

7 July 2023

Dear Adam,

RE: Mt Weld Potential SRE Taxa: Memo

Stantec Australia (Stantec) was engaged by Lynas Rare Earths (Lynas) to undertake a short-range endemic (SRE) invertebrate fauna survey of the Mt Weld Rare Earths Project (the Proposal) in the Goldfields region of Western Australia. This survey is the most recent terrestrial fauna survey completed for the Proposal, following those listed below:

- Lynas Corporation Ltd Mt Weld Rare Earths Project: Level 1 Terrestrial Fauna Assessment (Outback Ecology 2011)
- Lynas Corporation Ltd Mt Weld Rare Earths Project: Level 1 Vegetation, Flora and Fauna Assessment (Outback Ecology 2013)
- Lynas Corporation Ltd Mt Weld Rare Earths Project: Level 1 Vegetation, Flora and Fauna Survey (MWH 2014)
- Mt Weld Flora, Vegetation and Fauna Review (Stantec 2018)
- Mt Weld Rare Earths Project Level 2 and Targeted Terrestrial Fauna Survey (Stantec 2020)

Of these surveys, specimens from SRE invertebrate groups were collected during the MWH (2014) survey and during Phase 1 and Phase 2 surveys in 2020 (Stantec 2020). This memo summarises the SRE findings of these surveys including subsequent morphological and genetic identifications. All identifications undertaken for this memo were undertaken by Alacran Environmental Science (Alacran) and Genotyping Australia.

Previous Surveys Taxa identification:

In total, 13 taxa (28 specimens) were collected within the Proposal's Development Envelope over the course of the previous surveys (MWH 2014, Stantec 2020) to inform the impact assessment for the Life of Mine approvals. Nine of these taxa collected from the Survey Area were originally identified as potential SRE taxa (Stantec 2020) (**Table 1**).

Given potential impacts to the locations of these collections, additional morphological (WAM 2023) and genetic analysis (**Appendix A**) was undertaken in May 2023 to better understand the potential distributions of these taxa outside the Proposal Development Envelope. This analysis revealed that five of the nine potential SRE taxa were known to have distributions that extend outside the Survey Area (**Table 1, Figure 1**). These taxa were:

- *Aname* 'MYG629'
- *Idiosoma* 'MYG722'
- *Proshermacha* 'MYG715'
- *Buddelundia* '103'
- *Buddelundia* '106'

However, the remaining four taxa were only known from the Development Envelope. These taxa were:

- *Idiosoma* 'MW1'
- *Idiosoma* 'MW2'
- *Synsphyronus* 'weld'
- *Urodacus* sp. - (sequencing inconclusive prior to 2023 Targeted SRE Survey)

2023 Targeted SRE Survey

Stantec conducted a Targeted SRE Survey from 29th May – 2nd June 2023. The objectives of this survey were to:

- Collect new specimen(s) of *Urodacus* sp. for revised sequencing; and
- Determine whether the four taxa known only from the Development Envelope were present in the broader region, by surveying outside the Development Envelope.

24 specimens were collected during the 2023 Targeted SRE Survey. Genotyping Australia prepared and sequenced the specimens. Alacran (**Appendix A**) then amplified DNA sequence fragments where possible, in order to identify the specimens to species level. Two specimens collected outside the Development Envelope were not sequenced to species level: one being an unmatched *Idiosoma* specimen and the other being an outgroup of Barychellidae (a non-target family for this analysis).

The remaining 22 specimens were identified to species level (**Table 1**). The identification demonstrated that the following three taxa are confirmed present outside the Development Envelope (**Figure 1**):

- *Idiosoma* 'MW1'
- *Idiosoma* 'MW2'
- *Urodacus* 'MW1' (previously *Urodacus* sp.)

Synsphyronus 'weld' was not collected during the 2023 Targeted SRE Survey due to survey effort prioritising the three taxa listed above. This taxon is known from only one record within the Development Envelope (Non-priority Indicative Footprint), in Mulga on clay loam habitat which was assessed as having a low potential to support SRE taxa (**Figure 1**). This taxon has the potential to be widespread as the specimen was recorded from a widespread habitat type. Additional targeted searches for *Synsphyronus* 'weld' within Mulga on clay loam habitat outside the Development Envelope are needed to confirm this.

Please do not hesitate to contact the undersigned if you have any queries.

Yours sincerely



Joseph Sollis
Senior Zoologist
Stantec Australia Pty Ltd



Paul Bolton
Team Lead: Terrestrial Fauna



Table 1: Potential SRE specimens sampled from Mt Weld including their presence within and outside the Development Envelope (DE) and Indicative Footprint (IF), and the habitat from which they were collected. Habitats are coloured according to their potential to support SRE taxa (green – low potential, amber – moderate potential, red – high potential).

Group	Preliminary taxon name	Confirmed identification	Date of collection	Stantec codes	Alacran codes	Number	DE		IF		Habitats (SRE potential: green – low potential, amber – moderate potential, red – high potential).*							Comment
							In	Out	In	Out	Mulga on stony plain	Sparse shrubland on heavy clay	Mulga on clay loam	Low Mulga on clay loam	Shrub Plain	Stony Rise	Outcropping	
Mygalomorph Spiders	<i>Idiosoma</i> sp.	<i>Idiosoma</i> 'MW1'	2020	LN8970	AES230023	4	✓	✓	✓	✓	1				1	1		Confirmed outside Development Envelope (Appendix A)
				LN7479	AES230025													
				125	AES230052													
				145	AES230063													
	<i>Idiosoma</i> sp., <i>Idiopidae</i> sp. , <i>Aganippe</i> sp.	<i>Idiosoma</i> 'MYG722'	2020	LN9454	AES230022	18	✓	✓	✓	✓	1		11					Confirmed outside Development Envelope (Appendix A)
				LN7976	AES230026													
				LN8963	AES230024													
			2014	Site 2-2	T136170													
				Site 5-5	T136167													
			2023	100	AES230042													
				105	AES230043													
				107	AES230045													
				108	AES230046													
				113	AES230047													
				122	AES230049													
				131	AES230053													
				135	AES230057													
				136	AES230058													
				137	AES230059													
				138	AES230060													
				139	AES230061													
				144	AES230062													
	<i>Proshermacha</i> 'MYG715'	<i>Proshermacha</i> 'MYG715'	2020	647	AES200631	1	✓	✓	X	✓			1					Confirmed outside Development Envelope (Conspecific with regional records presented in Figure 1 (Stantec 2020, WAM 2023))
	<i>Aname</i> sp.	<i>Aname</i> 'MYG629'	2014	Site 1-1	T136178	1	✓	✓	X	✓			1					Confirmed outside Development Envelope (Appendix A)
	<i>Aganippe</i> sp.	<i>Idiosoma</i> 'MW2'	2014	Site 4-4	T136169	3	✓	✓	✓	✓			2					Confirmed outside Development Envelope (Appendix A)
				Site 6-6	T136168													
			2023	134	AES230056													



