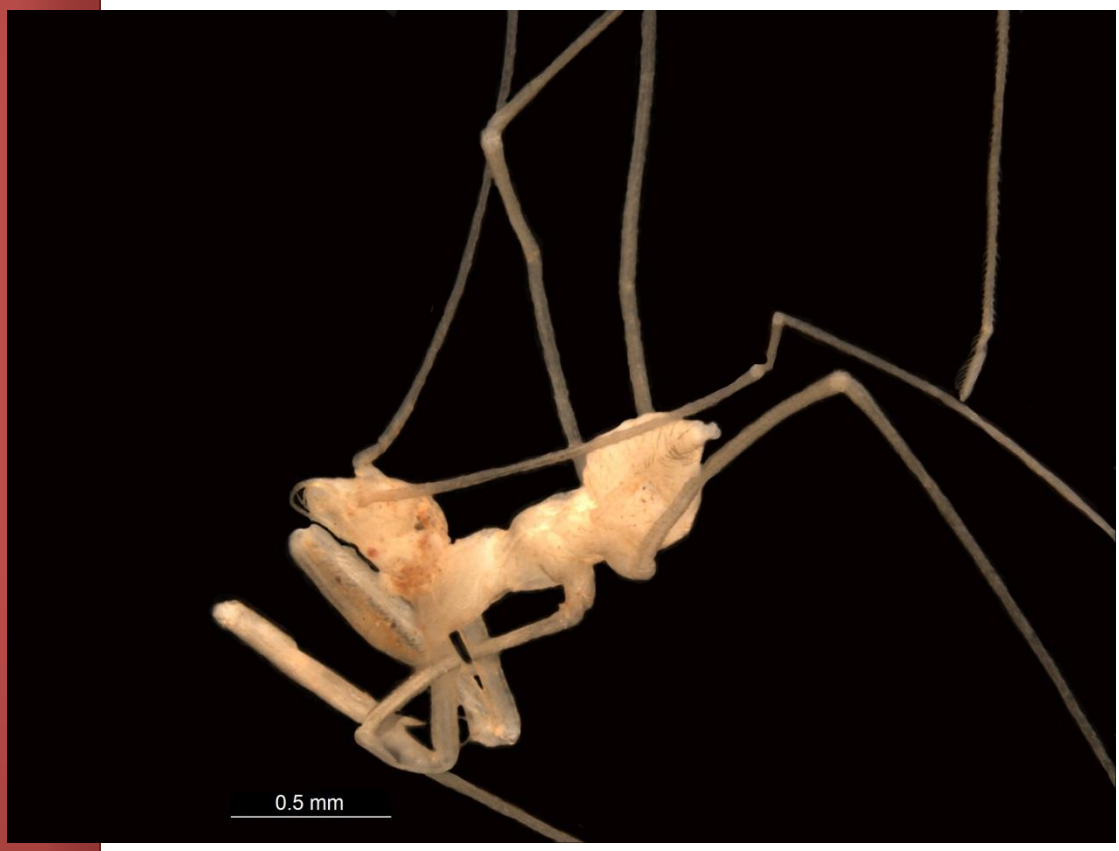
Subterranean Ecology

Scientific Environmental Services

BHP Billiton Iron Ore

Pilbara Regional Subterranean Fauna Survey

Area C North Mining Area - Ministers North



Prepared for BHP Billiton Iron Ore

November 2010

BHPBIO Billiton Iron Ore

Pilbara Regional Subterranean Fauna Survey

Area C North Mining Area - Ministers North

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COVER: Troglobitic assassin bug (Reduviidae: Emesinae) from Ministers North. Photo by Subterranean Ecology.

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.

Version	No.	Prepared by	Checked by	Submission Date
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Draft	2	N. Coen	S. Eberhard	14.12.2010

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EXECUTIVE SUMMARY

This report provides the results of a subterranean fauna survey conducted at BHP Billiton's Area C North Mining Area - Ministers North deposit - located approximately 140 kilometres north-west of Newman in the central Pilbara region of Western Australia. This survey forms part of BHP Billiton's ongoing regional studies of the diverse subterranean fauna of the Pilbara. The study was undertaken during February to May 2009, and February to April 2010.

The survey effort exceeded the EPA Guidance Statement 54A (2007), and comprised 42 samples of stygofauna from 20 drill holes. Troglofauna were surveyed in 50 drill holes, with a total of 128 scrape samples and 95 litter traps spanning four visits (two phases). Genetic analyses and DNA sequencing were used to confirm morpho-species and characterise genetic variation between populations and species at local and sub-regional scales.

The main results of the survey were:

- Stygofauna were not detected in any of the sampled drill holes during this survey..

- A total of 2,325 terrestrial invertebrate specimens were collected, but most of these were clearly of epigean (surface) origin and only 92 specimens were troglofauna.

- The troglofauna comprised a diverse assemblage of at least 24 morpho-species.

- The troglofauna was dominated by large numbers of a few species (particularly the cockroach *Nocticola sp.)*, with many species represented by just one or two specimens, a common pattern of species abundance in Pilbara troglofauna communities.

- One-third of the troglofauna species (8) have also been recorded from the nearby Mining Area C (Packsaddle Range and Jirrpalpar Range), while four of these species have also been recorded more widely in other BHP Pilbara tenements.

- Sixteen (16) new morpho-species were identified, and to-date, these species have only been collected in the Ministers North survey area. Based on other sub-regional patterns (eg. Mining Area C), some of these new species are likely to be short range endemic's (SRE's).

- This survey has confirmed the existence of a rich troglofauna at Ministers North, consistent with the findings from numerous other BHPBIO surveys recently undertaken in the Hamersley Ranges sub-region (including Mining Area C and Newman areas) and the wider Pilbara region (Goldsworthy).

Diverse troglofauna assemblages may be expected to occur in topographically elevated permeable BIF terrains throughout the Pilbara.

- The failure to detect stygofauna, despite a substantial survey effort over four sampling visits, supports the interpretation that stygofauna may be absent, or possibly occur in very low abundance, at Ministers North. This result is consistent with depauperate stygofauna recorded in topographically elevated BIF terrains including Mining Area C (which has a similar geology and is located to the south of the study area). In contrast, Marillana Creek located to the north of the study area contains a diverse and abundant stygofauna assemblage, consistent with its more permeable CID geology and topographically subdued fluvial setting.

- The species accumulation curve and richness estimators (EstimateS; Colwell, 2006) suggest that 63 to 84 % of troglofauna species have been collected and total richness lies between 29 to 37 species. Hence, further sampling is likely to reveal additional species of troglofauna.

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1. INTRODUCTION

1.1 Purpose and Scope

This report presents the results of a survey for troglofauna and stygofauna at Ministers North, a part of BHP Billiton's Area C North Mining Area in the Pilbara region of Western Australia. This survey forms part of BHP Billiton's ongoing regional studies of the diverse subterranean fauna of the Pilbara.

The objectives of this survey were to:

1. Sample terrestrial and aquatic invertebrate fauna in exploration drill holes and water monitoring bores at the Ministers North deposit;

2. Identify troglomorphic and stygomorphic species (fauna that exhibit morphological modifications suited to subterranean life);

3. Assess the distribution and conservation status of identified species, at local and sub-regional scales.

1.2 Existing Knowledge

No previous troglofauna or stygofauna surveys have been undertaken at the Ministers North deposit. However, recently completed troglofauna and stygofauna surveys at nearby Mining Area C include deposits in the Packsaddle Range (Subterranean Ecology 2008a, b, 2009a) and Jirrpalpar Range (Bennelongia 2008a, b, c) found troglofauna to be diverse and abundant. Troglofauna have also recently been sampled at Boundary Ridge (Subterranean Ecology 2009b) and Marillana (Ecologia 2010), although the fauna from these deposits were found at a lower diversity and abundance than those collected in the more northern sections of Mining Area C. The 'Marillana' tenement described is not the same as the BHP Marillana tenement – the former includes only a section of the creek bed southwest of Yandi Mine.

BHP Billiton is undertaking surveys of troglofauna at numerous locations across the Pilbara as part of its Regional Subterranean Fauna Study. The preliminary results of BHPBIO's Regional Troglofauna Sampling Program, combined with the results of other troglofauna surveys in the Pilbara, show that the majority of troglofauna species collected from boreholes at Mining Area C and other deposits near Newman are widespread in suitable geological habitats at local and sub-regional scales, however, a few species have, to date, been collected from only one or a few drill holes and/or appear to have narrowly circumscribed distribution ranges (Bennelongia 2008c, Subterranean Ecology 2008c, 2009a). Determining the distribution ranges of these species remains a subject of ongoing research in BHPBIO's program.

Stygofauna surveys have been completed at Boundary Ridge and Marillana (Ecologia 2009), which are located approximately 60km south-west and 16 km north-east of Ministers North, respectively. These surveys indicated a relatively low species diversity and abundance when compared to other areas within the Pilbara region (Subterranean Ecology 2009c, Ecologia 2009). However, stygofauna monitoring at Yandi, which is situated 11km northeast of Ministers North, supports a diverse and abundant stygofauna assemblage (Subterranean Ecology 2010).

1.3 Survey Area

Ministers North forms part of BHP Billiton's Area C North Mining Area tenements in the Pilbara region, Western Australia. Ministers North is situated approximately 11km southwest of Yandi Mine and 20km north of Mining Area C (Figure 1). Ministers North is a relatively small tenement, 4.5 km long and 2.3 km wide. The tenement comprises part of the Hancock Range in the catchment of both an unnamed creek and Yandicoogina Creek, both tributaries of Marillana Creek. This in turn drains into the Fortescue Marsh. The elevation ranges from 650 to 800 m and comprises two ridges (North Ridge and South Ridge).

