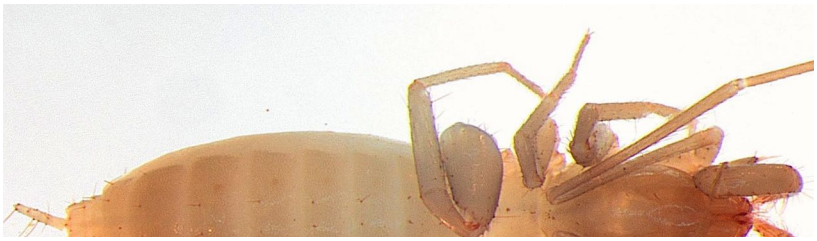
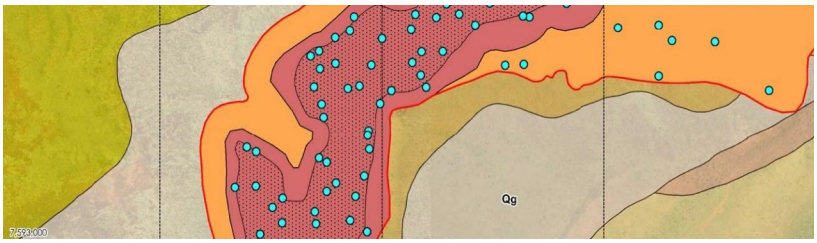
Biota Environmental Sciences

Robe Mesa Troglofauna Baseline Assessment Report (Phase 1-3)





Prepared for CZR Resources Ltd

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Robe Mesa Troglofauna Assessment (Phase 1-3)

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1.0 Executive Summary

CZR Resources Ltd (CZR) is proposing to develop the Robe Mesa Iron Ore Project (the project), located in the west Pilbara, 29 km southwest of Pannawonica. The Robe Mesa deposit and specifically, the conceptual pit, contains Robe Pisolite which is considered to represent primary habitat for troglofauna.

As the mine development has the potential to impact troglofauna, Biota Environmental Sciences (Biota) was commissioned to:

1. conduct a desktop study of relevant data available from the 'study area' (a 15 km buffer around and including the Robe Mesa deposit);
2. conduct a three-phase detailed survey to document troglofauna present within the 'survey area' (the deposit itself); and
3. place the recorded fauna into local and regional context and discuss their significance.

During the combined survey phases, 74 drillholes were sampled for troglofauna using baited colonisation traps, with a total of 368 traps installed. Molecular analysis was conducted for the specimens to determine the number of species present, and to compare the results with contextual data from the wider Pilbara region.

A total of 102 troglobitic fauna specimens were recorded in the survey area from 22 drillholes across the three phases of sampling. These represent a minimum of 17 putative species from seven orders; Pseudoscorpiones, Schizomida, Polydesmida, Diplura, Zygentoma, Symphyla and Isopoda.

Although species representing the same taxonomic orders have been recorded on other mesa formations in the Robe Valley, the great majority of species-level taxa recorded and identified via molecular sequencing from the survey area have not been previously documented. Considering the Robe Valley troglofauna have been extensively surveyed over the past 15 years and are well represented in genetic databases, it is evident that the troglobitic community recorded during the survey is essentially endemic to Mesa F.

The recorded taxa are all potentially significant as each:

- has a very short-range distribution, with apparently restricted to Mesa F;
- is representative of relictual fauna (Harvey 2002), with the Robe Valley troglofauna representing lineages derived from the late Miocene (at least the last 10 million years); and
- forms part of the Robe Valley PEC.

Seven of the 17 troglobitic taxa recorded during the survey are known only from within the project's conceptual pit outline. However, based on surface geology and modelled stratigraphic cross-sections, similarly suitable habitat occurs outside of the conceptual pit outline and throughout Mesa F. This is supported by the recorded locations of *Draculoides* sp. H-SCH200, which occurs both inside and outside the conceptual pit. Similar patterns of widespread distributions of endemic species within mesa landforms have been shown repeatedly in the Robe valley and it is likely that the seven taxa only known from the conceptual pit area occur more widely within Mesa F.

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2.0 Introduction

2.1 Project Background

CZR Resources Ltd (CZR) is proposing to develop the Robe Mesa Iron Ore Project (the project), located in the west Pilbara, 29 km southwest of Pannawonica. The Robe Mesa deposit adjoins Mesa F, which is located between the Mesa A and Mesa J-K channel iron deposit (CID) mines operated by Rio Tinto. Currently, there are no operational mines on the CZR tenements (E08/1060 and E08/1686) or anywhere on Mesa F.

The project area and conceptual pit, contains Robe Pisolite which is considered to represent primary habitat for troglofauna and requires survey for this fauna to inform environmental impact assessment (EIA). For the purposes of this report, the 'survey area' comprises the Robe Mesa deposit (274.4 ha) (Figure 2.1). The 'study area' comprises a 15 km buffer around and including the survey area (Figure 2.1).

CZR commissioned Biota Environmental Sciences (Biota) to complete the required troglobitic fauna assessment, which included three phases of troglofauna sampling, and a desktop study collating previous data from the study area.

2.2 Study Objectives and Scope

The scope of this study was to conduct a baseline assessment of troglobitic fauna and habitat within the survey area through three phases of sampling. The key objectives of this study were:

1. conduct a desktop study of relevant data available from the study area;
2. conduct a three-phase detailed survey to document troglofauna present within the survey area; and
3. place the recorded fauna into regional context and discuss their significance.

The study was planned and implemented as far as practicable in accordance with the following:

- Environmental Factor Guideline – Subterranean Fauna (EPA 2016a);
- Technical Guidance - Subterranean fauna survey (EPA 2016b) [1];
- Technical Guidance - Sampling methods for subterranean fauna (EPA 2016c); and
- Technical guidance - Subterranean fauna surveys for environmental impact assessment (EPA 2021).

2.2.1 Purpose of this Report

The purpose of this report is to inform feasibility studies and the future EIA of proposed mining activities within CZR tenements at Mesa F.

This report presents the findings of the desktop study and documents the methodology, sampling effort and results of the three-phase troglobitic fauna sampling program. Both the field sampling and report are subject to specific limitations that are discussed in Section 3.10.

[1] All survey phases were conducted in accordance with the updated subterranean fauna technical guidance (EPA 2021). However, this was officially released after completion of the Phase 1 and Phase 2 survey.

2.3 Troglofauna Background

Western Australia contains diverse subterranean fauna assemblages, particularly at Cape Range, Barrow Island, the Pilbara bioregion, the Yilgarn and the Nullarbor (Humphreys 2001, Page et al. 2008, Guzik et al. 2010). Subterranean fauna can be categorised into two distinct ecological groups based on habitat: troglofauna and stygofauna. Troglofauna survive only in air-filled cavities and interstices between the surface and the water table. Stygofauna comprise aquatic taxa occurring in groundwater aquifers and subterranean water bodies.

Troglofauna are obligatory subterranean habitat dwellers, and while they may occur close to surface environments, are strongly adapted to subterranean environments. This makes them unable or highly unlikely to survive surface conditions. A range of similarly adapted fauna occur that opportunistically use subterranean habitats but can survive outside these stable environments. Studies from the Pilbara bioregion have demonstrated that these suites of fauna; troglophiles, trogloxenes and edaphobites, are not similarly restricted in range and therefore unlikely to be impacted by small scale developments (Biota and Helix 2012, Helix 2012). Troglofauna have been collected from a range of geological units such as pisolitic iron formations, channel iron deposits, unconsolidated alluvium and sedimentary basalt (Biota 2004, 2006, 2010, 2011a, 2013), indicating that the suitability of a formation as habitat for troglofauna is mostly a function of the availability of habitable space (Biota 2006), rather than a specific geology unit.

Troglofauna in semi-arid Australia are thought to be relictual rainforest fauna; fauna adapted to humid environments, which retreated underground to cave systems during the aridification of Australia (In the late Miocene; Humphreys 1993). This is inferred from affinities of the taxonomic groups represented amongst the troglofauna with other extant taxa in tropical climates. Some invertebrate groups with troglobitic representatives include the Arachnida (e.g. Schizomida, Pseudoscorpiones and Araneae), Chilopoda (e.g. Scolopendrida), Diplopoda (e.g. Polydesmida and Haplodesmida), and Insecta (e.g. Diplura, Zygentoma, Coleoptera and Blattodea). A single troglobitic vertebrate species of blind snake (*Anilios longissimus*) is known from Australia, collected from Barrow Island (Aplin 1998, Humphreys et al. 2013).

Due to their dependence on constant humidity, the dispersal and distribution of troglofauna species tends to remain limited to individual blocks of inter-connected habitat, leading to long periods of population isolation and speciation. As a result, troglofauna often display extreme short-range endemism and are therefore vulnerable to localised extinction by relatively small - scale developments, such as mining and construction.

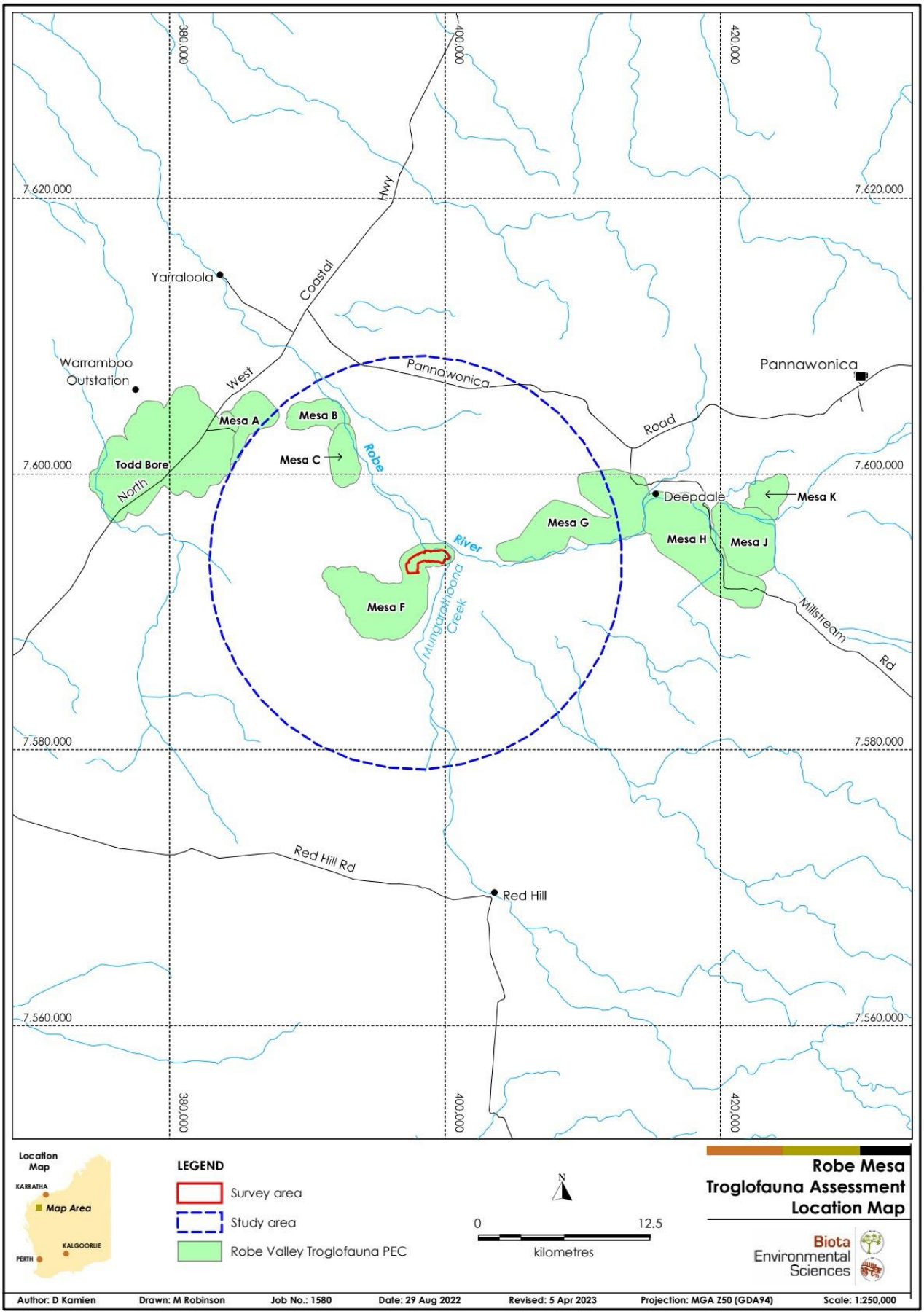

Figure 2.1: Study area and survey area location map.

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3.0 Methodology

3.1 Desktop Study

3.1.1 Review of Previous Relevant Studies

A literature review was completed to identify relevant previous surveys within the study area. These included searches of Biota's library database and the Environmental Protection Authority (EPA) website, in addition to the identification of previous surveys via database specimen records (Section 3.1.2).

3.1.2 Database Searches

The following databases were searched to assist with compilation of a list of potential troglobitic species in the study area:

1. **NatureMap**: a collaboration between the Department of Biodiversity Conservation and Attractions (DBCA) and the Western Australian Museum (WAM). This database represents the most comprehensive source of information on the distribution of Western Australia's fauna, comprising records from the Fauna Survey Returns database and WA Threatened Fauna Database (both maintained by DBCA) and the WAM Specimen Database.
2. **Atlas of Living Australia (ALA)**: a collaborative project between academic collecting institutions, private individual collectors, and community groups. ALA contains occurrence records, environmental data, images, and the conservation status of species throughout Australia.
3. **The Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC) Protected Matters Search Tool**: a database of federally listed fauna species and any other matters of national environmental significance (MNES) that may occur in the locality.
4. **WAM Arachnid and Myriapod, and Insect databases.**
5. **Biota Internal Data**: this includes subterranean fauna data collected by Biota within Western Australia.

Searches were centred on the coordinate 21.7586°S; 116.0123°E with search results requested from a 15 km radius.

3.1.3 Assessment of Likelihood of Occurrence in the Survey Area

Results from the literature review and database searches were used to compile a list of troglofauna species that had previously been recorded in the study area. An assessment of the likelihood that these troglofauna species occur within the survey area was conducted based on:

- assessment of presence of suitable habitat for troglofauna within the survey area (Section 4.2);
- distribution of troglofauna records within 15 km of the survey area (Section 4.1.2); and
- assessment of the likelihood that a given species would be geographically restricted to the survey area.

3.2 Habitat Characterisation

The likely troglofauna habitats within the survey area were initially characterised using a combination of regional information and site-specific geological data. Inputs considered in this analysis included:

1. the spatial extent of the survey area in order to define the geological units;
2. regional surface geology mapping;

3. previous sampling effort in the locality and the results of published and unpublished studies on troglobitic fauna (Section 3.1.1);
4. identification of rock types that have previously yielded troglobitic fauna from the study area (Section 4.1), including reviewing studies that have investigated the nature of troglofauna habitat in the Robe Valley (e.g. Biota and DC Blandford & Associates 2013); and
5. Geological review of troglofauna habitat (CZR Resources 2023).

The habitat units identified through this process were assigned a likelihood to support troglofauna based on the above inputs, and then revisited on completion of sampling, to further investigate if the spatial distribution of the fauna records provide support for the habitat model.

For troglofauna, we have categorised the prospectivity of the geological units within the survey area as low, medium and high, based on the following characteristics:

A) presence of mesocaverns, vugs and interstitial spaces;

B) known hydration, weathering or significant cavity zones (where available);

C) presence of clay lenses or impeding layers to maintain stable humidity;

D) the known occurrence of troglofauna communities from equivalent rock types during historic Pilbara surveys; and

E) occurrence above water table within the survey area.

Geological unit prospectivity was then determined as follows:

Low – Suitable geology only occurs below water table in the survey area. Rock type may have B), C) and E) characteristics but locally lacking suitable habitat space. Troglofauna not known from previous studies sampling of the same geology.

Medium – Suitable geology likely or known to occur above the water table in the survey area (E). Geology known to have interstices or vugs (A) and troglofauna have occasionally been detected in similar rock types previously (D). Geology may be subject to seasonal inundation (e.g. alluvium and colluvium). Where known, units of prospectivity were categorised as medium if less than 5 m in thickness.

High – Majority (four or five) categories confirmed for the geological unit, including E). Troglofauna routinely recorded from same rock type (D).

3.3 Sampling Methodology

Troglobitic fauna sampling was completed in three phases, under "Fauna Taking (Biological Assessment) Licence" No. BA27000433 (Appendix 1). Methodology and approach were consistent with those outlined in EPA Technical Guidance, Subterranean Fauna Surveys for Environmental Impact Assessment (EPA 2021). Similar methodologies have been used in previous Robe Valley subterranean fauna assessments (Biota 2006, 2009a, 2011b, 2017a, 2017b, 2019a).

Custom-built litter colonisation traps were suspended at intervals within drillholes located within the survey area. Traps were constructed from 60 mm internal diameter PVC pipe cut to a length of 180 mm. Each trap had a series of 20 mm holes drilled into the side, and traps remained open at the upper end. Up to four traps were installed such that they were in contact with the interior of the sampled drillhole, facilitating fauna entry into the trap.

Leaf litter was gathered locally from the ground surface in the study area, particularly from the base of *Acacia* shrubs. The collected litter was soaked in water and irradiated in a microwave oven for three minutes on maximum power setting. Irradiation acted to kill any surface invertebrates present and assisted in the breaking down of organic matter. Wet litter was added to the traps, which were then kept in sealed containers until immediately prior to insertion into the drillholes to avoid desiccation of leaf litter. After trap installation, the opening of each drillhole was sealed to maintain humidity and to avoid entry of surface fauna.

Traps were recovered after six weeks and stored in labelled zip lock bags to maintain humidity and prevent desiccation of potential troglofauna specimens during transportation to Perth.

Fauna specimens were recovered from the traps using specially designed Tullgren funnel units. Leaf litter from each trap was placed in a sieve under an aluminium lamp containing a 25-watt globe. This created a temperature of approximately 30°C at the surface of the leaf litter. A funnel situated below the leaf litter collected the fauna as they fell, directing them into an attached vial of 100% ethanol. Leaf litter was left in the Tullgren funnels for a period of 24 hours, or until dry, after which time the bulked invertebrate sample was removed.

3.4 Data and Specimen Management

Preliminary identification of troglofauna involved identification of specimens to order level, where possible, or separation of specimens into distinct morphotypes. Sorting was completed in Perth using dissecting microscopes with up to 40x magnification. Morphotypes were then assigned a unique number based on drill hole name, date, and method of collection. Specimens were preserved in 100% ethanol once separated out into morphotypes, which allows for molecular analyses.

3.5 Molecular Analysis

Molecular analysis of the collected troglofauna was conducted to determine the number of species present in each taxonomic group, and to compare the results to those obtained during previous surveys that have been undertaken for these groups elsewhere in the Pilbara. The collected specimens were sequenced by Helix Molecular Solutions (Helix) for variation at the mitochondrial cytochrome oxidase subunit I gene (CO1) (see Appendix 5).

Helix also provided analysis and interpretation of the molecular data. Maximum Likelihood analyses, with parameters determined in the Best Model analysis was adopted, comparing sequence data to representative sequences from regional context data sets. This resulted in specimens from the current survey being placed into genetic lineages, in context of specimens collected elsewhere in the region. Determination of putative species was then inferred based on the level of divergence between lineages of the same group (such as order or family), considering the variation within each lineage.

As phylogenetic analysis was based on sequence data from a single gene only, all putative species arrived at by this approach should be considered preliminary unless previously described and fully determined by taxonomic specialists. Where possible, species boundaries were inferred based on sequence variation within and between phylogenetic groups.

3.6 Categories of Conservation Significance

For this report, the conservation significance of identified troglofauna was categorised as per Table 3.1.

Table 3.1: Conservation classification used within this report.

Category	Description
Significant species	Species listed as priority or threatened at State or Federal levels.
Confirmed Short-range endemic (SRE) species	Species where sufficient taxonomic expertise is available, and with adequate representation in WAM collections or genetic databases, that are known to be limited in distribution based on geological characteristics.
Potential SRE species	Species where there is insufficient taxonomic knowledge or too few collections to determine SRE status. Habitat, morphology, molecular or taxonomic data deficient, but belonging to groups that may display short-range endemism.
Widespread (not SRE) species	Well-collected species, that are typically taxonomically well resolved. Species are not confined by geological barriers.

3.7 Survey Timing and Personnel

Five field mobilisations were conducted between June 2021 and January 2023 to achieve three sampling phases (Table 3.2).

Table 3.2: Summary of field mobilisations completed in survey area.

Mobilisation	Dates	Personnel	Purpose	Phase
1	16-18 June 2021	Dan Kamien Roxanne de Vos	Troglofauna habitat trap installation	1
2	16-18 August 2021	Dan Kamien Roxanne de Vos	Troglofauna habitat trap recovery	1
			Troglofauna habitat trap installation	2
3	29 September – 1 October 2021	Dan Kamien Roxanne de Vos	Troglofauna habitat trap recovery	2
4	29-30 November 2022	Michael Greenham Oliver Krumholz	Troglofauna habitat trap installation	3
5	19-20 January 2023	Michael Greenham Rob Hooper	Troglofauna habitat trap recovery	3

This study was managed by Dan Kamien. Preliminary sorting of collected troglofauna was completed by Jess Cairnes and Oliver Krumholtz, of Biota.

3.8 Climate and Weather

Long-term climate data (rainfall from 1885 – 2022, temperature data from 1956 – 2022) and recent weather data were obtained from the Bureau of Meteorological (BOM) weather station in Mardie (station number 5008), approximately 60 km north of the survey area.

Several major rainfall events occurred prior to the sampling period, particularly in the month of May, when triple the average rainfall fell (Figure 3.1). Past observations suggest the approximate timing of sampling after significant rainfall creates suitable conditions for sampling for troglofauna (Biota 2006).

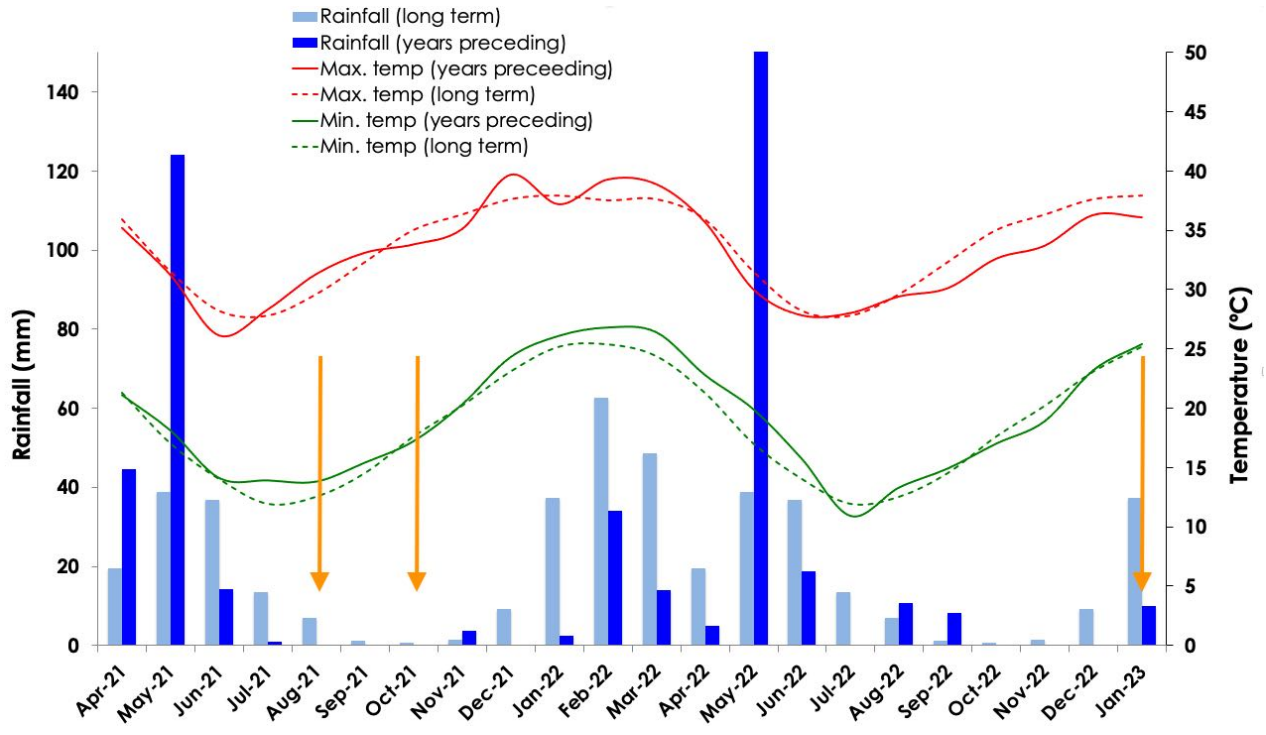

Figure 3.1: Climate and weather graph depicting long-term averages and 2021-2023 data.
Orange arrows indicate timing of trap extraction.

