Jimblebar North

Short- range Endemic Invertebrate Fauna

Molecular Systematics Analysis

Biologic Environmental Survey

Report to BHP Billiton Iron Ore Pty Ltd

February 2021

DOCUMENT STATUS				
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GLOSSARY

COI	Cytochrome Oxidase subunit 1, a mitochondrial gene commonly used in phylogenetic studies and used as a DNA barcode to identify species
Bootstrap	Value between 0 and 100 that indicates the robustness of the node in a phylogenetic tree
GenBank	Annotated open access sequence database of all publicly available nucleotide sequences and their protein translations
OTU	Operational taxonomic unit – species-equivalent taxonomic unit based on COI or 12S cluster similarity

1 INTRODUCTION

BHP commissioned Biologic Environmental Survey (Biologic) to undertake a molecular systematics analysis (DNA barcoding) of 31 specimens collected from Jimblebar North (the Study Area). In addition, we included nine isopod specimens that represented known morphospecies in the region for direct comparison with isopods collected from the Study Area.

1.1 Aims and objectives

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

- Undertake DNA sequencing of 40 subterranean fauna specimens (31 from Study Area, 9 for comparison with isopods) to obtain barcoding sequences of the mitochondrial gene Cytochrome Oxidase I (COI; Hebert, Ratnasingham, *et al.*, 2003).
- Investigate the interspecific and intraspecific relationships between sequences of each higher taxonomic group (*i.e.* use the results of the DNA analysis to indicate how many different species/ OTUs are likely to occur within each genus or relevant higher taxon, based on published species-thresholds); and
- Investigate the relationships between sequences from the Study Area and relevant previous sequences from the wider Pilbara region, using available DNA databases (*i.e.* compare the results of the current analysis with accessible DNA databases to assess whether any of the species/ OTUs from the Study Area have been collected previously or more widely beyond the Study Area).

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

2 METHODS

2.1 Sub-sample preparation

In 2019, Biologic undertook a Level 2 Short-range Endemic (SRE) invertebrate Survey at the Jimblebar North Study Area (Biologic, 2020b). A number of morphospecies required further analysis due to their taxonomic uncertainty; these were identified as Potential SRE species and required further clarification of species identification and regional distribution. These species were:

- Araneae, *Karaops* sp. indet;
- Pseudoscorpiones, *Austrohorus* sp. JIM 02;
- Pseudoscorpiones, *Austrohorus* sp. indet.;
- Pseudoscorpiones, *Indolpium* sp. JIM-03;
- Pseudoscorpiones, *Indolpium* sp. JIM-04;
- Pseudoscorpiones, *Indolpium* sp. indet.;

- Pseudoscorpiones, Olpiidae sp. indet.; and
- Isopoda, *Buddelundia* sp. indet.

Due to the general lack of available sequences for *Buddelundia* species in the public literature, additional specimens of previously collected species of *Buddelundia* from the Jimblebar/ Newman area were obtained from Dr Simon Judd's reference collection. Dr Simon Judd has been a consultant taxonomist for terrestrial Isopoda in Western Australia (WA) for the last 15 years. Dr Judd has published keys and descriptions of this group and has collected specimens from throughout the Pilbara and the rest of WA. Dr Judd also has a WA Museum endorsed reference collection of WA isopods from throughout the state. Reference specimens added to the current survey's specimens for molecular analysis.

A total of 31 specimens were selected for molecular systematics analysis (Table 2.1). These were collected from the Study Area as well as from the East Jimblebar and Caramulla SRE Invertebrate Survey (Biologic, 2020a) undertaken by Biologic. The specimens were chosen based on their geographic spread across the Jimblebar Study Areas to assist with understanding species distributions. Adequate redundancy in specimen selection was incorporated to account for any potential sequence generation failure. Specimens in good condition were chosen to increase their DNA extraction potential. Nine specimens of isopods were sourced from Dr Simon Judd's taxonomic collection. These specimens represented six morphospecies that may match the isopod sequences from the Study Area.

Where whole specimens were available, tissue preparation was undertaken by removing a leg or another body part less important for taxonomic identification, briefly drying off the ethanol, and placing the tissue in ATL buffer. In some instances, for very small and/or juvenile specimens, the entire animal was utilised. Again, these were briefly dried and placed in ATL buffer. Greatest care was taken to decontaminate all tools and equipment between samples, using bleach and repeated rinsing in deionised water. Table 2.1 provides details of the taxonomic orders chosen for molecular analysis. Further taxonomic clarification for each specimen included in the analysis can be found in Appendix A.

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

Class/Subclass	Order	Number of samples	PCR success	Sequence success	% sequence success
Arachnida	Pseudoscorpiones	29	23	22	75.8%
Arachnida	Araneae	1	1	1	100%
Malacostraca	Isopoda	1	1	1	100%
Malacostraca	Isopoda (comparative dataset)	9	8	8	88.9%
TOTAL		**40**	**33**	**32**	**80%**

2.2 DNA extraction, amplification and sequencing

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

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

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

2.3 Specimen selection for comparative analysis

DNA comparisons were typically conducted at the order level (Table 2.1). However, in cases where there was an abundance of sequences, secondary comparisons were also undertaken at the family level. For groups where taxonomic clarification at the order level was not possible due to low numbers of specimens (e.g. <10), comparisons between sequences remained at the class level. Please refer to the relevant section of the Results and Discussion for clarification of the taxonomic resolution for each group (Sections 3.1 -3.3.).

Comparative sequences were taken from different sources and analysed using two separate methods.

Sources

- Biologic Sequence Library: sequences produced from previous jobs undertaken by Biologic, which is comprised of 3,076 quality assured DNA sequences.
- GenBank: Annotated open access sequence database of all publicly available nucleotide sequences and their protein translations.
- Previous molecular data from the study area where available. The analysis of *Karaops* sp. (Araneae) included sequences obtained from a previous report (Dolman & Castalanelli, 2013).

Methods

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

The final datasets for each taxonomic group were supplemented with an appropriate outgroup sequence to root the final phylogeny, and any duplicate sequences (identical sequences from the same specimen found in multiple databases) were removed.

2.4 Analysis and interpretation of sequence alignments/divergence

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

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

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

2.5 Constraints and Limitations

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

The databases used for regional comparisons included GenBank. While these sequence databases, in combination, comprise a large portion of the subterranean fauna genetic work undertaken in the Pilbara region, it is acknowledged that there may be many other relevant sequences from third party project areas nearby or elsewhere in the region that were not available for comparison at the time of the study. GenBank is dynamic database, and the addition of new sequences and altered taxonomic classifications could not be included into this report if they occurred after the 16th June 2020.

DNA barcoding using the mitochondrial gene COI, while useful for explaining genetic differences between closely related or moderately related species, is limited in its ability to resolve deeper phylogenetic relationships among taxa at higher taxonomic levels (*e.g.* genus, family, order). In the current study, phylogenetic relationships among species/OTUs at >25% COI divergence are treated

with caution (Simon, 1994). If further resolution of deeper phylogeny is important for project goals, this could be investigated using a multiple gene approach.

3 RESULTS AND DISCUSSION

A total of 40 specimens were processed for sequencing by Biologic. Sequences were successfully derived for 33 of these specimens (80.25% of specimens), with seven failing to produce a PCR product. Of these 33 sequences, one did not produce a sequence of the target organism (likely contamination). This left 32 high quality sequences for analysis (80% of sequences), with 24 from the Study Area. The orders of the sequences are tabulated in Table 2.1.

In total, seven OTUs have been designated to specimens from the Study Area; six of these being specific to the Jimblebar area (Table 3.1). The results of each taxonomic group's analysis are described in the subsequent sections and the full list of OTUs per order can be found in Appendix A. Locations of the specimens from which the sequences were derived are shown in Figure 3.1.

Table 3.1: Summary of species and OTUs recovered from 24 samples successfully from the Study Area, organised by taxon.

Species/OTU	Number of specimens from Study Area	Unique to Study Area?	Linear Distance
Pseudoscorpiones	**22**		
Austrohorus `sp. Biologic-PSEU023`	1	Yes	-
Austrohorus `sp. Biologic-PSEU024`	4	Yes	8.01 km
Austrohorus `sp. Biologic-PSEU025`	4	Yes	14.6 km
Indolpium `sp. Biologic-PSEU026`	5	Yes	10.56 km
Indolpium `sp. Biologic-PSEU027`	8	Yes	14.4 km
Araneae	**1**		
Karaops `sp. WAM-ARA004`	1	No	23 km
Isopoda	**1**		
Buddelundia `sp. Biologic-ISOP014`	1	Yes	-

789500 793000 796500 800000 803500 807000 810500 814000 817500 821000 824500 828000 831500 835000 838500 842000 845500 849000

7429500 7426000 7422500 7419000 7415500 7412000 7408500 7405000 7401500 7398000

Legend

Project OTU Name, Legacy ID

- Karaops `sp. WAM-ARA004`;Karaops sp.

