Biota Environmental Sciences

Robe Mesa Project Short-range Endemic Invertebrate Fauna Survey

Prepared for CZR Resources Ltd

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Biota Environmental Sciences

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Robe Mesa Project SRE Fauna Survey

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1.0 Executive Summary

1.1 Background

CZR Resources Ltd (CZR) is proposing to develop the Robe Mesa Iron Ore Project (the project), located in the west Pilbara, 29 km southwest of Pannawonica. To service the planned mine, the project will require associated infrastructure and a haul road.

CZR commissioned Biota Environmental Sciences (Biota) to conduct a single-phase short-range endemic (SRE) invertebrate survey to inform the environmental impact assessment (EIA). The survey area comprised:

- the Mine Associated Infrastructure Area (MAIA);
- two haul road options that run west and northwest from the MAIA to meet the North West Coastal Highway; and
- the East Additional Area (EAA) located east of the MAIA.

1.2 Methodology

A desktop study of relevant literature, databases, and past survey reports from the locality (40 km from the survey area) was undertaken to identify biological features and potential constraints.

The targeted survey for potential SRE invertebrates was conducted two Biota zoologists from 14 to 20 June 2022, in accordance with relevant Environmental Protection Authority policy. Survey methods included:

- a habitat assessment that considered SRE invertebrate life histories and known important habitat types;
- a total of 80 person hours dedicated to SRE fauna searches at 34 sites; and
- molecular sequencing (DNA barcoding) to identify specimens to species level and to determine known distributions.

1.3 Results

Four broad fauna habitats were identified in the survey area:

- River/Flood Plains;
- Alluvial Plains;
- Colluvial Plains; and
- Low Stony Hills.

All four habitats were common in the locality, being contiguous between the survey area and surrounds.

Specimens were collected from two taxonomic groups that have a higher potential to contain SRE species; mygalomorph spiders (eight taxa) and land snails (two taxa). Of the 10 nominal species recorded during the survey, six of the mygalomorph spider taxa are potential SREs known solely from the survey area. However, the habitat attributes of the survey area and wider locality make it unlikely that these nominal species would be restricted to the survey area. The findings for the land snail specimens were consistent with this, with the two species represented being confirmed as widespread and not SREs.

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2.0 Introduction

2.1 Project Background

CZR Resources Ltd (CZR) is proposing to develop the Robe Mesa Iron Ore Project (the project), located in the west Pilbara, 29 km southwest of Pannawonica. To service the planned mine, the project will require associated infrastructure and a haul road.

CZR commissioned Biota Environmental Sciences (Biota) to conduct a single-phase short-range endemic (SRE) invertebrate survey to inform the environmental impact assessment (EIA) of the project.

As summarised in Table 2.1 and presented in (Figure 2.1), the 'survey area' comprised:

- Mine Associated Infrastructure Area (MAIA);
- an Upper Haul Road option that runs northwest from the MAIA to meet the North West Coastal Highway;
- a Lower Haul Road option that run west from the MAIA to meet the North West Coastal Highway; and
- the East Additional Area (EAA) located east of the MAIA.

Table 2.1: Spatial scope of the survey area.

Section	Area (ha)
MAIA	1,204.0
Upper Haul Rd option	477.5
Lower Haul Rd option	606.2
EAA	762.3
Total Area	**3,050.0**

2.2 Short-range Endemic Invertebrates

SREs include taxonomic groups that exhibit naturally small distributions (less than 10,000 km^2; Harvey 2002). Due to their predisposition to small spatial scales, SRE fauna are more likely to be at risk of population extinctions than more widely distributed taxa (Harvey 2002, Harvey et al. 2011, EPA 2016) and as such, the assessment of potential impact to SREs is an important component of EIA.

Certain groups of invertebrates are pre-disposed to short-range endemism through particular life history traits such as poor dispersal capabilities, confinement to disjunct habitats, slow reproduction and low fecundity (Harvey 2002, Ponder and Colgan 2002). In the Pilbara, these most commonly include:

- mygalomorph spiders (Mygalomorphae);
- millipedes (Diplopoda); and
- terrestrial snails (Pulmonata).

2.3 Study Scope and Objectives

The specific objectives of this study were to complete:

- a desktop study, to consolidate available relevant SRE data to identify biological features and constraints;
- a single-phase SRE fauna survey in accordance with relevant EPA guidance (EPA 2016);
- an SRE fauna habitat assessment and mapping; and
- a report detailing the findings of the desktop study and SRE fauna survey to identify any key values.

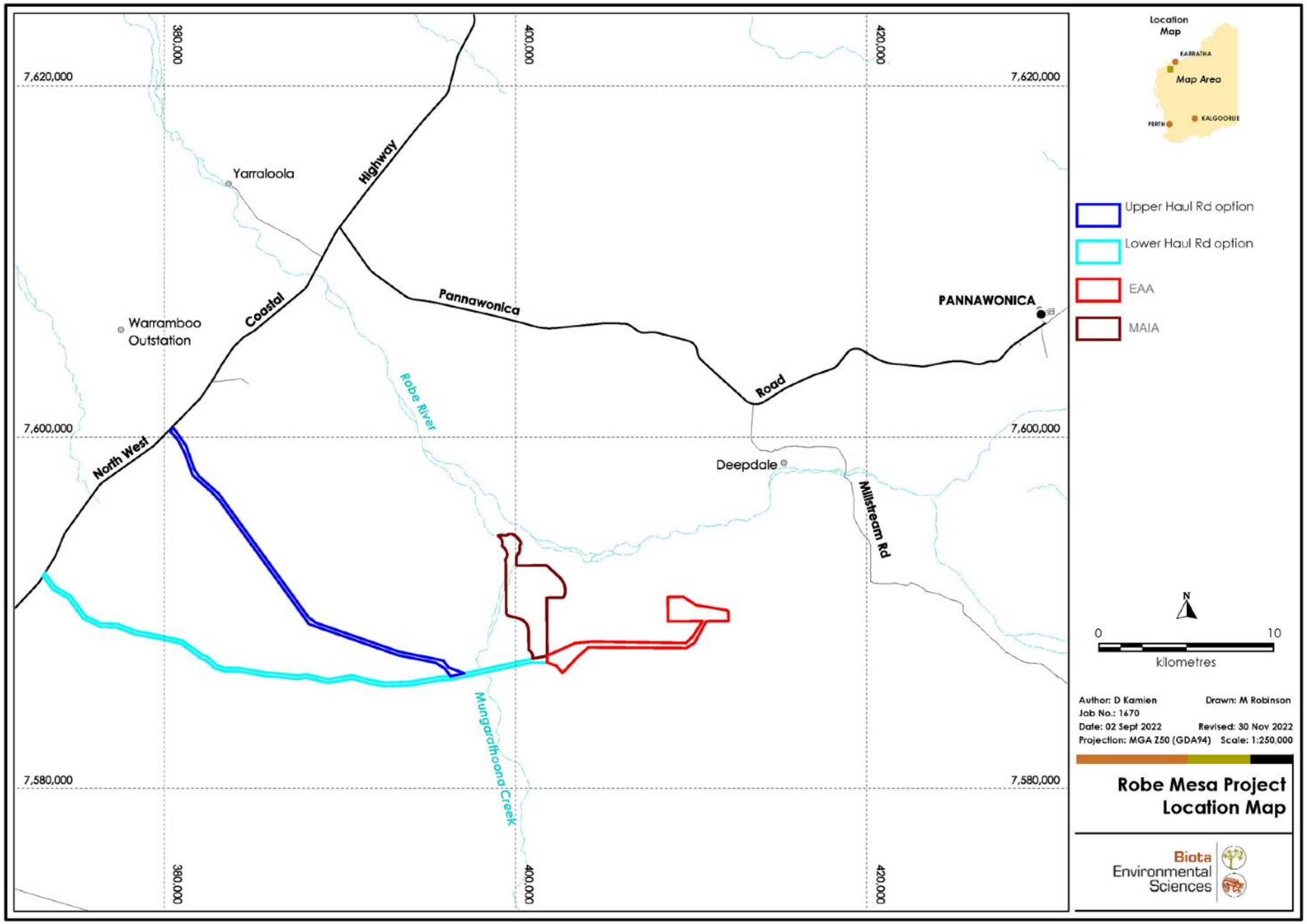

Figure 2.1: Survey area location map.

3.0 Methodology

3.1 Desktop Study

3.1.1 Literature Review

Reports and papers relevant to the study were reviewed. These included:

- a review of relevant biological surveys previously completed in the locality (within a 40 km of the survey area polygon; see Section 4.0);
- molecular and morphological characterisation of new species of the mygalomorph spider genus *Aname* (Harvey et al. 2012); and
- barcoding of mygalomorph spiders in the Pilbara bioregion of WA (Castalanelli et al. 2014a).

3.1.2 Database Searches

The following databases were searched to assist with compilation of a list of potential SRE species in the survey area:

1. **NatureMap**: a collaboration between the Department of Biodiversity Conservation and Attractions (DBCA) and the Western Australian Museum (WAM). This database represents the most comprehensive source of information on the distribution of Western Australia's fauna, comprising records from the Fauna Survey Returns database and WA Threatened Fauna Database (both maintained by DBCA) and the WAM Specimen Database.
2. **Atlas of Living Australia (ALA)**: a collaborative project between government and academic collecting institutions, private individual collectors and community groups. ALA contains occurrence records, environmental data, images, and information on the conservation status of species throughout Australia.
3. **The Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) Protected Matters Search Tool**: a database of federally listed fauna species and any other matters of national environmental significance (MNES) that may occur in the locality.
4. **WAM Arachnid and Myriapod, and Mollusc databases**: as DBCA licencing requirements stipulate that specimens are to be lodged with the WAM, this database represents a comprehensive depository of SRE records.
5. **Biota Internal Data**: this includes invertebrate fauna data collected by Biota within Western Australia.

All database searches were conducted from within a 40 km buffer on the survey area.

3.2 Habitat Assessment

Habitat descriptions were conducted at each sampling site within the survey area with elements recorded including landscape type, soil type, surface material, landform, any notable microhabitats present, any disturbances, broad vegetation characteristics and representative photographs. Habitat descriptions were then considered in the context of available aerial imagery, regional land systems mapping, detailed vegetation mapping (Biota in prep.) and geology, to validate and inform the extent of identified habitats.

A combination of field-based habitat descriptions and thematic layers was used to define fauna landscapes and landforms of the survey area based on Biota's fauna landscape approach (Biota 2013), which identifies functional landforms within a broader landscape and in consideration of EPA (2016) guidance.

3.3 Survey Timing and Weather

3.3.1 Survey Team

The survey was conducted by two Biota zoologists from 14 to 20 June 2022 (Table 3.1). The survey was completed under "Fauna taking (Biological Assessment) Licence" No. BA27000646, issued to Roxanne de Vos (Appendix 1).

Table 3.1: Summary of personnel involved in the SRE fauna survey.

Name	Position at Biota	Qualification	Years of Experience	Survey Role
Dan Kamien	Principal Zoologist	BSc. Hons	25	Project Manager Reporting
Michael Greenham	Senior Zoologist	BSc.	22	Field survey team leader
Roxanne de Vos	Zoologist	BSc.	3	Field team member

3.3.2 Climate and Weather

Long-term climate data (rainfall from 1885 – 2022, temperature data from 1956 – 2022) and recent weather data were obtained from the Bureau of Meteorological weather station in Mardie (station number 5008), approximately 60 km north of the survey area.

During the survey, initial weather conditions were warm and dry (Table 3.2) but cooled slightly on day five when 4.6 mm of rain was recorded (Table 3.2).

Comparison of the year preceding the survey with long-term climate averages indicated that the survey was conducted following a year of generally average weather conditions although it followed a month of considerably greater than average precipitation (Figure 3.1).

Table 3.2: Weather at Mardie during the 2022 survey period.

	14/06	15/06	16/06	17/06	18/06	19/06	20/06	Mean/Total
Maximum temperature (°C)	30.9	30.7	31.9	28.1	26	28.1	28.6	**16.7**
Minimum temperature (°C)	17.3	17.3	15.9	18	18.6	16.9	13.2	**29.2**
Rainfall (mm)	0	0	0	0	4.6	0.8	0	**5.4**

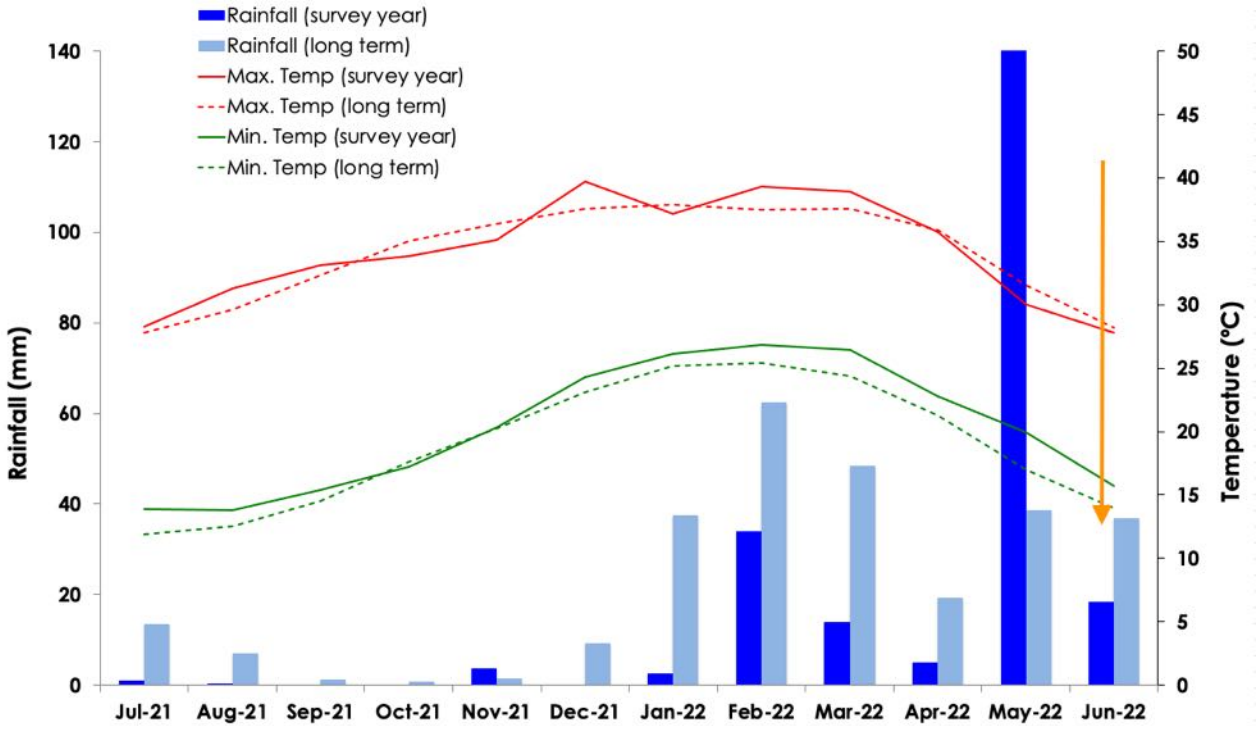

Figure 3.1: Climate and weather graph (Mardie Station) depicting long-term averages and 2022 data. Orange arrows indicate survey timing.

3.4 Fauna Sampling

In total, 80 person hours were dedicated to SRE fauna searches (Table 3.3). Search sites were located where SRE species most frequently occur (e.g. habitat with suitable soil profile and drainage depressions). A total of 34 SRE sites were sampled (Table 3.3 and Figure 3.2).

Mygalomorph spider burrows were located visually and were photographed prior to excavation. Holes were dug adjacent to each burrow, allowing the burrow to be followed until the spider was located. Two legs of each spider collected were removed and placed in 100% ethanol for molecular studies (Section 3.5), while the remainder of the spider was preserved in 70% ethanol suitable for morphological studies.

Searches for land snails were conducted by excavating leaf litter and soil around the base of trees and bushes and searching under rocks and in rock crevices. Live specimens were stored in calico bags prior to being lodged with Helix Molecular Solutions for molecular analysis. Millipede searches were conducted by raking through leaf litter and debris, but none were located.

Table 3.3: SRE site locations and search effort.

Site	Latitude	Longitude	Search Date	Habitat	Search Effort (minutes)
RMP01SRE_MG	-21.697575	115.845110	20/6/2022	Colluvial Plain	176
RMP02SRE_MG	-21.705843	115.851447	20/6/2022	Colluvial Plain	220
RMP03SRE_MG	-21.714095	115.854482	19/6/2022	Colluvial Plain	162
RMP04SRE_MG	-21.721237	115.858767	19/6/2022	Alluvial Plain	158
RMP05SRE_MG	-21.723157	115.861938	19/6/2022	Alluvial Plain	106
RMP07SRE_RD	-21.726002	115.864579	19/6/2022	Alluvial Plain	128
RMP08SRE_MG	-21.737444	115.875427	14/6/2022	Alluvial Plain	122
RMP09SRE_MG	-21.752589	115.886926	19/6/2022	Alluvial Plain	156
RMP10SRE_RD	-21.769488	115.899293	19/6/2022	Alluvial Plain	73
RMP11SRE_MG	-21.787786	115.913572	18/6/2022	Alluvial Plain	160
RMP12SRE_MG	-21.802148	115.941076	18/6/2022	Alluvial Plain	130
RMP13SRE_MG	-21.813262	115.978983	18/6/2022	Colluvial Plain	6
RMP14SRE_MG	-21.821471	116.007865	18/6/2022	Alluvial Plain	178
RMP15SRE_MG	-21.754446	116.027051	17/6/2022	Low Stony Hill	226
RMP16SRE_MG	-21.785496	116.035150	17/6/2022	River/Floodplain	186
RMP17SRE_MG	-21.807744	116.069638	18/6/2022	Alluvial Plain	188
RMP18SRE_MG	-21.808021	116.125674	17/6/2022	Alluvial Plain	112
RMP19SRE_MG	-21.786125	116.127977	16/6/2022	Alluvial Plain	154
RMP20SRE_MG	-21.771906	115.775254	15/6/2022	Colluvial Plain	246
RMP21SRE_MG	-21.779800	115.783991	15/6/2022	Colluvial Plain	184
RMP22SRE_MG	-21.788631	115.792338	15/6/2022	Alluvial Plain	8
RMP23SRE_MG	-21.795797	115.80911	15/6/2022	Alluvial Plain	80
RMP24SRE_MG	-21.799625	115.824289	15/6/2022	River/Floodplain	134
RMP25SRE_MG	-21.801098	115.829046	15/6/2022	Low Stony Hill	116
RMP26SRE_MG	-21.816661	115.866306	16/6/2022	Alluvial Plain	158
RMP27SRE_MG	-21.818417	115.870530	16/6/2022	Alluvial Plain	162
RMP28SRE_MG	-21.822232	115.897709	16/6/2022	Alluvial Plain	168
RMP29SRE_MG	-21.824808	115.926678	16/6/2022	Alluvial Plain	126
RMP30SRE_MG	-21.825418	115.979625	18/6/2022	Alluvial Plain	130
RMP31SRE_MG	-21.794750	116.047268	17/6/2022	Alluvial Plain	184
RMP32SRE_MG	-21.807646	116.103852	17/6/2022	Low Stony Hill	160
RMP33SRE_MG	-21.789892	116.121517	16/6/2022	Alluvial Plain	126
RMP34SRE_MG	-21.792182	116.141230	17/6/2022	Alluvial Plain	92
RMP35SRE_MG	-21.812704	116.045393	18/6/2022	Alluvial Plain	88
				Total	**4,803**

3.5 Genetic Analysis

Potential SRE specimens often cannot be assigned to species due to unresolved taxonomies or lack of defining morphological characters (particularly in the case of juveniles, females or species complexes). To address these limitations, the current study made use of molecular sequencing (DNA barcoding) to determine whether the putative species collected in the current survey have been collected more widely, as a means of establishing their SRE status (Table 3.4).

Helix Molecular Solutions (Helix) carried out the molecular analysis comparing sequences of specimens collected during the survey to those available in Helix's database and the publicly available GenBank database. From this, taxa were assigned to putative species based on Bayesian analysis of COX1 haplotypes. In regards to mygalomorph spiders, previous assessments have been based on combining samples into species if they show less than 9.5% sequence divergence, this being based on frequency distribution analysis of pairwise estimates of *P*-distance (Biota 2012). This threshold is accepted by arachnologist Dr Mark Harvey (WAM) as suitably conservative. However, there are occasionally exceptions to this when there is high intraspecific (within species) genetic variation, but even higher interspecific (between species) variation, resulting in higher species divergence thresholds.

