Bennelongia Environmental Consultants

Western Ridge Targeted Troglofauna Surveys: 2021 and 2022

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Final Report

Short-Range Endemics I Subterranean Fauna

Waterbirds I Wetlands

Western Ridge Targeted Troglofauna Surveys: 2021 and 2022

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EXECUTIVE SUMMARY

The Western Ridge Area (henceforth referred to as the 'Study Area') is located approximately 11 km west-southwest of Newman. It lies south of BHP's Orebody 35 mine site at the Whaleback Hub and extends 12 km in an east–west direction and 3.5 km north–south. BHP is investigating development of Western Ridge and any future mining of iron ore will involve excavation of several open mine pits.

The Study Area was surveyed for troglofauna by Bennelongia Environmental Consultants in 2010 and 2019-20. These baseline surveys resulted in the collection of five troglofauna species known only from proposed mine pit areas. Collecting these five target species was the aim of targeted troglofauna surveys undertaken in 2021 and 2022, the results of which are reported here. The five target species for the surveys were:

- the centipedes *Cryptops* `BSCOL064` and *Cryptops* `BSCOL065`;
- the symphylan *Hanseniella* `BSYM095`; and
- the pseudoscorpions *Indohya* `BPS294` and *Tyrannochthonius* `PSE053`.

Thirty-five exploration drill holes were sampled in 2021, yielding 227 troglofaunal animals that represent nine orders and at least 10 species. None of the five targeted species was collected. However, one new species known only from the Study Area was recorded, namely Coleoptera Genus 2 `BCO230`. Two other troglofauna species were recorded for the first time in the Study Area, *Prethopalpus* sp. and *Ptinella* sp. B01 (=MC). The former is not identified to species level (and hence its species distribution is unclear, albeit likely to be small) but the latter is known to be a widespread species in the Pilbara.

Seventy-seven drill holes were sampled in 2022, yielding 102 troglofaunal animals representing seven orders and at least eight species. The target species *Cryptops* `BSCOL064` was recorded from two additional holes in 2022. Two other species were collected from the Study Area for the first time in 2022: a symphylan *Symphylella* 'BSYM109' known only from the Study Area and a hemipteran Cixiidae sp. B02, which is widespread across the Pilbara. In addition, Coleoptera Genus 2 'BCO230' was recorded again (in a different drill hole) in the Study Area.

Genomic analysis of target species, as well as species of interest, was undertaken to provide or confirm identification of animals that might potentially show wider ranges of these species. Results included showing that juvenile specimens of silverfish identified as Dodecastyla sp. and dipluans identified as Japygidae sp. were, in fact, the relatively widespread *Dodecastyla* sp. B02 and Japygidae `BDP165`, respectively.

Overall, sampling in the Study Area from 2010 to 2022 has shown that at least 27 species of troglofauna occur at Western Ridge. Of these, 11 species are currently known only from Western Ridge. They are:

- oonopid spider *Prethopalpus* sp.;
- palpigrad *Eukoenenia* `BPAL046`;
- palpigrad Palpigradi sp. B07;
- pseudoscorpion *Indohya* `BPS294`;
- pseudoscorpion *Tyrannochthonius* `PSE053`;
- centipede *Cryptops* `BSCOL064`;
- centipede *Cryptops* `BSCOL065`;
- symphylan *Hanseniella* `BSYM095';
- symphylan *Symphylella* `BSYM109`;
- beetle Lathrobiina sp. B01; and
- beetle Coleoptera Genus 2 `BCO230`.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Western Ridge (henceforth referred to as the 'Study Area') is located approximately 11 km west-southwest of Newman, in the Pilbara region of Western Australia, directly south of BHP's Orebody 35 mine site at the Whaleback Hub. The Study Area extends 12 km in an east–west direction and 3.5 km north–south (Figure 1).

Surveys of troglofauna were undertaken at Western Ridge in 2010 and 2019/2020 by Bennelongia Environmental Consultants (Figure 1). The first survey, between April and September 2010, comprised a baseline subterranean fauna survey in the Study Area as part of BHP's Regional Subterranean Fauna Sampling Program (Bennelongia 2011). The second survey, between September 2019 and April 2020, comprised a two-season subterranean fauna survey and habitat assessment (Bennelongia 2021). When the data from the earlier surveys were examined by BHP, it was determined that five troglofauna species had been collected only from proposed mine pits at Western Ridge. These species were:

- centipedes *Cryptops* \`BSCOL064\` and *Cryptops* \`BSCOL065';
- symphylan *Hanseniella* \`BSYM095\`; and
- pseudoscorpions *Indohya* \`BPS294\` and *Tyrannochthonius* \`PSE053\`.

Targeted surveys were undertaken in 2021 and 2022 with the objective of collecting specimens of the above five target species. After completion of survey work in 2022, BHP requested Bennelongia consider an additional four species of interest with the aim of providing further taxonomic resolution and/or clarity on their potential distribution within the Study Area, principally through use of genetics. The species were:

- beetle Coleoptera Genus 2 \`BCO230\` (recorded in the 2021 survey);
- *Phaconeura* sp. (recorded during the 2010 survey);
- dipluran Japygidae sp. (recorded during the 2021 survey); and
- silverfish *Dodecastyla* sp. (recorded during the 2020 survey).

This report provides the results of the surveys in 2021 and 2022, as well as associated genomic analyses undertaken to provide further taxonomic resolution of morphological identifications to potentially show wider ranges of target species.

2. METHODS

The targeted troglofauna fauna surveys were conducted according to the general principles laid out for subterranean fauna sampling by the Environmental Protection Authority (EPA) in *Technical Guidance: subterranean fauna surveys for environmental impact assessment* (EPA 2021) and *Environmental Factor Guideline – subterranean fauna* (EPA 2016a)

2.1. Sampling Effort and Dates

In 2021, 35 drill holes were sampled (Table 1, Figure 1; see Appendix 1 for details of holes). Scraping holes and setting of traps (see section 2.2 for description of techniques) were undertaken from 29-30 March and traps were retrieved on 31 May 2021. This resulted in the collection of 34 scrape samples (at one hole the net snagged at the top of the hole) and 67 trap samples (in WSR1121R neither trap could be retrieved because of minor hole collapse, and in WSR1827R a single trap was set because of a blockage midway as a result of a rock dislodging during scraping). Sampling effort in this situation would usually be quantified as 34.5 samples that mostly consist of one scrape and two trap 'sub-samples'.

In 2022, 77 drill holes were sampled (Table 1, Figure 1, Appendix 1). These drill holes chosen for sampling by BHP because they were considered to be located in similar geology to the holes containing target species. Scraping and setting of traps were undertaken from 24-26 May and traps were retrieved on 9-10 August. Scraping and setting of traps occurred at every hole but both traps were detached from the retrieval cord at WSR0198R and the bottom traps detached during retrieval at WSR2822E and

WSR1983R. Sampling effort in this situation, as defined by EPA (2021), would usually be quantified as 76.5 samples that mostly consisted of one scrape and two trap 'sub-samples'.

No holes from the 2010 survey were available for re-sampling in later surveys but eight of the holes sampled in 2021 had been sampled in the baseline survey of 2019-2020. Thirty-five of the holes sampled in 2022 has been sampled previously (11 in 2019-2020 and 30 in 2021, with six holes sampled in both earlier periods).

Table 1. Sampling effort for troglofauna at Western Ridge in 2021 and 2022.

Year	Holes	Scrapes	Trap 1	Trap 2
2021	35	34	34	33
2022	77	77	76	74

2.2. Troglofauna Field and Laboratory Methods

Troglofauna samples were collected from vertical, uncased drill holes. As outlined above, samples consisted of two separate collecting techniques at each drill hole. Use of the two techniques contributes significantly to obtaining a representative sample of the troglofauna community in drill holes (Halse and Pearson 2014):

1. *Scraping* is a technique used prior to setting traps. It was undertaken using a conical net made of 150 µm mesh, with a diameter closely matched to 60% of the bore diameter. The net funnels into a brass cylinder containing a polycarbonate vial. The brass provides weight to facilitate lowering the net to the bottom of a bore or to the water table. The net was lowered and then scraped back to the surface at least four times. In each of these scrapes a different section of the wall of the hole was targeted (e.g., north, south, etc.) to maximise the number of organisms retrieved. The contents of each scrape were immediately transferred to cold 100% ethanol to preserve specimens in a manner suitable for genetic analysis and were transported to the laboratory for sorting and identification.
2. *Trapping*. Cylindrical traps made of PVC tube (115 x 80 mm) with holes drilled on the side to function as entrances were baited with microwaved leaf litter. Traps were lowered on nylon cord to a few metres above the end of the drill hole, which in this case varied between 9 and 60 m below ground level. If a second trap was set, it was positioned approximately halfway between the surface and the first trap. Traps were left in place for 8 weeks to allow troglofauna enough time to colonise them. Drill holes were sealed at the surface to minimise movement of surface animals into the troglofauna traps. On retrieval, trap contents were transferred to a zip-lock bag and transported (with animals alive) to the laboratory in Perth for sorting and identification.