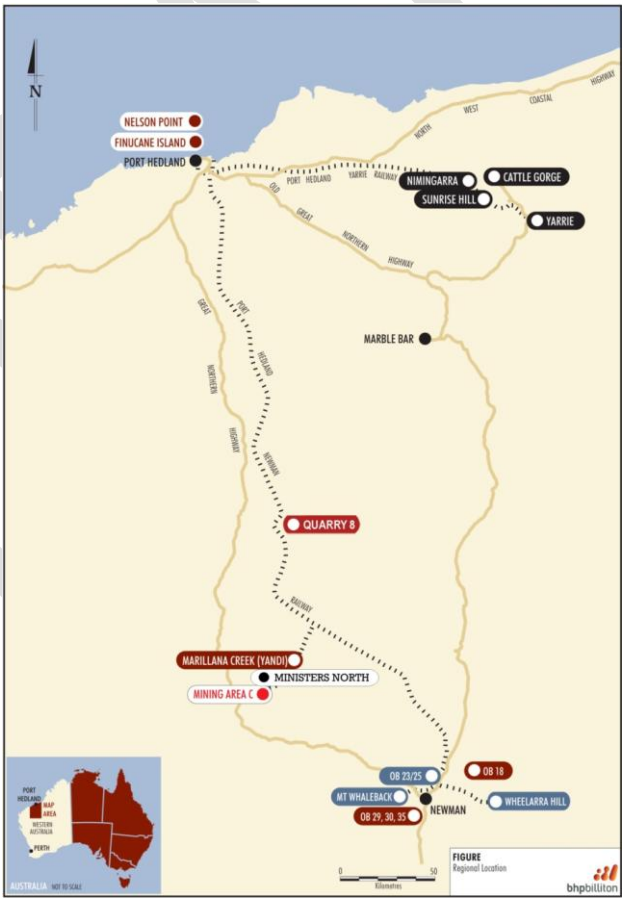

Figure 1: Location of Ministers North in relation to other BHPBIO Pilbara mining operations.

1.4 Geology

The geology and drill hole locations at Ministers North are shown in Figure 2. A description of the geology is reproduced below (summarised from BHPBIO):

Outcrop in the Area C North area is of Hamersley Group sediments from the Weeli Wolli Formation to the Brockman Iron Formation. As a host for iron ore, this has been the most economically important formation in the Province. It consists of an alternating sequence of BIF, shale and chert, and is subdivided into four Members.

1. *The Dales Gorge Member*
2. *The Mt Whaleback Shale Member*
3. *The Joffre Member*
4. *The Yandicoogina Shale Member*

Hardcap occurs on both Joffre and Brockman BIF and is typically 10 to 30m thick. The lower boundary of the hardcap is gradational, often semi-hardcap material is present. Hardcap is typically goethite or vitreous goethite with numerous cavities some filled with clay material.

1.5 Habitat Characterisation

Ministers North is developed in Brockman Iron Formation, the same geological formation as Packsaddle Range and a similar geology (Marra Mamba Iron Formation) to Jirrpalpar Range and Boundary Ridge, and which, excluding the latter, harbour diverse assemblages of troglofauna (Subterranean Ecology 2009b). Based on their similar geology and geographic proximity, it is expected that Ministers North contains similar geological habitat for troglofauna and stygofauna as occurs in the Packsaddle and Jirrpalpar Ranges. The drill holes accessible for sampling troglofauna and stygofauna at Ministers North were all located on elevated parts of the range. The holes were uncased for their length, but had a short collar at the top. The hardcap zone, which is typically 10 to 30m thick, contains numerous cavities as reported above, thus providing prospective habitat for subterranean fauna.

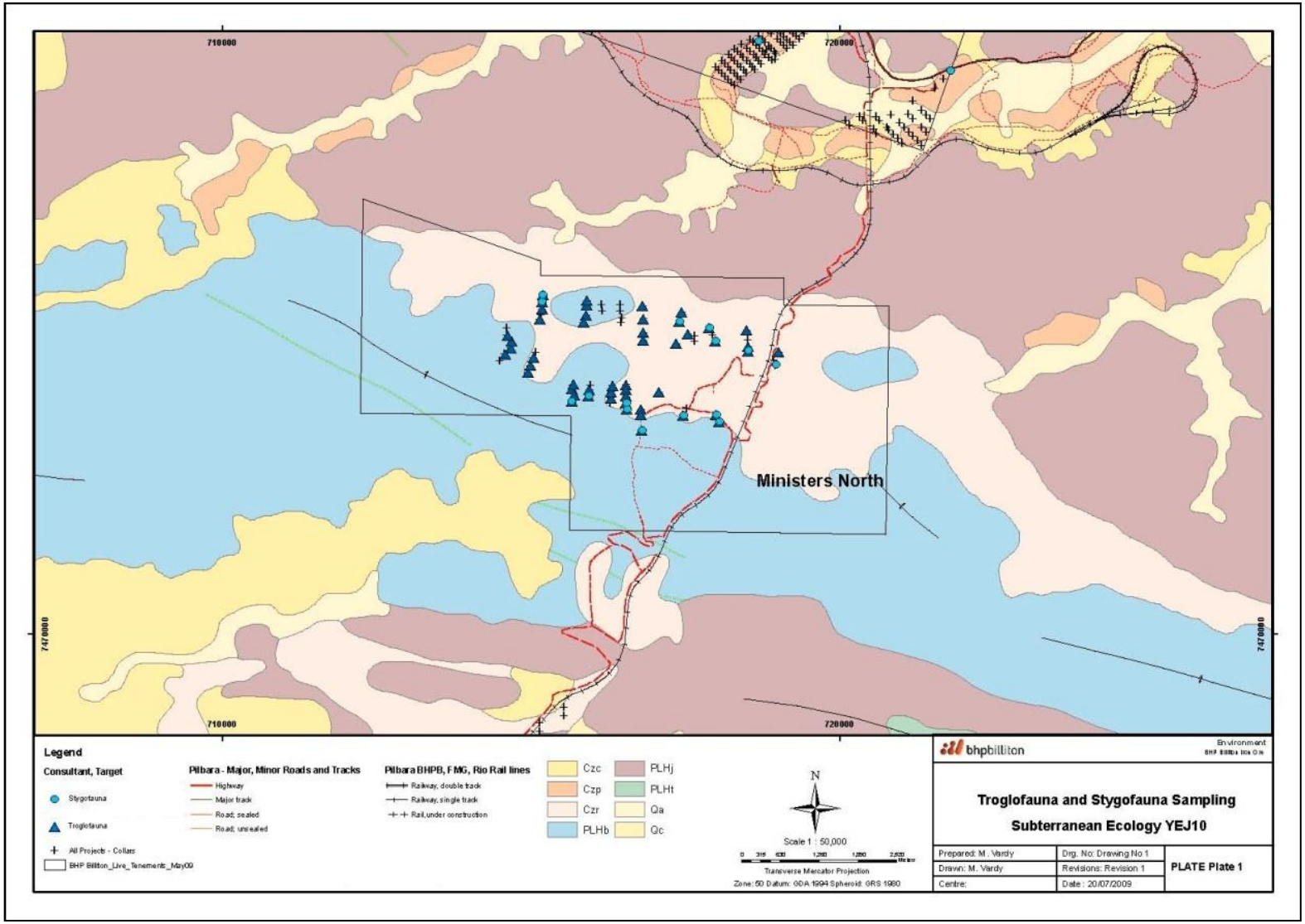

Figure 2: Ministers North geology showing existing drill holes and holes sampled for subterranean fauna

2. METHODS

The sampling methods conform with the Western Australian Environmental Protection Authority (EPA) Guidance Statements for subterranean fauna, No. 54 (EPA 2003) and 54a (EPA 2007), BHPBIO's (2008) Regional Subterranean Fauna Study – Troglofauna Sampling Program Methodology and the Department of Environment and Conservation (DEC) Stygofauna Sampling Protocol (Eberhard *et al.* 2005).

2.1 Field

Troglofauna was investigated using two different sampling techniques – trapping and scraping in accordance with the BHPBIO (2008) Regional Subterranean Fauna Study – Troglofauna Sampling Program Methodology. The first technique employed litter traps that were suspended in bore holes following the procedure adopted by BIOTA (2006). The traps were constructed of 32 or 55 mm diameter PVC pipe cut to a length of 140 mm. The top of the trap consisted of 10 mm aviary mesh to allow invertebrates to enter the trap. The bottom of the trap was PVC capped with a hole drilled in the middle to allow excess moisture to drain. The traps were loosely filled with sterilised organic matter derived from native vegetation in the Pilbara (typically Spinifex, *Acacia* spp. and *Eucalyptus* spp.). Leaf litter was soaked in water prior to being sterilised in a microwave on high power for 10 minutes. The traps were packed with litter and sealed in zip lock bags, to retain moisture and maintain sterile conditions. The litter traps were wetted again prior to installation in boreholes. Traps were left in place for at least six weeks during each sampling phase to allow adequate time for colonisation by fauna according to EPA (2007) guidelines. When traps were recovered they were sealed in zip lock bags for transport to the laboratory, where the fauna were extracted using Tulgren funnels, and preserved in 100% ethanol.

The second technique, scraping, was based on the net haul approach used for stygofauna, and involved a stygofauna 150 μm mesh net of diameter approximating two-thirds that of the drill hole. The net was lowered to the base of the borehole, or one metre below the water table, and hauled up in a manner that maximised contact with the wall surface, with the aim of dislodging and collecting invertebrates. This process was repeated four times per borehole with the aim of sampling each side of the hole.

Stygofauna was sampled by the net haul method. Two, 150 and 50 μm mesh plankton nets of an appropriate diameter (45-300 mm) to match the bore size, with a weighted vial attached, were each separately lowered into the bore and then hauled up through the water column. Each bore was sampled with six hauls (three hauls with each mesh size).

All scrape and stygofauna haul samples were elutriated on site then transferred to a 30mL vial and preserved in 100% ethanol. To minimise the possibility of faunal contamination between sites, nets were treated with 2-5% Decon 90 solution and thoroughly rinsed in water. Field work was conducted by Subterranean Ecology.

To characterise groundwater quality, physicochemical measurements were taken in the field using a 90-FLMV data logger at the same time as stygofauna sampling. A 100mL groundwater sample was collected with a bailer prior to stygofauna sampling and measured for temperature, salinity, electrical conductivity (EC), dissolved oxygen (DO mg/L and DO% saturation), pH and redox (mV).

2.2 Sample Processing and Analysis

All fauna collected were sorted and identified in the laboratory under a dissecting microscope. Each taxon was identified to the lowest taxonomic rank possible using published keys and descriptions, and the numbers of each taxon were recorded. Identification of microfauna and dissected macrofauna used a compound microscope. Potential troglofauna were distinguished by the possession of troglomorphic characters such as de-pigmentation, reduction or loss of eyes, elongation of appendages and sensory structures. New morpho-species (species distinguished on the basis of morphological characteristics) were described and photographed, and retained in a voucher collection for future reference. Identifications were also obtained from a specialist taxonomist, Dr. M. Harvey (Western Australian Museum), for both Araneae and Pseudoscorpionida. Specimens that could not be fully determined by morphology alone were sent to Dr. Terrie Finston of UWA for DNA sequencing and molecular phylogenetic analyses.

