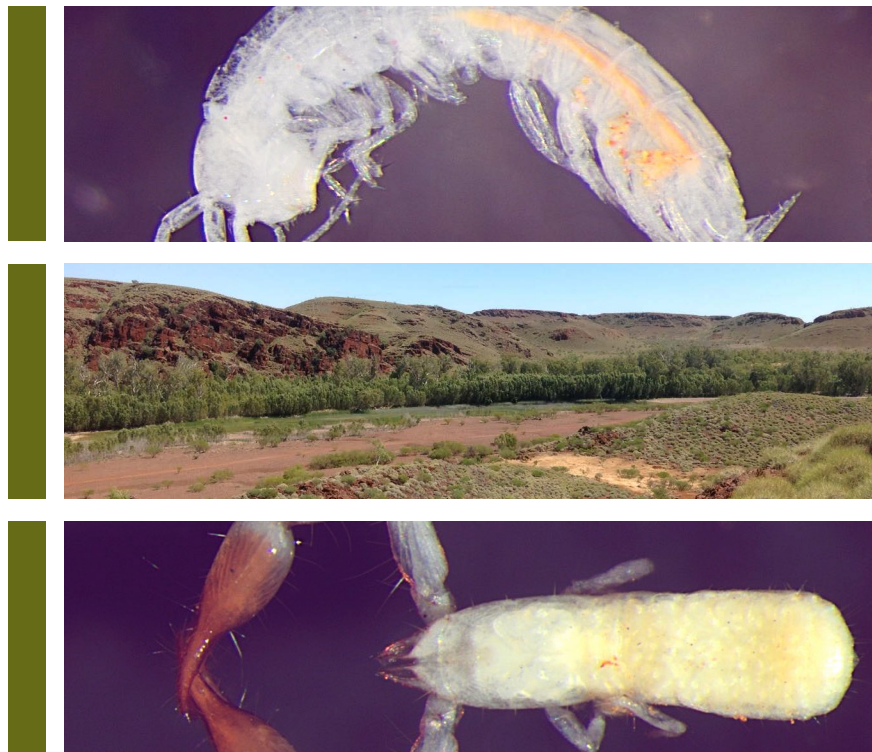




# Mesa H Subterranean Fauna Assessment



Prepared for Rio Tinto

February 2019





© Biota Environmental Sciences Pty Ltd 2018  
ABN 49 092 687 119  
Level 1, 228 Carr Place  
Leederville Western Australia 6007  
Ph: (08) 9328 1900 Fax: (08) 9328 6138

Job No.: 1146B

Prepared by: Nicola Watson, Dan  
Kamien, Garth Humphreys

**Document Quality Checking History**

|                |                                  |
|----------------|----------------------------------|
| Version: Rev A | Peer review: Jason Alexander     |
| Version: Rev 0 | Director review: Garth Humphreys |
| Version: Rev 0 | Format review: Fiona Hedley      |

Approved for issue: Garth Humphreys

This document has been prepared to the requirements of the client identified on the cover page and no representation is made to any third party. It may be cited for the purposes of scientific research or other fair use, but it may not be reproduced or distributed to any third party by any physical or electronic means without the express permission of the client for whom it was prepared or Biota Environmental Sciences Pty Ltd.

**This report has been designed for double-sided printing. Hard copies supplied by Biota are printed on recycled paper.**

This page intentionally blank.



# Mesa H Subterranean Fauna Assessment

## Contents

|            |  |           |
|------------|--|-----------|
| <b>1.0</b> | <b>Executive Summary</b>                       | <b>9</b>  |
| <b>2.0</b> | <b>Introduction</b>                            | <b>11</b> |
| 2.1        | Project Background                             | 11        |
| 2.2        | Terminology                                    | 11        |
| 2.3        | Objectives and Scope                           | 11        |
| 2.4        | Purpose of this Report                         | 13        |
| 2.5        | Subterranean Fauna Overview                    | 13        |
| <b>3.0</b> | <b>Methodology</b>                             | <b>15</b> |
| 3.1        | Desktop Review and Database Searches           | 15        |
| 3.2        | Survey Timing and Personnel                    | 15        |
| 3.3        | Stygofauna Sampling                            | 18        |
| 3.4        | eDNA Sampling                                  | 18        |
| 3.5        | Troglofauna Sampling                           | 18        |
| 3.6        | Morphological Identifications                  | 19        |
| 3.7        | Specimen Molecular Analysis                    | 19        |
| 3.8        | eDNA Sample Analysis                           | 20        |
| 3.9        | Categories of Conservation Significance        | 20        |
| 3.10       | Sampling Effort                                | 21        |
| 3.11       | Study Limitations                              | 30        |
| <b>4.0</b> | <b>Desktop Review</b>                          | <b>31</b> |
| 4.1        | Priority and Threatened Ecological Communities | 31        |
| 4.2        | Surface Geology                                | 31        |
| 4.3        | Previous Relevant Surveys                      | 35        |
| 4.4        | Desktop Review Results                         | 38        |
| <b>5.0</b> | <b>Survey Results</b>                          | <b>47</b> |
| 5.1        | Stygofauna                                     | 47        |
| 5.2        | eDNA   | 55        |
| 5.3        | Troglofauna                                    | 57        |
| <b>6.0</b> | <b>Discussion</b>                              | <b>69</b> |
| 6.1        | Known Subterranean Assemblage                  | 69        |
| 6.2        | Conservation Significant Fauna                 | 69        |
| 6.3        | Potential SRE Fauna                            | 70        |
| <b>7.0</b> | <b>Glossary</b>                                | <b>79</b> |
| <b>8.0</b> | <b>References</b>                              | <b>81</b> |

## Appendix 1

Licence to Take Fauna

## Appendix 2

Morphological Identifications by Bennelongia Environmental Consultants

**Appendix 3**

Genetic Analyses by Helix Molecular Solutions

**Appendix 4**

Curtin University eDNA Metabarcoding Report

**Appendix 5**

WAM Morphological Identification of Troglobitic Spiders

**Appendix 6**

Fauna Recorded

**Tables**

|             |  |    |
|-------------|--|----|
| Table 3.1:  | Summary of field sampling undertaken during the survey.  | 16 |
| Table 3.2:  | Stygofauna sampling sites at Mesa H (*site outside of the drawdown extent).  | 21 |
| Table 3.3:  | eDNA sites sampled during December 2017 (*site outside of the drawdown extent).  | 22 |
| Table 3.4:  | Troglofauna sampling sites at Mesa H.  | 25 |
| Table 4.1:  | Priority Ecological Communities within 50 km of the development envelope relevant to subterranean fauna.   | 31 |
| Table 4.2:  | Surface geology units of the development envelope and representative sampling effort (units previously identified as subterranean fauna habitat highlighted in yellow).  | 33 |
| Table 4.3:  | Summary of surveys completed within 15 km of the Mesa H development envelope (source: Biota database).   | 35 |
| Table 4.4:  | Stygofauna taxa and abundance (n) recorded from the desktop review (records highlighted in bold were recorded within the drawdown extent).   | 39 |
| Table 4.5:  | Troglofauna taxa and abundance (n) recorded during the desktop review (Taxa highlighted in bold were recorded within the Mesa H development envelope).   | 42 |
| Table 5.1:  | Summary of stygofauna recorded during the current survey (n= number of specimens).   | 47 |
| Table 5.2:  | Summary of water mite taxa recorded from the current survey (*denotes sites outside of the drawdown extent).   | 49 |
| Table 5.3:  | Summary of ostracod taxa recorded from the current survey (*denotes sites outside of the drawdown extent).   | 49 |
| Table 5.4:  | Summary of copepod taxa recorded from the current survey (*denotes sites outside of the drawdown extent).  | 51 |
| Table 5.5:  | Summary of thermosbaenaceans recorded from the current survey (*denotes sites outside of the drawdown extent).   | 51 |
| Table 5.6:  | Summary of stygal isopod taxa recorded from the current survey (*denotes sites outside of the drawdown extent).  | 51 |
| Table 5.7:  | Summary of amphipod taxa recorded from the current survey (*denotes sites outside of the drawdown extent).   | 53 |
| Table 5.8:  | Results of eDNA analysis of environmental samples ('Y' = positive detection of <i>Ophisternon</i> DNA; *denotes sites outside of the drawdown extent; †denotes detection only after refinement of metabarcoding methods (latter section of Appendix 4)). | 55 |
| Table 5.9:  | Summary of troglobitic fauna recorded from Mesa H during the current survey (n= number of specimens collected).  | 57 |
| Table 5.10: | Summary of dipluran taxa recorded from the current survey.   | 57 |

|  |    |
|--|----|
| Table 5.11: Summary of isopod taxa recorded from the current survey.   | 59 |
| Table 5.12: Summary of pseudoscorpion taxa recorded from the current survey.   | 60 |
| Table 5.13: Summary of schizomid taxa recorded from Mesa H during the current study.   | 63 |
| Table 5.14: Summary of centipede taxa recorded from the current survey.  | 64 |
| Table 5.15: Summary of spider taxa recorded from the current survey.   | 65 |
| Table 5.16: Summary of cockroach taxa recorded from the current survey.  | 65 |
| Table 5.17: Summary of beetle taxa recorded from the current survey.   | 66 |
| Table 5.18: Summary of silverfish taxa recorded from the current survey.   | 67 |
| Table 6.1: Summary of conservation significant and potential SRE stygofauna species from the Mesa H survey area (combined desktop review and survey results; desktop records highlighted in grey shading; species in bold recorded from within the drawdown extent). | 71 |
| Table 6.2: Summary of potential SRE troglifauna species recorded within Mesa H development envelope (combined desktop review and survey results; desktop records highlighted in grey shading).   | 76 |

## Figures

|   |    |
|---|----|
| Figure 2.1: Location map of the Mesa H survey area.   | 12 |
| Figure 3.1: Climate and weather graph depicting long-term and monthly averages for 6 months preceding and during subterranean fauna sampling.                                     | 17 |
| Figure 3.2: Overview of stygofauna sampling sites Phases 1 - 5.   | 23 |
| Figure 3.3: Stygofauna sampling sites within and adjacent to the Mesa H development envelope Phases 1-5.  | 24 |
| Figure 3.4: Troglifauna sampling sites at Mesa H.   | 28 |
| Figure 4.1: PECs relevant to subterranean fauna within 50 km of the Mesa H development envelope.  | 32 |
| Figure 4.2: Surface geology mapping of the Mesa H development envelope.   | 34 |
| Figure 4.3: Previous troglifauna surveys within 15 km of the Mesa H development envelope.   | 36 |
| Figure 4.4: Previous stygofauna surveys within 15 km of the Mesa H development envelope.  | 37 |
| Figure 4.5: Stygofauna records from the desktop review.   | 41 |
| Figure 4.6: Schizomid records from the desktop review.  | 44 |
| Figure 4.7: Pseudoscorpion records from the desktop review.   | 45 |
| Figure 4.8: Other troglifauna records from the desktop review.  | 46 |
| Figure 5.1: Lower abundance stygofauna taxa recorded from Mesa H during the current survey.   | 48 |
| Figure 5.2: Copepod taxa recorded from Mesa H during the current survey.  | 50 |
| Figure 5.3: Amphipoda taxa recorded from Mesa H during the current survey.  | 54 |
| Figure 5.4: Locations where positive eDNA results were recorded for <i>Ophisternon</i> DNA detection, in context with confirmed <i>Ophisternon</i> specimen collection locations. | 56 |
| Figure 5.5: Bayesian analysis of CO1 haplotypes of Diplura recorded from the Mesa H survey area.  | 58 |

|   |    |
|---|----|
| Figure 5.6: Excerpt of bayesian analysis of CO1 haplotypes of Philosciidae recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).  | 59 |
| Figure 5.7: Excerpt of Bayesian analysis of CO1 haplotypes of Armadillidae recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).  | 59 |
| Figure 5.8: Excerpt of bayesian analysis of CO1 haplotypes of Armadillidae recorded from the Mesa H survey area, showing Lineage ISA057 in context with Lineage ISA056 from Mesa C (see Appendix 3 for complete phylogenetic tree).           | 60 |
| Figure 5.9: Excerpt of bayesian analysis of CO1 haplotypes of Hyiidae recorded from survey area.  | 61 |
| Figure 5.10: Excerpt of bayesian analysis of CO1 haplotypes of Olpiidae and Atemnidae from the survey area.   | 61 |
| Figure 5.11: Excerpt of bayesian analysis of CO1 haplotypes of Chthoniidae recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).  | 62 |
| Figure 5.12: Excerpt of Bayesian analysis of CO1 haplotypes of Schizomida recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).   | 64 |
| Figure 5.13: Excerpt of Bayesian analysis of CO1 haplotypes of Scolopendrida recorded from the Mesa H study area (see Appendix 3 for complete phylogenetic tree).   | 65 |
| Figure 5.14: Excerpt of Bayesian analysis of CO1 haplotypes of Blattodea recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).  | 66 |
| Figure 5.15: Excerpt of Bayesian analysis of CO1 haplotypes of Coleoptera recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).   | 67 |
| Figure 5.16: Troglofauna taxa recorded from Mesa H during the current survey.   | 68 |
| Figure 6.1: Distribution of conservation significant and potential SRE stygofauna relative to the Mesa H drawdown extent, including specimen and eDNA records of <i>Ophisternon candidum</i> , from both the current survey and past records. | 73 |
| Figure 6.2: Potential SRE troglofauna recorded within the Mesa H development envelope (combined desktop review and survey results).   | 75 |

## Plates

|  |    |
|--|----|
| Plate 5.1: Paramelitidae sp. 'AMP035' from drillhole RC13MEH0007.                      | 52 |
| Plate 5.2: Parajapygidae sp. 'DPA001' from drillhole RC14MEH0308.                      | 58 |
| Plate 5.3: <i>Beierolpium</i> sp. 'PO014' from drillhole RC16JIM0026.                  | 62 |
| Plate 5.4: Hyidae sp. 'PH017/PH027' from drillhole RC14MRH0252.                        | 62 |
| Plate 5.5: Olpiidae sp. 'PO008' from drillhole RC15MEH0302.                            | 62 |
| Plate 5.6: Atemnidae sp. 'PA004' from drillhole RC15MEH0329.                           | 62 |
| Plate 5.7: Hubbardiidae sp. 'SCH015/SCH016' (lateral view) from drillhole RC12MEH0221. | 63 |
| Plate 5.8: Hubbardiidae sp. 'SCH015/SCH016' (dorsal view) from drillhole RC12MEH0221.  | 63 |

# 1.0 Executive Summary

Rio Tinto is evaluating the potential development of a number of iron ore deposits within the Robe Valley in the Pilbara region of Western Australia. This includes the development of the Mesa H deposit (15 km southwest of Pannawonica). The development has the potential to impact subterranean fauna, including both troglofauna (air-breathing subterranean fauna living above the water table) and stygofauna (aquatic fauna of the groundwater). Biota Environmental Sciences was therefore commissioned to conduct a detailed subterranean fauna survey at Mesa H. While the location of the proposed mine pits and other infrastructure, and the extent of the dewatering needed for the project to access below water table ore, are considered here, their use in this report is limited to a framework to categorise the subterranean fauna records arising from the survey.

A total of six phases of troglofauna sampling and five phases of stygofauna sampling were undertaken through nine field mobilisations occurring between October 2015 and December 2017. Stygofauna were sampled using modified plankton haul nets, and troglobitic fauna were sampled using two methods: baited colonisation traps and haul net scraping. A total of 53 sites were sampled for stygofauna across five phases and 136 sites were sampled for troglofauna using colonisation traps across six phases, with 57 sites sampled for troglofauna with haul nets. Environmental DNA (eDNA) sampling was also conducted at 16 sites in December 2017, with groundwater samples collected to target the specially protected Blind Cave Eel (*Ophisternon candidum*), which is not readily detectable with conventional sampling methods.

Molecular analysis was conducted to determine the number of species present, and to compare the results with data from elsewhere in the Pilbara. Morphological identifications of specimens were undertaken where mature, intact specimens and suitable taxonomic frameworks were available.

A total of 855 stygofauna specimens were recorded over the five phases of sampling, comprising at least 43 species-level taxa. As is typical of the Pilbara bioregion, the fauna recorded was numerically dominated by the Crustacea, which accounted for 98% of the specimens.

With the survey results, and the additional species previously known from desktop records, 46 stygofauna species are known from the immediate locality of Mesa H and the stygofauna of the drawdown extent itself currently stands at a total of 32 species, with half of these taxa recorded occur more widely in the locality or the bioregion. Thirteen of the species within the drawdown extent were identified as potential Short-range Endemic (SRE) fauna and six of these are restricted to the Mesa H drawdown extent based on the currently available data (the snail Hydrobiidae sp. 2, the ostracod Candoninae sp. 'BOS541', the copepod *Parastenocaris* sp. 'B28', the isopod *Haptolana* sp. 'B01', and the amphipod species Paramelitidae sp. 'AMP003' and Paramelitidae sp. 'AMP037').

Three stygofauna species of conservation significance have been recorded within the Mesa H groundwater drawdown extent during previous surveys: the Blind Cave Eel *Ophisternon candidum* (Vulnerable; Schedule 3), and the Schedule 3 amphipod species *Nedsia hurlberti* and *Nedsia sculptilis*. No additional records of the latter two species were obtained during the current survey, but further sampling targeting this species during the current study resulted in the detection of *Ophisternon* DNA at five locations, both along Jimmawurruda Creek and the Robe River, including at two sites within the drawdown extent and three outside of the drawdown extent along the Robe River. An additional specimen was also collected during surface water alluvium sampling along the Robe River, bringing the number of locations outside of the drawdown extent that the species has been recorded at to five (including Cape Range). The significantly improved distributional data indicate that the species occurs more widely in the locality, probably in association with the major alluvial aquifers of the Bungaroo-Jimmawurruda-Robe system.

A total of 150 troglobitic fauna specimens were collected across the six phases of sampling, representing five classes, nine orders and at least 32 species-level taxa. The orders Coleoptera and Schizomida were the greatest contributors to troglofaunal composition, accounting for 33% and 25% of the specimens collected, respectively. One other troglobitic taxon was previously known from the development envelope, bring the current known troglofauna assemblage to 33 species.

No troglofauna of conservation significance (i.e. listed as Priority, Schedule or Vulnerable at State or Federal levels) were recorded during the survey at Mesa H. A total of 27 troglofauna taxa classified as potential SREs are now known solely from within the Mesa H development envelope and do not currently have wider documented distributions (26 species from the current survey and one from historical records).

Twenty of these potential SRE taxa were represented by singleton records (single specimen records), making determination of their true distributions difficult. This overall pattern of short-range endemism is consistent with the findings of previous comprehensive surveys of the troglofauna of pisolitic mesas elsewhere within the Robe River valley.

## 2.0 Introduction

### 2.1 Project Background

Rio Tinto is evaluating the potential development of a number of iron ore deposits within the Robe River valley, in the Pilbara region of Western Australia. This includes the development of the Mesa H deposit (15 km southwest of Pannawonica) (Figure 2.1).

Previous studies have documented the occurrence of troglobitic and stygobitic fauna communities within the Robe River valley, with many of the documented troglofauna species isolated to individual mesa formations (Biota 2006a, Harvey et al. 2008). Troglofauna, in particular, demonstrate extreme short-range endemism as a result of geological barriers, and are therefore of significance to the environmental impact assessment (EIA) of any proposed developments affecting their habitat.

The development of the Mesa H deposit has the potential to impact subterranean fauna, and Rio Tinto therefore wishes to improve knowledge of these communities at Mesa H by collating existing data, determining information gaps and completing additional phases of sampling. Rio Tinto commissioned Biota Environmental Sciences (Biota) to complete the required subterranean fauna surveys to achieve this.

### 2.2 Terminology

This report primarily addresses the outcomes of the subterranean fauna surveys, with the assessment of impacts on subterranean fauna species, communities and habitats considered in a separate report (Biota 2018). While the location of the proposed mine pits and other infrastructure, and the extent of the dewatering needed for the project to access below water table ore, are considered here, their use in this report is limited to a framework to categorise subterranean fauna occurrence. For the purposes of this report then, the following terms are used as defined below:

- Development envelope – The Mesa H project boundary, which will accommodate the full extent of the physical components of the project (Figure 2.1);
- Drawdown extent – the spatial extent of the maximum groundwater dewatering influence for the Mesa H project, which extends beyond the development envelope to the east and southeast, and is primarily of relevance to stygofauna from an EIA perspective (Biota 2018) (Figure 2.1);
- Survey area – The area surveyed for subterranean fauna, including the development envelope and reference sites outside of the development envelope at the Pannawonica bore field and Pannawonica Road (Figure 2.1); and
- Desktop review area – An area encompassing the development envelope and a 15 km square around it to include previous surveys undertaken adjacent to Mesa H (Figure 2.1).

### 2.3 Objectives and Scope

The overall scope of this study was to document the subterranean fauna from the proposed Mesa H development envelope, consolidating the data with existing records from the locality, and placing the findings into wider regional context and assessing conservation status, where possible.

The specific objectives of this study were to:

1. conduct molecular analyses of troglobitic fauna specimens previously collected in the vicinity of Mesa H;
2. conduct a multiple phase sampling programme to document troglofauna and stygofauna present within the survey area; and
3. place the recorded fauna into a regional context and discuss their potential conservation significance.



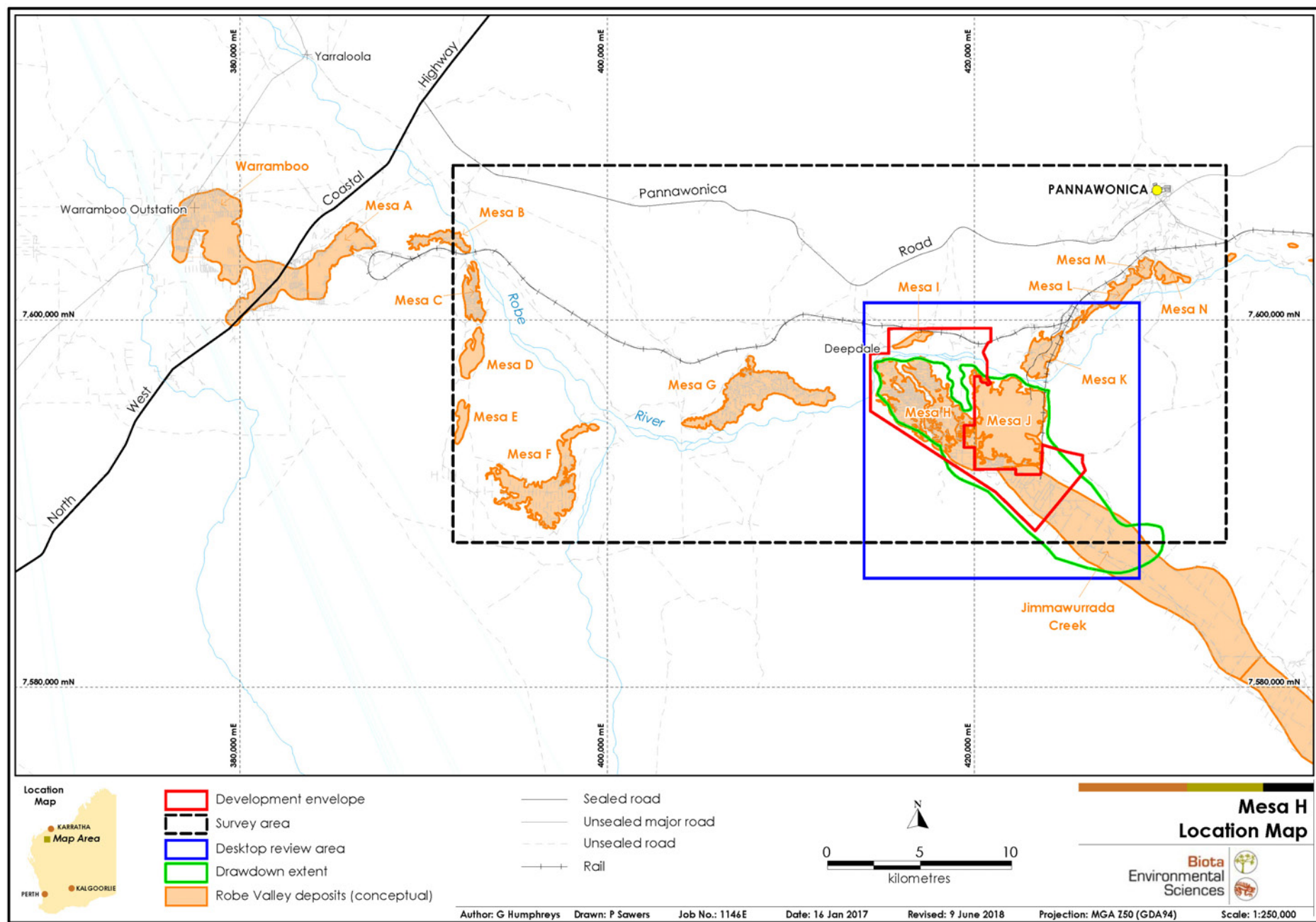


Figure 2.1: Location map of the Mesa H survey area.



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

- EPA Statement of Environmental Principles, Factors and Objectives (EPA 2016a);
- Environmental Factor Guideline – Subterranean Fauna (EPA 2016b);
- Technical Guidance - Subterranean fauna survey (EPA 2016c); and
- Technical Guidance - Sampling methods for subterranean fauna (EPA 2016d).

## 2.4 Purpose of this Report

The purpose of this report is to provide consolidated baseline survey information to inform the EIA of proposed mining activities within the development envelope.

This report documents the methodology, sampling effort and results of the subterranean fauna sampling programme completed within the survey area. Both the field sampling and report are subject to specific limitations that are discussed in Section 3.11.

## 2.5 Subterranean Fauna Overview

Until recently, Australia was thought to lack habitat suitable for subterranean fauna. However, recent research (mostly with an emphasis on impact assessment) has revealed Australia to be highly diverse in subterranean fauna, with numerous areas of interest throughout the continent (Guzik et al. 2010). In Western Australia these zones of subterranean biodiversity can be found at Cape Range, Barrow Island, the Pilbara bioregion, the Yilgarn and the Nullarbor (Humphreys 2001a, Page et al. 2008, Guzik et al. 2010).

Recent surveys in the Pilbara have collected subterranean fauna from a range of geological units such as pisolitic iron formations, channel iron deposits, unconsolidated alluvium and sedimentary basalt (Marmonier et al. 1993, Biota 2004, 2006a, 2010a, 2011a, 2013). This indicates that the suitability of a formation as habitat for subterranean fauna is mostly a function of the availability of habitable space (Marmonier et al. 1993, Humphreys 1999, Biota 2006a), rather than a specific geological unit.

Subterranean fauna habitats are characterised by shared physical parameters that include a lack of light, stable temperature, limited nutrient infiltration from surface environments and a constant humidity (Juberthie 2000, Romero 2009). These habitat characteristics have resulted in convergence in body morphology evolution amongst many subterranean fauna. Morphological characteristics common to most subterranean fauna include reduced or lack of pigmentation, reduced or lack of eyes, and elongate body morphology and appendages adapted for sensory movement (Culver and Pipan 2009, Romero 2009).

Subterranean fauna can be categorised into two distinct ecological groups based on habitat: troglofauna and stygofauna. Troglofauna are a suite of fauna that survive only in air-filled cavities and interstices between the surface and the water table. Stygofauna comprise aquatic taxa occurring in groundwater aquifers and subterranean water bodies. Troglobites and stygobites are obligatory subterranean habitat dwellers, and while they may occur close to surface environments, are strongly adapted to subterranean environments. This makes such fauna unable or highly unlikely to survive surface conditions. A range of similarly adapted fauna known as troglaphiles, troglloxenes and edaphobites also occur; these fauna use subterranean habitats opportunistically but are able to survive outside these stable environments. Studies from the Pilbara bioregion have demonstrated that these suites of fauna are not similarly restricted in range and are therefore unlikely to be impacted by small-scale developments (Biota and Helix 2012, Helix 2012).

### 2.5.1 Troglofauna

Troglofauna in semi-arid Australia are thought to be relictual rainforest fauna; fauna adapted to humid environments, which retreated underground to cave systems during the aridification of

Australia (In the late Miocene; Humphreys 1993). This is inferred from affinities of the taxonomic groups represented amongst the troglofauna with other extant taxa in tropical climates. Some invertebrate groups with troglobitic representatives include the Arachnida (e.g. Schizomida, Pseudoscorpiones and Araneae), Chilopoda (e.g. Scolopendrida), Diplopoda (e.g. Polydesmida and Haplodesmida), and Insecta (e.g. Diplura, Zygentoma, Coleoptera and Blattodea). A single troglobitic vertebrate species of blind snake (*Anilius longissimus*) is known from Australia, collected from Barrow Island (Aplin 1998, Humphreys et al. 2013).

Due to their dependence on constant humidity, the dispersal and distribution of troglobitic fauna species tends to remain limited to individual blocks of inter-connected habitat, leading to long periods of population isolation and speciation. As a result, troglobitic fauna are considered to be of conservation significance, given that species often display extreme short-range endemism and may therefore be affected by relatively small-scale developments such as mining and construction.

## **2.5.2 Stygofauna**

Stygol communities in Western Australia are predominantly Crustacean (e.g. Amphipoda, Isopoda, Decapoda, Syncarida, Ostracoda and Copepoda), however Annelida (e.g. Oligochaeta), Arachnida (e.g. Hydracarina) and Platyhelminthes are also commonly collected. Three species of stygal vertebrates have been recorded from Australia. This includes two species of blind gudgeon (*Milyeringa veritas* and *M. justitia*) as well as a blind cave eel, *Ophisternon candidum* (Humphreys 2001b, DSEWPac 2008, Foster and Humphreys 2011, Larson et al. 2013).

## 3.0 Methodology

### 3.1 Desktop Review and Database Searches

#### 3.1.1 Desktop Review

A desktop review of relevant existing information was undertaken in order to provide context to the assessment of subterranean fauna at Mesa H. The review considered:

1. previous relevant studies conducted within 40 km of the development envelope;
2. Threatened Ecological Communities (TECs) and Priority Ecological Communities (PECs) listed by the Department of Biodiversity, Conservation and Attractions (DBCA) that are of relevance to subterranean fauna and within 40 km of the development envelope; and
3. assessment of the surface geology of the development envelope in a local context.

#### 3.1.2 Database Searches

The following databases were searched to assist with compilation of a list of potential species for the development envelope:

1. **NatureMap:** a collaboration between DBCA and the Western Australian Museum (WAM). This database represents the most comprehensive source of information on the distribution of Western Australia's fauna, comprising records from the Fauna Survey Returns database and Western Australian Threatened Fauna Database (both maintained by the Department of Biodiversity, Conservation and Attractions) and the WAM Specimen Database. The search was conducted 06/02/2017 within a 20 km radius of a central point (421372 m E, 7597354 m S).
2. **Atlas of Living Australia (ALA):** a collaborative project between academic collecting institutions, private individual collectors and community groups. The atlas contains occurrence records, environmental data, images and the conservation status of species throughout Australia. The search was conducted 06/02/2017 within a 10 km radius of a central point (421372 m E, 7597354 m S).
3. **WAM's Arachnida, Myriapoda, Crustacea and Mollusca databases:** The search request was submitted 14/03/2017 using the following bounding co-ordinates: 405138 m E, 7607948 m S (top left corner), 435642 m E, 7582695 m S (bottom right corner).
4. **Biota Internal Database:** This database includes all of the subterranean fauna data collected by Biota within Western Australia, which includes the majority of the past records from the Robe River valley. The search was conducted 06/02/2017 within 15 km from a central point in the development envelope (421490 m E, 7593430 m S).

### 3.2 Survey Timing and Personnel

A total of six phases of troglafauna sampling and five phases of stygofauna sampling were undertaken over the course of nine field mobilisations occurring between October 2015 and December 2017.

The field work was completed under "Regulation 17: Licence to Take Fauna for Scientific Purposes", licence numbers SF010536 (30/09/2015 – 30/06/2016) and SF010915 (22/07/2016 – 30/06/2017), both issued to Mr Chris Cole of Biota (Appendix 1). Phases 4- 6 were completed under "Licence to Take Fauna for Scientific Purposes" No. 08-001539-1 issued to Jason Alexander (Appendix 1).

A summary of survey timing, survey components and survey personnel is presented in Table 3.1.

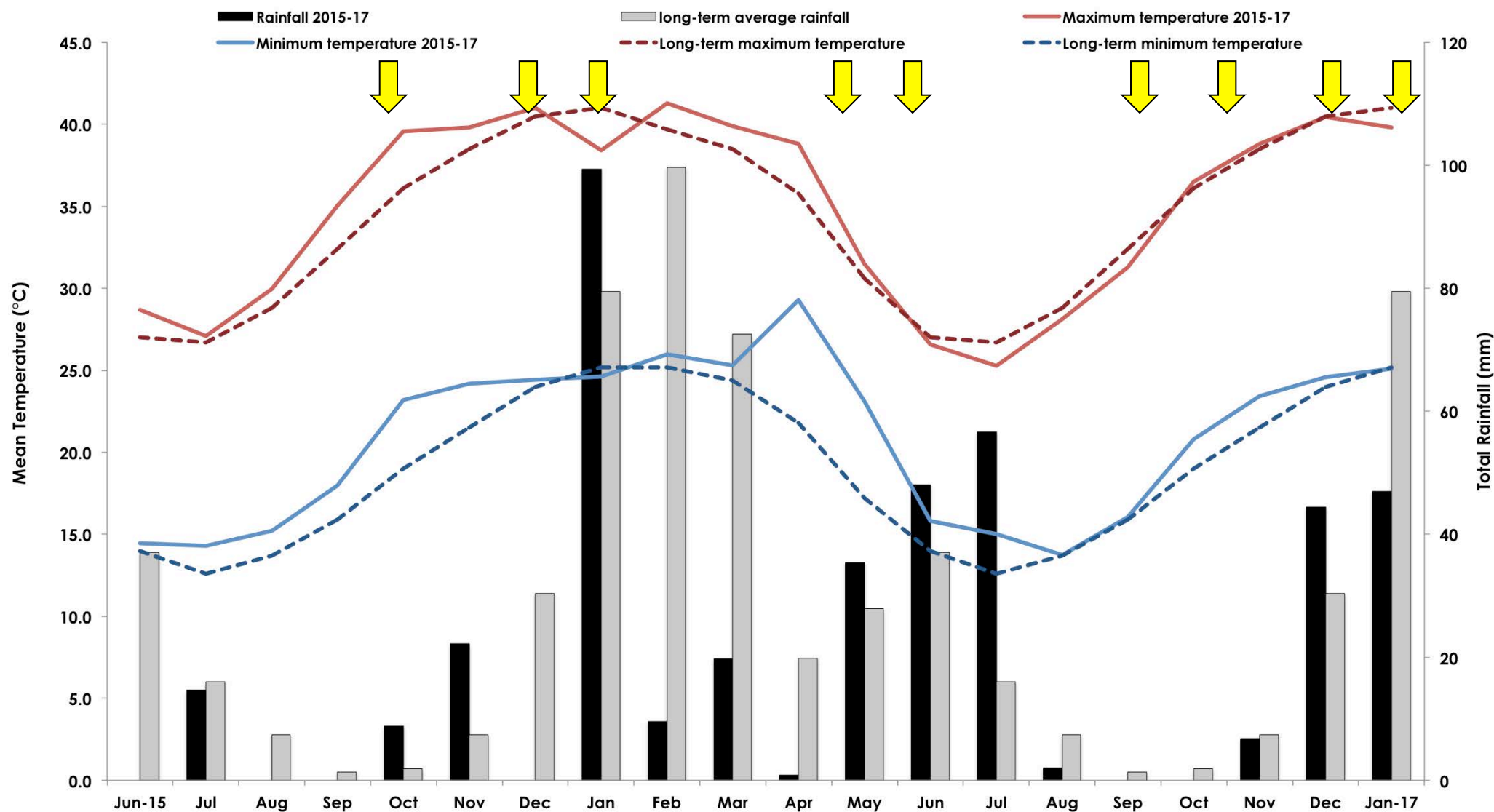
**Table 3.1: Summary of field sampling undertaken during the survey.**

| Sampling Dates        | Survey Component   | Survey Personnel                                |
|-----------------------|--|---|
| 23 - 29 October 2015  | Phase 1 trap installation<br>Phase 1 stygofauna sampling | David Keirle and Chris Cole                     |
| 9 -12 December 2015   | Phase 1 trap retrieval<br>Phase 2 trap installation      | David Keirle, Tim Sachse                        |
| 18 – 21 January 2016  | Phase 2 trap retrieval<br>Phase 2 stygofauna sampling    | Penny Brooshooft, Scott Werner                  |
| 3 – 7 May 2016        | Phase 3 trap installation<br>Phase 3 stygofauna sampling | Chris Cole, Nicola Watson                       |
| 27 – 28 June 2016     | Phase 3 trap retrieval                                   | Chris Cole, Nicola Watson                       |
| 9 – 14 September 2016 | Phase 4 trap installation<br>Phase 4 stygofauna sampling | Nicola Watson, Penny Brooshooft                 |
| 25 – 27 October 2016  | Phase 4 trap retrieval<br>Phase 5 trap installation      | Penny Brooshooft, Jason Alexander               |
| 12 – 14 December 2016 | Phase 5 trap retrieval<br>Phase 6 trap installation      | Nicola Watson, Jacinta King                     |
| 30 -31 January 2017   | Phase 6 trap retrieval                                   | Jason Alexander, Greg Richter (Rio Tinto)       |
| 11-15 December 2017   | eDNA and Phase 5 stygofauna sampling (reference areas)   | Jason Alexander, Jacinta King, Michael Greenham |

### 3.2.1 Weather Conditions

Temperature and rainfall data were obtained from the Mesa J mining operations adjacent to the east end of Mesa H (Figure 2.1). Long-term climate data (rainfall from 1971 – 2014, temperature data from 1971 – 2005) were obtained from the Bureau of Meteorological (BOM) weather station in Pannawonica (station number 5069), approximately 15 km northeast of Mesa H.

The phases of sampling within the development envelope occurred between October 2015 and the end of January 2017. Several major rainfall events occurred throughout that sampling period (Figure 3.1). Past observations suggest the approximate timing of sampling after significant rainfall creates suitable conditions for sampling for troglifauna (Biota 2006a). Weather data from Pannawonica show that no rainfall was recorded during the three months preceding the Phase 5 reference area stygofauna sampling in December 2017.



**Figure 3.1:** Climate and weather graph depicting long-term and monthly averages for 6 months preceding and during subterranean fauna sampling. Long-term temperature data 1971-2005, rainfall data 1971-2014; arrows indicate field mobilisation timing.

### 3.3 Stygofauna Sampling

Stygofauna were sampled using modified plankton haul nets, constructed from 70  $\mu$ m plankton mesh, with 50 mm and 100 mm apertures attached to a stainless steel catch cylinder. Nets were lowered to the bottom of water bores and drill holes before being hauled slowly through the water column to the surface, where the contents of the cylinder were flushed into a uniquely labelled container. Each monitoring site was sampled in this way a minimum of five times. On the final haul, the net was agitated gently to stir the benthos layer and mobilise any fauna present for more effective specimen collection. Specimens were stored in a shaded esky in order to keep the samples cool prior to sorting and identification.

Following sampling of each monitoring site, the nets were thoroughly rinsed with water, inspected and (where possible) left to dry before using on another site. This prevented cross-contamination of specimens between sampling sites.

### 3.4 eDNA Sampling

Environmental DNA (eDNA) sampling took place on completion of stygofauna haul net sampling during Phase 5 in December 2017 (Table 3.1). The environmental samples were collected to target the Blind Cave Eel (*Ophisternon candidum*) (Section 4.4.1.1), which is not readily detectable with conventional sampling methods (Biota and Helix 2014).

This sampling initially included agitation of the benthos at the base of the borehole, to mobilise DNA molecules and fragments of tissues within the water column. Then, wearing nitrile gloves, a 1 L bailer, attached to the same line used for haul net sampling at that specific site, was lowered into the bore until approximately 1 m from the bottom of the hole. The bailer was then removed from the bore and the contents of the bailer emptied into a brand new and uniquely labeled 1 L container. This step was repeated twice more. The line was then discarded after bailing to prevent contamination of eDNA between sites.

A further two 1 L containers were filled using a specialised downhole pump (a GEOsub 12 V purging pump) attached to brand new HDPE tubing. The pump was lowered to approximately 1 m from the benthos and activated. The pump was allowed to run for three minutes, which acts to purge the bore and draw in groundwater, and potential eDNA, from the surrounding aquifer. After this, the remaining two labeled containers were filled in the same fashion. All filled containers were stored in the field in an ice-filled esky until arrival at the on-site laboratory in Pannawonica.

### 3.5 Troglafauna Sampling

Troglobitic fauna were sampled using two methods; baited colonisation traps and haul net scraping.

#### 3.5.1 Colonisation Trapping

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

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



Traps were recovered from each drillhole after a minimum of six weeks and stored in labelled zip-lock bags in order to maintain humidity and prevent desiccation of potential troglofauna specimens during transportation to Perth.

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

### **3.5.2 Troglofauna Scraping**

Troglofauna were also sampled using reinforced stygofauna haul nets. These nets were constructed from 70 µm plankton mesh and had 100 mm apertures attached to a weighted catch jar. Sampling took place by lowering the net to the bottom of the site before being hauled slowly to the surface whilst scraping the edge of the drillhole. This dislodged any fauna on the vertical surface of the drillhole interior. Each site was scraped a minimum of four times, scraping each side of the drillhole. On completion, fauna specimens were preserved in-situ in 100% ethanol and individually labelled. The contents of the net, which included dry soil and root matter, were emptied into a uniquely labelled container. The container was then filled with 100% ethanol to preserve any specimens that may have been in the soil and root matter for later sorting in the field laboratory.

## **3.6 Morphological Identifications**

Preliminary identification of subterranean fauna involved identification of specimens to order level, where possible, or separation of specimens into distinct morphotypes. Sorting was completed using dissecting microscopes (Olympus SZ40 and SZ61, magnification up to 40x). Specimens were preserved in 100% ethanol once separated out into morphotypes, which allows for both morphological and molecular analyses. Specialist morphological identifications of copepods, ostracods and isopods were undertaken by Jane McRae and Stuart Halse of Bennelongia Environmental Consultants (Appendix 2).

## **3.7 Specimen Molecular Analysis**

Molecular analysis of selected groups was conducted to determine the number of species present, and to compare the results with data from elsewhere in the Pilbara. These were taxonomic groups for which past studies have shown morphological identifications to species level are generally not possible or reliable for most specimens, where inadequate taxonomic expertise is readily available for the groups in question, and/or where well established phylogenetic frameworks exist to more adequately place the specimens into regional context. Yvette Hitchen of Helix Molecular Solutions (Helix) completed DNA sequencing of the collected Amphipoda, Schizomida, Diplura, Thysanura, Isopoda and Pseudoscorpiones. Terrie Finston (Helix) and Rachael King (South Australian Museum) provided analysis and interpretation of the molecular data.

The collected specimens were sequenced for variation at the mitochondrial cytochrome oxidase subunit 1 gene (CO1). Where larger numbers of specimens of the same morphotype (i.e. more than five) were recorded from the same sampling site, a subsample of five specimens was typically taken for sequencing. Schizomid specimens recorded during previous surveys at Mesa J (Biota 2009) were also sequenced as part of the current study to improve local context. A complete account of the molecular analysis methodology is provided in Appendix 3.

On the basis of existing literature and past molecular studies of Pilbara troglofauna (Hebert et al. 2003a, 2003b, Harvey et al. 2008), lineages with a divergence of less than 4.0% were considered a single species, whereas lineages with a divergence greater than 6.0% were considered separate distinct species for the purpose of this report, subject to a review of the relative variation within each putative species, unless advised otherwise by Helix based on other data for the relevant taxonomic group. Lineages with intermediate divergences ranging from 4.0 – 6.0% were resolved to a preliminary level for the purposes of this report, with further input from Helix, and consideration to collection proximity, habitat, geology and past results from the same taxonomic groups.

## 3.8 eDNA Sample Analysis

### 3.8.1 Sample Processing

Groundwater samples collected for eDNA analysis were filtered through 0.45 µm sterile filter membranes, using both Sentino Microbiology Pumps and manually using specialised Nalgene Filter Flasks and a hand pump. One container of groundwater (1 L) was processed per membrane and on completion of this process, the membrane was folded in half then cut vertically. These halves were placed in separate sterile storage bags and frozen with the remaining filters for that site depending on which lab the membrane would be despatched to for further analysis (Section 3.8.2). All laboratory equipment was sterilised between samples using a bleach solution.

### 3.8.2 Sample Analysis

eDNA sample filters were analysed by two different molecular methods, each of which was undertaken independently by two separate laboratories. The objectives of both methods were to detect residual DNA from the Blind Cave Eel (*Ophisternon candidum*) (Section 4.4.1.1) in the environmental samples.

#### 3.8.2.1 qPCR

Helix Molecular Solutions analysed half of the replicate membranes from each sample site using a real-time qPCR method developed previously for the Blind Cave Eel (Biota and Helix 2014). Sequence data from past collections of *Ophisternon* from the Jimmawurrada and Bungaroo Creek locality was used to design a species-specific probe using the Integrated DNA Technology (IDT) design tool PrimerQuest and further edited using Oligo Primer analysis software version 6 (Molecular Biology Insights, Cascade, USA). The resulting assay amplified a diagnostic 80 bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) (Biota and Helix 2014).

Samples were extracted using the QIAGEN Blood and Tissue Kit (QIAGEN, Hilden, Germany) and as with previous studies all starting volumes were doubled to ensure that the filter membrane was covered during incubation. All samples had two elutes of 50 µl each.

The specifically designed qPCR assay was then used to detect the presence of *Ophisternon* DNA. Quantitative real-time PCR assays were performed using the Applied Biosystems StepOne Plus real-time PCR system and software (Biota and Helix 2014). The assay identifies the presence of the target species by the unique fluorescent signal produced during the polymerase chain reaction when both the species-specific primers as well as species-specific probes match DNA present in the sample. The fluorescent response can be visualised in real time as amplification proceeds. Only samples that showed a positive CT value (the number of cycles required for the fluorescent signal to exceed the background level of fluorescence) and amplification plot were considered to test positive for the target *Ophisternon* DNA.

#### 3.8.2.2 Next Generation Sequencing Metabarcoding

The matching halves of the filters from each site were despatched to the Trace and Environmental DNA laboratory at Curtin University. The molecular analysis undertaken by Curtin University also involved a QIAGEN Blood and Tissue Kit to extract eDNA from the samples, and then utilised a metabarcoding to simultaneously sequence the resultant environmental DNA. A fish 16S assay as used amplified using PCR followed by sequencing to obtain data relevant to *Ophisternon* (Appendix 4). This was then subsequently refined using specific assays developed for the purposes of this study (Appendix 4).

The sequence data were then analysed against both publicly available reference sequence data on GenBank and other sources, in addition to comparisons with sequences obtained from *Ophisternon* specimens collected previously from the locality (Appendix 4).

## 3.9 Categories of Conservation Significance

For the purpose of this report, the conservation significance of the fauna collected during this study, or records collated during the desktop review, was categorised as follows:



- **Confirmed short-range endemic (SRE) species:** Species where sufficient taxonomic expertise is available, and with adequate representation in WAM collections or genetic databases, that are known to be limited in distribution.
- **Potential SRE species:** Species where there is insufficient taxonomic knowledge or too limited a number of collections to determine SRE status. Habitat, morphology, molecular or taxonomic data deficient, but belonging to groups that may display short-range endemism.
- **Widespread species (Not an SRE):** Well-collected species, that are typically taxonomically well resolved.

SRE categories are based on WAM SRE guidelines (see Appendix 4 for more detail).

## 3.10 Sampling Effort

### 3.10.1 Stygofauna

A total of 53 sites were sampled for stygofauna across the five phases (Table 3.2, Figure 3.2 and Figure 3.3). The majority of sites (n=39) were sampled on at least two separate phases. Fourteen sites were sampled only once, 10 sites were sampled across three phases and one site (WB13MEJ003) was sampled across four of the five phases (Table 3.2). Eighteen of the 53 sites were located outside of the development envelope (denoted by an asterisk in Table 3.2) (Figure 3.2; Figure 3.3).

**Table 3.2: Stygofauna sampling sites at Mesa H (\*site outside of the drawdown extent).**

| Site Name   | Easting (m E) | Northing (m N) | Sampling Phase |   |   |   |   |
|-------------|---------------|----------------|----------------|---|---|---|---|
|             |               |                | 1              | 2 | 3 | 4 | 5 |
| DD13MEH0007 | 419635        | 7593002        | ✓              | ✓ |   | ✓ |   |
| MB12MEJ004  | 421046        | 7591683        | ✓              | ✓ |   | ✓ |   |
| RC13MEH0007 | 419642        | 7592603        | ✓              |   |   | ✓ |   |
| RC13MEH0040 | 419893        | 7592704        | ✓              | ✓ |   | ✓ |   |
| RC13MEH0041 | 419940        | 7592705        | ✓              | ✓ |   |   |   |
| RC13MEH0097 | 420086        | 7592803        | ✓              |   |   | ✓ |   |
| RC13MEH0099 | 420088        | 7592901        | ✓              |   |   | ✓ |   |
| RC14MEH0018 | 419088        | 7593299        | ✓              | ✓ |   | ✓ |   |
| RC14MEH0053 | 419092        | 7592752        | ✓              | ✓ |   | ✓ |   |
| WB13MEJ003  | 420717        | 7591683        | ✓              | ✓ | ✓ | ✓ |   |
| RC12JIM0019 | 421396        | 7591472        |                | ✓ |   |   |   |
| RC15MEJ0018 | 419893        | 7592052        |                | ✓ |   | ✓ |   |
| WB12MEJ004  | 421773        | 7591276        |                | ✓ | ✓ | ✓ |   |
| 158*        | 406804        | 7597112        |                |   | ✓ | ✓ |   |
| 199*        | 402701        | 7606147        |                |   | ✓ | ✓ |   |
| Dave Bore*  | 392512        | 7607436        |                |   | ✓ | ✓ | ✓ |
| 25*         | 432152        | 7602229        |                |   | ✓ | ✓ | ✓ |
| 31*         | 432929        | 7603179        |                |   | ✓ | ✓ | ✓ |
| 32*         | 432452        | 7602673        |                |   | ✓ | ✓ |   |
| 34*         | 432423        | 7602432        |                |   | ✓ | ✓ |   |
| 87*         | 410739        | 7604138        |                |   | ✓ | ✓ |   |
| MB12MEJ001  | 421763        | 7591266        |                |   | ✓ | ✓ |   |
| RC15MEH0178 | 415934        | 7595048        |                |   | ✓ | ✓ |   |
| RC15MEH0284 | 416632        | 7597202        |                |   | ✓ | ✓ |   |
| RC15MEH0286 | 416840        | 7597576        |                |   | ✓ | ✓ |   |
| RC15MEH0295 | 417235        | 7597402        |                |   | ✓ | ✓ |   |
| RC15MEH0296 | 418179        | 7595152        |                |   | ✓ | ✓ |   |
| RC15MEH0297 | 417884        | 7594252        |                |   | ✓ | ✓ |   |
| RC15MEH0298 | 419285        | 7594350        |                |   | ✓ | ✓ |   |
| RC15MEH0299 | 419291        | 7593101        |                |   | ✓ | ✓ |   |
| RC15MEH0349 | 415635        | 7597550        |                |   | ✓ | ✓ |   |
| RC15MEH0350 | 418585        | 7592350        |                |   | ✓ | ✓ |   |

| Site Name    | Easting (m E) | Northing (m N) | Sampling Phase |    |    |    |    |
|--------------|---------------|----------------|----------------|----|----|----|----|
|              |               |                | 1              | 2  | 3  | 4  | 5  |
| RC15MEH0351  | 417838        | 7593554        |                |    | ✓  | ✓  |    |
| RC15MEH0352  | 416535        | 7595450        |                |    | ✓  | ✓  |    |
| RC15MEH0353  | 416735        | 7595950        |                |    | ✓  | ✓  |    |
| RC15MEH0354  | 416335        | 7596450        |                |    | ✓  | ✓  |    |
| RC15MEH0355  | 415335        | 7596050        |                |    | ✓  | ✓  |    |
| RC15MEH0356  | 415335        | 7597550        |                |    | ✓  | ✓  |    |
| RC15MEJ0017  | 420087        | 7591940        |                |    | ✓  | ✓  |    |
| RR1*         | 419176        | 7597904        |                |    | ✓  | ✓  | ✓  |
| RC16JIM0019  | 423011        | 7590291        |                |    |    | ✓  |    |
| BC186        | 429578        | 7587212        |                |    |    |    | ✓  |
| BC401*       | 443563        | 7574126        |                |    |    |    | ✓  |
| Budgie Bore* | 382495        | 7615587        |                |    |    |    | ✓  |
| JW023        | 426138        | 7590140        |                |    |    |    | ✓  |
| JW024        | 427126        | 7590154        |                |    |    |    | ✓  |
| MB16MEC0008* | 393565        | 7600580        |                |    |    |    | ✓  |
| MB16MEC0009* | 393590        | 7601145        |                |    |    |    | ✓  |
| MB17MEH0007* | 417664        | 7600421        |                |    |    |    | ✓  |
| MB17MEH0008* | 417120        | 7600417        |                |    |    |    | ✓  |
| MB17MEH0009* | 416436        | 7599848        |                |    |    |    | ✓  |
| MB17MEH0010* | 416440        | 7599848        |                |    |    |    | ✓  |
| MB17MEH0015  | 416041        | 7597690        |                |    |    |    | ✓  |
| Total        |               |                | 10             | 10 | 29 | 39 | 16 |

Sixteen of the bores sampled for fauna were also sampled for eDNA during the December 2017 Phase 5 stygofauna sampling (Table 3.3; Figure 3.2; Figure 3.3). These were selected primarily on the basis of having been past confirmed eel record sites (Section 4.4.1.1) or being situated in equivalent hydrogeological settings along Bungaroo Creek, Jimmawurrada Creek and the Robe River (Table 3.3). In addition, a 3 L sample of surface water was taken as a Control sample from standing water within a pool along the Robe River (Table 3.3; Figure 3.2). The majority of the eDNA sampling sites were outside of the drawdown extent, within five located within (Table 3.3; Figure 3.3).

**Table 3.3: eDNA sites sampled during December 2017 (\*site outside of the drawdown extent).**

| Site Name    | Easting (m E) | Northing (m N) | Notes  |
|--------------|---------------|----------------|--|
| Control*     | 424478        | 7597147        | Equivalent hydrogeology to confirmed eel sites                       |
| 25*          | 432152        | 7602229        | Equivalent hydrogeology to confirmed eel sites                       |
| 31*          | 432929        | 7603179        | Equivalent hydrogeology to confirmed eel sites                       |
| Dave Bore*   | 392512        | 7607436        | Equivalent hydrogeology to confirmed eel sites                       |
| BC186        | 429578        | 7587212        | Confirmed eel record 2009  |
| BC401        | 443563        | 7574126        | Equivalent hydrogeology to confirmed eel sites                       |
| Budgie Bore* | 382495        | 7615587        | Equivalent hydrogeology to confirmed eel sites                       |
| JW023        | 426138        | 7590140        | Confirmed eel record 2016  |
| JW024        | 427126        | 7590154        | Confirmed eel record 2017  |
| MB16MEC0008* | 393565        | 7600580        | Equivalent hydrogeology to confirmed eel sites                       |
| MB16MEC0009* | 393590        | 7601145        | Equivalent hydrogeology to confirmed eel sites                       |
| MB17MEH0007* | 417664        | 7600421        | Equivalent hydrogeology to confirmed eel sites; Bailer only for eDNA |
| MB17MEH0008* | 417120        | 7600417        | Equivalent hydrogeology to confirmed eel sites; Bailer only for eDNA |
| MB17MEH0009* | 416436        | 7599848        | Equivalent hydrogeology to confirmed eel sites                       |
| MB17MEH0010* | 416440        | 7599848        | Equivalent hydrogeology to confirmed eel sites                       |
| MB17MEH0015  | 416041        | 7597690        | Equivalent hydrogeology to confirmed eel sites; Bailer only for eDNA |
| RR1*         | 419176        | 7597904        | Equivalent hydrogeology to confirmed eel sites; Bailer only for eDNA |

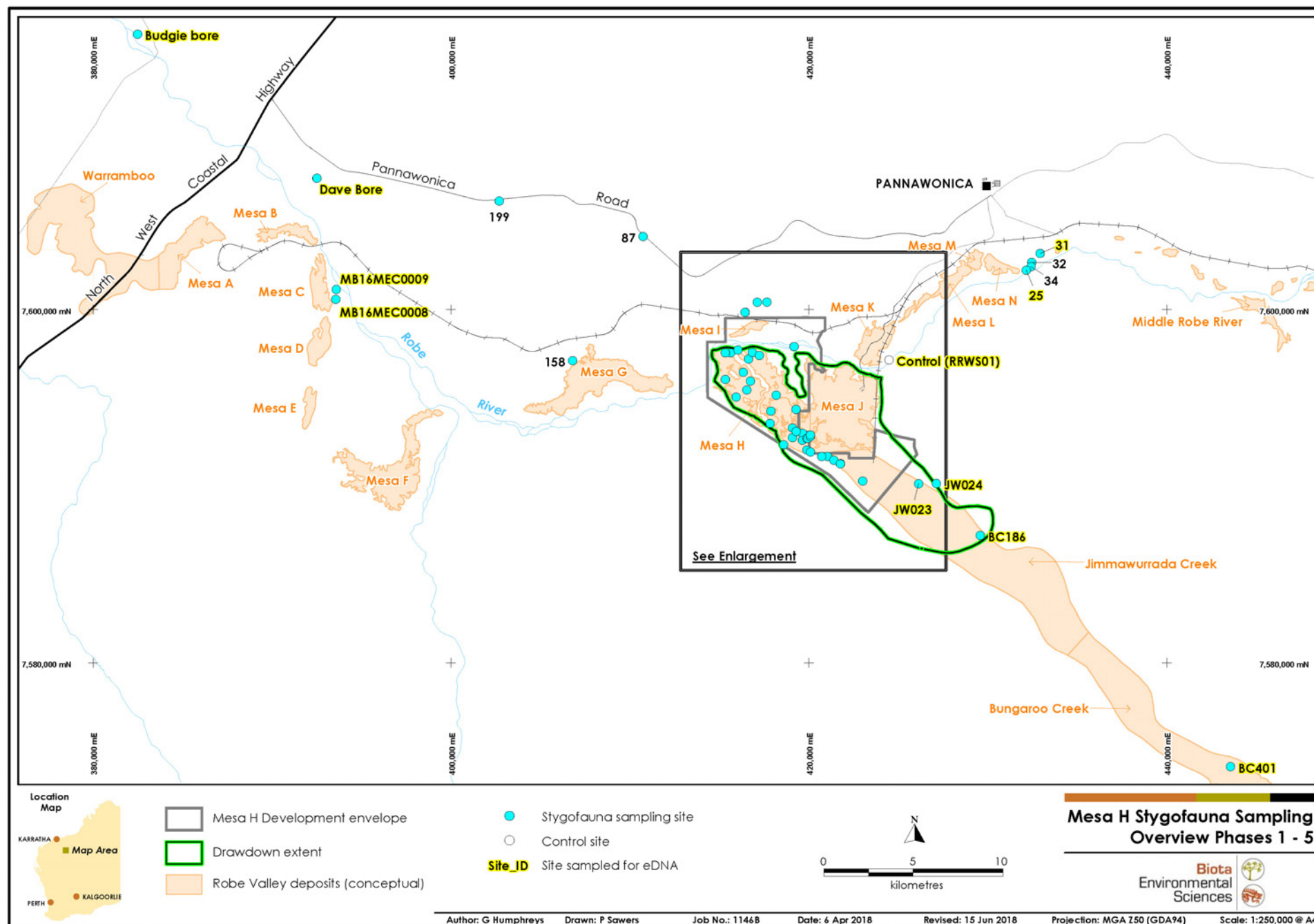


Figure 3.2: Overview of stygofauna sampling sites Phases 1 - 5.



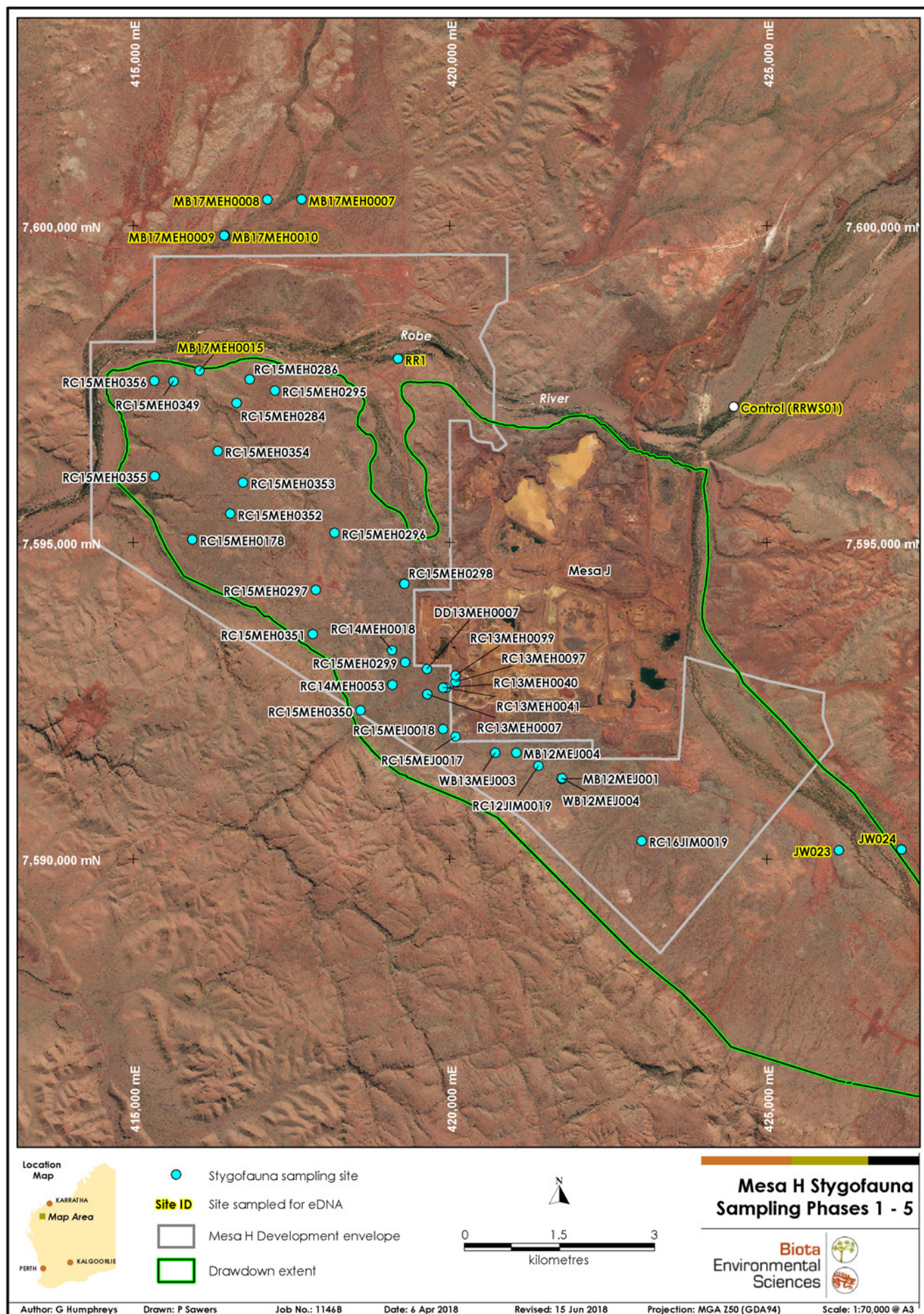


Figure 3.3: Stygofauna sampling sites within and adjacent to the Mesa H development envelope Phases 1-5.



### 3.10.2 Troglotauna

A total of 136 sites were sampled for troglotauna across six phases (Table 3.4, Figure 3.4). Two thirds of the sites were sampled were sampled at least twice (n=86), with the remaining 50 sampled once (Table 3.4). A total of 57 sites had additional haul net sampling completed (denoted by 'S' in Table 3.4), with nine sites being scraped with haul nets twice.

**Table 3.4: Troglotauna sampling sites at Mesa H.**

| Site Name   | Easting (m E) | Northing (m N) | Sampling Phase |      |   |   |      |   |
|-------------|---------------|----------------|----------------|------|---|---|------|---|
|             |               |                | 1              | 2    | 3 | 4 | 5    | 6 |
| DD15MEH0003 | 419342        | 7592303        | ✓              | ✓    |   |   |      |   |
| GR15MEH0013 | 415736        | 7595443        | ✓              | ✓    |   |   |      |   |
| MEHRC0957   | 415437        | 7596556        | ✓              | ✓    |   |   |      |   |
| MEHRD0386   | 415141        | 7596155        | ✓              | ✓    |   |   |      |   |
| RC12JIM0019 | 421396        | 7591472        | ✓(S)           | ✓(S) |   |   |      |   |
| RC12JIM0027 | 421626        | 7591084        | ✓              | ✓    |   |   |      |   |
| RC12MEH0075 | 419894        | 7592163        | ✓              | ✓    |   |   |      |   |
| RC12MEH0120 | 419988        | 7592302        | ✓(S)           | ✓(S) |   |   |      |   |
| RC12MEH0221 | 419349        | 7593105        | ✓              | ✓    |   | ✓ | ✓(S) |   |
| RC13MEH0007 | 419642        | 7592603        | ✓              | ✓    |   |   |      |   |
| RC13MEH0041 | 419940        | 7592705        | ✓              | ✓    |   |   |      |   |
| RC13MEH0135 | 419288        | 7593902        | ✓              | ✓    |   |   |      |   |
| RC14MEH0051 | 419391        | 7593448        | ✓              | ✓    |   |   |      |   |
| RC14MEH0053 | 419092        | 7592752        | ✓              | ✓    |   |   |      |   |
| RC14MEH0109 | 418837        | 7593804        | ✓              | ✓    |   |   |      |   |
| RC14MEH0169 | 418888        | 7593560        | ✓              | ✓    |   |   |      |   |
| RC14MEH0238 | 418846        | 7592802        | ✓(S)           | ✓(S) |   |   |      |   |
| RC14MEH0252 | 418789        | 7593304        | ✓              | ✓    |   | ✓ | ✓(S) |   |
| RC14MEH0270 | 418381        | 7593102        |                |      | ✓ | ✓ | ✓(S) |   |
| RC14MEH0308 | 418987        | 7592556        | ✓              | ✓    |   |   |      |   |
| RC14MEH0315 | 418894        | 7592510        |                |      | ✓ |   |      |   |
| RC14MEH0326 | 418389        | 7593354        | ✓              | ✓    |   |   |      |   |
| RC14MEH0339 | 418393        | 7594004        | ✓              | ✓    |   |   |      |   |
| RC14MEH0388 | 418088        | 7593504        |                |      | ✓ |   |      |   |
| RC15MEH0007 | 417793        | 7593659        | ✓              | ✓    |   |   |      |   |
| RC15MEH0060 | 418487        | 7594750        | ✓(S)           | ✓(S) |   |   |      |   |
| RC15MEH0083 | 417880        | 7594552        | ✓              | ✓    |   |   |      |   |
| RC15MEH0097 | 417536        | 7594447        | ✓              | ✓    |   |   |      |   |
| RC15MEH0114 | 416434        | 7594352        | ✓(S)           | ✓(S) |   |   |      |   |
| RC15MEH0166 | 416933        | 7594851        | ✓(S)           | ✓(S) |   |   |      |   |
| RC15MEH0175 | 416434        | 7594944        | ✓              | ✓    |   |   |      |   |
| RC15MEH0190 | 416733        | 7595253        | ✓              | ✓    |   |   |      |   |
| RC15MEH0196 | 416733        | 7595748        | ✓              | ✓    |   |   |      |   |
| RC15MEH0202 | 416934        | 7595650        | ✓              | ✓    |   |   |      |   |
| RC15MEH0230 | 416334        | 7595650        | ✓              | ✓    |   |   |      |   |
| RC15MEH0236 | 415737        | 7595850        | ✓              | ✓    |   |   |      |   |
| RC15MEH0261 | 418438        | 7595599        | ✓              | ✓    |   |   |      |   |
| RC15MEH0268 | 417695        | 7596007        | ✓              | ✓    |   |   |      |   |
| RC15MEH0272 | 417638        | 7596399        | ✓              | ✓    |   |   |      |   |
| RC15MEH0283 | 417234        | 7596996        | ✓(S)           | ✓(S) |   |   |      |   |
| RC15MEH0289 | 416840        | 7597402        | ✓              | ✓    |   |   |      |   |
| RC15MEH0302 | 416333        | 7596351        | ✓(S)           | ✓(S) |   |   |      |   |
| RC15MEH0317 | 415542        | 7596657        | ✓              | ✓    |   |   |      |   |
| RC15MEH0329 | 415435        | 7596950        | ✓              | ✓    |   |   |      |   |

| Site Name   | Easting (m E) | Northing (m N) | Sampling Phase |      |   |   |      |   |
|-------------|---------------|----------------|----------------|------|---|---|------|---|
|             |               |                | 1              | 2    | 3 | 4 | 5    | 6 |
| RC15MEH0375 | 415035        | 7597350        | ✓(S)           | ✓(S) |   |   |      |   |
| RC15MEH0379 | 417985        | 7594250        | ✓              | ✓    |   |   |      |   |
| RC15MEH0382 | 418985        | 7594650        | ✓              | ✓    |   |   |      |   |
| RC15MEJ0018 | 419893        | 7592052        | ✓              | ✓    |   |   |      |   |
| GR15MEH0009 | 415533        | 7594947        |                |      | ✓ |   |      |   |
| GR15MEH0015 | 416035        | 7596750        |                |      | ✓ |   |      |   |
| GR15MEH0029 | 417035        | 7594450        |                |      | ✓ |   |      |   |
| GR15MEH0032 | 419085        | 7594650        |                |      | ✓ |   |      |   |
| RC15MEH0014 | 419085        | 7595251        |                |      | ✓ |   |      |   |
| RC15MEH0048 | 417785        | 7594946        |                |      | ✓ |   |      |   |
| RC15MEH0049 | 417787        | 7594846        |                |      | ✓ |   |      |   |
| RC15MEH0087 | 418387        | 7594450        |                |      | ✓ |   |      |   |
| RC15MEH0100 | 418187        | 7594347        |                |      | ✓ |   |      |   |
| RC15MEH0109 | 416933        | 7594150        |                |      | ✓ |   |      |   |
| RC15MEH0127 | 416233        | 7594350        |                |      | ✓ |   |      |   |
| RC15MEH0132 | 415935        | 7594551        |                |      | ✓ |   |      |   |
| RC15MEH0153 | 415736        | 7594754        |                |      | ✓ |   |      |   |
| RC15MEH0227 | 416135        | 7596248        |                |      | ✓ |   |      |   |
| RC15MEH0245 | 415835        | 7596150        |                |      | ✓ |   |      |   |
| RC15MEH0274 | 418038        | 7596599        |                |      | ✓ |   |      |   |
| RC15MEH0284 | 416632        | 7597202        |                |      | ✓ |   |      |   |
| RC15MEH0286 | 416840        | 7597576        |                |      | ✓ |   |      |   |
| RC15MEH0306 | 416431        | 7596648        |                |      | ✓ |   |      |   |
| RC15MEH0315 | 415738        | 7596650        |                |      | ✓ |   |      |   |
| RC15MEH0335 | 415237        | 7597151        |                |      | ✓ |   |      |   |
| RC15MEH0339 | 415735        | 7597150        |                |      | ✓ |   |      |   |
| RC15MEH0349 | 415635        | 7597550        |                |      | ✓ |   |      |   |
| RC15MEH0362 | 415240        | 7597754        |                |      | ✓ |   |      |   |
| RC15MEH0371 | 417035        | 7594350        |                |      | ✓ |   |      |   |
| RC15MEH0373 | 414835        | 7597450        |                |      | ✓ |   |      |   |
| RC15MEH0381 | 418785        | 7595250        |                |      | ✓ |   |      |   |
| RC16JIM0001 | 420285        | 7591800        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0002 | 420662        | 7591812        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0003 | 420714        | 7591537        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0004 | 420900        | 7591450        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0005 | 421198        | 7591536        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0006 | 421360        | 7591830        | ✓              | ✓    |   |   | ✓    |   |
| RC16JIM0007 | 421774        | 7591708        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0008 | 422167        | 7591581        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0009 | 422061        | 7591411        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0010 | 422450        | 7591660        |                |      |   |   | ✓(S) |   |
| RC16JIM0013 | 422231        | 7591306        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0014 | 423237        | 7591031        |                |      |   |   | ✓(S) |   |
| RC16JIM0015 | 423273        | 7590714        |                |      |   |   | ✓(S) |   |
| RC16JIM0016 | 422909        | 7590883        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0017 | 422460        | 7590180        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0018 | 422698        | 7590543        |                |      |   |   | ✓(S) |   |
| RC16JIM0019 | 423011        | 7590291        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0020 | 423345        | 7590441        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0021 | 423110        | 7591582        |                |      |   | ✓ | ✓(S) |   |
| RC16JIM0022 | 422898        | 7591243        |                |      |   | ✓ | ✓(S) |   |

| Site Name   | Easting (m E) | Northing (m N) | Sampling Phase |   |   |   |      |   |
|-------------|---------------|----------------|----------------|---|---|---|------|---|
|             |               |                | 1              | 2 | 3 | 4 | 5    | 6 |
| RC16JIM0023 | 422125        | 7591136        |                |   |   | ✓ | ✓(S) |   |
| RC16JIM0024 | 421457        | 7591199        |                |   |   | ✓ | ✓(S) |   |
| RC16JIM0025 | 421176        | 7591102        |                |   |   | ✓ | ✓(S) |   |
| RC16JIM0026 | 420035        | 7591850        |                |   |   | ✓ | ✓(S) |   |
| RC16JIM0027 | 419885        | 7591750        |                |   |   | ✓ | ✓(S) |   |
| RC16JIM0028 | 419885        | 7591750        |                |   |   | ✓ | ✓(S) |   |
| RC16JIM0029 | 419585        | 7591850        |                |   |   | ✓ | ✓(S) |   |
| RC16JIM0030 | 419585        | 7591850        |                |   |   | ✓ | ✓(S) |   |
| RC16JIM0031 | 419789        | 7591694        |                |   |   | ✓ | ✓(S) |   |
| RC16JIM0032 | 419885        | 7591627        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0427 | 415131        | 7597113        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0428 | 414786        | 7597621        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0429 | 415037        | 7597616        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0430 | 414766        | 7597254        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0431 | 415143        | 7597821        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0432 | 415239        | 7597832        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0433 | 415439        | 7597649        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0434 | 415641        | 7597739        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0435 | 415662        | 7597625        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0436 | 415708        | 7597254        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0437 | 415892        | 7597032        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0572 | 415845        | 7594449        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0573 | 415455        | 7595243        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0574 | 419335        | 7593100        |                |   |   | ✓ | ✓(S) |   |
| RC16MEH0575 | 418785        | 7593300        |                |   |   | ✓ | ✓(S) |   |
| MEHRD0577   | 417838        | 7593855        |                |   |   |   |      | ✓ |
| MEHRD0757   | 415139        | 7596014        |                |   |   |   |      | ✓ |
| MEHRD0758   | 414937        | 7595953        |                |   |   |   |      | ✓ |
| MEHRD0834   | 418837        | 7592555        |                |   |   |   |      | ✓ |
| MEHRD1021   | 419587        | 7594252        |                |   |   |   |      | ✓ |
| DD16MEH0056 | 415646        | 7596307        |                |   |   |   |      | ✓ |
| RC15MEJ0019 | 419264        | 7592144        |                |   |   |   |      | ✓ |
| RC16MEH0041 | 417186        | 7594957        |                |   |   |   |      | ✓ |
| RC16MEH0042 | 417243        | 7594901        |                |   |   |   |      | ✓ |
| RC16MEH0255 | 417229        | 7594246        |                |   |   |   |      | ✓ |
| RC16MEH0258 | 417236        | 7594454        |                |   |   |   |      | ✓ |
| RC16MEH0261 | 417233        | 7594651        |                |   |   |   |      | ✓ |
| RC16MEH0264 | 417476        | 7594964        |                |   |   |   |      | ✓ |
| RC16MEH0707 | 415586        | 7596302        |                |   |   |   |      | ✓ |
| RC16MEH0712 | 415188        | 7596057        |                |   |   |   |      | ✓ |



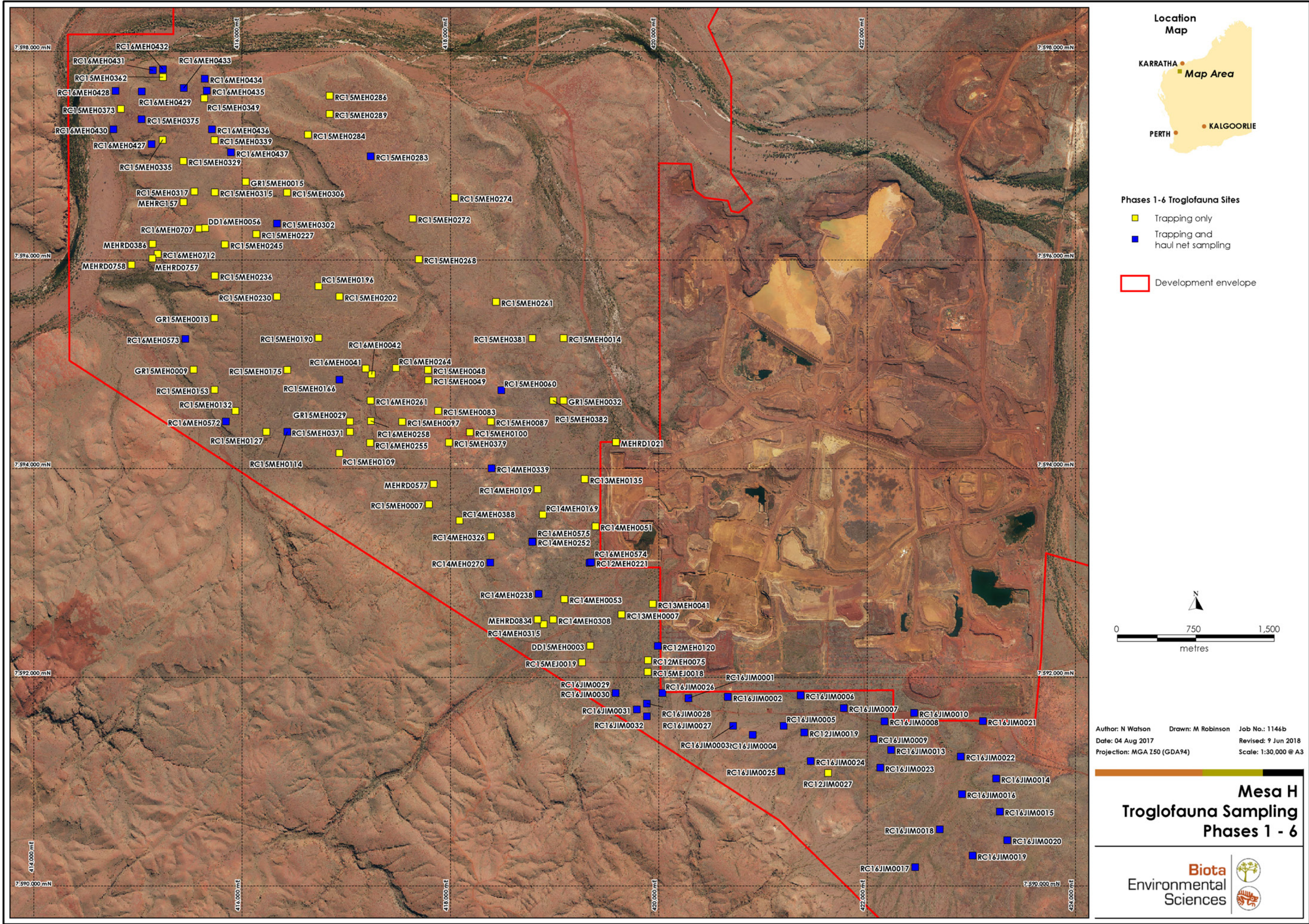


Figure 3.4: Troglafauna sampling sites at Mesa H.



This page intentionally blank.

## 3.11 Study Limitations

A number of limitations apply to this study that are not necessarily functions of this specific survey, but rather relate to work on subterranean fauna in general. These limitations include:

- There is a lack of adequate taxonomic framework and specialist expertise for some faunal groups, both within Australia and internationally. This often limits the conclusiveness of some of the findings in regards to both taxonomic placement and ecological status.
- Sampling points are often limited to locations of interest for geological exploration: as most drill holes have been sited in areas of mining interest, there is often a lack of sampling points in neighbouring geological units, which may limit interpretations of available subterranean fauna habitat.
- Conservation significance could not be assigned to many taxa which could not be identified to species level, as these specimens may correspond to taxa already known from elsewhere and/or may represent species complexes. The records for these indeterminate specimens are still presented from both desktop and field survey sources, but they are excluded from final tallies of taxa for stygofauna or troglafauna.
- All genetic analysis was completed using the CO1 gene only. This gene was used because it has proved to be relatively fast evolving across many taxonomic groups and a typically reliable indicator of likely species boundaries (Hebert et al. 2003b). Recent studies, however, have indicated that using specific genes for different taxa, or using multiple genes, may provide for more informative results.
- Detailed morphological analysis was not completed for the specimens subject to molecular analysis, and the phylogenetic analysis was based on sequence data from a single mitochondrial gene. Therefore, all species arrived at by this approach should be considered putative unless previously described and fully determined by taxonomic specialists.

Despite these limitations, the survey meets current EPA guidance and is adequate to inform the forthcoming EIA for the Mesa H proposal.

## 4.0 Desktop Review

### 4.1 Priority and Threatened Ecological Communities

Threatened Ecological Communities (TECs) are described by DBCA as biological assemblages occurring in a particular habitat, which are under threat of modification or destruction from various processes (DEC 2013). Priority Ecological Communities (PECs) are biological (flora or fauna) communities that are recognised to be of significance, but do not meet the criteria for a TEC.

A total of seven PECs occur within 50 km of the Mesa H development envelope, of which three are relevant to subterranean fauna; all of which overlap the development envelope (Figure 2.1, Table 4.1). No TECs are known within 50 km of the development envelope.

**Table 4.1: Priority Ecological Communities within 50 km of the development envelope relevant to subterranean fauna.**

| Community  | Priority   |
|--|------------|
| <b>Subterranean invertebrate community of pisolitic hills in the Pilbara</b><br>A series of isolated low undulating hills occur in the state's Pilbara region. The troglofauna are being identified as having very short range distributions. Threats include mining" (Department of Parks and Wildlife 2016).   | Priority 1 |
| <b>Subterranean invertebrate communities of mesas in the Robe Valley region</b><br>"A series of isolated mesas occur in the Robe Valley in the state's Pilbara Region. The mesas are remnants of old valley infill deposits of the paleo Robe River. The troglobitic faunal communities occur in an extremely specialised habitat and appear to require the particular structure and hydrogeology associated with mesas to provide a suitable humid habitat. Short range endemism is common in the fauna. The habitat is the humidified pisolitic strata. Threats include mining" (Department of Parks and Wildlife 2016). | Priority 1 |
| <b>Stygofaunal Community of the Bungaroo Aquifer</b><br>"A unique assemblage of aquatic subterranean fauna including eels, snails and other stygofauna. Threats include groundwater drawdown and mining" (Department of Parks and Wildlife 2016).  | Priority 1 |

All three PECs are classified as Priority One, "Poorly-known ecological communities", defined as: *"Ecological communities that are known from very few occurrences with a very restricted distribution (generally  $\leq 5$  occurrences or a total area of  $\leq 100$ ha). Occurrences are believed to be under threat either due to limited extent, or being on lands under immediate threat (e.g. within agricultural or pastoral lands, urban areas, active mineral leases) or for which current threats exist. May include communities with occurrences on protected lands. Communities may be included if they are comparatively well-known from one or more localities but do not meet adequacy of survey requirements, and/or are not well defined, and appear to be under immediate threat from known threatening processes across their range"* (DEC 2013).

### 4.2 Surface Geology

Mesa H is one of several channel iron deposits (CID), which form the Robe Pisolite Formation, a series of paleodrainage channels that meanders through the Robe River valley. CIDs are often preserved in an inverted topography as remnant mesas in the centre of the palaeochannels (Ramanaidou et al. 2003).

The Mesa H development envelope comprises 13 surface geological units (Table 4.2). By area, the majority of the development envelope is accounted for by just two surface geological units: colluvium, covering 34%, and Robe Pisolite Formation, covering 32%. Both of these geological units have been shown to represent suitable habitat for subterranean fauna in the Robe valley and the wider Pilbara region, with Robe Pisolite in particular recognised as core habitat for troglofauna. A more detailed review of the subterranean fauna habitats is presented in Biota (2018).

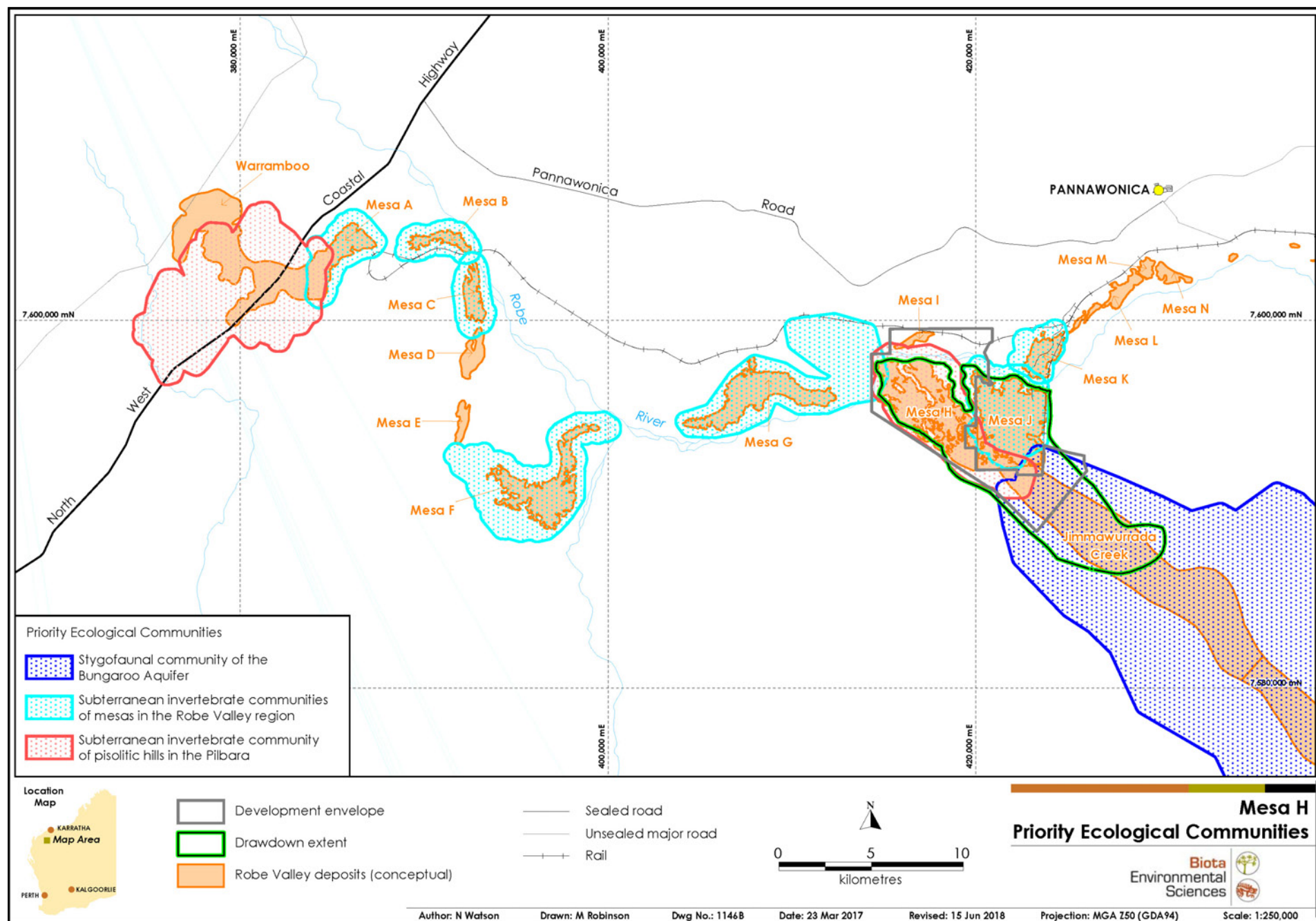
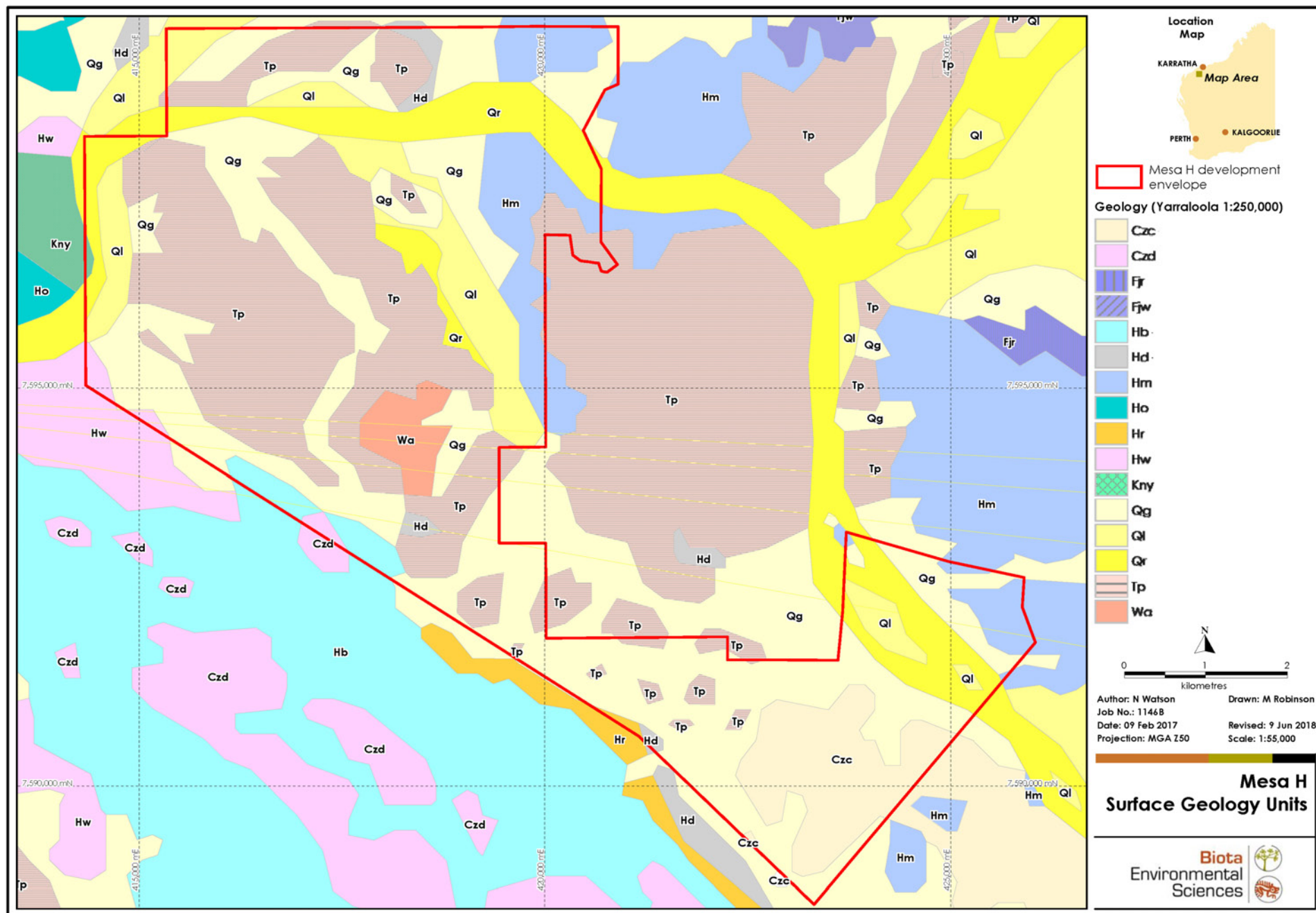


Figure 4.1: PECs relevant to subterranean fauna within 50 km of the Mesa H development envelope.

**Table 4.2: Surface geology units of the development envelope and representative sampling effort (units previously identified as subterranean fauna habitat highlighted in yellow).**

| Eon         | Era         | Unit code | Description   | Surface area (ha) | Proportion of development envelope (%) | Number of stygofauna sites | Number of troglafauna sites |
|-------------|-------------|-----------|---|-------------------|--|----------------------------|-----------------------------|
| Precambrian | Archaean    | Hm        | <b>Marra Mamba Iron Formation:</b> Chert, ferruginous chert and banded iron with minor shale; jaspilite with pronounced 'pinch and swell' structures, small occurrences of manganese. | 302.1             | 7.1                                    | -                          | -                           |
|             | Proterozoic | Hb        | <b>Brockman Iron Formation:</b> Banded jaspilite and chert with some shale dolomitic with riebeckite and crocidolite at Wittenoom Gorge and Dales Gorge. Contains stromatolites.      | 85.2              | 2.0                                    | -                          | -                           |
|             |             | Hd        | <b>Wittenoom Dolomite Formation:</b> Thin to medium-bedded grey crystalline dolomite, intercalations of chert, dolomitic shale, and metatuff in upper part.                           | 39.4              | 0.9                                    | -                          | 1                           |
|             |             | Hr        | <b>Mt Mcrae Shale Formation:</b> Shale, siltstone and dolomitic shale some and some chert.  | 44.4              | 1.0                                    | -                          | -                           |
|             |             | Hw        | <b>Woongarra Volcanics Formation:</b> rhyolitic and dacitic volcanics, commonly porphyritic, some tuff. Some phases are intrusive.  | 21.1              | 0.5                                    | -                          | -                           |
|             | -           | Wa        | <b>Ashburton Formation:</b> wacke, mudstone, ferruginous mudstone interbedded with sandstone and dolomite intruded by doleritic sills.  | 98.1              | 2.4                                    | 1                          | 6                           |
| Phanerozoic | Mesozoic    | Kny       | <b>Yarraloola Conglomerate:</b> Poorly sorted conglomerate with shale, claystone lenses and interbedded sandstone.  | 4.1               | 0.1                                    | -                          | -                           |
|             | Cenozoic    | Czc       | <b>Colluvium:</b> Partly consolidated valley-fill deposits.   | 51.6              | 1.2                                    | 1                          | 6                           |
|             |             | Czd       | <b>Duricrust:</b> Indurated crust on older rocks; relict texture and structure preserved in some places   | 10.1              | 0.2                                    | -                          | -                           |
|             |             | Qg        | <b>Colluvium:</b> Unconsolidated to loosely consolidated slope deposits.  | 1,471.6           | 34.5                                   | 20                         | 55                          |
|             |             | Ql        | <b>Lacustrine deposits:</b> Clay, silt; saline in part, flood deposits. Unconsolidated fluviatile and sheet - flood deposits in levees and river terraces.                            | 351.8             | 8.3                                    | 4                          | 2                           |
|             |             | Qr        | <b>Alluvium:</b> Unconsolidated fluviatile deposits, mostly sand.   | 382.0             | 8.9                                    | 2                          | -                           |
|             |             | Tp        | <b>Robe Pisolite Formation:</b> Pisolitic limonite deposits. Occurs along old river channels.   | 1,403.3           | 32.9                                   | 13                         | 66                          |
|             |             |           | <b>Total</b>  | <b>4,264.8</b>    | <b>100</b>                             | <b>41</b>                  | <b>136</b>                  |





**Figure 4.2:** Surface geology mapping of the Mesa H development envelope.

## 4.3 Previous Relevant Surveys

Extensive sampling for troglotauna and stygofauna has been undertaken in areas of proposed development throughout the Robe River valley. Multiple-phase sampling programmes have been undertaken on tenements neighbouring Mesa H, including at Bungaroo (Biota 2010b), Mesa K (Biota 2007) and on Australian Premium Iron's West Pilbara Iron Ore Project (Biota 2010c, 2010d).

Due to the highly localised distributions of subterranean fauna and often mesa-specific species distributions, searches for past survey records were limited to within a desktop review area of a 15 km square around a central point in the development envelope (see Figure 2.1).

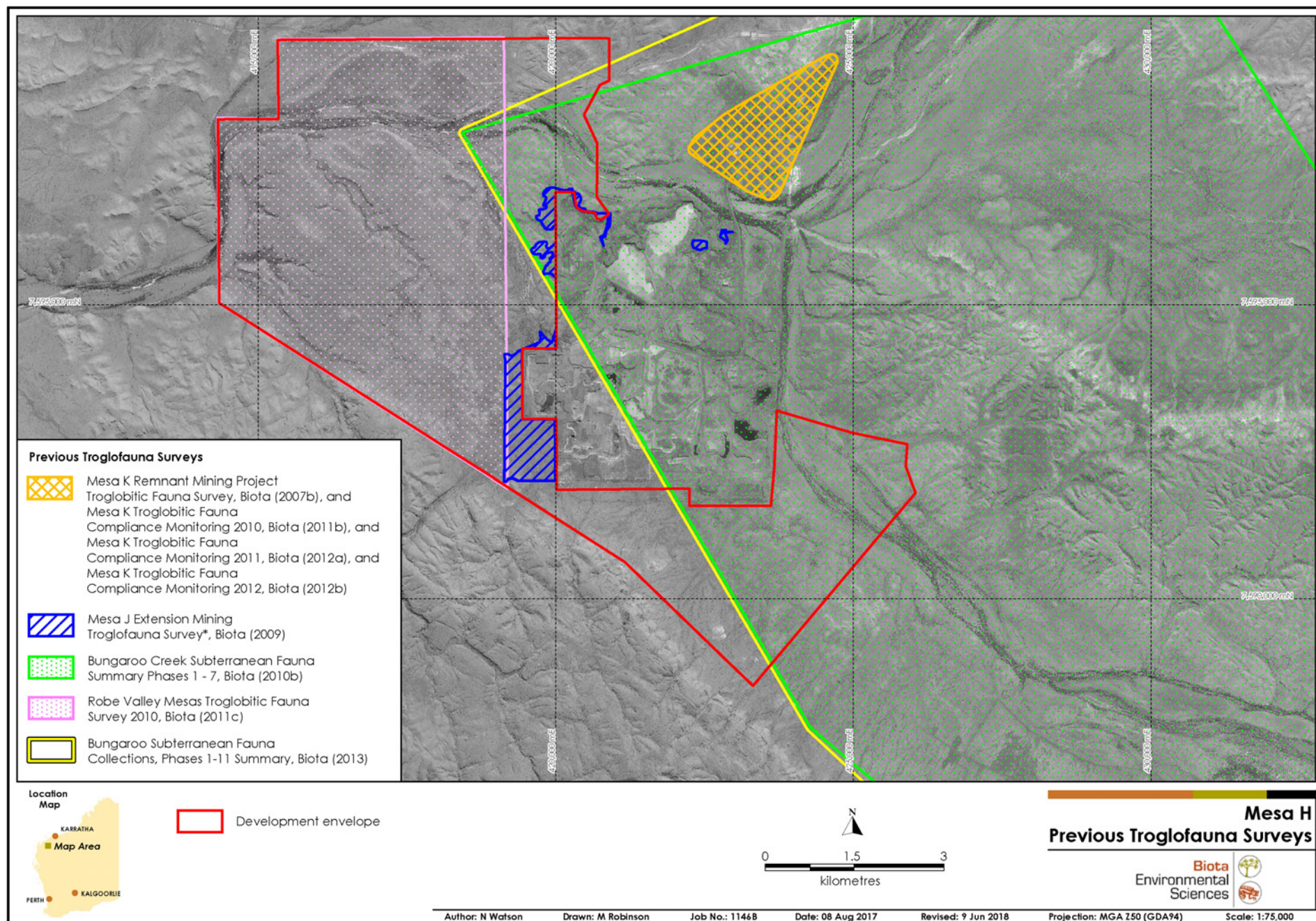
A total of seven troglobitic and eight stygobitic fauna surveys have been conducted within the search area (Table 4.3; Figure 4.3; Figure 4.4). The fauna recorded from these surveys within the desktop review area were reviewed and collated and are presented in Section 4.4.

**Table 4.3: Summary of surveys completed within 15 km of the Mesa H development envelope (source: Biota database).**

| Survey   | Number of sites within desktop search area | Number of records within desktop search area | Reference            |
|--|--|--|----------------------|
| <b>Stygofauna</b>  |  |  |                      |
| Bungaroo Trial Pit Stygofauna Assessment                     | 0  | 0  | Biota (2006b, 2006b) |
| Bungaroo Creek Subterranean Fauna Summary Phases 1 - 7       | 10   | 27   | Biota (2010b)        |
| Bungaroo Subterranean Fauna Collections, Phases 1-11 Summary | 22   | 246  | Biota (2013)         |
| Bungaroo Coastal Waters Project Stygofauna Monitoring 2014   | 7  | 232  | Biota (2014)         |
| Bungaroo Coastal Waters Project Stygofauna Monitoring 2015   | 8  | 131  | Biota (2016a)        |
| Bungaroo Coastal Waters Project Stygofauna Monitoring 2016   | 8  | 118  | Biota (2016b)        |
| Pilbara Stygofauna Survey                                    | 1  | 1  | Halse et al. (2014)  |
| <b>Troglotauna</b>   |  |  |                      |
| Mesa K Remnant Mining Project Troglobitic Fauna Survey       | 56   | 174  | Biota (2007)         |
| Mesa J Extension Mining Troglotauna Survey*                  | 33   | 0  | Biota (2009)         |
| Bungaroo Creek Subterranean Fauna Summary Phases 1 - 7       | 3  | 0  | Biota (2010b)        |
| Mesa K Troglobitic Fauna Compliance Monitoring 2010          | 30   | 22   | Biota (2011b)        |
| Mesa K Troglobitic Fauna Compliance Monitoring 2011          | 33   | 54   | Biota (2012a)        |
| Mesa K Troglobitic Fauna Compliance Monitoring 2012          | 33   | 26   | Biota (2012b)        |
| Robe Valley Mesas Troglobitic Fauna Survey 2010              | 23   | 26   | Biota (2011c)        |
| Bungaroo Subterranean Fauna Collections, Phases 1-11 Summary | 4  | 1  | Biota (2013)         |

\*Specimens from this survey were sequenced with specimens from the current survey and included in analysis in Section 5.3.4.