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Group	Preliminary taxon name	Confirmed identification	Date of collection	Stantec codes	Alacran codes	Number	DE		IF		Habitats (SRE potential: green – low potential, amber – moderate potential, red – high potential).*							Comment
							In	Out	In	Out	Mulga on stony plain	Sparse shrubland on heavy clay	Mulga on clay loam	Low Mulga on clay loam	Shrub Plain	Stony Rise	Outcropping	
Scorpions	<i>Urodacus</i> sp.	<i>Urodacus</i> 'MW1'	2014	Site 3-3	-	7	✓	✓	✓	✓			7					Confirmed outside Development Envelope (Appendix At)
			2023	114	AES230048													
				123	AES230050													
				124	AES230051													
				132	AES230054													
				133	AES230055													
				147	AES230065													
Pseudoscorpions	<i>Synsphyronus</i> 'weld'	<i>Synsphyronus</i> 'weld'	2020	LN6256	-	1	✓	X	X	✓			1					Analysis complete: No matches (Appendix A) Record is within Development Envelope, outside Indicative Footprint
Slaters	<i>Buddelundia</i> '103'	<i>Buddelundia</i> '103'	2020	LN7966	-	1	✓	✓	✓	✓					1			Confirmed outside Development Envelope (Conspecific with regional records presented in Figure 1 (S. Judd, pers comm. 19 Apr 2023))
	<i>Buddelundia</i> '106'	<i>Buddelundia</i> '106'	2020	LN7988	-	1	✓	✓	X	✓			1					Confirmed outside Development Envelope (Conspecific with regional records presented in Figure 1 (S. Judd, pers comm. 19 Apr 2023))

*Counts by habitat types are only provided for specimens collected within the Level 2 Fauna Survey Area.

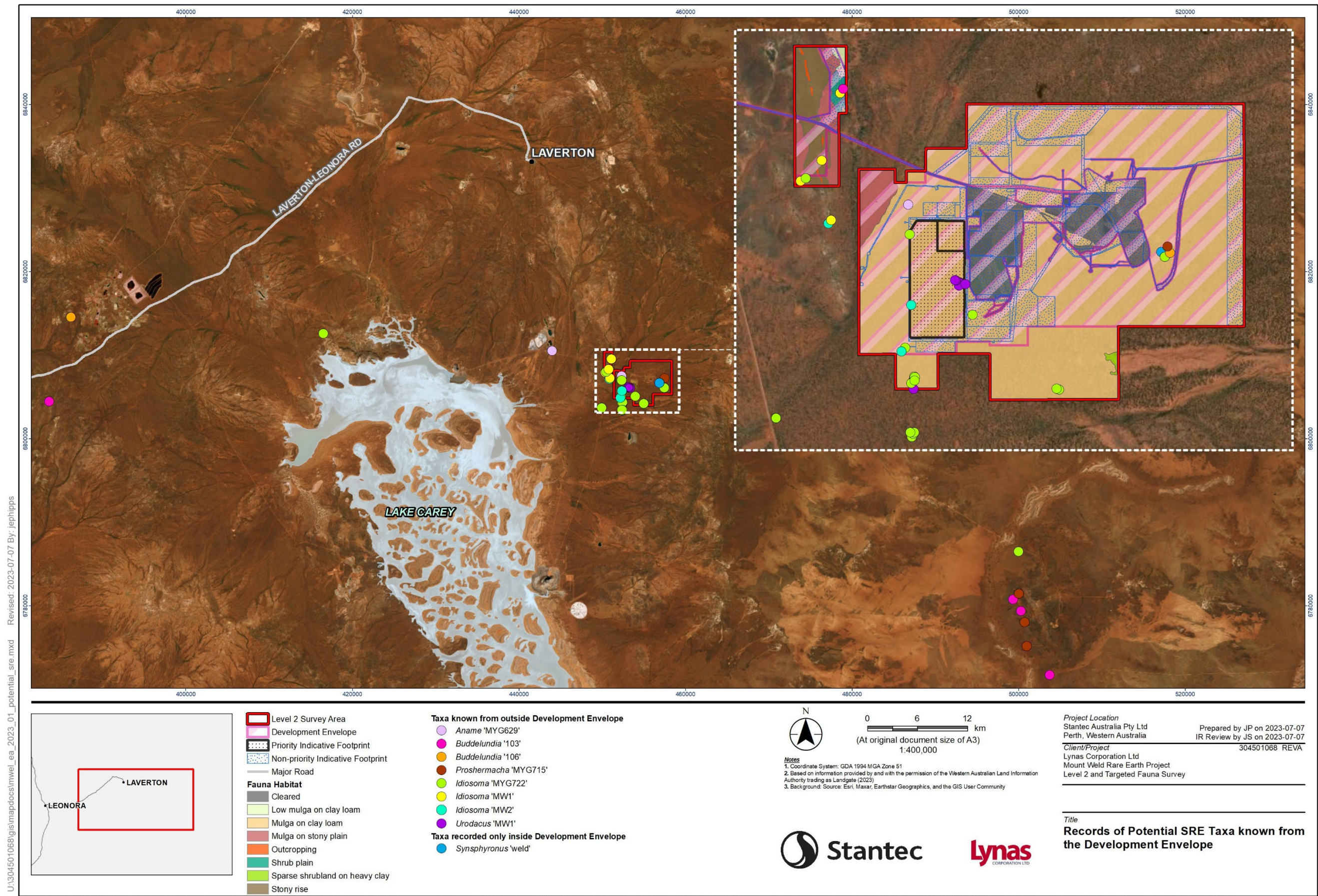


Figure 1: Records of potential SRE taxa known to occur within the Development Envelope, and regional records.

