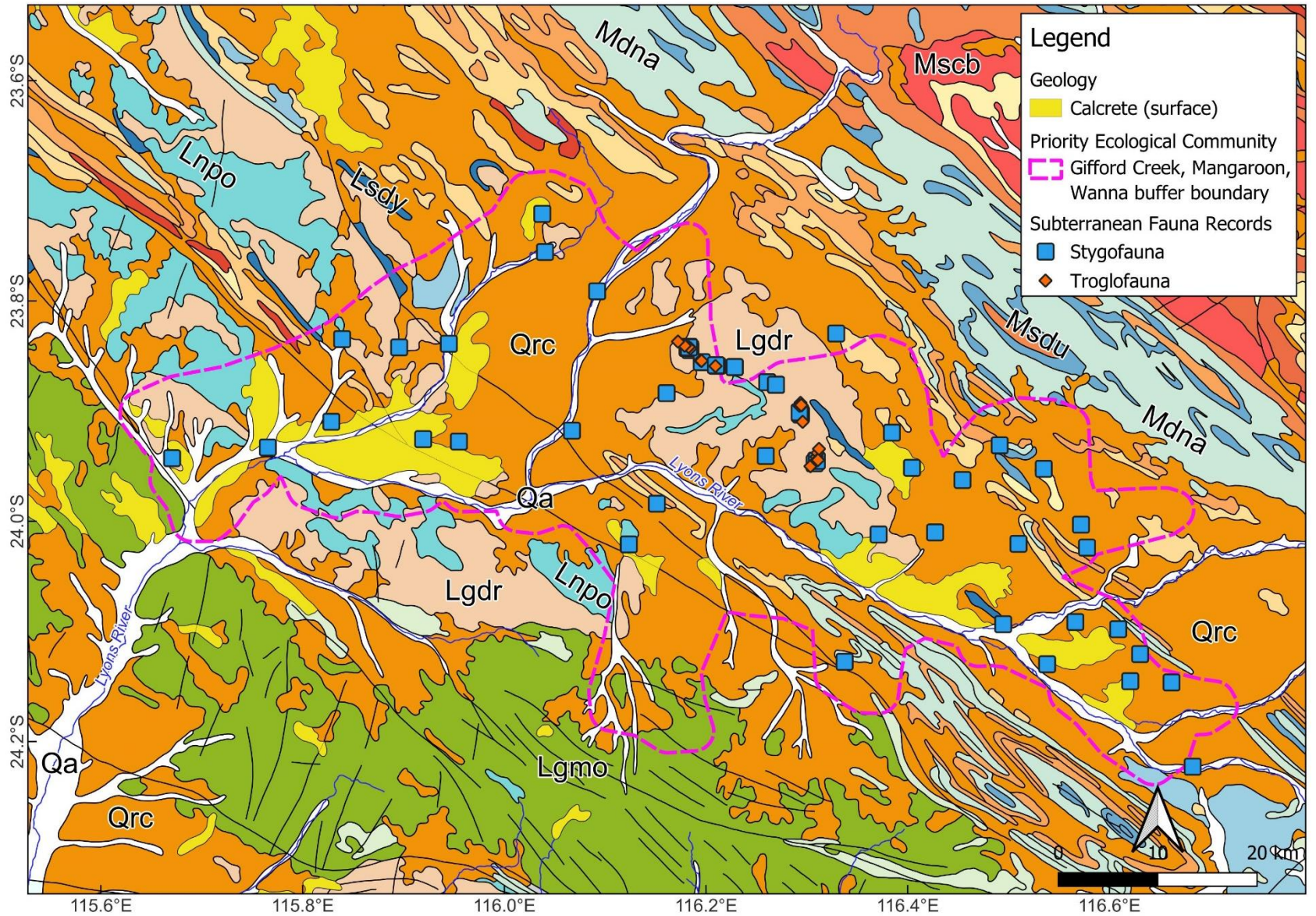


Figure 5-3: Gifford Creek PEC and subterranean fauna records in relation to surface geology. Refer Table 4-2 for relevant geology code descriptions.

Table 5-1: Stygofauna studies of relevance to the Project.

Locality / Project	Aquifer / Geological Unit/s Assessed	Distance & Direction from Malinda Prospect	Findings
Yangibana Rare Earths Project	Calcrete aquifer system associated with Gifford Creek PEC on the Lyons palaeodrainage. Fractured rock aquifer system hosted mostly within the granitoids of the Durlacher Supersuite. Surficial alluvial/ colluvial aquifer systems associated with the Gifford Creek PEC.	Sites sampled range in distance from 50 to 90 km to the north.	<p>Seven survey phases, collecting a total of 167 samples (63 impact, 104 reference): Phase 1— May 2015, 13 samples; Phase 2— September 2015, 18 samples; Phase 3— October 2016, 34 samples; Phase 4— December 2016, 1 sample; Phase 5— October 2017, 51 samples; Phase 6— December 2017, 12 samples; & Phase 7— May 2018, 19 samples.</p> <p>A total of 62 stygofauna species, representing nine higher level taxonomic groups were recorded: Acarina— 1sp.; Amphipoda— 10 spp.; Bathynellacea— 5 spp.; Coleoptera— 3 spp.; Copepoda— Cyclopoida, 10 spp., Harpacticoida, 13 spp.; Isopoda— 2 spp.; and Ostracoda— 18 spp.</p> <p>Forty-six of the stygofauna species recorded were considered likely to be SRE & endemic to the Gifford Creek PEC, with the remaining 16 spp. more widespread in distribution within the Gascoyne bioregion and/or beyond.</p> <p>Only 3 of the recorded 62 stygofauna species were collected from within the granite fractured rock aquifer system within the proposed impact area, each of which were also collected from beyond the impact area within the calcrete and associated alluvial/ colluvial aquifer systems.</p> <p>Fifteen stygoxene/ stygophile species were also recorded: 6 species of which are not considered in EIA (Nematoda*— 1 sp.; Rotifera*— 4 spp.; & Microturbellaria*— 1 sp.); 9 species are oligochaetes— 4 spp. of which were considered to not be SRE (Naididae & Aeolosoma spp.), with the SRE status unknown for the remaining 5 spp. Three of the recorded 15 stygoxene/ stygophile species were collected from within the granite fracture rock aquifer system within the proposed impact area, each of which were also collected from beyond the impact area within the calcrete and associated alluvial/ colluvial aquifer systems.</p> <p>No genetic analysis was done so species richness & distribution based on morphological assessment only.</p> <p>Data source: Bennelongia (2016, 2017, 2018), Ecoscape (2016).</p>
Wanna Station, Gifford Creek PEC	Surficial alluvial/ colluvial aquifer system associated with the Gifford Creek PEC.	Stone Tank Well, 65 km north.	<p>Stygobitic amphipod species, <i>Nedsia wanna</i>, holotype material collected in 2003. Data source: (King <i>et al.</i> 2022).</p> <p>Stygobitic isopod species, <i>Pygolabis gascoyne</i>, holotype material collected in 2003. Data source: Keable and Wilson (2006).</p> <p>ALA database, which contain WAM records, only contained records for both the described amphipod & isopod species mentioned above.</p>

* Nematodes, microturbellaria, and rotifers are not considered in EIA because they are ubiquitous in the environment. Nematodes occur in soil, surface-water, groundwater, on and in plants, and as parasites. Microturbellarian flatworm species are more diverse in marine environments, but do occur in freshwater environments, including in moist soil. Rotifers are common in fresh or saline waters, even present in damp moss or lichens, with some species parasitic. There is not the ecological or taxonomic framework (morphological and genetic) for these groups to determine what species, if any, are truly stygobitic, or even SRE.

Table 5-2: Troglifauna studies of relevance to the Project.

Location	Geological Unit/s Assessed	Distance & Direction from Malinda Prospect	Findings
Yangibana Rare Earths Project	Unsaturated substrate associated with weathered and fractured rock, as well as more widespread colluvium.	Sites sampled range in distance from 58 to 75 km to the north.	<p>Three survey phases, collecting a total of 64 litter trap and 32 scrape samples (10 impact, 30 reference): Phase 1— May to July 2015, 18 litter trap & 6 scrape samples; Phase 2— September to November 2015, 26 litter trap & 6 scrape samples; & Phase 3— October to December 2016, 20 litter trap & 20 scrape samples.</p> <p>A total of 12 troglifauna species, representing eight higher level taxonomic groups were recorded: Chilopoda— 2 sp.; Diplura— 2 sp.; Hemiptera— 1 spp.; Isopoda— 3 spp.; Palpigradi— 1 spp.; Polyxenida— 1 spp.; Symphyla— 1 spp.; and Zygentoma— 1 sp.</p> <p>Ten of the troglifauna species recorded were considered likely to be SRE & endemic to the Gifford Creek PEC area, with the remaining 2 spp. more widespread in distribution within the Gascoyne bioregion and/or beyond.</p> <p>Five troglifauna species were collected from within the proposed impact area, one of which was also collected from beyond the impact area.</p> <p>No genetic analysis was done so species richness & distribution based on morphological assessment only.</p> <p>Data source: Bennelongia (2016, 2017, 2018), Ecoscape (2016).</p>

5.3 Groundwater Properties

Groundwater properties have an important influence on the diversity and distribution of stygofauna. The more important parameters in regard to influencing stygofauna habitat are considered to be salinity, pH, dissolved oxygen, the depth to groundwater, and the elevation of the aquifer system in relation to the surrounding landscape (e.g., within valley system versus surrounding hills/ range). Recorded groundwater temperatures ranged from 25.1 to 32.1°C (**Appendix A**).

5.3.1 Salinity

The groundwater salinity, measured as specific electrical conductivity (EC), recorded from 92 groundwater samples taken from 49 sites within Malinda showed the salinity levels ranged from freshwater (<5 mS/cm) to mesosaline conditions (>30–70 mS/cm), with an average salinity of 16.5 mS/cm in the hyposaline range (5–30 mS/cm) (**Figure 5-4, Appendix A**). Within Malinda, sites located in the northern drainage area had a broader range in salinity levels compared to sites in the southern drainage area and were more saline on average, 21.7 mS/cm and 12.4 mS/cm, respectively. Regional reference sites generally had lower salinity levels than the mean levels for Malinda sites, with the exception of regional site 23MARC01 (25.6 mS/cm). The salinity levels recorded are likely to reflect the variation in groundwater recharge rates in the area, with fresher groundwater generally considered to have higher rates of recharge as larger volumes of water infiltrate more rapidly, thereby limiting evapo-concentration rates.

Salinity levels at sites that recorded stygofauna (range 2.5–36.2 mS/cm; mean 18.6 mS/cm), showed a broad overlap in levels with sites where no stygofauna were collected (range 1.3–30.9 mS/cm; mean 15.3 mS/cm). Both Harpacticoida sp. 'Biologic-HARP087' and Halicyclops sp. 'Biologic-CYCL099' were collected from the most saline conditions (36.2 mS/cm) recorded from the study area as well as from fresh and hyposaline conditions.

Stygofauna generally show a preference for salinities less than that of seawater (<35 ppt or <55 mS/cm) (Strayer 1994). However, some species can tolerate salinity levels up to 70 mS/cm, with fewer species, more commonly copepods, able to exist in salinities in excess of 70 mS/cm (Humphreys 2008, MWH 2015, 2016b, Outback Ecology 2011a, 2012b). In general, stygofauna diversity is considered to decline with increasing salinity above 5 mS/cm (Humphreys 2008, MWH 2016b), a similar trend to surface aquatic species (Pinder *et al.* 2005, Pinder *et al.* 2002). Within Malinda, stygofauna were recorded from freshwater to mesosaline groundwater environments.

5.3.2 pH

Within Malinda, groundwater pH was shown to range from circumneutral (≥ 6.5 to ≤ 7.5) to slightly alkaline (> 7.5) with an average of 7.23 (**Figure 5-4, Appendix A**). Malinda sites in the northern drainage area had a marginally lower mean pH (7.1) compared to southern drainage sites (7.3). Regional reference sites were more alkaline (7.6–8.3) compared to the mean for Malinda sites (7.2). There was little difference in the range and mean pH for sites that recorded stygofauna (6.7–8.1; mean 7.2) compared to sites that did not (6.95–8.3; mean 7.23).

The most diverse stygofauna assemblages inhabit calcareous environments with circumneutral to low alkaline pH generally between 7.2 and 8.2 (Humphreys 2008). Acidic groundwaters (pH<6.5), which are generally associated with igneous and metamorphic sedimentary rocks, generally provide less suitable conditions for stygofauna (Humphreys 2008). However, stygobitic ostracods within the Pilbara region have been recorded from acidic groundwaters with pH as low as 4.4 (Reeves *et al.*, 2007). Therefore, although stygofauna diversity may decline with increasing acidity, the occurrence of some stygofauna taxa in acidic waters cannot be fully discounted.

5.3.3 Dissolved Oxygen

For Malinda sites there was a broad range in dissolved oxygen (DO) levels recorded from bailer sampled groundwater (0.27–6.35 mg/L; mean 1.8 mg/L), from suboxic (<0.3 mg/L) to oxic (≥ 3 mg/L) conditions (**Figure 5-4, Appendix A**). The mean DO levels for Malinda sites in the northern drainage area (1.77 mg/L) were comparable to southern drainage sites (1.83 mg/L). Regional reference sites generally had lower DO levels than the mean levels for Malinda sites, with the exception of regional site YIN01 (2.1 mg/L). There was little difference in the range and mean DO for sites that recorded stygofauna (0.43–3.71 mg/L; mean 1.84 mg/L) compared to sites that did not (0.27–6.08 mg/L; mean 1.74 mg/L).

Dissolved oxygen concentrations are often patchy in the subterranean environment, commonly ranging from suboxic (<0.3 mg/L) to oxic (>3 mg/L) over time, in addition to small and large spatial scales. Given the natural variability of these environments, stygofauna tend to be more resistant to low levels of oxygen compared to surface water aquatic species (Malard and Hervant 1999, Strayer 1994). While concentrations below 5 mg/L may adversely affect surface aquatic biota, stygofauna have been

documented from DO conditions well below 1 mg/L (Chapman and Kimstach 1996, Humphreys 2008). Stygofauna species richness and abundance does begin to decline at levels below 1 mg/L DO, with levels below 0.5 mg/L considered to represent a critical threshold for long term persistence (Hahn 2006).

5.3.4 SWL

The variation in depth to standing water level (SWL) in the Malinda study area, measured as metres below ground level (mbgl), generally reflected local topography, with depth to groundwater much lower (11.2–22 mbgl) for sites below 329 m AHD elevation compared to more elevated sites (26.6–42.5 mbgl) (**Figure 5-5A, Appendix A**). The groundwater within the fractured rock aquifer system of the Malinda study area does appear to be relatively compartmentalised, particularly within the southern drainage area (**Figure 5-5B**). Recorded SWL for the Malinda northern drainage sites indicate a low groundwater gradient is present, with groundwater movement, principally through the geological structural features of the fractured rock aquifer system, from infiltration sites higher in the landscape to lower in the landscape. However, there is no indication from recorded SWL of a consistent widespread groundwater gradient for the southern drainage area, suggesting that the groundwater is more structurally controlled and compartmentalised than for the northern drainage area.

The depth to groundwater recorded from Malinda sites (range 11.2–42.5 mbgl, mean 22.5 mbgl) was deeper than for all four regional sites (4.9–10 mbgl) (**Figure 5-6**). There was a greater variation in the ground surface elevations for sites in the Malinda southern drainage area (318–344 mAHD) compared to Malinda northern drainage sites (320–334 mAHD), which is reflected in the variations in SWL. For sites that recorded stygofauna, the depth to groundwater (4.9–30.3 mbgl, mean 17.6 mbgl) was shallower on average compared to sites where no stygofauna were collected (6.7–45.2 mbgl, mean 24.7 mbgl). In unconfined aquifer conditions, stygofauna diversity is considered to decline with increasing depth to groundwater, particularly greater than 30 mbgl (Halse *et al.* 2014).

5.3.5 Groundwater Assessment

The recorded groundwater properties from all the sites sampled are mostly within the range that are considered suitable for stygofauna habitation. The salinity levels present at sites from which stygofauna were recorded. The recorded SWLs within the Malinda study area suggest a low hydraulic gradient with flow from areas located higher in the landscape, towards the surrounding aquifer systems lower in the adjoining landscape. At the local scale the anisotropic permeability of the fractured rock aquifer system would result in a limited and complex pattern of groundwater movement. This appears to be more pronounced in the southern drainage area of the Malinda study area, where groundwater movement appears to be more compartmentalised and structurally controlled, compared to the northern drainage area.

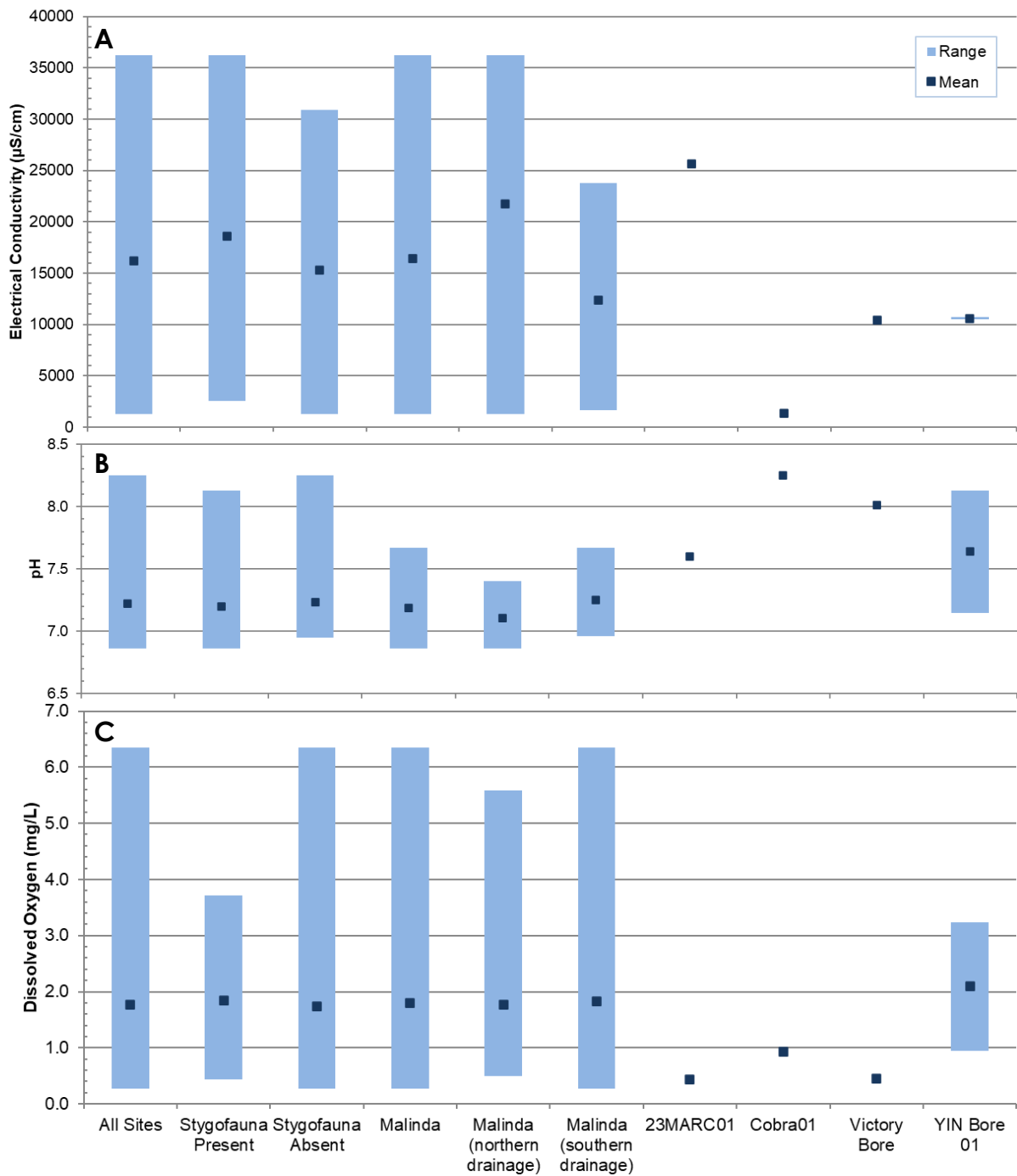


Figure 5-4: Minimum, maximum, and mean of recorded groundwater parameters: A) specific electrical conductivity (EC); B) pH; C) dissolved oxygen (DO).

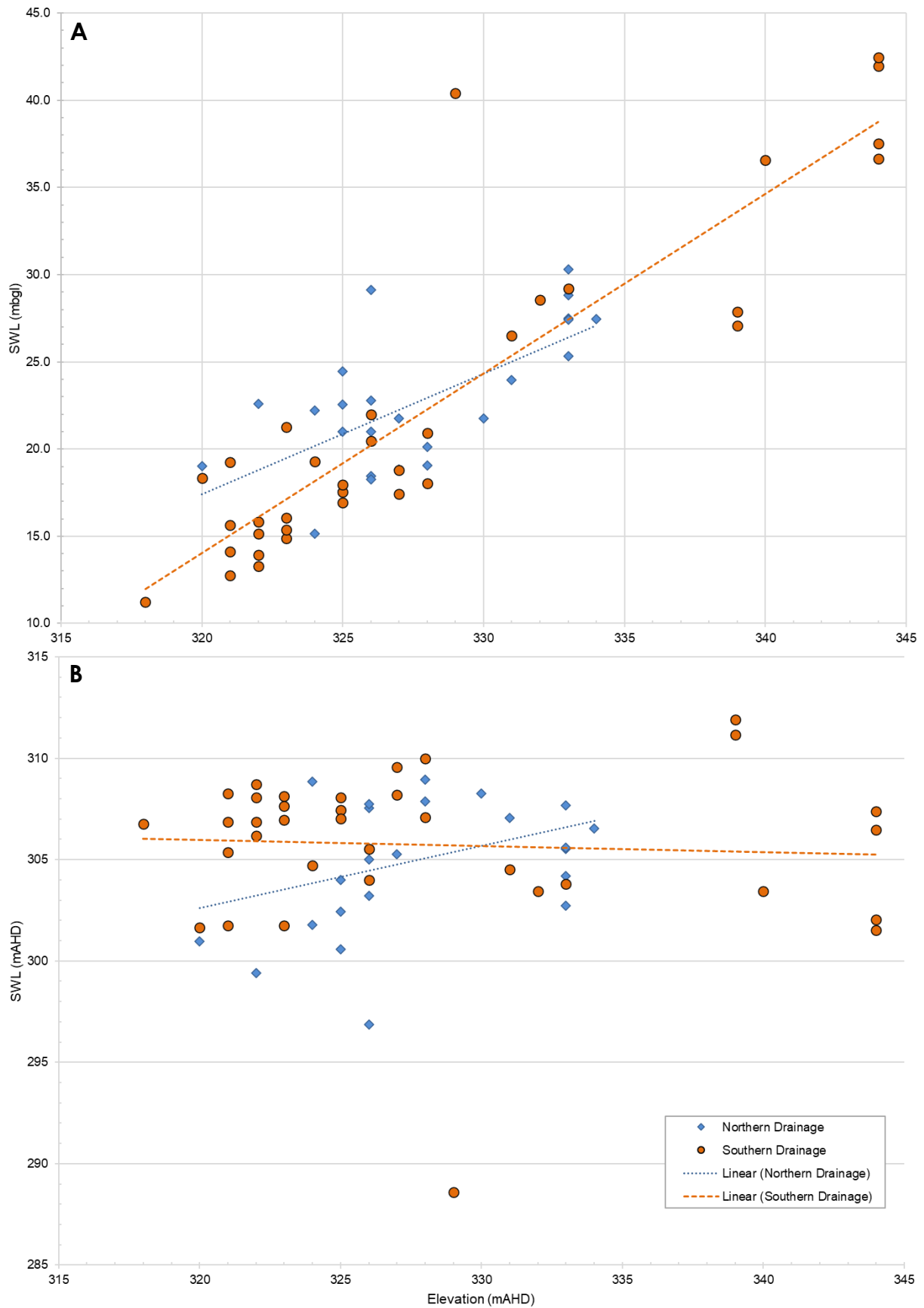


Figure 5-5: Groundwater standing water levels (SWL) recorded from Malinda sites relative to site surface elevation, as metres above sea level (Australian Height Datum (AHD)): A) SWL expressed as metres below ground level (mbgl); B) SWL expressed as mAHD.