3.6 Determining SRE Status

The SRE status of species is based on the extent of their geographic distribution (Harvey 2002, EPA 2016). Table 3.4 details the criteria used to determine the SRE status of putative species for the purposes of this report.

Table 3.4: Criteria used to determine SRE status.

SRE Status	Defining Criteria
Known SRE	• Species, morphotype or genetic type has a documented distribution of <10,000 km^2. • Species, morphotype or genetic type is well collected with numerous specimens typed and habitat preference understood.
Potential SRE	• Species, morphotype or genetic type has a documented distribution of <10,000 km^2 but is poorly sampled. • Specimen may not be formally described or assigned to a morphotype / genetic type. • Short-range endemism may be common in genus or family. • May have been collected from restricted, refugia or isolated habitats.
Unlikely to be an SRE	• Species, morphotype or genetic type has a documented distribution of <10,000 km^2 but is poorly sampled. • Specimen may not be formally described or assigned to a morphotype / genetic type. • Short-range endemism is not common in genus or family. • Taxon was not collected from restricted, refugia or isolated habitats. • Few other individuals of the taxon collected, but records are separated by long distances (>100 km).
Not an SRE	• Specimen formally described or assigned to a morphotype / genetic type. • Species, morphotype or genetic type has a documented distribution of >10,000 km^2.
Undetermined	• Taxa where there is insufficient taxonomic framework available to provide any informed comment on the species-level distribution of the fauna or, therefore, the risk of small-scale spatial restrictions.

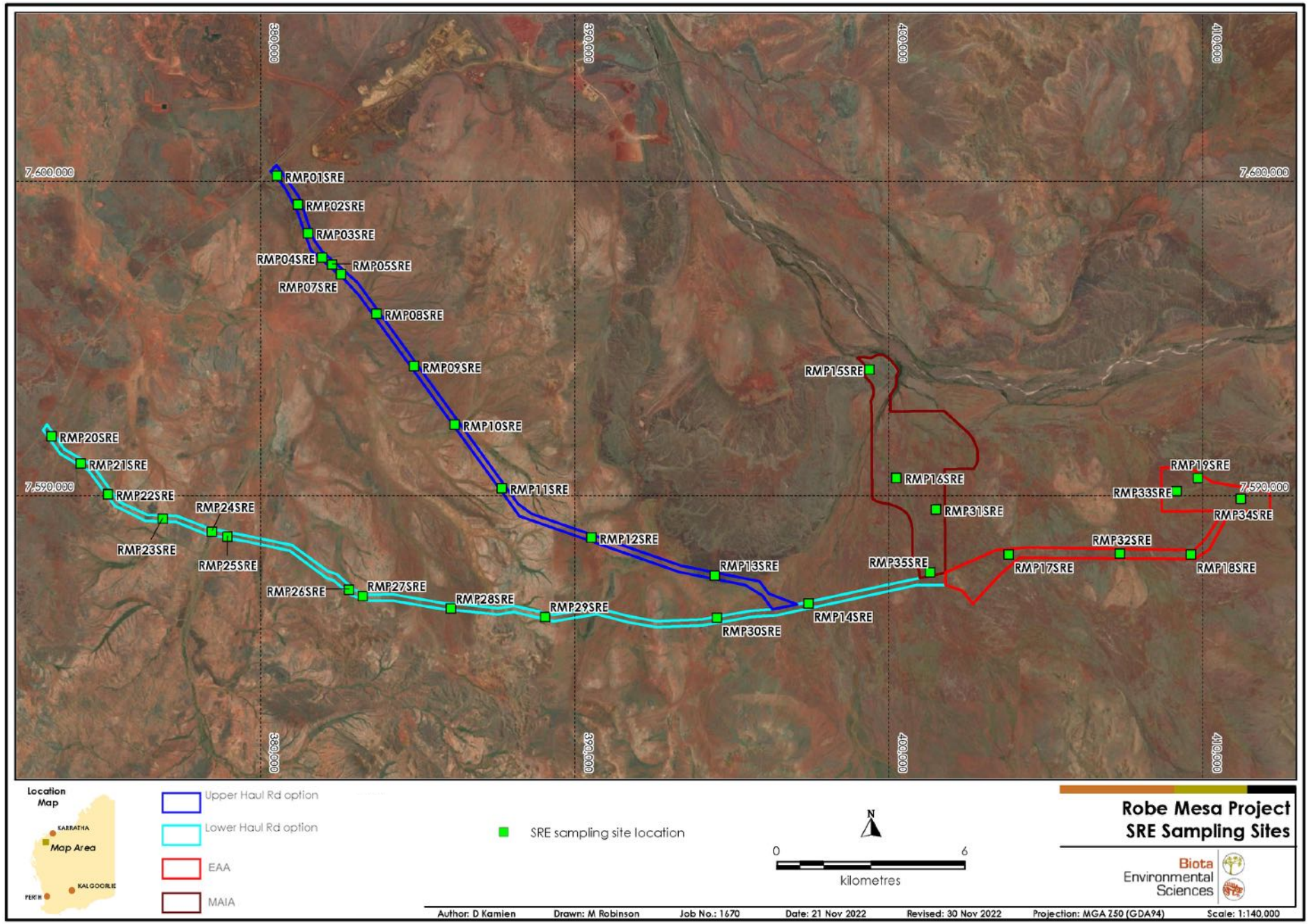

Figure 3.2: SRE sampling locations.

3.7 Study Limitations

The results presented within this report should be interpreted with consideration to the limitations outlined below.

- Not every section of the survey area was ground-truthed or sample. Sampling was, however, completed in habitats considered to represent the range of landforms and land systems present in the survey area.
- Many SRE taxa are difficult to sample. For example, mygalomorph spiders are time-consuming to locate and often construct cryptic burrows. Additionally, some rainfall occurred during the survey (Table 3.2), resulting in increased difficulty locating spider burrows .
- Specimens were assigned to lineages and then species-level taxa based on Bayesian analysis of COX1 haplotypes. These do not represent formal taxonomic groupings but have been used to infer nominal species equivalents based on current data.
- While up to date phylogenetic data are presented in this report, molecular work is ongoing on the SRE fauna of the Pilbara, and as additional data are incorporated into the DNA barcoding framework, species' distributions and SRE status may be revised in the future.

4.0 Desktop Study Results

4.1 Regional Context of the Survey Area

4.1.1 IBRA Bioregion and Subregion

The Interim Biogeographic Regionalisation for Australia (IBRA) recognises 89 bioregions and 419 subregions within Australia (DSEWPaC 2012). The survey area lies within the Pilbara bioregion and within the Hamersley subregion (PIL3), described by Kendrick (2003) as follows:

> "Mountainous area of Proterozoic sedimentary ranges and plateaus, dissected by gorges (basalt, shale and dolerite). Mulga low woodland over bunch grasses on fine textured soils in valley floors, and *Eucalyptus leucophloia* over *Triodia brizoides* on skeletal soils of the ranges. The climate is Semi-desert tropical. Total area is 6,215,092 ha."

4.1.2 Land Systems

Land systems are composed of repeating patterns of topography, soils and vegetation, which are described as a series of land units (Christian and Stewart 1953). A total of 105 land systems have been identified and mapped in the Pilbara bioregion by the then Department of Agriculture. Land systems mapping covering the survey area was prepared by van Vreeswyk et al. (2004).

A total of nine land systems are mapped within the survey area (Table 4.1, Figure 4.1). The most extensive of these is the Sherlock land system, which accounts for 38.5% of the survey area. However, the survey area intersects only a small proportion of the extent of each land system in the Pilbara bioregion.

4.1.3 Geology

Geological units for the locality were mapped at 1:250,000 scale by the Geological Survey of Western Australia (1968) as part of the Geological Survey of WA series. Thirteen geological units were mapped within the survey area, with the most widespread being the Qp unit comprising 32.9% of the survey area (Table 4.2, Figure 4.2).

4.1.4 Soils

Soil units have been mapped by Northcote et al. (1960). Five broad soil types have been mapped within the survey area, the most abundant of which is Oc66 (Table 4.3 and Figure 4.3) comprised of hard alkaline red soils with small areas associated with occasional patches of calcrete (Table 4.3).

4.1.5 Pre-European Vegetation

Broad-scale vegetation mapping for the locality has been prepared at the 1:1,000,000 scale based on the work of J.S. Beard for the Pilbara (Beard 1975a). The survey area includes four of Beard's vegetation associations, with 'Stewart Hills 29' encompassing most of the survey area (Table 4.4, Figure 4.4).

Table 4.1: Description of land systems in the survey area.
Data from Department of Agriculture WA (van Vreeswyk et al. 2004).

Land System	Description	Extent in Pilbara Bioregion (ha)	Extent in Survey Area		Extent in Survey Area as a Proportion of the Bioregion (%)
			Area (ha)	Proportion (%)	
Sherlock (SRK)	Stony alluvial plains supporting snakewood shrublands with patchy tussock grasses and spinifex grasslands.	38,638.9	1,173.8	38.5	3.0
Urandy (URY)	Stony plains, alluvial plains and drainage lines supporting shrubby soft spinifex grasslands.	131,975.6	734.6	24.1	0.6
Stuart (STT)	Undulating plains with snakewood; low hills with spinifex; stony chenopod and hard spinifex pastures in fair to excellent condition; no erosion.	276,684.8	687.2	22.5	0.2
Nanutarra (NNT)	Low mesas and hills of sedimentary rocks supporting soft and hard spinifex grasslands.	77,383.8	125.1	4.1	0.2
Robe (ROB)	Low plateaux, mesas and buttes of limonite supporting soft spinifex and occasionally hard spinifex grasslands.	128,859.4	98.5	3.2	<0.1
River (RIV)	Narrow, seasonally active flood plains and major river channels supporting moderately close, tall shrublands or woodlands of acacias and fringing communities of eucalypts sometimes with tussock grasses or spinifex.	482,175.6	94.2	3.1	<0.1
Capricorn (CPN)	Rugged sandstone hills and ridges; hard spinifex or stony short grass forb pasture in fair to good condition; no erosion.	698,530.6	65.2	2.1	<0.1
Peedamulla (PED)	Gravelly plains supporting hard spinifex grasslands and minor snakewood shrublands	59,200.7	55.8	1.9	<0.1
Boolgeeda (BGD)	Stony lower slopes and plains below hill systems supporting hard and soft spinifex grasslands or mulga shrublands.	961,634.8	15.5	0.5	<0.01
		Total	**3,050.0**	**100**	

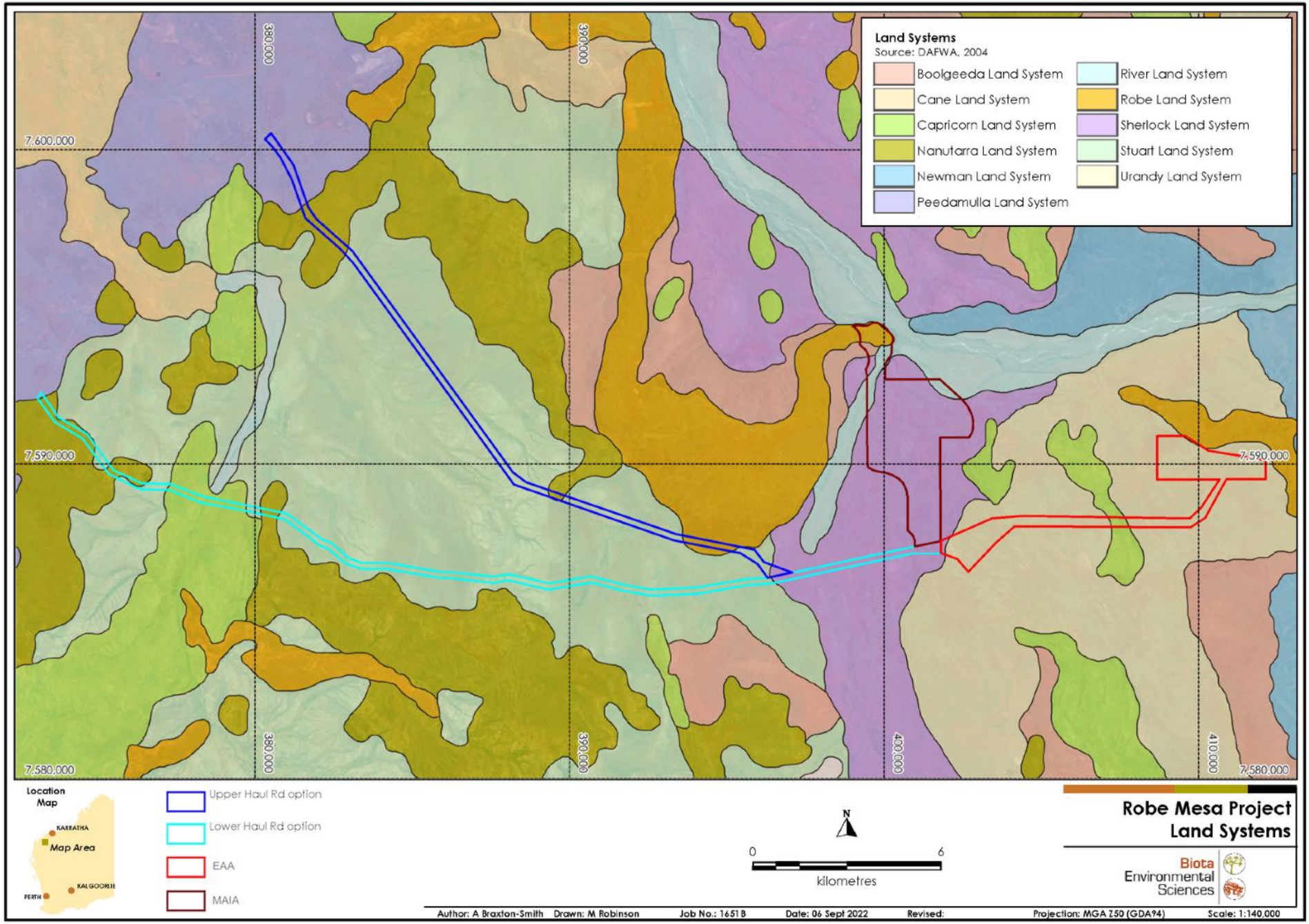

Figure 4.1: Land systems mapping of the survey area.

Table 4.2: Description and extent of geological units in the survey area.
Data from Geoscience Australia (Stewart et al. 2008).

Geological Unit	Description	Extent in Survey Area	
		Area (ha)	Proportion (%)
Qp	Eluvium and alluvium. Residual 'high level' clay and sandy clay plain with gilgais; intermittent veneer of alluvium; residual deposits of sand, gravel, and pebbles; sheet kunkar in places.	1,004.2	32.92
Qg	Colluvium. Unconsolidated to loosely consolidated slope deposits; calcareous and ferruginous cement in older parts.	813.4	26.67
Ql	Lacustrine deposits - clay, silt; saline in part, flood deposits. Unconsolidated fluviatile and sheet - flood deposits in levees and river terraces.	686.8	22.52
Qpt	Eluvium. Residual, unconsolidated or loosely consolidated, low angle slope deposits; angular to subrounded shale and ironstone fragments; quartz and quartzite pebbles	188.7	6.19
Tp	Pisolitic limonite deposits with fossil wood fragments. Occurs along old river channels. Contains iron ore.	102.1	3.35
Qr	Alluvium. Unconsolidated fluviatile deposits, mostly sand.	67.5	2.21
Kn	Nanutarra formation. Shale, siltstone, micaceous siltstone; ferruginous and glauconitic quartz sandstone; some conglomerate; contains plant and marine fossils	40.9	1.34
Wd	Duck Creek dolomite. Calcitic dolomite, minor shale; with Collenia	40.9	1.34
Ma	Warramboo sandstone. Interbedded massive and flaggy quartz sandstone, and shale	28.1	0.92
Wa	Ashburton formation. Interbedded shale, fine grained sandstone, greywacke; ferruginous and siliceous shale, thin dolomite; phyilite, quartz-mica schist and mica schist	24.0	0.79
Wdc	Chert, chert breccia.	25.8	0.85
Kny	Yarraloola conglomerate. Poorly sorted conglomerate with shale, claystone lenses and interbedded sandstone; contains plant fossils	22.1	0.72
Mk	Katanga conglomerate. Poorly sorted conglomerate with interbedded quartz sandstone	5.5	0.18
	Total	**3,050.0**	**100**

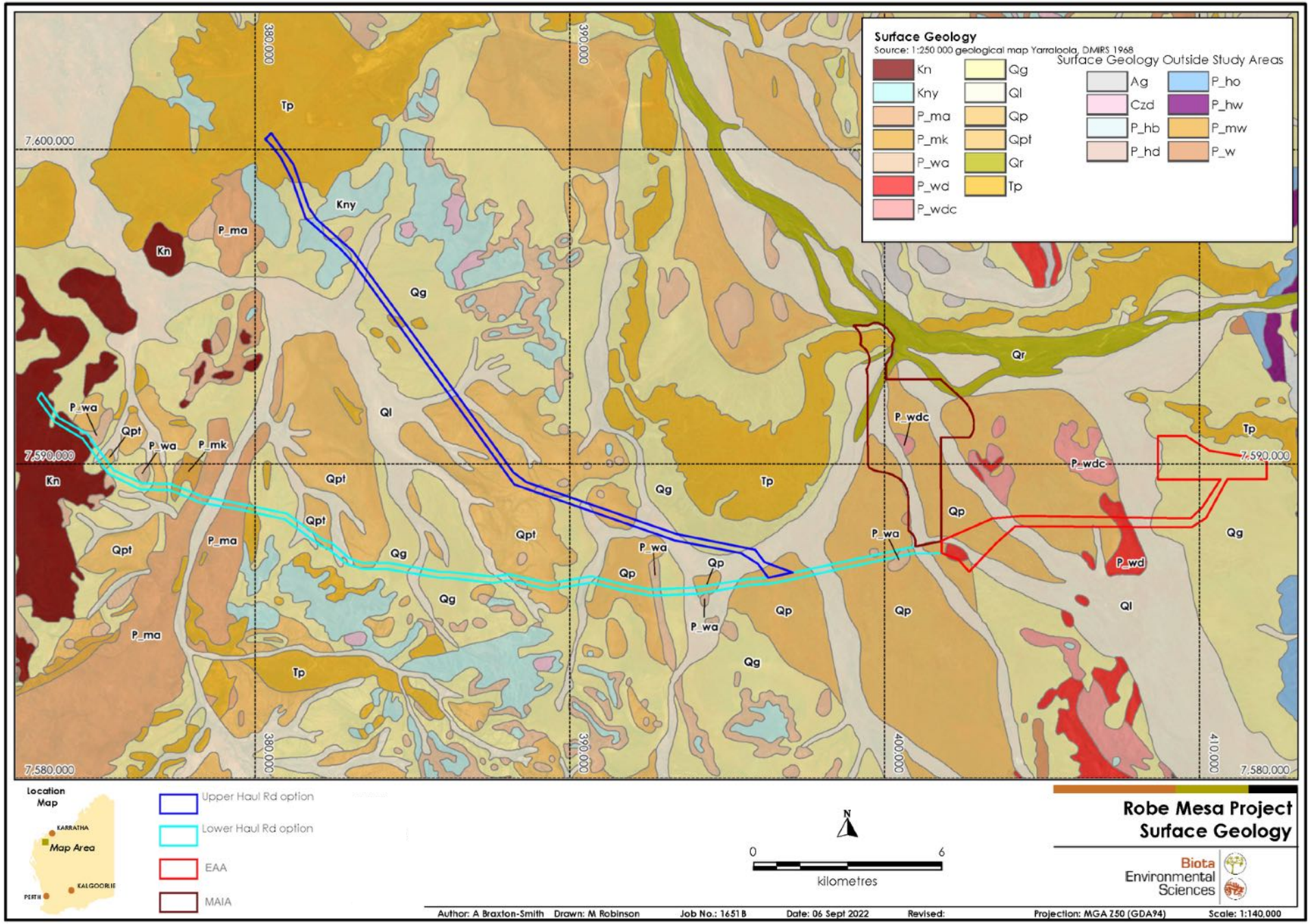

Figure 4.2: Geological units of the survey area.

