Bennelongia Environmental Consultants

Orebody 29, 30, 35 Expansion Stygofauna Desktop Assessment and Survey

Prepared for:

BHP WAIO

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Final Report

Short-Range Endemics I Subterranean Fauna

Waterbirds I Wetlands

Orebody 29, 30, 35 Expansion Stygofauna Desktop Assessment and Survey

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EXECUTIVE SUMMARY

BHP Western Australian Iron Ore (BHP WAIO) is investigating additional mining opportunities in the region between Newman Operations East and West at Orebodies 29, 30 and 35 in an area (the Survey Area) around two kilometres north of the town of Newman and 9.5 km west north-west of Ophthalmia Dam. At its closest point the Survey Area lies approximately three kilometres west north-west of the Ethel Gorge Aquifer Stygobiont Threatened Ecological Community (the Ethel Gorge TEC). Recognising there may be potential for impacts on subterranean fauna from mining operations in the Survey Area, BHP WAIO requested that Bennelongia Environmental Consultants (Bennelongia) undertake a desktop review of stygofauna and habitat in the Survey Area, as well as conduct a detailed field survey. The specific aims of this report are to:

- undertake an assessment of previous records of subterranean fauna in a 100 km^2 desktop assessment search area (the Study Area), and associated literature, to determine the likelihood based on sub-regional information of the Survey Area supporting stygofauna, including significant species and/or communities;
- provide the results of a stygofauna survey, covering parts of the Survey Area that have not previously been sampled, consisting of a detailed survey and targeted sampling for two species identified from previous surveys as being potentially restricted - Enchytraeidae sp. OB3 and Naididae sp. N08; and
- determine on the basis of current survey and historical records whether there are any further species that may be potentially restricted to the Survey Area and confirm the status of the two species of interest.

Desktop Assessment
To assess the likelihood of stygofauna occurring at the Survey Area a review of animal records and associated geologies was made within a 100 km x 100 km desktop Search Area (the Study Area), where a record is the presence of a species at a location on a specific date.

In total, 4,095 stygofauna records were retrieved from the desktop Search Area which equate to at least 183 different species of stygofauna. The majority of the records are arthropods (82.8%), followed by annelids (12.6%) and nematodes (3.9%) with some representation from rotifers (0.4%) and platyhelminthes (0.3%). Twenty-seven of the 183 desktop search area species were collected from the Survey Area, and seven of these are core endemic species of the Ethel Gorge TEC. In addition, two historically collected annelid worms have been identified as being potentially restricted and within previously assessed potential impact areas. These are Enchytraeidae sp. OB3 (Order: Enchytraeida), and Naididae sp. N08 (Order: Haplotaxida). One of these, Naididae sp. N08, falls outside the current Survey Area and is therefore not discussed within this report.

The desktop search has revealed an abundant and diverse regional stygofauna community. Considering habitat prospectivity at the Survey Area, geology is predominantly a combination of tertiary detritals and banded iron-formation (BIF), both of which can host aquifers, with the former hosting the most prospective valley-fill aquifers. Chemistry within groundwater is within ranges required for stygofauna to occur. Further, groundwater within the Survey Area appears to be relatively shallow ranging from 5.6 metres below ground level (mbgl) to 43.0 mbgl. Combined, it is likely that a moderate to rich stygofauna community exists at the Survey Area. However, understanding the depth of detrital and BIF layers would be useful to further characterise the resident aquifers and their prospectivity.

Survey
Three rounds of sampling were conducted during 2023. Eighteen net haul samples were taken from 6-7 February, 12 net haul samples taken from 14-15 June and 12 net haul samples taken from 3-4 October. A total of 42 stygofauna samples from 19 bore holes were collected in the three rounds of sampling. Stygofauna identifications were made using published, unpublished, and informal taxonomic keys, as well as species descriptions in the scientific literature. In addition, DNA sequencing was completed

successfully on 13 samples to help resolve taxonomic identifications and increase understanding of species distributions.

Seventeen out of a total of 19 drill holes sampled (90%) yielded stygofauna. Over the three rounds of survey, 341 stygofauna specimens from 52 records were collected. In addition, four specimens from four records (three additional drill holes) were collected within the Survey Area as part of an overlapping BHP survey in 2023 for orebodies 32 West, 28 and 33. Combined, 345 stygofauna specimens were collected from the Survey Area during 2023, from 56 records made up of at least 16 species. Stygofauna species collected were mostly arthropods (82.3 %), annelids (12.5 %) and nematodes (6.3 %) which is consistent with stygofauna communities in the Pilbara. Two species have distributions that are currently known from only within the Survey Area and are therefore potentially restricted - one amphipod, *Kruptus* \`BAM227\`, and one syncarid, \` *Billibathynella* \`BSY249\`. A further three species are considered core endemic species to the Ethel Gorge TEC – *Chydaekata acuminata* (amphipod), *Pygolabis humphreysi* (isopod) and *Pilbaracandona eberhardi* (ostracod). None of the species recorded from the Survey Area are conservation-listed. The potentially restricted annelid worm Enchytraeidae sp. OB3 (Order: Enchytraeida) was not recorded during this survey.

Further information on the three species potentially restricted to the current Survey Area is provided here:

***Kruptus* \`BAM227\`**

Kruptus \`BAM227\` is an amphipod from family Paramelitidae. It has been identified by both morphological and molecular evidence to be a unique species. It was recovered from three drill holes in the north-west of the Survey Area; ERCSGW0024, ERCSGW0025 and HEA0353 which are in relatively close proximity to a drainage line. The depth to water in these three holes at the time of survey was approximately 5.6, 8.2 and 22.0 m, respectively. The surface geology at all three is alluvium and, considering that groundwater depth is shallow, this species may reside in detritals, which are widespread and extend outside the Survey Area, likely following the adjacent drainage line. Reviewing detailed lithology of these sites would help confirm host geology.

***Billibathynella* \`BSY249\`**

Billibathynella \`BSY249\` is a syncarid of family Parabathynellidae. It has been identified by morphological evidence to be a unique species. It was recovered from one site, HWHB1665 (previously WBGWO11), on two different occasions – two specimens in June and one in October. This site lies in a drainage line and it also has alluvial host geology. Depth to groundwater at the time of survey was approximately 8.5 m. This suggests that this species lives in detritals that extend to outside the Survey Area.

Enchytraeidae sp. OB3

Enchytraeidae sp. OB3 is an enchytraeid worm of order Haplotaxida. This species, identified by both morphological and molecular evidence to be unique, is known from two specimens collected by Subterranean Ecology in 2011 from a single hole, HEOP0317 (previously called W13), in the eastern part of the Survey Area. This hole is inside the TEC buffer boundary but outside the area of mapped calcrete considered to be the core habitat of Ethel Gorge. At the time of the collection the depth to water in HEOP0317 was approximately 13–16 m. It is inferred that Enchytraeidae sp. OB3 inhabits detritals and, possibly, dolomite. Mapped surficial geology suggests that detrital habitat in alluvium and colluvium may extend from HEOP0317 beyond the Survey Area. The collection site for Enchytraeidae sp. OB3 is upstream of the main Fortescue River channel so it is possible that the species occupies suitable habitat further downstream.

Conclusion

The desktop assessment revealed an abundant and speciose stygofauna community in the region with 4,095 records consisting of at least 184 species. Based on surface geology and hydrogeology at the Survey Area, extrapolations from the desktop search predicted that an abundant stygofauna community should also occur there, which was confirmed as a total of 345 specimens of 16 species were recovered from 56 records. Two new species were described that are known only from within the Survey Area, *Kruptus* \`BAM227\` and *Billibathynella* \`BSY249\`. Both of these species occur in holes that likely reside in detrital layers that extend beyond the Survey Area and therefore are unlikely to be restricted within the local area.

One historically collected species, Enchytraeidae sp. OB3, remains potentially restricted as no other occurrences of this species were recorded after the current survey, including after genetic analysis of regionally occurring related specimens. However, as with the two more recently collected species, host geology is likely detrital layers which extend well beyond the Survey Area and therefore it is expected that this species is not restricted within the local area.

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1. INTRODUCTION

BHP Western Australian Iron Ore (WAIO) has extensive operations mining iron ore in the Newman region of the Pilbara, Western Australia. Continued mining requires exploration to identify new, economically viable iron deposits and assessment of the environmental impact of any proposed mining. BHP WAIO is currently investigating additional mining opportunities in the region between Newman Operations East and West at Orebodies 29, 30 and 35 in an area (the Survey Area) around two kilometres north of the town of Newman and 9.5 km west north-west of Ophthalmia Dam (Figure 1). At its closest point the Survey Area lies approximately three kilometres west north-west of the Ethel Gorge Aquifer Stygobiont Threatened Ecological Community (hereafter referred to as the Ethel Gorge TEC). The region has a high stygofauna species richness, likely reflective of the highly prospective habitat found in the region as river systems and palaeovalleys are widespread, and the calcrete aquifer associated with the Ethel Gorge TEC is in the vicinity. Calcrete aquifers are well known as prospective stygofauna habitat in Western Australia (Halse *et al.* 2014, Halse 2018b).

Subterranean fauna comprises mostly small invertebrates that can be divided into two groups: aquatic stygofauna that live in groundwater, and air-breathing troglofauna that occupy substrates between the water table and the lower layers of surface soil. As a result of living underground, a high proportion of subterranean species have localised distributions (Gibert and Deharveng 2002). Species with restricted ranges are vulnerable to extinction from anthropogenic activities that lead to habitat loss and other environmental changes (Fontaine *et al.* 2007; Ponder and Colgan 2002). Therefore, subterranean fauna is one of the factors examined by the Environmental Protection Authority (EPA) when assessing the environmental impacts of development projects in Western Australia (EPA 2016).

Recognising there may be potential impacts on subterranean fauna from mining operations in the Survey Area, BHP WAIO requested that Bennelongia Environmental Consultants (Bennelongia) undertake a desktop review of stygofauna and habitat in the Survey Area, as well as conduct a detailed field survey. All survey work followed guidelines for subterranean fauna surveys in Western Australia as described in EPA guidance (EPA 2016, 2018, 2021), as well as BHP WAIO's Subterranean Fauna Assessment Methods (0020346) and Biological Survey Spatial Data Requirements (SPR-IEN-EMS-015).

