Mesa A / Warramboo and Yarraloola Borefield Development

Baseline Stygofauna Assessment

Prepared for

Robe River Iron Associates

Prepared by

Biota Environmental Sciences Pty Ltd

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Mesa A / Warramboo Yarraloola Borefield Stygofauna Assessment

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1.0 Introduction

1.1 Project Background

Robe River Iron Associates (Robe) is currently undertaking a number of iron ore mining expansion projects in the vicinity of Pannawonica in the Pilbara region of Western Australia. Two of these deposits, Mesa A and Warramboo, are located approximately 43 km west of Pannawonica adjacent to the North West Coastal Highway. These deposits have been identified as the next priorities for the development of the company's operations in the Robe valley. The proposal to develop these deposits into operational mines is currently being formally assessed by the Environmental Protection Authority (EPA).

A new borefield is required to supply water for the proposed mining operations. The current proposed location for this is on Yarraloola Station on the eastern side of the Robe River, with an associated pipeline linking the bore field to Mesa A/Warramboo. Consideration is also being given to the alternative of sourcing water from the Waramboo area to meet project requirements. Figure 1.1 shows the locality of the project areas.

1.2 Scope and Aims of this Study

Extraction of groundwater has the potential to affect any stygofauna communities which may be present (groundwater dwelling fauna). As part of the assessment of potential impacts of this project, Biota Environmental Sciences was commissioned to undertake surveys for stygofauna in the areas under consideration for development of the borefield. No field stygofauna collections have previously been completed in this specific area as part of Pilbara Iron's ongoing stygofauna research programme. Sampling for stygofauna in the locality was first completed by Biota (2004) at Mesa A as part of exploration stage environmental surveys. No stygofauna were collected from any of the 14 boreholes sampled during this work. The only other nearby stygofauna sampling previously completed for Pilbara Iron was at Bungaroo some 70 km to the south east of the current study area (sampled in 2003 and again in 2005). CALM has sampled three bores at Yarraloola as part of ongoing regional survey work but most of the results of this work were not available at the time of preparing this document (some genetic analysis of amphipods from this collection has been completed; Section 3.2.1).

The scope of the current study was to address the potential impacts on stygofauna arising from the proposal to use local groundwater to supply production water for the proposed mining operations. The specific aims of the work were to:

1. sample available water monitoring sites, stock-wells and production bores within or near the Yarraloola and Warramboo areas for the presence of stygofauna;
2. identify the stygofauna collected to the species level wherever possible to place them into local and regional context; and
3. assess the potential impacts on stygofauna due to the proposed use of groundwater;

The term stygofauna is used in this report in reference to stygobites: obligate groundwater-dwelling, aquatic fauna. An overview of the ecology, taxonomy and status of subterranean fauna is provided in Appendix 1.

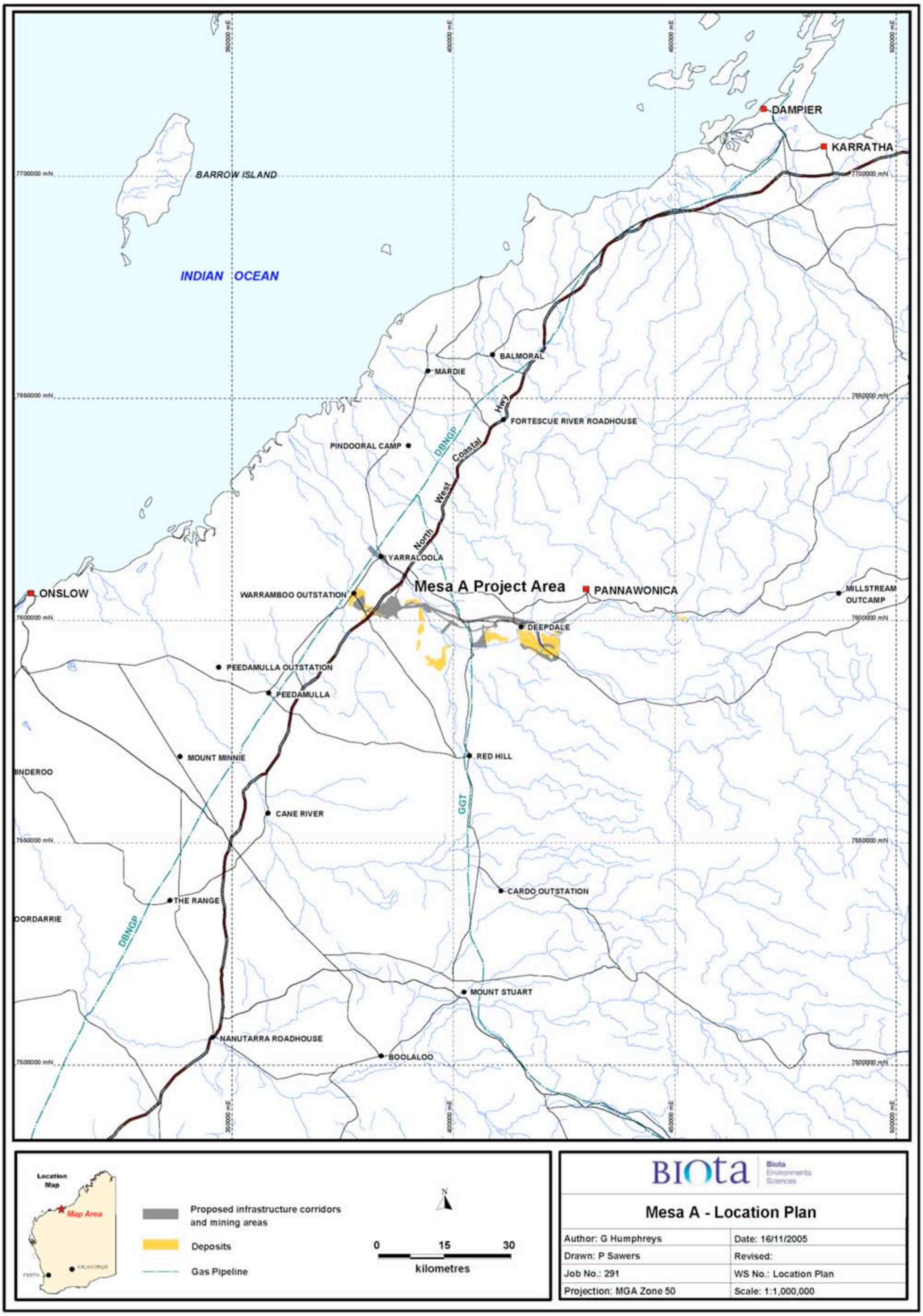

Figure 1.1: Location of study areas.

2.0 Methodology

2.1 Stygofauna Sampling Design

The boreholes selected for this study represent a combination of drill holles, existing monitoring bores and stock wells within the Yarraloola, Warramboo, Todd Bore and Dinner Camp areas. The majority of bores within the Warramboo area were exploration drill holes installed between 1982 and 2004. The Geological Survey of Western Australia (GSWA) carried out a drilling programme between 1983 and 1985, to locate groundwater supplies for coastal towns of the Pilbara region (Commander 1994). These bores were designated the Robe River and Robe River Coastal Plain series. Sampling was also carried out a Bungaroo Creek during the current study, but this will be reported in a separate document.

