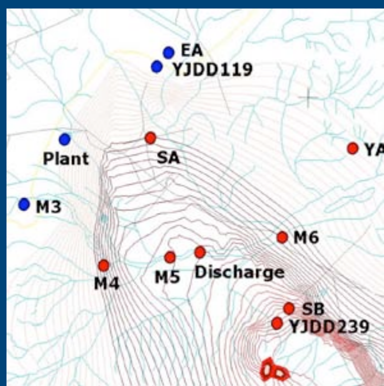
Biota Environmenta Sciences

Yandi Junction South East (JSE) Expansion

Baseline Stygofauna Assessment



Prepared for

Hamersley Iron Pty Ltd

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Yandi JSE Expansion Stygofauna Assessment

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1.0 Introduction

1.1 Project Background

Hamersley Iron Pty Ltd proposes to expand existing iron ore mining operations at its Yandi mine site, approximately 90 km north-west of Newman, in the Pilbara region of Western Australia. The expansion area is situated near the confluence of three major creek systems in the area; Marillana, Yandicoogina and Weeli Wolli (the Yandi Junction South East (JSE) Expansion). The planned expansion will include a new open-cut pit, waste dump and processing plant. The Western Australian Environmental Protection Authority (EPA) will review the environmental acceptability of this expansion under an Environmental Protection Statement (EPS) currently being prepared by Hamersley Iron.

1.2 Scope of this Study

As part of the assessment of potential biodiversity impacts of the project, Biota Environmental Sciences was commissioned to undertake an assessment of stygofauna occurring in the Yandi JSE area. Field stygofauna collections had already been completed in the Yandi area as part of Hamersley Iron's ongoing stygofauna research programme during November 2003 and earlier work completed for Hope Downs Management Services (Biota in prep.). These exercises had yielded good specimen sample sizes, representatives of most taxonomic groups and a spatial spread of sampling points across the proposed expansion area and surrounds. Given this, no additional field collecting was completed during 2004 and this report has been based on analysis of the existing specimens.

The scope of this study addresses the potential impacts on stygofauna arising from the proposal to dewater and mine a channel iron deposit between Marillana and Weeli Wolli Creeks (the Yandi JSE Expansion). This has been assessed in the context of current and future groundwater influences of existing mining operations at Yandi, with modelling producing drawdown contours that take account of these approved operations rather than being incremental (see Section 2.3).

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.

2.0 Methods

2.1 Stygofauna Survey and Analysis Team

Field stygofauna sampling was undertaken by Mr Kyle Armstrong and Mr Phil Runham of Biota Environmental Sciences. Sorting of stygofauna samples was completed by Kyle Armstrong and Mr Phil Runham, with further specimen identification conducted by Mr Garth Humphreys and Ms Jane McRae. DNA analyses of amphipod specimens were undertaken by Dr Terrie Finston, a post-doctoral researcher at the School of Animal Sciences at UWA. Cara Francis, PhD candidate at the UWA School of Animal Sciences, completed the preliminary DNA sequencing of isopod specimens presented here.

2.2 Stygofauna Sampling and Data Management

Stygofauna sampling 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).

Stygofauna were sampled from water bores, by means of specially modified plankton haul nets. Sampling nets were constructed from 150 μm plankton mesh, with a c. 100mm aperture 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 plastic bag 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 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 to facilitate genetic analysis at the School of Animal Sciences at UWA. Larger and more intact specimens, which were more likely to be identifiable by morphological features, were stored in 100% ethanol.

Site, sampling methods, borehole descriptions and specimen data were recorded on customised datasheets for later entry into a dedicated Microsoft Access database.