The specific aims of this report are to:

- undertake an assessment of previous records of subterranean fauna in a 100 km^2 desktop assessment search area (the Study Area), and associated literature, to determine the likelihood based on sub-regional information of the Survey Area supporting stygofauna, including significant species and/or communities;
- provide the results of a stygofauna survey, covering parts of the Survey Area that have not previously been sampled, consisting of a detailed survey and targeted sampling for two species identified from previous surveys as being potentially restricted - Enchytraeidae sp. OB3 and Naididae sp. N08; and
- determine on the basis of current survey and historical records whether there are any further species that may be potentially restricted to the Survey Area and confirm the status of the two species of interest.

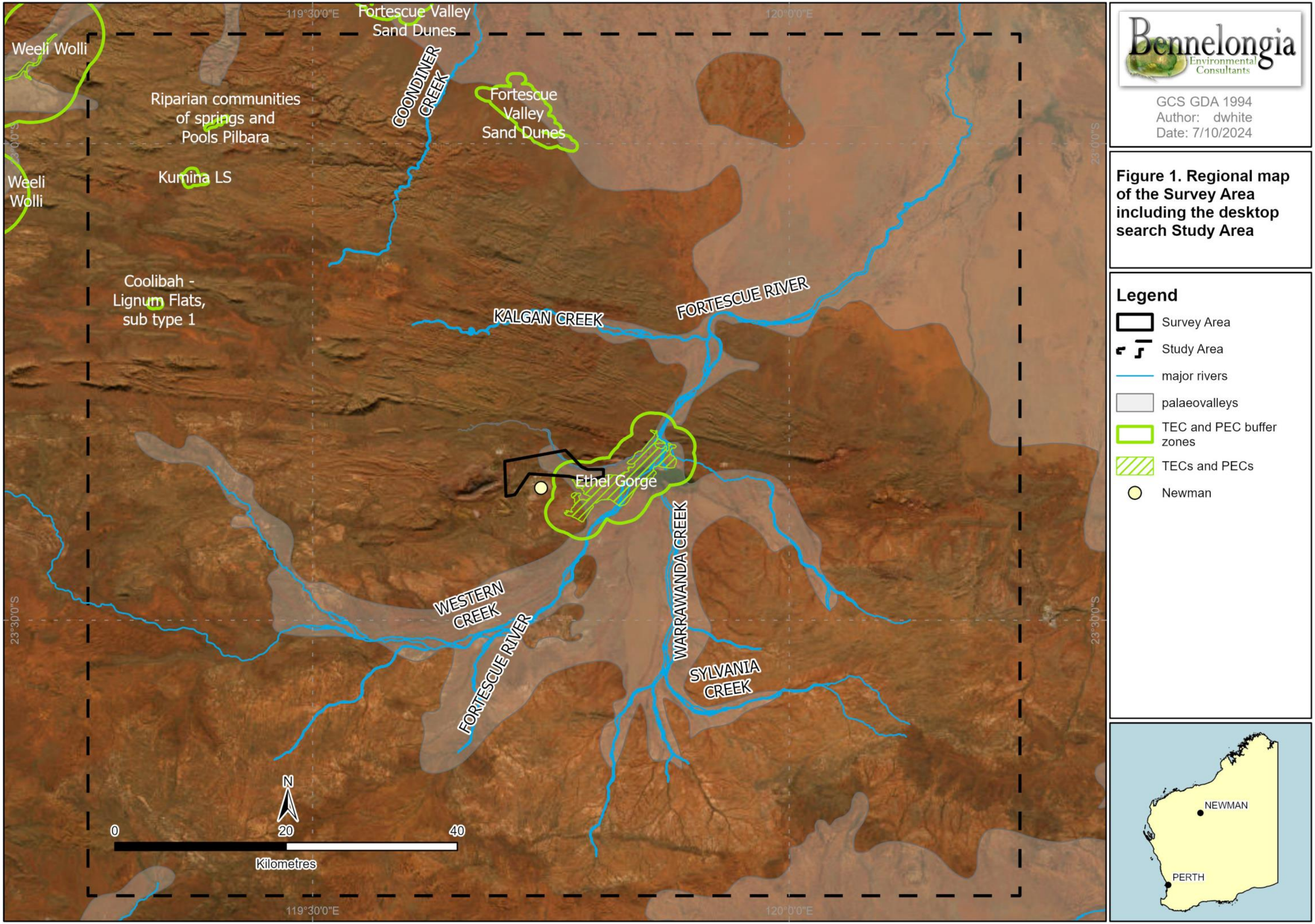
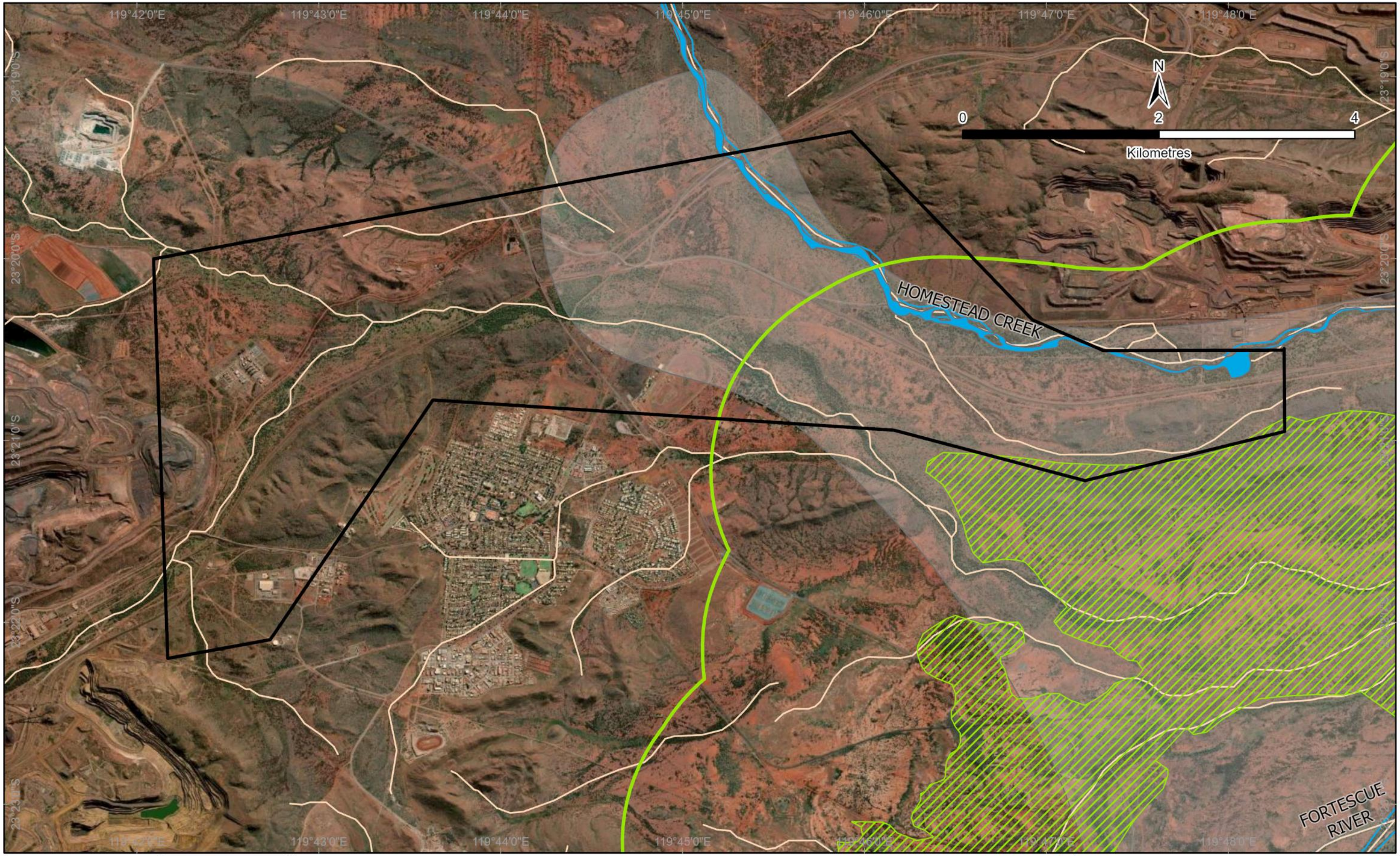


Figure 1. Regional map of the Survey Area including the desktop search Study Area



Bennelongia Environmental Consultants

GCS GDA 1994
Author: dwhite
Date: 10/07/2024

Legend

- Survey Area
- water body
- drainage lines
- palaeovalleys
- Ethel Gorge TEC Buffer Boundary
- Ethel Gorge TEC

Figure 2. Local map of the Survey Area

2. FRAMEWORK

2.1. Stygofauna

There are two main types of subterranean fauna – troglofauna that inhabit subterranean spaces above the water table, including interstices, voids, vugs, cavities and fissures; and stygofauna that inhabit the groundwater that fills these spaces. Stygofauna, like all subterranean fauna, usually exhibit adaptations to life underground that include the loss of eyes and skin pigmentation and the elongation of appendages and sensory structures. While most species in Western Australia are invertebrates, there is at least one record of a stygofaunal fish (Whitely 1945). The Pilbara region is recognised as globally significant for subterranean fauna, with at least 1,300 species of stygofauna (Halse 2018a, b), although reliable estimates are hindered by a developing and sometimes non-existent taxonomic framework for some of the subterranean animal groups.

2.2. Habitat

The occurrence and distribution of subterranean fauna is closely related to lithology and geology. Geologies that contain more of the required subterranean spaces - interstices, voids, vugs, cavities and fissures - represent the most prospective habitat. Stygofauna communities are usually richest in alluvial and calcrete aquifers, especially within palaeochannels, although they may also be found in iron formations, especially detrital and channel iron (Halse 2018a). Water chemistry (especially salinity and the amount of carbonate) also affects the occurrence of stygofauna (Halse 2018b).