2.3 Sample Sites and Survey Effort

Troglofauna sampling was conducted during two phases (four visits - Feb 09 to May 09, Feb 10 to Apr 10), using both litter traps and scraping. Sampling in the Ministers North deposit deployed 91 traps over all phases and an additional 127 scrape samples (Table 1, Appendix 1). In total, there were 46 bore holes that had both scrapes and traps deployed, with scrapes conducted at an additional three holes (Figure 3, Appendix 1).

Sampling for stygofauna was conducted during the same four visits (rounds) as the troglofauna, with a total of 41 net-haul sampling events spread over 16 sites (Table 1, Appendix 1). Thirteen of these sites were in common with the troglofauna sampling, while three holes were sampled for stygofauna only (Figure 3, Appendix 1).

The adequacy of the sampling effort was evaluated by plotting species accumulation curves and estimating the total species richness present using EstimateS (Colwell 2006).

Table 1: Ministers North survey effort.

	Troglofauna traps	Scrapes	Stygofauna net hauls
Visit 1 (Feb-09)		41	15
Visit 2 (May-09)	49	12	11
Visit 3 (Feb-10)		39	8
Visit 4 (Apr-10)	46	36	8
Total number samples	**95**	**128**	**42**

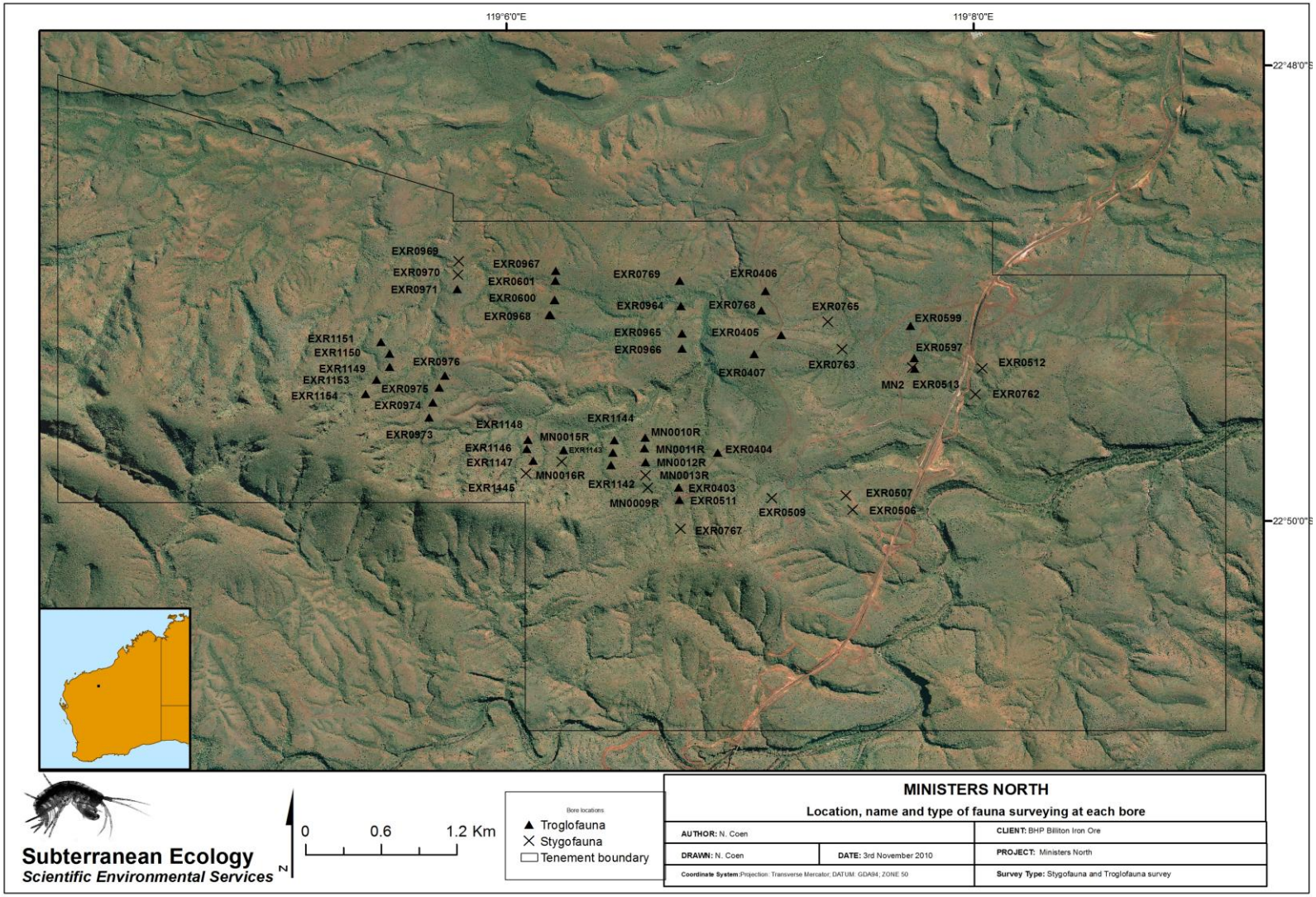

Figure 3: The location and names of the drill holes sampled for troglofauna and stygofauna at Ministers North.

3. Results

3.1 Troglofauna

A total of 2,325 invertebrate specimens were collected by the traps, scrapes and net hauls, but most of these were clearly of epigean (surface) origin and only 92 terrestrial invertebrate specimens were troglomorphic. The troglomorphic specimens were diverse however, with 24 morpho-species identified (Table 2, Appendix 2). The epigean species were dominated by springtails (36.44%), mites (21.25%) and ants (18.38%).

Invertebrate communities exhibit a common pattern consisting of a few, very abundant species, but most species are represented in collections by relatively few individuals. The troglomorphic community collected at Ministers North adhered to this general pattern with the eight most abundant species representing over 76% of individuals, while the ten least abundant species constituted only 10% of total individuals (Figure 4). The most abundant troglomorphic species was *Nocticola* sp.S1, representing 37% of all troglomorphic species collected. Two-thirds (16) of the troglomorphic species were each represented by only one or two specimens.

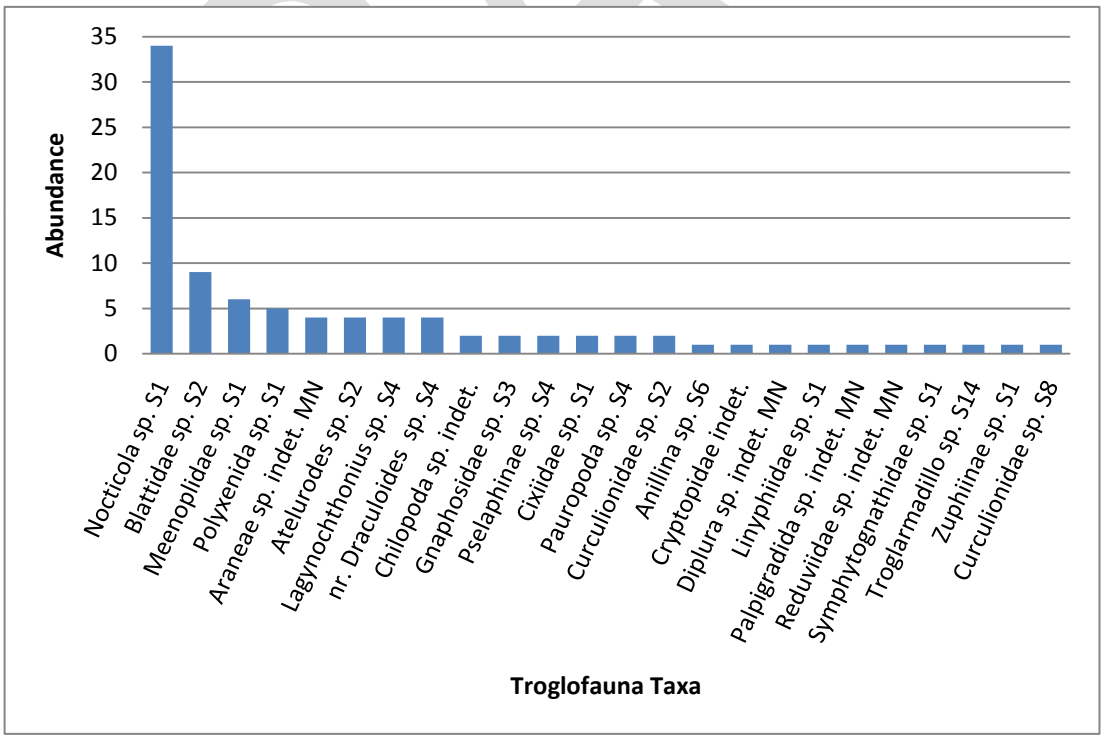

Figure 4: Abundance of troglomorphic taxa in samples from Ministers North, showing a few abundant species and most other species collected in low abundance.

In a regional context, some species were found to be distributed in more than one survey area (Table 3). Of the troglomorphic species recorded from Ministers North, 29% were also collected from Packsaddle deposits P1-3 and Jirrpalpar Range, and 38% were recorded from Packsaddle deposits P4-6. Boundary Ridge, a deposit characterised by low abundance and diversity (likely a sampling artefact related to drill holes located in sub-optimal geological habitat (Subterranean Ecology 2009b), shared only 8% of the troglomorphic taxa with Ministers North.

Table 2: Troglofauna morpho-species including number of individuals, and the number of bores each taxon was recorded from.