The sampling design for this study represented a combined total of 53 bores. Of this total, 23 were located in the Warramboo area and 30 in the Yarraloola area. This included bores and wells located off the Pannawonica access road and adjacent to North-West Coastal Highway. The selected sites for sampling are shown on Figure 2.1.

2.2 Stygofauna Survey and Analysis Team

The Field team that conducted the sampling consisted of Lee Mould and Jessica Lynas of Biota. The project was co-ordinated by Garth Humphreys of Biota. The genetic analysis of Amphipoda collected was undertaken by Dr Terrie Finston of the University of Western Australia (UWA) in Perth. Taxonomic identification of the Copepoda and Thermosbaenaceans collected was undertaken by Dr Russell Shiel of the University of South Australia.

2.3 Stygofauna Sampling Methods and Data Management

Stygofauna sampling at Warramboo and Yarraloola areas followed similar methodology to that applied to other stygofauna sampling work recently completed in the region. The approach adopted was consistent with that outlined in Environmental Protection Authority (EPA) Guidance Statement Number 54 (EPA 2003).

Sampling for stygofauna was undertaken between 05/10/05 and 07/10/05, with the team based and working from Pannawonica. Stygofauna were sampled from water bores, by means of specially modified plankton haul nets. Sampling nets were constructed from 150 μm plankton mesh, with c. 50 mm and 100 mm apertures attached to a weighted catch jar. Each hole was dragged a minimum of three times. Once the net reached the bottom on the third haul, it was agitated gently to bring the benthos and any fauna present above the net before dragging the water column. On the surface, the net was flushed thoroughly with water bailed from the same hole and the resultant sample placed in a labelled container within a shaded esky. A hygiene protocol was followed at the completion of each hole whereby nets and catch bottles were washed clean to address the risks of any specimens being moved between boreholes.

Samples were not fixed prior to sorting, as live stygofauna are more easily observed and recovered. Samples were sorted in a modified laboratory on-site, under a dissecting microscope (magnification up to 40x). Stygofauna specimens were tracked on Biota's standard tracking forms and preserved in either liquid nitrogen (to allow immediate preservation) or 100% ethanol (suitable for both morphological and DNA analyses). Amphipod specimens recovered alive were stored and shipped to Perth in liquid nitrogen, which would enable allozyme analysis at the School of Animal Biology at UWA.

2.4 Stygofauna Sampling Effort

2.4.1 Warramboo

Sites visited for stygofauna sampling in the Warramboo area are given in Table 2.1 and shown in Figure 2.1.

Table 2.1: Warramboo selected boreholes (DW=Estimated depth to water table; TD=Estimated total water depth to bottom of hole; All co-ordinates in GDA94 Zone 50).

Hole Reference	Sampled	Easting	Northing	DW	TD	Hauls	Comments
Dinner Camp							
MEARC4186	05/10/05	370933	7597956	10	22	3	
MEARC4189	05/10/05	370133	7598755	14	31	3	
MEARC4191	05/10/05	370933	7599961	13	17	3	
MEARC4197	05/10/05	370136	7600757	12	29	3	
MEARC4198	05/10/05	369733	7600756	11	27	3	
MEARC4206	05/10/05	368932	7602758	9	28	3	
Todd Bore							
MEARC3151	06/10/05	375040	7602814	3	14	2	
MEARC3167	06/10/05	375712	7600443	5.5	6	3	
MEARC3175	06/10/05	376163	7599052	13	19	3	
MEARC3180	05/10/05	379521	7600160	27	50	3	
MEARC3181	05/10/05	379909	7600573	20	25	3	
TBRC3152	05/10/05	375441	7602826		16		Dry
Warramboo							
MEARC3232	05/10/05	379140	7604395	18	23	3	
MEARC3296	05/10/05	378685	7602981				Could not be located
MEARC3380	05/10/05	378162	7603667	14	19.5	3	
MEARC3483	06/10/05	378059	7606861	8	20	3	
MEARC3947	06/10/05	377125	7604830	9	25	3	
MEARC3585	05/10/05	378617	7605079	4	17	3	
MEARC3655	06/10/05	379091	7605891	14	20	3	
MEARC3719	05/10/05	379666	7603613	21	40	3	
MEARC3996	06/10/05	377599	7605642	11	32	3	
MEAWO4094	06/10/05	379900	7605918	12	30	2	
WARWE4185	05/10/05	379288	7602985	7	39	3	Replaced 3296

2.4.2 Yarraloolola

The sites visited to sample for stygofauna in the Yarraloola area are given in Table 2.2 and shown on Figure 2.1.

Table 2.2: Yarraloola selected boreholes (DW=Estimated depth to water table; TD=Estimated total water depth; All co-ordinates in GDA94 Zone 50).

Borehole	Sampled	Easting	Northing	DW	TD	Hauls	Comments
Ashley Well	06/10/05	394884	7602322	3	5	3	
BF 2-1	07/10/05	382332	7615286	4	11	3	
BF 2-2	07/10/05	381808	7615784				Could not be located
BHP Town Bore	06/10/05	387433	7607902	7	13	3	
Camp Bore	07/10/05	382763	7612704	6	16	3	Same as RRCP2A
Five Mile Well 39Y	07/10/05	388612	7610781				No access
Garden Well	07/10/05	383810	7614405				No access to water
Lane Bore	07/10/05	381602	7613974				Being pumped
Macks Bore	06/10/05	392499	7607478	13	17	3	
Nr Five Mile Well	06/10/05	388529	7610565			3	

Borehole	Sampled	Easting	Northing	DW	TD	Hauls	Comments
Perseverance	07/10/05	384263	7614398	-	-	3	
Robe River 16A	07/10/05	384802	7614890	15	19	3	
Robe River 3A	07/10/05	382374	7616588	-	-	3	
Robe River 4A	07/10/05	380457	7614508	9	25	3	
Robe River 4P	06/10/05	380475	7614484	6	16	3	
Robe River 5A	07/10/05	379238	7613554				No access
Robe River 5B	07/10/05	379238	7613554				No access
Robe River 6A	07/10/05	381503	7618595	5	14	3	
Robe River 7A	07/10/05	379138	7617655				Being pumped
Robe River 8A	07/10/05	377138	7617055				Missing
Robe River Coastal Plain 1A	07/10/05	384325	7613752	11	17	3	
Robe River Coastal Plain 2A	07/10/05	382763	7612704				Same as Camp bore
Robe River Coastal Plain 4	07/10/05	380488	7614470	4	17	3	
Sheryl Bore 13Y	07/10/05	376642	7617778	3	7	3	
The Daisy Well	07/10/05	381745	7617580	-	-	3	
The Jubilee Bore	07/10/05	383416	7612535				No access
The Queen Well	07/10/05	374567	7617795	2	5	3	
Varanus Well	07/10/05	402696	7606151	4	5	3	
Wongoo Bore	06/10/05	387839	7607953	5	8	3	
Woolshed Well (Shearing Shed)	07/10/05	383525	7614582	4.5	7.5	3	