3.9 Sampling Effort

Seventy-four drillholes were sampled during the study with a total of 368 traps installed over the three sampling phases (Table 3.3, Figure 3.2 and Appendix 2). An additional 51 drillholes were considered for sampling, but these were either blocked or could not be located.

Table 3.3: Summary of troglofauna sampling.

Sampling Phase	Inside Conceptual Pit		Outside Conceptual Pit	
	No. Drill Holes Sampled	**No. of Traps Installed**	**No. Drill Holes Sampled**	**No. of Traps Installed**
Phase 1	19	61	14	41
Phase 2	19	61	14	39
Phase 3	42	126	15	40
Project Total	**56**	**248**	**18**	**120**

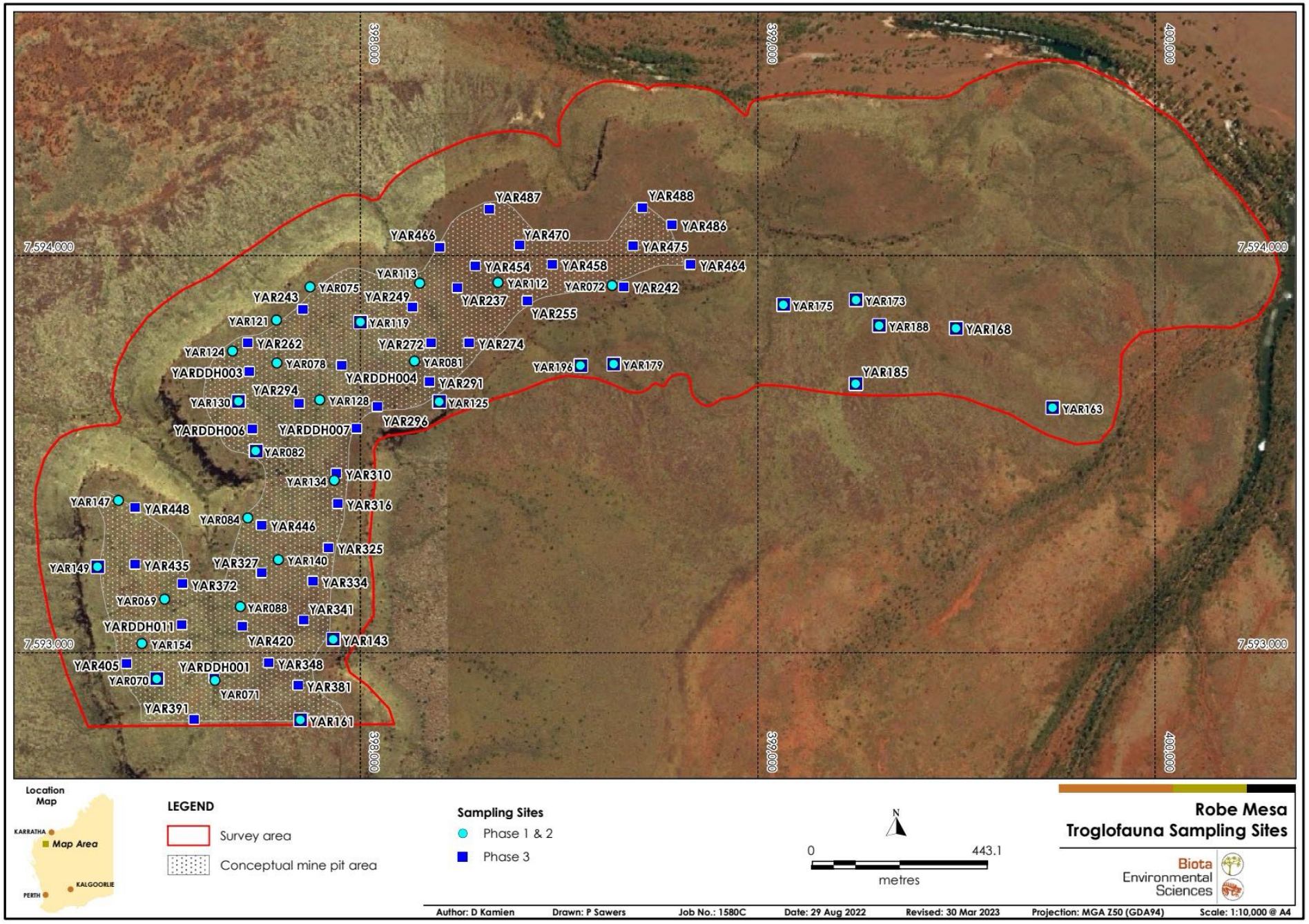

Figure 3.2: Troglofauna sampling site locations.

3.10 Study Limitations

Several limitations apply to this study, some of which are commonly associated with troglobitic fauna sampling, rather than being specific to this study. These limitations include:

1. Sampling for troglofauna within the region relies on drillholes, which are typically installed as part of exploration drilling and thus focus on geological units of economic importance. This creates a bias in the availability of drillholes for troglofauna sampling, as sampling tends to not extend far beyond the footprint of the target ore body.

   This places limitations on determining wider species distributions and potential barriers to dispersal. For the current study, most sampling was completed within the ore body (on top of Mesa F). As a result, most sampling was completed within habitat considered to be of high prospectivity for troglofauna.
2. Related to point 1, colluvial geological units were not sampled during the survey due to an absence of available drillholes. Colluvium is categorised as medium troglofauna habitat prospectivity, with troglofauna being infrequently recorded from this rock type in the Pilbara. In the Robe Valley, colluvium seems most likely to provide troglofauna habitat when in contact with pisolite, as is the case in the current survey area.
3. Surface geology mapping for the survey area was originally published in 1968 (Geological Survey of Western Australia (1968)) and later manually digitised. Due to the age of the surface geology mapping, there is a misalignment between surface geology mapping and conceptual pit outlines (as evident in Figure 4.1).
4. Genetic analysis was completed using the CO1 gene only. This gene was used because it has shown to be relatively fast evolving across many taxonomic groups and a typically reliable indicator of likely species boundaries. Recent studies, however, have indicated that using specific genes for different taxa, or using multiple genes, may increase accuracy.
5. Previously recorded Mesa F specimens were not identified to species level (Biota 2006, 2011b). Therefore, direct comparison of the specimens sequenced during the current survey were not made to previously recorded specimens (Biota 2006, 2011b).
6. Due to excessive rain, access was restricted during Phase 3 trap retrieval. As a result, traps from eight drillholes on the lower pisolite could not be retrieved (Appendix 2).

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4.0 Results

4.1 Desktop Study

4.1.1 Priority Ecological Communities

The subterranean invertebrate communities present within the Robe Valley mesas represent a Priority Ecological Community (PEC) (Table 4.1). This includes Mesa F and the survey area.

Table 4.1: Description of PEC overlapping the survey area and study area.

PEC Name	Description	Category
Subterranean invertebrate communities of mesas in the Robe Valley region	A series of isolated mesas occur in the Robe Valley in the state's Pilbara Region. The mesas are remnants of old valley infill deposits of the palaeo Robe River. The troglobitic faunal communities occur in an extremely specialised habitat and appear to require the particular structure and hydrogeology associated with mesas to provide a suitable humid habitat. Short range endemism is common in the fauna. The habitat is the humidified pisolitic strata (DBCA 2022).	Priority 1

The PEC is categorised as Priority One; Poorly-known ecological communities, which "are known from very few occurrences with a very restricted distribution (generally ≤5 occurrences or a total area of ≤ 100ha). Occurrences are believed to be under threat either due to limited extent, or being on lands under immediate threat (e.g. within active mineral leases) or for which current threats exist. Communities may be included if they are comparatively well-known from one or more localities but do not meet adequacy of survey requirements, and/or are not well defined, and appear to be under immediate threat from known threatening processes across their range" (DBCA 2022).

4.1.2 Previous Relevant Studies

Previous relevant surveys at Mesa F include two phases of troglofauna sampling of 29 sites (each sampled once only) (Biota 2006, 2011b), however none of these sites occur within the survey area. Extensive sampling has been conducted within the rest of the study area, with a total of 19 phases undertaken across Mesas B, C and G (Table 4.2).

Table 4.2: Summary of previous surveys completed within the study area.

	Area Surveyed				
	Mesa F		Mesa B	Mesa C	Mesa G
Citations	Current study	Biota 2006, 2011b	Biota 2006, 2011b, 2017a, 2019a, 2019b, 2020, Bennelongia 2017	Biota 2006, 2011b, 2017a, 2017b, 2019a, 2019b, 2020	Biota 2006, 2009a, 2009b, 2011b, 2012
Phases	3	2	7	6	6
Sites sampled	74	29	45	39	29

4.1.3 Previously Recorded Troglofauna

A total of 47 troglobitic species and nominal species have previously been recorded within the study area (Table 4.3 and Appendix 3). This total comprises 358 specimens belonging to 12 orders, (Table 4.3), of which many are considered to represent short-range endemic fauna (Biota 2011b). The most commonly collected specimens belong to the Schizomida, which are represented by three genetically distinct species within the study area (Biota 2006, 2011b, Harvey et al. 2008, Abrams et al. 2020):

- *Draculoides bythius* at Mesas B and C (Vulnerable under the Biodiversity Conservation (BC) Act 2016);
- *Draculoides gnophicola* at Mesa G (Vulnerable under the BC Act 2016); and
- *Draculoides* sp. nov. 'Mesa F' at Mesa F.

Table 4.3: Results of troglofauna database search for the study area.

Taxonomy		No. of Species and Nominal Species	No. of Significant Species	No. Collected	Mesa Recorded
Class	**Order**				
Arachnida	Araneae	6	0	9	B, C, G
	Opilionida	1	0	1	C
	Pseudoscorpiones	13	2	86	B, C, G
	Schizomida	3	3	133	B, C, F, G
Chilopoda	Scolopendrida	2	0	4	B, G
Diplopoda	Polydesmida	3	0	9	B, C, G
Entognatha	Diplura	2	0	3	F, G
Insecta	Blattodea	2	0	42	G
	Coleoptera	3	0	32	B, C
	Archeaognatha	1	0	1	C
	Zygentoma	2	0	4	B, C, G
Malacastraca	Isopoda	9	0	34	B, C, F, G
	Total	**47**	**5**	**358**	

4.2 Habitat Characterisation

Troglofauna habitat is primarily a function of available space, maintenance of a constantly high humidity and the potential for nutrient input from surface systems (Humphreys 1991, Wilkens et al. 2000, Biota and DC Blandford & Associates 2013). The following sections provide an initial description of the physical aspects of habitat relevant to troglofauna communities.

4.2.1 Geology and Habitat Prospectivity

The Robe Valley mesas were formed by the dissection and erosion of Robe Pisolite paleochannels by drainage systems (Ramanaidou et al. 2003), a process that started approximately 10 million years ago. The mesas are in fact CID deposits which rise above the surrounding Fortescue formation basement rock. Mesa F (which includes the survey area) is one of several such CID deposits that comprise the Robe Pisolite Formation in the Robe River valley. Pisolitic ore comprises a combination of the iron minerals goethite and hematite. The majority of the pisolite is made up of spherical accretions of iron minerals called pisoliths (Ramanaidou et al. 2003).

Robe Valley mesa formations are frequently vuggy, fractured and often contain small-scale caverns; thereby representing suitable habitat for subterranean fauna (Biota 2006, 2011b, 2016a). The gradual isolation of troglofauna habitat has led to the formation of unique troglobitic communities within each disconnected mesa within the Robe Valley (Biota 2006, Harvey et al. 2008, Biota 2011b, 2016a).

Troglobitic habitat suitability may also be associated with the hydrological function of the mesa such as impeding layers and clay lenses which store infiltrated water from recharge events, maintaining humidity in the system (Biota and DC Blandford & Associates 2013). Additionally, the combination of these geological units provides for percolation of water and associated nutrients from surface habitats. Based on the categories outlined in Section 3.2, Robe Pisolite is categorised as a highly prospective unit, which represents primary habitat for troglofauna in the survey area. The surface geology is presented in Figure 4.1 in relation to the conceptual mine pit area and sampling locations.

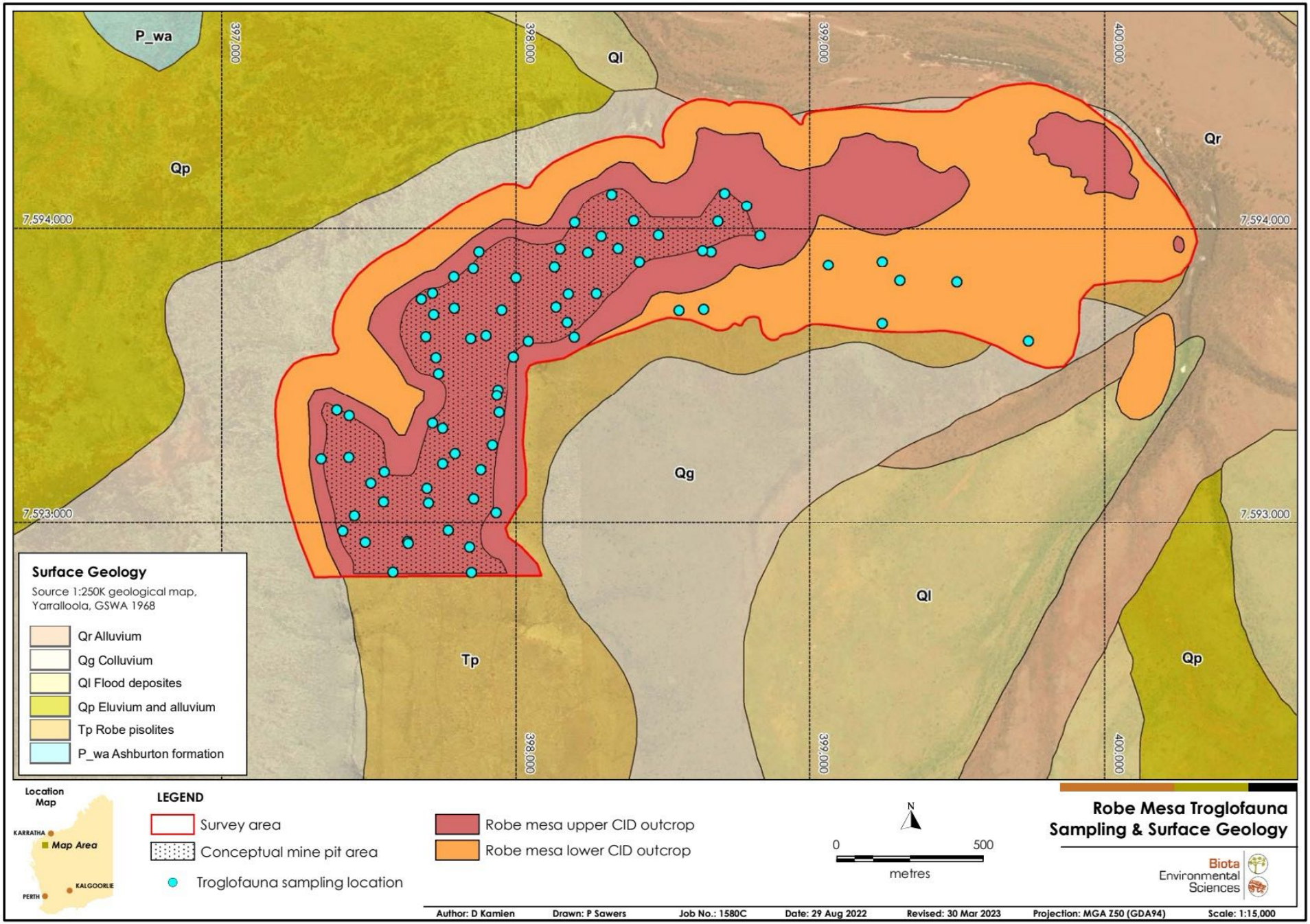

Figure 4.1: Sampling sites and surface geology of the survey area.

4.2.2 Geology and Stratigraphy

Stratigraphically, the survey area comprises two channels of pisolitic ironstone (Plate 4.1), which are separated by interstitial waste (Plate 4.2) and clay units that likely form a confining barrier between the upper pisolite and lower pisolite (CZR Resources 2023, Table 4.4 and Figure 4.2). The conceptual pit outline is located wholly within the Robe Pisolite upper CID outcrop (Figure 4.1 and Figure 4.2), but the mining will extract ore from both the upper and lower CID (Figure 4.3).

Table 4.4: Survey area stratigraphy (CZR Resources 2023).

Figure 4.2 Legend	Geology Code	Unit Name	Description	Habitat Suitability Characteristics
	MCU	CID – upper	Pisolitic ironstone - strongly mineralised	High
	MMU	Mixed zone – upper	Pisolitic ironstone - poorly sorted	High
	WII	Interstitial waste	Sandy ironstone with some mixed pisolite	Low
	WCI	Interstitial clay	Clay rich lenses within interstitial waste	Low
	MCL	CID – lower	Pisolitic ironstone - strongly mineralised	High
	MML	Mixed zone - lower	Pisolitic ironstone - poorly sorted	High
	WIB	Silty ironstone-basal	Clay rich ironstone	Low
	WCB	Basal clay	Claystone basal unit	Low

Plate 4.1: Diamond core of CID-upper layer depicting vugs and fractures (CZR Resources 2023).

Plate 4.2: Diamond core of interstitial waste layer depicting absence of vugs (CZR Resources 2023).

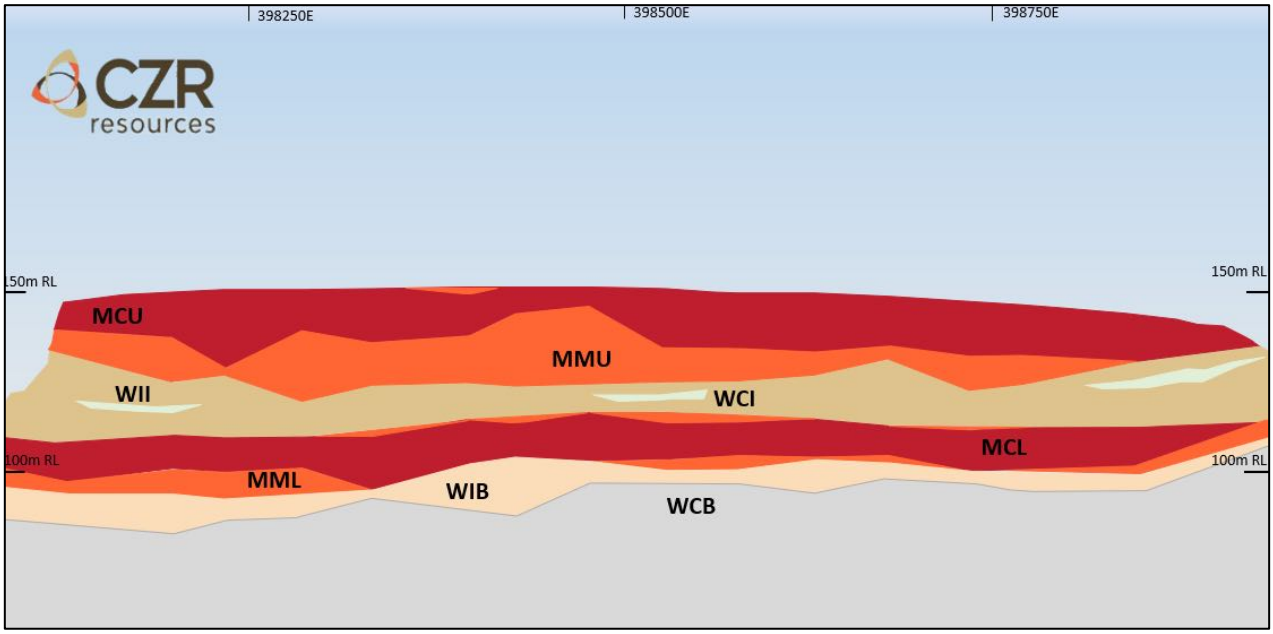

Figure 4.2: Schematic cross-section of the survey area (CZR Resources 2023).

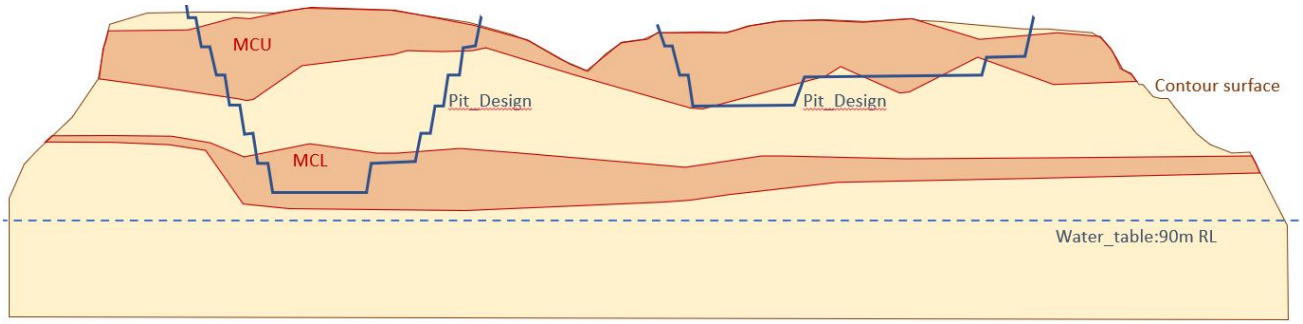

Figure 4.3: Schematic cross-section indicating nominal pit design (CZR Resources 2023).

4.3 Survey Results

A total of 102 troglomorphic specimens were collected from 22 drillholes across the three sampling phases. The specimens represented seven classes and eight orders (Table 4.5 and Appendix 4).

Table 4.5: Summary of troglomorphic fauna recorded from the survey area.

Taxonomy				n	Number of Sites
Phylum	**Subphylum**	**Class**	**Order**		
Arthropoda	Chelicerata	Arachnida	Pseudoscorpiones (Pseudoscorpion)	2	2
			Schizomida (Schizomid)	15	8
	Hexapoda	Entognatha	Diplura (Dipluran)	3	3
		Insecta	Zygentoma (Silverfish)	12	3
	Myriapoda	Symphyla	Cephalostigmata (Symphylan)	4	4
		Diplopoda	Polydesmida (Polydesmid millipede)	34	1
	Crustacea	Malacostraca	Isopoda (slater)	28	7
Mollusca	Gastropoda	Stylommatophora	Pupillidae (Chrysalis snail)	4	3
			Total:	**102**	

As is common during troglofauna surveys, many edaphobitic (deep soil adapted fauna) and epigean (surface) specimens were also collected. These specimens are not troglobitic and are therefore not discussed further in this report.

A detailed account of the troglobitic and potentially troglobitic fauna recorded, including discussion of the results of genetic analysis, follows in Sections 4.3.1 to 4.3.8. Record locations are presented in Figure 4.4.

4.3.1 Order Pseudoscorpiones

Two pseudoscorpion specimens of the family Olpiidae were recorded from the survey area at site YAR070 (during Phase 1) and site YAR296 (Phase 3) (Figure 4.4). Molecular analysis placed the Phase 1 specimen into a new and previously undocumented nominal species, Olpiidae sp. H-PO033 (Plate 4.3), with the most closely related specimen previously recorded at nearby Mesa B, having a sequence divergence of 5.7% (Appendix 5). Sequencing of the specimen recorded at YAR296 during Phase 3 confirmed its identity as the described species *Indohya alexanderi* (Appendix 5). *I. alexanderi* is not restricted to the survey area and has previously been recorded from several other Robe valley mesas (Harvey et al. 2023).

Plate 4.3: Olpiidae sp. H-PO033 recorded from drillhole YAR070.

