

Appendix G Subterranean Fauna Reports





Havieron Project:
Subterranean Fauna Survey

Biologic Environmental Survey
Report to Newcrest Mining Limited
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GLOSSARY

BC Act Biodiversity Conservation Act 2016

BoM Bureau of Meteorology

DBCA Department Biodiversity, Conservation and Attractions

EPA Western Australian Environmental Protection Authority

EP Act Environmental Protection Act 1986

EPBC Act Environment Protection and Biodiversity Conservation Act 1999

mbgl Metres below ground level

PEC Priority Ecological Communities

SRE Short-range endemic

TEC Threatened Ecological Communities

WAM Western Australian Museum



EXECUTIVE SUMMARY

The Havieron Project is a farm-in joint venture between Newcrest Mining Limited (Newcrest) and Greatland Gold Ltd. It is located 45 km east of Newcrest's Telfer Gold Mine in the Great Sandy Desert, Western Australia. The Havieron Project is targeting a gold-copper resource and will comprise an underground decline, waste rock landform, workshops, service corridor to the Telfer mine and road/construction bores along this corridor. This report assesses the potential impacts to subterranean fauna from construction of the underground decline to further define the resource. Construction will result in dewatering, excavation, and waste rock landforms, which may potentially have direct and indirect impacts on any subterranean fauna.

Biologic Environmental Survey (Biologic) undertook a desktop assessment and two-season subterranean fauna survey of the Havieron Project and Telfer region in accordance with the EPA guidelines. Four field trips took place between November 2019 and June 2020, sampling 33 holes at the Havieron Project and 41 in the Telfer region. In total, 181 subterranean fauna samples were collected. At the Havieron Project this comprised 60 stygofauna samples and 77 troglofauna samples. Samples were taken from holes of a variety of ages and a few contained drilling muds and/ or hydrocarbons. Subterranean fauna has some inherent limitations and it is difficult to determine whether some samples fully meet EPA guidelines. Sampling occurred both inside and outside the area of impact, and throughout all geologies and habitats present.

The current survey recorded a total of 573 subterranean fauna specimens, of which 15 stygofauna and 167 amphibious animals were recorded at the Havieron Project. Groups collected at the Havieron Project were identified to species level (including any occurring at both the Project and Telfer) and comprised four amphibious taxa (enchytraeid worms) and three stygofauna taxa (a copepod, an amphipod and an ostracod). No troglofauna were recorded.

The Havieron Project predominantly hosts widespread geologies and hydrogeological units that may offer limited habitat to subterranean fauna. The current survey results mostly agreed with this assessment, except for some small or localised areas of alluvials and calcareous cement occurring throughout the region, which may represent more suitable stygofauna habitat.

The Project has a small area of direct and indirect impacts within broad and continuous geologies that do not appear to host a rich community of subterranean fauna. One taxon, Humphreyscandonini sp. indet., is currently only known from the area of impact, however this taxon likely inhabits the unconfined aquifer in an area subject to minimal drawdown. The Project is unlikely to have a negative impact on the subterranean fauna of the region.



1 INTRODUCTION

1.1 Background

The Havieron Project (The Project), a farm-in joint venture between Newcrest Mining Limited (Newcrest) and Greatland Gold Ltd is located 45 km east of Newcrest's Telfer Gold Mine in the Great Sandy Desert, Western Australia (Figure 1.1). The Havieron Project is targeting a gold-copper resource within Proterozoic basement rocks, which are overlain by 410 m of Permian sedimentary cover. The Project will comprise an underground decline, waste rock landform, workshops, service corridor to the Telfer mine, and road/construction bores along this corridor.

To access the top of the resource, an underground decline will be constructed to a depth of approximately 430 m. This report assesses the potential impacts to subterranean fauna from:

- dewatering of the proposed underground decline on groundwater resources (hosted within the Permian sedimentary cover); and
- excavation (of Permian sedimentary rocks) to construct the underground decline.

The Project is geographically isolated and there has been historic sampling for subterranean fauna in the local region (referred to as the Study Area) that has largely occurred at and around the Telfer Gold Mine. Newcrest commissioned Biologic Environmental Survey (Biologic) to undertake a two-season subterranean fauna survey of the Project and vicinity (within the Study Area), in accordance with the EPA guidelines regarding subterranean fauna.

1.2 Objectives

The overarching objective of this assessment was to identify the occurrence of any subterranean fauna assemblages within the Study Area, and their supporting habitats. Specifically, the key objectives of the assessment were to provide:

- a desktop review of all previous subterranean fauna surveys in the vicinity of the Study Area and existing subterranean fauna databases on the local/ sub-regional scale
- results of a two-phase stygofauna and troglofauna survey throughout the Study Area, including detailed identifications of all species collected
- an assessment of the likely local occurrence of stygofauna and troglofauna species relative to key habitat units and proposed impact areas, and a discussion of their conservation status and wider potential distribution with reference to regional taxonomic comparisons
- a detailed risk assessment of key subterranean fauna values (species and habitat) in relation to the potential impacts of the proposed mining development.

1.3 Legislation and guidance

Western Australia's subterranean fauna is considered globally-significant due to an unprecedented richness of species and high levels of short-range endemism (EPA, 2016c). The EPA's environmental



objective for subterranean fauna is to "protect subterranean fauna so that biological diversity and ecological integrity are maintained" (EPA, 2016a, p2). In this context, the EPA defines ecological integrity as "the composition, structure, function and processes of ecosystems, and the natural range of variation of these elements" (EPA, 2016a, p2).

Protection for conservation significant subterranean species and/ or Threatened or Priority Ecological Communities (TECs and PECs) is provided under State and Federal legislation, comprising:

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

Most subterranean species and assemblages are not listed under these Acts, due to incomplete taxonomic or ecological knowledge. Consideration of range-restricted subterranean fauna is therefore also important, including species that only occur within restricted habitats, as these have a higher potential of being Short-Range Endemic (SRE) species (Eberhard *et al.*, 2009; Harvey, 2002).

This assessment has been undertaken in consideration of the following EPA guidance statements:

- EPA (2016a) Environmental Factor Guideline: Subterranean Fauna
- EPA (2016c) Technical Guidance: Subterranean Fauna Survey
- EPA (2016b) Technical Guidance: Sampling Methods for Subterranean Fauna.

1.4 Subterranean fauna

Subterranean fauna are animals that live underground. In Western Australia, subterranean fauna are mainly invertebrates such as crustaceans, insects, arachnids, myriapods, worms, and snails, but a small number of vertebrate taxa such as fish and reptiles have also been found (EPA, 2013; Humphreys, 1999). Subterranean fauna are grouped into two major ecological categories:

- stygofauna aquatic animals that inhabit groundwater in caves, aquifers, and water-saturated interstitial voids
- troglofauna air-breathing animals that inhabit air-filled caves and smaller voids above the water table.

Nevertheless, there are some taxa which cross-over between these categories and are known to occur in groundwater as well as air-filled subterranean habitats (e.g. enchytraeid worms), and yet other species that occur within subterranean habitats for only part of their lifecycles (stygoxenes/ stygophiles, and trogloxenes/ troglophiles respectively).

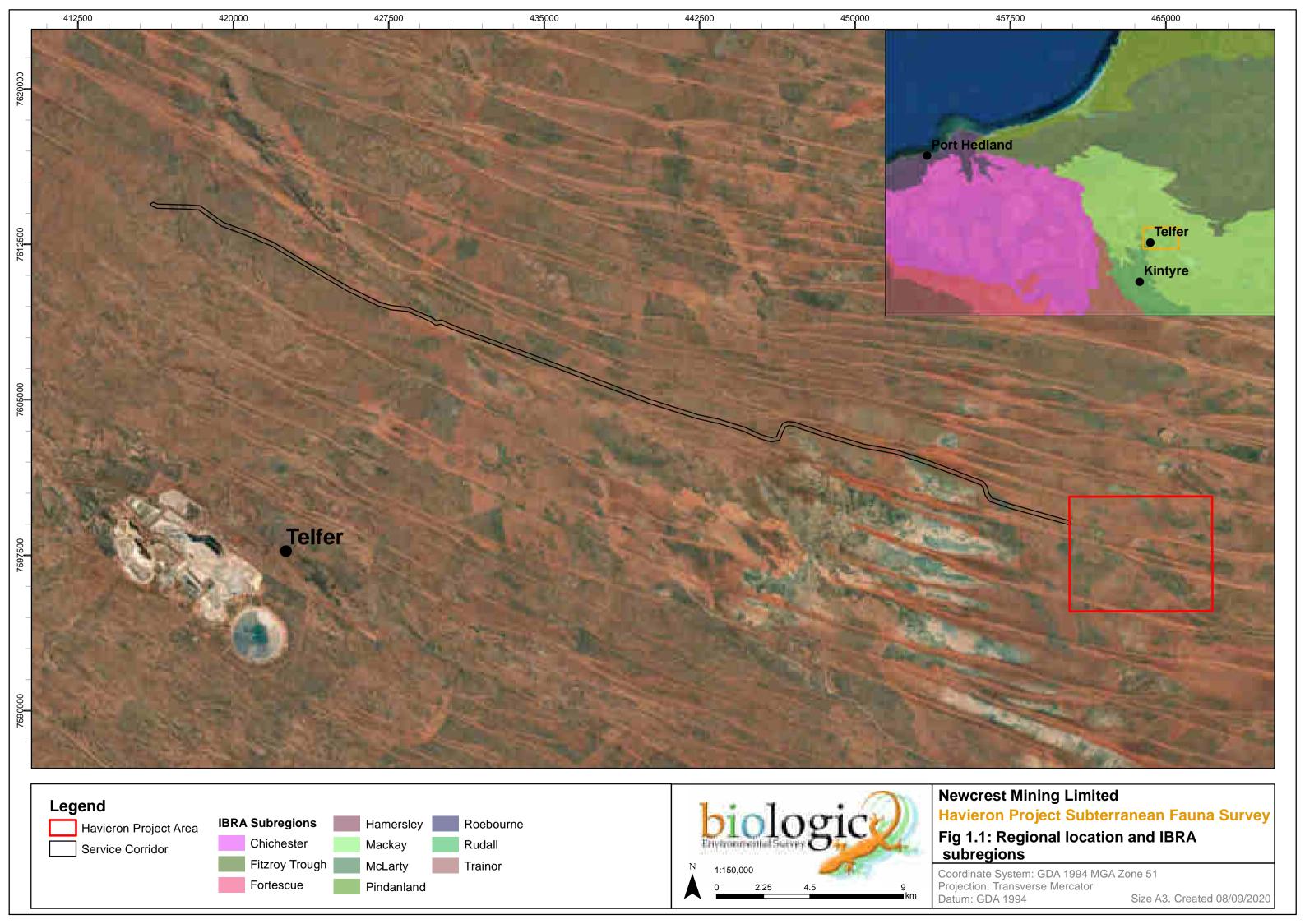
Following EPA (2016c) guidelines, obligate subterranean fauna (known respectively as stygobites and troglobites) are defined as species that live their entire lives underground and are completely dependent upon, or restricted to, subterranean habitats. Such species are considered to have a high likelihood of being limited to very narrow ranges (*i.e.* short-range endemic (SRE) species), and therefore may be at greater risk of impacts from proposed developments (EPA, 2016c). SRE species as described by Harvey



(2002), are species whose natural ranges are limited to <10,000 km² (or <100 km x 100 km), whereas Eberhard *et al.* (2009) regarded even this criterion as potentially too vast for range-restricted subterranean fauna, offering an alternative threshold of <1,000 km² for subterranean SRE species.

Troglobites and stygobites often display evolutionary adaptations to underground life; these include features such as reduced pigment, reduced or vestigial wings, reduced cuticle thickness, elongation of sensory appendages, and reduced eyes or eyelessness. Additional adaptations to underground life can include changes to physiology, lifecycle, metabolism, feeding and behaviour (Christiansen, 2005; Gibert & Deharveng, 2002).

As the darkness of hypogean environments precludes photosynthesis, subterranean ecosystems are generally dependent upon allochthonous inputs of nutrients and oxygen from the surface (except in cases where chemo-autotrophic bacteria are present) (Hahn, 2009). Energy and nutrients are generally transported into subterranean ecosystems by the infiltration of water, particularly via the roots of groundwater dependent vegetation (Howarth, 1983; Humphreys, 2006; Malard & Hervant, 1999; Poulson & Lavoie, 2000). Thus, the porosity (or otherwise) of the overlying geologies, the distance from the surface, and the presence/absence of caves or fissures that can provide a conduit for water and nutrients are important physical features that influence the suitability of underground habitats for subterranean fauna (Hahn & Fuchs, 2009; Strayer, 1994). Groundwater physicochemistry (including salinity, pH, dissolved oxygen and redox potential) is also an important determinant of habitat suitability for stygofauna (Eberhard *et al.*, 2009; Hahn, 2009; Humphreys, 2008; Watts & Humphreys, 2004).





2 ENVIRONMENT

2.1 Geology

Geology of the Great Sandy Desert is dominated by Phanerozoic sedimentary rock formations which are extensively mantled by reddish aeolian sands, characteristic of a large portion of the Canning Basin (Burbidge & McKenzie, 1983). Red sands dominate the Great Sandy Desert, often occurring as extensive sandplains or longitudinal sand dunes ranging from three to 25 m in height (Burbidge & McKenzie, 1983).

The Study Area is within the Paterson province. This includes the Paleoproterozoic to Mesoproterozoic Rudall Complex, the Neoproterozoic officer and Yeneena Basins, as well as the Phanerozoic Canning and Gunbarrel Basins (Rockwater, 2019).

The surface geology is dominated by Quaternary-aged aeolian sand dunes which is characteristic of the Great Sandy Desert (Figure 2.2). These sand dunes comprise predominantly quartz, minor feldspar and, in some locations, heavy mineral sands, including well rounded zircon and tourmaline. While the interdunal corridors comprise both weathering products (laterite, silcrete, ferricrete, and calcrete) and sediments (aeolian sand, alluvium, and evaporites). Below these dunes lie the Permian aged Sediments of the Kidson Sub-basin (of the Canning Basin) which are likely to belong to the fluvio-glacial Paterson Formation. The Canning Basin sediments overlie the Neoproterozoic Yeneena Basin which hosts the mineralisation for the project (Rockwater, 2019). The typical geological sequence at the Project is shown below in Table 2.2.

The Permian sediments comprise six subunits that include weathered mudstone, tillite, siltstone and sandstone (drill core photos shown below in Table 2.1). The Upper Mudstone (UWM) is dominantly a massive mudstone to clay zone with weathering close to the surface (upper saprolite, lower saprolite and sap-rock subunits). The Upper Tillite (UMT) is a permeable sandstone that comprises a laterally defined conglomerate unit and a broad vertical extent of poorly sorted poly-clastic tillite interbedded with minor thin beds of fine well-rounded sandstone and rare drop stones. The Lower Siltstone has an abundance of fine-grained sediment. The Lower Tillite is dominantly coarse, poorly sorted sandstone. The Proterozoic basement rocks host the mineralisation and are cemented and largely un-fractured. Notable exceptions include the contact with the Permian deposition.

The geologies at the Project are generally flat and become gradually shallower to the west (see cross section in Figure 2.1). The UWM underlies the Quaternary cover unit in the east, but due to the slight dip to the west this unit thins out, leaving Upper Tillite directly under the Quaternary-aged surface sediments (approximately in the Percival Palaeovalley). The basement rocks gradually shallow to the west and outcrop on the western side of the Percival Paleovalley near Telfer.



Table 2.1. Drill core photos of Permian and Permian sediments.





Table 2.2	. Typical Geolog	gical Sequence	(Rockwater, 2019).
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Age	Geological Formation	Unit	Average Thickness (m)	Average Depth to base of formation (m bgl)			
Quaternary	Superficial	Undifferentiated COVER (aeolian sand, alluvium, and evaporates)	5-15	5-15			
		QUATERNARY/PERMIAN UNCONFORMITY					
	Paterson	UWM - Upper Mudstone		100-110			
		UMT - Upper Tillite	60	170			
Permian		LCS - Upper Siltstone	85	255			
Permian		LST - Middle Sandstone	25	280			
		LSL - Lower Siltstone	35	315			
		LFT - Lower Tillite	95	410			
	PERMIAN/PROTEROZOIC UNCONFORMITY						
Proterozoic	Undifferentiated	Undifferentiated Basement	N/D	N/D			

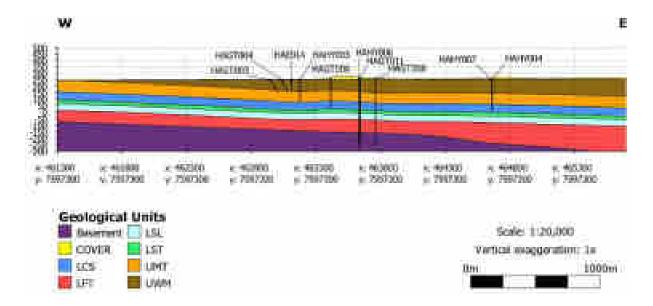


Figure 2.1 Cross-section parallel to the direction of the proposed mine decline.

2.2 Hydrogeology

The Study Area includes sandplains and linear sand dunes rising to 18 m above interdunal corridors. The landforms within the Project are predominantly influenced by Cenozoic erosion and deposition events resulting in a series of westerly to north-westerly trending longitudinal dunes (Ferguson *et al.*, 2005). The most significant valley near the Project is the Percival Palaeovalley (English *et al.*, 2015) and hosts



significant calcrete outcrops. The topography trends west towards the palaeovalley, which drains to Lake Dora that is located 34 km southwest of the Project. Lake Dora is the closest substantial surface water feature that lies within the Rudall River National Park and is recognised as a groundwater dependant ecosystem (BoM, 2020).

The water table is generally greater than 10 mbgl at the Project, becoming shallow to the west (up to 5 mbgl). Hydrogeological works by Rockwater (2019) have identified four key aquifer units at the Project, in order from the surface:

- 1. Unconfined/Perched Aquifer
- 2. Upper Confined Aquifer
- 3. Lower Confined Aquifer
- 4. Proterozoic Aquifer (fractured).

The aquifers are described below with key characteristics summarised in Table 2.3.

2.2.1 Unconfined/ perched aquifer

The unconfined aquifer is small and interpreted to comprise:

- superficial sediments (generally unsaturated at Havieron given their thickness of 5 15m and the average water table depth of 14m (Rockwater, 2020))
- part of the upper UWM (upper saprolite, lower saprolite and saprock subunits) where weathered units are underlain by a thick succession of very low-permeability claystone and mudstone of the upper UWM.

It has a low transmissivity (0.37 m²/day) and high salinity of 18,800 to 39,100 mg/L TDS, which is likely due to limited salt flushing and evapoconcentration occurring near playas.

2.2.2 Upper confined aquifer

This unit comprises the basal upper silt/sandstone beds of the UWM and entire UMT, with the top of the aquifer located at 15 mbgl in the west to up to 110 mbgl in the east. It is confined by the UWM except where UWM is absent (to the north west and south west of the Project). The average transmissivity is 2.0 m²/day. Groundwater in the Paterson Formation is generally fresh near recharge areas becoming saline with depth and distance down the flow system (Laws (1990). At the Project, low salinities (3,000 to 4,000 mg/L) were observed where the aquifer is shallow and increasing (15,000 to 20,000 mg/L) at greater depth.

