Bennelongia Environmental Consultants

East Jimblebar Targeted Troglofauna Survey Report

Prepared for:

BHP

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Final Report

Short-Range Endemics I Subterranean Fauna

Waterbirds I Wetlands

East Jimblebar Targeted Troglofauna Survey Report

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EXECUTIVE SUMMARY

BHP Western Australian Iron Ore (WAIO) are conducting environmental studies at the East Jimblebar area, located 50 km east of the town of Newman and immediately east of current mining operations at the BHP WAIO Jimblebar Hub. One such study included a baseline subterranean fauna survey conducted by Bennelongia Environmental Consultants (Bennelongia 2023) which resulted in the identification of five target troglofauna species of interest, including the:

- pseudoscorpion *Tyrannochthonius* 'PSE057',
- isopod nr *Andricophiloscia* 'BIS509',
- centipede *Cryptops* 'BSCOL066',
- symphylan *Scutigerella* 'BSYM113', and
- dipluran Japygidae 'BDP192'.

Bennelongia was subsequently commissioned by BHP to conducted a **targeted troglofauna survey** aiming to maximise the collection of these target species. In addition, Bennelongia also designed a **broadscale genetic sequencing program** of specimens both from within the East Jimblebar area and the surrounding landscape to increase the chances of extending the known distribution of these species based on previous specimen collections.

The targeted troglofauna survey resulted in the collection of 60 specimens of troglofauna, including two partial specimens of diplurans that were identified morphologically as possibly belonging to Japygidae 'BDP192`. These specimens were submitted for DNA sequencing but either were not a match (first sample) or could not successfully be sequenced (second sample). The status of Japygidae 'BDP192' thus remains unchanged.

The broadscale genetic sequencing programme was conducted using 40 individuals, of which 25 produced successful sequences. This resulted in a genetic match for the priority species *Scutigerella* 'BSYM113' and a reappraisal of its distribution. *Scutigerella* 'BSYM113' is now known from four bores and has a known linear distance of 11 km, extending the known range of this species significantly. All collection locations intersect colluvium or alluvium at the surface and intersect either bedrock Brockman Iron Formation or bedrock Marra Mamba Iron Formation which is supported by drill log data. DNA sequencing also resulted in additional records and expanded distributions for ten species previously identified from the Jimblebar area; including the amalgamation of two previous morphospecies of Symphyla into a single genetic species *Hanseniella* sp. B35.

No matches for the target species *Tyrannochthonius* 'PSE057', nr *Andricophiloscia* 'BIS509' and *Cryptops* 'BSCOL066' could be established through either the targeted survey or the extended genetic sequencing and analysis. Their exact distribution ranges remain unknown.

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1. INTRODUCTION

BHP Western Australian Iron Ore (WAIO) are exploring the option to expand operations at the East Jimblebar area (the Study Area) in the Pilbara bioregion. The Study Area encompasses the Hashimoto and East Jimblebar iron ore deposits, located 50 km east of the town of Newman and immediately east of current mining operations at the BHP WAIO Jimblebar Hub (Figure 1). To facilitate any future developments, environmental studies are being conducted and this includes detailed environmental assessments for Subterranean Fauna.

BHP WAIO engaged Bennelongia Environmental Consultants (Bennelongia) to conduct subterranean fauna surveys as a part of the environmental investigations at the Study Area. Subterranean fauna are considered to be a key Environmental Factor by the Environmental Protection Authority (EPA) of Western Australia (WA), due to their sensitivity to human-induced disturbance and importance in maintaining the natural biodiversity of WA (EPA 2023). Studies of subterranean fauna have been conducted at East Jimblebar since 2008-2009 (Bennelongia 2009; Ecowise 2009), and more recently in 2020-2022 (Bennelongia 2023). This report is the continuation of the most recent round of surveys from 2020-2022, specifically targeting troglofauna that currently have known distributions restricted to the Study Area (Bennelongia 2023).

1.1. Troglofauna

Troglofauna are the terrestrial (air-breathing) component of the subterranean fauna with the other being the aquatic stygofauna. Evolution in underground habitats has given species in these communities many convergent morphological and physiological characteristics such as reduced or absent eyes, reduced or absent body pigmentation, vermiform morphologies, elongate sensory structures, loss of wings in otherwise flying taxa, increased lifespans, a shift towards a K-selected breeding strategy resulting in low numbers of offspring, and decreased rates of metabolism (Gibert and Deharveng 2002). The overwhelming majority of troglofauna species in Western Australia are invertebrates, apart from a single snake species from Barrow Island (Aplin 1998). Troglofauna species belong to a wide variety of invertebrate groups such as arachnids, crustaceans, snails and insects. The most commonly collected groups are the isopods, palpigrades, spiders, schizomids, pseudoscorpions, harvestmen, millipedes, centipedes, pauropods, symphylans, bristletails, silverfish, cockroaches, true bugs, beetles, and fungus-gnats (Halse 2018b).

Although inconspicuous, subterranean fauna contribute markedly to the overall biodiversity of Australia. Most subterranean species satisfy Harvey's (2002) criterion for short-range endemism (SRE), having total geographic ranges of less than 10,000 km^2 and occupying patchy or discontinuous habitats within those ranges. Given that species with small ranges are more vulnerable to extinction following habitat degradation than widely distributed species (Ponder and Colgan 2002), it follows that subterranean taxa are highly susceptible to anthropogenic threats, particularly large-scale excavation such as open pit mining and groundwater abstraction.

Troglofauna can be divided into three sub-categories. These are troglobites that spend their entire life in subterranean habitats, troglophiles that move to the surface during one or more life stages (or have surface populations), and trogloxenes that use subterranean spaces only opportunistically (e.g. for hibernation and reproduction). Trogloxenes tend to have wider distribution ranges than obligate subterranean species, and troglophiles usually have larger distributions than troglobites.

Our understanding of the subterranean fauna in the Pilbara bioregion has progressed immensely since the late 1990s (Humphreys 1999; Eberhard *et al.* 2005), in large part due to extensive sampling as part of environmental impact assessments. At least 1,500 species of troglofauna are now thought to inhabit the Pilbara (Halse 2018b), although reliable estimates are hindered by poor taxonomic frameworks for some of the animal groups. It is also well established now that the diversity of subterranean fauna (troglofauna) is closely linked to local geology, because these animals can only colonise areas with

appropriate subterranean habitat and as a result, individual species or species communities are usually restricted to specific geological features. Geologies supporting rich troglofauna communities include mineralised or weathered iron formations, calcrete, alluvium, and sometimes mafic volcanic rocks (Halse 2018a). As a result of their dependence on the distribution of underground spaces, the composition and richness of troglofauna communities often vary significantly over short distances. Therefore, to achieve reliable estimates of the diversity and composition of the troglofauna of an area, knowledge of local geology needs to be coupled with detailed biological surveys that establish presence of such fauna and the distribution of the species recorded.

1.2. Conservation Framework

Native flora and fauna in Western Australia are protected at both State and Commonwealth levels. At the national level, the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) provides a legal framework to protect and manage nationally and internationally important flora, fauna and ecological communities. However, the threatened fauna lists of the EPBC Act currently place little emphasis on subterranean fauna. The legal framework for protection of flora and fauna at the state level in Western Australia is the *Biodiversity Conservation Act 2016* (BC Act). Most protection is provided for species listed under the BC Act as 'threatened' and this list includes some subterranean species. In addition to the list of threatened species under the BC Act, the Department of Biodiversity, Conservation and Attractions (DBCA) in Western Australia maintains a list of priority species that are of conservation importance but, for various reasons, do not meet the criteria for listing as threatened.

Both the EPBC and BC Acts provide frameworks for the protection of Threatened Ecological Communities (TECs). The DBCA also informally recognises communities of potential conservation concern, defined as Priority Ecological Communities (PECs), if there is not enough information to support listing them as TECs. The list of TECs and PECs recognised under the BC Act and by DBCA is more extensive than the EPBC Act TEC list and has much greater focus on subterranean communities.

1.3. Project Background

This report presents the results of the 2023 targeted troglofauna survey and subsequent round of genetic sequencing for the East Jimblebar area, commissioned by BHP WAIO. The geology of the region is briefly described in this report and additional details are found in Bennelongia (2023). For context, the results from previous sampling rounds are also summarised here but further results of the desktop analyses can be found in Bennelongia (2023).

Previous environmental assessments in the area were conducted by Bennelongia in 2008/09 and include a troglofauna survey of the Jimblebar Iron Ore Project (which included sampling of Hashimoto, South Jimblebar, Wheelara Hill, Jimblebar East, Caramulla, Jimblebar West and Mesa Gap areas). The Bennelongia (2008/09) survey included 301.5 troglofauna samples and resulted in the collection of 506 specimens of troglofauna that represented 29 species (Bennelongia 2009).

In 2020, Bennelongia conducted a further round of subterranean fauna surveys, including for troglofauna, which was extended into 2022 after the release of the most recent subterranean fauna technical guidance statement in 2021. The latter requires three rounds of survey "in areas where there is suitable habitat and a reasonable expectation of encountering troglofauna" (EPA 2021). This survey program resulted in the collection of 197 troglofauna samples. Genetic sequencing was conducted on 50 animals including 39 specimens from the Study Area (the remaining animals were from the surrounding landscape), in an attempt to identify specimens in a nondiagnostic life stage, and to enable comparison of Study Area specimens with those from other tenements. The subsequent report (Bennelongia 2023) collated all known data for troglofauna in the Study Area into a single dataset (i.e. sampling from 2008-2022) and compiled a list of troglofauna (and stygofauna) that are currently only known from within the boundary of the defined Study Area. The list of restricted troglofauna included

15 species of pseudoscorpions, isopods, centipedes, pauropods, symphylans, and a dipluran (Bennelongia 2023).

BHP WAIO subsequently identified five target species of interest for further assessment and commissioned Bennelongia to conduct a single targeted round of troglofauna surveys. Two of these species were also classified as priority species by BHP WAIO and these are the pseudoscorpion *Tyrannochthonius* 'PSE057' and symphylan *Scutigerella* 'BSYM113'. Details of the five species can be found in Table 1 and Figure 2. The present report compiles the results from the latest targeted troglofauna field survey.

Table 1: Troglofauna targeted during the 2023 survey.

Fauna Group	Species	Priority Species	Known collection Location(s)	Known Linear distribution
Pseudoscorpion	*Tyrannochthonius* 'PSE057'	Yes	PI012, EJR0014, EJ0791R and EJ0833R	2.75 km
Isopod	nr *Andricophiloscia* 'BIS509'		HH3076R	Singleton
Centipede	*Cryptops* 'BSCOL066'		EJ1211R	Singleton
Symphylan	*Scutigerella* 'BSYM113'	Yes	EJ0798R	Singleton
Dipluran	*Japygidae* 'BDP192'		PI031	Singleton

***Tyrannochthonius* 'PSE057' – priority species**

Tyrannochthonius `PSE057` is presently known from four sites in the Study Area (Figure 2). It was collected twice in 2008, a single specimen via troglofauna scraping at drill hole PI012 (Bennelongia 2009) and two specimens were collected as by-catch from a stygofauna net haul at site EJR0014 (Ecowise 2009). Bennelongia collected two additional individuals as by catch during stygofauna sampling at sites EJ0833R and EJ0791R in July 2020.

nr *Andricophiloscia* 'BIS509'

nr *Andricophiloscia* 'BIS509' is known from a single specimen collected from within the Study Area (Figure 2). This specimen is currently only known only from drill hole HH3076R and was collected in 2022 as by-catch in a stygofauna net haul. Depth to water at this location was 68 m below ground level (bgl) at the time of sampling.

