



Biologic Environmental Survey Pty Ltd  
PO Box 179 Floreat  
Western Australia

6 April 2017

Carly Nixon

Rio Tinto Iron Ore – Central Park

152-158 St Georges Tce

PERTH WA 6000

**RE: Results of DNA analysis of subterranean fauna collected at West Angelas Deposits C, D, and G**

Dear Carly,

Rio Tinto Iron Ore requested genetic identification of certain troglofauna and stygofauna taxa sampled throughout West Angelas Deposits C, D and G, and comparisons with pre-existing regional sequences to determine whether the species found at West Angelas matched others from elsewhere in the region.

- Based on the results of the West Angelas Deposits C, D and G subterranean fauna survey (Biologic 2016), as requested and approved by RTIO, 26 specimens from 10 taxonomic groups were chosen for sequencing, comprising Bathynellacea (4), Amphipoda (5), Haplotaxida (Enchytraeidae) (6), Oligochaeta (3), Isopoda (2), Hemiptera (Meenoplidae) (2), Thysanura (1), and Symphyla (3) (Table 1).
- Specimens and species were chosen based on the following criteria: A) taxa that have a broad regional context available in the form of multiple previous regional sequences throughout the Pilbara region, B) taxa that were only found within potential impact areas (pits and/ or potential drawdown areas), or taxa that were found both inside and outside of potential impact areas at West Angelas, and C) taxa that had some degree of taxonomic uncertainty regarding their morphological identifications at the species level.
- Analyses/ comparisons were limited to those that would most quickly and adequately address the following hypotheses: 1) does the material from West Angelas represent a single species or multiple different species; and 2) does the material from West Angelas match any previously recorded species from the Pilbara region (based on available sequences). Deeper phylogenetic relationships between material recorded at West Angelas and previous regional material were not investigated and cannot be inferred from the types of neighbour-joining cluster analysis used.

**Table 1. Details of specimens sent for DNA analysis (1 specimen per record)**

Specimen code	HIGHER TAXON Family	Preliminary ID	Bore/hole name	UTM E z50 GDA94	UTM N z50 GDA94
<b>BATHYNELLACEA</b>					
BES:1900	Parabathynellidae	<i>Atopobathynella</i> sp. 'WA'	RC15WAC0416	668584.57	7439798.37
BES:2297	Parabathynellidae	<i>Atopobathynella</i> sp. 'WA'	RC15WAC0413	669436.13	7439804.66
BES:1996	Bathynellidae	Bathynellidae sp. 'WA'	RC15WAC0413	669436.13	7439804.66
BES:2054	Bathynellidae	Bathynellidae sp. 'WA'	RC15WAC0384	667401.98	7440199.63
<b>AMPHIPODA</b>					
BES:1812	Paramelitidae	<i>Kruptus</i> sp. 'WA'	RC13WAD0287	667339.68	7436249.24
BES:1910	Paramelitidae	<i>Kruptus</i> sp. 'WA'	RC15WAC0387	666231.13	7439987.54
BES:1966	Paramelitidae	<i>Kruptus</i> sp. 'WA'	RC14WAD0346	664939.59	7436650.84
BES:1971	Paramelitidae	<i>Kruptus</i> sp. 'WA'	RC15WAC0276	672654.61	7438949.45
BES:2352	Paramelitidae	<i>Maarka</i> sp. 'WA'	RC12WAD0295	664742.89	7437505.38
<b>HAPLOTAXIDA</b>					
BES:1844	Enchytraeidae	Enchytraeidae sp. indet.	RC12WAD0189	671538.78	7436245.02
BES:1936	Enchytraeidae	Enchytraeidae sp. indet.	RC15WAC0387	666231.13	7439987.54
BES:2035	Enchytraeidae	Enchytraeidae sp. indet.	RD14WAF0003	689191.74	7433400.35
BES:2039	Enchytraeidae	Enchytraeidae sp. indet.	RC14WAF0066	689286	7433433.84
BES:2114	Enchytraeidae	Enchytraeidae sp. indet.	RC14WAD0350	665336.97	7437144.78
BES:2355	Enchytraeidae	Enchytraeidae sp. indet.	RC15WAC0380	671570.22	7439796.36
<b>OLIGOCHAETA</b>					
BES:1849		Oligochaeta sp. indet.	RC14WAD0346	664939.59	7436650.84
BES:2030		Oligochaeta sp. indet.	RC15WAC0384	667401.98	7440199.63
BES:2031		Oligochaeta sp. indet.	RC15WAC0413	669436.13	7439804.66
<b>ISOPODA</b>					
BES:1876	Armadillidae	Armadillidae sp. indet.	RC12WAD0295	664742.89	7437505.38
BES:2199		Isopoda sp. indet.	RC12WAD0295	664742.89	7437505.38
<b>HEMIPTERA</b>					
BES:2139	Meenoplidae	Meenoplidae sp. indet.	RC15WAC0377	667347.98	7439303.55
BES:2356	Meenoplidae	Meenoplidae sp. indet.	RC14WAD0217	665792.22	7436703.79
<b>THYSANURA</b>					
BES:1823	Nicoletiidae	Atelurinae sp. indet.	RC14WAD0350	665336.97	7437144.78
<b>SYMPHYLA</b>					
BES:2112	Scutigerellidae	Scutigerellidae sp. indet.	RC14WAF0072	692251.8	7434505.65
BES:2117	Scutigerellidae	Scutigerellidae sp. indet.	RC15WAC0276	672654.61	7438949.45
BES:2055		Symphyla sp. indet.	RC15WAC0197	668634.6	7439014.15

## METHODS

The DNA sequencing and comparisons were conducted by Helix Molecular Solutions. A full account of the methods and detailed results can be found in Appendix I. The following details are provided in summary (from Helix 2016):

- Sequencing of the mitochondrial gene cytochrome oxidase subunit 1 (COI) and the nuclear gene 12s was conducted using multiple primers.

- Sequences were edited using GENEIOUS software, while alignments were conducted using CLUSTAL W software.
- Genetic distances were calculated using uncorrected p-distances (total percentage of nucleotide differences between sequences) and trees were constructed using neighbour-joining in MEGA 6.0 software.
- Based on published data, lineages were defined as haplotypes (or groups of haplotypes) differing by >3% sequence divergence (COI), while the thresholds for species-level divergence were defined on a taxon-by taxon basis, acknowledging that a vast majority of species- pairs differ from each other by >8% (COI), following Hebert et al. (2003). For this reason, divergences <3% (COI) were regarded as the same lineage within a species, and >8% (COI) were regarded as likely to be different species, however divergences between 3% – 8% were generally regarded as an intermediate zone between interspecific and intraspecific variability.

## RESULTS

Table 2 shows the results of the genetic analyses within each of the groups of taxa. Overall, the analyses found that:

1. Several sequences in the Oligochaeta (*Pristina longiseta* and Phreodrilidae `OLP012`), Haplotaxida (Enchytraeidae `OLE026`), and Hemiptera (Meenoplidae `HEM003`) aligned to previously recorded species or lineages that are known to occur widely in the Pilbara;
2. Sequences of the Bathynellidae, Parabathynellidae, Amphipoda, Isopoda, and Symphyla were all unique regionally, with high levels of genetic divergence between any of the available regional material from these taxonomic groups and the samples from West Angelas;
3. Three specimens, respectively from the Bathynellidae, Oligochaeta, and Thysanura did not sequence successfully, therefore the identifications of these specimens have not changed; and
4. Additional putative species were revealed within the Amphipoda (*Kruptus* `AMP045`, Paramelitidae `AMP036`, and *Maarka* `AMP037`), Haplotaxida (Enchytraeidae `OLE026`, `OLE028`, `OLE029`, and `OLE030`), Oligochaeta (*Pristina longiseta* and Phreodrilidae `OLP012`), and Symphyla (Scutigerellidae `SYM027`, `SYM028`, and `SYM029`). The implications for the remaining specimens within these groups that have not been sequenced are discussed in further detail below.

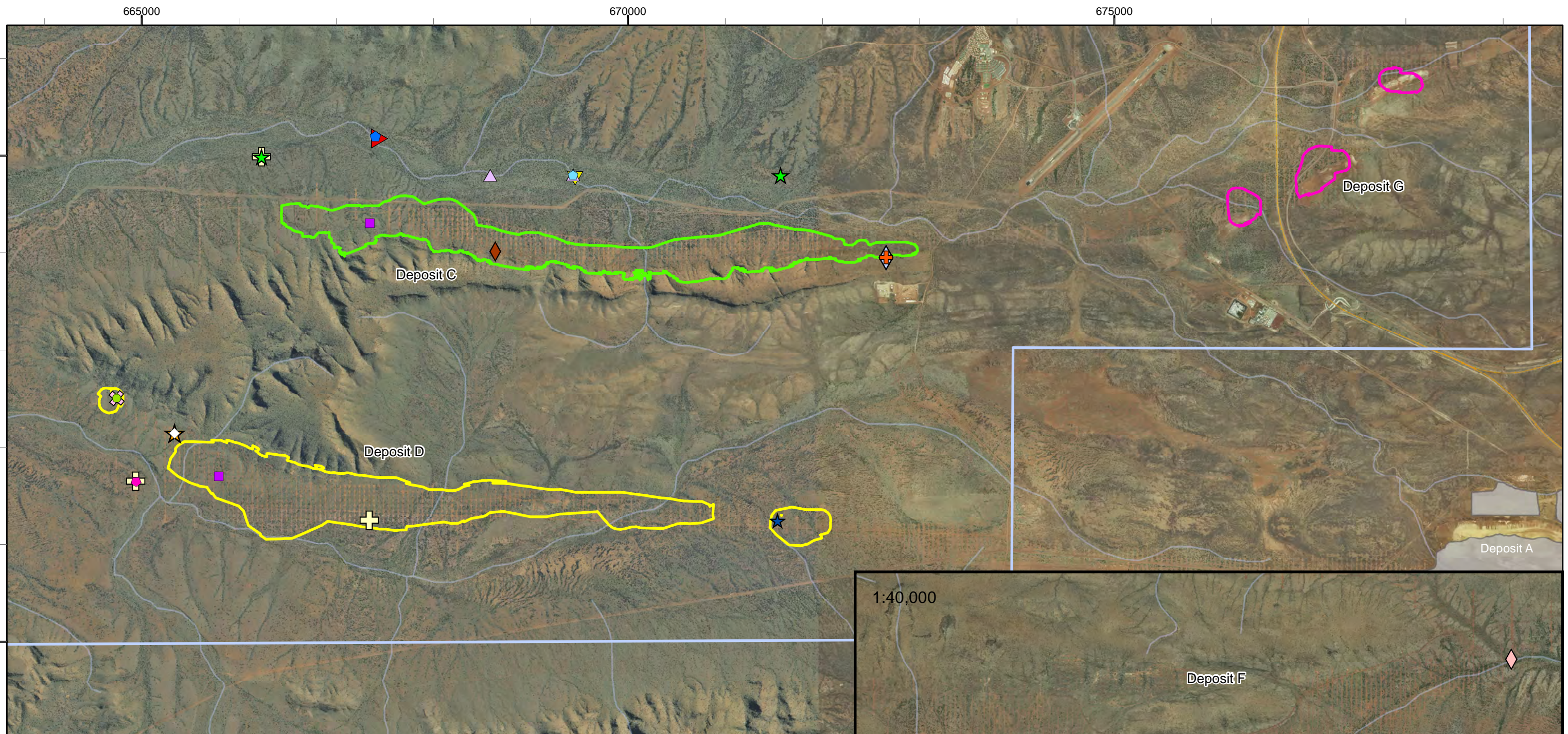
Figure 1 shows the locations of subterranean fauna sequences from Deposits C, D, and G, and the resulting genetic identifications of the taxa.

**Table 2: Summary of local and regional genetic alignment results.**

Specimen code	HIGHER TAXON Family	Preliminary ID	Bore/hole name	Local match	% div. COI	Regional match	% div. COI	Nominal species-level ID	Comment
<b>BATHYNELLACEA</b>									
BES:1900	Parabathynellidae	<i>Atopobathynella</i> sp. 'WA'	RC15WAC0416	BES:2297	0.3%	No	>10%	<b><i>Atopobathynella</i> 'BAP027'</b>	Regionally distinct species of Parabathynellidae. Likely <i>Atopobathynella</i> following G. Perina morphological ID, August 2016.
BES:2297	Parabathynellidae	<i>Atopobathynella</i> sp. 'WA'	RC15WAC0413	BES:1900					
BES:1996	Bathynellidae	Bathynellidae sp. 'WA'	RC15WAC0413	No		No	>19%	<b>Bathynellidae 'BAB018'</b>	Regionally distinct species of Bathynellidae
BES:2054	Bathynellidae	Bathynellidae sp. 'WA'	RC15WAC0384						Sequence failed
<b>AMPHIPODA</b>									
BES:1812	Paramelitidae	<i>Kruptus</i> sp. 'WA'	RC13WAD0287	BES: 1910, 1966	0.3 - 0.9%	No	>15%	<b><i>Kruptus</i> 'AMP035'</b>	Regionally distinct species of Paramelitidae. Likely <i>Kruptus</i> following G. Perina morphological ID, August 2016.
BES:1910	Paramelitidae	<i>Kruptus</i> sp. 'WA'	RC15WAC0387	BES: 1812, 1966					
BES:1966	Paramelitidae	<i>Kruptus</i> sp. 'WA'	RC14WAD0346	BES: 1812, 1910					
BES:1971	Paramelitidae	<i>Kruptus</i> sp. 'WA'	RC15WAC0276	No	>17%	No	>15%	<b>Paramelitidae 'AMP036'</b>	Regionally distinct species of Paramelitidae. Juvenile specimens only, uncertain morphological ID. Unable to be placed to genus on current information.
BES:2352	Paramelitidae	<i>Maarka</i> sp. 'WA'	RC12WAD0295	No	>17%	No	>15%	<b><i>Maarka</i> 'AMP037'</b>	Regionally distinct species of Paramelitidae. Likely <i>Maarka</i> following G. Perina morphological ID, August 2016.
<b>HAPLOTAXIDA</b>									
BES:1936	Enchytraeidae	Enchytraeidae sp. indet.	RC15WAC0387	BES:2355	1.2%	OLE26	2.8% - 3.4%	<b>Enchytraeidae 'OLE026'</b>	Genetic alignment to a species (OLE26) previously sampled elsewhere in the Pilbara (Helix 2016).
BES:2355	Enchytraeidae	Enchytraeidae sp. indet.	RC15WAC0380	BES:1936					
BES:1844	Enchytraeidae	Enchytraeidae sp. indet.	RC12WAD0189	No	>16%	No	>12%	<b>Enchytraeidae 'OLE028'</b>	Regionally distinct species of Enchytraeidae.
BES:2114	Enchytraeidae	Enchytraeidae sp. indet.	RC14WAD0350	No	>7.5%	Possibly	5.7%	<b>Enchytraeidae 'OLE029'</b>	Potentially distinct, but moderate divergences indicate more information required to separate distinct species. Occurs in a large species complex found across a wide area and multiple catchment boundaries (Helix 2016).
BES:2035	Enchytraeidae	Enchytraeidae sp. indet.	RD14WAF0003	BES:2039	1.6%	Possibly	7.8%	<b>Enchytraeidae 'OLE030'</b>	Potentially distinct, but moderate divergences indicate more information required to separate distinct species. Occurs in a large species complex found across a wide area and multiple catchment boundaries (Helix 2016).
BES:2039	Enchytraeidae	Enchytraeidae sp. indet.	RC14WAF0066	BES:2035					

Specimen code	HIGHER TAXON Family	Preliminary ID	Bore/hole name	Local match	% div. COI	Regional match	% div. COI	Nominal species-level ID	Comment
<b>OLIGOCHAETA</b>									
BES:1849		Oligochaeta sp. indet.	RC14WAD0346	No		OLP12	<2.8%	<b>Phreodrilidae</b> <b>'OLP12'</b>	Genetic alignment to a species (OLP12) previously sampled widely across four catchments in the Pilbara (Helix 2016).
BES:2030		Oligochaeta sp. indet.	RC15WAC0384	No		<i>Pristina longiseta</i>	1.7%	<b><i>Pristina longiseta</i></b>	Genetic alignment to a cosmopolitan species previously sampled worldwide (Helix 2016).
BES:2031		Oligochaeta sp. indet.	RC15WAC0413						Sequence failed
<b>ISOPODA</b>									
BES:1876		Armadillidae sp. indet.	RC12WAD0295	BES:2199	0.3%	No	>18%	<b>Armadillidae</b> <b>'ISA049'</b>	Regionally distinct species of Armadillidae.
BES:2199		Isopoda sp. indet.	RC12WAD0295	BES:1876					
<b>HEMIPTERA</b>									
BES:2139	Meenoplidae	Meenoplidae sp. indet.	RC15WAC0377	BES:2356	Identical	HEM003	1.6 - 2.3%	<b>Meenoplidae</b> <b>'HEM003'</b>	Genetic alignment to a widespread species previously sampled at Murrays Hill, Hardy River, and Upper South Fortescue (Helix 2016).
BES:2356	Meenoplidae	Meenoplidae sp. indet.	RC14WAD0217	BES:2139					
<b>THYSANURA</b>									
BES:1823	Nicoletiidae	Atelurinae sp. indet.	RC14WAD0350						Sequence failed
<b>SYMPHYLA</b>									
BES:2112	Scutigerellidae	Scutigerellidae sp. indet.	RC14WAF0072	No	>16%	No	>16%	<b>Scutigerellidae</b> <b>'SYM027'</b>	Regionally distinct species of Scutigerellidae
BES:2117	Scutigerellidae	Scutigerellidae sp. indet.	RC15WAC0276	Possibly 2055	4.9%	No	>16%	<b>Scutigerellidae</b> <b>'SYM028'</b>	Potentially distinct, but moderate divergence from local specimens indicates more information required to separate distinct species.
BES:2055		Symphyla sp. indet.	RC15WAC0197	Possibly 2117	4.9%	No	>16%	<b>Scutigerellidae</b> <b>'SYM029'</b>	Potentially distinct, but moderate divergence from local specimens indicates more information required to separate distinct species.





**Legend**

Pilbara Rail

West Angelas Current Mining Operations

Study Area (Deposits C, D, G)

Proposed Deposit C Pit

Proposed Deposit D Pit

Proposed Deposit G Pit

**Taxonomic group, Genetic ID**

Crustaceans, Atopobathynella sp. `BAP027`

Crustaceans, Bathynellidae sp. `BAB018`

Crustaceans, Bathynellidae sp. indet.

Crustaceans, Kruptus sp. `AMP035`

Crustaceans, Maarrka sp. `AMP037`

Crustaceans, Paramelitidae sp. `AMP036`

Crustaceans, Armadillidae sp. `ISA049`

Hexapods, Atelurinae sp. indet.

Hexapods, Meenoplidae sp. `HEM003`

Myriapods, Scutigereidae sp. `SYM027`

Myriapods, Scutigereidae sp. `SYM028`

Myriapods, Scutigereidae sp. `SYM029`

Worms, Enchytraeidae sp. `OLE026`

Worms, Enchytraeidae sp. `OLE028`

Worms, Enchytraeidae sp. `OLE029`

Worms, Enchytraeidae sp. `OLE030`

Worms, Phreodrilidae sp. `OLP012`

Worms, Pristina longiseta

Worms, Naididae sp. indet.