Table 4.3: Description and extent of soil units within the survey area.
Data from Northcote et al. (1960).

Soil Unit	Description	Extent in Survey Area (ha)	
		Area (ha)	Proportion (%)
Oc66	Gently undulating pediplains extending out from breakaways capped by Robe pisolite deposits and other related formations. There may be a few small flat-topped residuals rising above the pediplains: chief soils are hard alkaline red soils (Dr2.33). Small areas of (Um5.11) soils may be associated with occasional patches of calcrete (kunkar). Minor soil occurrences include (Uf6.71), (Ug5.37), (Gn2.13), and stony (Gn2.12) soils.	1,517.1	49.74
Oc67	Plains: dominant soils are hard alkaline red soils (Dr2.33). Associated are extensive areas of (Um5.52) soils with (Ug5.38) soils in central landscape positions. Small areas of (Gn2.12) soils also occur as well as (Um5.11) on calcrete (kunkar).	786.1	25.77
B27	Low terrace associated with main stream channels: chief soils are loose sands (Uc1.22) with some (Um5.11) soils on patches of calcrete (kunkar).	358.1	11.74
Oc65	Low stony hills and steeply dissected pediments in areas of fine-grained sandstone, shale, and dolomite. There may be small areas of ferruginous duricrust and Robe pisolite as a capping. The soils are often shallow and stony: chief soils are hard alkaline red soils (Dr2.33) with some (Uc5.11) soils. (Um5.11) soils may occur on calcrete (kunkar) in the narrow valley plains and on exposures of calcareous rocks. (KS-Gn2.11) soils occur on the small area of ferruginous duricrust and Robe pisolite.	344.7	11.30
MY1	Gently undulating plateau elements sometimes sharply incised by narrow valleys. The boundary of this unit is frequently formed by breakaways but it may at times merge beneath the adjacent plain. These areas are capped by the Robe pisolite iron ore formation. The chief soils are gravelly acid red earths (KS-Gn2.11) with (Dr2.33) soils on the pediments.	44.0	1.44
	Total	**3,050.0**	**100**

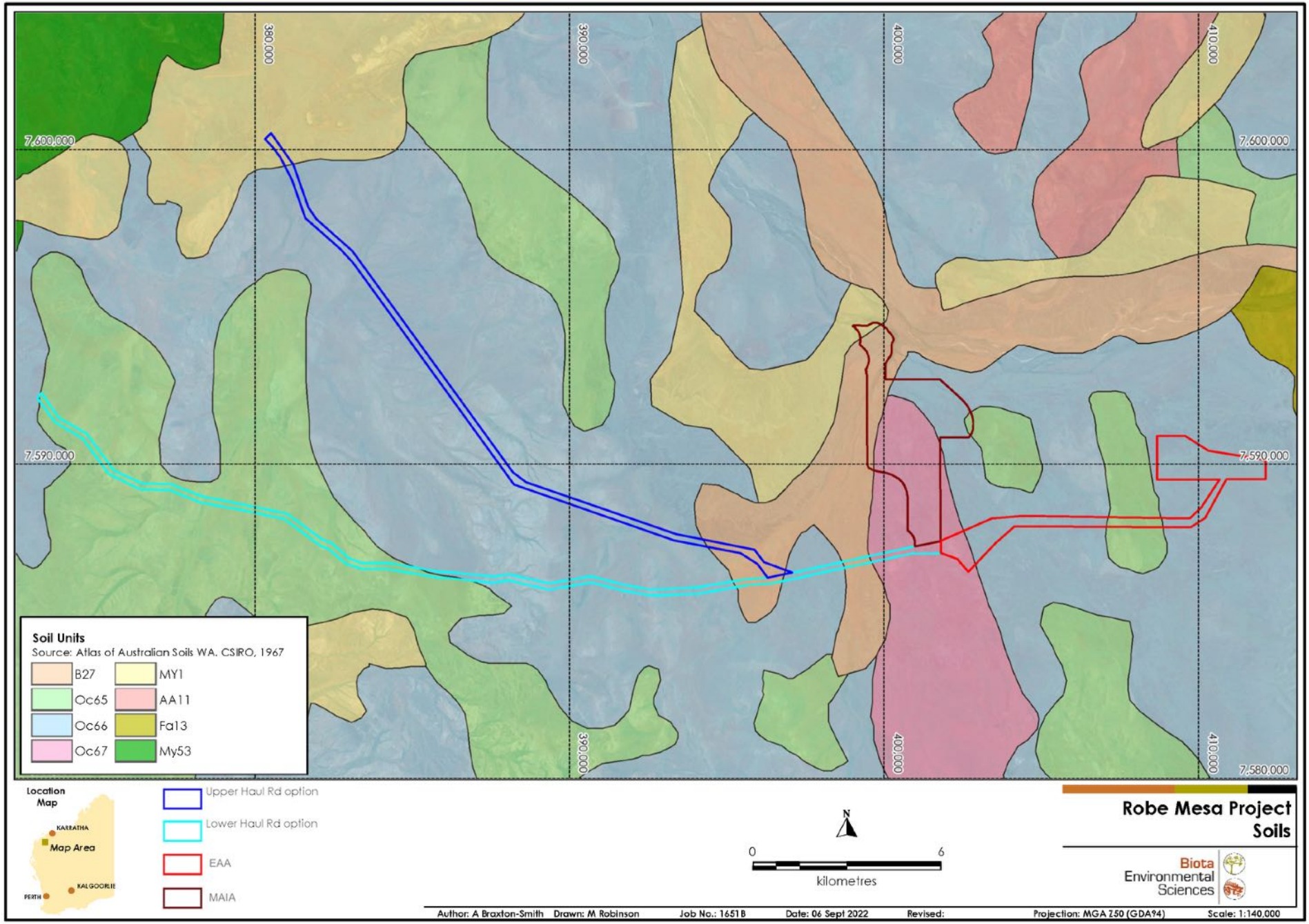

Figure 4.3 Soil units of the survey area.

Table 4.4: Description and extent of Beard's vegetation units in the survey area.
Data from Beard (1975b).

Vegetation Association	Description	Extent in Survey Area		Extent in Pilbara Bioregion (ha)	Extent in Survey Area as a Proportion of the Pilbara Bioregion (%)
		Area (ha)	Proportion (%)		
Stewart Hills 29	Sparse low woodland; mulga, discontinuous in scattered groups	1,395.6	45.8	17,221.6	8.1
Stewart Hills 583	Hummock grasslands, sparse shrub steppe; kanji & *Acacia bivenosa* over hard spinifex *Triodia basedowii* & *T. wiseana*	1,018.5	33.4	242,297.5	0.4
Stewart Hills 620	Hummock grasslands, shrub steppe; snakewood over soft spinifex	633.1	20.8	15,530.1	4.1
Stewart Hill 93	Hummock grasslands, shrub steppe; *Senna* sp. over soft spinifex	2.8	0.1	55,510.2	<0.01
	Total	**3,050.0**	**100**		

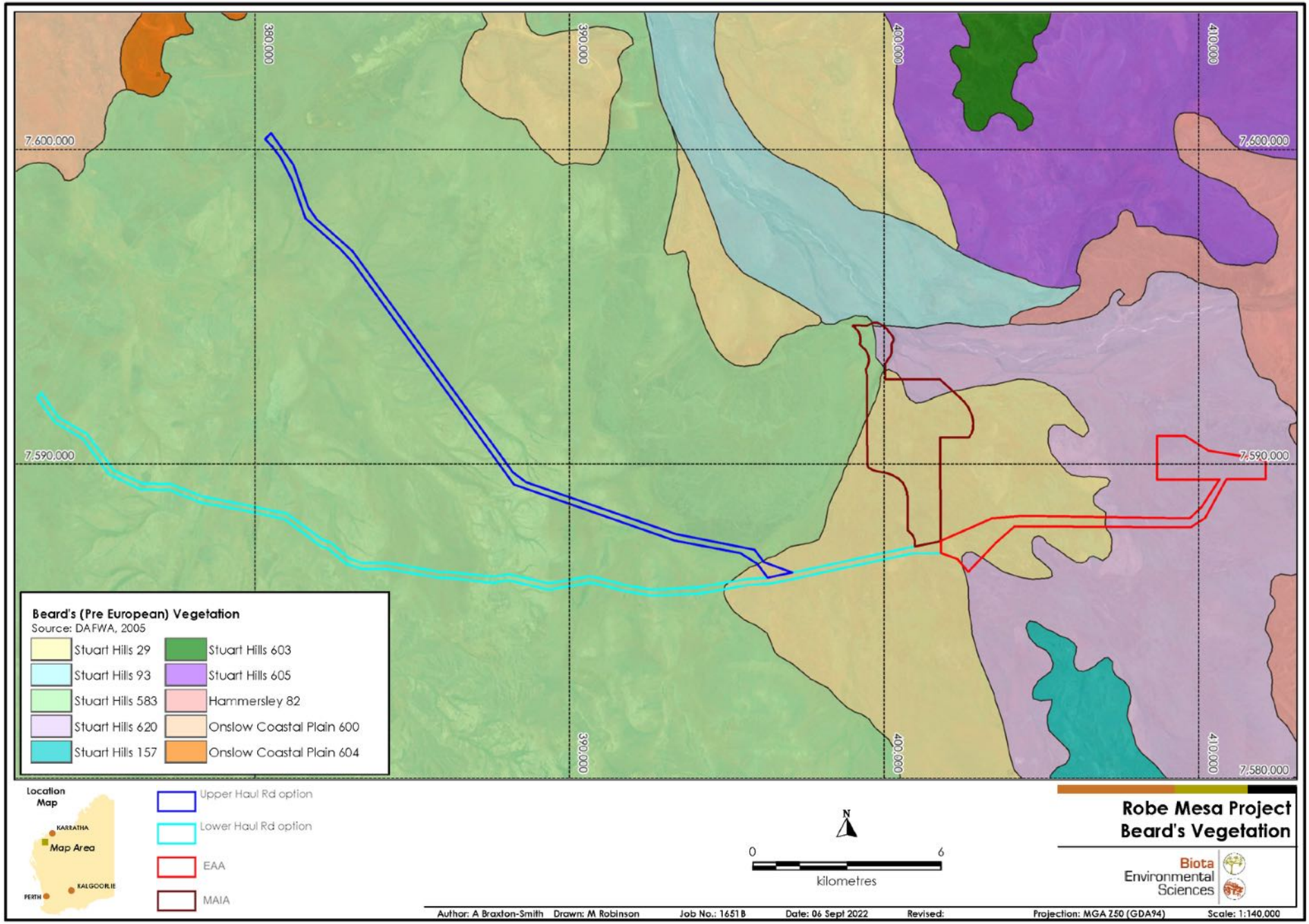

Figure 4.4: Beard's vegetation association mapping in the survey area and contextual area.

4.2 Previous Fauna Surveys

Several fauna surveys that have included an SRE fauna sampling component have been conducted in the survey area locality. However as molecular sequencing was not conducted prior to 2012, earlier reports did not provide species level identifications. Relevant past surveys include:

- Biota Environmental Sciences (2005) - Fauna Habitats and Fauna Assemblage of Mesa A and G, near Pannawonica, unpublished report to Robe River Iron Associates.
- Biota Environmental Sciences (2006) - Fauna Habitats and Fauna Assemblage of the Mesa A Transport Corridor and Warramboo, unpublished report to Robe River Iron Associates.
- Biota Environmental Sciences (2007) - Bungaroo Trial Pit and Transport Corridor to Mesa J, near Pannawonica - Fauna Assemblage Seasonal Survey.
- Biota Environmental Sciences (2009a) - West Pilbara Iron Ore Project Onslow Rail Corridor Terrestrial Fauna Survey, unpublished report to API Management.
- Biota Environmental Sciences (2010) - Greater Bungaroo Seasonal Fauna Survey, unpublished report to Rio Tinto Iron Ore
- Biota Environmental Sciences (2009b) - Mesa G Baseline Fauna Survey, unpublished report to Rio Tinto Iron Ore.
- Biota Environmental Sciences (2009c) - Warramboo Expansion Terrestrial Fauna Survey, unpublished report to Rio Tinto Iron Ore.
- Biota Environmental Sciences (2011) - Robe Valley Mesas Fauna Survey, unpublished report to Rio Tinto Iron Ore.
- Biota Environmental Sciences (2015a) - Buckland Hills Mine and Haul Road Targeted Fauna Survey, unpublished report to API Management.
- Biota Environmental Sciences (2015b) - WPIOP Stage 1 Extension Additional Areas Fauna Assessment, unpublished report to API Management.
- Astron Environmental Services (2016) - Mesa H Level 2 Fauna Assessment May 2016

4.3 Significant SRE Fauna

Database searches did not return any formally listed significant invertebrate species from the survey area locality. However, consolidated data from the database searches yielded a total of 44 species-level taxa recorded from the locality, comprising:

- five described and 25 nominal mygalomorph spider species from five families;
- three nominal harvestman species from two families;
- three described and four nominal millipede species from three families;
- one nominal slater beetle species; and
- one described and two nominal snail species from two families.

Of the 44 invertebrate taxa belonging to relevant groups that were retrieved from the database searches, one is a known SRE species. A further nine species are not considered SREs and the remaining further 34 species represent potential SREs (Table 4.5).

Table 4.5: Potential SRE taxa returned from database searches and the literature review.

Family	Taxon	Location in Survey Area	Distribution	Data Deficient	SRE Status	Source			
						WAM	ALA	EPBC	Other
Mygalomorph Spiders									
Actinopodidae	*Missulena* sp. MYG040	Outside	Indet.	Yes	Potential SRE	√			
	Missulena sp. 8	Outside	Indet.	Yes	Potential SRE	√			
	Missulena rutraspina	Outside	>10,000 km^2	No	Not an SRE	√			
Anamidae	*Aname* sp. N23	Outside	Indet.	Yes	Potential SRE				
	Aname sp. N25	Outside	Indet.	Yes	Potential SRE				Biota 2015a
	Aname sp. N141	Outside	Indet.	Yes	Potential SRE				Biota 2015a
	Aname sp. N148	Outside	Indet.	Yes	Potential SRE				Biota 2015b
	Aname sp. N149	Outside	Indet.	Yes	Potential SRE				Biota 2015b
	Aname sp. N150	Outside	Indet.	Yes	Potential SRE				Biota 2015b
	Aname sp. N151	Outside	Indet.	Yes	Potential SRE				Biota 2015b
	Aname sp. MYG271	Outside	Indet.	Yes	Potential SRE	√			
	Aname sp. MYG369	Outside	Indet.	Yes	Potential SRE	√			
	Aname sp. MYG413	Inside and outside	Indet.	Yes	Potential SRE	√			
	*Aname mellosa**	Outside	>10,000 km^2	No	Not an SRE	√	√		Biota 2015a Biota 2015b Astron 2016c
	Kwonkan sp. MYG374	Outside	Indet.	Yes	Potential SRE	√			
	Kwonkan sp. MYG375	Outside	Indet.	Yes	Potential SRE	√			
Barychelidae	Barychelidae sp. B7	Outside	Indet.	Yes	Potential SRE				Biota 2015a
	Barychelidae sp. B14	Outside	Indet.	Yes	Potential SRE				Biota 2015b
	Aurecocrypta chichester	Outside	>10,000 km^2	No	Not an SRE	√			Bennelongia 2012
	Aurecocrypta sp. MYG057	Outside	Indet.	Yes	Potential SRE	√			
	Aurecocrypta sp. MYG319	Outside	Indet.	Yes	Potential SRE	√			
	Synothele sp. DNA05	Outside	Indet.	Yes	Potential SRE	√			
	Synothele sp. MYG335	Outside	Indet.	Yes	Potential SRE	√			
	Synothele pseudidiommata	Outside	Indet.	Yes	Potential SRE	√			Phoenix 2012
	Synothele xkarara	Outside	>10,000 km^2	No	Not an SRE	√			Castalanelli et al. 2014b
Halonoproctidae	Ctenezidae sp. C12	Outside	Indet.	Yes	Potential SRE				Biota 2015a
	Conothele MYG536	Outside	Indet.	Yes	Potential SRE	√			Phoenix 2021

Family	Taxon	Location in Survey Area	Distribution	Data Deficient	SRE Status	Source			
						WAM	ALA	EPBC	Other
Idiopidae	*Bungulla bertmaini*	Outside	>10,000 km^2	No	Not an SRE	√	√		
	Euoplos MYG307	Outside	Indet.	Yes	Potential SRE	√			
	Idiosoma MYG083	Outside	Indet.	Yes	Potential SRE	√			
Harvestmen									
Assamiidae	*Dampetrus* DNA01	Outside	Indet.	Yes	Potential SRE	√			
	Dampetrus DNA07	Outside	Indet.	Yes	Potential SRE	√			
Triaenonychidae	Triaenonychidae sp. indet.	Outside	Indet.	Yes	Potential SRE	√			
Millipedes									
Paradoxosomatidae	*Antichiropus uvulus*	Inside and outside	>10,000 km^2	No	Not an SRE	√	√		
	Austrostrophus stictopygus	Outside	>10,000 km^2	No	Not an SRE	√	√		Biota 2015a
	Paradoxosomatidae sp. DNA02	Outside	Indet.	Yes	Potential SRE	√			
Haplodesmidae	Haplodesmidae sp. DNA03	Outside	Indet.	Yes	Potential SRE	√			
	Haplodesmidae sp. DNA04	Outside	Indet.	Yes	Potential SRE	√			
	Genus indet. DNA03	Outside	Indet.	Yes	Potential SRE	√			
Siphonotidae	Siphonotidae sp. indet.	Outside	Indet.	Yes	Potential SRE	√			
Slater Beetles									
Armadillidae	Buddelundia sp. 62	Outside	Indet.	Yes	Potential SRE				Biota 2015a Biota 2015b Astron 2016c
Land Snails		Outside							
Camaenidae	*Rhagada* convicta	Outside	>10,000 km^2	No	Not an SRE				Biota 2015b
	Rhagada sp. Pannawonica	Outside	<10,000 km^2	No	Known SRE	√			
Lymnaeidae	*Austropeplea* cf. *tomentosa*	Outside	>10,000 km^2	No	Not an SRE	√			

* Likely to represent a species complex (Castalanelli et al. 2014a); indet. = indeterminate.

5.0 Field Survey Results

5.1 Fauna Habitats

Four broad fauna habitats were identified in the survey area, from most common to least (Figure 5.1):

- **Alluvial Plains (AP):** This represents ~2,617 ha within the survey area. It comprises vegetation units dominated by either *Acacia xiphophylla* (Snakewood) shrubland or mixed *Acacia* spp., over soft *Triodia* sp. open hummock grassland (Biota in prep.). Associated with silty clay loam soil, that is common throughout the survey area (Plate 5.2).
- **Colluvial Plains (CP):** This represents ~201 ha within the survey area. It comprises vegetation units dominated by *Corymbia* spp., over hard *Triodia* sp. open hummock grassland (Biota in prep.). Associated with clay loam soils distributed in large patches through the survey area (Plate 5.3).
- **River/Flood Plains (RF):** This represents ~147 ha within the survey area. Large ephemeral rivers typically supported *Eucalyptus victrix* and *E. camaldulensis* woodland, over *Melaleuca* spp. woodland, over mixed *Acacia* spp., over **Cenchrus* spp. tussock grassland. Minor rivers and associated seasonally active flood plains primarily comprised *Corymbia hamersleyana* over *Acacia* spp. over *Triodia sp.* (Biota in prep.). Associated with clay loam, sandy clay loam and silty clay loam soils(Plate 5.1).
- **Low Stony Hills (SH):** Comprises low sandstone hills or low pisolitic mesa formations, representing ~85 ha within the survey area. It typically comprises *Eucalyptus leucophloia* scattered trees, over *Acacia* spp., over hard *Triodia* sp. open hummock grassland (Biota in prep.)(Plate 5.4).