**Figure 4.3: Previous troglofauna surveys within 15 km of the Mesa H development envelope.**



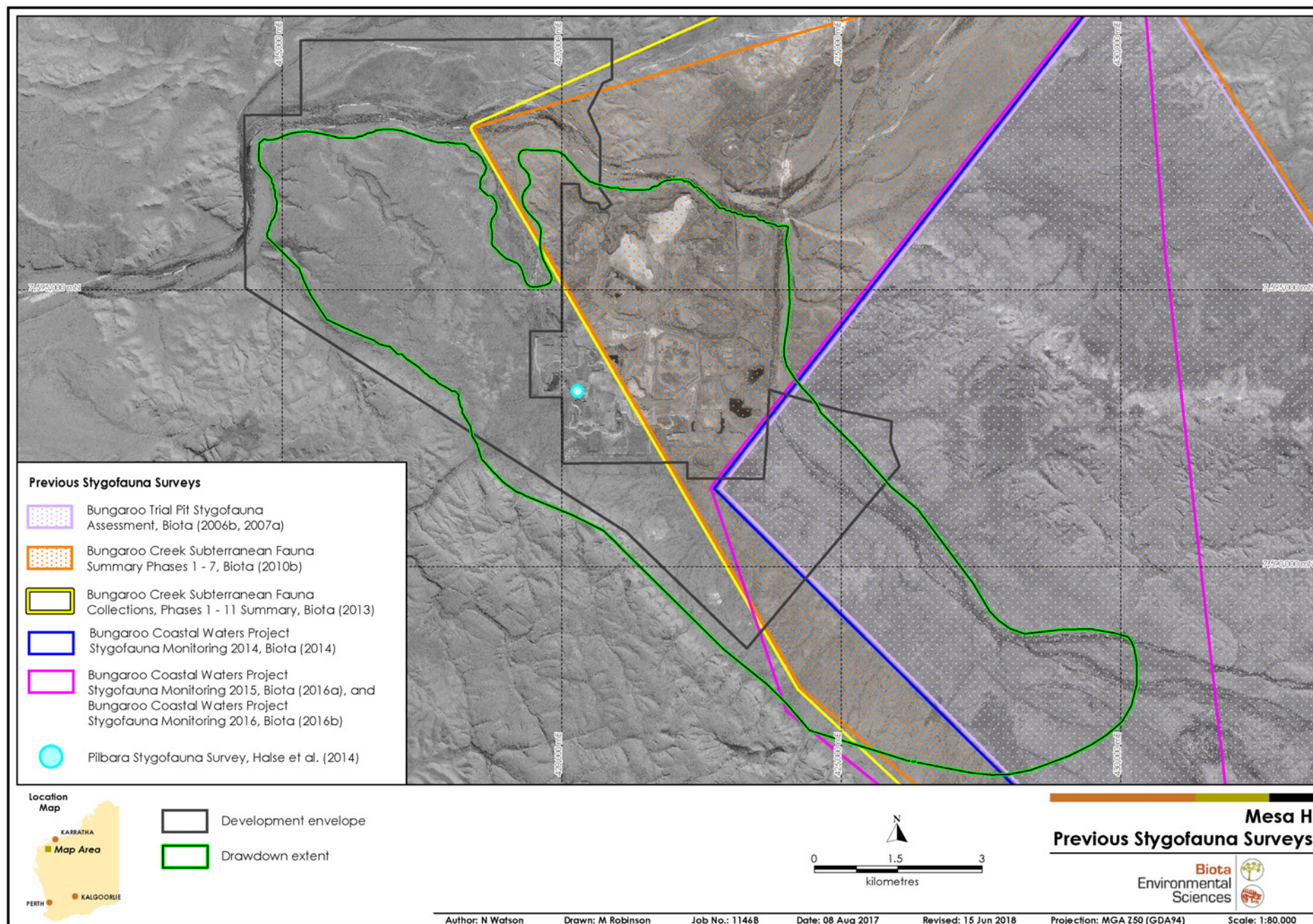


Figure 4.4: Previous stygofauna surveys within 15 km of the Mesa H development envelope.

## 4.4 Desktop Review Results

### 4.4.1 Stygofauna

A total of 15 orders were represented amongst the past stygofauna specimens recorded from within the desktop review area, the majority of which were recorded during stygofauna surveys at Bungaroo (Table 4.4; Figure 4.5).

Excluding indeterminate specimens, a total of 22 species-level taxa have been recorded during previous surveys occur within the Mesa H drawdown extent. This included three stygofauna species of conservation significance:

- Blind Cave Eel, *Ophisternon candidum* – Vulnerable; Schedule 3 (see Section 4.4.1.1);
- the amphipod species *Nedsia hurlberti* – Schedule 3; and
- the amphipod species *Nedsia sculptilis* – Schedule 3 (Table 4.4; Figure 4.5).

Five of the remaining 19 taxa are considered to represent potential SRE species (Neoniphargidae sp. 'B02', *Parastenocaris* sp. 'B28', *Haptolana* sp. 'B01', Candoninae sp. 'BOS541' and Hydrobiidae sp. 2; Table 4.4). These taxa have been included in the assessment of taxa recorded from within the drawdown extent in Table 6.1 (see Section 6.2).

#### 4.4.1.1 Blind Cave Eel

The Blind Cave Eel (*Ophisternon candidum*) is a de-pigmented, subterranean fish growing up to 40 cm in length, with a long slender body, no eyes, and a thin rayless membrane around the tip of the tail (DSEWPaC 2008). The Blind Cave Eel is the world's longest cave fish and one of only three vertebrate animals known from Australia that are restricted to subterranean waters (Humphreys 2001b). The species is specially protected under legislation at both State (Schedule 3) and Commonwealth (Vulnerable) levels.

The Blind Cave Eel inhabits groundwater systems in subterranean caves, transmissive geological formations, fissures and wells (Humphreys 2001b). On Cape Range, the Blind Cave Eel is known to utilise cave floor pool sediments characteristic of crustacean-rich cave habitats. It also occurs within the karst aquifers of Barrow Island, though it remains to be confirmed if this record represents the same species as present on Cape Range (Humphreys et al. 2013).

The Blind Cave Eel has also been previously collected from three sites in the Jimawurrada-Bungaroo Creek system, within the current study area. The first specimen was recorded in 2009 at Bungaroo Creek from borehole BC186, 5.6 km southeast of the development envelope and just outside of the current desktop review boundary (Figure 2.1) (Biota 2010e), with subsequent records coming from JW023 (1 km southeast of the development envelope) (Biota 2016b) and the immediately adjacent JW024 in 2017 (C. O'Neill, Rio Tinto, pers. comm. 2017). Tissue from the Bungaroo specimens has been sequenced at both the CO1 mitochondrial DNA and 16S ribosomal RNA markers, showing that the Bungaroo records are less than 1% divergent from the Cape Range specimens, indicating that they are the same species (Foster and Humphreys 2011).

The species is considered likely to be associated with the regional aquifer of the Robe River (Biota and Helix 2014) and it is probable that it occurs within at least the southeast portion of the development envelope in association with the alluvial aquifer of Jimawurrada Creek. Environmental DNA sampling was undertaken as part of the current study in an attempt to better understand the distribution of the species in the locality (Section 3.4).

### 4.4.2 Troglafauna

Eleven orders were represented amongst the troglafauna taxa previously been recorded within the desktop review area (Table 4.4; Figure 4.6; Figure 4.7; Figure 4.8). One species is of conservation significance; the schizomid species *Paradraculoides kryptus* (Schedule 3). This species is restricted to Mesa K and is very unlikely to occur at Mesa H.

Excluding indeterminate records, four taxa have been recorded from sites within the Mesa H development envelope and two of these are true troglobites considered to represent potential SRE fauna: the isopod species *Troglarmadillo* sp. 1 and the schizomid species *Paradraculoides* sp. nov. 'Mesa H' (Table 4.4; Figure 4.6).



**Table 4.4: Stygofauna taxa and abundance (n) recorded from the desktop review** (records highlighted in **bold** were recorded within the drawdown extent).

| Order                   | Family             | Species  | n          | Sites  | Record Location                       | Conservation Significance                    |
|-------------------------|--------------------|--|------------|--|---------------------------------------|--|
| Oligochaeta             | Naididae           | <i>Antipodrilus</i> sp. nov.                   | 2          | JW023  | Bungaroo                              | N/A  |
|                         | Phreodrilidae      | Phreodrilidae sp. dissimilar ventral chaetae   | 3          | JW023  | Bungaroo                              | Not SRE                                      |
|                         | Tubificidae        | <b>Tubificidae sp. group B</b>                 | <b>27</b>  | <b>JW011A, JW023</b>                                   | <b>Mesa J, Bungaroo</b>               | <b>Not SRE</b>                               |
| Turbellaria             | -                  | Platyhelminthes sp. indet.                     | 1          | JW024  | Bungaroo                              | N/A  |
|                         | -                  | Turbellaria sp. indet.                         | 1          | JW021  | Bungaroo                              | N/A  |
| Nematoda                | -                  | Nematoda sp. indet.                            | 4          | Hyporheic04  | Bungaroo                              | N/A  |
| Gastropoda              | Hydrobiidae        | <b>Hydrobiidae sp. 2</b>                       | <b>4</b>   | <b>JW023</b>   | <b>Jimmawurrada</b>                   | <b>Potential SRE</b>                         |
| Acari                   | -                  | Sarcoptiformes group 1 (PSW)                   | 2          | Hyporheic04  | Bungaroo                              | N/A  |
| Ostracoda               | Candonidae         | <b><i>Humphreyscandona fovea</i></b>           | <b>5</b>   | <b>JW011A, JW021</b>                                   | <b>Mesa J, Jimmawurrada</b>           | <b>Not SRE</b>                               |
|                         |                    | <b><i>Humphreyscandona waldockae</i></b>       | <b>1</b>   | <b>JW011A</b>  | <b>Mesa J</b>                         | <b>Not SRE</b>                               |
|                         |                    | <b><i>Humphreyscandona</i> sp. 2</b>           | <b>2</b>   | <b>JW011A</b>  | <b>Bungaroo</b>                       | <b>Not SRE</b>                               |
|                         |                    | <i>Pierrecandona</i> sp. indet.                | 1          | JW021  | Bungaroo                              | N/A  |
|                         |                    | <b><i>Areacandona brookanthana</i></b>         | <b>1</b>   | <b>JW023</b>   | <b>Jimmawurrada</b>                   | <b>Not SRE</b>                               |
|                         |                    | <b><i>Areacandona triangulum</i></b>           | <b>4</b>   | <b>JW011A</b>  | <b>Mesa J</b>                         | <b>Not SRE</b>                               |
|                         |                    | <b><i>Areacandona lepte</i></b>                | <b>4</b>   | <b>JW011A</b>  | <b>Mesa J</b>                         | <b>Not SRE</b>                               |
|                         |                    | <b><i>Pilbaracandona rosa</i></b>              | <b>10</b>  | <b>JW011A</b>  | <b>Mesa J</b>                         | <b>Not SRE</b>                               |
|                         |                    | <b><i>Candoninae</i> sp. 'BOS541'</b>          | <b>1</b>   | <b>JW024</b>   | <b>Jimmawurrada</b>                   | <b>Potential SRE</b>                         |
| Copepoda: Calanoida     | Ridgewayiidae      | <b><i>Stygoridgewayia trispinosa</i></b>       | <b>132</b> | <b>JIMDR094, JW011A, JW021, JW024</b>                  | <b>Mesa J, Bungaroo, Jimmawurrada</b> | <b>Not SRE</b>                               |
| Copepoda: Cyclopoida    | Cyclopidae         | <b><i>Diacyclops cockingi</i></b>              | <b>3</b>   | <b>JIMDD080, JW021</b>                                 | <b>Jimmawurrada</b>                   | <b>Not SRE</b>                               |
|                         |                    | <b><i>Diacyclops humphreysi humphreysi</i></b> | <b>6</b>   | <b>J154, JW023, PZ10BUN004</b>                         | <b>Bungaroo</b>                       | <b>Not SRE</b>                               |
|                         |                    | <i>Diacyclops</i> sp. indet.                   | 1          | JW024  | Bungaroo                              | N/A  |
|                         |                    | <b><i>Halicyclops calm</i></b>                 | <b>4</b>   | <b>JW011A</b>  | <b>Mesa J</b>                         | <b>Not SRE</b>                               |
|                         |                    | <b><i>Halicyclops rochai</i></b>               | <b>66</b>  | <b>JW023</b>   | <b>Jimmawurrada</b>                   | <b>Not SRE</b>                               |
| Copepoda: Harpacticoida | Parastenocarididae | <b><i>Parastenocaris</i> sp. 'B28'</b>         | <b>1</b>   | <b>JW023</b>   | <b>Jimmawurrada</b>                   | <b>Potential SRE</b>                         |
| Thermosbaenacea         | Halosbaenidae      | <b><i>Halosbaena tulki</i></b>                 | <b>251</b> | <b>JW021, JW024</b>                                    | <b>Jimmawurrada</b>                   | <b>Not SRE</b>                               |
| Bathynellacea           | Bathynellidae      | Bathynellidae sp. indet.                       | 1          | Unknown  | Mesa H                                | N/A  |
| Isopoda                 | Cirolanidae        | <b><i>Haptolana</i> sp. 'B01'</b>              | <b>1</b>   | <b>JW024</b>   | <b>Jimmawurrada</b>                   | <b>Potential SRE</b>                         |
|                         |                    | <b><i>Kagalana tonde</i></b>                   | <b>8</b>   | <b>JW021, JW023, JW024</b>                             | <b>Jimmawurrada</b>                   | <b>Not SRE</b>                               |
|                         | Tainisopidae       | <i>Pygolabis</i> sp. indet.                    | 1          | JW023  | Bungaroo                              | N/A  |
| Amphipoda               | Bogidiellidae      | Bogidiellidae sp. indet.                       | 13         | JW021, JW024   | Jimmawurrada                          | N/A  |
|                         | Melititidae        | Melitidae sp. 1 (PSS)                          | 3          | JW023  | Jimmawurrada                          | Not SRE                                      |
|                         |                    | Melitidae sp. 1 nr norcarpensis                | 1          | JW021  | Jimmawurrada                          | Not SRE                                      |
|                         |                    | <b><i>Nedsia hurlberti</i></b>                 | <b>134</b> | <b>JIMDD080, JIMDR094, JW011A, JW021, JW023, JW024</b> | <b>Mesa J, Bungaroo</b>               | <b>Conservation Significant - Schedule 3</b> |
|                         |                    | <b><i>Nedsia sculptilis</i></b>                | <b>86</b>  | <b>JIMDR094, JW011A, JW021</b>                         | <b>Mesa J, Bungaroo</b>               | <b>Conservation Significant - Schedule 3</b> |
|                         |                    | <i>Nedsia</i> sp. indet.                       | 4          | J154, JW023, JW024                                     | Bungaroo                              | N/A  |

| Order             | Family         | Species                            | n        | Sites                        | Record Location     | Conservation Significance                                |
|-------------------|----------------|------------------------------------|----------|------------------------------|---------------------|--|
|                   | Neoniphargidae | <b>Neoniphargidae sp. 'B02'</b>    | <b>3</b> | <b>JW021</b>                 | <b>Bungaroo</b>     | <b>Potential SRE</b>                                     |
|                   | Paramelitidae  | Paramelitidae Genus 2 sp.          | 1        | JW024                        | Jimmawurrada        | N/A  |
|                   |                | Paramelitidae sp. indet.           | 2        | JIMDR094                     | Bungaroo            | N/A  |
|                   |                | Amphipoda sp. indet.               | 42       | BUNUNK01, J154, JW023, JW024 | Bungaroo            | N/A  |
| Synbranchiiformes | Synbranchidae  | <b><i>Ophisternon candidum</i></b> | <b>2</b> | <b>JW023, JW024</b>          | <b>Jimmawurrada</b> | <b>Conservation Significant – Vulnerable; Schedule 3</b> |

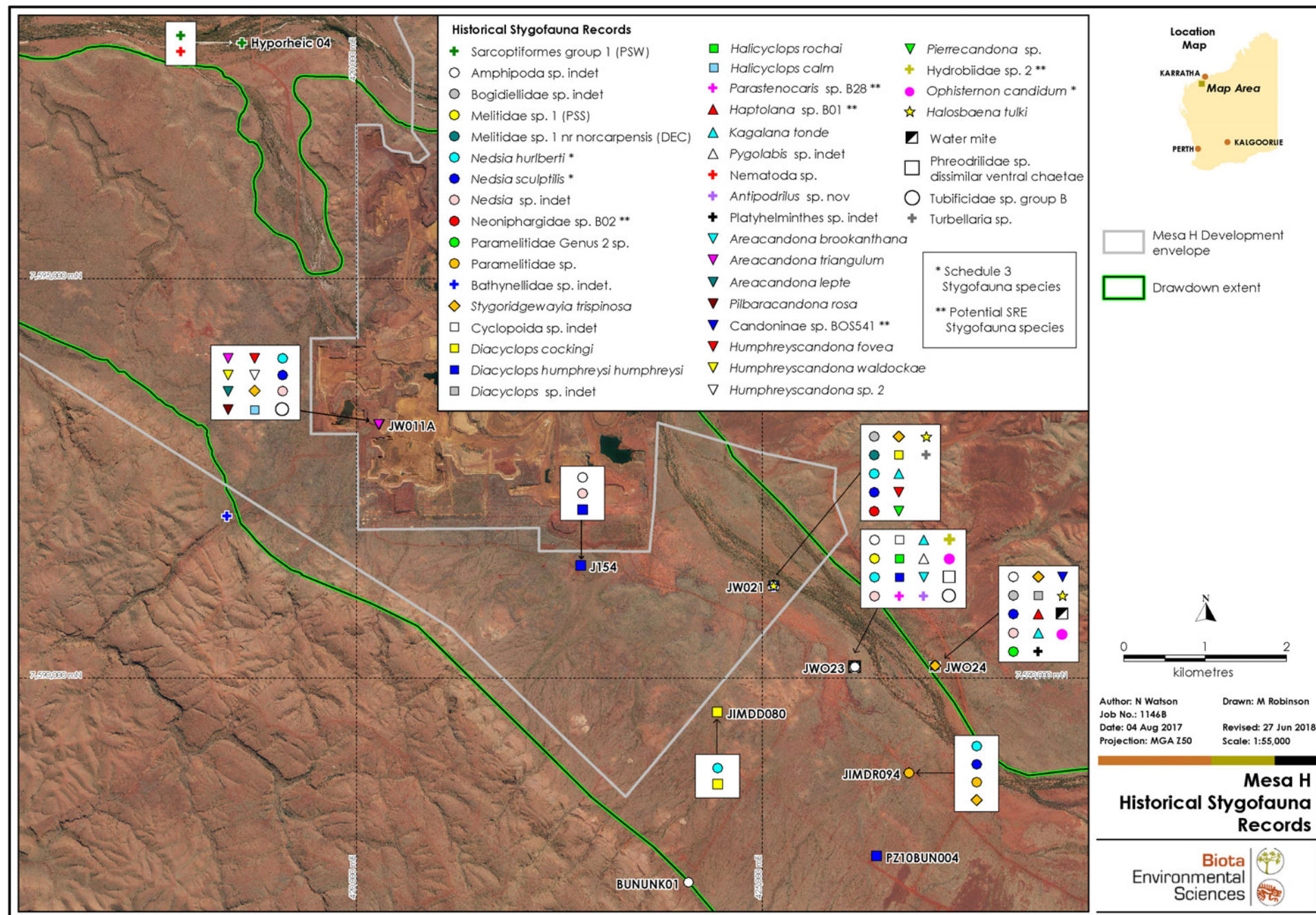


Figure 4.5: Stygofauna records from the desktop review.

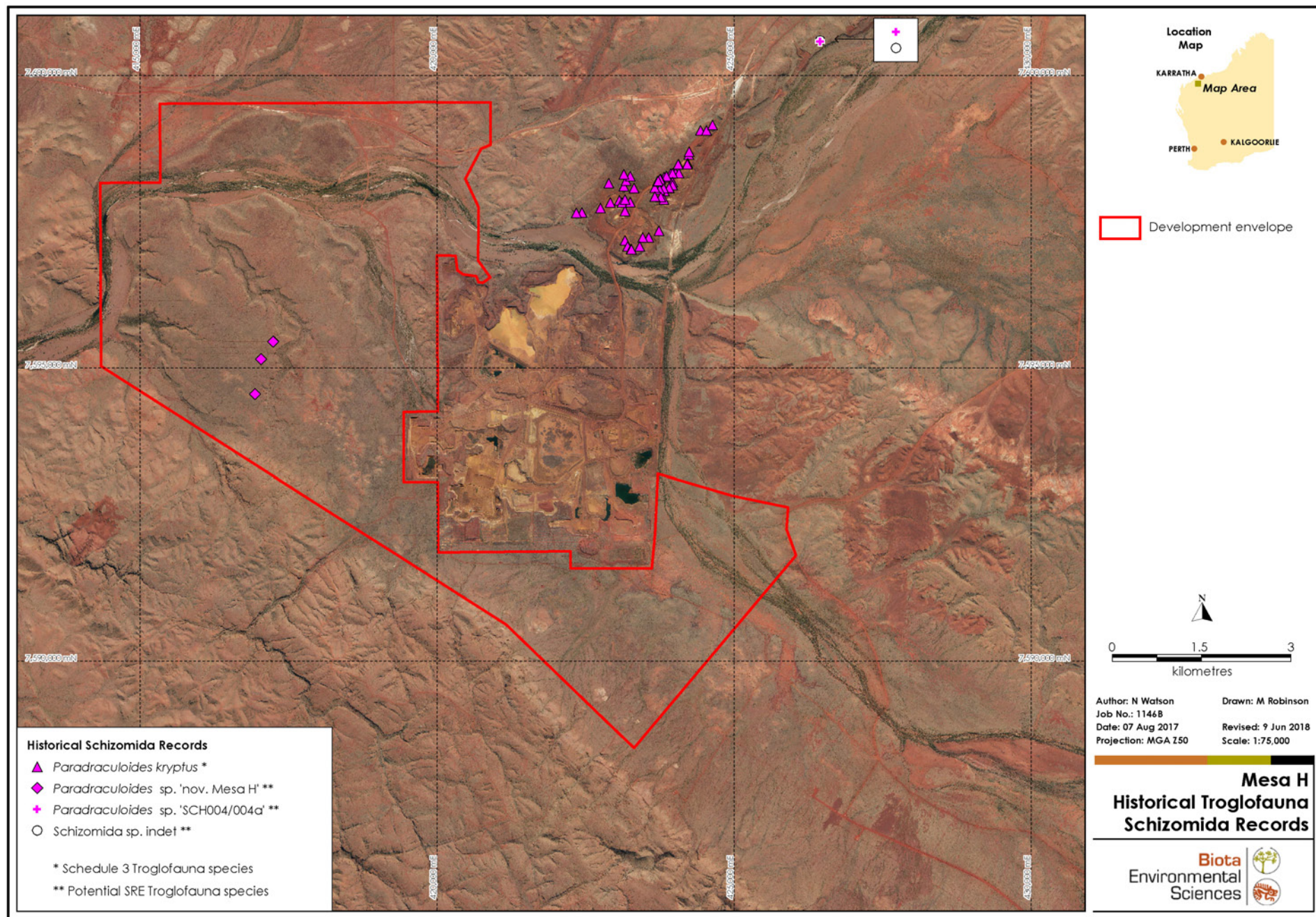


**Table 4.5: Troglofauna taxa and abundance (n) recorded during the desktop review** (Taxa highlighted in **bold** were recorded within the Mesa H development envelope).

| Order            | Family              | Species                              | n         | Sites   | Location              | Conservation Significance          |
|------------------|---------------------|--------------------------------------|-----------|---|-----------------------|------------------------------------|
| Araneae          | Micropholcommatidae | <i>Eperiella</i> 'ARA050'            | 1         | MEK1685A  | Mesa K                | Potential SRE                      |
| Blattodea        |                     | Blattodea sp. indet.                 | 1         | K1318   | Mesa K                | N/A                                |
| Chilopoda        | Scolopendridae      | Scolopendridae sp. 'CHI025'          | 1         | DD14MEL0001   | Mesa L                | Potential SRE                      |
|                  |                     | <i>Cryptops</i> sp. 1                | 1         | MEK1570   | Mesa K                | Potential SRE                      |
|                  |                     | <i>Cryptops</i> sp. indet.           | 1         | Unknown   | Mesa K                | N/A                                |
| Coleoptera       | Curculionidae       | Curculionidae sp. 'CCU007'           | 5         | MEK1478, MEK1718  | Mesa K                | Potential SRE                      |
|                  | Ptiliidae           | Ptiliidae sp. 'CPT003'               | 11        | K0968, MEK1721  | Mesa K                | Potential SRE                      |
|                  |                     | Coleoptera sp. indet                 | 76        | K1303, MEK1701, MEK1731, MEK1735, MEK1694, MEK1478, MEK1728, K0503, K0607 | Mesa K                | N/A                                |
| Diplura          | -                   | <i>Heterojapyx</i> sp. indet.        | 1         | MEK1478   | Mesa K                | N/A                                |
| Isopoda          | Armidillidae        | <b>Barrowdillo sp. 1</b>             | <b>11</b> | <b>MEHRD0752, MEHRD0776</b>   | <b>Mesa H</b>         | <b>Not troglobitic, Not an SRE</b> |
|                  |                     | <b>Troglarmadillo sp. 1</b>          | <b>1</b>  | <b>MEHDC0865</b>  | <b>Mesa H</b>         | <b>Potential SRE</b>               |
|                  | Oniscidae           | <i>Haloniscus</i> sp. 1              | 1         | MEK1337   | Mesa K                | Potential SRE                      |
|                  |                     | <i>Hanoniscus</i> sp. 'Mesa K1'      | 1         | Unknown   | Mesa K                | Potential SRE                      |
|                  |                     | <i>Hanoniscus</i> sp.                | 1         | Unknown   | Mesa K                | Potential SRE                      |
|                  | Philosciidae        | Philosciidae sp. 'ISP050'            | 2         | K0989, MEK1735A   | Mesa K                | Potential SRE                      |
|                  |                     | nr <i>Andricophiloscia</i> sp. 'B19' | 1         | Unknown   | Mesa K                | Potential SRE                      |
|                  | -                   | Isopoda sp. indet.                   | 3         | K0989, K1318, MEHDC0865   | Mesa K, Mesa H        | N/A                                |
| Polydesmida      | Polydesmidae        | Polydesmida sp. indet.               | 9         | K0741, K0949, K1315, MEK1712, K0502, MEK1685                              | Mesa K,               | N/A                                |
| Polyxenida       | Lophoproctidae      | Lophoproctidae sp. indet.            | 8         | MEK1478, MEK1712, MEK1718, MEK1731,                                       | Mesa K                | Not an SRE                         |
|                  |                     | <b>Polyxenida sp. indet.</b>         | <b>12</b> | <b>MEK1551, MEK1552, MEK1556, MEK1337, MEK1685A, MEK1712, MEHRC0955</b>   | <b>Mesa K, Mesa H</b> | <b>Not troglobitic, Not an SRE</b> |
| Pseudoscorpiones | Syrinidae           | <i>Ideoblothrus</i> sp. 'Mesa K'     | 1         | MEK1685A  | Mesa K                | Potential SRE                      |
|                  |                     | <i>Ideoblothrus</i> sp. nov          | 1         | MEK1721   | Mesa K                | Potential SRE                      |
|                  | Hyidae              | Hyidae sp. 'PH019'                   | 1         | K0996   | Mesa K                | Potential SRE                      |
|                  |                     | <i>Indohya</i> sp. Mesa K            | 1         | K0741   | Mesa K                | Potential SRE                      |
|                  |                     | <i>Indohya</i> sp. nov               | 1         | MEK1696   | Mesa K                | Potential SRE                      |
|                  |                     | <i>Indohya</i> sp. indet.            | 1         | BC314, K0607, K0502   | Mesa K                | Potential SRE                      |
|                  | Chthoniidae         | Chthoniidae sp. 'PCH009'             | 1         | DD14MEL0001   | Mesa L                | Potential SRE                      |
|                  |                     | Chthoniidae sp. 'PCH048'             | 2         | MEK1731   | Mesa K                | Potential SRE                      |
|                  |                     | Chthoniidae sp. 'PCH051'             | 1         | MEK1721   | Mesa K                | Potential SRE                      |

| Order      | Family       | Species   | n        | Sites  | Location      | Conservation Significance                                     |
|------------|--------------|---|----------|--|---------------|---|
|            |              | <i>Lagynochthonius</i> sp. 'Mesa K'   | 2        | MEK1685, MEK1735   | Mesa K        | Potential SRE   |
|            |              | <i>Lagynochthonius</i> sp. nov  | 1        | MEK1689  | Mesa K        | Potential SRE   |
|            |              | <i>Lagynochthonius</i> sp. indet.   | 1        | MEK1731  | Mesa K        | Potential SRE   |
|            | Olpiidae     | Olpiidae sp. indet.   | 1        | K0502  | Mesa K        | Potential SRE   |
|            | Atemnidae    | Paratemnoides sp. indet.  | 1        | K0607  | Mesa K        | Potential SRE   |
|            |              | <i>Anatemnus</i> 'PSE081'   | 1        | K1074  | Mesa K        | Potential SRE   |
|            |              | <i>Oratemnus</i> 'PSE081'   | 1        | Unknown  | Mesa K        | Potential SRE   |
|            | -            | Pseudoscorpion sp. indet.   | 2        | MEK1685, MEK1689   | Mesa K        | Potential SRE   |
| Schizomida | Hubbardiidae | <i>Paradraculoides kryptus</i>  | 36       | K0607, K0948, K0989, MEK0672, MEK0739, MEK1337, MEK1478, MEK1685, MEK1685A, MEK1689A, MEK1696, MEK1702, MEK1718, MEK1732, MEK1735A   | Mesa K        | Conservation Significant - Schedule 3                         |
|            |              | <i>Paradraculoides</i> sp. indet. (Juvenile or female individuals, likely to be <i>P. kryptus</i> ) | 166      | K0557, K0607, K0608, K0672, K0673, K0740, K0989, K0996, K1066, K1068, K1074, K1075, K1328, K1337, K1598, MEK1456, MEK1458, MEK1475, MEK1478, MEK1482, MEK1524, MEK1529, MEK1551, MEK1553, MEK1556, MEK1558, MEK1570, MEK1609, MEK1685, MEK1685A, MEK1689, MEK1689A, MEK1694, MEK1696, MEK1697, MEK1703, MEK1712, MEK1718, MEK1721, MEK1724, MEK1728, MEK1731, MEK1732, MEK1735, MEK1735A, MEK1757, OPK02 | Mesa K        | Conservation Significant - Schedule 3 (if <i>P. kryptus</i> ) |
|            |              | <i>Paradraculoides</i> sp. 'SCH004/004a'  | 1        | DD14MEL0001  | Mesa L        | Potential SRE   |
|            |              | <b><i>Paradraculoides</i> sp. nov. "Mesa H"</b>   | <b>8</b> | <b>MEHRC0724, MEHRC0870, MEHRC0894</b>   | <b>Mesa H</b> | <b>Potential SRE</b>  |
|            |              | Schizomida sp. indet.   | 2        | DD14MEL0001  | Mesa L        | N/A   |
|            |              |   |          |  |               |   |
| Zygentoma  | -            | <i>Zygentoma</i> sp. indet.   | 1        | MEK1731  | Mesa K        | N/A   |





**Figure 4.6:** Schizomid records from the desktop review.



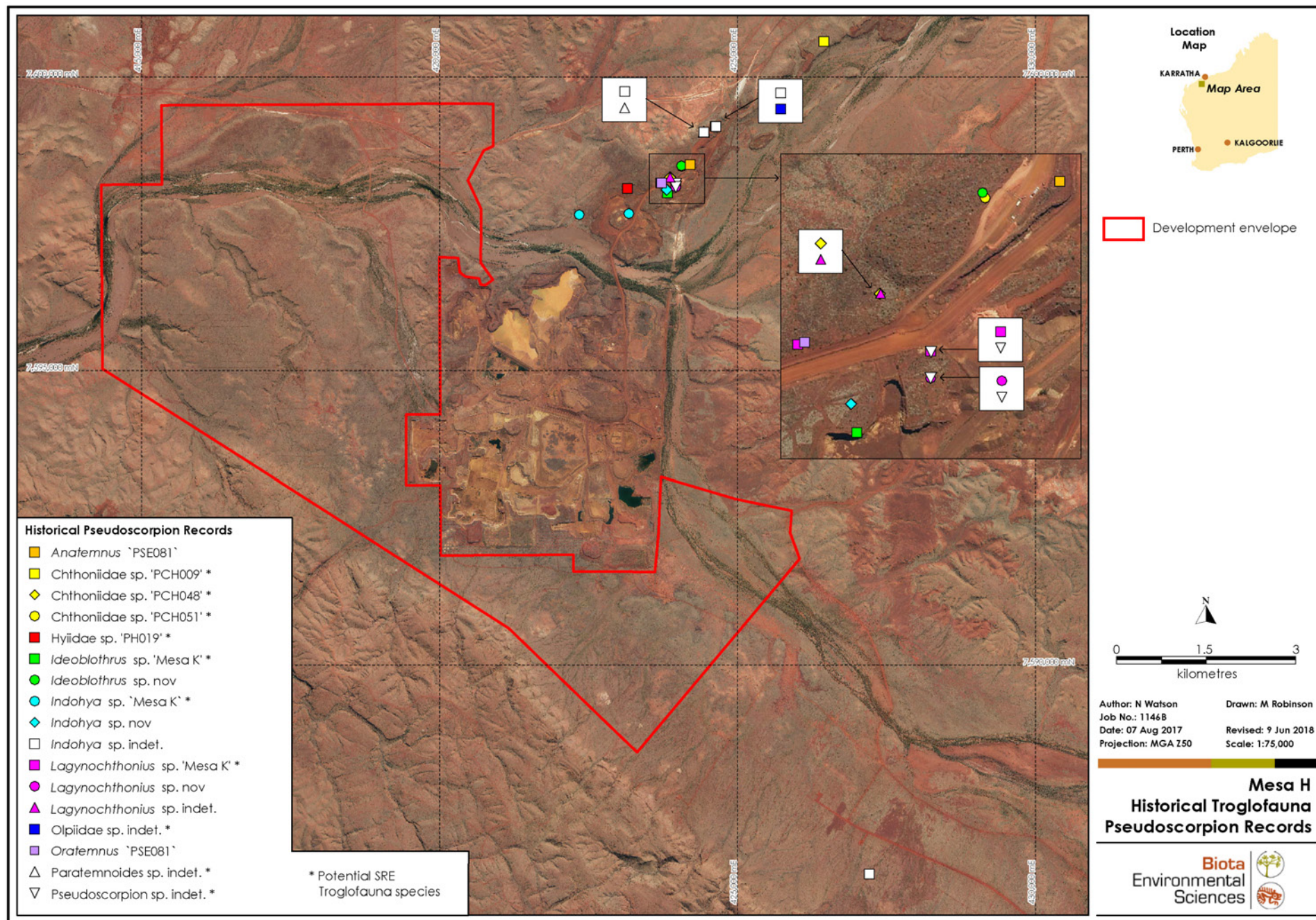


Figure 4.7: Pseudoscorpion records from the desktop review.



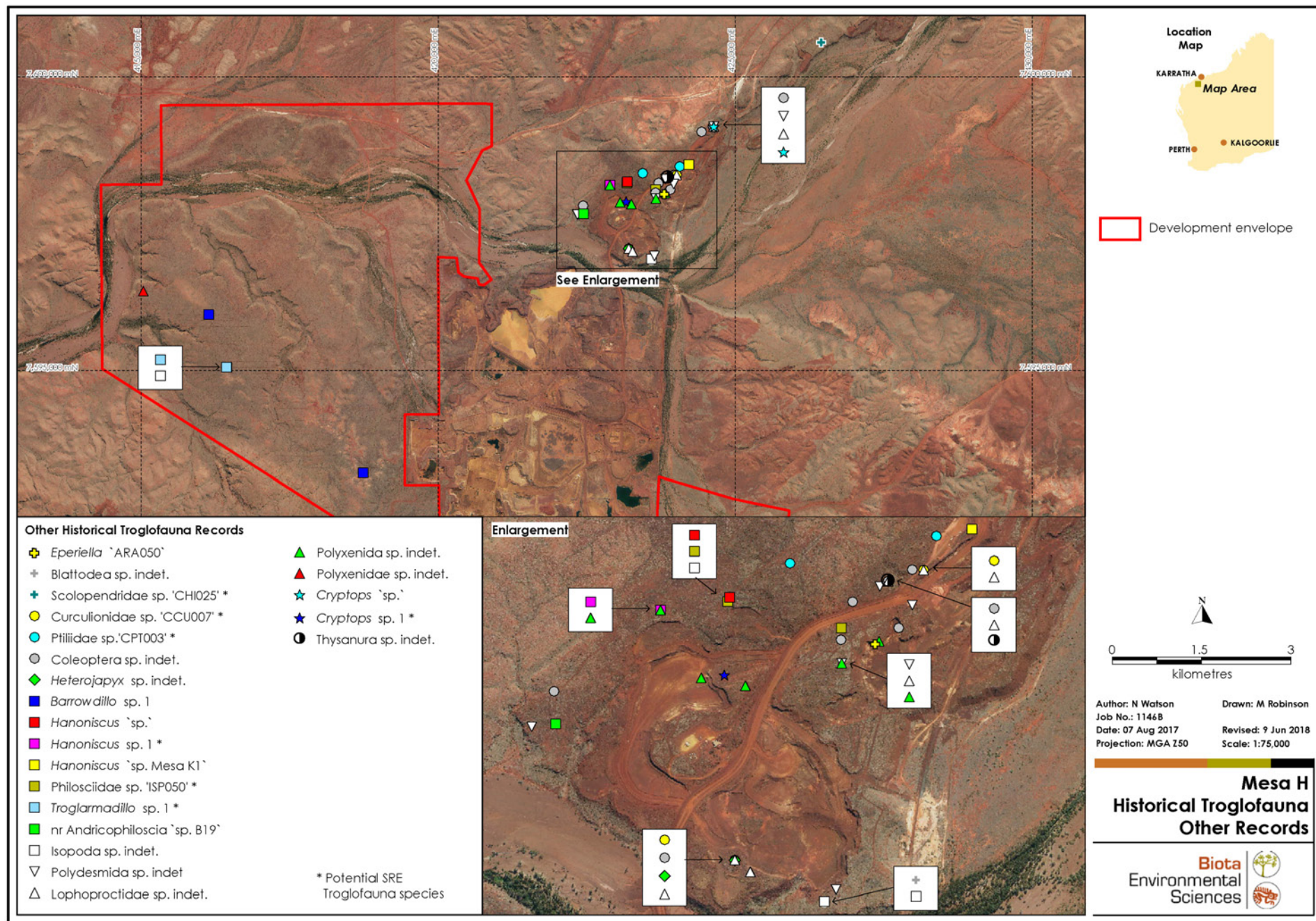


Figure 4.8: Other troglofauna records from the desktop review.

## 5.0 Survey Results

### 5.1 Stygofauna

A total of 855 stygofauna specimens were recorded over five phases of sampling, comprising at least 43 species-level taxa (excluding indeterminate specimens that could not be resolved to species level). The fauna was dominated by the Crustacea, which was represented by seven orders, with the Maxillopoda (copepods) and Amphipoda (amphipods) accounting for the most specimens (48% and 37% of total specimens respectively; Table 5.1). The least commonly recorded groups were the oligochaete and nematode worms (n=1 and 2 respectively), isopods (n=4), water mites (order Trombidiformes; n=4) and snails (order Hypsogastropoda; n=2) (Table 5.1).

**Table 5.1: Summary of stygofauna recorded during the current survey (n= number of specimens).**

| Taxonomy        |             |             |                 | n               | Number of Sites |
|-----------------|-------------|-------------|-----------------|-----------------|-----------------|
| Phylum          | Subphylum   | Class       | Order           |                 |                 |
| Annelida        | -           | Oligochaeta | Tubificida      | 1               | 1               |
| Platyhelminthes | -           | -           | -               | 9               | 2               |
| Nematoda        | -           | -           | -               | 2               | 2               |
| Mollusca        | -           | Gastropoda  | Hypsogastropoda | 2               | 1               |
| Arthropoda      | Chelicerata | Arachnida   | Trombidiformes  | 4               | 3               |
|                 | Crustacea   | Ostracoda   | Podocopida      | 83              | 9               |
|                 |             | Maxillopoda | Calanoida       | 236             | 20              |
|                 |             |             | Cyclopoida      | 138             | 17              |
|                 |             |             | Harpacticoida   | 36              | 3               |
|                 |             |             | Malacostraca    | Thermosbaenacea | 20              |
|                 |             | Isopoda     |                 | 4               | 2               |
|                 |             | Amphipoda   |                 | 320             | 24              |
|                 |             |             |                 | Total           | 855             |

A detailed account of the fauna recorded, including the results of genetic analysis, follows in Sections 5.1.10 to 5.1.3. Record locations for the lower abundance groups are shown in Figure 5.1, with the numerically dominant Maxillopoda in Figure 5.2 and Amphipoda in Figure 5.3.

#### 5.1.1 Class Oligochaeta (Earthworms)

A single oligochaete was recorded from site BC186, on the margin of the drawdown extent (Figure 5.1). The specimen was identified as *Enchytraeus* sp. 'AP PSS1', which has previously been recorded during the Pilbara Biodiversity Survey from approximately 39 km to the west (Halse et al. 2014).

#### 5.1.2 Phylum Platyhelminthes (Flatworms)

A total of nine platyhelminth specimens were recorded from sites 31 and 32, both of which were outside of the drawdown extent (Figure 5.1). These specimens are unable to be identified below phylum level due to a lack of taxonomic framework for this group.

#### 5.1.3 Phylum Nematoda (Nematode Worms)

Two nematode specimens were recorded from two sites within the drawdown extent during the survey (DD13MEH0007 and RC12JIM0019; Figure 5.1). These specimens are unable to be identified below phylum level due to a lack of taxonomic framework for this group.

#### 5.1.4 Class Gastropoda (Aquatic Snails)

Eight specimens of hydrobiid snails were recorded from site MB17MEH0007 to the north of the drawdown extent during the Phase 5 sampling (Figure 5.1). The specimens were identified to morphotype level as Hydrobiidae sp. 'B09', which appears to be distinct from other hydrobiid snail forms collected previously from the adjacent Bungaroo valley (Biota 2015a).



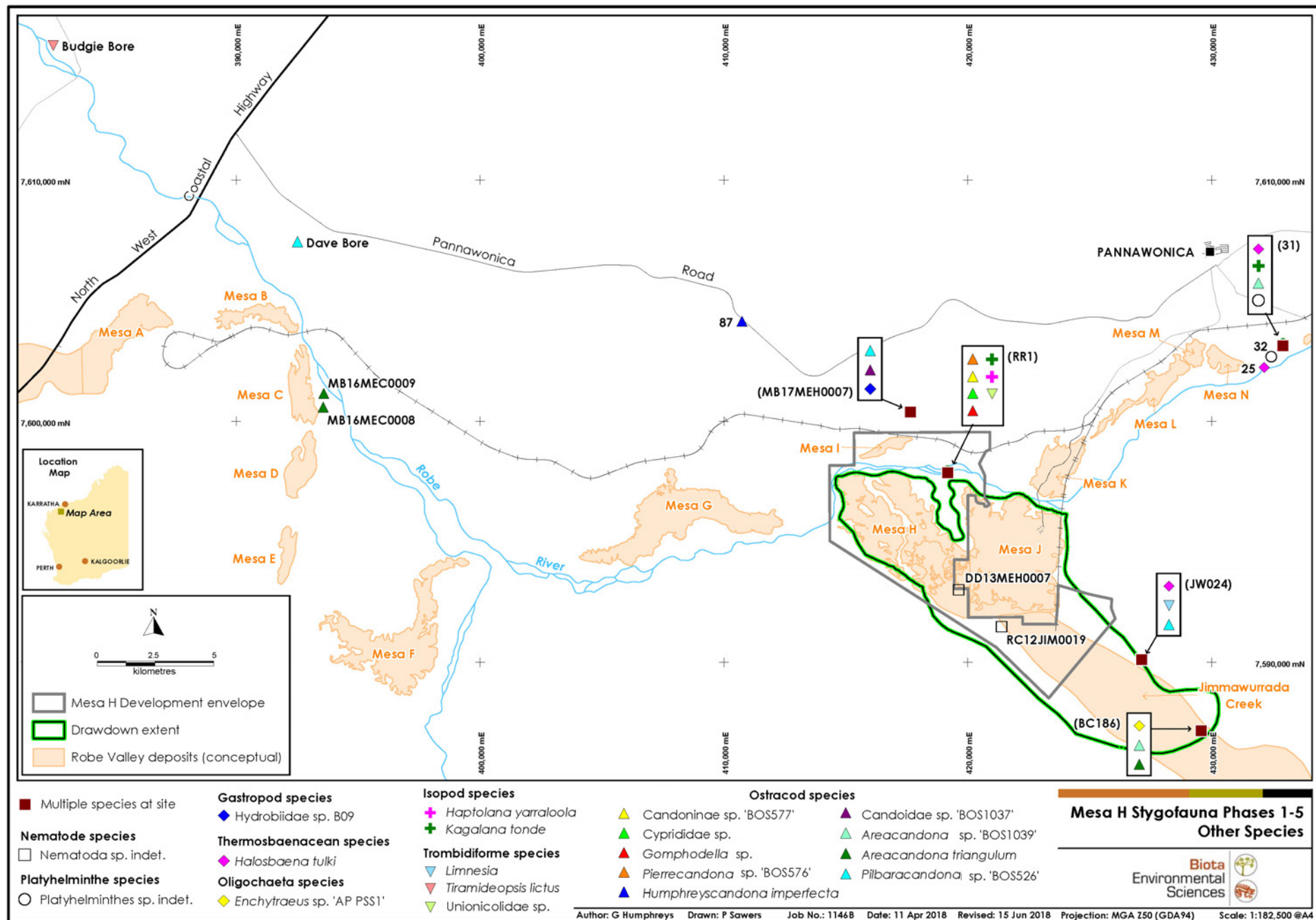


Figure 5.1: Lower abundance stygofauna taxa recorded from Mesa H during the current survey.

### 5.1.5 Order Trombidiformes (Water Mites)

Three water mite taxa were recorded during the survey, all during the Phase 5 sampling (Appendix 5; Table 5.2). Two of the taxa, Unionicolidae sp. indet and *Tiramideopsis lictus*, were recorded from sites outside of the drawdown extent (Figure 5.1).

**Table 5.2: Summary of water mite taxa recorded from the current survey (\*denotes sites outside of the drawdown extent).**

| Family        | Species                     | Site         | Number   |
|---------------|-----------------------------|--------------|----------|
| Limnesiidae   | <i>Limnesia</i> sp. indet.  | JW024        | 1        |
| Mideopsidae   | <i>Tiramideopsis lictus</i> | Budgie Bore* | 2        |
| Unionicolidae | Unionicolidae sp. indet.    | RR1*         | 1        |
| <b>Total</b>  |                             |              | <b>4</b> |

### 5.1.6 Order Ostracoda (Ostracods)

Eighty-three ostracod specimens were recorded during the Mesa H survey (Table 5.3; Figure 5.1), with one specimen from site RR1 unable to be resolved below family level (Cyprididae sp.). Two taxa represented were previously known species (*Humphreyscandona imperfecta* and *Areacandona triangulum*) were recorded (Table 5.3), which are both originally described from elsewhere in the Pilbara (Karanovic 2005).

*Gomphodella* sp. from site RR1 was the sole representative of the Limnocytheridae (Table 5.3), and all of the five remaining ostracod taxa belong to the family Candonidae and are currently undescribed. Two of these, *Pilbaracandona* sp. 'BOS526' and *Pierrecandona* sp. 'BOS576', have been recorded previously from Warramboe (Biota 2017) and Bungaroo (Biota 2016b), respectively. None of the ostracod taxa are known only from the drawdown extent (Table 5.3).

**Table 5.3: Summary of ostracod taxa recorded from the current survey (\*denotes sites outside of the drawdown extent).**

| Family          | Species                            | Site                              | Number    |
|-----------------|------------------------------------|-----------------------------------|-----------|
| Cyprididae      | Cyprididae sp.                     | RR1*                              | 1         |
| Limnocytheridae | <i>Gomphodella</i> sp.             | RR1*                              | 3         |
| Candonidae      | Candoninae sp. 'BOS577'            | RR1*                              | 3         |
|                 | <i>Humphreyscandona imperfecta</i> | 87*                               | 3         |
|                 | <i>Pierrecandona</i> sp. 'BOS576'  | RR1*                              | 2         |
|                 | <i>Pilbaracandona</i> sp. 'BOS526' | Dave Bore*, MB17MEH0007*, JW024   | 10        |
|                 | <i>Areacandona</i> sp. 'BOS1039'   | BC186, 31*                        | 9         |
|                 | <i>Areacandona triangulum</i>      | MB16MEC0008*, MB16MEC0009*, BC186 | 27        |
|                 | Candonidae sp. 'BOS1037'           | MB17MEH0007*                      | 25        |
| <b>Total</b>    |                                    |                                   | <b>83</b> |

### 5.1.7 Subclass Copepoda (Copepods)

Nine species of copepod were identified from amongst the 410 specimens collected during the survey, representing four families (Figure 5.2; Table 5.4). Seventeen juvenile specimens could only be morphologically determined to genus level (*Diacyclops* sp.; Table 5.4). All of the described species recorded are widespread and previously known from outside the survey area (Biota 2016b).

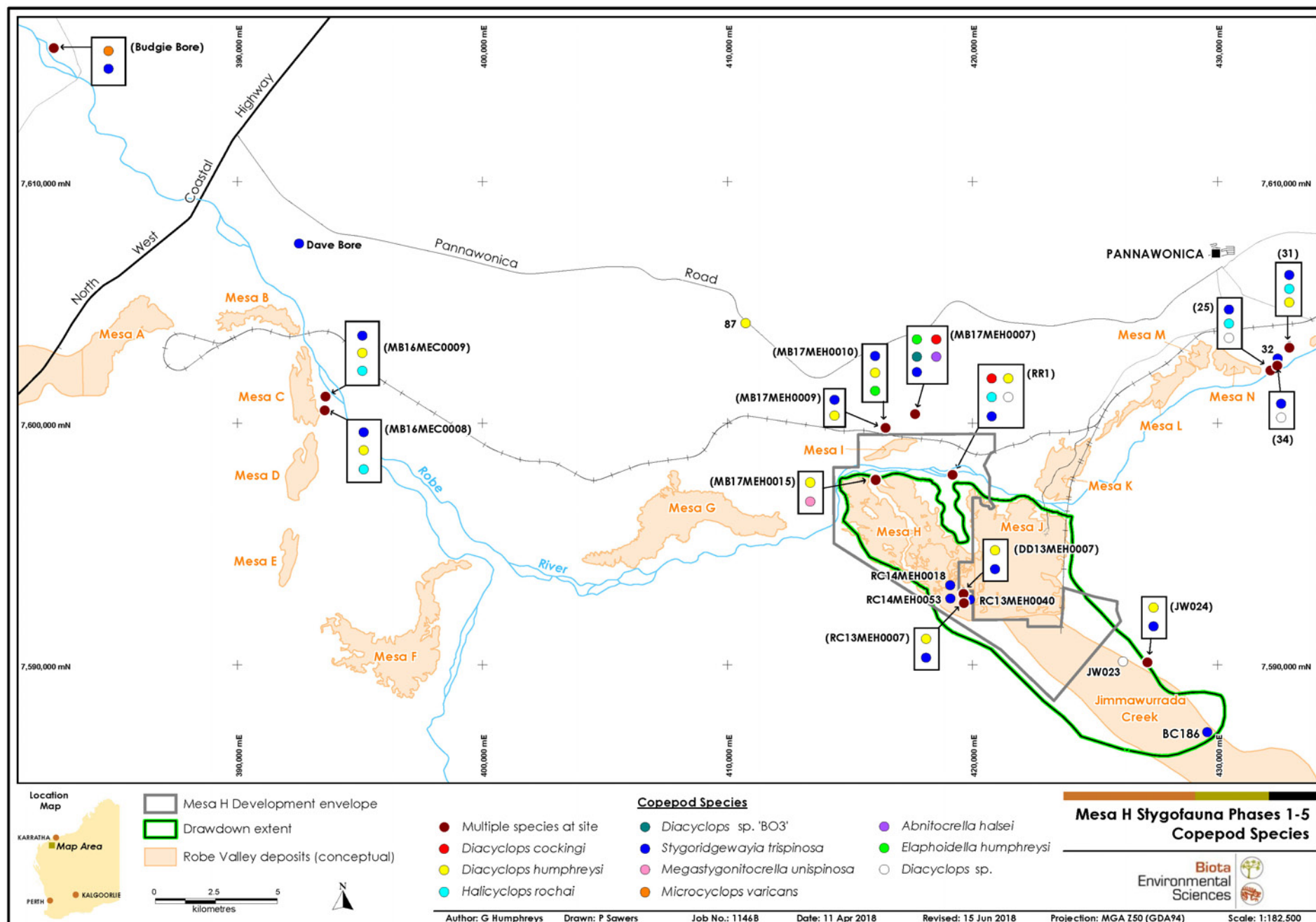


Figure 5.2: Copepod taxa recorded from Mesa H during the current survey.



**Table 5.4: Summary of copepod taxa recorded from the current survey (\*denotes sites outside of the drawdown extent).**

| Family          | Species                                 | Site   | Number     |
|-----------------|---|--|------------|
| Ridgewayiidae   | <i>Stygoridgewayia trispinosa</i>       | 25*, 31*, 32*, 34*, Dave Bore*, Budgie Bore*, RR1*, MB16MEC0008*, MB16MEC0009*, MB17MEH0007*, MB17MEH0009*, MB17MEH0010*, BC186, DD13MEH0007, JW024, RC13MEH0007, RC13MEH0040, RC14MEH0018, RC14MMEH0053 | 236        |
| Cyclopidae      | <i>Diacyclops cockingi</i>              | RR1*, MB17MEH0007*   | 24         |
|                 | <i>Diacyclops humphreysi humphreysi</i> | 31*, 87*, JW024*, RR1*, MB16MEC0008*, MB16MEC0009*, MB17MEH0007*, MB17MEH0009*, MB17MEH0010*, DD13MEH0007, RC13MEH0007, MB17MEH0015  | 68         |
|                 | <i>Diacyclops</i> sp. 'BO3'             | MB17MEH0007*   | 1          |
|                 | <i>Halicyclops rochai</i>               | 25*, 31*, RR1*, MB16MEC0008*, MB16MEC0009*, Budgie Bore*   | 20         |
|                 | <i>Microcyclops varicans</i>            | Budgie Bore*   | 8          |
|                 | <i>Diacyclops</i> sp.                   | 25*, 34*, RR1*, JW023  | 17         |
| Ameiridae       | <i>Abnitocrella halsei</i>              | MB17MEH0007*   | 1          |
|                 | <i>Megastygonitocrella unispinosa</i>   | MB17MEH0015  | 2          |
| Canthocamptidae | <i>Elaphoidella humphreysi</i>          | MB17MEH0007*   | 33         |
| <b>Total</b>    |   |  | <b>410</b> |

### 5.1.8 Order Thermosbaenacea (Thermosbaenaceans)

Twenty specimens of *Halosbaena tulki* were recorded from three sites; JW024, 25 and 31, the latter two of which are outside of the drawdown extent (Table 5.5; Figure 5.1). *H. tulki* is widespread and has been collected commonly from the Bungaroo Valley (Biota 2016b) as well as from locations around Cape Range, Barrow Island and Warrambo (Humphreys 1989, Humphreys et al. 2013, Biota 2017). Recent molecular work has indicated that this taxon may actually represent a species complex containing at least five separate species that reflect local geography (Page et al. 2016). Records of *H. tulki* from the Robe River would belong to the Pilbara high elevation species.

**Table 5.5: Summary of thermosbaenaceans recorded from the current survey (\*denotes sites outside of the drawdown extent).**

| Family            | Species                 | Site            | Number |
|-------------------|-------------------------|-----------------|--------|
| Thermosbaenacidae | <i>Halosbaena tulki</i> | 25*, 31*, JW024 | 20     |

### 5.1.9 Order Isopoda (Isopods)

Four stygal isopods belonging to two described species were recorded from site 31 and RR1, both of which are outside the drawdown extent (Table 5.6; Figure 5.1). *Haptolana yarraloola* was originally described from records from Yarraloola station (Bruce 2008) and has also been recorded from the Warrambo area (Biota 2017). *Kagalana tonde* is widespread in the west Pilbara (Biota 2010c) and has been recorded adjacent to the Mesa H survey area from Bungaroo (Biota 2016b).

**Table 5.6: Summary of stygal isopod taxa recorded from the current survey (\*denotes sites outside of the drawdown extent).**

| Family       | Species                     | Site      | Number   |
|--------------|-----------------------------|-----------|----------|
| Cirolanidae  | <i>Haptolana yarraloola</i> | RR1*      | 1        |
|              | <i>Kagalana tonde</i>       | RR1*, 31* | 3        |
| <b>Total</b> |                             |           | <b>4</b> |

### 5.1.10 Order Amphipoda (Amphipods)

A total of 320 amphipod specimens were recorded, comprising 19 taxa from three families (Table 5.7; Figure 5.3). Eighty-four specimens that were unable to be identified to species level remained as *Amphipoda* sp. indet (Table 5.7). These specimens were unable to be determined due to either a failure to sequence or where large numbers of specimens of the same morphotype were recorded from the same site (only a subsample of the specimens from such sites were sequenced; Section 3.7).

A total of 148 specimens recorded across 16 sites were assigned to lineage AMM001 (Table 5.7). This lineage was placed into a well-supported group which has been assigned the putative species name of *Nedsia* sp. 'AMM001' (family Eriopisidae), which is part of a species complex containing multiple other closely-related (<5% mean sequence divergence) lineages from the locality (Helix 2017a). This species complex has been recorded at multiple locations throughout the Robe River valley including from Warrambo, Budgie Bore and Camp Bore (Biota 2017, Helix 2017a) (Appendix 3).

An additional taxon belonging to family Eriopisidae, *Nedsia* sp. 'AMM031' was also recorded from a single site outside of the drawdown extent (Dave Bore; Table 5.7; Figure 5.3). This lineage was originally included within the *Nedsia* sp. 'AMM001' species complex but discussion with genetic and taxonomic specialists at Helix, WAM and the South Australian Museum have conservatively determined that *Nedsia* sp. 'AMM031' is likely to be a distinct species, though additional specimens and further analysis would be needed to more fully confirm this. This species has also been recorded from Dave Bore, Budgie Bore and Mesa A (Biota 2017, Helix 2017a) (Table 5.7).

Six other taxa from the family Eriopisidae, *Nedsia* sp. 'AMM004', sp. 'AMM06', sp. 'AMM022', sp. 'AMM031', sp. 'AMM032' and sp. 'AMM033', were also recorded during the survey, but none of these were detected within the drawdown extent (Table 5.7; Figure 5.3). Three of these taxa, *Nedsia* sp. 'AMM004', sp. 'AMM032' and sp. 'AMM033', represent new species not previously recorded in the Pilbara (Helix 2017a) (Appendix 3). The final erioposid taxon, *Nedsia* sp. 'AMM026' was recorded from within the drawdown extent, but also occurs outside (at site 31; Table 5.7; Figure 5.3).

Five species of Neoniphargidae amphipods were recorded from the survey area (Table 5.7), consisting of one species that has been recorded elsewhere in the Robe River valley; Neoniphargidae sp. 'AMN008' (Biota 2017), and three new species that had not previously been recorded in the region: Neoniphargidae sp. 'AMN002', sp. 'AMN003' and *Wesniphargus* sp. 'AMN004' (Helix 2017a) (Appendix 3). Additional sequencing of historical survey specimens by the WAM found that records of both Neoniphargidae sp. 'AMN003' and *Wesniphargus* sp. 'AMN004', had previously gone unrecognised as such from sampling at Bungaroo (Cullen and Harvey 2017). *Wesniphargus* sp. 'AMN004' was the only neoniphargid taxa to be recorded from the drawdown extent, but was also recorded from reference site 25 (Table 5.7), in addition to the earlier Bungaroo record. The fifth species, Neoniphargidae sp. 'B02', was recorded from site 32 on the basis of morphology. This species has also been recorded from a site within the Mesa H development envelope during a previous survey at Bungaroo (Biota 2013) (see Section 4.4.1).

The final family, Paramelitidae, was represented by six species, with three recorded from within the drawdown extent: Paramelitidae sp. 'AMP003' sp., 'AMP035' (Plate 5.1) and 'AMP037', two of which were collected as singletons (Table 5.7), and appear to represent new taxa not previously detected in the region (Helix 2017a) (Appendix 3).



**Plate 5.1:** Paramelitidae sp. 'AMP035' from drillhole RC13MEH0007.

**Table 5.7: Summary of amphipod taxa recorded from the current survey (\*denotes sites outside of the drawdown extent).**

| Family         | Lineage                             | Species                          | Site   | Number     |
|----------------|-------------------------------------|----------------------------------|--|------------|
| Eriopisidae    | AMM001                              | <i>Nedsia</i> sp. 'AMM001'       | 25*, 31*, 32*, 34*, Budgie Bore*, DD13MEH0007, JW024, RC12JIM0019, RC13MEH0040, RC13MEH0041, RC13MEH0097, RC14MEH0018, RC14MEH0053, RC16JIM0019, RR1 | 148        |
|                | AMM004                              | <i>Nedsia</i> sp. 'AMM004'       | 87*, Dave Bore*, MB16MEC0009*  | 16         |
|                | AMM006                              | <i>Nedsia</i> sp. 'AMM006'       | MB16MEC0008*, MB16MEC0009*   | 2          |
|                | AMM022                              | <i>Nedsia</i> sp. 'AMM022'       | MB17MEH0007*   | 1          |
|                | AMM026                              | <i>Nedsia</i> sp. 'AMM026'       | 31*, RC13MEH0097   | 6          |
|                | AMM031                              | <i>Nedsia</i> sp. 'AMM031'       | Dave Bore*   | 3          |
|                | AMM032                              | <i>Nedsia</i> sp. 'AMM032'       | 31*  | 1          |
|                | AMM033                              | <i>Nedsia</i> sp. 'AMM033'       | MB17MEH0007*, MB17MEH0009*, MB17MEH0010*   | 27         |
| Neoniphargidae | AMN002                              | Neoniphargidae sp. 'AMN002'      | RR1*   | 1          |
|                | AMN003                              | Neoniphargidae sp. 'AMN003'      | 32*  | 1          |
|                | AMN004                              | <i>Wesniphargus</i> sp. 'AMN004' | 25*, JW024   | 4          |
|                | AMN008                              | Neoniphargidae sp. 'AMN008'      | Dave Bore*, Budgie Bore*   | 11         |
|                | -                                   | Neoniphargidae sp. 'B02'         | 32*  | 1          |
| Paramelitidae  | AMP003                              | Paramelitidae sp. 'AMP003'       | RC13MEH0041  | 1          |
|                | AMP009                              | Paramelitidae sp. 'AMP009'       | Budgie Bore*   | 1          |
|                | AMP035                              | Paramelitidae sp. 'AMP035'       | RR1*, RC13MEH0007  | 8          |
|                | AMP036                              | <i>Chydaekata</i> sp. 'AMP036'   | RR1*   | 1          |
|                | AMP037                              | Paramelitidae sp. 'AMP037'       | BC186  | 1          |
|                | AMP038                              | Paramelitidae sp. 'AMP038'       | 25*, RR1*  | 2          |
| Indeterminate  | Failed to sequence or not sequenced | Amphipoda sp. indet.             | 31*, 34*, 87*, MB17MEH0010*, RR1*, BC186, DD13MEH0007, JW024, RC12JIM0019, RC16JIM0019, RC13MEH0007  | 84         |
| <b>Total</b>   |                                     |                                  |  | <b>320</b> |



**Figure 5.3: Amphipoda taxa recorded from Mesa H during the current survey.**



## 5.2 eDNA

Replicated samples from the 16 sites where groundwater was sampled for eDNA were analysed by two different molecular methods in an attempt to detect residual DNA from the Blind Cave Eel (*Ophisternon candidum*) (Section 3.4).

Six of the 16 eDNA sampling sites yielded positive results for *Ophisternon* DNA by at least one method (Figure 5.4; Table 5.8), with the results from sites 25, MB17MEH0015 and the Control site having an even higher degree of confidence being independently detected by both laboratories (Table 5.8). An additional site, JW021, which was previously sampled for groundwater as part of previous work (Figure 5.4; Table 5.8), also yielded a positive detection during the preliminary metabarcoding analysis undertaken by Curtin University (Appendix 4).

**Table 5.8: Results of eDNA analysis of environmental samples ('Y' = positive detection of *Ophisternon* DNA; \*denotes sites outside of the drawdown extent; †denotes detection only after refinement of metabarcoding methods (latter section of Appendix 4)).**

| Site         | Easting (m E) | Northing (m N) | Ophisternon DNA Detected? |                      | Notes              |
|--------------|---------------|----------------|---------------------------|----------------------|--------------------|
|              |               |                | Helix qPCR Assay          | Curtin Metabarcoding |                    |
| Control*     | 424478        | 7597147        | Y                         | Y                    | Surface pool site  |
| 25*          | 432152        | 7602229        | Y                         | Y                    |                    |
| MB17MEH0015  | 416041        | 7597690        | Y                         | Y†                   |                    |
| RR1*         | 419176        | 7597904        | -                         | Y                    |                    |
| 31*          | 432929        | 7603179        | -                         | -                    |                    |
| Dave Bore*   | 392512        | 7607436        | -                         | -                    |                    |
| BC186        | 429578        | 7587212        | -                         | Y†                   | Confirmed eel site |
| BC401*       | 443563        | 7574126        | -                         | -                    |                    |
| Budgie Bore* | 382495        | 7615587        | -                         | -                    |                    |
| JW023        | 426138        | 7590140        | -                         | -                    | Confirmed eel site |
| JW024        | 427126        | 7590154        | -                         | Y†                   | Confirmed eel site |
| MB16MEC0008* | 393565        | 7600580        | -                         | -                    |                    |
| MB16MEC0009* | 393590        | 7601145        | -                         | -                    |                    |
| MB17MEH0007* | 417664        | 7600421        | -                         | -                    |                    |
| MB17MEH0008* | 417120        | 7600417        | -                         | -                    |                    |
| MB17MEH0009* | 416436        | 7599848        | -                         | -                    |                    |
| MB17MEH0010* | 416440        | 7599848        | -                         | -                    |                    |
| JW021        | 424138        | 7589754        | Not sampled               | Y                    | Sampled previously |

The qPCR assay is a targeted molecular method that will only identify the presence of target DNA in an environmental sample when both the species-specific primer and the species-specific probe match the DNA present in the sample (Biota and Helix 2014). This means the positive results from the qPCR assay from sites 25, MB17MEH0015 and the Control sample can all be regarded with confidence as containing *Ophisternon candidum* DNA (Table 5.8) (Helix 2018).

The metabarcoding approach uses a high throughput approach to amplify DNA and in the case of this study the 16S gene was specifically targeted during amplification to compare against reference sequence data. The findings from the preliminary Curtin University analysis were that possible *Ophisternon* DNA was detected from site 25 and RR1, as well as site JW021 (Table 5.8; Figure 5.4). The sequences from the JW021 site were a 100% match for the known *O. candidum* sequences from JW024, and those from sites 25 and RR1 were a 97% match. Subsequent additional work using an assay specifically developed for *Ophisternon* resulted in further detections at three other sites, including MB17MEH0015; where the qPCR assay had independently detected eDNA belonging to the species (Table 5.8).

In addition to the desktop review records, a specimen was recently recorded from the phreatic zone of the Robe River during aquatic fauna sampling in a surface pool in the river (Biota 2018) (site RRD2; Figure 5.4). Considered in total then, the eDNA positive sites and specimen collection locations to date comprise five sites within the drawdown extent and four sites outside (Figure 5.4).



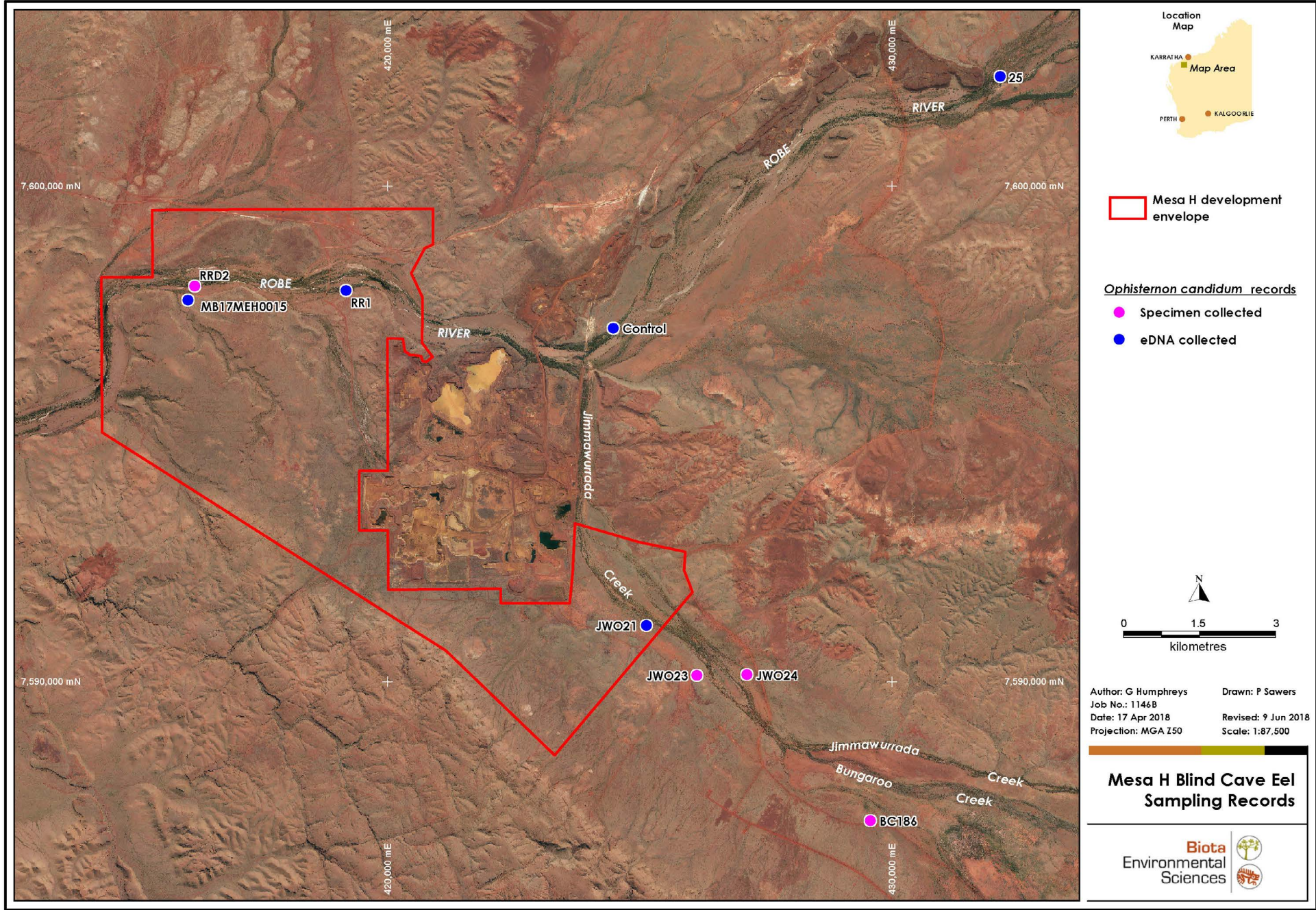


Figure 5.4: Locations where positive eDNA results were recorded for *Ophisternon* DNA detection, in context with confirmed *Ophisternon* specimen collection locations.



## 5.3 Troglafauna

A total of 150 troglobitic specimens were collected across the six phases of sampling of the Mesa H survey area (Table 5.9). The specimens represented five classes and nine orders and 32 taxa (Appendix 5). A total of 150 troglobitic specimens were collected across the six phases of sampling of the Mesa H survey area (Table 5.9). The specimens represented five classes and nine orders and 32 taxa (Appendix 5). The orders Coleoptera and Schizomida were the greatest contributors to faunal composition, accounting for 33% and 25% of the specimens, respectively.

**Table 5.9: Summary of troglobitic fauna recorded from Mesa H during the current survey (n= number of specimens collected).**

| Taxonomy   |             |              |                                    | n     | Number of Sites |
|------------|-------------|--------------|------------------------------------|-------|-----------------|
| Phylum     | Subphylum   | Class        | Order                              |       |                 |
| Arthropoda | Chelicerata | Arachnida    | Araneae (Spiders)                  | 2     | 2               |
|            |             |              | Pseudoscorpiones (Pseudoscorpions) | 14    | 12              |
|            |             |              | Schizomida (Schizomids)            | 37    | 22              |
|            | Hexapoda    | Entognatha   | Diplura (Bristletails)             | 12    | 11              |
|            |             | Insecta      | Blattodea (Cockroaches)            | 2     | 2               |
|            |             |              | Coleoptera (Beetles)               | 50    | 5               |
|            |             |              | Zygentoma (Silverfish)             | 2     | 2               |
|            | Crustacea   | Malacostraca | Isopoda (Slaters)                  | 30    | 4               |
|            | Myriapoda   | Chilopoda    | Scolopendromorpha (Centipedes)     | 1     | 1               |
|            |             |              |                                    | Total | 150             |

As is common during troglafauna surveys, a large number of edaphobitic (deep soil adapted fauna) and epigeal (surface) specimens were also collected. These specimens are not troglobitic and are therefore not discussed further in this report.

A detailed account of the troglobitic fauna recorded, including discussion of the results of genetic analysis, follows in Sections 5.3.1 to 5.3.6. Record locations are presented in Figure 5.16.

### 5.3.1 Order Diplura (Diplurans)

Twelve dipluran specimens were recorded and assigned to nine lineages based on molecular analysis (Helix 2017b) (Table 5.10; Figure 5.16). Two specimens failed to sequence and were therefore indeterminate (*Diplura* sp. indet.), while three lineages were closely related (3.5 % sequence divergence) and were combined into one species; *Projapygidae* sp. 'DPR008/009/011'. The remaining six lineages identified correspond to six new species (Helix 2017b) (Figure 5.5).

**Table 5.10: Summary of dipluran taxa recorded from the current survey.**

| Family        | Lineage            | Species                                 | Site  | Number |
|---------------|--------------------|---|---|--------|
| Parajapygidae | DPA001             | Parajapygidae sp. 'DPA001' (Plate 5.2). | RC14MEH0308   | 1      |
|               | DPA009             | Parajapygidae sp. 'DPA009'              | MEHRD0758   | 1      |
| Projapygidae  | DPR008             | Projapygidae sp. 'DPR008/DPR009/DPR011' | RC15MEH0166,<br>RC15MEH0175,<br>RC15MEH0261,<br>RC16JIM0005 | 4      |
|               | DPR009             |   |   |        |
|               | DPR011             |   |   |        |
|               | DPR010             | Projapygidae sp. 'DPR010'               | RC16JIM0005   | 1      |
| Japygidae     | DJA003             | Japygidae sp. 'DJA003'                  | RC14MEH0388   | 1      |
|               | DJA011             | Japygidae sp. 'DJA011'                  | DD13MEH0007   | 1      |
| Campodeidae   | DCA005             | Campodeidae sp. 'DCA005'                | GR15MEH0032   | 1      |
| Indeterminate | Failed to sequence | <i>Diplura</i> sp. indet.               | RC15MEH0114,<br>RC16MEH0264                                 | 2      |
| Total         |                    |   |   | 12     |



Plate 5.2: Parajapygidae sp. 'DPA001' from drillhole RC14MEH0308.

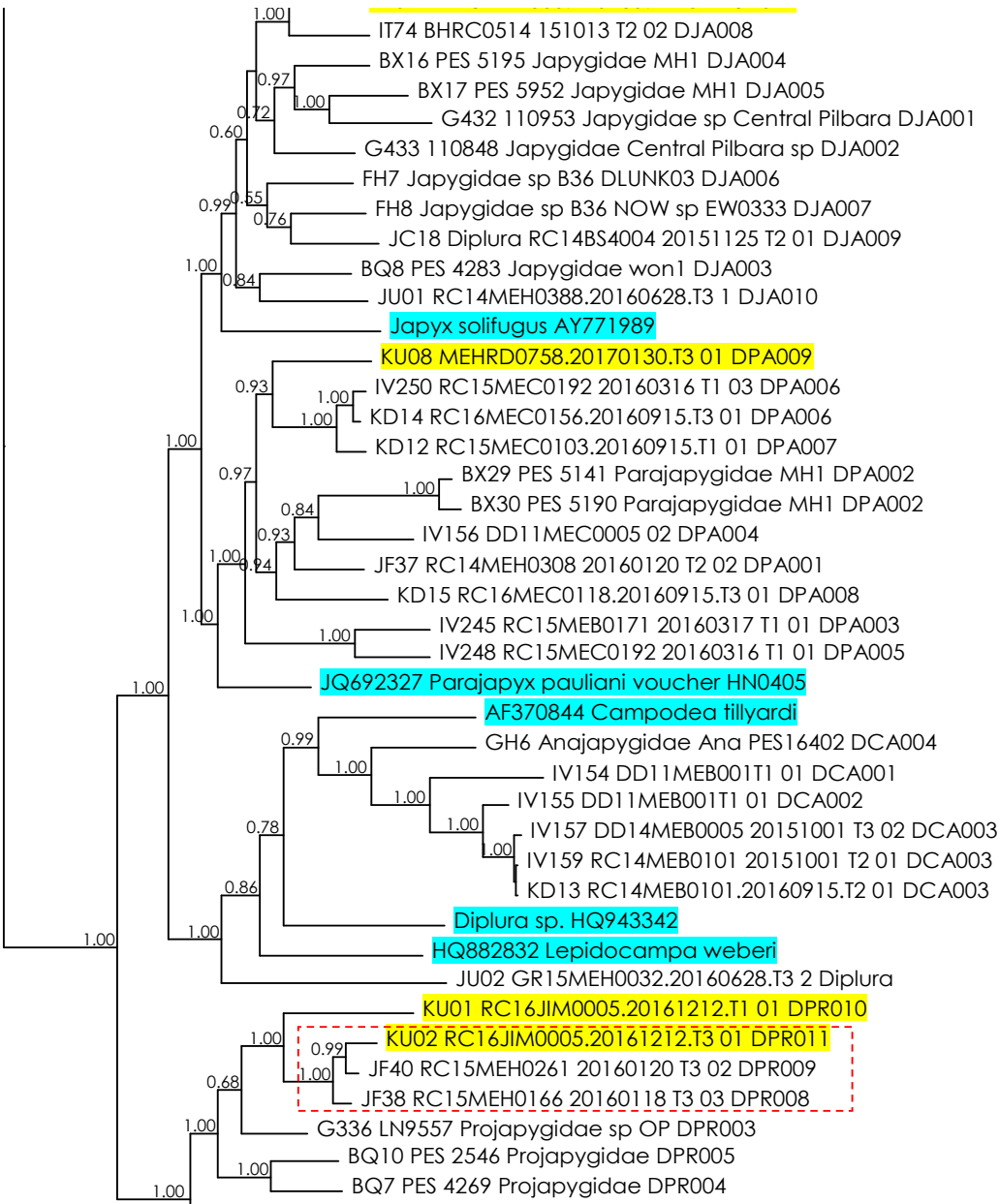


Figure 5.5: Bayesian analysis of CO1 haplotypes of Diplura recorded from the Mesa H survey area. (Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the current study are highlighted in yellow; GenBank specimens are highlighted in turquoise. Red boxes enclose species with high confidence. Red boxes with dashed lines indicate lineages combined to form single species for the purposes of this assessment.



5.3.2 Order Isopoda (Slaters)

A total of 30 troglomorphic isopod specimens were recorded from the survey area, 27 of which were sequenced. Ten of these specimens failed to yield a sequence and therefore remained indeterminate as *Isopoda* sp. indet., along with three specimens that were not sequenced as large numbers of specimens of the same morphotype were recorded from the same site (Table 5.11). The remaining 17 specimens were assigned to four lineages belonging to two families; *Philosciidae* and *Armadillidae* (Table 5.11; Figure 5.16).

All four collected lineages are new, however the two *philosciid* lineages (*Philosciidae* sp. 'ISP047' and 'ISP055') are closely related (5.1 % sequence divergence) (Figure 5.6) and may represent the same species, but confirming this requires further investigation using morphological analysis of adult specimens or sequencing of additional genes (Helix 2017b). In the interests of a conservative assessment, the two taxa are maintained as distinct species in the current report (Table 5.11).

Of the two *armadillid* lineages, Lineage ISA046 was assigned to the species *Troglarmadillo* sp. 'ISA046' and differed by 18.5% to its mostly closely related lineage (ISA054 from Mesa C) (Figure 5.7). The second *armadillid* Lineage, ISA057, was closely related (4.5 % sequence divergence) to a lineage previously recorded from Mesa C (lineage 'ISA056') (Figure 5.8) (Helix 2017b), and the two lineages were grouped to form a single putative species, *Armadillidae* sp. 'ISA056/ISA057' (Table 5.11).

Table 5.11: Summary of isopod taxa recorded from the current survey.

| Family        | Lineage            | Species                            | Site                    | Number |
|---------------|--------------------|------------------------------------|-------------------------|--------|
| Philosciidae  | ISP047             | Philosciidae sp. 'ISP047'          | RC15MEH0382             | 6      |
|               | ISP055             | Philosciidae sp. 'ISP055'          | RC15MEH0427             | 1      |
| Armadillidae  | ISA046             | <i>Troglarmadillo</i> sp. 'ISA046' | RC15MEH0382             | 1      |
|               | ISA057             | Armadillidae sp. 'ISA056/ISA057'   | MEHRD0577,<br>MEHRD0834 | 9      |
| Indeterminate | Failed to sequence | Isopoda sp. indet.                 | MEHRD0577,<br>MEHRD0834 | 13     |
| Total         |                    |                                    |                         | 30     |

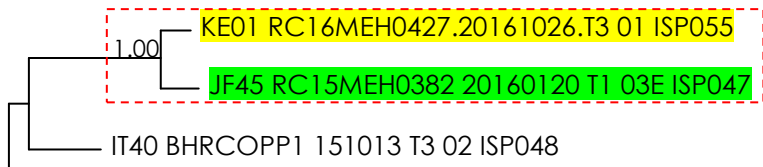


Figure 5.6: Excerpt of bayesian analysis of CO1 haplotypes of *Philosciidae* recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).  
(Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the current study are highlighted in yellow (Phase 1 – 3) and green (Phase 4 – 6. Red boxes enclose species with high confidence. Red boxes with dashed lines indicate lineages grouped into a single species for the purposes of this assessment.

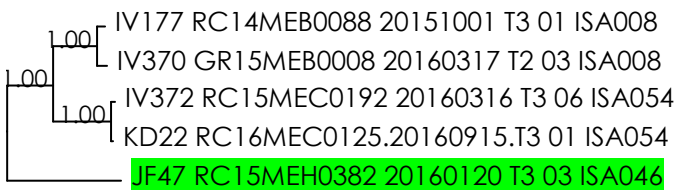
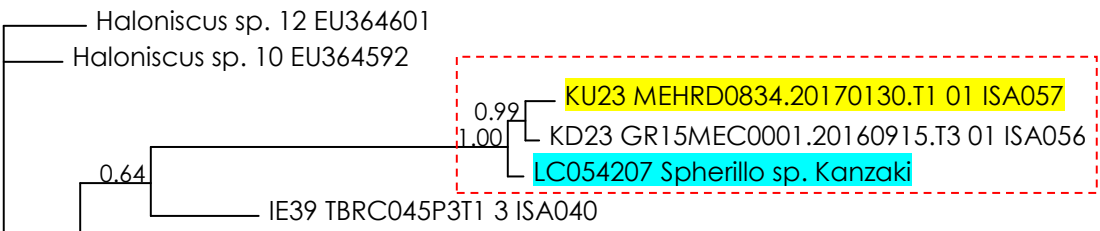


Figure 5.7: Excerpt of Bayesian analysis of CO1 haplotypes of *Armadillidae* recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).  
(Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the current study are highlighted in green.



**Figure 5.8:** Excerpt of bayesian analysis of CO1 haplotypes of Armadillidae recorded from the Mesa H survey area, showing Lineage ISA057 in context with Lineage ISA056 from Mesa C (see Appendix 3 for complete phylogenetic tree).

(Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the current study are highlighted in yellow; GenBank specimens are highlighted in turquoise. Red boxes enclose species with high confidence. Red boxes with dashed lines indicate groups requiring further investigation.

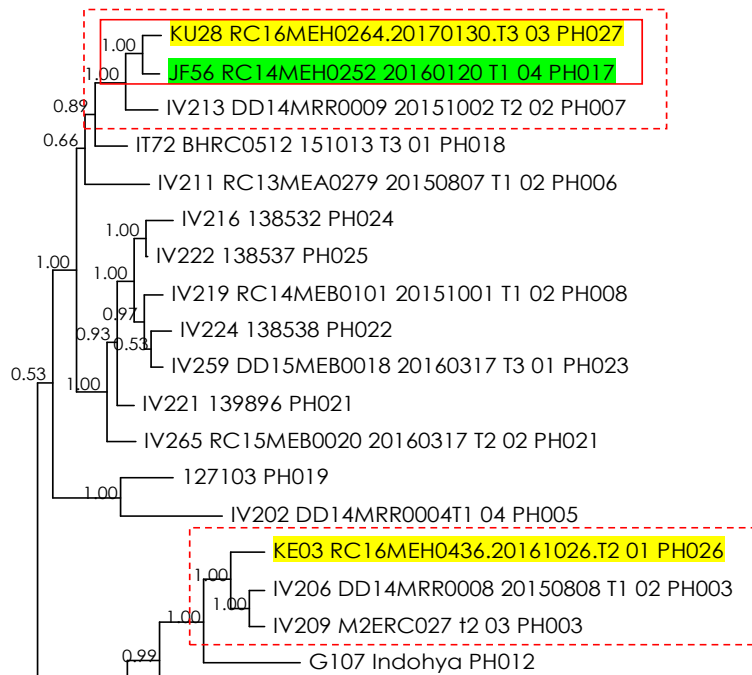
**5.3.3 Order Pseudoscorpiones (Pseudoscorpions)**

Fourteen pseudoscorpion specimens were recorded from the survey area. Molecular analysis placed the specimens into 10 lineages, which correspond to eight species across three families (Helix 2017b) (Table 5.12; Figure 5.9 to Figure 5.11; Figure 5.16).

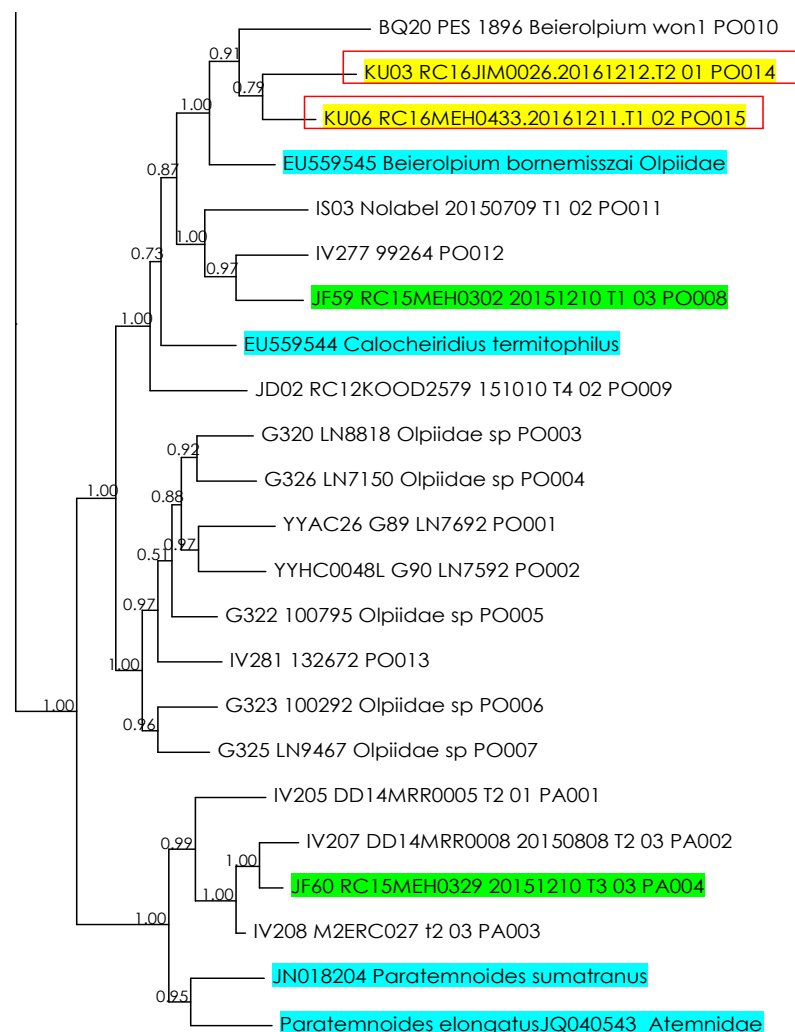
Representative photographs of four of the taxa are presented in Plate 5.3 to Plate 5.6. Two specimens failed to sequence and remained indeterminate as Pseudoscorpion sp. indet.. None of the eight species recorded have been previously detected in the Pilbara (Helix 2017b).

**Table 5.12:** Summary of pseudoscorpion taxa recorded from the current survey.

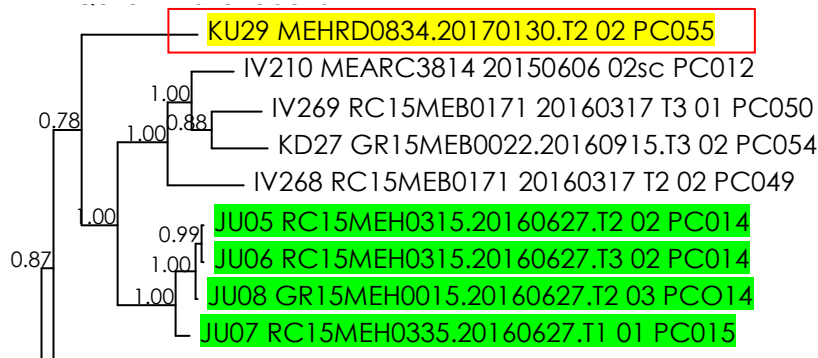
| Family        | Lineage            | Species                       | Site                                  | Number |
|---------------|--------------------|-------------------------------|---------------------------------------|--------|
| Hyidae        | PH017, PH027       | Hyidae sp. 'PH017/PH027'      | RC14MEH0252, RC16MEH0264              | 2      |
|               | PH026              | Hyidae sp. 'PH026'            | RC16MEH0436                           | 1      |
| Olpiidae      | PO008              | Olpiidae sp. 'PO008'          | RC15MEH0302                           | 1      |
|               | PO014              | Beierolpium sp. 'PO014'       | RC16JIM0026                           | 1      |
|               | PO015              | Beierolpium sp. 'PO015'       | RC16MEH0433                           | 1      |
| Atemnidae     | PA004              | Atemnidae sp. 'PA004'         | RC15MEH0329                           | 1      |
| Chthoniidae   | PC014, PC015       | Chthoniidae sp. 'PC014/PC015' | GR15MEH0015, RC15MEH0315, RC15MEH0335 | 4      |
|               | PC055              | Chthoniidae sp. 'PC055'       | MEHRD0834                             | 1      |
| Indeterminate | Failed to sequence | Pseudoscorpion sp. indet.     | RC12MEH0120                           | 2      |
| Total         |                    |                               |                                       | 14     |



**Figure 5.9: Excerpt of bayesian analysis of CO1 haplotypes of Hyiidae recorded from survey area.**  
(Numbers on major nodes are posterior probabilities; values <50% not shown. Specimens from the current survey highlighted in yellow (Phase 1 – 3) and green (Phase 4 – 6). Red boxes enclose species with high confidence. Boxes with dashed lines denote groups requiring further investigation).



**Figure 5.10: Excerpt of bayesian analysis of CO1 haplotypes of Olpiidae and Atemnidae from the survey area.**  
(Numbers on major nodes are posterior probabilities; values <50% not shown. Specimens from the current survey highlighted in yellow (Phase 1 – 3) and green (Phase 4 – 6). Red boxes enclose species with high confidence. Boxes with dashed lines denote groups requiring further investigation).



**Figure 5.11:** Excerpt of bayesian analysis of CO1 haplotypes of Chthoniidae recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).

(Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the current study are highlighted in yellow (Phase 1 – 3) and green (Phase 4 – 6). Red boxes enclose species with high confidence. Red boxes with dashed lines indicate groups requiring further investigation.



**Plate 5.3:** *Beierolpium* sp. 'PO014' from drillhole RC16JIM0026.



**Plate 5.4:** *Hyidae* sp. 'PH017/PH027' from drillhole RC14MRH0252.



**Plate 5.5:** *Olpiidae* sp. 'PO008' from drillhole RC15MEH0302.



**Plate 5.6:** *Atemnidae* sp. 'PA004' from drillhole RC15MEH0329.



5.3.4 Order Schizomida (Schizomids)

A total of 45 schizomid specimens were sequenced as part of the Mesa H assessment. Fourteen were collected during the current survey (Table 5.13), 23 were collected during a previous survey at Mesa J (Biota 2009), and WAM also separately sequenced, and morphologically identified, eight specimens from historically sampled sites at Mesa H previously identified as *Paradraculoides* sp. nov. 'Mesa H' (see Section 4.4.2) (Cullen and Harvey 2017). Five lineages corresponding to four species were identified, with six of the 45 specimens unable to be assigned to a lineage due to failure to sequence or missing historical specimens (Table 5.13; Figure 5.16).

Of the four species detected, Hubbardiidae sp. 'SCH011' was recorded within the Mesa H development envelope and also from several sites across Mesa J (Table 5.13). Two lineages recorded from Mesa H were found to be closely related (at 4.3% divergence) and were grouped together as a single species based on habitat proximity and geology (Helix 2017b) (Figure 5.12). This species, Hubbardiidae sp. 'SCH015/SCH016' (Plate 5.7 and Plate 5.8) has also been recorded previously, from approximately 6 km southwest of the development envelope (T. Finston, Helix, pers. comm. April 2017). The WAM analysis of historical material was consistent with the Helix analysis, indicating the presence of two distinct lineages corresponding to Hubbardiidae sp. 'SCH011' and Hubbardiidae sp. 'SCH015/SCH016' (Table 5.13) (Cullen and Harvey 2017). The remaining two lineages were each assigned to new species within the genus *Paradraculoides* (*P.* sp. 'SCH038' and *P.* sp. 'SCH039'; Table 5.13). Both species are previously unrecorded outside the development envelope.

**Table 5.13: Summary of schizomid taxa recorded from Mesa H during the current study.**  
(Includes sequencing of specimens from (Biota 2009) and Cullen and Harvey (2017)).

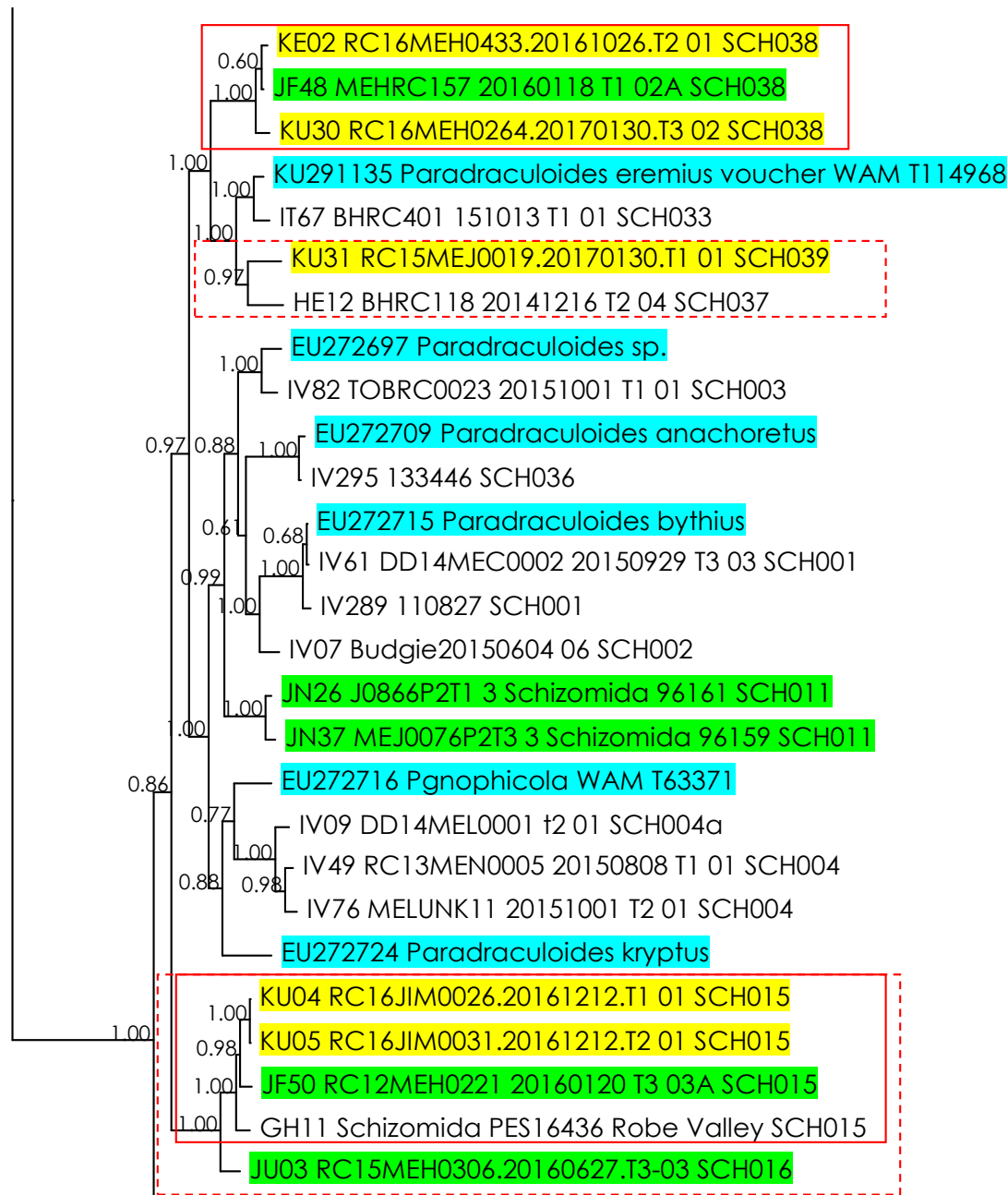
| Family       | Lineage            | Species                             | Site   | Number |
|--------------|--------------------|-------------------------------------|--|--------|
| Hubbardiidae | SCH011             | Hubbardiidae sp. 'SCH011'           | J0866, J0867, J0928, MEJ0007, MEJ0048, MEJ0076, MEJ0085, J0866, MEJ5548alt, RC08MEJ0015, RC08MEJ0004, RC08MEJ0023, MEHRC0724 | 23     |
|              | SCH015             | Hubbardiidae sp. 'SCH015/SCH016'    | DD13MEH0007, RC12MEH0221, RC14MEH0252, RC15MEH0306, RC16JIM0026, RC16JIM0031, MEHRD0870                                      | 10     |
|              | SCH016             |                                     |  |        |
|              | SCH038             | <i>Paradraculoides</i> sp. 'SCH038' | MEHRC157, RC15MEH0329, RC16MEH0264, RC16MEH0433  | 5      |
|              | SCH039             | <i>Paradraculoides</i> sp. 'SCH039' | RC15MEJ0019  | 1      |
|              | Failed to sequence | Schizomida sp. indet.               | RC15MEH0329, MEH, MEJ0007, MEJ0076, J0866, MEHRC0894   | 6      |
| Total        |                    |                                     |  | 45     |



**Plate 5.7:** Hubbardiidae sp. 'SCH015/SCH016' (lateral view) from drillhole RC12MEH0221.



**Plate 5.8:** Hubbardiidae sp. 'SCH015/SCH016' (dorsal view) from drillhole RC12MEH0221.



**Figure 5.12:** Excerpt of Bayesian analysis of CO1 haplotypes of Schizomida recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).

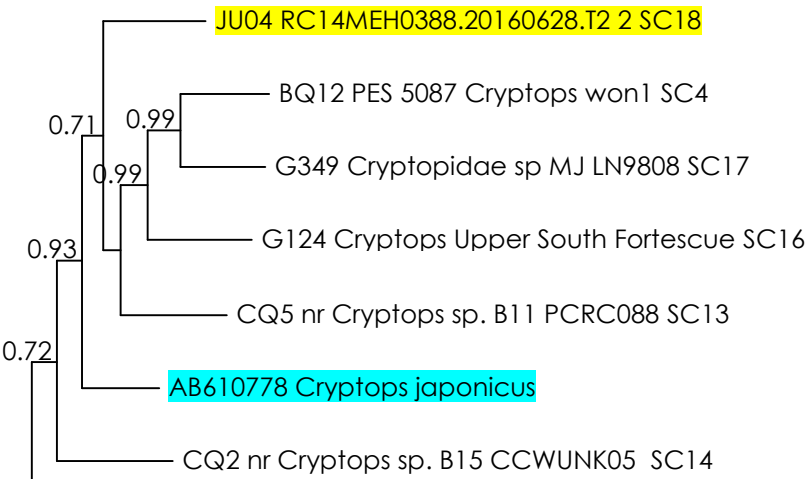
(Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the current study are highlighted in yellow (Phase 1 – 3) and green (Phase 4 – 6); GenBank specimens are highlighted in turquoise. Red boxes enclose species with high confidence. Red boxes with dashed lines indicate groups requiring further investigation.

### 5.3.5 Order Scolopendromorpha (Centipedes)

A single centipede specimen was sequenced and assigned to the family Cryptopidae (Table 5.14; Figure 5.16). This specimen differed from reference specimens by more than 15% and is a new species (Figure 5.13)(Helix 2016).

**Table 5.14:** Summary of centipede taxa recorded from the current survey.

| Family      | Lineage | Species                | Site        | Number |
|-------------|---------|------------------------|-------------|--------|
| Cryptopidae | SC18    | Cryptopidae sp. 'SC18' | RC14MEH0388 | 1      |



**Figure 5.13:** Excerpt of Bayesian analysis of CO1 haplotypes of Scolopendrida recorded from the Mesa H study area (see Appendix 3 for complete phylogenetic tree).  
(Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the current study are highlighted in yellow; GenBank specimens are highlighted in turquoise.)