In the laboratory, troglofauna scrape samples were elutriated to separate animals from sediment and sieved to fractionate the contents according to size (53, 90 and 250 µm) and thereby to improve searching efficiency. All potential troglofauna were separated from other specimens. The contents of trap samples were processed in Berlese funnels under halogen lamps for 72 hours, during which time the light and heat drives animals downwards and towards a vial containing 100% ethanol as a preservative. All potential troglofauna were removed from these vials and set aside. The litter in the top of the funnels was checked quickly to ensure the Tullgren system had functioned correctly and that no invertebrates remained in the litter.

Potential troglofaunal animals were distinguished from surface species by the presence of troglomorphic characteristics (e.g. reduced eyes and pigmentation, well developed sensory organs, slender appendages, vermiform body shape, etc.). They were then identified to species or morphospecies level, with particular emphasis on whether they might belong to one of the target species. Dissection of specimens and examination under binocular and compound microscopes were used as necessary.

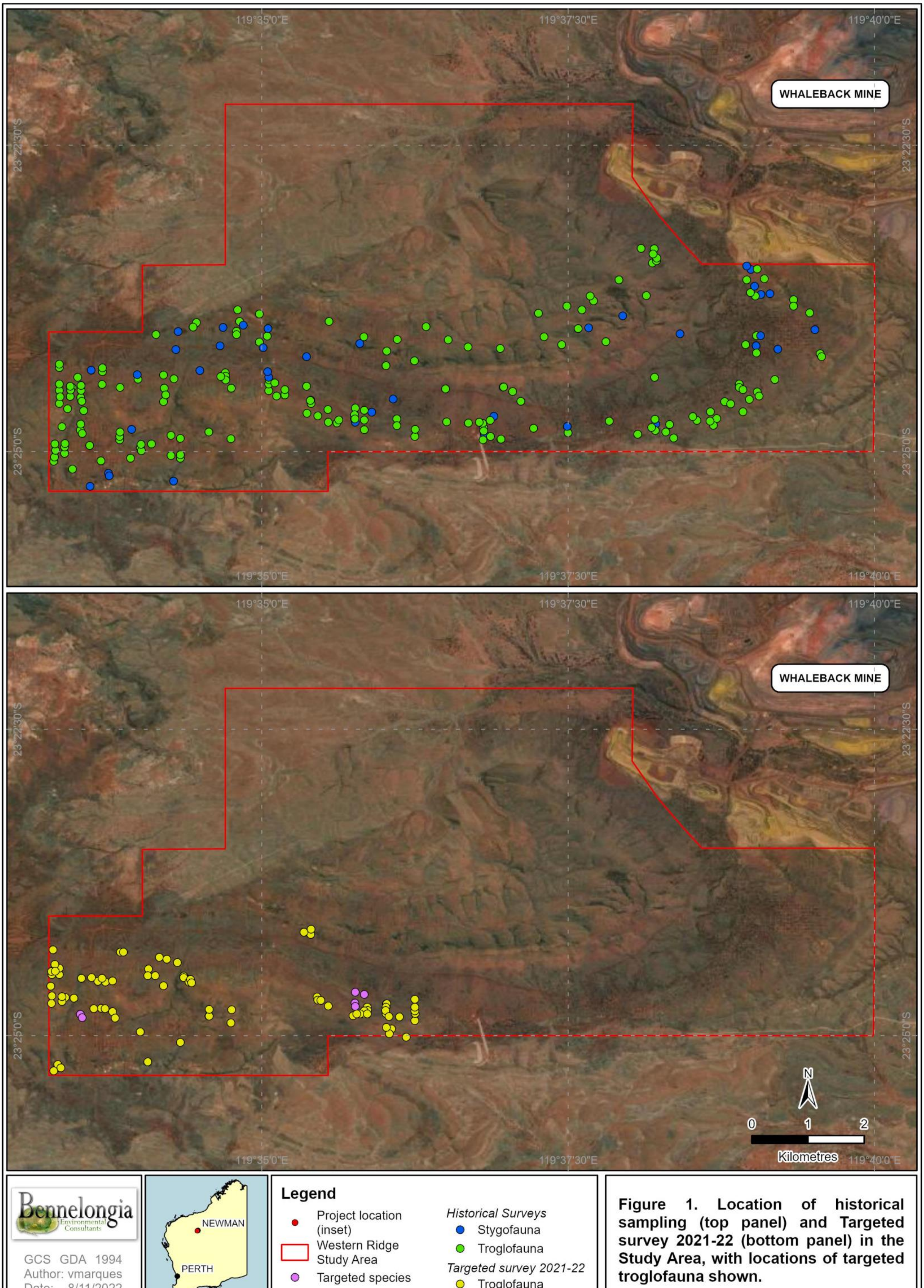


Figure 1. Location of historical sampling (top panel) and Targeted survey 2021-22 (bottom panel) in the Study Area, with locations of targeted troglofauna shown.

2.3. Genomic Analysis

Genomic analysis was undertaken, when material was available and suitable for analysis (i.e. not degraded), to provide or confirm identification of animals that might potentially show wider ranges of target species and the additional species of interest. Genomic analysis involves sequencing key sections of mitochondrial DNA, which mutates at a relatively constant rate, and comparing the number and/or site of mutations between target specimens. Sequences of the CO1 gene are widely used to provide a measure of the relatedness of specimens.

Twelve animals were sequenced using methods described by Bennelongia (2021). Sequencing was done by the Australian Genomic Research Facility. The aim was to compare sequences of two specimens of the target centipede *Cryptops* \`BSCOL064\` with a specimen of the morphologically similar *Cryptops* \`BSCOL091\` collected at Yandicoogina, 81 km north-northwest of Western Ridge. The symphylans *Symphylella* \`BSYM109\` and *Hanseniella* \`BSYM095\` and the hexapods *Dodecastyla* sp. and Japygidae sp. were compared with specimens in the Bennelongia database. Sequences of two specimens of the beetle Coleoptera Genus 2 \`BCO230\` were compared with one specimen each of the morphologically similar beetles Coleoptera sp. B07 and Coleoptera sp. B09 from elsewhere in the central Pilbara. The single specimen of *Eukoenenia* 'BPAL046' collected in 2022 was sequenced for identification.

2.4. Personnel

Fieldwork was conducted by Jim Cocking, Sam Chidgzey, Jaxon Haines and Ella Carstens. Samples were sorted by Heather McLetchie, Jane McRae, Monique Moroney, Jaxon Haines, Vitor Marques, Will Fleming and Melita Pennifold. All morphological identifications were made by Jane McRae. Genomic extraction, sequencing, and alignment were undertaken by Heather McLetchie and Melanie Fulcher. Melanie Fulcher made the phylogenetic trees. Vitor Marques assisted with mapping and data compilation.

2.5. Compiling Species Lists

In some cases, troglofauna specimens could not be identified to species level morphologically. Such specimens were identified to the lowest rank possible, but were counted as additional species only if no other species in that group (e.g. genus or family) had been recorded.

3. RESULTS

3.1. 2021 Survey Results

The targeted troglofauna survey in 2021 collected 227 troglofaunal animals, representing at least 10 species and nine orders (Table 2 and Table 3). These results include representatives of two orders of arachnids, two classes of myriapods, and five orders of hexapods. Note that the species names used throughout the report reflect the final identification of animals after DNA sequencing and morphological review rather than the names used at the time of collection and earlier reporting (Bennelongia 2021)

Troglofauna was collected in 11 of the 35 holes sampled, with between one and four species in the holes that yielded. Seven of the 10 species collected were found in single holes and often there was only one animal collected. In contrast, *Ptinella* sp. B01 (=MC) and *Allopnyxia* sp. B01 were collected from three and four holes, respectively, in large numbers (Table 2).

Five of the species collected during the 2021 survey are known to occur beyond Western Ridge, i.e. they are either widespread in the Pilbara, known from elsewhere in Western Australia, or are cosmopolitan. Four of these species were also recorded in earlier surveys: *Allopnyxia* sp. B01, *Phaconeura* sp. B04, *Dodecastyla* sp. B02 (=Atelurodes sp. S02), and *Lophoturus madecassus*.

Table 2. Troglofauna species and number of individuals collected during the 2021 targeted survey.
Species newly found in the Study Area are highlighted in blue.

Taxonomic placement	Species	No. of specimens	Holes	Distribution	Linear range (km)
Arachnida					
Araneae					
Oonopidae	*Prethopalpus* sp.	1	WSR2930R	Unknown	-
Hexapoda					
Insecta					
Coleoptera					
Ptiliidae	*Ptinella* sp. B01 (=MC)	168	WSR1921R, WSR3658R, WSR1794R	Pilbara widespread	400
Unknown family	Coleoptera Genus 2 `BCO230`	3	WSR1911R	Only record, new species	-
Diptera					
Sciaridae	*Allopnyxia* sp. B01	42	WSR1827R, WSR1893R, WSR3658R, WSR2905R	Pilbara widespread	480
Hemiptera					
Meenoplidae	*Phaconeura* sp. B04	2	WSR1813R	Pilbara widespread	>1000
Zygentoma					
Nicoletiidae	*Dodecastyla* sp. B02 (=Atelurodes sp. S02)	1	WSR1918R	Pilbara widespread	500
Entognatha					
Diplura					
Japygidae	Japygidae sp. `BDP165`	1	WSR3658R	Eastern Pilbara	65
Myriapoda					
Diplopoda					
Polyxenida					
Lophoproctidae	*Lophoturus madecassus*	7	WSR3658R, WSR3664R	Cosmopolitan	N/A

Table 3. Higher level identification collected during the 2021 survey that probably do not represent additional species.

