



Winu Project Rimfire Borefield Stygofauna Assessment



Prepared for

Rio Tinto Winu Pty Ltd

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Biota
Environmental
Sciences



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Rimfire Stygofauna Assessment

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1.0 Executive Summary

Rio Tinto Winu Pty Limited (RTW) plans to develop a new copper-gold mine, referred to as the Winu Project, approximately 320 km east of Port Hedland in the Pilbara region of Western Australia, within both the Nyangumarta and Martu Peoples' Native Title Determination Areas.

Considerable biological survey work has been completed to support the environmental impact assessment of the proposal. The current study provides an assessment of the potential stygofauna values of the Winu Project's Rimfire borefield area ('the survey area') and reports on seven phases of stygofauna sampling there. Prior to the survey effort detailed in this report, six phases of stygofauna sampling have previously been completed in other areas of the Winu Project.

A desktop study was undertaken to review previous records from the locality and to compile a list of stygofauna species potentially occurring in the survey area. This included database searches and a review of publicly available literature. The desktop study revealed a paucity of stygofauna surveys within the Winu locality. Where stygofauna have been recorded within the Great Sandy Desert, the host geological units are reported as alluvial or calcrete units associated with paleovalleys.

A review of hydrogeological and geological information relevant to the survey area was undertaken and the prospectivity of the geological units present at Rimfire to act as stygofauna habitat was determined to be low-moderate. Shallow aquifers within paleovalley settings were identified as representing the most prospective stygofauna habitat in the region.

This report details seven phases of stygofauna sampling at the Rimfire borefield. Three phases were undertaken of 12 drillholes in May, August and November 2024. A further 27 drillholes were then drilled in 2024 and subsequently sampled across four phases in March, May, August and November 2025.

Five orders of stygobitic crustaceans were recorded from the 2024 and 2025 stygofauna sampling at Rimfire. This total was represented by 22 molecular taxa, comprising; one amphipod taxa, two isopod taxa, 14 copepod taxa (12 harpacticoid and two cyclopoid) and five oligochaete taxa. Sixteen of the 22 taxa were only recorded from single sites. The records were all within or close to the Percival paleodrainage tributary, consistent with the available regional reference data.

Two groundwater abstraction cases for the Rimfire borefield are considered in this impact assessment:

1. Project water demand case (Expected water demand with a P90 level of confidence) – this represents a conservative estimate of the actual expected drawdown based on predicted water demand through the life of mine. A decreasing drawdown from 7 m to 2 m is predicted over a distance of approximately 6.4 - 8.8 km from the centre of the borefield. This would impact five of the 17 locations where stygofauna have been recorded. At these five locations the following six stygofauna taxa were recorded: three harpacticoid copepod species and three enchytraeid oligochaete worms (one of which is widespread). The remaining 16 stygofauna taxa recorded will be outside of the area of impact (< 2 m expected drawdown) under the Project water demand case.
2. Project licensing case (Maximum annual abstraction) – this represents a worst-case scenario where 2.8 GL may be required in a given year, assuming this happens every year for the life of mine, which is very unlikely, and has only been included as a conservative impact assessment. Here, the drawdown contours decrease from 10 m to 2 m across a

distance of approximately 11 to 17 km from the centre of the borefield. This would impact 15 of the 17 locations where stygofauna have been recorded. Eighteen stygofauna taxa have been recorded from these 15 locations, one of which has also been recorded from a site outside of the 2 m drawdown contour), and a further two are known to have a distribution beyond the Winu locality.

Of the two abstraction cases, the Project water demand case represents the most likely scenario for the implementation of the Winu Project, while the Project licensing case represents a 'worse-case scenario' and therefore has a greater impact on the paleovalley tributary. At most, the Project licensing case has potential to remove up to one-third of available saturated habitat at the single location where paleovalley lithology has been identified. Spatially, this case has potential to impact up to approximately 38 % of the length of the tributary (as mapped by Bell (2012)). However, stygofauna utilise a three-dimensional habitat below the water table, meaning that while a plan-view spatial assessment is useful, habitat impacts must consider that groundwater drawdown attenuates with distance from the borefield – this results in an increasing proportion of stygofauna habitat predicted to remain saturated at sites further southwest down the paleovalley tributary. Continuous suitable habitat extends southwards beyond the extent of the proposed groundwater drawdowns and a substantial volume of the paleovalley habitat in the tributary would remain unaffected by even the worst-case Project licensing case.

2.0 Introduction

2.1 Project Background

Rio Tinto Winu Pty Limited (RTW) plans to develop a new copper-gold mine, referred to as the Winu Project. This is located approximately 320 km east of Port Hedland in the Pilbara region of Western Australia, within both the Nyangumarta and Martu Peoples' Native Title Determination Areas. Key terminology for the Winu Project is defined in Table 2.1 and the location of the Winu Project is presented in Figure 2.1.

Table 2.1: Key terminology for the Winu Project.

Term	Definition
The project	Includes the development of a new below-water table copper -gold deposit and associated infrastructure and activities required to access, process and transport ore.
Development Envelope (DE)	Refers to the combined areas of the mine and infrastructure area, road access corridor and regional borefield, Rimfire. All activities will occur within this area.
Mine and Infrastructure Area (MIA)	Refers to the area containing the mineralised deposit and related infrastructure. This is the Winu mine site.
Road Access Corridor (RAC)	Refers to the Access Road, which encompasses the entire length of the existing Access Road from Great Northern Highway to the Winu Project Area.
Rimfire Borefield (Rimfire)	Refers to the regional borefield located at Rimfire, which is approximately 31 km from the MIA.

Considerable biological survey work has been completed to support the environmental impact assessment (EIA) of the proposal. As part of the original subterranean fauna assessment for the project, a desktop study was completed, along with two phases of troglofauna sampling and three phases of stygofauna sampling from 2019-2021, focussing on the MIA (Biota 2020, 2022a). No subterranean fauna were recorded.

The next phase of development for the project involved the evaluation of additional water sources to supply the project's operations. Desktop studies in relation to stygofauna for the potential borefield options were undertaken (Biota 2022b, 2023a, 2023b), as well as stygofauna sampling of available drillholes (Biota 2025).

The current study provides an assessment of the potential stygofauna values of the Rimfire borefield (hereafter, 'the survey area').

2.2 Purpose of this Report

The purpose of this report is to consolidate and review the existing data of relevance to stygofauna and stygofauna habitat in the survey area, and to report on the five phases of stygofauna sampling completed. This assessment was undertaken in accordance with the following:

- Environmental Protection Authority (EPA) (2021): *Technical Guidance – Subterranean Fauna Surveys for Environmental Impact Assessment*; and
- EPA (2016): *Environmental Factor Guideline – Subterranean Fauna*.

2.3 Stygofauna Overview

2.3.1 Stygobitic Fauna

Stygobitic fauna are groups of aquatic organisms which live in subterranean groundwater. Stygal communities in Western Australia are predominantly Crustacean (e.g. Amphipoda, Isopoda, Decapoda, Syncarida, Ostracoda and Copepoda); however, Annelida (e.g. Oligochaeta), Arachnida (e.g. Hydracarina) and Platyhelminthes are also commonly collected. In addition, three species of stygal vertebrates have been recorded from Western Australia, comprising two species of blind gudgeon (*Milyeringa veritas* and *M. justitia*) and a Blind Cave Eel, *Ophisternon candidum* (Humphreys 2001a, Larson et al. 2013).

2.3.2 Stygobitic Fauna Habitat

The suitability of any geological formation as habitat for subterranean fauna is predominantly determined by the availability of space and potential for nutrient infiltration.

In the case of stygofauna, this is further refined to where such rock formations occur below the water table. Several geological units are recognised as being core habitat for stygofauna as they characteristically form fractures, caverns and vugs, which allow for a greater rate of fauna dispersal and nutrient penetration. Stygofauna occur in all types of aquifers that have suitably sized voids: they occur variously in epikarst, limestone karst, pseudokarst (often sandstone, lava or laterite), fractured rock, calcretes and pisolites, and they occur widely in alluvial aquifers, except where the size of the interstices may limit the distribution of species (Humphreys 2009).

In Western Australia, diverse stygofauna assemblages have been recognised in the calcrete aquifers of the Yilgarn and Pilbara regions (Humphreys 2001b). In the Pilbara, non-karst environments have also been recognised as important habitats for subterranean fauna, notably banded iron formations, alluvial deposits and fractured rock aquifers (EPA 2012).

2.3.3 Conservation Significance

Protection for significant subterranean fauna species or communities is provided under State and Federal environmental legislation, namely the:

- *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act); and the
- *Biodiversity Conservation Act 2016* (BC Act).

The Department of Biodiversity, Conservation and Attractions (DBCA) also maintains a list of Priority species that have not been assigned statutory protection under the BC Act.

Subterranean fauna are evolutionary relics persisting from previous climatic conditions, and may be considered significant due to (but not limited to) the following reasons; being a new species, being locally endemic, being listed as threatened or priority species, occupying restricted habitats, and occurring as part of a Threatened or Priority Ecological Community (EPA 2016). Significant species are known from Barrow Island, Cape Range, Robe Valley and Bungaroo Valley; all recognised hotspots of diversity for subterranean fauna with WA. As new subterranean fauna species are routinely being discovered and for some groups taxonomic frameworks are not yet well developed, many fauna species remain undescribed and are often referred to as “data deficient”. These species may be classified into a morphospecies (based on morphology), or a putative species (based on genetic analysis). This incomplete knowledge can make assessment of their true conservation status difficult.

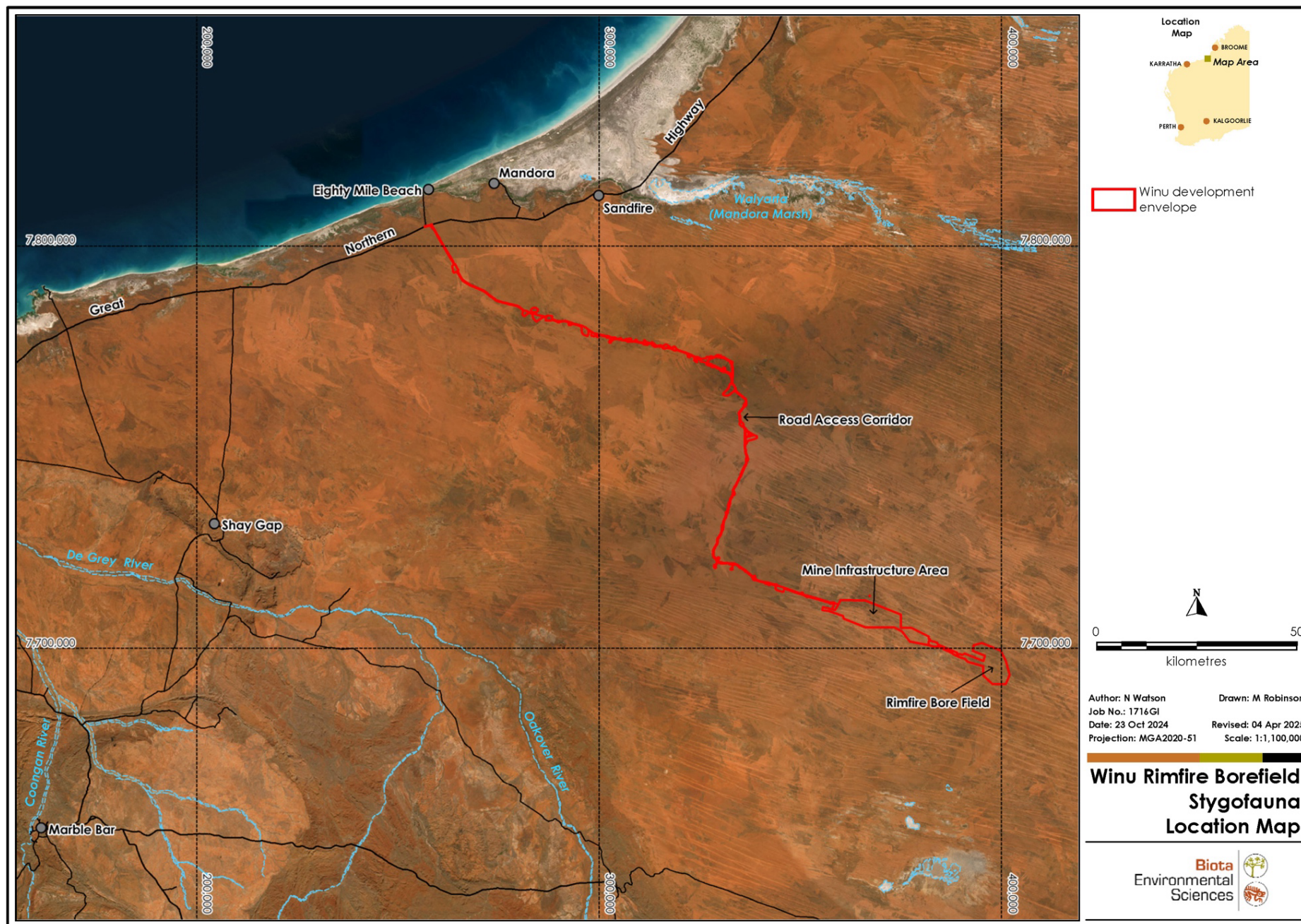


Figure 2.1: Location of the Winu Project and Rimfire borefield.

3.0 Methods

3.1 Database Searches

Database searches were undertaken in order to review previous records from the locality and to compile a list of species potentially occurring in the survey area. Based on the findings from previous desktop studies for Winu (Biota 2020, 2022a, 2022b), a large search area was used for the current study (a 200 km by 200 km square: 120.9044411, -19.96784685 top left; 122.8403847, -21.76179618 bottom right). This desktop search area was used to query the following databases:

- Dandjoo – DBCA’s biodiversity data repository;
- Index of Biodiversity Surveys for Assessments (IBSA) - data from biodiversity survey reports that support assessments and compliance under the Environmental Protection Act 1986 (the EP Act);
- The Western Australian Museum’s Crustacea and Worms database;
- the Atlas of Living Australia (ALA) database;
- Biota’s internal database of subterranean fauna records from surveys in the locality; and
- the Commonwealth EPBC Act Protected Matters search tool.

The records from these sources were collated and filtered for invertebrate groups known to represent stygobitic fauna. A discussion of previous records of stygofauna from within the desktop search area is presented in Section 4.8.

3.2 Literature Review

A review of publicly available literature was undertaken in 2022, focussing on previous stygofauna surveys conducted within Western Australian desert environs within the past 10 years (Biota 2022b). No new published surveys (except for those at Winu) were found to have been undertaken since that review.

3.3 Habitat Characterisation

Hydrogeological and geological information for the survey area was reviewed in order to identify potentially prospective geological units that may function as suitable habitat for stygofauna.

This information was used to appraise the prospectivity of the survey area to support stygofauna communities. The likely habitats for stygofauna were initially characterised using a combination of regional information and site-specific geological data sourced from drill logs (provided by RTW). Inputs considered in this process, where available, included:

- regional surface geology mapping;
- hydrological and hydrogeological information, including the continuity of the aquifer and transmissivity of the aquifer substrate;
- stratigraphic logging and information from drillholes in the study area;
- previous sampling effort in the locality and the results of published and unpublished studies on stygobitic fauna communities; and

- identification of rock types that have previously yielded stygofauna records from the study area and wider locality.

Given the above inputs, the habitat prospectivity of geological units present within the survey area was assessed. The following characteristics were used in determining likelihood categories:

- A) Presence of interstitial spaces or vugs.
- B) Continuity and transmissivity of the local occurrence of submerged geological units.
- C) The known occurrence of stygal communities from equivalent rock types during previous surveys.
- D) Absence of large amounts of fine sediments such as clays, silts and sands within the geological unit description.
- E) Substrate permits inflow of surface water and nutrients infiltration.
- F) Substrate submerged below the water table.

Following the assessment undertaken above, the geological units were assigned by Biota to a Low, Moderate or High prospectivity, based on the criteria outlined in Table 3.1.

Table 3.1: Criteria used to assess habitat prospectivity of geological units.

Likelihood	Criteria
Low	Suitable geological unit may occur only above the water table within the study area. Rock type might have interstitial spaces (A) however may have high levels of fine sediments which reduce usability of spaces. Stygofauna not known from previous studies sampling the same geology (C).
Moderate	Unit likely partially or completely submerged below the water table (F). Presence of interstitial spaces (A), low numbers of stygofauna have been recorded from this geology previously (C). Small amounts of fine sediments within the unit (D).
High	Majority (five or six) categories confirmed for the geological unit, including the presence of continuous, transmissive aquifer (A, B). Geology sufficiently porous to allow nutrient infiltration from surface water runoff (E). Stygofauna routinely recorded from same rock type (C) and partially or completely submerged below the water table (F).

3.4 Field Survey

3.4.1 Timing and Personnel

Seven phases of stygofauna sampling were undertaken in the survey area across 2024 and 2025 (Table 3.2). The work was undertaken under Regulation 27: Fauna Taking (Biological Assessment) Licences BA27000828 and BA27001925.

All sampling was completed by qualified and suitably experienced Biota zoologists. We acknowledge the support and guidance of all Traditional Owner rangers who participated in and assisted with surveys on country.

Table 3.2: Summary of stygofauna surveys at Rimfire.

Phase	Dates	Personnel
1	6-8/05/2024	Michael Greenham (Senior Zoologist) Hayley Winter (Zoologist)
2	21-25/08/2024	Michael Greenham (Senior Zoologist) Melanie McGellin (Senior Zoologist)

Phase	Dates	Personnel
3	25-30/11/2024	Michael Greenham (Senior Zoologist) Hayley Winter (Zoologist)
4	11-12/03/2025	Michael Greenham (Senior Zoologist) Ryan Boyer (Biologist)
5	19-23/05/2025	Michael Greenham (Senior Zoologist) Hayley Winter (Zoologist)
6	11-18/08/2025	Michael Greenham (Senior Zoologist) Madeline West (Biologist)
7	17-24/11/2025	Michael Greenham (Senior Zoologist) Sylvie Schmidt (Principal Zoologist)

3.4.2 Effort

3.4.2.1 2024 Survey Effort

In 2024, three phases of stygofauna sampling was undertaken of 12 reverse-circulation (RC) drillholes that were drilled specifically for stygofauna sampling in October 2023 (Table 3.3; Figure 3.1). Two sites (WINU1380 and WINU1381) were also sampled on 01/03/2024 before the original planned survey was demobilised due to heavy rain. Sampling was also undertaken at two historical bores located within the Percival paleovalley; AKD10, located 2.5 km south of Rimfire, and 'Old Bore', approximately 125 km southeast of Rimfire. Old Bore was identified during the desktop study as a potential reference site due to its location within the Percival paleovalley and having had stygofauna recorded from it previously (Bennelongia 2012). Table 3.3 details the three phases of stygofauna sampling at Rimfire in 2024.

3.4.2.2 2025 Survey Effort

An additional 27 RC drillholes were drilled in the survey area specifically for stygofauna sampling during October - December 2024. Fifteen of these (the most southerly sites) were sampled in March 2025 (representing Phase 4 at Rimfire) and all 27 (northern and southern sites) were sampled in May 2025 (Phase 5), August 2025 (Phase 6) and November 2025 (Phase 7). Table 3.4 details the four phases of stygofauna sampling at Rimfire in 2026.

Table 3.3: Rimfire 2024 stygofauna sampling sites (GDA 1994; MGA Zone 51).

Site	Easting (m)	Northing (m)	Drillhole stratigraphy* (unit intersecting water table underlined)	Sample Notes
WINU1371	397729	7695179	0 – 7 m: Aeolian sand 7 – 14 m: Ferricrete 14 – 17.5 m: Silcrete <u>17.5 – 70 m: Felsic intrusive</u>	Very sedimented
WINU1372	396781	7694720	0 – 8 m: Ferricrete 8 – 26 m: Silcrete <u>26 – 41 m: Claystone</u> 41 – 58 m: Mafic intrusive	Sedimented
WINU1373	391386	7695600	0 – 7 m: Aeolian sand 7 – 28 m: Ferricrete <u>28 – 42 m: Sandstone</u> 42 – 70 m: Gneiss	Sedimented

Site	Easting (m)	Northing (m)	Drillhole stratigraphy* (unit intersecting water table underlined)	Sample Notes
WINU1374	393007	7695728	0 – 3.5 m: Aeolian sand 3.5 – 21 m: Ferricrete 21 – 31 m: Silcrete 31 – 33 m: Ferricrete 33 – 38 m: Sandstone <u>38 – 43 m: Claystone</u> 43 – 62 m: Oxidised Granite 62 – 70 m: Mafic intrusive	Very sedimented
WINU1375	391314	7698953	0 – 15 m: Ferricrete 15 – 17.5 m: Silcrete <u>17.5 – 52 m: Sandstone</u> 52 – 70 m: Oxidised Granite	Sedimented
WINU1377	394270	7698963	0 – 20 m: Ferricrete 20 – 31.5 m: Silcrete 31.5 – 42 m: Sandstone <u>42 – 56 m: Claystone</u> 56 – 70 m: Fresh metasediment	Very sedimented
WINU1378	391988	7696680	0 – 7 m: Ferricrete 7 – 34 m: Silcrete 34 – 35 m: Sandstone <u>35 – 70 m: Claystone</u>	Sedimented
WINU1379	397460	7695792	0 – 15 m: Aeolian sand 15 – 20 m: Silcrete <u>20 – 29 m: Sandstone</u> 29 – 70 m: Oxidised Granite	Very sedimented
WINU1380 ^ψ	395538	7700080	0 – 8 m: Ferricrete 8 – 14 m: Silcrete <u>14 – 48 m: Sandstone</u> 48 – 51 m: Claystone 51 – 56 m: Mafic intrusive	Very sedimented
WINU1381 ^ψ	396008	7699080	0 – 10 m: Ferricrete 10 – 42 m: Sandstone <u>42 – 53 m: Claystone</u> 53 – 70 m: Granite	Very sedimented
WINU1383	396145	7695498	0 – 14 m: Ferricrete 14 – 23 m: Silcrete <u>23 – 43 m: Gneiss</u> 43 – 52 m: Granite	Very sedimented
WINU1384	391425	7696204	0 – 2 m: Aeolian sand 2 – 15 m: Ferricrete 15 – 24 m: Silcrete 24 – 28 m: Ferricrete <u>28 – 40 m: Sandstone</u> 40 – 46 m: Mafic intrusive	Very sedimented
AKD10	395380	7690092	Unknown – historical bore Depth to water – 4m Total depth – 50 m	Clear
Old Bore [#]	400420	7568055	Unknown – historical bore Depth to water – 6 m Total depth – 39 m	Sedimented

* Hydrogeological details from Rio Tinto.

^ψ Sampled four times.[#] Phase 3 only.

Table 3.4: Rimfire 2025 stygofauna sampling sites (GDA 1994, MGA Zone 51; - = site not available, ✖ = site could not be sampled, ✓ = site sampled).

Site	Easting (m)	Northing (m)	Drillhole stratigraphy* (unit intersecting water table underlined)	Sample Notes	Phase 4	Phase 5	Phase 6	Phase 7
WINU1422	397421	7699453	0 – 4 m: Sand 4 – 30 m: Sandstone 30 – 32 m: Claystone <u>32 – 51 m: Weathered Metasediments</u> 52 – 58 m: Sandstone	Very sedimented	-	✓	✓	✓
WINU1423	397338	7696648	0 – 9 m: Ferricrete <u>9 – 35 m: Sandstone</u> 35 – 57 m: Metasediment 57 – 60 m: Granite 60 – 70 m: Sandstone	Very sedimented	-	✓	✓	✓
WINU1424	400300	7697106	0 – 6 m: Residual soil 6 – 20 m: Ferricrete <u>21 – 32 m: Sandstone</u> 32 – 37 m: Metasediment 37 – 48 m: Granite 48 – 70 m: Metasediment	Sedimented	-	✓	✓	✓
WINU1425	399737	7693530	0 – 2 m: Sand <u>2 – 25 m: Sandstone</u> 25 – 37 m: Granite 37 – 46 m: Metasediment	Very sedimented	-	✓	✓	✓
WINU1426	401742	7694069	0 – 3 m: Sand <u>3 – 29 m: Sandstone</u> 29 – 31 m: Claystone 31 – 52 m: Weathered Metasediments	Sedimented	-	✓	✓	✓
WINU1427	399773	7698409	0 – 4 m: Sand 4 – 12 m: Ferricrete <u>12 – 42 m: Sandstone</u> 42 – 46 m: Metasediment 46 – 48 m: Claystone 48 – 60 m: Metasediment	Sedimented	-	✓	✓	✓

Site	Easting (m)	Northing (m)	Drillhole stratigraphy* (unit intersecting water table underlined)	Sample Notes	Phase 4	Phase 5	Phase 6	Phase 7
WINU1436	393787	7693875	0 – 2 m: Aeolian sand 2 – 5 m: Ferricrete 5 – 17 m: Sandstone 17 – 25 m: Ferricrete <u>25 – 32 m: Granite</u> 32 – 36 m: MetaSandstone 36 – 40 m: Granite 40 – 48 m: MetaSandstone	Sedimented	x	✓	✓	✓
WINU1437	392776	7694259	0 – 8 m: Sand 8 – 12 m: Ferricrete 12 – 19 m: Siltstone <u>19 – 26 m: Sandstone</u> 26 – 42 m: Granite	Sedimented	x	✓	✓	✓
WINU1438	391732	7694637	0 – 4 m: Aeolian sand 4 – 12 m: Ferricrete <u>12 – 33 m: Sandstone</u> 33 – 36 m: Claystone 36 – 38 m: MetaSandstone 38 – 44 m: Granite 44 – 46 m: MetaSandstone 46 – 48 m: Granite	Sedimented	x	✓	✓	✓
WINU1439	392364	7693056	0 – 4 m: Aeolian sand 4 – 8 m: Ferricrete 8 – 22 m: Sandstone <u>22 – 42 m: Granite</u>	Very sedimented	x	✓	✓	✓
WINU1440	393248	7692883	0 – 5 m: Aeolian sand 5 – 10 m: Ferricrete <u>10 – 21 m: Sandstone</u> 21 – 36 m: Granite 36 – 48 m: MetaSandstone	Very sedimented	x	✓	✓	✓

Site	Easting (m)	Northing (m)	Drillhole stratigraphy* (unit intersecting water table underlined)	Sample Notes	Phase 4	Phase 5	Phase 6	Phase 7
WINU1441	393736	7692786	0 – 4 m: Aeolian sand 4 – 12 m: Ferricrete <u>12 – 22 m: Silcrete</u> 22 – 30 m: Sandstone 30 – 32 m: Silcrete 32 – 34 m: Ferricrete 34 – 38 m: Claystone 38 – 60 m: Granite	Very sedimented	x	✓	✓	✓
WINU1442	394280	7692589	0 – 2 m: Ferricrete 2 – 8 m: Conglomerate 8 – 13 m: Sandstone 13 – 16 m: Conglomerate <u>16 – 21 m: Sandstone</u> 21 – 29 m: Granite 29 – 36 m: Sandstone 36 – 48 m: Granite	Sedimented	x	✓	✓	✓
WINU1443	392312	7690973	0 – 4 m: Sand 4 – 8 m: Ferricrete 8 – 24 m: Sandstone <u>24 – 36 m: Claystone</u> 36 – 44 m: MetaSandstone 44 – 48 m: Granite	Sedimented	✓	✓	✓	✓
WINU1444	394636	7690313	0 – 2 m: Sand <u>2 – 8 m: Ferricrete</u> 8 – 40 m: Sandstone 40 – 48 m: Granite	Sedimented	✓	✓	✓	✓
WINU1445	395112	7690866	0 – 2 m: Ferricrete 2 – 6 m: Sandstone 6 – 20 m: MetaSandstone <u>20 – 24 m: Granite</u> 24 – 32 m: MetaSandstone 32 – 58 m: Granite 58 – 60 m: MetaSandstone	Very sedimented	✓	✓	✓	✓

Site	Easting (m)	Northing (m)	Drillhole stratigraphy* (unit intersecting water table underlined)	Sample Notes	Phase 4	Phase 5	Phase 6	Phase 7
WINU1446	395843	7689965	0 – 1 m: Sand 1 – 10 m: <u>Claystone</u> 10 – 22 m: Sandstone 22 – 29 m: <u>Claystone</u> 29 – 40 m: Sand 40 – 55 m: MetaSandstone 55 – 57 m: Granite 57 – 59 m: MetaSandstone 59 – 63 m: Granite 63 – 66 m: MetaSandstone	Sedimented	✓	✓	✓	✓
WINU1447	392110	7688507	0 – 19 m: <u>Sandstone</u> 19 – 27 m: <u>Claystone</u> 27 – 29 m: Sandstone 29 – 37 m: <u>Claystone</u> 37 – 66 m: MetaSandstone	Sedimented	✓	✓	✓	✓
WINU1448	391931	7687535	0 – 2 m: Sand 2 – 21 m: <u>Sandstone</u> 21 – 48 m: Granite	Very sedimented	✓	✓	✓	✓
WINU1449	391311	7686514	0 – 4 m: Sand 4 – 12 m: Sandstone 12 – 42 m: <u>Granite</u>	Very sedimented	✓	✓	✓	✓
WINU1450	392665	7686117	0 – 18 m: <u>Sandstone</u> 18 – 51 m: MetaSandstone 51 – 54 m: Granite	Sedimented	✓	✓	✓	✓
WINU1451	393915	7685779	0 – 4 m: Sand 4 – 5 m: Ferricrete 5 – 16 m: <u>Sandstone</u> 16 – 17 m: Siltstone 17 – 28 m: Sandstone 28 – 48 m: MetaSediment	Sedimented	✓	✓	✓	✓
WINU1452	394055	7686608	0 – 2 m: Sand 2 – 34 m: <u>Granite</u>	Sedimented	✓	✓	✓	✓

Site	Easting (m)	Northing (m)	Drillhole stratigraphy* (unit intersecting water table underlined)	Sample Notes	Phase 4	Phase 5	Phase 6	Phase 7
WINU1453	399837	7684105	0 – 2 m: Sand 2 – 3 m: MetaSandstone <u>3 – 16 m: Granite</u> 16 – 54 m: MetaSandstone	Sedimented	✓	✓	✓	✓
WINU1454	399807	7683679	0 – 2 m: Sand <u>2 – 16 m: Sandstone</u> 16 – 33 m: MetaSandstone 33 – 36 m: Granite 36 – 48 m: MetaSandstone	Sedimented	✓	✓	✓	✓
WINU1455	400883	7683814	0 – 14 m: Ferricrete <u>14 – 36 m: Granite</u>	Very sedimented	✓	✓	✓	✓
WINU1456	401944	7683380	0 – 19 m: Ferricrete <u>19 – 42 m: Granite</u>	Very sedimented	✓	✓	✓	✓
AKD10	395380	7690092	Unknown – historical bore As the construction details for AKD10 were not available, a camera was run down the hole as part of hydrogeological investigations. The hole was found to be blank cased 0 – 40.1 m, with an estimated 1 mm slotted casing 40 – 46 m. Bottom of hole was filled with sediment (likely due to lack of gravel in the annulus) (information provided by RTW). Depth to water – 4 m Total depth -50 m	Clear	✓	✗	✗	✗

* Hydrogeological details from Rio Tinto.

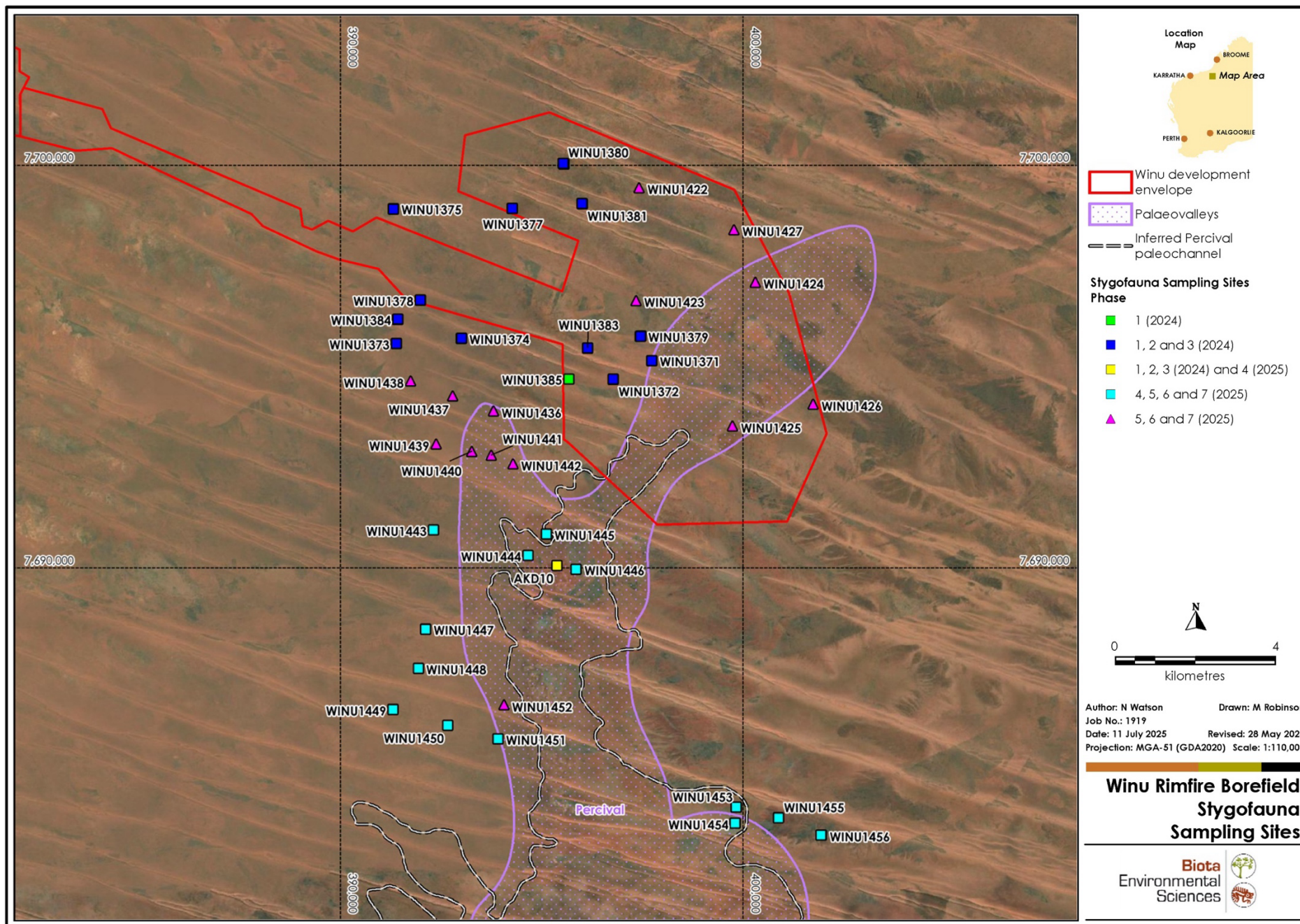


Figure 3.1: Stygofauna sampling sites in the survey area.

