Subterranean Ecology

Scientific Environmental Services

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Yeelirrie Subterranean Fauna Survey

BHP Billiton Proposed Yeelirrie Development

Prepared for BHP Billiton Yeelirrie Development Company Pty Ltd

March 2011

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YEELIRRIE SUBTERRANEAN FAUNA SURVEY
PROPOSED YEELIRRIE DEVELOPMENT
REVISION 0

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Project No. 2010/14
Prepared for BHP Billiton Yeelirrie Development Company Pty Ltd
Date: MARCH 2011

COVER: Seven species of stygal copepods sampled by net hauling at Yeelirrie bore YYD26, March 2010. 1) *Halicyclops cf. eberhardi*; 2) *Schizopera* 'uranusi' sp. n.; 3) *Schizopera* 'leptafurca' sp. n.; 4) *Schizopera* 'akation' sp. n.; 5) *Nitokra* 'yeelirrie' sp. n.; 6) *Pseudectinosoma* 'pentedicos' sp. n.; 7) *Kinnecaris* 'uranusi' sp. n. Photograph by Tom Karanovic, copyright Subterranean Ecology 2010.

LIMITATIONS: This survey was limited to the requirements specified by the client and the extent of information made available to the consultant at the time of undertaking the work. Information not made available to this study, or which subsequently becomes available may alter the conclusions made herein.

REVISION	AUTHOR	INTERNAL REVIEW	EXTERNAL REVIEW	DATE
0	S. Callan N. Coen	S. Eberhard S. Callan	URS Australia Pty Ltd	03/03/2011

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EXECUTIVE SUMMARY

BHP Billiton Yeelirrie Development Company Pty Ltd is proposing to mine uranium at Yeelirrie Station, approximately 70 km southwest of Wiluna, in the Northern Goldfields/ Yilgarn region of Western Australia. Subterranean Ecology Pty Ltd was commissioned to survey subterranean fauna (stygofauna and troglofauna) in relation to the Proposed Yeelirrie Development (the Project). The purpose of the survey was to:

1. Characterise the diversity and distribution of stygofauna and troglofauna species within the Study Area;
2. Provide a regional context for understanding and assessing the faunal results from the Study Area, based on pre-existing regional data;
3. Provide a conceptual understanding of the characteristics of the subterranean habitat relevant to environmental impact assessment of subterranean fauna; and
4. Identify any subterranean species of potential conservation concern in relation to the Project.

The Yeelirrie Subterranean Fauna Survey (the Survey) comprised six sampling trips for stygofauna and troglofauna from March 2009 to September 2010. Sampling effort and survey coverage exceeded the requirements outlined in EPA Guidance Statements no. 54 (2003) and 54a (2007) for baseline assessment of subterranean fauna. The Study Area for the Survey comprised seven zones representing different parts of the palaeochannel system from the margins to the centre and from the northwest (top) to the southeast (bottom) of the local catchment. A total of 641 stygofauna samples and 461 troglofauna samples were collected during the Survey from 259 bores throughout the Study Area. This sampling effort represents one of the most intensive subterranean fauna surveys undertaken in the Yilgarn region to date.

Forty-six stygofauna species and nine amphibious subterranean species were collected from the Study Area, comprising nine invertebrate orders. Identifications using morphology and genetic analyses confirmed rich stygofauna assemblages, including dytiscid diving beetles (three species), annelid worms (four species), amphipods (one species), ostracods (one species), bathynellaceans (eight species), and a high diversity of copepods (30 species from six families). Amphibious subterranean fauna comprised seven species of enchytraeid worms and two species of philosciid isopods (amphibious species are hereafter included as 'stygofauna'). Yeelirrie has recorded the highest number of stygofauna species from any comparable area in the Yilgarn region to date.

Forty-five troglofauna species representing 12 invertebrate orders were detected. Identifications using morphology and genetic analyses revealed a diverse assemblage of pseudoscorpions (eight species), spiders (four species), palpigrades (one species), isopods (10 species), myriapods (15 species), diplurans (three species), hemipterans (two species) and silverfish (two species). Yeelirrie has recorded the richest troglofauna assemblages currently known from any comparable area in the Yilgarn region.

Two stygofauna species (*Mesocyclops brooksi* and *Australocamptus hamondi*) recorded at Yeelirrie have previously been recorded from other calcretes in the Yilgarn region. In addition, two

troglofauna species (Polyxenida sp. S1, and Meenoplidae sp. Y1) are considered widespread; respectively, on the basis of genetic analyses, and morphological dispersal mechanisms (*i.e.* winged adult stage). The remaining subterranean species are all undescribed and currently known only from the Yeelirrie Study Area. The majority of species (62% of stygofauna and 66% of troglofauna) were not widespread throughout the palaeochannel, and many species were infrequently sampled. Twenty-two stygofauna species and 21 troglofauna species were recorded only from single calcrete habitats other than the central calcrete.

Seven species of stygofauna and six species of troglofauna were recorded only from sites within the deposit. The stygofauna include Enchytraeidae sp. Y6, Philosciidae sp. n. Y2, *Halicyclops* cf. *eberhardi* sp. B, *Schizopera* sp. 7439, *Schizopera* 'emphysema' sp. n., *Schizopera* 'akolos' sp. n., and *Novanitocrella* 'araia' sp. n. The troglofauna species only known from the deposit are *Troglarmadillo* sp. n. S9, *Trichorhina* sp. n. F, *Tyrannochthonius* sp. n. Y1, *Austrohorus* sp. n. Y1, Pauropoda sp. S6B and Symphyla sp. Y7. All other subterranean species collected from inside the deposit were also detected outside of the deposit, and the high similarity between assemblages inside and outside of the deposit suggests a continuous habitat with no apparent barriers to fauna dispersal. The potential distribution ranges of the species currently known only from inside the deposit may extend beyond their recorded occurrence, within the central calcrete.

An assessment of subterranean habitats was conducted, incorporating hydrology, drill logs, geological information, and vegetation maps to infer the location and extent of calcrete habitats. This assessment revealed a number of small, discontinuous calcrete bodies (approximately 42 - 224 ha) in the northwest, and larger calcretes around the Yeelirrie playa (approximately 300 - 3000 ha) to the southeast. These habitats are separated from the central calcrete (approximately 4300 ha), which extends continuously between the deposit and drill lines F, H, and G to the immediate northwest. Significant differences between the stygofauna assemblages of the central calcrete, compared to those in northwest, southeast and Yeelirrie playa, support the hypothesis that barriers to dispersal may exist between these areas.

The richness of stygofauna and troglofauna species at Yeelirrie is partially attributed to the occurrence of multiple, discontinuous calcrete habitats throughout the palaeochannel (as is known to occur throughout the region). The eco-hydrogeological model for the Study Area (based on regional knowledge and current investigations) indicates the importance of habitat discontinuities and physico-chemical variability (between and within calcretes) as key drivers of the richness of subterranean fauna found at Yeelirrie.

The rich stygofauna and troglofauna assemblages detected at Yeelirrie contribute greatly to the regional understanding of subterranean fauna in the Yilgarn. Based on the low intensity of sampling at most other calcretes in the region, it is unlikely that the high richness detected at Yeelirrie is unique. More detailed investigation (particularly utilising genetic analyses, and methods that target troglofauna) at other calcretes could be expected to reveal other rich assemblages. However, the established regional patterns of species distribution, known as the ‘calcrete island’ hypothesis, were reaffirmed at Yeelirrie, with the majority of species detected at single calcretes only. Based on these distribution patterns, it is unlikely that the majority of species detected at Yeelirrie to date would occur at other calcretes in the region.

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GLOSSARY

Abundance	Abundance describes the number of individuals collected per specified sampling unit, which could include a sample, a methodology, a site or sampling area, a deposit or over an entire survey.
Accidentals	Organisms or species that are normally surface or soil dwelling and which have wandered underground or fallen in to caves accidentally. Populations may survive underground for a period of time but further generations are not established underground.
Allopatric speciation	Process of speciation by which a population is fragmented by geographic barriers which leads to genetic differentiation and then to speciation, with the creation of new daughter species (Lieberman 2000).
Amphibious	Amphibious subterranean fauna are species that can move between terrestrial and aquatic subterranean habitats. Such species may need access to water for particular phases of their life cycle, however sufficient water may be obtained from water films within soils and highly humid terrestrial subterranean habitats.
Biodiversity	The totality of biological diversity in a given area, usually simply measured as species richness, but often incorporating measures of higher order taxonomic diversity, molecular genetic diversity, or ecosystem diversity.
Biogeography	The study of the distribution of species and other taxonomic groups throughout geographical space; also, the study of how spatial patterns in species distribution change over time.
Calcrete	Carbonate deposits that form in the soil or in the vicinity of the groundwater table as a result of the evaporation of soil water or groundwater respectively. Groundwater calcretes form in arid climates (annual rainfall less than 200 mm) with high potential evaporation (more than 3000 mm per year) (Mann and Horwitz 1979).
Cave	Subsurface cavity (not simply an over-hang) sufficiently large for a human to enter (see **macro-cavern**).
Craton	Part of the Earth's crust little deformed for a prolonged period (shield region).
Dewatering	Removal of water from a volume of ground, usually to facilitate mining or construction. Unsustainable water abstraction may result in dewatering.
Distribution (or distribution range)	In biology, the **distribution** of a species is the geographical area within which the species can be found. Within that area, species may vary greatly in local density and dispersion. The distribution pattern of a species may be subject to change over time, due to a variety of influences such as seasonality and climate, habitat quality, migration, or human impacts.
Diversity	The diversity, in relation to fauna, is the number of different species in a particular area (see **species richness**), weighted by some measure of abundance such as number of individuals.
Ecology	The scientific study of the relationships between living organisms and their environment.
Edaphofauna Note: edaphobites *vs.* troglobites	Soil-dwelling fauna (*i.e.* **Edaphobites** are obligate soil dwelling species) (see **troglofauna**, cf. **troglobites**). Soil fauna frequently display similar morphological traits to **troglobites** (troglomorphisms), such as reduced eyes and pigmentation. **Edaphobites** are sometimes found deeper underground as accidentals in caves (and meso-caverns) but their primary habitat is soil. Distinguishing edaphobites from troglobites may sometimes be difficult as soil dwelling animals frequently exhibit regressive traits such as reduced pigmentation and eyes. However, soil dwelling animals do not generally display progressive **troglomorphic** traits such as elongated appendages and increased body size, and they tend to have smaller and more robust, or vermiform, body form and shorter appendages. The expression of troglomorphic traits needs to be related to the life histories of lineages, and where possible, comparison of **epigean** and **hypogean** forms of closely related taxa.

Endemic	The ecological state or place of being unique to a particular geographic location, such as a specific island. To be endemic to a place or area means that an organism is found only in that particular part of the world, and nowhere else.
Endogean	Meaning 'of the soil'
Epigean	Meaning 'of the surface' (terrestrial organisms or habitats)
Frequency of Occurrence	A measure of the number of times a species was detected during a specified sampling unit (*i.e.* the number of samples within which a certain species was detected during sampling).
Habitat	An ecological or environmental area that is inhabited by a particular species. It is the natural environment in which an organism lives, or the physical environment that surrounds (influences and is utilized by) a species population.
Haplotype	A combination of DNA sequences at different places on the chromosome that are inherited together. Haplotypes can become divergent (resulting in different species) from **Allopatric**, **Sympatric** or **Parapatric** speciation.
Invertebrates	Multicellular animals without backbones (vertebral column). Includes terrestrial and aquatic animals such as insects, crustaceans and arachnids (all arthropods), as well as molluscs, annelid worms, nematodes, jellyfish, sponges and corals.
Karst	Soluble-rock landscape; terrain with distinctive hydrology and landforms arising from a combination of high rock solubility and well-developed secondary porosity. The distinctive landforms above and below ground that are the hallmark of karst result from the solution of rock (mainly by carbonic acid) along pathways provided by the structure. The unusual features of the Kras (Karst) region on the Italo-Slovenian border became known as 'karst phenomena'. Such areas are characterized by sinking streams, caves, enclosed depressions, fluted rock outcrops and large springs (Ford and Williams 1989).
Macro-cavern	Underground voids > 20 cm, especially caves and underground
Meso-cavern	Subsurface cavity generally too small for a human to enter. Underground voids in the size range 0.1-20 cm, especially in karst and volcanic substrates.
Micro-cavern	Underground voids less than 0.1cm wide
Morphology	The physical form and structure of an organism or one of its parts.
Morpho-species	A species distinguished from others only by observable morphology. Typically used during parataxonomic sorting, for specimens in unsuitable condition for the observation of species level characters, or for organisms where the current state of taxonomic knowledge is insufficient or unknown.
Palaeodrainage	An ancient river system evident in the landscape that was formed under different climatic conditions than those currently observed.
Parapatric speciation	Speciation arising from an environmental gradient rather than a geographic barrier.
Parataxonomy	Sorting of specimens into recognizable taxonomic units (*e.g.* higher taxonomic levels such as family or order, or morpho-species) based upon morphology, without the use of taxonomic keys or expert knowledge.
Species	A taxonomic rank that is the basic rank of biological classification. Commonly, a group of organisms capable of interbreeding and producing fertile offspring.
Project Footprint	The anticipated spatial area of physical disturbance for a project, comprising excavation/ mining and the construction of buildings and infrastructure.
Species richness	The total number of species in a given area or sample, the most commonly used measure of biodiversity.

SRE- Short-range Endemism	Organisms that are endemic to a locally-restricted area (between 10,000km^2 and 1,000 km^2 depending upon range criteria) are known as short-range endemics. In relation to SREs and subterranean fauna, the EPA (2007) Guidance Statement #54A states: *"Subterranean fauna are an important issue in EIA because a high proportion of subterranean species have geographically restricted **ranges**. It is also becoming apparent that subterranean habitats contain far more species than previously recognized and, in fact, contain a significant proportion of global biodiversity (Gibert and Deharveng 2002)".* *While many of the species occupying shallow groundwater are widespread (Halse et al., 2002), and the same is probably true of many terrestrial species in shallow subterranean habitats, species that occupy deeper subterranean habitats and never come to the surface tend to have localized distributions and to be short range endemics. Harvey (2002) defined short range endemism as having a range < 10,000 km^2, while Eberhard et al. (2007) suggested < 1000 km^2 constitutes a more appropriate range criterion. Ranges are difficult to determine precisely, however, and the characteristic that makes short range endemics vulnerable to extinction is being confined to highly restricted habitats or individual geological features. Examples of such vulnerable fauna are **troglofauna** in Channel Iron Deposit in mesas of the Pilbara and **stygofaunal** beetles.*"
Note: Troglofauna, Stygofauna and SRE	The terms **troglofauna** and **stygofauna** are often used as synonyms for **troglobites** and **stygobites** respectively. Application of the more explicit ecological subcategories may be useful when assessing the conservation status of species and potential impacts, because troglobites and stygobites in a strict sense are frequently **short range endemic** (**SRE**) species confined to narrowly delimited habitats. Because of their restricted distribution, SREs are more vulnerable to extinction from a range of threatening processes. In assessing the environmental impact of projects on subterranean species it may be useful to distinguish troglobites from other ecological categories of subterranean fauna, especially **endogean** (soil) fauna. However, it is important to recognize the habitat continuum between **epigean**, **endogean** and **hypogean** environments, and the likelihood that many species are not solely confined to either one or other of these habitats, and furthermore, that migration and mixing of fauna occurs between these habitats.
Surficial aquifer	Aquifers close to the surface, typically less than 15 m deep.
Sympatric speciation	Speciation occurring within a population by genetic polymorphism or behavioural differentiation. The species are not geographically isolated as in **Allopatric speciation,** and may continue to co-occur within the same habitats.
Stygo / Stygal	Prefix referring to groundwater habitats or organisms (Gibert *et al.* 1994).
Stygofauna	Aquatic fauna inhabiting the various types of groundwater. The term '**stygofauna'** embraces, implicitly, three categories (**stygobites**, **stygophiles**, **stygoxenes**) which can be used to further define aquatic subterranean fauna.
Troglofauna	Animals inhabiting air-filled caves or smaller cavities below the ground. As with stygofauna, the term '**troglofauna'** embraces, three further categories (**troglobites**, **troglophiles**, **trogloxenes,** see below).
Stygobite / Troglobite	Collectively, species that are specialised, obligate subterranean fauna, which are never found in epigean habitats. Stygobites are obligate subterranean aquatic fauna that occur in all kinds of groundwater habitats (both karst and alluvia), and sometimes may be found very close to the surface. Troglobites are animals that are specialised to inhabit air-filled caves and cavities underground, and are found exclusively in the dark zone.
Stygophile / Troglophile	Collectively, facultative subterranean fauna; organisms which can complete their life-cycles either underground or in epigean habitats. Hypogean and epigean populations may simultaneously exist, and individuals may be able to move between them (Trajano and Bichuette 2004). Such organisms may seek out and exploit resources in groundwater or underground habitats (Stanford and Ward 1993).

Stygoxene / Trogloxene	Collectively, organisms that are regularly found in subterranean habitats, but cannot complete their entire life-cycles underground. These species may frequently move between the surface and underground habitats for foraging and sheltering, as in cave-dwelling bats. Other species may move between the habitats infrequently or only once, to complete part of their reproductive cycles.
Taxa	(Singular, taxon) is any taxonomic group or rank, generally used as a grouping of organisms that belong to different species but the same family or genus.
Taxonomy	The scientific classification of organisms into specially named groups based either on shared characteristics or on evolutionary relationships.
Troglofauna	Animals inhabiting air-filled caves or smaller cavities (meso-caverns) below the ground. The term '**troglofauna'** embraces, implicitly, three categories (**troglobites**, **troglophiles**, **trogloxenes,** see above) which can be used to further define terrestrial subterranean fauna.
Troglomorphism / Troglomorphy	Any morphological, physiological, or behavioural feature that characterizes subterranean fauna (Christiansen 1962). Common morphological traits include: reduction of eyes, pigment, wings, elongation of appendages, specialization of sensory structures, pseudophysogastry (extreme distension of the abdomen), compressed or depressed body form (hexapods), elongate body form (Arachnida), increased size (Collembola, Arachnida), foot modification (Collembola, plant-hoppers), cuticle thinning (terrestrial arthropods). Not all cave animals display the complete set of troglomorphic traits, and their expression depends on the characteristics of the **epigean** ancestor (Aden 2005). Not every troglomorphic trait is adaptive, and comprises constructive (progressive) and regressive characteristics. Examples of constructive traits might include elongation of appendages and specialisation of sensory structures. Examples of regressive traits include reduction of eyes and wings. Though characteristic of cave animals, many of the regressive features are found in non-cave environments such as soil and the deep sea. Animals in these environments may exhibit "troglomorphic" traits but they are not **troglobites**. The term **troglomorph** serves to identify potential cave-adapted organisms without the possibly erroneous designation of **troglobite** (Christiansen 2005). Thus it is necessary to evaluate if a supposed troglomorphic trait is, in fact, **apomorphic** (derived) or merely a **plesiomorphy** (primitive character state) of the group or lineage (Aden 2005).

1. INTRODUCTION AND PROJECT OVERVIEW

BHP Billiton Yeelirrie Development Company Pty Ltd (BHP Billiton) is proposing to mine uranium at Yeelirrie, in the Yilgarn region of Western Australia. The Proposed Yeelirrie Development (hereafter "the Project") is located within the Yeelirrie Pastoral Station in the Shire of Wiluna, approximately 110 km northwest of the town of Leinster, 70 km south-west of the town of Wiluna and 60 km west of the Mt Keith Nickel Mine (Figure 1-1). BHP Billiton is preparing the Environmental Review and Management Programme (ERMP) for the Project. Subterranean Ecology has been commissioned to conduct a survey of subterranean fauna (the Survey) in the proposed Project area and to conduct a database review to establish a regional context, in order to obtain ecological information relevant to the environmental impact assessment for the Project. The subterranean fauna survey was designed in consultation with Department of Environment and Conservation (DEC) and Department of Water (DOW), under the guidance of the Environmental Protection Authority (EPA) Guidance Statements # 54 (2003) and # 54A (2007).

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600000 650000 700000 750000 800000 850000 900000 950000

7100000 7050000 7000000 6950000 6900000

MGA ZONE 50 | MGA ZONE 51

N

0 10 20 30 40

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Zone 50 and 51

Project Location

25 km 50 km 75 km 100 km

YALGAR POOL
MINDARAH CLAYPAN
KILEKILEGUNNA POOL
BERRIN POOL
PODOYNE POOL
COMBON CLAYPAN
CARBAR POOL
POCKINGNINGAR CLAYPAN
ILLGALARA POOL
JUNGAR POOL
NALLAN LAKE
LAKE AUSTIN
LAKE AUSTIN
LAKE AUSTIN
NULLAN LAKE
WIRRA WIRRA LAKE
BURAJINNI CLAYPAN
LAKE ANNEAN
PORLELL LAKE
TALVAL POOL
DENHAM POOL
BULLOCK POOL
NORTH POOL
LAKE MASON
LAKE VIOLET
URAMURDAH LAKE
LAKE WAY
DUCK SWAMP
LAKE WARD
LAKE MAITLAND
LAKE MIRANDA
YANDAL LAGOON
SALT LAGOON

Mooloogool
Diamond Well
Paroo
Yandil
Munarra
Greenwood
Killara
Belele
Yoothapina
Sherwood
MEEKATHARRA
Norie
Murchison Downs
Annean
Hill View
Polelle
Beebyn
Cullculli
Karbar
Yarrabubba
Younó Downs
No-ibla
Millgool Outcamp
Lake Way
Barwidgee
Wonganoo
Wanmulla
Taincrow
Nallan
CUE
Old Yarraquin
Cogla Downs
Barrambie
Old Gidgee
Lake Mason
Altona
Yakabindie
Yandal
Inglewood
Kaluwiri
Booylgoo Spring
Wanarie
Wondinong
Wynyangoo
Black Range
Depot Springs
Leinster Downs
LEINSTER
Windsor
Anketell
Hy Brazil
Black Hill
Dandaraga
Atley
Daly Outcamp
Munjeroo
Pinnacles
Wildara Outcamp
The Folly Outcamp
Challa
Windimurra
Iswia
Jundee
Millrose
Lake Violet
Desert Gold
Emu Farm
Millbillillie
WILUNA
Albion Downs
Mount Keith
MOUNT KEITH MINE
Yeelirrie
Mount Grey Outcamp

GREAT NORTHERN HIGHWAY
GOLDFIELDS HIGHWAY
NANNINE POLELLE ROAD
YOUNO DOWNS ROAD
MEEKATHARRA YEELIRRIE RD
WILUNA LEINSTER ROAD
WILUNA GRANITE PEAK ROAD
WONGAWOL ROAD
MOUNT FISHER WONGANOO ROAD
YJAWARN ROAD
DARLOTT WEEBO ROAD

DAMPIER
Western Australia
WILUNA
LEONORA
PERTH
ALBANY

Source: Landata 2005.

Legend

- Populated Places
- Homesteads
- Mine Locations
- 25 km Interval Radii from Yeelirrie Mineralisation
- Lake Miranda Catchment
- Key Flowpaths (DEM derived)
- Non-Perennial Lakes
- **Roads (GA, 2007)**
- Major Road
- Minor Road

BHP Billiton Yeelirrie Development Company Pty Ltd

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Project: **Proposed Yeelirrie Development**

Title: Regional Location and Lake Miranda Catchment

Drawn: DL/MR/CJT	Approved: DR	Date: 23/12/2010
Job No.: 42907132	File No.: 42907132-ERMP-103_RD.mxd	

Figure: 1-1 | Rev. D | A3

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1.1 Aims and Objectives

This report describes the methods and results of the Yeelirrie subterranean fauna survey. The results of hydrological investigations (URS 2011) and geological information are integrated with subterranean fauna results to produce a conceptual model of the subterranean ecosystems of the Study Area. Biogeographical patterns of subterranean fauna throughout the Yilgarn region were also investigated, to provide context for the results at Yeelirrie. The findings of these investigations form the basis for assessment of potential impacts on subterranean fauna in relation to the Project.

The specific objectives of the subterranean fauna assessment were to:

1. Characterise the diversity and distribution of subterranean fauna species within the Study Area;
2. Provide regional context for understanding and assessing the faunal results from the Study Area, based on existing data;
3. Provide a conceptual understanding of the characteristics of the subterranean habitat relevant to environmental impact assessment of subterranean fauna; and
4. Identify any subterranean fauna species of potential conservation concern in relation to the Project.

This report is structured as follows:

Chapter 1 – Introduction, project description and legislative framework;

Chapter 2 – Regional Context: Methods and results of investigation of existing subterranean fauna data from the Yilgarn region;

Chapter 3 – Existing environment at Yeelirrie: Interpretation of hydrogeology, geology, and vegetation data to infer location and extent of subterranean habitat;

Chapter 4 – Yeelirrie subterranean fauna survey: Study area and Survey approach;

Chapter 5 – Yeelirrie subterranean fauna survey: Methods;

Chapter 6 – Results of the survey; and

Chapter 7 – Discussion and Conclusions.

1.2 Legislative Framework

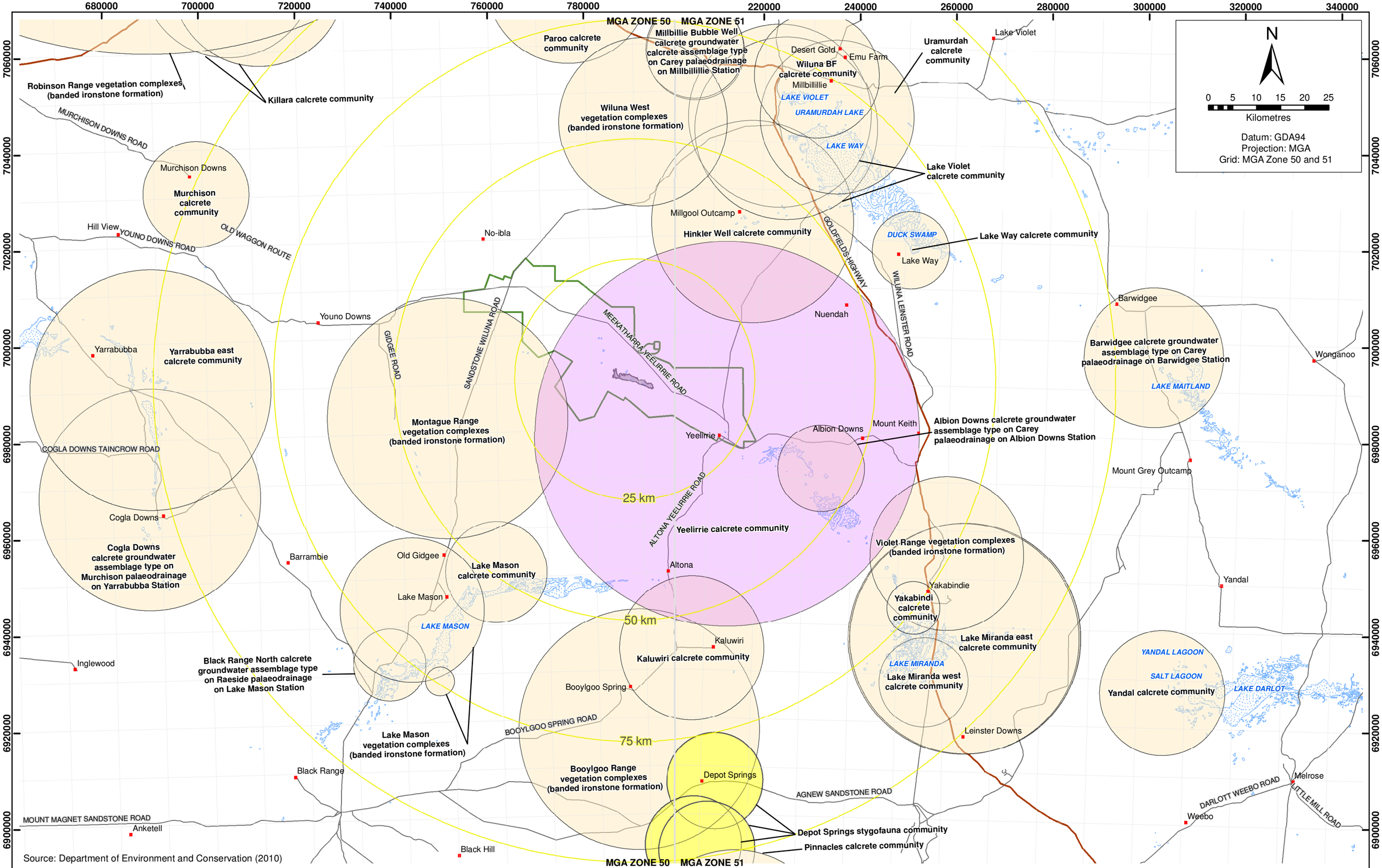
The subterranean fauna survey was designed to meet legislative requirements and guidelines for assessment of subterranean fauna, as follows:

- Guidance Statement 54: Consideration of Subterranean Fauna in Groundwater and Caves during Environmental Impact Assessment in Western Australia (EPA 2003);
- Guidance Statement 54a: Sampling Methods and Survey Considerations for Subterranean Fauna in Western Australia (EPA 2007);
- *Wildlife Conservation Act 1950* (State);
- *Environmental Protection Act 1986* (State); and
- *Environmental Protection and Biodiversity Conservation Act 1999* (Federal).

The Project is also subject to the provisions of the *Uranium (Yeelirrie) Agreement Act 1978*.

The *Federal Environment Protection and Biodiversity Conservation Act 1999*, is relevant where threatened species or threatened ecological communities (TECs) listed under this Act may be impacted by a proposed action. The Yeelirrie calcrete is listed by DEC as a Priority Ecological Community (PEC) under Priority One (DEC 2010). Priority One PECs are defined by DEC (2007) as, "poorly-known ecological communities, with apparently few, small occurrences; where all or most are not actively managed for conservation (*e.g.* within agricultural or pastoral lands, urban areas, active mineral leases), and for which current threats exist".

The boundary of the listed PEC at Yeelirrie is shown in Figure 1-2. The DEC (2010) list describes the PEC community as "Yeelirrie calcrete groundwater assemblage type on Carey palaeodrainage on Yeelirrie Station", which is interpreted to include the calcrete subterranean fauna assemblages of the Yeelirrie palaeochannel, within the boundary indicated by Figure 1-2. This boundary was defined prior to the current subterranean fauna results becoming available.



Source: Department of Environment and Conservation (2010)

Legend

- Homesteads
- Pit Outline
- Yeelirrie Calcrete Community (P1)
- Priority Ecological Communities (P1)
- Threatened Ecological Communities (Vulnerable)
- 25 km Interval Radii from Yeelirrie Mineralisation
- Non-Perennial Lakes

BHP Billiton Yeelirrie Development Company Pty Ltd	Project: **Proposed Yeelirrie Development**		Title: Priority and Threatened Ecological Communities
Subterranean Ecology, Scientific Environmental Services, www.subterraneanecology.com.au	Drawn: DL/MR/CJT	Approved: DR	Date: 23/12/2010
	Job No.: 42907132	File No.: 42907132-ERMP-105_RD.mxd	Figure: 1-2
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1.3 Project Overview

The Project is located within the Yeelirrie Pastoral Station in the Shire of Wiluna, Western Australia and is bounded by the Project Area (Figure 1-3). The proposed deposit contains mineralisation of uranium associated with calcrete and carbonated clay-quartz sediments. The deposit is approximately 9 km long, with an average width of 1 km and a maximum width of 1.5 km.

The Project is proposed to be developed as a conventional load and haul, open-pit mining operation, operating over a period of approximately 30 years. Major on-site infrastructure will include a quarry, pit-dewatering infrastructure, tailings disposal in-pit, a water supply wellfield, electricity supply, water treatment plant, waste and sewage disposal, and working and accommodation facilities. The disturbance footprint would be surrounded by a flood-protection bund which would divert surface water flows around the site. The Project will obtain water from a number of sources, including mine pit dewatering and water supply wellfields.

The subterranean fauna Study Area (Figure 1-3) was designed to encompass the disturbance footprint (focusing on the deposit), as well as other potential habitats outside of the disturbance footprint.

A comprehensive description of the project is available in the *Yeelirrie Project Environmental Review and Management Programme, Chapter 3 Project Description* (BHP Billiton 2011). Details regarding water supply are also available within the *URS Groundwater Report* (2011).

N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

MGA ZONE 50
MGA ZONE 51

Source: Landsat, 2005.

Legend

- Ministerial Temporary Reserve TR70/6899
- Pit Outline
- Project Area
- Indicative Project Footprint
- Sampling Bores
- Drill and Infill Traverses
- Study Area

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Project: **Proposed Yeelirrie Development**

Title: Subterranean Fauna Study Area

Drawn: CJT	Approved: JN	Date: 25/01/2011	Figure: 1-3	Rev. B
Job No.: 42907132	File No.: 42907132-ERMP-130_RB.mxd			A3

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2. REGIONAL CONTEXT – SUBTERRANEAN FAUNA OF THE YILGARN REGION

2.1 What are Subterranean Fauna?

Subterranean fauna include troglofauna (animals inhabiting air-filled caves or smaller cavities underground) and stygofauna (aquatic animals inhabiting groundwater). Certain subterranean species are amphibious, *i.e.* able to utilise both aquatic and terrestrial subterranean habitats. Most subterranean fauna are invertebrates, such as spiders, pseudoscorpions, millipedes, centipedes, beetles, bugs, crustaceans, and worms (Gibert *et al.* 1994). Obligate subterranean species, which spend their entire lives underground, are known as troglobites or stygobites, for terrestrial or aquatic forms respectively.

Subterranean fauna can be an important issue in environmental impact assessment because "a high proportion of subterranean species have geographically restricted ranges" (EPA 2007). Species confined to narrow distribution ranges (sometimes referred to as SRE - short-range endemics), are inherently more vulnerable to impacts than widely distributed species. Harvey (2002) provided a range criterion for SRE species as, "less than 10,000 km^2", while Eberhard *et al.* (2009) suggested that "< 1,000 km^2" may be more applicable for subterranean fauna SREs. Troglobites and stygobites frequently fit the criteria for SRE species, being restricted to narrowly-delimited distribution ranges that are limited by geological or hydrogeological discontinuities.

Troglobites and stygobites by definition exhibit certain morphological characteristics (troglomorphism) such as reduced or absent eyes, a lack of pigment, elongated appendages, and enhanced non-optic sensory structures (Christiansen 2005). These morphological features can indicate evolutionary adaptation to underground life. There are some species that utilise underground habitats but are not necessarily restricted to them, such as trogloxenes (or stygoxenes), and troglophiles (or stygophiles) (Christiansen 2005, Stanford and Ward 1993, Trajano and Bichuette 2004).

Occasionally surface (epigean) invertebrates accidentally occur underground (accidentals). Soil-dwelling fauna (edaphofauna) are also sometimes detected, and may show some degree of troglomorphy (*e.g.* reduced eyes, or lack of pigment). Morphological evidence is combined with ecological information in order to assess the ecological status of each species and its potential classification in reference to the terms above (*e.g.* Humphreys 2008, Platnick 2008). For full definition of terms, see the glossary.

The state of knowledge of the Yilgarn region's subterranean fauna is incomplete, and the vast majority of species collected are new or undescribed. This provides challenges for assessing the taxonomy, ecology and distribution of undescribed species. Molecular genetic (DNA) analyses are commonly used alongside morphological taxonomy to help resolve these issues (*e.g.* Harvey *et al*. 2008). Published information regarding closely-related species from the same region can also be useful for inferring ecology or potential habitat where information gaps exist.

2.2 Literature Review

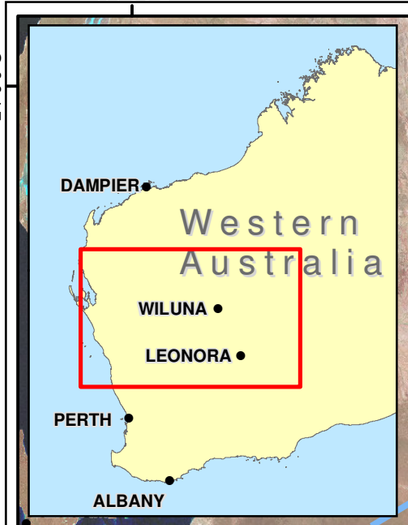
A high diversity of subterranean fauna has been recorded from calcrete deposits in the palaeodrainage channels of the Yilgarn region. The majority of stygofauna and troglofauna collected from calcretes in the region to date have been new, undescribed species. The majority of subterranean fauna in the Yilgarn region have only been recorded from single calcrete habitats. This characteristic pattern of locally-restricted species throughout the region is commonly known as the 'calcrete island' hypothesis, *i.e.* each calcrete aquifer is equivalent to a geographically-isolated island habitat, containing its own suite of range-restricted endemic species (Watts and Humphreys 1999, 2000, 2001, 2003, 2004, 2006, Humphreys 2001, 2006, 2008, Cooper *et al.* 2002, 2007, 2008, Leys *et al.* 2003, Guzik *et al.* 2008, 2009).

The high diversity and high proportion of range-restricted species, has led to the calcretes of the Yilgarn region being recognised as globally significant habitats for stygofaunal biodiversity (Culver and Pipan 2009, Cooper *et al.* 2002, Leys *et al.* 2003, Guzik *et al.* 2009). The majority of published studies to date have focussed on describing new species of stygofauna from calcretes across the region (*e.g.* Watts and Humphreys 1999, 2000, 2001, 2003, 2004, 2006, Bradford *et al.* 2010, Cho and Humphreys 2010). Comparatively few published studies have examined troglofauna, and the focus has also been on describing new species (Barranco and Harvey 2008, Edward and Harvey 2008, Platnick 2008). Few published studies have investigated the ecology of Yilgarn calcrete ecosystems as a whole, although some calcretes have been more intensively studied, such as Sturt Meadows, Uramurdah Lake, Hinkler Well, Lake Violet, and Paroo (Allford *et al.* 2008, Cooper *et al.* 2008, Guzik *et al.* 2008, Leys and Watts 2008, Outback Ecology 2008). Limited ecological information is available for most of the stygofauna and troglofauna species of the region, although some stygal groups such as dytiscid diving beetles (Watts and Humphreys 1999, 2000, 2001, 2003, 2004, 2006), copepods (De Laurentiis *et al*. 2001, Karanovic 2004) and *Haloniscus* isopods (Taiti and Humphreys 2001, Cooper *et al*. 2008) have received focussed study.

2.3 Investigation of Existing Records

In order to describe the regional patterns of subterranean fauna diversity and distribution, a combined dataset of 699 stygofauna and troglofauna records (excluding duplicates) from 68 calcrete deposits was compiled and examined. Records were obtained from the Western Australian Museum ('WAM'), published scientific literature and accessible unpublished reports (Outback Ecology 2008). The search area (approximately 485,000 km^2) for the combined data set is shown in Figure 2-1, bounded by the following coordinates: 24.5° S, 115.5° E; 24.5° S, 125° E; 29° S, 125° E; 29° S, 115.5° E.

114°0'0"E
117°0'0"E
120°0'0"E
123°0'0"E
24°0'0"S
27°0'0"S
30°0'0"S



N

0 25 50 75 100

Kilometres

Geographic Coordinate System
Datum: GDA94

Nabberu
Burnside
Murchison
Carnegie
Carey
Moore
Raeside

MEEKATHARRA
WILUNA
Project Location
CUE
KALBARRI
LEINSTER
MOUNT MAGNET
NORTHAMPTON
MULLEWA
LAVERTON
DRUMMOND COVE
GERALDTON
LEONORA
MINGENEW
MORAWA
DONGARA
THREE SPRINGS
CARNAMAH
MENZIES
ENEABBA
LEEMAN
GREEN HEAD
JURIEN BAY
DALWALLINU

Source: Landsat, 2005.

Legend

- Populated Places
- Catchment Boundary (SE, 2011)
- Yilgarn Palaeochannel (SE, 2011)
- Yilgarn Calcrete (SE, 2011)
- Search Area

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Project: **Proposed Yeelirrie Development**

Title: Regional Map

Drawn: DL	Approved: KD	Date: 01/03/2011	Figure: 2-1	Rev. A
Job No.: 42907132	File No.: 42907132-SUS-256.mxd			A3

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2.3.1 Data Handling

The WAM records represent specimens that have been vouchered by various collectors. Vouchering is not consistently undertaken, and may only include the minimum required amount of information. Comparisons of the sampling effort and faunal records are indicative estimates only, based upon WAM records (from Arachnida and Crustacea databases) and published literature as at September 2010. For a full list of data sources, see Appendix 1.

The combined incidence data (presence/ absence) for each calcrete contained many potential duplicate records, as well as undetermined records at a variety of taxonomic levels. The records were checked to validate ecological status (troglofauna or stygofauna *versus* potential accidental surface fauna), and update taxonomic changes. Where potential duplicate records appeared (*e.g.* 'Copepoda sp.', 'Cyclopoida sp.', and *Mesocyclops brooksi*, from the same calcrete), only the lowest ranked taxon was retained (*i.e. Mesocyclops brooksi*). Estimates of total richness included all taxa (excluding duplicates), while regional distributions and assemblage similarity were assessed using named species and species-level records only.

Calcretes were named in accordance with the DEC PEC list (DEC 2010), or the name of the nearest pastoral station if a calcrete was unnamed and not listed. Uncertain locations were verified by cross-referencing bore co-ordinates with geological survey information (Commander 1989). The sampling effort at each calcrete was represented by the numbers of bores sampled, as actual sample numbers and detailed sampling methods were often unavailable in the WAM records. The majority of samples were assumed to have been obtained by standard net hauling, except where the literature or WAM records noted otherwise. Threshold levels for categorising sampling effort (see Table 3-1) followed the minimum requirements of EPA (2007) Guidance Statement 54A:

- Less than 10 bores – below EPA minimum number of samples;
- More than 10 bores – minimum requirement for pilot survey; and
- More than 40 bores – minimum requirement for full baseline survey of stygofauna.

2.4 Sampling Effort and Coverage

Based on the combined data set, 68 of a potential 150 calcretes located within the literature search area (approximately 45 %) had been sampled for subterranean fauna. The sampling effort (number of bores sampled) at each calcrete varied considerably. Forty-nine calcretes had less than 10 bores sampled, while 14 calcretes had more than 10 bores sampled, and five calcretes (Sturt Meadows, Hinkler Well, Paroo, Lake Violet, and Uramurdah) had more than 40 bores sampled (Table 3-1). Records of net hauling and trap sampling were available for each of these five well-studied calcretes. The average number of bores sampled varied considerably between each group of calcretes. The majority of calcretes (49) received little sampling effort (average of 3 bores sampled). Sampling effort was highly variable between the well-sampled (>40 bores) calcretes (average 67.8 bores), which ranged between 41 bores (Urumurdah) and 137 bores sampled (Sturt Meadows) (Table 3-1).

Table 2-1: Number of bores sampled at each of the calcretes within the regional search area. Calcretes are arranged by position within the palaeodrainage catchment. Calcretes in bold font recorded troglofauna trap sampling in addition to net hauling. Data for Yeelirrie presented in this table does not include results of the current survey.

Catchment position		Map code	Calcrete name	Number of bores recorded		
				< 10	> 10	>40
Murchison	North arm	Mn1	Killara		16	
		Mn2	Murchison Downs	1		
		Mn3	Cogla Downs	2		
		Mn4	Yarrabubba East		12	
		Mn5	Hillview	2		
		Mn6	Karalundi	6		
		Mn7	Killara North	2		
		Mn8	Munarra	1		
		Mn9	Mt Padbury	1		
		Mn10	Yarrabubba West		13	
		Mn11	Polelle	3		
		Mn12	Belele	5		
		Mn13	Innouendy	1		
		Mn14	Meeberrie	2		
	South arm	Ms1	Windimurra		10	
		Ms2	Windsor	2		
		Ms3	Challa		10	
		Ms4	Challa North	5		
		Ms5	Madoonga	8		
		Ms6	Taincrow	2		
		Ms7	Cue	1		
		Ms8	Wondinong		12	
		Ms9	Austin Downs		12	
	Moore	MO1	Murrum	1		
Raeside	North arm	Rn1	Black Range S	3		
		Rn2	Black Range N	3		
		Rn3	Lake Mason	2		
		Rn4	Kaluwiri	2		
		Rn5	Depot Springs		27	
	Middle arm	Rm1	Yuinmery North	2		
		Rm2	Yuinmery South	1		
		Rm3	Dandaraga	2		
		Rm4	Pinnacles		13	
	South arm	Rs1	Perrinvale	2		
	Lower catchment	R1	**Sturt Meadows**			137
		R2	Melita	9		

Table 2-1: Continued.

Catchment position		Map code	Calcrete name	Number of bores recorded		
				< 10	> 10	>40
Carey	North arm	Cn1	Diamond Well	5		
		Cn2	**Paroo**			48
		Cn3	**Bubble Well**	8		
		Cn4	Wiluna BF	7		
		Cn5	**Lake Violet**			47
		Cn6	**Uramurdah**			41
		Cn7	Lake Way W	3		
		Cn8	**Hinkler Well**			66
		Cn9	Lake Way S	3		
		Cn10	**Barwidgee**		11	
	South arm	Cs1	Yeelirrie			
		Cs2	Albion Downs	2		
		Cs3	Yakabindie	1		
		Cs4	**Lake Miranda West**		18	
		Cs5	**Lake Miranda East**		18	
		Cs6	Yandal	7		
	Lower catchment	C1	**Melrose (Lake Darlot)**	7		
		C2	Banjawarn	5		
		C3	**Nambi**		24	
		C4	Windarra	1		
		C5	Laverton Downs	1		
		C6	Mt Morgan	1		
Nabberu		N1	Cunyu State Borefield	2		
		N2	Old Cunya	2		
		N3	Cunyu Sweetwater	1		
Carnegie		CG1	Jundee Homestead	3		
		CG2	Jundee South Hill		19	
		CG3	Lake Ward	2		
		CG4	Lorna Glen	6		
		CG5	Windidda	3		
Burnside		B1	Glenayle	2		
		B2	Carnegie Downs	1		
Number of calcretes per category				**48**	**14**	**5**
Mean # bores (± SD)				3.0 ± 2.24	15.36 ± 5.26	67.8 ± 39.8

2.5 Regional Subterranean Fauna Results

2.5.1 Richness (Number of Species)

The combined dataset contained a greater number of stygofauna records (562 records from 62 calcretes) than troglofauna records (137 records from 34 calcretes). High stygofauna richness was recorded at:

- Hinkler Well – 33 taxa;
- Uramurdah – 28 taxa;
- Lake Violet – 24 taxa;
- Paroo – 21 taxa;
- Depot Springs – 21 taxa; and
- Sturt Meadows – 8 taxa.

Excluding Depot Springs (Raeside), these calcretes are all situated within the Carey palaeochannel (Figure 2-1), and all, with the exception of Depot Springs, had more than 40 bores sampled.

Highest troglofauna richness was recorded at:

- Sturt Meadows – 17 taxa;
- Uramurdah – 15 taxa;
- Lake Violet – 8 taxa;
- Bubble Well – 8 taxa; and
- Nambi – 7 taxa.

Sturt Meadows occurs in Raeside catchment, while the other calcretes are all located in the Carey palaeochannel (Figure 2-1). The majority of these calcretes had more than 40 bores sampled, except for Nambi (24 bores) and Bubble Well (8 bores). All of the calcretes from which high diversities of troglofauna were recorded had been sampled for troglofauna by litter trapping.

In general, calcretes which had a higher number of bores sampled (particularly >40 bores) yielded higher richness of both stygofauna and troglofauna (particularly those which were surveyed using litter trap sampling in addition to net hauling). In general, subterranean fauna records from these calcretes had proportionally fewer undetermined taxa (particularly Sturt Meadows, Hinkler Well, Uramurdah, Lake Violet and Paroo), reflecting greater resolution of taxonomic identifications. In most situations, a higher number of samples combined with a greater degree of taxonomic attention were associated with a greater diversity recorded.

Figures 2-2 and 2-3 map the richness of stygofauna and troglofauna (respectively) recorded from each calcrete, with the number of taxa displayed as a colour gradient. Results from Yeelirrie and Albion Downs calcretes reflect the records of the WAM (prior to the current survey). Calcretes with more than 10 bores sampled are fully named, while calcretes with less than 10 bores are coded as in Table 2-1.

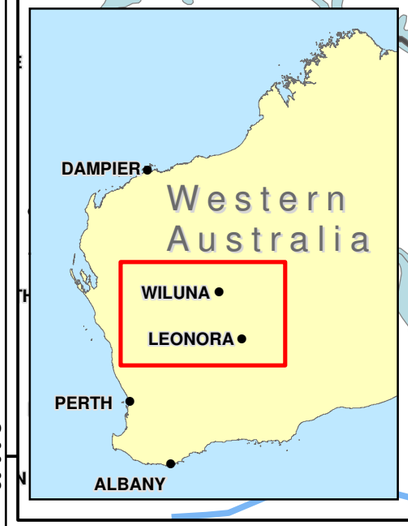
117°0'0"E
120°0'0"E
123°0'0"E
27°0'0"S
30°0'0"S

Nabberu
Burnside
Murchison
Carnegie
Carey
Moore
Raeside

B1/B2
N3
N1 / N2
CG3
CG1/ CG2
CG4
CG5
Mn09
Mn13
Mn08
Mn07
Mn06
Mn12
Mn01
Cn1
Cn2
Cn3
Cn4
Cn6
Cn7
Cn5
Cn8
Cn9
Cn11
Mn14
Mn02
Mn11
Mn05
Mn10
Mn04
Ms5
Ms6
Ms7
Ms9
Mn03
Ms8
Ms4
Ms2
Ms3
Ms1
MO1
Rn1
Rn2
Rn3
Rn4
Rn5
Rm1
Rm2
Rm3
Rm4
Cs1
Cs2
Cs3
Cs4
Cs5
Cs6
C1
C2
C3
C4
C5
C6
R1
R2
Rs1

MEEKATHARRA
WILUNA
CUE
MOUNT MAGNET
LEINSTER
LAVERTON
LEONORA
MENZIES

Yeelirrie Calcrete



N
0 25 50 75 100
Kilometres
Geographic Coordinate System
Datum: GDA94

Legend

- Populated Places
- Catchment Boundary (SE, 2011)
- Yilgarn Palaeochannel (SE, 2011)

Number of Taxa

- > 30
- 20 - 29
- 10 - 19
- 1 - 9
- No Data

BHP Billiton Yeelirrie Development Company Pty Ltd	Project: **Proposed Yeelirrie Development**		Title: Regional Stygofauna Diversity
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	Job No.: 42907132	File No.: 42907132-SUS-257.mxd	Figure: 2-2
			Rev. A, A3

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117°0'0"E
120°0'0"E
123°0'0"E
27°0'0"S
30°0'0"S

Nabberu
Burnside
Murchison
Carnegie
Carey
Moore
Raeside

B1/B2
N3
N1 / N2
CG3
CG1/ CG2
CG4
CG5
Mn09
Mn13
Mn08
Mn07
Mn06
Mn12
Mn01
MEEKATHARRA
Cn1
Cn2
Cn3
Cn4
Cn6
WILUNA
Cn7
Cn5
Cn8
Mn02
Mn14
Ms5
Mn11
Mn05
Mn10
Yeelirrie Calcrete
Cn9
Cn11
Cs1
Ms6
Mn04
Ms7
Ms9
CUE
Cs2
Mn03
Rn2
Rn3
Rn4
Cs3
Cs5
Cs4
Cs6
C1
C2
LEINSTER
Rn1
Rn5
Ms8
Ms4
Ms2
MOUNT MAGNET
C3
Ms3
Ms1
MO1
Rm4
Rm3
Rm1
Rm2
C4
C5
LAVERTON
R1
C6
LEONORA
R2
Rs1
MENZIES

DAMPIER
Western Australia
WILUNA
LEONORA
PERTH
ALBANY

N
0 25 50 75 100
Kilometres
Geographic Coordinate System
Datum: GDA94

Legend

- Populated Places
- Catchment Boundary (SE, 2011)
- Yilgarn Palaeochannel (SE, 2011)

Number of Taxa

- 1 - 4
- 10 - 15
- 5 - 9
- No Data

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Project: **Proposed Yeelirrie Development**

Title: Regional Troglofauna Diversity

Drawn: DL	Approved: KD	Date: 01/03/2011
Job No.: 42907132	File No.: 42907132-SUS-258.mxd	

Figure: 2-3

Rev. A
A3

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2.5.2 Species Distributions

The majority of identified stygofauna (112) and troglofauna (25) species were known only from single calcretes (henceforth 'unique' species), although in the stygofauna, a significant proportion of species were recorded from more than one calcrete (henceforth, 'shared' species). Approximately one-third of shared species were sampled from calcretes in close proximity to one another (neighbour distribution). The remaining shared species occurred within several calcretes far away from one another, or were regionally widespread (widely distributed). Table 2-2 shows the numbers of species within each distribution category. Distributions of some of the taxa were not able to be assessed due to unresolved identifications.

Table 2-2: Number of records of unique species, species with neighbour distribution, widely distributed species, and undetermined taxa within the combined data set.

	Stygofauna	Troglofauna
Unique species	111	16
Neighbour distribution	21	3
Widely distributed	32	0
Undetermined	18	15
Total sp.	**182**	**34**

Although unique species constituted over 60 % of the stygofauna diversity, each calcrete contained varying proportions of unique species and shared species. Widespread stygofauna made up a considerable proportion of the total diversity (21 - 52 %) in the five calcretes which recorded the richest assemblages (Table 2-3).

Table 2-3: Proportions of unique species, neighbouring species, and widely distributed species recorded from the most species-rich calcretes in the combined data set.

Stygofauna	**Hinkler Well**	**Uramurdah**	**Lake Violet**	**Paroo**	**Depot Springs**
Unique sp.	39 %	21 %	13 %	33 %	24 %
Neighbour sp.	18 %	43 %	46 %	10 %	0 %
Widely distributed	27 %	21 %	29 %	33 %	52 %
Undetermined	15 %	14 %	13 %	24 %	24 %
Total sp.	**32**	**27**	**24**	**21**	**21**
Troglofauna	**Sturt Meadows**	**Uramurdah**	**Bubble Well**	**Lake Violet**	**Lake Miranda E**
Unique sp.	69 %	0 %	20 %	20 %	0 %
Neighbour sp.	0 %	17 %	0 %	20 %	20 %
Widely distributed	0 %	0 %	0 %	0 %	0 %
Undetermined	31 %	83 %	80 %	60 %	80 %
Total sp.	**13**	**10**	**6**	**5**	**5**

Stygofauna species with neighbour distribution were particularly prevalent where calcretes were located geographically close to one another (*e.g.* Lake Violet and Uramurdah) (Table 2-3). Commonly detected, regionally widespread stygofauna included several cyclopoid copepods, and one species of syncarid. Some species were recorded from a few, widely-separated calcretes including cyclopoid and harpacticoid copepods, syncarids, ostracods, and three dytiscid diving beetles (*Limbodessus macrohinkleri*, *L. phoebeae*, and *L. yuinmeryensis*). Taxa detected only at neighbouring calcretes included several isopods (*Haloniscus*), harpacticoids, ostracods, syncarids, and several species of diving beetles. However, the overwhelming majority of species in these groups were unique to single calcretes.

Most calcretes recorded a considerable proportion of unique species, and the overall diversity of unique species throughout the region supports the calcrete island hypothesis to some degree. However, the common occurrence of widespread and neighbour-distributed species in many calcretes suggests that the hypothesis does not apply to all species of stygofauna.

Troglofauna distribution patterns were more difficult to categorise due to a general lack of data, and the poor state of taxonomic resolution. The majority of calcretes recorded a high proportion of undetermined troglofauna taxa which were unable to be assessed for distribution. Sturt Meadows was the exception, recording the highest number of troglofauna in the region, including mostly unique species (9 out of a total 16). Range-restricted troglobitic species are known from some of the taxonomic groups collected from calcretes in the Yilgarn, including spiders, palpigrades, pseudoscorpions, and some isopods (Barranco and Harvey 2008, Edward and Harvey 2008, Platnick 2008). No widespread troglofauna species were recorded, although three species were shared between neighbouring calcretes; a spider and two species of pseudoscorpions.

2.5.3 Similarity of Assemblages

To determine the relative similarity of the calcrete stygofauna assemblages, the records were analysed using Bray-Curtis similarity index (1-BC) (Bray and Curtis 1957). There were too few troglofauna records to make analysis possible. Calcrete assemblages with less than two stygofauna species were excluded. Unique species (singletons) were also excluded, so the analysis was based upon the composition of shared species in the assemblage.

Bray-Curtis similarity of the well-studied (>40 bores sampled) calcretes with their nearest neighbours, as well as with other calcretes in the same catchment, and with calcretes further afield in different catchments are shown in Table 3-4. Sturt Meadows is excluded as there were insufficient numbers of shared species for analysis. Of the five well-studied calcretes (>40 bores sampled), each assemblage was most similar to its nearest neighbouring calcrete (Table 3-4), and assemblage similarity generally declined with distance. The decline in shared species over geographical distance may reflect the current occurrence of barriers to species dispersal (including geological and hydrological discontinuity and increasing salinity) between historically connected habitats (Humphreys 2001).

Table 2-4: Bray-Curtis similarity (1-BC, as a percentage) of species assemblages recorded from well-studied calcretes in the Yilgarn region. Undetermined species excluded, the top two highest percentages reported. Sturt Meadows assemblage had no level of similarity to assemblages at other calcretes.

Calcrete	Similarity to Other Assemblages		
	Nearest neighbour	Same catchment	Different catchment
Uramurdah	Lake Violet (60 %)	Hinkler Well (45 %) Lake Way W (22 %)	Jundee Sth Hill (28 %) Taincrow (25 %)
Lake Violet	Uramurdah (60 %)	Hinkler Well (48 %) Paroo (35 %)	Austin Downs (32 %) Jundee Sth Hill (31 %)
Hinkler Well	Lake Violet (48 %)	Uramurdah (45 %) Paroo (33 %)	Austin Downs (38 %) Depot Springs (38 %)
Paroo	Diamond Well (50 %)	Lake Violet (35 %) Hinkler Well (33 %)	Killara (46 %) Windimurra (40 %)

2.5.4 Records from Yeelirrie

Very little sampling had been undertaken at Yeelirrie prior to the commencement of the current survey. The Western Australian Museum had not conducted extensive collections at Yeelirrie (W. Humphreys pers. comm.), although three taxa were recorded from three bores, as follows:

- Amphipoda indet.;
- Isopoda: Oniscidea indet.; and
- Copepoda*: Mesocyclops brooksi*.

Sampling of two bores in the neighbouring Albion Downs calcrete also revealed three taxa:

- Amphipoda: Chiltoniidae indet.;
- Copepoda: Harpacticoida indet.; and
- Bathynellacea: Parabathynellidae indet.

The low diversity recorded in both areas is likely due to the limited sampling effort. Regional trends have shown that high survey intensity tends to be associated with high species diversity. Based upon these trends, it could be inferred that intensive sampling for troglofauna and stygofauna at Yeelirrie could yield a greater richness of species, as has been recorded elsewhere. The indicative regional distribution trends would suggest that a proportion of species potentially occurring at Yeelirrie could be widespread, or found to occur in two or more neighbouring calcretes. It is also likely that a number of new, undescribed species could be expected, some of which will not be likely to occur at other calcretes in the region.

3 EXISTING ENVIRONMENT AT YEELIRRIE

3.1 Rainfall and Evaporation

The average annual rainfall for Yeelirrie is 238 mm, although the frequency and amount of rainfall during any given year is highly variable (URS 2011). Yeelirrie receives 67 % of mean annual rainfall in summer and autumn (URS 2011). Rainfall from thunderstorms during summer is regularly higher in intensity than winter rainfall. Mean annual pan evaporation is 2,412 mm at Yeelirrie, and potential evaporation exceeds average rainfall in every calendar month (URS 2011). Groundwater recharge is dependent upon infrequent, high-intensity rainfall events such as tropical cyclones in summer and frontal storms in winter (URS 2011).

3.2 Geomorphology and Geology

The Study Area is located in the north-eastern part of the Archaean Yilgarn craton. The Yilgarn craton formed a plateau during the Cretaceous (145 - 65 mya), which was eroded into a series of deep valleys during the early Tertiary period (URS 2011). Subsequent in-fill of the valleys has occurred over millions of years of erosion, deposition, and in-situ weathering of the bedrock, resulting in the current landscape.

The Yeelirrie palaeochannel comprises five major land systems identified by the WA Department of Agriculture and Food (Pringle *et al*. 1994, Payne *et al*. 1998, URS 2011):

1. Breakaways – granite outcrops occurring at the margins of the palaeochannel;
2. Wash Plains – rocky hard-pan plains immediately down-gradient of the breakaways;
3. Sand Plains – broad plains of alluvial and colluvial deposits, gently sloping into the valley centre;
4. Playas – hard-pan and silt/clay flats associated with the central valley floor, often including shallow saline depressions (Blandford 2010); and
5. Calcretes – carbonate deposits in the centre of the palaeochannel, in some areas forming outcropping domes with lower-relief sides.

3.3 Hydrology and Hydrogeology

Each of the five land systems of the palaeochannel has important hydrological characteristics, which influence subterranean fauna habitat. Surface runoff from the Breakaways and Wash Plains is high, typically characterised by short-lived sheet flows and shallow stream flows. Infiltration in the intermediate Sand Plains prevents most of this water reaching surficial aquifers in the centre of the palaeochannel (Blandford 2010, URS 2011). High-intensity rainfall events may result in surface flows from the periphery reaching the Playas in the centre of the palaeochannel, between the Sand Plains and the Calcrete. The Playas may hold surface water (ponding) after rainfall, and facilitate groundwater recharge in some circumstances (URS 2011). Recharge on the Calcrete via direct infiltration is likely to be high, due to soil porosity. However, the margins of many calcretes are elevated, resulting in runoff (URS 2011).

Discharge of the surficial aquifer is dominated by evapo-transpiration in the centre of the palaeochannel. In areas dominated by groundwater-dependant vegetation, such as *Eucalyptus gypsophila)* (G. Cockerton pers. comm.), evapo-transpiration is a major discharge mechanism (URS 2011). Discharge also occurs down-channel into large playas such as the Yeelirrie Playa and Lake Miranda. Longitudinal flows between surficial aquifers, and vertical transfer of groundwater between surficial and deeper palaeochannel aquifers is thought to be density-driven, dependent upon salinity (URS 2011).

3.4 Calcrete

Calcrete is a carbonate rock formed by the *in situ* replacement or displacement of the alluvial and colluvial deposits by magnesium and calcium carbonate precipitated from percolating carbonate-saturated groundwater (Mann and Horwitz 1979). The source of the carbonate-rich groundwater is related to the decomposition of ultramafic minerals in greenstone rocks, and the precipitated carbonate is generally calcite rather than dolomite (Geotechnics 1972). Bodies of calcrete generally occur at the margins of present-day salt lakes, in the main tributaries in the palaeodrainage systems (Sanders 1974). Dissolutional processes within groundwater (such as the rise and fall of the water table, and chemical/ biological weathering) continue to operate within the calcretes to the present day (Mann and Horwitz 1979, URS 2011).

The calcretes at Yeelirrie form a broad mound in the middle of the palaeochannel, covered in weathered material and alluvium. The size, shape, thickness and structure of calcrete varies throughout the palaeochannel, also between separate calcrete deposits (URS 2011). The internal structure of the calcretes is variably massive, to fractured and friable, with frequent dissolution features such as vugs and cavities (URS 2011). The permeability and transmissivity of calcrete is generally high, dependent upon the internal structure (URS 2011).

The lateral and vertical margins of calcrete grade into transitional calcrete: a friable aggregate of clay-quartz and calcrete. Transitional calcrete is further graded into carbonated clay-quartz and other superficial formations. These formations vary in porosity and transmissivity, and some show secondary dissolutional features such as vugs (URS 2011). Vuggy formations (such as carbonated clay-quartz and sandstone), or porous layers such as coarse alluvium (grit), may provide prospective habitats for subterranean fauna.

Certain subterranean species are able to inhabit micropores within soils (*e.g.* enchytraeid worms) or alluvial aquifers (*e.g.* some copepods and syncarids). However, the patterns of stygofauna distribution throughout the Yilgarn region suggest that less habitable geological layers such as consolidated alluvium and clay can form barriers to species dispersal between calcrete deposits (Humphreys 2001).

3.5 Subterranean Habitat Assessment

The habitat assessment herein is provisional, based upon the geological data (URS 2011 and WMC 1978), hydrological data (URS 2011), and vegetation data (Western Botanical 2011), available at the time of writing.

Figure 3-1 shows the inferred distribution of saturated calcrete (calcrete below water table), based on hydrogeological investigations (URS 2011). The cross-sectional drill lines formed the basis for the hydrological investigations; therefore the distribution of calcrete between these lines is inferred. There are a number of inferred breaks between calcrete bodies to the north western and south eastern ends of the palaeochannel. The distribution of unsaturated calcrete habitats (calcrete above water table) is shown in Figure 3-2. This data is based on Western Mining Corporation's 1978 auger drilling program (WMC 1978) and surface expression of calcrete. The map of unsaturated calcrete showed significantly larger and more continuous areas of calcrete than the recent hydrological program. The locations and extent of the WMC auger holes, relative to BHP Billiton's cross sectional drill lines are not known, and the extent of inference between sample sites is also unclear.