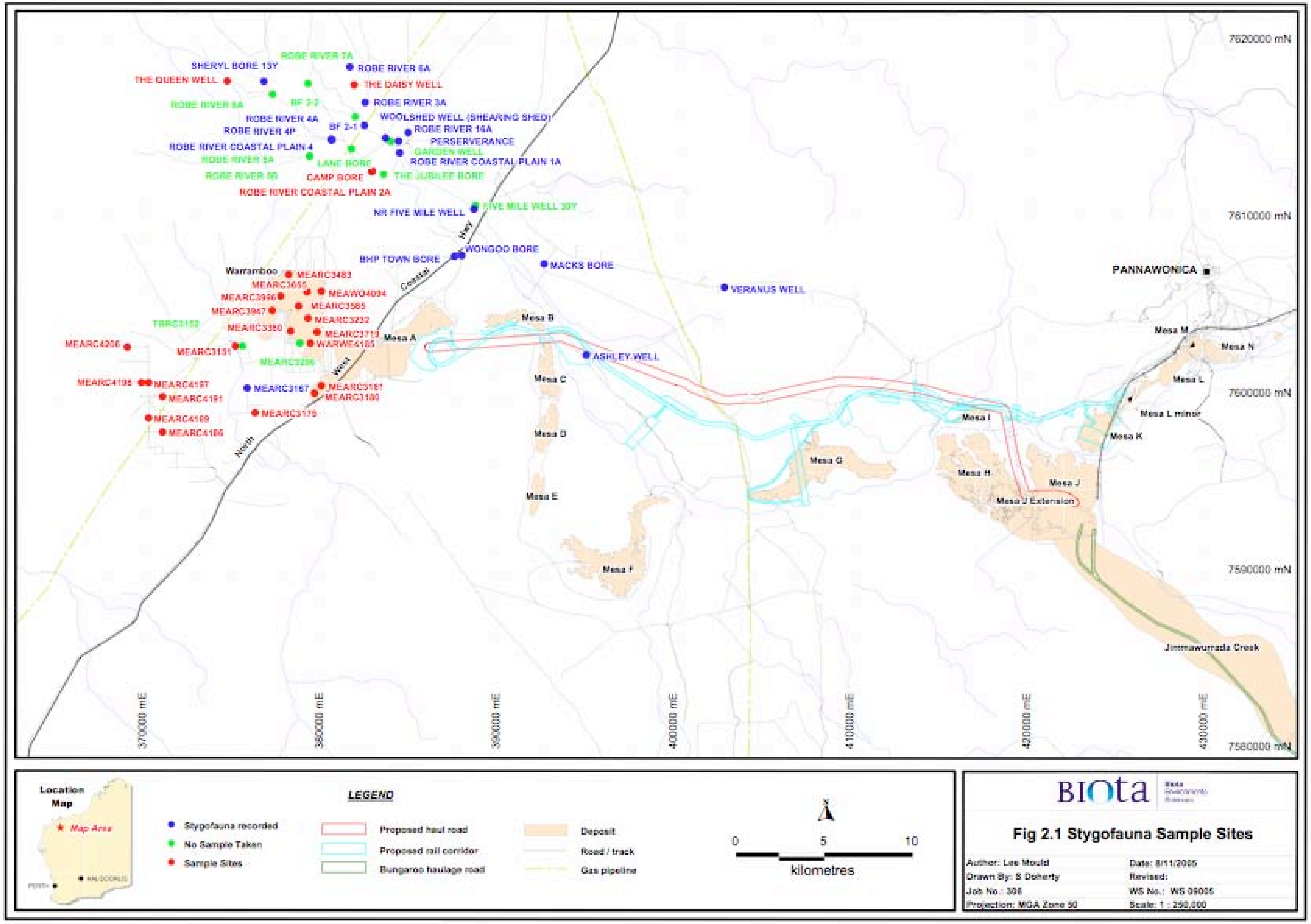

Figure 2.1: Stygofauna survey sites at Warramboo and Yarraloola.

3.0 Results

3.1 Sampling Results and Stygofauna Collected

3.1.1 Warramboo Area Results

Samples were obtained from 21 of the 23 total bores selected at Warramboo. One bore was no longer present and one bore dry at the time of sampling (see Table 2.1).

Stygofauna were recorded from only a single bore within the Todd Bore area; bore MEARC3167, where 20 oligochaetes were collected. More detailed identification of these specimens has not been completed at this time. The majority of the oligochaete specimens collected in recent Pilbara work appear to be relatively widespread at the species level (Biota 2005; Biota in prep.; A. Pinder, CALM, pers. comm.).

3.1.2 Yarraloola Area Results

Samples were collected from 20 of the 31 bores selected for sampling at Yarraloola. Two bores could not be located, one was dry, two bores shared the same location with different names, two bores were being pumped and no access was available for five of the selected bores (see Table 2.2).

Stygofauna were recorded from 17 bores and wells in the Yarraloola area as shown in Table 3.1 and Figure 2.1.

Table 3.1: Stygofauna recorded from the Yarraloola area (n=number of specimens).

Hole Reference	Sampled	Taxa	n
Ashley Well	06/10/05	Copepoda	1
BF 2-1	07/10/05	Copepoda	12
BHP Town Bore	06/10/05	Amphipoda	3
	06/10/05	Copepoda	14
	06/10/05	Thermosbaenacea	10
Macks Bore	06/10/05	Copepoda	3
Nr Five Mile Well	06/10/05	Amphipoda	6
	06/10/05	Copepoda	3
	06/10/05	Thermosbaenacea	3
Perseverance Well	07/10/05	Amphipoda	4
Robe River 16A	07/10/05	Amphipoda	37
	07/10/05	Oligochaeta	1
Robe River 3A	07/10/05	Amphipoda	6
	07/10/05	Copepoda	47
	07/10/05	Thermosbaenacea	14
Robe River 4A	07/10/05	Amphipoda	8
	07/10/05	Copepoda	30
Robe River 4P	06/10/05	Amphipoda	3
	06/10/05	Copepoda	7
	06/10/05	Ostracoda	13
Robe River 6A	07/10/05	Amphipoda	1
Robe River Coastal Plain 1A	07/10/05	Copepoda	26
	07/10/05	Thermosbaenacea	4
Robe River Coastal Plain 4	07/10/05	Amphipoda	20
	07/10/05	Copepoda	32
Sheryl Bore 13Y	07/10/05	Amphipoda	1
	07/10/05	Copepoda	2
Varanus Well	07/10/05	Amphipoda	1
Wongoo Bore	06/10/05	Amphipoda	1
Woolshed Well (Shearing Shed)	07/10/05	Copepoda	2
	07/10/05	Thermosbaenacea	2

3.2 Identification and Genetic Analyses of Yarraloola Fauna

3.2.1 Amphipoda

The draft document produced by Dr Terrie Finston of UWA 'Summary Report for the Robe River region, Genetic Variation in Stygobitic Amphipods' is given as Appendix 2 and should be read in conjunction with this section.

DNA sequencing analysis was completed on 1-2 individuals from 13 collecting sites (including specimens from the Bungaroo Creek area). This enabled genetic groups and divergence between genetic groups and previously studied areas to be determined. This analysis, and preliminary morphological review, indicated that all amphipods sequenced from Yarraloola belonged to the family Melitidae.