References

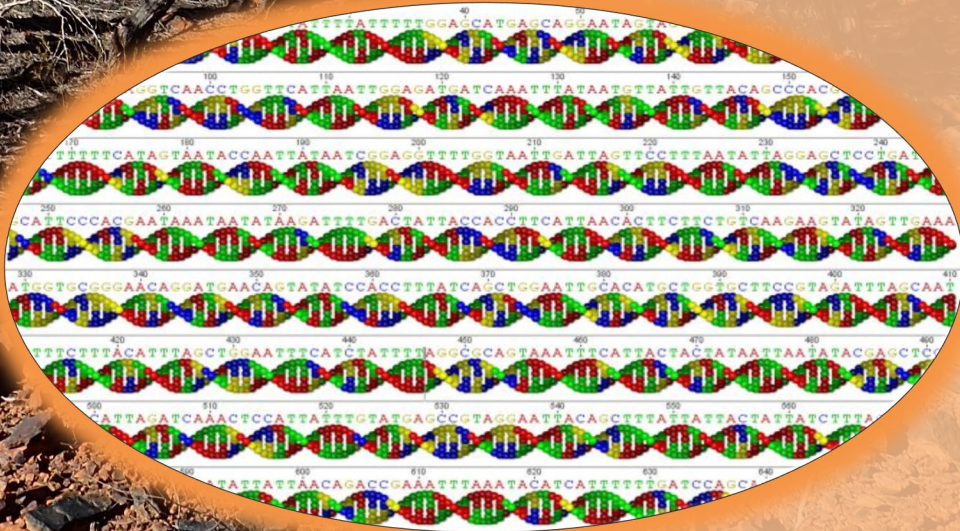
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- WAM, Western Australian Museum. (2023) *Arachnid and Diplopod Collection Database (custom search)*.

Appendix A: Troubleshooting of Short-range Endemic Category Arachnids From Mount Weld Mine Expansion Footprint

Troubleshooting of Short-range Endemic Category Arachnids From Mount Weld Mine Expansion Footprint

Prepared for
Stantec

6/07/2023



Troubleshooting of Short-range Endemic Category Arachnids From Mount Weld Mine Expansion Footprint

Report No. 2305

Submitted to Paul Bolton and Joseph Sollis (Stantec)

6/07/2023

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EXECUTIVE SUMMARY

The Mount Weld Mine is proposing an expansion of its mining footprint in the Eastern Goldfields (W.A.). Previous surveys for Short-range endemic (SRE) invertebrates yielded 12 invertebrates from the proposed expansion footprint:

- nine (9) specimens of Idiopidae (spiny trapdoor spiders), including species identified to the genera *Idiosoma* sp., *Aganippe* sp. (= *Idiosoma*)
- two (1) specimens of Anamidae (open-hole trapdoor spiders)
- one (1) specimen of Urodacidae (Australian burrowing scorpions), *Urodacus* sp.
- one (1) specimen of the pseudoscorpion family Garypidae.

The scorpion and spider samples were represented by female or juvenile samples; therefore, species level identification was not possible using morphology alone. Species level identification in most trapdoor spiders and Australian burrowing scorpions requires assessment of the adult males.

Additional samples were sourced from the W.A. Museum as well as more recently (2023) collected specimens, and DNA sequences were amplified from these in order to determine species identity assess their potential to be SREs.

The analyses determined matches with two morphospecies: *Aname* 'MYG629' and *Idiosoma* 'MYG722'.

No matches were found for morphospecies *Idiosoma* 'MW1', *Idiosoma* 'MW2' and *Urodacus* 'MW1'; however, each of these species was represented by several specimens including the target specimens.

No matches were found for the Pseudoscorpion *Synsphyronus* 'weld'.

1 AIMS

The Mount Weld Mine is proposing an expansion of its mining footprint in the Eastern Goldfields (W.A.). Previous surveys for Short-range endemic (SRE) invertebrates yielded 12 invertebrates from the proposed expansion footprint **Table 1.1**:

- nine (9) specimens of Idiopidae (spiny trapdoor spiders), including species identified to the genera *Idiosoma* sp., *Aganippe* sp. (= *Idiosoma*)
- two (1) specimens of Anamidæ (open-hole trapdoor spiders)
- one (1) specimen of Urodacidae (Australian burrowing scorpions), *Urodacus* sp.
- one (1) specimen of the pseudoscorpion family Garypidae.

The scorpion and spider samples were represented by female or juvenile samples; therefore, species level identification was not possible using morphology alone. Species level identification in most trapdoor spiders and Australian burrowing scorpions requires assessment of the adult males.