Taxonomic placement	Species	No. of specimens	Holes	Likely Species (and distribution)
Arachnida				
Palpigradi				
Unknown family	Palpigradi sp.	1	WSR1919R	See text in section 3.1
Myriapoda				
Pauropoda				
Tetramerocerata				
Pauropodidae	Pauropodidae sp.	1	WSR1921R	*Decapauropus tenuis* (cosmopolitan) Pauropodidae sp. B09 (11 km linear range)

Three species were recorded in the Study Area for the first time in the 2021 targeted sampling. These were the beetles Coleoptera Genus 2 `BCO230` and *Ptinella* sp. B01 (=MC) and the oonopid spider *Prethopalpus* sp. Coleoptera Genus 2 `BCO230`, which was collected from a trap at 30 mbgl at WSR1911R, represents a new species of beetle. The specimens collected are morphologically similar to animals recorded by Bennelongia at Mount Sheila, Mulga East, and Ministers North in the Pilbara but were considered likely to be distinct at the time of initial identification because of the distance between the Western Ridge record and the other locations (85-220 km).

Ptinella sp. B01 (=MC) is widespread in the Pilbara with a linear range of 400 km. No troglofaunal *Prethopalpus* have been previously collected at Western Ridge, so *Prethopalpus* sp., which was collected in a scrape from WSR2930R, represents an additional species for the Study Area (Table 3). Its distribution is uncertain. Another *Prethopalpus*, also identified only to species but clearly different from the 2021 animal and considered to be a surface species, was collected from a trap in WSR1321R in 2019. Similar species were found in leaf litter during surface sampling ground-dwelling invertebrates in the central Pilbara.

The likely identity of the single Palpigradi sp. collected in 2021 is complex (Table 3). Palpigrads collected in the Study Area in 2010 included males and were identified morphologically) as Palpigradi sp. B07. This species has 3 lateral organs and its affinities were not resolved below order level because in 2010 it was believed the generic diagnosis for the most likely genus, *Eukoenenia*, included possession of 6-8 lateral organs. It subsequently became clear that *Eukoenenia* species can have any number of lateral organs. The palpigrads collected in 2020, which also had 3 lateral organs, were all female or juvenile and could not be identified morphologically but sequencing suggested they are a new species of *Eukoenenia*, called *Eukoenenia* 'BPAL046'. Palpigradi sp. B07 and *Eukoenenia* 'BPAL046' may be the same species, although they have been collected from different parts of the Study Area. Thus, it is possible a single species of palpigrad occurs in the Study Area (notionally *Eukoenenia* 'BPAL046' with an expanded range). If two species occur, based on location Palpigradi sp. most likely belongs to Palpigradi sp. B07. The specimen was too damaged to identify morphologically or attempt sequencing.

3.2. 2022 Survey Results

The targeted troglofauna survey in 2022 collected 102 troglofaunal animals, comprising at least eight species (Table 4 and Table 5). Palpigrads were represented by one species, centipedes by one species of *Cryptops*, millipedes by the widespread *Lophoturus madecassus*, symphylans by one species, and insects by four species (two hemipterans, one beetle and the widespread fly *Allopnyxia* sp. B01).

Troglofauna was collected from 26 of the 77 holes sampled, with between one and five species in the holes that yielded. Four of the eight species collected (including *Eukoenenia* 'BPA046') were found in single holes, while *Lophoturus madecassus*, *Phaconeura* sp. B04 and *Allopnyxia* sp. B01 (probably the three most frequently encountered troglofauna species in the Pilbara), were collected from 12, 7 and 5 holes, respectively.

Two species were collected from the Study Area for the first time in 2022. The new species of symphylan *Symphylella* 'BSYM109' was collected from WSR3658R in a trap at 30 mbgl and was identified genetically. It was too juvenile to compare with *Symphylella* sp. B02 (BHP), which is known from 3 km away but has not been sequenced. *Symphylella* 'BSYM109' is not known from outside the Study Area (though see section 3.4.2). The hemipteran Cixiidae sp. B02 was also collected for the first time in the Study Area but it is widespread across the Pilbara, with many previous records. The 2022 collection of Coleoptera Genus 2 'BCO230', from WSR0303R in a trap at 10 mbgl, is the second time this species has been collected in the Study Area.

The target species, *Cryptops* 'BSCOL064' was collected from two holes by trapping (WSR0305R at 15 mbgl and WSR3130R at 10 mbgl). In 2019, it was collected from WSR1644R at 15 mbgl.

Table 4. Troglofauna species and number of individuals collected during the 2022 targeted survey.
Species newly found in the Study Area are highlighted in blue.

Taxonomy	Lowest ID	No. of specimens	Holes	Distribution	Linear range (km)
Arachnida					
Palpigradi					
Eukoeneniidae	*Eukoenenia* 'BPAL046'	1	WRS1606R	Known only from Study Area	1.6
Myriapoda					
Chilopoda					
Scolopendrida					
Cryptopidae	*Cryptops* `BSCOL064`	2	WSR3130R, WSR0305R	Known only from Study Area	-
Diplopoda					
Polyxenida					
Lophoproctidae	*Lophoturus madecassus*	35	WSR3663R, WSR3272R, WSR3775DG, WSR3772DG, WSR2930R, WSR2429R, WSR1781R, WSR2930R, WSR3214R, WSR3433R, WSR3658R, WSR3272R	Widespread	1,000
Symphyla					
Cephalostigmata					
Scolopendrellidae	*Symphylella* `BSYM109`	1	WSR3658R	Known only from Study Area	-
Hexapoda					
Insecta					
Hemiptera					
Meenoplidae	*Phaconeura* sp. B04	8	WSR0305R, WSR1911R, WSR1893R, WSR2929R, WSR0198R, WSR2956R, WSR1815R	Widespread	>1,000
Ciixidae	Cixiidae sp. B02	4	WSR1911R	Widespread	>1,000
Coleoptera	Coleoptera Genus 2 `BCO230`	1	WSR0303R	Known only from Study Area	-
Diptera					
Sciaridae	*Allopnyxia* sp. B01	49	WSR3187R, WSR2431R, WSR2904R, WSR2922R, WSR3434R	Widespread	700

Table 5. Higher level identification (immature or damaged specimens) collected during in 2022 survey.

Taxonomy	Lowest ID	No. of specimens	Holes	Likely Species (and distribution)
Myriapoda				
Diplopoda				
Polyxenida				
Unknown family	Polyxenida sp.	1	WSR1606R	*Lophoturus madecassus* (widespread)

3.3. Pattern of Specimen Collection

The average number of animals collected per hole in the Study Area varied substantially in different years (Table 6). Sampling efficiency in 2021 was higher than in 2022 but the yield in 2022 compared favourably with those of 2019 and 2020. Sampling results in 2010 were similar to 2021 and 2022.

Capture rates appear to have been lower in the Study Area during the late dry and early wet seasons than in the late wet and early dry seasons but this is not a clear pattern and neither is it a consistent one in the Pilbara. There was little overlap in the holes sampled except in 2021 and 2022 and it is likely that other factors than season, including habitat structure in the immediate vicinity of the hole, had significant influence on the pattern of capture in the Study Area even if rainfall had a role (WABSI 2021).

Table 6. Sampling effort and specimen collection rates at Western Ridge.

Year	Season	Holes sampled	Specimens	Species	Specimens /hole	No. of new species
2010	Apr - Jun	50	85	11	1.7	11
2010	Jul - Sep	47	148	14	3.1	5
2019	Sep - Nov	50	9	3	0.18	1
2020	Jan - Mar	77	63	10	0.82	5
2021	Mar - May	35	227	10	6.5	3
2022	May - Aug	77	102	8	1.4	2

Reflecting the very large numbers of samples required to fully document troglofauna communities, the species list for the Study Area continued to grow during the five rounds of sampling undertaken (Figure 2). The targeted sampling in 2021 and 2022 was deliberated constrained to areas where target species occurred and, therefore, probably increased the species list less than a program aimed at documenting the community of the whole Study Area.