Both vertical and lateral connectivity of subterranean spaces are important factors in determining the distribution of subterranean fauna. Vertical connectivity of spaces to the surface affects supply of carbon and nutrients to subterranean communities (Korbel and Hose 2011), while lateral connectivity of spaces enables underground dispersal of animals. Topographic and geological features such as valleys and dykes may act as barriers to dispersal of subterranean fauna and may lead to species having highly restricted distributions, but it is difficult to predict this from habitat information alone because the characteristics controlling distributions are finer scale than the available information. As a result of limited capacity for dispersal, many stygofauna species are short range endemics (SREs), and in fact have much smaller ranges than Harvey's (Harvey 2002) range criterion of 10,000 km^2.

2.3. Geology and Hydrogeology

2.3.1. Geology

The 1:250,000 geology maps from Geosciences Australia show that the majority of the Survey Area sits on the Upper and Lower subgroups of the Hamersley Group stratigraphic unit within the Hamersley Basin, with the north-west of the Survey Area intersecting sequence 5 of the Fortescue Group. The Upper Hamersley Group is characterised by metamorphosed rock including banded iron-formation (BIF), chert, mudstone, siltstone, rhyolite and numerous dolerite sills. The Lower Hamersley Group is also characterised by metamorphosed rock including BIF, carbonate sedimentary rocks, shale, siltstone and chert. Fortescue Group sequence 5, which also intersects with a small central region in the south of the Survey Area, includes the Jeerinah Formation that is characterised by siliciclastic sedimentary rocks, mafic volcanic rocks and minor felsic volcanic rocks, local carbonate rocks, chert and dolerite sills. Surface layers of BIF are typically weathered and form a vuggy carapace (hardcap) over much of the outcropping areas of rock in the Pilbara.

2.3.2. Hydrogeology

In a conceptual sense, the regional aquifers of the Pilbara can be divided into three general types:

- Unconsolidated sedimentary aquifers (i.e. occurring in alluvium, detrital iron);
- Chemically deposited aquifers of drainage channels (i.e. calcrete and channel iron); and
- Fractured-rock aquifers (i.e. BIF).

However, from a biological perspective, there is little distinction between sedimentary and chemically deposited aquifers. Chemically deposited aquifers can be regarded as a special kind of unconsolidated sedimentary aquifer and together these two aquifer types are often referred to as valley-fill aquifers in the Pilbara. Fractured rock aquifers are usually significantly less prospective for stygofauna than valley-fill aquifers although stygofauna can occur in all three aquifer types. The likelihood of stygofauna occurrence decreases significantly, however, where groundwater is more than 30 m below ground level (Halse *et al.* 2014) and abundant stygofauna communities are most likely to be in valley-fill aquifers (Halse 2018b).

In broad terms, there is abundant potential for groundwater re-charge in the vicinity of the Survey Area, with the Homestead Creek running through the eastern section, various drainage lines traversing the area and the Fortescue River running 2.5 km to the east. Further, the Sylvania palaeovalley surrounds part of the Survey Area and intersects the eastern side (Figure 2). Regional hydrogeological monitoring 5 to 10 km to the south-west of the Survey Area at individual orebodies 29, 30 and 35 showed that these orebodies are predominantly hosted in permeable Marra Mamba Formation, which is likely to form significant, localised aquifers where below the water table (Aquaterra 2012). The majority of detritals in the vicinity of these orebodies are above the water table (ranging from 30 metres below ground level (mbgl) at Orebody 29 to 90 mbgl at Orebody 35 (BHP 2023)) and, in general, only deeper aquifers in mineralised rock provide potential habitat for stygofauna in the Study Area (Bennelongia 2019). It is generally considered that mineralised, fractured and weathered rock formations are prospective for stygofauna, provided that ample subterranean spaces occur, and depth to water is not excessive (Halse *et al.* 2014). However, rich stygal assemblages are more commonly found in surficial Tertiary deposits such as calcrete and alluvium.

2.4. TECs and PECs

Legislation dealing with conservation of fauna in Western Australia consists of the *Biodiversity Conservation Act 2016* (BC Act) at the State level and the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) at the national level. While all subterranean fauna species are protected in a general sense under these acts, some stygofauna and troglofauna species have been listed for special protection under threatened species legislation, or by the Department of Biodiversity, Conservation and Attractions (DBCA) as priority species. Similarly, some subterranean communities have been listed as either threatened ecological communities (TECs) under the BC Act and EPBC Act, or as priority ecological communities (PECs) by DBCA.

There are currently six subterranean PECs in the Pilbara. Of these, four PECs pertain to stygofaunal communities. These include three Priority 1 PECs - the subterranean community of the Weeli Wolli Spring, the stygofauna of Barrow Island faunal communities and the stygofauna community of the Bungarooo Aquifer. In addition, the stygofaunal communities of the Western Fortescue Plains freshwater aquifer is a Priority 4 PEC. None of these are in close proximity to the Survey Area.

The sole subterranean TEC in the Pilbara is the Ethel Gorge aquifer stygobiont TEC (Ethel Gorge TEC). It is associated with the calcrete aquifer on the Fortescue River east of Newman and is listed only under the BC Act as Endangered. Groundwater levels in the Ethel Gorge TEC are currently affected by both abstraction and recharge from Ophthalmia Dam (Figure 2). The surface geology of the area is composed of carbonate calcrete, alluvium and colluvium. The rich stygofauna assemblage of the community includes oligochaetes, arachnids, ostracods, copepods, syncarids, isopods and amphipods. Thirty-seven core endemic species have been described (Bennelongia 2013), and recent estimates based on species accumulation rarefaction suggests there are between 44 and 58 species in the TEC. Monitoring done in 2017 and 2022 recovered 36 species with at least two core amphipod species of genus *Chydaekata* (Bennelongia 2022; Stantec 2017, 2022). The Survey Area is adjacent to the south-west of the Ethel Gorge TEC with a small overlap of the Survey Area's south-east border (Figure 2).

3. DESKTOP ASSESSMENT

To assess the likelihood of stygofauna occurring at the Survey Area a review of animal records and associated geologies was made within a 100 km x 100 km desktop search area (the Study Area), where a record is the presence of a species at a location on a specific date. The Study Area was centred on the centre of the Survey Area and was bounded by coordinates 22°53′6″S to 23°47′20″S and 119°15′52″E to 120°14′32″E, equating to a search area of approximately 10,000 km^2 (Figure 1).

3.1. Methods

A three-stepped approach was employed to assess habitat prospectivity. Initially, animal records from within the Study Area were retrieved from the Western Australia Museum (WAM) and Bennelongia databases, as well as scientific literature and previous subterranean fauna survey reports. Records from the Survey Area that were collected prior to 2023 are included in the desktop search area records. A search of any conservation-listed species was made using DBCA's most recent list of conservation codes for Western Australia fauna downloaded from https://www.dbca.wa.gov.au/wildlife-and-ecosystems/animals/list-threatened-and-priority-fauna (accessed 30/04/2024), with the view to incorporate results into the overall assessment of subterranean fauna conservation values.

Second, geology and hydrogeology at the Survey Area were assessed. Geology was assessed by mapping a Map Grid Australia (MGA) surface geology layer from zone 50, based on the Geocentric Datum of Australia 2020 (GDA), using ArcGISPro v2.9.5. This was compared to geological data from the 1:100,000 Geological Series, provided by the Geological Survey of Western Australia. Hydrogeology was assessed using previous reports and reviewing monitoring data collected during the current survey.

Finally, associations of historical stygofauna records with geology/hydrogeology of the wider Study Area were made so that habitat prospectivity and likelihood of stygofauna occurrence at the Survey Area could be inferred.

3.2. Results

3.2.1. Stygofauna

In total, 4,095 stygofauna records were retrieved from the desktop Search Area (Appendix 1). These 4,0954 records equate to at least 183 different species of stygofauna. The majority of the records are arthropods (82.8%), followed by annelids (12.6%) and nematodes (3.9%) with some representation from rotifers (0.4%) and platyhelminths (0.3%). Twenty-eight of the 183 desktop search area species have been historically collected from the Survey Area (Table 1). Seven of these are core endemic species of the Ethel Gorge TEC as described in Bennelongia (2013).

Table 1: Historically collected stygofauna from within the Survey Area.
Species shaded in orange indicate core endemic species of the Ethel Gorge TEC as described in (Bennelongia 2013).

Higher Taxonomic Order	Lowest ID	No. of specimens	Known Distribution
Annelida			
Clitellata			
Oligochaeta	Oligochaeta sp.	3	n/a
Enchytraeida			
Enchytraeidae	Enchytraeidae sp.	7	n/a
	Enchytraeidae sp. OB3	2	Pilbara
	Enchytraeidae sp. OB_MC†	10	Pilbara‡‡
	Enchytraeus sp. AP PSS1 s.l.	17	Westen Australia
Haplotaxida			
Phreodrilidae	Phreodrilidae sp.	2	n/a