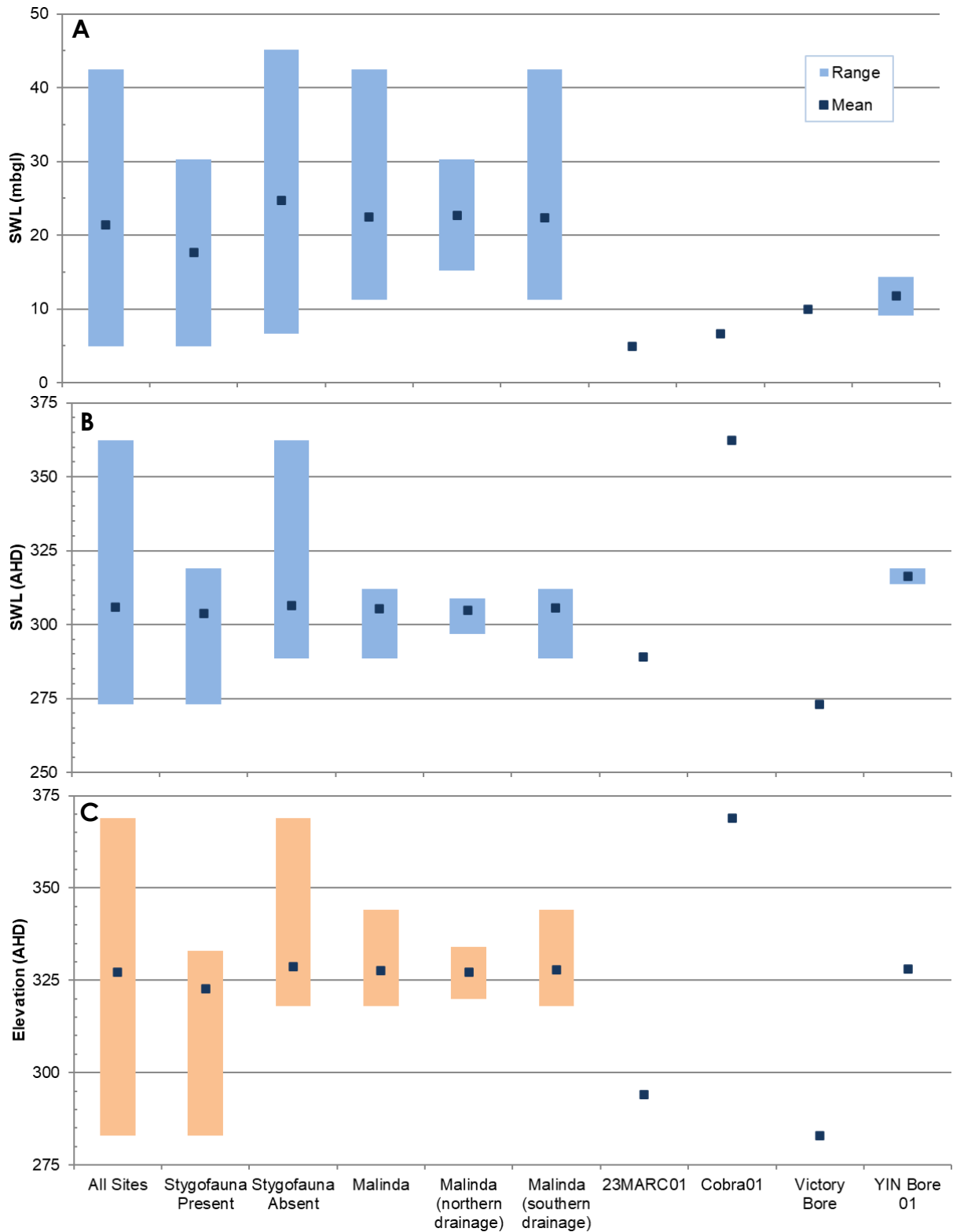


Figure 5-6: Minimum, maximum, and mean of recorded groundwater standing water levels (SWL) and site surface elevation, as metres above sea level (Australian Height Datum (AHD)): A) SWL expressed as metres below ground level (mbgl); B) SWL expressed as mAHd; C) site ground surface elevation.

5.4 Survey Results

5.4.1 Stygofauna

A total of 823 stygofauna specimens, representing six species from four higher level taxonomic groups (Amphipoda, Cyclopoida, Harpacticoida, and Isopoda), were collected from 18 of the 53 sites sampled across the three survey phases (**Table 5-3, Figure 5-7, Figure 5-8, Figure 5-9, Appendix B**). The harpacticoid copepods were the most diverse group with 777 specimens collected, representing two species. Four stygofauna species were collected in 89 haul net samples from 49 sites in the Malinda study area, compared to five species collected from four samples from three neighbouring regional sites. The regional site YIN01, nearly 5 km north of Malinda that intercepts alluvial/ colluvial aquifer environment, was the most species rich site with four of the six recorded species found to occur there, including three of the five species recorded from the study area. Three of the four species that were recorded from the Malinda study area, *Halicyclops* sp. 'Biologic-CYCL099', Harpacticoida sp. 'Biologic-HARP087', and Paramelitidae sp. 'Biologic-AMPH095', were also collected from regional reference sites. Only the microcerberid isopod, *Robustura* sp. YINN01, was not collected from beyond the Malinda study area.

Amphipoda

Stygobitic amphipod species are commonly collected in the Pilbara, Yilgarn and Gascoyne regions, with genetic studies showing amphipods often possess more widespread distributions compared to other stygobitic members of the same stygofauna assemblage, with distributions often shown to extend over 15 km, and even up to 70 km (Biologic 2022, Bradford *et al.* 2013, Cooper *et al.* 2007, Guzik *et al.* 2011, King *et al.* 2022, MWH 2015, Stantec 2017, 2018a, Subterranean Ecology 2011).

Important contributing factors to the relatively broad distributions documented for many stygobitic amphipod species would be the relatively broad habitat preferences often exhibited. Species of stygobitic amphipods that are known from a calcrete aquifer system often exhibit distributions that extend into and along surficial alluvial/ colluvial aquifers within the associated drainage system. They often display the ability to tolerate relatively wide variations in groundwater salinity, from fresh conditions (<5 mS/cm) at sites upstream of salt lake playas, to mesosaline conditions, often in excess of 50 mS/cm, from sites progressively closer to the hypersaline aquifers of a lake playa (MWH 2015, Stantec 2018b, Subterranean Ecology 2011).

Genetic analysis demonstrated that the distribution of Paramelitidae sp. 'Biologic-AMPH095', the only amphipod species recorded from within Malinda, at site YRRD101, extends beyond the study area for nearly 5 km, to regional site, YIN Bore01 (**Figure 5-7, Figure 5-8, Figure 5-9, Appendix C**). It is likely that the distribution of Paramelitidae sp. 'Biologic-AMPH095', extends for further than demonstrated when considering the documented broader distributions and habitat preferences of other stygobitic amphipod species from the Pilbara and Yilgarn.

Cyclopoida

The cyclopid genus *Halicyclops* occurs globally and is commonly found inhabiting interstitial groundwater habitats along coastlines, including anchialine and estuarine environments, as well as from inland aquifer systems, including in the Pilbara and Yilgarn regions (Karanovic 2006). The collection of *Halicyclops* sp. 'Biologic-CYCL099' appears to be the first known record of *Halicyclops* in the Gascoyne region, with none of the 11 Cyclopidae species recorded from the Gifford Creek stygofauna PEC belonging to the genus. Genetic analysis confirmed the species distribution to extend from four Malinda sites to regional site, YIN Bore01, nearly 5 km beyond the study area. *Halicyclops* sp. 'Biologic-CYCL099' was collected sympatrically with Harpacticoida sp. 'Biologic-HARP087' from four sites.

Harpacticoida

The Harpacticoida are a large and ecologically diverse order of copepods that are well represented in the groundwater habitats of Western Australia, including 13 species, representing six genera from five families, recorded from the Gifford Creek stygofauna PEC (Bennelongia 2018). Harpacticoida sp. 'Biologic-HARP087' was the most abundant and widespread stygofauna species recorded from the Project, and the only harpacticoid species recorded from within the Malinda study area. Genetic analysis confirmed the species distribution to extend for 15 km from among 14 Malinda sites to two regional sites, YIN Bore01 (5 km north) and 23MARC01 (10 km southwest).

Isopoda

Microcerberidae isopods occur globally and are tiny (commonly ~1 mm in length), slender, elongate species with most described species known to inhabit the interstitial sandy coastal marine habitats, with fewer described species found within fresh to hyposaline groundwater environments (Hutchins *et al.* 2021, Wägele 1983, Wägele *et al.* 1995). Globally, most freshwater specimens are collected from the hyporheic

zone of river and creek systems, with fewer species recorded from deeper sampling of groundwater wells. In Western Australia, microcerberids are not commonly collected in stygofauna assessments, but the few specimens to have been found have been recorded from alluvial/ colluvial and calcrete aquifer habitats in the Pilbara (Biologic 2022, Stantec 2017, Subterranean Ecology 2013) as well as a single specimen from within the Gifford Creek Stygofauna PEC (Bennelongia 2018).

The collection of three *Robustura* sp. YIN01 specimens from a single exploration hole, YRRD036, provides little insight into the likely distribution range of the species. Known microcerberid collection records from stygofauna assessments in the Pilbara and Gascoyne each consist of a single specimen (singleton) only, thus providing no opportunity to assess the likely distribution ranges of microcerberid species in these regions. This is also often the case from overseas studies as well, suggesting species occur at low population densities. The distribution extents of the few freshwater species known from multiple sites exhibit ranges of 3 to 13 km in alluvial groundwater environments, and up to approximately 40 km in the extensive karstic Edwards Aquifer system in Texas, a known global hot spot for stygofauna (Coineau and Albuquerque 2001, Hutchins *et al.* 2021, Wagele *et al.* 1995).

The distribution of *Robustura* sp. YIN01 is considered likely to extend for several kilometres at least beyond the localised fractured rock aquifer system sampled at site YRRD036, into associated alluvial/ colluvial aquifers lower in the landscape. The sympatric collection from YRRD036 of specimens of the more widely distributed copepod, Harpacticoida sp. 'Biologic-HARP087', demonstrates that the groundwater intercepted within the local area is not totally isolated by any geological barriers from the surrounding associated aquifer systems. The seemingly restricted distribution of a taxon to a single site is often an artefact of sampling a species occurring at low population densities that may have a patchy and irregular distribution in response to varying micro- and meso-habitat biotic and abiotic factors, temporal/seasonal fluctuations, biological interactions, and availability of energy resources, rather than the actual distribution being confined to one limited area that was intercepted by a single drilled hole. Although the broader distribution extent of *Robustura* sp. YIN01 has not been demonstrated, the distribution range is considered to be of a wider extent within the broader expanse of contiguous saturated subterranean habitat present, and not confined to the immediate vicinity of YRRD036.

Table 5-3: Stygofauna diversity recorded.

Taxon	Malinda		Regional	Comments
	Proposed Pit Areas	Outside Pit Areas		
Amphipoda				
Amphipoda indet.	1			Specimen was in very poor condition, body badly damaged, missing posterior segments & most appendages so not possible to morphologically identify below Order level; DNA sequencing attempted but PCR failed.
Paramelitidae sp. 'Biologic-AMPH094'			2	Detected from single site only. Was most closely related to Paramelitidae sp. 'Biologic-AMPH095', displaying 18.6-18.8 % CO1 sequence divergence.
Paramelitidae sp. 'Biologic-AMPH095'	3	1	2	Linear distribution range 4.9 km; Regional material sequenced displayed intraspecific divergence 3.5-3.7 % from Malinda material.
Cyclopoida: Cyclopidae				
<i>Halicyclops</i> sp. 'Biologic-CYCL099'	1	32	1	Linear distribution range 5.2 km; Only two of six specimens successfully sequenced, showing an intraspecific divergence of 0.2 % within a single site (YRRD147) from where most specimens (29) were collected.
Harpacticoida				
<i>Cletocamptus</i> sp. 'Biologic-HARP063'			3	Considered stygophile, non-SRE. Linear distribution range 350 km; Sequenced material from regional site, Victory Bore, matched databased sequenced material from the Pilbara, having an intraspecific divergence of 1.2 %.
Harpacticoida sp. 'Biologic-HARP087'	290	481	3	Linear distribution range 15 km; Sequenced material from regional sites, 23MARC01 & YIN Bore 01, showed intraspecific divergences from Malinda material ranging from 0.2–3.9 % and 2.1–5 %, respectively. Intraspecific divergences shown for Malinda material ranged from 0.0–4.6 %, with 3.9 % the highest divergence found for sequenced material from a single site (YRRD164).
Isopoda: Microcerberidae				
<i>Robustura</i> sp. YIN01	3			All 3 microcerberid specimens collected from a single site (YRRD036) in sympatry with Harpacticoida sp. 'Biologic-HARP087'.
Abundance	298	514	11	
Taxon Richness	4	3	5	

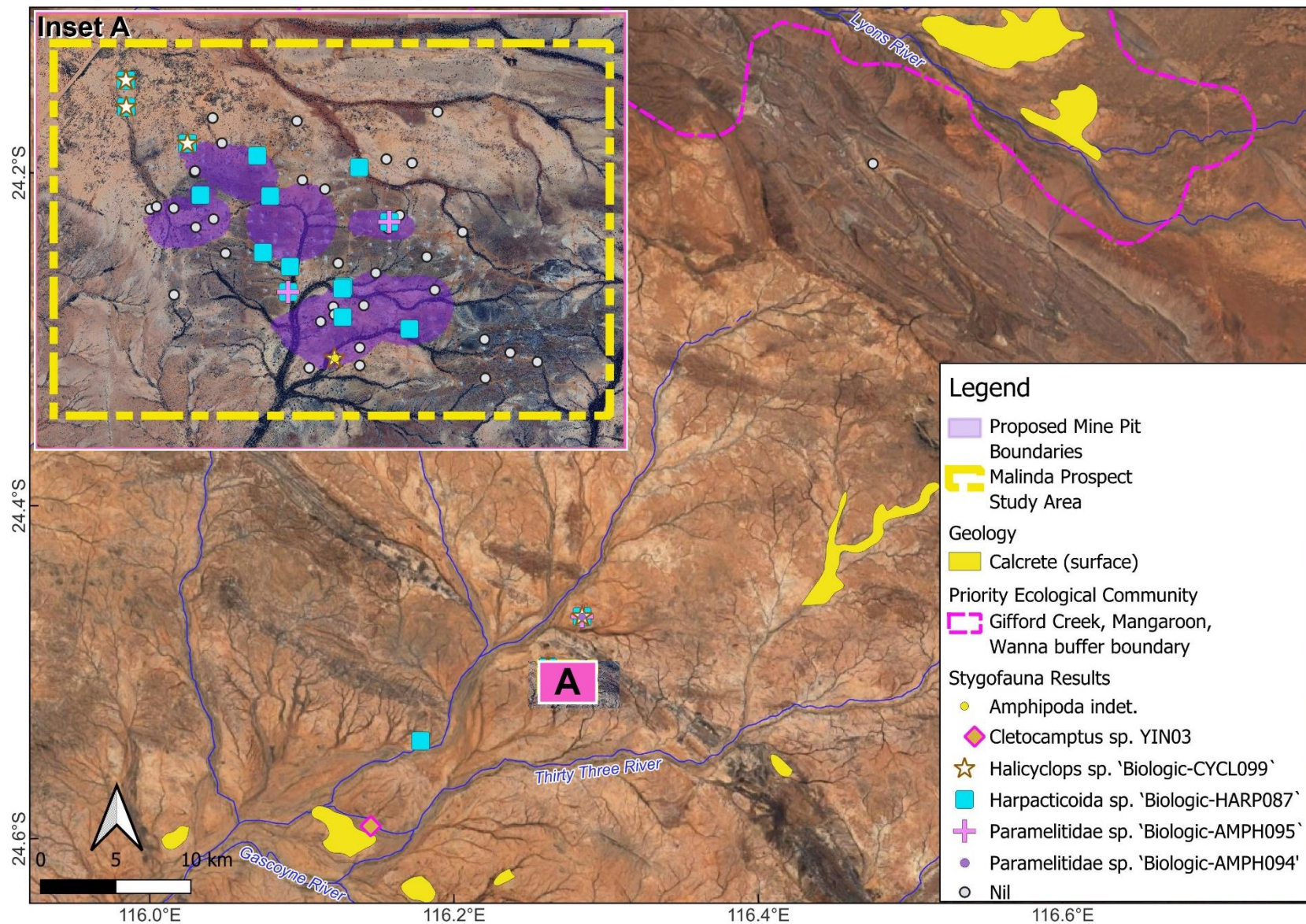


Figure 5-7: Distribution of stygofauna species recorded.

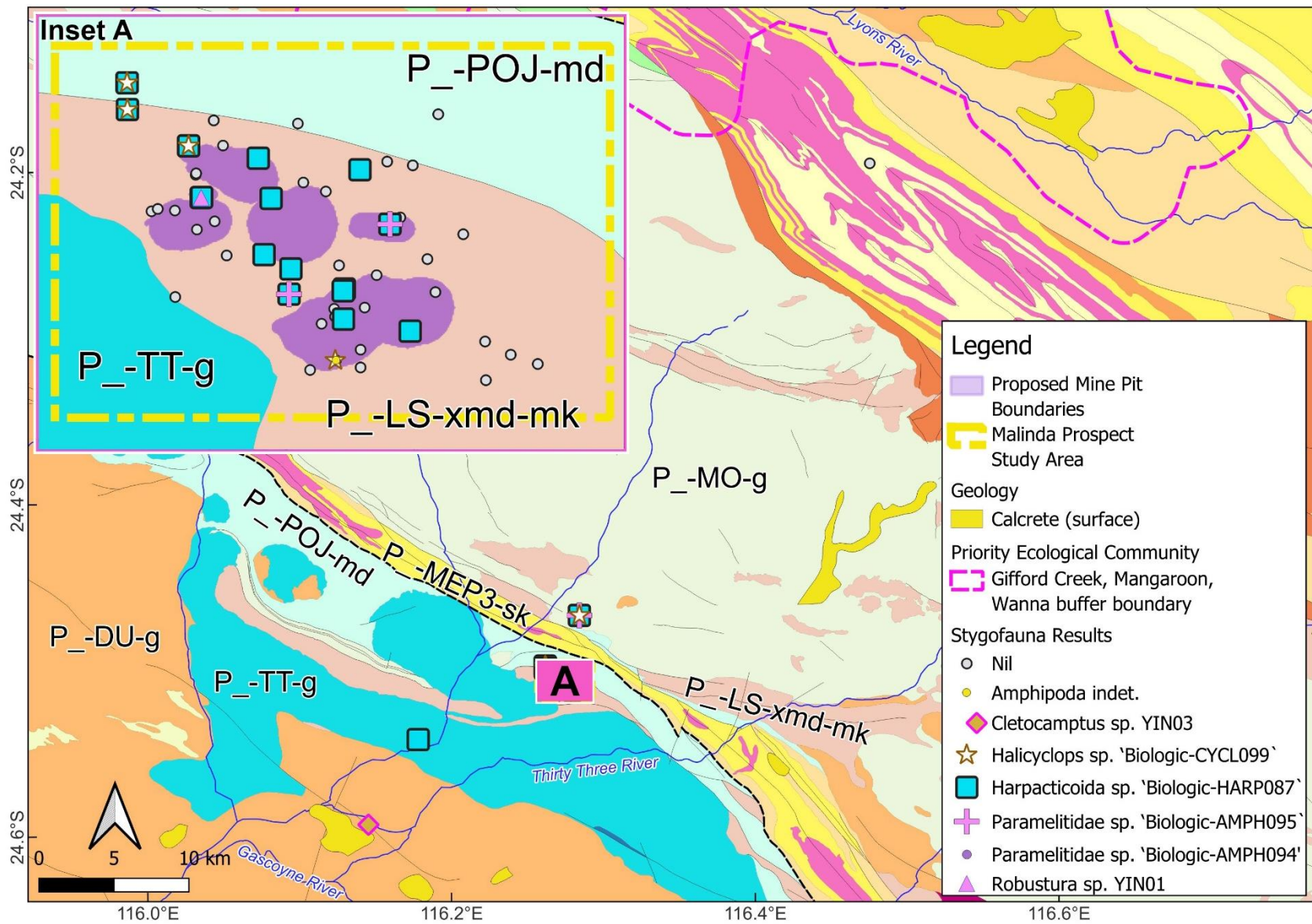


Figure 5-8: Distribution of stygofauna species recorded in relation to bedrock geology. Refer Table 4-1 for relevant geology code descriptions.

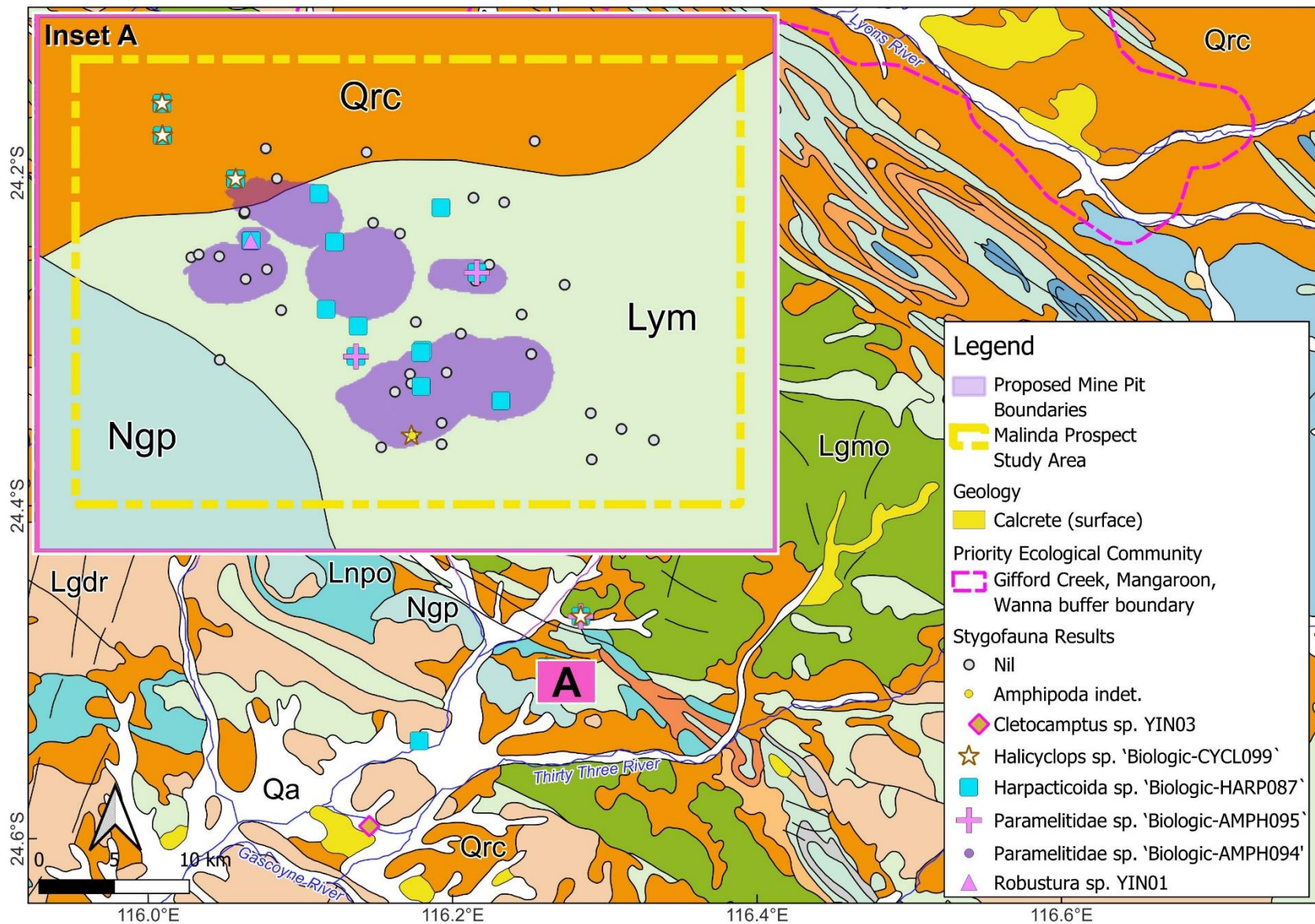


Figure 5-9: Distribution of stygofauna species recorded in relation to surface geology. Refer Table 4-2 for relevant geology code descriptions.