***Cryptops* 'BSCOL066'**

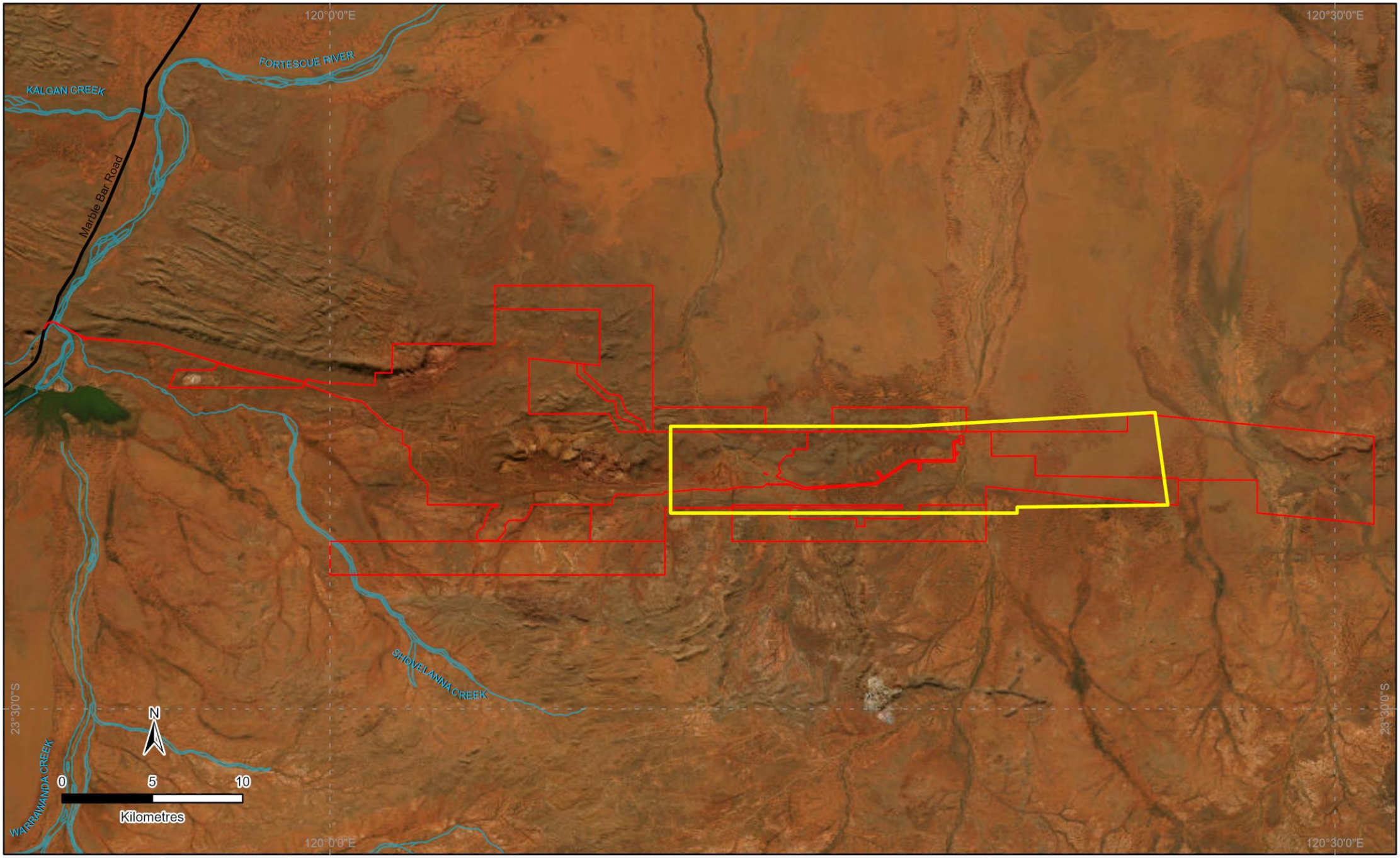
A single specimen of *Cryptops* `BSCOL066` was collected from drill hole EJ1211R in 2020 (Figure 2). This specimen was collected in a trap targeting troglofauna. The trap was set at a depth of 23 m bgl, providing a known collection depth for this species, and depth to water at this location at the time of sampling was 53 m bgl.

***Scutigerella* 'BSYM113' – priority species**

A single specimen of *Scutigerella* 'BSYM113' was collected from drill hole EJ0798R in 2022 using troglofauna scrape sampling methods (Figure 2). This hole had a depth to water of 65 m bgl.

Japygidae 'BDP192'

The dipluran Japygidae 'BDP192' was collected as a singleton in June 2020. It was collected as by-catch from a stygofauna net haul at bore hole PI031 (Figure 2). Depth to water at this site at the time of collection was 68 m bgl.



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Legend

- BHP Tenements
- Study Area
- Major roads
- Major drainage lines
- The Project (inset map)

Figure 1. Location of the Project

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Legend

- Study Area
- The Project (inset map)

Target Species

- Cryptops `BSCOL066`
- Japygidae `BDP192`
- Scutigerella `BSYM113`
- Tyrannochthonius `PSE057`
- nr Andricophiloscia `BIS509`

Figure 2. Distribution of Target Species at the Study Area

2. ENVIRONMENT

2.1. Geology

Two major iron bearing ridgelines run in an east west direction through the Study Area (Figure 3). One ridgeline runs along the southern border and contains the Jeerinah Formation of the Fortescue group and the Marra Mamba Iron Formation of the Hamersley Group. These two units outcrop throughout this ridgeline and can be up to 600 m and 110 m thick respectively (Williams and Tyler 1991). The second ridgeline runs through the northern third of the Study Area and similarly contains two sets of outcropping iron-rich geologies. These are the Brockman Iron Formation and the Weeli Wolli Formation (Figure 3). The Brockman Iron formation can be up the 450 m thick, while the Weeli Wolli Formation is known in places to be as thick as 420 m (Williams and Tyler 1991). The Jeerinah Formation is often absent of weathering, however, the three iron formations of these two ridgelines are known to be well weathered and therefore, prospective for troglofauna. It is important in the context of troglofauna that these ridgelines are not seemingly connected.

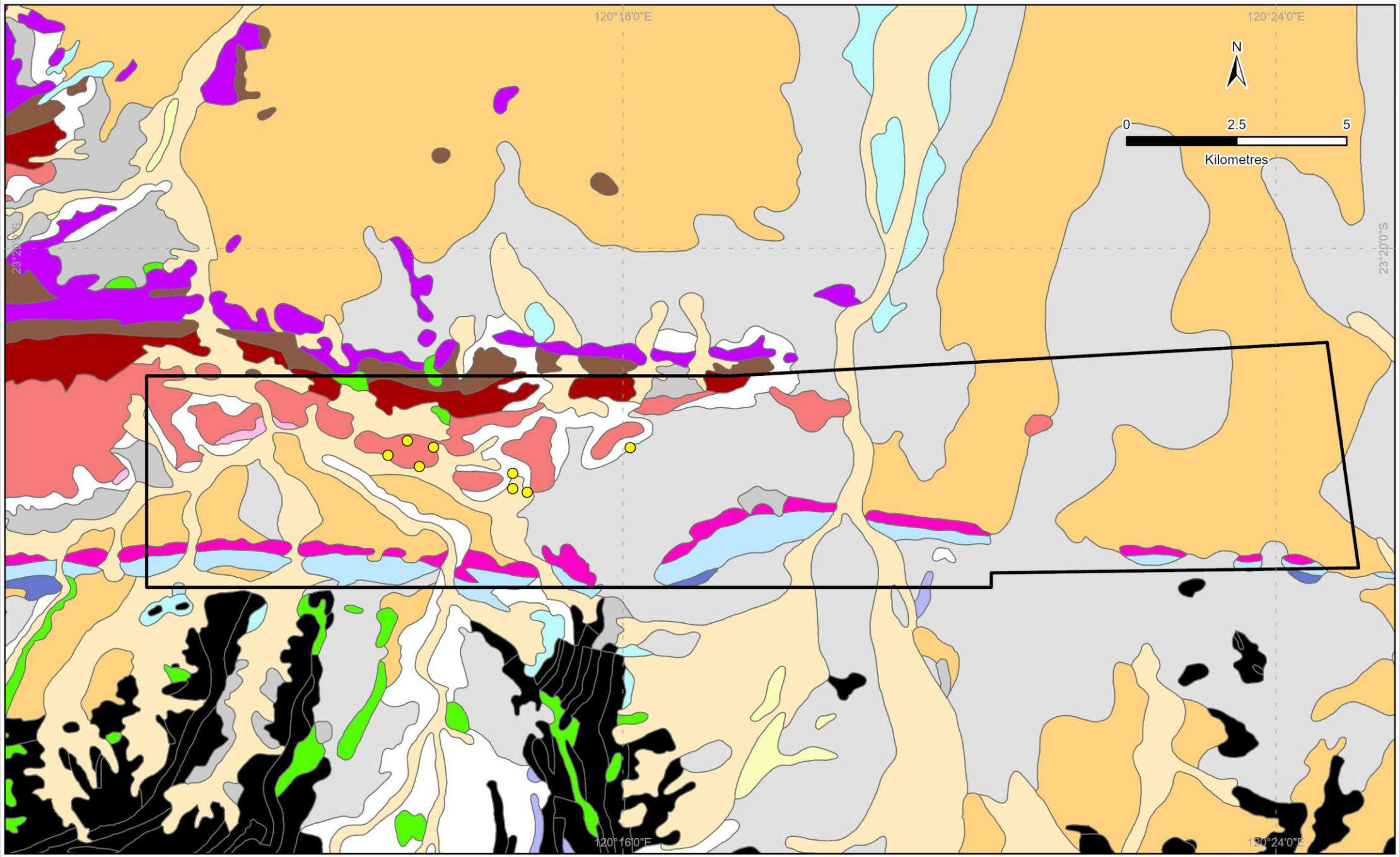
Alluvial deposits of silt, sand, and gravel are also present within the tenements along floodplains and drainage channels such as Jimblebar Creek and Copper Creek. Quaternary deposits of colluvium and minor alluvium adjacent to and derived from the bedrock are present on the scree slopes and talus slopes. These units are quite young geologically and were formed during the Cainozoic period (Williams and Tyler 1991). They overlie older bedrock more consistent with the iron rich outcropping ridgelines to the north and south of the Study Area.

Bedrock mapping indicates that the bedrock geologies, including weathered iron-rich units, run in strips in an east-westerly direction (Figure 4). A section of Woongarra Rhyolite separates the iron units of the Study Area from the broader Boolgeeda Iron Fomation to the North of the Study Area (Figure 4). While the Woongarra Rhyolite does contain some Banded Iron Formation (BIF), it is characterised as felsic rocks containing quartz as well as devitrified opaques (Williams and Tyler 1991), which may limit the amount of subterranean spaces that can support troglofauna. A thin strip of the Weeli Wolli Formation follows the northern boundary of the Study Area. In addition, two larger sections of the Brockman Iron Formation and Wittenoom Formation extend through the centre of the Study Area. The Wittenoom Formation consists primarily of dolomite and dolomitic shale (Williams and Tyler 1991), and tends to be less prospective than the surrounding iron rich geologies. the Brockman Iron Formation and Wittenoom Formation are separated by a thin, discontinuous strip of Mt McRae Shale. While shales are not necessarily conducive to the presence of subterranean fauna, the Mt McRae Shale is described as a pink and white weathering shale up to 23 m thick (Williams and Tyler 1991). To the south of the Wittenoom Fomation is a thin strip of the Marra Mamba Iron Fomation which in turn is bordered on its south by a strip of the Jeerinah Formation (Figure 4). The Jeerinah Formation is not normally associated with weathering and as a result may act as the southern edge to the weathering profile of the Study Area.

The bedrock units appear continuous throughout the Study Area and extend marginally both to the east and west (Figure 4). However, a series of faults throughout these units, particularly to the western end of the Study Area (Williams and Tyler 1991), may act as barriers and result in isolation of pockets of weathered, vuggy geologies suitable for troglofauna.

2.2. Assessment of Drill Logs

According to Bedrock mapping (Figure 4), each of the drill holes from which restricted fauna were collected, intersect BIF at some point. The drill logs of the bores from which the restricted animals were collected were also assessed to determine if site specific drilling and geological assessment confirm this conclusion. Each of these drill holes intersect BIF either through the Dales Gorge Member, Joffre Member, Whaleback Member, Yandicoogina Member, or the Mt Sylvia Formation (BHP drill Logs).



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Legend

- Study Area
- Target troglofauna

Geology

- Alluvium
- Boolgeeda Iron Formation
- Brockman Iron Formation
- Calcrete
- Colluvium
- Colluvium and alluvium
- Colluvium and minor alluvium
- Eolian sand
- Jeerinah Formation
- Laterite
- Marra Mamba Iron Formation
- Mount McRae Shale and Mount Sylvia Formation
- Mixed lacustrine and eolian deposits
- Weeli Wolli Formation
- Woongarra Volcanics
- Mafic Volcanics
- Serpentinite
- Other Units

Figure 3. Surface Geology of the Study Area

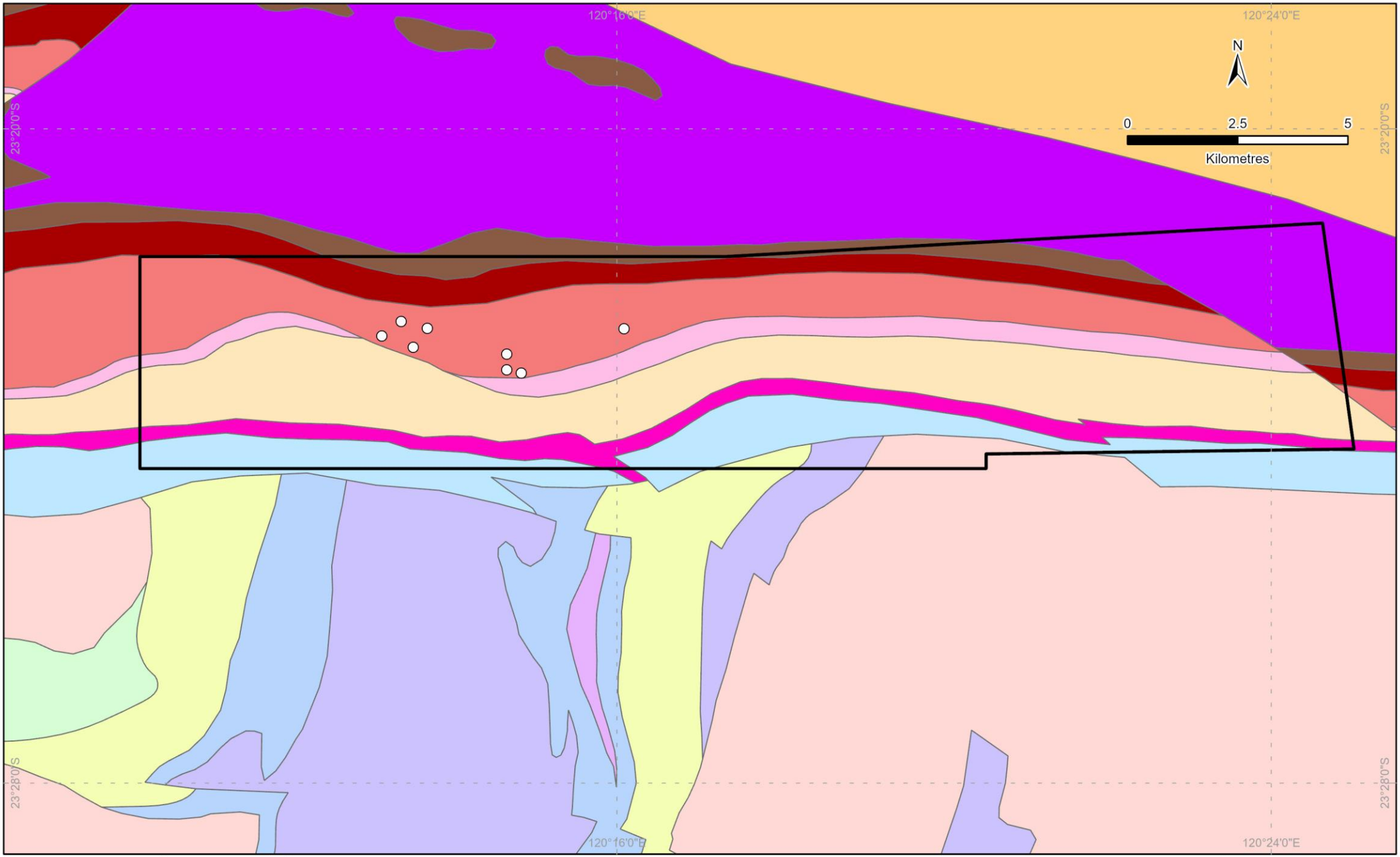

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Figure 4. Bedrock Geology of the Study Area

3. METHODS

A single round of target troglofauna survey was conducted in 2023 and this included two site visits. The first site visit was between 29th May 2023 and June 1st 2023 when troglofauna scrapes were collected and troglofauna traps were set. The second site visit occurred from August 9th 2023 to August 10th 2023 when troglofauna traps were collected for data analyses.