Table 1.1 Target samples from previous surveys at Mount Weld

Alacran / WA Museum Registration	Previous identification	Latitude S.	Longitude E.
AES230023	<i>Idiosoma</i> sp.	28°51'06.75	122°29'43.26
AES230025	<i>Idiosoma</i> sp.	28°50'44.57	122°29'39.16
AES230022	<i>Idiosoma</i> sp.	28°52'00.91	122°33'28.33
AES230026	<i>Idiosoma</i> sp.	28°52'34.32	122°31'19.99
AES230024	<i>Idiosoma</i> sp.	28°52'00.91	122°33'28.33
T136178	<i>Aname</i> sp.	28°51'32.90	122°30'40.60
T136170	<i>Aganippe</i> sp. (= <i>Idiosoma</i> sp.)	28°51'50.10	122°30'41.60
T136169	<i>Aganippe</i> sp. (= <i>Idiosoma</i> sp.)	28°52'31.30	122°30'42.40
T136167	<i>Aganippe</i> sp. (= <i>Idiosoma</i> sp.)	28°52'56.30	122°30'38.20
T136168	<i>Aganippe</i> sp. (= <i>Idiosoma</i> sp.)	28°52'58.40	122°30'35.90
T136195	<i>Urodacus</i> sp.	28°52'17.80	122°31'14.60
AES230029	<i>Synsphyronus</i> 'weld'	28°52'00.91	122°33'28.33

In order to obtain species level identifications and additional conspecific records for SRE assessment, Alacran attempted to amplify DNA sequence fragments of the gene Cytochrome C oxidase subunit I (COI) to enable species delimitation.

In order to obtain potential regional context for this study, an additional 42 samples representing families from the target taxa (**Table 1.1**) were also obtained from within 100 km of Mt Weld from the Western Australian Museum (**Table 1.2**):

- 13 Anamidæ
- 23 Idiopidae
- 6 Urodacidae.

Table 1.2 Western Australian Museum regional context samples

W. A. Museum Registration	Identification	Latitude	Longitude
T110326	<i>Aname</i> sp.	28°48'40	122°08'44
T110348	<i>Aname</i> sp.	28°48'49	122°08'49
T110341	<i>Aname</i> sp.	28°48'46	122°08'41
T110342	<i>Aname</i> sp.	28°48'46	122°08'41
T110343	<i>Aname</i> sp.	28°48'46	122°08'41
T110330	<i>Aname</i> sp.	28°48'40	122°08'44
T155078	<i>Aname</i> sp.	28°57'55.18	121°46'54.85
T155085	<i>Aname</i> sp.	28°56'06.50	121°48'09.15
T155098	<i>Aname</i> sp.	28°57'55.18	121°46'54.85
T155103	<i>Aname</i> sp.	28°58'16.70	121°44'41.50
T155105	<i>Aname</i> sp.	28°56'06.50	121°48'09.15
T155109	<i>Aname</i> sp.	28°56'06.50	121°48'09.15
T155110	<i>Aname</i> sp.	28°56'06.50	121°48'09.15
T110327	<i>Idiosoma</i> sp.	28°48'40	122°08'44
T110332	<i>Idiosoma</i> sp.	28°48'40	122°08'44
T110333	<i>Idiosoma</i> sp.	28°48'40	122°08'44
T110337	<i>Idiosoma</i> sp.	28°48'42	122°08'40
T110338	<i>Idiosoma</i> sp.	28°48'42	122°08'40
T110340	<i>Idiosoma</i> sp.	28°48'46	122°08'48
T110346	<i>Idiosoma</i> sp.	28°48'47	122°08'44
T110347	<i>Idiosoma</i> sp.	28°48'47	122°08'44
T110349	<i>Idiosoma</i> sp.	28°48'49	122°08'50
T110350	<i>Idiosoma</i> sp.	28°48'50	122°08'43
T110353	<i>Idiosoma</i> sp.	28°49'03	122°08'42
T151174	<i>Idiosoma</i> sp.	29°10'59.84	123°02'19.13
T151176	<i>Idiosoma</i> sp.	29°10'59.84	123°02'19.13
T151179	<i>Idiosoma</i> sp.	29°09'07.34	123°00'36.51
T151180	<i>Idiosoma</i> sp.	29°09'07.34	123°00'36.51
T151182	<i>Idiosoma</i> sp.	29°07'33.86	123°00'26.71
T151183	<i>Idiosoma</i> sp.	29°07'33.86	123°00'26.71
T151354b	<i>Idiosoma</i> sp.	29°06'50.33	123°00'11.53
T155083	<i>Idiosoma</i> sp.	28°44'04.70	121°52'13.37
T155089	<i>Idiosoma</i> sp.	28°48'19.92	121°54'00.12
T155108	<i>Idiosoma</i> sp.	28°43'34.89	121°53'11.29
T155113	<i>Idiosoma</i> sp.	28°48'19.92	121°54'00.12
T156445	<i>Idiosoma</i> sp.	28°09'16.80	122°22'00.15
T151410	<i>Urodacus</i> sp.	29°10'22.91	123°02'08.16
T156448	<i>Urodacus</i> sp.	28°11'05.96	122°21'47.34
T89474	<i>Urodacus</i> sp.	28°51'39.10	121°48'00.30
T151144	<i>Urodacus</i> sp.	29°09'05.82	123°01'53.00
T151145	<i>Urodacus</i> sp.	29°09'05.82	123°01'53.00
T151146	<i>Urodacus</i> sp.	29°09'05.82	123°01'53.00

A more recent (2023) collection survey was undertaken by Stantec to sample additional *Urodacus* and *Idiosoma* species from the Mt. Weld area. That survey yielded an additional 24 samples comprising 18 spiny trapdoor spiders and six (6) burrowing scorpions.