Plate 5.1: River/Flood Plain

Plate 5.2: Alluvial Plain

Plate 5.3: Colluvial Plain

Plate 5.4: Low Stony Hill (sandstone)

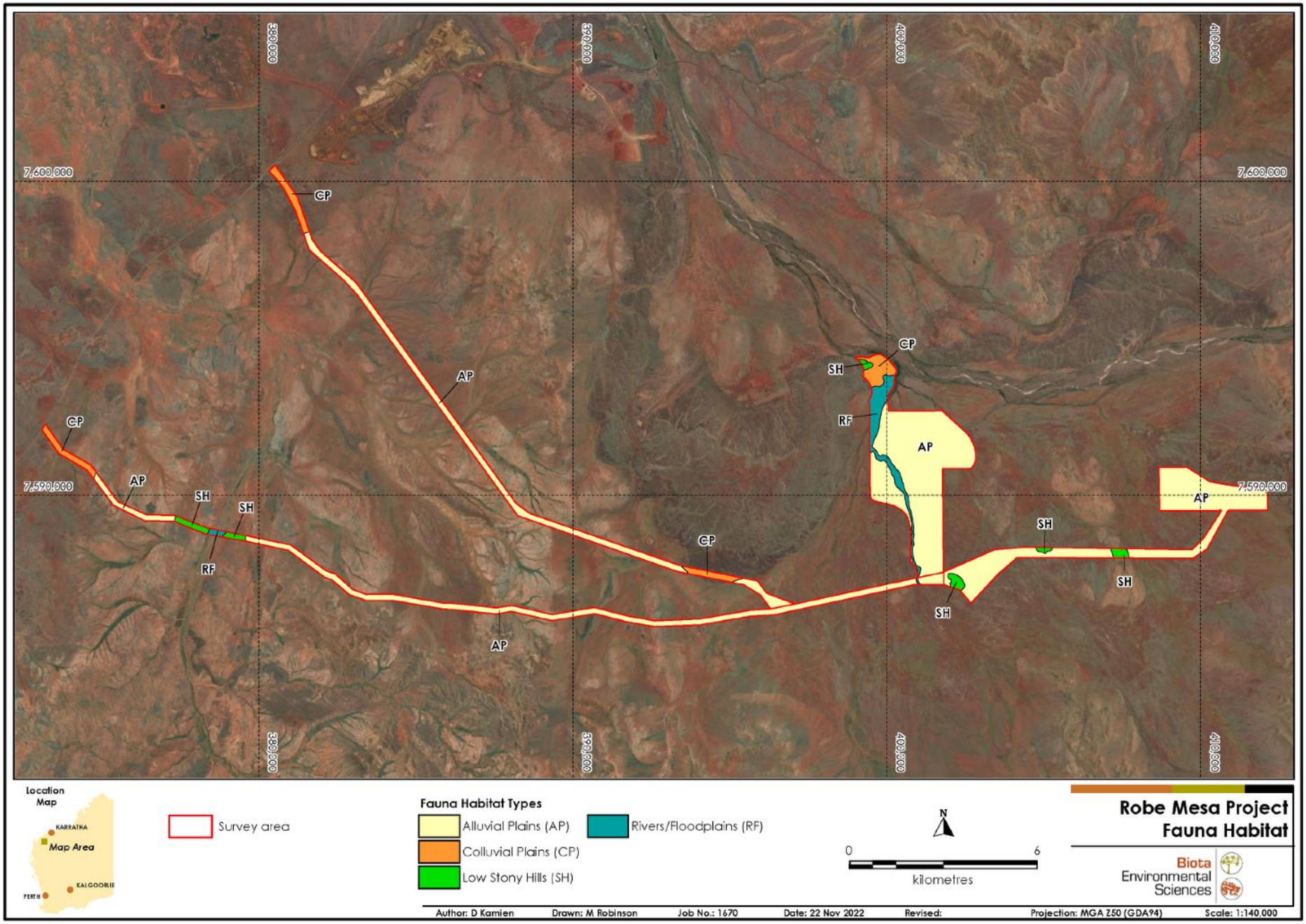

Figure 5.1: SRE fauna habitats of the survey area.

5.2 Short-Range Endemic Invertebrates

Two higher-order taxonomic groups with the potential to include SRE species were recorded within the survey area; mygalomorph spiders and land snails. Table 5.1 summarises the records by sites, with nominal species determined via molecular analysis (Appendix 2). Section 5.2.1 provides an account of each species.

Table 5.1: Summary of potential SRE invertebrate fauna recorded during the survey (species known only from the survey area highlighted).

Invertebrate Group & Family	Species	Number Recorded	Habitat †	Sites Recorded	Previously Recorded	Known to Occur Outside Survey area
Mygalomorph Spiders						
Anamidae	*Aname mellosa*	13	AP CP RF	RMP13SRE_MG RMP14SRE_MG RMP16SRE_MG RMP17SRE_MG RMP19SRE_MG RMP30SRE_MG RMP31SRE_MG RMP35SRE_MG	Yes	Yes
	Aname mellosa sp. H–N167	6	SH CP	RMP01SRE_MG RMP02SRE_MG RMP03SRE_MG RMP10SRE_RD RMP24SRE_MG	Yes	Yes
	Aname sp. H–N161	3	CP	RMP20SRE_MG RMP21SRE_MG	No	No
	Aname sp. H–N162	7	AP SH	RMP19SRE_MG RMP32SRE_MG RMP33SRE_MG RMP34SRE_MG	No	No
	Aname sp. H–N163	2	CP	RMP28SRE_MG	No	No
	Aname sp. H–N164	1	AP	RMP18SRE_MG	No	No
	Aname sp. H–N165	3	CP	RMP13SRE_MG	No	No
	Aname sp. H–N166	3	CP	RMP01SRE_MG RMP02SRE_MG	No	No
Land Snails						
Camaenidae	*Rhagada convicta*	25	AP CP RF	RMP04SRE_MG RMP08SRE_MG RMP11SRE_MG RMP12SRE_MG RMP14SRE_MG RMP17SRE_MG RMP20SRE_MG RMP26SRE_MG RMP30SRE_MG RMP35SRE_MG	Yes	Yes
Succineidae	*Succinea* sp. Pilbara	6	AP RF	RMP16SRE_MG RMP30SRE_MG	Yes	Yes

† River and Flood Plains (RF), Alluvial Plains (AP), Colluvial Plains (CP), Low Stony Hills (SH).

5.2.1 Mygalomorph Spiders

Two groups of mygalomorph spider from the family Anamidae, were collected during the survey:

1. the *Aname mellosa* species complex; and
2. a new monophyletic clade of 'sock' *Aname*.

5.2.1.1 *Aname mellosa* Species Complex

The *Aname mellosa* complex contains 10 identified genetic lineages showing distinct non-overlapping geographical (peripatric) clustering (Castalanelli et al. 2014a). These lineages cannot be distinguished morphologically (Castalanelli et al. 2014a). This species complex is distributed throughout the central Pilbara in a range of habitats such as river banks, loamy plains, mulga plains, hill slopes and plateaus (Harvey et al. 2012). Adults are approximately 2 cm in length and individuals construct a burrow that is characterised by an open hole with a 'hooded' entrance.

Aname mellosa

Distribution and SRE Status

Aname mellosa (Plate 5.5) was recorded on 13 occasions from eight sites within the survey area, (Table 5.1 and Figure 5.2). When first described, *Aname mellosa* was thought to be a widespread species, not representing an SRE (Harvey et al. 2012). However, due to high intraspecific molecular divergence between lineages, Castalanelli et al. (2014a) postulated that *A. mellosa* likely comprises several cryptic species, each representing potential SRE taxa. This hypothesis is congruent with the fact that the majority of identified clades occur in peripatry rather than sympatry (Castalanelli et al. 2014a). The 13 specimens assigned to *A. mellosa* here all grouped within a clade containing representatives of the previously known *A. mellosa* lineages and the records are taken to represent the described species which is a potential SRE but occurs outside the survey area.

Habitat

Specimens were recorded from the survey area in Colluvial Plain, Alluvial Plain and, River/Floodplain habitat on clay loam or silty clay loam substrate (Table 5.1). An example of the burrow entrance is depicted in Plate 5.6.

Plate 5.5: *Aname mellosa*.

Plate 5.6: *Aname mellosa* burrow.

***Aname mellosa* sp. H–N167**

Distribution and SRE Status

Aname mellosa sp. H–N167 was recorded on six occasions from five sites within the survey area, (Figure 5.2). Based on the available data, *A. mellosa* sp. H-N167 exhibited an intraspecific variation of only 3.1%, with an interspecific clade variation of 14.6% (*Aname mellosa* being its closest relative) (Appendix 2). As a result, *A. mellosa* sp. H–N167 should be considered a separate species from other taxa within the *A. mellosa complex,* although morphologically they are indistinguishable (Castalanelli et al. 2014a). This species is regarded as a potential SRE based on the criteria outlined in Table 3.4. *A. mellosa* sp. H-N167 sits within a clade that contains at least two representative individuals previously identified as *A. mellosa* (Appendix 2). *A. mellosa* sp. H-N167 is therefore shown to occur outside the survey area.

Habitat
Specimens were recorded from Colluvial Plain on clay loam substrate and in Low Stony Hill habitat. An example burrow entrance is shown in Plate 5.6.

5.2.1.2 Sock *Aname*

Sock *Aname* have been recorded throughout the Pilbara bioregion (Biota database). Members of this group are typically less than 1 cm long (Plate 5.7), with burrows characterised by a sand spoil over a sock-like silk opening (Plate 5.8). Different species cannot be readily distinguished morphologically.

Taxa recorded within the survey area formed a unique molecular clade consisting of six species showing between 9.5% – 20.7% inter-specific pairwise divergence (Appendix 2). Identified species were not recorded in sympatry.

Plate 5.7: *Aname* sp. H–N165.

Plate 5.8: *Aname* sp. H–N165 burrow.

All putative species of 'sock' *Aname* recorded during the survey are known solely from the survey area and have not been recorded previously (Appendix 2). species summary is presented in Table 5.2.

Table 5.2: Summary of 'sock' *Aname* recored during the survey.

Species	Number Recorded	Number of Sites	Habitat	Nearest Relative/ Divergence
Aname sp. H-N161	3	2	Colluvial Plains	*Aname* sp. H-N165 / 20.7%
Aname sp. H-N162	7	4	Alluvial Plains Low Stony Hills	*Aname* sp. H-N165 / 14.6%
Aname sp. H-N163	2	1	Alluvial Plains	*Aname* sp. H-N165 / 10.4%
Aname sp. H-N164	1	1	Alluvial Plains	*Aname* sp. H-N165 / 9.9%
Aname sp. H-N165	3	1	Colluvial Plains	*Aname* sp. H-N164 / 9.9%
Aname sp. H-N166	3	2	Colluvial Plains	*Aname* sp. H-N165 / 13.7%

5.2.2 Land Snails

Rhagada convicta

Distribution and SRE Status
Twenty-five *Rhagada convicta* specimens (Plate 5.9) were recorded from 10 sites within the survey area (Table 5.1, Figure 5.2 and Appendix 2). *Rhagada convicta* has one of the largest distributions of any *Rhagada* species (Solem 1997, Hamilton 2018) and does not constitute an SRE.

Habitat
During the survey *Rhagada convicta* was recorded in Colluvial Plain, Alluvial Plain and River/Floodplain habitats, found aestivating under *Triodia* species hummocks. However, after rain, active individuals were observed.

Plate 5.9: ***Rhagada convicta***

Succinea sp. Pilbara

Distribution and SRE Status

Succinea sp. Pilbara specimens (Plate 5.10) were recorded from two locations within the survey area (Table 5.1, Figure 5.2 and Appendix 2). This taxon has been recorded outside the survey area approximately 100 km to the southwest of the survey area (Biota 2019). The genus *Succinea* is taxonomically poorly known in Australia but all known species are considered to be widespread (Whisson 2012).

Habitat

Succinea sp. Pilbara was recorded in Alluvial Plain, and River/Floodplain habitats, primarily aestivating under *Triodia* species hummocks.

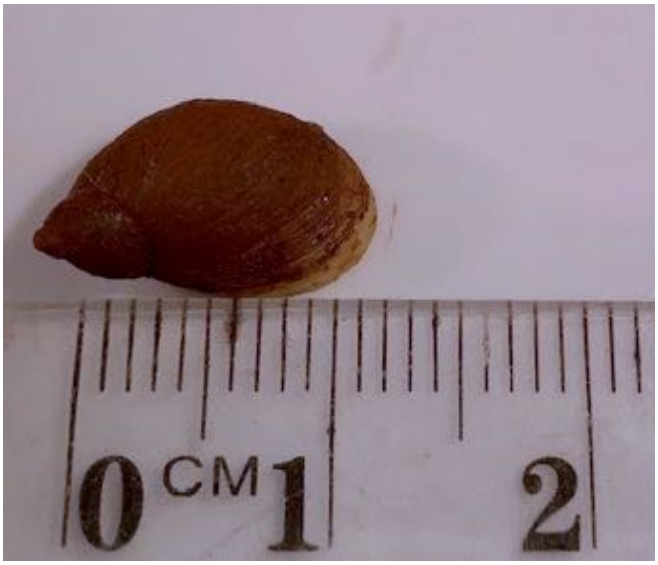

Plate 5.10: ***Succinea* sp. Pilbara**

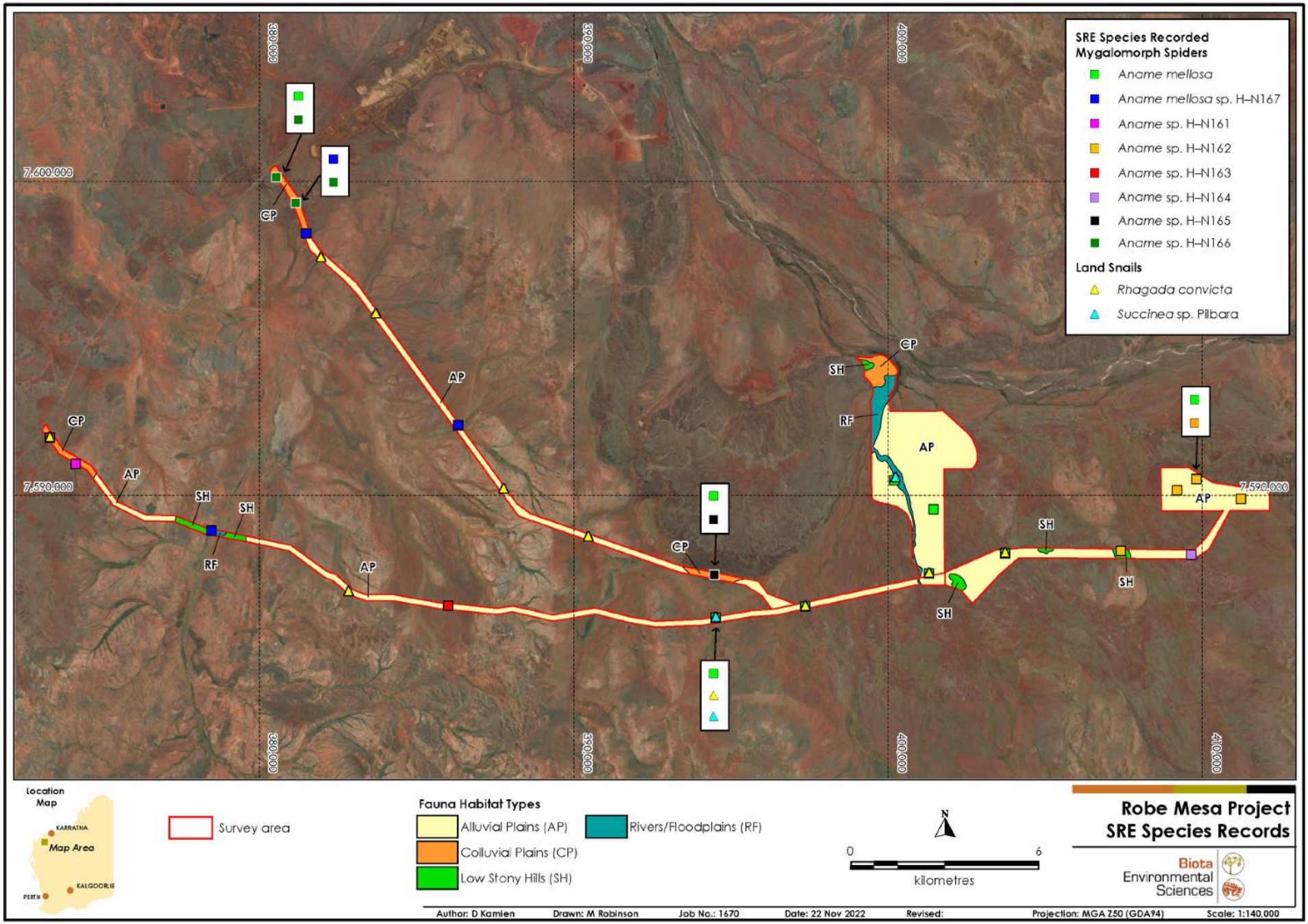

Figure 5.2: Potential SRE species records in the survey area.

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6.0 Discussion and Key Findings

Within the survey area, all four identified habitats support SRE species or potential SRE species. Although Low Stony Hills habitat may be regarded as a geomorphological isolate potentially facilitating short-range endemism, all four habitats are common in the locality, and occur contiguously with the survey area. That is, while they have habitat attributes important to SREs, their wider extent and geomorphological and hydrological connections make them unlikely to result in a high risk of species level distributions being restricted to the scale of the survey area. This is supported by the fact that four species recorded (two mygalomorph spider species and two snail species) have been recorded outside of the survey area, indicating that there is no mechanism limiting dispersal or gene flow at the locality scale.

Of the invertebrate taxa collected during the survey, six monophyletic nominal mygalomorph species of 'sock' *Aname* are known solely from the survey area (Table 5.1). Although survey records represent a small sample size, the limited data indicate that these species also occur in peripatry, potentially being isolated by distance at a small geographic scale, aided by a low dispersal life history (Schäfer et al. 2001, Bond et al. 2006).

Eighty-six percent of the survey area is represented by Alluvial Plains habitat, and therefore this habitat was subjected to the most survey effort. Despite this, most nominal mygalomorph spider species were recorded on Colluvial Plains habitat, perhaps indicating a habitat preference within the survey area. Of the 'sock' *Aname* taxa, two nominal species were recorded solely in the Upper Haul Road option (*Aname* sp. N165 and *Aname* sp. N166) and two nominal species were recorded solely within the Lower Haul Road option (*Aname* sp. N161 and *Aname* sp. N163). Additionally, *Aname* sp. N162 and *Aname* sp. N164 were recorded only within the EAA.

Despite significant phylogenetic divergence and the high potential for short-range endemism, it is unlikely that the six nominal species of 'sock' *Aname* are truly restricted to the survey area. The absence of records outside of the survey area may be due to inadequate sampling effort in analogous habitats, in combination with the difficulty locating spiders of this type. That is, most previous terrestrial SRE invertebrate surveys in the locality have focused primarily on iron ore deposits, rather than plains habitats (WAM database). The apparent absence of these six nominal species of 'sock' *Aname* beyond the survey area, may also be due to a lack of molecular species level resolution for many previously recorded taxa, thereby preventing direct comparison of the records from this study with past work.

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8.0 Glossary

BC Act	Western Australian *Biodiversity Conservation Act 2016.*
Biota	Biota Environmental Sciences.
Clade/ Monophyletic	A taxonomic group believed to comprise all the evolutionary descendants of a common ancestor.
DBCA	Department of Biodiversity, Conservation and Attractions.
EIA	Environmental Impact Assessment.
EPA	Environmental Protection Authority of Western Australia.
EPBC Act	Commonwealth *Environment Protection and Biodiversity Conservation Act 1999.*
Haplotype	A set of markers (polymorphisms) on a single chromosome that tend to be inherited together.
IBRA	Interim Biogeographic Regionalisation for Australia.
Landform	A geomorphological unit that is largely defined by its surface form and location in the survey area.
Lineage	A single, direct line of descent among taxa.
Mesic	Relating to an environment having a balanced supply of moisture.
MNES species	Species that are listed as Matters of National Environmental Significance under the EPBC Act.
Peripatry	When species evolve in contiguous, yet spatially segregated habitats.
Significant species	A species formally listed as threatened, migratory or priority.
sp. (plural: spp.)	Abbreviation of "species".
SRE	Short-range endemic.
Survey area	The area in which the on-ground survey was conducted.
Taxon (plural: taxa)	A taxonomic entity, typically at species level or below.
WAM	Western Australian Museum.