2.3 Sampling Design and Effort

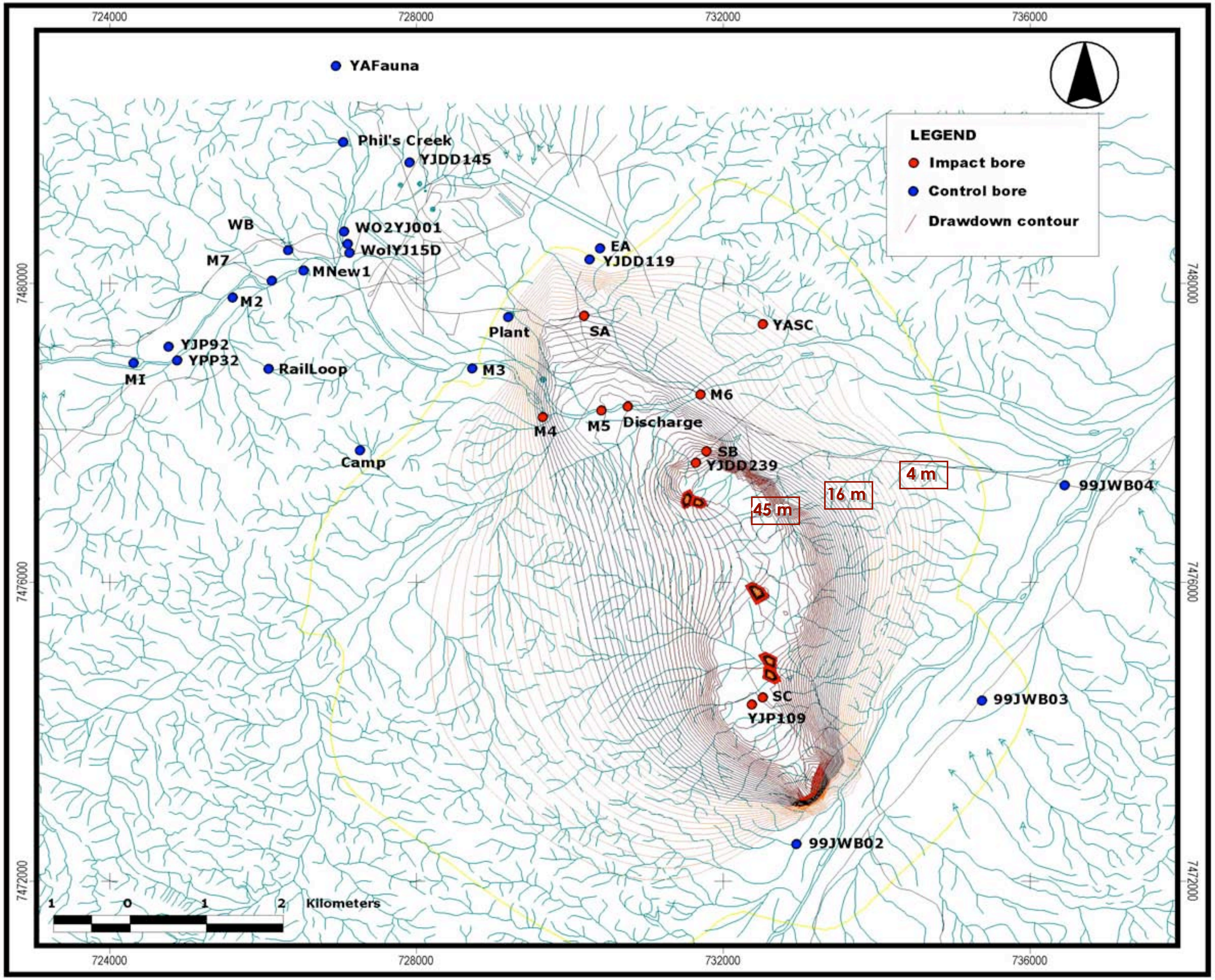
Thirty-eight boreholes situated in the vicinity of the proposed Yandi expansion area were selected for sampling between 3rd and 5th November 2003. This included 10 bores within the proposed expansion 'Impact' area and 28 bores in areas outside of the impact area for this proposal ('Reference' locations) (see Table 2.1). The impact area associated with the Yandi JSE project was identified by superimposing groundwater drawdown contours arising from hydrogeological modelling completed by Hamersley Iron. Note that these contours take into account the drawdown from existing approved operations and the modelled impacts are not incremental.

The location and spatial spread of these bores relative to the proposed development area is shown in Figure 2.1.

Table 2.1: Boreholes selected for sampling in the Yandi expansion area (Status = 'Impact' (inside the identified disturbance area) or 'Reference' outside; Easting and Northings given in UTMs WGS84 datum; **Impact** sites highlighted in bold).

Borehole	Easting	Northing	Status
99YJWB1	-	-	Reference
99YJWB2	-	-	Reference
99YJWB3	-	-	Reference
99YJWB4	-	-	Reference
Airport (EA)	729874	7480651	Reference
Camp (YMCC)	726744	7477949	Reference
Discharge (YMCD)	**730236**	**7478532**	**Impact**
Fauna	726431	7483093	Reference
Marillana 1 (M1)	723792	7479113	Reference
Marillana 2 (M2)	725082	7479990	Reference
Marillana 3 (M3)	728209	7479045	Reference
Marillana 4 (M4)	**729126**	**7478392**	**Impact**
Marillana 5 (M5)	**729890**	**7478482**	**Impact**
Marillana 6 (M6)	**731182**	**7478693**	**Impact**
Marillana 7 (M7)	725592	7480218	Reference
Phil's Creek	726523	7482071	Reference
Plant	728677	7479732	Reference
Rail Loop	725552	7479036	Reference
SA	**729664**	**7479749**	**Impact**
YASC	**731995**	**7479638**	**Impact**
SB	**731263**	**7477933**	**Impact**
SC	**731995**	**7474638**	**Impact**
Mnew1 (Upstream)	726009	7480355	Reference
W01YJ15D	726605	7480589	Reference
W02YJ001	726535	7480876	Reference
WB	725805	7480628	Reference
Weeli Wolli	-	-	Reference
YJDD239	**731122**	**7477778**	**Impact**
YJDD119	729735	7480499	Reference
YJDD145	727392	7481798	Reference
YJDD64	726586	7480707	Reference
YJP92	724247	7479332	Reference
YJP109	**731855**	**7474545**	**Impact**
YJDD262	-	-	Reference
YPP32	724363	7479150	Reference
YPP33	724363	7479150	Reference
YPP07	-	-	Reference
YPP08	-	-	Reference

Figure 2.1: Location of boreholes selected for stygofauna sampling in the vicinity of the proposed Yandi expansion (Impact bores = red; Reference bores = blue; Groundwater drawdown contours shown in dark red from 16-45 m into the central pit area, grading from to 4-16 m in from the outer cone of depression).



3.0 Results

3.1 Sampling Results

Fifteen of the boreholes visited as part of this study could not be sampled for a variety of reasons as outlined in Table 3.1. However, collections were obtained from the remaining 23 boreholes which were able to be sampled, and a good spatial spread of collection points across the project area was achieved. Seventeen of these final 23 bores contained stygofauna (five Impact bores and 12 Reference bores; Table 3.1). The stygofauna collected as identified in the field are listed in Table 3.1.