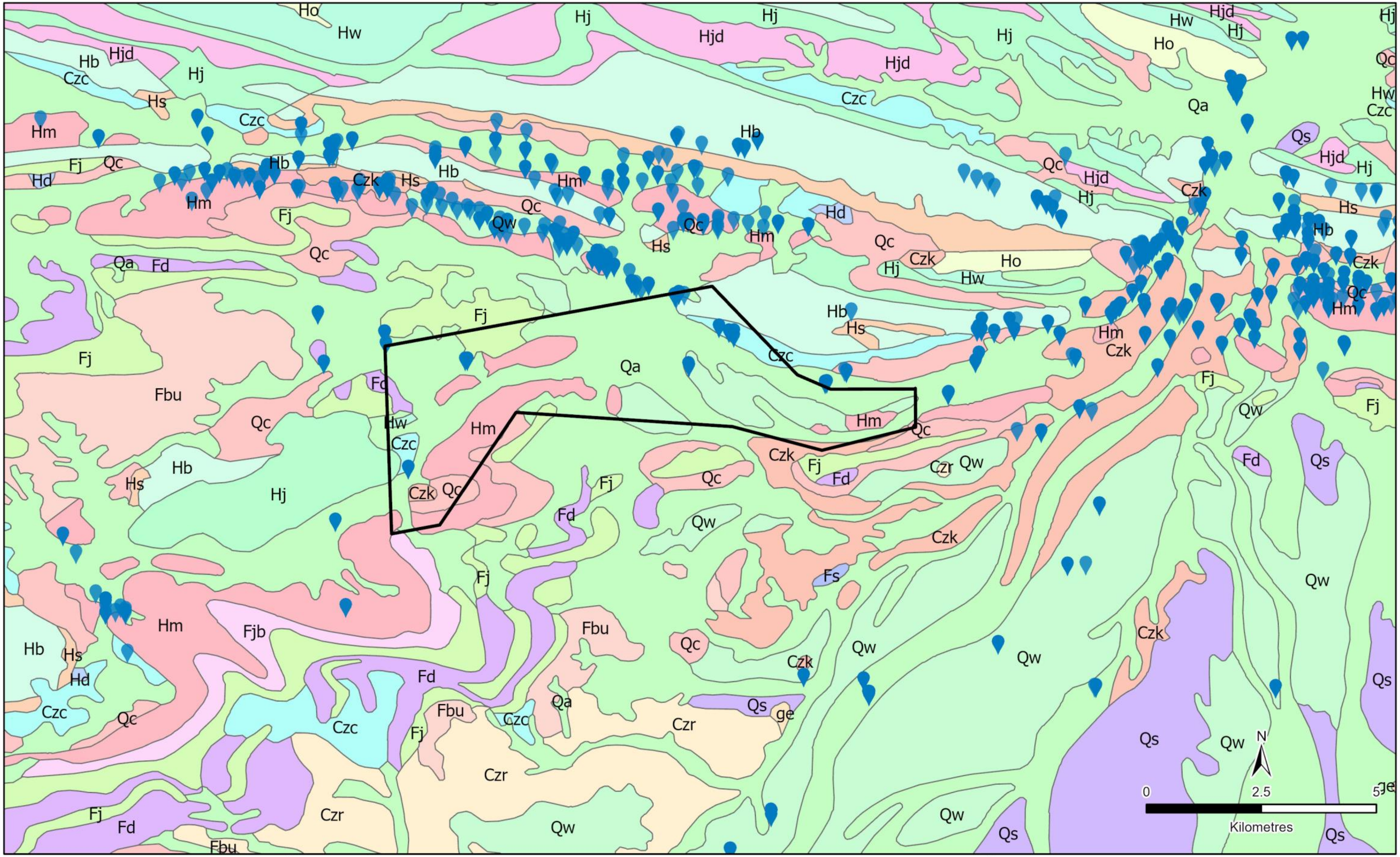
Higher Taxonomic Order	Lowest ID	No. of specimens	Known Distribution
	Phreodrilidae sp. AP DVC s.l.	2	Westen Australia
	Phreodrilidae sp. P11	5	Pilbara
	Phreodrilus sp.	20	n/a
Arthropoda			
Chelicerata			
Arachnida			
Trombidiformes			
Pezidae	*Peza* `ACA001`	1	Pilbara
Crustacea			
Malacostraca			
Syncarida			
Bathynellacea			
Bathynellidae	Bathynellidae sp.	8	n/a
	Pilbaranella `sp. A`	4	East Pilbara
	Pilbaranella `sp. B`	1	East Pilbara
	Pilbaranella ethelensis	6	East Pilbara
Parabathynellidae	*Billibathynella cassidis*	3	East Pilbara
Amphipoda	Amphipoda sp.	30	n/a
Paramelitidae	Paramelitidae gen. no. 1 `AMP001`	32	East Pilbara
	Paramelitidae `sp. OB1 (B33)`	1	East Pilbara
	Paramelitidae `sp. OB1`	2	East Pilbara
	Chydaekata acuminata	36	Pilbara
	Chydaekata sp.	14	n/a
Isopoda			
Tainisopidae	*Pygolabis humphreysi*	7	East Pilbara
Maxillopoda			
Copepoda			
Cyclopoida			
Cyclopidae	*Diacyclops humphreysi*	70	Pilbara
	Diacyclops humphreysi s.l.	623	Pilbara
	Diacyclops sobeprolatus	52	Pilbara
	Diacyclops sp.	4	n/a
Harpacticoida			
Ameiridae	*Archinitocrella newmanensis*	2	East Pilbara
Parastenocarididae	*Parastenocaris* `COP001`	5	East Pilbara
	Parastenocaris jane	9	Western Australia
	Parastenocaris sp.	9	n/a
Ostracoda	Ostracoda sp.	1	n/a
Podocopida			
Candonidae	Candoninae sp.	2	n/a
	Candonopsis tenuis	1	Western Australia
	Meridiescandona `3` (PSS)	1	Pilbara
	Origocandona `BOS099`	1	East Pilbara

Higher Taxonomic Order	Lowest ID	No. of specimens	Known Distribution
	Pilbaracandona `OST002`	1	South-east Pilbara
	Pilbaracandona kosmos	4	South-east Pilbara
Nematoda	Nematoda spp.	1	n/a

†This species name was synonymised with *Enchytraeus* sp. Ench6 in Bennelongia (2015) and then Enchytraeidae sp. E12 in Brown *et al* (2015). ‡‡This is the known distribution of Enchytraeidae sp. E12.

Table 2: Codes and definitions for surface geology associated with Figure 3.

Code	Definition
Czc	Colluvium - partly consolidated valley-fill deposits
Czk	Calcrete - sheet carbonate usually formed in major drainage lines
Czr	Laterite, includes surficial hematite-goethite deposits on banded iron-formation; forms Hamersley Surface
Fbu	Upper mafic volcanic unit; metabasalt with minor metadolerite sills
Fd	Metadolerite sills intruded into Fortescue Group; medium- to coarse-grained, massive grey-green rock, usually foliated
Fj	JEERINAH FORMATION: interbedded mudstone, siltstone and chert with minorfelsic tuff, dolomite and sandstone
Fjb	Metabasalt; pillows locally well developed
Hb	BROCKMAN IRON FORMATION: banded iron-formation, chert and minor shale (2490+-20 Ma, U-Pb)
Hd	WITTENOOM DOLOMITE: dolomite; interbedded thin chert, shale and dolomite in upper part
Hj	WEELI WOLLI FORMATION: interlayered banded iron-formation and metadoleritic sills, minor shale
Hjd	WEELI WOLLI FORMATION: Medium- to coarse-grained massive grey-green metadolerite sills, usually foliated
Hm	MARRA MAMBA IRON FORMATION: chert, ferruginous chert and banded iron-formation with minor shale
Ho	BOOLGEEDA IRON FORMATION: fine-grained, finely laminated, dark grey-brown to black, flaggy iron-formation; minor chert, jaspilite and shale
Hs	MOUNT McRAE SHALE and MOUNT SYLVIA FORMATION: interbedded shale, chert and banded iron-formation
Hw	WOONGARRA VOLCANICS: quartz - or feldspar-phyric rhyolite and rhyodacite as sills or flows; tuff and minor jaspilitic banded iron-formation (2470 +- 30 Ma, U-Pb; 2370 Ma, Rb-Sr)
Qa	Alluvium - unconsolidated silt, sand and gravel
Qc	Colluvium - unconsolidated quartz and rock fragments in soil
Qs	Eolian sand - in sheets and dunes
Qw	Alluvium and colluvium - red-brown sandy and clayey soil



Bennelongia Environmental Consultants

GCS GDA 1994
Author: dwhite
Date: 13/05/2024

Legend

Survey Area

historical stygofauna records

Figure 3. Desktop Study Area stygofauna records mapped on to surface geology

3.2.2. Geology and Hydrogeology of the Survey Area

Geology

Surface geology mapping shows the eastern regions of the Survey Area are primarily sedimentary detritals such as unconsolidated silt, sand and gravel alluvial layers with some exposed Marra Mamba and Brockman BIF (Figure 3, Table 2). The eastern boundary of the Survey Area also intersects exposed sheet carbonate calcrete. There is a greater prevalence of exposed Marra Mamba iron formation in the western areas of the Survey Area, as well as evidence of Jeerinah formation and metadolorite sills. There is also a smaller amount of exposed sheet carabonate calcrete.

Hydrogeology

Using our own hydrogeological measurements taken while collecting the 42 samples from 19 different drill holes for this survey, depth to ground water ranged from 5.6 metres below ground level (mbgl) to 43.0 mbgl (mean 18.4 mbgl, s.d. 8.1). Electrical conductivity, an indicator of salinity, ranged from 445.6 µs (microsiemens)/cm (mean 2,105.6 µs/cm, s.d. 1194.0) to 4,695 µs/cm which is equivalent to 245.1 mg/L total dissolved solids (TDS) to 2582.3 mg/L TDS (mean 1,158.1 mg/L TDS, s.d. 656.7). This range is well within the currently understood threshold for stygofauna tolerance of 25,000 mg/L TDS (Hose *et al.* 2015; Humphreys 2009). Water pH ranged from 3.5 (in a sole drillhole with site ID HEQ0012) to 7.7 (mean 7.0, s.d., 0.6). In summary, hydrogeological values are consistent with prospective stygofauna habitat.

3.2.3. Prospective Habitat

The desktop search has revealed an abundant and diverse regional stygofauna community. Geology at the Survey Area is predominantly a combination of tertiary detritals and BIF, both of which can host aquifers. Chemistry within groundwater is within ranges required for stygofauna to occur. Further, groundwater appears to be shallower at the Survey Area compared to orebodies 29, 30 and 35 and may occur in tertiary detritals, which host the most prospective valley-fill aquifers. Combined, it is likely that a moderate to rich stygofauna community exists at the Survey Area (greater than fracture-rock aquifer communities in terms of abundance and species richness, but less than calcrete aquifer communities) but understanding the depth of detrital and BIF layers would be useful to further characterise the resident aquifers and their prospectivity.

4. FIELD SURVEY

4.1. Methods

4.1.1. Sampling Effort

Three rounds of sampling were conducted during 2023. Eighteen net haul samples were taken from 6-7 February, 12 net haul samples taken from 14-15 June and 12 net haul samples taken from 3-4 October. A total of 42 stygofauna samples from 19 bore holes were collected in the three rounds of sampling (Table 3). The number of sites surveyed and samples taken is a reduction from the original sampling plan (22 sites per round and a total of 66 samples taken over the three rounds) due largely to the rehabilitation of holes and inaccessibility of sites. The final number of sites was communicated to and approved by BHP. The location of sampling sites is presented in Figure 4, and further information on the holes sampled and date of sampling is provided in Appendix 2.