Table 1.3 Samples from Stantec's 2023 targeted survey

Alacran Registration	Stantec Field Codes	Identification	Latitude	Longitude
AES230042	100	<i>Idiosoma</i> sp.	28°53'37.16	122°29'12.18
AES230043	105	<i>Idiosoma</i> sp.	28°53'13.18	122°30'44.13
AES230044	106	<i>Idiosoma</i> sp.	28°52'24.89	122°29'45.42
AES230045	107	<i>Idiosoma</i> sp.	28°53'21.02	122°32'20.34
AES230046	108	<i>Idiosoma</i> sp.	28°53'37.04	122°29'12.18
AES230047	113	<i>Idiosoma</i> sp.	28°53'17.06	122°30'42.26
AES230049	122	<i>Idiosoma</i> sp.	28°53'48.37	122°30'42.42
AES230052	125	<i>Idiosoma</i> sp.	28°51'18.72	122°29'29.20
AES230053	131	<i>Idiosoma</i> sp.	28°53'46.64	122°30'43.45
AES230056	134	<i>Idiosoma</i> sp.	28°51'43.52	122°29'47.53
AES230057	135	<i>Idiosoma</i> sp.	28°53'13.55	122°30'44.58
AES230058	136	<i>Idiosoma</i> sp.	28°53'15.48	122°30'44.48
AES230059	137	<i>Idiosoma</i> sp.	28°53'45.59	122°30'43.99
AES230060	138	<i>Idiosoma</i> sp.	28°53'45.71	122°30'41.45
AES230061	139	<i>Idiosoma</i> sp.	28°53'20.62	122°32'18.76
AES230062	144	<i>Idiosoma</i> sp.	28°51'17.21	122°29'32.53
AES230063	145	<i>Idiosoma</i> sp.	28°51'41.73	122°29'49.29
AES230064	146	<i>Idiosoma</i> sp.	28°52'24.11	122°29'45.12
AES230048	114	<i>Urodacus</i> sp.	28°52'20.10	122°31'14.19
AES230050	123	<i>Urodacus</i> sp.	28°52'17.23	122°31'12.20
AES230051	124	<i>Urodacus</i> sp.	28°53'20.44	122°30'43.76
AES230054	132	<i>Urodacus</i> sp.	28°52'19.25	122°31'18.44
AES230055	133	<i>Urodacus</i> sp.	28°53'15.38	122°30'43.57
AES230065	147	<i>Urodacus</i> sp.	28°52'17.02	122°31'11.68

This report presents the results of DNA sequencing and barcoding analyses undertaken for these samples.

2 METHODS

2.1 GENOMIC IDENTIFICATION (DNA BARCODING)

Species and morphospecies were initially determined using morphological characteristics and all identified by Dr E.S. Volschenk (Alacran).

Sequence fragments of the COI gene were amplified from muscle tissues obtained from specimens fixed and preserved in >95% ethanol or from samples fixed in Propylene glycol and later preserved in >95% ethanol. Sequence generation from tissues was undertaken by Xytogen Pty Ltd (<https://xytogen.com.au>) and AGRF Perth (<http://www.agrf.org.au>).

Sequence contigs were assembled from forward and reverse amplicons using Geneious R7 version 7.1.9 and were subsequently edited and pruned by eye, prior to consensus sequences being created. Consensus

sequences were then assessed for contamination using the NCBI Nucleotide Blast tool (<https://blast.ncbi.nlm.nih.gov>).

For analyses and alignment, additional sequences of species pairs were obtained from GenBank ([Benson et al. 2012](#)) or from Alacran's database of unpublished sequences, to better guide alignments and assess with determining species boundaries. Species pairs were selected from taxa within the same genus or family of the taxa under investigation, for which DNA sequence fragments were greater than 400 bases long.

Sequences sourced from samples previously studied by Alacran (unpublished) but subsequently lodged at the W.A. Museum are identified with the W.A. Museum registration codes:

- Arachnology dept. beginning with "T" and followed by a string of numbers;
- Crustaceans dept beginning with "C" and followed by a string of numbers;
- Molluscs dept beginning with "S" and followed by a string of numbers.

Alacran sequence registration codes use the format of "AES" or "ESV" followed by a string of six numbers. Sequences sourced from GenBank are identified with the Genbank accession code (comprised of two letters followed by a string of six numbers).

Sequence editing, alignment and phylogenetic analysis were performed with Geneious R7. For each analysis sequences were assembled and aligned using ClusallW or the Geneious alignment tool, implementing the following variable settings: Global alignment with free ends; Cost matrix, 70% similarity (IUB); Open gap penalty, 70; Gap extension penalty, 10; refinement iterations, 4.

Phylogenetic analyses were implemented using Geneious R7 and MrBayes ([Huelsenbeck et al. 2001](#); [Ronquist et al. 2012](#)). Outgroups were selected from (in order of preference): sister -genus, -family or -order, to the group under investigation, where systematic relationships were known. Species delimitation was based on an 8% sequence divergence threshold for most taxa ([Hebert et al. 2003a](#); [Hebert et al. 2003b](#); [Barrett et al. 2005](#); [Robinson et al. 2009](#)). For trapdoor spiders, the delimiting threshold of 9.5 % was used ([Castalanelli et al. 2014](#); [Huey et al. 2019](#)) and 4% was used for scorpions (Volschenk unpublished data).

Reference taxa were selected from Genbank in two ways, depending on the number of sequences available for each group. For small groups, all reference COI sequences were sourced from Genbank and a subset of two or three samples were selected to represent each species or morphospecies identified. For groups with many reference COI sequences on Genbank, reference sequences were selected from BLAST (Basic Local Alignment Search Tool) results undertaken for each query sequence. Sequence BLAST was undertaken using the Nucleotide BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Two or three reference sequences were selected from the 20 most similar sequences (using percentage identity) resulting from a BLAST of each query sequence.

3 RESULTS

Of the 13 target samples, all yielded DNA sequence data; however, the sequence data from *Urodacus* sp. (T136195) was very poor with numerous ambiguous (N) bases.

Of the comparative W.A. Museum samples attempted, three failed to yield useable DNA sequence data (T110330, T110337, T110350). Of the recently collected Stantec samples, One sample only yielded a clean sequence from the forward primer amplification (*Idiosoma* sp., AES230049).

Comparisons of the edited COI DNA sequence with Genbank and Alacran reference sequences are summarised in Table 3.1. Relevant parts of the phylogenetic trees are provided in the Figures section (Section 5) for visualisation of species' relationships. The raw sequence data is supplied for reference in Appendix 1.