Appendix 1

Fauna Taking Licence

Department of **Biodiversity, Conservation and Attractions**

GOVERNMENT OF WESTERN AUSTRALIA

FAUNA TAKING (BIOLOGICAL ASSESSMENT) LICENCE

Regulation 27, Biodiversity Conservation Regulations 2018

Licence Number: BA27000646

Licence Holder: Roxanne de Vos
Biota Environmental Sciences
Level 1, 228 Carr Place
Leederville WA 6007

Date of Issue: 16/05/2022

Date Valid From: 14/06/2022
Date of Expiry: 31/07/2022

LICENSED ACTIVITIES

Subject to the terms and conditions on this licence, the licence holder may –

1. Take or disturb fauna for short range endemic (SRE) survey for CZR Resources Ltd for environmental impact assessment using hand collection techniques (foraging / raking), Mygalomorph spiders will be targeted by visually locating burrows, and then excavating them with a shovel. Specimens to be lodged with the WA Museum upon completion of the project.

LOCATIONS

1. Robe Mesa Iron Ore Project, located in the West Pilbara, 29 km southwest of Pannawonica. The Robe Mesa deposit adjoins Mesa F, which is located between the Mesa A and Mesa J-K.

AUTHORISED PERSONS

The following persons or persons of the specified class may assist in carrying out the licensed activities:

1. Michael Greenham

CONDITIONS

1. Fauna must not be taken on CALM land, (as defined in the Conservation and Land Management Regulations 2002*),* unless authorised by a written notice of a lawful authority issued under regulations 4 and 8 of the Conservation and Land Management Regulations 2002.
2. If persons, other than the licence holder, are authorised to carry out/assist in carrying out the activities under the licence, the licence holder must ensure those persons have read and understand the licence terms and conditions.
3. The written authorisation of the person in possession or occupation of the land accessed and upon which fauna is taken, as required under regulation 101(2) and referred to in “Additional information” below, must:
   a) state location details (including lot or location number, street/road, suburb and local government authority);

b) state land owner or occupier name, and contact phone number;

c) specify the time period that the authorisation is valid for;

d) be signed and dated; and

e) be attached to this licence at all times.

4. This licence, and any written authorisation or lawful authority which authorises the take of fauna on specified locations must be carried at all times while conducting licensed activities and be produced on demand by a wildlife officer.

5. If a species of fauna listed as a threatened species under Section 19 of the *Biodiversity Conservation Act 2016* is inadvertently captured, that species is to be released immediately at the point of capture. If the fauna is injured or deceased, the licence holder shall contact the DBCA Wildlife Licensing Section (wildlifelicensing@dbca.wa.gov.au) for advice on treatment or disposal. Details of any capture of threatened fauna must be included in the "Return of Fauna Taken."

6. The licence holder must not:

   a) release any fauna in any area where it does not naturally occur;

   b) transfer fauna to any other person or authority (other than the Western Australian Museum) unless approved in writing by the CEO; or

   c) dispose of the remains of fauna in any manner likely to interfere the natural or present day distribution of the species.

7. The licence holder must not take and remove more than ten specimens of any one protected species of fauna from any location less than 20km apart. Where exceptional circumstances make it necessary to take a larger number of specimens from a particular location in order to obtain adequate statistical data, the collector must proceed with circumspection and justify their actions to the Director General in advance.

8. All holotypes and syntypes and a half share of paratypes of species or subspecies permitted to be permanently taken under this licence must be donated to the Western Australian Museum. Duplicates (one pair in each case) of any species collected, which represents a significant extension of geographic range must be offered to the Western Australian Museum.

9. All specimens and material retained under the authority of this licence must be offered to the Western Australian Museum for loan, for inclusion in its collection, or on request be made available to other persons involved in relevant scientific studies.

10. The licence holder must create, compile and maintain records and information as required in a DBCA approved "Return of Fauna Taken" of all fauna taking activities as they occur.

11. A DBCA approved "Return of Fauna Taken" must be completed in full (including nil taking details) and submitted to DBCA Wildlife Licensing Section (wildlifelicensing@dbca.wa.gov.au) prior to the end of each annual period of the licence (from the valid from date) (refer to "Additional Information" section

Danny Stefoni
LICENSING OFFICER
WILDLIFE PROTECTION BRANCH

Delegate of CEO

ADDITIONAL INFORMATION

1. It is an offence to take any species of fauna listed as a threatened species under Section 19 of the *Biodiversity Conservation Act 2016* unless the person is authorised under Section 40. The penalty ranges between $300 000 and $500 000; Section 150 Biodiversity Conservation Act 2016.
2. Regulation 82 empowers the CEO to add, substitute or delete a term or condition of a licence or to correct errors. Such power may be exercised on application of a licence holder or by the CEO's own initiative. If an amendment to a licence term or condition is required, please contact the CEO or the Licensing Section on wildlifelicensing@dbca.wa.gov.au in the first instance. The licence holder, if adversely affected by a condition imposed in this licence, may apply to the State Administrative Tribunal for review of the decision of the CEO to impose that condition on a licence: regulation 89(2) Biodiversity Conservation Regulations 2018.
3. A person must not contravene a condition of a licence. The penalty for an offence involving the contravention of a condition of a licence is a fine of $10 000: regulation 84 of the Biodiversity Conservation Regulations 2018.
4. It is an offence for persons authorised by this licence to enter land that is not in their possession or under their control without first having the *prior* written authorisation of the current owner or occupier of the land to:

   a) enter the land; and

   b) carry out the activity authorised by this licence.

   The penalty for this offence is a fine of $5 000: regulation 101(2) of the Biodiversity Conservation Regulations 2018.
5. The licence holder must be able to produce for inspection upon request any information or records required by regulation 85(2) of the Biodiversity Conservation Regulations 2018 Penalty $10 000. It is an offence to knowingly include false or misleading information or make statements in records: regulation 85(3) of the Biodiversity Conservation Regulations 2018 Penalty $10 000. It is an offence to include any information or make any statement in a return that the licence holder knows to be false or misleading in a material particular: regulation 86 (2) of the Biodiversity Conservation Regulations 2018 Penalty $10 000.
6. The approved DBCA "Return of Fauna Taken" data file can be downloaded from the DBCA webpage (https://www.dpaw.wa.gov.au/plants-and-animals/licences-and-authorities).
7. The issuing of a licence under the Biodiversity Conservation Regulations 2018 does not constitute an animal ethics approval or a licence to use animals for scientific purposes as required under the *Animal Welfare Act 2002,* Animal Welfare (Scientific Purposes) Regulations 2003. It is the responsibility of a licence applicant / licence holder to ensure that they comply with the requirements of all applicable legislation. Enquiries relating to the Animal Welfare Act licences and animal ethics approvals are to be directed to the Department of Primary Industries and Regional Development (https://www.agric.wa.gov.au/animalwelfare).
8. Threatened fauna can only be taken under a *Biodiversity Conservation Act 2016* Section 40 authorisation, Occurrences of threatened species must be reported to the CEO. For more information please see https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities/threatened-animals.
9. Any interaction involving Nationally Listed Threatened Fauna that may be invasive and/or harmful to the fauna may require approval from the Commonwealth Department of the Environment and Energy http://www.environment.gov.au/about-us/business-us/permits-assessments-licences. Interaction with such species is controlled by the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* and Environment Protection and Biodiversity Conservation Regulations 2000 as well as the *Biodiversity Conservation Act 2016* and Biodiversity Conservation Regulations 2018.

This is the content of the first appendix.

Appendix 2

Helix Molecular Sequencing Report

Helix Molecular Solutions

Molecular Systematics of the Robe Mesa Project Short-range Endemic Invertebrates

Prepared for Biota Environmental Sciences

November 2022

Author: Dr Karen Cullen
Dr Zoë Hamilton

Quality Checking History

Version: Peer review: Dr Zoë Hamilton
Version: Director review:

Approved for issue:

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Robe Mesa Project SRE Molecular Systematics'

Contents

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1.0 Executive Summary

Biota Environmental Sciences has conducted a targeted short-range endemic (SRE) invertebrate fauna survey in areas of nominal infrastructure relating to the Robe Mesa Project. The survey yielded 41 Mygalomorphae spider specimens and 31 land snail specimens representing potential SRE taxa. Helix Molecular Solutions to perform DNA extractions, sequencing of the mitochondrial cytochrome oxidase subunit I gene (COI mtDNA), and comparative analyses of sequences obtained from specimens previously recorded in the Robe Valley Area (from both the Helix database and GenBank) to gain information on the number of potential species and their apparent distributions, based on molecular genetic data currently available for comparison.

Of the 41 specimens collected, 40 yielded COI sequences and were analysed along with 702 reference specimens from both the Helix database (n=251) and GenBank (n=451). Two specimens (Helix ID RT21 and RT34) were identified through molecular sequencing as Araneomorph spiders and were removed from further analysis as they were not relevant to this project. The remaining 38 specimens were confirmed to belong within the genus *Aname* (Family Anamidae), with a total of six distinct species identified from a new *Aname* sp. clade, and two known species belonging within the *Aname mellosa* complex.

Thirty-one land snail specimens belonging to two families (Gastropoda: Camaenidae (n = 25) and Succineidae (n= 6)) from the study area were sequenced and assessed for variation at the COI mtDNA gene. Genetic analysis of the land snails found that the original morphological assessment for six specimens (Family: Bothryembryontidae, genus *Bothryembrion*) was misplaced, and all six specimens (RT67-RT72) belonged within the family Succineidae.

Twenty-five camaenid land snail specimens, yielded 13 unique haplotype specimens, which aligned with a previously described species *Rhagada convicta* (Camaenidae), with two distinct lineages geographically separated across the study area.

All six specimens of Succineidae belonged to one distinct genetic lineage within the genus *Succinea* and all specimens aligned closely to a previously sequenced undescribed species: *Succinea* sp. Pilbara (Helix OV161 – OV165).

Both species of land snail (*Rhagada convicta* and *Succinea* sp. Pilbara) have previously been recorded outside the study area. The two species belonging to the *Aname mellosa* species complex are known previously outside the study area, but the distribution of the six new species of *Aname* (**H-N161*** - **H-N166***) is uncertain. No sympatry was detected within this new *Aname* sp. clade, but there were instances where geographical overlap, or sympatry occurred between the two major clades. Based on the collecting information, species **H-N162*** and **H-N165*** occur in sympatry with *Aname mellosa* in the SE section of the study area, and **H-N166*** occurs in sympatry with *A. mellosa* species H-N167 in the NW corner of the study area.

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2.0 Background and Objective

Biota Environmental Sciences conducted a targeted short-range endemic (SRE) invertebrate fauna search of the following areas relating the Robe Mesa Project:

- the Development Envelope;
- two haul road options that run from the Development Envelope west and northwest to meet the North West Coastal Highway; and
- the East Additional Area located east of the Development Envelope.

These areas are collectively termed the 'study area' in this report (Figure 1).

Biota Environmental Sciences engaged Helix Molecular Solutions to perform DNA extractions, sequencing of the mitochondrial cytochrome oxidase subunit I gene (COI mtDNA), and analyses of the specimens from the Robe Valley locality to gain information on the number of potential species and their apparent distributions, based on the molecular genetic data available for comparison.

The infraorder Mygalomorphae, which includes trapdoor spiders and their kin, are frequently identified as SREs, as are many species of Camaenidae and *Bothriembryon* land snails. Targeted SRE sampling yielded a total of 72 specimens, which included 41 Mygalomorphae spider specimens and 31 land snail specimens belonging to two different families (Camaenidae and Bothriembryontidae) based on morphological character traits in the field (Table 2).

Of the 72 invertebrate specimens from 26 sites within the study area, 69 specimens were successfully sequenced. Molecular sequences were then assessed for variation to determine the number of taxa present and compare these results to previously sequenced specimens that have been undertaken elsewhere in the Pilbara (Helix database), and sequences publicly available on GenBank for context (see Section 3.0).

2.1 Fauna Background

2.1.1 Mygalomorphae

Mygalomorphae, includes trapdoor spiders and their kin, which are frequently identified as SREs, primarily due to their poor dispersal capabilities (e.g., Harvey *et al*., 2011; Castalanelli *et al*.,2014). This ancient group has a worldwide distribution that includes tarantulas, trapdoor spiders and funnel-web spiders (Opatova *et al*., 2020). Identification of species has traditionally been performed using morphological techniques, however, only adult males can be morphologically identified, as both females and juveniles lack the diagnostic characters. Even then, inaccuracies in morphological identification persist. Furthermore, there is a large backlog of undescribed taxa.

Mygalomorph spider systematics has received increasing attention over the past decade, as integrative molecular approaches have revolutionised understanding of speciation, classification and biogeography (Rix *et al*., 2018). Multi-locus approaches have revealed higher levels of diversity than previously determined from morphology alone (Rix *et al*., 2018).

DNA barcoding with the use of COI mtDNA has become a rapid and objective method aiding mygalomorph species identifications and their distributions and is recognised as providing important data to inform Environmental Impact Assessment (EIA) (Rix *et al*., 2008; Castalanelli *et al*., 2014). Extensive molecular work has been conducted on the Western Australian Mygalomorphae (Helix, 2009a and b; 2010; 2011 a - l; 2012a – l; 2013a and b; 2014a – d; 2015a – e; 2018, 2019, 2020, 2021 and 2022). The resulting dataset provides a molecular framework that can be used to provide regional context for localised sampling.

2.1.1.1 Family Anamidae

The family Anamidae (previously Nemesiidae) is one of the most diverse and speciose mygalomorph families in Australia (Castalanelli *et al*., 2014; 2017; 2020). Considered the most dominant trapdoor spider family in Australia (Castalanelli *et al*., 2020), it includes ten described genera (*Aname*, L. Koch, 1873, *Chenistonia* Hogg, 1901, *Hesperonatalius*, Castalanelli *et al*., 2017, *Kwonkan* (including *Yilgarnia* and *Aname turrigera*), Main, 1983, *Namea*, Raven, 1984, *Proshermacha*, Simon, 1908, *Swolnpes*, Main & Framenau, 2009, *Teyl*, Main 1975, *Teyloides*, Main 1985 and *Stanwellia* (Castalanelli *et al*., 2014; 2017; 2020; Harvey *et al*., 2018). The most speciose of these genera, *Aname*, is found across most of Australia and is currently represented by 48 described species (World Spider Catalog 2022).

The genus *Aname* is morphologically distinguishable by the presence of a small groove or depression in the male pedipalpal tibia. Females are rarely distinguishable from each other and require molecular analysis to assign species. The species complex *Aname mellosa* is known to contain several cryptic species, with high levels of intra-specific diversity but very conservative morphology (Castalanelli *et al*., 2014).

2.1.2 Gastropoda

2.1.2.1 Gastropoda – Family Camaenidae

The Camaenid genus *Rhagada* is the most species-rich genus of land snails in Western Australia's semi-arid Pilbara region, where it shows both morphological conservatism within and among species over large distances (Solem, 1997; Johnson *et al*., 2012; Hamilton & Johnson, 2015) and extreme morphological diversification of shell traits over relatively small areas (Stankowski, 2011, 2013, 2015; Stankowski & Johnson, 2014; Johnson *et al.,* 2015). Additionally, molecular studies have demonstrated the presence of distinct cryptic taxa, and the occurrence of narrow hybrid zones between them (Hamilton & Johnson, 2015). As a result, a combination of DNA barcoding in addition to morphological taxonomy, is required to aid species diagnosis within this complex group.

2.1.2.2 Gastropoda – Family Succineidae

The family Succineidae (amber snails) has a worldwide distribution (Patterson, 1971; Pilsbury, 1948) and the World Conservation Union (IUCN, 2006) considers five species or subspecies of Succineids as threatened: three species are categorised as Data Deficient, and two species are categorised as Near Threatened.

The Western Australian Succineids have not been researched since Iredales' review of land molluscs in 1939, where he recognised seven species. However, due to limited collections, poor morphological differentiation between species, and insufficient molecular data, distributional ranges of distinct populations or lineages have not been

documented (WA Museum 2006). Despite this, Succineids are still regarded as a potential SRE group (Whisson & Kirkendale 2014).

In Western Australia, the genus *Succinea* is found from the Kimberley to south-western Australia, including the Pilbara (Iredale 1930; Solem 1989; Stanisic *et al*. 2010). They are characterised by an amber-coloured thin, often fragile shell (1-2cm height), with little sculpture and often associated with moist environments, such as roadside ditches, river and creek banks, swampy swale areas of coastal dune systems and river estuaries.

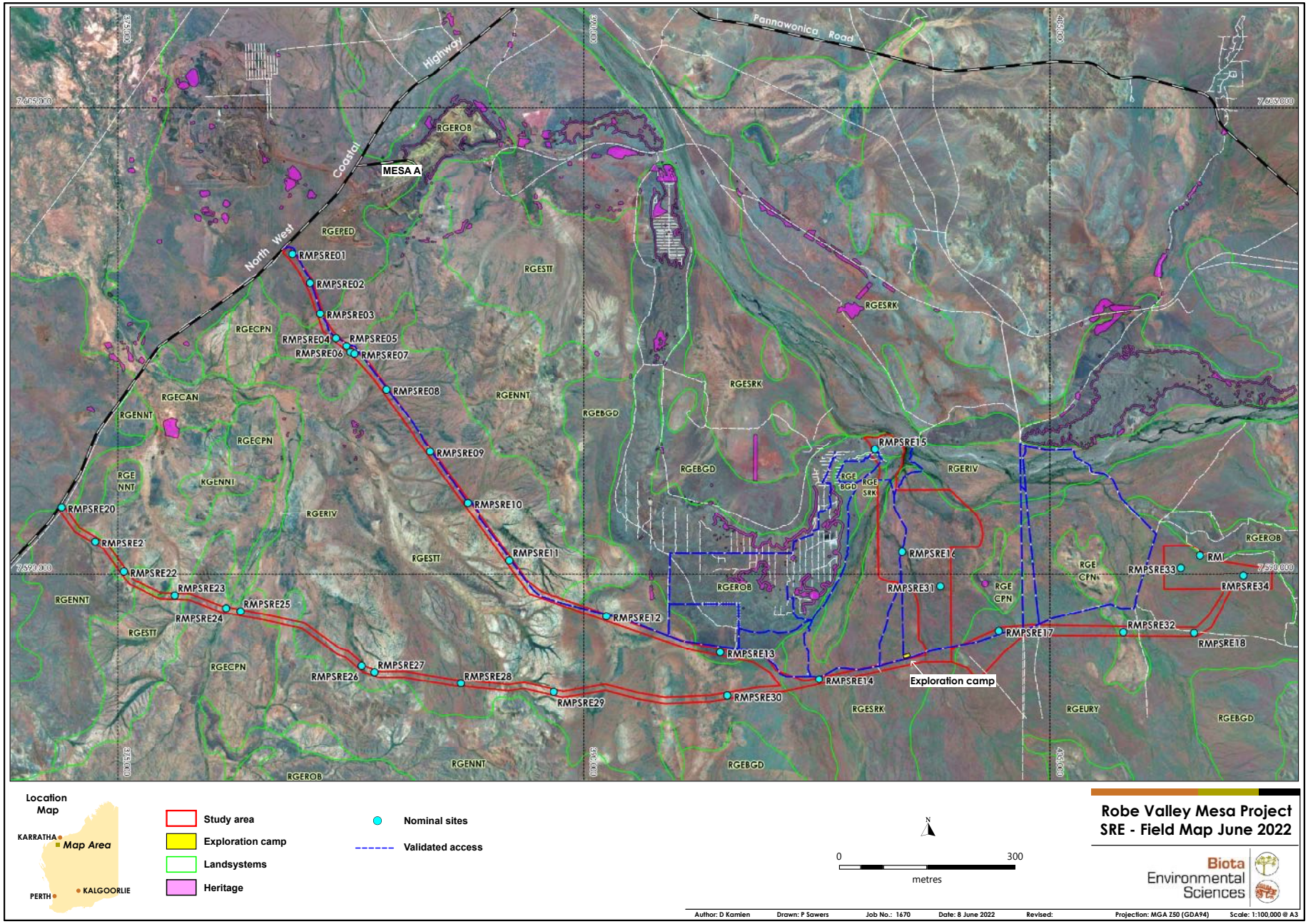

Figure 1: Targeted SRE sampling sites of the study area.