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N

0

700

1,400

2,800

4,200

km

**Rio Tinto Iron Ore - West Angelas Project**

**Deposits C, D, & G Subterranean Fauna Survey**

**Fig. 1: Locations of subterranean fauna DNA sequences and genetic identifications**

Coordinate System: GDA 1994 MGA Zone 50

Projection: Transverse Mercator

Datum: GDA 1994

Size A3. Created 24/05/2016



## CHANGES TO RISK ASSESSMENT

The risk assessment has not changed for any of the taxa that were not sequenced (or not successfully sequenced), including the beetles *Hydrobiomorpha* sp. indet., and Anillini sp. indet., the silverfish Atelurinae sp. indet., the springtail Cyphoderidae sp. indet., the copepods *Australocamptus* sp. `B13` and *Parastenocaris* sp. indet., and *Thermocyclops* sp. `WA`, and the worms Aeolosomatidae sp. indet., and Turbellaria sp. indet. Biologic (2016) contains relevant details for each of these taxa.

Table 2 provides a summary of changes to the risk assessment following the results of the DNA analysis. Figure 2 shows the locations of taxa now considered to be at risk, in the context of the subterranean habitats assessed by Biologic (2016). Four taxa were found to align genetically to widespread taxa, and therefore are now considered to be at negligible risk of impact, comprising:

- The phreodrilid worm Phreodrilidae `OLP12`, found near Deposit C;
- The cosmopolitan naidid worm *Pristina longiseta*, found near Deposit C;
- The enchytraeid worm Enchytraeidae `OLE026`, found near Deposit C; and
- The meenoplid bug Meenoplidae sp. `HEM003`, found within Deposits C and D.

The remaining 13 taxa shown in Table 3 are considered to range from high to moderate low risk based on what is known about the regional occurrence of the taxon from the genetic comparisons, and whether or not the taxon occurs locally outside of the direct impact area (comprising the pit boundaries for troglofauna, and the likely extent of drawdown for stygofauna).

The genetically determined identifications were applied to specimens from the same sample and the same bore/ hole where it was reasonable to do so (such as samples where there were 17 'Enchytraeidae sp. indet.' collected and one was sent for sequencing, or bores/ holes where there were 5 'Enchytraeidae sp. indet.' from the first trip and 2 from the second trip). Nevertheless, not all of the `sp. indet.` taxa were able to be treated this way, owing to the presence of multiple genetically determined species co-occurring in the same habitat/ deposit (particularly in the Enchytraeidae and Paramelitidae). Where a specimen from a different bore/ hole was unable to be reasonably allocated to either of multiple genetically determined species, it remained `sp. indet.` and the risk level did not change materially.

Note also that enchytraeids were assessed herein as stygofauna, because it was assumed that groundwater drawdown may possibly result in desiccation of the air-filled subterranean habitat as well as the loss of groundwater habitat within the drawdown zone.

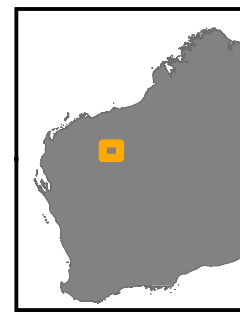
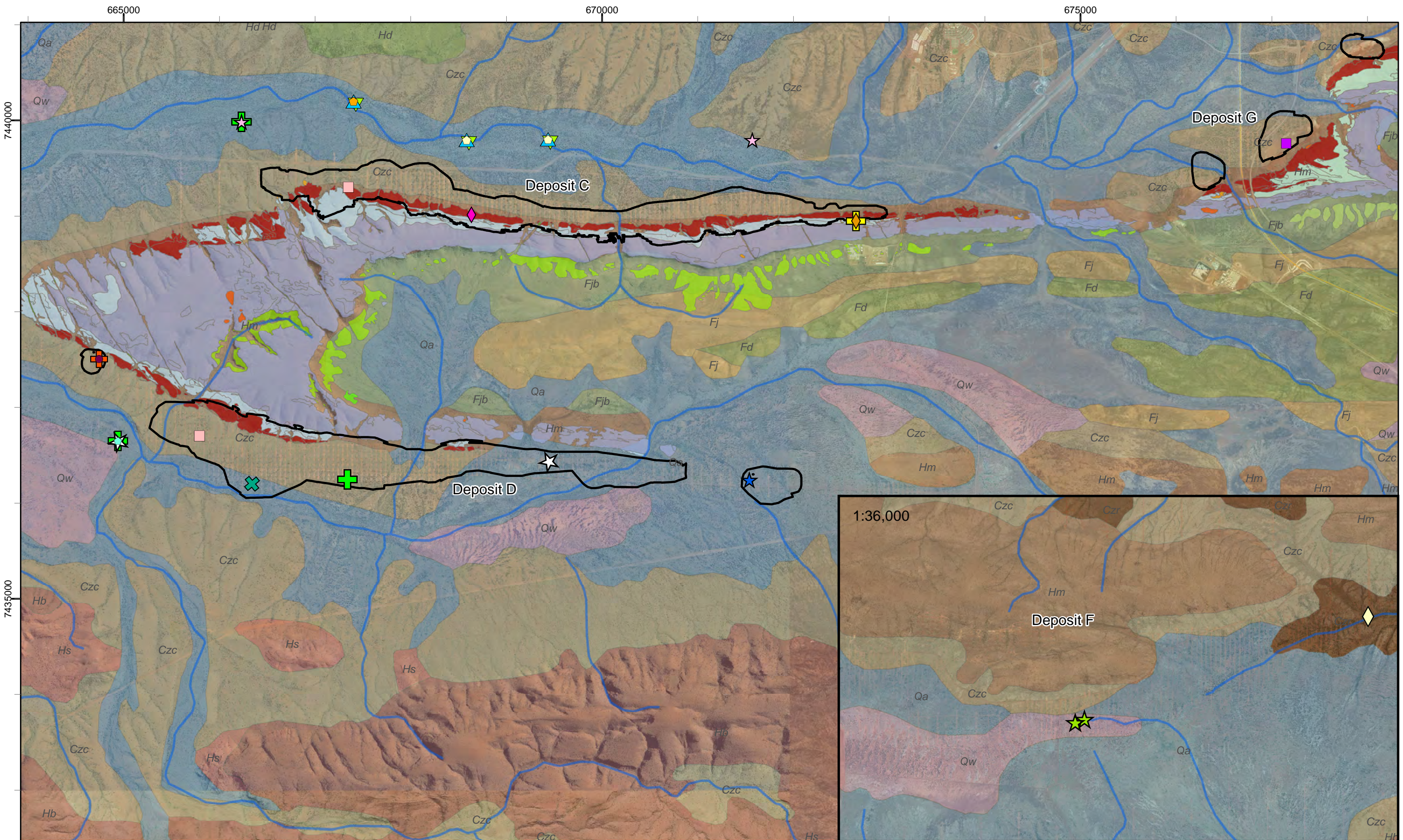
**Table 3: Changes to risk assessment of subterranean taxa following DNA analysis.**

Morphospecies	Current SRE status	Previous taxon	Within deposit/ impact	Out of deposit/ impact	Extent of habitat beyond deposit/ likely impact area	Risk of direct impacts
<b>Worms</b>						
Enchytraeidae `OLE026`	Widespread	Enchytraeidae sp. indet.		Near Dep. C (29)	Regional	<b>Negligible.</b> Regionally widespread species
Enchytraeidae `OLE028`	Confirmed SRE (D)	Enchytraeidae sp. indet.	Dep. D (2)		Likely beyond deposit within Calcrete/ Orebody/ Mt Newman Member, but within likely drawdown	<b>High.</b> Direct impacts may include mining and drawdown.
Enchytraeidae `OLE029`	Potential SRE (A&D)	Enchytraeidae sp. indet.		Near Dep. D (11)	Beyond deposit within Orebody/ Mt Newman Member, but within likely drawdown	<b>High.</b> Direct impacts may include mining and drawdown.
Enchytraeidae `OLE030`	Potential SRE (A&D)	Enchytraeidae sp. indet.	Dep. F (26)		Likely beyond deposit within Alluvials/ Mt Newman Member	<b>Negligible.</b> Unlikely to be affected by mining at Dep. C, D, G
Enchytraeidae sp. indet.	Potential SRE (A)	Enchytraeidae sp. indet.	Dep. D (75), F (38)	Near Dep D (1), C (6)	Likely beyond Deposit D within Calcrete/ Orebody/ Mt Newman Member, but within likely drawdown	<b>Mod / High.</b> Specimens cannot be allocated on current information. Records in/ near Dep. C and D may be at risk from mining or drawdown
Phreodrilidae `OLP12`	Widespread	Oligochaeta sp. indet.	Near Dep. D (1)		Regional	<b>Negligible.</b> Regionally widespread species
<i>Pristina longiseta</i>	Widespread	Oligochaeta sp. indet.	Near Dep. C (6)		Worldwide	<b>Negligible.</b> Cosmopolitan widespread species
<i>c.f. Pristina longiseta</i>	Widespread	Oligochaeta sp. indet.	Near Dep. C (22)		Worldwide	<b>Negligible.</b> Likely to represent <i>P. longiseta</i> owing to location and broad morphology. Widespread.
<b>Crustaceans</b>						
<i>Kruptus</i> sp. `AMP035`	Confirmed SRE (D&E)	<i>Kruptus</i> sp. `WA`	Near Dep. C (6), Dep D (3)		Beyond Deposit C/ D within Calcrete/ Alluvials, but all current records within drawdown	<b>High.</b> Species currently known only from within likely drawdown extent
<i>Maarrka</i> sp. `AMP037`	Confirmed SRE (D&E)	<i>Maarka</i> sp. `WA`	Dep. D (1)		Likely beyond deposit within Orebody/ Mt Newman, but all current records within drawdown	<b>High.</b> Species currently known only from within likely drawdown extent
Paramelitidae sp. `AMP036`	Confirmed SRE (D&E)	<i>Kruptus</i> sp. `WA`	Dep. C (1)		Likely beyond deposit within Orebody/ Mt Newman Member, but all current records within drawdown	<b>High.</b> Species currently known only from within likely drawdown extent
Paramelitidae sp. indet.	Potential SRE (A&E)	<i>Kruptus</i> sp. `WA`	Near Dep. C (1)		Likely beyond Deposit D within Calcrete/ Orebody/ Mt Newman Member, but within likely drawdown	<b>Mod / High.</b> Likely to be the same as <i>K. AMP035</i> , but would not extend range beyond likely drawdown
<i>Atopobathynella</i> sp. `BAP027`	Confirmed SRE (D&E)	<i>Atopobathynella</i> sp. `WA`	Near Dep. C (200)		Unknown, but all current records within likely drawdown	<b>High.</b> Species currently known only from within likely drawdown extent
Bathynellidae sp. `BAB018`	Confirmed SRE (D&E)	Bathynellidae sp. `WA`	Near Dep. C (87)		Unknown, but all current records within likely drawdown	<b>High.</b> Species currently known only from within likely drawdown extent
Armadillidae sp. `ISA049`	Confirmed SRE (D&E)	Armadillidae sp. indet., Isopoda sp. indet.	Dep. D (2)		Recorded close to boundary, habitat likely beyond Dep. D throughout Orebody/ Mt Newman Member	<b>Mod.</b> Current records only just within pit, habitat likely to extend beyond.



Morphospecies	Current SRE status	Previous taxon	Within deposit/ impact	Out of deposit/ impact	Extent of habitat beyond deposit/ likely impact area	Risk of direct impacts
<b>Myriapods</b>						
Scutigerellidae sp. 'SYM027'	Confirmed SRE (D&E)	Scutigerellidae sp. indet.		Outside Dep. F (1)	Locally beyond Deposit F	<b>Negligible.</b> Unlikely to be affected by mining at Dep. C, D, G
Scutigerellidae sp. 'SYM028'	Confirmed SRE (D&E)	Scutigerellidae sp. indet.	Dep. C (17)		Recorded close to boundary, habitat likely beyond Dep. C throughout Orebody/ Mt Newman Member	<b>Mod.</b> Current records only just within pit, habitat likely to extend beyond. Moderate genetic similarities to S. SYM029
Scutigerellidae sp. 'SYM029'	Confirmed SRE (D&E)	Symphyla sp. indet.	Dep. C (1)		Likely beyond Deposit C throughout Orebody/ Mt Newman Member	<b>Mod.</b> Current records within pit, but habitat may extend beyond. Moderate genetic similarities to S. SYM028
<b>Hexapods</b>						
Meenoplidae sp. 'HEM003'	Widespread	Meenoplidae sp. indet. (Biologic)	Dep. C (2), Dep. D (1)	Regional	Regional	<b>Negligible.</b> Regionally widespread species
Meenoplidae sp. indet. (Ecologia 2013)	Potential SRE (A)	Meenoplidae sp. indet. (Ecologia)	Dep. G (1)	Dep. H (1)	Likely beyond Deposit G throughout Orebody/ Mt Newman Member	<b>Mod / Low.</b> Possibly be the same as M. HEM003, which is regionally widespread
Atelurinae sp. indet. (Biologic 2016)	Potential troglobite	Atelurinae sp. indet. (Biologic)		Outside Dep. D (1)	Habitat outside of Dep D throughout Orebody/ Mt Newman Member	<b>Low.</b> Specimen does not occur within pit. Possibly be the same as Ecologia species, owing to proximity and connected habitats
Atelurinae sp. indet. (Ecologia 2013)	Potential troglobite	Atelurinae sp. indet. (Ecologia)	Dep D (1)		Habitat likely beyond Dep D throughout Orebody/ Mt Newman Member	<b>Mod.</b> Possibly same as Biologic species, owing to proximity and connected habitats.





**biologic**  
Environmental Survey

1:36,000

0 700 1,400 2,800 4,200 km

N

**Rio Tinto Iron Ore - West Angelas Project**  
**Deposits C, D, & G Subterranean Fauna Survey**  
**Fig. 2: Subterranean fauna risk assessment changes following DNA analysis**

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

Size A3. Created 24/10/2016



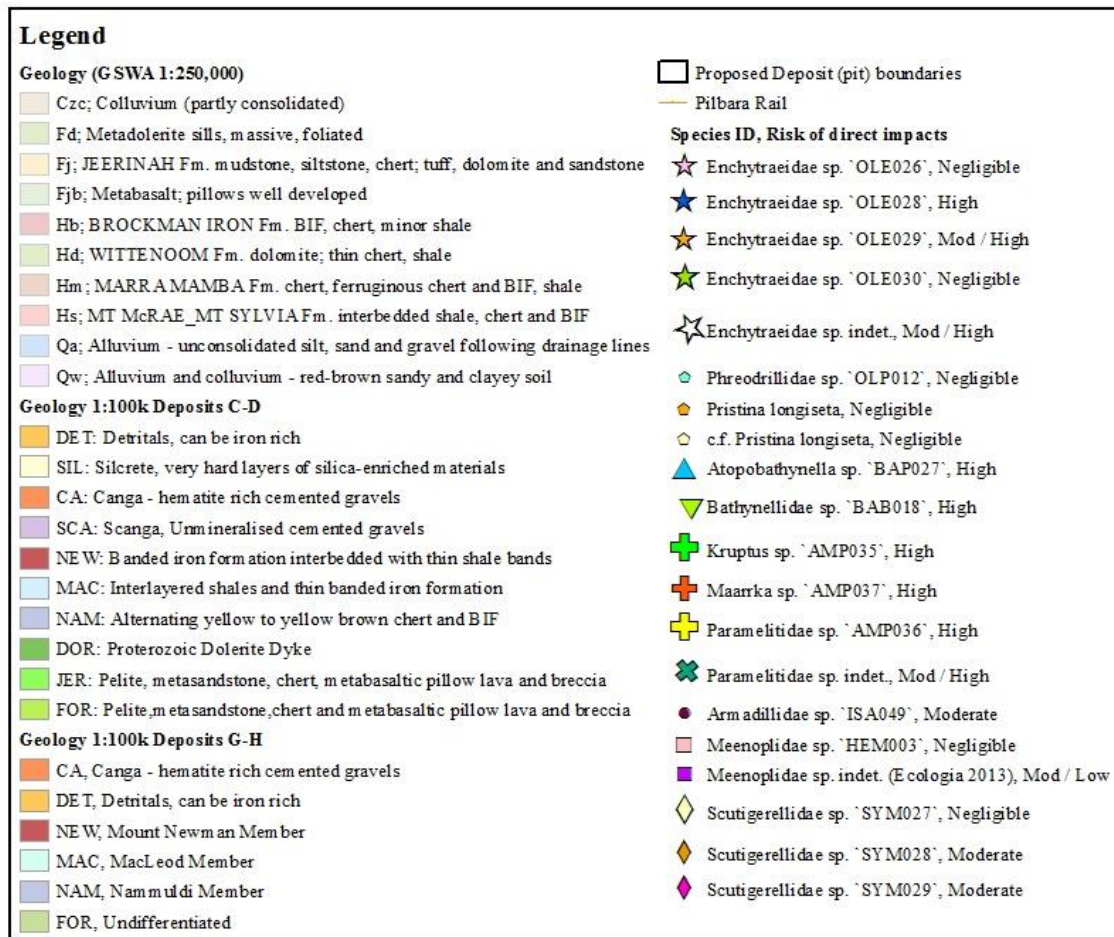


Figure 2 Legend.

## OVERALL RISK ASSESSMENT

This assessment considers the risk of direct impacts to subterranean fauna from the development of West Angelas Deposits C, D, and G. As defined in Biologic (2016), direct impacts on troglofauna occur as a result of the removal of habitat within the pit boundaries, while direct impacts on stygofauna include both direct removal of habitat within the pit and the associated drawdown of groundwater throughout permeable hydrogeological layers nearby (which is yet to be modelled precisely).