Table 3.1 Summary of species delimitation of query samples with supporting COI DNA divergence results; target sample codes in bold.

Sample Codes	Order	Family	Species or Morphospecies Name	Divergence % Between Conspecific Samples	Minimum Divergence % From Other Species Analysed	Reference Figure	Database Match	SRE status
T136178 , T110341, T110348, T110342, T110343, MW518644	Araneae	Anamidae	<i>Aname</i> sp. 'MYG629'	0-6.82	10.7	Figure 5.1	Genbank	Potential SRE
OQ865359, OQ865360, T155078, T155085, T155098, T155105, T155109, T155110,	Araneae	Anamidae	<i>Aname</i> sp. 'Phoenix0055'	0-10.6	16.43	-	Genbank	Potential SRE
T110326	Araneae	Anamidae	<i>Aname</i> sp. 'MW1'	-	15.97	-	-	Potential SRE
T155103	Araneae	Anamidae	<i>Aname</i> sp. 'MW2'	-	11.16	-	-	Potential SRE
AES200341, KJ745099, MW621136, T110340, T110346, T110347, T110349, T141017, T141019, T141020	Araneae	Idiopidae	<i>Idiosoma</i> sp. 'MYG256'	8.8	0-7.42	Figure 5.2	Genbank and Alacran	Widespread
AES200132, KY295319, MW199717, T151182, T151183	Araneae	Idiopidae	<i>Idiosoma</i> sp. 'MYG721'	0.23-6.4	10.92	Figure 5.2	Genbank	Widespread
AES230022 , AES230024 , AES230026 , AES230042, AES230043, AES230045, AES230046, AES230047, AES230049, AES230053, AES230057, AES230058, AES230059, AES230060, AES230061, AES230062, MW199716, KY295274, T110338, T155089, T155113, T136167 , T136170 , T151179 ,	Araneae	Idiopidae	<i>Idiosoma</i> sp. 'MYG722'	0-6.117	13.166	Figure 5.2	Genbank	Potential SRE

Sample Codes	Order	Family	Species or Morphospecies Name	Divergence % Between Conspecific Samples	Minimum Divergence % From Other Species Analysed	Reference Figure	Database Match	SRE status
T151180, T155083, T155108, T156445, MW199720, MW199721, T151174, T151176, T160697	Araneae	Idiopidae	<i>Idiosoma</i> sp. 'MYG723'	0-0.31	13.228		Genbank	Potential SRE
AES230023, AES230025, AES230052, AES230063,	Araneae	Idiopidae	<i>Idiosoma</i> sp. 'MW1'	0.16-1.28	7.937	Figure 5.2	-	Potential SRE
AES230056, T136168, T136169	Araneae	Idiopidae	<i>Idiosoma</i> sp. 'MW2'	1.07-3.61	7.94	Figure 5.2	-	Potential SRE
T110327, T110332, T110333, T110353, AES230044	Araneae	Idiopidae	Unmatched <i>Idiosoma</i> species	-	-	-	-	Potential SRE
AES230029	Pseudoscorpiones	Garypidae	<i>Synsphyronus</i> sp. 'weld'	-	16.615	Figure 5.3	-	Potential SRE
AES23048, AES23050 AES23051, AES23054 AES23055, AES23065 T136195	Scorpiones	Urodacidae	<i>Urodacus</i> sp. 'MW1'	0-1.4	5.42	Figure 5.4	-	Potential SRE: DDG

4 DISCUSSION AND SUMMARY

This study assessed COI sequences from 12 target taxa and assessed these against sequences from Genbank, Alacran, WA Museum and more recently collected samples from Mount Weld. This study determined matched the identity of 11 of the 12 samples with sequences from genbank and, or, sequenced generated from WA Museum samples or recently collected samples Table 3.1. Only the Pseudoscorpion *Synsphyronus* 'weld' was not matched with any reference sequences. Three species could not be matched to known morphospecies: *Idiosoma* 'MW1', *Idiosoma* 'MW2' and *Urodacus* 'MW1'.

Idiosoma 'MW1' and *Idiosoma* 'MW2' were at the upper limit of the threshold usually used to delimit trapdoor spider morphospecies using COI. These species appear to be maintaining this discrete threshold in relatively close proximity therefore it is likely that these represent recently diverged sister species.

The target sample of *Urodacus* 'MW1' (T136195) yielded poor sequence data; however, despite numerous ambiguous bases, this was matched to the additional samples collected by Stantec in 2023.

Sample AES230064 was placed among an outgroup of Barychellidae therefore no further attempt was made to determine the identity of that sequence.

5 FIGURES

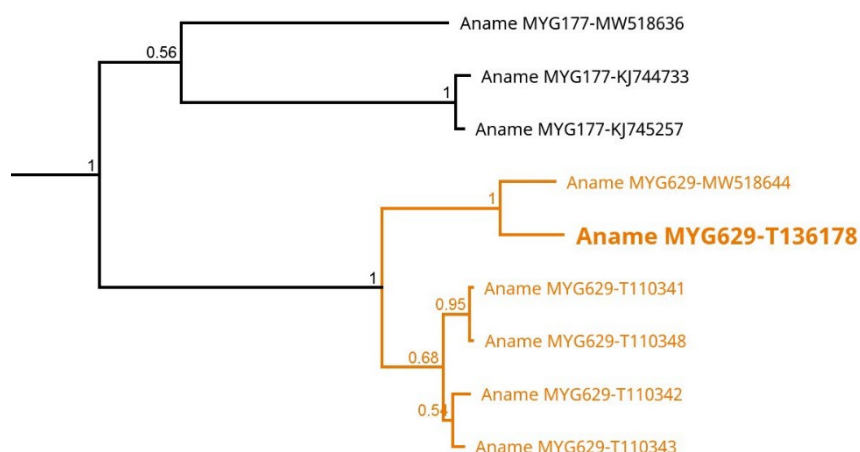


Figure 5.1. Subclade of Bayesian phylogeny of Anamidae showing *Aname* sp. 'MYG629' conspecific taxa highlighted in coloured text and query sample in bold. Branch labels represent posterior probabilities and scale bar represents substitutions per site.