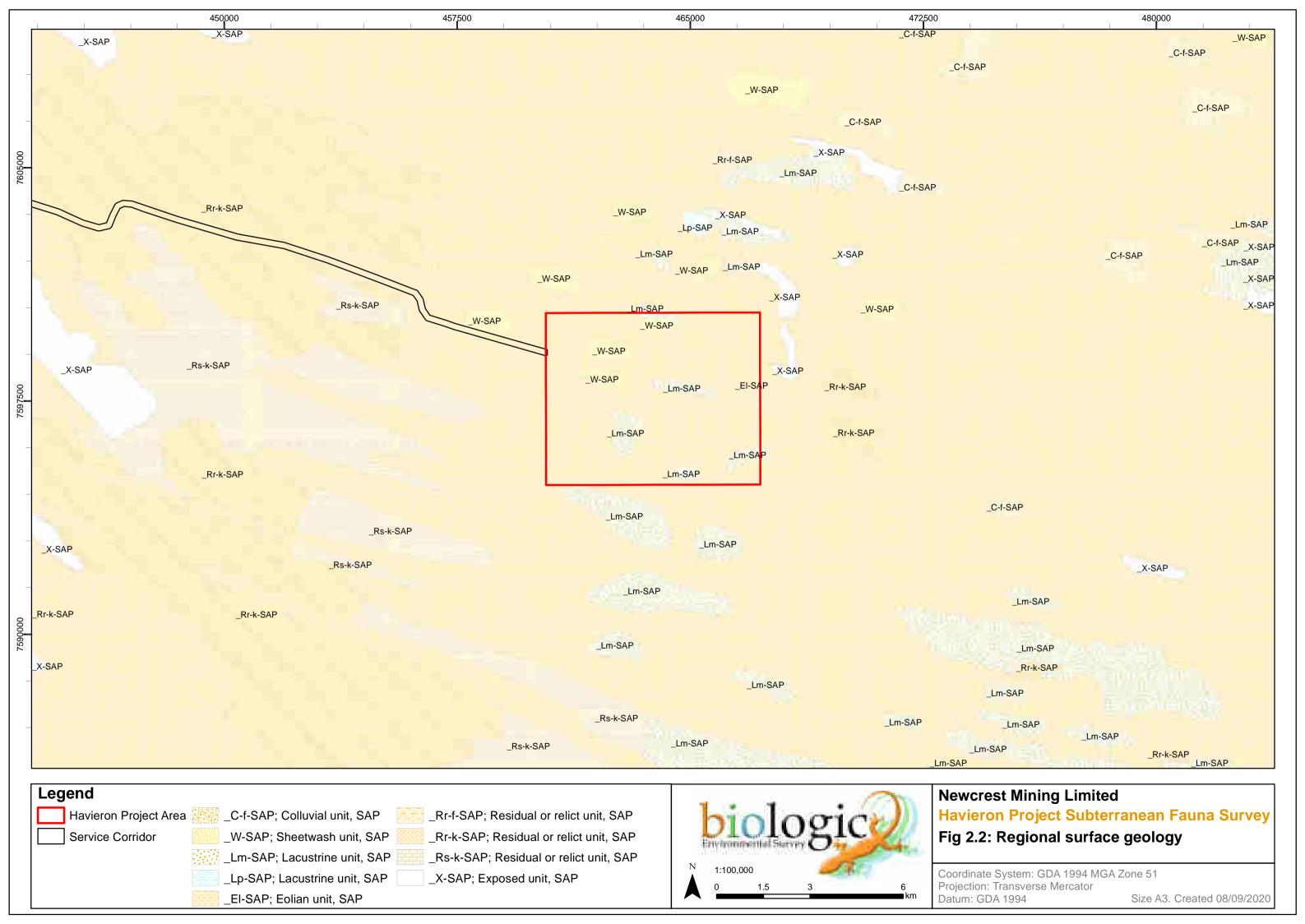
2.2.3 Lower confined aquifer

This aquifer comprises two units – the LST and entire LFT, which is typically ~150 mbgl deeper than the Upper Confined Aquifer. It is confined by the low permeability siltstones of the LCS and is underlain by basement Permian rocks. Salinity increases with depth and is recorded at up to 55,000 mg/L.



Table 2.3 Aquifer characteristics

Aquifer	Depth	Hydraulic Transmissivity	Typical Thickness of Aquifer	Salinity (mg/L TDS)
Unconfined/ perched	Within the uppermost 15 m and absent elsewhere	0.37 m²/day	0.0 – 19.2 m (av. 6.2 m)	18,800 to 39,100
Upper confined	Top of aquifer from 15 m in the west to up to 110 m in the east	2.0 m²/day	80 m	Shallow (3,000 to 4,000) At depth (15,000 to 20,000)
Lower Confined	Typically, about 150 m deeper than the Upper Confined Aquifer	0.9 m²/day	95 m	Up to 55,000





3 METHODS

3.1 Database search and review of previous reports

Five databases were searched for subterranean fauna records in April 2019 (Table 3.1):

- DBCA's NatureMap database (DBCA, 2020)
- Western Australian Museum (WAM) Arachnida/ Myriapoda database (WAM, 2019a)
- WAM Crustacea database (WAM, 2019b)
- WAM Mollusca database (WAM, 2019c)
- DBCA's Pilbara Stygofauna Survey species list (Halse et al., 2014).

All records were filtered based on collection methods and known stygofauna/ troglofauna taxonomic groups where information on subterranean status was not present in the data.

Table 3.1. Databases searched for subterranean fauna records

Database	Parameters
NatureMap	40 km radius around 21°43'22.1"S 122°38'55.0"E
WAM Arachnida/ Myriapoda WAM Crustacea WAM Mollusca	65 km radius around 21°43'22.1"S 122°38'55.0"E
DBCA's Pilbara Stygofauna Survey	65 km radius around 21°43'22.1"S 122°38'55.0"E

Reports from subterranean fauna surveys within 40 km of the Study Area were reviewed for local and regional context. Reports from Telfer region are listed below:

- Telfer Gold Mine Stygofauna Sampling Report, November 2001 (Biota, 2001)
- Newcrest Telfer Gold Mine Subterranean Fauna Monitoring Program Field Report November 2004 (Natural Resource Services, 2004)
- Telfer Project Subterranean Fauna Management Plan (TP-PRO-80-02-0001) (Newcrest, 2004)
- Stygofauna Taxonomy Report for Newcrest Mining Limited (NCM), Telfer Operations -Stygofauna Monitoring (Horwitz & Clarke, 2005)
- Newcrest Mining Limited: Telfer Gold Mine Stygofauna Monitoring, December 2009 (Bennelongia, 2010b)
- Preliminary subterranean fauna sampling, O'Callaghans Deposit, Telfer (Bennelongia, 2010a)
- Telfer Gold Mine. Monitoring program: taxonomic alignment of stygofauna species draft report (Bennelongia, 2010c)
- Stygofauna Monitoring: Telfer Gold Mine, September 2010. Final Report. (Bennelongia, 2011a)
- Telfer Gold Mine: baseline troglofauna survey (Bennelongia, 2011b)
- Stygofauna for the O'Callaghan's Project, Telfer (Bennelongia, 2012a)
- Stygofauna Monitoring: Telfer Gold Mine, September 2011 (Bennelongia, 2012b)
- Telfer Gold Mine: troglofauna monitoring in 2011 (Bennelongia, 2012d)
- Stygofauna Monitoring: Telfer Gold Mine, October 2012 (Bennelongia, 2013a)



- Stygofauna monitoring: Telfer Gold Mine, October 2013 (Bennelongia, 2013b)
- Troglofauna Monitoring: Telfer Gold Mine, October 2013 (Bennelongia, 2014b)
- 10 Years of Stygofauna Monitoring at Telfer Gold Mine (Bennelongia, 2014a)
- Final Report Subterranean Fauna 5 Year Review 2009-2013 (Newcrest, 2014)
- Havieron Project Hydrogeological Assessment (Rockwater, 2019).

One additional survey for subterranean fauna have been conducted beyond the 65 km search area and is included due to its relevance and paucity of subterranean fauna sampling within the search area:

• Subterranean fauna Assessment of the Kintyre Uranium Deposit (Bennelongia, 2012c).

3.2 Survey timing

A two-phase survey for subterranean fauna was undertaken in accordance with guidelines for subterranean fauna assessments (EPA, 2016a, 2016b, 2016c). The first phase of sampling was undertaken in November 2019 – February 2020, representing a wet season survey with the second phase undertaken during the dry season months April – June 2020. Each survey phase comprised two field trips as follows:

Phase 1

- Trip 1, 21st to 26th November 2019: trap deployment and scrape / haul / pump sampling; and
- Trip 2, 18th to 21st February 2020: trap retrieval and scrape / haul / pump sampling.

Phase 2

- Trip 3, 30th March to 3rd April 2020: trap deployment and scrape / haul / pump sampling; and
- Trip 4, 14th to 19th June 2020: trap retrieval and scrape / haul / pump sampling.

3.3 Site selection and survey effort

Within the Study Area, site selection for subterranean fauna sampling was limited to accessible, vertical bores (*i.e.* cased, production or monitoring bores) and drill holes (uncased holes). In general, suitable sampling sites are contingent on:

- drill hole construction (uncased required for troglofauna),
- angle (90° required for scraping and net hauling)
- time since drilling (>6 months required for stygofauna, following EPA 2016b), and
- whether the holes intercepted groundwater (required for stygofauna).

A total of 74 holes were sampled throughout the Study Area, 33 in Havieron and 41 in the Telfer region, over the course of the two survey phases (Figure 3.1). Stygofauna were sampled for in 63 holes (33 at the Project) and 42 for troglofauna (26 at the Project). Standard sampling methodology was utilised for stygofauna - hauling, dual haul-scraping, and pumps (filtering pump outflow) and troglofauna - scraping, dual haul-scraping, and trapping. Hole and sample details are provided in Appendix A.

A total of 181 subterranean fauna samples were collected during the survey, of which 93 come from the Project and 88 from Telfer (three troglofauna traps are not included in the tally as they were lost due to



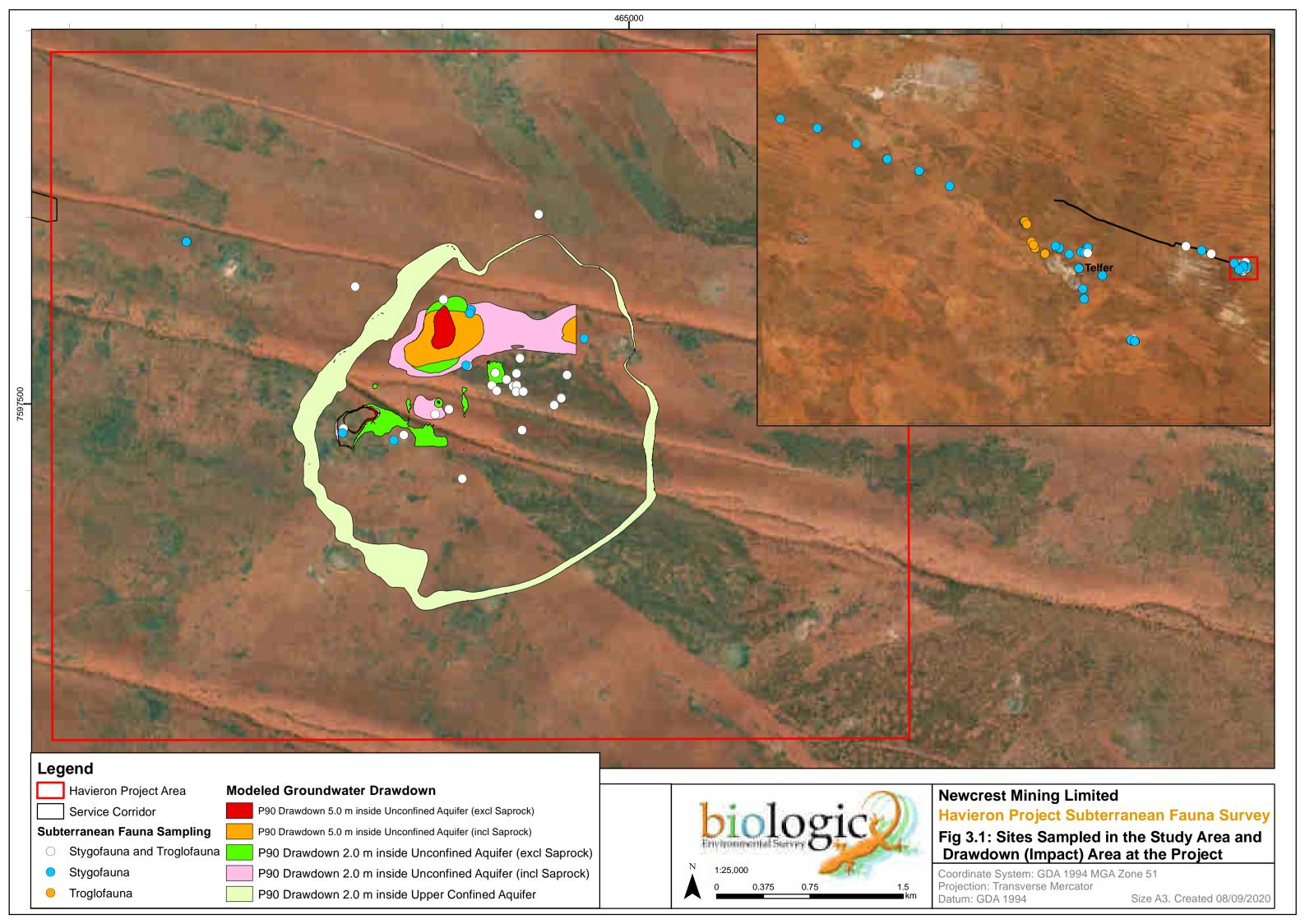
hole collapse or hole rehabilitation). The sampling at the Project comprised 60 stygofauna samples (12 hauls, 4 pumps and 44 scrape-hauls) and 77 troglofauna samples (44 scrape-hauls and 33 traps) (Table 3.2; Table 3.3). Samples were taken from holes of a variety of ages and a few contained drilling muds and/or hydrocarbons. Subterranean fauna has some inherent limitations and it is difficult to determine whether some samples fully meet EPA guidelines. See Appendix A for drill hole details and dates. Sampling occurred both inside and outside the area of impact, and throughout all geologies and habitats present.

Table 3.2: Number of subterranean fauna samples collected in the Study Area.

Locality	Trip Number	Haul	Scrape	Scrape-Haul	Pump	Trap Set	Trap Collected
	1	2		12	1	12	10
Havianan	2	2		3	1		
Havieron	3	3		18	1	23	23
	4	5		11	1		
Sub-total		12		44	4	35	33
	1	18	6	1	1	9	9
T-16	2	2	1	2			
Telfer	3	13	2	2	1	15	14
	4	9	4	3			
Sub-total		42	13	8	2	24	23
Total		54	13	52	6	59	56

Table 3.3: Number of subterranean fauna samples collected in the Project.

Method	Target Fauna	Impact	Reference	Total
Haul	Stygofauna	6	6	12
Pump	Stygofauna	4		4
Scrape-Haul	Stygofauna and Troglofauna	36	8	44
Trap	Troglofauna	28	5	33
Total		74	19	93





3.4 Sampling methods

The sampling methods used were consistent with EAG #12 (EPA, 2016c), Guidance Statement #54A (EPA, 2016b) and the Stygofauna Sampling Protocol developed for the Pilbara Biodiversity Study Subterranean Fauna Survey (Eberhard *et al.*, 2005; Eberhard *et al.*, 2009). The field work was undertaken by Dean Main, Syngeon Rodman, and Courtney Proctor. Laboratory sorting was undertaken by Syngeon Rodman, Mary van Wees, Juliana Pille Arnold, Stephanie Floeckner, Siobhan Paget, Kaylin Geelhoed, Isabelle Johansson, and Morgan Lythe.

3.4.1 Troglofauna trapping

Trapping utilised custom-made cylindrical PVC traps (approximately 50 mm x 300 mm) baited with decaying leaf litter (dead spinifex / acacia sourced from the Pilbara region), which were sterilised with boiling water. Traps were lowered *via* a nylon cord to a suitable depth and left in operation six to eight weeks, before being collected and transported back to the laboratory in Perth.

3.4.2 Troglofauna scraping

Scraping was undertaken at vertical, uncased drill holes using a reinforced 150 µm weighted stygofauna net, with a specialised scraping attachment used above the net to maximise gentle contact with the walls of the hole. The net was lowered and raised through the full length of the hole four times for holes where no water was present, with each haul being emptied into a sample bucket. Where the water table was intercepted, a combined net-haul / scrape sample was taken using the scraping attachment. Each combined net-haul / scrape sample comprised a total of six hauls from the bottom of the hole to the top (including AWT and BWT habitats), with three hauls using a 150 µm mesh and three hauls using a 50 µm mesh. The contents of the sample were elutriated, processed, and stored in 100 % ethanol.

3.4.3 Stygofauna net-hauling

Stygofauna were sampled by standard net-hauling methods, using a plankton net of a diameter to suit each bore or drill hole (in most cases 30-80 mm). Each haul sample comprised a total of six hauls from the bottom of the hole to the top, with three hauls using a 150 μ m mesh and three hauls using a 50 μ m mesh. The base of the net was fitted with a lead weight and a sample receptacle with a base mesh of 50 μ m. To stir up sediments, the net was raised and lowered at the bottom of the hole prior to retrieval and hauled at an even pace through the water column to maximise filtration of the water.

The sample from each haul was emptied into a bucket, which was elutriated after the final haul to remove coarse sediments and filtered back through the 50 µm net/ sample receptacle to remove as much water as possible. The sample was transferred to a 50-120 mL preservation vial (depending upon the quantity of sediment) and preserved in 100% ethanol. The ethanol and the samples were kept chilled on ice or in a refrigerator to facilitate cool-temperature DNA fixation.

3.4.4 Pumping (stygofauna)

Two sites were sampled by actively pumping water from bores and running the water from the pump release valve through a stygofauna net three times for approximately 10 minutes total at each site.



3.4.5 Water physicochemistry

Prior to stygofauna sampling, a groundwater sample was collected using a plastic cylindrical bailer (length: 1 m), for the purposes of physicochemical measurements. The bailer was lowered down the hole until reaching groundwater and a water sample was collected at a depth of 2 m below the surface. As such the results were not indicative of water parameters throughout the entire bore (or aquifer) but rather provide a general indication of near surface conditions. Conditions sampled during pumping were measured using a sample collected from the pump outflow, which would have artificially increased the dissolved oxygen readings. Groundwater physicochemical data (including EC, pH, TDS, Redox ORP, and dissolved O₂) was measured using a multi-parameter water meter. Constrictions in piezometer bores, blockages from root material, or excessive depths to groundwater inhibited the collection of physicochemical readings at some sites.

3.4.6 Sorting and taxonomy

Sorting and parataxonomy were undertaken in-house using dissecting microscopes. The personnel involved (Syngeon Rodman, Mary van Wees, Juliana Pille Arnold, Stephanie Floeckner, Siobhan Paget, Kaylin Geelhoed, Isabelle Johansson, Morgan Lythe, Michael Curran, and Giulia Perina) were all suitably trained and experienced in sorting and parataxonomy of subterranean fauna.