3.1. Troglofauna Sampling Methods

As far as possible, each troglofauna sample represented the combined results of two different, complementary sampling techniques: scraping and trapping.

Scraping is an active sampling technique that is used prior to setting traps. In each scraping event, a troglofauna net is prepared with a weighted ring net of 150-µm mesh, and a diameter closely matched to 60% of the bore diameter. This net is lowered to the bottom of a bore or to the water table, and subsequently scraped back to the surface along the hole wall at least four times. In each of these scrapes a unique and cardinally opposed section of the wall of the hole is targeted (e.g., north, south, east, and west) to maximize the number of organisms retrieved. The contents of each scrape are immediately transferred to 100% ethanol to preserve the sample and its DNA. The samples are then stored at ambient temperatures for taxonomic analyses in the lab.

Trapping is a passive sampling technique used after the drill hole has been scraped for troglofauna. Traps of cylindrical PVC (270 x 70 mm) with holes drilled on the side and top to function as entrances are baited with sterilized (microwaved) leaf litter. Traps are lowered on nylon cord to the end of the bore, or to a few metres above the water table. An additional second trap is set (at half the depth of the first trap) in approximately every fourth hole (where possible; Halse *et al.* 2018). The traps in this survey were left inside the bores for almost eleven weeks. During that period, the bores were sealed whenever possible to prevent the movement of surface animals into the troglofauna traps. When the traps were retrieved, their contents were transferred to a zip-lock bag and transported to the laboratory in Perth where living animals were extracted.

The sampling effort for troglofauna is calculated on the basis that one standard sample comprises both scraping and trapping, with scraping and trapping each comprising 50% (0.5) of a given sample. For example, if both trapping and scraping are carried out in a given hole, the sample effort would be 1. If, however, scraping is carried out and a trap is set but the trap is subsequently lost, the sample effort would be 0.5. Sample effort is calculated in this way irrespective of how many traps are set in a given hole.

3.2. Sample Effort

The 2023 targeted troglofauna survey event resulted in the collection of 70 troglofauna samples (Table 2 and Figure 5) from across the Study Area. BHP WAIO provided Bennelongia with a dataset of available sites to target the restricted fauna and Bennelongia sampled a subset of these available sites, targeting the best available holes (meaning easy and safe access and sampling feasibility) with the closest proximity to targeted taxa. With the aim of widening the known ranges of the targeted species, bore holes within which the targeted taxa had been collected in the previous surveys (2008-09 and 2020-2022) were not re-sampled. A total of 71 sites were visited (Figure 5), however two traps were lost during the survey, both from holes containing a single trap. This equates to a loss equal to one sample. This is not uncommon during troglofauna sampling events because tree roots and rocks might prevent recollection of traps. A complete list of sampled sites can be found in Appendix 1.

Table 2: Sample effort for the 2023 targeted troglofauna survey event.

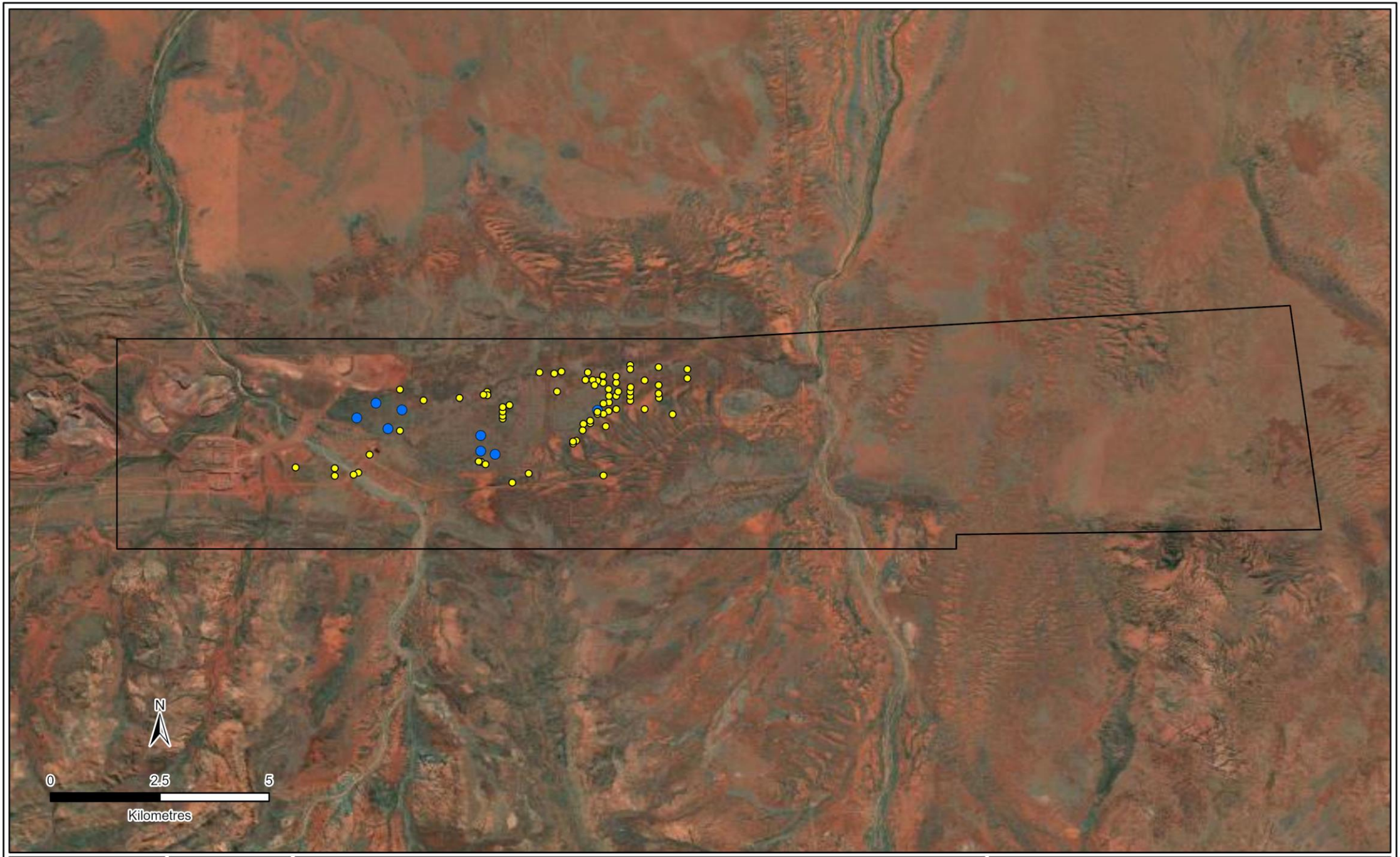
	Scrape	Trap 1	Trap 2
Number Collected	71	69	18
Total Samples		70	

3.3. Laboratory Processing

All samples were sorted in the Bennelongia laboratory. Leaf litter retrieved from traps was processed in Tullgren 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. Litter was checked after removal from the funnels to ensure no invertebrates (dead or alive) remained.

All samples (from scraping and trapping) were elutriated to separate out heavy sediment particles and sieved into size fractions using 250-, 90-, and 53-µm screens. Samples were sorted and identified under a dissecting microscope and, where necessary, dissected and examined under a differential interference contrast compound microscope. During the final phase of identification, dissecting and compound microscopes were used, with the process often requiring dissection of specimens.

Specimens were identified to described species where possible using published taxonomic keys and species descriptions. Most troglofauna species remain undescribed, however, and these species (whether identified morphologically or genetically) are usually assigned species codes (e.g. `BSY01`) to facilitate comparisons of species collected in different surveys. In other cases, when the taxonomic framework is exceptionally poor or the specimen in question is damaged or of a non-diagnostic life stage, the lowest level to which the specimen can be identified is usually genus or family (but can be of a higher order). After the taxonomic assessment was completed, representative animals were lodged with the WAM, a list of which and their respective WAM Registration Numbers can be found in Appendix 2



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Legend

- Study Area
- The Project (inset map)
- Target troglofauna locations
- Target survey sites

Figure 5. Sample effort during the Targeted Survey

3.4. Sequencing

The DNA sequencing program set out to identify conspecifics of target taxa from two sources:

1) any specimens that were collected during the present survey that might match the targeted troglofauna species as defined above were sequenced and compared to sequences available for these target taxa. All target taxa had available sequences from previous surveys, except *Tyrannochthonius* `PSE057`, of which two specimens were added to this sequencing program, and

2) the Bennelongia database was searched and any specimens held at Bennelongia that had a possibility of matching target taxa (either by taxonomy or sampling location) were also sequenced. This method was used in previous sequencing runs (Bennelongia 2023), and as a result, a broader selection of animals were targeted for this report, including animals from further afield, older animals that had not previously been sequenced and a greater variety of animals (i.e. comparing animals from closely related genera) were selected for sequencing.

A total of 40 animals were sequenced specifically for this report, comprising four specimens from the Study Area (two from this survey and two from previous survey events) and 36 from adjacent tenements. Selected animals were graded as to the volume of available tissue (i.e. on a scale of A to D where A is a lot of available tissue and D is a tiny amount of available tissue), and the condition of the specimens (i.e. on a scale of 1 to 4 where 1 is really good quality and 4 is very poor-quality specimens). The highest score available is 1A (a lot of high-quality tissue) and the lowest score available is 4D (a small amount of tissue and animals in poor-quality) with all available iterates between.

Depending on the size of the specimens, legs or whole animals were used for DNA extraction using a Qiagen DNeasy Blood & Tissue kit (Qiagen 2006). Elute volumes were set at 30 µL and determined by the age, condition, and quantity of material. Primer combinations used for PCR amplifications were LCOI490:HCO2198, C1J1718:HCO2198, and LCOI490:HCOoutout for the mitochondrial "barcoding" COI gene (Folmer *et al.* 1994; Schwendinger and Giribet 2005). Sanger sequencing for DNA strands in dual direction was undertaken for PCR products by the Australian Genome Research Facility (AGRF).

The returned sequences were edited and aligned in Geneious Prime v2022.2.2 (Geneious 2024) using the MAFFT alignment strategy (Katoh and Standley 2013). The sequences were then individually verified and compared to available sequences in GenBank using the Basic Local Alignment Tool (BLAST: Altschul *et al.* 1990), and to related sequences held in the Bennelongia database. To visualise genetic distances and phylogenetic relationships between taxa, unrooted Neighbour-Joining (NJ) trees (Saitou and Nei 1987) using bootstrap of 1000 replicates were generated, also in Geneious v2022.2.2 (Geneious 2024). Genetic distances (total percentage of nucleotide differences between sequences) within and between individuals or species groups were calculated in MEGA11 (Tamura *et al.* 2021) as uncorrected *p*-distances, and species intra- and interspecific boundaries were verified using published literature. For a further understanding of the phylogenetic relationships between related species, a Maximum Likelihood (ML) tree was generated using the RAxML plugin (Stamatakis 2014) in Geneious v2022.2.2 (Geneious 2024), with GTR + G + I as the best substitution model (MEGA11) (Tamura *et al.* 2021). Upon completion of sequencing and subsequent analysis, sequences were uploaded to GenBank and a list of these as well as their GenBank accession numbers can be found in Appendix 2.

3.5. Personnel Involved in the Survey

Table 3 below identifies the personnel involved in the project from conception to completion and lists their respective qualifications and experience. Table 4 lists the limitations associated with the current survey.

Table 3: Personnel involved in the project.