Table 3.1: Boreholes sampled and stygofauna collected from the Yandi area in November 2003 (Impact sites in bold).

Borehole	Easting	Northing	Results and other comments
99YJWB1	-	-	Sampled as part of the Hope Downs - Mining Area C stygofauna assessment (Biota 2004, Biota in prep). Pumped immediately prior to site visit and therefore not re-sampled
99YJWB2	-	-	
99YJWB3	-	-	
99YJWB4	-	-	
Airport (EA)	729874	7480651	Amphipoda (19 frozen).
Camp (YMCC)	726744	7477949	Amphipoda (58 frozen).
Discharge (YMCD)	730236	7478532	Amphipoda (7 frozen),
			Isopoda (13 frozen, 35 in 100% ethanol),
			Ostracoda (7 in 100% ethanol),
			Platyhelminthes (5 in 100% ethanol).
YAFauna	726431	7483093	Dry
Marillana 1 (M1)	723792	7479113	Amphipoda (33 frozen),
			Ostracoda (38 in 100% ethanol),
			Platyhelminthes (5 in 100% ethanol).
Marillana 2 (M2)	725082	7479990	Amphipoda (46 frozen, 19 100% ethanol),
			Ostracoda (47 in 100% ethanol),
			Platyhelminthes (3 in 100% ethanol).
Marillana 3 (M3)	728209	7479045	Amphipoda (23 frozen),
			Copepoda (observed but not collected),
			Isopoda (25 frozen, 26 in 100% ethanol).
Marillana 4 (M4)	729126	7478392	Pumped immediately prior to site visit and therefore not sampled.
Marillana 5 (M5)	729890	7478482	Amphipoda (23 frozen),
			Isopoda (11 in 100% ethanol),
			Oligochaeta (12 in 100% ethanol),
			Ostracoda (20 in 100% ethanol).
Marillana 6 (M6)	731182	7478693	Amphipoda (9 frozen),
			Copepoda (1 in 100% ethanol),
			Hydracarina (3 in 100% ethanol),
			Ostracoda (2 in 100% ethanol),
			Platyhelminthes (17 in 100% ethanol).
Marillana 7 (M7)	725592	7480218	Amphipoda (52 frozen, 29 100% ethanol),
			Copepoda (10 in 100% ethanol),
			Hydracarina (1 in 100% ethanol),
			Isopoda (86 in 100% ethanol),
			Ostracoda (11 in 100% ethanol),
			Oligochaeta (8 in 100% ethanol),
			Platyhelminthes (71 in 100% ethanol).
Phil's Creek	726523	7482071	Dry
Plant	728677	7479732	Amphipoda (11 frozen, 1 100% ethanol),
			Hydracarina (1 in 100% ethanol),
			Isopoda (4 in 100% ethanol),
			Oligochaeta (13 in 100% ethanol),
			Ostracoda (2 in 100% ethanol).
Rail Loop	725552	7479036	Destroyed by grader
SA	729664	7479749	Borehole YJDD106?
			Amphipoda (1 frozen).

Table 3.1: Boreholes sampled and stygofauna collected from the Yandi area in November 2003 (Impact sites in bold).

Borehole	Easting	Northing	Results and other comments
YASC	731995	7479638	Nil
SB	731263	7477933	Copepoda (19 in 100% ethanol),
			Isopoda (10 frozen, 6 in 100% ethanol)
SC	731995	7474638	Amphipoda (19 frozen).
Mnew1 (Upstream)	726009	7480355	Amphipoda (44 frozen, 36 in 100% ethanol),
			Copepoda (2 in 100% ethanol),
			Hydracarina (1 in 100% ethanol),
			Isopoda (20 in 100% ethanol),
			Ostracoda (34 in 100% ethanol),
			Platyhelminthes (24 in 100% ethanol)
W01YJ15D	726605	7480589	Platyhelminthes (1 in 100% ethanol)
W02YJ001	726535	7480876	Amphipoda (1 in 100% ethanol),
			Ostracoda (5 in 100% ethanol),
			Oligochaeta (10 in 100% ethanol)
WB	725805	7480628	Borehole YJP99?
			Amphipoda (2 frozen, 4 in 100% ethanol)
Weeli Wolli	-	-	Destroyed
YJDD239	731122	7477778	Could not locate
YJDD119	729735	7480499	
YJDD145	727392	7481798	
YJDD64	726586	7480707	
YJP92	724247	7479332	
YJP109	731855	7474545	
YJDD262	-	-	Mined out
YPP32	724363	7479150	Pump headworks attached and therefore not sampled
YPP33	724363	7479150	
YPP07	-	-	
YPP08	-	-	

3.2 Morphological Identifications

More detailed morphology-based identification of the collected specimens was completed on return to Perth (see Section 2.1). Slide-mounting of specimens, reference to published and unpublished keys and comparison with other material collected from the region were all used as part of this process. Some of the bores sampled only yielded a low number of specimens and/or material that was damaged or very small. Given the low probability of identification based on morphology, these specimens were frozen in liquid nitrogen for genetic analysis. This reduced the number of sites that intact specimens were collected from for morphological work to 13 (four Impact and nine Reference: Discharge, M1-3, M5-7, Plant, SB, Mnew1, W01YJ15D, W02YJ001, WB; see Table 3.1).

A summary of identifications completed to date is provided for major taxonomic groups in the following annotated list. Some material has been forwarded to specialist taxonomists for more completed diagnosis and to contribute to ongoing descriptive work.