Parataxonomy of the specimens utilised published literature and taxonomic keys where available. Each morphospecies from each sample was assigned a separate labelled vial and labelled with a specimen tracking code. Taxonomic groups were examined in as much detail as possible using in-house expertise, before sending a reference collection to specialist taxonomists for detailed taxonomic advice. Species comparisons and alignments were performed using regional specimens collected beyond the Study Area throughout the wider sub-regional area. Dr Giulia Perina provided specialist taxonomic identifications and regional alignments.

Genetic analysis (DNA barcoding using the mitochondrial gene COI) was conducted by Biologic on certain subterranean taxa to validate morphological identifications and provide a basis for species-level identifications and regional comparisons where taxonomic resolution was limited. Refer to Appendix C for further details regarding the methods of DNA extraction, choice of primers, sequencing, and analysis.

3.4.7 Conservation status and SRE classification

A few subterranean species and assemblages from the Pilbara region are listed under relevant legislation as threatened species, or as Threatened or Priority Ecological Communities in certain locations. Any listed subterranean species or community is regarded as conservation significant although, due to a lack of survey effort and taxonomic certainty for the majority of subterranean fauna in the Pilbara region, there are many potentially range-restricted (SRE) or conservation significant species and communities that do not appear on these lists.

The likelihood of taxa representing SRE species (*i.e.* distribution <10,000 km² following Harvey 2002, or <1,000 km² following Eberhard *et al.* 2009) was assessed based on the known local species distribution, and regional comparisons where data was available, following advice from the WAM and other relevant taxonomic specialists. The assessment of SRE status was highly dependent on:



- 1. the degree of taxonomic certainty at the genus and species levels
- the current state of taxonomic and ecological knowledge for each taxon (including whether a regional genetic context has been investigated)
- 3. the scale and intensity of the local and regional sampling effort
- 4. whether or not relevant taxonomic specialists were available to provide advice.

The SRE status categories used in this report follow the WAM's categorisation for SRE invertebrates. This system is based upon the 10,000 km² range criterion proposed by Harvey (2002), and uses three broad categories to deal with varying levels of taxonomic certainty that may apply to any given taxon (Table 3.3). Because most subterranean fauna are poorly known taxonomically, and the general limitations to sampling subterranean fauna, the majority of morphospecies invariably fall within one (or several) of the five Potential SRE sub-categories.

Table 3.4: SRE categorisation used by WAM taxonomists

	Taxonomic Certainty	Taxonomic Uncertainty	
Distribution <10 000km ²	 Confirmed SRE A known distribution of < 10,000km². The taxonomy is well known. The group is well represented in collections and/ or <i>via</i> comprehensive sampling. 	Potential SRE Patchy sampling has resulted in incomplete knowledge of geographic distribution. Incomplete taxonomic knowledge The group is not well represented in collections.	
Distribution >10 000km ²	 Widespread (not an SRE) A known distribution of > 10,000km². The taxonomy is well known. The group is well represented in collections and/ or <i>via</i> comprehensive sampling. 	Category applies where there are significant knowledge gaps. SRE Sub-categories may apply: A) Data Deficient B) Habitat Indicators C) Morphology Indicators D) Molecular Evidence E) Research & Expertise	

The degree of stygomorphy or troglomorphy (observable physical adaptations to subterranean habitats such as eyelessness, depigmentation, elongation of sensory appendages and thinning of the cuticle) assessed to determine each morphospecies' 'subterranean status', *i.e.* whether a taxon was more or less likely to be an obligate subterranean species (stygobite/ troglobite). It is acknowledged that the current EPA guideline for subterranean fauna does not account for non-obligate subterranean fauna, stating, "...subterranean fauna are defined as fauna which live their entire lives (obligate) below the surface of the earth.... Fauna that use a subterranean environment for only part of the day or season (e.g. soil-dwelling or burrowing species, cave-dwelling bats and birds) are not considered as subterranean fauna for this EAG" (EPA, 2013).

Nevertheless, there may be fauna with restricted distributions <10,000 km² following Harvey (2002), or <1,000 km² following Eberhard *et al.* (2009) that are of interest because of their SRE status, regardless of whether they can be definitively regarded as 'obligate' subterranean fauna. For this reason, this report



presents an assessment of both the subterranean status and the SRE status of each taxon collected, to the best available knowledge.

In some cases where thorough sampling has been conducted and sufficient habitat information and ecological information is available, the potential occurrence of a taxon at a local scale may be inferred *via* the extent of habitats, particularly where the rest of the assemblages are highly similar, and the habitats appear well-connected. Despite the suggestion within the current EPA (2013) guidelines that related species' ranges may be used as surrogates for poorly-known species' ranges, the level of evidence required to support the identification of an appropriate surrogate is almost prohibitively high for most subterranean fauna, therefore this would only be investigated as a last resort.

3.5 Constraints and limitations

Table 3.5: Survey limitations and constraints

Potential limitation or constraint	Applicability to this survey
Experience of personnel.	No constraint.
Site Selection	No constraint. Availability improved mid survey by drilling of holes specifically designed to target subterranean fauna.
Sampling Techniques	No constraint.
Survey Effort Troglofauna	Minor constraint – some holes contained biodegradable drilling muds/ hydrocarbons
Survey Effort Stygofauna	Minor constraint – some holes contained biodegradable drilling muds/ hydrocarbons or sampling occurred less than 6 months post drilling
Specimen Identification	No constraint, noting that identifications are inherently limited for shells/valves and molecular work was undertaken, providing a very high level of taxonomic resolution.
Level of Survey	No constraint considering small impact area within widespread, well connected geologies.
Sources of information (recent or historic) and availability of contextual information.	All previous surveys relevant to the planning of the survey were available and consulted. Historic, comparative genetic data were not available.



4 RESULTS

4.1 Previous survey and database search results

Reports from subterranean fauna surveys within the vicinity of the Study Area were reviewed for local and regional context. None of the surveys sampled bores or drill holes from within the Study Area. Five of the seven previous surveys were conducted at Telfer Gold Mine, approximately 45 km west of the Study Area, including nearby reference sites. One subterranean fauna survey was conducted at O'Callaghans Deposit just west of Telfer Gold Mine and another at Kintyre Uranium Deposit 83 km southwest of the Study Area.

The earliest subterranean fauna survey was conducted in 2001, sampling 17 water bores within Telfer Borefield and nearby reference sites (Biota, 2001). The six bores yielding stygofauna were located within Proterozoic sediments and tertiary calcrete. In total, eleven stygofauna taxa from six higher level taxonomic groups were recorded, comprising Amphipoda, Copepoda, Gastropoda, Nematoda, Ostracoda, and Polychaeta (this polychaete was possibly the first stygal member of this group recorded in Australia at the time). It should be noted that no lower-level taxonomy was conducted on any of the specimens found in this survey, with only Amphipoda being split into six distinct taxa using electrophoresis. The number of stygofauna taxa may therefore be higher, but this cannot be confirmed.

In 2004, sixteen bores within and nearby the Telfer Gold Mine corridor were sampled for the Subterranean Fauna Monitoring Program (Natural Resource Services, 2004) as outlined in the Telfer Gold Mine – Subterranean Fauna Management Plan (Newcrest, 2004). Stygofauna were recovered from four bores, three of which were reference sites 40 km to the west and 20 km to the east of the central mine corridor. In total, eight taxa (36 individuals) were obtained, comprising four copepod species, three amphipod species, and one oligochaete worm species. Each of the bores in which stygofauna were found intercepted different aquifer geologies, including Wilkie Quartzite, deep weathered siltstones, tertiary calcrete, and Permian Tillite, respectively.

In 2010, Newcrest Mining Ltd. was assessing the feasibility of developing a below-ground mine at O'Callaghans Deposit, situated west of Telfer Gold Mine. As part of this assessment, preliminary subterranean fauna sampling of 20 drill holes was undertaken at O'Callaghans and nearby reference sites (Bennelongia, 2010a). Three bores yielded stygal amphipods belonging to the Paramelitidae family which could not be identified further and may therefore represent more than one species. Only one troglobitic specimen was found, the symphylan *Scutigerella* `sp. B1`. The study concluded that these findings confirm that the geology at O'Callaghans (located in the Punta Punta Formation, consisting of dolomitic limestone) was prospective for troglofauna and stygofauna, but that more sampling would be needed to meet EPA guidelines.

In 2014, Newcrest Mining Ltd. released a review of the 5-year stygofauna monitoring (2009 – 2013) and the four-year troglofauna monitoring (2010 – 2013) conducted at Telfer Gold Mine and surrounds (Newcrest, 2014). Throughout these surveys, a total of 51 bores were sampled (most of them several times). The troglofauna monitoring surveys (Bennelongia, 2014b) revealed nine taxa (25 specimens total) belonging to seven higher level taxonomic groups, including Araneae, Isopoda, Pauropoda, Symphyla,



Diplura, Zygentoma, and Hemiptera. Such low specimen numbers from a total of 165 troglofauna samples across four years suggest that the Telfer area is characterised by a depauperate troglofauna community. The stygofauna monitoring surveys (Bennelongia, 2010b, 2011a, 2012b, 2013a, 2013b), on the other hand, revealed rich stygofauna assemblages, detecting 23 distinct taxa (possibly more due to a number of indeterminate taxa) from ten higher level taxonomic groups, comprising Acari, Amphipoda, Copepoda, Gastropoda, Isopoda, Nematoda, Oligochaeta, Ostracoda, Polychaeta, Rotifera, and Syncarida.

Subterranean fauna survey conducted at Kintyre Uranium Deposit (Bennelongia, 2012c), located 83 km south-west of the Study Area was the most distant assessment reviewed. This study sampled a total of 112 bores, collecting 190 samples across three sampling rounds. A total of 23 troglofauna species from twelve higher level taxonomic groups (Araneae, Blattodea, Diplura, Hemiptera, Isopoda, Palpigradi, Pauropoda, Polyxenida, Pseudoscorpiones, Scolopendromorpha, Symphyla, Zygentoma) were found, revealing a moderately rich troglofauna community for the Pilbara region, owing to the presence of good prospective troglofauna habitat such as tertiary detrital sediments, calcrete, and sheared Proterozoic rocks. The surveys also revealed fifteen stygofauna taxa from seven higher level taxonomic groups (Amphipoda, Copepoda, Isopoda, Nematoda, Oligochaeta, Rotifera, and Syncarida), representing a relatively sparse stygofauna community for the Pilbara region.

The database searches (WAM, NatureMap, and PSS) yielded no additional species that had not already been recorded from the survey review. In total, 61 stygofauna and potential stygofauna taxa were recorded within the greater regional area (Table 4.1). Twelve stygofauna taxa were recorded from the Kintyre region and 51 stygofauna taxa were recorded from the Telfer region (Table 4.1). Thirty-one troglofauna and potential troglofauna taxa were recorded from the greater regional area (Table 4.1). Twenty-two troglofauna taxa were recorded from the Kintyre region and nine troglofauna taxa were recorded from the Telfer region (Table 4.1).

Based on current knowledge, none of the stygofauna or troglofauna taxa recorded from the vicinity of the Study Area appear on any threatened species lists, however many of these taxa are potential short-range endemics (Table 4.1).



Table 4.1: Subterranean fauna morphospecies recorded in the databases within 65 km of the Study Area (search parameters as per Table 3.1). Note: based on current taxonomic information indeterminate taxa that appeared highly unlikely to represent a unique species within the search area are not included in this table.

Higher Taxonomy	Lowest Identification	Locale in Source Ta		Taxonomic Resolution	Distribution	Subterranean Status	SRE Status	
Stygofauna								
Nematoda	Nematoda sp. 12 (PSS)	Telfer	PSS	No taxonomic framework	Unknown	Unknown	Unknown	
	Nematoda spp.	Telfer, Kintyre	Cameco, Newcrest	No taxonomic framework	Unknown	Unknown	Unknown	
Rotifera								
Bdelloidea	Bdelloidea sp. 3:2	Telfer	Newcrest	No taxonomic framework	Unknown	Unknown	Unknown	
Flosculariacea	Filinia sp.	Kintyre	Cameco	No taxonomic framework	Unknown	Unknown	Unknown	
Mollusca	Hydrobiidae sp. B02	Telfer	Newcrest	Undescribed new species	One aquifer	Stygobite	Potential	
Annelida								
Polychaeta	Namanereis pilbarensis	Telfer	Newcrest	Described but lacks DNA, includes sp. B01	Pilbara wide	Stygobite	Potential	
Aphanoneura								
Aeolosomatidae	Aeolosoma sp. 1 (PSS)	Telfer	Newcrest	Complex	Unknown	Unknown	Unknown	
Clitellata								
Oligochaeta								
Enchytraeidae	Enchytraeus sp. PST1/PSS1	Telfer	Newcrest	Complex	Aquifer to catchment scale	Amphibious	Potential	
Phreodrilidae	Insulodrilus sp.	Telfer	Newcrest	Indeterminate	Aquifer to catchment scale	Unknown	Unknown	
	Phreodrilidae dissimilar ventral chaetae.	Telfer	Newcrest	Complex	Aquifer to catchment scale	Stygobite	Potential	
	Phreodrilidae similar ventral chaetae	Telfer	Newcrest	Complex	Aquifer to catchment scale	Stygobite	Potential	
Naididae (ex Tubificidae)	Tubificidae stygo type 1 (imm Ainudrilus ?WA25/26) (PSS)	Telfer	Newcrest, PSS	Likely new species, found in surface waters	Pilbara wide	Stygoxene	No	
	Tubificidae `stygo type 5` Kintyre		Cameco	Likely new species, found in surface waters	Pilbara wide	Stygoxene	No	
Arthropoda								
Crustacea								
Amphipoda								
Bogidiellidae	Bogidiella sp. B02	Telfer, Kintyre	Cameco, Newcrest	Likely new species	Unknown	Stygobite	Potential	
Melitidae	Melitidae sp.	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential	
Paramelitidae	Paramelitidae sp. B06	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential	
	Paramelitidae sp. B07	Kintyre	Cameco	Likely new species	Aquifer to catchment scale	Stygobite	Potential	
	Paramelitidae sp. B10	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential	
	Paramelitidae sp. B11	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential	
	Paramelitidae sp. B28	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential	





Higher Taxonomy	Lowest Identification	Locale in Search Area	Source	Taxonomic Resolution	Distribution	Subterranean Status	SRE Status
	Paramelitidae sp. B30	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	Pilbarus sp.	Telfer	Newcrest, PSS	Likely new species	Aquifer to catchment scale	Stygobite	Potential
Isopoda							
Microcerberidae	Microcerberidae sp.	Telfer	Newcrest, PSS	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	Microcerberidae sp. B04	Kintyre	Cameco	Likely new species	Aquifer to catchment scale	Stygobite	Potential
Olibrinidae	Adoniscus sp. B01	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential
Syncarida							
Parabathynellidae	Atopobathynella sp. B08	Telfer	Newcrest, WAM	Likely new species, ex Hexabathynella `A`	Aquifer to catchment scale	Stygobite	Potential
	Notobathynella sp. B06	Kintyre	Cameco	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	nr Billibathynella (Brevismobathynella) sp. B08	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential
Copepoda							
Cyclopoida							
Cyclopidae	Bryocyclops sp. 1 (PSS)	Telfer	PSS	Likely new species	Unknown	Stygo-phile/bite	Unknown
	Diacyclops cockingi	Telfer	Newcrest, PSS	Described but lacks DNA	Pilbara wide	Stygophile	Unknown
	Diacyclops einslei	Telfer	Newcrest	Described but lacks DNA	Pilbara wide	Stygophile	Unknown
	Diacyclops humphreysi	Telfer	Newcrest	Described but limited DNA suggests a complex	Pilbara wide	Stygophile	Unknown
	Diacyclops scanloni	Telfer	Newcrest	Described but lacks DNA	Pilbara wide	Stygophile	Unlikely
	Fierscyclops (Fierscyclops) fiersi	Telfer	Newcrest	Described but lacks DNA	Pilbara wide	Stygophile	Unlikely
	Halicyclops kieferi	Telfer	Newcrest, PSS	Described but lacks DNA	Pilbara wide	Stygophile	Unlikely
	Mesocyclops sp.	Telfer	Newcrest	Indeterminate	Unknown	Stygo-phile/bite	Unlikely
	Metacyclops sp.	Telfer	PSS	Indeterminate	Unknown	Stygo-phile/bite	Unlikely
	Microcyclops varicans	Telfer	Newcrest	Described but lacks DNA	Pilbara wide	Stygophile	Unlikely
	Orbuscyclops westaustraliensis	Kintyre	Cameco	Described but lacks DNA	Pilbara wide	Stygophile	Unlikely
	Pilbaracyclops frustratio	Telfer	Newcrest, PSS, WAM	Described but lacks DNA	Pilbara wide	Stygophile	Unlikely
Harpacticoida							
Ameiridae	Abnitocrella sp. 1 (TOK)	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	Abnitocrella sp. B02 (nr obesa)	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	Megastygonitocrella sp. B03 (nr ecowisei)	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	Megastygonitocrella trispinosa	Telfer	Newcrest	Described but lacks DNA	Pilbara wide	Stygophile	Potential
	Nitocrella knotti	Telfer	Newcrest	Described but lacks DNA	Aquifer to catchment scale	Stygobite	Potential
	Nitocrella sp. B04 (nr obesa)	Kintyre	Cameco	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	Nitocrella sp. B05	Kintyre	Cameco	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	nr Gordonitocrella sp. B01	Telfer	Newcrest	Likely new species	Aquifer to catchment scale	Stygobite	Potential
Parastenocarididae	Parastenocaris sp.	Telfer	Newcrest, WAM	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	Parastenocaris sp. B07	Kintyre	Cameco	Likely new species	Aguifer to catchment scale	Stygobite	Potential