A total of 18 individuals were successfully sequenced, of which 11 distinct haplotypes were identified. These haplotypes fall into five distinct groups. Four of the five groups or lineages (A, B, D, and F) are found at Yarraloola (see Table 3.2). The fifth, lineage C, was found at Bungaroo Creek. Within each lineage, sequence divergence among haplotypes was low, ranging from 0 to 0.4%. Sequence divergence between lineages was more than 10 times higher, ranging from 4.8% between lineages A and C, to 13.3 % between lineages B and D (see Appendix 2). This suggests that each lineage is effectively representative of a distinct melitid species. All are highly divergent from the *Nedsia* haplotypes from Barrow Island, ranging from 15.6 – 17.3% sequence divergence.

Table 3.2: Results of DNA analysis of amphipod specimens, showing distribution of melitid lineages (species) from bores and study areas (n=number of individuals sequenced).

Melitid Lineage	Bore (location)	n
A	RR3A (Yarraloola)	2
	RR4A (Yarraloola)	1
B	RRCP1A (Yarraloola)	1
	Nr 5-Mi Well (Yarraloola)	1
	Sheryl bore (Yarraloola)	1
C	BUN0731 (Bungaroo)	2
	BUN0863 (Bungaroo)	1
D	RR2A (Yarraloola – CALM collection)	8
E	MW13-102 (Barrow Island)	2
	X62-111 (Barrow Island)	2
F	RR6A (Yarraloola)	1

The results of this initial analysis indicate several species-level taxa are represented in the amphipod fauna of the Yarraloola area. The mechanisms separating gene flow amongst these lineages are currently not clear.

The most divergent haplotype of the group was recorded from bore RR6A (lineage F). This lineage differs from the rest of the melitid lineages by 40.0 – 46.9%. While the other lineages are clearly melitids, the inclusion of other reference families and a closer morphological examination will be necessary to identify the family to which the haplotype in lineage F belongs. Morphological comparison of specimens also showed the individual collected from RR6A to have two enlarged gnathopods, distinct from other species collected (Appendix 2).

3.2.2 Copepoda

Copepod specimens were identified by fixing and slide-mounting, followed by identification by reference to available keys. Some individuals were injected with dye to enable accurate identification and enhanced definition for photographic purposes. Some specimens were dissected and identified to species level under high magnification (>x40).

A total of 179 copepod specimens were collected from Yaraloola during the study. Six copepod taxa were identified from amongst this material (see Table 3.3).

Table 3.3: Records of copepod taxa from the Yarraloola study area.

	Borehole *											
Taxon	RRCP 1A	RR 3A	RR 4A	RRCP 4	WW	nr FMW	SB	BF2-1	RR 4P	BHP Town	MB	AW
Paracyclops chiltoni						2						
Thermocyclops decipiens								10				
indet. copepodites: [3-segmented P1-4]						1		2				
Centropagidae cf. *Gladioferens* sp. (sp. nov.?)												1
indet. family/genus/species (gen. et sp. nov?)	23	39	30	32	2				7	14	1	
indet. calanoid copepodites	3	8					2				2	

* Borehole codes: RRCP 1A:Robe River Coastal Plain 1A; RR 3A: Robe River Coastal Plain 3A; RR 4A:Robe River 4A; RRCP 4:Robe River Coastal Plain 4; WW:Woolshed Well; nr FMW:Near Five Mile Well; SB:Sheryl Bore; RR 4P: Robe River 4P; MB: Macks Bore; AW:Ashley Well.

Three families were represented by the six taxa, as outlined in the following annotated list.

FAMILY EUCYCLOPINAE

Paracyclops chiltoni (Plate 3.1, Plate 3.2)
Recorded from a single site: near Five Mile Well at Yarraloola (Table 3.3). Also known from other records in the Pilbara region.

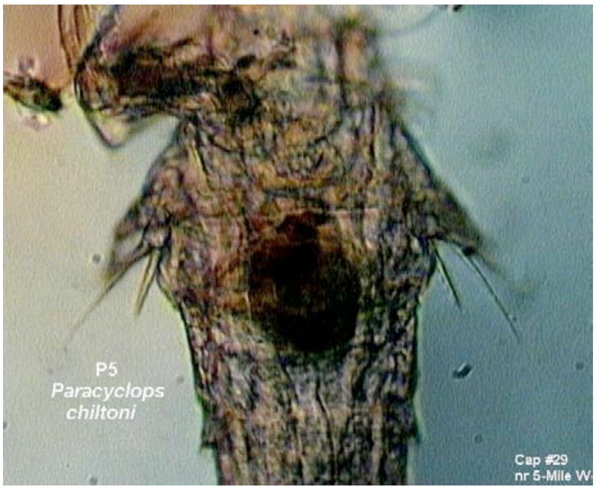

Plate 3.1: Maxilla of *Paracyclops chiltoni* Copepod from Nr Five Mile Well.

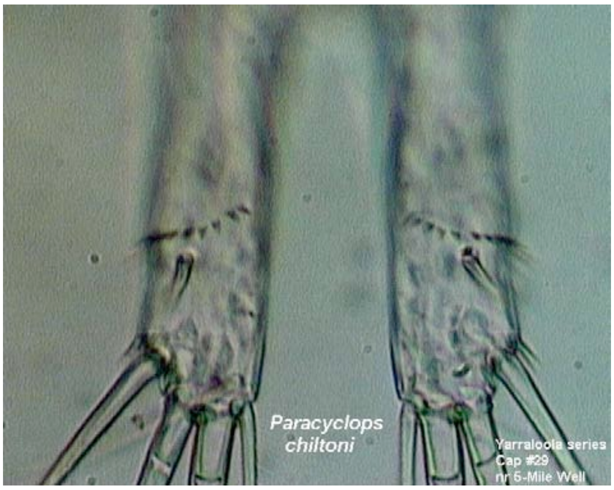

Plate 3.2: Caudal ramus of *Paracyclops chiltoni* Copepod from Nr Five Mile Well.

FAMILY CYCLOPINAE

Thermocyclops decipiens Kiefer, 1929
Recorded from a single site at Yarraloola: bore BF-2 (see Table 3.3). This species was also collected from Bungaroo Creek during the current study.

indet. copepodites: [3-segmented P1-4]
Immature, therefore only identifiable to family level. Recorded from two sites in the Bungaroo area; Near Five Mile Well and BF2-1 (Table 3.3). Cyclopoid copepodites were also recorded from two sites in the Bungaroo Creek area, sites: BUNMD887 and BUNW00769

FAMILY CALANOIDA

Centropagidae cf. *Gladioferens* sp. (sp. nov.?) (Plate 3.3)
Recorded from one site: Ashley well at Yarraloola (see Table 3.3).

indet. family/genus/species (gen. et sp. nov?) (Plate 3.4)
Previously undescribed species. Recorded from eight sites in the Yarraloola are (Table 3.3).

indet. calanoid copepodites
Previously undescribed species. Recorded from four sites in the Yarraloola area, Robe River Coastal Plain 1A, Robe River 3A, Sheryl Bore 13Y (see Plate 3.5) and Macks Bore.

Plate 3.3: Diagnostic features of Centropagidae cf. *Gladioferens* from Ashley Well.