CLASS TURBELLARIA

Stygal flatworms (Platyhelminthes:Turbellaria), were collected from six bores during the study (four Reference and two Impact sites; Table 3.1). The turbellarian specimens, whilst all superficially similar, do not resemble those collected from elsewhere in the region. These have been forward to a specialist on this group for more detailed review. It is unlikely that species level identification of this material will be available in the near future.

CLASS OLIGOCHAETA

Oligochaete worms were collected from three Reference bores (W02YJ001, M7 and Plant) and one Impact bores (M5; Table 3.1). All specimens collected belonged to the family Tubificidae and resembled the genus *Ainudrilus* (Adrian Pinder, pers. comm.).

Unfortunately all specimens were immature and adult specimens are required for confirmed species identifications.

ORDER ACARINA

Hydracarina (water mites) were collected from three Reference bores and one Impact bore during the field survey (Table 3.1). Identification work is incomplete on this group at present, awaiting input from Dr Mark Harvey of the WA Museum. Identification work to date however, suggests that there are at least two taxa amongst the specimens. Most have been assigned to the genus *Recifella*, whilst one specimen belonging to the genus *Arrenurus* is clearly a previously uncollected species (see Plate 3.1; Table 3.2).

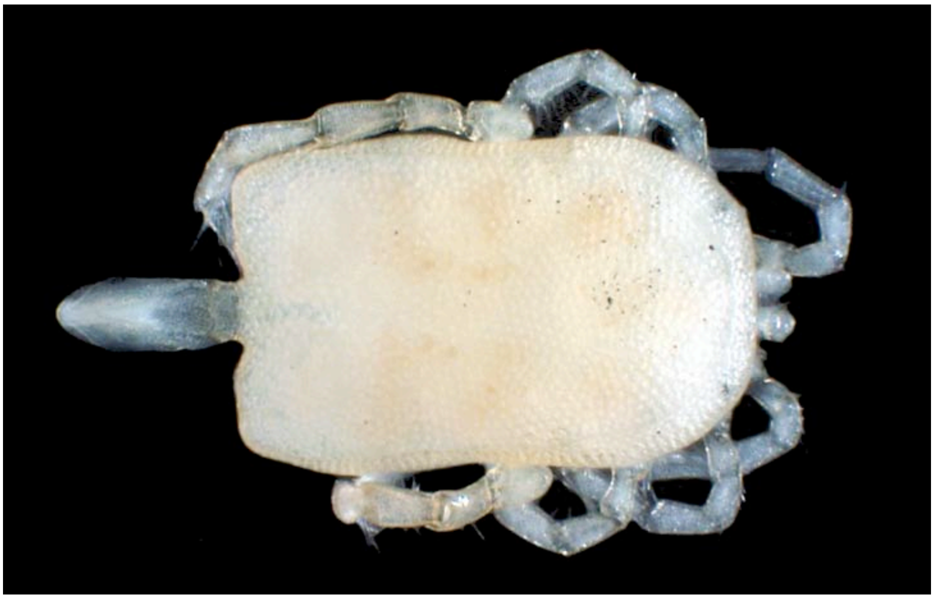


Plate 3.1: Male *Arrenurus* hydracarine specimen collected from bore Mnew1 (Photo: J. McRae).

Table 3.2: Distribution of hydracarine specimens across the Yandi expansion project area (sample sizes given as 'n=' in brackets for each collection location).

Species	Locations	Status	Comments
Recifella sp.	M6 (n=3)	Impact	Species level identifications yet to be completed
Arrenurus sp. nov.	Mnew1 (n=1)	Reference	Male specimen of a previously uncollected taxon, elongate proboscis
Hydracarina indet.	Plant (n=1) M7 (n=1)	Reference Reference	Species level identifications yet to be completed

CLASS OSTRACODA

Ostracods were collected from nine of the sixteen bores that yielded stygofauna (three Impact and six Reference; Table 3.1). All ostracods collected belonged to the family Candonidae, with most specimens assigned to two genera; *Pilbaracandona* and *Meridiescandona*. Full descriptive work on these two genera is still being completed by Dr Ivana Karanovic. Whilst the *Pilbaracandona* sp. and *Meridiescandona* sp. specimens do not belong to any named taxa, they correspond to species currently in the process of being described from material collected from the wider region.

Twelve specimens were also collected from bore M5 in the Impact area that belong to the genus *Gomphodella*. These appear to be a previously uncollected taxon, not corresponding to any of the species being described by Dr. Karanovic. A selection of the specimens has been forwarded to her for further review. The distribution and abundance of the Ostracoda collected from the project area is provided in Table 3.3.

Table 3.3: Distribution of ostracod specimens across the Yandi expansion project area (sample sizes given as 'n=' in brackets for each collection location).

Species	Locations	Status	Comments
Pilbaracandona sp.	Discharge (n=2) M7 (n=7) Mnew1 (n=2)	Impact Reference Reference	Specimens correspond to a species currently being described. Identifications to be confirmed.
Meridiescandona sp.	Discharge (n=2) M5 (n=4) M6 (n=2) M1 (n=38) M2 (n=47) M7 (n=2) Plant (n=1) W02YJ001 (n=5) Mnew1 (n=33)	Impact Impact Impact Reference Reference Reference Reference Reference Reference	Specimens correspond to a species currently being described. Identifications to be confirmed.
Gomphodella sp.	M5 (n=12)	Impact	Specimens belong to a species not represented amongst the taxa currently being described by Dr Ivana Karanovic

CLASS COPEPODA

Copepods were collected from only four of the bores sampled during the study (two Impact and two Reference; Table 3.1), with all specimens belonging to the order Cyclopidea. Abundances were generally low, with many juvenile specimens, which limited species level identifications. Two species were, however, confirmed from the material; *Diacyclops cockingi* (bore M7; n=4) and *Mesocyclops darwini* (bore M6; n=1) (see Table 3.4). The remaining specimens were all determined to the genus *Diacyclops*, but most specimens were juveniles and could not be identified to species (see Table 3.4).