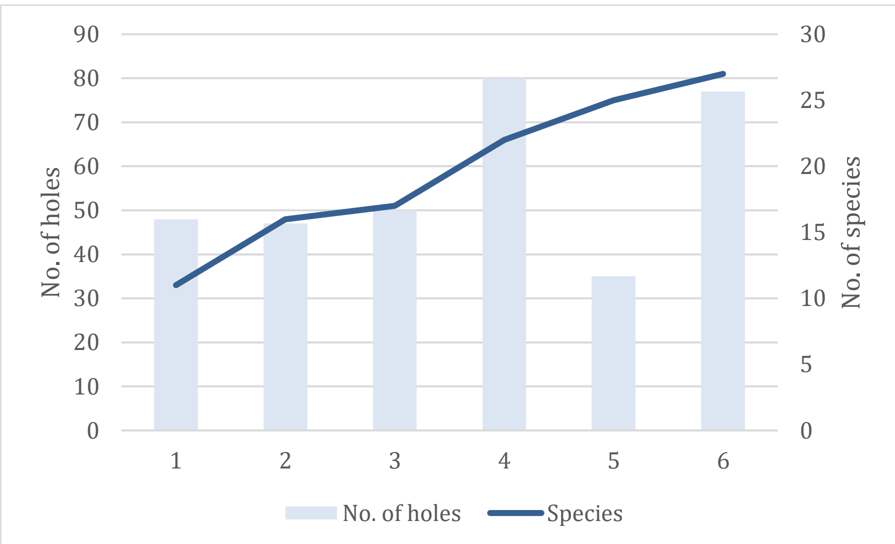


Figure 2. Pattern of species accumulation in relation to rounds of sampling.
Numbers on x-axis reflect order of surveys in Table 6. Higher order identifications not included.

3.4. Genomic Analysis

3.4.1. *Cryptops* Specimens

As outlined in Section 2.3, the *Cryptops* `BSCOL064` specimens were sequenced to examine whether the morphologically similar *Cryptops* `BSCOL091` (collected at Yandicoogina) is the same species. The two specimens of *Cryptops* `BSCOL064` collected in 2022 returned highly similar sequences for the three primers used, diverging from each other by only 0-0.5% and forming a distinct monophyletic clade.

The sequences of *Cryptops* `BSCOL064` were not similar enough to *Cryptops* `BSCOL091` or to any other sequenced species of *Cryptops* to assign them an existing species (Figure 3).

Morphological similarities between the target species *Cryptops* `BSCOL064` and *Cryptops* 'BSCOL078', another referent 15 km north of Orebodies 28, 32 and 33 (Appendix 3), suggested they might be the same species. However, there was divergence of 19.3% in the Mt-CO1 gene, suggesting they are different species (Wesener *et al.* 2016).

Based on the above analysis, *Cryptops* `BSCOL064` is retained as a discrete species in this analysis. Note that *Cryptops* 'BSCOL065' (near the bottom of the tree) is distinct from *Cryptops* `BSCOL064`, as was apparently even more clearly from morphology.

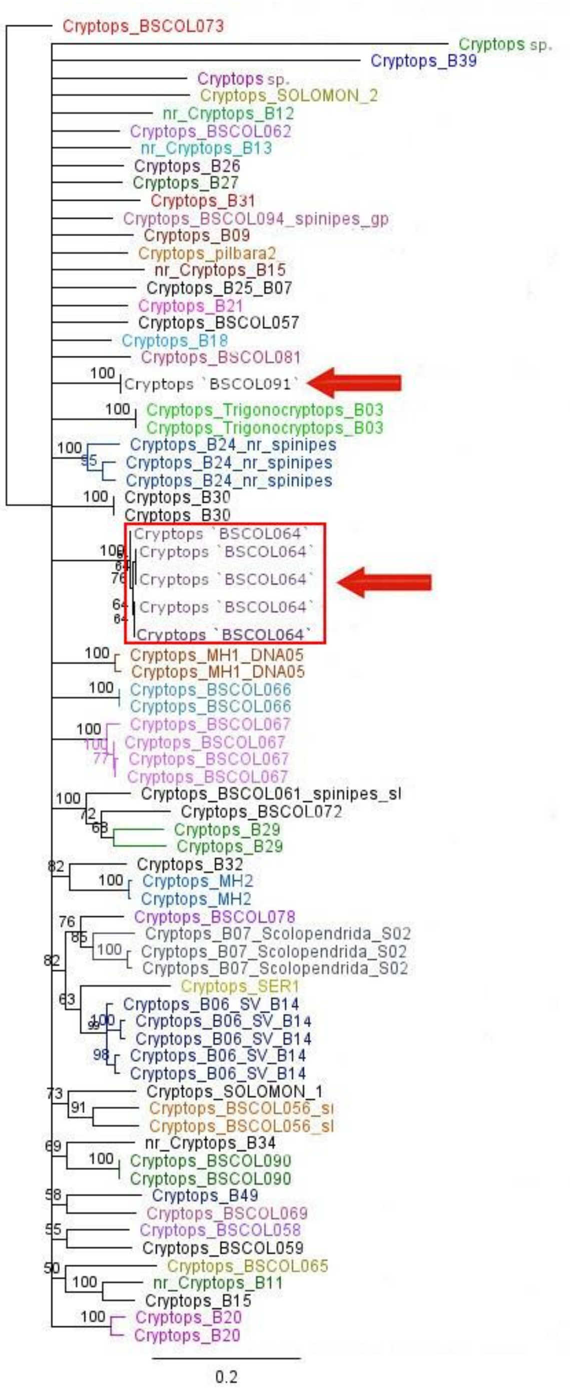


Figure 3. Maximum likelihood tree for *Cryptops*. The target species are flagged with red arrows.

3.4.2. Symphylan Specimens

A BLAST search of Genbank indicated that sequences from the symphylan *Symphylella* `BSYM109` (recorded in the 2022 survey) most closely resembled those of *Symphylella* `EW`, a species occurring 300 km west-northwest of the Study Area. However, the sequences of *Symphylella* `BSYM109` and *Symphylella* `EW` differed by 15.4-15.7%, suggesting they are separate species. In a phylogenetic analysis of related species in the Bennelongia database, *Symphylella* `BSYM109` occurs in polytomy with multiple other undescribed species, the closest of which is *Symphyla* sp. B04 from Boundary Ridge at 15.5% CO1 difference (Figure 5). Previous attempts to sequence *Symphylella* sp. B02 collected at Western Ridge in 2010 (and possibly the same species as *Symphylella* `BSYM109`) failed and all specimens are now >10 years old. The one animal of *Symphylella* `BSYM109` could not be compared morphologically to *Symphylella* sp. B02, because the former was very juvenile.

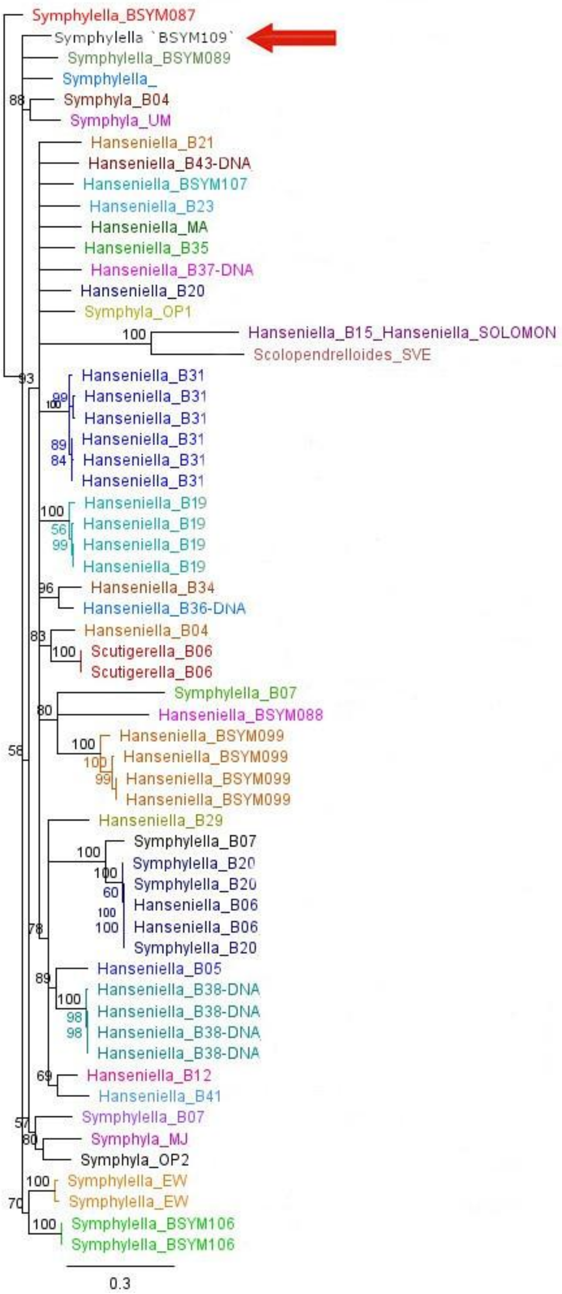


Figure 5. Maximum likelihood tree for *Symphylella* `BSYM109` and related species. Specimens sequenced for this report are flagged with red arrows.