4.3.2 Order Schizomida

Fifteen schizomid specimens of the family Hubbardiidae were recorded across the three sampling phases at sites YAR072, YAR125, YAR143, YAR161, YAR296, YAR466, YARDDH001 and ARDDH007 (Figure 4.4). Molecular analysis placed all of the specimens into the same previously undocumented nominal species *Draculoides* sp. H-SCH200 (Plate 4.4) (Appendix 5), with the most closely related species being *Draculoides cochranus*, previously recorded at Red Hill Station approximately 22 km southeast of the survey area (Abrams et al. 2020). *D. cochranus* shows 9.3% sequence divergence from *Draculoides* sp. H-SCH200 (Appendix 5). The specimen recorded at site YAR072 failed to sequence.

Six schizomid specimens have previously been recorded adjacent to the survey area on Mesa F (Biota 2011b). These previously recorded specimens are juveniles, and cannot be morphologically identified to species level (Biota 2011b).

Plate 4.4: *Draculoides* sp. H-SCH200 recorded from drillhole YAR161.

4.3.3 Order Diplura

Three dipluran specimens of the family Japygidae were recorded from the survey area at sites YAR149, YAR173 and YAR296 (Figure 4.4.). One specimen was recorded during Phase 3, but DNA sequencing was not successful, with the remaining two specimens recorded during Phase 2 (from sites YAR149 and YAR173) being successfully sequenced and placed into separate lineages (H-DJA022 and H-DJA023 respectively). These lineages were 4.5% divergent from each other but sit within the same well-supported clade (Appendix 5).

As each lineage is represented by a single specimen, genetic divergence within lineages cannot be determined to inform potential species boundaries. However, we have conservatively treated them here as two separate species-equivalent taxa.

Japygidae sp. H-DJA022 (Plate 4.5) and Japygidae sp. H-DJA023 are most closely related to the Japygidae species recorded on nearby Mesa C, at 7.3% and 7.9% sequence divergence respectively, indicating that the Mesa C specimens represent different species to those recorded in the survey area (Appendix 5).

Plate 4.5: Japygidae sp. H-DJA022 recorded from drillhole YAR149.

4.3.4 Order Zygentoma

Twelve troglobitic silverfish specimens were recorded from three drillholes during the Phase 3 sampling (Figure 4.4.). These represent the first Zygentoma recorded at Mesa F. Two morphotypes were recognised on initial identification: an elongate body type (Plate 4.6) and a more dorsoventrally flattened (Plate 4.7).

Plate 4.6: Nicoletiinae sp. H-TN001 recorded from drillhole YAR125.

Plate 4.7: Ateluriinae sp. H-TA010 recorded from drillhole YAR143.

Molecular analysis revealed that these morphological differences corresponded to different families, with the elongate specimens belonging to family Nicoletiinae and the other morphotypes being Ateluriinae (Appendix 5; Table 4.6). Phylogenetic analysis of the six successfully sequenced specimens identified four putative species (Table 4.6). None of the species have been recorded from outside the survey area based on available reference data (Appendix 1).

Table 4.6: Zygentoma taxa recorded during the survey.

Family	Taxon	Site	Number
Nicoletiinae	Nicoletiinae sp. H-TN001	YAR486	1
	Nicoletiinae sp. H-TN002	YAR125	1
	Nicoletiinae sp. H-TN003	YAR143	1
Ateluriinae	Ateluriinae sp. H-TA010	YAR143	3

4.3.5 Order Cephalostigmata

Four Symphyla specimens (order Cephalostigmata; family Scolopendrellidae) were recorded from the survey area during Phase 1 and 2 at sites YAR134, YAR163, YAR173, and a single specimen during Phase 3 at YAR291 (Figure 4.4). Molecular analysis placed the three specimens recorded in Phases 1 and 2 in three distinct but closely related lineages (H-SYM038, H-SYM039 (Plate 4.8) and H-SYM040), with 3.1% to 6.7% sequence divergence amongst them (Appendix 5). The Phase 3 specimen could not be successfully sequenced. As each lineage is represented by a single specimen, it is unclear if this level of divergence represents three distinct species (Appendix 5). However, again we have conservatively treated each lineage as a species-equivalent taxon.

The recorded specimens are most closely related to lineages H-SYM027 and H-SYM028, which were previously recorded at Mesa C, and Warramboo respectively (Biota database). Sequence divergence indicates that taxa recorded from the survey area represent different species to those recorded at Mesa C and Warramboo (Table 4.7 and Appendix 5).

Table 4.7: Comparison of sequence divergence of recorded Symphyla (Phase 1 and Phase 2) to neighbouring specimens.

Species within Survey Area	Site	Sequence Divergence	
		H-SYM027 (Mesa C)	H-SYM028 (Warramboo)
Scolopendrellidae sp. H-SYM038	YAR134	12.8%	8.8%
Scolopendrellidae sp. H-SYM039	YAR163	8.5%	12.0%
Scolopendrellidae sp. H-SYM040	YAR173	6.0%	9.5%

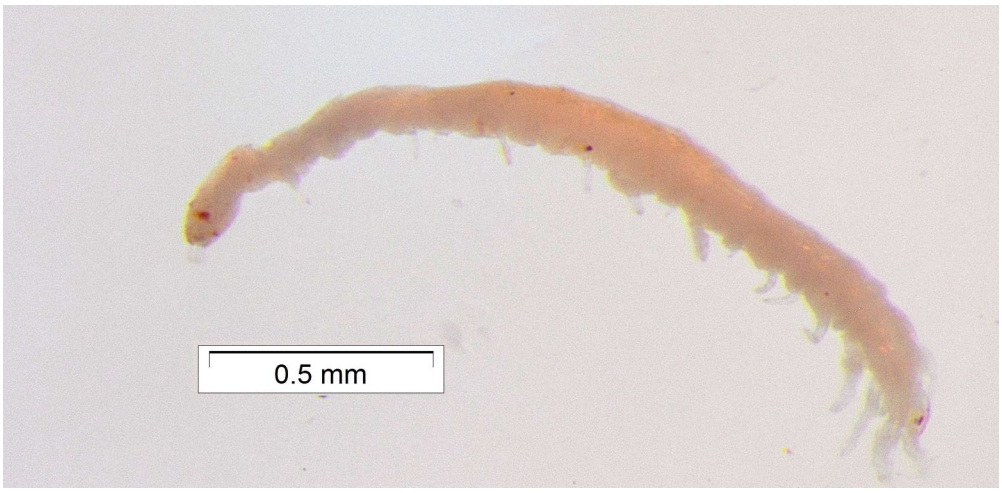

Plate 4.8: Scolopendrellidae sp. H-SYM039 recorded from drillhole YAR163.

4.3.6 Order Polydesmida

Forty-four polydesmid millipedes (family Haplodesmidae) were recorded from a single drillhole during the Phase 3 sampling; site YAR487 (Figure 4.4 and Plate 4.9). These represent the first Polydesmida recorded at Mesa F.

Plate 4.9: Haplodesmidae sp. recorded from drillhole YAR487.

Molecular analysis of the Phase 3 specimens found that all represented the same species, Haplodesmidae sp. DIHAP001, which has not been previously recorded (Appendix 1).

4.3.7 Order Isopoda

Seven isopod specimens belonging to the family Armadillidae were recorded from three sites during Phase 2 and Phase 3, and 21 specimens belonging to the family Philosciidae were recorded from four sites across all three phases (Figure 4.4).

Within the family Philosciidae, two new lineages separated by a 4.3% sequence divergence were recorded during Phase 1 and 2 (lineages H-ISP059 and H-ISP060) (Appendix 5). Twenty-one of the total 24 Isopoda specimens from Phase 3 sampling were successfully sequenced, yielding a third philosciid lineage (H-ISP061) (Plate 4.10), all of which have been conservatively treated as individual taxa (Appendix 5; Table 4.6). The single armadillid taxon, Armadillidae sp. H-ISA064, has only been recorded from site YAR249 (Table 4.6; Figure 4.4).

Table 4.8: Isopoda taxa recorded during the survey.

Family	Taxon	Site	Number
Philosciidae	Philosciidae sp. H-ISP059	YAR082, YARDDH004	1
	Philosciidae sp. H-ISP060	YAR161	1
	Philosciidae sp. H-ISP061	YAR237, YAR249	1
Armadillidae	Armadillidae sp. H-ISA064	YAR249	3

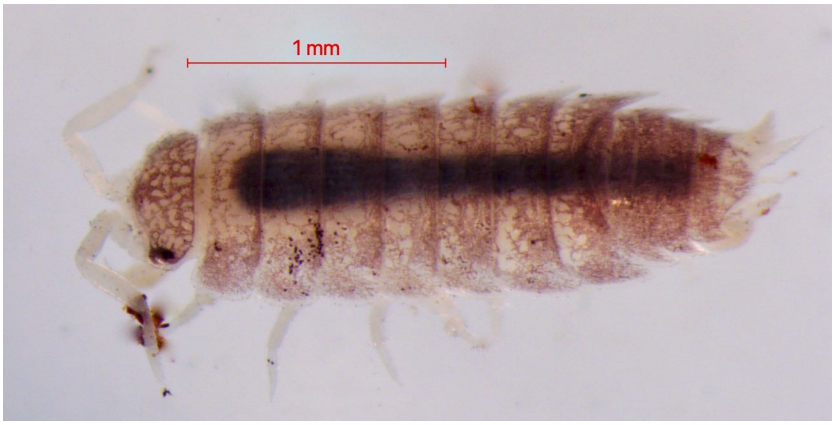

Plate 4.10: Philosciidae sp. H-ISP061 recorded at YAR237.

4.3.8 Order Stylommatophora

Four Stylommatophora snail specimens of the family Pupillidae were recorded from the survey area at sites YAR075, YAR124 and YAR251 (Figure 4.4.). However, sequencing failed for two of the specimens. The remaining two specimens were genetically identical and both identified as *Gastrocopta servilis* (Plate 4.11); a widespread terrestrial snail in Asia and Australia (ALA database). It is not troglobitic and does not represent an SRE.

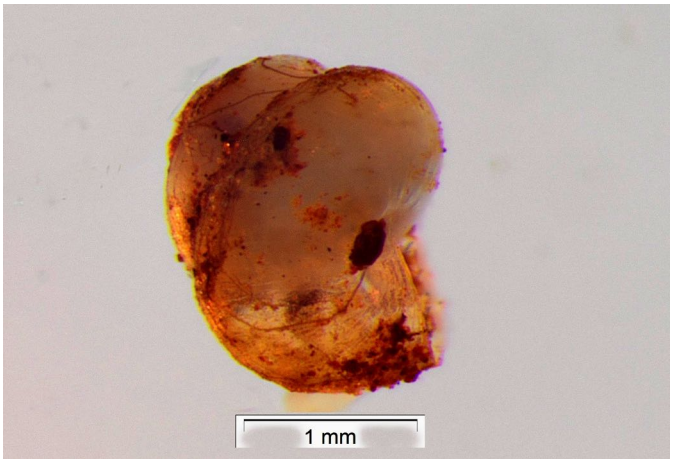

Plate 4.11: *Gastrocopta servilis* recorded from site YAR075.

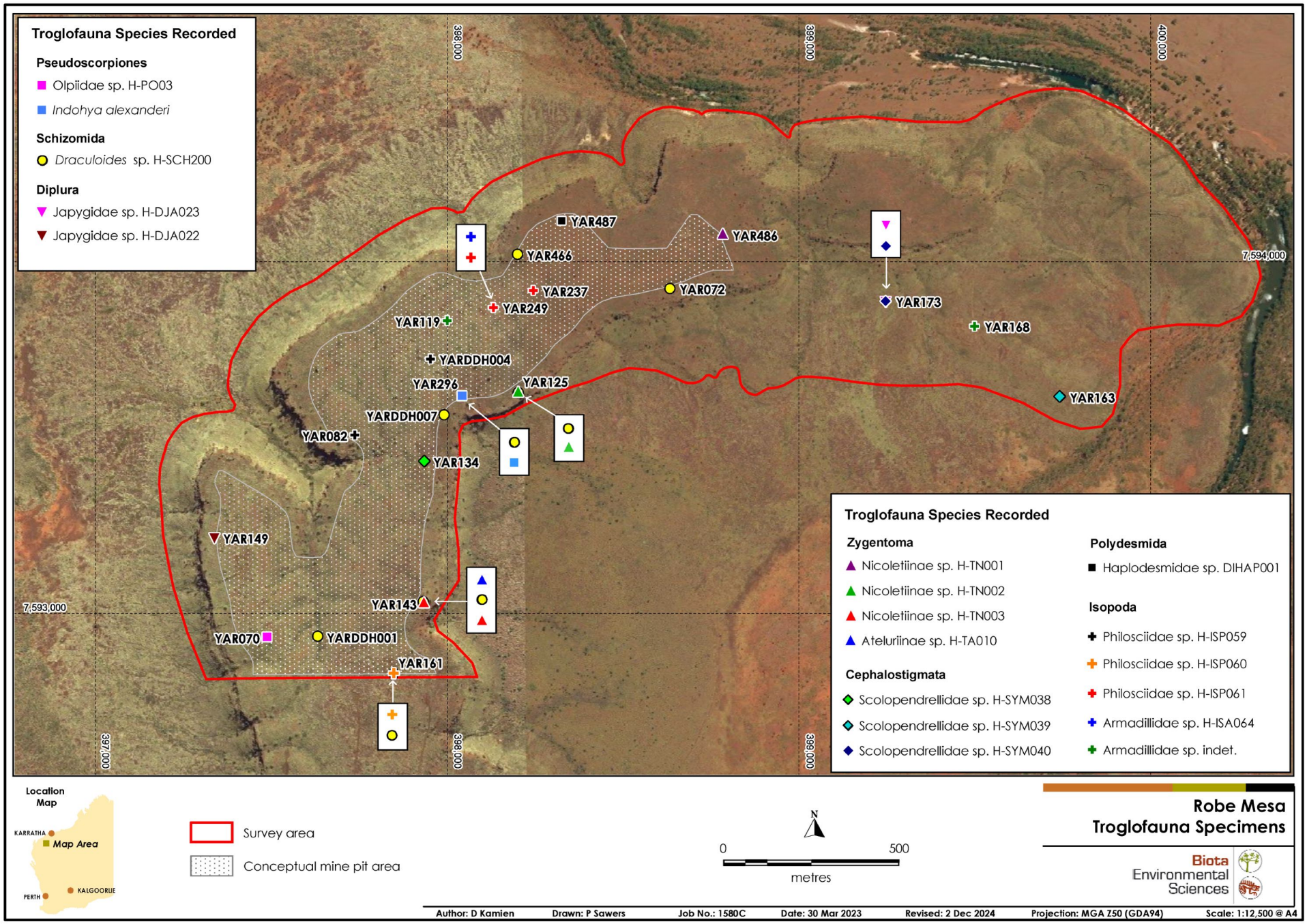

Figure 4.4: Troglobitic taxa recorded during the survey.

5.0 Discussion

A total of 102 troglobitic fauna specimens were recorded from the three phases of sampling. These represent a minimum of 17 putative troglobitic species from seven orders; Pseudoscorpiones, Schizomida, Polydesmida, Diplura, Zygentoma, Symphyla and Isopoda.

Although species representing the same taxonomic orders have been recorded on other mesa formations in the Robe Valley (Biota 2012, 2016b, 2017b, 2017a, 2019c), the great majority of species-level taxa recorded and identified via molecular sequencing from the survey area have not been previously documented. Only one of the 17 species, the pseudoscorpion *Indohya alexanderi*, has been recorded from another nearby mesa. Considering the Robe Valley troglofauna have been extensively surveyed over the past 15 years and are well represented in genetic databases, it is evident that the troglobitic community recorded during the survey is essentially endemic to Mesa F.

The recorded taxa are all potentially significant as each:

- has a very short-range distribution, with apparently restricted to Mesa F;
- is representative of relictual fauna (Harvey 2002), with the Robe Valley troglofauna representing lineages derived from the late Miocene (at least the last 10 million years); and
- forms part of the Robe Valley PEC.

The sequenced specimens recorded from Mesa F are genetically distinct from similar specimens recorded at other mesas in the Robe Valley. This corroborates previous studies that demonstrate that mesas in the Robe Valley are habitat isolates, and there is no gene flow between mesas. Although the species recorded in the survey area are currently undescribed, it is probable that if formally described, they would be designated as significant, as is the case for many troglobitic species in the Robe Valley, including five *Draculoides* species.

Seven of the 17 troglobitic taxa recorded during the survey are known only from within the conceptual pit outline (Table 5.1 and Figure 4.4).

Table 5.1: Troglobitic fauna recorded solely within the conceptual pit outline.

Taxon	Sites	Significance
Olpiidae H-PO03	YAR070	Confirmed SRE species
Scolopendrellidae sp. H-SYM038	YAR134	Confirmed SRE species
Philosciidae sp. H-ISP059	YAR082, YARDDH004	Confirmed SRE species
Philosciidae sp. H-ISP060	YAR161	Confirmed SRE species
Philosciidae sp. H-ISP061	YAR237, YAR249	Confirmed SRE species
Armadillide sp. H-ISA064	YAR249	Confirmed SRE species
Haplodesmidae sp. DIHAP001	YAR487	Confirmed SRE species

However, based on surface geology (Figure 4.1) and modelled stratigraphic cross-sections (Figure 4.2), equally suitable habitat is likely to occur outside of the conceptual pit outline within the survey area. This is supported by the recorded locations of *Draculoides* sp. H-SCH200, which occurs both inside and outside the conceptual pit. Similarly, suitable habitat is likely to occur throughout Mesa F, which is contiguous with that of the survey area (Figure 4.1 and Biota 2006, 2011b). It is therefore very likely that the species recorded during the survey would also occur throughout Mesa F. This is implicitly supported by collections of unsequenced *Draculoides* sp. nov. 'Mesa F'. Japygidae sp. and Armadillidae sp. obtained from previous surveys at Mesa F (Biota 2006, 2011b), that possibly represent the same species as those recorded during the current survey. Data from other, more extensively sampled, mesas in the locality demonstrate the same patterns, with any given species typically occurring across the extent of each landform (Biota 2006, 2011b).

Stratigraphic data shows the upper and lower pisolite (troglofauna habitat) to be separated by Interstitial waste and clay layers that may form a vertical barrier to troglofauna (CZR Resources 2023). To date, this has not been substantiated or disproven by the troglofauna data.

Additionally, troglofauna are known to move up or down drillhole walls, meaning it cannot be stated with certainty at what depth they naturally occur[2]. However, data from other Robe mesas with similar stratigraphic arrangements have shown that troglofauna occur in deeper pisolite layers, below clay-dominated impeding layers, and that any vertical barriers are typically not complete (Biota and DC Blandford & Associates 2013, Biota 2019d, 2020b).

[2] Five shallow drillholes (<30 m depth), penetrating only the upper pisolite were sampled, but no fauna were recorded.

6.0 Glossary

Edaphobitic / Edaphobite	Soil dwelling fauna that can often display troglomorphic characteristics. Edaphobites are unlikely to have limited distributions and therefore unlikely to be classified as short-range endemics.
EPA	Environmental Protection Authority.
Interstices	An opening or space, especially a small or narrow one between mineral grains in a rock or within sediments or soil.
Short-Range Endemic (SRE)	A species that has a naturally small distribution and is often characterised by having poor dispersal capabilities, confinement to disjunct habitats and low fecundity.
Taxonomy	Theory and practice of biological classification.
Troglomorphic	Pertaining to morphological, behavioural or physiological characters that are adaptions to living in subterranean environments, such as loss of pigments and reduced eyes.
Troglophilic / Troglophile	Species able to live and reproduce underground as well as in the epigean environment (Wilkens et al. 2000).
Troglobite / Troglofauna	Species living obligatory in caves; also blind, depigmented and often having an elongate body morphology.
Trogloxene	Organisms that can come and go from a cave environment but use it during certain periods of their life cycles, such as nesting and hibernation.
Vug	A small to medium-sized cavity inside rock.
WAM	Western Australian Museum

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Appendix 1

DBCA Fauna Taking Licence

Department of **Biodiversity, Conservation and Attractions**

GOVERNMENT OF WESTERN AUSTRALIA

FAUNA TAKING (BIOLOGICAL ASSESSMENT) LICENCE

Regulation 27, Biodiversity Conservation Regulations 2018

Licence Number: BA27000433

Licence Holder: Mr Daniel Kamien
Biota Environmental Pty Ltd
PO Box 155
LEEDERVILLE WA 6903

Date of Issue: 07/05/2021

Date Valid From: 04/06/2021
Date of Expiry: 03/06/2022

LICENSED ACTIVITIES

Subject to the terms and conditions on this licence, the licence holder may –

1. Take or disturb fauna for Robe Mesa project subterranean fauna survey to inform feasibility studies and future environmental impact assessment (EIA) using troglofaunal traps Customised PVC irrigation pipes with a series of holes drilled into the sides, a screw cap at one end and permanent opening at the other. 80 mm diameter, 200 mm length and stygofauna haul nets Modified plankton haul net made out of 150 µm plankton mesh with a 100 mm aperture attached to a weighted catch jar. Specimens to be lodgement with the WA Museum.

LOCATIONS

1. Mesa F, Robey Walley (Pilbara Region).

AUTHORISED PERSONS

The following persons or persons of the specified class may assist in carrying out the licensed activities:

1. Nathan Beerkens
2. Joshua Keen
3. Michael Greenham
4. John Graff
5. Sylvie Schmidt
6. Jason Alexander
7. De Vos Roxanne

CONDITIONS

1. Fauna must not be taken on CALM land, (as defined in the Conservation and Land Management Regulations 2002*),* unless authorised by a written notice of a lawful authority issued under regulations 4 and 8 of the Conservation and Land Management Regulations 2002.
2. If persons, other than the licence holder, are authorised to carry out/assist in carrying out the activities under the licence, the licence holder must ensure those persons have read and understand the licence terms and conditions.

3. The written authorisation of the person in possession or occupation of the land accessed and upon which fauna is taken, as required under regulation 101(2) and referred to in "Additional information" below, must:
   a) state location details (including lot or location number, street/road, suburb and local government authority);
   b) state land owner or occupier name, and contact phone number;
   c) specify the time period that the authorisation is valid for;
   d) be signed and dated; and
   e) be attached to this licence at all times.
4. This licence, and any written authorisation or lawful authority which authorises the take of fauna on specified locations must be carried at all times while conducting licensed activities and be produced on demand by a wildlife officer.
5. If a species of fauna listed as a threatened species under Section 19 of the *Biodiversity Conservation Act 2016* is inadvertently captured, that species is to be released immediately at the point of capture. If the fauna is injured or deceased, the licence holder shall contact the DBCA Wildlife Licensing Section (wildlifelicensing@dbca.wa.gov.au) for advice on treatment or disposal. Details of any capture of threatened fauna must be included in the "Return of Fauna Taken."
6. The licence holder must not:
   a) release any fauna in any area where it does not naturally occur;
   b) transfer fauna to any other person or authority (other than the Western Australian Museum) unless approved in writing by the CEO; or
   c) dispose of the remains of fauna in any manner likely to interfere the natural or present day distribution of the species.
7. The licence holder must not take and remove more than ten specimens of any one protected species of fauna from any location less than 20km apart. Where exceptional circumstances make it necessary to take a larger number of specimens from a particular location in order to obtain adequate statistical data, the collector must proceed with circumspection and justify their actions to the Director General in advance.
8. All holotypes and syntypes and a half share of paratypes of species or subspecies permitted to be permanently taken under this licence must be donated to the Western Australian Museum. Duplicates (one pair in each case) of any species collected, which represents a significant extension of geographic range must be offered to the Western Australian Museum.
9. All specimens and material retained under the authority of this licence must be offered to the Western Australian Museum for loan, for inclusion in its collection, or on request be made available to other persons involved in relevant scientific studies.
10. The licence holder must create, compile and maintain records and information as required in a DBCA approved "Return of Fauna Taken" of all fauna taking activities as they occur.
11. A DBCA approved "Return of Fauna Taken" must be completed in full (including nil taking details) and submitted to DBCA Wildlife Licensing Section (wildlifelicensing@dbca.wa.gov.au) prior to the end of each annual period of the licence (from the valid from date) (refer to "Additional Information" section

Danny Stefoni
LICENSING OFFICER
WILDLIFE PROTECTION BRANCH

Delegate of CEO

ADDITIONAL INFORMATION

1. It is an offence to take any species of fauna listed as a threatened species under Section 19 of the *Biodiversity Conservation Act 2016* unless the person is authorised under Section 40. The penalty ranges between $300 000 and $500 000; Section 150 Biodiversity Conservation Act 2016.
2. Regulation 82 empowers the CEO to add, substitute or delete a term or condition of a licence or to correct errors. Such power may be exercised on application of a licence holder or by the CEO's own initiative. If an amendment to a licence term or condition is required, please contact the CEO or the Licensing Section on wildlifelicensing@dbca.wa.gov.au in the first instance. The licence holder, if adversely affected by a condition imposed in this licence, may apply to the State Administrative Tribunal for review of the decision of the CEO to impose that condition on a licence: regulation 89(2) Biodiversity Conservation Regulations 2018.
3. A person must not contravene a condition of a licence. The penalty for an offence involving the contravention of a condition of a licence is a fine of $10 000: regulation 84 of the Biodiversity Conservation Regulations 2018.
4. It is an offence for persons authorised by this licence to enter land that is not in their possession or under their control without first having the *prior* written authorisation of the current owner or occupier of the land to:

   a) enter the land; and

   b) carry out the activity authorised by this licence.