Including all survey results to date, the following eight troglofauna taxa were regarded to be potentially at risk from mining at Deposits C, D, and G (Figure 3):

### Moderate risk of direct impact:

- Isopoda: Armadillidae sp. 'ISA049' (Deposit D);
- Symphyla: Scutigerellidae sp. 'SYM028' (Deposit C);
- Symphyla: Scutigerellidae sp. 'SYM029' (Deposit C);
- Coleoptera: Anillini 'sp. indet.' (Ecologia 2013) (Deposit C);
- Coleoptera: *Hydrobiomorpha* 'sp. indet.' (Ecologia 2013) (Deposit D);
- Thysanura: Atelurinae 'sp. indet.' (Ecologia 2013) (Deposit D);

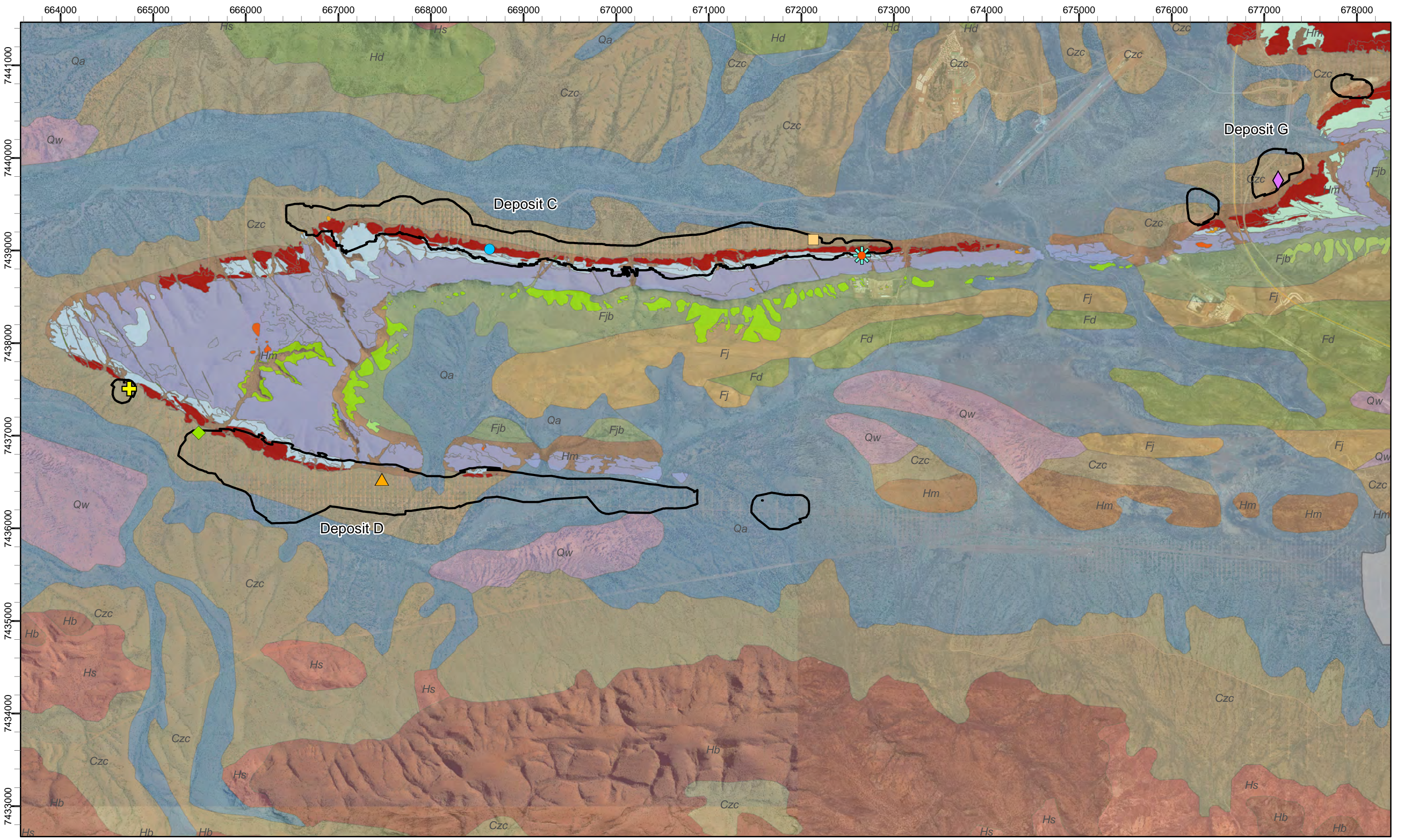
**Moderate / Low risk of direct impact:**

- Collembola: Cyphoderidae `sp. indet.` (Deposit C); and
- Hemiptera: Meenoplidae `sp. indet.` (Ecologia 2013) (Deposit G).

Each of these taxa are known only from locations within the proposed mining deposits, and were therefore regarded as being potentially at risk. The potential risk level was moderated by the location of many of the records (particularly Armadillidae sp. `ISA049`, Scutigerellidae sp. `SYM028`, Cyphoderidae `sp. indet.`, Anillini `sp. indet.`, and *Hydrobiomorpha* `sp. indet.`) very close to the deposit boundaries and the lack of any clear geological or geomorphological barriers between suitable habitat layers inside and outside of the deposits (as detailed in Biologic 2016). The current records of these taxa are likely to underestimate the actual distributions of these species throughout the subterranean habitat, owing to both the inherent difficulties in sampling rarely occurring fauna with limited means of dispersal, as well as the physical limitations of accessing the wider subterranean habitat using only suitable and available drill holes.

The potential risk level for Atelurinae `sp. indet.` and Meenoplidae `sp. indet.` was moderated by the likelihood that these taxa (originally collected by Ecologia, and unable to be compared with current specimens) could represent the same species as other representatives of these taxa found to occur outside of the deposits in the current survey. There also remains some doubt as to the likelihood that Cyphoderidae `sp. indet.` and Meenoplidae `sp. indet.` represent obligate (troglobitic) subterranean fauna or potentially facultative subterranean fauna, which may be less likely to be restricted to habitats within the deposits.





**Rio Tinto Iron Ore - West Angelas Project**  
**Deposits C, D, & G Subterranean Fauna Survey**  
**Fig. 3: Troglofauna taxa overall risk assessment**

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

Size A3. Created 24/10/2016



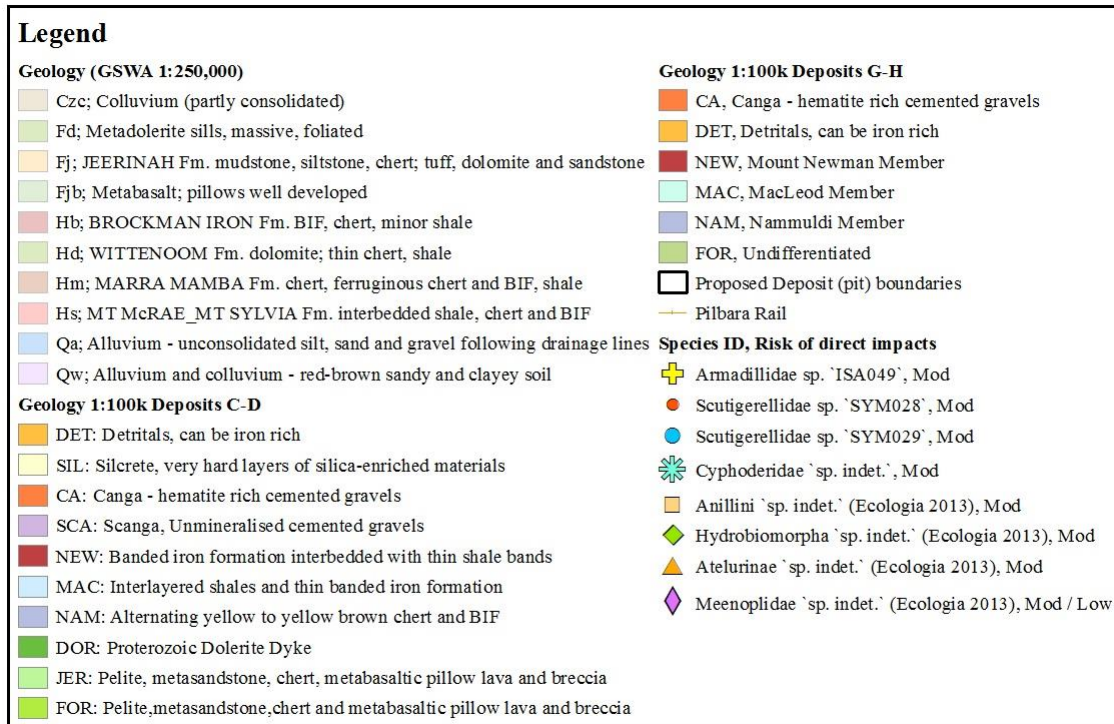


Figure 3 Legend.

The following 14 stygofauna taxa were regarded to be potentially at risk from mining and the associated groundwater drawdown at Deposits C and D (Figure 4):

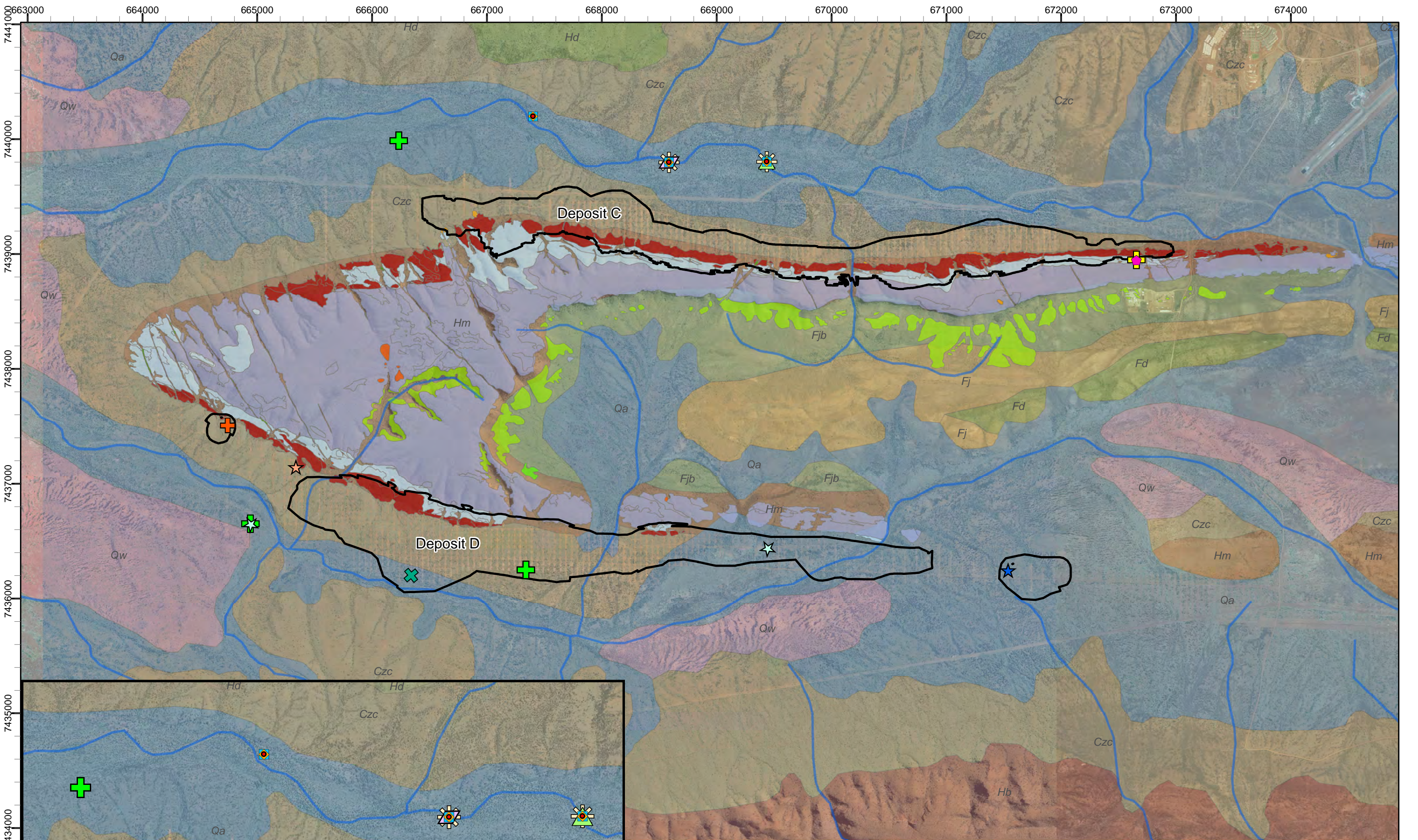
**High risk of direct impact:**


- Haplotaxida: Enchytraeidae sp. 'OLE028' (Deposit D);
- Haplotaxida: Enchytraeidae sp. 'OLE029' (near Deposit D);
- Amphipoda: *Kruptus* sp. 'AMP035' (near Deposit C and D);
- Amphipoda: *Maarrka* sp. 'AMP037' (Deposit D);
- Amphipoda: Paramelitidae sp. 'AMP036' (Deposit C);
- Bathynellacea: *Atopobathynella* sp. 'BAP027' (near Deposit C);
- Bathynellacea: Bathynellidae sp. 'BAB018' (near Deposit C);
- Harpacticoida: *Australocamptus* sp. 'B13' (near Deposit C); and

**Moderate / High risk of direct impact:**


- Haplotaxida: Enchytraeidae 'sp. indet.' (near Deposit D);
- Turbellaria: Turbellaria 'sp. indet.' (near Deposit C);
- Polychaeta: Aeolosomatidae 'sp. indet.' (Deposit C);
- Amphipoda: Paramelitidae 'sp. indet.' (Deposit D);
- Cyclopoida: *Thermocyclops* sp. 'WA' (near Deposit C); and
- Harpacticoida: *Parastenocaris* 'sp. indet.' (near Deposit C).







biologic  
Environmental Survey



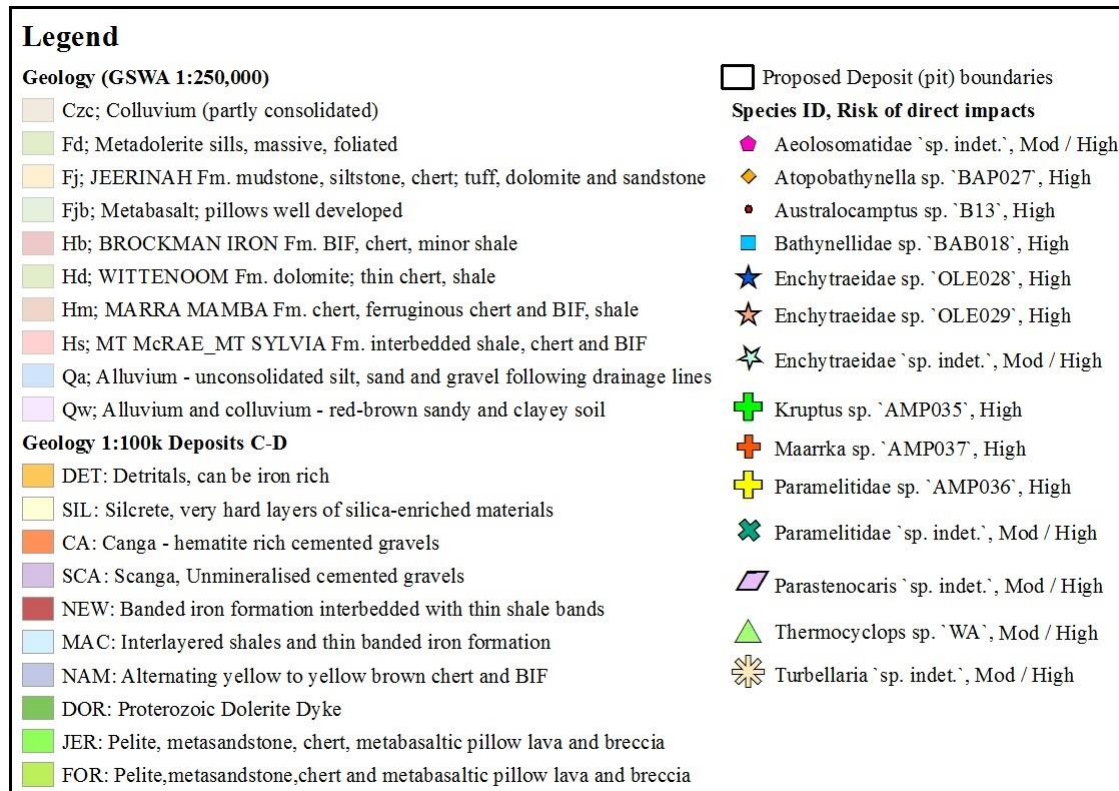
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0 700 1,400 2,800 4,200 km  
1:30,000

**Rio Tinto Iron Ore - West Angelas Project**  
**Deposits C, D, & G Subterranean Fauna Survey**  
**Fig. 4: Stygofauna taxa overall risk assessment**

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

Size A3. Created 24/10/2016





**Figure 4 Legend.**

The risk level for the five indeterminate taxa (identified as 'sp. indet.') was moderated by the taxonomic uncertainties regarding these taxa (or groups of specimens). Each of these nominal taxa was unable to be allocated to other existing morphospecies (or genetically determined species) on current information, and the possibility remains that they could occur more widely than the other 'high' risk taxa, as some widely occurring members of these groups are known regionally. Nevertheless, for Enchytraeidae 'sp. indet.' and Paramelitidae 'sp. indet.' in particular, it is also reasonably likely that some of the specimens could represent one of the other existing 'high' risk taxa in their respective groups.

The risk level for the 'high' risk stygofauna was primarily dependent upon the magnitude and extent of groundwater drawdown, which is yet to be modelled in detail, but which is inferred to extend throughout the orebody aquifers within the deposits and the alluvial/ detrital aquifers of the flanking valleys nearby (Biologic 2016). This is inferred to include all of the locations of stygofauna records from the current survey (which are all within 1 km of the deposit boundaries), on the basis of the available hydrogeological information. Nevertheless, it is possible that the risk of direct impact for stygofauna species may be subject to change with further information regarding groundwater drawdown.

It is also possible that the current species distributions may be partly attributed to sampling artefacts, as there has been no sampling for subterranean fauna to the immediate west of the current deposits within the same hydrogeological catchment (Turee Creek East Branch). Previous

stygo fauna collected further afield (approximately 15 km to the south west at Turee Creek Borefield), and to the immediate east within the Central Plateau area of West Angelas are not able to be compared with current specimens due to old/ incomplete identifications, incompatible specimen preservation, and a lack of genetic information.

Owing to the position of the deposits within the local catchment and the direction of flow from the Central Plateau to the northern flanking valley between Deposits C and G (as discussed in Biologic 2016), there is a reasonable likelihood that some of the current stygo fauna species could also occur in the Central Plateau, and/or further downstream to the west within the Turee Creek East sub-catchment.

Yours sincerely,



**Brad Durrant**

Principal Zoologist / Managing Director

[brad@biologicenv.com.au](mailto:brad@biologicenv.com.au)

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## REFERENCES

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- Ecologia Environmental Consultants (Ecologia) (2013). Greater West Angelas Subterranean Fauna Assessment. Unpublished report prepared for Rio Tinto Iron Ore
- Helix Molecular Solutions (2017) Report on the molecular systematics of subfauna. Unpublished report prepared for Biologic Environmental Survey c/o Alacran Environmental (Appendix I to this document)

## **Appendix I – Helix Molecular Solutions DNA Report**



# Helix

## Molecular Solutions

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2 April, 2017

Erich Volschenk

Via email

### **Re. Report on the molecular systematics of subfauna**

Dear Erich,

Following is a summary of the results of the subfauna study we have completed on eight taxonomic groups. Sixteen distinct genetic lineages were detected among the seven groups for which sequences were obtained. We did not obtain a sequence from the one Thysanura specimen. Four of the 16 lineages have been detected previously (one each of Enchytraeidae, Naididae, Phreodrilidae and Meenoplidae), whereas the remainder appear to be new or require further investigation.

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-six specimens of subfauna (troglofauna and stygofauna) belonging to eight taxonomic groups were sequenced for variation at the mitochondrial COXI or 12s genes. 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.

## Methods

Twenty-six specimens of subfauna were sequenced for variation at the mitochondrial cytochrome oxidase subunit I gene (COXI) using multiple pairs of primers (LCOI/HCO2, LCOI-long/HCO2-long, NemF1/NemR1 and LCOI/CIN2341). The 12s region was amplified and sequenced using primers 12-ai and 12s-bi (Simon et al., 1994).

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).

Genetic distances were calculated using p-distances, and neighbour-joining trees were constructed from those distances using MEGA 6.0 (Tamura et al., 2013).

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).

COXI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003a). In a comparison of COXI 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.