5.4.2 Troglifauna

Only three troglifauna specimens, representing two species from two higher level taxonomic groups (Diplura and Polyxenida), were collected from two of the 56 sites sampled across the three survey Phases (**Table 5-4, Figure 5-10, Figure 5-11, Figure 5-12, Appendix B**). The dipluran specimen was collected by a scrape sample and the polyxenid specimens were collected in the same litter trap sample. Both species were each recorded from a single sample only, collected from different sites within or near to the same proposed pit area.

Table 5-4: Troglifauna diversity recorded.

Taxon	Malinda		Regional	Comments
	Proposed Pit Areas	Outside Pit Areas		
Diplura	1			
Diplura indet.	1			Detected from single site only (YRRD139), located close to a proposed pit boundary (~10 m). Specimen in very poor condition, missing posterior abdominal segments and terminal antennae segments, so cannot be identified below order level & had no viable tissue for genetic analysis. Trichobothria and claw characteristics present indicate either Japydidae or Campodeidae species.
Polyxenida: Lophoproctidae				
<i>Lophoturus madecassus</i>	2			Detected from single site only (GASRC0003) located within proposed pit boundary. Is a globally widespread species, relatively commonly collected in subterranean fauna surveys in Pilbara & Yilgarn regions, & was recorded from Gifford Creek PEC. Not an SRE and not confined to Malinda study area.
Abundance	3			
Taxon Richness	2			

Diplura

All dipluran species have evolved to be largely unpigmented with no eye development and rely on highly specialised antennae for sensing their environment. Most species are known to be soil dwelling (edaphofauna), particularly in more mesic environments (Naumann 1991). Dipluran taxa are often recorded in subterranean fauna assessments from cavernous subterranean habitats (Subterranean Ecology 2010) as well as alluvial soil profiles (Outback Ecology 2011a). Not enough is known about the group to conclusively determine if specimens collected are true troglobites or if they are edaphofauna, particularly as all species are pale and blind. A dipluran taxonomist has considered that most diplurans collected in Western Australia are likely to be soil dwelling species (A. Sendra pers. comm. in Subterranean Ecology 2011).

An important factor for distinguishing between the two ecological niches relates to the differences in likely distribution range; i.e., true troglobites are more likely to have restricted distributions and be SREs compared to soil dwelling species that may also be a SRE but would be less likely to have as restricted a distribution as a troglobite. Dipluran specimens have been recorded from many subterranean fauna EIA surveys in the Pilbara and northern Yilgarn regions but typically in low numbers, often as single specimens (singletons) or from single sites only, making it difficult to assess their likely distribution range.

The median area of distribution of 15 Pilbara dipluran species (established on morphological assessments) collected from three or more sites was estimated to be around 16 km² (Halse and Pearson 2014). A later study recorded a linear distribution of 327 km for another Pilbara species, established on morphological (Bennelongia 2021).

A recent and large subterranean fauna study in the Hammersley region of the Pilbara, covering around 640 km² and amassing nearly 1,500 samples from 683 sites, recorded 20 dipluran species, 17 of which were determined by genetic analysis (Biologic 2022). The large majority of the species (16) were singletons, or from a single site only, so provided little in terms of assessing potential distribution range. Genetic results for the four species recorded from multiple sites established the linear distribution distances of 0.6 km, 2.4 km, 20 km, and 27 km (Biologic 2022).

For this assessment, the badly damaged diplura specimen is cautiously regarded as a potential troglofauna, because although it could likely be a soil dwelling species, this cannot be conclusively determined. Although the broader distribution extent of the indeterminate Diplura species has not been demonstrated, the distribution range is considered to be of a wider extent within the broader expanse of contiguous subterranean habitat present, and not confined to the immediate vicinity of YRRD139.

Polyxenida

Lophoturus madecassus is a widespread species with a circum-tropical distribution, occurring in Africa, the Caribbean, Florida, Pacific islands and Australia, with some suggesting the species may likely have been synanthropically dispersed (Car *et al.* 2013). The species is relatively commonly collected from subterranean fauna sampling across the Pilbara and Yilgarn regions with genetics data showing little sequence divergence between specimens from both regions (Outback Ecology 2011a, 2013). This species is considered a troglophile and is not an SRE.

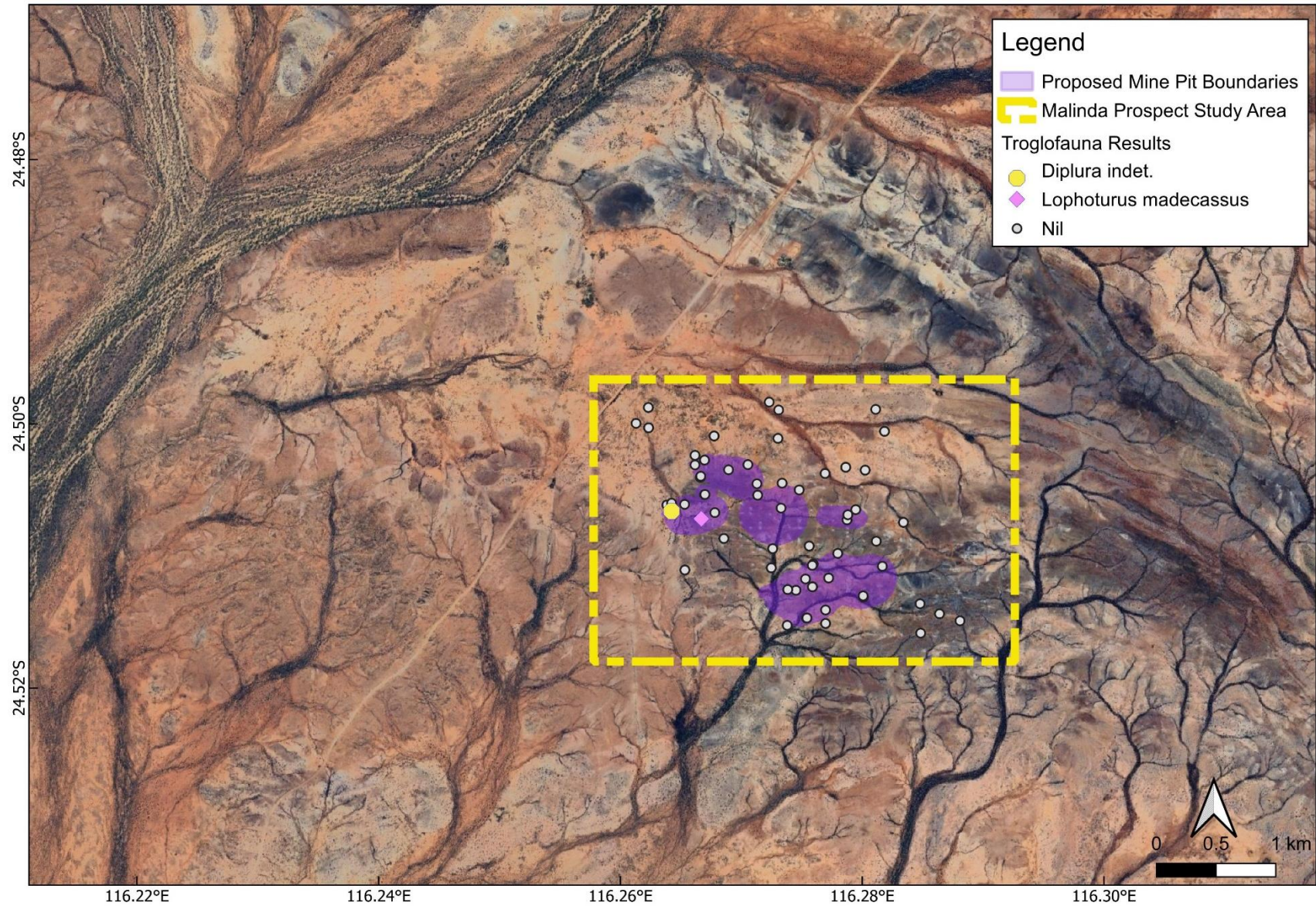


Figure 5-10: Distribution of troglofauna species recorded.

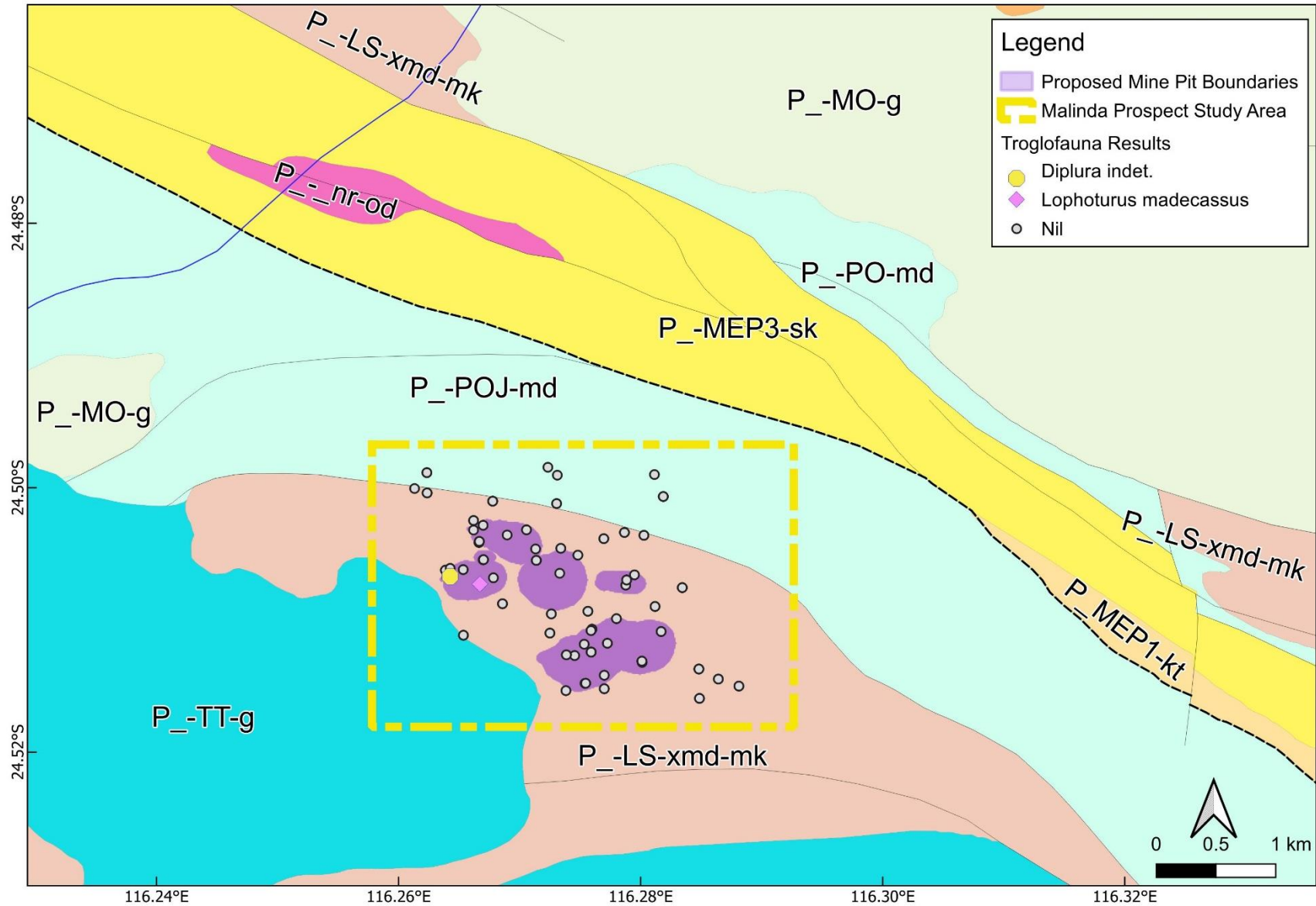


Figure 5-11: Distribution of troglofauna species recorded in relation to bedrock geology. Refer Table 4-1 for relevant geology code descriptions.

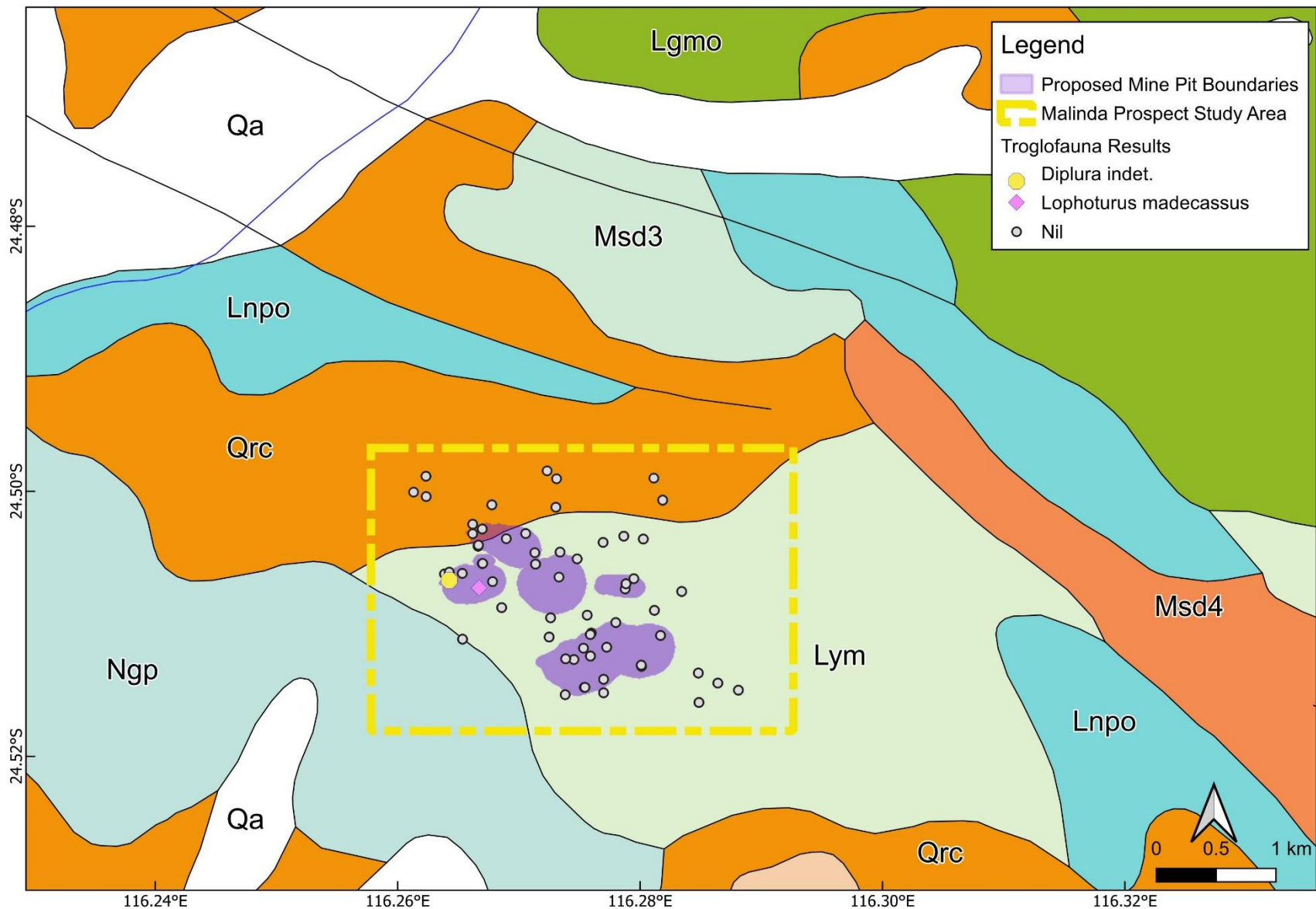


Figure 5-12: Distribution of troglofauna species recorded in relation to surface geology. Refer Table 4-2 for relevant geology code descriptions.

6 Discussion

Key factors influencing subterranean fauna diversity and distribution are the presence of extensive interconnected porosity within suitable geological and hydrogeological units that are in connection with adequate hydrological regimes to ensure pathways for the infiltration (vertical and/ or lateral) of resources such as oxygen and nutrients (Humphreys 2008, Sacco *et al.* 2019, Saccò *et al.* 2022, Strayer 1994). Within the Gascoyne bioregion, the palaeodrainage channel calcrete habitats, as well as associated alluvial and colluvial aquifer systems, are considered to host diverse stygofauna and troglifauna assemblages, however, these have not yet been as extensively studied as many Pilbara and Yilgarn subterranean fauna assemblages.

Studies of the Gifford Creek stygofauna PEC found that most of the recorded stygofauna diversity (abundance and species richness) occurred within the calcrete and associated surficial alluvial/ colluvial aquifer habitat. The occurrence of stygofauna from such environments is not unexpected as such habitats are known to provide optimal conditions that host diverse assemblages (refer Section 2.1). The associated fractured rock groundwater environments hosted a much lower diversity with only three of the recorded 62 stygofauna species collected from within granite fractured rock aquifer habitats, each of which were also present in the calcrete and associated alluvial/ colluvial aquifer systems.

The Project findings show that the more prospective, higher value stygofauna habitat in the region occurs along the main drainage channels within calcrete aquifers and associated alluvial/ colluvial aquifer environments. The target ore resource of the Malinda Prospect occurs in an elevated fractured rock aquifer system that is surrounded by more extensive surficial alluvial/ colluvial aquifers lower in the landscape that are associated with the larger drainage systems, which in areas host calcrete aquifer systems. The elevated fractured rock aquifer environment would provide less optimal habitat conditions for stygofauna, fringing the more optimal alluvial/ colluvial and calcrete aquifer habitats lower in the landscape.

It is considered unlikely that any stygofauna species are restricted to the fractured rock aquifer system present in the Malinda study area. Three of the four stygofauna species recorded from the fractured rock aquifer system within the Malinda study area were found to have distributions that extended to neighbouring regional sites that intercepted or were closer to the higher value alluvial/ colluvial aquifer habitats. These regional sites recorded a higher stygofauna species richness compared to Malinda from a much lower sampling intensity.

It is considered highly unlikely that any troglifauna species are restricted to the Malinda study area. The troglifauna values of the weathered and fractured rock habitat within Malinda were found to be very low, to potentially zero, considering the single potential troglifauna species collected could more likely be edaphofauna and not troglobitic. Unfortunately, there were no regional sites available that were suitable for troglifauna sampling so no comparison of diversity could be made.

7 Conclusion

The total sample effort completed, primarily from within the focussed exploration drilling area of the Malinda Prospect, along with the habitat assessment and subterranean fauna records from the broader region, is considered more than sufficient to provide a reliable characterisation of the subterranean fauna values present in the Malinda study area. The Project findings showed that the fractured rock habitat present in Malinda hosts low stygofauna values and very low, to potentially zero, troglifauna values. Higher valued stygofauna habitats are considered to occur in surficial alluvial/ colluvial aquifers associated with the main drainage channels in the neighbouring area as well as the broader region, particularly where larger calcrete bodies have formed.

8 Reference

- Aplin, K. (1998) Three new blindsnakes (Squamata: Typhlopidae) from northwestern Australia. *RECORDS-WESTERN AUSTRALIAN MUSEUM* 19: 1-12.
- Barranco, P. and Harvey, M. S. (2008) The first indigenous palpi-grade from Australia: a new species of *Eukoenia* (Palpi-gradi : Eukoeniidae). *Invertebrate Systematics* 22: 227-233.
- Bennelongia. (2009) *Yilgarn Iron Ore Project: Carina Deposit, Subterranean Fauna Assessment*. Report prepared for Polaris Metals NL, Western Australia.
- Bennelongia. (2015) *Yeelirrie Subterranean Fauna Assessment* Prepared for Cameco Australia, Perth, Western Australia.
- Bennelongia. (2016) *Subterranean Fauna Identifications for Hastings Rare Metals (Yangibana)* Letter report prepared for Ecoscape, included as Appendix 7 in Ecoscape (2016) *Yangibana Subterranean Fauna Assessment* Perth, Western Australia.
- Bennelongia. (2017) *Yangibana Project: Subterranean Fauna Assessment* Report prepared for Hastings Technology Metals Limited, Perth, Western Australia.
- Bennelongia. (2018) *Yangibana Rare Earths Project: Subterranean Fauna Assessment* Report prepared for Hastings Technology Metals Ltd, Perth, Western Australia.
- Bennelongia. (2021) *Western Ridge Subterranean Fauna Survey and Habitat Assessment* Report prepared for BHP Western Australian Iron Ore by Bennelongia Environmental Consultants, Perth, Western Australia.
- Biologic. (2022) *Greater Brockman: Subterranean Fauna Survey* Report prepared for Rio Tinto Iron Ore by Biologic Environmental Survey, Perth, Western Australia.
- Biologic. (2024) *Bestiolas Consulting Yinnetharra Subterranean Fauna Systematics Analysis* Report prepared for Bestiolas Consulting and Delta Lithium, Perth, Western Australia.
- Biota. (2006) *BHP Billiton Iron Ore Regional Subterranean Fauna Study: Research Programme Design* Biota Environmental Science Pty Ltd, Perth, WA.
- Bishop, R. E., Humphreys, W. and Jaume, D. (2020) Subterranean and anchialine waters. In: *Evolution and Biogeography: Volume 8*, p 331
- Boulton, A. J. (2000) The Subsurface Macrofauna. In: B. J. Jones and P. J. Mulholland (eds) *Streams and Ground Waters*. Academic Press, San Diego, pp 337-361
- Boulton, A. J., Findlay, S., Marmonier, P., Stanley, E. H. and Valett, H. M. (1998) The functional significance of the hyporheic zone in streams and rivers. *Annual Review of Ecology and Systematics* 29: 59-81.
- Bradford, T., Adams, M., Guzik, M. T., Humphreys, W. F., Austin, A. D. and Cooper, S. J. B. (2013) Patterns of population genetic variation in sympatric chiltoniid amphipods within a calcrete aquifer reveal a dynamic subterranean environment. *Heredity*: 1-9.
- Bureau of Meteorology. (2024) *Climate Data: Burringurrah Airstrip (#007210) and Gascoyne Junction (#006022)*. Bureau of Meteorology. Australian Government. Available online at <http://www.bom.gov.au>. Accessed on July, 2024.
- Car, C. A., Short, M., Huynh, C. and Harvey, M. S. (2013) The millipedes of Barrow Island, Western Australia (Diplopoda). *Records of the Western Australian museum: supplement 83: the terrestrial invertebrate fauna of Barrow Island* 83: 209-219.
- Chapman, D. and Kimstach, V. (1996) Selection of water quality variables. In: D. Chapman (ed) *Water Quality Assessments: A Guide to the Use of Biota, Sediments and Water in Environmental Monitoring*. E and FN Spon London, United Kingdom
- Coineau, N. and Albuquerque, E. (2001) Palaeobiogeography of the freshwater isopods Microcerberidae (Crustacea) from Caribbean and North America. In 13th International Congress of Speleology, 4th Speleological Congress of Latin America and Caribbean, 26th Brazilian Congress of Speleology. Brasilia, Brazil, July. City, pp 15-22
- Cooper, S. J. B., Bradbury, J. H., Saint, K. M., Leys, R., Austin, A. D. and Humphreys, W. F. (2007) Subterranean archipelago in the Australian arid zone: mitochondrial DNA phylogeography of amphipods from central Western Australia. *Molecular Ecology* 16: 1533-1544.
- Cooper, S. J. B., Hinze, S., Leys, R., Watts, C. H. S. and Humphreys, W. F. (2002) Islands under the desert: molecular systematics and evolutionary origins of stygobitic water beetles (Coleoptera: Dytiscidae) from central Western Australia. *Invertebrate Systematics* 16: 589-598.
- Cowan, M. (2001) Gascoyne 2 (GAS2- Carnegie subregion). In: J. May and N. McKenzie (eds) *A Biodiversity Audit of Western Australia's 53 Biogeographical Subregions in 2002*. Department of Conservation and Land Management, Kensington, Western Australia, pp 233-239
- Culver, D. C. and Sket, B. (2000) Hotspots of subterranean biodiversity in caves and wells. *Journal of Cave and Karst Studies* 62(1): 11-17.
- Danielopol, D. L. and Pospisil, P. (2000) Biodiversity in groundwater: a large-scale view. *TREE* 15: 223-224.
- DBCA (2022) *Threatened and Priority Fauna Database Search for Mt Ida Lithium-Copper-Gold Project*. Department of Biodiversity, Conservation, and Attractions. Available online at

<https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities/threatened-animals>.