3.4.3 Sampling Methods

Stygobitic fauna were sampled using modified plankton haul nets, constructed from 70 μ m plankton mesh, with 50 mm and 100 mm apertures attached to a stainless steel catch cylinder. Nets were lowered to the bottom of drillholes before being slowly hauled through the water column to the surface, where the contents of the cylinder were flushed into a uniquely labelled container. Each bore was sampled in this way a total of six times. On the final haul the net was agitated gently to stir the benthos layer and mobilise any fauna present for more effective specimen collecting. Samples were stored in a shaded esky in order to keep the sample cool prior to sorting and identification.

3.4.4 Specimen Identifications

Fauna specimens, where collected, were identified to Order level using dissecting microscopes (Olympus SZ61, magnification up to 40x). Fauna specimens were then assigned a unique number based on drill hole name and location, and were tracked on customised data sheets. Specimens were preserved in 100% ethanol, which allowed for both morphological and molecular analysis.

3.4.4.1 Morphological Identifications

Morphological identifications to order level were completed during the field survey by Biota staff. Specialist identifications were undertaken by Jane McRae of Bennelongia Environmental Consultants.

3.4.4.2 Molecular Systematics and Genetic Analysis of Specimens

Molecular (DNA) analysis was undertaken to determine the number of species present, place them into local and regional context where possible. Molecular analyses were undertaken by Helix Molecular Solutions Pty Ltd (Helix), with methodology detailed in Appendix 1.

3.5 Limitations

A number of limitations apply to this study that relate to work on subterranean fauna in general, including:

- Sampling points are often limited to locations of interest for geological or hydrological exploration, which may limit interpretations of faunal distributions and available habitat.
- There is a lack of adequate taxonomic framework and specialist expertise for some faunal groups, both within Australia and internationally. This often limits the conclusiveness of some of the findings in regard to both taxonomic placement and ecological status. Molecular analysis, as used in this study, can overcome this to some degree, however the power of molecular analysis is limited by the comprehensiveness of the reference data set available.

The following limitations should be recognised with regard to this report:

- The Great Sandy Desert region is under-surveyed compared to other regions known for high levels of stygofauna diversity (e.g. the Pilbara and Yilgarn regions). Contextual records are limited but indicate generally low subterranean diversity. This is likely a reflection of both the lower suitability of Great Sandy Desert geological units as stygofauna habitat, and the limited survey effort expended in the region.
- The majority of the stygofauna samples retrieved from the survey area were very sedimented during all phases of sampling. This may simply be a reflection of the stratigraphy, or it may have been turbidity persisting from the drilling of the bores.

4.0 Existing Environment

4.1 IBRA

The Interim Biogeographic Regionalisation of Australia (IBRA) identifies 89 bioregions across Australia (DCCEE 2025). Winu is located within the Great Sandy Desert IBRA bioregion, which is divided into six subregions. Of the six subregions, Rimfire occurs within the Mackay subregion, described by Kendrick (2003) as follows: *“The Mackay subregion (GSD2) (18,636,695 ha) comprises the “tropical inland ‘red-centre’ desert, and includes the ‘Percival’ and ‘Auld’ palaeoriver systems”. Mainly tree steppe grading to shrub steppe in south; comprising hummock grassland of *Triodia pungens* and *Triodia schinzii* with scattered trees of *Owenia reticulata* and bloodwood (*Corymbia* spp.), and shrubs of *Acacia* spp., *Grevillea wickhamii* and *G. refracta*, on Quaternary red longitudinal sand dune fields. The climate is arid tropical with summer rainfall, and monsoonal influences apparent in the northwestern sector of this region”* (Kendrick 2003).

Kendrick (2003), when describing the Mackay subregion, observed for stygofauna in the subregion that the *“underground waters, associated with calcrete deposits along palaeodrainage lines”* may hold special values *“possibly stygofauna – some stygofauna known from vicinity of Canning Stock Route”*.

4.2 Geology

Regional geology is described as being *“broadly similar to the main Winu project area 30 km to the west, albeit with added complexities at a local scale arising from the intrusion of a granitic pluton into the metasedimentary basement”* (Worley 2025a). Bedrock geology of the Rimfire area lies wholly within the Anketell Formation, which comprises *“Fine-grained sandstone and siltstone; lesser coarse-grained sandstone and conglomerate”* (Geological Survey of Western Australia 2022) (Figure 4.1). Surface geology at Rimfire is represented by dunes (Qd) and sandplain (Czs), with calcrete units occurring further south (Figure 4.2).

4.3 Paleovalleys

The Winu Project is located between two major paleovalleys; the Percival and Wallal paleovalleys (Graaff et al. 1977) and the survey area overlaps a tributary of the Percival paleovalley (as mapped by Bell et al. (2012)) (Figure 4.1; Figure 4.2). This is described by Worley (2025e) as follows: *“The palaeovalley in the south Rimfire area appears to be an infilled tributary valley for a river that once discharged into the main Percival Palaeoriver 40 km to the south. The ancient Percival Palaeoriver flowed west across the Canning Basin to the Indian Ocean, and in doing so, incised into the underlying Permian sediments. The Rimfire area however is situated northeast of the Anketell fault, which separates the Anketell Shelf to the east (where Rimfire is located) from the Barnicarndy Graben. The Proterozoic-age metasedimentary bedrock is elevated in this area as a result of the fault displacement, and subcrops beneath shallow Quaternary sediments. The infilled tributary valley at Rimfire incises into this bedrock as a result.”*

In an attempt to refine the broad-scale mapping of the Percival paleovalley by Bell et al. (2012), Worley used airborne transient electromagnetics data (TEM) for the Rimfire area to delineate an ‘Inferred Percival Palaeochannel’, which shows *“an area of high conductivity lithology (interpreted as clay infill) within the palaeovalley boundary, comprising a 1.5 km wide main-channel, with elongate branches extending to the east and west”* (Worley 2025e). This ‘inferred paleochannel’ suggests a more limited extent of the paleovalley within the proposed Rimfire borefield than is mapped by Bell et al. (2012) (see Figure 4.1).

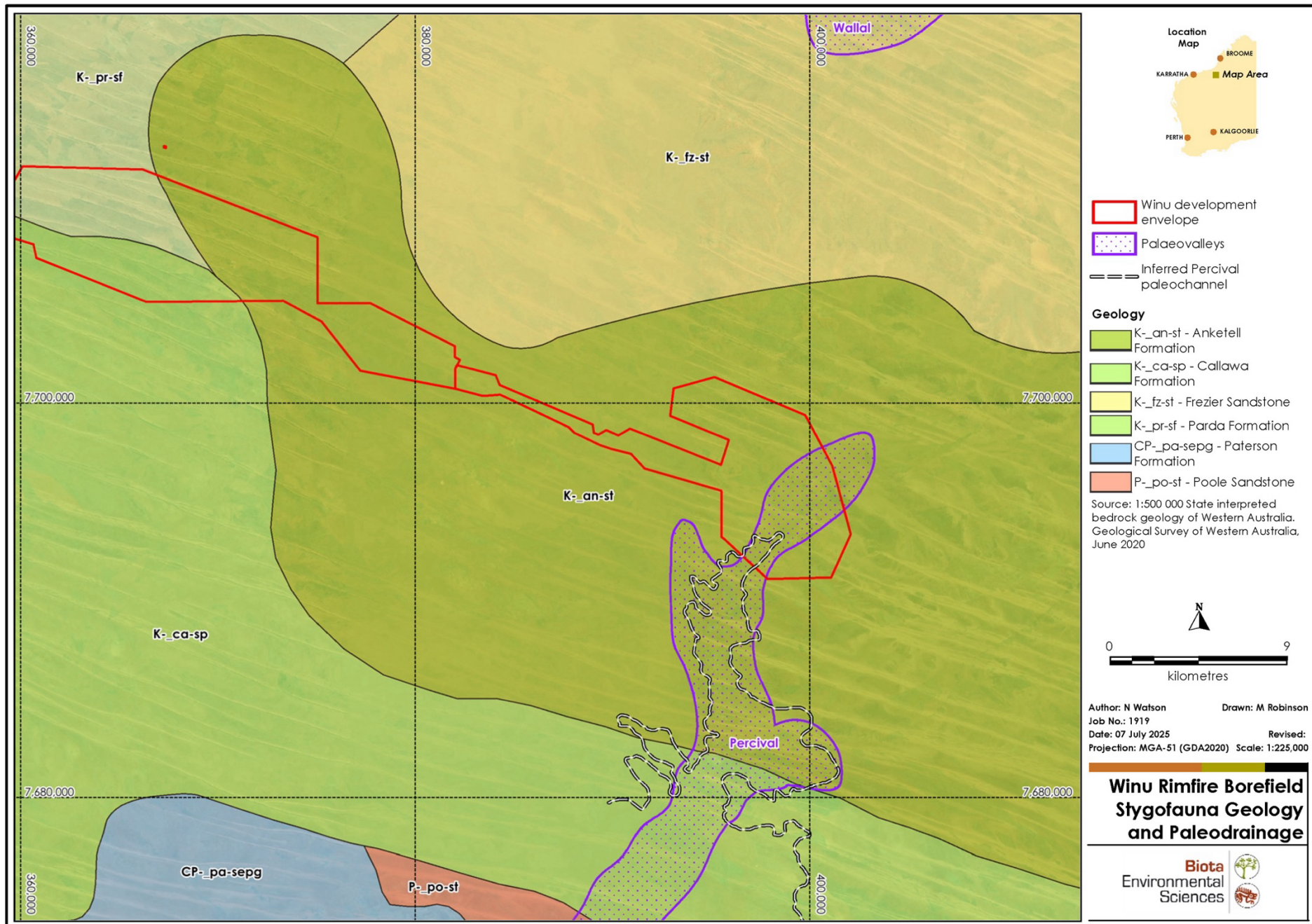


Figure 4.1: Bedrock geology and location of paleovalleys in relation to Rimfire.

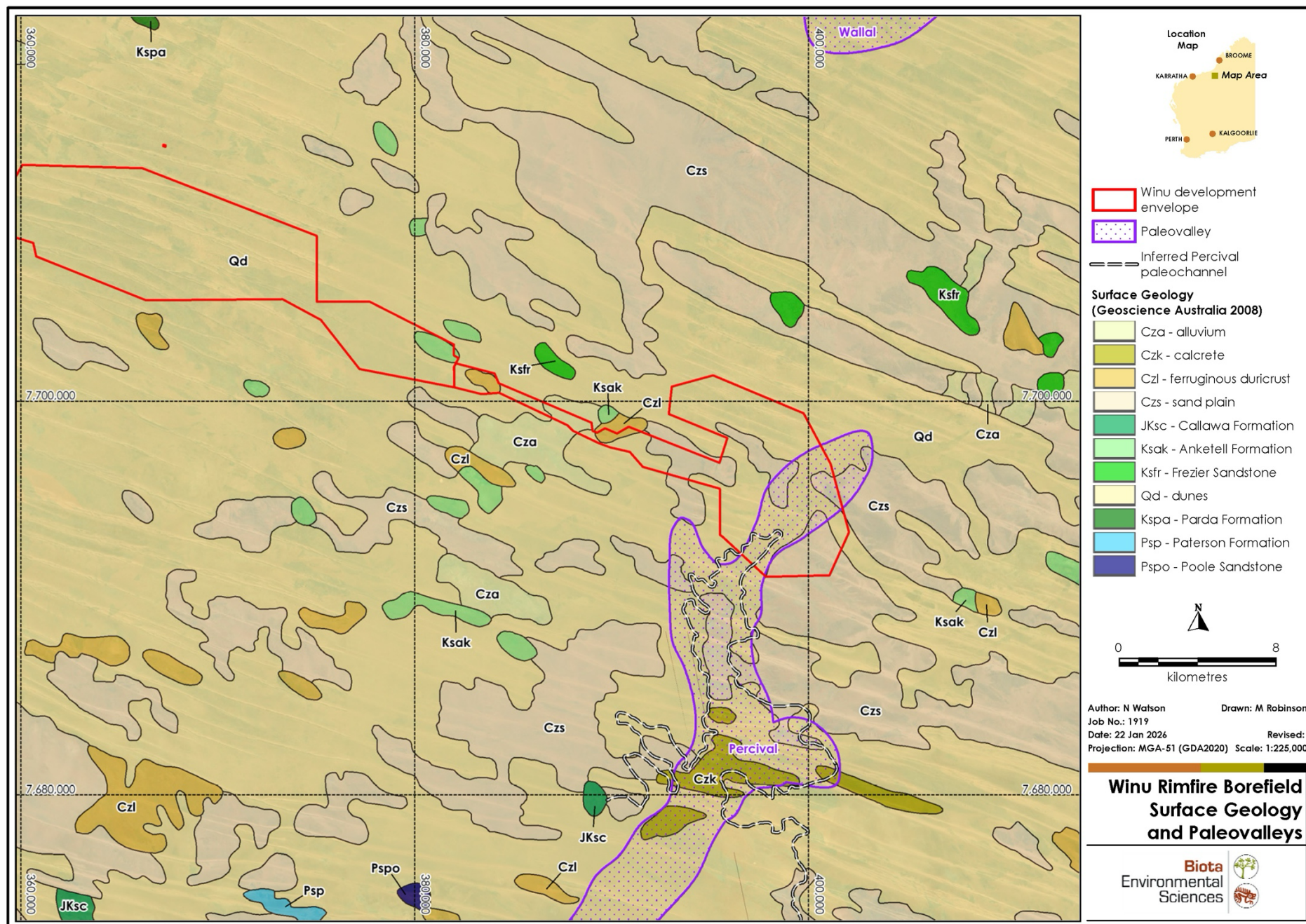


Figure 4.2: Surface geology and location of paleovalleys in relation to Rimfire.

4.4 Lithology

Worley (2025a) identified five geological units at Rimfire, which are described from surface to deepest (youngest to oldest) in Table 4.1 (sourced from Worley (2025a)). A conceptual cross section across the Rimfire area, showing the spatial configuration of the various units is provided in Figure 4.3 (sourced from Worley (2025a)).

Table 4.1: Main geological units of the Rimfire area (sourced from Worley (2025a)).

Lithology	Thickness (m)	Description
Quaternary aeolian sand	0 to 10	Very fine to medium grained aeolian sand, typically reddish-brown, well sorted, minor silt.
Unconfined sandstone and silcrete	10 to 30	Silty sandstone with high silt content reducing with depth, quartz sand grains, colour ranges between white to pink, fine to coarse grained, poorly sorted, subangular to subrounded grains. Silcrete and hematitic silcrete (ferricrete) typically occur within the top 10 to 20 m of the interval, colour ranges between white to reddish brown.
Mafic intrusions within the Proterozoic metasediments	5 to 40	Black to grey, fine grained, slightly weathered to fresh, hornblende phenocrysts, micaceous/schistose in some intervals.
Granite and felsic intrusions within the Proterozoic metasediments	5 to 80	Moderately weathered to highly weathered granite/felsic intervals, typically occurring between 20 and 80 m BGL, light brown to tan, occurs as variably consolidated sandy silt comprising quartz and feldspar grains, little to no biotite visible, fines content reducing with depth as weathering decreases.
Proterozoic metasediment	> 300	Moderately weathered to fresh metasedimentary intervals, medium grained, dark grey, biotitic, Metasiltstones and Metasandstones.

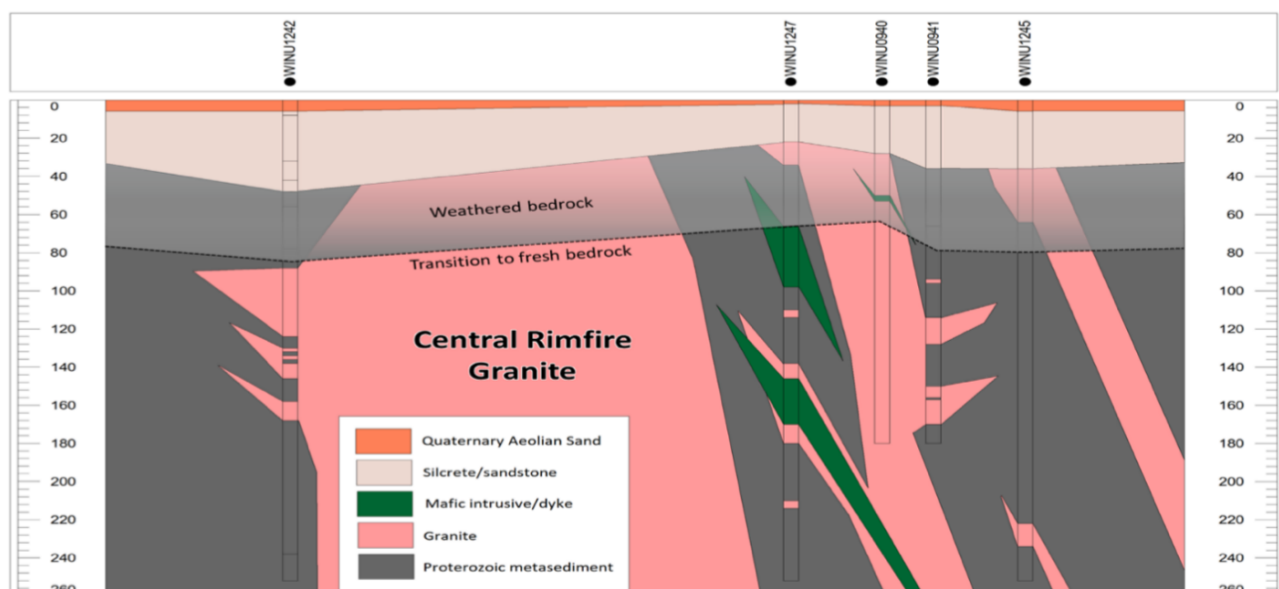


Figure 4.3: Conceptual cross-section through Rimfire (sourced from Worley (2025a)).

4.5 Hydrostratigraphic Units

A hydrostratigraphic unit can be defined as a part of a body of rock that forms a distinct hydrologic unit with respect to the flow of groundwater (Maxey 1964). Worley (2026) describe six hydrostratigraphic units at Rimfire (including the Percival paleovalley), based on their lithology and hydrogeological function (see Table 4.2).

Table 4.2: Summary of hydrostratigraphic units at Rimfire (sourced from Worley (2026)).

Hydrostratigraphic Unit	Lithology	Thickness (m)	Hydrogeological Function	Description
Quaternary aeolian sand	Silty sand, very fine to medium grained.	0 to 10	Unsaturated	Occurs over majority of the study area, unsaturated except following periods of increased rainfall.
Unconfined Sandstone and Silcrete	Fine to coarse grained silcretised sandstone with variable ferricrete	10 to 30	Unsaturated or partially saturated unconfined aquifer	Occurs over majority of the study area, unsaturated or partially saturated. Variable spatial coverage and thickness.
Percival paleovalley	Highly weathered claystone, sandstone and fine sand	0 to 40	Unconfined sedimentary aquifer	Unconfined aquifer overlaying the basal metasediments and granites
Weathered Granite	Moderately to highly weathered granite	20 to 80	Unconfined aquifer	The weathered zones function as an unconfined primary aquifer.
Weathered Metasediment	Moderately to highly weathered metasediment	20 to 80	Unconfined Aquifer	The weathered zones function as an unconfined primary aquifer.
Metasediment Granite	Slightly weathered to fresh metasediment, massive, fine to coarse	>80	Fractured aquifer Intrusive aquitard	Divided into different zones due to faulting and weathering. The fresh zones are inferred to act as a fractured aquifer.
	Slightly weathered granite, generally occurs as quartz, feldspar, and biotite in a matrix.	>80		These are interpreted to function as low permeability facies and presumably inhibit groundwater flow in the metasediment aquifer.

4.6 Groundwater

Worley (2025a) describe the groundwater at Rimfire as “fresh, with an EC (electrical conductivity) ranging between 580 and 1,330 $\mu\text{S}/\text{cm}$, and a neutral to slightly basic pH between 7.4 to 8.0”. Groundwater EC and pH do not appear to have an obvious spatial or vertical trend, nor lithological association.”

“The primary water supply targets in the Rimfire area are the weathered granites and metasediments, particularly where they are coincident with lineaments...Lineaments also likely comprise faults and fractures within the weathered metasediment and granite have the potential to store and convey water... The mafic intrusive units encountered at several locations are considered relatively sparsely distributed and unlikely to act as significant confining units. However, locally, they may behave as partially impermeable flow barriers and partly compartmentalise groundwater flow” (Worley 2025a).

In relation to groundwater-surface water interactions at Rimfire, Worley (2025a) state that “in general, no significant groundwater surface water interaction is likely based on the observed water levels >20 m BGL and the absence of any surface hydrological features (i.e., soaks and claypans). This may be different to the southeast where groundwater level in bore AKD10 is 3.82 m BTOC. The shallow groundwater occurrence is currently poorly understood; however, it is considered likely a combination of topography and potentially perched groundwater levels within the Percival Palaeovalley. Groundwater surface water interaction is likely to be spatially constrained to minor areas with shallow groundwater levels (below 5 m BGL), and temporally constrained during and immediately following periods of intense rainfall and/or cyclonic events” (Worley 2025a).

4.7 Groundwater Dependent Ecosystems

Groundwater Dependent Ecosystems (GDEs) are natural ecosystems that require access to groundwater to meet all, or some, of their water requirements on a permanent or intermittent basis, so as to maintain their communities of plants and animals, ecosystem processes and ecosystem services (Richardson et al. 2011).

Western Botanical (2025) identified a strong correlation between the occurrence of a *Melaleuca* community and the boundaries of the Percival paleovalley, suggesting that the vegetation is utilising the groundwater source there. *Eucalyptus victrix*, a potential indicator of GDEs, was also recorded to the South of Rimfire outside the DE (Western Botanical 2025). Though there is a lack of contextual data in the Great Sandy Desert region, and the GDE indicator species for the Winu area are currently undefined, the recorded communities along the Percival paleochannel may represent a GDE (Western Botanical 2025).

Given the common requirement for shallow groundwater between vegetation GDEs and stygal GDEs, the mapping of the *Melaleuca* community serves as an additional line of evidence supporting the likely extent of stygofauna habitat associated with the paleochannel.

4.8 Previous Records of Stygofauna

The desktop study returned a total of 147 records, collated from database searches and previous surveys within the desktop search area (see Appendix 2). The assemblage comprised copepods (28%), ostracods (20%), worms (21%), amphipods (17%), isopods

(6%), syncarids (5%), aquatic snails (1%), water mites (1%) and rotifers (<1%), with the majority of taxa being undescribed species. None of the desktop stygofauna records were recorded within 50 km of Winu. With the exception of surveys undertaken for the Winu Project, no new surveys were found to have been undertaken in the desktop search area since the bioregional review of subterranean fauna surveys conducted in WA desert environments was presented in Biota (2022b).

Nearly half of the records in the desktop search area were from surveys conducted for EIA purposes for the Telfer mine (100 km southeast), Kintyre Uranium Project (170 km south), and Havieron Gold-Copper Project (130 km southeast). In addition to these, 53% of the total records were from the Pilbara Stygofauna Survey (PSS) conducted between 2003 - 2005 (Halse et al. 2014). These records were from 27 sites sampled as part of the PSS, however only one-third (nine) of these sites are located within the Great Sandy Desert bioregion (as mapped by IBRA; DCCEEW 2025), and all of the PSS records are located more than 50 km to the south and southwest, therefore not providing ideal local context.

The Great Sandy Desert has been poorly surveyed for subterranean fauna, and previous desktop studies for the Winu Project have revealed a lack of stygofauna surveys in proximity to Winu (Biota 2020, 2022a, 2022b). A small number of stygofauna surveys undertaken in the Great Sandy Desert have recorded moderately diverse stygofauna communities, with the records being from calcrete or alluvial geological units occurring within a broader setting of the sandstone and mudstone units common to the bioregion. Table 4.3 presents a summary of previous subterranean fauna surveys undertaken within the Great Sandy Desert bioregion

Prior to the survey effort detailed in this report, six phases of stygofauna sampling had been undertaken at other locations at Winu and no stygofauna had been recorded (Biota 2022a, 2023a, 2025)¹.

¹ Records from site AKD10 reported on in Biota (2025) are included in the results of this report as the site is located within the paleovalley and was sampled at the same time as Rimfire Phase 2.

Table 4.3: Summary of previous subterranean fauna surveys undertaken within the Great Sandy Desert bioregion.

Report (Author)	Description	Subregion	Geological Attributes	Survey Dates	Stygofauna Recorded
Winu Project Texas Borefield Stygofauna Assessment (Biota 2025)	Desktop study and two phase stygofauna survey	Mackay	Silcrete/ferricrete Sandstone Claystone Metasediments (occurrence of mafic intrusive units)	Phase 1: 10-13/05/2024 Phase 2: 23-25/08/2024	Isopoda (Microcerberidae) (in AKD10 paleovalley site, reported on in this report) Rotifera
Winu Texas Stygofauna Desktop Study (Biota 2023b)	Desktop study only	Mackay	Silcrete/ferricrete Sandstone Claystone Metasediments (occurrence of mafic intrusive units)	N/A	N/A
Winu Project Wallal Borefield Stygofauna Assessment (Biota 2023a)	Desktop study and basic stygofauna survey of Wallal	McLarty	Sandstone Mudstone Siltstone Sandstone Wallal Sandstone aquifer	3-4/05/2023	Nil recorded
Winu Production Bores Stygofauna Desktop Study (Biota 2022b)	Desktop study of Wallal, Texas and Rimfire	McLarty, Mackay	Geology units as listed in three studies above.	N/A	N/A
Winu Project Subterranean Fauna Desktop Study, Verification and Basic Surveys (Biota 2020, 2022a)	Three phase stygofauna and two phase troglotauna survey of MIA	Mackay	Mudstone Tillite Sandstone	Phase 1: 25-26/09/2019 Phase 2: 14/11/2019 Phase 3: 9-13/09/2021	Nil recorded
Havieron Project Stage 1 and 2 Subterranean Fauna Surveys (Biologic 2020, 2021)	Three phase subterranean fauna	Mackay	Mudstone Tillite Siltstone Sandstone Percival paleovalley	Stage 1 (Phases 1 and 2): 21-26/11/2019 18-21/02/2020 30/03 – 03/04/2020 14 – 19/06/2020 Stage 2 (Phase 3): 3-7 May 2021	Oligochaeta (Enchytraedidae), Amphipoda (Paramelitidae) Isopoda (Microcerberidae) Copepoda Ostracoda Stygobitic records from alluvium/calcrete

Report (Author)	Description	Subregion	Geological Attributes	Survey Dates	Stygofauna Recorded
Pilbara Stygofauna Survey (Halse et al. 2014)	Pilbara-wide stygofauna survey conducted 2003 -2005. Mostly Pilbara but 34 sites in GSD.	1 GSD site within desktop search area - Mackay subregion	Not discussed	2003 - 2005	Site PSS226 records: Tubificida, Amphipoda, Isopoda, Copepoda, Bdelloidea, Ostracoda
Canning Basin Project Stygofauna Survey and Assessment (Subterranean Ecology 2012)	Level 1 stygofauna survey – desktop assessment and pilot survey.	Pindanland	Wallal Sandstone aquifer	Phase 1: 21-24/02/2012 Phase 2: 28-30/03/2012	1 individual from 1 stygofauna order (Polychaeta)*
BHP Billiton Regional Subterranean Fauna Study (Stygofauna) 2005-2007 (Biota 2008)	2 sites sampled at Shay Gap Borefield by Biota (2008) as part of regional study. Same sites sampled 3 times by Subterranean Ecology (2009) (source report unavailable).	McLarty	Wallal Sandstone aquifer	2005 – 2007 for BHP regional sampling 2007 – 2008 for Goldsworthy Mine compliance	Nil
Two new species of <i>Leicacandona</i> Karanovic (Ostracoda) from the GSD (Karanovic and McKay 2010)	Sampling conducted at the Telfer Gold Mine for a species taxonomic description publication.	Mackay	Not discussed	2008 dates unknown, 19 bores sampled	2 stygofauna species (Ostracoda)

* Record not conclusively attributed to the Wallal Sandstone Aquifer, due to the level of deterioration of the bore – possibly colonised the bore from shallower groundwater or even surface waters (Subterranean Ecology 2012).

5.0 Stygofauna Habitat Assessment

5.1 Lithology

As part of hydrogeological investigations undertaken to inform the location of the proposed production bores at Rimfire, eight RC drillholes and one water bore were initially drilled. These drillholes varied in depth from 80 m to 324 m, with the depth to groundwater ranging between 26 - 66 m; this intercepted either granite, sandstone or mudstone geology units in the eight drillholes for which information is available.

A further 12 RC drillholes were drilled in 2024 for the purposes of stygofauna sampling (see Table 3.3 and Figure 3.1). The 12 drillholes sampled for stygofauna were all drilled to a depth of approximately 70 m below ground level (mbgl) and the depth to water was approximately 40 mbgl, which generally intercepted either granite, sandstone or claystone geology units (Table 3.3). An additional 27 RC drillholes were drilled at Rimfire during 2024, these holes varied in depth from 42 – 70 mbgl, with depth to water ranging from 13 – 46 mbgl, this water level intercepted either granite, sandstone, silcrete, and claystone (see Table 3.4; Figure 3.1). Twelve of these were drilled within the mapped WASANT paleovalley extent (Bell et al. 2012), including five within the ‘inferred paleochannel’ (Worley 2025e).

5.2 Stygofauna Habitat Prospectivity

The assessment of habitat prospectivity for stygofauna is presented in Table 5.1, and utilises the generalised hydrostratigraphic sequence presented in Section 4.5, along with summarised drill logs of the drillholes sampled for stygofauna (Table 3.3 and Table 3.4), and drill logs of the exploration drillholes.

Without the inclusion of the Percival paleovalley, the stratigraphic units at Rimfire are categorised as having Low-Moderate prospectivity to act as stygofauna habitat (Table 5.1). The aeolian sand and silcrete units present immediately below-ground to approximately 30 mbgl occur for the most part, above the water table and as a result, would not represent habitat for stygofauna. Beneath these units, the sandstone and granite units are considered to have a Moderate prospectivity to act as stygofauna habitat, and the remaining metasediment units have Low prospectivity.

Sandstone can demonstrate some porosity and stygofauna have been recorded from sandstone units previously, therefore where the sandstone unit occurs below the water table at Rimfire it could potentially support stygofauna, especially where these units are highly weathered. However, there is a high proportion of fine sediments present in this unit, which reduces its suitability.

Despite a lack of stygofauna records from sandstone geologies in Western Australia, there is some evidence from eastern Australia of stygofauna occurring in fractured sandstone aquifers (Hose et al. 2015). Stygofauna assemblages found in the Triassic Hawkesbury sandstone to the north and south of Sydney were found to be dominated by copepods (and some syncarids), and limited to shallow water-bearing zones (Hose et al. 2015).

In relation to the granite unit, depending on the level of fracturing, this could also provide suitable cavities for stygofauna to occupy. The availability of pore space is a critical factor for the presence of stygofauna habitat, and the size of the pore spaces in fractured rock is a feature dependent on the parent rock and also the processes that have led to the fracturing (Hose et al. 2015). Sand and silt dominated sediments typically have small pore spaces and few invertebrates, while coarse sand and gravel deposits, and large fractures have pore spaces large enough for invertebrate fauna (Hose et al. 2015). In the Gascoyne region, stygofauna have been recorded from granite units where such suitable fracturing occurs (Ecoscape 2016), however the assemblage recorded from these units was determined to be of low significance, with the granite and granitoid units concluded to be “*generally uncondusive to stygofauna*” (Bennelongia 2017a). At Rimfire, the granite units occur at depths where nutrient and primary production energy inputs are beginning to attenuate; this reduces the likelihood that a stygofauna community of any significance could be supported, even if sufficient fracturing is present. Previous studies have found that fractured rock aquifers typically yield depauperate stygofauna communities, which are often dominated by widespread copepod species (discussed in Bennelongia 2017b).

Previous desktop studies for the Winu project identified the shallow aquifers beneath paleovalleys as the most prospective stygofauna habitat within the region (Biota 2022b), with these geological units described broadly as “Alluvial and lacustrine sand, silt, mud and calcrete” (Geological Survey of Western Australia 2022). Inclusion of this unit in the lithological sequence at Rimfire, and assessment against the criteria outlined in Table 3.1, finds that it meets the most criteria for prospectivity as stygofauna habitat of all of the Rimfire lithologies (Table 5.1). However, of the seven drillholes located between the ‘inferred paleodrainage’ and the WASANT paleovalley extent, “*none intercepted lithology consistent with a palaeovalley...(they) intersected ferricrete, silcrete and sandstone typical of the Rimfire cover sequence. Inside the geophysics-inferred palaeovalley, WINU1446 was the only bore in which the characteristic palaeovalley sequence of interbedded clay and sandstone was encountered, and is the only bore located within the main channel inferred from the geophysics. Other bores located within the geophysics-inferred extent and on the branches adjacent to this main channel encountered either outcropping bedrock (WINU1445, WINU1452, WINU1453) or ferricrete and sandstone (WINU1444, WINU1454), consistent with the surrounding cover. These observations suggest that the actual extent of the palaeovalley is more limited than indicated by the geophysics, and it may potentially be confined only to the main high-conductivity channel area*” (Worley 2025e). Drill logs “*indicate that at WINU1446 the tributary palaeovalley is 40 m deep, overlying micaceous metasandstone and granite. The basal unit of the palaeovalley strata is a 10 metre thickness of fine-grained sand, overlain by highly weathered claystone and sandstone*” (Worley 2025e).

Calcrete deposits are typically associated with paleovalleys and are well recognised as providing highly prospective subterranean fauna habitat, these deposits “*occur widely in palaeovalleys across the arid zone, particularly in the upper nearsurface sediments. They are deposited from laterally migrating shallow groundwater...Calcrete deposits are typically up to 10 metres thick, although thicker deposits may exist...Calcrete deposits may extend across the entire width of the host-palaeovalley, typically beyond the limits of the underlying, narrower palaeochannel sand facies*” (Magee 2009). Based on the drill logs examined for the drillholes drilled for stygofauna sampling at Rimfire, no calcrete units have been intercepted within or near to the paleovalley tributary. However, calcrete outcropping has been identified on the ground surface further south along the Percival paleovalley tributary (Western Botanical 2025), so there is a possibility that there are deeper calcrete deposits in isolated pockets that have not yet been intercepted by existing drillholes. Calcrete units have not been included in the stygofauna habitat assessment in Table 5.1.

Table 5.1: Assessment of stygofauna habitat prospectivity at Rimfire.