Table 3.4: Distribution of copepod specimens across the Yandi expansion project area (sample sizes given as 'n=' in brackets for each collection location).

Species	Locations	Status	Comments
Diacyclops cockingi	M7 (n=4)	Reference	This species has been described from collections elsewhere in the region
Mesocyclops darwini	M6 (n=1)	Impact	This species has been described from collections elsewhere in the region
Diacyclops sp. indet.	Mnew1 (n=2) SB (n=19)	Reference Impact	Juveniles

ORDER AMPHIPODA

Amphipods were collected from almost every bore that stygofauna were recovered from (five Impact and 11 Reference; Table 3.1). Due to the high proportion of small and damaged specimens, and giving consideration to ongoing genetic research focused on this order, material from only eight of these bores was preserved in 100% ethanol for morphological identifications (see Table 3.1).

Two families were represented amongst the collected amphipods; the Paramelitidae (two taxa identified amongst the specimens examined), and the Melitidae (genus *Chydakata*). A number of the specimens were juvenile or missing key features and could not be identified below family or genus level. The distribution and abundance of the identified Amphipoda is provided in Table 3.5.

Table 3.5: Distribution of amphipod specimens across the Yandi expansion project area (sample sizes given as 'n=' in brackets for each collection location).

Species	Locations	Status	Comments
Paramelitidae sp. 2	M7 (n=24) WB (n=4) Mnew1 (n=31)	Reference Reference Reference	These specimens correspond to a morphotype nominal species collected as part of the ongoing CALM regional survey
Chydakata sp.	M2 (n=16) Plant (n=1) Mnew1 (n=1)	Reference Reference Reference	A taxonomically difficult genus, possible synonymous with *Pilbarus millsi* (see Section 4.1). Many juveniles amongst the specimens.
Paramelitidae sp. indet.	W02YJ001 (n=1)	Reference	Too damaged to identify any further

ORDER ISOPODA

Isopods were collected from seven of the sampled bores (three Impact and four Reference; Table 3.1). Specimens were available for morphological identification from all of these bores. Comparison with keys indicated that all isopods collected belonged to the genus *Pygolabis*, recently described by Dr. Buz Wilson of the Australian Museum (see Plate 3.2).

Plate 3.2: The isopod *Pygolabis* sp. 3 'Weeli Wolli' (Photo: J. McRae).

Morphological differences indicated that two species were present amongst the material (in addition to some specimens which could not be determined). The locations from which these were recorded are provided Table 3.6, along with comments on the isopod taxa.

Table 3.6: Distribution of isopod specimens across the Yandi expansion project area (sample sizes given as 'n=' in brackets for each collection location).

Species	Locations	Status	Comments
Pygolabis sp. nr. Wilson Sp. 3	M5 (n=11) SB (n=6)	Impact Impact	These specimens appear to correspond to *Pygolabis* sp. 3 ('Weeli Wolli') of Wilson's working key for this group (Plate 3.2). This species is currently being described.
Pygolabis sp. No. 1	Discharge (n=35) M3 (n=27) M7 (n=86) Mnew1 (n=18)	Impact Reference Reference Reference	Differences in telson morphology separated these specimens from *Pygolabis* sp. (nr Wilson Sp. 3).
Pygolabis sp. indet.	Plant (n=4)	Reference	Female and immature specimens only.

3.3 Genetic Analyses

3.3.1 Order Amphipoda

Genetic analysis of amphipod specimens was completed by Dr Terrie Finston of the School of Animal Sciences, UWA (see Section 2.1). Her complete report is provided as Appendix 2 of this document, with a summary of key findings presented below.

DNA was extracted from one to two individuals from each of 12 bores sampled in the Yandi JSE project area. Eleven of these specimens representing eight bores were successfully amplified for the CO1 gene (four Impact bores and four Reference). Analyses were carried out to examine relationships among the specimens and to identify groupings indicative of species. Three clear groups were found amongst the specimens from the eight sample locations, indicating the presence of three species (nominally A, B and C; see Table 3.7; Appendix 2).

Table 3.7: Distribution of stygal amphipod nominal species groups based on DNA sequencing (families based on distance clustering with other material from the region; Appendix 2).

Amphipod species	Borehole and Status
Sp. A (Paramelitidae)	SA (Impact)
Sp. B (Melitidae)	SC (Impact) M5 (Impact) Mnew1 (Reference) M2 (Reference)
Sp. C (Paramelitidae)	M6 (Impact) Camp (Reference) WB (Reference)

The Yandi amphipods were also compared with specimens from elsewhere in the region. The dendrogram arising from this work suggested that Species A and C belong to the family Paramelitidae, while Species B is a melitid (see Appendix 2). Species A shows very close affinities to amphipods collected from two bores in the Weeli Wolli Springs area during the Hope downs stygofauna surveys (Biota in prep.). With sequence divergences ranging from 1% – 2.8% between specimens from the three locations, they almost certainly belong to the same species. The analysis indicated that Species B belongs to the *Pilbarus millsi* group, a widespread melitid species complex that shows regional variation in association with contemporary surface water catchments. Species B also shows close phylogeographic links with Weeli Wolli Springs, with the individuals found in Weeli Wolli and Marillana Creeks clearly belonging to the same species (sequence divergences from 0.2% - 2.3%; Appendix 2).