   The penalty for this offence is a fine of $5 000: regulation 101(2) of the Biodiversity Conservation Regulations 2018.
5. The licence holder must be able to produce for inspection upon request any information or records required by regulation 85(2) of the Biodiversity Conservation Regulations 2018 Penalty $10 000. It is an offence to knowingly include false or misleading information or make statements in records: regulation 85(3) of the Biodiversity Conservation Regulations 2018 Penalty $10 000. It is an offence to include any information or make any statement in a return that the licence holder knows to be false or misleading in a material particular: regulation 86 (2) of the Biodiversity Conservation Regulations 2018 Penalty $10 000.
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7. The issuing of a licence under the Biodiversity Conservation Regulations 2018 does not constitute an animal ethics approval or a licence to use animals for scientific purposes as required under the *Animal Welfare Act 2002,* Animal Welfare (Scientific Purposes) Regulations 2003. It is the responsibility of a licence applicant / licence holder to ensure that they comply with the requirements of all applicable legislation. Enquiries relating to the Animal Welfare Act licences and animal ethics approvals are to be

directed to the Department of Primary Industries and Regional Development (https://www.agric.wa.gov.au/animalwelfare).

8. Threatened fauna can only be taken under a *Biodiversity Conservation Act 2016* Section 40 authorisation, Occurrences of threatened species must be reported to the CEO. For more information please see https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities/threatened-animals.

9. Any interaction involving Nationally Listed Threatened Fauna that may be invasive and/or harmful to the fauna may require approval from the Commonwealth Department of the Environment and Energy http://www.environment.gov.au/about-us/business-us/permits-assessments-licences. Interaction with such species is controlled by the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* and Environment Protection and Biodiversity Conservation Regulations 2000 as well as the *Biodiversity Conservation Act 2016* and Biodiversity Conservation Regulations 2018.

Department of **Biodiversity, Conservation and Attractions**

GOVERNMENT OF WESTERN AUSTRALIA

FAUNA TAKING (BIOLOGICAL ASSESSMENT) LICENCE

Regulation 27, Biodiversity Conservation Regulations 2018

Licence Number: BA27000433-2

Licence Holder: Mr Daniel Kamien
Biota Environmental Pty Ltd
PO Box 155
LEEDERVILLE WA 6903

Date of Issue: 23/11/2022

Date Valid From: 28/11/2022
Date of Expiry: 27/11/2023

LICENSED ACTIVITIES

Subject to the terms and conditions on this licence, the licence holder may –

1. Take or disturb fauna for Robe Mesa project subterranean fauna survey to inform feasibility studies and future environmental impact assessment (EIA) using troglofaunal traps Customised PVC irrigation pipes with a series of holes drilled into the sides, a screw cap at one end and permanent opening at the other. 80 mm diameter, 200 mm length and stygofauna haul nets Modified plankton haul net made out of 150 µm plankton mesh with a 100 mm aperture attached to a weighted catch jar. Specimens to be lodgement with the WA Museum.

LOCATIONS

1. Mesa F, Robey Walley (Pilbara Region).

AUTHORISED PERSONS

The following persons or persons of the specified class may assist in carrying out the licensed activities:

1. Nathan Beerkens
2. Joshua Keen
3. Michael Greenham
4. John Graff
5. Sylvie Schmidt
6. Jason Alexander
7. De Vos Roxanne
8. Oliver Krumholz

CONDITIONS

1. Fauna must not be taken on CALM land, (as defined in the Conservation and Land Management Regulations 2002*),* unless authorised by a written notice of a lawful authority issued under regulations 4 and 8 of the Conservation and Land Management Regulations 2002.

2. If persons, other than the licence holder, are authorised to carry out/assist in carrying out the activities under the licence, the licence holder must ensure those persons have read and understand the licence terms and conditions.
3. The written authorisation of the person in possession or occupation of the land accessed and upon which fauna is taken, as required under regulation 101(2) and referred to in "Additional information" below, must:
    a) state location details (including lot or location number, street/road, suburb and local government authority);
    b) state land owner or occupier name, and contact phone number;
    c) specify the time period that the authorisation is valid for;
    d) be signed and dated; and
    e) be attached to this licence at all times.
4. This licence, and any written authorisation or lawful authority which authorises the take of fauna on specified locations must be carried at all times while conducting licensed activities and be produced on demand by a wildlife officer.
5. If a species of fauna listed as a threatened species under Section 19 of the *Biodiversity Conservation Act 2016* is inadvertently captured, that species is to be released immediately at the point of capture. If the fauna is injured or deceased, the licence holder shall contact the DBCA Wildlife Licensing Section (wildlifelicensing@dbca.wa.gov.au) for advice on treatment or disposal. Details of any capture of threatened fauna must be included in the "Return of Fauna Taken."
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Danny Stefoni
LICENSING OFFICER
WILDLIFE PROTECTION BRANCH

Delegate of CEO

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   a) enter the land; and

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Appendix 2

Troglofauna Sampling Locations and Effort

Drillhole	Latitude	Longitude	Drillhole Diameter	Casing Type	Location	Surface Geology	End of Hole	Depth to Water	Number of Traps Installed			Sampling Depths (m)			
									Phase 1	Phase 2	Phase 3	Trap 1	Trap 2	Trap 3	Trap 4
YAR069	-21.762447	116.008728	150mm	None	Conceptual Pit	Upper Pisolite	10	Indet.	1	1	–	10	–	–	–
YAR070	-21.764263	116.008528	150mm	Collared	Conceptual Pit	Upper Pisolite	70	60	3	3	3	20	30	40	–
YAR071	-21.764303	116.009942	150mm	None	Conceptual Pit	Upper Pisolite	64	Indet.	4	4	–	10	20	30	40
YAR072	-21.755376	116.019672	150mm	None	Reference	Upper Pisolite	64	Indet.	3	3	–	15	30	45	–
YAR075	-21.755361	116.012323	150mm	None	Reference	Upper Pisolite	70	Indet.	3	3	–	15	30	45	–
YAR078	-21.757089	116.011500	150mm	None	Conceptual Pit	Upper Pisolite	88	Indet.	4	4	–	15	30	45	60
YAR081	-21.757069	116.014847	150mm	None	Conceptual Pit	Upper Pisolite	52	Indet.	3	3	–	15	30	45	–
YAR082	-21.759090	116.010971	150mm	Collared	Conceptual Pit	Upper Pisolite	6	Indet.	3	3	3	15	30	45	–
YAR084	-21.760614	116.010767	150mm	None	Conceptual Pit	Upper Pisolite	58	Indet.	3	3	–	15	30	45	–
YAR088	-21.762624	116.010572	150mm	None	Conceptual Pit	Upper Pisolite	58	Indet.	3	3	–	15	30	45	–
YAR112	-21.755291	116.016896	150mm	None	Conceptual Pit	Upper Pisolite	70	Indet.	4	4	–	15	30	45	60
YAR113	-21.755296	116.014991	150mm	None	Conceptual Pit	Upper Pisolite	72	Indet.	3	3	–	15	30	45	–
YAR119	-21.756169	116.013536	150mm	Collared	Conceptual Pit	Upper Pisolite	72	Indet.	3	3	3	20	40	60	–
YAR121	-21.756114	116.011501	150mm	None	Reference	Upper Pisolite	96	Indet.	4	4	–	10	30	50	70
YAR124	-21.756807	116.010420	150mm	None	Conceptual Pit	Upper Pisolite	78	Indet.	4	4	–	15	30	45	60
YAR125	-21.757995	116.015442	150mm	Collared	Reference	Upper Pisolite	48	Indet.	3	3	3	15	30	45	–
YAR128	-21.757933	116.012542	150mm	None	Conceptual Pit	Upper Pisolite	60	Indet.	3	3	–	15	30	45	–
YAR130	-21.757956	116.010559	150mm	Collared	Conceptual Pit	Upper Pisolite	78	Indet.	4	4	3	15	30	45	60
YAR134	-21.759771	116.012881	150mm	None	Conceptual Pit	Upper Pisolite	48	Indet.	3	3	–	15	30	45	–
YAR140	-21.761562	116.011503	150mm	None	Conceptual Pit	Upper Pisolite	66	Indet.	3	3	–	15	30	45	–
YAR143	-21.763384	116.012831	150mm	Collared	Reference	Upper Pisolite	48	Indet.	3	3	3	15	30	45	–
YAR147	-21.760196	116.007625	150mm	None	Conceptual Pit	Upper Pisolite	66	Indet.	3	3	–	15	30	45	–
YAR149	-21.761700	116.007102	150mm	Collared	Reference	Upper Pisolite	63	Indet.	3	3	3	15	30	45	–
YAR154	-21.763449	116.008175	150mm	None	Conceptual Pit	Upper Pisolite	70	Indet.	3	3	–	15	30	45	–
YAR161	-21.765214	116.012012	150mm	Collared	Conceptual Pit	Upper Pisolite	51	Indet.	4	4	3	10	20	30	40
YAR163	-21.758216	116.030372	150mm	None	Reference	Lower Pisolite	25	21	4	4	2	5	10	15	20
YAR168	-21.756394	116.028035	150mm	None	Reference	Lower Pisolite	19	Indet.	2	2	2	5	10	0	–
YAR173	-21.755738	116.025602	150mm	None	Reference	Lower Pisolite	19	17	3	3	2	5	10	15	–
YAR175	-21.755837	116.023831	150mm	Collared	Reference	Lower Pisolite	30	Indet.	3	2	3	10	20	30	–
YAR179	-21.757162	116.019699	150mm	None	Reference	Lower Pisolite	37	28	3	3	3	5	15	25	–
YAR185	-21.757647	116.025587	150mm	None	Reference	Lower Pisolite	28	9	2	2	2	3	8	–	–
YAR188	-21.756328	116.026164	150mm	None	Reference	Lower Pisolite	19	17	2	2	3	5	10	15	–

Drillhole	Latitude	Longitude	Drillhole Diameter	Casing Type	Location	Surface Geology	End of Hole	Depth to Water	Number of Traps Installed			Sampling Depths (m)			
									Phase 1	Phase 2	Phase 3	Trap 1	Trap 2	Trap 3	Trap 4
YAR196	-21.757194	116.018892	150mm	None	Reference	Lower Pisolite	25	17	3	2	3	5	10	15	–
YAR237	-21.755409	116.015904	150mm	Collared	Conceptual Pit	Upper Pisolite	70	Indet.	–	–	3	20	40	60	–
YAR242	-21.755406	116.019952	150mm	Collared	Conceptual Pit	Upper Pisolite	45	Indet.	–	–	3	20	30	40	–
YAR243	-21.755869	116.012135	150mm	Collared	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	20	40	60	–
YAR249	-21.755836	116.014809	150mm	Collared	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	20	40	60	–
YAR255	-21.755709	116.017597	150mm	Collared	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	20	40	60	–
YAR262	-21.756623	116.010793	150mm	Collared	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	20	40	60	–
YAR272	-21.756664	116.015254	150mm	Collared	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	20	40	50	–
YAR274	-21.756661	116.016176	150mm	Collared	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	20	40	60	–
YAR291	-21.757544	116.015213	150mm	Collared	Conceptual Pit	Upper Pisolite	48	Indet.	–	–	3	10	20	30	–
YAR294	-21.758020	116.012043	150mm	Collared	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	20	40	60	–
YAR296	-21.758103	116.013935	150mm	Collared	Conceptual Pit	Upper Pisolite	48	Indet.	–	–	4	10	20	30	40
YAR310	-21.759611	116.012929	150mm	Collared	Conceptual Pit	Upper Pisolite	50	Indet.	–	–	3	15	30	45	–
YAR316	-21.760300	116.012954	150mm	Collared	Conceptual Pit	Upper Pisolite	30	Indet.	–	–	3	10	20	30	–
YAR325	-21.761300	116.012724	150mm	Collared	Conceptual Pit	Upper Pisolite	24	Indet.	–	–	3	5	10	15	–
YAR327	-21.761864	116.01109	150mm	Collared	Conceptual Pit	Upper Pisolite	30	Indet.	–	–	3	10	20	30	–
YAR334	-21.762056	116.012338	150mm	Collared	Conceptual Pit	Upper Pisolite	30	Indet.	–	–	3	10	20	30	–
YAR341	-21.762954	116.012104	150mm	Collared	Conceptual Pit	Upper Pisolite	30	Indet.	–	–	3	10	20	30	–
YAR348	-21.763912	116.011254	150mm	Collared	Conceptual Pit	Upper Pisolite	30	Indet.	–	–	3	10	20	30	–
YAR372	-21.762105	116.009171	150mm	Collared	Conceptual Pit	Upper Pisolite	20	Indet.	–	–	2	10	20	–	–
YAR381	-21.764428	116.011958	150mm	Collared	Conceptual Pit	Upper Pisolite	30	Indet.	–	–	3	10	20	30	–
YAR391	-21.765192	116.009428	150mm	Collared	Conceptual Pit	Upper Pisolite	36	Indet.	–	–	3	10	20	30	–
YAR405	-21.763912	116.007799	150mm	Collared	Conceptual Pit	Upper Pisolite	60	55	–	–	3	10	30	50	–
YAR420	-21.763072	116.010617	150mm	Collared	Conceptual Pit	Upper Pisolite	66	Indet.	–	–	3	10	20	30	–
YAR435	-21.761655	116.008009	150mm	Collared	Conceptual Pit	Upper Pisolite	66	Indet.	–	–	3	20	40	60	–
YAR446	-21.760775	116.011096	150mm	Collared	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	15	30	45	–
YAR448	-21.760366	116.00803	150mm	Collared	Reference	Upper Pisolite	66	Indet.	–	–	3	20	40	60	–
YAR454	-21.754903	116.016348	150mm	Collared	Conceptual Pit	Upper Pisolite	45	Indet.	–	–	3	15	30	45	–
YAR458	-21.754880	116.018223	150mm	Collared	Conceptual Pit	Upper Pisolite	66	Indet.	–	–	3	20	40	60	–
YAR464	-21.754914	116.021574	150mm	Collared	Reference	Upper Pisolite	50	Indet.	–	–	3	15	30	45	–
YAR466	-21.754477	116.015483	150mm	Collared	Conceptual Pit	Upper Pisolite	66	Indet.	–	–	3	20	40	60	–
YAR470	-21.754447	116.017423	150mm	Collared	Conceptual Pit	Upper Pisolite	66	Indet.	–	–	3	20	40	60	–
YAR475	-21.754472	116.020186	150mm	Collared	Conceptual Pit	Upper Pisolite	50	Indet.	–	–	3	15	30	45	–

Drillhole	Latitude	Longitude	Drillhole Diameter	Casing Type	Location	Surface Geology	End of Hole	Depth to Water	Number of Traps Installed			Sampling Depths (m)			
									Phase 1	Phase 2	Phase 3	Trap 1	Trap 2	Trap 3	Trap 4
YAR486	-21.754003	116.021136	150mm	Collared	Reference	Upper Pisolite	60	Indet.	–	–	3	20	40	60	–
YAR487	-21.753621	116.016695	150mm	Collared	Conceptual Pit	Upper Pisolite	62	Indet.	–	–	3	20	40	60	–
YAR488	-21.753608	116.020411	150mm	Collared	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	20	40	60	–
YARDDH001	-21.764254	116.009919	100mm	None	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	4	15	30	45	60
YARDDH003	-21.757277	116.010830	100mm	None	Conceptual Pit	Upper Pisolite	20	Indet.	–	–	2	10	20	–	–
YARDDH004	-21.757147	116.013066	150mm	None	Conceptual Pit	Upper Pisolite	60	Indet.	–	–	3	20	40	60	–
YARDDH006	-21.758591	116.010887	100mm	None	Conceptual Pit	Upper Pisolite	50	Indet.	–	–	3	15	30	45	–
YARDDH007	-21.758590	116.013432	100mm	None	Reference	Upper Pisolite	30	Indet.	–	–	2	15	25	–	–
YARDDH011	-21.763031	116.009143	100mm	Collared	Conceptual Pit	Upper Pisolite	102	Indet.	–	–	3	20	40	60	–

Coordinate datum GDA94.
Shaded cells = traps not retrieved during Phase 3 sampling.

Appendix 3

Desktop Study Troglofauna Records

Class	Order	Family	Species	Location	Number	Sites
Arachnida	Araneae	Indet.	Araneae sp. *AUN001*	Mesa C	1	RC17MEC0029
		Gnaphosidae	Gnaphosidae sp. *AGN005*	Mesa C	1	RC15MEC0192
		Oonopidae	Oonopidae sp. *AOO012*	Mesa C	4	GR15MEC0009, GR15MEC0010, RC15MEC0084, RC17MEC0017
			Prethopalpus sp. AO01	Mesa B	1	MEBRC0068
			Prethopalpus sp. *AO016*	Mesa B	1	RC15MEB0153
			Opopaea ectognophus	Mesa G	1	MEGRC0130
	Opilionida	Assamiidae	Assamiidae sp. *OAS003*	Mesa C	1	RC17MEC0034
	Pseudoscorpiones	Indet.	Pseudoscorpiones sp. *indet.*	Mesa B	11	DD17MEB0002, MEBRC0021, RC14MEB0038, RC14MEB0076, RC14MEB0101, RC14MEB0115
				Mesa C	7	GR15MEC0017
		Atemnidae	*Oratemnus ORAT001 / PA015*	Mesa B	1	GR15MEB0011
		Chthoniidae	*Tyrannochthonius basme / PC050*	Mesa B	1	GR15MEB0011
				Mesa C	1	GR15MEC0008
			Tyrannochthonius sp. *CH003-A / PC070*	Mesa C	1	RC15MEC0192
			Tyrannochthonius sp. *CH005 / PC076*	Mesa C	1	GR15MEC0008
		Hyidae	Hyidae sp. *`PH001a`*	Mesa B	2	RC15MEB0040
			Hyidae sp. *`PH001b`*	Mesa B	1	GR15MEB0013
		Olpiidae	*Indolpium* sp. *PSE016*	Mesa G	1	MEGPL04463
		Syarinidae	*Ideoblothrus pisolitus*	Mesa B	46	DD17MEB0004, GR15MEB0013, RC14MEB0038, RC14MEB0076, RC14MEB0101, RC14MEB0115, RC14MEB0150, RC15MEB0020, RC15MEB0031, RC15MEB0040, RC15MEB0153
		Syarinidae	*Ideoblothrus* sp. *IDE001 / PS039*	Mesa B	1	GR15MEB0013
			Ideoblothrus sp. *IDE006 / PSE038*	Mesa B	2	RC14MEB0038, RC14MEB0150
			Ideoblothrus sp. *IDEO004 / PS029*	Mesa C	9	GR15MEC0017
			Syarinidae sp. *IDE002 / PS035*	Mesa C	1	GR15MEC0010
	Schizomida	Hubbardiidae	*Draculoides bythius*	Mesa B	34	DD15MEB0018, DD17MEB0002, DD17MEB0004, GR15MEB0016, GR15MEB0009, MEBRC0021, RC14MEB0010, RC15MEB0020, RC15MEB0031, RC15MEB0040, RC15MEB0153, DD15MEB0018,

Class	Order	Family	Species	Location	Number	Sites
						DD17MEB0002, DD17MEB0004, GR15MEB0016, MEBRC0021, RC14MEB0010, RC14MEB0076, RC15MEB0031, RC15MEB0040, GR15MEB0011, MEBRC0018, RC14MEB0150, RC15MEB0001
			Draculoides bythius	Mesa C	31	GR15MEB0011, MEBRC0018, RC14MEB0150, RC15MEB0001, GR15MEC0001, GR15MEC0009, GR15MEC0017, GR15MEC0019, RC15MEC0009, RC15MEC0014, RC15MEC0121, RC15MEC0186, RC17MEC0016
			Draculoides sp. *nov. Mesa F*	Mesa F	6	MEFRC0197, MEFRC0226, MEFRC0231
			Draculoides gnophicola	Mesa G	72	MEGRC0163, MEGDC0014A, MEGDC0057A, MEGRC0163, MEGDC0202, MEGPL09055, MEGRC0130, MEG-Unamed02
Chilopoda	Scolopendrida	Cryptopidae	*Cryptops* sp. nov.	Mesa G	3	MEGDC0057A
		Indet.	Chilopoda sp. Indet.	Mesa B		GR15MEB0004
Diplopoda	Polydesmida	Haplodesmidae	Haplodesmidae sp. DIHAP001	Mesa B	3	DD17MEB0004, GR15MEB0011
		Haplodesmidae		Mesa C	2	RC17MEC0016, RC17MEC0029
		Haplodesmidae	Haplodesmidae sp. POLYD001	Mesa C	1	GR15MEC0001
		Indet.	Polydesmida sp. Indet.	Mesa G	1	MEGDC0108A
			Polydesmida sp. Indet.	Mesa B	2	DD17MEB0004
Entognatha	Diplura	Japygidae	Japyx sp. DPLJ001 / DJA020	Mesa C	1	RC17MEC0030
			Japyx sp. indet.	Mesa F	1	MEF0075 T1-8
				Mesa F	1	MEFRC0158P1T2-2
				Mesa G	1	MEGDC0108A
Insecta	Blattodea	Blatellidae	Blatellidae sp. 1	Mesa G	1	MEGDC0014A
		Blatellidae	Blatellidae sp. 2	Mesa G	1	MEGDC0014A
		Nocticolidae	Nocticolidae sp. Indet.	Mesa G	40	MEGDC0014A, MEGDC0202, MEGDC0057A, MEGOP01
	Coleoptera	Indet.	Coleoptera sp. CUN004	Mesa C	1	GR15MEC0001
			Coleoptera sp. indet.	Mesa C	4	GR15MEC0010, GR15MEC0017, RC15MEC0121

Class	Order	Family	Species	Location	Number	Sites
				Mesa B	13	RC14MEB0038, RC15MEB0001, RC15MEB0031, RC15MEB0040
		Circulionidae	Circulionidae sp. CCU012	Mesa B	7	GR15MEB0013, RC14MEB0038, RC15MEB0020, RC15MEB0031, RC15MEB0040
				Mesa C	7	GR15MEC0010, GR15MEC0016, GR15MEC0017, RC15MEC0084, RC15MEC0186, RC15MEC0192, RC17MEC0004,
	Archeaognatha	Indet.	indet.	Mesa C	1	RC17MEC0004
	Zygentoma		Zygentoma sp. indet.	Mesa B	1	RC14MEB0150
				Mesa G	1	MEGRC0104
			Zygentoma TX004	Mesa C	2	RC15MEC0014
		Subnicoletiidae	Subnicoletiidae sp. TN010	Mesa B	1	RC14MEB0076
Malacostraca	Isopoda	Indet.	Isopoda sp. indet.	Mesa B	6	MEBRC0018, RC14MEB0076, RC14MEB0101
				Mesa C	6	RC17MEC0003, RC17MEC0017
		Armadillidae	Armadillidae sp. ISA008	Mesa B	9	GR15MEB0001, GR15MEB0004, RC14MEB0076
			Armadillidae sp. ISA055	Mesa B	2	RC14MEB0101, RC14MEB0115
			Armadillidae sp. ISA054	Mesa C	3	RC15MEC0192, RC17MEC0017, RC17MEC0034
			Troglarmadillo sp. 2	Mesa F	3	MEFRC0226P1T1-2
			Troglarmadillo sp. indet.	Mesa G	1	MEGRC0104
		Philoscidae	Philoscidae sp. ISP004	Mesa B	2	MEBRC0018, MEBRC0021
			Philoscidae sp. ISP057	Mesa C	1	GR15MEC0001
			Philosciidae sp. indet.	Mesa G	1	MEGRC0537