		Subterranean Habitat Characteristics						Criteria Met	Likelihood
Stratigraphic Unit/ Formation*	Lithology*	A Presence of interstitial spaces or vugs	B Continuity and transmissivity of the local occurrence of submerged geological units	C The known occurrence of stygal communities from equivalent rock types during previous surveys	D Absence of large amounts of fine sediments such as clays, silts and sands within the geological unit description	E Substrate permits inflow of surface water and nutrients infiltration	F Substrate submerged below the water table		
Quaternary aeolian sand	Silty sand, very fine to medium grained	✖	✖	✓	✖	✓	✖	C, E	Nil – occurs above water table
Silcrete/Sandstone	Fine to coarse grained silcretised sandstone with variable ferricrete	✖	✖	✓	✖	✓	✓	C, E, F	Moderate
Percival paleovalley	Highly weathered claystone, sandstone and fine sand	✓	✓	✓	✖	✓	✓	A, B, C, E, F	High (where highly weathered)
Weathered granite	Moderately to highly weathered granite, generally occurs as quartz, feldspar, and biotite in a matrix	✓	✖	✓	✓	✖	✓	A, C, D, F	Moderate
Weathered metasediment	Moderately to highly weathered metasediment	✓	✓	✖	✖	✖	✓	A, B, F	Moderate (where highly weathered)
Metasediment Granite	Slightly weathered to fresh metasediment, massive, fine to coarse grained	✖	✖	✖	✖	✖	✓	F	Low Metasediment geologies typically cemented and high portion of fine sediments
	Slightly weathered to fresh, granite, generally occurs as quartz, feldspar, and biotite in a matrix	✖	✖	✖	✓	✖	✓	D, F	Low

*From Worley (2026).

6.0 Stygofauna Results

6.1 Rimfire

Five higher order groups (order or subclass level) of stygobitic fauna were recorded from the seven phases of sampling at Rimfire through 2024 and 2025.

The stygofauna community recorded was represented predominantly by copepods, which constituted half of the records (42% harpacticoid, 8% cyclopoids). The remaining half of the assemblage was shared by isopods (16%) and oligochaete worms (24%) and lastly, amphipods, represented one-tenth of the recorded assemblage (Figure 6.1).

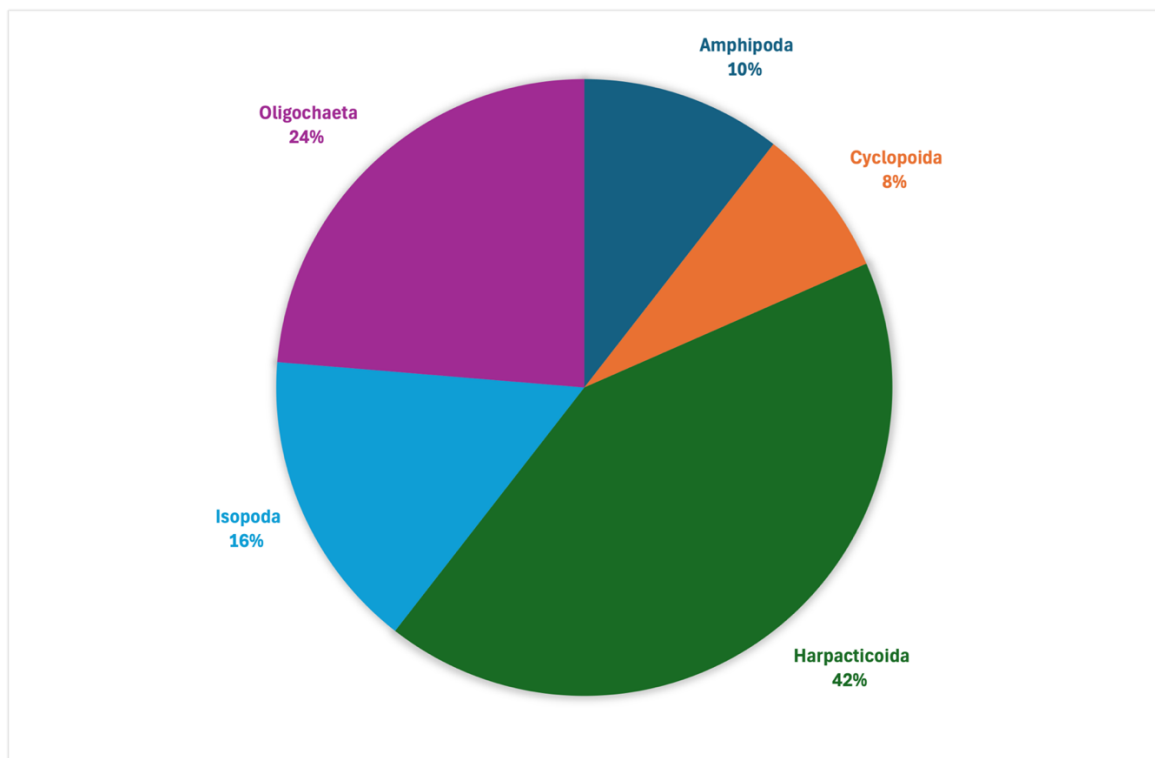


Figure 6.1: Stygofauna community composition at Rimfire.

The stygofauna records are summarised in Table 6.1 and the locations of stygofauna records are presented in Figure 6.2. Only species-level records are mapped in Figure 6.2. Two additional groups of aquatic fauna belonging to the phyla Rotifera and Nematoda were also recorded however no further identification was sought for these groups as they are generally widespread, occurring in surface waters and soil strata, and are not typically assessed as part of EIA. The Rotifera and Nematoda records are therefore not discussed further.

Twenty-two species-level taxa were recorded across the seven phases of sampling at Rimfire (Table 6.1) which are discussed further in Sections 6.1.1 - 6.1.5. None of these were assigned to formally described species, thus all were assigned to a molecular species at either the order, family or genus level (Table 6.1). For detailed discussion on the molecular species assignments see Helix (2026) (Appendix 1).

Table 6.1: Summary of stygofauna records from Rimfire.

Order	Family	Species	Phase (number of specimens) Sites (number of specimens)
Amphipoda	Paramelitidae	Paramelitidae sp. H-AMP051	Phase 5 (1): WINU1452 (1) Phase 6 and 7 (19): WINU1451 (4, 15)
Isopoda	Microcerberidae	Microcerberidae sp. H-ISM003	Phases 1, 3, 4 (18): AKD10 (10, 4, 4) Phase 5 (1): WINU1446 (1)
		Microcerberidae sp. H-ISM005	Phase 5, 6, 7 (18): WINU1451 (2, 9, 7)
		Microcerberidae sp. indet.	Phase 2 (16): AKD10
Harpacticoida	Parastenocarididae	<i>Parastenocaris</i> sp. H-HAR010A	Phase 2 (1): WINU1372 (1) Phase 6 (2): WINU1437 (2) Phase 7 (7): WINU1423 (7)
		<i>Parastenocaris</i> sp. H-HAR010B	Phase 7(12): WINU1425 (12)
		<i>Parastenocaris</i> sp. H-HAR024	Phase 6 (4): WINU1437 (4)
		<i>Parastenocaris</i> sp. H-HAR026	Phase 7 (3): WINU1425 (3)
		<i>Parastenocaris</i> sp. H-HAR028	Phase 7 (3): WINU1451 (3)
		<i>Parastenocaris</i> sp. H-HAR029	Phase 7 (1): WINU 1450
		<i>Parastenocaris</i> sp. indet.	Phase 2 (15): WINU1372 (15)
	Indeterminate	Harpacticoida sp. H-HAR015	Phase 4 (2): WINU1454 (2)
		Harpacticoida sp. H-HAR016	Phase 4 (1): WINU1447 (1) Phase 7 (2): WINU1447 (2)
		Harpacticoida sp. H-HAR017A	Phase 4 (2): WINU1453 (2) Phase 5 (1): WINU1450 (1) Phase 6(7): WINU1450 (6) WINU1454 (1) Phase 7 (7) WINU1450 (3) WINU1454 (4)
		Harpacticoida sp. H-HAR017B	Phase 4 (1): WINU1453 (1) Phase 7 (1): WINU1453 (1)

Order	Family	Species	Phase (number of specimens) Sites (number of specimens)
		Harpacticoida sp. H-HAR023	Phase 6 (13): WINU1444 (13)
		Harpacticoida sp. H-HAR027	Phase 7 (10): WINU1444 (10)
Cyclopoida	Indeterminate	Cyclopoida sp. H-CYC010	Phase 5, 6, 7: WINU1453 (1, 8, 5)
		Cyclopoida sp. H-CYC036	Phase 6 (1): WINU1447 (1)
		Cyclopoida sp.	Phase 5 (1): WINU1447 (1)
Subclass Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026	Phase 6 (25): WINU1436 (23) WINU1437 (1) WINU1445 (1) Phase 7 (1): WINU1451 (1)
		Enchytraeidae sp. H-OLE063	Phase 6 (4): WINU1438 (3) WINU1452 (1)
		Enchytraeidae sp. H-OLE064	Phase 6 (13): WINU1424 (13)
		Enchytraeidae sp. H-OLE065	Phase 6 (1): WINU1424 (1)
		Enchytraeidae sp. H-OLE066	Phase 6 (1): WINU1451 (1)

6.1.1 Amphipoda

One new amphipod taxon was recorded from two sites (WINU1451 and WINU1452; distance of ~830 m between sites) (Table 6.1; Figure 6.2). The taxon was assigned to a new molecular species within the family Paramelitidae (Paramelitidae sp. H-AMP051). The nearest closest relatives to this taxon were two Paramelitidae taxa recorded from subterranean fauna surveys for the Havieron project (Biologic 2020) (Appendix 1), but these were clearly distinct species.

6.1.2 Isopoda

Two new isopod taxa were recorded; one from a single site (Microcerberidae sp. H-ISM005 from WINU1451), and another from two sites (Microcerberidae sp. H-ISM003 from AKD10 and WINU1446; distance of ~480 m between sites) (Table 6.1; Figure 6.2).

The two taxa were assigned distinct molecular species as they showed approximately 8% divergence from each other, suggesting they represent two different species (Helix 2026). There are no Australian molecular systematic studies published for this family and undescribed species from Australia have yet to be formally documented (Helix 2026) (Appendix 1).

6.1.3 Harpacticoida

Harpacticoid copepods were recorded from five sites at Rimfire (Table 6.1; Figure 6.2). Molecular analysis revealed 12 previously undetected taxa; six were assigned to the genus *Parastenocaris*, while the remaining six taxa could only be placed to order level (Harpacticoida sp.) (Helix 2026)(Appendix 1).

6.1.4 Cyclopoida

Two cyclopoid copepod taxa were recorded from a single site each: Cyclopoida sp. H-CYC010 from WINU1453 and Cyclopoidae sp. H-CYC036 from WINU1447 (Table 6.1). Both were new putative species and shared the same nearest relative *Dussartcyclops* sp. Biologic-CYCL007, from which they were clearly distinct (Helix 2026) (Appendix 1).

6.1.5 Oligochaeta

Five putative species of oligochaete worms were recorded from seven sites (Table 6.1; Figure 6.2). Two of the five species have been recorded previously; Enchytraeidae sp. H-OLE026 is a widespread species, and Enchytraeidae sp. H-OLE063 has been previously recorded from the Havieron project and wider Telfer area (as Biologic-OLIG024). The remaining three taxa are newly detected species, each recorded from a single site (Helix 2026)(Appendix 1). This group of fauna was not recorded from the Rimfire survey area prior to Phase 6 of sampling.

6.2 Old Bore

Three orders of stygofauna were recorded from the reference site, Old Bore, located approximately 125 km southeast of Rimfire within the Percival paleovalley (Table 6.2). These records are not presented in Figure 6.2, as none of the taxa represent the same species recorded from Rimfire.

Table 6.2: Summary of stygofauna records from Old Bore in the Percival paleovalley.

Order	Family	Species	Phase (number of specimens) Sites (number of specimens)
Amphipoda	Paramelitidae	Paramelitidae sp. H-AMP050	Phase 5 (4): Old Bore (4)
Isopoda	Microcerberidae	Microcerberidae sp. H-ISM004	Phase 3 (20): Old Bore (20)
Harpacticoida	Indeterminate	Harpacticoida sp. H-HAR011	Phase 3 (3): Old Bore (3)

6.2.1 Amphipoda

One new species of amphipod was recorded from Old Bore, Paramelitidae sp. H-AMP050. This taxon was 16.8% divergent from the Rimfire amphipod taxon (Paramelitidae sp. H-AMP051) and 15-16% divergent from two Paramelitidae taxa recorded from subterranean fauna surveys for the Havieron project (Biologic 2020), indicating it represents a newly detected species (Helix 2026) (Appendix 1).

6.2.2 Isopoda

One new molecular species of microcerberid isopod was recorded from Old Bore, Microcerberidae sp. H-ISM004. This taxon is approximately 16-17% divergent from the Rimfire microcerberid taxa (sp. H-ISM003 and sp. H-ISM005), and represents a different species (Helix 2026) (Appendix 1).

6.2.3 Harpacticoida

One new molecular species of harpacticoid copepod was recorded from Old Bore; Harpacticoida sp. H-HAR011. This taxon is approximately 26-27% divergent from the Rimfire harpacticoid taxa and likely belongs to a different genus (Helix 2026) (Appendix 1).

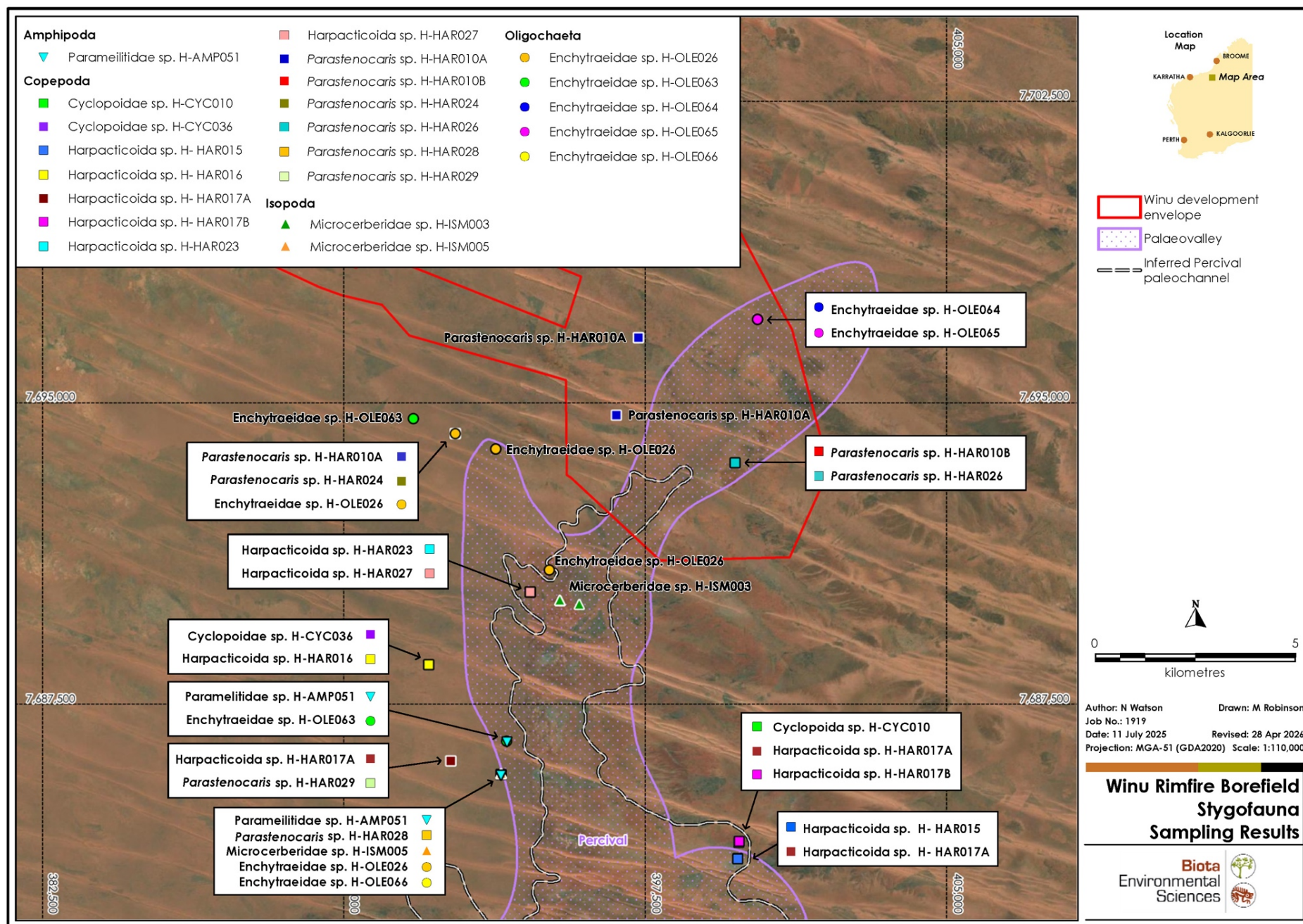


Figure 6.2: Location of stygofauna records from Rimfire.

7.0 Impact Assessment

7.1 Proposed Groundwater Abstraction

Two groundwater abstraction scenarios for the Rimfire borefield are considered in this impact assessment: a Project water demand case - the actual expected drawdown with a P90 level of confidence based on predicted water demand through the life of mine – and a Project licensing case. The second case is to ensure sufficient water in any given year, but is very unlikely to be required in reality and has been included as a conservative impact assessment scenario. The drawdown contours for the two cases are presented in Figure 7.2 and Figure 7.3, and summarised below in Section 7.1.1 and 7.1.2.

7.1.1 Project Water Demand Case

A project water balance was developed as a representation of the proposed water management system for the project, to evaluate the future performance in terms of water supply reliability and discharge risk (Worley 2025b). The model was developed to quantify the timing and magnitude of water requirements from the Rimfire borefield, and the timing and magnitude of excess water discharges from the MIA (Worley 2025b).

For the purpose of the water balance, a nominal 10.0 Mtpa processing rate was assumed (Worley 2025b). The P90 case of the water balance has been used to ensure a conservative estimate of water demand and therefore a conservative assessment of potential environmental impacts, based on the site-wide water balance, *“the proposed Rimfire Borefield will have a staged operation, with varying flows from 0 to approximately 2,000 Megalitres per year (ML/yr). Abstraction is anticipated to start after 13 years of mining, and peak in 26 years”*(Worley 2026) (see Figure 7.1).

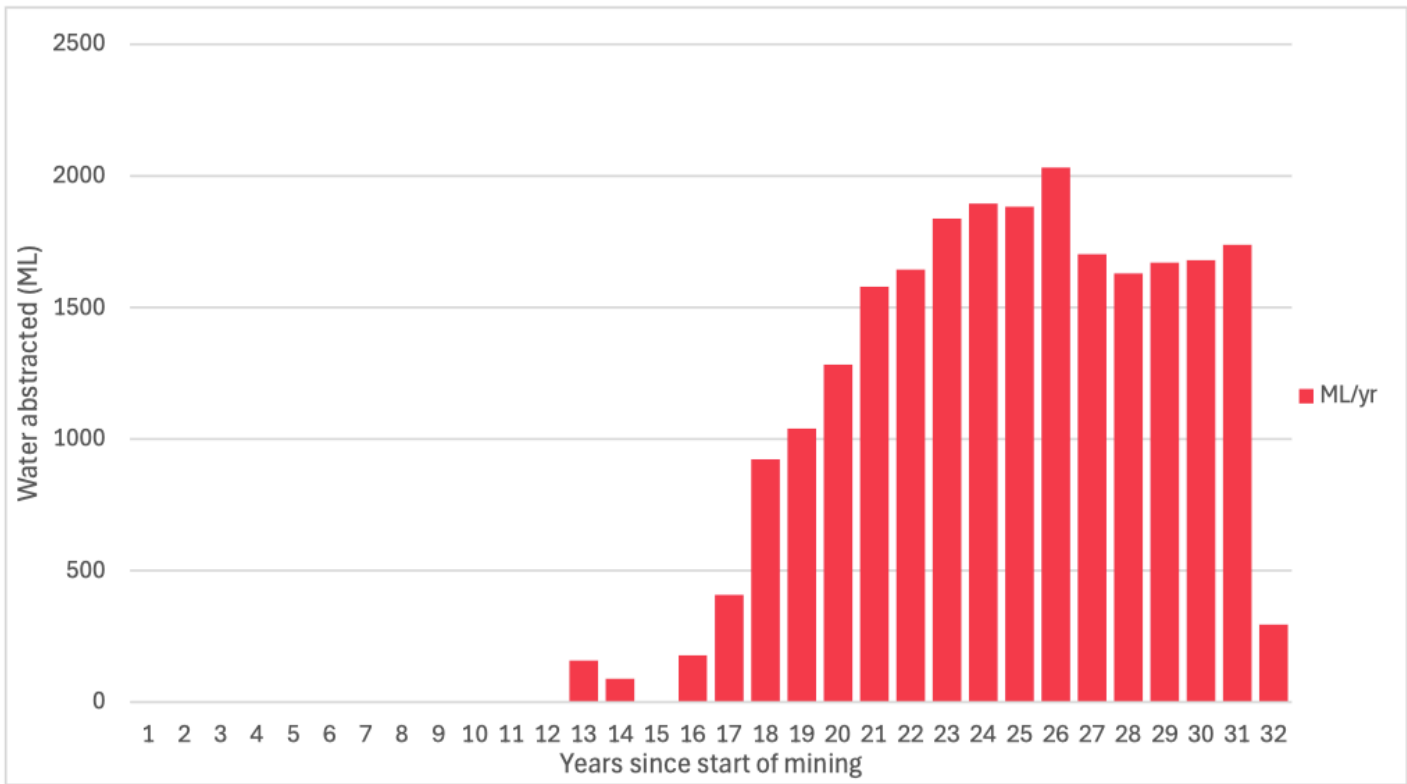


Figure 7.1: Proposed Rimfire borefield extraction for the Project water demand case (P90) (reproduced from Worley(2026)).

Modelling of the Project water demand case represents a conservative estimate of the actual expected drawdown based on predicted water demand through the life of mine. It predicts a decreasing drawdown of 7 m to 2 m from the centre of the drawdowns to the outermost drawdown contour over a distance of approximately 6.4 - 8.8 km from the centre of the borefield (Figure 7.2).

This would impact 5 of the 17 locations where stygofauna have been recorded (Table 7.1). At these 5 locations, six stygofauna taxa were recorded, comprising: three harpacticoid copepod species and three enchytraeid oligochaete worm species (one of which is widespread). All other stygofauna taxa recorded from Rimfire (16 taxa as listed in Table 6.1) will be outside of the predicted area of impact (outside of the 2 m expected drawdown contour) under the Project water demand case (Figure 7.2; Table 7.1).

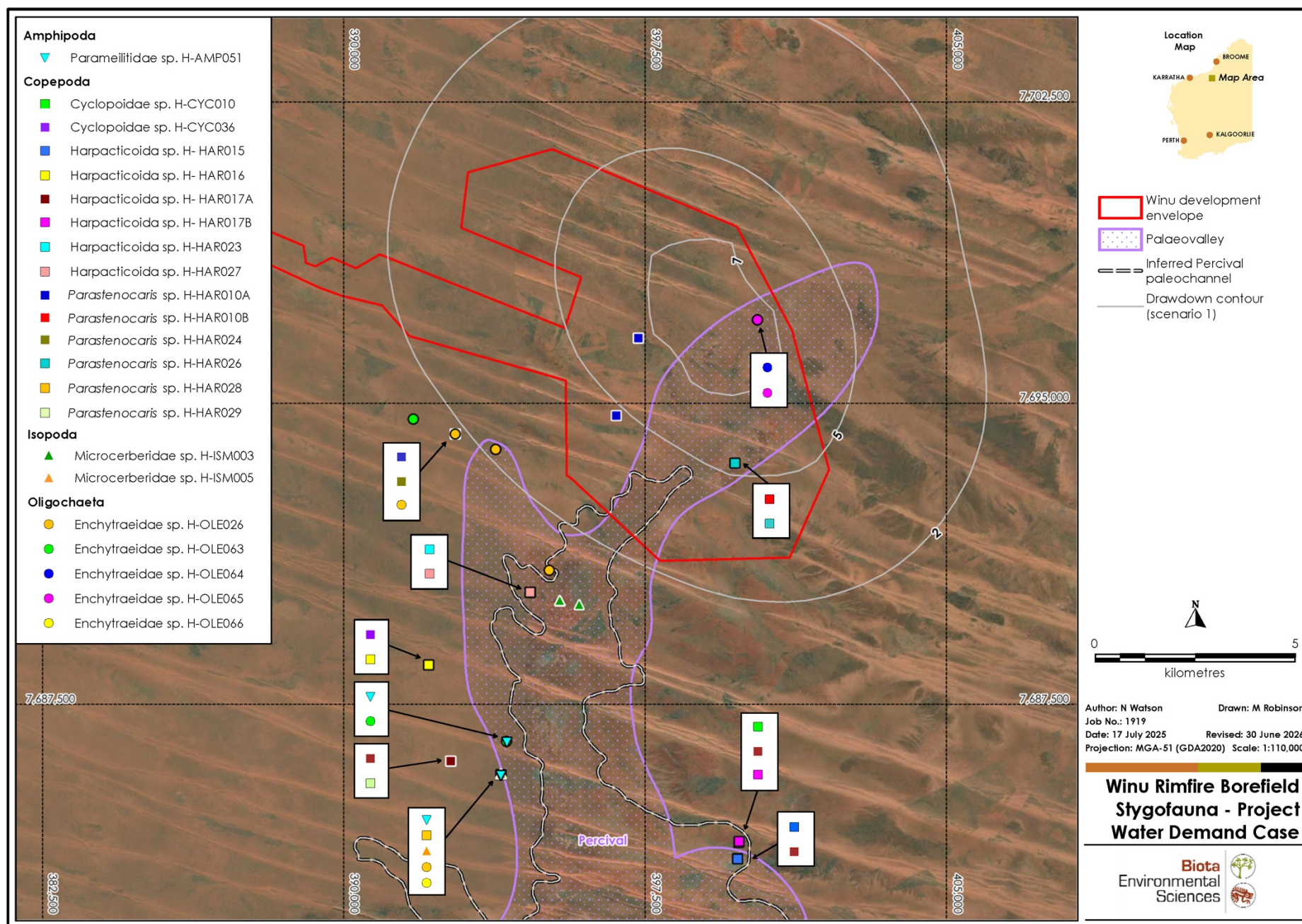


Figure 7.2: Stygofauna records relative to predicted drawdown contours for the Project water demand case (maximum rate shown – at Year 26).

7.1.2 Project Licensing Case

To conservatively assess drawdown impacts on local and regional aquifers, the Project licensing case considered an extreme worst-case pumping scenario, whereby borefield abstraction was simulated at 2.8 GL per annum for the full life of mine (32 years) (Worley 2025a).

The modelling of this case is as follows: *“The model allows to examine the groundwater response to abstraction from the current production bore, including drawdown and mounding from pumping discharge. The numerical block model was used to simulate a nominal nine-bore borefield intersecting the highly weathered fractured rock aquifer incorporating the granite and metasediments. Forward predictions of groundwater abstraction from these nine locations show the nominal borefield is potentially capable of sustaining the estimated worst-case peak demand of 2.8 GL/a (8 to 11 L/s, per bore). Groundwater abstraction will create a cone of depression radially extending from the borefield. The 2 m drawdown contour, as a measure pumping effect, after simulation of 32 years of continuous abstraction from nine bores (which far exceeds what is forecast to be actually required) is predicted to extend in the order of 15 km from the borefield”* (Worley 2025a). Due to lack of information on the tributary palaeovalley, the palaeovalley was not included in the model as a feature with its own distinct hydraulic properties (Worley 2025a).

Under the Project licensing case, the drawdown contours decrease from 10 m to 2 m across a maximum distance of approximately 17 km from the centre of the borefield to the outermost drawdown (Figure 7.3).

This would impact 15 locations where stygofauna have been recorded (see Figure 7.3 and Table 7.1). At these 15 sites, the recorded stygal assemblage comprises:

- 11 copepod species (10 harpacticoid, one copepod species);
- five enchytraeid oligochaete worms (two of which have been recorded outside of Winu);
- two microcerberid isopod species; and
- one paramelitid amphipod species.

An additional two sites where stygofauna have been recorded from lie outside of the predicted drawdowns under the project licence case. Four of the 22 recorded species were recorded from these two sites outside of the 2 m drawdown contour under the Project licensing case (Figure 7.3 and Table 7.1).

Of the four taxa recorded outside of the predicted drawdowns, three were recorded solely outside of the 2 m expected drawdown contour, while one was also recorded inside the drawdown contours.

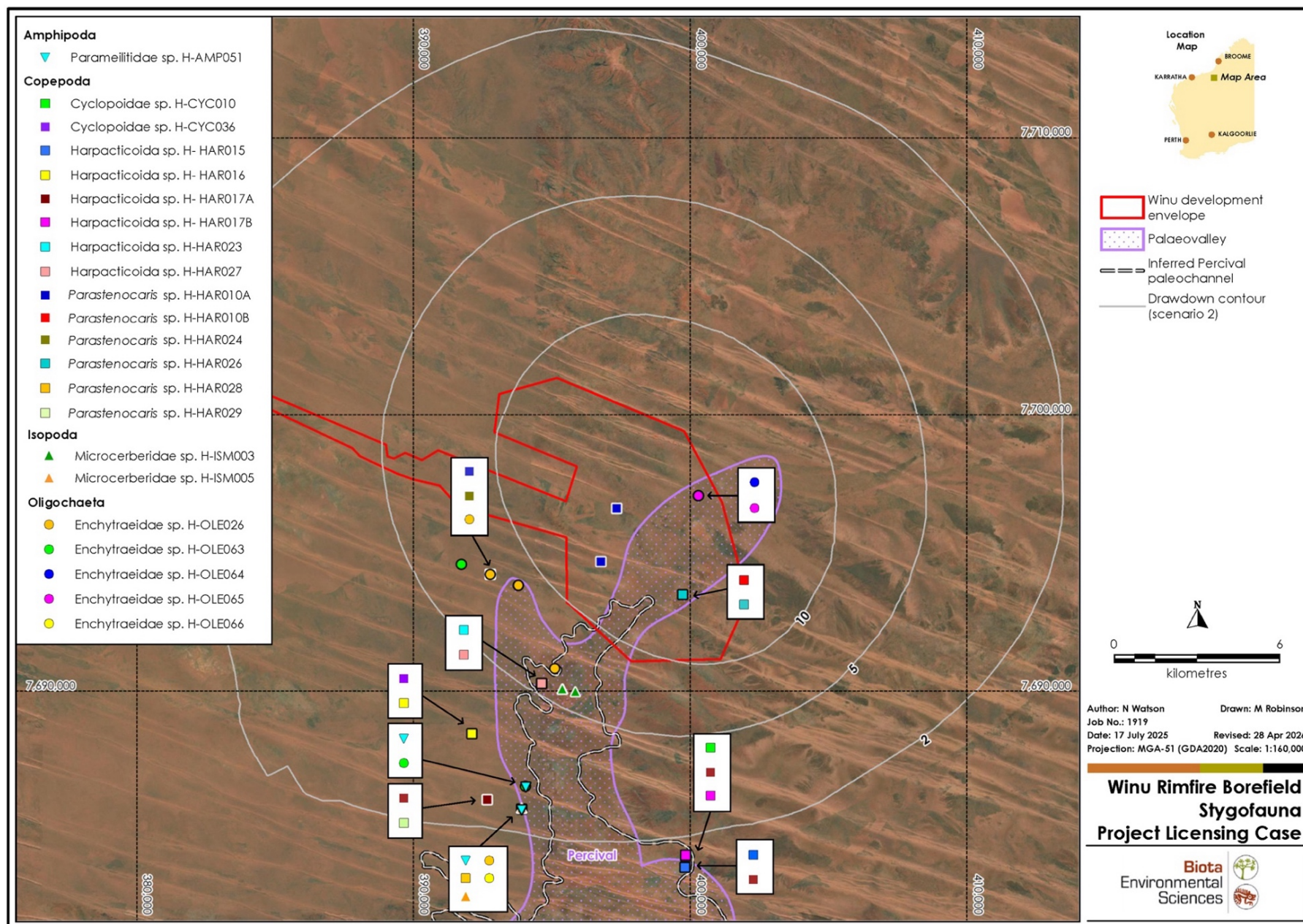


Figure 7.3: Stygofauna records relative to predicted drawdown contours for the Project licensing case (maximum at the end of 32 years simulation at 2.8 GL/a).

7.2 Potential Impacts

Impacts on stygofauna are the ultimate consequences of a proposed projects' activities, and at a generic level the types of impacts are reasonably well recognised. Direct impacts include the removal of habitat, drawdown of groundwater, inundation, and water quality changes (EPA 2016). Indirect impacts include changes to hydrology, siltation, void collapse, alteration to nutrient balance and contamination (EPA 2016). The potential consequences of these impacts on stygal species and habitats include:

- individual mortality;
- habitat loss;
- habitat fragmentation and population subdivision;
- reduced population viability; and
- local extinction.

There is limited information in the literature on changes to stygofauna as a result of groundwater abstraction or variations in flow rates (Humphreys 2009). Most impact assessments have focused on large scale advance dewatering rather than localised abstraction bores. The effect of water abstraction will depend on whether the drawdown proceeds to a level below the zone of voids suitable as habitat, or whether a species is restricted to the area of impact, as well as the overall magnitude of the dewatering operations (Humphreys 2009). There is some evidence that the response of stygofauna to water abstraction may be taxon-specific, with those restricted to larger pore spaces being affected more immediately by a lowering of the water table, since larger pores drain first while water may remain in smaller pores (Stumpp and Hose 2013). It has also been suggested that the fate of groundwater fauna in response to changes in water level may be closely related to their mode of locomotion, with crawling fauna (e.g. syncarids) being able to actively hold on to the sediment, compared to swimming fauna (e.g. copepods) that prefer to be in open water spaces (Stumpp and Hose 2013). The rate of lowering is also likely to be a factor in an organism's ability to adapt to the changing water level.

The prediction of impacts should ideally be evidence-based, but the documentation of stygofauna impacts is often subjective or anecdotal and there is a general paucity of empirical data from field programs that adequately quantify the impacts on subterranean ecosystems. In order to assess potential impacts, the spatial extent of the predicted impact needs to be quantified. This therefore requires consideration of the following:

- A. the documented, or inferred distribution of the species or assemblage (see Section 7.3); and,
- B. the spatial and vertical extent of suitable subterranean fauna habitats (see Section 7.4).

7.3 Potential Wider Distributions

Twenty of the 22 stygofauna taxa recorded from the Rimfire surveys were found to be newly detected molecular species, based on available reference data. This is not surprising given the paucity of stygofauna sampling in the Winu locality, and more broadly in the Great Sandy Desert region. Discussion of potential wider distributions for these 20 taxa is challenging, given they are all newly recorded species, and also that 18 of the 20 new taxa were singleton records (only recorded from a single location).