**5.3.6 Order Araneae (Spiders)**

Two troglotitic spider specimens were recorded from Mesa H (Table 5.15; Figure 5.16).  
After morphological examination by the WAM (Appendix 4), one adult male specimen from the family Oonopidae was assigned to a new species within the genus *Prethopalpus* (Table 5.15). No specimens from this genus have been previously collected from Mesa H and this species is considered likely to be an SRE species (Cullen et al. 2017).  
The remaining specimen was a juvenile of the family Gnaphosidae and could not be identified to genus level. This specimen is considered to represent a potential SRE species (Cullen et al. 2017).

**Table 5.15:** Summary of spider taxa recorded from the current survey.

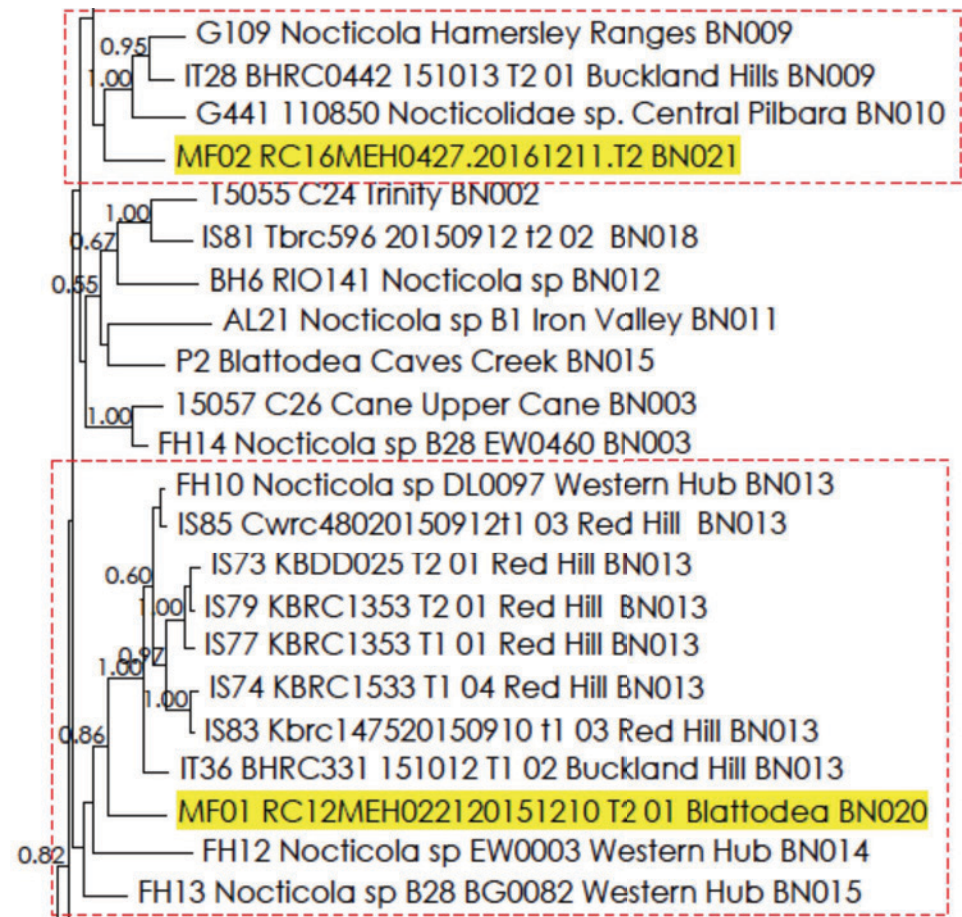
| Family      | Species                      | Site        | Number |
|-------------|------------------------------|-------------|--------|
| Oonopidae   | <i>Prethopalpus</i> 'ARA052' | RC16JIM0019 | 1      |
| Gnaphosidae | Genus indef. sp. 'Mesa H'    | RC14MEH0270 | 1      |
| Total       |                              |             | 2      |

**5.3.7 Order Blattodea (Cockroaches)**

Two troglotitic cockroach specimens were recorded from Mesa H (Table 5.16; Figure 5.16). Sequencing identified these as belonging to two separate lineages, which form part of a potential West Pilbara species complex: ?*Nocticola* sp. 'West Pilbara Complex' (family Nocticolidae; Table 5.16; Appendix 3). The two specimens from the current study differed from each other by 6.3%, which is greater than normally expected within a species, but the lineages within this potential species complex also show an equivalent average genetic distance of approximately 6% over a very large geographic range, including a record from Red Hill (some 60 km to the south) which varied from the RC16MEH0427 specimen from the current survey by only 4.2% (divergence more consistent with within-species variation; Appendix 3). The records have therefore been retained here as representatives of the ?*Nocticola* species complex until further work can clearly identify where any species boundaries should be ascribed within the complex.

**Table 5.16:** Summary of cockroach taxa recorded from the current survey.

| Family       | Species                               | Site        | Number |
|--------------|---------------------------------------|-------------|--------|
| Nocticolidae | ?Nocticola sp. 'West Pilbara Complex' | RC12MEH0221 | 1      |
|              |                                       | RC16MEH0427 | 1      |
|              |                                       | Total       | 2      |



**Figure 5.14:** Excerpt of Bayesian analysis of CO1 haplotypes of Blattodea recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).

(Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the current study are highlighted in yellow. Red boxes with dashed lines indicate groups requiring further investigation.

5.3.8 Order Coleoptera (Beetles)

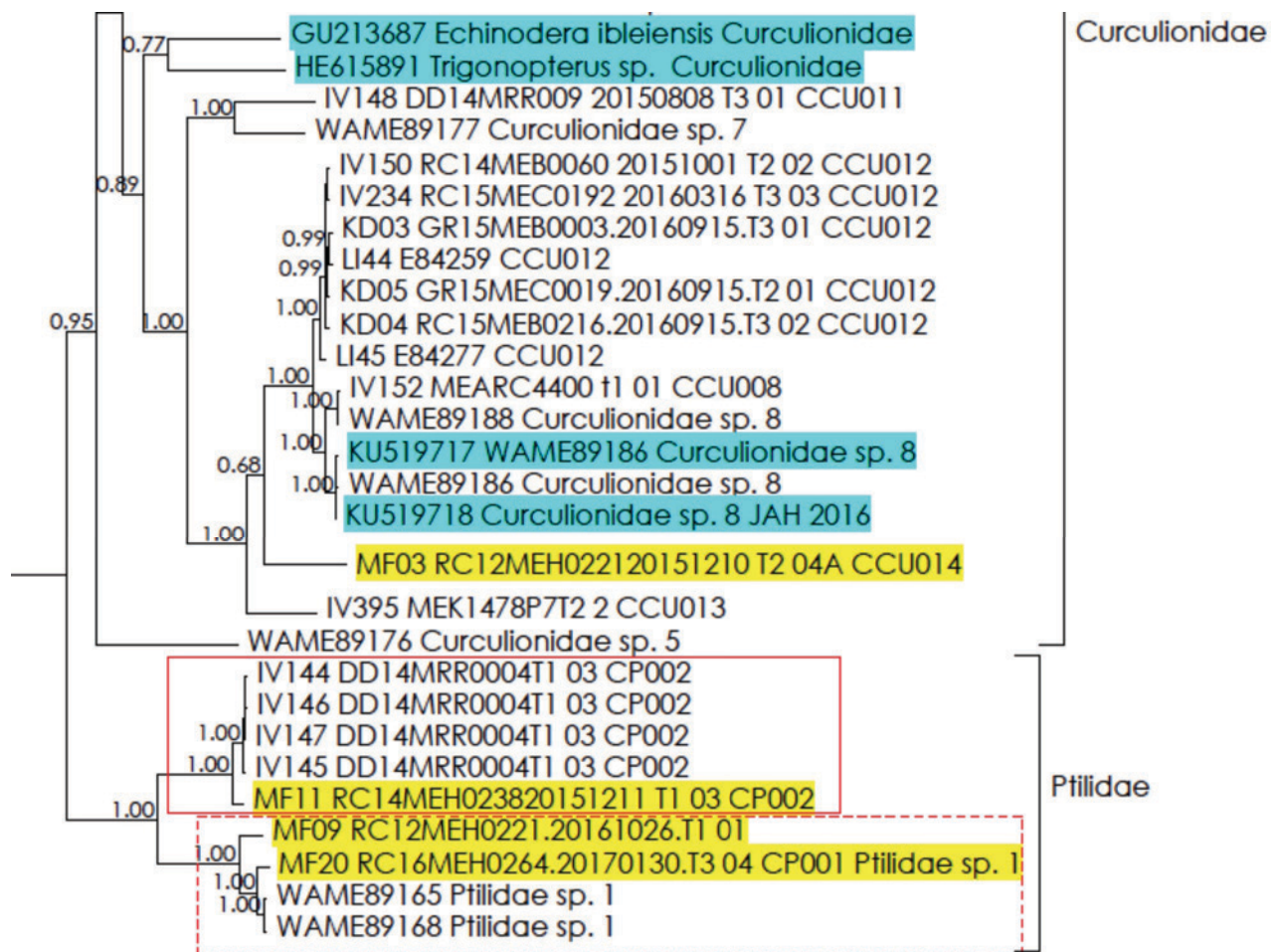
Eighteen specimens of Coleoptera from Mesa H were sequenced, with the specimens found to represent four phylogenetic lineages (Appendix 3). These correspond to three putative species, representing two families (Table 5.17). Two of the specimens could not be assigned to a lineage due to failure to sequence.

Curculionidae sp. 'CCU014' is a new species currently only known from the development envelope, but was recorded from three locations (Table 5.17). The remaining two beetle species are known from outside of the development envelope: Ptilidae sp. 'Robe Valley' was only recorded from the development envelope during the current survey, but it has previously been recorded elsewhere in the Robe Valley (lineage CP002 on Figure 5.15). Although the last taxon, Ptilidae sp. 'CP003' was recorded solely from site RC12MEH0221 within the development envelope, it is likely to represent the same species as Ptilidae sp. 1, as divergence from that taxon was only 4.4% (Figure 5.15). Ptilidae sp. 1 has been previously recognised by WAM from outside of the development envelope.

**Table 5.17:** Summary of beetle taxa recorded from the current survey.

| Family        | Species                            | Site                     | Number |
|---------------|------------------------------------|--------------------------|--------|
| Curculionidae | Curculionidae sp. 'CCU014'         | RC12MEH0221              | 4      |
|               |                                    | RC14MEH0252              | 1      |
|               |                                    | RC15MEH0302              | 1      |
| Ptilidae      | Ptilidae sp. 1/ 'CP003'            | RC12MEH0221, RC16MEH0264 | 34     |
|               | Ptilidae sp. 'Robe Valley'/'CP002' | RC14MEH0238              | 10     |
| Total         |                                    |                          | 50     |





**Figure 5.15:** Excerpt of Bayesian analysis of CO1 haplotypes of Coleoptera recorded from the Mesa H survey area (see Appendix 3 for complete phylogenetic tree).

(Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the current study are highlighted in yellow. GenBank specimens are highlighted in turquoise. Red boxes enclose species with high confidence. Red boxes with dashed lines indicate groups requiring further investigation.

### 5.3.9 Order Zygentoma (Silverfish)

Two troglobitic silverfish specimens, representing two different species, were recorded from Mesa H (Table 5.18). Both taxa were recorded from only within the development envelope (Figure 5.16).

**Table 5.18:** Summary of silverfish taxa recorded from the current survey.

| Family       | Species                  | Site        | Number |
|--------------|--------------------------|-------------|--------|
| Nicoletiinae | Nicoletiinae sp. 'TN019' | RC16JIM0006 | 1      |
|              | Nicoletiinae sp. 'TN020' | RC14MEH0252 | 1      |
| Total        |                          |             | 2      |



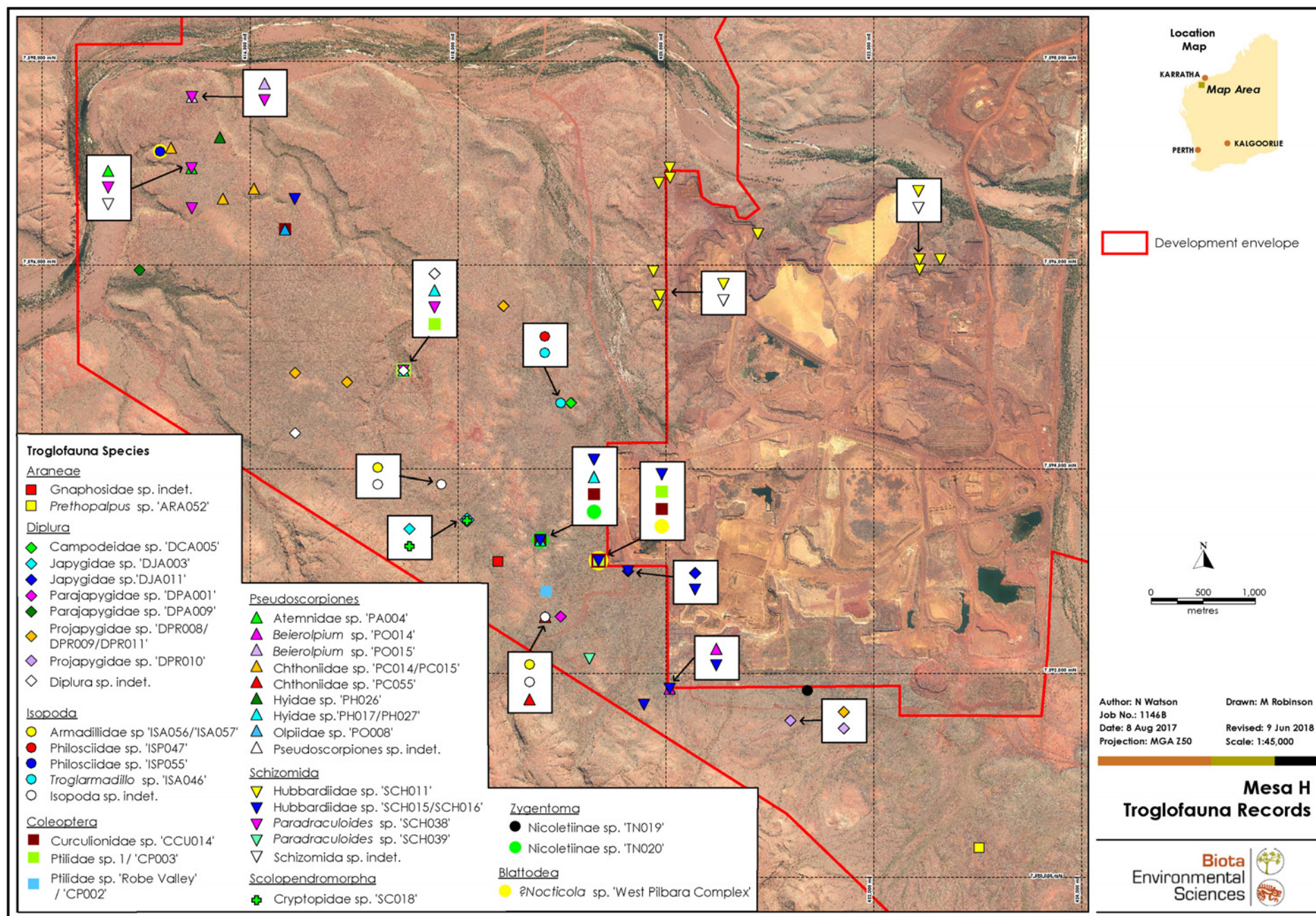


Figure 5.16: Troglofauna taxa recorded from Mesa H during the current survey.

## 6.0 Discussion

### 6.1 Known Subterranean Assemblage

#### 6.1.1 Stygofauna

At least 43 species of stygofauna were recorded during the current survey (Section 5.1), with an additional 13 species-level taxa known from previous records (Section 4.4.1), bringing the total known assemblage of the survey area to 56 species (removing duplicated species in common to both the survey and the desktop review data set, and excluding indeterminate specimens).

Forty-six species occur in the immediate Mesa H locality (Rio Tinto 2019), and a subset of 32 of these species occurs within the drawdown extent: 14 recorded during the current survey plus an additional 18 from the desktop review (again, excluding indeterminate specimens and removing overlaps in the two data sets). The majority of these species (26 out of 32 taxa; 81%) are either widespread regionally or have records outside of the drawdown extent. The remaining seven taxa that are currently only known from the drawdown extent are not formally listed as being of conservation significance but are potential SRE taxa, the majority of which are represented by single specimens (see Section 6.3.1).

#### 6.1.2 Troglofauna

Thirty-two troglobitic taxa were recorded from the Mesa H development envelope during the current survey, which, with one additional desktop taxon<sup>1</sup> (Section 4.4.2), brings the known total fauna of the development envelope to at least 33 troglobitic species.

Six of the 33 taxa have been recorded from outside of the development envelope and have demonstrated wider distributions, with the remaining 27 taxa currently only known from within the development envelope. Twenty of these latter were singleton taxa, making comment on their potential wider distributions difficult, but they have been retained as potential SRE taxa here to provide a precautionary assessment.

### 6.2 Conservation Significant Fauna

#### 6.2.1 Stygofauna

No stygofauna specimens of conservation significance (i.e. listed as Priority, Schedule or Vulnerable at State or Federal levels) were collected during the current survey. Records of *Ophisternon candidum* were, however, obtained from eDNA analysis of environmental samples (Section 5.2).

Specimens of the Blind Cave Eel have now been collected from three sites in the Jimmawurruda and Bungaroo Creeks locality (BC186, JW023 and JW024; Figure 6.1), and a recent additional site on the Robe River immediately north of Mesa H (Biota 2018), in addition to occurring at Cape Range (Section 4.4.1.1).

Sampling targeting this species was conducted during this study using eDNA techniques (Section 3.4). This resulted in the detection of *Ophisternon* DNA at five additional locations, both along Jimmawurruda Creek and the Robe River, including at one site within the development envelope and two to the immediate southeast (Figure 6.1). This indicates that the species occurs more widely in the locality, having been recorded from nine locations in total, and it probably occurs in association with the major alluvial aquifers of the Bungaroo-Jimmawurruda-Robe system (Biota 2018).

<sup>1</sup> *Paradraculoides* sp. nov. 'Mesa H' was also identified from the Development Envelope during the desktop review in Section 4.4.2, but subsequent sequencing reassigned those historical specimens to other Schizomida taxa (Section 5.3.4), removing it from this final tally.



Two Amphipoda species of conservation significance have been recorded from multiple locations within the Mesa H drawdown extent during past surveys: *Nedsia hurlberti* and *Nedsia sculptilis*, both of which are Schedule 3 species collected during a survey at Bungaroo (Biota 2013) (Figure 6.1).

It is worth noting that these two conservation significant amphipod species recorded from within the Mesa H development envelope have been described morphologically with no genetic analysis completed as part of their formal description (Bradbury and Williams 1996), whereas specimens from this survey were identified based on molecular analysis. Multiple studies in the Pilbara have indicated that amphipod species described solely on the basis of morphology may represent species complexes, or multiple species (Biota 2015b). Equally, past difficulties have arisen with resolving Amphipoda taxonomic boundaries based on morphology alone, where differences in characters has actually represented variation within a species rather than delineating separate species (Biota 2001). The large number of specimens that are juvenile and damaged, and are therefore not informative to morphological analysis, compounds these issues, reducing the power of morphological approaches and limiting determinations of species distribution based on this.

Given these considerations, genetic analysis is likely to be more effective and objective approach to assigning species, reviewing distributions, and thereby assessing conservation significance of putative species within this group. It is possible that taxa identified in this way on a molecular basis during the current study may represent either *Nedsia hurlberti* or *N. sculptilis* but a fuller taxonomic and phylogeographic review would be required to adequately resolve this.

## 6.2.2 Troglotauna

No troglotauna of conservation significance (i.e. those listed as Priority, Schedule or Vulnerable at State or Federal levels) were recorded during the survey at Mesa H.

One species of conservation significance was recorded from the desktop assessment, the schizomid *Paradraculoides kryptus* (Schedule 3), which to date has been recorded solely from Mesa K. It is unlikely that this species would occur at Mesa H.

## 6.3 Potential SRE Fauna

### 6.3.1 Stygofauna

Twenty-nine stygofauna species recorded from the Mesa H survey area are not formally listed as being of conservation significance but are considered to represent potential SRE fauna (Table 6.1). Eleven of these taxa are singletons, making comment on their true wider distribution difficult (Table 6.1). Specimens belonging to one higher rank taxon were unable to be identified to species level (*Gomphodella* sp. indet.) but were still retained as potential SRE stygofauna given distributional patterns in other members of this genus (Biota 2015c).

Thirteen of the 29 potential SRE taxa have been recorded within the drawdown extent (Figure 6.1), with seven of these either also recorded from sites outside of the drawdown extent or known to occur more widely in the region due to previous surveys (Table 6.1).

The remaining six potential SRE taxa are currently only known from the drawdown extent (Hydrobiidae sp. 2, Candoninae sp. 'BOS541', *Parastenocaris* sp. 'B28', *Haptolana* sp. 'B01', Paramelitidae sp. 'AMP003' and Paramelitidae sp. 'AMP037') (Figure 6.1; Table 6.1).

**Table 6.1: Summary of conservation significant and potential SRE stygofauna species from the Mesa H survey area (combined desktop review and survey results; desktop records highlighted in grey shading; species in bold recorded from within the drawdown extent).**

| Species Name                                | Site Recorded with Drawdown Extent      | Distribution (km <sup>2</sup> ) | Collection Representation (Total number sites)* | New Species | Singleton Record | SRE Sub-categories <sup>^</sup>                             | Location of Records Outside Drawdown extent |
|---|---|---------------------------------|---|-------------|------------------|---|---|
| <b>CONSERVATION SIGNIFICANT FAUNA</b>       |   |                                 |   |             |                  |   |   |
| <u>Amphipoda</u>                            |   |                                 |   |             |                  |   |   |
| <i>Nedsia hurlberti</i>                     | JW021, JW011A                           | 169.3                           | Good (Good)                                     | -           | -                | -   | Bungaroo, Barrow Island                     |
| <i>Nedsia sculptilis</i>                    | JW021, JW011A                           | 19.1                            | Good (Good)                                     | -           | -                | -   | Bungaroo, Barrow Island                     |
| <u>Vertebrata</u>                           |   |                                 |   |             |                  |   |   |
| <i>Ophisternon candidum</i> <sup>Ψ</sup>    | JW021, JW023, JW024, MB17MEH0015, BC186 | 108.3                           | Poor (Poor)                                     | -           | -                | -   | RR1, Robe River (site 25), RRD2, Cape Range |
| <b>POTENTIAL SRE FAUNA</b>                  |   |                                 |   |             |                  |   |   |
| <u>Gastropoda</u>                           |   |                                 |   |             |                  |   |   |
| <b>Hydrobiidae sp. 2</b>                    | JW023                                   | -                               | Poor (Poor)                                     | X           | -                | <b>A. Data Deficient</b><br><b>C. Morphology Indicators</b> | -   |
| Hydrobiidae sp. 'B09'                       | -                                       | -                               | Poor (Poor)                                     | X           | -                | A. Data Deficient<br>C. Morphology Indicators               | MB17MEH0007                                 |
| <u>Ostracoda</u>                            |   |                                 |   |             |                  |   |   |
| <i>Gomphodella</i> sp.                      | -                                       | -                               | Poor (Poor)                                     | -           | -                | A. Data Deficient<br>C. Morphology Indicators               | RR1   |
| <i>Pierrecandona</i> sp. 'BOS576'           | -                                       | -                               | Poor (Poor)                                     | -           | -                | A. Data Deficient<br>C. Morphology Indicators               | RR1, Bungaroo, Waramboo                     |
| <b><i>Pilbaracandona</i> sp. 'BOS526'</b>   | JW024                                   | 90.2                            | <b>Poor (Poor)</b>                              | -           | -                | <b>A. Data Deficient</b><br><b>C. Morphology Indicators</b> | <b>Dave Bore, MB17MEH0007</b>               |
| <b><i>Areacandona</i> sp. 'BOS1039'</b>     | BC186                                   | -                               | <b>Poor (Poor)</b>                              | X           | -                | <b>A. Data Deficient</b><br><b>C. Morphology Indicators</b> | <b>Robe River (site 31)</b>                 |
| <i>Candonidae</i> sp. 'BOS577'              | -                                       | -                               | Poor (Poor)                                     | -           | -                | A. Data Deficient<br>C. Morphology Indicators               | RR1   |
| <i>Candonidae</i> sp. 'BOS1037'             | -                                       | -                               | Poor (Poor)                                     | X           | -                | A. Data Deficient<br>C. Morphology Indicators               | MB17MEH0007                                 |
| <b><i>Candonidae</i> sp. 'BOS541'</b>       | JW024                                   | -                               | <b>Poor (Poor)</b>                              | X           | X                | <b>A. Data Deficient</b><br><b>C. Morphology Indicators</b> | -   |
| <u>Copepoda</u>                             |   |                                 |   |             |                  |   |   |
| <b><i>Megastygonoicrella unispinosa</i></b> | MB17MEH0015                             | 5.2                             | <b>Poor (Poor)</b>                              | -           | -                | <b>A. Data Deficient</b>                                    | <b>Robe River valley</b>                    |
| <b><i>Parastenocaris</i> sp. 'B28'</b>      | JW023                                   | -                               | <b>Poor (Poor)</b>                              | X           | X                | <b>A. Data Deficient</b>                                    | -   |
| <u>Isopoda</u>                              |   |                                 |   |             |                  |   |   |
| <i>Haptolana yarraloola</i>                 | -                                       | 107.4                           | Poor (Poor)                                     | -           | -                | A. Data Deficient   | RR1, Yarraloola, Budgie Bore                |
| <b><i>Haptolana</i> sp. 'B01'</b>           | JW024                                   | -                               | <b>Poor (Poor)</b>                              | X           | X                | <b>A. Data Deficient</b><br><b>C. Morphology Indicators</b> | -   |

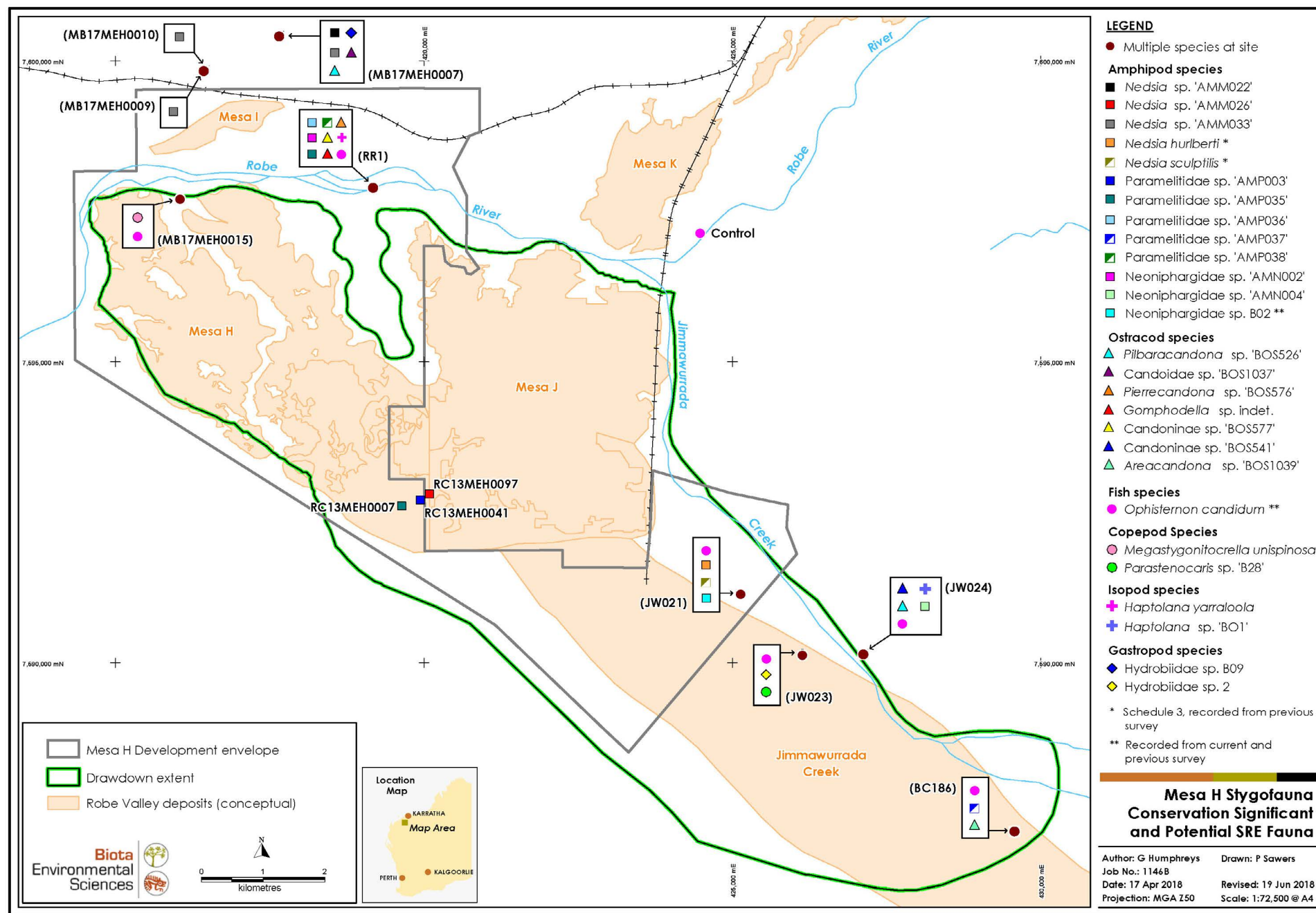
| Species Name   | Site Recorded with Drawdown Extent | Distribution (km <sup>2</sup> ) | Collection Representation (Total number sites)* | New Species | Singleton Record | SRE Sub-categories <sup>^</sup>                    | Location of Records Outside Drawdown extent |
|--|------------------------------------|---------------------------------|---|-------------|------------------|--|---|
| <u>Amphipoda</u>                                     |                                    |                                 |   |             |                  |  |   |
| <i>Nedsia</i> sp. 'AMM004'                           | -                                  | -                               | Poor (Poor)                                     | -           | -                | A. Data Deficient<br>D. Molecular Evidence         | 87, Dave Bore, MB16MEC0009                  |
| <i>Nedsia</i> sp. 'AMM006'                           | -                                  | -                               | Poor (Poor)                                     | -           | -                | A. Data Deficient<br>D. Molecular Evidence         | MB16MEC0008, MB16MEC0009                    |
| <i>Nedsia</i> sp. 'AMM022'                           | -                                  | -                               | Poor (Poor)                                     | -           | X                | A. Data Deficient<br>D. Molecular Evidence         | MB17MEH0007                                 |
| <b><i>Nedsia</i> sp. 'AMM026'</b>                    | <b>RC13MEH0097</b>                 | -                               | <b>Poor (Poor)</b>                              | <b>X</b>    |                  | <b>A. Data Deficient<br/>D. Molecular Evidence</b> | <b>Robe River (site 31)</b>                 |
| <i>Nedsia</i> sp. 'AMM032'                           | -                                  | -                               | Poor (Poor)                                     | X           | X                | A. Data Deficient<br>D. Molecular Evidence         | Robe River (site 31)                        |
| <i>Nedsia</i> sp. 'AMM033'                           | -                                  | -                               | Poor (Poor)                                     | X           | -                | A. Data Deficient<br>D. Molecular Evidence         | MB17MEH0007, MB17MEH0009, MB17MEH0010       |
| Neoniphargidae sp. 'AMN002'                          | -                                  | -                               | Poor (Poor)                                     | X           | X                | A. Data Deficient<br>D. Molecular Evidence         | RR1   |
| Neoniphargidae sp. 'AMN003'                          | -                                  | -                               | Poor (Poor)                                     | X           | X                | A. Data Deficient<br>D. Molecular Evidence         | Robe River (site 32)                        |
| Neoniphargidae sp. 'AMN008'                          | -                                  | -                               | Poor (Poor)                                     | X           | -                | A. Data Deficient<br>D. Molecular Evidence         | Dave Bore, Budgie Bore                      |
| <b><i>Wesniphargus</i> sp. 'AMN004' <sup>Ψ</sup></b> | <b>JW024</b>                       | -                               | <b>Poor (Poor)</b>                              | -           | -                | <b>A. Data Deficient<br/>D. Molecular Evidence</b> | <b>Robe River (site 25)</b>                 |
| <b>Neoniphargidae sp. 'B02' <sup>Ψ</sup></b>         | <b>JW021</b>                       | -                               | <b>Poor (Poor)</b>                              | -           | -                | <b>A. Data Deficient</b>                           | <b>Robe River (site 32)</b>                 |
| <b>Paramelitidae sp. 'AMP003'</b>                    | <b>RC13MEH0041</b>                 | -                               | <b>Poor (Poor)</b>                              | <b>X</b>    | <b>X</b>         | <b>A. Data Deficient<br/>D. Molecular Evidence</b> | -   |
| <b>Paramelitidae sp. 'AMP035'</b>                    | <b>RC13MEH0007</b>                 | -                               | <b>Poor (Poor)</b>                              | <b>X</b>    | <b>X</b>         | <b>A. Data Deficient<br/>D. Molecular Evidence</b> | RR1   |
| Paramelitidae sp. 'AMP036'                           | -                                  | -                               | Poor (Poor)                                     | X           | X                | A. Data Deficient<br>D. Molecular Evidence         | RR1   |
| <b>Paramelitidae sp. 'AMP037'</b>                    | <b>BC186</b>                       | -                               | <b>Poor (Poor)</b>                              | <b>X</b>    | <b>X</b>         | <b>A. Data Deficient<br/>D. Molecular Evidence</b> | -   |
| Paramelitidae sp. 'AMP038'                           | -                                  | -                               | Poor (Poor)                                     | X           | -                | A. Data Deficient<br>D. Molecular Evidence         | RR1, Robe River (site 25)                   |

<sup>Ψ</sup> Same taxa recorded during current survey and previous nearby surveys.

\* Poor: <10 specimens/sites, Fair: 10 – 25, Good: >25 (estimated from available data).

<sup>^</sup> A. Data Deficient, B. Habitat Indicators, C. Morphology indicators, D. Molecular Evidence, E. Research and Expertise (more detail in Appendix 4).





**Figure 6.1:** Distribution of conservation significant and potential SRE stygofauna relative to the Mesa H drawdown extent, including specimen and eDNA records of *Ophisternon candidum*, from both the current survey and past records.

### 6.3.2 Troglafauna

Specimens belonging to five higher rank troglobitic taxa could not be identified to species level (Diplura sp. indet., Pseudoscorpiones sp. indet., Isopoda sp. indet., Schizomida sp. indet. and Gnaphosidae sp. indet. (Section 4.4.2)). While it is highly likely that these specimens represent range-restricted species, it is not possible to reconcile whether they represent the same species as other records of their respective groups from within or outside the development envelope. The specimens still represent spatial records of potential SRE troglafauna (Figure 6.2), but most have not been recognised as distinct taxa in our conclusions (and are therefore not listed in Table 6.2). The only exception to this is the spider Gnaphosidae sp. indet. as this was the only specimen of its family and is clearly a discrete troglobitic taxon (Section 5.3.6) (Table 6.2).

Excluding indeterminate records, a total of 32 troglobitic taxa were recorded during the survey at Mesa H and all are regarded as potential SRE fauna (Figure 6.2; Table 6.2). Six of the potential SRE taxa have been recorded from both inside and outside of the development envelope: the schizomid species Hubbardiidae sp. 'SCH011' and Hubbardiidae sp. 'SCH015/SCH016', the isopod species Armadillidae sp. 'ISA056/ISA057', the blattodean *Nocticola* sp. 'West Pilbara Complex', and the beetles Ptilidae sp. 1/'CP003' and P. sp. 'Robe Valley'/'CP002' (Table 6.2).

The remaining 26 taxa recorded during the survey have been recorded solely from within the Mesa H development envelope based on current data (Figure 6.2; Table 6.2).

One additional potential SRE species recorded from previous surveys (Biota 2011c) occurs solely within the development envelope (Section 4.4.2); the isopod *Troglarmadillo* sp. 1, which is considered to represent a potential SRE species (Figure 6.2 and Table 6.2). It is probable that it represents the same species as other *Troglarmadillo* taxa recorded during the current survey.



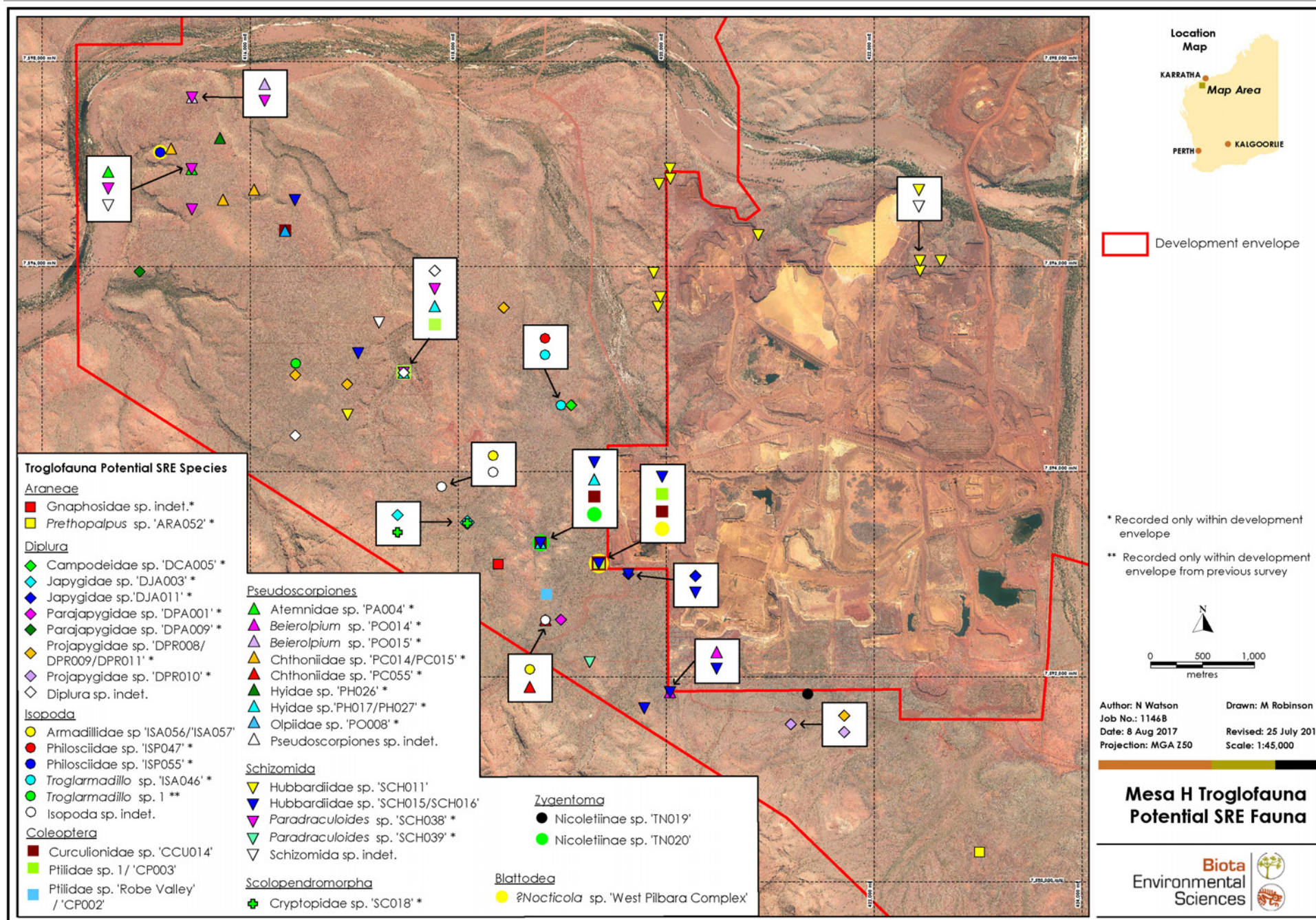


Figure 6.2: Potential SRE troglofauna recorded within the Mesa H development envelope (combined desktop review and survey results).



**Table 6.2: Summary of potential SRE troglofauna species recorded within Mesa H development envelope (combined desktop review and survey results; desktop records highlighted in grey shading).**

| Species Name                     | Site Recorded  | Distribution (km <sup>2</sup> ) | Collection Representation (Total number of sites) * | New Species | Singleton Record | SRE Sub-categories <sup>Λ</sup>            | Location of records outside Development Envelope |
|----------------------------------|--|---------------------------------|---|-------------|------------------|--|--|
| <b>POTENTIAL SRE FAUNA</b>       |  |                                 |   |             |                  |  |  |
| <u>Pseudoscorpiones</u>          |  |                                 |   |             |                  |  |  |
| Hyidae sp. 'PH017/PH027'         | RC14MEH0252, RC16MEH0264   | -                               | Poor (Poor)   | X           | -                | A. Data Deficient<br>D. Molecular Evidence | -  |
| Hyidae sp. 'PH026'               | RC16MEH0436  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence | -  |
| Olpiidae sp. 'PO008'             | RC15MEH0302  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence | -  |
| Beierolpium sp. 'PO014'          | RC16JIM0026  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence | -  |
| Beierolpium sp. 'PO015'          | RC16MEH0433  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence | -  |
| Atemnidae sp. 'PA004'            | RC15MEH0329  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence | -  |
| Chthoniidae sp. 'PC014/PC015'    | GR15MEH0015, RC15MEH0315, RC15MEH0335  | 0.1                             | Poor (Poor)   | X           | -                | A. Data Deficient<br>D. Molecular Evidence | -  |
| Chthoniidae sp. 'PC055'          | MEHRD0834  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence | -  |
| <u>Schizomida</u>                |  |                                 |   |             |                  |  |  |
| Paradraculoides sp. 'SCH038'     | MEHRC0957, RC15MEH0329, RC16MEH0264, RC16MEH0433   | 1.1                             | Poor (Poor)   | X           | -                | A. Data Deficient<br>D. Molecular Evidence | -  |
| Paradraculoides sp. 'SCH039'     | RC15MEJ0019  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence | -  |
| Hubbardiidae sp. 'SCH011'        | J0866, J0867, J0928, MEJ0007, MEJ0048, MEJ0076, MEJ0085, J0866, MEJ5548alt, RC08MEJ0015, RC08MEJ0004, RC08MEJ0023, MEHRC0724 | 2.0                             | Fair (Fair)   | -           | -                | A. Data Deficient<br>D. Molecular Evidence | Mesa J   |
| Hubbardiidae sp. 'SCH015/SCH016' | DD13MEH0007, RC12MEH0221, RC14MEH0252, RC15MEH0306, RC16JIM0026, RC16JIM0031, MEHRD0870                                      | 2.0                             | Poor (Poor)   | -           | -                | A. Data Deficient<br>D. Molecular Evidence | Redgate, 6 km SW                                 |
| <u>Myriapoda</u>                 |  |                                 |   |             |                  |  |  |
| Cryptopidae sp. 'SC018'          | RC14MEH0329  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence | -  |
| <u>Diplura</u>                   |  |                                 |   |             |                  |  |  |
| Parajapygidae sp. 'DPA001'       | RC14MEH0308  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence | -  |

| Species Name                            | Site Recorded                                      | Distribution (km <sup>2</sup> ) | Collection Representation (Total number of sites) * | New Species | Singleton Record | SRE Sub-categories <sup>^</sup>                | Location of records outside Development Envelope |
|---|--|---------------------------------|---|-------------|------------------|--|--|
| Parajapygidae sp. 'DPA009'              | MEHRD0758  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Projapygidae sp. 'DPR008/DPR009/DPR011' | RC15MEH0166, RC15MEH0175, RC15MEH0261, RC16JIM0005 | 5.0                             | Poor (Poor)   | X           | -                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Projapygidae sp. 'DPR010'               | RC16JIM0005  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Japygidae sp. 'DJA003'                  | RC14MEH0388  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Japygidae sp. 'DJA011'                  | DD13MEH0007  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Campodeidae sp. 'DCA005'                | GR15MEH0032  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| <u>Isopoda</u>                          |  |                                 |   |             |                  |  |  |
| Philosciidae sp. 'ISP047'               | RC15MEH0382  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Philosciidae p. 'ISP055'                | RC15MEH0427  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Troglarmadillo sp. 1                    | MEHDC0865  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>C. Morphological Evidence | -  |
| Troglarmadillo sp. 'ISA046'             | RC15MEH0382  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Armadillidae sp. 'ISA056/ISA057'        | MEHRD0577, MEHRD0834                               | -                               | Poor (Poor)   | -           | -                | A. Data Deficient<br>D. Molecular Evidence     | Mesa C   |
| <u>Coleoptera</u>                       |  |                                 |   |             |                  |  |  |
| Curculionidae sp. 'CCU014'              | RC12MEH0221, RC14MEH0252, RC15MEH0302              | 0.7                             | Poor (Poor)   | X           | -                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Ptilidae sp. 1/'CP003'                  | RC16MEH0264, RC12MEH0221                           | 17.1                            | Good (Poor)   | -           | -                | A. Data Deficient<br>D. Molecular Evidence     | Warramboo  |
| Ptilidae sp. 'Robe Valley'/'CP002'      | RC14MEH0238  | 19.3                            | Good (Poor)   | -           | -                | A. Data Deficient<br>D. Molecular Evidence     | Robe Valley, Middle Robe                         |
| <u>Zygentoma</u>                        |  |                                 |   |             |                  |  |  |
| Nicoletiinae sp. 'TN019'                | RC16JIM0006  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| Nicoletiinae sp. 'TN020'                | RC14MEH0252  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>D. Molecular Evidence     | -  |
| <u>Araneae</u>                          |  |                                 |   |             |                  |  |  |
| <i>Prethopalpus</i> 'ARA052'            | RC16JIM0019  | -                               | Poor (Poor)   | X           | X                | E. Research and Expertise                      | -  |
| Gnaphosidae sp. indet.                  | RC14MEH0270  | -                               | Poor (Poor)   | X           | X                | A. Data Deficient<br>E. Research and Expertise | -  |

| Species Name                                  | Site Recorded            | Distribution (km <sup>2</sup> ) | Collection Representation (Total number of sites) * | New Species | Singleton Record | SRE Sub-categories ^                       | Location of records outside Development Envelope |
|---|--------------------------|---------------------------------|---|-------------|------------------|--|--|
| <u>Blattodea</u>                              |                          |                                 |   |             |                  |  |  |
| ? <i>Nocticola</i> sp. 'West Pilbara Complex' | RC12MEH0221, RC16MEH0427 | 114.9                           | Poor (Fair)   | -           | -                | A. Data Deficient<br>D. Molecular Evidence | Red Hill, 60 km S                                |

\* Poor: <10 specimens/sites, Fair: 10 – 25, Good: >25 (estimated from available data).

^ A. Data Deficient, B. Habitat Indicators, C. Morphology indicators, D. Molecular Evidence, E. Research and Expertise (more detail in Appendix 4).



## 7.0 Glossary

|                            |   |
|----------------------------|---|
| Conservation significance  | Listed as Priority, Schedule or Vulnerable at State level.  |
| Edaphobitic / Edaphobite   | Soil-dwelling fauna that can often display troglomorphic characteristics. Edaphobites are unlikely to have limited distributions and are therefore unlikely to be classified as short-range endemics. |
| EPA                        | Environmental Protection Authority of Western Australia.  |
| Interstices                | An opening or space, especially a small or narrow one between mineral grains in a rock or within sediments or soil.   |
| Karst                      | Soluble-rock landscape; terrain with distinctive hydrology and landforms arising from a combination of high rock solubility and well-developed secondary porosity (Wilkins et al. 2000).              |
| Short-Range Endemic (SRE)  | A species that has a naturally small distribution and is often characterised by having poor dispersal capabilities, confinement to disjunct habitats and low fecundity.                               |
| Stygobite / Stygofauna     | Obligate groundwater fauna.   |
| Taxonomy                   | Theory and practice of biological classification.   |
| Troglobite / Troglifauna   | Species living obligatory in caves; also blind, depigmented and often having an elongate body morphology.   |
| Troglomorphic              | Pertaining to morphological, behavioural and physiological characters that are convergent in subterranean populations (Christiansen 1962).  |
| Troglophilic / Troglophile | Species able to live and reproduce underground as well as in the epigeal environment (Wilkins et al. 2000).   |
| Vug                        | A small cavity in a rock or vein, often with a mineral lining of different composition from that of the surrounding rock.   |

This page intentionally blank.

## 8.0 References

- Aplin, K. P. (1998). Three new blindsnakes (Squamata: Typhlopidae) from northwestern Australia. *Records of the Western Australian Museum* 19:1–12.
- Biota (2001). Orebody 23 Stygofauna Investigations: Morphological and Molecular Variation. Unpublished report prepared for BHP Iron Ore Pty Ltd, July 2001, Biota Environmental Sciences, Western Australia.
- Biota (2004). Mesa A and Bungaroo Creek Exploration Areas Subterranean Fauna Survey. Unpublished report prepared for Robe River Iron Mining Company, August 2004, Biota Environmental Sciences, Western Australia.
- Biota (2006a). Mesa A and Robe Valley Mesas Troglobitic Fauna Survey. Unpublished report prepared for Robe River Iron Associates, March 2006, Biota Environmental Sciences, Western Australia.
- Biota (2006b). Bungaroo Trial Pit Stygofauna Assessment. Unpublished report prepared for Robe River Iron Associates, December 2006, Biota Environmental Sciences, Western Australia.
- Biota (2007). Mesa K Remnant Mining Project Troglobitic Fauna Survey. Unpublished report prepared for Pilbara Iron, June 2007, Biota Environmental Sciences, Western Australia.
- Biota (2009). Mesa J Extension Troglifauna Survey. Unpublished report prepared for Pilbara Iron Company Pty Ltd, November 2009, Biota Environmental Sciences, Western Australia.
- Biota (2010a). Yandicoogina Subterranean Fauna Assessment Phases I - V. Unpublished report prepared for Rio Tinto Iron Ore, December 2010, Biota Environmental Sciences, Western Australia.
- Biota (2010b). Bungaroo Creek Subterranean Fauna Summary Phases I - VII. Unpublished report prepared for Rio Tinto Iron Ore, November 2010, Biota Environmental Sciences, Western Australia.
- Biota (2010c). West Pilbara Iron Ore Project Stygofauna Assessment. Unpublished report prepared for API Management, March 2010, Biota Environmental Sciences, Western Australia.
- Biota (2010d). West Pilbara Iron Ore Project Troglobitic Fauna Assessment. Unpublished report prepared for API Management, April 2010, Biota Environmental Sciences, Western Australia.
- Biota (2010e). Bungaroo Creek Subterranean Fauna Summary Phases 1 - 7. Unpublished Report for Rio Tinto Iron Ore Pty Ltd, Biota Environmental Sciences.
- Biota (2011a). WPIOP Basalt Quarry Troglobitic Fauna Pilot Survey Results. Unpublished letter report prepared for API Management, 21 January 2011, Biota Environmental Sciences, Western Australia.
- Biota (2011b). Mesa K Troglobitic Fauna Compliance Report 2010. Unpublished report prepared for Rio Tinto Iron Ore, February 2011, Biota Environmental Sciences, Western Australia.
- Biota (2011c). Robe Valley Mesas Troglobitic Fauna Survey 2010. Unpublished report prepared for Rio Tinto Iron Ore, March 2011, Biota Environmental Sciences, Western Australia.
- Biota (2012a). Mesa K Troglobitic Fauna Compliance Report 2011. Unpublished report prepared for Rio Tinto, February 2012, Biota Environmental Sciences, Western Australia.
- Biota (2012b). Mesa K Troglobitic Fauna Compliance Monitoring 2012. Unpublished report prepared for Rio Tinto, December 2012, Biota Environmental Sciences, Western Australia.
- Biota (2013). Bungaroo Subterranean Fauna Collections Summary; Phases 1 - 11. Unpublished report prepared for Rio Tinto, March 2013, Biota Environmental Sciences, Western Australia.



- Biota (2014). Bungaroo Coastal Waters Project Stygofauna Monitoring 2014. Unpublished report prepared for Rio Tinto, December 2014, Biota Environmental Sciences, Western Australia.
- Biota (2015a). Bungaroo Subterranean Fauna Desktop Assessment. Unpublished report prepared for Rio Tinto, June 2015, Biota Environmental Sciences, Western Australia.
- Biota (2015b). Yandi Operations Stygofauna Data Consolidation; 2003 - 2014. Unpublished report prepared for Rio Tinto, Biota Environmental Sciences.
- Biota (2015c). Yandi Operations Stygofauna Data Consolidation 2003 - 2014. Unpublished report prepared for Rio Tinto, April 2015, Biota Environmental Sciences, Western Australia.
- Biota (2016a). Bungaroo Coastal Waters Project Stygofauna Monitoring 2015. Unpublished report prepared for Pilbara Iron, Biota Environmental Sciences, Western Australia.
- Biota (2016b). Bungaroo Coastal Waters Project Stygofauna Monitoring 2016. Unpublished report prepared for Rio Tinto, Biota Environmental Sciences.
- Biota (2017). Mesa A Hub: Warrambo and Mesa C Stygofauna Assessment. Unpublished report prepared for Pilbara Iron, Biota Environmental Sciences, Western Australia.
- Biota (2018). Mesa H Subterranean Fauna Habitat and Impact Assessment. Unpublished report for Rio Tinto, Biota Environmental Sciences, Western Australia.
- Biota, and Helix (2012). Polyxenid Millipede Regional Molecular Analysis. Unpublished report prepared for Rio Tinto Iron Ore, December 2012, Biota Environmental Sciences and Helix Molecular Solutions.
- Biota, and Helix (2014). Blind Cave Eel Environmental DNA Pilot Study. Unpublished report prepared for Rio Tinto, .
- Bradbury, J. H., and W. D. Williams (1996). Freshwater Amphipods from Barrow Island, Western Australia. *Records of the Western Australian Museum* 48:33–74.
- Bruce, N. L. (2008). New species and a new genus of Cirolanidae (Isopoda: Cymothoidea: Crustacea) from groundwater in calcretes in the Pilbarra, northern Western Australia. *Zootaxa* 1823:51–64.
- Christiansen, K. (1962). Proposition for the Classification of Cave Animals. *Spelunca* 2:76–78.
- Cullen, K., and M. S. Harvey (2017). Molecular Analysis of Historical Specimens from the Robe Valley, Western Australia. Western Australian Museum.
- Cullen, K., M. S. Harvey, and J. M. Waldock (2017). Troglotic Spiders from Mesa H, Robe Valley, Western Australia. Troglotic Spiders from Mesa H, Robe Valley, Western Australia, Western Australian Museum.
- Culver, D. C., and T. Pipan (2009). *The Biology of Caves and Other Subterranean Habitats*. Oxford University Press.
- DEC (2013). Definitions, Categories and Criteria for Threatened and Priority Ecological Communities - SUPERCEDED. Species and Communities Branch, Department of Environment and Conservation, January 2013.
- Department of Parks and Wildlife (2016). Priority Ecological Communities for Western Australia, Version 26. Species and Communities Branch, Department of Parks and Wildlife, November 2016.
- DSEWPaC (2008). Approved Conservation Advice for *Ophisternon candidum* (Blind Cave Eel). *EPBC Act 1999*, s266B.

EPA (2016a). *Statement of Environmental Principles, Factors and Objectives*. Environmental Protection Authority, Western Australia.

EPA (2016b). *Environmental Factor Guideline: Subterranean Fauna*. Environmental Protection Authority, Western Australia.

EPA (2016c). *Technical Guidance: Subterranean Fauna Survey*. Environmental Protection Authority, Western Australia.

EPA (2016d). *Technical Guidance - Sampling methods for subterranean fauna*. Environmental Protection Authority, Western Australia.

Foster, R., and B. Humphreys (2011). Report on the genetic identification of a Blind Cave Eel collected near Pannawonica, Western Australia. Unpublished report prepared for Biota Environmental Sciences, 2011, Western Australian Museum.

Guzik, M. T., A. D. Austin, S. J. B. Cooper, M. S. Harvey, W. F. Humphrey, T. Bradford, S. M. Eberhard, R. A. King, R. Leys, K. A. Muirhead, and M. Tomlinson (2010). Is the Australian subterranean fauna uniquely diverse? *Invertebrate Systematics* 24:407–418.

Halse, S. A., M. D. Scanlon, J. S. Cocking, H. J. Barron, J. B. Richardson, and S. M. Eberhard (2014). Pilbara stygofauna: deep groundwater of an arid landscape contains globally significant radiation of biodiversity. *Records of the Western Australian Museum Supplement* 78:443–483.

Harvey, M. S., O. Berry, K. L. Edward, and G. Humphreys (2008). Molecular and morphological systematics of hypogean schizomids (Schizomida: Hubbardiidae) in semi-arid Australia. *Invertebrate Systematics* 22:1–28.

Hebert, P. D. N., A. Cywinska, S. Ball, and J. R. deWaard (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B* 270:313–321.

Hebert, P. D. N., S. Ratnasingham, and J. R. deWaard (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of The Royal Society of London Series B-Biological Sciences* 270:S96–S99.

Helix (2012). Report on the molecular systematics of Polyxenida from the Pilbara - update. Unpublished report prepared for Biota Environmental Sciences, 20 December 2012, Helix Molecular Solutions, Western Australia.

Helix (2016). Report on the molecular systematics of subfauna from Mesa H, Phase 2. Unpublished report prepared for Biota Environmental Sciences, Helix Molecular Solutions, Western Australia.

Helix (2017a). Report on the molecular systematics of Amphipoda from Mesa H - Phase 4. Unpublished report prepared for Biota Environmental Sciences, Helix Molecular Solutions, Western Australia.

Helix (2017b). Report on the molecular systematics of subfauna from Mesa H, Phases 4 - 6. Unpublished report prepared for Biota Environmental Sciences, Helix Molecular Solutions, Western Australia.

Helix (2018). Report for the analysis of environmental water samples collected during December 2017 for the presence of blind eel DNA. Unpublished report prepared for Biota Environmental Sciences, Helix Molecular Solutions, Western Australia.

Humphreys, G., J. Alexander, M. S. Harvey, and W. F. Humphreys (2013). The subterranean fauna of Barrow Island, northwestern Australia: 10 years on. *Records of the Western Australian Museum Supplement* 83:145–158.

Humphreys, W. F. (1989). Status of relict Cave Fauna of Cape Range, WA, especially the Schizomidae.

Humphreys, W. F. (1993). Cave fauna in semi-arid tropical Western Australia : a diverse relict wet-forest litter fauna. *Memoires de Biospeologie* 20:105–110.

Humphreys, W. F. (1999). Relict stygofaunas living in sea salt, karst and calcrete habitats in arid northwestern Australia contain many ancient lineages. Pages 219–227 in W. Ponder and D. Lumney, editors. *The Other 99% -The Conservation and Biodiversity of invertebrates*. Royal Zoological Society of New South Wales, Mosman.

Humphreys, W. F. (2001a). The subterranean fauna of Barrow Island, northwestern Australia, and its environment. *Memoires de Biospeologie (International Journal of Subterranean Biology)* 28:107–127.

Humphreys, W. F. (2001b). *Milyeringa veritas* (Eleotridae), a remarkably versatile cave fish from the arid tropics of northwestern Australia. *Environmental Biology of Fishes* 62:297–313.

Juberthie, C. (2000). Chapter 1: The Diversity of the Karstic and Pseudokarstic Hypogean Habitats in the World. Pages 17–40 in H. Wilkens, D. C. Culver, and W. F. Humphreys, editors. *Ecosystems of the World 30: Subterranean Ecosystems*. Elsevier Science, Amsterdam.

Karanovic, I. (2005). Towards a revision of Candoninae (Crustacea: Ostracoda): Australian representatives of the subfamily, with descriptions of three new genera and seven new species. *New Zealand Journal of Marine and Freshwater Research* 39:29–75.

Larson, H. K., R. Foster, W. F. Humphreys, and M. L. Stevens (2013). A new species of the blind cave gudgeon *Milyeringa* (Pisces: Gobioidae, Eleotridae) from Barrow Island, Western Australia, with a redescription of *M. veritas* Whitley. *Zootaxa* 3616(2):135–150.

Marmonier, P., P. Vervier, J. Giber, and M. J. Dole-Olivier (1993). Biodiversity in ground waters. *Trends in Ecology & Evolution* 8:392–395.

Page, T. J., J. M. Hughes, K. Real, M. I. Stevens, R. A. King, and W. F. Humphrey (2016). Allegory of a cave crustacean: systematic and biogeographic reality of Halosbaena (Peracarida: Thermosbaenacea) sought with molecular data at multiple scales. *Marine Biodiversity*:1–18.

Page, T. J., W. F. Humphreys, and J. M. Hughes (2008). Shrimps Down Under: Evolutionary Relationships of Subterranean Crustaceans from Western Australia (Decapoda: Atyidae: Stygiocaris). *PLoS One* 3:e1618. doi: 10.1371/journal.pone.0001618.

Ramanaidou, E. R., R. C. Morris, and R. C. Horwitz (2003). Channel iron deposits of the Hamersley Province, Western Australia. *Australian Journal of Earth Sciences* 50(5):669–690.

Rio Tinto (2019). Mesa H Project Environmental Review Document. Environmental Review Document, Rio Tinto, Perth.

Romero, A. (2009). *Cave Biology: Life in Darkness*. Cambridge University Press.

Wilkens, H., D. C. Culver, and W. F. Humphreys (Eds.) (2000). *Ecosystems of the World 30: Subterranean Ecosystems*. Elsevier Science, Amsterdam.



# Appendix 1

---

## Licence to Take Fauna





# DEPARTMENT OF PARKS AND WILDLIFE



Department of  
Parks and Wildlife



Enquiries:  
Telephone:  
Facsimile:

17 DICK PERRY AVE, KENSINGTON, WESTERN AUSTRALIA  
08 9219 9000  
08 9219 8242

Correspondence:

Locked Bag 30  
Bentley Delivery Centre WA 6983

PAGE 2  
NO. SF010536  
PERSON NO. 165096

## AUTHORISED PERSONS

ROY TEALE  
GARTH HUMPHREYS  
MICHAEL GREENHAM  
DANIEL KAMIEN  
JASON ALEXANDER  
TIM SACHSE  
JESSICA CAIRNES  
PENNY BROOSHOOFT  
DAVID KEIRLE  
VICKIE CARTLEDGE  
CHRIS COLE  
NICOLA WATSON  
SYLVIE SCHMIDT  
STEWART FORD  
JACINTA KING  
ANDREW SHEPPARD  
SCOTT WERNER

DATE OF ISSUE 24/09/2015  
VALID FROM 30/09/2015  
DATE OF EXPIRY 30/06/2016

  
LICENSING OFFICER

RESIDENTIAL ADDRESS: 22 HILLTOP PLACE  
KELMSCOTT WA 6111

LICENSEE: MR CF COLE  
ADDRESS C/- BIOTA ENVIRONMENTAL  
PO BOX 155  
LEEDERVILLE WA 6903

(CHRISTOPHER FITZPANE)

-----



# DEPARTMENT OF PARKS AND WILDLIFE



Department of  
Parks and Wildlife



Enquiries:  
Telephone:  
Facsimile:

17 DICK PERRY AVE, KENSINGTON, WESTERN AUSTRALIA  
08 9219 9000  
08 9219 8242

Correspondence:

Locked Bag 30  
Bentley Delivery Centre WA 6983

PAGE NO. 1  
PERSON NO. SF010536  
165096

RECEIPT NO. AMOUNT  
\$0.00

## WILDLIFE CONSERVATION ACT 1950 REGULATION 17

### LICENCE TO TAKE FAUNA FOR SCIENTIFIC PURPOSES

THE UNDERMENTIONED PERSON MAY TAKE FAUNA FOR RESEARCH OR OTHER SCIENTIFIC PURPOSES AND WHERE AUTHORISED, KEEP IT IN CAPTIVITY, SUBJECT TO THE FOLLOWING AND ATTACHED CONDITIONS, WHICH MAY BE ADDED TO, SUSPENDED OR OTHERWISE VARIED AS CONSIDERED FIT.

DIRECTOR GENERAL

#### CONDITIONS

- 1 The licensee shall comply with the provisions of the Wildlife Conservation Act 1950, Wildlife Conservation Regulations 1970 and any notices in force under this legislation.
- 2 Unless specifically authorised in the Conditions of this Licence or otherwise in writing by the Director General, species of fauna declared as likely to become extinct, rare or otherwise in need of special protection shall not be captured or otherwise taken.
- 3 No fauna shall be taken from any Nature Reserve, National Park, Marine Park, Marine Nature Reserve, Timber Reserve or State Forest without prior written approval of the Director General. No fauna shall be taken from any other public land without the written approval of the Government Authority managing that land.
- 4 No entry or collection of fauna to be undertaken on any private property or pastoral lease without the consent in writing of the owner or occupier, or from any Aboriginal lands without the written approval of the Department of Aboriginal Affairs.
- 5 No fauna or their progeny shall be released in any area where it does not naturally occur, nor be handed over to any other person or authority unless approved by the Director General, nor shall the remains of such fauna be disposed of in such manner as to confuse the natural or present day distribution of the species.
- 6 This licence and the written permission referred to at conditions 3 & 4 must be carried by the licensee or authorised agent at all times for the purpose of proving their authority to take fauna when questioned as to their right to do so by a Wildlife Officer, any other State or Local Government employee or any member of the public.
- 7 Any interaction involving Gazetted Threatened Fauna that may be harmful and/or invasive may require approval from the Department of the Environment ph 02 6274 1111. Interaction with such species is controlled by the Commonwealth Government's "Environment Protection and Biodiversity Conservation Act 1999" and "Environment Protection and Biodiversity Conservation Regulations 2000" as well as this Department's Wildlife Conservation Act 1950 and Wildlife Conservation Regulations 1970.
- 8 No bioprospecting involving the removal of sample aquatic and terrestrial organisms (both flora and fauna) for chemical extraction and bioactivity screening is permitted to be conducted without specific written approval by the Director General.
- 9 Further conditions (numbered 1 to 9) are attached.

#### PURPOSE

SUBTERRANEAN FAUNA SURVEY, USING REINFORCED STYGOFAUNA HAUL NETS AND TROGLOFAUNA COLONISATION TRAPS PLUS SCRAPING OF PLANKTON HAUL NETS, IN ACCORDANCE WITH EPA GUIDANCE STATEMENT 54A, FOR ENVIRONMENTAL IMPACT ASSESSMENT AT RIO TINTO MESAS J AND H 18KMS SOUTH WEST OF PANNAWONICA.

## WILDLIFE CONSERVATION REGULATIONS 1970

### Regulation 17:- Licence to Take Fauna for Scientific Purposes

FURTHER CONDITIONS (OF LICENCE NUMBER SF010536)

1. The licensee shall take fauna only in the manner stated on the endorsed Regulation 17 licence application form and endorsed related correspondence.
2. Except in the case of approved lethal traps, the licensee shall ensure that measures are taken in the capture and handling of fauna to prevent injury or mortality resulting from that capture or handling. Where traps or other mechanical means or devices are used to capture fauna these shall be deployed so as to prevent exposure of trapped animals to ants and debilitating weather conditions and inspected at regular intervals throughout each day of their use. At the conclusion of research all markers etc and signs erected by the licensee and all traps shall be removed, all pitfalls shall be refilled or capped and the study area returned to the condition it was in prior to the research/capture program. During any break in research, cage traps should be removed and pitfalls either removed, capped or filled with sand.
3. No collecting is to be undertaken in areas where it would impinge on pre-existing scientific research programs.
4. Any form of colour marking of birds or bats shall only be undertaken in accordance with the requirements of the Australian Bird and Bat Banding Scheme.
5. Any inadvertently captured specimen of fauna which is declared as likely to become extinct, rare or otherwise in need of special protection is to be released immediately at the point of capture. Where such a specimen is injured or deceased, the licensee shall contact Department of Parks and Wildlife licensing staff at Kensington (08 9219 9831) for advice on disposal. Records are to be kept of any fauna so captured and details included in the report required under further condition 6 below.
6. Within one month of the expiration of this licence, the holder shall submit an electronic return detailing the locality, site, geocode, date and number of each species captured, sighted or vouchered during the currency of the licence, into the Department of Parks and Wildlife Fauna Survey Database (FSD). A copy of any paper, report or thesis resulting from the research shall on completion be lodged with the Director General. If a renewal of this licence is required, the licensee shall submit a written progress report for activities undertaken during this licence period prior to the expiry of this licence.
7. Not more than ten specimens of any one protected species shall be taken and removed from any location less than 20km apart. Where exceptional circumstances make it necessary to take large series in order to obtain adequate statistical data the collector will proceed with circumspection and justify their actions to the Director General in advance.
8. All holotypes and syntypes and a half share of paratypes of species or subspecies permitted to be permanently taken under this licence shall be donated to the Western Australian Museum. Duplicates (one pair in each case) of any species collected which represents a significant extension of geographic range shall be donated on request to the Western Australian Museum.
9. To prevent any unnecessary collecting in this State, all specimens and material collected under the authority of this license shall, on request, be loaned to the Western Australian Museum. Also, the unused portion or portions of any specimen collected under the authority of this license shall be offered for donation to the Western Australian Museum or made available to other scientific workers if so required.



# DEPARTMENT OF PARKS AND WILDLIFE



Department of  
Parks and Wildlife



Enquiries:  
Telephone:  
Facsimile:

17 DICK PERRY AVE, KENSINGTON, WESTERN AUSTRALIA  
08 9219 9000  
08 9219 8242

Correspondence:

Locked Bag 30  
Bentley Delivery Centre WA 6983

**PAGE** 2  
**NO.** SF010915  
**PERSON NO.** 165096

## AUTHORISED PERSONS

ROY TEALE  
GARTH HUMPHREYS  
MICHAEL GREENHAM  
DANIEL KAMIEN  
JASON ALEXANDER  
TIM SACHSE  
JESSICA CAIRNES  
PENNY BROOSHOOFT  
DAVID KEIRLE  
VICKIE CARTLEDGE  
CHRIS COLE  
NICOLA WATSON  
SYLVIE SCHMIDT  
STEWART FORD  
JACINTA KING  
ANDREW SHEPPARD  
SCOTT WERNER

**DATE OF ISSUE** 11/07/2016  
**VALID FROM** 22/07/2016  
**DATE OF EXPIRY** 30/06/2017

  
**LICENSING OFFICER**

**RESIDENTIAL ADDRESS:** 22 HILLTOP PLACE  
KELMSCOTT WA 6111

**LICENSEE:** MR CF COLE  
**ADDRESS** C/- BIOTA ENVIRONMENTAL  
PO BOX 155  
LEEDERVILLE WA 6903

(CHRISTOPHER FITZPANE)

-----

# DEPARTMENT OF PARKS AND WILDLIFE



Department of  
Parks and Wildlife



Enquiries:  
Telephone:  
Facsimile:

17 DICK PERRY AVE, KENSINGTON, WESTERN AUSTRALIA  
08 9219 9000  
08 9219 8242

Correspondence:

Locked Bag 30  
Bentley Delivery Centre WA 6983

|             |                    |
|-------------|--------------------|
| PAGE NO.    | 1                  |
| PERSON NO.  | SF010915<br>165096 |
| RECEIPT NO. | AMOUNT<br>\$0.00   |

## WILDLIFE CONSERVATION ACT 1950 REGULATION 17

### LICENCE TO TAKE FAUNA FOR SCIENTIFIC PURPOSES

THE UNDERMENTIONED PERSON MAY TAKE FAUNA FOR RESEARCH OR OTHER SCIENTIFIC PURPOSES AND WHERE AUTHORISED, KEEP IT IN CAPTIVITY, SUBJECT TO THE FOLLOWING AND ATTACHED CONDITIONS, WHICH MAY BE ADDED TO, SUSPENDED OR OTHERWISE VARIED AS CONSIDERED FIT.

DIRECTOR GENERAL

#### CONDITIONS

- 1 The licensee shall comply with the provisions of the Wildlife Conservation Act 1950, Wildlife Conservation Regulations 1970 and any notices in force under this legislation.
- 2 Unless specifically authorised in the Conditions of this Licence or otherwise in writing by the Director General, species of fauna declared as likely to become extinct, rare or otherwise in need of special protection shall not be captured or otherwise taken.
- 3 No fauna shall be taken from any Nature Reserve, National Park, Marine Park, Marine Nature Reserve, Timber Reserve or State Forest without prior written approval of the Director General. No fauna shall be taken from any other public land without the written approval of the Government Authority managing that land.
- 4 No entry or collection of fauna to be undertaken on any private property or pastoral lease without the consent in writing of the owner or occupier, or from any Aboriginal lands without the written approval of the Department of Aboriginal Affairs.
- 5 No fauna or their progeny shall be released in any area where it does not naturally occur, nor be handed over to any other person or authority unless approved by the Director General, nor shall the remains of such fauna be disposed of in such manner as to confuse the natural or present day distribution of the species.
- 6 This licence and the written permission referred to at conditions 3 & 4 must be carried by the licensee or authorised agent at all times for the purpose of proving their authority to take fauna when questioned as to their right to do so by a Wildlife Officer, any other State or Local Government employee or any member of the public.
- 7 Any interaction involving Gazetted Threatened Fauna that may be harmful and/or invasive may require approval from the Department of the Environment ph 02 6274 1111. Interaction with such species is controlled by the Commonwealth Government's "Environment Protection and Biodiversity Conservation Act 1999" and "Environment Protection and Biodiversity Conservation Regulations 2000" as well as this Department's Wildlife Conservation Act 1950 and Wildlife Conservation Regulations 1970.
- 8 No bioprospecting involving the removal of sample aquatic and terrestrial organisms (both flora and fauna) for chemical extraction and bioactivity screening is permitted to be conducted without specific written approval by the Director General.
- 9 Further conditions (numbered 1 to 9) are attached.

#### PURPOSE

SUBTERRANEAN FAUNA SURVEY, USING REINFORCED STYGOFUNA HAUL NETS AND TROGLOFAUNA COLONISATION TRAPS PLUS SCRAPING OF PLANKTON HAUL NETS, IN ACCORDANCE WITH EPA GUIDANCE STATEMENT 54A, FOR ENVIRONMENTAL IMPACT ASSESSMENT AT RIO TINTO MESAS J AND H 18KMS SOUTH WEST OF PANNAWONICA.



# WILDLIFE CONSERVATION REGULATIONS 1970

## Regulation 17:- Licence to Take Fauna for Scientific Purposes

FURTHER CONDITIONS (OF LICENCE NUMBER SF 010915)

1. The licensee shall take fauna only in the manner stated on the endorsed Regulation 17 licence application form and endorsed related correspondence.
2. Except in the case of approved lethal traps, the licensee shall ensure that measures are taken in the capture and handling of fauna to prevent injury or mortality resulting from that capture or handling. Where traps or other mechanical means or devices are used to capture fauna these shall be deployed so as to prevent exposure of trapped animals to ants and debilitating weather conditions and inspected at regular intervals throughout each day of their use. At the conclusion of research all markers etc and signs erected by the licensee and all traps shall be removed, all pitfalls shall be refilled or capped and the study area returned to the condition it was in prior to the research/capture program. During any break in research, cage traps should be removed and pitfalls either removed, capped or filled with sand.
3. No collecting is to be undertaken in areas where it would impinge on pre-existing scientific research programs.
4. Any form of colour marking of birds or bats shall only be undertaken in accordance with the requirements of the Australian Bird and Bat Banding Scheme.
5. Any inadvertently captured specimen of fauna which is declared as likely to become extinct, rare or otherwise in need of special protection is to be released immediately at the point of capture. Where such a specimen is injured or deceased, the licensee shall contact Department of Parks and Wildlife licensing staff at Kensington (08 9219 9831) for advice on disposal. Records are to be kept of any fauna so captured and details included in the report required under further condition 6 below.
6. Within one month of the expiration of this licence, the holder shall submit an electronic return detailing the locality, site, geocode, date and number of each species captured, sighted or vouchered during the currency of the licence, into the Department of Parks and Wildlife Fauna Survey Database (FSD). A copy of any paper, report or thesis resulting from the research shall on completion be lodged with the Director General. If a renewal of this licence is required, the licensee shall submit a written progress report for activities undertaken during this licence period prior to the expiry of this licence.
7. Not more than ten specimens of any one protected species shall be taken and removed from any location less than 20km apart. Where exceptional circumstances make it necessary to take large series in order to obtain adequate statistical data the collector will proceed with circumspection and justify their actions to the Director General in advance.
8. All holotypes and syntypes and a half share of paratypes of species or subspecies permitted to be permanently taken under this licence shall be donated to the Western Australian Museum. Duplicates (one pair in each case) of any species collected which represents a significant extension of geographic range shall be donated on request to the Western Australian Museum.
9. To prevent any unnecessary collecting in this State, all specimens and material collected under the authority of this license shall, on request, be loaned to the Western Australian Museum. Also, the unused portion or portions of any specimen collected under the authority of this license shall be offered for donation to the Western Australian Museum or made available to other scientific workers if so required.

## Appendix 2

---

### Morphological Identifications by Bennelongia Environmental Consultants







| Phase | Site         | Date      | Class        | Subclass       | Order           | Suborder      | Superfamily | Family            | LowestID                                 | n  | IDBy | Sex  | LifeStage | Comments   |
|-------|--------------|-----------|--------------|----------------|-----------------|---------------|-------------|-------------------|--|----|------|------|-----------|--|
| 1     | RC13MEH0007  | 24-Oct-15 | Maxillopoda  | Copepoda       | Cyclopoida      |               |             | Cyclopidae        | <i>Diacyclops humphreysi humphreysi</i>  | 19 | JMM  |      |           |  |
| 1     | RC14MMEH0053 | 24-Oct-15 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 3  | JMM  |      |           |  |
| 1     | DD13MEH0007  | 24-Oct-15 | Maxillopoda  | Copepoda       | Cyclopoida      |               |             | Cyclopidae        | <i>Diacyclops humphreysi humphreysi</i>  | 1  | JMM  | Male | Adult     |  |
| 1     | DD13MEH0007  | 24-Oct-15 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 16 | JMM  |      |           |  |
| 2     | RC14MEH0018  | 19-Jan-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 1  | JMM  |      |           |  |
| 1     | RC13MEH0007  | 24-Oct-15 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 14 | JMM  |      |           |  |
| 3     | 200          | 05-May-16 | Ostracoda    | Popocopa       | Popocopida      | Cypridocopina | Cypridoidea | Candonidae        | <i>Pilbaracandona</i> sp. BOS526         | 1  | SAH  |      |           | BOS575   |
| 3     | RR1          | 05-May-16 | Ostracoda    | Popocopa       | Popocopida      | Cypridocopina | Cypridoidea | Candonidae        | <i>Pierrecandona</i> sp. BOS576          | 1  | SAH  |      |           | BOS576   |
| 3     | RR1          | 05-May-16 | Malacostraca | Eumalacostraca | Isopoda         |               |             | Cirolanidae       | <i>Haptolana yarraloola</i>              | 1  | JMM  |      |           |  |
| 3     | RR1          | 05-May-16 | Malacostraca | Eumalacostraca | Isopoda         |               |             | Cirolanidae       | <i>Kagalana tonde</i>                    | 1  | JMM  |      |           |  |
| 3     | RR1          | 05-May-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 10 | JMM  |      |           |  |
| 3     | RR1          | 05-May-16 | Maxillopoda  | Copepoda       | Cyclopoida      |               |             | Cyclopidae        | <i>Halicyclops roachi</i>                | 2  | JMM  |      |           |  |
| 3     | RR1          | 05-May-16 | Maxillopoda  | Copepoda       | Cyclopoida      |               |             | Cyclopidae        | <i>Diacyclops cockingi</i>               | 1  | JMM  |      |           |  |
| 3     | RR1          | 05-May-16 | Ostracoda    | Popocopa       | Popocopida      | Cypridocopina | Cypridoidea | Candonidae        | Candoninae n. gen sp. BOS577             | 3  | SAH  |      |           | BOS577   |
| 3     | 87           | 05-May-16 | Ostracoda    | Popocopa       | Popocopida      | Cypridocopina | Cypridoidea | Candonidae        | <i>Humphreyscandona imperfecta</i>       | 1  | SAH  | Male | Adult     | BOS578. male. species described from juvenile female at Mardie, fortescue River. prob same species |
| 3     | 32           | 06-May-16 | Malacostraca | Eumalacostraca | Amphipoda       |               |             | Neoniphargidae    | Neoniphargidae sp. B02 (nr Wesniphargus) | 1  | JMM  | Male | Adult     | also found at Bungaroo   |
| 3     | 31           | 07-May-16 | Maxillopoda  | Copepoda       | Cyclopoida      |               |             | Cyclopidae        | <i>Halicyclops roachi</i>                | 2  | JMM  |      |           |  |
| 3     | 31           | 07-May-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 4  | JMM  |      |           |  |
| 4     | 87           | 10-Sep-16 | Ostracoda    | Popocopa       | Popocopida      | Cypridocopina | Cypridoidea | Candonidae        | <i>Humphreyscandona imperfecta</i>       | 2  | SAH  |      |           | BOS805. see note on earlier id   |
| 4     | 200          | 10-Sep-16 | Ostracoda    | Popocopa       | Popocopida      | Cypridocopina | Cypridoidea | Candonidae        | <i>Pilbaracandona</i> sp. BOS526         | 1  | SAH  |      |           | BOS801   |
| 4     | 31           | 10-Sep-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 18 | JMM  |      |           |  |
| 4     | 34           | 10-Sep-16 | Maxillopoda  | Copepoda       | Cyclopoida      |               |             | Cyclopidae        | <i>Diacyclops</i> sp.                    | 2  | JMM  |      | Juvenile  |  |
| 4     | 34           | 10-Sep-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 2  | JMM  |      |           |  |
| 4     | 25           | 10-Sep-16 | Malacostraca | Eumalacostraca | Thermosbaenacea |               |             | Thermosbaenacidae | <i>Halosbaena tulki</i>                  | 1  | JMM  |      |           |  |
| 4     | DD13MEH0007  | 14-Sep-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 7  | JMM  |      |           |  |
| 4     | RC13MEH0040  | 14-Sep-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 1  | JMM  |      |           |  |
| 4     | RC13MEH0007  | 14-Sep-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 27 | JMM  |      |           |  |
| 4     | 25           | 10-Sep-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 1  | JMM  |      |           |  |
| 4     | 32           | 10-Sep-16 | Maxillopoda  | Copepoda       | Calanoida       |               |             | Ridgewayiidae     | <i>Stygoridgewayia trispinosa</i>        | 5  | JMM  |      |           |  |
| 4     | RR1          | 10-Sep-16 | Ostracoda    | Popocopa       | Popocopida      | Cypridocopina | Cypridoidea | Candonidae        | <i>Pierrecandona</i> sp. BOS576          | 1  | SAH  |      |           |  |
| 4     | RR1          | 10-Sep-16 | Ostracoda    | Popocopa       | Popocopida      | Cytherocopina | Cytheroidea | Limnocytheridae   | <i>Gomphodella</i> sp.                   | 3  | SAH  |      |           | dead, damaged BOS802, BOS803   |
| 4     | RR1          | 10-Sep-16 | Ostracoda    | Popocopa       | Popocopida      | Cypridocopina | Cypridoidea | Cyprididae        | <i>Cyprididae</i> sp.                    | 1  | SAH  |      |           | dead, damaged BOS804   |
| 4     | 87           | 10-Sep-16 | Maxillopoda  | Copepoda       | Cyclopoida      |               |             | Cyclopidae        | <i>Diacyclops humphreysi humphreysi</i>  | 2  | JMM  |      |           |  |



## Appendix 3

---

### Genetic Analyses by Helix Molecular Solutions









# Helix

## Molecular Solutions

School of Animal Biology The University of Western Australia  
Hackett Entrance No. 4 Hackett Drive Crawley WA 6009

PO Box 155  
Leederville WA 6903

t: [08] 6488 4509

f: [08] 6488 1029

abn: 32 133 230 243

w: [www.helixsolutions.com.au](http://www.helixsolutions.com.au)

29 July, 2017

Nicola Watson  
Biota Environmental Sciences  
Level 1, 228 Carr Place  
Leederville, WA 6007

Via email

### **Re. Report on the molecular systematics of Amphipoda from Mesa H – Phase 4**

Dear Nicola,

Following is a summary of the results of the Amphipoda study we have completed from Mesa H phase 4 collections. Eight distinct genetic lineages from three families (Melitidae, Niphargidae, Paramelitidae) were detected in the phase 4 collections, four of which have been detected previously in the Pilbara, and four of which appear to be new, based on the material available for comparison.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston, Yvette Hitchen and Dr. Oliver Berry  
Helix Molecular Solutions



## Background and Objective

Forty-two specimens of Amphipoda were collected from Mesa H during phase 4 and sequenced for variation at the mitochondrial COI gene. The molecular data were assessed in order to determine the number of species present in each group and compare the results to those obtained during previous surveys at Mesa H and throughout the Pilbara.

## Executive summary

- Forty-two specimens of amphipods from Mesa H phase 4 were sequenced for variation at the COI gene.
- Eight distinct genetic lineages from three families (Melitidae, Niphargidae, Paramelitidae) were detected among the 38 specimens for which sequences were obtained.
- Four of the eight lineages have been detected previously in the Pilbara.

## Methods

Forty-two specimens of Amphipoda from 14 drillholes at Mesa H were sequenced for variation at the mitochondrial cytochrome oxidase subunit I gene (COI) using primers LCOI/HCO2.

Sequences were edited using GENEIOUS software (Drummond *et al.* 2011). Alignment was performed with CLUSTAL W (Thompson *et al.* 1994) using default parameters. Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotide differences between sequences).

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data for each taxonomic group. Bayesian analysis was used to construct the phylogenetic trees, incorporating the model as identified in MODELTEST for each taxonomic group. The phylogeny, branch lengths and posterior probabilities were obtained by running two trees simultaneously, each running four simultaneous MCMC chains. The number of cycles needed was determined by the standard deviation of the split frequencies of the two trees. The analysis was paused after every  $1 \times 10^6$  generations and when the standard deviation fell below 0.01, the analysis was stopped. A majority rule consensus tree was constructed after discarding the "burn-in" trees in both analyses. The burn-in value was determined by plotting the posterior probabilities obtained after every generation and identifying the point at which the values reach stationarity (= the asymptote). Trees produced prior to stationarity were discarded.

For the purposes of this report, lineages were defined as haplotypes or groups of haplotypes differing from other such groups by >3% sequence divergence. This cut-off was selected based on bar-coding data, which indicates that intra-specific variation rarely exceeds 3% (Hebert *et al.*, 2003b).

## Results

### *Preliminary analysis - Reference sequences and outgroups*

Forty-two specimens of amphipods from phase 4 were sequenced for COI (Table 1; Appendix 1). In order to reduce analysis time and to simplify the presentation of results, a preliminary neighbour-joining (NJ) analysis was conducted on the 38 Robe Valley amphipods for which sequences were obtained, and haplotypes were selected from each genetic lineage to include in a model-based phylogenetic analysis.

The neighbour-joining analysis placed the 38 specimens of amphipods into eight lineages (Figure 1). Comparisons to the GenBank sequence database indicated that four lineages were most similar to sequences of Niphargidae and one was most similar to Paramelitidae, whereas three lineages were most similar to sequences of Melitidae. The Paramelitidae and Niphargidae lineages each contained single specimens, whereas the Melitidae lineages contained between one and 25 specimens (Figure 1). The three lineages of Melitidae differed from one another by between 5.8 and 9.8% mean sequence divergence and the four lineages of Niphargidae differed from one another by between 12.1 and 18.9% sequence divergence (Table 2).

Differentiation within lineages for all three families ranged from 0 to 0.5% mean sequence divergence (Table 3).

The Paramelitidae and Niphargidae were analysed in a single phylogenetic analysis. For the Paramelitidae, 32 reference sequences were included in the phylogenetic analysis, six GenBank vouchers of Paramelitidae, *Maarrka etheli* (Genbank accession #DQ838031), two *Maarrka weeliwoollii* (Genbank accession # DQ838032 and # DQ838033), Paramelitidae sp. 3 (Genbank accession # EF558852) and two references of 'Yilgarus' sp. (Genbank accession # EF118232 and EF118194) as well as 26 specimens from previous surveys of the Robe Valley, Central Pilbara, Barrow Island, Lower Shaw River, Fortescue River, and Marillana Creek, including one representative from a previous survey at Mesa H. For the Niphargidae, eight reference sequences were included in the analysis, six specimens from previous surveys in the Robe Valley, including one representative from Mesa H, and two Genbank reference sequences of *Niphargus*: *N. fontanus* (Genbank accession number KC315635) and *N. glenniei* (Genbank accession number KC315646). Two sequences of Melitidae, *Nedsia* sp. 1 (Genbank accession #EU304458), and *Norcapensis mandibulis* (Genbank accession # JQ608487)

For the Melitidae, 43 reference sequences were included in the phylogenetic analysis, two GenBank vouchers of Melitidae, *Nedsia* sp. 1 (Genbank accession #EU304458), and *Norcapensis mandibulis* (Genbank accession # JQ608487) as well as 41 specimens from previous surveys of the Robe Valley, Central Pilbara, Barrow Island, Lower Shaw River, Fortescue River, and Marillana Creek, including five representatives from previous surveys at Mesa H. Two sequences of the amphipod genus *Niphargus* were used as outgroups as follows: *N. fontanus* (Genbank accession number KC315635) and *N. glenniei* (Genbank accession number KC315646).

#### *Phylogenetic analysis- Paramelitidae and Niphargidae*

The phylogenetic analysis, which included the one representative of Paramelitidae and the four representatives of Niphargidae from Mesa H phase 4, in addition to 32 and eight reference specimens, respectively, revealed the presence of two well-supported major clades, corresponding to the two families (Figure 2). The phase 4 Mesa H specimen of Paramelitidae was placed in a single distinct lineage, which did not contain any reference specimens and thus was assigned to the new lineage AMP035 (Figure 2). The Mesa H phase 4 Niphargidae specimens were placed in four distinct genetic lineages, one of which also contained reference specimens from previous surveys at Mesa H and the Robe Valley (Figure 2). Specifically, specimen KE07 from bore 200, was placed in a lineage containing specimens from previous surveys in the Robe Valley and Mesa H (Daves, Budgie and bore 200) and thus was assigned to the existing lineage AMN008 (Figure 2). The remaining three lineages of Niphargidae from Mesa H phase 4 (KE18, KE28 and KE33) did not contain any reference specimens and thus were assigned to the new lineages AMN002, AMN003 and AMN004, respectively (Figure 2). Lineage KE28/AMN003 formed a well-supported clade with reference specimen IV129 from the Robe Valley (Figure 2).

#### *Differentiation between lineages – Paramelitidae and Niphargidae*

The Robe Valley Paramelitidae lineage represented by specimen KE16/AMP035 differed from the nearest reference specimen GK62 by 17.4% sequence divergence (Table 4). The Mesa H Niphargidae lineage represented by specimen KE07/AMN008 differed from the nearest reference specimens KJ64, KD39, IV100 and JN08 (lineage AMN008) by between 0.0 and 0.6% sequence divergence (Table 4). Phase 4 specimen KE28/AMN003 differed from the nearest reference specimen (IV129) by 6.3% sequence divergence and the remaining two lineages of Niphargidae from phase 4, KE18/AMN002 and KE33/AMN004 differed from the nearest reference specimens by >12% sequence divergence (Table 4).

#### *Phylogenetic analysis- Melitidae*

The phylogenetic analysis, which included the three representatives of Melitidae from Mesa H phase 4, in addition to 43 reference specimens, placed the Mesa H specimens in three distinct genetic lineages, all of which also contained reference specimens from previous surveys in the Pilbara (Figure 3). Specifically, specimen KE31 was placed in a lineage containing reference



specimens KJ65, IV97, IV126, JN17, KD49, JF02 and rr3a and thus was assigned to the existing lineage AMM001, and specimen KE05 was placed in a lineage containing reference specimens KJ73, KD45 and KD55 and thus was assigned to the existing lineage AMM031 (Figure 3), Specimen KE10 was placed in a lineage with reference specimens IV128 and JN05, and thus was assigned to the existing lineage AMM004 (Figure 3).

Specimens KE05 and KE031 were placed in a larger clade containing eight closely related lineages from the Robe Valley (AMM001, AMM002, AMM024, AMM025, AMM027, AMM029, AMM030 and AMM031; Figure 3).

#### *Differentiation between lineages - Melitidae*

All three specimens representing the three lineages collected in phase 4 were assigned to existing lineages and differed from the nearest reference specimens by <2.5% (Table 5). Specifically, KE031/AMM001 differed from the nearest reference specimens IV97, IV126, JN17, KD49, KJ65, JF02 and rr3a by between 0.2 and 2.2% sequence divergence and KE05/AMM031 differed from the nearest reference specimens KJ73, KD45 and KD55 by between 2.2 and 3.5% sequence divergence (Table 5). KE10/AMM004 differed from the nearest reference specimens IV128 and JN05 by between 0.0 and 0.2% sequence divergence (Table 5).

The genetic distance between specimens in the large well-supported clade comprising the eight closely related lineages (enclosed by a dashed red box in Figure 3) ranged from 0 – 7.8% and the mean was 4.9% sequence divergence.

### **Conclusions**

COI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003a). In a comparison of COI sequences for over 13,000 pairs of taxa, Hebert et al (2003b) found a mean of 11.1% sequence divergence between distinct species. Nearly 80% of the comparisons showed that species pairs differed from one another by greater than 8% sequence divergence. However, a taxon by taxon approach, examining the amount of phylogenetic variation within and between species is the most widely accepted method of defining species.

#### *Paramelitidae*

One lineage of Paramelitidae (AMP035) was detected at Mesa H during phase 4. The lineage differed from the nearest reference specimens by 17% or greater, indicating that it represents a distinct lineage and species that has not been detected previously.

AMP035 = one species from RR1, so far not detected previously in the Pilbara, based on the material available for comparison.

#### *Niphargidae*

Four lineages of Niphargidae were detected at Mesa H during phase 4. One lineage, AMN008, has been detected previously at Mesa H and other sites in the Robe Valley, whereas two (AMN002 and AMN004) have not been detected previously, based on the high level of sequence divergence between them and the nearest reference specimens (>12%). The fourth lineage requires further consideration. Lineage AMN003 differed from the nearest reference specimen (IV129/AMN007 from Daves bore in the Robe Valley) by 6.3% sequence divergence. This level of genetic divergence, while moderately low, is more than would be expected to be observed between individuals of the same species and particularly from the same region. For example, lineage AMN008 shares a similar distribution, being detected at Mesa H and Daves and Budgie bores, however differentiation between specimens in lineage AMN008 is <1%. Nevertheless, lineages AMN003 and AMN007 are closely related. Because sample sizes are low and the specimens were collected at different deposits in the Robe Valley, further sampling at intermediate sites may help resolve the relationship among these lineages.

AMN008 = one species from bore 200, detected previously in the Robe Valley at Daves and Budgie bores.

AMN002 = new species

AMN003 = new species; closely related to AMN007 and further investigation may help resolve the relationship between the two lineages

AMN004 = new species

## Melitidae

Three lineages of Melitidae were detected at Mesa H, differing from one another by between 5.8 and 9.8% mean sequence divergence, indicating that each is likely to represent a distinct species (however see following comments). All three of the lineages were assigned to existing lineages (AMM001, AMM004, and AMM031).

Two of the three lineages detected in phase 4 at Mesa H (AMM001 and AMM031) were placed in a well-supported clade containing a group of closely related specimens (enclosed by a dashed red box in Figure 3), which differed by <5% mean sequence divergence (range = 0 – 7.8%). Thus this group may represent several closely related species (as shown by solid red boxes in Figure 3), or may represent a single species showing genetic differentiation across its range. Further investigation may be required to resolve relationships among the lineages, but the most likely explanation would be to consider them a single species, owing to the fact that they have a relatively wide distribution (Robe Valley to Barrow Island) and the observed genetic variation may reflect geographic variation associated with a 'stepping stone' pattern of gene exchange – populations that are near-by would be expected to exchange genes, but those on the geographic extremes are not exchanging genes. This clade forms a larger clade with additional lineages, including the Genbank reference specimen of *Nedsia* sp. 1, allowing us to assign the lineages to the genus *Nedsia* with some confidence.

AMM001 } AMM001 was detected in eight bores at Mesa H as well as previously at Dave's  
AMM031 } and Budgie bores in the Robe Valley. AMM031 was detected in bore 200 at  
Mesa H as well as Budgie bore. May correspond to one species of *Nedsia*, but  
relationships among the clade members requires further investigation.

AMM004 = one species from bores 87 and 200, detected previously in the Robe Valley.

## References

- Alexander, J. B. , Burger M. A.A., and Harvey, M.S. (2014). A new species of troglobitic *Anatemnus* (Pseudoscorpiones: Atemnidae) from the Pilbara bioregion of Australia. *Records of the Western Australian Museum* 29: 141 – 148.
- Australian Faunal Directory. <http://www.environment.gov.au/biodiversity/abrs/online-resources/>. Accessed 27 Dec., 2015.
- Bayly, I. A.E, Ellis p. (1969). *Haloniscus searlei* chilton: An aquatic "terrestrial" isopod with remarkable powers of osmotic regulation. *Comparative Biochemistry and Physiology* 31: 523-528
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2011) Geneious v5.4, <http://www.geneious.com/>
- Harvey, M. S., Berry, O. Edward, K. L., Humphreys, G. (2008) Molecular and morphological systematics of hypogean schizomids (Schizomida: Hubbardiidae) in semiarid Australia. *Invertebrate Systematics* 22: 167-194.
- Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard J.R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* 270: 313-321.
- Hebert, P.D.N., Ratnasingham, S., deWaard J.R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B (supplement)* 270: S96-S99.

- Helix Molecular Solutions (2015). Report on the molecular systematics of Schizomida from The Robe River Valley. Prepared for Biota Environmental Sciences, 5 February.
- Posada, D., Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- Thompson, J., Higgins, D., and Gibson, T. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.  
doi:10.1093/nar/ 22.22.4673

Table 1. Specimens of Amphipoda used in the present study and the lineage to which they were assigned, based on variation at the COI gene. \*=new lineage

| Tracking Number            | Cap # | N | Helix ID | Lineage |
|----------------------------|-------|---|----------|---------|
| RC16MEH0427.20161026.T3-01 | 56    | 1 | KE01     | AMM001  |
| RC16MEH0433.20161026.T2-01 | 71    | 1 | KE02     | AMM001  |
| RC16MEH0436.20161026.T2-01 | 85    | 1 | KE03     | AMM001  |
| DD13MEH0007-20160914-01    | 22    | 1 | KE04     | AMM001  |
| 200-20160910-01            | 12    | 4 | KE05     | AMM031  |
| 200-20160910-01            | 12    |   | KE06     | AMM004  |
| 200-20160910-01            | 12    |   | KE07     | AMN008  |
| 200-20160910-01            | 12    |   | KE08     | AMM031  |
| 87-20160919-02             | 10    | 5 | KE09     | AMM004  |
| 87-20160919-02             | 10    |   | KE10     | AMM004  |
| 87-20160919-02             | 10    |   | KE11     | AMM004  |
| 87-20160919-02             | 10    |   | KE12     | AMM004  |
| 87-20160919-02             | 10    |   | KE13     | AMM004  |
| RR1-20160910-01            | 7     | 5 | KE14     | AMM001  |
| RR1-20160910-01            | 7     |   | KE15     | No data |
| RR1-20160910-01            | 7     |   | KE16     | AMP035* |
| RR1-20160910-01            | 7     |   | KE17     | AMM001  |
| RR1-20160910-01            | 7     |   | KE18     | AMN002* |
| 34-20160910-01             | 18    | 3 | KE19     | AMM001  |
| 34-20160910-01             | 18    |   | KE20     | No data |
| 34-20160910-01             | 18    |   | KE21     | No data |
| 31-20160910-04             | 17    | 5 | KE22     | AMM001  |
| 31-20160910-04             | 17    |   | KE23     | AMM001  |
| 31-20160910-04             | 17    |   | KE24     | AMM001  |
| 31-20160910-04             | 17    |   | KE25     | AMM001  |
| 31-20160910-04             | 17    |   | KE26     | AMM001  |
| 32-20160910-02             | 5     | 4 | KE27     | AMM001  |
| 32-20160910-02             | 5     |   | KE28     | AMN003* |
| 32-20160910-02             | 5     |   | KE29     | AMM001  |
| 32-20160910-02             | 5     |   | KE30     | AMM001  |
| 25-20160910-01             | 1     | 3 | KE31     | AMM001  |
| 25-20160910-01             | 1     |   | KE32     | AMM001  |
| 25-20160910-01             | 1     |   | KE33     | AMN004* |
| RC16JIM0019.20160912-01    | 20    | 5 | KE34     | AMM001  |
| RC16JIM0019.20160912-01    | 20    |   | KE35     | AMM001  |
| RC16JIM0019.20160912-01    | 20    |   | KE36     | AMM001  |
| RC16JIM0019.20160912-01    | 20    |   | KE37     | AMM001  |
| RC16JIM0019.20160912-01    | 20    |   | KE38     | AMM001  |
| DD13MEH0007-20160914-02    | 23    | 5 | KE39     | AMM001  |
| DD13MEH0007-20160914-02    | 23    |   | KE40     | AMM001  |
| DD13MEH0007-20160914-02    | 23    |   | KE41     | No data |
| DD13MEH0007-20160914-02    | 23    |   | KE42     | AMM001  |
| DD13MEH0007-20160914-02    | 23    |   | KE43     | AMM001  |
| RC13MEH0040.20160914-01    | 25    | 3 | KE44     | AMM001  |
| RC13MEH0040.20160914-01    | 25    |   | KE45     | AMM001  |
| RC13MEH0040.20160914-01    | 25    |   | KE46     | AMM001  |



Table 2. Mean genetic distance (p-distances) between lineages of amphipods as shown in Figure 1. Distances between lineages within families are highlighted in yellow. Para=Paramelitidae.

| Lineage | Melitidae |       |       | Para  | Niphargidae |       |       |       |
|---------|-----------|-------|-------|-------|-------------|-------|-------|-------|
|         | 1         | 2     | 3     | 4     | 5           | 6     | 7     | 8     |
| Gp_1    |           | 0.009 | 0.010 | 0.018 | 0.016       | 0.017 | 0.016 | 0.016 |
| Gp_2    | 0.058     |       | 0.011 | 0.017 | 0.016       | 0.017 | 0.016 | 0.017 |
| Gp_3    | 0.098     | 0.098 |       | 0.016 | 0.016       | 0.016 | 0.016 | 0.017 |
| Gp_4    | 0.330     | 0.322 | 0.331 |       | 0.013       | 0.014 | 0.014 | 0.014 |
| Gp_5    | 0.308     | 0.306 | 0.312 | 0.231 |             | 0.015 | 0.014 | 0.013 |
| Gp_6    | 0.305     | 0.300 | 0.303 | 0.228 | 0.184       |       | 0.012 | 0.013 |
| Gp_7    | 0.307     | 0.307 | 0.322 | 0.243 | 0.189       | 0.156 |       | 0.012 |
| Gp_8    | 0.305     | 0.300 | 0.310 | 0.246 | 0.183       | 0.156 | 0.121 |       |

Table 3. Mean genetic distance (p-distances) and standard errors within lineages of amphipods as shown in Figure 1. N= number of specimens in the lineage and rep= specimen selected to represent the lineage in the model-based phylogenetic analysis.

| Lineage | D     | s.e.  | N  | Rep  |
|---------|-------|-------|----|------|
| Gp 1    | 0.005 | 0.001 | 25 | KE31 |
| Gp 2    | 0.000 | 0.000 | 2  | KE05 |
| Gp 3    | 0.001 | 0.001 | 6  | KE10 |
| Gp 4    | n/c   | n/c   | 1  | KE16 |
| Gp 5    | n/c   | n/c   | 1  | KE18 |
| Gp 6    | n/c   | n/c   | 1  | KE28 |
| Gp 7    | n/c   | n/c   | 1  | KE07 |
| Gp 8    | n/c   | n/c   | 1  | KE33 |

Table 4 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Niphargidae and Paramelitidae (Amphipoda) detected at Robe Valley and the reference lineages as shown in Figure 2. Distances between the Robe Valley specimens and the reference specimens are highlighted in yellow.