Sequencing of another symphylan in the Study Area, *Hanseniella* `BSYM095` (recorded in the 2020 survey), to see whether it had occurrences elsewhere showed it occurring in a polytomy with multiple other undescribed species (Figure 4). Sequences from *Hanseniella* `BSYM095` most closely matched those from an undescribed species of *Hanseniella* 'Biologic-SYMP003' in Genbank (accession number MT902549), but it differed from *Hanseniella* `BSYM095` by 15.8%, indicating probable species level divergence.

Based on the above analysis, *Hanseniella* `BSYM095` is retained as a discrete species in this analysis.

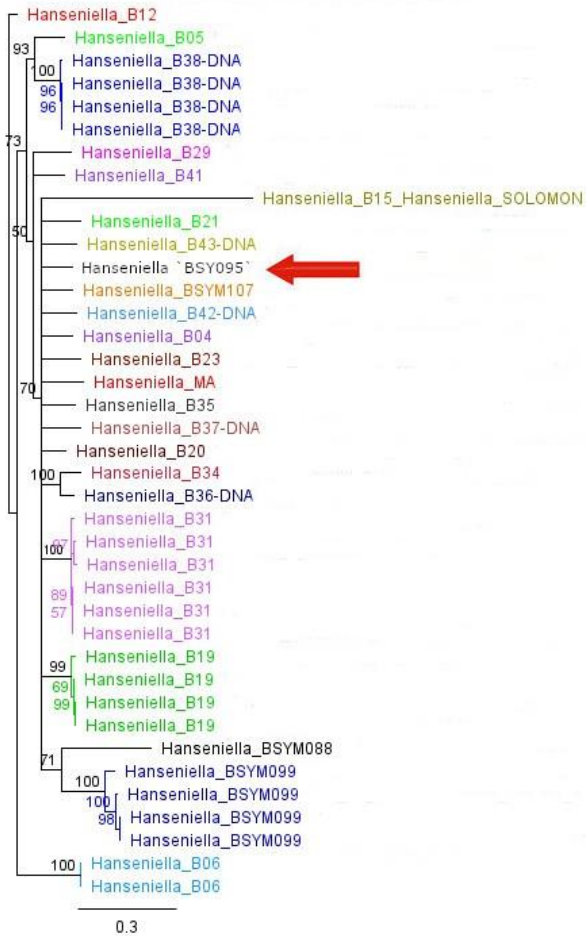


Figure 4. Maximum likelihood tree for *Hanseniella* `BSYM095` and related species. The specimen sequenced for this report is flagged with red arrows.

3.4.3. Hexapod Specimens

The specimen of silverfish *Dodecastyla* sp. collected in 2020 was sequenced to provide species level identification. The specimen differed from *Dodecastyla* sp. B02 specimens collected in the 2021 targeted survey by as little as 4% and is considered to belong to *Dodecastyla* sp. B02, which is a widespread species (Table 7).

Comparisons of the sequences of the dipluran specimen Japygidae sp. collected in 2021 with other available sequences (mostly from the Bennelongia database) found divergences of <8.5% among the Western Ridge animal and specimens assigned to Japygidae \`BDP165\`, a species known from outside the Study Area over a distance of 70 km (Appendix 4).

Sequences of these animals form a well-supported clade and are treated as one species (Appendix 2). The first record of sequenced Japygidae \`BDP165\` was from the Study Area in 2010.

3.4.4. Coleoptera Specimens

Two specimens of the beetle Coleoptera Genus 2 \`BCO230\` (collected in 2021 and 2022) were sequenced and were found to differ by 0.2%. The specimens diverged from the morphologically similar species Coleoptera sp. B07 and Coleoptera sp. B09 by 15-16% (see Figure 6 and Appendix 3). This confirmed the morphologically identified Coleoptera Genus 2 \`BCO230\` is a new species known only from the Study Area but provided no more information than morphology on its higher level taxonomy.

A summary of the genomic analysis is provided in Table 7.

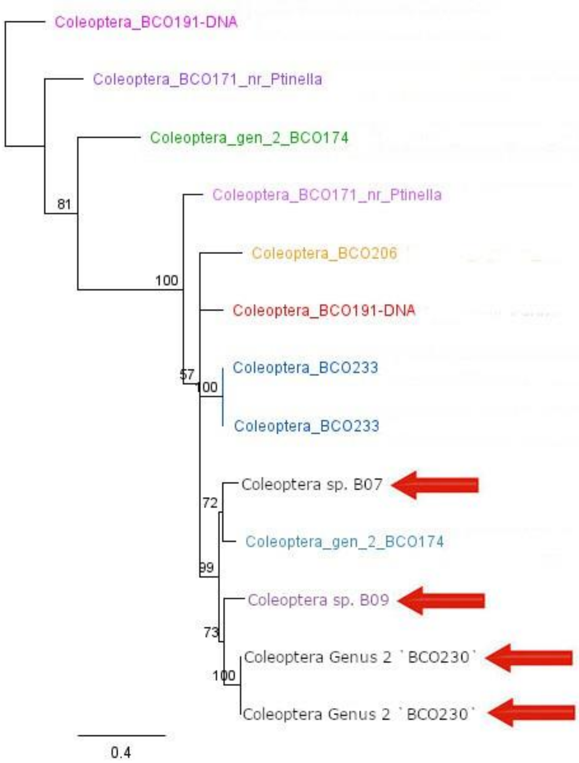


Figure 6. Maximum likelihood tree for Coleoptera Genus 2 \`BCO230\` and related species. Specimens sequenced for this report are flagged with red arrows.

3.4.5. Palpigrad Specimen

Although it could not be identified morphologically, the specimen of palpigrade collected from WRS1606R in 2022 was successfully sequenced and shown by a BLAST search to be only 2.3% different from previously sequenced *Eukoenenia* 'BPAL046' specimens in the western part of the Study Area.

Table 7. Summary of the results of the genomic analyses.

Original identification	Holes	Year Collected	Conclusions
Palpigradi sp.	WRS1606R	2022	Specimen differs by up to 2.5 % from other *Eukoenenia* 'BPAL046' specimens in Study Area, represents *Eukoenenia* 'BPAL046'
Cryptops `BSCOL064`	WSR3130R, WSR0305R	2022	Specimens differ by 0-0.5% from each other; differ substantially from *Cryptops* `BSCOL091`
Symphylella `BSYM109`	WSR3658R	2022	Differs from closest match by 15.5%
Hanseniella `BSYM095`	WSR1917R	2020	Differs from *Hanseniella* sp. Biologic-SYMP003 by 15.8%
Dodecastyla sp.	WSR1974R	2020	<4% divergence from *Dodecastyla* sp. B02, a widespread species Therefore, this record represents *Dodecastyla* sp. B02.
Japygidae sp.	WSR3658R	2021	Sits in clade with <8.5% divergence among specimens. This record represents Japygidae `BDP1651.
Coleoptera Genus 2 `BCO230`	WSR1911R, WSR0303R	2021, 2022	0.2% divergence among specimens; 15-16% divergence from other species, is a new species

4. DISCUSSION

The targeted troglofauna sampling in 2021 yielded 227 animals representing nine orders and at least 10 species, and the survey in 2022 yielded 102 animals representing seven orders and at least eight species. Of the five target species, only one was recorded during the additional targeted sampling. *Cryptops* 'BSCOL064' was collected at two holes in the 2022 survey, bringing the number of records for the species within the Study Area to three.

The surveys in 2021 and 2022 collected five species in the Study Area that were not recorded during previous surveys. These were beetles Coleoptera Genus 2 `BCO230` (known only from the Study Area) and *Ptinella* sp. B01 (=MC) (widespread distribution in the Hamersley Range), the oonopid spider *Prethopalpus* sp. (known only from the Study Area); the symphylan *Symphylella* 'BSYM109' (known only from the Study Area), and the hemipteran Cixiidae sp. B02 (widespread distribution in Pilbara).

DNA sequencing of specimens representing the target species or species of interest provided clarity on identifications. The results of DNA sequencing have not been reported fully; instead information has been provided only when it resolved issues of species identification or distribution, such as confirming that specimens identified morphologically as *Dodecastyla* sp. and Japygidae sp. were *Dodecastyla* sp. B02 and Japygidae `BDP1651, respectively.

Overall, sampling in the Study Area since 2010 has shown that at least 27 species of troglofauna occur at the Study Area (Appendix 3). The community contains cockroaches, pseudoscorpions, and beetles, which are groups that are indicative of a well developed troglofauna community, although species richness is substantially lower than farther west in the Hamersley Range. Eight of the species recorded are widespread, occurring across a substantial portion of the Pilbara or more widely. Seven species have more localised occurrence outside the Study Area, with four of these species having ranges extending well east of Newman and three having occurrences elsewhere in the Newman Hub. Eleven species are known only from the Study Area. Further discussion is provided below of the target species and species known only from Study Area (Figure 7).