Higher Taxonomy	Lowest Identification	Locale in Search Area	Source	Taxonomic Resolution	Distribution	Subterranean Status	SRE Status
	Parastenocaris sp. B20	Kintyre	Cameco	Likely new species	Aquifer to catchment scale	Stygobite	Potential
Ostracoda							
Candonidae	Areacandona `4` (PSS)	Telfer	PSS	Likely new species	Aquifer to catchment scale	Stygobite	Potential
	Areacandona arteria	Telfer	PSS	Described but lacks DNA	Pilbara wide	Stygophile	Potential
	Areacandona cf. iuno	Telfer	PSS	Described but lacks DNA	Aquifer to catchment scale	Stygo-phile/bite	Potential
	Leicacandona desserti	Telfer	Newcrest	Described but lacks DNA	One aquifer	Stygobite	Potential
	Leicacandona jula	Telfer	Newcrest, Karanovic & McKay 2010	Described but lacks DNA	One aquifer	Stygobite	Potential
	Leicacandona pinkajartinyi	Telfer	Newcrest, Karanovic & McKay 2010	Described but lacks DNA	One aquifer	Stygobite	Potential
	Leicacandona quasilite	Telfer	Newcrest	Described but lacks DNA	One aquifer	Stygobite	Potential
	Leicacandona yandagoogeae	Telfer	PSS	Described but lacks DNA	One aquifer	Stygobite	Potential
Cyprididae	Cypretta seurati	Telfer	Newcrest	Described, found in surface waters	Pilbara wide	Stygoxene	No
Limnocytheridae	Gomphodella `BOS354`	Telfer	Newcrest	Likely new species	One aquifer	Stygo-phile/bite	Potential
Troglofauna							
Arthropoda							
Arachnida							
Araneae							
Oonopidae	Prethopalpus sp. B20	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Prethopalpus sp. B28 (nr kintyre)	Telfer	Newcrest	Undescribed new species	One deposit	Troglobite	Potential
Palpigradi	Palpigradi sp. B01	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
Pseudoscorpiones	Lechytia sp. B03	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
Crustacea							
Isopoda							
Armadillidae	Armadillidae sp. B10	Telfer	Newcrest	Undescribed new species	One deposit	Troglobite	Potential
	Troglarmadillo sp. B19	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Troglarmadillo sp. B33	Telfer	Newcrest	Undescribed new species	One deposit	Troglobite	Potential
Oniscidae	Hanoniscus sp. B05	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
Hexapoda							
Diplura	Japygidae sp.	Kintyre	Cameco	Likely new species	One deposit	Troglobite	Potential
	Parajapygidae sp. B13	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Projapygidae sp. B03	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Projapygidae sp. B07	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Projapygidae sp. B15	Telfer	Newcrest	Undescribed new species	One deposit	Troglobite	Potential
Insecta				·			
Blattodea	Nocticola sp.	Kintyre	Cameco	Likely new species	One deposit	Troglobite	Potential
Hemiptera					·		
Enicocephalidae	Systelloderes sp.	Kintyre	Cameco	Undescribed new species	Unknown	Troglo-phile/bite	Unknown





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Higher Taxonomy	Lowest Identification	Locale in Search Area	Source	Taxonomic Resolution	Distribution	Subterranean Status	SRE Status
Meenoplidae	Meenoplidae sp.	Telfer	Newcrest	Likely new species	Pilbara wide	Troglo-phile/bite	Potential
Zygentoma							
Nicoletiidae	Atelurinae sp. B16	Telfer	Newcrest	Undescribed new species	One deposit	Troglobite	Potential
	Trinemura sp. B07	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Trinemura sp. B12	Telfer	Newcrest	Undescribed new species	One deposit	Troglobite	Potential
Myriapoda							
Chilopoda							
Cryptopidae	Cryptops sp. B19	Kintyre	Cameco	Likely new species	One deposit	Troglobite	Potential
	nr Cryptops sp. B12	Kintyre	Cameco	Likely new species	One deposit	Troglobite	Potential
	nr Cryptops sp. B13	Kintyre	Cameco	Likely new species	One deposit	Troglobite	Potential
Diplopoda							
Polyxenida	Lophoturus madecassus	Kintyre	Cameco	Complex, ex Lophoproctidae sp. B01	Pilbara wide	Troglo-phile/bite	Potential
Pauropoda	Pauropodidae sp. B21	Telfer	Newcrest	Undescribed new species	One deposit	Troglobite	Potential
	Pauropodidae sp. B24	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Pauropodidae sp. B25	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Pauropodidae sp. B26	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
Symphyla							
Scutigerellidae	Scutigerella sp. B01	Telfer	Newcrest	Undescribed new species	One deposit	Troglobite	Potential
	Scutigerella sp. B02	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Symphyella sp. B06	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential
	Symphyella sp. B08	Kintyre	Cameco	Undescribed new species	One deposit	Troglobite	Potential



4.2 Current survey results

The current survey recorded a total of 573 subterranean fauna specimens, comprising approximately 27% stygofauna (154 specimens), 69% amphibious (398 specimens) and 4% troglofauna (24 specimens). The samples collected from the Havieron Project Area included 15 stygofauna and 167 amphibious animals. No troglofauna were recorded from the Project Area. The records were collected from 74 bores and holes throughout the Study Area, of which 33 are within the Project.

4.2.1 Amphibious Animals

A total of 395 amphibious animals were collected during the current survey, all of which belong to the Oligochaeta family Enchytraeidae. Of these, 386 were identified as one of five distinct morphospecies supported by molecular analysis (Appendix C). The remaining nine specimens were either immature or in too poor condition for proper identification. Enchytraeids collected in this survey were classed as amphibious because they were collected in samples from both above and below the water table, i.e. troglofauna traps and either hauls or haul-scrapes.

Two morphospecies, Enchytraeidae `sp. Biologic-OLIG026` and Enchytraeidae `sp. 12`, were only collected from regional reference sites. The collections of Enchytraeidae `sp. E12` (see Brown *et al.*, 2015) represent the easternmost records for this morphospecies and it now has a known distribution throughout the central and eastern Pilbara and into the Great Sandy Desert. One other morphospecies, Enchytraeidae `sp. Biologic-OLIG024` was recorded from the Project Area and at regional reference sites at Telfer. Two morphospecies, Enchytraeidae `sp. Biologic-OLIG023` and Enchytraeidae `sp. Biologic-OLIG025`, were only collected from within the Project Area, however neither of these were from sites with significant projected drawdown (Figure 4.1).

4.2.2 Stygofauna

A total of 154 stygofauna specimens were collected during the current survey belonging to nine taxonomic groups: nematodes, tubificid and naidid oligochaetes, ostracods, harpacticoid and cyclopoid copepods, syncarids, amphipods, and isopods (Table 4.1)

Within the Project Area only two morphospecies was recorded: an amphipod, Paramelitidae `sp. Biologic-AMPH027`, and an ostracod, Humphreyscandonini sp. indet. (Table 4.1, Figure 4.1). Paramelitidae `sp. Biologic-AMPH027` was represented by 11 specimens collected from a single water bore outside of drawdown impacts (Figure 4.1). Humphreyscandonini sp. indet. was represented by two valves (shells) collected in a single sample from within projected drawdown impacts. As only valves were collected and not the entire organism, this specimen could only be identified to tribe. Live, mature animals and extremely intricate dissections of genitalia are needed for accurate identification of ostracods to species level.

See Section 6 for an assessment of potential impacts and risks to subterranean fauna.



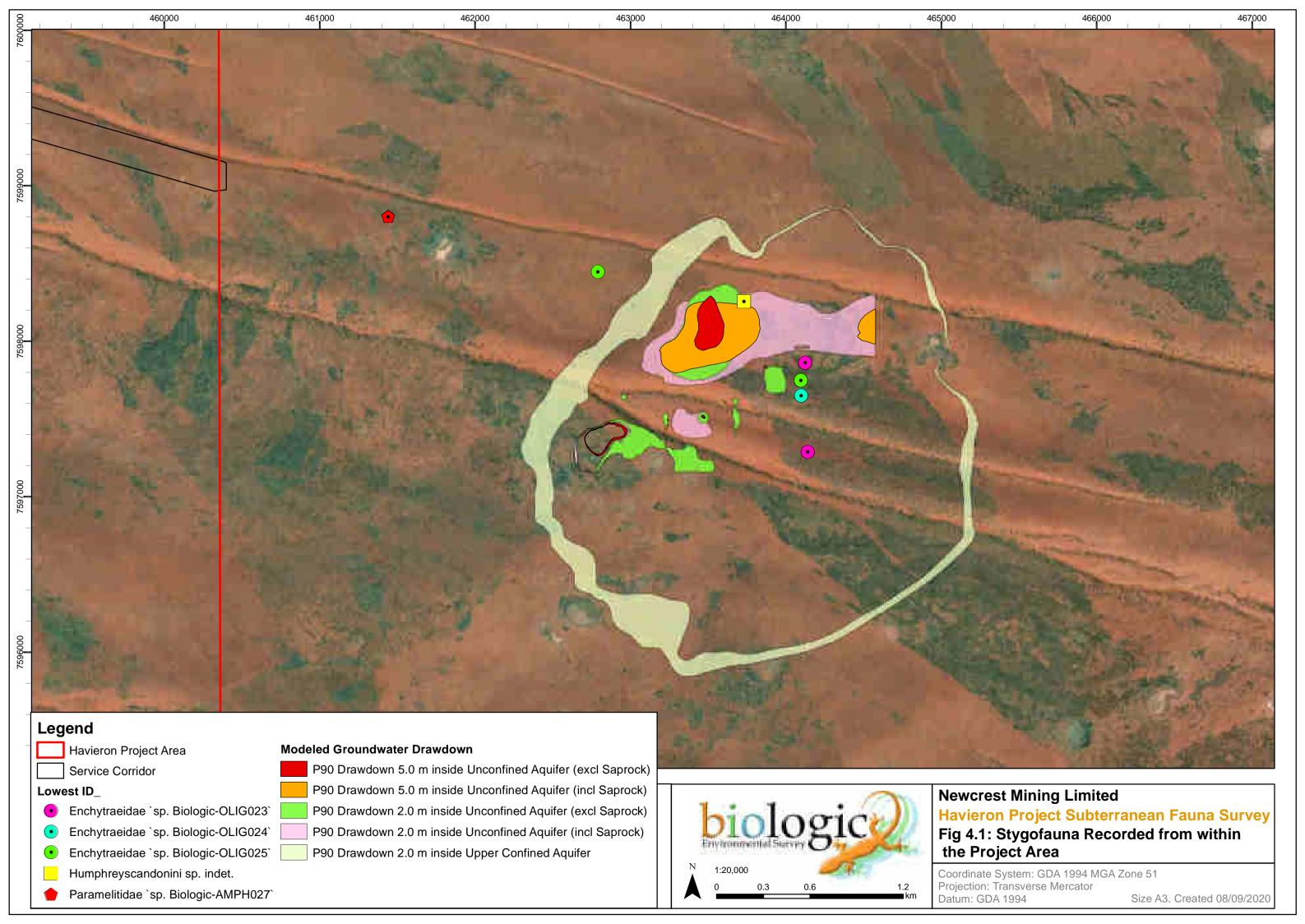
Table 4.2:Subterranean results to date, taxonomic and distribution comments, known linear ranges and collection locations

Taxonomy	Havieron (impact)	Havieron (outside impact)	Regional (reference)	Total	Taxonomic resolution	Subterranean status	SRE status	Distribution comments, Known linear range (km)
Stygofauna								
NEMATODA								
Nematoda sp. indet.			1	1	No taxonomic framework; not assessed in EIAs	Unknown	Unknown	Singleton
OLIGOCHAETA								
Naididae								
Naididae sp. indet.			4	4	Indeterminate (family-level)	Stygoxene	Unlikely	Single site
Indeterminate								
Oligochaeta sp. indet.			1	1	Indeterminate (subclass-level)	Unknown	Unknown	Singleton
OSTRACODA								
Candonidae								
Humphreyscandonini sp. indet.	2			2	Indeterminate (tribe-level)	Stygobite	Potential	Single site
ndeterminate								
Ostacoda sp. indet.			17	17	Indeterminate (class-level)	Unknown	Unknown	Known from 5 sites
CYCLOPOIDA								
Cyclopidae								
Fierscyclops (Pilbaracyclops) ?frustatio			1	1	Indeterminate (genus-level)	Stygophile	Unlikely	Singleton
ndeterminate		,	,		,	,	,	
Cyclopoida sp. indet*			61	61	Indeterminate (order-level)	Unknown	Unknown	Known from 3 sites
HARPACTICOIDA							·	
Harpacticoida sp. indet.			8	8	Indeterminate (order-level)	Unknown	Unknown	Known from 4 sites
SYNCARIDA		,				1		
Parabathynellidae								
Parabathynellidae sp. indet.			4	4	Indeterminate (family-level)	Stygobite	Potential	Single site
AMPHIPODA		,				,		
Paramelitidae								
Paramelitidae `sp. Biologic-AMPH025`			10	10	Genetically identified (unique lineage)	Stygobite	Potential	Locally restricted (<0.01 km)
Paramelitidae `sp. Biologic-AMPH026`			1	1	Genetically identified (unique lineage)	Stygobite	Potential	Singleton
Paramelitidae `sp. Biologic-AMPH027`		11		11	Genetically identified (unique lineage)	Stygobite	Potential	Single site
Paramelitidae `sp. Biologic-AMPH028`			4	4	Genetically identified (unique lineage)	Stygobite	Potential	Single site
Paramelitidae `sp. Biologic-AMPH029`			15	15	Genetically identified (unique lineage)	Stygobite	Potential	Locally restricted (0.4 km)
Paramelitidae `sp. Biologic-AMPH030`		<u> </u>	3	3	Genetically identified (unique lineage)	Stygobite	Potential	Single site
Paramelitidae `sp. Biologic-AMPH031`			1	1	Genetically identified (unique lineage)	Stygobite	Potential	Singleton
Paramelitidae sp. indet.			6	6	Indeterminate (family-level)	Stygobite	Potential	Known from 4 sites





					ISOPODA			
				M	icrocerberidae			
Microcerberidae sp. indet.			4	4		Stygobite	Potential	Known from 2 sites
TOTAL	2	11	141	154				
Amphibious								
OLIGOCHAETA								
Enchytraeidae								
Enchytraeidae `sp. Biologic-OLIG023`		142		142	Genetically identified (unique lineage)	Amphibious	Potential	Locally restricted (0.58 km)
Enchytraeidae `sp. Biologic-OLIG024`		18	60	78	Genetically identified (unique lineage)	Amphibious	No	Regionally widespread (77.2 km)
Enchytraeidae `sp. Biologic-OLIG025`		7		7	Genetically identified (unique lineage)	Amphibious	Potential	Locally restricted (1.48 km)
Enchytraeidae `sp. Biologic-OLIG026`			10	10	Genetically identified (unique lineage)	Amphibious	Potential	Single site
Enchytraeidae `sp. E12`			149	149	Genetically identified (previously detected lineage)	Amphibious	No	Regionally widespread (100+ km ¹)
Enchytraeidae sp. indet.			9	9	Indeterminate (family-level)	Amphibious	Potential	Single site
TOTAL	0	167	228	395				
Troglofauna								
ARACHNIDA								
Palpigradi								
Palpigradi sp. indet.			1	1	Indeterminate (order-level)	Troglobite	Potential	Singleton
MYRIAPODA								
Polyxenida								
Polyxenida sp. indet.			8	8	Indeterminate (order-level)	Troglophile / Troglobite	Potential	Known from 2 sites
Symphyla								
Symphyla sp. indet.			1	1	Indeterminate (class-level)	Troglobite	Potential	Singleton
INSECTA								
Zygentoma								
Atelurinae sp. indet.			12	12	Indeterminate (family-level)	Troglobite	Potential	Single site
ISOPODA								
Armadillidae								
Armadillidae sp. indet.			2	2	Indeterminate (family-level)	Troglobite	Potential	Known from 2 sites
TOTAL	0	0	24	24				





5 SUBTERRANEAN HABITAT ASSESSMENT

The habitat assessment for potentially restricted species within the Study Area is based upon available geological and hydrogeological reports, surface geology maps (GSWA, 2020) and three-dimensional geological mapping based on drill-hole logging data in the program Leapfrog® (provided by Rockwater). Groundwater physicochemical measurements taken during the survey were incorporated into the stygofauna habitat assessment where appropriate.

Subterranean fauna habitats are predicated on the occurrence and interconnectedness of subterranean voids, cavities, cracks, porosity, aperture spaces, and caverns, both below and above the water table. The occurrence and distribution of subterranean fauna is influenced or limited by the geology in which they occur. Small and fragmented species ranges, leading to high levels of endemism (EPA, 2003), result from such dispersal limitations. Thus, it is important to identify the type and extent of habitats that are likely to host subterranean fauna.

5.1 Troglofauna habitats (AWT)

Potential AWT habitats for troglofauna (*i.e.* caves, cavities, fractures, vugs, and pore spaces) appear to be very low to absent within the Project. The Study Area lies within the Canning Basin, which was under water during the Jurassic (199.6 – 145.5 Ma) and experienced marine sedimentation (Kellner *et al.*, 2010; Turner *et al.*, 2009). At the Project, the upper 15 m comprises unconsolidated, superficial sediments, which are made of aeolian sand, alluvium and evaporates. The water table lies between 6 and 14 mbgl (metres below ground level).

Troglofauna rely on the presence and continuity of places to live (caves, cavities, fractures, vugs, and pore spaces), high humidity (saturated) and vertical connectivity to supply nutrients and oxygen. The superficial sediments may provide a lot of small pore spaces with high humidity and moderate vertical connectivity; however, they are unconsolidated and highly unlikely to provide continuity across the landscape. The landscape is very flat comprising extensive dunes and together with a shallow water table, may reduce the chances of species persisting during historically wetter times when the water table is elevated.

5.2 Stygofauna habitats

Stygofauna appear in a variety of aquifers, springs, and hyporheos across the world and their existence relies on several current and historical factors. Their persistence depends on vertical connectivity to allow ingress of carbon and nitrogen (Saccò *et al.*, 2019) and lateral connectivity to enable movement. Stygofauna comprise a very diverse range of groups, life histories and adaptations, and although they have been recorded in an expansive range of physiochemical parameters the tolerances are understudied. The first efforts to investigate tolerances are relatively recent and looked at sensitivity of hypogean copepods to agricultural pollutants (Di Lorenzo *et al.*, 2014) and toxicity of arsenic, zinc and chromium to groundwater copepods (Hose *et al.*, 2016). Stygofauna sampling throughout WA has recorded animals from salinities as high as 100,000 mg/L TDS (Outback Ecology, 2012). Stygofauna are known to occur more than 2 km below the surface (Sendra & Reboleira, 2012), although such cave



systems are not common in Australia. These very deep communities were found to be generally similar to shallow upper communities, with local ecological and structural factors explaining their distributions (Trontelj et al., 2019).

At the Project, the upper unconfined aquifer appears to represent moderately prospective stygofauna habitat, whereas the two confined aquifers by their nature are unlikely to be prospective, except where they outcrop (away from the Project) and are no longer covered by impermeable layers. The unconsolidated nature of the upper unconfined aquifer is likely to provide reasonable vertical and lateral connectivity and although pore spaces are small, there may be localised patches of calcrete or shallow alluvium that acts as refuges to small communities. The only records of true stygofauna (stygobites) from the Project come from three bores/holes that targeted alluvium/calcrete.

5.3 Groundwater characteristics

Salinity at the Project ranged from fresh to saline (492 to 37,146 mg/L TDS), compared to fresh to brackish around Telfer (120 to 2,874 mg/L TDS). The water at the Project was circum-neutral, ranging from 6.57 to 7.72, compared with a greater range at Telfer (5.94 to 8.77). Similar ORP (redox potential) and oxygen saturation values were recorded at Telfer and the Project, with ORP ranging from -312 to 222 mV and oxygen saturation from 1.7% to 82.4%. Groundwater temperatures ranged from 24.9 to 33.9 degrees. Dissolved oxygen levels varied from 0.13 to 59 ppm. These levels are well within the range suitable for stygofauna.

Redox and DO measurements are typically variable between sites due to individual bore conditions rather than overall aquifer conditions. All bores contained groundwater with sufficient dissolved oxygen for stygofauna to occur (>1 ppm). The redox potential of groundwater is a measure of the system's capacity to oxidise materials through chemical reactions and has important implications for metal mobility, bioavailability and toxicity (Schuring *et al.*, 1999). Stygofauna were only recorded in holes with ORP values greater than -133.7 mV, below salinities of 10,176 mg/L and below dissolved oxygen of 6.7 ppm.