Table 5 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Melitidae (Amphipoda) detected at Robe Valley and the reference lineages as shown in Figure 3. Distances between the Robe Valley specimens and the reference specimens are highlighted in yellow.

Appendix 1. All specimens, assigned to lineages, from phases 1-6. Lineages with name changes are highlighted in yellow.

| Specimen ID               | Taxon     | Cap_No | Helix ID | phase | old lineage | new lineage |
|---------------------------|-----------|--------|----------|-------|-------------|-------------|
| DD13MEH0007-20151024-01A  | Amphipoda | 11     | JF01     | 1     | A1          | AMM001      |
| DD13MEH0007-20151024-01B  | Amphipoda | 11     | JF02     | 1     | A1          | AMM001      |
| DD13MEH0007-20151024-01C  | Amphipoda | 11     | JF03     | 1     | A1          | AMM001      |
| DD13MEH0007-20151024-01D  | Amphipoda | 11     | JF04     | 1     | A1          | AMM001      |
| DD13MEH0007-20151024-01E  | Amphipoda | 11     | JF05     | 1     | A1          | AMM001      |
| DD13MEH0007-20160119-01A  | Amphipoda | 20     | JF06     | 1     | A1          | AMM001      |
| DD13MEH0007-20160119-01B  | Amphipoda | 20     | JF07     | 1     | A1          | AMM001      |
| DD13MEH0007-20160119-01C  | Amphipoda | 20     | JF08     | 1     | A1          | AMM001      |
| DD13MEH0007-20160119-01D  | Amphipoda | 20     | JF09     | 1     | A1          | AMM001      |
| DD13MEH0007-20160119-01E  | Amphipoda | 20     | JF10     | 1     | A1          | AMM001      |
| RC12JIM0019-20160119-01A  | Amphipoda | 14     | JF11     | 1     | A1          | AMM001      |
| RC12JIM0019-20160119-01B  | Amphipoda | 14     | JF12     | 1     | A1          | AMM001      |
| RC12JIM0019-20160119-01C  | Amphipoda | 14     | JF13     | 1     | No data     | No data     |
| RC12JIM0019-20160119-01D  | Amphipoda | 14     | JF14     | 1     | A1          | AMM001      |
| RC12JIM0019-20160119-01E  | Amphipoda | 14     | JF15     | 1     | A1          | AMM001      |
| RC13MEH00400-20160119-01A | Amphipoda | 19     | JF16     | 1     | A1          | AMM001      |
| RC13MEH00400-20160119-01B | Amphipoda | 19     | JF17     | 1     | A1          | AMM001      |
| RC13MEH0007-20151024-03   | Amphipoda | 3      | JF18     | 1     | A3          | AMP003      |
| RC13MEH0041-20160119-01   | Amphipoda | 13     | JF19     | 1     | A3          | AMP003      |
| RC13MEH0041-20151024-01   | Amphipoda | 5      | JF20     | 1     | A1          | AMM001      |
| RC13MEH0097-20151024-01A  | Amphipoda | 7      | JF21     | 1     | A2          | AMM026      |
| RC13MEH0097-20151024-01B  | Amphipoda | 7      | JF22     | 1     | A1          | AMM001      |
| RC13MEH0097-20151024-01C  | Amphipoda | 7      | JF23     | 1     | A1          | AMM001      |
| RC14MEH0018-20151025-01   | Amphipoda | 6      | JF24     | 1     | A1          | AMM001      |
| RC14MEH0018-20160119-01A  | Amphipoda | 16     | JF25     | 1     | A1          | AMM001      |
| RC14MEH0018-20160119-01B  | Amphipoda | 16     | JF26     | 1     | A1          | AMM001      |
| RC14MEH0018-20160119-01C  | Amphipoda | 16     | JF27     | 1     | A1          | AMM001      |
| RC14MEH0018-20160119-01D  | Amphipoda | 16     | JF28     | 1     | A1          | AMM001      |
| RC14MEH0018-20160119-01E  | Amphipoda | 16     | JF29     | 1     | A1          | AMM001      |
| RC14MEH0053-20151024-01A  | Amphipoda | 9      | JF30     | 1     | A1          | AMM001      |
| RC14MEH0053-20151024-01B  | Amphipoda | 9      | JF31     | 1     | A1          | AMM001      |
| RC14MEH0053-20160119-01A  | Amphipoda | 24     | JF32     | 1     | A1          | AMM001      |
| RC14MEH0053-20160119-01B  | Amphipoda | 24     | JF33     | 1     | A1          | AMM001      |
| RC14MEH0053-20160119-01C  | Amphipoda | 24     | JF34     | 1     | A1          | AMM001      |
| RC14MEH0053-20160119-01D  | Amphipoda | 24     | JF35     | 1     | A1          | AMM001      |
| RC14MEH0053-20160119-01E  | Amphipoda | 24     | JF36     | 1     | A1          | AMM001      |
| 87-20160505-02A           | Amphipoda | 12A    | JN01     | 2     | A2(AMP004)  | AMM004      |
| 87-20160505-02B           | Amphipoda | 12B    | JN02     | 2     | A2(AMP004)  | AMM004      |
| 87-20160505-02C           | Amphipoda | 12C    | JN03     | 2     | A2(AMP004)  | AMM004      |
| 87-20160505-02D           | Amphipoda | 12D    | JN04     | 2     | A2(AMP004)  | AMM004      |
| 87-20160505-02E           | Amphipoda | 12E    | JN05     | 2     | A2(AMP004)  | AMM004      |
| 200-20160505-02A          | Amphipoda | 15A    | JN06     | 2     | A2(AMP004)  | AMM004      |

|                  |           |     |      |   |            |            |
|------------------|-----------|-----|------|---|------------|------------|
| 200-20160505-02B | Amphipoda | 15B | JN07 | 2 | A4(AMP008) | AMN008     |
| 200-20160505-02C | Amphipoda | 15C | JN08 | 2 | A4(AMP008) | AMN008     |
| 200-20160505-02D | Amphipoda | 15D | JN09 | 2 | A4(AMP008) | AMN008     |
| 200-20160505-02E | Amphipoda | 15E | JN10 | 2 | A2(AMP004) | AMM004     |
| RR120160505-01A  | Amphipoda | 1A  | JN11 | 2 | A1(AMP001) | AMM001     |
| RR120160505-01B  | Amphipoda | 1B  | JN12 | 2 | A1(AMP001) | AMM001     |
| RR120160505-01C  | Amphipoda | 1C  | JN13 | 2 | A1(AMP001) | AMM001     |
| RR120160505-01D  | Amphipoda | 1D  | JN14 | 2 | Failed PCR | Failed PCR |
| RR120160505-01E  | Amphipoda | 1E  | JN15 | 2 | Failed PCR | Failed PCR |
| 34-20160506-01   | Amphipoda | 18  | JN16 | 2 | A1(AMP001) | AMM001     |
| 32-20160506-01A  | Amphipoda | 16A | JN17 | 2 | A1(AMP001) | AMM001     |
| 32-20160506-01B  | Amphipoda | 16B | JN18 | 2 | A1(AMP001) | AMM001     |
| 32-20160506-01C  | Amphipoda | 16C | JN19 | 2 | A1(AMP001) | AMM001     |
| 32-20160506-01D  | Amphipoda | 16D | JN20 | 2 | A1(AMP001) | AMM001     |
| 32-20160506-01E  | Amphipoda | 16E | JN21 | 2 | A1(AMP001) | AMM001     |
| 3120160507-03A   | Amphipoda | 10A | JN22 | 2 | A3(AMP010) | AMM026     |
| 3120160507-03B   | Amphipoda | 10B | JN23 | 2 | A1(AMP001) | AMM001     |
| 3120160507-03C   | Amphipoda | 10C | JN24 | 2 | A1(AMP001) | AMM001     |
| 3120160507-03D   | Amphipoda | 10D | JN25 | 2 | A1(AMP001) | AMM001     |
| 200-20160910-01  | Amphipoda | 12  | KE05 | 4 | AMM031     | AMM031     |
| 200-20160910-01  | Amphipoda | 12  | KE06 | 4 | AMM004     | AMM004     |
| 200-20160910-01  | Amphipoda | 12  | KE07 | 4 | AMN008     | AMN008     |
| 200-20160910-01  | Amphipoda | 12  | KE08 | 4 | AMM031     | AMM031     |
| 87-20160919-02   | Amphipoda | 10  | KE09 | 4 | AMM004     | AMM004     |
| 87-20160919-02   | Amphipoda | 10  | KE10 | 4 | AMM004     | AMM004     |
| 87-20160919-02   | Amphipoda | 10  | KE11 | 4 | AMM004     | AMM004     |
| 87-20160919-02   | Amphipoda | 10  | KE12 | 4 | AMM004     | AMM004     |
| 87-20160919-02   | Amphipoda | 10  | KE13 | 4 | AMM004     | AMM004     |
| RR1-20160910-01  | Amphipoda | 7   | KE14 | 4 | AMM001     | AMM001     |
| RR1-20160910-01  | Amphipoda | 7   | KE15 | 4 | No data    | No data    |
| RR1-20160910-01  | Amphipoda | 7   | KE16 | 4 | AMP035*    | AMP035*    |
| RR1-20160910-01  | Amphipoda | 7   | KE17 | 4 | AMM001     | AMM001     |
| RR1-20160910-01  | Amphipoda | 7   | KE18 | 4 | AMN002*    | AMN002*    |
| 34-20160910-01   | Amphipoda | 18  | KE19 | 4 | AMM001     | AMM001     |
| 34-20160910-01   | Amphipoda | 18  | KE20 | 4 | No data    | No data    |
| 34-20160910-01   | Amphipoda | 18  | KE21 | 4 | No data    | No data    |
| 31-20160910-04   | Amphipoda | 17  | KE22 | 4 | AMM001     | AMM001     |
| 31-20160910-04   | Amphipoda | 17  | KE23 | 4 | AMM001     | AMM001     |
| 31-20160910-04   | Amphipoda | 17  | KE24 | 4 | AMM001     | AMM001     |
| 31-20160910-04   | Amphipoda | 17  | KE25 | 4 | AMM001     | AMM001     |
| 31-20160910-04   | Amphipoda | 17  | KE26 | 4 | AMM001     | AMM001     |
| 32-20160910-02   | Amphipoda | 5   | KE27 | 4 | AMM001     | AMM001     |
| 32-20160910-02   | Amphipoda | 5   | KE28 | 4 | AMN003*    | AMN003*    |
| 32-20160910-02   | Amphipoda | 5   | KE29 | 4 | AMM001     | AMM001     |
| 32-20160910-02   | Amphipoda | 5   | KE30 | 4 | AMM001     | AMM001     |

|                         |           |    |      |   |         |         |
|-------------------------|-----------|----|------|---|---------|---------|
| 25-20160910-01          | Amphipoda | 1  | KE31 | 4 | AMM001  | AMM001  |
| 25-20160910-01          | Amphipoda | 1  | KE32 | 4 | AMM001  | AMM001  |
| 25-20160910-01          | Amphipoda | 1  | KE33 | 4 | AMN004* | AMN004* |
| RC16JIM0019.20160912-01 | Amphipoda | 20 | KE34 | 4 | AMM001  | AMM001  |
| RC16JIM0019.20160912-01 | Amphipoda | 20 | KE35 | 4 | AMM001  | AMM001  |
| RC16JIM0019.20160912-01 | Amphipoda | 20 | KE36 | 4 | AMM001  | AMM001  |
| RC16JIM0019.20160912-01 | Amphipoda | 20 | KE37 | 4 | AMM001  | AMM001  |
| RC16JIM0019.20160912-01 | Amphipoda | 20 | KE38 | 4 | AMM001  | AMM001  |
| DD13MEH0007-20160914-02 | Amphipoda | 23 | KE39 | 4 | AMM001  | AMM001  |
| DD13MEH0007-20160914-02 | Amphipoda | 23 | KE40 | 4 | AMM001  | AMM001  |
| DD13MEH0007-20160914-02 | Amphipoda | 23 | KE41 | 4 | No data | No data |
| DD13MEH0007-20160914-02 | Amphipoda | 23 | KE42 | 4 | AMM001  | AMM001  |
| DD13MEH0007-20160914-02 | Amphipoda | 23 | KE43 | 4 | AMM001  | AMM001  |
| RC13MEH0040.20160914-01 | Amphipoda | 25 | KE44 | 4 | AMM001  | AMM001  |
| RC13MEH0040.20160914-01 | Amphipoda | 25 | KE45 | 4 | AMM001  | AMM001  |
| RC13MEH0040.20160914-01 | Amphipoda | 25 | KE46 | 4 | AMM001  | AMM001  |



Figure 1. Neighbour-joining analysis of specimens of Amphipoda from the present study. Numbers on major nodes correspond to bootstrap support over 100 iterations; values <50% are not shown. Scale bar= genetic distance. The specimens used to represent each lineage in the model-based phylogenetic analysis are highlighted in yellow.

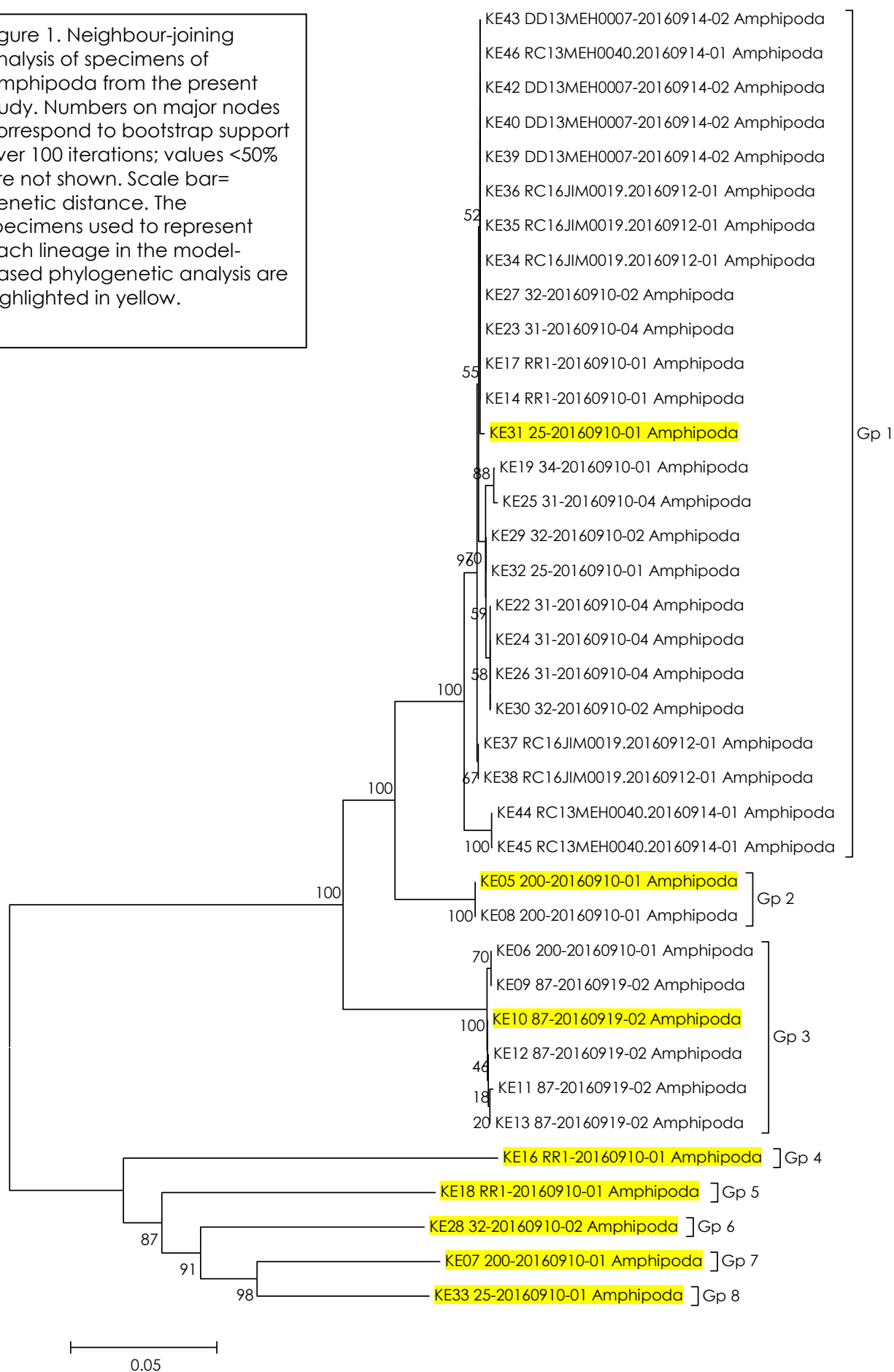


Figure 2. Bayesian analysis of COI haplotypes of Paramelitidae and Niphargidae (Amphipoda) from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; specimens from previous phases at Mesa H are highlighted in green; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes enclose species with high confidence.

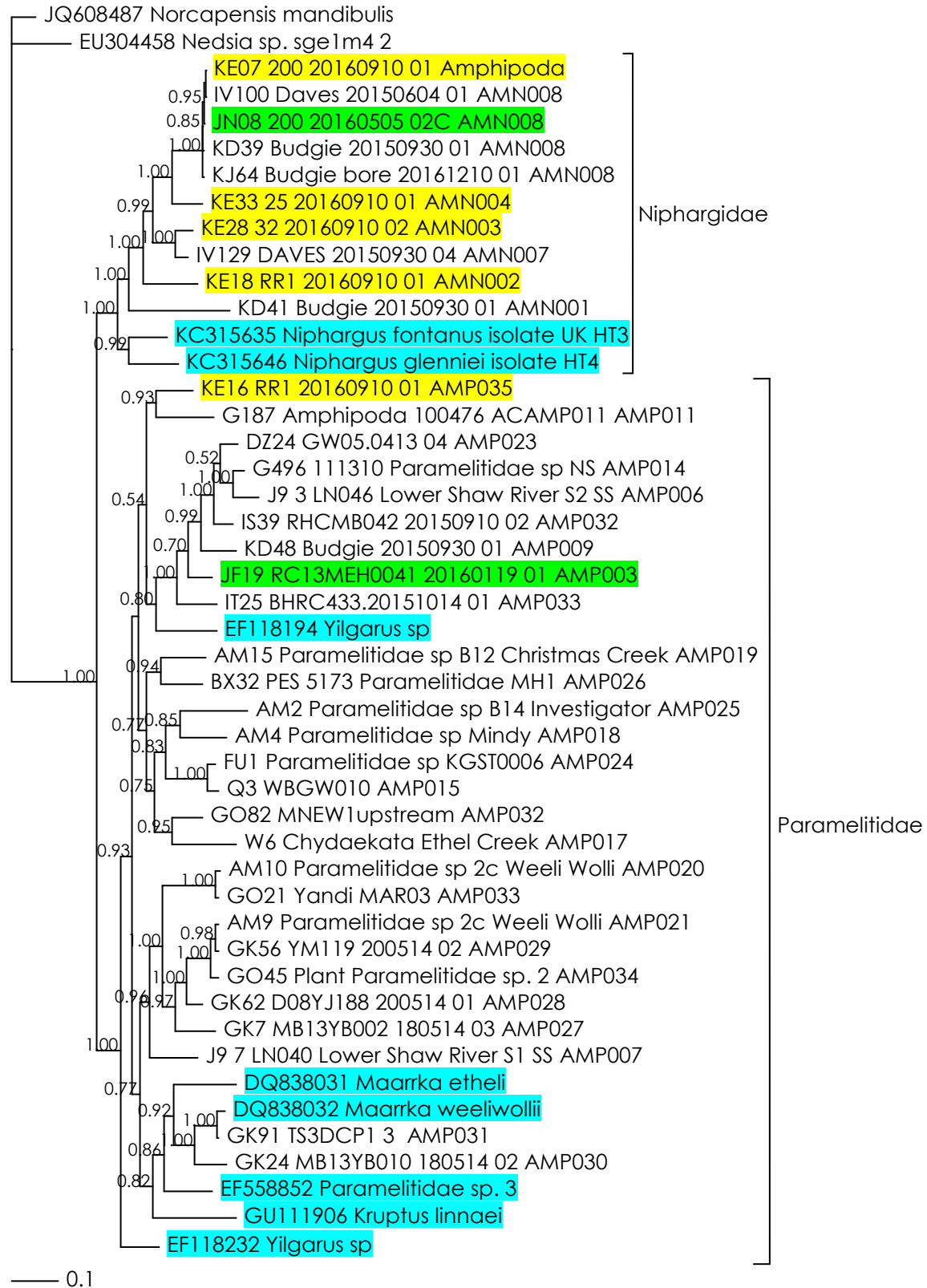
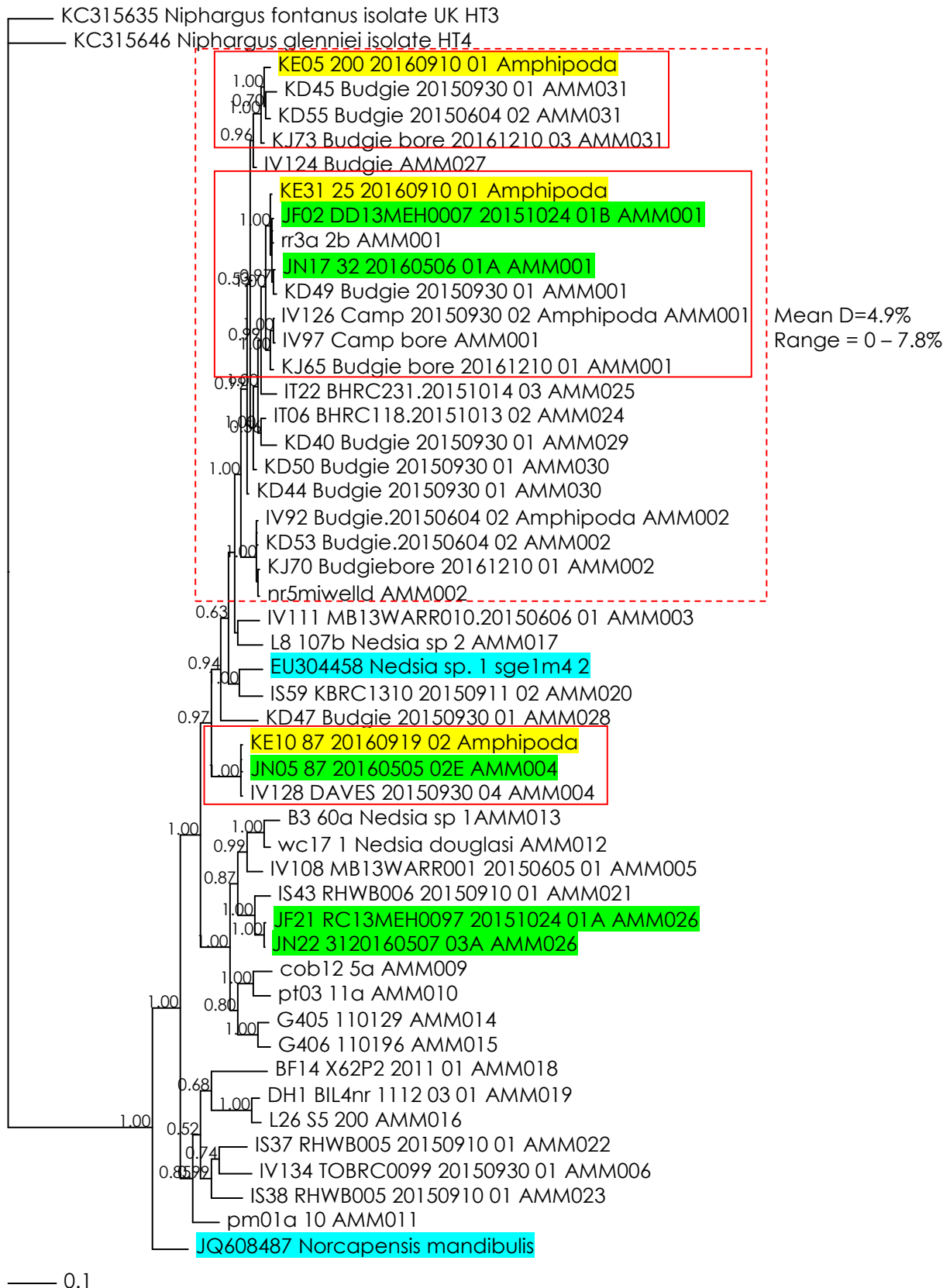


Figure 3. Bayesian analysis of COI haplotypes of Melitidae (Amphipoda) from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; specimens from previous phases at Robe Valley are highlighted in green; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes with dashed lines represent groups requiring further investigation.





# Helix

## Molecular Solutions

School of Animal Biology The University of Western Australia  
Hackett Entrance No. 4 Hackett Drive Crawley WA 6009

PO Box 155  
Leederville WA 6903

t: [08] 6488 4509

f: [08] 6488 1029

abn: 32 133 230 243

w: [www.helixsolutions.com.au](http://www.helixsolutions.com.au)

29 July, 2017

Nicola Watson  
Biota Environmental Sciences  
Level 1, 228 Carr Place  
Leederville, WA 6007

Via email

**Re. Report on the molecular systematics of additional subfauna from the Mesa H – Phases 4 -6**

Dear Nicola,

Following is a summary of the results of the subfauna study we have completed on four additional taxonomic groups (Diplura, Isopoda, Pseudoscorpiones, Schizomids) collected during Mesa H phases 4 - 6. Fourteen distinct genetic lineages were detected among the four groups. Two of the 14 lineages have been detected previously in the Pilbara, whereas the remaining 12 are new, based on the material available for comparison.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston, Yvette Hitchen and Dr. Oliver Berry  
Helix Molecular Solutions





## Background and Objective

Thirty-five specimens of subfauna belonging to four taxonomic groups (Diplura, Isopoda, Pseudoscorpiones, Schizomida) were collected from Mesa H, phases 4 - 6 and sequenced for variation at the mitochondrial COI gene. The molecular data were assessed in order to determine the number of species present in each group and compare the results to those obtained during previous surveys that have been undertaken for these groups elsewhere in the Pilbara.

## Executive summary

- Thirty-five specimens from Mesa H belonging to four taxonomic groups (Diplura, Isopoda, Pseudoscorpiones, Schizomida) were sequenced for variation at the COI gene.
- Fourteen lineages were detected among the four groups.
- Two of the lineages have been detected previously in the Pilbara, whereas the remaining 12 appear to be new, based on the material available for comparison.

## Methods

Thirty-five specimens of subfauna from four taxonomic groups (Diplura, Isopoda, Pseudoscorpiones, Schizomida) collected from Mesa H, phases 4 - 6 were sequenced for variation at the mitochondrial cytochrome oxidase subunit I gene (COI) using multiple pairs of primers (LCOI/HCO2, LCOI-long/HCO2-long, NemF1/NemR1 and LCOI/CIN2341).

Sequences were edited using GENEIOUS software (Drummond *et al.* 2011). Alignment was performed with CLUSTAL W (Thompson *et al.* 1994) using default parameters. Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotide differences between sequences).

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data for each taxonomic group. Bayesian analysis was used to construct the phylogenetic trees, incorporating the model as identified in MODELTEST for each taxonomic group. The phylogeny, branch lengths and posterior probabilities were obtained by running two trees simultaneously, each running four simultaneous MCMC chains. The number of cycles needed was determined by the standard deviation of the split frequencies of the two trees. The analysis was paused after every  $1 \times 10^6$  generations and when the standard deviation fell below 0.01, the analysis was stopped. A majority rule consensus tree was constructed after discarding the "burn-in" trees in both analyses. The burn-in value was determined by plotting the posterior probabilities obtained after every generation and identifying the point at which the values reach stationarity (= the asymptote). Trees produced prior to stationarity were discarded.

For the purposes of this report, lineages were defined as haplotypes or groups of haplotypes differing from other such groups by >3% sequence divergence. This cut-off was selected based on bar-coding data, which indicates that intra-specific variation rarely exceeds 3% (Hebert *et al.*, 2003b).

We are currently developing a consistent and unique lineage and species naming system, so all lineages from previous phases will be consolidated in this report to aid in comparisons between phases.

## Results

### Isopoda

#### *Preliminary analysis, reference specimens and outgroups*

Twenty specimens of Isopoda were sequenced from Mesa H phases 4 - 6 (Table 1). Ten of the 20 specimens yielded a DNA sequence; the remainder could not be amplified, despite trying three different primer pairs. A preliminary neighbour-joining analysis of the ten specimens revealed the presence of two distinct genetic groups (Figure 1). One group contained nine specimens from phase 6 and the second group contained a single specimen from phase 4 (Figure 1). A search of similar sequences on Genbank and in the Helix database indicated that the specimens from the first group were most closely matched with Armadillidae and the specimen from the second

group to the Philosciidae. Hence two separate phylogenetic analyses were conducted; the first contained the one representative from the phase 6 Mesa H Armadillidae and included two Genbank voucher specimens of Armadillidae: *Troglarmadillo* sp. 6 (Genbank accession # KX656276) and *Spherillo* sp. Kazaki, as well as 37 reference specimens of Armadillidae from previous surveys in the Pilbara. Two specimens of the isopod genus *Haloniscus*, *Haloniscus* sp. 10 (Genbank accession #EU364592) and *Haloniscus* sp. 12 (Genbank accession #EU364601) were used as outgroups.

The second analysis contained the single specimen of Philosciidae from phase 4 and included GenBank voucher specimens of *Haloniscus* sp. 21 (Genbank accession #EU364622), *Haloniscus* sp. 10 (Genbank accession #EU364592), *Haloniscus* sp. 12 (Genbank accession #EU364601), which are assigned by different authors to either the Philosciidae or the Onisceidae and *Laevophiloscia yalgoorensis* (Genbank accession #EU364629) from the family Philosciidae, as well as 19 specimens of Philosciidae from previous surveys in the Pilbara. Two specimens of Armadillidae, *Armadillidium nasatum* (Genbank accession # FN824099) and *Troglarmadillo* sp. 6 (Genbank accession # KX656276) were used as outgroups.

#### Phylogenetic analysis

The phylogenetic analysis of the Armadillidae, which included the representative specimen from Mesa H (KU23), in addition to 39 reference specimens, placed the Mesa H specimen in a single distinct lineage (Figure 2). The lineage did not contain any of the reference specimens, so was assigned to the new lineage ISA057, however, specimen KU23 did form a well-supported clade with reference specimen KD23 from the Robe Valley, and the Genbank voucher specimen of *Spherillo* from Japan (Figure 2).

The phylogenetic analysis of the Philosciidae, which included the specimen from Mesa H (KE01), in addition to 23 reference specimens, placed the Mesa H specimen in a single distinct genetic lineage (Figure 3). The lineage did not contain any of the reference specimens, so was assigned to the new lineage ISP055, however, specimen KE01 did form a well-supported clade with reference specimen JF45 from a previous survey at Mesa H (Figure 3).

#### Differentiation within and between lineages

Specimens within the Mesa H lineage of Armadillidae differed from one another by 0.1% mean sequence divergence (Table 2). Specimen KU23 differed from the nearest reference specimen, KD23, by 4.5% sequence divergence and from the Genbank specimen of *Spherillo* by 6.1% sequence divergence (Table 3). Specimen KU23 differed from the remaining reference specimens by >30% sequence divergence (Table 3).

The Mesa H lineage of Philosciidae differed from the nearest reference specimen, JF45 from a previous survey at Mesa H, by 5.1% sequence divergence and from the remaining reference specimens by >15% sequence divergence (Table 4).

#### Diplura

##### Reference specimens and outgroups

Five specimens of Diplura from Mesa H phases 4 – 6 were sequenced for COI (Table 5). The four specimens for which sequences were obtained were analysed with 36 specimens of Diplura from the Pilbara belonging to five families: Anajapygidae, Campodeiidae, Japygidae, Parajapygidae and Projapygidae, as well as five Genbank voucher specimens, *Diplura* sp. (Genbank accession #HQ943342), *Japyx solifugus* (Japygidae; Genbank accession #AY771989) *Campodea tillyardi* (Campodeidae; Genbank accession # AF370844), *Lepidocampa weberi* (Campodeidae; Genbank accession #HQ882832) and *Parajapyx pauliani* (Genbank accession # JQ692327). The planthopper *Lycorma delicatula* (Genbank accession # FJ456942) and a specimen of Fulgoridae (Hemiptera; Genbank accession #GU671563) were used as outgroups.

#### Phylogenetic analysis

The phylogenetic analysis, which included the four specimens of Diplura from Mesa H, in addition to 41 reference specimens, placed the Mesa H specimens in four distinct lineages

(Figure 4). The four lineages were placed in three well-supported clades containing Genbank and Pilbara reference specimens of Japygidae (one lineage), Projapygidae (two lineages) and Parajapygidae (three lineages; Figure 4). None of the four Mesa H specimens were placed in lineages containing reference specimens, and thus were assigned to the new lineages DJA011, DPR010, DPR011 and DPA009 for KE04, KU01, KU02 and KU08, respectively (Figure 4). However, one, DPR011, was placed in a well-supported clade that also contained reference specimen JF40 from a previous survey at Mesa H (Figure 4).

#### *Differentiation within and between lineages*

The four lineages of Diplura from Mesa H phases 4 - 6 differed from one another by between 16.4 and 27.0% sequence divergence (Table 6). The four lineages differed from the nearest reference specimens by between 3.6 and 15.5% sequence divergence (Table 6). Specifically, DPR011 (KU02) differed from specimens JF40 and JF38 from a previous survey at Mesa H by 3.6% and 5.1% sequence divergence, respectively (Table 6). In contrast, the remaining lineages detected at Mesa H (DPR010, DPA009 and DJA011) differed from the nearest reference specimens by >12% (Table 6).

#### Pseudoscorpions

##### *Reference sequences and outgroups*

Five pseudoscorpions from phases 4 – 6 were sequenced from Mesa H (Table 7). A search of similar sequences on Genbank and in the Helix database indicated that one of the specimens most closely matched reference specimens of Chthoniidae, two of the specimens most closely matched reference specimens of Olpiidae and two of the specimens most closely matched reference specimens of Hyiidae. Based on those results, three separate phylogenetic analyses were conducted, one for each family. In addition, specimens of Atemnidae were analysed with the Olpiidae, because Atemnidae were detected in a previous survey at Mesa H.

For the Hyiidae, one GenBank voucher sequence of Hyiidae, *Indohya* sp JM 2008 (Genbank accession # EU559564) and 27 specimens of Hyiidae from previous surveys in the Pilbara were included in the analysis.

For the Olpiidae and Atemnidae, two Genbank voucher specimens of Olpiidae, *Beierolpium bornemisszai* (Genbank accession # EU559545) and *Calocheiridius termitophilus* (Genbank accession #EU559544) and two Genbank vouchers of Atemnidae, *Paratemnoides sumatranus* (Genbank accession # JN018204) and *Paratemnoides elongates* (Genbank accession # JQ040543) as well as 13 and four specimens from the Pilbara, respectively were included in the analysis.

For the Chthoniidae, a reference dataset of 76 Genbank and Pilbara specimens was reduced to a more manageable and relevant dataset by selecting only the 40 reference specimens that occurred in the same clades as the phase 4 – 6 specimens, based on a preliminary neighbour joining analysis. Two Genbank voucher sequences of Chthoniidae were included in the analysis, *Paraliochthonius* sp JM 2008 (Genbank accession #EU5595505) and *Tyrannochthonius aridus* (GenBank accession # KJ659959 from Western Australia) and in addition, as local references, 38 specimens of Chthoniidae from previous surveys in the Pilbara. Sequences of the scorpion *Pandinus imperator* (Genbank accession # AY1565821) and harvestman spider *Siro rubens* (Genbank accession # DQ5131111) were used as outgroups.

##### *Phylogenetic analyses*

The phylogenetic analysis of the Hyiidae, which included one specimen from Mesa H phase 4 (KE03) and one from phase 6 (KU28), in addition to 28 reference specimens of Hyiidae, placed the Mesa H specimens in two distinct genetic lineages, neither of which contained reference specimens (Figure 5). KE03 therefore was assigned to the new lineage PH026 and KU28 was assigned to the new lineage PH027 (Figure 5). KE03/PH026 formed a well-supported clade with specimens IV206 and IV209 from the Robe Valley (lineage PH001) and KU28 formed a well-supported clade with specimen JF56 (lineage PH017) from a previous survey at Mesa H and IV213 (lineage PH007) from the Robe Valley (Figure 5).

The phylogenetic analysis of the Olpiidae, which included the two specimens from Mesa H phase 5 (KU03, KU06), in addition to 15 reference specimens, placed the Mesa H specimens in two distinct genetic lineages, neither of which contained reference specimens (Figure 6). KU03 therefore was assigned to the new lineage PO014 and KU06 was assigned to the new lineage PO015 (Figure 6). KU03/PO014 and KU06/PO015 formed a well-supported clade with one another and two specimen of *Beierolpium*: specimen BQ20 from Wonmunna and the Genbank voucher specimen of *Beierolpium bornemisszai* (Figure 6).

The phylogenetic analysis of the Chthoniidae, which included the one Chthoniidae specimen from Mesa H phase 6 (KU29), in addition to 40 reference specimens, placed the Mesa H specimen in a distinct genetic lineage that did not contain any reference specimens (Figure 7). KU29 was therefore assigned to the new lineage PC055 (Figure 7). However the lineage showed significant although distant relationships with some reference specimens (Figure 7). Specifically, specimen KU29/PC055 formed a well-supported clade with specimens JU05, JU06 and JU08 from a previous survey at Mesa H and specimens IV210, IV268, IV269 and KD27, from previous surveys at Mesas A and B in the Robe Valley (Figure 7).

#### *Differentiation within and between lineages*

The two specimens of Hyiidae from Mesa H (KE03 and KU28) differed from one another by 24.9% sequence divergence (Table 8). Specimen KE03 differed from the nearest reference specimens IV206 and IV209 by 7.3 sequence divergence (Table 8). Specimen KU28 differed from the nearest reference specimens JF56 from Mesa H and IV213 from the Robe Valley by 4.0 and 7.1% sequence divergence, respectively (Table 8).

The two specimen of Olpiidae from the Mesa H (KU03 and KU06) differed from one another by 17.0% sequence divergence and from the reference specimens of *Beierolpium* (*B. bornemisszai* and BQ20) by between 16.6 and 23.8% sequence divergence (Table 9).

The specimen of Chthoniidae from Mesa H (KU29) differed from the nearest references (three specimens from a previous survey at Mesa H, JU05, JU06, JU08 and four specimens from Mesa A and B in the Robe Valley, IV210, IV268, IV269, KD27) by more than 16% sequence divergence (Table 10).

#### Schizomida

##### *Preliminary analysis - Reference sequences and outgroups*

Five schizomids from phases 4 – 6 were sequenced from Mesa H (Table 11). The specimens were analysed with >700 reference sequences of schizomids from previous surveys in the Pilbara to compile a manageable and relevant reference data set. The reference sequences were selected based on the criteria that they showed  $\leq 15\%$  sequence divergence from at least one of the Mesa H lineages. A reference dataset of 28 reference specimens was selected, 20 from previous surveys in the Pilbara and eight Genbank voucher sequences as follows:

*Paradraculoides anachoretus* (Genbank accession # EU272709), *Paradraculoides eremius* (Genbank accession # KU291135), *Paradraculoides* sp. (Genbank accession # EU272697), *Paradraculoides bythius* (Genbank accession # EU272715), *Paradraculoides gnophicola* (Genbank accession # EU272716), *Paradraculoides kryptus* (Genbank accession # EU272724), *Draculoides vinei* (Genbank accession # EU272694), *Draculoides julianneae* (Genbank accession # EU272696) A specimen of schizomid from the family Hubbardiidae, *Brignolozomus woodwardi* (GenBank accession # EU272675) was used as an outgroup.

##### *Phylogenetic analysis*

The phylogenetic analysis, which included the five Mesa H specimens, in addition to 28 selected reference specimens of schizomids, placed the Mesa H specimens in three distinct lineages, two of which also contained reference specimens from previous surveys at Mesa H and the Robe Valley (Figure 8). Thus two of the specimens (KE02, KU30) were assigned to the existing lineage SCH038 and two (KU04, KU05) were assigned to the existing lineage SCH015 (Figure 8). The last



specimen KU3, was placed in a lineage that did not contain any reference specimens and thus was assigned to the new lineage SCH039 (Figure 8). Lineages SCH038 and SCH039 were placed in a well-supported clade containing the six Genbank reference specimens of *Paradraculoides* (Figure 8). Lineage SCH015 was placed in a clade that was distinct from both the *Paradraculoides* and *Draculoides* clades (Figure 8).

#### *Differentiation within and between lineages*

The five specimen of schizomids detected at Mesa H differed from one another by between 0 and 13.0% sequence divergence (Table 12). The two specimens in lineage SCH038 (KE02, KU30) differed from one another by 2.4% sequence divergence and the two specimens in lineage SCH015 (KU04, KU05) were genetically identical (Table 12).

Two of the lineages differed from the closest reference specimens by <3% sequence divergence (Table 12). Specifically, specimens KE02 and KU30 (lineage SCH038) differed from reference specimen JF48 by 0.8% sequence divergence but from the next nearest reference specimens (IT67 and HE12) by >10% (Table 12). Specimens KU04 and KU05 differed from the nearest reference specimens JF50 and GH11 by 2.1% and 2.7%, respectively, and from JU03 by 4.3% sequence divergence (Table 12). Specimens KU04 and KU05 differed from the remaining reference specimens by >12% sequence divergence (Table 12).

### **Conclusions**

COI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003a). In a comparison of COI sequences for over 13,000 pairs of taxa, Hebert et al (2003b) found a mean of 11.1% sequence divergence between distinct species. Nearly 80% of the comparisons showed that species pairs differed from one another by greater than 8% sequence divergence. However, a taxon by taxon approach, examining the amount of phylogenetic variation within and between species is the most widely accepted method of defining species.

#### Isopoda

Two distinct genetic lineages of Isopoda were detected at Mesa H, one of which was most similar to reference specimens of Armadillidae, and one of which was most similar to reference specimens of Philosciidae. Both lineages were assigned to new lineages, ISA057 and ISP055, respectively, because they differed from the reference lineages by > 3%.

While each is a new lineage, the Mesa H lineages did show moderately close relationships to reference specimens, which require further consideration. The Mesa H Armadillidae lineage ISA057 differed from a reference specimen at Mesa C by 4.5% sequence divergence and from the *Spherillo* specimen from Japan by 6.1%. These values are greater than the intraspecific variation generally observed within Isopoda lineages (<1%; Hutchins, 2007; Finston et al., 2009), however, given the geographic distance between sites where this clade was detected, this could represent a pattern of genetic diversity associated with geographic distance. Sampling intermediate sites may help determine whether there is a sharp break between haplotypes (this might indicate the presence two different species) or if there is a gradual shift from one haplotype to the next over that geographical space (this would suggest connectivity among populations and imply that we are observing variation within a single species). It is worth noting that lineage ISA057 (KU23) was highly divergent from the remaining reference specimens of Armadillidae, so it is only tentatively placed in that family, however, it was most closely related to Armadillidae in both the Helix and GenBank databases.

Mesa H Philosciidae lineage ISP055 differed from reference specimen JF45 from a previous survey at Mesa H by 5.1%. In contrast to the Armadillidae, this level of genetic variation is detected over a relatively short geographic distance – both samples were taken from Mesa H. This would suggest a lack of gene flow between populations despite being geographically close, and may indicate the presence of two recently-separated but distinct species. Again, sampling of intermediate areas may help resolve this relationship.

#### *Armadillidae*

**ISA057** = new lineage; species likely to have been detected previously at Mesa C but requires further investigation.

*Philosciidae*

**ISP055** = new lineage; species may be new but requires further investigation

Diplura

Four distinct genetic lineages of Diplura were detected at Mesa H, one of which was most similar to reference specimens of Japygidae, two of which were most similar to reference specimens of Projapygidae, and one of which was most similar to reference specimens of Parajapygidae. All four lineages were assigned to new lineages, DJA011, DPR010, DPR011 and DPA009, because they differed from the reference lineages by > 3%.

While each is a new lineage, one Mesa H lineage did show a moderately close relationship to a reference specimen, which requires further consideration. The Mesa H Projapygidae lineage DPR011 differed from reference specimen JF40 from a previous survey at Mesa H by 3.6% sequence divergence and from JF38 by 5.1% sequence divergence. These values are greater than the intraspecific variation normally observed within Diplura lineages (mean = 1.9% for species in the genus *Japyx*; Bu et al, 2012). This would suggest limited gene flow between populations in this clade despite being geographically close, although we may be observing a 'stepping-stone' pattern of genetic diversity, where nearby populations exchange genes, whereas those at the extreme ends of the continuum do not. Again, sampling of intermediate areas may help resolve this relationship.

*Japygidae*

**DJA011**= new lineage; new species

*Parajapygidae*

**DPA009**= new lineage, new species

*Projapygidae*

**DPR010**= new lineage, new species

**DPR011**= new lineage; species may have been detected previously at Mesa H, but requires further investigation

Pseudoscorpiones

Five distinct genetic lineages of pseudoscorpions were detected at Mesa H phases 4 - 6: one Chthoniidae, two Olpiidae and two Hyiidae.

The two lineages of Hyiidae detected at Mesa H (PH026 and PH027) differed from one another by 24.9% sequence divergence, indicating that they represent two distinct species. While both lineages were new, differing from the reference specimens by >3% sequence divergence, one, PH027, is likely to belong to a species that has been detected previously at Mesa H, differing from reference specimen JF56 (lineage PH017) by 4.0% sequence divergence. This level of genetic differentiation however, indicates that gene flow may be low between sampling sites, perhaps owing to the poor dispersal ability of the taxon or geographic barriers. Lineages PH027 and PH017 in turn form a well-supported clade with specimen IV213 (lineage PH007) from the Robe Valley. The three specimens differ from one another by between 6.9 and 7.1% sequence divergence. This suggests that while closely related, the Robe Valley lineage is likely to represent a distinct species from the Mesa H lineage, although sampling intermediate sites may help resolve relationships between these lineages.

The second lineage of Hyiidae, PH026, formed a relatively close relationship with lineage PH003 (specimens IV206 and IV209) from the Robe Valley, forming a well-supported clade, and differing by 7.3% sequence divergence. Distances between specimens assigned to the same species in previous studies in the Pilbara (i.e. PC047, PH002) differ by  $\leq 2.0\%$ , so the distance between PH026 and PH003 is higher than appears typical for the Pseudoscorpiones. This suggests that while closely related, the two lineages are likely to represent distinct species, although sampling intermediate sites may help resolve relationships between these lineages.

The two lineages of Olpiidae detected at Mesa H, PO014 and PO015, represent both new lineages and new species, as they differed from the nearest reference specimens by >16% sequence divergence. However, we can tentatively assign both lineages to the genus *Beierolpium*, as PO014 and PO015 were both placed in a clade containing reference specimens of that genus.

The lineage of Chthoniidae detected at Mesa H, PC055, represents both a new lineage and a new species, as it differed from the nearest reference specimens by >16% sequence divergence.

#### *Hyiidae*

**PH026** = new lineage, new species

**PH027** = new lineage, species detected previously at Mesa H

#### *Olpiidae*

**PO014** = new lineage, new species of *Beierolpium*

**PO015** = new lineage, new species of *Beierolpium*

#### *Chthoniidae*

**PC055** = new lineage, new species

#### Schizomida

Previous analyses of genetic variation between morphologically distinct species of Schizomida can be used as a genetic 'yardstick' to interpret the current data set. The five described species of *Paradraculoides* (Harvey et al, 2008) differ from one another by between 8.4 to 12.1% sequence divergence (uncorrected p-distances; calculated by us from the Harvey et al., 2008 data). Similarly, the four described species of *Draculoides* differ from one another by between 4.5 to 13.7% sequence divergence (uncorrected p-distances calculated by us from Harvey et al., 2008).

Three lineages of Schizomida were detected at Mesa H, phases 4 – 6, two *Paradraculoides* (SCH038, SCH039), and one not assigned to a family (SCH015). Two of the lineages (SCH015, SCH038) have been detected previously at Mesa H and in the Robe Valley, whereas one (SCH039) is new.

One of the *Paradraculoides* lineages, SCH038, is a lineage and species that has been detected previously at Mesa H, however, the new lineage, SCH039, likely represents a new species, as it differs from the nearest reference specimen by >7%. However, this level of sequence divergence is only moderate, and the nearest reference specimen was collected in Buckland Hills, thus sampling intermediate sites may resolve the relationship between these two lineages.

The lineage that we could not assign to a family (SCH015) has been detected previously at Mesa H and a second site in the Robe Valley. It does not appear to belong to *Draculoides*, *Paradraculoides* or *Bamazomus* (*Bamazomus* not shown), as it was placed in a clade that was distinct from the clades of these families. A closely related lineage (SCH016) was detected during a previous survey at Mesa H. Lineages SCH015 and SCH016 differ from one another by 4.3% and are likely to represent the same species, however, this level of divergence is somewhat elevated, given that the two lineages were both collected at Mesa H. This suggests there may be a barrier to gene flow at Mesa H between RC16JIM and RC15MEH.

#### *Paradraculoides*

**SCH038** = lineage and species detected previously at Mesa H

**SCH039** = new lineage, new species

#### *Unknown family*

**SCH015** lineage and species detected previously at Mesa H and the Robe Valley

## References

- Bu Y., Gao Y, Potapov M. B., Luan Y. (2012). Redescription of arenicolous dipluran *Parajapyx pauliani* (Diplura, Parajapygidae) and DNA barcoding analyses of *Parajapyx* from China. *ZooKeys* 221: 19-29.
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2011) Geneious v5.4, <http://www.geneious.com/>
- Finston, Francis, Johnson (2009). Biogeography of the stygobitic isopod *Pygolabis* (Malacostraca: Tainisopidae) in the Pilbara, Western Australia: evidence for multiple colonisations of the groundwater. *Molecular Phylogenetics and Evolution* 52:448-460.
- Harvey, M. S., Berry, O. Edward, K. L., Humphreys, G. (2008) Molecular and morphological systematics of hypogean schizomids (Schizomida: Hubbardiidae) in semiarid Australia. *Invertebrate Systematics* 22: 167-194.
- Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard J.R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* 270: 313-321.
- Hebert, P.D.N., Ratnasingham, S., deWaard J.R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B (supplement)* 270: S96-S99.
- Posada, D., Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- Thompson, J., Higgins, D., and Gibson, T. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680. doi:10.1093/nar/ 22.22.4673



Table 1. Specimens of Isopoda used in the present study and the lineage to which they were assigned, based on variation at the COI gene. \*=new lineage this study.

| Specimen ID                | Phase | Cap no. | New cap no. | Helix ID | Lineage |
|----------------------------|-------|---------|-------------|----------|---------|
| RC16MEH0427.20161026.T3-01 | 4     | 56      | -           | KE01     | ISP055* |
| MEHRD0577.20170130.T1-02   | 6     | 13      | 13          | KU09     | No amp  |
| MEHRD0577.20170130.T1-02   | 6     |         | 13c         | KU10     | No amp  |
| MEHRD0577.20170130.T1-02   | 6     |         | 13b         | KU11     | No amp  |
| MEHRD0577.20170130.T2-01   | 6     | 15      | 15          | KU12     | No amp  |
| MEHRD0577.20170130.T2-01   | 6     |         | 15b         | KU13     | ISA057* |
| MEHRD0577.20170130.T2-01   | 6     |         | -           | KU14     | ISA057* |
| MEHRD0577.20170130.T3-01   | 6     | 17      | 17          | KU15     | No amp  |
| MEHRD0577.20170130.T3-01   | 6     |         | 17b         | KU16     | No amp  |
| MEHRD0577.20170130.T3-01   | 6     |         | 17c         | KU17     | No amp  |
| MEHRD0577.20170130.T3-01   | 6     |         | -           | KU18     | No amp  |
| MEHRD0577.20170130.T3-01   | 6     |         | -           | KU19     | ISA057* |
| MEHRD0577.20170130.T3-01   | 6     |         | -           | KU20     | ISA057* |
| MEHRD0577.20170130.T3-01   | 6     |         | -           | KU21     | No amp  |
| MEHRD0834.20170130.T1-01   | 6     | 21      | 21          | KU22     | ISA057* |
| MEHRD0834.20170130.T1-01   | 6     |         | 21b         | KU23     | ISA057* |
| MEHRD0834.20170130.T1-01   | 6     |         | -           | KU24     | ISA057* |
| MEHRD0834.20170130.T1-01   | 6     |         | -           | KU25     | ISA057* |
| MEHRD0834.20170130.T1-01   | 6     |         | -           | KU26     | No amp  |
| MEHRD0834.20170130.T2-01   | 6     | 23      | 23          | KU27     | ISA057* |

Table 2. Mean genetic distance (D) and standard error (s.e.) within lineages of Isopoda as shown in Figure 1. N= number of specimens in the lineage; Rep= specimen used to represent the lineage in the model-based phylogenetic analysis. n/c= not calculated where the number of specimens in the lineage is 1.

| Lineage | D     | s.e.  | N | Rep  |
|---------|-------|-------|---|------|
| 1       | 0.001 | 0.001 | 9 | KU23 |
| 2       | n/c   | n/c   | 1 | KE01 |

Table 3 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Armadillidae (Isopoda) detected at Mesa H and the reference lineages as shown in Figure 2. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

Table 4 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Philosciidae (Isopoda) detected at Mesa H and the reference lineages as shown in Figure 3. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

Table 5. Specimens of Diplura used in the present study and the lineage to which they were assigned, based on variation at the COI gene. \*=new lineage this study.

| Specimen ID                | Phase | Helix ID | Cap no. | New cap no. | Lineage |
|----------------------------|-------|----------|---------|-------------|---------|
| DD13MEH0007-20160914-01    | 4     | KE04     | 22      | 22          | DJA011* |
| RC16JIM0005.20161212.T1-01 | 5     | KU1      | 48      | 48          | DPR010* |
| RC16JIM0005.20161212.T3-01 | 5     | KU2      | 50      | 50          | DPR011* |
| RC16MEH0264.20170130.T3-01 | 6     | KU7      | 3       | 3           | No amp  |
| MEHRD0758.20170130.T3-01   | 6     | KU8      | 8       | 8           | DPA009* |

Table 6 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Diplura detected at Mesa H and the reference lineages as shown in Figure 4. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

Table 7. Specimens of Pseudoscorpiones used in the present study and the lineage to which they were assigned, based on variation at the COI gene. \*=new lineage this study.

| Specimen ID                | Phase | Cap no. | New cap no. | Helix ID | Lineage |
|----------------------------|-------|---------|-------------|----------|---------|
| RC16MEH0436.20161026.T2-01 | 4     | 85      | 1           | KE03     | PH026*  |
| RC16JIM0026.20161212.T2-01 | 5     | 21      | 21          | KU03     | PO014*  |
| RC16MEH0433.20161211.T1-02 | 5     | 47      | 47          | KU06     | PO015*  |
| RC16MEH0264.20170130.T3-03 | 6     | 5       | 5           | KU28     | PH027*  |
| MEHRD0834.20170130.T2-02   | 6     | 24      | 24          | KU29     | PC055*  |

Table 8 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Hyiidae detected at Mesa H and the reference lineages as shown in Figure 5. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

Table 9 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Ophiidae detected at Mesa H and the reference lineages as shown in Figure 6. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

Table 10 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Chthoniidae detected at Mesa H and the reference lineages as shown in Figure 7. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

Table 11. Specimens of Schizomida used in the present study and the lineage to which they were assigned, based on variation at the COI gene. \*=new lineage this study.

| Specimen ID                | Phase | Helix ID | Old cap no. | New cap no. | Lineage |
|----------------------------|-------|----------|-------------|-------------|---------|
| RC16MEH0433.20161026.T2-01 | 4     | KE02     | 71          | 71          | SCH038  |
| RC16JIM0026.20161212.T1-01 | 5     | KU04     | 23          | 23          | SCH015  |
| RC16JIM0031.20161212.T2-01 | 5     | KU05     | 16          | 16          | SCH015  |
| RC16MEH0264.20170130.T3-02 | 6     | KU30     | 4           | 4           | SCH038  |
| RC15MEJ0019.20170130.T1-01 | 6     | KU31     | 18          | 18          | SCH039* |

Table 12. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Schizomida detected at Mesa H and the reference lineages as shown in Figure 8. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

Figure 1. Neighbour-joining analysis of specimens of Isopoda from the present study. Numbers on major nodes correspond to bootstrap support over 100 iterations; values <50% are not shown. Scale bar= genetic distance. The specimens used to represent each lineage in the model-based phylogenetic analysis are highlighted in yellow.

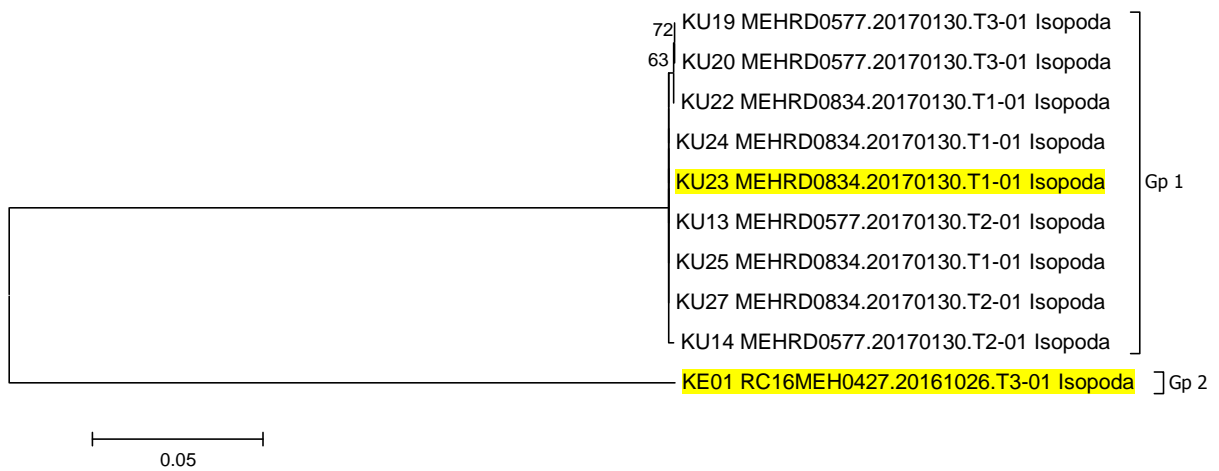
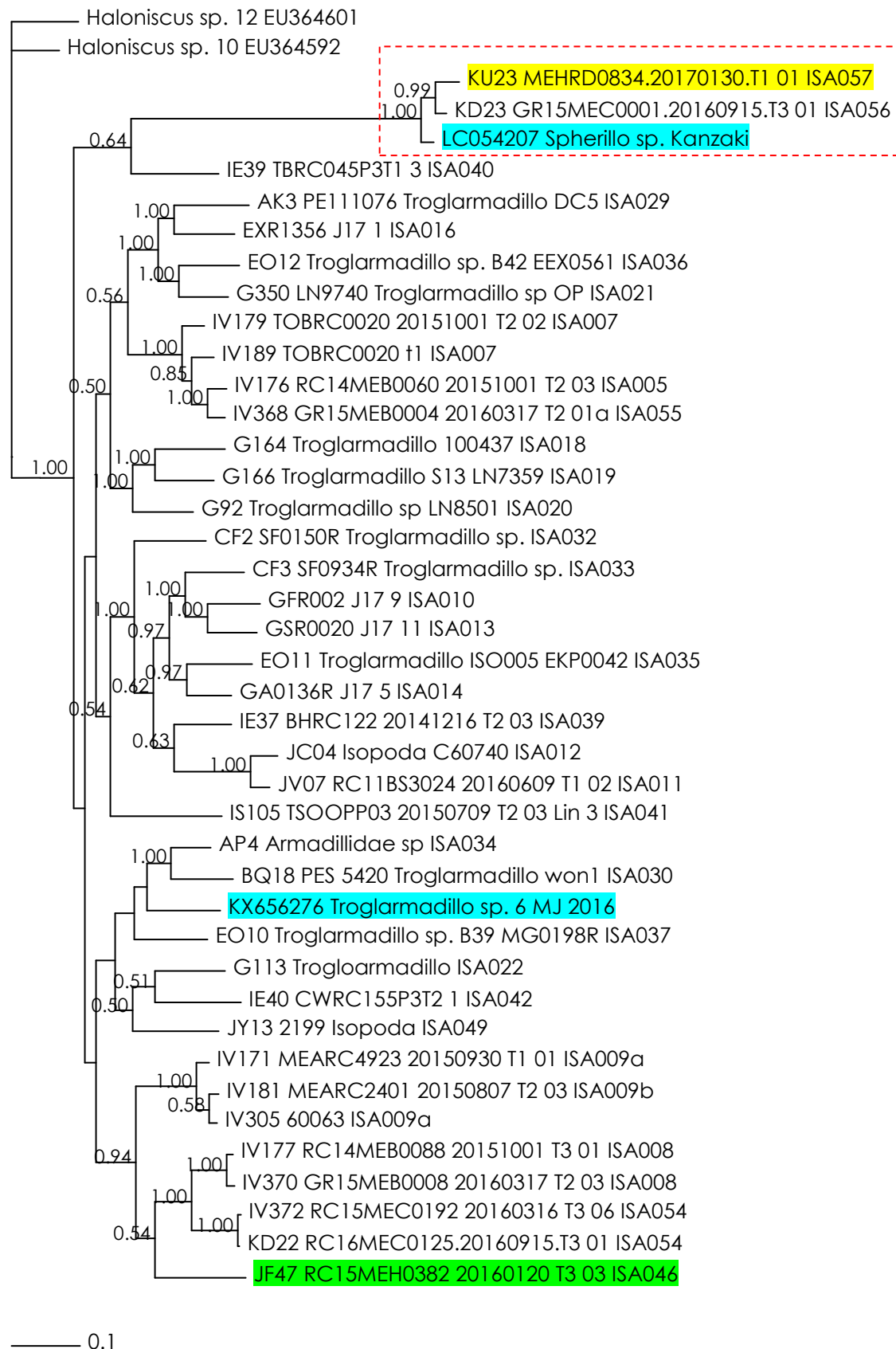




Figure 2. Bayesian analysis of COI haplotypes of Armadillidae from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; specimens from previous phases at Mesa H are highlighted in green; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes enclose species with high confidence; red boxes with dashed lines represent groups requiring further investigation.



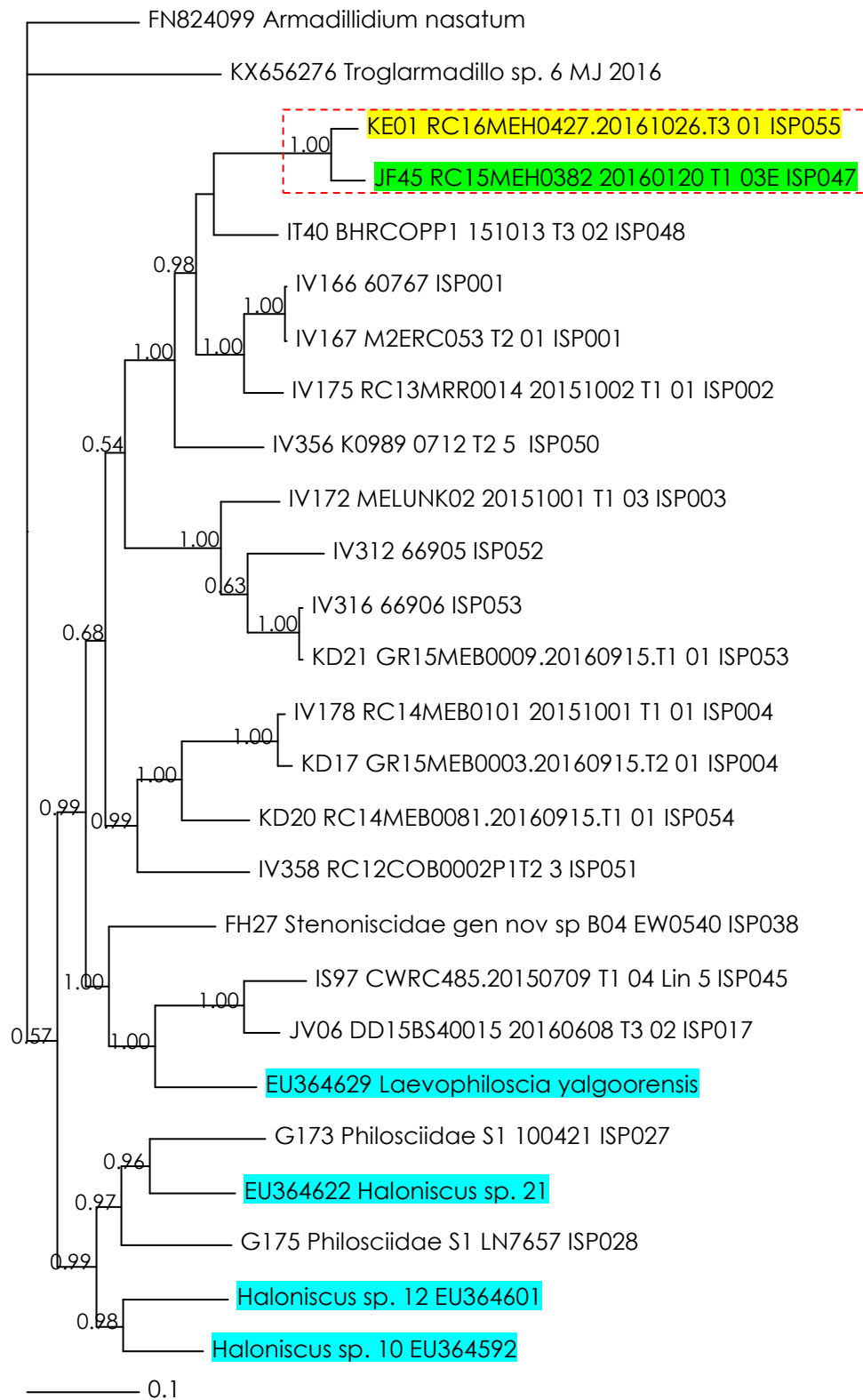


Figure 4. Bayesian analysis of COI haplotypes of Diplura from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; specimens from previous phases at Mesa H are highlighted in green; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes with solid lines enclose species with high confidence; red boxes with dashed lines represent groups requiring further investigation.

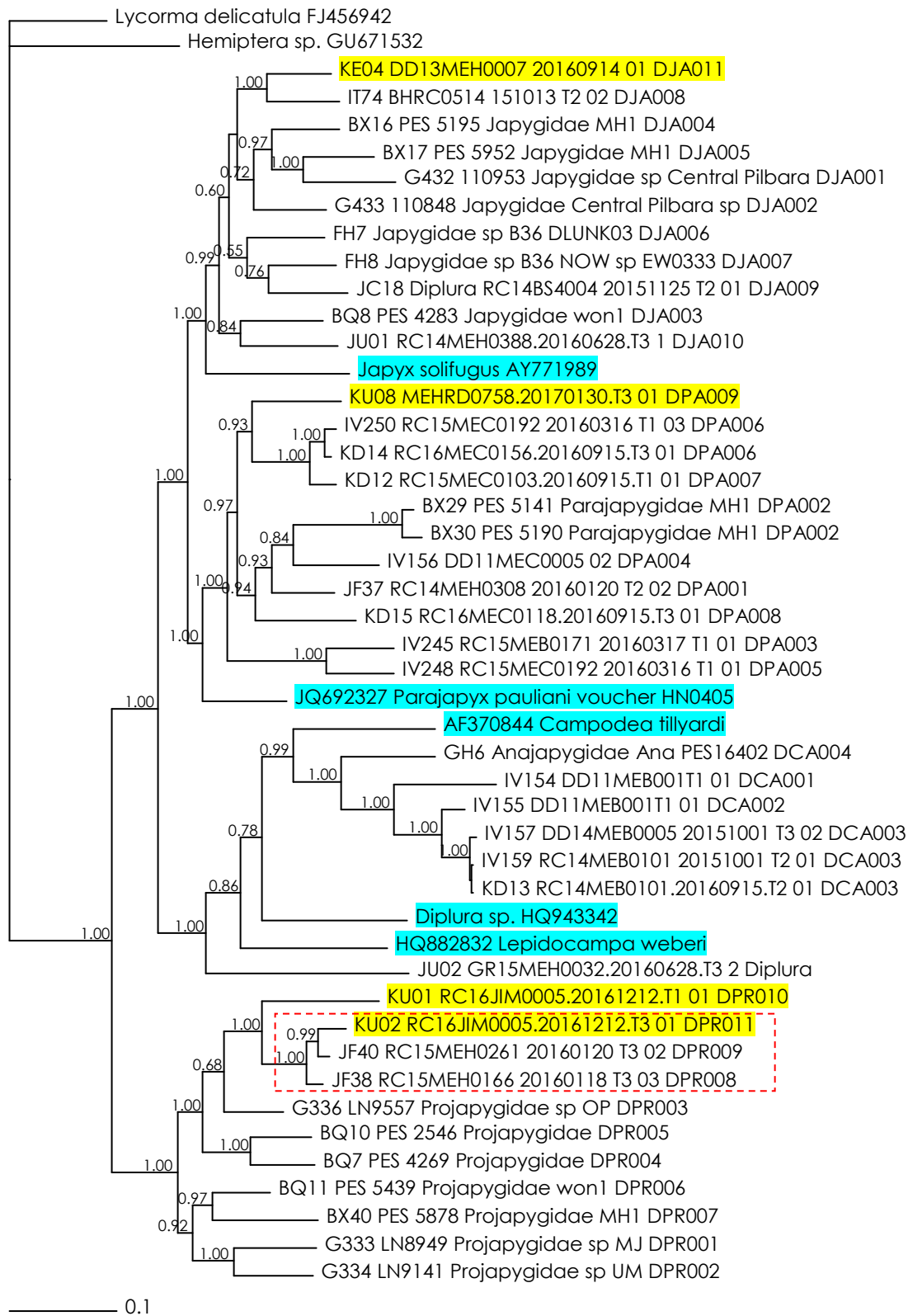






Figure 6. Bayesian analysis of COI haplotypes of Olpiidae and Atemnidae (Pseudoscorpiones) from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; specimens from previous phases at Mesa H are highlighted in green; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes with solid lines enclose species with high confidence.

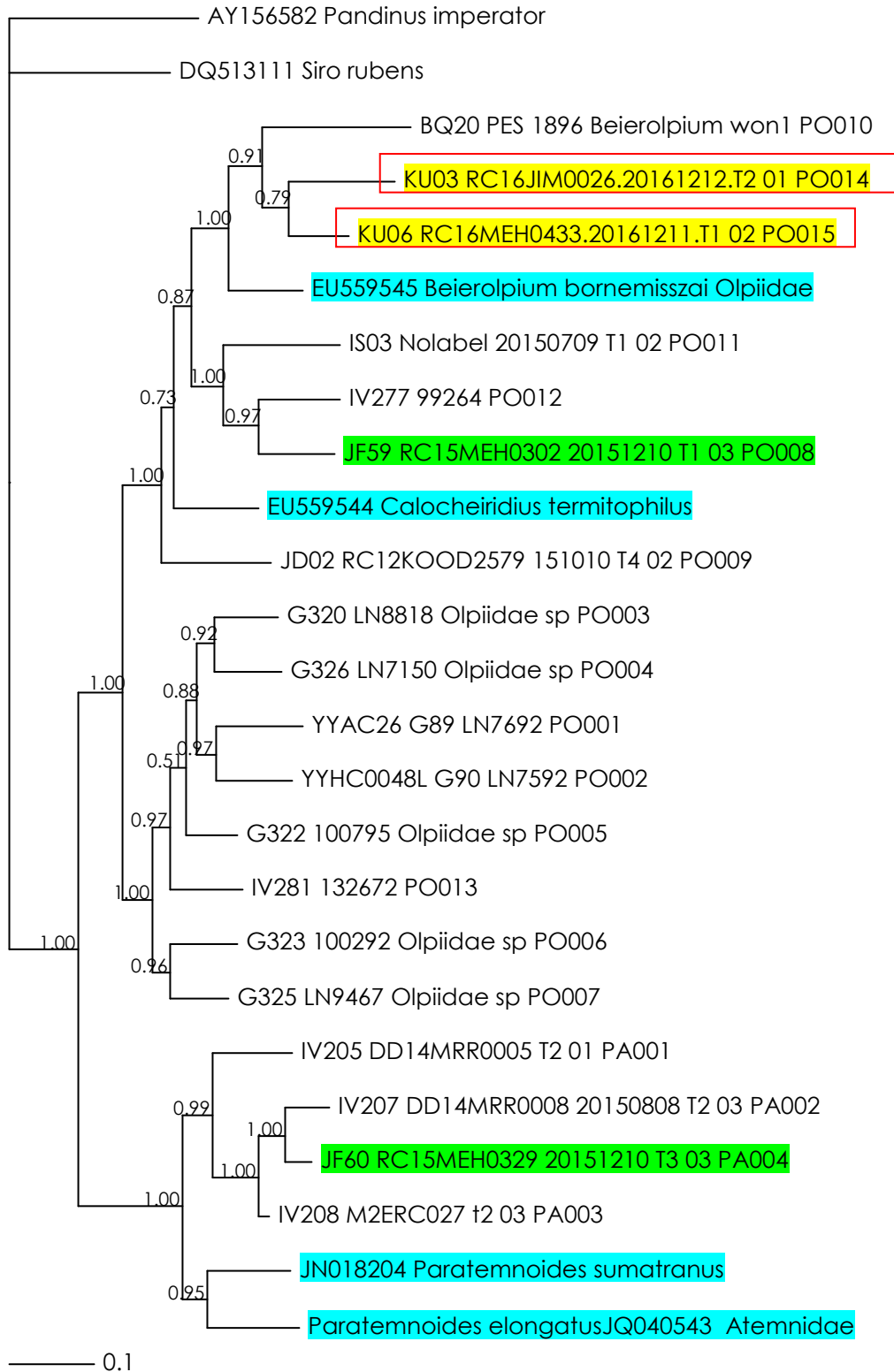


Figure 7. Bayesian analysis of COI haplotypes of Chthoniidae (Pseudoscorpiones) from the present study and reference specimens from Genbank and the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; specimens from previous phases at Mesa H are highlighted in green; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes with solid lines enclose species with high confidence.

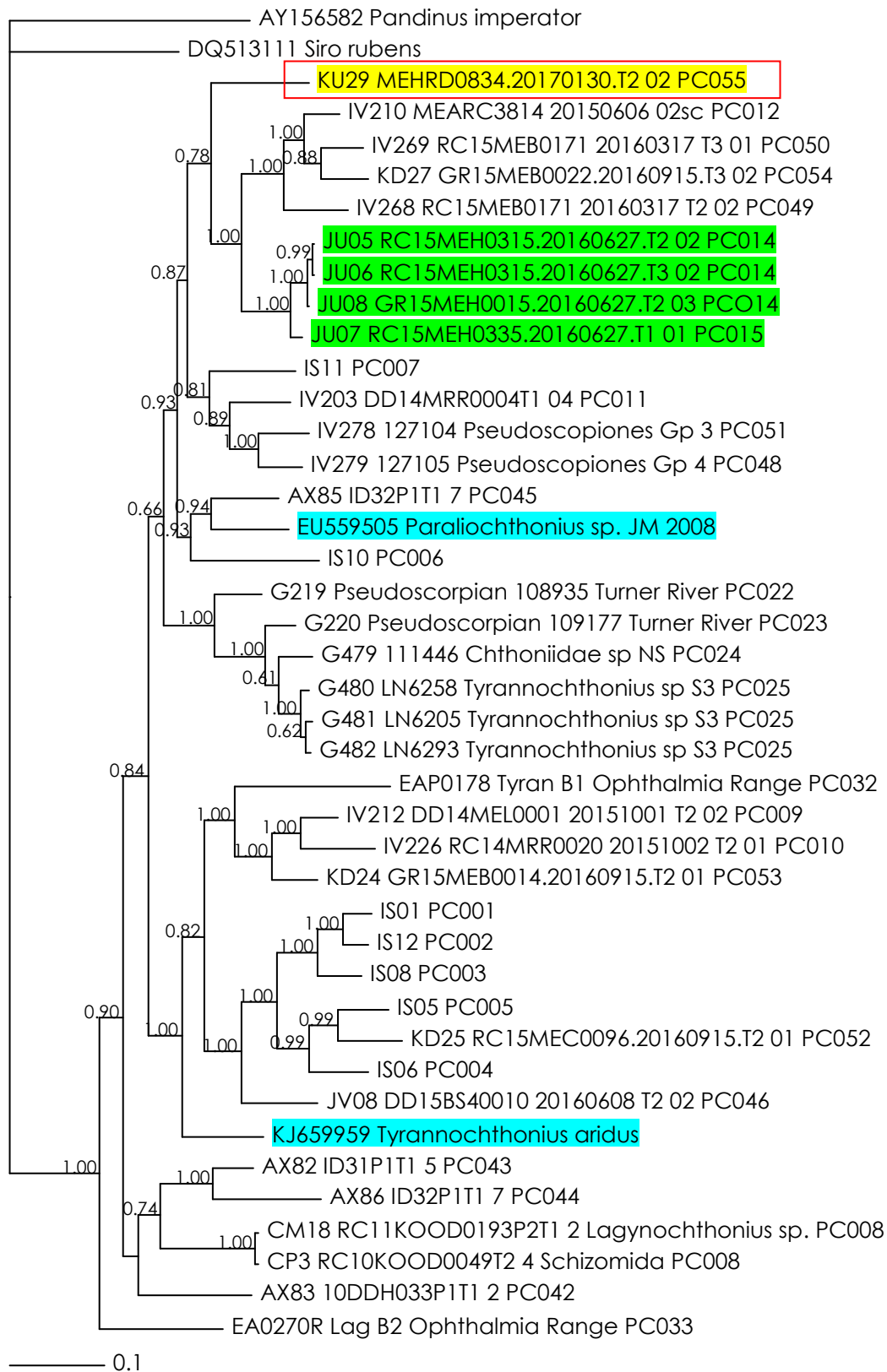
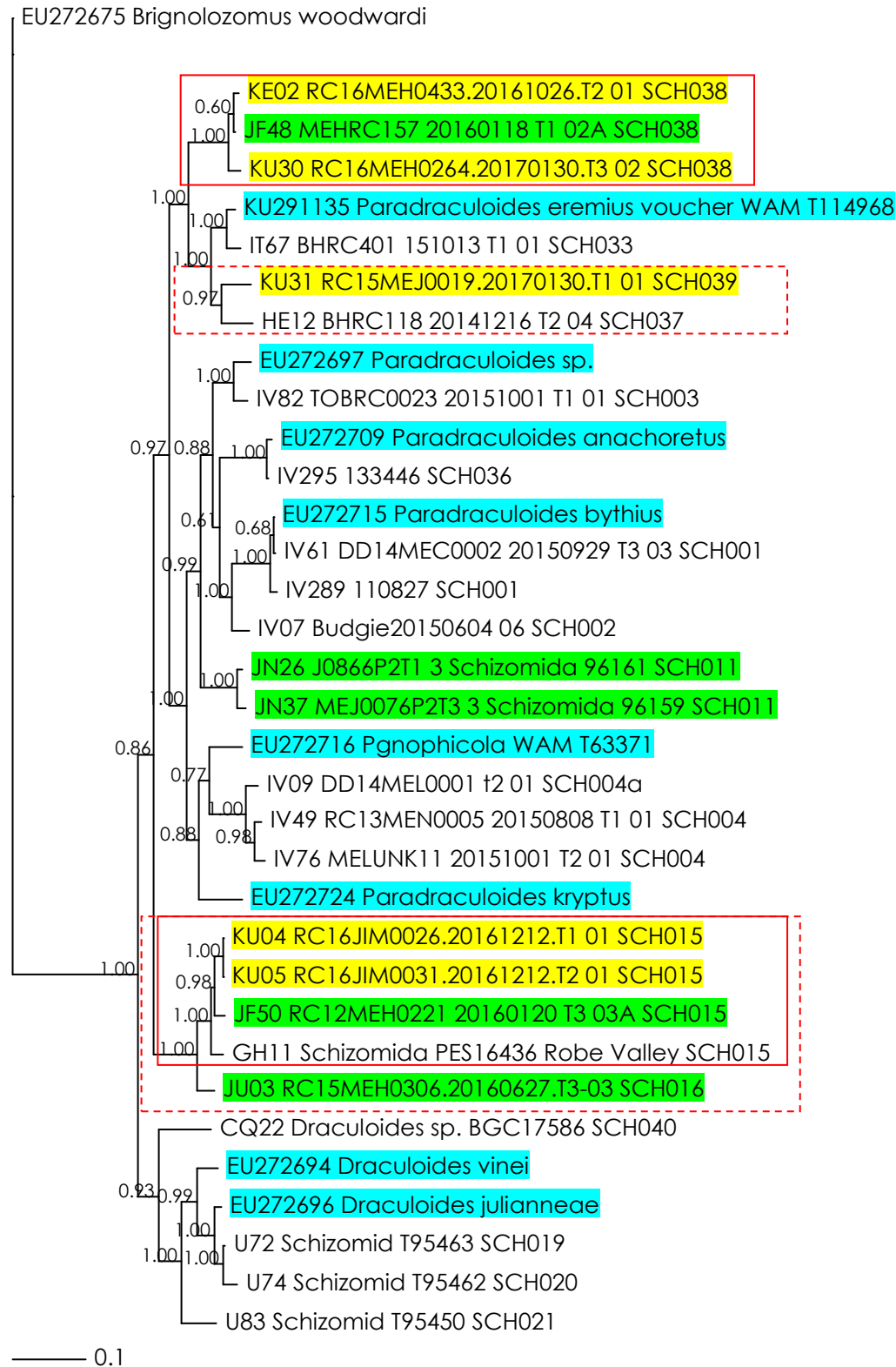


Figure 8. Bayesian analysis of COI haplotypes of Schizomida from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; specimens from previous phases at Mesa H are highlighted in green; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes with solid lines enclose species with high confidence.



Appendix 1. All specimens of Isopoda from Mesa H phases 1-6, and the lineage to which they were assigned. Lineages with name changes between phases are highlighted in yellow.

| Specimen ID                 | Phase | Helix ID | Old lineage | New Lineage |
|-----------------------------|-------|----------|-------------|-------------|
| RC15MEH0382-20160120-T1-03A | 1     | JF41     | I1          | ISP047      |
| RC15MEH0382-20160120-T1-03B | 1     | JF42     | I1          | ISP047      |
| RC15MEH0382-20160120-T1-03C | 1     | JF43     | I1          | ISP047      |
| RC15MEH0382-20160120-T1-03D | 1     | JF44     | I1          | ISP047      |
| RC15MEH0382-20160120-T1-03E | 1     | JF45     | I1          | ISP047      |
| RC15MEH0382-20160120-T2-03  | 1     | JF46     | I1          | ISP047      |
| RC15MEH0382-20160120-T3-03  | 1     | JF47     | I2          | ISA046      |
| RC16MEH0427.20161026.T3-01  | 4     | KE01     |             | ISP055*     |
| MEHRD0577.20170130.T1-02    | 6     | KU09     |             | No amp      |
| MEHRD0577.20170130.T1-02    | 6     | KU10     |             | No amp      |
| MEHRD0577.20170130.T1-02    | 6     | KU11     |             | No amp      |
| MEHRD0577.20170130.T2-01    | 6     | KU12     |             | No amp      |
| MEHRD0577.20170130.T2-01    | 6     | KU13     |             | ISA057*     |
| MEHRD0577.20170130.T2-01    | 6     | KU14     |             | ISA057*     |
| MEHRD0577.20170130.T3-01    | 6     | KU15     |             | No amp      |
| MEHRD0577.20170130.T3-01    | 6     | KU16     |             | No amp      |
| MEHRD0577.20170130.T3-01    | 6     | KU17     |             | No amp      |
| MEHRD0577.20170130.T3-01    | 6     | KU18     |             | No amp      |
| MEHRD0577.20170130.T3-01    | 6     | KU19     |             | ISA057*     |
| MEHRD0577.20170130.T3-01    | 6     | KU20     |             | ISA057*     |
| MEHRD0577.20170130.T3-01    | 6     | KU21     |             | No amp      |
| MEHRD0834.20170130.T1-01    | 6     | KU22     |             | ISA057*     |
| MEHRD0834.20170130.T1-01    | 6     | KU23     |             | ISA057*     |
| MEHRD0834.20170130.T1-01    | 6     | KU24     |             | ISA057*     |
| MEHRD0834.20170130.T1-01    | 6     | KU25     |             | ISA057*     |
| MEHRD0834.20170130.T1-01    | 6     | KU26     |             | No amp      |
| MEHRD0834.20170130.T2-01    | 6     | KU27     |             | ISA057*     |



Appendix 2. All specimens of Diplura from Mesa H phases 1-6, and the lineage to which they were assigned. Lineages with name changes between phases are highlighted in yellow.

| Specimen ID                | Phase | Helix ID | Old lineage | New lineage |
|----------------------------|-------|----------|-------------|-------------|
| RC14MEH0308-20160120-T2-02 | 1     | JF37     | D1          | DPA001      |
| RC15MEH0166-20160118-T3-03 | 1     | JF38     | D3          | DPR008      |
| RC15MEH0175-20151210-T1-01 | 1     | JF39     | D3          | DPR008      |
| RC15MEH0261-20160120-T3-02 | 1     | JF40     | D2          | DPR009      |
| RC14MEH0388.20160628.T3-1  | 3     | JU01     | DU          | DJA003      |
| GR15MEH0032.20160628.T3-2  | 3     | JU02     | DV          | DCA005      |
| DD13MEH0007-20160914-01    | 4     | KE04     |             | DJA011*     |
| RC16JIM0005.20161212.T1-01 | 5     | KU1      |             | DPR010*     |
| RC16JIM0005.20161212.T3-01 | 5     | KU2      |             | DPR011*     |
| RC16MEH0264.20170130.T3-01 | 6     | KU7      |             | No amp      |
| MEHRD0758.20170130.T3-01   | 6     | KU8      |             | DPA009*     |

Appendix 3. All specimens of Pseudoscorpiones from Mesa H phases 1-6, and the lineage to which they were assigned. Lineages with name changes between phases are highlighted in yellow.

| Specimen ID                 | Phase | Helix ID | Old lineage | New lineage |
|-----------------------------|-------|----------|-------------|-------------|
| RC14MEH0252-20160120-T1-04  | 1     | JF56     | P1          | PH017       |
| RC12MEH0120-20151211-T1-01A | 1     | JF57     | No amp      | No amp      |
| RC12MEH0120-20151211-T1-01B | 1     | JF58     | No amp      | No amp      |
| RC15MEH0302-20151210-T1-03  | 1     | JF59     | P2          | PO008       |
| RC15MEH0329-20151210-T3-03  | 1     | JF60     | P3          | PA004       |
| RC15MEH0315.20160627.T2-02  | 3     | JU05     | P14         | PC014       |
| RC15MEH0315.20160627.T3-02  | 3     | JU06     | P14         | PC014       |
| RC15MEH0335.20160627.T1-01  | 3     | JU07     | P15         | PC015       |
| GR15MEH0015.20160627.T2-03  | 3     | JU08     | P14         | PC014       |
| RC16MEH0436.20161026.T2-01  | 4     | KE03     |             | PH026*      |
| RC16JIM0026.20161212.T2-01  | 5     | KU03     |             | PO014*      |
| RC16MEH0433.20161211.T1-02  | 5     | KU06     |             | PO015*      |
| RC16MEH0264.20170130.T3-03  | 6     | KU28     |             | PH027*      |
| MEHRD0834.20170130.T2-02    | 6     | KU29     |             | PC055*      |

Appendix 4. All specimens of Schizomida from Mesa H phases 1-6, and the lineage to which they were assigned. Lineages with name changes between phases are highlighted in yellow.

| Specimen ID                 | Phase | Helix ID | Old lineage | New lineage |
|-----------------------------|-------|----------|-------------|-------------|
| MEHRC157-20160118-T1-02A    | 1     | JF48     | S2          | SCH038      |
| MEHRC157-20160118-T1-02B    | 1     | JF49     | S2          | SCH038      |
| RC12MEH0221-20160120-T3-03A | 1     | JF50     | S1          | SCH015      |
| RC12MEH0221-20160120-T3-03B | 1     | JF51     | S1          | SCH015      |
| RC14MEH0252-20160120-T3-02  | 1     | JF52     | S1          | SCH015      |
| RC15MEH0329-20160118T3-01A  | 1     | JF53     | S2          | SCH038      |
| RC15MEH0329-20160118T3-01B  | 1     | JF54     | No amp      |             |
| DD13MEH0007-20160119-03     | 1     | JF55     | S1          | SCH015      |
| J0866P2T1-3                 | 2     | JN26     | S1          | SCH011      |
| J0866P2T2-1                 | 2     | JN27     | S1          | SCH011      |
| J0867P2T1-1                 | 2     | JN28     | S1          | SCH011      |
| J0867P2T3-1                 | 2     | JN29     | S1          | SCH011      |
| J0928P2T1-5                 | 2     | JN30     | S1          | SCH011      |
| J0928P2T1-5A                | 2     | JN31     | S1          | SCH011      |
| MEJ0007P1T1-1               | 2     | JN32     | S1          | SCH011      |
| MEJ0007P1T2-1               | 2     | JN33     | S1          | SCH011      |
| MEJ0007P1T2-1A              | 2     | JN34     | No amp      |             |
| MEJ0048P2T2-3               | 2     | JN35     | S1          | SCH011      |
| MEJ0076P2T2-4               | 2     | JN36     | No amp      |             |
| MEJ0076P2T3-3               | 2     | JN37     | S1          | SCH011      |
| MEJ0085P2T3-3               | 2     | JN38     | S1          | SCH011      |
| MEJ0085P2T3-3A              | 2     | JN39     | S1          | SCH011      |
| J0866P1T1-1                 | 2     | JN40     | S1          | SCH011      |
| J0866P1T2-1                 | 2     | JN41     | No amp      |             |
| MEJ5548altP1T2-2            | 2     | JN42     | S1          | SCH011      |
| MEJ5548altP1T3-2            | 2     | JN43     | S1          | SCH011      |
| MEJ5548altP1T3-2A           | 2     | JN44     | S1          | SCH011      |
| RC08MEJ0015P4T1-5           | 2     | JN45     | S1          | SCH011      |
| RC08MEJ0015P4T2-3           | 2     | JN46     | S1          | SCH011      |
| RC08MEJ0004P4T2-4           | 2     | JN47     | S1          | SCH011      |
| RC08MEJ0023P4T2-3           | 2     | JN48     | S1          | SCH011      |
| RC15MEH0306.20160627.T3-03  | 3     | JU03     |             | SCH016      |
| RC16MEH0433.20161026.T2-01  | 4     | KE02     |             | SCH038      |
| RC16JIM0026.20161212.T1-01  | 5     | KU04     |             | SCH015      |
| RC16JIM0031.20161212.T2-01  | 5     | KU05     |             | SCH015      |
| RC16MEH0264.20170130.T3-02  | 6     | KU30     |             | SCH038      |
| RC15MEJ0019.20170130.T1-01  | 6     | KU31     |             | SCH039      |



# Helix

## Molecular Solutions

School of Animal Biology The University of Western Australia  
Hackett Entrance No. 4 Hackett Drive Crawley WA 6009

PO Box 155  
Leederville WA 6903

t: (08) 6488 4509

f: (08) 6488 1029

abn: 32 133 230 243

w: [www.helixsolutions.com.au](http://www.helixsolutions.com.au)

29 Aug, 2016

Penny Brooshooft  
Biota Environmental Sciences  
Level 1, 228 Carr Place  
Leederville, WA 6007

Via email

**Re. Report on the molecular systematics of subfauna from Mesa H, phase 2 (Biota job 1146B)**

Dear Penny,

Following is a summary of the results of the subfauna study we have completed on the additional specimens from Mesa H from four taxonomic groups (Diplura, Pseudoscorpiones, Scolopendrida and Schizomida). Six distinct genetic lineages from the four taxonomic groups were detected. The six lineages likely represent five species. None of the species appear to have been detected previously in the Pilbara, based on the material available for comparison, although the species of Schizomida is closely related to a species detected previously at Mesa H and Pannawonica.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston, Yvette Hitchen and Dr. Oliver Berry  
Helix Molecular Solutions



## Background and Objective

Eight specimens of subfauna (belonging to four taxonomic groups (Diplura, Pseudoscorpiones, Scolopendrida, and Schizomida) were collected from Mesa H and sequenced for variation at the mitochondrial COI gene. The molecular data were assessed in order to determine the number of species present in each group and compare the results to those obtained during previous surveys that have been undertaken for these groups in the Pilbara.

## Executive summary

- Eight specimens from Mesa H belonging to four taxonomic groups were sequenced for variation at the COI gene.
- Six lineages were detected among the four groups.
- The six lineages likely represent five species.
- None of the species appear to have been detected previously in the Pilbara, based on the material available for comparison, although the species of Schizomida is closely related to a species detected previously at Mesa H and Pannawonica.

## Methods

Eight specimens of subfauna collected from Mesa H were sequenced for variation at the mitochondrial cytochrome oxidase subunit I gene (COI) using primers LCOI/HCO2 and LCOI/CIN2341.

Sequences were edited using GENEIOUS software (Drummond *et al.* 2011). Alignment was performed with CLUSTAL W (Thompson *et al.* 1994) using default parameters. Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotide differences between sequences).

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data for each taxonomic group. Bayesian analysis was used to construct the phylogenetic trees, incorporating the model as identified in MODELTEST for each taxonomic group. The phylogeny, branch lengths and posterior probabilities were obtained by running two trees simultaneously, each running four simultaneous MCMC chains. The number of cycles needed was determined by the standard deviation of the split frequencies of the two trees. The analysis was paused after every  $1 \times 10^6$  generations and when the standard deviation fell below 0.01, the analysis was stopped. A majority rule consensus tree was constructed after discarding the "burn-in" trees in both analyses. The burn-in value was determined by plotting the posterior probabilities obtained after every generation and identifying the point at which the values reach stationarity (= the asymptote). Trees produced prior to stationarity were discarded.

For the purposes of this report, lineages were defined as haplotypes or groups of haplotypes differing from other such groups by >3% sequence divergence. This cut-off was selected based on bar-coding data, which indicates that intra-specific variation rarely exceeds 3% (Hebert *et al.*, 2003b).

## Results

### Diplura

#### *Reference specimens and outgroups*

Two specimens of Diplura from two drillholes at Mesa H were sequenced (Table 1). The two specimens were analysed with 30 specimens of Diplura from the Pilbara belonging to four families: Anajapygidae, Japygidae, Parajapygidae and Projapygidae, as well as four Genbank voucher specimens, *Diplura* sp. (Genbank accession #HQ943342), *Japyx solifugus* (Japygidae; Genbank accession #AY771989) *Campodea tillyardi* (Campodeidae; Genbank accession #AF370844), *Lepidocampa weberi* (Campodeidae; Genbank accession #HQ882832). The planthopper *Lycorma delicatula* (Genbank accession # FJ456942) and a specimen of Fulgoridae (Hemiptera; Genbank accession #GU671563) were used as outgroups.

#### *Phylogenetic analysis*



The phylogenetic analysis, which included the two specimens of Diplura from Mesa H, in addition to 34 reference specimens, placed the Mesa H specimens in two distinct lineages (Figure 1). The Mesa H specimens showed no close relationship to any of the reference specimens, and thus were assigned to the new lineages DU and DV for JU01 and JU02, respectively (Figure 1). However, one, DU, was placed in a well-supported clade containing the Pilbara and GenBank reference specimens of Japygidae, while DV was placed in a well-supported clade containing the Pilbara and GenBank reference specimens of Campodeidae (Figure 1).

#### *Differentiation within and between lineages*

The two lineages of Diplura differed from one another by 25% sequence divergence (Table 2). DU (JU01) differed from the reference specimens by between 16.2 and 27.2% sequence divergence (Table 2). DV (JU02) differed from the reference specimens by between 23.5 and 28.6% sequence divergence (Table 2).

#### *Distribution of lineages*

Lineages DU and DV were each detected in single drillholes.

### Pseudoscorpiones

#### *Reference sequences and outgroups*

Four pseudoscorpions were sequenced from three drillholes at Mesa H (Table 1). In order to reduce analysis time and to simplify the presentation of results, a preliminary search of similar sequences on Genbank and in the Helix database indicated that the specimen most closely matched specimens in the family Chthoniidae. Based on those results, GenBank voucher sequences were included as references as follows: four sequences belonging to three genera of Chthoniidae: *Tyrannochthonius* sp JM 2008 (Genbank accession # EU559506) from South America (Columbia), *Lagynochthonius johni* (Genbank accession # EU559503; from Indonesia), *Austrochthonia* sp JM 2008 (Genbank accession # EU559513; from Australia) and *Tyrannochthonius aridus* (GenBank accession # KJ659959 from Western Australia). As local references, 27 specimens of Chthoniidae from 12 sites in the Pilbara (Helix, 2012) were included in the analysis. Sequences of the scorpion *Pandinus imperator* (Genbank accession # AY1565821) and harvestman spider *Siro rubens* (Genbank accession # DQ5131111) were used as outgroups.

#### *Phylogenetic analyses*

The phylogenetic analysis, which included the four specimens from Mesa H, in addition to 31 reference specimens, placed the Mesa H specimens in two distinct genetic lineages that formed a well-supported clade (Figure 2). The Mesa H lineages did not show a close relationship to any of the Pilbara reference specimens, and were assigned to the new lineages P14 and P15; however they formed a well-supported clade with a specimen from the Highway and Tod bore deposit in the Robe Valley (Figure 2). Specimens assigned to *Tyrannochthonius* and *Lagynochthonius* on the basis of morphology did not form monophyletic clades (Figure 2).

#### *Differentiation within and between lineages*

The two lineages of pseudoscorpion from Mesa H differed from one another by 3.0% mean sequence divergence. The three specimens within lineage P14 differed from one another by 0.1% mean sequence divergence. Mesa H lineages P14 and P15 differed the reference specimens by between 13.3 and 28.0% and 13.6 and 28.8% sequence divergence, respectively (Table 3).

#### *Distribution of lineages*

The Mesa H pseudoscorpion lineage P14 was detected in two drillholes: RC15MEH0315 and GR15MEH0015, and lineage P15 was detected in one drillhole, RC15MEH0335.

### Schizomids

#### *Preliminary analysis - Reference sequences and outgroups*

One schizomid was sequenced from drillhole RC12MEH0221 at Mesa H (Table 1). In order to reduce analysis time and to simplify the presentation of results, a preliminary neighbour-joining (NJ) analysis was conducted with the new Mesa H specimen and >500 reference sequences of schizomids from previous surveys in the Pilbara to compile a manageable and relevant reference data set. The reference sequences were selected based on the criteria that they showed  $\leq 15\%$  sequence divergence from the Mesa H lineage. In addition, representatives of the five described species of *Paradraculoides* and the four described species of *Draculoides* were included in the analysis. Two specimens of schizomids from the family Hubbardiidae, *Brignolozomus woodwardi* (GenBank accession # EU272675) and *Bamazomus* sp. (GenBank accession # EU272676) were used as outgroups.

#### Phylogenetic analysis

The phylogenetic analysis, which included the single Mesa H specimen, in addition to 11 reference specimens of schizomids, placed the Mesa H specimen in a distinct lineage within a well-supported clade containing reference specimens from previous surveys at Mesa H and Pannawonica (Figure 3). It was assigned to the new lineage SCH016 (Figure 3).

#### Differentiation within and between lineages

The Mesa H specimen differed from the closest reference specimens (JF50 and GH11, from Mesa H and Pannawonica, respectively) by 5.2% and 5.3% sequence divergence (Table 4).

#### Distribution of lineages

The new Mesa H specimen was detected in drillhole RC12MEH0221.

### Scolopendrida

One specimen of Scolopendrida from Mesa H was sequenced (Table 1). A search of similar sequences on GenBank and the Helix database indicated that the specimen belongs to the family Cryptopidae, thus the specimen was analysed with a Genbank vouchers of Cryptopidae as follows: *Cryptops japonicas* (Genbank accession # AB610778 as well as 15 reference specimens of Cryptopidae from nine sites in the Pilbara. Two specimens of Onychophora, *Metaperipatus inae* (Genbank accession # HQ453464) and *Opisthopatus cinctipes* (Genbank accession # NC014273) were used as outgroups.

#### Phylogenetic analysis

The phylogenetic analysis, which included the specimen from Mesa H, in addition to 16 reference specimens, placed the Mesa H specimen in a genetically distinct lineage and thus was assigned to the new lineage SC18 (Figure 4). The specimen formed a well-supported clade with the Genbank specimen of *Cryptops japonicas* as well as reference specimens from the Pilbara assigned to the genus *Cryptops* (Figure 4).

#### Differentiation within and between lineages

The Mesa H lineage of Scolopendrida differed from the reference specimens by between 16.4 and 22.8% sequence divergence (Table 5).

#### Distribution of lineages

The Mesa H lineage of Scolopendrida was detected at drillhole RC14MEH0388.

### Conclusions

COI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003a). In a comparison of COI sequences for over 13,000 pairs of taxa, Hebert et al (2003b) found a mean of 11.1% sequence divergence between distinct species. Nearly 80% of the comparisons showed that species pairs differed from one another by greater than 8% sequence divergence. However, a taxon by taxon approach examining the amount of phylogenetic variation within and between species is the most widely accepted method of defining species.

### Diplura

Two distinct lineages of Diplura were detected at Mesa H, representing two species, owing to the high level of sequence divergence between them. Lineage DU belongs to the Japygidae, however it differed from the nearest reference specimens by >16% sequence divergence, indicating that it is likely to belong to a new species, which has so far not been detected in the Pilbara, based on the material available for comparison. Lineage DV may belong to the Campodeidae, but differs from the nearest reference specimen by >20% and in the phylogenetic analysis, was the most external branch in the Campodeidae clade. Thus it is only tentatively assigned to this family, until further reference sequences become available. The large sequence divergence between it and the reference specimens indicates that it is a new species, which has so far not been detected in the Pilbara, based on the material available for comparison.