Taxon	Number of individuals	Number bore records
Insecta		
Anillina sp_ S6	1	1
Blattidae sp_ S2	9	6
Cixiidae sp_ S1	2	2
Curculionidae sp_ S8	1	1
Curculionidae sp_ S2	2	2
Meenoplidae sp_ S1	6	4
Nocticola sp_ S1	34	16
Pselaphinae sp_ S4	2	1
Reduviidae sp_ indet_ MN	1	1
Zuphiinae sp_ S1	1	1
Arachnida		
Araneae sp_ indet_ MN	4	3
Gnaphosidae sp_ S3	2	2
Lagynochthonius sp_ S4	4	3
Linyphiidae sp_ S1	1	1
nr_ *Draculoides* sp_ S4	4	4
Palpigradida sp_ indet_ MN	1	1
Symphytognathidae sp_ S1	1	1
Entognatha		
Atelurodes sp_ S2	4	3
Diplura sp_ indet_ MN	1	1
Chilopoda		
Chilopoda sp_ indet_	2	2
Cryptopidae indet_	1	1
Pauropoda		
Pauropoda sp_ S4	2	2
Diplopoda		
Polyxenida sp_ S1	5	4
Isopoda		
Troglarmadillo sp_ S14	1	1
24 morpho-species	**92 individuals**	

Table 3: Troglofauna collected at Ministers North, Mining Area C, and other BHP Pilbara tenements. Presence and absence data for other deposits situated in the Pilbara derived from Bennelongia (2008a) and Subterranean Ecology (2008a, b, 2009a).

Taxon	Ministers North (# specimens)	Packsaddle		Jirrpalpar	Boundary Ridge	Mudlark		Other Records	Notes
		P1-3	P4-6			Alligator Jaws	Governor West		
Insecta									
Anillina sp_ S6	1								
Blattidae sp_ S2	9	X	X	X					T. Finston 6-Oct-09 Blattodea
Cixiidae sp_ S1	2								
Curculionidae sp_ S8	1								
Curculionidae sp_ S2	2	X	X	X					
Meenoplidae sp_ S1	6	X	X	X	X		X	Yarri, Jinaryi, Jimblebar, OB18	T. Finston 13-Oct-09 Hemiptera
Nocticola sp_ S1	34	X	X	X			S3	Southern Flank, jinaryi	T. Finston 6-Oct-09 Blattodea
Pselaphinae sp_ S4	2								
Reduviidae sp_ indet_ MN	1								
Zuphiinae sp_ S1	1		X	X					
Arachnida									
Araneae sp_ indet_ MN	4		?						
Gnaphosidae sp_ S3	2								
Lagynochthonius sp_ S4	4								
Linyphiidae sp_ S1	1	X							T. Finston, 5-Oct-09, Araneae
nr_ Draculoides sp_ S4	4	S3	S2, S3	S2					
Palpigradida sp_ indet_ MN	1								
Symphytognathidae sp_ S1	1								

Taxon	Ministers North (# specimens)	Packsaddle P1-3	Packsaddle P4-6	Jirrpalpar	Boundary Ridge	Mudlark Alligator Jaws	Mudlark Governor West	Other Records	Notes
Entognatha									
Atelurodes sp_ S2	4	X	X	X		X		Jinaryi, OB18, 24, Jimblebar, Hashimoto.	T. Finston, 14-Oct-09, Thysanura
Diplura sp_ indet_ MN	1								
Chilopoda									
Chilopoda sp_ indet_MN	2	?	?						
Cryptopidae indet_MN	1								
Pauropoda									
Pauropoda sp_ S4	2		S1	S1					
Diplopoda									
Polyxenida sp_ S1	5	X	X	X	X	X	X	Hashimoto, Jinaryi, OB23, 14, 21, 22, 24, 25, Jimblebar, Goldsworthy, Quarry 8	T. Finston, 28-Oct-09, Diplopoda
Isopoda									
Troglarmadillo sp_ S14	1								

Note: 'S' number in the column equates to a species that was found in the area of survey, however it was a different species than the one collected at Ministers North. '?' equates to a species that may be the same as the one recorded from Ministers North. Those morpho-species that underwent DNA sequencing are highlighted in grey.

3.2 Genetic Diversity and Distribution Patterns

Genetic analyses and DNA sequencing confirmed morpho-species designations and characterised the genetic variation between populations and species at local and sub-regional scales (Appendices 5-12). Six of the 12 morpho-species identified at Ministers North shared identical or similar haplotypes (a combination of DNA sequences that are inherited together) with populations collected from other local or sub-regional sites (Table 3).

Blattodea (cockroaches):

Six specimens of the cockroach *Nocticola* collected from Ministers North were found, from genetic analyses, to belong to the species *Nocticola* S1. This species shows some geographic variation (*i.e.* 0.3 - 8.3% sequence divergence) and has also been collected at Packsaddle, Area C, Southern Flank and Jinaryi (Appendix 5, Figure 5).

Figure 5: Lateral aspect of *Nocticola* sp. S1. Scale bar 0.5mm. Photo by Subterranean Ecology.

The two specimens of Blattidae (a cockroach) belong to the same haplotype (*i.e.* species), Blattidae sp. S2, which was additionally found at Packsaddle deposits (Appendix 5, Figure 6).

Figure 6: Ventral aspect of Blattidae sp. S2. Scale bar 2.0mm. Photo by Subterranean Ecology.

Cixiidae and Meenoplidae (Planthoppers):

Genetic analyses were carried out on two specimens of the planthopper Meenoplidae, which showed that the two specimens belong to the same haplotype, Meenoplidae sp. S1. This species is widespread, and has so far been collected at Packsaddle, Area C, Jinaryi, Boundary Ridge, Jimblebar, Orebody 18 and Yarri (Appendix 6, Figure 7).

Figure 7: Lateral aspect of Meenoplidae sp. S1. Photo by Subterranean Ecology.

One specimen of Cixiidae that was DNA sequenced differed from the two other Cixiidae taxa by 15.6 - 17.9% sequence divergence, indicating that Cixiidae sp. S1 from Ministers North is a distinct species (Appendix 6, Figure 8).

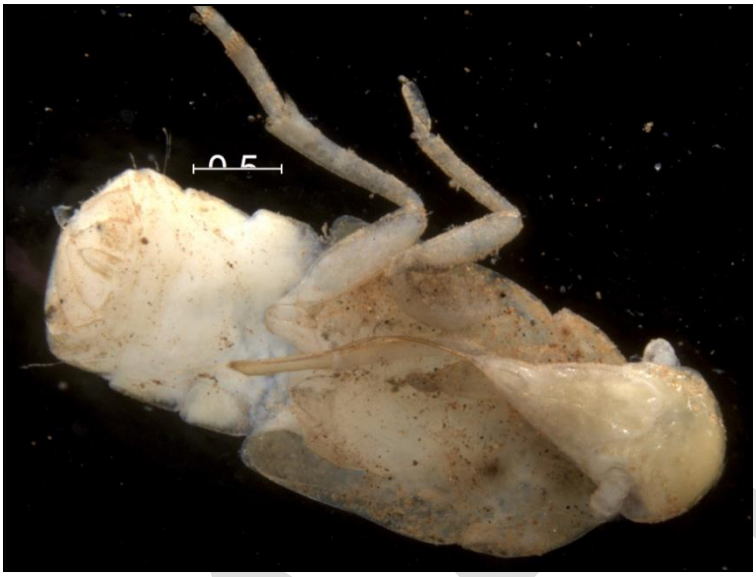

Figure 8: Ventral aspect of Cixiidae sp. S1. Scale bar 0.5mm. Photo by Subterranean Ecology.

Diplopoda (Millipedes):

The pincushion millipede Polyxenida sp. S1 is geographically widespread, as it has been recorded at Ministers North, as well as Packsaddle, Area C and Boundary Ridge and many other Pilbara sites. Genetic analysis concluded that the three specimens of Polyxenid found at Ministers North belonged to the same haplotype, and that this morpho-species contained geographic variants of a single species (Appendix 7, Figure 9).

Figure 9: Dorsal aspect of Polyxenida sp. S1. Scale bar 0.2mm. Photo by Subterranean Ecology.

Thysanura (Silverfish):

Molecular analysis of the mitochondrial 12s region was undertaken on a specimen of *Atelurodes* from Ministers North in order to determine if this represented a previously known species. The Ministers North specimen of *Atelurodes* showed affinities to specimens from Packsaddle and Area C, and is likely to belong to the widespread species *Atelurodes* sp. S2, whose distribution ranges from Packsaddle to Hashimoto. This species showed moderate genetic variation over this range (up to 8.5%) (Appendix 8, Figure 10).

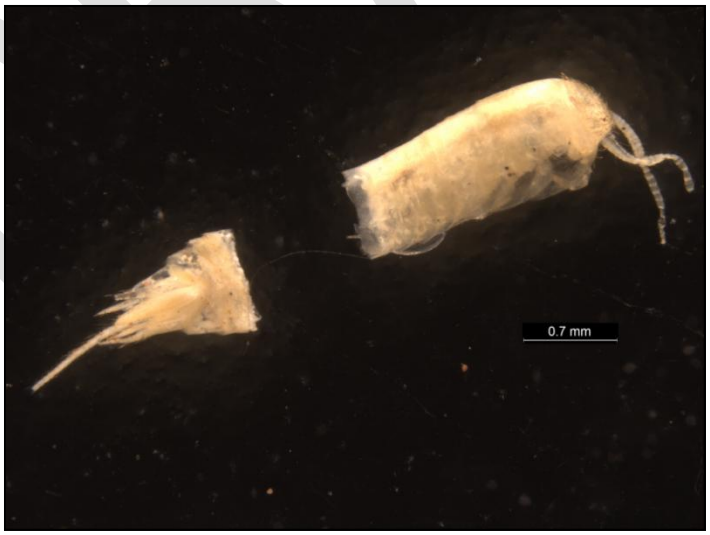

Figure 10: Lateral aspect of *Atelurodes* sp. S2. Photo by Subterranean Ecology.

Schizomida (Schizomids):

Two specimens of schizomid that were recorded from Ministers North were found to belong to the same species, nr. Draculoides sp. S4. Genetic analysis on this species showed a strong relationship with specimens from Packsaddle and Area C. However, the moderately high genetic divergence, at 8.6 - 9.8%, suggests that the Ministers North species is distinct from those recorded at Packsaddle P6 and Area C (Appendix 9, Figure 11).

Figure 11: Lateral aspect of nr. *Draculoides* sp. S4. Scale bar 1 mm. Photo by Subterranean Ecology.

Araneae (Spiders):

Three species of Araneae were identified from four specimens sent for DNA sequencing. It is likely that Linyphiidae sp. S1 from Ministers North is the same as several specimens collected from Packsaddle P2 (Figure 12). However, Gnaphosidae sp. S3 and Symphytognathidae sp. S1 are both distinct species that are only represented, so far, in the deposit of Ministers North. The genetic sequence divergence of each of these species is high, ranging from 11 – 31% (Appendix 10, Figures 13 and 14).