Western Botanical (a division of Landcare Holdings Pty Ltd) conducted a vegetation survey for the Project. The survey identified a number of calcrete-associated vegetation complexes (Figure 3-3a) in the centre of the palaeochannel, including groundwater-dependant species such as *E. gypsophila* (G. Cockerton pers. comm.), which are associated with calcrete aquifers. The calcrete associated vegetation complexes have finer-scale distribution boundaries than the calcrete revealed by geological and hydrogeological data, particularly between drill lines.

Figure 3-4 shows the inferred boundaries of subterranean (below surface expression) calcrete habitats, based on a combination of hydrogeological data, auger data, drill logs, and vegetation data. The various data sources were mostly in agreement regarding the extent of the central calcrete, from the deposit to the immediate northwest (ending north of line G). Vegetation data indicated an extension of the central calcrete between lines G and A that was not drilled and sampled (Figure 3-4). The vegetation data also showed discontinuities between calcretes in the northwest study zone, on lines A, E, 312, and P. Vegetation communities between these calcretes showed affinities with sandplain and hardpan land systems, which may indicate potential barriers to dispersal between these calcretes.

Southeast of the deposit, auger data and vegetation data suggested the continuation of calcrete through lines C and K (Figures 3-2, 3-3a). However, hydrological data showed a discontinuity between the deposit and line C (Figure 3-1). Drill logs and hydrogeological cross sections for lines C and K (URS 2011) showed no calcrete, despite some vuggy carbonated material (up to 20%) in hardpan and sandstone units.

There was a clear separation between the central calcrete and the calcrete surrounding the Yeelirrie Playa (lines L, N, SB14). The connectivity of calcrete between lines L, N, and SB14 is poorly resolved, although all of the data sources suggested large calcrete habitats at lines L and SB14, with smaller or more patchy deposits under the Playa at line N (Figure 3-4).

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

MGA ZONE 50
MGA ZONE 51

INFERRED BREAK

INFERRED BREAK

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses
- Submerged Wet Calcrete (URS, 2010)

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Inferred Saturated Calcrete

Drawn: DL/AK/CJT	Approved: JN	Date: 25/01/2011
Job No.: 42907132	File No.: 42907132-SUS-190_RG.mxd	

Figure: 3-1

Rev. G

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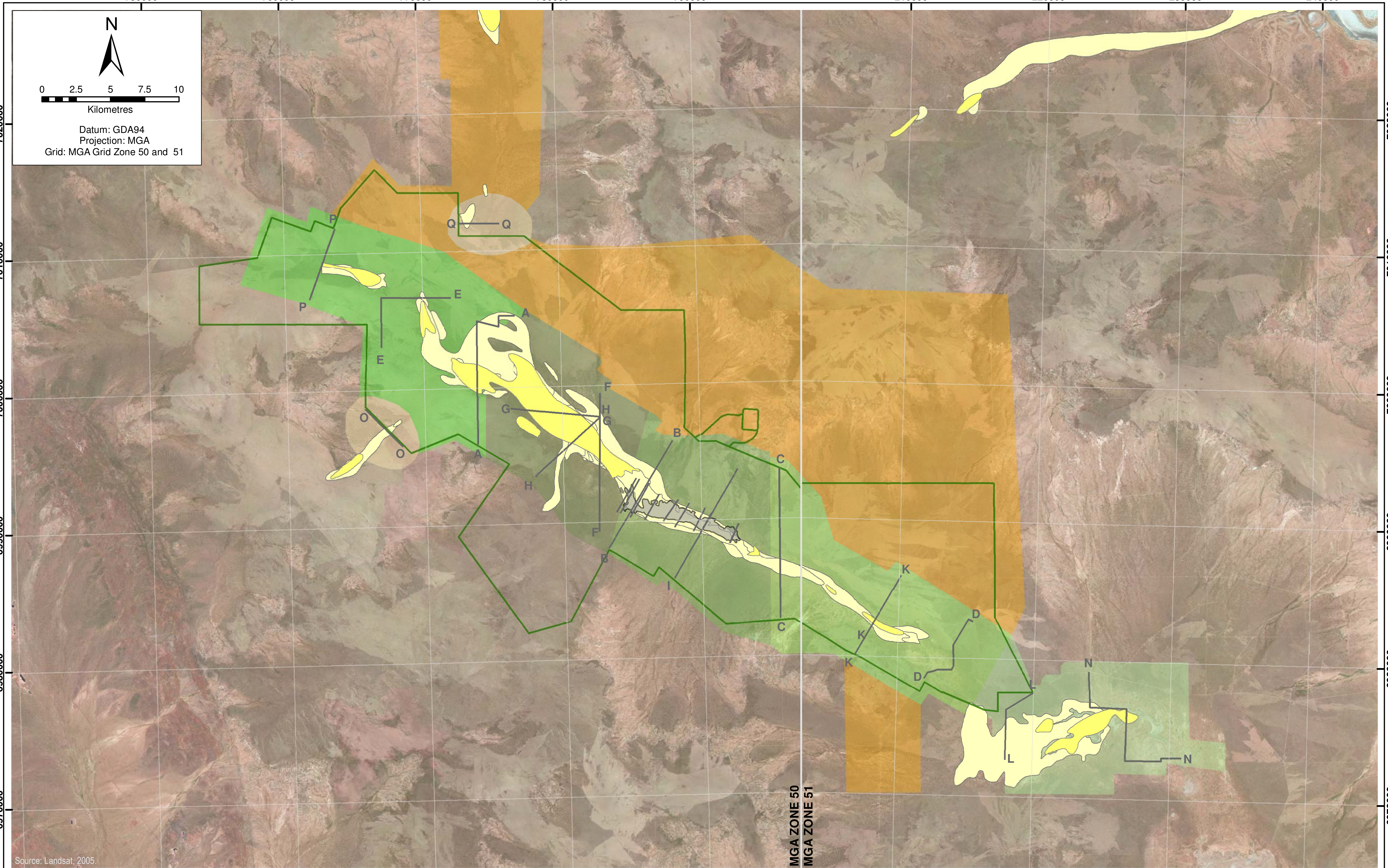

Legend

- Pit Outline
- Project Area

Unsaturated Calcrete (WMC, 1978)

- Calcrete
- Other Carbonated Material
- Drill and Infill Traverses

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

BHP Billiton Yeelirrie Development Company Pty Ltd	Project: Proposed Yeelirrie Development	Title: Unsaturated Calcrete
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	Job No.: 42907132; File No.: 42907132-SUS-195_RF.mxd	A3

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N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

Inset

MGA ZONE 50
MGA ZONE 51

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

BHP Billiton Yeelirrie Development Company Pty Ltd	Project: **Proposed Yeelirrie Development**		Title: Calcrete Inferred from Vegetation Mapping
Subterranean Ecology Scientific Environmental Services www.subterraneanecology.com.au	Drawn: CJT/DL	Approved: JN / Date: 09/03/2011	Figure: 3-3 / Rev. 0
	Job No.: 42907132	File No.: 42907132-SUS-247_R0.mxd	A3

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Legend

- CMGbS, Mulga Grevillea berryana Shrubland on Calcrete
- CMiS, Melaleuca interioris Shrubland on Calcrete
- CAbS, Acacia burkittii Shrubland on Calcrete
- CErG and CAbS and CEgW, Mosaic of Eragrostis sp. Grassland on Calcrete and Acacia burkittii Shrubland on Calcrete and Eucalyptus gypsophila Woodland on Calcrete
- CAbS and CCpW, Mosaic of Acacia burkittii Shrubland on Calcrete and Casuarina pauper Woodland on Calcrete
- CEgW, Eucalyptus gypsophila Woodland on Calcrete
- CMpS, Maireana pyramidata Shrubland on Calcrete
- CCpW, Casuarina pauper Woodland on Calcrete
- CAbS and CEgW, Mosaic of Acacia burkittii Shrubland on Calcrete and Eucalyptus gypsophila Woodland on Calcrete
- CRsS, Rhagodia sp. Yeelirrie Station Shubland on Calcrete
- CApS, Atriplex sp. Yeelirrie Station Shrubland on Calcrete
- CErG, Eragrostis sp. Grassland on Calcrete
- CMxS, Melaleuca xerophila Shrubland on Calcrete
- CErG and CLaS, Mosaic of Eragrostis sp. Grassland on Calcrete and Lycium australe Shrubland on Calcrete
- CLaS, Lycium australe Shrubland on Calcrete
- CsMp, Cratystylis subspinescens and Maireana pyramidata Shrubland

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Title
Calcrete Inferred from Vegetation Mapping - Legend

Drawn: CJT/DL	Approved: JN	Date: 09/03/2011	Figure: 3-3	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-247B_R0.mxd			A3

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750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

MGA ZONE 50
MGA ZONE 51

Calcrete - Lines A, E, 312 and P

Central Calcrete

Central Calcrete

Inferred potential habitat line C and K

Calcrete - SB14

Calcrete - Line L

Inferred potential habitat line N

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Inferred Boundaries of Subterranean Fauna Calcrete Habitat (SE, 2011)

- Inferred potential subterranean fauna habitat
- Inferred subterranean fauna calcrete habitat

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Title
Inferred Boundaries of Subterranean Fauna Calcrete Habitat

Drawn: CJT	Approved: JN	Date: 25/01/2011	Figure: 3-4	Rev. B
Job No.: 42907132	File No.: 42907132-SUS-249_RB.mxd			A3

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3.6 Conceptual Eco-hydrogeological Model for Yeelirrie

A conceptualisation of the subterranean habitats of the Yeelirrie palaeochannel, based upon the habitat assessment is presented in Figure 3-3a. The codes for geological layers (URS 2011) are as follows:

- PCH SAND - Basal palaeochannel sand;
- PCH CLAY - Palaeochannel clay;
- GRIT - Coarse, tertiary palaeochannel sand;
- CCQ - Carbonated clay quartz;
- SS & AL - Sandy alluvium, silty sand, and silty sandstone;
- CALCRETE – Calcrete, ranging from porcellanous to earthy/ transitional calcrete;
- AL - Superficial soils and alluvium;
- PLAYA – Playa land system; and
- WT - Conceptual groundwater level (water table).

The geology and hydrology of the palaeochannel is represented in long-section (vertical scale exaggerated) from the northwest (on the left-hand side of Figure 3-5) to the southeast (right). Inferred hydrogeological inputs, flows, and outputs are shown by arrows. The vegetation interactions are indicated in the calcretes, although phreatophytic vegetation may not be limited to calcrete only. Bore holes are represented by vertical black lines. The conceptual location of most of the cross sectional drill lines is indicated by letters or numbers above bore symbols.

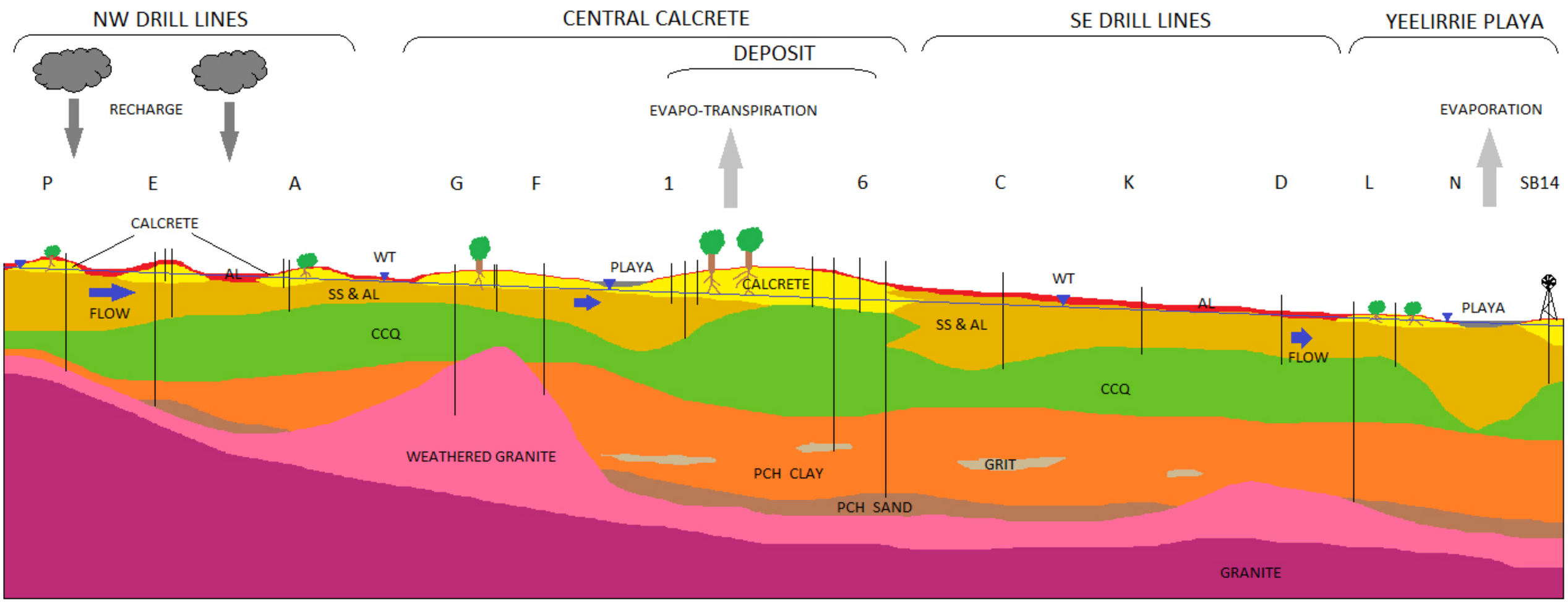
The interpreted stratigraphy (long-section) in the Yeelirrie Groundwater Report (URS 2011) shows some differences to the eco-hydrogeological model in the level of connectivity between calcrete habitats. Figure 3-5 shows discontinuous calcrete bodies to the northwest and south east of the central calcrete, inferred from Figures 3-1 and 3-2. Information regarding the suitability of geological layers such as sandstone, alluvium, and carbonated clay quartz for subterranean fauna habitat is incomplete at this time.

A summary of the main points of the eco-hydrogeological model:

1. Calcretes occur throughout the palaeochannel, forming the primary habitats for troglofauna (in the humid zone above the water table) and stygofauna (in the saturated zone below the watertable),
2. Physical and chemical characteristics of the calcretes influence the subterranean habitat. Important factors include the size and three-dimensional shape of calcrete bodies, internal structure (heterogeneous porosity, geological intrusions), hydrological processes (groundwater levels, flows, recharge and discharge), water physicochemistry (pH, salinity, redox), nutrient and oxygen cycles, and interactions with the surface (*e.g.* infiltration, vegetation roots);
3. Heterogeneity within calcrete bodies (*e.g.* variable thickness of calcrete, variable salinity throughout the water profile) is almost certain, providing many ecological niches for various species (Humphreys 2001);
4. Some geological layers other than calcrete (*e.g.* transitional calcrete, porous sandstone, alluvium) may provide potential habitat for certain species; and

5. The potential for unique species and assemblages occurring only within single calcrete habitats arises from physical and chemical differences between habitats (*e.g.* increasing salinity down-channel), and physical barriers to species dispersal (*i.e.* discontinuous calcretes) (Humphreys 2001).

Regional comparisons have revealed a relationship between the proximity of calcrete habitats and the similarity of their stygofauna assemblages (shared species) (Table 3-4). This is likely a result of climatic or geological factors isolating previously continuous habitats over evolutionary time-scales (Humphreys 2001). Subterranean habitats, which currently remain connected, would be expected to share the majority of their species and to contain relatively similar assemblages, except where heterogeneity within the habitat provides a spatially limited niche (Humphreys 2001).



KEY:
WT - Water table
AL - Alluvium (sand, loam)
SS & AL - Sandstone & alluvium
CCQ - Carbonated clay/ quartz
PCH - Palaeochannel
FLOW - Inferred hydrological flow paths

~ 10km

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Job No.:	File No.:			A3

4 YEELIRRIE SUBTERRANEAN FAUNA SURVEY EFFORT

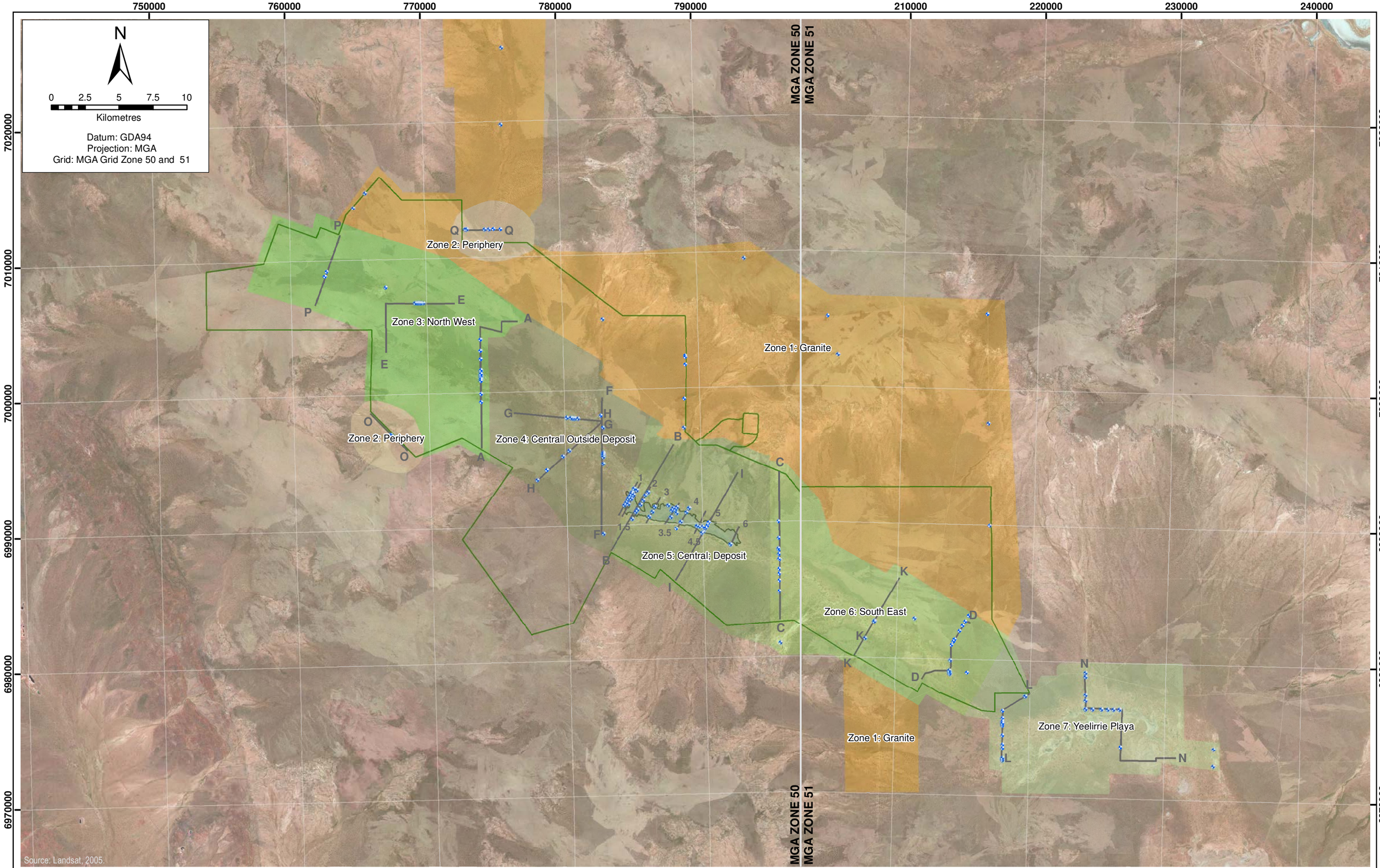
4.1 Study Area

The Study Area (approximately 1497 km^2) was designed to encompass multiple calcrete habitats throughout the length of the palaeochannel, with special reference to the Project footprint (Figure 1-3), which encompasses direct and indirect impact, such as excavation, dewatering, and drawdown due to mining activities. The sites of investigation within the Study Area comprised of pre-existing pastoral bores and wells, as well as purpose-built subterranean fauna sampling bores, drilled along cross-sectional lines throughout the palaeochannel.

The cross-sectional drill lines, labelled by a letter in Figure 4-1, were strategically located to provide hydrogeological information, as well as intercepting calcrete bodies for subterranean fauna sampling. The Study Area was divided into seven zones reflecting geography and geology. The seven study zones, are referred to throughout this report, are as follows:

1. Granite Pastoral Bores
2. Periphery (Lines O, Q)
3. North West (Lines P, 312, E, A)
4. Central, Outside Deposit (Lines F, H, G)
5. Central, Deposit (Lines 1 - 6)
6. South East (Lines C, K, D)
7. Yeelirrie Playa (L, N, SB14)

Figure 4-1 shows the approximate boundary of each zone in the Study Area.



Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses
- Sampling Bores

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Title: Subterranean Fauna Study Zones

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Figure: 4-1 | Rev. G | A3

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4.2 Study Approach

Field sampling for stygofauna and troglofauna was conducted over six sampling events:

1. March 2009 – pilot survey (16 pastoral bores and wells);
2. August 2009 – stygofauna survey of the deposit and central calcrete (31 bores);
3. November 2009 – stygofauna and troglofauna survey of all accessible drill lines (78 bores);
4. January 2010 – second round survey of accessible drill lines (148 bores);
5. March 2010 – third round survey of accessible drill lines (193 bores); and
6. September 2010 – fourth round stygofauna and troglofauna survey, regional drill lines only, and pastoral bores in granite aquifers (134 bores).

Each sampling event was designed to make use of the available bores to achieve adequate representation of subterranean habitats inside and outside of the deposit. The bores were located to target the primary calcrete habitats and secondary porous geological habitats along each drill line. The majority of samples were obtained from bores occurring within the central palaeochannel (*i.e.* dedicated BHP Billiton stygofauna and troglofauna bores, air-core exploration bores and pastoral bores in calcrete). Some sampling was undertaken in sub-prime habitats such as peripheral granite aquifers (pastoral bores), or in deeper palaeochannel aquifers (BHP Billiton hydro bores) as these areas targeted possible wellfield locations.

As results became available, the focus of each sampling event was adapted to address knowledge gaps identified from the previous surveys. The number of bores available for sampling was a constraint in the early stages of the survey, until the dedicated BHP Billiton stygofauna and troglofauna bores were developed between August 2009 and January 2010. Where bores were sampled prior to the EPA (2007) recommended six-month colonisation period, each site was re-sampled up to seven months after the initial sampling. Pumping of the central calcrete aquifer (for hydrological testing) provided an opportunity to achieve stygofauna results prior to colonisation of the bores.

The numbers of samples for each study zone are listed in Table 4-1. The greatest number of samples was obtained from the deposit (242 samples), although the combined total from palaeochannel zones outside of the deposit was significantly greater (527 samples excluding granite wellfield). The northern drill lines and Playa zones, which had the highest numbers of bores outside of the deposit, were the next most intensively sampled zones (153 and 167 samples respectively; Table 5-1).

Table 4-1: The total numbers of samples of each type of sampling method per study zone.

Study Zone.	Pump	Net haul	Scrape	Trap	Oppor-tunistic	Total samples	# bores
Granite Pastoral Wellfield (PW)		21	3		1	25	24
Peripheral drill lines (O, Q)		16	25	6		47	18
Northern Drill Lines (P, 312, E, A)		67	58	26	2	153	42
Central calcrete (F, H, G)		47	36	12		95	31
Central calcrete (deposit)	26	56	94	68		244	64
Southern Drill Lines (C, K, D)		42	20	1	1	64	31
Playa Drill Lines (L, N, SB14)		68	63	36		167	47
Total	**26**	**317**	**299**	**149**	**4**	**795**	**259**

The total number of stygofauna and troglofauna samples along each drill line is represented in Figure 4-2. Opportunistic samples such as vegetation roots from bore holes (3 samples), and a net sample from a stock water trough were excluded. Comparison of sample numbers (Figure 4-2) *versus* number of available bores (Figure 4-3) shows a greater ratio of troglofauna samples than stygofauna samples along most drill lines. This is attributed to simultaneous trapping and net scraping methods for troglofauna over the course of the survey.

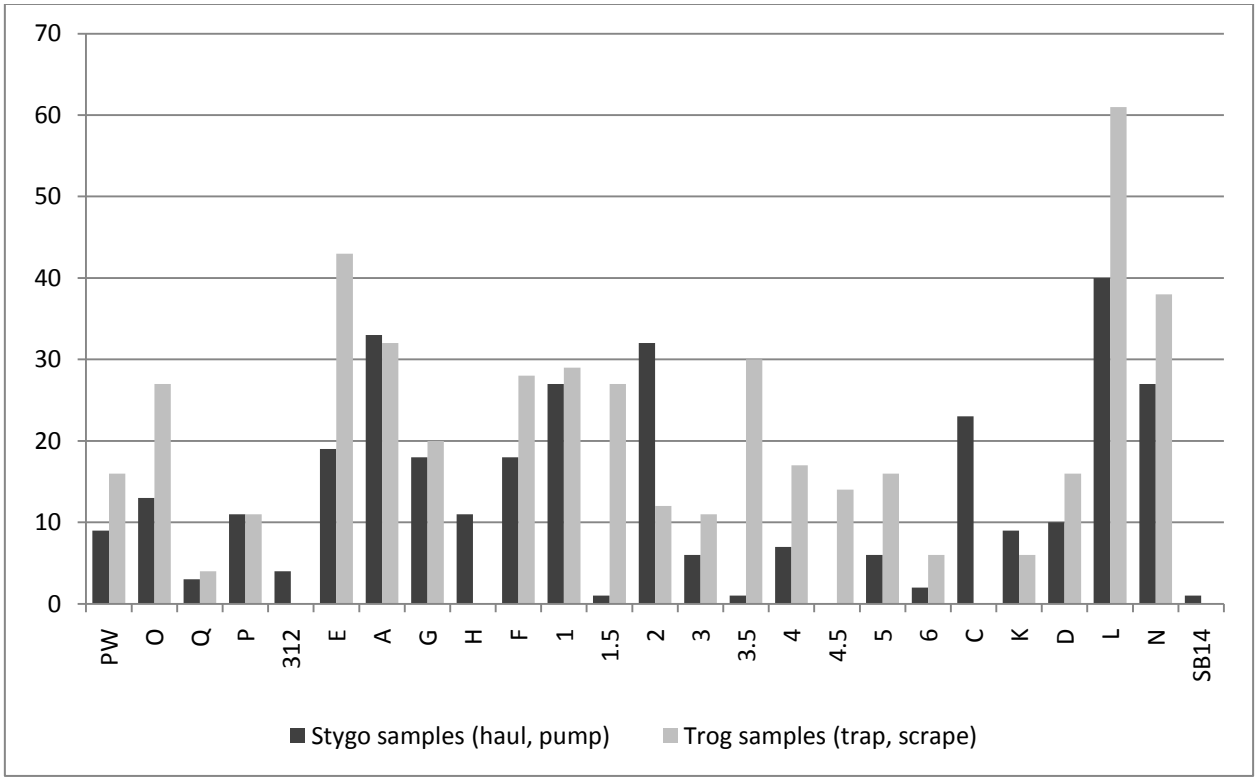


Figure 4-2: Total number of samples targeting stygofauna and troglofauna per drill line (excluding opportunistic samples).

The overall sampling effort exceeds EPA (2007) minimum requirements for consideration of stygofauna and troglofauna. Despite differences in sampling effort, almost all of the zones exceeded the EPA (2007) minimum sampling requirements also, except in a few cases (*e.g.* sampling in the granite wellfield). The high number of samples has provided a good basis for comparison of faunal results between each zone. Species accumulation curves were used as the survey progressed, to assess the adequacy of the overall sampling effort for troglofauna and stygofauna (Subterranean Ecology 2009a). Overall, the sampling at Yeelirrie represents one of the most intensive survey efforts for subterranean fauna of any calcrete in the Yilgarn Region.

4.3 Number of Bores and Construction Details

Figure 4-3 shows the total number of bores and wells sampled for stygofauna and troglofauna on each line during the survey.

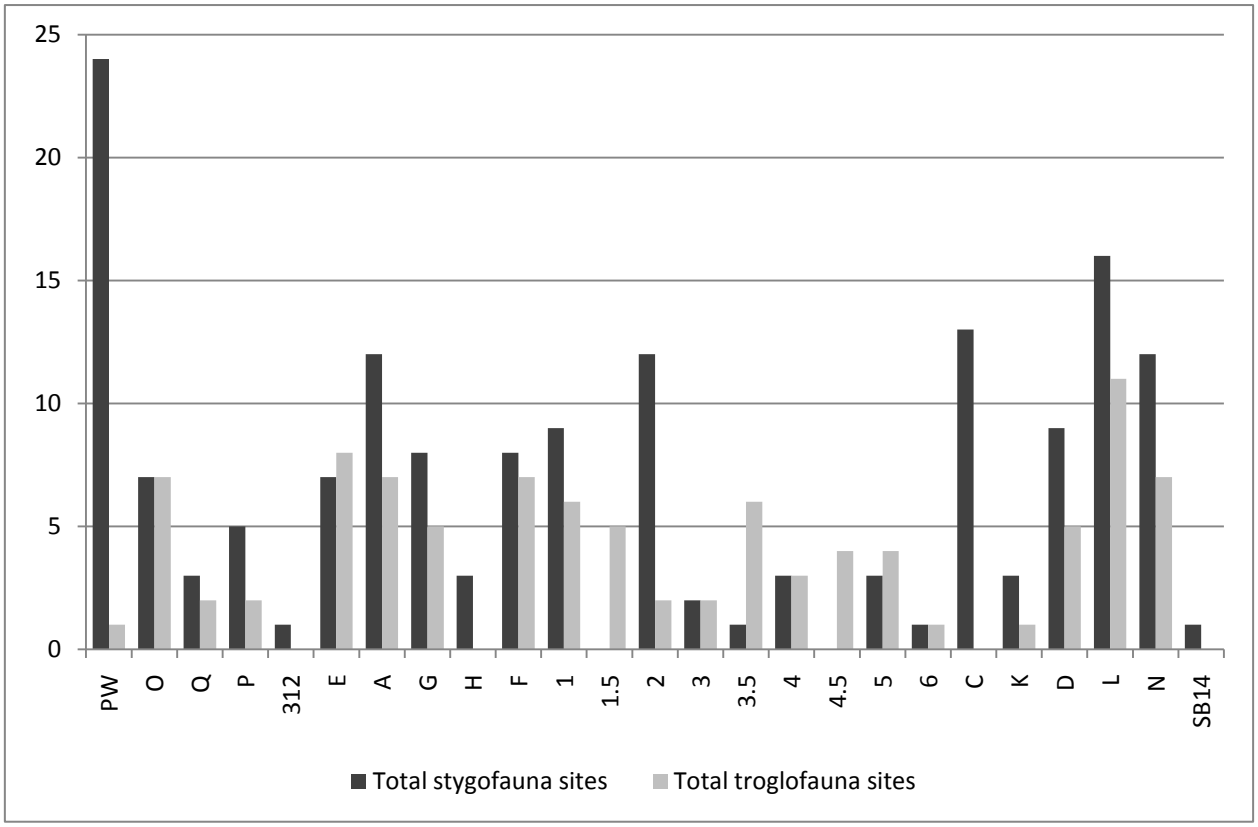


Figure 4-3: The number of bores and wells sampled for subterranean fauna within each of the drill lines.

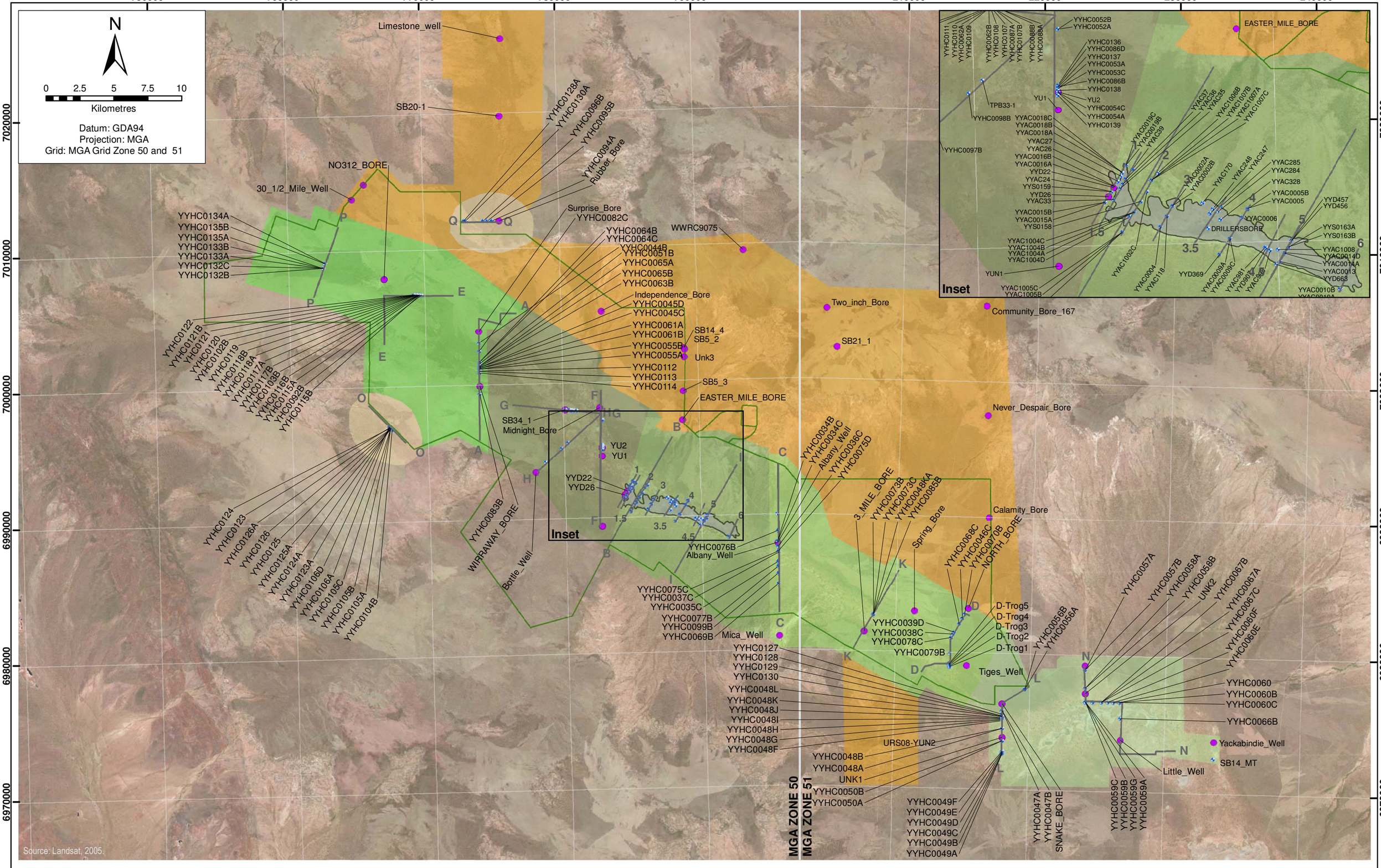
A total of 259 wells and bores were sampled throughout the Study Area, as follows:

- Pre-existing wells and bores – (22) pre-existing bores and wells of various sizes and construction, targeting the surficial aquifer, throughout the central part of the palaeochannel. Some 25 pre-existing bores and wells in granite aquifers at the periphery of the palaeochannel were also sampled. Pastoral bores in granite aquifers were sampled using a combined net hauling and scraping methodology, as trapping was not viable in these types of bores.

- BHP Billiton bores – new bores drilled between August 2009 and January 2010 for hydrological investigation and subterranean fauna sampling. Drill lines were generally located perpendicular across the centre of the palaeochannel, intercepting calcrete. At each drill site, a number of bores were constructed, including deep hydrological bores, a shallow stygofauna bore, and an uncased troglofauna bore. Further details follow:
    - *Hydrological bores* – (19) targeting various aquifers deep in the palaeochannel. 50 mm PVC construction, slotted with 1 mm slots. Sampled by pumping and net hauling to test presence of stygofauna in deeper aquifers.
    - *Dedicated stygofauna bores* – (98) targeting saturated calcrete, surficial aquifers. Air-core drilled to maximum depth of calcrete or transitional calcrete. 50 mm PVC case, 1.6 mm slots throughout the calcrete and transitional calcrete layers, packed with 3 mm – 5 mm gravel, steel outer collar, concrete base, and PVC cap.
    - *Dedicated troglofauna bores* – (74) targeting unsaturated calcrete above water table. Air-core drilled (100 mm diameter) to a depth approximately 1 m below water table, uncased below the surface, with steel or PVC collar.
    - *Air-core and diamond exploration bores* – (21) drilled throughout the uranium deposit only, to a maximum depth of 25 m. Air-core (100 mm) or diamond drilled (150 mm), uncased below the surface, collared with PVC collar, and capped.

Bores targeted for stygofauna included cased BHP stygofauna and hydrological bores, pre-existing cased bores and open wells. Bores targeted for troglofauna included uncased BHP trog bores, air-core and diamond bores, and pre-existing uncased exploration bores. Open wells were not considered suitable for troglofauna sampling due to potential desiccation.

The potential for troglofauna by-catch from pre-existing bores, and the high potential for stygofauna by-catch from uncased bores meant that there were potentially more sample sites for each group than represented in Figure 4-3. Figure 4-4 shows the location of each bore within the Yeelirrie palaeochannel.



Legend

- Project Area
- Pit Outline
- Bore Location
- Pastoral Bore
- Drill and Infill Traverses

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

BHP Billiton Yeelirrie Development Company Pty Ltd

Subterranean Ecology
Scientific Environmental Services
www.subterraneanecology.com.au

Project: **Proposed Yeelirrie Development**

Title: Bore Locations and Traverse Lines

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5. SURVEY METHODS

The sampling methods conform to the recommendations of EPA (2007) Guidance Statement # 54a, and are similar to methods developed by DEC for the Pilbara Regional Subterranean Fauna Survey (Eberhard *et al*. 2004). Survey personnel are listed in Appendix 2.

5.1 Stygofauna Sampling

Stygofauna were targeted by net hauling and pumping.

Net hauling utilised:

- Six hauls of the entire water column with a 50 µm mesh plankton net;
- Aperture of diameter to suit each bore (25 - 150 mm);
- Lead weight and sample vial (35 - 10 mL) to suit each net;
- Sediments agitated by raising and lowering the net at the bottom of the bore;
- Net retrieved at an even pace to avoid bow-wave;
- Each haul emptied into a jug of water; and, following the sixth haul; and
- Sample elutriated, and filtered back through the net to remove water.

To allow for cool-temperature DNA fixation, each sample was preserved in chilled 100 % ethanol, labelled, sealed, and stored on ice. After each field day, samples were transferred into a refrigerator (approximately 2 °C). The samples were transported back to the laboratory in insulated boxes containing ice packs, and stored in refrigerators.

Pumping of the aquifer was conducted at newly constructed bores by URS and BHP Billiton personnel, for hydrological testing. An opportunity to sample for stygofauna during pumping was taken, as the bores had not yet undergone the EPA (2007) recommended 6-month colonisation period (pumping is suggested as a way to overcome the need for bore colonisation). Net hauling of the bores was conducted immediately prior to pumping, and each bore was re-sampled on multiple occasions between 5 and 7 months after pumping occurred.

The water out-flow from the pump was sampled using a 50 µm mesh plankton net. Pumping utilised:

- Two types of pumps; a high flow Grundfos® MP1 submersible pump, and a low flow air-bladder pump;
- Bores were initially purged by airlifting, followed by high flow pumping (aquifer yield test) and low flow pumping (water quality test);
- High flow pump used a 2 mm stone-guard over the intake;
- Low-flow pump had a 6 mm intake;
- The pump was positioned at the top of the slotted interval in each bore;
- High flow pumping rates were 3 - 20 L/ min;
- Low flow pumping rate was approx. 0.12 L/ min;
- Volume from high flow pumping was 7 - 600 L depending upon aquifer yield;
- Volume from low flow pumping was 0.45 - 0.6 L; and

- Water quality measurements taken during low flow pumping were used in place of bailer sampling.

Incidental captures of stygofauna (by-catch) during net scraping of uncased bores formed a considerable supplement to the stygofauna results. The diversity of stygofauna by-catch from scraping was often equivalent to the diversity sampled by net hauling, thus scraping was considered as an additional methodology for targeting stygofauna.

5.2 Troglofauna Sampling

Troglofauna were targeted by net scraping and litter trap sampling.

Net scraping utilised:

- A standard 50 µm plankton net and scraping attachment attached to the main line above the net;
- Scraping attachment comprised many strands of line, radiating out from a central point;
- The scraping attachment and net were used to gently agitate the sides of the bore, dislodging any invertebrates clinging to the bore wall;
- Four hauls of the net from the bottom of the uncased bore (often beneath water table) to the top;
- Each haul emptied into a jug of water;
- Sample elutriated, and filtered back through the net to remove water; then
- Sample preserved in chilled 100 % ethanol, labelled, sealed, and stored on ice.

When utilised at pastoral bores in granite aquifers, or in air-core and diamond bores in the deposit as a combined methodology, scraping sampled the entire water column.

Litter trap sampling utilised:

- PVC traps comprising of a cylinder approx. 100 mm length by 50 mm diameter;
- Traps were filled with moist, decaying leaf litter (Spinifex), sterilised by microwave cooking;
- Lowered via string into uncased bore approx. 1 m above water table (or bottom of bore);
- Left for a duration of six to eight weeks, with the bore capped;
- Traps then retrieved into paper bags to reduce condensation, sealed in plastic snap-lock bags; and
- Labelled and stored in an insulated box for transport back to the laboratory.

At the laboratory, fauna were extracted from leaf litter using Tullgren funnels under heat lamps, for a period of 48 hours, or until the litter had completely dried out. Preservation vials under the funnels contained 100 % ethanol. Following extraction, the samples were sealed, labelled, and stored in a refrigerator at 2 °C.

5.3 Groundwater Data

To characterise the physicochemical parameters of the groundwater, bailer samples were taken from bores prior to stygofauna sampling. One litre of groundwater was sampled using a bailer, from a depth of approximately 1 m below the water's surface. Physicochemical parameters were measured using either a "TPT" brand or a "WTW" brand multi functional water meter. The parameters recorded were temperature (°C), acidity (pH), dissolved oxygen (DO in mg/L), electrical conductivity (EC in mS/cm), and reduction-oxidation potential (Eh in mV). The depth of water in each of the bores, and the total depth of the bores was calculated in the field by counting the number of revolutions of the line reel during hauling. The number of revolutions was then multiplied by the average circumference of the line spool (0.63 m).

5.4 Sorting, Identification and Genetic Analyses

Sorting of preserved samples was conducted in the laboratory, using dissecting microscopes. Each target taxon was identified to the lowest taxonomic rank achievable, and abundance within each sample was recorded. Further identification of specimens was undertaken in-house using published keys and descriptions, under high resolution dissecting or compound microscopes. Personnel and specialist taxonomists involved in sorting and identification are listed in Appendix 2. New taxa were described to morpho-species level using a standard parataxonomic approach.

Images of voucher specimens were compiled by high resolution auto-montage photography using Leica® digital microscope cameras. Some of these images were sent to remote taxonomists to verify identifications, when the specimens themselves were not able to be sent. Voucher specimens are retained in Subterranean Ecology's voucher collection. A reference collection of specimens is lodged with the Western Australian Museum.

Tissue samples from selected specimens were sent to specialists for molecular genetic analyses (DNA analyses). DNA analysis was used to confirm morpho-species identifications and provide information on species distribution, and the potential for morphologically-cryptic divergent haplotypes. Tissue samples were dissected from specimens under hygienic conditions and transported to specialists in Trehalose enzyme; a naturally produced compound that preserves DNA from deterioration. DNA analysis was conducted by Dr T. Finston of Helix Molecular Solutions, Dr S. Cooper and Dr. R. Leys at the South Australian Museum (Appendix 2). The methods of the DNA analyses varied for each group, depending upon the taxa and the hypotheses tested. Where available, sequences from Pilbara specimens and from the online genetic database GenBank® (http://www.ncbi.nlm.nih.gov/genbank/) were used as out groups during the analyses. The methods and results of genetic analyses have been included as appendices to this report.

5.5 Databasing

Quality control of data was maintained through the use of a dedicated relational database. The database was the central repository for site and location data, sampling data, and faunal records. The database was developed by Dr E.S. Volschenk at Subterranean Ecology, using a Microsoft® Access platform (version 2007).

5.6 Survey Adequacy

The adequacy of the survey effort was evaluated by constructing species accumulation curves, from diversity estimators in EstimateS® (Version 8.2, R. K. Colwell, http://purl.oclc.org/estimates).

Results of trapping and net scraping samples were used for troglofauna, while net hauling, scraping and pumping results were used for stygofauna. Low-flow and high-flow pumping samples from the same bore were combined. Species were determined by available genetic and morphological information. Incidence (presence/ absence) data was used rather than abundance data, in order to provide more consistent comparisons between stygofauna (which included some highly abundant species) and troglofauna (low abundance relative to stygofauna).

Predicted total diversity was calculated by diversity estimators in EstimateS® including ICE (Incidence-based Coverage Estimator), Chao 2, Jackknife 1 and Bootstrap. The analyses were run over 1000 replications, with the upper limit for rare species set to five (5). Predicted diversity was plotted against observed species richness (Sobs Mao Tau) as a percentage. Species accumulation curves were then plotted for the predicted Jackknife 1 and Bootstrap estimates, against the observed (Sobs Mao Tau).

5.7 Statistical Analysis

Multivariate, non-parametric data analyses are commonly used in invertebrate ecology to validate the significance of differences in species composition (Clarke 1993). Two descriptive techniques; non-metric multi-dimensional scaling (NMDS) and cluster analysis, were used to compare the subterranean fauna assemblages sampled on each drill line. Analysis of Similarities (ANOSIM) were used to test hypotheses at a defined level of significance (p=0.05). Multivariate statistics were conducted using the software package PRIMER® v6 (Clarke and Gorley 2006).

The aims of the analyses were to determine:

A. Whether the assemblages sampled within each drill line were homogeneous (*i.e.* likely to represent the same subterranean fauna community); and
B. Whether the assemblages sampled between drill lines were homogeneous, thereby representing a similar or continuous community.

NMDS shows the similarity between samples (in this case, drill line assemblages) by representing it as distance in a two-dimensional ordination chart. Cluster analysis assigns samples into hierarchical groups that reflect their relative similarity to one another. The analysis constructed clusters by group-averaging. Significant clusters were determined at 5 % (p=0.05), using the SIMPROF (similarity profile) test; a technique that looks for structure in samples that were unstructured prior to analysis.

The Bray-Curtis dissimilarity measure (inverse of the Sørensen similarity coefficient) was used to assess similarity or dissimilarity in species composition between samples (as in Gauch 1973). The

Bray-Curtis measure is useful in assessing noisy data sets, as it gives less weight to outliers and retains sensitivity with non-normally distributed data (Clarke 1993). All of the multivariate statistical techniques used for the Yeelirrie survey were defined by Bray-Curtis dissimilarity.

The sampling results were organised into a matrix of species by samples. Incidence (presence/ absence data were used in the analyses to standardize the data from different collecting methods. Troglofauna and stygofauna data were analysed separately, and amphibious organisms were included in the stygofauna data. Singletons (species recorded only from a single sample) were excluded from the data set. Species were defined by the available genetic and morphological information at the time of the analysis.

In some cases, species were identified by genetic analysis of a sub-sampled individual. In the absence of contrary evidence, all other similar individuals from the same sample were assumed to belong to the genetically defined species. For example (hypothetical), bore YYD22 sampled in January 2010 resulted in 10 specimens of *Halicyclops eberhardi*, and DNA from one of these specimens was determined to belong to *H. eberhardi* sp. B. In this case, the remaining nine *H. eberhardi* specimens from this sample would also be considered to belong to *H. eberhardi* sp. B.

5.8 Limitations

Subterranean fauna occur in cryptic habitats which renders them inherently difficult to study. Many characteristics of their habitat and ecology offer challenges for sampling and analysis of results. Some of these challenges are listed below:

- Many species are difficult to detect and it is often unknown whether a species is truly rare or simply difficult to detect;
- There is a high proportion of new, undescribed species, which are poorly known taxonomically or ecologically;
- Detection rates are often variable and unpredictable (particularly troglofauna), even at the same bore over multiple samples;
- Bore age and construction affect the potential for colonisation, and this in turn affects sampling results as new bores age over the course of a survey; and
- Bores provide only a minute, artificial window into potentially extensive underground habitats. The habitat within the bore may have slightly different characteristics to the remainder of the subterranean habitat, which may favour some species over others (Hahn and Matzke 2005).

These factors were taken into account when designing the survey and analysing the data, although some of the issues resulting from these factors were unavoidable. Where such issues were encountered, independent advice from specialists was obtained. The results and conclusions of the study are based upon the best information available under these conditions.

6. RESULTS

6.1 Stygofauna Results

A total of 8188 invertebrate specimens were collected over the course of the six surveys, which revealed 46 species of stygofauna and nine species of amphibious subterranean fauna. The combined stygofauna and amphibious fauna assemblages comprised nine orders and 16 invertebrate families. Amphibious subterranean fauna are species that can move between terrestrial and aquatic subterranean habitats, but may need access to water for particular phases of their life cycle.

Indeterminate taxa, *i.e.* those which could not be identified to species or distinguishable morpho-species were excluded due to potential duplication. These records are shown at the bottom of Tables 6-1 and 6-3. These data may be subject to revision pending further identifications of copepod species. In cases where DNA analyses revealed divergent, morphologically cryptic species, genetically undetermined records from the same sample were treated as the same species (see section 5.7). Records that had not been successfully sequenced for DNA were then treated as indeterminate.

Specimen images are shown in Appendix 3, taxonomic reports and genetic information is presented in Appendices 8, 9, 10 and 11, and maps showing the distribution of taxa within the Study Area are shown in Appendices 5 and 6.

Table 6-1: Amphibious subterranean fauna species recorded on each drill line. Shading indicates species recorded only within the deposit.

AMPHIBIOUS SUBTERRANEAN FAUNA		PERIPHERY			NORTH WEST				OUT OF DEPOSIT			CENTRAL CALCRETE DEPOSIT									SOUTH EAST			YEELIRRIE PLAYA		
		PW	O	Q	P	312	E	A	G	H	F	1	1.5	2	3	3.5	4	4.5	5	6	C	K	D	L	N	SB14
Oligochaeta																										
Enchytraeidae	Enchytraeidae sp. Y1																							19		
	Enchytraeidae sp. Y2							31																		
	Enchytraeidae sp. Y3				14																					
	Enchytraeidae sp. Y4																						38			
	Enchytraeidae sp. Y5																				1	2				
	Enchytraeidae sp. Y6											4														
	Enchytraeidae sp. Y7							8																2		
Isopoda																										
Philosciidae	Philosciidae sp. n. S1																								3	
	Philosciidae sp. n. Y2											5	1													
Number of individuals					14			39				9	1								1	2	38	21	3	
Number of species					1			2				2	1								1	1	1	2	1	
Undetermined/ Indeterminate																										
Enchytraeidae	Enchytraeidae sp. indet. YL *	26	144		64	1	372	22			14	32	88	5	7	22	5		145		1		11	27	19	

* Enchytraeidae sp. indet. YL includes all enchytraeid specimens detected from samples where no DNA data was obtained.

Table 6-2: Stygofauna species (other than Copepoda) recorded on each drill line. Shading indicates species recorded only within the deposit.

STYGOFAUNA		PERIPHERY			NORTH WEST				CENTRAL CALCRETE												SOUTH EAST			YEELIRRIE PLAYA		
									OUT OF DEPOSIT			DEPOSIT														
		PW	O	Q	P	312	E	A	G	H	F	1	1.5	2	3	3.5	4	4.5	5	6	C	K	D	L	N	SB14
Aphanoneura																										
Aeolosomatidae	*Aeolosoma* sp. S1																								101	
Oligochaeta																										
Naididae	Naididae sp. S4				1		1				1					1										
	Naididae sp. S5										3															
Phreodrillidae	Phreodrillidae sp. S8				1						1	4	4													2
Amphipoda																										
Chiltoniidae	nr. *Phreatochiltonia* sp. n. S1				1		7		3	84	70	22	19	42	15	73	1		3			11		31		5
Bathynellacea																										
Bathynellidae	Bathynellidae sp. S2									7	14	1										1				
	Bathynellidae sp. S4																							9		
Parabathynellidae	*Atopobathynella* sp. `line K`																					2				
	Atopobathynella sp. S4					2																				14
	Atopobathynella sp. S5							1		41	55	69	19	375	2	12			1							
	Atopobathynella sp. Y1				6																					
	Atopobathynella sp. Y2							1																		
	Atopobathynella sp. Y3				4			1																		
Ostracoda																										
Candonidae	*Candonopsis* sp. n. Y1									5	4	2		1												
Coleoptera																										
Dytiscidae	*Limbodessus* sp. n. 'yeelirriensis'									1	6	9	5	2		15										
	Limbodessus sp. S1						2			12	4	3	2	5	3	2			2			1				
	Paroster sp. n. 'angustus'									5	1	5	6	3		7										
Number of individuals					13	2	10	3	3	155	159	115	55	428	20	110	1		6			15		40	101	21
Number of species					5	1	3	3	1	7	10	8	6	6	3	6	1		3			4		2	1	3

Table 6-3: Stygofaunal copepod species recorded on each drill line. Shading indicates species recorded only within the deposit.

STYGOFAUNA: COPEPODA		PERIPHERY			NORTH WEST				CENTRAL CALCRETE											SOUTH EAST			YEELIRRIE PLAYA		
									OUT OF DEPOSIT			DEPOSIT													
		PW	O	Q	P	312	E	A	G	H	F	1	1.5	2	3	3.5	4	4.5	5	6	C	K	D	L	N	SB14
Cyclopoida																										
Cyclopidae	*Dussartcyclops* 'dostoyevskyi' sp. n.				11	211	83			3	3	23		23		1			10		4			59		
	Halicyclops cf. *eberhardi* sp. A									17	394			9		8						71				
	Halicyclops cf. *eberhardi* sp. B												372													
	Halicyclops cf. *eberhardi* sp. C																									130
	Mesocyclops brooksi																						1 †			
Harpacticoida																										
Ameiridae	*Nitokra* 'esbe' sp. n.																									15
	Nitokra `yeelirrie` sp. n.									1	36	83	103	16	2	8	1		1			2			7	30
	Novanitocrella 'araia linec' ssp. n.																				123					
	Novanitocrella 'araia' sp. n.												1													
Canthocamptidae	*Australocamptus hamondi*					344																				
Ectinosomatidae	*Pseudectinosoma* 'pentedicos' sp. A																									18
	Pseudectinosoma 'pentedicos' sp. B																							25		
	Pseudectinosoma 'pentedicos' sp. C *									5	4	16		1												
Miraciidae	*Schizopera* 'akation' sp. n.										6	39	2	5	2	2						26		24		6
	Schizopera 'akolos' sp. n.											4														
	Schizopera 'analspinulosa linel' ssp. n.																							40		
	Schizopera 'analspinulosa' sp. n.																									34
	Schizopera 'emphysema' sp. n.													8												
	Schizopera 'kronosi' sp. n.									9				2	1	3			1			3				
	Schizopera 'leptafurca' sp. n.										1	222	339	116	17	7			17			77				
	Schizopera 'linen' sp. n.																								1	
	Schizopera 'uranusi' sp. n.		1				9			36	131	59	8	130		6										
	Schizopera sp. 7439															5										
Parastenocaridae	Dussartstenocaris` `idioxenos` Gen. n. sp. n.				195	72	15	205																		
	Kinnecaris 'esbe' sp. n.																									19
	Kinnecaris 'linea' sp. n.						13	19																		
	Kinnecaris 'lined' sp. n.																						100			
	Kinnecaris 'linel' sp. n.																							159		
	Kinnecaris 'linep' sp. n.				2																					
	Kinnecaris 'uranusi' sp. n.									14	13	62		10								73				
Number of individuals			1		208	627	120	224		85	588	508	825	320	22	40	1		29		127	252	100	307	8	252
Number of species			1		3	3	4	2		7	8	8	6	10	4	8	1		4		2	6	1	5	2	7
Undetermined/ Indeterminate																										
	Halicyclops cf. *eberhardi*										11	624	24	273	42	42			4					62		
	Pseudectinosoma `pentedicos` sp. indet. YL							1																		
	Kinnecaris sp. indet. YL																				1					

* The haplotype *Pseudectinosoma* 'pentedicos' sp. C was detected at line F. Specimens occurring within the deposit are inferred as belonging to the same haplotype.

† The copepod *Mesocyclops brooksi* was detected previously during WAM sampling at a homestead bore near line D. This species has not been included in further analysis.

The taxa in Table 6-2 constitute represent amphibious subterranean fauna. Genetic analysis on the Enchytraeidae detected at Yeelirrie revealed an unusually high number of species (Subterranean Ecology unpub. data). Due to limited taxonomic information, all of the species detected at Yeelirrie were indistinguishable by external morphology (Appendix 3 for plates). Enchytraeids are known to occur in water films in soil and in terrestrial and aquatic subterranean habitats (A. Pinder pers. comm.). At Yeelirrie they were detected in troglofauna traps as well as in net scrape and net haul samples (Appendix 5 for distribution maps and Appendix 8 for DNA).

The isopod family Philosciidae is known to include terrestrial, amphibious and aquatic subterranean taxa, such as *Haloniscus* (S. Taiti pers. comm. 2010). Genetic analysis (Appendix 9) confirmed that two morphologically cryptic species of Philosciidae occur at Yeelirrie: Philosciidae sp. n. S1 and Philosciidae sp. n. Y2 (Appendix 3 for plates, Appendix 5 for distribution maps).

Taxa in Table 6-3 represent typical Yilgarn stygofauna taxa, with the exception of *Aeolosoma*, which was sampled from an open well, and may represent an accidental epigean species or stygophile. The amphipod nr. *Phreatochiltonia* sp. n. S1 was morphologically conservative despite genetic divergences between populations on different drill lines from the different study zones. Individual amphipods within the central calcrete (both inside and outside of the deposit) were genetically very similar (average 0.8% divergence COI), indicating a single population occurring throughout the habitat (Appendices 3 for plates, Appendix 6 for distribution maps and Appendix 9 for DNA).

The diving beetles *Limbodessus* sp. n. 'yeelirriensis', *L*. sp. n. S1 and *Paroster* sp. n. 'angustus' (Appendices 3 for plates) occurred mostly in the central calcrete, between lines H and 3.5. The species *Limbodessus* sp. S1 was detected more widely; larvae were recorded at Line E in the Northwest study zone, and adults were detected at line K in the Southeast study zone (Appendix 6 for distribution maps). This wide local distribution between several calcrete bodies is atypical for diving beetles, which are generally considered to be restricted to single calcrete aquifers (as in the calcrete-island hypothesis, Cooper 2002). However, the records of the WAM (as revealed in Chapter 2) include a few species of *Limbodessus* that are known from more than one calcrete.

The syncarids (families Bathynellidae and Parabathynellidae) were highly diverse, and a variety of distribution patterns were recorded (Appendix 3 for plates and Appendix 6 for distribution maps). Bathynellidae sp. S2 and S4, which were identified as morphologically and genetically distinct species, were recorded from different study zones (the central calcrete and the playa respectively). The population of Bathynellidae on line K, although morphologically similar to B. sp. S2, showed a moderate level of genetic divergence (7 %) from the population of B. sp. S2 recorded from the central calcrete, indicating a long historical separation of both populations (Appendix 9).

Six species of the parabathynellid *Atopobathynella* were recorded, comprising a high diversity in comparison with other calcretes in the region (WAM unpub. data; Guzik *et al.* 2008). Four species were recognised morphologically; while a further two morphologically cryptic species were revealed by DNA (K. Abrams pers. comm.; Appendix 9). The distribution of *Atopobathynella* species included sympatric species (*A*. sp. Y1, Y2, Y3, S5) and allopatric species (*A*. sp. 'line k'), as well as one species with an unusual disjunct distribution, despite intense sampling in the intermediate areas (*A*. sp. S4 on lines 312 and SB14) (Appendices 3 for plates and Appendix 6 for distribution maps).

Yeelirrie's stygal copepod fauna is the richest known from the Yilgarn region to date. Karanovic (2004) reported 24 species from extensive collections throughout the whole region, while the sampling at Yeelirrie revealed 30 species. Prior to the current survey, genetic analyses were not intensively used to support morphological identifications of copepods. The DNA analyses of Yeelirrie copepods (Appendix 10) confirmed that the majority of taxa identified represented new, undescribed species (approximately 19 species), and there were seven additional taxa revealed. Yeelirrie's copepod assemblages include members of almost all taxonomic groups found throughout the region, while the harpacticoid genus *Schizopera* was particularly rich (10 species) (Appendix 3 for plates, Appendix 6 for distribution maps). Karanovic (2010, Appendix 11) suggested that the diversity and distribution of copepod species at Yeelirrie has resulted from multiple colonisations of the discontinuous calcrete habitats, and periods of rapid adaptive evolution within the calcretes.

6.1.1 Richness (Number of Species)

Figure 6-1 shows the species richness of both stygofauna and amphibious subterranean fauna per drill line. Indeterminate records were excluded; however, when there were occurrences of indeterminate fauna without the duplication of corresponding identified fauna, the indeterminate fauna were included. For example, the specimens of Enchytraeidae sp. indet. YL recorded in line PW were included in the richness, as there was no duplication with identified enchytraeids recorded from that line. Drill lines in the central calcrete (inside and outside of the deposit) were generally rich, including the two highest species totals per line; 20 species (line F) and 19 species (line 1). No stygal or amphibious species were recorded from lines Q, 4.5 and 6.

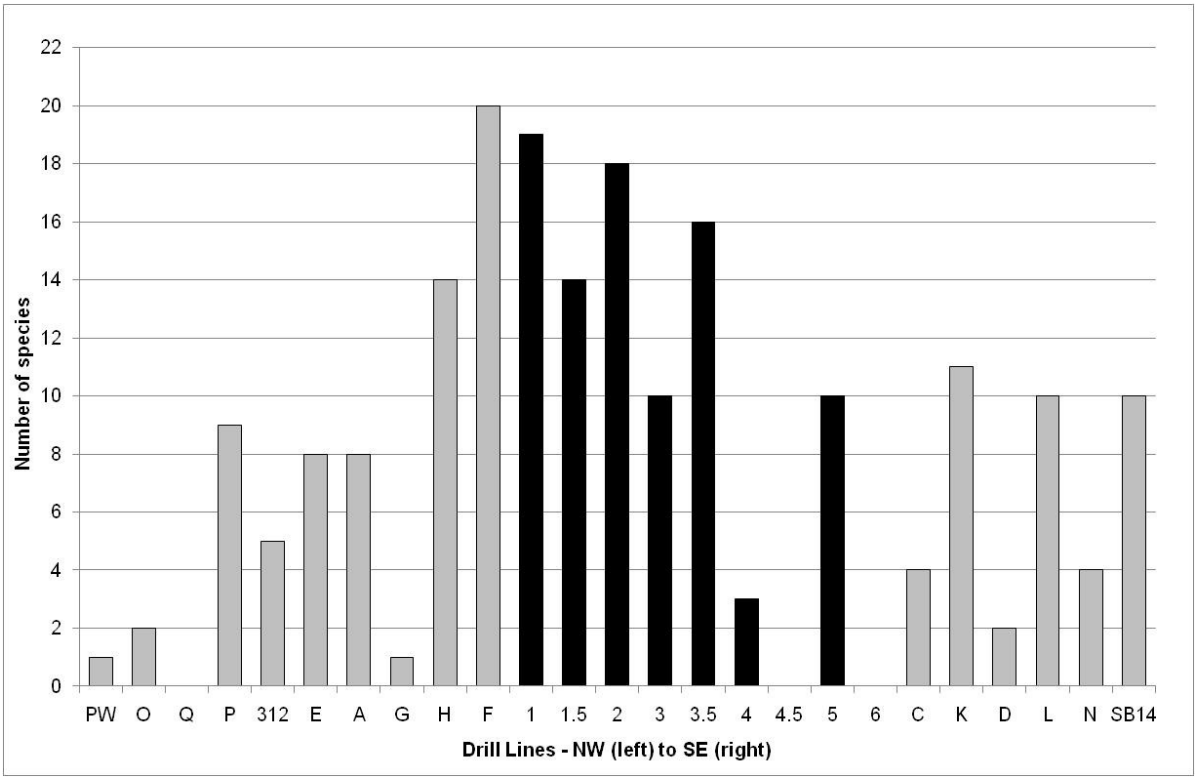


Figure 6-1: Total number of stygofauna and amphibious species per drill line. Deposit drill lines are shaded dark.