WAM Database Karaops records

- Karaops `ARA003-DNA`
- Karaops `ARA004-DNA`

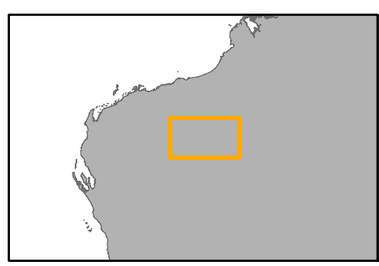
Isopoda OTU_Name/species

- Buddelundia `sp. Biologic-ISOP014`
- Buddelundia 10nm
- Buddelundia 14cr
- Buddelundia 16NM
- Buddelundia 36nm
- Buddelundia 49
- Buddelundiinae wn

Legend

Project OTU Name; Legacy ID

- Austrohorus `sp. Biologic-PSEU023`;Austrohorus sp. JIM 02
- Austrohorus `sp. Biologic-PSEU024`;Austrohorus sp. JIM 01
- Austrohorus `sp. Biologic-PSEU024`;Austrohorus sp. JIM 02
- Austrohorus `sp. Biologic-PSEU025`;Austrohorus sp.
- Austrohorus `sp. Biologic-PSEU025`;Austrohorus sp. JIM 01
- Austrohorus `sp. Biologic-PSEU025`;Austrohorus sp. JIM 02
- Austrohorus `sp. Biologic-PSEU025`;Indolpium sp.
- Indolpium `sp. Biologic-PSEU026`;Indolpium sp.
- Indolpium `sp. Biologic-PSEU026`;Indolpium sp. JIM 04
- Indolpium `sp. Biologic-PSEU026`;Olpiidae sp.
- Indolpium `sp. Biologic-PSEU027`;Indolpium sp.
- Indolpium `sp. Biologic-PSEU027`;Indolpium sp. JIM 03
- Indolpium `sp. Biologic-PSEU027`;Indolpium sp. JIM 04
- N/A;Indolpium sp.
- N/A;Indolpium sp. JIM 04



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N

1:152,333

0 1 2 4 6 8 km

BHP Western Australian Iron Ore

North Jimblebar SRE Invertebrate Survey

Figure 3.1: Locations of specimens from which project sequences were generated.

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994

Size A3. Created 14/01/2020

3.1 Pseudoscorpiones

The 22 pseudoscorpion sequences were BLASTed against 3, 076 sequences in Biologic's sequence databases, which included sequences from 216 pseudoscorpion specimens. The closest matches were combined with 31 olpiid sequences from GenBank to create an alignment of 103 sequences. The 22 sequences from the Study Area were not genetically close enough to be considered conspecific with any sequences outside of the Study Area. The alignment was then reduced down to just include sequences from GenBank and the Study Area for the presented tree (Fig 3.1.1) and pairwise distance table (Table 3.1.1).

The 22 pseudoscorpion sequences revealed 5 OTUs, from two separate genera (Table 3.1). The three *Austrohorus* OTUs (*Austrohorus* `sp. Biologic-PSEU023`. *Austrohorus* `sp. Biologic-PSEU024`, and *Austrohorus* `sp. Biologic-PSEU025`) were all closely related (8.7-15.2%), and had some genetic structure within these species (up to 7.0% in *Austrohorus* `sp. Biologic-PSEU025`, and up to 7.7% in *Austrohorus* `sp. Biologic-PSEU024`). Only *Austrohorus* `sp. Biologic-PSEU023` was a singleton sequence that did not match either of the other two *Austrohorus* OTUs and was only found in the Jimblebar North Study Area.

The two *Indolpium* OTUs were more divergent from other OTUs and each other (>13.1%), and similarly revealed some intraspecific divergence (up to 7.8% in *Indolpium* `sp. Biologic-PSEU026` and up to 7.0% in *Indolpium* `sp. Biologic-PSEU027`). Both Indolpium OTU's occurred in both the Jimblebar North Study Area and the East Jimblebar and Caramulla Study Area. Where more than one specimen was sampled for an OTU, geographic linear distances ranged between 8 and 14km (Table 3.1).

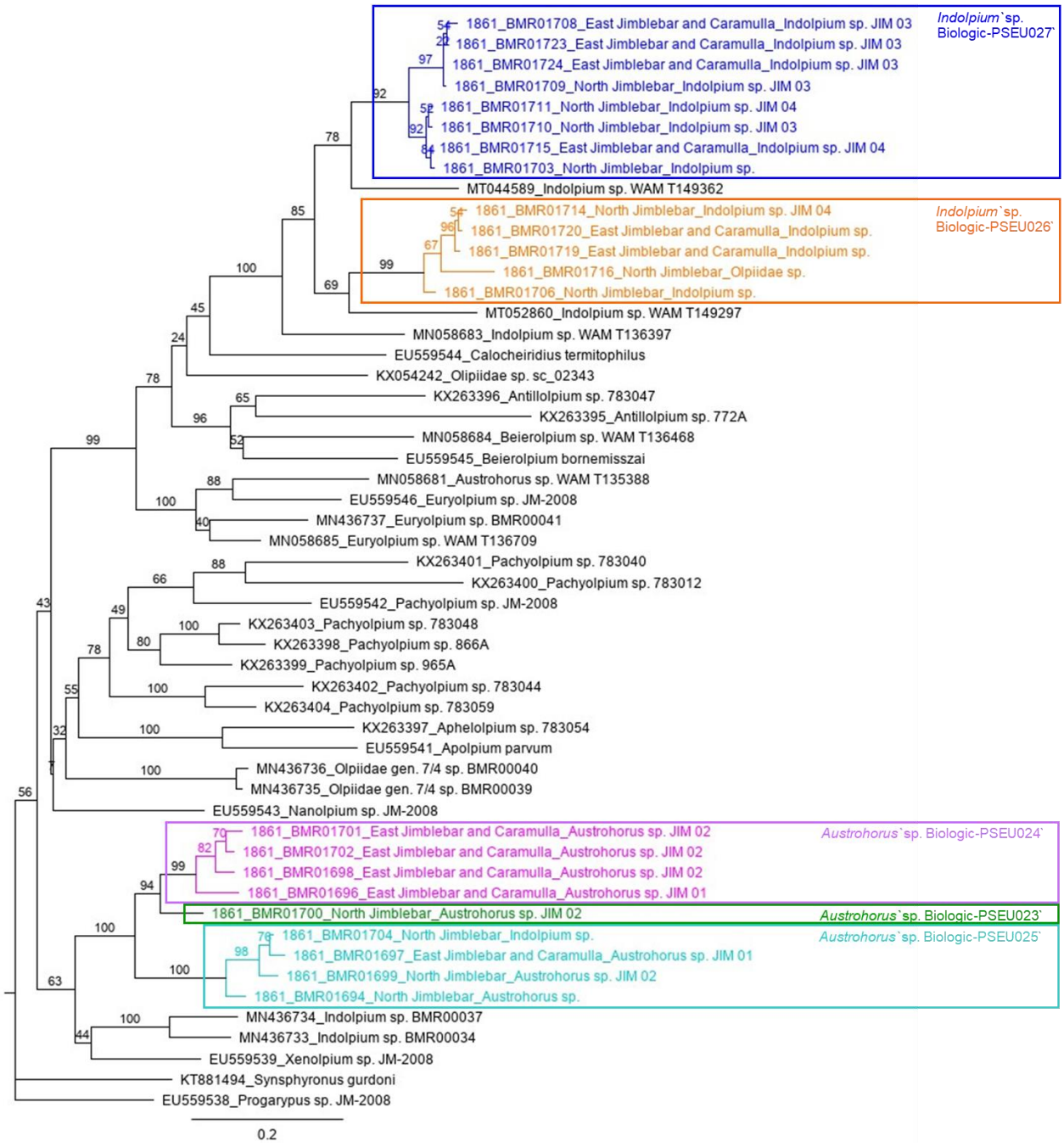

Figure 3.1.1: Maximum Likelihood phylogeny, with bootstrap values.