- DBCAs (2023a) *Conservation Category Definitions for Western Australian Ecological Communities*. Available online at.
- DBCAs (2023b) *Priority Ecological Communities for Western Australia (Version 35)*. Available online at <https://www.dbca.wa.gov.au/wildlife-and-ecosystems/threatened-ecological-communities>.
- DBCAs (2023c) *Threatened and Priority Ecological Communities Database Search for Yinnetharra Lithium Project*. Available online at <https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities/wa-s-threatened-ecological-communities>.
- DCCEEW. (2024) *Australia's Bioregions (IBRA)*. Australian Government, Department of Climate Change, Energy, the Environment and Water. Available online at <https://www.dcceew.gov.au/environment/land/nrs/science/ibra>. Accessed on July, 2024.
- Delta Lithium (2024) *ASX release: Drilling update from Yinnetharra*. Available online at.
- Desmond, A., Kendrick, P. and Chant, A. (2001) Gascoyne 3 (GAS3 - Augustus subregion). In: J. May and N. McKenzie (eds) *A Biodiversity Audit of Western Australia's 53 Biogeographical Subregions in 2002*. Department of Conservation and Land Management, Kensington, Western Australia, pp 240-252
- Eberhard, S. M., Halse, S. A. and Humphreys, W. F. (2005) Stygofauna in the Pilbara region, north-west Western Australia: a review. *Journal of the Royal Society of Western Australia* 88: 167-176.
- Ecoscope. (2016) *Yangibana Project Biological Assessment: Subterranean Fauna Report* prepared for Hastings Technology Metals Limited, Perth, Western Australia.
- EPA (2016) *Environmental Factor Guideline -Subterranean Fauna*. Guideline prepared by the Environmental Protection Authority of Western Australia. Available online at.
- EPA. (2021a) *Statement of environmental principles, factors, objectives and aims of EIA Statement* prepared by the Environmental Protection Authority of Western Australia.
- EPA. (2021b) *Technical Guidance - Subterranean Fauna Surveys for Environmental Impact Assessment* Technical Guidance prepared by the Environmental Protection Authority of Western Australia, Perth, Western Australia.
- Framenau, V. W., McMains, C. and Campos, M. (2021) *Subterranean Fauna Survey Review Project - Optimising Species Detection* Report prepared by Murdoch University for the Western Australian Biodiversity Science Institute (WABSI), Perth Western Australia.
- GDC. (2023) *Gascoyne Development Commission Annual Report 2022-23* Government of Western Australia, Gascoyne Development Commission, Carnarvon, Western Australia.
- Geological Survey of Western Australia (2020) *1:500 000 State interpreted bedrock geology of Western Australia*. Available online at www.dmp.wa.gov.au/datacentre.
- Geological Survey of Western Australia (2022) *1:500 000 tectonic units of Western Australia*, Geological Survey of Western Australia. Available online at.
- Gorički, Š., Niemiller, M. L., Fenolio, D. B. and Gluesenkamp, A. G. (2019) Salamanders. In: *Encyclopedia of caves*. Elsevier, pp 871-884
- Guzik, M. T., Austin, A. D., Cooper, S. J. B., Harvey, M. S., Humphreys, W. F., Bradford, T., Eberhard, S. M., King, R. A., Leys, R., Muirhead, K. A. and Tomlinson, M. (2010) Is the Australian subterranean fauna uniquely diverse? *Invertebrate Systematics* 24: 407-418.
- Guzik, M. T., Cooper, S. J. B., Humphreys, W. F., Ong, S., Kawakami, T. and Austin, A. D. (2011) Evidence for population fragmentation within a subterranean aquatic habitat in the Western Australian desert. *Heredity*: 1-16.
- Hahn, H. J. (2006) The GW-Fauna-Index: A first approach to a quantitative ecological assessment of groundwater habitats. *Limnologica* 36(2): 119-137.
- Halse, S. and Pearson, G. B. (2014) Troglifauna in the vadose zone: comparison of scraping and trapping results and sampling adequacy. *Subterranean Biology* 13: 17-34.
- Halse, S. A., Scanlon, M. D. and Cocking, J. S. (2002) *Do springs provide a window to the groundwater fauna of the Australian arid zone?* Department of Conservation and Land Management, Perth.
- Halse, S. A., Scanlon, M. D., Cocking, J. S., Barron, H. J., Richardson, J. B. and Eberhard, S. (2014) Pilbara stygofauna: deep groundwater of an arid landscape contains globally significant radiation of biodiversity. *Records of the Western Australian Museum. Supplement* 78: 443-483.
- Hamilton-Smith, E. and Eberhard, S. (2000) The diversity of the karstic and pseudokarstic hypogean habitats in the world. In: H. Wilkens, D. C. Culver and W. F. Humphreys (eds) *Subterranean Ecosystems*. Elsevier, Amsterdam, The Netherlands, pp 647-664
- Harrison, S. E., Guzik, M. T., Harvey, M. S. and Austin, A. D. (2014) Molecular phylogenetic analysis of Western Australian troglobitic chthoniid pseudoscorpions (Pseudoscorpiones : Chthoniidae) points to multiple independent subterranean clades. *Invertebrate Systematics* 28: 386-400.
- Harvey, M. E., Rix, M. G., Volker, W. F., Hamilton, Z. R., Johnson, M. S., Teale, R. J., Humphreys, G. and Humphreys, W. F. (2011) Protecting the innocent: studying short-range endemic taxa enhances conservation outcomes. *Invertebrate Systematics* 25: 1-10.

- Harvey, M. S. (2002) Short-range endemism among the Australian fauna: some examples from non-marine environments. *Invertebrate Systematics* 16: 555-570.
- Humphreys, G., Alexander, J., Harvey, M. S. and Humphreys, W. F. (2013) The subterranean fauna of Barrow Island, north-western Australia: 10 years on. *Records of the Western Australian Museum* 83(145-158).
- Humphreys, W. F. (1991) Experimental re-establishment of pulse-driven populations in a terrestrial troglobite community. *Journal of Animal Ecology* 60: 609-623.
- Humphreys, W. F. (2000a) The hypogean fauna of the Cape Range Peninsula and Barrow Island, northwestern Australia. In: H. Wilkens, D. C. Culver and W. F. Humphreys (eds) *Subterranean Ecosystems*. Elsevier, Amsterdam, The Netherlands, pp 581-602
- Humphreys, W. F. (2000b) Relict faunas and their derivation. In: H. Wilkens, D. C. Culver and W. F. Humphreys (eds) *Subterranean Ecosystems*. Elsevier, Amsterdam, The Netherlands, pp 417-432
- Humphreys, W. F. (2006) Aquifers: the ultimate groundwater-dependent ecosystems. *Australian Journal of Botany* 54: 115-132.
- Humphreys, W. F. (2008) Rising from Down Under: developments in subterranean biodiversity in Australia from a groundwater fauna perspective. *Invertebrate Systematics* 22: 85-101.
- Humphreys, W. F. (2009) Hydrogeology and groundwater ecology: Does each inform the other? *Hydrogeology Journal* 17(1): 5-21.
- Humphreys, W. F. (2017) Australasian subterranean biogeography. In: *Handbook of Australasian biogeography*. CRC Press, pp 295-320
- Humphreys, W. F. (2019) Biodiversity patterns in Australia. In: *Encyclopedia of caves*. Elsevier, pp 109-126
- Hutchins, B. T., Schwartz, B. F. and Coleman, W. T. (2021) Three new microcerberids (Isopoda: Microcerberidae) from subterranean freshwater habitats in Texas, USA. *Journal of Natural History* 55(35-36): 2261-2278.
- Johnson, S., Sheppard, S., Rasmussen, B., Wingate, M., Kirkland, C., Muhling, J., Fletcher, I. and Belousova, E. (2011) Two collisions, two sutures: punctuated pre-1950 Ma assembly of the West Australian Craton during the Ophthalmian and Glenburgh Orogenies. *Precambrian Research* 189(3-4): 239-262.
- Johnson, S. P., Sheppard, S., Groenewald, P. B. and Farrell, T. R. (2012) *Yinnetharra, WA Sheet 2148*. Geological Survey of Western Australia. Available online at.
- Juberthie, C. (2000) The diversity of the karstic and pseudokarstic hypogean habitats in the world. In: H. Wilkens, D. C. Culver and W. F. Humphreys (eds) *Subterranean Ecosystems*. Elsevier, Amsterdam, The Netherlands, pp 17-40
- Jurado-Rivera, J. A., Pons, J., Alvarez, F., Botello, A., Humphreys, W. F., Page, T. J., Iliffe, T. M., Willassen, E., Meland, K. and Juan, C. (2017) Phylogenetic evidence that both ancient vicariance and dispersal have contributed to the biogeographic patterns of anchialine cave shrimps. *Scientific Reports* 7(1): 1-11.
- Karanovic, T. (2006) Subterranean copepods (Crustacea, Copepoda) from the Pilbara region in Western Australia. *Records of the Western Australian Museum Supplement* 70.
- Keable, S. J. and Wilson, G. D. (2006) New species of *Pygolabis* Wilson, 2003 (Isopoda, Tainisopidae, Crustacea) from Western Australia. *Zootaxa* 1116(1): 1-27.
- King, R. A., Fagan-Jeffries, E. P., Bradford, T. M., Stringer, D. N., Finston, T. L., Halse, S. A., Eberhard, S. M., Humphreys, G., Humphreys, B. F. and Austin, A. D. (2022) Cryptic diversity down under: defining species in the subterranean amphipod genus *Nedsia* Barnard & Williams, 1995 (Hadzioidea: Eriopisidae) from the Pilbara, Western Australia. *Invertebrate Systematics* 36(2): 113-159.
- Korbel, K. L. and Hose, G. C. (2015) Habitat, water quality, seasonality, or site? Identifying environmental correlates of the distribution of groundwater biota. *Freshwater Science* 34(1): 329-343.
- Korbel, K. L., Stephenson, S. and Hose, G. C. (2019) Sediment size influences habitat selection and use by groundwater macrofauna and meiofauna. *Aquatic sciences* 81(2): 1-10.
- Larson, H. K., Foster, R., Humphreys, W. F. and Stevens, M. I. (2013) A new species of the blind cave gudgeon *Milyeringa* (Pisces: Gobioidae, Eleotridae) from Barrow Island, Western Australia, with a redescription of *M. veritas* Whitley. *Zootaxa* 3616(2): 135-150.
- Malard, F. and Hervant, F. (1999) Oxygen supply and the adaptations of animals in groundwater. *Freshwater Biology* 41: 1-30.
- Moore, G. I., Humphrey, W. F. and Foster, R. (2018) New populations of the rare subterranean blind cave eel *Ophisternon candidum* (Synbranchidae) reveal recent historical connections throughout north-western Australia. *Marine and Freshwater Research* 69: 1517-1524.
- MWH. (2015) *Wiluna Uranium Project: Millipede Targeted Subterranean Fauna Assessment Report* prepared for Toro Energy Ltd.
- MWH. (2016a) *Mount Keith Satellite Operations Subterranean Fauna Assessment Report* prepared for BHP Billiton Nickel West.
- MWH. (2016b) *Salinity Tolerance of Ethel Gorge Stygofauna TEC Report* prepared for BHP Billiton Iron Ore.

- Naumann, I. D. (ed) (1991). *The Insects of Australia. A textbook for students and research workers* (Second edn). Melbourne University Press, Carlton.
- Outback Ecology. (2011a) *Wiluna Uranium Project Subterranean Fauna Assessment, March 2011*. Prepared for Toro Energy Ltd, Perth, Western Australia.
- Outback Ecology. (2011b) *Wingellina Nickel Project Subterranean Fauna Assessment*. Prepared for Metals X Ltd, Perth, Western Australia.
- Outback Ecology. (2012a) *BHP Billiton Nickel West NDS1 Project: Lake Way Borefield Subterranean Fauna Assessment* Prepared for BHP Billiton Nickel West, Perth, Western Australia.
- Outback Ecology. (2012b) *Lake Maitland Uranium Project Level 2 Stygofauna Assessment* Prepared for Mega Lake Maitland Pty Ltd, Perth, Western Australia.
- Outback Ecology. (2012c) *Lake Maitland Uranium Project Level 2 Troglifauna Assessment* Prepared for Mega Lake Maitland Pty Ltd, Perth, Western Australia.
- Outback Ecology. (2013) *Murchison Goldfield: Moyagee Project. Subterranean Fauna Assessment*. Report prepared for Silver Lake Resources, Western Australia.
- Outback Ecology. (2014) *Browns Range Project Subterranean Fauna Assessment* Report prepared for Northern Minerals Ltd, Perth, Western Australia.
- Page, T. J., Humphreys, W. F. and Hughes, J. M. (2008) Shrimps down under: evolutionary relationships of subterranean crustaceans from Western Australia (Decapoda: Atyidae: Stygiocarid). *PLoS One* 3(2): e618.
- Page, T. J., Stevens, M. I., Adams, M., Foster, R., Velasco-Castrillón, A. and Humphreys, W. F. (2018) Multiple molecular markers reinforce the systematic framework of unique Australian cave fishes (Milyeringa: Gobioidae). *Australian Journal of Zoology* 66(2): 115-127.
- Phelps-Barber, Z., Trench, A. and Groves, D. I. (2022) Recent pegmatite-hosted spodumene discoveries in Western Australia: insights for lithium exploration in Australia and globally. *Applied Earth Science* 131(2): 100-113.
- Pinder, A. M., Halse, S. A., McRae, J. M. and Shiel, R. J. (2005) Occurrence of aquatic invertebrates of the wheatbelt region of Western Australia in relation to salinity. *Hydrobiologia* 543: 1-24.
- Pinder, A. M., Halse, S. A., Shiel, R. J., Cale, D. J. and McRae, J. M. (2002) Halophile aquatic invertebrates in the wheatbelt region of south-western Australia. *Verh. Internat. Verein. Limnol.* 28: 1-8.
- Platnick, N. I. (2008) A new subterranean ground spider genus from Western Australia (Araneae: Trochanteriidae) *Invertebrate Systematics* 22: 295-299.
- Poore, G. C. and Humphreys, W. F. (1992) First record of Thermosbaenacea (Crustacea) from the Southern Hemisphere: A new species from a cave in tropical Western Australia. *Invertebrate Taxonomy* 6: 719 - 725.
- Raymond, O. L., Liu, S., Gallagher, R., Highet, L. M. and Zhang, W. (2012) *Surface Geology of Australia, 1:1 000 000 scale*. Available online at <http://www.ga.gov.au>.
- Rockwater. (2023) *Yinnetharra Lithium Project Water Supply Bore Completion Report* Report prepared for Delta Lithium Limited, Perth, Western Australia.
- Sacco, M., Blyth, A. J., Humphreys, W. F., Kuhl, A., Mazumder, D., Smith, C. and Grice, K. (2019) Elucidating stygofaunal trophic web interactions via isotopic ecology. *PLoS One* 16: 1-25.
- Saccò, M., Campbell, M. A., Nevill, P., Humphreys, W. F., Blyth, A. J., Grierson, P. F. and White, N. E. (2022) Getting to the Root of Organic Inputs in Groundwaters: Stygofaunal Plant Consumption in a Calcrete Aquifer. *Frontiers in Ecology and Evolution* 10.
- Schmidt, S. I., Hahn, H. J., Hatton, T. J. and Humphreys, W. F. (2007) Do faunal assemblages reflect the exchange intensity in groundwater zones? *Hydrobiologia* 583: 1-19.
- Sheppard, S., Bodorkos, S., Johnson, S., Wingate, M. and Kirkland, C. (2010) The Paleoproterozoic Capricorn Orogeny: intracontinental reworking not continent-continent collision. *Geological Survey of Western Australia, Report* 108: 33.
- Sheppard, S., Farrell, T. R., Martin, D. M., Thorne, A. M. and Bagas, L. (2008) *Mount Phillips, WA, Sheet 2149*. Geological Survey of Western Australia. Available online at.
- Smith, R. J., Paterson, J. S., Launer, E., Tobe, S. S., Morello, E., Leijts, R., Marri, S. and Mitchell, J. G. (2016) Stygofauna enhance prokaryotic transport in groundwater ecosystems. *Scientific reports* 6(1): 32738.
- Stantec. (2017) *Ethel Gorge Stygofauna Monitoring Program: 2017 Report* prepared for BHP Iron Ore, Perth, Western Australia.
- Stantec. (2018a) *Abra Subterranean Fauna Level 2 Assessment Report* prepared for Galena Minerals, Perth, Western Australia.
- Stantec. (2018b) *Camelot Subterranean Fauna Level 2 Assessment Report* prepared for BHP Nickel West, Perth, Western Australia.
- Strayer, D. L. (1994) Limits to biological distributions in groundwater. In: J. Gibert, D. L. Danielopol and J. A. Stanford (eds) *Groundwater Ecology*. Academic Press, San Diego, pp 287-310
- Subterranean Ecology. (2008) *Goldsworthy Iron ore Mining Operations Cundaline and Callawa mining Operations Troglifauna Assessment Report* prepared for BHPBIO.

- Subterranean Ecology. (2010) *Fortescue Metals Group Solomon Project: Kings Deposits Subterranean Fauna Survey & Assessment Report* prepared for Fortescue Metal Group, Perth, Western Australia.
- Subterranean Ecology. (2011) *Yeelirrie Subterranean Fauna Survey* Prepared for BHP Billiton Yeelirrie Development Company Pty Ltd, Perth, Western Australia.
- Subterranean Ecology. (2013) *Ethel Gorge Aquifer Threatened Ecological Community Consolidated Taxonomy Report* prepared for BHP, Perth, Western Australia.
- Voltaic Strategic Resources (2024) *ASX Release: High Priority targets identified at Ti Tree (North)*. Available online at.
- Wägele, J. W. (1983) On the origin of the Microcerberidae (Crustacea: Isopoda). *Journal of Zoological Systematics and Evolutionary Research* 21 (4): 249-262.
- Wägele, J. W., N.J. V. and McArthur, V. (1995) Older than the Atlantic Ocean: Discovery of a fresh-water Microcerberus (Isopoda) in North America and erection of Coxicerberus, New Genus. *Journal of Crustacean Biology* 15(4): 733-745.
- White, N. E., Guzik, M. T., Austin, A. D., Moore, G. I., Humphreys, W. F., Alexander, J. and Bunce, M. (2020) Detection of the rare Australian endemic blind cave eel (*Ophisternon candidum*) with environmental DNA: implications for threatened species management in subterranean environments. *Hydrobiologia*.
- Yager, J. and Humphreys, W. (1996) *Lasionectes exleyi*, sp. nov., the first remipede crustacean recorded from Australia and the Indian Ocean, with a key to the world species. *Invertebrate Systematics* 10(1): 171-187.

APPENDICES

Appendix A: Yinnetharra Subterranean Fauna Site Details, Sample Effort, & Groundwater Details.

Table A-1: Stygofauna and troglifauna sample site details, sample effort, and recorded groundwater properties.

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	GASRC0003	11/06/2023		Haul Net	60	330	21.74	27.2	7.3	1.13	-211.3	6964	-24.5075	116.2669
MALINDA	GASRC0003	11/06/2023	2/08/2023	Litter Trap	60	330	21.74						-24.5075	116.2669
MALINDA	GASRC0003	11/06/2023		Scrape	60	330	21.74						-24.5075	116.2669
MALINDA	GASRC0003	12/11/2023	11/01/2024	Litter Trap	66	330							-24.5075	116.2668
MALINDA	GASRC0003	8/03/2024	Not Retrieved	Litter Trap	66	330							-24.5075	116.2668
MALINDA	GASRC0005	11/06/2023		Haul Net	60	328	19.07	28.8	7.13	1.29	160.5	22979	-24.5043	116.2668
MALINDA	GASRC0005	11/06/2023	2/08/2023	Litter Trap	60	328	19.07						-24.5043	116.2668
MALINDA	GASRC0005	11/06/2023		Scrape	60	328	19.07						-24.5043	116.2668
MALINDA	GASRC0005	12/11/2023		Haul Net	65	328	20.14	31.1	7.03	5.58	187.4	24897	-24.5042	116.2668
MALINDA	GASRC0005	12/11/2023	11/01/2024	Litter Trap	65	328							-24.5042	116.2668
MALINDA	GASRC0005	12/11/2023		Scrape	65	328	20.14						-24.5042	116.2668
MALINDA	GASRC0005	8/03/2024		Haul Net	65	328		32	7.25	0.56	-111.3	24747	-24.5042	116.2668
MALINDA	GASRC0005	8/03/2024	6/05/2024	Litter Trap	65	328							-24.5042	116.2668
MALINDA	GASRC0005	8/03/2024		Scrape	65	328							-24.5042	116.2668
MALINDA	GASRC0008	10/06/2023		Haul Net	60	331	23.95	29.3	7.13	1.56	-92.8	12180	-24.5070	116.2680
MALINDA	GASRC0008	10/06/2023	2/08/2023	Litter Trap	60	331	23.95						-24.5070	116.2680
MALINDA	GASRC0008	10/06/2023		Scrape	60	331	23.95						-24.5070	116.2680
MALINDA	GASRC0016	9/06/2023		Haul Net	60	325	16.94	29.3	7.3	2.69	181.1	14469	-24.5127	116.2760
MALINDA	GASRC0016	9/06/2023	2/08/2023	Litter Trap	60	325	16.94						-24.5127	116.2760
MALINDA	GASRC0016	9/06/2023		Scrape	60	325	16.94						-24.5127	116.2760
MALINDA	GASRC0016	11/11/2023		Haul Net	75	325	19.58	30.4	7.2	1.63	163.2	16892	-24.5127	116.2760
MALINDA	GASRC0016	11/11/2023	11/01/2024	Litter Trap	75	325	19.58						-24.5127	116.2760
MALINDA	GASRC0016	11/11/2023		Scrape	75	325	19.58						-24.5127	116.2760