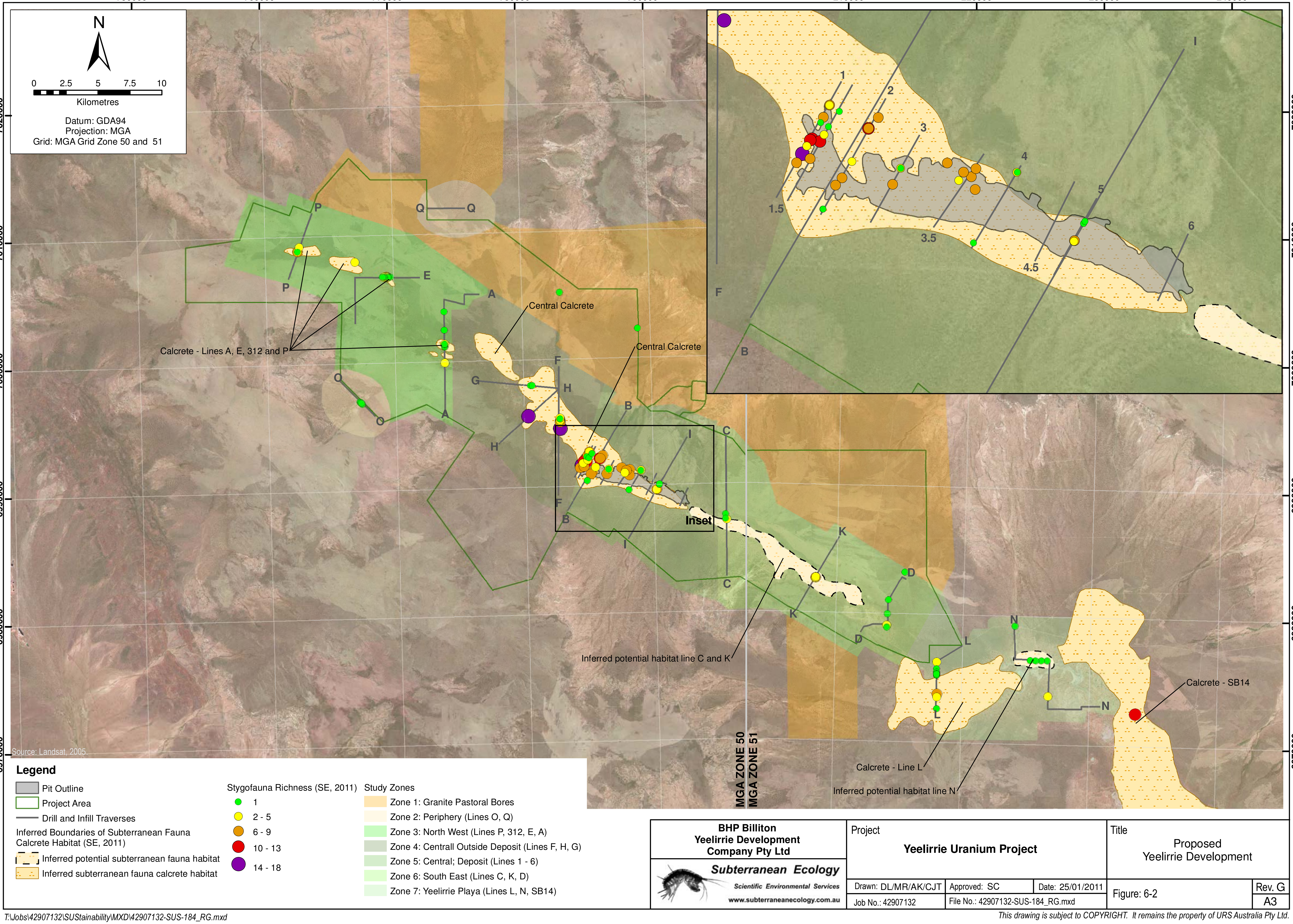
Table 6-4: Summary table - number of stygofauna species, amphibious species and indeterminate taxa occurring on each drill line and totals per study zone.

Study Zone		Drill line	Stygofauna species	Amphibious species	Indeterminate taxa	Total per zone	
						Stygo	Amph
PERIPHERY		PW			1		
		O	1		1	1	1*
		Q					
NORTH WEST		P	8	1	1		
		312	4		1	15	3
		E	7		1		
		A	5	2	2		
CENTRAL CALCRETE	OUTSIDE DEPOSIT	G	1				
		H	14			19	1*
		F	19		1		
	DEPOSIT	1	16	2	2		
		1.5	13	1	1		
		2	17		1		
		3	7		2		
		3.5	15		1	25	2
		4	2		1		
		4.5					
		5	7		2		
		6					
SOUTH EAST		C	2	1	2		
		K	10	1		13	2
		D	1	1	1		
YEELIRRIE PLAYA		L	7	2	2		
		N	3	1	1	17	3
		SB14	10				
Overall total			**46**	**9**	**4**		

* Single species assumed from undetermined taxa

The periphery (*i.e.* drill lines PW, O and Q) was relatively depauperate, recording only one species of stygofauna and undetermined enchytraeids (Table 6-4). The study zones northwest and southeast of the central calcrete recorded moderate richness (respectively 15 and 13 species of stygofauna, and several amphibious species), particularly from lines P, E and K. The greatest species richness was recorded in the deposit (24 species), which also had the highest number of drill holes and the highest number of samples from any zone. The central calcrete outside of the deposit contained the second highest richness (20 species), followed by the Yeelirrie Playa (18 species) (Table 6-4).

Stygofauna richness 'hotspots' (species richness recorded from each bore) are shown in Figure 6-2. The size and colour of the circle at each bore reflects the number of species detected. A cluster of moderately to highly rich bores was present in the north-western part of the deposit, and also on line L. The richest bores were old pastoral bores on lines F (YU1) and H (TPB-33-1). It is inferred that these bores contained well-established stygofauna assemblages, which accounted for a high proportion of the richness recorded from their respective drill lines.


N
0 2.5 5 7.5 10
Kilometres
Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51
750000
760000
770000
780000
790000
210000
220000
230000
240000
7020000
7010000
7000000
6990000
6980000
6970000
Calcrete - Lines A, E, 312 and P
Central Calcrete
Central Calcrete
Inset
Inferred potential habitat line C and K
Calcrete - Line L
Inferred potential habitat line N
Calcrete - SB14
MGA ZONE 50
MGA ZONE 51
Source: Landsat, 2005
Legend
Pit Outline
Project Area
Drill and Infill Traverses
Inferred Boundaries of Subterranean Fauna Calcrete Habitat (SE, 2011)
Inferred potential subterranean fauna habitat
Inferred subterranean fauna calcrete habitat
Stygofauna Richness (SE, 2011)
1
2 - 5
6 - 9
10 - 13
14 - 18
Study Zones
Zone 1: Granite Pastoral Bores
Zone 2: Periphery (Lines O, Q)
Zone 3: North West (Lines P, 312, E, A)
Zone 4: Centrall Outside Deposit (Lines F, H, G)
Zone 5: Central; Deposit (Lines 1 - 6)
Zone 6: South East (Lines C, K, D)
Zone 7: Yeelirrie Playa (Lines L, N, SB14)
BHP Billiton
Yeelirrie Development
Company Pty Ltd
Subterranean Ecology
Scientific Environmental Services
www.subterraneanecology.com.au
Project
Yeelirrie Uranium Project
Title
Proposed
Yeelirrie Development
Drawn: DL/MR/AK/CJT
Approved: SC
Date: 25/01/2011
Job No.: 42907132
File No.: 42907132-SUS-184_RG.mxd
Figure: 6-2
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6.1.2 Abundance and Frequency

Species abundance refers to the total number of individuals collected, whilst the frequency is the number of samples from which a species was detected. In general, stygofauna and amphibious fauna were highly abundant with a few species frequently sampled. Seventeen species numbered more than 100 individuals, while 21 species numbered between 100 and ten individuals. The copepod *Schizopera* 'leptafurca' sp. n. (796 individuals) and the parabathynellid *Atopobathynella* sp. S5 (575 individuals) were the most abundant species recorded (Tables 6-2 and 6-3). In contrast, there were three species recorded only from single individuals; Atopobathynella sp. Y2, *Novanitocrella* 'araia' sp. n. and *Schizopera* 'line n' sp. n. (Tables 6-2 and 6-3).

Figure 6-3 shows the number of occurrences of each species of stygofauna and amphibious fauna from the survey. The amphipod, nr. *Phreatochiltonia* sp. n. S1, was the most frequently recorded species (86 samples), along with the copepods *Nitokra* `yeelirrie` sp. n. and *Schizopera* 'leptafurca' sp. n., which were both sampled 46 times. Overall, there were few common species (10 species > 20 occurrences), with the majority of species (45) recorded from less than 16 samples, and 17 of these species were sampled only once (Figure 6-3). This result of many species recorded only once or twice is not unusual for subterranean fauna surveys (Eberhard *et al.* 2009).

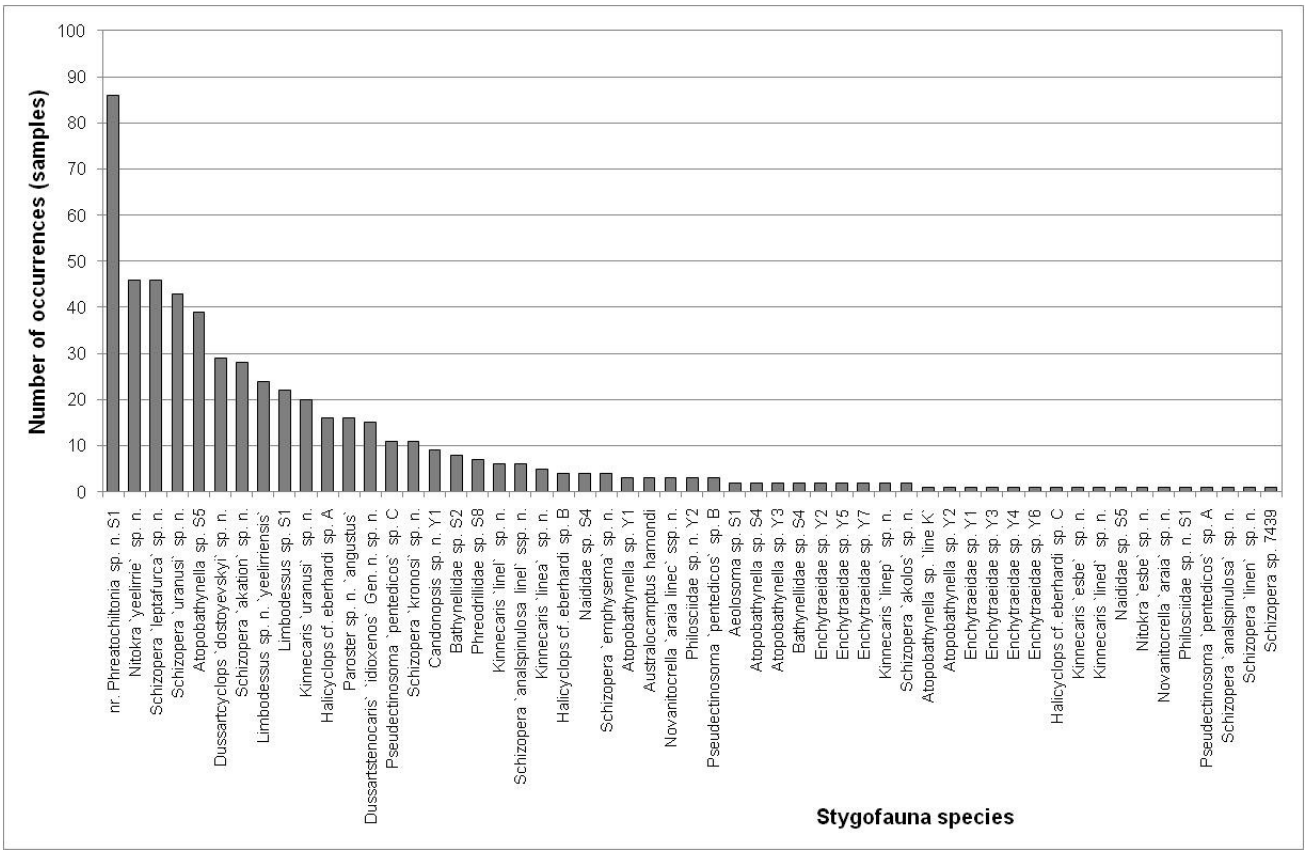

Figure 6-3: Occurrence of stygofauna species in all samples.

6.1.3 Species Distribution

Stygofauna and amphibious subterranean species were not evenly distributed throughout the Yeelirrie palaeochannel. There was a concentration of species and taxonomic diversity within the central calcrete and at the playa (Figures 6-1 and 6-2, Table 6-4). However, it is likely that the recorded occurrences of most of these species represent only a part of their actual distribution ranges within the subterranean habitat.

The stygofauna and amphibious fauna showed a wide variety of distribution patterns throughout the Study Area. The main distribution patterns of stygofauna and amphibious species included:

- Regionally widespread species;
- Species widespread throughout the Study Area, *i.e.* occurred in two or more study zones;
- Species occurring only in a few, adjacent drill lines, *i.e.* occurred in only one study zone;
- Species recorded from single drill lines only; and
- Singletons, species recorded once on one sampling occasion.

Table 6-5 shows the distribution patterns of the stygofauna and amphibious species recorded from the discrete Yeelirrie calcretes. Some species were widespread, such as *Mesocyclops brooksi*, a stygophile recorded by the previous WAM survey, which is known to occur in both subterranean (Humphreys *et al.* 2009) and epigean habitats (Holynska and Brown 2003) around much of southern Australia. *Australocamptus hamondi*, another potential stygophile (Karanovic 2010, Appendix 11), was also recorded from calcrete at Depot Springs and Wondinong (refer section 2). *Halicyclops* cf. *eberhardi*, was recorded from Paroo, Depot Springs and Melrose Station (Lake Darlot). Interestingly, these species were not widespread throughout the Study area, except *Halicyclops* cf. *eberhardi,* which probably represents a group of genetically-divergent species, on the basis of its DNA (Appendix 10). Morpho-species letters numbers identify morphologically-cryptic species revealed by DNA; *e.g. Pseudectinosoma* 'pentedicos' sp. A, sp. B and sp. C.

The majority of copepod species (18) were less commonly detected, being recorded only from single calcretes or single drill lines. In certain groups (*e.g.* the genera *Kinnecaris* and *Pseudectinosoma)*, different species were sampled in almost each different location, suggesting allopatric speciation, related to disconnected calcrete habitats (Appendix 11). Other species such as *Schizopera* 'line N' sp. n., *S*. 'akolos' sp. n., *S*. 'emphysema' sp. n., and *Novanitocrella* 'araia' sp. n. represent potentially rare or difficult to sample taxa, which are currently only known from certain locations within wider calcrete habitats. There were some genetically identified species that were recorded from single locations only (including *Halicyclops* cf. *eberhardi* sp. B and C, and *Schizopera* sp. 7439), although these records were contingent upon the extent of material sent for DNA sequencing. It is possible that these species may occur more widely than is currently recorded.

Table 6-5: The distribution patterns of the stygofauna and amphibious subterranean species recorded from the multiple Yeelirrie calcretes.

AMPHIBIOUS FAUNA AND STYGOFAUNA				
Regionally widespread	**Widespread multiple calcretes**	**Throughout single calcrete**	**Single location outside deposit**	**Inside deposit only**
Aphanoneura				
			Aeolosoma sp. S1	
Oligochaeta				
	Naididae sp. S4	Enchytraeidae sp. Y5	Enchytraeidae sp. Y1	**Enchytraeidae sp. Y6**
	Phreodrillidae sp. S8		Enchytraeidae sp. Y2	
	Enchytraeidae sp. Y7		Enchytraeidae sp. Y3	
			Enchytraeidae sp. Y4	
			Naididae sp. S5	
Amphipoda				
	nr. *Phreatochiltonia* sp. n. S1			
Bathynellacea				
	Bathynellidae sp. S2		Bathynellidae sp. S4	
	Atopobathynella sp. S4		*Atopobathynella* sp. `line K`	
	Atopobathynella sp. S5		*Atopobathynella* sp. Y1	
	Atopobathynella sp. Y3		*Atopobathynella* sp. Y2	
Isopoda				
			Philosciidae sp. n. S1	**Philosciidae sp. n. Y2**
Ostracoda				
		Candonopsis sp. n. Y1		
Coleoptera				
		Limbodessus sp. n. 'yeelirriensis'		
	Limbodessus sp. S1			
		Paroster sp. n. 'angustus'		
Cyclopoida				
Mesocyclops brooksi	*Dussartcyclops* 'dostoyevskyi' sp. n.		*Halicyclops* cf. *eberhardi* sp. C	***Halicyclops* cf. *eberhardi* sp. B**
	Halicyclops cf. *eberhardi* sp. A			
Harpacticoida				
Australocamptus hamondi	*Nitokra* `yeelirrie` sp. n.	*Novanitocrella* 'araia linec' ssp. n.	*Nitokra* 'esbe' sp. n.	***Novanitocrella* 'araia' sp. n.**
	Schizopera 'akation' sp. n.	*Schizopera* 'analspinulosa linel' ssp. n.	*Pseudectinosoma* 'pentedicos' sp. A	***Schizopera* 'akolos' sp. n.**
	Schizopera 'kronosi' sp. n.	*Pseudectinosoma* 'pentedicos' sp. C *	*Pseudectinosoma* 'pentedicos' sp. B	***Schizopera* 'emphysema' sp. n.**
	Schizopera 'leptafurca' sp. n.	*Kinnecaris* 'linel' sp. n.	*Schizopera* 'analspinulosa' sp. n.	***Schizopera* sp. 7439**
	Schizopera 'uranusi' sp. n.		*Schizopera* 'line N' sp. n.	
	Kinnecaris 'uranusi' sp. n.		*Kinnecaris* 'esbe' sp. n.	
	Kinnecaris 'linea' sp. n.		*Kinnecaris* 'lined' sp. n.	
	`*Dussartstenocaris* ' 'idioxenos' Gen n. sp. n.		*Kinnecaris* 'linep' sp. n.	
2	**19**	**8**	**20**	**7**

* Pseudectinosoma 'pentedicos' sp. C is assumed to occur throughout the central calcrete, although occurrence inside the deposit has not been verified by genetic data

Seven of a total 25 stygofauna and amphibious subterranean species recorded from inside the deposit were not recorded outside of the deposit (Tables 6-1, 6-2 and 6-3). Six of these species were found only on one line, while one occurred on two adjacent drill lines. Four of the species were morphologically cryptic (marked MC) and could only be identified by DNA analysis. These species were:

- Enchytraeidae sp. Y6 (MC) – amphibious, recorded on line 1 (Table 6-1, Appendix 6). Undetermined specimens were recorded from outside of the deposit on line F (Enchytraeidae sp. indet. YL);
- Philosciidae sp. n. Y2 (MC) – amphibious, recorded on lines 1 and 1.5. Genetically distinct from a single sister species, recorded only from line N (Philosciidae sp. n. S1) (Appendix 6, 9);
- *Halicyclops* cf. *eberhardi* sp. B (MC) – stygal, recorded on line 1.5. Genetically distinct from sympatric sister species recorded from lines H to K (*Halicyclops* cf. *eberhardi* sp. A), and SB14 (*Halicyclops* cf. *eberhardi* sp. C) (Appendices 9, 10 and 11). Morphologically conforms to *Halicyclops eberhardi*, a regionally-widespread species. Further genetic information required to assess variance from regional populations of *H. eberhardi*;
- *Schizopera* sp. 7439 (MC) – stygal, recorded on line 1.5 (Appendices 9, 10 and 11). Genetically distinct from all other *Schizopera* species;
- *Schizopera* 'emphysema' sp. n. – stygal, morphologically distinct, confirmed by DNA analysis (Appendices 9, 10 and 11). Recorded from several samples at the same location on line 3.5 (Table 6-3);
- *Schizopera* 'akolos' sp. n. – stygal, morphologically distinct, confirmed by DNA analysis (Appendices 9, 10 and 11). Recorded from several samples at the same location on line 1; and
- *Novanitocrella* 'araia' sp. n. – stygal, morphologically distinct (Appendices 9, 10 and 11). Recorded from a single specimen on line 1.5. Genetic analysis of the specimen failed. A sub-species occurs on line C, *Novanitocrella* 'araia linec' ssp. n.

The location of each of these seven species within the deposit of the central calcrete is shown in Figure 6-4. All the species are located on the western side of the calcrete, from lines 1 to 3.5 (Figure 6-4).

775000 780000 785000 790000 795000 205000

6995000 6990000 6985000

MGA ZONE 50
MGA ZONE 51

N

0 0.5 1 1.5 2
Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Central Calcrete
G
H
B
Central Calcrete
H
C
I
1
2
3
4
5
6
1.5
3.5
4.5
YYD22
YYAC35
YYAC33
YYAC1004C
YYAC248
F
B
I
C
Inferred potential habitat line C and K
MGA ZONE 50
MGA ZONE 51

Source: Landsat (2010)

Legend

- Project Area
- Indicative Project Footprint
- Pit Outline
- Drill and Infill Traverses BHP

Inferred Boundaries of Subterranean Fauna Calcrete Habitat (SE, 2011)

- Inferred potential subterranean fauna habitat
- Inferred subterranean fauna calcrete habitat

Stygofauna Species (Subterranean Ecology, 2010)

- *Enchytraeidae* sp. Y6
- *Halicyclops cf. eberhardi* sp. B
- *Novanitocrella 'araia'* sp. n.
- *Schizopera 'akolos'* sp. n.
- *Schizopera 'emphysema'* sp. n.
- *Schizopera* sp. 7439

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)

BHP Billiton Yeelirrie Development Company Pty Ltd

Subterranean Ecology
Scientific Environmental Services
www.subterraneanecology.com.au

Project
Proposed Yeelirrie Development

Title
Stygofauna species recorded only within the deposit

Drawn: CJT	Approved: JN	Date: 09/03/2011	Figure: 6-4	Rev. 0
Job No.: 42907132	File No.: 4290712-SUS-244_R0.mxd			A3

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6.1.4 Analysis of Stygofauna Assemblages

Test within Drill Lines

The total number of species detected within each bore was compared to the other bores along each drill line by using the ANOSIM test (Appendix 4 Table 3), to investigate heterogeneity of the stygofauna assemblages. Ten of the 24 drill lines sampled contained insufficient records of stygofauna or insufficient numbers of bores to be tested (including lines 312, 4, 4.5, 6, G, H, O, PW, Q, and SB14).

Of the 14 drill lines that could be tested, six recorded significant results, indicating different assemblages were detected at some of the bores on the drill line. The majority of these drill lines (4 out of 6) occurred within the deposit (Appendix 4 Table 3). However, subsequent global ANOSIM of all drill lines from the deposit (excluding lines 4.5 and 6, which recorded no stygofauna) revealed no significant differences between stygofauna assemblages (ANOSIM Global R= -0.011, p= 0.54, Appendix 4 Table 5). The results indicate localised heterogeneity between the assemblages of certain bores in the deposit, although in the broader context of the deposit as a whole, these differences are relatively minor.

The other two drill lines which showed heterogeneity were lines F and L. Consideration of bore construction as a factor in the ANOSIM analysis revealed a significant difference between assemblages from cased *versus* uncased bores on line L (Appendix 4 Table 4). Other factors such as differences in water physicochemistry and physical habitat characteristics may also have influenced the composition of stygofauna assemblages in these cases.

The analysis revealed that the assemblages recorded from the majority of drill lines were relatively uniform, despite some heterogeneity at a localised scale. The occurrence of relatively uniform assemblages along the majority of drill lines (particularly the regional drill lines) supports the current understanding of the subterranean habitat throughout the palaeochannel (refer Figure 4-3). These results provide a basis for comparing the total number and composition of species detected between each of the drill lines.

Tests between Drill Lines

The presence of stygofauna species per drill line was analysed by NMDS ordination, cluster analysis and ANOSIM, in order to investigate differences in the assemblages recorded on different drill lines.

The global ANOSIM result (Appendix 4 Table 2) for all drill lines was statistically significant ($p<0.05$), despite a modest global R-value of 0.340. Pair-wise tests showed that many of the lines were significantly different to each other in terms of their stygofauna community (Appendix 4 Table 1). Figure 6-5 shows a non-metric multidimensional scaling (NMDS) ordination (using Bray-Curtis Similarity) of stygofauna and amphibious subterranean assemblages per drill line, excluding outliers (drill lines D and N).

A group of similar assemblages from deposit drill lines (1, 1.5, 2, and 3.5) and nearby regional lines (F and H) is circled in the centre of the ordination. ANOSIM of the assemblages within this group showed no significant differences (R-value 0.046, p=0.203; Appendix 4 Table 6). The group of statistically similar drill line assemblages (within the circle of Figure 6-5) all occurred within the central calcrete, indicating potential connectivity of habitat, and no restrictions to gene flow or species movement. Pair-wise comparisons between assemblages inside and outside the group are represented by straight lines radiating from the circle. The lines indicate a significant difference between assemblages within the group and certain assemblages outside the group. No line indicates no significant difference, or that pair-wise ANOSIM comparisons were not possible due to low numbers of records (*e.g.* lines SB14, 4, G, and 312) (Appendix 4 Table 7).

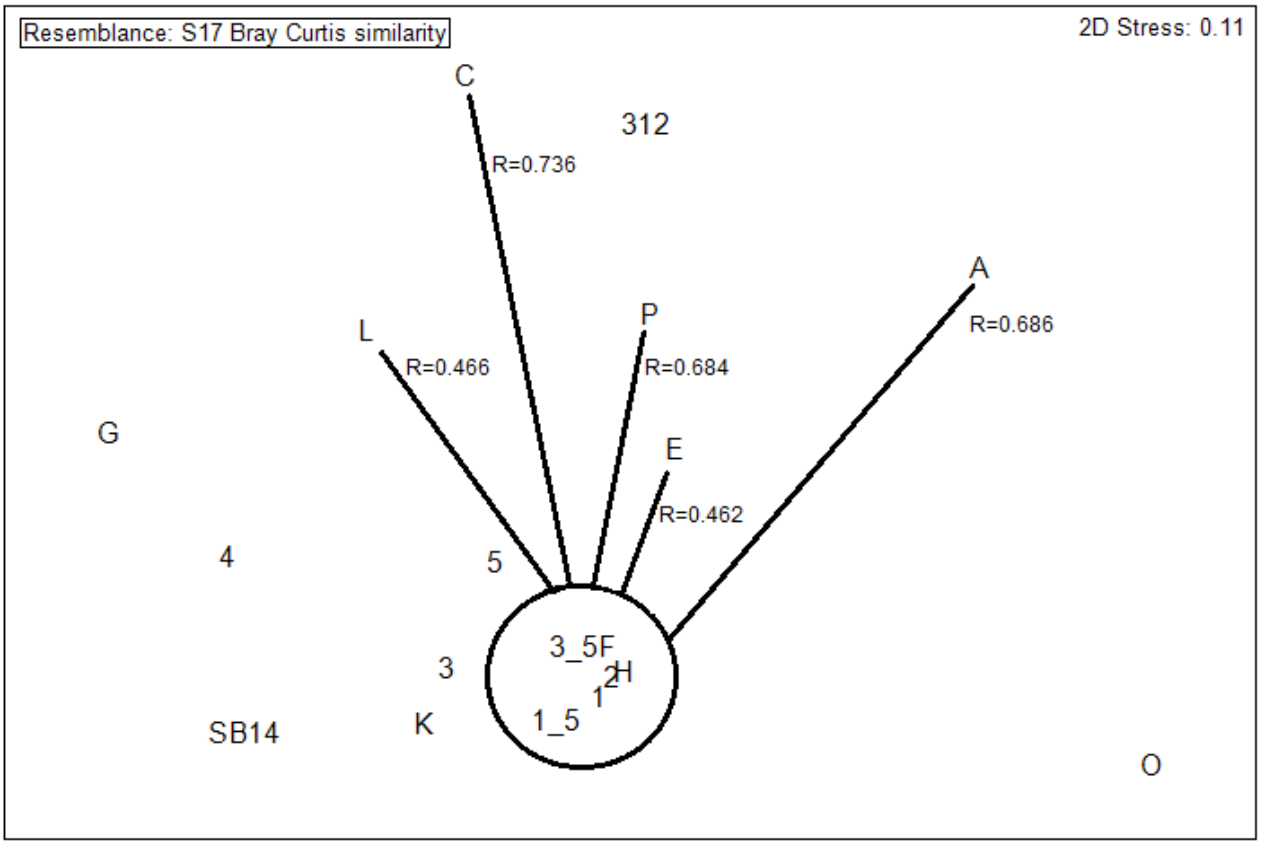

Figure 6-5: MDS of stygofauna assemblages per drill line. ANOSIM results indicated by lines. Drill lines within the circle were statistically similar. No lines indicate non-significant results or insufficient data for ANOSIM comparison.

The assemblages at drill lines 3, 5, and K were similar to the NMDS group, despite being outside the statistical threshold (p=0.05) of the SIMPROF test and ANOSIM. The cluster analysis and the NMDS showed modest differences between assemblages at these lines and the others in the deposit and central calcrete. The results indicate some variability within the central calcrete, for example stygofauna assemblages on line G and line 4 were considerably different from the remaining lines, while lines 3 and 5 were slightly different. Although line K is geographically separated from the central calcrete, the stygofauna assemblages are highly similar, which may suggest relatively recent opportunities for gene flow or species movement between these areas.

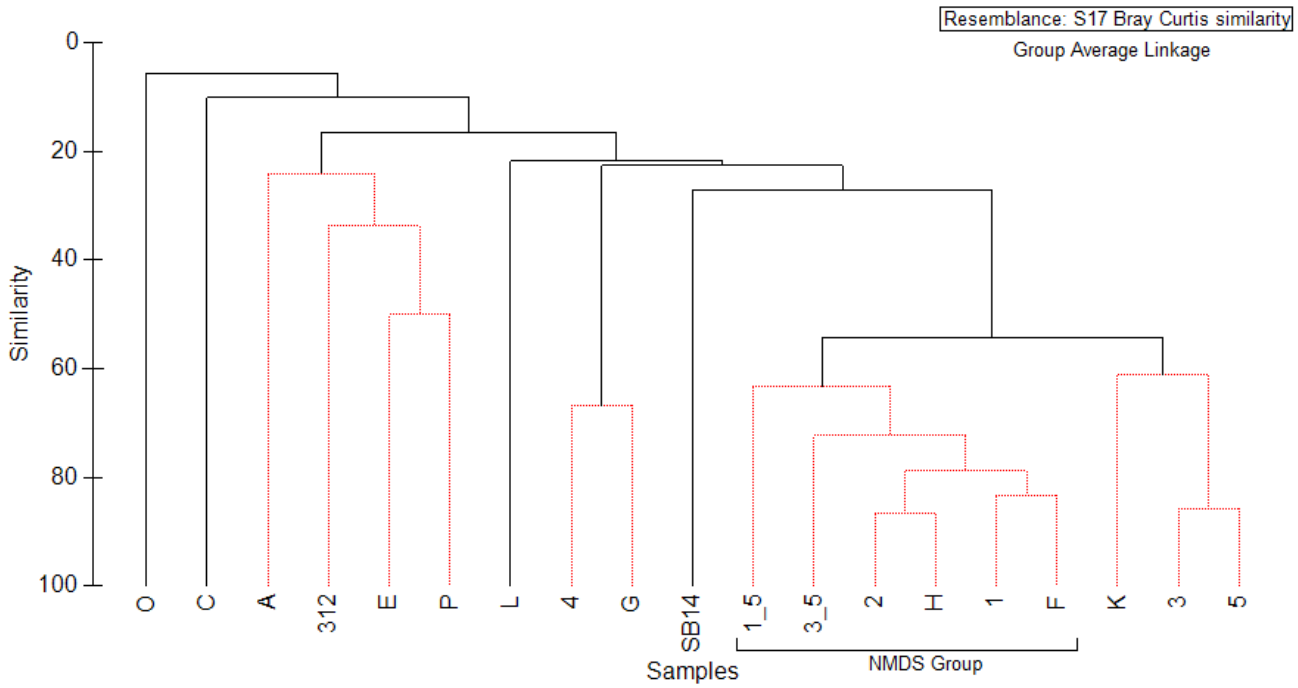

Figure 6-6: Cluster analysis of stygofauna assemblages, comparisons between drill lines. The SIMPROF test indicates those drill lines that do not contain significantly different assemblages, shown by the red lines.

Combined CLUSTER and SIMPROF analysis (Figure 6-6) showed the same group of drill lines (1, 1.5, 2, 3.5, F and H), had relatively similar assemblages (>60% similar). The next most similar assemblages occurred at lines 3, 5, and K, which were considerably closer to the NMDS group than any other drill lines. The results indicate that the majority of drill lines within the central calcrete, both inside and outside of the deposit are similar in their species composition. This high degree of similarity supports the hypothesis of habitat connectivity for stygofauna within the central calcrete, inside and outside of the boundaries of the deposit.

CLUSTER showed assemblages on lines N, O, C, L, and SB14 as distinct from all other assemblages, while lines A, E, 312 and P (the northwest study zone) were similar, as were lines 4 and G, and lines K, 3 and 5 (Figure 6-6). The patterns of association differed slightly between CLUSTER and MDS; however both analyses indicated that the assemblages in the main Yeelirrie calcrete were highly similar to one another (Figures 6-5 and 6-6).

6.1.5 Survey Adequacy

The combined survey effort for stygofauna at Yeelirrie comprised a total of 641 pumping, net hauling and scraping samples, from six sampling events (March 2009 to September 2010). Four species richness estimators within the program EstimateS v8.0 (Colwell 2005) determined that the current sampling for stygofauna (including results from pumping, net hauling and scraping) detected between 76.4% and 87.6% of the total number of species predicted to occur at Yeelirrie. The

proportion of species detected to date (55 species, excluding indeterminate morpho-species) *versus* the total number predicted by the species richness estimators is represented in Figure 6-7.

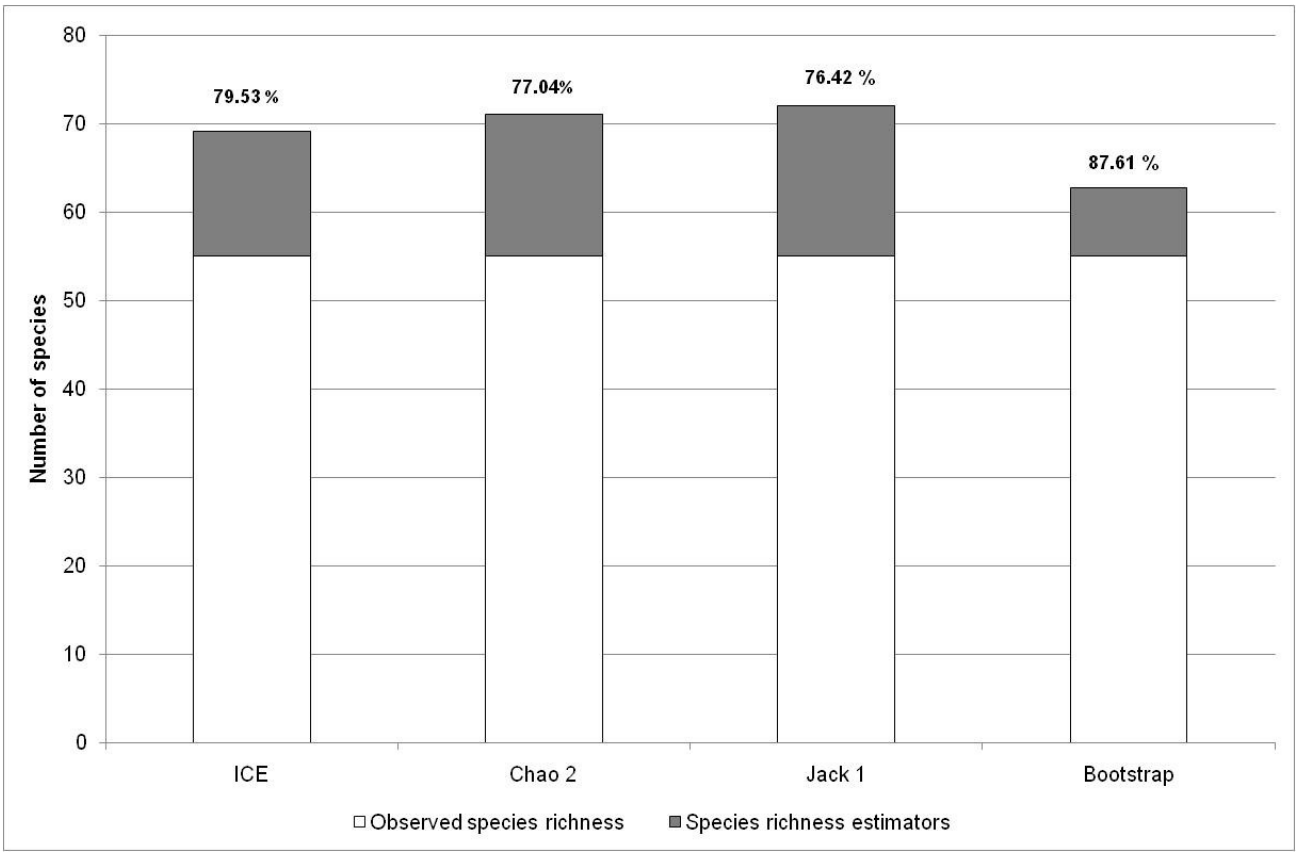

Figure 6-7: Observed stygofauna species richness, relative to predicted species richness, across multiple species richness estimators. Observed richness, as a proportion of each predicted total, is indicated by data labels.

A smoothed curve, representing the accumulation of new species as the sampling effort increased, is shown in Figure 6-8. Observed species richness shows a continuing, albeit less steep, rise towards the end of the sampling effort (> 600 samples). This indicates that there was no decline in the rate of new species detected with each additional monitoring round. The curves and estimates suggest that further sampling is likely to reveal additional species. These results are entirely consistent with species accumulation curves for other subterranean fauna surveys in Australia (*e.g.* Eberhard *et al.* 2009) and overseas, many of which do not plateau, even after many years of intensive survey effort (Culver and Pipan 2009).

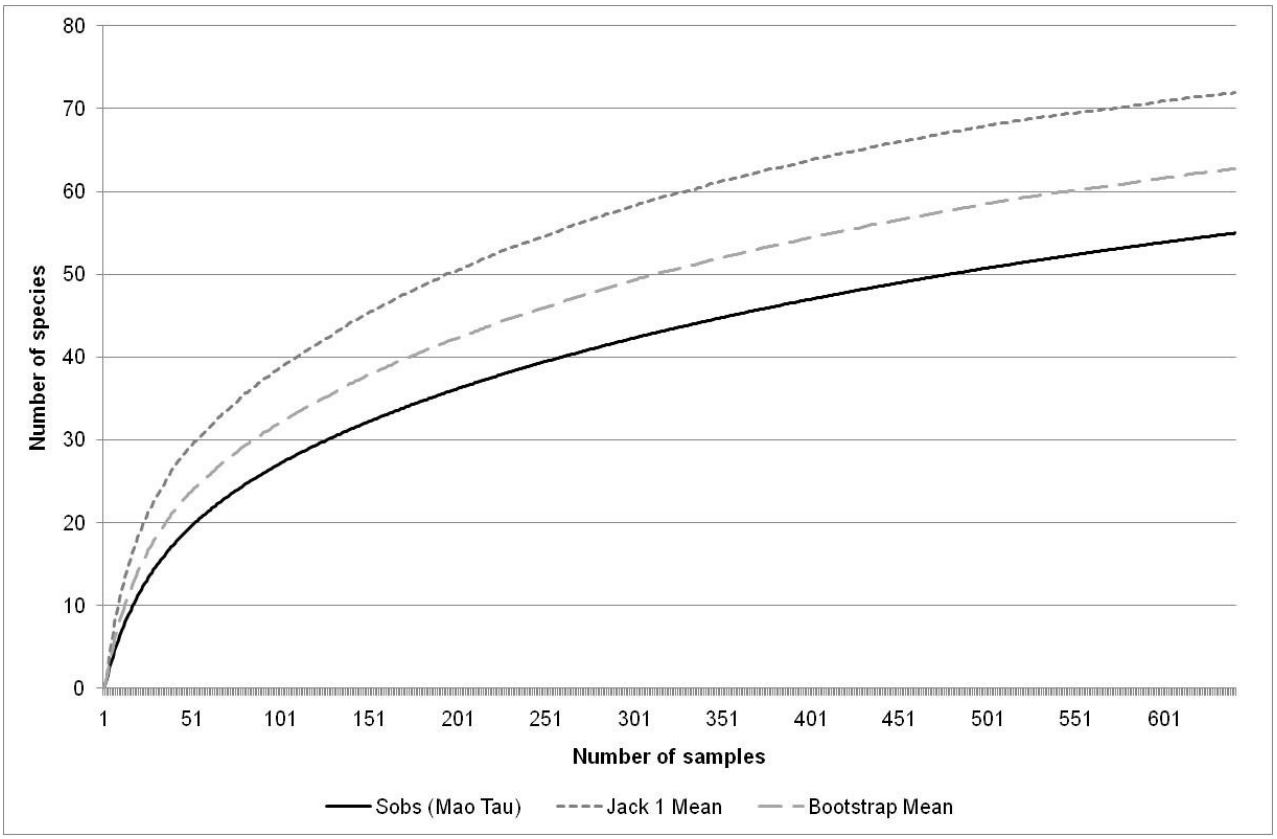

Figure 6-8: Stygofauna species accumulation over 641 samples. Curve generated using EstimateS v8.0 (Colwell 2005).

6.1.6 Water Quality Results

Physicochemical parameters of groundwater sampled at Yeelirrie (drill line averages or ranges) are presented in Figure 6-9. In general, the groundwater at Yeelirrie ranged from circum-neutral to alkaline (pH 6.28 – 9.81), had moderate oxygen levels (DO 2.14 – 6.41 mg/L), and was fresh to brackish (EC 1.6 – 58.69 mS/cm). These conditions are well within the range recorded for calcrete aquifers supporting stygofauna in the Yilgarn region (Humphreys 2008).

While temperature was consistent between most drill lines, dissolved oxygen, electrical conductivity, pH and redox potential was highly variable between drill lines and within drill lines. The minimum average DO observed was 2.14 mg/L at the outlier line PW, which may have contributed to the low abundance of stygofauna there. In addition, line G and line N also contained a relatively low diversity of stygofauna and lower levels of dissolved oxygen in some of their bores. However, a rich stygofauna assemblage (14 species) was recorded at line H despite low dissolved oxygen (Figure 6-1).

Six of the drill lines (*i.e.* PW, E, A, G, H and N) recorded negative Redox potential, suggesting a reducing (as opposed to oxidising) chemical environment in some of the bores. The pH was variable within many drill lines, particularly between bores within calcrete (generally pH >7.5) and those in granite or alluvial aquifers (generally pH 6.5 - 6.9. Redox and pH were also affected by the condition of some bores (*e.g.* organic debris and oxidized steel casings of some pastoral bores).

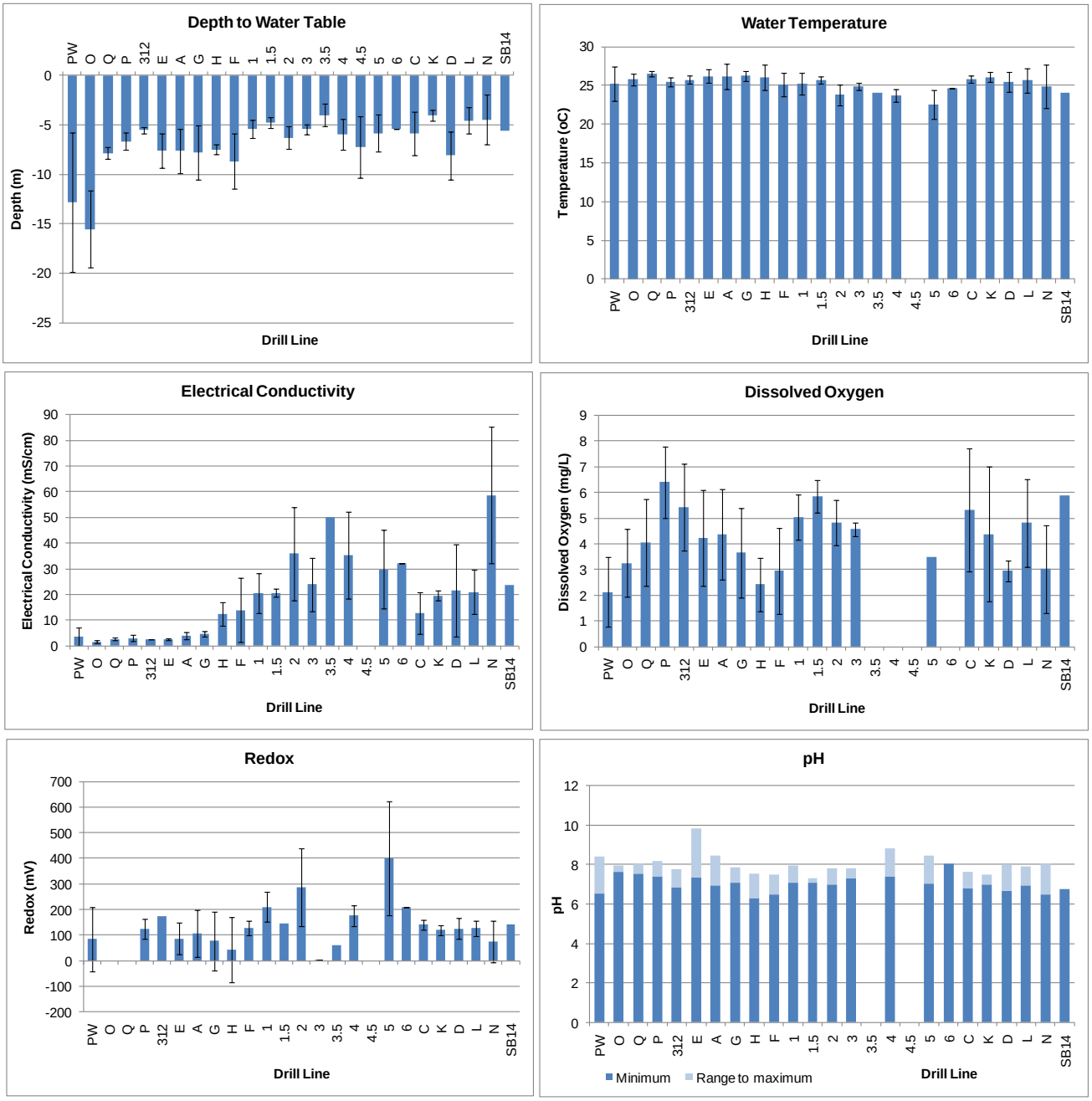

Figure 6-9: Mean water physicochemistry (± SD) per drill line (range for pH). Parameters were measured on site, via bailer or pumping, during stygofauna sampling.

Conductivity generally increased from the top of the catchment (lines PW, O, Q, P, 312, E, A and G, EC < 5 mS/cm) to the bottom of the catchment, beneath the playas at line N (EC > 58 mS/cm) (Figure 6-9). There was an increase in EC at the deposit, particularly on lines 2 and 3.5, while line C was comparatively fresh, suggesting discontinuity of groundwater flow between the deposit and areas immediately downstream. This may be related to the absence of saturated calcrete downstream of the deposit (refer Figure 3-1). The depth to the groundwater varied between drill lines, with the greatest depth to the groundwater occurring at the outlier lines PW and O. This may account for the low diversity of stygofauna recorded from line O, as the calcrete recorded on this line may be predominantly above water table (URS 2011).

6.2 Troglofauna Results

6.2.1 Richness (Number of Species)

The troglofauna surveys revealed 45 species of troglofauna from Yeelirrie, representing 12 invertebrate orders. A total of 498 individuals were collected over the six surveys. The richness of troglofauna per drill line is shown in Figure 6-10. High richness was recorded at lines L in the playa zone (18 species), A in the north-west zone, and 1 in the deposit (13 and 11 species, respectively). Several drill lines, including Q, 2, 4.5 and C, did not record any troglofauna species.

Indeterminate taxa, *i.e.* those that could not be identified to species or distinguishable morpho-species were excluded from these analyses due to potential duplication. These records are shown at the bottom of Table 6-6. These data may be subject to revision pending further identifications. In cases where DNA analyses revealed divergent, morphologically cryptic species, genetically undetermined records from the same sample were treated as the same species. Records that had not been successfully sequenced for DNA were then treated as indeterminate.

Specimen images are shown in Appendix 3, genetic information is presented in Appendices 8 and 9, and maps showing the distribution of the taxa within the Study Area are shown in Appendix 7.

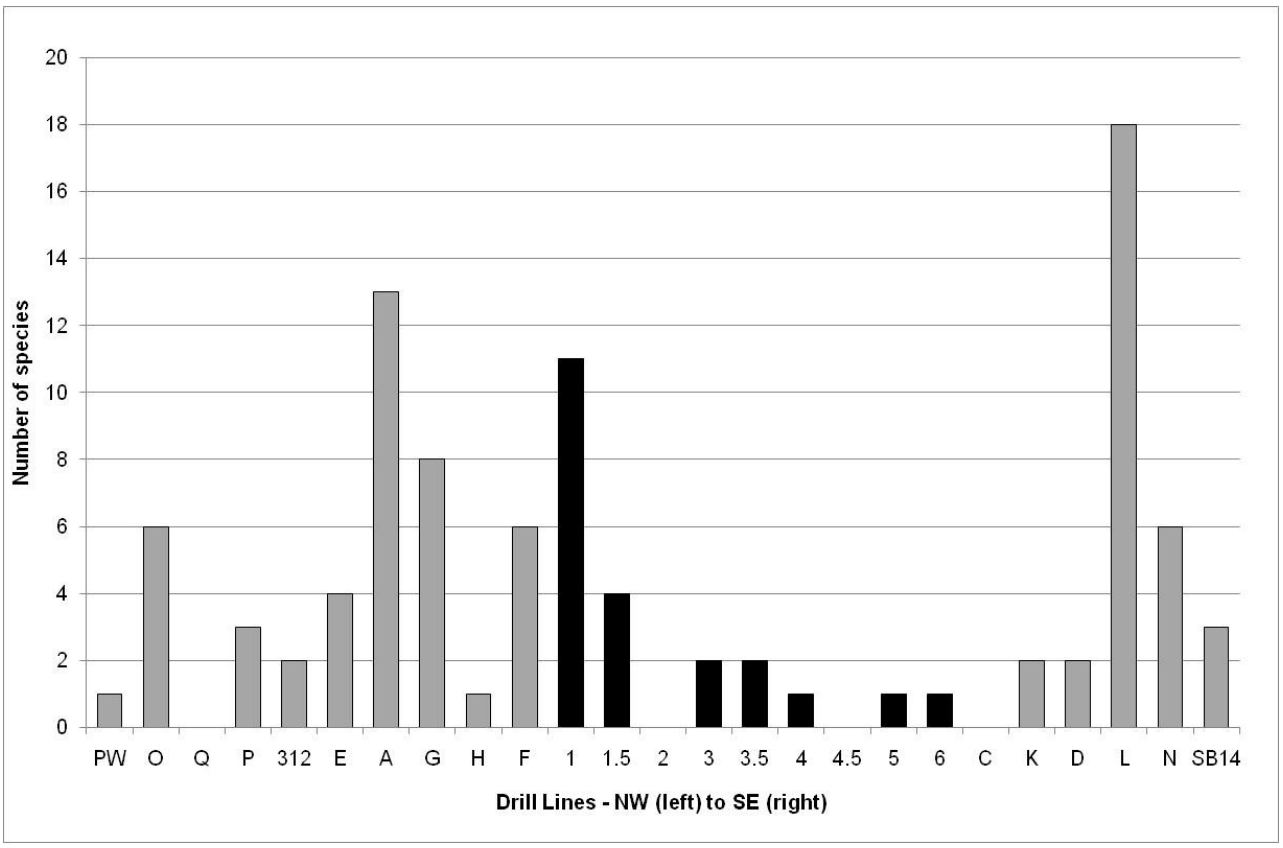

Figure 6-10: Total number of species of troglofauna per drill line. Indeterminate taxa have been excluded. Deposit drill lines are shaded dark.

Table 6-6: Troglofauna species (Crustacea and Arachnida) recorded on each drill line. Shading indicates species recorded only within the deposit.

TROGLOFAUNA		PERIPHERY			NORTH WEST				CENTRAL CALCRETE											SOUTH EAST			YEELIRRIE PLAYA		
									OUTSIDE DEPOSIT			DEPOSIT													
		PW	O	Q	P	312	E	A	G	H	F	1	1.5	2	3	3.5	4	4.5	5	6	C	K	D	L	N	SB14
Isopoda																										
Armadillidae	*Troglarmadillo* sp. n. S12																							1		
	Troglarmadillo sp. n. S13											1												7		
	Troglarmadillo sp. n. S7A										9															
	Troglarmadillo sp. n. S7C							3																		
	Troglarmadillo sp. n. S9											7														
Platyarthridae	*Trichorhina* sp. n. F														1											
	Trichorhina sp. n. G	2	1		1	3	1																			
	Trichorhina sp. n. H							5																		
	Trichorhina sp. n. I																					1				
Stenoniscidae	Stenoniscidae sp. n. Y1																							3		
Araneae																										
Oonopidae	*Opopaea* sp. n. Y2																							1		
	`*Prethopalpus*` `*callani*` Gen. n. sp. n.							1	1		1					1				1						
	`*Prethopalpus*` Gen. n. sp. n. B																							1		
Trochanteriidae	*Desognanops* sp. n. Y1								2			2												5		
Palpigradi																										
Eukoeneniidae	*Eukoenenia* sp. n. S2					2																		3	1	15
Pseudoscorpiones																										
Chthoniidae	*Tyrannochthonius* sp. n. Y1											1	1													
	Tyrannochthonius sp. n. Y2A							4				1	1													
	Tyrannochthonius sp. n. Y2C																								1	
	Tyrannochthonius sp. n. Y3																									1
	Tyrannochthonius sp. n. Y4																		1					1		
	Tyrannochthonius sp. n. Y5																							2		
Olpiidae	*Austrohorus* sp. n. Y1											1														
	Austrohorus sp. n. Y2																							2		
Chilopoda																										
Geophilidae	Geophilidae sp. Y1		1					6															4	2	1	
Cryptopidae	*Cryptops* sp. Y1				1			1	3		1															
Total number of individuals		2	1		1	5	1	13	3		10	13	2		1	1			1	1		1		26	2	16
Total number of species		1	1		1	2	1	4	2		2	6	2		1	1			1	1		1		10	2	2

Table 6-7: Troglofauna species recorded on each drill line (continued). Shading indicates species recorded only within the deposit.

TROGLOFAUNA		PERIPHERY			NORTH WEST				CENTRAL CALCRETE											SOUTH EAST			YEELIRRIE PLAYA		
									OUTSIDE DEPOSIT			DEPOSIT													
		PW	O	Q	P	312	E	A	G	H	F	1	1.5	2	3	3.5	4	4.5	5	6	C	K	D	L	N	SB14
Diplopoda																										
Polyxenida	Polyxenida sp. S1 (Yeelirrie)		2						4	1	5	10	5		3	5	1							22		
Pauropoda	Pauropoda sp. S6A							1																		
	Pauropoda sp. S6B											3														
	Pauropoda sp. Y1						4																			
	Pauropoda sp. Y2		1																							
	Pauropoda sp. Y3																							46		
Symphyla	Symphyla sp. Y1								8																	
	Symphyla sp. Y2										2															
	Symphyla sp. Y3		1																							
	Symphyla sp. Y4							3																		
	Symphyla sp. Y5																								2	
	Symphyla sp. Y6																							1		
	Symphyla sp. Y7											1														
Diplura																										
Japygidae	Japygidae sp. Y3						2	1																3		1
Parajapygidae	Parajapygidae sp. Y1							1	1															1		
Projapygidae	Projapygidae sp. Y2		1					1				1	1											3	4	
Hemiptera																										
Enicocephalidae	Enicocephalidae sp. Y1																								1	
Meenoplidae	Meenoplidae sp. Y1						70	1	1		2											1		8		
Zygentoma																										
Ateluridae	Atopatelurini sp. n. Y2																						1			
Nicoletiidae	*Hemitrinemura* sp. n. Y1				1			1	1			1														
Total number of individuals			8		4		78	33	23	1	22	36	11.5		8	10.5	5					3	5	122	24	19
Total number of species			7		4		5	11	8	1	6	8	5		4	4	2					3	2	10	7	3
Undetermined/ Indeterminate																										
Araneae																										
Oonopidae	Oonopidae sp. indet. YL							1	3	1	3	1		1	1									1	1	
Pseudoscorpiones																										
Chthoniidae	*Tyrannochthonius* sp. grp. Y2																							3		
	Tyrannochthonius sp. indet. YL							1				2												3	4	
Isopoda																										
Armadillidae	*Troglarmadillo* sp. grp. S7										1	12														
Platyarthridae	*Trichorhina* sp. indet. YL										1	5														
Pauropoda	Pauropoda sp. indet. YL						6	3	4			4		1			2		1		1		2	30	2	
Symphyla	Symphyla sp. indet. YL						1	5	7		4				5		6	2	1				1	4		
Indeterminate individuals							7	10	14	1	9	24		2	6		8	2	2		1		3	41	7	
Indeterminate species							2	4	3	1	4	5		2	2		2	1	2		1		2	5	3	

Yeelirrie's troglofauna assemblages are among the most diverse known from the Yilgarn region to date, although few other calcretes have been sampled as intensively for troglofauna. The richest drill line at Yeelirrie (18 species, line L) recorded an equivalent number of species to the previous highest total known from the region (17 at the Sturt Meadows calcrete).

Isopods were diverse in the Study area, with 10 species detected from three families. The taxonomy and ecology of Western Australia's Isopoda is currently undergoing major revisions (S. Taiti pers. comm.). The genera *Troglarmadillo* and *Trichorhina* have been widely recorded in the Pilbara and Yilgarn (Subterranean Ecology unpub. data), although few species have been described to date (S. Taiti pers. comm.). Stenoniscid isopods are previously only known from marine coastal environments, and the recent discovery of inland subterranean species in the region is unusual (S. Taiti pers. comm.). There is limited regional precedent for the rich isopod fauna at Yeelirrie, due to low sampling intensity for troglofauna throughout the region, and little genetic work being conducted to date (Appendices 3 for plates, 7A, 7B and 9).

Arachnida (Araneae, Palpigradi and Pseudoscorpiones) were highly diverse at Yeelirrie, including 13 new species which represent intriguing new records from the Yilgarn (Harvey and Edward 2007, Barranco and Harvey 2008, Platnick 2008, M. Harvey pers. comm.). Two species of spiders in the new genus 'Prethopalpus' were detected (Baehr *et al.* in prep), which has previously been recorded from Cape Range and the Pilbara (Harvey and Edward 2007, Subterranean Ecology unpub. data). New species of spiders in the genera *Desognanops* (previously recorded from Uramurdah calcrete) and *Opopaea* (previously recorded from the Pilbara and Kimberley) were also detected (Harvey and Edward 2007, Platnick 2008) (Appendices 3 for plates, 7C and 9). The palpigrade *Eukoenenia* sp. n. S2 is likely to represent the second subterranean palpigrade species recorded from the Yilgarn region (the other being *Eukoenenia guzikae* from Sturt Meadows) (Appendices 3 for plates and 7D).

Troglobitic pseudoscorpions were particularly rich at Yeelirrie, with eight new species from two genera, including the first records of troglobitic species in the genus *Austrohorus* (M. Harvey pers. comm.). Species in the genus *Tyrannochthonius* were morphologically distinct, and showed high genetic variability (Appendix 9) between populations from different calcretes (Appendices 3 for plates, 7E). This genus is highly diverse and widespread throughout the Pilbara and Yilgarn (Edward and Harvey 2008), although the richness of species detected at Yeelirrie was higher than expected (M. Harvey pers. comm.).

All of the arachnid and isopod species from Yeelirrie are considered troglobitic (excluding previously mentioned Philosciidae). These species have limited powers of dispersal, and their occurrence in restricted calcrete habitats suggests high potential for SRE species at a landscape scale (S. Taiti pers. comm., M. Harvey pers. comm.). The majority of these species were infrequently sampled and detected in low abundance, while some taxa were only able to be identified by genetic analyses. This has likely had an effect on the species distributions recorded, which are probably underestimates of the actual distribution ranges of taxa within the subterranean habitat.

There were other troglofauna species that may occur more widely than currently recorded, possibly even outside of the Study Area. DNA analysis revealed that Polyxenida sp. S1 was genetically similar to a species sampled widely throughout the Pilbara region (Appendices 3 for plates, 7G and

9), although the mechanisms for long-distance dispersal in this group are unknown at this time. Meenoplidae sp. Y1 is considered trogloxenic and unlikely to be restricted to the Study Area, due to the development of dispersal adaptations in the adult stage of the life-cycle (*e.g.* eyes, wings and pigment) (Appendices 3 for plates and 7K). The remaining taxonomic groups (Pauropoda, Symphyla, Geophilidae, Diplura and Zygentoma) are known to include species that inhabit soils as well as cavernous subterranean habitats. However, as the Yeelirrie species are all undescribed, little is known about their specific habitat requirements (Appendices 3 for plates, 7F, 7G, 7H, 7I, 8 and 9).

Both the pastoral wells in granite aquifers and the southeast study zone were depauperate in troglofauna, recording one and four species respectively (Table 6-8). Sixteen species were recorded from the Northwest drill lines, while the greatest number of species (23) was distributed within the playa zone at the southern end of the study site (Table 6-8). The central calcrete contained 14 troglofauna species (Table 6-8).

Table 6-8: Summary table: number of troglofauna species and indeterminate taxa occurring on drill lines and in study zones.

Study Zone		Drill line	Troglofauna	Indeterminate	Study Zone Total
PERIPHERY		PW	1	0	
		O	6	0	6
		Q	0	0	
NORTH WEST		P	3	0	
		312	2	0	
		E	4	2	16
		A	13	4	
CENTRAL CALCRETE	OUTSIDE DEPOSIT	G	8	3	
		H	1	1	10
		F	6	4	
	DEPOSIT	1	11	5	
		1.5	4	0	
		2	0	2	
		3	2	2	
		3.5	2	0	14
		4	1	2	
		4.5	0	1	
		5	1	2	
		6	1	0	
SOUTH EAST		C	0	1	
		K	2	0	4
		D	2	2	
YEELIRRIE PLAYA		L	18	5	
		N	6	3	22
		SB14	3	0	
Overall total			**45**	**7**	

The pattern of troglofauna species richness, on a bore by bore basis, is shown in Figure 6-11. The map shows high richness bores surrounded by low richness bores on lines L, A, and G. This is a considerably different pattern to that of the stygofauna diversity hotspots (Figure 6-2), which showed high diversity bores clustered together in the northwest of the deposit and on lines F and H. The deposit exhibited an evenly-dispersed low richness, except for some moderately diverse bores on line 1 (Figure 6-11).

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Central Calcrete

Central Calcrete

Calcrete - Lines A, E, 312 and P

Inset

Inferred potential habitat line C and K

Calcrete - SB14

Calcrete - Line L

Inferred potential habitat line N

MGA ZONE 50
MGA ZONE 51

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Inferred Boundaries of Subterranean Fauna Calcrete Habitat (SE, 2011)

- Inferred potential subterranean fauna habitat
- Inferred subterranean fauna calcrete habitat

Troglofauna Richness (SE, 2011)

- 1
- 2 - 3
- 4 - 5
- 6 - 7
- 9

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

BHP Billiton Yeelirrie Development Company Pty Ltd

Subterranean Ecology
Scientific Environmental Services
www.subterraneanecology.com.au

Project: **Yeelirrie Uranium Project**

Title: Proposed Yeelirrie Development

Drawn: DL/MR/AK/CJT | Approved: SC | Date: 25/01/2011

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6.2.2 Abundance and Frequency

In general, troglofauna were sparse, with most species found in low numbers (*i.e.* from one to ten individuals collected over all six surveys; Tables 6-6 and 6-7). Seven species numbered more than ten individuals. The plant bug Meenoplidae sp. Y1 and the pin cushion millipede Polyxenida sp. S1 (YL) were the most abundant species (83 and 58 individuals collected, respectively; Table 6-7). On the other hand, 15 species were recorded once on only one occasion, which correlates to the difficulty in capturing troglofauna species.

Figure 6-12 shows the number of occurrences of each species of troglofauna from the survey. Polyxenida sp. S1 (YL), was the most frequently occurring species (31 samples), along with Meenoplidae sp. Y1 which was sampled 17 times. Overall, there were very few common species (four species with ten or more occurrences), with 21 species sampled only once (Figure 6-12).