## Results and Conclusions

### Amphipoda

#### *Neighbour-joining analysis - Reference sequences and outgroups*

Five specimens of amphipods, identified on the basis of morphology as belonging to the Paramelitidae, were sequenced for COI. A search of similar sequences on Genbank indicated that the specimens were most similar to sequences of Paramelitidae, thus the sequences were analysed with reference specimens representing 49 distinct genetic lineages of Paramelitidae from the Pilbara. Two specimens of Niphargidae, *Niphargus fontanus* (Genbank accession #KC315635) and *Niphargus glenniei* (Genbank accession # KC315646) were used as outgroups. In order to reduce analysis time and simplify the presentation of results, a sub-set of reference lineages were selected based on the criteria that they were placed in clades containing the five target sequences in a preliminary NJ analysis.

The neighbour-joining analysis placed the five specimens into three lineages, which did not contain any reference lineages, therefore they were assigned to the new lineages AMP035 – AMP037 (Figure 1). Lineage AMP035 contained three specimens, whereas the remaining two lineages, AMP036 and AMP037, each contained a single specimen (Figure 1).

#### *Differentiation within and between lineages*

The three lineages of Paramelitidae detected in the present study differed from one another by between 17.5 and 18.7% sequence divergence (Table 1). Individuals within lineage AMP035 differed from one another by between 0.3 and 0.9% sequence divergence (Table 1).

All three lineages differed from the closest reference lineages by >15% sequence divergence (Table 1).

### Conclusions

Three lineages of amphipods were detected in the present study, belonging to the family Paramelitidae. The three lineages differed from one another and from the reference specimens by >15% sequence divergence, indicating that the three lineages represent three species that have so far not been detected in the Pilbara, based on the material available for comparison.

#### Paramelitidae

AMP035 = one species, new Paramelitidae

AMP036 = one species, new Paramelitidae

AMP037 = one species, new Paramelitidae

### Bathynellacea

#### *Neighbour-joining analysis - Reference sequences and outgroups*

Three specimens of Bathynellacea, assigned to the families Bathynellidae and Parabathynellidae on the basis of morphology, were sequenced for COI. The sequences were analysed with 47 reference lineages of Bathynellacea from the Pilbara, representing 65 specimens. Two species of Amphipoda were used as outgroups: *Chydaekata acuminata* Genbank accession #DQ838024 and *Maarka etheli* GenBank accession #DQ838031.

The neighbour-joining analysis placed the three specimens into two lineages, which did not contain any reference lineages, therefore they were assigned to the new lineages BAB018 and BAP027 (Figure 2). Lineage BAB018 contained a single specimen, whereas lineage BAP027 each contained two specimens (Figure 2). Lineage BAP027 was placed in a large clade containing reference specimens of Parabathynellidae, and lineage BAB018 was placed in a clade containing reference lineages of Bathynellidae.

#### *Differentiation within and between lineages*

Individuals within lineage BAP027 differed from one another by 0.3% sequence divergence (Table 2). Lineage BAP027 differed from the closest reference lineage (CA0084 from Callawa) by 10.8% sequence divergence (Table 2). Lineage BAB018 differed from the closest reference lineage (G456 from the Central Pilbara) by 19.6% sequence divergence (Table 2).

### Conclusions

Two lineages of Bathynellacea were detected in the present study, one each belonging to the families Bathynellidae and Parabathynellidae. The two lineages differed from the reference specimens by >10% sequence divergence, indicating that the two lineages represent two species that have so far not been detected in the Pilbara, based on the material available for comparison.

#### Bathynellidae

BAB018 = one species, new Bathynellidae

#### Parabathynellidae

BAP027 = one species, new Parabathynellidae

### Enchytraeidae

#### *Neighbour-joining analysis - Reference sequences*

Six specimens of Enchytraeidae were sequenced for COI. The sequences were analysed with 55 reference specimens of Enchytraeidae from Genbank and the Pilbara, representing 26 lineages.

The neighbour-joining analysis placed the six specimens into four lineages, three of which did not contain any reference lineages, therefore the three were assigned to new lineages OLE028 – OLE030 (Figure 3). One lineage also contained reference specimens from a previous survey in the Pilbara, and therefore was assigned to the existing lineage OLE026 (Figure 3). Lineages OLE028 and OLE029 each contained a single specimen, whereas lineage OLE030 contained two specimens (Figure 3).

#### *Differentiation within and between lineages*

The four lineages of Enchytraeidae detected in the present study differed from one another by between 7.4 and 17.1% sequence divergence (Table 3.) Individuals within lineage OLE026, which contained the two specimens from this study as well as two specimens from a previous survey, differed from one another by between 1.2 and 3.4%, and individuals within lineage OLE030 differed from one another by 1.6% sequence divergence (Table 3). Lineage OLE028 differed from the closest reference lineage (382 from Robe River) by 12.9% sequence divergence (Table 3). Lineage OLE029 differed from the closest reference lineage (EQ15) by 5.7% sequence divergence and OLE differed from the nearest reference specimen (EQ7 and GD10) by 7.8% (Table 3).

### Conclusions

Four lineages of Enchytraeidae were detected in the present study. They differed from one another by >7% sequence divergence. One of the lineages (OLE026) has been detected previously in the Pilbara. One lineage, OLE028, differed from the reference specimens by >approximately 13% indicating that it likely represents a species that has so far not been detected in the Pilbara, based on the material available for comparison. The remaining two lineages require further consideration. Lineages OLE029 and OLE030 were placed in a large clade including lineage OLE026 and containing specimens of the morphologically-defined species PST1 (Brown et al., 2015). Haplotypes within the lineage averaged 7.4% sequence divergence and cover a large geographical area covering six creek catchments and three river basins (Brown et al. 2015). Further investigation is required to resolve relationships among members of this group.

### Enchytraeidae

OLE028 = one species, new

OLE026 + OLE029 + OLE030 = species complex requiring further investigation; lineage OLE026 has been detected previously

### Naididae and Phreodrilidae

#### *Neighbour-joining analysis - Reference sequences*

Three additional specimens of Oligochaeta, which were not assigned to a taxonomic group, were sequenced for COI. A search of similar sequences on Genbank indicated that two belonged to the Naididae and one to the Phreodrilidae. For this reason, the sequences were analysed with 15 reference lineages of Naididae, representing five lineages from the Pilbara and Genbank, and 52 specimens of Phreodrilidae, representing 22 lineages from the Pilbara.

The neighbour-joining analysis placed the three specimens into two lineages, both of which also contained reference specimens (Figure 4). Two specimens were genetically identical and were placed in a lineage containing the reference specimen of *Pristina longiseta*, in the family Naididae (Figure 4). The second lineage was placed within Phreodrilidae lineage OLP012, which contained specimens from four drainage basins in the Pilbara (Brown et al., 2015).

#### *Differentiation within and between lineages*

The two specimens of Naididae from the present study were genetically identical, and differed from the reference specimen of *Pristina longiseta* by 1.7% sequence divergence (Table 4). The specimen of Phreodrilidae from the present study differed from the remaining specimens within lineage OLP012 by between 1.9 and 2.8% sequence divergence (Table 4).

### Conclusions

One lineage each of Naididae and Phreodrilidae were detected in the present study, each corresponding to a single species. Both belong to species that have been detected previously, one to the widespread Naididae species *Pristina longiseta*, and the second to the Phreodrilidae lineage OLP012, distributed widely in the Pilbara (Brown et al., 2015). The genus *Pristina* is known from North America, Central America, Europe, Asia and Australia (Rodrigues et al., 2015).

### Naididae

*Pristina longiseta*, world-wide distribution

### Phreodrilidae

OLP012 = one species, previously detected



### Hemiptera (Meenoplidae)

#### *Neighbour-joining analysis - Reference sequences*

Two specimens of Hemiptera, assigned to the family Meenoplidae, were sequenced for COI. The sequences were analysed with 26 reference specimens of Meenoplidae from the Pilbara.

The neighbour-joining analysis placed the two specimens in a single genetic lineage, which also contained reference specimens from previous surveys at Murrays Hill, Hardy River and Upper South Fortescue (Figure 5). Therefore the specimens from the present study were assigned to the existing lineage HEM003 (Figure 5).

#### *Differentiation within and between lineages*

The two specimens of Meenoplidae from the present study were genetically identical at COI and differed from the reference specimens from Murrays Hill, Hardy River and Upper South Fortescue by between 1.6 and 2.3% sequence divergence (Table 5).

#### *Conclusions*

The two specimens of Meenoplidae from the present study belonged to a single species, that has been detected previously at Murrays Hill, Hardy River and Upper South Fortescue.

#### Meenoplidae

HEM003 = one species, detected previously

### Isopoda

#### *Neighbour-joining analysis - Reference sequences*

Two specimens of Isopoda were sequenced for COI. The specimens were not further assigned to family. A search of similar sequences on Genbank indicated that the specimens were most similar to sequences of Troglarmadillo, thus the sequences were analysed with reference specimens representing 48 distinct genetic lineages of presumptive Armadillidae from the Pilbara, representing 66 specimens. In order to reduce analysis time and simplify the presentation of results, a sub-set of reference lineages were selected based on the criteria that they were placed in clades or near-by clades containing the two target sequences in a preliminary NJ analysis. Thirty-five lineages representing 46 specimens were used in the final NJ analysis.

The neighbour-joining analysis placed the two specimens into a single lineage, which did not contain any reference lineages, therefore it was assigned to the new lineage ISA049 (Figure 6). The lineage was placed in a clade containing reference specimens of Troglarmadillo from the Pilbara (Figure 6).

#### *Differentiation within and between lineages*

The two specimens in lineage ISA049 differed from one another by 0.3% sequence divergence (Table 6). Lineage ISA049 differed from the closest reference lineages by >18% sequence divergence (Table 6).

#### *Conclusions*

A single lineage of Isopoda was detected in the present study, likely belonging to the family Armadillidae. The lineage differed from the reference specimens by >18% sequence divergence, indicating that it likely represents a new species that has so far not been detected in the Pilbara, based on the material available for comparison.

#### Armadillidae

ISA049 = one species, new

### Symphyla

#### *Neighbour-joining analysis - Reference sequences*

Three specimens of Symphyla were sequenced for COI. Two were assigned to the family Scutigerellidae, on the basis of morphology, whereas the third was not further assigned. A search of similar sequences on Genbank and the Helix database indicated that the specimens were most similar to sequences of Scutigerellidae, thus the sequences were analysed with reference specimens representing 26 distinct genetic lineages of presumptive Scutigerellidae from the Pilbara.

The neighbour-joining analysis placed the three specimens into three distinct genetic lineages, which did not contain any reference lineages, and were in fact most similar to one another (Figure 7). The three lineages were therefore assigned to the new lineages SYM027, SYM028 and SYM029 (Figure 7).

*Differentiation within and between lineages*

The three lineages from the present study differed from one another by between 4.9 and 17.5% sequence divergence (Table 7). The three lineages differed from the closest reference lineages by >16% sequence divergence (Table 7).

*Conclusions*

Three distinct lineages of Symphyla were detected in the present study, likely belonging to the family Scutigerellidae. Two of the lineages, SYM028 and SYM029, showed moderately low genetic differentiation from one another (approximately 5%), thus require further consideration. The two lineages may represent incipient species, or differentiation within a single species between geographically separate sites, arising from the poor dispersal abilities of the taxon. Sampling of intermediate sites may help resolve their relationships. The three lineages differed from the reference specimens by >16% sequence divergence, indicating that they are likely to represent new species that have so far not been detected in the Pilbara, based on the material available for comparison.

Scutigerellidae:

SYM027 = one species, new

SYM028 + SYM029 = one or two new species, requiring further investigation

Thysanura

A DNA sequence was not obtained from the specimen of Thysanura.

**References**

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- Simon C, Frati F, Beckenbach AT, Crespi B, Liu H, et al. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am* 87: 651–701.
- Koichiro Tamura, Glen Stecher, Daniel Peterson, Alan Filipski, and Sudhir Kumar (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*:30 2725–2729.



Table 1. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Amphipoda detected in the present study and the reference lineages as shown in Figure 1.

Table 2. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Bathynellacea detected in the present study and the reference lineages as shown in Figure 2.

Table 3. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Enchytraeidae detected in the present study and the reference lineages as shown in Figure 3.

Table 4. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Naididae and Phreodrilidae detected in the present study and the reference lineages as shown in Figure 4.

Table 5. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Meenoplidae detected in the present study and the reference lineages as shown in Figure 5.

Table 6. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Isopoda detected in the present study and the reference lineages as shown in Figure 6.

Table 7. (attached). Genetic distances (below diagonal) and standard error (above diagonal, in blue) between specimens of Symphyla detected in the present study and the reference lineages as shown in Figure 7.

Figure 1. Neighbour-joining analysis of specimens of Amphipoda from the present study. Numbers on major nodes correspond to bootstrap support over 100 iterations. Scale bar= genetic distance. The specimens from the present study are highlighted in yellow; Genbank voucher specimens are highlighted in turquoise.