Comparison of the Rimfire assemblage with that recorded from subterranean surveys for the Havieron project (which is also located on, or adjacent to the Percival paleovalley) reveals a very similar suite of stygofauna (17 stygofauna taxa from six orders) (Biologic 2021). The five higher level groups recorded at Rimfire were also recorded at Havieron, with the additional order Ostracoda recorded at Havieron (Biologic 2021). There was, however, only one shared species-level taxon between the two project areas (the oligochaete worm Enchytraeidae sp. H-OLE063).

Knowledge of the stygofauna groups recorded from Rimfire (harpacticoid and cyclopoid copepods, paramelitid amphipods, microcerberid isopods and oligochaete worms) from other regions of Western Australia can be used to infer the likelihood of short-range endemism or restriction to small spatial scales for the Rimfire taxa. For stygal copepods, in the Pilbara, the majority have been found to be widespread (Karanovic 2006), however some do have distributions limited to single catchments or tributaries (Bennelongia 2013). None of these latter would be as small an extent as the predicted groundwater impact area at Winu, under either modelling scenario being considered.

In relation to harpacticoid copepods of the genus *Parastenocaris* – again, some species are widespread, whereas others have catchment-scale ranges (Bennelongia 2013). Similarly, paramelitid amphipods in the Pilbara have been found to demonstrate catchment-scale distributions, with tributary boundaries apparently forming hydrological barriers to gene flow and resulting in localised isolation of populations and divergence (Finston et al. 2007). Less is known about microcerberid isopods, as they are a cryptic group (Kim et al. 2021), which can render morphological identification ineffective at delimiting species (see Kim et al. (2018)) and validating the molecular approach used in this study. Their predisposition to short-range endemism is not yet clear, though it has been suggested that while marine microcerberids are typically widespread, freshwater taxa may have more restricted distributions (Coineau and Albuquerque 2001). Similarly, Wilson (2008) purports that “*most species of freshwater isopods species and many genera are narrow range endemics*”, and Hartebrodt (2024) also states that “*wide-ranging freshwater species (of isopods) are rare*”. However, they are extremely small animals (<1 mm) that live interstitially (within the spaces between sand or sediment), possibly suggesting that, while they may not travel far as individuals, it is also likely that geological units with only small interstices may not represent barriers to gene flow.

In relation to the oligochaete worms, a genetic study of oligochaetes recorded from multiple catchments in the Pilbara found that while “*the majority of species appeared to be restricted to individual catchments, highlighting the role of hydrological structure in shaping current distributions*”, some species were found to be widespread, with the authors of the study suggesting that “*intrinsic characteristics of oligochaetes, such as body size, shape, reproductive strategy and ecological requirements, may have allowed them greater dispersal within the subterranean biome*” and that, “*oligochaetes may occupy subterranean and surface waters, increasing their opportunities for dispersal*” (Brown et al. 2015).

Based on the above review, a conservative approach would be to assume a tributary-scale distribution for the newly recorded stygofauna taxa at Rimfire until additional specimens are recorded or sites further south along the paleovalley tributary can be sampled. A major geological fault (Anketell Fault) exists southwest of Rimfire and separates the Anketell Shelf from the Barnicarndy Graben and bisects the paleovalley tributary approximately where it meets the main body of the Percival paleovalley (see Figure 7.4). It is not known if this fault could act as a barrier to stygofauna distributions within the tributary.

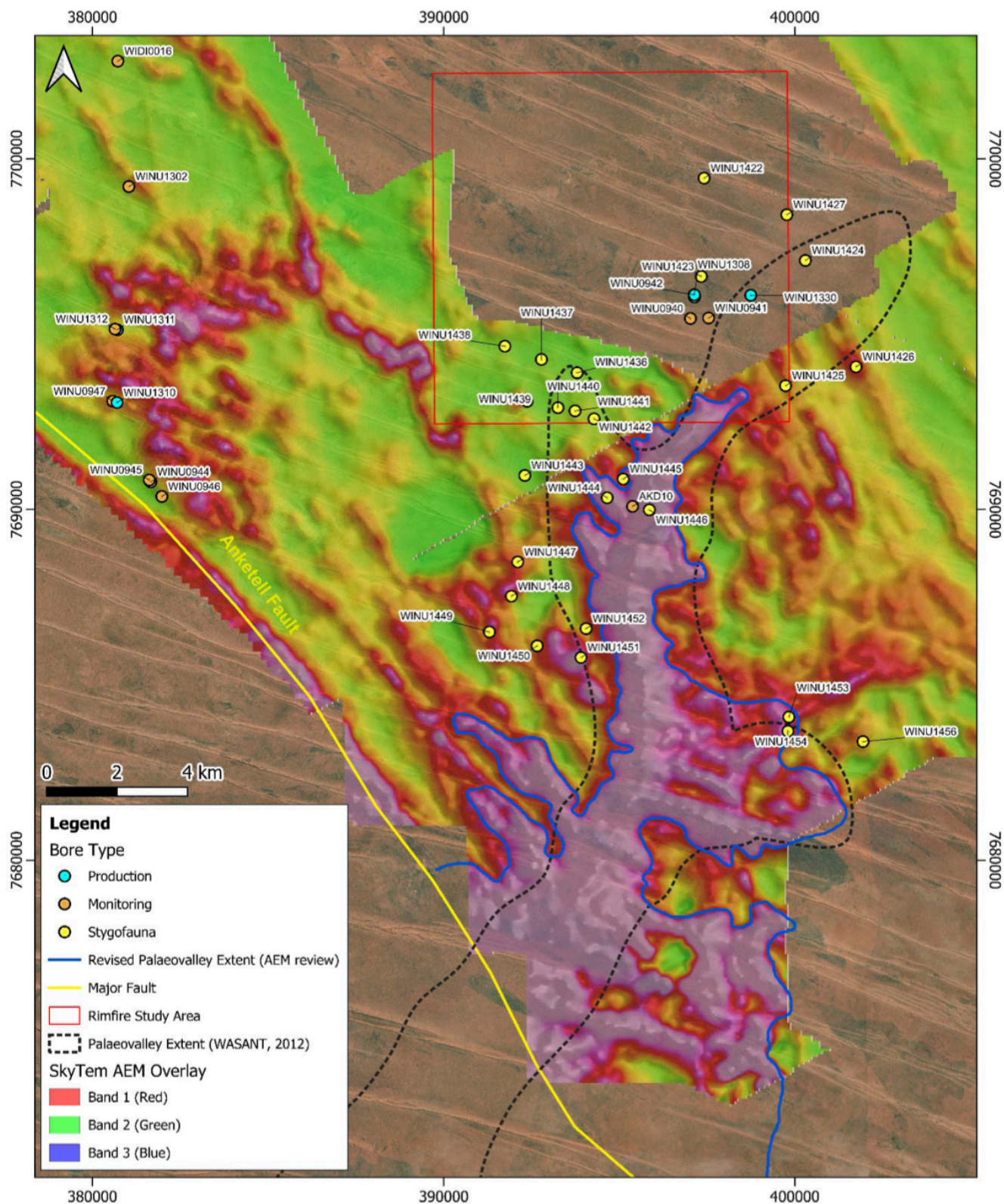


Figure 7.4: Percival Palaeovalley extent within the Rimfire area, with existing bore locations and Anketell Fault shown (from Worley (2025e)).

7.4 Extent of Stygofauna Habitat

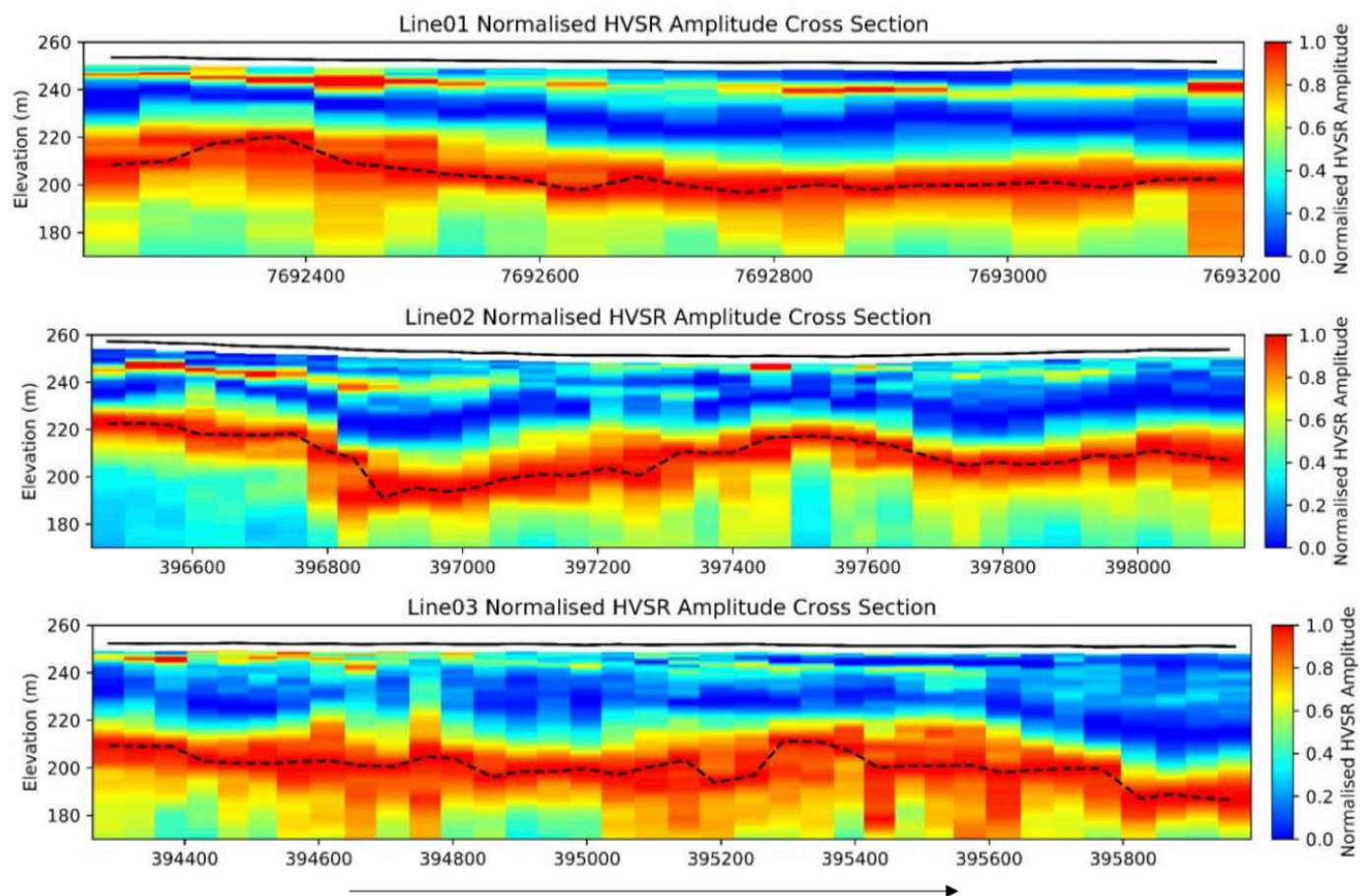
The findings of this study support previous assessments that the most prospective stygofauna habitat in the Winu area is in and around the Percival paleochannel (Biota 2022b). No stygofauna has been recorded from three phases of sampling at the MIA (Biota 2020, 2022a) or from two phases of sampling at Texas (Biota 2025)(another borefield option southwest of Rimfire that is no longer being investigated). At Winu, stygofauna have only been recorded within, and in close proximity to, the Percival paleovalley tributary (as mapped by Bell et al. (2012)), which overlaps the southern portion of the Rimfire borefield. However, defining the extent of stygofauna habitat at the paleovalley tributary remains a challenge.

Compared to paleovalley systems in other regions of WA, such as the channel-iron deposits of the Pilbara or paleovalleys of the Yilgarn, where prospective stygofauna habitat appears well stratified and typically identified as a saturated layer of alluvium and/or calcrete, the stygofauna habitat in the paleovalley tributary overlapping Rimfire is less well-defined, with the distinction between the lithologies (in terms of prospectivity for stygofauna) less clear. Compared with the paleovalleys of the Yilgarn and Pilbara, the Percival paleovalley is in general, much less prospective due to the fine-grained nature of the subterranean geologies (i.e. sandstone, claystone) and also the impervious nature of the rock types (i.e. granite), but it is the highly weathered nature of these lithologies which allow enough pore space for stygofauna to inhabit, occurring in conjunction with the paleohistory of these ancient rivers, which still flow beneath the ground surface.

Since stygofauna were recorded from geological units seemingly not overly prospective for stygofauna, it is difficult to quantify the extent of stygofauna habitat available and therefore assess potential impacts on the stygofauna that have been recorded. As there is no clear distinction in prospectivity between the lithologies at the Percival tributary (stygofauna were recorded from drillholes with groundwater intercepting sandstone, claystone and granite), it is not possible to identify which units specifically the stygofauna are inhabiting. As a result of this, a prudent approach is to assume the whole Percival paleovalley tributary hydrostratigraphic unit represents potential stygofauna habitat. There are however, still limitations on characterisation of this broader unit itself. Seismic investigations undertaken by Worley (2025c) into the boundaries and depths of the paleovalley tributary are re-presented in Figure 7.5 and summarised by the following findings:

- *“There is no discernible difference between the acoustic signature of the inferred palaeovalley sediments and the Permian sandstone which the palaeovalley has incised into, making it difficult to identify the true edges of the palaeovalley;*
- *The palaeovalley deepens southwards towards the broader Percival palaeovalley;*
- *The palaeovalley deepens to the east (in the vicinity of Line 3, middle of inferred palaeovalley, (see Figure 7.5);*
- *The maximum estimated intersected depth is 65 m bgl at the most easterly point on Line 3, however the thalweg is potentially deeper across the tenement boundary to the east.*
- *At the inferred edges of palaeovalley, depth to bedrock is ~30 m bgl” Worley (2025c).*

This information provides the best available characterisation of the paleovalley tributary – that the maximum depth of the paleovalley (where it meets bedrock) is 65 mbgl, and only 30 m bgl at the edges of the paleovalley.



HVSR = *Horizontal-to-Vertical Spectral Ratio* – a measure used for estimating the resonance frequency of strata

Survey results

Interpreted acoustic bedrock depth (palaeovalley base)

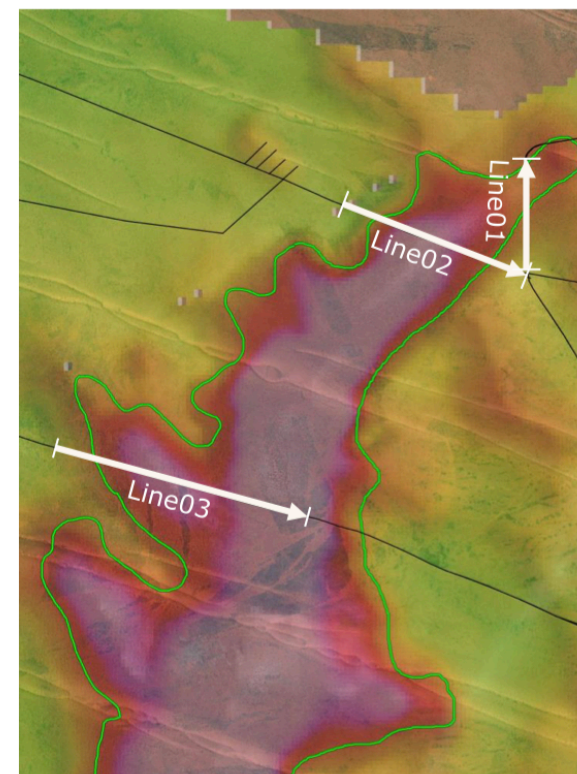


Figure 7.5: Interpreted bedrock depth of base of paleovalley (Worley (2025c)).

Worley's (2025e) review of drill log lithology identified only one drillhole (WINU1446) which intercepts a paleovalley lithology sequence (see Table 7.1). However, at Rimfire, stygofauna were recorded from 17 locations in and around the Percival paleovalley tributary, including from locations outside of Worley's (2025e) inferred paleovalley boundaries and outside the WASANT paleovalley mapping (as mapped by Bell et al. (2012)). At the WINU1446 location, currently available information indicates that the saturated thickness of the paleovalley sequence at WINU1446 is approximately 30 m (Worley 2025e) and the groundwater level sits at 4 m below ground level. Stygofauna abundance and diversity is expected to be higher where groundwater is closer to the ground surface, where oxygen levels and organic matter are more abundant (Serov 2017), thus providing sufficient energy inputs to support a stygofaunal community. The findings from Rimfire are consistent with this, with two of the five higher level groups recorded - the two orders of malacostracan crustaceans, the Amphipoda and Isopoda, only being recorded from sites where groundwater was very close to the ground surface (between 4-6 m below ground level). This was in contrast to the oligochaetes and two orders of copepods, which were found throughout a broader range of groundwater levels (including from depths >28 mbgl).

The most prospective stygofauna habitat therefore occurs where the groundwater level occurs closest to the ground surface. Figure 7.6 shows the groundwater contours around the Rimfire area including the paleovalley tributary, and the broader Winu area. Groundwater levels range from a minimum 1 m to a maximum 125 m below ground level across the whole Winu area. However, along the paleovalley tributary (as mapped by Bell et al. (2012) and Worley (2025e)) groundwater levels only range from 1 – 10 mbgl. These levels then drop to 20 m, 30 m and 40 m over a much shorter distance than that on the paleovalley tributary and we assume that this is where the alluvial aquifer reaches its limits and is replaced by the deeper metasedimentary aquifer.

The vertical connection between the alluvial groundwater and any distinct, deeper fractured rock aquifer is not yet clearly defined (although full connection was assumed in the groundwater modelling). Worley (2025d) state that *“the level of hydraulic connection between the palaeovalley aquifer and the metasedimentary aquifer has not been sufficiently assessed, although the 30 day constant rate pumping test at WINU1308 (2.8 km north of inferred palaeovalley) induced a drawdown of 0.12 m at AKD10 and suggests a potential hydraulic connection”*. In addition, *“There is some limited evidence that groundwater flow in the palaeovalley is southwards, in contrast to the north-northeasterly flow direction in the metasedimentary aquifer”* (Worley 2025f).

Figure 7.6 shows that the shallow groundwater levels (1- 10 m) along the paleovalley tributary extend both to the east of the tributary and also to the south where the tributary joins the main body of the Percival paleodrainage. These groundwater level contours map the likely extent of the most prospective stygofauna habitat (atleast in a plan-view) along the paleodrainage tributary and where it overlaps the Rimfire borefield. Figure 7.6 shows how the stygofauna habitat extends continuously southwards along the paleovalley tributary.

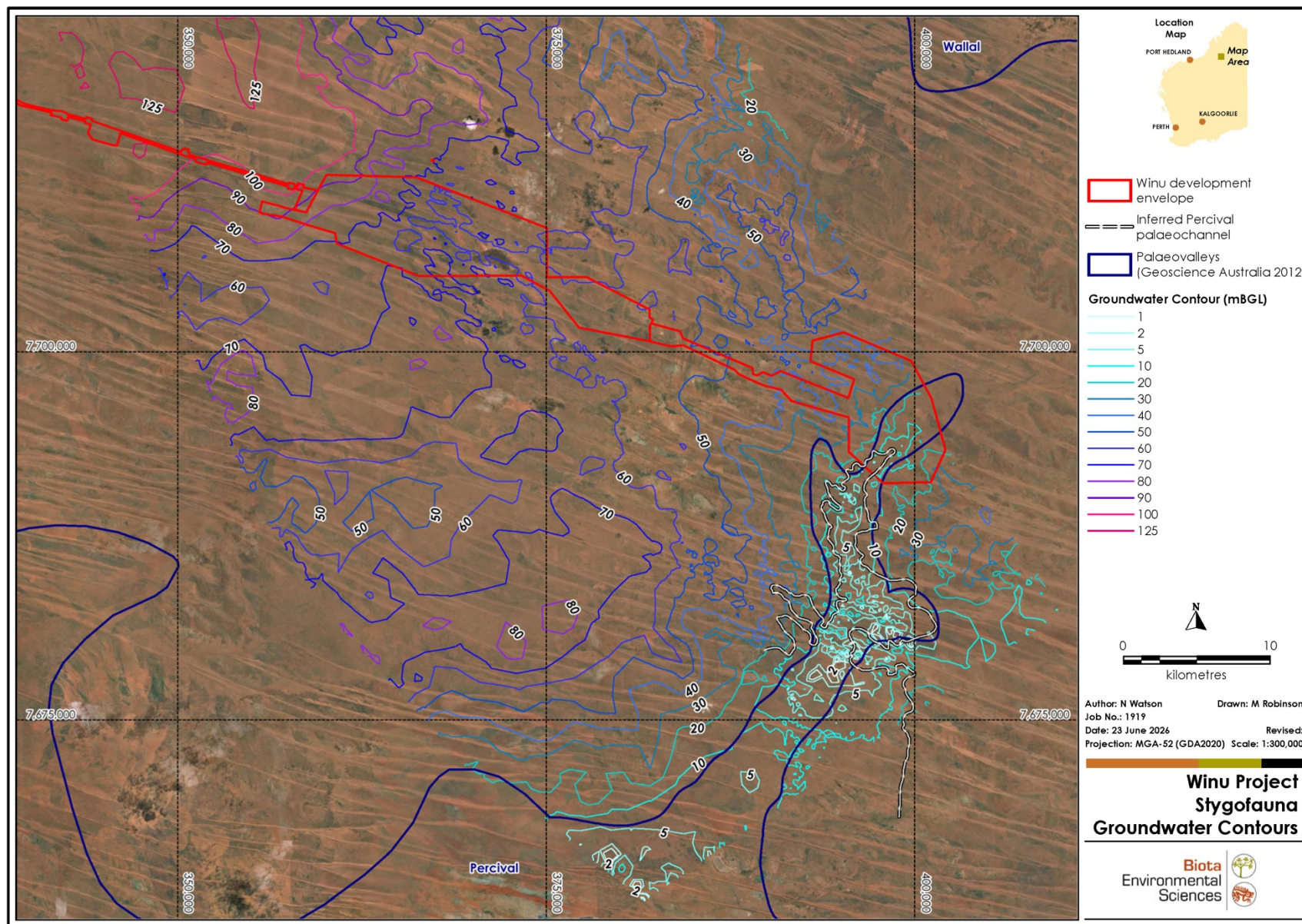


Figure 7.6: Groundwater levels (mbgl) for the Rimfire area and wider Winu area.

Parallel evidence from vegetation mapping along the paleovalley tributary found the extent of a *Melaleuca*-dominated vegetation community “to correlate well with the Percival Paleovalley, with minor variations in floristics driven by proximity to underlying geomorphological and groundwater features” (Western Botanical 2025). This *Melaleuca* community likely represents a Groundwater-Dependent Ecosystem, whose boundaries may provide a surrogate for the lateral extent of the alluvial aquifer beneath the paleovalley tributary and the area which surrounds it (see Western Botanical (2025)).

Despite the current limitations in assessing the extent of stygofauna habitat within the paleovalley tributary, we have drawn together all available relevant information to provide a general assessment of the potential impact to stygofauna as a result of the proposed groundwater abstraction scenarios. We have utilised the geological stratigraphy provided in the drill logs along with the depths to groundwater (as measured during stygofauna sampling) and additional information provided in the hydrogeological reports, in an attempt to quantify the available saturated stygofauna habitat along the paleovalley tributary (see Table 7.1).

Reliable calculations of the amount of stygofauna habitat that may be removed (or desaturated) at specific locations is not currently possible given the difficulties in assessing exactly which of the lithologies represents core stygofauna habitat. However, an estimate of the available saturated habitat can be approximated based on the extent of groundwater within prospective lithologies (in this case, all lithologies below the water level to approximately 50 m). Table 7.1 lists the drillhole stratigraphy for sites which yielded stygofauna along with the species recorded from them and the expected drawdown under each of the abstraction scenarios.

Table 7.1: Drillhole lithology, groundwater levels and predicted drawdown under each abstraction model for sites which yielded stygofauna.

Site	Drillhole stratigraphy (unit intersecting water table underlined)	Depth to water (mbgl)	Predicted drawdown extent (mbgl)		ORDER Species	Distribution notes (number of Winu sites)
			Water demand case (P90)	Water licensing case		
WINU1372	0 – 8 m: Ferricrete 8 – 26 m: Silcrete <u>26 – 41 m: Claystone</u> 41 – 58 m: Mafic intrusive	26	2-5	10	HARPACTICOIDA: • <i>Parastenocaris</i> sp. H-HAR010A	• New species (3)
WINU1423	0 – 9 m: Ferricrete <u>9 – 35 m: Sandstone</u> 35 – 57 m: Metasediment 57 – 60 m: Granite 60 – 70 m: Sandstone	30	5-7	10	HARPACTICOIDA: • <i>Parastenocaris</i> sp. H-HAR010A	• New species (3)
WINU1424	0 – 6 m: Residual soil 6 – 20 m: Ferricrete <u>21 – 32 m: Sandstone</u> 32 – 37 m: Metasediment 37 – 48 m: Granite 48 – 70 m: Metasediment	28	7	10	Subclass OLIGOCHAETA • Enchytraeidae sp. H-OLE064 • Enchytraeidae sp. H-OLE065	• New species (1) • New species (1)
WINU1425	0 – 2 m: Sand <u>2 – 25 m: Sandstone</u> 25 – 37 m: Granite 37 – 46 m: Metasediment	15	5-7	10	HARPACTICOIDA: • <i>Parastenocaris</i> sp. H-HAR010B • <i>Parastenocaris</i> sp. H-HAR026	• New species (1) • New species (1)
WINU1436	0 – 2 m: Aeolian sand 2 – 5 m: Ferricrete 5 – 17 m: Sandstone 17 – 25 m: Ferricrete <u>25 – 32 m: Granite</u> 32 – 36 m: MetaSandstone 36 – 40 m: Granite 40 – 48 m: MetaSandstone	28	2-5	5-10	Subclass OLIGOCHAETA • Enchytraeidae sp. H-OLE026	• Widespread (4)
WINU1437	0 – 8 m: Sand 8 – 12 m: Ferricrete 12 – 19 m: Siltstone <u>19 – 26 m: Sandstone</u> 26 – 42 m: Granite	22	Outside of drawdowns	5-10	Subclass OLIGOCHAETA • Enchytraeidae sp. H-OLE026 HARPACTICOIDA: • <i>Parastenocaris</i> sp. H-HAR010A • <i>Parastenocaris</i> sp. H-HAR024	• Widespread (4) • New species (3) • New species (1)
WINU1438	0 – 4 m: Aeolian sand 4 – 12 m: Ferricrete <u>12 – 33 m: Sandstone</u> 33 – 36 m: Claystone 36 – 38 m: MetaSandstone 38 – 44 m: Granite 44 – 46 m: MetaSandstone 46 – 48 m: Granite	28	Outside of drawdowns	5-10	Subclass OLIGOCHAETA • Enchytraeidae sp. H-OLE063	• Previously recorded from Havieron & Telfer (as Enchytraeidae sp. Biologic- OLIG024); 2 Winu sites
WINU1444	0 – 2 m: Sand <u>2 – 8 m: Ferricrete</u> 8 – 40 m: Sandstone 40 – 48 m: Granite	5	Outside of drawdowns	5-10	HARPACTICOIDA: • Harpacticoida sp. H-HAR023 • Harpacticoida sp. H-HAR027	• New species (1) • New species (1)

Site	Drillhole stratigraphy (unit intersecting water table underlined)	Depth to water (mbgl)	Predicted drawdown extent (mbgl)		ORDER Species	Distribution notes (number of Winu sites)
			Water demand case (P90)	Water licensing case		
WINU1445	0 – 2 m: Ferricrete 2 – 6 m: Sandstone <u>6 – 20 m: MetaSandstone</u> 20 – 24 m: Granite 24 – 32 m: MetaSandstone 32 – 58 m: Granite 58 – 60 m: MetaSandstone	7	Outside of drawdowns	5-10	Subclass OLIGOCHAETA: • Enchytraeidae sp. H-OLE026	• Widespread (4)
AKD10	Blank cased to 46 m Total depth – 50 m	4	Outside of drawdowns	5-10	ISOPODA: Microcerberidae sp. H-ISM003	• New species (2)
WINU1446	0 – 1 m: Sand <u>1 – 10 m: Claystone</u> 10 – 22 m: Sandstone 22 – 29 m: Claystone 29 – 40 m: Sand 40 – 55 m: MetaSandstone 55 – 57 m: Granite 57 – 59 m: MetaSandstone 59 – 63 m: Granite 63 – 66 m MetaSandstone	4	Outside of drawdowns	5-10	ISOPODA: • Microcerberidae sp. H-ISM003	• New species (2)
WINU1447	<u>0 – 19 m: Sandstone</u> 19 – 27 m: Claystone 27 – 29 m: Sandstone 29 – 37 m: Claystone 37 – 66 m: MetaSandstone	16	Outside of drawdowns	2-5	HARPACTICOIDA: • Harpacticoida sp. H-HAR016 CYCLOPOIDA: • Cyclopoidae sp. H-CYC036	• New species (1) • New species (1)
WINU1450	<u>0 – 18 m: Sandstone</u> 18 – 51 m: MetaSandstone 51 – 54 m: Granite	13	Outside of drawdowns	2-5	HARPACTICOIDA: • Harpacticoida sp. H-HAR017A • <i>Parastenocaris</i> sp. H-HAR029	• New species (3) • New species (1)
WINU1451	0 – 4 m: Sand 4 – 5 m: Ferricrete <u>5 – 16 m: Sandstone</u> 16 – 17 m: Siltstone 17 – 28 m: Sandstone 28 – 48 m: MetaSediment	6	Outside of drawdowns	2-5	AMPHIPODA: • Paramelitidae sp. H-AMP051 ISOPODA: • Microcerberidae sp. H-ISM005 HARPACTICOIDA: • <i>Parastenocaris</i> sp. H-HAR028 Subclass OLIGOCHAETA: • Enchytraeidae sp. H-OLE026 • Enchytraeidae sp. H-OLE066	• New species (2) • New species (1) • New species (1) • Widespread (4) • New species (1)
WINU1452	0 – 2 m: Sand <u>2 – 34 m: Granite</u>	6	Outside of drawdowns	2-5	AMPHIPODA: • Paramelitidae sp. H-AMP051 Subclass OLIGOCHAETA: • Enchytraeidae sp. H-OLE063	• New species (2) • Previously recorded from Havieron & Telfer (as Enchytraeidae sp. Biologic- OLIG024); 2 Winu sites
WINU1453	0 – 2 m: Sand 2 – 3 m: MetaSandstone <u>3 – 16 m: Granite</u> 16 – 54 m: MetaSandstone	14	Outside of drawdowns	Outside of drawdowns	HARPACTICOIDA: • Harpacticoida sp. H-HAR017A • Harpacticoida sp. H-HAR017B CYCLOPOIDA: • Cyclopoida sp. H-CYC010	• New species (3) • New species (1) • New species (1)
WINU1454	0 – 2 m: Sand <u>2 – 16 m: Sandstone</u> 16 – 33 m: MetaSandstone 33 – 36 m: Granite 36 – 48 m: MetaSandstone	10	Outside of drawdowns	Outside of drawdowns	HARPACTICOIDA: • Harpacticoida sp. H-HAR015 • Harpacticoida sp. H- HAR017A	• New species (1) • New species (3)

8.0 Conclusions

8.1 Overview

After seven phases of sampling at Rimfire, five orders of stygobitic fauna have been recorded. Prior to the sampling at Rimfire, the six phases of stygofauna sampling undertaken at other locations at Winu recorded no stygofauna specimens (Biota 2022a, 2023a, 2025).

Previous desktop studies undertaken for the Winu project identified the Great Sandy Desert region as a relatively under-surveyed part of Western Australia. Where stygofauna have been recorded within the Great Sandy Desert (and also the Little Sandy Desert) bioregion, they have been modestly diverse stygofauna communities associated with the occurrence of calcrete or alluvial geology units associated with paleovalleys, occurring within a broader setting of sandstone and mudstone units common to these bioregions. The findings from Winu support this, with the only records of stygofauna being recorded in and in proximity to the Rimfire borefield, where the Percival paleovalley tributary overlaps the Winu DE.

Twenty of the 22 stygofauna taxa recorded are newly detected species, based on data available for comparison, which limits conclusion on their wider distributions. However, inferences from Pilbara species belonging to the same taxonomic groups – and sharing a high level of similarity in ecology, life history and general morphology – suggest that a precautionary approach would assume at least tributary-scale distributions; an extent considerably greater than the zone of influence of the proposed Rimfire borefield.

Whether the Anketell Fault has implications for the stygofauna species' distributions is not yet known, but there is potential it could act as a barrier to stygofauna distributions from the tributary to the main body of the Percival paleovalley.

8.2 Predicted Impacts

8.2.1 Project Water Demand Case

The Project water demand case would impact 5 locations where stygofauna have been recorded and these locations are predicted to be impacted by a drawdown of 2 - 7 m. Six stygofauna taxa were recorded at these 5 locations: three harpacticoid copepod species and three enchytraeid oligochaete worms (one of which is considered widespread). All other stygofauna taxa recorded from the survey area (a further 16 taxa) will be outside of the area of impact under the Project water demand case.

8.2.2 Project Water Licensing Case

Under the Project licensing case, 15 of the 17 locations where stygofauna have been recorded from would be impacted by drawdowns of 2 - 10 m. At these 15 locations, 19 stygofauna taxa have been recorded, comprising 11 copepod species (10 harpacticoid and one cyclopoid copepod species), five enchytraeid oligochaete worms (two of which have been recorded outside of Winu), two microcerberid isopod species, and one paramelitid amphipod species. At the two locations where stygofauna have been recorded outside of the predicted drawdown under the Project licensing case, four taxa were present: three of which were recorded solely outside of the impact area, while one was also recorded inside the predicted area of impact.

Of the two abstraction cases, the Project water demand case represents the most likely scenario for the implementation of the Winu Project, while the Project licensing case represents a ‘worse-case scenario’ and therefore has a greater impact on the paleovalley tributary. At most, the Project licensing case has potential to remove up to one-third of available saturated habitat at the single location where paleovalley lithology has been identified. Spatially, this case has potential to impact up to approximately 38 % of the length of the tributary (as mapped by Bell (2012)). However, stygofauna utilise a three-dimensional habitat below the water table, meaning that while a plan-view spatial assessment is useful, habitat impacts must consider that groundwater drawdown attenuates with distance from the borefield – this results in an increasing proportion of stygofauna habitat predicted to remain saturated at sites further southwest down the paleovalley tributary.

Continuous suitable habitat extends southwards beyond the extent of the proposed groundwater drawdowns and a substantial volume of the paleovalley habitat in the tributary would remain unaffected by even the worst-case Project licensing case. We predict that stygofauna habitat increases in prospectivity further south along the tributary where calcrete outcropping has been noted (Geoscience Australia 2008, Western Botanical 2025), and beyond this where the tributary connects to the main body of the Percival paleovalley system.