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

COI divergences (%) of Olpiidae (Pseudoscorpiones)	BMR01694	BMR01697	BMR01704	BMR01699	BMR01696	BMR01698	BMR01701	BMR01702	BMR01700	EU559539	MN436733	MN436734	MN436735	MN436736	EU559542	EU559543	KX263398	KX263403	KX263399	KX263402	KX263404	EU559538	KT881494	KX263401	KX263400	EU559541	KX263397	BMR01703	BMR01715	BMR01710	BMR01711	BMR01708	BMR01709	BMR01723	BMR01724	MT044589	BMR01706	BMR01714	BMR01720	BMR01719	BMR01716	MT052860	MN058683	EU559544	EU559545	KX054242	EU559546	MN058685	MN436737	MN058681	MN058684	KX263396	KX263395
1861_BMR01694_North Jimblebar_Austrohorus sp.		6.7	6.0	7.0	13.5	13.9	14.2	13.9	13.1	16.9	17.3	17.8	19.8	19.7	19.8	19.4	17.6	18.3	20.0	19.4	18.3	18.9	19.7	22.6	23.2	21.7	23.6	22.3	22.1	22.1	22.4	23.0	22.3	22.0	21.5	23.8	22.1	23.4	23.4	23.4	23.4	22.9	23.2	20.9	24.0	21.5	24.6	21.7	21.5	23.1	25.2	22.6	26.0
1861_BMR01697_East Jimblebar and Caramulla_Austrohorus sp. JIM 01	6.7		2.0	3.8	15.3	15.1	14.5	14.8	15.3	17.9	18.3	18.1	20.9	20.8	20.9	19.2	20.8	20.3	21.2	20.5	21.2	19.1	19.8	23.5	25.2	23.1	25.5	23.2	23.1	23.4	23.7	23.7	23.4	22.7	22.6	24.3	22.4	24.0	24.0	24.0	23.4	23.5	24.0	21.8	24.4	22.7	25.8	22.9	22.4	24.0	26.6	23.2	26.5
1861_BMR01704_North Jimblebar_Indolpium sp.	6.0	2.0		3.4	15.3	14.5	14.5	14.8	14.4	17.1	18.2	18.4	21.2	21.1	21.2	19.2	19.7	20.5	20.8	19.8	20.5	19.2	19.8	23.4	24.9	21.8	25.0	22.6	22.4	22.7	23.1	23.7	23.1	22.7	22.3	24.0	21.8	24.0	24.0	24.0	22.9	23.4	23.5	21.6	23.8	22.1	25.5	22.6	22.0	24.0	25.8	23.1	26.0
1861_BMR01699_North Jimblebar_Austrohorus sp. JIM 02	7.0	3.8	3.4		14.7	14.5	14.8	14.5	13.6	16.8	17.9	17.6	21.4	21.2	20.8	18.9	18.8	19.1	20.0	20.2	19.5	18.3	19.7	22.7	24.0	22.4	25.4	22.4	22.3	22.6	22.9	23.4	22.8	22.4	22.0	24.1	22.1	24.0	24.0	24.0	22.9	22.4	23.4	21.5	24.1	22.0	24.6	22.3	22.2	22.7	25.2	22.9	25.7
1861_BMR01696_East Jimblebar and Caramulla_Austrohorus sp. JIM 01	13.5	15.3	15.3	14.7		7.7	7.2	6.9	10.2	18.3	16.4	17.6	18.8	19.1	23.0	17.2	18.8	19.1	19.0	20.2	20.3	19.4	22.1	24.5	26.3	23.7	22.7	23.8	24.1	24.4	24.4	24.4	23.8	23.4	22.9	23.6	23.6	23.9	23.7	23.9	23.1	21.8	24.7	22.1	24.1	24.0	24.2	21.4	22.0	23.1	24.4	22.8	26.4
1861_BMR01698_East Jimblebar and Caramulla_Austrohorus sp. JIM 02	13.9	15.1	14.5	14.5	7.7		4.0	4.0	8.7	16.8	15.9	17.2	19.1	18.9	21.1	16.6	18.9	18.5	17.4	20.2	19.5	18.9	19.8	23.4	23.5	22.7	22.3	23.7	23.8	24.4	24.0	25.0	24.7	24.0	23.8	24.6	23.4	24.0	23.5	23.4	23.7	22.7	24.6	21.0	24.0	22.9	23.8	21.1	21.3	22.6	24.7	21.4	27.1
1861_BMR01701_East Jimblebar and Caramulla_Austrohorus sp. JIM 02	14.2	14.5	14.5	14.8	7.2	4.0		2.6	9.9	17.7	16.5	17.0	18.5	18.6	21.1	16.8	20.0	19.5	17.9	20.8	19.8	19.4	20.5	23.6	24.0	22.3	22.8	24.0	24.1	24.1	24.0	24.8	24.1	23.8	23.4	24.1	22.9	22.4	22.6	22.7	24.1	22.9	24.0	21.8	24.1	23.5	23.4	21.1	21.7	22.7	24.9	22.4	26.9
1861_BMR01702_East Jimblebar and Caramulla_Austrohorus sp. JIM 02	13.9	14.8	14.8	14.5	6.9	4.0	2.6		9.2	17.3	15.9	17.0	18.8	18.6	21.5	17.1	19.4	19.4	18.5	20.6	19.8	19.8	20.2	22.9	24.4	22.7	22.1	23.2	23.4	23.7	23.4	24.2	23.4	23.2	22.7	24.3	23.2	23.8	23.7	23.5	24.1	22.3	24.1	21.5	24.1	23.7	24.0	21.1	21.8	23.2	24.9	22.1	26.2
1861_BMR01700_North Jimblebar_Austrohorus sp. JIM 02	13.1	15.3	14.4	13.6	10.2	8.7	9.9	9.2		14.8	17.1	17.9	18.9	19.1	20.9	17.4	18.5	18.5	17.3	18.0	18.2	19.4	20.0	23.9	24.1	21.8	22.4	22.7	22.6	22.9	22.7	23.7	23.1	22.9	22.4	24.7	22.3	22.9	22.9	23.1	23.1	22.7	22.3	21.6	21.8	22.6	22.3	21.1	21.8	22.9	24.4	22.3	27.3
EU559539_Xenolpium sp. JM-2008	16.9	17.9	17.1	16.8	18.3	16.8	17.7	17.3	14.8		16.0	16.0	17.4	17.3	19.5	18.3	18.5	17.7	18.8	19.5	18.8	18.6	18.9	22.7	24.4	21.1	20.1	22.4	22.1	22.0	22.0	23.4	22.6	22.3	21.8	22.9	22.9	24.6	24.4	24.4	23.7	21.2	21.4	20.6	20.9	21.8	21.7	20.3	20.2	21.1	23.7	22.6	25.9
MN436733_Indolpium sp. BMR00034	17.3	18.3	18.2	17.9	16.4	15.9	16.5	15.9	17.1	16.0		10.3	16.3	16.5	22.4	19.5	19.5	19.2	18.9	20.3	19.7	20.9	20.2	23.2	24.9	22.7	20.9	23.7	23.7	23.7	23.8	23.9	23.9	22.9	23.1	24.9	22.4	22.7	22.6	23.1	22.7	21.2	23.1	18.3	21.7	22.1	22.7	20.6	20.1	22.1	25.0	23.2	25.3
MN436734_Indolpium sp. BMR00037	17.8	18.1	18.4	17.6	17.6	17.2	17.0	17.0	17.9	16.0	10.3		17.8	17.5	20.7	19.3	19.2	18.2	19.9	20.5	19.5	18.2	18.7	23.6	24.5	24.2	22.4	23.1	23.3	23.1	23.3	24.1	23.5	23.3	22.8	23.6	22.5	23.1	23.3	23.4	23.0	21.0	23.4	21.0	22.7	23.6	21.8	19.5	21.2	22.2	24.7	23.9	26.7
MN436735_Olpiidae gen. 7/4 sp. BMR00039	19.8	20.9	21.2	21.4	18.8	19.1	18.5	18.8	18.9	17.4	16.3	17.8		1.8	20.6	19.4	20.0	18.5	19.2	20.9	20.8	20.8	20.5	23.9	24.3	21.1	20.0	25.2	25.0	25.0	25.2	26.5	26.3	25.5	25.3	25.0	22.9	23.4	22.9	23.4	22.7	24.1	23.8	20.0	23.2	23.7	22.3	19.8	22.4	21.5	23.7	22.9	26.3
MN436736_Olpiidae gen. 7/4 sp. BMR00040	19.7	20.8	21.1	21.2	19.1	18.9	18.6	18.6	19.1	17.3	16.5	17.5	1.8		20.2	19.5	20.2	18.0	19.4	21.1	21.2	21.2	20.2	24.2	24.1	21.1	20.0	25.8	25.6	25.6	25.8	27.0	26.7	26.0	25.8	26.0	22.6	23.1	22.6	23.4	23.1	24.6	24.3	20.6	23.7	23.1	22.7	20.5	22.2	21.4	24.1	23.1	26.5
EU559542_Pachyolpium sp. JM-2008	19.8	20.9	21.2	20.8	23.0	21.1	21.1	21.5	20.9	19.5	22.4	20.7	20.6	20.2		19.8	17.3	18.0	17.1	18.0	16.8	20.6	21.4	20.7	20.2	21.4	22.6	22.9	22.6	23.1	22.6	25.7	25.2	24.7	24.3	22.9	22.4	21.4	21.7	21.8	23.1	23.7	23.5	21.2	22.7	22.0	23.4	22.1	22.6	23.8	25.0	25.2	27.6
EU559543_Nanolpium sp. JM-2008	19.4	19.2	19.2	18.9	17.2	16.6	16.8	17.1	17.4	18.3	19.5	19.3	19.4	19.5	19.8		18.3	17.7	16.8	20.6	19.7	17.9	19.4	25.1	23.5	22.4	22.8	23.2	23.1	23.4	22.9	25.0	24.2	23.8	23.4	23.1	23.1	23.1	22.7	22.9	22.4	22.1	22.9	21.0	23.5	22.0	21.2	20.2	21.1	23.1	24.9	23.4	26.8