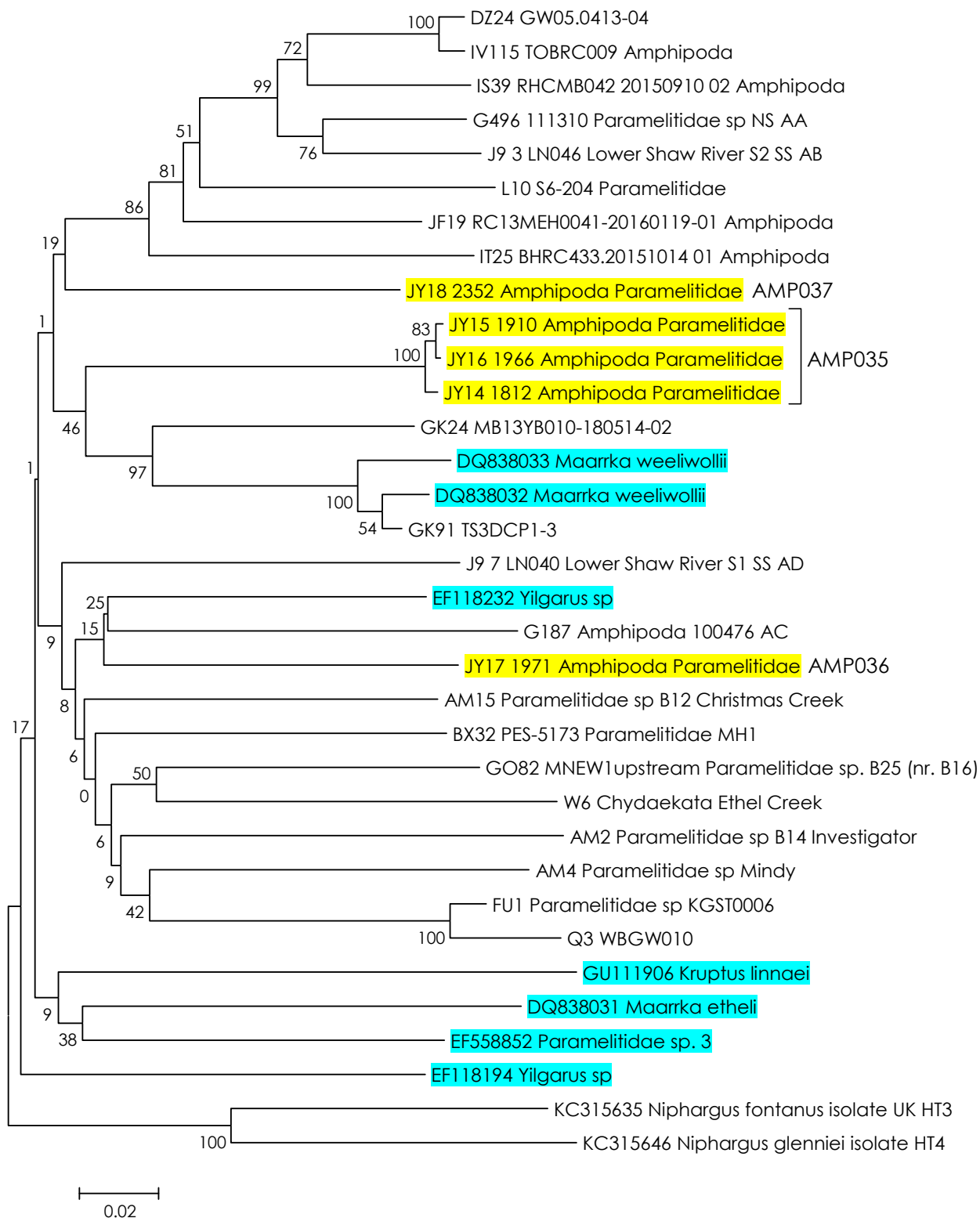




TABLE 1. Amphipod Distance Matrix																																		
Specimen ID		JY14 1812 Amphipoda Paramelitidae	JY15 1910 Amphipoda Paramelitidae	JY16 1966 Amphipoda Paramelitidae	JY17 1971 Amphipoda Paramelitidae	JY18 2352 Amphipoda Paramelitidae	AM15 Paramelitidae sp B12 Christmas Creek	AM2 Paramelitidae sp B14 Investigator	AM4 Paramelitidae sp Mindy	BX32 PES-5173 Paramelitidae MH1	DQ838031 Maarka etheli	DQ838032 Maarka weeliwolli	DQ838033 Maarka weeliwolli	DZ24 GW05.0413-04	EF118194 Yilgarus sp	EF118232 Yilgarus sp	EF558852 Paramelitidae sp. 3	FU1 Paramelitidae sp KGST0006	G187 Amphipoda 100476 AC	G496 111310 Paramelitidae sp NS AA	GK24 MB13YB010-180514-02	GK91 TS3DCP1-3	GO82 MNEW1upstream Paramelitidae sp. B25 (nr. B16)	GU111906 Kruptus linnaei	IS39 RHCMB042 20150910 02 Amphipoda	IT25 BHRC-433.20151014 01 Amphipoda	IV115 TOBRC009 Amphipoda	J9 3 LN046 Lower Shaw River S2 SS AB	J9 7 LN040 Lower Shaw River S1 SS AD	JF19 RC13MEH0041-20160119-01 Amphipoda	L10 S6-204 Paramelitidae	Q3 WBGW010	W6 Chykaeta Ethel Creek	
JY14 1812 Amphipoda Paramelitidae			0.003	0.003	0.015	0.014	0.015	0.015	0.015	0.016	0.016	0.015	0.015	0.016	0.017	0.014	0.016	0.014	0.015	0.015	0.014	0.014	0.015	0.018	0.014	0.019	0.015	0.016	0.016	0.015	0.017	0.019	0.015	
JY15 1910 Amphipoda Paramelitidae	0.009			0.002	0.016	0.014	0.016	0.015	0.015	0.015	0.016	0.015	0.016	0.016	0.016	0.015	0.016	0.014	0.015	0.015	0.015	0.014	0.014	0.015	0.018	0.014	0.019	0.015	0.016	0.016	0.015	0.017	0.019	0.015
JY16 1966 Amphipoda Paramelitidae	0.006	0.003			0.016	0.014	0.015	0.015	0.015	0.015	0.016	0.015	0.016	0.016	0.016	0.015	0.016	0.014	0.015	0.015	0.015	0.014	0.014	0.015	0.018	0.014	0.019	0.015	0.016	0.016	0.015	0.017	0.019	0.015
JY17 1971 Amphipoda Paramelitidae	0.181	0.181	0.181			0.014	0.015	0.015	0.015	0.015	0.016	0.015	0.015	0.015	0.015	0.014	0.015	0.015	0.015	0.015	0.015	0.014	0.014	0.015	0.016	0.014	0.019	0.015	0.016	0.017	0.014	0.015	0.020	0.014
JY18 2352 Amphipoda Paramelitidae	0.177	0.178	0.175	0.187			0.014	0.015	0.015	0.015	0.015	0.015	0.015	0.014	0.014	0.015	0.014	0.016	0.014	0.013	0.013	0.014	0.013	0.016	0.013	0.018	0.014	0.014	0.017	0.013	0.016	0.021	0.015	
AM15 Paramelitidae sp B12 Christmas Creek	0.207	0.208	0.205	0.180	0.188			0.013	0.016	0.016	0.016	0.014	0.016	0.016	0.015	0.016	0.016	0.014	0.015	0.015	0.012	0.013	0.014	0.018	0.016	0.020	0.016	0.018	0.016	0.013	0.017	0.021	0.016	
AM2 Paramelitidae sp B14 Investigator	0.219	0.223	0.220	0.219	0.226	0.214			0.016	0.015	0.017	0.016	0.015	0.015	0.018	0.015	0.018	0.015	0.017	0.015	0.014	0.015	0.015	0.017	0.015	0.020	0.015	0.017	0.018	0.015	0.015	0.021	0.016	
AM4 Paramelitidae sp Mindy	0.226	0.228	0.225	0.207	0.222	0.193	0.211			0.016	0.015	0.014	0.015	0.017	0.017	0.017	0.015	0.016	0.017	0.017	0.016	0.014	0.016	0.019	0.015	0.019	0.017	0.016	0.017	0.017	0.018	0.020	0.017	
BX32 PES-5173 Paramelitidae MH1	0.202	0.199	0.201	0.194	0.191	0.178	0.204	0.195			0.014	0.016	0.016	0.018	0.017	0.016	0.015	0.018	0.015	0.017	0.016	0.015	0.015	0.016	0.017	0.019	0.018	0.017	0.017	0.014	0.015	0.020	0.016	
DQ838031 Maarka etheli	0.233	0.236	0.236	0.245	0.214	0.231	0.229	0.231	0.233			0.017	0.018	0.018	0.014	0.015	0.014	0.016	0.016	0.015	0.015	0.015	0.017	0.015	0.017	0.017	0.022	0.018	0.018	0.017	0.017	0.019	0.024	0.016
DQ838032 Maarka weeliwolli	0.170	0.173	0.173	0.205	0.184	0.199	0.234	0.225	0.216	0.194			0.006	0.014	0.015	0.015	0.016	0.013	0.017	0.015	0.013	0.005	0.015	0.019	0.015	0.021	0.015	0.016	0.016	0.013	0.018	0.021	0.014	
DQ838033 Maarka weeliwolli	0.178	0.183	0.182	0.219	0.190	0.201	0.234	0.229	0.224	0.201	0.030			0.015	0.016	0.015	0.016	0.015	0.017	0.015	0.013	0.007	0.016	0.018	0.015	0.021	0.015	0.017	0.016	0.014	0.018	0.021	0.015	
DZ24 GW05.0413-04	0.206	0.209	0.209	0.206	0.201	0.212	0.239	0.237	0.224	0.230	0.214	0.218			0.016	0.015	0.017	0.015	0.014	0.011	0.016	0.014	0.016	0.019	0.011	0.017	0.005	0.010	0.017	0.012	0.014	0.023	0.016	
EF118194 Yilgarus sp	0.197	0.199	0.199	0.217	0.191	0.216	0.254	0.243	0.202	0.236	0.211	0.221	0.209			0.016	0.015	0.017	0.015	0.017	0.014	0.014	0.016	0.019	0.016	0.019	0.016	0.016	0.018	0.016	0.016	0.021	0.016	
EF118232 Yilgarus sp	0.212	0.213	0.212	0.172	0.190	0.194	0.223	0.212	0.176	0.238	0.200	0.205	0.209	0.197			0.015	0.015	0.014	0.017	0.015	0.015	0.015	0.017	0.015	0.021	0.015	0.018	0.015	0.017	0.016	0.020	0.016	
EF558852 Paramelitidae sp. 3	0.199	0.196	0.198	0.222	0.191	0.213	0.242	0.236	0.219	0.202	0.194	0.203	0.207	0.211	0.202			0.018	0.015	0.015	0.016	0.015	0.014	0.018	0.015	0.022	0.017	0.016	0.015	0.017	0.017	0.025	0.014	
FU1 Paramelitidae sp KGST0006	0.204	0.202	0.202	0.210	0.223	0.175	0.217	0.184	0.194	0.226	0.217	0.224	0.225	0.233	0.197	0.211			0.016	0.015	0.015	0.012	0.014	0.019	0.017	0.021	0.015	0.017	0.014	0.015	0.019	0.010	0.016	
G187 Amphipoda 100476 AC	0.219	0.223	0.221	0.192	0.215	0.203	0.227	0.210	0.222	0.244	0.224	0.224	0.208	0.213	0.184	0.212	0.213			0.014	0.018	0.016	0.015	0.017	0.014	0.021	0.014	0.015	0.017	0.015	0.017	0.021	0.015	
G496 111310 Paramelitidae sp NS AA	0.208	0.211	0.210	0.208	0.205	0.212	0.233	0.215	0.212	0.226	0.220	0.226	0.079	0.219	0.205	0.211	0.229	0.208				0.015	0.015	0.015	0.018	0.011	0.020	0.011	0.009	0.016	0.013	0.014	0.023	0.015
GK24 MB13YB010-180514-02	0.178	0.175	0.175	0.208	0.183	0.190	0.218	0.221	0.204	0.205	0.140	0.145	0.208	0.202	0.187	0.179	0.212	0.207	0.212			0.011	0.014	0.019	0.014	0.019	0.016	0.016	0.016	0.014	0.019	0.023	0.016	
GK91 TS3DCP1-3	0.158	0.161	0.161	0.199	0.174	0.193	0.224	0.218	0.205	0.193	0.017	0.046	0.210	0.205	0.197	0.184	0.210	0.219	0.211	0.117			0.013	0.019	0.014	0.021	0.014	0.016	0.014	0.013	0.018	0.021	0.014	
GO82 MNEW1upstream Paramelitidae sp. B25 (nr. B16)	0.222	0.219	0.221	0.190	0.199	0.178	0.215	0.193	0.172	0.225	0.198	0.198	0.221	0.227	0.166	0.208	0.180	0.218	0.221	0.191	0.180			0.018	0.015	0.020	0.016	0.018	0.016	0.016	0.016	0.018	0.015	
GU111906 Kruptus linnaei	0.242	0.244	0.244	0.230	0.248	0.253	0.248	0.251	0.234	0.250	0.240	0.240	0.232	0.244	0.253	0.226	0.234	0.253	0.226	0.238	0.236	0.257			0.018	0.024	0.018	0.018	0.019	0.017	0.019	0.020	0.016	
IS39 RHCMB042 20150910 02 Amphipoda	0.207	0.211	0.210	0.196	0.189	0.217	0.228	0.216	0.219	0.236	0.220	0.223	0.081	0.216	0.186	0.214	0.241	0.203	0.089	0.219	0.211	0.216	0.244			0.016	0.011	0.013	0.016	0.012	0.014	0.023	0.015	
IT25 BHRC433.20151014 01 Amphipoda	0.195	0.195	0.195	0.200	0.173	0.238	0.240	0.228	0.209	0.214	0.183	0.195	0.155	0.195	0.224	0.214	0.231	0.244	0.173	0.195	0.180	0.221	0.244	0.166			0.017	0.017	0.023	0.016	0.017	0.022	0.023	
IV115 TOBRC009 Amphipoda	0.199	0.202	0.202	0.209	0.204	0.218	0.232	0.240	0.220	0.229	0.213	0.217	0.013	0.218	0.208	0.208	0.218	0.209	0.083	0.213	0.210	0.219	0.228	0.081	0.149			0.011	0.017	0.012	0.014	0.022	0.016	
J9 3 LN046 Lower Shaw River S2 SS AB	0.215	0.217	0.217	0.204	0.219	0.230	0.245	0.206	0.220	0.228	0.217	0.217	0.108	0.221	0.197	0.200	0.226	0.223	0.076	0.228	0.210	0.199	0.239	0.111	0.173	0.109		0.016	0.013	0.014	0.021	0.015		
J9 7 LN040 Lower Shaw River S1 SS AD	0.219	0.219	0.219	0.210	0.200	0.191	0.245	0.230	0.192	0.213	0.195	0.204	0.215	0.215	0.182	0.208	0.182	0.219	0.221	0.193	0.189	0.213	0.243	0.232	0.223	0.215	0.224		0.017	0.018	0.018	0.018		
JF19 RC13MEH0041-20160119-01 Amphipoda	0.206	0.211	0.208	0.203	0.183	0.205	0.221	0.201	0.189	0.223	0.201	0.201	0.126	0.199	0.174	0.217	0.210	0.207	0.125	0.204	0.191	0.203	0.217	0.128	0.151	0.130	0.134	0.204		0.013	0.021	0.016		
L10 S6-204 Paramelitidae	0.231	0.233	0.233	0.212	0.219	0.234	0.238	0.217	0.222	0.228	0.210	0.2																						

Estimates of Evolutionary Divergence between Sequences  
The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 34 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for [1.] Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. (2013). MEGA6 : Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.  
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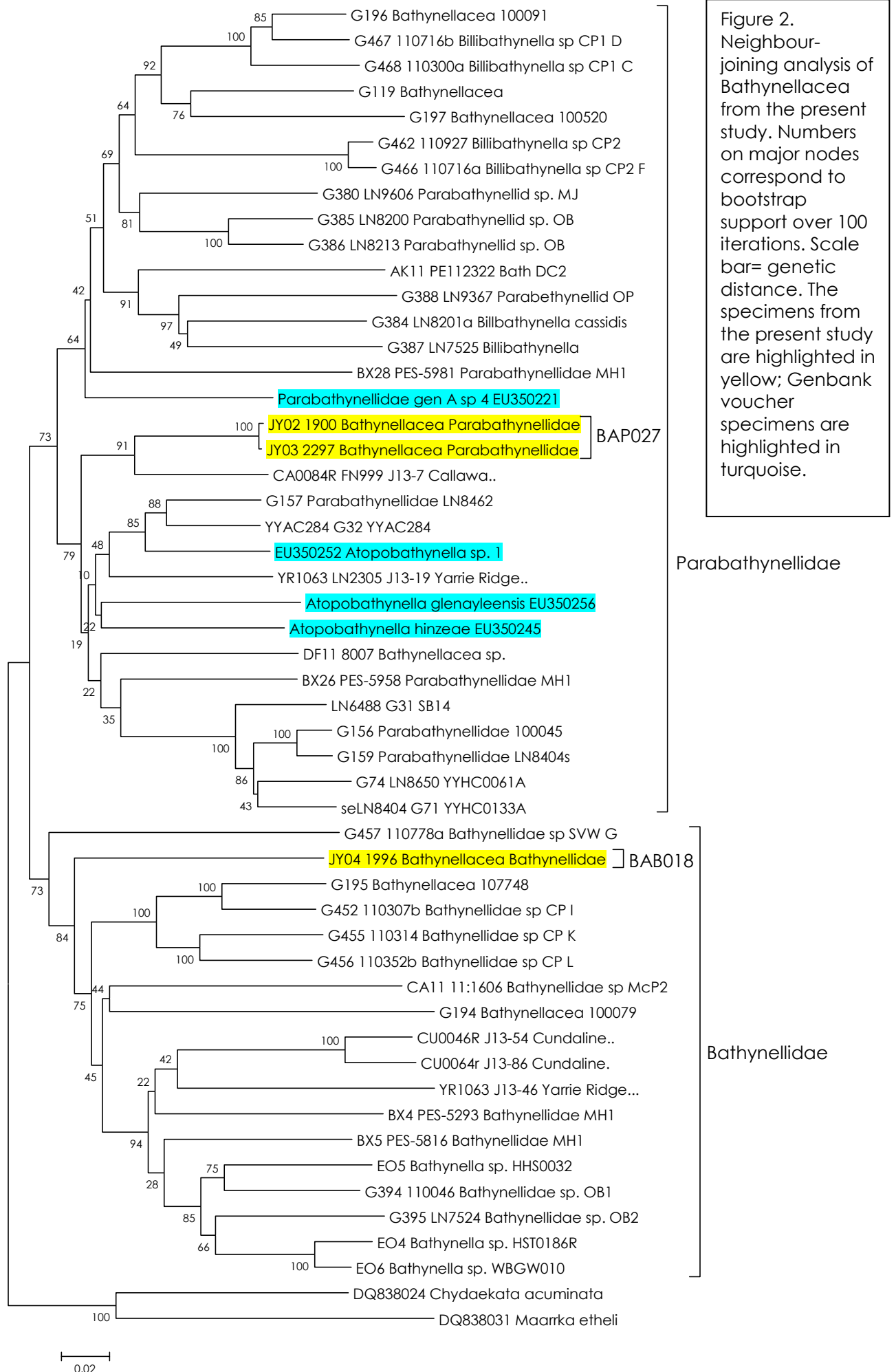




TABLE 2. Bathynellacea Distance Matrix

Specimen ID	Y02 1900 Bathynellacea Parabathynellidae	YJ03 2297 Bathynellacea Parabathynellidae	YJ04 1996 Bathynellacea Bathynellidae	AK11 PE112322 Bath DC2	Apobathynella glenyleensis EU350256	Apobathynella hizeae EU350245	BX26 PES-5958 Parabathynellidae MH1	BX28 PES-5981 Parabathynellidae MH1	BX4 PES-5293 Bathynellidae MH1	BX5 PES-3814 Bathynellidae MH1	CA0084R FN999 J13-7 Callawa	CA11 11:1606 Bathynellidae sp McP2	CU0046R J13-54 Cundaline	CU0064r J13-86 Cundaline	DF11 8007 Bathynellacea sp.	E04 Bathynella sp. HST0186R	E05 Bathynella sp. HHS0032	E06 Bathynella sp. WBGW010	EU350252 Apobathynella sp. 1	G119 LCOI Plate1C4	G156 Parabathynellidae 100045	G157 Parabathynellidae LN8442	G159 Parabathynellidae LN8404s	G194 Bathynellacea 100079	G195 Bathynellacea 107748	G196 Bathynellacea 100091	G197 Bathynellacea 100520	G380 LN9604 Parabathynellid sp. MJ	G384 LN8201 a Billibathynella cossidis	G385 LN8200 Parabathynellid sp. OB	G386 LN8213 Parabathynellid sp. OB	G387 LN7525 Billibathynella	G388 LN9367 Parabathynellid OP	G394 110046 Bathynellidae sp. OB1	G395 LN7524 Bathynellidae sp. OB2	G452 110307b Bathynellidae sp CP I	G455 110314 Bathynellidae sp CP K	G456 110352b Bathynellidae sp CP L	G457 110778a Bathynellidae sp SVW G	G462 110927 Billibathynella sp CP2	G466 110716a Billibathynella sp CP2 F	G467 110714b Billibathynella sp CP1 D	G468 110300a Billibathynella sp CP1 C	G74 LN8650 YHHC0061A	N6488 G31 3814	Parabathynellidae gen A sp 4 EU350221	seLN8404 G71 YHHC0133A	IR1063 J13-46 Yarnie Ridge	IR1063 LN2005 J13-19 Yarnie Ridge	YYAC284 G32 YYAC284				
JY02 1900 Bathynellacea Parabathynellidae	0.002	0.016	0.019	0.017	0.015	0.014	0.016	0.018	0.016	0.013	0.017	0.023	0.024	0.018	0.017	0.016	0.017	0.016	0.018	0.020	0.017	0.020	0.020	0.017	0.018	0.019	0.017	0.015	0.016	0.015	0.017	0.018	0.016	0.015	0.017	0.015	0.015	0.015	0.015	0.014	0.016	0.016	0.016	0.016	0.017	0.018	0.017	0.018	0.018	0.017	0.016			
JY03 2297 Bathynellacea Parabathynellidae	0.003	0.016	0.019	0.017	0.014	0.016	0.018	0.016	0.013	0.017	0.023	0.024	0.017	0.018	0.016	0.017	0.015	0.018	0.019	0.017	0.019	0.019	0.017	0.019	0.017	0.018	0.019	0.017	0.015	0.016	0.015	0.017	0.018	0.016	0.015	0.017	0.015	0.015	0.015	0.014	0.016	0.016	0.016	0.017	0.018	0.017	0.018	0.018	0.017	0.016				
JY04 1996 Bathynellacea Bathynellidae	0.236	0.236	0.018	0.019	0.018	0.014	0.016	0.016	0.016	0.015	0.016	0.020	0.019	0.020	0.017	0.016	0.018	0.019	0.021	0.017	0.020	0.018	0.014	0.018	0.020	0.016	0.018	0.020	0.017	0.015	0.015	0.015	0.015	0.014	0.016	0.017	0.018	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.017	0.018	0.017	0.018	0.018	0.017	0.016			
AK11 PE112322 Bath DC2	0.233	0.231	0.261	0.019	0.021	0.016	0.017	0.020	0.021	0.018	0.021	0.024	0.024	0.024	0.021	0.019	0.020	0.021	0.019	0.020	0.021	0.019	0.020	0.021	0.019	0.020	0.021	0.017	0.017	0.015	0.016	0.016	0.017	0.020	0.020	0.020	0.023	0.019	0.019	0.020	0.018	0.018	0.018	0.019	0.019	0.019	0.020	0.019	0.021	0.017	0.019			
Apobathynella glenyleensis EU350256	0.177	0.175	0.232	0.224	0.016	0.016	0.018	0.017	0.024	0.019	0.021	0.021	0.021	0.018	0.021	0.018	0.021	0.021	0.018	0.020	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.019	0.017	0.017	0.020	0.020	0.020	0.023	0.019	0.019	0.020	0.018	0.018	0.018	0.019	0.019	0.019	0.020	0.019	0.021	0.015	0.015				
Apobathynella hizeae EU350245	0.144	0.142	0.242	0.240	0.159	0.017	0.020	0.022	0.023	0.019	0.020	0.024	0.024	0.021	0.022	0.022	0.022	0.016	0.018	0.021	0.018	0.020	0.025	0.024	0.019	0.020	0.022	0.022	0.022	0.022	0.021	0.019	0.019	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018			
BX26 PES-5958 Parabathynellidae MH1	0.168	0.168	0.228	0.236	0.180	0.178	0.015	0.017	0.017	0.015	0.017	0.021	0.021	0.017	0.018	0.017	0.018	0.016	0.017	0.016	0.017	0.015	0.018	0.017	0.015	0.018	0.017	0.015	0.016	0.015	0.016	0.013	0.015	0.014	0.015	0.015	0.016	0.015	0.016	0.015	0.016	0.015	0.016	0.015	0.016	0.015	0.016	0.015	0.016	0.015	0.016			
BX28 PES-5981 Parabathynellidae MH1	0.219	0.222	0.262	0.241	0.219	0.207	0.216	0.018	0.019	0.017	0.016	0.022	0.023	0.022	0.018	0.017	0.018	0.018	0.020	0.020	0.020	0.019	0.017	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018		
BX4 PES-5293 Bathynellidae MH1	0.259	0.258	0.230	0.265	0.267	0.263	0.244	0.296	0.014	0.018	0.017	0.019	0.020	0.021	0.014	0.013	0.015	0.018	0.021	0.019	0.019	0.019	0.020	0.018	0.021	0.018	0.021	0.018	0.018	0.017	0.019	0.018	0.017	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019		
BX5 PES-5816 Bathynellidae MH1	0.236	0.236	0.215	0.266	0.253	0.240	0.238	0.280	0.018	0.014	0.021	0.021	0.024	0.013	0.014	0.014	0.020	0.021	0.021	0.021	0.021	0.021	0.018	0.018	0.021	0.018	0.021	0.019	0.018	0.019	0.018	0.019	0.018	0.019	0.018	0.017	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016		
CA0084R FN999 J13-7 Callawa	0.108	0.108	0.235	0.223	0.174	0.171	0.152	0.219	0.261	0.243	0.019	0.021	0.022	0.020	0.019	0.017	0.019	0.020	0.021	0.020	0.019	0.020	0.019	0.016	0.018	0.021	0.015	0.016	0.016	0.016	0.016	0.017	0.017	0.017	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
CA11 11:1606 Bathynellidae sp McP2	0.264	0.264	0.232	0.295	0.259	0.253	0.263	0.274	0.234	0.235	0.279	0.023	0.024	0.024	0.018	0.016	0.019	0.022	0.020	0.018	0.019	0.022	0.020	0.018	0.019	0.021	0.021	0.021	0.017	0.017	0.017	0.017	0.018	0.019	0.016	0.019	0.017	0.018	0.019	0.017	0.018	0.019	0.017	0.018	0.019	0.017	0.018	0.019	0.017	0.018	0.019	0.017	0.018	
CU0046R J13-54 Cundaline	0.274	0.271	0.226	0.273	0.260	0.279	0.267	0.307	0.210	0.214	0.245	0.264	0.010	0.025	0.021	0.020	0.021	0.021	0.023	0.024	0.022	0.022	0.025	0.021	0.022	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	
CU0064r J13-86 Cundaline	0.283	0.281	0.224	0.282	0.262	0.279	0.265	0.312	0.210	0.219	0.260	0.262	0.057	0.025	0.020	0.019	0.021	0.021	0.022	0.022	0.022	0.022	0.021	0.024	0.021	0.022	0.025	0.024	0.023	0.022	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	
DF11 8007 Bathynellacea sp.	0.189	0.147	0.226	0.252	0.178	0.163	0.176	0.243	0.288	0.255	0.167	0.281	0.266	0.269	0.026	0.025	0.025	0.020	0.019	0.019	0.018	0.018	0.024	0.022	0.021	0.020	0.021	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	
E04 Bathynella sp. HST0186R	0.252	0.253	0.216	0.275	0.240	0.255	0.242	0.291	0.200	0.157	0.254	0.247	0.202	0.207	0.012	0.007	0.021	0.021	0.022	0.020	0.021	0.018	0.017	0.020	0.022	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	
E05 Bathynella sp. HHS0032	0.246	0.244	0.219	0.268	0.244	0.255	0.233	0.291	0.197	0.168	0.249	0.251	0.205	0.202	0.272	0.130	0.013	0.020	0.020	0.021	0.021	0.021	0.019	0.016	0.020	0.023	0.017	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018
E06 Bathynella sp. WBGW010	0.237	0.239	0.216	0.268	0.238	0.240	0.236	0.281	0.199	0.153	0.248	0.235	0.188	0.193	0.255	0.039	0.133	0.021	0.020	0.022	0.020	0.021	0.018	0.016	0.021	0.022	0.021	0.017	0.018	0.017	0.018	0.017	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018
EU350252 Apobathynella sp. 1	0.154	0.152	0.213	0.231	0.159	0.144	0.153	0.198	0.273	0.232	0.150	0.245	0.255	0.164	0.121	0.248	0.238	0.232	0.018	0.019	0.013	0.018	0.025	0.020	0.017	0.017	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	
G119 LCOI Plate1C4	0.208	0.205	0.275	0.223	0.225	0.221	0.234	0.214	0.277	0.268	0.225	0.299	0.294	0.294	0.219	0.277	0.290	0.268	0.214	0.020	0.018	0.020	0.025	0.021	0.019	0.017	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	
G156 Parabathynellidae 100045	0.200	0.198	0.225	0.242	0.182	0.198	0.161	0.244	0.276	0.230	0.195	0.253	0.276	0.267	0.168	0.248	0.238	0.208	0.182	0.241	0.015	0.008	0.022	0.022	0.019	0.019	0.020	0.020	0.018	0.019	0.020	0.020	0.018																					