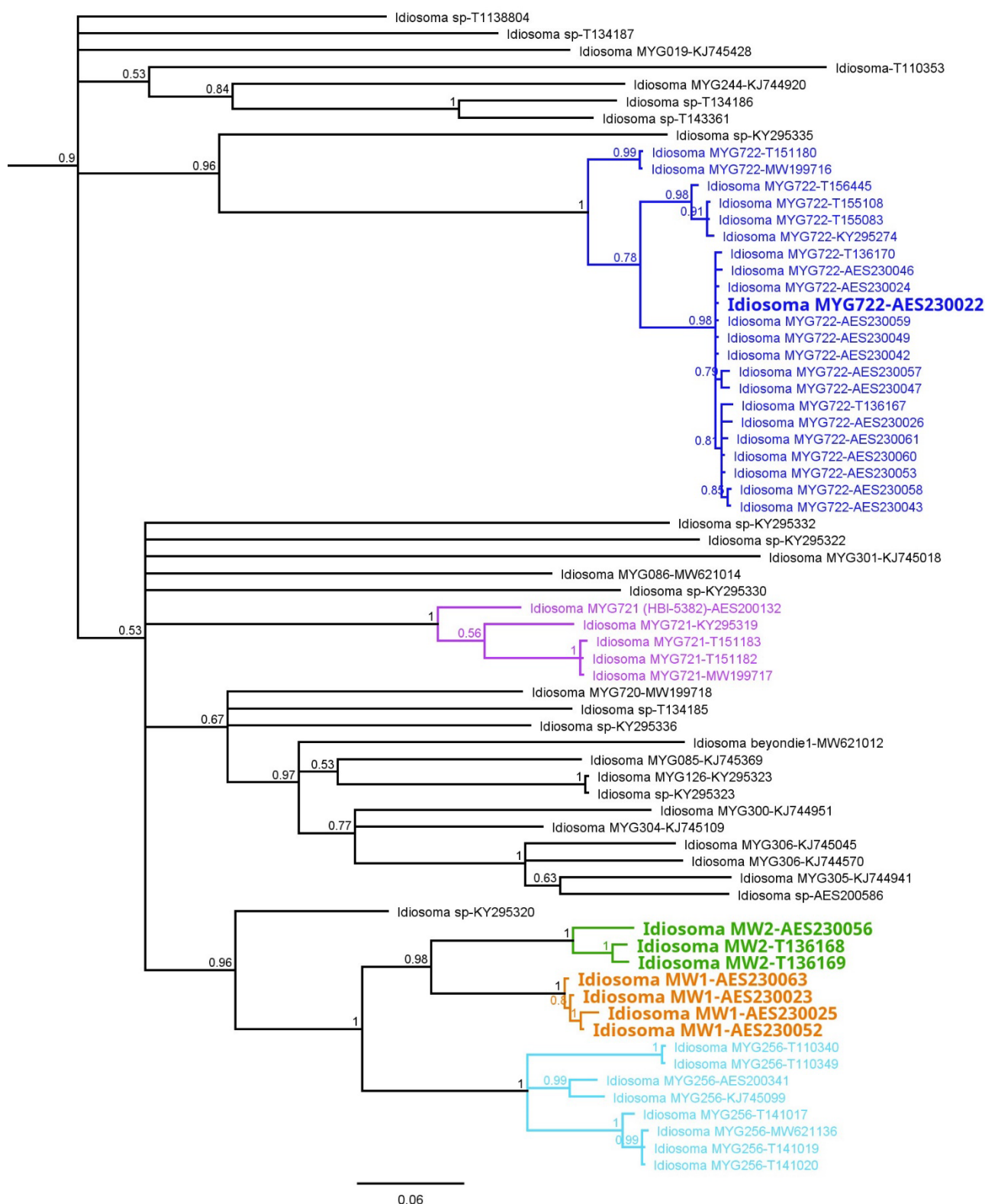


Figure 5.2. Subclade of Bayesian phylogeny of Idiopidae showing *Idiosoma* species with query taxa highlighted in coloured text and target samples from this project in bold. Branch labels represent posterior probabilities and scale bar represents substitutions per site.