### Pseudoscorpiones

Two lineages, of Pseudoscorpiones, P14, P15, were detected at Mesa H, differing from one another by 3% mean sequence divergence. The two lineages belong to the family Chthoniidae and are likely to represent a single species, showing genetic divergence owing to their occurrence in different drillholes and poor dispersal abilities of the taxon. The species differs from the reference specimens by >13% sequence divergence, and hence is likely to be a new species based on the material available for comparison. The taxonomy of the Chthoniidae is in need of revision (M. Harvey, pers. comm.), so we did not attempt to place the Mesa H specimen into a genus.

### Schizomida

Previous analyses of genetic variation between morphologically distinct species of Schizomida can be used as a genetic 'yardstick' to interpret the current data set. The five described species of *Paradraculoides* (Harvey et al, 2008) differ from one another by between 8.4 to 12.1% sequence divergence (uncorrected p-distances; calculated by us from the Harvey et al., 2008 data). Similarly, the four described species of *Draculoides* differ from one another by between 4.5 to 13.7% sequence divergence (uncorrected p-distances calculated by us from Harvey et al., 2008).

One lineage of schizomid was detected at Mesa H, SCH016. The lineage was placed in a clade with specimens from a previous survey at Mesa H and the Pannawonica area. It shows a moderately low level of sequence divergence from these reference specimens (approximately 5%), but more than is usually detected between species of schizomids as described above. Thus it may represent a new species, however sampling of intermediate sites and or morphological data are needed to confirm the relationship between these specimens. We cannot assign the specimen to a genus, as it was placed outside the clade containing the reference specimens of both *Draculoides* and *Paradraculoides*.

### Scolopendrida

A single genetically distinct lineage of Scolopendrida, SC18, was detected at Mesa H, likely belonging to the family Cryptopidae. The lineage differed from the nearest reference specimen by >15% sequence divergence, and is likely to be a new species, based on the material available for comparison.

### Summary

Six lineages from four taxonomic groups (Diplura, Pseudoscorpiones, Scolopendrida, Schizomida) were detected at Mesa H, as summarised below. The six lineages likely represent five species. None of the species appear to have been detected previously in the Pilbara, based on the material available for comparison, although the species of Schizomida is closely related to a species detected previously at Mesa H and Pannawonica.

### Diplura

Two lineages of Diplura were detected at Mesa H, one Japygidae and one Campodeidae. The lineages differed from the reference specimens by >15% sequence divergence, and hence both are likely to be new species, based on the material available for comparison.

#### Pseudoscorpiones

Two lineages of Pseudoscorpiones were detected at Mesa H, belonging to the family Chthoniidae. They likely represent a single species, which appears to be new, based on the material available for comparison.

#### Schizomida

A single lineage was detected at Mesa H, corresponding to a single species. The lineage likely cannot be placed in a genus. The species is similar to one detected previously at Mesa H and Pannawonica, however shows enough genetic divergence from these reference specimens to warrant further investigation.

#### Scolopendrida

A single genetically distinct lineage of Scolopendrida was detected at Mesa H, likely belonging to the family Cryptopidae. The lineage differed from the nearest reference specimen by >15% sequence divergence, and is likely to be a new species, based on the material available for comparison.

### **References**

- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2011) Geneious v5.4, <http://www.geneious.com/>
- Harvey, M. S., Berry, O. Edward, K. L., Humphreys, G. (2008) Molecular and morphological systematics of hypogean schizomids (Schizomida: Hubbardiidae) in semiarid Australia. *Invertebrate Systematics* 22: 167-194.
- Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard J.R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* 270: 313-321.
- Hebert, P.D.N., Ratnasingham, S., deWaard J.R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B (supplement)* 270: S96-S99.
- Posada, D., Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- Thompson, J., Higgins, D., and Gibson, T. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680. doi:10.1093/nar/ 22.22.4673



Table 1. Specimens used in the present study and the lineage to which they were assigned, based on variation at the COI gene.

| Tracking Number            | Taxonomic group | Cap # | Helix ID | Lineage |
|----------------------------|-----------------|-------|----------|---------|
| RC14MEH0388.20160628.T3-1  | Diplura         | 7     | JU01     | DU      |
| GR15MEH0032.20160628.T3-2  | Diplura         | 20    | JU02     | DV      |
| RC15MEH0306.20160627.T3-03 | Schizomida      | 71    | JU03     | SCH016  |
| RC14MEH0388.20160628.T2-2  | Scolopendrida   | 6     | JU04     | SC18    |
| RC15MEH0315.20160627.T2-02 | Pseudoscorpion  | 56    | JU05     | P14     |
| RC15MEH0315.20160627.T3-02 | Pseudoscorpion  | 59    | JU06     | P14     |
| RC15MEH0335.20160627.T1-01 | Pseudoscorpion  | 60    | JU07     | P15     |
| GR15MEH0015.20160627.T2-03 | Pseudoscorpion  | 47    | JU08     | P14     |

Table 2 (attached). Mean genetic distance (below diagonal) and standard error (above diagonal, in blue) between lineages of Diplura detected in the present study and the reference lineages as shown in Figure 1. Distances between the lineages from the present study and the reference lineages are highlighted in yellow.

Table 3. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Pseudoscorpiones detected at in the present study and the reference lineages as shown in Figure 2. Distances between the lineages from the present study and the reference lineages are highlighted in yellow.

Table 4. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Schizomida detected at in the present study and the reference lineages as shown in Figure 3. Distances between the lineages from the present study and the reference lineages are highlighted in yellow.

Table 5. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Scolopendrida detected at in the present study and the reference lineages as shown in Figure 4. Distances between the lineages from the present study and the reference lineages are highlighted in yellow.

Figure 1. Bayesian analysis of COI haplotypes of Diplura from the present study and reference specimens from previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site.

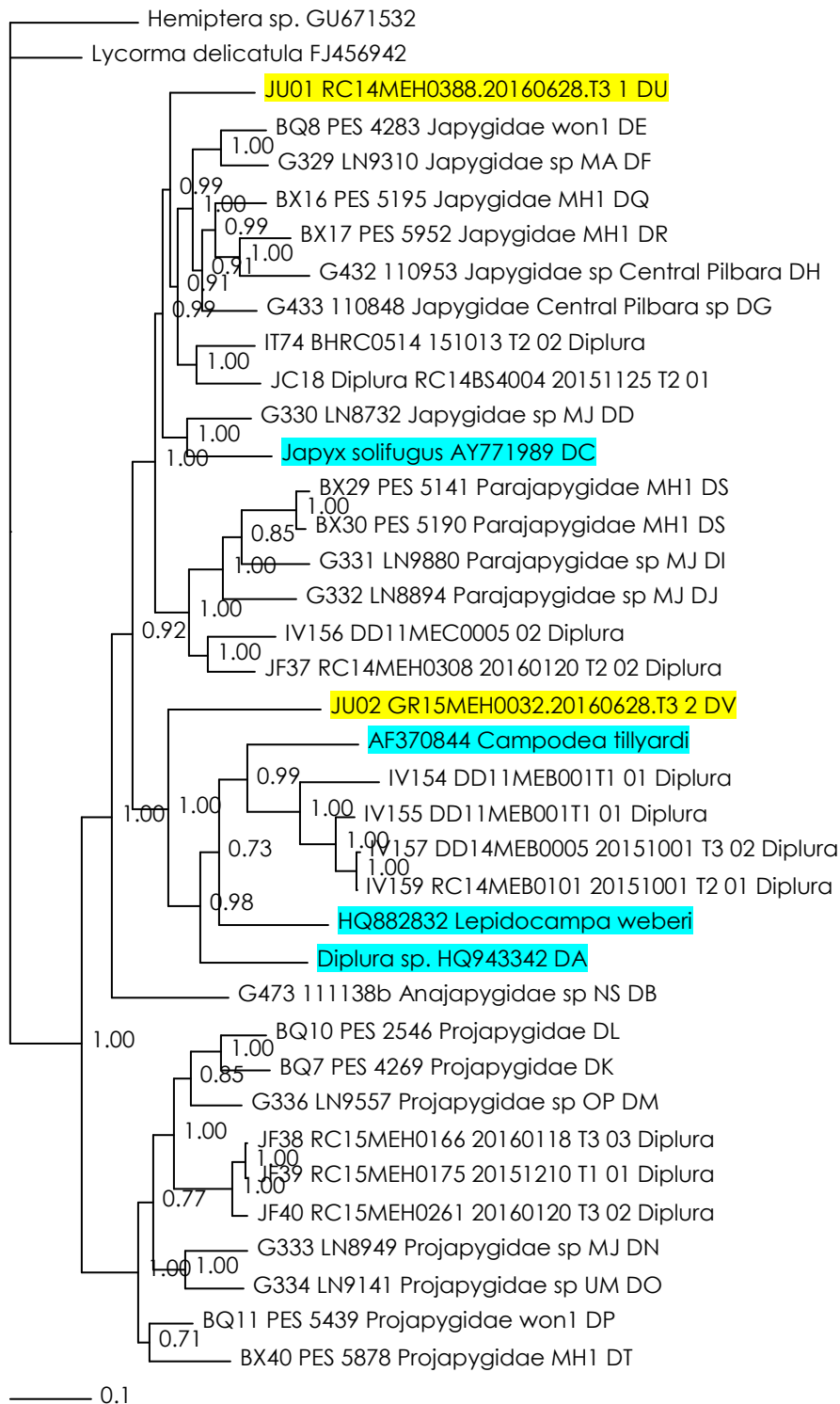


Figure 2. Bayesian analysis of COI haplotypes of Pseudoscorpiones from the present study and reference specimens from previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site.

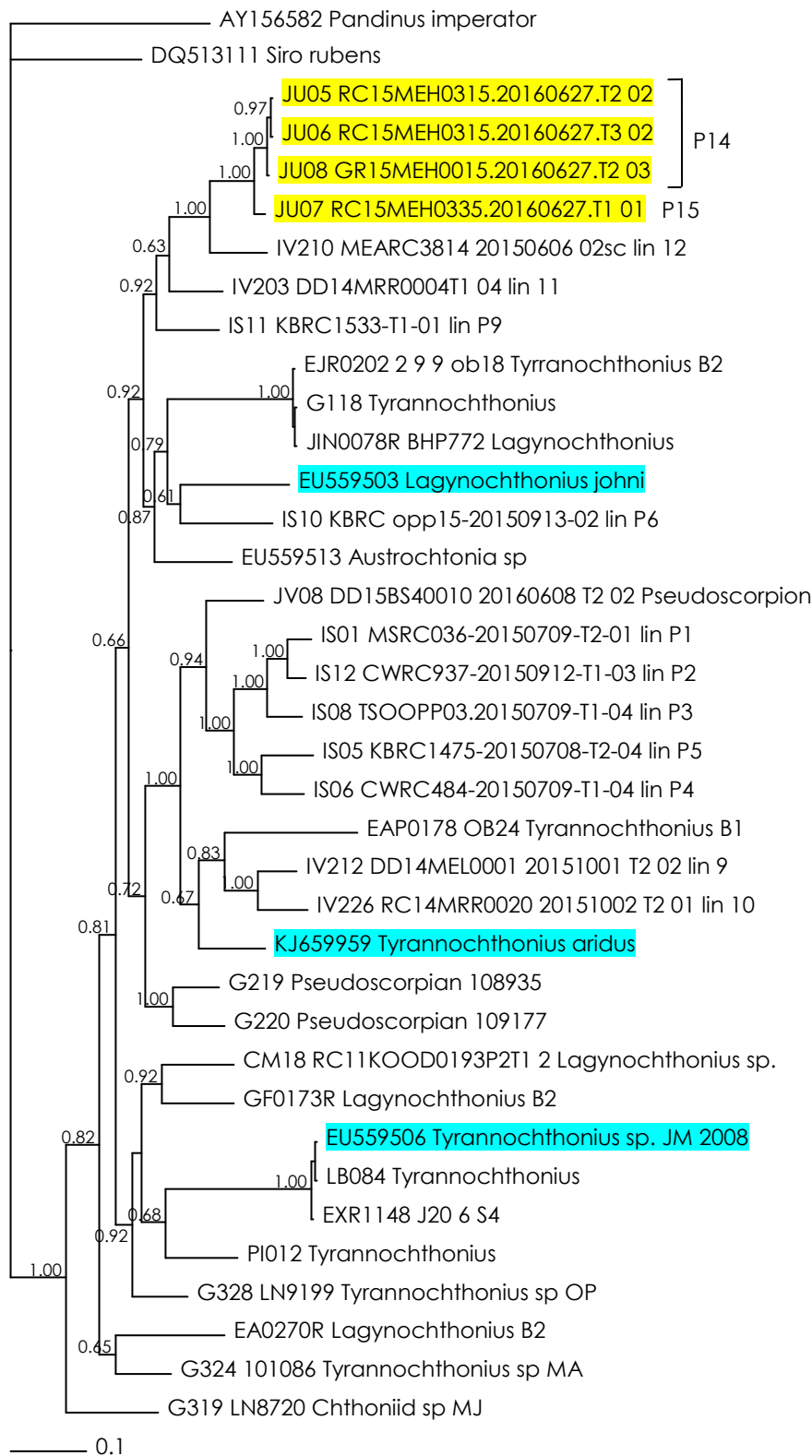


Figure 3. Bayesian analysis of COI haplotypes of Schizomida from the present study and reference specimens from previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site.

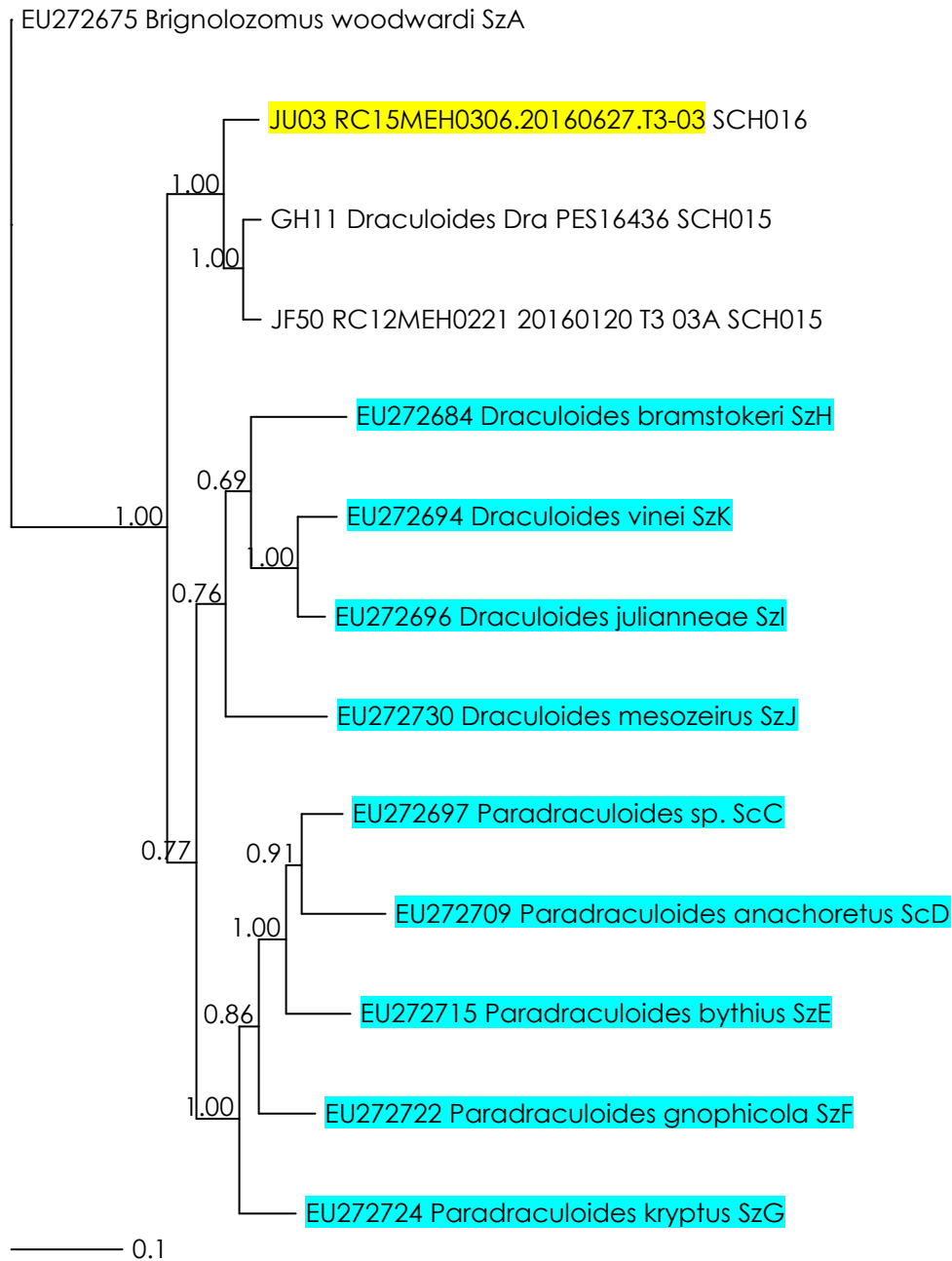
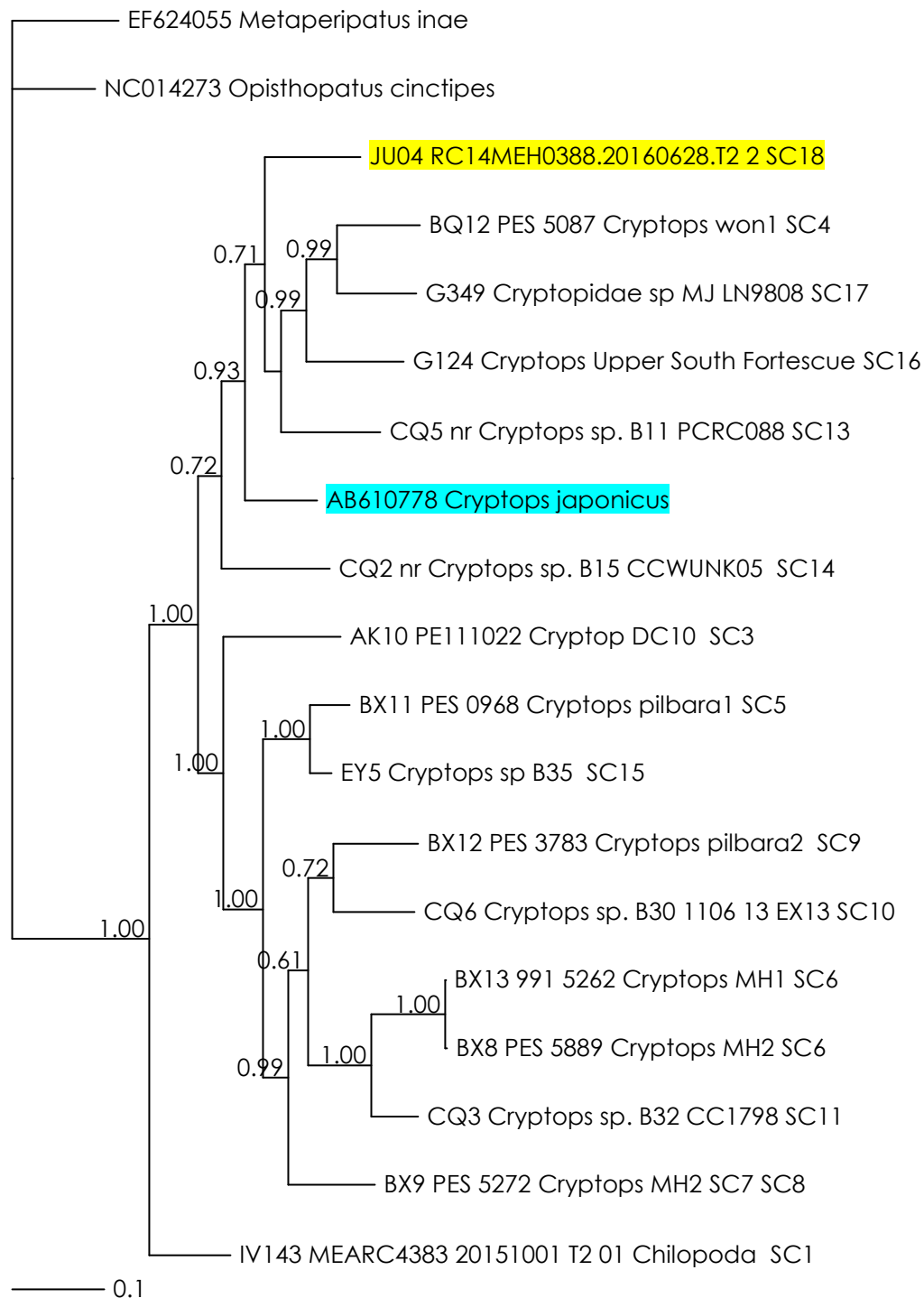




Figure 4. . Bayesian analysis of COI haplotypes of Scolopendrida from the present study and reference specimens from previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site.





# Helix

## Molecular Solutions

School of Animal Biology The University of Western Australia  
Hackett Entrance No. 4 Hackett Drive Crawley WA 6009

PO Box 155  
Leederville WA 6903

t: [08] 6488 4509

f: [08] 6488 1029

abn: 32 133 230 243

w: [www.helixsolutions.com.au](http://www.helixsolutions.com.au)

23 August, 2017

Nicola Watson  
Biota Environmental Sciences  
Level 1, 228 Carr Place  
Leederville, WA 6007

Via email

### **Re. Report on the molecular systematics of additional subfauna from Mesa H**

Dear Nicola,

Following is a summary of the results of the subfauna study we have completed on three additional taxonomic groups (Blattodea, Coleoptera, Thysanura) collected at Mesa H. Eight distinct genetic lineages were detected among the three groups. Two of the eight lineages have been detected previously in the Pilbara, whereas the remaining six are new, based on the material available for comparison.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston, Yvette Hitchen and Dr. Oliver Berry  
Helix Molecular Solutions



## Background and Objective

Twenty-two specimens of subfauna belonging to three taxonomic groups (Blattodea, Coleoptera, Thysanura) were collected from Mesa H and sequenced for variation at the mitochondrial COI or 12s gene. The molecular data were assessed in order to determine the number of species present in each group and compare the results to those obtained during previous surveys that have been undertaken for these groups elsewhere in the Pilbara.

## Executive summary

- Twenty-two specimens of subfauna belonging to three taxonomic groups (Blattodea, Coleoptera, Thysanura) were collected from Mesa H and sequenced for variation at the mitochondrial COI or 12s gene
- Eight lineages were detected among the three groups
- Two of the lineages have been detected previously in the Pilbara, whereas the remaining six appear to be new, based on the material available for comparison

## Methods

Twenty-two specimens of subfauna belonging to three taxonomic groups (Blattodea, Coleoptera, Thysanura) were collected from Mesa H and sequenced for variation at the mitochondrial COI or 12s gene using multiple pairs of primers (LCOI/HCO2, LCOI/HCO-out, L2020/H2619 for COI and 12Sai/12SRJ for 12s).

Sequences were edited using GENEIOUS software (Drummond *et al.* 2011). Alignment was performed with CLUSTAL W (Thompson *et al.* 1994) using default parameters. Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotide differences between sequences).

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data for each taxonomic group. Bayesian analysis was used to construct the phylogenetic trees, incorporating the model as identified in MODELTEST for each taxonomic group. The phylogeny, branch lengths and posterior probabilities were obtained by running two trees simultaneously, each running four simultaneous MCMC chains. The number of cycles needed was determined by the standard deviation of the split frequencies of the two trees. The analysis was paused after every  $1 \times 10^6$  generations and when the standard deviation fell below 0.01, the analysis was stopped. A majority rule consensus tree was constructed after discarding the "burn-in" trees in both analyses. The burn-in value was determined by plotting the posterior probabilities obtained after every generation and identifying the point at which the values reach stationarity (= the asymptote). Trees produced prior to stationarity were discarded.

For the purposes of this report, lineages were defined as haplotypes or groups of haplotypes differing from other such groups by >3% sequence divergence. This cut-off was selected based on bar-coding data, which indicates that intra-specific variation rarely exceeds 3% (Hebert *et al.*, 2003b).

We are currently developing a consistent and unique lineage and species naming system, so all lineages from previous phases will be consolidated in this report to aid in comparisons between phases.

## Results

### Blattodea

#### *Reference specimens and outgroups*

Two specimens of Blattodea from two bores were sequenced from Mesa H (Table 1). A search of the reference database GenBank indicated that both specimens were most closely matched to the genus *Nocticola*. A reference database of 35 *Nocticola* specimens from previous surveys in the Pilbara were analysed with the specimens from Mesa H. Two Genbank reference specimens of *Nocticola* from the Pilbara were also included as references, *N. quartermaini* (Genbank accession #KU258365) and *N. cockingi* (Genbank accession #KU258378). Two specimens of the closely related Blattodea family Coryiidae, *Latindia* sp. 13

FL2015 (Genbank accession # KP986417) and *Eupoyphaga sinensis* (Genbank accession #NC014274) were used as outgroups.

#### Phylogenetic analysis

The phylogenetic analysis, which included the two specimens from Mesa H in addition to 37 reference specimens, placed the Mesa H specimens in two distinct lineages (Figure 2). Neither of those lineages contained reference specimens and thus were assigned to the new lineages BN020 and BN021 (Figure 2). Specimen MF01 (lineage BN020) formed a moderately well-supported clade with seven specimens of Nocticolidae from Western Hub, Red Hill and Buckland Hills (Figure 1). Specimen MF02 (lineage BN021), formed a clade with three specimens from the Central Pilbara, Buckland Hills and the Hamersley Range, however the relationship was not well-supported (Figure 1). The two lineages were placed in a large widespread clade containing the Genbank reference specimen *N. quartermaini* and reference specimens of *Nocticola* and Nocticolidae from the Pilbara (Figure 1).

#### Differentiation within and between lineages

The two Mesa H lineages of *Nocticola* differed from one another by 6.3% sequence divergence (Table 2). The Mesa H specimens of *Nocticola* differed from the reference specimens by between 4.2 and 8.1% sequence divergence (Table 2). Specifically, lineage BN020 differed from the nearest reference specimens at Western Hub, Red Hill and Buckland Hills by between 4.2 and 5.1% sequence divergence and lineage BN021 differed from the nearest reference specimens from the Central Pilbara, Buckland Hills and the Hamersley Range by between 5.0 and 6.0% (Table 2). Specimens in the large, widespread clade differed from one another by between 0.2 and 9.1% sequence divergence (Table 2).

### Coleoptera

#### Reference specimens and outgroups

Eighteen specimens of Coleoptera from Mesa H were sequenced for COI (Table 1). In order to reduce analysis time and to simplify the presentation of results, a preliminary neighbour-joining (NJ) analysis was conducted on the Mesa H specimens in order to identify the number of lineages present. Four distinct genetic lineages were detected among the 16 specimens for which sequences were obtained (Figure 2). A representative of each lineage was compared to the GenBank sequence database. One lineage was most closely matched to reference specimens of Curculionidae and three lineages were most closely matched to reference specimens of Ptilidae. Thus, four haplotypes, representing the four lineages were analysed with 44 reference sequences of Curculionidae and Ptilidae, including 14 provided by the WAM and 30 from previous surveys in the Pilbara, including Mesas B and C. Two specimens of Coleoptera from the family Dermestidae, *Dermestes maculatus* (Genbank accession # HM909035) and *Dermestes frischii* (Genbank accession # KM578824) were used as outgroups.

#### Phylogenetic analysis

The phylogenetic analysis, which included the four specimens from Mesa H, in addition to 44 reference specimens, placed the Mesa H specimens in four distinct genetic lineages, two of which also contained reference specimens (Figure 3). Specifically one lineage contained reference specimens of Ptilidae sp. 1 from the WAM and one lineage contained reference specimens of Ptilidae from the Robe Valley, and thus were assigned to the existing lineages CP001 (Ptilidae sp. 1) and CP002 (Figure 3). The remaining two lineages did not contain reference specimens, and thus were assigned to the new lineages CP003 and CCU014 (Figure 3).

#### Differentiation within and between lineages

Differentiation within the four lineages of Coleoptera detected at Mesa H ranged from 0 to 1.6% mean sequence divergence (Table 3). The three lineages of Ptilidae from Mesa H differed from one another by between 4.6 and 15.9% mean sequence divergence (Table 4).

The one lineage of Curculionidae detected at Mesa H, CCU014, differed from the nearest reference specimens by  $\geq 13.0\%$  (Table 5).



The three lineages of Ptilidae differed from the reference specimens by between 1.6 and 16.1% sequence divergence (Table 6). In particular, lineage CP001 differed from the nearest reference specimens (WAM Ptilidae sp. 1) by 1.7% sequence divergence and lineage CP002 differed from the nearest reference specimens (IV44 – IV47) by 1.6% sequence divergence (Table 6). Lineage CP003 differed from the nearest reference specimens (WAM Ptilidae sp. 1) by 4.4% sequence divergence (Table 6).

### Thysanura

#### *Reference specimens and outgroups*

Two specimens of Thysanura from Mesa H were sequenced for 12s (Table 1). A search of similar sequences on Genbank and in the Helix database indicated that the specimens most closely matched database specimens of the subfamily Nicoletiinae. Based on those results, GenBank voucher sequences were included as reference sequences of Nicoletiinae (two specimens) as follows: *Trinemura callawa* and *T. cundalin* (GenBank accession # JQ282164 and JQ282165, respectively). In addition, as local references, 43 specimens of Nicoletiinae were included in the analysis. A specimen of Atelurinae was used as an outgroup: *Atelura formicaria* (Genbank accession # EU084035).

#### *Phylogenetic analyses*

The phylogenetic analysis, which included the two new specimens from Mesa H, in addition to 45 reference specimens, placed the Mesa H specimens in two distinct genetic lineages, neither of which contained reference specimens, thus the two were assigned to the new lineages TN019 and TN020 (Figure 4). Mesa H lineage TN020 formed a well-supported clade with lineage TN011 from the Robe Valley, and lineage TN019 formed a clade with lineage TN008 from Christmas Creek, but the relationship was not well-supported (Figure 4).

#### *Differentiation within and between lineages*

Mesa H lineage TN019 differed from the nearest reference specimens (CQ8, CQ10) by >10% sequence divergence, and lineage TN020 differed from nearest reference specimen (IV334) by 7.2% sequence divergence (Table 7).

### **Conclusions**

COI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003a). In a comparison of COI sequences for over 13,000 pairs of taxa, Hebert et al (2003b) found a mean of 11.1% sequence divergence between distinct species. Nearly 80% of the comparisons showed that species pairs differed from one another by greater than 8% sequence divergence. However, a taxon by taxon approach, examining the amount of phylogenetic variation within and between species is the most widely accepted method of defining species.

### Blattodea

Two distinct genetic lineages of Nocticolidae (Blattodea) were detected at Mesa H (BN020, BN021). The two lineages differed from one another by 6.3 % sequence divergence. This is generally more sequence divergence than would be expected within a single species, however, it suggests that the lineages are closely related and may represent incipient species or a species complex. Morphological evidence or sampling additional specimens from intermediate areas may help resolve this relationship. The two Mesa H lineages are placed within a large clade containing reference specimens of *Nocticola* and Nocticolidae from Pannawonica, the Western Hamersley Range and Central Pilbara. This widely distributed clade has an average genetic distance of approximately 6% (range 0.2 – 9.1) across a large geographical range, thus it may represent a species complex of closely related, recently diverged species (possibly in the genus *Nocticola*).

#### *Nocticola*

**BN020** = new lineage, may belong to a species complex detected at Western Hub, Red Hill and Buckland Hills

**BN021** = new lineage, may belong to a species complex detected at Buckland Hills, the Central Pilbara and Hamersley Range.

### Coleoptera

A single genetic lineage of Curculionidae and three lineages of Ptilidae were detected at Mesa H. They differed from the nearest reference specimens by between 1.6 and >13% sequence divergence.

For comparison, differentiation within the four WAM species of Curculionidae for which there are multiple sequences (species 1, 3, 4, 8) ranges from a mean of 0.9% (species 1) to a mean of 5.2% (species 4). The eight species differed by a mean of 11.3 to 24.3% sequence divergence. The two specimens of Ptilidae sp. 1 shared the same haplotype.

The Curculionidae lineage from Mesa H differed from the nearest reference specimens by  $\geq 13\%$ , indicating that the specimen likely represents a new lineage and species of Curculionidae that has not been detected previously.

Two of the Mesa H lineages of Ptilidae were placed in a clade containing reference specimens from the WAM, assigned to the species Ptilidae sp. 1. The Mesa H lineages CP001 and CP003 differed from the WAM reference specimens of Ptilidae sp. 1 by between 1.6 and 4.4% sequence divergence, suggesting that the lineages from Mesa H belong to Ptilidae sp. 1. However, owing to the moderate level of sequence divergence (4.4%) morphological characters may be useful to confirm this placement.

### Curculionidae

**CCU014** = new lineage and species, not detected previously in the Pilbara

### Ptilidae

**CP001** = WAM Ptilidae sp. 1, previously detected in the Pilbara

**CP002** = lineage and species detected previously in the Robe Valley

**CP003** = new lineage; likely to belong to previously detected species WAM Ptilidae sp. 1, but morphology should be used to confirm.

### Thysanura

The mitochondrial gene 12S is widely used in insect systematics (Simon, 1991; Caterino et al., 2000), although in contrast to the mitochondrial gene COI, fewer broadscale comparative studies are available to provide a basis for species discrimination. Nonetheless, 12S has proven useful for establishing phylogenetic relationships in many insect groups (Caterino et al., 2000). The 12S gene evolves approximately 1.5 times (Mueller, 2006) more slowly than COI. Hence we would expect that the threshold for species discrimination using 12S would be, by inference, lower than COI.

Two distinct genetic lineages of Thysanura were detected at Mesa H, Nicoletiinae lineages TN019 and TN020. Both differed from the nearest reference specimens by >7% sequence divergence.

For comparison, the six specimens of *Trimenura callawa* differ by a mean of 0.4% sequence divergence (range = 0 to 0.9% at 12s) and *T. callawa* and *T. cundaline* differ by 6.5% mean sequence divergence at 12s. Given the relatively high genetic distance between Mesa H lineages TN019 and TN020 and the reference specimens (>7%), but keeping in mind that our conclusions are based on current understanding of 12s evolution rates, it is likely that both new lineages from Mesa H represent new species of Nicoletiinae.

### Nicoletiinae

**TN019** = new lineage and species

**TN020** = new lineage and species

## **References**

- Bu Y., Gao Y, Potapov M. B., Luan Y. (2012). Redescription of arenicolous dipluran *Parajapyx pauliani* (Diplura, Parajapygidae) and DNA barcoding analyses of *Parajapyx* from China. *ZooKeys* 221: 19-29.
- Caterino, M.S., Cho, S., Sperling, F.A.H. (2000). The current state of insect molecular systematics: a thriving Tower of Babel. *Annu. Rev. Entomol.* 45, 1–54.
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2011) Geneious v5.4, <http://www.geneious.com/>
- Finston, Francis, Johnson (2009). Biogeography of the stygobitic isopod *Pygolabis* (Malacostraca: Tainisopidae) in the Pilbara, Western Australia: evidence for multiple colonisations of the groundwater. *Molecular Phylogenetics and Evolution* 52:448-460.
- Harvey, M. S., Berry, O. Edward, K. L., Humphreys, G. (2008) Molecular and morphological systematics of hypogean schizomids (Schizomida: Hubbardiidae) in semiarid Australia. *Invertebrate Systematics* 22: 167-194.
- Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard J.R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* 270: 313-321.
- Hebert, P.D.N., Ratnasingham, S., deWaard J.R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B (supplement)* 270: S96-S99.
- Posada, D., Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- Simon C (1991) Appendix 3. In: *Molecular Techniques in Taxonomy* (eds Hewitt GM, Johnston A, Young JP), pp. 345–355. SpringerVerlag, United Kingdom.
- Thompson, J., Higgins, D., and Gibson, T. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680. doi:10.1093/nar/ 22.22.4673

Table 1. Specimens of troglotauna used in the present study and the lineage to which they were assigned, based on variation at COI or 12s (for Thysanura). \*=new lineage this study.

| Tracking Number            | Taxonomy   | Cap # | Helix ID | Lineage              |
|----------------------------|------------|-------|----------|----------------------|
| RC12MEH022120151210-T2-01  | Blattodea  | 163   | MF01     | BN020                |
| RC16MEH0427.20161211.T2    | Blattodea  | 36    | MF02     | BN021                |
| RC12MEH022120151210-T2-04A | Coleoptera | 166   | MF03     | CCU014               |
| RC12MEH022120151210-T2-04B | Coleoptera | 166   | MF04     | CCU014               |
| RC12MEH0221.20161026.T1-01 | Coleoptera | 61    | MF05     | CP003                |
| RC12MEH0221.20161026.T1-01 | Coleoptera |       | MF06     | No data              |
| RC12MEH0221.20161026.T1-01 | Coleoptera |       | MF07     | CP003                |
| RC12MEH0221.20161026.T1-01 | Coleoptera |       | MF08     | CP003                |
| RC12MEH0221.20161026.T1-01 | Coleoptera |       | MF09     | CP003                |
| RC14MEH023820151211-T1-03  | Coleoptera | 182   | MF10     | CP002                |
| RC14MEH023820151211-T1-03  | Coleoptera |       | MF11     | CP002                |
| RC14MEH023820151211-T1-03  | Coleoptera |       | MF12     | CP002                |
| RC14MEH023820151211-T1-03  | Coleoptera |       | MF13     | CP002                |
| RC14MEH023820151211-T1-03  | Coleoptera |       | MF14     | No d                 |
| RC14MEH025220151210-T1-04  | Coleoptera | 154   | MF15     | CCU014               |
| RC15MEH030220151210-T2-02  | Coleoptera | 58    | MF16     | CCU014               |
| RC16MEH0264.20170130.T3-04 | Coleoptera | 6     | MF17     | CP001/Ptilidae sp. 1 |
| RC16MEH0264.20170130.T3-04 | Coleoptera |       | MF18     | CP001/Ptilidae sp. 1 |
| RC16MEH0264.20170130.T3-04 | Coleoptera |       | MF19     | CP001/Ptilidae sp. 1 |
| RC16MEH0264.20170130.T3-04 | Coleoptera |       | MF20     | CP001/Ptilidae sp. 1 |
| RC12JIM000620160119T2-02   | Thysanura  | 115   | MF21     | TN019                |
| RC14MEH025220151210-T1-03  | Thysanura  | 153   | MF22     | TN020                |



Table 2. Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Nocticolidae (Blattodea) detected at Mesa H and the reference lineages as shown in Figure 1. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

| Specimen ID                        | MF01  | MF02  | 14705.000 | 15055.000 | 15057.000 | 15137.000 | 8785.000 | AL14  | AL15  | AL21  | BH6   | BH8   | BQ23  | C21-  | EJR0201 | FH10  |
|------------------------------------|-------|-------|-----------|-----------|-----------|-----------|----------|-------|-------|-------|-------|-------|-------|-------|---------|-------|
| MF01 RC12MEH022120151210-T2-01     |       | 0.009 | 0.012     | 0.010     | 0.009     | 0.010     | 0.011    | 0.009 | 0.010 | 0.010 | 0.010 | 0.009 | 0.011 | 0.011 | 0.012   | 0.008 |
| MF02 RC16MEH0427.20161211.T2       | 0.063 |       | 0.010     | 0.009     | 0.010     | 0.009     | 0.009    | 0.009 | 0.010 | 0.010 | 0.010 | 0.009 | 0.010 | 0.010 | 0.012   | 0.009 |
| 14705 C9 Yaratoola Station         | 0.062 | 0.060 |           | 0.011     | 0.011     | 0.012     | 0.011    | 0.012 | 0.012 | 0.013 | 0.011 | 0.010 | 0.012 | 0.005 | 0.012   | 0.011 |
| 15055 C24 Trinity                  | 0.068 | 0.058 | 0.068     |           | 0.010     | 0.010     | 0.009    | 0.008 | 0.009 | 0.011 | 0.010 | 0.009 | 0.008 | 0.010 | 0.010   | 0.010 |
| 15057 C26 Cane Upper Cane          | 0.054 | 0.062 | 0.068     | 0.066     |           | 0.011     | 0.012    | 0.010 | 0.012 | 0.012 | 0.010 | 0.011 | 0.010 | 0.011 | 0.012   | 0.010 |
| 15137 C41 Upper Cane               | 0.065 | 0.051 | 0.065     | 0.071     | 0.073     |           | 0.010    | 0.011 | 0.011 | 0.012 | 0.011 | 0.010 | 0.011 | 0.011 | 0.011   | 0.011 |
| 8785 C105 Trinity                  | 0.066 | 0.056 | 0.062     | 0.060     | 0.072     | 0.053     |          | 0.010 | 0.011 | 0.012 | 0.010 | 0.010 | 0.010 | 0.010 | 0.012   | 0.010 |
| AL14 Nocticola sp B1 Deposit E     | 0.062 | 0.060 | 0.078     | 0.050     | 0.060     | 0.079     | 0.072    |       | 0.008 | 0.010 | 0.008 | 0.008 | 0.005 | 0.011 | 0.010   | 0.008 |
| AL15 Nocticola sp B1 Orebody 18    | 0.060 | 0.063 | 0.066     | 0.050     | 0.058     | 0.065     | 0.064    | 0.042 |       | 0.010 | 0.009 | 0.008 | 0.009 | 0.010 | 0.005   | 0.010 |
| AL21 Nocticola sp B1 Iron Valley   | 0.072 | 0.076 | 0.083     | 0.080     | 0.080     | 0.088     | 0.082    | 0.076 | 0.071 |       | 0.009 | 0.011 | 0.011 | 0.013 | 0.012   | 0.011 |
| BH6 RIO141 Nocticola sp            | 0.073 | 0.071 | 0.074     | 0.060     | 0.070     | 0.079     | 0.074    | 0.067 | 0.067 | 0.073 |       | 0.009 | 0.009 | 0.012 | 0.011   | 0.009 |
| BH8 RIO090 Nocticola sp            | 0.057 | 0.052 | 0.060     | 0.064     | 0.054     | 0.067     | 0.064    | 0.050 | 0.052 | 0.070 | 0.058 |       | 0.010 | 0.010 | 0.009   | 0.009 |
| BQ23 PES 5535 Nocticola sp. indet. | 0.062 | 0.068 | 0.076     | 0.052     | 0.062     | 0.073     | 0.070    | 0.017 | 0.041 | 0.082 | 0.066 | 0.052 |       | 0.011 | 0.009   | 0.010 |
| C21-Robe Valley                    | 0.062 | 0.062 | 0.016     | 0.062     | 0.072     | 0.067     | 0.064    | 0.070 | 0.054 | 0.083 | 0.074 | 0.052 | 0.064 |       | 0.011   | 0.010 |
| EJR0201                            | 0.062 | 0.068 | 0.070     | 0.054     | 0.062     | 0.061     | 0.070    | 0.047 | 0.016 | 0.080 | 0.068 | 0.050 | 0.045 | 0.058 |         | 0.011 |
| FH10 Nocticola sp DL0097           | 0.042 | 0.063 | 0.060     | 0.066     | 0.056     | 0.065     | 0.060    | 0.057 | 0.054 | 0.065 | 0.065 | 0.052 | 0.058 | 0.056 | 0.056   |       |
| FH11 Nocticola sp EW0528           | 0.057 | 0.067 | 0.070     | 0.062     | 0.062     | 0.065     | 0.054    | 0.060 | 0.063 | 0.073 | 0.068 | 0.050 | 0.060 | 0.066 | 0.058   | 0.054 |
| FH12 Nocticola sp EW0003           | 0.072 | 0.081 | 0.076     | 0.078     | 0.082     | 0.081     | 0.076    | 0.075 | 0.075 | 0.081 | 0.076 | 0.065 | 0.070 | 0.068 | 0.076   | 0.060 |
| FH13 Nocticola sp B28 BG0082       | 0.047 | 0.054 | 0.064     | 0.054     | 0.054     | 0.065     | 0.060    | 0.052 | 0.052 | 0.062 | 0.055 | 0.047 | 0.056 | 0.060 | 0.054   | 0.034 |
| FH14 Nocticola sp B28 EW0460       | 0.052 | 0.055 | 0.068     | 0.060     | 0.017     | 0.069     | 0.062    | 0.055 | 0.057 | 0.070 | 0.058 | 0.047 | 0.058 | 0.068 | 0.058   | 0.049 |
| G109 Nocticola                     | 0.060 | 0.055 | 0.064     | 0.062     | 0.058     | 0.059     | 0.066    | 0.062 | 0.060 | 0.075 | 0.057 | 0.049 | 0.062 | 0.066 | 0.058   | 0.058 |
| G131 Mount Dove                    | 0.067 | 0.060 | 0.072     | 0.070     | 0.066     | 0.075     | 0.076    | 0.068 | 0.062 | 0.084 | 0.083 | 0.067 | 0.072 | 0.072 | 0.068   | 0.065 |
| G391 LN9122a Nocticola indet OP    | 0.062 | 0.062 | 0.078     | 0.058     | 0.062     | 0.075     | 0.064    | 0.052 | 0.055 | 0.078 | 0.067 | 0.054 | 0.052 | 0.066 | 0.054   | 0.055 |
| G441 110850 Nocticolidae sp.       | 0.055 | 0.060 | 0.066     | 0.060     | 0.060     | 0.061     | 0.058    | 0.062 | 0.055 | 0.075 | 0.067 | 0.055 | 0.062 | 0.072 | 0.060   | 0.062 |
| GF0215R 1 SH1                      | 0.066 | 0.068 | 0.083     | 0.056     | 0.066     | 0.081     | 0.078    | 0.010 | 0.049 | 0.085 | 0.074 | 0.058 | 0.023 | 0.076 | 0.052   | 0.062 |
| IS73 KBDD025 T2 01 sp              | 0.049 | 0.070 | 0.072     | 0.074     | 0.064     | 0.073     | 0.066    | 0.065 | 0.062 | 0.070 | 0.077 | 0.058 | 0.070 | 0.068 | 0.064   | 0.016 |
| IS74 KBRC1533 T1 04 sp             | 0.049 | 0.067 | 0.072     | 0.070     | 0.066     | 0.069     | 0.063    | 0.060 | 0.063 | 0.070 | 0.072 | 0.054 | 0.064 | 0.064 | 0.063   | 0.016 |

|   |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| IS77 KBRC1353 T1 01 sp                      | 0.046 | 0.069 | 0.070 | 0.076 | 0.059 | 0.071 | 0.064 | 0.063 | 0.060 | 0.069 | 0.076 | 0.056 | 0.068 | 0.066 | 0.063 | 0.014 |
| IS79 KBRC1353 T2 01 sp                      | 0.046 | 0.067 | 0.068 | 0.074 | 0.061 | 0.069 | 0.063 | 0.062 | 0.058 | 0.067 | 0.074 | 0.054 | 0.066 | 0.064 | 0.061 | 0.012 |
| IS81 TbrC596 20150912 t2 02 sp              | 0.063 | 0.062 | 0.070 | 0.033 | 0.055 | 0.069 | 0.057 | 0.065 | 0.062 | 0.079 | 0.065 | 0.063 | 0.059 | 0.061 | 0.061 | 0.060 |
| IS83 KbrC147520150910 t1 03 sp              | 0.051 | 0.069 | 0.070 | 0.072 | 0.066 | 0.071 | 0.064 | 0.060 | 0.063 | 0.072 | 0.074 | 0.056 | 0.064 | 0.063 | 0.063 | 0.014 |
| IS85 Cwrc48020150912t1 03 sp                | 0.042 | 0.063 | 0.063 | 0.068 | 0.059 | 0.067 | 0.061 | 0.058 | 0.058 | 0.067 | 0.065 | 0.054 | 0.061 | 0.059 | 0.059 | 0.002 |
| IT28 BHRC0442-151013-T2-01                  | 0.052 | 0.050 | 0.060 | 0.054 | 0.052 | 0.063 | 0.060 | 0.062 | 0.059 | 0.073 | 0.054 | 0.052 | 0.056 | 0.066 | 0.062 | 0.049 |
| IT36 BHRC331-151012-T1-02                   | 0.045 | 0.061 | 0.068 | 0.064 | 0.062 | 0.071 | 0.062 | 0.062 | 0.059 | 0.072 | 0.064 | 0.054 | 0.060 | 0.060 | 0.058 | 0.013 |
| IT37 BHRCOPP1-151013-T4-01                  | 0.063 | 0.063 | 0.070 | 0.064 | 0.074 | 0.053 | 0.050 | 0.065 | 0.060 | 0.091 | 0.067 | 0.062 | 0.066 | 0.068 | 0.062 | 0.062 |
| KU258365 Nocticola quartermaini isolate EP3 | 0.060 | 0.058 | 0.076 | 0.049 | 0.058 | 0.077 | 0.070 | 0.002 | 0.041 | 0.075 | 0.065 | 0.049 | 0.016 | 0.068 | 0.045 | 0.055 |
| KU258378 Nocticola cockingi isolate EP14    | 0.156 | 0.163 | 0.163 | 0.171 | 0.165 | 0.157 | 0.167 | 0.170 | 0.163 | 0.167 | 0.172 | 0.159 | 0.173 | 0.160 | 0.161 | 0.147 |
| P2  | 0.050 | 0.054 | 0.060 | 0.058 | 0.052 | 0.063 | 0.068 | 0.057 | 0.054 | 0.067 | 0.063 | 0.055 | 0.054 | 0.056 | 0.056 | 0.050 |
| PSF0065R LN6278A P6 S3                      | 0.066 | 0.076 | 0.083 | 0.060 | 0.070 | 0.084 | 0.082 | 0.021 | 0.050 | 0.087 | 0.078 | 0.066 | 0.023 | 0.076 | 0.054 | 0.062 |

| FH11  | FH12  | FH13  | FH14  | G109  | G131  | G391  | G441  | GF0215R 1 | IS73  | IS74  | IS77  | IS79  | IS81  | IS83  | IS85  | IT28  | IT36  | IT37  | KU258365 | KU258378 | P2    | PSF0065R |
|-------|-------|-------|-------|-------|-------|-------|-------|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|----------|-------|----------|
| 0.010 | 0.010 | 0.008 | 0.009 | 0.010 | 0.009 | 0.009 | 0.009 | 0.011     | 0.009 | 0.009 | 0.009 | 0.009 | 0.009 | 0.010 | 0.008 | 0.008 | 0.008 | 0.009 | 0.010    | 0.014    | 0.008 | 0.011    |
| 0.010 | 0.011 | 0.008 | 0.008 | 0.009 | 0.009 | 0.009 | 0.009 | 0.010     | 0.010 | 0.009 | 0.010 | 0.010 | 0.010 | 0.010 | 0.009 | 0.008 | 0.009 | 0.008 | 0.009    | 0.014    | 0.009 | 0.010    |
| 0.012 | 0.013 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.013 | 0.012     | 0.012 | 0.012 | 0.012 | 0.011 | 0.013 | 0.012 | 0.011 | 0.011 | 0.010 | 0.012 | 0.012    | 0.015    | 0.010 | 0.012    |
| 0.010 | 0.012 | 0.008 | 0.010 | 0.010 | 0.011 | 0.009 | 0.010 | 0.008     | 0.012 | 0.010 | 0.011 | 0.011 | 0.008 | 0.011 | 0.010 | 0.009 | 0.010 | 0.010 | 0.008    | 0.015    | 0.010 | 0.009    |
| 0.011 | 0.012 | 0.010 | 0.005 | 0.011 | 0.010 | 0.011 | 0.011 | 0.010     | 0.011 | 0.011 | 0.010 | 0.010 | 0.009 | 0.011 | 0.010 | 0.010 | 0.010 | 0.012 | 0.010    | 0.015    | 0.009 | 0.011    |
| 0.012 | 0.011 | 0.010 | 0.011 | 0.010 | 0.011 | 0.010 | 0.010 | 0.012     | 0.012 | 0.010 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.009 | 0.012 | 0.008 | 0.011    | 0.015    | 0.011 | 0.012    |
| 0.011 | 0.012 | 0.009 | 0.011 | 0.010 | 0.010 | 0.009 | 0.009 | 0.010     | 0.011 | 0.010 | 0.011 | 0.011 | 0.010 | 0.010 | 0.010 | 0.009 | 0.011 | 0.009 | 0.010    | 0.016    | 0.011 | 0.010    |
| 0.010 | 0.010 | 0.009 | 0.008 | 0.010 | 0.010 | 0.007 | 0.009 | 0.004     | 0.010 | 0.009 | 0.010 | 0.010 | 0.009 | 0.009 | 0.009 | 0.010 | 0.009 | 0.010 | 0.002    | 0.015    | 0.009 | 0.006    |
| 0.011 | 0.011 | 0.009 | 0.010 | 0.010 | 0.010 | 0.009 | 0.009 | 0.010     | 0.011 | 0.010 | 0.011 | 0.011 | 0.009 | 0.010 | 0.010 | 0.010 | 0.009 | 0.010 | 0.008    | 0.014    | 0.010 | 0.011    |
| 0.011 | 0.010 | 0.010 | 0.011 | 0.009 | 0.010 | 0.010 | 0.010 | 0.011     | 0.011 | 0.010 | 0.011 | 0.011 | 0.010 | 0.011 | 0.011 | 0.010 | 0.010 | 0.011 | 0.010    | 0.014    | 0.009 | 0.012    |
| 0.009 | 0.011 | 0.009 | 0.009 | 0.009 | 0.011 | 0.009 | 0.009 | 0.010     | 0.011 | 0.009 | 0.010 | 0.011 | 0.010 | 0.010 | 0.010 | 0.008 | 0.009 | 0.009 | 0.008    | 0.013    | 0.010 | 0.011    |
| 0.009 | 0.009 | 0.008 | 0.009 | 0.009 | 0.009 | 0.008 | 0.009 | 0.010     | 0.011 | 0.010 | 0.011 | 0.011 | 0.010 | 0.010 | 0.010 | 0.010 | 0.009 | 0.010 | 0.008    | 0.015    | 0.009 | 0.011    |
| 0.012 | 0.010 | 0.010 | 0.010 | 0.010 | 0.012 | 0.009 | 0.010 | 0.006     | 0.011 | 0.010 | 0.011 | 0.011 | 0.010 | 0.010 | 0.010 | 0.010 | 0.009 | 0.010 | 0.005    | 0.017    | 0.010 | 0.006    |
| 0.012 | 0.011 | 0.010 | 0.011 | 0.012 | 0.010 | 0.011 | 0.012 | 0.011     | 0.011 | 0.011 | 0.011 | 0.011 | 0.012 | 0.011 | 0.010 | 0.012 | 0.010 | 0.012 | 0.011    | 0.015    | 0.009 | 0.012    |
| 0.012 | 0.013 | 0.010 | 0.012 | 0.011 | 0.011 | 0.010 | 0.010 | 0.011     | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.010    | 0.016    | 0.010 | 0.012    |
| 0.010 | 0.010 | 0.007 | 0.009 | 0.010 | 0.009 | 0.008 | 0.009 | 0.010     | 0.006 | 0.006 | 0.005 | 0.005 | 0.010 | 0.005 | 0.002 | 0.009 | 0.005 | 0.010 | 0.008    | 0.014    | 0.008 | 0.010    |
|       | 0.011 | 0.009 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.011     | 0.011 | 0.011 | 0.012 | 0.012 | 0.010 | 0.012 | 0.011 | 0.010 | 0.010 | 0.011 | 0.010    | 0.014    | 0.009 | 0.011    |
| 0.073 |       | 0.009 | 0.010 | 0.010 | 0.010 | 0.011 | 0.011 | 0.012     | 0.012 | 0.011 | 0.012 | 0.012 | 0.012 | 0.011 | 0.011 | 0.012 | 0.010 | 0.010 | 0.010    | 0.015    | 0.010 | 0.013    |
| 0.054 | 0.062 |       | 0.007 | 0.009 | 0.008 | 0.008 | 0.009 | 0.010     | 0.008 | 0.008 | 0.008 | 0.008 | 0.009 | 0.008 | 0.007 | 0.009 | 0.008 | 0.009 | 0.009    | 0.014    | 0.009 | 0.010    |
| 0.057 | 0.067 | 0.042 |       | 0.009 | 0.010 | 0.008 | 0.009 | 0.010     | 0.010 | 0.009 | 0.009 | 0.009 | 0.009 | 0.009 | 0.009 | 0.009 | 0.009 | 0.010 | 0.008    | 0.015    | 0.009 | 0.011    |
| 0.057 | 0.063 | 0.052 | 0.052 |       | 0.010 | 0.009 | 0.008 | 0.011     | 0.011 | 0.011 | 0.012 | 0.012 | 0.010 | 0.012 | 0.011 | 0.006 | 0.010 | 0.009 | 0.009    | 0.014    | 0.009 | 0.012    |
| 0.073 | 0.076 | 0.062 | 0.067 | 0.068 |       | 0.010 | 0.010 | 0.011     | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.010 | 0.009 | 0.010 | 0.010 | 0.010    | 0.014    | 0.009 | 0.012    |
| 0.062 | 0.073 | 0.055 | 0.049 | 0.057 | 0.071 |       | 0.009 | 0.009     | 0.010 | 0.009 | 0.009 | 0.009 | 0.009 | 0.009 | 0.009 | 0.009 | 0.008 | 0.009 | 0.007    | 0.014    | 0.010 | 0.009    |
| 0.065 | 0.076 | 0.059 | 0.054 | 0.037 | 0.068 | 0.065 |       | 0.010     | 0.010 | 0.010 | 0.011 | 0.011 | 0.010 | 0.011 | 0.010 | 0.008 | 0.010 | 0.008 | 0.009    | 0.014    | 0.009 | 0.011    |
| 0.064 | 0.082 | 0.060 | 0.062 | 0.066 | 0.072 | 0.060 | 0.066 |           | 0.011 | 0.010 | 0.011 | 0.011 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.011 | 0.003    | 0.016    | 0.010 | 0.006    |
| 0.065 | 0.074 | 0.048 | 0.053 | 0.070 | 0.074 | 0.060 | 0.070 | 0.074     |       | 0.007 | 0.003 | 0.003 | 0.010 | 0.006 | 0.006 | 0.010 | 0.007 | 0.011 | 0.010    | 0.014    | 0.009 | 0.011    |
| 0.062 | 0.067 | 0.042 | 0.053 | 0.067 | 0.074 | 0.056 | 0.074 | 0.068     | 0.021 |       | 0.006 | 0.006 | 0.010 | 0.002 | 0.006 | 0.009 | 0.007 | 0.010 | 0.009    | 0.015    | 0.010 | 0.010    |

Table 3. Mean genetic distance (D) and standard error (s.e.) within lineages of Coleoptera as shown in Figure 2. N= number of specimens in the lineage; Rep= specimen used to represent the lineage in the model-based phylogenetic analysis. n/c= not calculated where the number of specimens in the lineage is 1.

| Lineage | D     | s.e.  | N | rep  |
|---------|-------|-------|---|------|
| 1       | 0.000 | 0.000 | 4 | MF09 |
| 2       | 0.003 | 0.002 | 4 | MF20 |
| 3       | 0.009 | 0.003 | 4 | MF11 |
| 4       | 0.016 | 0.003 | 4 | MF03 |

Table 4. Mean genetic distance (D; below diagonal) and standard error (s.e.; above diagonal, in blue) between lineages of Coleoptera as shown in Figure 2.

| Lineage | 1     | 2     | 3     | 4     |
|---------|-------|-------|-------|-------|
| 1       |       | 0.007 | 0.012 | 0.016 |
| 2       | 0.046 |       | 0.013 | 0.015 |
| 3       | 0.159 | 0.157 |       | 0.016 |
| 4       | 0.264 | 0.265 | 0.260 |       |



Table 5. Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Curculionidae (Coleoptera) detected at Mesa H and the reference lineages as shown in Figure 3. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

| Specimen ID | MF03  | CF10  | CF13  | CF5   | CF6   | CF7   | CF8   | CF9   | G115  | GU213687 | HE615891 | IV148 | IV150 | IV152 | IV234 | IV395 | KD03  | KD04  | KD05  | KU519717 | KU519718 |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|----------|-------|-------|-------|-------|-------|-------|-------|-------|----------|----------|
| MF03        |       | 0.018 | 0.018 | 0.017 | 0.017 | 0.017 | 0.017 | 0.018 | 0.021 | 0.016    | 0.017    | 0.017 | 0.012 | 0.013 | 0.012 | 0.013 | 0.012 | 0.013 | 0.012 | 0.013    | 0.013    |
| CF10        | 0.218 |       | 0.004 | 0.008 | 0.005 | 0.008 | 0.007 | 0.004 | 0.017 | 0.016    | 0.016    | 0.014 | 0.018 | 0.018 | 0.018 | 0.016 | 0.017 | 0.018 | 0.018 | 0.018    | 0.018    |
| CF13        | 0.223 | 0.014 |       | 0.008 | 0.005 | 0.007 | 0.007 | 0.004 | 0.018 | 0.017    | 0.017    | 0.014 | 0.017 | 0.018 | 0.018 | 0.016 | 0.017 | 0.017 | 0.017 | 0.018    | 0.018    |
| CF5         | 0.223 | 0.038 | 0.035 |       | 0.007 | 0.008 | 0.008 | 0.007 | 0.017 | 0.017    | 0.017    | 0.014 | 0.017 | 0.018 | 0.017 | 0.017 | 0.017 | 0.017 | 0.017 | 0.017    | 0.017    |
| CF6         | 0.224 | 0.024 | 0.021 | 0.036 |       | 0.008 | 0.007 | 0.005 | 0.017 | 0.017    | 0.017    | 0.014 | 0.017 | 0.017 | 0.017 | 0.016 | 0.016 | 0.017 | 0.017 | 0.017    | 0.017    |
| CF7         | 0.220 | 0.039 | 0.038 | 0.046 | 0.045 |       | 0.005 | 0.007 | 0.016 | 0.016    | 0.015    | 0.014 | 0.018 | 0.018 | 0.018 | 0.015 | 0.018 | 0.018 | 0.018 | 0.018    | 0.018    |
| CF8         | 0.218 | 0.035 | 0.033 | 0.042 | 0.038 | 0.013 |       | 0.007 | 0.018 | 0.016    | 0.016    | 0.014 | 0.018 | 0.019 | 0.019 | 0.016 | 0.018 | 0.018 | 0.018 | 0.018    | 0.018    |
| CF9         | 0.218 | 0.014 | 0.014 | 0.029 | 0.024 | 0.041 | 0.039 |       | 0.018 | 0.017    | 0.016    | 0.014 | 0.017 | 0.017 | 0.017 | 0.016 | 0.017 | 0.017 | 0.017 | 0.017    | 0.017    |
| G115        | 0.252 | 0.152 | 0.158 | 0.154 | 0.152 | 0.158 | 0.165 | 0.152 |       | 0.018    | 0.020    | 0.020 | 0.022 | 0.022 | 0.022 | 0.021 | 0.022 | 0.022 | 0.022 | 0.022    | 0.022    |
| GU213687    | 0.218 | 0.208 | 0.207 | 0.208 | 0.202 | 0.198 | 0.199 | 0.205 | 0.189 |          | 0.015    | 0.014 | 0.016 | 0.016 | 0.016 | 0.014 | 0.016 | 0.016 | 0.016 | 0.015    | 0.015    |
| HE615891    | 0.218 | 0.193 | 0.195 | 0.209 | 0.202 | 0.190 | 0.201 | 0.192 | 0.208 | 0.198    |          | 0.017 | 0.014 | 0.014 | 0.014 | 0.015 | 0.014 | 0.014 | 0.014 | 0.015    | 0.015    |
| IV148       | 0.190 | 0.176 | 0.171 | 0.173 | 0.180 | 0.177 | 0.177 | 0.167 | 0.224 | 0.214    | 0.224    |       | 0.015 | 0.015 | 0.015 | 0.016 | 0.015 | 0.015 | 0.015 | 0.015    | 0.015    |
| IV150       | 0.130 | 0.220 | 0.220 | 0.224 | 0.217 | 0.215 | 0.217 | 0.209 | 0.235 | 0.190    | 0.196    | 0.190 |       | 0.006 | 0.000 | 0.011 | 0.001 | 0.002 | 0.000 | 0.006    | 0.006    |
| IV152       | 0.139 | 0.221 | 0.218 | 0.228 | 0.218 | 0.223 | 0.224 | 0.214 | 0.240 | 0.195    | 0.204    | 0.190 | 0.032 |       | 0.006 | 0.013 | 0.007 | 0.007 | 0.006 | 0.005    | 0.005    |
| IV234       | 0.130 | 0.222 | 0.222 | 0.226 | 0.219 | 0.217 | 0.219 | 0.212 | 0.232 | 0.190    | 0.196    | 0.192 | 0.000 | 0.033 |       | 0.011 | 0.001 | 0.002 | 0.000 | 0.006    | 0.006    |
| IV395       | 0.141 | 0.217 | 0.220 | 0.226 | 0.219 | 0.213 | 0.217 | 0.216 | 0.252 | 0.213    | 0.224    | 0.179 | 0.129 | 0.135 | 0.129 |       | 0.012 | 0.011 | 0.011 | 0.013    | 0.013    |
| KD03        | 0.131 | 0.217 | 0.217 | 0.221 | 0.214 | 0.215 | 0.217 | 0.206 | 0.238 | 0.191    | 0.198    | 0.189 | 0.001 | 0.034 | 0.001 | 0.130 |       | 0.003 | 0.001 | 0.006    | 0.006    |
| KD04        | 0.130 | 0.218 | 0.218 | 0.220 | 0.213 | 0.217 | 0.215 | 0.208 | 0.237 | 0.193    | 0.196    | 0.190 | 0.003 | 0.035 | 0.003 | 0.132 | 0.004 |       | 0.002 | 0.006    | 0.006    |
| KD05        | 0.130 | 0.219 | 0.219 | 0.223 | 0.216 | 0.215 | 0.216 | 0.209 | 0.235 | 0.190    | 0.196    | 0.190 | 0.000 | 0.032 | 0.000 | 0.129 | 0.001 | 0.003 |       | 0.006    | 0.006    |
| KU519717    | 0.131 | 0.228 | 0.228 | 0.236 | 0.225 | 0.229 | 0.231 | 0.220 | 0.226 | 0.193    | 0.207    | 0.198 | 0.029 | 0.021 | 0.029 | 0.140 | 0.030 | 0.032 | 0.029 |          | 0.000    |
| KU519718    | 0.131 | 0.228 | 0.228 | 0.236 | 0.225 | 0.229 | 0.231 | 0.220 | 0.226 | 0.193    | 0.207    | 0.198 | 0.029 | 0.021 | 0.029 | 0.140 | 0.030 | 0.032 | 0.029 | 0.000    |          |
| LI44        | 0.131 | 0.216 | 0.216 | 0.221 | 0.213 | 0.215 | 0.216 | 0.206 | 0.238 | 0.191    | 0.198    | 0.189 | 0.001 | 0.034 | 0.001 | 0.130 | 0.000 | 0.004 | 0.001 | 0.030    | 0.030    |
| LI45        | 0.131 | 0.226 | 0.226 | 0.230 | 0.223 | 0.221 | 0.226 | 0.215 | 0.253 | 0.192    | 0.198    | 0.198 | 0.006 | 0.034 | 0.005 | 0.129 | 0.009 | 0.011 | 0.006 | 0.027    | 0.027    |
| WAME89091   | 0.227 | 0.036 | 0.040 | 0.049 | 0.041 | 0.017 | 0.018 | 0.040 | 0.129 | 0.198    | 0.198    | 0.184 | 0.226 | 0.234 | 0.226 | 0.223 | 0.228 | 0.226 | 0.226 | 0.233    | 0.233    |
| WAME89114   | 0.231 | 0.038 | 0.035 | 0.044 | 0.043 | 0.041 | 0.033 | 0.035 | 0.136 | 0.201    | 0.204    | 0.184 | 0.229 | 0.234 | 0.229 | 0.217 | 0.228 | 0.233 | 0.229 | 0.233    | 0.233    |
| WAME89115   | 0.228 | 0.035 | 0.032 | 0.041 | 0.040 | 0.035 | 0.033 | 0.029 | 0.129 | 0.195    | 0.196    | 0.175 | 0.225 | 0.229 | 0.225 | 0.216 | 0.223 | 0.228 | 0.225 | 0.228    | 0.228    |
| WAME89116   | 0.222 | 0.036 | 0.040 | 0.052 | 0.047 | 0.014 | 0.015 | 0.040 | 0.134 | 0.198    | 0.195    | 0.182 | 0.216 | 0.223 | 0.216 | 0.222 | 0.217 | 0.219 | 0.216 | 0.222    | 0.222    |

|           |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| WAME89146 | 0.220 | 0.116 | 0.120 | 0.122 | 0.117 | 0.126 | 0.128 | 0.114 | 0.002 | 0.181 | 0.190 | 0.193 | 0.211 | 0.216 | 0.211 | 0.225 | 0.213 | 0.208 | 0.211 | 0.213 | 0.213 |
| WAME89147 | 0.223 | 0.114 | 0.119 | 0.123 | 0.119 | 0.125 | 0.129 | 0.112 | 0.014 | 0.175 | 0.187 | 0.191 | 0.208 | 0.216 | 0.208 | 0.225 | 0.210 | 0.208 | 0.208 | 0.210 | 0.210 |
| WAME89148 | 0.222 | 0.117 | 0.122 | 0.123 | 0.119 | 0.128 | 0.129 | 0.116 | 0.014 | 0.176 | 0.189 | 0.193 | 0.208 | 0.216 | 0.208 | 0.226 | 0.210 | 0.205 | 0.208 | 0.210 | 0.210 |
| WAME89151 | 0.218 | 0.119 | 0.119 | 0.116 | 0.122 | 0.103 | 0.106 | 0.114 | 0.132 | 0.190 | 0.209 | 0.199 | 0.210 | 0.207 | 0.210 | 0.217 | 0.211 | 0.213 | 0.210 | 0.210 | 0.210 |
| WAME89154 | 0.218 | 0.138 | 0.141 | 0.140 | 0.144 | 0.137 | 0.135 | 0.137 | 0.139 | 0.185 | 0.183 | 0.201 | 0.213 | 0.217 | 0.213 | 0.213 | 0.214 | 0.213 | 0.213 | 0.216 | 0.216 |
| WAME89157 | 0.223 | 0.117 | 0.116 | 0.123 | 0.119 | 0.125 | 0.123 | 0.116 | 0.018 | 0.181 | 0.187 | 0.193 | 0.213 | 0.220 | 0.213 | 0.231 | 0.214 | 0.210 | 0.213 | 0.214 | 0.214 |
| WAME89172 | 0.223 | 0.128 | 0.126 | 0.132 | 0.132 | 0.126 | 0.129 | 0.123 | 0.127 | 0.190 | 0.181 | 0.167 | 0.216 | 0.220 | 0.216 | 0.216 | 0.217 | 0.216 | 0.216 | 0.222 | 0.222 |
| WAME89176 | 0.241 | 0.219 | 0.219 | 0.217 | 0.217 | 0.208 | 0.214 | 0.222 | 0.215 | 0.208 | 0.215 | 0.233 | 0.242 | 0.237 | 0.242 | 0.234 | 0.240 | 0.239 | 0.242 | 0.237 | 0.237 |
| WAME89177 | 0.202 | 0.204 | 0.198 | 0.199 | 0.199 | 0.198 | 0.195 | 0.195 | 0.224 | 0.217 | 0.198 | 0.138 | 0.191 | 0.191 | 0.191 | 0.195 | 0.190 | 0.191 | 0.191 | 0.191 | 0.191 |
| WAME89186 | 0.131 | 0.228 | 0.228 | 0.236 | 0.225 | 0.229 | 0.231 | 0.220 | 0.226 | 0.193 | 0.207 | 0.198 | 0.029 | 0.021 | 0.029 | 0.140 | 0.030 | 0.032 | 0.029 | 0.000 | 0.000 |
| WAME89188 | 0.144 | 0.229 | 0.226 | 0.237 | 0.226 | 0.231 | 0.233 | 0.222 | 0.229 | 0.193 | 0.204 | 0.198 | 0.032 | 0.002 | 0.032 | 0.137 | 0.033 | 0.035 | 0.032 | 0.021 | 0.021 |

| /18   | L144  | L145  | WAME89091 | WAME89114 | WAME89115 | WAME89116 | WAME89146 | WAME89147 | WAME89148 | WAME89151 | WAME89154 | WAME89157 | WAME89172 | WAME89176 | WAME89177 | WAME89186 | WAME89188 |
|-------|-------|-------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 0.012 | 0.012 | 0.017 | 0.018     | 0.017     | 0.017     | 0.017     | 0.017     | 0.017     | 0.017     | 0.017     | 0.017     | 0.018     | 0.016     | 0.017     | 0.013     | 0.013     |           |
| 0.017 | 0.018 | 0.007 | 0.007     | 0.006     | 0.007     | 0.011     | 0.011     | 0.011     | 0.014     | 0.013     | 0.012     | 0.013     | 0.015     | 0.016     | 0.018     | 0.019     |           |
| 0.017 | 0.017 | 0.008 | 0.006     | 0.006     | 0.008     | 0.012     | 0.012     | 0.012     | 0.014     | 0.013     | 0.012     | 0.012     | 0.015     | 0.015     | 0.018     | 0.018     |           |
| 0.017 | 0.017 | 0.010 | 0.008     | 0.008     | 0.009     | 0.013     | 0.012     | 0.013     | 0.012     | 0.014     | 0.013     | 0.013     | 0.015     | 0.015     | 0.017     | 0.018     |           |
| 0.016 | 0.017 | 0.008 | 0.007     | 0.006     | 0.008     | 0.012     | 0.011     | 0.011     | 0.014     | 0.013     | 0.012     | 0.013     | 0.015     | 0.015     | 0.017     | 0.018     |           |
| 0.018 | 0.018 | 0.005 | 0.007     | 0.006     | 0.005     | 0.012     | 0.012     | 0.012     | 0.013     | 0.013     | 0.012     | 0.012     | 0.014     | 0.015     | 0.018     | 0.019     |           |
| 0.018 | 0.018 | 0.006 | 0.007     | 0.007     | 0.004     | 0.013     | 0.013     | 0.013     | 0.013     | 0.013     | 0.012     | 0.013     | 0.015     | 0.015     | 0.018     | 0.019     |           |
| 0.016 | 0.017 | 0.008 | 0.006     | 0.005     | 0.007     | 0.012     | 0.012     | 0.012     | 0.013     | 0.013     | 0.012     | 0.012     | 0.014     | 0.015     | 0.017     | 0.018     |           |
| 0.022 | 0.022 | 0.015 | 0.016     | 0.016     | 0.015     | 0.002     | 0.005     | 0.005     | 0.015     | 0.017     | 0.006     | 0.016     | 0.020     | 0.020     | 0.022     | 0.022     |           |
| 0.016 | 0.016 | 0.016 | 0.017     | 0.016     | 0.017     | 0.016     | 0.016     | 0.016     | 0.017     | 0.016     | 0.016     | 0.017     | 0.015     | 0.016     | 0.015     | 0.015     |           |
| 0.014 | 0.014 | 0.016 | 0.016     | 0.016     | 0.015     | 0.015     | 0.015     | 0.015     | 0.016     | 0.014     | 0.015     | 0.015     | 0.015     | 0.015     | 0.015     | 0.014     |           |
| 0.015 | 0.016 | 0.015 | 0.014     | 0.013     | 0.015     | 0.016     | 0.016     | 0.016     | 0.016     | 0.014     | 0.016     | 0.013     | 0.017     | 0.013     | 0.015     | 0.015     |           |
| 0.001 | 0.003 | 0.018 | 0.018     | 0.017     | 0.019     | 0.017     | 0.018     | 0.018     | 0.017     | 0.016     | 0.017     | 0.015     | 0.016     | 0.013     | 0.006     | 0.007     |           |
| 0.007 | 0.007 | 0.019 | 0.018     | 0.018     | 0.019     | 0.017     | 0.018     | 0.018     | 0.018     | 0.016     | 0.018     | 0.015     | 0.016     | 0.014     | 0.005     | 0.001     |           |
| 0.001 | 0.002 | 0.018 | 0.018     | 0.017     | 0.019     | 0.017     | 0.018     | 0.018     | 0.017     | 0.016     | 0.017     | 0.015     | 0.016     | 0.013     | 0.006     | 0.007     |           |
| 0.012 | 0.012 | 0.016 | 0.017     | 0.017     | 0.016     | 0.015     | 0.016     | 0.016     | 0.017     | 0.014     | 0.015     | 0.015     | 0.016     | 0.016     | 0.013     | 0.013     |           |
| 0.000 | 0.004 | 0.018 | 0.018     | 0.017     | 0.019     | 0.018     | 0.018     | 0.018     | 0.017     | 0.016     | 0.018     | 0.015     | 0.016     | 0.013     | 0.006     | 0.007     |           |
| 0.003 | 0.004 | 0.019 | 0.018     | 0.017     | 0.019     | 0.018     | 0.018     | 0.018     | 0.017     | 0.016     | 0.018     | 0.015     | 0.016     | 0.014     | 0.006     | 0.007     |           |
| 0.001 | 0.003 | 0.018 | 0.018     | 0.017     | 0.019     | 0.017     | 0.018     | 0.018     | 0.017     | 0.016     | 0.017     | 0.015     | 0.016     | 0.013     | 0.006     | 0.007     |           |
| 0.006 | 0.006 | 0.018 | 0.017     | 0.017     | 0.018     | 0.017     | 0.017     | 0.017     | 0.017     | 0.015     | 0.017     | 0.015     | 0.016     | 0.013     | 0.000     | 0.005     |           |
| 0.006 | 0.006 | 0.018 | 0.017     | 0.017     | 0.018     | 0.017     | 0.017     | 0.017     | 0.017     | 0.015     | 0.017     | 0.015     | 0.016     | 0.013     | 0.000     | 0.005     |           |
|       | 0.004 | 0.018 | 0.018     | 0.017     | 0.019     | 0.018     | 0.018     | 0.018     | 0.017     | 0.016     | 0.018     | 0.015     | 0.016     | 0.013     | 0.006     | 0.007     |           |
| 0.009 |       | 0.018 | 0.018     | 0.017     | 0.019     | 0.018     | 0.018     | 0.018     | 0.017     | 0.016     | 0.017     | 0.015     | 0.017     | 0.013     | 0.006     | 0.007     |           |
| 0.228 | 0.228 |       | 0.007     | 0.007     | 0.005     | 0.012     | 0.012     | 0.012     | 0.014     | 0.013     | 0.012     | 0.013     | 0.015     | 0.016     | 0.018     | 0.019     |           |
| 0.228 | 0.228 | 0.036 |           | 0.004     | 0.007     | 0.013     | 0.012     | 0.012     | 0.014     | 0.013     | 0.013     | 0.013     | 0.015     | 0.015     | 0.017     | 0.018     |           |
| 0.223 | 0.223 | 0.033 | 0.012     |           | 0.006     | 0.012     | 0.012     | 0.012     | 0.013     | 0.013     | 0.012     | 0.012     | 0.014     | 0.015     | 0.017     | 0.018     |           |
| 0.217 | 0.217 | 0.015 | 0.040     | 0.033     |           | 0.012     | 0.012     | 0.012     | 0.012     | 0.013     | 0.012     | 0.013     | 0.014     | 0.015     | 0.018     | 0.019     |           |

Table 6. Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Ptilidae (Coleoptera) detected at Mesa H and the reference lineages as shown in Figure 3. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

| Specimen ID | MF09  | MF11  | MF20  | IV144 | IV145 | IV146 | IV147 | WAME89165 | WAME89168 |
|-------------|-------|-------|-------|-------|-------|-------|-------|-----------|-----------|
| MF09        |       | 0.014 | 0.007 | 0.014 | 0.014 | 0.014 | 0.014 | 0.008     | 0.008     |
| MF11        | 0.160 |       | 0.014 | 0.005 | 0.005 | 0.005 | 0.005 | 0.015     | 0.015     |
| MF20        | 0.047 | 0.156 |       | 0.015 | 0.015 | 0.015 | 0.015 | 0.004     | 0.004     |
| IV144       | 0.161 | 0.016 | 0.160 |       | 0.000 | 0.000 | 0.000 | 0.015     | 0.015     |
| IV145       | 0.161 | 0.016 | 0.160 | 0.000 |       | 0.000 | 0.000 | 0.015     | 0.015     |
| IV146       | 0.161 | 0.016 | 0.160 | 0.000 | 0.000 |       | 0.000 | 0.015     | 0.015     |
| IV147       | 0.161 | 0.016 | 0.160 | 0.000 | 0.000 | 0.000 |       | 0.015     | 0.015     |
| WAME89165   | 0.044 | 0.158 | 0.017 | 0.166 | 0.166 | 0.166 | 0.166 |           | 0.000     |
| WAME89168   | 0.044 | 0.158 | 0.017 | 0.166 | 0.166 | 0.166 | 0.166 | 0.000     |           |



Table 7. Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Thysanura detected at Mesa H and the reference lineages as shown in Figure 4. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.

| Specimen ID                            | MF21  | MF22  | AY2   | AY3   | BX42  | BX43  | CA0006 2 | CA0006 3 | CA0006 4 | CA0124 | CA0144 | CA35  | CA36  | CA37  | CA38  |
|--|-------|-------|-------|-------|-------|-------|----------|----------|----------|--------|--------|-------|-------|-------|-------|
| MF21 RC12JIM000620160119T2-02          |       | 0.018 | 0.025 | 0.020 | 0.019 | 0.017 | 0.024    | 0.024    | 0.024    | 0.024  | 0.024  | 0.016 | 0.016 | 0.016 | 0.017 |
| MF22 RC14MEH025220151210-T1-03         | 0.125 |       | 0.025 | 0.023 | 0.022 | 0.021 | 0.023    | 0.023    | 0.023    | 0.023  | 0.023  | 0.019 | 0.019 | 0.019 | 0.019 |
| AY2 BES16311 B10 TN005                 | 0.220 | 0.204 |       | 0.003 | 0.024 | 0.022 | 0.023    | 0.023    | 0.023    | 0.023  | 0.023  | 0.022 | 0.022 | 0.022 | 0.021 |
| AY3 BES15706 S9 Trinemura TN005        | 0.249 | 0.253 | 0.003 |       | 0.020 | 0.020 | 0.021    | 0.021    | 0.021    | 0.021  | 0.021  | 0.021 | 0.021 | 0.021 | 0.020 |
| BX42 PES-5266 Trinemura MH1 TN006      | 0.160 | 0.170 | 0.243 | 0.244 |       | 0.016 | 0.022    | 0.022    | 0.022    | 0.022  | 0.023  | 0.017 | 0.017 | 0.017 | 0.017 |
| BX43 PES-5964 Trinemura MH1 TN007      | 0.171 | 0.192 | 0.190 | 0.200 | 0.146 |       | 0.021    | 0.021    | 0.021    | 0.020  | 0.021  | 0.019 | 0.019 | 0.019 | 0.019 |
| CA0006 2 Trinemura callawa             | 0.188 | 0.171 | 0.173 | 0.160 | 0.178 | 0.169 |          | 0.000    | 0.000    | 0.003  | 0.005  | 0.020 | 0.020 | 0.020 | 0.021 |
| CA0006 3 Trinemura callawa             | 0.188 | 0.171 | 0.173 | 0.160 | 0.178 | 0.169 | 0.000    |          | 0.000    | 0.003  | 0.005  | 0.020 | 0.020 | 0.020 | 0.021 |
| CA0006 4 Trinemura callawa             | 0.188 | 0.171 | 0.173 | 0.160 | 0.178 | 0.169 | 0.000    | 0.000    |          | 0.003  | 0.005  | 0.020 | 0.020 | 0.020 | 0.021 |
| CA0124 Trinemura callawa               | 0.191 | 0.174 | 0.177 | 0.163 | 0.181 | 0.172 | 0.003    | 0.003    | 0.003    |        | 0.004  | 0.020 | 0.020 | 0.020 | 0.020 |
| CA0144 LN1020 Trinemura callawa        | 0.191 | 0.177 | 0.180 | 0.169 | 0.187 | 0.178 | 0.009    | 0.009    | 0.009    | 0.006  |        | 0.020 | 0.020 | 0.020 | 0.021 |
| CA35 11 1513 Trinemura indet. TN013    | 0.142 | 0.141 | 0.185 | 0.212 | 0.156 | 0.166 | 0.149    | 0.149    | 0.149    | 0.152  | 0.158  |       | 0.000 | 0.000 | 0.004 |
| CA36 11 1456 Trinemura indet. TN013    | 0.142 | 0.141 | 0.185 | 0.212 | 0.156 | 0.166 | 0.149    | 0.149    | 0.149    | 0.152  | 0.158  | 0.000 |       | 0.000 | 0.004 |
| CA37 12 0096 Trinemura indet. TN013    | 0.142 | 0.141 | 0.185 | 0.212 | 0.156 | 0.166 | 0.149    | 0.149    | 0.149    | 0.152  | 0.158  | 0.000 | 0.000 |       | 0.004 |
| CA38 12 0107 Trinemura indet. TN013    | 0.149 | 0.147 | 0.182 | 0.210 | 0.159 | 0.171 | 0.152    | 0.152    | 0.152    | 0.155  | 0.162  | 0.008 | 0.008 | 0.008 |       |
| CA39 11 1511 Trinemura indet. TN013    | 0.149 | 0.147 | 0.182 | 0.210 | 0.159 | 0.171 | 0.152    | 0.152    | 0.152    | 0.155  | 0.162  | 0.008 | 0.008 | 0.008 | 0.000 |
| CA40 11 1527 Trinemura indet. TN013    | 0.149 | 0.147 | 0.182 | 0.210 | 0.159 | 0.171 | 0.152    | 0.152    | 0.152    | 0.155  | 0.162  | 0.008 | 0.008 | 0.008 | 0.000 |
| CQ10 Trinemura sp. B18 CC1886 TN008    | 0.121 | 0.128 | 0.211 | 0.246 | 0.181 | 0.203 | 0.179    | 0.179    | 0.179    | 0.182  | 0.185  | 0.153 | 0.153 | 0.153 | 0.156 |
| CQ11 Trinemura sp. B15 CC0179 TN009    | 0.175 | 0.161 | 0.170 | 0.171 | 0.171 | 0.148 | 0.163    | 0.163    | 0.163    | 0.167  | 0.167  | 0.164 | 0.164 | 0.164 | 0.168 |
| CQ12 Trinemura sp. B15 CCMUNK11 TN009  | 0.167 | 0.146 | 0.173 | 0.173 | 0.174 | 0.142 | 0.150    | 0.150    | 0.150    | 0.154  | 0.154  | 0.162 | 0.162 | 0.162 | 0.167 |
| CQ13 Trinemura sp. B19 CC0748 TN016    | 0.161 | 0.185 | 0.205 | 0.234 | 0.183 | 0.155 | 0.172    | 0.172    | 0.172    | 0.175  | 0.178  | 0.166 | 0.166 | 0.166 | 0.172 |
| CQ8 Trinemura sp. GNGCUNK011 TN008     | 0.107 | 0.120 | 0.211 | 0.244 | 0.170 | 0.203 | 0.179    | 0.179    | 0.179    | 0.182  | 0.185  | 0.144 | 0.144 | 0.144 | 0.147 |
| CQ9 Trinemura sp. B20 CC3527 TN009     | 0.172 | 0.160 | 0.173 | 0.201 | 0.157 | 0.143 | 0.159    | 0.159    | 0.159    | 0.155  | 0.155  | 0.160 | 0.160 | 0.160 | 0.163 |
| EJ0245R BHP165 J2 36 OB18 TN011        | 0.206 | 0.194 | 0.217 | 0.206 | 0.218 | 0.171 | 0.211    | 0.211    | 0.211    | 0.215  | 0.215  | 0.184 | 0.184 | 0.184 | 0.191 |
| EXR0524 OB21 OB22 TN016                | 0.198 | 0.171 | 0.205 | 0.202 | 0.202 | 0.167 | 0.207    | 0.207    | 0.207    | 0.210  | 0.204  | 0.189 | 0.189 | 0.189 | 0.196 |
| EXR0919 J16-1 TN001                    | 0.180 | 0.189 | 0.115 | 0.117 | 0.193 | 0.158 | 0.142    | 0.142    | 0.142    | 0.145  | 0.151  | 0.174 | 0.174 | 0.174 | 0.178 |
| EXR0983 BHP821 J8 13 Jimblebar W TN015 | 0.134 | 0.120 | 0.201 | 0.193 | 0.166 | 0.174 | 0.169    | 0.169    | 0.169    | 0.172  | 0.172  | 0.137 | 0.137 | 0.137 | 0.143 |

|  |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| EXR1136 BHP1364 J8 15 Mudlark TN016        | 0.190 | 0.193 | 0.180 | 0.174 | 0.182 | 0.160 | 0.172 | 0.172 | 0.172 | 0.175 | 0.175 | 0.172 | 0.172 | 0.172 | 0.172 |
| FL3 TOBRC0011PIT2-1 A TN014                | 0.128 | 0.128 | 0.208 | 0.228 | 0.146 | 0.160 | 0.172 | 0.172 | 0.172 | 0.175 | 0.181 | 0.104 | 0.104 | 0.104 | 0.109 |
| FL4 TOBRC0011PIT2-1 B TN014                | 0.128 | 0.128 | 0.208 | 0.228 | 0.146 | 0.160 | 0.172 | 0.172 | 0.172 | 0.175 | 0.181 | 0.104 | 0.104 | 0.104 | 0.109 |
| FL5 TOBRC0011-02 TN014                     | 0.128 | 0.128 | 0.208 | 0.228 | 0.146 | 0.160 | 0.172 | 0.172 | 0.172 | 0.175 | 0.181 | 0.104 | 0.104 | 0.104 | 0.109 |
| G126 TN003                                 | 0.181 | 0.205 | 0.209 | 0.192 | 0.170 | 0.134 | 0.213 | 0.213 | 0.213 | 0.210 | 0.216 | 0.174 | 0.174 | 0.174 | 0.180 |
| G205 100101 TN003                          | 0.175 | 0.193 | 0.212 | 0.217 | 0.168 | 0.129 | 0.205 | 0.205 | 0.205 | 0.208 | 0.215 | 0.166 | 0.166 | 0.166 | 0.171 |
| G206 108042 TN004                          | 0.169 | 0.188 | 0.223 | 0.225 | 0.157 | 0.123 | 0.198 | 0.198 | 0.198 | 0.201 | 0.207 | 0.173 | 0.173 | 0.173 | 0.178 |
| G207 107781 TN003                          | 0.171 | 0.193 | 0.208 | 0.212 | 0.161 | 0.127 | 0.211 | 0.211 | 0.211 | 0.208 | 0.215 | 0.166 | 0.166 | 0.166 | 0.171 |
| G317 LN8923 Nicoletiidae sp. OP TN002      | 0.172 | 0.214 | 0.207 | 0.216 | 0.159 | 0.098 | 0.206 | 0.206 | 0.206 | 0.209 | 0.209 | 0.170 | 0.170 | 0.170 | 0.175 |
| G318 LN8588 Nicoletiidae sp. MC TN001      | 0.206 | 0.226 | 0.115 | 0.108 | 0.212 | 0.170 | 0.132 | 0.132 | 0.132 | 0.135 | 0.141 | 0.190 | 0.190 | 0.190 | 0.193 |
| IV227 MEARC4383 TN010                      | 0.132 | 0.122 | 0.203 | 0.225 | 0.154 | 0.167 | 0.149 | 0.149 | 0.149 | 0.152 | 0.159 | 0.128 | 0.128 | 0.128 | 0.133 |
| IV333 MEBRC0020T1-20 TN010                 | 0.127 | 0.125 | 0.199 | 0.210 | 0.137 | 0.141 | 0.140 | 0.140 | 0.140 | 0.143 | 0.149 | 0.128 | 0.128 | 0.128 | 0.133 |
| IV334 M2ERC0057T1-21 TN011                 | 0.111 | 0.072 | 0.224 | 0.238 | 0.152 | 0.171 | 0.183 | 0.183 | 0.183 | 0.186 | 0.186 | 0.129 | 0.129 | 0.129 | 0.134 |
| IV338 GR15MEC0001-20160316-T2-06 TN012     | 0.116 | 0.122 | 0.185 | 0.214 | 0.168 | 0.177 | 0.155 | 0.155 | 0.155 | 0.158 | 0.164 | 0.117 | 0.117 | 0.117 | 0.119 |
| IV339 RC15MEC0197-20160316-T2-01 TN012     | 0.113 | 0.128 | 0.188 | 0.213 | 0.161 | 0.170 | 0.155 | 0.155 | 0.155 | 0.158 | 0.164 | 0.114 | 0.114 | 0.114 | 0.116 |
| JIN0037R J8 25 Jinariyi TN002              | 0.167 | 0.164 | 0.197 | 0.183 | 0.174 | 0.119 | 0.173 | 0.173 | 0.173 | 0.170 | 0.170 | 0.170 | 0.170 | 0.170 | 0.177 |
| JIN0182R BHP465 J2 37 Jinariyi TN016       | 0.166 | 0.180 | 0.194 | 0.180 | 0.174 | 0.139 | 0.178 | 0.178 | 0.178 | 0.181 | 0.187 | 0.136 | 0.136 | 0.136 | 0.142 |
| JQ282164 Trinemura callawa CA0006          | 0.188 | 0.171 | 0.173 | 0.160 | 0.178 | 0.169 | 0.000 | 0.000 | 0.000 | 0.003 | 0.009 | 0.149 | 0.149 | 0.149 | 0.152 |
| JQ282165 Trinemura cundalin CU0060R LN1093 | 0.199 | 0.187 | 0.187 | 0.171 | 0.180 | 0.175 | 0.065 | 0.065 | 0.065 | 0.062 | 0.062 | 0.164 | 0.164 | 0.164 | 0.167 |
| LI33 MEAWO4086-20170308-04 12S             | 0.122 | 0.125 | 0.196 | 0.210 | 0.136 | 0.136 | 0.137 | 0.137 | 0.137 | 0.140 | 0.146 | 0.128 | 0.128 | 0.128 | 0.133 |
| PROP003 J16-2 Trimenura TN002              | 0.183 | 0.202 | 0.205 | 0.205 | 0.190 | 0.125 | 0.201 | 0.201 | 0.201 | 0.204 | 0.204 | 0.189 | 0.189 | 0.189 | 0.196 |

| CA39  | CA40  | CQ10  | CQ11  | CQ12  | CQ13  | CQ8   | CQ9   | EJ0245R | EXR0524 | EXR0919 | EXR0983 | EXR1136 | FL3   | FL4   | FL5   | G126  | G205  | G206  | G207  | G317  | G318  | N227  | N333  |
|-------|-------|-------|-------|-------|-------|-------|-------|---------|---------|---------|---------|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0.017 | 0.017 | 0.016 | 0.017 | 0.019 | 0.017 | 0.015 | 0.017 | 0.023   | 0.022   | 0.022   | 0.019   | 0.021   | 0.016 | 0.016 | 0.016 | 0.019 | 0.018 | 0.017 | 0.017 | 0.019 | 0.019 | 0.016 | 0.016 |
| 0.019 | 0.019 | 0.017 | 0.019 | 0.020 | 0.019 | 0.018 | 0.019 | 0.024   | 0.023   | 0.023   | 0.018   | 0.025   | 0.016 | 0.016 | 0.016 | 0.021 | 0.019 | 0.020 | 0.020 | 0.023 | 0.021 | 0.016 | 0.017 |
| 0.021 | 0.021 | 0.022 | 0.025 | 0.025 | 0.026 | 0.022 | 0.025 | 0.025   | 0.024   | 0.020   | 0.025   | 0.022   | 0.022 | 0.022 | 0.022 | 0.024 | 0.025 | 0.025 | 0.024 | 0.023 | 0.020 | 0.021 | 0.022 |
| 0.020 | 0.020 | 0.022 | 0.024 | 0.024 | 0.023 | 0.022 | 0.022 | 0.023   | 0.023   | 0.019   | 0.024   | 0.020   | 0.020 | 0.020 | 0.020 | 0.020 | 0.023 | 0.023 | 0.022 | 0.021 | 0.017 | 0.020 | 0.020 |
| 0.017 | 0.017 | 0.022 | 0.019 | 0.021 | 0.018 | 0.024 | 0.018 | 0.023   | 0.021   | 0.021   | 0.020   | 0.020   | 0.019 | 0.019 | 0.019 | 0.017 | 0.017 | 0.017 | 0.016 | 0.018 | 0.020 | 0.018 | 0.017 |
| 0.019 | 0.019 | 0.022 | 0.019 | 0.019 | 0.018 | 0.023 | 0.017 | 0.021   | 0.020   | 0.021   | 0.021   | 0.019   | 0.018 | 0.018 | 0.018 | 0.017 | 0.016 | 0.016 | 0.016 | 0.014 | 0.019 | 0.020 | 0.018 |
| 0.021 | 0.021 | 0.021 | 0.023 | 0.022 | 0.024 | 0.021 | 0.021 | 0.024   | 0.023   | 0.019   | 0.020   | 0.021   | 0.022 | 0.022 | 0.022 | 0.022 | 0.022 | 0.024 | 0.022 | 0.022 | 0.019 | 0.019 | 0.020 |
| 0.021 | 0.021 | 0.021 | 0.023 | 0.022 | 0.024 | 0.021 | 0.021 | 0.024   | 0.023   | 0.019   | 0.020   | 0.021   | 0.022 | 0.022 | 0.022 | 0.022 | 0.022 | 0.024 | 0.022 | 0.022 | 0.019 | 0.019 | 0.020 |
| 0.021 | 0.021 | 0.021 | 0.023 | 0.022 | 0.024 | 0.021 | 0.021 | 0.024   | 0.023   | 0.019   | 0.020   | 0.021   | 0.022 | 0.022 | 0.022 | 0.022 | 0.022 | 0.024 | 0.022 | 0.022 | 0.019 | 0.019 | 0.020 |
| 0.020 | 0.020 | 0.021 | 0.023 | 0.022 | 0.024 | 0.021 | 0.022 | 0.024   | 0.023   | 0.020   | 0.020   | 0.021   | 0.022 | 0.022 | 0.022 | 0.022 | 0.022 | 0.024 | 0.022 | 0.022 | 0.020 | 0.019 | 0.020 |
| 0.021 | 0.021 | 0.021 | 0.023 | 0.022 | 0.024 | 0.021 | 0.022 | 0.023   | 0.023   | 0.020   | 0.020   | 0.021   | 0.022 | 0.022 | 0.022 | 0.023 | 0.023 | 0.024 | 0.023 | 0.022 | 0.020 | 0.020 | 0.021 |
| 0.004 | 0.004 | 0.017 | 0.020 | 0.021 | 0.021 | 0.018 | 0.019 | 0.024   | 0.023   | 0.021   | 0.019   | 0.022   | 0.013 | 0.013 | 0.013 | 0.020 | 0.018 | 0.020 | 0.018 | 0.018 | 0.020 | 0.017 | 0.017 |
| 0.004 | 0.004 | 0.017 | 0.020 | 0.021 | 0.021 | 0.018 | 0.019 | 0.024   | 0.023   | 0.021   | 0.019   | 0.022   | 0.013 | 0.013 | 0.013 | 0.020 | 0.018 | 0.020 | 0.018 | 0.018 | 0.020 | 0.017 | 0.017 |
| 0.004 | 0.004 | 0.017 | 0.020 | 0.021 | 0.021 | 0.018 | 0.019 | 0.024   | 0.023   | 0.021   | 0.019   | 0.022   | 0.013 | 0.013 | 0.013 | 0.020 | 0.018 | 0.020 | 0.018 | 0.018 | 0.020 | 0.017 | 0.017 |
| 0.000 | 0.000 | 0.017 | 0.020 | 0.022 | 0.021 | 0.018 | 0.019 | 0.024   | 0.023   | 0.021   | 0.019   | 0.022   | 0.014 | 0.014 | 0.014 | 0.020 | 0.018 | 0.020 | 0.018 | 0.018 | 0.020 | 0.017 | 0.017 |
|       | 0.000 | 0.017 | 0.020 | 0.022 | 0.021 | 0.018 | 0.019 | 0.024   | 0.023   | 0.021   | 0.019   | 0.022   | 0.014 | 0.014 | 0.014 | 0.020 | 0.018 | 0.020 | 0.018 | 0.018 | 0.020 | 0.017 | 0.017 |
| 0.000 |       | 0.017 | 0.020 | 0.022 | 0.021 | 0.018 | 0.019 | 0.024   | 0.023   | 0.021   | 0.019   | 0.022   | 0.014 | 0.014 | 0.014 | 0.020 | 0.018 | 0.020 | 0.018 | 0.018 | 0.020 | 0.017 | 0.017 |
| 0.156 | 0.156 |       | 0.021 | 0.022 | 0.020 | 0.008 | 0.019 | 0.026   | 0.025   | 0.023   | 0.017   | 0.025   | 0.016 | 0.016 | 0.016 | 0.022 | 0.022 | 0.019 | 0.021 | 0.023 | 0.022 | 0.015 | 0.016 |
| 0.168 | 0.168 | 0.183 |       | 0.006 | 0.015 | 0.021 | 0.007 | 0.021   | 0.019   | 0.024   | 0.020   | 0.020   | 0.017 | 0.017 | 0.017 | 0.017 | 0.018 | 0.019 | 0.017 | 0.019 | 0.023 | 0.019 | 0.019 |
| 0.167 | 0.167 | 0.181 | 0.011 |       | 0.016 | 0.021 | 0.009 | 0.021   | 0.019   | 0.023   | 0.020   | 0.020   | 0.018 | 0.018 | 0.018 | 0.020 | 0.020 | 0.018 | 0.020 | 0.022 | 0.023 | 0.020 | 0.020 |
| 0.172 | 0.172 | 0.181 | 0.107 | 0.099 |       | 0.020 | 0.015 | 0.021   | 0.019   | 0.023   | 0.022   | 0.018   | 0.017 | 0.017 | 0.017 | 0.016 | 0.016 | 0.018 | 0.016 | 0.018 | 0.022 | 0.019 | 0.019 |
| 0.147 | 0.147 | 0.031 | 0.186 | 0.184 | 0.175 |       | 0.018 | 0.026   | 0.025   | 0.023   | 0.017   | 0.026   | 0.018 | 0.018 | 0.018 | 0.022 | 0.021 | 0.020 | 0.021 | 0.024 | 0.022 | 0.016 | 0.017 |
| 0.163 | 0.163 | 0.172 | 0.016 | 0.021 | 0.105 | 0.174 |       | 0.021   | 0.020   | 0.022   | 0.021   | 0.020   | 0.017 | 0.017 | 0.017 | 0.017 | 0.017 | 0.017 | 0.016 | 0.019 | 0.020 | 0.018 | 0.017 |
| 0.191 | 0.191 | 0.207 | 0.142 | 0.139 | 0.153 | 0.201 | 0.146 |         | 0.014   | 0.023   | 0.024   | 0.021   | 0.022 | 0.022 | 0.022 | 0.021 | 0.022 | 0.021 | 0.022 | 0.020 | 0.023 | 0.023 | 0.022 |
| 0.196 | 0.196 | 0.212 | 0.138 | 0.139 | 0.165 | 0.218 | 0.145 | 0.086   |         | 0.022   | 0.022   | 0.020   | 0.023 | 0.023 | 0.023 | 0.021 | 0.022 | 0.022 | 0.022 | 0.017 | 0.021 | 0.022 | 0.021 |
| 0.178 | 0.178 | 0.203 | 0.153 | 0.144 | 0.173 | 0.199 | 0.143 | 0.186   | 0.187   |         | 0.022   | 0.022   | 0.022 | 0.022 | 0.022 | 0.024 | 0.025 | 0.024 | 0.024 | 0.022 | 0.005 | 0.021 | 0.021 |
| 0.143 | 0.143 | 0.137 | 0.157 | 0.154 | 0.170 | 0.134 | 0.156 | 0.184   | 0.173   | 0.159   |         | 0.023   | 0.017 | 0.017 | 0.017 | 0.021 | 0.022 | 0.021 | 0.021 | 0.021 | 0.022 | 0.014 | 0.014 |

Figure 1. Bayesian analysis of COI haplotypes of Blattodea (Nocticolidae) from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; specimens from previous phases at Mesa H are highlighted in green; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes enclose species with high confidence; red boxes with dashed lines represent groups requiring further investigation.