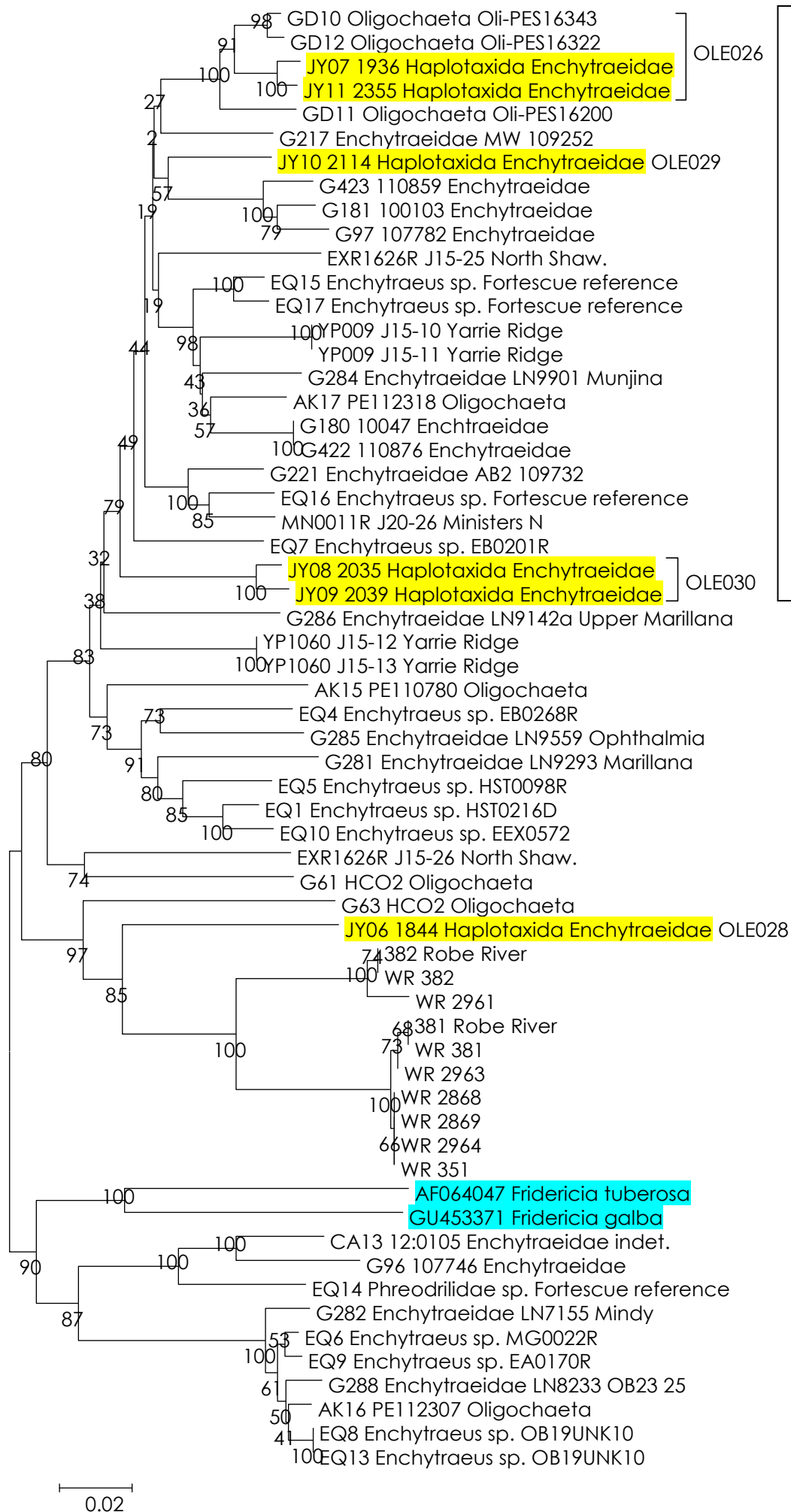


Figure 3. Neighbour-joining analysis of specimens of Enchytraeidae (Haplotaxida) from the present study. Numbers on major nodes correspond to bootstrap support over 100 iterations. Scale bar= genetic distance. The specimens from the present study are highlighted in yellow; Genbank voucher specimens are highlighted in turquoise.



TABLE 3. Enchytraeidae Distance Matrix

[illegible]

Table. Estimates of Evolutionary Divergence between Sequences  
The number of base differences per site from the reference sequences are shown. Standard error estimates (s.e.) are shown above each value. The analysis involved 61 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 721 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [1].

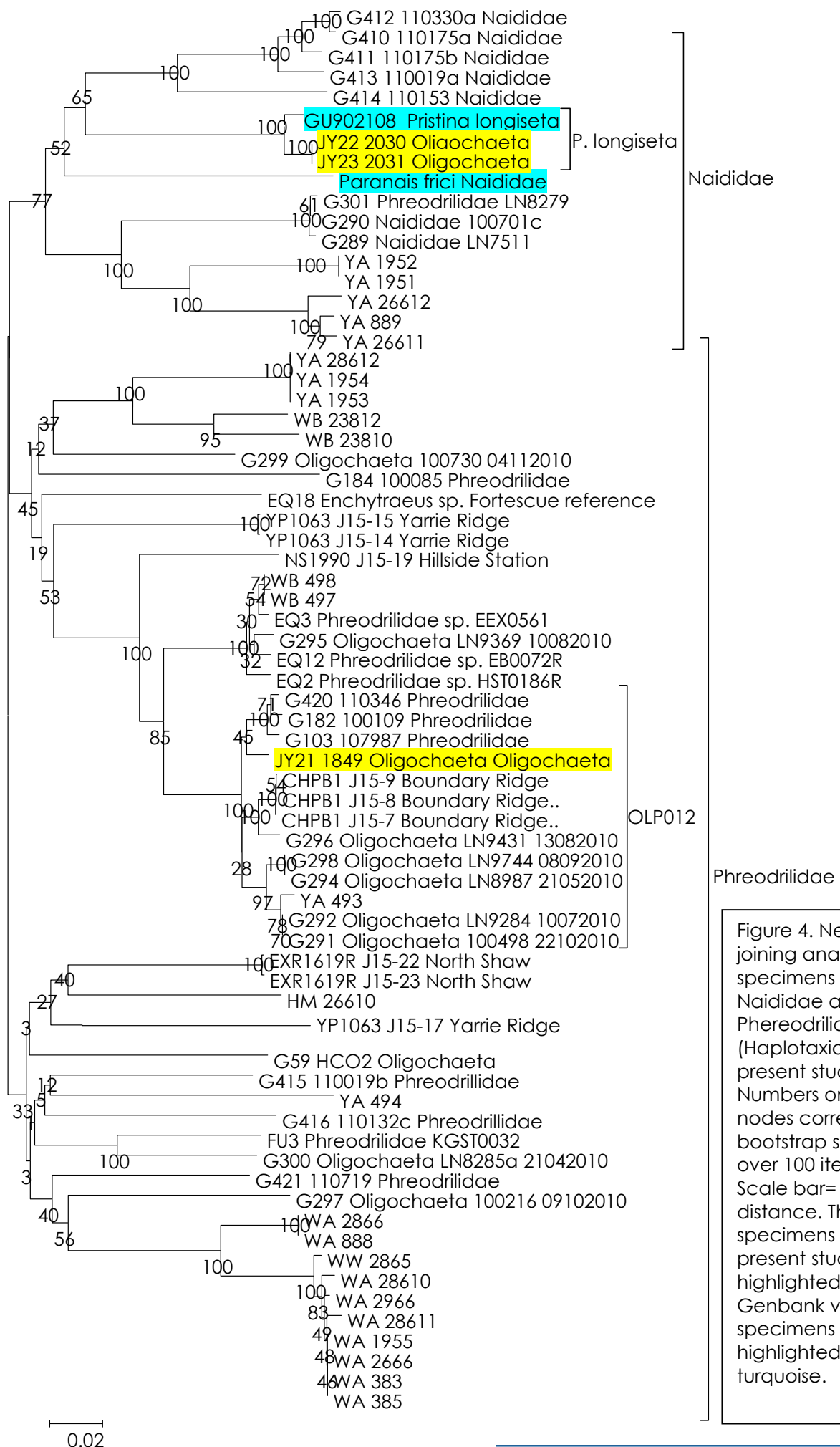


Figure 4. Neighbour-joining analysis of specimens of Naididae and Phreodrilidae (Haplotaxida) from the present study. Numbers on major nodes correspond to bootstrap support over 100 iterations. Scale bar = genetic distance. The specimens from the present study are highlighted in yellow; Genbank voucher specimens are highlighted in turquoise.



TABLE 4. Naididae Distance Matrix.

[illegible]

1. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*: 2725-2729.

Figure 5. Neighbour-joining analysis of specimens of Meenoplidae (Hemiptera) from the present study. Numbers on major nodes correspond to bootstrap support over 100 iterations. Scale bar= genetic distance. The specimens from the present study are highlighted in yellow; Genbank voucher specimens are highlighted in turquoise.