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	GASRC0016	9/03/2024		Haul Net	75	325		29.6	7.41	1.91	140.7	16234	-24.5127	116.2760
MALINDA	GASRC0016	9/03/2024	6/05/2024	Litter Trap	75	325							-24.5127	116.2760
MALINDA	GASRC0016	9/03/2024		Scrape	75	325							-24.5127	116.2760
MALINDA	MARC008	10/06/2023		Haul Net	60	323	16.05	27.8	7.14	2.04	148.9	10618	-24.5121	116.2754
MALINDA	MARC008	10/06/2023	2/08/2023	Litter Trap	60	323	16.05						-24.5121	116.2754
MALINDA	MARC008	10/06/2023		Scrape	60	323	16.05						-24.5121	116.2754
MALINDA	MARC011	10/06/2023		Haul Net	60	321	14.13	27.1	7.37	1.59	131.3	18262	-24.5129	116.2747
MALINDA	MARC011	10/06/2023	2/08/2023	Litter Trap	60	321	14.13						-24.5129	116.2747
MALINDA	MARC011	10/06/2023		Scrape	60	321	14.13						-24.5129	116.2747
MALINDA	MARC012	10/06/2023		Haul Net	60	323	14.86	28.9	7.22	2.23	46.3	6224	-24.5109	116.2761
MALINDA	MARC012	10/06/2023	2/08/2023	Litter Trap	60	323	14.86						-24.5109	116.2761
MALINDA	MARC012	10/06/2023		Scrape	60	323	14.86						-24.5109	116.2761
MALINDA	MARC012	12/11/2023		Haul Net	75	323	21.25	29	7.38	2.13	100.2	7380	-24.5110	116.2760
MALINDA	MARC012	12/11/2023	11/01/2024	Litter Trap	75	323							-24.5110	116.2760
MALINDA	MARC012	12/11/2023		Scrape	75	323	21.25						-24.5110	116.2760
MALINDA	MARC012	9/03/2024		Haul Net	75	323		29.8	7.25	1.41	126.6	11423	-24.5110	116.2760
MALINDA	MARC012	9/03/2024	6/05/2024	Litter Trap	75	323							-24.5110	116.2760
MALINDA	MARC012	9/03/2024		Scrape	75	323							-24.5110	116.2760
MALINDA	MARC013	10/06/2023		Haul Net	60	333	25.33	28.8	7.19	1.78	25.2	18520	-24.5068	116.2796
MALINDA	MARC013	10/06/2023	2/08/2023	Litter Trap	60	333	25.33						-24.5068	116.2796
MALINDA	MARC013	10/06/2023		Scrape	60	333	25.33						-24.5068	116.2796
MALINDA	MARC014	10/06/2023		Haul Net	60	328	18.01	29.9	7.17	1.58	154.6	10245	-24.5057	116.2715
MALINDA	MARC014	10/06/2023	2/08/2023	Litter Trap	60	328	18.01						-24.5057	116.2715
MALINDA	MARC014	10/06/2023		Scrape	60	328	18.01						-24.5057	116.2715
MALINDA	MARC014	12/11/2023		Haul Net	75	328	20.92	30.6	7.23	1.85	-21	21796	-24.5057	116.2715
MALINDA	MARC014	12/11/2023	11/01/2024	Litter Trap	75	328							-24.5057	116.2715
MALINDA	MARC014	12/11/2023		Scrape	75	328	20.92						-24.5057	116.2715
MALINDA	MARC014	9/03/2024		Haul Net	75	328		32	7.37	0.74	-14.2	20349	-24.5057	116.2715
MALINDA	MARC014	9/03/2024	6/05/2024	Litter Trap	75	328							-24.5057	116.2715

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	MARC014	9/03/2024		Scrape	75	328							-24.5057	116.2715
MALINDA	MARC017	11/06/2023		Haul Net	60	324	15.16	27.7	7.31	3.69	120.4	1309	-24.5012	116.2679
MALINDA	MARC017	11/06/2023	2/08/2023	Litter Trap	60	324	15.16						-24.5012	116.2679
MALINDA	MARC017	11/06/2023		Scrape	60	324	15.16						-24.5012	116.2679
MALINDA	MARC017	14/11/2023		Haul Net	75	324	22.22	30	7.17	2.1	138.4	22209	-24.5012	116.2679
MALINDA	MARC017	14/11/2023	11/01/2024	Litter Trap	75	324							-24.5012	116.2679
MALINDA	MARC017	14/11/2023		Scrape	75	324	22.22						-24.5012	116.2679
MALINDA	MARC017	8/03/2024		Haul Net	75	324		30.6	7.21	1.34	115.1	22205	-24.5012	116.2679
MALINDA	MARC017	8/03/2024	6/05/2024	Litter Trap	75	324							-24.5012	116.2679
MALINDA	MARC017	8/03/2024		Scrape	75	324							-24.5012	116.2679
MALINDA	YDRD007	14/11/2023		Haul Net	65	326		28.8	7.63	0.72	-74.2	20396	-24.5053	116.2750
MALINDA	YDRD007	14/11/2023	11/01/2024	Litter Trap	65	326							-24.5053	116.2750
MALINDA	YDRD007	14/11/2023		Scrape	65	326							-24.5053	116.2750
MALINDA	YDRD010	9/11/2023		Haul Net	75	320	18.33	0	7.3	0.27	100.7	20061	-24.5125	116.2755
MALINDA	YDRD010	9/11/2023	Not Retrieved	Litter Trap	75	320	18.33						-24.5125	116.2755
MALINDA	YDRD010	9/11/2023		Scrape	75	320	18.33						-24.5125	116.2755
MALINDA	YDRD010	9/03/2024		Haul Net	75	320		29.1	7.29	3.68	138.3	19178	-24.5125	116.2755
MALINDA	YDRD010	9/03/2024	1/07/2024	Litter Trap	75	320							-24.5125	116.2755
MALINDA	YDRD010	9/03/2024		Scrape	75	320							-24.5125	116.2755
MALINDA	YNRD017	11/06/2023		Haul Net	54.76	327	18.82	28.1	7.15	1.49	82.8	27856	-24.5064	116.2655
MALINDA	YNRD017	11/06/2023	2/08/2023	Litter Trap	54.76	327	18.82						-24.5064	116.2655
MALINDA	YNRD017	11/06/2023		Scrape	54.76	327	18.82						-24.5064	116.2655
MALINDA	YNRD023	11/06/2023		Haul Net	55.44	326	18.45	28.4	6.95	1.11	74.4	29482	-24.5064	116.2640
MALINDA	YNRD023	11/06/2023	2/08/2023	Litter Trap	55.44	326	18.45						-24.5064	116.2640
MALINDA	YNRD023	11/06/2023		Scrape	55.44	326	18.45						-24.5064	116.2640
MALINDA	YNRD024	10/06/2023		Haul Net	56.12	333	27.48	25.1	7.18	1.72	133.2	11367	-24.5076	116.2789
MALINDA	YNRD024	10/06/2023	2/08/2023	Litter Trap	56.12	333	27.48						-24.5076	116.2789
MALINDA	YNRD024	10/06/2023		Scrape	56.12	333	27.48						-24.5076	116.2789

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	YREX032	10/11/2023		Haul Net	70	334	27.47	28.5	7.19	0.96	83	10634	-24.5078	116.2836
MALINDA	YREX032	10/11/2023	11/01/2024	Litter Trap	70	334	27.47						-24.5078	116.2836
MALINDA	YREX032	10/11/2023		Scrape	70	334	27.47						-24.5078	116.2836
MALINDA	YREX032	9/03/2024		Haul Net	70	334		30.7	7.31	5.09	65.5	10037	-24.5078	116.2836
MALINDA	YREX032	9/03/2024	6/05/2024	Litter Trap	70	334							-24.5078	116.2836
MALINDA	YREX032	9/03/2024		Scrape	70	334							-24.5078	116.2836
MALINDA	YRRD002	9/06/2023		Haul Net	54.06	325	17.97	29	7.14	0.52	-43.2	6921	-24.5120	116.2774
MALINDA	YRRD002	9/06/2023	2/08/2023	Litter Trap	54.06	325	17.97						-24.5120	116.2774
MALINDA	YRRD002	9/06/2023		Scrape	54.06	325	17.97						-24.5120	116.2774
MALINDA	YRRD014	9/06/2023		Haul Net	55.77	318	11.24	28.1	7.46	1.63	105.7	7185	-24.5156	116.2739
MALINDA	YRRD014	9/06/2023	2/08/2023	Litter Trap	55.77	318	11.24						-24.5156	116.2739
MALINDA	YRRD014	9/06/2023		Scrape	55.77	318	11.24						-24.5156	116.2739
MALINDA	YRRD019	9/06/2023		Haul Net	56.34	321	12.73	28.9	7.24	1.35	121.2	2541	-24.5150	116.2755
MALINDA	YRRD019	9/06/2023	2/08/2023	Litter Trap	56.34	321	12.73						-24.5150	116.2755
MALINDA	YRRD019	9/06/2023		Scrape	56.34	321	12.73						-24.5150	116.2755
MALINDA	YRRD019	11/11/2023		Haul Net	70	321	19.26	30	7.36	5.66	99.1	2645	-24.5150	116.2755
MALINDA	YRRD019	11/11/2023	11/01/2024	Litter Trap	70	321	19.26						-24.5150	116.2755
MALINDA	YRRD019	11/11/2023		Scrape	70	321	19.26						-24.5150	116.2755
MALINDA	YRRD019	7/03/2024		Haul Net	70	321	15.64	31.2	7.16	1.71	67.6	3391	-24.5150	116.2755
MALINDA	YRRD019	7/03/2024	6/05/2024	Litter Trap	70	321	15.64						-24.5150	116.2755
MALINDA	YRRD019	7/03/2024		Scrape	70	321	15.64						-24.5150	116.2755
MALINDA	YRRD021	10/06/2023		Haul Net	56.09	326	21.99	29.1	7.23	1.3	109.8	10586	-24.5101	116.2781
MALINDA	YRRD021	10/06/2023	2/08/2023	Litter Trap	56.09	326	21.99						-24.5101	116.2781
MALINDA	YRRD021	10/06/2023		Scrape	56.09	326	21.99						-24.5101	116.2781
MALINDA	YRRD025	9/06/2023		Haul Net	80	327	18.81	26.7	7.12	2.83	145	14053	-24.5135	116.2802
MALINDA	YRRD025	9/06/2023	2/08/2023	Litter Trap	80	327	18.81						-24.5135	116.2802
MALINDA	YRRD025	9/06/2023		Scrape	80	327	18.81						-24.5135	116.2802
MALINDA	YRRD025	9/11/2023		Haul Net	80	327	17.44	31.6	7.06	1.54	98.5	13659	-24.5134	116.2802
MALINDA	YRRD025	9/11/2023	11/01/2024	Litter Trap	80	327	17.44						-24.5134	116.2802

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	YRRD025	9/11/2023		Scrape	80	327	17.44						-24.5134	116.2802
MALINDA	YRRD025	7/03/2024		Haul Net	80	327		32.1	7.12	1.41	102.2	10446	-24.5134	116.2802
MALINDA	YRRD025	7/03/2024	6/05/2024	Litter Trap	80	327							-24.5134	116.2802
MALINDA	YRRD025	7/03/2024		Scrape	80	327							-24.5134	116.2802
MALINDA	YRRD027	9/06/2023		Haul Net	80	323	15.36	27.8	7.67	1.33	130	4465	-24.5144	116.2771
MALINDA	YRRD027	9/06/2023	Not Retrieved	Litter Trap	80	323	15.36						-24.5144	116.2771
MALINDA	YRRD027	9/06/2023		Scrape	80	323	15.36						-24.5144	116.2771
MALINDA	YRRD036	11/06/2023		Haul Net	60	333	27.41	28	6.91	2.05	42.4	24724	-24.5056	116.2671
MALINDA	YRRD036	11/06/2023	2/08/2023	Litter Trap	60	333	27.41						-24.5056	116.2671
MALINDA	YRRD036	11/06/2023		Scrape	60	333	27.41						-24.5056	116.2671
MALINDA	YRRD036	12/11/2023		Haul Net	65	333	28.80	31.1	6.91	2.72	183.6	25657	-24.5056	116.2672
MALINDA	YRRD036	12/11/2023	11/01/2024	Litter Trap	65	333	28.80						-24.5056	116.2672
MALINDA	YRRD036	12/11/2023		Scrape	65	333	28.80						-24.5056	116.2672
MALINDA	YRRD036	8/03/2024		Haul Net	65	333		Not Recorded	Not Recorded	Not Recorded	Not Recorded	Not Recorded	-24.5056	116.2672
MALINDA	YRRD036	8/03/2024	6/05/2024	Litter Trap	65	333							-24.5056	116.2672
MALINDA	YRRD036	8/03/2024		Scrape	65	333							-24.5056	116.2672
MALINDA	YRRD049	11/11/2023		Haul Net	60	331	26.50	30.5	6.96	1.15	157.5	21038	-24.5090	116.2687
MALINDA	YRRD049	11/11/2023	11/01/2024	Litter Trap	60	331	26.50						-24.5090	116.2687
MALINDA	YRRD049	11/11/2023		Scrape	60	331	26.50						-24.5090	116.2687
MALINDA	YRRD049	8/03/2024		Haul Net	60	331		31.4	7.04	3.9	154	20954	-24.5090	116.2687
MALINDA	YRRD049	8/03/2024	6/05/2024	Litter Trap	60	331							-24.5090	116.2687
MALINDA	YRRD049	8/03/2024		Scrape	60	331							-24.5090	116.2687
MALINDA	YRRD061	11/11/2023		Haul Net	55	332	28.56	30.6	6.99	0.9	140.5	19009	-24.5113	116.2655
MALINDA	YRRD061	11/11/2023	11/01/2024	Litter Trap	55	332	28.56						-24.5113	116.2655
MALINDA	YRRD061	11/11/2023		Scrape	55	332	28.56						-24.5113	116.2655
MALINDA	YRRD061	8/03/2024		Haul Net	55	332		30.4	7.03	1.56	123.7	18682	-24.5113	116.2655
MALINDA	YRRD061	8/03/2024	6/05/2024	Litter Trap	55	332							-24.5113	116.2655

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	YRRD061	8/03/2024		Scrape	55	332							-24.5113	116.2655
MALINDA	YRRD080	14/11/2023	11/01/2024	Litter Trap	65	330							-24.5038	116.2691
MALINDA	YRRD097	11/11/2023		Haul Net	60	322	13.28	28.9	7.29	0.77	137	14905	-24.5098	116.2727
MALINDA	YRRD097	11/11/2023	11/01/2024	Litter Trap		322							-24.5098	116.2727
MALINDA	YRRD097	11/11/2023		Scrape		322							-24.5098	116.2727
MALINDA	YRRD097	8/03/2024		Haul Net	60	322		31.3	7.29	2.96	135.8	14490	-24.5098	116.2727
MALINDA	YRRD097	8/03/2024	6/05/2024	Litter Trap	60	322							-24.5098	116.2727
MALINDA	YRRD097	8/03/2024		Scrape	60	322							-24.5098	116.2727
MALINDA	YRRD099	11/11/2023		Haul Net	70	325	22.55	29.9	7.05	1.14	Not recorded	20196	-24.5089	116.2711
MALINDA	YRRD099	11/11/2023	Not Retrieved	Litter Trap	70	325	22.55						-24.5089	116.2711
MALINDA	YRRD099	11/11/2023		Scrape	70	325	22.55						-24.5089	116.2711
MALINDA	YRRD099	8/03/2024		Haul Net	70	325		32.1	7.13	1.66	133	20584	-24.5089	116.2711
MALINDA	YRRD099	8/03/2024	6/05/2024	Litter Trap	70	325							-24.5089	116.2711
MALINDA	YRRD099	8/03/2024		Scrape	70	325							-24.5089	116.2711
MALINDA	YRRD101	11/11/2023		Haul Net	70	322	13.95	28.7	7.38	3.02	131.5	10807	-24.5112	116.2726
MALINDA	YRRD101	11/11/2023	11/01/2024	Litter Trap	70	322	13.95						-24.5112	116.2726
MALINDA	YRRD101	11/11/2023		Scrape	70	322	13.95						-24.5112	116.2726
MALINDA	YRRD101	8/03/2024		Haul Net	70	322		31.2	7.18	2.16	129.9	13628	-24.5112	116.2726
MALINDA	YRRD101	8/03/2024	6/05/2024	Litter Trap	70	322							-24.5112	116.2726
MALINDA	YRRD101	8/03/2024		Scrape	70	322							-24.5112	116.2726
MALINDA	YRRD112	14/11/2023	11/01/2024	Litter Trap	65	326							-24.5067	116.2734
MALINDA	YRRD112	9/03/2024	6/05/2024	Litter Trap	65	326							-24.5067	116.2734
MALINDA	YRRD113	12/11/2023		Haul Net	70	326	20.47	30.9	7.03	1.24	149.5	23751	-24.5048	116.2735
MALINDA	YRRD113	12/11/2023	11/01/2024	Litter Trap	70	326	20.47						-24.5048	116.2735
MALINDA	YRRD113	12/11/2023		Scrape	70	326	20.47						-24.5048	116.2735
MALINDA	YRRD123	10/11/2023		Haul Net	75	324	19.28	31.7	7.07	3.54	113.5	17003	-24.5096	116.2758
MALINDA	YRRD123	10/11/2023	11/01/2024	Litter Trap	75	324	19.28						-24.5096	116.2758

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	YRRD123	10/11/2023		Scrape	75	324	19.28						-24.5096	116.2758
MALINDA	YRRD123	9/03/2024		Haul Net	75	324		31.9	7.14	0.76	106	15277	-24.5096	116.2758
MALINDA	YRRD123	9/03/2024	6/05/2024	Litter Trap	75	324							-24.5096	116.2758
MALINDA	YRRD123	9/03/2024		Scrape	75	324							-24.5096	116.2758
MALINDA	YRRD136	12/11/2023		Haul Net	90	325	21.00	29.8	7.4	0.49	78.3	25892	-24.5027	116.2685
MALINDA	YRRD139	11/11/2023		Haul Net	90	326	18.26	30.6	6.97	0.5	165.6	30842	-24.5063	116.2644
MALINDA	YRRD139	11/11/2023	11/01/2024	Litter Trap	90	326	18.26						-24.5063	116.2644
MALINDA	YRRD139	11/11/2023		Scrape	90	326	18.26						-24.5063	116.2644
MALINDA	YRRD139	8/03/2024		Haul Net	90	326		29.9	6.98	1.92	134	30172	-24.5063	116.2644
MALINDA	YRRD139	8/03/2024	6/05/2024	Litter Trap	90	326							-24.5063	116.2644
MALINDA	YRRD139	8/03/2024		Scrape	90	326							-24.5063	116.2644
MALINDA	YRRD140	14/11/2023	11/01/2024	Litter Trap	65	327							-24.4993	116.2813
MALINDA	YRRD143	14/11/2023	11/01/2024	Litter Trap		324							-24.4993	116.2733
MALINDA	YRRD144	14/11/2023	11/01/2024	Litter Trap	65	323							-24.4987	116.2725
MALINDA	YRRD147	14/11/2023		Haul Net	65	320	19.03	29	6.95	1.14	116.5	36191	-24.4990	116.2625
MALINDA	YRRD147	14/11/2023	11/01/2024	Litter Trap	65	320	19.03						-24.4990	116.2625
MALINDA	YRRD147	14/11/2023		Scrape	65	320	19.03						-24.4990	116.2625
MALINDA	YRRD147	8/03/2024		Haul Net	65	320		31.8	7.02	3.04	113.8	35912	-24.4990	116.2625
MALINDA	YRRD147	8/03/2024	6/05/2024	Litter Trap	65	320							-24.4990	116.2625
MALINDA	YRRD147	8/03/2024		Scrape	65	320							-24.4990	116.2625
MALINDA	YRRD162	9/11/2023		Haul Net	65	340	36.56	31.7	7.29	1.02	-101.6	8009	-24.5140	116.2849
MALINDA	YRRD162	9/11/2023	11/01/2024	Litter Trap	65	340							-24.5140	116.2849
MALINDA	YRRD162	9/11/2023		Scrape	65	340							-24.5140	116.2849
MALINDA	YRRD162	7/03/2024		Haul Net	65	340		31.9	7.18	1.25	81.5	7877	-24.5140	116.2849
MALINDA	YRRD162	7/03/2024	6/05/2024	Litter Trap	65	340							-24.5140	116.2849
MALINDA	YRRD162	7/03/2024		Scrape	65	340							-24.5140	116.2849
MALINDA	YRRD164	14/11/2023	11/01/2024	Litter Trap	70	327							-24.5041	116.2771
MALINDA	YRRD164	9/03/2024		Haul Net	70	327		32	7.14	0.99	99.8	21605	-24.5041	116.2771
MALINDA	YRRD164	9/03/2024	6/05/2024	Litter Trap	60	327							-24.5041	116.2771

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	YRRD164	9/03/2024		Scrape		327							-24.5041	116.2771
MALINDA	YRRD170	14/11/2023		Haul Net	70	326	29.13	28.8	7.05	1.99	109.4	22473	-24.5036	116.2788
MALINDA	YRRD170	14/11/2023	11/01/2024	Litter Trap	70	326							-24.5036	116.2788
MALINDA	YRRD170	14/11/2023		Scrape	70	326							-24.5036	116.2788
MALINDA	YRRD170	9/03/2024		Haul Net	70	326		30.2	7.18	3.09	122.9	21781	-24.5036	116.2788
MALINDA	YRRD170	9/03/2024	6/05/2024	Litter Trap	70	326							-24.5036	116.2788
MALINDA	YRRD170	9/03/2024		Scrape	70	326							-24.5036	116.2788
MALINDA	YRRD180	9/11/2023		Haul Net	65	344	37.52	29.5	7.32	1.01	-6.6	1661	-24.5148	116.2865
MALINDA	YRRD180	9/11/2023	11/01/2024	Litter Trap		344							-24.5148	116.2865
MALINDA	YRRD180	9/11/2023		Scrape		344							-24.5148	116.2865
MALINDA	YRRD180	7/03/2024		Haul Net	65	344	36.62	30.1	7.39	1.07	-140	2356	-24.5148	116.2865
MALINDA	YRRD180	7/03/2024	6/05/2024	Litter Trap	65	344	36.62						-24.5148	116.2865
MALINDA	YRRD180	7/03/2024		Scrape	65	344	36.62						-24.5148	116.2865
MALINDA	YRRD190	14/11/2023		Haul Net	65	327	21.75	30.3	7.02	0.7	157.8	24830	-24.5034	116.2707
MALINDA	YRRD190	14/11/2023	11/01/2024	Litter Trap	65	327	21.75						-24.5034	116.2707
MALINDA	YRRD190	14/11/2023		Scrape	65	327	21.75						-24.5034	116.2707
MALINDA	YRRD191	9/11/2023		Haul Net	70	344	41.95	31.4	7.44	0.37	-233.5	6959	-24.5162	116.2849
MALINDA	YRRD191	9/11/2023	11/01/2024	Litter Trap	70	344	41.95						-24.5162	116.2849
MALINDA	YRRD191	9/11/2023		Scrape	70	344	41.95						-24.5162	116.2849
MALINDA	YRRD191	7/03/2024		Haul Net	70	344	42.46	31.6	7.53	2.04	-246.6	6936	-24.5162	116.2849
MALINDA	YRRD191	7/03/2024	6/05/2024	Litter Trap	70	344	42.46						-24.5162	116.2849
MALINDA	YRRD191	7/03/2024		Scrape	70	344	42.46						-24.5162	116.2849
MALINDA	YRRD195	9/11/2023		Haul Net	80	339	27.08	28.2	7.41	6.35	69.4	11210	-24.5153	116.2882
MALINDA	YRRD195	9/11/2023	11/01/2024	Litter Trap	80	339	27.08						-24.5153	116.2882
MALINDA	YRRD195	9/11/2023		Scrape	80	339	27.08						-24.5153	116.2882
MALINDA	YRRD195	7/03/2024		Haul Net	80	339	27.86	30.2	7.47	3.53	-157.8	7967	-24.5153	116.2882
MALINDA	YRRD195	7/03/2024	6/05/2024	Litter Trap	80	339	27.86						-24.5153	116.2882
MALINDA	YRRD195	7/03/2024		Scrape	80	339	27.86						-24.5153	116.2882
MALINDA	YRRD207	10/11/2023		Haul Net	75	326	21.00	30.4	7.16	0.83	92.7	12274	-24.5009	116.2820