Plate 3.4: Undescribed calanoid copepod collected from Robe River Coastal Plain 1A.

Plate 3.5: Calanoid copepod collected from Sheryl Bore.

Plate 3.6: The thermosbaenacean *Halosbaena tulki*.

The Yaraloola area appears to have an interesting and relatively abundant copepod fauna. This is likely to be associated with the alluvial aquifer of the Robe River (Commander 1994), which is intersected by many of the bores sampled.

3.2.3 Ostracoda

Ostracods were only collected from a single site at Yarraloola during this study: Robe River 4P (see Table 3.1). Thirteen specimens were collected, with the results of detailed identifications still pending at the time of preparing this draft.

3.2.4 Thermosbaenacea

Thermosbaenaceans were recorded from seven sites at Yarraloola during the current study; Robe River Coastal Plain 1A, Robe River 3A, Woolshed Well, nr Five Mile Well, BHP town Bore and Mack's Bore (see Table 3.1). Detailed identification confirmed that all specimens were the species *Halosbaena tulki* (Plate 3.6). In addition to these local records, this species is also more widely distributed in the Pilbara and on Barrow Island (Poore and Humphreys 1992, Humphreys 2002).

4.0 Conclusions and Potential Impacts

4.1 Warramboo

The available data suggest that there appears to be little in the way of a stygal community present at Warramboo. The bores sampled have been installed for a considerable period (see Section 2.4.1), so it therefore appears unlikely that any issue relating to the period elapsed since bore installation are affecting the result. No stygofauna were recorded from the Warramboo deposit or the area that would be affected by water abstraction (see Figure 4.1).

The only stygofauna recorded to date from this locality were oligochaetes from a single site at Todd Bore, which are typically widespread at the species level (see Section 3.1.1; Figure 4.1). The results to date therefore suggest that there is little impediment to sourcing process water from the bores sampled at Warramboo to meet project requirements. This finding will be confirmed by an additional sampling phase at Warramboo to be conducted in 2006, if this area is still considered to be a suitable water source for the Mesa A/Warramboo mine site.

4.2 Yarraloola

The main potential impact on stygofauna arising from the proposed development would be the effect on groundwater levels arising from the development of a borefield at Yarraloola. Figure 4.1 shows the predicted water table drawdown over the life expectancy of the project and the location of the sample sites included in this study. Sites where fauna have been recorded have been classified as either 'Impact' (inside water draw-down area) or 'Reference' (outside water draw-down area). For the purposes of this exercise, sites have been considered 'Impact' if they are predicted to experience a groundwater reduction between 0.7 m and 0.3 m (i.e. any sites beyond the 0.3 m drawdown contour are treated as 'Reference' locations. Adopting this approach, there were eight bores where stygofauna were recorded that would experience some level of influence from the borefield development (see Table 4.1).

Table 4.1: 'Impact' boreholes located within the area of influence of the proposed borefield at Yarraloola.

Borehole	Stygofauna Collected	Status
BHP Town Bore	Amphipod: Melitidae sp.	No sequence data obtained from this specimen
	Copepoda: indet. family/genus/species (gen. et sp. nov?)	Undescribed taxon; Also collected from 4 other Reference bores in the locality
	Themosbaenacea: *Halosbaena tulki*	Known from other sites in the bioregion
Nr Five Mile Well	Amphipod: Melitidae sp. B	Known from 'Reference' site RRCP1A
	Copepoda: *Paracyclops chiltoni*	Known from other sites in the bioregion
	Themosbaenacea: *Halosbaena tulki*	Known from other sites in the bioregion
Robe River 3A	Amphipod: Melitidae sp. A	Only known from borefield area to date
	Copepoda: indet. family/genus/species (gen. et sp. nov?)	Undescribed taxon; Also collected from 4 other Reference bores in the locality
	Themosbaenacea: *Halosbaena tulki*	Known from other sites in the bioregion
Robe River 4A	Amphipod: Melitidae sp. A	Only known from borefield area to date
	Copepoda: indet. family/genus/species (gen. et sp. nov?)	Undescribed taxon; Also collected from 4 other Reference bores in the locality
Robe River 4P	Amphipod: Melitidae sp. A	No sequence data obtained from this specimen
	Copepoda: indet. family/genus/species (gen. et sp. nov?)	Undescribed taxon; Also collected from 4 other Reference bores in the locality
	Ostracoda	Not identified to date
Robe River 6A	Amphipod: Melitidae sp. F	Divergent lineage; Only known from borefield area to date
Sheryl Bore 13Y	Amphipod: Melitidae sp. B	Also known from 'Reference' site RRCP1A
	Copepoda: Indet. calanoid copepodites	Also recorded from three other 'Reference' bores locally
Wongoo Bore	Amphipod: Melitidae sp.	No sequence data obtained from this specimen