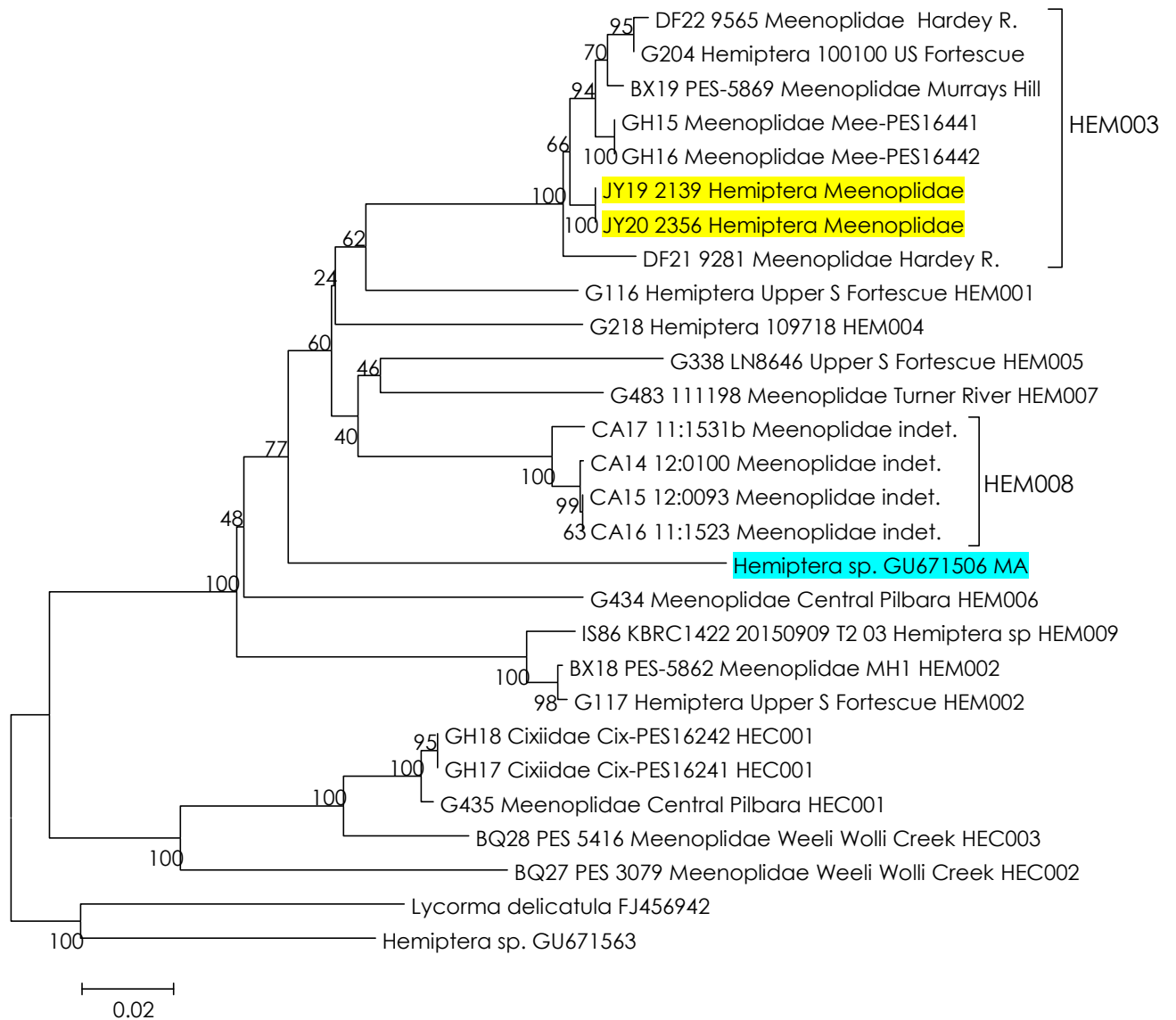




TABLE 5. Meenoplidae Distance Matrix																											
Specimen ID	JY19 2139 Hemiptera Meenoplidae	JY20 2356 Hemiptera Meenoplidae	BQ27 PES 3079 Meenoplidae Weeli Walli Creek MH	BQ28 PES 5416 Meenoplidae Weeli Walli Creek MI	BX18 PES-5862 Meenoplidae MH1 MG	BX19 PES-5869 Meenoplidae MH1 MB	CA14 12:0100 Meenoplidae indet.	CA15 12:0093 Meenoplidae indet.	CA16 11:1523 Meenoplidae indet.	CA17 11:1531b Meenoplidae indet.	DF21 9281 Meenoplidae sp.	DF22 9565 Meenoplidae sp.	G116 Hemiptera Upper S Fortescue MC(2)	G117 Hemiptera Upper S Fortescue MG(2)	G204 Hemiptera 100100 widespread MB(2)	G218 Hemiptera 109718(2)	G338 Hemiptera LN8646 Upper S Fortescue ME(2)	G434 Meenoplidae Central Pilbara MF	G435 Meenoplidae Central Pilbara MJ	G483 111198 Meenoplidae Turner River MD	GH15 Meenoplidae Mee-PES16441	GH16 Meenoplidae Mee-PES16442	GH17 Cixiidae Cix-PES16241	GH18 Cixiidae Cix-PES16242	Hemiptera sp. GU671506 MA	IS86 KBRC1422 20150909 T2 03 Hemiptera sp	
JY19 2139 Hemiptera Meenoplidae		0.000	0.014	0.016	0.013	0.005	0.012	0.012	0.012	0.012	0.005	0.008	0.010	0.013	0.006	0.012	0.014	0.014	0.015	0.014	0.005	0.005	0.016	0.016	0.015	0.014	
JY20 2356 Hemiptera Meenoplidae	0.000		0.014	0.016	0.013	0.005	0.012	0.012	0.012	0.012	0.005	0.008	0.010	0.013	0.006	0.012	0.014	0.014	0.015	0.014	0.005	0.005	0.016	0.016	0.015	0.014	
BQ27 PES 3079 Meenoplidae Weeli Walli Creek MH	0.220	0.221		0.012	0.016	0.014	0.015	0.015	0.015	0.015	0.014	0.019	0.015	0.016	0.015	0.015	0.017	0.014	0.013	0.017	0.014	0.014	0.014	0.014	0.019	0.017	
BQ28 PES 5416 Meenoplidae Weeli Walli Creek MI	0.217	0.216	0.127		0.015	0.016	0.016	0.016	0.016	0.015	0.016	0.020	0.016	0.015	0.016	0.016	0.015	0.015	0.008	0.017	0.016	0.016	0.008	0.008	0.017	0.017	
BX18 PES-5862 Meenoplidae MH1 MG	0.146	0.146	0.216	0.206		0.014	0.015	0.015	0.015	0.015	0.014	0.018	0.015	0.002	0.014	0.015	0.015	0.014	0.015	0.016	0.014	0.014	0.016	0.016	0.015	0.005	
BX19 PES-5869 Meenoplidae MH1 MB	0.016	0.016	0.226	0.224	0.149		0.012	0.012	0.012	0.012	0.007	0.005	0.010	0.014	0.004	0.012	0.015	0.014	0.015	0.014	0.004	0.004	0.016	0.016	0.015	0.015	
CA14 12:0100 Meenoplidae indet.	0.115	0.115	0.210	0.211	0.149	0.120		0.001	0.001	0.004	0.012	0.015	0.012	0.015	0.012	0.012	0.013	0.014	0.015	0.014	0.010	0.010	0.016	0.016	0.015	0.014	
CA15 12:0093 Meenoplidae indet.	0.115	0.115	0.210	0.211	0.147	0.120	0.001		0.000	0.004	0.012	0.015	0.012	0.015	0.012	0.012	0.013	0.013	0.015	0.014	0.010	0.010	0.016	0.016	0.015	0.014	
CA16 11:1523 Meenoplidae indet.	0.115	0.115	0.210	0.211	0.147	0.120	0.001	0.000		0.004	0.012	0.015	0.012	0.015	0.012	0.012	0.013	0.013	0.015	0.014	0.010	0.010	0.016	0.016	0.015	0.015	
CA17 11:1531b Meenoplidae indet.	0.115	0.115	0.209	0.207	0.144	0.118	0.015	0.013	0.013		0.012	0.014	0.012	0.016	0.012	0.013	0.012	0.013	0.015	0.013	0.012	0.012	0.016	0.016	0.015	0.016	
DF21 9281 Meenoplidae sp.	0.023	0.023	0.228	0.227	0.153	0.031	0.117	0.117	0.117	0.117		0.010	0.010	0.014	0.007	0.012	0.015	0.014	0.016	0.014	0.006	0.006	0.017	0.017	0.015	0.015	
DF22 9565 Meenoplidae sp.	0.022	0.022	0.241	0.226	0.168	0.012	0.130	0.130	0.130	0.135	0.036		0.013	0.018	0.002	0.016	0.018	0.020	0.020	0.019	0.006	0.006	0.020	0.020	0.018	0.019	
G116 Hemiptera Upper S Fortescue MC(2)	0.095	0.095	0.221	0.203	0.132	0.101	0.111	0.111	0.111	0.105	0.107	0.111		0.015	0.010	0.012	0.016	0.014	0.014	0.014	0.010	0.010	0.015	0.015	0.014	0.014	
G117 Hemiptera Upper S Fortescue MG(2)	0.148	0.148	0.218	0.204	0.003	0.151	0.148	0.150	0.150	0.148	0.156	0.168	0.130		0.014	0.015	0.015	0.014	0.015	0.017	0.014	0.014	0.016	0.016	0.015	0.005	
G204 Hemiptera 100100 widespread MB(2)	0.019	0.019	0.231	0.224	0.153	0.009	0.117	0.117	0.117	0.117	0.031	0.002	0.104	0.153		0.012	0.014	0.014	0.016	0.014	0.004	0.004	0.016	0.016	0.015	0.015	
G218 Hemiptera 109718(2)	0.110	0.110	0.218	0.209	0.148	0.116	0.107	0.107	0.107	0.108	0.119	0.125	0.110	0.147	0.119		0.014	0.016	0.016	0.015	0.012	0.012	0.016	0.016	0.016	0.015	
G338 Hemiptera LN8646 Upper S Fortescue ME(2)	0.136	0.136	0.223	0.189	0.168	0.137	0.112	0.112	0.112	0.114	0.142	0.139	0.128	0.167	0.136	0.127		0.016	0.014	0.014	0.014	0.014	0.014	0.014	0.015	0.015	
G434 Meenoplidae Central Pilbara MF	0.153	0.154	0.210	0.207	0.138	0.156	0.152	0.150	0.150	0.146	0.162	0.171	0.138	0.144	0.159	0.147	0.158		0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.014	
G435 Meenoplidae Central Pilbara MJ	0.206	0.205	0.137	0.045	0.204	0.216	0.195	0.194	0.194	0.192	0.213	0.204	0.203	0.206	0.213	0.200	0.187	0.208		0.015	0.015	0.015	0.003	0.003	0.018	0.017	
G483 111198 Meenoplidae Turner River MD	0.117	0.117	0.227	0.212	0.162	0.120	0.109	0.108	0.108	0.104	0.126	0.135	0.107	0.166	0.121	0.124	0.111	0.142	0.191		0.014	0.014	0.016	0.016	0.016	0.016	
GH15 Meenoplidae Mee-PES16441	0.016	0.016	0.229	0.226	0.150	0.012	0.110	0.110	0.112	0.116	0.025	0.014	0.103	0.150	0.010	0.116	0.139	0.163	0.214	0.121		0.000	0.016	0.016	0.015	0.013	
GH16 Meenoplidae Mee-PES16442	0.016	0.016	0.229	0.226	0.150	0.012	0.110	0.110	0.112	0.116	0.025	0.014	0.103	0.150	0.010	0.116	0.139	0.163	0.214	0.121	0.000		0.016	0.016	0.015	0.013	
GH17 Cixiidae Cix-PES16241	0.204	0.205	0.133	0.050	0.204	0.215	0.200	0.200	0.200	0.190	0.212	0.200	0.202	0.205	0.210	0.202	0.184	0.206	0.006	0.193	0.219	0.219		0.000	0.019	0.017	
GH18 Cixiidae Cix-PES16242	0.204	0.205	0.133	0.050	0.204	0.215	0.200	0.200	0.200	0.190	0.212	0.200	0.202	0.205	0.210	0.202	0.184	0.206	0.006	0.193	0.219	0.219	0.000		0.019	0.017	
Hemiptera sp. GU671506 MA	0.169	0.169	0.250	0.234	0.191	0.167	0.178	0.178	0.178	0.179	0.170	0.161	0.163	0.193	0.167	0.158	0.163	0.178	0.236	0.157	0.169	0.169	0.234	0.234		0.016	
IS86 KBRC1422 20150909 T2 03 Hemiptera sp	0.155	0.154	0.223	0.199	0.020	0.158	0.145	0.143	0.146	0.152	0.166	0.178	0.130	0.017	0.159	0.151	0.169	0.152	0.199	0.166	0.147	0.147	0.212	0.212	0.194		

Table. Estimates of Evolutionary Divergence between Sequences

The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 28 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 834 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [1].

1. Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution30: 2725-2729.

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Figure 6. Neighbour-joining analysis of specimens of Isopoda from the present study. Numbers on major nodes correspond to bootstrap support over 100 iterations. Scale bar= genetic distance. The specimens from the present study are highlighted in yellow; Genbank voucher specimens are highlighted in turquoise.

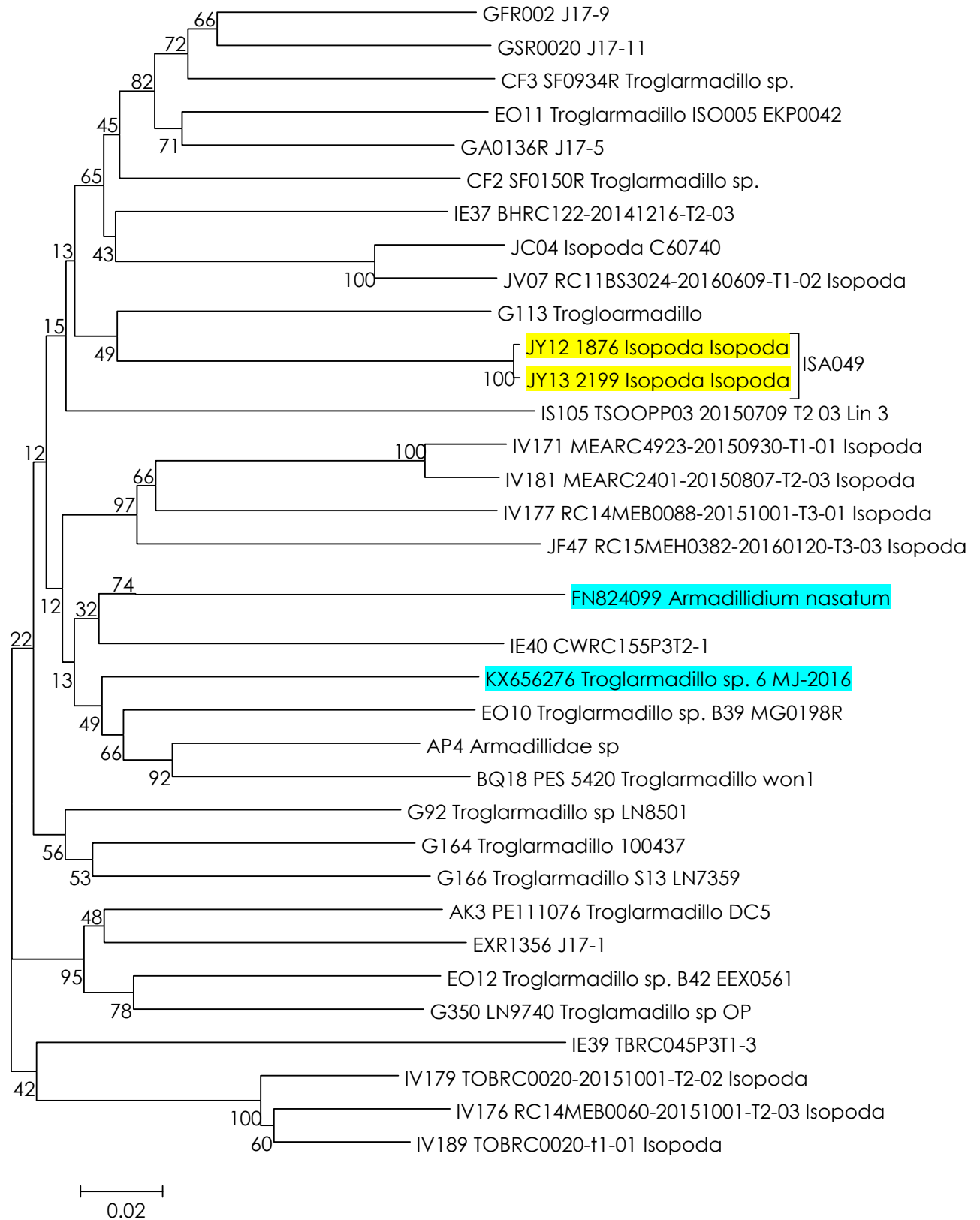




TABLE 6. Isopoda Distance Matrix

Lineage ID	JY12 1876 Isopoda Isopoda	JY13 2199 Isopoda Isopoda	AK3 PE11076 Troglarm DC5	AP4 Armadillidae sp	BQ18 PES 5420 Troglarmadillo won1	CF2 SF0150R Troglarmadillo sp.	CF3 SF0934R Troglarmadillo sp.	EO10 Troglarmadillo sp. B39 MGO198R	EO11 Troglarmadillo ISO005 EKP0042	EO12 Troglarmadillo sp. B42 EEX0561	EXR1356 J17-1	FN824099 Armadillidium nasatum	G113 Troglarmadillo	G164 Troglarmadillo 100437	G166 Troglarmadillo S13 LN7359	G350 LN9740 Troglarmadillo sp OP	G92 Troglarmadillo sp LN8501	GA0136R J17-5	GFR002 J17-9	GSR0020 J17-11	IE37 BHRC122-20141216-T2-03	IE39 TBRC045P3T1-3	IE40 CWRC155P3T2-1	IS105 TSOOPP03 20150709 T2 03 Lin 3	IV171 MEARC4923-20150930-T1-01 Isopoda	IV176 RC14MEB0060-20151001-T2-03 Isopoda	IV177 RC14MEB0088-20151001-T3-01 Isopoda	IV179 TOBRC0020-20151001-T2-02 Isopoda	IV181 MEARC2401-20150807-T2-03 Isopoda	IV189 TOBRC0020-T1-01 Isopoda	JC04 Isopoda C60740	JF47 RC15MEH0382-20160120-T3-03 Isopoda	JV07 RC11BS3024-20160609-T1-02 Isopoda	KX656276 Troglarmadillo sp. 6 MJ-2016
JY12 1876 Isopoda Isopoda		0.002	0.017	0.016	0.016	0.014	0.017	0.016	0.017	0.015	0.016	0.014	0.015	0.016	0.016	0.016	0.013	0.016	0.015	0.017	0.015	0.020	0.015	0.016	0.018	0.015	0.014	0.015	0.018	0.014	0.016	0.016	0.016	0.017
JY13 2199 Isopoda Isopoda	0.003		0.017	0.015	0.016	0.014	0.017	0.016	0.017	0.015	0.016	0.014	0.015	0.016	0.016	0.016	0.013	0.016	0.015	0.017	0.015	0.020	0.015	0.016	0.018	0.015	0.014	0.015	0.018	0.014	0.016	0.016	0.016	0.017
AK3 PE11076 Troglarm DC5	0.224	0.224		0.014	0.016	0.015	0.016	0.016	0.016	0.014	0.016	0.015	0.015	0.017	0.016	0.015	0.015	0.015	0.015	0.017	0.015	0.024	0.017	0.015	0.016	0.016	0.016	0.015	0.016	0.015	0.016	0.014	0.015	0.015
AP4 Armadillidae sp	0.186	0.187	0.183		0.014	0.013	0.016	0.016	0.013	0.015	0.015	0.014	0.015	0.014	0.013	0.016	0.016	0.013	0.015	0.014	0.014	0.021	0.015	0.015	0.016	0.013	0.015	0.012	0.015	0.013	0.012	0.013	0.012	0.014
BQ18 PES 5420 Troglarmadillo won1	0.191	0.190	0.199	0.133		0.015	0.015	0.016	0.015	0.017	0.017	0.014	0.016	0.016	0.016	0.017	0.014	0.015	0.016	0.015	0.016	0.025	0.016	0.017	0.018	0.016	0.017	0.015	0.018	0.016	0.015	0.015	0.013	0.017
CF2 SF0150R Troglarmadillo sp.	0.203	0.203	0.199	0.193	0.213		0.014	0.015	0.015	0.013	0.016	0.014	0.016	0.013	0.014	0.016	0.013	0.014	0.014	0.013	0.012	0.020	0.017	0.015	0.016	0.017	0.015	0.015	0.016	0.015	0.012	0.016	0.015	
CF3 SF0934R Troglarmadillo sp.	0.196	0.197	0.204	0.205	0.205	0.164		0.016	0.015	0.016	0.016	0.015	0.015	0.015	0.016	0.015	0.016	0.014	0.013	0.013	0.014	0.022	0.018	0.015	0.017	0.019	0.015	0.016	0.017	0.017	0.014	0.015	0.015	0.017
EO10 Troglarmadillo sp. B39 MGO198R	0.225	0.225	0.221	0.160	0.168	0.217	0.225		0.015	0.014	0.016	0.014	0.016	0.013	0.015	0.016	0.015	0.015	0.015	0.016	0.015	0.024	0.017	0.015	0.016	0.014	0.016	0.014	0.016	0.013	0.015	0.014	0.014	0.015
EO11 Troglarmadillo ISO005 EKP0042	0.221	0.221	0.204	0.190	0.208	0.172	0.165	0.197		0.015	0.016	0.015	0.015	0.015	0.014	0.015	0.014	0.013	0.016	0.015	0.015	0.021	0.018	0.014	0.018	0.017	0.016	0.016	0.018	0.015	0.015	0.014	0.015	0.015
EO12 Troglarmadillo sp. B42 EEX0561	0.219	0.222	0.187	0.183	0.220	0.226	0.229	0.190	0.210		0.017	0.016	0.014	0.015	0.014	0.014	0.015	0.014	0.014	0.015	0.013	0.023	0.015	0.015	0.014	0.015	0.015	0.014	0.014	0.015	0.014	0.015	0.014	0.014
EXR1356 J17-1	0.237	0.237	0.170	0.193	0.220	0.220	0.209	0.231	0.176		0.016	0.017	0.016	0.015	0.014	0.017	0.016	0.015	0.013	0.013	0.014	0.022	0.018	0.017	0.019	0.015	0.016	0.014	0.016	0.014	0.016	0.016	0.016	0.016
FN824099 Armadillidium nasatum	0.242	0.242	0.245	0.212	0.213	0.227	0.236	0.209	0.230	0.235	0.228		0.014	0.012	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.021	0.015	0.017	0.016	0.014	0.015	0.013	0.016	0.014	0.014	0.015	0.015	0.015
G113 Troglarmadillo	0.189	0.189	0.215	0.174	0.205	0.197	0.197	0.225	0.217	0.225	0.230	0.235		0.016	0.014	0.016	0.015	0.015	0.016	0.014	0.016	0.020	0.014	0.015	0.017	0.014	0.017	0.014	0.017	0.014	0.015	0.016	0.014	0.015
G164 Troglarmadillo 100437	0.216	0.215	0.191	0.203	0.200	0.178	0.204	0.199	0.215	0.215	0.220	0.228	0.206		0.014	0.015	0.012	0.015	0.014	0.013	0.016	0.022	0.014	0.017	0.016	0.016	0.014	0.015	0.015	0.014	0.015	0.015	0.015	0.015
G166 Troglarmadillo S13 LN7359	0.222	0.222	0.197	0.182	0.210	0.203	0.203	0.215	0.193	0.201	0.200	0.235	0.210	0.161		0.014	0.015	0.015	0.015	0.015	0.015	0.021	0.014	0.016	0.015	0.013	0.014	0.013	0.016	0.014	0.015	0.013	0.014	0.015
G350 LN9740 Troglarmadillo sp OP	0.233	0.233	0.173	0.178	0.210	0.222	0.216	0.198	0.204	0.145	0.164	0.210	0.222	0.189	0.182		0.016	0.015	0.015	0.015	0.015	0.020	0.015	0.015	0.016	0.014	0.015	0.015	0.016	0.015	0.017	0.014	0.016	0.015
G92 Troglarmadillo sp LN8501	0.215	0.215	0.205	0.203	0.197	0.190	0.212	0.196	0.201	0.182	0.215	0.234	0.210	0.175	0.162	0.204		0.015	0.014	0.015	0.015	0.021	0.015	0.016	0.015	0.015	0.014	0.015	0.015	0.015	0.014	0.013	0.012	0.013
GA0136R J17-5	0.209	0.211	0.213	0.193	0.194	0.170	0.148	0.196	0.141	0.203	0.217	0.229	0.213	0.203	0.182	0.193	0.183		0.013	0.013	0.015	0.023	0.017	0.014	0.017	0.016	0.016	0.015	0.017	0.015	0.015	0.014	0.013	0.016
GFR002 J17-9	0.203	0.205	0.205	0.195	0.206	0.180	0.153	0.203	0.168	0.209	0.217	0.252	0.193	0.195	0.182	0.221	0.194	0.148		0.012	0.015	0.022	0.017	0.015	0.017	0.014	0.017	0.015	0.017	0.013	0.014	0.015	0.015	0.016
GSR0020 J17-11	0.205	0.205	0.219	0.191	0.217	0.174	0.140	0.218	0.174	0.235	0.232	0.251	0.202	0.195	0.186	0.223	0.208	0.150	0.130		0.014	0.021	0.016	0.014	0.018	0.017	0.016	0.017	0.017	0.016	0.014	0.014	0.014	0.015
IE37 BHRC122-20141216-T2-03	0.191	0.189	0.207	0.187	0.208	0.169	0.174	0.196	0.170	0.204	0.207	0.227	0.199	0.196	0.182	0.209	0.191	0.167	0.162	0.166		0.022	0.016	0.015	0.016	0.016	0.015	0.015	0.016	0.014	0.014	0.014	0.014	0.016
IE39 TBRC045P3T1-3	0.246	0.243	0.245	0.232	0.243	0.246	0.262	0.253	0.251	0.243	0.249	0.262	0.243	0.222	0.253	0.236	0.243	0.222	0.262	0.258	0.239		0.018	0.019	0.022	0.019	0.021	0.020	0.022	0.021	0.023	0.019	0.021	0.020
IE40 CWRC155P3T2-1	0.217	0.217	0.213	0.189	0.201	0.207	0.225	0.192	0.214	0.211	0.212	0.221	0.188	0.226	0.214	0.204	0.207	0.222	0.227	0.217	0.218	0.243		0.018	0.014	0.014	0.015	0.014	0.014	0.015	0.017	0.014	0.016	0.015
IS105 TSOOPP03 20150709 T2 03 Lin 3	0.227	0.228	0.248	0.228	0.236	0.208	0.205	0.231	0.226	0.245	0.238	0.242	0.216	0.207	0.222	0.235	0.201	0.198	0.213	0.213	0.216	0.265	0.258		0.016	0.017	0.017	0.016	0.017	0.016	0.016	0.014	0.015	0.014
IV171 MEARC4923-20150930-T1-01 Isopoda	0.237	0.237	0.243	0.222	0.219	0.217	0.228	0.204	0.219	0.224	0.237	0.215	0.225	0.213	0.203	0.225	0.197	0.206	0.221	0.215	0.223	0.267	0.214	0.216		0.017	0.016	0.014	0.006	0.015	0.015	0.014	0.015	0.015
IV176 RC14MEB0060-20151001-T2-03 Isopoda	0.229	0.231	0.230	0.198	0.210	0.225	0.244	0.215	0.250	0.198	0.209	0.223	0.225	0.188	0.207	0.188	0.201	0.236	0.220	0.234	0.218	0.219	0.223	0.230	0.231		0.015	0.010	0.016	0.010	0.014	0.016	0.015	0.016
IV177 RC14MEB0088-20151001-T3-01 Isopoda	0.221	0.219	0.235	0.201	0.201	0.207	0.232	0.200	0.233	0.232	0.248	0.219	0.216	0.204	0.207	0.220	0.204	0.217	0.226	0.224	0.214	0.258	0.218	0.232	0.166	0.218		0.013	0.016	0.014	0.014	0.015	0.015	0.017
IV179 TOBRC0020-20151001-T2-02 Isopoda	0.211	0.211	0.213	0.198	0.213	0.204	0.229	0.198	0.227	0.181	0.187	0.212	0.222	0.194	0.191	0.200	0.181	0.216	0.211	0.220	0.195	0.217	0.204	0.218	0.201	0.082	0.207		0.013	0.009	0.015	0.015	0.015	0.015
IV181 MEARC2401-20150807-T2-03 Isopoda	0.236	0.236	0.232	0.216	0.214	0.220	0.237	0.198	0.219	0.219	0.236	0.212	0.225	0.215	0.200	0.216	0.193	0.205	0.218	0.215	0.217	0.267	0.217	0.220	0.038	0.228	0.169	0.207		0.014	0.016	0.013	0.015	0.015
IV189 TOBRC0020-T1-01 Isopoda	0.221	0.221	0.221	0.195	0.210	0.207	0.234	0.201	0.227	0.190	0.203	0.212	0.224	0.191	0.197	0.197	0.185	0.214	0.206	0.224	0.202	0.229	0.205	0.226	0.216	0.076	0.211	0.067	0.207		0.014	0.016	0.013	0.015
JC04 Isopoda C60740	0.214	0.215	0.205	0.192	0.219	0.192	0.185	0.210	0.192	0.210	0.222	0.252	0.199	0.193	0.212	0.233	0.223	0.200	0.192	0.192	0.180	0.251	0.226	0.213	0.230	0.228	0.224	0.229	0.230	0.213		0.014	0.010	0.017
JF47 RC15MEH0382-20160120-T3-03 Isopoda	0.246	0.246	0.237	0.211	0.216	0.219	0.228	0.220	0.224	0.229	0.239	0.236	0.227	0.219	0.219	0.225	0.210	0.219	0.220	0.224	0.220	0.267	0.221	0.231	0.191	0.245	0.185	0.224	0.185	0.226	0.238		0.013	0.014
JV07 RC11BS3024-201																																		