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	YRRD207	10/11/2023	11/01/2024	Litter Trap	75	326	21.00						-24.5009	116.2820
MALINDA	YRRD207	10/11/2023		Scrape	75	326	21.00						-24.5009	116.2820
MALINDA	YRRD207	9/03/2024		Haul Net	75	326		30.6	7.26	1.26	105.3	10941	-24.5009	116.2820
MALINDA	YRRD207	9/03/2024	6/05/2024	Litter Trap	75	326							-24.5009	116.2820
MALINDA	YRRD207	9/03/2024		Scrape	75	326							-24.5009	116.2820
MALINDA	YRRD209	10/11/2023		Haul Net	80	326	22.79	30.2	7.04	1.38	123.8	16531	-24.5038	116.2804
MALINDA	YRRD209	10/11/2023	11/01/2024	Litter Trap	80	326	22.79						-24.5038	116.2804
MALINDA	YRRD209	10/11/2023		Scrape	80	326	22.79						-24.5038	116.2804
MALINDA	YRRD209	9/03/2024		Haul Net	80	326		30.9	7.23	0.71	107.1	16409	-24.5038	116.2804
MALINDA	YRRD209	9/03/2024	6/05/2024	Litter Trap	80	326							-24.5038	116.2804
MALINDA	YRRD209	9/03/2024		Scrape	80	326							-24.5038	116.2804
MALINDA	YRRD210	10/11/2023		Haul Net	80	325	17.61	30.5	7.1	1.11	107	18150	-24.5014	116.2732
MALINDA	YRRD210	10/11/2023	11/01/2024	Litter Trap	80	325							-24.5014	116.2732
MALINDA	YRRD210	10/11/2023		Scrape	80	325							-24.5014	116.2732
MALINDA	YRRD213	14/11/2023	11/01/2024	Litter Trap	60	329							-24.5048	116.2715
MALINDA	YRRD217	10/11/2023		Haul Net	75	333	30.29	31.1	6.94	2.29	111.8	22078	-24.5072	116.2790
MALINDA	YRRD217	10/11/2023	11/01/2024	Litter Trap	75	333	30.29						-24.5072	116.2790
MALINDA	YRRD217	10/11/2023		Scrape	75	333	30.29						-24.5072	116.2790
MALINDA	YRRD217	9/03/2024		Haul Net	75	333		31.4	7.06	1.5	110.6	21564	-24.5072	116.2790
MALINDA	YRRD217	9/03/2024	6/05/2024	Litter Trap	75	333							-24.5072	116.2790
MALINDA	YRRD217	9/03/2024		Scrape	75	333							-24.5072	116.2790
MALINDA	YRRD219	10/11/2023		Haul Net	80	333	29.20	29.6	7.32	1.41	-219.7	19100	-24.5092	116.2813
MALINDA	YRRD219	10/11/2023	11/01/2024	Litter Trap	80	333	29.20						-24.5092	116.2813
MALINDA	YRRD219	10/11/2023		Scrape	80	333	29.20						-24.5092	116.2813
MALINDA	YRRD219	9/03/2024		Haul Net	80	333		30.6	7.39	1.03	-155.3	19700	-24.5092	116.2813
MALINDA	YRRD219	9/03/2024	6/05/2024	Litter Trap	80	333							-24.5092	116.2813
MALINDA	YRRD219	9/03/2024		Scrape	80	333							-24.5092	116.2813
MALINDA	YRRD222	14/11/2023		Haul Net	75	322	22.60	29.4	7.14	0.69	4.3	30929	-24.5006	116.2625
MALINDA	YRRD222	14/11/2023	11/01/2024	Litter Trap	75	322	22.60						-24.5006	116.2625

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
MALINDA	YRRD222	14/11/2023		Scrape	75	322	22.60						-24.5006	116.2625
MALINDA	YRRD222	8/03/2024		Haul Net	75	322		30.5	7.15	1.04	127.5	30246	-24.5006	116.2625
MALINDA	YRRD222	8/03/2024	Not Retrieved	Litter Trap	75	322							-24.5006	116.2625
MALINDA	YRRD222	8/03/2024		Scrape	75	322							-24.5006	116.2625
MALINDA	YRRD223	14/11/2023	11/01/2024	Litter Trap	60	322							-24.5002	116.2615
MALINDA	YRRD223	8/03/2024	6/05/2024	Litter Trap	60	322							-24.5002	116.2615
MALINDA	YRRD233	10/11/2023		Haul Net	70	329	40.41	29.3	7.29	0.72	40.9	3525	-24.5111	116.2818
MALINDA	YRRD233	10/11/2023	11/01/2024	Litter Trap	70	329	40.41						-24.5111	116.2818
MALINDA	YRRD233	10/11/2023		Scrape	70	329	40.41						-24.5111	116.2818
MALINDA	YRRD233	7/03/2024		Haul Net	70	329		31.8	7.21	1.42	80.2	3601	-24.5111	116.2818
MALINDA	YRRD233	7/03/2024	6/05/2024	Litter Trap	70	329							-24.5111	116.2818
MALINDA	YRRD233	7/03/2024		Scrape	70	329							-24.5111	116.2818
MALINDA	YRRD240	9/11/2023		Haul Net	80	322	15.15	30.7	7.02	1.84	94.4	14078	-24.5154	116.2771
MALINDA	YRRD240	9/11/2023	Not Retrieved	Litter Trap	80	322	15.15						-24.5154	116.2771
MALINDA	YRRD240	9/11/2023		Scrape	80	322	15.15						-24.5154	116.2771
MALINDA	YRRD240	7/03/2024		Haul Net	80	322	15.84	30.8	6.98	0.69	8.6	13694	-24.5154	116.2771
MALINDA	YRRD240	7/03/2024	6/05/2024	Litter Trap	80	322	15.84						-24.5154	116.2771
MALINDA	YRRD240	7/03/2024		Scrape	80	322	15.84						-24.5154	116.2771
MALINDA	YRRD246	14/11/2023	11/01/2024	Litter Trap	65	325							-24.5034	116.2664
MALINDA	YRRD246	8/03/2024	6/05/2024	Litter Trap	65	325							-24.5034	116.2664
MALINDA	YRRD248	14/11/2023		Haul Net	70	325	24.43	30.4	6.86	1.73	132.5	30628	-24.5027	116.2664
MALINDA	YRRD248	14/11/2023	11/01/2024	Litter Trap	60.38	325	22.6						-24.5027	116.2664
MALINDA	YRRD248	14/11/2023		Scrape	60.38	325	22.6						-24.5027	116.2664
MALINDA	YRRD248	8/03/2024		Haul Net	70	325		31.9	6.95	3.71	93	29980	-24.5027	116.2664
MALINDA	YRRD248	8/03/2024	6/05/2024	Litter Trap	70	325							-24.5027	116.2664
MALINDA	YRRD248	8/03/2024		Scrape	0	325							-24.5027	116.2664
Regional	23MARC01	13/11/2023		Haul Net	90	294	4.94	28.3	7.6	0.43	-113	25646	-24.5428	116.1787

Area	Site	Sample Date	Trap Retrieval Date	Collection Method	Hole Inclination	Elevation (mAHD)	SWL (mbgl)	Water Temp (°C)	pH	DO (mg/L)	Redox (mV)	EC (SPC: µS/cm)	Latitude	Longitude
Regional	Cobra01	13/11/2023		Haul Net	90	369	6.65	29.9	8.25	0.93	66.1	1333	-24.1973	116.4772
Regional	Victory Bore	13/11/2023		Haul Net	90	283	10.00	27	8.01	0.45	-111	10446	-24.5938	116.1390
Regional	YIN Bore 01	11/06/2023		Haul Net	90	328	9.10	27.7	8.13	0.95	85.7	10705	-24.4686	116.2851
Regional	YIN Bore 01	13/11/2023		Haul Net	90	328	14.39	29.2	7.15	3.24	117.7	10496	-24.4686	116.2851

Appendix B: Subterranean Fauna Sample Results

Table B-1: Stygofauna haul net sample results.

Area	SITE	Sample Date	Group	Taxon	Abundance	Latitude	Longitude
MALINDA	GASRC0003	11/06/2023			0	-24.50747	116.26685
MALINDA	GASRC0005	11/06/2023			0	-24.50432	116.26679
MALINDA	GASRC0005	12/11/2023			0	-24.50424	116.26682
MALINDA	GASRC0005	8/03/2024			0	-24.50424	116.26682
MALINDA	GASRC0008	10/06/2023			0	-24.50700	116.26797
MALINDA	GASRC0016	9/06/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087'	1	-24.51266	116.27602
MALINDA	GASRC0016	11/11/2023			0	-24.51266	116.27602
MALINDA	GASRC0016	9/03/2024			0	-24.51266	116.27602
MALINDA	MARC008	10/06/2023			0	-24.51206	116.27545
MALINDA	MARC011	10/06/2023			0	-24.51291	116.27465
MALINDA	MARC012	10/06/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087'	1	-24.51092	116.27609
MALINDA	MARC012	12/11/2023			0	-24.51104	116.27602
MALINDA	MARC012	9/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087'	22	-24.51104	116.27602
MALINDA	MARC013	10/06/2023			0	-24.50683	116.27963
MALINDA	MARC014	10/06/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087'	1	-24.50570	116.27152
MALINDA	MARC014	12/11/2023			0	-24.50570	116.27152
MALINDA	MARC014	9/03/2024			0	-24.50570	116.27152
MALINDA	MARC017	11/06/2023			0	-24.50120	116.26795
MALINDA	MARC017	14/11/2023			0	-24.50120	116.26795
MALINDA	MARC017	8/03/2024			0	-24.50120	116.26795
MALINDA	YDRD007	14/11/2023			0	-24.50532	116.27496
MALINDA	YDRD010	9/11/2023			0	-24.51251	116.27550
MALINDA	YDRD010	9/03/2024			0	-24.51251	116.27550
MALINDA	YNRD017	11/06/2023			0	-24.50637	116.26548
MALINDA	YNRD023	11/06/2023			0	-24.50640	116.26401
MALINDA	YNRD024	10/06/2023			0	-24.50760	116.27891
MALINDA	YREX032	10/11/2023			0	-24.50782	116.28357

Area	SITE	Sample Date	Group	Taxon	Abundance	Latitude	Longitude
MALINDA	YREX032	9/03/2024			0	-24.50782	116.28357
MALINDA	YRRD002	9/06/2023			0	-24.51200	116.27736
MALINDA	YRRD014	9/06/2023			0	-24.51556	116.27391
MALINDA	YRRD019	9/06/2023	Cyclopoida	Halicyclops sp. 'Biologic-CYCL099`	1	-24.51502	116.27550
MALINDA	YRRD019	11/11/2023			0	-24.51501	116.27554
MALINDA	YRRD019	7/03/2024	Amphipoda	Amphipoda indef.	1	-24.51501	116.27554
MALINDA	YRRD021	10/06/2023			0	-24.51014	116.27812
MALINDA	YRRD025	9/06/2023			0	-24.51345	116.28022
MALINDA	YRRD025	9/11/2023			0	-24.51337	116.28020
MALINDA	YRRD025	7/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	17	-24.51337	116.28020
MALINDA	YRRD027	9/06/2023			0	-24.51441	116.27709
MALINDA	YRRD036	11/06/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	22	-24.50563	116.26713
MALINDA	YRRD036	11/06/2023	Isopoda	Robustura sp. YIN01	3	-24.50563	116.26713
MALINDA	YRRD036	12/11/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	11	-24.50563	116.26716
MALINDA	YRRD036	8/03/2024			0	-24.50563	116.26716
MALINDA	YRRD049	11/11/2023			0	-24.50896	116.26871
MALINDA	YRRD049	8/03/2024			0	-24.50896	116.26871
MALINDA	YRRD061	11/11/2023			0	-24.51133	116.26547
MALINDA	YRRD061	8/03/2024			0	-24.51133	116.26547
MALINDA	YRRD097	11/11/2023			0	-24.50975	116.27274
MALINDA	YRRD097	8/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	1	-24.50975	116.27274
MALINDA	YRRD099	11/11/2023			0	-24.50893	116.27106
MALINDA	YRRD099	8/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	28	-24.50893	116.27106
MALINDA	YRRD101	11/11/2023	Amphipoda	Paramelitidae sp. 'Biologic-AMPH095`	1	-24.51121	116.27262
MALINDA	YRRD101	11/11/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	12	-24.51121	116.27262
MALINDA	YRRD101	8/03/2024			0	-24.51121	116.27262
MALINDA	YRRD113	12/11/2023			0	-24.50480	116.27355
MALINDA	YRRD123	10/11/2023			0	-24.50956	116.27577
MALINDA	YRRD123	9/03/2024			0	-24.50956	116.27577
MALINDA	YRRD136	12/11/2023			0	-24.50265	116.26852

Area	SITE	Sample Date	Group	Taxon	Abundance	Latitude	Longitude
MALINDA	YRRD139	11/11/2023			0	-24.50627	116.26440
MALINDA	YRRD139	8/03/2024			0	-24.50627	116.26440
MALINDA	YRRD147	14/11/2023	Cyclopoida	Halicyclops sp. 'Biologic-CYCL099`	3	-24.49902	116.26252
MALINDA	YRRD147	14/11/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	26	-24.49902	116.26252
MALINDA	YRRD147	14/11/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	250	-24.49902	116.26252
MALINDA	YRRD147	8/03/2024	Cyclopoida	Halicyclops sp. 'Biologic-CYCL099`	26	-24.49902	116.26252
MALINDA	YRRD147	8/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	113	-24.49902	116.26252
MALINDA	YRRD147	8/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	20	-24.49902	116.26252
MALINDA	YRRD162	9/11/2023			0	-24.51398	116.28491
MALINDA	YRRD162	7/03/2024			0	-24.51398	116.28491
MALINDA	YRRD164	9/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	36	-24.50408	116.27711
MALINDA	YRRD170	14/11/2023			0	-24.50362	116.27882
MALINDA	YRRD170	9/03/2024			0	-24.50362	116.27882
MALINDA	YRRD180	9/11/2023			0	-24.51475	116.28651
MALINDA	YRRD180	7/03/2024			0	-24.51475	116.28651
MALINDA	YRRD190	14/11/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	30	-24.50338	116.27073
MALINDA	YRRD191	9/11/2023			0	-24.51620	116.28495
MALINDA	YRRD191	7/03/2024			0	-24.51620	116.28495
MALINDA	YRRD195	9/11/2023			0	-24.51529	116.28821
MALINDA	YRRD195	7/03/2024			0	-24.51529	116.28821
MALINDA	YRRD207	10/11/2023			0	-24.50092	116.28204
MALINDA	YRRD207	9/03/2024			0	-24.50092	116.28204
MALINDA	YRRD209	10/11/2023			0	-24.50384	116.28042
MALINDA	YRRD209	9/03/2024			0	-24.50384	116.28042
MALINDA	YRRD210	10/11/2023			0	-24.50141	116.27323
MALINDA	YRRD217	10/11/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	32	-24.50722	116.27897
MALINDA	YRRD217	9/03/2024	Amphipoda	Paramelitidae sp. 'Biologic-AMPH095`	3	-24.50722	116.27897
MALINDA	YRRD217	9/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	125	-24.50722	116.27897
MALINDA	YRRD219	10/11/2023			0	-24.50923	116.28131
MALINDA	YRRD219	9/03/2024			0	-24.50923	116.28131

Area	SITE	Sample Date	Group	Taxon	Abundance	Latitude	Longitude
MALINDA	YRRD222	14/11/2023			0	-24.50055	116.26253
MALINDA	YRRD222	8/03/2024	Cyclopoida	Halicyclops sp. 'Biologic-CYCL099`	2	-24.50055	116.26253
MALINDA	YRRD222	8/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	1	-24.50055	116.26253
MALINDA	YRRD222	8/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	9	-24.50055	116.26253
MALINDA	YRRD233	10/11/2023			0	-24.51113	116.28181
MALINDA	YRRD233	7/03/2024			0	-24.51113	116.28181
MALINDA	YRRD240	9/11/2023			0	-24.51544	116.27708
MALINDA	YRRD240	7/03/2024			0	-24.51544	116.27708
MALINDA	YRRD248	14/11/2023	Cyclopoida	Halicyclops sp. 'Biologic-CYCL099`	1	-24.50266	116.26636
MALINDA	YRRD248	14/11/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	9	-24.50266	116.26636
MALINDA	YRRD248	8/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	4	-24.50266	116.26636
Pastoral	YIN Bore 01	11/06/2023	Cyclopoida	Halicyclops sp. 'Biologic-CYCL099`	1	-24.46863	116.28512
Pastoral	YIN Bore 01	11/06/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	2	-24.46863	116.28512
Regional	23MARC01	13/11/2023	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	1	-24.54280	116.17865
Regional	Cobra01	13/11/2023			0	-24.19735	116.47718
Regional	Victory Bore	13/11/2023	Harpacticoida	Cletocamptus sp. 'Biologic-HARP063`	3	-24.59381	116.13904
Regional	YIN Bore 01	13/11/2023	Amphipoda	Paramelitidae sp. 'Biologic-AMPH094`	2	-24.46863	116.28512
Regional	YIN Bore 01	13/11/2023	Amphipoda	Paramelitidae sp. 'Biologic-AMPH095`	2	-24.46863	116.28512
MALINDA	YRRD219	10/11/2023			0	-24.50923	116.28131
MALINDA	YRRD219	9/03/2024			0	-24.50923	116.28131
MALINDA	YRRD222	14/11/2023			0	-24.50055	116.26253
MALINDA	YRRD222	8/03/2024	Cyclopoida	Halicyclops sp. 'Biologic-CYCL099`	2	-24.50055	116.26253
MALINDA	YRRD222	8/03/2024	Harpacticoida	Harpacticoida sp. 'Biologic-HARP087`	1	-24.50055	116.26253

Table B-2: Troglifauna collection records.

Area	Site	Sample date	Trap Retrieval Date	Collection Method	Group	Taxon	Abundance	Latitude	Longitude
MALINDA	GASRC0003	11/06/2023	2/08/2023	Litter Trap	Polyxenida	Lophoturus madecassus	2	-24.5075	116.2669
MALINDA	YRRD139	11/11/2023		Scrape	Diplura	Diplura indet.	1	-24.5063	116.2644

Appendix C: Genetic Analysis

Biologic (2024) Bestiolas Consulting Yinnetharra Subterranean Fauna Molecular Systematics Analysis.



Biologic
ENVIRONMENTAL
SURVEY

Bestiolas Consulting
Yinnetharra
Subterranean Fauna
Molecular
Systematics
Analysis

Report to Bestiolas Consulting

3 July 2024

Document Status				
Revision No.	Author	Review / Approved for Issue	Approved for Issue to Name	Date
1	Stephanie Floeckner, Shriya Bhattacharya, Joel Huey	Joel Huey	Nick Stevens	2/07/24
2				
3				

“IMPORTANT NOTE”

Biologic Environmental Survey Pty Ltd (“Biologic”) has prepared this report for Bestiolas Consulting (“Client”), in accordance with the Client’s specific instructions and solely for the purposes for which it is required by the Client (“Purpose”). This report and its content are only pertinent to the Purpose and any matters, facts or results contained in this report are not to be used for any purpose other than the Purpose.

The information contained in this report is not financial advice and Biologic is not licenced to provide financial advice. The report does not take into account the investment objectives, financial situation or specific investment needs of the Client and should not form the basis of an investment decision by the Client.

In preparing this report Biologic has assumed the accuracy and completeness of all the information and documents received or obtained from the Client and all information and documents received or obtained as a result of any request or enquiry made to a government department, authority, government register or database. Biologic has not independently verified any such assumptions.

Apart from fair dealing for the purposes of private study, research, criticism, or review as permitted under the Copyright Act, no part of this report, its attachments or appendices may be reproduced by any process, released, or distributed without the written consent of Biologic. All enquiries should be directed to Biologic.

This report is presented without the assumption of a duty of care to any other person (other than the Client) (“Third Party”). The report may not contain sufficient information for the purposes of a Third Party or for other uses and may not be relied on by a Third Party without Biologic’s prior written consent.

Biologic will not be liable to a Third Party for any loss, damage, liability, or claim arising out of or incidental to a Third-Party publishing, using or relying on the facts, content, opinions or subject matter contained in this report.

If a Third Party uses or relies on the facts, content, opinions, or subject matter contained in this report with or without the consent of Biologic, Biologic disclaims all risk, and the Third Party assumes all risk and releases and indemnifies and agrees to keep Biologic indemnified from any Loss, Damage, claim or liability arising directly or indirectly from the use of or reliance on this report.

For the purpose of this document, a reference to “Loss” and “Damage” includes past and prospective economic loss, loss of profits, damage to property, injury to any person (including death) costs and expenses incurred in taking measures to prevent, mitigate or rectify any harm, loss of opportunity, legal costs, compensation, interest and any other direct, indirect, consequential, or financial or other loss.

Glossary

Bootstrap	Value between 0 and 100 that indicates the robustness of the node in a phylogenetic tree.
COI	Cytochrome Oxidase subunit 1, a mitochondrial gene commonly used in phylogenetic studies and used as a DNA barcode to identify species.
GenBank	Annotated open access sequence database of all publicly available nucleotide sequences and their protein translations.
Monophyletic	A grouping of specimens that all share a common ancestor, inferred by sequence data. The sequences within the monophyletic group will all be more closely related to each other, relative to sequences outside of the monophyletic group. This grouping is often referred to as a lineage or clade, and is graphically represented in phylogenies/trees by sharing a single node with a high bootstrap value.
OTU	Operational taxonomic unit – species-equivalent taxonomic unit based on COI or 12S cluster similarity.
Study specimens	Specimens collected by Bestiolas Consulting and run for molecular barcoding by Biologic for analysis in this report.

Table of Contents

Glossary	3
1 Introduction	6
1.1 Background.....	6
2 Methods	7
2.1 Sub-sample Preparation	7
2.2 DNA Extraction, Amplification and Sequencing	7
2.3 Specimen Selection for Comparative Analysis.....	8
2.4 Analysis and Interpretation of Alignments and Phylogenies	8
2.5 Constraints and Limitations	9
3 Results and Discussion	11
3.1 Amphipoda	11
3.2 Cyclopoida.....	14
3.3 Harpacticoida	16
4 Summary	19
5 References	20
Appendix A: Specimen Data	21

Tables

Table 2.1: Taxonomic groups included in the analysis, with a summary of PCR and sequencing success.....	7
Table 3.1: Summary of species and OTUs recovered from samples successfully sequenced in this study, organised by taxon	11
Table 3.2: Pairwise distances (%) for the Amphipoda dataset.....	13
Table 3.3: Pairwise distances (%) for the Cyclopoida dataset	15
Table 3.4: Pairwise distances (%) for the Harpacticoida dataset	18

Figures

Figure 2.1. Example phylogeny showing delimited OTUs with internode distances and bootstrap values indicated.....	9
Figure 3.1. Phylogeny for the Amphipoda dataset, with bootstrap values.....	12
Figure 3.2. Phylogeny for the Cyclopoida dataset, with bootstrap values.....	14
Figure 3.3. Phylogeny for the Harpacticoida dataset, with bootstrap values.....	17

Appendices

Appendix A: Specimen Data..... 21

1 Introduction

1.1 Background

Bestiolas Consulting commissioned Biologic Environmental Survey Pty Ltd (Biologic) to undertake a molecular systematics analysis (DNA barcoding) of 48 specimens collected from Yinnetharra (the Study Area).

The aims and objectives of the molecular systematics analysis were to:

- Undertake DNA sequencing of 48 subterranean fauna specimens to obtain barcoding sequences of the mitochondrial gene Cytochrome Oxidase subunit 1 (COI; Hebert *et al.*, 2003b).
- Investigate the interspecific and intraspecific relationships among sequences of each higher taxonomic group (i.e. use the results of the DNA analysis to indicate how many different OTU/species are likely to occur within each genus or relevant higher taxon)
- Investigate the relationships among sequences from the Study Area and relevant previous sequences from the wider region, using available DNA databases (i.e. compare the results of the current analysis with accessible DNA databases to assess whether any of the species/ OTUs from the Study Area have been collected previously or more widely beyond the Study Area).

This document reports the methods and results of the molecular systematics analysis. All sequence data will be uploaded to GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) as per Biologic Molecular Systematics standard procedure.

2 Methods

2.1 Sub-sample Preparation

Where whole specimens were available, tissue preparation was undertaken by removing a leg or another body part less important for taxonomic identification, briefly drying off the ethanol, and placing the tissue in ATL buffer. In some instances, for very small and/or juvenile specimens, the entire animal was utilised. Again, these were briefly dried and placed in ATL buffer. Greatest care was taken to decontaminate all tools and equipment between samples, using bleach and repeated rinsing in deionised water. Table 2.1 provides details of the taxonomic orders chosen for molecular analysis. Further taxonomic clarification for each specimen included in the analysis can be found in Appendix A.