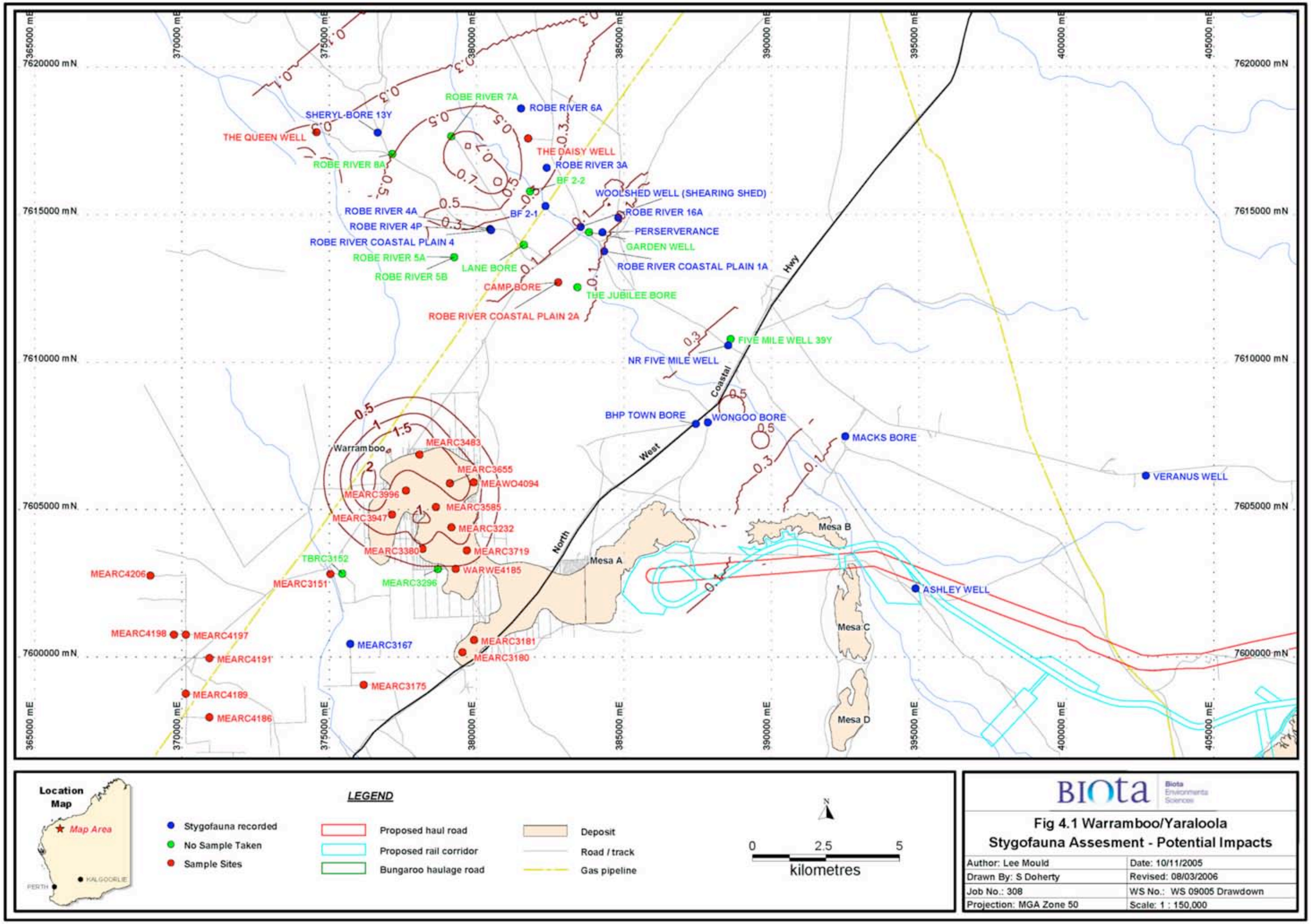

Figure 4.1: **Stygofauna sampling locations and predicted drawdown for the development of the Yarraloola borefield.**

This analysis suggests that there are few taxa that are currently only known from the area of potential influence of a borefield development at Yarraloola (see Table 4.1). These are:

- the amphipod Melitidae sp. A (currently only known from bores Robe River 3A and 4A) – predicted to be drawndown by 0.5 m and 0.3 m respectively;
- the amphipod Melitidae sp. F (currently only known from bores Robe River 6A) – predicted to be drawndown by 0.5 m; and
- the currently unidentified ostracods from bore Robe River 4P – predicted to be drawndown by 0.5 m.

It should be noted however that, while these collection sites have been treated as 'Impact' for the purposes of this report, there is actually a very low probability that the predicted level of change would have any effect on stygofauna in the Robe alluvium. A drawdown of less than 1 m is well within annual and long-term groundwater level variation (Commander 1994). The distribution of some of the better collected and identified species, such as *Halosbaena tulki* (see Table 4.1), suggest that the less well collected taxa may also be more widespread. Therefore there would appear to be a low risk of the conservation status of any of these apparently localised taxa being altered by the proposed development.

The results to date do, however, indicate that the western Robe River contains an interesting stygal fauna. Additional sampling will be carried out at Yarraloola as part of Pilbara Iron's ongoing regional stygofauna programme, in the event that the Yarraloola borefield option is selected for project water supply requirements. This will include further work to investigate the genetic structure of local amphipod populations and the description of the new calanoid copepod occurring along this system.

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Appendix 1

Overview of Subterranean Fauna Systematics and Ecology

Overview of Subterranean Ecosystems

Subterranean fauna have been recorded from Western Australia since the 1940s. Stygofauna is a general term used to describe the subterranean fauna occurring in the groundwater of a given area (Humphreys 2000). True subterranean fauna may be divided into two main categories (Humphreys 2000):

- Stygobites: obligate groundwater-dwelling, aquatic fauna; and
- Troglobites: obligate cave or karst-dwelling, terrestrial subterranean fauna occurring above the watertable.

The term stygofauna is used in this report in reference to stygobites. An overview of the typical ecological characteristics of subterranean fauna and their environment is provided in Table 1. Groundwater food webs studied in other countries are typically almost entirely heterotrophic, with bioproduction primarily dependent on the transport of resources (biomass, detritus) from the surface (allotrophy; Gibert et al. 1994). There are few primary producers (e.g. chemolithotrophic bacteria; Danielopol et al. 1994). Groundwater microbes (i.e. bacteria, fungi and protozoans) are the primary consumers, with short direct trophic links to most meiofauna in the system.

Stygofauna are those fauna that inhabit groundwater, sometimes occurring very close to the surface. They tend to be highly specialised to, and obligate dwellers of, subterranean groundwater habitats ('stygobites'; Humphreys 2000). Stygofauna are known to be present in a variety of rock types including karst (limestones), fissured rock (e.g. granite) and porous rock (e.g. alluvium) (Marmonier et al. 1993). The types of animals that have become stygal (groundwater-inhabiting) in Western Australia include platyhelminthes, oligochaetes, crustaceans, water mites and water beetles (e.g. Humphreys 1999; Watts and Humphreys 1999, 2000; Biota unpublished data). Much attention has been directed to the crustacean fauna, which are usually the most abundant and widespread fauna and include ostracods, copepods, remipedes, bathynellid syncarids, spelaeogriphaceans, thermosbaenaceans, isopods and amphipods. A systematic resume of these groups is presented in Table 2.

Table 1: Characteristics of subterranean ecosystems and their components (adapted from Gibert et al. 1994).

Environment	Constant darkness
	Physical inertia which increases with depth
	Predictability: hydrologic and chemical variation usually low
	Restricted variety of habitats: lack of vegetation, reduction of space
	Habitat heterogeneity results from arrangement of grains, void size, physical and chemical characteristics of aquifers within the pore space
Organisms	Obligate groundwater dwellers (stygofauna) or obligate cave or karst dwelling terrestrial subterranean fauna (troglofauna)
	Morphological, physiological and behavioural specialisations to subterranean environment:
	general lack of pigmentation
	ocular regression and lengthened appendages
	highly developed chemical and mechanical receptors
	convergence of vermiform body shape for different taxa
Biocenosis	Dominance of one species
	Richness, diversity and density low and variable
	A-type strategy of Greenslade (1983):
	slower metabolic rates and growth, reduced motor output
	lengthening of lifecycle stages, late maturity, greater longevity
	less frequent reproduction, lower fecundity
	unique behaviour such as stereotropism, thigmotropism, thigmotactism
Functional Characteristics	Heterotrophy and allotrophy
	Short, simple food webs with few trophic links
	Detritus feeders dominant
	System with low productivity
	Invertebrate diets not specialised, polyphagous

Table 2: Taxonomic summary of stygofauna recorded from Western Australia (sources: Humphreys 1999; Watts and Humphreys 1999; Biota unpublished data).

Phylum	Class	Order	Common name
Platyhelminthes	Turbellaria	-	Flatworms
Annelida	Oligochaeta	Tubificina	Oligochaetes
Chelicerata	Arachnida	Acarina	Water mites
Crustacea	Ostracoda	Cypridacea	Seed shrimp, ostracods
	Copepoda	Cyclopidea	Copepods
	Remipedia	Nectiopoda	Remipedes
	Malacostraca (Syncarida)	Bathynellacea	Bathynellaceans
	Malacostraca (Peracarida)	Spelaeogriphacea	Spelaeogriphaceans
		Thermosbaenacea	Thermosbaenacean s
		Decapoda	Shrimp
		Isopoda	Isopods, slaters
		Amphipoda	Amphipods, hoppers
Uniramia	Insecta	Coleoptera	Water beetles

The distribution of subterranean fauna often appears to be more restricted than that of surface fauna analogues. Higher levels of endemicity have been found to be characteristic of subterranean taxa, and endemic species tend to be concentrated in habitats that support relatively diverse communities, rather than being distributed randomly (see review in Strayer 1994). Some taxa do have large ranges (e.g. the polychaete worm *Troglochaetus* sp. which has been recorded across central Europe and parts of North America; Strayer 1994) but appear to be the exception. Also, whilst genera can be widespread, a species within a particular genus is more likely to have a spatially restricted distribution.