Figure 12: Dorsal aspect of Linyphidae sp. S1. Scale bar 0.5mm. Photo by Subterranean Ecology.

Figure 13: Dorsal aspect of Gnaphosidae sp. S3. Scale bar 0.5mm. Photo by Subterranean Ecology.

Figure 14: Dorsal aspect of Symphytognathidae sp. S1. Scale bar 0.2mm. Photo by Subterranean Ecology.

Coleoptera (Beetles):

One specimen of carabid beetle *Anillina* was recorded in Ministers North and through DNA sequencing of the gene Cytochrome Oxidase Subunit One (COI) the Ministers North species, *Anillina* sp. S6, was found to be a different haplotype to specimens from Packsaddle and Area C, with a sequence divergence of 12.1 – 13.4%. There were also both morphological and genetic differences between haplotypes recorded from Yarrie and Quarry 8. Therefore *Anillina* sp. S6 represents a distinct species in Ministers North (Appendix 11, Figure 15).

Figure 15: Dorsal aspect of *Anillina* sp. S6. Photo by Subterranean Ecology.

Chilopoda (Centipedes):

The centipede specimen collected from Ministers North, Cryptopidae indet. MN, has been shown to be a distinct species. Although this haplotype occurred in an already recognised clade (a group of organisms considered as having evolved from a common ancestor), the high sequence divergence between this haplotype and the others in the clade (at 15.7 – 16.9%), indicates the Ministers North specimen to be distinct (Appendix 12, Figure 16).

Figure 16: Dorsal aspect of Cryptopidae indet. MN. Photo by Subterranean Ecology.

3.3 Survey Completeness

At the Ministers North sites 57% of sampled drill holes yielded troglofauna. The species accumulation curve, which was based on the troglomorphic species collected from traps, scrapes and net hauls, indicated that, although there was a total of 266 troglofauna samples, there was no plateau in the curve (Figure 17) and there was no decline in the rate of new species detected with each additional monitoring round. In fact, the species richness estimators in EstimateS (Colwell 2006) predicted between 63 and 84% of troglofauna species have been collected to date, with a mean of 76% (± 7.4) (Appendices 3 and 4). These estimators suggest the total species richness may be between 29 and 37 species for the Ministers North deposit (Appendix 4).

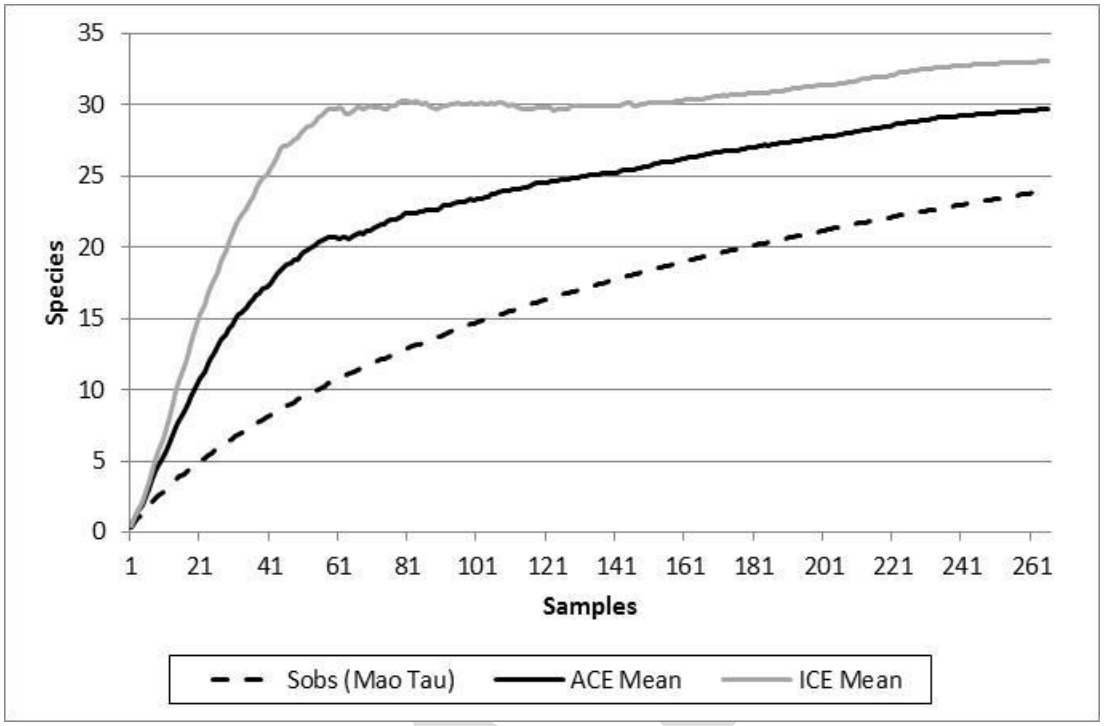

Figure 17: Species accumulation curve (Sobs Mao Tau, EstimateS Colwell 2006) for troglofauna species in Ministers North.

3.4 Stygofauna

No stygofauna were collected at Ministers North during the four rounds of sampling.

3.5 Groundwater Quality

Groundwater quality parameters (conductivity, salinity, dissolved oxygen, temperature and pH) measured across four rounds of stygofauna sampling at the Ministers North sites are shown (Figures 18-21; Table 5).

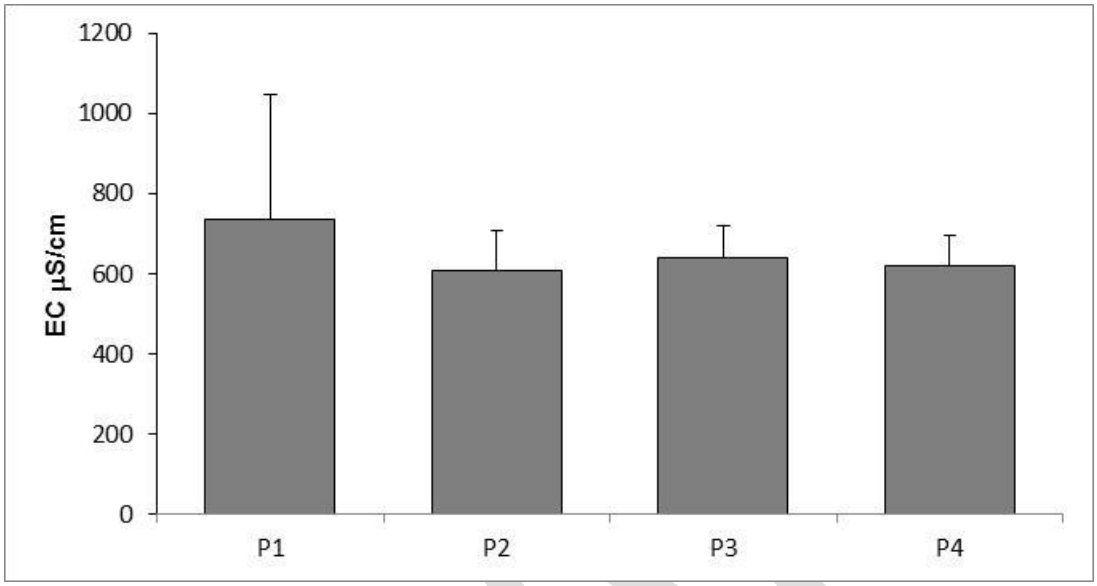

Figure 18: Mean (+ SE) electrical conductivity EC µs/cm at all bores sampled in each of the four phases.

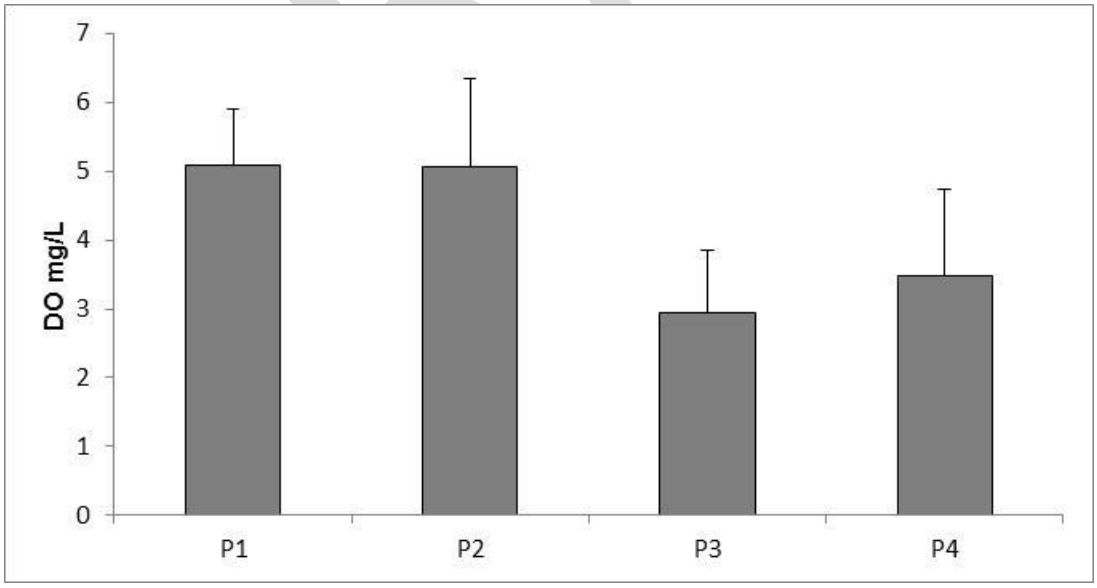

Figure 19: Mean (+SE) dissolved oxygen DO mg/L at all bores sampled in each of the four phases.