Species C appears to be the only species at Yandi that was not previously recorded at Weeli Wolli Creek or elsewhere in the region. It shows some affiliation with specimens from Weeli Wolli, but sequence divergences are high (~24% – 25%), eliminating the possibility that they are the same species.

3.3.2 Order Isopoda

DNA sequencing was also completed for isopod specimens from selected sites by Cara Francis of UWA (see Section 2.1). Similar to work completed on the Amphipoda, the CO1 gene was again selected as a reliable indicator of species boundaries and subdivision in gene flow amongst populations.

These analyses were preliminary only, but still indicated two discrete taxa within the isopod specimens assessed. Specimens from two sites (M3 (Reference) and Discharge (Impact); Table 3.1; Figure 3.5), appeared to represent the same species (Species '1') based on sequence divergence (>3%). The remaining specimens analysed, from bore M5 within the Impact area, had an identical sequence for the analysed gene to specimens from Weeli Wolli Springs (nominally Species '2'). Further genetic work is ongoing on this group.

4.0 Discussion and Conclusions

4.1 Consolidation of Morphological and Genetics Studies

Suitable datasets to examine the concordance between morphological and DNA-based species determinations exist for two orders; the Amphipoda and the Isopoda. The results of these two independently completed studies show a high degree of correlation where comparisons have been possible, providing confidence that a robust assessment of the collected material has been completed.

A comparison of the morphologically determined amphipod taxa (Section 3.2) with the DNA sequence species 'groups' from the same location (Section 3.3.1), is presented in Table 4.1.

Table 4.1: Comparison of morphological determinations with DNA sequencing species groups for amphipod specimens collected from the same site.

Genetic 'species'	Borehole and Status	Morphologically identified taxa from the same site
Sp. B (Melitidae)	Mnew1 (Reference) M2 (Reference)	*Chydakata* sp. (n=1) *Chydakata* sp. (n=16)
Sp. C (Paramelitidae)	WB (Reference)	Paramelitidae sp. 2 (n=4)

As can be seen from Table 4.1, morphological identifications and clustering based on genetic data are in agreement at the family level. The two data sets also appear consistent at genus and nominal species level (as determined from sequence divergence and morphological similarities), with the same number of taxa distinguished by both methods. Amphipod Sp. B distinguished from the DNA sequencing appears to equate to *Chydakata* sp., while Sp. C is the nominal Paramelitidae sp. 2 (Table 4.1; Section 3.2). Specimens from other sites that cluster out as Sp. B based on DNA sequences have also been morphologically identified as belonged to the genus *Chydakata* Bradbury (2000) (notably Ethel Gorge and Weeli Wolli Springs material; Appendix 2).

Note that this assessment is not entirely conclusive as it is based on results from individuals from the same borehole rather than paired data where morphology and genetic analyses have been completed on the same individual. Further work is underway to attempt to extract DNA from the specimens identified as Paramelitidae sp. 2 to confirm this assessment.

Morphologically determined isopod species are compared with the DNA analysis species groups from the same borehole in Table 4.2.

Table 4.2: Comparison of morphological determinations with DNA sequencing species groups for isopod specimens collected from the same site.

Genetic 'species'	Borehole and Status	Morphologically identified taxa from the same site
Sp. 1	M3 (Reference) Discharge (Impact)	*Pygolabis* sp. No. 1 *Pygolabis* sp. No. 1
Sp. 2	M5 (Impact)	*Pygolabis* sp. nr. Wilson sp. 3 (sp. 'Weeli Wolli')

The morphological and genetic groupings correspond within the Isopoda specimens examined. Specimens grouped as *Pygolabis* sp. 1 based on DNA sequencing match the sites where *Pygolabis* sp. No. 1 was identified from morphological characters. Genetically determined *Pygolabis* sp. 2 was only identified from a specimen from M5 at Yandi; the same borehole where *Pygolabis* sp. nr. Wilson sp. 3 (sp. 'Weeli Wolli') was identified based on morphology. This specimen had an identical sequence to another *Pygolabis* specimen collected from Weeli Wolli Springs (the type locality for Wilson's species), further supporting the match between the two data sets.

Again, this work has not to date included any cases were genetic and morphological data are paired within a specimen. However, this appears unlikely to be a significant issue as both morphological (Section 3.2) and genetic data (Section 3.3.2) indicate that only a single taxon occurs within each bore sampled. Given this, and the good sample sizes collected, it appears the risk of multiple isopod species within a single bore (and therefore errors in the dataset matching suggested above), is low.

It is interesting to note the connectedness between Marillana Creek and Weeli Wolli Creek suggested by both the genetic and morphological data sets. The DNA analysis of amphipod specimens showed two species (Genetic Sp. A and B) that occur both in Marillana Creek and at Weeli Wolli Springs. This pattern was repeated in the isopod fauna, with one of the isopods from Marillana Creek (Genetic Sp. 2) analysed as the same species represented by specimens from Weeli Wolli. This may be indicative of larger-scale connection of stygal populations than previous studies have demonstrated (e.g. Humphreys 1999, Finston and Johnson in review). These distribution patterns will be further investigated as part of Hamersley Iron's ongoing stygofauna research programme, and in conjunction with the forthcoming Hope Downs project stygofauna assessment (Biota in prep.).