The high levels of endemism that this fauna can exhibit may be due in part to poor dispersal capabilities. The dispersal of fauna inhabiting groundwater may be extremely slow and may be limited by the geological formation in which they occur. Relative transmissivity and the nature of groundwater flows and energy inputs differ between aquifers and are likely to influence the occurrence and distribution of stygofauna (Marmonier et al. 1993; Gibert et al. 1994). Many species have not been able to disperse a significant distance from their place of origin (e.g. Strayer 1994). Physical variables such as dissolved oxygen have also been shown to control the distribution of some subterranean species on a local and microhabitat scale (e.g. some isopods; Danielopol et al. 1994). Furthermore, distribution patterns and evolutionary processes can be closely linked, for example amphipods such as freshwater Crangonyctoidea and the mostly marine Hadzioidea. Consequently, distribution patterns can be a useful indicator of evolutionary processes in these groups (Holsinger 1994).

Subterranean fauna in Western Australia are regarded as relicts, descendents from ancient pre-Gondwanan lineages. Some stygofauna species such as those inhabiting Cape Range represent relict lineages that are closely related to fauna of Gondwana, the ancient Tethys Sea and epigean ancestors that occurred prior to the break-up of Pangaea (see review in Humphreys 1999). Limited information is available in the published literature on stygofauna in the inland areas of the Pilbara, although work is in progress (Finston and Johnson, in review; CALM regional survey).

Subterranean fauna has become a key issue for several new developments in Western Australia as recent surveys and research have suggested that relatively localised impacts such as mining have the potential to significantly change the conservation status of locally endemic species (Eberhard and Humphreys 1999; Biota Environmental Sciences 2001). It is unclear at present whether the occurrence of stygofauna as documented by recent surveys in Western Australia reflect the true distribution of the fauna, and hence potential impact, or whether this is more a function of the current limitations on sampling and understanding of stygal systems

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Appendix 2

Outcomes of Amphipod Genetic Analyses

Summary report for the Robe River region:

Genetic variation in stygobitic amphipods

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Introduction

Individuals of the same species can differ in their physical appearance due to differences in the environments in which they live. Because genetic variation is generally free from the influence of the environment, it can be used to identify distinct species when differences in morphology may or may not be important.

DNA sequencing of mitochondrial genes has become an increasingly popular tool in evolutionary and systematic studies. Each base pair in the sequence represents a character, providing hundreds or thousands of characters in which to analyse differences between individuals. A further advantage is that DNA can be extracted from minute or poorly-preserved specimens, and amplified in quantities large enough for analysis. This represents a real advantage over protein electrophoresis, where the small size of individuals limits the amount of information that can be gathered. Lastly, it eliminates the need for side-by-side comparisons of specimens. This is especially critical when samples are limited.

mtDNA sequence analysis was conducted on samples of amphipods from 13 bores in the Robe River/Pannawonica area to determine the number of genetic groups present. Genetic groups can then be examined morphologically, to confirm their status as distinct species, and to begin the process of species descriptions.

Methods

To expedite the process of obtaining the best first-look estimate of biodiversity in the area, 1-2 individuals were selected from each of 13 bores for analysis. DNA was extracted from ethanol-preserved specimens using a proteinase K protocol (Schwenk, 1996; See Table 1 for samples used in the analysis). Because preliminary morphological identifications indicated the majority of specimens belonged to the amphipod family Melitidae, specially designed primers were used to sequence the target region of the COI gene using standard PCR protocols. Samples were sequenced in both the forward and reverse directions. Sequences were edited and aligned using Genedoc (Nicholas and Nicholas 1997). Genetic distances were calculated between samples using Kimura's (1980) two-parameter distance model in PHYLIP (Felsenstein, 1995). In order to identify distinct lineages, a phylogenetic tree was constructed in PHYLIP on the sequence data, using neighbour joining (NJ). Support for the branches was obtained using 100 replicate bootstraps. Specimens of *Nedsia* from the type locality on Barrow Island were included as reference sequences. In addition, sequences obtained from the South Australian Museum from individuals collected by CALM from bore RR2A were also included.

Results

Some problems were encountered while trying to sequence particular specimens. However, sequences were obtained from specimens from all but five bores: RRCP4-1, RR16A, Wongoo bore, Veranus Well, and BUNWO0766.

Based on a 476 base-pair fragment of the CO1 gene, there were 11 distinct haplotypes among the 18 individuals for which sequences were obtained. These haplotypes fall into five distinct groups (lineages A – D, and F; Table 1; Fig. 1). Four of the five lineages (A, B, D, and F) are found at Yarraloola. The fifth, lineage C, is found at Bungaroo. Within each lineage, sequence divergence among haplotypes is low, ranging from 0 to 0.4 %. Sequence divergence among lineages is more than 10 times higher, ranging from 4.8% between lineages A and C, to 13.3 % between lineages B and D. All are highly divergent from the *Nedsia* haplotypes from Barrow Island, ranging from 15.6 – 17.3 % sequence divergence. The most divergent haplotype of the group came from bore RR6a (lineage F). This lineage differs from the rest of the melitid lineages by 40.0 – 46.9%. While the other lineages are clearly melitids, the inclusion of other reference families and a closer morphological examination will be necessary to identify the family to which the haplotype in lineage F belongs

Discussion

The Robe River region possesses haplotypes belonging to five distinct genetic groups. Much debate has arisen over which genes provide the best taxonomic information, and the amount of sequence divergence that corresponds to distinct species (see Moritz and Cicero 2004 for a summary). The CO1 gene has been identified as having great potential to aid in species identification, due to its rapid evolutionary rate, and consistent levels of divergence among known species. Based on extensive studies across many major taxonomic groups, a 'yard-stick' has been proposed that provides a measure of the amount of sequence divergence indicative of species (Hebert *et al*., 2004). This yard-stick defines individuals or haplotypes as "very likely to belong to different species" when divergence among lineages is 10x greater than that within lineages. Using that criterion, each of the five distinct lineages in the Robe River specimens would qualify as separate species. Indeed, based on a 2% per million years rate of sequence divergence (Knowlton *et al*., 1993; Knowlton and Weigt 1998; Shubart *et al*., 1998) these lineages have been isolated from one another for a minimum of approximately 2.4 my between lineages A and C, to 6.65 my between lineages B and D. Particularly compelling evidence is the occurrence of four distinct lineages at a single site, Yarraloola. It would be difficult to explain how individuals with the potential for contact could maintain such divergent haplotypes if they were interbreeding. Such biodiversity over short distances has been observed in regions where the underlying geology is complex (Finston and Johnson, 2004). Discrete aquifers contained in differing geological formations with little opportunity for contact can lead to divergence among populations that are geographically close. Biodiversity may also be elevated due to the hydrological history of the area. The Robe has been, at times, captured by both the Fortescue and the Ashburton Rivers (Bill Humphreys, pers. comm.).