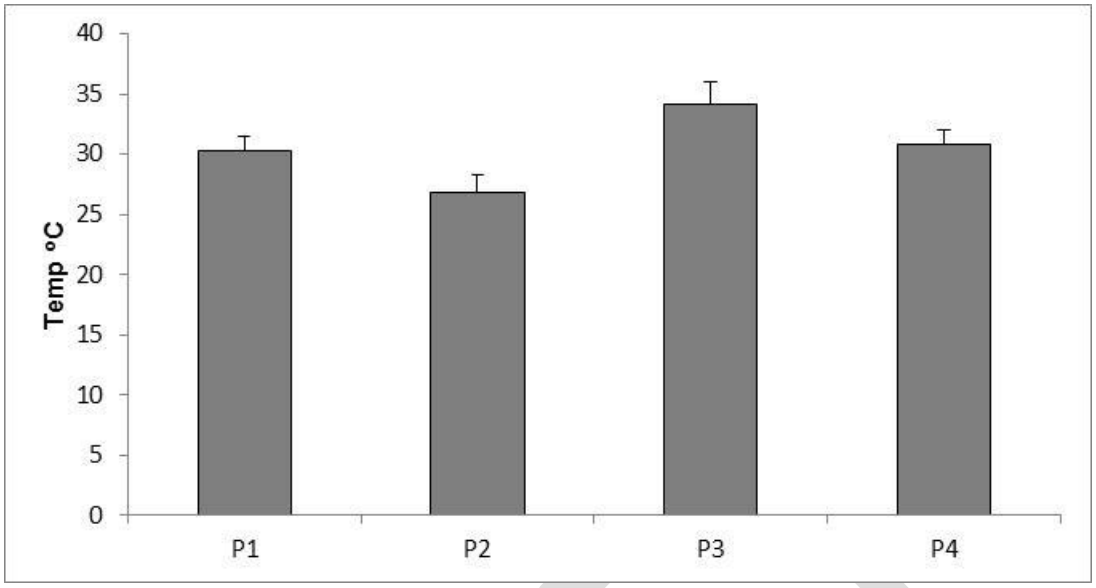

Figure 20: Mean (+SE) temperature ^{o}C at all bores sampled in each of the four phases.

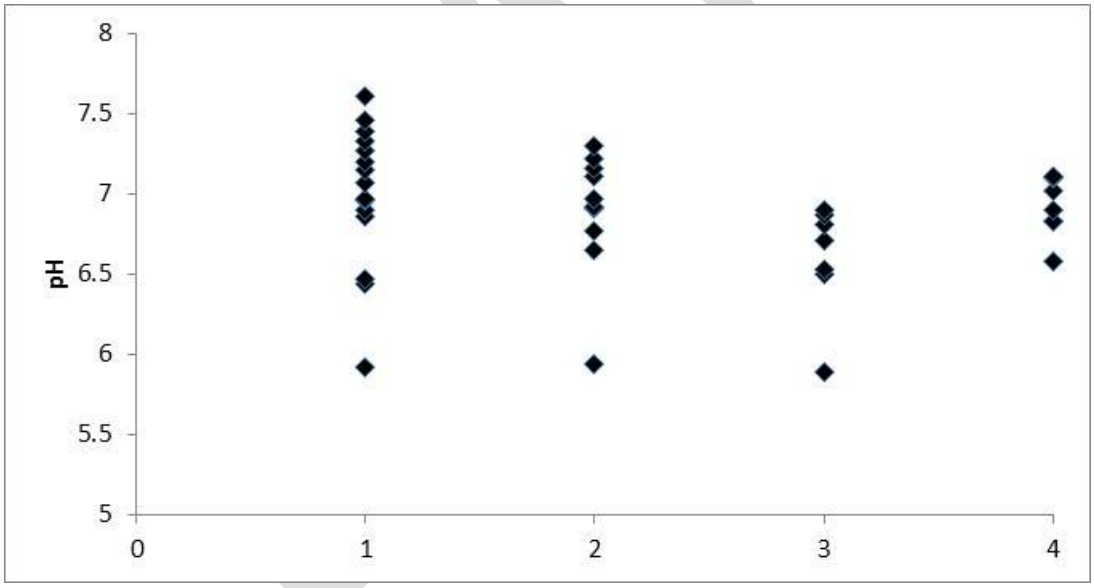

Figure 21: The range of pH measured at each bore in each of the four phases (1-4).

Table 4: Groundwater parameters (Mean ± SE) measured at Ministers North from four phases of sampling.

Groundwater parameters	P1		P2		P3		P4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
EC ms/cm	735.9	310.8	608.2	101.6	641.6	78.9	619.9	75.9
Salinity mg/L	-	-	-	-	339.1	42.8	346.0	44.6
DO mg/L	5.1	0.8	5.1	1.3	2.9	0.9	3.5	1.3
DO % sat	72.9	12.8	63.3	14.9	42.3	13.9	47.1	16.9
Temp ^{o}C	30.3	1.2	26.8	1.4	34.1	2.0	30.8	1.1
pH (range)	5.92 - 7.61	-	5.94 - 7.30	-	5.89 - 6.90	-	6.58 - 7.11	-
Total # of samples	**15**		**10**		**7**		**7**	

Over the four sampling phases, the conductivity/salinity did not vary significantly and the measurements indicated fresh groundwater conditions at the water table (Figure 18). The dissolved oxygen levels did not differ significantly between sampling phases, however the levels were noticeably lower during phase three (Feb 2010) when compared to the other phases (Figure 19). The temperatures of the bores were quite high in all four phases, with phase three showing the greatest recorded temperature (34.09^{o}C) and phase two (May 09) the lowest, at 26.83^{o}C (Figure 20). The ranges of pH were similar for all four phases, with phase one recording the largest pH range, and phase four the smallest (Figure 21; Table 4).

4. DISCUSSION

4.1 Genetic Diversity and Distribution Patterns

Twelve of the 24 morpho-species recorded from Ministers North were subjected to DNA sequencing, which established that six of those 12 species have distribution ranges extending beyond the immediate survey area on a local or sub-regional scale. The genetic studies also provided sufficient evidence that at least four species, *i.e. Nocticola* sp. S1, Meenoplidae sp. S1, Polyxenida sp. S1 and *Atelurodes* sp. S2, are even more widely distributed, and occur in other distant Pilbara regional sites, such as Newman and Yarri.

The DNA analyses revealed geographic variation between populations within a species, in some cases indicating considerable genetic diversity and complexity at local and sub-regional scales (Appendices 5-11). The Ministers North populations of the cockroach *Nocticola* sp. S1, millipede Polyxenida sp. S1 and the thysanuran *Atelurodes* sp. S2 represent locally divergent populations of these species.

It is possible, based upon distribution patterns observed for the majority of sub-regional taxa, that wider distributions of the remaining 18 species collected at Ministers North may be demonstrated with further sampling in other areas, and further taxonomic studies including DNA sequencing, however, some taxa, particularly the arachnids (schizomids, pseudoscorpions, spiders), are likely to exhibit restricted distribution ranges as found in other surveys, for example at Packsaddle Range (Subterranean Ecology 2008a, 2009a).

4.2 Regional Patterns and Comparisons

This survey has confirmed the existence of a rich troglofauna at Ministers North, which is consistent with the findings from numerous other surveys recently undertaken in the Hamersley Ranges sub-region (including Mining Area C and Newman areas) and the wider Pilbara region. Diverse troglofauna assemblages may be anticipated to occur in topographically elevated permeable BIF terrains throughout the Pilbara.

Ministers North recorded a significant troglofauna assemblage comprising at least 24 species. One third of those species were also collected from the nearby Mining Area C, *i.e.* Packsaddle and Jirrpalpar Ranges. The molecular studies (see 3.2 above) clearly demonstrates that some species (e.g. Polyxenida sp. S1, *Nocticola* sp. S1, Meenoplidae sp. S1 and *Atelurodes* sp. S2) are even more widely distributed and occur in other Pilbara survey areas distant from Ministers North. These species do not appear to pose a conservation concern.

Not all species detected at Ministers North are known to occur more widely, with 16 species only collected from Ministers North to date. Thirteen of these species were singletons or doubletons, known only from one or two specimens (Table 3). It is highly

unlikely that the distribution of each of these species is restricted to the immediate vicinity of the bore holes from which they were collected. Instead, the seemingly limited distribution range is more likely a reflection of the difficulty in detecting such species that probably exist at low population density levels. Therefore the range of these uncommonly encountered species is incompletely known and will be difficult to fully determine given their low occurrence in samples. Furthermore, five of these species (*i.e. Anillina* sp. S6, Cixiidae sp. S1, Gnaphosidae sp. S3, Symphytognathidae sp. S1 and Cryptopidae sp. MN) all had molecular work conducted on them, and were all found to be distinct from other areas where these taxa have been collected (Appendices 6, 9-11). Based on other sub-regional patterns (eg. Mining Area C), some of these new species are likely to be short range endemic's (SRE's) (Subterranean Ecology 2008a,b; 2009a,b,c).

Three species so far collected only at Ministers North were not rare in samples. The pseudoscorpion *Lagynochthionus* sp. S4, the spider Araneae sp. MN and the schizomid nr. *Draculoides* sp. S4 were all recorded from multiple bore holes. It is not known how accurately the sampling records reflect the true distribution range for these species. For example, species of *Lagynochthionus* have been collected from Jirrpalpar, Jinaryi, Alligator Jaws and Orebody 24 (Bennelongia, unpub. data). Further taxonomy and molecular work on this genus would highlight if the species are the same or different from the other areas sampled. Additional taxonomy is also required on the unidentified Araneae sp. in order to classify this species to a lower taxon, and thus determine if this species is located in any areas other than Ministers North. Molecular analysis on the schizomid indicated that this species was very similar to those recorded from Packsaddle and Jirrpalpar Ranges, but a greater sequence diversity showed that nr. *Draculoides* sp. S4 was a distinct species found only, so far, at Ministers North.

The failure to detect stygofauna, despite a substantial survey effort over four sampling visits, supports the interpretation that stygofauna may be absent, or possibly occur in very low abundance, at Ministers North. This result is consistent with depauperate stygofauna recorded in topographically elevated BIF terrains including Mining Area C (which has a similar geology and is located to the south of the study area) (Subterranean Ecology 2009c). Groundwater quality at Ministers North was found to be within an optimum range for stygofauna, and there were no extreme physico-chemical conditions recorded that would preclude stygofauna. In contrast to elevated BIF terrains, Marillana Creek located to the north of the study area contains a diverse and abundant stygofauna assemblage, consistent with its more permeable CID geology and topographically subdued fluvial setting (Subterranean Ecology 2010).

4.2 Survey Completeness

The sampling of troglofauna and stygofauna at Ministers North complied with the EPA (2007) guidelines, and the number of samples collected exceeded the minimum recommended for an EIA. The species accumulation curve indicated that there is almost certainly a greater number of species not yet collected. Species richness estimators in EstimateS (Colwell, 2006) provided a range of estimates for total species richness of between 29 and 37 species, in which case sampling to date had collected 84 to 63% of estimated total species richness.