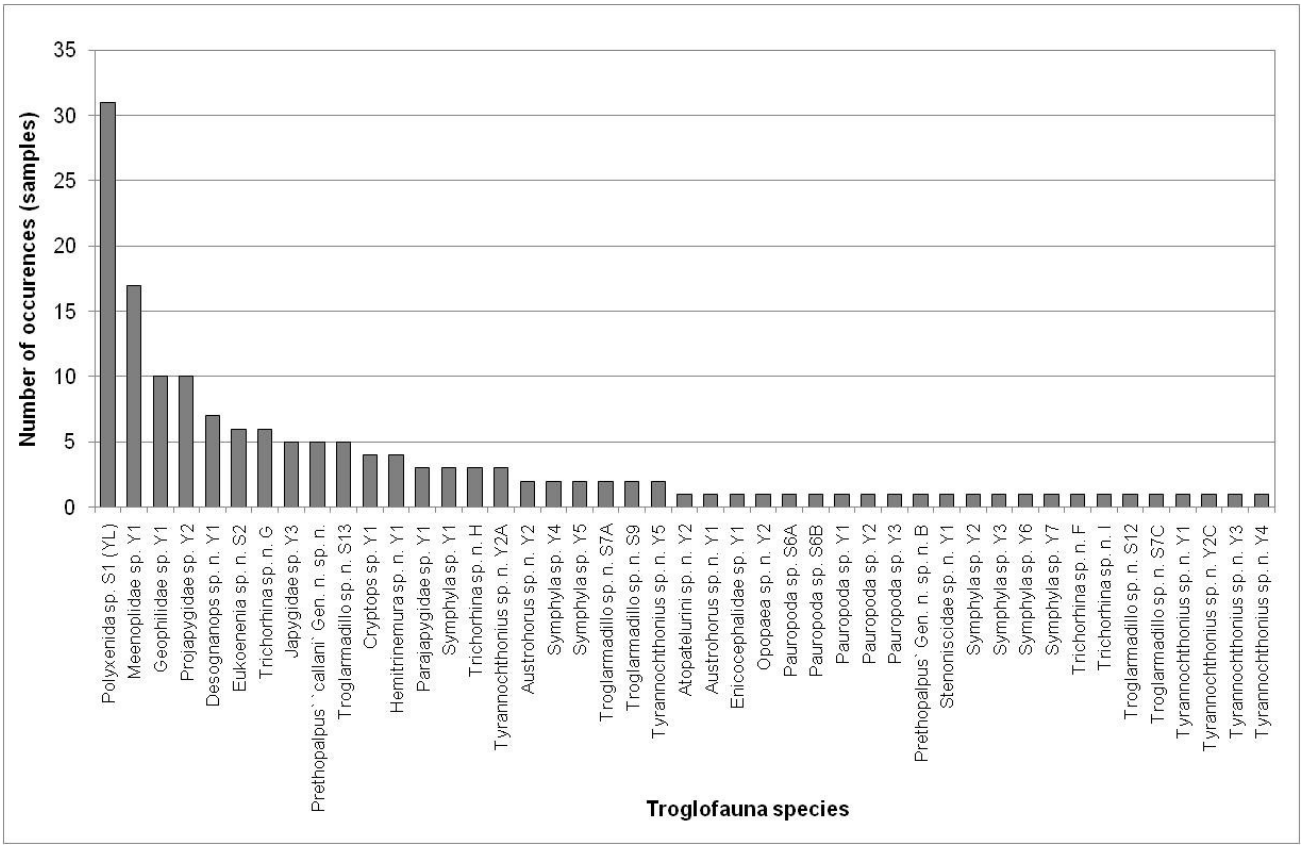

Figure 6-12: Occurrence of troglofauna species in all samples.

Most troglofauna samples contained only one or two individuals of each species, even for commonly-detected species. Infrequently, exceptions to this pattern occurred, for example; pauropods (46 specimens per sample) and polyxenids (10 specimens per sample). The overall pattern indicated that troglofauna populations were inherently sparser than stygofauna, and their assemblages were not as concentrated in the bores. Due to the low frequency and abundance of most troglofauna species, it is difficult to infer population characteristics and the extent of distributions ranges for many of the species.

6.2.3 Species Distribution

It is highly likely that the recorded occurrences of most of these species represent only a part of their actual distribution ranges within the subterranean habitat. However, it can be seen that the troglofauna species were not evenly distributed throughout the Yeelirrie palaeochannel (Tables 6-6 and 6-7).

The troglofauna showed a wide variety of distribution patterns throughout the Study Area. The main distribution patterns included:

- Regionally widespread species,
- Species widespread throughout the Study Area, *i.e.* occurred in two or more study zones,
- Species recorded from single drill lines only, and
- Singletons, species recorded once on one sampling occasion.

Table 6-9 shows the distribution patterns of the troglofauna recorded from the multiple Yeelirrie calcretes. Those species that were named the same, with only a letter or number differentiating between them, refers to morphologically-cryptic species, *e.g. Trichorhina* sp. n. F, G, H and I.

Table 6-9: The distribution patterns displayed by the troglofauna recorded at Yeelirrie.

TROGLOFAUNA				
Regionally widespread	**Widespread multiple calcretes**	**Throughout single calcrete**	**Single location outside deposit**	**Inside deposit only**
Isopoda				
	Troglarmadillo sp. n. S13		*Troglarmadillo* sp. n. S12	***Troglarmadillo* sp. n. S9**
	Trichorhina sp. n. G		*Troglarmadillo* sp. n. S7A	***Trichorhina* sp. n. F**
			Troglarmadillo sp. n. S7C	
			Trichorhina sp. n. H	
			Trichorhina sp. n. I	
			Stenoniscidae sp. n. Y1	
Araneae				
	`*Prethopalpus*` `*callani*` Gen. n. sp. n.		*Opopaea* sp. n. Y2	
	Desognanops sp. n. Y1		`*Prethopalpus*` Gen. n. sp. n. B	
Palpigradi				
	Eukoenenia sp. S2			
Pseudoscorpiones				
	Tyrannochthonius sp. n. Y2A		*Tyrannochthonius* sp. n. Y2C	***Tyrannochthonius* sp. n. Y1**
	Tyrannochthonius sp. n. Y4		*Tyrannochthonius* sp. n. Y3	***Austrohorus* sp. n. Y1**
			Tyrannochthonius sp. n. Y5	
			Austrohorus sp. n. Y2	
Chilopoda				
	Geophilidae sp. Y1			
	Cryptops sp. Y1			
Diplopoda				
Polyxenida sp. S1 (YL)				
Pauropoda				
			Pauropoda sp. S6A	**Pauropoda sp. S6B**
			Pauropoda sp. Y1	
			Pauropoda sp. Y2	
			Pauropoda sp. Y3	
Symphyla				
			Symphyla sp. Y1	**Symphyla sp. Y7**
			Symphyla sp. Y2	
			Symphyla sp. Y3	
			Symphyla sp. Y4	
			Symphyla sp. Y5	
			Symphyla sp. Y6	
Diplura				
	Japygidae sp. Y3			
	Parajapygidae sp. Y1			
	Projapygidae sp. Y2			
Hemiptera				
Meenoplidae sp. Y1*			Enicocephalidae sp. Y1	
Zygentoma				
	Hemitrinemura sp. n. Y1		Atopatelurini sp. n. Y2	
2	**13**	**0**	**24**	**6**

* Meenoplidae sp. Y1 is assumed widespread because of its likely ecological status as a trogloxene and the ability for adult forms to disperse by flight.

Six of a total 14 troglofauna species recorded from the deposit were not recorded outside of the deposit (Tables 6-6 and 6-7). These species were:

- *Troglarmadillo* sp. n. S9 – recorded from line 1 (Table 6-6). Genetically distinct from several sister species recorded on lines 1, F, A and L (Appendix 9);
- *Trichorhina* sp. n. F – recorded once on one sampling occasion from Line 3. Genetically distinct from all other *Trichorhina* species. Undetermined specimens were recorded from outside of the deposit on line F (*Trichorhina* sp. indet. YL) (Appendix 9);
- *Tyrannochthonius* sp. n. Y1 - species identified by distinct morphology, confirmed by DNA analysis (Appendix 9). Recorded from one sample at two locations on lines 1 and 1.5. Undetermined specimens were recorded from outside of the deposit on lines F, L and N (*Tyrannochthonius* sp. indet. YL);
- *Austrohorus* sp. n. Y1 – recorded once from Line 1. Genetically distinct from *Austrohorus* sp. n. Y2 found on line L (Appendix 9);
- Pauropoda sp. S6B - recorded from several samples at the same location on line 1. Genetically distinct from all other pauropod species (Appendices 8 and 9). Undetermined specimens were recorded from outside of the deposit on lines E, A, G, C, D, L and N (Pauropoda sp. indet. YL); and
- Symphyla sp. Y7 – genetically distinct from all other symphylan species (Appendices 8). Recorded from Line 1 once. Undetermined specimens were recorded from outside of the deposit on lines E, A, G, F, D, and L (Symphyla sp. indet. YL).

The location of each of these species within the central calcrete is shown in Figure 6-13. In a similar distribution to the stygofauna (Figure 6-4), the six troglofauna species are located in the western section of the deposit in the central calcrete, from lines 1 to 3.

775000 780000 785000 790000 795000 205000

6995000 6990000 6985000

MGA ZONE 50
MGA ZONE 51

N

0 0.5 1 1.5 2
Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Central Calcrete
G
H
H
B
Central Calcrete
C
I
1
2
3
4
5
6
1.5
3.5
4.5
YYAC0018C
YYAC26
YYAC36
YYD22
YYAC33
YYAC0015A
YYAC118
F
B
I
Inferred potential habitat line C and K
C
MGA ZONE 50
MGA ZONE 51

Source: Landsat (2010)

Legend

- Project Area
- Indicative Project Footprint
- Pit Outline
- Drill and Infill Traverses BHP

Inferred Boundaries of Subterranean Fauna Calcrete Habitat (SE, 2011)

- Inferred potential subterranean fauna habitat
- Inferred subterranean fauna calcrete habitat

Troglofauna Species (Subterranean Ecology, 2010)

- *Austrohorus* sp. Y2-1
- *Pauropoda* sp. S6B
- *Symphyla* sp. Y7
- *Trichorhina* sp. F
- *Troglarmadillo* sp. S9
- *Tyrannochthonius* sp. Y1

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)

BHP Billiton Yeelirrie Development Company Pty Ltd

Subterranean Ecology
Scientific Environmental Services
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Project
Proposed Yeelirrie Development

Title
Troglofauna species recorded only within the deposit

Drawn: CJT	Approved: JN	Date: 09/03/2011
Job No.: 42907132	File No.: 4290712-SUS-245_R0.mxd	

Figure: 6-13

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6.2.4 Survey Adequacy

The total survey effort for troglofauna comprised 452 trapping and bore scraping samples over the course of six sampling events from March 2009 to September 2010. Four species richness estimator models in EstimateS (Colwell 2005) indicated that the sampling to date collected between 67.2% and 83% of the total number of species predicted to occur at Yeelirrie. The proportion of species detected (37 species) *versus* the total number predicted by species richness estimators is shown in Figure 6-14. The discrepancy between the number of species between that recorded previously and now (45 troglofauna species compared to 37) results from the fact that eight troglofauna species were excluded from the survey effort as they were by-catch from stygofauna net hauls and not collected by troglofauna methods.

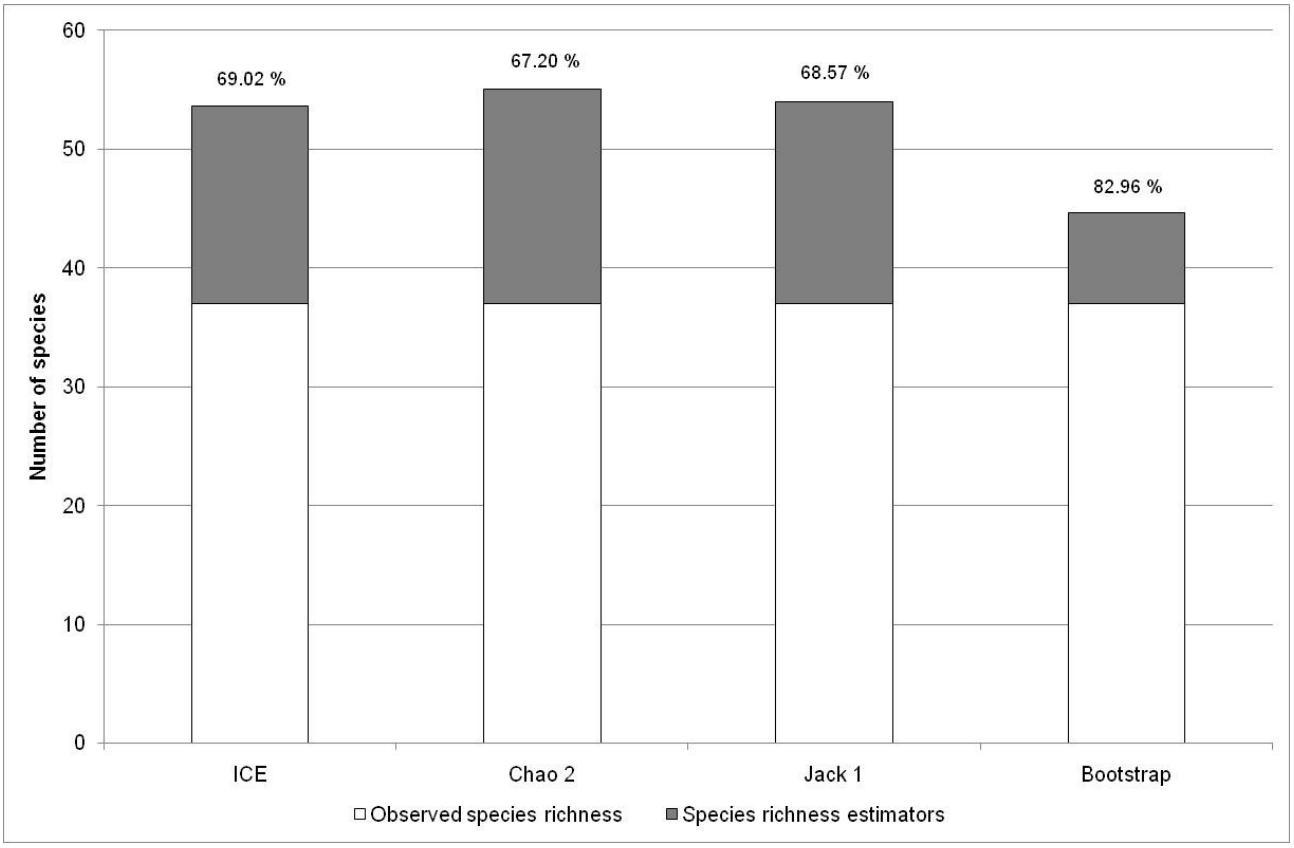

Figure 6-14: Observed troglofauna species richness, relative to predicted species richness, across multiple species richness estimators. Observed richness, as a proportion of each predicted total, is indicated by data labels.

A smoothed species accumulation curve, representing new species detection over the course of the sampling, is shown in Figure 6-15. The rate of new species detection (represented by Sobs Mau Tao) was more gradual than that of stygofauna (Figure 6-8). The observed species curve is rising towards the end of the survey and not reaching a plateau, indicating that the sampling to date for troglofauna at Yeelirrie has been thorough, but not every species predicted to occur has been collected.

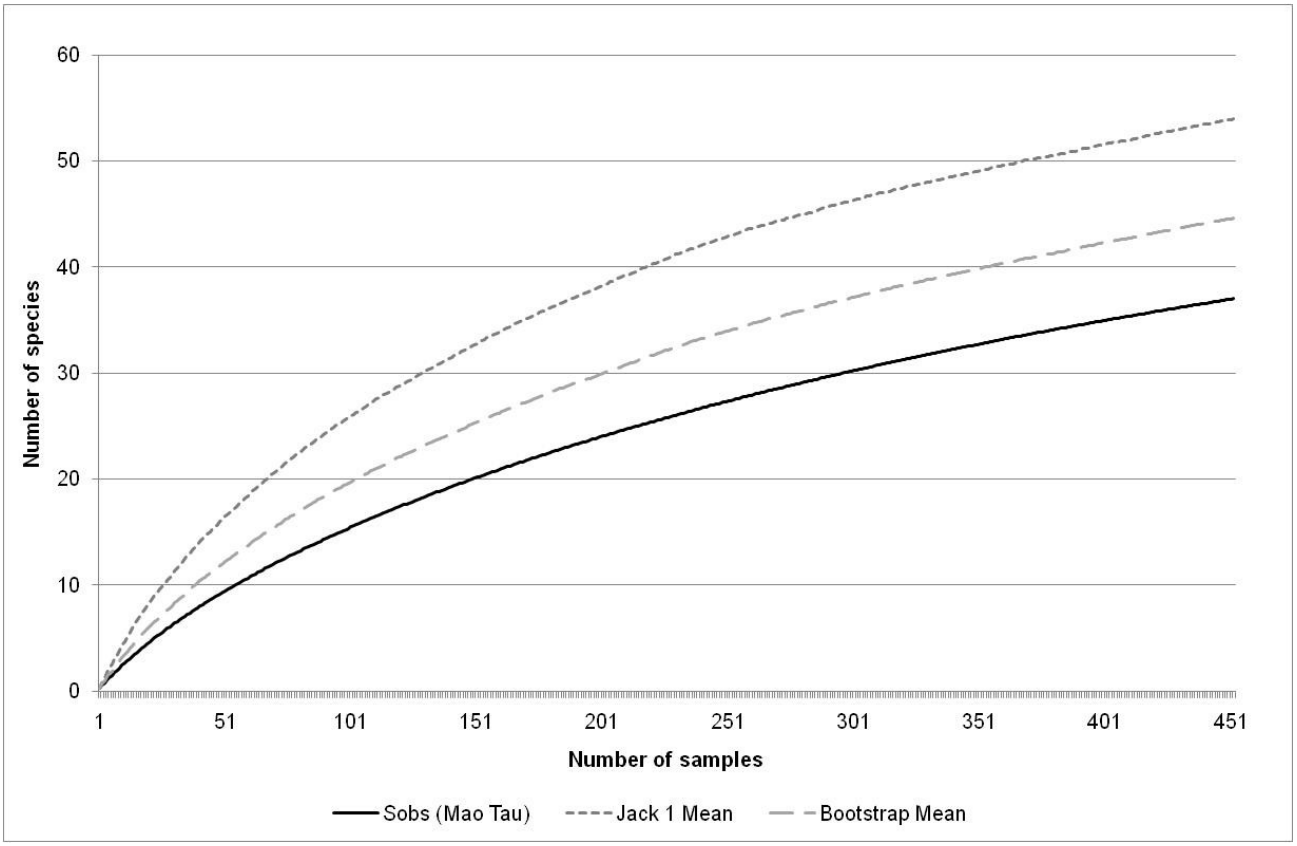

Figure 6-15: Observed troglofauna species accumulation over 452 samples. Curve generated using EstimateS v8.0 (Colwell 2005).

7. DISCUSSION

The survey revealed a high richness of subterranean fauna in the Study Area, with a total of 47 stygofauna species, nine amphibious species and 45 troglofauna species. The richness of stygofauna and troglofauna from the Yeelirrie Study Area is greater than any previous total recorded from other calcretes in the Yilgarn region (based on WAM records and published literature). However, the species rich stygofauna assemblages recorded during the DEC Pilbara survey (Eberhard *et al.* 2009) eclipse even the rich assemblages recorded from Yeelirrie (*e.g.* up to 54 species recorded from a single bore in calcrete in the Pilbara).

The detection of highly diverse subterranean fauna assemblages at Yeelirrie can be partially attributed to high numbers of bores available for sampling (259 bores and wells), the use of multiple methodologies, and high sampling intensity throughout the Study Area. With 641 stygofauna samples and 461 troglofauna samples, the Survey represents the most intensive sampling program for subterranean fauna in the region to date. Detailed morphological and genetic investigations resulted in a high level of taxonomic resolution (only 17% of stygofauna and 27% of troglofauna taxa remained indeterminate). All of these factors have contributed to the detection of rich stygofauna and troglofauna assemblages, beyond what has previously been recorded from the region (based on WAM records and published literature).

Across the Yilgarn region, the few calcretes that have been intensively sampled to date (*e.g.* Uramurdah, Sturt Meadows, Hinkler Well) have recorded significantly higher numbers of species than the remainder, largely in proportion to the level of sampling undertaken (Table 2-1, Figures 2-2 and 2-3). It is not surprising that the results at Yeelirrie follow this trend. It is unlikely that Yeelirrie is unique in the richness of its subterranean fauna assemblages, and further investigation (particularly genetic analysis of species identifications) at other calcretes would be expected to reveal other similarly rich assemblages.

Reasons for the detection of high diversity at Yeelirrie are straightforward; however, the evolutionary origins of the rich assemblages are more complex. Throughout the Yilgarn region, a number of hydrogeological and geographic factors are thought to have influenced the current patterns of species distributions and diversity, including;

- The existence of multiple, hydrologically and geologically discontinuous ‘calcrete island’ habitats (Cooper 2002);
- The gradual onset of aridity from north to south, driving aquatic and terrestrial invertebrate species to take refuge in subterranean habitats (Humphreys 2001);
- Variable salinity within parts of the palaeochannels, producing a range of physicochemical characteristics which provide niche habitats, and potentially, barriers to dispersal for some species (Humphreys 2001, Humphreys *et al.* 2009);
- Historical changes in sea levels and opportunities for invasion of marine species (Humphreys 2001); and

- Historical changes to flow paths, volumes, and direction over time, facilitating successive invasion and re-invasion of epigean aquatic taxa and stygofauna (Humphreys 2001).

Groundwater physicochemical parameters at Yeelirrie indicated the potential for a wide variety of physicochemical niches, due to variable salinity (as represented by EC recorded at the top 1 m of the water table) (Figure 6-9). URS (2011) indicated a complex hydrological system, with variable salinity in lateral, longitudinal, and vertical scales, particularly in the central calcrete. Karanovic (Appendix 11) suggested heterogeneity in water quality as a potential reason for the high richness of copepod species at Yeelirrie. Other potential reasons include the existence of barriers to species movement and gene flow, multiple invasions and changes to flow paths and direction over time (Humphreys 2001, Humphreys *et al.* 2009).

Species accumulation curves indicated adequate survey coverage for stygofauna and troglofauna within the Study Area, despite species estimators indicating that additional species may result from additional sampling. This is not unusual for subterranean fauna surveys; even for the most intensive sampling programs (*e.g.* Eberhard *et al.* 2009). Species accumulation curves may also have been affected by the inclusion of genetically identified species (from only a sub-sample of the total material collected).

The stygofauna and troglofauna assemblages of the Study Area are not evenly distributed between drill lines, or even within drill lines. To date, the richest stygofauna assemblages (12-19 species per line) have been recorded from the central calcrete, specifically in the northwest part of the deposit on lines 1, 1.5, 2, 3, and 3.5, and outside of the deposit on lines F and H. This stygofauna 'hotspot' does not correlate to the most intensively sampled area (40 stygofauna samples at line L and 33 samples at line A (Figure 4-2), and appears to be concentrated around pre-existing bores on lines 1, F and H (Figure 6-1, Figure 6-2). The richest troglofauna assemblage was recorded on line L (18 species), followed by line A (13 species). These records align with two highly sampled areas (61 samples on Line L, 32 on line A), although the results at other well-sampled lines (*e.g.* lines N and E) did not record high troglofauna richness.

The richness of subterranean species in the central calcrete may be attributed to the geological, hydrological and biological characteristics of the habitat. The central calcrete is the largest calcrete body in the palaeochannel, which may provide potential variability in habitat niches due to its size. The central location of this habitat provides fresh to brackish groundwater conditions, and may have made it accessible to colonisations from saline-tolerant species (dispersing upstream), and fresh water species (dispersing downstream).

The high degree of similarity of the stygofauna assemblages within the central calcrete (Figure 6-5 and 6-6) suggests that the habitat is currently continuous, and there is no restriction to the movement of species (or genes) between areas outside the deposit (lines H and F) and areas inside the deposit (lines 1 - 5). However, the stygofauna assemblages outside of the central calcrete are markedly different from assemblages inside the central calcrete (except at line K), and from each other (Figure 6-5 and 6-6). These findings are consistent with a series of discontinuous 'calcrete

islands' (Cooper *et al.* 2002, Leys and Watts 2008) occurring throughout the palaeochannel, of which the central calcrete comprises the largest consolidated unit.

The majority of drill lines including calcrete and transitional calcrete habitats recorded rich to moderately rich subterranean assemblages, supporting the regional patterns (Humphreys 2001, Cooper *et al.* 2002). The occurrence of a rich stygofauna assemblage on line K (11 species, including typical 'calcrete island endemics' such as dytiscid diving beetles), in the absence of saturated calcrete, is atypical. It is possible that the vuggy, carbonated hardpan at line K may provide habitat for the stygofauna species sampled there. Alternatively, fine-scale habitat heterogeneity may occur at line K, including localised calcrete bodies in the immediate vicinity of the current bores.

In general, drill lines where the habitat did not include calcrete or transitional calcrete (*e.g.* lines C, D, Q, and 'PW', the pastoral wells in granite aquifers), recorded comparatively few stygofauna and troglofauna species. Certain species (such as enchytraeid worms, polyxenid millipedes, silverfish, some syncarids and some copepods) may be adapted to exploit interstitial habitats (soils and silt) as well as subterranean habitats. However, for the majority of species considered true stygobites and troglobites, interstitial areas in-between calcrete bodies may form a barrier for dispersal.

There were a variety of spatial patterns in the recorded distribution of subterranean species in the Study Area. A small proportion of stygofauna species (5%) were known to occur in other calcretes in the Yilgarn region (including *M. brooksi, H. eberhardi, A. hamondi*). There were also some troglofauna taxa which would be expected to be widespread on the basis of their ecology (*e.g.* Meenoplidae sp. Y1, a possible trogloxene with a fully-winged adult stage), or from regional results (Polyxenida sp. S1 was genetically similar to populations sampled in the Pilbara, Appendix 9). However, the majority of species detected in the Study Area have not been recorded elsewhere in the region, and are considered unique to the Yeelirrie palaeochannel at this time.

The most south-easterly site within the Study Area (SB14) constitutes part of the Albion Downs calcrete, which is recognised by DEC as a Priority 1 PEC in its own right (DEC 2010). Five species of stygofauna (Phreodrillidae sp. S8, nr. *Phreatochiltonia* sp. n. S1, *Atopobathynella* sp. S4, *Nitokra* sp. `yeelirrie`), and two species of troglofauna (*Eukoenenia* sp. n. S2, and Japygidae sp. Y3) sampled at Yeelirrie were also detected at SB14. The distribution of these species ranges extends beyond the Yeelirrie calcretes.

The regional records revealed a small proportion of 'neighbour' species (11% of stygofauna and 9% of troglofauna) that are known from adjacent calcretes. The Yeelirrie results indicated approximately 28% of amphibious and stygofauna and 32% of troglofauna species were recorded at multiple calcrete habitats (or locally-widespread) at Yeelirrie (Table 6-5 and 6-9). The high proportions of 'neighbour' species in the Study Area may be attributed to the relatively small distances between some calcrete habitats (particularly in the Northwest), and the potential for some species to inhabit geological layers in between calcrete habitats.

The majority of stygofauna and troglofauna species at Yeelirrie, and throughout the region, are known from single calcretes only, *i.e.* calcrete island hypothesis (Cooper *et al.* 2002, Leys and Watts 2008). Yeelirrie recorded high proportions of stygofauna and troglofauna species (respectively 56% and 64% of the total richness) from single drill lines only. Regarding these species:

- Singletons (species from one sample or one bore only) may represent chance detections of species that are rare or difficult to detect. In these cases, the current recorded distribution is likely to be an under-estimate of the actual distribution range throughout the habitat;
- Morphologically cryptic taxa identified by genetic analyses constitute only a sub-sample of the potential wider distribution records for the species. In these cases, the indeterminate specimens which have not been analysed for DNA may indeed represent additional records of the species currently only detected from a single sub-sample; and
- Species recorded from multiple bores along the same drill line may be difficult to detect (as for singletons), or may occur within habitats or niches that are not well-represented in the layout of bores.

There is a possibility that some of the species currently recorded only from single calcretes might be restricted to these habitats (*i.e.* range-restricted species). The majority of undescribed subterranean species detected at Yeelirrie may technically fit the spatial criterion for SRE species (as defined by Harvey 2002 or Eberhard *et al.* 2009). However, at the spatial scales which apply to most species distribution ranges at Yeelirrie, the occurrence of discontinuous habitats is more relevant to conservation significance than the technical definition of what constitutes an SRE species.

At the current time, 35 stygofauna species and 29 troglofauna species were recorded only from single drill lines in the Study Area (Tables 6-1, 6-2, 6-3, 6-5, and 6-6). The majority of these species were detected from calcretes in the Northwest, Southeast and Yeelirrie Playa zones. The recorded occurrence of these species may only represent a proportion of their actual distribution range throughout the available habitat.

Two amphibious species, five stygofauna species and six troglofauna species are currently only known from inside the deposit. Most of these are morphologically cryptic species that were detected by genetic analyses; including, Enchytraeidae sp. Y6, Philosciidae sp. n. Y2, Pauropoda sp. S6B, Symphyla sp. Y7, *Halicyclops* cf. *eberhardi* sp. B, *Trichorhina* sp. n. F and *Schizopera* sp. 7439. Based on the connectivity of subterranean habitats in the central calcrete inside and outside of the deposit, it is likely that these species are more widely distributed throughout the central calcrete than the current data suggests.

Several other species including *Troglarmadillo* sp. n. S9, *Tyrannochthonius* sp. n. Y1, *Austrohorus* sp. n. Y1, *Novanitocrella* 'araia' sp. n, *Schizopera* 'emphysema' sp. n., and *Schizopera* 'akolos' sp. n. represent rare, but morphologically distinct, species. The precise habitat requirements for each of these species are not well-documented. However, the lack of any geological, hydrological or geographic barriers to their dispersal within the central calcrete (inside and outside of the deposit)

would suggest that their potential distribution ranges may extend beyond their current occurrence inside the deposit.

8. CONCLUSIONS

The Yeelirrie subterranean fauna survey revealed a high richness of subterranean fauna in the Study Area, with a total of 47 stygofauna species, nine amphibious species (hereafter included with 'stygofauna') and 45 troglofauna species. The detection of highly diverse assemblages was attributed to the sampling intensity (641 stygofauna samples and 461 troglofauna samples) and the high degree of investigation in the morphological and genetic identifications.

The Survey represents one of the most intensive sampling programs for subterranean fauna in the Yilgarn, and it is not surprising that this has resulted in the greatest richness of stygofauna and troglofauna from any comparable area in the region to date. The results at Yeelirrie fit regional trends in terms of more intensive surveys detecting higher diversity. More detailed investigations at other calcretes (particularly incorporating genetic analyses) would be expected to reveal other rich subterranean assemblages.

The richness of the Yeelirrie subterranean fauna assemblages is a result of the size and variability of discontinuous calcrete habitats throughout the palaeochannel. Saturated and unsaturated calcrete provides the primary habitat, surrounded by a matrix of other geological units which grade from habitable (*e.g.* transitional calcrete) to less habitable (*e.g.* alluvium) for subterranean species. The less habitable geological units between calcretes present barriers to dispersal for the majority of subterranean species. There are a variety of habitat niches within each calcrete which support a diversity of species, due to variable size and structure of the habitat, and differences in physicochemical characteristics (*e.g.* salinity and dissolved oxygen).

The majority of stygofauna and troglofauna species were recorded from single calcrete habitats only. This conforms to the established regional patterns of species distribution, known as the 'calcrete island' hypothesis. A proportion of the stygofauna and troglofauna assemblages were distributed between several calcretes in the Yeelirrie palaeochannel, while only a few species were known to be widespread elsewhere in the region. The distribution patterns displayed by the majority of subterranean fauna species are consistent with the habitat assessment, indicating geographical barriers to dispersal between certain calcretes.

The central calcrete, including the deposit and lines F, H, and G to the immediate northwest, forms a continuous habitat with no apparent barriers to species dispersal or gene flow. The smaller calcrete habitats to the Northwest (separately occurring on lines A, E, 312, and P) support distinct species assemblages, and are considered discontinuous from the central calcrete. To the southeast of the deposit there is an area between lines C and K which has distinct stygofauna assemblages, despite the apparent absence of substantial calcrete units. At the bottom of the palaeochannel, the calcretes (and their assemblages) surrounding the Yeelirrie playa (lines L, N, and SB14) are distinct

from all others in the palaeochannel, and from each other. The periphery of the palaeochannel (lines O, Q, and the pastoral bores in granite aquifers) did not record significant stygofauna, due to the absence of saturated calcrete. However, troglofauna were recorded in unsaturated calcrete at line O.

Although some of the species at Yeelirrie are distributed in multiple calcretes throughout the system, each discontinuous calcrete appears to support some species which have not been recorded elsewhere. The majority of these species were rare, *i.e.* detected only once, and many of them were morphologically cryptic (identified only by a sub-sample sent for genetic analyses). This suggests that the potential distribution ranges of many of these species may extend beyond their recorded occurrence throughout their habitat. Based on regional results, it is highly likely that many of the species detected only in isolated calcrete habitats at Yeelirrie do not occur elsewhere in the region.

Seven species of stygofauna and six species of troglofauna are currently known only from within the boundaries of the deposit. The stygofauna species include; an oligochaete, Enchytraeidae sp. Y6; an isopod, Philosciidae sp. n. Y2; and copepods, *Halicyclops* cf. *eberhardi* sp. B, *Schizopera* sp. 7439, *Schizopera* 'emphysema' sp. n., *Schizopera* 'akolos' sp. n., and *Novanitocrella* 'araia' sp. n. The troglofauna species detected only inside the deposit include isopods, *Troglarmadillo* sp. n. S9, and *Trichorhina* sp. n. F; pseudoscorpions, *Tyrannochthonius* sp. n. Y1 and *Austrohorus* sp. n. Y1; and myriapods, Pauropoda sp. S6B and Symphyla sp. Y7. The connectivity of the central calcrete and the high similarity of the central calcrete assemblages inside and outside of the deposit suggest that these species are either rare or difficult to detect, or they may have specific habitat requirements as yet undefined.

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APPENDIX 1:

Yilgarn regional subterranean fauna records:

WA Museum & published literature

Table 1: Yilgarn regional subterranean fauna records

Calcrete	WAM records	Literature & Reports		Total exc. overlap	
		Unpublished	Published	Stygofauna	Troglofauna
Hinkler Well	27	32	4	37	4
Paroo	24		6	24	2
Lake Violet	35		5	27	8
Uramurdah Lake	38		7	29	15
Millbillillie	20		2	13	8
Cunyu	2		4	4	1
Depot Springs	24		3	23	2
Austin Downs	13		4	15	1
Yarrabubba East	11		1	8	3
Jundee	6		4	6	0
Carnegie Downs	0		1	1	0
Mt Padbury	1		4	4	0
Cunyu Sweetwaters	0		4	7	0
Lorna Glen	14		1	13	1
Sturt Meadows	27		17	13	17
Challa North	10		3	11	0
Cue	0		4	4	0
Lake Mason	11		2	11	0
Windarra	0		3	3	0
Yuinmery South	7		2	9	0
Belele	5		2	7	0
Innouendy	0		4	4	0
Melrose station (Lake Darlot)	11		2	11	1
Pinnacles	12		4	14	1
Killara	9		1	10	0
Windimurra	14		1	12	3
Karalundi	5		3	8	0
Wiluna	6		1	5	1
Killara North	5		1	6	0
Hillview	5		1	5	0
Barwidgee	13		2	9	4
Mt Morgan	0		3	3	0
Perrinvale	10		1	9	2
Melita	4		1	4	1
Yakabindie	2		2	4	1
Nambi	16		1	9	7
Yandal	7		1	6	1

Appendix 2:

Subterranean Ecology personnel & specialist taxonomists

Survey Team

Members of the baseline survey team are indicated in the tables below.

Table 1: Subterranean Ecology personnel involved with Yeelirrie Subterranean Fauna Survey.

Role	Names
Project Manager	S. Callan
Survey Design	S. Eberhard, S. Callan
Field Team	P. Bell, S. Callan, G. Perina, T. Karanovic, N. Krawczyk, S. Catomore
Sorting	N. Krawczyk, S. Callan, S. Voss, G. Perina
Identifications	G. Perina, T. Karanovic, S. Callan, E.S. Volschenk
Data Management and Analysis	S. Callan, N. Coen, E.S. Volschenk
Mapping	P. Bell, URS Australia Pty Ltd
Report compilation	S. Callan, N. Coen
Review	M. Weerheim, S. Blane, S. Danti, S. Eberhard

Table 2: Specialist taxonomists consulted during the Yeelirrie Subterranean Fauna Survey.

Taxonomist	Taxon	Affiliations
Dr. P. Barranco	Palpigradi (palpigrades)	Universidad de Almeria, Spain
Dr S. Cooper	Molecular genetic analyses (copepods)	SA Museum
Dr. R. Leys	Molecular genetic analyses (enchytraeids, dytiscids, symphyla, pauropoda)	SA Museum
Dr T. Finston	Molecular genetic analyses (all other taxa)	Helix Solutions
Dr M.S. Harvey;	Pseudoscorpiones (pseudoscorpions)	WA Museum
Dr M.S. Harvey	Araneae (spiders)	WA Museum
Dr M.S. Harvey	Diplopoda (millipedes)	WA Museum
Dr I. Karanovic	Ostracoda (ostracods)	University of Hamburg
Dr T. Karanovic	Copepoda (copepods)	Subterranean Ecology
Dr. M. Moir	Hemiptera (true bugs)	University of Melbourne
Dr S. Taiti	Isopoda (slaters)	CNR Institute for Ecosystem Studies, Italy
Dr E.S. Volschenk	Chilopoda (centipedes)	Subterranean Ecology
Dr C. Watts	Dytiscidae (diving beetles)	SA Museum
Dr R. King	Amphipoda (amphipods)	SA Museum

APPENDIX 3:

Taxonomic Information

ANNELIDA

Plate 1. Yeelirrie oligochaetes; Naididae sp. S4 (top), Enchytraeidae sp. indet. YL (middle), Phreodrillidae sp. S8 (bottom). Photo copyright Subterranean Ecology 2010.

The Naididae (formerly Tubificidae) and Phreodrillidae sampled at Yeelirrie (Plate 1) are potentially stygobitic, as stygobitic members of these groups are known from the Pilbara and South-west (Pinder *et al.* 2006, Pinder 2008). It is uncertain whether the Aeolosomatidae and Enchytraeidae represent obligate or facultative stygofauna, or incidental by-catch.

DNA analyses of Phreodrillidae (Helix 2010A, Appendix 4) confirmed the presence of a single phreodrillid species collected in the north, central calcrete and playa zones. Enchytraeidae analyses (Leijs 2010, Appendix 5, 6) showed that these amphibious fauna exhibited a high level of genetic divergence between specimens in the four zones they were recorded from, which suggests the occurrence of morphologically cryptic species. In total, seven species of enchytraeid were recorded from Yeelirrie, with sequence divergences ranging from 15.29 – 21.86%.

AMPHIPODA

Plate 2. Yeelirrie Amphipoda: nr. *Phreatochiltonia* sp. n. S1. Photo copyright Subterranean Ecology 2010.

A single, locally widespread species of amphipod was identified, belonging to an undescribed species from a new genus near *Phreatochiltonia* (Plate 2). DNA analyses confirmed this morphological designation, revealing a low to moderate level of genetic variability between geographically separated populations occurring throughout the Yeelirrie palaeochannel, from the north drill lines through to the playa drill lines (Helix 2010C, Appendix 7).

Morphological and genetic analysis of the Yeelirrie amphipods revealed a phylogenetic relationship to taxa that have been sampled from the Great Artesian Basin, rather than other taxa known from the Yilgarn region (King 2009, R. King pers. comm., Helix 2010C).

BATHYNELLIDAE

The morphological identification of the Yeelirrie bathynellids (e.g. Bathynellidae sp. S2, Plate 3) was conducted by G. Perina, revealing two undescribed species, from an unknown genus. The limited state of bathynellid taxonomy prevents identification to generic or specific level at the current time. DNA analyses indicated three genetic lineages at Yeelirrie, corresponding to two geographically-separated species, with one species found only on one line (Bathynellidae sp. S4 in line L; Helix 2010D, Appendix 8).

Further investigation is required to fully document the distribution ranges of the Yeelirrie bathynellid species; although the current information suggests that they conform to the calcrete island paradigm (Guzik *et al.* 2008).

Plate 3. Yeelirrie Bathynellidae: Bathynellidae sp. S2. Photo copyright Subterranean Ecology 2010.

PARABATHYNELLIDAE

Plate 4. Yeelirrie Parabathynellidae: *Atopobathynella* sp. S5. Photo copyright Subterranean Ecology 2010.

Morphological identification of parabathynellids was conducted by G. Perina, resulting in 3 new, undescribed species from the genus *Atopobathynella* (e.g. *Atopobathynella* sp. S5, Plate 4). DNA analyses verified the morphological identifications, while suggesting an allopatric lineage resulting in an additional three species (located in the northern satellite calcretes, lines P and A) (Helix 2010E, Appendix 9).

In the Yilgarn region, the known distribution of stygal parabathynellids conforms to the “calcrete island” hypothesis. The overall pattern of occurrence of *Atopobathynella* species at Yeelirrie supports this hypothesis, although there are some knowledge gaps remaining in the level of gene flow and genetic differences between geographically separated populations (Helix 2010E).

OSTRACODA

Plate 5. Yeelirrie Ostracoda: *Candonopsis* sp. S1. Photo copyright Subterranean Ecology 2010.

The taxonomy of the Yeelirrie ostracods was conducted by Dr T. Karanovic, with assistance from Dr I. Karanovic. An undescribed species of *Candonopsis* (Plate 5) was identified, which was considered morphologically distinct from other known candonids in the Yilgarn region (I. Karanovic pers. comm.). *Candonopsis* sp. S1 was recorded from the central calcrete and is considered likely to be SRE (I. Karanovic pers. comm.).

An additional epigean species (*Sarscypridopsis ochracea*) was detected from various open pastoral wells; this was considered incidental by-catch.

DYTISCIDAE

Plate 6. Yeelirrie Dytiscidae: *Paroster* sp. S1 (left), *Limbodessus* sp. S2 (middle), *Limbodessus* sp. S1 (right). Photo copyright Subterranean Ecology 2010.

The Yeelirrie dytiscid species exhibited distinct morphological characters (including differentiation by size, as seen in Plate 6) that clearly separated them into three undescribed species (C. Watts pers. comm.). The occurrence of three unique, endemic dytiscid species at Yeelirrie conforms to the known distribution pattern of Dytiscidae in the Yilgarn calcretes, *i.e*. SRE's (Cooper *et al.* 2002, Leys *et al.* 2003, Watts and Humphreys 1999, 2000, 2001, 2004, 2006). The detection of a specimen morphologically and genetically conforming to *Limbodessus* sp. S1 (C. Watts pers. comm., Leijs 2010, Appendix 10) outside of the main Yeelirrie calcrete at lines A and K was an unusual discovery in this context.

Genetic analyses confirmed the morphological identifications, and also placed the juvenile specimens that were collected into their respective species (Leijs 2010).

COPEPODA

Plate 7. Yeelirrie Copepoda: *Schizopera* 'akation' (larger, some females with eggs) and *S*. 'akolos' (smaller, middle). Photo copyright Subterranean Ecology 2010.

Morphological taxonomy of the copepods was conducted by Dr T. Karanovic, and verified by DNA analyses of multiple specimens from each morpho-species (conducted by Dr. S. Cooper at the South Australian Museum). Overall, the DNA analyses confirmed the morphological identifications and phylogeny (Cooper 2010 and Karanovic 2010), which resulted in a total of 34 species.

Copepods were widespread and highly abundant in the samples, particularly *Halicyclops eberhardi*, *Schizopera* 'akation' spp. (Plate 7), *S.* 'leptafurca' sp. n., and *S.* 'uranusi' sp. n. Some taxa were sampled at one location only, suggesting the potential for range-restricted species; for example, *Novanitocrella* 'araia linec' sp. n., *Schizopera* 'emphysema' sp. n., *S.* 'akolos' sp. n. (Plate 7), and *Nitokra* 'esbe' sp. n. (Karanovic 2010).

The Yeelirrie copepod species exhibited an unusual trend in the co-occurrence of multiple, closely related species (particularly in the genus *Schizopera*) within the same habitat; sometimes even within the same bore (Karanovic 2010). As many as seven copepod species were recorded from a single sample (such as YYD22 and YYD26 in March 2010). Evidence of sympatric, parapatric, and allopatric distributions within the *Schizopera* species suggests a complex evolutionary history (Karanovic 2010).

In addition to the 19 species identified by morphology, genetic analyses indicated the possibility of additional, morphologically-cryptic species in six copepod taxa (*Halicyclops eberhardi,*

Pseudectinosoma 'pentedicos' sp. n., *Schizopera* 'analspinulosa' sp. n., *S*. 'akation' sp. n., *Nitokra* 'yeelirrie' sp. n. and *Kinnecaris* sp. n.). Four of these genetically distinct haplotypes occurred within the direct impact area, however the limited genetic sampling to date does not adequately characterise their true distribution ranges.

The morphological taxonomy of *Schizopera* 'akolos' sp. n., and *S*. 'emphysema' sp. n was verified by DNA analysis (S. Cooper 2010). A sub-species of *Novanitocrella* 'araia' sp. n. was recorded outside the direct impact area at line C (*N*. 'araia linec' ssp. n.), although genetic analysis was not able to verify the identification of these two sub-species, as their DNA did not amplify (Karanovic 2010).

ISOPODA

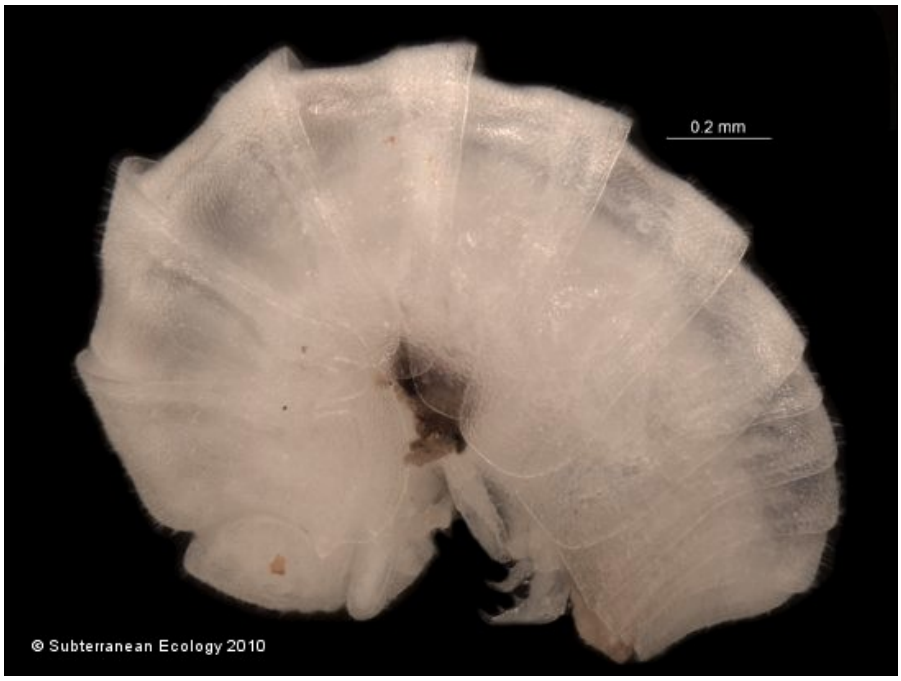

Plate 8. Yeelirrie troglobitic Isopoda: *Troglarmadillo* sp. S13. Photo copyright Subterranean Ecology 2010.

Plate 9. Yeelirrie stygobitic Isopoda: Philosciidae sp. indet. S1. Photo copyright Subterranean Ecology 2010.

Sampling revealed a diverse isopod fauna at Yeelirrie. The genus *Troglarmadillo* (e.g. Plate 8 shows *T*. sp. S13) is well-represented in the Pilbara region, where multiple troglobitic taxa have been revealed by morphological and genetic identification (Subterranean Ecology unpub. data.). The

collection records of Yeelirrie *Troglarmadillo* species are shown in Appendix 11. Genetic testing revealed five species of *Troglarmadillo* (Helix 2010B).

Prior to detection at Yeelirrie, the genus *Trichorhina* was not known from subterranean ecosystems in the Yilgarn, and the poor state of taxonomy within this group prevented species-level identifications (S. Taiti pers. comm.). Genetic testing of the Yeelirrie *Trichorhina* revealed the presence of four highly divergent haplotypes on eight of the drill lines (Helix 2010B, Appendix 11).

The amphibious philosciid isopods have been previously recorded from calcretes at Lake Way and Tenindewa (Taiti and Humphreys 2001). The poor condition of the Yeelirrie specimens prevented identification to genus-level; however, the two philosciid isopods detected by genetic analysis (e.g. Plate 9) did not appear to conform to any other previously-recorded species (S. Taiti pers. comm., Helix 2010B).

Each of the isopod species is regarded as potential SRE, based on the known distribution of isopod species in the Yilgarn, which conform to the 'calcrete island' hypothesis (S. Taiti pers. comm., Cooper *et al.* 2008, Taiti and Humphreys 2001).

ARANEAE

Plate 10. Yeelirrie Araneae: *Desognanops* sp. n. Y1. Photo copyright Subterranean Ecology 2010.

Four species of spiders were collected at Yeelirrie, representing two families; the Trochanteriidae (*Desognanops* sp. n. Y1), and the Oonopidae (*Opopaea* sp. n. Y2, `*Prethopalpus*` `*callani*` Gen. n. sp. n. and *P.* Gen. n. sp. B). Juvenile spiders cannot be identified past family level; therefore a number of indeterminate specimens were recorded as 'Oonopidae sp. indet. YL'. The spiders detected at Yeelirrie belong to undescribed, troglobitic species (M. Harvey pers. comm.), which represent important new records from calcrete habitats in the Yilgarn region. They are key predators of the subterranean ecosystem, and have a high potential to be SRE.

Desognanops is a new, troglobitic trochanteriid genus described from Uramurdah calcrete, near Lake Way (Platnick 2008). The Yeelirrie species, *Desognanops* sp. n. Y1 (Plate 10), was recorded from the main Yeelirrie calcrete (line G and line 1), and the Yeelirrie playa (line L). Direct comparison of DNA sequences for specimens of *Desognanops* indicated they were one species, although genetic variation was detected within the lineage (Helix 2010F, Appendix 12).

Troglomorphic oonopid spiders are well-known from the Pilbara, Kimberley and Yilgarn regions (Harvey and Edward 2007, Subterranean Ecology unpub. data), and are generally considered highly likely to be SRE (M. Harvey pers. comm.). `*Prethopalpus*` `*callani*` Gen. n. sp. n (Plate 11) was detected at a number of locations throughout the Yeelirrie palaeochannel and DNA analysis determined another species of *Prethopalpus*, *P.* Gen. n. sp. B (Helix 2010F; Appendix 12). *Opopaea* sp. n. Y2 was detected only at one location, outside the direct impact area at the Yeelirrie playa (line

L). The DNA of this specimen did not amplify, however Dr. M. Harvey identified the specimen as belonging to this genus from morphological characteristics.

Plate 11. Yeelirrie Araneae: `*Prethopalpus*` `*callani*` Gen. n. sp. n. Photo copyright Subterranean Ecology 2010.

The Araneae specimens are currently undergoing morphological descriptions at the WAM as all species from Yeelirrie are new, undescribed specimens. This, however, will not change the results presented within this report.

PSEUDOSCORPIONIDA

Plate 12. Yeelirrie Pseudoscorpionida: *Tyrannochthonius* sp. Y1 (large) and *Tyrannochthonius* sp. Y2A (small). Photo copyright Subterranean Ecology 2010.

Plate 13. Yeelirrie Pseudoscorpiones: *Austrohorus* sp. Y2-2. Photo copyright Subterranean Ecology 2010.

Six species of pseudoscorpions, from two families; Chthoniidae and Olpiidae, were sampled at Yeelirrie. Troglobitic pseudoscorpions well-known from the Pilbara, Kimberley, Yilgarn and

Southwest regions of WA, and are generally considered to be SRE (Edward and Harvey 2008). The taxonomy and ecology of troglobitic pseudoscorpions is incomplete, although there has recently been a great deal of investigation into the morphological taxonomy and patterns of genetic divergence of Chthoniidae throughout the Pilbara region (Helix 2010G, M. Harvey pers. comm., Subterranean Ecology unpub. data, T. Finston pers. comm.).

The genus *Tyrannochthonius* is known to include subterranean species from all over the world, while in Western Australia, troglobitic species have been recorded from Cape Range, Barrow Island, the Pilbara (Robe River Mesas) and the Yilgarn (Sturt Meadows calcrete and Austin Downs station) (Edward and Harvey 2008).

Two morpho-species of *Tyrannochthonius* (Plate 12) were collected at Yeelirrie, which are clearly differentiated by their adult size, and the dentition of their chelal finger (E. Volschenk pers. comm.). The two species show sympatric (co-occurring) distribution ranges and occurrence inside and outside of the direct impact area. DNA analyses indicated that there were, in fact, six species of *Tyrannochthonius* present at Yeelirrie (Helix 2010G, Appendix 13).

The genus *Austrohorus* has not previously been recorded from subterranean habitats, but the degree of troglomorphism (eyelessness, depigmentation) in the Yeelirrie specimens is high (M. Harvey pers. comm., Plate 13). *Austrohorus* sp. Y2 was sampled over a disjunct distribution range from line 1 inside the deposit, to line L (south eastern Playa calcrete). DNA analysis revealed a high level of genetic divergence between the disjunct populations, indicating morphologically cryptic species within *Austrohorus* at Yeelirrie and resulting in two species (Helix 2010G; Appendix 14).

The pseudoscorpion specimens are currently undergoing morphological descriptions at the WAM as all species from Yeelirrie are new, undescribed specimens. This, however, will not change the results presented within this report.

PALPIGRADI

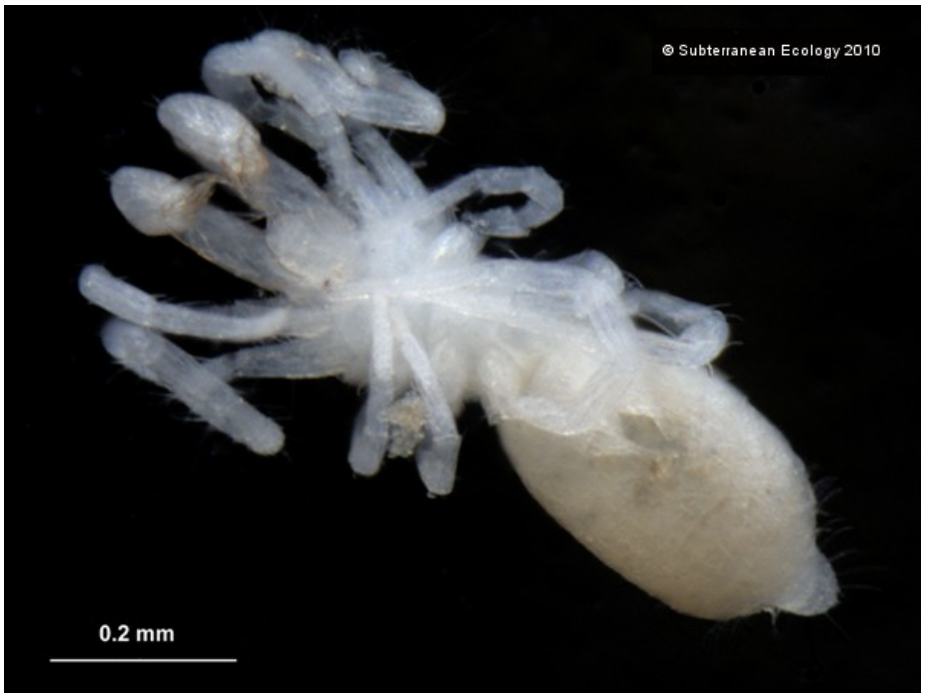

Plate 14. Yeelirrie Palpigradi: *Eukoenenia* sp. S2. Photo copyright Subterranean Ecology 2010.

Palpigrades are poorly represented and rarely collected in Australia, except for non-native epigean species (Barranco and Harvey 2008). The taxonomy of the group is poorly known, although the first indigenous species, *Eukoenenia guzikae,* was described from Sturt Meadows calcrete in the Yilgarn (Barranco and Harvey 2008).

Identification of the Yeelirrie palpigrades is incomplete, although specimens have been sent to a specialist (P. Barranco, Universidad de Almeria) for verification. The record of the Yeelirrie morpho-species, tentatively identified as *Eukoenenia* sp. S2 (Plate 14), is a potentially significant discovery, representing only the second record of the group in the Yilgarn region. The Yeelirrie palpigrade is considered a potential troglobitic SRE species (M. Harvey pers. comm.).

PAUROPODA

Plate 15. Yeelirrie Pauropoda: Pauropoda sp. Indet. YL. Photo copyright Subterranean Ecology 2010.

Pauropods are a poorly studied taxonomic group that is regularly sampled during subterranean fauna surveys. Troglomorphic features (eyelessness, de-pigmentation) always occur throughout the group, and it is uncertain whether pauropods represent troglofauna or soil fauna. The lack of taxonomic and ecological knowledge of Western Australia's pauropod fauna is a serious impediment to their classification and identification.

DNA analyses revealed five highly divergent species from a limited number of specimens that were able to be sequenced (Helix 2010I, Leijs 2010; Appendix 15), with one haplotype occurring on line A and the other on line 1, inside the direct impact area. The high level of genetic divergence indicated the occurrence of morphologically-cryptic species (Plate 15).

SYMPHYLA

Plate 16. Yeelirrie Symphyla: Symphyla sp. indet. YL. Photo copyright Subterranean Ecology 2010.

Symphyla are a poorly studied taxonomic group and although despite a lack of ecological knowledge concerning its status, it is regularly collected. Troglomorphic features (eyelessness, de-pigmentation) generally occur throughout the group, and it is often unclear whether Symphyla species represent troglobites, trogloxenes, or troglophiles.

A lack of taxonomic resources precluded species-level identification of the Symphyla sampled at Yeelirrie (Plate 16). DNA sequencing (Leijs 2010; Appendix 16) revealed highly divergent haplotypes from seven specimens sampled across five zones. These results indicated the occurrence of morphologically-cryptic species throughout the Yeelirrie palaeochannel, some of which co-occur in the same habitat.

SCOLOPENDROMORPHA

Plate 17. Yeelirrie Scolopendromorpha: *Cryptops* sp. indet. YL. Photo copyright Subterranean Ecology 2010.

Scolopendromorph centipedes are represented by the families Cryptopidae and Scolopendridae in Western Australia, with only a single genus in the Cryptopidae: *Cryptops* (Koch 1983, E. Volschenk pers. comm.). Troglomorphic scolopendromorphs have been recorded in Western Australia (Edgecombe 2005, Subterranean Ecology unpub. data), although ecological knowledge of subterranean cryptopids is limited. *Cryptops* specimens are generally pale and always eyeless (Koch 1983), therefore regressive troglomorphisms are not useful for inferring their ecological status.

Cryptopids can grow to a considerable size (several centimetres long), and it follows that larger animals would inhabit larger fractures and meso-caverns, rather than interstitial spaces in soil habitats. The adult size of *Cryptops* sp. indet. YL (Plate 17) indicates it would probably inhabit cavernous habitats such as subterranean calcrete, rather than soil. The Yeelirrie specimens were generally in poor condition, which prevented morphological identification to species level. No DNA analysis was conducted on the cryptopids from Yeelirrie, as they were not detected inside the direct impact area.

GEOPHILIDA

Plate 18. Yeelirrie Geophilida: Geophilidae sp. indet. YL. Photo copyright Subterranean Ecology 2010.

Geophilids are the most diverse order of centipedes, and the most poorly known (M. Harvey pers. comm.). Geophilids are eyeless and de-pigmented, with an elongated (vermiform) body shape, therefore it is difficult to distinguish soil fauna from troglofauna. The current understanding of geophilid centipedes is that a high proportion of species are SREs, regardless of being edaphofauna or troglofauna (M. Harvey pers. comm., Foddai 1999). Due to the high diversity in the group and specific habitat requirements, Geophilidae sp. indet. YL (Plate 18) is considered likely to represent an undescribed SRE species. No DNA analysis was conducted on the Yeelirrie geophilids as they were not detected inside the direct impact area.

POLYXENIDA

Polyxenid millipedes are known from throughout the Pilbara and Yilgarn regions, where they can at times be found in large aggregations (Koch 1983). The morphological taxonomy of the group is not well developed, but genetic analyses have shown some species to be widespread on a regional scale (T. Finston pers. comm.).

The Yeelirrie Polyxenida conformed to a single morpho-species (Polyxenids sp. S1 (YL), Plate 19), with low levels of genetic divergence between geographically dispersed areas (Helix 2010M; Appendix 17). The Yeelirrie haplotypes aligned genetically to the morpho-species Polyxenida sp. S1, which was sampled at many locations throughout the Hamersley Ranges in the Pilbara region (Helix 2010H; Subterranean Ecology unpub. data). The results indicate that Polyxenida sp. S1 (YL) is not a SRE species.

Plate 19. Yeelirrie Polyxenida: Polyxenida sp. S1 (YL). Photo copyright Subterranean Ecology 2010.

DIPLURA

Plate 20. Yeelirrie Diplura: Japygidae sp. Y3. Photo copyright Subterranean Ecology 2010.

Diplurans are commonly sampled in subterranean habitats; however, troglomorphisms (eyelessness, de-pigmentation) occur throughout the group, which causes confusion regarding their ecological status. Troglobitic diplurans are known from Spain (Sendra *et al*. 2006), and Christmas Island (Humphreys and Eberhard 2001). Most diplurans collected in Western Australia are considered to be soil-dwelling species (A. Sendra pers. comm.), although undescribed troglobitic species may occur.

Identification of the Yeelirrie Diplura was hampered by the poor condition of most specimens; the characters used to identify Diplura are easily damaged (e.g. tail segments, antennae). There were three species detected (e.g. Japygidae sp. Y3, Plate 20), none of which were able to be sequenced for genetic analysis. The Yeelirrie dipluran morpho-species were found to have disjunct distributions over geographically-separated areas, suggesting that none of the species are range restricted.

MEENOPLIDAE

Plate 21. Yeelirrie Meenoplidae: Meenoplidae sp. Y1, in adult form (left), and two stages of nymph (middle and right). Photo copyright Subterranean Ecology 2010.

Subterranean meenoplid bugs are commonly sampled throughout the Pilbara, Kimberley, Yilgarn, and Southwest regions of Western Australia (Subterranean Ecology unpub. data). They are highly troglomorphic, with reduced or absent eyes, a lack of pigment, and elongated legs, although many adult meenoplids (including the Yeelirrie specimens) have fully formed wings. Meenoplids feed on plant roots underground, and are generally considered troglophiles or trogloxenes (facultative subterranean species). As such, they are an important part of the subterranean ecosystem, but unlikely to be SRE species. The Yeelirrie meenoplids conformed morphologically to a single morpho-species, Meenoplidae sp. Y1 (Plate 22) (M. Moir pers. comm.), and were not tested for genetic divergence due to their occurrence only outside of the direct impact area.

ENICOCEPHALIDAE

The Enicocephalidae are a family of reduvioid assassin bugs. Very little information regarding troglofaunal enicocephalids is available, however the family has been recorded from caves in Tasmania and Queensland (Eberhard 1996, Howarth 1988). The degree of troglomorphy in the Yeelirrie species is high, including reduced eyes, depigmentation, narrow body shape, and loss of wings, indicative of troglofauna (M. Moir pers. comm.). Enicocephalidae sp. Y1 (Plate 23) is known from a single specimen only (line N) and is considered a potential SRE species.

Plate 22. Yeelirrie Enicocephalidae: Enicocephalidae sp. Y1. Photo copyright Subterranean Ecology 2010.

ZYGENTOMA

Plate 23. Yeelirrie Zygentoma: *Hemitrinemura* sp. Y1. Photo copyright Subterranean Ecology 2010.

Subterranean species of Zygentoma (silverfish, also known as Thysanura) are commonly recorded from caves, karst, and other subterranean ecosystems worldwide (Smith 1998, Harvey 2002). Silverfish in the subfamily Atelurinae are known to inhabit ant nests or termite nests underground, while the Nicoletiinae are detritivores, living in leaf litter, soil or caves (Smith 1998).