***Cryptops* 'BSCOL064' and *Cryptops* `BSCOL065`**

Although *Cryptops* species in the Pilbara have been observed to have variable distributions, work on these centipedes suggests they tend to have small ranges. Bennelongia (2021) reported the estimated median linear range of troglofaunal *Cryptops* in the Pilbara is about 6 km. Additionally, while it is noted that the collection of most specimens as singletons makes ranges uncertain, collection sites in the Pilbara separated by about 5 km appear mostly to support different species. As an example, at Orebodies 28, 32, and 33, *Cryptops* \`BSCOL078\` is replaced by *Cryptops* sp. B43 and *Cryptops* sp. B10 at intervals of about 6 km moving eastward (albeit in different geologies). Within the Study Area, the collection of *Cryptops* \`BSCOL064\` and *C.* \`BSCOL065\` (which are morphologically and genetically distinct species, Figure 3) at single localities 4 km apart may be another example of two species with ranges of only a few kilometres. Together, this suggests that the linear range of the target species *Cryptops* \`BSCOL064\` may only be several kilometres (Figure 7).

***Hanseniella* 'BSYM095' and *Symphylella* 'BSYM109'**

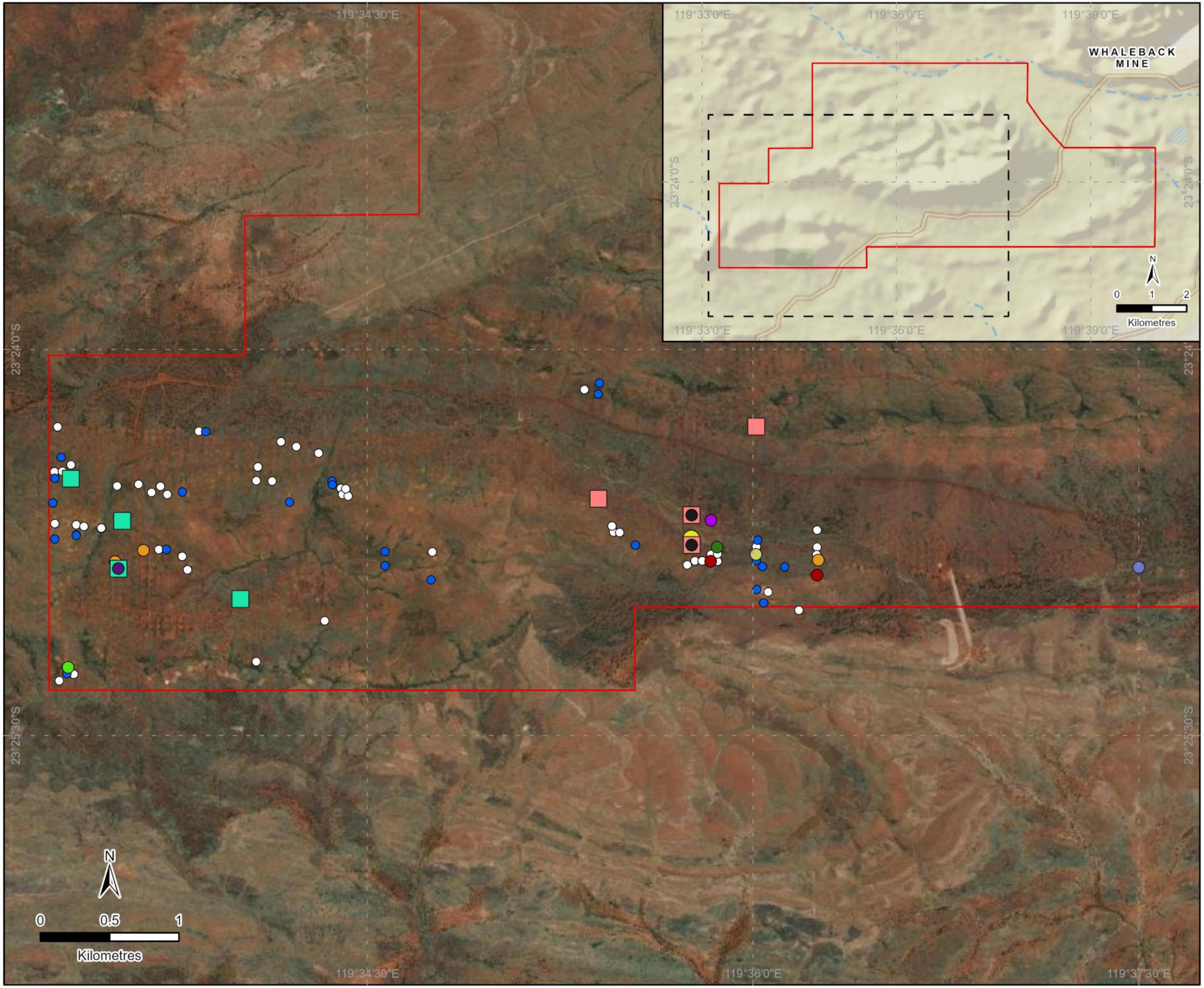
The local range for symphylans in the genus *Hanseniella* is poorly understood and there is currently no information on the life history and habits of the target species *Hanseniella* \`BSYM095\`. However, Halse and Pearson (2014) have found that most troglofaunal symphylans in the Pilbara have a range of only a few kilometres, although the sampling on which this is based was clustered and there may have been bias against assigning distant specimens to the same species. Several *Hanseniella* species have been recorded in baseline surveys commissioned by BHP and these were reviewed to provide additional context regarding the potential range of *Hanseniella* \`BSYM095\`. One species at Orebodies 28, 32, and 33 (*Hanseniella* sp. B19) has an approximate linear range of 6 km, although the range estimate may be constrained as a result of the limited geographic extent of sampling. The three records of *Hanseniella* sp. B06 at Orebody 35, which is 6 km northeast of where *Hanseniella* 'BSYM095' was collected at Western Ridge, extend over a distance of 0.8 km but it has been shown genetically that the species has a fourth occurrence 280 km northwest. *Hanseniella* sp. B06 is likely to be a troglophile (i.e. spending most but not all of its life cycle underground) with surface dispersal and such large ranges are not expected in species that spend their entire life cycle below ground (troglobites). The likely range of *Hanseniella* \`BSYM095\` can only be discussed in terms of probabilities: it most likely has a small range (i.e., <10 km) but may be a widespread symphylan with a range of hundreds of kilometres. An intermediate range is unlikely.

The specimen of the target species *Symphylella* \`BSYM109\` found at Western Ridge was a juvenile, necessitating genomic analysis for identification. It is possibly *Symphylella* 'BPAL046', which has a linear range of 70 km in the eastern Pilbara but this would need morphological confirmation, which was not possible. It is genetically most closely aligned with the Pilbara species *Symphylella* \`EW\`, which has a known linear distribution of approximately 12 km. Some other species of *Symphylella* known from singletons are replaced by other species over distances of <3 km, suggesting the species have very short ranges. Thus, the situation with *Symphylella* 'BSYM109' is similar to *Hanseniella* 'BSYM095': *Symphylella* 'BSYM109' probably has a linear range of a few kilometres but may be widespread.

***Indohya* \`BPS294\` and *Tyrannochthonius* \`PSE053\`**

Indohya 'BPS294' was collected from the Study Area in 2020 from hole WSR1659R (which has not been re-sampled). Troglofaunal species of the pseudoscorpion genus *Indohya* in the Pilbara have been collected from single holes in two-thirds of the surveys where they have been recorded. Where found in multiple holes their linear ranges have varied from 200 m to 22 km (Bennelongia records). Given the relatively large amount of sampling that has failed to re-collect *Indohya* 'BPS294', this species appears to fall into the 'difficult to collect' group of *Indohya* species, either because of low abundance or having a very small range.

Tyrannochthonius 'PSE053' was collected in 2010 from two holes, EXR1659R and EXR1666R that were 210 m apart. The holes have not been sampled since. More than half the troglofaunal species of *Tyrannochthonius* in Western Australia have been recorded from single holes. Most of the species with multiple records have known linear ranges from 0.2 – 5 km, but two species have linear ranges of 18 km and one has a range of 50 km. While the current linear range of *Tyrannochthonius* 'PSE053' is likely to be an underestimate, the true linear range of *Tyrannochthonius* 'PSE053' is likely to be <5 km.



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GCS GDA 1994
Author: vmarques
Date: 21/12/2022

Figure 7. Locations of troglofauna sampling in 2021 and 2022 and species known only from the Survey Area.

Legend

- Project location (inset)
- Western Ridge Study Area

Targeted survey 2021-22

- Trog present
- Trog absent

Species known only from the Survey Area

- Coleoptera Genus 2 `BCO230`
- Cryptops `BSCOL064`
- Cryptops `BSCOL065`
- Eukoenenia `BPAL046`
- Hanseniella `BSYM095`
- Indohya `BPS294`
- Lathrobiina sp. B01
- Palpigradi sp. B07
- Palpigradi sp.
- Prethopalpus sp.
- Symphylella `BSYM109`
- Tyrannochthonius `PSE053`

Coleoptera Genus 2 `BCO230` and Lathrobiina sp. B01

Of the species that seem morphologically closest to the beetle Coleoptera Genus 2 `BCO230`, Coleoptera sp. B07 has a linear range of 25 km and Coleoptera sp. B09 is represented by a single record. These are species, like Coleoptera Genus 2 `BCO230`, for which the family (and associated life history information) are unknown. The analysis by Halse and Pearson (2014) suggests most troglofaunal beetles have linear ranges of <10 km, but some species may be even more restricted (e.g. Baehr 2014a; Baehr 2014b). While so little is known about Coleoptera Genus 2 `BCO230`, a precautionary approach would be to treat its linear range as being only a few kilometres.