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).

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7. APPENDICES

Appendix 1. Bore names and locations and the sampling methods used during each visit.

The names and locations of the bores sampled at Ministers North during sampling in 2009 and 2010 and the type of sampling undertaken during each visit. Note S = scrape, T = litter trap and N = net haul. Whether any target fauna were recorded is also shown.

Bore name	Latitude	Longitude	V1 Feb 09	V2 May 09	V3 Feb 10	V4 Ap 10	Subterranean Fauna recorded
EXR0403	-22.83089	119.11237	S	T	not accessible		
EXR0404	-22.82831	119.11509	S	T	S	T	
EXR0405	-22.81971	119.11964	S	T	S	S, T	
EXR0406	-22.81650	119.11852	S	S, T	S	S, T	
EXR0407	-22.82111	119.11771	S	T	S	S, T	
EXR0506	-22.83252	119.12473	N	N, T	S	S, T	
EXR0507	-22.83146	119.12423	N	N, T	N	N, T	
EXR0509	-22.83165	119.11897	N	N, T	S	S, T	X
EXR0511	-22.83164	119.11234	S		S	S, T	
EXR0512	-22.82220	119.13399	S	S, T	N	S, T	X
EXR0513	-22.82218	119.12917	S	T	S	S, T	X
EXR0597	-22.82142	119.12913	S	T	S	S, T	X
EXR0599	-22.81905	119.12887	S	T	S	S, T	X
EXR0600	-22.81716	119.10349	S	T	plug stuck		
EXR0601	-22.81576	119.10351	S	T	S	S, T	X
EXR0762A	-22.82406	119.13350	N	N	N	N	
EXR0763	-22.82077	119.12398	N	T	N	N, T	
EXR0765	-22.81876	119.12296	N	N, T	N	N, T	X
EXR0767	-22.83392	119.11246	N	N, T	S	T	
EXR0768	-22.81791	119.11822	N, S	T	S	S, T	X
EXR0769	-22.81574	119.11241	S	T	S	S, T	
EXR0964	-22.81761	119.11248	S	T	S	S, T	
EXR0965	-22.81961	119.11254	S	T	S	S, T	X
EXR0966	-22.82072	119.11255	S	T	S	S, T	X
EXR0967	-22.81504	119.10354	S	T	S	S, T	
EXR0968	-22.81825	119.10310	S	T	S	S, T	X
EXR0969	-22.81432	119.09663	N	N, T	N	N, T	X
EXR0970	-22.81533	119.09657	N	N, T	N	N, T	
EXR0971	-22.81637	119.09652	S	S, T	S	S, T	X
EXR0973	-22.82575	119.09450	S	T	S	S, T	X
EXR0974	-22.82470	119.09486	S	T	S	S, T	
EXR0975	-22.82355	119.09524	S	T	S	S, T	X
EXR0976	-22.82269	119.09565			S	S, T	
EXR0977	-22.81785	119.09620	S				
EXR1142	-22.82928	119.10756	S	T	S	S, T	X
EXR1143	-22.82836	119.10759	S	S, T	S	S, T	X

Bore name	Latitude	Longitude	V1 Feb 09	V2 May 09	V3 Feb 10	V4 Ap 10	Subterranean Fauna recorded
EXR1144	-22.82747	119.10778	S	S, T	S	S, T	X
EXR1145	-22.82985	119.10142	N				
EXR1146	-22.82800	119.10141	S	T	S	S, T	X
EXR1147	-22.82889	119.10184	S	T	S	S, T	X
EXR1148	-22.82733	119.10162	S	T	S	S, T	X
EXR1149	-22.82216	119.09165	S	S, T	S	S, T	X
EXR1150	-22.82118	119.09175	S	S, T	S	S, T	X
EXR1151	-22.82031	119.09111	S	S, T	S	S, T	X
EXR1153	-22.82307	119.09078	S	T	S	S, T	X
EXR1154	-22.824	119.08997	S	S, T	S	S, T	X
MN0009R	-22.83089	119.11011	N	N, T	S	N, T	
MN0010R	-22.82736	119.10997	S	S			
MN0011R	-22.82805	119.10992	S	S, T	S	S, T	X
MN0012R	-22.82898	119.10997	S	T	S	S, T	
MN0013R	-22.82998	119.10996	N	N, T	N	T	X
MN0015	-22.82811	119.10413	S	S			X
MN0016R	-22.82900	119.10399	N	N, T	S	S, T	
MN2	-22.82206	119.12910	N			N	X

Appendix 2. Abundance and distribution of troglomorphic species among the bore holes at Ministers North.

The abundance of identified troglomorphic species collected at Ministers North sites during sampling in 2009 and 2010. Note NR and SR denote the location of the bore in the Ministers North deposit, *i.e.* NR = North Ridge and SR = South Ridge.

Taxon	NR	SR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	EXR0406	EXR0509	EXR0512	EXR0513	EXR0597	EXR0599	EXR0601	EXR0765	EXR0768	EXR0965	EXR0966	EXR0968	EXR0969
Insecta													
Anillina sp_ S6													
Blattidae sp_ S2													
Cixiidae sp_ S1					1								
Curculionidae sp_ S8								1					
Curculionidae sp_ S2								1					1
Meenoplidae sp_ S1									1				
Nocticola sp_ S1		1						2		2	2		
Pselaphinae sp_ S4													
Reduviidae sp_ indet_ MN					1								
Zuphiinae sp_ S1													
Arachnida													
Araneae sp_ indet_ MN		1			1								
Gnaphosidae sp_ S3												1	
Lagynochthonius sp_ S4													
Linyphiidae sp_ S1													
nr_ *Draculoides* sp_ S4					1		1						
Palpigradida sp_ indet_ MN	1												
Symphytognathidae sp_ S1													1
Entognatha													
Atelurodes sp_ S2											1		
Diplura sp_ indet_ MN			1										
Chilopoda													
Chilopoda sp_ indet_			1										

Taxon	NR	SR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	EXR0406	EXR0509	EXR0512	EXR0513	EXR0597	EXR0599	EXR0601	EXR0765	EXR0768	EXR0965	EXR0966	EXR0968	EXR0969
Cryptopidae indet_													
Pauropoda													
Pauropoda sp_ S4													
Diplopoda													
Polyxenida sp_ S1				2	1	1	1						
Isopoda													
Troglarmadillo sp_ S14													
Total Number of Taxa	**1**	**2**	**2**	**2**	**5**	**1**	**2**	**4**	**1**	**2**	**3**	**1**	**2**

Taxon	NR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR
	EXR0971	EXR0973	EXR0975	EXR1142	EXR1143	EXR1144	EXR1146	EXR1147	EXR1148	EXR1149	EXR1150	EXR1151	EXR1153	EXR1154	MN0011R
Insecta															
Anillina sp_ S6														1	
Blattidae sp_ S2			2					1			1	1			3
Cixiidae sp_ S1															
Curculionidae sp_ S8															
Curculionidae sp_ S2															
Meenoplidae sp_ S1								3						1	
Nocticola sp_ S1			2	2	3	2	1	1		3	4	3	1		4
Pselaphinae sp_ S4											2				
Reduviidae sp_ indet_ MN															
Zuphiinae sp_ S1											1				
Arachnida															
Araneae sp_ indet_ MN									2						
Gnaphosidae sp_ S3									1						
Lagynochthonius sp_ S4					1	1			2						
Linyphiidae sp_ S1									1						
nr_ *Draculoides* sp_ S4		1												1	
Palpigradida sp_ indet_ MN															
Symphytognathidae sp_ S1															
Entognatha															
Atelurodes sp_ S2										1	2				
Diplura sp_ indet_ MN															
Chilopoda															
Chilopoda sp_ indet_	1														
Cryptopidae indet_															
Pauropoda															
Pauropoda sp_ S4			1												

Taxon	NR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR
	EXR0971	EXR0973	EXR0975	EXR1142	EXR1143	EXR1144	EXR1146	EXR1147	EXR1148	EXR1149	EXR1150	EXR1151	EXR1153	EXR1154	MN0011R
Diplopoda															
Polyxenida sp_ S1															
Isopoda															
Troglarmadillo sp_ S14										1					
Total Number of Taxa	**1**	**1**	**4**	**2**	**4**	**3**	**1**	**5**	**6**	**5**	**9**	**4**	**1**	**3**	**6**

Taxon	SR	SR	NR
	MN0012R	**MN0013R**	**MN2**
Insecta			
Anillina sp_ S6			
Blattidae sp_ S2		1	
Cixiidae sp_ S1		1	
Curculionidae sp_ S8			
Curculionidae sp_ S2			
Meenoplidae sp_ S1		1	
Nocticola sp_ S1	1		
Pselaphinae sp_ S4			
Reduviidae sp_ indet_ MN			
Zuphiinae sp_ S1			
Arachnida			
Araneae sp_ indet_ MN			
Gnaphosidae sp_ S3			
Lagynochthonius sp_ S4			
Linyphiidae sp_ S1			
nr_ *Draculoides* sp_ S4			
Palpigradida sp_ indet_ MN			
Symphytognathidae sp_ S1			
Entognatha			
Atelurodes sp_ S2			
Diplura sp_ indet_ MN			
Chilopoda			
Chilopoda sp_ indet_			
Cryptopidae indet_		1	
Pauropoda			
Pauropoda sp_ S4			1
Diplopoda			
Polyxenida sp_ S1			
Isopoda			
Troglarmadillo sp_ S14			
Total Number of Taxa	**1**	**2**	**1**