KX263398_Pachyolpium sp. 866A	17.6	20.8	19.7	18.8	18.8	18.9	20.0	19.4	18.5	18.5	19.5	19.2	20.0	20.2	17.3	18.3		6.3	12.7	17.9	16.4	18.2	20.2	22.1	21.2	21.8	21.8	21.7	21.7	22.4	22.1	23.6	23.0	22.4	22.0	23.2	24.0	24.4	24.1	24.3	24.1	22.1	22.9	20.9	23.7	21.8	23.2	21.4	21.7	22.7	24.6	24.1	27.1
KX263403_Pachyolpium sp. 783048	18.3	20.3	20.5	19.1	19.1	18.5	19.5	19.4	18.5	17.7	19.2	18.2	18.5	18.0	18.0	17.7	6.3		11.2	17.5	16.3	17.3	17.7	20.2	20.5	21.8	21.4	23.8	23.8	23.7	23.8	25.0	24.5	24.1	23.7	24.1	24.1	25.5	25.2	25.5	24.7	21.7	23.5	20.9	23.4	22.4	22.9	21.1	22.6	23.1	24.6	24.2	27.0
KX263399_Pachyolpium sp. 965A	20.0	21.2	20.8	20.0	19.0	17.4	17.9	18.5	17.3	18.8	18.9	19.9	19.2	19.4	17.1	16.8	12.7	11.2		16.7	15.5	18.3	20.2	20.5	20.0	20.8	20.3	24.3	24.3	24.3	24.3	25.1	24.5	24.1	23.7	22.1	22.9	22.9	22.4	22.9	23.5	23.4	22.4	22.1	23.1	22.3	21.1	20.8	21.5	22.4	24.0	24.1	26.6
KX263402_Pachyolpium sp. 783044	19.4	20.5	19.8	20.2	20.2	20.2	20.8	20.6	18.0	19.5	20.3	20.5	20.9	21.1	18.0	20.6	17.9	17.5	16.7		11.7	20.8	20.9	22.2	22.3	20.6	21.6	22.7	22.6	22.7	22.6	24.3	23.9	23.2	22.9	22.3	21.5	22.3	22.3	22.6	21.8	22.1	23.2	22.4	23.5	22.7	21.4	21.4	21.3	23.2	24.7	23.5	26.2
KX263404_Pachyolpium sp. 783059	18.3	21.2	20.5	19.5	20.3	19.5	19.8	19.8	18.2	18.8	19.7	19.5	20.8	21.2	16.8	19.7	16.4	16.3	15.5	11.7		19.1	19.8	22.3	21.5	19.8	21.8	22.1	22.0	21.8	22.0	24.3	23.7	23.2	22.7	22.6	22.9	22.9	22.4	23.1	22.3	21.7	22.9	21.5	22.7	20.8	22.1	21.1	21.7	22.3	23.7	25.0	25.1
EU559538_Progarypus sp. JM-2008	18.9	19.1	19.2	18.3	19.4	18.9	19.4	19.8	19.4	18.6	20.9	18.2	20.8	21.2	20.6	17.9	18.2	17.3	18.3	20.8	19.1		16.9	23.4	23.2	22.1	21.4	24.7	24.7	24.9	24.7	25.3	24.5	24.4	24.0	23.5	25.0	24.3	24.4	24.7	24.4	23.2	25.0	22.7	24.3	24.4	22.6	20.8	22.6	22.9	25.5	24.6	28.2
KT881494_Synsphyronus gurdoni	19.7	19.8	19.8	19.7	22.1	19.8	20.5	20.2	20.0	18.9	20.2	18.7	20.5	20.2	21.4	19.4	20.2	17.7	20.2	20.9	19.8	16.9		23.9	22.4	22.9	24.2	25.0	24.9	25.0	25.2	26.8	26.3	25.6	25.2	25.2	26.6	26.6	26.9	27.2	26.0	26.3	23.4	22.9	24.9	22.9	22.4	21.7	22.2	24.4	26.4	26.3	26.9
KX263401_Pachyolpium sp. 783040	22.6	23.5	23.4	22.7	24.5	23.4	23.6	22.9	23.9	22.7	23.2	23.6	23.9	24.2	20.7	25.1	22.1	20.2	20.5	22.2	22.3	23.4	23.9		22.3	24.6	26.0	26.2	26.4	26.1	25.7	27.4	27.1	27.4	27.1	28.7	26.5	27.4	27.9	27.9	27.6	29.7	26.7	24.2	25.5	28.0	26.4	24.5	25.3	28.6	27.1	27.2	31.5
KX263400_Pachyolpium sp. 783012	23.2	25.2	24.9	24.0	26.3	23.5	24.0	24.4	24.1	24.4	24.9	24.5	24.3	24.1	20.2	23.5	21.2	20.5	20.0	22.3	21.5	23.2	22.4	22.3		25.2	23.9	26.7	26.4	26.9	26.4	28.0	27.5	27.0	26.6	26.4	25.8	25.0	25.3	25.0	26.1	27.6	26.4	25.8	26.4	26.4	25.2	23.2	28.1	26.4	27.5	27.7	29.8
EU559541_Apolpium parvum	21.7	23.1	21.8	22.4	23.7	22.7	22.3	22.7	21.8	21.1	22.7	24.2	21.1	21.1	21.4	22.4	21.8	21.8	20.8	20.6	19.8	22.1	22.9	24.6	25.2		17.3	25.6	25.5	25.5	25.5	27.6	26.9	26.6	26.1	25.0	24.4	24.6	24.7	25.0	25.5	25.6	24.1	23.0	26.4	23.5	24.3	23.5	23.8	23.7	27.6	26.5	29.4
KX263397_Aphelolpium sp. 783054	23.6	25.5	25.0	25.4	22.7	22.3	22.8	22.1	22.4	20.1	20.9	22.4	20.0	20.0	22.6	22.8	21.8	21.4	20.3	21.6	21.8	21.4	24.2	26.0	23.9	17.3		24.7	24.6	24.2	23.9	24.5	24.2	23.2	23.1	23.2	23.6	22.1	22.6	22.6	22.9	23.7	23.6	22.7	23.6	24.9	24.7	20.3	24.3	24.6	24.1	25.1	28.1
1861_BMR01703_North Jimblebar_Indolpium sp.	22.3	23.2	22.6	22.4	23.8	23.7	24.0	23.2	22.7	22.4	23.7	23.1	25.2	25.8	22.9	23.2	21.7	23.8	24.3	22.7	22.1	24.7	25.0	26.2	26.7	25.6	24.7		0.5	1.7	1.7	7.0	6.0	6.1	5.6	14.0	14.2	15.7	15.3	15.0	15.0	17.7	15.9	21.6	22.3	21.2	23.1	21.7	22.0	25.0	22.6	24.1	23.9
1861_BMR01715_East Jimblebar and Caramulla_Indolpium sp. JIM 04	22.1	23.1	22.4	22.3	24.1	23.8	24.1	23.4	22.6	22.1	23.7	23.3	25.0	25.6	22.6	23.1	21.7	23.8	24.3	22.6	22.0	24.7	24.9	26.4	26.4	25.5	24.6	0.5		1.2	1.2	6.9	5.8	6.0	5.5	13.9	14.0	15.6	15.1	14.8	14.8	17.9	15.7	21.5	22.1	20.8	22.9	21.7	22.4	25.0	22.6	24.3	23.9
1861_BMR01710_North Jimblebar_Indolpium sp. JIM 03	22.1	23.4	22.7	22.6	24.4	24.4	24.1	23.7	22.9	22.0	23.7	23.1	25.0	25.6	23.1	23.4	22.4	23.7	24.3	22.7	21.8	24.9	25.0	26.1	26.9	25.5	24.2	1.7	1.2		0.9	6.4	5.5	6.0	5.5	13.3	14.2	15.4	15.0	14.7	14.7	17.9	15.6	21.5	22.3	21.2	22.6	21.2	22.2	25.3	22.4	24.6	23.7
1861_BMR01711_North Jimblebar_Indolpium sp. JIM 04	22.4	23.7	23.1	22.9	24.4	24.0	24.0	23.4	22.7	22.0	23.8	23.3	25.2	25.8	22.6	22.9	22.1	23.8	24.3	22.6	22.0	24.7	25.2	25.7	26.4	25.5	23.9	1.7	1.2	0.9		6.6	5.5	5.8	5.3	13.4	13.9	15.1	14.7	14.0	14.7	17.6	15.6	21.5	22.3	20.6	22.7	21.4	22.4	25.2	22.3	24.3	23.9
1861_BMR01708_East Jimblebar and Caramulla_Indolpium sp. JIM 03	23.0	23.7	23.7	23.4	24.4	25.0	24.8	24.2	23.7	23.4	23.9	24.1	26.5	27.0	25.7	25.0	23.6	25.0	25.1	24.3	24.3	25.3	26.8	27.4	28.0	27.6	24.5	7.0	6.9	6.4	6.6		0.5	1.1	1.5	14.1	15.9	16.8	16.4	15.8	16.5	17.6	17.6	22.2	23.6	22.5	24.0	22.5	22.7	25.3	23.9	24.1	24.0
1861_BMR01709_North Jimblebar_Indolpium sp. JIM 03	22.3	23.4	23.1	22.8	23.8	24.7	24.1	23.4	23.1	22.6	23.9	23.5	26.3	26.7	25.2	24.2	23.0	24.5	24.5	23.9	23.7	24.5	26.3	27.1	27.5	26.9	24.2	6.0	5.8	5.5	5.5	0.5		0.8	0.3	13.7	15.1	16.2	15.7	15.3	16.0	17.0	16.7	21.8	23.3	22.0	23.1	21.9	22.7	24.5	23.4	24.2	24.0
1861_BMR01723_East Jimblebar and Caramulla_Indolpium sp. JIM 03	22.0	22.7	22.7	22.4	23.4	24.0	23.8	23.2	22.9	22.3	22.9	23.3	25.5	26.0	24.7	23.8	22.4	24.1	24.1	23.2	23.2	24.4	25.6	27.4	27.0	26.6	23.2	6.1	6.0	6.0	5.8	1.1	0.8		0.5	13.1	15.0	15.9	15.4	14.8	15.6	16.5	16.5	21.3	22.4	21.5	23.1	21.4	22.7	24.3	22.9	23.5	22.8
1861_BMR01724_East Jimblebar and Caramulla_Indolpium sp. JIM 03	21.5	22.6	22.3	22.0	22.9	23.8	23.4	22.7	22.4	21.8	23.1	22.8	25.3	25.8	24.3	23.4	22.0	23.7	23.7	22.9	22.7	24.0	25.2	27.1	26.6	26.1	23.1	5.6	5.5	5.5	5.3	1.5	0.3	0.5		13.1	14.8	15.7	15.3	14.7	15.4	16.2	16.0	21.2	22.3	21.2	22.6	20.9	22.7	23.8	22.7	23.7	23.0