3.0 Methods

Specimens were sequenced for variation at the cytochrome oxidase subunit I gene (COI) using primers LCOI & HCO2 (Folmer *et al*., 1994). The COI mtDNA gene is widely considered to show suitable variation to distinguish species (Hebert *et al*., 2003a), and the use of this gene can be extremely effective for 'DNA barcoding' in taxa where clear differentiation exists between intraspecific (within species) and interspecific (between species) levels of divergence (e.g., Hebert *et al*., 2004a; 2004b). However, it is prudent to adopt a taxon by taxon approach, examining both intraspecific and interspecific variation for each taxon. This is the most widely accepted method of delineating species and their distributions, especially in areas where rapidly expanding mining operations outpace taxonomic treatment of unresolved taxa.

The resulting 69 successful COI sequences comprised 10 species (13 lineages) belonging to three families. One specimen (RT09) was not successfully sequenced and could not be assigned to known or unknown species. Sequence failure could be due to degradation of the DNA, primer mismatches, or contamination by other DNA in the sample. Based on genetic analysis, two other specimens (RT21 and RT34) were identified as Araneomorphae and were removed from subsequent analyses. Additionally, all specimens morphologically identified as *Bothriembryon* (RT67 – RT72) were identified as species of *Succinea* (Family Succineidae) following molecular analysis.

Sequences were edited using Geneious version 6.1.8 software (https://www.geneious.com) performed within MEGA version 5.05 (Tamura *et al.,* 2011) using the built-in alignment tool in CLUSTAL W (Thompson *et al*., 1994), with default parameters. DNA nucleotide sequences were translated into protein sequences to ensure that the amplified sequences corresponded to the target mtDNA. The translated protein sequences were then checked for the presence of stop codons, to ensure that pseudogenes hadn't been amplified. Pseudogenes have a DNA sequence that is similar to the functional gene (e.g., COI) however, they do not code for a functioning protein despite the shared ancestry with the functional gene. The presence of pseudogenes can complicate molecular analyses, producing odd results.

DNA sequences were translated into proteins with ExPASy using the invertebrate genetic code. All sequences analysed were of high quality with no evidence of heterogeneous peaks. We then employed the Basic Local Alignment Search Tool (BLAST) available from the National Centre for Biotechnology Information (NCBI) to compare DNA nucleotide sequences with a library of sequences. This program identifies sequences within the database that resemble the query sequences above a certain threshold. Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances the total percentage of nucleotides different between sequences. To account for polymorphism within lineages, the net genetic diversity of Nei (1987) was calculated to give a 'corrected' distance between lineages.

For phylogenetic analysis, likelihood ratio tests using the Bayesian Information Criterion were calculated in MEGA 7.0 (Kumar *et al*., 2016) to determine the best-fit model of evolution. The phylogenetic analyses were conducted in Geneious Prime version 2022.2.2 (https://www.geneious.com) using the best-fit model of evolution calculated for each family (Table 1). Maximum likelihood (ML) analyses with 100 rapid bootstrap

replicates, were performed in RAxML (Randomised Accelerated Maximum Likelihood) version 8.2.11 software (Stamatakis, 2014), using default settings.

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). Species were determined based on taxonomic group, molecular data available and previously published literature for each taxonomic group. For Mygalomorph spiders, determining a species cut-off value is problematic, particularly for the genus *Aname*. Castalanelli *et al* (2014) showed 92% of the morphological species of Mygalomorphae were congruent with molecular species boundary based on 9.5% inter-specific pairwise divergence. One exception was *Aname mellosa* species complex, which showed 16.7% intra-specific diversity based on morphological identifications. However, COI data from the study determined 10 distinct genetic clades in the Pilbara and northern Gascoyne, showing distinct geographical clustering (Castalanelli *et al*., 2014), with 5 - 9% inter-clade genetic diversity. Rix *et al*. (2017) found the mean intra-specific (within species) divergence ranged from 0% - 4.2% and mean inter-specific (between species) divergence ranged from 10.6% - 16.3% for a range of species belonging to the family Idiopidae. Based on these results we generally used a cut-off value of 5.0% as a conservative starting point.

To summarise intra- and inter specific divergences between species determined within this study, we used the Species Delimitation Plugin (Masters *et al*. 2011) within Geneious Prime version 2022.2.2. This is an exploratory tool that allows the user to assess putative species in phylogenetic trees, by summarising measures of phylogenetic support and diagnosability of species. This analysis was only conducted for taxa where more than one new species was detected.

NB. For most groups, Helix species names have been updated for the current report to reflect new knowledge and current Helix nomenclature. This update includes the prefix ‘H-‘.

Table 1: Best-fit model of evolution for each of the taxonomic groups and families analysed. Abbreviations: GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; G: Gamma; I: Invariant sites.

Analysis Group	Family/Genus	Best Model	Gamma Value
Mygalomorphae	Anamidae/*Aname*	GTR + G + I	0.95
Pulmonata	Camaenidae/*Rhagada*	HYK + G + I	0.88
Pulmonata	Succineidae/*Succinea*	HYK + G + I	0.43

Table 2: Invertebrate specimens used in the present study (n=72), and the genetic lineage/species to which they were assigned. Shaded cells represent samples that failed to sequence. N/A indicates specimens that are not relevant to the present study (i.e., Araneomorphae). Molecular lineages in bold text and marked with '*' represent newly detected species.

Specimen ID	Site	Lat dec	Long dec	Morphological ID	Helix ID	Species ID	Lineages	Molecular Sp. ID
M20220615.RMP20SRE_MG-01	RMP20SRE_MG	-21.77232771	115.7751411	Anamidae	RT01	*Aname* sp.	**A01**	**H – N161***
M20220615.RMP21SRE_MG-01	RMP21SRE_MG	-21.77995129	115.7828579	Anamidae	RT02	*Aname* sp.	**A02**	**H – N161***
M20220615.RMP21SRE_MG-02	RMP21SRE_MG	-21.77994571	115.7828367	Anamidae	RT03	*Aname* sp.	**A02**	**H – N161***
M20220615.RMP24SRE_MG-01	RMP24SRE_MG	-21.79940405	115.8244897	Anamidae	RT04	*Aname mellosa*	A08	H – N167
M20220616.RMP28SRE_MG-01	RMP28SRE_MG	-21.8214223	115.8971479	Anamidae	RT05	*Aname* sp.	**A03**	**H – N163***
M20220616.RMP28SRE_MG-02	RMP28SRE_MG	-21.82228297	115.8973185	Anamidae	RT06	*Aname* sp.	**A03**	**H – N163***
M20220616.RMP33SRE_MG-01	RMP33SRE_MG	-21.7897126	116.1217243	Anamidae	RT07	*Aname* sp.	**A04**	**H – N162***
M20220616.RMP33SRE_MG-02	RMP33SRE_MG	-21.78971535	116.1217258	Anamidae	RT08	*Aname* sp.	**A04**	**H – N162***
M20220616.RMP19SRE_MG-01	RMP19SRE_MG	-21.78634973	116.1277152	Anamidae	RT09	No Data		No Data
M20220616.RMP19SRE_MG-02	RMP19SRE_MG	-21.78660257	116.1276261	Anamidae	RT10	*Aname* sp.	**A04**	**H – N162***
M20220616.RMP19SRE_MG-03	RMP19SRE_MG	-21.78608348	116.1280033	Anamidae	RT11	*Aname mellosa*	A10	*Aname mellosa*
M20220617.RMP16SRE_MG-01	RMP16SRE_MG	-21.78527388	116.0348259	Anamidae	RT12	*Aname mellosa*	A10	*Aname mellosa*
M20220617.RMP16SRE_MG-02	RMP16SRE_MG	-21.78618853	116.0348102	Anamidae	RT13	*Aname mellosa*	A10	*Aname mellosa*
M20220617.RMP31SRE_MG-01	RMP31SRE_MG	-21.79473033	116.0466953	Anamidae	RT14	*Aname mellosa*	A10	*Aname mellosa*
M20220617.RMP32SRE_MG-01	RMP32SRE_MG	-21.80692644	116.1042608	Anamidae	RT15	*Aname* sp.	**A04**	**H – N162***
M20220617.RMP32SRE_MG-02	RMP32SRE_MG	-21.80692625	116.1042832	Anamidae	RT16	*Aname* sp.	**A04**	**H – N162***
M20220617.RMP18SRE_MG-01	RMP18SRE_MG	-21.80811848	116.1259473	Anamidae	RT17	*Aname* sp.	**A05**	**H – N164***
M20220617.RMP34SRE_MG-01	RMP34SRE_MG	-21.7921997	116.1413148	Anamidae	RT18	*Aname* sp.	**A04**	**H – N162***
M20220617.RMP34SRE_MG-02	RMP34SRE_MG	-21.79214213	116.1411555	Anamidae	RT19	*Aname* sp.	**A04**	**H – N162***
M20220618.RMP17SRE_MG-01	RMP17SRE_MG	-21.80779343	116.0684116	Anamidae	RT20	*Aname mellosa*	A10	*Aname mellosa*
M20220618.RMP11SRE_MG-01	RMP11SRE_MG	-21.788555	115.9142846	~~Anamidae~~ Araneomorphae	RT21	N/A		N/A
M20220618.RMP13SRE_MG-01	RMP13SRE_MG	-21.81309604	115.9791504	Anamidae	RT22	*Aname* sp.	**A06**	**H – N165***
M20220618.RMP13SRE_MG-02	RMP13SRE_MG	-21.81305936	115.9784207	Anamidae	RT23	*Aname mellosa*	A10	*Aname mellosa*
M20220618.RMP13SRE_MG-04	RMP13SRE_MG	-21.81306677	115.979231	Anamidae	RT24	*Aname* sp.	**A06**	**H – N165***
M20220618.RMP13SRE_MG-03	RMP13SRE_MG	-21.81299207	115.9787436	Anamidae	RT25	*Aname* sp.	**A06**	**H – N165***
M20220618.RMP30SRE_MG-01	RMP30SRE_MG	-21.82615984	115.9795448	Anamidae	RT26	*Aname mellosa*	A10	*Aname mellosa*
M20220618.RMP30SRE_MG-02	RMP30SRE_MG	-21.82614475	115.9795027	Anamidae	RT27	*Aname mellosa*	A10	*Aname mellosa*
M20220618.RMP14SRE_MG-01	RMP14SRE_MG	-21.82139937	116.0089015	Anamidae	RT28	*Aname mellosa*	A10	*Aname mellosa*
M20220618.RMP14SRE_MG-02	RMP14SRE_MG	-21.82138039	116.0088621	Anamidae	RT29	*Aname mellosa*	A10	*Aname mellosa*
M20220618.RMP14SRE_MG-03	RMP14SRE_MG	-21.82109326	116.0078205	Anamidae	RT30	*Aname mellosa*	A10	*Aname mellosa*
M20220618.RMP14SRE_MG-04	RMP14SRE_MG	-21.82108657	116.0077764	Anamidae	RT31	*Aname mellosa*	A10	*Aname mellosa*
M20220618.RMP35SRE_MG-01	RMP35SRE_MG	-21.81296273	116.0450899	Anamidae	RT32	*Aname mellosa*	A10	*Aname mellosa*
M20220619.RMP10SRE_MG-01	RMP10SRE_RD	-21.76957992	115.90062	Anamidae	RT33	*Aname mellosa*	A09	H – N167
M20220619.RMP07SRE_MG-01	RMP07SRE_RD	-21.72500814	115.8632911	~~Anamidae~~ Araneomorphae	RT34	N/A		N/A
M20220619.RMP03SRE_MG-02	RMP03SRE_MG	-21.71417848	115.8542497	Anamidae	RT35	*Aname mellosa*	A09	H – N167

Specimen ID	Site	Lat dec	Long dec	Morphological ID	Helix ID	Species ID	Lineages	Molecular Sp. ID
M20220620.RMP02SRE_MG-01	RMP02SRE_MG	-21.70535275	115.8511916	Anamidae	RT36	*Aname mellosa*	A09	H – N167
M20220620.RMP02SRE_MG-02	RMP02SRE_MG	-21.7058899	115.8511481	Anamidae	RT37	*Aname* sp.	**A07**	**H – N166***
M20220620.RMP02SRE_MG-03	RMP02SRE_MG	-21.70592841	115.8511431	Anamidae	RT38	*Aname mellosa*	A09	H – N167
M20220620.RMP01SRE_MG-02	RMP01SRE_MG	-21.69800762	115.845274	Anamidae	RT39	*Aname* sp.	**A07**	**H – N166***
M20220620.RMP01SRE_MG-01	RMP01SRE_MG	-21.69774366	115.8455936	Anamidae	RT40	*Aname mellosa*	A09	H – N167
M20220620.RMP01SRE_MG-03	RMP01SRE_MG	-21.69801692	115.8453301	Anamidae	RT41	*Aname* sp.	**A07**	**H – N166***
Sn20220615.RMP20SRE_MG-01	RMP20SRE_MG	-21.77234081	115.7751399	Camaenidae	RT42	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220615.RMP20SRE_MG-01	RMP20SRE_MG	-21.77234081	115.7751399	Camaenidae	RT43	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220615.RMP20SRE_MG-01	RMP20SRE_MG	-21.77234081	115.7751399	Camaenidae	RT44	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220617.RMP12SRE_MG-01	RMP12SRE_MG	-21.80199428	115.9404388	Camaenidae	RT45	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220616.RMP26SRE_MG-01	RMP26SRE_MG	-21.81726318	115.8665249	Camaenidae	RT46	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220618.RMP17SRE_MG-01	RMP17SRE_MG	-21.80735084	116.06872	Camaenidae	RT47	*Rhagada convicta*	R01	*Rhagada convicta*
Sn20220618.RMP17SRE_MG-01	RMP17SRE_MG	-21.80735084	116.06872	Camaenidae	RT48	*Rhagada convicta*	R01	*Rhagada convicta*
Sn20220618.RMP17SRE_MG-01	RMP17SRE_MG	-21.80735084	116.06872	Camaenidae	RT49	*Rhagada convicta*	R01	*Rhagada convicta*
Sn20220617.RMP11SRE_MG-01	RMP11SRE_MG	-21.78800171	115.9145127	Camaenidae	RT50	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220617.RMP11SRE_MG-01	RMP11SRE_MG	-21.78800171	115.9145127	Camaenidae	RT51	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220617.RMP11SRE_MG-01	RMP11SRE_MG	-21.78800171	115.9145127	Camaenidae	RT52	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220618.RMP30SRE_MG-01	RMP30SRE_MG	-21.82538034	115.9795643	Camaenidae	RT53	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220618.RMP30SRE_MG-01	RMP30SRE_MG	-21.82538034	115.9795643	Camaenidae	RT54	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220618.RMP30SRE_MG-01	RMP30SRE_MG	-21.82538034	115.9795643	Camaenidae	RT55	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220618.RMP14SRE_MG-01	RMP14SRE_MG	-21.82239135	116.0070348	Camaenidae	RT56	*Rhagada convicta*	R01	*Rhagada convicta*
Sn20220618.RMP35SRE_MG-01	RMP35SRE_MG	-21.81301964	116.045186	Camaenidae	RT57	*Rhagada convicta*	R01	*Rhagada convicta*
Sn20220618.RMP35SRE_MG-01	RMP35SRE_MG	-21.81301964	116.045186	Camaenidae	RT58	*Rhagada convicta*	R01	*Rhagada convicta*
Sn20220618.RMP35SRE_MG-01	RMP35SRE_MG	-21.81301964	116.045186	Camaenidae	RT59	*Rhagada convicta*	R01	*Rhagada convicta*
Sn20220619.RMP04SRE_MG-01	RMP04SRE_MG	-21.72123653	115.8587768	Camaenidae	RT60	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220619.RMP04SRE_MG-01	RMP04SRE_MG	-21.72123653	115.8587768	Camaenidae	RT61	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220619.RMP04SRE_MG-01	RMP04SRE_MG	-21.72123653	115.8587768	Camaenidae	RT62	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220614.RMP08SRE_MG-01	RMP08SRE_MG	-21.73754889	115.8755018	Camaenidae	RT63	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220614.RMP08SRE_MG-01	RMP08SRE_MG	-21.73754889	115.8755018	Camaenidae	RT64	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220614.RMP08SRE_MG-01	RMP08SRE_MG	-21.73754889	115.8755018	Camaenidae	RT65	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220614.RMP08SRE_MG-01	RMP08SRE_MG	-21.73754889	115.8755018	Camaenidae	RT66	*Rhagada convicta*	R02	*Rhagada convicta*
Sn20220618.RMP30SRE_MG-02	RMP30SRE_MG	-21.82538351	115.9795609	~~Bothriembryontidae~~/ Succineidae	RT67	*Succinea* sp.		Succinea sp. Pilbara
Sn20220618.RMP30SRE_MG-02	RMP30SRE_MG	-21.82538351	115.9795609	~~Bothriembryontidae~~/ Succineidae	RT68	*Succinea* sp.		Succinea sp. Pilbara
Sn20220618.RMP30SRE_MG-02	RMP30SRE_MG	-21.82538351	115.9795609	~~Bothriembryontidae~~/ Succineidae	RT69	*Succinea* sp.		Succinea sp. Pilbara
Sn20220617.RMP16SRE_MG-01	RMP16SRE_MG	-21.78633564	116.0347727	~~Bothriembryontidae~~/ Succineidae	RT70	*Succinea* sp.		Succinea sp. Pilbara
Sn20220617.RMP16SRE_MG-01	RMP16SRE_MG	-21.78633564	116.0347727	~~Bothriembryontidae~~/ Succineidae	RT71	*Succinea* sp.		Succinea sp. Pilbara
Sn20220617.RMP16SRE_MG-01	RMP16SRE_MG	-21.78633564	116.0347727	~~Bothriembryontidae~~/ Succineidae	RT72	*Succinea* sp.		Succinea sp. Pilbara

4.0 Results

Amongst the total 69 analysed specimens 10 species (13 lineages) from three families were identified. Of these six were previously undetected (new) species, according to the molecular data currently available for comparison.

4.1 Mygalomorphae (Family Anamidae)

4.1.1 Reference Specimens and Outgroups

The forty-one specimens identified as Mygalomorphae were collected from 25 sampling locations within the study area. Of these, 40 yielded COI sequences and were initially analysed with 702 reference specimens from both the Helix database (n=251) and GenBank (n=451). Two specimens (Helix ID RT21 and RT34) were identified through molecular sequencing as Araneomorph spiders and were removed from further analysis as they were not relevant to this project are not potential SREs. A secondary analysis was undertaken with a reduced dataset to simplify the phylogenetic tree, due to the large number of samples examined (Figure 2). However, the full dataset was examined for relationships to reference specimens. Both *Anamidae* trees were rooted using two outgroups, *Centuroides vittatus* (GenBank Accession #EU404114) and *Mesobuthus martensii* (GenBank Accession #JF00146).

4.1.2 Phylogenetic Analyses

There were 28 unique haplotypes detected amongst 38 mygalomorph specimens sequenced from the current survey. Based on sequence divergence, these haplotype specimens represent eight species, being spread amongst 10 distinct lineages (based on 3% sequence divergence) (Table 2, Figure 2). Two species (lineages = 3) fall within the *Aname mellosa* species complex, whilst six species (lineages= 7) formed a new clade of *Aname*.

Specifically, within the *Aname mellosa* complex, one lineage/species containing nine representative haplotype specimens (n=13) aligned with the majority of *Aname mello*sa reference specimens from GenBank (Figure 2). Most of the reference specimens showed 0.26 – 0.77% divergence from the project specimens (e.g., GB# KJ745432, WAM# T96553, Table 3). A further five haplotype specimens (n=5), belonging to one species (lineages = 2), aligned with additional "*Aname mellosa*" vouchers from GenBank (GB#KJ744474, WAM#T102865 & GB# KJ744486, WAM# T102888), which formed a separate well supported clade (Figure 2).

The other six species (lineages = 7) formed a well-supported clade and were not aligned closely with any other reference specimens. Molecular genetic distances ranged between 16.1 – 20.4% between this clade compared to those from the *Aname mellosa* complex clade.