Staphylinid beetles are often collected as troglofauna in most parts of the world, although less commonly in the Pilbara. The four specimens of Lathrobiina collected from EXR0992 in 2010 provides the only record of this group of staphylinids in the Pilbara. Halse and Pearson (2014) suggested beetles mostly have linear ranges <10 km and, in the absence of other information, this may be a preliminary guide to the likely range of Lathrobiina sp.

***Prethopalpus* sp.**

A damaged specimen of goblin spider *Prethopalpus* sp. collected from hole WSR2930R during targeted sampling in 2021. It was not considered to be a species of interest and was not sequenced. Several troglofaunal species of *Prethopalpus* occur in the eastern Pilbara (Bennelongia 2021) but it is unlikely that the Study Area specimen belongs to these species. *Prethopalpus* species mostly have small linear ranges, with the three species in the vicinity with multiple occurrences having linear ranges of 1.2 – 5.4 km. Most *Prethopalpus* species are known from single sites or have small ranges, although *Prethopalpus maini* has a known linear range of 18 km (Bennelongia records). *Prethopalpus* sp. is likely to have a linear range of ≤5 km.

***Eukoenenia* 'BPAL046', Palpigradi sp. B07 and Palpigradi sp.**

As discussed in Section 3.1, Palpigradi sp. B07 was collected in 2010 at holes EXR0648R, EXR1446R, EXR1659R and EXR1666R. In 2020, additional palpigrads identified as *Eukoenenia* 'BPAL046' were collected further west in the Study Area at holes WSR1659R, WSR1661R and WSR1749R. During targeted sampling in 2022, another *Eukoenenia* 'BPAL046' was collected at WRS1606R. In 2021, an animal that could be identified only as Palpigradi sp. was collected from WRS1919R. Based on its location, this animal appears likely to belong to Palpigradi sp. B07 (Figure 7). However, it remains unclear whether *Eukoenenia* 'BPAL046' and Palpigradi sp. B07 are separate or the same species. If two species are present, existing data suggest their linear ranges are approximately 2 km. Less than a quarter of the troglofaunal palpigrad species in the Pilbara are represented by multiple records and those that are mostly have linear ranges of approximately 1 km, although Palpigradi sp. B03 has a range of 24 km and Palpigradi sp. B01 occurs throughout the Pilbara.

5. REFERENCES

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APPENDICES

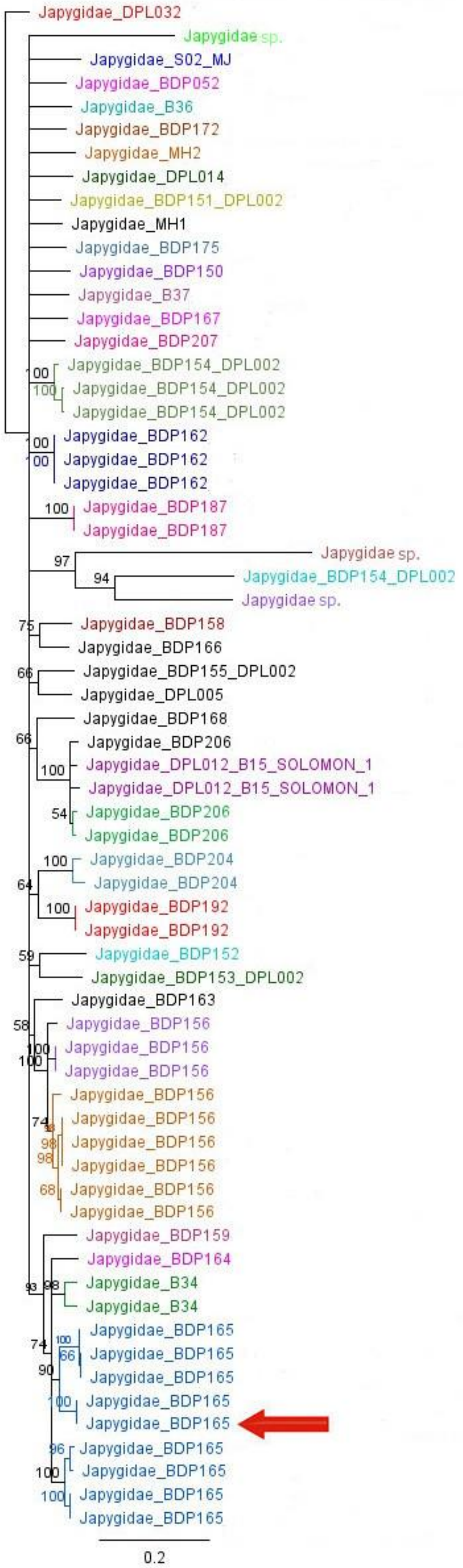
Appendix 1. Drill holes sampled during the 2021 and 2022 surveys

Hole ID	Latitude	Longitude	2021 survey	2022 survey	Depth to water (m)	End of hole (m)
WSR1121R	-23.405004	119.554947	30/03/2021			9
WSR0198R	-23.4122795	119.55474	30/03/2021	26/05/2022		87
WSR0303R	-23.4146188	119.604137	30/03/2021	24/05/2022		96
WSR0305R	-23.41363	119.60422		24/05/2022		71
WSR0307R	-23.41279	119.60416		24/05/2022		21
WSR0309R	-23.4117	119.60416		25/05/2022		33
WSR1606R	-23.4083728	119.555793	30/03/2021	26/05/2022		45
WSR1608R	-23.40749	119.55581		26/05/2022		54
WSR1618R	-23.42103	119.55598		25/05/2022		69
WSR1657R	-23.40885	119.5588		26/05/2022		44
WSR1663R	-23.40875	119.56017		26/05/2022		39
WSR1694R	-23.40887	119.56159		26/05/2022		39
WSR1741R	-23.40531	119.56408		26/05/2022		67
WSR1749R	-23.4161617	119.566785	30/03/2021			28
WSR1781R	-23.40988	119.56995		25/05/2022		37
WSR1799R	-23.41757	119.57223		25/05/2022		37
WSR1807R	-23.40901	119.5733		26/05/2022		32
WSR1813R	-23.4140143	119.576146	30/03/2021	25/05/2022		41
WSR1815R	-23.4130844	119.576155	30/03/2021	25/05/2022		39
WSR1827R	-23.4149156	119.57913	30/03/2021	25/05/2022		51
WSR1835R	-23.4131231	119.579217	30/03/2021	25/05/2022		39
WSR1883R	-23.4118158	119.590961	30/03/2021	25/05/2022		45
WSR1884R	-23.41143	119.59085		26/05/2022		21
WSR1893R	-23.4126577	119.592378	30/03/2021	25/05/2022		33
WSR1911R	-23.4137109	119.597247	29/03/2021	25/05/2022		45
WSR1912R	-23.4132752	119.597271	29/03/2021	25/05/2022		63
WSR1918R	-23.4136785	119.600218	29/03/2021	25/05/2022		81
WSR1919R	-23.4132718	119.600192	29/03/2021	25/05/2022		20
WSR1920R	-23.4127791	119.600211	29/03/2021	25/05/2022		38
WSR1921R	-23.4123503	119.600295	29/03/2021	25/05/2022		28
WSR2426R	-23.4026	119.58907		25/05/2022		49
WSR2429R	-23.40219	119.59005		25/05/2022		33
WSR2431R	-23.4028778	119.589964	30/03/2021	25/05/2022		11
WSR2822RE	-23.4132969	119.60412	30/03/2021	24/05/2022		63
WSR2903R	-23.41128	119.55475		26/05/2022		37
WSR2904R	-23.40995	119.55463		26/05/2022		32
WSR2905R	-23.408338	119.554764	29/03/2021			32
WSR2906R	-23.40793	119.55472		26/05/2022		33
WSR2909R	-23.42146	119.55503		25/05/2022		43
WSR2920R	-23.4079	119.55525		26/05/2022		37
WSR2922R	-23.40699	119.55517		26/05/2022		33
WSR2929R	-23.4210199	119.555538	29/03/2021	25/05/2022		81
WSR2930R	-23.4206002	119.555606	29/03/2021	25/05/2022		75
WSR2956R	-23.4120276	119.556133	30/03/2021	26/05/2022		39