Task	Personnel	Qualifications/Experience
Fieldwork	Jim Cocking	B.Sc. Grad Dip. Over 20 years of experience conducting subterranean fauna field work throughout WA.
	Jaxon Haines	B.Sc. One year experience conducting subterranean fauna surveys throughout WA
Sample Sorting	Blake Wyber	B.Sc. M.Sc. Ph.D. 1 years' experience at Bennelongia
	Jaxon Haines	B.Sc. 1.5 years' experience at Bennelongia
	Georgia Rice	B.Sc. 1 years' experience at Bennelongia
	Ashley Browse	B.Sc. M.Sc. 1 years' experience at Bennelongia
	Melita Pennifold	BSc. (Hons). Over 25 years of research and taxonomic identification experience
	Megan Lewis	B.Sc. M.Sc. 1 years' experience at Bennelongia
	Ella Carstens	B.Sc. 2 years' experience at Bennelongia
	Vitor Marques	B.Sc. 4 years' experience at Bennelongia
Species Identification	Jane McRae	30 years of identification experience at Australian Museum, British Museum, DBCA, Bennelongia, author/co-author of 14 taxonomic papers and nine papers on species inventory/ecology
DNA analysis	Daniel White	B.Sc. (Hons). M.Sc. Ph.D. 16 years' experience performing genetic sequencing and analysis
	Veera Haslam	B.Sc. (Hons) Ph.D. candidate 17 years' experience performing genetic sequencing and analysis
	Heather McLetchie	B.Sc. (Hons). 22 years' experience performing genetic sequencing and analysis
Mapping	Huon Clark	B.Sc. (Hons) Ph.D. Over 10 years' experience in environmental consulting including the use of multiple mapping programs
Reporting	Huon Clark	B.Sc. (Hons) Ph.D. Over 10 years' experience in environmental consulting including extensive report writing experience
Review	Robin Hare	B.Sc. (Hons), Ph.D.

Table 4: Limitations of the survey

Limitation	Rationale	Mitigation	Severity
Loss of sample	Samples (or part thereof) can be lost for multiple reasons. This survey resulted in the loss of two traps which equates to one sample. This is 1.5% of the total targeted samples.	All effort is taken to ensure that samples are well protected and the integrity of the samples are maintained.	This is quite a low loss rate and therefore it is considered to have a very small to negligible impact on the success of the survey.
Taxonomic difficulties	Some groups have a poor taxonomic framework making it difficult to identify some specimens to species level, particularly for juvenile and female specimens.	Genetic analyses were used on 40 individuals from the survey and surrounding area in an attempt to identify conspecific individuals.	This had a small to negligible impact on the results as specimens were selected to maximise the success rate of finding a match.
Sequencing difficulties	Sequences can be hard to obtain particularly if they belong to samples with a low volume of body tissue, specimens of poor quality and/or old age or imperfect preservation.	Where possible, material selected for sequencing is new, and in good condition. This provides the best possibility of obtaining a successful sequence for comparison.	This had a moderate impact on the sequencing run where by design there was a higher percentage of poor and/or low volume material.

4. RESULTS

Results presented below outline the results of the targeted troglofauna survey only and detail the success or otherwise of collecting and identifying conspecific specimens to targeted taxa.

4.1. Troglofauna Results

The 2023 targeted troglofauna survey resulted in the collection of 60 specimens of troglofauna (Table 5). Groups represented included isopods (Isopoda), millipedes (Diplopoda), pauropods (Pauropoda), diplurans (Diplura), beetles (Coleoptera) and a fly (Diptera) (Table 5). Unfortunately, based on morphology, only two specimens of Diplura were identified as possibly belonging to a target species. The other target taxa were not collected and ruled out based on either belonging to the wrong taxonomic group or strong morphological differences between the target taxa and collected specimens.

A total of 67 specimens of stygofauna were also collected as by-catch during the targeted troglofauna survey. These represent six distinct taxonomic units (species), the details of which can be found in Appendix 3.

Table 5: Troglofauna specimens collected during the targeted survey at the Study Area in 2023

Higher Order Identification	Lowest Identification	No. of Specimens	Sequenced
Arthropoda			
Malacostraca			
Isopoda			
Armadillidae	*Troglarmadillo* sp. B07	3	No
Diplopoda			
Polyxenida			
Lophoproctidae	*Lophoturus madecassus*	51	No
Pauropoda			
Tetramerocerata			
Pauropodidae	*Decapauropus tenuis*	1	No
Entognatha			
Diplura			
Japygidae	Japygidae `BDP165`	1	Yes
	Japygidae sp. (fragment)	1	Yes
Insecta			
Coleoptera			
Staphylinidae	Ctenistini `BCO249`	1	No
	Staphylinidae sp.	1	No
Diptera			
Sciaridae	*Allopnyxia* sp. B01	1	No
Total	8	60	2

4.2. Sequencing Results

Of the 40 individuals sent for sequencing, 25 produced successful sequences (Table 6). A total of 10 matches were identified (based on thresholds from published literature or determined by inhouse within species genetic distances) and the taxonomy of these individuals updated (Table 5 and Table 6). While none of these identified a match for targeted species, comparing specimens with other sequences held at Bennelongia resulted in finding a match to the restricted species *Scutigerella* 'BSYM113'.

Table 6: Specimens sent for sequencing and the results obtained.

Original ID	Bennelongia specimen number	BHP Survey ID	Location of sample relative to Study Area	Collection Date	Target Taxa	Material quality (1-4) and quantity (A-D)	Sequence Result	Outcome	Final Name
Tyrannochthonius `BPS498`	752770	NA	55 km north east	27/07/2023	*Tyrannochthonius* 'PSE057'	2C	Success	Retain name	*Tyrannochthonius* 'BPS498'
Tyrannochthonius `BPS499`	752906	10522	125 km north west	2/08/2023	*Tyrannochthonius* 'PSE057'	2C	Success	Retain name	*Tyrannochthonius* `BPS499`
Tyrannochthonius `PSE057`	709009	10262	Study Area	1/07/2020	Target Animal	2D	Failed	NA	*Tyrannochthonius* `PSE057`
Tyrannochthonius `PSE057`	709901	10262	Study Area	1/07/2020	Target Animal	2D	Failed	NA	*Tyrannochthonius* `PSE057`
Philosciidae sp.	649094	NA	115 km north	10/08/2017	nr *Andricophiloscia* 'BIS509'	2B	Failed	NA	Philosciidae sp.
nr *Andricophiloscia* sp. B20	653891	NA	125 km north west	1/11/2017	nr *Andricophiloscia* 'BIS509'	2C	Failed	NA	nr *Andricophiloscia* sp. B20
nr *Andricophiloscia* sp.	654141	NA	105 km north	4/11/2017	nr *Andricophiloscia* 'BIS509'	2B	Failed	NA	nr *Andricophiloscia* sp.
Cryptops sp.	649325	NA	110 km north west	7/09/2017	*Cryptops* 'BSCOL066'	4A	Success	New species	*Cryptops* `BSCOL110`
Cryptops sp.	652662	NA	125 km north west	7/11/2017	*Cryptops* 'BSCOL066'	2B	Success	New species	*Cryptops* `BSCOL112`

Original ID	Bennelongia specimen number	BHP Survey ID	Location of sample relative to Study Area	Collection Date	Target Taxa	Material quality (1-4) and quantity (A-D)	Sequence Result	Outcome	Final Name
Cryptops sp.	749071	10439	135 km north west	2/05/2023	*Cryptops* 'BSCOL066'	2B	Success	New species	*Cryptops* `BSCOL111`
Cryptops `BSCOL091`	735805	10439	120 km north west	10/05/2022	*Cryptops* 'BSCOL066'	2A	Success	Retain name	*Cryptops* 'BSCOL091
Cryptops `BSCOL091`	746840	10439	120 km north west	9/03/2023	*Cryptops* 'BSCOL066'	2A	Success	Retain name	*Cryptops* 'BSCOL091
Symphyla sp.	649150	NA	125 km north west	5/08/2017	*Scutigerella* 'BSYM113'	2B	Failed	NA	Symphyla sp.
Symphyla sp.	649539	NA	125 km north west	4/09/2017	*Scutigerella* 'BSYM113'	2B	Success	New species	*Hanseniella* `BSYM130`
Symphyla sp.	649341	NA	125 km north west	3/09/2017	*Scutigerella* 'BSYM113'	4B	Failed	NA	Symphyla sp.
Symphyla sp.	649384	NA	115 km north	5/09/2017	*Scutigerella* 'BSYM113'	2C	Failed	NA	Symphyla sp.
Symphyla sp.	649273	NA	125 km north west	4/09/2017	*Scutigerella* 'BSYM113'	2B	Success	New species	*Hanseniella* `BSYM130`
Symphyla sp.	653007	NA	125 km north west	7/11/2017	*Scutigerella* 'BSYM113'	2C	Success	New species	*Hanseniella* `BSYM130`
Symphyla sp.	653010	NA	120 km north west	7/11/2017	*Scutigerella* 'BSYM113'	2B	Success	New species	*Hanseniella* `BSYM130`
Symphyla sp.	653014	NA	125 km north west	7/11/2017	*Scutigerella* 'BSYM113'	2B	Success	New species	*Hanseniella* `BSYM130`
Symphyla sp.	653711	NA	105 km north	5/11/2017	*Scutigerella* 'BSYM113'	2B	Success	New species	*Hanseniella* `BSYM131`
Symphyla sp.	653766	NA	125 km north west	7/11/2017	*Scutigerella* 'BSYM113'	2B	Success	New species	*Hanseniella* `BSYM130`

Original ID	Bennelongia specimen number	BHP Survey ID	Location of sample relative to Study Area	Collection Date	Target Taxa	Material quality (1-4) and quantity (A-D)	Sequence Result	Outcome	Final Name
Symphyla sp.	653799	NA	125 km north west	1/11/2017	*Scutigerella* 'BSYM113'	2B	Success	New species	*Hanseniella* `BSYM130`
Symphyla sp.	653850	NA	125 km north west	1/11/2017	*Scutigerella* 'BSYM113'	2B	Failed	NA	Symphyla sp.
Symphyla sp.	653892	NA	125 km north west	1/11/2017	*Scutigerella* 'BSYM113'	2B	Failed	NA	Symphyla sp.
Symphyla sp.	654076	NA	125 km north west	2/11/2017	*Scutigerella* 'BSYM113'	2B	Failed	NA	Symphyla sp.
Symphyla sp.	750240	10439	125 km north west	27/04/2023	*Scutigerella* 'BSYM113'	2B	Success	Match. Update species name	*Hanseniella* sp. B42-DNA
Hanseniella sp.	745862	10525	45 km west	7/02/2023	*Scutigerella* 'BSYM113'	2B	Success	Match. Update species name	*Hanseniella* sp. B19
Scutigerella sp. B03	637354	10057	130 km north west	17/02/2016	*Scutigerella* 'BSYM113'	4C	Success	Match. Update species name	*Hanseniella* sp. B35
Japygidae sp.	703406	NA	95 km north	19/03/2020	Japygidae 'BDP192'	4B	Failed	NA	Japygidae sp.
Japygidae sp.	703414	NA	90 km north	19/03/2020	Japygidae 'BDP192'	4C	Failed	NA	Japygidae sp.
Japygidae sp.	713587	10475	120 km north west	24/11/2020	Japygidae 'BDP192'	2B	Success	Match. Update species name	Japygidae sp. B34

Original ID	Bennelongia specimen number	BHP Survey ID	Location of sample relative to Study Area	Collection Date	Target Taxa	Material quality (1-4) and quantity (A-D)	Sequence Result	Outcome	Final Name
Japygidae sp.	715711	10475	125 km north west	25/02/2021	Japygidae 'BDP192'	2B	Success	Match. Update species name	Japygidae sp. B34
Japygidae sp.	751310	10547	Study Area	30/05/2023	Japygidae 'BDP192'	4C – pincer only	Failed	NA	Japygidae sp.
Japygidae sp.	751329	10547	Study Area	1/06/2023	Japygidae 'BDP192'	3C	Success	Match. Update species name	Japygidae `BDP165`
Japygidae sp.	752911	10522	120 km north west	2/08/2023	Japygidae 'BDP192'	4B	Failed	NA	Japygidae sp.
Japygidae sp.	755426	10312	28 km west	21/09/2023	Japygidae 'BDP192'	2B	Success	Match. Update species name	Japygidae `BDP165`
Japygidae `BDP194`	751691	10522	125 km north west	27/06/2023	Japygidae 'BDP192'	2A	Success	Match. Update species name	Japygidae sp. B34
Japygidae `BDP194`	752123	10522	130 km north west	27/06/2023	Japygidae 'BDP192'	2B	Success	Match. Update species name	Japygidae sp. B34
Japygidae `BDP194`	752896	10522	125 km north west	1/08/2023	Japygidae 'BDP192'	2B	Success	Match. Update species name	Japygidae sp. B34

5. DISCUSSION

5.1. Targeted fauna species

Of the five target taxa, only one match was identified (*Scutigerella* 'BSYM113'), extending the known distribution of this species. Details for each of the target taxa are listed below and pair-wise genetics distance tables can be found in Appendix 4.