Table 3: Sampling Effort

OB29/30/35	R1 (Feb 2023)	R2 (June 2023)	R3 (Oct 2023)	**TOTAL**
Stygofauna net haul	18	12	12	**42**

4.1.2. Sampling Techniques

Stygofauna was collected by lowering a small, weighted plankton net to the bottom of the hole and then agitated vigorously to stir benthic and epibenthic fauna into the water column, where animals were then captured as the net was slowly retrieved. Six separate net hauls were made in each hole (three with 50 µm mesh net and three with 150 µm mesh net). The contents of the net were transferred to 100% ethanol for preservation after each haul. Contamination between sites was avoided by washing the nets between the sampling of different drill holes.

4.1.3. Laboratory Processing

Stygofauna identifications were made using published, unpublished, and informal taxonomic keys, as well as species descriptions in the scientific literature. Morphospecies identifications based on the characters of existing species keys were used for undescribed species, and the lowest level of identification possible was reached given the constraints of sex, maturity of the specimens (juveniles and females are often impossible to identity to species level) and possible damage to body parts. During the final phase of identification, dissecting and compound microscopes were used, with the process often requiring dissection of specimens. After the taxonomic assessment was completed, representative animals were lodged with the WAM.

DNA sequencing was completed successfully on eight out of 19 stygofauna samples (i.e. 11 failures) from the current survey. In addition, DNA sequencing was completed successfully on five out of seven stygofauna samples (two failures) that were collected from outside the Survey Area as part of reference sampling. To summarise, 13 out of 26 samples were successfully sequenced for this work (Appendix 3). Depending on the size of the specimens, legs or whole animals were used and micro-pestled for DNA extractions using a Qiagen DNeasy Blood & Tissue kit (Qiagen 2006). Final elute volumes varied from 60 µL to 100 µL depending on the quantity and of dissected material. Primer combinations used for polymerase chain reaction (PCR) amplifications were LCO1490:HCO2198 and C1J1718:HC02198 for the MT-CO1 gene (Folmer *et al.* 1994; Simon *et al.* 1994). Next, dual-direction, Sanger sequencing was undertaken for PCR products by the Australian Genome Research Facility (AGRF). Sequences returned were edited and aligned in Geneious Prime v2022.2.2 (https://www.geneious.com) (Geneious 2024). Pairwise genetic distances to related sequences in the Bennelongia database were calculated as uncorrected p-distances (total percentage of nucleotide differences between sequences). Similarity to all sequences in the non-redundant nucleotide database at GenBank was determined using the Basic Local Alignment Search Tool nucleotide (BLAST) suite of applications (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al.* 1990). To visualise genetic distances and phylogenetic relationships between taxa, distance based phylogenetic trees were generated, also in Geneious v2022.2.2. Publicly available sequences on GenBank were included in phylogenetic analysis to provide a framework for assessing intra- versus interspecific variation and determining species boundaries. All validated DNA sequences were uploaded to GenBank, and accession numbers assigned (Appendix 3).

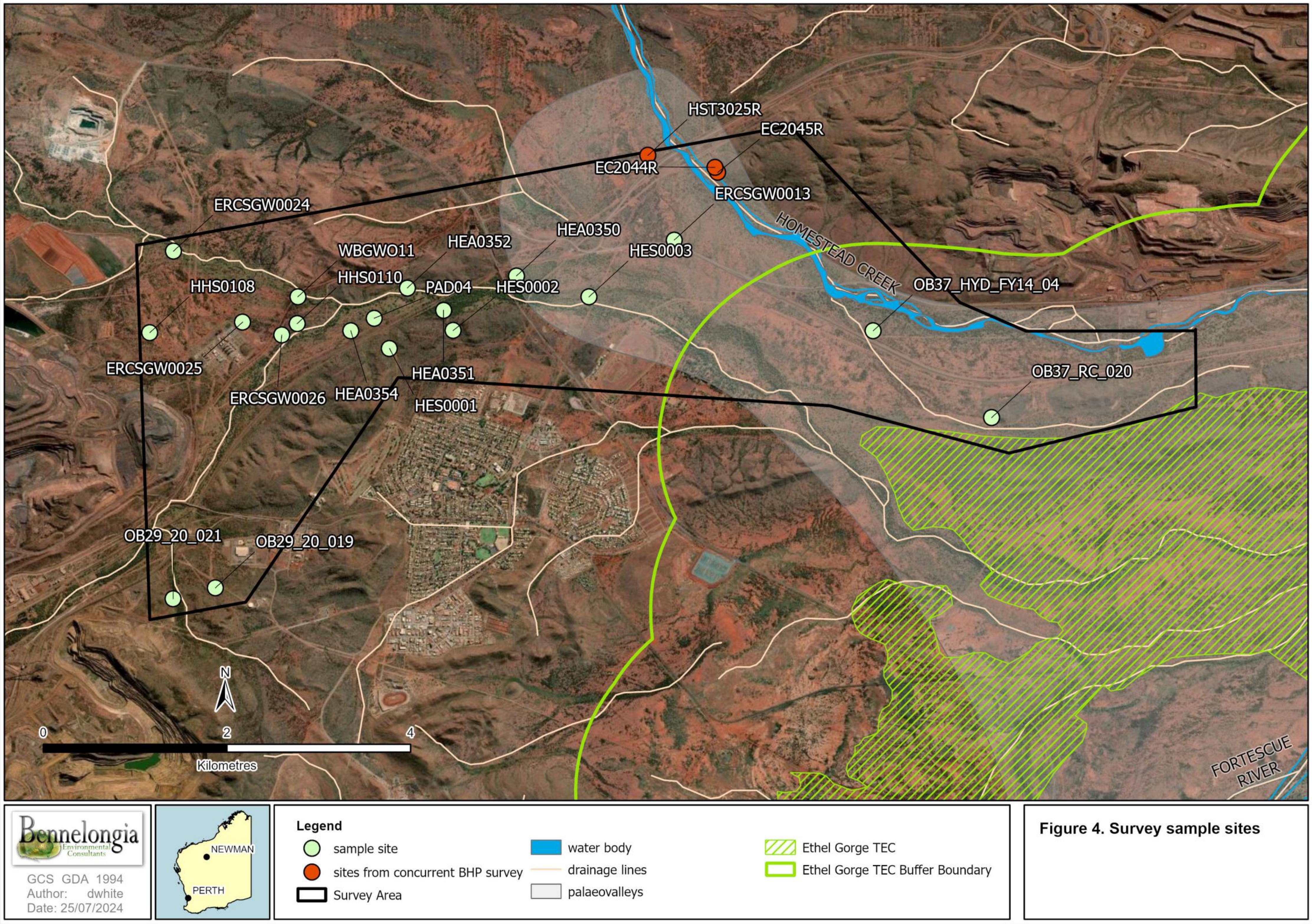


Figure 4. Survey sample sites

4.1.4. Fieldwork Personnel

Fieldwork was led by Jim Cocking and assisted by Monique Moroney in round 1, Georgia Rice in round 2 and Jaxon Haines in round 3. Sample sorting was done by Melita Pennifold, Ella Carstens and Jaxon Haines. Identifications were made by Jane McRae. Molecular laboratory work was done by Heather McLetchie and Veera Haslam. DNA analysis and report writing was carried out by Daniel White. Jim Cocking has more than 16 years of experience sampling and identifying subterranean fauna in the Pilbara and Yilgarn, while Monique, Georgia and Jaxon had a combined 2 years of experience conducting subterranean fauna surveys. Jane has been the primary taxonomic identifier for Bennelongia's subterranean fauna surveys for 15 years and has undertaken regional surveys of aquatic invertebrates in wetlands and rivers across WA. Heather has over 15 years' experience in molecular ecology labs and Veera Haslam has recently completed her PhD which focused on the genetics and ecology of *Drupella cornus*, the main invertebrate corallivore snail in WA. Jane has written or co-authored 20 papers describing new invertebrate species or their distributions. Daniel White has 14 years' experience of lecturing and undertaking research at Australian and New Zealand universities and institutes. His research, which has culminated in over 20 publications, has focused on using genetic analysis to understand species' ecology and to discriminate between species.

4.2. Survey Results

Seventeen out of a total of 19 drill holes sampled (90%) yielded stygofauna. The two drill holes that did not yield are HEA0354 and EEG1720DTM (Figure 4). Over the three rounds of survey, 341 stygofauna specimens from 52 records were collected, where a record is a species collected from a site on a specific date. In addition, four specimens from four records (three additional drill holes) were collected within the Survey Area as part of an overlapping BHP survey in 2023 for orebodies 32 West, 28 and 33 (Figure 4, Bennelongia 2024). Combined, 345 stygofauna specimens were collected from the Survey Area during 2023, from 56 records made up of at least 16 species (Table 4). Stygofauna species collected were mostly arthropods (82.3 %), annelids (12.5 %) and nematodes (6.3 %) which is consistent with stygofauna communities in the Pilbara. Two of the species are currently known only from the Survey Area – the amphipod *Kruptus* \`BAM227\` and the syncarid \`*Billibathynella* \`BSY249\`. A further three are considered core endemic species of the Ethel Gorge TEC as described in Bennelongia (2013) – *Chydaekata acuminata* (amphipod), *Pygolabis humphreysi* (isopod) and *Pilbaracandona eberhardi* (ostracod). None of the species recorded from the Survey Area are conservation-listed. Table 4 lists all species recorded from the Survey Area during 2023 (i.e. this survey and Bennelongia 2024) and their known distribution. Interpreted results of molecular analysis are presented in Appendix 3.

4.3. Survey Area Community

In terms of abundance, the stygofauna community within the Survey Area appears to be dominated by copepods and ostracods. Species richness is also high in these groups with three species of copepod and four species of ostracod. Indeed, species richness is relatively high across all the crustaceans including the amphipods (3), scynacrids (2) and isopods (1). A large number (341) of stygofauna specimens were recovered from a relatively small number of samples taken (42) in the current survey. Further, only two of the 19 different drill holes did not yield stygofauna over the three rounds of survey (HEA0354 and EEG1720DTM). The three drill holes sampled for the overlapping BHP survey in 2023 for orebodies 32 West, 28 and 33 (Bennelongia 2024) both yielded stygofauna. Taken together, evidence provided here suggests there is an abundant and rich stygofauna community in the Survey Area. This is not surprising considering the Survey Area is immediately adjacent to the Ethel Gorge TEC which hosts an aquifer stygobiont community, it is bisected by the Homestead Creek which is a potential source of groundwater re-charge and it hosts a palaeovalley and multiple drainage lines which are associated with stygofauna occurrence. Further, it appears both the depth and chemistry of the groundwater is prospective for stygofauna.