8.3 Management Strategies

Management of impacts to stygofauna from the Rimfire borefield should focus on limiting abstraction from the alluvial aquifer within the paleodrainage. Drawdown of deeper groundwater alone would not be expected to impact stygofauna or stygofauna habitat, however, the level of hydraulic connection between the alluvial and deeper aquifer/s is not well understood.

RTW proposes to drill six monitoring bores along the paleodrainage channel to monitor groundwater levels, with four of these bores screened in the shallow aquifer and two screened in the deep aquifer. Running the groundwater abstraction models outlined in Section 7.1 and forecasting expected drawdown levels at the proposed monitoring bores can provide some guidance for monitoring impacts and developing thresholds to inform management triggers.

Figure 8.1 presents the expected drawdown levels for the proposed monitoring bores along the paleodrainage channel under three groundwater abstraction scenarios (the two presented here plus a median water demand, or P50, case). These predicted drawdown levels can be used to inform a trigger threshold, beyond which specific impact investigation and management responses would be implemented. Trigger threshold levels also need to be placed into context with the natural fluctuation in the water table. Groundwater abstraction that leads to drawdown levels that are within the natural fluctuations in the water table are not expected to cause significant impacts to stygofauna or stygofauna habitat. However, limited data are currently available on the natural fluctuations in groundwater levels in the Rimfire area. Data loggers monitoring groundwater levels in some bores over a 12 month period (2025-2026) showed that water levels remained stable, only fluctuating within 0.5 m (Worley, unpublished data). This is, however, a relatively short monitoring period and in a region such as the Great Sandy Desert groundwater fluctuations are likely to be episodic in response to large recharge events. Continued monitoring of baseline groundwater levels will provide further confidence in this data. A range of 2 m is expected from longer-term monitoring (advice from RTW).

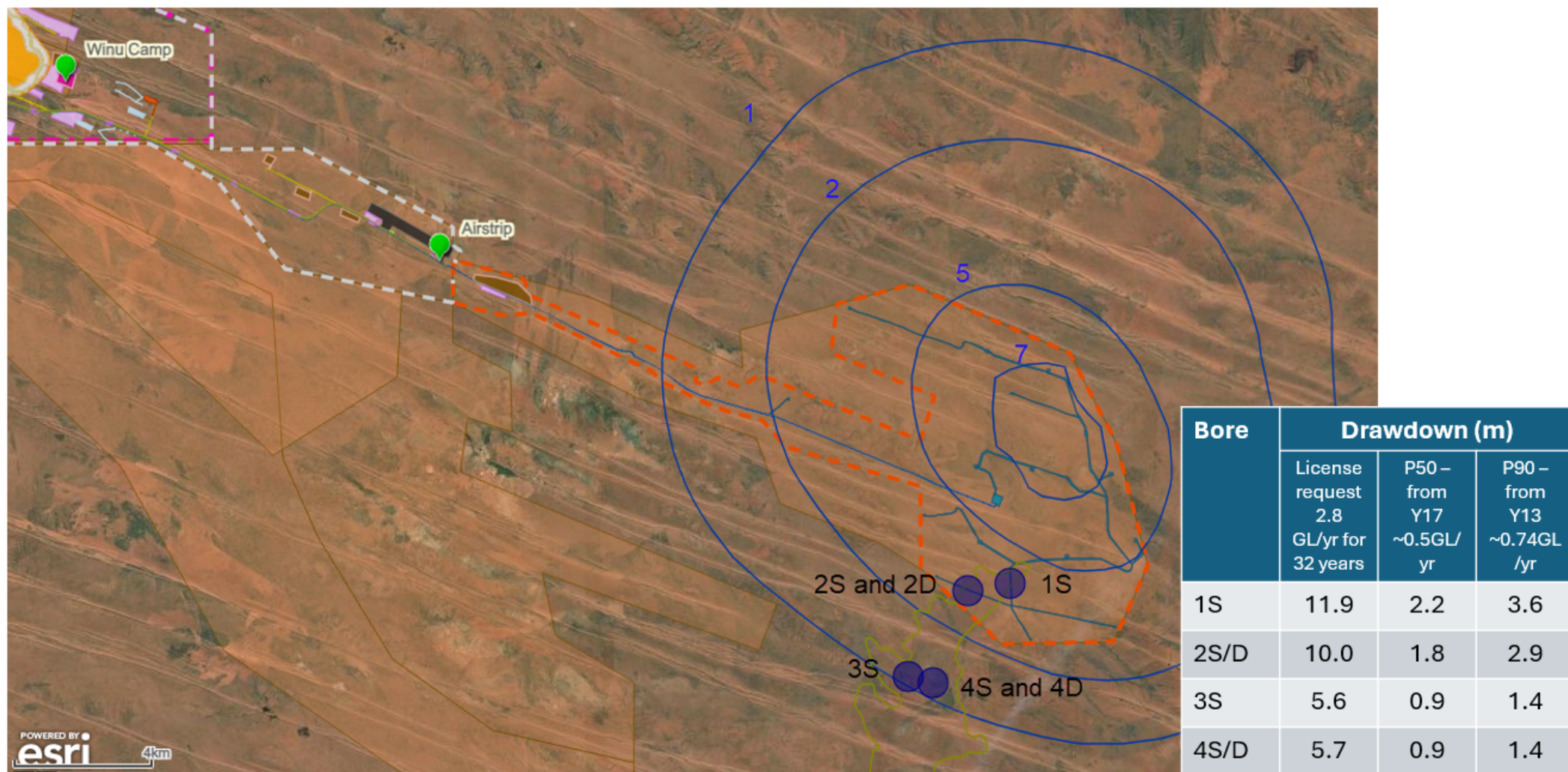


Figure 8.1: Proposed Rimfire paleochannel monitoring bores and predicted drawdown extent under modelled scenarios with Water demand case drawdowns mapped (P90) (provided by RTW).

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Appendix 1

Helix Molecular Solutions Report

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Molecular Sequencing of the Winu Rimfire Stygofauna (Phases 6 and 7)



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Molecular sequencing of the Winu Rimfire Stygofauna (P6 – P7)

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1.0 Executive Summary

Helix Molecular Solutions was engaged by Biota Environmental Sciences to perform DNA extractions, sequencing of the mitochondrial cytochrome oxidase subunit I gene (COI) and analyses of 184 stygofauna specimens belonging to the Oligochaeta, Amphipoda, Isopoda, and Copepoda collected during the phases 6 and 7 survey of the Winu Rimfire Borefield survey area (current study). This report provides a synopsis of the relationships between the successfully sequenced specimens and the reference specimens available, including the number of putative species based on the phylogenetic analysis. Analyses in this report include the previously collected specimens from Winu (Helix 2024a, 2024b, 2025a, 2025b, 2025c) where relevant. Following initial molecular analysis, an individual symphyllan (Myriapoda) specimen was detected during phase 6 which could be either edaphobitic or troglobitic. Although not the target of this survey, the results are also reported for this species.

The objective of this study was to gain information on the number of putative species and their apparent distributions, based on the molecular genetic data available for comparison.

Amongst the 167 specimens sequenced (phase 6 n=93, phase 7 n=74), phylogenetic analysis revealed a total of 22 putative species (phase 6 n=16, phase 7 n=13). Twelve of these were previously detected taxa and 10 were newly detected taxa, based on the molecular sequence data currently available for comparison.

Annelida: Clitellata, Oligochaeta: Family Enchytraeidae

Forty-five of the 48 specimens identified as oligochaetes were successfully sequenced for COI during this project (phase 6 and 7). Previous survey phases did not include any Oligochaete specimens for comparison. Phylogenetic analysis confirmed the specimens represented one Oligochaete family: Enchytraeidae. A total of five putative species were detected, corresponding to two previously detected but undescribed taxa: Enchytraeidae sp. H-OLE026 and Enchytraeidae sp. H-OLE063, and three newly detected taxa: Enchytraeidae sp. **H-OLE064**, Enchytraeidae sp. **H-OLE065** and Enchytraeidae sp. **H-OLE066**.

Specimens of Enchytraeidae sp. H-OLE026 detected from both phase 6 (n=24) and 7 (n=1) sampling (historically known as Enchytraeidae sp. E6) have been previously detected from multiple catchments within the Pilbara bioregion and the taxon appears to be widespread. The closest reference specimen genetically was Enchytraeidae sp. E6-B03 (GenBank PP940020), which differed by 5.5 – 7.0% pairwise molecular sequence divergence. This reference specimen was collected from a location approximately 360 km southwest of the project specimens.

Specimens of Enchytraeidae sp. **H-OLE063** (also known as Enchytraeidae sp. Biologic-OLIG024) were detected in phase 6 only (n=4) and have been previously detected from an area ranging from approximately 70 km south to 120 km southeast of the project specimens. Reference specimens (Enchytraeidae sp. Biologic-OLIG024: GenBank#s MW021316, MW021317, MW021322, MW021319 and OR940732) differed from the project specimens by 7.1 – 8.2% pairwise sequence divergence.

Enchytraeidae sp. **H-OLE064** (n=13), Enchytraeidae sp. **H-OLE065** (n=1) and Enchytraeidae sp. **H-OLE066** (n=1) are currently only known from this project ('WJ' prefix) all detected during phase 6 sampling, based on current reference specimens available for comparison.

Crustacea: Malacostraca: Amphipoda: Family Paramelitidae

Nineteen amphipod specimens sequenced from the current Winu survey were collected from a single site (Winu1451) across phases 6 (n=4) and 7 (n=15). All specimens from this site were found to belong to Paramelitidae sp. H-AMP051, also detected during a phase 5 at site Winu1452.

Crustacea: Malacostraca: Isopoda: Family Microcerberidae

Sixteen isopod specimens from site Winu1451 were analysed along with 13 previously sequenced isopod specimens from five previously sampled sites at Winu, ADK10 and Old Bore, (Helix 2024a, 2025a, 2025b), Winu1446, 1447, 1451 and 1452, (Helix 2025). All phase 6 (n=9) and 7 (n=7) specimens, represented by three closely related haplotypes, aligned with previously detected microcerberid isopods from the same site, Winu1451, and were assigned to the existing taxon, Microcerberidae sp. H-ISM005. The two additional microcerberid isopod species known from Winu, Microcerberidae sp. H-ISM003 (from sites ADK10, Winu1446 and 1447) and Microcerberidae sp. H-ISM004 (from site Old Bore) were not detected in the current sampling phases.

Crustacea: Maxillopoda: Cyclopoida

Fourteen cyclopoid specimens (phase 6 n=9, phase 7 n=5) were sequenced during the current project. Thirteen specimens belong to the previously detected taxon Cyclopidae sp. H-CYC010 (phase 5), all from the same site at Winu1453. There was no divergence (0.00%) amongst the all 15 specimens of Cyclopidae sp. H-CYC010. The closest relative, according to the molecular data currently available for comparison, was *Dussartcyclops* sp. Biologic-CYCL007 (GenBank accession # PV442707) with 19.3% molecular sequence divergence.

A single cyclopoid specimen (WJ130) from site Winu1447 did not align with any previously detected cyclopoid sequences, based on the molecular sequence data currently available for comparison. This specimen was assigned to the newly detected taxon Cyclopidae sp. **H-CYC036** and was detected during phase 6 only. This taxon showed 19.1% from the only other cyclopoid detected at Winu, Cyclopidae H-CYC010. The closest relative, according to the molecular sequence data currently available for comparison was *Dussartcyclops* sp. Biologic-CYCL007 (GenBank accession # PV442765).

Crustacea: Maxillopoda: Harpacticoida

Seventy-two harpacticoid specimens (phase 6 n=26, phase 7 n=46) were sequenced, amongst which were ten putative taxa (phase 6 n=4, phase 7 n=8). Amongst these ten harpacticoid putative taxa there was between 2.7 and 29.9% molecular sequence divergence. Three of these taxa have been detected previously, Harpacticoida sp. H- HAR017A (phases 4,5, 6 and 7), Harpacticoida sp. H- HAR017B (phases 4 and 7), *Parastenocaris* sp. H-HAR010A (phases 2, 5, 6 and 7) and Harpacticoida sp. H- HAR016 (phases 4 and 7). Whilst seven were newly detected taxa, based on the molecular sequence data currently available for comparison. These new taxa include *Parastenocaris* sp. H-HAR010B (phase 7 only), Harpacticoida sp. **H-HAR023** (phase 6 only), *Parastenocaris* sp. **H-HAR024** (phase 6 only), *Parastenocaris* sp. **H-HAR026** (phase 7 only), Harpacticoida sp. **H-HAR027** (phase 7 only), *Parastenocaris* sp. **H-HAR028** (phase 7 only) and *Parastenocaris* sp. **H-HAR029** (phase 7 only). These Winu harpacticoid taxa showed between 1.9 and 24.0 % sequence divergence from the closest relative harpacticoid reference specimen sequences.

Myriapoda: Symphyla: Family Scolopendrellidae

One specimen (WJ172) collected from site Winu 1450, originally assigned to the Isopoda, was reassigned to the non-target taxa Myriapoda: Symphyla based on the resulting sequence data. This individual symphylan specimen was identified as a newly detected putative species *Symphylella* sp. **H-SYM041** (family Scolopendrellidae), which differed from the closest reference specimen (Helix# QY02 *Symphylella* sp. H-SYM027b) by 12.2%. Symphylans are blind soil dwelling myriapods that have a sub-cosmopolitan distribution and are known to be edaphobitic. Although edaphobites frequently display similar morphological traits to troglobites, such as loss of eyes and pigmentation and are frequently found deeper underground in caves, their primary habitat is soil. Despite the difficulties distinguishing edaphobites from troglobites, troglofauna surveys across Western Australia have detected numerous undescribed species belonging to both families of symphylan: Scolopendrellidae and Scutigereidae.

2.0 Introduction

2.1 Project Background

There have been seven phases of subterranean surveys for Winu Rimfire project area to date with stygofauna specimen collections and sequencing.

The first, phase 1 detected an Isopoda in the family Microcerberidae, molecular analyses revealed this to be a previously undetected taxon, based on the molecular data currently available for comparison. This taxon was recognised as Microcerberidae sp. H-ISM003 and was detected only from site [AKD10](#) (specimen code prefix 'TT' - 2024a Molecular Systematics of Winu).

The second phase, phase 2 sampled an additional bore site ([RIMFIRE STYGO2](#)), and sampled Copepoda belonging to the Harpacticoida. Molecular analyses revealed this to be a previously undetected taxon, based on the molecular data currently available for comparison. This taxon was recognised as *Parastenocaris* sp. H-HAR010(A) and is known only from site RIMFIRE STYGO2 (specimen code prefix 'UD' - 2024b - Molecular Systematics of Winu Copepoda).

The third phase of subterranean sampling, phase 3 resampled the bore site sampled in phase 1 ([ADK10](#)) and detected an Isopoda in the family Microcerberidae, molecular analyses confirmed this to be the taxon detected in phase 1 (Helix, 2024a), Microcerberidae sp. H-ISM003. An additional bore site was sampled, site [OldBore](#), which detected both Isopoda and Copepoda specimens. Molecular analyses revealed these to be previously undetected taxa, based on the molecular data currently available for comparison. These taxa were referred to as Microcerberidae sp. H-ISM004 and Harpacticoida sp. H-HAR011 both from the site OldBore (specimen code prefix 'UT' - 2025a - Winu Rimfire Isopoda and Copepoda).

The fourth phase of subterranean sampling, phase 4 resampled bore site [ADK10](#) that had been previously sampled in phases 1 (Helix, 2024a) and 3 (Helix, 2025a) and also sampled an additional bore site ([Winu1447](#)) (Helix, 2025b). Specimens from this phase of sampling included both Isopoda and Copepoda. Molecular analyses, based on the molecular data currently available for comparison, redetected Microcerberidae sp. H-ISM003 from site ADK10. The additional sites also revealed a previously undetected copepod, Harpacticoida sp. H- HAR015 from [Winu1454](#), Harpacticoida sp. H- HAR016 from site [Winu1447](#) and Harpacticoida sp. H- HAR017 from site [Winu1453](#) (specimen code prefix 'VB' - 2025b -Winu Rimfire Isopoda and Copepoda).

The fifth phase of subterranean sampling, phase 5, resampled site [OldBore](#) site as well as sampling the additional sites [Winu1446](#), [Winu1447](#), [Winu1450](#), [Winu1451](#), [Winu1452](#) and [Winu1453](#) (Helix, 2025c). Specimens from this survey included Amphipoda, Isopoda and Copepoda (Helix, 2025c). Based on the molecular data currently available for comparison, the molecular analyses detected the Amphipoda specimens detected belong to the were the highly differentiated newly detected taxa Parameilitidae sp. H-AMP050 ([OldBore](#)) and Parameilitidae sp. H-AMP051 ([Winu1452](#)). The Isopoda taxa detected included Microcerberidae sp. H-ISM003, redetected from the same site [ADK10](#), and a highly new previously undetected taxon highly differentiated taxon Microcerberidae sp. H-ISM005 from site [Winu1451](#) (Helix, 2025c). The Copepoda specimens, based on the molecular data currently available for comparison, were revealed as a Harpacticoida sp. H- HAR017 from site [Winu1450](#), previously detected from site [Winu1453](#) (Helix, 2025b). The remaining analysed

Copepoda from site Winu1453 belonged to the Cyclopoida was assigned to Cyclopidae sp. H-CYC010. This was found to occur in sympatry with Harpacticoida sp. H-HAR017 at Winu1453, however these collections were undertaken on separate surveys. All phase 5 specimens were assigned the specimen code prefix 'VJ' (see Helix, 2025c).

3.0 Methods

DNA was extracted from the Winu Rimfire specimens submitted using the Qiagen DNeasy Blood & Tissue kit (QIAGEN) following the spin-column protocol for *purification of total DNA from animal tissues*. This methodology was modified slightly by performing the final elution step twice, using 30µl and 50µl of buffer AE. The resulting purified DNA was then stored at -20°C prior to use. PCR amplification then was performed on an Eppendorf Thermocycler using COI primers LCOI and HCO2 (Folmer et al., 1994). Final PCR products were sequenced at the Australian Genome Research Facility (AGRF) using Sanger Sequencing.

Sequences were edited and aligned using Geneious Prime version 2025.1.2 (<http://www.geneious.com>). DNA nucleotide sequences were translated into protein sequences with ExPASy using the invertebrate genetic code to ensure that the amplified sequences corresponded to the target mtDNA. The translated protein sequences were then checked for the presence of stop codons, to ensure that pseudogenes had not been amplified. Pseudogenes have a DNA sequence that is analogous to the functional gene (e.g. COI) however, they do not code for a functioning protein despite the shared ancestry with the functional gene. The presence of pseudogenes can complicate molecular analyses, producing misleading results.

All sequences analysed were of high quality with no evidence of heterogeneous peaks. All resulting sequences were 'BLAST'ed (Basic Local Alignment Search Tool) with the NCBI (National Centre for Biotechnology Information) which is a publicly available database. The program compares DNA nucleotide sequences with a library of sequences and identifies sequences within the database that resemble the query sequences above a certain threshold.

Genetic distance between unique genetic sequences were measured using uncorrected p-distances (total percentage of nucleotides different between sequences). Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance. To account for polymorphism within lineages, the net genetic diversity of Nei (1987) was calculated to give a 'corrected' distance between lineages.

For phylogenetic analysis, likelihood ratio tests using the Bayesian Information Criterion were calculated in MEGA 11 (Tamura et al., 2021) to determine the best-fit model of evolution. The phylogenetic analyses were then conducted in Geneious Prime version 2025.1.2 (<https://www.geneious.com>) using the best-fit model of evolution calculated (Table 1). Maximum likelihood (ML) analyses with 100 rapid bootstrap replicates were performed in RAxML (Randomised Accelerated Maximum Likelihood) version 8.2.11 (Stamatakis, 2014), using default settings.

Table 3-1 **Best-fit model of evolution for the Amphipoda, Isopoda, Copepoda and Oligochaeta analysed from Winu.** Abbreviations: HKY: Hasegawa-Kishino-Yano; GTR: General Time Reversible; G: Gamma; I: Invariant sites.

Taxonomic Group	Analysis Group	Best Model	Gamma Value	Invariant sites
Annelida	Oligochaeta	GTR+G+I	0.61	0.42
Crustacea	Malacostraca: Amphipoda	GTR+G+I	0.92	0.39
	Malacostraca: Isopoda	HKY+G+I	0.43	0.36
	Maxillopoda: Harpacticoida and Cyclopoida	GTR+G+I	0.58	0.28
Myriapoda	Symphyla	GTR+G+I	0.64	0.40

For the purposes of this report, lineages were defined as haplotypes or groups of haplotypes differing from other such groups between 3 to 5% sequence divergence. This cut-off was selected based on meta-reviews of bar-coding data, which indicate that intra-specific (within species) variation rarely exceeds 3% (Hebert et al., 2003). Species were determined based on taxonomic group, molecular and morphological data available and previously published literature for each taxonomic group.

Helix species names have been updated for the current report to reflect our most current data and follow current Helix nomenclature (with nominal species names using the prefix 'H-').

4.0 Results

One hundred and sixty-seven subterranean specimens submitted to Helix from this survey were successfully sequenced for the cytochrome oxidase subunit I gene (COI) (Table 4-1, Supplementary material). This total included 45 oligochaetes, 19 amphipods, 16 isopods, 14 cyclopoids, 72 harpacticoids and one symphylan specimen. Only three oligochaete specimens (WJ04, WJ43, WJ44) failed to sequence. One specimen (WJ172) initially identified based on morphology as an isopod was reassigned as a symphylan following preliminary molecular analysis.

Tables of genetic p-distance and associated standard errors between the project specimens are provided in Appendix 1 (Table A1.1). Tables of genetic p-distances and associated standard errors of reference specimens showing less than 15 - 20% sequence divergence from project specimens are provided as additional supplementary material due to the size of the datasets.

4.1 Annelida

4.1.1 Clitellata: Oligochaeta: Family Enchytraeidae

Oligochaetes have not undergone particular morphological changes in the subterranean environment, in part this may be due to the elongated, segmented and flexible body shape that already characterises all oligochaetes and enables them to move in reduced spaces, whether in interstitial surface habitats or subterranean environments (Creuzé des Châtelliers et al., 2009). Moreover, with these characters they are morphologically pre-adapted to such environments. There has been widespread colonisation of subterranean environments among oligochaetes, with seven families showing stygobitic representatives (Creuzé des Châtelliers et al., 2009). There is a strong tendency for endemism amongst stygobitic oligochaetes, with more than half of the species known only from their type locality (Creuzé des Châtelliers et al., 2009). Molecular studies have revealed numerous cryptic species, which cannot be identified by standard morphological means (Trontelj and Douady, 2004; Verovnik et al., 2004; Lefébure et al., 2006a, b, 2007).

The oligochaete fauna of the Pilbara is known to be diverse but lacking in formal description (Brown et al., 2015). A comprehensive phylogenetic study of three oligochaete families (Enchytraeidae, Phreodrilidae and Naididae) within the Pilbara found that over 89% of putative species detected were restricted to individual catchments with no evidence of historical gene flow between catchments (Brown et al., 2015). However, 10% of species were shown to be more widespread and were detected in multiple catchments (Brown et al., 2015).

Reference Specimens and Outgroups

Forty-eight oligochaete specimens were collected from the survey area during phase 6 (n=46) and 7 (n=2) of which 45 were successfully sequenced for COI (Table 4-1). Oligochaetes were not collected in any prior phases. The COI sequenced specimens were analysed along with a total of 371 reference specimens for COI from both the Helix database (n=85), GenBank (n=260), and BOLD systems (n=26), including the outgroups *Oxytychus* sp. AFOF-2012 (GenBank#JX203383) and *Orobodella ketagalan* (GenBank # AB704787). A reduced dataset was used to simplify the phylogenetic tree for this report.

Phylogenetic Analyses

A standard 687 bp fragment of COI was isolated for the 45 oligochaete specimens. Preliminary analysis confirmed the resulting sequences sit within the family Enchytraeidae and represent 10 unique haplotypes (Table 4-2). The specimens likely represent five taxa, three of which were newly detected putative species and two previously detected but undescribed, according to the molecular sequence data currently available for comparison.

Twenty-six specimens (n=3 haplotypes) representing one species Enchytraeidae sp. H-OLE026 aligned with several reference specimens from both the Helix database (Enchytraeidae sp. H-OLE026: Helix #GD10, GD12, JY10) and GenBank (Enchytraeidae sp. E6), but do not form a clear, well-resolved clade (Figure 4-1, Table 4-2). This putative species was collected from three sites in Phase 6 (Winu1436, 1445 and 1437) and one site in Phase 7 (Winu 1451; Table 4-1).

Four specimens representing putative species Enchytraeidae sp. H-OLE063 (n= 2 haplotypes) were collected from two sites (Winu1438 and Winu 1452) and formed a well-supported clade with reference specimens of Enchytraeidae sp. Biologic-OLIG024, which is likely conspecific to the project lineage (Figure 4-1).

Table 4-1: Invertebrate specimens used in the present survey (‘WJ’ prefix, n = 184) and those collected during previous surveys at Winu (prefix ‘TT’, ‘UD’, ‘UT’, ‘VB’, and ‘VJ’), and the genetic species to which they were assigned. Molecular lineages in bold text represent a newly detected species. Specimens that were not successfully sequenced are not included. Please refer to Supplementary Material for additional information.

Helix Job No.	Phase	Specimen number	Site	Latitude	Longitude	Helix ID	Taxonomic Group	Family	Molecular ID
910	6	WINU_1436_01	Winu1436	-20.852177	121.97906	WJ05	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_01	Winu1436	-20.852177	121.97906	WJ06	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_01	Winu1436	-20.852177	121.97906	WJ07	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_01	Winu1436	-20.852177	121.97906	WJ08	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_01	Winu1436	-20.852177	121.97906	WJ09	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ10	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ11	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ12	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ13	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ14	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ15	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ16	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ17	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ18	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ19	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ20	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1436-20250812-01	Winu1436	-20.852177	121.97906	WJ21	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_02	Winu1436	-20.852177	121.97906	WJ22	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_02	Winu1436	-20.852177	121.97906	WJ23	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_02	Winu1436	-20.852177	121.97906	WJ24	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_02	Winu1436	-20.852177	121.97906	WJ25	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_02	Winu1436	-20.852177	121.97906	WJ26	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1436_02	Winu1436	-20.852177	121.97906	WJ27	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU_1445_01	Winu1445	-20.879443	121.991618	WJ28	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	Winu1437-20250812-03	Winu1437	-20.848652	121.969372	WJ47	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
910	6	WINU1438_1	Winu1438	-20.845181	121.959359	WJ01	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE063
910	6	WINU1438_1	Winu1438	-20.845181	121.959359	WJ02	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE063
910	6	WINU1438_1	Winu1438	-20.845181	121.959359	WJ03	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE063
910	6	WINU_1452_01	Winu1452	-20.917852	121.981195	WJ45	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE063
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ29	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ31	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ32	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ33	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ34	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ35	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ36	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ37	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ38	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ39	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ40	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ41	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ42	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE064
910	6	WINU1424	Winu1424	-20.823351	122.041846	WJ30	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE065
910	6	Winu1451-20250813-02	Winu1451	-20.925334	121.979797	WJ46	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE066
910	7	Winu1451-20251121-04	Winu1451	-20.925334	121.979797	WJ179	Annelida: Clitellata, Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026
886	5	OldBore-20241130-02	OldBore	-21.98918656	122.03538	VJ01	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP050
886	5	OldBore-20241130-03	OldBore	-21.98918656	122.03538	VJ02	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP050
886	5	OldBore-20241130-03	OldBore	-21.98918656	122.03538	VJ03	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP050
886	5	OldBore-20241130-03	OldBore	-21.98918656	122.03538	VJ04	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP050
886	5	Winu1452-20250521-01	Winu1452	-20.91788483	121.9812496	VJ05	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP051
910	6	Winu1451-20250813-04	Winu1451	-20.925334	121.979797	WJ144	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP051
910	6	Winu1451-20250813-04	Winu1451	-20.925334	121.979797	WJ145	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP051
910	6	Winu1451-20250813-04	Winu1451	-20.925334	121.979797	WJ146	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP051
910	6	Winu1451-20250813-04	Winu1451	-20.925334	121.979797	WJ147	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP051
910	7	Winu1451-20251121-02	Winu1451	-20.925334	121.979797	WJ148	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP051
910	7	Winu1451-20251121-02	Winu1451	-20.925334	121.979797	WJ149	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP051
910	7	Winu1451-20251121-02	Winu1451	-20.925334	121.979797	WJ150	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP051
910	7	Winu1451-20251121-02	Winu1451	-20.925334	121.979797	WJ151	Crustacea: Malacostraca, Amphipoda	Paramelitidae	Parameilitidae sp. H-AMP051

Helix Job No.	Phase	Specimen number	Site	Latitude	Longitude	Helix ID	Taxonomic Group	Family	Molecular ID
910	6	WINU_1444_01	Winu1444	-20.884407	121.987007	WJ70	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR023
910	6	WINU_1444_01	Winu1444	-20.884407	121.987007	WJ71	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR023
910	6	WINU_1444_01	Winu1444	-20.884407	121.987007	WJ72	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR023
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ106	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ107	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ108	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ109	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ110	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ111	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ112	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ113	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ114	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
910	7	Winu1444-20251120-01	Winu1444	-20.884407	121.987007	WJ115	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpactcoida sp. H-HAR027
870	4	Winu1454.20250311-02	Winu1454	-20.7329	121.7593	VB06	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR015
870	4	Winu1454.20250311-02	Winu1454	-20.7329	121.7593	VB07	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR015
870	4	Winu1447.20250311-01	Winu1447	-20.7329	121.7593	VB01	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR016
910	7	Winu1447-20251120-01	Winu1447	-20.900583	121.962613	WJ116	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR016
910	7	Winu1447-20251120-01	Winu1447	-20.900583	121.962613	WJ117	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR016
870	4	Winu145320250311-01	Winu1453	-20.7328	121.7594	VB09	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
870	4	Winu145320250311-01	Winu1453	-20.7328	121.7594	VB10	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
886	5	Winu1450-20250523-01	Winu1450	-20.92220171	121.9678097	VJ09	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	7	Winu1450-20251121-01	Winu1450	-20.922202	121.967804	WJ121	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	7	Winu1450-20251121-01	Winu1450	-20.922202	121.967804	WJ122	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	7	Winu1450-20251121-01	Winu1450	-20.922202	121.967804	WJ124	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	7	Winu1454-20251122-01	Winu1454	-20.94463	122.036335	WJ127	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	7	Winu1454-20251122-01	Winu1454	-20.94463	122.036335	WJ128	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	7	Winu1454-20251122-01	Winu1454	-20.94463	122.036335	WJ129	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	7	Winu1454-20251122-02	Winu1454	-20.94463	122.036335	WJ181	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	6	Winu1454-20250815-01	Winu1454	-20.94463	122.036335	WJ48	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	6	Winu1450-20250814-01	Winu1450	-20.922202	121.967804	WJ73	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	6	Winu1450-20250814-01	Winu1450	-20.922202	121.967804	WJ74	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	6	Winu1450-20250814-01	Winu1450	-20.922202	121.967804	WJ75	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	6	Winu1450-20250814-01	Winu1450	-20.922202	121.967804	WJ76	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	6	Winu1450-20250814-01	Winu1450	-20.922202	121.967804	WJ77	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
910	6	Winu1450-20250814-01	Winu1450	-20.922202	121.967804	WJ78	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017A
870	4	Winu145320250311-01	Winu1453	-20.7328	121.7594	VB08	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017 B
910	7	Winu1453-20251122-02	Winu1453	-20.940783	122.036647	WJ126	Crustacea: Maxillopoda, Harpacticoida (Copepod)	undetermined	Harpacticoida sp. H- HAR017B
910	6	Winu1450-20250814-03	Winu1450	-20.922202	121.967804	WJ172	Myriapoda: Symphyla	Scolopendrellidae	Symphylella sp. H-SYM041

Thirteen specimens assigned to putative species *Enchytraeidae* sp. H-OLE064 (n=3 haplotypes) did not align closely with any reference sequences available for comparison and likely represent a newly detected species (Figure 4-1). All 13 specimens were collected from a single site, Winu1424 (Table 4-1).

The remaining two specimens represent two new putative taxa not previously detected according to the molecular data currently available for comparison. These were *Enchytraeidae* sp. H-OLE065 and *Enchytraeidae* sp. H-OLE066 (Table 4-2, Figure 4-1). *Enchytraeidae* sp. H-OLE065 was detected at site Winu1424 and *Enchytraeidae* sp. H-OLE066 was detected at site Winu1451 (Table 4-1).

Table 4-2: Summary of the *Enchytraeidae* Oligochaeta species detected, showing number of specimens collected, number of unique haplotypes sequenced for COI, levels of Intra-specific (within species) pairwise genetic divergence (%) between project specimen and reference specimens, and inter-specific pairwise genetic divergence (between project specimen and next closest reference specimens). Newly detected species shown in ‘bold’.

Species	Phase (Sites detected)	No. specimens	No. haplotypes	Intra-specific divergence (%) Project specimens	Intra-specific divergence (%) Reference specimens	Inter-specific divergence (%)
<i>Enchytraeidae</i> sp. H-OLE026	P6/P7 (Winu1436, 1445, 1437, 1451)	26	3	0.0 – 5.8	5.5 – 7.0 (GB#PP940020, E06-B03/ sp. H-OLE026)	9.5 (GB#MW021262, WAM ENCH002)
<i>Enchytraeidae</i> sp. H-OLE063	P6 (Winu1438, 1452)	4	2	0.0 – 7.4	7.1 – 8.2 (GB#MW021316, Biologic – OLIG024)	10.0 – 10.1 (Helix #G61 H-OLE035)
<i>Enchytraeidae</i> sp. H-OLE064	P6 (Winu1424)	13	3	0.0 – 2.5	N/A	16.1 – 17.1 (GB#OR940722, Biologic-OLIG065)
<i>Enchytraeidae</i> sp. H-OLE065	P6 (Winu1424)	1	1	N/A	N/A	10.5 (GB# OR940722.1: Biologic-OLIG065)
<i>Enchytraeidae</i> sp. H-OLE066	P6 (Winu1451)	1	1	N/A	N/A	17.5 (BOLD# GAHAP497-13 Marionina)

Differentiation within and between lineages/species

Phylogenetic analyses of the *Enchytraeidae* suggest five species were present amongst the 45 project specimens (Table 4-2), which exhibited between 0.0 - 24.3% molecular sequence divergence (Table A1.1).