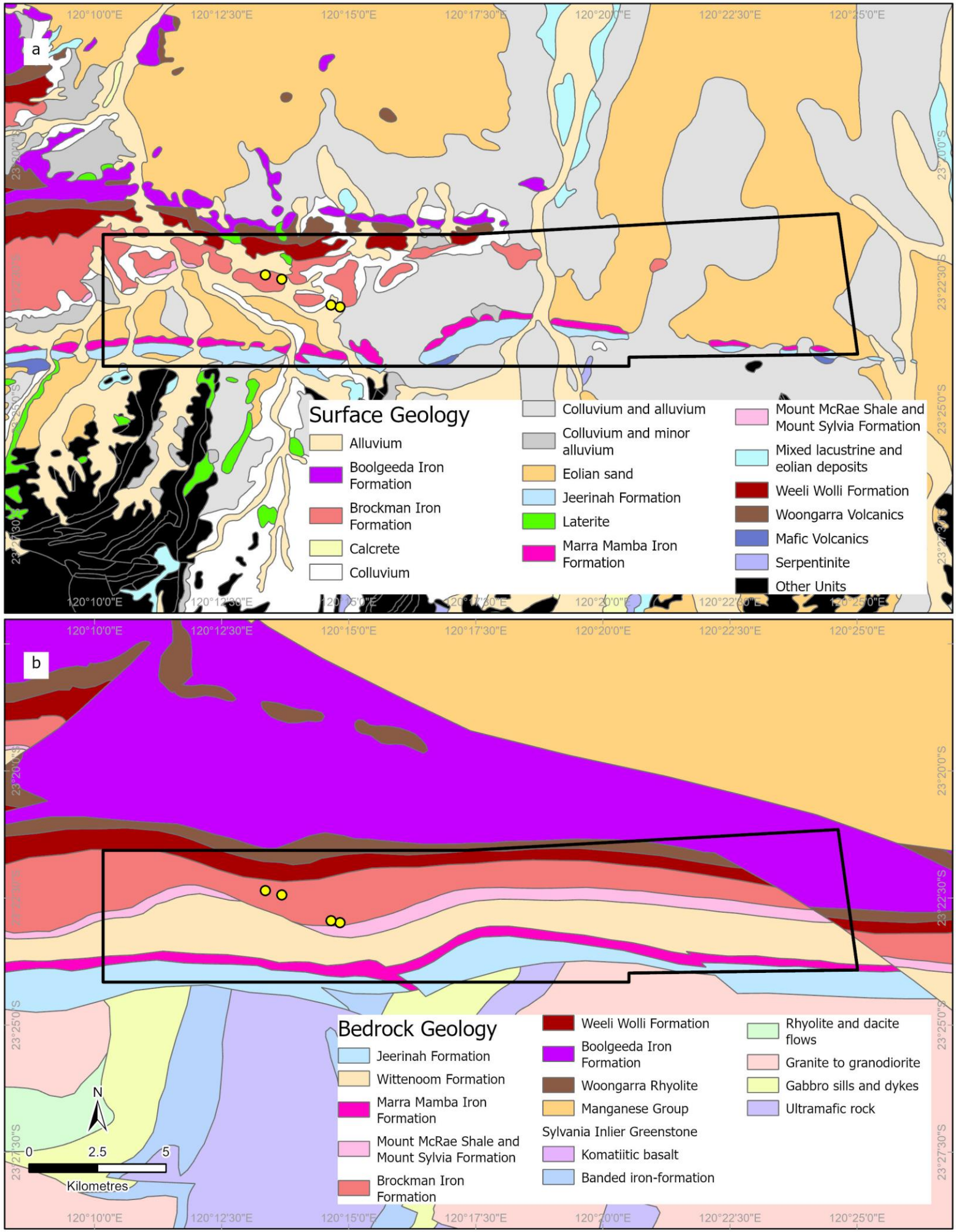
***Tyrannochthonius* 'PSE057' – priority species**

The targeted survey failed to collect any further *Tyrannochthonius* specimens or other pseudoscorpions (Table 5). Past attempts at finding a match to the target species, *Tyrannochthonius* 'PSE057', have failed in part due to challenges of obtaining a sequence for *Tyrannochthonius* 'PSE057'. This sequence run included further attempts to obtain sequences from two *Tyrannochthonius* 'PSE057' specimens (Table 6). Unfortunately, these specimens did not return successful sequences (Table 6), making this a minimum of six times between 2008 and now, that sequencing has been attempted on this species to no avail. Each of these samples were classed as 2D, meaning there was very little material usable for sequencing, perhaps due to previous sequencing attempts. *Tyrannochthonius* 'PSE057' is still restricted to the Study Area, known from four drill holes, with a known linear distribution of 2.75 km (Figure 6).

All specimens were collected either through troglofauna scrape samples or as by-catch during stygofauna net hauling making a determination on collection depth challenging. The most likely geology from which these specimens were collected is BIF, with two holes intersecting the Joffre Member of the Brockman Iron Formation (PI012 from 21 m bgl to the water table at 75 m bgl and EJR0014 between 10 m bgl to the water table at 70 m bgl). The other two collection locations intersect the Dales Gorge Member (EJ0833R between 17 m bgl to the water table at 60 m bgl and EJ0791R between from the surface to the water table at 60 m bgl). This is supported by the broader scale geological mapping which shows the BIF geology extending east/west through the Study Area (Figure 6). According to the drill logs, sites PI012 and EJR0014 both contained secondary weathered geologies above the main BIF, Vuggy Breccia and Yandicoogina Shale Member of the Brockman Iron Formation, respectively. These also have the potential to be available habitat for *Tyrannochthonius* 'PSE057'.

nr *Andricophiloscia* 'BIS509'

The targeted survey did not collect any other specimens from this genus (Table 5). Three specimens from the Bennelongia database were identified as having the potential to be conspecifics to nr *Andricophiloscia* 'BIS509' and as a result were sent for sequencing. Unfortunately, none of these returned a successful sequence. Each of these specimens were classified as having relatively good quality material (2 on a scale of 1-4) and ranged in quantity from B to C (on a scale of A-D). All three specimens were from 2017, which is on the old side for sequencing and may explain why these individuals failed to return a sequence. The species nr *Andricophiloscia* 'BIS509' remains as a singleton specimen collected from within outcropping Brockman Iron Formation (Figure 7) which appears to be consistent all the way to the water table at approximately 68 m bgl. This is supported by the bore logs for this site which indicate that this hole contains Dales Gorge Member from the surface all the way to the water table, providing a known geology from within which this specimen was collected. This specimen was collected as by-catch during stygofauna sampling, and as a result, it is difficult to determine a collection depth for this species.



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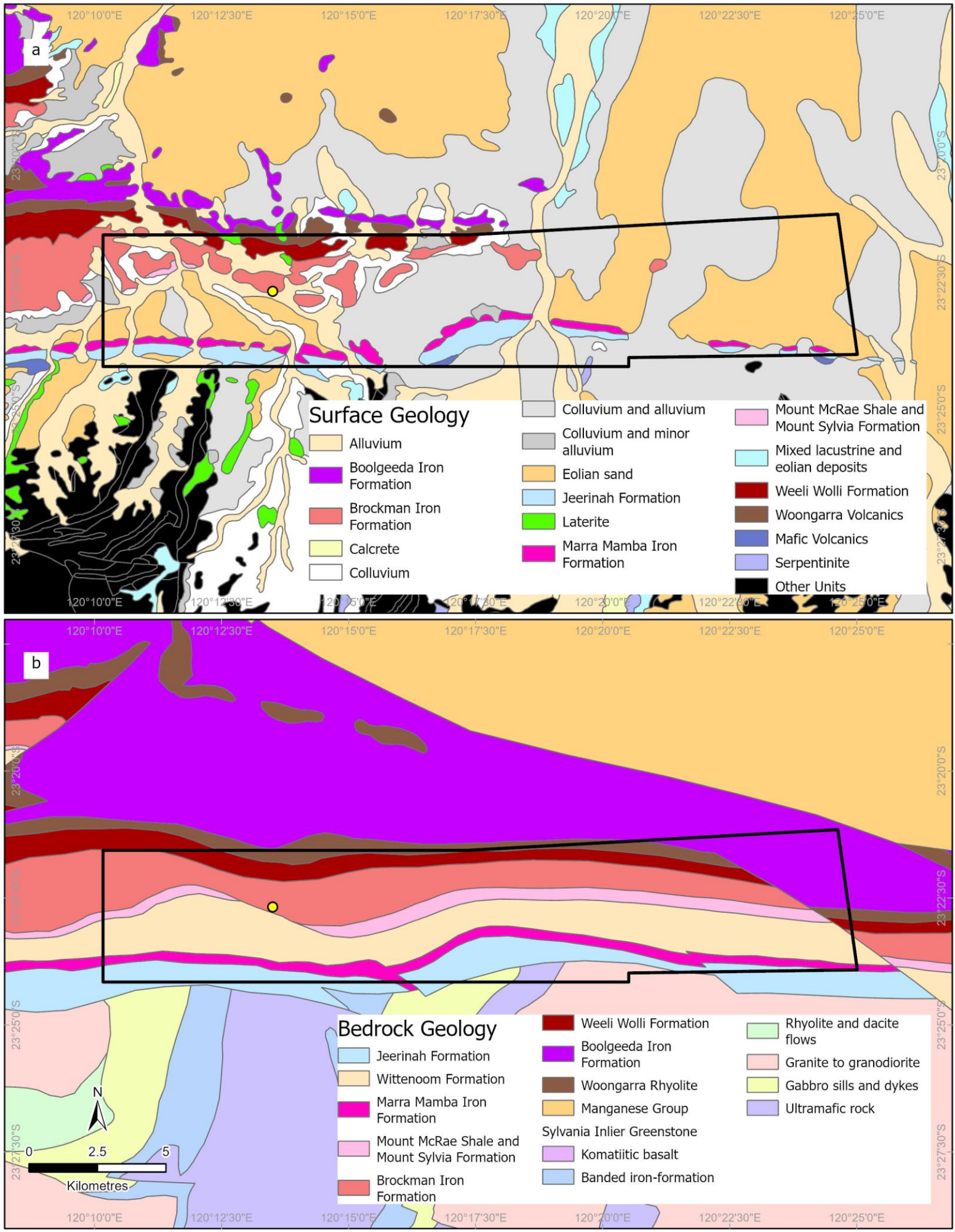
Legend

- Tyrannochthonius `PSE507`
- Study Area

Figure 6. Known collection locations of *Tyrannochthonius* `PSE057` in relation to a) Surface Geology (1:250000) and b) Bedrock Geology (1:500000)



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Legend

- nr Andricophiloscia `BIS509`
- Study Area

Figure 7. Known collection locations of nr *Andricophiloscia* `BIS509` in relation to a) Surface Geology (1:250000) and b) Bedrock Geology (1:500000)

***Cryptops* 'BSCOL066'**

No centipedes were collected during the 2023 targeted survey (Table 5) however five specimens held at Bennelongia were identified for sequencing in an attempt to find a match to *Cryptops* `BSCOL066` (Table 6). While all of these returned a successful sequence, none of these specimens were found to be a match to *Cryptops* `BSCOL066` with genetic distances ranging between 18.8 % and 24.1 %, which is well outside of current known within species divergence (Wesener *et al.* 2016; Figure 8 and Appendix 4). *Cryptops* `BSCOL066` remains as a singleton known from the Study Area (Figure 9).

Cryptops `BSCOL066` was collected from a drill hole (EJ1211R) that intersects colluvium and subsequently the bedrock Brockman Iron Formation (Figure 9). This geological unit appears to be deep at this location with the drill hole at the time of sampling reaching 53 m bgl without intersecting water. The drill logs for this location indicate that the first 16 m bgl are made up of Surface Scree and Tertiary Detritals. From 16 to 24 m bgl the geology is Mt McRae Shale and from 24 m bgl to the water table at 59.5 m bgl, the geology consists of the Mt Sylvia Formation. This individual was collected from a troglofaunal trap which was set at approximately 23 m bgl. The most likely geology from which this individual was collected is the Mt Sylvia Formation. However the weathered Mt McRae shale is also a possibility. Each of these units extend outside of the Study Area both to the east and west.