Appendix 4

Survey Data

Specimen Code	SiteYAR372	Latitude	Longitude	Phylum	Class	Order	Family	Molecular Taxon/Species	Morphological Taxon	Phase Collected	n	Troglobitic
YAR082.20210817.T1-01	YAR082	-21.75909	116.010971	Crustacea	Malacostraca	Isopoda	Philosciidae	Philosciidae sp. H-ISP059	Philosciidae sp.	P1	1	yes
YAR134.20210817.T2-01	YAR134	-21.759771	116.012881	Arthropoda	Symphyla	Symphyla	Scolopendrellidae	Scolopendrellidae sp. H-SYM038	Scolopendrellidae sp.	P1	1	yes
YAR149.20210930.T3-01	YAR149	-21.761700	116.007102	Arthropoda	Insecta	Diplura	Japygidae	Japygidae sp. H-DJA022	Japygidae sp.	P2	2	yes
YAR173.20210940.T2-01	YAR173	-21.755738	116.025602	Arthropoda	Insecta	Diplura	Japygidae	Japygidae sp. H-DJA023	Japygidae sp.	P2	1	yes
YAR168.20210931.T1-01	YAR168	-21.756394	116.028035	Crustacea	Malacostraca	Isopoda	Armadillidae	Armadillidae sp. indet.	Armadillidae sp.	P2	1	yes
YAR119.20210930.T3-01	YAR119	-21.756169	116.013536	Crustacea	Malacostraca	Isopoda	Armadillidae	Armadillidae sp. indet.	Armadillidae sp.	P2	1	yes
YAR161.20210930.T4-01	YAR161	-21.765214	116.012012	Crustacea	Malacostraca	Isopoda	Philosciidae	Philosciidae sp. H-ISP060	Philosciidae sp.	P2	1	yes
YAR125.20210930.T1-01	YAR125	-21.757995	116.015442	Mollusca	Gastropoda	Mollusca	Pupillidae	*Gastrocopta servilis*	*Gastrocopta servilis*	P2	1	no
YAR075.20210930.T3-01	YAR075	-21.755361	116.012323	Mollusca	Gastropoda	Mollusca	Pupillidae	*Gastrocopta servilis*	*Gastrocopta servilis*	P2	1	no
YAR075.20210930.T1-01	YAR075	-21.755361	116.012323	Mollusca	Gastropoda	Mollusca	Pupillidae	*Gastrocopta servilis*	*Gastrocopta servilis*	P2	1	no
YAR124.20210930.T1-01	YAR124	-21.799237	115.968522	Mollusca	Gastropoda	Mollusca	Pupillidae	*Gastrocopta servilis*	*Gastrocopta servilis*	P2	1	no
YAR070.20210930.T1-01	YAR070	-21.764263	116.008528	Arthropoda	Arachnida	Pseudoscorpiones	Olpiidae	Olpiidae sp. H-PO033	Olpiidae sp.	P2	1	yes
YAR072.20210930.T3-01	YAR072	-21.755376	116.019672	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P2	1	yes
YAR161.20210930.T4-02	YAR161	-21.765214	116.012012	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P2	1	yes
YAR143.20210930.T3-01	YAR143	-21.763384	116.012831	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P2	1	yes
YAR163.20210930.T1-01	YAR163	-21.758216	116.030371	Arthropoda	Symphyla	Symphyla	Scolopendrellidae	Scolopendrellidae sp. H-SYM039	Scolopendrellidae sp.	P2	1	yes
YAR173.20210930.T2-02	YAR173	-21.755738	116.025602	Arthropoda	Symphyla	Symphyla	Scolopendrellidae	Scolopendrellidae sp. H-SYM040	Scolopendrellidae sp.	P2	1	yes
YAR296.20230119.T4-02	YAR296	-21.758103	116.013935	Arthropoda	Insecta	Diplura	Japygidae	Indet.	Japygidae sp.	P3	1	yes
YAR249.20230119.T3-01	YAR249	-21.755836	116.014809	Crustacea	Malacostraca	Isopoda	Armadillidae	Indet	Armadillidae sp.	P3	1	yes
YAR249.20230119.T2-01	YAR249	-21.755836	116.014809	Crustacea	Malacostraca	Isopoda	Armadillidae	Armadillidae sp. H-ISA064	Armadillidae sp.	P3	3	yes
YAR249.20230119.T3-01	YAR249	-21.755836	116.014809	Crustacea	Malacostraca	Isopoda	Armadillidae	Indet	Armadillidae sp.	P3	1	yes
YARDDH004.20230119.T3-02	YARDDH004	-21.757147	116.013066	Crustacea	Malacostraca	Isopoda	Philosciidae	Philosciidae sp. H-ISP059	Philosciidae sp.	P3	3	yes
YARDDH004.20230119.T1-01	YARDDH004	-21.757147	116.013066	Crustacea	Malacostraca	Isopoda	Philosciidae	Indet.	Philosciidae sp.	P3	2	yes
YAR237.20230119.T3-01	YAR237	-21.755409	116.015904	Crustacea	Malacostraca	Isopoda	Philosciidae	Indet.	Philosciidae sp.	P3	14	yes
YAR487.20239119.T3-91	YAR487	-21.753621	116.016695	Arthropoda	Diplopoda	Polydesmida	Haplodesmidae	Haplodesmidae sp. DIHAP001	Haplodesmidae sp.	P3	30	yes
YAR487.20239119.T2-01	YAR487	-21.753621	116.0167	Arthropoda	Diplopoda	Polydesmida	Haplodesmidae	Haplodesmidae sp. DIHAP001	Haplodesmidae sp.	P3	4	yes
YAR487.20230119.T1-01	YAR487	-21.753621	116.016695	Arthropoda	Diplopoda	Polydesmida	Haplodesmidae	Haplodesmidae sp. DIHAP001	Haplodesmidae sp.	P3	10	yes
YAR296.20230119.T4-01	YAR296	-21.758103	116.013935	Arthropoda	Arachnida	Pseudoscorpiones	Olpiidae	*Indohya alexanderi*	Olpiidae sp.	P3	1	yes
YARDDH007.20230119.T2-01	YARDDH007	-21.758590	116.013432	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P3	2	yes
YAR125.20230119.T3-01	YAR125	-21.758030	116.015483	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P3	3	yes
YAR161.20230118.T2-01	YAR161	-21.765179	116.012027	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P3	1	yes
YARDDH001.20230118.T3-02	YARDDH001	-21.764254	116.00992	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P3	1	yes
YAR296.20230119.T1-01	YAR296	-21.758103	116.01393	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P3	2	yes
YAR296.20230119.T4-03	YAR296	-21.758103	116.01393	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P3	1	yes
YAR466.20230116.T1-01	YAR466	-21.754477	116.015483	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P3	1	yes
YAR466.20230119.T2-01	YAR466	-21.754477	116.015483	Arthropoda	Arachnida	Schizomida	Hubbardiidae	*Draculoides* sp. H-SCH200	*Draculoides* sp. Mesa F	P3	1	yes
YAR291.20230119.T1-01	YAR291	-21.757544	116.015213	Arthropoda	Symphyla	Symphyla	Scolopendrellidae	Indet.	Scolopendrellidae sp.	P3	1	yes
YAR143.20230119.T3-01	YAR143	-21.763419	116.012846	Arthropoda	Insecta	Zygentoma	indet.	Nicoletiinae sp. H-TN003.	Zygentoma sp.	P3	3	yes
YAR125.20230119.T2-01	YAR125	-21.75803	116.015482	Arthropoda	Insecta	Zygentoma	Nicoletiidae	Nicoletiinae sp. H-TN002	*Trinemura* sp.	P3	1	yes
YAR486.20230119.T3-01	YAR486	-21.754003	116.021136	Arthropoda	Insecta	Zygentoma	Nicoletiidae	Nicoletiinae sp. H-TN001	*Trinemura* sp.	P3	1	yes
YAR143.20230119.T3-02	YAR143	-21.763419	116.012846	Arthropoda	Insecta	Zygentoma	Nicoletiidae	Ateluriinae sp. H-TA010	*Trinemura* sp.	P3	6	yes
YAR143.20230119.T1-01	YAR143	-21.763419	116.012846	Arthropoda	Insecta	Zygentoma	Ateluriinae	Ateluriinae sp. H-TA010	*Trinemura* sp.	P3	1	yes

Appendix 5

Helix Molecular Analysis Results

Helix Molecular Solutions

Molecular Systematics of the Robe Mesa Troglobitic invertebrates (Phase 1-3)

Prepared for

Biota Environmental Sciences

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Molecular Systematics of the Robe Mesa Troglobitic invertebrates (Phase 1-3)

Contents

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1.0 Executive Summary

Helix Molecular Solutions was engaged by Biota Environmental Sciences to perform DNA extractions, sequencing of the mitochondrial cytochrome oxidase subunit I gene (COI) and analyses of specimens collected during the troglobitic invertebrate fauna surveys of the Robe Mesa Valley project area. Across the three Phases, a total of 102 specimens from eight taxonomic groups (Diplura, Isopoda, Polydesmida, Pseudoscorpiones, Schizomida, Symphyla, Gastropoda and Zygentoma) were collected. Data from Phase 1 (August 2021) and Phase 2 (September 2021) have been analysed and assessed previously (see Helix, 2022). These previous data are incorporated into the current report and all three Phases are discussed and referred to here as 'project specimens'.

The objective of this report is to summarise information on the number of potential troglobitic species from the Robe Mesa project area across the three survey Phases and their apparent distributions, based on the molecular genetic data available for comparison.

CHELICERATA

ARACHNIDA: Schizomida

The 15 project specimens, comprising three specimens from Phase 2 and 12 from Phase 3, all belonged to the same taxon; *Draculoides* sp. H-SCH200. This putative species has not been detected elsewhere according to the molecular data available for comparison.

ARACHNIDA: Pseudoscorpiones

Two families of pseudoscorpions were detected from the project specimens.

One pseudoscorpion belonging to the family Olpiidae was detected during Phase 2 sampling. Analyses revealed it to be a previously undetected putative species, Olpiidae sp. H-PSO033, according to the molecular data currently available for comparison.

A single pseudoscorpion specimen belonging to the family Hyidae was collected in Phase 3 and identified as a previously described species *Indohya alexanderi* (also known as *I.* sp. H-PH003a).

CRUSTACEA

MALACOSTRACA: Isopoda

Twenty-eight Isopoda specimens were collected during the survey. This included one individual during Phase 1, three during Phase 2, and 24 individuals during Phase 3. The specimens represented three newly detected species belonging to the family Philosciidae and one new species of Armadillidae were detected.

Philosciidae sp. H-ISP059 and H-ISP060 were collected from Phase 2, with Philosciidae sp. H-ISP059 also detected in Phase 3. An additional new lineage of Philosciidae (H-ISP061) was also detected in Phase 3, which was had not been detected previously in Phases 1 or 2. One new species of Armadillidae sp. H-ISA064 was detected in Phase 3. None of the four taxa could be assigned to a genus.

HEXAPODA

INSECTA: Zygentoma (Thysanura)

No specimens of Zygentoma were collected during Phase 1 and 2 but 12 specimens were collecting during Phase 3. Six of these were successfully sequenced and assigned to four lineages, one Ateluriinae (H-TA010) and three Nicoletiinae (H-TN001- H-TN003). All four lineages were new, having not been detected previously, based on the material available for comparison.

ENTOGNATHA: Diplura

Three dipluran specimens were recorded across the three Phases of sampling. Two specimens from Phase 2 belonged to a previously undetected putative species Japygidae sp. H-DJA022. The single specimen of Diplura from Phase 3 failed to sequence and was unable to be further analysed.

MYRIAPODA

DIPLOPODA: Polydesmida: Haplodesmidae

Thirty-four millipede specimens were collected during Phase 3 sampling only. Thirty-one sequences were obtained from the 34 specimens belonging to the family Haplodesmidae from Phase 3. All 31 specimens belonged to a single haplotype, a previously detected species Haplodesmidae sp. H-DIHAP001, which has been recorded previously in the Robe valley and the Hardey River locality.

SYMPHYLA (CEPHALOSTIGMATA)

Four symphylan specimens were obtained across the three Phases of sampling. Three symphylan specimens, one from Phase 2 and two from Phase 3, all belong to the family Scolopendrillidae. The specimens from Phase 2 each represented a separate putative taxon, not previously detected (Scolopendrilllidae sp. H-SYM038, Scolopendrilllidae sp. H-SYM039 and Scolopendrilllidae sp. H-SYM040, Helix 2022). The single symphylan from Phase 3 did not amplify and could not be analysed further for species identification.

GASTROPODA

GASTROPODA: Pupillidae

Four gastropod specimens belonging to the family Pupilidae were collected during Phase 2 sampling. Two specimens failed to amplify and could not be analysed further for species identification. Analyses for the remaining two project specimens identified them as the described and previously detected species *Gastrocopta servilis*. No gastropods were collected during Phase 3.

2.0 Introduction

2.1 Project Background

CZR Resources (CZR) is proposing to develop the Robe Mesa iron ore Project (the project), located in the West Pilbara, 29 km southwest of Pannawonica. The Robe Mesa deposit (the study area) adjoins Mesa F, which is located between the Mesa A and Mesa J-K channel iron deposit (CID) mines operated by Rio Tinto Ltd. Currently there are no developed mines on the CZR tenements (E08/1060 and E08/1686).

Biota Environmental Sciences has sampled the troglofauna and stygofauna in the project area for CZR over three Phases (Helix, 2022). Extensive sampling and sequencing for both troglofauna and stygofauna has previously been undertaken in the Robe valley (Biota 2006a, 2006b, 2011, 2017) and studies have documented short-range endemic troglobitic fauna that are restricted to individual mesas (Biota 2006a, 2011, Helix 2020, 2021, 2022).

2.2 Study Scope

Across the three Phases of sampling, a total of 102 invertebrate fauna specimens were collected from 23 sites from the project area. These repredsented eight higher rank taxonomic groups, comprising Diplura n=3, Isopoda=28, Polydesmida n=34, Pseudoscorpiones n=2, Schizomida n=15, Symphyla n=4, Gastropoda n=4 and Zygentoma n=12.

Biota Environmental Sciences (Biota) engaged Helix Molecular Solutions (Helix) to undertake DNA Extractions of subterranean invertebrate fauna collected from the survey of the project area. These specimens were sequenced for variation at the mitochondrial cytochrome oxidase subunit I gene (COI), with the exception of the Zygentoma which were sequenced at the 12S ribosomal rRNA region. Analysis was undertaken to elucidate the taxonomic/phylogenetic relationships amongst the subterranean invertebrate fauna and to determine the number of taxa present and their current known distributions or closest known locality outside the survey area based on comparisons with available genetic reference data.

This report provides a synopsis of the relationships between the successfully sequenced specimens from all three Phases of sampling, updating the previous report on the project (Helix, 2022), using all relevant reference specimens publicly available, including the number of putative species based on the phylogenetic analysis.

3.0 Methods

The 102 specimens, comprising eight taxonomic groups based on morphology (Schizomida, Pseudoscorpiones, Isopoda, Zygentoma, Polydesmida, Diplura, Gastropoda and Symphyla) were collected from 23 sampling sites (Table 4-1). The specimens were sequenced for variation at COI using primers LCOI and HCO2 (Folmer et al., 1994), and for the Zygentoma at 12S using primers 12Sai/12SRJ (Simon *et al*., 1994)

Sequences were edited using Geneious version 6.1.8 (http://www.geneious.com) performed within MEGA version 5.05 (Tamura et al., 2011) using the build-in alignment tool using CLUSTAL W (Thompson et al., 1994) default parameters. DNA nucleotide sequences were translated into protein sequences to ensure that the amplified sequences corresponded to the target mtDNA. The translated protein sequences were then checked for the presence of stop codons, to ensure that pseudogenes had not been amplified. Pseudogenes have a DNA sequence that is similar to the functional gene (e.g. COI) however, they do not code for a functioning protein despite the shared ancestry with the functional gene. The presence of pseudogenes can complicate molecular analyses, producing misleading results.

DNA sequences were translated into proteins with ExPASy using the invertebrate genetic code. All sequences analysed were of high quality with no evidence of heterogeneous peaks. All resulting sequences were 'BLAST'ed (Basic Local Alignment Search Tool) with the NCBI (National Centre for Biotechnology Information). The program compares DNA nucleotide sequences with a library of sequences and identifies sequences within the database that resemble the query sequences above a certain threshold. Genetic distance between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotides different between sequences). Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance. To account for polymorphism within lineages, the net genetic diversity of Nei (1987) was calculated to give a 'corrected' distance between lineages.

For phylogenetic analysis, likelihood ratio tests using the Bayesian Information Criterion were calculated in MEGA 11 (Tamura et al., 2021) to determine the best-fit model of evolution (Nei, Kumar 2000; Tamura et al., 2013). The phylogenetic analyses were conducted in Geneious Prime version 2024.0.5 (https://www.geneious.com) using the best-fit model of evolution calculated for each family (Table 3-1). Maximum likelihood (ML) analyses with 100 rapid bootstrap replicates were performed in RAxML (Randomised Accelerated Maximum Likelihood) version 8.2.11 (Stamatakis, 2014), using default settings.

For the purposes of this report, lineages were defined as haplotypes or groups of haplotypes differing from other such groups between 3 to 5% sequence divergence. This cut-off was selected based on meta-reviews of bar-coding data, which indicate that intra-specific (within species) variation rarely exceeds 3% (Herbert et al., 2003b). Species were determined based on taxonomic group, molecular and morphological data available and previously published literature for each taxonomic group. Helix species names have been updated for the current report to reflect our most current data and follow current Helix nomenclature (with nominal species names using the prefix 'H-').

Table 3-1. **Best-fit model of evolution for each of the taxonomic groups and families analysed (Phase 3).** Abbreviations: GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; G: Gamma; I: Invariant sites.

Analysis Group	Best Model	Gamma Value
CHELICERATA		
Arachnida: Pseudoscorpiones	GTR + G +I	0.68
Arachnida: Schizomida	GTR + G +I	0.92
CRUSTACEA		
Malacostraca: Isopoda	GTR + G +I	0.52
HEXAPODA		
Insecta: Zygentoma	GTR + G +I	1.29
Entognatha: Diplura	n/a	n/a
MYRIAPODA		
Diplopoda: Polydesmida	TN93 + G +I	0.80
Symphyla: Cephalostigmata	n/a	n/a
MOLLUSCA		
Gastropoda	GTR + G + I	1.05

4.0 Results

Of the 102 specimens, 19 failed to amplify strongly enough to yield a viable sequence (one each of Diplura, Schizomida and Symphyla, three Polydesmida, five Isopoda, six Zygentoma and two Gastropoda; Table 4-1). The remaining 83 successful molecular sequences were then assessed to determine the number of taxa present by comparing these results to those molecular sequences publicly available on GenBank, as well as those already in Helix's database for context.

Seventeen specimens were collected during Phase 1 and 2 sampling (Helix, 2022). Ten taxa were detected amongst the 14 that were successfully sequenced, representing seven new putative species and three that had been detected previously (Helix, 2022).

Amongst the 85 specimens analysed from Phase 3, 71 were successfully sequenced for COI (Table 4-1). The symphylan (UH19) and dipluran (UH17) specimens did not amplify a DNA sequence and could therefore not be further identified or analysed. Furthermore, three Haplodesmidae, three Isopoda, and six Zygentoma failed to amplify (Table 4-1).

To provide a thorough synopsis of the relationships between the Phase 1 – 3 specimens, the 14 successfully sequenced specimens from Phases 1 and 2 (Schizomida n=2, Pseudoscorpiones n=1, Isopoda n=2, Diplura n= 2, Symphyla n=3 and Gastropoda n=2) (all 'QY' prefix) were included in this updated analysis (Helix, 2022).

Tables of genetic p-distance and associated standard errors between survey area specimens from Phase 1 – Phase 3 are provided in Appendix 1. Tables of genetic p-distances and associated standard errors of reference specimens showing less than 15 to 20% sequence divergence from survey specimens are provided in Appendix 2 (supplementary data).

Table 4-1. **Invertebrate specimens collected from Phase 1, 2 and 3 (n=102) and the genetic species to which they were assigned. Molecular lineages in bold text represent a newly detected lineage or species. Grey shading represents specimens that failed to produce a usable sequence.**

Sampling Phase	Specimen ID	Helix ID	Site	Latitude	Longitude	Taxonomic Group	Molecular Family ID	Molecular Genus ID	Molecular Species ID
2	YAR070.20210930.T1-01	**QY08**	YAR070	-21.764263	116.0085279	Arachnida: Pseudoscorpiones	Olpiidae	unknown	Olpiidae sp. H-PO033
3	YAR296.20230119.T4-01	**UH16**	YAR296	-21.75810333	116.0139351	Arachnida: Pseudoscorpiones	Hyidae	*Indohya*	*Indohya alexanderi*
2	YAR072.20210930.T3-01	**QY15**	YAR072	-31.937396	115.8422345	Arachnida: Schizomida	Hubbardiidae	No data	No data
2	YAR161.20210930.T4-02	**QY16**	YAR161	-21.76517922	116.0120266	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	*Draculoides* sp. H-SCH200
2	YAR143.20210930.T3-01	**QY17**	YAR143	-21.76341877	116.0128456	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	*Draculoides* sp. H-SCH200
3	YARDDH007.20230119.T2-01	**UH01**	YARDDH007	-21.7585905	116.0134319	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YARDDH007.20230119.T2-01	**UH02**	YARDDH007	-21.7585905	116.0134319	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR125.20230119.T3-01	**UH03**	YAR125	-21.75803037	116.0154826	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR125.20230119.T3-01	**UH04**	YAR125	-21.75803037	116.0154826	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR125.20230119.T3-01	**UH05**	YAR125	-21.75803037	116.0154826	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR161.20230118.T2-01	**UH07**	YAR161	-21.76517922	116.0120266	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	**Draculoides sp. H-SCH200**
3	YARDDH001.20230118.T3-02	**UH08**	YARDDH001	-21.76425403	116.0099189	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR296.20230119.T1-01	**UH14**	YAR296	-21.75810333	116.0139351	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR296.20230119.T1-01	**UH15**	YAR296	-21.75810333	116.0139351	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR296.20230119.T4-03	**UH18**	YAR296	-21.75810333	116.0139351	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR466.20230116.T1-01	**UH60**	YAR466	-21.75447695	116.015483	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR466.20230119.T2-01	**UH61**	YAR466	-21.75447695	116.015483	Arachnida: Schizomida	Hubbardiidae	*Draculoides*	***Draculoides* sp. H-SCH200**
3	YAR487.20239119.T3-01	**UH33**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	No data	No data
3	YAR487.20239119.T3-01	**UH40**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	No data	No data
3	YAR487.20239119.T3-01	**UH43**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	No data	No data
3	YAR487.20239119.T3-01	**UH31**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH32**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH34**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH35**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH36**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH37**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH38**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH39**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH41**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH42**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH44**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH45**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH46**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH47**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH48**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH49**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T3-01	**UH50**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T2-01	**UH51**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T2-01	**UH52**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T2-01	**UH53**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20239119.T2-01	**UH54**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20230119.T1-01	**UH76**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20230119.T1-01	**UH77**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20230119.T1-01	**UH78**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20230119.T1-01	**UH79**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20230119.T1-01	**UH80**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20230119.T1-01	**UH81**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001