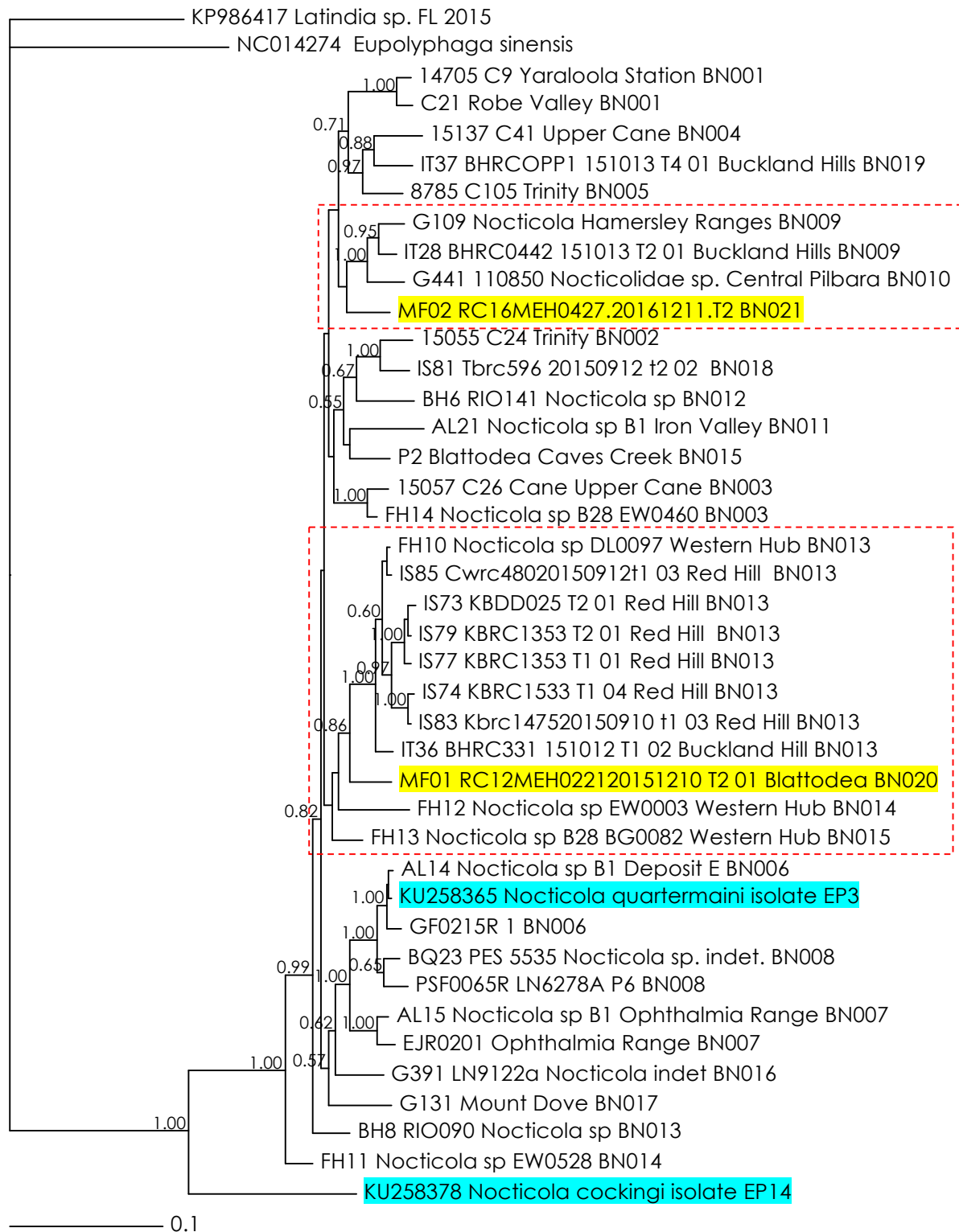




Figure 2. Neighbour-joining analysis of specimens of Coleoptera from the present study. Numbers on major nodes correspond to bootstrap support over 100 iterations; values <50% are not shown. Scale bar= genetic distance. The specimens used to represent each lineage in the model-based phylogenetic analysis are highlighted in yellow.

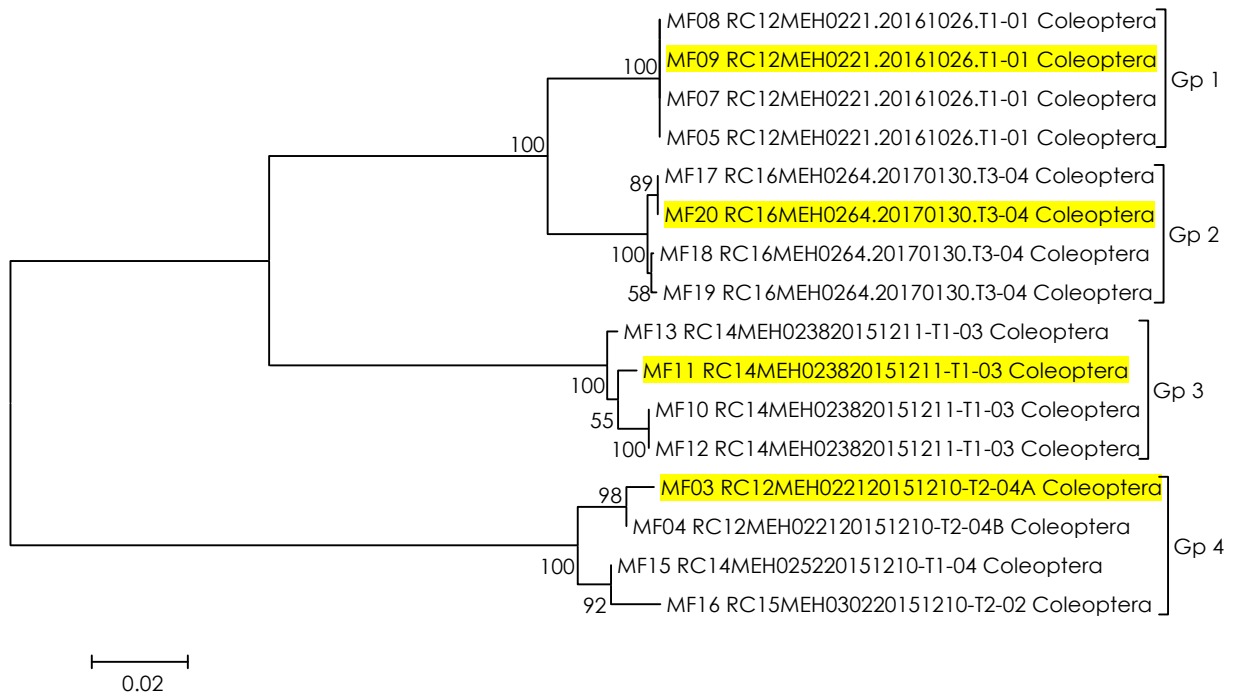
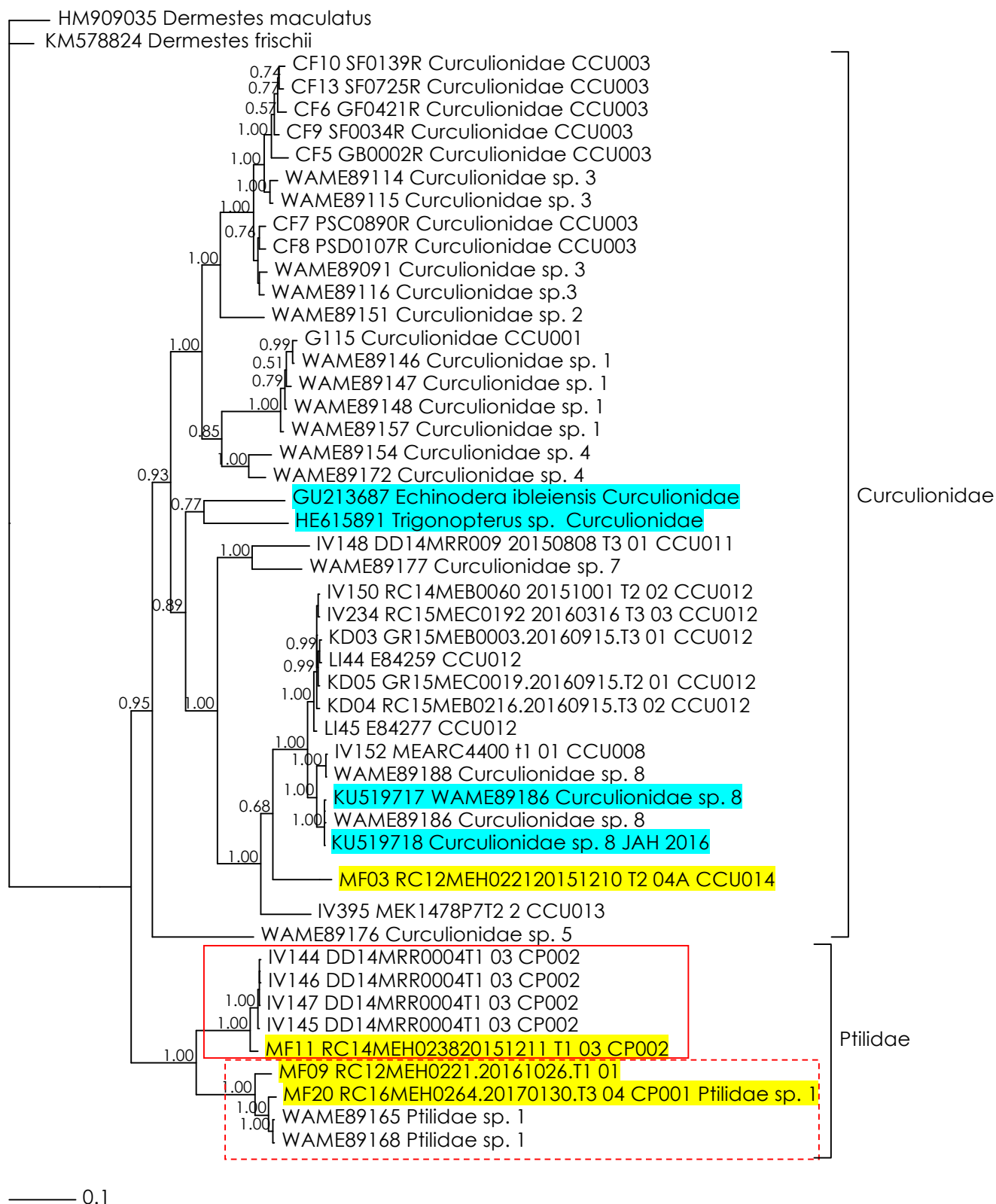


Figure 3. Bayesian analysis of COI haplotypes of Ptilidae and Curculionidae (Coleoptera) from the present study and reference specimens from the WAM and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; specimens from previous phases at Mesa H are highlighted in green; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes with solid lines enclose species with high confidence; red boxes with dashed lines represent groups requiring further investigation.







# Helix

## Molecular Solutions

School of Animal Biology The University of Western Australia  
Hackett Entrance No. 4 Hackett Drive Crawley WA 6009

PO Box 155  
Leederville WA 6903

t. [08] 6488 4509

f. [08] 6488 1029

abn. 32 133 230 243

w. [www.helixsolutions.com.au](http://www.helixsolutions.com.au)

12 April, 2017

Garth Humphreys  
Biota Environmental Sciences  
Level 1, 228 Carr Place  
Leederville, WA 6007

Via email

### **Re. Report on the molecular systematics of Amphipoda from Mesa H (Biota 1146f)**

Dear Garth,

Following is a summary of the results of the Amphipoda study we have completed from Mesa H. Fifteen distinct genetic lineages from three families (Melitidae/Eriopisidae, Neoniphargidae, Paramelitidae) were detected in the collections, nine of which have been detected previously in the Pilbara.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston, Yvette Hitchen and Dr. Oliver Berry  
Helix Molecular Solutions



## Background and Objective

One hundred twenty-one specimens of Amphipoda were collected from Mesa H and sequenced for variation at the mitochondrial COI gene. The molecular data were assessed in order to determine the number of species present and compare the results to those obtained during previous surveys in the Pilbara.

## Executive summary

- One hundred twenty-one specimens of amphipods from Mesa H were sequenced for variation at the COI gene.
- Fifteen distinct genetic lineages (eight Melitidae/ Eriopisidae, two Neoniphargidae and five Paramelitidae) were detected among the 95 specimens for which sequences were obtained.
- Nine of the lineages have been detected previously in the Pilbara, whereas six (two Melitidae/Eriopisidae and four Paramelitidae) appear to be new, based on the material available for comparison.

## Methods

One hundred twenty-one specimens of Amphipoda from 12 drillholes at Mesa H were sequenced for variation at the mitochondrial cytochrome oxidase subunit I gene (COI) using primers LCOI/HCO2.

Sequences were edited using GENEIOUS software (Drummond *et al.* 2011). Alignment was performed with CLUSTAL W (Thompson *et al.* 1994) using default parameters. Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotide differences between sequences).

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data for each taxonomic group. Bayesian analysis was used to construct the phylogenetic trees, incorporating the model as identified in MODELTEST for each taxonomic group. The phylogeny, branch lengths and posterior probabilities were obtained by running two trees simultaneously, each running four simultaneous MCMC chains. The number of cycles needed was determined by the standard deviation of the split frequencies of the two trees. The analysis was paused after every  $1 \times 10^6$  generations and when the standard deviation fell below 0.01, the analysis was stopped. A majority rule consensus tree was constructed after discarding the "burn-in" trees in both analyses. The burn-in value was determined by plotting the posterior probabilities obtained after every generation and identifying the point at which the values reach stationarity (= the asymptote). Trees produced prior to stationarity were discarded.

For the purposes of this report, lineages were defined as haplotypes or groups of haplotypes differing from other such groups by >3% sequence divergence. This cut-off was selected based on bar-coding data, which indicates that intra-specific variation rarely exceeds 2-3% (Hebert *et al.*, 2003b).

## Results

### *Preliminary analysis - Reference sequences and outgroups*

One hundred twenty-one specimens of amphipods from Mesa H were sequenced for COI (Table 1). In order to reduce analysis time and to simplify the presentation of results, a preliminary neighbour-joining (NJ) analysis was conducted on the 95 amphipods for which sequences were obtained, to identify unique haplotypes to include in a model-based phylogenetic analysis. Thirty-eight unique haplotypes were detected among the 95 sequences; these haplotypes were further sorted into 15 lineages. Comparisons to the GenBank sequence database indicated that five lineages were most similar to Paramelitidae and two were most similar to Niphargidae (Figure 1). Eight lineages were most similar to sequences of Melitidae/ Eriopisidae (Figure 2).

The three families were analysed in separate phylogenetic analyses with relevant reference specimens. The Niphargidae are not found in Australia, thus the lineages are likely to belong to



the closely related family Neoniphargidae, which is represented in Australia by seven genera (World Register of Marine Species – WORMS). Genbank lacks sequences of Neoniphargidae for COI, thus the closest matches appear to be from the family Niphargidae. Ten reference sequences of presumptive Neoniphargidae from previous surveys in the Robe Valley were included in the analysis, one from a borehole in which other specimens were identified as *Wesniphargus* sp. on the basis of morphology. Two Genbank reference sequences of Niphargidae, *Niphargus*: *N. fontanus* (Genbank accession number KC315635) and *N. glenniei* (Genbank accession number KC315646) were also included. Two sequences of Melitidae/Eriopisidae, *Nedsia* sp. 1 (Genbank accession #EU304458), and *Norcapensis mandibulis* (Genbank accession # JQ608487) were used as outgroups.

For the Paramelitidae, 48 reference sequences were included in the phylogenetic analysis, nine GenBank vouchers of Paramelitidae, as well as 39 specimens from previous surveys of the Robe Valley, Central Pilbara, Barrow Island, Lower Shaw River, Fortescue River, and Marillana Creek. Two sequences of the amphipod genus *Niphargus* were used as outgroups as follows: *N. fontanus* (Genbank accession number KC315635) and *N. glenniei* (Genbank accession number KC315646). Two sequences of Melitidae/Eriopisidae, *Nedsia* sp. 1 (Genbank accession #EU304458), and *Norcapensis mandibulis* (Genbank accession # JQ608487) were used as outgroups.

For the Melitidae/Eriopisidae, 52 reference sequences were included in the phylogenetic analysis. The genus *Nedsia* has been reassigned to the family Eriopisidae (WAM, pers. comm.) so GenBank vouchers of both Melitidae and Eriopisidae, *Nedsia* sp. 1 (Genbank accession #EU304458), and *Norcapensis mandibulis* (Genbank accession # JQ608487) as well as 50 specimens from previous surveys of the Robe Valley, Central Pilbara, Barrow Island, Lower Shaw River, Fortescue River, and Marillana Creek were included in the phylogenetic analysis. Two sequences of the amphipod genus *Niphargus* were used as outgroups as follows: *N. fontanus* (Genbank accession number KC315635) and *N. glenniei* (Genbank accession number KC315646).

#### Phylogenetic analysis- Neoniphargidae

The phylogenetic analysis, which included the five haplotypes of Neoniphargidae from Mesa H in addition to 13 reference specimens, placed the Mesa H haplotypes in two distinct genetic lineages. One lineage contained several reference specimens from Dave's and Budgie bores, and thus was assigned to the existing lineage AMN008 (Figure 3). The second lineage contained a reference specimen from a bore where other individuals were identified as *Wesniphargus* sp. on the basis of morphology and thus was assigned to the existing lineage *Wesniphargus* sp. AMN004 (Figure 3). Lineages AMN004 and AMN008 formed a well-supported clade (Figure 3).

#### Differentiation between lineages –Neoniphargidae

The Mesa H Neoniphargidae assigned to lineage AMN008, represented by three haplotypes (NH20, NH29 and NH32), differed from the nearest reference specimens KJ64, KD39, KE07, IV100, JN08 and LI20 (reference lineage AMN008) by between 0.4 and 1.4% sequence divergence, and from the remaining reference specimens by >12% sequence divergence (Table 2). The Mesa H Neoniphargidae assigned to lineage AMN004, represented by two haplotypes (NH67 and NH69) differed from the nearest reference specimen KE33 (reference lineage *Wesniphargus* sp. AMN004) by between 0.5 and 0.6% sequence divergence, and from the remaining reference specimens by >12 sequence divergence (Table 2). Lineages AMN004 and AMN008 differed from one another by ≥12% sequence divergence (Table 2).

#### Phylogenetic analysis- Paramelitidae

The phylogenetic analysis, which included the seven haplotypes of Paramelitidae from Mesa H in addition to 48 reference specimens, placed the Mesa H haplotypes in five distinct genetic lineages (Figure 4). One of the lineages also contained reference specimens. Specifically, haplotype NH12 was placed in the existing lineage AMP009 containing two haplotypes from Barrow Island and Budgie bore (Figure 4). The remaining six haplotypes were placed in four lineages that did not contain reference specimens, so were assigned to the new lineages AMP035, AMP036, AMP037 and AMP038 (Figure 4). Three of those new lineages, AMP036, AMP037 and AMP038, were placed in well supported clades with other reference lineages

(Figure 4). Specifically, lineage AMP036 was placed in a moderately well-supported clade containing reference specimens of *Chydaekata*, and lineage AMP037 was placed in a well-supported clade with lineage AMP032 from Mesa B (Figure 4). Lineage AMP038 was placed in a well-supported clade containing lineage AMP033 from Mesa C (Figure 4). The remaining new lineage, AMP035, did not show close relationships to reference specimens (Figure 4).

#### *Differentiation between lineages –Paramelitidae*

The Mesa H haplotype NH12 that was assigned to lineage AMP009 differed from the reference specimens in lineage AMP009 by 0.9 to 1.1% sequence divergence and from the remaining reference specimens by >12% sequence divergence (Table 3). Haplotype NH53D, which was assigned to the new lineage AMP037, differed from the nearest reference specimen, IS39 in lineage AMP032, by 4.1% sequence divergence and from the remaining reference specimens by approximately 8% sequence divergence (Table 3). Specifically, it differed from specimens in lineage AMP023 by between 7.9 and 8.0% sequence divergence (Table 3). Haplotypes NH54 and NH90, assigned to the new lineage AMP038, differed from the nearest reference specimen, IT25 in lineage AMP033 by between 3.7 and 4.0% sequence divergence and from the remaining reference specimens by >14% (Table 3). The remaining two new lineages, AMP035 and AMP036 differed from the nearest reference specimens by >17% sequence divergence (Table 3).

#### *Phylogenetic analysis- Melitidae/ Eriopisidae*

The phylogenetic analysis, which included the 26 haplotypes of Melitidae/ Eriopisidae from Mesa H, in addition to 52 reference specimens, placed the Mesa H specimens in eight distinct genetic lineages, all but two of which also contained reference specimens from previous surveys in the Pilbara (Figure 5). Specifically, haplotypes NH37 and NH39 were placed in existing lineage AMM006, containing reference specimen IV134 from Warrambo, and haplotype NH104 was placed in lineage AMM022, containing reference specimen IS37 from Red Hill (Figure 5). Haplotype NH63 was placed in existing lineage AMM026, containing reference specimens JN22, JF21 and LI10 from Mesas C and H, and haplotypes NH33, NH35 and NH38 were placed in lineage AMM004, containing reference specimens IV128, JN05, KE10 and LI11 from Mesa C and Red Hill (Figure 5). Haplotype NH36 was placed in existing lineage AMM031 with specimens LI25, KE05, KD45 and KD55 from Budgie bore and Mesa C (Figure 5). Fifteen haplotypes were placed in existing lineage AMM001 with eight reference specimens from Budgie bore, Mesa H and other bores in the Robe River valley (Figure 5).

Three haplotypes from Mesa H were placed in two lineages that did not contain any reference specimens. Specifically, haplotype NH62 was placed in a distinct lineage that did not contain any reference specimens and was assigned to the new lineage AMM032 (Figure 5). Likewise, NH46 and NH 110 were placed in a second distinct lineage that did not contain any reference specimens and were assigned to lineage AMM033 (Figure 5).

Three of the lineages, AMM001, AMM026, and AMM031 were placed in well supported clades with other reference lineages (Figure 5). Specifically, lineage AMM001 was placed in a large, well-supported clade containing multiple lineages, AMM025, AMM024, AMM029, AMM030 and AMM002 (Figure 5). Lineage AMM026 was placed in a well-supported clade containing reference lineage AMM021 from Red Hill, and lineage AMM031 was placed in a well-supported clade with lineage AMP027 from Budgie bore, and more distantly, with the AMM001 lineage complex (Figure 5). The remaining lineages did not show close relationships to reference specimens (Figure 5), although both lineage AMM026 and AMM004 showed significant relationships with reference specimens of *Nedsia*, suggesting they may belong to that genus.

#### *Differentiation between lineages – Melitidae/Eriopisidae*

The six Melitidae/Eriopisidae lineages detected at Mesa H that were assigned to existing lineages AMM001, AMM004, AMM006, AMM022, AMM026 and AMM031 differed from the nearest reference specimens by  $\leq 2.5\%$ , with the exception of lineage AMM031 where distances between haplotypes ranged from 0 – 3.5% (Table 4). Lineages AMM004, AMM006 and AMM022 differed from the remaining reference specimens by >9.5% (Table 4). Lineage AMM026 formed a well-supported clade with AMM021 and differed from that lineage by 4.6%, and from the remaining reference specimens by >8% (Table 4).

Lineage AMM031 formed a well-supported clade with AMM027 and differed from that lineage by 4% (Table 5). Lineage AMM001 formed a well-supported clade with multiple lineages (=AMM001 lineage complex) as well as with lineage complex AMM031 + AMM027 (Figure 5). Genetic distances between specimens in lineage complex AMM001 (enclosed by a dashed red box in Figure 5), ranged from 0 – 7.8% and the mean was 4.9% sequence divergence.

Haplotype NH62, in the new lineage AMM032, differed from the reference specimens by >14% sequence divergence (Table 4). Haplotypes NH46 and NH110, in the new lineage AMM033, differed from the reference specimens by >9% sequence divergence (Table 4).

## Conclusions

COI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003a). In a comparison of COI sequences for over 13,000 pairs of taxa, Hebert et al (2003b) found a mean of 11.1% sequence divergence between distinct species. Nearly 80% of the comparisons showed that species pairs differed from one another by greater than 8% sequence divergence. However, a taxon by taxon approach, examining the amount of phylogenetic variation within and between species is the most widely accepted method of defining species.

## Neoniphargidae

Two lineages of Neoniphargidae, AMN004 and AMN008, were detected at Mesa H during the current collection phase, both of which have been detected previously in the Pilbara. The two lineages differ from other reference lineages by >12% sequence divergence, thus they are likely to correspond to two distinct species. Lineage AMN004 is tentatively assigned to the genus *Wesniphargus*, owing to it containing a reference specimen from a bore at Mesa H where another individual was assigned to that genus, while lineage AMN008 has been detected previously at Mesa C and other sites in the Robe Valley.

**AMN004** = species detected previously at Mesa H; may belong to the genus *Wesniphargus*.

**AMN008** = species detected previously in the Robe Valley at Dave's and Budgie bores in the Robe Valley.

## Paramelitidae

Five lineages of Paramelitidae were detected at Mesa H, differing from one another by >15%, indicating that each is likely to represent a distinct species. One of the lineages has been detected previously (AMP009) and four appear to be new (AMP035, AMP036, AMP037 and AMP038), based on the material available for comparison. Of the four new lineages, two (AMP037 and AMP038) showed close relationships with other lineages. Specifically, lineage AMP037 formed a well-supported clade with lineage AMP032 from Mesa B and differed from it by 4.1% sequence divergence. While there are two lineages present, we cannot say if they represent one or two species, based on the current information; for this reason we will refer to the clade as a species complex. Similarly, lineage AMP038 formed a well-supported clade with lineage AMP033 from Mesa C, and differed from it by between 3.7 and 4.1%, again suggesting the presence of a species complex – two distinct lineages represented by an unknown number of species. Further research into these groups is necessary to define species boundaries.

**AMP009**: species previously detected at Barrow Island and Budgie bore

**AMP035**; new lineage and species, forms poorly supported clade with a reference specimen of *Yilgarus*

**AMP036**; new lineage and species, forms moderately well-supported clade with reference specimens of *Chydaekata*

**AMP037**; new lineage; belongs to a species complex with lineage AMP032 detected previously at Red Hill

**AMP038**; new lineage; belongs to a species complex with lineage AMP033 detected previously at Buckland Hill

### Melitidae/Eriopisidae

Eight lineages of Melitidae/Eriopisidae were detected at Mesa H, six of which were assigned to existing lineages. With the exception of distances between lineages AMM001 and AMM031, the lineages differed from one another by >9.0% sequence divergence, indicating that each is likely to represent a distinct species. One of the lineages, AMM026, formed a well-supported clade with reference lineage AMM021 and differed from that lineage by 4.6%. While there are two lineages present, we cannot say if they represent one or two species, based on the current information; for this reason we will refer to the clade as a species complex. Further research into this group is necessary to define species boundaries.

Lineages AMM001 and AMM031 formed a well-supported clade with one another and with multiple, closely related lineages that require further consideration (enclosed by a dashed red box in Figure 5). The specimens in this group differed by <5% mean sequence divergence (range = 0 – 7.8%). While there are clearly multiple lineages present, we cannot say how many species they represent, based on the current information; for this reason we will refer to the clade as a species complex. Further research into this group is necessary to define species boundaries.

**AMM004** = species detected previously at Mesa H and Dave's bore in the Robe Valley.

**AMM006** = species detected previously at Warramboo.

**AMM022** = species detected previously at Red Hill.

**AMM026** = lineage detected previously at Mesa C and Mesa H; forms a species complex with lineage AMM021 from Red Hill

**AMM001** } = large species complex comprised of multiple lineages; relationships among  
**AMM031** } the clade members requires further investigation.

**AMM032** = new lineage and species based on the material available for comparison

**AMM033** = new lineage and species based on the material available for comparison

### References

- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2011) Geneious v5.4, <http://www.geneious.com/>
- Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard J.R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* 270: 313-321.
- Hebert, P.D.N., Ratnasingham, S., deWaard J.R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B (supplement)* 270: S96-S99.
- Posada, D., Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- Thompson, J., Higgins, D., and Gibson, T. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680. doi:10.1093/nar/ 22.22.4673

Table 1. Specimens of Amphipoda used in the present study and the lineage to which they were assigned, based on variation at the COI gene. \* = new lineage this study

| Cap # | Site name   | Tracking number         | Helix ID | Lineage |
|-------|-------------|-------------------------|----------|---------|
| 1     | Budgie      | Budgie-20171212-01      | NH01     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH02     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH03     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH04     | AMM001  |
| 1     | Budgie      | Budgie-20171212-01      | NH05     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH06     | AMM001  |
| 1     | Budgie      | Budgie-20171212-01      | NH07     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH08     | AMN008  |
| 1     | Budgie      | Budgie-20171212-01      | NH09     | AMM001  |
| 1     | Budgie      | Budgie-20171212-01      | NH10     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH11     | AMM001  |
| 1     | Budgie      | Budgie-20171212-01      | NH12     | AMP009  |
| 1     | Budgie      | Budgie-20171212-01      | NH13     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH14     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH15     | AMM001  |
| 1     | Budgie      | Budgie-20171212-01      | NH16     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH17     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH18     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH19     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH20     | AMN008  |
| 1     | Budgie      | Budgie-20171212-01      | NH21     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH22     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH23     | AMM001  |
| 1     | Budgie      | Budgie-20171212-01      | NH24     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH25     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH26     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH27     | AMN008  |
| 1     | Budgie      | Budgie-20171212-01      | NH28     | AMN008  |
| 1     | Budgie      | Budgie-20171212-01      | NH29     | AMN008  |
| 1     | Budgie      | Budgie-20171212-01      | NH30     | AMM001  |
| 1     | Budgie      | Budgie-20171212-01      | NH31     | no data |
| 1     | Budgie      | Budgie-20171212-01      | NH32     | AMN008  |
| 7     | 200         | 200-20171212-03         | NH33     | AMM004  |
| 7     | 200         | 200-20171212-03         | NH34     | AMM004  |
| 7     | 200         | 200-20171212-03         | NH35     | AMM004  |
| 7     | 200         | 200-20171212-03         | NH36     | AMM031  |
| 9     | MB16MEC0009 | MB16MEC0009-20171212-02 | NH37     | AMM006  |
| 9     | MB16MEC0009 | MB16MEC0009-20171212-02 | NH38     | AMM004  |
| 14    | MB16MEC0008 | MB16MEC0008-20171212-03 | NH39     | AMM006  |
| 16    | MB17MEH0010 | MB17MEH0010-20171212-01 | NH40     | AMM033* |
| 16    | MB17MEH0010 | MB17MEH0010-20171212-01 | NH41     | AMM033* |
| 16    | MB17MEH0010 | MB17MEH0010-20171212-01 | NH42     | AMM033* |



|    |             |                         |      |         |
|----|-------------|-------------------------|------|---------|
| 16 | MB17MEH0010 | MB17MEH0010-20171212-01 | NH43 | no data |
| 19 | MB17MEH0009 | MB17MEH0009-20171212-02 | NH44 | AMM033* |
| 19 | MB17MEH0009 | MB17MEH0009-20171212-02 | NH45 | AMM033* |
| 19 | MB17MEH0009 | MB17MEH0009-20171212-02 | NH46 | AMM033* |
| 19 | MB17MEH0009 | MB17MEH0009-20171212-02 | NH47 | AMM033* |
| 19 | MB17MEH0009 | MB17MEH0009-20171212-02 | NH48 | AMM033* |
| 19 | MB17MEH0009 | MB17MEH0009-20171212-02 | NH49 | AMM033* |
| 19 | MB17MEH0009 | MB17MEH0009-20171212-02 | NH50 | AMM033* |
| 19 | MB17MEH0009 | MB17MEH0009-20171212-02 | NH51 | AMM033* |
| 22 | BC186       | BC186-20171213-03       | NH52 | no data |
| 22 | BC186       | BC186-20171213-03       | NH53 | AMP037* |
| 25 | 25          | 25-20171213-02          | NH54 | AMP038* |
| 27 | 31          | 31-20171213-02          | NH55 | AMM001  |
| 27 | 31          | 31-20171213-02          | NH56 | AMM001  |
| 27 | 31          | 31-20171213-02          | NH57 | AMM001  |
| 27 | 31          | 31-20171213-02          | NH58 | AMM001  |
| 27 | 31          | 31-20171213-02          | NH59 | AMM001  |
| 27 | 31          | 31-20171213-02          | NH60 | AMM001  |
| 27 | 31          | 31-20171213-02          | NH61 | AMM001  |
| 27 | 31          | 31-20171213-02          | NH62 | AMM032* |
| 27 | 31          | 31-20171213-02          | NH63 | AMM026  |
| 27 | 31          | 31-20171213-02          | NH64 | AMM026  |
| 36 | JW024       | JW024-20171213-06       | NH65 | AMM001  |
| 36 | JW024       | JW024-20171213-06       | NH66 | no data |
| 36 | JW024       | JW024-20171213-06       | NH67 | AMN004  |
| 36 | JW024       | JW024-20171213-06       | NH68 | AMN004  |
| 36 | JW024       | JW024-20171213-06       | NH69 | AMN004  |
| 36 | JW024       | JW024-20171213-06       | NH70 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH71 | no data |
| 41 | RR01        | RR01-20171214-05        | NH72 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH73 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH74 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH75 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH76 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH77 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH78 | AMP036* |
| 41 | RR01        | RR01-20171214-05        | NH79 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH80 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH81 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH82 | AMP035* |
| 41 | RR01        | RR01-20171214-05        | NH83 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH84 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH85 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH86 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH87 | AMM001  |

|    |             |                         |       |         |
|----|-------------|-------------------------|-------|---------|
| 41 | RR01        | RR01-20171214-05        | NH88  | AMP035* |
| 41 | RR01        | RR01-20171214-05        | NH89  | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH90  | AMP038* |
| 41 | RR01        | RR01-20171214-05        | NH91  | no data |
| 41 | RR01        | RR01-20171214-05        | NH92  | AMP035* |
| 41 | RR01        | RR01-20171214-05        | NH93  | no data |
| 41 | RR01        | RR01-20171214-05        | NH94  | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH95  | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH96  | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH97  | AMP035* |
| 41 | RR01        | RR01-20171214-05        | NH98  | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH99  | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH100 | AMM001  |
| 41 | RR01        | RR01-20171214-05        | NH101 | no data |
| 41 | RR01        | RR01-20171214-05        | NH102 | AMP035* |
| 41 | RR01        | RR01-20171214-05        | NH103 | AMP035* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH104 | AMM022  |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH105 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH106 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH107 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH108 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH109 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH110 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH111 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH112 | no data |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH113 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH114 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH115 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH116 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH117 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH118 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH119 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH120 | AMM033* |
| 45 | MB17MEH0007 | MB17MEH0007-20171214-02 | NH121 | AMM033* |

Table 2 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between haplotypes of Neoniphargidae (Amphipoda) detected at Mesa H and the reference lineages as shown in Figure 3. Distances between the Mesa H haplotypes and the reference specimens are highlighted in yellow.

| Specimen ID                                | NH20  | NH29  | NH32  | NH67  | NH69  | IV100 | IV129 | JN08  | KC315635 | KC315646 | KD39  | KD41  | KE07  | KE18  | KE28  | KE33  | KJ64  | LI20  |
|--|-------|-------|-------|-------|-------|-------|-------|-------|----------|----------|-------|-------|-------|-------|-------|-------|-------|-------|
| NH20 Budgie-20171212-01 AMN008             |       | 0.002 | 0.001 | 0.012 | 0.012 | 0.004 | 0.013 | 0.003 | 0.017    | 0.017    | 0.003 | 0.018 | 0.002 | 0.015 | 0.012 | 0.012 | 0.003 | 0.002 |
| NH29 Budgie-20171212-01 AMM008             | 0.003 |       | 0.003 | 0.013 | 0.012 | 0.004 | 0.013 | 0.003 | 0.017    | 0.016    | 0.002 | 0.018 | 0.003 | 0.015 | 0.012 | 0.013 | 0.004 | 0.002 |
| NH32 Budgie-20171212-01 AMM008             | 0.001 | 0.004 |       | 0.013 | 0.012 | 0.002 | 0.013 | 0.002 | 0.017    | 0.017    | 0.003 | 0.018 | 0.002 | 0.015 | 0.012 | 0.012 | 0.004 | 0.003 |
| NH67 JW024 20171213 06 AMM004              | 0.120 | 0.121 | 0.122 |       | 0.002 | 0.013 | 0.012 | 0.012 | 0.016    | 0.017    | 0.013 | 0.018 | 0.012 | 0.014 | 0.012 | 0.003 | 0.012 | 0.013 |
| NH69 JW024 20171213 06 AMM004              | 0.119 | 0.120 | 0.121 | 0.001 |       | 0.013 | 0.012 | 0.012 | 0.016    | 0.017    | 0.013 | 0.018 | 0.012 | 0.014 | 0.013 | 0.002 | 0.012 | 0.013 |
| IV100 Daves-20150604-01 AMN008             | 0.014 | 0.014 | 0.004 | 0.132 | 0.131 |       | 0.013 | 0.000 | 0.017    | 0.016    | 0.002 | 0.018 | 0.000 | 0.015 | 0.012 | 0.012 | 0.003 | 0.003 |
| IV129 DAVES-20150930-04 AMN007             | 0.155 | 0.156 | 0.148 | 0.157 | 0.156 | 0.149 |       | 0.013 | 0.017    | 0.016    | 0.014 | 0.017 | 0.013 | 0.015 | 0.008 | 0.012 | 0.013 | 0.013 |
| JN08 200-20160505-02C AMN008               | 0.007 | 0.007 | 0.004 | 0.126 | 0.125 | 0.000 | 0.149 |       | 0.017    | 0.017    | 0.002 | 0.018 | 0.000 | 0.015 | 0.012 | 0.012 | 0.003 | 0.003 |
| KC315635 Niphargus fontanus isolate UK HT3 | 0.205 | 0.205 | 0.205 | 0.200 | 0.202 | 0.205 | 0.190 | 0.205 |          | 0.015    | 0.018 | 0.020 | 0.017 | 0.015 | 0.017 | 0.016 | 0.016 | 0.017 |
| KC315646 Niphargus glenniei isolate HT4    | 0.215 | 0.213 | 0.213 | 0.210 | 0.210 | 0.213 | 0.190 | 0.213 | 0.167    |          | 0.017 | 0.018 | 0.017 | 0.016 | 0.016 | 0.017 | 0.017 | 0.017 |
| KD39 Budgie-20150930-01 Amphipoda          | 0.006 | 0.003 | 0.005 | 0.127 | 0.125 | 0.003 | 0.156 | 0.003 | 0.216    | 0.216    |       | 0.018 | 0.002 | 0.015 | 0.013 | 0.013 | 0.003 | 0.002 |
| KD41 Budgie-20150930-01 AMN001             | 0.242 | 0.242 | 0.238 | 0.251 | 0.250 | 0.240 | 0.246 | 0.240 | 0.251    | 0.246    | 0.237 |       | 0.018 | 0.018 | 0.017 | 0.018 | 0.018 | 0.018 |
| KE07 200-20160910-01 AMN008                | 0.006 | 0.006 | 0.004 | 0.124 | 0.123 | 0.000 | 0.149 | 0.000 | 0.205    | 0.213    | 0.003 | 0.241 |       | 0.015 | 0.012 | 0.012 | 0.003 | 0.003 |
| KE18 RR1-20160910-01 AMN002                | 0.192 | 0.193 | 0.188 | 0.187 | 0.190 | 0.189 | 0.173 | 0.189 | 0.196    | 0.215    | 0.191 | 0.258 | 0.189 |       | 0.015 | 0.013 | 0.015 | 0.015 |
| KE28 32-20160910-02 AMN003                 | 0.159 | 0.161 | 0.155 | 0.161 | 0.160 | 0.156 | 0.063 | 0.156 | 0.197    | 0.199    | 0.163 | 0.253 | 0.156 | 0.184 |       | 0.012 | 0.012 | 0.013 |
| KE33 25-20160910-01 AMN004                 | 0.124 | 0.126 | 0.122 | 0.006 | 0.005 | 0.121 | 0.151 | 0.121 | 0.199    | 0.210    | 0.123 | 0.245 | 0.121 | 0.183 | 0.156 |       | 0.012 | 0.013 |
| KJ64 Budgiebore-20161210-01 AMN008         | 0.007 | 0.007 | 0.007 | 0.123 | 0.122 | 0.006 | 0.151 | 0.006 | 0.205    | 0.211    | 0.006 | 0.243 | 0.006 | 0.187 | 0.158 | 0.122 |       | 0.003 |
| LI20 MEAWO4086-20170308-01 AMN008          | 0.005 | 0.003 | 0.006 | 0.128 | 0.127 | 0.005 | 0.159 | 0.005 | 0.209    | 0.219    | 0.003 | 0.245 | 0.005 | 0.199 | 0.163 | 0.128 | 0.008 |       |

Table 3 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between haplotypes of Paramelitidae (Amphipoda) detected at Mesa H and the reference lineages as shown in Figure 4. Distances between the Mesa H haplotypes and the reference specimens are highlighted in yellow.



| Specimen ID   | NH12  | NH53D | NH54  | NH78  | NH90  | NH92  | NH97  | AM10  | AM15  | AM2   | AM4   | AM9   | BX32  | DF3   | DQ255962 | DQ255989 | DQ490126 | DQ838024 | DQ838031 | DQ838032 | DZ24  | EF118194 | EF118232 | EF558852 | FU1   |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|----------|----------|----------|----------|----------|-------|----------|----------|----------|-------|
| NH12 Budgie-201 AMP00971212-01                          |       | 0.012 | 0.016 | 0.017 | 0.016 | 0.016 | 0.015 | 0.015 | 0.015 | 0.016 | 0.015 | 0.015 | 0.018 | 0.016 | 0.020    | 0.016    | 0.019    | 0.017    | 0.015    | 0.015    | 0.013 | 0.018    | 0.015    | 0.015    | 0.016 |
| NH53D BC186-20171213-03 AMP037                          | 0.149 |       | 0.013 | 0.015 | 0.013 | 0.016 | 0.015 | 0.015 | 0.017 | 0.016 | 0.016 | 0.015 | 0.019 | 0.016 | 0.020    | 0.015    | 0.020    | 0.015    | 0.018    | 0.018    | 0.011 | 0.017    | 0.015    | 0.017    | 0.017 |
| NH54 25-20171213-02 AMP038                              | 0.165 | 0.150 |       | 0.018 | 0.002 | 0.014 | 0.014 | 0.013 | 0.016 | 0.017 | 0.018 | 0.016 | 0.018 | 0.017 | 0.019    | 0.018    | 0.019    | 0.018    | 0.018    | 0.016    | 0.013 | 0.016    | 0.015    | 0.018    | 0.018 |
| NH78 RR01-20171214-05 AMP036                            | 0.227 | 0.240 | 0.244 |       | 0.018 | 0.016 | 0.015 | 0.016 | 0.015 | 0.016 | 0.016 | 0.019 | 0.017 | 0.015 | 0.018    | 0.015    | 0.014    | 0.014    | 0.017    | 0.016    | 0.017 | 0.016    | 0.018    | 0.017    | 0.016 |
| NH90 RR01-20171214-05 AMP038                            | 0.165 | 0.150 | 0.003 | 0.244 |       | 0.014 | 0.014 | 0.013 | 0.016 | 0.017 | 0.018 | 0.016 | 0.018 | 0.018 | 0.019    | 0.018    | 0.019    | 0.018    | 0.018    | 0.016    | 0.013 | 0.017    | 0.015    | 0.018    | 0.018 |
| NH92 RR01-20171214-05 AMP                               | 0.220 | 0.215 | 0.206 | 0.229 | 0.206 |       | 0.004 | 0.016 | 0.015 | 0.015 | 0.016 | 0.017 | 0.018 | 0.017 | 0.021    | 0.017    | 0.021    | 0.017    | 0.018    | 0.016    | 0.016 | 0.016    | 0.014    | 0.017    | 0.016 |
| NH97 RR01-20171214-05                                   | 0.206 | 0.219 | 0.210 | 0.220 | 0.210 | 0.008 |       | 0.016 | 0.014 | 0.014 | 0.015 | 0.016 | 0.018 | 0.016 | 0.021    | 0.016    | 0.021    | 0.017    | 0.017    | 0.015    | 0.016 | 0.016    | 0.013    | 0.017    | 0.015 |
| AM10 Paramelitidae sp 2c Weeli Wolli AMP020             | 0.256 | 0.258 | 0.218 | 0.233 | 0.219 | 0.214 | 0.207 |       | 0.015 | 0.016 | 0.014 | 0.014 | 0.016 | 0.016 | 0.020    | 0.016    | 0.018    | 0.017    | 0.017    | 0.017    | 0.014 | 0.015    | 0.015    | 0.016    | 0.014 |
| AM15 Paramelitidae sp B12 Christmas Creek AMP019        | 0.226 | 0.246 | 0.209 | 0.218 | 0.208 | 0.209 | 0.207 | 0.217 |       | 0.016 | 0.015 | 0.015 | 0.015 | 0.015 | 0.020    | 0.015    | 0.016    | 0.015    | 0.017    | 0.015    | 0.017 | 0.017    | 0.015    | 0.014    | 0.014 |
| AM2 Paramelitidae sp B14 Investigator AMP025            | 0.224 | 0.245 | 0.232 | 0.245 | 0.232 | 0.256 | 0.248 | 0.238 | 0.214 |       | 0.016 | 0.017 | 0.015 | 0.016 | 0.019    | 0.018    | 0.020    | 0.016    | 0.015    | 0.018    | 0.016 | 0.018    | 0.014    | 0.016    | 0.015 |
| AM4 Paramelitidae sp Mindy AMP018                       | 0.211 | 0.242 | 0.227 | 0.235 | 0.227 | 0.240 | 0.238 | 0.245 | 0.193 | 0.211 |       | 0.014 | 0.016 | 0.014 | 0.019    | 0.016    | 0.017    | 0.016    | 0.015    | 0.017    | 0.017 | 0.015    | 0.015    | 0.016    | 0.015 |
| AM9 Paramelitidae sp 2c Weeli Wolli AMP021              | 0.226 | 0.243 | 0.213 | 0.253 | 0.214 | 0.217 | 0.214 | 0.190 | 0.208 | 0.238 | 0.245 |       | 0.017 | 0.015 | 0.017    | 0.015    | 0.018    | 0.016    | 0.015    | 0.013    | 0.015 | 0.014    | 0.015    | 0.017    | 0.015 |
| BX32 PES-5173 Paramelitidae MH1 AMP026                  | 0.219 | 0.233 | 0.207 | 0.221 | 0.209 | 0.227 | 0.219 | 0.226 | 0.178 | 0.204 | 0.195 | 0.207 |       | 0.016 | 0.018    | 0.016    | 0.016    | 0.018    | 0.017    | 0.018    | 0.018 | 0.017    | 0.014    | 0.019    | 0.016 |
| DF3 9776 Amphipoda D2                                   | 0.230 | 0.236 | 0.215 | 0.205 | 0.215 | 0.237 | 0.237 | 0.235 | 0.229 | 0.250 | 0.221 | 0.210 | 0.209 |       | 0.017    | 0.014    | 0.016    | 0.017    | 0.016    | 0.017    | 0.017 | 0.016    | 0.015    | 0.017    | 0.015 |
| DQ255962 Molina pleobranchos                            | 0.230 | 0.241 | 0.221 | 0.217 | 0.223 | 0.232 | 0.232 | 0.226 | 0.243 | 0.278 | 0.250 | 0.219 | 0.226 | 0.199 |          | 0.017    | 0.016    | 0.018    | 0.017    | 0.020    | 0.018 | 0.020    | 0.018    | 0.020    | 0.018 |
| DQ255989 Chydaekata sp. Marillana                       | 0.236 | 0.255 | 0.208 | 0.201 | 0.207 | 0.216 | 0.219 | 0.231 | 0.207 | 0.260 | 0.234 | 0.226 | 0.210 | 0.190 | 0.193    |          | 0.017    | 0.013    | 0.016    | 0.015    | 0.016 | 0.015    | 0.016    | 0.017    | 0.015 |
| DQ490126 Pilbarus millsii                               | 0.241 | 0.256 | 0.219 | 0.191 | 0.221 | 0.249 | 0.249 | 0.245 | 0.226 | 0.245 | 0.217 | 0.223 | 0.234 | 0.197 | 0.210    | 0.174    |          | 0.015    | 0.018    | 0.019    | 0.019 | 0.017    | 0.017    | 0.016    | 0.017 |
| DQ838024 Chydaekata acuminata                           | 0.230 | 0.244 | 0.208 | 0.158 | 0.207 | 0.229 | 0.236 | 0.248 | 0.212 | 0.244 | 0.230 | 0.225 | 0.224 | 0.219 | 0.215    | 0.164    | 0.161    |          | 0.017    | 0.016    | 0.016 | 0.017    | 0.018    | 0.017    | 0.016 |
| DQ838031 Maarrka etheli                                 | 0.225 | 0.252 | 0.224 | 0.236 | 0.221 | 0.265 | 0.261 | 0.254 | 0.231 | 0.229 | 0.231 | 0.229 | 0.233 | 0.228 | 0.230    | 0.240    | 0.236    | 0.228    |          | 0.016    | 0.018 | 0.018    | 0.016    | 0.014    | 0.016 |
| DQ838032 Maarrka weeliwollii                            | 0.202 | 0.241 | 0.197 | 0.233 | 0.196 | 0.226 | 0.219 | 0.243 | 0.199 | 0.234 | 0.225 | 0.203 | 0.216 | 0.226 | 0.237    | 0.221    | 0.232    | 0.184    | 0.194    |          | 0.018 | 0.016    | 0.016    | 0.014    | 0.016 |
| DZ24 GW05.0413-04 AMP023                                | 0.132 | 0.079 | 0.160 | 0.224 | 0.160 | 0.215 | 0.210 | 0.233 | 0.212 | 0.239 | 0.237 | 0.231 | 0.224 | 0.224 | 0.230    | 0.236    | 0.243    | 0.230    | 0.230    | 0.214    |       | 0.017    | 0.015    | 0.015    | 0.016 |
| EF118194 Yilgarus sp                                    | 0.240 | 0.236 | 0.196 | 0.214 | 0.196 | 0.203 | 0.199 | 0.228 | 0.216 | 0.254 | 0.243 | 0.228 | 0.202 | 0.213 | 0.234    | 0.216    | 0.224    | 0.222    | 0.236    | 0.211    | 0.209 |          | 0.015    | 0.015    | 0.015 |
| EF118232 Yilgarus sp                                    | 0.195 | 0.197 | 0.196 | 0.217 | 0.196 | 0.168 | 0.166 | 0.220 | 0.194 | 0.223 | 0.212 | 0.217 | 0.176 | 0.223 | 0.237    | 0.223    | 0.223    | 0.215    | 0.238    | 0.200    | 0.209 | 0.197    |          | 0.015    | 0.015 |
| EF558852 Paramelitidae sp. 3                            | 0.231 | 0.229 | 0.211 | 0.213 | 0.213 | 0.226 | 0.223 | 0.222 | 0.213 | 0.242 | 0.236 | 0.204 | 0.219 | 0.217 | 0.224    | 0.216    | 0.215    | 0.210    | 0.202    | 0.194    | 0.207 | 0.211    | 0.202    |          | 0.012 |
| FU1 Paramelitidae sp KGST0006 AMP024                    | 0.233 | 0.256 | 0.216 | 0.223 | 0.214 | 0.212 | 0.215 | 0.220 | 0.175 | 0.217 | 0.184 | 0.208 | 0.194 | 0.221 | 0.236    | 0.208    | 0.223    | 0.219    | 0.226    | 0.217    | 0.225 | 0.233    | 0.197    | 0.211    |       |
| G186 Amphipoda 100152a AE                               | 0.222 | 0.243 | 0.216 | 0.183 | 0.216 | 0.242 | 0.240 | 0.253 | 0.217 | 0.229 | 0.209 | 0.222 | 0.221 | 0.189 | 0.205    | 0.173    | 0.051    | 0.177    | 0.229    | 0.220    | 0.230 | 0.221    | 0.211    | 0.203    | 0.222 |
| G187 Amphipoda 100476 ACAMP011 AMP011                   | 0.211 | 0.215 | 0.218 | 0.248 | 0.219 | 0.207 | 0.205 | 0.235 | 0.203 | 0.227 | 0.210 | 0.232 | 0.222 | 0.237 | 0.250    | 0.252    | 0.247    | 0.247    | 0.244    | 0.224    | 0.208 | 0.213    | 0.184    | 0.212    | 0.213 |
| G193 Amphipoda 100083b                                  | 0.244 | 0.244 | 0.224 | 0.211 | 0.224 | 0.211 | 0.214 | 0.230 | 0.233 | 0.251 | 0.251 | 0.209 | 0.228 | 0.214 | 0.199    | 0.201    | 0.213    | 0.195    | 0.247    | 0.216    | 0.230 | 0.218    | 0.225    | 0.233    | 0.235 |
| G444 110717 Paramelitidae sp AF                         | 0.227 | 0.251 | 0.232 | 0.196 | 0.232 | 0.251 | 0.251 | 0.266 | 0.230 | 0.241 | 0.221 | 0.226 | 0.226 | 0.192 | 0.200    | 0.184    | 0.085    | 0.181    | 0.219    | 0.209    | 0.249 | 0.243    | 0.218    | 0.213    | 0.226 |
| G496 111310 Paramelitidae sp NS AMP014                  | 0.144 | 0.103 | 0.164 | 0.223 | 0.164 | 0.224 | 0.218 | 0.235 | 0.212 | 0.233 | 0.215 | 0.241 | 0.212 | 0.223 | 0.247    | 0.242    | 0.256    | 0.233    | 0.226    | 0.220    | 0.079 | 0.219    | 0.205    | 0.211    | 0.229 |
| GK24 MB13YB010-180514-02 AMP030                         | 0.217 | 0.234 | 0.186 | 0.255 | 0.184 | 0.229 | 0.230 | 0.229 | 0.190 | 0.218 | 0.221 | 0.202 | 0.204 | 0.204 | 0.224    | 0.210    | 0.219    | 0.219    | 0.205    | 0.140    | 0.208 | 0.202    | 0.187    | 0.179    | 0.212 |
| GK48 99YJWB02-200514-01                                 | 0.236 | 0.253 | 0.215 | 0.204 | 0.214 | 0.223 | 0.226 | 0.230 | 0.205 | 0.256 | 0.229 | 0.223 | 0.209 | 0.181 | 0.199    | 0.006    | 0.182    | 0.167    | 0.243    | 0.223    | 0.238 | 0.220    | 0.222    | 0.217    | 0.208 |
| GK56 YM119-200514-02 AMP029                             | 0.230 | 0.243 | 0.217 | 0.256 | 0.218 | 0.213 | 0.213 | 0.192 | 0.211 | 0.236 | 0.247 | 0.010 | 0.210 | 0.205 | 0.213    | 0.225    | 0.219    | 0.222    | 0.233    | 0.201    | 0.229 | 0.222    | 0.213    | 0.207    | 0.202 |
| GK62 D08YJ188-200514-01 AMP028                          | 0.233 | 0.226 | 0.207 | 0.240 | 0.206 | 0.192 | 0.194 | 0.171 | 0.190 | 0.239 | 0.229 | 0.100 | 0.190 | 0.197 | 0.212    | 0.216    | 0.224    | 0.216    | 0.237    | 0.210    | 0.213 | 0.200    | 0.207    | 0.207    | 0.201 |
| GK7 MB13YB002-180514-03 AMP027                          | 0.217 | 0.203 | 0.206 | 0.224 | 0.204 | 0.216 | 0.216 | 0.196 | 0.205 | 0.215 | 0.210 | 0.156 | 0.177 | 0.207 | 0.217    | 0.210    | 0.237    | 0.218    | 0.226    | 0.204    | 0.192 | 0.220    | 0.207    | 0.204    | 0.208 |
| GK91 TS3DCP1-3 AMP031                                   | 0.206 | 0.229 | 0.191 | 0.237 | 0.189 | 0.223 | 0.219 | 0.236 | 0.193 | 0.224 | 0.218 | 0.198 | 0.205 | 0.211 | 0.236    | 0.223    | 0.230    | 0.189    | 0.193    | 0.017    | 0.210 | 0.205    | 0.197    | 0.184    | 0.210 |
| GO21 Yandi MAR03 Paramelitidae Genus 2 sp. B2 AMP033    | 0.255 | 0.253 | 0.215 | 0.230 | 0.217 | 0.212 | 0.207 | 0.012 | 0.217 | 0.236 | 0.244 | 0.187 | 0.224 | 0.231 | 0.219    | 0.228    | 0.241    | 0.244    | 0.255    | 0.242    | 0.231 | 0.228    | 0.220    | 0.219    | 0.221 |
| GO29 Yandi WW3 Paramelitidae Genus 2 sp. B2             | 0.235 | 0.245 | 0.220 | 0.253 | 0.222 | 0.220 | 0.219 | 0.192 | 0.211 | 0.236 | 0.245 | 0.007 | 0.207 | 0.205 | 0.221    | 0.231    | 0.224    | 0.227    | 0.231    | 0.199    | 0.234 | 0.228    | 0.217    | 0.205    | 0.207 |
| GO42 Marillana01 Paramelitidae sp. 2 (DEC code)         | 0.235 | 0.248 | 0.210 | 0.201 | 0.209 | 0.218 | 0.222 | 0.227 | 0.204 | 0.253 | 0.230 | 0.223 | 0.207 | 0.182 | 0.195    | 0.002    | 0.176    | 0.163    | 0.242    | 0.220    | 0.234 | 0.217    | 0.222    | 0.217    | 0.205 |
| GO45 Plant Paramelitidae sp. 2 AMP034                   | 0.243 | 0.248 | 0.228 | 0.250 | 0.230 | 0.216 | 0.216 | 0.196 | 0.214 | 0.239 | 0.257 | 0.031 | 0.207 | 0.208 | 0.221    | 0.236    | 0.236    | 0.236    | 0.239    | 0.214    | 0.226 | 0.225    | 0.223    | 0.211    | 0.211 |
| GO82 MNEW1upstream Paramelitidae sp. B25 nr. B16 AMP032 | 0.220 | 0.228 | 0.218 | 0.197 | 0.218 | 0.207 | 0.206 | 0.207 | 0.178 | 0.215 | 0.193 | 0.195 | 0.172 | 0.208 | 0.234    | 0.210    | 0.230    | 0.207    | 0.225    | 0.198    | 0.221 | 0.227    | 0.166    | 0.208    | 0.180 |
| GU111906 Krupus linnaei                                 | 0.230 | 0.238 | 0.234 | 0.246 | 0.232 | 0.263 | 0.261 | 0.259 | 0.253 | 0.248 | 0.251 | 0.251 | 0.234 | 0.246 | 0.243    | 0.215    | 0.249    | 0.246    | 0.250    | 0.240    | 0.232 | 0.244    | 0.253    | 0.226    | 0.234 |
| IS39 RHCMB042 20150910 02 AMP032                        | 0.135 | 0.041 | 0.146 | 0.222 | 0.146 | 0.201 | 0.193 | 0.231 | 0.217 | 0.228 | 0.216 | 0.226 | 0.219 | 0.226 | 0.247    | 0.245    | 0.241    | 0.231    | 0.236    | 0.220    | 0.081 | 0.216    | 0.186    | 0.214    | 0.241 |
| IT25 BHRC433.20151014 01 AMP033                         | 0.161 | 0.188 | 0.037 | 0.240 | 0.040 | 0.220 | 0.219 | 0.233 | 0.238 | 0.240 | 0.228 | 0.212 | 0.209 | 0.248 | 0.232    | 0.226    | 0.246    | 0.216    | 0.214    | 0.183    | 0.155 | 0.195    | 0.224    | 0.214    | 0.231 |
| IV115 TOBRC009 Amphipoda AMP023                         | 0.128 |       |       |       |       |       |       |       |       |       |       |       |       |       |          |          |          |          |          |          |       |          |          |          |       |

|                                    |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| KD63 TOBRC0099-20150930-01 AMP023  | 0.131 | 0.080 | 0.160 | 0.227 | 0.160 | 0.218 | 0.215 | 0.241 | 0.220 | 0.235 | 0.241 | 0.232 | 0.223 | 0.232 | 0.226 | 0.233 | 0.239 | 0.232 | 0.230 | 0.216 | 0.013 | 0.217 | 0.207 | 0.209 | 0.219 |
| KJ43 MB16WARR0009-02 lin 1 Paramel | 0.131 | 0.079 | 0.162 | 0.226 | 0.162 | 0.213 | 0.210 | 0.235 | 0.215 | 0.241 | 0.241 | 0.230 | 0.227 | 0.226 | 0.228 | 0.235 | 0.243 | 0.231 | 0.232 | 0.217 | 0.005 | 0.212 | 0.209 | 0.209 | 0.223 |
| L10 S6-204 Paramelitidae AMP009    | 0.009 | 0.138 | 0.166 | 0.245 | 0.166 | 0.221 | 0.217 | 0.259 | 0.234 | 0.238 | 0.217 | 0.231 | 0.222 | 0.224 | 0.228 | 0.228 | 0.241 | 0.231 | 0.228 | 0.210 | 0.136 | 0.236 | 0.193 | 0.228 | 0.240 |
| Q1 HCO2                            | 0.255 | 0.255 | 0.242 | 0.175 | 0.242 | 0.239 | 0.237 | 0.255 | 0.215 | 0.263 | 0.261 | 0.212 | 0.231 | 0.220 | 0.240 | 0.185 | 0.176 | 0.067 | 0.223 | 0.212 | 0.266 | 0.242 | 0.220 | 0.215 | 0.247 |
| Q3 WBGW010 AMP015                  | 0.247 | 0.255 | 0.225 | 0.241 | 0.225 | 0.241 | 0.241 | 0.223 | 0.210 | 0.218 | 0.196 | 0.218 | 0.225 | 0.241 | 0.251 | 0.215 | 0.246 | 0.236 | 0.236 | 0.231 | 0.239 | 0.241 | 0.225 | 0.249 | 0.037 |
| W6 Chykaeta Ethel Creek AMP017     | 0.229 | 0.240 | 0.234 | 0.257 | 0.234 | 0.238 | 0.233 | 0.250 | 0.232 | 0.218 | 0.215 | 0.232 | 0.217 | 0.230 | 0.252 | 0.246 | 0.254 | 0.245 | 0.237 | 0.220 | 0.221 | 0.233 | 0.205 | 0.205 | 0.220 |

| G186  | G187  | G193  | G444  | G496  | GK24  | GK48  | GK56  | GK62  | GK7   | GK91  | GO21  | GO29  | GO42  | GO45  | GO82  | GUI11906 | IS39  | IT25  | IV115 | J9 3  | J9 7  | JF19  | KD48  | KD63  | KJ43  | L10   | Q1    | Q3    | W6    |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0.019 | 0.015 | 0.017 | 0.015 | 0.012 | 0.014 | 0.016 | 0.016 | 0.015 | 0.015 | 0.015 | 0.015 | 0.016 | 0.016 | 0.016 | 0.013 | 0.022    | 0.011 | 0.019 | 0.012 | 0.016 | 0.017 | 0.014 | 0.004 | 0.012 | 0.013 | 0.004 | 0.023 | 0.024 | 0.016 |
| 0.019 | 0.015 | 0.018 | 0.018 | 0.011 | 0.016 | 0.016 | 0.015 | 0.016 | 0.016 | 0.018 | 0.014 | 0.016 | 0.015 | 0.015 | 0.016 | 0.020    | 0.007 | 0.019 | 0.011 | 0.013 | 0.017 | 0.015 | 0.013 | 0.010 | 0.010 | 0.013 | 0.025 | 0.023 | 0.017 |
| 0.019 | 0.015 | 0.019 | 0.019 | 0.015 | 0.017 | 0.018 | 0.016 | 0.016 | 0.018 | 0.016 | 0.013 | 0.016 | 0.018 | 0.017 | 0.016 | 0.021    | 0.013 | 0.011 | 0.013 | 0.016 | 0.016 | 0.015 | 0.017 | 0.013 | 0.013 | 0.017 | 0.024 | 0.022 | 0.016 |
| 0.014 | 0.018 | 0.015 | 0.014 | 0.017 | 0.017 | 0.015 | 0.018 | 0.016 | 0.017 | 0.016 | 0.015 | 0.018 | 0.015 | 0.018 | 0.016 | 0.020    | 0.016 | 0.024 | 0.017 | 0.019 | 0.020 | 0.016 | 0.019 | 0.017 | 0.016 | 0.019 | 0.020 | 0.023 | 0.018 |
| 0.019 | 0.015 | 0.019 | 0.019 | 0.015 | 0.017 | 0.018 | 0.016 | 0.016 | 0.018 | 0.016 | 0.014 | 0.016 | 0.018 | 0.017 | 0.016 | 0.020    | 0.013 | 0.010 | 0.014 | 0.016 | 0.017 | 0.015 | 0.017 | 0.014 | 0.013 | 0.017 | 0.024 | 0.022 | 0.016 |
| 0.018 | 0.016 | 0.016 | 0.019 | 0.018 | 0.017 | 0.017 | 0.017 | 0.016 | 0.018 | 0.017 | 0.015 | 0.018 | 0.017 | 0.017 | 0.015 | 0.020    | 0.016 | 0.021 | 0.016 | 0.018 | 0.016 | 0.016 | 0.017 | 0.016 | 0.016 | 0.017 | 0.023 | 0.022 | 0.015 |
| 0.018 | 0.016 | 0.016 | 0.018 | 0.017 | 0.015 | 0.016 | 0.016 | 0.015 | 0.017 | 0.015 | 0.015 | 0.017 | 0.016 | 0.016 | 0.014 | 0.020    | 0.015 | 0.021 | 0.016 | 0.018 | 0.016 | 0.014 | 0.017 | 0.016 | 0.016 | 0.017 | 0.023 | 0.022 | 0.014 |
| 0.016 | 0.016 | 0.016 | 0.016 | 0.014 | 0.015 | 0.016 | 0.014 | 0.013 | 0.013 | 0.016 | 0.004 | 0.014 | 0.016 | 0.014 | 0.015 | 0.018    | 0.015 | 0.021 | 0.014 | 0.016 | 0.014 | 0.015 | 0.016 | 0.014 | 0.014 | 0.016 | 0.024 | 0.019 | 0.015 |
| 0.015 | 0.014 | 0.015 | 0.015 | 0.017 | 0.014 | 0.015 | 0.015 | 0.015 | 0.016 | 0.014 | 0.015 | 0.015 | 0.015 | 0.015 | 0.014 | 0.019    | 0.017 | 0.023 | 0.017 | 0.019 | 0.016 | 0.016 | 0.017 | 0.017 | 0.017 | 0.018 | 0.020 | 0.022 | 0.015 |
| 0.016 | 0.014 | 0.018 | 0.015 | 0.015 | 0.015 | 0.017 | 0.017 | 0.016 | 0.015 | 0.017 | 0.016 | 0.017 | 0.017 | 0.017 | 0.014 | 0.017    | 0.016 | 0.022 | 0.016 | 0.018 | 0.017 | 0.016 | 0.017 | 0.016 | 0.016 | 0.017 | 0.025 | 0.022 | 0.013 |
| 0.016 | 0.015 | 0.016 | 0.016 | 0.015 | 0.015 | 0.015 | 0.015 | 0.016 | 0.016 | 0.016 | 0.014 | 0.014 | 0.015 | 0.015 | 0.014 | 0.020    | 0.016 | 0.022 | 0.017 | 0.017 | 0.017 | 0.014 | 0.016 | 0.017 | 0.017 | 0.017 | 0.024 | 0.022 | 0.015 |
| 0.016 | 0.018 | 0.016 | 0.016 | 0.015 | 0.015 | 0.015 | 0.004 | 0.012 | 0.014 | 0.013 | 0.014 | 0.003 | 0.015 | 0.006 | 0.015 | 0.019    | 0.015 | 0.022 | 0.016 | 0.017 | 0.013 | 0.015 | 0.015 | 0.016 | 0.015 | 0.016 | 0.022 | 0.020 | 0.016 |
| 0.016 | 0.016 | 0.017 | 0.015 | 0.019 | 0.017 | 0.016 | 0.016 | 0.016 | 0.015 | 0.017 | 0.016 | 0.017 | 0.016 | 0.016 | 0.015 | 0.019    | 0.018 | 0.021 | 0.018 | 0.020 | 0.018 | 0.016 | 0.018 | 0.018 | 0.018 | 0.020 | 0.023 | 0.022 | 0.016 |
| 0.016 | 0.015 | 0.016 | 0.017 | 0.016 | 0.016 | 0.013 | 0.015 | 0.015 | 0.014 | 0.016 | 0.015 | 0.015 | 0.013 | 0.016 | 0.014 | 0.020    | 0.015 | 0.023 | 0.018 | 0.020 | 0.017 | 0.014 | 0.017 | 0.017 | 0.017 | 0.018 | 0.022 | 0.021 | 0.015 |
| 0.016 | 0.019 | 0.017 | 0.016 | 0.019 | 0.017 | 0.017 | 0.017 | 0.019 | 0.018 | 0.019 | 0.020 | 0.018 | 0.017 | 0.018 | 0.018 | 0.020    | 0.020 | 0.027 | 0.018 | 0.018 | 0.016 | 0.020 | 0.019 | 0.018 | 0.018 | 0.019 | 0.022 | 0.022 | 0.020 |
| 0.016 | 0.015 | 0.015 | 0.015 | 0.018 | 0.017 | 0.003 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 0.001 | 0.015 | 0.014 | 0.019    | 0.016 | 0.024 | 0.016 | 0.019 | 0.016 | 0.017 | 0.018 | 0.016 | 0.015 | 0.018 | 0.021 | 0.020 | 0.016 |
| 0.008 | 0.018 | 0.017 | 0.010 | 0.019 | 0.017 | 0.017 | 0.018 | 0.017 | 0.021 | 0.018 | 0.018 | 0.018 | 0.017 | 0.019 | 0.016 | 0.020    | 0.022 | 0.027 | 0.019 | 0.020 | 0.018 | 0.019 | 0.019 | 0.019 | 0.019 | 0.019 | 0.021 | 0.023 | 0.019 |
| 0.014 | 0.018 | 0.014 | 0.016 | 0.017 | 0.017 | 0.013 | 0.017 | 0.016 | 0.017 | 0.015 | 0.017 | 0.017 | 0.013 | 0.017 | 0.016 | 0.019    | 0.016 | 0.023 | 0.016 | 0.018 | 0.018 | 0.016 | 0.018 | 0.016 | 0.016 | 0.018 | 0.012 | 0.022 | 0.015 |
| 0.017 | 0.016 | 0.017 | 0.016 | 0.017 | 0.016 | 0.016 | 0.015 | 0.016 | 0.016 | 0.016 | 0.017 | 0.015 | 0.016 | 0.017 | 0.017 | 0.020    | 0.017 | 0.023 | 0.017 | 0.018 | 0.017 | 0.016 | 0.016 | 0.017 | 0.017 | 0.016 | 0.023 | 0.023 | 0.015 |
| 0.016 | 0.017 | 0.017 | 0.016 | 0.018 | 0.014 | 0.015 | 0.013 | 0.014 | 0.015 | 0.005 | 0.017 | 0.013 | 0.015 | 0.014 | 0.015 | 0.018    | 0.017 | 0.020 | 0.018 | 0.019 | 0.016 | 0.016 | 0.016 | 0.018 | 0.017 | 0.017 | 0.022 | 0.023 | 0.016 |
| 0.018 | 0.015 | 0.018 | 0.018 | 0.010 | 0.016 | 0.015 | 0.016 | 0.016 | 0.016 | 0.017 | 0.015 | 0.016 | 0.015 | 0.015 | 0.016 | 0.018    | 0.011 | 0.017 | 0.004 | 0.013 | 0.014 | 0.014 | 0.013 | 0.004 | 0.002 | 0.014 | 0.024 | 0.023 | 0.018 |
| 0.017 | 0.015 | 0.016 | 0.017 | 0.017 | 0.015 | 0.015 | 0.014 | 0.015 | 0.016 | 0.016 | 0.015 | 0.014 | 0.015 | 0.015 | 0.015 | 0.021    | 0.017 | 0.020 | 0.017 | 0.019 | 0.017 | 0.017 | 0.020 | 0.017 | 0.017 | 0.020 | 0.021 | 0.020 | 0.014 |
| 0.017 | 0.015 | 0.017 | 0.016 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.017 | 0.016 | 0.015 | 0.015 | 0.016 | 0.015 | 0.013 | 0.020    | 0.015 | 0.021 | 0.015 | 0.016 | 0.017 | 0.014 | 0.015 | 0.015 | 0.015 | 0.015 | 0.024 | 0.023 | 0.014 |
| 0.015 | 0.016 | 0.017 | 0.016 | 0.017 | 0.015 | 0.017 | 0.017 | 0.017 | 0.018 | 0.014 | 0.016 | 0.017 | 0.017 | 0.017 | 0.017 | 0.016    | 0.017 | 0.021 | 0.015 | 0.019 | 0.017 | 0.017 | 0.015 | 0.015 | 0.015 | 0.015 | 0.022 | 0.020 | 0.015 |
| 0.016 | 0.014 | 0.016 | 0.015 | 0.016 | 0.014 | 0.015 | 0.015 | 0.015 | 0.016 | 0.015 | 0.014 | 0.015 | 0.015 | 0.016 | 0.014 | 0.018    | 0.017 | 0.022 | 0.017 | 0.017 | 0.016 | 0.015 | 0.016 | 0.017 | 0.016 | 0.017 | 0.022 | 0.009 | 0.015 |
|       | 0.015 | 0.016 | 0.010 | 0.019 | 0.017 | 0.016 | 0.017 | 0.015 | 0.018 | 0.016 | 0.016 | 0.016 | 0.016 | 0.017 | 0.015 | 0.019    | 0.020 | 0.025 | 0.018 | 0.020 | 0.018 | 0.019 | 0.020 | 0.018 | 0.018 | 0.020 | 0.019 | 0.023 | 0.018 |
| 0.229 |       | 0.016 | 0.018 | 0.016 | 0.016 | 0.015 | 0.018 | 0.018 | 0.017 | 0.016 | 0.016 | 0.018 | 0.015 | 0.019 | 0.015 | 0.018    | 0.015 | 0.024 | 0.015 | 0.017 | 0.015 | 0.014 | 0.015 | 0.015 | 0.015 | 0.014 | 0.023 | 0.019 | 0.014 |
| 0.203 | 0.248 |       | 0.015 | 0.016 | 0.016 | 0.015 | 0.016 | 0.016 | 0.019 | 0.017 | 0.016 | 0.016 | 0.015 | 0.016 | 0.015 | 0.020    | 0.018 | 0.022 | 0.018 | 0.021 | 0.016 | 0.016 | 0.019 | 0.017 | 0.018 | 0.020 | 0.022 | 0.022 | 0.016 |
| 0.090 | 0.243 | 0.196 |       | 0.018 | 0.016 | 0.014 | 0.016 | 0.018 | 0.018 | 0.016 | 0.016 | 0.016 | 0.014 | 0.017 | 0.016 | 0.019    | 0.018 | 0.022 | 0.018 | 0.020 | 0.019 | 0.018 | 0.016 | 0.018 | 0.018 | 0.017 | 0.022 | 0.022 | 0.016 |
| 0.237 | 0.208 | 0.241 | 0.254 |       | 0.016 | 0.017 | 0.016 | 0.016 | 0.017 | 0.017 | 0.014 | 0.016 | 0.017 | 0.015 | 0.017 | 0.018    | 0.010 | 0.019 | 0.010 | 0.011 | 0.016 | 0.013 | 0.013 | 0.011 | 0.010 | 0.013 | 0.022 | 0.022 | 0.015 |
| 0.202 | 0.207 | 0.206 | 0.204 | 0.212 |       | 0.016 | 0.015 | 0.015 | 0.016 | 0.012 | 0.015 | 0.015 | 0.016 | 0.015 | 0.013 | 0.019    | 0.015 | 0.019 | 0.016 | 0.018 | 0.017 | 0.015 | 0.015 | 0.016 | 0.016 | 0.017 | 0.022 | 0.021 | 0.014 |
| 0.179 | 0.254 | 0.199 | 0.181 | 0.242 | 0.190 |       | 0.015 | 0.015 | 0.015 | 0.014 | 0.015 | 0.015 | 0.002 | 0.015 | 0.013 | 0.018    | 0.016 | 0.023 | 0.016 | 0.019 | 0.017 | 0.016 | 0.018 | 0.015 | 0.015 | 0.018 | 0.020 | 0.020 | 0.016 |
| 0.220 | 0.229 | 0.208 | 0.226 | 0.245 | 0.187 | 0.208 |       | 0.012 | 0.013 | 0.013 | 0.014 | 0.003 | 0.015 | 0.005 | 0.015 | 0.019    | 0.016 | 0.023 | 0.017 | 0.017 | 0.013 | 0.015 | 0.016 | 0.016 | 0.016 | 0.017 | 0.022 | 0.020 | 0.016 |
| 0.209 | 0.215 | 0.205 | 0.218 | 0.227 | 0.180 | 0.200 | 0.099 |       | 0.012 | 0.014 | 0.012 | 0.011 | 0.015 | 0.012 | 0.015 | 0.018    | 0.015 | 0.021 | 0.016 | 0.018 | 0.016 | 0.015 | 0.017 | 0.016 | 0.016 | 0.018 | 0.023 | 0.023 | 0.016 |
| 0.225 | 0.211 | 0.232 | 0.235 | 0.187 | 0.186 | 0.198 | 0.149 | 0.128 |       | 0.015 | 0.012 | 0.013 | 0.015 | 0.013 | 0.016 | 0.018    | 0.015 | 0.021 | 0.016 | 0.017 | 0.017 | 0.015 | 0.016 | 0.016 | 0.016 | 0.016 | 0.023 | 0.023 | 0.017 |
| 0.224 | 0.219 | 0.212 | 0.207 | 0.211 | 0.117 | 0.209 | 0.184 | 0.193 | 0.184 |       | 0.016 | 0.012 | 0.014 | 0.013 | 0.014 | 0.017    | 0.016 | 0.020 | 0.017 | 0.018 | 0.015 | 0.015 | 0.016 | 0.017 | 0.017 | 0.017 | 0.022 | 0.023 | 0.015 |
| 0.250 | 0.235 | 0.224 | 0.262 | 0.233 | 0.216 | 0.214 | 0.177 | 0.159 | 0.184 | 0.222 |       | 0.013 | 0.015 | 0.013 | 0.014 | 0.019    | 0.015 | 0.020 | 0.014 | 0.016 | 0.014 | 0.015 | 0.015 | 0.014 | 0.014 | 0.016 | 0.024 | 0.020 | 0.015 |
| 0.224 | 0.234 | 0.211 | 0.227 | 0.244 | 0.190 | 0.211 | 0.008 | 0.096 | 0.151 | 0.183 | 0.176 |       | 0.015 | 0.005 | 0.015 | 0.018    | 0.016 | 0.022 | 0.017 | 0.017 | 0.013 | 0.015 | 0.016 | 0.016 | 0.016 | 0.017 | 0.022 | 0.020 | 0.016 |
| 0.174 | 0.253 | 0.199 | 0.181 | 0.238 | 0.191 | 0.004 | 0.208 | 0.200 | 0.194 | 0.207 | 0.209 | 0.212 |       | 0.014 | 0.013 | 0.019    | 0.016 | 0.024 | 0.016 | 0.019 | 0.017 | 0.016 | 0.018 | 0.015 | 0.015 | 0.018 | 0.020 | 0.020 | 0.016 |
| 0.232 | 0.232 | 0.214 | 0.241 | 0.247 | 0.194 | 0.218 | 0.028 | 0.098 | 0.151 | 0.197 | 0.180 | 0.028 | 0.216 |       | 0.015 | 0.019    | 0.016 | 0.023 | 0.016 | 0.018 | 0.013 | 0.015 | 0.016 | 0.015 | 0.015 | 0.018 | 0.022 | 0.020 | 0.016 |
| 0.225 | 0.218 | 0.227 | 0.215 | 0     |       |       |       |       |       |       |       |       |       |       |       |          |       |       |       |       |       |       |       |       |       |       |       |       |       |

|       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0.229 | 0.211 | 0.230 | 0.241 | 0.087 | 0.213 | 0.235 | 0.229 | 0.216 | 0.194 | 0.211 | 0.238 | 0.233 | 0.230 | 0.227 | 0.221 | 0.228 | 0.084 | 0.157 | 0.000 | 0.109 | 0.215 | 0.129 | 0.133 |       | 0.003 | 0.013 | 0.025 | 0.022 | 0.018 |
| 0.232 | 0.213 | 0.230 | 0.248 | 0.085 | 0.211 | 0.236 | 0.227 | 0.213 | 0.192 | 0.213 | 0.231 | 0.231 | 0.231 | 0.225 | 0.222 | 0.230 | 0.082 | 0.162 | 0.008 | 0.104 | 0.215 | 0.126 | 0.133 | 0.008 |       | 0.014 | 0.024 | 0.023 | 0.018 |
| 0.221 | 0.202 | 0.245 | 0.224 | 0.143 | 0.214 | 0.228 | 0.233 | 0.233 | 0.210 | 0.207 | 0.260 | 0.238 | 0.226 | 0.247 | 0.219 | 0.236 | 0.136 | 0.166 | 0.133 | 0.158 | 0.232 | 0.143 | 0.005 | 0.134 | 0.134 |       | 0.023 | 0.024 | 0.017 |
| 0.168 | 0.242 | 0.215 | 0.185 | 0.242 | 0.226 | 0.188 | 0.212 | 0.218 | 0.223 | 0.210 | 0.253 | 0.210 | 0.188 | 0.218 | 0.210 | 0.263 | 0.253 | 0.242 | 0.266 | 0.240 | 0.235 | 0.247 | 0.250 | 0.266 | 0.266 | 0.255 |       | 0.021 | 0.023 |
| 0.233 | 0.223 | 0.279 | 0.239 | 0.241 | 0.252 | 0.223 | 0.215 | 0.223 | 0.220 | 0.231 | 0.231 | 0.215 | 0.218 | 0.220 | 0.204 | 0.239 | 0.268 | 0.228 | 0.231 | 0.240 | 0.191 | 0.233 | 0.249 | 0.231 | 0.236 | 0.255 | 0.245 |       | 0.022 |
| 0.250 | 0.213 | 0.245 | 0.256 | 0.223 | 0.214 | 0.245 | 0.233 | 0.224 | 0.214 | 0.208 | 0.247 | 0.232 | 0.244 | 0.226 | 0.183 | 0.263 | 0.222 | 0.243 | 0.224 | 0.210 | 0.236 | 0.223 | 0.217 | 0.225 | 0.222 | 0.226 | 0.266 | 0.239 |       |

Table 4 (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between haplotypes of Melitidae/Eriopisidae (Amphipoda) detected at Mesa H and the reference lineages as shown in Figure 5. Distances between the Mesa H specimens and the reference specimens are highlighted in yellow.



| Specimen ID                            | NH37  | NH39  | NH104 | NH62  | NH63  | NH46  | NH110 | NH33  | NH35  | NH38  | NH36  | NH65  | NH70  | NH15  | NH06  | NH09  | NH23  | NH74  | NH83  | NH86  | NH60  | NH30  | NH58  | NH84  | NH89  | NH98  | KC315x |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| NH37 MB16MEC0009-20171212-02 AMM006    |       | 0.001 | 0.011 | 0.013 | 0.013 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.013 | 0.013 | 0.014 | 0.013 | 0.013 | 0.014 | 0.014 | 0.013 | 0.015 | 0.013 | 0.013 | 0.013 | 0.012 | 0.013 | 0.013 | 0.013 | 0.017  |
| NH39 MB16MEC0009-20171212-02 AMM006    | 0.001 |       | 0.011 | 0.013 | 0.013 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.013 | 0.013 | 0.014 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.015 | 0.013 | 0.013 | 0.013 | 0.012 | 0.013 | 0.013 | 0.013 | 0.017  |
| NH104 MB17MEH0007-20171214-02 AMM022   | 0.104 | 0.104 |       | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.014 | 0.013 | 0.014 | 0.013 | 0.013 | 0.014 | 0.014 | 0.013 | 0.015 | 0.013 | 0.014 | 0.014 | 0.013 | 0.013 | 0.013 | 0.013 | 0.018  |
| NH62 31-20171213-02 AMM032             | 0.145 | 0.143 | 0.135 |       | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.015 | 0.014 | 0.014 | 0.015 | 0.015 | 0.014 | 0.016 | 0.014 | 0.015 | 0.015 | 0.014 | 0.014 | 0.014 | 0.014 | 0.018  |
| NH63 31-20171213-02 AMM026             | 0.164 | 0.164 | 0.143 | 0.177 |       | 0.013 | 0.013 | 0.012 | 0.012 | 0.012 | 0.013 | 0.012 | 0.013 | 0.012 | 0.012 | 0.013 | 0.013 | 0.012 | 0.014 | 0.012 | 0.013 | 0.013 | 0.012 | 0.012 | 0.012 | 0.012 | 0.018  |
| NH46 MB17MEH0009-20171212-02 AMM033    | 0.170 | 0.168 | 0.165 | 0.180 | 0.152 |       | 0.002 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.012 | 0.011 | 0.011 | 0.012 | 0.012 | 0.012 | 0.013 | 0.011 | 0.012 | 0.012 | 0.012 | 0.011 | 0.011 | 0.011 | 0.018  |
| NH110 MB17MEH0007-20171214-02 AMM033   | 0.171 | 0.170 | 0.165 | 0.182 | 0.152 | 0.003 |       | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.012 | 0.011 | 0.011 | 0.012 | 0.012 | 0.012 | 0.013 | 0.011 | 0.012 | 0.012 | 0.011 | 0.011 | 0.011 | 0.011 | 0.018  |
| NH33 200-20171212-03 AMM004            | 0.149 | 0.148 | 0.157 | 0.174 | 0.133 | 0.098 | 0.098 |       | 0.001 | 0.002 | 0.012 | 0.011 | 0.012 | 0.011 | 0.011 | 0.012 | 0.012 | 0.011 | 0.014 | 0.011 | 0.012 | 0.012 | 0.011 | 0.011 | 0.011 | 0.011 | 0.019  |
| NH35 200-20171212-03 AMM004            | 0.148 | 0.146 | 0.155 | 0.173 | 0.132 | 0.097 | 0.097 | 0.001 |       | 0.002 | 0.012 | 0.011 | 0.012 | 0.011 | 0.011 | 0.012 | 0.012 | 0.011 | 0.014 | 0.011 | 0.012 | 0.012 | 0.011 | 0.011 | 0.011 | 0.011 | 0.019  |
| NH38 MB16MEC0009-20171212-02 AMM004    | 0.146 | 0.145 | 0.154 | 0.171 | 0.130 | 0.095 | 0.095 | 0.003 | 0.001 |       | 0.012 | 0.011 | 0.012 | 0.011 | 0.011 | 0.012 | 0.012 | 0.011 | 0.013 | 0.011 | 0.012 | 0.012 | 0.011 | 0.011 | 0.011 | 0.011 | 0.019  |
| NH36 200-20171212-03 AMM031            | 0.146 | 0.145 | 0.151 | 0.170 | 0.143 | 0.104 | 0.104 | 0.101 | 0.100 | 0.098 |       | 0.009 | 0.010 | 0.009 | 0.009 | 0.010 | 0.010 | 0.009 | 0.010 | 0.009 | 0.010 | 0.010 | 0.009 | 0.009 | 0.009 | 0.009 | 0.019  |
| NH65 JW024-20171213-06 AMM001          | 0.143 | 0.142 | 0.149 | 0.174 | 0.135 | 0.107 | 0.107 | 0.094 | 0.092 | 0.091 | 0.061 |       | 0.002 | 0.003 | 0.003 | 0.004 | 0.003 | 0.005 | 0.005 | 0.004 | 0.005 | 0.005 | 0.004 | 0.004 | 0.004 | 0.004 | 0.018  |
| NH70 JW024-20171213-06 AMM001          | 0.145 | 0.144 | 0.156 | 0.185 | 0.130 | 0.106 | 0.106 | 0.102 | 0.100 | 0.099 | 0.064 | 0.002 |       | 0.004 | 0.004 | 0.003 | 0.003 | 0.006 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.019  |
| NH15 Budgie-20171212-01 AMM001         | 0.145 | 0.143 | 0.152 | 0.176 | 0.139 | 0.108 | 0.108 | 0.098 | 0.097 | 0.095 | 0.066 | 0.007 | 0.010 |       | 0.001 | 0.003 | 0.002 | 0.006 | 0.006 | 0.005 | 0.006 | 0.006 | 0.005 | 0.005 | 0.005 | 0.005 | 0.018  |
| NH06 Budgie-20171212-01 AMM001         | 0.146 | 0.145 | 0.151 | 0.174 | 0.141 | 0.110 | 0.110 | 0.100 | 0.098 | 0.097 | 0.064 | 0.006 | 0.009 | 0.001 |       | 0.002 | 0.002 | 0.005 | 0.006 | 0.005 | 0.006 | 0.006 | 0.005 | 0.004 | 0.005 | 0.005 | 0.018  |
| NH09 Budgie-20171212-01 AMM001         | 0.150 | 0.149 | 0.158 | 0.179 | 0.137 | 0.108 | 0.108 | 0.103 | 0.102 | 0.100 | 0.066 | 0.010 | 0.007 | 0.005 | 0.003 |       | 0.002 | 0.006 | 0.006 | 0.005 | 0.005 | 0.005 | 0.006 | 0.005 | 0.006 | 0.005 | 0.018  |
| NH23 Budgie-20171212-01 AMM001         | 0.150 | 0.149 | 0.158 | 0.179 | 0.139 | 0.110 | 0.110 | 0.102 | 0.100 | 0.099 | 0.068 | 0.008 | 0.007 | 0.003 | 0.002 | 0.002 |       | 0.006 | 0.006 | 0.005 | 0.005 | 0.005 | 0.006 | 0.005 | 0.005 | 0.005 | 0.018  |
| NH74 RR01-20171214-05 AMM001           | 0.148 | 0.146 | 0.154 | 0.173 | 0.136 | 0.114 | 0.114 | 0.104 | 0.102 | 0.101 | 0.061 | 0.016 | 0.019 | 0.023 | 0.022 | 0.026 | 0.024 |       | 0.003 | 0.002 | 0.004 | 0.004 | 0.003 | 0.003 | 0.003 | 0.003 | 0.018  |
| NH83 RR01-20171214-05 AMM001           | 0.156 | 0.155 | 0.162 | 0.177 | 0.128 | 0.111 | 0.111 | 0.116 | 0.115 | 0.113 | 0.061 | 0.013 | 0.013 | 0.023 | 0.021 | 0.021 | 0.021 | 0.004 |       | 0.000 | 0.002 | 0.002 | 0.002 | 0.002 | 0.003 | 0.002 | 0.020  |
| NH86 RR01-20171214-05 AMM001           | 0.146 | 0.145 | 0.152 | 0.171 | 0.135 | 0.111 | 0.111 | 0.101 | 0.100 | 0.098 | 0.057 | 0.012 | 0.014 | 0.019 | 0.018 | 0.021 | 0.019 | 0.004 | 0.000 |       | 0.002 | 0.003 | 0.001 | 0.002 | 0.002 | 0.002 | 0.018  |
| NH60 31-20171213-02 AMM001             | 0.149 | 0.147 | 0.160 | 0.173 | 0.134 | 0.111 | 0.111 | 0.100 | 0.099 | 0.097 | 0.063 | 0.015 | 0.014 | 0.023 | 0.021 | 0.021 | 0.019 | 0.008 | 0.002 | 0.003 |       | 0.003 | 0.002 | 0.003 | 0.003 | 0.003 | 0.018  |
| NH30 Budgie-20171212-01 AMM001         | 0.149 | 0.147 | 0.157 | 0.173 | 0.132 | 0.107 | 0.107 | 0.099 | 0.097 | 0.095 | 0.065 | 0.016 | 0.016 | 0.024 | 0.023 | 0.023 | 0.021 | 0.010 | 0.004 | 0.005 | 0.005 |       | 0.003 | 0.003 | 0.003 | 0.003 | 0.018  |
| NH58 31-20171213-02 AMM001             | 0.145 | 0.143 | 0.154 | 0.170 | 0.136 | 0.113 | 0.113 | 0.100 | 0.098 | 0.097 | 0.059 | 0.013 | 0.016 | 0.020 | 0.019 | 0.023 | 0.021 | 0.006 | 0.002 | 0.001 | 0.002 | 0.006 |       | 0.003 | 0.003 | 0.003 | 0.018  |
| NH84 RR01-20171214-05 AMM001           | 0.146 | 0.145 | 0.149 | 0.170 | 0.136 | 0.111 | 0.111 | 0.101 | 0.100 | 0.098 | 0.057 | 0.012 | 0.016 | 0.016 | 0.015 | 0.019 | 0.018 | 0.007 | 0.002 | 0.003 | 0.005 | 0.006 | 0.004 |       | 0.001 | 0.001 | 0.018  |
| NH89 RR01-20171214-05 AMM001           | 0.148 | 0.146 | 0.148 | 0.171 | 0.135 | 0.113 | 0.113 | 0.100 | 0.098 | 0.097 | 0.059 | 0.013 | 0.017 | 0.018 | 0.016 | 0.021 | 0.019 | 0.009 | 0.004 | 0.004 | 0.006 | 0.008 | 0.006 | 0.001 |       | 0.002 | 0.018  |
| NH98 RR01-20171214-05 AMM001           | 0.148 | 0.146 | 0.148 | 0.168 | 0.135 | 0.113 | 0.113 | 0.102 | 0.101 | 0.100 | 0.059 | 0.013 | 0.017 | 0.018 | 0.016 | 0.021 | 0.019 | 0.009 | 0.004 | 0.004 | 0.006 | 0.008 | 0.006 | 0.001 | 0.003 |       | 0.018  |
| LI10 MB16MEC0009-170308-2 Amphipoda    | 0.167 | 0.167 | 0.150 | 0.179 | 0.013 | 0.153 | 0.153 | 0.144 | 0.142 | 0.140 | 0.136 | 0.128 | 0.127 | 0.131 | 0.132 | 0.133 | 0.134 | 0.129 | 0.124 | 0.128 | 0.131 | 0.129 | 0.129 | 0.128 | 0.126 | 0.126 | 0.316  |
| LI11 MB16MEC0009-170308-2 Amphipoda    | 0.148 | 0.147 | 0.156 | 0.171 | 0.132 | 0.096 | 0.096 | 0.003 | 0.002 | 0.000 | 0.099 | 0.091 | 0.099 | 0.096 | 0.097 | 0.100 | 0.099 | 0.100 | 0.113 | 0.097 | 0.097 | 0.095 | 0.096 | 0.099 | 0.097 | 0.100 | 0.324  |
| LI25 RC15MEC0200-20170308-01 Amphipoda | 0.147 | 0.145 | 0.153 | 0.169 | 0.140 | 0.104 | 0.104 | 0.102 | 0.100 | 0.099 | 0.000 | 0.062 | 0.062 | 0.067 | 0.065 | 0.065 | 0.066 | 0.064 | 0.061 | 0.059 | 0.061 | 0.063 | 0.061 | 0.057 | 0.059 | 0.059 | 0.322  |
| B3 60a Nedsia sp 1AMM013               | 0.156 | 0.156 | 0.164 | 0.182 | 0.100 | 0.183 | 0.183 | 0.147 | 0.145 | 0.144 | 0.152 | 0.147 | 0.149 | 0.149 | 0.151 | 0.152 | 0.152 | 0.152 | 0.149 | 0.151 | 0.151 | 0.152 | 0.149 | 0.149 | 0.151 | 0.147 | 0.337  |
| BF14 X62P2-2011-01 AMM018              | 0.153 | 0.153 | 0.157 | 0.184 | 0.176 | 0.189 | 0.191 | 0.188 | 0.186 | 0.184 | 0.186 | 0.183 | 0.185 | 0.181 | 0.183 | 0.183 | 0.184 | 0.192 | 0.195 | 0.188 | 0.187 | 0.189 | 0.186 | 0.186 | 0.188 | 0.188 | 0.324  |
| cob12 5a AMM009                        | 0.160 | 0.160 | 0.158 | 0.191 | 0.105 | 0.172 | 0.172 | 0.158 | 0.155 | 0.153 | 0.143 | 0.143 | 0.143 | 0.145 | 0.147 | 0.147 | 0.147 | 0.155 | 0.155 | 0.151 | 0.153 | 0.149 | 0.153 | 0.149 | 0.147 | 0.149 | 0.330  |
| DH1 BIL4nr-1112-03-01 AMM019           | 0.148 | 0.148 | 0.150 | 0.166 | 0.189 | 0.174 | 0.175 | 0.156 | 0.155 | 0.155 | 0.161 | 0.162 | 0.168 | 0.166 | 0.164 | 0.165 | 0.166 | 0.159 | 0.168 | 0.159 | 0.160 | 0.162 | 0.158 | 0.158 | 0.159 | 0.156 | 0.317  |
| EU304458 Nedsia sp. 1 sge1m4 2         | 0.167 | 0.165 | 0.165 | 0.216 | 0.150 | 0.104 | 0.104 | 0.116 | 0.114 | 0.112 | 0.112 | 0.106 | 0.106 | 0.108 | 0.110 | 0.110 | 0.110 | 0.104 | 0.106 | 0.106 | 0.108 | 0.106 | 0.108 | 0.104 | 0.101 | 0.104 | 0.334  |
| G405 110129 AMM014                     | 0.174 | 0.174 | 0.162 | 0.180 | 0.110 | 0.174 | 0.174 | 0.150 | 0.148 | 0.147 | 0.140 | 0.134 | 0.133 | 0.136 | 0.137 | 0.139 | 0.141 | 0.142 | 0.135 | 0.140 | 0.145 | 0.144 | 0.142 | 0.139 | 0.137 | 0.140 | 0.317  |
| G406 110196 AMM015                     | 0.167 | 0.167 | 0.170 | 0.183 | 0.110 | 0.178 | 0.178 | 0.147 | 0.145 | 0.144 | 0.145 | 0.140 | 0.138 | 0.142 | 0.144 | 0.142 | 0.144 | 0.151 | 0.149 | 0.147 | 0.149 | 0.147 | 0.148 | 0.145 | 0.144 | 0.147 | 0.320  |
| IS37 RHWB005 20150910 01 AMM022        | 0.106 | 0.106 | 0.016 | 0.136 | 0.153 | 0.169 | 0.170 | 0.155 | 0.153 | 0.151 | 0.150 | 0.151 | 0.154 | 0.155 | 0.153 | 0.155 | 0.155 | 0.155 | 0.156 | 0.153 | 0.157 | 0.153 | 0.155 | 0.151 | 0.150 | 0.150 | 0.326  |
| IS38 RHWB005 20150910 01 AMM023        | 0.109 | 0.110 | 0.114 | 0.148 | 0.181 | 0.178 | 0.177 | 0.167 | 0.166 | 0.164 | 0.156 | 0.162 | 0.166 | 0.166 | 0.164 | 0.165 | 0.166 | 0.161 | 0.166 | 0.161 | 0.162 | 0.162 | 0.159 | 0.159 | 0.158 | 0.161 | 0.326  |
| IS43 RHWB006 20150910 01 AMM021        | 0.189 | 0.189 | 0.150 | 0.188 | 0.046 | 0.159 | 0.159 | 0.148 | 0.147 | 0.145 | 0.148 | 0.137 | 0.133 | 0.139 | 0.140 | 0.139 | 0.141 | 0.142 | 0.135 | 0.137 | 0.139 | 0.137 | 0.139 | 0.136 | 0.134 | 0.134 | 0.325  |
| IS59 KBRC1310 20150911 02 AMM020       | 0.177 | 0.175 | 0.169 | 0.178 | 0.145 | 0.091 | 0.091 | 0.109 | 0.107 | 0.106 | 0.095 | 0.103 | 0.106 | 0.104 | 0.103 | 0.105 | 0.107 | 0.103 | 0.097 | 0.099 | 0.105 | 0.103 | 0.101 | 0.098 | 0.099 | 0.099 | 0.326  |
| IT06 BHRC118.20151013-02 AMM024        | 0.150 | 0.148 | 0.151 | 0.184 | 0.132 | 0.103 | 0.103 | 0.098 | 0.097 | 0.095 | 0.060 |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |        |