Table 2.1: Taxonomic groups included in the analysis, with a summary of PCR and sequencing success

Order	Fail	Pass	Total
Amphipoda	1	5	6
Cyclopoida	4	2	6
Harpacticoida	3	33	36
Total	8	40	48

2.2 DNA Extraction, Amplification and Sequencing

DNA extraction and sequencing methods followed standard methods (e.g. Edgecombe *et al.*, 2019; Framenau *et al.*, 2018; Huey *et al.*, 2019; Perina *et al.*, 2018), as follows:

Subsampled tissue/specimen was placed directly into ATL buffer for extraction using the QIAGEN DNeasy Blood and Tissue extraction kit, and DNA extraction followed the manufacturer's protocols. DNA extractions were amplified by Polymerase Chain Reaction (PCR) using Folmer PCR primers (LCO1490, HCO2198; Folmer *et al.*, 1994) to assess the variability of COI. For some specimens that did not amplify using the Folmer primers, alternative primers amplifying the same part of COI were used, such as C1-J2329 and C1-J1718 (Perina *et al.*, 2018; Simon *et al.*, 1994).

The resulting PCR product was cleaned up and sequenced by the Australian Genomic Research Facility (AGRF) Perth node. Molecular laboratory workflows were managed using GENEIOUS Prime (Kearse *et al.*, 2012) with the Biocode plugin (<http://www.mooreabiocode.org>). Raw sequence data were edited and assembled in GENEIOUS, and final consensus sequences were then available for downstream analysis.

2.3 Specimen Selection for Comparative Analysis

DNA comparisons were typically conducted at the order level (Table 2.1). Comparative sequences were from GenBank (a publicly available DNA sequence database) and Biologic's unpublished DNA sequence libraries, using two separate methods.

- BLAST (Basic Local Alignment Search Tool): a method for rapidly searching a DNA sequence library to identify similar sequences. Sequences were searched using the “blastn” function, which returns similar matches.
- Taxonomic Curation: BLAST occasionally fails to identify sequences that could be considered useful for comparison, such as species that might be genetically distant, but are required to be included in the analysis for comparison. Taxonomically relevant specimens were identified using the available taxonomic classifications and identifications in those databases.

The final phylogenies and distance matrices in this report were pruned back to those sequences that can be provided to the Client, with any matches to sequences that cannot be provided to the Client discussed in the relevant sections.

2.4 Analysis and Interpretation of Alignments and Phylogenies

For each taxonomic group, the selected sequences were aligned using the MAFFT (Multiple Alignment using Fast Fourier Transform) algorithm (Katoh *et al.*, 2002). Trees were constructed on resulting alignments using the RaxML (Stamatakis, 2014) plugin in GENEIOUS Prime, using 1,000 bootstrap replicates and the GTR+G substitution model.

To delimit taxonomic units using molecular data, we integrated multiple lines of evidence, including:

- Genetic distance threshold method (~8% pairwise distances at COI, see below);
- Morphological identifications, where available;
- Geographic information; and
- Interpretation of phylogenetic topology.

Fauna-specific genetic distance thresholds for delimiting species and OTUs were used wherever possible, based on published literature and available previous reports. Where these thresholds were not available, the assessment used average divergence thresholds for related groups or higher taxa developed by broad-level studies (e.g. Hebert *et al.*, 2003a). In general, $\leq 8\%$ COI divergence is seen as appropriate to determine OTUs (Hebert *et al.*, 2003a), however, higher or lower divergences are sometimes justified depending on the organism studied. Unless otherwise stated, we considered sequences that exhibited COI divergences $\leq 8\%$ to belong to the same OTU.

The branching pattern and statistical robustness of the nodes (measured using bootstrap support) is also used to inform OTU delimitation. OTUs form monophyletic groups (or lineages), and so if an unknown sequence falls within a lineage comprised of other sequences that have already been identified as a single OTU or species, then that unknown sequence likely shares the same OTU/species as those sequences it is nested within. Additionally, distinct OTUs typically have large internode distances separating OTUs, with short internode distances within the OTU/species.

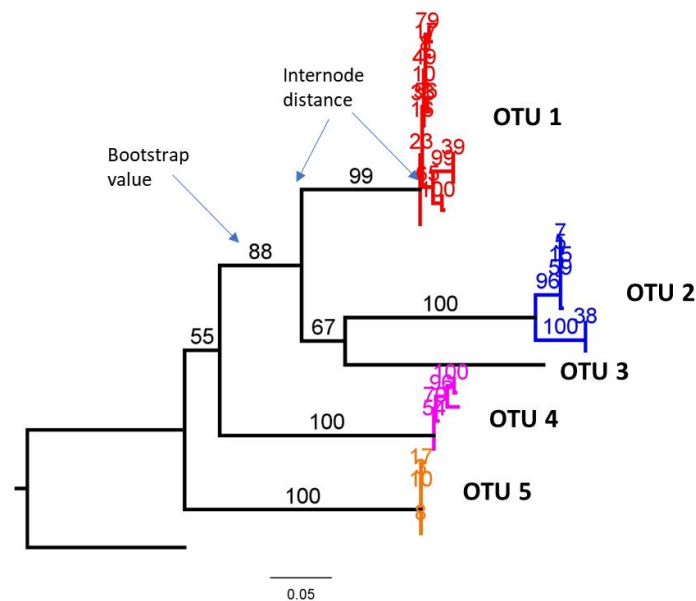


Figure 2.1. Example phylogeny showing delimited OTUs with internode distances and bootstrap values indicated

2.5 Constraints and Limitations

The analysis was constrained by the breadth of data available to undertake comparisons, the accessibility of pre-existing regional sequences, and the success rate of genetic sequencing, which can be affected by specimen collection, preservation, storage methods and contamination. All care was taken to ensure that the risks of laboratory contamination, data handling issues, and specimen management issues were minimised within Biologic's laboratories throughout the subsampling, processing and genetic analysis.

The databases used for regional comparisons included GenBank and Biologic's sequence libraries. While these sequence databases, in combination, comprise a large portion of the subterranean fauna genetic work undertaken in the Pilbara region, it is acknowledged that there may be many other relevant sequences from third party project areas nearby or elsewhere in the region that were not available for comparison at the time of the study.

GenBank is dynamic database, and the addition of new sequences and altered taxonomic classifications could not be included into this report if they occurred after 27/6/2024.

DNA barcoding using the mitochondrial gene COI, while useful for explaining genetic differences between closely related or moderately related species, is limited in its ability to resolve deeper phylogenetic relationships among taxa at higher taxonomic levels (e.g. genus, family, order). In the current study, phylogenetic relationships among species/OTUs >25% COI divergence are treated with caution. If further resolution of deeper phylogeny is important for project goals, this could be investigated using a multiple gene approach.

3 Results and Discussion

A total of 48 specimens were processed for sequencing by Biologic (Table 2.1). Sequences were successfully derived for 46 of these, with two specimens failing to produce a PCR product. Six specimens did not produce a high-quality sequence (less than 80% of untrimmed bases in the sequence were of high quality) or were high quality sequences of an organism that was not the target organism (likely contamination). This left 40 high quality sequences for analysis (83.3% of specimens). The taxonomic orders of the sequences are tabulated in Table 2.1.

In total, five OTUs have been designated to specimens from this Study Area, four of these being specific to this study (Table 3.1). The results of each taxonomic group's analysis are described in the subsequent sections.

Table 3.1: Summary of species and OTUs recovered from samples successfully sequenced in this study, organised by taxon

OTU (genetic taxon)	Sequenced specimens	Matches to external sequences	Linear Range
Amphipoda			
Paramelitidae `sp. Biologic-AMPH094`	1	no	singleton
Paramelitidae `sp. Biologic-AMPH095`	4	no	4.8 km
Maxillopoda			
Cyclopoida			
<i>Halicyclops</i> `sp. Biologic-CYCL099`	2	no	single site
Harpacticoida			
<i>Cletocamptus</i> `sp. Biologic-HARP063`	2	yes	347.0 km
Harpacticoida `sp. Biologic-HARP087`	31	no	5.0 km

3.1 Amphipoda

Five amphipod sequences formed two new OTUs. All five specimens were morphologically identified as potentially belonging to the paramelitid genus *Chydaekata*. The analysis supports their placement within Paramelitidae, however the OTUs are not closely related to other sequenced specimens of *Chydaekata* (Figure 3.1). Paramelitidae `sp. Biologic-AMPH095` consisted of four sequences with an intraspecific divergence of 3.7%, and was more than 18.5% divergent from all other sequences in the analysis (Table 3.2). This OTU had a linear distance of 4.8 km (Table 3.1). Paramelitidae `sp. Biologic-AMPH094` was represented by one sequence and was most closely related to the other Paramelitidae OTU from this study (with approximately 19% divergence, Table 3.2).

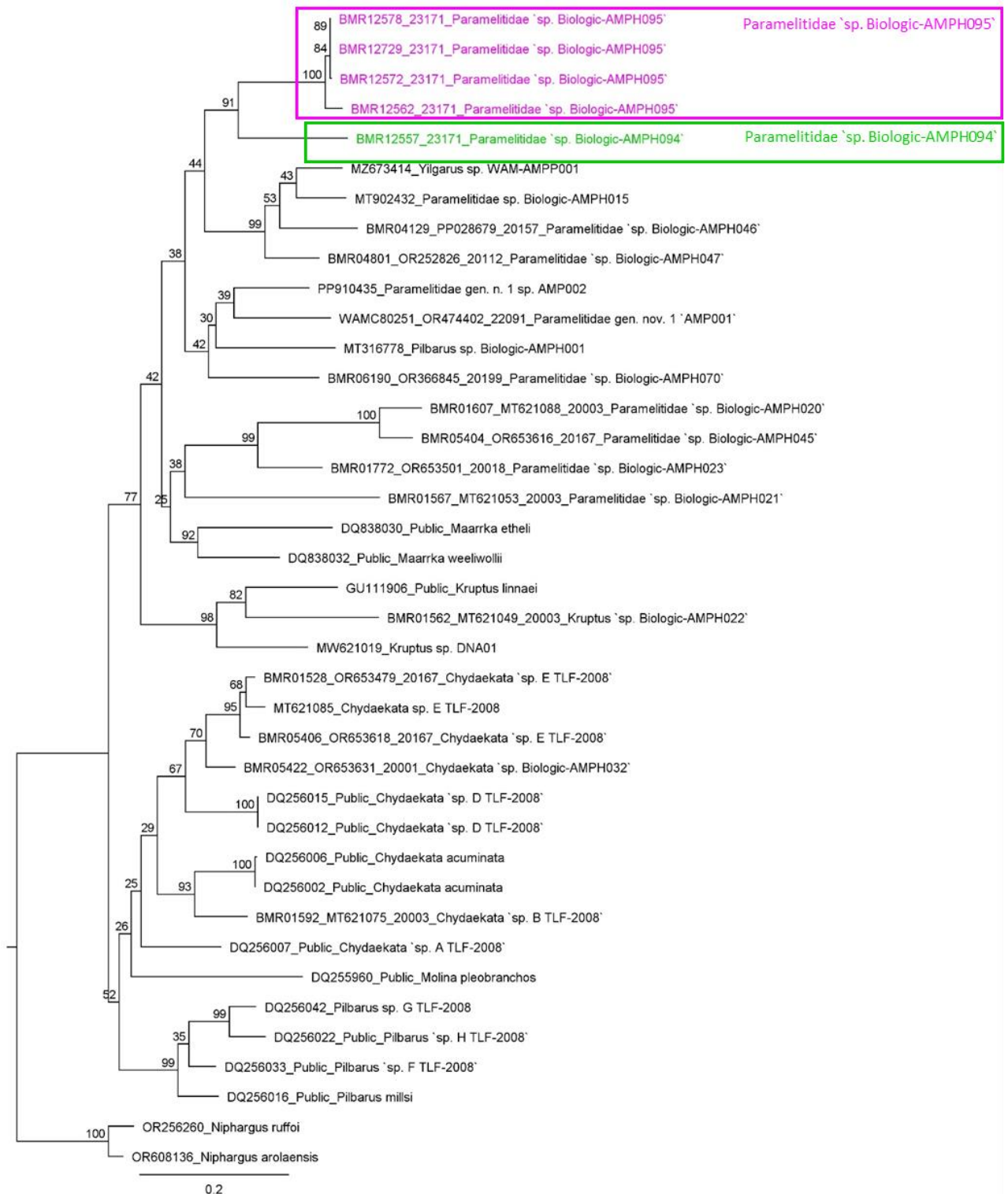


Figure 3.1. Phylogeny for the Amphipoda dataset, with bootstrap values

Table 3.2: Pairwise distances (%) for the Amphipoda dataset

COI Pairwise Distances (%)	MM621019	GU111906	BMR01562	MZ673414	MT902432	BMR04801	BMR04129	BMR12729	BMR12578	BMR12572	BMR12562	BMR12557	MT316778	PP910435	WAMC80251	BMR06190	BMR01772	BMR05404	BMR01607	DQ256042	DQ256022	DQ256033	DQ256016	DQ256006	DQ256002	BMR01592	DQ256007	DQ255960	DQ838032	DQ838030	OR608136	OR256260	BMR01567						
MW621019_Krampus sp. DNA01	19.4	21.0	21.2	21.9	22.5	22.5	22.9	22.9	23.6	22.2	23.4	21.6	23.3	24.0	23.7	26.3	25.1	22.2	22.9	23.1	26.1	22.8	23.1	21.0	23.4	23.4	21.6	21.3	21.1	22.3	24.7	23.7	23.7	23.7	24.5	23.9			
GU111906_Public_Krampus linnaei	19.4	20.0	21.5	22.8	23.1	24.0	23.8	23.8	24.0	24.8	25.7	25.5	23.2	23.8	24.6	23.2	26.7	24.8	24.6	23.6	24.0	23.4	22.5	21.7	23.0	21.1	24.2	24.2	24.8	24.6	23.0	21.9	24.0	24.0	24.8	25.9	25.5	26.1	
BMR01562_MT621049_20003_Krampus 'sp. Biologic-AMPH022'	21.0	20.0	24.8	26.6	27.5	26.3	24.8	24.8	25.0	26.1	26.4	27.8	25.8	26.4	27.1	25.2	28.9	29.2	26.0	25.4	24.8	29.4	26.0	26.0	25.7	25.1	26.3	26.3	28.4	28.2	26.0	26.3	23.0	25.7	23.1	28.1	28.0	24.9	
MZ673414_Yilgarus sp. WAM-AMPP001	21.2	21.5	24.8	10.8	13.0	14.5	20.7	20.7	20.6	21.2	22.2	21.7	18.3	19.9	19.0	19.5	20.7	20.9	20.9	23.1	22.6	27.0	20.0	21.0	21.2	20.3	21.0	21.0	20.0	19.7	21.0	20.7	21.5	22.1	23.4	20.9	21.9	22.4	
MT902432_Paramellitidae sp. Biologic-AMPH015	21.9	22.8	26.6	10.8	13.0	13.8	20.7	20.7	20.6	21.6	21.0	19.3	18.8	19.6	18.5	21.1	21.1	20.2	22.3	23.6	23.9	26.5	22.3	22.9	23.6	22.0	22.3	22.3	21.3	21.0	22.3	22.3	23.9	20.8	23.1	21.4	22.9	22.3	
BMR04801_OR252826_20112_Paramellitidae 'sp. Biologic-AMPH047'	22.5	23.1	27.5	13.0	13.0	16.1	20.5	20.5	20.4	21.8	21.4	21.4	18.1	19.6	18.3	19.8	22.9	21.6	20.5	22.7	22.7	27.5	22.0	22.0	22.0	20.9	21.1	21.1	22.0	21.8	20.7	19.2	23.8	22.7	26.2	21.6	22.9	22.9	
BMR04129_PP028679_20157_Paramellitidae 'sp. Biologic-AMPH046'	22.5	24.0	26.3	14.5	13.8	16.1	20.4	20.4	20.3	21.0	19.9	21.9	19.0	20.7	22.2	22.3	25.1	23.6	23.6	23.7	25.4	27.8	23.6	24.6	24.8	22.8	22.8	22.8	21.6	21.3	23.6	22.3	25.3	21.6	24.2	23.9	24.5	23.4	
BMR12729_23171_Paramellitidae 'sp. Biologic-AMPH095'	22.9	23.8	24.8	20.7	20.7	20.5	20.4	0.0	0.2	3.5	18.7	21.7	19.0	19.8	20.9	22.2	25.4	25.1	25.4	25.5	22.9	28.3	23.3	23.7	23.1	20.2	24.6	24.6	25.2	25.0	22.9	23.4	25.8	21.9	22.6	23.1	22.8	22.5	
BMR12578_23171_Paramellitidae 'sp. Biologic-AMPH095'	22.9	23.8	24.8	20.7	20.7	20.5	20.4	0.0	0.2	3.5	18.7	21.7	19.0	19.8	20.9	22.2	25.4	25.1	25.4	25.5	22.9	28.3	23.3	23.7	23.1	20.2	24.6	24.6	25.2	25.0	22.9	23.4	25.8	21.9	22.6	23.1	22.8	22.5	
BMR12572_23171_Paramellitidae 'sp. Biologic-AMPH095'	22.9	24.0	25.0	20.6	20.6	20.4	20.3	0.2	0.2	3.7	18.6	22.0	18.9	20.2	20.8	22.0	25.2	24.8	25.6	25.8	23.2	28.2	23.2	23.6	23.0	20.4	24.5	24.5	25.2	24.9	22.9	23.6	25.7	21.7	22.6	23.0	23.0	22.4	
BMR12562_23171_Paramellitidae 'sp. Biologic-AMPH095'	23.6	24.8	26.1	21.2	21.6	21.8	21.0	3.5	3.5	3.7	18.8	21.4	19.6	21.6	21.6	22.3	24.9	25.2	26.3	26.1	24.2	29.1	24.2	24.3	24.3	21.7	25.4	25.4	26.6	26.3	24.9	23.4	25.8	22.2	22.8	23.7	23.4	23.4	
BMR12557_23171_Paramellitidae 'sp. Biologic-AMPH094'	22.2	25.7	26.4	22.2	21.0	21.4	19.9	18.7	18.7	18.6	18.8	21.1	20.2	20.7	20.7	23.7	24.3	23.9	23.4	25.4	23.4	27.0	24.0	24.8	24.9	25.1	24.0	25.1	25.1	23.9	23.4	23.7	25.2	26.1	21.4	22.9	25.2	26.3	22.8
MT316778_Pilbarus sp. Biologic-AMPH001	23.4	25.5	27.8	21.7	19.3	21.4	21.9	21.7	21.7	22.0	21.4	21.1	17.5	19.8	20.5	21.3	23.4	24.2	22.8	23.6	22.6	27.8	22.6	21.9	22.6	19.8	23.1	23.1	21.0	20.7	21.7	21.0	23.5	19.9	22.0	23.7	24.9	22.8	
PP910435_Paramellitidae gen. n. 1 sp. AMP002	21.6	23.2	25.8	18.3	18.8	18.1	19.0	19.0	19.0	18.9	19.6	20.2	17.5	17.2	18.3	20.5	21.1	22.0	21.6	21.4	21.1	26.4	22.2	22.0	22.3	20.4	21.9	21.9	20.5	20.2	19.8	20.4	23.2	21.0	22.8	22.6	22.6	22.8	
WAMC80251_OR474402_22091_Paramellitidae gen. nov. 1 'AMP001'	23.3	23.8	26.4	19.9	19.6	19.6	20.7	19.8	19.8	20.2	21.6	20.7	19.8	17.2	17.8	21.1	22.5	21.1	22.2	21.8	22.2	29.5	21.6	21.8	21.8	20.3	22.2	22.2	22.2	22.0	22.7	20.9	24.4	22.9	23.6	24.2	24.4	25.6	
BMR06190_OR366845_20199_Paramellitidae 'sp. Biologic-AMPH070'	24.0	24.6	27.1	19.0	18.5	18.3	22.2	20.9	20.9	20.8	21.6	20.7	20.5	18.3	17.8	18.3	22.5	22.7	19.6	19.8	20.0	28.0	19.6	19.6	19.8	19.8	18.1	18.1	20.3	20.0	18.9	20.5	24.9	19.4	23.1	21.1	22.0	24.4	
BMR01772_OR653501_20018_Paramellitidae 'sp. Biologic-AMPH023'	23.7	23.2	25.2	19.5	21.1	19.8	22.3	22.2	22.2	22.0	22.3	23.7	21.3	20.5	21.1	18.3	17.8	18.5	22.3	23.6	23.6	26.4	22.0	21.7	21.7	21.6	18.8	18.8	21.6	21.6	23.6	20.8	21.9	21.7	23.1	23.1	23.6	24.9	
BMR05404_OR653616_20167_Paramellitidae 'sp. Biologic-AMPH045'	26.3	26.7	28.9	20.7	21.1	22.9	25.1	25.4	25.4	25.2	24.9	24.3	23.4	21.1	22.5	22.5	17.8	9.0	25.8	26.9	26.6	30.2	24.0	23.4	22.9	22.9	21.1	21.1	25.4	25.0	24.0	22.8	23.5	25.1	26.1	22.3	23.9	25.1	
BMR01607_MT621088_20003_Paramellitidae 'sp. Biologic-AMPH020'	25.1	24.8	29.2	20.9	20.2	21.6	23.6	25.1	25.1	24.8	25.2	23.9	24.2	22.0	21.1	22.7	18.5	9.0	25.2	26.0	26.4	30.2	24.2	24.6	24.3	23.7	22.5	22.5	25.4	25.3	25.8	23.1	25.0	23.7	26.9	23.9	25.1	25.2	
DQ256042_Pilbarus sp. G TLF-2008	22.2	24.6	26.0	20.9	22.3	20.5	23.6	25.4	25.4	25.6	26.3	23.4	22.8	21.6	22.2	19.6	22.3	25.8	25.2	7.9	11.6	17.1	19.1	19.5	19.8	17.6	19.6	19.6	17.6	17.2	18.8	15.5	21.5	22.6	23.7	22.5	22.9	25.7	
DQ256022_Public_Pilbarus 'sp. F TLF-2008'	22.9	23.6	25.4	23.1	23.6	22.7	23.7	25.5	25.5	25.8	26.1	25.4	23.6	21.4	21.8	19.8	23.6	26.9	26.0	7.9	11.7	18.2	18.7	20.2	20.2	18.2	19.9	19.9	18.8	18.4	19.5	17.5	21.0	22.2	23.6	23.9	23.7	26.6	
DQ256033_Public_Pilbarus 'sp. F TLF-2008'	23.1	24.0	24.8	22.6	23.9	22.7	25.4	22.9	22.9	23.2	24.2	23.4	22.6	21.1	22.2	20.0	23.6	26.6	26.4	11.6	11.7	13.9	19.9	19.6	20.2	17.2	19.0	19.0	18.2	17.8	17.5	17.0	19.9	21.7	23.6	23.9	24.2	25.5	
DQ256016_Public_Pilbarus millsi	26.1	23.4	29.4	27.0	26.5	27.5	27.8	28.3	28.3	28.2	29.1	27.0	27.8	26.4	29.5	28.0	26.4	30.2	30.2	17.1	18.2	13.9	22.4	22.1	21.8	21.0	23.1	23.1	23.1	22.9	22.3	21.5	25.4	26.9	27.2	27.8	27.6	31.0	
MT621085_Chydaekata sp. E TLF-2008	22.8	22.5	26.0	20.0	22.3	22.0	23.6	23.3	23.2	24.2	24.8	22.6	22.2	21.6	19.6	22.0	24.0	24.2	19.1	18.7	19.9	22.4	4.1	5.0	9.6	14.6	14.6	16.3	15.8	16.3	16.6	20.4	22.5	24.5	21.3	21.4	26.7		
BMR01528_OR653479_20167_Chydaekata 'sp. E TLF-2008'	23.1	21.7	26.0	21.0	22.9	22.0	24.6	23.7	23.7	23.6	24.3	24.9	21.9	22.0	21.8	19.6	21.7	23.4	24.6	19.5	20.2	19.6	22.1	4.1	3.6	8.8	14.7	17.0	16.6	15.0	17.6	19.0	23.1	24.2	21.1	21.7	25.5		
BMR05406_OR653618_20167_Chydaekata 'sp. E TLF-2008'	23.1	23.0	25.7	21.2	23.6	22.0	24.8	23.1	23.1	23.0	24.3	25.1	22.6	22.3	21.8	19.8	21.7	22.9	24.3	19.8	20.2	20.2	21.8	5.0	3.6	8.8	13.8	13.8	17.0	16.6	15.5	17.2	19.8	23.4	23.9	20.7	21.1	25.1	
BMR05422_OR653631_20001_Chydaekata 'sp. Biologic-AMPH032'	21.0	21.1	25.1	20.3	22.0	20.9	22.8	20.2	20.2	20.4	21.7	24.0	19.8	20.4	20.3	19.8	21.6	22.9	23.7	17.6	18.2	17.2	21.0	9.6	8.8	8.8	13.4	13.4	16.4	16.1	14.1	15.7	20.2	21.4	22.6	19.1	19.0	24.6	
DQ256015_Public_Chydaekata 'sp. D TLF-2008'	23.4	24.2	26.3	21.0	22.3	21.1	22.8	24.6	24.6	24.5	25.4	25.1	23.1	21.9	22.2	18.1	18.8	21.1	22.5	19.6	19.9	20.0	14.6	14.7	13.8	13.4	0.0	16.1	15.7	16.3	14.4	20.7	22.9	23.4	20.4	21.4	25.4		
DQ256012_Public_Chydaekata 'sp. D TLF-2008'	23.4	24.2	26.3	21.0	22.3	21.1																																	

3.2 Cyclopoida

The two successful cyclopoid sequences formed one new OTU. Analysis of the phylogenetic tree suggests that this OTU can be placed within the *Halicyclops* genus, given the strong node support and clustering of this taxonomic group (Figure 3.2). *Halicyclops* `sp. Biologic-CYCL099` was closest related to several Cyclopoida sp. TB-2009 sequences accessed from GenBank (19% divergent, Table 3.3) although upon investigation no further taxonomic resolution could be obtained from these sequences. The OTU was only found from one location and had less than 1% intraspecific divergence (Table 3.3).