All members of the family Nicoletiidae are blind and de-pigmented, and the Nicoletiinae generally have an elongate body form (G. Smith pers. comm.). For this reason, troglomorphisms are unreliable indicators of ecological status. Based upon current knowledge, it is likely that *Hemitrinemura* sp. Y1 (Plate 21) is troglofaunal, while Atopatelurini sp. Y2 may be edaphofauna or troglofauna. Species-level identifications were not possible due to the poor condition of the specimens, and DNA failed to amplify. Sampling detected only one of these morpho-species from a single location, while *Hemitrinemura* sp. Y1 was recorded from three zones. There were a number of fragmented Zygentoma remains which were unable to be identified and it is likely that both of these species have a wider distribution range than the current data suggests.

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APPENDIX 4:

Statistical Information

Table 1: ANOSIM (PRIMER v6) between drill lines (pair-wise results)

Yeelirrie stygofauna data Singletons excluded, species incidence per bore, significant results only					
Drill Lines	**R-value**	**Significance (<5.0% = sig)**	**Possible permutations**	**Actual # perm.**	**# perm. >= R**
F, L	0.454	0.1	5005	999	0
F, A	0.795	0.1	715	715	1
F, C	0.879	0.5	220	220	1
1, E	0.53	0.1	43758	999	0
1, P	0.573	0.4	1001	999	3
1, L	0.453	0.3	8008	999	2
1, A	0.712	0.1	1001	999	0
1, C	0.785	0.3	286	286	1
1, G	0.443	4.5	66	66	3
1.5, E	0.392	0.6	165	165	1
2, E	0.34	1	6435	999	9
2, P	0.496	1.2	330	330	4
2, L	0.322	0.8	1716	999	7
2, A	0.586	0.9	330	330	3
2, C	0.669	0.8	120	120	1
3.5, K	0.645	1.2	84	84	1
3.5, E	0.431	1.1	3003	999	10
3.5, P	0.708	1	210	210	2
3.5, L	0.294	2.2	462	462	10
3.5, A	0.817	0.5	210	210	1
3.5, C	0.88	1.2	84	84	1
K, E	0.431	0.6	165	165	1
E, L	0.237	3.9	3003	999	38
P, L	0.379	1.9	210	210	4
L, A	0.454	1	210	210	2
L, C	0.395	2.4	84	84	2

Table 2: ANOSIM (PRIMER v6) all drill lines (Global results)

Yeelirrie stygofauna data Singletons excluded, species incidence per bore, significant results only				
Drill lines included	**Global R-value**	**Significance (<5.0% = sig)**	**# Permutations**	**# perm. >= R**
All	0.351	0.10%	999	0

Table 3: ANOSIM (PRIMER v6) within drill lines (global results)

Yeelirrie stygofauna data Singletons excluded, species incidence per bore, significant results only				
Drill line	**Global R-value**	**Significance (<5.0% = sig)**	**# Permutations**	**# perm. >= R**
1	0.483	0.10%	999	0
3	0	60.00%	15	9
1.5	0.961	0.30%	999	2
3.5	0.314	2.10%	999	20
2	0.694	0.10%	999	0
4	N/A			
4.5	N/A			
A	0.239	19.60%	56	11
5	-0.114	68.30%	60	41
C	0.4	50.00%	6	3
6	N/A			
E	0.419	19.70%	630	124
F	0.405	0.30%	999	2
K	0.567	2.10%	280	6
G	N/A			
L	0.778	0.1	999	0
H	N/A			
N	N/A			
O	N/A			
312	N/A			
P	-0.296	79.00%	105	83
SB14	N/A			
Q	N/A			

Table 4: ANOSIM (PRIMER v6) within drill lines (construction uncased vs cased)

Yeelirrie stygofauna data Singletons excluded, species incidence per bore				
Drill line	**Global R-value**	**Significance (<5.0% = sig)**	**# Permutations**	**# perm. >= R**
1	0.151	2.90%	999	28
1.5	N/A			
2	0.144	6.7% (ns)	999	66
3.5	N/A			
F	0.106	9.2% (ns)	999	91
K	63	2.90%	35	1
L	0.797	2.20%	45	1

Table 5: ANOSIM (PRIMER v6) within deposit lines (Global results)

Yeelirrie stygofauna data Singletons excluded, species incidence per bore, significant results only				
Drill lines included	**Global R-value**	**Significance (<5.0% = sig)**	**# Permutations**	**# perm. >= R**
1, 1.5, 2, 3, 3.5, 4, 5	0.054	23.40%	999	233

Table 6: ANOSIM (PRIMER v6) within NMDS group (Global result)

Yeelirrie stygofauna data Singletons excluded, species incidence per bore, significant results only				
Drill lines included	**Global R-value**	**Significance (<5.0% = sig)**	**# Permutations**	**# perm. >= R**
1, 1.5, 2, 3, 3.5, 5, F, H	0.085	8.90%	999	88

Table 7: ANOSIM (PRIMER v6) between NMDS group and drill lines (pairwise results)

Yeelirrie stygofauna data Singletons excluded, species incidence per bore				
Drill lines included	**Global R-value**	**Significance (<5.0% = sig)**	**# Permutations**	**# perm. >= R**
Group vs E	0.496	0.1	999	0
Group vs P	0.511	0.7	999	6
Group vs L	0.406	0.6	999	5
Group vs 312	0.735	4.7	43	2
Group vs A	0.717	0.1	999	0
Group vs C	0.768	0.2	999	1
K vs E	0.431	0.6	165	1
E vs L	0.237	3.3	999	32
P vs L	0.379	1.9	210	4
L vs A	0.454	1	210	2
L vs C	0.395	2.4	84	2

APPENDIX 5: Amphibious fauna maps

N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Haplotaxida

- Enchytraeidae sp. Y1
- Enchytraeidae sp. Y2
- Enchytraeidae sp. Y3
- Enchytraeidae sp. Y4
- Enchytraeidae sp. Y5
- Enchytraeidae sp. Y6
- Enchytraeidae sp. Y7
- Enchytraeidae sp. indet. YL

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Amphibious Enchytraeidae

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 5A	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-180R_R0.mxd			A3

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N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses
- Philosciidae sp. n. S1
- Philosciidae sp. n. Y2

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Amphibious Philosciidae

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 5B	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-181M_R0.mxd			A3

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APPENDIX 6: Stygofauna maps

N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Haplotaxida
- Aeolosoma sp. S1
- Naididae sp. S4
- Naididae sp. S5
- Phreodrillidae sp. S8

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Stygofauna Annelida

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 6A	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-180I_R0.mxd			A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

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7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Amphipoda

- nr. Phreatochiltonia sp. n. S1

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Stygofauna Amphipoda

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 6B	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-180C_R0.mxd			A3

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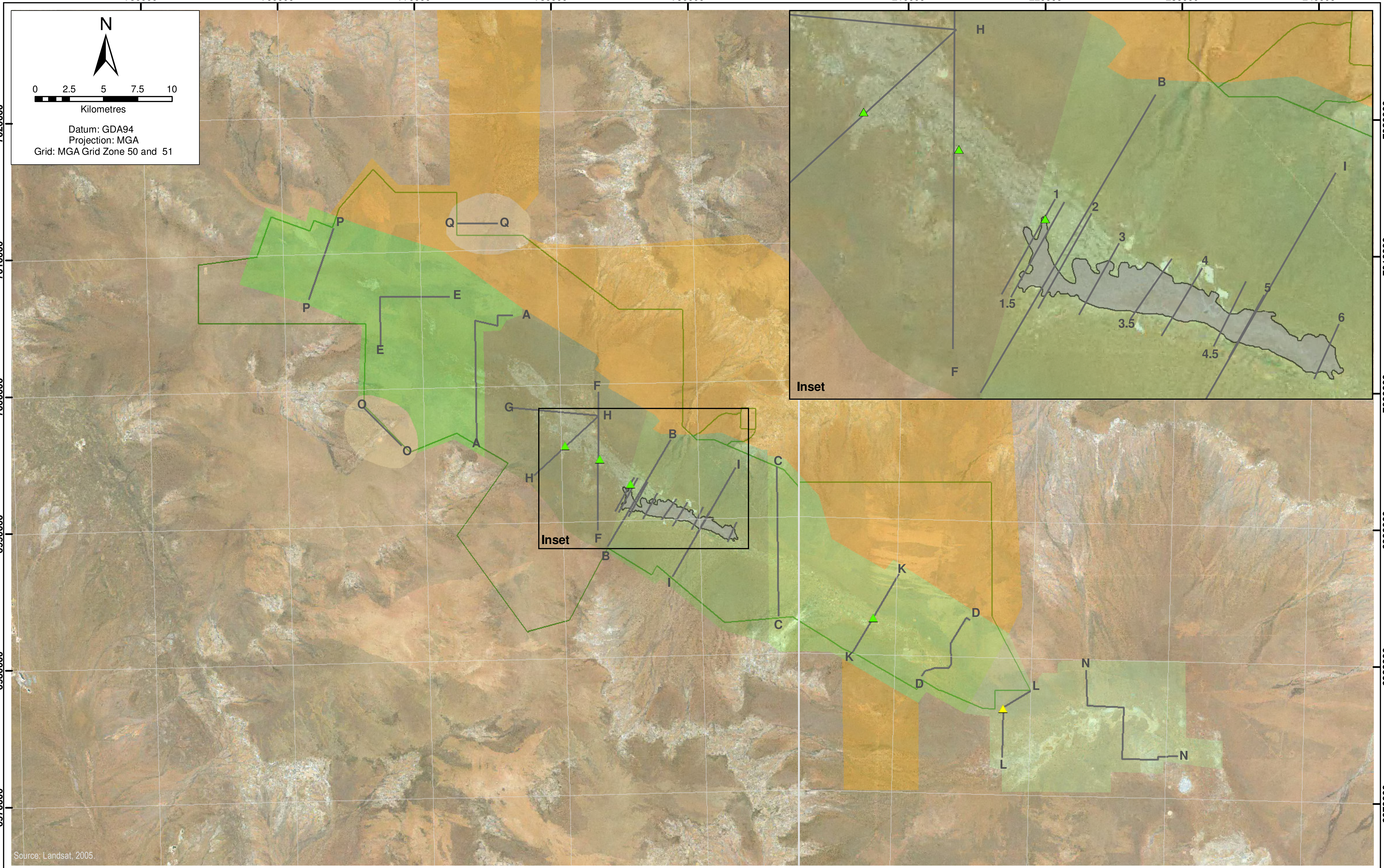

Legend

- Pit Outline
- Drill and Infill Traverses

Bathynellacea

- Bathynellidae sp. S2
- Bathynellidae sp. S4

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

BHP Billiton Yeelirrie Development Company Pty Ltd	Project: **Proposed Yeelirrie Development**		Title: Location of Stygofauna Bathynellidae
Subterranean Ecology Scientific Environmental Services www.subterraneanecology.com.au	Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011
	Job No.: 42907132	File No.: 42907132-SUS-180_R0.mxd	Appendix 6C — Rev. 0 — A3

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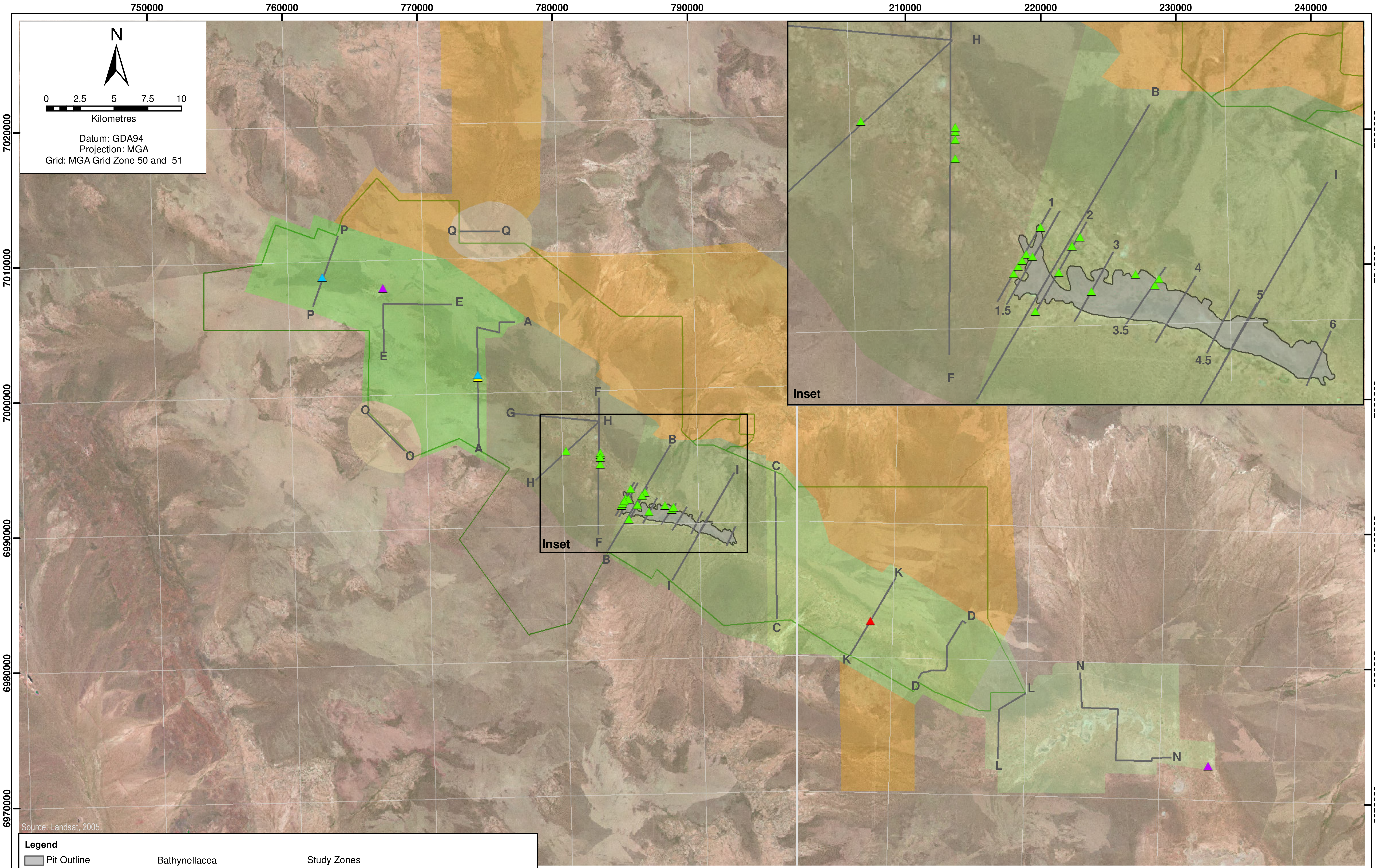

Legend

Pit Outline
Project Area
Drill and Infill Traverses

Bathynellacea
- Atopobathynella sp. S4
- Atopobathynella sp. S5
- Atopobathynell sp. Y1
- Atopobathynella sp. Y2
- Atopobathynella sp. Y3
- Atopobathynella sp. `line K`

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

BHP Billiton Yeelirrie Development Company Pty Ltd	Project: **Proposed Yeelirrie Development**		Title: Location of Stygofauna Parabathynellidae	
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	Job No.: 42907132	File No.: 42907132-SUS-180B_R0.mxd	Appendix 6D	A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

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7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Harpacticoida
- Australocamptus hamondi
- Novanitocrella `araia linec` ssp. n.
- Novanitocrella `araia` sp. n.
- Pseudectinosoma `pentedicos` sp. A
- Pseudectinosoma `pentedicos` sp. B
- Pseudectinosoma `pentedicos` sp. C
- Pseudectinosoma sp. grp. `pentedicos`

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Stygofauna Harpacticoida

Drawn: AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 6E	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-180S_R0.mxd			A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

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7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Harpacticoida
- Schizopera `akation` sp. n.
- Schizopera `leptafurca` sp. n.
- Schizopera `uranusi` sp. n.

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Stygofauna Schizopera Akation

Drawn: DL/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 6F	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-180G_R0.mxd			A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Inset

Source: Landsat, 2005

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Harpacticoida

- Schizopera `akolos` sp. n.
- Schizopera `analspinulosa linel` ssp. n.
- Schizopera `analspinulosa` sp. n.
- Schizopera `emphysema` sp. n.
- Schizopera `kronosi` sp. n.
- Schizopera `line N` sp. n.
- Schizopera sp. 7439

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project
Proposed Yeelirrie Development

Title
Location of
Stygofauna Harpacticoida

Drawn: DL./AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 6G	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-180M_R0.mxd			A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Harpacticoida

- Kinnecaris `esbe` sp. n.
- Kinnecaris `linea` sp. n.
- Kinnecaris `lined` sp. n.
- Kinnecaris `linel` sp. n.
- Kinnecaris `linep` sp. n.
- Kinnecaris `uranusi` sp. n.
- Kinnecaris sp. indet. YL

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Stygofauna Harpacticoida

Drawn: DL/MR/AK/CJT | Approved: SC | Date: 09/03/2011

Job No.: 42907132 | File No.: 42907132-SUS-180F_R0.mxd

Appendix 6H | Rev. 0 | A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

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7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Harpacticoida

- Nitokra `esbe` sp. n.
- Nitokra `yeelirrie` sp. n.

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Stygofauna Harpacticoida

Drawn: DL/MR/AK/CJT | Approved: SC | Date: 09/03/2011

Job No.: 42907132 | File No.: 42907132-SUS-180E_R0.mxd

Appendix 6I | Rev. 0 | A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

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Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses
- Dussartcyclops `dostoyevskyi` sp. n.

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

BHP Billiton Yeelirrie Development Company Pty Ltd	Project: **Proposed Yeelirrie Development**		Title: Location of Stygofauna Cyclopoida
Subterranean Ecology — Scientific Environmental Services — www.subterraneanecology.com.au	Drawn: DL/MR/AK/CJT	Approved: SC / Date: 09/03/2011	Appendix 6J — Rev. 0 — A3
	Job No.: 42907132	File No.: 42907132-SUS-180K_R0.mxd	

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

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7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat: 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Cyclopoida
- Halicyclops cf. eberhardi
- Halicyclops cf. eberhardi sp. A
- Halicyclops cf. eberhardi sp. B
- Halicyclops cf. eberhardi sp. C

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Stygofauna Cyclopoida

Drawn: DL/MR/AK/CJT | Approved: SC | Date: 09/03/2011

Job No.: 42907132 | File No.: 42907132-SUS-180D_R0.mxd

Appendix 6K | Rev. 0 | A3

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Kilometres

Datum: GDA94
Projection: Lambert Conformal Grid: MGA Zone 50 and 51

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7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Podocopida
- Candonopsis sp. n. Y1

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Stygofauna Coleoptera

Drawn: DL/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 6L	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-180J_R0.mxd			A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Inset

Source: Landsat 2005

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Coleoptera

- Limbodessus sp. n. `yeelirriensis`
- Limbodessus sp. S1
- Paroster sp. n. `angustus`

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Stygofauna Coleoptera

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 6M	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-180H_R0.mxd			A3

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APPENDIX 7: Troglofauna maps

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Inset

Source: Landsat, 2005.

Legend

Project Area
Pit Outline
Drill and Infill Traverses

Isopoda
- Troglarmadillo sp. grp. S7
- Troglarmadillo sp. S12
- Troglarmadillo sp. S13
- Troglarmadillo sp. S7A
- Troglarmadillo sp. S7C
- Troglarmadillo sp. S9

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project
Proposed Yeelirrie Development

Title
Location of
Troglofauna Isopoda

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 7A	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-181D_R0.mxd			A3

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N

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Isopoda
- Trichorhina sp. F
- Trichorhina sp. G
- Trichorhina sp. H
- Trichorhina sp. I
- Trichorhina sp. indet. YL
- Stenoniscidae sp. Y1

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Isopoda

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 7B	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-181I_R0.mxd			A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

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7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Aranae

- Oonopidae sp. indet. YL
- Opopaea sp. n. Y2
- Prethopalpus` `callani` Gen. n. sp. n.
- Prethopalpus` Gen. n. sp. B
- Desognanops sp. n. Y1

Subterranean Fauna Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Araneae

Drawn: DL/MR/AK/CJT | Approved: SC | Date: 24/01/2011

Job No.: 42907132 | File No.: 42907132-SUS-181_R0.mxd

Appendix 7C | Rev. 0 | A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

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7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Pseudoscorpiones
- Eukoenenia sp. S2
- Austrohorus sp. Y2-1
- Austrohorus sp. Y2-2

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Arachnida

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 7D	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-181E_R0.mxd			A3

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Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Pseudoscorpiones

- Tyrannochthonius sp. indet. YL
- Tyrannochthonius sp. grp. Y2
- Tyrannochthonius sp. Y1
- Tyrannochthonius sp. Y2A
- Tyrannochthonius sp. Y2C
- Tyrannochthonius sp. Y3
- Tyrannochthonius sp. Y4
- Tyrannochthonius sp. Y5

Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Pseudoscorpiones

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 7E	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-181J_R0.mxd			A3

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N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Geophilida and Scolopendromorpha

- Geophilidae sp. Y1
- Cryptops sp. Y1

Subterranean Fauna Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Myriapoda

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 7F	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-181G_R0.mxd			A3

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N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Polyxenida

- Polyxenida sp. S1 (YL)

Subterranean Fauna Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Polyxenida

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 7G	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-181L_R0.mxd			A3

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N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Pauropoda
- Pauropoda sp. indet. YL
- Pauropoda sp. S6A
- Pauropoda sp. S6B
- Pauropoda sp. Y1
- Pauropoda sp. Y2
- Pauropoda sp. Y3

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Pauropoda

Drawn: DL/MR/AK/CJT | Approved: SC | Date: 09/03/2011

Job No.: 42907132 | File No.: 42907132-SUS-181F_R0.mxd

Appendix 7H | Rev. 0 | A3

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750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Symphyla

- Symphyla sp. indet. YL
- Symphyla sp. Y1
- Symphyla sp. Y2
- Symphyla sp. Y3
- Symphyla sp. Y4
- Symphyla sp. Y5
- Symphyla sp. Y6
- Symphyla sp. Y7

Subterranean Fauna Study Zones

- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Symphyla

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 7I	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-181K_R0.mxd			A3

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750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Diplura
- Japygidae sp. Y3
- Parajapygidae sp. Y1
- Projapygidae sp. Y2

Subterranean Fauna Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Diplura

Drawn: DL/MR/AK/CJT	Approved: SC	Date: 09/03/2011	Appendix 7J	Rev. 0
Job No.: 42907132	File No.: 42907132-SUS-181H_R0.mxd			A3

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750000 760000 770000 780000 790000 210000 220000 230000 240000

7020000 7010000 7000000 6990000 6980000 6970000

N

0 2.5 5 7.5 10

Kilometres

Datum: GDA94
Projection: MGA
Grid: MGA Grid Zone 50 and 51

Inset

Source: Landsat, 2005.

Legend

- Project Area
- Pit Outline
- Drill and Infill Traverses

Hemiptera
- Enicocephalidae sp. Y1
- Meenoplidae sp. Y1

Study Zones
- Zone 1: Granite Pastoral Bores
- Zone 2: Periphery (Lines O, Q)
- Zone 3: North West (Lines P, 312, E, A)
- Zone 4: Centrall Outside Deposit (Lines F, H, G)
- Zone 5: Central; Deposit (Lines 1 - 6)
- Zone 6: South East (Lines C, K, D)
- Zone 7: Yeelirrie Playa (Lines L, N, SB14)

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Project: **Proposed Yeelirrie Development**

Title: Location of Troglofauna Hemiptera

Drawn: DL/MR/AK/CJT | Approved: SC | Date: 09/03/2011

Job No.: 42907132 | File No.: 42907132-SUS-181C_R0.mxd

Appendix 7K | Rev. 0 | A3

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APPENDIX 8: Dr R. Leys (SA Museum) DNA Reports

Enchytraeidae

Pauropoda

Symphyla

Dytiscidae

For Subterranean Ecology

Molecular biodiversity assessment of the subterranean fauna of the Yeelirrie calcrete

Methods

Biodiversity assessment of a selection of the collected fauna from the Yeelirrie calcrete (Table 1) was performed by PCR amplification and sequencing of a 677 bp fragment of CO1, commonly used for DNA barcoding (Hebert et al. 2003). The sequences were added to datasets that consists of related taxa from the region (Dytiscidae; Leys et al. 2003, 2008) complemented with unpublished sequence data at the SA-Museum and data from Genbank.

	Genus	SA-Museum id.	sample	Code		Extr.date	Coll.Date	locality	CO1
ST1190	Enchytraeidae sp. indet. YL		10:0412	YYHC0133B	P	15-Nov-10	22-Sep-10	Yeelirrie	no PCR
ST1191	Enchytraeidae sp. indet. YL	sp. 7	10:0071	WIRRAWAY_BORE	A	15-Nov-10	19-Sep-10	Yeelirrie	
ST1192	Enchytraeidae sp. indet. YL	sp. 7	LN:7316	UNK1	L	15-Nov-10	14-Nov-09	Yeelirrie	
ST1193	Enchytraeidae sp. indet. YL		LN:8381	YYHC0120	E	15-Nov-10	16-Mar-10	Yeelirrie	no PCR
ST1194	Enchytraeidae sp. indet. YL	sp. 3	LN:8429	YYHC0133B	P	15-Nov-10	17-Mar-10	Yeelirrie	
ST1195	Enchytraeidae sp. indet. YL	sp. 1	LN:8452	YYHC0048L	L	15-Nov-10	17-Mar-10	Yeelirrie	
ST1196	Enchytraeidae sp. indet. YL	sp. 5	LN:8456	YYHC0048KA	K	15-Nov-10	18-Mar-10	Yeelirrie	
ST1197	Enchytraeidae sp. indet. YL	sp.4	LN:8460	NORTH_BORE	D	15-Nov-10	19-Mar-10	Yeelirrie	
ST1198	Enchytraeidae sp. indet. YL		LN:8490	YU1	F	15-Nov-10	15-Mar-10	Yeelirrie	no PCR
ST1199	Enchytraeidae sp. indet. YL		LN:8529	NO312_BORE	312	15-Nov-10	16-Mar-10	Yeelirrie	no PCR
ST1200	Enchytraeidae sp. indet. YL	sp. 2	LN:8559	WIRRAWAY_BORE	A	15-Nov-10	18-Mar-10	Yeelirrie	
ST1201	Enchytraeidae sp. indet. YL		LN:8567	YU1	F	15-Nov-10	18-Mar-10	Yeelirrie	no PCR
ST1202	Enchytraeidae sp. indet. YL		LN:8573	YYHC0078C	D	15-Nov-10	19-Mar-10	Yeelirrie	no PCR
ST1203	Enchytraeidae sp. indet. YL		LN:8596	YYHC0037C	C	15-Nov-10	19-Mar-10	Yeelirrie	no PCR
ST1204	Enchytraeidae sp. indet. YL	sp. 5	LN:9994	YYHC0037C	C	15-Nov-10	23-Sep-10	Yeelirrie	
ST1205	Enchytraeidae sp. indet. YL		LN:9997	YYHC0121B	E	15-Nov-10	22-Sep-10	Yeelirrie	no PCR
ST1206	Pauropoda sp. indet. YL	sp.1	10:0376	YYHC0118A	E	15-Nov-10	22-Sep-10	Yeelirrie	
ST1207	Pauropoda sp. indet. YL		10:0397	D-Trog5	D	15-Nov-10	23-Sep-10	Yeelirrie	no PCR
ST1208	Pauropoda sp. indet. YL		10:0399	D-Trog4	D	15-Nov-10	23-Sep-10	Yeelirrie	no PCR
ST1209	Pauropoda sp. indet. YL		10:0033	YYHC0120	E	15-Nov-10	19-Sep-10	Yeelirrie	no PCR
ST1210	Pauropoda sp. indet. YL	sp.2	10:0038	YYHC0105C	O	15-Nov-10	19-Sep-10	Yeelirrie	
ST1211	Pauropoda sp. indet. YL		LN:7084	YYHC0111	G	15-Nov-10	14-Jan-10	Yeelirrie	no PCR
ST1212	Pauropoda sp. indet. YL	sp.3	LN:7577	YYHC0048I	L	15-Nov-10	12-Jan-10	Yeelirrie	
ST1213	Pauropoda sp. indet. YL		LN:7603	YYHC0059G	N	15-Nov-10	12-Jan-10	Yeelirrie	no PCR
ST1214	Pauropoda sp. indet. YL		LN:7611	YYHC0048F	L	15-Nov-10	12-Jan-10	Yeelirrie	no PCR
ST1215	Pauropoda sp. indet. YL		LN:7617	YYHC0114	A	15-Nov-10	11-Jan-10	Yeelirrie	no PCR
ST1216	Pauropoda sp. indet. YL		LN:7637	YYHC0110	G	15-Nov-10	14-Jan-10	Yeelirrie	no PCR
ST1217	Pauropoda sp. indet. YL		LN:8470	YYHC0114	A	15-Nov-10	16-Mar-10	Yeelirrie	no PCR
ST1218	Symphyla sp. indet. YL		10:0433	YYHC0053C	F	15-Nov-10	17-Sep-10	Yeelirrie	no PCR
ST1219	Symphyla sp. indet. YL		10:0459	YYHC0113	A	15-Nov-10	19-Sep-10	Yeelirrie	no PCR
ST1220	Symphyla sp. indet. YL		FN:8112b	YYHC0111	G	15-Nov-10	14-Jan-10	Yeelirrie	no PCR
ST1221	Symphyla sp. indet. YL		LN:7337	WIRRAWAY_BORE	A	15-Nov-10	15-Nov-09	Yeelirrie	no PCR
ST1222	Symphyla sp. indet. YL	sp.3	LN:7368	YYHC0105C	O	15-Nov-10	13-Jan-10	Yeelirrie	
ST1223	Symphyla sp. indet. YL	sp.1	LN:7627	YYHC0109	G	15-Nov-10	14-Jan-10	Yeelirrie	
ST1224	Symphyla sp. indet. YL	sp.1	LN:7630	YYHC0107	G	15-Nov-10	14-Jan-10	Yeelirrie	
ST1225	Symphyla sp. indet. YL		LN:8196	D-Trog5	D	15-Nov-10	19-Mar-10	Yeelirrie	contam.
ST1226	Symphyla sp. indet. YL		LN:8397	YYHC0136	F	15-Nov-10	17-Mar-10	Yeelirrie	no PCR
ST1227	Symphyla sp. indet. YL		LN:8433	YYHC0053C	F	15-Nov-10	17-Mar-10	Yeelirrie	no PCR
ST1228	Symphyla sp. indet. YL	sp.5	LN:8436	YYHC0059G	N	15-Nov-10	17-Mar-10	Yeelirrie	
ST1229	Symphyla sp. indet. YL		LN:8440	YYHC0048H	L	15-Nov-10	17-Mar-10	Yeelirrie	no PCR
ST1230	Symphyla sp. indet. YL		LN:8467	YYHC0115B	E	15-Nov-10	16-Mar-10	Yeelirrie	no PCR
ST1231	Symphyla sp. indet. YL		LN:9763	YYHC0048G	L	15-Nov-10	23-Sep-10	Yeelirrie	no PCR
ST1232	Dytiscidae larva	*Limbodessus* sp. nov.1	10:0377	YYHC0118A	E	15-Nov-10	22-Sep-10	Yeelirrie	
ST1233	Dytiscidae larva	*Limbodessus* sp. nov.1	10:0429	YYHC0118B	E	15-Nov-10	19-Sep-10	Yeelirrie	
ST1234	Limbodessus sp. S1	*Limbodessus* sp. nov.1	10:0011a	YU1	F	15-Nov-10	17-Sep-10	Yeelirrie	
ST1235	Limbodessus sp. S1		LN:7682	YYAC328	3.5	15-Nov-10	11-Jan-10	Yeelirrie	no PCR
ST1236	Limbodessus sp. S1	*Limbodessus* sp. nov.1	LN:8507	TPB33-1	H	15-Nov-10	15-Mar-10	Yeelirrie	
ST1237	Limbodessus sp. S2	*Limbodessus* sp. nov.2	10:0456	YYHC0054C	F	15-Nov-10	17-Sep-10	Yeelirrie	
ST1238	Limbodessus sp. S2	*Limbodessus* sp. nov.2	10:0011b	YU1	F	15-Nov-10	17-Sep-10	Yeelirrie	
ST1239	Limbodessus sp. S2		LN:8353	YYAC0019C	1	15-Nov-10	16-Mar-10	Yeelirrie	no PCR
ST1240	Limbodessus sp. S2	*Limbodessus* sp. nov.2	LN:8367	YYAC248	3.5	15-Nov-10	16-Mar-10	Yeelirrie	
ST1241	Paroster sp. S1	*Paroster* sp. nov 1	LN:6655	YYAC0018C	1	15-Nov-10	12-Nov-09	Yeelirrie	
ST1242	Paroster sp. S2 1leg		LN:7435	YYAC248		15-Nov-10	12-Nov-09	Yeelirrie	contam.

Table 1. Overview of the analysed specimens. The first column gives the DNA extraction number, the last column indicates whether the PCR was successful. All specimens were tried with two different sets of PCR primers

Phylogenetic analyses using neighbour joining of uncorrected sequence distances in PAUP* (Swofford 1998) were used to estimate the number of species among the received specimens from the Yeelirrie area, as well as for checking whether these species were found at other localities in the region. Results of phylogenetic analyses are presented as partial phylogenetic trees showing the target species with some closest related species as well as a matrix of uncorrected ("p") pairwise distances between target species and relevant taxa in the phylogenetic trees. The target species are yellow highlighted in the phylogenetic trees. *Intra*specific pairwise distances (cut off value > 4%) are yellow highlighted.

Results

Oligochaete worms – Enchytraeidae

For the analysis of this group data from Genbank was added to sequence data generated from specimens of the Yeelirrie calcrete, including data produced by Helix. These data includes undescribed species from the Sturt Meadows calcrete published by Bradford et al. (2009). Eigth out of 16 enchytraeid specimens produced successful PCRs that resulted in clear sequences. Phylogenetic analysis (Figure and matrix 1) showed that these eight specimens represent six rather divergent lineages, represented by long branches in the phylogeny (Figure 1) and pairwise sequence divergences ranging 15.29 – 21.86% (Matrix 1). The specimens ST1195 and ST1200, are species that have been sequenced by Helix, respectively G61-LN7711 (sequence divergence 0.0%) and G63-LN7595 (sequence divergence 0.685%). There are two pairs of specimens, ST1191 and ST1192 respectively ST1196 and ST1204, that are conspecific (pairwise sequence divergences are <3.2%). Specimens ST1196 and ST1204 are grouping with Enchytraeidae from the Sturt Meadows calcrete, of which the pairwise sequence divergence (highlighted in green in matrix 1) indicates probably relationships at the genus level. Each of the six divergent lineages should be treated as individual, probably, undescribed taxa. The current sequencing resulted in an additional four deep lineages, bringing the total of deep lineages of Oligochaeta in the Yeelirrie calcrete to seven. The seven deep lineages in the Yeelirrie calcrete and four in the Sturt Meadows calcrete show that Oligochaete biodiversity in single calcrete aquifers can be extensive.

Uncorrected ("p") distance matrix

		70	71	72	73	74	75	76	77	78	79	80	82	83
70	>G62 LN6608 Heli	-												
71	>ST1191 M414	0.21943	-											
72	>ST1192 M423	0.22138	0.00000	-										
73	>ST1194 M414	0.21644	0.21453	0.21613	-									
74	>G63 LN7595A Hel	0.20857	0.20103	0.21074	0.17434	-								
75	>ST1200 M414	0.21897	0.20090	0.21100	0.16211	0.00685	-							
76	>ST1196 M409	0.22574	0.19418	0.19709	0.17643	0.18360	0.18012	-						
77	>ST1204 M409	0.21927	0.19239	0.19715	0.17309	0.17163	0.17382	0.03175	-					
78	>G61 LN7711 Heli	0.20872	0.20259	0.20421	0.16527	0.15627	0.15304	0.15860	0.16793	-				
79	>ST1195 M423	0.21430	0.21455	0.20471	0.16606	0.15914	0.15543	0.15796	0.16934	0.00000	-			
80	>ST1197 M410	0.21999	0.21864	0.21663	0.15299	0.20034	0.20272	0.17216	0.17032	0.17908	0.18221	-		
82	>GU014149Enchytr	0.22502	0.19403	0.19192	0.19400	0.17749	0.18336	0.18050	0.16933	0.17263	0.17071	0.18207	-	
83	>FJ785780ligoch	0.22596	0.19682	0.19488	0.18063	0.19700	0.20117	0.10540	0.10394	0.18165	0.18385	0.18286	0.17194	-
84	>FJ785779Oligoch	0.22104	0.19022	0.19118	0.17883	0.20368	0.20600	0.12305	0.11999	0.18468	0.18345	0.16739	0.17516	0.12199
85	>FJ785778Oligoch	0.23430	0.20830	0.21009	0.22249	0.20973	0.22314	0.18097	0.17625	0.20784	0.20883	0.22788	0.18773	0.20867
86	>FJ785777Oligoch	0.22104	0.19022	0.19118	0.17883	0.20368	0.20600	0.12305	0.11999	0.18468	0.18345	0.16739	0.17516	0.12199
87	>FJ785776Oligoch	0.22282	0.18872	0.18614	0.17909	0.20733	0.20777	0.12307	0.12638	0.19121	0.19000	0.17099	0.17195	0.12520
88	>FJ785775Oligoch	0.21161	0.20804	0.21148	0.20453	0.19844	0.19617	0.17124	0.16818	0.20417	0.20571	0.18982	0.22027	0.20867

Matrix 1 of uncorrected 'p' distances of Enchytraeidae (Oligochaeta).

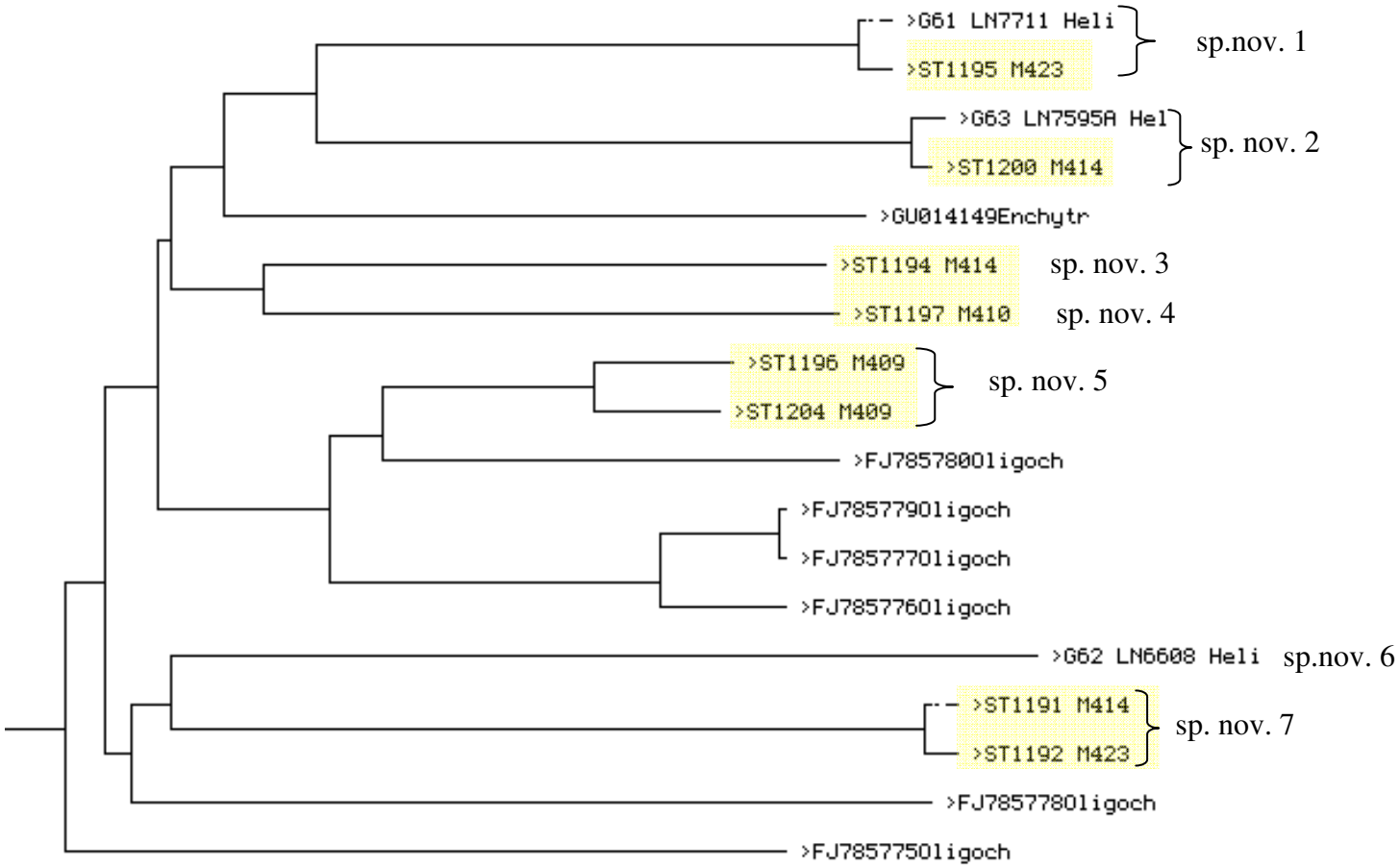

Figure 1. Partial neighbourjoining phylogram of Enchytraeidae (Oligochaeta).

Pauropoda

There is no pre-existing CO1 data for Pauropoda on GenBank. The three out of 12 specimens that produced successful PCRs and resulted in good sequences will therefore be compared amongst each other. Phylogenetic analysis shows that the specimens ST1206, ST1210 and ST1212 each belong to rather divergent lineages (Figure 2), with sequence divergence > 13.1% (Matrix 2). These taxa should be considered as three different, most likely undescribed species.

Symphyla

Four out of 14 specimens produced successful PCRs that resulted in good sequences. The generated sequence data was compared with data from Helix. Specimens ST1223 and ST1224 appear to be conspecific (Figure 2) and are of the same species as specimen G44-LN7626 sequenced by Helix, with sequence divergences ranging 0.155-0.902% (Matrix 2). Specimen ST1228 is conspecific with G42-LN7602, sequence divergence 0.0%. The remaining specimens are represented by divergent taxa, indicated by long branches in the phylogeny, and pairwise sequence divergences with values >10% (Matrix 2). Based on the here presented data there is evidence for five species of Symphyla that judged from their sequence divergence may consist of a number of different genera.

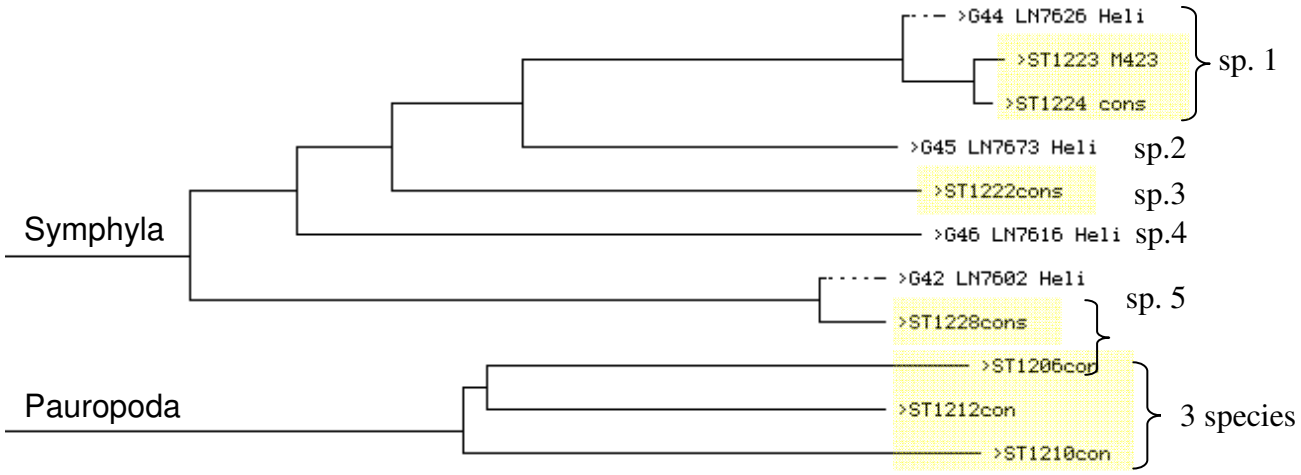

Figure 2. Partial neighbourjoining phylogram of Pauropoda and Symphyla.

		57	58	59	60	61	62	63	64	65	66	67
57	>ST1206con	-										
58	>ST1212con	0.13083	-									
59	>ST1210con	0.14578	0.14990	-								
60	>G45 LN7673 Heli	0.27687	0.28915	0.26676	-							
61	>G46 LN7616 Heli	0.32914	0.30285	0.27904	0.16796	-						
62	>G43 LN7590 Heli	0.29925	0.26524	0.30658	0.26870	0.26805	-					
63	>G44 LN7626 Heli	0.27446	0.28009	0.27971	0.12206	0.18031	0.24204	-				
64	>ST1222cons	0.30079	0.30226	0.29836	0.16059	0.19863	0.25360	0.15196	-			
65	>ST1223 M423	0.30252	0.29466	0.29445	0.11516	0.18243	0.24828	0.00392	0.15678	-		
66	>ST1224 cons	0.29614	0.29006	0.28806	0.10969	0.17104	0.24935	0.00902	0.15430	0.00155	-	
67	>G42 LN7602 Heli	0.27010	0.26236	0.28276	0.17734	0.21044	0.24477	0.18698	0.20717	0.19456	0.19315	-
68	>ST1228cons	0.28875	0.27820	0.30139	0.17396	0.21366	0.24238	0.18726	0.20721	0.19431	0.19308	0.00000

Matrix 2 of uncorrected 'p' distances of Pauropoda and Symphyla.

Subterranean diving beetles (Dytiscidae)

The subterranean diving beetles from the Yilgarn area have been studied intensively (Watts & Humphreys 2009, Cooper et al. 2002, Leys et al. 2003, Leys & Watts 2008). A sequence data set at the SA-Museum comprising more than 90 subterranean diving species was used to analyse the subterranean diving beetle adults and larvae from the Yeelirrie calcrete. This data set consists of sequences from the 5' end of the CO1 gene, which does not overlap with the part of the CO1 gene that is commonly used for barcoding. Therefore the specimens were also PCR amplified using primers for the barcoding gene, to enable comparisons with sequencing by Helix. Eight out of ten of the extracted diving beetles resulted in successful PCR and clean sequences. These eight consist of three species (Figure 3). Two undescribed species of *Limbodessus* (Figure 3) and one still undescribed species of *Paroster*. Two beetle larvae, specimens ST1232 and ST1233 could be matched with *Limbodessus* sp. nov. 1. Specimens ST1232-ST1234 and ST1236 are also conspecific with a specimen (ST582) that was sequenced for Subterranean Ecology on an earlier occasion. The two Yeelirrie *Limbodessus* species correspond as follows with the specimens sequenced by Helix: *Limbodessus* sp. nov. 1 with LN6506-G22-TPB-33-1 and *Limbodessus* sp. nov. 2 with LN8454-G65-YYHC0049K and LN6593-G23-YYAC0016A (see matrix 3c, yellow shaded areas). The third species of Dytyscid waterbeetle from the Yeelirrie calcrete appear to be an undescribed species of *Paroster* (sample ST1241). This specimen in the phylogenetic analyses groups with *Paroster melrosensis*, *P. darlotensis*, and a still undescribed species from a calcrete near Belele (Figure 3). The pairwise sequence divergences of the Yeelirrie *Paroster* with the other taxa is >7.8%.

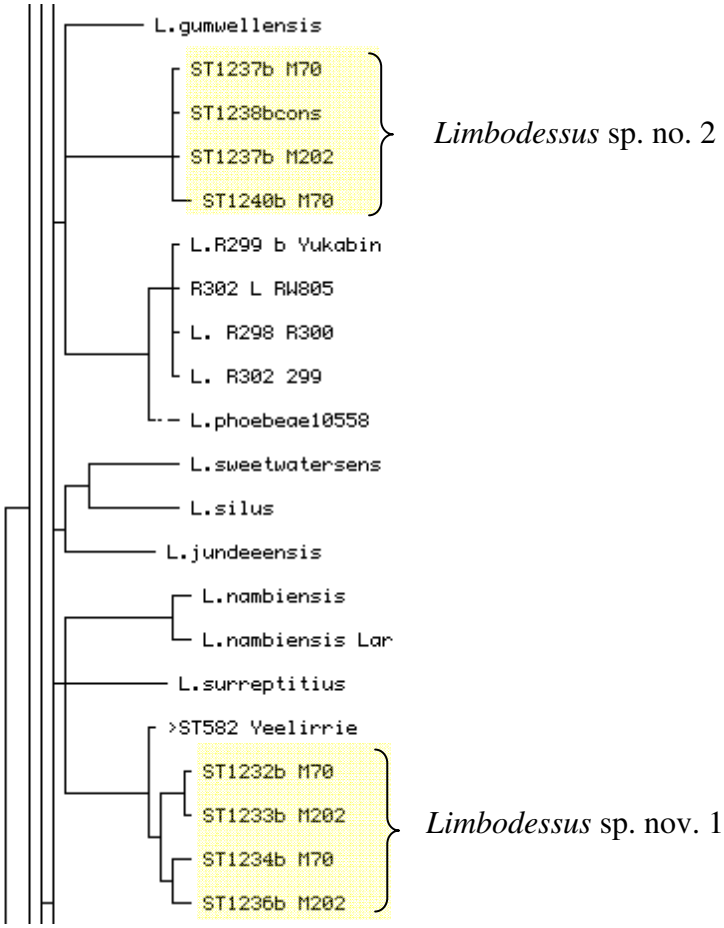

Figure 3. Partial neighbourjoining phylogram of *Limbodessus* (Dytiscidae).

Uncorrected ("p") distance matrix
35 characters are excluded

	177	254	316	317	318	319
177 L.gumwellensis	-					
254 L.phoebeae10558	0.05167	-				
316 ST1237b M70	0.05592	0.06158	-			
317 ST1238bcons	0.05652	0.06412	0.00000	-		
318 ST1240b M70	0.05907	0.06709	0.00497	0.00498	-	
319 ST1237b M202	0.05726	0.06476	0.00000	0.00000	0.00508	-

Matrix 3a of uncorrected 'p' distances of species closely related to *Limbodessus* sp. nov. 2.

Uncorrected ("p") distance matrix
35 characters are excluded

	181	185	310	312	313	314	315
181 L.nambiensis	-						
185 L.surreptitius	0.06637	-					
310 >ST582 Yeelirrie	0.06405	0.05651	-				
312 ST1232b M70	0.08064	0.06624	0.01840	-			
313 ST1234b M70	0.07676	0.06031	0.00872	0.01489	-		
314 ST1233b M202	0.08253	0.06691	0.01999	0.00127	0.01627	-	
315 ST1236b M202	0.07861	0.06396	0.01035	0.01533	0.00507	0.01382	-

Matrix 3b of uncorrected 'p' distances of species closely related to *Limbodessus* sp. nov. 1.

Uncorrected ("p") distance matrix

		1	2	3	4	5	6	7	8
1	>ST1237 M409	-							
2	>ST1238 M410	0.00000	-						
3	>6506 G22 TPB331	0.01120	0.01224	-					
4	>ST1232 M410	0.08300	0.08221	0.09642	-				
5	>ST1236 M410	0.07573	0.07544	0.09082	0.01609	-			
6	>seLN8454 G65 YV	0.08459	0.07694	0.09227	0.02133	0.00496	-		
7	>6593 G23 YVAC00	0.09984	0.09266	0.10648	0.04006	0.02482	0.01195	-	
8	>6620 G21 YU2	0.20707	0.20997	0.20176	0.19494	0.18709	0.19232	0.16983	-

Matrix 3c. Uncorrected p distances of CO1 of 'barcoding' region. Comparison of current sequences with those of Helix.

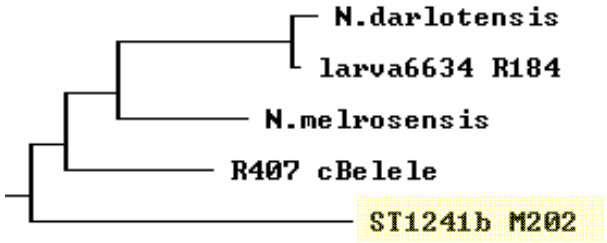

Figure 4. Partial neighbourjoining phylogram of species closely related to *Paroster* sp. nov. 1.

		129	130	149	275	322
129	N.darlotensis	-				
130	N.melrosensis	0.05378	-			
149	larva6634 R184	0.00379	0.04574	-		
275	R407 cBelele	0.06421	0.05442	0.06912	-	
322	ST1241b M202	0.09207	0.07894	0.09409	0.09438	-

Matrix 4 of uncorrected 'p' distances of species closely related to *Paroster* sp. nov. 1.

Summary of results

- Three undescribed species of subterranean diving beetles were recorded, which have not been found anywhere elseand could therefor be considered as endemic for the Yeelirrie calcrete
- There is a high biodiversity of enchytraeid oligochaeta in the Yeelirrie calcrete. Seven deep lineages were found, which are likely different genera.
- The biodiversity of Pauropoda and Symphyla is represented by respectively three and five deep lineages. Sequencing success of these groups was low, so the results here may not recover the total biodiversity in the Yeelirrie calcrete. .

References

Bradford T, Adams M, Humphreys WF et al. (2009). DNAbarcoding of stygofauna uncovers cryptic amphipod diversity in a calcrete aquifer in Western Australia's arid zone. *Molecular Ecology* Resources (2009) doi: 10.1111/j.1755-0998.2009.02706.x

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SA-Museum, Remko Leijs, 18 December 2010

APPENDIX 9: HELIX Molecular Solutions DNA Reports

Phreodrillidae

Amphipoda

Bathynellidae

Parabathynellidae

Isopoda

Araneae

Pseudoscorpiones

Pauropoda

Polyxenida

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14 February 2011

Shae Callan
Subterranean Ecology

Via email

Re. Report on the molecular systematics of Phreodrilidae

Dear Shae,

Following is a summary of the results of the oligochaete study we have completed. Our data suggest that your collections contain a single species of Phreodrilidae. The species is genetically distinct from species of Phreodrilidae from the Pilbara, suggesting it is a new species.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston
Helix Molecular Solutions

Background
The oligochaetes fauna of the Pilbara is diverse, with eight species known, and many yet to be described (Pinder, pers. comm.). This study focuses on the Phreodrilidae from Yeelirrie, near Wiluna, Western Australia, in the Yilgarn. Sequencing of oligochaete specimens for the mitochondrial gene 12s from four drainage basins in the Pilbara revealed multiple divergent lineages, belonging to four families (Finston, unpublished data). Four species of Phreodrilidae were supported, based on a combination of morphology and genetic data (species S1, S3, S6, S7). At least one species of Enchytraeidae was supported, based on morphological and genetic data (species S1). Species of Tubificidae and Haplotaxidae were also represented.

Objective
Two specimens of oligochaetes belonging to the Phreodrilidae were sequenced to test morphological identifications, assess the likely number of species in the collections and their affinities to Pilbara oligochaetes collected from the Fortescue, De Gray and Ashburton drainage basins.

Executive summary
- The two samples of Phreodrilidae, both identified morphologically as species S8, differed from one another by 1.3% sequence divergence at 12s,, indicating that they belong to the same species
- Phreodrilidae S8 is genetically distinct from the previously recognised species of Phreodrilidae from the Pilbara at both COI and 12s, indicating it is likely to be a new species

Methods
Two specimens of Phreodrilidae species S8 were collected from two bores from Yeelirrie (Table 1). The specimens were sequenced for variation at the mitochondrial gene COI, using primers LCO1490 and HCO2198 (Folmer et al., 1994). Because not all samples could be amplified for COI, they were also amplified for the mitochondrial gene 12s using the primers SR-N-14588 and SR-J-14233 (Simon et al., 1994).

Genetic distances between unique genetic types (haplotypes) were measured using uncorrected p-distances (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.

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data. Bayesian analysis was used to construct the phylogenetic tree, incorporating the General Time Reversible (GTR+I+G) model for COI and GTR+G model for 12s, as identified in MODELTEST. Posterior probabilities were obtained by running four MCMC chains, each of 10^6 cycles. A majority rule consensus tree was constructed after discarding the first 300 ("burn-in") trees for the Enchytraeidae and the first 400 for Phreodrilldiae. Two specimens from the Tubificidae (EF675214 *Tubificodes amplivasatus* and EF675225 *Tubificodes heterochaetus*) were used as outgroups in the COI analysis. A voucher sequence of Phreodrilidae (*Insulodrilus bifidus* DQ459882) was included as a reference sequence and a specimen of *Tubificoides pseudogaster* DQ459922 was included as an outgroup for the 12s phylogenetic analyses. In addition, sequences of undescribed specimens of Enchytraeidae and Phreodrilidae from the Pilbara were included as reference specimens.

Results
One Phreodrilidae specimen failed to amplify (LN6489) for COI. Both samples were successfully amplified for 12s. Sequences are shown in Appendices 1 and 2. The two samples of Phreodrilidae species S8 had unique haplotypes, which differed from one another by 1.3% sequence divergence at 12s.

Both phylogenetic analyses showed that the specimens of species S8 formed a well-supported lineage (Figures 1, 2). The S8 lineage differed from the Pilbara lineages by between 16.5 and 20.5% net sequence divergence at COI (Table 2) and 14.0 to 19.2% sequence divergence at

12s (Table 3). Variation within lineages was lower, ranging from 0.0 to 2.4% mean sequence divergence (Table 4).

Conclusions

The two samples of S8 differ from one another by 1.3% sequence divergence at 12s, indicating they are likely to belong to the same species.

Phreodrilidae S8 is genetically distinct from the previously recognised (but undescribed) species of Phreodrilidae from the Pilbara, indicating it is likely to be a new species.

References

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochormoe c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3, 294-299.

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Nei, M. (1987) DNA polymorphism within and between populations. In: Molecular Evolutionary Genetics. pp.254-286. Columbia University Press New York, NY.

Posada, D., Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Simon, C., Fratl, F, Beckenbach, A., Crespi, B., Liu, H., Flook, P. (1996) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society 87: 651-701.

Table 1. Samples used in the current study, and the genetic lineages to which they belong.

Species ID	Specimen ID	Bore	Lab ID	12s lineage	COI lineage
Phreodrilidae sp. S8	LN7693	YYAC33	G59	S8	S8
Phreodrilidae sp. S8	LN6489	SB14-MT	G60	S8	

Table 2. Net COI sequence divergence between morphological species of Phreodrilidae. This method 'corrects' for variation within groups.

	S1	S3	S6	S7	S8
S1	X				
S3	18.2	X			
S6	15.6	13.5	X		
S7	18.0	18.7	16.1	X	
S8	20.5	19.6	16.5	18.0	X

Table 3. Net 12s sequence divergence between morphological species of Phreodrilidae. This method 'corrects' for variation within groups.

	S1	S3	S6	S7	S8
S1	X				
S3	20.4	X			
S6	16.8	11.2	X		
S7	12.5	18.9	16.6	X	
S8	14.0	19.1	19.2	15.0	X

Table 4. Mean within group 12s distances for five lineages of Phreodrilidae.

morphospecies	distance
S1	0.7
S3	0.0
S6	1.1
S7	2.4
S8	1.3

Figure 1. Bayesian analysis of COI haplotypes of Phreodrilidae oligochaetes. Numbers on nodes correspond to posterior probabilities, with values greater than 0.90 considered to be strong support. Lineages are labelled with morphological assignments as in the text and Table 2.

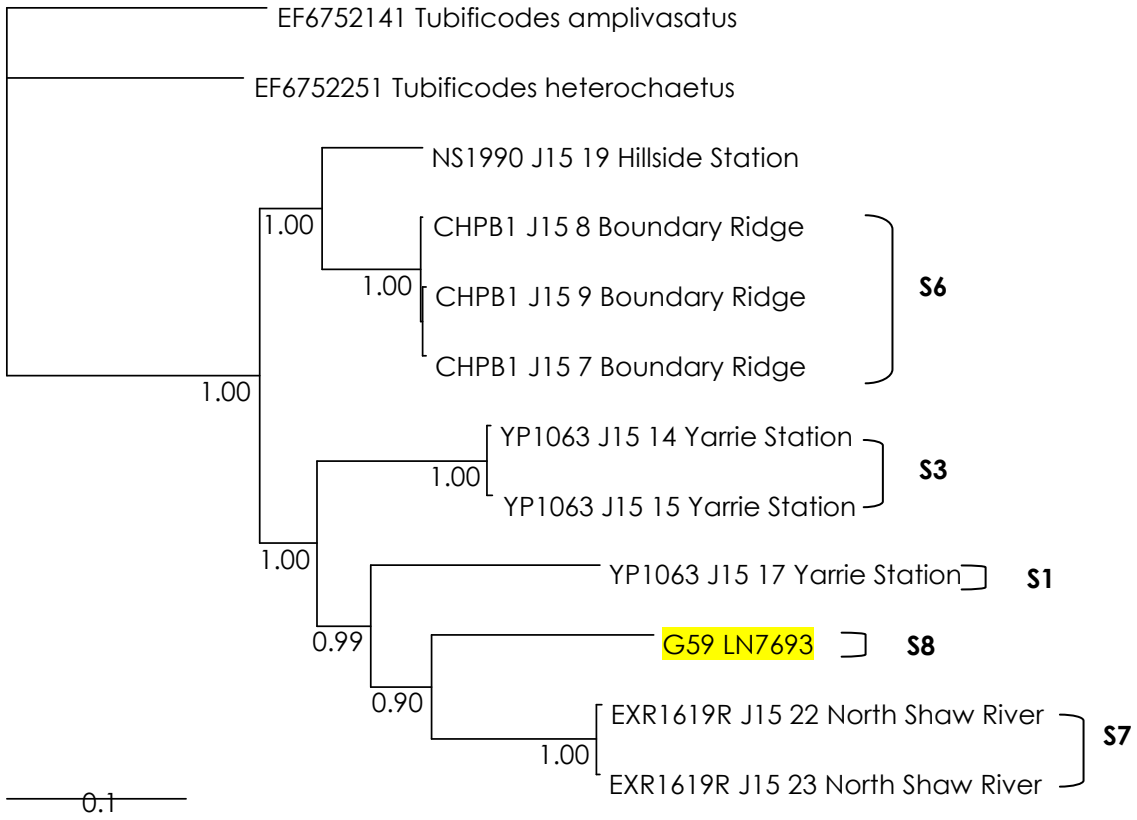
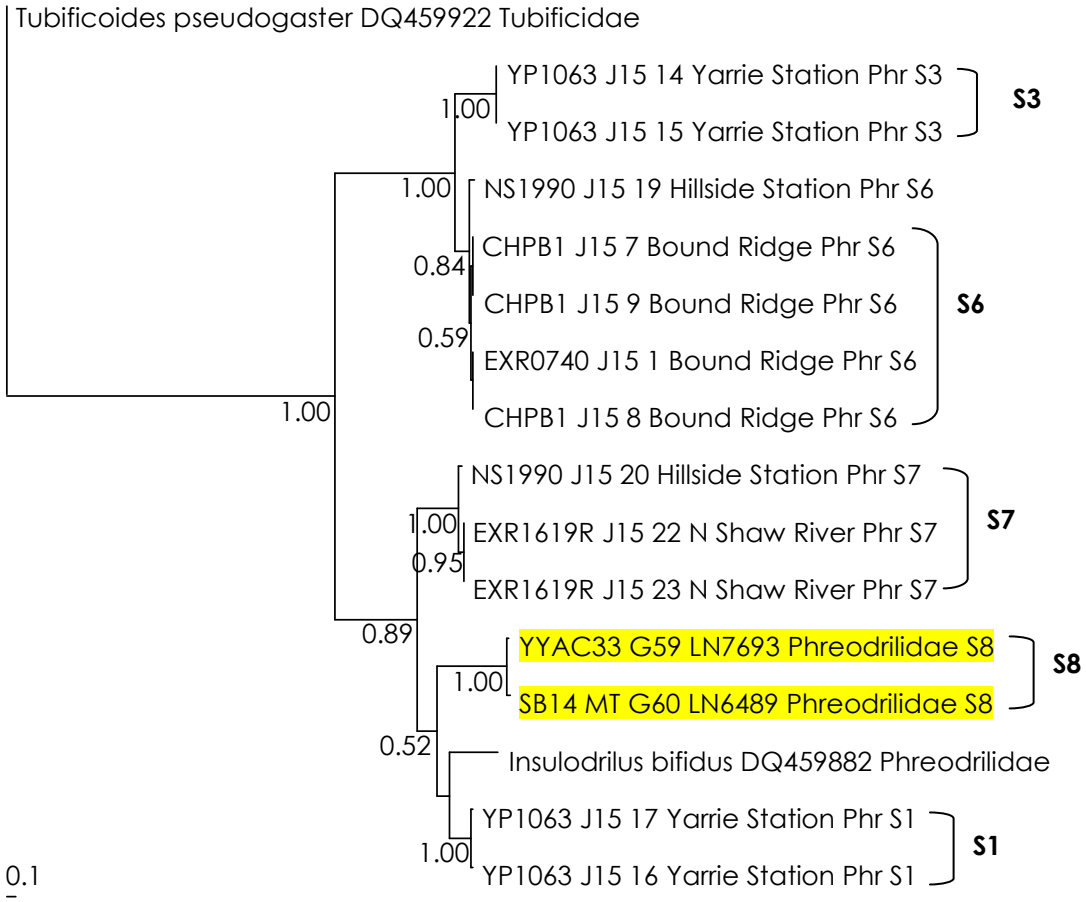


Figure 1. Bayesian analysis of 12s haplotypes of Phreodrilidae oligochaetes. Numbers on nodes correspond to posterior probabilities, with values greater than 0.90 considered to be strong support. Lineages are labelled with morphological identifications, as referred to in the text and Table 3.