4.2 Analysis of Potential Impacts on Stygal Taxa

The abundance and diversity of stygofauna collected from the Yandi area was considerable, exceeding even Paraburdoo, which has given the greatest yields of all Hamersley Iron sites previously sampled (Biota 2003, Biota unpublished data). Taxonomic richness was also comparable with, or exceeded, other sites. Seven higher-level taxa were collected: Amphipoda, Copepoda, Hydracarina, Isopoda, Ostracoda, Oligochaeta and Platyhelminthes.

The relatively high diversity is probably attributable to the extent of alluvial and calcrete aquifer habitats associated with the major drainages in the locality. A review of available drill log information supports this, indicating that almost all stygofauna were collected from shallow boreholes that intersected superficial alluvials, consistent with findings from other similar sites in the region.

An assessment of potential impacts on stygofauna has been completed by overlaying the modelled groundwater drawdown contours on the locations where fauna were collected (see Figure 2.1). This enabled categorisation of sites into Impact and Reference (see Section 2.2). The key question for this assessment relates to the potential for any of the taxa recorded to be restricted in distribution to the Yandi JSE impact area. This arises from the statutory requirement to ensure the project does not impact on the conservation status of any fauna species protected by the *Wildlife Conservation Act 1950-1979* (see Biota 2002 for a fuller account). For some groups the nature of the specimens and the limits of current taxonomy do not permit this species level analysis to be completed. The turbellarian and oligochaete specimens collected could not be identified to species level, but are generally considered less likely to show such extreme short-range endemism (A. Pinder, pers. comm.).

Stygal water mite specimens collected are still undergoing more detailed examination, but it appears that the majority belong to the described genus *Recifella* (see Section 3.2). One hydracarine specimen collected clearly represents a new taxon but this was collected from Reference bore Mnew1, outside of the Yandi JSE drawdown area. Table 4.3 below provides a species level review of the remaining taxonomic groups recorded from bores within the project impact area. Comment on their status and wider distribution is also provided.

Table 4.3: Stygal taxa collected from locations within the proposed Yandi JSE groundwater drawdown Impact area, with comments on their wider distribution.

Taxa	Impact Boreholes	Status and wider distribution
Ostracoda		
Pilbaracandona sp.	Discharge	Previously described from specimens collected elsewhere in the region – not restricted to Impact area
Meridiescandona sp.	Discharge, M5-6	Also recorded locally from Reference bores M1, M2, W02YJ001 and Mnew1; previously described from specimens collected elsewhere in the region – not restricted to Impact area
Gomphodella sp.	M5	Specimens belong to a species not represented amongst known taxa currently being described – only collected from Impact area
Copepoda		
Mesocyclops darwini	M6	Previously described from specimens collected elsewhere in the region – not restricted to Impact area
Amphipoda		
Chydakata sp. (Genetic sp. B)	M5	Also recorded locally from Reference bores SC, M2, Mnew1 and WB. The same species also occurs in Weeli Wolli Creek – not restricted to Impact area
Paramelitidae sp. 2 (Genetic sp. C)	M6	Also recorded locally from Reference bores Camp and WB. The same species has been recorded during the CALM regional survey – not restricted to Impact area
Isopoda		
Pygolabis sp. No. 3 'Weeli Wolli'	M5, SB	The same species also occurs in Weeli Wolli Creek – not restricted to Impact area
Pygolabis sp. No. 1	Discharge	Also recorded locally from Reference bore M3 – not restricted to Impact area

As can be seen from Table 4.3, almost all of the stygal taxa recorded from the Yandi JSE Expansion impact area were also recorded from Reference bores or previously from elsewhere in the region. These species are therefore unlikely to be at risk of extinction or significant change in conservation status if the proposed expansion proceeds.

Only one of the 14 stygal taxa identified in this study is known from the Impact area only based on current sampling data. This is the ostracod *Gomphodella* sp., which does not match any previously collected specimens from the region. These specimens were collected from bore M5 in the northern part of the Impact area; a location within the dewatering influence of the already approved Yandi Junction Central operation. As all other stygal taxa collected from the Impact area occur more widely, it is possible that this apparent restricted distribution is due to sampling effects. The distribution of the other constituents of the stygal community at Yandi (and the apparent close connections with the fauna of Weeli Wolli Creek; Section 4.1), suggests that the risk of *Gomphodella* sp. being truly restricted to vicinity of M5 is likely to be low. The species is probably distributed at least locally along the alluvial aquifer of Marillana Creek. Additional sampling will be carried out in the Yandi area during 2005 in an attempt to better document the distribution of this species.

It is also worth noting that the Yandi JSE deposit is a Channel Iron Deposit (CID), and modelling suggests that dewatering influence will be somewhat spatially confined (see Figure 2.1). Observations from the drilling programme also suggest limited connectedness between the superficial alluvial aquifer (where most stygofauna were collected) and the deeper underlying CID aquifer along Weeli Wolli Creek. The development will dewater this deeper structure and this lack of vertical interaction may limit disruption to the superficial aquifer, reducing hydrological impacts on local stygal communities.

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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.

Appendix 2

UWA Stygal Amphipod Genetics Report

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Summary of results for 2004 – DNA sequence variation among samples from Yandi

DNA was extracted from 1 - 2 individuals from each of 12 bores at Yandi. From these 22 extractions, a total of 11 specimens from eight bores were successfully amplified for the CO1 gene. A maximum likelihood phylogenetic analysis was used to examine relationships among the samples, and look for groups indicative of species.