|                                      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|--------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| IV97 Camp bore AMM001                | 0.152 | 0.150 | 0.160 | 0.181 | 0.139 | 0.112 | 0.112 | 0.104 | 0.102 | 0.101 | 0.069 | 0.011 | 0.012 | 0.006 | 0.005 | 0.006 | 0.005 | 0.027 | 0.025 | 0.022 | 0.024 | 0.026 | 0.024 | 0.021 | 0.022 | 0.022 | 0.321 |
| JF02 DD13MEH0007-20151024-01B AMM001 | 0.148 | 0.147 | 0.155 | 0.172 | 0.134 | 0.110 | 0.110 | 0.103 | 0.101 | 0.099 | 0.057 | 0.013 | 0.016 | 0.017 | 0.016 | 0.019 | 0.018 | 0.006 | 0.002 | 0.002 | 0.005 | 0.006 | 0.003 | 0.000 | 0.002 | 0.002 | 0.314 |
| JF21 RC13MEH0097-20151024-01A AMM026 | 0.170 | 0.170 | 0.147 | 0.178 | 0.002 | 0.153 | 0.153 | 0.137 | 0.136 | 0.134 | 0.140 | 0.131 | 0.128 | 0.139 | 0.137 | 0.136 | 0.137 | 0.132 | 0.126 | 0.131 | 0.132 | 0.131 | 0.132 | 0.132 | 0.131 | 0.131 | 0.322 |
| JN05 87-20160505-02E AMM004          | 0.147 | 0.145 | 0.155 | 0.172 | 0.133 | 0.095 | 0.095 | 0.003 | 0.002 | 0.003 | 0.100 | 0.092 | 0.099 | 0.096 | 0.098 | 0.100 | 0.099 | 0.101 | 0.113 | 0.098 | 0.097 | 0.095 | 0.096 | 0.100 | 0.098 | 0.101 | 0.325 |
| JN17 32-20160506-01A AMM001          | 0.147 | 0.145 | 0.158 | 0.170 | 0.134 | 0.110 | 0.110 | 0.099 | 0.098 | 0.096 | 0.060 | 0.013 | 0.016 | 0.021 | 0.019 | 0.023 | 0.021 | 0.006 | 0.002 | 0.002 | 0.002 | 0.006 | 0.000 | 0.003 | 0.005 | 0.005 | 0.315 |
| JN22 3120160507-03A AMM026           | 0.169 | 0.169 | 0.148 | 0.180 | 0.000 | 0.151 | 0.151 | 0.136 | 0.134 | 0.132 | 0.142 | 0.132 | 0.130 | 0.137 | 0.139 | 0.137 | 0.139 | 0.134 | 0.128 | 0.132 | 0.134 | 0.132 | 0.134 | 0.134 | 0.132 | 0.132 | 0.320 |
| JQ608487 Norcapensis mandibulis      | 0.166 | 0.164 | 0.170 | 0.196 | 0.189 | 0.190 | 0.190 | 0.176 | 0.176 | 0.175 | 0.171 | 0.164 | 0.166 | 0.168 | 0.166 | 0.168 | 0.168 | 0.170 | 0.176 | 0.170 | 0.170 | 0.170 | 0.168 | 0.170 | 0.168 | 0.171 | 0.315 |
| KD40 Budgie-20150930-01 AMM029       | 0.157 | 0.155 | 0.158 | 0.187 | 0.135 | 0.094 | 0.094 | 0.110 | 0.109 | 0.107 | 0.071 | 0.052 | 0.054 | 0.057 | 0.055 | 0.057 | 0.057 | 0.060 | 0.056 | 0.057 | 0.061 | 0.063 | 0.059 | 0.055 | 0.057 | 0.057 | 0.331 |
| KD44 Budgie-20150930-01 AMM030       | 0.146 | 0.144 | 0.149 | 0.176 | 0.129 | 0.092 | 0.092 | 0.102 | 0.100 | 0.098 | 0.039 | 0.037 | 0.040 | 0.042 | 0.041 | 0.043 | 0.043 | 0.041 | 0.034 | 0.036 | 0.039 | 0.041 | 0.037 | 0.034 | 0.036 | 0.036 | 0.320 |
| KD45 Budgie-20150930-01 AMM031       | 0.145 | 0.144 | 0.151 | 0.175 | 0.145 | 0.102 | 0.102 | 0.105 | 0.103 | 0.102 | 0.035 | 0.067 | 0.064 | 0.072 | 0.070 | 0.070 | 0.070 | 0.067 | 0.063 | 0.063 | 0.065 | 0.063 | 0.065 | 0.061 | 0.063 | 0.063 | 0.340 |
| KD47 Budgie-20150930-01 AMM028       | 0.170 | 0.170 | 0.172 | 0.202 | 0.129 | 0.135 | 0.135 | 0.131 | 0.129 | 0.127 | 0.129 | 0.127 | 0.130 | 0.129 | 0.131 | 0.134 | 0.134 | 0.127 | 0.126 | 0.122 | 0.127 | 0.125 | 0.124 | 0.120 | 0.118 | 0.118 | 0.324 |
| KD49 Budgie-20150930-01 AMM001       | 0.148 | 0.146 | 0.158 | 0.176 | 0.134 | 0.108 | 0.108 | 0.110 | 0.108 | 0.106 | 0.063 | 0.017 | 0.019 | 0.026 | 0.024 | 0.026 | 0.026 | 0.010 | 0.006 | 0.005 | 0.009 | 0.004 | 0.007 | 0.007 | 0.009 | 0.009 | 0.320 |
| KD50 Budgie-20150930-01 AMM030       | 0.142 | 0.140 | 0.149 | 0.169 | 0.121 | 0.092 | 0.092 | 0.097 | 0.095 | 0.094 | 0.041 | 0.027 | 0.025 | 0.029 | 0.027 | 0.025 | 0.025 | 0.034 | 0.026 | 0.029 | 0.029 | 0.031 | 0.031 | 0.027 | 0.029 | 0.029 | 0.317 |
| KD53 Budgie.20150604-02 AMM002       | 0.147 | 0.146 | 0.152 | 0.177 | 0.131 | 0.106 | 0.106 | 0.113 | 0.111 | 0.110 | 0.070 | 0.069 | 0.074 | 0.072 | 0.070 | 0.073 | 0.073 | 0.069 | 0.073 | 0.067 | 0.071 | 0.069 | 0.069 | 0.065 | 0.067 | 0.067 | 0.324 |
| KD55 Budgie.20150604-02 AMM031       | 0.142 | 0.139 | 0.147 | 0.170 | 0.130 | 0.102 | 0.102 | 0.099 | 0.097 | 0.095 | 0.024 | 0.061 | 0.065 | 0.066 | 0.064 | 0.067 | 0.067 | 0.061 | 0.058 | 0.054 | 0.060 | 0.062 | 0.057 | 0.052 | 0.054 | 0.052 | 0.348 |
| KE05 200-20160910-01 Amphipoda       | 0.147 | 0.145 | 0.153 | 0.169 | 0.142 | 0.104 | 0.104 | 0.103 | 0.101 | 0.099 | 0.000 | 0.062 | 0.064 | 0.066 | 0.065 | 0.066 | 0.068 | 0.063 | 0.061 | 0.058 | 0.063 | 0.065 | 0.060 | 0.057 | 0.058 | 0.058 | 0.322 |
| KE10 87-20160919-02 Amphipoda        | 0.147 | 0.145 | 0.155 | 0.172 | 0.132 | 0.095 | 0.095 | 0.003 | 0.002 | 0.003 | 0.099 | 0.091 | 0.099 | 0.096 | 0.098 | 0.100 | 0.099 | 0.101 | 0.113 | 0.098 | 0.097 | 0.095 | 0.096 | 0.099 | 0.098 | 0.101 | 0.325 |
| KE31 25-20160910-01 Amphipoda        | 0.150 | 0.148 | 0.156 | 0.170 | 0.136 | 0.109 | 0.109 | 0.101 | 0.099 | 0.098 | 0.058 | 0.014 | 0.017 | 0.019 | 0.017 | 0.021 | 0.019 | 0.008 | 0.004 | 0.003 | 0.006 | 0.008 | 0.005 | 0.002 | 0.003 | 0.003 | 0.316 |
| KJ65 Budgiebore-20161210-01          | 0.146 | 0.144 | 0.157 | 0.177 | 0.135 | 0.106 | 0.106 | 0.098 | 0.097 | 0.095 | 0.068 | 0.010 | 0.009 | 0.002 | 0.003 | 0.003 | 0.002 | 0.025 | 0.023 | 0.021 | 0.021 | 0.023 | 0.022 | 0.019 | 0.021 | 0.021 | 0.315 |
| KJ70 Budgiebore-20161210-01          | 0.147 | 0.146 | 0.151 | 0.171 | 0.125 | 0.101 | 0.101 | 0.111 | 0.109 | 0.108 | 0.070 | 0.071 | 0.073 | 0.074 | 0.073 | 0.071 | 0.073 | 0.071 | 0.073 | 0.070 | 0.071 | 0.069 | 0.071 | 0.068 | 0.070 | 0.070 | 0.314 |
| KJ73 Budgiebore-20161210-03          | 0.146 | 0.144 | 0.146 | 0.165 | 0.130 | 0.094 | 0.094 | 0.097 | 0.095 | 0.094 | 0.022 | 0.059 | 0.055 | 0.063 | 0.062 | 0.060 | 0.061 | 0.062 | 0.055 | 0.057 | 0.058 | 0.057 | 0.059 | 0.055 | 0.057 | 0.057 | 0.320 |
| L26 S5-200 AMM016                    | 0.149 | 0.149 | 0.159 | 0.173 | 0.194 | 0.176 | 0.178 | 0.170 | 0.168 | 0.168 | 0.164 | 0.171 | 0.173 | 0.175 | 0.173 | 0.175 | 0.175 | 0.170 | 0.172 | 0.170 | 0.170 | 0.171 | 0.168 | 0.168 | 0.170 | 0.166 | 0.315 |
| L8 107b Nedsia sp 2 AMM017           | 0.159 | 0.159 | 0.156 | 0.192 | 0.136 | 0.113 | 0.113 | 0.125 | 0.124 | 0.122 | 0.088 | 0.082 | 0.082 | 0.081 | 0.082 | 0.082 | 0.082 | 0.086 | 0.086 | 0.082 | 0.081 | 0.084 | 0.081 | 0.081 | 0.079 | 0.082 | 0.324 |
| nr5miwelld AMM002                    | 0.141 | 0.139 | 0.153 | 0.181 | 0.120 | 0.109 | 0.109 | 0.122 | 0.120 | 0.118 | 0.082 | 0.080 | 0.080 | 0.084 | 0.082 | 0.082 | 0.082 | 0.078 | 0.076 | 0.078 | 0.080 | 0.078 | 0.080 | 0.076 | 0.078 | 0.076 | 0.324 |
| pm01a 10 AMM011                      | 0.111 | 0.109 | 0.101 | 0.158 | 0.153 | 0.158 | 0.158 | 0.143 | 0.141 | 0.139 | 0.151 | 0.149 | 0.149 | 0.153 | 0.151 | 0.151 | 0.151 | 0.153 | 0.148 | 0.149 | 0.151 | 0.149 | 0.151 | 0.147 | 0.145 | 0.147 | 0.303 |
| pt03 11a AMM010                      | 0.174 | 0.174 | 0.172 | 0.193 | 0.118 | 0.176 | 0.176 | 0.164 | 0.162 | 0.160 | 0.141 | 0.147 | 0.147 | 0.149 | 0.151 | 0.151 | 0.151 | 0.158 | 0.157 | 0.155 | 0.158 | 0.153 | 0.158 | 0.153 | 0.155 | 0.153 | 0.334 |
| rr3a 2b AMM001                       | 0.141 | 0.139 | 0.162 | 0.176 | 0.130 | 0.116 | 0.116 | 0.116 | 0.113 | 0.111 | 0.059 | 0.019 | 0.019 | 0.025 | 0.023 | 0.023 | 0.023 | 0.008 | 0.002 | 0.004 | 0.006 | 0.008 | 0.006 | 0.002 | 0.004 | 0.002 | 0.321 |
| wc17 1 Nedsia douglasi AMM012        | 0.153 | 0.153 | 0.157 | 0.189 | 0.089 | 0.174 | 0.174 | 0.142 | 0.140 | 0.138 | 0.142 | 0.142 | 0.142 | 0.144 | 0.146 | 0.146 | 0.146 | 0.151 | 0.148 | 0.146 | 0.144 | 0.146 | 0.144 | 0.144 | 0.142 | 0.144 | 0.323 |

| 35    | KC315646 | L110  | L111  | L125  | B3    | BF14  | Cob12 | DH1   | EU304458 | G405  | G406  | IS37  | IS38  | IS43  | IS59  | IT06  | IT22  | IV108 | IV111 | IV124 | IV126 | IV128 | IV134 | IV92  | IV97  | JF02  | JF21  | JN05  | JN17  | JN22  | JQ608487 | KD40  | KD44  | KD45  | KD47  | KD49  |
|-------|----------|-------|-------|-------|-------|-------|-------|-------|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|-------|-------|-------|-------|-------|
| 0.018 | 0.014    | 0.015 | 0.013 | 0.014 | 0.014 | 0.016 | 0.013 | 0.017 | 0.015    | 0.015 | 0.012 | 0.012 | 0.015 | 0.015 | 0.014 | 0.018 | 0.014 | 0.014 | 0.015 | 0.013 | 0.014 | 0.003 | 0.014 | 0.014 | 0.013 | 0.014 | 0.014 | 0.013 | 0.014 | 0.015 | 0.015    | 0.014 | 0.014 | 0.016 | 0.014 |       |
| 0.018 | 0.014    | 0.015 | 0.013 | 0.014 | 0.014 | 0.016 | 0.013 | 0.017 | 0.015    | 0.015 | 0.012 | 0.012 | 0.015 | 0.015 | 0.014 | 0.017 | 0.014 | 0.014 | 0.015 | 0.013 | 0.014 | 0.002 | 0.014 | 0.014 | 0.013 | 0.014 | 0.014 | 0.013 | 0.014 | 0.015 | 0.015    | 0.014 | 0.014 | 0.016 | 0.014 |       |
| 0.018 | 0.014    | 0.014 | 0.014 | 0.015 | 0.014 | 0.016 | 0.014 | 0.016 | 0.014    | 0.015 | 0.005 | 0.012 | 0.013 | 0.014 | 0.014 | 0.018 | 0.014 | 0.014 | 0.015 | 0.014 | 0.014 | 0.012 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.015 | 0.015    | 0.014 | 0.015 | 0.015 | 0.015 |       |
| 0.019 | 0.015    | 0.015 | 0.014 | 0.015 | 0.015 | 0.017 | 0.014 | 0.018 | 0.015    | 0.015 | 0.014 | 0.014 | 0.015 | 0.015 | 0.015 | 0.020 | 0.015 | 0.015 | 0.017 | 0.015 | 0.015 | 0.013 | 0.014 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.017 | 0.016    | 0.015 | 0.015 | 0.017 | 0.015 |       |
| 0.019 | 0.004    | 0.013 | 0.014 | 0.012 | 0.015 | 0.013 | 0.015 | 0.016 | 0.012    | 0.012 | 0.014 | 0.014 | 0.008 | 0.013 | 0.013 | 0.016 | 0.011 | 0.013 | 0.013 | 0.013 | 0.013 | 0.014 | 0.012 | 0.013 | 0.012 | 0.002 | 0.013 | 0.012 | 0.000 | 0.014 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 |       |
| 0.018 | 0.014    | 0.012 | 0.011 | 0.015 | 0.015 | 0.017 | 0.015 | 0.013 | 0.015    | 0.015 | 0.014 | 0.015 | 0.014 | 0.011 | 0.011 | 0.015 | 0.014 | 0.012 | 0.012 | 0.012 | 0.012 | 0.015 | 0.012 | 0.012 | 0.012 | 0.014 | 0.011 | 0.012 | 0.014 | 0.016 | 0.012    | 0.011 | 0.012 | 0.014 | 0.012 |       |
| 0.018 | 0.014    | 0.011 | 0.011 | 0.016 | 0.015 | 0.017 | 0.015 | 0.013 | 0.015    | 0.015 | 0.014 | 0.015 | 0.014 | 0.011 | 0.011 | 0.015 | 0.014 | 0.012 | 0.012 | 0.012 | 0.011 | 0.015 | 0.012 | 0.012 | 0.012 | 0.014 | 0.011 | 0.012 | 0.014 | 0.016 | 0.012    | 0.011 | 0.012 | 0.014 | 0.012 |       |
| 0.019 | 0.013    | 0.002 | 0.012 | 0.014 | 0.015 | 0.017 | 0.014 | 0.014 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.012 | 0.012 | 0.016 | 0.013 | 0.013 | 0.014 | 0.012 | 0.001 | 0.015 | 0.013 | 0.012 | 0.012 | 0.013 | 0.002 | 0.012 | 0.013 | 0.016 | 0.013    | 0.013 | 0.013 | 0.014 | 0.013 |       |
| 0.019 | 0.013    | 0.002 | 0.012 | 0.014 | 0.015 | 0.017 | 0.014 | 0.014 | 0.014    | 0.013 | 0.014 | 0.014 | 0.013 | 0.012 | 0.012 | 0.016 | 0.013 | 0.013 | 0.014 | 0.012 | 0.000 | 0.015 | 0.013 | 0.012 | 0.012 | 0.013 | 0.002 | 0.012 | 0.013 | 0.016 | 0.013    | 0.012 | 0.013 | 0.014 | 0.013 |       |
| 0.019 | 0.013    | 0.000 | 0.012 | 0.014 | 0.015 | 0.016 | 0.014 | 0.014 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.012 | 0.012 | 0.015 | 0.013 | 0.013 | 0.014 | 0.012 | 0.002 | 0.015 | 0.012 | 0.012 | 0.012 | 0.013 | 0.002 | 0.012 | 0.013 | 0.016 | 0.013    | 0.012 | 0.013 | 0.014 | 0.013 |       |
| 0.018 | 0.014    | 0.012 | 0.000 | 0.015 | 0.015 | 0.016 | 0.014 | 0.014 | 0.014    | 0.013 | 0.014 | 0.014 | 0.014 | 0.011 | 0.010 | 0.013 | 0.013 | 0.011 | 0.009 | 0.010 | 0.012 | 0.013 | 0.010 | 0.010 | 0.009 | 0.014 | 0.012 | 0.009 | 0.014 | 0.016 | 0.011    | 0.008 | 0.007 | 0.014 | 0.010 |       |
| 0.018 | 0.012    | 0.011 | 0.010 | 0.014 | 0.015 | 0.016 | 0.014 | 0.013 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.012 | 0.007 | 0.010 | 0.013 | 0.011 | 0.009 | 0.004 | 0.011 | 0.013 | 0.010 | 0.004 | 0.004 | 0.012 | 0.011 | 0.004 | 0.012 | 0.015 | 0.009    | 0.008 | 0.010 | 0.014 | 0.005 |       |
| 0.019 | 0.013    | 0.012 | 0.010 | 0.014 | 0.015 | 0.016 | 0.015 | 0.013 | 0.013    | 0.013 | 0.014 | 0.015 | 0.013 | 0.012 | 0.007 | 0.010 | 0.013 | 0.011 | 0.009 | 0.004 | 0.012 | 0.014 | 0.011 | 0.004 | 0.005 | 0.013 | 0.012 | 0.005 | 0.013 | 0.015 | 0.009    | 0.008 | 0.010 | 0.014 | 0.006 |       |
| 0.018 | 0.013    | 0.012 | 0.010 | 0.014 | 0.015 | 0.016 | 0.014 | 0.014 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.012 | 0.007 | 0.011 | 0.013 | 0.011 | 0.010 | 0.003 | 0.012 | 0.013 | 0.010 | 0.003 | 0.005 | 0.013 | 0.012 | 0.005 | 0.013 | 0.015 | 0.009    | 0.008 | 0.011 | 0.014 | 0.006 |       |
| 0.018 | 0.013    | 0.012 | 0.010 | 0.014 | 0.015 | 0.016 | 0.014 | 0.014 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.012 | 0.007 | 0.010 | 0.013 | 0.011 | 0.009 | 0.002 | 0.012 | 0.013 | 0.010 | 0.003 | 0.005 | 0.013 | 0.012 | 0.005 | 0.013 | 0.015 | 0.009    | 0.008 | 0.011 | 0.014 | 0.006 |       |
| 0.018 | 0.013    | 0.012 | 0.010 | 0.015 | 0.015 | 0.016 | 0.014 | 0.014 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.012 | 0.007 | 0.011 | 0.013 | 0.011 | 0.009 | 0.003 | 0.012 | 0.013 | 0.010 | 0.003 | 0.005 | 0.013 | 0.012 | 0.006 | 0.013 | 0.015 | 0.009    | 0.008 | 0.011 | 0.014 | 0.006 |       |
| 0.018 | 0.012    | 0.012 | 0.010 | 0.015 | 0.015 | 0.017 | 0.014 | 0.013 | 0.013    | 0.014 | 0.014 | 0.014 | 0.013 | 0.012 | 0.007 | 0.011 | 0.013 | 0.011 | 0.009 | 0.006 | 0.012 | 0.013 | 0.010 | 0.006 | 0.003 | 0.012 | 0.012 | 0.003 | 0.012 | 0.015 | 0.009    | 0.008 | 0.010 | 0.014 | 0.004 |       |
| 0.020 | 0.014    | 0.013 | 0.010 | 0.015 | 0.017 | 0.017 | 0.015 | 0.014 | 0.014    | 0.015 | 0.015 | 0.016 | 0.014 | 0.013 | 0.008 | 0.012 | 0.015 | 0.012 | 0.009 | 0.006 | 0.014 | 0.015 | 0.011 | 0.006 | 0.002 | 0.014 | 0.013 | 0.002 | 0.014 | 0.016 | 0.010    | 0.008 | 0.011 | 0.015 | 0.003 |       |
| 0.018 | 0.012    | 0.012 | 0.009 | 0.014 | 0.015 | 0.016 | 0.014 | 0.013 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.011 | 0.007 | 0.011 | 0.013 | 0.011 | 0.009 | 0.005 | 0.012 | 0.013 | 0.010 | 0.006 | 0.002 | 0.012 | 0.012 | 0.002 | 0.012 | 0.015 | 0.009    | 0.008 | 0.010 | 0.014 | 0.003 |       |
| 0.018 | 0.013    | 0.012 | 0.010 | 0.014 | 0.015 | 0.016 | 0.014 | 0.014 | 0.014    | 0.014 | 0.014 | 0.014 | 0.013 | 0.012 | 0.007 | 0.011 | 0.013 | 0.012 | 0.009 | 0.006 | 0.012 | 0.014 | 0.010 | 0.006 | 0.003 | 0.013 | 0.012 | 0.002 | 0.013 | 0.015 | 0.010    | 0.008 | 0.010 | 0.014 | 0.004 |       |
| 0.018 | 0.013    | 0.012 | 0.010 | 0.014 | 0.015 | 0.016 | 0.014 | 0.013 | 0.014    | 0.014 | 0.014 | 0.014 | 0.013 | 0.012 | 0.007 | 0.011 | 0.013 | 0.012 | 0.009 | 0.006 | 0.012 | 0.013 | 0.010 | 0.006 | 0.003 | 0.013 | 0.012 | 0.003 | 0.013 | 0.015 | 0.010    | 0.008 | 0.010 | 0.014 | 0.002 |       |
| 0.018 | 0.013    | 0.012 | 0.010 | 0.014 | 0.015 | 0.016 | 0.014 | 0.014 | 0.013    | 0.014 | 0.014 | 0.014 | 0.013 | 0.012 | 0.007 | 0.010 | 0.013 | 0.011 | 0.009 | 0.005 | 0.012 | 0.013 | 0.010 | 0.006 | 0.002 | 0.012 | 0.012 | 0.000 | 0.012 | 0.015 | 0.009    | 0.008 | 0.010 | 0.014 | 0.003 |       |
| 0.018 | 0.012    | 0.012 | 0.009 | 0.014 | 0.015 | 0.016 | 0.014 | 0.013 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.011 | 0.007 | 0.011 | 0.013 | 0.011 | 0.009 | 0.005 | 0.012 | 0.013 | 0.009 | 0.005 | 0.000 | 0.012 | 0.012 | 0.002 | 0.012 | 0.015 | 0.009    | 0.008 | 0.010 | 0.014 | 0.003 |       |
| 0.018 | 0.012    | 0.012 | 0.010 | 0.014 | 0.015 | 0.016 | 0.014 | 0.013 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.011 | 0.007 | 0.011 | 0.013 | 0.011 | 0.009 | 0.005 | 0.012 | 0.013 | 0.010 | 0.006 | 0.002 | 0.012 | 0.012 | 0.003 | 0.012 | 0.015 | 0.009    | 0.008 | 0.010 | 0.014 | 0.003 |       |
| 0.327 |          | 0.013 | 0.014 | 0.012 | 0.014 | 0.013 | 0.015 | 0.016 | 0.012    | 0.012 | 0.014 | 0.015 | 0.008 | 0.013 | 0.013 | 0.016 | 0.011 | 0.013 | 0.014 | 0.013 | 0.013 | 0.014 | 0.012 | 0.013 | 0.012 | 0.004 | 0.013 | 0.013 | 0.004 | 0.014 | 0.014    | 0.013 | 0.015 | 0.014 | 0.013 |       |
| 0.332 | 0.140    |       | 0.012 | 0.014 | 0.015 | 0.016 | 0.014 | 0.014 | 0.013    | 0.013 | 0.014 | 0.014 | 0.013 | 0.012 | 0.011 | 0.015 | 0.013 | 0.013 | 0.014 | 0.012 | 0.002 | 0.015 | 0.013 | 0.012 | 0.012 | 0.013 | 0.002 | 0.012 | 0.013 | 0.015 | 0.013    | 0.012 | 0.013 | 0.014 | 0.013 |       |
| 0.325 | 0.136    | 0.099 |       | 0.015 | 0.015 | 0.016 | 0.014 | 0.014 | 0.014    | 0.013 | 0.014 | 0.014 | 0.014 | 0.012 | 0.010 | 0.013 | 0.013 | 0.011 | 0.009 | 0.010 | 0.012 | 0.013 | 0.010 | 0.010 | 0.009 | 0.014 | 0.012 | 0.010 | 0.014 | 0.016 | 0.011    | 0.008 | 0.007 | 0.015 | 0.010 |       |
| 0.337 | 0.101    | 0.142 | 0.153 |       | 0.016 | 0.015 | 0.017 | 0.016 | 0.013    | 0.013 | 0.015 | 0.015 | 0.013 | 0.015 | 0.014 | 0.018 | 0.010 | 0.015 | 0.015 | 0.015 | 0.014 | 0.014 | 0.014 | 0.015 | 0.014 | 0.012 | 0.014 | 0.014 | 0.012 | 0.015 | 0.015    | 0.014 | 0.015 | 0.015 | 0.014 |       |
| 0.313 | 0.172    | 0.185 | 0.185 | 0.175 |       | 0.016 | 0.014 | 0.018 | 0.014    | 0.015 | 0.014 | 0.014 | 0.015 | 0.014 | 0.015 | 0.019 | 0.014 | 0.015 | 0.017 | 0.015 | 0.015 | 0.014 | 0.015 | 0.015 | 0.015 | 0.015 | 0.014 | 0.015 | 0.015 | 0.015 | 0.016    | 0.016 | 0.016 | 0.016 | 0.016 |       |
| 0.309 | 0.109    | 0.153 | 0.143 | 0.128 | 0.174 |       | 0.017 | 0.017 | 0.014    | 0.014 | 0.016 | 0.016 | 0.014 | 0.016 | 0.015 | 0.018 | 0.013 | 0.014 | 0.016 | 0.016 | 0.017 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 0.013 | 0.016 | 0.016 | 0.013 | 0.016    | 0.015 | 0.015 | 0.016 | 0.014 | 0.016 |
| 0.319 | 0.185    | 0.156 | 0.161 | 0.197 | 0.169 | 0.187 |       | 0.018 | 0.015    | 0.015 | 0.013 | 0.013 | 0.015 | 0.015 | 0.014 | 0.019 | 0.016 | 0.015 | 0.017 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.015 | 0.014 | 0.014 | 0.015 | 0.015    | 0.016 | 0.015 | 0.015 | 0.017 | 0.015 |
| 0.326 | 0.150    | 0.112 | 0.112 | 0.152 | 0.199 | 0.163 | 0.186 |       | 0.016    | 0.016 | 0.017 | 0.016 | 0.016 | 0.012 | 0.013 | 0.016 | 0.016 | 0.013 | 0.013 | 0.014 | 0.014 | 0.017 | 0.013 | 0.014 | 0.013 | 0.016 | 0.014 | 0.014 | 0.016 | 0.017 | 0.013    | 0.013 | 0.013 | 0.014 | 0.014 |       |
| 0.317 | 0.110    | 0.145 | 0.140 | 0.111 | 0.180 | 0.109 | 0.192 | 0.152 |          | 0.007 | 0.014 | 0.014 | 0.012 | 0.013 | 0.013 | 0.017 | 0.012 | 0.014 | 0.015 | 0.013 | 0.014 | 0.014 | 0.013 | 0.014 | 0.013 | 0.014 | 0.013 | 0.012 | 0.013 | 0.013 | 0.012    | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 |
| 0.317 | 0.112    | 0.144 | 0.144 | 0.123 | 0.    |       |       |       |          |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |          |       |       |       |       |       |

|       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0.331 | 0.133 | 0.099 | 0.067 | 0.154 | 0.184 | 0.149 | 0.168 | 0.114 | 0.141 | 0.145 | 0.157 | 0.168 | 0.144 | 0.107 | 0.036 | 0.051 | 0.139 | 0.091 | 0.052 | 0.002 | 0.102 | 0.150 | 0.077 |       | 0.005 | 0.013 | 0.012 | 0.006 | 0.013 | 0.015 | 0.009 | 0.008 | 0.011 | 0.014 | 0.007 |
| 0.328 | 0.128 | 0.099 | 0.057 | 0.149 | 0.186 | 0.149 | 0.158 | 0.104 | 0.139 | 0.145 | 0.151 | 0.159 | 0.136 | 0.098 | 0.031 | 0.047 | 0.139 | 0.087 | 0.040 | 0.019 | 0.101 | 0.147 | 0.066 | 0.021 |       | 0.012 | 0.012 | 0.002 | 0.012 | 0.015 | 0.009 | 0.008 | 0.010 | 0.014 | 0.003 |
| 0.333 | 0.014 | 0.134 | 0.139 | 0.102 | 0.178 | 0.107 | 0.188 | 0.152 | 0.112 | 0.112 | 0.151 | 0.180 | 0.047 | 0.144 | 0.130 | 0.113 | 0.085 | 0.132 | 0.119 | 0.137 | 0.136 | 0.167 | 0.126 | 0.137 | 0.132 |       | 0.013 | 0.012 | 0.002 | 0.014 | 0.014 | 0.013 | 0.014 | 0.014 | 0.013 |
| 0.329 | 0.140 | 0.003 | 0.099 | 0.147 | 0.185 | 0.153 | 0.156 | 0.112 | 0.147 | 0.144 | 0.152 | 0.164 | 0.145 | 0.106 | 0.095 | 0.106 | 0.141 | 0.107 | 0.105 | 0.100 | 0.002 | 0.148 | 0.109 | 0.101 | 0.100 | 0.134 |       | 0.012 | 0.013 | 0.015 | 0.013 | 0.012 | 0.013 | 0.014 | 0.013 |
| 0.330 | 0.129 | 0.096 | 0.061 | 0.149 | 0.186 | 0.153 | 0.158 | 0.108 | 0.142 | 0.148 | 0.155 | 0.159 | 0.139 | 0.101 | 0.034 | 0.045 | 0.142 | 0.087 | 0.044 | 0.022 | 0.098 | 0.148 | 0.069 | 0.024 | 0.003 | 0.132 | 0.096 |       | 0.012 | 0.015 | 0.009 | 0.008 | 0.010 | 0.014 | 0.003 |
| 0.331 | 0.013 | 0.132 | 0.140 | 0.100 | 0.176 | 0.105 | 0.189 | 0.150 | 0.110 | 0.110 | 0.153 | 0.181 | 0.046 | 0.145 | 0.132 | 0.116 | 0.084 | 0.134 | 0.121 | 0.139 | 0.134 | 0.166 | 0.128 | 0.139 | 0.134 | 0.002 | 0.133 | 0.134 |       | 0.014 | 0.013 | 0.013 | 0.014 | 0.014 | 0.013 |
| 0.294 | 0.184 | 0.173 | 0.172 | 0.180 | 0.176 | 0.162 | 0.170 | 0.171 | 0.178 | 0.182 | 0.171 | 0.168 | 0.197 | 0.173 | 0.165 | 0.168 | 0.182 | 0.152 | 0.175 | 0.168 | 0.176 | 0.168 | 0.159 | 0.170 | 0.170 | 0.187 | 0.175 | 0.168 | 0.189 |       | 0.015 | 0.015 | 0.015 | 0.016 | 0.015 |
| 0.324 | 0.128 | 0.106 | 0.072 | 0.151 | 0.185 | 0.145 | 0.173 | 0.099 | 0.139 | 0.149 | 0.160 | 0.167 | 0.137 | 0.100 | 0.037 | 0.066 | 0.133 | 0.069 | 0.046 | 0.055 | 0.109 | 0.155 | 0.071 | 0.057 | 0.055 | 0.133 | 0.107 | 0.059 | 0.135 | 0.178 |       | 0.008 | 0.011 | 0.013 | 0.009 |
| 0.332 | 0.122 | 0.098 | 0.039 | 0.140 | 0.186 | 0.137 | 0.169 | 0.093 | 0.139 | 0.147 | 0.144 | 0.151 | 0.132 | 0.088 | 0.031 | 0.045 | 0.124 | 0.068 | 0.020 | 0.044 | 0.100 | 0.144 | 0.044 | 0.046 | 0.034 | 0.127 | 0.098 | 0.037 | 0.129 | 0.168 | 0.041 |       | 0.008 | 0.013 | 0.008 |
| 0.335 | 0.140 | 0.102 | 0.035 | 0.150 | 0.203 | 0.143 | 0.172 | 0.101 | 0.130 | 0.135 | 0.149 | 0.159 | 0.156 | 0.100 | 0.060 | 0.072 | 0.135 | 0.091 | 0.032 | 0.072 | 0.103 | 0.144 | 0.049 | 0.072 | 0.061 | 0.144 | 0.102 | 0.065 | 0.145 | 0.175 | 0.070 | 0.044 |       | 0.014 | 0.010 |
| 0.309 | 0.125 | 0.127 | 0.131 | 0.149 | 0.187 | 0.126 | 0.199 | 0.117 | 0.144 | 0.140 | 0.174 | 0.174 | 0.140 | 0.112 | 0.113 | 0.119 | 0.131 | 0.116 | 0.111 | 0.131 | 0.129 | 0.170 | 0.107 | 0.134 | 0.120 | 0.131 | 0.127 | 0.124 | 0.129 | 0.178 | 0.118 | 0.105 | 0.120 |       | 0.014 |
| 0.329 | 0.128 | 0.105 | 0.063 | 0.149 | 0.195 | 0.151 | 0.169 | 0.108 | 0.143 | 0.151 | 0.157 | 0.163 | 0.137 | 0.103 | 0.040 | 0.050 | 0.139 | 0.094 | 0.044 | 0.028 | 0.108 | 0.146 | 0.073 | 0.030 | 0.007 | 0.132 | 0.106 | 0.007 | 0.134 | 0.170 | 0.062 | 0.040 | 0.061 | 0.124 |       |
| 0.317 | 0.115 | 0.094 | 0.040 | 0.135 | 0.182 | 0.134 | 0.160 | 0.097 | 0.126 | 0.133 | 0.144 | 0.153 | 0.126 | 0.092 | 0.025 | 0.053 | 0.124 | 0.072 | 0.020 | 0.031 | 0.095 | 0.140 | 0.054 | 0.031 | 0.027 | 0.119 | 0.094 | 0.031 | 0.121 | 0.164 | 0.041 | 0.016 | 0.042 | 0.119 | 0.034 |
| 0.326 | 0.124 | 0.109 | 0.071 | 0.154 | 0.183 | 0.134 | 0.175 | 0.101 | 0.142 | 0.154 | 0.155 | 0.155 | 0.139 | 0.087 | 0.057 | 0.074 | 0.139 | 0.080 | 0.044 | 0.074 | 0.111 | 0.146 | 0.000 | 0.076 | 0.065 | 0.129 | 0.110 | 0.069 | 0.131 | 0.159 | 0.071 | 0.044 | 0.049 | 0.107 | 0.073 |
| 0.345 | 0.127 | 0.094 | 0.024 | 0.148 | 0.201 | 0.141 | 0.161 | 0.104 | 0.139 | 0.142 | 0.144 | 0.163 | 0.135 | 0.099 | 0.047 | 0.066 | 0.137 | 0.076 | 0.036 | 0.064 | 0.097 | 0.139 | 0.066 | 0.067 | 0.052 | 0.128 | 0.095 | 0.057 | 0.130 | 0.182 | 0.069 | 0.035 | 0.027 | 0.118 | 0.061 |
| 0.327 | 0.136 | 0.099 | 0.000 | 0.152 | 0.186 | 0.143 | 0.161 | 0.112 | 0.141 | 0.145 | 0.150 | 0.156 | 0.148 | 0.095 | 0.060 | 0.077 | 0.136 | 0.079 | 0.040 | 0.066 | 0.101 | 0.145 | 0.068 | 0.069 | 0.057 | 0.141 | 0.100 | 0.060 | 0.142 | 0.171 | 0.071 | 0.039 | 0.035 | 0.129 | 0.063 |
| 0.329 | 0.140 | 0.003 | 0.099 | 0.147 | 0.185 | 0.153 | 0.156 | 0.112 | 0.147 | 0.144 | 0.152 | 0.164 | 0.145 | 0.106 | 0.095 | 0.106 | 0.141 | 0.107 | 0.105 | 0.100 | 0.002 | 0.148 | 0.109 | 0.101 | 0.100 | 0.134 | 0.000 | 0.096 | 0.133 | 0.175 | 0.107 | 0.098 | 0.102 | 0.127 | 0.106 |
| 0.330 | 0.129 | 0.097 | 0.059 | 0.147 | 0.185 | 0.151 | 0.156 | 0.106 | 0.141 | 0.147 | 0.153 | 0.158 | 0.137 | 0.096 | 0.032 | 0.050 | 0.141 | 0.088 | 0.042 | 0.021 | 0.100 | 0.148 | 0.068 | 0.022 | 0.002 | 0.134 | 0.098 | 0.005 | 0.136 | 0.168 | 0.057 | 0.036 | 0.063 | 0.122 | 0.009 |
| 0.323 | 0.131 | 0.096 | 0.067 | 0.151 | 0.180 | 0.145 | 0.165 | 0.108 | 0.136 | 0.139 | 0.154 | 0.165 | 0.136 | 0.106 | 0.034 | 0.053 | 0.133 | 0.090 | 0.050 | 0.006 | 0.097 | 0.144 | 0.074 | 0.006 | 0.019 | 0.136 | 0.095 | 0.022 | 0.135 | 0.170 | 0.059 | 0.044 | 0.072 | 0.132 | 0.028 |
| 0.319 | 0.121 | 0.108 | 0.069 | 0.156 | 0.178 | 0.134 | 0.168 | 0.101 | 0.141 | 0.147 | 0.154 | 0.151 | 0.133 | 0.086 | 0.061 | 0.077 | 0.135 | 0.079 | 0.044 | 0.076 | 0.109 | 0.146 | 0.002 | 0.077 | 0.068 | 0.124 | 0.108 | 0.071 | 0.125 | 0.161 | 0.073 | 0.046 | 0.049 | 0.109 | 0.075 |
| 0.323 | 0.126 | 0.094 | 0.021 | 0.145 | 0.180 | 0.139 | 0.157 | 0.099 | 0.139 | 0.138 | 0.143 | 0.147 | 0.133 | 0.098 | 0.047 | 0.066 | 0.127 | 0.079 | 0.022 | 0.063 | 0.095 | 0.144 | 0.060 | 0.064 | 0.055 | 0.128 | 0.094 | 0.059 | 0.130 | 0.170 | 0.055 | 0.032 | 0.030 | 0.122 | 0.056 |
| 0.317 | 0.191 | 0.166 | 0.165 | 0.187 | 0.171 | 0.183 | 0.029 | 0.178 | 0.190 | 0.202 | 0.151 | 0.152 | 0.202 | 0.178 | 0.165 | 0.182 | 0.189 | 0.175 | 0.167 | 0.175 | 0.168 | 0.152 | 0.171 | 0.176 | 0.168 | 0.192 | 0.170 | 0.168 | 0.194 | 0.168 | 0.167 | 0.166 | 0.166 | 0.195 | 0.172 |
| 0.317 | 0.131 | 0.122 | 0.088 | 0.154 | 0.186 | 0.134 | 0.170 | 0.082 | 0.147 | 0.145 | 0.151 | 0.152 | 0.138 | 0.111 | 0.077 | 0.076 | 0.143 | 0.086 | 0.076 | 0.082 | 0.124 | 0.163 | 0.086 | 0.082 | 0.081 | 0.138 | 0.122 | 0.081 | 0.136 | 0.163 | 0.072 | 0.068 | 0.085 | 0.111 | 0.086 |
| 0.315 | 0.120 | 0.118 | 0.082 | 0.147 | 0.195 | 0.137 | 0.181 | 0.099 | 0.139 | 0.149 | 0.158 | 0.155 | 0.137 | 0.092 | 0.067 | 0.080 | 0.139 | 0.086 | 0.048 | 0.086 | 0.120 | 0.139 | 0.002 | 0.086 | 0.076 | 0.118 | 0.118 | 0.080 | 0.120 | 0.155 | 0.080 | 0.050 | 0.048 | 0.109 | 0.080 |
| 0.317 | 0.158 | 0.139 | 0.151 | 0.168 | 0.166 | 0.164 | 0.147 | 0.159 | 0.158 | 0.162 | 0.099 | 0.120 | 0.162 | 0.160 | 0.137 | 0.148 | 0.164 | 0.149 | 0.135 | 0.153 | 0.141 | 0.105 | 0.137 | 0.153 | 0.147 | 0.151 | 0.139 | 0.151 | 0.153 | 0.164 | 0.149 | 0.134 | 0.137 | 0.166 | 0.151 |
| 0.319 | 0.122 | 0.160 | 0.141 | 0.118 | 0.181 | 0.067 | 0.195 | 0.167 | 0.111 | 0.109 | 0.172 | 0.187 | 0.128 | 0.158 | 0.145 | 0.151 | 0.101 | 0.143 | 0.139 | 0.153 | 0.162 | 0.170 | 0.147 | 0.153 | 0.153 | 0.120 | 0.160 | 0.158 | 0.118 | 0.160 | 0.155 | 0.141 | 0.145 | 0.145 | 0.155 |
| 0.324 | 0.130 | 0.111 | 0.059 | 0.145 | 0.202 | 0.151 | 0.164 | 0.106 | 0.139 | 0.145 | 0.158 | 0.164 | 0.139 | 0.101 | 0.040 | 0.054 | 0.139 | 0.095 | 0.037 | 0.027 | 0.113 | 0.139 | 0.076 | 0.027 | 0.002 | 0.128 | 0.111 | 0.006 | 0.130 | 0.172 | 0.061 | 0.036 | 0.059 | 0.120 | 0.011 |
| 0.312 | 0.091 | 0.138 | 0.142 | 0.038 | 0.174 | 0.115 | 0.185 | 0.147 | 0.091 | 0.087 | 0.161 | 0.178 | 0.096 | 0.159 | 0.136 | 0.142 | 0.068 | 0.142 | 0.132 | 0.149 | 0.140 | 0.153 | 0.149 | 0.149 | 0.144 | 0.091 | 0.142 | 0.144 | 0.089 | 0.166 | 0.153 | 0.134 | 0.144 | 0.126 | 0.149 |

| KD50  | KD53  | KD55  | KE05  | KE10  | KE31  | KJ65  | KJ70  | KJ73  | L26   | L8    | nr5miwella | pm01a | pf03  | r3a 2b | wc17 1 |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|------------|-------|-------|--------|--------|
| 0.014 | 0.014 | 0.016 | 0.013 | 0.014 | 0.013 | 0.013 | 0.013 | 0.013 | 0.014 | 0.015 | 0.015      | 0.014 | 0.016 | 0.015  | 0.016  |
| 0.014 | 0.014 | 0.016 | 0.013 | 0.014 | 0.013 | 0.013 | 0.013 | 0.013 | 0.014 | 0.015 | 0.015      | 0.014 | 0.016 | 0.015  | 0.016  |
| 0.014 | 0.014 | 0.017 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.015 | 0.015 | 0.015      | 0.013 | 0.017 | 0.016  | 0.016  |
| 0.015 | 0.015 | 0.018 | 0.014 | 0.015 | 0.015 | 0.015 | 0.014 | 0.014 | 0.015 | 0.016 | 0.017      | 0.016 | 0.017 | 0.017  | 0.018  |
| 0.013 | 0.013 | 0.017 | 0.014 | 0.013 | 0.012 | 0.013 | 0.012 | 0.013 | 0.015 | 0.014 | 0.014      | 0.016 | 0.014 | 0.015  | 0.013  |
| 0.012 | 0.012 | 0.014 | 0.011 | 0.011 | 0.012 | 0.012 | 0.012 | 0.011 | 0.016 | 0.013 | 0.014      | 0.016 | 0.016 | 0.014  | 0.017  |
| 0.012 | 0.012 | 0.014 | 0.011 | 0.011 | 0.012 | 0.012 | 0.012 | 0.011 | 0.016 | 0.013 | 0.014      | 0.016 | 0.016 | 0.014  | 0.017  |
| 0.012 | 0.013 | 0.014 | 0.013 | 0.002 | 0.012 | 0.012 | 0.013 | 0.012 | 0.015 | 0.014 | 0.015      | 0.016 | 0.016 | 0.015  | 0.015  |
| 0.012 | 0.013 | 0.014 | 0.012 | 0.002 | 0.012 | 0.012 | 0.013 | 0.012 | 0.015 | 0.014 | 0.015      | 0.016 | 0.016 | 0.015  | 0.015  |
| 0.012 | 0.012 | 0.014 | 0.012 | 0.002 | 0.012 | 0.012 | 0.012 | 0.012 | 0.015 | 0.014 | 0.015      | 0.016 | 0.016 | 0.015  | 0.015  |
| 0.008 | 0.010 | 0.008 | 0.000 | 0.012 | 0.009 | 0.010 | 0.010 | 0.006 | 0.014 | 0.012 | 0.012      | 0.016 | 0.015 | 0.011  | 0.016  |
| 0.006 | 0.010 | 0.011 | 0.010 | 0.011 | 0.005 | 0.004 | 0.010 | 0.009 | 0.015 | 0.011 | 0.012      | 0.016 | 0.015 | 0.006  | 0.016  |
| 0.006 | 0.011 | 0.012 | 0.010 | 0.012 | 0.006 | 0.004 | 0.011 | 0.010 | 0.015 | 0.011 | 0.012      | 0.016 | 0.015 | 0.006  | 0.016  |
| 0.007 | 0.010 | 0.012 | 0.010 | 0.012 | 0.005 | 0.002 | 0.011 | 0.010 | 0.015 | 0.011 | 0.013      | 0.016 | 0.015 | 0.007  | 0.016  |
| 0.007 | 0.010 | 0.011 | 0.010 | 0.012 | 0.005 | 0.002 | 0.010 | 0.010 | 0.015 | 0.011 | 0.012      | 0.016 | 0.015 | 0.006  | 0.016  |
| 0.006 | 0.010 | 0.012 | 0.010 | 0.012 | 0.006 | 0.002 | 0.010 | 0.010 | 0.015 | 0.011 | 0.012      | 0.016 | 0.015 | 0.006  | 0.016  |
| 0.006 | 0.010 | 0.012 | 0.010 | 0.012 | 0.005 | 0.002 | 0.010 | 0.010 | 0.015 | 0.011 | 0.012      | 0.016 | 0.015 | 0.006  | 0.016  |
| 0.007 | 0.010 | 0.011 | 0.010 | 0.012 | 0.004 | 0.006 | 0.010 | 0.009 | 0.015 | 0.012 | 0.011      | 0.016 | 0.016 | 0.004  | 0.017  |
| 0.007 | 0.011 | 0.012 | 0.010 | 0.013 | 0.003 | 0.006 | 0.011 | 0.010 | 0.016 | 0.012 | 0.012      | 0.016 | 0.016 | 0.002  | 0.017  |
| 0.007 | 0.010 | 0.011 | 0.009 | 0.012 | 0.002 | 0.005 | 0.010 | 0.009 | 0.015 | 0.012 | 0.012      | 0.016 | 0.015 | 0.003  | 0.016  |
| 0.007 | 0.010 | 0.011 | 0.010 | 0.012 | 0.003 | 0.005 | 0.010 | 0.009 | 0.015 | 0.011 | 0.012      | 0.016 | 0.015 | 0.004  | 0.016  |
| 0.007 | 0.010 | 0.011 | 0.010 | 0.012 | 0.004 | 0.005 | 0.010 | 0.009 | 0.015 | 0.012 | 0.012      | 0.016 | 0.015 | 0.004  | 0.016  |
| 0.007 | 0.010 | 0.011 | 0.009 | 0.012 | 0.003 | 0.006 | 0.010 | 0.009 | 0.015 | 0.011 | 0.012      | 0.016 | 0.015 | 0.004  | 0.016  |
| 0.006 | 0.010 | 0.010 | 0.009 | 0.012 | 0.002 | 0.005 | 0.010 | 0.009 | 0.015 | 0.011 | 0.011      | 0.016 | 0.015 | 0.002  | 0.016  |
| 0.007 | 0.010 | 0.011 | 0.009 | 0.012 | 0.002 | 0.006 | 0.010 | 0.009 | 0.015 | 0.011 | 0.012      | 0.015 | 0.016 | 0.003  | 0.016  |
| 0.007 | 0.010 | 0.010 | 0.009 | 0.012 | 0.002 | 0.006 | 0.010 | 0.009 | 0.015 | 0.011 | 0.011      | 0.016 | 0.015 | 0.002  | 0.016  |
| 0.013 | 0.013 | 0.017 | 0.014 | 0.013 | 0.012 | 0.013 | 0.012 | 0.013 | 0.015 | 0.014 | 0.014      | 0.016 | 0.014 | 0.015  | 0.013  |
| 0.012 | 0.012 | 0.014 | 0.012 | 0.002 | 0.012 | 0.012 | 0.013 | 0.012 | 0.015 | 0.014 | 0.015      | 0.016 | 0.016 | 0.015  | 0.015  |
| 0.008 | 0.010 | 0.008 | 0.000 | 0.012 | 0.009 | 0.010 | 0.010 | 0.005 | 0.014 | 0.012 | 0.012      | 0.016 | 0.015 | 0.011  | 0.016  |
| 0.014 | 0.014 | 0.017 | 0.015 | 0.014 | 0.014 | 0.015 | 0.015 | 0.014 | 0.016 | 0.015 | 0.016      | 0.017 | 0.014 | 0.016  | 0.008  |
| 0.016 | 0.015 | 0.019 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.016 | 0.018      | 0.016 | 0.017 | 0.018  | 0.017  |
| 0.015 | 0.016 | 0.017 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 0.015 | 0.017 | 0.015 | 0.016      | 0.017 | 0.011 | 0.016  | 0.014  |
| 0.015 | 0.015 | 0.017 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.007 | 0.015 | 0.017      | 0.015 | 0.017 | 0.016  | 0.018  |
| 0.013 | 0.013 | 0.015 | 0.014 | 0.014 | 0.013 | 0.014 | 0.013 | 0.013 | 0.017 | 0.012 | 0.013      | 0.016 | 0.016 | 0.013  | 0.016  |
| 0.014 | 0.014 | 0.016 | 0.014 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.016 | 0.015 | 0.015      | 0.016 | 0.014 | 0.016  | 0.013  |
| 0.014 | 0.014 | 0.016 | 0.013 | 0.013 | 0.013 | 0.013 | 0.014 | 0.013 | 0.016 | 0.015 | 0.015      | 0.016 | 0.014 | 0.016  | 0.012  |
| 0.014 | 0.014 | 0.017 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.016      | 0.013 | 0.017 | 0.016  | 0.016  |
| 0.015 | 0.014 | 0.018 | 0.014 | 0.014 | 0.014 | 0.014 | 0.013 | 0.014 | 0.015 | 0.014 | 0.015      | 0.014 | 0.017 | 0.017  | 0.016  |
| 0.013 | 0.014 | 0.017 | 0.014 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.016 | 0.014 | 0.016      | 0.016 | 0.014 | 0.015  | 0.014  |
| 0.012 | 0.010 | 0.014 | 0.011 | 0.012 | 0.011 | 0.012 | 0.010 | 0.012 | 0.015 | 0.013 | 0.012      | 0.015 | 0.016 | 0.013  | 0.016  |
| 0.006 | 0.009 | 0.010 | 0.010 | 0.011 | 0.007 | 0.007 | 0.009 | 0.008 | 0.015 | 0.011 | 0.011      | 0.015 | 0.014 | 0.008  | 0.015  |
| 0.011 | 0.013 | 0.012 | 0.013 | 0.015 | 0.011 | 0.011 | 0.013 | 0.012 | 0.020 | 0.013 | 0.014      | 0.018 | 0.017 | 0.012  | 0.019  |
| 0.013 | 0.013 | 0.016 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.012 | 0.016 | 0.014 | 0.015      | 0.016 | 0.013 | 0.015  | 0.011  |
| 0.011 | 0.011 | 0.013 | 0.011 | 0.013 | 0.011 | 0.011 | 0.011 | 0.011 | 0.015 | 0.011 | 0.013      | 0.016 | 0.015 | 0.013  | 0.016  |
| 0.006 | 0.009 | 0.010 | 0.009 | 0.014 | 0.009 | 0.010 | 0.009 | 0.006 | 0.016 | 0.012 | 0.010      | 0.016 | 0.015 | 0.009  | 0.016  |
| 0.007 | 0.010 | 0.011 | 0.010 | 0.012 | 0.005 | 0.003 | 0.010 | 0.010 | 0.015 | 0.011 | 0.012      | 0.016 | 0.016 | 0.007  | 0.016  |
| 0.012 | 0.013 | 0.014 | 0.012 | 0.002 | 0.012 | 0.012 | 0.013 | 0.012 | 0.015 | 0.014 | 0.015      | 0.016 | 0.016 | 0.015  | 0.015  |
| 0.014 | 0.014 | 0.016 | 0.013 | 0.014 | 0.013 | 0.013 | 0.013 | 0.013 | 0.014 | 0.015 | 0.015      | 0.014 | 0.016 | 0.015  | 0.016  |
| 0.009 | 0.000 | 0.011 | 0.010 | 0.012 | 0.010 | 0.011 | 0.002 | 0.009 | 0.015 | 0.011 | 0.002      | 0.015 | 0.015 | 0.011  | 0.016  |



|       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0.007 | 0.011 | 0.012 | 0.010 | 0.012 | 0.006 | 0.003 | 0.010 | 0.010 | 0.015 | 0.011 | 0.012 | 0.016 | 0.016 | 0.007 | 0.016 |
| 0.006 | 0.010 | 0.010 | 0.009 | 0.012 | 0.002 | 0.005 | 0.010 | 0.009 | 0.015 | 0.011 | 0.011 | 0.016 | 0.015 | 0.002 | 0.016 |
| 0.013 | 0.013 | 0.016 | 0.014 | 0.013 | 0.012 | 0.013 | 0.012 | 0.013 | 0.015 | 0.014 | 0.014 | 0.016 | 0.014 | 0.015 | 0.013 |
| 0.012 | 0.012 | 0.014 | 0.012 | 0.000 | 0.012 | 0.011 | 0.012 | 0.012 | 0.015 | 0.013 | 0.014 | 0.016 | 0.016 | 0.014 | 0.015 |
| 0.007 | 0.010 | 0.011 | 0.009 | 0.012 | 0.003 | 0.006 | 0.010 | 0.009 | 0.015 | 0.011 | 0.012 | 0.016 | 0.015 | 0.004 | 0.016 |
| 0.013 | 0.013 | 0.017 | 0.014 | 0.013 | 0.012 | 0.013 | 0.012 | 0.013 | 0.015 | 0.014 | 0.014 | 0.016 | 0.014 | 0.015 | 0.013 |
| 0.015 | 0.014 | 0.018 | 0.016 | 0.015 | 0.015 | 0.015 | 0.014 | 0.015 | 0.015 | 0.015 | 0.015 | 0.016 | 0.016 | 0.017 | 0.016 |
| 0.008 | 0.011 | 0.012 | 0.011 | 0.013 | 0.009 | 0.009 | 0.011 | 0.009 | 0.016 | 0.011 | 0.012 | 0.016 | 0.015 | 0.010 | 0.016 |
| 0.005 | 0.008 | 0.009 | 0.008 | 0.012 | 0.008 | 0.008 | 0.008 | 0.007 | 0.015 | 0.010 | 0.010 | 0.016 | 0.014 | 0.008 | 0.015 |
| 0.009 | 0.009 | 0.007 | 0.007 | 0.013 | 0.010 | 0.011 | 0.009 | 0.007 | 0.015 | 0.012 | 0.010 | 0.016 | 0.015 | 0.011 | 0.015 |
| 0.014 | 0.013 | 0.015 | 0.014 | 0.014 | 0.014 | 0.014 | 0.013 | 0.014 | 0.016 | 0.013 | 0.014 | 0.016 | 0.015 | 0.015 | 0.014 |
| 0.007 | 0.010 | 0.011 | 0.010 | 0.013 | 0.004 | 0.006 | 0.011 | 0.009 | 0.015 | 0.012 | 0.012 | 0.016 | 0.016 | 0.004 | 0.016 |
|       | 0.009 | 0.009 | 0.008 | 0.012 | 0.007 | 0.007 | 0.009 | 0.007 | 0.015 | 0.010 | 0.010 | 0.016 | 0.014 | 0.007 | 0.016 |
| 0.054 |       | 0.011 | 0.010 | 0.012 | 0.010 | 0.011 | 0.002 | 0.010 | 0.015 | 0.011 | 0.002 | 0.015 | 0.015 | 0.011 | 0.016 |
| 0.038 | 0.066 |       | 0.008 | 0.014 | 0.011 | 0.012 | 0.012 | 0.007 | 0.017 | 0.012 | 0.012 | 0.017 | 0.016 | 0.011 | 0.018 |
| 0.041 | 0.070 | 0.024 |       | 0.012 | 0.009 | 0.010 | 0.010 | 0.006 | 0.014 | 0.012 | 0.012 | 0.016 | 0.015 | 0.011 | 0.016 |
| 0.094 | 0.110 | 0.095 | 0.099 |       | 0.012 | 0.011 | 0.012 | 0.012 | 0.015 | 0.013 | 0.014 | 0.016 | 0.016 | 0.014 | 0.015 |
| 0.029 | 0.067 | 0.054 | 0.058 | 0.098 |       | 0.006 | 0.010 | 0.009 | 0.015 | 0.011 | 0.012 | 0.016 | 0.015 | 0.003 | 0.016 |
| 0.027 | 0.074 | 0.069 | 0.068 | 0.095 | 0.021 |       | 0.010 | 0.010 | 0.015 | 0.011 | 0.013 | 0.016 | 0.015 | 0.007 | 0.016 |
| 0.052 | 0.002 | 0.069 | 0.070 | 0.108 | 0.070 | 0.073 |       | 0.009 | 0.015 | 0.011 | 0.002 | 0.015 | 0.015 | 0.011 | 0.016 |
| 0.029 | 0.063 | 0.021 | 0.022 | 0.094 | 0.057 | 0.062 | 0.059 |       | 0.014 | 0.011 | 0.011 | 0.016 | 0.014 | 0.010 | 0.015 |
| 0.162 | 0.171 | 0.165 | 0.164 | 0.170 | 0.166 | 0.176 | 0.173 | 0.161 |       | 0.015 | 0.017 | 0.015 | 0.017 | 0.016 | 0.018 |
| 0.069 | 0.086 | 0.075 | 0.088 | 0.122 | 0.082 | 0.081 | 0.086 | 0.073 | 0.165 |       | 0.012 | 0.016 | 0.016 | 0.012 | 0.016 |
| 0.061 | 0.002 | 0.071 | 0.082 | 0.118 | 0.078 | 0.084 | 0.002 | 0.067 | 0.170 | 0.090 |       | 0.015 | 0.015 | 0.011 | 0.016 |
| 0.139 | 0.137 | 0.144 | 0.151 | 0.139 | 0.145 | 0.153 | 0.137 | 0.141 | 0.139 | 0.158 | 0.134 |       | 0.017 | 0.016 | 0.016 |
| 0.139 | 0.147 | 0.141 | 0.141 | 0.160 | 0.151 | 0.149 | 0.147 | 0.137 | 0.187 | 0.149 | 0.149 | 0.174 |       | 0.016 | 0.014 |
| 0.029 | 0.076 | 0.058 | 0.059 | 0.111 | 0.004 | 0.025 | 0.076 | 0.053 | 0.164 | 0.082 | 0.078 | 0.149 | 0.155 |       | 0.016 |
| 0.136 | 0.149 | 0.141 | 0.142 | 0.142 | 0.146 | 0.144 | 0.149 | 0.136 | 0.178 | 0.142 | 0.149 | 0.157 | 0.106 | 0.146 |       |

Figure 1. Neighbour-joining analysis of specimens of Neoniphargidae and Paramelitidae from the present study to aid in the identification of unique haplotypes and lineages. Numbers on major nodes correspond to bootstrap support over 100 iterations; values <50% are not shown. Scale bar= genetic distance. Unique haplotypes used to represent each lineage in the model-based phylogenetic analysis are highlighted in yellow.

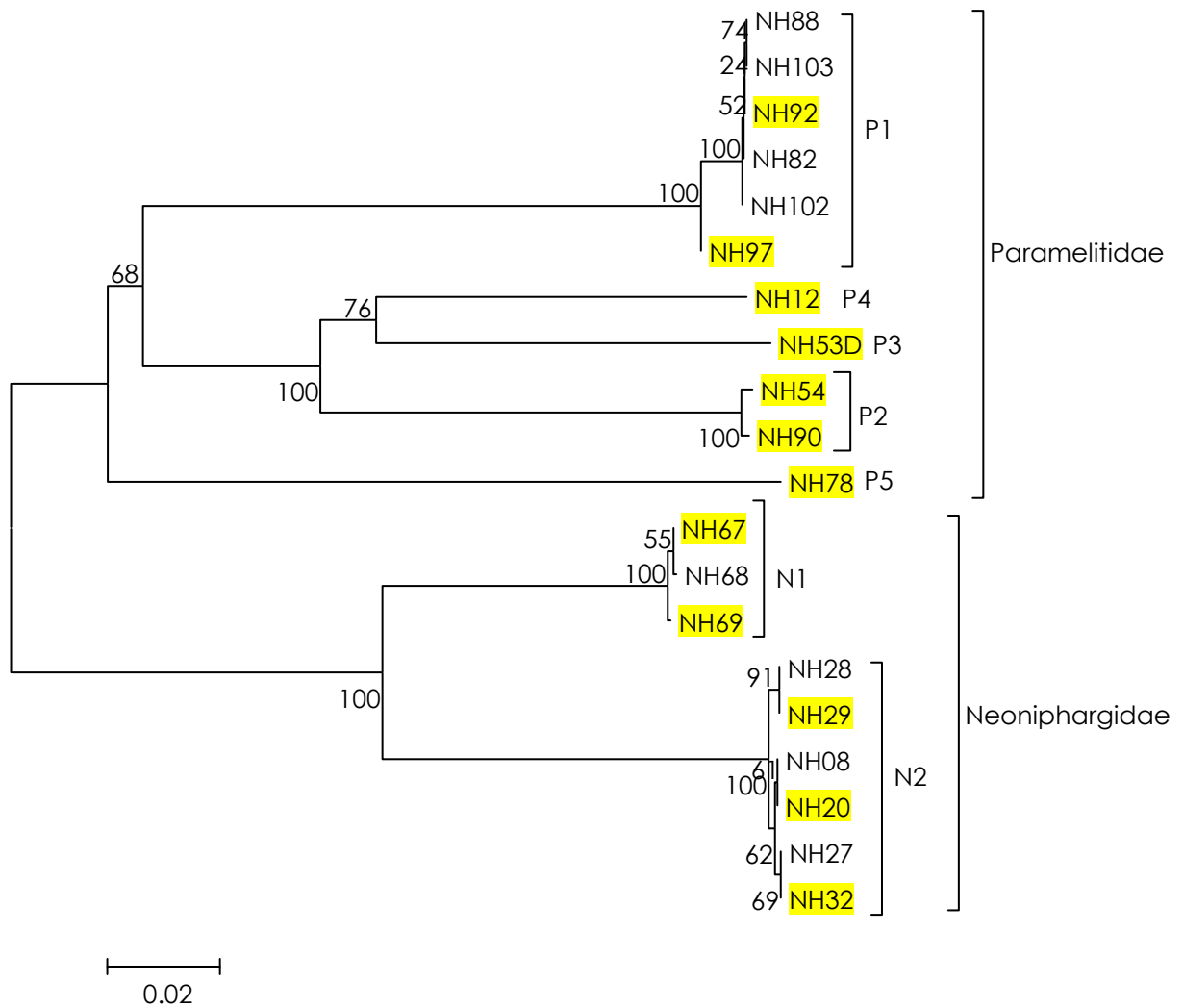


Figure 2. Neighbour-joining analysis of specimens of Melitidae/Eriopisidae from the present study, to aid in the identification of unique haplotypes and lineages. Numbers on major nodes correspond to bootstrap support over 100 iterations; values <50% are not shown. Scale bar= genetic distance. Unique haplotypes used to represent each lineage in the model-based phylogenetic analysis are highlighted in yellow.

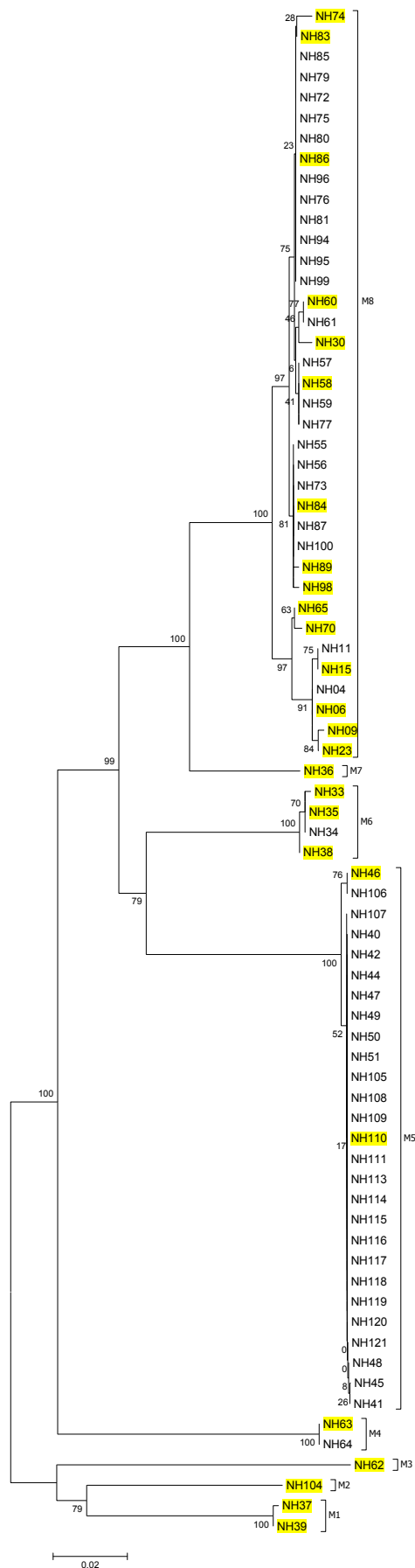


Figure 3. Bayesian analysis of COI haplotypes of Neoniphargidae (Amphipoda) from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes enclose species with high confidence.

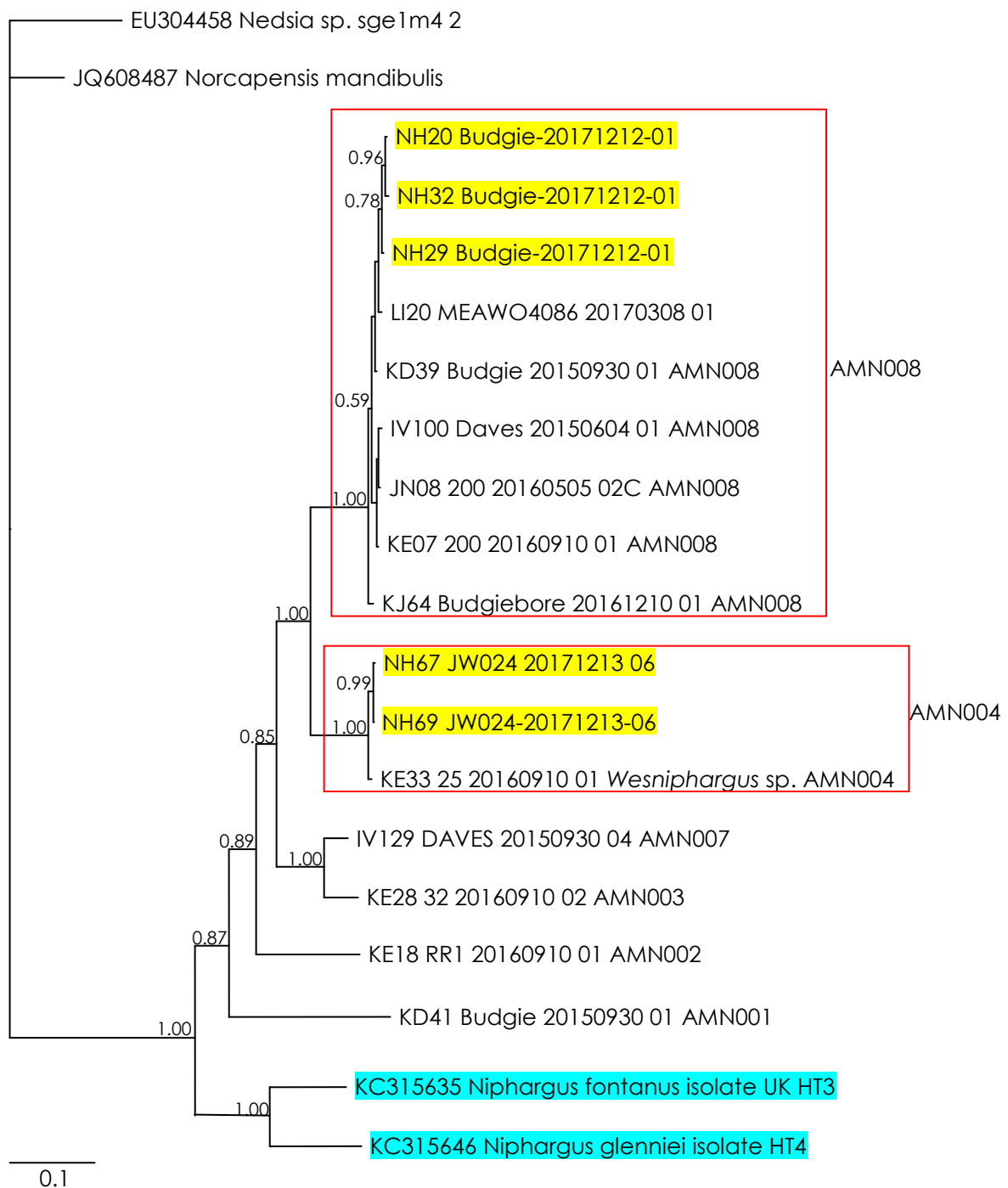


Figure 4. Bayesian analysis of COI haplotypes of Paramelitidae (Amphipoda) from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes enclose species with high confidence; dashed lines indicate groups that require further consideration.

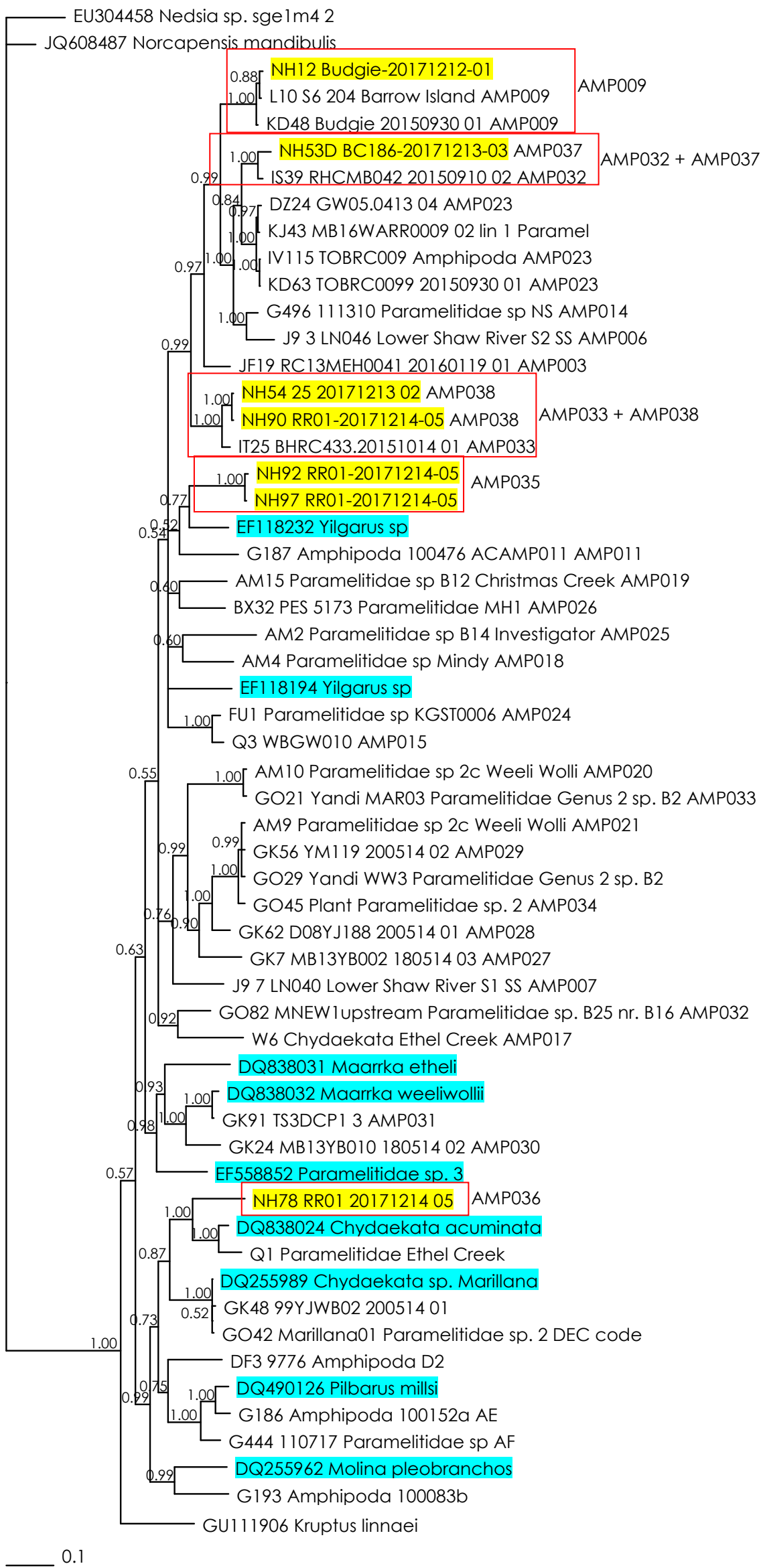
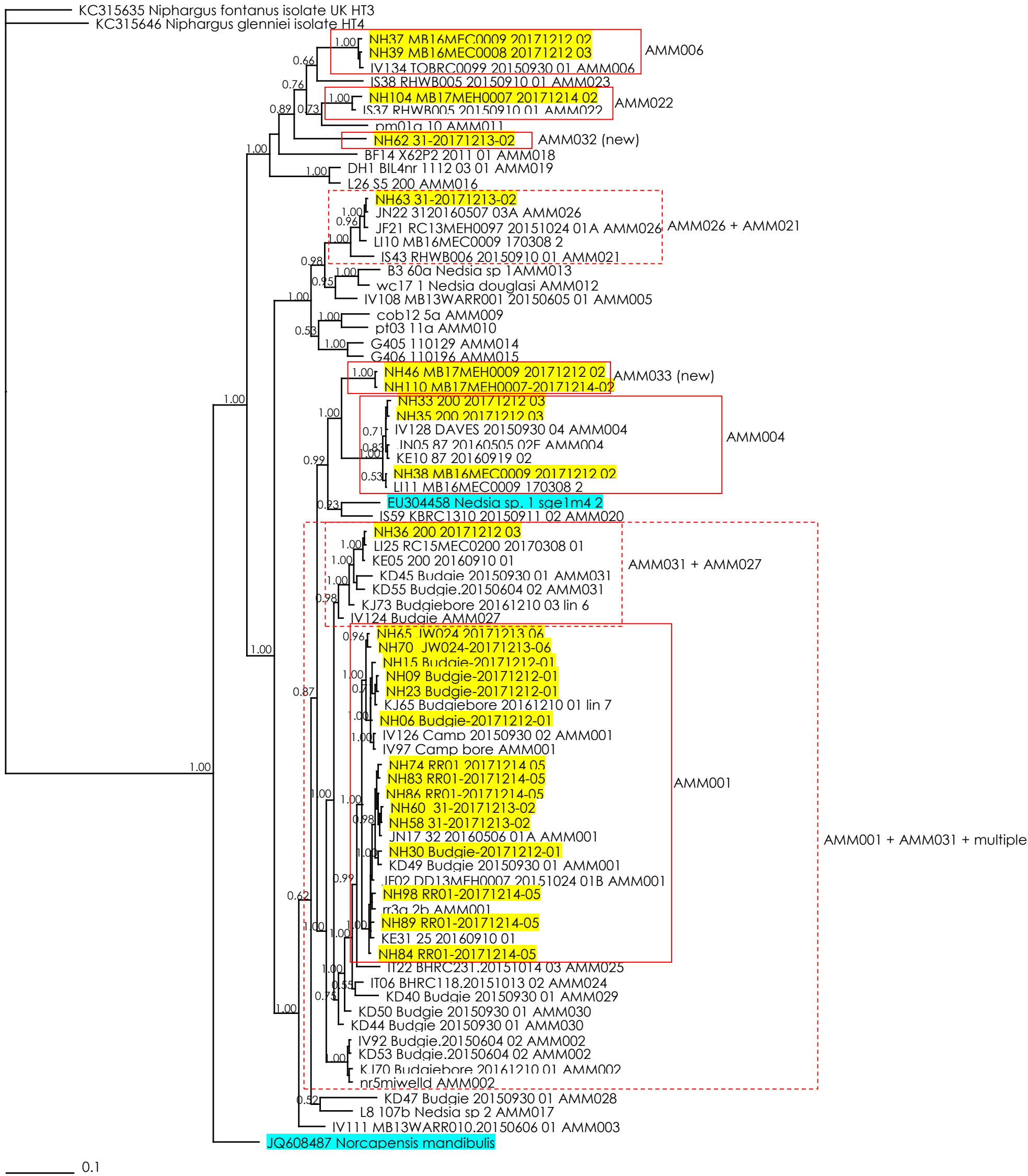




Figure 5. Bayesian analysis of COI haplotypes of Melitidae/Eriopisidae (Amphipoda) from the present study and reference specimens from Genbank and previous surveys in the Pilbara. Numbers on major nodes correspond to posterior probabilities; values <50% are not shown. Specimens from the present study are highlighted in yellow; GenBank voucher specimens highlighted in turquoise. Scale bar= number of substitutions per site. Red boxes enclose species with high confidence; dashed lines indicate groups that require further consideration.





# Helix

## Molecular Solutions

School of Animal Biology The University of Western Australia  
Hackett Entrance No. 4 Hackett Drive Crawley WA 6009

PO Box 155  
Leederville WA 6903

t: 0459 459 481

f: [08] 6488 1029

abn: 32 133 230 243

w: [www.helixsolutions.com.au](http://www.helixsolutions.com.au)

11 April 2017

Biota Environmental Sciences

Via email

**Re. Report for the analysis of environmental water samples collected during December 2017 for the presence of blind eel DNA (Helix Job 484)**

**Executive Summary**

- Eighty-nine samples from 17 sites were extracted and analysed using the *O. candidum* probe.
- Amplification was observed in seven samples from three sites. The control site (n=3), 25 site (n=1) and MB17MEH0015 site (n=3).
- All samples were run with the 16S mammal probe as an extraction positive control. Of the 89 samples only 59 samples amplified for the presence of mammal DNA.
- All samples that amplified with the *O. candidum* probe also amplified for the mammal probe

**Methods**

A modification to previous collections methods was in how filters were stored during transport. In previous collection filter papers were stored in 100% ethanol. A modification in this study was filter papers were stored in a sterile plastic bag at -20°C for transport. Furthermore only half of the filter paper was sent for analysis. Upon reaching the lab all samples were stored at -80°C.

Samples were extracted using the QIAGEN Blood and Tissue Kit (QIAGEN, Hilden, Germany) and as with previous studies all starting volumes were doubled to ensure that the filter was covered during incubation. All samples had two elutes of 50µl each.

The extracted DNA was run with the species diagnostic assay for *Ophisternon candidum* and the mammal control assay (16S) as per previous studies.

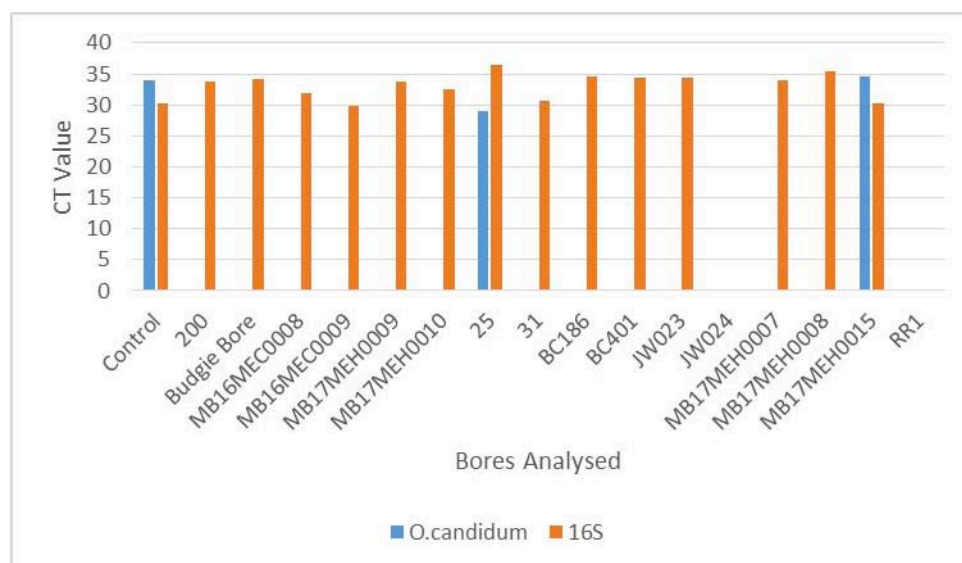
**Results**

Seventeen sites were sampled with three to six filters per site using two collection methods (bailer and pump) (Appendix 1). Of the 89 filters tested seven amplified from three different sites for the presence of *O. candidum* DNA (Figure 1). These sites were the control site, site 25 and site MB17MEH0015. For the control site three filters were collected all of which amplified for the presence of *O. candidum*. For site 25 only one of the five filters collected amplified, and for MB17MEH0015 three of the five filters amplified.

From the 89 filters sent for analysis, 59 filters amplified with the control probe. The 20 filters that failed to amplify may have been due to no mammal DNA present in the bore or the presence of only degraded DNA. Two sites were noted to have failed across all filters collected (JW024 and RR1).

All samples had three replicates for the *O. candidium* probe and one replicate for the control probe. Amplification of the *O. candidium* was noted in all replicates except NI84 in which the third replicate failed to amplify. This was most likely the result of degradation.

Figure 1: Sites sampled during the December 2017 collection and the results for *O. candidium* specific qPCR assay. No bar indicates no amplification.



### **Conclusion**

In conclusion, we confirm that *O. candidium* DNA was observed in three sites. The control site (n=3), 25 site (n=1) and MB17MEH0015 site (n=3). Both sampling methods (bailer and pump) collected *O. candidium* DNA even though varying volumes of water were used (500-1000ml). The modified methods of storing filters at -20°C for transport and -80°C for storage appear to be effective in addition to using only half the filters.

Three replicates were done for each sample and a range of 5.09 - 7.68% increase in the CT value was noted between replicates. As expected eDNA is susceptible to degradation even via limited freezer thaw cycles. For future work it is suggested that freeze thaw cycles be limited as much as possible.

### **Reference**

Helix\_Job\_258\_Blind\_eel\_DNA\_update\_v1.2 report

Thank you once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

**Helix Molecular Solutions Pty Ltd**

| Site        | Easting     | Northing    | Sampled date | Biota Code              | Hole depth | Drilled      | Notes                     | Post Notes   | Stygo hauls | Type | Notes  | eDNA | # EDNA filters | Lab ID | Extraction Notes | Positive                       | O. Candidum rep 1 | O. Candidum rep 2 | O. Candidum rep 3 |
|-------------|-------------|-------------|--------------|-------------------------|------------|--------------|---------------------------|--|-------------|------|--------|------|----------------|--------|------------------|--------------------------------|-------------------|-------------------|-------------------|
| Control     | 424478      | 7597147     | 2017-12-11   | Control-20171211-1      |            |              |                           | eDNA only, Taken from surface water at the site of RRW501    | -           |      |        | Yes  |                |        | N101             |                                | 32.73             | 33.68             | 35.99             |
| Control     | 424478      | 7597147     | 2017-12-11   | Control-20171211-2      |            |              |                           | eDNA only, Taken from surface water at the site of RRW501    | -           |      |        | Yes  |                |        | N102             |                                | 32.91             | 30.94             | 33.77             |
| Control     | 424478      | 7597147     | 2017-12-11   | Control-20171211-3      |            |              |                           | eDNA only, Taken from surface water at the site of RRW501    | -           |      |        | Yes  | 3              |        | N103             |                                | 30                | 32.14             | 32.36             |
| 200         | 392512.0324 | 7607435.55  | 2017-12-12   | 200-20171212-B1         |            |              | PSS089                    | Stygo net stuck and lost after 2 hauls                       | 5           | B1   |        | Yes  |                |        | N104             |                                | -                 | -                 | -                 |
| 200         | 392512.0324 | 7607435.55  | 2017-12-12   | 200-20171212-B2         |            |              | PSS089                    | Stygo net stuck and lost after 2 hauls                       | 5           | B2   |        | Yes  |                |        | N105             |                                | 33.5              | -                 | -                 |
| 200         | 392512.0324 | 7607435.55  | 2017-12-12   | 200-20171212-B3         |            |              | PSS089                    | Stygo net stuck and lost after 2 hauls                       | 5           | B3   |        | Yes  |                |        | N106             |                                | 34.55             | -                 | -                 |
| 200         | 392512.0324 | 7607435.55  | 2017-12-12   | 200-20171212-P4         |            |              | PSS089                    | Stygo net stuck and lost after 2 hauls                       | 5           | P4   |        | Yes  |                |        | N107             |                                | 32.72             | -                 | -                 |
| 200         | 392512.0324 | 7607435.55  | 2017-12-12   | 200-20171212-P5         |            |              | PSS089                    | Stygo net stuck and lost after 2 hauls                       | 5           | P5   |        | Yes  |                |        | N108             |                                | 33.44             | -                 | -                 |
| 200         | 392512.0324 | 7607435.55  | 2017-12-12   | 200-20171212-P6         |            |              | PSS089                    | Stygo net stuck and lost after 2 hauls                       | 5           | P6   |        | Yes  | 6              |        | N109             |                                | 34.48             | -                 | -                 |
| Budgie Bore | 382494.7379 | 7615587.299 | 2017-12-12   | Budgie Bore-20171212-B1 |            |              |                           |  | 5           | B1   |        | Yes  |                |        | N110             |                                | -                 | -                 | -                 |
| Budgie Bore | 382494.7379 | 7615587.299 | 2017-12-12   | Budgie Bore-20171212-B2 |            |              |                           |  | 5           | B2   | 500ml  | Yes  |                |        | N111             | whole filter in bag, half used | -                 | -                 | -                 |
| Budgie Bore | 382494.7379 | 7615587.299 | 2017-12-12   | Budgie Bore-20171212-B3 |            |              |                           |  | 5           | B3   |        | Yes  |                |        | N112             |                                | 34.52             | -                 | -                 |
| Budgie Bore | 382494.7379 | 7615587.299 | 2017-12-12   | Budgie Bore-20171212-P4 |            |              |                           |  | 5           | P4   |        | Yes  |                |        | N113             |                                | 34.84             | -                 | -                 |
| Budgie Bore | 382494.7379 | 7615587.299 | 2017-12-12   | Budgie Bore-20171212-P5 |            |              |                           |  | 5           | P5   |        | Yes  |                |        | N114             |                                | -                 | -                 | -                 |
| Budgie Bore | 382494.7379 | 7615587.299 | 2017-12-12   | Budgie Bore-20171212-P6 |            |              |                           |  | 5           | P6   | 500ml  | Yes  | 6              |        | N115             | whole filter in bag, half used | -                 | -                 | -                 |
| MB16MEC0008 | 393565      | 7600580     | 2017-12-12   | MB16MEC0008-20171212-B1 | 36         | Drilled 2016 |                           |  | 5           | B1   |        | Yes  |                |        | N116             |                                | 31.28             | -                 | -                 |
| MB16MEC0008 | 393565      | 7600580     | 2017-12-12   | MB16MEC0008-20171212-B2 | 36         | Drilled 2016 |                           |  | 5           | B2   |        | Yes  |                |        | N117             |                                | 31.29             | -                 | -                 |
| MB16MEC0008 | 393565      | 7600580     | 2017-12-12   | MB16MEC0008-20171212-B3 | 36         | Drilled 2016 |                           |  | 5           | B3   |        | Yes  |                |        | N118             |                                | 30.91             | -                 | -                 |
| MB16MEC0008 | 393565      | 7600580     | 2017-12-12   | MB16MEC0008-20171212-P4 | 36         | Drilled 2016 |                           |  | 5           | P4   |        | Yes  |                |        | N119             |                                | 31.96             | -                 | -                 |
| MB16MEC0008 | 393565      | 7600580     | 2017-12-12   | MB16MEC0008-20171212-P5 | 36         | Drilled 2016 |                           |  | 5           | P5   |        | Yes  |                |        | N120             |                                | 33.83             | -                 | -                 |
| MB16MEC0008 | 393565      | 7600580     | 2017-12-12   | MB16MEC0008-20171212-P6 | 36         | Drilled 2016 |                           |  | 5           | P6   |        | Yes  | 6              |        | N121             |                                | 32.32             | -                 | -                 |
| MB16MEC0009 | 393590.062  | 7601144.506 | 2017-12-12   | MB16MEC0009-20171212-B1 | 43         | Drilled 2016 |                           |  | 5           | B1   |        | Yes  |                |        | N122             |                                | 29.64             | -                 | -                 |
| MB16MEC0009 | 393590.062  | 7601144.506 | 2017-12-12   | MB16MEC0009-20171212-B2 | 43         | Drilled 2016 |                           |  | 5           | B2   |        | Yes  |                |        | N123             |                                | 30.33             | -                 | -                 |
| MB16MEC0009 | 393590.062  | 7601144.506 | 2017-12-12   | MB16MEC0009-20171212-B3 | 43         | Drilled 2016 |                           |  | 5           | B3   |        | Yes  |                |        | N124             |                                | 29.96             | -                 | -                 |
| MB16MEC0009 | 393590.062  | 7601144.506 | 2017-12-12   | MB16MEC0009-20171212-P4 | 43         | Drilled 2016 |                           |  | 5           | P4   |        | Yes  |                |        | N125             |                                | 35.17             | -                 | -                 |
| MB16MEC0009 | 393590.062  | 7601144.506 | 2017-12-12   | MB16MEC0009-20171212-P5 | 43         | Drilled 2016 |                           |  | 5           | P5   |        | Yes  |                |        | N126             |                                | 28.24             | -                 | -                 |
| MB16MEC0009 | 393590.062  | 7601144.506 | 2017-12-12   | MB16MEC0009-20171212-P6 | 43         | Drilled 2016 |                           |  | 5           | P6   | 500ml  | Yes  | 6              |        | N127             | whole filter in bag, half used | -                 | -                 | -                 |
| MB17MEH0009 | 416435.8    | 7599848     | 2017-12-12   | MB17MEH0009-20171212-B1 | 43         | 30-Aug-17    |                           |  | 5           | B1   |        | Yes  |                |        | N128             |                                | 34.48             | -                 | -                 |
| MB17MEH0009 | 416435.8    | 7599848     | 2017-12-12   | MB17MEH0009-20171212-B2 | 43         | 30-Aug-17    |                           |  | 5           | B2   |        | Yes  |                |        | N129             |                                | 35.79             | -                 | -                 |
| MB17MEH0009 | 416435.8    | 7599848     | 2017-12-12   | MB17MEH0009-20171212-B3 | 43         | 30-Aug-17    |                           |  | 5           | B3   |        | Yes  |                |        | N130             |                                | 33.94             | -                 | -                 |
| MB17MEH0009 | 416435.8    | 7599848     | 2017-12-12   | MB17MEH0009-20171212-P4 | 43         | 30-Aug-17    |                           |  | 5           | P4   |        | Yes  |                |        | N131             |                                | -                 | -                 | -                 |
| MB17MEH0009 | 416435.8    | 7599848     | 2017-12-12   | MB17MEH0009-20171212-P5 | 43         | 30-Aug-17    |                           |  | 5           | P5   |        | Yes  |                |        | N132             |                                | -                 | -                 | -                 |
| MB17MEH0009 | 416435.8    | 7599848     | 2017-12-12   | MB17MEH0009-20171212-P6 | 43         | 30-Aug-17    |                           |  | 5           | P6   |        | Yes  | 6              |        | N133             |                                | 35.28             | -                 | -                 |
| MB17MEH0010 | 416440      | 7599848     | 2017-12-12   | MB17MEH0010-20171212-B1 | 30         | 31-Aug-17    |                           |  | 5           | B1   |        | Yes  |                |        | N134             |                                | 30.36             | -                 | -                 |
| MB17MEH0010 | 416440      | 7599848     | 2017-12-12   | MB17MEH0010-20171212-B2 | 30         | 31-Aug-17    |                           |  | 5           | B2   |        | Yes  |                |        | N135             |                                | 31.1              | -                 | -                 |
| MB17MEH0010 | 416440      | 7599848     | 2017-12-12   | MB17MEH0010-20171212-B3 | 30         | 31-Aug-17    |                           |  | 5           | B3   |        | Yes  |                |        | N136             |                                | 32.06             | -                 | -                 |
| MB17MEH0010 | 416440      | 7599848     | 2017-12-12   | MB17MEH0010-20171212-P4 | 30         | 31-Aug-17    |                           |  | 5           | P4   |        | Yes  |                |        | N137             |                                | 33.8              | -                 | -                 |
| MB17MEH0010 | 416440      | 7599848     | 2017-12-12   | MB17MEH0010-20171212-P5 | 30         | 31-Aug-17    |                           |  | 5           | P5   |        | Yes  |                |        | N138             |                                | 35.6              | -                 | -                 |
| MB17MEH0010 | 416440      | 7599848     | 2017-12-12   | MB17MEH0010-20171212-P6 | 30         | 31-Aug-17    |                           |  | 5           | P6   |        | Yes  | 6              |        | N139             |                                | 34.09             | -                 | -                 |
| 25          | 432152      | 7602229     | 2017-12-13   | 25-20171213-B1          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | B1   |        | Yes  |                |        | N140             |                                | 34.91             | -                 | -                 |
| 25          | 432152      | 7602229     | 2017-12-13   | 25-20171213-B2          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | B2   |        | Yes  |                |        | N141             |                                | -                 | -                 | -                 |
| 25          | 432152      | 7602229     | 2017-12-13   | 25-20171213-P3          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | P3   |        | Yes  |                |        | N142             |                                | -                 | -                 | -                 |
| 25          | 432152      | 7602229     | 2017-12-13   | 25-20171213-P4          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | P4   | 500ml  | Yes  |                |        | N143             |                                | 35.32             | 28.35             | 28.79             |
| 25          | 432152      | 7602229     | 2017-12-13   | 25-20171213-P5          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | P5   |        | Yes  | 5              |        | N144             |                                | -                 | -                 | -                 |
| 31          | 432929      | 7603179     | 2017-12-13   | 31-20171213-B1          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | B1   |        | Yes  |                |        | N145             |                                | 29.37             | -                 | -                 |
| 31          | 432929      | 7603179     | 2017-12-13   | 31-20171213-B2          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | B2   |        | Yes  |                |        | N146             |                                | 32.71             | -                 | -                 |
| 31          | 432929      | 7603179     | 2017-12-13   | 31-20171213-P3          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | P3   |        | Yes  |                |        | N147             |                                | -                 | -                 | -                 |
| 31          | 432929      | 7603179     | 2017-12-13   | 31-20171213-P4          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | P4   |        | Yes  |                |        | N148             |                                | -                 | -                 | -                 |
| 31          | 432929      | 7603179     | 2017-12-13   | 31-20171213-P5          |            |              |                           | Site Locked - Needed key from utilities in Panna (near mess) | 5           | P5   | 500ml  | Yes  | 5              |        | N149             |                                | -                 | -                 | -                 |
| BC186       | 429578      | 7587212     | 2017-12-13   | BC186-20171213-B1       |            |              | Confirmed eel record 2009 |  | 5           | B1   |        | Yes  |                |        | N150             |                                | -                 | -                 | -                 |
| BC186       | 429578      | 7587212     | 2017-12-13   | BC186-20171213-B2       |            |              | Confirmed eel record 2009 |  | 5           | B2   | 500ml  | Yes  |                |        | N151             | whole filter in bag, half used | 34.61             | -                 | -                 |
| BC186       | 429578      | 7587212     | 2017-12-13   | BC186-20171213-B3       |            |              | Confirmed eel record 2009 |  | 5           | B3   |        | Yes  |                |        | N152             |                                | -                 | -                 | -                 |
| BC186       | 429578      | 7587212     | 2017-12-13   | BC186-20171213-P4       |            |              | Confirmed eel record 2009 |  | 5           | P4   | 500ml  | Yes  |                |        | N153             | whole filter in bag, half used | -                 | -                 | -                 |
| BC186       | 429578      | 7587212     | 2017-12-13   | BC186-20171213-P5       |            |              | Confirmed eel record 2009 |  | 5           | P5   |        | Yes  |                |        | N154             |                                | -                 | -                 | -                 |
| BC401       | 443563      | 7574126     | 2017-12-13   | BC401-20171213-B1       |            |              |                           |  | 5           | B1   | 500ml  | Yes  | 5              |        | N155             | whole filter in bag, half used | 32.7              | -                 | -                 |
| BC401       | 443563      | 7574126     | 2017-12-13   | BC401-20171213-B2       |            |              |                           |  | 5           | B2   |        | Yes  |                |        | N156             |                                | 35.32             | -                 | -                 |
| BC401       | 443563      | 7574126     | 2017-12-13   | BC401-20171213-P3       |            |              |                           |  | 5           | P3   | 500ml  | Yes  |                |        | N157             | whole filter in bag, half used | -                 | -                 | -                 |
| BC401       | 443563      | 7574126     | 2017-12-13   | BC401-20171213-P4       |            |              |                           |  | 5           | P4   |        | Yes  |                |        | N158             |                                | 34.74             | -                 | -                 |
| BC401       | 443563      | 7574126     | 2017-12-13   | BC401-20171213-P5       |            |              |                           |  | 5           | P5   |        | Yes  |                |        | N159             |                                | 37.06             | -                 | -                 |
| JW023       | 426138      | 7590140     | 2017-12-13   | JW023-20171213-B1       |            |              | Confirmed Eel record 2016 | Hydrocarbons   | 5           | B1   |        | Yes  | 5              |        | N160             |                                | 35.34             | -                 | -                 |
| JW023       | 426138      | 7590140     | 2017-12-13   | JW023-20171213-B2       |            |              | Confirmed Eel record 2016 | Hydrocarbons   | 5           | B2   | 500ml? | Yes  |                |        | N161             |                                | 36.56             | -                 | -                 |
| JW023       | 426138      | 7590140     | 2017-12-13   | JW023-20171213-B3       |            |              | Confirmed Eel record 2016 | Hydrocarbons   | 5           | B3   |        | Yes  |                |        | N162             |                                | 36.64             | -                 | -                 |
| JW023       | 426138      | 7590140     | 2017-12-13   | JW023-20171213-B4       |            |              | Confirmed Eel record 2016 | Hydrocarbons   | 5           | B4   |        | Yes  |                |        | N163             |                                | 33.33             | -                 | -                 |
| JW023       | 426138      | 7590140     | 2017-12-13   | JW023-20171213-B5       |            |              | Confirmed Eel record 2016 | Hydrocarbons   | 5           | B5   | 500ml  | Yes  | 5              |        | N164             | whole filter in bag, half used | 35.01             | -                 | -                 |
| JW024       | 427126      | 7590154     | 2017-12-13   | JW024-20171213-B1       |            |              | Confirmed Eel record 2017 |  | 5           | B1   |        | Yes  |                |        | N165             |                                | -                 | -                 | -                 |
| JW024       | 427126      | 7590154     | 2017-12-13   | JW024-20171213-B2       |            |              | Confirmed Eel record 2017 |  | 5           | B2   |        | Yes  |                |        | N166             |                                | -                 | -                 | -                 |
| JW024       | 427126      | 7590154     | 2017-12-13   | JW024-20171213-P3       |            |              | Confirmed Eel record 2017 |  | 5           | P3   |        | Yes  |                |        | N167             |                                | -                 | -                 | -                 |
| JW024       | 427126      | 7590154     | 2017-12-13   | JW024-20171213-P4       |            |              | Confirmed Eel record 2017 |  | 5           | P4   |        | Yes  |                |        | N168             |                                | -                 | -                 | -                 |
| JW024       | 427126      | 7590154     | 2017-12-13   | JW024-20171213-P5       |            |              | Confirmed Eel record 2017 |  | 5           | P5   | 500ml  | Yes  | 5              |        | N169             |                                | -                 | -                 | -                 |
| MB17MEH0007 | 417663.8    | 7600421     | 2017-12-14   | MB17MEH0007-20171214-B1 | 30         | 29-Aug-17    |                           | Bailer only for eDNA, Pump stopped working                   | 5           | B1   |        | Yes  |                |        | N170             | whole filter in bag, half used | 34.84             | -                 | -                 |
| MB17MEH0007 | 417663.8    | 7600421     | 2017-12-14   | MB17MEH0007-20171214-B2 | 30         | 29-Aug-17    |                           | Bailer only for eDNA, Pump stopped working                   | 5           | B2   |        | Yes  |                |        | N171             |                                | -                 | -                 | -                 |
| MB17MEH0007 | 417663.8    | 7600421     | 2017-12-14   | MB17MEH0007-20171214-B3 | 30         | 29-Aug-17    |                           | Bailer only for eDNA, Pump stopped working                   | 5           | B3   |        | Yes  |                |        | N172             |                                | -                 | -                 | -                 |
| MB17MEH0007 | 417663.8    | 7600421     | 2017-12-14   | MB17MEH0007-20171214-B4 | 30         | 29-Aug-17    |                           | Bailer only for eDNA, Pump stopped working                   | 5</         |      |        |      |                |        |                  |                                |                   |                   |                   |





## Appendix 4

# Curtin University eDNA Metabarcoding Report







## **eDNA Metabarcoding Preliminary Report: Blind Cave Eel survey in the Pilbara WA**

*Prepared for*

**Rio Tinto  
Level 24, 152-158 St Georges Terrace  
Perth, WA, 6000**

*Prepared by*

**Trace and Environmental DNA (TrEnD) Laboratory  
Curtin University**

21<sup>st</sup> March 2018

**Report Authors:** Dr Nicole White and Prof. Michael Bunce (TrEnD laboratory)

**Signature:**

**(Dr Nicole White)      Date: 21<sup>st</sup> March 2018**

### **Disclaimer:**

The Trace and Environmental DNA (TrEnD) laboratory offers DNA services across a number of biological applications. While TrEnD stands by the validity of its work and the science that underpins it, stakeholders should use the information contained within the report at their own risk. We suggest using DNA results we report as a line of evidence in decision making processes and it may be appropriate to repeat results, re-sample at sites, corroborate data using other DNA markers or use other non-molecular methods. TrEnD accepts no liability or responsibility for it in respect of any use of or reliance upon this report by any third party. Copying this report without prior written consent of TrEnD is not permitted. © Copyright 2018 TrEnD Laboratory, Curtin University.