Enchytraeidae sp. H-OLE026 was represented by 26 project specimens which exhibited 0.0 – 5.9% genetic divergence between specimens collected across four sites. Of the 26 specimens collected, 24 specimens collected from sites Winu1436 (WJ05 – WJ27), Winu1437 (WJ47) and Winu1451 (WJ179) were identical showing 0.0% genetic divergence. One specimen from site Winu1436 (WJ27) differed by 0.4% and one specimen from Winu1445 (WJ28) differed by 5.8 - 5.9% divergence (Table 4-2, Table A1.1, Supplementary Information). This species has previously been detected and likely conspecific with several reference specimens listed as *Enchytraeidae* sp. H-OLE026 (Helix database #GB10 - GB12, JY10) and various sequences on GenBank referred to as *Enchytraeidae* sp. E6, *Enchytraeidae* sp. E6-B03, *Enchytraeidae* sp. WAM ENCH003, etc. The closest reference sequence was *Enchytraeidae* sp. E6-B03 (GenBank #PP940020) with 5.5% pairwise sequence divergence. It is likely that this species is conspecific with reference specimens of *Enchytraeidae* sp. E6 and

similar sequences, which range between 5.5 – 8.4% genetic divergence, but it is unclear where the species boundary ends (Table 4-2, supplementary data).

Enchytraeidae sp. H-OLE063 was represented by four project specimens which exhibited 0.0% sequence divergence between specimens collected from site Winu1438 (WJ01 – WJ03) and 7.4% intra-specific genetic divergence between sites Winu1438 and Winu1452 (WJ45 ; Table 4-2, Table A1.1, Supplementary Information). Despite the pairwise sequence divergence >5%, it is likely that this species is conspecific with reference specimens of Enchytraeidae sp. Biologic-OLIG024 (GenBank #'s MW021316, MW021317, MW021322, MW021319, MW021311, OR940732), showing 7.1 – 8.2% sequence divergence. The next closest reference specimen was Enchytraeidae sp H-OLE035 (Helix #G61), showing 10.0 – 10.1% inter-specific genetic divergence (Table 4-2, supplementary data).

Enchytraeidae sp H-OLE064 was represented by 13 project specimens which exhibited between 0.0 – 2.5% intra-specific pairwise genetic divergence all collected from a single site (Winu 1424). Project specimens did not closely align with any reference specimens available for analysis, with the next closest reference specimen Enchytraeidae sp. Biologic OLIGO65 (GB # OR940722) showing 16.1 – 17.1% sequence divergence (Table 4-2, Supplementary Information).

The closest relative of Enchytraeidae sp. H-OLE065 (WJ30), according to the molecular data currently available for comparison, was Enchytraeidae sp. Biologic-OLIGO65 showing 10.5% genetic differentiation (GenBank # OR940722), followed by 15.7 – 16.4% from Enchytraeidae sp. Biologic-OLIG024 (GenBank #'s MW021319, MW021316) (Table 4-2, Supplementary Information). This species was collected from the same site (Winu1424) as Enchytraeidae sp. H-OLE064 but differed between 16.8 – 17.2% genetically.

The closest relative of the Enchytraeidae sp. H-OLE066 (WJ46) was an Enchytraeidae reference specimen *Marionina* sp. (BOLD # GAHAP497-13), with 17.5% genetic differentiation, followed by Enchytraeidae sp. Biologic-OLIG040 (GenBank#OR653516) with 17.9% differentiation (Table 4-2, Supplementary material).

Summary

Five species of Enchytraeidae were detected from the project specimens, corresponding to two previously detected but undescribed taxa: Enchytraeidae sp. H-OLE026 and Enchytraeidae sp. H-OLE063, and three newly detected taxa: Enchytraeidae sp H-OLE064, Enchytraeidae sp H-OLE065 and Enchytraeidae sp H-OLE066.

Specimens of Enchytraeidae sp. H-OLE026 (also known as Enchytraeidae sp. E6) have been previously detected from multiple catchments within the Pilbara bioregion and appears to be widespread (Brown et al., 2015). Enchytraeidae sp. E6-B03 (GenBank: PP940020), which differed from project specimens by 5.5 – 7.0% genetically, is known from a location approximately 360 km southwest of the project specimens. Specimens of Enchytraeidae sp. H-OLE063 (also recognised as Enchytraeidae sp. Biologic-OLIG024) have been previously detected from an area ranging from approximately 70 km south to 120 km southeast of the project specimens. Reference specimens (Enchytraeidae sp. Biologic-OLIG024: GenBank accession #'s MW021316, MW021317, MW021322, MW021319 and OR940732) differed from the project specimens by 7.1 – 8.2% genetically.

Enchytraeidae sp H-OLE064, Enchytraeidae sp H-OLE065 and Enchytraeidae sp H-OLE066 are currently only known from this project, based on current reference specimens available for comparison.

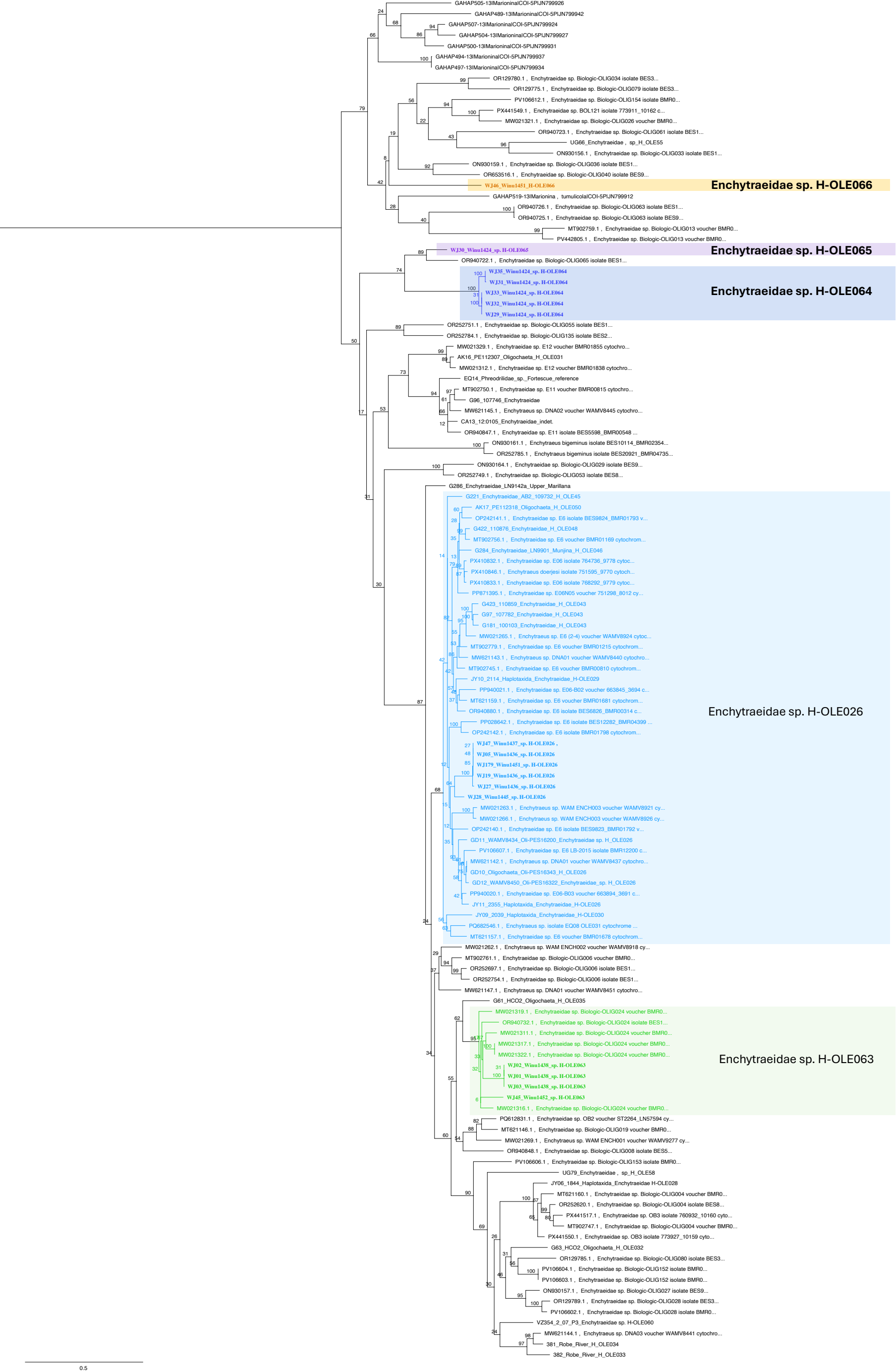


Figure 4-1: Maximum Likelihood analysis of Oligochaete COI mtDNA sequences, showing the placement of the survey area specimens (‘WJ’ prefix) and the five species designations, within a reduced taxonomic framework of the family Enchytraeidae (original analysis included 371 reference specimens). Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site). ML Tree for illustrative purposes only with outgroups removed.

4.2 Crustacea

Despite its arid setting, north-west Western Australia appears to contain one of the world's more diverse subterranean faunas, dominated by crustaceans and including many relict obligate groundwater species with affinities to Tethys, and to Pangaea and its derived landmasses (Humphreys 1993ab, 1999, 2001; Knott 1993) (Eberhardt *et al.*, 2005).

4.2.1 Malacostraca: Amphipoda

Amphipod crustaceans comprise a significant and enigmatic component of Australian groundwater ecosystems, particularly in the Pilbara region of Western Australia. The Amphipoda is one of the largest orders of the Crustacea, Malacostraca with 223 families, 1618 genera and a little less than 10,000 species (Lowry and Myers, 2013).

Four diverse stygobiotic families and 35 species have been recorded from the Pilbara, the adjacent North-West Cape Peninsula and Barrow Island, including the Bogidiellidae (Hertzog, 1936); Eriopisidae (Lowry & Myers, 2013); Hadziidae (S. Karaman, 1943) and the Paramelitidae (Bousfield, 1977) (King *et al.*, 2022). Eriopisids are considered primitive melitids, and their present disjunct distribution (Figure 3b) is suggestive of an ancestry based in the Tethys (Knott 1993).

Stygobiotic amphipods brood young with specialised ventral pouches, are considered to not swim long distances and subject to the confinement of subterranean habitats (i.e. aquifers, springs, hyporheic habitats or catchment boundaries that may contain physical barriers inhibiting dispersal). As such, many are likely to be short range endemic (SRE) species (Harvey 2002; Eberhardt *et al.* 2009) and are significant in terms of biodiversity conservation (Harvey *et al.* 2011) and management of groundwater, including as potential ecosystem providers (Boulton *et al.* 2008).

Many stygobiotic amphipod genera described from Western Australia are monotypic or contain few species. Furthermore, molecular analyses have indicated that some described genera contain highly divergent lineages (=putative species) across and within catchments.

Reference Specimens and Outgroups

A 687 bp fragment of COI was isolated for the 19 phase 6 and 7 Winu amphipod specimens (WJ144 – WJ162). All 19 amphipod specimens were collected from the 1451 sampling location (Table 4-1). Thirteen of these shared the same haplotype (0.00% sequence divergence). Two additional amphipods (WJ144, WJ150) shared a second haplotype whilst the remaining two amphipods (WJ151 and WJ154) represented two additional haplotypes. The four haplotypes differed by between 0.3 and 0.9 % sequence divergence, indicating they belong to a single lineage/species (Table 4-3).

A representative of each of the four haplotypes from the Winu phase 6 and 7 amphipod specimens (WJ144, WJ145, WJ151, WJ154) were analysed along with 120 reference sequences, including the two haplotypes from two drillholes from phase 5: VJ01/H-AMP050 from Old Bore and VJ05/H-AMP051 from 1452. Two shrimp species from the family Mysidae were used as outgroups *Paramesopodopsis rufa* (GenBank# AF052393) and *Tenagomysis australis* (AF052393). Reference specimen sequences were sourced from the Helix database (n=62) and Genbank (n=58).

Phylogenetic Analyses

Phylogenetic analyses revealed all four amphipod haplotypes from phases 6 and 7 were placed in a single existing lineage, H-AMP051, detected previously in phase 5 at Winu1452, within the family Paramelitidae (Figure 4-2).

Differentiation within and between lineages/species

The specimens from phases 6 and 7 differed from the haplotype VJ05/H-AMP051 from phase 5 (Winu1452) by between 0.6 and 1.2% sequence divergence, and from the haplotype VJ01/H-AMP050 from phase 5 (Old Bore) by between 17.8 and 18.4% sequence divergence.

Specimens from Winu lineage H-AMP051 (from sites 1451 and 1452) differed from the nearest reference specimen, Biologic AMPH025 (GenBank accession # MW021304) by between 14.1 and 14.3% sequence divergence (Table 4-3, Supplementary material).

The haplotype from Winu lineage H-AMP050 (site Old Bore only) differed from the nearest reference specimen, Biologic AMPH028 by 15.7% sequence divergence (Table 4-3).

Summary

The two Paramelitidae amphipod lineages from Winu H-AMP050 and H-AMP051, are genetically distinct (based on the >5% sequence divergence criterion), with enough divergence from one another and from the available reference specimens to be considered two distinct species that have so far not been detected outside the Winu area.

Table 4-3: Summary of the Paramelitidae Amphipoda species detected, showing number of specimens collected, number of unique haplotypes sequenced for COI, levels of Intra-specific (within species) pairwise genetic divergence (%) between project specimen and reference specimens, and inter-specific pairwise genetic divergence (between project specimen and next closest reference specimens). Newly detected species shown in bold.

Species	Phase (Site)	No. specimens	No. haplotypes	Intra-specific divergence (%) - Project specimens	Intra-specific divergence (%) - Reference specimens	Inter-specific divergence (%)
Paramelitidae sp. H-AMP050	P5 (OldBore)	4	1	0.0	N/A	15.7 (Biologic AMPH028)
Paramelitidae sp. H-AMP051	P5/P6/P7 (Winu1452, Winu1451)	20	4	0.0 – 0.9 ('WJ' prefix)	0.4 – 0.7 (Helix#VJ05/H-AMP051)	14.1 – 14.3 (Biologic AMPH025)

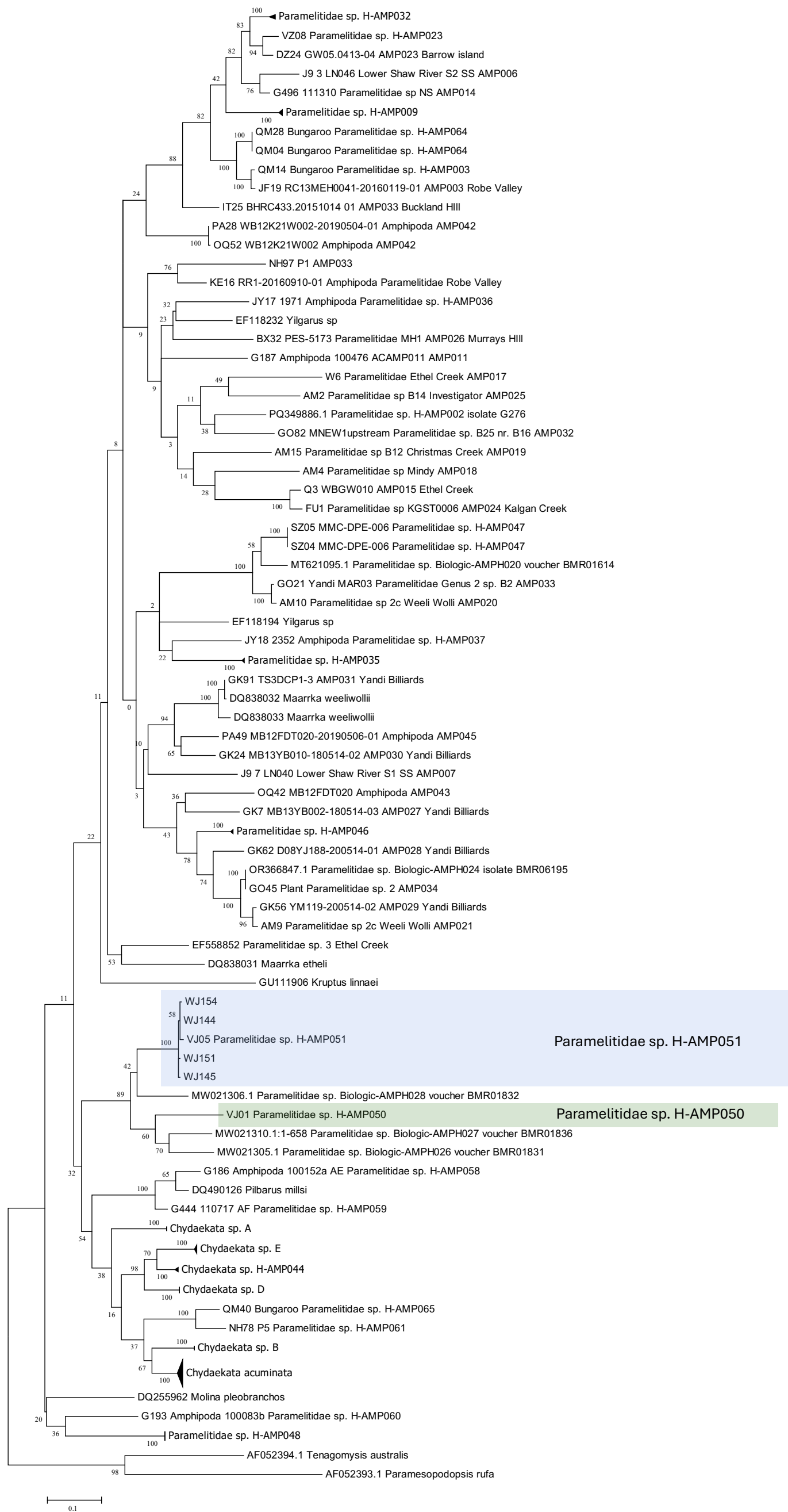


Figure 4-2: Maximum Likelihood analysis of Amphipoda COI mtDNA sequences, showing the placement of the phase 6 and 7 survey specimens (‘WJ’ prefix) and phase 5 survey specimens (‘VJ’ prefix), in relation to reference sequences of amphipods and their species designation (highlighted in shaded boxes) within a reduced taxonomic framework of the taxonomic group Amphipoda (Paramelitidae). Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).

4.2.2 Malacostraca: Isopoda

4.2.2.1 Family Microcerberidae

Microcerberids are small isopod crustaceans living in the interstitial groundwater of both marine littoral zones and freshwater aquifers and interstitial spaces. The family Microcerberidae Karaman 1940 includes eight genera and 45 species, with the greatest diversity in marine littoral ecosystems (Hutchins et al, 2021). The marine genus *Coxicerberus* has a worldwide distribution (Coineau and Albuquerque, 2001; Kim et al, 2018). In contrast, freshwater species occur in a few localized areas such as South Africa, Morocco, southern Europe, southern USA and Cuba (Coineau and Albuquerque, 2001; Hutchins et al, 2021). Within Australia there are no documented genera or species (Australian Faunal Directory). Currently there are no Australian molecular systematic studies published for this family and undescribed species from Australia have yet to be formally documented. Limited molecular work has been carried out on Japanese and South Korean populations of *Coxicerberus*, revealing the likelihood of cryptic species with no discernible morphological differences (Kim et al, 2018).

Reference Specimens and Outgroups

A 687 bp fragment of COI was isolated for the 16 phase 6 and 7 Winu isopod specimens ('WJ' prefix). There were three COI haplotypes among the 16 specimens. These three phase 6 and 7 isopod haplotypes were analysed along with 20 reference specimens including those from prior Winu survey phases ('VJ', 'VB', 'UT' and 'TT', n=13), the Helix database (n=5) and GenBank (n=2) as well as the outgroups *Asellus aquaticus* (GenBank# DQ144755) and *Lirceus ouachitaensis* (OR292084).

Species H-ISM005 showed 9.4 to 9.5% sequence divergence from the closest relative, H-ISM003 from three bores, ADK10, 1446, 1447, from previous collecting phases at Winu. H-ISM005 differed by between 15.5 – 16.0 % from the next closest relative, Microcerberidae sp. H-ISM001 (EY01, EY02) from the Helix database (Supplementary Material). Winu species H-ISM003 and H-ISM005 differed from a third Microcerberidae species detected at Winu from Old Bore, H-ISM004, by between 15.6 and 17.4% sequence divergence. (Helix 2024a, Helix 2024b, Helix 2025a, Helix 2025b).

Phylogenetic Analyses

The phylogenetic analysis confirmed that all three isopod haplotypes from phases 6 and 7 were placed in a single existing lineage, H-ISM005 within the family Microcerberidae, detected previously in phase 5 at the same bore (Figure 4-3).

The placement of the three Winu microcerberid Isopoda species, detected among the 29 microcerberid isopod specimens successfully sequenced across seven sampling phases, are well supported within the family Microcerberidae, based on the molecular sequence data currently available for comparison (Figure 4-3). The three species, H-ISM003, H-ISM004, and H-ISM005, were collected from five bores at Winu (Table 4-1, Table 4-4; Figure 4-3). Species H-ISM004 and H-ISM005 were each detected in single bores, Old Bore and 1451, respectively, whereas the third species, H-ISM003, was detected in two bores, ADK10 and 1446 (Figure 4-3, Table 4-1, Table 4-4)

Differentiation within and between lineages/species

The three isopod haplotypes (WJ163, WJ174, WJ177) from the current survey shared the same haplotype or very similar haplotypes to those collected previously at Winu and assigned to

the taxon Microcerberidae sp. H-ISM005 (Table 4-1, Table 4-4). There was between 0.0 and 0.3% sequence divergence between all individuals within this lineage, which were all sampled from a single bore, 1451, over two phases (Table 4-4).

Summary

The species Microcerberidae sp. H-ISM003 was collected in phases 3, Microcerberidae sp H-ISM005 was detected in phases 5 – 7. The species Microcerberidae sp H-ISM004 has to date been collected in only a single sampling phase during phase 3 (Table 4-1, Table 4-4).

The three Microcerberidae isopod lineages from Winu (H-ISM003, H-ISM004 and H-ISM005) are genetically distinct (based on the >5% sequence divergence criterion), with enough divergence from one another and from the available reference specimens to be considered three distinct species that have so far not been detected outside the Winu area.

Table 4-4: Summary of the Microcerberidae Isopoda species detected, showing number of specimens collected, number of unique haplotypes sequenced for COI, levels of intra-specific (within species) pairwise genetic divergence (%) between project specimen (Phase 6/7 only) and reference specimens, and inter-specific pairwise genetic divergence (between project specimen and next closest reference specimens). Recently detected species shown in 'bold'.

Species	Phase (Site)	No. specimens	No. haplotypes	Intra-specific divergence (%) Project specimens (P6/7)	Intra-specific divergence (%) Reference specimens	Inter-specific divergence (%)
Microcerberidae sp. H-ISM003	P1/ P3 -P5 (AKD10, Winu1446)	12	1	N/A	0.0 (P1/P3-5 H-ISM003)	8.6 – 9.1 (H-ISM005)
Microcerberidae sp. H-ISM004	P4 (Old Bore)	20	1	N/A	0.0 (P3 H-ISM004)	16.1 – 16.2 (H-ISM003)
Microcerberidae sp. H-ISM005	P5/P6/P7 (Winu1451)	18	4	0.0 – 0.29	0.0 – 0.29 (P5 H-ISM005)	8.6 – 9.1 (H-ISM003)

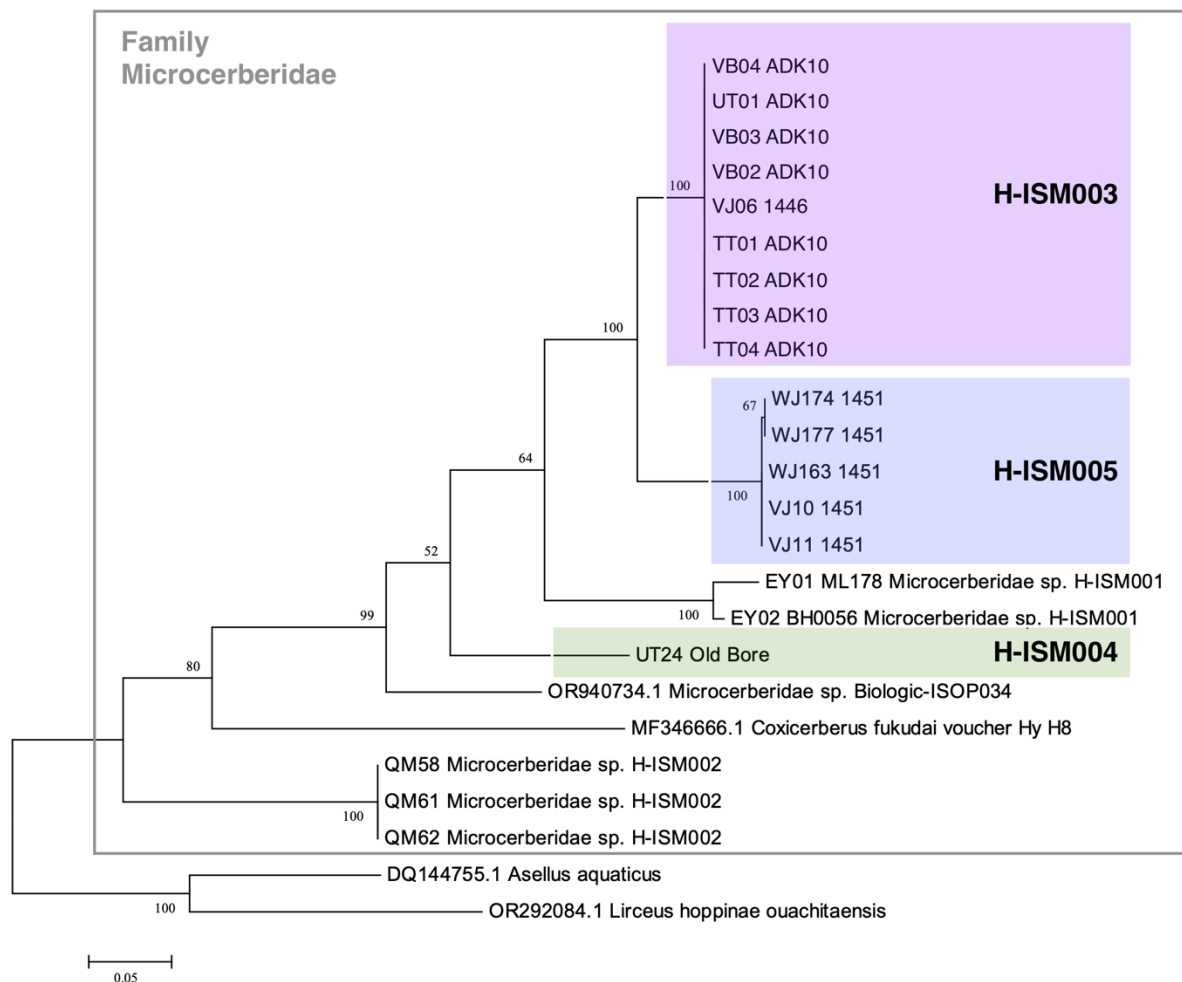


Figure 4-3: Maximum Likelihood analysis of Isopoda COI mtDNA sequences, showing the placement of the phase 6 and 7 survey specimens ('WJ' prefix) in relation to previously detected isopods from Winu ('WJ' 'VJ' 'VB', TT' and 'UT' prefix) and their species designation (highlighted in shaded boxes) within a reduced taxonomic framework of the family Microcerberidae. ML tree is for illustrative purposes only with a complete ML tree available on request. Numbers on nodes indicate nodal support by means of ML bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).

4.2.3 Maxillopoda: Copepoda

Copepods are amongst the most commonly collected stygofauna in northern Australia and previous systematic reviews conducted in north-western Australia have documented substantial biodiversity (e.g. 41 species reported by Karanovic (2006)). Many stygobitic copepod species are widely distributed (Karanovic 2006, Halse et al. 2014), while others are endemic at bioregional scale or appear to have ranges of only a few kilometres (e.g. Karanovic and Cooper 2012).

4.2.3.1 Harpacticoida

Reference Specimens and Outgroups

A 687 bp fragment of COI was sequenced for 72 harpacticoid specimens from phase 6 and 7 sampling (see Table 4-1). This total includes 26 harpacticoid specimens from phase 6 sampling from four sites (Winu1450, Winu1454, Winu1444 and Winu1437, see Table 4-1) and 46 harpacticoid specimens from phase 7 sampling from eight sites (Winu1423, Winu1425, Winu1437, Winu1444, Winu1447, Winu1450, Winu1451 and Winu1454, see Table 4-1).

These 72 phase 6 and 7 harpacticoid specimens were analysed along with 11 harpacticoid sequences previously detected from Winu (phase 2 n=1, phase 3 n=3, phase 4 n=6, phase 5 n=1), the Winu cyclopoid sequences, harpacticoid and cyclopoid reference sequence data from Genbank and the outgroup *Allanaspides helonomus* also obtained from GenBank (GenBank accession # OQ721880). Preliminary analyses included a total of 175 reference specimens. Whilst not all reference sequence data was necessarily informative due to the large divergences, the placement of cyclopoid and harpacticoid specimen sequences showed greater resolution when both groups were analysed together. The reference sequence data included both Helix and GenBank reference sequences (total n=175). With such a large reference sequence database, phylogenetic clades for reference groups that did not show a strong affinity to the focus sequences were collapsed. This meant that for the analyses of the Harpacticoida, the Cyclopoida clades were collapsed, and vice versa.

The fourteen phase 6 and 7 cyclopoid specimen sequences were analysed along with the single phase 5 cyclopoid specimen (VJ08) and Preliminary analyses included 175 reference specimens including project and reference harpacticoid reference sequence data. Whilst not all reference sequence data was necessarily informative due to the large divergences, the placement of cyclopoid and harpacticoid specimen sequences showed greater resolution when both groups were analysed together.

Phylogenetic Analyses

Phylogenetic analyses revealed 12 differentiated harpacticoid clades amongst the Winu harpacticoid specimens (Figure 4-4). Five clades (H-HAR016, H-HAR017A, H-HAR017B, H-HAR027 and H-HAR029) are closely aligned with one another with high statistical support (Figure 4-4). There is also strong support for the placement of these clades with other taxa from the Parastenocarididae (Figure 4-4). Five more Winu clades (*Parastenocaris* sp. H-HAR010A, H-HAR010B, H-HAR024, H-HAR026 and H-HAR028, Figure 4-4) and closely aligned with strong statistical support, with their placement with many taxa from the Parastenocarididae, and the genus *Parastenocaris* (Figure 4-4). The two taxa, not detected during phases 6 or 7 sampling (Harpacticoida H-HAR011 and H-HAR016) remain ambiguous in relation to the placement amongst other Harpacticoida taxa (Figure 4-4).

Differentiation within and between lineages/species

Amongst all Winu harpacticoid specimens there existed between 0.00% (those belonging to the same taxa) to 23.5% (between the Winu taxa *Harpacticoida* sp. **H-HAR027** and *Harpacticoida* sp. **H-HAR029**, see Table 4-5 and [Supp Info](#)).

Parastenocaris sp. H-HAR010A had 0.00 to 0.44% intraspecific divergence (see Table 4-5 and [Supp Info](#)). The closest relative at 2.25% sequence divergence was *Parastenocaris* sp. H-HAR010B (current project), followed by the Winu taxon *Parastenocaris* sp. **H-HAR026** (current project with 4.5% sequence divergence and then *Parastenocaris* sp. **H-HAR028** at 14.2 % (current project, see Table 4-5 and [Supp Info](#)) based on the molecular sequence data currently available for comparison.

Parastenocaris sp. H-HAR010B had 0.00 to 0.18% intraspecific divergence (see Table 4-5 and [Supp Info](#)). The closest relative at 2.25% sequence divergence was *Parastenocaris* sp. H-HAR010A, followed by another Winu taxon *Parastenocaris* sp. **H-HAR026** (current project with 4.1% sequence divergence and then *Parastenocaris* sp. **H-HAR028** at 13.1 % (current project, see Table 4-5 and [Supp Info](#)) based on the molecular sequence data currently available for comparison.

Whilst not detected this survey, *Harpacticoida* sp. H-HAR011 had no intraspecific divergence (0.00%, see Table 4-5 and [Supp Info](#)). The closest relative at 24.0% sequence divergence was a Helix database specimen (RO38) *Nikotra* sp. H-HAU003 from the family Ameiridae, followed by *Australocamptus* sp. Biologic-HARP060 (GenBank accession # OR252839) at 24.8% (see Table 4-5 and [Supp Info](#)) based on the molecular sequence data currently available for comparison.

Also not detected this survey, but an existing Winu harpacticoid taxon, *Harpacticoida* sp. H-HAR015, had 1.9% intraspecific divergence (see Table 4-5 and [Supp Info](#)). The closest relative at 20.4% sequence divergence was *Dussartstenocaris idioxenos* from the family Parastenocarididae (GenBank accession # JN039168) (see Table 4-5 and [Supp Info](#)), followed closely by *Dussartstenocaris* sp. Biologic-HARP024 (GenBank accession # OR004442) (see Table 4-5 and [Supp Info](#)), based on the molecular sequence data currently available for comparison.

Harpacticoida sp. H-HAR016 had 0.00 to 0.15% intraspecific divergence (see Table 4-5 and [Supp Info](#)). The closest relative at 3.5% sequence divergence was another Winu taxon, *Harpacticoida* sp. **H-HAR029**, (see Table 4-5 and [Supp Info](#)), followed by another Winu taxon *Harpacticoida* sp. H-HAR017A at 7.3% (see Table 4-5 and [Supp Info](#)), based on the molecular sequence data currently available for comparison.

Harpacticoida sp. H-HAR017A had 0.00 to 0.73% intraspecific divergence (see Table 4-5 and [Supp Info](#)). The closest relative at 1.7% sequence divergence was another Winu taxon, *Harpacticoida* sp. H-HAR017B, (see Table 4-5 and [Supp Info](#)), followed by another Winu taxon *Harpacticoida* sp. **H-HAR029** at 7.1% (see Table 4-5 and [Supp Info](#)), based on the molecular sequence data currently available for comparison.

Harpacticoida sp. H-HAR017B had no intraspecific divergence (0.00%, see Table 4-5 and [Supp Info](#)). The closest relative at 1.7% sequence divergence was another Winu taxon, *Harpacticoida* sp. H-HAR017A, (see Table 4-5 and [Supp Info](#)), followed by another Winu taxon *Harpacticoida* sp. **H-HAR029** at 7.8% (see Table 4-5 and [Supp Info](#)), based on the molecular sequence data currently available for comparison.

Parastenocaris sp. **H-HAR024** had no intraspecific divergence (0.00%, see Table 4-5 and Supp Info). The closest relative at 10.6% sequence divergence was another Winu taxon, *Parastenocaris* sp. **H-HAR028** (see Table 4-5 and [Supp Info](#)), followed by another Winu taxon *Parastenocaris* sp. H-HAR010B at 13.5.8% (see Table 4-5 and [Supp Info](#) both from the family Parastenocaridida, based on the molecular sequence data currently available for comparison.

Parastenocaris sp. **H-HAR026** had no intraspecific divergence (0.00%, see Table 4-5 and [Supp Info](#)). The closest relative at 4.2% sequence divergence was another Winu taxon *Parastenocaris* sp. H-HAR010B (see Table 4-5 and [Supp Info](#)), both from the family Parastenocarididae, based on the molecular sequence data currently available for comparison.