Table 4: Survey Area species list from 2023.
Species shaded orange indicate core endemic species of the Ethel Gorge TEC as described in Bennelongia (2013). Species shaded grey are potentially restricted. + Indicates the species was recorded from the current survey and Bennelongia (2024). Note, only a single specimen of each of the three species was recorded in the Bennelongia (2024) drill holes. ^ Indicates the species was recorded only from the Bennelongia (2024) drill holes.

Higher Taxonomic Order	Lowest ID	No. Specimens	Known Distribution
Annelida			
Oligochaeta			
Enchytraeida	Enchytraeidae `1 bundle`(long thin 2 per seg)	3	Australia
Haplotaxida	Phreodrilidae sp. P11	8	Pilbara
Arthropoda			
Amphipoda	*Chydaekata acuminata*+	12	Pilbara
	Paramelitidae gen. nov. 1 `AMP001`+	4	Pilbara
	Kruptus `BAM227`	3	Survey Area
Isopoda	*Pygolabis humphreysi*	5	Pilbara
Syncarida	*Billibathynella* `BSY249`	3	Survey Area
	Pilbaranella B	8	East Pilbara
Copepoda			
Cyclopoida	*Diacyclops humphreysi* s.l. +	16	Pilbara
	Diacyclops sobeprolatus	77	Pilbara
Harpacticoida	*Parastenocaris* `COP001`	111	Pilbara
Ostracoda			
	Origocandona grommike	30	Pilbara
	Origocandona inanitas	61	Pilbara
	Notacandona modesta	2	East Pilbara
	Pilbaracandona eberhardi	1	East Pilbara
Nematoda			
	Nematoda spp.^	1	n/a

While none of the stygofauna species recorded from the Survey Area are conservation-listed, several species have distributions that are currently known from only within the Survey Area and are therefore potentially restricted. The current survey has identified one amphipod, *Kruptus* `BAM227`, and one syncarid, ` *Billibathynella* `BSY249` (Figure 5). In addition, the historically collected annelid worm Enchytraeidae sp. OB3 (Order: Enchytraeida) is known only from the Survey Area (Bennelongia 2019). Locations of these three species is shown in Figure 5.

Further information on the three species potentially restricted to the current Survey Area is provided here:

***Kruptus* `BAM227`**

Kruptus `BAM227` is an amphipod from family Paramelitidae. It has been identified by both morphological and molecular evidence to be a unique species. It was recovered in June from three drill holes in the north-west of the Survey Area, ERCSGW0024, ERCSGW0025 and HEA0353 which are in relatively close proximity to a drainage line. The depth to water in these three holes at the time of survey was approximately 5.6, 8.2 and 22.0 m, respectively. The surface geology at all three is alluvium and, considering that groundwater depth is shallow, this species may reside in detritals, which are widespread and extend outside the Survey Area, likely following the adjacent drainage line. Reviewing detailed lithology of these sites would help confirm host geology.

***Billibathynella* `BSY249`**

Billibathynella `BSY249` is a syncarid of family Parabathynellidae. It has been identified by morphological evidence to be a unique species. It was recovered from one site, HWHB1665 (previously ENVPad_0001, WBGWO11), on two different occasions – two specimens in June and one in October. This site lies in a drainage line, and it also has alluvial host geology. Depth to groundwater at the time of survey was approximately 8.5 m. Again, this suggests that this species lives in detritals that extend to outside the Survey Area.

Enchytraeidae sp. OB3

Enchytraeidae sp. OB3 is an enchytraeid worm of order Haplotaxida. This species, identified by both morphological and molecular evidence to be unique, is known from two specimens collected by Subterranean Ecology in 2011 from a single hole, HEOP0317 (previously called W13), in the eastern part of the Survey Area. This hole is inside the TEC buffer boundary but outside the area of mapped calcrete considered to be the core habitat of Ethel Gorge (Figure 5). At the time of the collection the depth to water in HEOP0317 was approximately 13–16 m and the end of hole was approximately 35 m. The geological logs provided by BHP for HEOP0137 and a neighbouring hole (production bore W2) document alluvium, shale, calcrete and some dolomite in the top 30 m overlying dolomite and it is inferred that Enchytraeidae sp. OB3 inhabits detritals and, possibly, dolomite (Bennelongia 2019). Mapped surficial geology suggests that detrital habitat in alluvium and colluvium may extend from HEOP0317 beyond the Survey Area (Bennelongia 2019). The collection site for Enchytraeidae sp. OB3 is upstream of the main Fortescue River channel so it is possible that the species occupies suitable habitat further downstream.

4.4. Limitations

A possible limitation of this survey was the inaccessibility to a number of planned sites. This changed the total number of samples taken from 66 to 42, a reduction of 36%. Nonetheless, a relatively large number of animals were recovered, and high species richness revealed. It is expected that a greater number of samples would lead to greater yields.

5. CONCLUSION

The desktop assessment revealed an abundant and speciose stygofauna community in the region with 4,095 records consisting of at least 184 species. Based on surface geology and hydrogeology at the Survey Area, extrapolations from the desktop search predicted that an abundant stygofauna community should also occur there. The survey confirmed these predictions as 341 specimens of 15 species were recorded from 52 records. When including additional records collected within the Survey Area as part of an overlapping BHP survey in 2023 for orebodies 32 West, 28 and 33 (Bennelongia 2024), a total of 345 specimens of 16 species were recorded from 56 records.

Two new species were described that are known only from within the Survey Area, *Kruptus* `BAM227` and *Billibathynella* `BSY249`. Both of these species occur in holes that likely reside in detrital layers that extend beyond the Survey Area and therefore are unlikely to be restricted within the local area.

One historically collected species, Enchytraeidae sp. OB3, remains potentially restricted as no other occurrences of this species were recorded after the current survey, including after genetic analysis of regionally occurring related specimens. However, as with the two more recently collected species, host geology is likely detrital layers which extend well beyond the Survey Area and therefore it is expected that this species is not restricted within the local area.

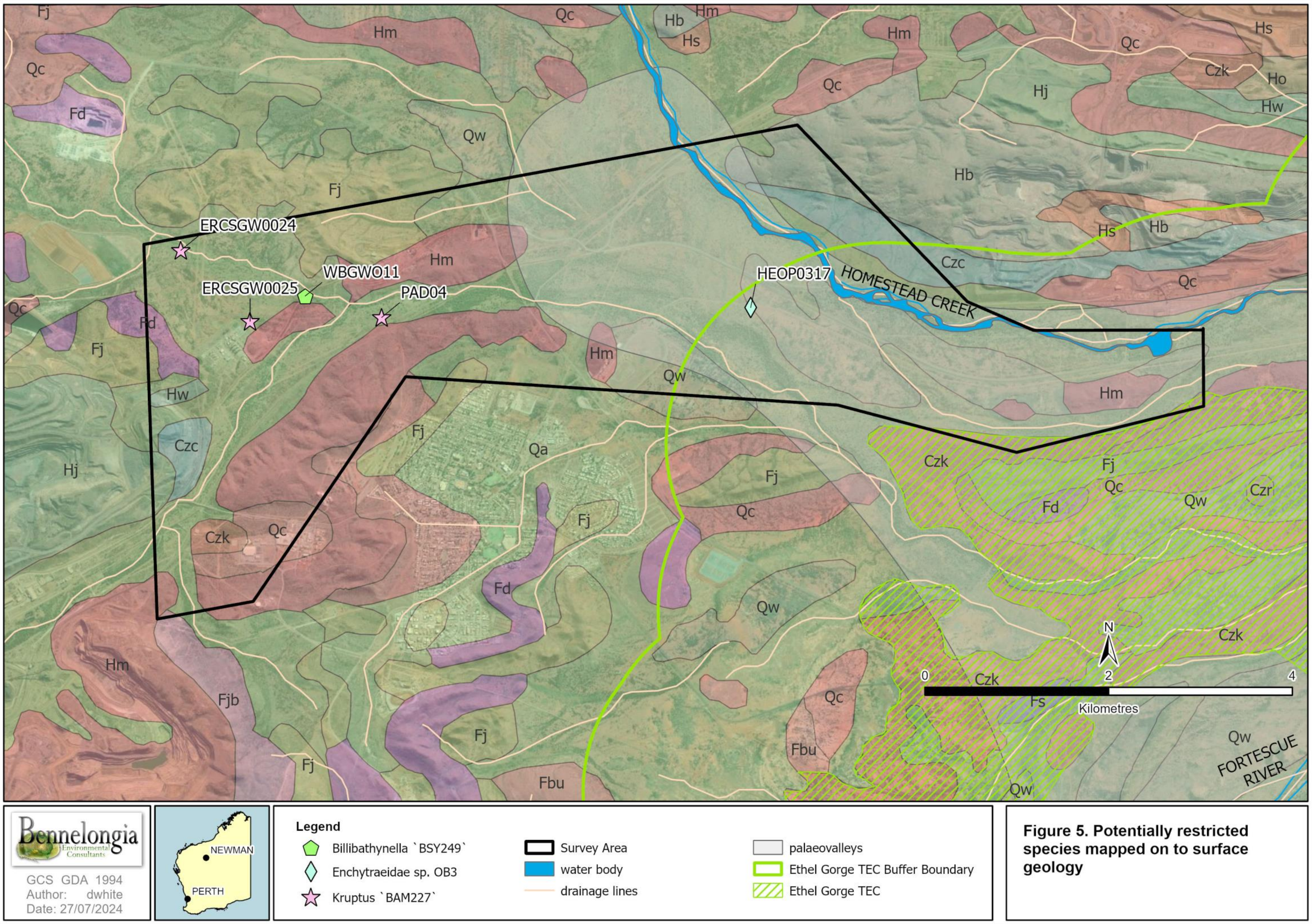


Figure 5. Potentially restricted species mapped on to surface geology

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Appendix 1 – Study Area species list from desktop search