Sampling Phase	Specimen ID	Helix ID	Site	Latitude	Longitude	Taxonomic Group	Molecular Family ID	Molecular Genus ID	Molecular Species ID
3	YAR487.20230119.T1-01	**UH82**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20230119.T1-01	**UH83**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	Not assigned	Haplodesmidae sp. DIHAP001
3	YAR487.20230119.T1-01	**UH84**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	unknown	Haplodesmidae sp. DIHAP001
3	YAR487.20230119.T1-01	**UH85**	YAR487	-21.75362125	116.0166955	Diplopoda: Polydesmida	Haplodesmidae	unknown	Haplodesmidae sp. DIHAP001
3	YAR296.20230119.T4-02	**UH17**	YAR296	-21.75810333	116.0139351	Entognatha: Diplura	no data	No data	No data
3	YAR143.20230119.T3-01	**UH21**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	Nicoletiidae	unknown	**Ateluriinae sp. H-TA010**
3	YAR143.20230119.T3-01	**UH22**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	Nicoletiidae	unknown	**Ateluriinae sp. H-TA010**
3	YAR143.20230119.T3-01	**UH23**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	Nicoletiidae	unknown	**Ateluriinae sp. H-TA010**
3	YAR486.20230119.T3-01	**UH20**	YAR486	-21.75400345	116.0211358	Insecta: Zygentoma	Nicoletiidae	unknown	**Nicoletiinae sp. H-TN001**
3	YAR125.20230119.T2-01	**UH06**	YAR125	-21.75803037	116.0154826	Insecta: Zygentoma	Nicoletiidae	unknown	**Nicoletiinae sp. H-TN002**
3	YAR143.20230119.T3-02	**UH29**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	Nicoletiidae	unknown	**Nicoletiinae sp. H-TN003**
3	YAR143.20230119.T3-02	**UH24**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	no data	No data	No data
3	YAR143.20230119.T3-02	**UH25**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	no data	No data	No data
3	YAR143.20230119.T3-02	**UH26**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	no data	No data	No data
3	YAR143.20230119.T3-02	**UH27**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	no data	No data	No data
3	YAR143.20230119.T3-02	**UH28**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	no data	No data	No data
3	YAR143.20230119.T1-01	**UH30**	YAR143	-21.76341877	116.0128456	Insecta: Zygentoma	no data	No data	No data
1	YAR082.20210817.T1-01	**QY04**	YAR082	-31.937208	115.8423714	Malacostraca: Isopoda	Philosciidae	unknown	Philosciidae sp. H-ISP059
2	YAR168.20210931.T1-01	**QY05**	YAR168	-31.937349	115.8422193	Malacostraca: Isopoda	no data	No data	Bacterial contaminant
2	YAR119.20210930.T3-01	**QY06**	YAR119	-31.936988	115.8425566	Malacostraca: Isopoda	no data	No data	Bacterial contaminant
2	YAR161.20210930.T4-01	**QY07**	YAR161	-31.937323	115.8422186	Malacostraca: Isopoda	Philosciidae	unknown	Philosciidae sp. H-ISP060
3	YAR249.20230119.T3-01	**UH55**	YAR249	-21.75583569	116.0148089	Malacostraca: Isopoda	Armadillidae	unknown	**Armadillidae sp. H-ISA064**
3	YAR249.20230119.T2-01	**UH56**	YAR249	-21.75583569	116.0148089	Malacostraca: Isopoda	No data	No data	No data
3	YAR249.20230119.T2-01	**UH57**	YAR249	-21.75583569	116.0148089	Malacostraca: Isopoda	No data	No data	No data
3	YAR249.20230119.T2-01	**UH58**	YAR249	-21.75583569	116.0148089	Malacostraca: Isopoda	No data	No data	No data
3	YARDDH004.20230119.T3-02	**UH09**	YARDDH004	-21.75714739	116.0130657	Malacostraca: Isopoda	Philosciidae	unknown	Philosciidae sp. H-ISP059
3	YARDDH004.20230119.T3-02	**UH10**	YARDDH004	-21.75714739	116.0130657	Malacostraca: Isopoda	Philosciidae	unknown	Philosciidae sp. H-ISP059
3	YARDDH004.20230119.T3-02	**UH11**	YARDDH004	-21.75714739	116.0130657	Malacostraca: Isopoda	Philosciidae	unknown	Philosciidae sp. H-ISP059
3	YARDDH004.20230119.T1-01	**UH12**	YARDDH004	-21.75714739	116.0130657	Malacostraca: Isopoda	Philosciidae	unknown	Philosciidae sp. H-ISP059
3	YARDDH004.20230119.T1-01	**UH13**	YARDDH004	-21.75714739	116.0130657	Malacostraca: Isopoda	Philosciidae	unknown	Philosciidae sp. H-ISP059
3	YAR249.20230119.T1-01	**UH59**	YAR249	-21.75583569	116.0148089	Malacostraca: Isopoda	Philosciidae	unknown	Philosciidae sp. H-ISP059
3	YAR237.20230119.T3-01	**UH62**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH63**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH64**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH65**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH66**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH67**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH68**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH69**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH70**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH71**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH72**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH73**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH74**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
3	YAR237.20230119.T3-01	**UH75**	YAR237	-21.75540929	116.0159043	Malacostraca: Isopoda	Philosciidae	unknown	**Philosciidae sp. H-ISP061**
1	YAR134.20210817.T2-01	**QY01**	YAR134	-31.937204	115.8424364	Symphyla	Scolopendrillidae	unknown	**Scolopendrillidae sp. H-SYM038**
2	YAR163.20210930.T1-01	**QY02**	YAR163	-21.758216	116.0303715	Symphyla	Scolopendrillidae	unknown	**Scolopendrillidae sp. H-SYM039**
2	YAR173.20210930.T2-02	**QY03**	YAR173	-31.937309	115.8422034	Symphyla	Scolopendrillidae	unknown	**Scolopendrillidae sp. H-SYM040**
3	YAR291.20230119.T1-01	**UH19**	YAR291	-21.75754384	116.0152129	Symphyla	No data	No data	No data

Sampling Phase	Specimen ID	Helix ID	Site	Latitude	Longitude	Taxonomic Group	Molecular Family ID	Molecular Genus ID	Molecular Species ID
2	YAR149.20210930.T3-01	**QY09**	YAR149	-31.937213	115.8423483	Diplura	Japygidae	unknown	Japygidae sp. H- DJA022
2	YAR173.20210940.T2-01	**QY10**	YAR173	-31.937432	115.8422069	Diplura	Japygidae	unknown	Japygidae sp. H- DJA022
2	YAR125.20210930.T1-01	**QY11**	YAR125	-31.937277	115.8422129	Stylommatophora	Pupillidae	*Gastrocopta*	*Gastrocopta servilis*
2	YAR075.20210930.T3-01	**QY12**	YAR075	-31.937343	115.8422087	Stylommatophora	Pupillidae	*Gastrocopta*	*Gastrocopta servilis*
2	YAR075.20210930.T1-01	**QY13**	YAR075	-31.937343	115.8422087	Stylommatophora	No data	No data	No data
2	YAR124.20210930.T1-01	**QY14**	YAR124	-31.937396	115.8422345	Stylommatophora	No data	No data	No data

4.1 CHELICERATA

4.1.1 ARACHNIDA: Schizomida

Schizomids are small arachnids, largely confined to the tropical and subtropical regions of the world. They are highly dependent on humid habitats due to their weakly sclerotized integuments which make them vulnerable to desiccation (Humphreys et al., 1989). Consequently, they may have difficulty in dispersing between non-humid habitats. In Australia, schizomids are well-known from above-ground wet forests, particularly in tropical northern Australia where they are diverse and highly restricted to forest patches (Harvey, 1992a, 2000).

The arid Pilbara region in Western Australia, however, is currently recognised as the most diverse area for schizomids in the world. Whilst the Pilbara is not currently a humid environment, it has retained these relictual organisms from before the aridification that occurred during the Miocene. This diverse schizomid specification has been demonstrated to have been epigean in origin followed by subsequent subterranean colonisation and ongoing diversification below ground (Abrams et al., 2019). In Western Australia, schizomids have been described as a 'flagship' subterranean group (Harms et al., 2018) as they are known almost exclusively from subterranean habitats (Abrams et al., 2019). The one exception, *Draculoides* sp. SCH053, is thought to have returned to an epigean habitat, or could possibly move between epigean and subterranean environments. Schizomids are limited in their dispersal abilities, vulnerable to desiccation, demonstrate substantial diversity and have a relatively high occurrence in subterranean habitats (Abrams et al., 2019). The Western Australian genera *Paradraculoides* and *Draculoides* have recently been synonymised due detailed molecular investigations and a lack of consistent clear-cut morphological differences and are now recognized as *Draculoides* (Abrams et al., 2019). Just four species (*D. bramstrokeri, D. brooksi, D. julianneae* and *D. vinei*) occur outside of the Pilbara bioregion, whilst the remaining 68 species recognised to date occur only in the iron-bearing geologies in the Pilbara. Currently, there are 72 species of *Draculoides* recognised in Western Australia (Abrams et al., 2019).

Previous analyses of genetic variation between morphologically distinct species of schizomids can be used as a genetic 'yardstick' to assist in interpreting the molecular divergences in current data set. Five of the described species of *Draculoides* from the West Pilbara (Harvey et al., 2008), each occurring on a separate isolated mesas, differ from one another by between 8.4 to 12.1% sequence divergence (uncorrected p-distances; calculated by us from the Harvey et al., 2008 data). Similarly, the four described species of *Draculoides* that occur only on Cape Range and Barrow Island differ from one another by between 4.5 to 13.7% sequence divergence (uncorrected p-distances calculated by us from Harvey et al., 2008). Furthermore, amongst 66 of the *Draculoides* species examined by Abrams et al., 2019 there was between 3.2% and 18.8% interspecific sequence divergence (see Appendix 1).

Across the three Phases of sampling, a single schizomid taxon of was detected, which all 14 specimen from seven sites were identified as. One site (YAR161) yielded a schizomid specimen both in the current and previous survey (Table 4-1, Helix, 2022).

Reference Specimens and Outgroups

The 12 schizomids collected from six sites were assigned to the family Hubbardiidae. Project specimen sequences were analysed along with 277 reference specimens from both GenBank and the Helix database, which included two specimens from Phase 2 (Helix, 2022) The

outgroups *Stenochrus* sp. (GenBank # KY573310) and *Brignolozomus woowardi* (GenBank # EU272675) were used to root the phylogenetic tree.

Phylogenetic Analyses

A 687 base-pair (bp) fragment of COI was isolated for each specimen from the survey and used to analyse specimens. According to the molecular sequence data currently available for comparison, specimens were confirmed to sit within the family Hubbarbiidae (Subfamily Hubbardiinae, and the genus *Draculoides* (Figure 4-1). Given the existing comprehensive phylogenetic framework for Pilbara schizomids (Abrams et al., 2018), and its geographical locality, it was expected that the schizomids detected in this survey would sit amongst clades previously detected from the West Pilbara.

The 12 schizomid specimens from Phase 3 (UG) aligned with the two QY specimens from Phase 2 and all represent a single putative species *Draculoides* sp. H-SCH200 (Figure 4-1). This species forms a well-supported West Pilbara clade along with *Draculoides cochranus* (WAMT93232) and *Draculoides* sp. H-SCH103 (Figure 4-1). One of these species, *D. cochranus*, was included in in Abrams et al. (2018) assessment and was placed in Clade '*G*', of the West Pilbara. The use of the one mtDNA gene (COI) restricts the statistical support of the deeper nodes of the tree, often supported by slower evolving genes such as 12S, 18S and 28S.

Differentiation within and between lineages/species

Amongst the individuals belonging to this putative species, *Draculoides* sp. H-SCH200, there exists 0.0 to 1.6% sequence divergence (see Appendix 1). *Draculoides* sp. H-SCH200, showed 5.2% from its closest relative, *Draculoides* sp. SCH103 (GB # OQ001455) (see Appendix 1), followed by 9.8% sequence divergence from *Draculoides cochranus* (GB # MG913087) from the West Pilbara according to data publicly available on Genbank and the Helix database.

Conclusions

All schizomid specimens collected from the project area were divergent from reference schizomid sequence data currently available for comparison. Whilst there have been numerous species of schizomids recorded in the Robe valley mesas (Helix 2021, 2022), the putative taxon, *Draculoides* sp. H-SCH200 has not been detected previously. (see Appendix 1).

Draculoides sp. H-SCH200, is currently known from seven sites (YARDH007, YAR125, YAR161, YARDH001, YAR296, YAR466 and YAR143; Appendix 1; Helix, 2022).

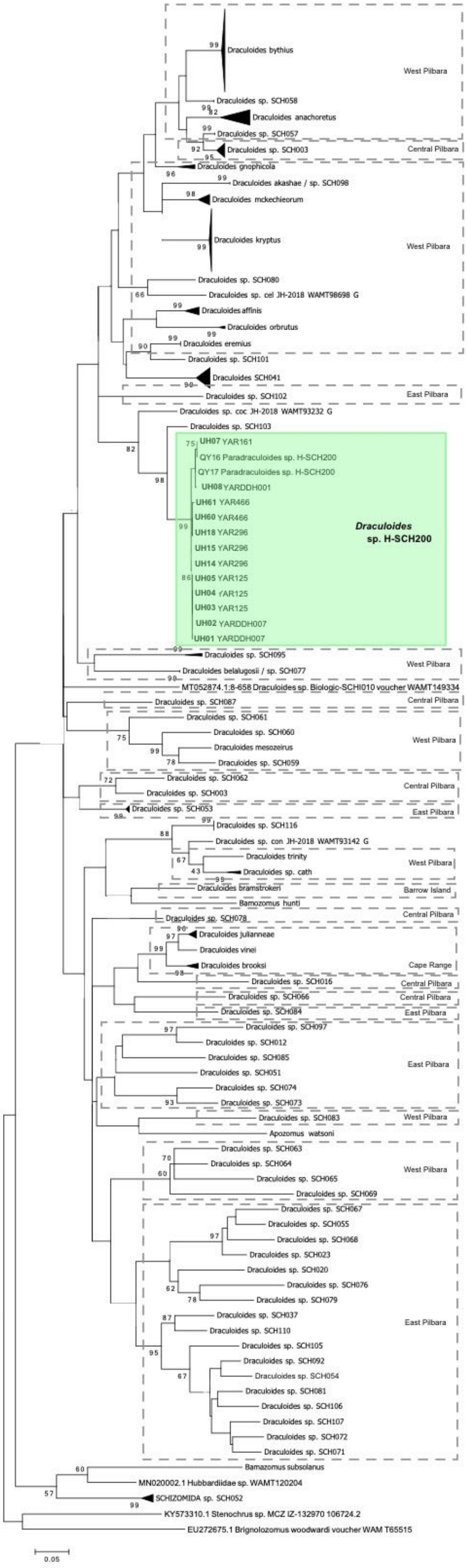

Figure 4-1. **Maximum Likelihood analysis of *Draculoides* COI mtDNA sequences, showing the placement of the Phase 1 and 2 specimens ('QY' prefix) and the Phase 3 survey area specimens ('UH' prefix) and their species designation (depicted as shaded colour), within the taxonomic framework of the Schizomida and the family Hubbardiidae including 277 reference specimens. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).**

4.1.2 ARACHNIDA: Pseudoscorpiones

The arachnid order Pseudoscorpiones is one of the oldest lineages of terrestrial organisms with the group (Murienne et al., 2008). They occupy virtually all terrestrial habitats on earth, but are most common in leaf litter, soil or under bark of trees and logs (Murienne et al., 2008).). They can also be frequently found in caves, and many species occur in littoral habitats along the coast (Murienne et al., 2008). The first molecular analysis of pseudoscorpion relationships was undertaken by Murienne et al (2008) with resulting groups mostly congruent with early classifications of Chamberlin (1931) and Harvey (1992b). There are troglobitic representatives of multiple families documented around the world (e.g. Mahnert, 2001; Ćurčić et al., 2014; Hou et al., 2023; Feng et al., 2023; Turbanov et al., 2024) including Australia (Harms and Harvey 2013).

A single trogolobitic pseudoscorpion specimen (QY08) from site YAR070 in Phase 2 was placed in the family Olpiidae and assigned to a newly detected putative taxon Olpiidae sp. H-PO033 (Helix, 2022).

A single troblobitic pseudoscorpion specimen (UH16) belonging to the family Hyidae was collected within the survey area from site YAR296 during Phase 3 and subsequently sequenced for variation at the COI mtDNA gene.

Reference Specimens and Outgroups

For the Phase 2 project specimen, 184 additional reference specimens were used during analyses (Helix, 2022).

The COI sequence of the Phase 3 project specimen UH16 was analysed along with 194 specimens including 29 additional representatives from the family Hyiidae from both GenBank and the Helix database. The outgroups *Pandinus dictator* (GenBank #AY156582) and *Siro rubens* (GenBank #DQ513111) were used to root the COI phylogenetic tree.

Phylogenetic Analyses

The single pseudoscorpion specimen from Phase 2 (QY08) was placed with other reference specimens belonging to the family Olpiidae (Figure 4-2), It did not, however, align closely with any previously detected lineages according to the molecular data currently available for comparison. This new olpiid pseudoscorpion specimen (QY08) from the Robe Valley has not been detected previously, based on the molecular data currently available for comparison and was assigned to a new lineage (H-PO033).

A 689 bp fragment of the COI mitochondrial gene was isolated for specimen UH16 from Phase 3 sampling. Phylogenetic analyses found that the pseudoscorpion specimen UH16 from the current (third) Phase of sampling, clearly sits within the family Hyiidae, amongst sequences identified as *Indohya alexanderi* / Hyiidae sp. H-PH003a according to the molecular sequence data currently available for comparison (Figure 4-3). There was strong statistical support for both the family and species placement.

Differentiation within and between lineages/species

Amongst the sequenced specimens, the pseudoscorpion lineage detected from Phase 2 sampling (H-PO033**)** showed 5.7% sequence divergence from the closest lineage H-PO031. The remaining 181 reference specimens showed greater than 15% sequence divergence and are therefore not presented in the table.

Analysis of the COI sequence data places the Phase 3 pseudoscorpion specimen (UH16) with other sequences belonging to the species *Indohya alexanderi* / Hyiidae sp. H-PH003a. The closest reference specimen to this Phase 3 specimen (UH16) showed 4.8% sequence divergence (H-PH003a, see Appendix 1) followed by *Indohya alexanderi* (GB # OR067347, WAM T139895) with 5.1% sequence divergence (Appendix 1). These both represent the same species, as depicted in the phylogenetic placement. These reference specimens have ≤ 3% sequence divergence between them, most liklely representing the same taxon. The next closest relative to the Phase 3 specimen UH16 was *Indohya arnoldstrongi* with 15.2% sequence divergence (Appendix 1).

Conclusions

The single lineage of olpiid pseudoscorpion detected during Phase 2 (H- PO033) has not been detected previously according to the molecular data currently available for comparison. The specimen was collected from site YAR070 (Helix, 2022).

A single specimen of a previously detected and described species *Indohya alexanderi* (also referred to as *I.* sp. H-PH003a) was identified from site YAR296 during Phase 3 sampling. The representative of this species on GenBank (# OR067356), included as a reference specimen during analyses, was collected from Mesa H, 20 km east southeast of Pannawonica (Harvey et al., 2023). The species is also known from two additional records located at the eastern end of the Robe Valley (Harvey et al., 2023). There have been two taxa belonging to the family Hyidae previously detected (Helix, 2021), from Mesa A (Hyidae sp. H-PH001b, n=1) and Mesa B (Hyidae sp. H-PH001a, n=2).

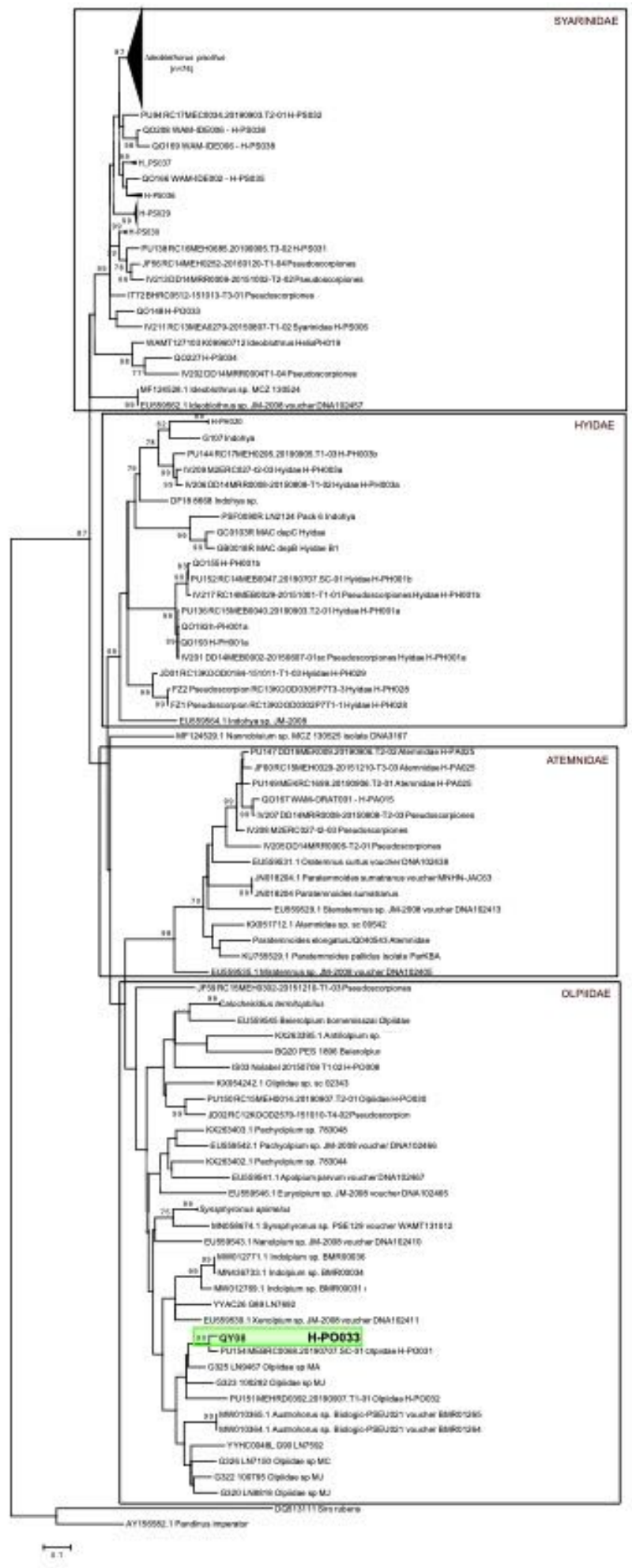

Figure 4-2. **Maximum Likelihood analysis of Pseudoscorpiones COI mtDNA sequences, showing the placement of the Mesa F pseudoscorpion Phase 3 specimen ('QY' prefix), within the taxonomic framework of the Pseudoscorpiones, including 184 reference specimens from four families. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site)**

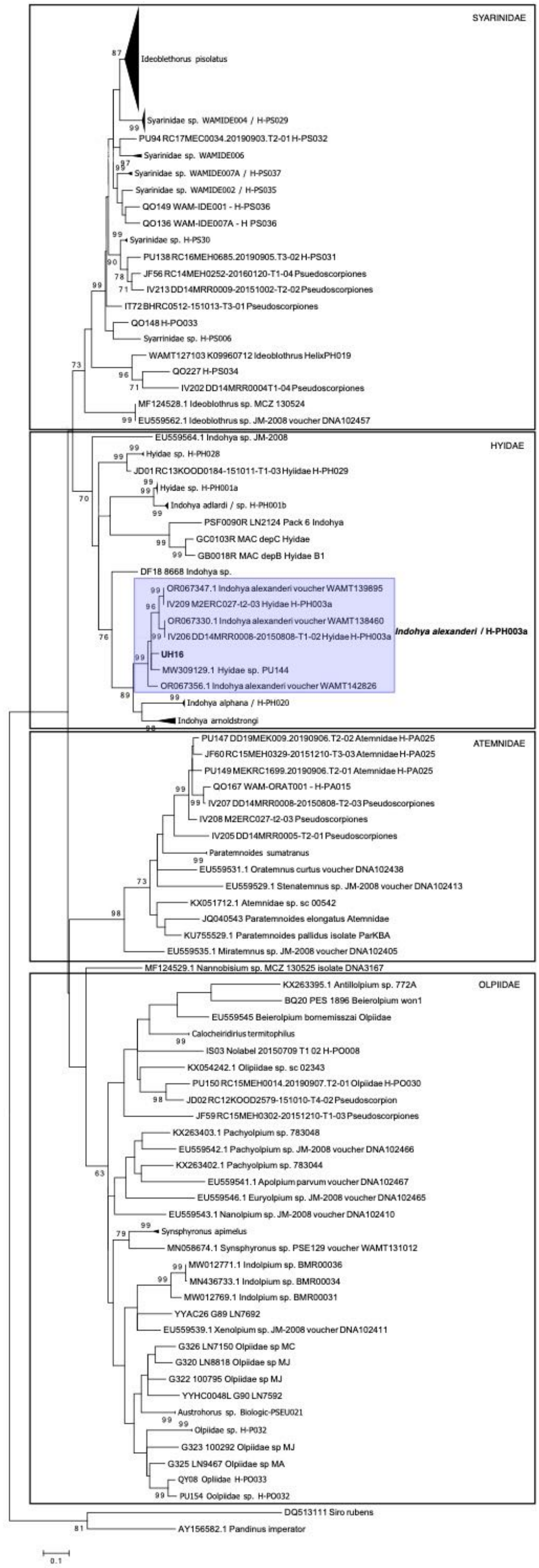

Figure 4-3. **Maximum Likelihood analysis of *Indohya* COI mtDNA sequences, showing the placement of the survey area specimen ('UH' prefix) and species designation (depicted as shaded colour), within the taxonomic framework of the Pseudoscorpiones and the family Hyidae including 194 reference specimens. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).**

4.2 CRUSTACEA

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

4.2.1 MALACOSTRACA: Isopoda

Isopods are a diverse group of crustaceans found in marine, freshwater and terrestrial environments (Austin 2022). Several genera and species have been described from groundwater habitats (Wilson and Ponder, 1992) but troglobitic species are less well documented. Of the troglobitic groups, Armadillidae and Philosciidae appear to be among the most commonly represented (Helix; pers. obs.). Five genera of Philosciidae and 24 genera of Armadillidae are described from Australia (Australian faunal Directory, accessed 29 October, 2024).