4.1.3 Differentiation within and between species/lineages

Thirty-eight specimens of Anamidae collected from the study area show between 0.00% and 25.1% sequence divergence from each other (Table 3) and phylogenetic analyses suggest that several species are present in the study area belonging to two main clades (*Aname mellosa* complex and a new *Aname* spp. clade). Most of the species clades

showed between 9.7% and 25% inter-specific pairwise genetic distance, the exception being species **H-N163***, **H-N164***, and **H-N165*** which ranged between 7.4% - 10.7% inter-specific pairwise genetic distance.

Within the *Aname mellosa* species complex clade, sequence divergence ranged from 0.00% - 24.3%. Within this species complex, there appears to be two main species clades: one species H-N167 (lineages = 2), showing 0.00% - 4.3% intra-specific divergence, and one species that was aligned with the majority of *Aname mellosa* reference sequences, with 0.00 – 2.6% intra-specific divergence. The sequence divergence between these two major *Aname mellosa* clades ranged from 11.8% - 13%.

The second major *Aname* sp. clade consisted of six species (lineages = 7) showing between 0.00 – 15.1% genetic divergence and were not recorded in sympatry. Mean inter-specific pairwise divergence between species were high (9.5% – 15.1%). Intra-specific pair-wise divergence ranged from 0.00% - 9.2%. Within **H- N161*** the specimens RT01 and RT02/RT03, which represent two distinct lineages showed 4.6% divergence from each other. This divergence is relatively high considering the two sampling localities occur less than 170m apart.

Although no direct sympatry was detected within each of these two major clades, there was geographical overlap, or sympatry, between them. Based on the collecting information, species **H-N162*** and **H-N165*** occur in sympatry with *Aname mellosa* in the SE section of the study area (Sites RMP19SRE_MG and RMP13SRE_MG respectively), and **H-N166*** occurs in sympatry with *A. mellosa* species H-N167 in the NW corner of the study area (sites RMP01SRE_MG and RMP02SRE_MG). Genetic divergence between these groups in sympatry is high ranging from 20.2%-22.3% between *Aname mellosa* and H-N162/H-N165, and 19.9% – 21% between **H-N166*** and H-N167.

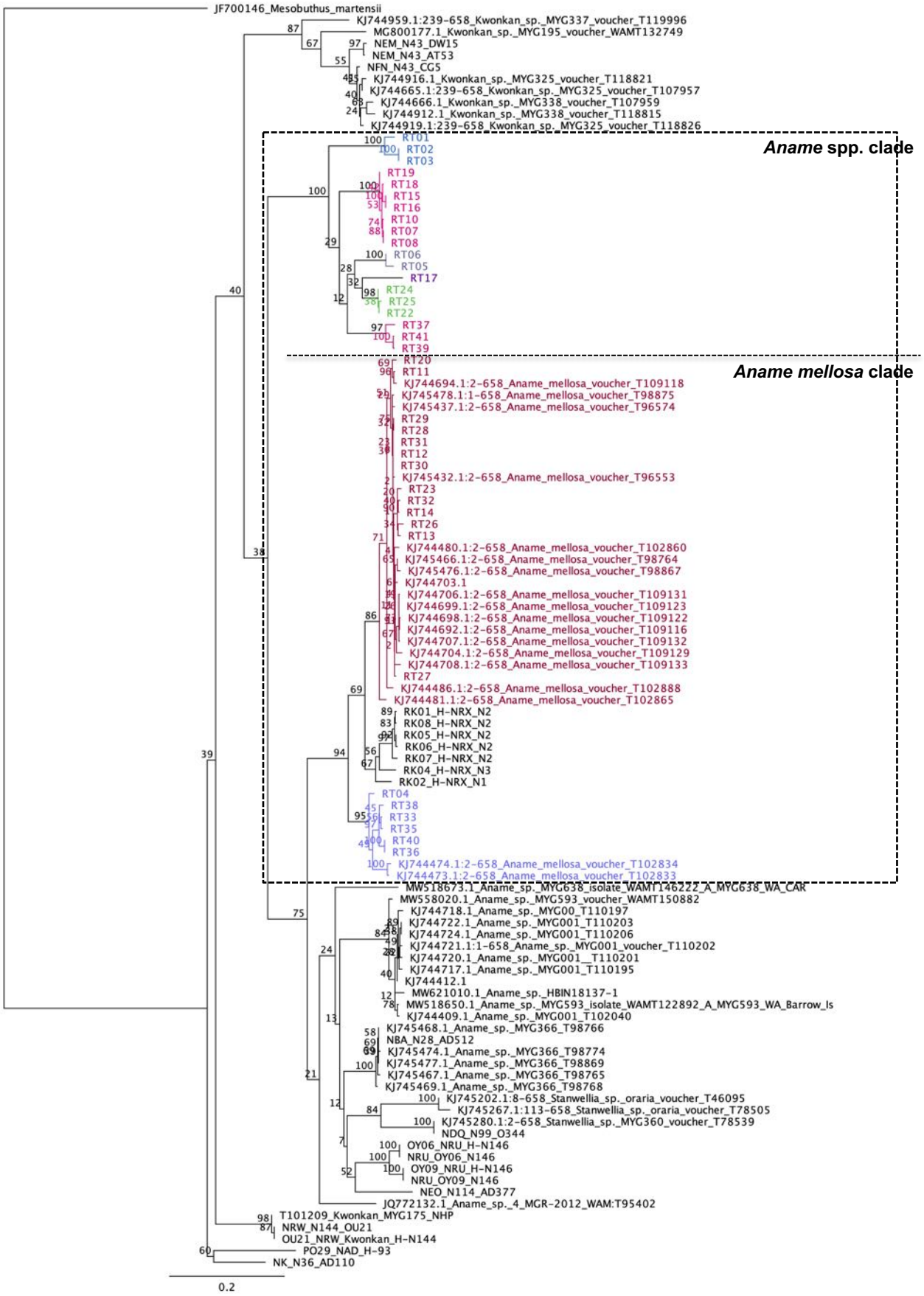

Figure 2: **Maximum Likelihood analysis of Anamidae COI mtDNA sequences, showing the placement of the 28 haplotypes ('RT' prefix) and eight species designations (coloured), representing the 38 successfully sequenced Mygalomorph specimens within the taxonomic framework of the family Anamidae, including 70 reference specimens. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).**

Table 3: Genetic p-distance (below) and the associated standard error (above - blue text) between the 38 Anamidae specimens from this study ('RT' prefix). Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance

	RT01	RT03	RT02	RT16	RT15	RT19	RT10	RT07	RT08	RT18	RT37	RT41	RT39	RT17	RT25	RT24	RT22	RT06	RT05	RT26	RT13	RT23	RT32	RT14	RT20	RT11	RT27	RT31	RT30	RT12	RT29	RT28	RT04	RT40	RT36	RT38	RT35	RT33
RT01		0.011	0.011	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.018	0.018	0.018	0.017	0.017	0.017	0.017	0.018	0.018	0.022	0.022	0.022	0.022	0.022	0.021	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.021	0.021	0.021	0.021	0.021	0.021
RT03	0.046		0.000	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.018	0.018	0.018	0.017	0.017	0.017	0.017	0.017	0.017	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.021	0.020	0.020	0.021	0.020	0.020
RT02	0.046	0.000		0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.018	0.018	0.018	0.017	0.017	0.017	0.017	0.017	0.017	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.021	0.020	0.020	0.021	0.020	0.020
RT16	0.133	0.138	0.138		0.000	0.005	0.004	0.004	0.004	0.003	0.016	0.015	0.015	0.016	0.015	0.016	0.016	0.015	0.016	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT15	0.133	0.138	0.138	0.000		0.005	0.004	0.004	0.004	0.003	0.016	0.015	0.015	0.016	0.015	0.016	0.016	0.015	0.016	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT19	0.123	0.128	0.128	0.010	0.010		0.004	0.004	0.004	0.004	0.016	0.015	0.015	0.015	0.015	0.015	0.015	0.014	0.015	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.019	0.020	0.020	0.020	0.019	0.019
RT10	0.128	0.133	0.133	0.005	0.005	0.005		0.003	0.003	0.003	0.016	0.015	0.015	0.015	0.015	0.016	0.016	0.015	0.015	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT07	0.130	0.133	0.133	0.008	0.008	0.008	0.003		0.000	0.004	0.016	0.015	0.016	0.015	0.016	0.016	0.016	0.015	0.015	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT08	0.130	0.133	0.133	0.008	0.008	0.008	0.003	0.000		0.004	0.016	0.015	0.016	0.015	0.016	0.016	0.016	0.015	0.015	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT18	0.130	0.136	0.136	0.003	0.003	0.008	0.003	0.005	0.005		0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT37	0.143	0.146	0.146	0.107	0.107	0.107	0.107	0.110	0.110	0.105		0.009	0.009	0.017	0.014	0.015	0.015	0.016	0.016	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.021	0.020	0.020
RT41	0.148	0.151	0.151	0.102	0.102	0.097	0.102	0.105	0.105	0.100	0.033		0.003	0.017	0.015	0.015	0.015	0.015	0.016	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT39	0.151	0.153	0.153	0.105	0.105	0.100	0.105	0.107	0.107	0.102	0.036	0.003		0.017	0.015	0.015	0.015	0.016	0.016	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT17	0.130	0.123	0.123	0.107	0.107	0.102	0.102	0.105	0.105	0.105	0.136	0.136	0.138		0.014	0.015	0.015	0.015	0.016	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.021	0.021	0.021	0.020	0.020
RT25	0.128	0.128	0.128	0.105	0.105	0.100	0.105	0.107	0.107	0.102	0.090	0.097	0.100	0.090		0.003	0.003	0.013	0.014	0.021	0.020	0.020	0.021	0.020	0.020	0.020	0.020	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.019
RT24	0.130	0.130	0.130	0.107	0.107	0.102	0.107	0.110	0.110	0.105	0.092	0.100	0.102	0.092	0.003		0.000	0.013	0.014	0.021	0.020	0.021	0.020	0.020	0.020	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT22	0.130	0.130	0.130	0.107	0.107	0.102	0.107	0.110	0.110	0.105	0.092	0.100	0.102	0.092	0.003	0.000		0.013	0.014	0.021	0.020	0.021	0.020	0.020	0.020	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.020	0.020	0.020	0.020
RT06	0.141	0.130	0.130	0.100	0.100	0.090	0.095	0.097	0.097	0.097	0.110	0.105	0.107	0.100	0.074	0.077	0.077		0.004	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.021	0.021	0.021	0.021	0.021
RT05	0.143	0.133	0.133	0.107	0.107	0.097	0.102	0.105	0.105	0.105	0.115	0.110	0.113	0.107	0.082	0.084	0.084	0.008		0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021
RT26	0.246	0.251	0.251	0.225	0.225	0.223	0.228	0.228	0.228	0.225	0.225	0.228	0.230	0.217	0.215	0.212	0.212	0.223	0.230		0.007	0.007	0.007	0.006	0.008	0.008	0.007	0.006	0.006	0.006	0.006	0.006	0.017	0.017	0.017	0.017	0.017	0.017
RT13	0.240	0.240	0.240	0.217	0.217	0.215	0.220	0.220	0.220	0.217	0.217	0.220	0.223	0.210	0.202	0.205	0.205	0.215	0.223	0.018		0.005	0.006	0.005	0.007	0.007	0.006	0.006	0.006	0.006	0.006	0.006	0.016	0.017	0.017	0.017	0.016	0.017
RT23	0.243	0.248	0.248	0.217	0.217	0.215	0.220	0.220	0.220	0.217	0.217	0.220	0.223	0.215	0.207	0.210	0.210	0.220	0.228	0.018	0.010		0.006	0.005	0.008	0.007	0.006	0.006	0.006	0.006	0.006	0.006	0.016	0.017	0.017	0.017	0.016	0.017
RT32	0.238	0.246	0.246	0.220	0.220	0.217	0.223	0.223	0.223	0.220	0.225	0.228	0.230	0.223	0.210	0.207	0.207	0.223	0.230	0.018	0.015	0.015		0.004	0.007	0.008	0.007	0.006	0.006	0.006	0.006	0.006	0.016	0.017	0.017	0.017	0.016	0.017
RT14	0.240	0.251	0.251	0.223	0.223	0.220	0.225	0.225	0.225	0.223	0.223	0.225	0.228	0.220	0.207	0.205	0.205	0.220	0.228	0.013	0.010	0.010	0.005		0.006	0.007	0.006	0.004	0.004	0.004	0.004	0.004	0.016	0.017	0.017	0.017	0.017	0.017
RT20	0.235	0.243	0.243	0.217	0.217	0.215	0.220	0.220	0.220	0.217	0.212	0.215	0.217	0.215	0.205	0.202	0.202	0.217	0.225	0.026	0.018	0.023	0.020	0.015		0.006	0.008	0.007	0.007	0.007	0.007	0.007	0.016	0.016	0.016	0.017	0.016	0.016
RT11	0.240	0.246	0.246	0.212	0.212	0.210	0.215	0.215	0.215	0.212	0.215	0.217	0.220	0.215	0.207	0.210	0.210	0.215	0.223	0.026	0.018	0.018	0.023	0.018	0.015		0.007	0.007	0.007	0.007	0.007	0.007	0.016	0.016	0.016	0.017	0.016	0.016
RT27	0.243	0.248	0.248	0.220	0.220	0.217	0.223	0.223	0.223	0.220	0.220	0.217	0.220	0.223	0.207	0.210	0.210	0.217	0.225	0.020	0.013	0.013	0.018	0.013	0.026	0.020		0.005	0.005	0.005	0.005	0.005	0.016	0.017	0.017	0.016	0.016	0.017
RT31	0.243	0.248	0.248	0.220	0.220	0.217	0.223	0.223	0.223	0.220	0.225	0.223	0.225	0.217	0.215	0.212	0.212	0.223	0.230	0.015	0.013	0.013	0.013	0.008	0.020	0.020	0.010		0.000	0.000	0.000	0.000	0.016	0.017	0.017	0.017	0.016	0.017
RT30	0.243	0.248	0.248	0.220	0.220	0.217	0.223	0.223	0.223	0.220	0.225	0.223	0.225	0.217	0.215	0.212	0.212	0.223	0.230	0.015	0.013	0.013	0.013	0.008	0.020	0.020	0.010	0.000		0.000	0.000	0.000	0.016	0.017	0.017	0.017	0.016	0.017
RT12	0.243	0.248	0.248	0.220	0.220	0.217	0.223	0.223	0.223	0.220	0.225	0.223	0.225	0.217	0.215	0.212	0.212	0.223	0.230	0.015	0.013	0.013	0.013	0.008	0.020	0.020	0.010	0.000	0.000		0.000	0.000	0.016	0.017	0.017	0.017	0.016	0.017
RT29	0.243	0.248	0.248	0.220	0.220	0.217	0.223	0.223	0.223	0.220	0.225	0.223	0.225	0.217	0.215	0.212	0.212	0.223	0.230	0.015	0.013	0.013	0.013	0.008	0.020	0.020	0.010	0.000	0.000	0.000		0.000	0.016	0.017	0.017	0.017	0.016	0.017
RT28	0.243	0.248	0.248	0.220	0.220	0.217	0.223	0.223	0.223	0.220	0.225	0.223	0.225	0.217	0.215	0.212	0.212	0.223	0.230	0.015	0.013	0.013	0.013	0.008	0.020	0.020	0.010	0.000	0.000	0.000	0.000		0.016	0.017	0.017	0.017	0.016	0.017
RT04	0.217	0.210	0.210	0.192	0.192	0.182	0.187	0.187	0.187	0.189	0.199	0.199	0.202	0.202	0.184	0.187	0.187	0.207	0.215	0.123	0.115	0.118	0.120	0.120	0.113	0.113	0.113	0.118	0.118	0.118	0.118	0.118		0.010	0.010	0.010	0.010	0.010
RT40	0.212	0.205	0.205	0.194	0.194	0.184	0.189	0.189	0.189	0.192	0.205	0.205	0.207	0.210	0.187	0.189	0.189	0.212	0.220	0.125	0.123	0.125	0.123	0.128	0.120	0.120	0.123	0.125	0.125	0.125	0.125	0.125	0.043		0.000	0.008	0.006	0.007
RT36	0.212	0.205	0.205	0.194	0.194	0.184	0.189	0.189	0.189	0.192	0.205	0.205	0.207	0.210	0.187	0.189	0.189	0.212	0.220	0.125	0.123	0.125	0.123	0.128	0.120	0.120	0.123	0.125	0.125	0.125	0.125	0.125	0.043	0.000		0.008	0.006	0.007
RT38	0.220	0.212	0.212	0.194	0.194	0.184	0.189	0.189	0.189	0.192	0.210	0.199	0.202	0.210	0.187	0.189	0.189	0.217	0.225	0.128	0.125	0.128	0.125	0.130	0.123	0.123	0.120	0.123	0.123	0.123	0.123	0.123	0.041	0.023	0.023		0.006	0.006
RT35	0.215	0.207	0.207	0.192	0.192	0.182	0.187	0.187	0.187	0.189	0.202	0.202	0.205	0.202	0.184	0.187	0.187	0.210	0.217	0.125	0.118	0.120	0.118	0.123	0.115	0.115	0.118	0.120	0.120	0.120	0.120	0.120	0.038	0.015	0.015	0.013		0.004
RT33	0.220	0.207	0.207	0.189	0.189	0.179	0.184	0.184	0.184	0.187	0.205	0.199	0.202	0.205	0.182	0.184	0.184	0.212	0.220	0.130	0.123	0.125	0.123	0.128	0.120	0.120	0.123	0.125	0.125	0.125	0.125	0.125	0.038	0.020	0.020	0.013	0.005	

Table 4: **Robe Valley ('RT' prefix) p-distance and the associated standard error (blue text) from reference specimens showing less than 15% sequence divergence. Lineages in bold text represent lineages detected during the current survey. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.**

Table 4 attached separately as a PDF document due to the size and detail

Species Delimitation

Based on current phylogenetic data and those previously published species delimitations in mygalomorph spiders, it is likely up to eight species are present within the study area. Based on the framework discussed at the start of this section, the 10 lineages fall into eight "species" delimitations. Table 5 shows the Species Delimitation summary output for the eight species, showing mean intra-specific distance (within species) and mean inter-specific distance (between focal species and the closest species). All species were monophyletic.

Table 5 : **Summary of Species Delimitation showing mean intraspecific (within species) and Interspecific (between next closest species) p-distance of 10 lineages from the study area, representing eight species.**

Inter/Inter: ratio of Intra Dist to Inter Dist. P ID: mean probability (95% CI) for the prediction, of making a correct identification of an unknown specimen of the focal species using best sequence alignment, closest genetic distance or placement on a tree, with criterion that it falls sister to or within a monophyletic species clade. P (Randomly Distinct): probability that a clade has the observed degree of distinctiveness. Clade Support: Bootstrap support (%).

Species	Closest Species	Intra Dist	Inter Dist - Closest	Intra/Inter	P ID (Liberal)	P (Randomly Distinct)	Clade Support
H-N161*	**H-N165***	0.028	0.207	0.13	0.93 (0.79, 1.0)	0.050	100
H-N162*	**H-N165***	0.007	0.146	0.04	0.99 (0.93, 1.0)	0.050	100
H-N163*	**H-N165***	0.013	0.104	0.13	0.90 (0.75, 1.0)	0.050	100
H-N164*	**H-N165***	0.000	0.099	0.00	0.96 (0.83, 1.0)	NA	NA
H-N165*	**H-N164***	0.003	0.099	0.03	0.99 (0.84, 1.0)	0.090	98
H-N166*	**H-N165***	0.021	0.137	0.16	0.92 (0.78, 1.0)	0.050	97
A. mellosa	H-N-167	0.018	0.146	0.12	0.98 (0.96, 1.0)	0.050	86
H-N-167	*A. mellosa*	0.031	0.146	0.21	0.94 (0.88, 1.0)	0.950	95

4.1.4 Conclusion

A total of 10 *Aname* lineages have been detected in the study area, likely corresponding to at least eight distinct species. Six of the eight species detected during the current survey were new (**H-N161* - H-N166***), as they differed from the nearest reference specimens by >20%. None of the new *Aname* species were found in sympatry. The remaining two species (Lineages A08-A10), align with the *Aname mellosa* species complex, which is suspected to encompass several currently undescribed species.