6 RISK ASSESSMENT

Impacts on subterranean fauna may be direct or indirect. Direct impacts cause direct habitat loss and include the removal of habitat, groundwater drawdown, inundation, and water quality changes. Indirect impacts reduce the quality of subterranean habitat and include changes to hydrology, siltation, void collapse, alteration to nutrient balance and contamination (EPA, 2016a). Direct impacts can lead to the extinction of SRE subterranean species, whilst indirect impacts may possibly reduce the population size.

The Project comprises excavation of an underground decline and associated dewatering, both of which may potentially have direct impacts to stygofauna and amphibious fauna, whilst the decline may potentially have a direct impact to troglofauna. The Project includes a waste rock landform that may potentially have an indirect impact on subterranean fauna, through siltation, contamination, or changes to hydrology or nutrient balance.

6.1 Impacts to troglofauna

The decline may potentially have a direct impact to troglofauna, whilst the waste rock landform may potentially have an indirect impact through siltation, contamination, or changes to hydrology or nutrient balance.

6.2 Risks to troglofauna species

Of the 24 troglofauna specimens collected during the current survey, none were recorded from within the Project Area. Most sample sites met EPA guidelines (see Constraints, Section 3.5) and were distributed throughout the Project providing sufficient characterisation of the geologies present. The habitat assessment concluded limited troglofauna habitat is likely to be present at the Project owing to the combination of three factors - low relief, a relatively shallow water table in a geology that lacks structure, continuity and large spaces, and historic marine sedimentation. This habitat assessment is supported by the current survey finding no troglofauna at the Project, when compared with troglofauna records from nearby areas with elevated land features (Telfer, current and historic surveys; Kintyre, historic surveys). The Project appears to be unsuitable for troglofauna species and habitat, though a greatly depauperate community may be present.

Considering the small area of direct and indirect impacts, the lack of troglofauna collected, and lack of suitable troglofauna habitat, the Project is unlikely to pose any risks to troglofauna species.

6.3 Impacts to stygofauna and amphibious species

The Project comprises excavation of an underground decline and associated dewatering, both of which may potentially have direct impacts to stygofauna and amphibious fauna. The waste rock landform may potentially have an indirect impact through siltation, contamination, or changes to hydrology or nutrient balance.

6.4 Risks to stygofauna and amphibious species

It should be noted that some sampling limitations and hole characteristics (See Section 3.5) may have impeded the detection of stygofauna and amphibious fauna within impact areas.



The only records of true stygofauna (stygobites) from the Project came from holes that targeted alluvials and calcareous cement, whereas the large number of amphibious worms collected at the Project and Telfer were collected from various geologies and aquifers. The richness of stygofauna and amphibious species recorded at the Project during the current survey is very low compared to the nearest results at Telfer and Kintyre.

The low richness of stygofauna and amphibious species during the current survey provide strong support that the aquifers in the Project are not prospective for stygofauna, although there appears to be small, localised patches of higher prospect stygofauna habitat present in the Study Area, which are represented by alluvials and calcareous cement, which seem to be associated with claypans in the area. The Claypans may present a unique environment due to the salinity, interactions with significant rainfall events and clayey materials. The Claypan habitat appears associated with the perched aquifer and represents a smaller groundwater system associated with rainfall events. Some interactions with the Upper Confined Aquifer may occur. The amphibious species, on the other hand, appear to be occurring consistently throughout the Study Area, irrespective of geology or aquifer, although likely close to the water table.

Under current groundwater drawdown modelling scenarios, only one taxon appears to be at risk of direct drawdown impact: the ostracod Humphreyscandonini sp. indet. The distributions of stygobitic ostracods in Western Australia range from widespread to highly range-restricted (Reeves *et al.*, 2007). While the identification of Humphreyscandonini sp. indet. is tentative, the family Candonidae is known to contain Confirmed SREs as well as widespread species. This taxon was found from a borehole targeting the patches of alluvials and calcareous cements. While this borehole did extend through both the Upper Unconfined and Upper Confined Aquifers, bore logs strongly suggest the specimen comes from the upper unconfined aquifer, where the geology is highly porous and not under pressure. The Upper Unconfined Aquifer is extensive throughout the Project Area and beyond. Additionally, nearby bore logs indicate approximately 16.5 m of saturated saprolite and saprock at this location. The saprock is modelled to experience 2 - 5 m of drawdown, which will reduce the overall saturated thickness to approximately 11.5 – 14.5 m. This level of impact is likely to be lessened by annual recharge, as the hole is located very close to a claypan, which is a low point in the landscape and likely a greater area of recharge.

Based on current taxonomic and ecological information, modelling of groundwater drawdown and the likely extent of suitable habitats for stygofauna fauna beyond these impacts, Humphreyscandonini sp. indet. was assessed as 'low risk' (Table 6.1). All stygofauna/ amphibious fauna risk levels are contingent upon the extent of groundwater drawdown as modelled. Any new information or modelling that changes the spatial extent or magnitude of drawdown, the duration of drawdown, or the duration of subsequent recovery of aquifer habitats following the end of project may result in changes to the potential risks to stygofauna and amphibious taxa.



Table 6.1: Stygofauna risk assessment based on current taxonomic and habitat factors, and distribution relative to impacts

Potentially restricted taxon	Taxonomic factors	Distribution factors	bution factors Habitat factors Risk level			
Ostracoda						
Humphreyscandonini sp. indet.	Potential Stygobite. Represented by two valves (dead shells). Indeterminate tribelevel taxon identification (Ivana Karanovic), likely unique species.	Known from a single site within modelled groundwater drawdown. Family includes stygophiles with catchment distributions and stygobites only known from single aquifers. Potential SRE (C- Morphology Indicators, E- Research and Expertise). Family includes both widespread and restricted species.	Modelled drawdown at location of record from 2 – 5 m within the Upper Unconfined Aquifer. Modelled drawdown within the Upper Confined Aquifer is 2 m. Habitat (alluvials and calcareous concrete) occurs patchily within the Quaternary cover that forms part of the extensive Upper Unconfined Aquifer. Some interactions between the Upper Unconfined and Upper Confined Aquifers may occur.	LOW	LOW Due to uncertainty of identification, extent of habitat, limited impact (2 – 5 m) and record from a single site.	



7 KEY FINDINGS

The key findings are based on the sampling results of the current survey, available habitat information and current knowledge of the impacts to subterranean fauna:

- There are currently no troglofauna taxa known from the Project Area.
- Geology within the Project Area does not appear to be suitable for troglofauna.
- One stygofauna taxon, Humphreyscandonini sp. indet., is known only from within modelled groundwater drawdown of 2 – 5 m.
- Based on current taxonomic and ecological information, modelling of groundwater drawdown and the likely extent of suitable habitats for stygofauna fauna beyond impacts, the risk to Humphreyscandonini sp. indet. is considered Low.
- Impact to the available stygofauna habitat of the Upper Confined Aquifer, which extends throughout and beyond the Project Area, is likely to be minimal.
- The overall impact to the wider extent of subterranean fauna habitat, and any assemblages occurring therein, is considered negligible.



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9 APPENDICES

Appendix A: Drill and bore holes sampled during the current survey

Hole Name	Latitude	Longitude	Drill Date	Broad Locality	Fine Locality	Site Type
411-BO-117	-21.66467	122.13341	27/05/05	Telfer	Passmore	Referenc
BTB5	-21.8862473	122.3759652	Unknown	Telfer	Big Tree	Referenc
BTC206001	-21.8896513	122.3851531	Unknown	Telfer	Big Tree	Referenc
HAC0301	-21.72578424	122.6557641	10/05/03	Havieron		Impact
HAC9101	-21.72433568	122.6525514	26/08/91	Havieron		Impact
HAC9502	-21.72235922	122.6531041	01/10/95	Havieron		Impact
HAD001	-21.72428227	122.6528487	22/06/19	Havieron		Impact
				·		
HAD002	-21.72343188	122.6511956	22/06/19	Havieron		Impact
HAD003	-21.72388803	122.6521318	22/06/19	Havieron		Impact
HAD004	-21.72339361	122.6528467	22/06/19	Havieron		Impact
HAD005	-21.7242942	122.6509157	22/06/19	Havieron		Impact
HAD006	-21.72470424	122.6528248	22/06/19	Havieron		Impact
HAD008	-21.72472012	122.6533363	22/06/19	Havieron		Impact
HAD009A	-21.72520844	122.6562334	22/06/19	Havieron		Impact
				···•		
HAD010A	-21.72471102	122.6513225	09/06/19	Havieron		Impact
HAD027	-21.72288022	122.6490118	17/09/19	Havieron		Impact
HAD030	-21.72634941	122.6464783	25/09/19	Havieron		Impact
HAE001	-21.72755741	122.6532572	22/03/20	Havieron		Impact
HAE002	-21.68421483	122.5472892	26/03/20	Havieron		Referenc
HAE003	-21.69295239	122.5714711	25/03/20	Havieron		Reference
HAE004	-21.71705945	122.6402291	24/03/20	Havieron		Reference
	b	······································		}		.\$
HAE005	-21.71803869	122.6471031	23/03/20	Havieron		Impact
HAE006	-21.71188451	122.6546223	24/03/20	Havieron		Reference
HAE007	-21.72353655	122.6567953	23/03/20	Havieron		Impact
HAE008	-21.72600068	122.6475414	22/03/20	Havieron		Impact
HAE009	-21.67505996	122.5112941	??/03/20	Havieron		Reference
HAE010	-21.72743466	122.6393908	01/04/20	Havieron		Impact
HAE011	-21.72790923	122.6440575	01/04/20	Havieron		Impact
	,,			}		·\$
HAE012	-21.73108323	122.6485882	31/03/20	Havieron		Impact
HAE014	-21.72823596	122.6433176	10/04/20	Havieron		Impact
HAHY002	-21.72767245	122.6391829	11/04/20	Havieron		Impact
HAHY007	-21.72093784	122.6582853	17/05/20	Havieron		Impact
HAVUNK02	-21.8873701	122.3796527	Unknown	Telfer	Big Tree	Reference
HAVUNK03	-21.69197262	122.1660277	Unknown	Telfer	gcc	Reference
	-21.886917	122.3793304		Telfer	Dia Troo	· } ·····
IAVUNK03_2			Unknown		Big Tree	Reference
HAVUNK04	-21.67906196	122.2008553	Unknown	Telfer		Reference
HAVWB01	-21.71880564	122.6493049	Unknown	Havieron		Impact
HAVWB02	-21.7190348	122.6492181	Unknown	Havieron		Impact
HAVWB03	-21.7138192	122.6271789	22/09/19	Havieron		Reference
HAVWB04	-21.7228138	122.6490065	29/09/19	Havieron		Impact
HB106	-21.6884548	122.2537054	??/??/88	Telfer	Wilkie Glen	Reference
HB10B	-21.61938784	122.114753	17/03/84	Telfer	Thompsons	Reference
HB144	-21.69270219	122.2252264	Unknown	Telfer		Reference
HB157	-21.7250731	122.2490239	02/07/93	Telfer	Staggers	Reference
HB164	-21.67876958	122.2689355	09/11/93	Telfer	Wilkie Glen	Reference
HB165	-21.67883439	122.2704724	10/11/93	Telfer	Wilkie Glen	Reference
HB225	-21.67927613	122.2011041	01/02/02	Telfer	3.0.1	Reference
HB227		122.1915715		Telfer		
	-21.67455999		05/02/02		C+	Reference
HB23A	-21.7411884	122.3057203	15/02/93	Telfer	Staggers	Reference
HB243	-21.77179681	122.2587267	27/02/02	Telfer	Staggers	Reference
HB281	-21.67937997	122.1419646	28/03/08	Telfer		Reference
HB326	-21.7941169	122.2615609	01/08/08	Telfer	Prices Fault	Reference
HB400	-21.53877467	121.9340038	10/08/02	Telfer		Reference
HB401	-21.50436285	121.8595696	Unknown	Telfer		Reference
		·············				•
HB402	-21.50430042	121.8596585	26/07/99	Telfer		Reference
HB406	-21.477845	121.7814067	29/09/91	Telfer		Reference
HB407	-21.44265585	121.7073823	05/10/91	Telfer		Reference
HB445	-21.8886664	122.3798653	01/08/91	Telfer	Big Tree	Reference
HB446	-21.8901671	122.3849207	11/08/91	Telfer	Big Tree	Referenc
HB447	-21.88464291	122.3794966	13/08/91	Telfer	Big Tree	Reference
HB448	-21.8862437	122.3759739	17/08/91	Telfer	Big Tree	Reference
		······•				
HB449	-21.8901966	122.3849189	28/08/91	Telfer	Big Tree	Reference
HB67	-21.6892676	122.2619372	17/08/93	Telfer	Wilkie Glen	Reference
HB71	-21.690824	122.2697215	30/09/83	Telfer	Wilkie Glen	Reference
Telfer 73/1	-21.40756755	121.6112586	Unknown	Telfer		Referenc
	-21.38624244	121.5215411	Unknown	Telfer		Reference



Hole Name	Latitude	Longitude	Drill Date	Broad Locality	Fine Locality	Site Type
TelfUnkT3001	-21.6764433	122.1403883	Unknown	Telfer	Passmore	Reference
TelfUnkT3002	-21.67938024	122.1418494	Unknown	Telfer		Reference
TelfUnkT3003	-21.6721054	122.1379849	Unknown	Telfer		Reference
TelfWindmill	-21.4777417	121.7820067	Unknown	Telfer		Reference
TMR0001	-21.61869209	122.1158671	Unknown	Telfer		Reference
TMR0002	-21.62223048	122.118996	Unknown	Telfer		Reference
TMUNK001	-21.61910151	122.1175142	Unknown	Telfer		Reference
TR103	-21.62561879	122.1216991	Unknown	Telfer		Reference

Appendix B: Sampling effort during the current survey

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Borehole	Method	Trin	Sampling Date	Trap Collected
HAD008	Trap	3	1/04/2020	15/06/2020
HAD009A	Scrape-Haul		23/11/2019	13/00/2020
HAD009A	Trap	1	23/11/2019	19/02/2020
HAD009A	Scrape-Haul		1/04/2020	15/02/2020
	†	3	1/04/2020	15/06/2020
HAD009A	Trap			15/06/2020
HAD010A	Scrape-Haul		24/11/2019	
HAD027		2	19/02/2020	
HAD027	Scrape-Haul		31/03/2020	/ /
HAD027	Trap	3	31/03/2020	15/06/2020
HAD030	Scrape-Haul	·····	19/02/2020	
HAE001	Scrape-Haul		30/03/2020	
HAE001	Trap	3	30/03/2020	15/06/2020
HAE001	Scrape-Haul	4	15/06/2020	
HAE002	Haul	3	31/03/2020	
HAE002	Trap	3	31/03/2020	15/06/2020
HAE002	Haul	4	15/06/2020	
HAE003	Scrape-Haul	3	31/03/2020	
HAE003	Trap	3	31/03/2020	15/06/2020
HAE003	Scrape-Haul	4	15/06/2020	
HAE004	Scrape-Haul	3	31/03/2020	
HAE004	Trap	3	31/03/2020	15/06/2020
HAE004	Scrape-Haul	4	15/06/2020	
HAE005	Scrape-Haul	}	31/03/2020	
HAE005	Trap	3	31/03/2020	15/06/2020
HAE005	Scrape-Haul	4	15/06/2020	
HAE006	Scrape-Haul		31/03/2020	
HAE006	Trap	3	31/03/2020	15/06/2020
HAE006	†	4	15/06/2020	25, 00, 2020
HAE007	Scrape-Haul		30/03/2020	
HAE007	Trap	3	30/03/2020	15/06/2020
HAE007	Scrape-Haul		15/06/2020	13/00/2020
HAE008	Scrape-Haul		30/03/2020	
HAE008	İ	3	30/03/2020	15/06/2020
= = = =	Trap			15/06/2020
HAE008	Scrape-Haul		15/06/2020	
HAE009	Scrape-Haul		31/03/2020	15 /06 /2020
HAE009	Trap	3	31/03/2020	15/06/2020
HAE009	Scrape-Haul	4	15/06/2020	1 = 10 C 10 00 0
HAE010	Trap	3	3/04/2020	15/06/2020
HAE010		4	15/06/2020	1= /00/0
HAE011	Trap	3	3/04/2020	15/06/2020
HAE011	·	4	15/06/2020	
HAE012	Trap	3	3/04/2020	15/06/2020
HAE012	Scrape-Haul	4	15/06/2020	
HAE014	Haul	4	16/06/2020	
HAHY002	Haul	4	16/06/2020	
HAHY007	Pump	4	15/06/2020	



Borehole	Method	Trip	Sampling Date	Trap Collected
HAVUNK02	Trap	1	22/11/2019	20/02/2020
HAVUNK02	Scrape-Haul	2	20/02/2020	
HAVUNK02	Trap	3	2/04/2020	16/06/2020
HAVUNK02	Scrape-Haul	4	16/06/2020	
HAVUNK03	Scrape	1	23/11/2019	
HAVUNK03	Trap	1	23/11/2019	20/02/2020
HAVUNK03	Trap	3	3/04/2020	17/06/2020
HAVUNK03	Scrape	4	17/06/2020	
HAVUNK03 2	Trap	1	22/11/2019	19/02/2020
HAVUNK03_2	Scrape	2	20/02/2020	
HAVUNK03 2	Trap	3	2/04/2020	16/06/2020
HAVUNK04	Scrape	1	23/11/2019	
HAVUNK04	Trap	1	23/11/2019	20/02/2020
HAVUNK04	Trap	3	3/04/2020	17/06/2020
HAVUNK04	Scrape	4	17/06/2020	, ,
HAVWB01	Haul	1	25/11/2019	
HAVWB01	Haul	2	19/02/2020	
HAVWB01	Haul	3	31/03/2020	
HAVWB01	Haul	4	16/06/2020	
HAVWB02	Pump	2	19/02/2020	
HAVWB02	Pump	3	31/03/2020	
HAVWB03	Haul	1	24/11/2019	
HAVWB03	Haul	2	19/02/2020	
HAVWB03	Haul	3	31/03/2020	
HAVWB03	Haul	4	16/06/2020	
HAVWB04	Pump	1	25/11/2019	
HB106	Haul	1	22/11/2019	
HB106	Haul	3	1/04/2020	
HB10B	Haul	2	20/02/2020	
HB10B	Haul	3	2/04/2020	
HB144	Haul	4	17/06/2020	
HB157	Haul	1	22/11/2019	
HB157	Haul	3	1/04/2020	
HB164	Haul	1	22/11/2019	
HB164	Haul	4	17/06/2020	
HB165	Haul	1	22/11/2019	
HB165	Haul	3	1/04/2020	
HB165	Haul	4	17/06/2020	
HB225	Haul	1	23/11/2019	
HB225	Haul	4	17/06/2020	
HB227	Haul	1	23/11/2019	
HB227	Haul	4	17/06/2020	
HB23A	Haul	1	22/11/2019	
HB23A	Haul	3	1/04/2020	
HB243	Haul	3	2/04/2020	