Hole ID	Latitude	Longitude	2021 survey	2022 survey	Depth to water (m)	End of hole (m)
WSR2957R	-23.41137	119.55614		26/05/2022		33
WSR2978R	-23.41147	119.55663		26/05/2022		6
WSR3006R	-23.4115838	119.557767	30/03/2021	26/05/2022		27
WSR3130R	-23.413	119.5605		26/05/2022		44
WSR3157R	-23.4092687	119.561027	29/03/2021	26/05/2022		39
WSR3168R	-23.4129522	119.561486	29/03/2021	25/05/2022		33
WSR3187R	-23.412972	119.561989	29/03/2021	25/05/2022		21
WSR3189R	-23.4093897	119.562038	30/03/2021	26/05/2022		33
WSR3213R	-23.4134	119.56301		26/05/2022		18
WSR3214R	-23.40924	119.56302		26/05/2022		27
WSR3235R	-23.41427	119.56336		26/05/2022	92	
WSR3272R	-23.40532	119.56452		26/05/2022		61
WSR3339R	-23.42022	119.56781		25/05/2022		6
WSR3350R	-23.40851	119.56781		26/05/2022		23
WSR3352R	-23.40761	119.56791		26/05/2022		33
WSR3363R	-23.40852	119.56884		26/05/2022		31
WSR3384	-23.40599	119.56941		26/05/2022		54
WSR3402R	-23.40629	119.5704		26/05/2022		46
WSR3428R	-23.40673	119.57184		26/05/2022		33
WSR3433R	-23.40876	119.57273		26/05/2022		42
WSR3434R	-23.40854	119.57271		26/05/2022		19
WSR3438R	-23.4094	119.5734		26/05/2022		21
WSR3439R	-23.4095	119.57377		26/05/2022		29
WSR3440R	-23.40902	119.57361		26/05/2022		30
WSR3599R	-23.4118667	119.591349	30/03/2021	25/05/2022		39
WSR3640R	-23.413947	119.595727	29/03/2021			63
WSR3641R	-23.4136905	119.59624	29/03/2021	25/05/2022		39
WSR3648R	-23.4136916	119.59671	29/03/2021	25/05/2022		39
WSR3656R	-23.41371	119.59769		25/05/2022		29
WSR3657R	-23.4132554	119.597703	29/03/2021	25/05/2022		45
WSR3658R	-23.4128038	119.597683	29/03/2021	25/05/2022		33
WSR3663R	-23.41407	119.60062		25/05/2022		43
WSR3664R	-23.4140889	119.602064		30/03/2021		51
WSR3687R	-23.41688	119.60298		24/05/2022	34	
WSR3772DG	-23.41556	119.60025		24/05/2022		5
WSR3773DG	-23.41572	119.60099		24/05/2022		19
WSR3775DG	-23.41642	119.6007		24/05/2022		18

Appendix 2. Additional genomic results

Analysis of sequences of species of Japygidae



Appendix 3. Troglofauna species in the Study Area

From sampling in 2010, 2019/2020, 2021, and 2022. Taxa marked with * do not add to species total.

Classes, Subclasses, Orders, and Species	No. of animals	No. of records	Distribution
Arachnida			
Chelicerata			
Araneae			
Prethopalpus sp.	1	1	Study Area
Palpigradi			
Eukoenenia `BPAL046`	4	4	Study Area
Palpigradi sp.*	1	1	-
Palpigradi sp. B07	9	4	Study Area
Pseudoscorpiones			
Indohya `BPS294`	1	1	Study Area
Lechytia `PSE019`	1	1	Eastern Pilbara
Tyrannochthonius `PSE053`	2	2	Study Area
Myriapoda			
Chilopoda			
Cryptops `BSCOL064`	3	3	Study Area
Cryptops `BSCOL065`	1	1	Study Area
Chilopoda sp.*	1	1	
Diplopoda			
Lophoturus madecassus	163	30	Cosmopolitan
Polyxenida sp.*	1	1	
Pauropoda			
Decapauropus tenuis	13	2	Cosmopolitan
Pauropodidae sp. B09	2	1	Newman area
Pauropodidae sp. *	1	1	
Symphyla			
Hanseniella `BSYM095`	1	1	Study Area
Symphylella `BSYM109`	1	1	Study Area
Symphylella sp. B02 (BHP)	1	1	Eastern Pilbara
Crustacea			
Isopoda			
Troglarmadillo `ISO005`	3	2	Newman area
Hexapoda			
Entognatha			
Japygidae `BDP165`	2	2	Eastern Pilbara
Japygidae `DPL002` s.l.	4	2	Hamersley Range
Parajapygidae sp.	1	1	-
Insecta			
Trinemura sp. B04	1	1	Eastern Pilbara
Dodecastyla sp. B02 (=*Atelurodes* sp. S02)	83	4	Pilbara
Nocticola `BBL040` (*quartermaini* s.l.)	6	2	Newman area
Phaconeura sp. B04	11	6	Pilbara
Phaconeura sp.*	18	10	-
Cixiidae sp. B02	4	1	Pilbara
Ptinella sp. B01 (=MC)	168	3	Hamersley Range
Lathrobiina sp. B01	4	1	Study Area
Coleoptera Genus 2 `BCO230`	4	2	Study Area
Allopnyxia sp. B01	117	13	Pilbara

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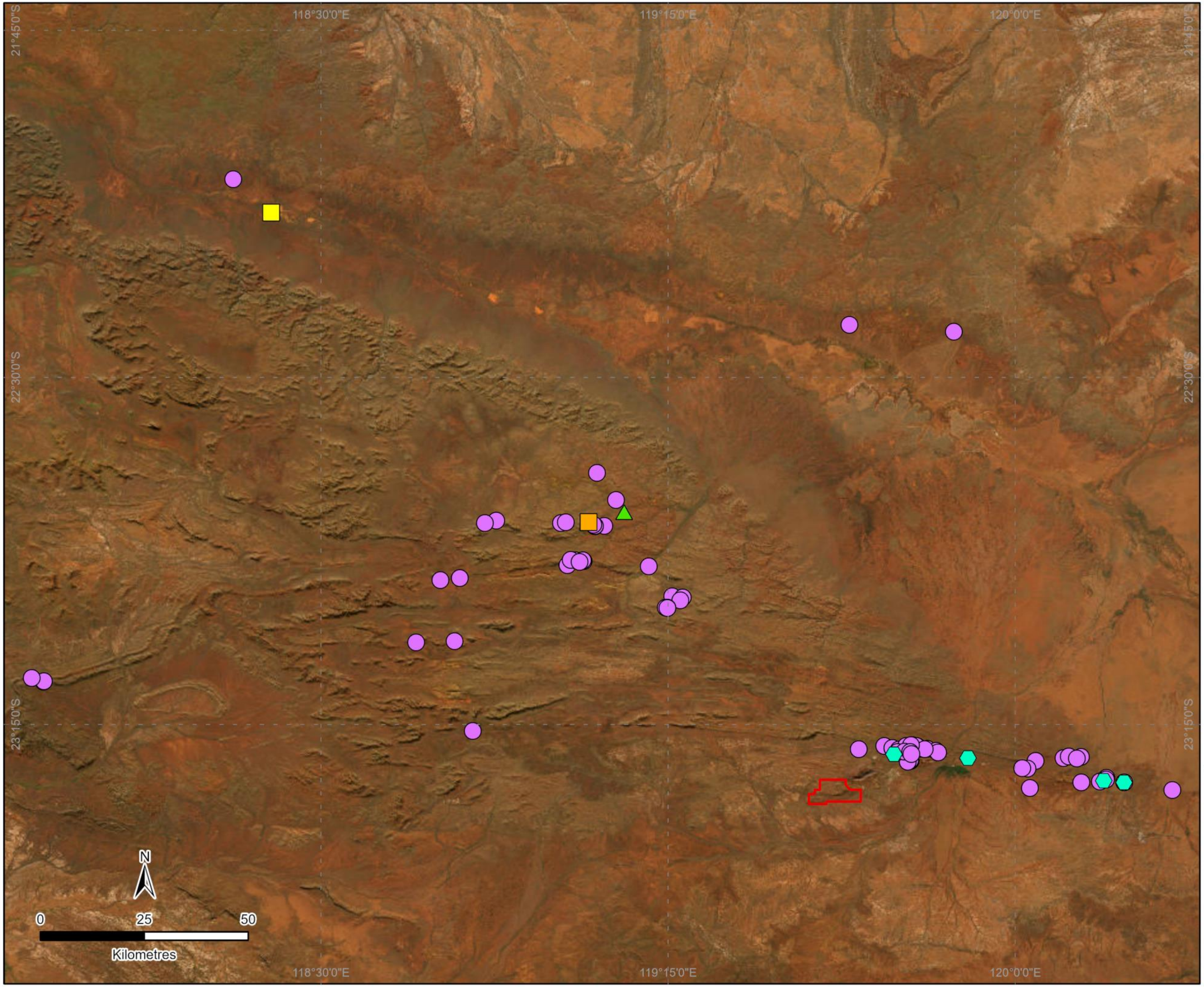
GCS GDA 1994
Author: mfulcher
Date: 22/11/2022

Appendix 4. Location of reference species in relation to the Western Ridge Study Area.



Legend

- Project location (inset)
- Western Ridge Study Area

Reference species

- Coleoptera sp. B07
- Coleoptera sp. B09
- Cryptops `BSCOL091`
- Dodecastyla sp. B02 (=Atelurodes sp. S02)
- Japygidae `BDP165`