MT044589_Indolpium sp. WAM T149362	23.8	24.3	24.0	24.1	23.6	24.6	24.1	24.3	24.7	22.9	24.9	23.6	25.0	26.0	22.9	23.1	23.2	24.1	22.1	22.3	22.6	23.5	25.2	28.7	26.4	25.0	23.2	14.0	13.9	13.3	13.4	14.1	13.7	13.1	13.1		16.9	16.8	16.5	16.0	17.6	16.9	18.0	20.3	21.1	23.1	23.2	20.8	24.0	24.7	23.4	24.0	23.9
1861_BMR01706_North Jimblebar_Indolpium sp.	22.1	22.4	21.8	22.1	23.6	23.4	22.9	23.2	22.3	22.9	22.4	22.5	22.9	22.6	22.4	23.1	24.0	24.1	22.9	21.5	22.9	25.0	26.6	26.5	25.8	24.4	23.6	14.2	14.0	14.2	13.9	15.9	15.1	15.0	14.8	16.9		4.6	5.2	4.9	7.8	14.5	15.9	21.3	20.8	21.5	23.2	21.2	22.0	23.2	23.5	22.6	24.2
1861_BMR01714_North Jimblebar_Indolpium sp. JIM 04	23.4	24.0	24.0	24.0	23.9	24.0	22.4	23.8	22.9	24.6	22.7	23.1	23.4	23.1	21.4	23.1	24.4	25.5	22.9	22.3	22.9	24.3	26.6	27.4	25.0	24.6	22.1	15.7	15.6	15.4	15.1	16.8	16.2	15.9	15.7	16.8	4.6		1.2	1.8	6.9	16.6	17.3	21.5	22.1	21.4	22.7	21.2	23.1	23.4	24.3	23.7	25.6
1861_BMR01720_East Jimblebar and Caramulla_Indolpium sp.	23.4	24.0	24.0	24.0	23.7	23.5	22.6	23.7	22.9	24.4	22.6	23.3	22.9	22.6	21.7	22.7	24.1	25.2	22.4	22.3	22.4	24.4	26.9	27.9	25.3	24.7	22.6	15.3	15.1	15.0	14.7	16.4	15.7	15.4	15.3	16.5	5.2	1.2		1.2	7.2	16.2	17.6	21.5	22.1	21.4	22.9	21.5	22.6	23.1	24.4	23.4	24.8
1861_BMR01719_East Jimblebar and Caramulla_Indolpium sp.	23.4	24.0	24.0	24.0	23.9	23.4	22.7	23.5	23.1	24.4	23.1	23.4	23.4	23.4	21.8	22.9	24.3	25.5	22.9	22.6	23.1	24.7	27.2	27.9	25.0	25.0	22.6	15.0	14.8	14.7	14.0	15.8	15.3	14.8	14.7	16.0	4.9	1.8	1.2		7.2	16.2	17.7	21.5	22.1	21.7	22.9	21.2	22.9	23.4	24.1	23.5	24.5
1861_BMR01716_North Jimblebar_Olpiidae sp.	23.4	23.4	22.9	22.9	23.1	23.7	24.1	24.1	23.1	23.7	22.7	23.0	22.7	23.1	23.1	22.4	24.1	24.7	23.5	21.8	22.3	24.4	26.0	27.6	26.1	25.5	22.9	15.0	14.8	14.7	14.7	16.5	16.0	15.6	15.4	17.6	7.8	6.9	7.2	7.2		15.9	16.2	22.3	22.1	21.2	23.2	21.4	22.0	23.7	23.8	23.2	23.3
MT052860_Indolpium sp. WAM T149297	22.9	23.5	23.4	22.4	21.8	22.7	22.9	22.3	22.7	21.2	21.2	21.0	24.1	24.6	23.7	22.1	22.1	21.7	23.4	22.1	21.7	23.2	26.3	29.7	27.6	25.6	23.7	17.7	17.9	17.9	17.6	17.6	17.0	16.5	16.2	16.9	14.5	16.6	16.2	16.2	15.9		17.9	20.7	20.5	20.3	23.5	21.7	22.6	22.4	25.0	23.4	23.9
MN058683_Indolpium sp. WAM T136397	23.2	24.0	23.5	23.4	24.7	24.6	24.0	24.1	22.3	21.4	23.1	23.4	23.8	24.3	23.5	22.9	22.9	23.5	22.4	23.2	22.9	25.0	23.4	26.7	26.4	24.1	23.6	15.9	15.7	15.6	15.6	17.6	16.7	16.5	16.0	18.0	15.9	17.3	17.6	17.7	16.2	17.9		20.3	21.5	21.4	22.4	20.9	21.5	23.5	23.1	22.8	23.9
EU559544_Calocheiridius termitophilus	20.9	21.8	21.6	21.5	22.1	21.0	21.8	21.5	21.6	20.6	18.3	21.0	20.0	20.6	21.2	21.0	20.9	20.9	22.1	22.4	21.5	22.7	22.9	24.2	25.8	23.0	22.7	21.6	21.5	21.5	21.5	22.2	21.8	21.3	21.2	20.3	21.3	21.5	21.5	21.5	22.3	20.7	20.3		18.0	19.8	23.0	20.4	21.7	21.6	22.1	20.2	24.3
EU559545_Beierolpium bornemisszai	24.0	24.4	23.8	24.1	24.1	24.0	24.1	24.1	21.8	20.9	21.7	22.7	23.2	23.7	22.7	23.5	23.7	23.4	23.1	23.5	22.7	24.3	24.9	25.5	26.4	26.4	23.6	22.3	22.1	22.3	22.3	23.6	23.3	22.4	22.3	21.1	20.8	22.1	22.1	22.1	22.1	20.5	21.5	18.0		20.5	22.3	18.9	21.5	20.0	18.8	19.8	21.3
KX054242_Olipiidae sp. sc_02343	21.5	22.7	22.1	22.0	24.0	22.9	23.5	23.7	22.6	21.8	22.1	23.6	23.7	23.1	22.0	22.0	21.8	22.4	22.3	22.7	20.8	24.4	22.9	28.0	26.4	23.5	24.9	21.2	20.8	21.2	20.6	22.5	22.0	21.5	21.2	23.1	21.5	21.4	21.4	21.7	21.2	20.3	21.4	19.8	20.5		22.4	18.9	20.1	21.7	23.8	22.8	25.1
EU559546_Euryolpium sp. JM-2008	24.6	25.8	25.5	24.6	24.2	23.8	23.4	24.0	22.3	21.7	22.7	21.8	22.3	22.7	23.4	21.2	23.2	22.9	21.1	21.4	22.1	22.6	22.4	26.4	25.2	24.3	24.7	23.1	22.9	22.6	22.7	24.0	23.1	23.1	22.6	23.2	23.2	22.7	22.9	22.9	23.2	23.5	22.4	23.0	22.3	22.4		13.9	16.0	16.1	22.4	21.8	25.4
MN058685_Euryolpium sp. WAM T136709	21.7	22.9	22.6	22.3	21.4	21.1	21.1	21.1	21.1	20.3	20.6	19.5	19.8	20.5	22.1	20.2	21.4	21.1	20.8	21.4	21.1	20.8	21.7	24.5	23.2	23.5	20.3	21.7	21.7	21.2	21.4	22.5	21.9	21.4	20.9	20.8	21.2	21.2	21.5	21.2	21.4	21.7	20.9	20.4	18.9	18.9	13.9		12.3	15.4	19.4	21.7	22.7
MN436737_Euryolpium sp. BMR00041	21.5	22.4	22.0	22.2	22.0	21.3	21.7	21.8	21.8	20.2	20.1	21.2	22.4	22.2	22.6	21.1	21.7	22.6	21.5	21.3	21.7	22.6	22.2	25.3	28.1	23.8	24.3	22.0	22.4	22.2	22.4	22.7	22.7	22.7	22.7	24.0	22.0	23.1	22.6	22.9	22.0	22.6	21.5	21.7	21.5	20.1	16.0	12.3		16.7	21.8	22.0	24.6
MN058681_Austrohorus sp. WAM T135388	23.1	24.0	24.0	22.7	23.1	22.6	22.7	23.2	22.9	21.1	22.1	22.2	21.5	21.4	23.8	23.1	22.7	23.1	22.4	23.2	22.3	22.9	24.4	28.6	26.4	23.7	24.6	25.0	25.0	25.3	25.2	25.3	24.5	24.3	23.8	24.7	23.2	23.4	23.1	23.4	23.7	22.4	23.5	21.6	20.0	21.7	16.1	15.4	16.7		20.9	21.7	24.2
MN058684_Beierolpium sp. WAM T136468	25.2	26.6	25.8	25.2	24.4	24.7	24.9	24.9	24.4	23.7	25.0	24.7	23.7	24.1	25.0	24.9	24.6	24.6	24.0	24.7	23.7	25.5	26.4	27.1	27.5	27.6	24.1	22.6	22.6	22.4	22.3	23.9	23.4	22.9	22.7	23.4	23.5	24.3	24.4	24.1	23.8	25.0	23.1	22.1	18.8	23.8	22.4	19.4	21.8	20.9		20.0	23.3
KX263396_Antillolpium sp. 783047	22.6	23.2	23.1	22.9	22.8	21.4	22.4	22.1	22.3	22.6	23.2	23.9	22.9	23.1	25.2	23.4	24.1	24.2	24.1	23.5	25.0	24.6	26.3	27.2	27.7	26.5	25.1	24.1	24.3	24.6	24.3	24.1	24.2	23.5	23.7	24.0	22.6	23.7	23.4	23.5	23.2	23.4	22.8	20.2	19.8	22.8	21.8	21.7	22.0	21.7	20.0		20.8
KX263395_Antillolpium sp. 772A	26.0	26.5	26.0	25.7	26.4	27.1	26.9	26.2	27.3	25.9	25.3	26.7	26.3	26.5	27.6	26.8	27.1	27.0	26.6	26.2	25.1	28.2	26.9	31.5	29.8	29.4	28.1	23.9	23.9	23.7	23.9	24.0	24.0	22.8	23.0	23.9	24.2	25.6	24.8	24.5	23.3	23.9	23.9	24.3	21.3	25.1	25.4	22.7	24.6	24.2	23.3	20.8	