Borehole	Method	Trip	Sampling Date	Trap Collected
HB281	Haul	4	17/06/2020	
HB326	Haul	1	22/11/2019	
HB326	Haul	3	2/04/2020	
HB400	Haul	1	21/11/2019	
HB400	Haul	3	2/04/2020	
HB401	Haul	1	21/11/2019	
HB402	Haul	1	21/11/2019	
HB406	Haul	3	2/04/2020	
HB407	Pump	1	21/11/2019	
HB445	Haul	1	22/11/2019	
HB445	Haul	3	2/04/2020	
HB445	Haul	4	16/06/2020	
HB446	Haul	1	22/11/2019	
HB446	Haul	2	20/02/2020	
HB446	Haul	3	2/04/2020	
HB447	Scrape-Haul	2	20/02/2020	
HB447	Scrape-Haul	3	2/04/2020	
HB447	Trap	3	2/04/2020	16/06/2020
HB447	Scrape-Haul		16/06/2020	
HB448	Haul	1	22/11/2019	
HB449	Haul	1	22/11/2019	
HB449	Haul	4	16/06/2020	
HB67	Haul	3	1/04/2020	
HB71	Scrape-Haul	3	1/04/2020	
HB71	Trap	3	1/04/2020	16/06/2020
Telfer 73/1	Haul	1	21/11/2019	
Telfer 84/2	Haul	1	21/11/2019	
	Trap	3	2/04/2020	17/06/2020
TelfUnkT3002	Trap	3	2/04/2020	17/06/2020
TelfUnkT3002	•	4	17/06/2020	
TelfUnkT3003	 	3	3/04/2020	17/06/2020
TelfUnkT3003	Scrape	4	17/06/2020	
TelfWindmill	Pump	3	2/04/2020	
TMR0001	Scrape	1	21/11/2019	
TMR0001	Trap	1	21/11/2019	20/02/2020
TMR0002	Scrape-Haul	1	22/11/2019	
TMR0002	Trap	1	22/11/2019	20/02/2020
TMR0002	Trap	3	2/04/2020	16/06/2020
TMR0002	Scrape-Haul	4	16/06/2020	
TMUNK001	Scrape	1	22/11/2019	
TMUNK001	Trap	1	22/11/2019	20/02/2020
TMUNK001	Scrape	3	2/04/2020	
TMUNK001	Trap	3	2/04/2020	16/06/2020
TR103	Trap	3	2/04/2020	16/06/2020



Appendix C: Molecular Report





Havieron Project – Pilot Subterranean Fauna Survey Molecular Systematics Analysis

Biologic Environmental Survey
Report to Newcrest Mining Limited
August 2020



DOCUMENT	DOCUMENT STATUS												
Version	Authors	Review / Approved	Approved for Issue to										
No.	Additors	for Issue	Name	Date									
1	Joel Huey, Stephanie Floeckner	Nihara Gunawardene	Michael Curran	21/08/2020									

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GLOSSARY

12S Mitochondrially encoded 12S ribosomal RNA, a component of the small subunit of the

mitochondrial ribosome, which is useful in phylogenetic studies

Bootstrap Value between 0 and 100 that indicates the robustness of the node in a phylogenetic

tree

COI Cytochrome Oxidase subunit 1, a mitochondrial gene commonly used in phylogenetic

studies and used as a DNA barcode to identify species

GenBank Annotated open access sequence database of all publicly available nucleotide

sequences and their protein translations

OTU Operational taxonomic unit – species-equivalent taxonomic unit based on COI or 12S

cluster similarity



1 INTRODUCTION

Newcrest Mining Limited commissioned Biologic Environmental Survey (Biologic) to undertake a molecular systematics analysis (DNA barcoding) of 28 specimens collected from the Havieron Project Area (the Study Area).

1.1 Aims and objectives

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

- Undertake DNA sequencing of 28 subterranean fauna specimens to obtain barcoding sequences of the mitochondrial gene Cytochrome Oxidase I (COI; Hebert et al., 2003b). These specimens were comprised of 19 oligochaetes and 9 amphipods;
- Investigate the interspecific and intraspecific relationships between sequences of each higher taxonomic group (i.e. use the results of the DNA analysis to indicate how many different species/ OTUs are likely to occur within each group based on published species-thresholds);
 and
- Investigate the relationships between sequences from the Study Area and relevant previous sequences from the wider Pilbara region, using available DNA databases (i.e. compare the results of the current analysis with accessible DNA databases to assess whether any of the species/ OTUs from the Study Area have been collected previously or more widely beyond the Study Area).

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



2 METHODS

2.1 Sub-sample preparation

Specimens were selected for sequencing from survey work undertaken by Biologic. Specimens of oligochaetes and amphipods were to represent geographic and morphological variation across the Study Area. A total of 28 specimens collected from the Study Area by Biologic were selected for molecular systematics analysis (Table 2.1). Adequate redundancy in specimen selection was incorporated to account for any potential sequence generation failure. Specimens in good condition were chosen to increase their DNA extraction potential. Specimen selection was undertaken by Michael Curran.

Whole animals were selected for DNA extraction, where multiple specimens of a putative morpho-type had been collected from a site. Where only a single specimen was available, tissue preparation was undertaken by removing a leg of amphipods, or bisecting the oligochaete specimen, thus retaining taxonomically informative body parts. Tissue was dried briefly to remove ethanol and placed in ATL buffer. Greatest care was taken to decontaminate all tools and equipment between samples, using bleach and repeated rinsing in deionised water. Table 2.1 provides details of the taxonomic orders chosen for molecular analysis. Further taxonomic clarification for each specimen included in the analysis can be found in Appendix A.

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

Class/Subclass	Order	Family	Number of samples	PCR success	Sequence success	% sequence success
Oligochaeta	Haplotaxida	Enchytraeidae	19	19	19	100%
Malacostraca	Amphipoda	Paramelitidae	9	9	9	100%
TOTAL			28	28	28	100%

2.2 DNA extraction, amplification and sequencing

DNA extraction and sequencing methods followed Cullen and Harvey (2017, 2018), as follows:

Subsampled tissue/specimen was placed directly into ATL buffer for extraction using the *QIAGEN DN-easy* Blood and Tissue extraction kit, and DNA extraction followed the manufacturer's protocols. DNA extractions were amplified by Polymerase Chain Reaction (PCR) using Folmer PCR primers (LCO1490, HCO2198; Folmer et al., 1994) to assess the variability of COI.

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



and assembled in GENEIOUS, and final consensus sequences were then available for downstream analysis.

2.3 Specimen selection for comparative analysis

DNA comparisons were typically conducted at the order level (Table 2.1). Comparative sequences were from GenBank (a publicly available DNA sequence database) and Biologic's unpublished DNA sequence libraries (3,135 sequences), using two separate methods.

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

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

2.4 Analysis and interpretation of sequence alignments/divergence

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

To delimit taxonomic units using molecular data, we applied a genetic distance-based threshold method, combined with our morphological identifications. Fauna-specific genetic distance thresholds for delimiting species and OTUs were used wherever possible, based on published literature and available previous reports. Where these thresholds were not available, the assessment used average divergence thresholds for related groups or higher taxa developed by broad-level studies (e.g. Hebert et al., 2003a).

In general, ≤8% COI divergence is seen as appropriate to determine OTUs (Hebert et al., 2003a), however, higher or lower divergences are sometimes justified depending on the organism studied. Unless otherwise stated, we considered sequences that exhibited COI divergences ≤8% to belong to the same OTU.



2.5 Constraints and Limitations

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

The databases used for regional comparisons included GenBank and Biologic's Sequence Library. While these sequence databases, in combination, comprise a large portion of the subterranean fauna genetic work undertaken in Western Australia, it is acknowledged that there may be many other relevant sequences from third party project areas nearby or elsewhere in the region that were not available for comparison at the time of the study. GenBank is dynamic database, and the addition of new sequences and altered taxonomic classifications could not be included into this report if they occurred after the 14th of August 2020.

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



3 RESULTS AND DISCUSSION

A total of 28 specimens were processed for sequencing by Biologic. Sequences were successfully derived for all of these specimens (100% of specimens). Of these 28 sequences, all produced a high-quality sequence of the target organism. Therefore, 28 high quality sequences were available for analysis (100% of sequences). The orders of the sequences are tabulated in Table 2.1.

In total, 12 OTUs have been designated to specimens from the Study Area, 11 of these being unique to this survey (Table 3.1). The results of each taxonomic group's analysis are described in the subsequent sections.

Table 3.1: Summary of species and OTUs recovered from 28 samples successfully sequenced in this study, organised by taxon.

Species/OTU	Number of specimens from Study Area	Unique to Study Area?	Linear Distance
Oligochaeta	19		
Enchytraeidae `sp. E12`	5	No	>>100 km
Enchytraeidae `sp. Biologic-OLIG023`	4	Yes	0.58 km
Enchytraeidae `sp. Biologic-OLIG024`	5	Yes	77.20 km
Enchytraeidae `sp. Biologic-OLIG025`	4	Yes	1.48 km
Enchytraeidae `sp. Biologic-OLIG026`	1	Yes	-
Amphipoda	9		
Paramelitidae `sp. Biologic-AMPH025`	2	Yes	<0.01 km
Paramelitidae `sp. Biologic-AMPH026`	1	Yes	-
Paramelitidae `sp. Biologic-AMPH027`	1	Yes	-
Paramelitidae `sp. Biologic-AMPH028`	1	Yes	-
Paramelitidae `sp. Biologic-AMPH029`	2	Yes	0.4 km
Paramelitidae `sp. Biologic-AMPH030`	1	Yes	-
Paramelitidae `sp. Biologic-AMPH026`	1	Yes	-

3.1 Oligochaeta

The 19 sequenced oligochaete specimens fell into five OTUs (Fig 3.1.1, Table 3.1), which were all ≥9.27% divergent from all other specimens in the analysis (Table 3.1.1).

Five specimens matched a previously sequenced OTU, Enchytraeidae `sp. E12` (Fig. 3.1.1), which has been sampled in the upper Fortescue catchment of the Pilbara region (Brown *et al.*, 2015). This OTU therefore has a large geographic distribution (Table 3.1) and exhibited <4% intraspecific genetic divergence (Table 3.1.1).

Three OTUs were comprised of multiple specimens from multiple sites. Enchytraeidae `sp. Biologic-OLIG023` and Enchytraeidae `sp. Biologic-OLIG025` had small geographic ranges (<2 km, Table .1),



but showed moderate intraspecific genetic divergence (7.14% and 5.78%, respectively, Table 3.1.1). Enchytraeidae `sp. Biologic-OLIG024` had a much larger geographic range (77 km, Table 3.1), and displayed moderate intraspecific genetic variation (≤7.3%, Table 3.1.1). A single specimen formed a singleton lineage, Enchytraeidae `sp. Biologic-OLIG026`, which was >18% divergent from all other specimens in the analysis (Table 3.1.1).

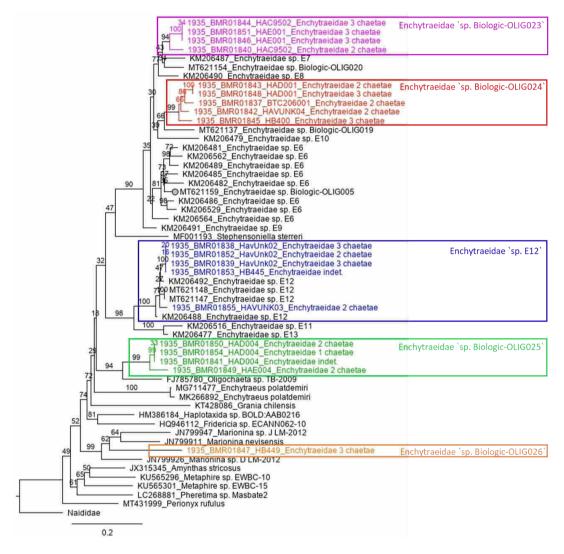


Figure 3.1.1: Maximum Likelihood phylogeny, with bootstrap values.



Table 3.1.1: Pairwise distances for the dataset included in Fig 3.1.1. Colours of OTUs match those in Fig 3.1.1.

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MG71477_Enchytraeus polatdemiri 13.0 19.45 19.00 19.61 2.037 20.52 20.52 20.52 20.52 20.52 20.52 20.52 20.52 20.52 20.52 20.67 20.61 18.54 19.45 19.00 19.16 19.95 20.66 19.65 19.95 19.61 19.95 19.06 19.65 19.90 19.95 19.65 19.95 19.		
MK268892_Enchytraeus polatdemiri		
MT431999 Perionyx raffulus 19.48 19.48 19.71 19.33 19.94 20.25 20.25 20.25 20.25 21.12 19.48 20.01 20.06 20.01 20.21 20.21 20.15 20.40 19.16 20.97 21.57 19.46 18.85 20.86 20.09 19.33 19.		
1935_BMR01847_HB449_Enchytraeidae 3 chaetae 1905_BMR01847_HB449_Enchytraeidae 3 chaetae 1906_BMR01847_HB449_Enchytraeidae 3 chaetae 1906_BMR01847_HB449_En	MT431999_Perionyx rufulus	
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N799947_Marionina sp. 1 LM-2012 20.70 2.070	JN799911_Marionina nevisensis	
KT428086_Grania chilensis 20,97 19.61 19.61 19.76 20,37 19.91 19.15 19.15 20,67 18.85 19.30 19.61 19.76 19.45 19.45 19.1	JN799947_Marionina sp. J LM-2012	
	JN799926_Marionina sp. D LM-2012	
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	Naididae	25.40 25.25 25.25 22.80 25.10 22.19 25.10



3.2 Amphipoda

The nine amphipod sequences represented seven OTUs, all unique to the Study Area (Fig. 3.2.1, Table 3.1). All these OTUs showed interspecific genetic distances over 12% within the Study Area (Table 3.2.1). Only two OTUs had more than one specimen, and in both cases, they were sampled from two sites, less than 400 m apart (Table 3.1), with no genetic variation within the OTUs.

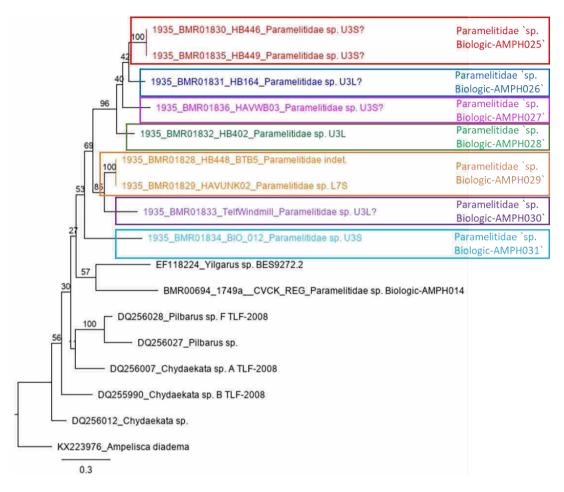


Figure 3.2.1: Maximum Likelihood phylogeny, with bootstrap values.



Table 3.2.1: Pairwise distances for the dataset included in Fig 3.2.1. Colours of OTUs match those in Fig 3.2.1.

COI Pairwise Distances (%)	BMR01828	BMR01829	BMR01833	BMR01830	BMR01835	BMR01831	BMR01832	BMR01836	DQ255990	DQ256007	DQ256012	DQ256027	DQ256028	KX223976	BMR01834	EF118224	BMR00694
1935_BMR01828_HB448_BTB5_Paramelitidae indet.		0.00	14.13	17.93	17.93	17.63	16.87	19.45	17.02	16.26	17.37	18.81	19.61	23.56	21.13	19.90	21.81
1935_BMR01829_HAVUNK02_Paramelitidae sp. L7S	0.00		14.13	17.93	17.93	17.63	16.87	19.45	17.02	16.26	17.37	18.81	19.61	23.56	21.13	19.90	21.81
1935_BMR01833_TelfWindmill_Paramelitidae sp. U3L?	14.13	14.13		20.06	20.06	20.67	19.15	21.13	20.67	21.28	21.75	22.88	21.73	28.12	21.88	22.99	25.35
1935_BMR01830_HB446_Paramelitidae sp. U3S?	17.93	17.93	20.06		0.00	12.31	13.98	14.59	20.06	20.37	20.50	20.53	20.52	25.68	19.45	22.99	22.58
1935_BMR01835_HB449_Paramelitidae sp. U3S?	17.93	17.93	20.06	0.00		12.31	13.98	14.59	20.06	20.37	20.50	20.53	20.52	25.68	19.45	22.99	22.58
1935_BMR01831_HB164_Paramelitidae sp. U3L?	17.63	17.63	20.67	12.31	12.31		14.13	13.98	20.21	20.67	18.62	19.91	20.82	23.40	21.73	22.13	23.96
1935_BMR01832_HB402_Paramelitidae sp. U3L	16.87	16.87	19.15	13.98	13.98	14.13		15.96	22.95	20.67	18.62	22.10	21.88	23.10	22.34	22.47	25.35
1935_BMR01836_HAVWB03_Paramelitidae sp. U3S?	19.45	19.45	21.13	14.59	14.59	13.98	15.96		20.06	22.80	20.34	21.16	20.67	23.25	21.28	24.70	23.35
DQ255990_Chydaekata sp. B TLF-2008	17.02	17.02	20.67	20.06	20.06	20.21	22.95	20.06		17.93	15.96	19.75	17.63	22.49	19.76	20.07	22.73
DQ256007_Chydaekata sp. A TLF-2008	16.26	16.26	21.28	20.37	20.37	20.67	20.67	22.80	17.93		14.87	17.71	17.02	21.88	22.19	19.38	21.20
DQ256012_Chydaekata sp.	17.37	17.37	21.75	20.50	20.50	18.62	18.62	20.34	15.96	14.87		19.84	19.25	17.84	20.81	21.44	21.80
DQ256027_Pilbarus sp.	18.81	18.81	22.88	20.53	20.53	19.91	22.10	21.16	19.75	17.71	19.84		12.07	21.63	23.82	22.13	23.04
DQ256028_Pilbarus sp. F TLF-2008	19.61	19.61	21.73	20.52	20.52	20.82	21.88	20.67	17.63	17.02	19.25	12.07		21.28	20.67	20.76	22.43
KX223976_Ampelisca diadema	23.56	23.56	28.12	25.68	25.68	23.40	23.10	23.25	22.49	21.88	17.84	21.63	21.28		25.53	24.36	25.81
1935_BMR01834_BIO_012_Paramelitidae sp. U3S	21.13	21.13	21.88	19.45	19.45	21.73	22.34	21.28	19.76	22.19	20.81	23.82	20.67	25.53		24.53	22.58
EF118224_Yilgarus sp. BES9272.2	19.90	19.90	22.99	22.99	22.99	22.13	22.47	24.70	20.07	19.38	21.44	22.13	20.76	24.36	24.53		21.27
BMR00694_1749aCVCK_REG_Paramelitidae sp. Biologic-AMPH014	21.81	21.81	25.35	22.58	22.58	23.96	25.35	23.35	22.73	21.20	21.80	23.04	22.43	25.81	22.58	21.27	



4 SUMMARY

Using well-established DNA extraction and sequencing methods, this molecular systematics analysis designated 12 distinct species/ OTUs to 28 high quality sequences from the Study Area. All OTUs, the areas in which they were found, and the specimen numbers per OTU are shown in Appendix A. The following are the key findings at the species/ OTU level:

- Oligochaeta (COI): five OTUs, four unique lineages, one matching a external sequences,
- Amphipoda (COI): seven OTUs, all unique lineages.



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Appendix 1: All Operational Taxonomic Units (OTUs) found in the Study Area.