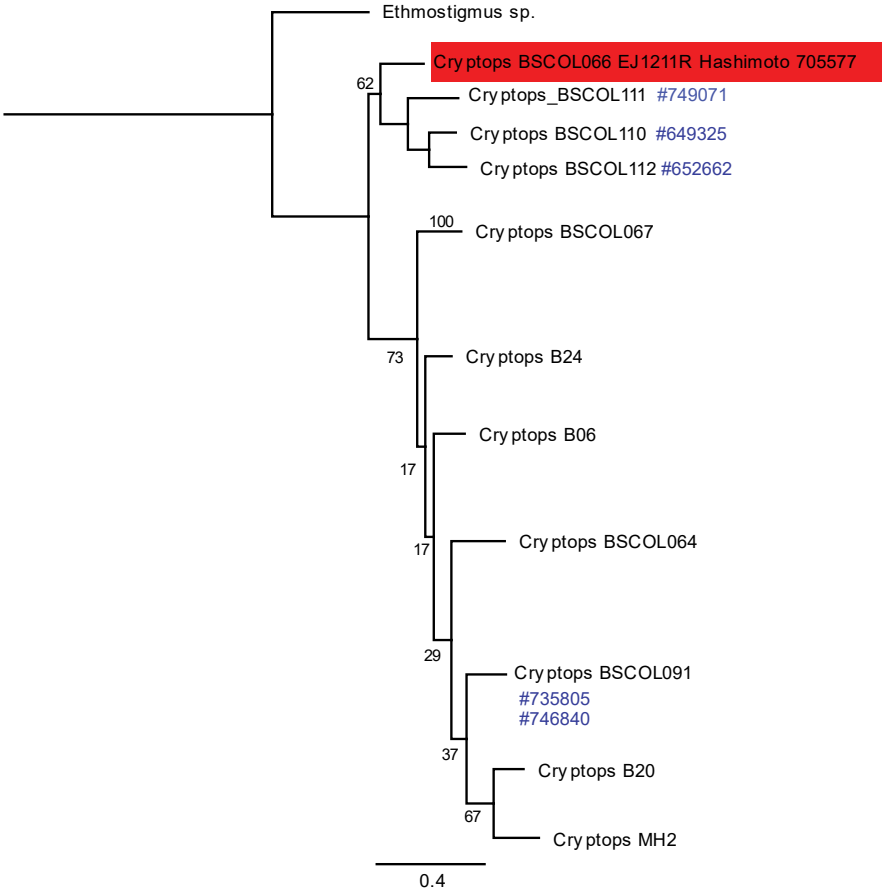
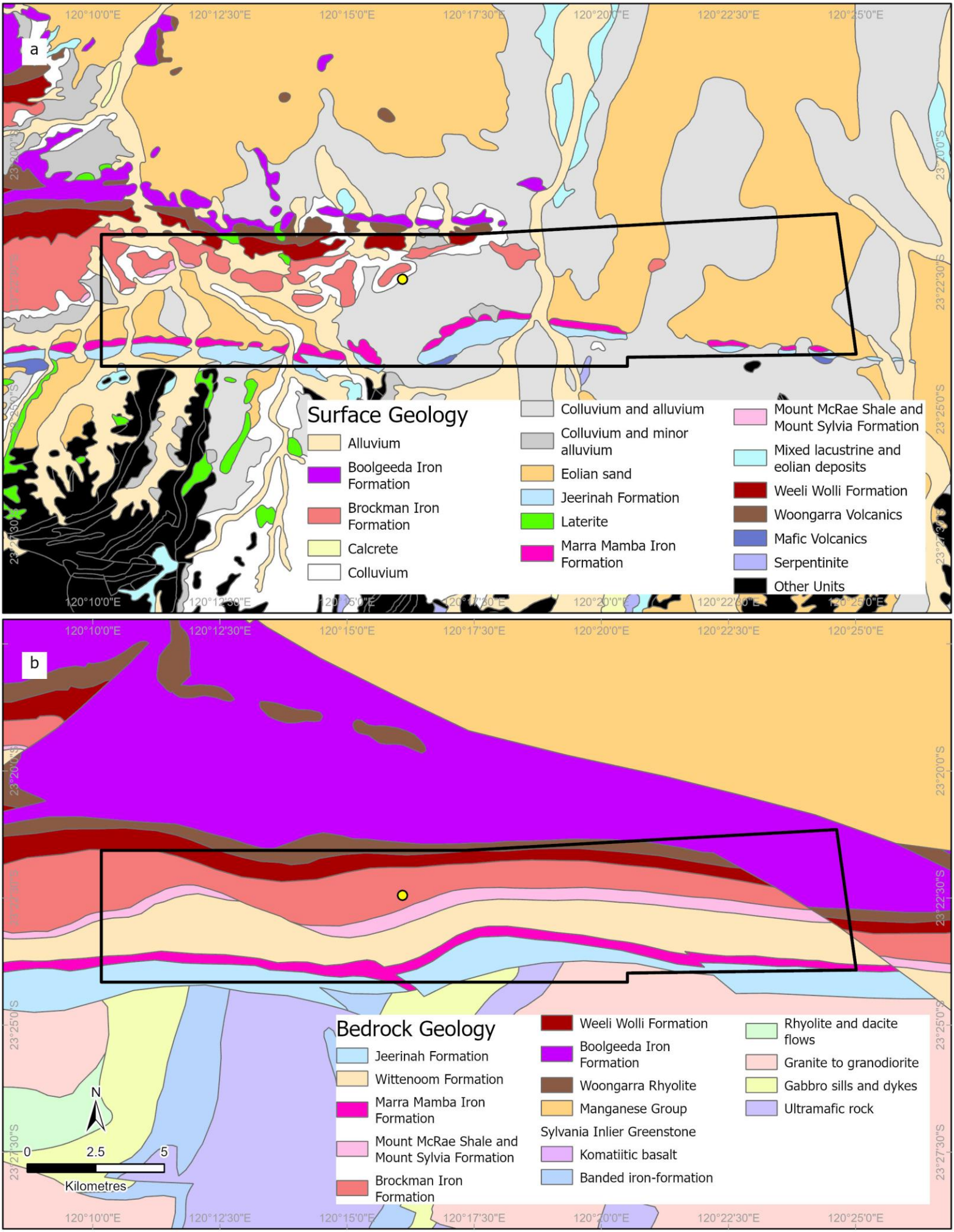


Figure 8: Maximum Likelihood (ML) tree of *Cryptops* spp. including all sampled individuals (in blue), using *Ethmostigmus* sp. as an outgroup. The bootstrap values of major divergences are shown and the target species is highlighted in red.



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Date: 22/05/2024

Legend

- Cryptops `BSCOL066`
- Study Area

Figure 9. Known collection locations of *Cryptops* `BSCOL066` in relation to a) Surface Geology (1:250000) and b) Bedrock Geology (1:500000)

***Scutigerella* 'BSYM113' – priority species**

The targeted survey did not collect any symphylan species (Table 5). However, 17 specimens of Symphylans held at Bennelongia were identified for sequencing in an attempt to find a match to *Scutigerella* 'BSYM113'. Of these 17 specimens, six failed to return a successful sequence and all of these specimens were old or had low volume or bad quality material (Table 6). The remaining 11 sequences were then compared to the target species along with other sequences from the Bennelongia database. This resulted in the identification of a genetic match with *Scutigerella* 'BSYM113'.

A sequence from a specimen of *Hanseniella* sp. B04 was identified as being a genetic match to *Scutigerella* 'BSYM113' with a pairwise genetic difference of 4% (Figure 10 and included in the distance table in Appendix 4 as *Scutigerella* 'BSYM113'), which is within the current knowledge of intraspecific genetic variability (Jin *et al.* 2023). *Hanseniella* sp. B04 is a species also restricted to the Study Area (Bennelongia 2023) but had a known linear distribution of 5.5 km and was not identified by BHP WAIO as a target species. The genetic works have resulted in these two species (*Scutigerella* 'BSYM113' and *Hanseniella* sp. B04) being synonymised into a single species. The analyses place this species within the genus *Scutigerella* (Figure 10) and as a result, the name *Scutigerella* 'BSYM113' is applied to here and in future reports.

All representatives of *Scutigerella* 'BSYM113' were collected via troglofauna scrape samples making it difficult to surmise a collection depth for these individuals. Investigations of drill logs indicate that drill hole EXR0626 intersects the Dales Gorge Member from 41 m bgl to the water table while drill hole EJ0798R intersects the Joffre Member from 18 m bgl to 51 m bgl, followed by the Whaleback Member all the way to the water table at 65 m bgl. Each of these units are members of the Brockman Iron Formation and are the most likely collection locations within these drill holes, and broadscale mapping shows Brockman Iron formation running in an east/west direction through the Study Area (Figure 11). Drill logs of the other two drill holes (EXR1462R and EXP0012) demonstrate these both intersect the Marra Mamba Formation, the first from 30 m bgl to the water table at 55 m bgl and the latter from 13 m bgl to the end of hole at 58 m bgl. Again as demonstrated in Figure 11, this unit extends throughout the Study Area in an east/west running band. The distribution of these animals implies that there is at least some habitat connectivity between the Brockman Iron Formation in the north and the Marra Mamba Iron Formation in the south, and the geologies that lie between them (Mt McRae Shale and the Wittenoom Formation).

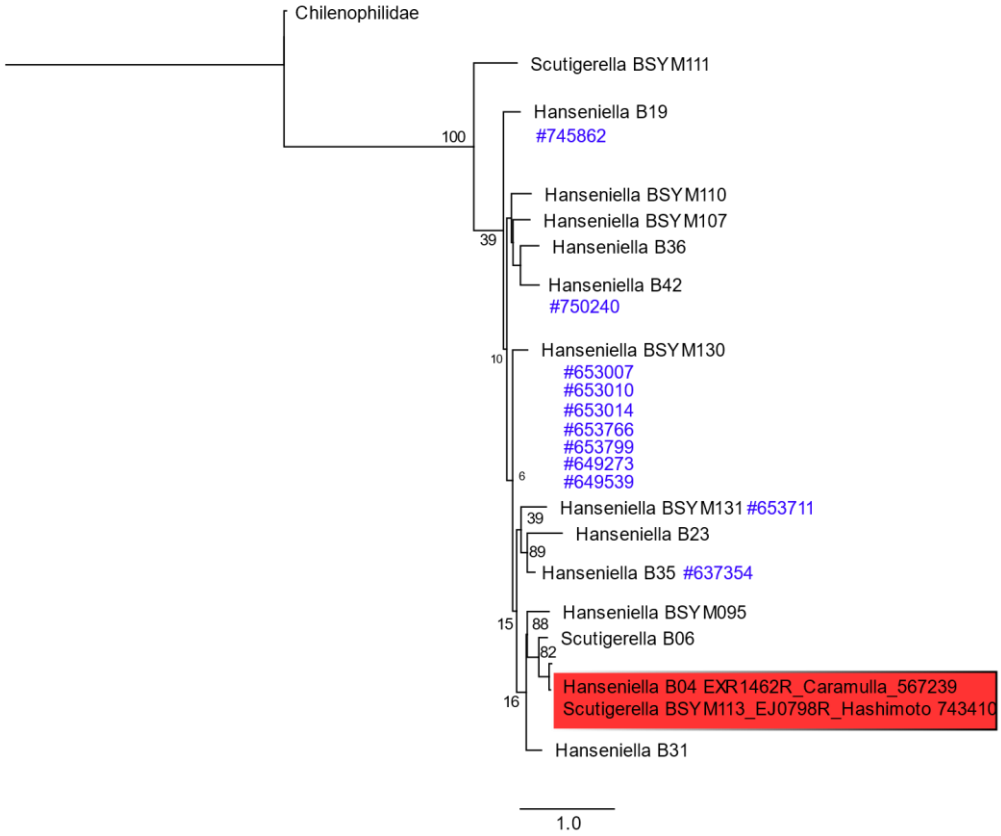
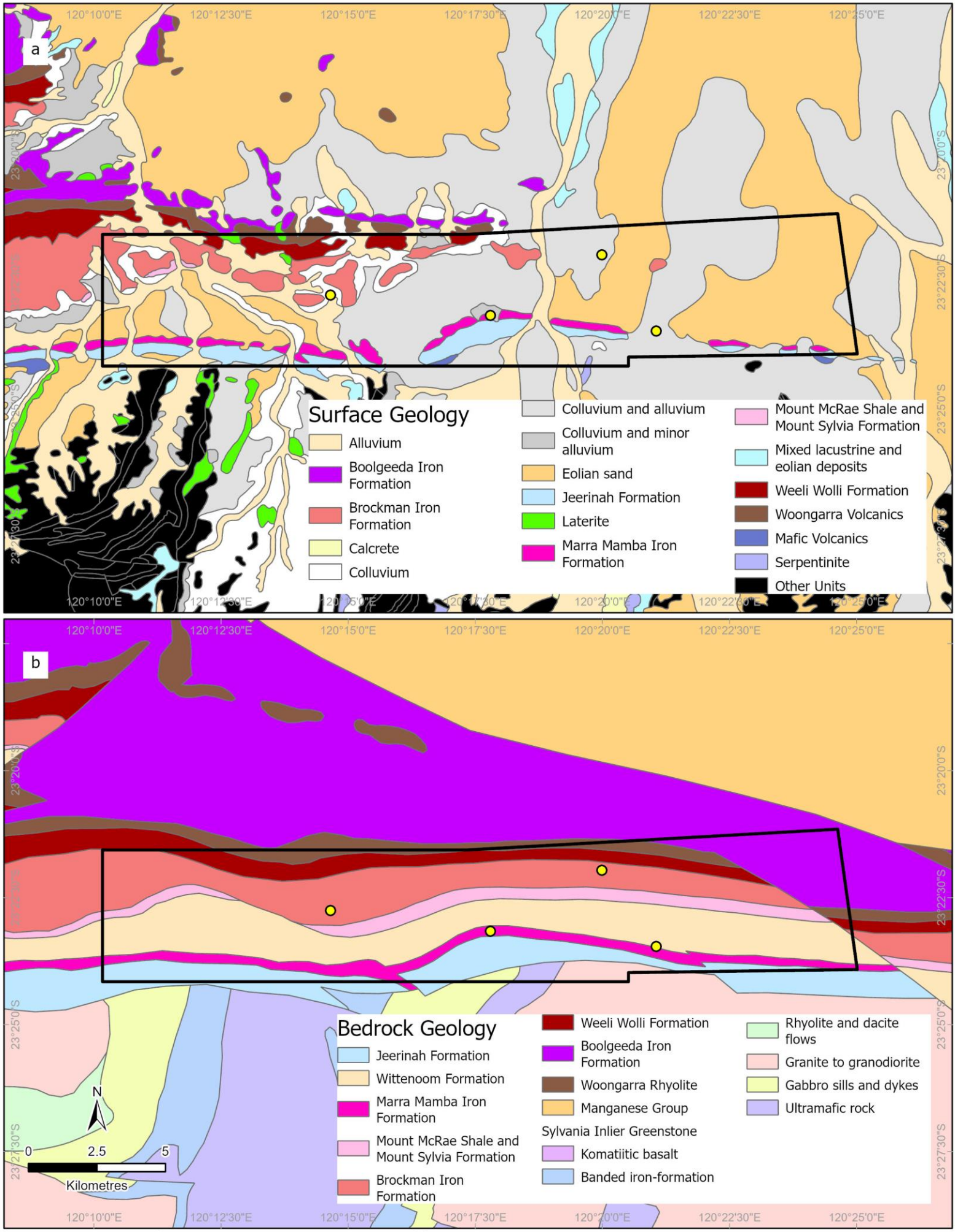


Figure 10: Maximum Likelihood (ML) tree of *Scutigerella* spp. and *Hanseniella spp.*, including all sampled individuals (in blue), and using Chilenophilidae sp. as an outgroup. Bootstrap values of major divergences are shown and the target species is highlighted in red.