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24 January 2011

Shae Callan
Subterranean Ecology

Via email

Re. Report on the molecular systematics of the Chiltoniidae

Dear Shae,

Following is a summary of the results of the Chiltoniidae study we have completed. Our data suggest that two of the three new specimens you provided to us belong to genetic lineages detected in the first two phases of sampling. The remaining specimen formed a new genetic lineage not detected during phase 1 and 2. The lineages show moderate levels of divergence from one-another, which in the absence of other data, we deem as likely to represent intra-specific variation.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston and Dr. Oliver Berry
Helix Molecular Solutions

Background

Amphipods from the family Chiltoniidae, identified as resembling the genus *Phreatochiltonia,* were collected from 12 bores at Yeelirrie, over an approximate 70 km distance. Genetic analysis of the mitochondrial gene COI from the two phases of sampling revealed the presence of five genetic lineages (Helix report 27 November, 2009; Helix report 25 February, 2010). The lineages differed from one another by between 2.9 and 6.2% net sequence divergence (Helix report 25 February, 2010). The sequence divergence observed between lineages is lower than is generally observed between crustacean species, and was assessed as likely representing intra-specific variation. This conclusion was also supported by a positive correlation between genetic distance and geographic distance (isolation by distance), indicating recent or current gene flow is occurring between bores that are geographically close (Helix report 25 February, 2010).

Objective

Three specimens from three additional bores were collected during a third phase of sampling. These were also identified as resembling *Phreatochiltonia*. The new samples were sequenced for variation at the mitochondrial DNA gene cytochrome oxidase subunit 1 (COI), with the aim of testing whether they were likely to belong to the species identified in the first two phases of sampling.

Executive summary

- Three new specimens of chiltoid amphipods were added to an existing phylogeny.
- Two of the new specimens occurred in previously recognised lineages.
- One specimen formed a new lineage, not detected in the first studies.
- The new lineage differed from the other lineages by 5.2 to 7.6% net sequence divergence.
- Divergence between the six lineages is lower than that generally seen between crustacean species.
- In the absence of ecological or morphological differentiation, the variation among Chiltoniidae at Yeelirrie is likely to represent intra-specific variation.

Methods

Three new specimens of Chiltoniidae (Table 1) were sequenced for variation at the mitochondrial COI gene using primers M735 and M737 (Murphy et al., 2009). Genetic distances between unique genetic types (haplotypes) were measured using uncorrected p-distances (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.

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data. Bayesian analysis was used to construct the phylogenetic tree, incorporating the general time reversible model (GTR+I+G), as identified in MODELTEST. Posterior probabilities were obtained by running four MCMC chains, each of 10^6 cycles. A majority rule consensus tree was constructed after discarding the first 600 ("burn-in") trees. Voucher sequences of Chiltoniidae amphipods were included as reference sequences, including *Austrochiltonia* sp Clade F (EU886891), *Austrochiltonia dalhousiensis* (EU886885), *Austrochiltonia* sp Clade B (EU886950), *Austrochiltonia* sp Clade A2 (EU886939) and *Phreatochiltonia anophthalma* (EU8868881). These specimens were collected from the Great Artesian Basin in South Australia (Murphy et al., 2009), and are likely to represent the nearest known relatives to the Yeelirrie specimens. Two specimens of Hyalellidae (*Exhyalella natalensis* AF5204361) and Hyalidae (*Hyale nilssoni* AF5204351) were included as outgroups.

Results

In addition to the five lineages detected in the previous analyses, the phylogenetic tree revealed the presence of one additional lineage (Figure 1). Pair-wise net distances between lineages A-F ranged from 2.9 to 7.6% sequence divergence (Table 2).

Two of the phase 3 specimens occurred in previously recognised lineages. Specimen LN8376 YYHC0107 occurred in lineage A and specimen LN8457 YYHC0049K occurred in lineage D (Figure 1). The mean genetic distance between haplotypes within lineage A was 0.4% and the mean genetic distance between haplotypes within lineage D was 0.2% (Table 3).

The third specimen (LN8334) formed a new lineage (F; Figure 1), not detected in the previous two phases.

The relationship of the Yeelirrie specimens to species of *Austrochiltonia* and *Phreatochiltonia anophthalma* from the Great Artesian Basin were poorly resolved, however, the Yeelirrie specimens formed a well-supported clade to the exclusion of all GAB species. The Yeelirrie specimens and the GAB specimens differed from each other by 11.5 to 16.3% sequence divergence.

Conclusions

In the previous report, differentiation between lineages A-E was assessed as likely being intra-specific, due to the relatively low level of sequence divergence between them, as well as the finding of isolation by distance among haplotypes (Helix report 25 February, 2010). In the present study, lineage F showed similar levels of differentiation from the remaining lineages. Based on comparisons between over 1700 crustacean species, Hebert et al (2003) found an average of 15.4 % sequence divergence between species pairs. Only 18% of pair-wise comparisons showed less than 8% sequence divergence. Hence the differentiation between clades A-F is less than that generally seen between crustacean species, and less that those seen between clades of Chiltoniidae from the Great Artesia Basin (Murphy et al., 2009). Bradford et al (2010) found 11-13% sequence divergence among three lineages of Chiltoniidae in a single calcrete in the Yilgarn. Reproductive isolation between lineages was confirmed with nuclear (allozyme) markers. Hence, in the absence of morphological or ecological differentiation, we would regard the observed differences as intra-specific. However, regardless of their taxonomic status, gene flow is restricted among geographically distant populations of Chiltoniidae at Yeelirrie. Using a standard molecular clock for arthropods (Brower, 1994), this corresponds to approximately 3 million of years of isolation among the most divergent lineages.

While the specimens from Yeelirrie show greatest similarity to Genbank sequences of *Phreatochiltonia* and *Austrochiltonia*, they are significantly genetically divergent from them and are likely to represent new, undescribed species.

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Nei, M. (1987) DNA polymorphism within and between populations. In: Molecular Evolutionary Genetics. pp.254-286. Columbia University Press New York, NY.

Table 1. The specimens used in the present study and the genetic lineage to which they belong.

Site	Specimen ID	Helix ID	Morphological ID	Lineage
L-UNK1	LN7313A	G33	Chiltoniidae	C
L-UNK1	LN7358A	G35	Chiltoniidae	C
SB14	LN7597A	G36	Chiltoniidae	E
SB14	LN7597B	G37	Chiltoniidae	E
SB14-MT (MKC-06)	LN6485	G11	nr. *Phreatochiltonia* n. sp. S1	No data
TPB-33-1	LN6482A	G16	nr. *Phreatochiltonia* n. sp. S1	No data
TPB-33-1	LN6482B	G17	nr. *Phreatochiltonia* n. sp. S1	No data
YU1	seLN6611A	G14	nr. *Phreatochiltonia* n. sp. S1	A
YU1	seLN6611B	G15	nr. *Phreatochiltonia* n. sp. S1	No data
YU1	LN7348A	G34	Chiltoniidae	A
YYAC0002A	seLN7309A	G19	nr. *Phreatochiltonia* n. sp. S1	A
YYAC0002A	seLN7309B	G20	nr. *Phreatochiltonia* n. sp. S1	A
YYAC0005	seLN6592	G7	nr. *Phreatochiltonia* n. sp. S1	A
YYAC0014A	seLN6598	G10	nr. *Phreatochiltonia* n. sp. S1	A
YYAC0016A	seLN6595A	G8	nr. *Phreatochiltonia* n. sp. S1	A
YYAC0016A	seLN6595B	G9	nr. *Phreatochiltonia* n. sp. S1	No data
YYAC1006B	seLN7293A	G12	nr. *Phreatochiltonia* n. sp. S1	No data
YYAC1006B	seLN7293C	G13	nr. *Phreatochiltonia* n. sp. S1	A
YYAC1007A	seLN7306A	G5	nr. *Phreatochiltonia* n. sp. S1	A
YYAC1007A	seLN7306D	G6	nr. *Phreatochiltonia* n. sp. S1	A
YYD26	seLN6600	G18	nr. *Phreatochiltonia* n. sp. S1	No data
YYHC0049K	LN7671A	G40	Chiltoniidae	D
YYHC0049K	LN7671B	G41	Chiltoniidae	D
YYHC0049K	seLN8457	G77	nr. Phreatochiltonia sp.	D
YYHC0107	seLN8376	G76	nr. Phreatochiltonia sp.	A
YYHC0118B (ETR5)	LN7668	G38	Chiltoniidae	B
YYHC0133A	seLN8334	G75	nr. Phreatochiltonia sp.	F
YYHC0139	LN7625	G39	Chiltoniidae	A

Table 2. Net sequence divergence between lineages for a 405 bp fragment of COI for specimens of Chiltoniidae.

	A	**B**	**C**	**D**	**E**	**F**
A	x					
B	3.8	x				
C	3.8	5.5	x			
D	6.0	6.9	2.9	x		
E	6.1	6.4	4.1	4.8	x	
F	5.2	5.2	5.8	7.6	7.5	x

Table 3. Mean within group distances for six lineages of Chiltoniidae. na= not applicable

	distance
A	0.4
B	na
C	0.0
D	0.2
E	0.0
F	na

Figure 1. Bayesian analysis of COI haplotypes of Chiltoniidae. Numbers on nodes correspond to posterior probabilities. Lineages are labelled A-F, as referred to in the text and Tables 1 and 2; coloured bars refer to map shown in Figure 2. New phase 3 samples are highlighted in yellow.

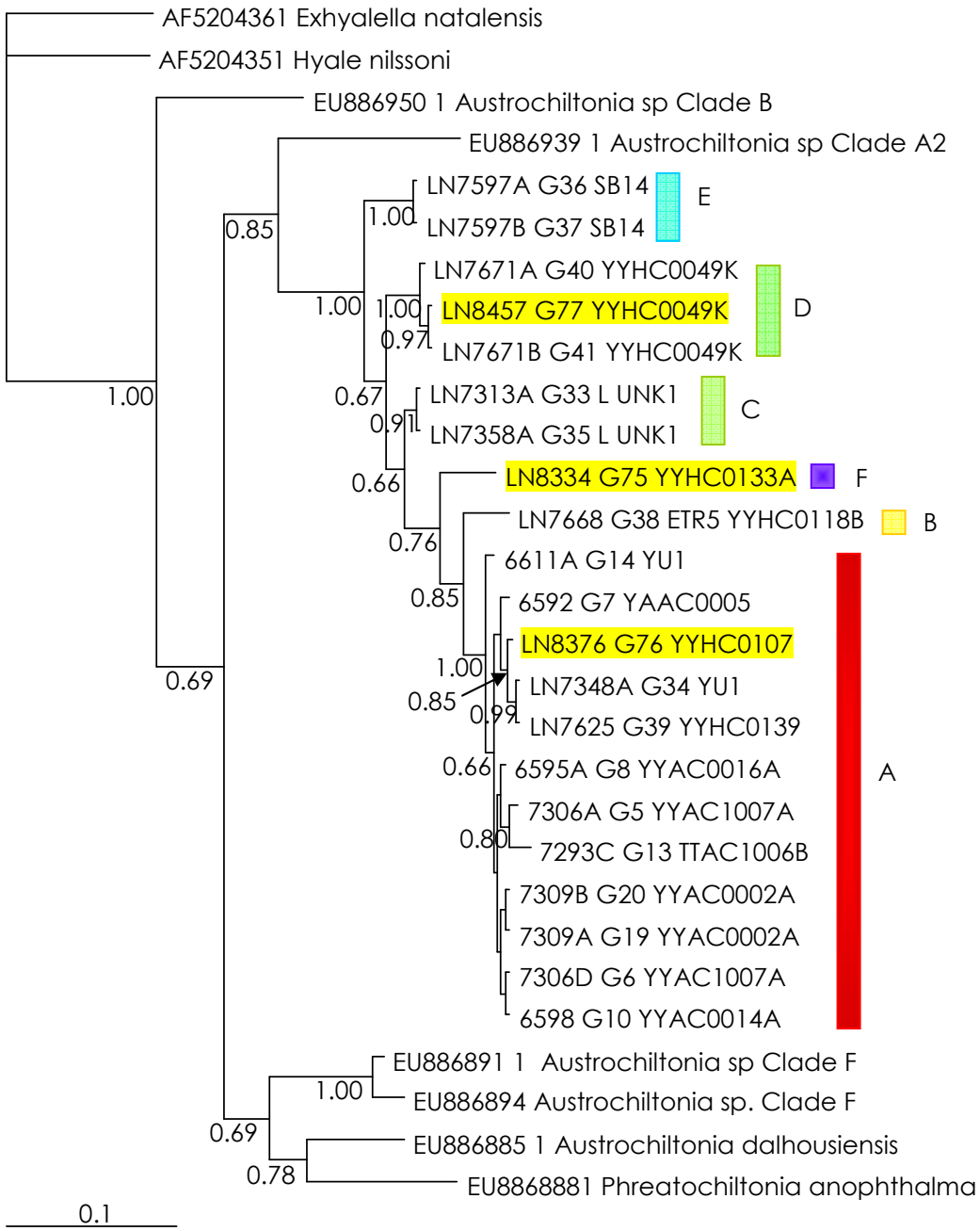
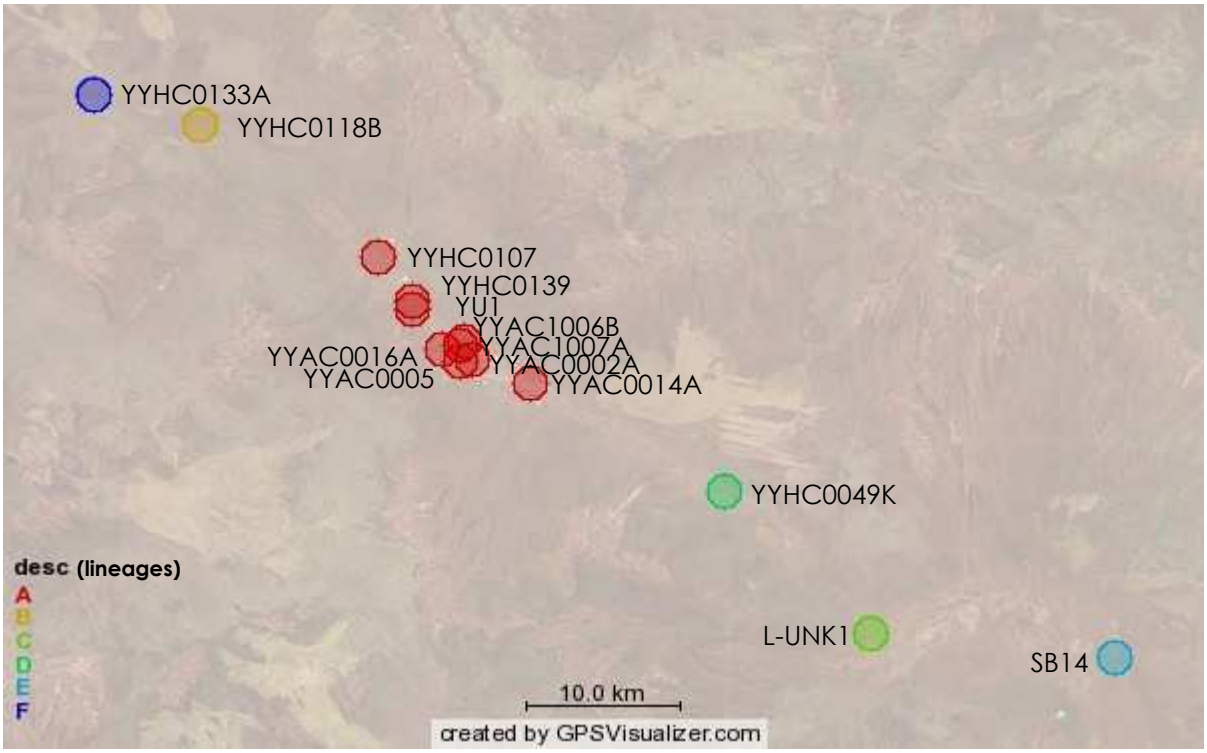


Figure 2. Distribution of lineages as shown in Figure 1.



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24 January 2011

Shae Callan
Subterranean Ecology
Via email

Re. Report on the molecular systematics of the Bathynellidae from Yeelirrie

Dear Shae,

Following is a summary of the work we have completed. The specimens of Bathynellidae you provided appear to belong to at least two distinct species. There are no COI sequences for bathynellids on Genbank, so we were unable to comment on similarities to other species.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston and Dr. Oliver Berry
Helix Molecular Solutions

Objective
Five specimens of Bathynellidae were recently collected from Yeelirrie, Western Australia and sequenced for the mitochondrial COI gene to assess the likely number of species in the collection and their distributions.

Executive summary
- Phylogenetic analysis of the five specimens revealed three distinct genetic lineages.
- The three genetic lineages differed by between 7.0 and 23.2% net sequence divergence.
- Lineage A is highly genetically divergent from lineages B and C, indicating that it is a distinct species.
- Lineages B and C show moderate level of sequence divergence (mean = 7%). This may indicate that they are separate species, or they may represent a single species showing a high degree of geographical variation, likely caused by long term isolation of populations.
- Lineage C contained three specimens, differing by a mean of 0.6% sequence divergence, indicating that they belong to the same species.

Methods
Five specimens of Bathynellidae were collected from four bores at Yeelirrie (Table 1). The four bores span approximately 30 km. The specimens were sequenced for variation at the COI gene using primers LCO1490 and HCO2198 (Folmer et al., 1994). Mean genetic distances between genetic types (haplotypes) were estimated from uncorrected p-distances (total percentage of nucleotides different between sequences). To account for polymorphism within lineages, the net genetic diversity of Nei (1987) was calculated to provide a 'corrected' distance between lineages.

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data. Bayesian analysis was used to construct the phylogenetic tree, incorporating the Tamura-Nei model (TrN+G), as identified in MODELTEST. Posterior probabilities were obtained by running four MCMC chains, each of 10^6 cycles. A majority rule consensus tree was constructed after discarding the first 200 ("burn-in") trees. Voucher sequences of Parabathynellidae belonging to three genera (*Atopobathynella*, *Billibathynella*, and Parabathynellidae gen. A) were included in the phylogenetic analysis(Guzik et al., 2008). Because there are no sequences of bathynellids on the international DNA sequenced database, Genbank, these species represent the nearest available relatives to the study specimens. The syncarid Psammaspididae sp 1 was used as an outgroup (Guzik et al., 2008).

Results
The five Yeelirrie specimens differed from one another by between 0.4 and 24.2% sequence divergence (Table 2). The specimens formed genetically divergent lineages with generally poor support (A-C: Figure 1). Lineages A and B were represented by single specimens whereas lineage C comprised three specimens. Lineage A differed from lineages B and C by 24.2 and 23.2% net sequence divergence, respectively (Table 3). Lineages B and C showed moderate divergence, differing from one another by 7.0% net sequence divergence (Table 3). The specimens within lineage C differed from one another by between 0.4 and 0.8% sequence divergence, with a mean divergence of 0.6%.

Discussion
The extremely high level of divergence between lineage A with respect to lineages B and C indicates clearly that it is a distinct species. The moderate level of divergence between lineages B and C is more difficult to interpret, particularly with limited geographic sampling. Based on comparisons between over 1700 crustacean species, Hebert et al (2003) found an average of 15.4 % sequence divergence between species pairs. Only 18% of pair-wise comparisons showed less than 8% sequence divergence. Therefore, while the observed divergence is lower than the majority of crustacean species pairs, it is within the realm of species-level divergence, and certainly indicative of a long period of geographic isolation.

Using a standard arthropod molecular clock for COI, lineage A diverged from lineages B and C approximately 10 million years ago (Brower 1994). Lineages B and C share a closer relationship. Thus while the level of divergence between lineages B and C is moderate and lower than is often seen between crustacean species (Hebert et al., 2003), the two lineages have been evolving independently for approximately 3 million years. This is not surprising given the geographic distance between the two specimens (approximately 20 km; Figure 2). Dispersal is generally limited in stygobitic group by the disconnected nature of groundwater aquifers and the poor dispersal ability of the fauna (Gibert et al., 1994; Holsinger, 1991).

The poor level of support in the phylogeny (owing to the high divergences and few specimens) makes taxonomic relationships between the lineages difficult to interpret. Nevertheless, based on the observed levels of sequence divergence, there are at least two species of Bathynellidae in the collection, and moderate genetic distance and geographic separation between lineages B and C suggests that there may be up to three species present. The fact that the two populations where lineages B and C are found are allopatric (geographically separated) means dispersal is unlikely, but the meaning of the observed genetic differentiation is less clear than if two divergent lineages were found at the same site, as the latter would demonstrate reproductive isolation. The two scenarios are summarised as follows:

Scenario 1
Species 1: Lineage A
Species 2: Lineages B and C represent a single species showing geographic variation

Scenario 2
Species 1: Lineage A
Species 2: Lineage B
Species 3: Lineage C

In order to differentiate between the two scenarios, further sampling of intermediate sites between those that provided specimens bearing lineages B and C is required.

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Posada, D., Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Table 1. Collection sites for the specimens used in the present study and the genetic lineage to which the specimens belong.

Site	**Specimen ID**	**Helix ID**	**Morphological ID**	**Genetic lineage**
YYAC0019B	seLN8300	G66	Bathynellidae sp.	C
SNAKE BORE	seLN8308	G67	Bathynellidae sp.	A
YYHC0049K	seLN8455	G68	Bathynellidae sp.	B
YU1	seLN8488a	G69	Bathynellidae sp.	C
YU1	seLN8488j	G70	Bathynellidae sp.	C

Table 2. Uncorrected p-distances between specimens of Bathynellidae from Yeelirrie for COI.

	LN8300 G66	LN8308 G67	LN8455 G68	LN8488a G69	LN8488j G70
LN8300 G66	x				
LN8308 G67	23.6	x			
LN8455 G68	7.0	24.2	x		
LN8488a G69	0.4	23.4	7.4	x	
LN8488j G70	0.4	23.6	7.4	0.8	x

Table 3. Net sequence divergence between lineages of Bathynellidae as shown in Figure 1.

	A	B	C
A	x		
B	24.2	x	
C	23.2	7.0	x

Figure 1. Bayesian analysis of Bathynellidae specimens from Yeelirrie based on a 506 bp fragment of COI. Numbers on branches represent posterior probabilities. The inset shows the lineages labelled A-C, as referred to in the text; coloured bars correspond to the map in Figure 2.

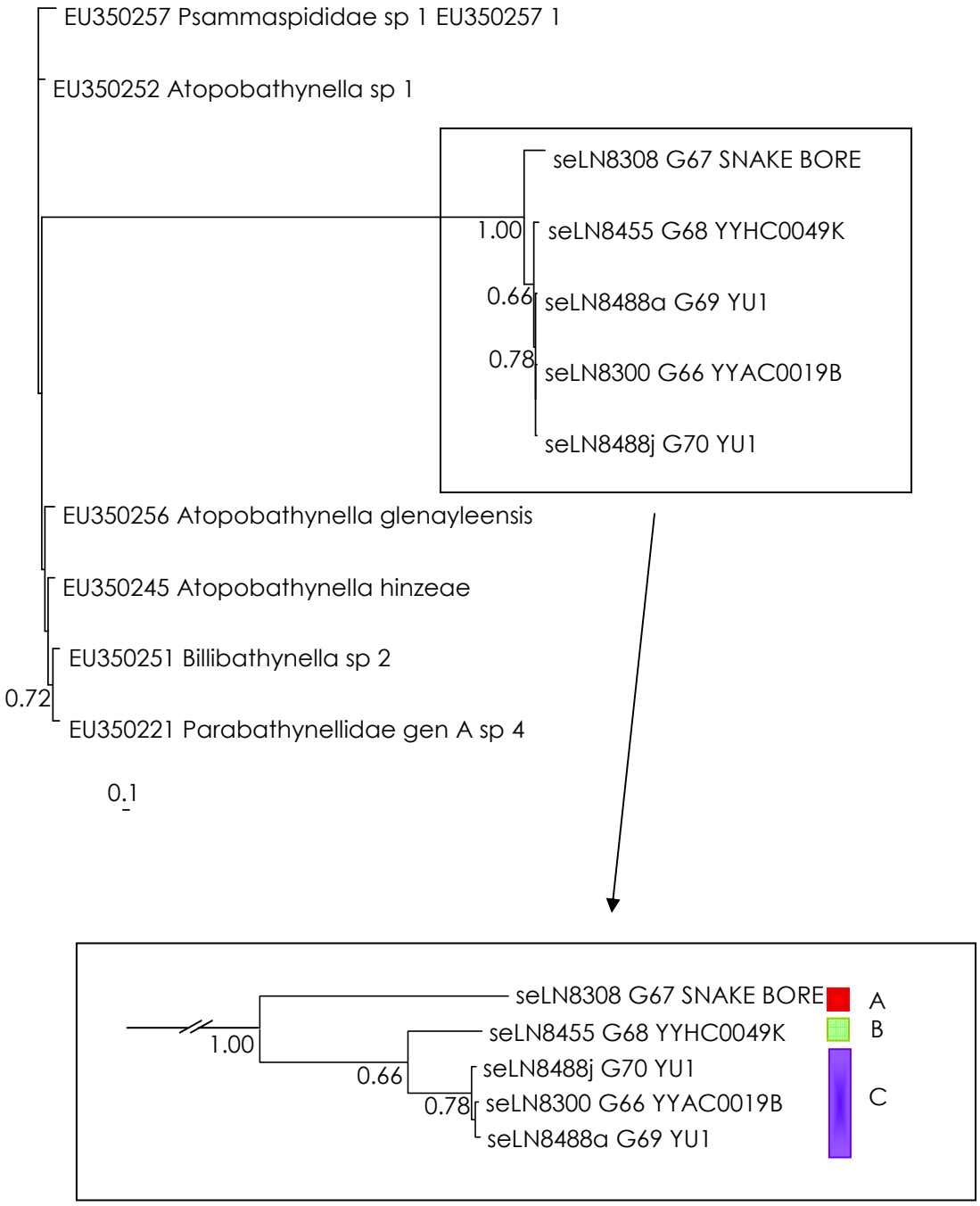

Figure 2. Map showing the distribution of lineages as detected by phylogenetic analysis from Figure 1.

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18 January 2011

Shae Callan
Subterranean Ecology Pty. Ltd.
Scientific Environmental Services
Suite 8, 37 Cedric St, Stirling, WA 6021, AUSTRALIA

Via email

Re. Report on the molecular systematics of Parabathynellidae

Dear Shae,

Following is a summary of the results of the *Atopobathynella* study we have completed. Our results place the new specimens into the two previously recognised major lineages, but the new specimens form two additional geographical lineages within them. While the genetic data support the presence of two species as indicted by morphology, variation within each species is moderately high, indicating that gene flow is limited to near-by areas.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston and Dr. Oliver Berry
Helix Molecular Solutions

Background

Thirteen specimens identified as *Atopobathynella* sp. (family Parabathynellidae), collected from Yeelirrie, near Wiluna, W.A., were sequenced for variation at the mitochondrial DNA gene cytochrome oxidase subunit 1(COI) to assess the number of species present and test morphological identifications (Helix, 2010a; Helix 2010b). The molecular results revealed two genetically distinct lineages, which was interpreted to indicate the presence of two species in the collections. Moderate genetic variation associated with sampling sites within one of the lineages was observed, and raised questions regarding whether splitting of the lineage into additional species was necessary. This report concerns the analysis of additional specimens of *Atopobathynella* from Yeelirrie.

Objective

Five new specimens identified as belonging to two morphospecies of *Atopobathynella* sp. (S5, S6; Shae Callan, pers. comm.) were sequenced with the aim of:

- testing morphological hypotheses,
- testing their genetic affinities to the two species detected during previous phases of sampling at Yeelirrie, and
- increasing the geographical range of samples in order to assess the patterns of genetic variation within species.

Executive summary

- Five new specimens from four new locations at Yeelirrie were sequenced for variation at the mitochondrial gene COI.
- A phylogenetic analysis, which included 18 specimens from all surveys, revealed the presence of two distinct lineages.
- The two lineages differed from one another by 12.7% net sequence divergence.
- Lineage 1 contained specimens of morphospecies S6 and lineage 2 contained a specimen of morphospecies S5.
- The observed level of sequence variation, coupled with morphological associations, indicates the presence of two species.
- Both species show moderately high genetic variation between sites, suggesting that gene flow is limited to near-by areas.

Methods

Five specimens of Parabathynellidae, collected from four bores at Yeelirrie were identified as belonging to two morphospecies of *Atopobathynella* (S5, S6; Shae Callan, pers. comm.; Table 1). The specimens were sequenced for variation at the mitochondrial COI gene using primers LCO1490 and HCO2198 (Folmer *et al.,* 1994).

Sequences were edited using SEQUENCHER software (Gene Codes Corporation, Ann Arbor, MI, USA). Alignment was performed with CLUSTAL W (Thompson *et al*. 1994) using default parameters. To test that the amplified COI sequences were the target mtDNA gene, sequences were translated into proteins and checked for the presence of stop codons. Genetic distances between unique genetic types (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotides different between sequences).

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data. Bayesian analysis was used to construct the phylogenetic tree, incorporating Kimura model (K8luf+G), as identified in MODELTEST. The phylogeny, branch lengths and posterior probabilities were obtained by running two trees simultaneously, each running four simultaneous MCMC chains. The number of cycles needed was determined by the standard deviation of the split frequencies of the two trees. The analysis was paused after every 1 x 10^6 generations and when the standard deviation fell below 0.01, the analysis was stopped. A majority rule consensus tree was constructed after discarding the first 3000 ("burn-in") trees. The burn-in value was assessed by plotting the posterior probabilities obtained after every generation and identifying the point at which the values reach stationarity (= the asymptote). Trees produced prior to stationarity were discarded.

Three voucher sequences of *Atopobathynella* were included in the phylogenetic analysis (*Atopobathynella* sp. 1 from the Murchison River drainage (EU350252), *A. glenayleensis* from the

Burnside drainage basin (EU350256), and *A. hinzae* from the Raeside drainage basin (EU350245); Guzik et al., 2008). These species were collected in the Yilgarn and are likely to represent the nearest relatives to the study specimens. The syncarid Psammaspididae sp 1 was used as an outgroup (Guzik et al., 2008).

Results

Phylogenetic analysis

The phylogenetic analysis, which included 13 specimens from previous surveys, as well as the five new specimens analysed here, placed the Yeelirrie specimens in two distinct, well-supported genetic lineages (1, 2; Figure 1). Four of the new specimens were placed in lineage 1 and one was placed in lineage 2 (Figure 1).

The two lineages were composed of multiple, well-supported lineages (Figure 1). Lineage 1 contained four lineages (B, C, D, E), and lineage 2 contained two lineages (A, F; Figure 1). Lineages E and F were not detected previously.

Lineage 2 formed a well-supported clade with the Genbank voucher specimen *Atopobathynella* sp. 1 from the Murchison River drainage.

Genetic differentiation within and between lineages

Lineages 1 and 2 differed from one another by 12.7% net sequence divergence.

Within lineage 1, net sequence divergence between lineages B, C, D, and E ranged from 5.7 to 9.4% (Table 2). Within lineage 2, net sequence divergence between lineages A and F was 9.1% (Table 2). Mean genetic divergence within lineages A – F ranged from 0.0 to 3.1% (Table 3).

Lineages containing the Yeelirrie specimens differed from the Genbank voucher sequences of *Atopobathynella* by between 9.3 and 20.8% net sequence divergence (Table 2). In particular, lineages A and F differed from *Atopobathynella* sp. 1 by 9.3 and 10.1% net sequence divergence, respectively (Table 2).

Morphological associations

Four specimens, identified as belonging to morphospecies S6, occurred in lineage 1. The specimens were placed in two lineages, C and E (Figure 1). One specimen, identified as belonging to morphospecies S5, occurred in lineage 2. The specimen was placed in lineage F (Figure 1).

Geographic distribution of lineages

Lineage 1: Lineage B was found in two bores, approximately 70 km apart, and lineage C was found in two bores approximately 10 m apart (Figure 2). Lineage E was found in two bores, approximately 14 km apart. Lineage D was found in a single bore, 170 m from a site containing lineage E (Figure 2).

Lineage 2: There was a strong geographic component to the distribution of lineages A and F. Lineage A was found in six bores, over a distance of approximately 17 km (Figure 3). Lineage F was found in a single bore, approximately 17 km southeast from the nearest bore containing lineage A.

Conclusions

There is a strong geographical component to the phylogeny, in that each lineage was detected at single or near-by bores (distances < 20 km). The one exception was lineage B, which was found in two bores approximately 70 km apart. So far, sampling of intermediate areas has not turned up further individuals belonging to lineage B, however limited sampling prevents making firm conclusions about the distributions of the lineages.

COI is considered to be an appropriate marker for species level molecular differentiation (Hebert et al., 2003). Based on comparisons between over 1700 crustacean species, Hebert et al (2003) found an average of 15.4 % sequence divergence between species pairs. Only 18% of pair-wise comparisons showed less than 8% sequence divergence.

Genetic differentiation between lineages 1 and 2 is sufficiently high that when coupled with the morphological association with morphospecies S6 and S5 respectively, indicates that each is a distinct species.

Differentiation within lineages 1 and 2 falls into a 'grey' area with divergences higher than that usually seen between individuals of the same species, but lower than that generally seen between species. Within lineage 1, variation between lineages B – E surpasses 8% in two of the six comparisons, but all values fall below the mean of 15.4%. In lineage 2, differentiation between lineages A and F exceeds 8% divergence (9.1%), but falls below the mean of 15.4%.

Lefébure et al (2006) defined 0.16 substitutions per site as the benchmark for species delineation in Crustacea using COI. Under this criterion, the individual lineages within lineages 1 and 2 are not sufficiently divergent to merit species status, with the number of substitutions per site ranging from 0.05 to 0.06.

Taking the two delineation methods together, and in the absence of morphological or ecological differentiation, we would likely regard the observed differences within lineages 1 and 2 as intra-specific. However, based on the data available, gene flow appears to be limited between sites that are geographically distant, resulting in lineages that have been isolated for millions of years, based on the molecular clock for COI for arthropods (Brower, 1994).

The Yeelirrie specimens are most similar to *Atopobathynella* sp. 1 from the Murchison River drainage, but divergence between them is moderately high, reflecting several millions of years of isolation.

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Posada, D., Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Table 1. Specimens from Yeelirrie used in the present study and the genetic lineage to which they belong

Bore ID	Specimen ID	lineage	Taxonomic ID	Lab ID
NO312 BORE	LN7082	B	Parabathynellidae	G27
SB14	LN7598A	B	Parabathynellidae	G29
SB14	LN7598B	B	Parabathynellidae	G30
SB14	LN6488	B	Parabathynellidae	G31
SB14-MT	seLN6487	B	nr. *Atopobathybnella* sp. S2	G4
YU1	LN7349	A	Parabathynellidae	G28
YYAC0016A	seLN8414	A	Atopobathynella sp.	G72
YYAC1005A	seLN7290		nr. *Atopobathybnella* sp. S2	G2
YYAC1007A	seLN7307A	A	nr. *Atopobathybnella* sp. S2	G1
YYAC284	LN7646	A	Parabathynellidae	G32
YYD26	seLN6601A	A	nr. *Atopobathybnella* sp. S2	G3
YYHC0048K	seLN8462		Atopobathynella sp.	G73
YYHC0048KA	LN:8462	F	Atopobathynella sp. S5	G157
YYHC0055A	LN7341	A	Parabathynellidae	G26
YYHC0061A	seLN8560	D	Atopobathynella sp.	G74
YYHC0065B	10:0045	E	Atopobathynella sp. S6	G156
YYHC0132B	LN:8406	C	Atopobathynella sp. S6	G158
YYHC0133A	LN:8404s	E	Atopobathynella sp. S6	G159
YYHC0133A	LN:8404b	C	Atopobathynella sp. S6	G160
YYHC0133A	seLN8404	C	Atopobathynella sp.	G71

Table 2 Net sequence divergence (un-corrected p-distances) between lineages as shown in Figure 1. Above diagonal=standard error. Aglen= A glenayleensis, Ahinz= A hinzeae, Asp= Atopobathynella sp. 1.

	A	B	C	D	E	F	Aglen	Ahinz	Asp
A		0.018	0.019	0.020	0.019	0.015	0.020	0.019	0.015
B	0.164		0.013	0.015	0.013	0.019	0.019	0.020	0.019
C	0.166	0.069		0.015	0.012	0.019	0.021	0.021	0.020
D	0.187	0.094	0.087		0.014	0.020	0.021	0.021	0.020
E	0.176	0.071	0.057	0.073		0.018	0.020	0.021	0.020
F	0.091	0.156	0.165	0.180	0.154		0.021	0.018	0.016
Aglen	0.186	0.162	0.183	0.199	0.180	0.208		0.020	0.020
Ahinz	0.151	0.178	0.189	0.202	0.189	0.146	0.171		0.018
Asp	0.093	0.162	0.168	0.189	0.172	0.106	0.177	0.134	

Table 3. Mean sequence divergence (D) within lineages as shown in Figure 1. s.e.=standard error; n/c=not calculated.

lineage	D	s.e.
A	0.010	0.003
B	0.002	0.002
C	0.000	0.000
D	n/c	n/c
E	0.031	0.009
F	n/c	n/c
A glenayleensis	n/c	n/c
A hinzeae	n/c	n/c
A sp. 1	n/c	n/c

Figure 1. Bayesian analysis of COI variation for specimens of Parabathynellidae from Yeelirrie. Letters correspond to lineages as referred to in the text. Numbers on branches correspond to posterior probabilities, indicating the reliability of the branching pattern. New specimens are highlighted in yellow.

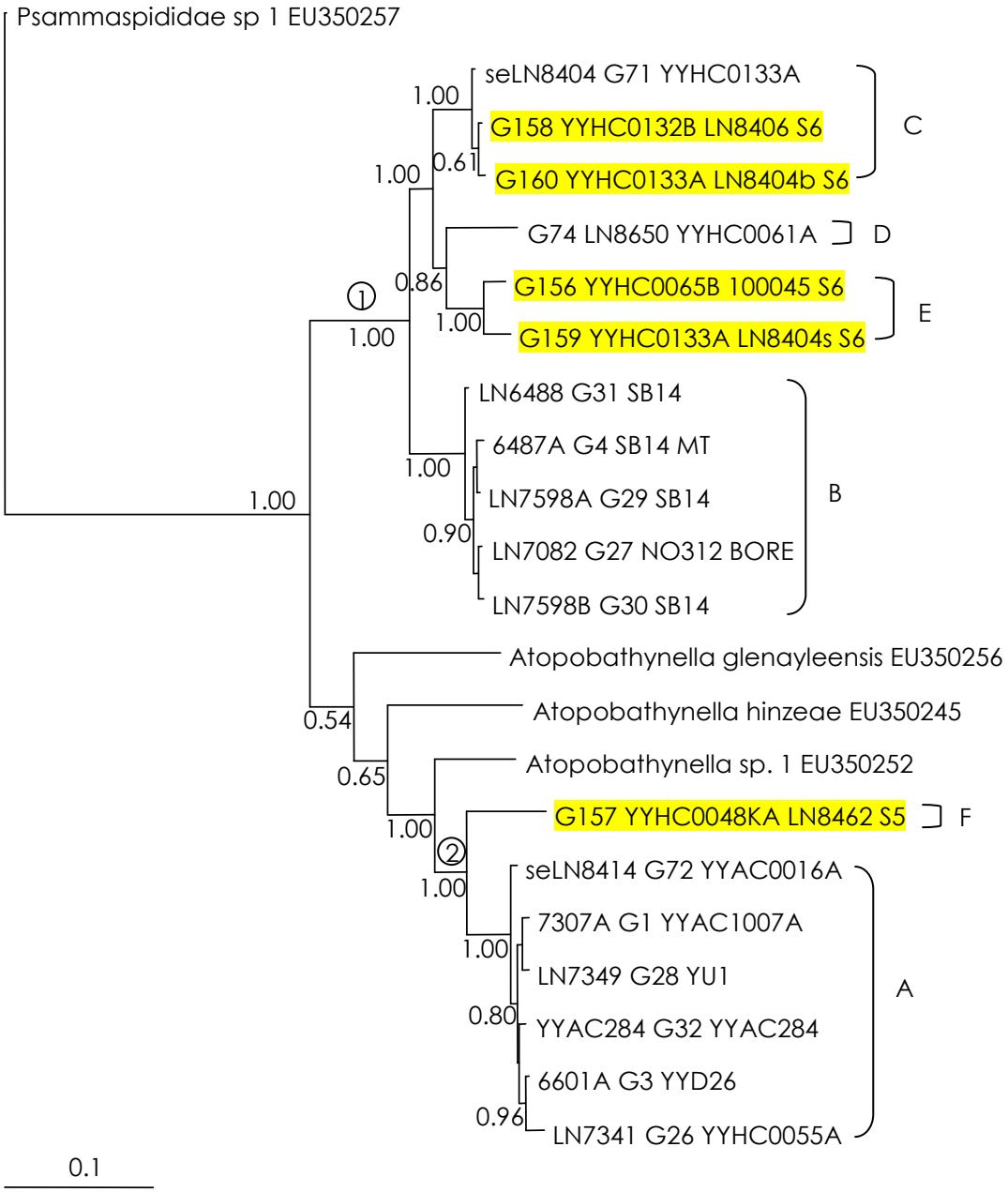
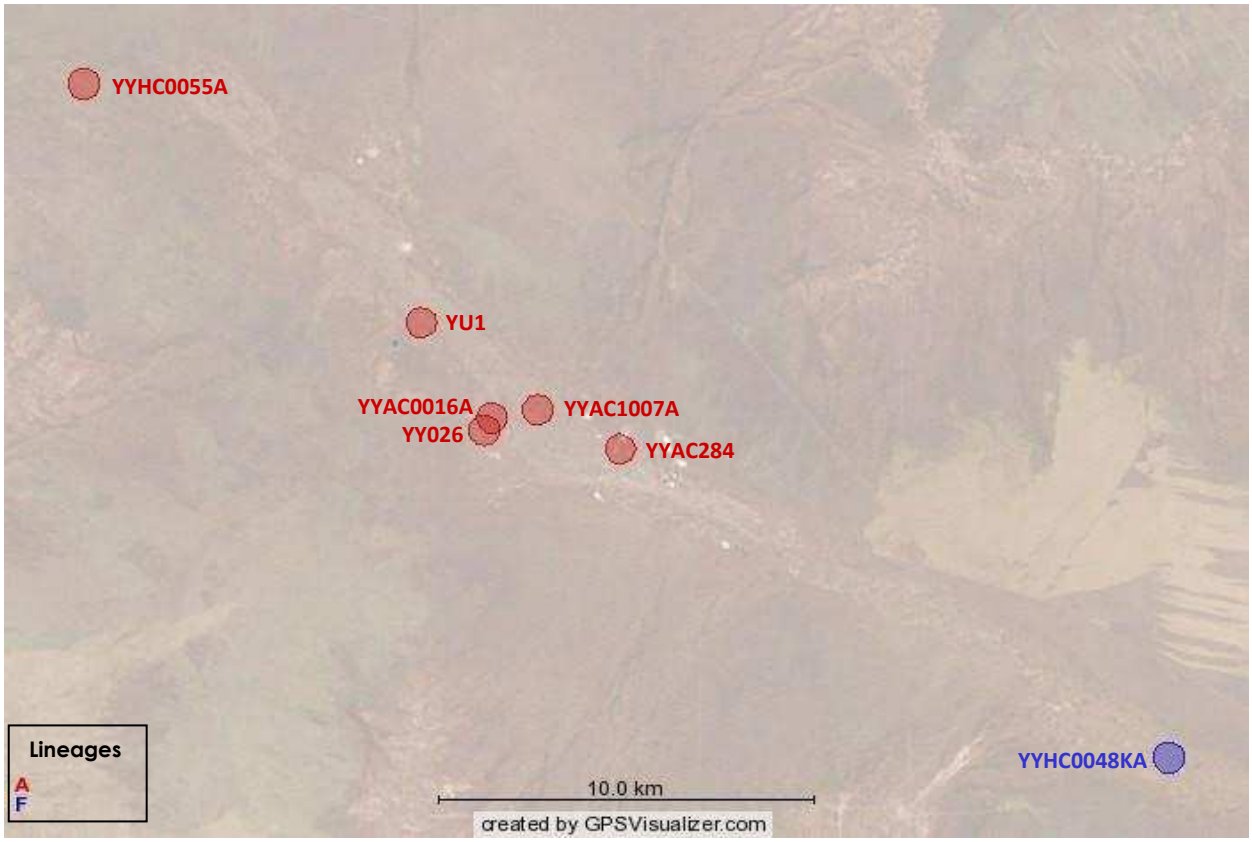


Figure 2. Map showing distribution of lineages B – E (components of lineage 1), as shown in Figure 1.

Figure 3. Map showing distribution of lineages A and F (components of lineage 2), as shown in Figure 1.



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18 January 2011

Shae Callan
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Via email

Re. Report on the molecular systematics of Isopoda

Dear Shae,

Following is a summary of the results of the isopod study we have completed. Our data revealed the presence of nine distinct genetic lineages, each of which is likely to represent a distinct species, owing to the high level of sequence divergence between them. Four of the lineages were detected previously, and five are new. From the current data set, we can't rule out the possibility that some lineages may represent populations that are connected by continuously breeding populations, owing to the disjunct distribution of lineages and limited sampling.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston
Helix Molecular Solutions

Background
In a previous collecting phase (Helix, 2010), specimens of troglobitic isopods were collected from sites at Yeelirrie and assigned by morphology to species within three isopod families (Philosciidae, Platyarthridae, Armadillidae). Molecular sequences of four specimens indicated that each was likely to be a distinct species, owing to the high levels of sequence divergence between specimens (Helix, 2010). The present study concerns an additional 19 specimens of Isopoda from Yeelirrie.

Objective
Nineteen specimens of isopods collected at Yeelirrie, Western Australia were sequenced for variation at the mitochondrial gene COI to:

- assess the number of species likely to be in the collection and test morphological assignments; and
- establish the genetic relationships of the Yeelirrie isopod specimens with those collected in phase 1 at Yeelirrie.

Executive summary

- Thirteen specimens of isopoda were sequenced for variation at the mitochondrial gene COI.
- The phylogenetic analysis, which included a total of 17 specimens from phases 1 and 2, showed the presence of nine divergent genetic lineages, which differed from one another by between 11.9 and 27.0% net sequence divergence.
- Four of the lineages were detected during phase 1, whereas five lineages were new to this study.
- Owing to the high levels of sequence divergence between them, each lineage is likely to represent a distinct species.
- Each lineage was found in single or near-by bores, and only one bore contained specimens belonging to more than one lineage.

Methods
Nineteen isopod specimens were collected from 11 bores at Yeelirrie (Table 1). The specimens were identified morphologically as belonging to three families, Aramadillidae, Philosciidae, and Platyarthridae (Shae Callan, pers. comm.). Some specimens were identified morphologically to genus (Table 1). The specimens were sequenced for the mitochondrial gene COI, using primers LCO1490 and HCO2198 (Folmer et al., 1993) or LCO1490 and HCOoutout (G. Giribet, pers. comm.)

Sequences were edited using SEQUENCHER software (Gene Codes Corporation, Ann Arbor, MI, USA). Alignment was performed with CLUSTAL W (Thompson *et al*. 1994) using default parameters. To test that the amplified COI sequences were the target mtDNA gene, sequences were translated into proteins and checked for the presence of stop codons. Genetic distances between unique genetic types (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotides different between sequences).

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data. Bayesian analysis was used to construct the phylogenetic tree, incorporating TVM+G model, as identified in MODELTEST. The phylogeny, branch lengths and posterior probabilities were obtained by running two trees simultaneously, each running four simultaneous MCMC chains. The number of cycles needed was determined by the standard deviation of the split frequencies of the two trees. The analysis was paused after every 1 x 10^6 generations and when the standard deviation fell below 0.01, the analysis was stopped. A majority rule consensus tree was constructed after discarding the first 3000 ("burn-in") trees. The burn-in value was assessed by plotting the posterior probabilities obtained after every generation and identifying the point at which the values reach stationarity (= the asymptote). Trees produced prior to stationarity were discarded. Two amphipod species (*Metacrangonyx lonipes* Genbank accession # NC013032 and *Parawaldeckia kidderi*, Genbank accession # FJ608920) were used as outgroups.

Results
Thirteen specimens yielded COI sequences of 681-831 base pairs (Appendix 1).

Phylogenetic analyses

The COI analysis, which included 17 specimens from phases 1 and 2, revealed the presence of nine distinct, well-supported genetic lineages (A – I; Figure 1). Each of the three families formed well-supported clades. There were four lineages of Platyarthridae (F, G, H, I), three lineages of Armadillidae (A, B, C) and two lineages of Philosciidae (D, E). Four of the lineages were detected in phase 1 (C, E, G, H), and five were new (A, B, D, F, I).

Genetic divergence within and between lineages

The nine lineages differed from one another by between 11.9 and 27.0% net sequence divergence (Table 2). Within families, genetic divergence between lineages ranged from 11.9 to 19.0% (Table 2). Mean divergence within lineages was lower, ranging from 0.0 to 2.6% (Table 3).

Geographic distribution of lineages

Each lineage was found in single or near-by bores. The three lineages of Armadillidae (A, B, C) were found in three bores, which were separated by between about 14 and 46 km (Figure 2). The two lineages of Philosciidae (D, E) were found in three bores (Figure 3). Lineage E was found in two bores separated by less than 0.5 km, whereas lineage D was found in a bore approximately 40 km away (Figure 3). The four lineages of Platyarthridae (F, G, H, I) were found in nine bores (Figure 4). Lineages F and I were each found in a single bore. Lineage H was found in two bores that were approximately 1 km apart (Figure 4). Lineage G was found in five bores, separated by between approximately 3 and 13 km (Figure 4).

Only a single bore (Wirraway) had specimens from two different lineages (C, H). All other bores contained specimens from a single lineage.

Conclusions

COI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003). Based on comparisons between over 1700 crustacean species, Hebert et al (2003) found an average of 15.4 % sequence divergence between species pairs. Only 18% of pair-wise comparisons showed less than 8% sequence divergence. Divergence values between all nine lineages in the present study exceed the minimum of 8% and only one pair, F and I, fall below the average of 15% suggesting that each lineage is likely to represent a distinct species.

Limited sampling prevents making firm conclusions about the distributions of the lineages. There is a strong geographical component to the phylogeny, in that each lineage was detected at single or near-by bores. Because sampling of intermediate areas may reveal intermediate haplotypes, we cannot discount the possibility that some pairs or groups of distinct lineages may represent the extremes of populations that are connected by continuously breeding populations. One such example is the case between lineages F and I, where sequence divergence is 11.9% and the two bores are 18 km apart. Sampling of intermediate sites is necessary to determine if there is a genetic break between the two lineages, or whether there are intermediate haplotypes in the intermediate areas. Regardless, if the samples in lineages F and I are representative of their populations, the two populations have been reproductively isolated for approximately 5 million years, using the COI molecular clock for arthropods (Brower, 1994).

Similarly Troglarmadillo lineages A, B, C were sampled from geographically distant sites. Sampling of intermediate sites would help determine if the observed genetic breaks are 'real'. Further, morphological characters that differentiate the genetic lineages would offer strong support for their status as separate species. Nevertheless, based on the data currently available, this study would suggest that with the exception of *Trichorhina* lineage G, dispersal abilities in the other groups are poor, and limited to areas that are geographically close.

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Table 1A. Samples used in the current study (phase 2).

Specimen ID	Bore ID	Lab ID	Taxonomic ID	Genetic lineage
LN:8409	YYD22	G161	Troglarmadillo sp. ?	
LN:6607a	YYD22	G162	Troglarmadillo sp. S9	
LN:7596	YU2	G163	Troglarmadillo sp. ?	
10:0437	YUN1	G164	Troglarmadillo sp. ?	COI-A
LN:7354	UNK1	G165	Troglarmadillo sp. S12	
LN:7359	UNK1	G166	Troglarmadillo sp. S13	COI-B
10:0458	Surprise Bore	G167	Trichorhina sp.	COI-G
10:0070	WIRRAWAY BORE	G168	Trichorhina sp.	COI-H
LN:8371	YYAC118	G169	Trichorhina sp.	COI-F
10:0384	YYHC0117A	G170	Trichorhina sp.	COI-G
LN:8465	YYHC0048KA	G171	Trichorhina sp.	COI-I
10:0408	YYHC0135B	G172	Trichorhina sp.	COI-G
10:0421	YYD22	G173	Philosciidae sp.	COI-E
10:0420a	YYD22	G174	Philosciidae sp.	
LN:7657	YYHC0060C	G175	Philosciidae sp.	COI-D
LN:7582	YYHC0060C	G176	Philosciidae sp.	
LN:7079		G177	Isopoda	GOI-G
LN:8465		G178	Isopoda	COI-I
LN:8530		G179	Isopoda	COI-G

Table 1B. Samples used in the phase 1 study.

Specimen	Bore	Lab ID	Species	Genetic lineage
LN7657	YYHC60C	G57	Philosciidae sp. S1	
LN6607B	YYD22	G58	Philosciidae indet.	
LN6607A	YYD22	G91	Troglarmadillo sp.	
LN8501	WIRRAWAY BORE	G92	Troglarmadillo sp.	COI-C
LN8373	YYAC36	G93	Philosciidae sp. Y2	COI-E
LN8473	YYHC0061B	G94	Trichorhina sp.	COI-H
LN8471	YYHC0104B	G95	Trichorhina sp.	COI-G

Table 2. Net sequence divergence (uncorrected p-distances) for COI between lineages of Isopoda at Yeelirrie. Yellow shaded areas denote distances among lineages within families.

	Armadillidae			Philosciidae		Platyarthridae			
	A	B	C	D	E	F	G	H	I
A		0.016	0.017	0.018	0.017	0.019	0.018	0.018	0.019
B	0.166		0.016	0.018	0.017	0.018	0.017	0.018	0.018
C	0.181	0.171		0.018	0.018	0.018	0.018	0.018	0.018
D	0.241	0.242	0.251		0.016	0.017	0.017	0.017	0.016
E	0.235	0.230	0.238	0.175		0.017	0.017	0.018	0.016
F	0.254	0.242	0.246	0.232	0.206		0.015	0.017	0.014
G	0.246	0.246	0.248	0.228	0.221	0.169		0.016	0.015
H	0.270	0.271	0.271	0.227	0.221	0.191	0.190		0.016
I	0.239	0.251	0.251	0.217	0.195	0.119	0.161	0.189	

Table 3. Mean sequence divergence (uncorrected p-distances) for COI within lineages of Isopoda at Yeelirrie.

lineage	D	s.e
A	n/c	n/c
B	n/c	n/c
C	n/c	n/c
D	n/c	n/c
E	0.005	0.003
F	n/c	n/c
G	0.026	0.004
H	0.000	0.000
I	0.000	0.000

Figure 1. Bayesian analysis of COI haplotypes of Isopoda. Numbers on nodes correspond to posterior probabilities. Lineages are labelled with family designations and specimens are labelled with morphospecies identifications as referred to in the text. New Yeelirrie specimens are highlighted in yellow.

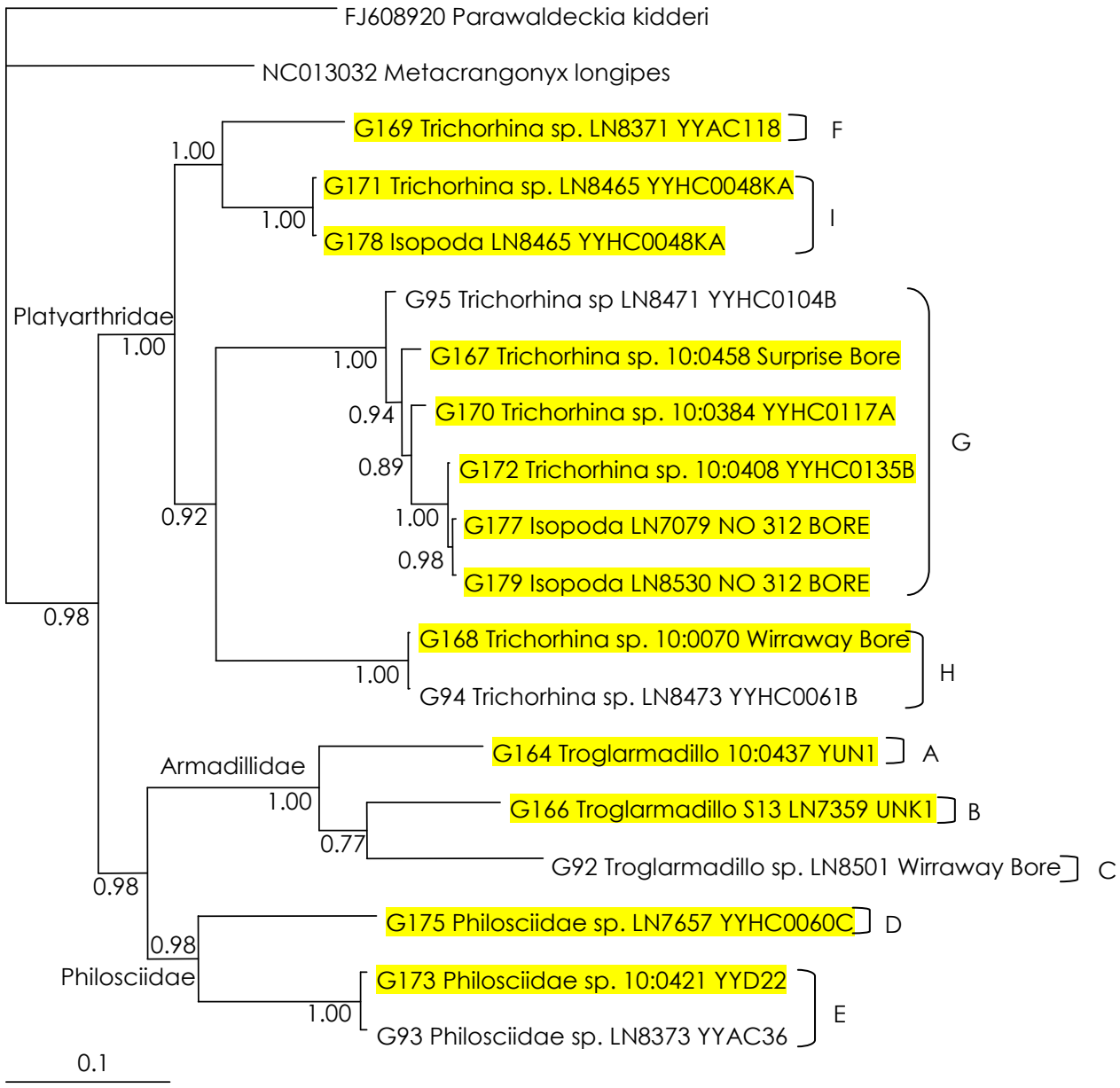


Figure 2. Geographic distribution of genetic lineages of Armadillidae.

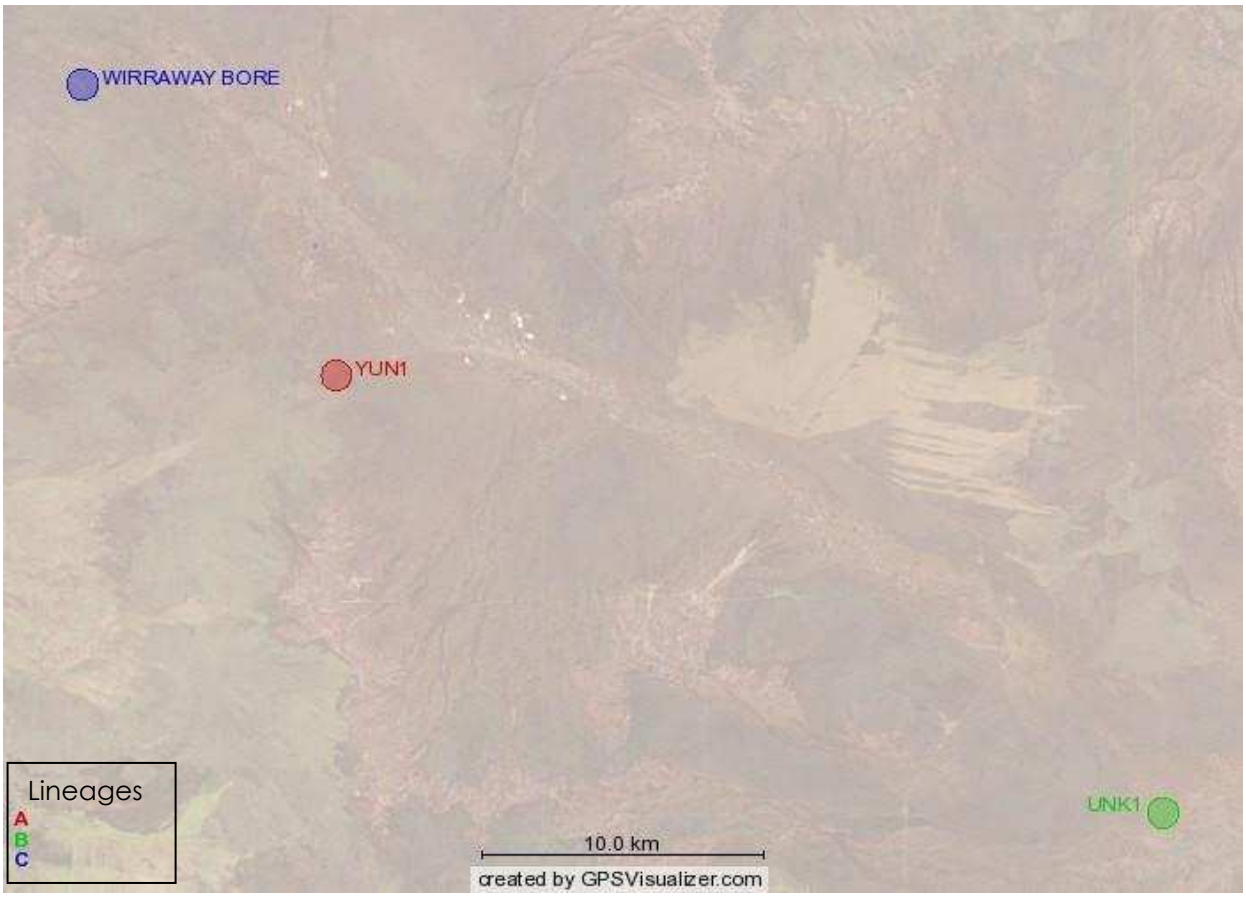
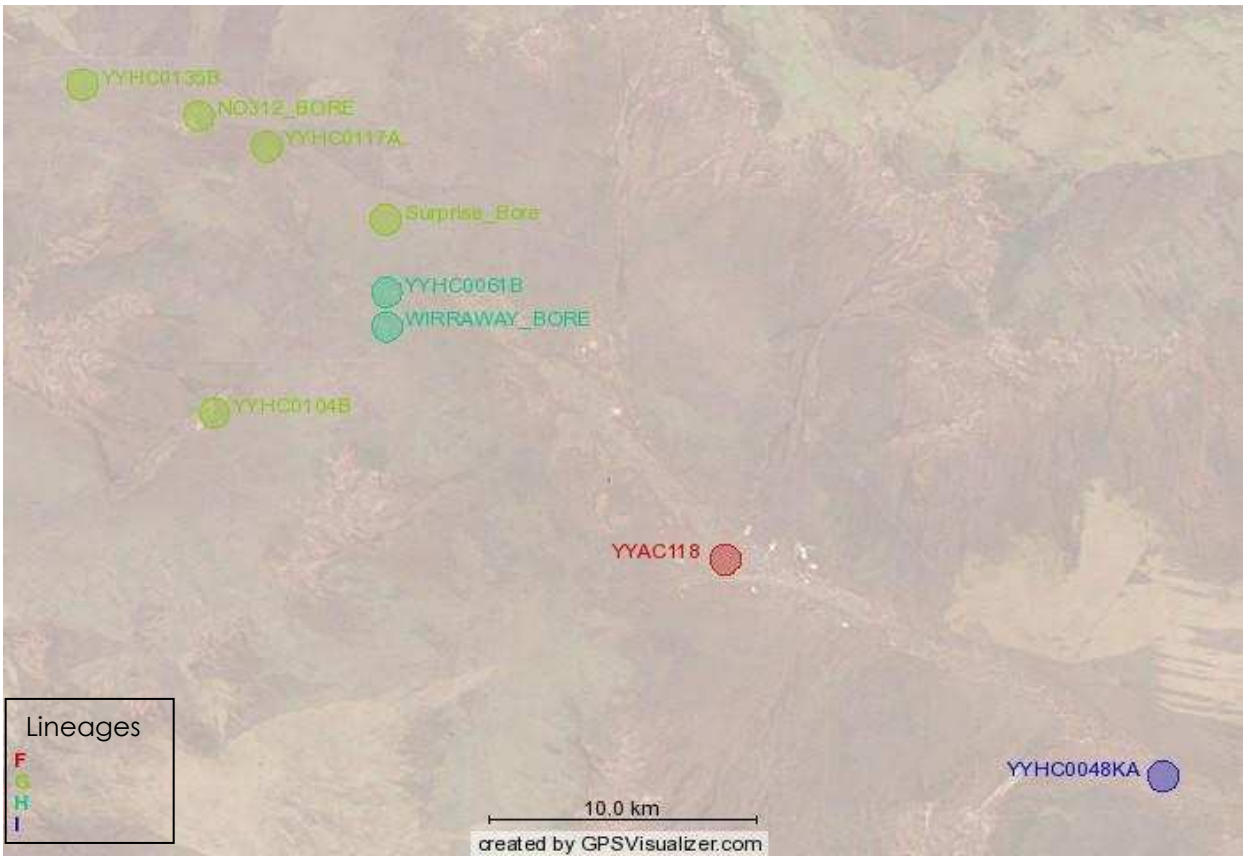


Figure 3. Geographic distribution of genetic lineages of Philosciidae.