BMR	Unique ID code	Site	Latitude	Longitude	Family	Lowest ID Legacy	OTU Name	Reaction State
Oligochaeta								
BMR01837	7361	BTC206001	-21.88965	122.38515	Enchytraeidae	Enchytraeidae 2 chaetae	Enchytraeidae `sp. Biologic-OLIG024`	PASS
BMR01838	6362	HavUnk02	-21.88737	122.37965	Enchytraeidae	Enchytraeidae 3 chaetae	Enchytraeidae `sp. E12`	PASS
BMR01839	6373	HavUnk02	-21.88737	122.37965	Enchytraeidae	Enchytraeidae 3 chaetae	Enchytraeidae `sp. E12`	PASS
BMR01840	6418	HAC9502	-21.72233	122.65311	Enchytraeidae	Enchytraeidae 2 chaetae	Enchytraeidae `sp. Biologic-OLIG023`	PASS
BMR01841	6438	HAD004	-21.72342	122.65285	Enchytraeidae	Enchytraeidae indet.	Enchytraeidae `sp. Biologic-OLIG025`	PASS
BMR01842	6511	HAVUNK04	-21.67906	122.20086	Enchytraeidae	Enchytraeidae 2 chaetae	Enchytraeidae `sp. Biologic-OLIG024`	PASS
BMR01843	6684	HAD001	-21.72430	122.65286	Enchytraeidae	Enchytraeidae 2 chaetae	Enchytraeidae `sp. Biologic-OLIG024`	PASS
BMR01844	7085	HAC9502	-21.72233	122.65311	Enchytraeidae	Enchytraeidae 3 chaetae	Enchytraeidae `sp. Biologic-OLIG023`	PASS
BMR01845	7183	HB400	-21.53877	121.93400	Enchytraeidae	Enchytraeidae 3 chaetae	Enchytraeidae `sp. Biologic-OLIG024`	PASS
BMR01846	7275	HAE001	-21.72754	122.65326	Enchytraeidae	Enchytraeidae 3 chaetae	Enchytraeidae `sp. Biologic-OLIG023`	PASS
BMR01847	7390	HB449	-21.89020	122.38492	Enchytraeidae	Enchytraeidae 3 chaetae	Enchytraeidae `sp. Biologic-OLIG026`	PASS
BMR01848	7854	HAD001	-21.72430	122.65286	Enchytraeidae	Enchytraeidae 3 chaetae	Enchytraeidae `sp. Biologic-OLIG024`	PASS
BMR01849	7950	HAE004	-21.71708	122.64032	Enchytraeidae	Enchytraeidae 2 chaetae	Enchytraeidae `sp. Biologic-OLIG025`	PASS
BMR01850	8478	HAD004	-21.72342	122.65285	Enchytraeidae	Enchytraeidae 2 chaetae	Enchytraeidae `sp. Biologic-OLIG025`	PASS
BMR01851	8550	HAE001	-21.72754	122.65326	Enchytraeidae	Enchytraeidae 3 chaetae	Enchytraeidae `sp. Biologic-OLIG023`	PASS
BMR01852	8557	HavUnk02	-21.88737	122.37965	Enchytraeidae	Enchytraeidae 2 chaetae	Enchytraeidae `sp. E12`	PASS
BMR01853	8570	HB445	-21.88867	122.37987	Enchytraeidae	Enchytraeidae indet.	Enchytraeidae `sp. E12`	PASS
BMR01854	8574	HAD004	-21.72342	122.65285	Enchytraeidae	Enchytraeidae 1 chaetae	Enchytraeidae `sp. Biologic-OLIG025`	PASS
BMR01855	8583	HAVUNK03	-21.69197	122.16603	Enchytraeidae	Enchytraeidae 2 chaetae	Enchytraeidae `sp. E12`	PASS
Amphipoda								
BMR01828	7893	BTB5	-21.88625	122.37597	Paramelitidae	Paramelitidae indet.	Paramelitidae `sp. Biologic-AMPH029`	PASS
BMR01829	6094	HavUnk02	-21.88737	122.37965	Paramelitidae	Paramelitidae sp. L7S	Paramelitidae `sp. Biologic-AMPH029`	PASS
BMR01830	6115	HB446	-21.89017	122.38492	Paramelitidae	Paramelitidae sp. U3S?	Paramelitidae `sp. Biologic-AMPH025`	PASS
BMR01831	6186	HB164	-21.67877	122.26894	Paramelitidae	Paramelitidae sp. U3L?	Paramelitidae `sp. Biologic-AMPH026`	PASS
BMR01832	6762	HB402	-21.50430	121.85966	Paramelitidae	Paramelitidae sp. U3L	Paramelitidae `sp. Biologic-AMPH028`	PASS
BMR01833	7886	TelfWindmill	-21.47774	121.78201	Paramelitidae	Paramelitidae sp. U3L?	Paramelitidae `sp. Biologic-AMPH030`	PASS
BMR01834	8544	HAE009	-21.67508	122.51007	Paramelitidae	Paramelitidae sp. U3S	Paramelitidae `sp. Biologic-AMPH031`	PASS
BMR01835	8561	HB449	-21.89020	122.38492	Paramelitidae	Paramelitidae sp. U3S?	Paramelitidae `sp. Biologic-AMPH025`	PASS
BMR01836	8568	HAVWB03	-21.71380	122.62719	Paramelitidae	Paramelitidae sp. U3S?	Paramelitidae `sp. Biologic-AMPH027`	PASS





Havieron Project Stage 2: Subterranean Fauna Survey

Biologic Environmental Survey

Preliminary Report to Newcrest Mining Limited

July 2021





Havieron Project: Subterranean Fauna Survey

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1. INTRODUCTION

The Havieron Project Joint Venture (The Project) is a farm-in joint venture agreement between Newcrest Mining Limited (Newcrest) and Greatland Gold Ltd. The Project is located approximately 45 km east of the Telfer Gold Mine and has the potential to deliver a high-grade underground gold deposit. The first stage (Stage 1) of the Project has environmental approval for development; it comprises a boxcut and decline development to 400 m below ground level, a service road and supporting infrastructure. Newcrest is seeking information to inform environmental approvals of the final stage (Stage 2) of the Havieron Project, which comprises the following:

- Infrastructure corridor to Telfer;
- Treatment of tailings at the Telfer Processing Plant;
- Open stope or sub level cave underground mine;
- Waste rock landforms;
- Evaporation ponds; and
- Associated infrastructure.

To inform environmental approvals of Stage 2, Newcrest requested that Biologic undertake a singlephase stygofauna survey within the Stage 2 Havieron Project Area. The aims of this survey are:

- Gather sufficient data and information on the subterranean fauna values within the Project Area to facilitate environmental approvals; and
- Provide sufficient information to accurately assess the likely impact, including cumulative impacts, of mining activities on subterranean fauna values of conservation significance within the Project Area in a local and regional context.
- Refine the extent of the distribution of any stygofauna species within the Project Area.



2. METHODS

2.1 Survey Effort and Timing

A single-phase (single trip) subterranean fauna survey (Stage 2 Survey) was undertaken within the Havieron Project Area and Service Corridor (Figure 2-1), in accordance with guidelines for subterranean fauna assessments (EPA, 2016a, 2016b, 2016c). The survey was undertaken from the 3rd to the 7th of May (fauna collection licence BA27000177-2).

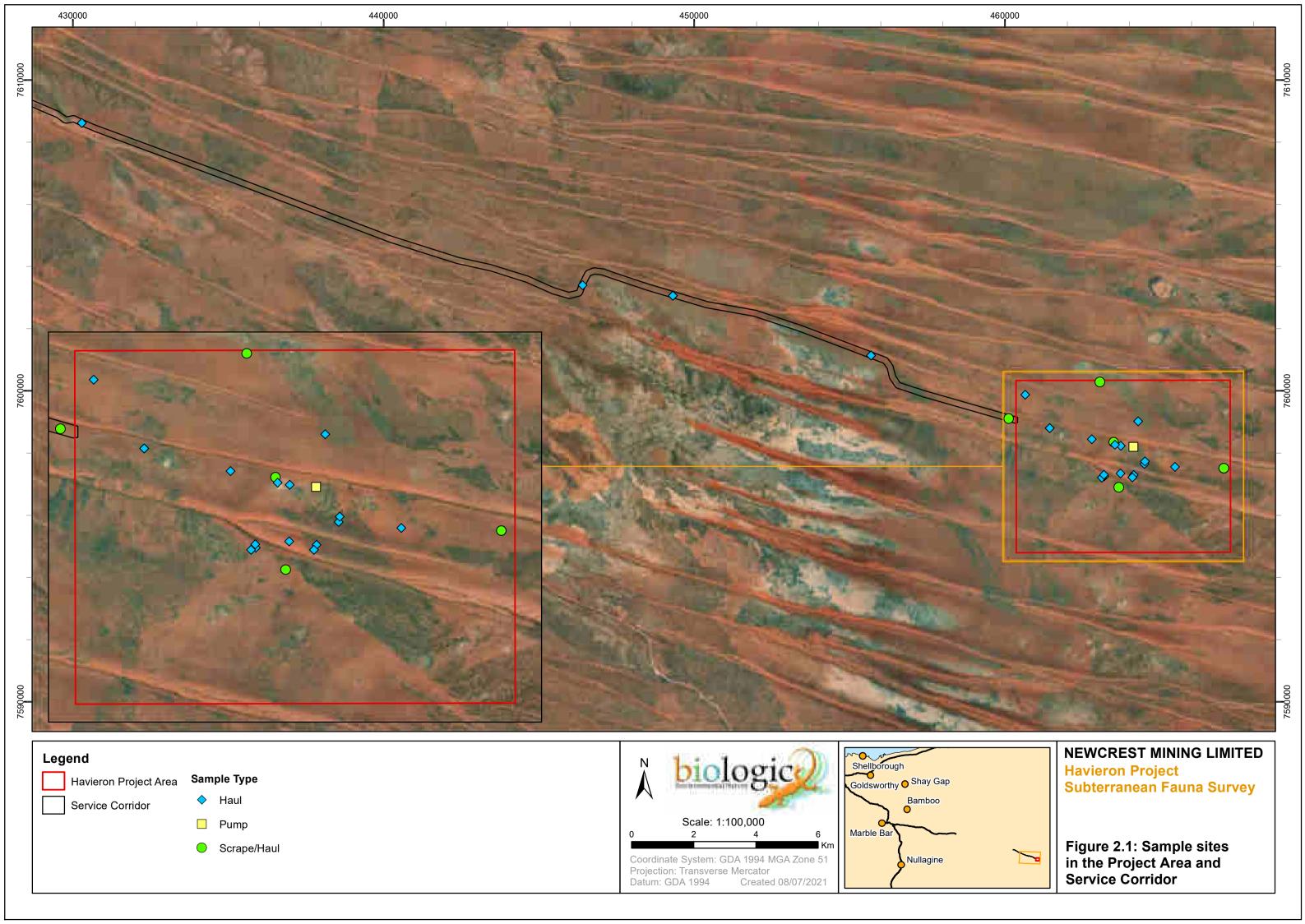
A total of 25 samples were collected from the Project Area during this survey, consisting of haul, scrape haul and pump samples (Figure 2-1). Stygofauna sampling was undertaken at 25 sites and troglofauna sampling at five sites. Including Stage 1 survey work, a total of 85 stygofauna samples (hauls, scrape/hauls or pump) and 82 troglofauna samples (scrape/hauls and traps) have been collected from the Project Area to date (Table 2-1).

Standard methodology was utilised for sampling stygofauna (hauling, dual haul-scraping, and pump outflow sampling) and troglofauna (dual haul-scraping). Samples were taken from holes of a variety of ages and throughout all geologies and habitats where drilling was undertaken in the Project Area.

Table 2-1: Total sample effort undertaken at the Project Area.

Locality	Survey Stage	Trip Number	Haul	Scrape-Haul	Pump	Trap
	Stage 1	1	2	12	1	10
		2	2	3	1	
Havieron		3	3	18	1	23
		4	5	11	1	
	Stage 2 5		19	5	1	
	Total			49	5	33

^{*}Survey effort does not in include the Telfer regional sampling undertaken during the Stage 1 survey work.





2.2 Sampling Methods

The sampling methods used were consistent with EAG #12 (EPA, 2016c), Guidance Statement #54A (EPA, 2016b) and the Stygofauna Sampling Protocol developed for the Pilbara Biodiversity Study Subterranean Fauna Survey (Eberhard *et al.*, 2005; Eberhard *et al.*, 2009). The field work was undertaken by Syngeon Rodman and Isabelle Johansson.

Stygofauna net-hauling

Stygofauna were sampled by standard net-hauling methods, using a plankton net of a diameter to suit each bore or drill hole (in most cases 30-80 mm). Each haul sample comprised a total of six hauls from the bottom of the hole to the top, with three hauls using a 150 μ m mesh and three hauls using a 50 μ m mesh. The base of the net was fitted with a lead weight and a sample receptacle with a base mesh of 50 μ m. To stir up sediments, the net was raised and lowered at the bottom of the hole prior to retrieval and hauled at an even pace through the water column to maximise filtration of the water.

The sample from each haul was emptied into a bucket, which was elutriated after the final haul to remove coarse sediments and filtered back through the 50 µm net/ sample receptacle to remove as much water as possible. The sample was transferred to a 50-120 mL preservation vial (depending upon the quantity of sediment) and preserved in 100% ethanol. The ethanol and the samples were kept chilled on ice or in a refrigerator to facilitate cool-temperature DNA fixation.

Pumping (stygofauna)

One site was sampled by actively pumping water from bores and running the water from the pump release valve through a stygofauna net three times for approximately 10 minutes total at each site.

Troglofauna scraping

Scraping was undertaken at vertical, uncased drill holes using a reinforced 150 μ m weighted stygofauna net, with a specialised scraping attachment used above the net to maximise gentle contact with the walls of the hole. The net was lowered and raised through the full length of the hole four times for holes where no water was present, with each haul being emptied into a sample bucket. Where the water table was intercepted, a combined net-haul / scrape sample was taken using the scraping attachment. Each combined net-haul / scrape sample comprised a total of six hauls from the bottom of the hole to the top (including AWT and BWT habitats), with three hauls using a 150 μ m mesh and three hauls using a 50 μ m mesh. The contents of the sample were elutriated, processed, and stored in 100 % ethanol.

Water physicochemistry

Prior to stygofauna sampling, a groundwater sample was collected using a plastic cylindrical bailer (length: 1 m), for the purposes of physicochemical measurements. The bailer was lowered down the hole until reaching groundwater and a water sample was collected at a depth of 2 m below the surface. As such the results were not indicative of water parameters throughout the entire bore (or aquifer) but rather provide a general indication of near surface conditions. Conditions sampled during pumping were measured using a sample collected from the pump outflow, which would have artificially increased the dissolved oxygen readings. Groundwater physicochemical data (including EC, pH, TDS, Redox ORP,



and dissolved O₂) was measured using a multi-parameter water meter. Constrictions in piezometer bores, blockages from root material, or excessive depths to groundwater inhibited the collection of physicochemical readings at some sites.

Sorting and taxonomy

Sorting and parataxonomy were undertaken in-house using dissecting microscopes. The personnel involved (Mary van Wees, Juliana Pille Arnold, Stephanie Floeckner, Siobhan Paget, Isabelle Johansson, Courtney Wilkins, and Giulia Perina) were all suitably trained and experienced in sorting and parataxonomy of subterranean fauna.

Parataxonomy of the specimens utilised published literature and taxonomic keys where available. Each morphospecies from each sample was assigned a separate labelled vial and labelled with a specimen tracking code. Taxonomic groups were examined in as much detail as possible using in-house expertise, before sending a reference collection to specialist taxonomists for detailed taxonomic advice. Species comparisons and alignments were performed using regional specimens collected beyond the Study Area throughout the wider sub-regional area. Dr Giulia Perina provided specialist taxonomic identifications and regional alignments.

Genetic analysis (DNA barcoding using the mitochondrial gene COI) is used for certain subterranean taxa to validate morphological identifications and provide a basis for species-level identifications and regional comparisons where taxonomic resolution was limited. Genetic analysis currently underway, as such the results of the analysis have not been provided in this memorandum.

Conservation status and SRE classification

Very few subterranean species and assemblages from the Pilbara region are listed under relevant legislation as threatened species, or as Threatened or Priority Ecological Communities in certain locations. Any listed subterranean species or community is regarded as conservation significant although, due to a lack of survey effort and taxonomic certainty for the majority of subterranean fauna in the Pilbara region, there are many potentially range restricted (SRE) or conservation significant species and communities that do not appear on these lists.

The likelihood of taxa representing SRE species (*i.e.* distribution <10,000 km² following Harvey 2002, or <1,000 km² following Eberhard *et al.* 2009) was assessed based on the known local species distribution, and regional comparisons where data was available, following advice from the WAM and other relevant taxonomic specialists. The assessment of SRE status was highly dependent on:

- 1. the degree of taxonomic certainty at the genus and species levels
- 2. the current state of taxonomic and ecological knowledge for each taxon (including whether a regional genetic context has been investigated)
- 3. the scale and intensity of the local and regional sampling effort
- 4. whether or not relevant taxonomic specialists were available to provide advice.

The SRE status categories used in this report follow the WAM's categorisation for SRE invertebrates. This system is based upon the 10,000 km² range criterion proposed by Harvey (2002), and uses three



broad categories to deal with varying levels of taxonomic certainty that may apply to any given taxon (Table 2.2). Because most subterranean fauna are poorly known taxonomically, and the general limitations to sampling subterranean fauna, the majority of morphospecies invariably fall within one (or several) of the five Potential SRE sub-categories.

Table 2.2: SRE categorisation used by WAM taxonomists

	Taxonomic Certainty	Taxonomic Uncertainty
Distribution <10 000km ²	 Confirmed SRE A known distribution of < 10,000km². The taxonomy is well known. The group is well represented in collections and/ or <i>via</i> comprehensive sampling. 	Potential SRE Patchy sampling has resulted in incomplete knowledge of geographic distribution. Incomplete taxonomic knowledge. The group is not well represented in collections. Category applies where there are
Distribution >10 000km ²	 Widespread (not an SRE) A known distribution of > 10,000km². The taxonomy is well known. The group is well represented in collections and/ or <i>via</i> comprehensive sampling. 	significant knowledge gaps. SRE Sub-categories may apply: A) Data Deficient B) Habitat Indicators C) Morphology Indicators D) Molecular Evidence E) Research & Expertise

The degree of stygomorphy or troglomorphy (observable physical adaptations to subterranean habitats such as eyelessness, depigmentation, elongation of sensory appendages and thinning of the cuticle) assessed to determine each morphospecies' 'subterranean status', *i.e.* whether a taxon was more or less likely to be an obligate subterranean species (stygobite/ troglobite). It is acknowledged that the current EPA guideline for subterranean fauna does not account for non-obligate subterranean fauna, stating, "...subterranean fauna are defined as fauna which live their entire lives (obligate) below the surface of the earth.... Fauna that use a subterranean environment for only part of the day or season (e.g. soil-dwelling or burrowing species, cave-dwelling bats and birds) are not considered as subterranean fauna for this EAG" (EPA, 2013).