Aname mellosa (lineage A10) specimens have been detected previously based on reference GenBank sequences from published research (Castalanelli *et al*., 2014), with project specimen RT12 differing by <0.3% from KJ745432.1 *Aname mellosa* voucher (WAM T96553). The species "*Aname mellosa"* H-N167 (consisting of lineages A08 and A09), were closely related to reference sequences KJ744473 *Aname mellosa* voucher (WAM T102833) and KJ744474 *Aname mellosa* voucher (WAM T102834) from GenBank, differing by <4.3% and 5.4% respectively. Deep genetic structuring of haplotypes of *A. mellosa* may be indicative of cryptic speciation within the *A. mellosa* - complex (Harvey *et al.*, 2012). Based on current data from this study we consider that two species of *A. mellosa* are present within the study area.

The two species within the *Aname mellosa* species complex were not recorded in sympatry (i.e. none of the sites recorded more than a single lineage (A08, A09 or A10). Lineage A08 was recorded from one locality in the SW corner of the study area, A09 was found from four sampling localities in a band running from NW to the centre of the study area, and A10 was found in the eastern section of the study area (eight sampling localities). Approximately 500m separates the closest collecting locality of A08 A09, and A09 from A10.

4.2 Gastropoda (Family Camaenidae)

4.2.1 *Reference Specimens and Outgroups*

The 25 Camaenidae specimens were collected from 10 sampling locations within the study area (Table 2). All specimens yielded successful COI sequences and were initially analysed with 122 reference specimens from both the Helix database (n=22) and GenBank (n=100). A secondary analysis was undertaken with a reduced dataset to simplify the phylogenetic tree, due to the large number of samples examined (Figure 3). However, the full dataset was examined for relationships to reference specimens. The preliminary tree was rooted using *Centuroides vittatus* (GenBank Accession #EU404114) and *Mesobuthus martensii* (GenBank Accession #JF00146), with the subsequent tree rooted using *Baudinella sp.* (WAM S37063).

4.2.2 Phylogenetic Analyses

Thirteen unique haplotypes were detected amongst 25 sequenced individuals. These aligned with a previously described species *Rhagada convicta* (Camaenidae), but were spread amongst two lineages (based on 3% sequence divergence; Figure 3, Table 2 & Table 6).

Two representative haplotype specimens (n=7) from one lineage (R01) aligned closely with *Rhagada convicta* specimens from GenBank, with reference specimens showing 0.00 – 0.17% divergence from the project specimens (e.g., GB#s KM405443 - KM405447; Table 6). A further 11 haplotype specimens (n=18), belonging to separate lineage (R02), did not align closely with reference specimens but differed from *Rhagada convicta* sequences from GenBank (GB#JQ362695-JQ362696) by 5.2% and 5.4% respectively (Figure 3). Both lineages formed well supported clades.

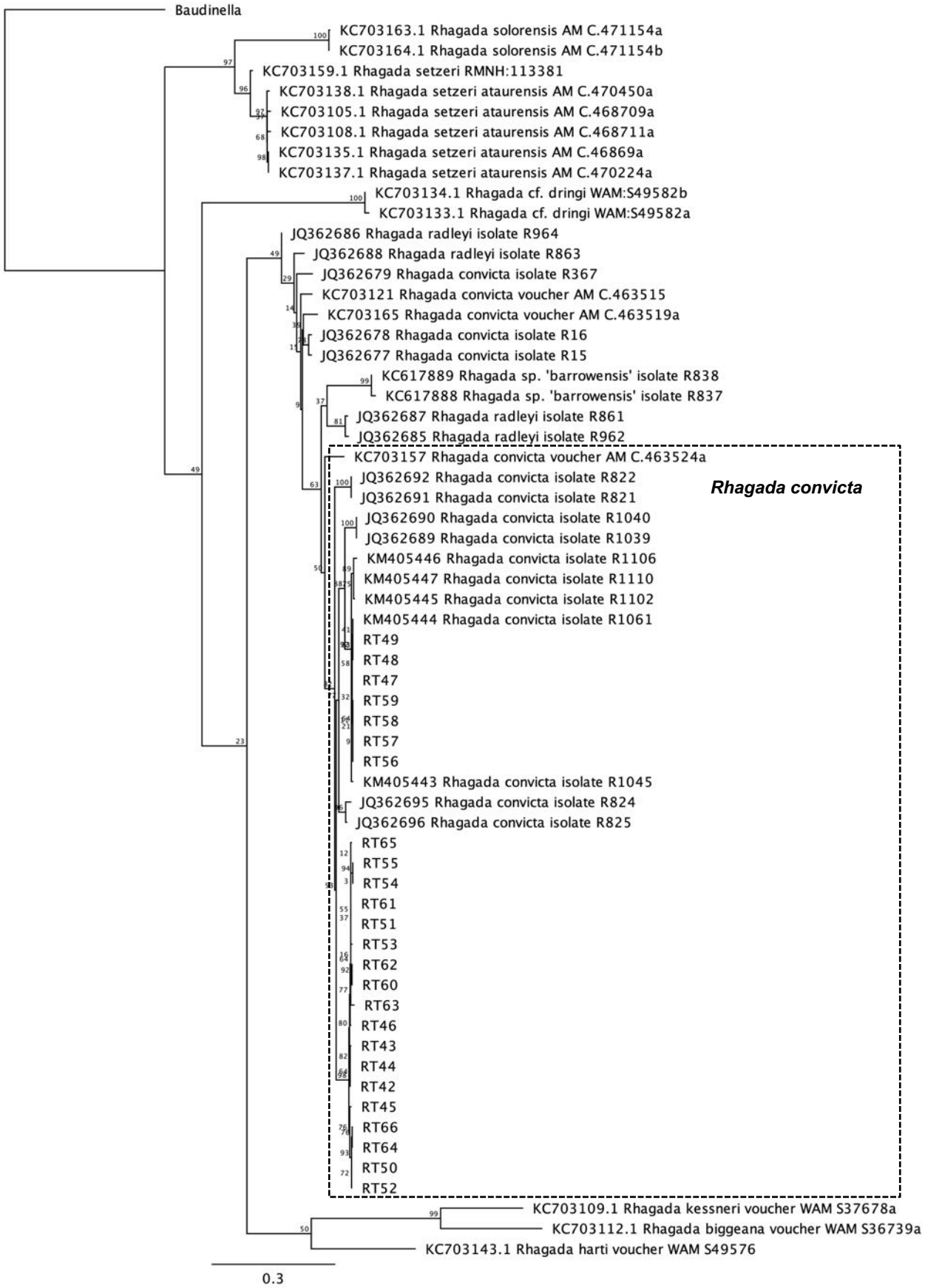

Figure 3: Maximum Likelihood analysis of 62 COI mtDNA sequences, showing the placement of 25 successfully sequenced *Rhagada convicta* specimens ('RT' prefix, 13 unique haplotypes), within the taxonomic framework of the family Camaenidae, including 36 reference specimens of *Rhagada* (Camaenidae).

Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).

Table 6: **Genetic p-distance (below) and the associated standard error (above - blue text) between the 25 Camaenid specimens from this study ('RT' prefix).**

Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

	RT45	RT50	RT52	RT64	RT66	RT63	RT42	RT44	RT43	RT54	RT55	RT60	RT62	RT53	RT46	RT65	RT51	RT61	RT56	RT57	RT58	RT59	RT47	RT48	RT49
RT45		0.004	0.004	0.004	0.004	0.005	0.003	0.003	0.003	0.005	0.005	0.004	0.004	0.004	0.004	0.004	0.003	0.003	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT50	0.009		0.000	0.002	0.002	0.005	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.003	0.004	0.004	0.004	0.004	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT52	0.009	0.000		0.002	0.002	0.005	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.003	0.004	0.004	0.004	0.004	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT64	0.010	0.002	0.002		0.000	0.006	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.004	0.005	0.005	0.004	0.004	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT66	0.010	0.002	0.002	0.000		0.006	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.004	0.005	0.005	0.004	0.004	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT63	0.012	0.017	0.017	0.019	0.019		0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.004	0.003	0.004	0.004	0.004	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT42	0.007	0.009	0.009	0.010	0.010	0.012		0.000	0.000	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.002	0.002	0.010	0.010	0.010	0.010	0.009	0.009	0.009
RT44	0.007	0.009	0.009	0.010	0.010	0.012	0.000		0.000	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.002	0.002	0.010	0.010	0.010	0.010	0.009	0.009	0.009
RT43	0.007	0.009	0.009	0.010	0.010	0.012	0.000	0.000		0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.002	0.002	0.010	0.010	0.010	0.010	0.009	0.009	0.009
RT54	0.012	0.014	0.014	0.016	0.016	0.014	0.009	0.009	0.009		0.000	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT55	0.012	0.014	0.014	0.016	0.016	0.014	0.009	0.009	0.009	0.000		0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT60	0.010	0.012	0.012	0.014	0.014	0.012	0.007	0.007	0.007	0.009	0.009		0.000	0.003	0.003	0.003	0.002	0.002	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT62	0.010	0.012	0.012	0.014	0.014	0.012	0.007	0.007	0.007	0.009	0.009	0.000		0.003	0.003	0.003	0.002	0.002	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT53	0.009	0.007	0.007	0.009	0.009	0.010	0.005	0.005	0.005	0.007	0.007	0.005	0.005		0.002	0.002	0.002	0.002	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT46	0.009	0.010	0.010	0.012	0.012	0.007	0.005	0.005	0.005	0.007	0.007	0.005	0.005	0.003		0.002	0.002	0.002	0.010	0.010	0.010	0.010	0.009	0.009	0.009
RT65	0.009	0.010	0.010	0.012	0.012	0.010	0.005	0.005	0.005	0.007	0.007	0.005	0.005	0.003	0.003		0.002	0.002	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT51	0.007	0.009	0.009	0.010	0.010	0.009	0.003	0.003	0.003	0.005	0.005	0.003	0.003	0.002	0.002	0.002		0.000	0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT61	0.007	0.009	0.009	0.010	0.010	0.009	0.003	0.003	0.003	0.005	0.005	0.003	0.003	0.002	0.002	0.002	0.000		0.010	0.010	0.010	0.010	0.010	0.010	0.010
RT56	0.061	0.063	0.063	0.065	0.065	0.059	0.056	0.056	0.056	0.063	0.063	0.058	0.058	0.059	0.056	0.059	0.058	0.058		0.000	0.000	0.000	0.002	0.002	0.002
RT57	0.061	0.063	0.063	0.065	0.065	0.059	0.056	0.056	0.056	0.063	0.063	0.058	0.058	0.059	0.056	0.059	0.058	0.058	0.000		0.000	0.000	0.002	0.002	0.002
RT58	0.061	0.063	0.063	0.065	0.065	0.059	0.056	0.056	0.056	0.063	0.063	0.058	0.058	0.059	0.056	0.059	0.058	0.058	0.000	0.000		0.000	0.002	0.002	0.002
RT59	0.061	0.063	0.063	0.065	0.065	0.059	0.056	0.056	0.056	0.063	0.063	0.058	0.058	0.059	0.056	0.059	0.058	0.058	0.000	0.000	0.000		0.002	0.002	0.002
RT47	0.059	0.061	0.061	0.063	0.063	0.058	0.054	0.054	0.054	0.061	0.061	0.056	0.056	0.058	0.054	0.058	0.056	0.056	0.002	0.002	0.002	0.002		0.000	0.000
RT48	0.059	0.061	0.061	0.063	0.063	0.058	0.054	0.054	0.054	0.061	0.061	0.056	0.056	0.058	0.054	0.058	0.056	0.056	0.002	0.002	0.002	0.002	0.000		0.000
RT49	0.059	0.061	0.061	0.063	0.063	0.058	0.054	0.054	0.054	0.061	0.061	0.056	0.056	0.058	0.054	0.058	0.056	0.056	0.002	0.002	0.002	0.002	0.000	0.000	

4.2.3 Differentiation within and between lineages

The specimens collected from this survey could be considered all *Rhagada convicta*, which is a previously known and described species. Intra-specific divergence between specimens from this study ranged from 0.00 – 6.46%, with a mean intra-specific divergence of 5.2%. Within this species, there appears to be two distinct lineages amongst the sequenced specimens from the study area (R01 and R02). Both lineages had low levels of intraspecific sequence variation. Lineage R01 showed 0.00 % - 0.17% genetic divergence between specimens. Lineage R02 showed 0.00% – 1.92% genetic divergence between specimens (Table 6). Genetic divergence between these two lineages ranged from 5.41 % to 6.46%. Lineages were separated geographically across the study area, with lineage R01 occurring in three eastern sites and lineage R02 occurring in seven northern and western sites. Only 162m separates the closest sites RMP30SRE_MG (lineage R02, specimens RT53-RT55) and RMP14SRE_MG (lineage R01, RT56), with genetic divergences ranging from 5.9%-6.3% between lineages.

4.2.4 Conclusion

Two lineages of *Rhagada* have been detected within the study area likely corresponding to one previously described species, *Rhagada convicta.* This species has been found outside the study area and has a widespread distribution. Previous research, however, has shown apparent polyphyly of *Rhagada convicta*, which has two phylogenetic and geographical groups, with a mean sequence divergence of approximately 7% (Johnson *et al.,* 2012). Further taxonomic re-examination of this complex group is required.

4.3 Gastropoda (Family Succineidae)

4.3.1 Reference Specimens and Outgroups

Six individuals of *Succinea* sp. were successfully sequenced from two sites (Table 2). During the BLAST analysis, all specimens showed a 100% match to previously sequenced *Succinea sp.* Pilbara (OV161), so only a basic phylogenetic analysis was undertaken. Thirty-six reference specimens were used during analyses, including six sequences from this project, five sequences from the Helix database, and 24 sequences representing four genera of Succineidae, as well as the outgroup *Lymnaea aulacospira* (AY150091.1) obtained from GenBank (Figure 4).

4.3.2 Phylogenetic Analyses

The six representative specimens clearly belonged to one distinct lineage belonging to the genus *Succinea* (Table 2). This lineage has been detected previously from the Pilbara. The six specimens (one unique haplotype) aligned with other specimens sequenced by Helix belonging to the previously detected *Succinea* sp. Pilbara (Figure 4).

4.3.3 Differentiation within and between lineages

There were no molecular genetic divergences within the specimens (0.0%), with only one species/lineage detected from the study area. Within this lineage there were very low levels of intraspecific sequence variation, between 0.0% - 0.02%, with a mean divergence of 0.01% between project specimens and previously sequenced OV161-OV165.

Levels of mean interspecific sequence variation between the lineage detected during the current study and the closest reference lineage within the phylogenetic analysis was 12.9% (GB# KY904740.1 *Succinea* sp. Voucher 2287).

4.3.4 Conclusion

Succinea sp. Pilbara has been detected previously from outside the study area, with sequences differing from the nearest reference specimens by <0.2%. The Succineidae are adept at dispersal across vast geographic distances, as demonstrated by their worldwide distribution. However, it is possible that some species may exhibit short-range endemism. Further resolution is likely with additional sequencing of specimens collected elsewhere in the Pilbara.

The *Succinea* specimens were originally identified as *Bothryembrion* (Family Bothriembryontidae) in the field. However, *Succinea* spp. have a small, thin, fragile shell (height 1-2cm), which is often amber in colour and lacking sculpture (i.e., smooth). Whereas *Bothryembrion* spp. have a thicker, larger globose shell (height 1- 6cm), with a punctate sculpture on the shell tip (Whisson & Kirkendale, 2014).

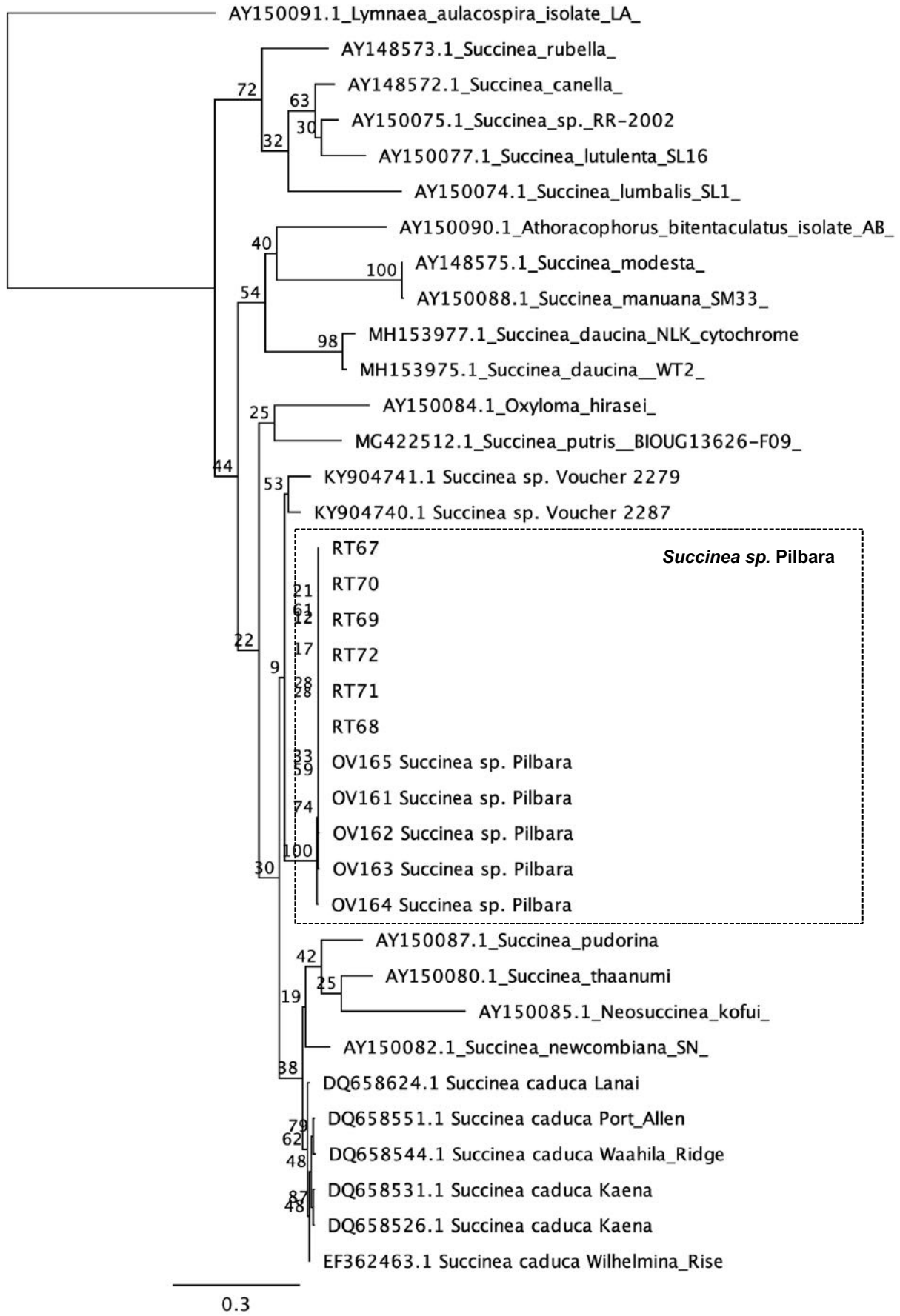

Figure 4: **Maximum Likelihood analysis of 36 COI mtDNA sequences, showing the placement of the six successfully sequenced *Succinea* specimens ('RT' prefix) within the taxonomic framework of the family Succineidae, including 30 reference specimens. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Scale indicates inferred evolutionary distance (substitutions/site).**

5.0 Summary

Through molecular sequencing and phylogenetic anlayses we detected a total of 10 species (13 lineages) belonging to three families. Anamidae (n=8), Camaenidae (n=1) and Succineidae (n=1), amongst the 69 analysed specimens from 25 sites in the study area. Of the 10 species, six were new and have not been detected previously, according to the molecular data available for comparison. The remaining four species aligned with lineages recorded previously, which included two species within the *Aname mellosa* species complex (Family Anamidae), *Rhagada convicta* (Family Camaenidae), and *Succinea* sp. Pilbara (Family Succineidae).

Based on known geographical distributions, it is possible that the six new species of Anamidae (genus *Aname*) detected in this study represent SREs. Further resolution is likely with more widespread sampling and sequencing of specimens from elsewhere in the Pilbara.

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