3.2 Araneae

A single specimen of the selenopid spider genus *Karaops* was successfully sequenced. This specimen matched sequences from a previously published report, which had been identified as the undescribed OTU, *Karaops* `sp. WAM-ARA004` (Dolman & Castalanelli, 2013). *Karaops* `sp. WAM-ARA004` had low intraspecific genetic divergences (>0.06%) and was closely related to a *Karaops* `sp. WAM-ARA003` (>2.7%), a lineage that was identified as being distinct by Dolman and Castalanelli (2013). *Karaops* `sp. WAM-ARA004` has been found in Wheelarra North, approximately 23km from the specimen collected in the Study Area.

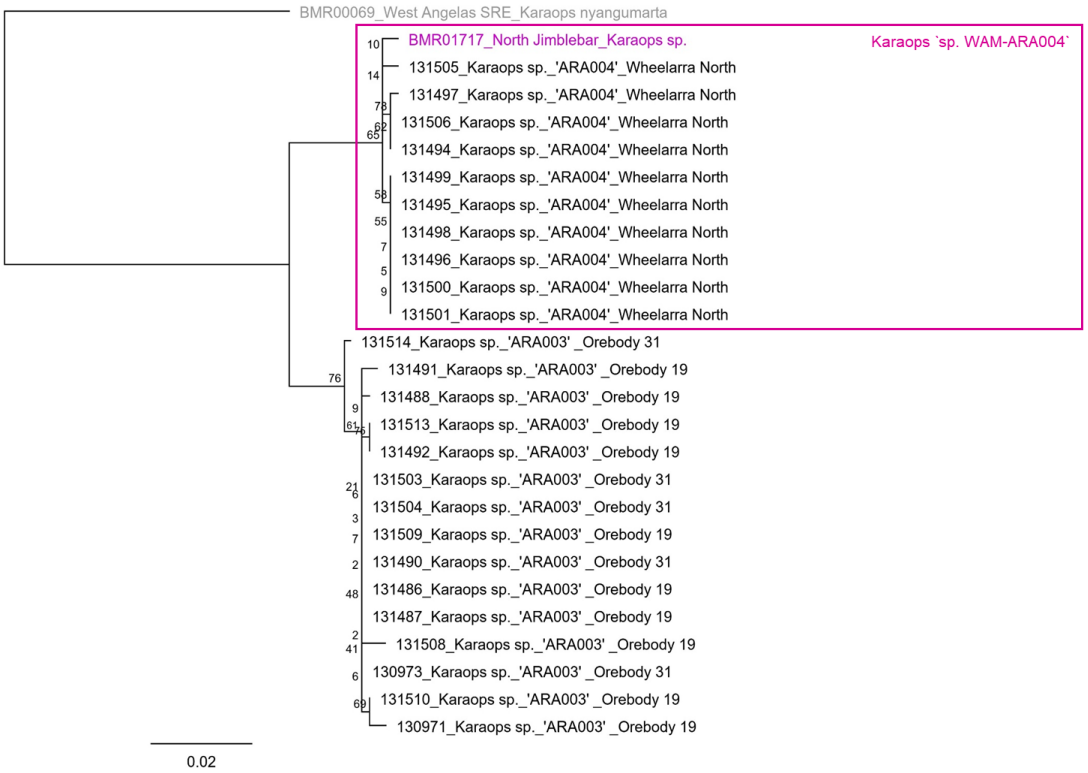

Figure 3.2.1: Maximum Likelihood phylogeny, with bootstrap values.

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

COI divergences (%) of *Karaops* (regional comparisons)	BMR 00069	BMR 01717	131505	131497	131506	131494	131499	131495	131498	131496	131500	131501	131514	131491	131488	131513	131492	131503	131504	131509	131490	131486	131487	131508	130973	131510	130971
BMR00069_West Angelas SRE_Karaops nyangumarta		7.0	6.7	6.7	6.8	6.8	6.7	6.5	6.5	6.5	6.5	6.5	6.8	6.4	6.8	6.8	6.8	6.7	6.7	6.7	6.7	6.9	7.0	6.7	7.3	6.8	7.0
BMR01717_North Jimblebar_Karaops sp.	7.0		0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	2.9	3.0	3.2	3.2	3.2	3.0	3.0	3.0	3.0	3.1	3.0	3.5	3.6	3.2	3.4
131505_'ARA004'_Wheelarra North	6.7	0.6		0.6	0.5	0.5	0.3	0.5	0.5	0.5	0.5	0.5	2.9	3.0	3.2	3.2	3.2	3.0	3.0	3.0	3.0	3.0	2.8	3.5	3.3	3.2	3.2
131497_'ARA004'_Wheelarra North	6.7	0.6	0.6		0.2	0.2	0.5	0.5	0.5	0.5	0.5	0.5	2.9	3.0	3.2	3.2	3.2	3.0	3.0	3.0	3.0	3.1	3.0	3.5	3.6	3.2	3.4
131506_'ARA004'_Wheelarra North	6.8	0.5	0.5	0.2		0.0	0.3	0.3	0.3	0.3	0.3	0.3	2.7	2.9	3.0	3.0	3.0	2.9	2.9	2.9	2.9	3.0	2.8	3.3	3.3	3.0	3.2
131494_'ARA004'_Wheelarra North	6.8	0.5	0.5	0.2	0.0		0.3	0.3	0.3	0.3	0.3	0.3	2.7	2.9	3.0	3.0	3.0	2.9	2.9	2.9	2.9	3.0	2.8	3.3	3.3	3.0	3.2
131499_'ARA004'_Wheelarra North	6.7	0.5	0.3	0.5	0.3	0.3		0.0	0.0	0.0	0.0	0.0	2.8	3.0	3.1	3.1	3.1	3.0	3.0	3.0	3.0	3.0	2.8	3.4	3.3	3.1	3.2
131495_'ARA004'_Wheelarra North	6.5	0.5	0.5	0.5	0.3	0.3	0.0		0.0	0.0	0.0	0.0	2.7	2.9	3.0	3.0	3.0	2.9	2.9	2.9	2.9	3.0	2.8	3.3	3.3	3.0	3.2
131498_'ARA004'_Wheelarra North	6.5	0.5	0.5	0.5	0.3	0.3	0.0	0.0		0.0	0.0	0.0	2.7	2.9	3.0	3.0	3.0	2.9	2.9	2.9	2.9	3.0	2.8	3.3	3.3	3.0	3.2
131496_'ARA004'_Wheelarra North	6.5	0.5	0.5	0.5	0.3	0.3	0.0	0.0	0.0		0.0	0.0	2.7	2.9	3.0	3.0	3.0	2.9	2.9	2.9	2.9	3.0	2.8	3.3	3.3	3.0	3.2
131500_'ARA004'_Wheelarra North	6.5	0.5	0.5	0.5	0.3	0.3	0.0	0.0	0.0	0.0		0.0	2.7	2.9	3.0	3.0	3.0	2.9	2.9	2.9	2.9	3.0	2.8	3.3	3.3	3.0	3.2
131501_'ARA004'_Wheelarra North	6.5	0.5	0.5	0.5	0.3	0.3	0.0	0.0	0.0	0.0	0.0		2.7	2.9	3.0	3.0	3.0	2.9	2.9	2.9	2.9	3.0	2.8	3.3	3.3	3.0	3.2
131514_'ARA003'_Orebody 31	6.8	2.9	2.9	2.9	2.7	2.7	2.8	2.7	2.7	2.7	2.7	2.7		0.8	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.9	0.6	0.6	1.0
131491_'ARA003'_Orebody 19	6.4	3.0	3.0	3.0	2.9	2.9	3.0	2.9	2.9	2.9	2.9	2.9	0.8		0.5	0.5	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.8	0.2	0.5	0.8
131488_'ARA003'_Orebody 19	6.8	3.2	3.2	3.2	3.0	3.0	3.1	3.0	3.0	3.0	3.0	3.0	0.6	0.5		0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.6	0.2	0.3	0.7
131513_'ARA003'_Orebody 19	6.8	3.2	3.2	3.2	3.0	3.0	3.1	3.0	3.0	3.0	3.0	3.0	0.6	0.5	0.3		0.0	0.2	0.2	0.2	0.2	0.2	0.2	0.6	0.2	0.3	0.7
131492_'ARA003'_Orebody 19	6.8	3.2	3.2	3.2	3.0	3.0	3.1	3.0	3.0	3.0	3.0	3.0	0.6	0.5	0.3	0.0		0.2	0.2	0.2	0.2	0.2	0.2	0.6	0.2	0.3	0.7
131503_'ARA003'_Orebody 31	6.7	3.0	3.0	3.0	2.9	2.9	3.0	2.9	2.9	2.9	2.9	2.9	0.5	0.3	0.2	0.2	0.2		0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.2	0.5
131504_'ARA003'_Orebody 31	6.7	3.0	3.0	3.0	2.9	2.9	3.0	2.9	2.9	2.9	2.9	2.9	0.5	0.3	0.2	0.2	0.2	0.0		0.0	0.0	0.0	0.0	0.5	0.0	0.2	0.5
131509_'ARA003'_Orebody 19	6.7	3.0	3.0	3.0	2.9	2.9	3.0	2.9	2.9	2.9	2.9	2.9	0.5	0.3	0.2	0.2	0.2	0.0	0.0		0.0	0.0	0.0	0.5	0.0	0.2	0.5
131490_'ARA003'_Orebody 31	6.7	3.0	3.0	3.0	2.9	2.9	3.0	2.9	2.9	2.9	2.9	2.9	0.5	0.3	0.2	0.2	0.2	0.0	0.0	0.0		0.0	0.0	0.5	0.0	0.2	0.5
131486_'ARA003'_Orebody 19	6.9	3.1	3.0	3.1	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.5	0.3	0.2	0.2	0.2	0.0	0.0	0.0	0.0		0.0	0.5	0.0	0.2	0.5
131487_'ARA003'_Orebody 19	7.0	3.0	2.8	3.0	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	0.5	0.3	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0		0.5	0.0	0.2	0.2
131508_'ARA003'_Orebody 19	6.7	3.5	3.5	3.5	3.3	3.3	3.4	3.3	3.3	3.3	3.3	3.3	0.9	0.8	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5	0.5		0.2	0.6	1.0
130973_'ARA003'_Orebody 31	7.3	3.6	3.3	3.6	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	0.6	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2		0.2	0.2
131510_'ARA003'_Orebody 19	6.8	3.2	3.2	3.2	3.0	3.0	3.1	3.0	3.0	3.0	3.0	3.0	0.6	0.5	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.6	0.2		0.3
130971_'ARA003'_Orebody 19	7.0	3.4	3.2	3.4	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	1.0	0.8	0.7	0.7	0.7	0.5	0.5	0.5	0.5	0.5	0.2	1.0	0.2	0.3	