Harpacticoida sp. **H-HAR027** had no intraspecific divergence (0.00%, see Table 4-5 and Supp Info). The closest relative at 11.4% sequence divergence was another Winu taxon, Harpacticoida sp. H HAR016 (see Table 4-5 and [Supp Info](#)), followed by another Winu taxon, Harpacticoida sp. H HAR017A, at 11.5% (see Table 4-5 and [Supp Info](#)), based on the molecular sequence data currently available for comparison.

Parastenocaris sp. **H-HAR028** had no intraspecific divergence (0.00%, see Table 4-5 and [Supp Info](#)). The closest relative at 10.6% sequence divergence, based on the molecular sequence data currently available for comparison, was another Winu taxon, *Parastenocaris* sp. **H-HAR024** (see Table 4-5 and [Supp Info](#)), followed by another Winu taxon *Parastenocaris* sp. **H-HAR026** at 12.6% sequence divergence (see Table 4-5 and [Supp Info](#)), all of the family Parastenocarididae

Harpacticoida sp. **H-HAR029** had no intraspecific divergence (0.00%, see Table 4-5 and [Supp Info](#)). The closest relative at 3.5% sequence divergence was another Winu taxon, Harpacticoida sp. H HAR016 (see Table 4-5 and [Supp Info](#)), followed by another Winu taxon Harpacticoida sp. H-HAR017A at 7.1% (see Table 4-5 and [Supp Info](#)), based on the molecular sequence data currently available for comparison.

Summary

The taxa Harpacticoida sp. H-HAR015 and Harpacticoida H-HAR011 detected on prior Winu survey phases were not detected during the current phase 6 and 7 sampling. The following 10 potential harpacticoid taxa were detected during the phase 6 and 7 surveys at Winu:

- *Parastenocaris* sp. H-HAR010A: (n=8) (n=7 Winu1423 phase 7, n=1 RIMFIRE02 phase 2);
- *Parastenocaris* sp. H-HAR010B: (n=10) Winu1425 phase 7, n=1 Winu1437 phase 6);
- Harpacticoid sp. H-HAR016: (n=1, Winu1447, phase 4), (n=2, Winu1447, phase 7);
- Harpacticoida sp. H-HAR017A (**NB** updated nomenclature, previously referred to as HAR017 and HAR019): (n=2 Winu1454 phase 7), (n=2 Winu1450 phase 7), (n=3 Winu1450 phase 6), (n=1 Winu1454 phase 6), (n=3 Winu1450 phase 6), (n=1 Winu1450 phase 5), (n=2 Winu1453 phase 4);
- Harpacticoida sp. H-HAR017B (**NB** updated nomenclature, previously referred to as HAR018): (n=1 Winu1453 phase 4), (n=1 Winu1453 phase 7).
- *Parastenocaris* sp. **H-HAR024**: (n=4) Winu1437 phase 6;
- *Parastenocaris* sp. **H-HAR026**: (n=3) Winu1425 phase 7;

- Harpacticoida sp. **H-HAR027**: (n=10, Winu1444, phase 7);
- *Parastenocaris* sp. **H-HAR028**: (n=3) Winu1451 phase 7; and
- Harpacticoida sp. **H-HAR029**: (n=1, Winu1450, phase 7);

Taking a conservative approach, the taxa *Parastenocaris* sp. H-HAR010A and *Parastenocaris* sp. H-HAR010B are treated here as separate lineages and taxa, however the molecular sequence divergence levels between them is relatively low (~2%) and may represent intraspecific variation with geographical differentiation (particularly as they were detected at separate bore sites). Further sampling may assist in delineating the taxa or may provide evidence for these lineages to be considered a single taxon.

Similarly, also taking a conservative approach, the taxa Harpacticoida sp. H-HAR017A (NB updated nomenclature - previously referred to as H-HAR017 and H-HAR019) and Harpacticoida sp. H-HAR017B (NB updated nomenclature - previously referred to as H-HAR018) are treated here as separate lineages and taxa. However, the sequence divergence between these harpacticoid lineages is relatively low (~2%) and it is possible this could represent intraspecific variation with retained mitochondrial signals of historic geographical isolation and differentiation (as both detected during phase 4 sampling from bore site Winu1453). Additional sampling may assist in delineating the taxa or may provide evidence for these lineages to be considered a single taxon.

Table 4-5: Summary of the Harpacticoida copepod species detected, showing number of specimens collected, number of unique haplotypes sequenced for COI, levels of intra-specific (within species) pairwise genetic divergence (%) between project specimen and reference specimens, and inter-specific pairwise genetic divergence (between project specimen and next closest reference specimens). Newly detected species (current/phase 6 or 7 detections) shown in **bold.**

Species	Phase (Site)	No. specimens	No. haplotypes	Intra-specific divergence (%) Project specimens	Intra-specific divergence (%) Reference specimens	Inter-specific divergence (%)
<i>Parastenocaris</i> sp. H-HAR010A	P2/P6/P7 (STYGO 02, Winu1423, Winu1437)	13	2	0.00-0.43	n/a	2.2 (<i>Parastenocaris</i> sp. H-HAR010B)
<i>Parastenocaris</i> sp. H-HAR010B	P7 (Winu1425)	12	5	0.00-0.18	n/a	2.2 (<i>Parastenocaris</i> sp. H-HAR010B)
Harpacticoida sp. H-HAR011	P3 (OldBore)	3	1	0.00	n/a	24.0 (RO38- <i>Nitokra</i> sp. H HAU003)
Harpacticoida sp. H-HAR015	P4 (Winu1454)	2	2	1.9%	n/a	20.4 (<i>Dussartstenocaris idioxenos</i> , JN039168)
Harpacticoida sp. H-HAR016	P4/P7 (Winu1447)	3	1	0.00 to 0.14	n/a	3.5 (Harpacticoida sp. H HAR029)
Harpacticoida sp. H-HAR017A	P4/P6/P7 (P4-Winu1453, P6 & P7-1454, P6 & P7-1450)	21	5	0.00 to 0.72	n/a	1.7 (Harpacticoida sp. H HAR017B)
Harpacticoida sp. H- HAR017B	P4/P7 (Winu1453)	2	1	0.00	n/a	1.6 (Harpacticoida sp. H HAR017A)
<i>Parastenocaris</i> sp. H-HAR024	P6 (Winu1437)	4	1	0.00	n/a	10.5 (<i>Parastenocaris</i> sp. H-HAR028)
<i>Parastenocaris</i> sp. H-HAR026	P7 (Winu1425)	3	1	0.00	n/a	4.1 (<i>Parastenocaris</i> sp. H-HAR010B)
Harpacticoida sp. H-HAR027	P7 (Winu1444)	21	1	0.00	n/a	11.4 (Harpacticoida sp. H-HAR016)
<i>Parastenocaris</i> sp. H-HAR028	P7 (Winu1451)	3	1	0.00	n/a	10.5 (<i>Parastenocaris</i> sp. H-HAR024)
<i>Parastenocaris</i> sp. H-HAR029	P7 (Winu1450)	1	1	n/a	n/a	3.5 (Harpacticoida sp. H-HAR016)

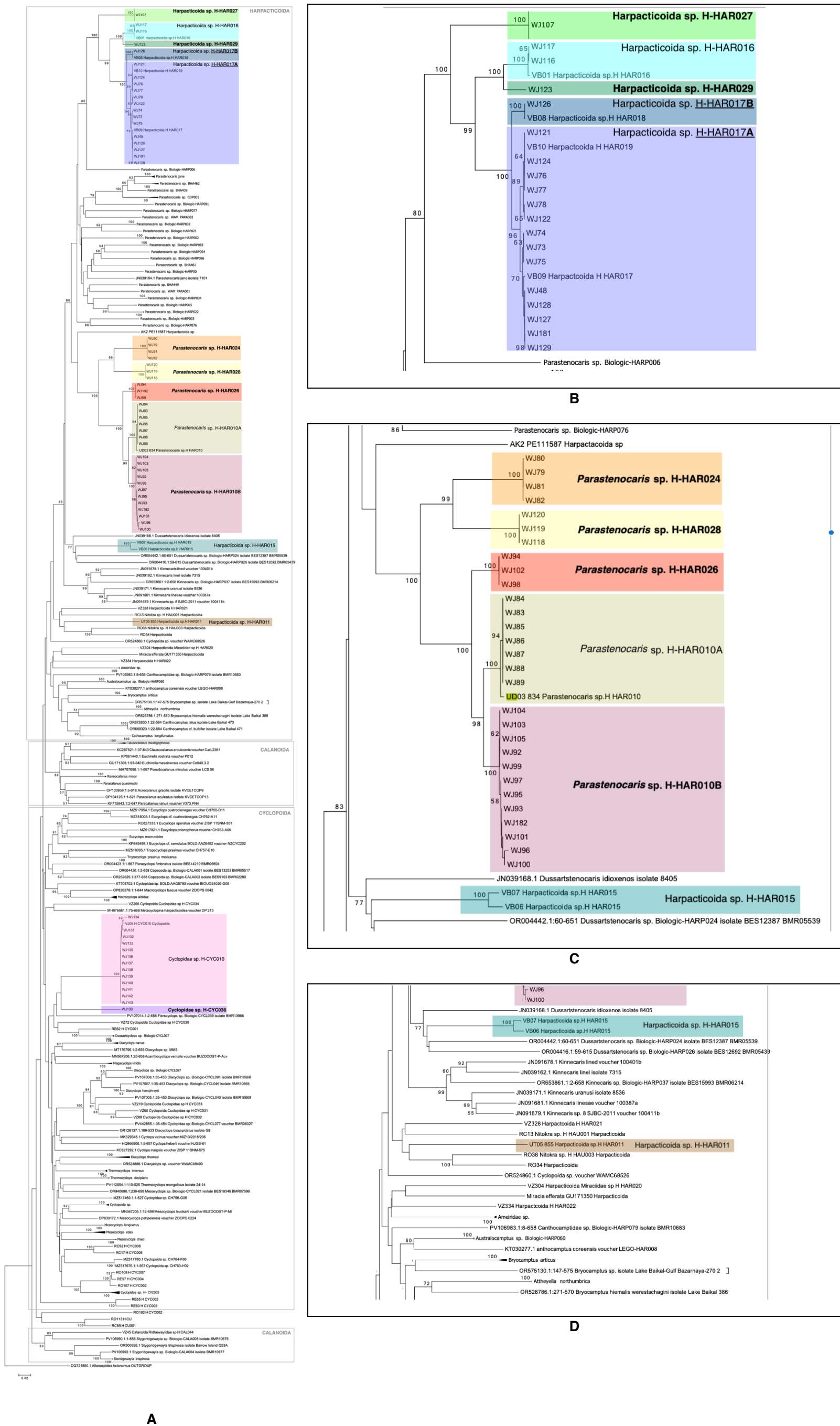


Figure 4-4: Maximum Likelihood analysis of copepod COI mtDNA sequences, showing the placement of the phase 6 and 7 survey specimens (‘WJ’ prefix) in relation to previously detected copepods from Winu (‘VJ’ ‘VB’, ‘UD’ and ‘UT’ prefix) and their species designation (highlighted in shaded boxes) reference Harpacticoida, Calanoida and Cyclopoida (A) and previously detected Harpacticoida (B,C,D) from phases 3, 4 and 5 within a reduced taxonomic framework of the taxonomic group Copepoda. ML tree is for illustrative purposes only with a complete ML tree available on request. Numbers on nodes indicate nodal support by means of ML bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).

4.2.3.2 Harpacticoida

A 687 bp fragment of COI was isolated using a combination of three sets of primers including LCOI and HCO2 (Folmer et al, 1994), Nem_120126 (Helix, unpublished) and Lep_130126 (Ratnasingham and Hebert (2007)). During phase 6 and 7 there were a total of 14 cyclopoid specimens sequenced from two sites (see Table 4-1). This total includes nine cyclopoid specimens from phase 6 sampling from two sites (CYC036 - Winu1447, CYC010 - Winu1453, see Table 4-1) and five cyclopoid specimens from phase 7 sampling from a single bore site (CYC010 - Winu1453, see Table 4-1).

Reference Specimens and Outgroups

The fourteen phase 6 and 7 cyclopoid specimen sequences were analysed along with the single phase 5 cyclopoid specimen (VJ08) and the outgroup *Allanaspides helonomus* obtained from GenBank (GenBank accession # OQ721880). Preliminary analyses included 175 reference specimens including project and reference harpacticoid reference sequence data. Whilst not all reference sequence data was necessarily informative due to the large divergences, the placement of cyclopoid and harpacticoid specimen sequences showed greater resolution when both groups were analysed together.

The reference sequence data included both Helix and GenBank reference sequences (total n=175). With such a large reference sequence database, phylogenetic clades for reference groups that did not show a strong affinity to the focus sequences were collapsed. This meant that for the analyses of the Cyclopoida, the Harpacticoida clades were collapsed, and vice versa.

Phylogenetic Analyses

Phylogenetic analyses confirmed the placement of the 14 Winu phase 6 and 7 specimens in the Cyclopoida, along with other sequence data from species belonging to the family Cyclopidae (Figure 4-5). This family of copepods contains more than half of the species in the order Cyclopoida in over 70 genera (Stoch, 2009). Thirteen specimens aligned with the phase 4 Winu specimen ('VJ' prefix) belonging to the taxon Cyclopidae sp. H-CYC010, previously detected at Winu. Amongst these, eight specimens were from phase 6 (Winu1453) and five were from phase 7 sampling (see Table 4-1). All fourteen samples (phases 5, 6 and 7) were from the same sampling bore site, namely Winu1453 (see Table 4-1).

One specimen (WJ130), from phase 6 sampling (site Winu1447), did not align closely with any previously detected cyclopoid specimen sequences, based on the molecular data currently available for comparison, instead it formed a novel taxon Cyclopidae sp. H-CYC036 (see Figure 4-5). Placement of both these taxa, with respects to other cyclopoids is ambiguous, reflected in the low statistical support for the nodes of the phylogenetic tree (Figure 4-5).

Differentiation within and between lineages/species

The taxon Cyclopidae sp. H-CYC010 had no (0.00%) intraspecific divergence amongst its fourteen specimens from three phases of sampling at site Winu1453 (Table 4-6). Whilst intraspecific divergence could not be determined for the taxon Cyclopidae sp. H-CYC036, being represented by a single individual. These two Winu cyclopoid taxa were highly differentiated from one another with 19.6% sequence divergence existing between them (see Table 4-6).

According to the molecular data currently available for comparison, the closest relative to Cyclopidae sp. H-CYC010 at 19.5% sequence divergence was *Dussartcyclops* sp. Biologic-

CYCL007 (GenBank accession # PV442707), followed by the Winu taxon Cyclopidae sp. H-CYC036 (WJ130) with 19.6% sequence divergence (Table 4-6, and [Supp Info](#)).

The closest relative to the Cyclopidae sp. H-CYC036 taxon, based on the molecular data currently available for comparison, at 18.1% sequence divergence, was *Dussartcyclops* sp. Biologic-CYCL007 (GenBank accession # PV442765), followed by *Mesocyclops pehpeiensis* (GenBank accession # OP830172) see [Supp Info](#)).

Summary

A total of fourteen Cyclopoida specimens detected during phases 6 and 7 from two sites. Nine cyclopoids were detected during phase 6 from two sites (Cyclopidae sp H- CYC036- Winu1447 and Cyclopidae sp H-CYC010 Winu1453) and five cyclopoid specimens (all Cyclopidae sp. H-CYC010) during phase 7 from one site (Winu1453).

There has been a total of two cyclopoid taxa detected from the Winu Rimfire stygofauna surveys to date. The taxon assigned to the single phase 5 cyclopoid specimen successfully sequenced (VJ08) was detected again in phases 6 and 7 at the same bore site (Winu1453). At this site (Winu1453) the Cyclopidae sp. H-CYC010 taxon has been detected on three phases of sampling (phases 5, 6 and 7). One additional cyclopoid taxon was detected from phase 6 sampling from a separate site, Winu1447.

Two taxa, each detected from only a single site, albeit different sites, have been detected during stygofauna sampling at Winu. These taxa include:

- Cyclopidae sp. H-CYC010 (n=14, phases 4 ('VJ' prefix, 6 and 7 (both 'WJ' prefix', site Winu1453); and
- Cyclopidae sp. **H-CYC036** (n=1 (WJ130), phase 6, site Winu1447).

Table 4-6: Summary of the Cyclopoida taxa detected, showing number of specimens collected, number of unique haplotypes sequenced for COI, levels of intra-specific (within species) pairwise genetic divergence (%) between project specimen and reference specimens, and inter-specific pairwise genetic divergence (between project specimen and next closest reference specimens). Newly detected species (from current surveys- P6 and P7) shown in **bold.**

Species	Phase (Site)	No. specimens	No. haplotypes	Intra-specific divergence (%) Project specimens	Intra-specific divergence (%) Reference specimens	Inter-specific divergence (%)
Cyclopoida sp. H-CYC010	P5/P6/P7 (Winu1453)	14	1	0.00 (VJ and WJ)	n/a	19.5% (<i>Dussartcyclops</i> sp. Biologic-CYCL007, GB# PV442707)
Cyclopidae sp. H- CYC036	P6 (Winu 1447)	1	1	n/a	n/a	18.1 % (<i>Dussartcyclops</i> sp. Biologic-CYCL007, GB# PV442765)

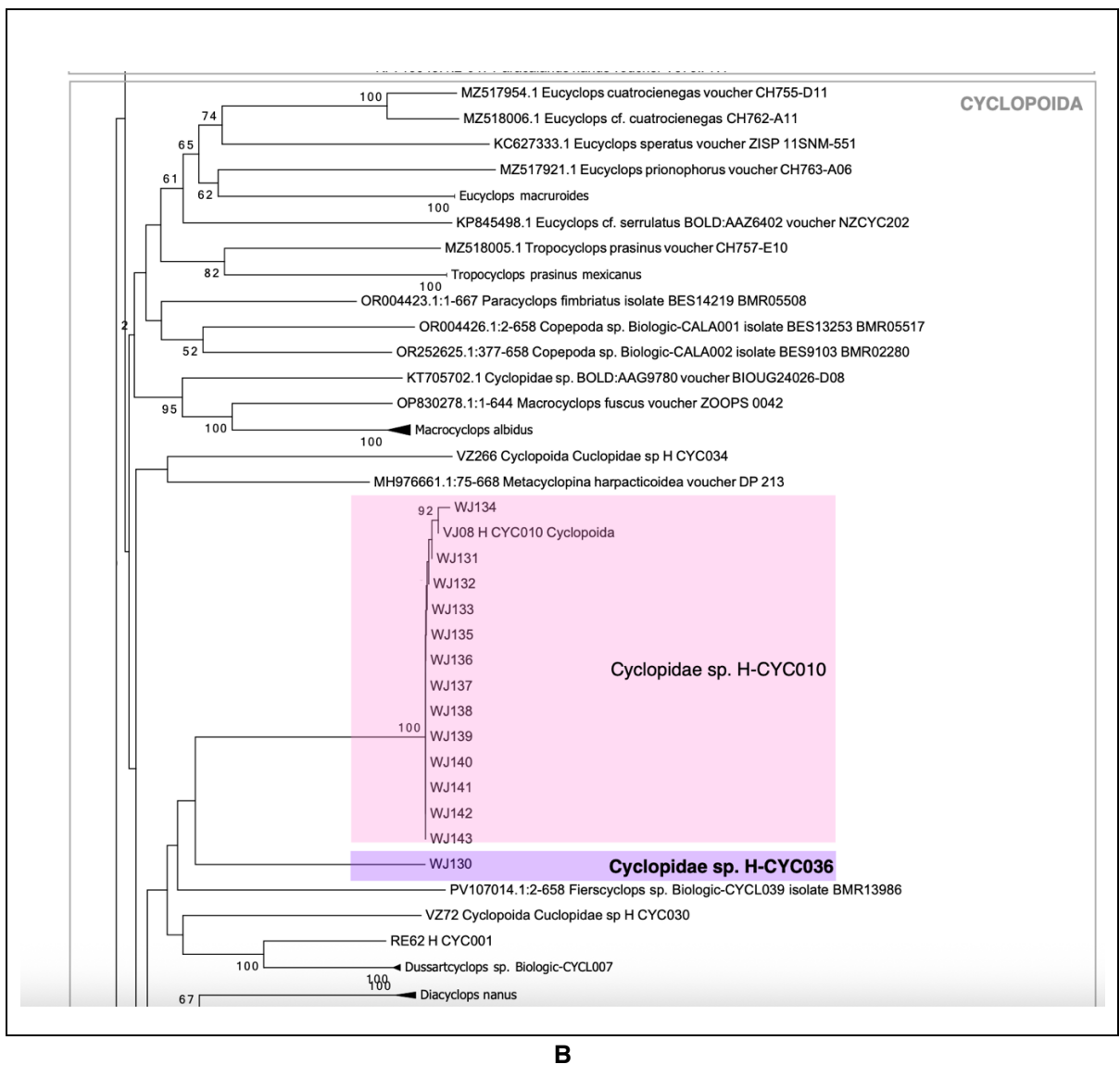
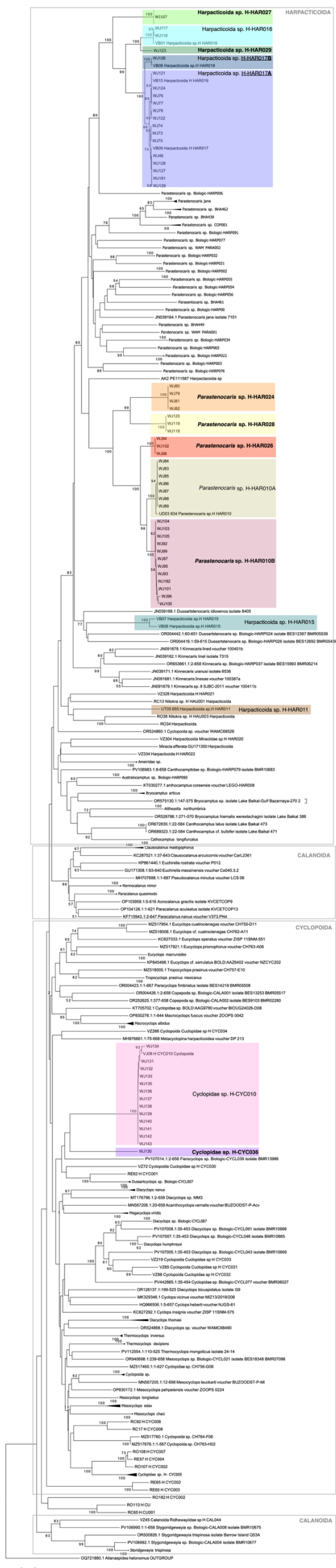


Figure 4-5: Maximum Likelihood analysis of Cyclopoida copepod COI mtDNA sequences, showing the placement of the phase 6 and 7 survey specimens ('WJ' prefix) in the framework of reference Harpacticoida, Calanoida and Cyclopoida (A) and previously detected Cyclopoida (B) from phase 5 Winu ('VJ' prefix) and their species designation (highlighted in shaded boxes) within a reduced taxonomic framework of the taxonomic group Copepoda. ML tree is for illustrative purposes only with a complete ML tree available on request. Numbers on nodes indicate nodal support by means of ML bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).

4.3 Myriapoda

4.3.1 Symphyla: Cephalostigmata: Family Scolopendrellidae

Symphylans are blind soil dwelling myriapods that have a sub-cosmopolitan distribution and are known to be generally edaphobitic. Although edaphobites frequently display similar morphological traits to troglobites, such as loss of eyes and pigmentation and are frequently found deeper underground in caves, their primary habitat is soil (Eberhard 2007). Despite the difficulties distinguishing edaphobites from troglobites, troglofauna surveys across Western Australia have detected numerous undescribed species belonging to both families of symphylan: Scolopendrellidae and Scutigerellidae (Clark et al., 2021, Helix 2020, 2021, 2022, 2024c Bennelongia 2022, Biota Environmental Sciences 2024). Symphylans are known to be speciose within the Pilbara and amongst the most short-range subterranean taxa in the Pilbara region (Bennelongia ,2015), identified as belonging to three described genera: *Hanseniella*, *Scutigerella* within the family Scutigerellidae, and *Symphylella* within the family Scolopendrellidae. The species and generic-level taxonomy is poorly described, and it is expected that multiple new genera and species are present.

Reference Specimens and Outgroups

One specimen (WJ172) collected from site Winu1450 during phase 6 sampling was originally identified as an isopod then subsequently re-assigned as a symphylan following sequencing. The COI sequenced specimen was analysed along with a total of 175 reference specimens for COI from the Helix database (n=48), GenBank (n=125) and BOLD systems (n=2), including the outgroup *Polydesmus angustus* (Family Polydesmidae) voucher_BC_ZSM_MYR_00252 (GenBank# HQ966174). A reduced dataset was used to simplify the phylogenetic tree for this report.

Phylogenetic Analyses

A standard 687 bp fragment of COI was isolated for the individual symphylan specimen (WJ172). Preliminary analysis confirmed the resulting sequence aligns with the family Scolopendrellidae and likely the genus *Symphylella* (Table 4-7, Figure 4-6). The specimen represents a newly detected taxa, *Symphylella* sp. H-SYM041, according to the molecular sequence data currently available for comparison (Table 4-2, Table 4-7).

Symphylella sp. H-SYM041 was detected from a single bore (Winu1450) during phase 6 and did not align closely with any reference specimens currently available. However, the project sequence formed a well-supported clade with other *Symphylella* species previously collected from the Pilbara bioregion (Figure 4-6, Table 4-1).

Table 4-7: Summary of the Scolopendrellidae Symphyla species detected, showing number of specimens collected, number of unique haplotypes sequenced for COI, levels of Intra-specific (within species) pairwise genetic divergence (%) between project specimen and reference specimens, and inter-specific pairwise genetic divergence (between project specimen and next closest reference specimens). Newly detected species shown in ‘bold’.

Species	Phase (Sites detected)	No. specimens	No. haplotypes	Intra-specific divergence (%) Project specimens	Intra-specific divergence (%) Reference specimens	Inter-specific divergence (%)
<i>Symphylella</i> sp. H-SYM041	P6 (Winu1450)	1	1	n/a	n/a	12.2 – 14.5 (<i>Symphylella</i> sp. H-SYM027b)

Differentiation within and between lineages/species

Symphylella sp. H-SYM041 was represented by a single project specimen (WJ172), which did not closely align with any reference specimens available for comparison. The closest relative of *Symphylella* sp. H-SYM041, according to the molecular data currently available for comparison, was *Symphylella* sp. H-SYM027b showing between 12.2 – 14.5% pairwise interspecific genetic differentiation (Helix #OY02, ML48, MO07; GenBank#MT457864, PV106570; Table 4-7, Supplementary Information).

Summary

One species of *Symphylella* (Family: Scolopendrellidae) was detected from the project area, representing a newly detected taxon: *Symphylella* sp H-SYM041. This specimen did not align closely with any reference sequences available for comparison, with the next closest species *Symphylella* sp. H-SYM027b differing from *S. sp. H-SYM041* by more than 12.2% genetically. Specimens considered to belong to the putative species *Symphylella* sp. H-SYM027b are known from the Robe Valley in the Pilbara, specifically from Mesa C (Helix 2020, 2021) and Mesa F landforms (Helix 2022). The taxon appears to exhibit high levels of genetic variation across its geographical range (Helix 2022).

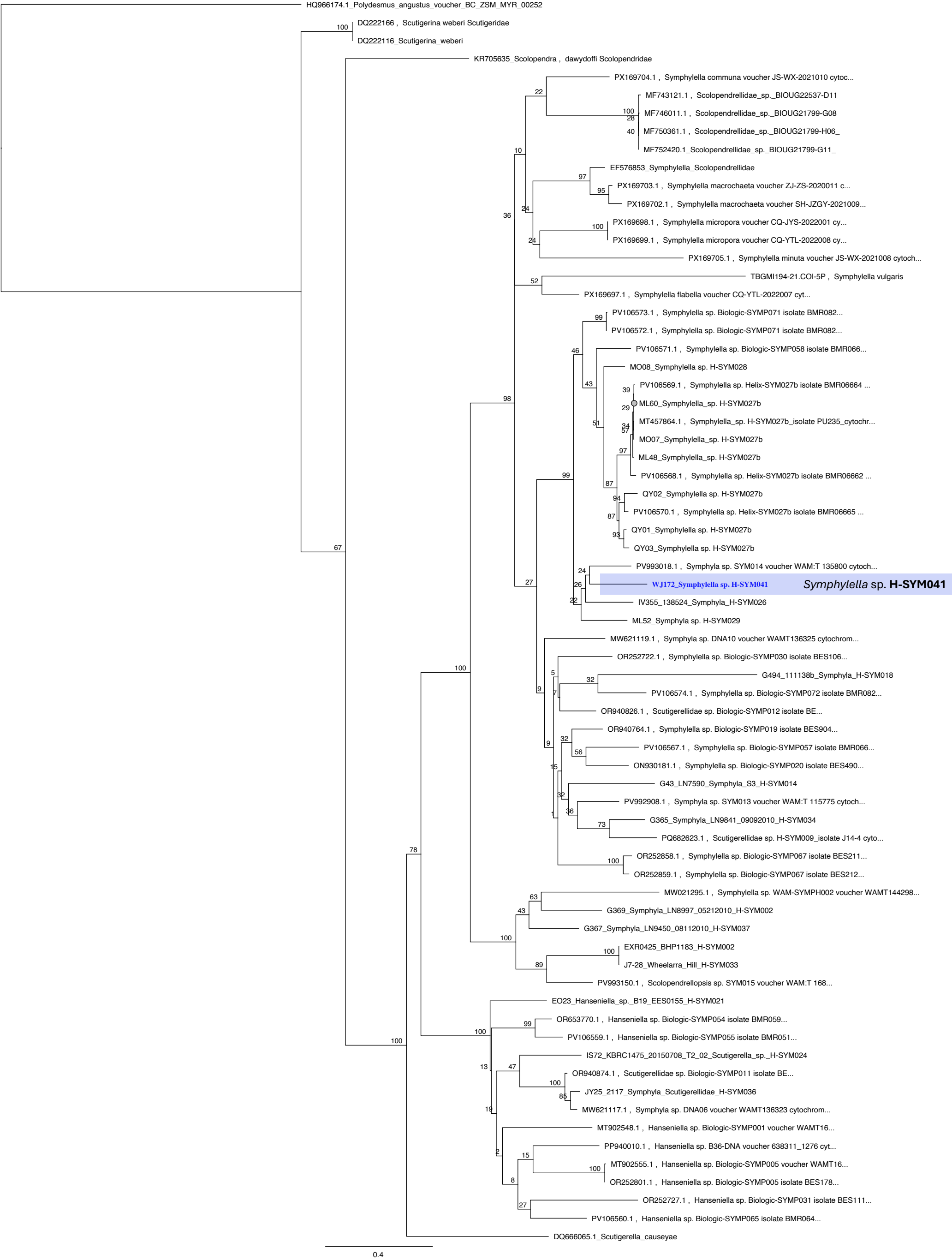


Figure 4-6: Maximum Likelihood analysis of Symphyla COI mtDNA sequences, showing the placement of the phase 6 survey specimen (‘WJ’ prefix) within a reduced taxonomic framework of the families Scolopendrellidae and Scutigereidae (original analysis included 175 reference specimens) and the species designation (highlighted in shaded boxes) . ML tree is for illustrative purposes only with a complete ML tree available on request. Numbers on nodes indicate nodal support by means of ML bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).

5.0 Discussion

COI is widely considered to show suitable variation to distinguish species (Hebert et al. 2003a) and the use of this gene can be extremely effective for DNA barcoding in taxa where clear differentiation exists between intra and interspecific levels of divergence (e.g. Hebert et al. 2004a; 2004b). In a comparison of COI sequences for over 13,000 pairs of taxa, Hebert et al. (2003b) found a mean of 11.1% sequence divergence between distinct species. Nearly 80% of these comparisons found that species pairs differed from one another by >8% sequence divergence. Despite its merits in barcoding, a taxon-by-taxon approach, examining the amount of phylogenetic variation within and between taxa is the most widely accepted method of delineating species and their distributions, especially in areas where rapidly expanding development outpaces taxonomic treatment of unresolved taxa.

In summary, amongst the 167 specimens successfully sequenced for COI from the Winu Borefields project area during phases 6 and 7 (Table 4-1), we detected a total of 22 taxa (1 amphipod (phase 6 and 7), one isopod (phase 6 and 7), six oligochaete (phase 6 only n=5 taxa, phase 6 and 7 n=1), two cyclopoid (phase 6 n=2, phase 7=1), 11 harpacticoid and one symphylan putative species from greater than six families. Of the total 22 taxa detected, only 10 of these (see Table 5-1) align with previously detected species, including those from prior phases of sampling at Winu. Whilst 12 were newly detected putative taxa unique to the Winu project area, see Table 5-1), based on the molecular sequence data currently available for comparison.