Figure 7. Neighbour-joining analysis of specimens of Symphyla from the present study. Numbers on major nodes correspond to bootstrap support over 100 iterations. Scale bar= genetic distance. The specimens from the present study are highlighted in yellow; Genbank voucher specimens are highlighted in turquoise.

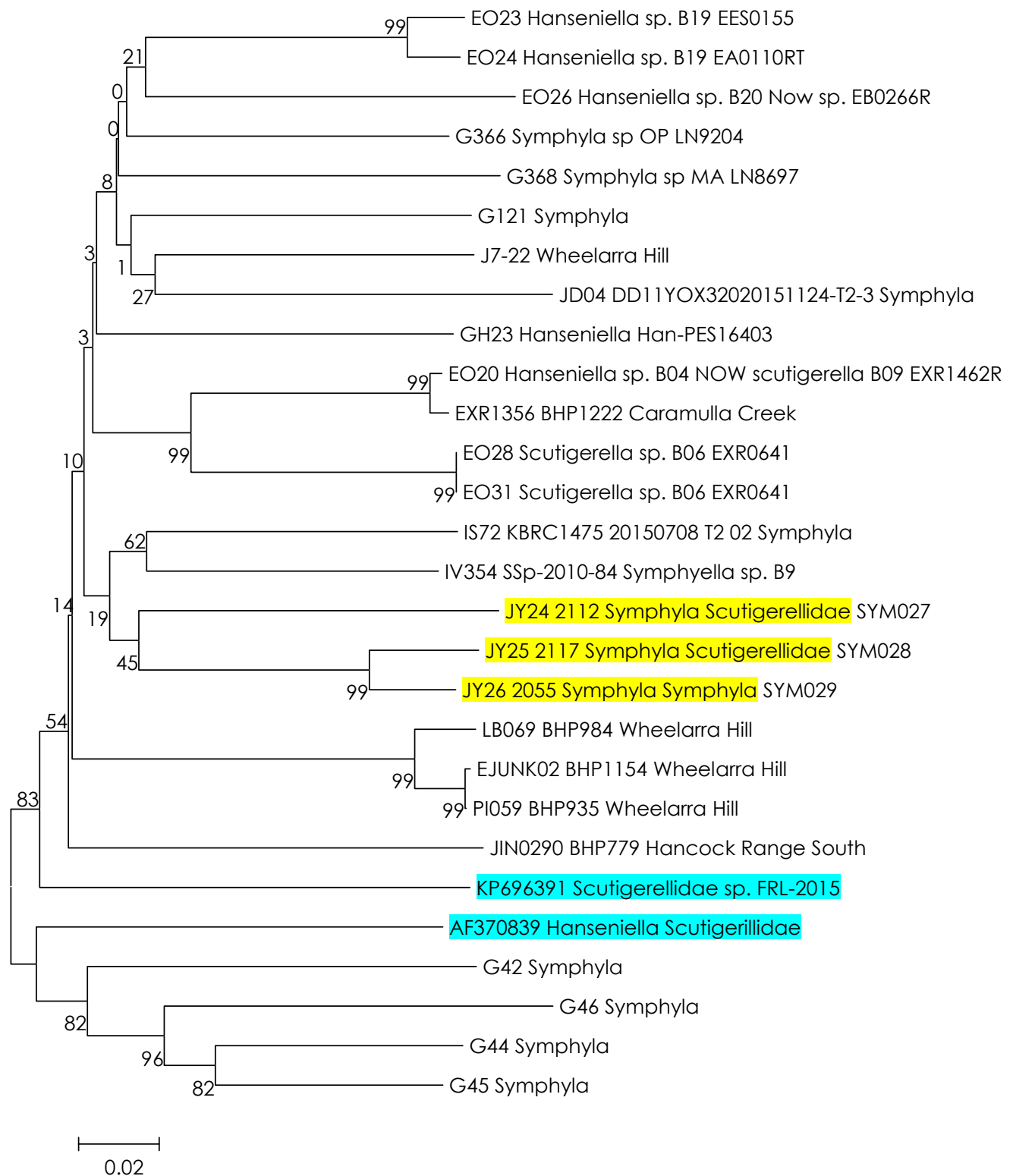




TABLE 7. Symphyla Distance Matrix

Specimen ID	JY24 2112 Symphyla Scutigereillidae	JY25 2117 Symphyla Scutigereillidae	JY26 2055 Symphyla Symphyla	AF370839 Hanseniella Scutigereillidae	EJUNK02 BHP1154 Jimblebar E	EO20 Hanseniella sp. B04 NOW scutigereilla B09 EXR1462R	EO23 Hanseniella sp. B19 EES0155	EO24 Hanseniella sp. B19 EA0110RT	EO26 Hanseniella sp. B20 Now sp. EB0266R	EO28 Scutigereilla sp. B06 EXR0641	EO31 Scutigereilla sp. B06 EXR0641	EXR1356 BHP1222 Caramulla Creek	G121 Symphyla	G366 Symphyla sp OP LN9204	G368 Symphyla sp MA LN8697	G42 Symphyla	G44 Symphyla	G45 Symphyla	G46 Symphyla	GH23 Hanseniella Han-PES16403	IS72 KBRC1475 20150708 T2 02 Symphyla	IV354 SSp-2010-84 Symphyella sp. B9	J7-22 Wheelarra Hill	JD04 DD11YOX32020151124-T2-3 Symphyla	JIN0290 BHP7779 Hancock Range South	KP696391 Scutigereillidae sp. FRL-2015	LB069 BHP984 Hashimoto	PI059 BHP935 Hashimoto	
JY24 2112 Symphyla Scutigereillidae		0.014	0.014	0.015	0.012	0.013	0.015	0.014	0.013	0.017	0.017	0.013	0.013	0.012	0.015	0.018	0.017	0.013	0.021	0.015	0.014	0.011	0.012	0.015	0.016	0.018	0.011	0.012	
JY25 2117 Symphyla Scutigereillidae	0.175		0.010	0.017	0.012	0.016	0.012	0.013	0.013	0.013	0.016	0.014	0.014	0.012	0.014	0.019	0.019	0.013	0.023	0.013	0.014	0.015	0.014	0.020	0.017	0.020	0.014	0.013	
JY26 2055 Symphyla Symphyla	0.166	0.049		0.017	0.013	0.014	0.012	0.012	0.012	0.013	0.013	0.015	0.013	0.013	0.015	0.018	0.019	0.015	0.025	0.010	0.014	0.015	0.012	0.020	0.018	0.018	0.013	0.013	
AF370839 Hanseniella Scutigereillidae	0.211	0.224	0.220		0.014	0.017	0.015	0.013	0.013	0.016	0.016	0.017	0.012	0.014	0.014	0.019	0.017	0.015	0.017	0.011	0.017	0.015	0.015	0.018	0.014	0.016	0.014	0.014	
EJUNK02 BHP1154 Wheelarra Hill	0.194	0.192	0.198	0.209		0.013	0.016	0.013	0.014	0.016	0.016	0.014	0.014	0.017	0.016	0.021	0.017	0.016	0.018	0.014	0.016	0.015	0.013	0.017	0.016	0.017	0.006	0.001	
EO20 Hanseniella sp. B04 NOW scutigereilla B09 EXR1462R	0.172	0.209	0.203	0.226	0.192		0.015	0.015	0.012	0.012	0.012	0.003	0.012	0.014	0.014	0.020	0.019	0.014	0.021	0.013	0.016	0.012	0.015	0.017	0.014	0.018	0.015	0.014	
EO23 Hanseniella sp. B19 EES0155	0.202	0.178	0.172	0.223	0.197	0.181		0.005	0.014	0.013	0.013	0.017	0.013	0.012	0.011	0.019	0.017	0.016	0.022	0.015	0.012	0.012	0.014	0.017	0.015	0.018	0.013	0.015	
EO24 Hanseniella sp. B19 EA0110RT	0.206	0.175	0.166	0.218	0.194	0.183	0.027		0.013	0.013	0.013	0.017	0.015	0.014	0.012	0.019	0.016	0.014	0.023	0.013	0.013	0.013	0.013	0.016	0.016	0.018	0.012	0.014	
EO26 Hanseniella sp. B20 Now sp. EB0266R	0.211	0.195	0.192	0.240	0.228	0.181	0.173	0.168		0.015	0.015	0.013	0.014	0.012	0.014	0.015	0.020	0.011	0.017	0.012	0.012	0.015	0.015	0.015	0.016	0.020	0.014	0.014	
EO28 Scutigereilla sp. B06 EXR0641	0.203	0.187	0.191	0.217	0.195	0.125	0.176	0.180	0.197		0.000	0.013	0.013	0.011	0.015	0.020	0.016	0.016	0.017	0.013	0.014	0.012	0.012	0.015	0.015	0.020	0.017	0.017	
EO31 Scutigereilla sp. B06 EXR0641	0.203	0.187	0.191	0.217	0.195	0.125	0.176	0.180	0.197	0.000		0.013	0.013	0.011	0.015	0.020	0.016	0.016	0.017	0.013	0.014	0.012	0.012	0.015	0.015	0.020	0.017	0.017	
EXR1356 BHP1222 Caramulla Creek	0.179	0.212	0.206	0.226	0.191	0.008	0.183	0.183	0.180	0.133	0.133		0.012	0.014	0.016	0.022	0.019	0.015	0.021	0.014	0.018	0.014	0.015	0.017	0.013	0.018	0.015	0.014	
G121 Symphyla	0.196	0.191	0.176	0.216	0.205	0.177	0.183	0.180	0.186	0.177	0.177	0.176		0.013	0.013	0.020	0.016	0.012	0.021	0.011	0.016	0.012	0.013	0.014	0.016	0.018	0.014	0.013	
G366 Symphyla sp OP LN9204	0.203	0.193	0.190	0.221	0.190	0.178	0.162	0.164	0.177	0.171	0.171	0.180	0.174		0.012	0.020	0.015	0.016	0.020	0.014	0.015	0.012	0.014	0.014	0.015	0.020	0.016	0.016	
G368 Symphyla sp MA LN8697	0.186	0.211	0.206	0.220	0.202	0.209	0.184	0.188	0.190	0.196	0.196	0.212	0.185	0.175		0.016	0.018	0.016	0.017	0.015	0.015	0.014	0.014	0.017	0.014	0.017	0.016	0.016	
G42 Symphyla	0.238	0.216	0.201	0.216	0.260	0.224	0.219	0.212	0.215	0.228	0.228	0.220	0.241	0.226	0.239		0.017	0.014	0.017	0.018	0.020	0.022	0.017	0.028	0.019	0.017	0.021	0.021	
G44 Symphyla	0.241	0.231	0.227	0.208	0.218	0.226	0.228	0.221	0.221	0.245	0.245	0.224	0.207	0.202	0.224	0.187		0.012	0.018	0.017	0.017	0.021	0.019	0.021	0.030	0.021	0.021	0.018	0.017
G45 Symphyla	0.226	0.227	0.224	0.200	0.236	0.222	0.213	0.206	0.219	0.231	0.231	0.224	0.215	0.206	0.220	0.182	0.118		0.017	0.015	0.015	0.017	0.017	0.020	0.015	0.017	0.017	0.016	
G46 Symphyla	0.253	0.267	0.258	0.217	0.240	0.232	0.244	0.248	0.235	0.216	0.216	0.235	0.242	0.252	0.234	0.215	0.178	0.158		0.020	0.025	0.023	0.019	0.026	0.018	0.022	0.020	0.018	
GH23 Hanseniella Han-PES16403	0.191	0.203	0.191	0.219	0.189	0.170	0.180	0.179	0.181	0.182	0.182	0.176	0.178	0.185	0.190	0.224	0.219	0.227	0.252		0.013	0.013	0.015	0.020	0.013	0.018	0.014	0.014	
IS72 KBRC1475 20150708 T2 02 Symphyla	0.174	0.175	0.163	0.221	0.205	0.170	0.168	0.163	0.179	0.187	0.187	0.177	0.197	0.196	0.217	0.207	0.229	0.210	0.241	0.181		0.014	0.012	0.020	0.016	0.017	0.015	0.016	
IV354 SSp-2010-84 Symphyella sp. B9	0.194	0.174	0.163	0.221	0.186	0.163	0.176	0.185	0.194	0.177	0.177	0.169	0.159	0.174	0.195	0.239	0.209	0.218	0.251	0.186	0.150		0.014	0.017	0.014	0.017	0.015	0.015	
J7-22 Wheelarra Hill	0.221	0.197	0.191	0.241	0.202	0.191	0.162	0.165	0.170	0.185	0.185	0.191	0.171	0.169	0.185	0.254	0.232	0.223	0.274	0.175	0.197	0.173		0.017	0.013	0.017	0.015	0.014	
JD04 DD11YOX32020151124-T2-3 Symphyla	0.189	0.212	0.210	0.228	0.216	0.195	0.208	0.195	0.203	0.199	0.199	0.193	0.187	0.189	0.201	0.255	0.229	0.228	0.284	0.212	0.210	0.195	0.178		0.020	0.021	0.017	0.017	
JIN0290 BHP7779 Hancock Range South	0.221	0.195	0.197	0.229	0.211	0.200	0.192	0.195	0.226	0.203	0.203	0.197	0.199	0.186	0.202	0.212	0.241	0.224	0.269	0.196	0.197	0.208	0.205	0.226		0.017	0.017	0.016	
KP696391 Scutigereillidae sp. FRL-2015	0.237	0.207	0.201	0.220	0.215	0.195	0.211	0.216	0.229	0.207	0.207	0.195	0.216	0.211	0.211	0.221	0.247	0.238	0.251	0.217	0.208	0.204	0.198	0.241	0.217		0.019	0.017	
LB069 BHP984 Wheelarra Hill	0.187	0.196	0.201	0.199	0.029	0.193	0.193	0.192	0.235	0.201	0.201	0.192	0.209	0.184	0.201	0.258	0.228	0.240	0.244	0.194	0.199	0.196	0.212	0.226	0.195	0.219		0.006	
PI059 BHP935 Wheelarra Hill	0.192	0.191	0.197	0.209	0.002	0.191	0.195	0.195	0.228	0.194	0.194	0.189	0.203	0.189	0.200	0.260	0.218	0.236	0.240	0.189	0.203	0.185	0.200	0.216	0.209	0.214	0.028		

Table. Estimates of Evolutionary Divergence between Sequences

The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. The analysis involved 28 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 14667 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [1].

1. Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution30: 2725-2729.

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