Nevertheless, there may be fauna with restricted distributions <10,000 km² following Harvey (2002), or <1,000 km² following Eberhard *et al.* (2009) that are of interest because of their SRE status, regardless of whether they can be definitively regarded as 'obligate' subterranean fauna. For this reason, this report presents an assessment of both the subterranean status and the SRE status of each taxon collected, to the best available knowledge.

In some cases where thorough sampling has been conducted and sufficient habitat information and ecological information is available, the potential occurrence of a taxon at a local scale may be inferred *via* the extent of habitats, particularly where the rest of the assemblages are highly similar, and the habitats appear well-connected. Despite the suggestion within the current EPA (2013) guidelines that related species' ranges may be used as surrogates for poorly-known species' ranges, the level of



evidence required to support the identification of an appropriate surrogate is almost prohibitively high for most subterranean fauna, therefore this would only be investigated as a last resort.

3. RESULTS

3.1 Previous survey results (Stage 1)

A two-phase survey was undertaken in the Study Area in 2019-2020 in which a total of 74 holes were sampled throughout the Study Area, 33 within the Havieron Project Area and 41 in the Telfer region (Biologic, 2020). A total of 573 subterranean fauna specimens were recorded in the overall Study Area, comprising approximately 27% stygofauna, 69% amphibious and 4% troglofauna. Two specimens, representing two stygofauna taxa (*Fierscyclops* (*Pilbaracyclops*)? *frustatio* and Paramelitidae `sp. Biologic-AMPH031`) were recorded from within the Service Corridor (Figure 3-1). Within the Havieron Project Area, 15 stygofauna and 167 amphibious fauna were collected. No troglofauna were recorded within the Project Area and an assessment of the geology confirms that it does not appear to be suitable troglofaunal habitat.

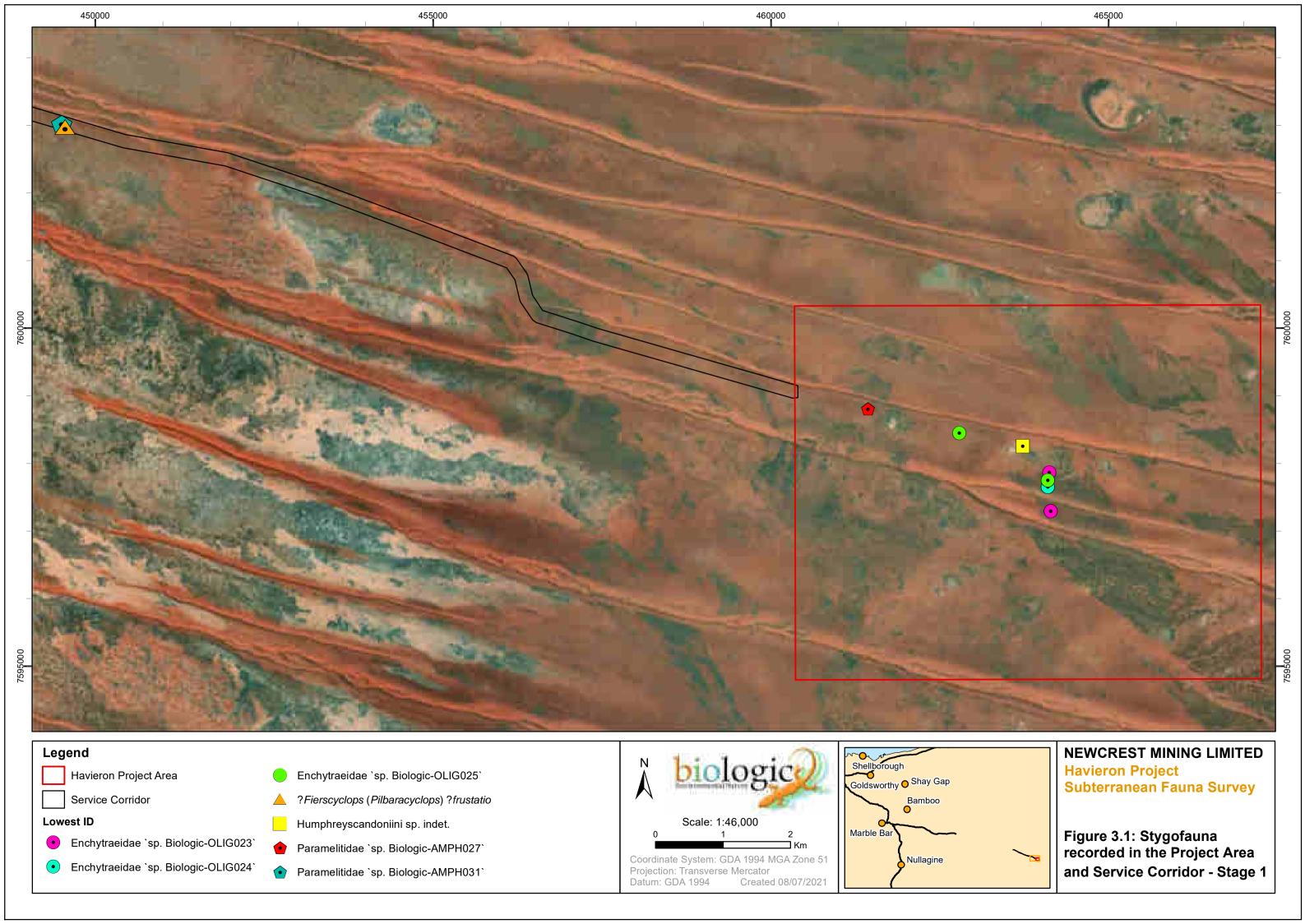
A total of 108 amphibious fauna, belonging to the Oligochaeta family Enchytraeidae, were collected within the Project Area. Enchytraeids were represented by two morphospecies, Enchytraeidae `sp. Biologic-OLIG023` and Enchytraeidae `sp. Biologic-OLIG025`, collected from four sites with the Project Area. The Enchytraeids collected were classed as amphibious as they were collected in samples from both above and below the water table. Two, were only collected from within the Project Area (Figure 3-1).

A total of 154 stygofauna specimens were recorded in the broader Study Area belonging to nine taxonomic groups. However only two morphospecies were recorded within the Project Area: an amphipod, Paramelitidae `sp. Biologic-AMPH027` and an ostracod, Humphreyscandonini sp. indet. Paramelitidae `sp. Biologic-AMPH027` was represented by 11 specimens collected from a single water bore (Figure 3-1). Humphreyscandonini sp. indet. was represented by two valves (shells) collected in a single sample (Figure 3-1). As only valves were collected and not the entire organism, this specimen could only be identified to tribe. The distributions of stygobitic ostracods in Western Australia range from widespread to highly range-restricted (Reeves *et al.*, 2007). While the identification of Humphreyscandonini sp. indet. is tentative, the family Candonidae is known to contain Confirmed SREs as well as widespread species.

The only records of true stygofauna (stygobites) from the Project Area came from holes that targeted alluvials and calcareous cement. The richness of stygofauna and amphibious species recorded from the Project Area to date is very low compared to the nearest results from Telfer and Kintyre (Bennelongia, 2014) (Bennelongia, 2012). The low richness of stygofauna and amphibious species during the Stage 1 survey provide strong support that the aquifers in the Project are not prospective for stygofauna, although there appears to be small, localised patches of higher prospect stygofauna habitat present in the Study Area, which are represented by alluvials and calcareous cement, which seem to be associated with claypans in the area. The Claypans may present a unique environment due to the salinity, interactions with significant rainfall events and clayey materials. The Claypan habitat appears associated with the



perched aquifer and represents a smaller groundwater system associated with rainfall events. Some interactions with the Upper Confined Aquifer may occur. The amphibious species, on the other hand, appear to be occurring consistently throughout the Study Area, irrespective of geology or aquifer, although likely close to the water table.





3.2 Current Survey Results (Stage 2)

The current survey recorded a total of 329 subterranean fauna specimens, comprising approximately 73% stygofauna (240 specimens), 26% amphibious (88 specimens) and less than 1% troglofauna (one specimen). Approximately 70 % (222 specimens) of the total specimens were recorded from within the Service Corridor. They consisted almost entirely of stygofauna and a single amphibious specimen (Enchytraeidae sp.). Of the stygofauna, the cyclopoid copepod *Fierscyclops* cf. *fiersi* was the most abundantly recorded, with over 200 specimens recorded from a single site. Two harpacticoid taxa, *Parapseudoleptomesocra* cf. *tureei* and *Schizopera* sp. and an amphipod taxon, Paramelitidae sp. U3S were also recorded, in lower abundances (Figure 3-2). Following molecular analysis, it is suspected that *Fierscyclops* cf *fiersi* and Paramelitidae sp. U3S, will align with *Fierscyclops* (*Pilbaracyclops*)? *frustatio* and Paramelitidae `sp. Biologic-AMPH031` respectively. These taxa were recorded from the same site during the Stage 1 survey.

Within the Havieron Project Area, 98 subterranean fauna, representing five taxa (identified taxonomically), were recorded, consisting of 10 stygofauna, 87 amphibious fauna and one troglofauna. The taxa recorded from the Havieron Project Area are discussed in further detail below.

Amphipods

One amphipod taxon, Paramelitidae sp. U3S, was recorded within the Project Area (Figure 3-2). This taxon is a stygobite (true stygofauna) and was recorded from a single site (HAVWB03) located within a localised patch of higher prospective stygofauna habitat represented by alluvials and calcareous cement. A paramelitid amphipod, Paramelitidae `sp. Biologic-AMPH027`, was also recorded from this site during the Stage 1 survey, and it is likely that molecular analysis will align with this morphospecies (Biologic, 2020). The Paramelitidae are a diverse family of amphipods, containing several SRE representatives as well as representatives with larger local distributions (Finston *et al.*, 2011).

Microcerberid Isopods

One isopod from the family Microcerberidae (Microcerberidae sp.) was recorded within the Project Area (Figure 3-2). This taxon is a stygobite (true stygofauna), recorded from the same site as Paramelitidae sp. U3S (HAVWB03) located within a localised patch of higher prospective stygofauna habitat. Microcerberids were not recorded from the Project Area during the Stage 1 survey, however several specimens were collected in the Telfer region (Biologic, 2020) and will be used for molecular comparison. Microcerberids are adapted to subterranean and interstitial (inhabiting saturated aquatic sediments) habitats and might be able to disperse during flood events (Bishop *et al.*, 2020; Coineau & Albuquerque, 2001). Very little work has been done on this group in Australia, and from international research, this group is known to be locally restricted (Coineau & Albuquerque, 2001). As such this taxon is a potential SRE.

Enchytraeid annelid worms

A total of 88 specimens belonging to the Oligochaeta family Enchytraeidae (Enchytraeidae sp.) were collected within the Project Area (Figure 3-2). These were classed as amphibious based on results of



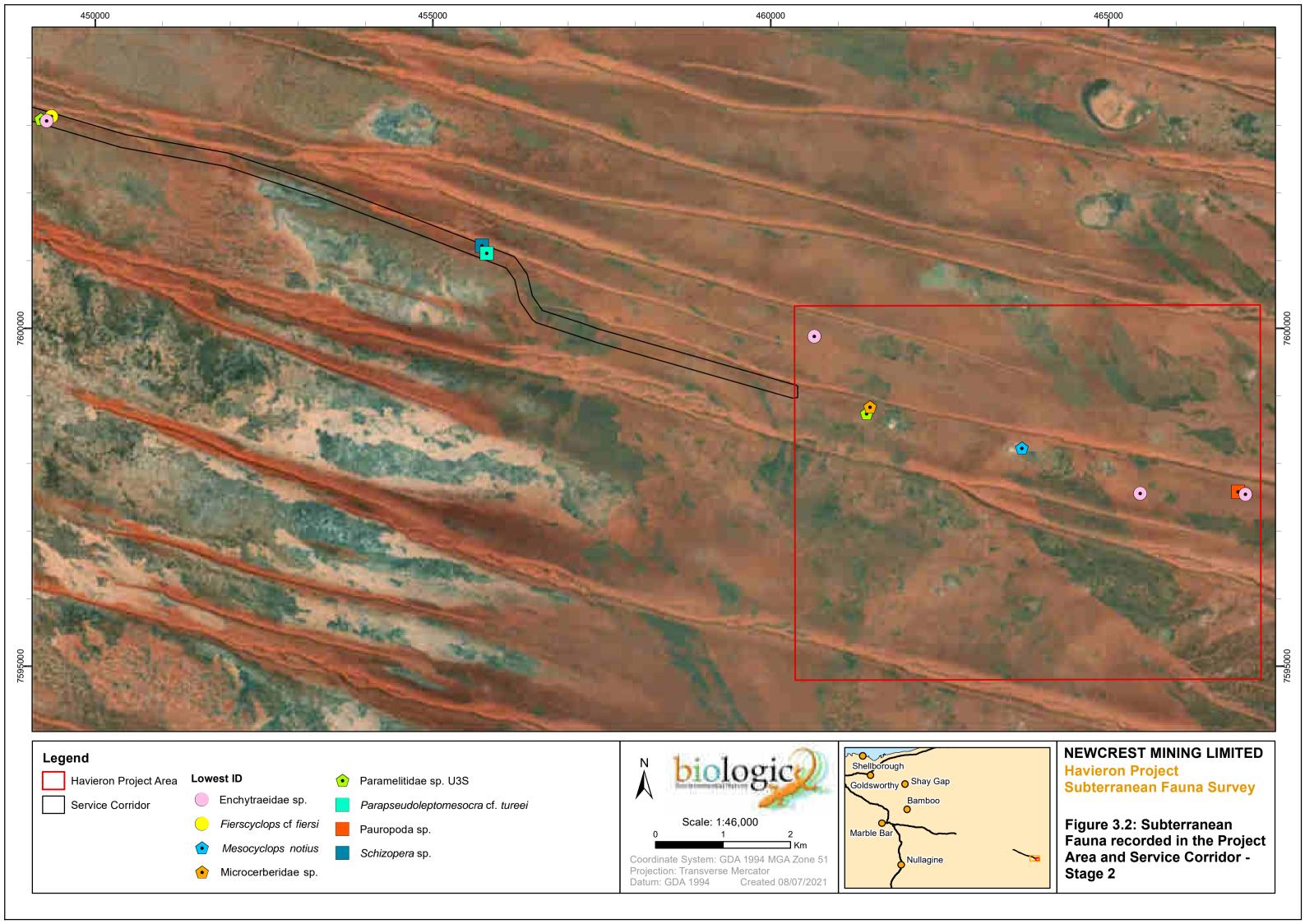
other subterranean surveys, grey literature and the result of the Stage 1 survey, as specimens from this family are frequently collected in samples from both above and below the water table, i.e. troglofauna traps and either hauls or haul-scrapes (Biologic, 2020). As there is no taxonomic framework for this family in Australia, identification past family level is not possible (Pinder pers. comm. 2020). Molecular analysis is currently underway, with the aim of aligning these specimens with Enchytraeid morphospecies recorded during Stage 1 survey work, including Enchytraeidae `sp. Biologic-OLIG023` and Enchytraeidae `sp. Biologic-OLIG025`. Taxa in this family are highly likely to have distributions of catchment-scale to basin-scale, or greater. They appear to tolerate extremes in temperature, salinity and depth and are generalists, principally living off plant residues (Didden, 1991; Ponge, 1991). There is mounting evidence that enchytraeids should not be considered SREs. Therefore, any 'new' species are very likely to be artefacts of a significant lack of knowledge, both in terms of taxonomy and collection records.

Cyclopoid copepods

One cyclopoid copepod species, *Mesocyclops notius*, was recorded from a single site (HAVWB02) within the Project Area (Figure 3-2). This species is likely to be a stygoxene, known to occur in surface waters including lakes, dams, and reservoirs (Ueda & Reid, 2003). As this species was recorded from a water bore less than 100m from a claypan, it is likely that this species would also persist in the surface water claypans within the Project Area. *Mesocyclops notius* has an Australia wide distribution and has been recorded from Western Australia, New South Whales, Northern Territory and Queensland (Pesce *et al.*, 1996) (Ueda & Reid, 2003).

Pauropods

One troglofauna taxon, from the class Pauropoda (Pauropoda sp.) was recorded from a single site (BIO_014) within the Project Area (Figure 3-2). This represents the only troglofauna taxa recorded from the Project Area to date. The taxonomic and ecological framework for pauropods in Australia is limited, though many species are likely to have limited ranges. The taxon collected from the Project Area is a potential SRE.





3.3 Stage 1 and Stage 2 Result Summary

Table 3-1 summarises the subterranean fauna results recorded within the Project area during Stage 1 and 2 survey work.

Table 3-1: Subterranean fauna recorded within the Project Area during Stage 1 and 2 surveys

Taxonomy	Morphospecies / Taxon	Level of identification	Abundance	Survey	Distribution / Range	Unique to Project Area	Subterranean Status, SRE Status	Taxonomic / habitat / distribution comments
Stygofauna Oligochaeta								
Enchytraeidae	Enchytraeidae sp.	Taxonomic	87	Stage 2	3 sites (BIO_014, BIO_015, BIO_017)	Unknown	Amphibious, unlikely SRE	Present throughout aquatic and subterranean habitats, catchment or basin scale distribution
	Enchytraeidae `sp. Biologic- OLIG023`	Molecular	101	Stage 1	2 sites (HAC9502, HAE001)	Yes	Amphibious, unlikely SRE	Present throughout aquatic and subterranean habitats, catchment or basin scale distribution
	Enchytraeidae `sp. Biologic- OLIG025`	Molecular	7	Stage 1	2 sites (HAD004, HAE004)	Yes	Amphibious, unlikely SRE	Present throughout aquatic and subterranean habitats, catchment or basin scale distribution
Arthropoda								
Crustacea Malacostraca								
Amphipoda	Paramelitidae sp. U3S	Taxonomic	6	Stage 2	1 site (HAVWB02)	Unknown	Stygobite, potential SRE	Habitat (alluvials and calcareous concrete). Likely to represent Paramelitidae `sp. Biologic-AMPH027`
	Paramelitidae `sp. Biologic-AMPH027`	Molecular	11	Stage 1	1 site (HAVWB02)	Yes	Stygobite, potential SRE	Habitat (alluvials and calcareous concrete).
Isopoda	Microcerberidae sp.	Taxonomic	2	Stage 2	1 site (HAVWB02)	Unknown	Stygobite, potential SRE	Habitat (alluvials and calcareous concrete). Cryptic group with uncertain distribution trends.
Copepoda								
Cyclopoida	Mesocyclops notius	Taxonomic	2	Stage 2	Australia	No	Stygoxene/phile, widespread	Present throughout aquatic and subterranean habitats
Ostracoda								
Popocopida	Humphreyscandonini sp. indet.	Taxonomic	1	Stage 1	1 site (HAVWB01)	Unknown	Potential stygobite, potential SRE	Habitat (alluvials and calcareous concrete). Occurs patchily within the Quaternary cover that forms part of the extensive Upper Unconfined Aquifer. Represented by two valves (dead shells). Indeterminate tribe-level taxon identification (Ivana Karanovic), likely unique species.
Troglofauna								
Myriapoda								
Pauropoda	Pauropoda sp.	Taxonomic	1	Stage 2	1 site (BIO_014)	Unknown	Troglofauna, Potential SRE	Cryptic group. Taxon has the potential to have a limited range.
Total Abundance Total Richness			218 9					