3.3 Isopoda

A single isopod specimen was successfully sequenced from the Study Area, which was compared to eight isopods from the region, that represented five known morphospecies (S Judd, unpublished data). The target isopod did not match to any of the known morphospecies, or any other isopod included in Biologic's Sequence Library, and was assigned to a new OTU, *Buddelundia* `sp. Biologic-ISOP014` (Fig 3.3.1). *Buddelundia* `sp. Biologic-ISOP014` was over 17% divergent from all morphospecies included in the analysis (Table 3.3.1).

The three morphospecies that had more than one specimen included in the analysis all showed some within-species divergence, as high as 7.6% in *Buddelundia* `sp. 14cr`. This is likely to reflect geographic genetic structure within species and will be a useful guide for delimiting isopod species in the future.

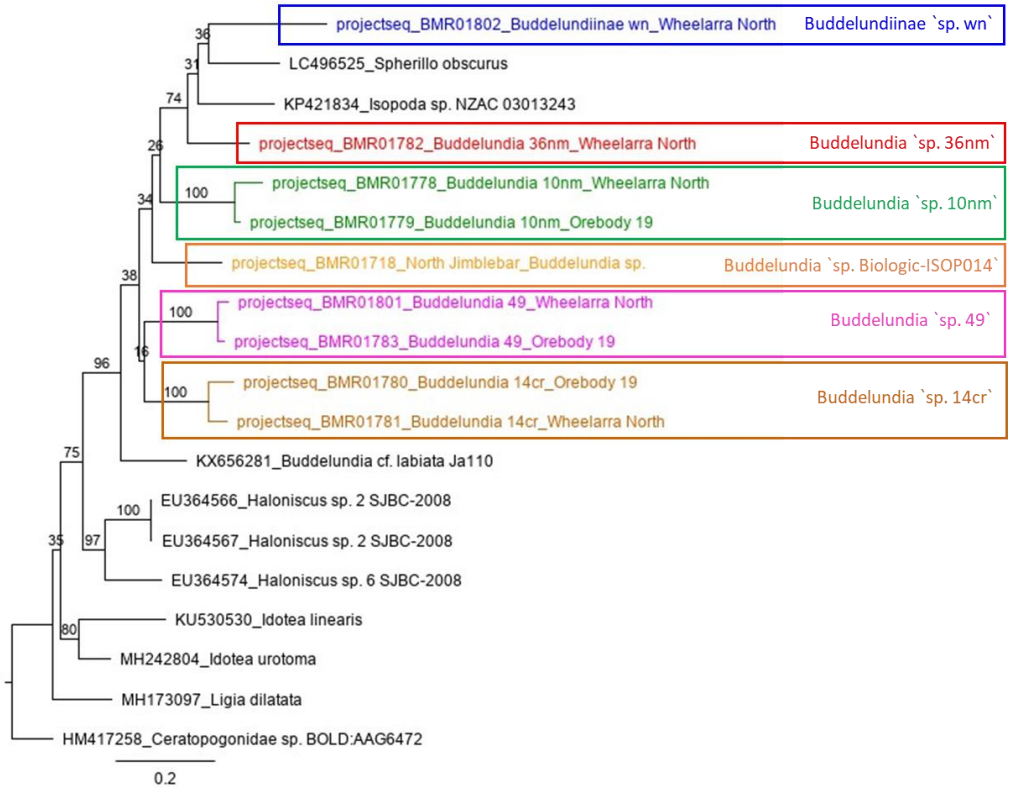

Figure 3.3.1: Maximum Likelihood phylogeny, with bootstrap values.

Table 3.3.1: Pairwise genetic distances for the dataset included in Fig 3.3.2. Colours of OTUs match those in Fig 3.3.2.

COI Genetic Distance (%)	BMR01802	BMR01801	BMR01783	BMR01718	BMR01779	BMR01778	BMR01782	LC496525	KP421834	BMR01781	BMR01780	KX656281	MH242804	KU530530	EU364574	EU364567	EU364566	MH173097	HM417258
projectseq_BMR01802_Buddelundiinae wn_Wheelarra North		21.88	21.88	22.49	20.82	21.28	18.69	22.20	21.88	22.64	22.49	21.79	19.94	23.74	22.17	20.19	20.19	20.70	24.77
projectseq_BMR01801_Buddelundia 49_Wheelarra North	21.88		3.34	18.24	18.54	20.97	20.97	21.57	22.34	18.24	18.09	18.86	21.92	22.37	20.80	20.96	21.11	21.92	24.62
projectseq_BMR01783_Buddelundia 49_Orebody 19	21.88	3.34		17.17	18.85	19.76	20.06	21.09	21.43	18.39	17.63	19.02	22.37	20.85	21.41	20.96	21.11	21.77	24.32
projectseq_BMR01718_North Jimblebar_Buddelundia sp.	22.49	18.24	17.17		19.30	19.61	19.30	20.29	18.85	18.54	19.30	18.54	21.31	22.68	21.41	21.42	21.26	21.77	22.95
projectseq_BMR01779_Buddelundia 10nm_Orebody 19	20.82	18.54	18.85	19.30		6.84	17.78	19.97	20.37	19.00	19.45	20.33	22.83	24.05	22.78	21.11	21.11	23.29	24.16
projectseq_BMR01778_Buddelundia 10nm_Wheelarra North	21.28	20.97	19.76	19.61	6.84		18.24	19.49	21.43	19.00	20.82	22.11	23.29	24.66	23.85	22.50	22.50	23.59	24.32
projectseq_BMR01782_Buddelundia 36nm_Wheelarra North	18.69	20.97	20.06	19.30	17.78	18.24		17.89	18.24	18.09	18.24	18.05	20.70	23.29	21.56	19.88	19.88	21.92	20.97
LC496525_Spherillo obscurus	22.20	21.57	21.09	20.29	19.97	19.49	17.89		18.85	20.13	20.77	20.13	21.41	23.48	22.20	22.68	22.68	22.05	24.28
KP421834_Isopoda sp. NZAC 03013243	21.88	22.34	21.43	18.85	20.37	21.43	18.24	18.85		21.73	21.43	22.93	21.46	22.98	23.55	22.65	22.50	21.92	25.08
projectseq_BMR01781_Buddelundia 14cr_Wheelarra North	22.64	18.24	18.39	18.54	19.00	19.00	18.09	20.13	21.73		7.60	16.42	19.03	22.22	21.10	20.49	20.65	18.57	17.78
projectseq_BMR01780_Buddelundia 14cr_Orebody 19	22.49	18.09	17.63	19.30	19.45	20.82	18.24	20.77	21.43	7.60		16.59	19.18	22.37	21.41	20.19	20.49	19.18	19.61
KX656281_Buddelundia cf. labiata Ja110	21.79	18.86	19.02	18.54	20.33	22.11	18.05	20.13	22.93	16.42	16.59		18.37	20.49	21.30	19.02	19.02	19.84	21.63
MH242804_Idotea urotoma	19.94	21.92	22.37	21.31	22.83	23.29	20.70	21.41	21.46	19.03	19.18	18.37		15.53	16.36	17.87	18.18	15.68	15.83
KU530530_Idotea linearis	23.74	22.37	20.85	22.68	24.05	24.66	23.29	23.48	22.98	22.22	22.37	20.49	15.53		19.73	20.65	20.34	18.27	23.29
EU364574_Haloniscus sp. 6 SJBC-2008	22.17	20.80	21.41	21.41	22.78	23.85	21.56	22.20	23.55	21.10	21.41	21.30	16.36	19.73		14.95	15.02	20.49	21.87
EU364567_Haloniscus sp. 2 SJBC-2008	20.19	20.96	20.96	21.42	21.11	22.50	19.88	22.68	22.65	20.49	20.19	19.02	17.87	20.65	14.95		0.16	17.87	19.72
EU364566_Haloniscus sp. 2 SJBC-2008	20.19	21.11	21.11	21.26	21.11	22.50	19.88	22.68	22.50	20.65	20.49	19.02	18.18	20.34	15.02	0.16		18.34	20.03
MH173097_Ligia dilatata	20.70	21.92	21.77	21.77	23.29	23.59	21.92	22.05	21.92	18.57	19.18	19.84	15.68	18.27	20.49	17.87	18.34		18.87
HM417258_Ceratopogonidae sp. BOLD:AAG6472	24.77	24.62	24.32	22.95	24.16	24.32	20.97	24.28	25.08	17.78	19.61	21.63	15.83	23.29	21.87	19.72	20.03	18.87	