Japygidae 'BDP192'

Two specimens of the family Japygidae were collected during the targeted troglofauna survey of 2023. One of these (Bennelongia specimen number: 751329) was the back half of an animal collected in a scrape sample taken from drill hole CM0149R and the other was a rear body fragment (Bennelongia specimen number: 751310) taken from drill hole CM0129R, also from a scrape. Each of these were sequenced in an attempt to match to Japygidae 'BDP192' although the sample from CM0129R was categorised as a 4C genetic sample (very low quality with low quantity). As such, this fragment failed to return a successful sequence (Table 6). The second individual did return a sequence but could not be matched with Japygidae 'BDP192' (Table 6; Figure 12 and Appendix 4) because high sequence divergences (18.1 % genetic distance) indicated a separate species. This individual belongs to the species Japygidae `BDP165` which has a known linear distribution of approximately 70 km.

A further nine specimens from the Bennelongia collections were sent for sequencing and six of these successfully returned sequences (Table 6). The three that failed to return a sequence were all classed as either 4B or 4C prior to sequencing (low volume and low quality) making it unsurprising that they did not produce successful sequences. The six successful sequences were then compared to the target species (Japygidae 'BDP192') along with other sequences from the Japygidae family held at Bennelongia. Unfortunately, none of these matched the sequence from Japygidae 'BDP192' (Figure 12 and Appendix 4) which remains as a singleton. The genetic divergence to Japygidae 'BDP192' ranged from 18.1 % to 15.8 % pairwise distances. We consider these distances to be indicative of two distinct species.



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Legend

- Scutigerella `BSYM113`
- Study Area

Figure 11. Known collection locations of *Scutigerella* `BSYM113` in relation to a) Surface Geology (1:250000) and b) Bedrock Geology (1:500000)

Japygidae 'BDP192' was collected in a drill hole (PI031) that intersected outcropping Brockman Iron Formation, which also forms the bedrock at this site (Figure 13). This specimen was collected as by-catch from a stygofauna net sample making it difficult to determine a collection depth. The standing water level at this location at the time of sampling was 68 m bgl. The drill log for this site has a recorded end of hole of approximately 111 m bgl. The geology recorded at the time of drilling includes the Whaleback Shale of the Brockman Iron Formation from the surface to 8 m bgl, followed by the Dales Gorge Member of the Brockman Iron Formation to a depth of approximately 82 m bgl. Below this are layers of the Mt McRae Shale. The Brockman Iron Formation extends beyond the boundaries of the Study Area both to the east and west interrupted in part by the presence of some faults (Figure 13).

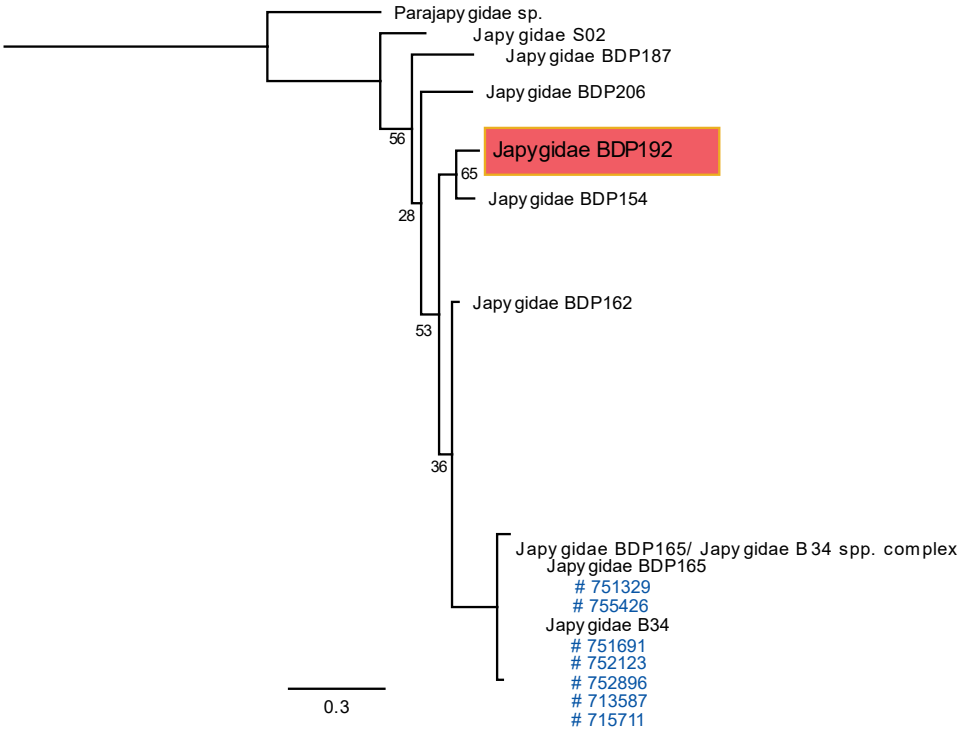
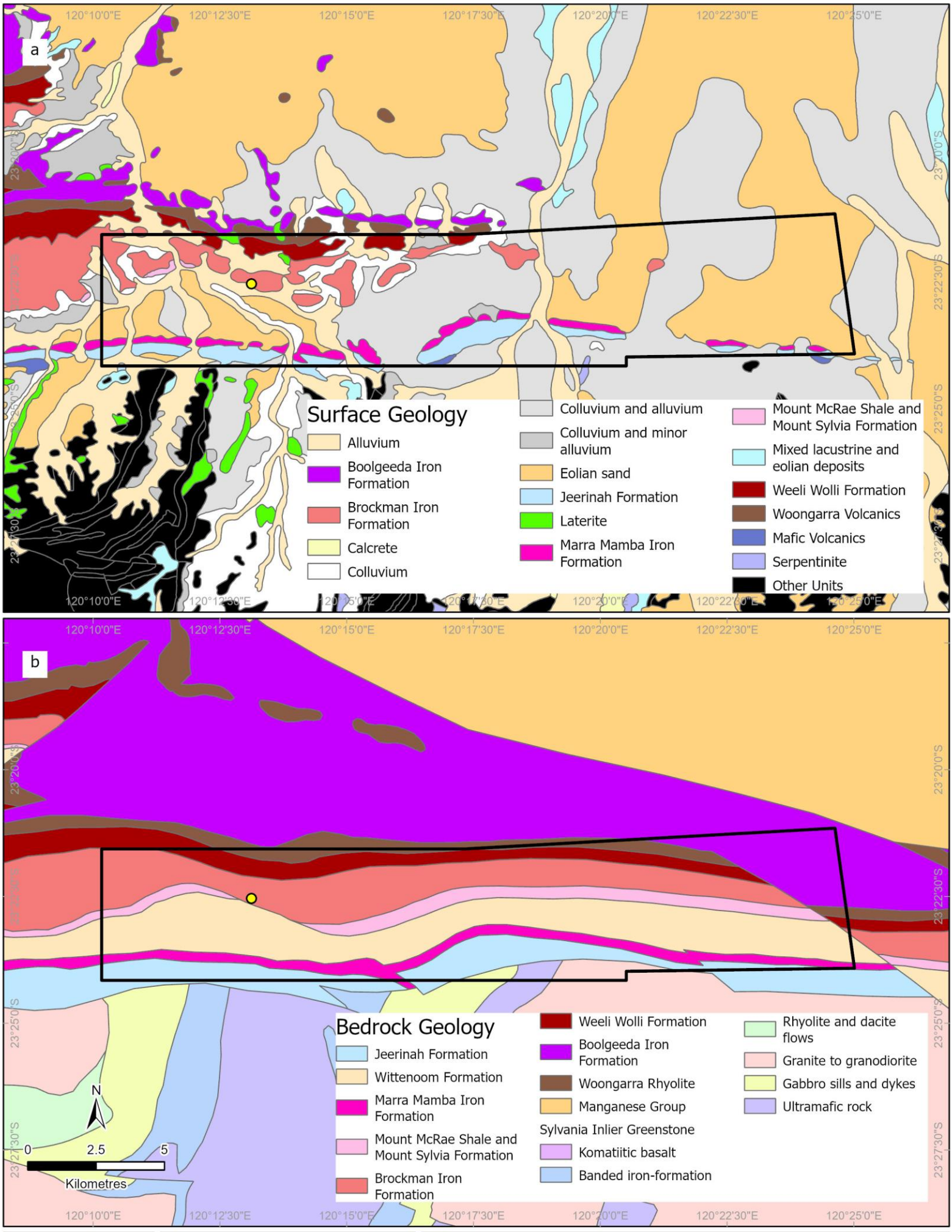


Figure 12: Maximum Likelihood (ML) tree of Japygidae spp. including all sampled individuals (in blue), and using Parajapygidae sp. as an outgroup. The bootstrap values of major divergences are shown and target species is highlighted in red.

Other taxonomic updates to species collected on behalf of BHP

The sequencing run associated with the current effort to locate matches to target taxa has resulted in the identification of ten further genetic matches for non-priority species (Table 6). The vast majority of these have been collected in the last 18 months and have either not yet been reported to BHP, or have been reported here and in intervening reports (e.g. Bennelongia 2024) with taxonomic updates included.

The taxonomic updates also include a new species synonymy that reduces the total count of troglofauna species known from the search area. A Symphyla specimen of the species *Scutigerella* sp. B03 collected in 2016 from South Flank (drill hole: SF3712R) was sequenced in an attempt to find a match to the target species *Scutigerella* 'BSYM113'. During the subsequent analysis, the *Scutigerella* sp. B03 sequence was observed to be a match with *Hanseniella* sp. B35. These two specimens have a genetic divergence of 9.4 % which is within the current knowledge of intraspecific genetic variability (Jin *et al.* 2023). These two species have now been synonymised into *Hanseniella* sp. B35 and are represented by nine specimens from five sample sites. For a list of all known specimens of this species and their collection locations, please refer to Appendix 5.



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Date: 22/05/2024

Legend

- Japygidae `BDP192`
- Study Area

Figure 13. Known collection locations of Japygidae `BDP192` in relation to a) Surface Geology (1:250000) and b) Bedrock Geology (1:500000)

A further nine specimens were found to match sequences held in the Bennelongia database and these are detailed below. They are not included in Appendix 5 however as they are all collections from ongoing work and have not yet been reported. They will be reported with their updated identifications upon completion of the respective projects.

A higher order symphylan sp. (Bennelongia specimen number: 750240) from Ministers North matched with and has been updated to *Hanseniella* sp. B42-DNA which now has a known linear distribution of approximately 2.85 km.

A specimen originally identified as the higher order *Hanseniella* sp. (Bennelongia specimen number: 745862) matched to *Hanseniella* sp. B19 from Orebody 29-30-35. This specimen identification has been updated to *Hanseniella* sp. B19.

Five specimens of Japygids (two higher order Japydigae sp. and three originally identified as Japygidae `BDP194`), all from Ministers North were found to match Japygidae sp. B34 and have thus been updated to reflect this. Japygidae sp. B34 now has a linear distribution of approximately 26 km.

Two specimens of higher order Japygid (Japygidae sp.) were found to match Japygidae `BDP165`. One from Caramulla (Bennelongia specimen number: 751329) and one from Orebody 42 (Bennelongia specimen number: 755426). Each of these have had their identification updated.

5.2. Conclusions

Previous desktop analysis and historical sampling in the Jimblebar area indicated that further surveys for subterranean fauna were required. As a result three rounds of survey were conducted between 2020 and 2022 (Bennelongia 2023), prompting BHP to identify five restricted troglofauna species for detailed assessment. These species, *Tyrannochthonius* 'PSE057', nr *Andricophiloscia* 'BIS509', *Cryptops* 'BSCOL066', *Scutigerella* 'BSYM113', and Japygidae 'BDP192', were then targeted in a fourth round of sampling in 2023 and are the focus of this report. In addition to the 2023 targeted troglofauna survey, a broadscale sequencing program was conducted to maximise the likelihood that matches would be found to target species, thereby extending their known distributions.

The field survey resulted in the collection of 60 specimens of troglofauna (Table 5). Only two of these specimens, both Diplura, were identified as possibly being members of target taxa. Each of these were collected as body fragments, including one which was a rear body fragment only. These two individuals, along with an additional 38 specimens from previous survey events and the surrounding landscape held in the Bennelongia collections were sent for sequencing and genetic analyses. Of the sequenced animals, 25 returned successful sequences (Table 6), resulting in a total of ten genetic matches with species already known from the Study Area and surrounding landscape (Table 5 and Table 6) and one species synonymy.

While none of these identified a match for the five targeted species, comparing specimens with other sequences held at Bennelongia resulted in finding a match to the priority species *Scutigerella* 'BSYM113' and a reassessment of its distribution. *Scutigerella* 'BSYM113' is now known from four locations at the Study Area (Figure 11) and has a known linear distance of 11 km. All collection locations intersect colluvium or alluvium at the surface and intersect either bedrock Brockman Iron Formation or bedrock Marra Mamba Iron Formation (Figure 11).

No other matches to target species were identified during either the targeted survey or through the extended genetic sequencing and analysis. The exact distribution of these species remains unknown.