Higher Taxonomic Order	Lowest ID	No. of records
Annelida		
Aphanoneura	*Aeolosoma* sp.	3
	Aeolosoma sp. 1 (PSS)	2
	Aeolosoma sp. OB	4
Clitellata		
Oligochaeta	Oligochaeta sp.	109
Enchytraeida		
Enchytraeidae	Enchytraeidae `2 bundle` s.l. (long thin 2 per seg)	4
	Enchytraeidae `2 bundle` s.l. (short sclero 2 per seg)	1
	Enchytraeidae `2 bundle` s.l. (short sclero 4 per seg)	6
	Enchytraeidae `3 bundle` s.l. (short sclero)	6
	Enchytraeidae `Biota1`	2
	Enchytraeidae `PST1` s.l. (E06)	2
	Enchytraeidae sp. E04	1
	Enchytraeidae sp. E05	1
	Enchytraeidae sp. E06-05	1
	Enchytraeidae sp. E06-B05	1
	Enchytraeidae sp. E08	2
	Enchytraeidae sp. E12	10
	Enchytraeidae sp. E12-02	2
	Enchytraeidae sp. OB_MC†	1
	Enchytraeidae sp. OB2	2
	Enchytraeidae sp. OB3	2
	Enchytraeidae sp. OB4	1
	Enchytraeidae sp. OP	5
	Enchytraeidae sp.	66
	Achaeta sp.	2
	Enchytraeus sp. AP PSS1 s.l.	8
	Enchytraeus sp. AP PSS2 s.l.	39
	Enchytraeus sp.	18
Haplotaxida		
Naididae	Naididae sp. N03	31
	Naididae sp. N08	2
	Naididae sp.	10
	Allonais pectinata	2
	Dero (*Dero*) *niea*	2
	Dero furcata	1
	Pristina aequiseta	2
	Pristina longiseta	3
	Tubificidae `stygo type 1A`	12
	Tubificidae `stygo type 5`	1

Higher Taxonomic Order	Lowest ID	No. of records
	Tubificinae `BOL092`	3
	Tubificinae sp.	3
	Ainudrilus sp. WA27 (PSS)	1
Phreodrilidae	Phreodrilidae `BOL084`	2
	Phreodrilidae sp. ?P11	2
	Phreodrilidae sp. AP DC s.l.	43
	Phreodrilidae sp. AP SC s.l.	4
	Phreodrilidae sp. B05	1
	Phreodrilidae sp. OB3	2
	Phreodrilidae sp. P10	1
	Phreodrilidae sp. P11	14
	Phreodrilidae sp. P15	4
	Phreodrilidae sp. WAM indet. 1	1
	Phreodrilidae sp.	43
	Antarctodrilus sp. OB4	1
	Insulodrilus `WA31`	3
	Insulodrilus sp.	2
	Phreodrilus peniculus	1
	Phreodrilus sp.	18
Arthropoda		
Chelicerata		
Arachnida		
Trombidiformes		
Halacaridae	Halacaridae sp.	1
Mideopsidae	*Guineaxonopsis* sp. S01 group (PSS)	2
Pezidae	Pezidae sp.	1
	Peza `ACA001`	28
	Pezidae sp. OB	6
	Peza sp.	1
Crustacea		
Malacostraca		
Syncarida	Syncarida sp.	9
Bathynellacea	Bathynellacea sp.	4
Bathynellidae	Bathynellidae sp. MC	1
	Bathynella sp. B03	2
	Pilbaranella `sp. A`	38
	Pilbaranella `sp. B`	14
	Pilbaranella `sp. C`	6
	Pilbaranella ethelensis	71
	Pilbaranella sp. B12 (=`poss sp. D`)	2
	Pilbaranella sp.	45
	Bathynellidae `sp. OB1`	4
	Bathynellidae sp.	24

Higher Taxonomic Order	Lowest ID	No. of records
Parabathynellidae	Parabathynellidae sp. MC	3
	Parabathynellidae sp. OP	1
	Parabathynellidae sp.	5
	Atopobathynella `BSY241`	1
	Atopobathynella `sp. DC1`	1
	Atopobathynella `sp. DNA02`	2
	Atopobathynella sp. B18	2
	Billibathynella `BSY280`	3
	Billibathynella `SYN001`	6
	Billibathynella cassidis	13
	Billibathynella sp.	1
	Breisomabathynella `BSY253`	1
	Breisomabathynella `BSY279`	1
	Breisomabathynella pilbaraensis	21
	Breisomabathynella sp.	2
	Notobathynella sp.	2
	nr *Billibathynella* sp.	9
Amphipoda	Amphipoda sp.	90
Melitidae	Melitidae sp.	1
Paramelitidae	*Chydaekata* `AMP005`	17
	Chydaekata `E`	1
	Chydaekata `sp. indet. OB`	3
	Chydaekata `sp.?OB1`	2
	Chydaekata acuminata	214
	Chydaekata simulata	1
	Chydaekata sp.	77
	Kruptus `AMP004`	23
	Maarrka etheli	32
	Maarrka weeliwollii	4
	Maarrka sp.	2
	Paramelitidae gen. no. 1 `AMP001`	118
	Paramelitidae gen. no. 1 `AMP001`/`AMP002`	8
	Paramelitidae gen. no. 1 `AMP002`	6
	Paramelitidae gen. no. 1 `AMP003`	40
	Paramelitidae Genus 2 sp.	3
	Paramelitidae Genus 2 sp. B02	5
	Pilbarus millsi s.l.	1
	Paramelitidae `sp. 2 (DEC)`	41
	Paramelitidae `sp. Coondiner`	16
	Paramelitidae `sp. indet .OB`	16
	Paramelitidae `sp. nr. 2 (DEC)`	19
	Paramelitidae `sp. OB1 (B33)`	1
	Paramelitidae `sp. OB1_AMP001`	2

Higher Taxonomic Order	Lowest ID	No. of records
	Paramelitidae `sp. OB1`	1
	Paramelitidae `sp. OB3_AMP003`	6
	Paramelitidae `sp. OP1`	5
	Paramelitidae `sp. OP2`	3
	Paramelitidae sp. 2 s.l. (PSS)	34
	Paramelitidae `sp. B34`	1
	Paramelitidae sp.	144
Isopoda	Isopoda sp.	8
Microcerberidae	Microcerberidae sp.	4
	Coxicerberus `ISO019`	7
	Coxicerberus `sp. OB2`	1
	Coxicerberus sp.	1
	Microcerberidae `sp. OB`	2
Tainisopidae	*Pygolabis humphreysi*	182
	Pygolabis sp.	24
	Tainisopidae sp.	1
Maxillopoda		
Copepoda	Copepoda sp.	16
Calanoida		
Ridgewayiidae	*Stygoridgewayia trispinosa*	1
Cyclopoida	Cyclopoida sp.	89
Cyclopidae	Cyclopidae sp.	1
	Anzcyclops sp. B06	1
	Anzcyclops sp. OB	1
	Diacyclops cockingi	15
	Diacyclops humphreysi	93
	Diacyclops humphreysi s.l.	434
	Diacyclops scanloni	2
	Diacyclops sobeprolatus	101
	Diacyclops sp.	9
	Dussartcyclops uniarticulatus s.l.	1
	Mesocyclops brooksi	6
	Mesocyclops notius	4
	Mesocyclops sp.	2
	Microcyclops aricans	39
	Microcyclops sp. P1 (PSW)	1
	nr *Pilbaracyclops* sp. OB	2
	Orbuscyclops westaustraliensis	20
	Pescecyclops pilbaricus	14
	Pilbaracyclops sp. B03 (nr *frustratio*)	2
	Pilbaracyclops supersensus	25
	Thermocyclops aberrans	7
	Thermocyclops decipiens	4

Higher Taxonomic Order	Lowest ID	No. of records
	Thermocyclops sp.	2
	Diacyclops cf. sobeprolatus	6
	Tropocyclops prasinus	1
Harpacticoida	Harpacticoida sp.	40
Ameiridae	Ameiridae sp.	1
	Archinitocrella newmanensis	124
	Megastygonitocrella bispinosa	1
	Nitocrella `ophthalmia`	10
	Nitocrella karanoici	74
	Nitocrella sp. 1 (PSS)	2
Canthocamptidae	*Australocamptus* sp.	1
	Australocamptus sp. B10	1
Parastenocarididae	Parastenocarididae sp.	3
	Dussartstenocaris `BHA303`	1
	Parastenocaris `COP001`	30
	Parastenocaris `COP002`	2
	Parastenocaris `outbacki`	15
	Parastenocaris `sp. OB1 (B02)`	2
	Parastenocaris `sp. OB1`	2
	Parastenocaris jane	80
	Parastenocaris jane s.l.	8
	Parastenocaris sp.	34
Ostracoda	Ostracoda sp.	104
Podocopida		
Candonidae	Candonidae `sp. OP1`	2
	Areacandona `7` (PSS)	2
	Areacandona `outbacki`	29
	Areacandona mulgae	2
	Areacandona newmani	1
	Areacandona nr *iuno*	1
	Areacandona sp.	4
	Candonopsis tenuis	8
	Meridiescandona `3` (PSS)	1
	Meridiescandona lucerna	1
	Meridiescandona marillanae	1
	Meridiescandona nr *facies* (PSS)	6
	Notacandona `BOS119`	1
	Notacandona gratia	26
	Notacandona modesta	1
	Notacandona sp.	1
	Origocandona `BOS099`	9
	Origocandona gratia	1
	Origocandona grommike	6

Higher Taxonomic Order	Lowest ID	No. of records
	Origocandona inanitas	40
	Pilbaracandona `OST001`	8
	Pilbaracandona `OST002`	7
	Pilbaracandona colonia	37
	Pilbaracandona eberhardi	82
	Pilbaracandona kosmos	43
	Pilbaracandona rhabdote	2
	Pilbaracandona sp.	3
	Pilbaracandona temporaria	6
	Candonidae sp.	12
	Candoninae sp.	12
Cyprididae	Cyprididae sp.	3
	Bennelongia sp.	1
	Cypretta seurati	3
	Cypretta sp.	1
	Cypridopsis idua	1
	Cypridopsis sp.	1
	Cyprinotus kimberleyensis s.l.	1
	Ilyodromus sp.	2
	Riocypris fitzroyi	3
	Stenocypris bolieki	1
	Stenocypris malcolmsoni	1
	Strandesia `466`	1
	Strandesia sp.	1
	Cyprinopsinae sp.	1
	Sarscypridopsis ochracea	1
Darwinulidae	*Darwinulidae* sp.	1
	Estalenula marmonieri	2
Limnocytheridae	*Gomphodella hirsuta*	73
	Limnocythere stationis	2
Nematoda		
	Nematoda spp.	159
Platyhelminthes		
Turbellaria	Turbellaria sp.	13
Rotifera		
	Rotifera sp.	1
Bdelloidea	Bdelloidea sp.	1
	Bdelloidea sp. 2:2	14
Philodinidae	Philodinidae sp.	2

[†]This species name was synonymised with Enchytraeidae sp. Ench6 in Bennelongia (2015) and then Enchytraeidae sp. E12 in Brown *et al* (2015).