5.1 Summary

The phase 6 and 7 Winu subterranean sampling and subsequent sequencing revealed:

- Five oligochaete species belonging to the family Enchytraeidae detected, corresponding to two previously detected, but undescribed taxa:
 - Enchytraeidae sp. H-OLE026 (phase 6 and 7, sites Winu1436 n=23, Winu1445 n=1, Winu 1437 n=1); and
 - Enchytraeidae sp. H-OLE063 (phase 6 only, site Winu1438 n=3, and Winu1452 n=1); as well as three new putative taxa, not detected previously:
 - Enchytraeidae sp **H-OLE064** (phase 6 only, site Winu1424, n=13);
 - Enchytraeidae sp **H-OLE065** (phase 6 only, site Winu1424, n=1); and
 - Enchytraeidae sp **H-OLE066** (phase 6 only, site Winu1451, n=1);
- One previously detected amphipodan species Paramelitidae sp. H-AMP051 from Winu1452 (n=1), detected in phases 6 and 7 from bore Winu1451 (n=19). The second previously detected species of Paramelitidae from Winu, namely Paramelitidae sp. H-AMP050 from the Old Bore collection locality (n=4) was not detected in phases 6 and 7;
- The Microcerberidae isopod sp. H-ISM005 detected in phase 5 from site Winu 1451 (n=2) was again detected in phases 6 and 7 from site Winu 1451 (n=16). The two additional species previously detected during prior sampling phases at Winu, Microcerberidae sp. H-ISM003 detected at Winu1446, 1447 and ADK10 during phase

- 2- 5 sampling (n=10) and H-ISM004, detected in phase 3 from Old Bore (n=1), were not detected during phase 6 and 7 sampling;
- The taxa Harpacticoida sp. H-HAR015 and Harpacticoida H-HAR011 detected on prior Winu survey phases were not detected during the phase 6 and 7 sampling. However, the following 10 potential harpacticoid taxa were detected during the phase 6 and 7 surveys at Winu:
 - *Parastenocaris* sp. H-HAR010A: (n=8) (n=7 Winu1423 phase 7, n=1 RIMFIRE02 phase 2);
 - *Parastenocaris* sp. H-HAR010B: (n=10) Winu1425 phase 7, n=1 Winu1437 phase 6);
 - Harpacticoid sp. H-HAR016: (n=1, Winu1447, phase 4), (n=2, Winu1447, phase 7);
 - Harpacticoida sp. H-HAR017A (**NB** updated nomenclature, previously referred to as HAR017 and HAR019): (n=2 Winu1454 phase 7), (n=2 Winu1450 phase 7), (n=3 Winu1450 phase 6), (n=1 Winu1454 phase 6), (n=3 Winu1450 phase 6), (n=1 Winu1450 phase 5), (n=2 Winu1453 phase 4);
 - Harpacticoida sp. H-HAR017B (**NB** updated nomenclature, previously referred to as HAR018): (n=1 Winu1453 phase 4), (n=1 Winu1453 phase 7);
 - *Parastenocaris* sp. **H-HAR024**: (n=4) Winu1437 phase 6;
 - *Parastenocaris* sp. **H-HAR026**: (n=3) Winu1425 phase 7;
 - Harpacticoida sp. **H-HAR027**: (n=10, Winu1444, phase 7);
 - *Parastenocaris* sp. **H-HAR028**: (n=3) Winu1451 phase 7; and
 - Harpacticoida sp. **H-HAR029**: (n=1, Winu1450, phase 7);
 - 15 Cyclopoida specimens were detected during phases 6 and 7 from two sites:
 - Cyclopidae sp. H-CYC010 (n=14, phases 4 ('VJ' prefix, 6 and 7 (both 'WJ' prefix', site Winu1453); and
 - Cyclopidae sp. **H-CYC036** (n=1 (WJ130), phase 6, site Winu1447); and
 - One symphyllan species (Family: Scolopendrellidae, genus *Symphylella*) detected during phase 6 sampling from site Winu1450 (n=1), representing a newly detected taxon, *Symphylella* sp. **H-SYM041**. This specimen did not align closely with any reference sequences available for comparison, however assignment to the subterranean environment is not compelling.

Table 5-1. Summary of the putative species detected from the project specimens. Species and lineages in '**bold**' are newly detected taxa in phase 6 and/or phase 7.

Taxonomic Group	Taxonomic Group	Molecular Family ID	Molecular species ID	Lineage	Detection Phase	Site
Annelida: Clitella	Oligochaeta	Enchytraeidae	Enchytraeidae sp. H-OLE026	H-OLE026	P6/P7	Winu1436, 1445, 1437, 1451)
			Enchytraeidae sp. H-OLE063	H-OLE063	P6	(Winu1438, 1452)
			Enchytraeidae sp. H-OLE064	H-OLE064	P6	(Winu1424)
			Enchytraeidae sp. H-OLE065	H-OLE065	P6	(Winu1424)
			Enchytraeidae sp. H-OLE066	H-OLE066	P6	(Winu1451)
Crustacea: Malacostraca	Isopoda	Microcerberidae	Microcerberidae sp. H-ISM003	H-ISM003	P1/P3/P4/P5	AKD10 Winu1446
			Microcerberidae sp. H-ISM004	H-ISM004	P4	Old Bore
			Microcerberidae sp. H-ISM004	H-ISM005	P5/P6/P7	Winu1451
	Amphipoda	Paramelitidae	Paramelitidae sp. H-AMP050	H-AMP050	P5	OldBore
			Paramelitidae sp. H-AMP051	H-AMP051	P5/P6/P7	Winu1452, Winu1451
Crustacea: Maxillopoda	Cyclopoida	Cyclopidae	Cyclopoidae sp. H-CYC010	H-CYC010	P5/P6/P7	Winu1453
			Cyclopidae sp. H- CYC036	H- CYC036	P6	Winu 1447
	Harpacticoida	Parastenocaridae	<i>Parastenocaris</i> sp. H-HAR010A	H-HAR010A	P2/P6/P7	STYGO 02 Winu1423

Taxonomic Group	Taxonomic Group	Molecular Family ID	Molecular species ID	Lineage	Detection Phase	Site
						Winu1437
			<i>Parastenocaris</i> sp. H-HAR010B	H-HAR010B	P7	Winu1425
			Harpacticoida sp. H-HAR011	H-HAR011	P3	OldBore
			Harpacticoida sp. H-HAR015	H-HAR015	P4	Winu1454
			Harpacticoida sp. H-HAR016	H-HAR016	P4/P7	Winu1447
			Harpacticoida sp. H-HAR017A	H-HAR017A	P4/P6/P7	Winu1453 Winu1454, Winu1450
			Harpacticoida sp. H- HAR017B	H- HAR017B	P4/P7	Winu1453
			Harpacticoida sp. H-HAR023	H-HAR023	P6	Winu1444
		Parastenocaridae	<i>Parastenocaris</i> sp. H-HAR024	H-HAR024	P6	Winu1437
			<i>Parastenocaris</i> sp. H-HAR026	H-HAR026	P7	Winu1425
			Harpacticoida sp. H-HAR027	H-HAR027	P7	Winu1444
		Parastenocaridae	<i>Parastenocaris</i> sp. H-HAR028	H-HAR028	P7	Winu1451
			<i>Parastenocaris</i> sp. H-HAR029	H-HAR029	P7	Winu1450
Myriapoda	Symphyla	Scolopendrellidae	<i>Symphylella</i> sp. H- SYM041	H- SYM041	P6	Winu1450

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Appendix 1 Genetic p-distances between project specimens

Table A1.1: Genetic p-distance (below) and associated standard error (above- **blue text**) between the representative haplotypes of the Winu Rimfire Enchytraeid *Oligochaeta* specimens from this study ('WJ' prefix).

Molecular ID	Helix ID	H-OLE063				H-OLE026						H-OLE064					H-OLE065	H-OLE066
		WJ01	WJ02	WJ03	WJ45	WJ27	WJ47	WJ05	WJ179	WJ19	WJ28	WJ29	WJ32	WJ33	WJ31	WJ35	WJ30	WJ46
	Winu site	1438			1452	1436	1437	1436	1451	1436	1445	1424					1424	1451
Enchytraeidae sp. H-OLE063	WJ01		0.000	0.000	0.010	0.014	0.013	0.013	0.013	0.014	0.013	0.015	0.015	0.015	0.015	0.016	0.019	0.016
	WJ02	0.000		0.000	0.010	0.014	0.013	0.013	0.013	0.014	0.013	0.015	0.015	0.015	0.015	0.016	0.019	0.016
	WJ03	0.000	0.000		0.010	0.014	0.013	0.013	0.013	0.014	0.013	0.015	0.015	0.015	0.015	0.016	0.019	0.016
	WJ45	0.074	0.074	0.074		0.013	0.013	0.013	0.013	0.013	0.013	0.015	0.015	0.015	0.015	0.017	0.019	0.016
Enchytraeidae sp. H-OLE026	WJ27	0.148	0.148	0.148	0.135		0.003	0.003	0.003	0.003	0.009	0.015	0.015	0.015	0.015	0.017	0.020	0.016
	WJ47	0.146	0.146	0.146	0.134	0.004		0.000	0.000	0.000	0.009	0.015	0.015	0.015	0.015	0.017	0.020	0.015
	WJ05	0.146	0.146	0.146	0.134	0.004	0.000		0.000	0.000	0.009	0.015	0.015	0.015	0.015	0.017	0.020	0.015
	WJ179	0.146	0.146	0.146	0.134	0.004	0.000	0.000		0.000	0.009	0.015	0.015	0.015	0.015	0.017	0.020	0.015
	WJ19	0.146	0.146	0.146	0.135	0.004	0.000	0.000	0.000		0.009	0.015	0.015	0.015	0.015	0.017	0.020	0.016
	WJ28	0.146	0.146	0.146	0.134	0.058	0.058	0.058	0.058	0.059		0.015	0.015	0.015	0.015	0.017	0.019	0.015
Enchytraeidae sp. H-OLE064	WJ29	0.178	0.178	0.178	0.197	0.195	0.197	0.197	0.197	0.198	0.195		0.000	0.000	0.006	0.006	0.018	0.016
	WJ32	0.178	0.178	0.178	0.197	0.195	0.197	0.197	0.197	0.198	0.195	0.000		0.000	0.006	0.006	0.018	0.016
	WJ33	0.178	0.178	0.178	0.197	0.195	0.197	0.197	0.197	0.198	0.195	0.000	0.000		0.006	0.006	0.018	0.016
	WJ31	0.185	0.185	0.185	0.201	0.202	0.204	0.204	0.204	0.205	0.202	0.025	0.025	0.025		0.002	0.018	0.016
	WJ35	0.196	0.196	0.196	0.204	0.224	0.226	0.226	0.226	0.226	0.223	0.020	0.020	0.020	0.002		0.019	0.018
Enchytraeidae sp. H-OLE065	WJ30																	
		0.182	0.182	0.182	0.182	0.203	0.203	0.203	0.203	0.203	0.198	0.167	0.167	0.167	0.172	0.171		
Enchytraeidae sp. H-OLE066	WJ46																	
		0.218	0.218	0.218	0.214	0.211	0.208	0.208	0.208	0.209	0.202	0.227	0.227	0.227	0.233	0.243	0.208	

Table A1. 2: Genetic p-distance (below) and associated standard error (above- **blue text**) between the unique haplotypes of Winu Rimfire amphipod specimens, collected from phase 5 ('VJ' prefix) and phases 6 and 7 ('WJ' prefix).

		H-AMP051					H-AMP050
		WJ154 1451	WJ151 1451	WJ145 1451	WJ144 1451	VJ05 1452	VJ01 Old Bore
H-AMP051	WJ154 1451		0.005	0.005	0.003	0.004	0.022
	WJ151 1451	0.009		0.004	0.005	0.006	0.021
	WJ145 1451	0.009	0.006		0.005	0.005	0.021
	WJ144 1451	0.003	0.006	0.006		0.004	0.021
	VJ05 1452	0.009	0.012	0.012	0.006		0.021
H-AMP050	VJ01 Old Bore	0.181	0.178	0.181	0.184	0.178	

Table A1. 3: Genetic p-distance (below) and associated standard error (above- **blue text**) between the Winu Rimfire microcerberid isopod specimens ('WJ', 'VJ', 'VB', 'UT' and 'TT' prefix).

		H-ISM005				H-ISM003				H-ISM004
		WJ163	WJ174	WJ177	VJ10	VJ06	VB02	UT01	TT01	UT24
H-ISM005	WJ163		0.001	0.002	0.000	0.014	0.014	0.014	0.014	0.015
	WJ174	0.001		0.001	0.002	0.014	0.014	0.014	0.014	0.015
	WJ177	0.003	0.001		0.002	0.014	0.014	0.014	0.014	0.015
	VJ10	0.000	0.002	0.002		0.014	0.014	0.014	0.014	0.015
H-ISM003	VJ06	0.094	0.095	0.095	0.094		0.000	0.000	0.000	0.013
	VB02	0.094	0.095	0.095	0.094	0.000		0.000	0.000	0.013
	UT01	0.094	0.095	0.095	0.094	0.000	0.000		0.000	0.013
	TT01	0.094	0.095	0.095	0.094	0.000	0.000	0.000		0.013
H-ISM004	UT24	0.173	0.174	0.174	0.173	0.156	0.156	0.156	0.156	

Table A1. 4: Genetic p-distance (below) and associated standard error (above- blue text) between the Winu Rimfire harpacticoid copepod specimens (‘WJ’, ‘VJ’, ‘VB’, ‘UD’ and ‘UT’ prefix).

	WJ97	WJ96	WJ94	WJ84	WJ80	WJ74	WJ134	WJ131	WJ130	WJ129	WJ126	WJ123	WJ122	WJ121	WJ120	WJ116	WJ107	WJ104	WJ100	VJ08	VB10	VB09	VB08	VB07	VB06	VB01	UT05	UD03
WJ97 <i>Parastenocaris</i> sp. HAR010B		0.0000	0.0077	0.0063	0.0171	0.0153	0.0208	0.0167	0.0220	0.0154	0.0153	0.0155	0.0152	0.0152	0.0170	0.0154	0.0154	0.0015	0.0000	0.0168	0.0152	0.0153	0.0154	0.0166	0.0167	0.0155	0.0170	0.0063
WJ96 <i>Parastenocaris</i> sp. HAR010B	0.0000		0.0086	0.0064	0.0204	0.0178	0.0265	0.0192	0.0270	0.0179	0.0178	0.0181	0.0178	0.0178	0.0204	0.0180	0.0176	0.0019	0.0000	0.0192	0.0178	0.0178	0.0178	0.0191	0.0192	0.0181	0.0193	0.0067
WJ94 <i>Parastenocaris</i> sp. HAR026	0.0422	0.0413		0.0079	0.0165	0.0153	0.0208	0.0167	0.0220	0.0154	0.0154	0.0155	0.0153	0.0153	0.0159	0.0156	0.0156	0.0078	0.0077	0.0168	0.0153	0.0153	0.0155	0.0168	0.0170	0.0158	0.0173	0.0081
WJ84 <i>Parastenocaris</i> sp. HAR010A	0.0277	0.0225	0.0451		0.0171	0.0153	0.0212	0.0169	0.0223	0.0154	0.0153	0.0156	0.0152	0.0152	0.0169	0.0155	0.0151	0.0064	0.0063	0.0169	0.0152	0.0153	0.0154	0.0167	0.0168	0.0156	0.0171	0.0025
WJ80 <i>Parastenocaris</i> sp. HAR024	0.1494	0.1352	0.1379	0.1494		0.0195	0.0216	0.0216	0.0221	0.0197	0.0194	0.0198	0.0193	0.0193	0.0147	0.0199	0.0198	0.0171	0.0171	0.0218	0.0193	0.0195	0.0196	0.0211	0.0211	0.0200	0.0220	0.0172
WJ74 <i>Harpacticoida</i> sp. HAR017A	0.2023	0.2158	0.2023	0.2009	0.2092		0.0222	0.0172	0.0221	0.0015	0.0056	0.0101	0.0029	0.0032	0.0195	0.0102	0.0122	0.0153	0.0153	0.0173	0.0032	0.0021	0.0056	0.0162	0.0164	0.0103	0.0171	0.0154
WJ134 <i>Cyclopidae</i> sp. H-CYC010	0.2529	0.2705	0.2529	0.2667	0.2851	0.3103		0.0000	0.0193	0.0223	0.0222	0.0223	0.0221	0.0221	0.0216	0.0223	0.0222	0.0208	0.0208	0.0000	0.0221	0.0222	0.0224	0.0209	0.0209	0.0225	0.0224	0.0212
WJ131 <i>Cyclopidae</i> sp. H-CYC010	0.2591	0.2702	0.2591	0.2664	0.2851	0.2853	0.0000		0.0193	0.0173	0.0173	0.0174	0.0172	0.0172	0.0216	0.0174	0.0172	0.0167	0.0167	0.0000	0.0172	0.0172	0.0174	0.0167	0.0167	0.0175	0.0173	0.0169
WJ130 <i>Cyclopidae</i> sp. H-CYC036	0.2877	0.2883	0.2901	0.3019	0.2925	0.2948	0.1958	0.1958		0.0221	0.0222	0.0221	0.0221	0.0221	0.0225	0.0225	0.0222	0.0220	0.0220	0.0193	0.0221	0.0221	0.0222	0.0213	0.0214	0.0225	0.0222	0.0222
WJ129 <i>Harpacticoida</i> sp. HAR017A	0.2050	0.2176	0.2050	0.2035	0.2111	0.0015	0.3132	0.2884	0.2948		0.0054	0.0101	0.0025	0.0029	0.0197	0.0101	0.0124	0.0154	0.0154	0.0173	0.0029	0.0015	0.0054	0.0163	0.0164	0.0102	0.0171	0.0154
WJ126 <i>Harpacticoida</i> sp. HAR017B	0.2023	0.2158	0.2052	0.2009	0.2069	0.0218	0.3126	0.2911	0.2995	0.0205		0.0102	0.0048	0.0050	0.0197	0.0103	0.0123	0.0153	0.0153	0.0174	0.0050	0.0056	0.0000	0.0162	0.0163	0.0104	0.0171	0.0154
WJ123 <i>Harpacticoida</i> sp. HAR029	0.2096	0.2251	0.2096	0.2125	0.2184	0.0757	0.3149	0.2940	0.2948	0.0747	0.0771		0.0099	0.0098	0.0195	0.0070	0.0122	0.0155	0.0155	0.0175	0.0098	0.0101	0.0102	0.0162	0.0164	0.0072	0.0171	0.0157
WJ122 <i>Harpacticoida</i> sp. HAR017A	0.1994	0.2139	0.2023	0.1980	0.2046	0.0058	0.3057	0.2853	0.2948	0.0044	0.0160	0.0728		0.0015	0.0193	0.0100	0.0122	0.0152	0.0152	0.0173	0.0015	0.0029	0.0048	0.0162	0.0164	0.0101	0.0171	0.0153
WJ121 <i>Harpacticoida</i> sp. HAR017A	0.1994	0.2139	0.2023	0.1980	0.2046	0.0073	0.3057	0.2853	0.2948	0.0059	0.0175	0.0713	0.0015		0.0193	0.0099	0.0122	0.0152	0.0152	0.0173	0.0000	0.0032	0.0050	0.0163	0.0164	0.0101	0.0171	0.0153
WJ120 <i>Parastenocaris</i> sp. HAR028	0.1471	0.1352	0.1264	0.1448	0.1057	0.2092	0.2828	0.2828	0.3113	0.2111	0.2161	0.2092	0.2046	0.2046		0.0197	0.0197	0.0170	0.0170	0.0218	0.0193	0.0195	0.0199	0.0210	0.0210	0.0198	0.0218	0.0168
WJ116 <i>Harpacticoida</i> sp. HAR016	0.2052	0.2233	0.2140	0.2082	0.2207	0.0771	0.3172	0.2940	0.3137	0.0761	0.0786	0.0349	0.0742	0.0728	0.2138		0.0122	0.0154	0.0154	0.0175	0.0099	0.0102	0.0103	0.0164	0.0165	0.0015	0.0171	0.0156
WJ107 <i>Harpacticoida</i> sp. HAR027	0.2038	0.2101	0.2111	0.1951	0.2184	0.1164	0.3126	0.2853	0.2995	0.1186	0.1179	0.1150	0.1164	0.1150	0.2161	0.1150		0.0153	0.0154	0.0173	0.0122	0.0124	0.0124	0.0163	0.0161	0.0122	0.0170	0.0152
WJ104 <i>Parastenocaris</i> sp. HAR010B	0.0015	0.0019	0.0437	0.0291	0.1494	0.2009	0.2529	0.2576	0.2877	0.2035	0.2009	0.2082	0.1980	0.1980	0.1471	0.2038	0.2023		0.0015	0.0168	0.0152	0.0153	0.0154	0.0166	0.0168	0.0155	0.0170	0.0065
WJ100 <i>Parastenocaris</i> sp. HAR010B	0.0000	0.0000	0.0422	0.0277	0.1494	0.2023	0.2529	0.2591	0.2877	0.2050	0.2023	0.2096	0.1994	0.1994	0.1471	0.2052	0.2038	0.0015		0.0168	0.0152	0.0153	0.0154	0.0166	0.0167	0.0155	0.0170	0.0063
VJ08 <i>Cyclopidae</i> sp. H-CYC010	0.2606	0.2702	0.2606	0.2679	0.2877	0.2870	0.0000	0.0000	0.1958	0.2884	0.2928	0.2958	0.2870	0.2870	0.2854	0.2958	0.2870	0.2592	0.2606		0.0173	0.0173	0.0174	0.0167	0.0167	0.0175	0.0173	0.0169
VB10 <i>Harpactcoida</i> H-HAR017A	0.1994	0.2139	0.2023	0.1980	0.2046	0.0073	0.3057	0.2853	0.2948	0.0059	0.0175	0.0713	0.0015	0.0000	0.2046	0.0728	0.1150	0.1980	0.1994	0.2870		0.0032	0.0050	0.0163	0.0164	0.0101	0.0171	0.0153
VB09 <i>Harpactcoida</i> H-HAR017A	0.2023	0.2158	0.2023	0.2009	0.2092	0.0029	0.3103	0.2853	0.2948	0.0015	0.0218	0.0757	0.0058	0.0073	0.2092	0.0771	0.1194	0.2009	0.2023	0.2870	0.0073		0.0056	0.0163	0.0164	0.0103	0.0171	0.0154
VB08 <i>Harpacticoida</i> sp. H-HAR017B	0.2035	0.2158	0.2064	0.2020	0.2088	0.0220	0.3155	0.2928	0.2995	0.0205	0.0000	0.0776	0.0161	0.0176	0.2181	0.0791	0.1186	0.2020	0.2035	0.2928	0.0176	0.0220		0.0162	0.0163	0.0104	0.0171	0.0154
VB07 <i>Harpacticoida</i> sp. H-HAR015	0.2518	0.2645	0.2606	0.2562	0.2575	0.2357	0.2529	0.2548	0.2594	0.2372	0.2328	0.2357	0.2357	0.2372	0.2552	0.2445	0.2372	0.2533	0.2518	0.2548	0.2372	0.2387	0.2328		0.0052	0.0165	0.0169	0.0167
VB06 <i>Harpacticoida</i> sp. H-HAR015	0.2577	0.2683	0.2694	0.2592	0.2599	0.2416	0.2529	0.2548	0.2642	0.2430	0.2387	0.2416	0.2416	0.2430	0.2552	0.2474	0.2313	0.2592	0.2577	0.2548	0.2430	0.2445	0.2387	0.0190		0.0165	0.0170	0.0167
VB01 <i>Harpacticoida</i> sp. H-HAR016	0.2079	0.2251	0.2167	0.2108	0.2227	0.0791	0.3202	0.2958	0.3137	0.0776	0.0805	0.0366	0.0761	0.0747	0.2158	0.0015	0.1142	0.2064	0.2079	0.2958	0.0747	0.0791	0.0805	0.2460	0.2460		0.0170	0.0156
UT05 <i>Harpacticoida</i> sp. H HAR011	0.2709	0.2739	0.2840	0.2753	0.2947	0.2738	0.3179	0.2840	0.2995	0.2753	0.2738	0.2738	0.2738	0.2738	0.2854	0.2738	0.2723	0.2723	0.2709	0.2840	0.2738	0.2738	0.2738	0.2650	0.2709	0.2723		0.0170
UD03 <i>Parastenocaris</i> sp. H-HAR010	0.0278	0.0244	0.0469	0.0044	0.1508	0.2035	0.2645	0.2650	0.2972	0.2050	0.2035	0.2152	0.2006	0.2006	0.1415	0.2108	0.1977	0.0293	0.0278	0.2650	0.2006	0.2035	0.2035	0.2548	0.2548	0.2123	0.2723	

Table A1. 5: Genetic p-distance (below) and associated standard error (above- blue text) between the Winu Rimfire Cyclopoida copepod specimens (‘WJ’, and ‘VJ’ prefix).



Appendix 2

Desktop Stygofauna Records for the Rimfire Area

Common Group	Phylum	Class	Order	Lowest ID	Record Location (PSS = Pilbara Stygofauna Survey)
Amphipods	Arthropoda	Malacostraca	Amphipoda	Bogidiella `sp. B02`	Access Road, ca. 44 km WSW Telfer
Amphipods	Arthropoda	Malacostraca	Amphipoda	Bogidiellidae sp. 1	PSS220
Amphipods	Arthropoda	Malacostraca	Amphipoda	Bogidiellidae sp. 1	PSS224
Amphipods	Arthropoda	Malacostraca	Amphipoda	Bogidiellidae sp. 1	PSS228
Amphipods	Arthropoda	Malacostraca	Amphipoda	Bogidiellidae sp. 1	PSS228
Amphipods	Arthropoda	Malacostraca	Amphipoda	Melitidae sp.	PSS226
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae `sp. B10`	Access Road, ca. 44 km WSW Telfer
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae `sp. B28`	Access Road, ca. 51 km W. Telfer
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae `sp. B30`	Access Road, ca. 51 km W. Telfer
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae `sp. Biologic-AMPH026`	Havieron Project
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae `sp. Biologic-AMPH027`	Havieron Project
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae `sp. Biologic-AMPH028`	Havieron Project
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae `sp. Biologic-AMPH030`	Havieron Project
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae `sp. Biologic-AMPH031`	Havieron Project
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp.	PSS220
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp.	PSS221
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp.	PSS226
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp.	PSS227
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp.	PSS228
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp. 6	PSS228
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp. 6	PSS228
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp. 6	PSS305
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp. 6	PSS306
Amphipods	Arthropoda	Malacostraca	Amphipoda	Paramelitidae sp. 6	PSS481
Amphipods	Arthropoda	Malacostraca	Amphipoda	Pilbarus millsii Bradbury and Williams	PSS305
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Bryocyclops sp. 1	PSS220
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops cockingi Karanovic	PSS220

Common Group	Phylum	Class	Order	Lowest ID	Record Location (PSS = Pilbara Stygofauna Survey)
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops cockingi Karanovic	PSS221
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops cockingi Karanovic	PSS228
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops humphreysi humphreysi Pesce et	PSS227
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops humphreysi humphreysi Pesce et	PSS306
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops scanloni Karanovic	PSS226
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops scanloni Karanovic	PSS306
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops scanloni Karanovic	PSS306
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops scanloni Karanovic	PSS481
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops sobeprolatus Karanovic	PSS306
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Elaphoidella humphreysi Karanovic	PSS227
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Elaphoidella humphreysi Karanovic	PSS228
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Elaphoidella*	PSS228
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Halicyclops kieferi Karanovic	PSS221
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida*	PSS220
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida*	PSS227
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida*	PSS492
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Mesocyclops brooksi Pesce et al.	PSS305
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Metacyclops*	PSS214
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Metacyclops*	PSS221
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Metacyclops*	PSS224
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Microcyclops varicans (Sars)	PSS305
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Nitocrella sp. 2	PSS221
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Nitocrella*	PSS221
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Parastenocaris*	PSS226
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Parastenocaris*	PSS228
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Pilbaracyclops frustratio (Karanovic)	PSS214
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Stygonitocrella bispinosa Karanovic	PSS492

Common Group	Phylum	Class	Order	Lowest ID	Record Location (PSS = Pilbara Stygofauna Survey)
Copepods	Arthropoda	Maxillopoda	Calanoida	Stygoridgewayia trispinosa Tang et al.	PSS226
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops cockingi	Access Road, ca. 51 km W. Telfer
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops humphreysi	Access Road, ca. 44 km WSW Telfer
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Diacyclops scanloni	Access Road, ca. 44 km WSW Telfer
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Fierscyclops frustratio	Wilkie Glenn, ca. 30 km STelfer
Copepods	Arthropoda	Maxillopoda	Cyclopoida	Halicyclops kieferi	Access Road, ca. 51 km W. Telfer
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Abnitocrella `sp. B02`	Access Road, ca. 51 km W. Telfer
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida `sp. B01`	Kintyre
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Nitocrella `sp. B03`	Access Road, ca. 51 km W. Telfer
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Nitocrella knotti	Telfer Road, 48 km NW of Telfer Mine
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Nitocrella knotti	Telfer Road, 48 km NW of Telfer Mine
Copepods	Arthropoda	Maxillopoda	Harpacticoida	Nitocrella knotti	Telfer Road, 48 km NW of Telfer Mine
Isopods	Arthropoda	Malacostraca	Isopoda	Adoniscus `sp. B01`	Access Road, ca. 51 km W Telfer
Isopods	Arthropoda	Malacostraca	Isopoda	Adoniscus sp.	PSS220
Isopods	Arthropoda	Malacostraca	Isopoda	Microcerberidae `sp. B04`	Kintyre
Isopods	Arthropoda	Malacostraca	Isopoda	Microcerberidae sp.	Access Road, ca. 44 km WSW Telfer
Isopods	Arthropoda	Malacostraca	Isopoda	Microcerberidae sp.	Oakover River, bore WW12-4
Isopods	Arthropoda	Malacostraca	Isopoda	Microcerberidae sp.	PSS226
Isopods	Arthropoda	Malacostraca	Isopoda	Microcerberidae sp.	PSS492
Isopods	Arthropoda	Malacostraca	Isopoda	Microcerberidae sp.	PSS492
Isopods	Arthropoda	Malacostraca	Isopoda	Oniscoidea sp.	Lower Carawing Gorge
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona arteria Karanovic	PSS220
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona arteria Karanovic	PSS221
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona arteria Karanovic	PSS227
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona iuno Karanovic	PSS220
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona iuno Karanovic	PSS221
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona sp. 4	PSS220

Common Group	Phylum	Class	Order	Lowest ID	Record Location (PSS = Pilbara Stygofauna Survey)
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona sp. 4	PSS221
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona sp. 4	PSS221
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona sp. 4	PSS227
Ostracods	Arthropoda	Ostracoda	Podocopida	Areacandona sp. 4	PSS228
Ostracods	Arthropoda	Ostracoda	Podocopida	Cypretta seurati	Access Road, ca. 51 km W Telfer
Ostracods	Arthropoda	Ostracoda	Podocopida	Gomphodella*	PSS221
Ostracods	Arthropoda	Ostracoda	Podocopida	Humphreyscandona capillus	Ragged Hills Mine, Pilbara Region
Ostracods	Arthropoda	Ostracoda	Podocopida	Humphreyscandona capillus	Ragged Hills Mine, Pilbara Region
Ostracods	Arthropoda	Ostracoda	Podocopida	Humphreyscandona capillus	Ragged Hills Mine, Pilbara Region
Ostracods	Arthropoda	Ostracoda	Podocopida	Humphreyscandona capillus Karanovic	PSS227
Ostracods	Arthropoda	Ostracoda	Podocopida	Humphreyscandona capillus Karanovic	PSS227
Ostracods	Arthropoda	Ostracoda	Podocopida	Humphreyscandona capillus Karanovic	PSS306
Ostracods	Arthropoda	Ostracoda	Podocopida	Leicacandona jula	Access Road, ca. 51 km W Telfer
Ostracods	Arthropoda	Ostracoda	Podocopida	Leicacandona pinkajartinyi	Access Road, ca. 51 km W Telfer
Ostracods	Arthropoda	Ostracoda	Podocopida	Leicacandona sp.	Access Road, ca. 44 km WSW Telfer
Ostracods	Arthropoda	Ostracoda	Ostracoda	Ostracoda (Unident.)*	PSS219
Ostracods	Arthropoda	Ostracoda	Ostracoda	Ostracoda (Unident.)*	PSS226
Ostracods	Arthropoda	Ostracoda	Ostracoda	Ostracoda (Unident.)*	PSS228
Ostracods	Arthropoda	Ostracoda	Ostracoda	Ostracoda (Unident.)*	PSS305
Ostracods	Arthropoda	Ostracoda	Ostracoda	Ostracoda (Unident.)*	PSS306
Ostracods	Arthropoda	Ostracoda	Ostracoda	Ostracoda (Unident.)*	PSS307
Ostracods	Arthropoda	Ostracoda	Ostracoda	Ostracoda (Unident.)*	PSS307
Ostracods	Arthropoda	Ostracoda	Ostracoda	Ostracoda (Unident.)*	PSS481
Rotifers	Rotifera	Rotifera	Bdelloidea	Bdelloidea sp.	PSS226
Snails	Mollusca	Gastropoda	Basommatophora	Planorbidae sp.	PSS221
Snails	Mollusca	Gastropoda	Basommatophora	Planorbidae sp.	PSS221
Syncarids	Arthropoda	Malacostraca	Bathynellacea	Atopobathynella `sp. B08`	Gardens, ca. 35 km S. Telfer

Common Group	Phylum	Class	Order	Lowest ID	Record Location (PSS = Pilbara Stygofauna Survey)
Syncarids	Arthropoda	Malacostraca	Bathynellacea	Atopobathynella sp. A	PSS227
Syncarids	Arthropoda	Malacostraca	Bathynellacea	Hexabathynella sp. A	PSS214
Syncarids	Arthropoda	Malacostraca	Bathynellacea	Hexabathynella sp. A	PSS214
Syncarids	Arthropoda	Malacostraca	Bathynellacea	Parabathynellidae nr. Billibathynella `sp. B08	Access Road, ca. 51 km W. Telfer
Syncarids	Arthropoda	Malacostraca	Bathynellacea	Parabathynellidae nr. Billibathynella `sp. B08	Access Road, ca. 51 km W. Telfer
Syncarids	Arthropoda	Malacostraca	Bathynellacea	Parabathynellidae nr. Billibathynella `sp. B08	Access Road, ca. 51 km W Telfer
Water mites	Arthropoda	Arachnida	Acariformes	Guineaxonopsis sp. S1	PSS227
Water mites	Arthropoda	Arachnida	Acariformes	Oribatida group 4	PSS222
Worms	Annelida	Aphanoneura	Aphaneura	Aeolosoma sp. 1	PSS305
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG023`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG023`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG023`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG023`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG024`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG024`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG024`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG024`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG025`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG025`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG025`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG025`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. Biologic-OLIG025`	Havieron Project
Worms	Annelida	Oligochaeta	Oligochaeta	Enchytraeidae `sp. E12`	Havieron Project
Worms	Annelida	Clitellata	Tubificida	Allonais ranauana (Michaelson and Boldt)	PSS307
Worms	Annelida	Clitellata	Tubificida	Phreodrilid with dissimilar ventral chaetae	PSS227
Worms	Annelida	Clitellata	Tubificida	Phreodrilid with dissimilar ventral chaetae	PSS227
Worms	Annelida	Clitellata	Tubificida	Phreodrilid with dissimilar ventral chaetae	PSS228

Common Group	Phylum	Class	Order	Lowest ID	Record Location (PSS = Pilbara Stygofauna Survey)
Worms	Annelida	Clitellata	Tubificida	Phreodrilid with similar ventral chaetae	PSS306
Worms	Annelida	Clitellata	Tubificida	Phreodrilidae sp.	Telfer
Worms	Annelida	Clitellata	Tubificida	Phreodrilidae sp. WA36	PSS306
Worms	Annelida	Clitellata	Tubificida	Pristina longiseta Ehrenberg	PSS226
Worms	Annelida	Clitellata	Tubificida	Tubificidae `sp. stygo type 1`	Telfer
Worms	Annelida	Clitellata	Tubificida	Tubificidae stygo type 1	PSS220
Worms	Annelida	Clitellata	Tubificida	Tubificidae stygo type 1	PSS220
Worms	Annelida	Clitellata	Tubificida	Tubificidae stygo type 1	PSS226
Worms	Annelida	Polychaeta	Phyllodocida	Namanereis `sp. B01`	Telfer
Worms	Nematoda	-	-	Nematoda `sp.`	Telfer
Worms	Nematoda	-	-	Nematoda sp. 12	PSS223
Worms	Nematoda	-	-	Nematoda sp. 14	PSS482
Worms	Nematoda	-	-	Nematoda*	PSS228