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Appendix 1: Target Troglofauna Survey Locations

Bore Name	Latitude	Longitude	Sample Type
CM0076R	-23.37427	120.2705	Scrape
			Trap 1
			Trap 2
CM0078R	-23.37247	120.27051	Scrape
			Trap 1
			Trap 2
CM0081R	-23.37111	120.27054	Scrape
CM0084R	-23.36972	120.27048	Scrape
			Trap 1
CM0103R	-23.37483	120.26936	Scrape
			Trap 1
CM0106R	-23.37266	120.26938	Scrape
			Trap 1
			Trap 2
CM0111R	-23.36841	120.26935	Scrape
			Trap 1
CM0113R	-23.36696	120.26933	Scrape
			Trap 1
CM0116R	-23.37382	120.272	Scrape
			Trap 1
CM0119R	-23.37112	120.27204	Scrape
			Trap 1
			Trap 2
CM0120R	-23.37019	120.27247	Scrape
			Trap 1
CM0122R	-23.36843	120.27202	Scrape
			Trap 1
CM0124R	-23.36708	120.27201	Scrape
			Trap 1
CM0129R	-23.37213	120.27493	Scrape
			Trap 1
CM0130R	-23.37114	120.27494	Scrape
			Trap 1
CM0131R	-23.37022	120.27494	Scrape
			Trap 1
			Trap 2
CM0132R	-23.36932	120.27496	Scrape
			Trap 1
CM0136R	-23.36563	120.27494	Scrape
			Trap 1
CM0137R	-23.36481	120.2749	Scrape
			Trap 1

Bore Name	Latitude	Longitude	Sample Type
CM0138R	-23.37381	120.27785	Scrape
			Trap 1
			Trap 2
CM0143R	-23.36794	120.27784	Scrape
			Trap 1
CM0148R	-23.37153	120.28079	Scrape
			Trap 1
CM0149R	-23.37063	120.28077	Scrape
			Trap 1
CM0151R	-23.36887	120.28077	Scrape
			Trap 1
CM0155R	-23.36525	120.28076	Scrape
			Trap 1
			Trap 2
CM0156R	-23.37487	120.28361	Scrape
			Trap 1
CM0173R	-23.36747	120.28662	Scrape
			Trap 1
CM0175R	-23.36566	120.28661	Scrape
			Trap 1
			Trap 2
EJ0523R	-23.37151	120.23986	Scrape
			Trap 1
EJ0546R	-23.37583	120.24869	Scrape
			Trap 1
EJ0573R	-23.36624	120.25625	Scrape
			Trap 1
EJ0584R	-23.3665	120.25927	Scrape
			Trap 1
			Trap 2
EJ0606R	-23.36801	120.26817	Scrape
			Trap 1
EJ0609R	-23.3703	120.24551	Scrape
			Trap 1
EJ0643R	-23.37201	120.23246	Scrape
			Trap 1
EJ0788R	-23.38468	120.24423	Scrape
			Trap 1
			Trap 2
EJ0829R	-23.37095	120.24558	Scrape
			Trap 1
EJ0864R	-23.37527	120.24867	Scrape
			Trap 1
EJ0865R	-23.37433	120.24867	Scrape

Bore Name	Latitude	Longitude	Sample Type
			Trap 1
			Trap 2
EJ0866R	-23.37341	120.24868	Scrape
			Trap 1
EJ0889R	-23.37299	120.25007	Scrape
			Trap 1
EJ0953R	-23.38704	120.25401	Scrape
			Trap 1
			Trap 2
EJ1165R	-23.37691	120.26531	Scrape
			Trap 1
			Trap 2
EJ1183R	-23.37671	120.26672	Scrape
			Trap 1
			Trap 2
EJ1184R	-23.37621	120.26668	Scrape
EJ1209R	-23.37486	120.26813	Scrape
			Trap 1
EJ1210R	-23.37443	120.26814	Scrape
			Trap 1
EJ1249R	-23.38035	120.26378	Scrape
			Trap 1
EJ1250R	-23.37817	120.26516	Scrape
			Trap 1
EJ1254R	-23.36614	120.26081	Scrape
			Trap 1
EJ1566R	-23.38456	120.24374	Scrape
			Trap 1
EJ1619R	-23.37086	120.24474	Scrape
			Trap 1
			Trap 2
EJ1620R	-23.38518	120.2452	Scrape
			Trap 1
EJ2140R	-23.37019	120.25984	Scrape
			Trap 1
EJ2287R	-23.36784	120.26571	Scrape
			Trap 1
EJ2301R	-23.36624	120.2662	Scrape
			Trap 1
EJ2312R	-23.36784	120.26719	Scrape
			Trap 1
EJ2323R	-23.36894	120.26763	Scrape
			Trap 1
			Trap 2

Bore Name	Latitude	Longitude	Sample Type
EJ2375R	-23.38087	120.26314	Scrape
			Trap 1
EJ2376R	-23.38045	120.26314	Scrape
			Trap 1
HCM0073DG	-23.38891	120.25069	Scrape
			Trap 1
HCM0074DG	-23.3875	120.2694	Scrape
			Trap 1
HH0859R	-23.38585	120.20616	Scrape
			Trap 1
HH0880R	-23.38596	120.2142	Scrape
			Trap 1
			Trap 2
HH0881R	-23.38759	120.21417	Scrape
			Trap 1
HH2034R	-23.3783	120.22763	Scrape
			Trap 1
HH2053R	-23.36977	120.22759	Scrape
			Trap 1
			Trap 2
HH3571DG	-23.37733	120.26987	Scrape
			Trap 1
HH3572DG	-23.38318	120.22133	Scrape
			Trap 1
HUB0028DG	-23.3869	120.21905	Scrape
			Trap 1
HUB0029DG	-23.38727	120.21803	Scrape
			Trap 1

Appendix 2: Animals submitted to WAM and their registration numbers and sequences uploaded to GenBank and their accession numbers

Species	Collection Location	Latitude	Longitude	Bennelongia Specimen Number	WAM Reg Number	GenBank Accession Number
Troglarmadillo sp. B07	HCM0073DG	-23.38891	120.25069	753653	C82234	
Ctenistini `BCO249`	HH0880R	-23.38596	120.2142	753780	I84296	
Tyrannochthonius `BPS499`	MN0675R	-22.81668	119.12642	752906		PP759356
Cryptops `BSCOL111`	YW3955DG	-22.74039	119.04099	749071		PP759357
Cryptops 'BSCOL091	YE2029R	-22.78892	119.15508	735805		PP759358
Cryptops 'BSCOL091	YE2033R	-22.79168	119.15498	746840		PP759359
Hanseniella sp. B42-DNA	MN2307R	-22.82479	119.09358	750240		PP759360
Hanseniella sp. B19	HES0001	-23.34342	119.72628	745862		PP759361
Hanseniella sp. B35	SF3712R	-23.01647	118.98569	637354		PP759362
Japygidae sp. B34	MN1472R	-22.82647	119.12997	713587		PP759363
Japygidae sp. B34	MN2662R	-22.83889	119.10877	715711		PP759364
Japygidae `BDP165`	CM0149R	-23.37063	120.28077	751329		PP759365
Japygidae `BDP165`	EOP0928R	-23.32733	119.90198	755426		PP759366
Japygidae sp. B34	MN0310R	-22.82274	119.09145	751691		PP759367
Japygidae sp. B34	MN2627R	-22.81157	119.07987	752123		PP759368
Japygidae sp. B34	MN2534R	-22.83089	119.11572	752896		PP759369

Appendix 3: Stygofauna bycatch from the 2023 Targeted Troglofauna survey

Taxon Identification	Number Of Specimens	Collection Bore Name(s)
Annelida		
Enchytraeidae		
Enchytraeidae `2 bundle` s.l. (short sclero 2 per seg)	1	HH3572DG
Enchytraeidae `2 bundle` s.l. (short sclero 4 per seg)	54	CM0156R, EJ1209R, EJ0788R, CM0129R, and CM0175R
Tubificida		
Tubificinae sp.	3	HUB0029DG
Arthropoda		
Cyclopoida		
Microcyclops varicans	1	HUB0029DG
Thermocyclops sp.	2	HCM0074DG
Nematoda		
Nematoda spp.	6	HH3571DG, EJ2140R, EJ0953R, EJ0606R, EJ2312R, CM0129R and EJ2323R

Appendix 4: Distance tables resulting from genetic analysis

Table 1: *Cryptops* species distance table with target individual coloured red. Blue indicates within species variation if more than one specimen compared to each other

	Cryptops BSCOL067	*Cryptops* B24	*Cryptops* B06	*Cryptops* BSCOL091	*Cryptops* B20	*Cryptops* MH2	*Cryptops* BSCOL064	***Cryptops* BSCOL066**	*Cryptops* BSCOL111	*Cryptops* BSCOL112	*Cryptops* BSCOL111
Cryptops BSCOL067	0.02										
Cryptops B24	0.18	0.07									
Cryptops B06	0.17	0.17	0.03								
Cryptops BSCOL091	0.21	0.17	0.18	0.01							
Cryptops B20	0.18	0.19	0.19	0.18	0.03						
Cryptops MH2	0.20	0.22	0.20	0.18	0.17	0.00					
Cryptops BSCOL064	0.21	0.20	0.19	0.18	0.19	0.21	0.01				
***Cryptops* BSCOL066**	0.23	0.20	0.22	0.24	0.24	0.26	0.23	n/a			
Cryptops BSCOL111	0.21	0.23	0.23	0.24	0.24	0.24	0.24	0.19	n/a		
Cryptops BSCOL112	0.22	0.23	0.25	0.24	0.21	0.22	0.24	0.20	0.18	n/a	
Cryptops BSCOL111	0.24	0.24	0.24	0.24	0.23	0.24	0.21	0.19	0.19	0.19	n/a

Table 2: *Scutigerella* species distance table with target individual coloured red. Blue indicates within species variation if more than one specimen compared to each other

	Scutigerella BSYM111	*Hanseniella* BSYM110	*Hanseniella* BSYM130	*Hanseniella* B35	*Scutigerella* B06	***Scutigerella* BSYM113**	*Hanseniella* BSYM095	*Hanseniella* B31	*Hanseniella* B19	*Hanseniella* B23	*Hanseniella* BSYM131	*Hanseniella* BSYM107	*Hanseniella* B36	*Hanseniella* B42
Scutigerella BSYM111	n/a													
Hanseniella BSYM110	0.25	n/a												
Hanseniella BSYM130	0.27	0.17	0.01											
Hanseniella B35	0.29	0.21	0.17	0.09										
Scutigerella B06	0.25	0.20	0.22	0.21	n/a									
***Scutigerella* BSYM113**	0.25	0.20	0.19	0.19	0.13	0.04								
Hanseniella BSYM095	0.24	0.21	0.21	0.22	0.18	0.20	n/a							
Hanseniella B31	0.26	0.19	0.19	0.19	0.18	0.20	0.18	0.02						
Hanseniella B19	0.24	0.19	0.20	0.20	0.19	0.20	0.20	0.21	0.01					
Hanseniella B23	0.26	0.21	0.20	0.21	0.22	0.20	0.20	0.19	0.21	n/a				
Hanseniella BSYM131	0.26	0.22	0.20	0.19	0.20	0.19	0.19	0.21	0.22	0.21	n/a			
Hanseniella BSYM107	0.26	0.18	0.19	0.19	0.21	0.19	0.20	0.20	0.20	0.19	0.19	n/a		
Hanseniella B36	0.24	0.20	0.21	0.20	0.22	0.20	0.21	0.20	0.20	0.21	0.19	0.19	n/a	
Hanseniella B42	0.26	0.21	0.20	0.23	0.21	0.20	0.20	0.20	0.21	0.22	0.18	0.18	0.18	0.01

Table 3: Japygidae species distance table with target individual coloured red. Blue indicates within species variation if more than one specimen compared to each other

	BDP165	B34	BDP165	BDP162	BDP156	BDP154	BDP204	**BDP192**	BDP206	BDP187	S02
BDP165	0.02										
B34	0.10	0.01									
BDP165	0.09	0.09	0.04								
BDP162	0.15	0.16	0.17	0.00							
BDP156	0.17	0.17	0.18	0.11	0.03						
BDP154	0.17	0.16	0.18	0.16	0.15	0.02					
BDP204	0.20	0.19	0.22	0.16	0.19	0.15	n/a				
BDP192	0.20	0.21	0.21	0.19	0.17	0.15	0.13	n/a			
BDP206	0.18	0.20	0.22	0.20	0.19	0.18	0.21	0.21	0.02		
BDP187	0.19	0.20	0.21	0.20	0.19	0.16	0.22	0.18	0.20	n/a	
S02	0.20	0.22	0.22	0.18	0.22	0.22	0.25	0.21	0.21	0.20	n/a

Appendix 5: Updated list of *Hanseniella* sp. B35 Records

Bore Name	Latitude	Longitude	Sample Type	Collection Date	Updated Species Name	Original Name	Number of Specimens
SF3712R	-23.01647	118.98569	Scrape	17/02/2016	*Hanseniella* sp. B35	*Scutigerella* sp. B03	2
SF0119R	-23.00935	118.85387	Scrape	16/02/2016	*Hanseniella* sp. B35	*Hanseniella* sp. B35	1
SF0141R	-23.01052	118.9826	Trap 1	28/04/2016	*Hanseniella* sp. B35	*Scutigerella* sp. B03	1
SF0146R	-23.01269	118.98848	Net	16/02/2010	*Hanseniella* sp. B35	*Scutigerella* sp. B03	4
SF0363R	-22.97961	118.84171	Scrape	19/03/2010	*Hanseniella* sp. B35	*Scutigerella* sp. B03	1