Appendix 2 – Drillholes sampled for survey and sampling date

Hole ID	Sample Date	Sample Name	Sampling Round	Total stygo specimens
ERCSGW0013	07/02/2023	Net	R1	31
ERCSGW0024	06/02/2023	Net	R1	8
ERCSGW0024	14/06/2023	Net	R2	1
ERCSGW0024	03/10/2023	Net	R3	3
ERCSGW0025	06/02/2023	Net	R1	1
ERCSGW0025	14/06/2023	Net	R2	1
ERCSGW0025	03/10/2023	Net	R3	0
ERCSGW0026	06/02/2023	Net	R1	0
ERCSGW0026	14/06/2023	Net	R2	0
ERCSGW0026	03/10/2023	Net	R3	3
HEA0350	07/02/2023	Net	R1	3
HEA0351	07/02/2023	Net	R1	1
HEA0351	15/06/2023	Net	R2	6
HEA0351	04/10/2023	Net	R3	1
HEA0352	07/02/2023	Net	R1	0
HEA0352	15/06/2023	Net	R2	0
HEA0352	03/10/2023	Net	R3	4
HEA0354	07/02/2023	Net	R1	0
HEA0354	15/06/2023	Net	R2	0
HEA0354	04/10/2023	Net	R3	0
HES0001	07/02/2023	Net	R1	1
HES0001	15/06/2023	Net	R2	3
HES0001	04/10/2023	Net	R3	0
HES0002	07/02/2023	Net	R1	12
HES0002	15/06/2023	Net	R2	26
HES0002	04/10/2023	Net	R3	28
HES0003	07/02/2023	Net	R1	16
HHS0108	06/02/2023	Net	R1	32
HHS0108	14/06/2023	Net	R2	30
HHS0108	03/10/2023	Net	R3	29
HHS0110	06/02/2023	Net	R1	1
HHS0110	14/06/2023	Net	R2	2
HHS0110	03/10/2023	Net	R3	1
EEG1720DTM	07/02/2023	Net	R1	0
EEG1722DTM	07/02/2023	Net	R1	7
HEQ0012	07/02/2023	Net	R1	4
EQ0018R	06/02/2023	Net	R1	1
HEA0353	15/06/2023	Net	R2	3
HEA0353	04/10/2023	Net	R3	1
HWHB1665	06/02/2023	Net	R1	30
HWHB1665	14/06/2023	Net	R2	32
HWHB1665	03/10/2023	Net	R3	19

Appendix 3 - Results of molecular analyses for stygofauna at Orebody 29, 30, 35, including seven reference samples collected outside these orebodies

Location	Year	Bore Code	Final Identification	Identification before DNA	Comments	GenBank Accession No.†
Jimblebar	11/10/2020	EOP0334R	*Achaeta* sp.	*Achaeta* sp.	Failed – low DNA yield	n/a
Jimblebar	11/10/2020	EOP0301R	*Achaeta* `BOL094`	*Achaeta* sp.	No species-level match was found with this sequence, with the closest match being 10.8% distant to Enchytraeidae sp. Biologic-OLIG016 which is stored in GenBank.	PP871389
Newman	15/06/2023	HEA0353	Enchytraeidae `1 bundle`(long thin 2 per seg)	Enchytraeidae `1 bundle`(long thin 2 per seg)	No species-level match was found either in GenBank or the Bennelongia database, therefore its higher order identification was retained.	PP871390
East Jimblebar	29/05/2023	HUB0029DG	Enchytraeidae sp. E06-05	Tubificinae sp.	This sequence is 2.1% distant to Enchytraeidae sp. E06-05 in the Bennelongia database and 5.4% to Enchytraeidae sp. E6 isolate G284 in GenBank.	PP871395
Newman	24/02/2021	HST3247R	Enchytraeidae sp. E12	Enchytraeidae sp.	This sequence is only 1.6% distant from Enchytraeidae E12-01 in the Bennelongia database and 1.5% distant from Enchytraeidae sp. E12 isolate G282 in GenBank. It is 17.9% distant to Enchytraeidae sp. OB3.	PP871391
Round Hill	5/02/2024	HR0124	Enchytraeidae sp. Biologic-OLIGO20	Enchytraeidae sp.	This sequence is 2.4% distant to Enchytraeidae sp. Biologic-OLIG020 in GenBank and 8.7% distant to Enchytraeidae sp. E08 in the Bennelongia database. It is 14.5% to Enchytraeidae sp. OB3.	n/a

Location	Year	Bore Code	Final Identification	Identification before DNA	Comments	GenBank Accession No.†
Newman	15/02/2024	EOP0829R	Enchytraeidae sp. OB2	Oligochaeta sp.	This sequence is only 1.4% distant to Enchytraeidae sp. OB2 in the Bennelongia database but 18.5% distant to Enchytraeidae sp. OB3. No species-level match was found in GenBank.	PP871392, PP829291 (12S)
Newman	14/06/2023	ERCSGW0025	*Kruptus* \`BAM227\`	*Kruptus* sp.	This sequence does not have a species-level match to any sequence stored in GenBank or the Bennelongia databases. It is only 1.6% distant to the *Kruptus* specimen from bore HEA0353. Consequently, these two animals were assigned the same, new species code.	PP851807
Newman	15/06/2023	HEA0353	*Kruptus* \`BAM227\`	*Kruptus* sp.	This sequence does not have a species-level match to any sequence stored in GenBank or the Bennelongia databases. It is only 1.6% distant to the *Kruptus* specimen from bore ERCSGW0025. Consequently, these two animals were assigned the same, new species code.	PP851808
Newman	14/06/2023	ERCSGW0024	*Kruptus* \`BAM227\`	*Kruptus* sp.	Failed – low DNA yield. Updated identification based on morphology.	n/a
Eastern Ridge	5/11/2014	HST0071R	Oligochaeta sp.	Oligochaeta sp.	Failed – low DNA yield.	n/a
Newman	6/02/2023	HHS0108	*Origocandona inanitas*	Ostracoda sp. indet.	Failed – low DNA yield. Identification updated based on morphology.	n/a

Location	Year	Bore Code	Final Identification	Identification before DNA	Comments	GenBank Accession No.†
Newman	14/06/2023	HHS0108	*Origocandona inanitas*	Ostracoda sp. unident.	Failed – low DNA yield. Identification updated based on morphology.	n/a
Newman	3/10/2023	HHS0108	*Origocandona grommike*	Ostracoda sp. indet.	This sequence is at least 15.1% distant to sequences stored in GenBank and Bennelongia databases. It is 4.4% distant to the ostracod from bore ERCSGW0013, consequently these records were assigned the same taxonomic identification.	PP869357
Newman	7/02/2023	ERCSGW0013	*Origocandona grommike*	Ostracoda sp. indet.	This sequence is at least 14.0% distant to sequences stored in GenBank and Bennelongia databases. It is 4.4% distant to the ostracod from bore HHS0108, consequently these records were assigned the same taxonomic identification.	PP869358
Newman	7/02/2023	HES0003	*Parastenocaris* \`COP001\`	*Parastenocaris* sp.	Failed – low DNA yield. Identification updated based on morphology and co-occurrence of con-specifics.	n/a
Newman	7/02/2023	HES0001	Phreodrilidae sp. P11	Phreodrilidae sp. AP DVC s.l.	This sequence is 0.4% distant to Phreodrilidae sp. P11 in the Bennelongia database and 1.2% distant to Phreodrilidae sp. P11 in GenBank.	PP871393, PP829292 (12S)
Newman	7/02/2023	HES0003	Phreodrilidae sp. P11	Phreodrilidae sp. AP DVC s.l.	Failed – low DNA yield. Identification updated based on morphology.	n/a

Location	Year	Bore Code	Final Identification	Identification before DNA	Comments	GenBank Accession No.†
Newman	7/02/2023	HEQ0012	*Pilbaranella* B	*Pilbaranella* sp.	Failed – low DNA yield. Identification updated based on morphological similarity to animal from site HEA0351.	n/a
Newman	15/06/2023	HEA0351	*Pilbaranella* B	*Pilbaranella* sp.	This sequence is 0.9% distant or less to *Pilbaranella* B stored in Bennelongia and GenBank databases.	PP871394
Newman	7/02/2023	HES0003	*Pilbaranella* B	*Pilbaranella* sp.	Failed – low DNA yield. Identification updated based on morphological similarity to animal from site HEA0351.	n/a
Newman	3/10/2023	HWHB1665	*Pilbaranella* B	*Pilbaranella* sp.	Failed – low DNA yield. Identification updated based on morphological similarity to animal from site HEA0351.	n/a
Newman	7/02/2023	HEA0350	*Pilbaranella* B	*Pilbaranella* sp.	Failed – low DNA yield. Identification updated based on morphological similarity to animal from site HEA0351.	n/a
Newman	14/06/2023	HHS0110	*Pygolabis humphreysi*	*Pygolabis* sp.	Failed – low DNA yield. Identification updated based on morphological similarity to animal from the same site collected on 03/10/2023.	n/a
Newman	3/10/2023	HHS0110	*Pygolabis humphreysi*	*Pygolabis* sp.	This sequence is 1.9% distant or less to *Pygolabis humphreysi* stored in Bennelongia and GenBank databases.	PP843555

Location	Year	Bore Code	Final Identification	Identification before DNA	Comments	GenBank Accession No.†
Newman	6/02/2023	ERCSGW0025	*Pygolabis humphreysi*	*Pygolabis* sp.	Failed – low DNA yield. Identification updated based on morphological similarity to animals from the site HHS0110.	n/a

† COI unless otherwise stated.