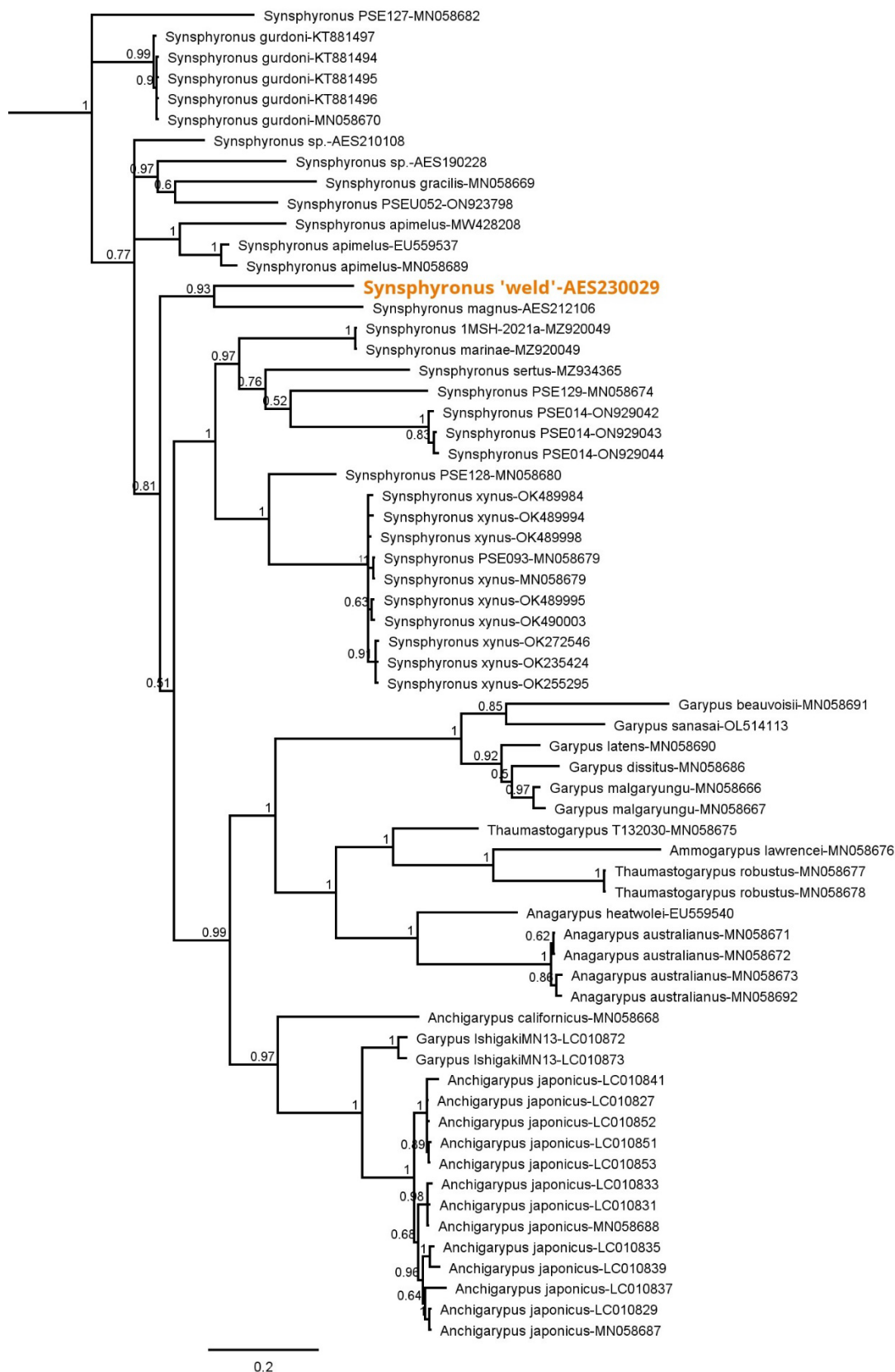


Figure 5.3. Subclade of Bayesian phylogeny of Garypidae showing *Synsphyronus* clade with query taxon highlighted. Branch labels represent posterior probabilities and scale bar represents substitutions per site.

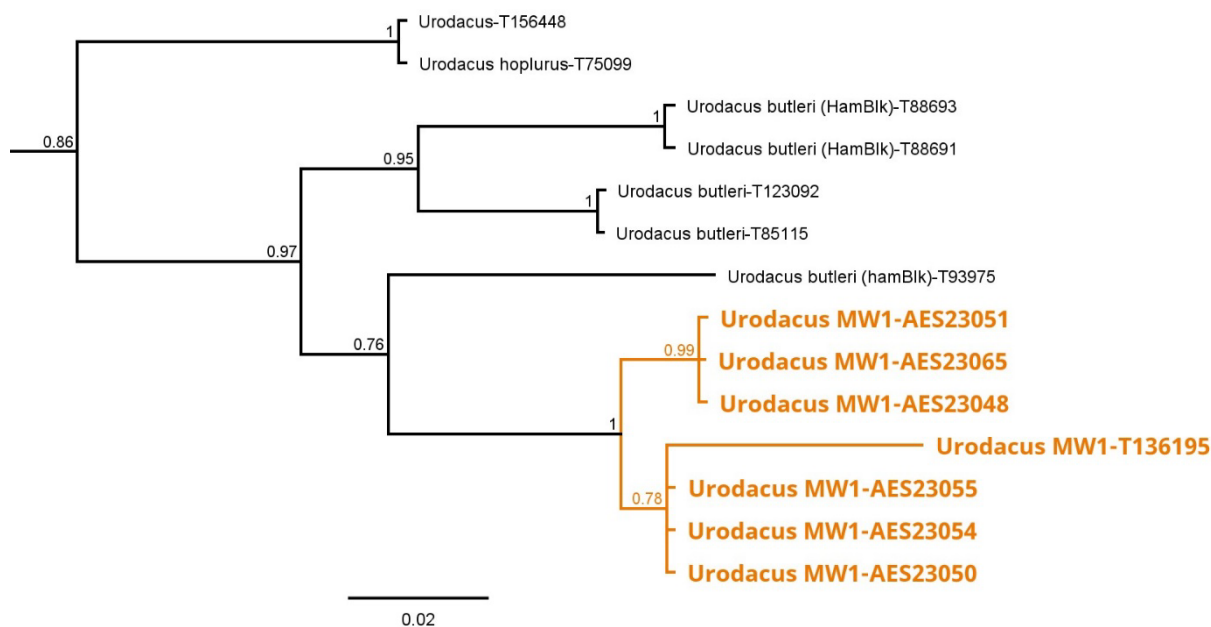


Figure 5.4. Subclade of Bayesian phylogeny of Urodacidae showing *Urodacus* clade with query taxon and sample from this project highlighted in coloured and bold text. Branch labels represent posterior probabilities and scale bar represents substitutions per site.

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7 APPENDIX

Appendix 1. DNA Sequences (COI) generated for this report.

>Aname_MW1-T110326

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>Aname_MW2-T155103

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>Aname_MYG629-T110341

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>Aname_MYG629-T110342

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>Barychellidae-AES230064

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>Idiosoma_MW1-AES230052

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>Idiosoma_MW2-AES230056

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>Idiosoma_MW2-T136168

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