Across the three Phases of sampling, twenty-eight isopod specimens were collected (Table 4-1). Of these five failed to amplify a usable sequence for species identification.

Twenty-one of the total 24 Isopoda specimens from Phase 3 sampling were successfully sequenced for COI. Four haplotypes were present among the 21 specimens. These were analysed with the one haplotype detected in Phase 1/Phase 2.

Reference Specimens and Outgroups

The five Isopoda haplotypes, representing 26 specimens (two from Phase 1 and 21 from Phase 3), were analysed along with 108 reference specimens of Isopoda, 30 from GenBank and 78 from the Helix database. Two specimens of Malacostraca were used as outgroups – the Louisiana crayfish *Procambarus clarki* (GenBank #JN000898) and the mudshrimp *Callianassa ceramica* (GenBank #NC031154).

Phylogenetic Analyses

The phylogenetic analysis, which included the five haplotypes from Phase 1 and Phase 3, and 108 additional reference specimens, placed the five haplotypes in four distinct lineages (Figure 4-4). One haplotype was placed within the Armadillidae whilst three haplotypes were placed within the Philosciidae (Figure 4-4). Specifically, representative haplotype specimen UH55 from Phase 3 was placed in a new lineage within the clade containing reference specimens of Armadillidae (H-ISA064; Figure 4-4). It was placed in a clade that clustered outside of the clades containing specimens identified as *Troglamadillo*, so it cannot be assigned to a known genus, based on the molecular sequence data currently available for comparison. The remaining four haplotypes were placed in three lineages within the clade containing reference specimens of Philosciidae (Figure 4-4). Two of the three Philosciidae lineages were detected previously in Phase 1 (H-ISP059, H-ISP060) and one lineage was newly detected in Phase 3 (H-ISP061). Lineage H-ISP059 contained a haplotype that was detected during both Phases 1 and 3, representing six specimens. The remaining lineages contained haplotypes from single Phases, representing between one (lineages H-ISA064, H-ISP060) and 14 specimens (H-ISP061).

Differentiation within and between lineages/species

The four lineages from Phases 1 and 3 differed from one another by between 4.7 and 24.1% sequence divergence (Appendix 1). Lineage H-ISA064 (represented by haplotype UH55 and belonging to the family Armadillidae) was highly distinct from the remaining lineages, differing between 23.9 and 24.1% sequence divergence (Appendix 1). The remaining three lineages, belonging to the family Philosciidae, differed from one another by between 4.7 and 6.8% sequence divergence (Appendix 1).

Lineage H-ISA064 clustered with Armadillidae reference lineage H-ISA046 (specimen JF47 from Mesa H) and differed from that specimen by 18.6% sequence divergence and from specimens in Armadillidae lineage H-ISA008 by between 17.8 and 18.6% sequence divergence (Appendix 1). Lineages H-ISP059 – H-ISP061 all clustered most closely with one another as reported above; the next nearest reference lineage to all three lineages was lineage H-ISP050, from the Robe Valley, differing between 14.4 and 16.2% sequence divergence (Appendix 1).

Conclusions

Lineages H-ISP059 and H-ISP060 require further examination. The two lineages are each represented by a single haplotype, which differ by 4.7% sequence divergence. Haplotype/lineage H-ISP059 was detected in two bores, YAR082 (Phase 1) and YARDDH004 (Phase 3) and haplotype/lineage H-ISP060 was detected in a single bore, YAR082. Because both lineages were detected in a single bore, YAR082, we suggest there are (or have recently been) genetic barriers between the two lineages. The two lineages are closely related but appear to be maintaining separation. To understand whether that separation is occurring at the species level requires further sampling in intermediate areas as well as thorough morphological examination. Sequencing at other gene regions may also aid species delineation. Distances and clustering patterns between the Robe valley lineages of Philosciidae and the reference specimens prevent us from assigning these new lineages to a genus.

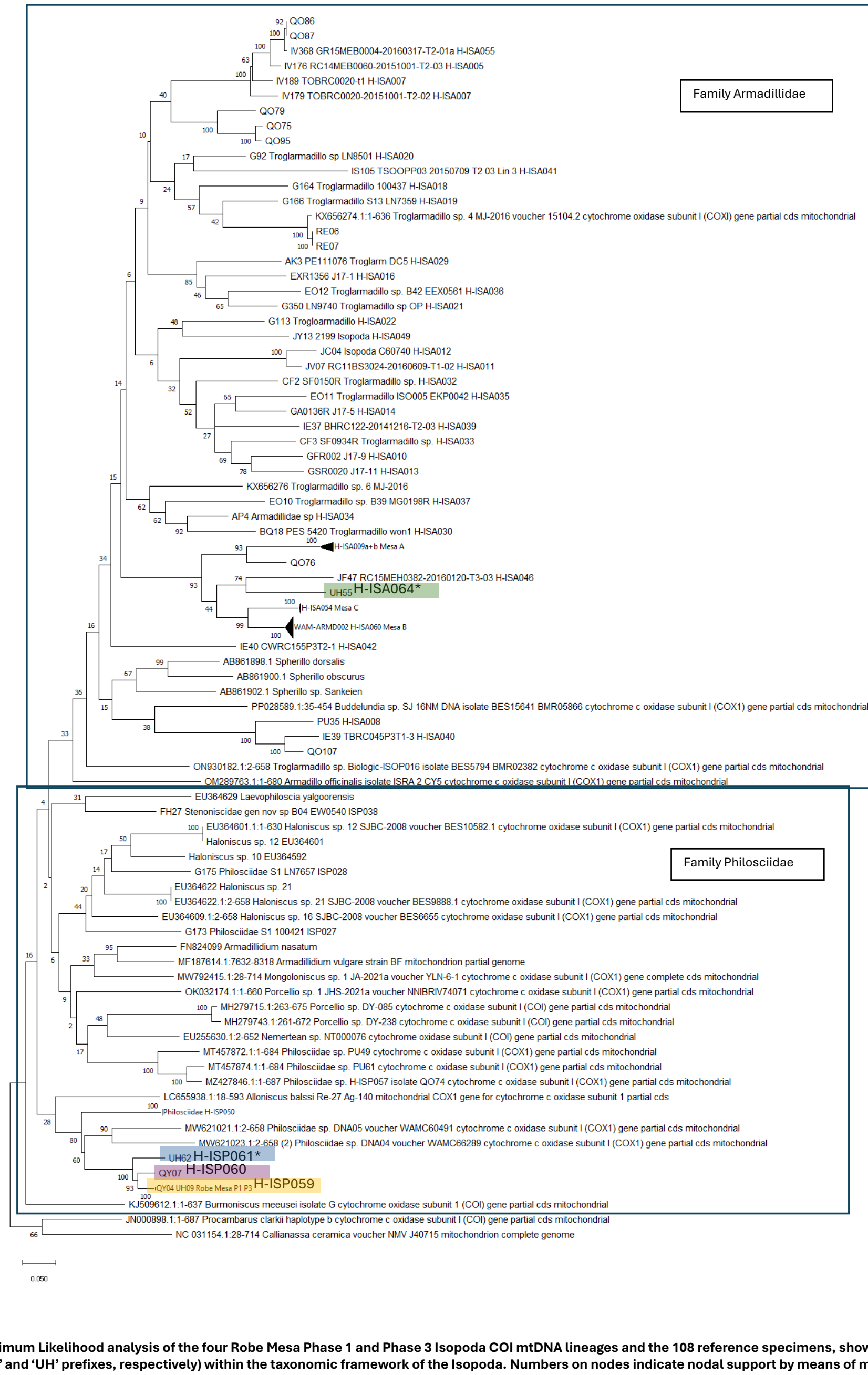

Figure 4-4. **Maximum Likelihood analysis of the four Robe Mesa Phase 1 and Phase 3 Isopoda COI mtDNA lineages and the 108 reference specimens, showing their placement ('QY' and 'UH' prefixes, respectively) within the taxonomic framework of the Isopoda. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).**

4.3 INSECTA

4.3.1 ARCHEOGNATHA: Zygentoma

Zygentoma (silverfish, bristletails and firebrats) are found worldwide, comprising about 400 species. Four families have been described, two of which are known from Australia, the Lepismatidae, which contains twelve genera, and the Nicoletiidae, which contains fourteen genera (Australian Faunal Directory, accessed 31 October, 2024). Australian species from both families have been the subject of recent studies, which have included both morphological and molecular examination (Smith et al., 2012; Smith, 2017; Smith et al, 2022).

Zygentoma were detected only during Phase 3 sampling. Six of twelve Zygentoma specimens from Phase 3 were successfully sequenced for COI. Four haplotypes were present amongst the six specimens.

The mitochondrial gene 12S is widely used in insect systematics (Simon *et al.,* 1996; Caterino *et al*., 2000), although in contrast to the mitochondrial gene COI, fewer broadscale comparative studies are available to provide a basis for species discrimination. Nonetheless, 12S has proven useful for establishing phylogenetic relationships in many insect groups (Caterino *et al*., 2000). The 12S gene evolves approximately 1.5 times (Mueller, 2006) more slowly than COI. Hence, we would expect that the threshold for species discrimination using 12S would be, by inference, lower than COI. Again, examination of genetic differentiation within and between established species provides the best method of establishing genetic "thresholds" and delineating species boundaries. In a recent study of two new species of Nicoletiidae from the Pilbara, Smith *et al.,* (2012) found that six specimens of *Trimenura callawa* differed by a mean of 0.4% sequence divergence at the 12S gene (range = 0 to 0.9%), and that they differed from *T. cundalin*, which is described from an adjacent ridge separated by a creek valley by 6.2 to 6.5% sequence divergence.

Reference Specimens and Outgroups

The four Zygentoma haplotypes, representing six specimens (all from Phase 3), were analysed at 12S using primers 12Sai/12SRJ (Simon *et al*., 1994) with 32 reference specimens of Zygentoma, 32 from GenBank (five of which originated from the Helix database). Two specimens of termites were used as outgroups – *Tauritermes* sp. (GenBank #OQ998963) and *Tauritermes triceromegas* (GenBank #OK506056).

Phylogenetic Analyses

The phylogenetic analysis, which included the four haplotypes from Phase 3, and 32 reference specimens, placed the four Phase 3 haplotypes in four distinct lineages (Figure 4-5). One lineage was placed within the Ateluriinae and three were placed within the Nicoletiinae (Figure 4-5). Specifically, haplotype UH21 was placed in a new lineage (H-TA010; Figure 4-5) within the Nicoletiidae, in a clade containing reference specimens of the subfamily Atelurinae sp. H-TA009 from the Robe Valley. The remaining three haplotypes were also placed within the Nicoletiidae, but in subfamily Nicoletiinae (Figure 4-5). Two haplotypes, UH20 and UH06 were each assigned to new lineages, H-TN001 and H-TN002, respectively, which were placed together in a clade with species of *Trimenura* (Biol-ZYGE043, WAM-ZYGS001) and Nicoletiinae sp. Biol-ZYGE006 and Biol-ZYGE007 (Figure 4-5). The final haplotype, UH29 was placed in a

new lineage, H-TN003, and clustered most closely with specimens of Nicoletiinae sp. Biol-ZYGE005 (Figure 4-5).

Differentiation within and between lineages/species

The four Zygentoma lineages from Phase 3 differed from one another by between 7.0 and 25.8% sequence divergence (Appendix 1). Lineage H-TA010 (represented by haplotype UH21) differed from the nearest reference lineage H-TA009 from the Robe Valley by 10.1-10.8% sequence divergence (Appendix 1). Zygentoma lineages H-TN001 and H-TN002, represented by haplotypes UH20 and UH06, respectively, differed from one another by 7% (Appendix 1) and from the nearest reference specimens of *Trimenura* Biol-ZYGE043 and WAM-ZYGS001 and Nicoletiinae Biol-ZYGE006 and Biol-ZYGE007 by between 16.1 and 19.8% (Table 6.11). The remaining Zygentoma lineage from Phase 3, H-TN003, differed from three specimens of Nicoletiinae belonging to the lineage-species ZYGE005 by between 15.7 and 16.3% sequence divergence (Appendix 1).

Conclusions

Four distinct lineages of Zygentoma were detected from Phase 3, a single Ateluriinae lineage (H-TA010) and three Nicoletiinae lineages (H-TN001 – H-TN003), all within the family Nicoletiidae. No Zygentoma specimens were collected in Phases 1 and 2 for comparison. All four lineages were newly detected in Phase 3 and have not been detected outside the survey area and represent new species, differing from reference specimens by >7%.

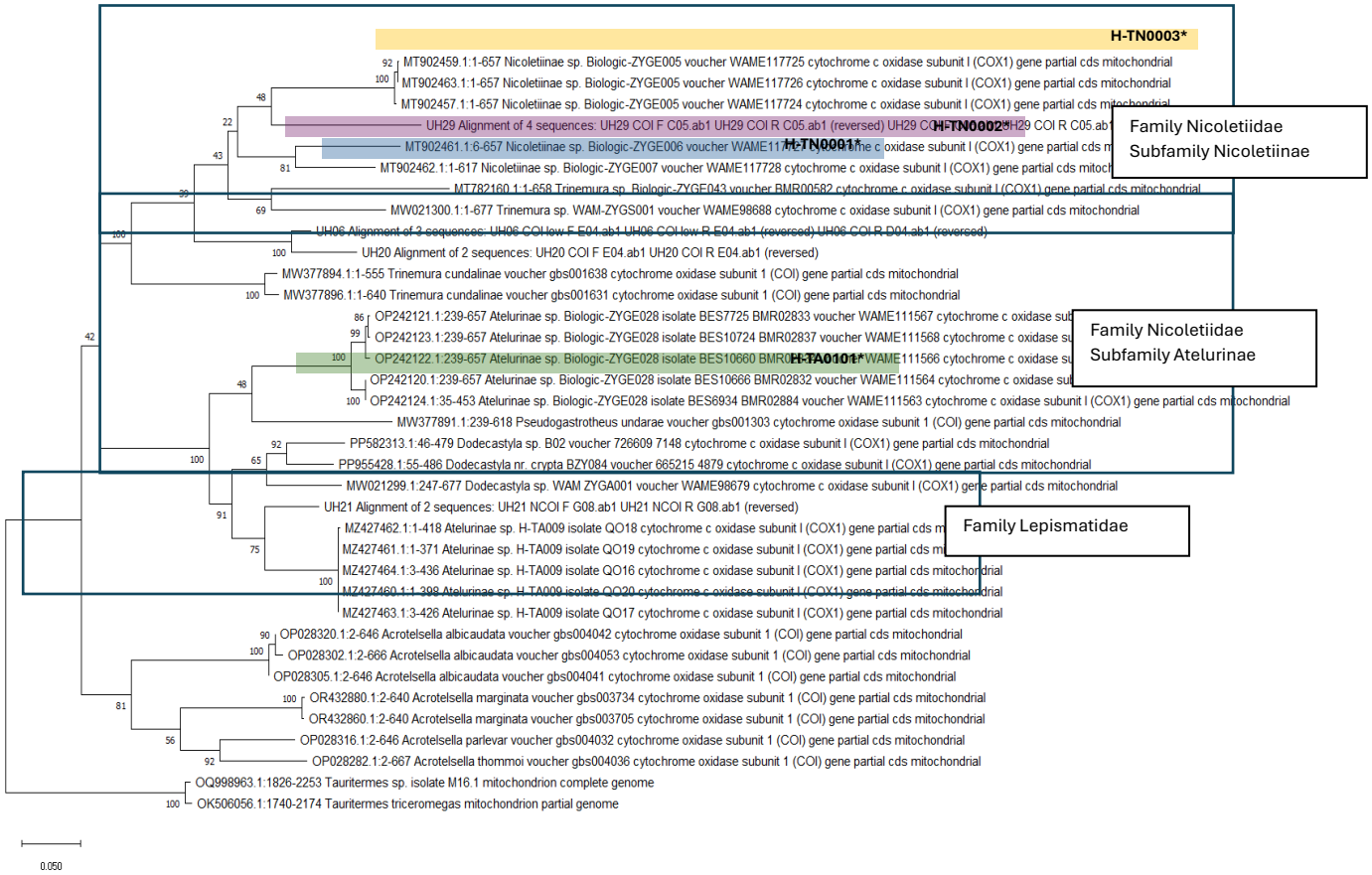

Figure 4-5. Maximum Likelihood analysis of the four Robe Valley Phase 3 Zygentoma COI mtDNA lineages and the 32 reference specimens, showing their placement ('UH' prefixes) within the taxonomic framework of the Zygentoma.

Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).

4.4 MYRIAPODA

4.4.1 DIPLOPODA: Polydesmida

The order Polydesmida is placed in the Class Diplopoda (millipedes). Polydesmids represent the largest order of millipedes and in Australia are represented by five families, 51 genera and 112 species (Key to Australian Freshwater and Terrestrial Invertebrates, accessed 31 October, 2024). In particular, the family Haplodesmidae contains three genera and 27 species (Australian Faunal Directory, accessed 31 October, 2024).

Diplopoda were detected only during Phase 3 sampling. Thirty-one of 34 specimens of Diplopoda from Phase 3, assigned to the family Haplodesmidae on the basis of morphology, were successfully sequenced for COI. One haplotype was detected among the 31 specimens. Haplodesmidae were not collected during Phases 1 and 2 for comparison.

Reference Specimens and Outgroups

The one Haplodesmida haplotype, representing 31 specimens (all from Phase 3), were analysed with 45 reference specimens of Haplodesmida, 25 from GenBank and 19 from the Helix database. Two specimens of Diplopoda were used as outgroups – *Polydesmida* sp. (GenBank #HQ979247) and *Polydesmida angustus* (GenBank #HQ966174).

Phylogenetic Analyses

The phylogenetic analysis, which included the one haplotype from Phase 3, (UH31) and 45 reference specimens, placed the Phase 3 specimen within the existing Haplodesmidae lineage H-DIHAP001, which has been detected previously in the Robe Valley, at Mesas B, C and K, as well as at Hardey River (Figure 4-6).

Differentiation within and between lineages/species

The Haplodesmidae specimen UH31 matched most closely with 22 GenBank sequences of Haplodesmidae sp. H-DIHAP001 (GenBank accession #s MT457866, MW621069, MZ427783-MZ427801, and PP582307), originating from Helix specimens from the Robe Valley, and showing 100% similarity. There was little to no genetic variation among the 15 individuals within lineage H-DIHAP001, with p-distances ranging from 0 to 0.15% (Appendix 1). The next nearest match among the reference specimens was to two GenBank specimens of the haplodesmid *Desmoxytes delphae* (GenBank accession #OR765875 and #OR765876) at 20.1% and 20.4% sequence divergence (Appendix 1).

Conclusions

The Haplodesmidae specimen UH31, representing 31 specimens, was placed in an existing lineage H-DIHAP001, which has been detected previously in the Robe Valley, at Mesas B, C and K, as well as the Hardey River region. The lineage/species does not show close relationships with any other Helix or GenBank specimens, based on the material available for comparison.

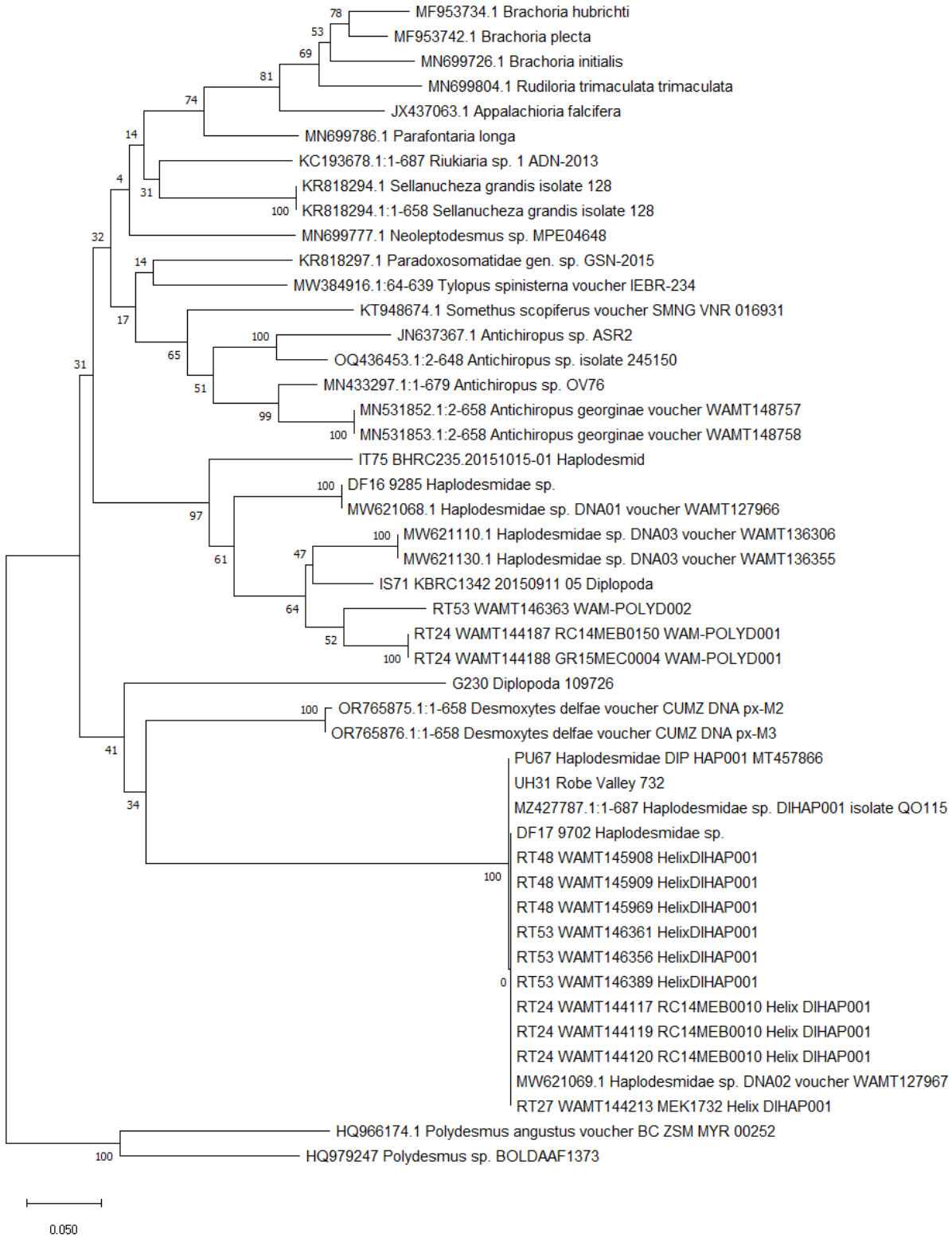

Figure 4-6. **Maximum Likelihood analysis of the Robe Valley Phase 3 Polydesmida COI mtDNA lineages and the 108 reference specimens, showing their placement ('UH' prefixes) within the taxonomic framework of the Diplopoda family Haplodesmidae. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).**

4.4.2 SYMPHYLA

Three specimens of Symphyla were collected during the Phase 1 and 2 sampling survey from Mesa F and the Robe Valley (Helix, 2022).

The single Phase 3 symphylan specimen (UH19, Table 4-1) failed to amplify and therefore could not be analysed or further identified. No further classification for this specimen was possible without a useable DNA sequence.

Reference Specimens and Outgroups

The COI sequences of the three specimens from Mesa F and the Robe Valley from Phase 1/Phase 2 were analysed along with 143 specimens. The outgroups *Euchorthippus pulvinatus* (GenBank #KR005925), *Polydesmus angustus* (GenBank #HQ966174), *Scutigerini weberi* (GenBank #DQ222116) and *Scutigerella causeyae* (GenBank #DQ666065) were all used to root the COI phylogenetic tree.