4 SUMMARY

Using well-established DNA extraction and sequencing methods, this molecular systematics analysis designated seven distinct species/ OTUs to 24 high quality sequences from the Study Area (see sections 3.1 – 3.3). All OTUs, the areas in which they were found, and the specimen numbers per OTU are shown in Appendix A. The following are the key findings at the species/ OTU level:

- Pseudoscorpiones (COI): 5 OTUs, all unique lineages to the North Jimblebar and South Jimblebar and Caramulla Study Areas. All OTUs had up to 7.7% intraspecific divergence and >8.7% interspecific divergence. One OTU was represented by a singleton *Austrohorus* `sp. Biologic-PSEU023.
- Araneae (COI): 1 OTU, matching with external sequences (<0.5 % divergent) beyond the North Jimblebar Study Area.
- Isopoda (COI): 1 OTU, unique lineage currently only found in the North Jimblebar Study Area.

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Appendix A: Specimens included in report with updated OTU identifications based on molecular data. Reactions states Fail;PCR indicates no DNA product was produced from the extraction and FAIL: CON indicates DNA product produced was not of the target organism.

BMR	Unique ID code	WAM Reg No	Site	Study Area	Latitude	Longitude	Family	Lowest ID Legacy	OTU Name	Reaction State
Pseudoscorpiones										
BMR01694	6252		SJIM-080	North Jimblebar	-23.3529	120.2071	Olpiidae	Austrohorus sp.	Austrohorus `sp. Biologic-PSEU025`	PASS
BMR01696	5144		SJIM-037	East Jimblebar and Caramulla	-23.3781	120.2805	Olpiidae	Austrohorus sp. JIM 01	Austrohorus `sp. Biologic-PSEU024`	PASS
BMR01697	5313		SJIM-044	East Jimblebar and Caramulla	-23.3909	120.3028	Olpiidae	Austrohorus sp. JIM 01	Austrohorus `sp. Biologic-PSEU025`	PASS
BMR01698	5686		SJIM-019	East Jimblebar and Caramulla	-23.3558	120.2848	Olpiidae	Austrohorus sp. JIM 02	Austrohorus `sp. Biologic-PSEU024`	PASS
BMR01699	5800		SJIM-061	North Jimblebar	-23.3559	120.1833	Olpiidae	Austrohorus sp. JIM 02	Austrohorus `sp. Biologic-PSEU025`	PASS
BMR01700	5998		SJIM-066	North Jimblebar	-23.3595	120.1710	Olpiidae	Austrohorus sp. JIM 02	Austrohorus `sp. Biologic-PSEU023`	PASS
BMR01701	6250		SJIM-106	East Jimblebar and Caramulla	-23.3642	120.2314	Olpiidae	Austrohorus sp. JIM 02	Austrohorus `sp. Biologic-PSEU024`	PASS
BMR01702	6767		SJIM-133	East Jimblebar and Caramulla	-23.3738	120.2496	Olpiidae	Austrohorus sp. JIM 02	Austrohorus `sp. Biologic-PSEU024`	PASS
BMR01703	5263		SJIM-075	North Jimblebar	-23.3587	120.2072	Olpiidae	Indolpium sp.	Indolpium `sp. Biologic-PSEU027`	PASS
BMR01704	7792		SJIM-075	North Jimblebar	-23.3587	120.2072	Olpiidae	Indolpium sp.	Austrohorus `sp. Biologic-PSEU025`	PASS
BMR01705	5382		SJIM-044	East Jimblebar and Caramulla	-23.3909	120.3028	Olpiidae	Indolpium sp.		FAIL;PCR
BMR01706	5745		SJIM-062	North Jimblebar	-23.3576	120.1630	Olpiidae	Indolpium sp.	Indolpium `sp. Biologic-PSEU026`	PASS
BMR01707	5928		SJIM-106	East Jimblebar and Caramulla	-23.3642	120.2314	Olpiidae	Indolpium sp.		FAIL;PCR
BMR01695	5144b		SJIM-037	East Jimblebar and Caramulla	-23.3781	120.2805	Olpiidae	Indolpium sp.		FAIL;PCR
BMR01719	6395b		SJIM-127	East Jimblebar and Caramulla	-23.3566	120.2663	Olpiidae	Indolpium sp.	Indolpium `sp. Biologic-PSEU026`	PASS
BMR01720	8573		SJIM-127	East Jimblebar and Caramulla	-23.3566	120.2663	Olpiidae	Indolpium sp.	Indolpium `sp. Biologic-PSEU026`	PASS
BMR01723	5014		SJIM-011	East Jimblebar and Caramulla	-23.3769	120.2622	Olpiidae	Indolpium sp. JIM 03	Indolpium `sp. Biologic-PSEU027`	PASS
BMR01724	8571		SJIM-011	East Jimblebar and Caramulla	-23.3769	120.2622	Olpiidae	Indolpium sp. JIM 03	Indolpium `sp. Biologic-PSEU027`	PASS
BMR01708	5798		SJIM-032	East Jimblebar and Caramulla	-23.3533	120.3078	Olpiidae	Indolpium sp. JIM 03	Indolpium `sp. Biologic-PSEU027`	PASS
BMR01709	6003		SJIM-070	North Jimblebar	-23.3535	120.2160	Olpiidae	Indolpium sp. JIM 03	Indolpium `sp. Biologic-PSEU027`	PASS
BMR01710	5139b		SJIM-057	North Jimblebar	-23.3571	120.1705	Olpiidae	Indolpium sp. JIM 03	Indolpium `sp. Biologic-PSEU027`	PASS
BMR01711	4129		SJIM-126	North Jimblebar	-23.3560	120.1796	Olpiidae	Indolpium sp. JIM 04	Indolpium `sp. Biologic-PSEU027`	PASS
BMR01712	5433		SJIM-007	East Jimblebar and Caramulla	-23.3992	120.2371	Olpiidae	Indolpium sp. JIM 04		FAIL;CON
BMR01713	5677		SJIM-096	East Jimblebar and Caramulla	-23.4024	120.3138	Olpiidae	Indolpium sp. JIM 04		FAIL;PCR
BMR01714	6121		SJIM-077	North Jimblebar	-23.3533	120.1903	Olpiidae	Indolpium sp. JIM 04	Indolpium `sp. Biologic-PSEU026`	PASS
BMR01715	6225		SJIM-104	East Jimblebar and Caramulla	-23.3694	120.1957	Olpiidae	Indolpium sp. JIM 04	Indolpium `sp. Biologic-PSEU027`	PASS
BMR01721	6712		SJIM-022	East Jimblebar and Caramulla	-23.3647	120.3852	Olpiidae	Indolpium sp. JIM 04		FAIL;PCR
BMR01722	8572		SJIM-022	East Jimblebar and Caramulla	-23.3647	120.3852	Olpiidae	Indolpium sp. JIM 04		FAIL;PCR
BMR01716	5132		SJIM-066	North Jimblebar	-23.3595	120.1710	Olpiidae	Olpiidae sp.	Indolpium `sp. Biologic-PSEU026`	PASS
Aranae										
BMR01717	5556		SJIM-076	North Jimblebar	-23.3598	120.2034	Selenopidae	Karaops sp.	Karaops `sp. WAM-ARA004`	PASS
Isopoda										
BMR01718	6745		SJIM-125	North Jimblebar	-23.3539	120.2015	Armadillidae	Buddelundia sp.	Buddelundia `sp. Biologic-ISOP014`	PASS
BMR01778	1024B	C65885	Station 148	Wheelarra North	-23.3541	120.1471	Armadillidae	Buddelundia 10nm		PASS
BMR01779	SJ4222	C65902	Station 155	Orebody 19	-23.3326	120.0013	Armadillidae	Buddelundia 10nm		PASS
BMR01780	0242	C65977	Station 28	Orebody 19	-23.3291	119.9997	Armadillidae	Buddelundia 14cr		PASS
BMR01781	1211	C66011	Station 96	Wheelarra North	-23.3518	120.1419	Armadillidae	Buddelundia 14cr		PASS
BMR01782	0060A	C66020	Station 75	Wheelarra North	-23.3480	120.1437	Armadillidae	Buddelundia 36nm		PASS
BMR01783	1164	C66039	Station 23	Orebody 19	-23.3314	120.0187	Armadillidae	Buddelundia 49		PASS
BMR01801	1207B	C66076	Station 96	Wheelarra North	-23.3518	120.1419	Armadillidae	Buddelundia 49		PASS
BMR01802	0732A	C66087	Station 98	Wheelarra North	-23.3534	120.1398	Armadillidae	Buddelundiinae wn		PASS
BMR01803	0178	C66147	Station 86	Orebody 24	-23.2728	119.8469	Armadillidae	Buddelundia 16NM		FAIL; PCR