## Executive Summary:

### Overall Project objective:

The aim of this pilot project was to use an environmental DNA (eDNA) metabarcoding approach to determine presence/absence of Blind Cave Eel (*Ophisternon candidum*) from within and outside the Mesa H project area in the Pilbara region of Western Australia. The data was to be incorporated into an environmental impact statement (EIS) by Rio Tinto. This interim report documents the results to date and will be superseded by the final report on the project which involves replication of data and design of new assays for eel detection.

### Outputs:

- Two collection trips in 2017 were conducted (i) June 2017: 91 water samples from 18 bores were collected and filtered by Michelle Guzik (University of Adelaide). (ii) December 2017: 85 water samples were collected and filtered by Jason Alexander (Biota) from 16 bores and one open surface water sample from the Robe River (5x 1 liter replicates). The TrEnD lab at Curtin University successfully isolated DNA from 122 of these samples.
- As a preliminary first-pass to determine the presence of Blind Cave Eel (*O. candidum*) from 122 water membranes the TrEnD labs Fish 16S assay was used. Although this DNA assay (Fish 16S) isn't specific for Blind Cave Eel our previous research has demonstrated successful amplification of eel DNA.
- The June 2017 water membranes detected the possibility of Blind Cave Eel (*O. candidum*) from bore JW021. The December 2017 water membranes detected the possibility of Blind Cave Eel (*Ophisternon candidum*) from bore 25 and bore RR1. This result is preliminary in nature – detections are currently un-replicated. Additional sites could yield eel signatures as assays are repeated and modified.
- Blind Cave Eel (*O.candidum*) tissue was sourced from the Western Australia Museum (WAM) for comparative purposes. We have sequenced the 16S barcode from this sample.
- Comparisons of the potential Blind Cave Eel (*O. candidum*) DNA sequences from bore JW021 with the National Centre for Biotechnology Information (NCBI) database showed an 85.9% match to *O. bengalense*, an eel-like fish found in northern Australia and 85.9% match to *Synbranchid* sp., a freshwater eel-like fish.
- Comparisons of the potential Blind Cave Eel (*O. candidum*) DNA sequences from bore 25 and RR1 with the NCBI database showed an 87.7% match to *O. bengalense* and 87.1% match to *Synbranchid* sp.
- Comparisons of the potential Blind Cave Eel (*O. candidum*) DNA sequences from bore JW021, 25 and RR1 with the reference sample obtained from the WAM showed 100% match to the DNA sequences obtained from bore JW021 and 97% match to the DNA sequences from bore 25 and RR1. The genetic sequences in bores 25 and RR1 are 100% similar to each other but distinctly different from the WAM reference or the JW021 sequence. The significance of this genetic difference is unknown.

### Recommendations:

- An eDNA approach using a Fish 16S assay has successfully detected Blind Cave Eel from three bores within and outside the Mesa H project area. The sensitivity and reproducibility of these findings still need to be confirmed by the ongoing research program. The successful detection of Blind eel from a non-invasive sample speaks to the promise of eDNA approaches for the detection of taxa in subterranean environments.

# 1. Introduction

---

eDNA refers to all genetic material that is recovered from environmental substrates (such as water or sediment). DNA directly from an organism (e.g. microorganisms) as well as trace amounts of DNA that are shed by all organisms is collectively termed eDNA. This preserved, but often degraded, genetic material provides a means to audit species composition and communities at a given location. When combined with recently developed DNA sequencing technologies (termed 'next generation' or 'high-throughput' sequencing), eDNA can provide a wealth of information for studies of introduced marine pests (IMP), biodiversity surveys, food web dynamics, and diet analysis.

Potential applications for monitoring using eDNA include:

- Biosecurity: to detect the presence/absence of introduced species
- Environmental Impact Assessment: baseline/ongoing monitoring and assessment; audit
- Conservation: to detect the presence/absence of threatened species

Rio Tinto has partnered with the Trace and Environmental DNA (TrEnD) Laboratory at Curtin University to develop eDNA tools for Blind Cave Eel (*Ophisternon candidum*) as a means to use DNA-based bio surveys in groundwater monitoring and management.

eDNA sampling has benefits over other traditional survey methods including:

- highly cost-effective monitoring compared to traditional survey methods
- rapid sample collection that is non destructive to habitats, flora and fauna
- non-reliance on individuals with taxonomic expertise, provision of consistent results between monitoring programs, independent of surveyors

The objectives of this project, albeit preliminary, were to use an environmental DNA (eDNA) metabarcoding approach to determine the presence/absence of Blind Cave Eel (*Ophisternon candidum*) a threatened species within the groundwater systems of the Pilbara in Western Australia, specifically the Mesa H area (Rio Tinto). The data was to be incorporated into an environmental impact statement.



## 2. Methods and Samples

Water samples were collected by Michelle Guzik (University of Adelaide; June 2017) and Jason Alexander (Biota; December 2017) and filtered using a Sentino pump (Pall corporation) as outlined in TrEnD standard operating procedures (available upon request). Turbidity in the water resulted in varying volumes of water that could be filtered between the bores and replicates (see table 1 below and attached electronic data file).

Table 1

| Bore water collected by Michelle Guzik (June 2017)             |               |               |               |              |              |              |                           |              |
|--|---------------|---------------|---------------|--------------|--------------|--------------|---------------------------|--------------|
| AWFS ID  | Bore Hole     | Collection me | Date filtered | Sample 1 (A) | Sample 2 (B) | Sample 3 (C) | Sample 4 (D)              | Sample 5 (E) |
| AWFS-I17-106 (A, B, C, D, E)                                   | JIMWE003      | Bailer        | 13/06/2017    | 250mL        | 750mL        | 1L           | 1L                        | 650mL        |
| AWFS-I17-107 (A, B, C, D, E)                                   | JW021         | Bailer        | 13/06/2017    | 700mL        | 700mL        | 600mL        | 600mL                     | 800mL        |
| AWFS-I17-108 (A, B, C, D, E)                                   | BC186         | Bailer        | 13/06/2017    | 1L           | 1L           | 1L           | 1L                        | 1L           |
| AWFS-I17-109 (A, B, C, D, E)                                   | JW024         | Bailer        | 13/06/2017    | 1L           | 1L           | 1L           | 700mL                     | 1L           |
| AWFS-I17-110 (A, B, C, D, E)                                   | BC276         | Bailer        | 14/06/2017    | 1L           | 500mL        | 500mL        | 500mL                     | 250mL        |
| AWFS-I17-111 (A, B, C, D, E)                                   | BC667         | Bailer        | 14/06/2017    | 250mL        | 250mL        | 250mL        | 1L                        | 1L           |
| AWFS-I17-112 (A, B, C, D, E)                                   | BCP001        | Bailer        | 14/06/2017    | 250mL (colle | 250mL (colle | 250mL        | 1L                        | 1L           |
| AWFS-I17-113 (A, B, C, D, E)                                   | BC458         | Bailer        | 15/06/2017    | 1L           | 1L           | 1L           | 1L                        | 1L           |
| AWFS-I17-114   | Robe River    | Container     | 14/06/2017    | 700mL        | NA           | NA           | NA                        | NA           |
| AWFS-I17-115 (A, B, C, D, E)                                   | BUNWO1108     | Bailer        | 15/05/2017    | 700mL        | 1L           | 1L           | 1L                        | 500mL        |
| AWFS-I17-116 (A, B, C, D)                                      | BUNWB13       | Bailer        | 15/05/2017    | 1L           | 750mL        | 750mL        | 750mL                     | NA           |
| AWFS-I17-117 (A, B, C, D, E)                                   | BUNWB09       | Bailer        | 15/06/2017    | 1L           | 800mL        | 800mL        | 800mL                     | 1L           |
| AWFS-I17-118 (A, B, C, D, E)                                   | BC401         | Bailer        | 16/06/2017    | 1L           | 600mL        | 500mL (colle | 1L                        | 1L           |
| AWFS-I17-119 (A, B, C, D, E)                                   | BUNWB08       | Bailer        | 16/06/2017    | 1L           | 1L           | 1L           | 1L                        | 1L           |
| AWFS-I17-120 (A, B, C, D, E)                                   | BC225         | Bailer        | 15/06/2017    | 1L           | 1L           | 1L           | 1L                        | 1L           |
| AWFS-I17-121 (A, B, C, D, E)                                   | JIMWE004      | Bailer        | 16/06/2017    | 1L           | 1L           | 1L           | 800mL                     | 800mL        |
| AWFS-I17-122 (A, B, C, D, E)                                   | BC292         | Bailer        | 16/06/2017    | 250mL        | 1L           | 1L           | 1L                        | 1L           |
| AWFS-I17-123 (A, B, C, D, E)                                   | BUNWO0773     | Bailer        | 16/06/2017    | 500mL        | 500mL        | 500mL        | 1L                        | 1L           |
| AWFS-I17-124 (A, B, C, D, E)                                   | PZ11BUN008    | Bailer        | 16/06/2017    | 600mL        | 250mL        | 250mL        | 250mL                     | 250mL        |
| Bore water collected by Jason Alexander (Biota; December 2017) |               |               |               |              |              |              |                           |              |
| AWFS ID  | Site          | Eastings      | Northing      | Date         | Hole depth   | Drilled      | Notes                     |              |
| AWFS-I18-001 (A,B,C,D,E)                                       | 25            | 432152        | 7602229       | 2017-12-13   |              |              |                           |              |
| AWFS-I18-002 (A,B,C,D,E)                                       | 31            | 432929        | 7603179       | 2017-12-13   |              |              |                           |              |
| AWFS-I18-003 (A,B,C,D,E)                                       | 200/Dave Bore | 392512.032    | 7607435.55    | 2017-12-12   |              |              | PSS089                    |              |
| AWFS-I18-004 (A,B,C,D,E)                                       | BC186         | 429578        | 7587212       | 2017-12-13   |              |              | Confirmed eel record 2009 |              |
| AWFS-I18-005 (A,B,C,D,E)                                       | BC401         | 443563        | 7574126       | 2017-12-13   |              |              |                           |              |
| AWFS-I18-006 (A,B,C,D,E)                                       | Budgie Bore   | 382494.738    | 7615587.3     | 2017-12-12   |              |              |                           |              |
| AWFS-I18-007 (A,B,C,D,E)                                       | JW023         | 426138        | 7590140       | 2017-12-13   |              |              | Confirmed Eel record 2016 |              |
| AWFS-I18-008 (A,B,C,D,E)                                       | JW024         | 427126        | 7590154       | 2017-12-13   |              |              | Confirmed Eel record 2017 |              |
| AWFS-I18-009 (A,B,C,D,E)                                       | MB16MEC0009   | 393590.062    | 7601144.51    | 2017-12-12   | 43           | Drilled 2016 |                           |              |
| AWFS-I18-010 (A,B,C,D,E)                                       | MB17MEH0008   | 417119.5      | 7600417       | 2017-12-14   | 18           | 29-Aug-17    |                           |              |
| AWFS-I18-011 (A,B,C,D,E)                                       | MB17MEH0010   | 416440        | 7599848       | 2017-12-12   | 30           | 31-Aug-17    |                           |              |
| AWFS-I18-012 (A,B,C,D,E)                                       | MB17MEH0015   | 416041        | 7597690       | 2017-12-14   | 37           | 3-Nov-17     |                           |              |
| AWFS-I18-013 (A,B,C,D,E)                                       | RR1           | 419176        | 7597904       | 2017-12-14   | 17.2         |              | depth to water 1.87       |              |
| AWFS-I18-014 (A,B,C,D,E)                                       | MB16MEC0008   | 393565        | 7600580       | 2017-12-12   | 36           | Drilled 2016 |                           |              |
| AWFS-I18-015 (A,B,C,D,E)                                       | MB17MEH0007   | 417663.8      | 7600421       | 2017-12-14   | 30           | 29-Aug-17    |                           |              |
| AWFS-I18-016 (A,B,C,D,E)                                       | MB17MEH0009   | 416435.8      | 7599848       | 2017-12-12   | 43           | 30-Aug-17    |                           |              |
| AWFS-I18-017 (A,B,C,D,E)                                       | Control       | 424478        | 7597147       | 2017-12-11   |              |              |                           |              |

eDNA was extracted using Qiagen Blood & Tissue DNeasy kits in the TrEnD laboratory at Curtin University in dedicated facilities (inclusive of laboratory clean rooms) suited for eDNA workflows. eDNA derived from the Fish 16S assay was amplified using PCR, followed by sequencing on an Illumina MiSeq in the TrEnD Laboratory. Negative laboratory controls were included (and sequenced if identified as positive) to control for environmental laboratory contamination.

The 16S DNA sequences recovered were compared to the National Centre for Biotechnology Information (NCBI) database for taxonomic identification and to reference Blind Cave Eel (*Ophisternon candidum*) obtained from the WAM (P.34817-001).

### 3. Results and Discussion

The following table outlines the taxonomic assignments from each bore water sample for which suspected Blind Cave Eel (*Ophisternon candidum*) was amplified and sequenced. Taxonomic assignments are to genera and species with the per cent match genetic similarity to the DNA sequences contained on the reference database (NCBI). At the time of this report, the taxonomic assignments for the DNA sequences obtained from the bore water samples (i.e. DNA 'barcodes') are listed in the table below and the electronic file accompanying this report. The environmental laboratory controls for this work were identified as negative and not included in the Table.

Table 2

| Taxonomic Assignments                       |       |                |  |
|---|-------|----------------|--|
| AWFS ID                                     | Bore  | % match (NCBI) | Species  |
| AWFS-I17-107B                               | JW021 | 85.9           | Synbranchid sp. VietNam-TC-2002 haplotype 1      |
|   |       | 85.9           | Ophisternon bengalense voucher MRS haplotype OB1 |
| AWFS-I18-001A                               | 25    | 87.1           | Synbranchid sp. VietNam-TC-2002 haplotype 1      |
|   |       | 87.1           | Ophisternon bengalense voucher MRS haplotype OB1 |
| AWFS-I18-013A                               | RR1   | 87.1           | Synbranchid sp. VietNam-TC-2002 haplotype 1      |
|   |       | 87.1           | Ophisternon bengalense voucher MRS haplotype OB1 |
| AWFS-I18-013C                               | RR1   | 87.1           | Synbranchid sp. VietNam-TC-2002 haplotype 1      |
|   |       | 87.1           | Ophisternon bengalense voucher MRS haplotype OB1 |
| AWFS-F18-005<br>BlindEel_WAM<br>P.34817-001 |       | 85.9           | Synbranchid sp. VietNam-TC-2002 haplotype 1      |
|   |       | 85.9           | Ophisternon bengalense voucher MRS haplotype OB1 |

Fish DNA was detected in a number of sampling locations. This preliminary report is only reporting on, and presenting data from, those bores that yielded eel DNA.

Comparisons of the potential Blind Cave Eel (*O. candidum*) DNA sequences from bore JW021 with the NCBI database showed an 85.9% match to *O. bengalense*, an eel-like fish found in northern Australia and 85.9% match to *Synbranchid* sp., a freshwater eel-like fish (see Table 2). The DNA barcode recovered from JW021 was a 100% match to the reference DNA sequence from the WAM Blind Cave Eel (*O. candidum*) sample. Eel DNA was recovered in one of two replicates from bore JW021

Comparisons of the potential Blind Cave Eel (*O. candidum*) DNA sequences from bore 25 and RR1 with the NCBI database showed an 87.7%% match to *O. bengalense* and 87.1% match to *Synbranchid* sp. (see table 2). The DNA barcode recovered from bore RR1 and bore 25 was a 97% match to the reference DNA sequence from the WAM Blind Cave Eel (*O. candidum*) sample. Eel DNA was recovered in two of the five replicates from bore RR1 and one of five replicates from bore 25.

The genetic sequences in bores 25 and RR1 are 100% similar to each other but distinctly different from the WAM reference or the JW021 sequence. The significance of this genetic difference is

unknown it may represent variation within the species or a distinct lineage. There is a distinctive DNA 'insertion' in the sequences obtained from JW021 and the WAM reference that is the major distinguishing feature (see Figure 1)



Figure 1: 16S DNA sequences from bore JW021, RR1, 25 and *O. candidum* (WAM P.34817-001)

## 4. Findings, Recommendations and Concluding remarks

The Fish 16S metabarcoding assay utilised on the bore water samples collected in June and December 2017 detected the potential Blind Cave Eel (*O. candidum*) in three of 34 bores. To our knowledge this represents the first time an eDNA based approach has been used in the ground water system(s) in the Pilbara for the detection of a restricted species. The gaps in the reference databases often prevent definitive assignments to genus and/or species at the time of this report. Although comparisons of the suspected Blind Cave Eel (*O. candidum*) DNA sequences with the DNA sequences obtained from a tissue sample of Blind Cave Eel (*O. candidum*) from the WAM indicate the strong likelihood of Blind Cave Eel (*O. candidum*). Current laboratory work is underway to sequence the complete mitochondrial genome from the WAM reference sample and develop a metabarcoding assay specific for Blind Cave Eel (*O. candidum*). Once completed, the Blind Cave Eel (*Ophisternon candidum*) metabarcoding assay will be used to re-screen the 122 water samples to validate the findings from the Fish 16S assay and potentially find additional bores that may contain Blind Cave Eel (*O. candidum*).

The DNA yields recovered from the bores was low and, as expected, not every replicate from positive wells yielded a positive detection. The sensitivity of the assays and the level of replication appropriate is yet to be determined for bores in the Pilbara. The development of additional assays and optimisation of existing assays (and sampling protocols) is ongoing and will likely enhance the likelihood of detections.

## 5. Appendices and additional information

---

- An electronic file titled; Appendix\_BlindCaveEel\_March2018.xlsx is attached to this report.
- Additional information on sampling and methods are available from TrEnD upon request however the exact nature of the assays and workflow are proprietary.
- Below is a list of acronyms, abbreviations and definitions that may apply to aspects of this report.

| % value in data            | Represents the percentage similarity of a DNA sequence recovered from a sample compared to reference sequences in a database (e.g. compared to DNA databases such as GenBank or references generated in-house)  |
|----------------------------|---|
| (x) value in data          | Represents the frequency the % value was recorded in the dataset  |
| 16S rRNA                   | The 16S rRNA refers to a conserved gene region of mitochondrial DNA, which codes for a subunit of the ribosome. 16S rRNA is found in all eukaryotes making it a good candidate for DNA barcoding and is used extensively to detect vertebrate taxa such as fish and mammals.  |
| 18S rRNA                   | The 18S rRNA refers to a conserved gene region of nuclear DNA, which codes for a subunit of the ribosome. 18S rRNA is found in all eukaryotes making it a good candidate for DNA barcoding  |
| 18S IMS reference database | Reference 18S rRNA sequences of invasive marine species that are available in DNA databases   |
| Assay                      | In the context of metabarcoding an assay is a molecular test (using PCR) that is implemented to target a group of taxa within a mixed biological substrate. It is akin to using a magnet to selective enrich for needles (the target) in the context of a haystack (the total DNA from a sample).   |
| COI                        | The gene region that is being used as the standard barcode for almost all animal groups is a 648 base-pair region of the mitochondrial cytochrome c oxidase 1 gene ("CO1"). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. COI is not an effective barcode region in plants because it evolves too slowly, but two gene regions in the chloroplast, matK and rbcL, have been approved as the barcode regions for plants |
| CO1 IMS reference database | Reference COI sequences of invasive marine species that are available in DNA databases  |
| DNA                        | Deoxyribonucleic Acid (DNA) is the hereditary material that contains the genetic information of an organism   |
| DNA metabarcoding          | Is a genetic technique that simultaneously amplifies and sequences barcode regions  |

|                     |  |
|---------------------|--|
|                     | (e.g. COI, 18S, 16S) of many different species in parallel   |
| eDNA                | Environmental DNA (eDNA) refers to genetic material that is recovered from an environmental substrate (e.g. water, sediment, air)  |
| eukaryotes          | An organism where cells contain a nucleus surrounded by a membrane and has the DNA bound together by proteins (histones) into chromosomes. The cells of eukaryotes also contain an endoplasmic reticulum and numerous specialised organelles not present in prokaryotes, especially mitochondria, golgi bodies, and lysosomes  |
| Fisheries           | Department of Primary Industries and Regional Development, Fisheries Division, Aquatic Biosecurity Section   |
| GenBank             | Publically available repository of genetic information. Contains the barcode information of genes that have previously been sequenced  |
| Genome              | A genome is all the genetic material of an organism. It consists of DNA (or RNA in RNA viruses). The genome includes both the genes (the coding regions) and the noncoding DNA. In eukaryotes it refers to the genomes of the nucleus, mitochondria and chloroplasts. In prokaryotes, there is a single genome (as they do not contain mitochondria or chloroplasts) |
| Illumina MiSeq      | Next generation sequencing platform developed by the company Illumina  |
| IMP                 | Introduced marine pests  |
| IMS                 | Introduced marine species  |
| Metabarcoding assay | A PCR reaction using a specific set of primers that simultaneously amplifies the same gene target from multiple species  |
| Mitochondria        | The mitochondrion (plural mitochondria) is a double membrane-bound organelle found in all eukaryotic organisms, although some cells in some organisms may lack them (e.g. Red blood cells). It contains its own genome   |
| Mitogenomes         | Refers to the mitochondrial genome   |
| NGS                 | Next generation sequencing or second generation sequencing refers to massively parallel sequencing technology, as opposed to first generation sequencing or sanger sequencing where only a single template is sequenced at one time  |
| Nucleotide          | A compound consisting of a nucleoside linked to a phosphate group. Nucleotides form the basic structural unit of nucleic acids such as DNA   |
| PCR                 | Polymerase chain reaction (PCR) is the technique that is used to amplify (akin to photocopying DNA) specific regions of the genome from specific groups of taxa  |
| Primer              | A short DNA strand (≈20bp in size) used in PCR to target particular groups of organisms and genes. Two of them are required for PCR (a forward and a reverse)  |
| Primer binding site | A primer-binding site is the target region of a genome where the primer attaches to start replication. The primer binding site is on one of the two complementary strands of a double-stranded nucleotide polymer, in the strand which is to be copied, or is within a single-stranded nucleotide polymer sequence   |
| prokaryote          | Any of the typically unicellular microorganisms that lack a distinct nucleus and   |



|                    |  |
|--------------------|--|
|                    | membrane-bound organelles and that are classified as a kingdom (Prokaryotae syn. Monera) or into two domains (Bacteria and Archaea)  |
| RNA                | Ribonucleic acid (RNA) is a polymeric molecule implicated in various biological roles in coding, decoding, regulation, and expression of genes   |
| rRNA               | ribosomal ribonucleic acid is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms  |
| Sequence           | DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA |
| Shotgun sequencing | Refers to randomly sequencing short pieces of DNA ( $\approx 150$ bp in size) after shearing or cutting DNA (e.g. fragmenting a genome)  |
| TrEnD              | Trace and Environmental DNA laboratory, Curtin University  |



**Follow-up Report: eDNA metabarcoding for Blind Cave Eel survey in the Pilbara  
WA**

*Prepared for*

**Rio Tinto  
Level 24, 152-158 St Georges Terrace  
Perth, WA, 6000**

*Prepared by*

**Trace and Environmental DNA (TrEnD) Laboratory  
Curtin University**

13<sup>th</sup> August 2018

**Report Authors:** Dr Nicole White (TrEnD laboratory)

**Signature:**

**Dr Nicole White**

**Date: 13 August 2018**

**Disclaimer:**

The Trace and Environmental DNA (TrEnD) laboratory offers DNA services across a number of biological applications. While TrEnD stands by the validity of its work and the science that underpins it, stakeholders should use the information contained within the report at their own risk. We suggest using DNA results we report as a line of evidence in decision making processes and it may be appropriate to repeat results, re-sample at sites, corroborate data using other DNA markers or use other non-molecular methods. TrEnD accepts no liability or responsibility for it in respect of any use of or reliance upon this report by any third party. Copying this report without prior written consent of TrEnD is not permitted. © Copyright 2018 TrEnD Laboratory, Curtin University.

## Executive Summary:

### Overall Project objective:

The aim of this pilot project was to use an environmental DNA (eDNA) metabarcoding approach to determine presence/absence of Blind Cave Eel (*Ophisternon candidum*) from within and outside the Mesa H project area in the Pilbara region of Western Australia. The data was to be incorporated into an environmental impact statement (EIS) by Rio Tinto.

### Outputs:

- This report is a follow-up of the results from the EelFishCOI 'molecular magnet' designed for Blind Cave Eel (*Ophisternon candidum*) detection. This report presents the eDNA results following on from the report issued to RioTinto on the 13<sup>th</sup> July 2018 by the TrEnD lab.
- The presence of Blind Cave Eel (*Ophisternon candidum*) DNA was confirmed from two sample sites of the Robe River and bores JW021, JW024, BC186, 25, RR1 and MB17MEH0015. Blind Cave Eel DNA was not previously detected from MB17MEH0015 with the EelFish16S assay reported on 13<sup>th</sup> July 2018. Re-screening all 122 eDNA water membranes collected in June and December 2017 with Fish16S and EelFishCOI 'molecular magnets' confirms the presence of *O. candidum* from this additional bore.

### Recommendations:

- This is the first time eDNA metabarcoding methods have been implemented in the Pilbara groundwater systems. The Fish16S, EelFish16S and EelFishCOI metabarcoding assays designed by the TrEnD lab indicates that an eDNA approach is able to detect Blind Cave Eel (*Ophisternon candidum*) from the survey area as a non-invasive method. Blind Cave Eel (*Ophisternon candidum*) DNA was isolated and DNA sequenced from 6 bores sampled, in addition to two open surface water samples collected from the Robe River, which indicates eDNA metabarcoding is capable of detecting threatened species. Finally it is worth noting that these eDNA assays are performing at the limits of detection and further gains in sensitivity may be achievable with optimised sampling.
-

# 1. Introduction

---

eDNA refers to all genetic material that is recovered from environmental substrates (such as water or sediment). DNA directly from an organism (e.g. microorganisms) as well as trace amounts of DNA that are shed by all organisms is collectively termed eDNA. This preserved, but often degraded, genetic material provides a means to audit species composition and communities at a given location. When combined with recently developed DNA sequencing technologies (termed 'next generation' or 'high-throughput' sequencing), eDNA can provide a wealth of information for studies of introduced marine pests (IMP), biodiversity surveys, food web dynamics, and diet analysis.

Potential applications for monitoring using eDNA include:

- Biosecurity: to detect the presence/absence of introduced species
- Environmental Impact Assessment: baseline/ongoing monitoring and assessment; audit
- Conservation: to detect the presence/absence of threatened species

Rio Tinto has partnered with the Trace and Environmental DNA (TrEnD) Laboratory at Curtin University to develop eDNA tools for Blind Cave Eel (*Ophisternon candidum*) as a means to use DNA-based bio surveys in groundwater monitoring and management.

eDNA sampling has benefits over other traditional survey methods including:

- highly cost-effective monitoring compared to traditional survey methods
- rapid sample collection that is non destructive to habitats, flora and fauna
- non-reliance on individuals with taxonomic expertise, provision of consistent results between monitoring programs, independent of surveyors

The objectives of this project were to use an environmental DNA (eDNA) metabarcoding approach to determine the presence/absence of Blind Cave Eel (*Ophisternon candidum*) a threatened species within the groundwater systems of the Pilbara in Western Australia, specifically the Mesa H area (Rio Tinto). The data is to be incorporated into an environmental impact statement.

## 2. Methods and Samples

Please refer to report “eDNA Metabarcoding Report: Blind Cave Eel survey in the Pilbara WA” issued to RioTinto by the TrEnD lab on 13<sup>th</sup> July 2018.

## 3. Results and Discussion

Table 1 list the water samples from each bore and those for which Blind Cave Eel (*Ophisternon candidum*) were detected with the Fish16S, EelFish16S and EelFishCOI metabarcoding assays. DNA sequences obtained for *O. candidum* can be found in the Supplementary Information accompanying this report.

**Table 1:** Results of eDNA metabarcoding (Fish16S, EelFish16S, EelFishCOI) from groundwater for Blind Cave Eel (*Ophisternon candidum*) detection.

| TrEnD Sample ID | Site (bore code) | Field Trip | Ophisternon candidum DNA detected? |
|-----------------|------------------|------------|------------------------------------|
| AWFS-I17-106    | JIMWE003         | Jun-17     | No                                 |
| AWFS-I17-107    | JW021            | Jun-17     | Yes                                |
| AWFS-I17-108    | BC186            | Jun-17     | No                                 |
| AWFS-I17-109    | JW024            | Jun-17     | No                                 |
| AWFS-I17-110    | BC276            | Jun-17     | No                                 |
| AWFS-I17-111    | BC667            | Jun-17     | No                                 |
| AWFS-I17-112    | BCP001           | Jun-17     | No                                 |
| AWFS-I17-113    | BC458            | Jun-17     | No                                 |
| AWFS-I17-114    | Robe River (RR)  | Jun-17     | Yes                                |
| AWFS-I17-115    | BUNWO1108        | Jun-17     | No                                 |
| AWFS-I17-116    | BUNWB13          | Jun-17     | No                                 |
| AWFS-I17-117    | BUNWB09          | Jun-17     | No                                 |
| AWFS-I17-118    | BC401            | Jun-17     | No                                 |
| AWFS-I17-119    | BUNWB08          | Jun-17     | No                                 |
| AWFS-I17-120    | BC225            | Jun-17     | No                                 |
| AWFS-I17-121    | JIMWE004         | Jun-17     | No                                 |
| AWFS-I17-122    | BC292            | Jun-17     | No                                 |
| AWFS-I17-123    | BUNWO0773        | Jun-17     | No                                 |
| AWFS-I17-124    | PZ11BUN008       | Jun-17     | No                                 |
| AWFS-I18-001    | 25               | Dec-17     | Yes                                |
| AWFS-I18-002    | 31               | Dec-17     | No                                 |
| AWFS-I18-003    | 200/Dave Bore    | Dec-17     | No                                 |
| AWFS-I18-004    | BC186            | Dec-17     | Yes                                |
| AWFS-I18-005    | BC401            | Dec-17     | No                                 |
| AWFS-I18-006    | Budgie Bore      | Dec-17     | No                                 |
| AWFS-I18-007    | JW023            | Dec-17     | No                                 |
| AWFS-I18-008    | JW024            | Dec-17     | Yes                                |
| AWFS-I18-009    | MB16MEC0009      | Dec-17     | No                                 |
| AWFS-I18-010    | MB17MEH0008      | Dec-17     | No                                 |
| AWFS-I18-011    | MB17MEH0010      | Dec-17     | No                                 |
| AWFS-I18-012    | MB17MEH0015      | Dec-17     | Yes                                |
| AWFS-I18-013    | RR1              | Dec-17     | Yes                                |



|              |              |        |     |
|--------------|--------------|--------|-----|
| AWFS-I18-014 | MB16MEC0008  | Dec-17 | No  |
| AWFS-I18-015 | MB17MEH0007  | Dec-17 | No  |
| AWFS-I18-016 | MB17MEH0009  | Dec-17 | No  |
| AWFS-I18-017 | Control (RR) | Dec-17 | Yes |

#### 4. Findings, Recommendations and Concluding remarks

---

At the time of report “**eDNA Metabarcoding Report: Blind Cave Eel survey in the Pilbara WA**” issued to RioTinto by the TrEnD lab on 13<sup>th</sup> July 2018, the water samples identified as positive from the EelFishCOI and Fish16S metabarcoding assays had not been DNA sequenced and bioinformatically processed.

The Fish16S, EelFish16S and EelFishCOI assays utilised on bore water samples collected in June and December 2017 detected Blind Cave Eel (*Ophisternon candidum*) from two sample locations of the Robe River, in addition to bores JW021, JW024, BC186, 25, RR1 and MB17MEH0015. To our knowledge this represents the first time an eDNA based metabarcoding approach, specific for *Ophisternon candidum*, has been applied to samples from ground water system(s) in the Pilbara, Western Australia. Sequencing the mitochondrial genome of *Ophisternon candidum* (WAM P.34817-001) has provided definitive assignments of DNA sequences from eDNA groundwater samples to genus and species.

## 5. Appendices and additional information

- An electronic file titled; SupplementaryInformation\_EelFish16SandCOI\_August2018.xlsx is attached to this report.
- Additional information on sampling and methods are available from TrEnD upon request however the exact nature of the assays and workflow are proprietary.
- Below is a list of acronyms, abbreviations and definitions that may apply to aspects of this report.

| % value in data            | Represents the percentage similarity of a DNA sequence recovered from a sample compared to reference sequences in a database (e.g. compared to DNA databases such as GenBank or references generated in-house)  |
|----------------------------|---|
| (x) value in data          | Represents the frequency the % value was recorded in the dataset  |
| 18S rRNA                   | The 18S rRNA refers to a conserved gene region of nuclear DNA, which codes for a subunit of the ribosome. 18S rRNA is found in all eukaryotes making it a good candidate for DNA barcoding  |
| 18S IMS reference database | Reference 18S rRNA sequences of invasive marine species that are available in DNA databases   |
| Assay                      | In the context of metabarcoding an assay is a molecular test (using PCR) that is implemented to target a group of taxa within a mixed biological substrate. It is akin to using a magnet to selective enrich for needles (the target) in the context of a haystack (the total DNA from a sample).   |
| COI                        | The gene region that is being used as the standard barcode for almost all animal groups is a 648 base-pair region of the mitochondrial cytochrome c oxidase 1 gene ("CO1"). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. COI is not an effective barcode region in plants because it evolves too slowly, but two gene regions in the chloroplast, matK and rbcL, have been approved as the barcode regions for plants |
| CO1 IMS reference database | Reference COI sequences of invasive marine species that are available in DNA databases  |
| DNA                        | Deoxyribonucleic Acid (DNA) is the hereditary material that contains the genetic information of an organism   |
| DNA metabarcoding          | Is a genetic technique that simultaneously amplifies and sequences barcode regions (e.g. COI, 18S, 16S) of many different species in parallel   |
| eDNA                       | Environmental DNA (eDNA) refers to genetic material that is recovered from an environmental substrate (e.g. water, sediment, air)   |

|                     |  |
|---------------------|--|
| eukaryotes          | An organism where cells contain a nucleus surrounded by a membrane and has the DNA bound together by proteins (histones) into chromosomes. The cells of eukaryotes also contain an endoplasmic reticulum and numerous specialised organelles not present in prokaryotes, especially mitochondria, golgi bodies, and lysosomes  |
| Fisheries           | Department of Primary Industries and Regional Development, Fisheries Division, Aquatic Biosecurity Section   |
| GenBank             | Publically available repository of genetic information. Contains the barcode information of genes that have previously been sequenced  |
| Genome              | A genome is all the genetic material of an organism. It consists of DNA (or RNA in RNA viruses). The genome includes both the genes (the coding regions) and the noncoding DNA. In eukaryotes it refers to the genomes of the nucleus, mitochondria and chloroplasts. In prokaryotes, there is a single genome (as they do not contain mitochondria or chloroplasts) |
| Illumina MiSeq      | Next generation sequencing platform developed by the company Illumina  |
| IMP                 | Introduced marine pests  |
| IMS                 | Introduced marine species  |
| Metabarcoding assay | A PCR reaction using a specific set of primers that simultaneously amplifies the same gene target from multiple species  |
| Mitochondria        | The mitochondrion (plural mitochondria) is a double membrane-bound organelle found in all eukaryotic organisms, although some cells in some organisms may lack them (e.g. Red blood cells). It contains its own genome   |
| Mitogenomes         | Refers to the mitochondrial genome   |
| NGS                 | Next generation sequencing or second generation sequencing refers to massively parallel sequencing technology, as opposed to first generation sequencing or sanger sequencing where only a single template is sequenced at one time  |
| Nucleotide          | A compound consisting of a nucleoside linked to a phosphate group. Nucleotides form the basic structural unit of nucleic acids such as DNA   |
| PCR                 | Polymerase chain reaction (PCR) is the technique that is used to amplify (akin to photocopying DNA) specific regions of the genome from specific groups of taxa  |
| Primer              | A short DNA strand ( $\approx 20$ bp in size) used in PCR to target particular groups of organisms and genes. Two of them are required for PCR (a forward and a reverse)   |
| Primer binding site | A primer-binding site is the target region of a genome where the primer attaches to start replication. The primer binding site is on one of the two complementary strands of a double-stranded nucleotide polymer, in the strand which is to be copied, or is within a single-stranded nucleotide polymer sequence   |
| prokaryote          | Any of the typically unicellular microorganisms that lack a distinct nucleus and membrane-bound organelles and that are classified as a kingdom (Prokaryotae syn. Monera) or into two domains (Bacteria and Archaea)   |
| RNA                 | Ribonucleic acid (RNA) is a polymeric molecule implicated in various biological roles in coding, decoding, regulation, and expression of genes   |

|                    |  |
|--------------------|--|
| rRNA               | ribosomal ribonucleic acid is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms  |
| Sequence           | DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA |
| Shotgun sequencing | Refers to randomly sequencing short pieces of DNA ( $\approx 150$ bp in size) after shearing or cutting DNA (e.g. fragmenting a genome)  |
| TrEnD              | Trace and Environmental DNA laboratory, Curtin University  |





## Appendix 5

---

### WAM Morphological Identification of Troglobitic Spiders





# **WAM Report**

## **Troglobitic Spiders from Mesa H, Robe Valley, Western Australia**

**(Project Mesa H Troglobitic Spiders)**

Report to Biota

16 Mar 2017

Karen Cullen, Mark S. Harvey, Julianne Waldock

Department of Terrestrial Zoology, Western Australian Museum,  
Locked Bag 49, Welshpool DC, Western Australia 6986, Australia



*Although identifications in this report were consistent with the best available information and current scientific thinking at the time of identification the use of this report is at the risk of the user. Any liability to users of this report for loss of any kind arising out of the use of this report or the information and identifications it contains is expressly disclaimed.*

## SUMMARY

---

Specimens were submitted to the Western Australian Museum on the 13<sup>th</sup> March 2017. The project contained: A Spider from the family Oonopidae (n=1) and a spider from the family Gnaphosidae (n=1). A summary of specimen identifications together with their SRE status may be found in Table 1. A full explanation of the SRE categories used by the Western Australian Museum may be found in Appendix 1.

**Table 1. Summary of specimen identifications and SRE status.**

| ORDER   | FAMILY      | GENUS               | SPECIES             | # OF SPECIMENS | SRE STATUS    | SRE SUB-CATEGORY            |
|---------|-------------|---------------------|---------------------|----------------|---------------|-----------------------------|
| Araneae | Oonopidae   | <i>Prethopalpus</i> | `ARA052`            | 1              | Potential SRE | (E) Research and experience |
| Araneae | Gnaphosidae | Genus indet.        | `sp. indet. (juv.)` | 1              | Potential SRE | (A) Juvenile                |

## **SHORT-RANGE ENDEMISM**

---

The terrestrial invertebrate fauna of inland Australia contains a plethora of species, and just the arthropods were recently estimated to consist of more than 250,000 species (Yeates, Harvey et al. 2004; Chapman 2009). The vast majority of these are found within the Insecta and Arachnida, although significant numbers of millipedes are to be expected. For many years, the prospect of including invertebrates in assessments of biological systems subject to modification proved daunting because of the large numbers of unknown species. These animals were largely ignored, as they were too diverse and their taxonomy too little known for them to be considered in environmental surveys that require a rapid turn-around time.

In a recent publication, the issue of Short-Range Endemism in the Australian invertebrate fauna was examined (Harvey 2002). Species that could be defined as Short-Range Endemics (SRE) were those that had a naturally small range of less than 10,000 km<sup>2</sup>. Harvey (2002) found that those species possessed a series of distinct ecological and life-history traits that contributed to their limited distributions, including:

- poor powers of dispersal;
- confinement to discontinuous habitats;
- usually highly seasonal, only active during cooler, wetter periods; and
- low levels of fecundity.

A number of major invertebrate groups have a high proportion of individual species that show these traits and can be considered SRE's. The Western Australian fauna contains a number of SRE taxa, including millipedes, land snails, trap-door spiders, some pseudoscorpions, slaters, and onychophorans and these represent focal groups in Environmental Impact Assessment studies in the state (EPA 2009). The south coast region is relatively well known compared with other regions of the state (Framenau, Moir et al. 2008), but there are many poorly known species and gaps in our understanding of the distributions of many species.

## **METHODS**

---

Specimens collected by Biota were submitted to the Western Australian Museum on the 13<sup>th</sup> March 2017. The specimens were examined at the WA museum using Leica dissecting microscopes (MZ6, MZ16). The SRE status of the taxonomic group was given using the SRE categorisation system developed and implemented by the Western Australian Museum. A full explanation of the WAM SRE categories is available in Appendix 1.



## SIGNIFICANT OUTCOMES

---

This project has found one species that may have significant SRE outcomes.

The significant findings are summarised below.

### ARACHNIDA

#### ORDER ARANEAE

##### Family Oonopidae

1. *Prethopalpus* 'ARA052'

- **SRE Category: Potential SRE; (B) Habitat indicators; (E) Research and experience.**
- Examination of the adult male morphology, specifically the shape of the male pedipalp and abdominal scutae, combined with the locality would indicate that it is likely an undescribed species within *Prethopalpus* and has been allocated the WAM species code 'ARA052'.

Baehr *et al.* (2014) described 14 troglobitic *Prethopalpus* species from the arid zone of Western Australia, all of which are SREs and many known only from a single bore. Two species have been recorded from the Robe River Valley, *Prethopalpus scanloni* from bore DBRC089 and the other *P.* 'ARA051' from bore MEBRC0073, Mesa B. No specimens of this genus have yet been collected from Mesa H. This species is similar to that of *P. scanloni* in having a bulging epigastric plate in lateral view, but differs by having a dorsal scute, whereas *P. scanloni* does not. Further, given the limited distributional ability of this taxa, and its location within an isolated mesa (Mesa H), makes this species likely to be an SRE. Collections of adult troglobitic spiders are patchy and occur rarely, so this find is particularly significant.

## APPENDIX 1. WAM SHORT-RANGE ENDEMIC CATEGORIES

|                                      | Taxonomic Certainty  | Taxonomic Uncertainty  |
|--------------------------------------|--|--|
| Distribution < 10 000km <sup>2</sup> | <b>Confirmed SRE</b> <ul style="list-style-type: none"> <li>• A known distribution of &lt; 10 000km<sup>2</sup>.</li> <li>• The taxonomy is well known.</li> <li>• The group is well represented in collections and/ or via comprehensive sampling.</li> </ul>           | <b>Potential SRE</b> <ul style="list-style-type: none"> <li>• Patchy sampling has resulted in incomplete knowledge of the geographic distribution of the group.</li> <li>• We have incomplete taxonomic knowledge.</li> <li>• The group is not well represented in collections.</li> <li>• This category is most applicable to situations where there are gaps in our knowledge of the taxon.</li> </ul> |
| Distribution > 10 000km <sup>2</sup> | <b>Widespread (not an SRE)</b> <ul style="list-style-type: none"> <li>• A known distribution of &gt; 10 000km<sup>2</sup>.</li> <li>• The taxonomy is well known.</li> <li>• The group is well represented in collections and/ or via comprehensive sampling.</li> </ul> | <b>Sub-categories for this SRE designation are outlined below</b>  |

### SRE SUB-CATEGORIES

If a taxon is determined to be a "Potential SRE", the following sub-categories will further elucidate this status.

#### A. Data Deficient:

- There is insufficient data available to determine SRE status.
- Factors that fall under this category include:
  - New species.
  - Lack of geographic information.
  - Lack of taxonomic information.
  - The group may be poorly represented in collections.
  - The individuals sampled (e.g. juveniles) may prevent identification to species level.

#### B. Habitat Indicators:

- It is becoming increasingly clear that habitat data can elucidate SRE status.
- Where habitat is known to be associated with SRE taxa and vice versa, it will be noted here.

#### C. Morphology Indicators:

- A suite of morphological characters are characteristic of SRE taxa.
- Where morphological characters are known to be associated with SRE taxa and vice-versa, it will be noted here.

#### D. Molecular Evidence:

- If molecular work has been done on this taxon (or a close relative), it may reveal patterns congruent or incongruent with SRE status.

#### E. Research & Expertise:

- Previous research and/ or WAM expertise elucidates taxon SRE status.
- This category takes into account the expert knowledge held within the WAM.

**APPENDIX 2. SPECIMEN DATA FOR [TAXA] COLLECTED FROM [GEOGRAPHIC LOCATION]**

| REGNO  | FLDNO                      | ORDER   | INFRAORDER    | FAMILY      | GENUS               | SPECIES             | AUTHORITY | STATE | SITE                               | LATITUDE   | LONGITUDE   | M | F | JUV | TOT |
|--------|----------------------------|---------|---------------|-------------|---------------------|---------------------|-----------|-------|------------------------------------|------------|-------------|---|---|-----|-----|
| 142823 | RC16JIM0019.20161027-01    | Araneae | Araneomorphae | Oonopidae   | <i>Prethopalpus</i> | `ARA052`            |           | W.A.  | Mesa H, ca. 18 km SSE. Pannawonica | 21°47'22"S | 116°15'18"E | 1 |   |     | 1   |
| 142824 | RC14MEH0270.20161212.T4-02 | Araneae | Araneomorphae | Gnaphosidae |                     | `sp. indet. (juv.)` |           | W.A.  | Mesa H, ca. 18 km SE. Pannawonica  | 21°45'50"S | 116°12'38"E |   |   | 1   | 1   |

## BIBLIOGRAPHY

- Chapman, A. D. (2009). Numbers of Living Species in Australia and the World. 2nd Edition. Toowoomba, Australian Biodiversity Information Services: 84 pp.
- Baehr, B.C., M.S. Harvey, M. Burger, M. Thoma (2012). "The New Australian Goblin Spider Genus *Prethopalpus* (Araneae, Oonopidae)". Bulletin of the American Museum of Natural History 369: 1- 113.
- EPA (2009). Guidance for the Assessment of Environmental Factors (in accordance with the Environmental Protection Act 1986). Sampling of Short Range Invertebrate Fauna for Environmental Impact Assessment in Western Australia. No. 20. Perth: 31 pp.
- Framenau, V. W., M. L. Moir, et al. (2008). Terrestrial Invertebrates of the South Coast NRM Region of Western Australia: Short-range Endemics in Gondwanan Relictual Habitats. Report to South Coast NRM. Welshpool, Western Australian Museum: 184pp.
- Harvey, M. S. (2002). "Short-range endemism among the Australian fauna: some examples from non-marine environments." Invertebrate Systematics 16: 555-570.
- Yeates, D. K., M. S. Harvey, et al. (2004). "New estimates for terrestrial arthropod species-richness in Australia." Records of the South Australian Museum, Monograph Series 7: 231-241.





# Appendix 6



## Fauna Recorded





| Site        | Phase      | Easting    | Northing    | Date     | Phylum     | Class        | Order      | Family          | Genus           | Species      | n  | Field code               | WAM registration | Preservation | Identifier       | Identification method | Lineage     |
|-------------|------------|------------|-------------|----------|------------|--------------|------------|-----------------|-----------------|--------------|----|--------------------------|------------------|--------------|------------------|-----------------------|-------------|
| DD13MEH0007 | Phase 1    | 419635.017 | 7593002.209 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20151024-01B | C60931           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| DD13MEH0007 | Phase 1    | 419635.017 | 7593002.209 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20151024-01C | C60932           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| DD13MEH0007 | Phase 1    | 419635.017 | 7593002.209 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20151024-01D | C60933           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| DD13MEH0007 | Phase 1    | 419635.017 | 7593002.209 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20151024-01E | C60934           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| DD13MEH0007 | Phase 1    | 419635.017 | 7593002.209 | 24/10/15 | Arthropoda | Maxillopoda  | Cyclopoida | Cyclopidae      | Diacyclops      | humphreysi   | 10 | DD13MEH000720151024-02   |                  |              | Stuart Halse     | Morphologic           |             |
| DD13MEH0007 | Phase 1    | 419635.017 | 7593002.209 | 24/10/15 | Arthropoda | Maxillopoda  | Calanoida  | Ridgewayiidae   | Stygoridgewayia | trispinosa   | 10 | DD13MEH000720151024-02   |                  |              | Jane McRae       | Morphologic           |             |
| DD13MEH0007 | Phase 1    | 419635.017 | 7593002.209 | 24/10/15 | Arthropoda | Amphipoda    | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20151024-01A | C60930           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC13MEH0007 | Phase 1    | 419641.545 | 7592602.94  | 24/10/15 | Arthropoda | Maxillopoda  | Cyclopoida | Cyclopidae      | Diacyclops      | humphreysi   | 10 | RC13MEH000720151024-01   |                  |              | Stuart Halse     | Morphologic           |             |
| RC13MEH0007 | Phase 1    | 419641.545 | 7592602.94  | 24/10/15 | Arthropoda | Maxillopoda  | Calanoida  | Ridgewayiidae   | Stygoridgewayia | trispinosa   | 14 | RC13MEH000720151024-02   |                  |              | Jane McRae       | Morphologic           |             |
| RC13MEH0007 | Phase 1    | 419641.545 | 7592602.94  | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae   |                 | sp. 'AMP035' | 1  | RC13MEH0007-20151024-03  | C60949           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMP035 |
| RC13MEH0041 | Phase 1    | 419940.332 | 7592705.239 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC13MEH0041-20151024-01  | C60951           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC13MEH0097 | Phase 1    | 420085.762 | 7592803.224 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC13MEH0097-20151024-01C | C60954           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC13MEH0097 | Phase 1    | 420085.762 | 7592803.224 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM026' | 1  | RC13MEH0097-20151024-01A | C60952           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM026 |
| RC13MEH0097 | Phase 1    | 420085.762 | 7592803.224 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC13MEH0097-20151024-01B | C60953           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0018 | Phase 1    | 419087.88  | 7593298.882 | 25/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0018-20151025-01  | C60955           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0053 | Phase 1    | 419091.838 | 7592752.187 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0053-20151024-01B | C60963           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0053 | Phase 1    | 419091.838 | 7592752.187 | 24/10/15 | Arthropoda | Maxillopoda  | Calanoida  | Ridgewayiidae   | Stygoridgewayia | trispinosa   | 9  | RC14MEH005320151024-02   |                  |              | Jane McRae       | Morphologic           |             |
| RC14MEH0053 | Phase 1    | 419091.838 | 7592752.187 | 24/10/15 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0053-20151024-01A | C60962           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| DD13MEH0007 | Phase 2    | 419635.017 | 7593002.209 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20160119-01B | C60937           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| DD13MEH0007 | Phase 2    | 419635.017 | 7593002.209 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20160119-01C | C60938           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| DD13MEH0007 | Phase 2    | 419635.017 | 7593002.209 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20160119-01D | C60939           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| DD13MEH0007 | Phase 2    | 419635.017 | 7593002.209 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20160119-01E | C60940           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| DD13MEH0007 | Phase 2    | 419635.017 | 7593002.209 | 19/1/16  | Nematoda   |              |            |                 |                 | sp.          | 1  |                          | C8560            | 100% Ethanol | Penny Brooshooft | Morphologic           |             |
| DD13MEH0007 | Phase 2    | 419635.017 | 7593002.209 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | DD13MEH0007-20160119-01A | C60936           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC12IJM0019 | Phase 2    | 421395.839 | 7591472.302 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC12IJM0019-20160119-01B | C60943           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC12IJM0019 | Phase 2    | 421395.839 | 7591472.302 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC12IJM0019-20160119-01D | C60945           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC12IJM0019 | Phase 2    | 421395.839 | 7591472.302 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC12IJM0019-20160119-01E | C60946           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC12IJM0019 | Phase 2    | 421395.839 | 7591472.302 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  |                 |                 | sp.          | 1  | RC12IJM0019-20160119-01C | C60944           | 100% Ethanol | Nicola Watson    | Morphologic           | Failed      |
| RC12IJM0019 | Phase 2    | 421395.839 | 7591472.302 | 19/1/16  | Nematoda   |              |            |                 |                 | sp.          | 1  |                          | C8661            | 100% Ethanol | Penny Brooshooft | Morphologic           |             |
| RC12IJM0019 | Phase 2    | 421395.839 | 7591472.302 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC12IJM0019-20160119-01A | C60942           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC13MEH0040 | Phase 2    | 419892.868 | 7592704.088 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC13MEH0040-20160119-01B | C60948           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC13MEH0040 | Phase 2    | 419892.868 | 7592704.088 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC13MEH0040-20160119-01A | C60947           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC13MEH0041 | Phase 2    | 419940.332 | 7592705.239 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae   |                 | sp. 'AMP003' | 1  | RC13MEH0041-20160119-01  | C60950           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMP003 |
| RC14MEH0018 | Phase 2    | 419087.88  | 7593298.882 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0018-20160119-01B | C60958           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0018 | Phase 2    | 419087.88  | 7593298.882 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0018-20160119-01C | C60959           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0018 | Phase 2    | 419087.88  | 7593298.882 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0018-20160119-01D | C60960           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0018 | Phase 2    | 419087.88  | 7593298.882 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0018-20160119-01E | C60961           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0018 | Phase 2    | 419087.88  | 7593298.882 | 19/1/16  | Arthropoda | Maxillopoda  | Calanoida  | Ridgewayiidae   | Stygoridgewayia | trispinosa   | 1  | RC14MEH0018-20160119-02  |                  |              | Jane McRae       | Morphologic           |             |
| RC14MEH0018 | Phase 2    | 419087.88  | 7593298.882 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0018-20160119-01A | C60957           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0053 | Phase 2    | 419091.838 | 7592752.187 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0053-20160119-01B | C60966           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0053 | Phase 2    | 419091.838 | 7592752.187 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0053-20160119-01C | C60967           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0053 | Phase 2    | 419091.838 | 7592752.187 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0053-20160119-01D | C60968           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0053 | Phase 2    | 419091.838 | 7592752.187 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0053-20160119-01E | C60969           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| RC14MEH0053 | Phase 2    | 419091.838 | 7592752.187 | 19/1/16  | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | RC14MEH0053-20160119-01A | C60965           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
| Dave Bore   | Phase 3    | 392512     | 7607436     | 5/5/16   | Arthropoda | Malacostraca | Amphipoda  | Neoniphargiidae |                 | sp. 'AMN008' | 1  | 200-20160505-02B         | C70336           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMN008 |
| Dave Bore   | Phase 3    | 392512     | 7607436     | 5/5/16   | Arthropoda | Malacostraca | Amphipoda  | Neoniphargiidae |                 | sp. 'AMN008' | 1  | 200-20160505-02C         | C70337           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMN008 |
| Dave Bore   | Phase 3    | 392512     | 7607436     | 5/5/16   | Arthropoda | Malacostraca | Amphipoda  | Neoniphargiidae |                 | sp. 'AMN008' | 1  | 200-20160505-02D         | C70338           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMN008 |
| Dave Bore   | Phase 3    | 392512     | 7607436     | 5/5/16   | Arthropoda | Malacostraca | Amphipoda  | Neoniphargiidae |                 | sp. 'AMN008' | 1  | 200-20160505-02E         | C70339           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMN008 |
| Dave Bore   | Phase 3    | 392512     | 7607436     | 5/5/16   | Arthropoda | Ostracoda    | Podocopida | Candonidae      | Pilbaracandona  | sp. 'BOS526' | 1  | 200-20160505-01          | C70410           | 100% Ethanol | Stuart Halse     | Morphologic           |             |
| Dave Bore   | Phase 3    | 392512     | 7607436     | 5/5/16   | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM004' | 1  | 200-20160505-02A         | C70335           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM004 |
|             | 31 Phase 3 | 432924.921 | 7603180.965 | 7/5/16   | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | 3120160507-03B           | C70326           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
|             | 31 Phase 3 | 432924.921 | 7603180.965 | 7/5/16   | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | 3120160507-03C           | C70327           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
|             | 31 Phase 3 | 432924.921 | 7603180.965 | 7/5/16   | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | 3120160507-03D           | C70328           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
|             | 31 Phase 3 | 432924.921 | 7603180.965 | 7/5/16   | Arthropoda | Maxillopoda  | Cyclopoida | Cyclopidae      | Halicyclops     | rochai       | 2  | 3120160507-01            |                  |              | Jane McRae       | Morphologic           |             |
|             | 31 Phase 3 | 432924.921 | 7603180.965 | 7/5/16   | Arthropoda | Maxillopoda  | Calanoida  | Ridgewayiidae   | Stygoridgewayia | trispinosa   | 4  | 3120160507-01            |                  |              | Jane McRae       | Morphologic           |             |
|             | 31 Phase 3 | 432924.921 | 7603180.965 | 7/5/16   | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM026' | 1  | 3120160507-03A           | C70325           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM026 |
|             | 32 Phase 3 | 432449.703 | 7602669.071 | 6/5/16   | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | 32-20160506-01B          | C70341           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
|             | 32 Phase 3 | 432449.703 | 7602669.071 | 6/5/16   | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | 32-20160506-01C          | C70342           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |
|             | 32 Phase 3 | 432449.703 | 7602669.071 | 6/5/16   | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae     | Nedsia          | sp. 'AMM001' | 1  | 32-20160506-01D          | C70343           | 100% Ethanol | Terrie Finston   | Molecular             | HelixAMM001 |

| Site      | Phase | Easting | Northing   | Date        | Phylum  | Class           | Order        | Family          | Genus          | Species          | n            | Field code | WAM registration | Preservation | Identifier   | Identification method | Lineage     |             |
|-----------|-------|---------|------------|-------------|---------|-----------------|--------------|-----------------|----------------|------------------|--------------|------------|------------------|--------------|--------------|-----------------------|-------------|-------------|
|           | 32    | Phase 3 | 432449.703 | 7602669.071 | 6/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 32-20160506-01E  | C70344       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 32    | Phase 3 | 432449.703 | 7602669.071 | 6/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Neoniphargidae |                  | sp. 802      | 1          | 32-20160506-02   |              |              | Jane McRae            | Morphologic |             |
|           | 32    | Phase 3 | 432449.703 | 7602669.071 | 6/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 32-20160506-01A  | C70340       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 34    | Phase 3 | 432417.972 | 7602437.654 | 6/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 34-20160506-01   | C70324       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 87    | Phase 3 | 410739     | 7604138     | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160505-02B  | C70330       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
|           | 87    | Phase 3 | 410739     | 7604138     | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160505-02C  | C70331       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
|           | 87    | Phase 3 | 410739     | 7604138     | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160505-02D  | C70332       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
|           | 87    | Phase 3 | 410739     | 7604138     | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160505-02E  | C70333       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
|           | 87    | Phase 3 | 410739     | 7604138     | 5/5/16  | Arthropoda      | Ostracoda    | Podocopida      | Candonidae     | Humphreyscandona | imperfecta   | 1          | 87-20160505-01   | C70409       | 100% Ethanol | Staurt Halse          | Morphologic |             |
|           | 87    | Phase 3 | 410739     | 7604138     | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160505-02A  | C70329       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | RR120160505-01B  | C70346       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | RR120160505-01C  | C70347       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       |                |                  | sp.          | 1          | RR120160505-01D  | C70348       | 100% Ethanol | Nicola Watson         | Morphologic | Failed      |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       |                |                  | sp.          | 1          | RR120160505-01E  | C70394       | 100% Ethanol | Nicola Watson         | Morphologic | Failed      |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Ostracoda    | Podocopida      | Candonidae     | Pierrecandona    | sp. 'BOS576' | 1          | RR1201605-06B    | C70408       | 100% Ethanol | Stuart Halse          | Morphologic |             |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Malacostraca | Isopoda         | Cirolanidae    | Haptolana        | yarraloola   | 1          | RR120160505-04   |              |              | Jane McRae            | Morphologic |             |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Malacostraca | Isopoda         | Cirolanidae    | Kagalana         | tonde        | 1          | RR120160505-04   |              |              | Jane McRae            | Morphologic |             |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Maxillopoda  | Calanoida       | Ridgewayiidae  | Stygoridgewayia  | trispinosa   | 10         | RR120160505-05   |              |              | Jane McRae            | Morphologic |             |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Maxillopoda  | Cyclopoida      | Cyclopidae     | Halicyclops      | rochai       | 2          | RR120160505-05   |              |              | Jane McRae            | Morphologic |             |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Maxillopoda  | Cyclopoida      | Cyclopidae     | Diacyclops       | cockingi     | 1          | RR120160505-05   | C70412       | 100% Ethanol | Jane McRae            | Morphologic |             |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Ostracoda    | Podocopida      | Candonidae     |                  | sp. 'BOS577' | 3          | RR120160505-06A  | C70407       | 100% Ethanol | Stuart Halse          | Morphologic |             |
| RR1       |       | Phase 3 | 419178.96  | 7597902.88  | 5/5/16  | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | RR120160505-01A  | C70345       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
| Dave Bore |       | Phase 4 | 392512     | 7607436     | 10/9/16 | Arthropoda      | Ostracoda    | Podocopida      | Candonidae     | Pilbaracandona   | sp. 'BOS526' | 1          | 200-20160910-02  | C70415       | 100% Ethanol | Stuart Halse          | Morphologic |             |
| Dave Bore |       | Phase 4 | 392512     | 7607436     | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM031' | 1          | 200-20160910-01A | C70360       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM031 |
| Dave Bore |       | Phase 4 | 392512     | 7607436     | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 200-20160910-01B | C70361       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
| Dave Bore |       | Phase 4 | 392512     | 7607436     | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Neoniphargidae |                  | sp. 'AMN008' | 1          | 200-20160910-01C | C70362       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMN008 |
| Dave Bore |       | Phase 4 | 392512     | 7607436     | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM031' | 1          | 200-20160910-01D |              |              | Terrie Finston        | Molecular   | HelixAMM031 |
|           | 25    | Phase 4 | 432150.74  | 7602224.467 | 10/9/16 | Arthropoda      | Malacostraca | Thermosbaenacea | Halosbaenidae  | Halosbaena       | tulki        | 1          | 25-20160910-02   | C70349       | 100% Ethanol | Jane McRae            | Morphologic |             |
|           | 25    | Phase 4 | 432150.74  | 7602224.467 | 10/9/16 | Arthropoda      | Maxillopoda  | Calanoida       | Ridgewayiidae  | Stygoridgewayia  | trispinosa   | 1          | 25-20160910-03   | C70398       | 100% Ethanol | Jane McRae            | Morphologic |             |
|           | 25    | Phase 4 | 432150.74  | 7602224.467 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 25-20160910-01A  | C70371       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 25    | Phase 4 | 432150.74  | 7602224.467 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 25-20160910-01B  | C70372       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 25    | Phase 4 | 432150.74  | 7602224.467 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Neoniphargidae | Wesniphargus     | sp. 'AMN004' | 1          | 25-20160910-01C  |              |              | Terrie Finston        | Molecular   | HelixAMN004 |
|           | 31    | Phase 4 | 432924.921 | 7603180.965 | 10/9/16 | Arthropoda      | Maxillopoda  | Calanoida       | Ridgewayiidae  | Stygoridgewayia  | trispinosa   | 18         | 31-20160910-02   | C70401       | 100% Ethanol | Jane McRae            | Morphologic |             |
|           | 31    | Phase 4 | 432924.921 | 7603180.965 | 10/9/16 | Arthropoda      | Malacostraca | Thermosbaenacea | Halosbaenidae  | Halosbaena       | tulki        | 16         | 31-20160910-03   | C70351       | 100% Ethanol | Jane McRae            | Morphologic |             |
|           | 31    | Phase 4 | 432924.921 | 7603180.965 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       |                |                  | sp.          | 17         | 31-20160910-04   |              |              | Penny Brooshooft      | Morphologic |             |
|           | 31    | Phase 4 | 432924.921 | 7603180.965 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 31-20160910-04A  | C70363       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 31    | Phase 4 | 432924.921 | 7603180.965 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 31-20160910-04B  | C70364       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 31    | Phase 4 | 432924.921 | 7603180.965 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 31-20160910-04C  | C70365       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 31    | Phase 4 | 432924.921 | 7603180.965 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 31-20160910-04D  | C70366       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 31    | Phase 4 | 432924.921 | 7603180.965 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 31-20160910-04E  | C70367       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 31    | Phase 4 | 432924.921 | 7603180.965 | 10/9/16 | Platyhelminthes |              |                 |                |                  | sp.          | 8          | 31-20160910-01   | V9010        | 100% Ethanol | Penny Brooshooft      | Morphologic |             |
|           | 32    | Phase 4 | 432449.703 | 7602669.071 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 32-20160910-02A  | C70379       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 32    | Phase 4 | 432449.703 | 7602669.071 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Neoniphargidae |                  | sp. 'AMN003' | 1          | 32-20160910-02B  | C70380       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMN003 |
|           | 32    | Phase 4 | 432449.703 | 7602669.071 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 32-20160910-02C  |              |              | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 32    | Phase 4 | 432449.703 | 7602669.071 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 32-20160910-02D  |              |              | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 32    | Phase 4 | 432449.703 | 7602669.071 | 10/9/16 | Platyhelminthes |              |                 |                |                  | sp.          | 1          | 32-20160910-03   | V9009        | 100% Ethanol | Penny Brooshooft      | Morphologic |             |
|           | 32    | Phase 4 | 432449.703 | 7602669.071 | 10/9/16 | Arthropoda      | Maxillopoda  | Calanoida       | Ridgewayiidae  | Stygoridgewayia  | trispinosa   | 5          | 32-20160910-01   | C70399       | 100% Ethanol | Jane McRae            | Morphologic |             |
|           | 34    | Phase 4 | 432417.972 | 7602437.654 | 10/9/16 | Arthropoda      | Maxillopoda  | Cyclopoida      | Cyclopidae     | Diacyclops       | sp.          | 2          | 34-20160910-02A  | C70402       | 100% Ethanol | Jane McRae            | Morphologic |             |
|           | 34    | Phase 4 | 432417.972 | 7602437.654 | 10/9/16 | Arthropoda      | Maxillopoda  | Calanoida       | Ridgewayiidae  | Stygoridgewayia  | trispinosa   | 2          | 34-20160910-02B  | C70403       | 100% Ethanol | Jane McRae            | Morphologic |             |
|           | 34    | Phase 4 | 432417.972 | 7602437.654 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM001' | 1          | 34-20160910-01A  | C70368       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM001 |
|           | 34    | Phase 4 | 432417.972 | 7602437.654 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       |                |                  | sp.          | 1          | 34-20160910-01B  | C70369       | 100% Ethanol | Nicola Watson         | Morphologic | Failed      |
|           | 34    | Phase 4 | 432417.972 | 7602437.654 | 10/9/16 | Arthropoda      | Malacostraca | Amphipoda       |                |                  | sp.          | 1          | 34-20160910-01C  | C70370       | 100% Ethanol | Nicola Watson         | Morphologic | Failed      |
|           | 87    | Phase 4 | 410739     | 7604138     | 19/9/16 | Arthropoda      | Malacostraca | Amphipoda       |                |                  | sp.          | 14         | 87-20160919-02   |              |              | Penny Brooshooft      | Morphologic |             |
|           | 87    | Phase 4 | 410739     | 7604138     | 19/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160919-02A  | C70355       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
|           | 87    | Phase 4 | 410739     | 7604138     | 19/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160919-02B  | C70356       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
|           | 87    | Phase 4 | 410739     | 7604138     | 19/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160919-02C  | C70357       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
|           | 87    | Phase 4 | 410739     | 7604138     | 19/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160919-02D  | C70358       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
|           | 87    | Phase 4 | 410739     | 7604138     | 19/9/16 | Arthropoda      | Malacostraca | Amphipoda       | Eriopisidae    | Nedsia           | sp. 'AMM004' | 1          | 87-20160919-02E  | C70359       | 100% Ethanol | Terrie Finston        | Molecular   | HelixAMM004 |
|           | 87    | Phase 4 | 410739     | 7604138     | 19/9/16 | Arthropoda      | Ostracoda    | Podocopida      | Candonidae     | Humphreyscandona | imperfecta   | 2          | 87-20160919-03   | C70414       | 100% Ethanol | Stuart Halse          | Morphologic |             |





| Site        | Phase   | Easting | Northing | Date     | Phylum     | Class        | Order     | Family         | Genus  | Species      | n | Field code         | WAM registration | Preservation | Identifier     | Identification method | Lineage     |
|-------------|---------|---------|----------|----------|------------|--------------|-----------|----------------|--------|--------------|---|--------------------|------------------|--------------|----------------|-----------------------|-------------|
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda | Eriopisidae    | Nedsia | sp. 'AMM001' | 1 | Budgie-20171212-01 |                  |              | Terrie Finston | Molecular             | HelixAMM001 |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda | Neoniphargidae |        | sp. 'AMM008' | 1 | Budgie-20171212-01 |                  |              | Terrie Finston | Molecular             | HelixAMN008 |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda | Neoniphargidae |        | sp. 'AMN008' | 1 | Budgie-20171212-01 |                  |              | Terrie Finston | Molecular             | HelixAMN008 |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda | Neoniphargidae |        | sp. 'AMN008' | 1 | Budgie-20171212-01 |                  |              | Terrie Finston | Molecular             | HelixAMN008 |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda | Neoniphargidae |        | sp. 'AMN008' | 1 | Budgie-20171212-01 |                  |              | Terrie Finston | Molecular             | HelixAMN008 |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda | Neoniphargidae |        | sp. 'AMN008' | 1 | Budgie-20171212-01 |                  |              | Terrie Finston | Molecular             | HelixAMN008 |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda | Neoniphargidae |        | sp. 'AMN008' | 1 | Budgie-20171212-01 |                  |              | Terrie Finston | Molecular             | HelixAMN008 |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda | Paramelitidae  |        | sp. 'AMP009' | 1 | Budgie-20171212-01 |                  |              | Terrie Finston | Molecular             | HelixAMN009 |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  | 7615587  | 12/12/17 | Arthropoda | Malacostraca | Amphipoda |                |        | sp.          | 1 | Budgie-20171212-01 |                  |              | J Alexander    | Morphologic           | Failed      |
| Budgie Bore | Phase 5 | 382495  |          |          |            |              |           |                |        |              |   |                    |                  |              |                |                       |             |



| Site | Phase   | Easting   | Northing   | Date     | Phylum     | Class        | Order      | Family        | Genus           | Species      | n | Field code       | WAM registration | Preservation | Identifier     | Identification method | Lineage     |
|------|---------|-----------|------------|----------|------------|--------------|------------|---------------|-----------------|--------------|---|------------------|------------------|--------------|----------------|-----------------------|-------------|
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae   | Nedsia          | sp. 'AMM001' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMM001 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae   | Nedsia          | sp. 'AMM001' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMM001 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae   | Nedsia          | sp. 'AMM001' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMM001 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Eriopisidae   | Nedsia          | sp. 'AMM001' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMM001 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae |                 | sp. 'AMP035' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMP035 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae |                 | sp. 'AMP035' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMP035 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae |                 | sp. 'AMP035' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMP035 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae |                 | sp. 'AMP035' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMP035 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae |                 | sp. 'AMP035' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMP035 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae |                 | sp. 'AMP035' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMP035 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae | Chydaekata      | sp. 'AMP036' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMP036 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  | Paramelitidae |                 | sp. 'AMP038' | 1 | RR01-20171214-05 |                  |              | Terrie Finston | Molecular             | HelixAMP038 |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  |               |                 | sp.          | 1 | RR01-20171214-05 |                  |              | J Alexander    | Morphologic           | Failed      |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  |               |                 | sp.          | 1 | RR01-20171214-05 |                  |              | J Alexander    | Morphologic           | Failed      |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Amphipoda  |               |                 | sp.          | 1 | RR01-20171214-05 |                  |              | J Alexander    | Morphologic           | Failed      |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Malacostraca | Isopoda    | Cirolanidae   | Kagalana        | tonde        | 1 | RR01-20171214-04 |                  |              | J Alexander    | Morphologic           |             |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Maxillopoda  | Calanoida  | Ridgewayiidae | Stygoridgewayia | trispinosa   | 1 | RR01-20171214-01 |                  |              | J Alexander    | Morphologic           |             |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Maxillopoda  | Cyclopoida | Cyclopidae    | Diacyclops      | humphreysi   | 1 | RR01-20171214-01 |                  |              | J Alexander    | Morphologic           |             |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Maxillopoda  | Cyclopoida | Cyclopidae    | Diacyclops      | sp.          | 2 | RR01-20171214-01 |                  |              | J Alexander    | Morphologic           |             |
| RR1  | Phase 5 | 419178.96 | 7597902.88 | 14/12/17 | Arthropoda | Maxillopoda  | Cyclopoida | Cyclopidae    | Halicyclops     | rochai       | 2 | RR01-20171214-01 |                  |              | J Alexander    | Morphologic           |             |

| Site        | Phase   | Eastin      | Northing    | Date     | Phylum     | Class        | Order             | Family        | Genus           | Species             | n | Field code                  | WAM registration | Preservation | Identifier       | Identification method | Lineage     |
|-------------|---------|-------------|-------------|----------|------------|--------------|-------------------|---------------|-----------------|---------------------|---|-----------------------------|------------------|--------------|------------------|-----------------------|-------------|
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           | Armadiillidae |                 | sp 'ISA056/ISA057'  | 1 | MEHRD0577.20170130.T2-01    | C70386           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISA057 |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           | Armadiillidae |                 | sp 'ISA056/ISA057'  | 1 | MEHRD0577.20170130.T2-01    |                  |              | Terrie Finston   | Molecular             | HelixISA057 |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           | Armadiillidae |                 | sp 'ISA056/ISA057'  | 1 | MEHRD0577.20170130.T3-01    |                  |              | Terrie Finston   | Molecular             | HelixISA057 |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           | Armadiillidae |                 | sp 'ISA056/ISA057'  | 1 | MEHRD0577.20170130.T3-01    |                  |              | Terrie Finston   | Molecular             | HelixISA057 |
| MEHRD0834   | Phase 6 | 418837.0531 | 7592555.134 | 20170130 | Arthropoda | Malacostraca | Isopoda           | Armadiillidae |                 | sp 'ISA056/ISA057'  | 1 | MEHRD0834.20170130.T1-01    | C70391           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISA057 |
| MEHRD0834   | Phase 6 | 418837.0531 | 7592555.134 | 20170130 | Arthropoda | Malacostraca | Isopoda           | Armadiillidae |                 | sp 'ISA056/ISA057'  | 1 | MEHRD0834.20170130.T1-01    | C70392           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISA057 |
| MEHRD0834   | Phase 6 | 418837.0531 | 7592555.134 | 20170130 | Arthropoda | Malacostraca | Isopoda           | Armadiillidae |                 | sp 'ISA056/ISA057'  | 1 | MEHRD0834.20170130.T1-01    |                  |              | Terrie Finston   | Molecular             | HelixISA057 |
| MEHRD0834   | Phase 6 | 418837.0531 | 7592555.134 | 20170130 | Arthropoda | Malacostraca | Isopoda           | Armadiillidae |                 | sp 'ISA056/ISA057'  | 1 | MEHRD0834.20170130.T2-01    | C70393           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISA057 |
| RC15MEH0329 | Phase 1 | 415438.227  | 7596951.285 | 20151210 | Arthropoda | Arachnida    | Pseudoscorpiones  | Atemnidae     |                 | sp. 'PA004'         | 1 | RC15MEH0329.20151210-T3-03  | T143625          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPA004  |
| RC16JIM0026 | Phase 5 | 420040.245  | 7591853.293 | 20161212 | Arthropoda | Arachnida    | Pseudoscorpiones  | Olpiidae      | Beierolpium     | sp. 'PO014'         | 1 | RC16JIM0026.20161212.T2-01  | T142827          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPO014  |
| RC16MEH0433 | Phase 5 | 415439.259  | 7597649.364 | 20161212 | Arthropoda | Arachnida    | Pseudoscorpiones  | Olpiidae      | Beierolpium     | sp. 'PO015'         | 1 | RC16MEH0433.20161211.T1-02  | T142828          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPO015  |
| GR15MEH0032 | Phase 3 | 419071.543  | 7594646.228 | 20160628 | Arthropoda | Entognatha   | Diplura           | Campodeidae   |                 | sp. 'DCA005'        | 1 | GR15MEH0032.20160628.T3-2   | E84290           | 100% Ethanol | Terrie Finston   | Molecular             | HelixDCA005 |
| MEHRD0834   | Phase 6 | 418837.0531 | 7592555.134 | 20170130 | Arthropoda | Arachnida    | Pseudoscorpiones  | Chthoniidae   |                 | sp. 'PC055'         | 1 | MEHRD0834.20170130.T2-02    | TT142829         | 100% Ethanol | Terrie Finston   | Molecular             | HelixPC055  |
| GR15MEH0015 | Phase 3 | 416033.7    | 7596749.003 | 20160627 | Arthropoda | Arachnida    | Pseudoscorpiones  | Chthoniidae   |                 | sp.'PC014/PC015'    | 1 | GR15MEH0015.20160627.T2-03  | T142833          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPC014  |
| RC15MEH0315 | Phase 3 | 415737.557  | 7596649.798 | 20160627 | Arthropoda | Arachnida    | Pseudoscorpiones  | Chthoniidae   |                 | sp.'PC014/PC015'    | 1 | RC15MEH0315.20160627.T2-02  | T143809          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPC015  |
| RC15MEH0315 | Phase 3 | 415737.557  | 7596649.798 | 20160627 | Arthropoda | Arachnida    | Pseudoscorpiones  | Chthoniidae   |                 | sp.'PC014/PC015'    | 1 | RC15MEH0315.20160627.T3-02  | T143810          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPC015  |
| RC15MEH0335 | Phase 3 | 415234.691  | 7597151.756 | 20160627 | Arthropoda | Arachnida    | Pseudoscorpiones  | Chthoniidae   |                 | sp.'PC014/PC015'    | 1 | RC15MEH0335.20160627.T1-01  | T143811          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPC015  |
| RC14MEH0388 | Phase 3 | 418087.615  | 7593503.838 | 20160628 | Arthropoda | Chilopoda    | Scolopendromorpha | Cryptopidae   |                 | sp. 'SC18'          | 1 | RC14MEH0388.20160628.T2-2   | T143812          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSC18   |
| RC15MEH0114 | Phase 2 | 416434.05   | 7594352.361 | 20160118 | Arthropoda | Entognatha   | Diplura           |               |                 | sp.                 | 1 | RC15MEH0114.20160118.T2-03  | E84292           | 100% Ethanol | Penny Brooshooft | Morphological         | Failed      |
| RC16MEH0264 | Phase 6 | 417476.021  | 7594964.208 | 20170130 | Arthropoda | Entognatha   | Diplura           |               |                 | sp.                 | 1 | RC16MEH0264.20170130.T3-01  | E84288           | 100% Ethanol | Nicola Watson    | Morphological         | Failed      |
| RC16MEH0270 | Phase 5 | 418380.862  | 7593101.951 | 20161212 | Arthropoda | Arachnida    | Araneae           | Gnaphosidae   |                 | sp.                 | 1 | RC14MEH0270.20161212.T4-02  | T142824          | 100% Ethanol | Karen Cullen     | Morphological         |             |
| DD13MEH0007 | Phase 1 | 419635.017  | 7593002.209 | 20151023 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  |                 | sp. 'SCH015/SCH016' | 1 | DD13MEH0007.20160119-03     | T143628          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH015 |
| RC12MEH0021 | Phase 2 | 419349.389  | 7591034.572 | 20160120 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  |                 | sp. 'SCH015/SCH016' | 1 | RC12MEH0021.20160120-T3-03A | T143618          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH015 |
| RC12MEH0021 | Phase 2 | 419349.389  | 7591034.572 | 20160120 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  |                 | sp. 'SCH015/SCH016' | 1 | RC12MEH0021.20160120-T3-03B | TT143619         | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH015 |
| RC14MEH0252 | Phase 2 | 418788.505  | 7593304.312 | 20160120 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  |                 | sp. 'SCH015/SCH016' | 1 | RC14MEH0252.20160120-T3-02  | T143621          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH015 |
| RC15MEH0306 | Phase 3 | 416430.841  | 7596647.612 | 20160627 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  |                 | sp. 'SCH015/SCH016' | 1 | RC15MEH0306.20160627.T3-03  | T133452          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH016 |
| RC16JIM0026 | Phase 5 | 420040.245  | 7591853.293 | 20161212 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  |                 | sp. 'SCH015/SCH016' | 1 | RC16JIM0026.20161212.T1-01  | T133453          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH015 |
| RC16JIM0031 | Phase 5 | 419780.264  | 7591698.526 | 20161212 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  |                 | sp. 'SCH015/SCH016' | 1 | RC16JIM0031.20161212.T2-01  | T133454          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH015 |
| RC15MEH0329 | Phase 2 | 415438.227  | 7596951.285 | 20160118 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  |                 | sp.                 | 1 | RC15MEH0329.20160118T3-01B  | T143627          | 100% Ethanol | Penny Brooshooft | Morphological         | Failed      |
| RC16MEH0436 | Phase 4 | 415708.444  | 7597253.62  | 20161026 | Arthropoda | Arachnida    | Pseudoscorpiones  | Hyidae        |                 | sp. 'PH026'         | 1 | RC16MEH0436.20161026.T2-01  | T142826          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPH026  |
| RC16MEH0264 | Phase 6 | 417476.021  | 7594964.208 | 20170130 | Arthropoda | Arachnida    | Pseudoscorpiones  | Hyidae        |                 | sp. 'PH017/PH027'   | 1 | RC16MEH0264.20170130.T3-03  | T142832          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPC027  |
| RC14MEH0252 | Phase 2 | 418788.505  | 7593304.312 | 20160120 | Arthropoda | Arachnida    | Pseudoscorpiones  | Hyidae        |                 | sp. 'PH017/PH027'   | 1 | RC14MEH0252.20160120-T1-04  | T143620          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPC017  |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0577.20170130.T1-02    | C70383           | 100% Ethanol | Nicola Watson    | Morphological         | Failed      |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0577.20170130.T1-02    | C70384           | 100% Ethanol | Nicola Watson    | Morphological         | Failed      |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0577.20170130.T1-02    | C70385           | 100% Ethanol | Nicola Watson    | Morphological         | Failed      |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0577.20170130.T2-01    | C70387           | 100% Ethanol | Nicola Watson    | Morphological         | Failed      |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0577.20170130.T3-01    | C70388           | 100% Ethanol | Nicola Watson    | Morphological         | Failed      |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0577.20170130.T3-01    | C70389           | 100% Ethanol | Nicola Watson    | Morphological         | Failed      |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0577.20170130.T3-01    | C70390           | 100% Ethanol | Nicola Watson    | Morphological         | Failed      |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0577.20170130.T3-01    |                  |              | Nicola Watson    | Morphological         | Failed      |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0577.20170130.T3-01    |                  |              | Nicola Watson    | Morphological         | Failed      |
| MEHRD0834   | Phase 6 | 418837.0531 | 7592555.134 | 20170130 | Arthropoda | Malacostraca | Isopoda           |               |                 | sp.                 | 1 | MEHRD0834.20170130.T1-01    |                  |              | Nicola Watson    | Morphological         | Failed      |
| RC14MEH0388 | Phase 3 | 418087.615  | 7593503.838 | 20160628 | Arthropoda | Entognatha   | Diplura           | Japygidae     |                 | sp. 'DJA003'        | 1 | RC14MEH0388.20160628.T3-1   | E84289           | 100% Ethanol | Terrie Finston   | Molecular             | HelixDJA003 |
| DD13MEH0007 | Phase 4 | 419635.017  | 7593002.209 | 20160914 | Arthropoda | Entognatha   | Diplura           | Japygidae     |                 | sp. 'DJA011'        | 1 | DD13MEH0007.20160914-01     | E84284           | 100% Ethanol | Terrie Finston   | Molecular             | HelixDJA011 |
| RC15MEH0302 | Phase 1 | 416333.034  | 7596351.403 | 20151210 | Arthropoda | Arachnida    | Pseudoscorpiones  | Olpiidae      |                 | sp. 'PO008'         | 1 | RC15MEH0302.20151210-T1-03  | T143624          | 100% Ethanol | Terrie Finston   | Molecular             | HelixPO008  |
| MEHRC0957   | Phase 2 | 415437.8502 | 7596554.182 | 20160118 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  | Paradraculoides | sp. 'SCH038'        | 1 | MEHRC157-20160118-T1-02A    | T143615          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH038 |
| MEHRC0957   | Phase 2 | 415437.8502 | 7596554.182 | 20160118 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  | Paradraculoides | sp. 'SCH038'        | 1 | MEHRC157-20160118-T1-02B    | T143616          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH038 |
| RC15MEH0329 | Phase 2 | 415438.227  | 7596951.285 | 20160118 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  | Paradraculoides | sp. 'SCH038'        | 1 | RC15MEH0329.20160118T3-01A  | T143626          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH038 |
| RC16MEH0264 | Phase 6 | 417476.021  | 7594964.208 | 20170130 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  | Paradraculoides | sp. 'SCH038'        | 1 | RC16MEH0264.20170130.T3-02  | T142831          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH038 |
| RC16MEH0433 | Phase 4 | 415439.259  | 7597649.364 | 20161026 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  | Paradraculoides | sp. 'SCH038'        | 1 | RC16MEH0433.20161026.T2-01  | T142825          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH038 |
| RC15MEJ0019 | Phase 6 | 419263.833  | 7592144.266 | 20170130 | Arthropoda | Arachnida    | Schizomida        | Hubbardiidae  | Paradraculoides | sp. 'SCH039'        | 1 | RC15MEJ0019.20170130.T1-01  | T142830          | 100% Ethanol | Terrie Finston   | Molecular             | HelixSCH039 |
| RC14MEH0308 | Phase 2 | 418986.673  | 7592555.537 | 20160120 | Arthropoda | Entognatha   | Diplura           | Parajapygidae |                 | sp. 'DPA001'        | 1 | RC14MEH0308.20160120-T2-02  | E84291           | 100% Ethanol | Terrie Finston   | Molecular             | HelixDPA001 |
| MEHRD0758   | Phase 6 | 414937.0889 | 7595953.467 | 20170130 | Arthropoda | Entognatha   | Diplura           | Parajapygidae |                 | sp. 'DPA009'        | 1 | MEHRD0758.20170130.T3-01    | E84287           | 100% Ethanol | Terrie Finston   | Molecular             | HelixDPA009 |
| RC15MEH0382 | Phase 2 | 418994.937  | 7594660.173 | 20160120 | Arthropoda | Malacostraca | Isopoda           | Philosciidae  |                 | sp. 'ISP047'        | 1 | RC15MEH0382.20160120-T1-03A | G60922           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISP047 |
| RC15MEH0382 | Phase 2 | 418994.937  | 7594660.173 | 20160120 | Arthropoda | Malacostraca | Isopoda           | Philosciidae  |                 | sp. 'ISP047'        | 1 | RC15MEH0382.20160120-T1-03B | G60923           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISP047 |
| RC15MEH0382 | Phase 2 | 418994.937  | 7594660.173 | 20160120 | Arthropoda | Malacostraca | Isopoda           | Philosciidae  |                 | sp. 'ISP047'        | 1 | RC15MEH0382.20160120-T1-03C | G60924           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISP047 |
| RC15MEH0382 | Phase 2 | 418994.937  | 7594660.173 | 20160120 | Arthropoda | Malacostraca | Isopoda           | Philosciidae  |                 | sp. 'ISP047'        | 1 | RC15MEH0382.20160120-T1-03D | G60925           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISP047 |
| RC15MEH0382 | Phase 2 | 418994.937  | 7594660.173 | 20160120 | Arthropoda | Malacostraca | Isopoda           | Philosciidae  |                 | sp. 'ISP047'        | 1 | RC15MEH0382.20160120-T1-03E | G60926           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISP047 |
| RC15MEH0382 | Phase 2 | 418994.937  | 7594660.173 | 20160120 | Arthropoda | Malacostraca | Isopoda           | Philosciidae  |                 | sp. 'ISP047'        | 1 | RC15MEH0382.20160120-T2-03  | G60927           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISP047 |
| RC16MEH0427 | Phase 4 | 415130.678  | 7597112.585 | 20161026 | Arthropoda | Malacostraca | Isopoda           | Philosciidae  |                 | sp. 'ISP055'        | 1 | RC16MEH0427.20161026.T3-01  | C70354           | 100% Ethanol | Terrie Finston   | Molecular             | HelixISP055 |
| RC16JIM0019 | Phase 4 | 423026.712  | 7590287.794 | 20161027 | Arthropoda | Arachnida    | Araneae           | Oonopidae     | Prethopalpus    | sp. 'ARA052'        | 1 | RC16JIM0019.20161027-01     | T142823          | 100% Ethanol | Karen Cullen     | Morphological         |             |

| Site        | Phase   | Eastin      | Northing    | Date     | Phylum     | Class        | Order            | Family          | Genus           | Species                    | n  | Field code                  | WAM registration | Preservation | Identifier     | Identification method | Lineage     |
|-------------|---------|-------------|-------------|----------|------------|--------------|------------------|-----------------|-----------------|----------------------------|----|-----------------------------|------------------|--------------|----------------|-----------------------|-------------|
| RC15MEH0175 | Phase 1 | 416434.343  | 7594944.106 | 20151210 | Arthropoda | Entognatha   | Diplura          | Projapygidae    |                 | sp. 'DPR008/DPR009/DPR011' | 1  | RC15MEH0175-20151210-T1-01  | E84294           | 100% Ethanol | Terrie Finston | Molecular             | HelixDPR008 |
| RC15MEH0166 | Phase 2 | 416932.656  | 7594851.369 | 20160118 | Arthropoda | Arachnida    | Diplura          | Projapygidae    |                 | sp. 'DPR008/DPR009/DPR011' | 1  | RC15MEH0166-20160118-T3-03  | E84293           | 100% Ethanol | Terrie Finston | Molecular             | HelixDPR008 |
| RC15MEH0261 | Phase 2 | 418437.926  | 7595598.632 | 20160120 | Arthropoda | Entognatha   | Diplura          | Projapygidae    |                 | sp. 'DPR008/DPR009/DPR011' | 1  | RC15MEH0261-20160120-T3-02  | E84295           | 100% Ethanol | Terrie Finston | Molecular             | HelixDPR009 |
| RC16JIM0005 | Phase 5 | 421202.357  | 7591531.346 | 20161212 | Arthropoda | Entognatha   | Diplura          | Projapygidae    |                 | sp. 'DPR010'               | 1  | RC16JIM0005.20161212-T1-01  | E84285           | 100% Ethanol | Terrie Finston | Molecular             | HelixDPR010 |
| RC16JIM0005 | Phase 5 | 421202.357  | 7591531.346 | 20161212 | Arthropoda | Entognatha   | Diplura          | Projapygidae    |                 | sp. 'DPR008/DPR009/DPR011' | 1  | RC16JIM0005.20161212-T3-01  | E84286           | 100% Ethanol | Terrie Finston | Molecular             | HelixDPR011 |
| RC12MEH0120 | Phase 1 | 419988.44   | 7592306.75  | 20151211 | Arthropoda | Arachnida    | Pseudoscorpiones |                 |                 | sp.                        | 1  | RC12MEH0120-20151211-T1-01B | T143623          | 100% Ethanol | Nicola Watson  | Morphological         | Failed      |
| RC12MEH0120 | Phase 1 | 419988.44   | 7592306.75  | 20151211 | Arthropoda | Arachnida    | Pseudoscorpiones |                 |                 | sp.                        | 1  | RC12MEH0120-20151211-T1-01A | T143622          | 100% Ethanol | Nicola Watson  | Morphological         | Failed      |
| RC15MEH0382 | Phase 2 | 418994.937  | 7594660.173 | 20160120 | Arthropoda | Malacostraca | Isopoda          | Armadillidae    | Trogglarmadillo | sp. 'ISA046'               | 1  | RC15MEH0382-20160120-T3-03  | C60928           | 100% Ethanol | Terrie Finston | Molecular             | HelixISA046 |
| MEHRD0577   | Phase 6 | 417838.1501 | 7593854.922 | 20170130 | Arthropoda | Malacostraca | Isopoda          |                 |                 | sp.                        | 3  | MEHRD0577.20170130-T3-01    |                  |              | Nicola Watson  | Morphological         |             |
| RC12MEH0221 | Phase 1 | 419349.389  | 7593104.572 | 20151210 | Arthropoda | Insecta      | Coleoptera       | Curculionidae   |                 | sp. 'CCU014'               | 2  | RC12MEH022120151210-T2-04A  | E34038           | 100% Ethanol | Terrie Finston | Molecular             | HelixCCU014 |
| RC12MEH0221 | Phase 1 | 419349.389  | 7593104.572 | 20151210 | Arthropoda | Insecta      | Coleoptera       | Curculionidae   |                 | sp. 'CCU014'               | 2  | RC12MEH022120151210-T2-04B  | E34039           | 100% Ethanol | Terrie Finston | Molecular             | HelixCCU014 |
| RC14MEH0252 | Phase 1 | 418788.505  | 7593304.312 | 20151210 | Arthropoda | Insecta      | Coleoptera       | Curculionidae   |                 | sp. 'CCU014'               | 1  | RC14MEH025220151210-T1-04   | E34042           | 100% Ethanol | Terrie Finston | Molecular             | HelixCCU014 |
| RC15MEH0302 | Phase 1 | 416333.034  | 7596351.403 | 20151210 | Arthropoda | Insecta      | Coleoptera       | Curculionidae   |                 | sp. 'CCU014'               | 1  | RC15MEH030220151210-T2-02   | E34043           | 100% Ethanol | Terrie Finston | Molecular             | HelixCCU014 |
| RC12MEH0221 | Phase 4 | 419349.389  | 7593104.572 | 20161026 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 1/ 'CP003'             | 25 | RC12MEH0221.20161026-T1-01  | E34040           | 100% Ethanol | Nicola Watson  | Morphological         |             |
| RC14MEH0238 | Phase 1 | 418845.591  | 7592802.363 | 20151211 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 'Robe Valley'/'CP002'  | 5  | RC14MEH023820151211-T1-03   | E34041           | 100% Ethanol | Nicola Watson  | Morphological         |             |
| RC16MEH0264 | Phase 6 | 417476.021  | 7594964.208 | 20170130 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 1/ 'CP003'             | 1  | RC16MEH0264.20170130-T3-04  |                  |              | Terrie Finston | Molecular             | HelixCP003  |
| RC16MEH0427 | Phase 5 | 415130.678  | 7597112.585 | 20161211 | Arthropoda | Insecta      | Blattodea        | Nocticolionidae | ?Nocticola      | sp. 'West Pilbara Complex' | 1  | RC16MEH0427.20161211-T2     | E34037           | 100% Ethanol | Terrie Finston | Molecular             | HelixBN021  |
| RC12MEH0221 | Phase 1 | 419349.389  | 7593104.572 | 20151210 | Arthropoda | Insecta      | Blattodea        | Nocticolionidae | ?Nocticola      | sp. 'West Pilbara Complex' | 1  | RC12MEH022120151210-T2-01   | E34036           | 100% Ethanol | Terrie Finston | Molecular             | HelixBN020  |
| RC16JIM0006 | Phase 2 | 421366.083  | 7591828.359 | 20160120 | Arthropoda | Entognatha   | Zygentoma        | Nicoletidae     |                 | sp. 'TN019'                | 1  | RC12JIM000620160119T2-02    | E34044           | 100% Ethanol | Terrie Finston | Molecular             | HelixTN019  |
| RC14MEH0252 | Phase 1 | 418788.505  | 7593304.312 | 20151210 | Arthropoda | Entognatha   | Zygentoma        | Nicoletidae     |                 | sp. 'TN020'                | 1  | RC14MEH025220151210-T1-03   | E34045           | 100% Ethanol | Terrie Finston | Molecular             | HelixTN020  |
| RC16MEH0264 | Phase 6 | 417476.021  | 7594964.208 | 20170130 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 1/ 'CP003'             | 1  | RC16MEH0264.20170130-T3-04  |                  |              | Terrie Finston | Molecular             | HelixCP003  |
| RC16MEH0264 | Phase 6 | 417476.021  | 7594964.208 | 20170130 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 1/ 'CP003'             | 1  | RC16MEH0264.20170130-T3-04  |                  |              | Terrie Finston | Molecular             | HelixCP003  |
| RC16MEH0264 | Phase 6 | 417476.021  | 7594964.208 | 20170130 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 1/ 'CP003'             | 1  | RC16MEH0264.20170130-T3-04  |                  |              | Terrie Finston | Molecular             | HelixCP003  |
| RC12MEH0221 | Phase 4 | 419349.389  | 7593104.572 | 20161026 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 1/ 'CP003'             | 1  | RC12MEH0221.20161026-T1-01  |                  |              | Terrie Finston | Molecular             | HelixCP003  |
| RC12MEH0221 | Phase 4 | 419349.389  | 7593104.572 | 20161026 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 1/ 'CP003'             | 1  | RC12MEH0221.20161026-T1-01  |                  |              | Terrie Finston | Molecular             | HelixCP003  |
| RC12MEH0221 | Phase 4 | 419349.389  | 7593104.572 | 20161026 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 1/ 'CP003'             | 1  | RC12MEH0221.20161026-T1-01  |                  |              | Terrie Finston | Molecular             | HelixCP003  |
| RC14MEH0238 | Phase 1 | 418845.591  | 7592802.363 | 20151211 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 'Robe Valley'/'CP002'  | 1  | RC14MEH023820151211-T1-03   |                  |              | Terrie Finston | Molecular             | HelixCP002  |
| RC14MEH0238 | Phase 1 | 418845.591  | 7592802.363 | 20151211 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 'Robe Valley'/'CP002'  | 1  | RC14MEH023820151211-T1-03   |                  |              | Terrie Finston | Molecular             | HelixCP002  |
| RC14MEH0238 | Phase 1 | 418845.591  | 7592802.363 | 20151211 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 'Robe Valley'/'CP002'  | 1  | RC14MEH023820151211-T1-03   |                  |              | Terrie Finston | Molecular             | HelixCP002  |
| RC14MEH0238 | Phase 1 | 418845.591  | 7592802.363 | 20151211 | Arthropoda | Insecta      | Coleoptera       | Ptiliidae       |                 | sp. 'Robe Valley'/'CP002'  | 1  | RC14MEH023820151211-T1-03   |                  |              | Terrie Finston | Molecular             | HelixCP002  |
| J0866       | Phase 1 | 422437.97   | 7596054.499 | 20080710 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | J0866P2T1-3                 | T96T161          | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| J0866       | Phase 2 | 422437.97   | 7596054.499 | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | J0866P2T2-1                 | T96160           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| J0866       | Phase 1 | 422437.97   | 7596054.499 | 20080710 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp.                        | 1  | J0866P1T2-1                 | T92212           | 100% Ethanol | Nicola Watson  | Morphological         | Failed      |
| J0866       | Phase 2 | 422437.97   | 7596054.499 | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | J0866P1T1-1                 | T92211           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| J0867       | Phase 2 | 422437.97   | 7595954.499 | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | J0867P2T3-1                 | T96167           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| J0867       | Phase 2 | 422437.97   | 7595954.499 | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | J0867P2T1-1                 | T96162           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| J0928       | Phase 2 | 422637.97   | 7596054.5   | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | J0928P2T1-5A                |                  |              | Terrie Finston | Molecular             | HelixSCH011 |
| J0928       | Phase 2 | 422637.97   | 7596054.5   | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | J0928P2T1-5                 | T96166           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| MEJ0076     | Phase 2 | 419946.7082 | 7595698.905 | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp.                        | 1  | MEJ0076P2T2-4               | T96164           | 100% Ethanol | Nicola Watson  | Morphological         | Failed      |
| MEJ0076     | Phase 2 | 419946.7082 | 7595698.905 | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | MEJ0076P2T3-3               | T96159           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| MEJ0048     | Phase 2 | 419879.9633 | 7595939.9   | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | MEJ0048P2T2-3               | T96165           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| MEJ0085     | Phase 2 | 419941.6808 | 7595604.879 | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | MEJ0085P2T3-3A              |                  |              | Terrie Finston | Molecular             | HelixSCH011 |
| MEJ0085     | Phase 2 | 419941.6808 | 7595604.879 | 20081202 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | MEJ0085P2T3-3               | T96163           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| MEJ5548alt  | Phase 1 | 420888.294  | 7596304.607 | 20080710 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | MEJ5548altP1T3-2            | T92210           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| MEJ5548alt  | Phase 1 | 420888.294  | 7596304.607 | 20080710 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | MEJ5548altP1T3-2A           |                  |              | Terrie Finston | Molecular             | HelixSCH011 |
| MEJ5548alt  | Phase 1 | 420888.294  | 7596304.607 | 20080710 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | MEJ5548altP1T2-2            | T92208           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| RC08MEJ0004 | Phase 4 | 420036.6506 | 7596954.526 | 20091022 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | RC08MEJ0004P4T2-4           | T99567           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| RC08MEJ0015 | Phase 4 | 420033.4152 | 7596851.769 | 20091022 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | RC08MEJ0015P4T2-3           | T99568           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| RC08MEJ0015 | Phase 4 | 420033.4152 | 7596851.769 | 20091022 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | RC08MEJ0015P4T1-5           | T99570           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| RC08MEJ0023 | Phase 4 | 419930.7698 | 7596804.544 | 20091022 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | RC08MEJ0023P4T2-3           | T99569           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| MEJ0007     | Phase 1 | 419891.6269 | 7596957.681 | 20080710 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | MEJ0007P1T2-1               | T92209           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |
| MEJ0007     | Phase 1 | 419891.6269 | 7596957.681 | 20080710 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp.                        | 1  | MEJ0007P1T2-1A              |                  |              | Nicola Watson  | Morphological         | Failed      |
| MEJ0007     | Phase 1 | 419891.6269 | 7596957.681 | 20080710 | Arthropoda | Arachnida    | Schizomida       | Hubbardiidae    |                 | sp. 'SCH011'               | 1  | MEJ0007P1T1-1               | T92207           | 100% Ethanol | Terrie Finston | Molecular             | HelixSCH011 |