Phylogenetic Analyses

Comparisons to the symphylan data to the molecular data currently available for comparison from GenBank and to those from the Helix database revealed that the specimens were most closely related to the family Scolopendrellidae. Analyses place the Phase 1 and 2 specimens, all from separate sampling locations (YAR134, YAR163 and YAR173, Appendix 1). with other symphylan specimens previously recorded from the Robe Mesa Valley (Figure 4-7).

Differentiation within and between lineages/species

There was between 3.1 to 6.7% molecular sequence divergence amongst the three specimens from three sites (YAR134, YAR163 and YAR173) from Mesa F during Phases 1 and 2 sampling (Appendix 1, Helix 2022).

Amongst the scolopendrellid specimens from Phase 1 and 2 sampling from Mesa F there were three individual haplotypes which showed 4.8 to 8.7% sequence divergence from the closest relative, the taxon H-SYM027 (Helix, 2022). This closest reference lineage H-SYM027 has been recorded previously from the Robe Valley area from Mesa C (Helix, 2020; 2021).

Conclusions

Three closely related lineages of Scolopendrellidae were detected in Phases 1 and 2 of the survey. Each lineage was represented by a single specimen thus we cannot estimate genetic divergence within lineages. Further sampling in the area is necessary to increase sample sizes, in order to determine whether the three lineages represent three distinct species or a single species showing genetic variation due to geographical distance.

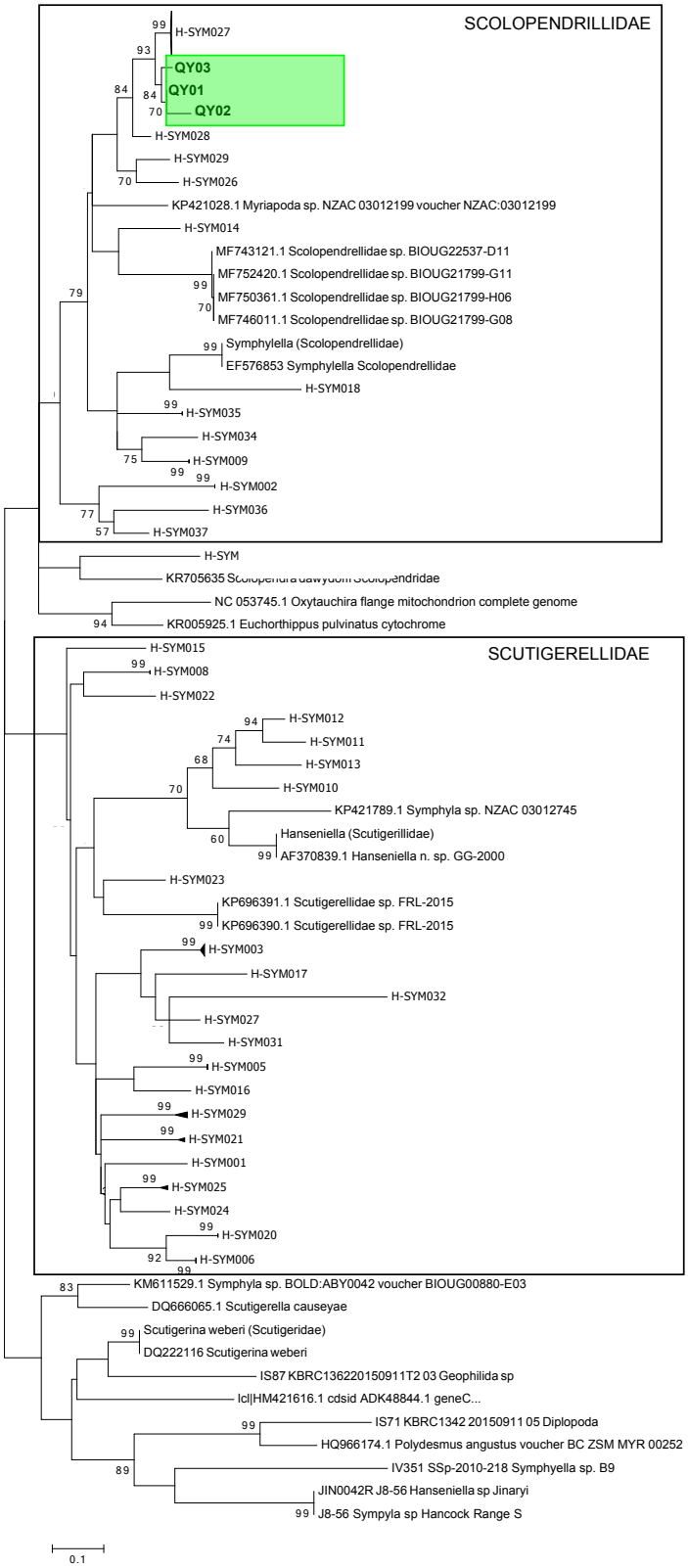

Figure 4-7. **Maximum Likelihood analysis of COI mtDNA sequences, showing the placement of the three Mesa F Symphylan specimens from Phase 1 and 2 ('QY' prefix), within the taxonomic framework of the Symphyla, including 143 reference specimens from GenBank and the Helix database. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).**

4.5 HEXAPODA

4.5.1 DIPLURA

Two dipluran specimens were detected during Phase 2 sampling. The single Phase 3 dipluran specimen (UH17, Table 4-1) failed to amplify and therefore could not be analysed or further identified. No further classification for this specimen was possible without a useable DNA sequence.

Reference Specimens and Outgroups

The COI sequences of the Phase 2 specimens were analysed along with 28 reference specimens from both GenBank and the Helix database. The outgroups *Lycorma delicatula* (GenBank #FJ456942) and *Geisha distinctissima* (GenBank #JN087438) were used to root the COI phylogenetic tree. Additional reference specimens from other families were also used for context and to exclude placement of project specimens with an alternative family.

Phylogenetic Analyses

There is high statistical support for the placement of these Phase 2 dipluran specimens with other reference specimens belonging to the family Japygidae (Figure 4 8).

Differentiation within and between lineages/species

The two dipluran specimens from Mesa F (from sites 'YAR149' and 'YAR173') collected during Phase 2 sampling (see Appendix 1) show substantial sequence divergence from one another (4.5%). This level of divergence may represent levels of divergence in line with species-level (interspecific) distances or large divergence within a single species (intraspecific) that could be found over a geographical distances.

The closest relative for the two Phase 2 specimens shows 7.3% to 7.9% sequence divergence (Helix, 2022). This closest relative, the Japygidae lineage H-DJA020 / WAM-DPLJ001 has been recorded on previously on two occasions from Mesa C (Helix, 2020; 2021).

Conclusions

The two dipluran specimens collected during Phase 2 sampling in the Robe Mesa Valley from sites YAR149 and YAR173 (see Appendix 1) represent a previously undetected putative taxon, Japygidae sp. H-DJA022, based on the molecular sequence data currently available.

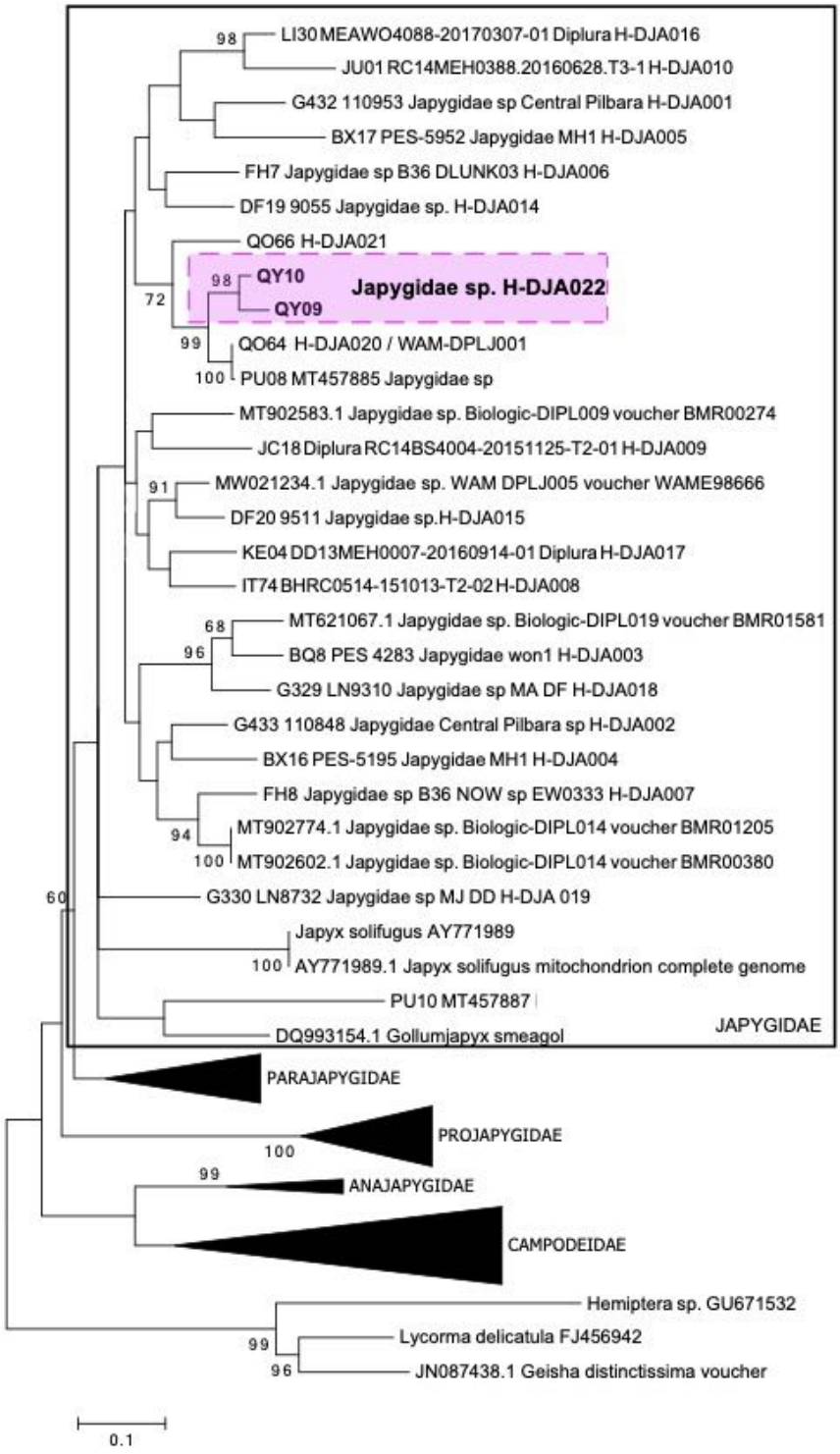

Figure 4-8. **Maximum Likelihood analysis of COI mtDNA sequences, showing the placement of the Phase 2 dipluran specimens from Mesa F ('QY' prefix), within the taxonomic framework of the Diplura, showing placement in the family Japygidae. Clades for the families Parajapygidae, Projapygidae, Anajapygidae and Campodeidae have been collapsed to simplify interpretation of the relationships. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).**

4.6 GASTROPODA

4.6.1 PUPILLIDAE

No gastropod specimens were collected during Phase 3 sampling. Four specimens were collected during Phase 2 sampling, with two successfully sequenced.

Reference Specimens and Outgroups

The Robe valley specimens from the Phase 2 survey were collected from sites YAR125 and YAR075 (see Appendix 1 and Helix, 2022). These specimens were analysed along with 46 reference specimens all obtained from GenBank. This total included 28 from the family Pupillidae, 14 from Valloniidae, 1 Helicarionidae, 1 Dyakiidae and 2 Ariphantidae. Specimens belonging to these families were all used as outgroups for the analyses (see Figure 4-9).

Phylogenetic Analyses

There was strong statistical support for the placement of the two specimens from Phase 2 sampling with other specimens belonging to the family Pupillidae, specifically the genus *Gastrocopta* (Figure 4-9). Both specimens aligned with representatives of the species *G. servillis* (GenBank #JN941062 and #JN941048) with very high statistical support (Figure 4-9).

Differentiation within and between lineages

There was no molecular sequence divergence between the two *Gastrocopta* specimens from Phase 2 sampling (0.00%, Helix 2022). These specimens demonstrated just 0.7% molecular sequence divergence from a specimen of *Gastrocopta servilis* obtained from GenBank (#JN941048) (Helix, 2022). The next closest relative is the congeneric species *G. pellucida* (GenBank #N941057) at 8.5% (Helix, 2022).

Conclusions

There is very high statistical support for the placement of the Robe Valley gastropoda specimens within the described and previously detected species *Gastrocopta servilis*. The species is well known throughout the Pacific (Nekola et al., 2012).

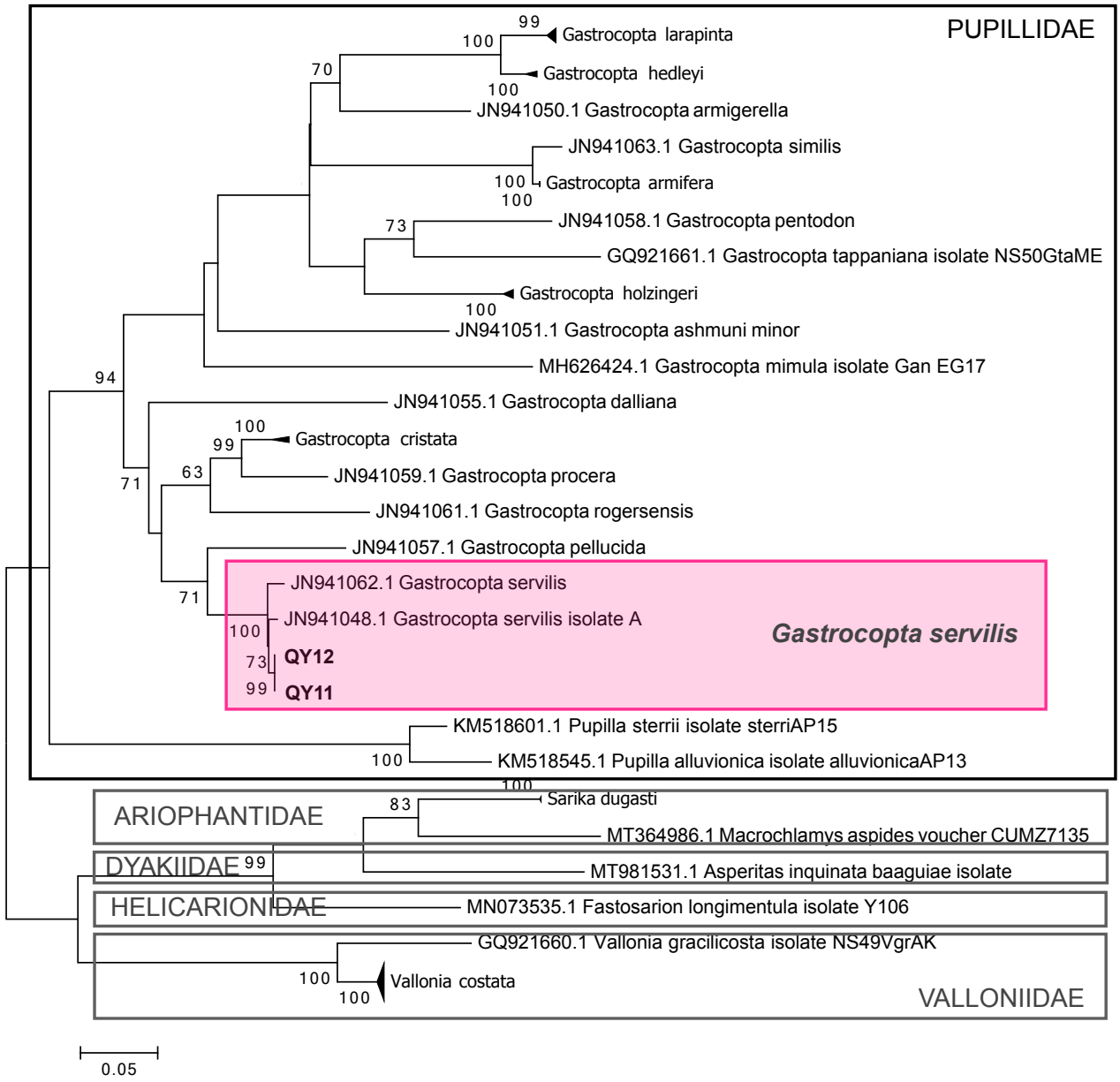

Figure 4-9. **Maximum Likelihood analysis of COI mtDNA sequences, showing the placement of the two gastropod specimens from Phase 2 sampling ('QY' prefix), showing placement in the family Pupillidae. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site)**

5.0 Discussion

COI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003a) and the use of this gene can be extremely effective for DNA barcoding in taxa where clear differentiation exists between intra and interspecific levels of divergence (Hebert et al., 2004a; 2004b). In a comparison of COI sequences for over 13,000 pairs of taxa, Hebert et al., (2003b) found a mean of 11.1% sequence divergence between distinct species. Nearly 80% of these comparisons found that species pairs differed from one another by >8% sequence divergence.

Despite its merits in barcoding, a taxon-by-taxon approach, examining the amount of phylogenetic variation within and between taxa is the most widely accepted method of delineating species and their distributions, especially in areas where rapidly expanding development outpaces taxonomic treatment of unresolved taxa.

Of the total 102 specimens collected from the Robe Mesa project area, 77 were sequenced for COI, and six for 12S (Table 4-1). In summary, we detected a total of 17 putative species from eight taxonomic groups, belonging to 11 families across the three Phases of sampling (see summary in Table 5-1).

Of the 17 species detected, three align with previously detected widespread species, two of which were known described species, and 14 are newly detected putative species (Table 5-1).

Table 5-1. Summary information for species detected during the three survey Phases for the eight taxonomic groups, including the sites and Phases where detections were found. Species in 'bold' text refer to putative taxa not previously detected elsewhere.

Taxonomic Group	Family	Species	Sites Recorded	Phase detected
Schizomida	Hubbardiidae	***Draculoides* sp. H-SCH200**	YARDH007, YAR125, YAR161, YARDH001, YAR296, YAR466	2 and 3
Pseudoscorpiones	Hyidae	*Indohya alexanderi* / Hyiidae sp. H-PH003a	YAR296	3
Pseudoscorpiones	Olpiidae	**Olpiidae sp. H-PO033**	YAR070	1
Isopoda	Armadillidae	**Armadillidae sp. H-ISA064**	YAR 249	3
Isopoda	undetermined	undetermined	YAR249	3
Isopoda	undetermined	undetermined	YAR168, YAR169	2
Isopoda	Philosciidae	**Philosciidae sp. H-ISP059**	YARDH004	2
Isopoda	Philosciidae	**Philosciidae sp. H-ISP060**	YAR161	1 and 2
Isopoda	Philosciidae	**Philosciidae sp. H-ISP061**	YAR 237	3
Zygentoma	Nicoletiidae	**Ateluriinae sp. H-TA010**	YAR143	3
Zygentoma	Nicoletiinae	**Nicoletiinae sp. H-TN001**	YAR486	3
Zygentoma	Nicoletiinae	**Nicoletiinae sp. H-TN002**	YAR125	3

Taxonomic Group	Family	Species	Sites Recorded	Phase detected
Zygentoma	Nicoletiinae	**Nicoletiinae sp. H-TN003**	YAR143	3
Zygentoma	undetermined	undetermined	YAR143	3
Polydesmida	Haplodesmidae	Haplodesmidae sp. DIHAP001	YAR487	3
Symphyla	Scolopendrillidae	**Scolopendrillidae sp. H-SYM038**	YAR134	1
Symphyla	Scolopendrillidae	**Scolopendrillidae sp. H-SYM039**	YAR163	2
Symphyla	Scolopendrillidae	**Scolopendrillidae sp. H-SYM040**	YAR173	2
Symphyla	undetermined	undetermined	YAR291	2
Diplura	Japygidae	**Japygidae sp. H-DJA022**	YAR149, YAR173	2
Diplure	undetermined	undetermined	YAR296	3
Gastropoda	Pupillidae	*Gastrocopta servillis*	YAR125, YAR075	2

Defining species boundaries requires an understanding of variation within and between phylogenetic groups. Each lineage may not necessarily represent a species; species may be composed of multiple lineages showing low levels of intraspecific sequence divergence. Because sample sizes were relatively small for each taxonomic group in this study, we do not have enough information to determine intraspecific molecular genetic variation. Further sampling would be required to determine species boundaries in this collection where lineages show moderate levels of sequence divergence. However, we can confidently determine that three species detected in this group have been detected previously, based on the molecular data currently available for comparison, whilst 14 putative taxa have not been detected previously.

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Appendix 1 Genetic p-distances between Robe Mesa survey specimens

Table A1. 1 **Genetic p-distance (below) and associated standard error (above- blue text) between the twelve schizomid specimens sequenced for COI from this study (Phase 3, prefix 'UH').**

	UH61 YAR466	**UH60** YAR466	**UH18** YAR296	**UH15** YAR296	**UH14** YAR296	**UH08** YARDDH001	**UH07** YAR161	**UH05** YAR125	**UH04** YAR125	**UH03** YAR125	**UH02** YARDDH007	**UH01** YARDDH007
UH61 YAR466		0.0020	0.0020	0.0014	0.0020	0.0060	0.0040	0.0014	0.0014	0.0014	0.0020	0.0020
UH60 YAR466	0.0029		0.0000	0.0014	0.0000	0.0051	0.0035	0.0014	0.0014	0.0014	0.0020	0.0000
UH18 YAR296	0.0029	0.0000		0.0014	0.0000	0.0051	0.0035	0.0014	0.0014	0.0014	0.0020	0.0000
UH15 YAR296	0.0014	0.0014	0.0014		0.0014	0.0055	0.0038	0.0000	0.0000	0.0000	0.0014	0.0014
UH14 YAR296	0.0029	0.0000	0.0000	0.0014		0.0051	0.0035	0.0014	0.0014	0.0014	0.0020	0.0000
UH08 YARDDH001	0.0160	0.0114	0.0114	0.0137	0.0114		0.0039	0.0055	0.0055	0.0055	0.0060	0.0051
UH07 YAR161	0.0116	0.0087	0.0087	0.0101	0.0087	0.0068		0.0038	0.0038	0.0038	0.0040	0.0035
UH05 YAR125	0.0014	0.0014	0.0014	0.0000	0.0014	0.0137	0.0101		0.0000	0.000	0.0014	0.0014
UH04 YAR125	0.0014	0.001	0.0014	0.0000	0.0014	0.0137	0.0101	0.0000		0.0000	0.0014	0.0014
UH03 YAR125	0.0014	0.0014	0.0014	0.0000	0.0014	0.0137	0.0101	0.0000	0.0000		0.0014	0.0014
UH02 YARDDH007	0.0029	0.0029	0.0029	0.0014	0.0029	0.0160	0.0116	0.0014	0.0014	0.0014		0.0020
UH01 YARDDH007	0.0029	0.0000	0.0000	0.0014	0.0000	0.0114	0.0087	0.0014	0.0014	0.0014	0.0029	

Table A1. 2 **Genetic p-distance (below) and associated standard error (above- blue text) between the three isopoda haplotypes sequenced for COI from this study (Phase 3, prefix 'UH'), in addition to the single isopoda haplotype from previous sampling (prefix 'QY').**

	UH55– YAR249, H-ISA064	**UH09**- YARDH004, H-ISP059	QY07 H-ISP060	**UH62**-YAR237, H-ISP061
UH55 – YAR249, H-ISA064		0.0205	0.0222	0.0238
UH09 - YARDH004, H-ISP059	0.2391		0.0081	0.0095
QY07 H-ISP060	0.2391	0.0466		0.0099
UH62 -YAR237, H-ISP061	0.2414	0.0655	0.0684	

Table A1. 3 **Genetic p-distance (below) and associated standard error (above- blue text) between the four Zygentoma haplotypes sequenced for COI from this study (Phase 3, prefix 'UH').**

	UH29– YAR143, H-TN003	**UH21** -YAR143, H-TA010	**UH20**– YAR486, H-TN001	**UH06**– YAR125, H-TN002
UH29 – YAR143, H-TN003		0.0175	0.0140	0.0139
UH21 -YAR143, H-TA010	0.2368		0.0247	0.0231
UH20 – YAR486, H-TN001	0.1831	0.2577		0.0105
UH06 – YAR125, H-TN002	0.1793	0.2207	0.0697	