Figure 4. Geographic distribution of genetic lineages of Platyarthridae.



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18 January 2011

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Via email

Re. Further reporting on the molecular systematics of Araneae

Dear Shae,

Following is a summary of the results of the Araneae study we have completed on your new samples from Yeelirrie. Our data revealed two divergent genetic groups in the Oonopidae, indicating the presence of two species, and one lineage of Trochanteriidae. Furthermore, variation detected within the Trochanteriidae suggests that further species may be present, but this result cannot be confirmed on the basis of the limited samples available and without further morphological work.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston and Oliver Berry
Helix Molecular Solutions

Background
Seven specimens of Araneae, belonging to two families (Oonopidae, Trochanteriidae) were collected from Yeelirrie and sequenced for variation at the mitochondrial genes COI and 12s during a first phase of sampling (Helix 2010). The phase 1 genetic data suggested there were two species of Oonopidae and up to two species of Trochanteriidae present in the collection. Owing to incomplete data for some specimens and some genes, it was impossible to determine whether there were one or two species of Trochanteriidae present in the sample (Helix 2010).

The present study concerns a molecular analysis of an additional 11 specimens of Oonopidae and Trochanteriidae from a second phase of sampling in the Yeelirrie region.

Objective
Eleven specimens of Araneae collected at Yeelirrie were sequenced for variation at the mitochondrial genes COI and 12s to:

- compare the results to those from the first phase of sampling to test morphological assignments; and
- assess the number of species likely to be in the collection.

Executive summary

- Collectively, 18 specimens of Araneae from phases 1 and 2 were sequenced for variation at the mitochondrial genes COI and 12s, including 11 new specimens.
- The difficulty of obtaining sequences from these new spider groups mean that in total, 12 specimens were successfully sequenced for COI and seven specimens were successfully sequenced for 12s. Four specimens were sequenced for both COI and 12s.
- Phylogenetic analyses of both genes showed a clear genetic division between families.
- The COI phylogenetic analysis revealed two distinct, well-supported genetic lineages of Oonopidae and one lineage of Trochanteriidae.
- The two COI lineages of Oonopidae differed from one another by 11.2% net sequence divergence, indicating the presence of two species.
- The lineage of Trochanteriidae represents at least one species, but moderate genetic variation among specimens may merit the recognition of additional species.
- Owing to a strong geographic component associated with genetic lineages, sampling of intermediate sites is required to determine if there are clear genetic breaks between lineages or whether intermediate haplotypes exist in intermediate regions.
- The 12s analysis supported the results of the COI analysis.

Methods
Eleven specimens of araneomorph spiders, from two families (Oonopidae, Trochanteriidae) were collected from ten bores at Yeelirrie (Table 1). Some specimens were identified morphologically as belonging to the genus *Prethopalpus* in the family Oonopidae while juveniles of the family were given the label Oonopidae sp. indet. (Shae Callan, Subterranean Ecology, pers. comm.). Specimens of the family Trochanteriidae were identified morphologically as belonging to the genus *Desognanops* (Shae Callan, Subterranean Ecology, pers. comm.). The samples were amplified for the mitochondrial gene COI using either primers LCO1490 and HCO2198 (Folmer et al., 1993) or C1-J-1751 and C1-N-2191 (Simon et al., 1994). Because CO1 sequence could not be amplified from all specimens, the samples were also amplified for the mitochondrial gene 12s using primers SR-N-14588 and SR-J-14233 (Simon et al., 1994).

Sequences were edited using SEQUENCHER software (Gene Codes Corporation, Ann Arbor, MI, USA). Alignment was performed with CLUSTAL W (Thompson *et al*. 1994) using default parameters. To test that the amplified COI sequences were the target mtDNA, sequences were translated into proteins and checked for the presence of stop codons. Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (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.

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data. Bayesian analysis was used to construct the phylogenetic trees, incorporating GTR+G model for COI and the TrN model for 12s, as identified in MODELTEST. Posterior probabilities were obtained by running four MCMC chains, each of 10^6 cycles. A majority rule consensus tree was constructed after discarding the first 3000 ("burn-in") trees. Specimens of the mygalomorphs *Antrodiaetus cerberus* (Genbank accession # DQ981635) and *Apomastus kristenae* (Genbank accession # DQ389886) were included as outgroups for COI. For 12s, two specimens of an undescribed species of Araneomorpha (sp B1) from the Ophthalmia Ranges were used as outgroups.

Results

Eight of the new specimens were successfully sequenced for COI and four new specimens were successfully sequenced for 12s. Two specimens were sequenced for both COI and 12s. Sequences for each specimen are given in Appendix 1 and 2.

Phylogenetic analyses

The COI phylogenetic analysis revealed the presence of three distinct, well-supported lineages (Figure 1). Lineages A and B corresponded to specimens of Oonopidae, and lineage C corresponded to specimens of Trochanteriidae (Figure 1). Lineage C was composed of two well-supported internal lineages (C-1, C-2; Figure 1).

The 12s phylogenetic analysis revealed the presence of two distinct, well-supported lineages (Figure 2). Lineage A corresponded to specimens of Oonopidae, and lineage C corresponded to specimens of Trochanteriidae (Figure 2). Lineage C was composed of two internal lineages (C-1, C-2; Figure 2). Lineage B was not recovered in the 12s analysis, owing to the failure to amplify specimens identified as lineage B in COI.

Genetic differentiation within and between lineages

COI lineages A – C differed from one another by between 11.2 and 17.5% net sequence divergence (Table 2). Mean genetic variation within lineages ranged from 0 to 5.8% (Table 3). Lineages C-1 and C-2 differed from one another by an average of 8.7% sequence divergence.

12s lineages A and C differed from one another by 41.1% net sequence divergence (Table 4). Mean genetic variation within the lineages ranged from 1.1 to 2.4% (Table 5). Lineages C-1 and C-2 differed from one another by an average of 5.7% sequence divergence.

Morphological associations

CO1 lineage A contained specimens identified as Oonopidae sp. indet. and *Prethopalpus* sp. and lineage B contained specimens identified as *Prethopalpus* sp.. Lineage C contained the specimens identified as *Desognanops* sp.

12S lineage A was composed of specimens identified as Oonopidae sp. indet. and *Prethopalpus* sp.. Lineage C was composed of specimens identified as *Desognanops* sp..

Geographic distribution of lineages

There was a clear geographic pattern to the distribution of lineages. Oonopidae lineage A was found in five bores at the northern end of the sampling area, spanning a distance of approximately 22 km, whereas lineage B was found in the southern-most bore of the sampling area, approximately 24 km away (Figure 3). The distribution of Trochanteriidae lineages C-1 and C-2 also showed a geographic pattern. Lineage C-1 was found in three bores at the northern end of the sampling area, spanning a distance of approximately 8 km, whereas lineage C-2 was found in a bore approximately 41 km to the south (Figure 4).

Conclusions

Species diversity

COI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003a). In a comparison of over 1200 chelicerate species, pairwise distances averaged 14.4% (s.e.=3.6), with over 97% of the pairwise comparisons showing > 8% sequence divergence (Hebert et al., 2003b). Barrett and Hebert (2005) focussed on the Araneae, and found that congeners differed by between 6.5 and 23.1% sequence divergence (mean= 16.4%, s.e.= 0.13) and conspecifics

differed by between 0 and 3.6% sequence divergence (mean=1.4%, s.e.=0.16). Using these values as a guide, the high level of sequence divergence between lineages A – B indicates that each is likely to be a distinct species. Lineage C is likely to represent a single species of *Desognanops*, although genetic variation detected within the lineage requires closer examination. Two groupings are apparent within the lineage, which differ by a mean of 5.8% sequence divergence at COI. This value is higher than the mean noted by Barrett and Hebert (2005) for different individuals of the same species, but lower than that noted between individuals of different species. The observed level of differentiation represents approximately 2.5 million years of isolation using a standard COI molecular clock for arthropods (Brower, 1994).

Whilst our DNA sequence data strongly suggest the presence of at least three species in the samples analysed, it should be noted that a lack of samples from intermediate sites limits the conclusions that can be drawn regarding species boundaries. Using the current data set it is impossible to determine if there is a clear genetic break between closely related lineages such as C-1 and C-2 and A and B, or whether intermediate haplotypes exist in intermediate areas.

Morphological implications

Lineages A and B both contain specimens identified morphologically as *Prethopalpus* sp. or unidentified juveniles (Oonopidae sp. Indet). The combined genetic and morphological results suggest that each lineage is likely to be a distinct species within the genus *Prethopalpus*, and the genetic results can be used to seek characters useful for distinguishing between the two species.

Lineage C contains specimens identified morphologically as *Desognanops* sp.. Variation within the lineage may indicate incipient speciation, and the genetic results can be used to seek characters differences between the lineages, which may merit the elevation to separate species status.

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Table 1. Specimens used in the phase 1 (G78 – G84) and the present study (G135 – G145), and the genetic lineages to which they belong.

Family	Species	Lab ID	Specimen ID	Bore	COI lineage	12s lineage
Oonopidae	Oonopidae sp. indet.	G81	LN7327	YYAC0010B	A	A
Oonopidae	Oonopidae sp. indet.	G136	LN7327	YYAC0010B	A	
Oonopidae	Oonopidae sp. indet.	G82	LN7371	YYAC0018C		
Oonopidae	Prethopalpus sp.	G79	LN7377	YYAC285	A	A
Oonopidae	Prethopalpus sp.	G135	LN7377	YYAC285	A	
Trochanteriidae	Desognanops sp.	G142	LN8482	YYD26	C	C
Oonopidae	Prethopalpus sp.	G78	LN8445	YYHC0048H	B	
Oonopidae	Prethopalpus sp.	G141	LN8445	YYHC0048H	B	
Oonopidae	Opopaea sp.	G80	LN8446	YYHC0048H		
Trochanteriidae	Desognanops sp.	G84	LN6635	YYHC0049E	C	
Trochanteriidae	Desognanops sp.	G145	LN6635	YYHC0049E	C	C
Oonopidae	Oonopidae sp. indet.	G139	LN8432	YYHC0086D	A	
Trochanteriidae	Desognanops sp.	G143	LN7676	YYHC0108		C
Oonopidae	Oonopidae sp. indet.	G140	LN8185	YYHC0110	A	
Trochanteriidae	Desognanops sp.	G83	LN7085	YYHC0111		C
Trochanteriidae	Desognanops sp.	G144	LN7085	YYHC0111		C
Oonopidae	Oonopidae sp. indet.	G137	10:0075	YYHC0112	A	
Oonopidae	Prethopalpus sp.	G138	LN7622	YYHC0112		

Table 2. Net sequence divergence between lineages for COI. Above diagonal=standard error.

	A	B	C
A		0.014	0.016
B	0.112		0.016
C	0.154	0.175	

Table 3. Mean within lineage distances (D) for COI. s.e.=standard error.

	D	s.e.
A	0.036	0.005
B	0.000	0.000
C	0.058	0.009

Table 4. Net sequence divergence between lineages for 12s. Above diagonal=standard error.

	A	C
A		0.029
C	0.411	

Table 5. Mean within lineage distances (D) for 12s. s.e.=standard error.

	D	s.e.
A	0.011	0.006
C	0.024	0.006

Figure 1. Bayesian analysis of COI haplotypes of Yeelirrie araneomorphs. Lineages are labelled as in text and Tables. Numbers on nodes correspond to posterior probabilities. New Yeelirrie specimens are highlighted in yellow.

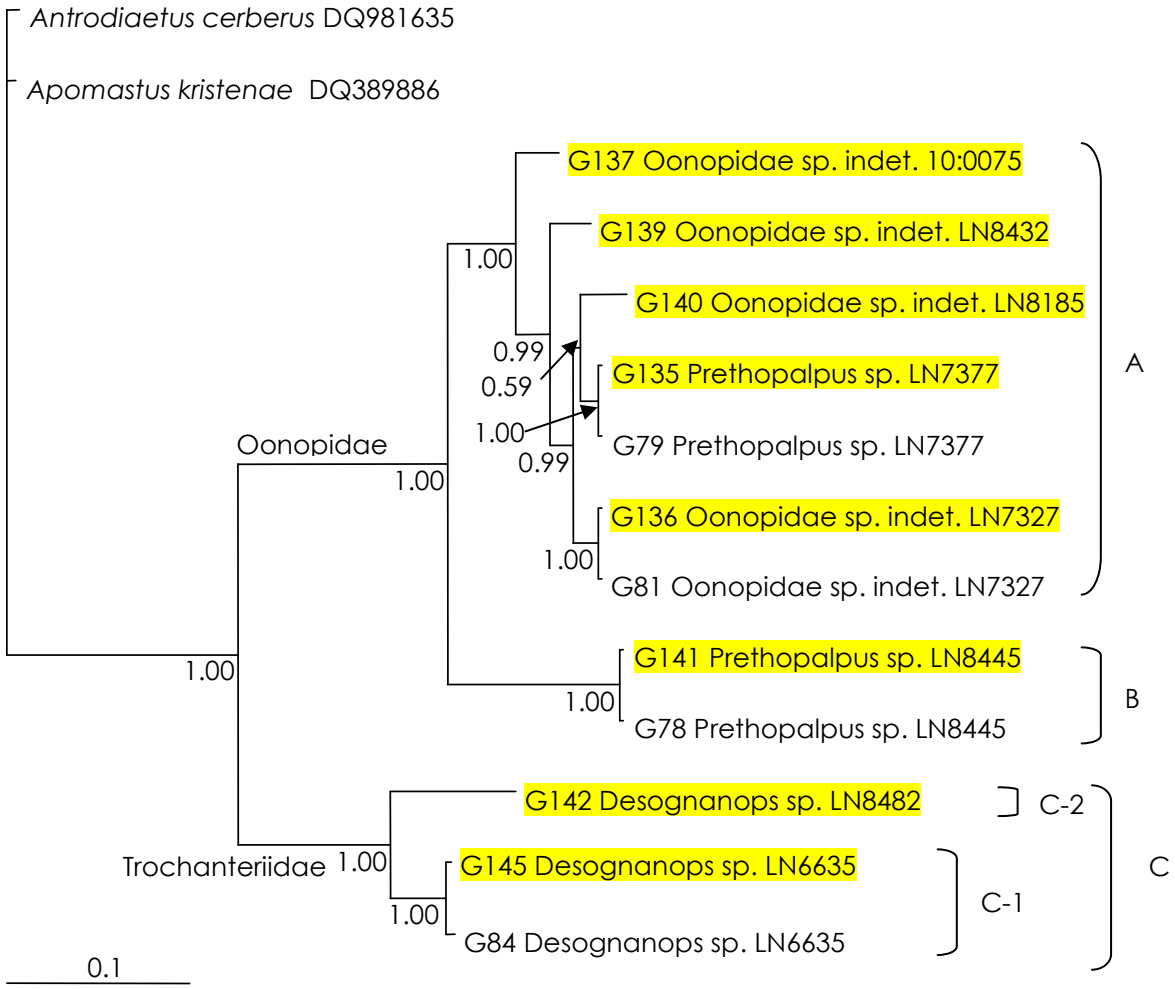


Figure 2. Bayesian analysis of 12s haplotypes of Yeelirrie araneomorphs. Lineages are labelled as in text and Tables. Numbers on nodes correspond to posterior probabilities. New Yeelirrie specimens are highlighted in yellow.

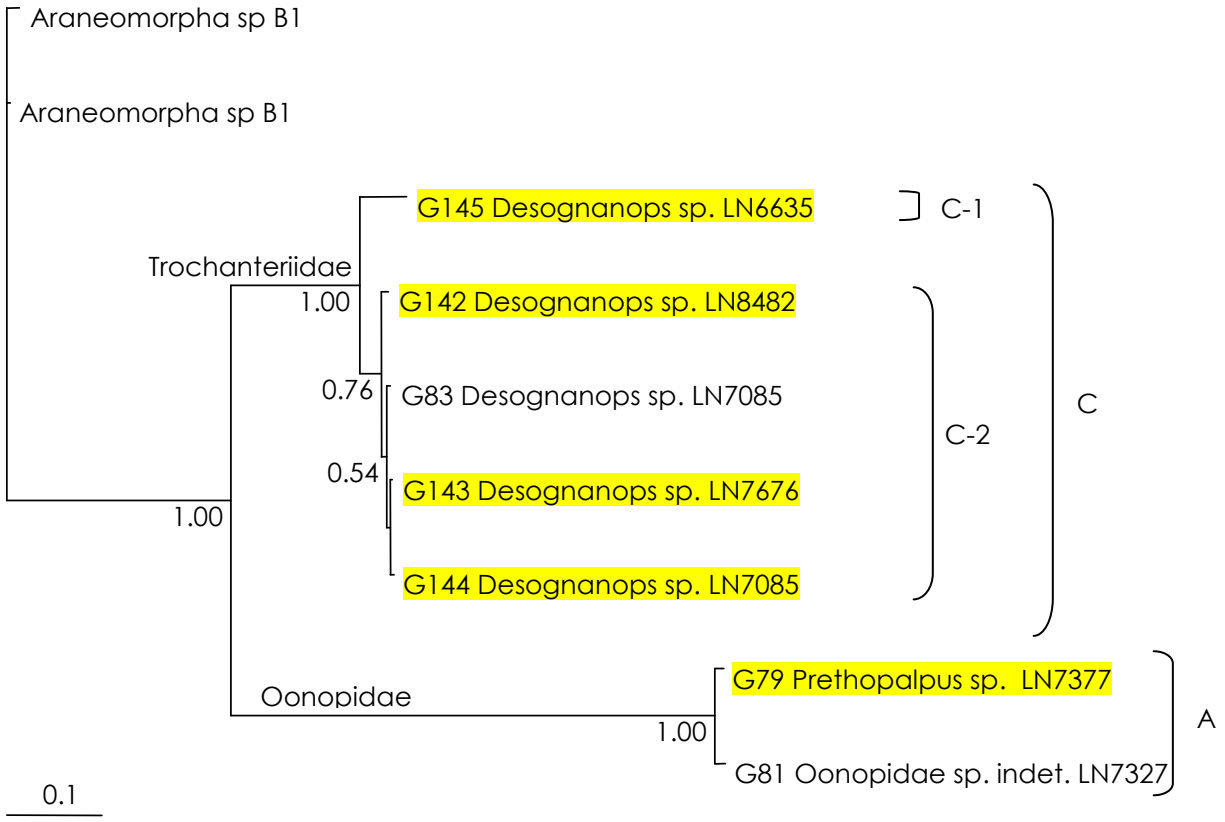


Figure 3. Distribution of lineages of Oonopidae as shown in Figures 1 and 2.

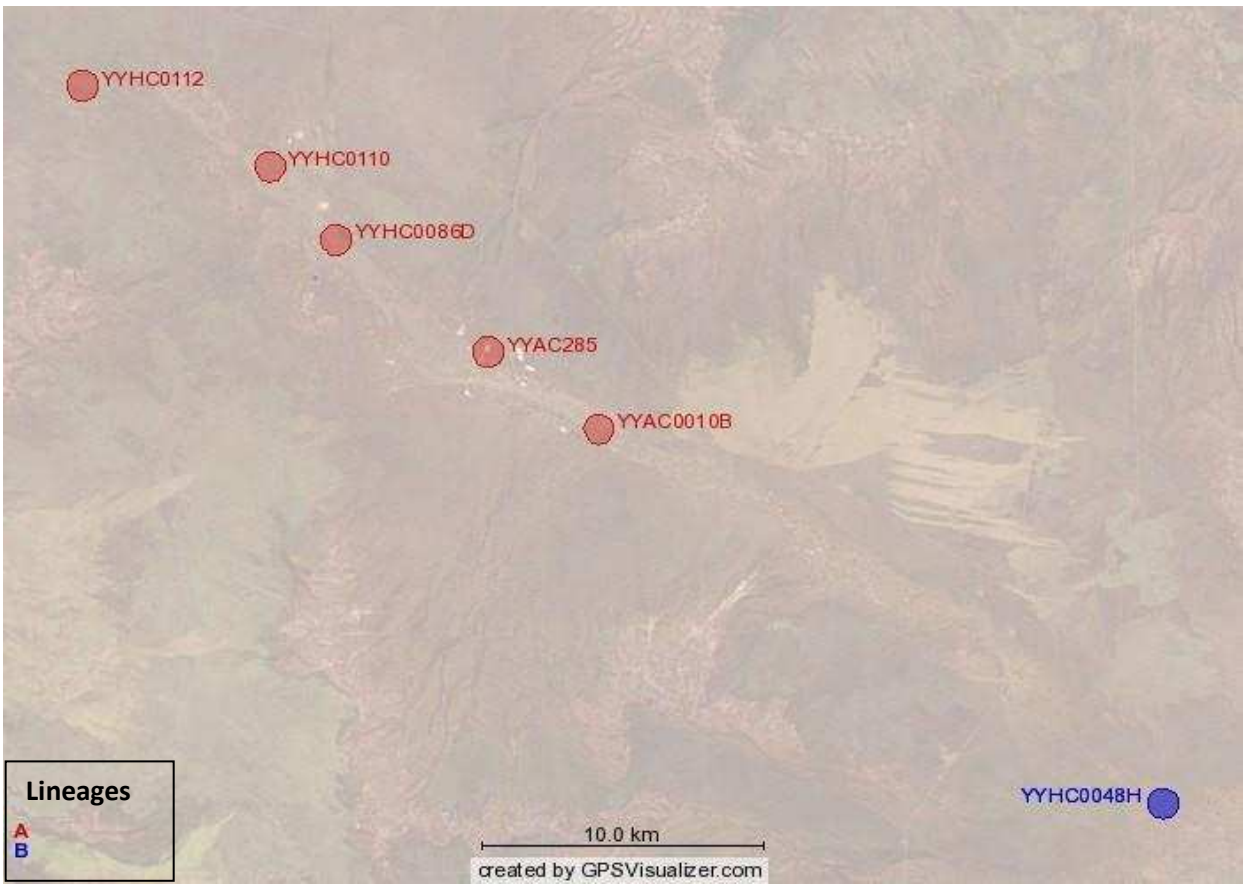
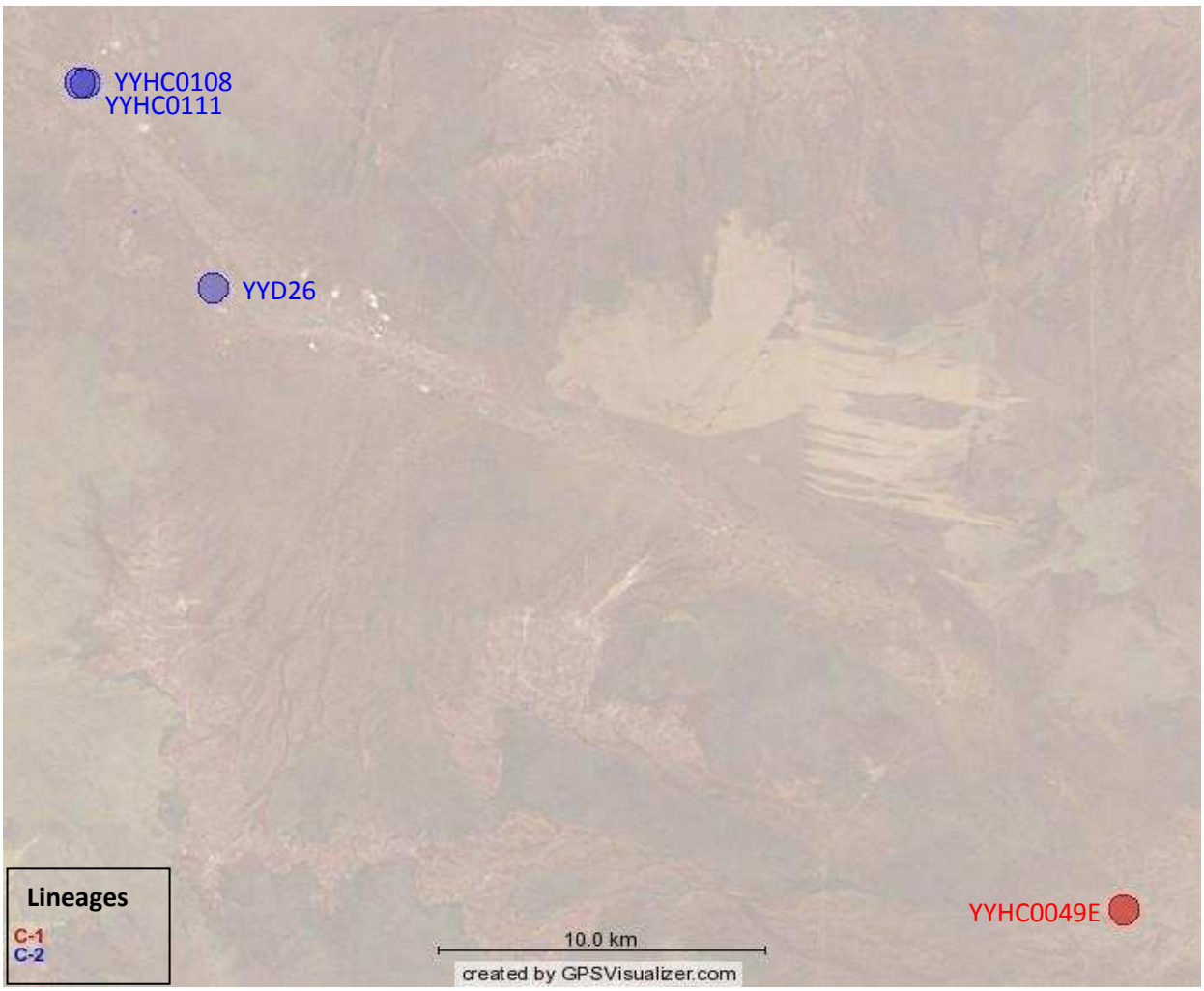


Figure 4. Distribution of lineages of Trochanteriidae as shown in Figures 1 and 2.



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18 January 2011

Shae Callan
Subterranean Ecology Pty. Ltd.
Scientific Environmental Services
Suite 8, 37 Cedric St, Stirling, WA 6021, AUSTRALIA

Via email

Re. Report on the molecular systematics of Pseudoscorpiones

Dear Shae,

Following is a summary of the results of the Pseudoscorpion study we have completed on your new samples from Yeelirrie. Our data revealed seven distinct genetic groups, which when combined with the morphological data, provides evidence for the recognition of seven species of Chthoniidae at Yeelirrie.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston and Dr. Oliver Berry
Helix Molecular Solutions

Background
Previous molecular data collected for the pseudoscorpion fauna of Yeelirrie indicated the presence of four genetic lineages of Chthoniidae, which were inferred to represent at least three species. In addition, two divergent lineages of Olpiidae were inferred to represent the presence of two species of (Helix, 2010). The current study was designed to compare new specimens from Yeelirrie to those from the first phase of sequencing to further test morphological identifications of species of Chthoniidae.

Objective
Ten pseudoscorpions collected at Yeelirrie, identified morphologically as belonging to six morphospecies were sequenced for variation at the mitochondrial gene cytochrome oxidase subunit I (COI) to:

- test morphological hypotheses and
- compare the specimens to those sequenced in the previous study.

Executive summary

- Seven specimens of pseudoscorpions were successfully sequenced for variation at the mitochondrial COI gene.
- The specimens were analysed with six specimens from the first phase of sequencing at Yeelirrie.
- Among the two sampling phases, seven morphospecies were identified.
- A phylogenetic analysis of both Yeelirrie data sets placed the Yeelirrie specimens of Chthoniidae in seven divergent genetic lineages.
- The two specimens of Olpiidae from the previous study were placed in an eighth genetic lineage.
- The seven lineages of Chthoniidae differed from one another by between 4.7 and 15.6% net sequence divergence.
- The seven genetic lineages of Chthoniidae each corresponded to distinct morphospecies, hence each lineage is likely to correspond to a distinct species.

Methods
Ten specimens of pseudoscorpions from the Chthoniidae, collected from ten bores at Yeelirrie were identified morphologically using established morphological characters for pseudoscorpions, and verified by taxonomists at the Western Australian Museum (WAM) (Table 1). The specimens were sequenced for the mitochondrial gene cytochrome oxidase subunit I (COI) using primers LCO1490 (Folmer et al., 1994) and HCOoutout (Murriene et al 2008).

Sequences were edited using SEQUENCHER software (Gene Codes Corporation, Ann Arbor, MI, USA). Alignment was performed with CLUSTAL W (Thompson *et al*. 1994) using default parameters. To test that the amplified sequences were the target mtDNA, sequences were translated into proteins and checked for the presence of stop codons. Genetic distances between unique genetic types (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotides different between sequences).

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data. Bayesian analysis was used to construct the phylogenetic tree, incorporating the TIM+I+G model, as identified in MODELTEST. The phylogeny was obtained by running four MCMC chains, each of 10^6 cycles. A majority rule consensus tree was constructed after discarding the first 2500 ("burn-in") trees. The burn-in value was assessed by plotting the posterior probabilities obtained after every generation and identifying the point at which the values reach stationarity (= the asymptote). Trees produced prior to the asymptote were discarded. Voucher sequences of species of Chthoniidae were included as reference sequences including a specimen of *Tyrannochthonius* sp JM 2008 (Genbank accession # EU559506) from South America (Columbia). Other Chthoniidae sequences included were: *Pseudotyrannochthonius* sp JM 2008 (Genbank accession # EU559508; from Australia), *Lagynochthonius johni* (Genbank accession # EU559503; from Indonesia), *Paraliochthonius* sp JM 2008 (Genbank accession # EU559505; from Australia), *Austrochtonia* sp JM 2008 (Genbank accession # EU559513; from Australia). A sequence of *Indohya* sp JM 2008 (Genbank accession # EU559564; from Australia) from the Hyidae was also included. No COI sequences of Olpiidae were available on Genbank. Sequences of the scorpion *Pandinus imperator* (Genbank

accession # AY1565821) and harvestman spider *Siro rubens* (Genbank accession # DQ5131111) were included as outgroups. A second phylogenetic analysis was run that included the above specimens as well as 42 specimens from other surveys in the Pilbara (Finston, unpublished data). The phylogenetic analysis required 5×10^6 cycles. The first 10000 trees were discarded as burn-in.

Results

Seven of the ten new specimens yielded DNA sequences (Appendix 1). The sequences differed from one another by between 0.0 and 15.4% (Table 2).

Phylogenetic analysis

The phylogenetic analysis, which included the seven specimens analysed here, as well as six from the first phase of sequencing, and six Genbank sequences of Chthoniidae, placed the Yeelirrie specimens of Chthoniidae in seven distinct, well-supported genetic lineages (Figure 1). Three of the lineages (1, 2A, 3, 4) were detected previously (Helix, 2010). Two specimens from the first phase belonged to the family Olpiidae and were placed in a fifth lineage (Figure 1). Lineages 1, 2A and 2B formed a well-supported clade (posterior probability = 1.00; Figure 1). Lineages 2C and 3 formed a well-supported clade (posterior probability = 1.00) and lineages 4 and 5 formed a well-supported clade (posterior probability=0.99; Figure 1).

The phylogenetic analysis that contained specimens from the wider Pilbara revealed the presence of three major clades that corresponded to three families (Chthoniidae, Olpiidae, Hyidae; Figure 2), plus one clade of unknown familial status. The analysis placed all of the Yeelirrie Chthoniidae specimens in a well-supported clade (posterior probability=1.00; Figure 2). Relationships between the Yeelirrie specimens and other Pilbara and voucher specimens were not well-supported.

The seven Chthoniidae lineages from Yeelirrie differed from one another by between 4.7 and 14.6% net sequence divergence (Table 3). The Yeelirrie lineages differed from the Genbank voucher sequences of Chthoniidae by between 17.9 and 22.6% net sequence divergence (Table 3). Mean genetic distances within the seven Yeelirrie lineages ranged from 0.0 to 0.2 % (Table 4).

Morphological hypotheses

There was good correspondence between genetic lineages and morphospecies assignments (Figure 1). Each genetic lineage corresponded to a discrete morphospecies. There were however some inconsistencies at higher taxonomic levels. The three morphospecies in morphological group 2, (2A, 2B, 2C) were not placed in the same clade. Morphospecies 2A and 2B formed a well supported clade, but morphospecies 2C was placed in a clade with morphospecies 3.

Geographic distribution of lineages

All but two lineages were found at single sites (Figure 3). Two lineages, 2B and 5, were found at more than one site, but the sites were geographically close (<2 km). No sites contained individuals from more than one lineage, however two sites that were geographically close, YYAC36 and YYAC0015, which are < 1 km apart, contained two different lineages (1, 2B).

Conclusions

COI is widely considered to show suitable variation to distinguish species (Hebert et al., 2003). In comparisons between over 1200 pairs of chelicerates, Hebert et al (2003) found a mean of 14.4% sequence divergence between distinct species. Over 97% of the comparisons showed that species pairs differed from one another by greater than 8% sequence divergence.

Using these values, as well as the morphological evidence to help guide the delineation of species, the seven lineages of Chthoniidae from Yeelirrie are likely to represent distinct species. While the genetic distance values between some Yeelirrie lineages were lower than the averages found by Hebert et al., (2003), good correspondence between morphological characters and genetic lineages provides strong evidence for their recognition as distinct species.

The Yeelirrie group forms a well-supported clade to the exclusion of other Pilbara specimens, however its relationship to the other Pilbara fauna cannot be resolved using the present data set. Despite the lack of resolution at higher taxonomic levels, the Yeelirrie fauna represents new species which are genetically distinct from the Pilbara fauna.

References

Brower, A.V.Z., 1994. Rapid morphological radiation and convergence among races of the butterfly Heliconius erato inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences USA 91: 6491-6495.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294-299.

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Murienne J, Harvey M.S., Giribet G. (2008). First molecular phylogeny of the major clades of Pseudocorpiones (Arthropoda: Chelicerata). Molecular Phylogenetics and Evolution 49: 170-184.

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Thompson, J., Higgins, D., and Gibson, T. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680. doi:10.1093/nar/ 22.22.4673

Table 1. Samples used in the current study and the genetic lineage to which they belong.

Helix ID	Specimen ID	WAM morphospecies	Bore ID	Genetic lineage
G85	LN8520	Tyrannochthonius sp.	SB14-MT	3
G88	LN8500	Tyrannochthonius sp.	WIRRAWAY BORE	2A
G149	LN:8500	Tyrannochthonius sp. 2A	WIRRAWAY_BORE	2A
G148	LN:7335	Tyrannochthonius sp.	WIRRAWAY_BORE	No data
G146	LN:6643	Tyrannochthonius sp. 2B	YYAC0015A	2B
G89	LN7692	Austrohorus sp.	YYAC26	Olpiidae
G147	LN:7695	Tyrannochthonius sp. 2B	YYAC33	2B
G154	LN:8374	Tyrannochthonius sp. 1	YYAC36	1
G86	LN8374	Tyrannochthonius sp.	YYAC36	1
G87	LN8425	Tyrannochthonius sp.	YYHC0048I	4
G150	LN:7591	Tyrannochthonius sp. 4	YYHC0048L	No data
G90	LN7592	Austrohorus sp.	YYHC0048L	Olpiidae
G155	LN:6634	Tyrannochthonius sp. 5	YYHC0049E	5
G151	LN:8451	Tyrannochthonius sp.	YYHC0049F	5
G152	LN:7608	Tyrannochthonius sp. 2C	YYHC0060D	2C
G153	0.689583333	Tyrannochthonius sp.	YYHC0060E	No data

Table 2. Sequence divergence at COI between haplotypes of pseudoscorpions at Yeelirrie. Above diagonal=standard error. Distances between haplotypes in the same lineage are highlighted in yellow.

lineage	2B		2A		1		3	2C	4	5	
	G146	G147	G149	G88	G86	G154	G85	G152	G87	G151	G155
G146 LN6643		0.002	0.010	0.010	0.011	0.011	0.015	0.015	0.016	0.014	0.014
G147 LN7695	0.002		0.010	0.010	0.012	0.011	0.015	0.016	0.016	0.014	0.014
G149 LN8500	0.045	0.047		0.000	0.012	0.012	0.016	0.015	0.016	0.015	0.015
G88 LN8500	0.045	0.047	0.000		0.012	0.012	0.016	0.015	0.016	0.015	0.015
G86 LN8374	0.069	0.071	0.078	0.078		0.002	0.016	0.016	0.015	0.016	0.016
G154 LN8374	0.067	0.069	0.076	0.076	0.002		0.016	0.016	0.016	0.016	0.016
G85 LN8520	0.136	0.138	0.141	0.141	0.141	0.138		0.014	0.017	0.017	0.017
G152 LN7608	0.138	0.141	0.129	0.129	0.134	0.132	0.094		0.016	0.017	0.017
G87 LN8425	0.129	0.132	0.129	0.129	0.127	0.129	0.141	0.138		0.015	0.015
G151 LN8451	0.123	0.125	0.123	0.123	0.136	0.134	0.147	0.152	0.114		0.002
G155 LN6634	0.125	0.127	0.125	0.125	0.138	0.136	0.150	0.154	0.116	0.002	

Table 3. Mean sequence divergence between lineages of Chthoniidae as shown in Figure 1. Above diagonal=standard error. Aust= Austrochthonius sp., Lagy= Lagynochthonius johni, Para= Paraliochthonius sp JM2008, Pseu= Pseudotyrannochthonius sp JM2008, Tyra= Tyrannochthonius sp JM2008.

	1	2A	2B	2C	3	4	5	Aust	Lagy	Para	Pseu	Tyra
1		0.013	0.012	0.017	0.016	0.017	0.016	0.018	0.019	0.018	0.019	0.019
2A	0.078		0.011	0.016	0.017	0.016	0.015	0.017	0.019	0.017	0.020	0.019
2B	0.068	0.047		0.017	0.016	0.016	0.015	0.018	0.019	0.018	0.019	0.019
2C	0.137	0.132	0.142		0.014	0.017	0.018	0.019	0.019	0.019	0.019	0.020
3	0.146	0.146	0.142	0.097		0.017	0.016	0.018	0.018	0.018	0.019	0.020
4	0.134	0.132	0.132	0.142	0.146		0.016	0.019	0.019	0.018	0.019	0.019
5	0.139	0.125	0.125	0.160	0.153	0.116		0.019	0.019	0.018	0.020	0.020
Aust	0.184	0.179	0.182	0.179	0.182	0.193	0.196		0.018	0.017	0.020	0.019
Lagy	0.226	0.210	0.219	0.208	0.208	0.210	0.210	0.198		0.018	0.019	0.019
Para	0.193	0.189	0.189	0.196	0.193	0.198	0.196	0.158	0.200		0.019	0.019
Pseu	0.226	0.224	0.212	0.205	0.217	0.210	0.217	0.208	0.222	0.215		0.021
Tyra	0.210	0.212	0.204	0.219	0.226	0.198	0.203	0.170	0.226	0.189	0.236	

Table 4. Mean within lineage distances (D). Above diagonal=standard error (s.e.). n/c=not calculated.

lineage	D	s.e.
1	0.000	0.000
2A	0.000	0.000
2B	0.002	0.002
2C	n/c	n/c
3	n/c	n/c
4	n/c	n/c
5	0.002	0.002

Figure 1. Bayesian analysis of COI haplotypes of pseudoscorpions. Numbers on nodes correspond to posterior probabilities. Lineages are labelled with morphospecies identifications as referred to in the text. New Yeelirrie specimens are highlighted in yellow.

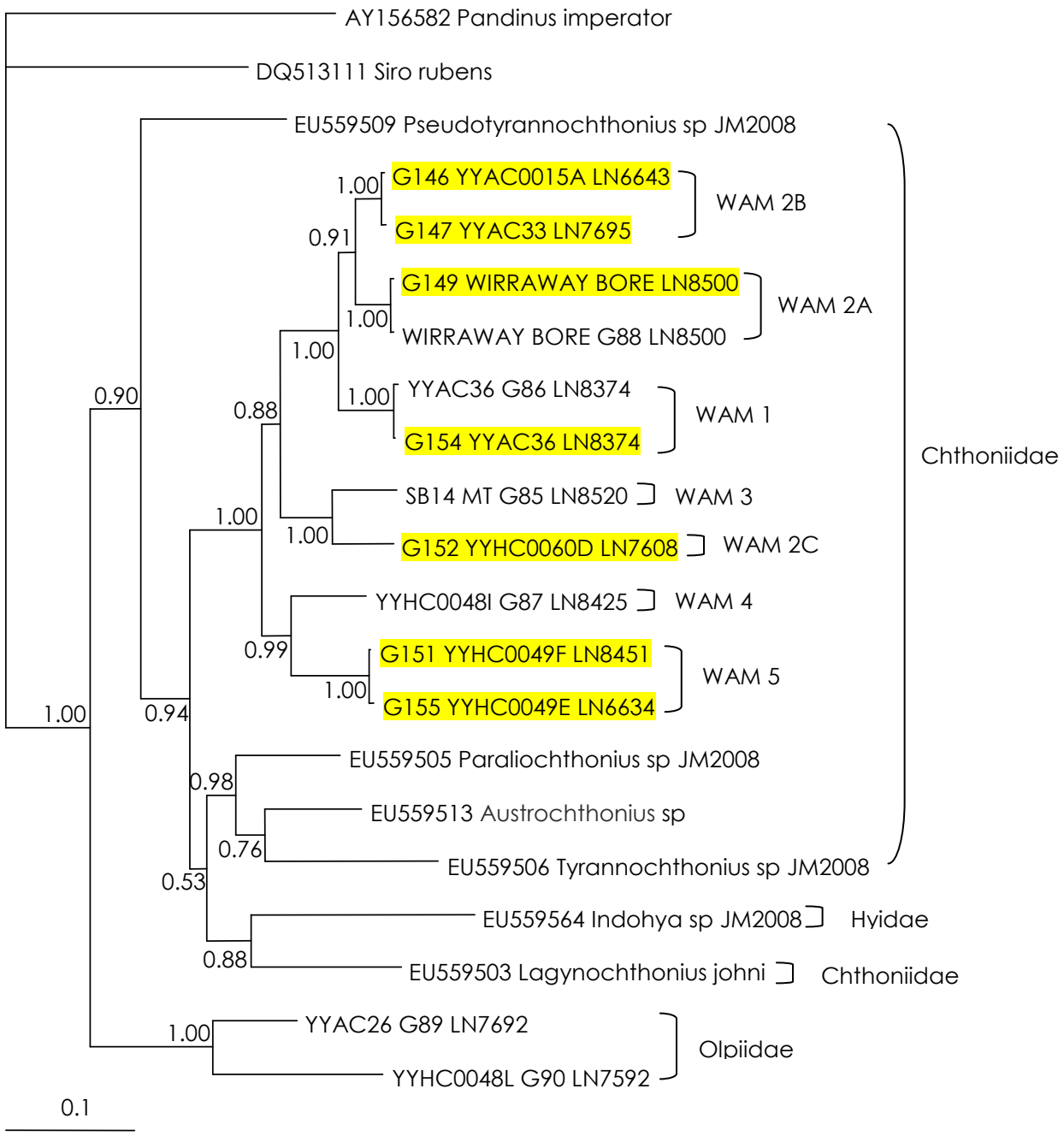

Figure 2. Bayesian analysis of COI haplotypes of Yeelirrie and Pilbara pseudoscorpions. Numbers on nodes correspond to posterior probabilities. Yeelirrie specimens indicated by yellow bars. Genbank vouchers indicated by turquoise bars; all others are Pilbara specimens.

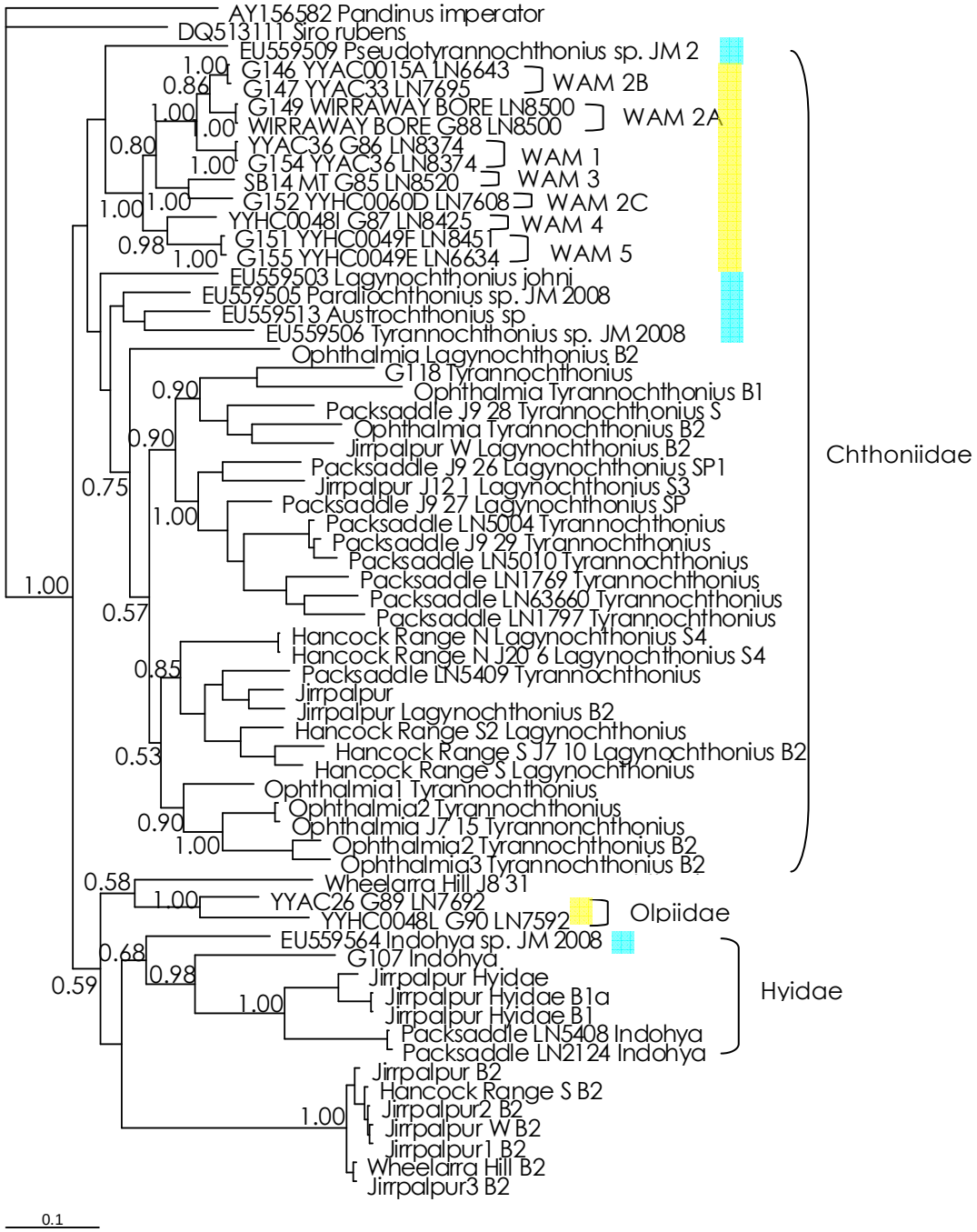

Figure 3. Distribution map of genetic lineages as detected in the phylogenetic analysis of COI.

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14 February 2011

Shae Callan
Subterranean Ecology

Via email

Re. Report on the molecular systematics of Pauropoda

Dear Shae,

Following is a summary of the results of the Pauropoda study we have completed. Regrettably we could not obtain DNA sequences from two of the specimens.. Nevertheless, our data suggest that there are two species present within the two specimens from which we obtained successful sequences.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston and Dr. Oliver Berry
Helix Molecular Solutions

Background
Four specimens identified on the basis of morphology as belonging to a singles species (species S6) of Pauropoda were collected from Yeelirrie, Western Australia.

Objective
DNA sequences were obtained from the specimens with the objective of assessing:

- the number of likely species in the collection; and
- the relationships of these specimens to Pauropoda species previously collected in the Pilbara.

Executive summary

- Two specimens of Pauropoda species S6 were successfully sequenced for the mtDNA 12s gene, while two failed to yield sequences.
- The two specimens are highly distinct, differing by 42% sequence divergence.
- The two specimens represent different species of Pauropoda.

Methods

Four specimens of Pauropoda, identified as morphological species S6 (Subterranean Ecology, pers. comm.), were collected from four bores at Yeelirrie (Table 1). The samples were sequenced for variation at the small subunit ribosomal RNA (12s) gene, using primers 12s ai and 12s bi (Simon et al., 1996).

Genetic distances between unique genetic types (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotides different between sequences).

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data. Bayesian analysis was used to construct the phylogenetic tree, incorporating TVM model, as identified in MODELTEST. Posterior probabilities were obtained by running four MCMC chains, each of 10^6 cycles. A majority rule consensus tree was constructed after discarding the first 200 ("burn-in") trees. A specimen of *Narceus americanus* (Genbank accession # FJ213764.1), a millipede species from the eastern US and the closest match to the Yeelirrie specimens on the international database Genbank, was included as an outgroup.

Results
Two specimens failed to amplify (LN7661, LN7084). The phylogenetic analysis placed the two specimens in two distinct lineages (A, B; Figure 1). The two specimens were highly genetically distinct, differing from one another by 42% sequence divergence (Table 2).

Conclusions
The high level of sequence divergence between the two specimens indicates that they are different species.

The specimens show little similarity to other specimens on the international database Genbank, but this is most likely due to a lack of available sequences of appropriate relatives in this little-known group.

The fact that two specimens failed to amplify may have taxonomic significance – failure to amplify may be due to significant differences in primer region. Alternatively, it may represent degradation of the specimens during storage. Further DNA analysis would be required to verify the taxonomic identity of these specimens.

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Posada, D., Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Simon, C., Fratl, F, Beckenbach, A., Crespi, B., Liu, H., Flook, P. (1996) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society 87: 651-701.

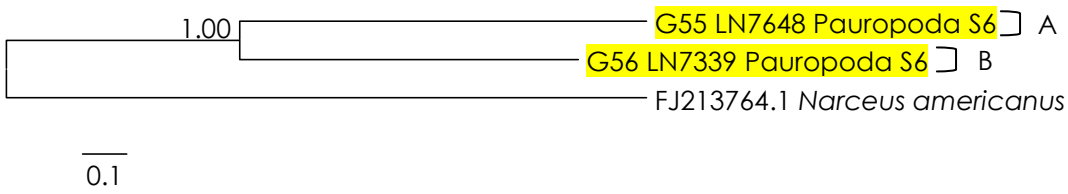
Table 1. Specimens used in the present study and the genetic lineage to which they belong.

Bore	Lab ID	Morphological ID	Specimen ID	Lineage
LTROG1 YYHC48F	G53	Pauropoda sp. S6	LN7661	No data
YYHC0111	G54	Pauropoda sp. S6	LN7084	No data
YYAC0018C	G55	Pauropoda sp. S6	LN7648	A
WIRRAWAY BORE	G56	Pauropoda sp. S6	LN7339	B

Table2. Percent sequence divergence (uncorrected p-distances) between two specimens of Pauropoda species S6 at 12s.

	LN7648	LN7339
LN7648	x	
LN7339	42.0	x

Figure 1. Bayesian analysis of 12s haplotypes of Pauropoda. Numbers on nodes correspond to posterior probabilities. The Yeelirrie specimens are highlighted in yellow. The millipede *Narceus americanus* from the US is used as an outgroup. Scale bar = number of substitutions per site.



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14 February 2011

Shae Callan
Subterranean Ecology

Via email

Re. Report on the molecular systematics of Polyxenida

Dear Shae,

Following is a summary of the results of the Polyxenida study we have completed. Our data suggest that the specimens you provided belong to a single species of polyxenid. The specimens also show close affinities to the widespread Pilbara species S1, and may represent a geographic isolate of that species.

Thanks once again for collaborating on this project with Helix. We hope we can continue to provide you with useful information, and feel free to contact us if you have any questions or would like to discuss the results in detail.

Sincerely,

Dr. Terrie Finston and Dr. Oliver Berry
Helix Molecular Solutions

Background
Mitochondrial (12s) and nuclear (28s) sequence data for Polyxenida millipedes from multiple sites in the Fortescue and DeGray basins in the Pilbara revealed the presence of what is likely to be a widespread species showing little mitochondrial variation and virtually no nuclear variation (species S1; Finston, unpublished data). Genetic evidence further suggests that species S1 is found in conjunction with a second species, so far known only from sites in the Packsaddle Ranges (species S5; Finston, unpublished data). Specimens of Polyxenida were recently collected at Yeelirrie and assigned a morphological identification of species S9 by Subterranean Ecology. Four species of Polyxenida have been described from Australia, with two surface species recorded from Western Australia (source: Australian Faunal Directory). The present study evaluates the genetic structure of five Polyxenida from five sites from the Yeelirrie.

Objective
Specimens of Polyxenida millipedes identified as morphological species S9 from Yeelirrie were sequenced for variation at the 12s mitochondrial gene to assess:

- the likely number of species in the collection; and
- the relationship of the Yeelirrie specimens to Polyxenida species recently identified on the basis of genetics and morphology from the Pilbara.

Executive summary

- The five samples of Polyxenida S9 differ from one another by <1% sequence divergence, indicating they belong to the same species.
- In the phylogenetic analysis including existing specimens from the Pilbara, the new S9 specimens were placed in the same evolutionary lineage as specimens of S1 from multiple sites in the Fortescue and DeGray basins.
- The five samples of S9 differ from species S1 from the Pilbara by 4.2% net sequence divergence.
- Low-moderate levels of sequence divergence between S1 and S9 suggest they could be the same species of Polyxenida, with S9 representing a geographic isolate of the S1 species.

Methods
Five specimens of Polyxenida identified as Polyxenida species S9 were collected from five bores at Yeelirrie (Table 1). The specimens were sequenced for the mitochondrial small subunit rRNA (12s) using primers 12s ai and 12s bi (Simon et al., 1996).

Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (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.

MODELTEST software (Posada and Crandall, 1998) was used to determine the model of sequence evolution that best fitted the data. Bayesian analysis was used to construct the phylogenetic tree, incorporating General Time Reversible model (GTR+G), as identified in MODELTEST. Posterior probabilities were obtained by running four MCMC chains, each of 10^6 cycles. A majority rule consensus tree was constructed after discarding the first 200 ("burn-in") trees. Voucher sequences of Polyxenida millipede sequences from the Pilbara (Finston, unpublished data) were included as reference sequences. A surface species of Polyxenida from Wheelarra Hill was included as an outgroup.

Results
All five specimens were successfully sequenced (Appendix 1). The five samples of Polyxenida sp. S9 from Yeelirrie differed from one another by <1% sequence divergence (Table 2), forming a well-supported lineage (Figure 1).

The Yeelirrie specimens occurred within the lineage containing haplotypes of species S1 from the Pilbara (Figure 1). Species S1 shows some evidence of genetic structure, consisting of multiple shallow lineages that show little geographical association (Figure 1). The five samples of S9 differ from species S1 from the Pilbara by 4.2% net sequence divergence (Table 3).

Conclusions

All specimens of species S9 collected at Yeelirrie show highly similar sequences, indicating they are likely one species.

While COI is considered to have appropriate levels of sequence evolution for species delineation by some authours (Hebert et al., 2003a), 12s is widely used, particularly among insect groups (Simon et al., 1994). The rate of sequence evolution varies along the 12s gene, with the 'upstream' half of the gene being more variable than the highly conserved 'downstream' half (Simon et al., 1994). The portion of the gene used in the present study arises from the 'upstream' end, and indeed, where 12s and COI data are available for the same set of samples, sequence divergence for the two genes is similar (Appendix 2). Thus if we assume that the particular region of 12s used in the present study is approximately equivalent to the bar-coding segment of COI with respect to its rate of sequence evolution, then the genetic 'yardstick' employed for COI would be appropriate for interpreting 12s variation in this study. Based on comparisons between over 1400 arthropod species, Hebert et al (2003b) found an average of 10% sequence divergence between species pairs. Furthermore, over 75% of pair-wise comparisons showed more than 4% sequence divergence.

Excluding the Yeelirrie specimens, variation among 12s haplotypes of S1 ranged from 0.0 to 5.4% sequence divergence (Finston, unpublished data). Net sequence divergence between the Yeelirrie specimens and the Pilbara specimens was 4.2%, hence, the inclusion of the Yeelirrie specimens within the clade containing the Pilbara specimens, in conjunction with the low-moderate levels of sequence divergence between them suggests they could be the same species of Polyxenida. However, using a standard molecular clock for arthropods (Brower, 1994), the Pilbara and Yeelirrie specimens have been isolated from one another for over a million years. Thus it is likely that the Yeelirrie specimens represent a geographical variant of S1.

S5 is highly distinct, suggesting it is a second species of Polyxenida, found in the Packsaddle Ranges of the Pilbara.

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Table 1. Identification codes, geographic origin and morphological assignments five Polyxenida examined in this report.

Bore	Lab ID	Specimen ID	Morphological ID
L-UNK1	G48	LN7318	Polyxenida sp. S9
YYAC0015B	G49	LN6642	Polyxenida sp. S9
YYHC0108	G50	LN7674	Polyxenida sp. S9
YYAC33	G51	LN7906	Polyxenida sp. S9
YYHC049T3	G52	LN7660	Polyxenida sp. S9

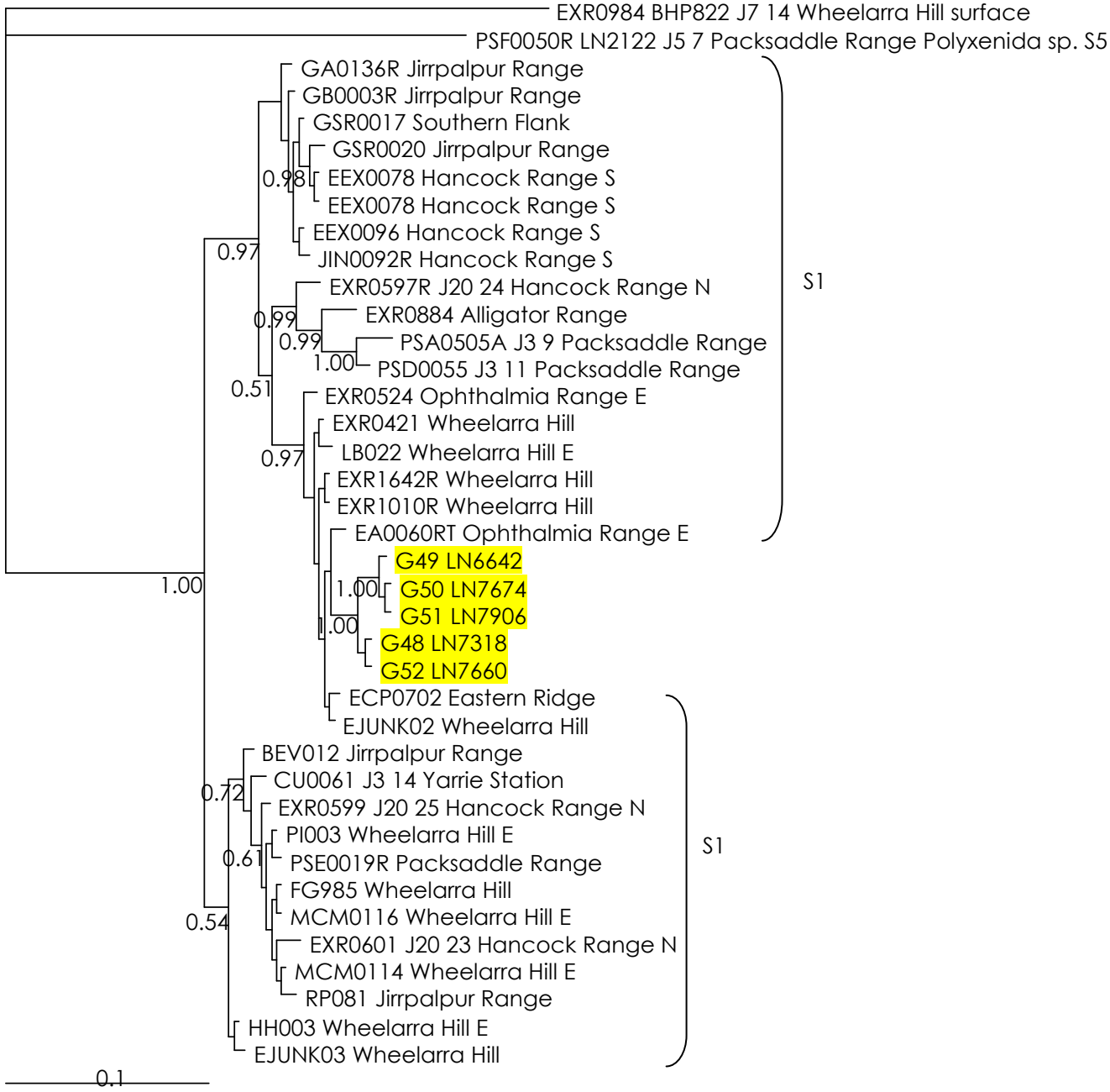
Table 2. Percent sequence divergence (uncorrected p-distances) for the mtDNA 12s gene among five Polyxenida specimens examined in this report.

	LN7318	LN6642	LN7674	LN7906	LN7660
LN7318					
LN6642	0.8				
LN7674	0.8	0.0			
LN7906	0.8	0.0	0.0		
LN7660	0.0	0.8	0.8	0.8	

Table 3. Percent net sequence divergence among morphological groups of Polyxenida as described in the text. Net sequence divergence corrects for variation within groups when calculating between group means.

	S1 (Pilbara)	S5 (Pilbara)	S9 (Yeelirrie)	surface
S1 (Pilbara)	x			
S5 (Pilbara)	22.3	x		
S9 (Yeelirrie)	4.2	26.0	x	
surface	18.8	28.0	22.0	x

Figure 1. Bayesian analysis of 12s haplotypes of Polyxenida millipedes from Yeelirrie and specimens collected in the Pilbara. The new specimens from Yeelirrie are highlighted in yellow. Numbers on nodes correspond to posterior probabilities, and values greater that 0.90 are generally accepted as well-supported. Species S1 and S5 as determined with 12s and 28s data, are labelled and refer to the text and Table 2. A surface species of Polyxenida from Wheelarra Hlll was used as an outgroup.



APPENDIX 10: Dr S. Cooper (SA Museum) DNA Report

Copepoda

Report to Subterranean Ecology for copepod sequencing work December 2009 to December 2010

by Assoc. Prof. Steven Cooper

Principal Scientist and Head of Biological Science
Evolutionary Biology Unit
South Australian Museum

Summary

One hundred and fifty nine copepod specimens were analysed to obtain DNA sequence data from the mitochondrial cytochrome oxidase subunit 1 gene (*COI*) and investigate species diversity in the Yeelirrie calcrete. Of these specimens, 78 copepod *COI* sequences were obtained, with 71 sequences obtained from specimens of the Yeelirrie calcrete. Phylogenetic analyses showed the presence of at least 19 divergent groups or lineages of *COI* sequences among the Yeelirrie specimens. Eighteen of the 19 groups corresponded to distinct morpho-species that had been characterized by T. Karanovic. One additional *COI* lineage most likely represents an additional species of the genus *Schizopera.* Four of the morpho-species (*Pseudectinosoma pentedicos, Schizopera akation, Kinnecaris solitaria and Halicyclops eberhardi*) were also found to have additional highly divergent (p-distance>13.7%, Kimura 2-parameter distances> 15.5%) *COI* lineages. Some of these lineages may represent additional cryptic species, but further investigation is required to rule out that they are geographic variants within species. Phylogenetic analyses also provided evidence that three morpho-species, *K. solitaria, S. uranusi* and *S. leptafurca*, are represented both inside and outside the resource area. One divergent lineage of *H. eberhardi,* which may represent a cryptic species, was found inside the resource area, but currently this lineage has not been detected outside the resource area.