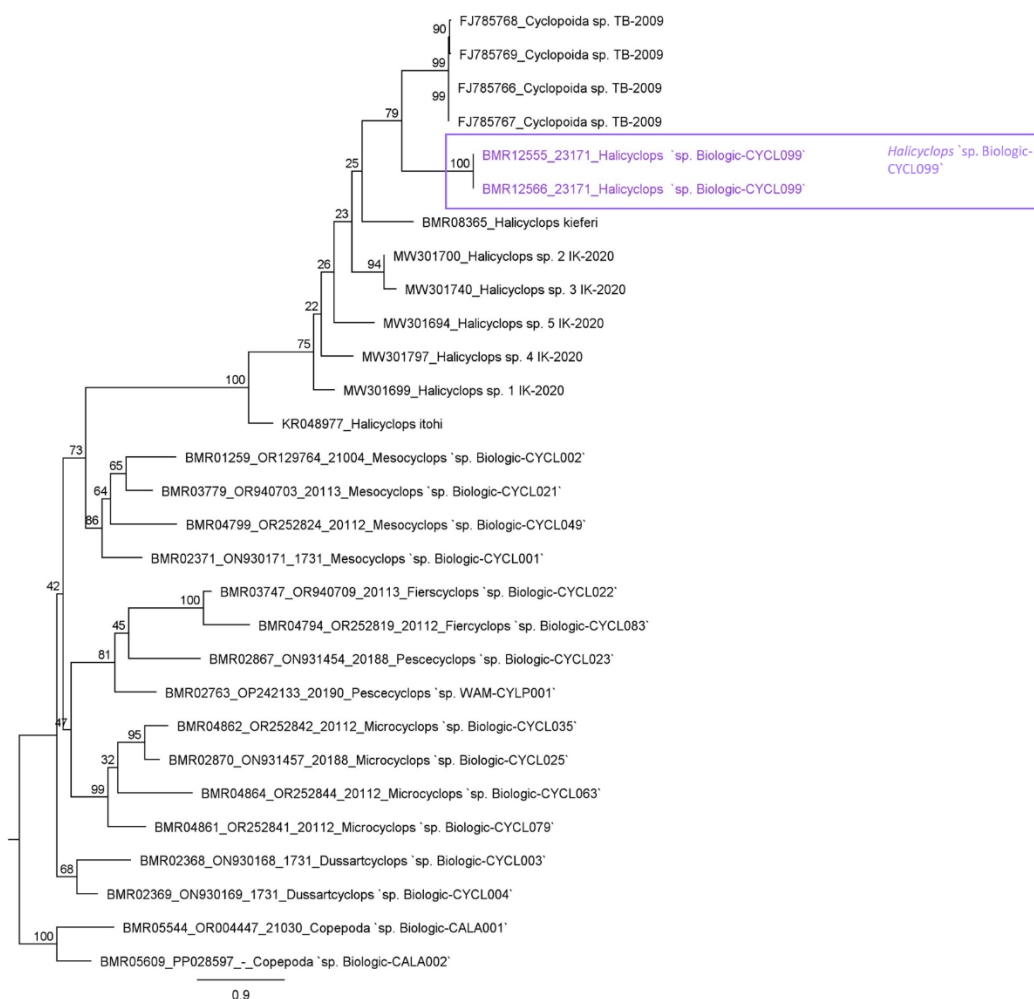


Figure 3.2. Phylogeny for the Cyclopoida dataset, with bootstrap values

Table 3.3: Pairwise distances (%) for the Cyclopoida dataset

COI Pairwise Distances (%)	BMR01259	BMR03779	BMR02371	BMR04799	BMR02368	BMR02369	BMR02870	BMR04862	BMR04861	BMR04864	BMR02763	BMR05544	BMR05609	BMR03747	BMR04794	BMR02867	BMR08365	MW301700	MW301740	MW301694	MW301699	MW301797	BMR12555	BMR12566	FJ785766	FJ785767	FJ785769	FJ785768	KR048977
BMR01259_OR129764_21004_Mesocyclops `sp. Biologic-CYCL002`																													
BMR03779_OR940703_20113_Mesocyclops `sp. Biologic-CYCL021`	14.8																												
BMR02371_ON930171_1731_Mesocyclops `sp. Biologic-CYCL001`	15.9	15.2																											
BMR04799_OR252824_20112_Mesocyclops `sp. Biologic-CYCL049`	18.1	16.7	18.2																										
BMR02368_ON930168_1731_Dussartcyclops `sp. Biologic-CYCL003`	23.1	19.5	19.8	20.9																									
BMR02369_ON930169_1731_Dussartcyclops `sp. Biologic-CYCL004`	21.1	19.5	19.1	20.9	15.2																								
BMR02870_ON931457_20188_Microcyclops `sp. Biologic-CYCL025`	21.6	20.7	20.1	21.8	21.3	18.8																							
BMR04862_OR252842_20112_Microcyclops `sp. Biologic-CYCL035`	22.7	19.9	20.5	24.9	23.4	20.2	12.0																						
BMR04861_OR252841_20112_Microcyclops `sp. Biologic-CYCL079`	20.3	19.8	20.5	21.6	20.3	18.5	17.6	18.5																					
BMR04864_OR252844_20112_Microcyclops `sp. Biologic-CYCL063`	20.9	22.0	23.0	22.0	23.9	22.5	19.3	20.4	18.1																				
BMR02763_OP242133_20190_Pescecyclops `sp. WAM-CYLP001`	23.6	23.1	23.0	25.6	21.6	21.1	24.2	23.8	22.7	21.8																			
BMR05544_OR004447_21030_Copepoda `sp. Biologic-CALA001`	24.4	22.9	20.8	25.8	23.7	22.3	24.9	23.9	23.1	24.6	25.3																		
BMR05609_PP028597_ Copepoda `sp. Biologic-CALA002`	22.9	20.9	21.2	23.6	23.6	19.8	22.9	24.4	20.9	24.2	25.6	18.5																	
BMR03747_OR940709_20113_Fierscyclops `sp. Biologic-CYCL022`	25.8	24.9	25.5	25.8	25.5	23.1	24.5	24.0	24.7	27.1	22.5	26.9	27.5																
BMR04794_OR252819_20112_Fierscyclops `sp. Biologic-CYCL083`	27.3	27.1	26.6	28.2	27.8	26.4	27.3	26.4	26.1	26.6	24.1	29.8	30.3	17.0															
BMR02867_ON931454_20188_Pescecyclops `sp. Biologic-CYCL023`	26.1	26.1	23.9	28.1	25.6	24.1	25.5	25.6	27.9	25.6	23.9	29.1	29.8	23.5	25.3														
BMR08365_Halicyclops kieferi	25.6	25.9	24.6	26.9	29.0	25.9	27.7	30.0	28.0	29.2	26.2	28.6	28.9	29.3	32.8	29.6													
MW301700_Halicyclops sp. 2 IK-2020	26.0	25.3	24.7	29.2	28.4	25.7	26.3	25.9	27.4	27.6	28.5	28.2	28.3	26.9	31.4	29.6	17.5												
MW301740_Halicyclops sp. 3 IK-2020	27.2	26.8	28.0	29.5	31.4	27.2	29.1	28.7	26.8	27.2	31.8	28.7	28.4	28.4	31.4	32.2	18.8	6.9											
MW301694_Halicyclops sp. 5 IK-2020	27.4	26.6	25.2	25.5	26.2	24.1	26.0	25.6	27.1	24.7	25.5	26.2	25.8	27.0	30.6	30.3	18.4	18.3	18.0										
MW301699_Halicyclops sp. 1 IK-2020	26.2	26.8	25.4	27.6	26.8	25.3	26.8	27.4	27.4	28.3	28.3	28.7	27.4	28.3	30.0	28.2	20.3	17.9	18.4	15.9									
MW301797_Halicyclops sp. 4 IK-2020	28.4	27.6	27.6	28.7	29.9	28.0	28.7	29.9	29.9	28.4	26.1	28.7	26.1	29.5	30.7	30.7	19.5	19.5	20.7	19.2	16.5								
BMR12555_23171_Halicyclops `sp. Biologic-CYCL099`	26.7	26.6	28.6	28.6	31.0	28.3	29.3	28.9	29.2	28.9	29.8	28.4	29.3	26.0	29.9	22.5	23.0	24.5	24.6	23.5	24.1								
BMR12566_23171_Halicyclops `sp. Biologic-CYCL099`	26.4	26.5	28.5	28.4	30.9	28.1	29.2	29.2	28.6	29.0	28.6	29.7	28.2	29.2	25.9	29.7	22.4	22.9	24.5	24.4	24.1	0.2							
FJ785766_Cyclopoida sp. TB-2009	26.2	28.1	28.0	26.7	29.4	26.7	28.6	29.1	28.5	29.1	29.1	29.4	26.5	28.3	30.3	30.3	22.0	20.8	24.1	22.5	21.9	21.1	19.4	19.2					
FJ785767_Cyclopoida sp. TB-2009	26.0	27.9	28.0	26.4	29.5	26.6	28.6	29.2	28.4	28.7	29.1	29.2	26.4	28.4	30.3	30.3	21.8	20.8	24.1	22.5	21.9	21.1	19.3	19.1	0.0				
FJ785769_Cyclopoida sp. TB-2009	26.4	28.6	28.6	26.7	29.5	26.8	28.3	29.5	28.6	29.2	28.9	29.9	26.4	27.8	30.7	30.3	21.8	21.0	23.0	22.5	22.1	19.9	20.1	19.9	1.3	1.3			
FJ785768_Cyclopoida sp. TB-2009	26.0	27.9	28.3	27.1	29.7	26.8	28.6	29.2	28.2	28.7	28.9	29.5	26.7	28.7	30.3	29.9	22.2	21.0	23.4	22.9	21.9	20.7	19.6	19.4	1.5	1.4	1.4		
KR048977_Halicyclops itohi	24.6	22.9	23.7	23.6	25.6	22.9	24.9	24.9	22.4	26.3	26.3	28.7	24.3	27.0	29.9	30.4	23.8	21.7	23.8	21.4	19.6	21.5	22.9	22.9	24.3	24.3	24.5	24.3	

3.3 Harpacticoida

Thirty-three harpacticoid sequences resolved into two OTUs, one new and one matching an existing Biologic OTU. *Cletocamptus* sp. Biologic-HARP063 includes two sequences from this study and a sequence from the unpublished Biologic sequence library. The location of the matching sequence cannot be shared with the Client, but it was included in the phylogenetic tree for ease of interpretation (Figure 3.3). The intraspecific divergence of this OTU was small (1.2%, Table 3.4), with a large linear distribution of 347 km (Table 3.1), the matching sequence being from the Pilbara region of Western Australia.

Harpacticoida sp. Biologic-HARP087 comprised thirty-one sequences from this study. The OTU had a small linear range of 5km (Table 3.1) and a maximum of 5% intraspecific variation (Table 3.4). Although a range of sequences from representative harpacticoid families likely for the area were included in the analysis, the OTU was conservatively retained at order level. The topology of the phylogenetic tree may indicate potential for this OTU to be placed within the Ameiridae family (Figure 3.3) however further morphologic information and/or more sequence data would be valuable to confirm this.

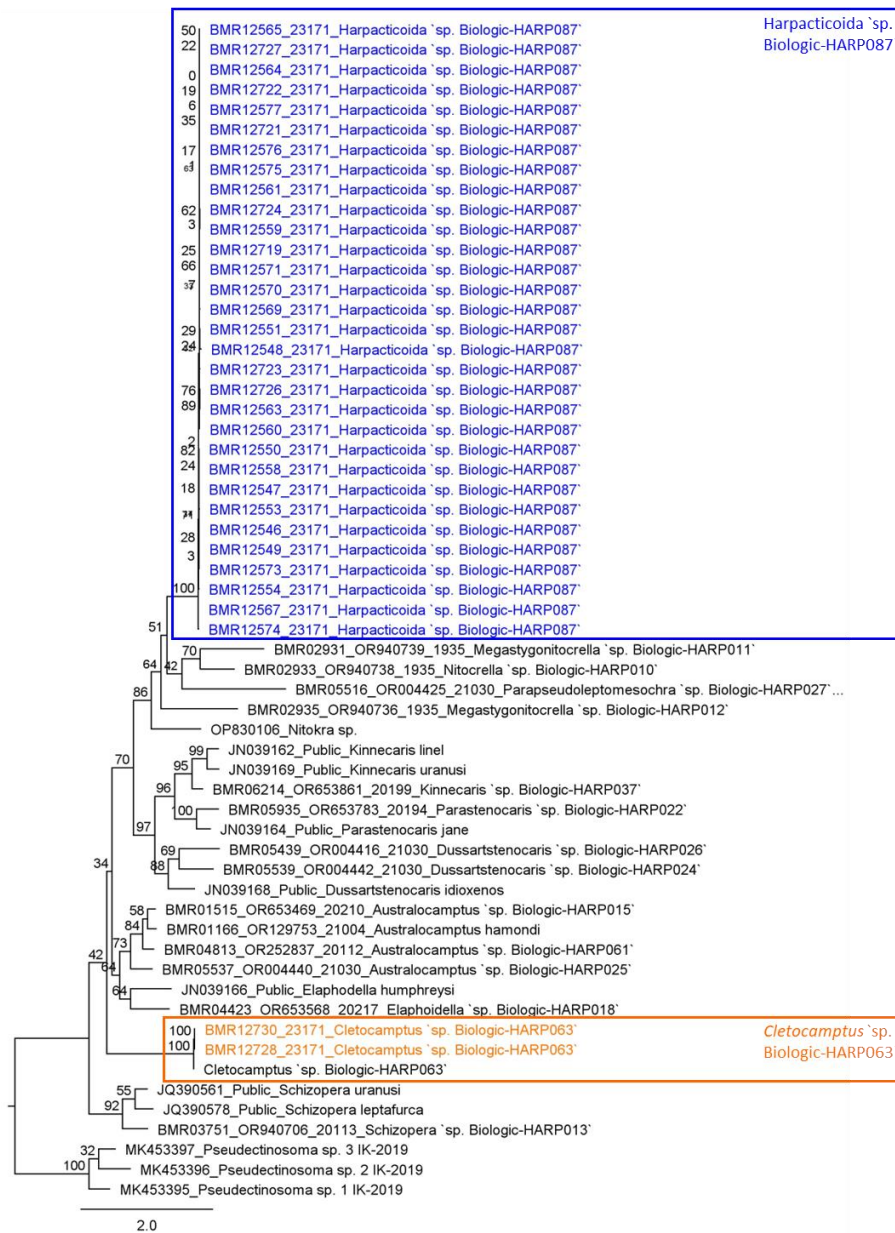


Figure 3.3. Phylogeny for the Harpacticoida dataset, with bootstrap values

4 Summary

Using well-established DNA extraction and sequencing methods, this molecular systematics analysis designated five distinct OTU/species to 40 high quality sequences from the Study Area. All OTUs, the areas in which they were found, and the specimen numbers per OTU are shown in Appendix A. The following are the key findings at the OTU/species level:

- Amphipoda (COI): 2 OTUs, both unique lineages,
- Cyclopoida (COI): 1 unique OTU, and
- Harpacticoida (COI): 2 OTUs, 1 unique lineage, 1 matching external sequences.

5 References

- Edgecombe, G. D., Huey, J. A., Humphreys, W. F., Hillyer, M., Burger, M. A., Volschenk, E. S., & Waldock, J. M. (2019). Blind scolopendrid centipedes of the genus *Cormocephalus* from subterranean habitats in Western Australia (Myriapoda: Scolopendromorpha: Scolopendridae). *Invertebrate Systematics*, 3(6), 807-824.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5), 294-299.
- Framenau, V. W., Hamilton, Z. R., Finston, T., Humphreys, G., Abrams, K. M., Huey, J. A., & Harvey, M. S. (2018). Molecular and morphological characterization of new species of hypogean *Paradraculoides* (Schizomida: Hubbardiidae) from the arid Pilbara bioregion of Western Australia. *Journal of Arachnology*, 46, 507-537. doi:<http://zoobank.org:8080/References/11121056-B8A3-486A-9CFA-0C248701D4F4>
- Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B*, 270(1512), 313-321.
- Hebert, P. D., Ratnasingham, S., & deWaard, J. R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B*, 270 Suppl 1, S96-99.
- Huey, J. A., Hillyer, M. J., & Harvey, M. S. (2019). Phylogenetic relationships and biogeographic history of the Australian trapdoor spider genus *Conothele* (Araneae: Mygalomorphae: Halonoproctidae): diversification into arid habitats in an otherwise tropical radiation. *Invertebrate Systematics*, 33(4), 628-643.
- Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30(14), 3059-3066.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., . . . Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647-1649.
- Perina, G., Camacho, A. I., Huey, J., Horwitz, P., & Koenders, A. (2018). Understanding subterranean variability: the first genus of Bathynellidae (Bathynellacea, Crustacea) from Western Australia described through a morphological and multigene approach. *Invertebrate Systematics*, 32, 423-447.
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, 87(6), 651-701.
- Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312-1313.

Appendix A: Specimen Data

BMR	Unique ID code	Site	Dec_Lat	Dec_Long	Lowest_ID_Legacy	OTU_Name	Reaction_State
Malacostraca							
Amphipoda: Paramelitidae							
BMR12543	BC_LN00939	YRRD019	-24.5150	116.2755	Amphipoda		FAIL; PCR
BMR12557	BC_LN01075	YIN Bore 01	-24.4686	116.2851	?Chydaekata sp. YIN01	Paramelitidae `sp. Biologic-AMPH094`	PASS
BMR12562	BC_LN01076	YIN Bore 01	-24.4686	116.2851	?Chydaekata sp. YIN01	Paramelitidae `sp. Biologic-AMPH095`	PASS
BMR12572	BC_LN01071	YRRD101	-24.5112	116.2726	?Chydaekata sp. YIN01	Paramelitidae `sp. Biologic-AMPH095`	PASS
BMR12578	BC_LN01072	YRRD217	-24.5072	116.2790	?Chydaekata sp. YIN01	Paramelitidae `sp. Biologic-AMPH095`	PASS
BMR12729	BC_LN01074	YRRD217	-24.5072	116.2790	?Chydaekata sp. YIN01	Paramelitidae `sp. Biologic-AMPH095`	PASS
Maxillopoda							
Cyclopoida							
BMR12545	BC_LN01079	YRRD222	-24.5006	116.2625	Cyclopoida sp. YIN01		FAIL; bad seq
BMR12555	BC_LN01077	YRRD147	-24.4990	116.2625	Cyclopoida sp. YIN01	<i>Halicyclops</i> `sp. Biologic-CYCL099`	PASS
BMR12566	BC_LN01078	YRRD147	-24.4990	116.2625	Cyclopoida sp. YIN01	<i>Halicyclops</i> `sp. Biologic-CYCL099`	PASS
BMR12568	BC_LN01080	YRRD147	-24.4990	116.2625	Cyclopoida sp. YIN01		FAIL; bad seq
BMR12720	BC_LN01081	YRRD147	-24.4990	116.2625	Cyclopoida sp. YIN01		FAIL; bad seq
BMR12725	BC_LN01082	YRRD147	-24.4990	116.2625	Cyclopoida sp. YIN01		FAIL; bad seq
Harpacticoida							
BMR12544	BC_LN00792	23MARC01	-24.5428	116.1787	Harpacticoida sp. YIN01		FAIL; bad seq
BMR12546	BC_LN00860	MARC012	-24.5110	116.2760	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12547	BC_LN00861	MARC012	-24.5110	116.2760	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12548	BC_LN00853	YIN Bore 01	-24.4686	116.2851	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12549	BC_LN00846	YRRD025	-24.5134	116.2802	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12550	BC_LN00847	YRRD025	-24.5134	116.2802	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12551	BC_LN00866	YRRD036	-24.5056	116.2671	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12552	BC_LN00867	YRRD036	-24.5056	116.2671	Harpacticoida sp. YIN01		FAIL; bad seq
BMR12553	BC_LN00849	YRRD099	-24.5030	116.2671	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12554	BC_LN00850	YRRD099	-24.5030	116.2671	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS

BMR	Unique ID code	Site	Dec_Lat	Dec_Long	Lowest_ID_Legacy	OTU_Name	Reaction_State
BMR12556	BC_LN00869	YRRD101	-24.5112	116.2726	Harpacticoida sp. YIN01		FAIL; PCR
BMR12558	BC_LN00870	YRRD101	-24.5112	116.2726	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12559	BC_LN01083	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12560	BC_LN01084	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12561	BC_LN01085	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12563	BC_LN01086	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12564	BC_LN01087	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12565	BC_LN01088	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12567	BC_LN00854	YRRD164	-24.5041	116.2771	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12569	BC_LN00855	YRRD164	-24.5041	116.2771	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12570	BC_LN01297	YRRD190	-24.5034	116.2707	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12571	BC_LN01298	YRRD190	-24.5034	116.2707	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12573	BC_LN00857	YRRD217	-24.5072	116.2790	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12574	BC_LN00858	YRRD217	-24.5072	116.2790	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12575	BC_LN01303	YRRD222	-24.5006	116.2625	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12576	BC_LN01304	YRRD222	-24.5006	116.2625	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12577	BC_LN01300	YRRD248	-24.5027	116.2664	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12719	BC_LN01301	YRRD248	-24.5027	116.2664	Harpacticoida sp. YIN01	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12721	BC_LN01093	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN02	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12722	BC_LN01094	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN02	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12723	BC_LN01095	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN02	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12724	BC_LN01090	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN02	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12726	BC_LN01091	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN02	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12727	BC_LN01092	YRRD147	-24.4990	116.2625	Harpacticoida sp. YIN02	Harpacticoida `sp. Biologic-HARP087`	PASS
BMR12728	BC_LN00863	Victory Bore	-24.5938	116.1390	Harpacticoida sp. YIN03	<i>Cletocamptus</i> `sp. Biologic-HARP063`	PASS
BMR12730	BC_LN00864	Victory Bore	-24.5938	116.1390	Harpacticoida sp. YIN03	<i>Cletocamptus</i> `sp. Biologic-HARP063`	PASS