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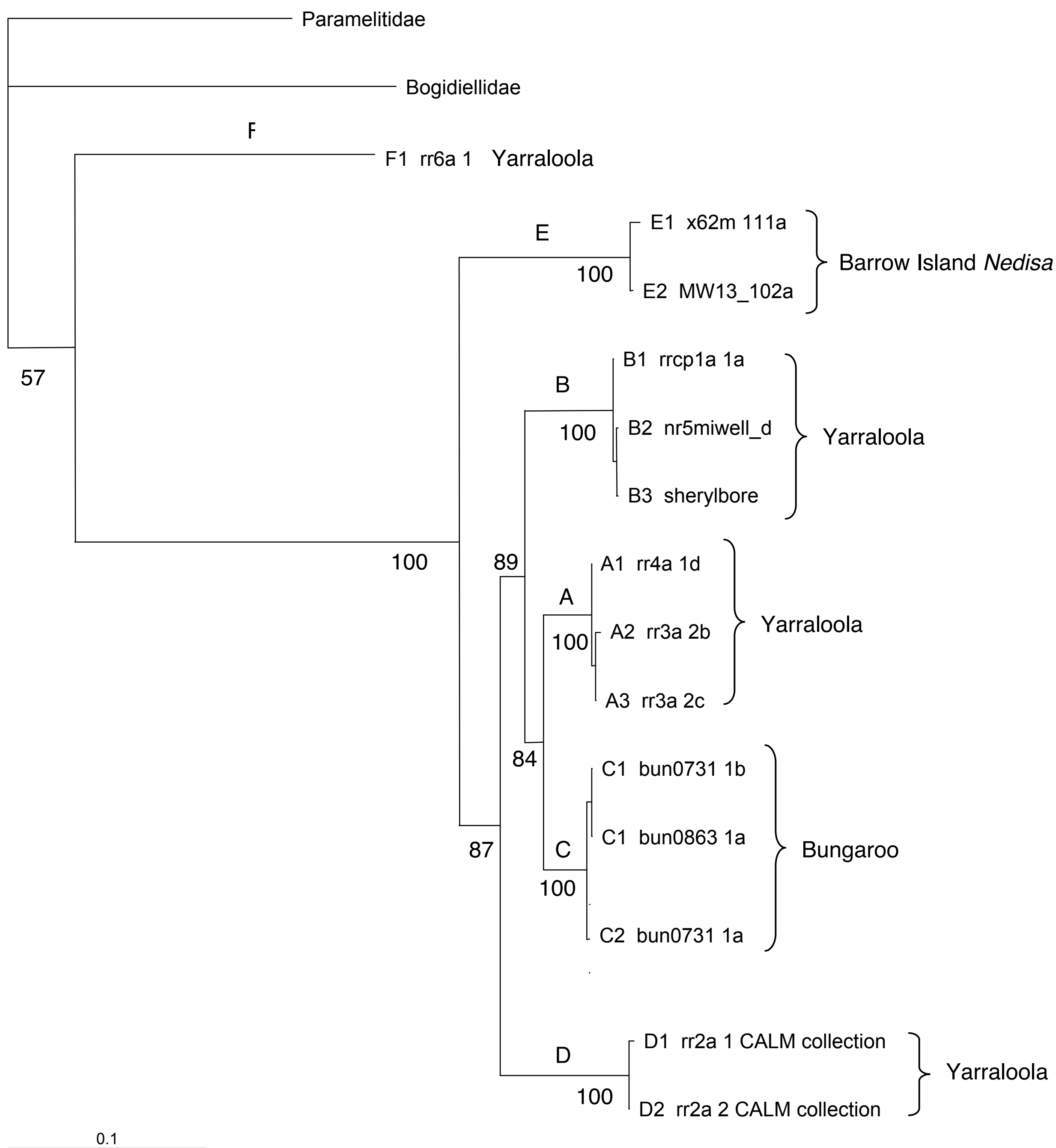
Table 1. Specimens used in mtDNA analysis.

Bore (location)	No. specimens extracted	No. specimens successfully sequenced	lineage	haplotype
RR3A (Yarraloola)	2	2	A	A2, A3
RR4A (Yarraloola)	2	1	A	A1
RR6A (Yarraloola)	1	1	F	F1
RR16A (Yarraloola)	2	0	-	
RRCP1A (Yarraloola)	1	1	B	B1
RRCP4 (Yarraloola)	2	0	-	
Nr 5-Mi Well (Yarraloola)	2	1	B	B2
Sheryl bore (Yarraloola)	1	1	B	B3
Wongoo bore (Yarraloola)	1	0	-	
Veranus Well (Yarraloola)	1	0	-	
BUN0731 (Bungaroo)	2	2	C	C1, C2
BUNWO0766 (Bungaroo)	1	0	-	
BUN0863 (Bungaroo)	1	1	C	C1
RR2A (Yarraloola – CALM collection)	9	8	D	D1, D2
MW13-102 (Barrow Island)	2	2	E	E2
X62-111 (Barrow Island)	2	2	E	E1

Table 2. Distance matrix for haplotypes from Robe River.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. rr2a_1	*													
2. rr2a_2	0.002	*												
3. bun0731_1b	0.111	0.108	*											
4. rr3a_2b	0.123	0.120	0.052	*										
5. rr3a_2c	0.120	0.118	0.050	0.002	*									
6. bun0731_1a	0.111	0.108	0.004	0.052	0.050	*								
7. rrcp1a_1a	0.128	0.126	0.082	0.080	0.077	0.077	*							
8. nr5miwelld	0.131	0.128	0.084	0.082	0.080	0.080	0.002	*						
9. rr4a_1d	0.118	0.115	0.048	0.004	0.002	0.048	0.075	0.077	*					
10. bun0863_1	0.111	0.108	0.004	0.052	0.050	0.000	0.077	0.080	0.048	*				
11. sherylbore	0.133	0.131	0.082	0.080	0.077	0.082	0.004	0.002	0.075	0.082	*			
12. rr6a_1	0.469	0.465	0.406	0.419	0.415	0.399	0.407	0.411	0.411	0.399	0.415	*		
13. x62m_111a	0.163	0.165	0.166	0.166	0.164	0.161	0.168	0.170	0.161	0.161	0.173	0.448	*	
14. mw13_102	0.163	0.165	0.161	0.161	0.158	0.156	0.162	0.165	0.156	0.156	0.168	0.448	0.006	*


Paramelitidae
Bogidiellidae
F
F1 rr6a 1 Yarraloola
E
E1 x62m 111a
E2 MW13_102a
100
Barrow Island *Nedisa*
57
B
B1 rrcp1a 1a
B2 nr5miwell_d
B3 sherylbore
100
Yarraloola
100
89
A
A1 rr4a 1d
A2 rr3a 2b
A3 rr3a 2c
100
Yarraloola
84
C1 bun0731 1b
C1 bun0863 1a
C2 bun0731 1a
C
100
Bungaroo
87
D
D1 rr2a 1 CALM collection
D2 rr2a 2 CALM collection
100
Yarraloola
0.1