Introduction

The Yilgarn region of Western Australia (~700,000 km^2) contains hundreds of physically isolated calcrete (carbonate) aquifers (reviewed in Humphreys *et al*. 2009 and references therein). Previous studies of these aquifers suggest there is a substantial diversity of macro-invertebrate (dytiscid beetles, amphipods, bathynellids, isopods) stygofauna, with evidence from molecular studies suggesting that species are highly restricted in their distribution to single calcrete bodies (Leys *et al*. 2003; Cooper *et al*. 2002, 2007, 2008; Guzik *et al*. 2008). However, little work has been done on other components of the stygofauna (e.g., copepods) from the Yilgarn calcretes, with the exception of a morphological study of copepods by Karanovic (2004) and a recent molecular study of copepods from a single calcrete by Bradford *et al*. (2009). Both studies suggest that there are likely to be a considerable diversity of copepod species within the calcretes, but whether species have highly restricted or widespread distributions remains to be investigated.

Recently, DNA-based species identification methods, referred to as 'DNA barcoding', have been widely employed to estimate levels of species diversity, with the 5'end of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*COI*) proposed as the 'barcode' for all animal species (Hebert *et al.* 2003). The advantage of the COI gene is that it often shows low levels of genetic variation within species, but high levels of divergence (usually >15% for crustacean species, Lefébure *et al.* 2006) between species. The availability of so-called 'universal' primers developed by Folmer *et al*. (1994) for the PCR-amplification of *COI* also greatly facilitates the use of this marker to investigate species boundaries in animals, and these primers have previously been employed successfully by Bradford *et al*. (2009) to PCR-amplify copepod DNA.

The aim of the project reported here was to use DNA barcoding analyses, based on the mitochondrial *COI* gene, to:

1. Derive a molecular phylogeny of copepod specimens from the Yeelirrie calcrete.
2. Estimate the number of distinct phylogenetic groups or evolutionary lineages that are likely to represent distinct species.
3. Assess whether the phylogenetic groups or evolutionary lineages correspond to distinct morpho-species recently characterised by T. Karanovic.

Methods

Samples

Whole copepod specimens stored in 100% alcohol were provided by Tom Karanovic following field surveys of the Yeelirrie calcrete in 2009 and 2010. A total of 159 copepod specimens were analysed, of which 152 were obtained from boreholes of the Yeelirrie calcrete, six specimens were from the Pilbara region and one was from the Margaret River WA (see Appendix 1). Also included in the phylogenetic analyses were seven *COI* sequences from two undescribed species of the Sturt Meadows calcrete (28˚41'S 120˚ 58'E) in WA (Bradford et al. 2009).

DNA extractions, PCR-amplifications and sequencing

Laboratory molecular analyses were carried out by Ms. Kathleen Saint from the SA Museum (March-May 2010, Nov-Dec 2010) and Ms. Tessa Bradford from the University of Adelaide (December 2009).

DNA was extracted using the GENTRA method (Puregene) according to the manufacturer's protocol for fresh tissues. PCR amplifications of a 623-bp fragment from the mitochondrial *COI* gene were generally carried out with the "universal" primers LCOI490 and HCO2198 (Folmer *et al.* 1994). However, the use of these primers proved problematic in many cases and hence additional 'nested' primers were designed from preliminary copepod *COI* sequence data by Ms. Kathleen Saint and used in combination with the Folmer *et al*. (1994) primers to improve the PCR-amplification efficiency (Table 1). An initial PCR-amplification used the combination LCOI490/HCO2198, then 1 µl of product was used to seed nested PCRs in the following combinations: M1323/ HCO2198 or M1321/M1322 (see Table 1 for codes). PCR-amplifications were carried out in 25 µl volumes containing, 4 mM $MgCl_2$, 0.20 mm dNTPs, 1× PCR buffer (Applied Biosystems), 6 pmol of each primer and 0.5 U of Ampli*Taq* Gold (Applied Biosystems). PCR amplification was performed under the following conditions: 94 °C 9 min, then 34 cycles of 94 °C 45 s; annealing 48 °C 45 s; 72 °C, 60 s; with a final elongation step at 72 °C for 6 min. PCR products were purified using a vacuum plate method and sequencing was undertaken using the ABI prism Big Dye Terminator Cycle sequencing kit (PE Applied Biosystems, Foster City, CA). Sequencing was carried out on an ABI 3700 DNA analyser and sequences were edited and manually aligned in SeqEd version 1.0.3 (Applied Biosystems).

Table 1. Oligonucleotide primers[1] used to PCR-amplify the 5' end of *COI*

Primer code	Primer sequence (5'-3')	Designed by:
LCOI490 (M414)	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. (2004)
HCO2198 (M423)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*. (2004)
M1321	TRRNGAYGAYCARRTTTATAATGT	K. Saint
M1322	TCAAAATARRTGYTGRTAWARHAC	K. Saint
M1323	GAYGAYCARRTTTATAATGT	K. Saint

1. three additional primers were also developed but were unsuccessful in PCR-amplifications of *COI.*

MtDNA analyses

The edited *COI* data were imported into the program MEGA v. 4 (Kumar *et al.* 2008) and aligned using CLUSTAL W. The aligned sequences were also translated to protein sequences using MEGA and the invertebrate mitochondrial genetic code, to check for the presence of nuclear paralogs. Exemplar sequences were also checked by an NCBI BLAST search of GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) available through MEGA in order to confirm that the sequences were copepod in origin and not contaminants (e.g. human *COI* sequence).

Phylogenetic analyses were conducted using a Neighbour Joining (NJ) analysis available in the program MEGA, with HKY85 distances (Hasegawa *et al.* 1985), aiming to cluster similar sequences into taxonomic groups rather than investigate their phylogenetic relationships (Hebert *et al.* 2003). The NJ tree was bootstrapped with 1000 pseudoreplicates, carried out using the same model of evolution as given above.

Average DNA sequence divergences (p-distance) within groups (morpho-taxa) and between groups (p-distance and Kimura 2-parameter (K2P) distance; Kimura 1980) were estimated using the program MEGA. Both distance estimates are usually very similar, with the K2P model correcting for multiple mutations at the same site, and they are both often used in comparisons among taxa.

Results

DNA was extracted from 159 copepod specimens and the *COI* fragment was successfully PCR-amplified and sequenced from 84 of these specimens using a nested combination of primers given in Table 1. BLAST analyses of GenBank revealed that five of the *COI* sequences were highly similar to human *COI,* and a second sequence (10:0436a) matched fish *COI* sequences. These contaminants were excluded from further phylogenetic analyses. The remaining sequences were found to be most closely related to copepod sequences on GenBank. One of the GenBank *COI* sequences (#AF315010.1) from the species *Cletocamptus deitersii* (Harpacticoida, Canthocamptidae) was included in the phylogenetic analyses. All the sequences were translated into protein using MEGA and were shown to have no evidence of stop codons indicative of non-functional copies of *COI*. Sequences were obtained from all the morpho-species characterised by T. Karanovic with the exception of *Dussartcyclops dostoyevskyi*. DNA from this latter species failed to PCR-amplify for all combinations of primers used.

NJ phylogenetic analyses of the *COI* sequence data revealed the presence of at least 19 divergent groups or evolutionary lineages within the Yeelirrie calcrete, each group showing very strong (>98%) bootstrap support (Fig 1). Eighteen of the 19 evolutionary lineages directly corresponded to morpho-species characterised by T. Karanovic. One specimen (seLN7439) preliminarily identified as *Schizopera uranusi*, formed a separate lineage in the NJ tree and is likely to represent another uncharacterised species of *Schizopera*. The NJ tree generally supported the monophyly of genera, with one exception (*Nitokra*), although relationships among the genera were not resolved.

Several species were found to contain multiple divergent lineages, including *Halicyclops eberhardi* (3 lineages >15.2% p-distance, >17.2% K2P distance), *Pseudectinosoma pentedicos* (3 lineages > 16.7% p-distance, >19.1% K2P distance), *Kinnecaris solitaria* (8.1% to 15.9% p-distance among lineages), *S. akation* (10.8% and 13.7% p-distances among lineages) and *Nitokra yeelirrie* (~9.6% p-distance, 10.4% K2P).

Average pairwise distances (p-distance and K2P distances) between morpho-species were found to be very high and generally >15% (Table 2). There were also some very high average divergences among *COI* sequences within several morpho-species, though the majority showed generally low values (Table 2). Four morpho-species were notable for their high average within species divergences: *S. akation* (9.4%), *H. eberhardi* (10.5%), *P. pentedicos* (17.8%) and *K. solitaria* (10.8%).

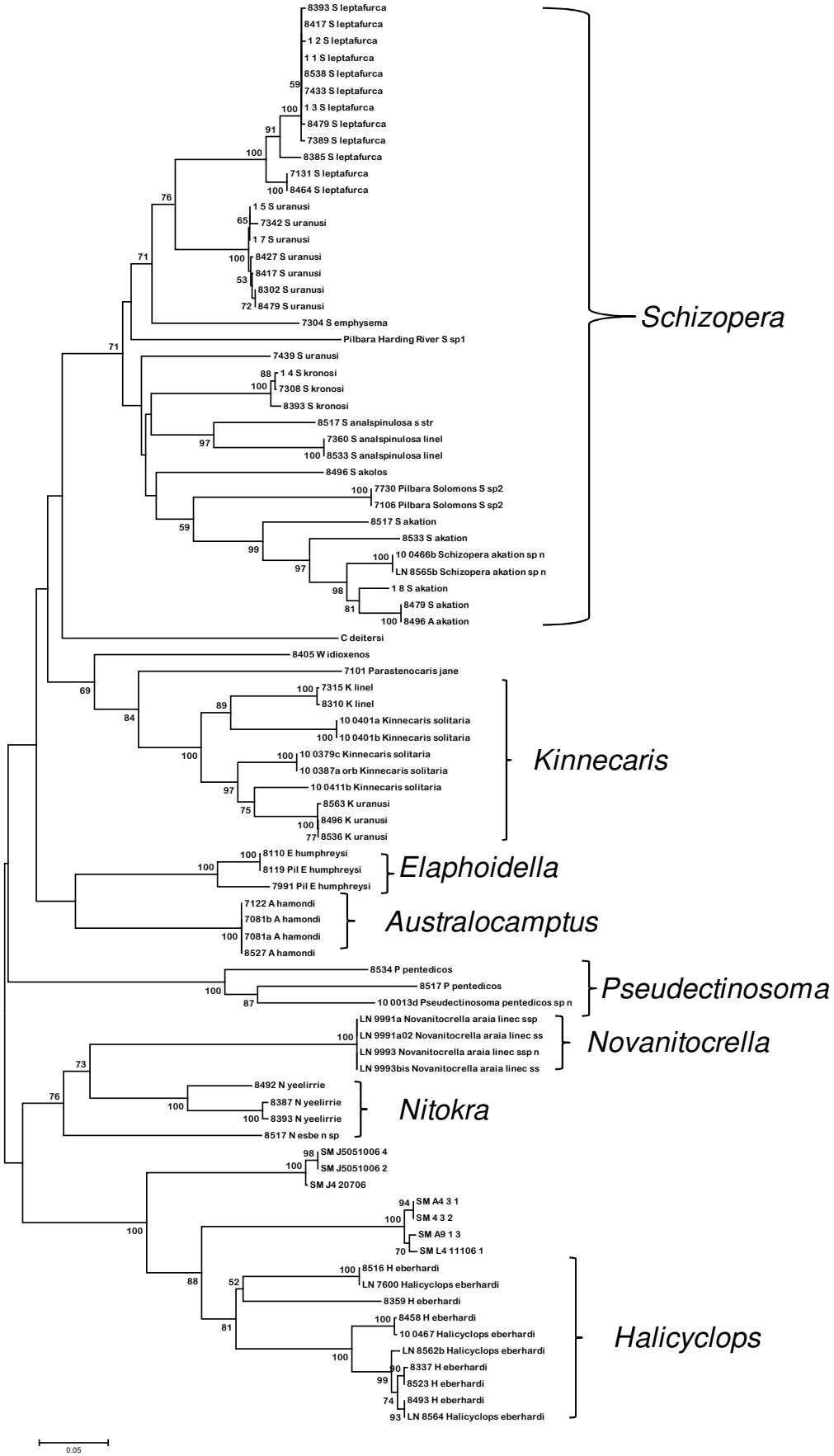


Fig. 1. Neighbour Joining tree based on *COI* distance data (HKY-85 model) from copepod specimens of the Yeelirrie calcrete, Sturt Meadows calcrete (SM numbers) and Pilbara region (Pilbara Harding River S. sp1, Pilbara Solomons S. sp2, *Parastenocaris jane* and *E. humphreysi*). Numbers adjacent to branches are bootstrap proportions from an analysis of 1000 pseudoreplicates.

Table 2. Average pairwise distances (p-distances below the diagonal, K2P distances above the diagonal) among *COI* sequences between each morpho-species and within morpho-species (p-distance-diagonal) calculated using the program MEGA v. 4 (Kumar *et al.* 2008).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. A_hamondi	0	0.234	0.309	0.289	0.267	0.263	0.351	0.241	0.26	0.381	0.3	0.334	0.291	0.284	0.328	0.273	0.282	0.31	0.251	0.303	0.275	0.287	0.319
2. E_humphreysi	0.199	0.042	0.359	0.343	0.34	0.364	0.362	0.294	0.307	0.407	0.379	0.343	0.297	0.326	0.327	0.289	0.307	0.325	0.313	0.302	0.288	0.248	0.316
3. H_eberhardi	0.252	0.284	0.105	0.35	0.356	0.363	0.439	0.353	0.335	0.405	0.403	0.409	0.388	0.399	0.411	0.39	0.423	0.411	0.361	0.401	0.396	0.349	0.386
4. K_linel	0.238	0.273	0.278	0.002	0.151	0.147	0.361	0.343	0.319	0.391	0.239	0.379	0.287	0.338	0.346	0.316	0.342	0.342	0.308	0.331	0.325	0.327	0.272
5. K_solitaria	0.224	0.271	0.282	0.132	0.108	0.139	0.366	0.305	0.298	0.408	0.271	0.347	0.328	0.329	0.35	0.336	0.327	0.357	0.311	0.329	0.311	0.317	0.285
6. K_uranusi	0.221	0.285	0.287	0.13	0.122	0.002	0.359	0.28	0.314	0.393	0.234	0.355	0.329	0.344	0.321	0.335	0.334	0.352	0.3	0.338	0.305	0.324	0.298
7. N_araia_linec_ssp	0.277	0.283	0.329	0.284	0.288	0.283	0	0.32	0.283	0.418	0.349	0.374	0.4	0.412	0.433	0.419	0.399	0.423	0.395	0.395	0.37	0.348	0.379
8. N_esbe_nsp	0.206	0.242	0.281	0.272	0.249	0.233	0.257	n/c[1]	0.255	0.387	0.322	0.348	0.336	0.328	0.342	0.312	0.31	0.32	0.286	0.345	0.334	0.312	0.367
9. N_yeelirrie	0.219	0.251	0.27	0.258	0.244	0.255	0.233	0.213	0.065	0.39	0.339	0.393	0.326	0.353	0.396	0.333	0.376	0.357	0.328	0.348	0.312	0.315	0.325
10. P_pentedicos	0.298	0.313	0.313	0.304	0.314	0.305	0.319	0.302	0.304	0.178	0.422	0.437	0.388	0.392	0.378	0.405	0.399	0.408	0.373	0.409	0.402	0.373	0.392
11. Parastenocaris_jane	0.245	0.294	0.31	0.202	0.223	0.199	0.277	0.26	0.27	0.322	n/c	0.398	0.34	0.348	0.357	0.323	0.312	0.359	0.337	0.345	0.327	0.344	0.281
12. Pil_Harding_R_S_sp1	0.267	0.273	0.313	0.293	0.275	0.279	0.292	0.277	0.303	0.327	0.301	n/c	0.286	0.29	0.273	0.282	0.273	0.254	0.25	0.264	0.218	0.236	0.353
13. Pil_Solomons_S_sp2	0.24	0.243	0.302	0.238	0.264	0.265	0.306	0.27	0.264	0.301	0.27	0.233	0	0.239	0.219	0.265	0.307	0.273	0.239	0.302	0.232	0.22	0.307
14. S_akation	0.236	0.262	0.308	0.271	0.265	0.274	0.315	0.265	0.28	0.304	0.276	0.237	0.203	0.094	0.281	0.274	0.267	0.281	0.233	0.286	0.266	0.267	0.318
15. S_akolos	0.265	0.263	0.316	0.276	0.277	0.26	0.326	0.275	0.307	0.294	0.282	0.225	0.189	0.234	n/c	0.225	0.226	0.281	0.198	0.236	0.231	0.206	0.337
16. S_analspinulosa_linel	0.228	0.239	0.304	0.256	0.269	0.268	0.316	0.255	0.267	0.311	0.26	0.233	0.221	0.228	0.194	0	0.153	0.253	0.192	0.24	0.246	0.192	0.311
17. S_analspinulosa_s_str	0.233	0.249	0.322	0.271	0.262	0.266	0.304	0.252	0.292	0.308	0.252	0.225	0.245	0.223	0.194	0.135	n/c	0.258	0.21	0.239	0.23	0.224	0.284
18. S_emphysema	0.252	0.262	0.316	0.273	0.282	0.279	0.319	0.26	0.282	0.312	0.282	0.211	0.225	0.232	0.23	0.213	0.216	n/c	0.221	0.209	0.184	0.231	0.355
19. S_kronosi	0.212	0.254	0.286	0.252	0.253	0.246	0.304	0.238	0.264	0.293	0.27	0.209	0.203	0.199	0.172	0.168	0.181	0.189	0.008	0.214	0.22	0.19	0.316
20. S_leptafurca	0.248	0.248	0.31	0.265	0.264	0.27	0.304	0.275	0.276	0.313	0.274	0.218	0.244	0.236	0.201	0.203	0.202	0.177	0.183	0.018	0.149	0.213	0.302
21. S_uranusi	0.229	0.238	0.307	0.261	0.253	0.249	0.29	0.268	0.254	0.309	0.262	0.186	0.199	0.222	0.198	0.207	0.195	0.159	0.187	0.131	0.005	0.187	0.314
22. S_uranusi_cryptic	0.238	0.21	0.278	0.263	0.257	0.262	0.277	0.255	0.257	0.292	0.275	0.201	0.189	0.222	0.179	0.167	0.189	0.196	0.166	0.184	0.165	n/c	0.277
23. W_idioxenos	0.257	0.256	0.3	0.227	0.235	0.243	0.294	0.287	0.261	0.304	0.23	0.277	0.25	0.257	0.27	0.252	0.235	0.279	0.256	0.246	0.255	0.23	n/c

1. n/c- not calculated (n=1)

Discussion

The phylogenetic analyses of *COI* sequence data from copepod specimens of the Yeelirrie calcrete, provided support for the existence of at least 18 morpho-species as characterized by T. Karanovic. An additional divergent *COI* sequence (for specimen seLN7439) provided evidence for the existence of a further species that grouped with other *Schizopera* taxa. However, currently there is no voucher specimen associated with this sample to enable morphological re-assessment. Four of the morpho-species, *H. eberhardi, S. akation*, *K. solitaria* and *P. pentedicos* were found to contain multiple highly divergent (p-distances between 13.7 to 17.4%; K2P distances>15.5%) *COI* haplotypes. Such high divergence values are often indicative of distinct species (by comparison with other crustacean species, see Lefébure *et al*. 2006). It is, however, also possible that some of the divergence haplotypes have resulted from long term isolation of populations of the same species within different geographic regions of the Yeelirrie calcrete or by a mechanism known as isolation by distance. Isolation by distance has been found at very small spatial scales in beetles of the Sturt Meadows calcrete (Guzik et al. 2009) and it possibly explains the divergence within *S. akation* as three of the *COI* lineages were found in different regions of the calcrete, separated by large distances (~20 km; T. Karanovic, Pers. Comm.). New sequence data (December 2010), was found to break up some of the long branches, in particular, only 6.4% divergence is now found among haplotypes within the resource area and outside. This level of divergence is indicative of intra-specific levels of variation within copepod species studied here. However, uncertainty remains for *H. eberhardi* and *P. pentedicos*, the latter showing the highest divergence values of 17.4% and the group most likely to comprise two or more distinct species. Further analyses using additional specimens for *COI*, morphological and nuclear DNA sequence data are required to verify this possibility.

One question of interest is whether morpho-species inside the resource area are clearly represented by specimens outside the resource area or whether the latter are divergent lineages that may represent cryptic species. Sequences were obtained from the morpho-species *N. yeelirrie, K. solitaria, S. uranusi, S. leptafurca, S. akation* and *H. eberhardi* from both inside and outside the resource area. For four of these six species, *K. solitaria, S. uranusi*, *S. leptafurca* and *S. akation*, the phylogenetic analyses and genetic distance data support the existence of the morpho-species being represented both inside and outside the resource area. For *N. yeelirrie* the resource area was found to have a divergent lineage (~9.6% p-distance, 10.4% K2P) compared to a lineage outside the resource area and it is not possible to entirely rule out that these lineages represent cryptic species. However, the *COI* data were limited and the alternative hypothesis that the divergences resulted from isolation by distance is also possible (see above). For *H. eberhardi*, one divergent lineage (seLN8359, p-distance>15.2%, K2P>17.2% divergence) found within the resource area, may well represent a cryptic species and the current data are insufficient to verify whether this lineage is found outside the resource area. However, phylogenetic analyses clearly show that a second divergent lineage of *H. eberhardi* is found both inside and outside the resource area.

Overall, there was a relatively poor success rate for the samples analysed, with only ~50% of the samples PCR-amplifying and giving reliable sequence data. This rate was an improvement on earlier work in December 2009, when only 30% of specimens could be amplified. The low success rate has most likely resulted from the very small size of the specimens, and low DNA yield subsequently obtained. However, the finding that human contaminating DNA was PCR-amplified and sequenced for five specimens suggests that the PCR primers did not work optimally for the copepods or that DNA from the copepod specimens was degraded. Following some modifications to the collecting protocol in January and February, the latter is deemed unlikely, as samples were collected in 100% ethanol and kept as cool as possible to avoid DNA degradation. We developed additional primers to PCR-amplify the copepod samples and these improved the sequencing results in April/May and December 2010. However, time did not permit further development of the PCR-methods and we were unable to obtain sequence data from the morpho-species *Dussartcyclops dostoyevskyi*.

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APPENDIX 11: Dr T. Karanovic Taxonomic Report

Copepoda

COMMENTS ON YEELIRRIE COPEPODA - Updated

By

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In Seoul, 24 December 2010

Introduction

In my former role as a Principal Scientist at Subterranean Ecology Pty Ltd, I was entrusted with morphological identification of all Yeelirrie copepods, as well as their preparation for DNA analysis. As I worked on this material after each field trip, discovering the Yeelirrie copepod diversity was a gradual process. This was also a consequence of the increased number of suitable bores over time and their maturity, as well as an increased sampling effort. We know very little about Western Australian stygofauna (Humphreys, 2008), especially its ecology and seasonal dynamics. It was very interesting that I had more vials of copepods from the March 2010 field trip than from all previous field trips combined. Especially poor results we had after the January field trip, which could only be explained with strong seasonality of copepod stygofauna, as the climatic conditions were very similar to those in March. In fact the water level in the aquifer was even slightly lower in March, and there were no significant rain events that could bring surface carbon into the subterranean environment, so we have to presume that even the amount of food was smaller than in January. For a morphological taxonomist these are important issues. Correctly identifying different species is much easier when one is presented with numerous specimens and both sexes of all species, while it becomes much harder when one deals with one or two specimens, and almost impossible when only juveniles are present. Taxonomic task becomes even harder when two closely related species from the same genus are present in one area, and in Yeelirrie we had up to four copepod species from the same genus in a single bore, in addition to three or four other copepod species. Having the help of DNA barcoding for this project, as a test of morphological species hypotheses, was indispensable (Cooper, 2010)

Regional perspective

The Yilgarn region of Western Australia is known for its rich copepod stygofauna, with 24 species reported (most as new) by Karanovic (2004). This fauna proved to be uniquely distinct from the neighbouring Pilbara region (Karanovic, 2006), and the differences were not limited just to copepods. Diving beetles are completely absent from the Pilbara (Leys & Watts, 2008) and the ostracod fauna is also very different (I. Karanovic, 2007). Although one has to assume that the zoogeography of some of the most ancient landscapes on earth would be very complex, what does not stop to amaze are the regional differences in stygofauna assemblages in Australia. The discovery that Australian regions have different relationships to other Gondwanan areas was already anticipated by Weston & Crisp (1994). Giribet & Edgecombe (2006) showed the importance of looking at small-scale patterns when inferring Gondwanan biogeography for terrestrial invertebrates. Recent results of phylogenetic analysis of different copepod groups and genera reinforce this notion (Karanovic & Hancock, 2009; Karanovic, submitted). This was already discussed in Karanovic (2006), where a “pulsating desert hypothesis” was proposed as a novel dynamic model that may explain some of the

differences observed. Other, published (Karanovic, 2008; 2010; Karanovic & Hancock, 2009; Karanovic T., Eberhard S.M., Murdoch A., in press) and unpublished research, done recently on subterranean waters in eastern Australia showed a similar dividing line between the stygofaunas of Queensland and New South Wales, although we are not sure yet where this boarder lies precisely. In short, copepods found in Queensland are more closely related to those from the Western Australian Pilbara region, than to the neighbouring New South Wales. A strong connection between the Pilbara region, tropical Queensland and New Zealand was observed, which may even predate Gondwanan regionality.

Arid Western Australia, and especially the Yilgarn region, is well known for its numerous isolated calcrete aquifers that lie along palaeodrainage channels, and range in diameter from tens of kilometres to hundreds of meters (Humphreys, 2006). Highly porous and carbonate rich sediments here represent an ideal habitat for various groups of stygofauna (aquatic subterranean fauna), including dytiscid beetles, amphipods, isopods, bathynellids, ostracods, and copepods. Previous genetic and morphological studies suggested that individual calcretes are equivalent to closed island habitats, which have been isolated for millions of years (Cooper et al., 2008). Majority of stygobitic species evolved within individual calcretes following independent colonisation by epigean ancestors (Guzik et al., 2008). The diversity of stygofauna is mostly dependent on the size of the calcrete, and typically includes one to three species from each major group, most of them endemic to that site. For example, in Karanovic (2004) five new Schizopera species were described, but each species was restricted to a single calcrete, or a group of neighbouring calcretes, and they were all allopatric (no species overlap). Although sampling effort in Karanovic (2004) was very low in any given calcrete, some recent copepod identifications I did for other consulting agencies in other Yilgarn calcretes were very thorough, and they did not increase the number of species significantly (unpublished data).

Results

This survey of the Yeelirrie area, which contains one of the largest calcretes in the uppermost reaches of its palaeochannel, revealed an unprecedented diversity of copepod crustaceans. Using morphological methods we were able to distinguish 22 different species and subspecies, from six copepod families. Mitochondrial DNA sequence data from cytochrome C oxidase subunit one (CO1) confirmed all these and revealed additional three cryptic species. The harpacticoid genus *Schizopera* is especially rich, containing nine different species in this calcrete (almost twice the number of species previously known from this whole region), and up to four species in a single sampling bore. This is usually associated with a remarkable size differentiation (Fig. 1), comparable only to that previously observed in some dytiscid beetles (Leys & Watts, 2008). However, both morphological and molecular data confirm that other major groups in this calcrete are much less diverse, containing the usual number of one to three species, which would suggest different age and colonization history for different groups. Detailed hydrological and geological data provided to Subterranean Ecology by URS, as well as fine level distribution of different species, revealed that what was initially perceived as a single calcrete is in reality a very complex and patchy habitat. This translates into high speciation potential and is probably responsible for the high copepod diversity, in combination with fresher waters in the upper reaches of this palaeochannel and their role in a long accumulation history. Some copepod lineages here, like the harpacticoid genus *Kinnecaris*, probably postdate the Permo-Carboniferous glaciation (≤300 million years) (Karanovic, 2005). Phylogenies of different groups, reconstructed using DNA sequence data (Cooper, 2010), show that both explosive radiation and multiple invasions are responsible for this diversity (see the discussion section below).

Systematic list of the Yeelirrie copepod morpho-species

Class Copepoda Milne-Edwards, 1840
Order Cyclopoida Rafinesque, 1815
Family Cyclopidae Rafinesque, 1815
Subfamily Halicyclopinae Kiefer, 1927
Genus *Halicyclops* Norman, 1903
1. *Halicyclops eberhardi* De Laurentiis, Pesce & Humphreys, 2001
Subfamily Cyclopinae Rafinesque, 1815
Genus *Dussartcyclops* Karanovic, Eberhard & Murdoch, in press
2. *Dussartcyclops dostoyevskyi* n. sp.
Order Harpacticoida Sars, 1903
Family Ectinosomatidae Sars, 1903
Genus *Pseudectinosoma* Kunz, 1935
3. *Pseudectinosoma pentedicos* n. sp.
Family Canthocamptidae Sars, 1906
Genus *Australocamptus* Karanovic, 2004
4. *Australocamptus hamondi* Karanovic, 2004
Family Ameiridae Monard, 1927
Genus *Nitokra* Boeck, 1865
5. *Nitokra yeelirrie* n.sp.
6. *Nitokra esbe* n. sp.
Genus Novanitocrella Karanovic, 2004
7. *Novanitocrella araia* n. sp.
8. *Novanitocrella araia linec* n. ssp.
Family Paractenocarididae Chappuis, 1940
Genus Kinnecaris Jakobi, 1972
9. *Kinnecaris esbe* n. sp.
10. *Kinnecaris linel* n. sp.
11. *Kinnecaris uranusi* n. sp.
12. *Kinnecaris linea* n.sp.
Genus *Dussartstenocaris* n. gen.
13. *Dussartstenocaris idioxenos* n. sp.
Family Miraciidae Sars, 1906
Genus *Schizopera* Sars, 1905
14. *Schizopera analspinulosa* n. sp.
15. *Schizopera analspinulosa linel* n. ssp.
16. *Schizopera akation* n. sp.
17. *Schizopera akolos* n. sp.
18. *Schizopera emphysema* n. sp.
19. *Schizopera leptafurca* n. sp.
20. *Schizopera kronosi* n. sp.
21. *Schizopera uranusi* n. sp.
22. *Schizopera linen* n. sp. (IDed by Giulia Perina)

Comments on genera and species

Genus *Halicyclops* Norman, 1903

This is mostly marine genus, with over 80 described species living in littoral and interstitial waters all around the world (Boxshall & Halsey, 2004). Australia is the only place where this genus has representatives in inland subterranean waters. There are four species described so

far from the Yilgarn region (Karanovic, 2004) and two from the Pilbara, although the latter belong to a separate subgenus (Karanovic, 2006). In Australian marine interstitial this genus is not so successful as in other parts of the world, with only two species present (Karanovic, 2008). Here it has been replaced with a closely related genus *Neocyclops* Gurney, 1927.

1. *Halicyclops eberhardi* De Laurentiis, Pesce & Humphreys, 2001

This morpho-species is very widely distributed in the Yilgarn region, and is one of the most common copepods in many places. Even though morphological differences between different species in this genus are very minute, and especially those between the four Yilgarn representatives, it always seems that morphological variability between different individuals in one sample is not smaller than that between different populations. Size is the main character that is variable, but in all samples with enough specimens there is a grade of different sizes and never a clear distinction between two size groups. However, size in cyclopoid copepods varies considerably with the way the somites (= body segments) extend or retract within each other, and sometimes it is possible to change their overall length just by transferring them from alcohol to glycerol and back several times. The possibility of cryptic species in this morpho-species indicated by the CO1 data (Cooper, 2010) would need to be verified by other genes and more samples, but if confirmed would represent a real challenge for future species discrimination in this genus. I shall point out that the clade of this morpho-species that is not questionable (consisting of 8337, 8523, 8493, and 8458) is widely distributed in Yeelirrie, being present in the resource area (lines 2 and 3.5), and on the lines F and K. One of the two questionable clades (8516) is present only in the bore SB14, which is off tenement and thus irrelevant for this project (see Cooper, 2010). The other one is from the resource area (8359), from the bore YYAC 33. This can possibly be a contamination (note that we did not get any sequence data from the second cyclopoid species), but a possibility of a cryptic species cannot be excluded.

Genus *Dussartcyclops* Karanovic, Eberhard & Murdoch, in press

Two species originally described in the genus *Goniocyclops* Kiefer, 1955 from the Yilgarn region by Karanovic (2004) were recently transferred into a newly erected genus *Dussartcyclops* by Karanovic et al. (in press). There are two other undescribed but closely related species from this genus, also from the Yilgarn. Thus, this new member from Yeelirrie represents the fifth known species. The genus belongs to a Gondwanan group of genera, all known just from freshwaters and most of them living in subterranean or interstitial environments.

2. *Dussartcyclops dostoyevskyi* n. sp.

This species differs from other members of the genus *Dussartcyclops* by the proportion of caudal rami and their armature elements, as well as a slightly different armature formula of the swimming legs. Unfortunately, we did not get any sequence data from this interesting small cyclopoid, and cannot comment on the haplotype variability. Morphologicly, I could not see any significant difference between different bores and lines. This species is widely distributed in Yeelirrie, being recorded so far from the resource area, as well as from the lines E, H, F, and L.

Genus *Pseudectinosoma* Kunz, 1935

The family Ectinosomatidae presently encompasses about 233 species and subspecies, classified into 20 valid genera (Karanovic and Pesce 2001). Only the genera *Rangabradya* Karanovic and Pesce, 2001 and *Pseudectinosoma* Kunz, 1935 are predominantly freshwater; all others are marine genera, some of which contain a few freshwater species. At present, the genus *Pseudectinosoma* includes six species: *P. galassiae* Karanovic, 2006 from the Pilbara

region in Western Australia; *P. janineae* Galassi, Dole-Olivier and De Laurentiis, 1999 from France; *P. kunzi* Galassi, 1997 from Italy, *P. minor* Kunz, 1935 from Germany; *P. reductum* Galassi and De Laurentiis, 1977 from Italy; and *P. vandeli* (Rouch, 1969) from France (see Karanovic, 2006). Because *P. minor* can be found in brackish waters, it is quite clear that the genus has a marine origin, and the timing could well be as early as the Jurassic and certainly by the Middle Cretaceous (Galassi *et al.* 1999, Karanovic, 2006). At that time the plate that is now Western Australia, formed the eastern shore of the Greater Tethys (Yager and Humphreys 1996). I have discovered two other new species from this genus in Western Australia, but they remain as yet undescribed.

3. *Pseudectinosoma pentedicos* n. sp.

This is the first record of the genus *Pseudectinosoma* in the Yilgarn region. The new species has a more plesiomorphic morphology of the fifth leg than the only described Australian representative (*P. galassiae* Karanovic, 2006), but a more apomorphic armature of the swimming legs. This means that they could have not evolved from each other, but from a different common ancestor, which was morphologically probably most similar to the only species from this genus that can be found in brackish waters: *P. minor*. Thus, the invasion of freshwaters in Australia by this genus can be an independent event from that in the Mediterranean region. DNA sequence data for CO1 gene by Cooper (2010) suggest the presence of at least one cryptic species in this morpho-species complex. This is quite possible. Representatives of this genus are extremely small (see Fig. 1) and have a very conservative external morphology, and given the amount of time for this project I was not able to dissect specimens from each bore and study their appendages in detail. Two genetically distinct species recognised by Cooper (2010) come from the southernmost part of the Yeelirrie group of calcretes, one from the bore L-UNK1 (line L), while the other comes from the bore SB14MT (off tenement). What is their relationship to specimens from further upstream in the palaeochannel (resource area, lines F, H, and A) remains unresolved. To solve this properly we would need DNA data from most lines. Also, a closer morphological re-examination of most of the collected specimens would be needed. Given the information from other copepod species and other stygofauna groups, I would speculate that indeed one species is present in bore SB14MT, one on line L, while all other specimens belong to a third species. A lack of gene flow (or more probably its reduction) between SB14MT and line L is obvious also in *Schizopera analspinulosa* n. sp. and *S. akation* n. sp., while another barrier is present between line L and the rest of the Yeelirrie calcrete, as exemplified by the haplotype variation of four *Kinnecaris* species (see below and Cooper, 2010)

Genus *Australocamptus* Karanovic, 2004

Karanovic (2004) established this genus for three presumably subterranean species from the Yilgarn region, but there are at least seven or eight undescribed surface-water species from the Weatbelt region (collected during a CALM survey and entrusted to me for taxonomic description), one subterranean species from the Kimberley region, and one subterranean species from Pioneer Valley in Queensland (all in preparation). Mysteriously, this widely distributed Australian genus is absent from the Pilbara region, probably displaced by a closely related genus *Elaphoidealla* Chappuis, 1929.

4. *Australocamptus hamondi* Karanovic, 2004

This species is widely distributed in the Yilgarn region (Karanovic, 2004), and I have found it also in many different calcretes after its original publication (unpublished data). In Yeelirrie, the species was only found in the bore No.312 (line E). This bore is actually an old pastoral well, and thus open to elements. This, combined with the relatively wide distribution of the species, makes me to believe that *A. hamondi* is not a proper stygobiont, but rather a

stygophile. Surface water is rather scarce in the Yilgarn region, and I have no information that this species has even been found in surface water habitats. I suspect that it is able to disperse between different calcretes during rare flooding events, and maybe even capable of surviving in resting stages after surface water habitats dry out. These resting stages could then be carried by the wind and can "infect" open bores. Some of the undescribed species from the Weatbelt region come from small pools on top of granite outcrops, which certainly dry out frequently. Very little genetic variability between four different specimens sequenced for CO1 (Cooper, 2010) confirm that this population went through a bottleneck faze, which is characteristic of a new invasion. On the other hand, there is so much morphological variability in this population, that probably species boundaries would have to be redefined for *A. hamondi* and another Yilgarn species (*A. similis* Karanovic, 2004) will have to be synonymized with it.

Genus *Nitokra* Boeck, 1865

This is also a predominantly marine genus, with many species in littoral and interstitial environments around the world (Boxshall & Halsey, 2004). In Australian continental waters only two species have been reported so far: *Nitokra humphreysi* Karanovic & Pesce, 2002 from the Cape Range anchialine waters (Karanovic & Pesce, 2002), and *N. lacustris pacifica* Yeatman, 1983, a stygphile from the Yilgarn region (Karanovic, 2004). The latter taxon has been previously known from crab holes of Western Samoa, Tonga and Fiji (Yeatman, 1983),as well as from two temporary brackish water pools on the Laing Island, Papua New Guinea (Fiers, 1986). Both new species from Yeelirrie are closely related to *N. lacustris pacifica,* and I have fund four other new species (all undescribed) from this complex in the Yilgarn. They all probably originated independently in different calcretes from invading populations of their marine ancestor, which was invading subterranean waters of increased salinity upstream along the palaochannel. Tha fact that *N. esbe* n. sp. has a basal position on the CO1 tree (Cooper, 2010) supports this hypothesis, as this species is found in the bore SM14MT (off tennement). This situation is similar to that found in *Schizopera akation* n. sp. (see below).

5. *Nitokra yeelirrie* n.sp.

This species is widely distributed in Yeelirrie, from line F in the north-west, to line K in the south-east (more than 20 km appart). In the resource area it is very frequent, and was recorded from lines 1, 1.5, 2, 3, and 3.5. DNA sequence data for CO1 confirms that specimens from lines F and 3.5 belong to the same species (Cooper, 2010). Morphological differences between *N. yeelirrie* and *N. esbe* are mostly confined to the armature formula of the swimming legs, as well as the urosome ornamentation patterns.

As for the two potentially cryptic Nitokra species from lines 3.5 and F, that differ by 10 % in the CO1, I think that is still intraspecific variability. Given the clear morphological difference between *Nitokra yelirrie* and *Nitokra esbe*, as well as two cases of significant size differentiation between closely related species from this complex in two areas not far away from Yeelirrie (Lake Way and Regis; work I did for Outback Ecology and Bennelongia), I am not sure that this complex is morphologically conservative at all. Despite the molecular data, I would still consider those specimens from lines 3.5 and F to belong to one species.

6. *Nitokra esbe* n. sp.

This species is found so far only in the bore SB14MT, and ony in the March 2010 lot of samples. This shows how important is multiple sampling for stygofauna surveys, and also suggest a possibility of seasonal dynamics. Further confirmation for this is that *N. esbe* was the second dominant harpacticoid species in the bore SB14MT in March, not a rare element.

Genus *Novanitocrella* Karanovic, 2004

This endemic Yilgarn genus was so far known after a single species, *Novanitocrella aboriginesi* Karanovic, 2004, recorded only from the old Cue water supply bores and Austin Downs Borefiled (Karanovic, 2004). New species found in Yeelirrie fits perfectly into the generic diagnosis, showing that it is relatively closely related to *N. aboriginesi.*

7. *Novanitocrella araia* n. sp.

Only one female specimen of this interesting species was found in the bore YYAC35 (resource area, line 1.5) in January 2010, together with four other copepod species. Our increased sampling effort in March (two samples taken from that bore!) did not produce any more specimens unfortunately, although we found all other species again. I think this is a case of a rare species, with very specific habitat requirements.

8. *Novanitocrella araia linec* n. ssp.

Numerous specimens of this taxon were found in the bore YYAC37C (line C), which is about 10 km down the palaeochannel from the nominotypical population. Morphological differences between the two are mostly notable in the ornamentation of urosome and number of spines on the anal operculum, plus some smaller difference in the size and proportions of some armature elements on the swimming legs. All this would suggest to me a subspecific, rather than a specific status. Unfortunately, we did not get any DNA sequence data from the line C population, while only one specimen was collected from the resource area and dissected for morphological observation. However, DNA data about stygofauna connections between line C and the resource area from other groups show a moderate level of genetic divergence (about 4%), which means that they cannot be considered as distinct species (Hebert et al, 2003). Of course, we cannot apply this knowledge directly to our harpacticoid populations, but it is a god indication in support of the morphological data.

Genus *Kinnecaris* Jakobi, 1972

Parastenocarididae are a unique group of harpacticoids, with no marine connections, found on all continents (except New Zealand), and almost exclusively in interstitial habitats (Karanovic, 2006). In this regard they are only comparable to bathynellids. Both groups are thought to be prime examples of vicariance zoogeography, with almost no surface water representatives and possibilities for a passive dispersal. The genus *Kinnecaris* was recently reinstated and redefined by Schminke (2008), for a group of *Parastenocaris* Kessler, 1913 species, defined as a monophyletic Gondwanan lineage by Karanovic (2005). Consequently, the Australian *Parastenocaris solitaria* Karanovic, 2004 was transferred into this genus. *Kinnecaris* has 18 species so far, but some of them are incompletely described and their generic status is questionable (Ranga Reddy & Schminke, 2009). Note that in my previous report I regarded all Yeelirrie *Kinnecaris* to belong to a widely distributed *Kinnecaris solitaria* (Karanovic, 2004), but continuing work on studying microcharacters (especially with the aid of a Scanning Electron Microcope) and variability among a greater number of specimens, revealed at least four short range endemics here. I submitted all my drawings and most of the SEMs of these new species to Subterranean Ecology, while working in the Zoologisches Museum in Hamburg, as well as Hanyang University in Seoul.

9. *Kinnecaris esbe* n. sp.

This morpho-species is only present in the bore SB14MT (off tenement). It has the longest caudal rami of all known congeners, which are also very divergent. Additionally, it can be distinguished from other three Yeelirrie species by the presence of two parallel lateral rows of spinules on the genital double somite in female, and one ventrolateral row in the next somite.

10. *Kinnecaris linel* n. sp.

Shorter caudal rami and presence of only one lateral row of spinules on the genital double somite in female easily distinguish *K.linel* from *K. esbe.* On the other hand, numerous minute spinules on all urosomite, presence of large spinules on genital and third urosomal somites, and the length of the caudal rami easily distinguish *K. linel* from *K. linea* and *K. solitaria* (Karanovic, 2004). Morphologically, *K. linel* is most similar to *K. uranusi*, but the latter has no large spinules on the genital double somite in female and also has a much narrow apical part of the third leg apophysis in male. These two closely related species were found to diverge by 14.7% in CO1 gene, which is right on the border of what is normally considered as two distinct species. The second one (*K. uranuis*) comes from the resource area (line 1), as well as lines F and H, while the first one (*K. linel*) comes from line L (i.e. some 30 km to the south-east). This is another discontinuity between line L and the northern part of Yeelirrie, already recognized for many other stygofauna taxa. In my previous report I suspected that these may be belong to the same species, but careful study of microcharacters of numerous animals revealed that this is not the case. While some characters show a gradual transformation from north-west to south-east (caudal rami length and intensity of ornamentation for example), others show clear discontinuities (shape of the male third leg apohysis for example). Also, a careful examination of another congener from the Lake Way area (not far away from Yeelirrie, but in a parallel palaeochannel; material collected by Outback Ecology) helped to put all these characters into a broader perspective and to recognize short range endemism in the *Kinnecaris solitaria* complex.

11. *Kinnecaris uranusi* n. sp.

This is the most widely distributed *Kinnecaris* species in Yeelirrie, being present on the following lines: K, 1, H, and F. Some small morphological differences between the specimens found on line K and on other lines were initially thought to represent species or subspecies characters, but after examining more material from the resource area and from lines H and F, it became clear that this is just intraspecific variability. Major differences between *K. uranusi* and the most closely congener (*K. linel*) are outlined above. It differs from *K. esbe* by shorter caudal rami and absence of any large spinules on the genital double somite in female. On the other hand, it differs from *K. linea* by my much longer caudal rami and presence of large spinules on the third urosomal somite in female, as well as many minute spinules on other urosomal somites. Ornamentation of urosomal somites of *K. uranusi* is in fact very similar to that found in *K. solitaria* (Karanovic, 2004), but the two species differ by the size, shape, and armature of the caudal rami (much shorter in the latter species, and with only two lateral setae). Unfortunately males of *K. solitaria* are still unknown, so the male characters cannot be compared. However, this species was described from Depot Springs Station, which is some 60 km due south from Yeelirrie (see Karanovic, 2004: p. 9), and is probably only remotely related to any of the Yeelirrie members (which in turn seem to represent a closely related species flock). For example, even though *K. solitaria* has shorter caudal rami than *K. uranusi*, the principal apical setae are much longer, showing that we are not observing related characters and a simple reduction in size of a certain appendage, but a different evolutionary path from a common (and probably relatively distant) ancestor.

12. *Kinnecaris linea* n. sp.

Unlike any other Australian congener, this species has a completely smooth cuticulum on all somites. This means no minute spinules on any of the somites and no large spinules on the genital double somite or third urosomal somite in female. Caudal rami are also significantly shorter than in any other Yeelirrie species, as are the ensory aestsacs on the antennula. I think these short rami are a plesiomorphic character in Australian *Kinnecaris*, as they can be found in *K. solitaria* (Karanovic, 2004), as well as in as yet undescribed new species from Lake Way (not far away from Yeelirrie, but in a parallel palaeochannel). Both species differ from *K*

linea by somite ornamentation, and even by the exact shape of the rami, which are almost perfectly cylindrical in *K. sollitaria*, more inflated in lateral view in *K. linea*, and with dorsal posterior corner characteristically produced in lateral view in the new species from lake Way. The latter species additionally differs from both by two rows of large spinules on the third urosomal somite, as well as on the fourth urosomal somite. The latter feature is not preset in any of the Yeelirrie congeners.

Genus *Dussartstenocaris* n. gen.

This is a third parastenocarid genus in Australia, and the first endemic one. In the previous report it was listed as *Parastenocaris* only provisionally. In the meantime, I had the opportunity to make line drawings and a lot of SEM photos, and to study these specimens in detail. Initial assumption that this new genus was possibly most closely related to some endemic South American genera, also proved to be wrong. The genus has no close relatives among the living parastenocarids, of which more than 250 species have been described so far (Schminke, 2010). Below (Table 1) is a list of the most important morphological characters that distinguish the three Australian genera:

Table 1. Major morphological diffences between three Australian parastenocarid ganera. Cuticular windows are those on abdominal somites. Note that several as yet undescribed species of both *Kinnecaris* Jakobi, 1972 and *Parastenocris* Kessler, 1913 were consulted. Autapomorphic features of *Dussartstenocaris* n. gen. are given in bold.

CHARACTERS \ GENERA	***Dussartstenocaris***	***Kinnecaris***	***Parastenocaris***
Lateral cuticular windows	absent	present	absent
Dorsal cuticular windows	absent	absent	present
Lateral caudal setae	2	3	2
Exopodal setae on leg 5	2	3	2
Inner bulb on male leg 3	**present**	absent	absent
Exopodal spine on male leg 3	**complex**	simple	simple
Basal processes on male leg 4	absent	absent	present
Spinules on inner margin of leg 5	**present**	absent	absent
Spiniform distal process of male leg 5	**small**	large	absent
Spiniform distal process of female leg 5	**small**	large	large
Endopod of female leg 3	**linguiform**	spiniform	spiniform
Long setules on male endopod of leg 4	absent	absent	present

13. *Dussartstenocaris idioxenos* n. sp.

From the preliminary morphological examination, it is clear that this species is not closely related to *Kinnecaris*, or indeed to any other Australian parastenocarid. This is also confirmed by the DNA sequence data (Cooper, 2010), which shows that *D. idioxenos* is less closely related to two endemic Yeelirrie *Kinnecaris* species than to a representative from the Pilbara region (*P. jane* Karanovic, 2006), which belongs to a completely different genus (Schminke, 2008). This all suggest that *D. idioxenos* represent a different phyletic line and probably a new genus. As mentioned above, preliminary morphological data that suggested a relatively close relationship with some endemic South American genera were wrong. The fact that *D. idioxenos* and a species of *Kinnecaris* can be found sympatrically (together in a single bore), tells us that they are also not in a strong ecological competition. This is the first time in Australia that we have more than one parastenocarid in one sample, which is quite common in many other parts of the world. The inclusion of the Pilbara species in the phylogenetic analysis was very important in order to disclose a very remote relationship between the two lineages of Yeelirrie parastenocarids.

Genus *Schizopera* Sars, 1905

The genus *Schizopera* presently encompasses about 75 species and subspecies, mostly inhabiting marine littoral, estuaries, coastal lagoons and interstitial waters of beaches around the world. In Australia there are five species described from the Yilgarn region (Karanovic, 2004) and two from the Pilbara region (Karanovic, 2006). All of them are relatively closely related, probably originating from an ancient common ancestor. Another large, but morphologically distinct, freshwater flock is known from the Lake Tanganyika, although this flock will probably be separated into a new genus in the future (Karanovic 2004). Looking for an outgroup for the numerous *Schizopera* species from Yeelirrie, we decided to sequence several apparently not very closely related populations from the Pilbara region, from Harding River and Solomons. These were provisionally identified as *Schizopera cf. roberiverensis*, but proved to be two distinct species (Cooper, 2010). The inclusion of „outgroups" from the Pilbara region exposed one of the Yeelirrie species (*S. akation*) as completely unrelated to other congeners from this area, and gave our CO1 analysis a deeper phylogenetic meaning. These were all important steps in solving properly this taxonomic case, as this was the first time ever we had more than one *Schizopera* species per calcrete. As mentioned above, here sometimes we have four species living in the same bore, and very often three (see Fig. 1). All Yeelirrie species, except *S. akation*, represent a monopyletic line, which means that they most probably originated from a common ancestor and most probably also in the very same place where they live today (or close to it). This would imply either sympatric speciation, or a complexity of what we originally percived as a single calcrete comparable to that of a island archipelago (which would allow for allopatric speciation and subsequent sympatric occurence). Whatever modes of evoulution were responsible for the diversity of *Schizopera* species in Yeelirrie, there is no doubt about their separate specific statuses. Both DNA and morphological differences are indicative of well defined species, and they are also consistent. In addition to different morphological features of each species, there is a strong size diffentiation, especially between species that live sympatrically (Fig. 1). This is also an indication of their close relationship, but distinct specific statuses. One of the most obvious morphological differences between these species is in the caudal rami shape and size, and this is not by accident. During the copulation process, males first grab female caudal rami with their first antennae, which makes them the most important body part for species recognition. This is very important in subterraenan habitats (although not limited to them), and in situations where many closely related species live in the same place and chemical signals perhaps did not evolve in parallel to reproductive isolation (most of this is general knowledge, so I am not giving any references).

14. *Schizopera analspinulosa* n. sp.

This is a large species, with rectangular caudal rami (about 1.6 times as long as wide), and two characteristicly enlarged spinules at dorsolateral corners of the anal somite. It was only found on line L and in the bore SB14MT (off tenement), which is some 20 km south-east from the resource area. In both locations it lives simpatrically with the much smaller *S. akation*. It is morphologically most similar to *S. kronosi*, which lives in the resource area, and DNA data confirm their closest phylogenetic relationship (Cooper, 2010).

15. *Schizopera analspinulosa linel* n. ssp.

The CO1 sequence data suggest that populations on line L and in the bore SB14MT represent two distinct haplotypes, with 15.5% divergence (again right on the border of what we normally consider different species), but we have to keep in mind that no populations were collected in between as no suitable bores were available for sampling. I speculate that those, if found, would slowly fill the gap between the two currently disjunct populations. Minute morphological differences in ornamentation of different appendages would suggest a separate

subspecific status. One of the easiest ways to distinguish *S. analspinulosa linel* from the nominotypical subspecies is by the number of groups of large spinules on the first leg basis (two and three respectively).

16. *Schizopera akation* n. sp.

Much smaller in size than the previous species, with shorter caudal rami (1.2x L/W), and absence of enlarged spinules on anal somite. Additionally, *S. akation* differs from all other Yeelirrie congeners by the 2-segmented endopod of the first swimming leg (3-segmented in other species). This morpho-species is most widely distributed of all *Schizopera* represenatives, but CO1 data show a strong genetic divergence between some of these populations (Cooper, 2010). Populations from the bore SB14MT (off tenement, 8517) appear to be quite distinct from those from line L (8533), while those from the resource area represent a third group (1_8, 8479, and 8496). What is interesting is that this the first population is the most plesiomorphic and appears most further down the palaeochannel, while the last one is also terminal and lives most upstream in the palaeochannel. This probably reflects its colonisation path, from a marine ancestor that was invading freshwaters dispersing slowly and actively upstream. Thus, I believe, these divergences have resulted from long term isolation of populations of the same species within different geographic regions of the Yeelirrie calcrete. Intensive sampling between these three areas may reveal intermediate populations, with smaller genetic divergences, which would mean that some limited gene flow is still possible. I could not find any morphological differences between these three populations, and if they are reproductively isolated they would be proper cryptic species. It is interesting that *S. akation* is more closely related to a species from the Pilbara region than to any of the Yeelirrie congeners. This implies their not very close phylogenetic relationship, and suggests that they colonized this calcrete independently.

17. *Schizopera akolos* n. sp.

This is the smallest of all Yeelirrie congeners (Fig. 1), with the shortest caudal rami. However, unlike in *S. akation*, its endopod of the first leg is 3-segmented, which shows that size reduction is not necessarily related to swimming legs oligomerization. This species differs from all others by its 2-segmented endopod of the fourth leg, as well as a somewhat different armature formula of the fifth leg and some swimming legs. The species was collected so far only twice (1 September 2009 and 15 March 2010) from the same bore (YYD22) in the resource area, on line 1.

18. *Schizopera emphysema* n. sp.

This is another rare species, being collected so far three times (27 August 2009, 16 March 2010, and 21 March 2010), but only from the bore YYAC1004C in the resource area, on line 2. However this is a large species, with extremely elongated and inflated caudal rami. It lives sympatrically in that bore with relatively closely related *S. leptafurca*, and not so closely related *S. akation*.

19. Schizopera leptafurca n. sp.

A very common species in the resource area, and also found on line K (to the south-east). Specimens from line K also have the most basal position on the phylogenetic tree (Cooper, 2010), so it is quite possible that the species originated here and later on invaded northern parts of the calcrete (up to line 1). It can be distinguished from all Yeelirrie members by its characteristically constricted caudal rami (Fig. 1). This feature has never been observed before in the genus *Schizopera*. It is most closely related to *S. uranusi.*

20. *Schizopera kronosi* n. sp.

Most closely related to *S. analspinulosa*, which was obvious morphologically, and also confirmed by CO1 sequence data (Cooper, 2010). Its caudal rami are cylindrical and there are also enlarged dorsal spinules on the anal somites, but the setae on the fifth leg and some swimming legs are extremely short and spiniform. It was only found in the resource area and on lines H and K, but is never very frequent. It seems that this is a more primitive and older species, and it has slowly being sidelined by the younger and better adapted *S. leptafurca* and *S. uranusi.*

21. *Schizopera uranusi* n. sp.

Its caudal rami are cylindrical in the anterior half and conical in the posterior, and it is most closely related to *S. leptafurca*. Males are very hard to distinguish indeed, and those of the latter species possess somewhat concave dorsal margin of the caudal rami. *Schizopera uranusi* is frequent in the resource area, but has also been found on the lines F, H, and O (to the north-west). Interestingly, *S. uranusi* and *S. leptafurca* overlap only in the resource area, and even here when they live together in a single bore it is usually that one is the most dominant and the other represented with only a few specimens. In some bore-lines this transition from top to bottom is quite apparent, suggesting that these two closely related species are trying to avoid competition by specializing for slightly different conditions (salinity?). *Schizopera uranusi* probably originated in the northern part of the calcrete (lines F and H) or even in some smaller calcrete (line O), and is now slowly invading the resource area and starting to overlap with its sister species, *S. leptafurca.* Their current area of overlap probably represents less than 20% of their respective ranges (note that *S. uranusi* is not yet present in the southernmost part of the resource area, on line 5). One of the specimens (7439), preliminary morphologically identified as *S. uranusi*, was revealed to be genetically quite distant from others and probably represents a cryptic species. Unfortunatey, that specimen was the only adult female from that bore and it is now destroyed, so I do not have suitable material to recheck morphological characters. We sampled the same locality twice in March, but unfortunately no copepods were present.

22. *Schizopera linen* n. sp.

I did not have a chance to examine this species myself, but have examined photos that Ms Giulia Perina sent. It was apparently collected in the last round of sampling in Yeelirrie, and on line N. It has a slightly inflated caudal rami shape, but nothing like that in *S. emphysema*. Judging from a few available photos, this new species seems to be most similar (at least morphologically) to an as yet undescribed new species from Lake Way (material collected by Outback Ecology). Both species have a reduced urosomal armature, very slender habitus, and proportion and placement of armature elements on the caudal rami, and are probably closely related. They differ from *S. emphysema* buy the absence of characteristic two parallel dorsal rows of large spinules along caudal rami, which are also shorter, less inflated and with different placement of armature elements.

Discussion

The mitochondrial DNA sequence data from cytochrome C oxidase subunit one (CO1) confirmed all morpho-species as monophyletic and genetically distinct, except *Dussartcyclps dostoyevskyi* n. sp. and *Novanitocrella araia* n. sp. for which not enough DNA could be amplified (Cooper, 2010). Additional three cryptic species were revealed within these morpho-species: *Halicyclops eberhardi* De Laurentiis et al., 2001; *Pseudectinosoma pentedicos* n. sp., and *Schizpera uranusi* n. sp. They are discussed in detail above. Reconstructed phylogenies from CO1 sequence data also revealed that both multiple

colonisations and explosive radiations are responsible for the unprecedented copepod diversity in Yeelirrie. I think that this is partly due to the geographical position of this calcrete, in the uppermost reaches its palaeochannel, with more freshwater habitats than in lower parts, and more complex freshwater/saline-water interactions. Another important factor is the physical complexity of this calcrete, which through space and time probably acted more like a subterranean archipelago, rather than an island. Geological and hydrological data will probably explain this in more detail, but I just want to emphasize that current copepod diversity and fine scale distributions are strongly supportive of this view, and probably reflective of a similar complexity well into the past. As mentioned above, previous genetic studies on other groups suggested that individual calcretes are equivalent to closed island habitats, which have been isolated for millions of years (Cooper et al., 2008), and the diversity of stygofauna is mostly dependent on the size of the calcrete. Majority of stygobitic species evolved within individual calcretes following independent colonisation by epigean ancestors (Guzik et al., 2008), and typically in Yilgarn each major group is represented with one to three endemic species.

Copepod DNA and morphological data confirm the presence of at least two major barriers to gene flow in the Yeelirrie calcrete, forming three separate zoogeographical areas. The first barrier lies between the bore SB14MT and line L, which is apparent in the relatively large genetic divergence between populations of *Schizopera annalspinulosa* n. sp., *S. akation* n. sp., and *Pseudectinosoma pentedicos* n.sp., while *Nitokra esbe* n.sp. is an endemic morpho-species for the bore SB14MT and clearly genetically distinct. *Kinnecaris esbe* n. sp. is also endemic to the bore SB14MT. Another major barrier is present somewhere between bore lines L and K, with a lot of genetic divergence i between *Kinnecaris linel* n. sp. and *K. uranusi* n. sp., but also absence of many species south from line K: *Nitokra yeelirrie* n.sp., *Novanitocrella araia* n.sp. (including its subspecies *linec* n. ssp.), *Dussartstenocaris idioxenos* n.sp., *Schizopera uranusi* n.sp., *S. leptafurca* n. sp., *S. emphysema* n. sp., *S. kronosi* n. sp., and *S. akolos* n. sp. Another smaller barrier is probably present somewhere between the resource area and line C, as there are small morphological differences (on subspecific level) between populations of *Novanitocrella araia*, and there is also some smaller genetic divergence between populations of *Halicyclops eberhardi* (sample 8458),and *Schizopera leptafurca* (samples 7131 and 8464).

Thre resource area (lines 1, 1.5, 2, 3, 3.5, and 5) and norhtern lines F and H seem to be a rather well conected habitat, with an unobstructed gene flow. For example, there is hardly any genetic divergence between popuations of *Halicyclops eberhardi* (sample 8493 from line F, 8337 and 8523 from the resource area), *Kinnecaris uranusi* (8536 line F, 8563 line H, 8496 resource), and *Schizopera uranusi* (8427 line F, all others resource area) (see Cooper, 2010). My morphological studies only confirm this, and also suggest that lines O, E, P and A would be part of this unit, although they do not have a very rich stygofauna. For example, in the March survey we discovered *Schizopera uranusi* on line O (sample 8394). *Dussartstenocaris idioxenos* n. sp. was found on lines P (sample 8405), E (8527 & 7122), and A (8505), but not in the resource area, and *Austalocamptus hamondi* Karanovic, 2004 was recorded only from line E. However, I think this is an ecological rather than a zoogeographic division, as both species come from freshwater ancestors and probably prefer habitats with lower salinity. The latter species is widespread (although not very frequent) in the Yilgarn region, and the reason may well be the lack of suitable freshwater habitats.

Genetic divergence between different populations of the widely distributed *Schizopera akation* is very interesting, as its most plesiomorphic population appears most further down the palaeochannel (bore SB14MT), the next one is further upstream (line L), while the most terminal clade lives most upstream in the palaeochannel (resource area). This probably reflects its colonisation path, from a marine ancestor that was invading freshwaters and dispersing slowly and actively upstream. This pattern can be observed in some other taxa of

marine origin (*Nitokra esbe* and *N. yeelirrie* for example, and maybe even *Schizopera analspinulsa* and *S. kronosi*), although here speciation is complete and is well expressed morphologically. This is an interesting case for further studies, especially between Yeelirrie stygofauna and other calcretes further downstream in the palaeochannel, as well as between parallel palaechannels.

Morphological and some molecular evidence would suggest a different colonisation history in the genus *Kinnecaris*, were the most plesiomorphic form (*K. linea*) lives in the uppermost reaches of the palaeochannel and the trend in the caudal rami elongation and denser somite ornamentation is obvious down the channel. This makes perfect sense, as parastenocarids are copepods of freshwater origin, and one can easily imagine dispersal of this group down the paleochannel during periods of increased rainfall, which then evolve into separate species during periods of increased aridity. Two different colonisation paths along this palaeochannel, one upstream for copepods of marine origin and the other downstream for freshwater representatives, may also be a mechanism that is partly responsible for this unusual species richness, as more species create more ecological niches and more opportunities for new colonisations.

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Figure 1. Seven copepod species collected in the bore YYD22 in March 2010 (from left to right): *Kinnecaris uranusi* n. sp., adult female; *Pseudectinosoma pentedicos* n. sp., adult female; *Nitokra yeelirrie* n. sp., adult female with an egg-sack; *Schizopera akolos* n. sp., adult female; *Schizopera akation* n. sp., adult female with egg-sacks; *Schizopera leptafurca* n. sp., adult female; *Halicyclops eberhardi* De Laurentiis et al., 2001, adult male. Photo T. Karanovic.