Three clear groups were found (Figure 1), indicating the presence of three species.

The three groups are labeled species A, B, and C, and the following table shows their distributions among the bores.

Species	location
A	SA-1
B	SC MNEW1 M5 M2
C	CAMP WB-1 M6

The samples were then included in an analysis of regional variation, to see how they relate to amphipods from other areas. Figure 2 shows their relationships to other species in the Pilbara. Species A shows very close affinities to amphipods collected from two bores in Weeli Wolli Creek, and with sequence divergences ranging from 1% – 2.8% between the three samples, they almost certainly belong to the same species. Species B belongs to the *Pilbarus millsi* group, a widespread group that shows regional variation in association with catchments. Whether each catchment contains a distinct species will need to be determined in conjunction with morphological evidence. Even if each lineage of *P. millsi* represents a distinct species, the individuals found in Weeli Wolli and Marillana Creeks clearly belong to the same species, with divergences ranging from 0.2% - 2.3%. Species C appears to be the only species at Yandi that is not found at Weeli Wolli or anywhere else so far examined. It shows some affiliation with specimens from Weeli Wolli, but sequence divergences are high (~24% – 25%), eliminating the possibility that they are the same species.

Fig. 1. Maximum likelihood tree for 11 specimens of amphipods from eight bores at Yandi. The number in parentheses = number of specimens analysed.

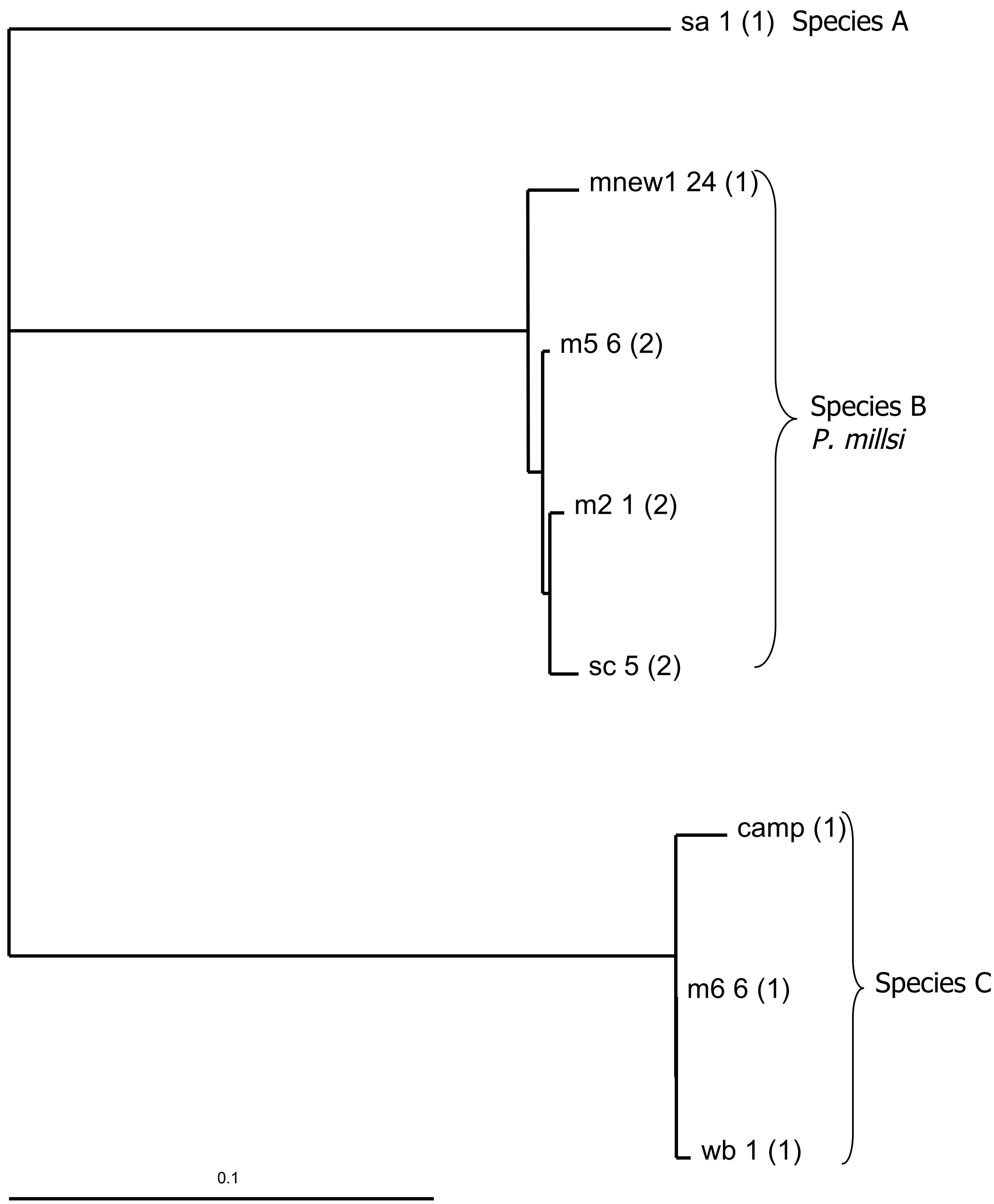

Fig. 2. Kimura's two-parameter distance and neighbour joining tree of representatives of all specimens of amphipods so far analysed from the Pilbara.