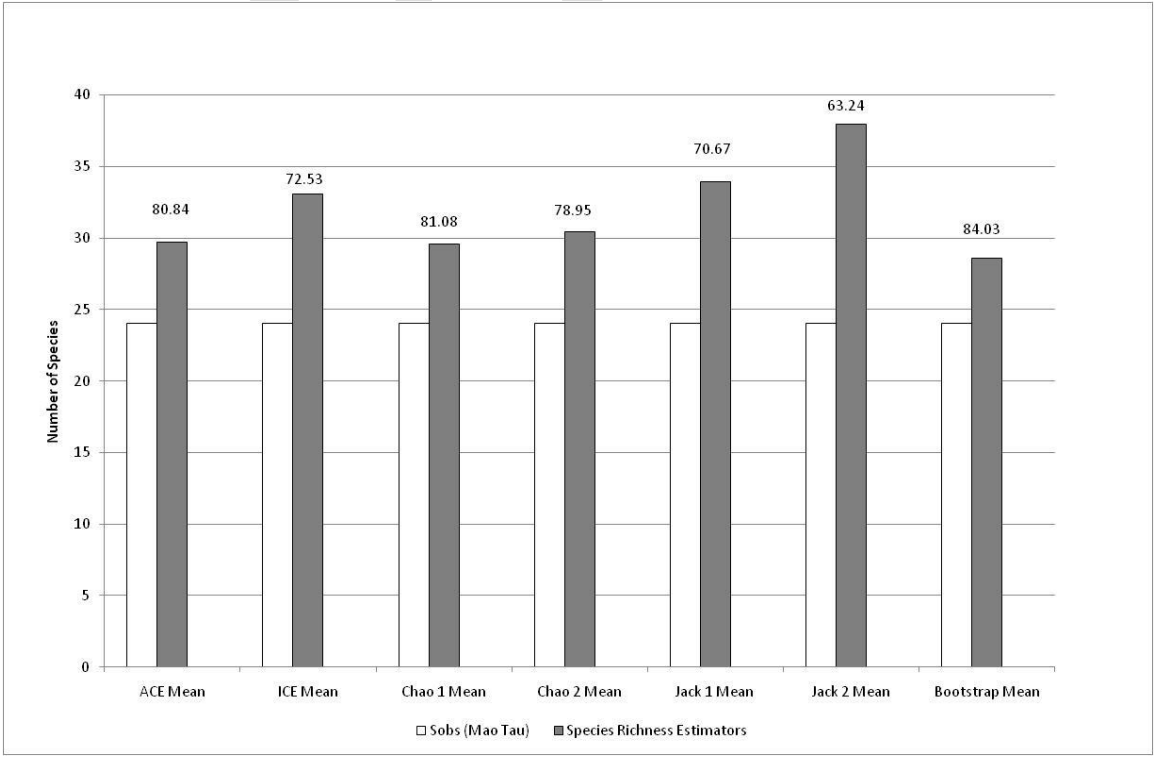
Appendix 3. Tabulation of species richness estimates.

Estimated number of troglofauna species in Ministers North for 50, 100, 150, 200, 250 and 266 samples, versus observed species richness (Sobs Mao Tau). Estimates calculated using EstimateS (Colwell 2006).

Species richness estimators	50	100	150	200	250	266	% detected
Sobs (Mao Tau)	9.41	14.67	18.34	21.14	23.38	24	
ACE Mean	19.43	23.31	25.68	27.73	29.42	29.69	80.84
ICE Mean	28.04	30.1	30.12	31.42	32.89	33.09	72.53
Chao 1 Mean	15.59	21.85	25.59	28.45	29.77	29.6	81.08
Chao 2 Mean	19.69	25.76	28.95	30.96	30.89	30.4	78.95
Jack 1 Mean	15.57	23.08	27.68	30.91	33.44	33.96	70.67
Jack 2 Mean	20.33	28.59	33.16	36.41	38.14	37.95	63.24
Bootstrap Mean	11.84	18.19	22.4	25.43	27.93	28.56	84.03
					Mean % detected		**75.91**
					SD detected		**7.37**

Appendix 4. Figure of species richness estimates.

Estimated number of troglofauna species in Ministers North for 266 samples, versus observed species richness (Sobs Mao Tau). Estimates calculated using EstimateS (Colwell 2006). Percentages shown indicate the number of species that have been collected to date compared to the estimated number.



Appendix 5. Blattodea (*Nocticola* and Blattidae) Molecular Genetic Analyses. Dr. T. Finston 6-Oct-2009.

Methods:

- Six specimens of *Nocticola* S1 from six bores
- Two specimens of Blattidae S2 from two bores
- 395 bp fragment of 12s
- Uncorrected p-distances
- Neighbour-joining tree
- Genbank voucher specimens *Nocticola australiensis*, *Blatta orientalis*, *Periplaneta australasiae*

Results:

Nocticola

The new specimens from Minister's North fell into the previously recognised clade S1 (Figure 1). Variation within this group ranged from 0.3 to 8.3% sequence divergence (Table 1). This species is found at Packsaddle, Area C, Southern Flank, Jinaryi, and Minister's North. There is variation associated with geographic locations evident in this clade. There are three well-supported sub-clades within S1, corresponding to Packsaddle/Area C/Southern Flank, Jinaryi, and Jinaryi/Minister's North.

Blattidae:

The new specimens from Minister's North fell into the previously recognised clade S3 (Figure 1). Variation between haplotypes of S3 ranged from 0 to 2.8% sequence divergence (Table 1). Based on the genetic data, this species is found at Packsaddle and Minister's North.

Conclusions:

The Ministers North specimens of *Nocticola* belong to *Nocticola* species S1. This species shows some geographic variation. The specimens of Blattidae belong to Blattidae species S3.

Table 1. Range of uncorrected p-distances within each of the major clades for a 395 bp alignment of 12s.

clade	% divergence
Nocticola S1	0.3 – 8.3
Nocticola S3	0.0 – 7.2
Blattidae S3	0.0 – 2.8

Figure 1. Neighbour-joining tree of uncorrected p-distances for a 395 bp alignment of 12s for *Nocticola* only. Bootstrap values >50% shown on branch nodes, new specimens highlighted in yellow. Species designations for S1 and S3 shown on branches.

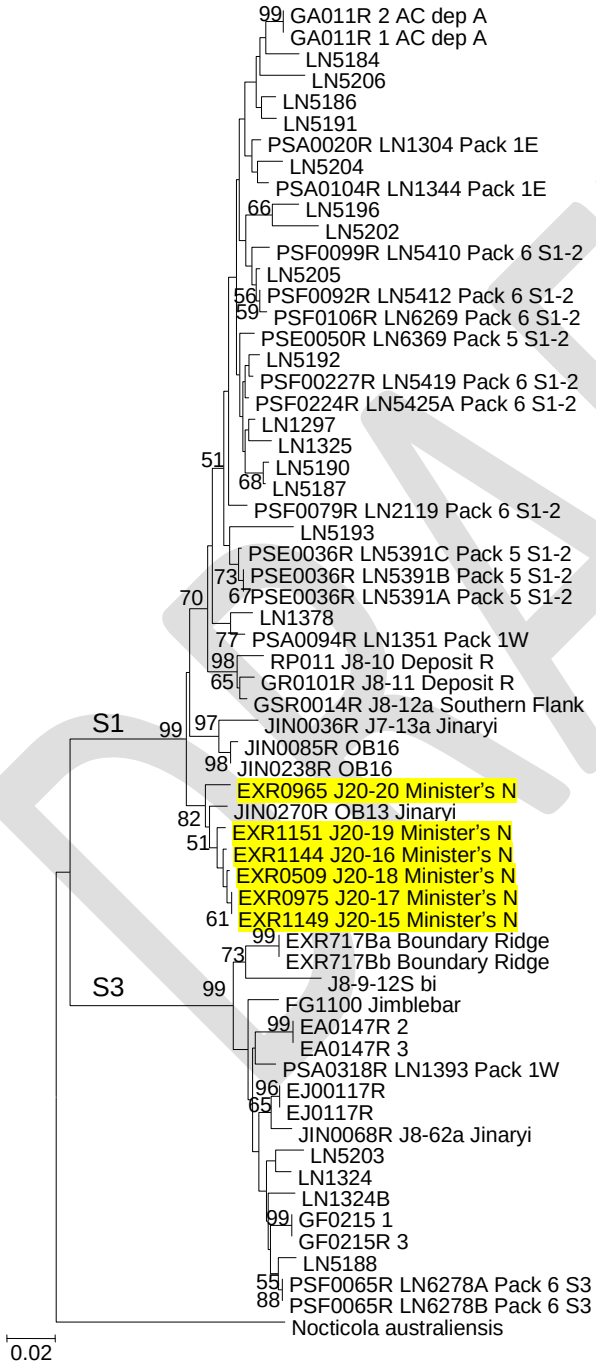
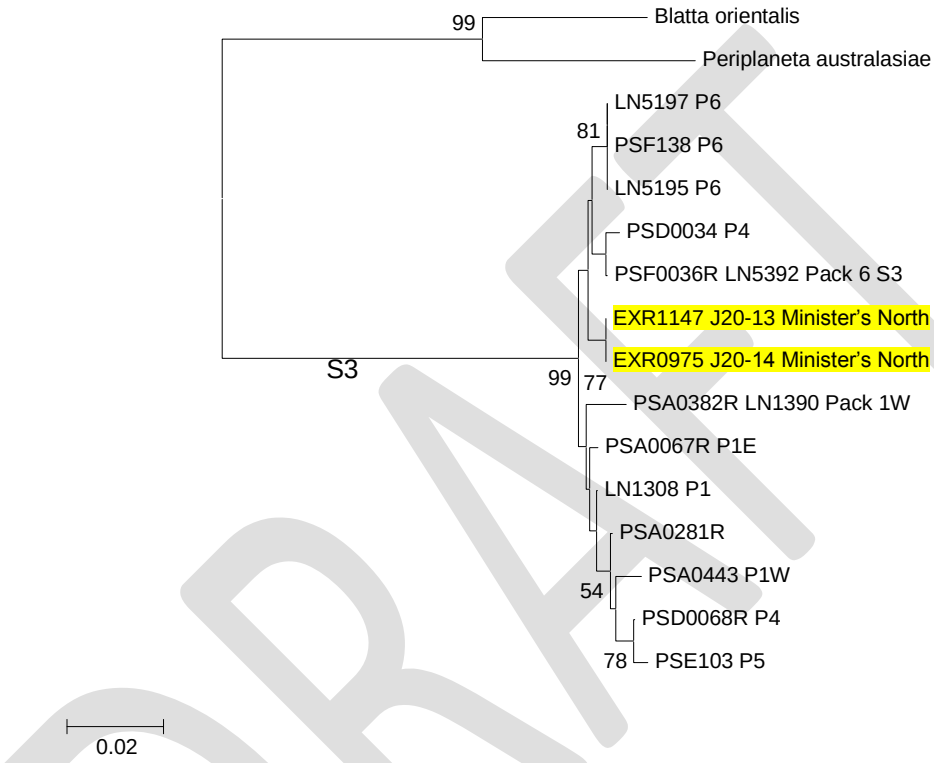


Figure 2. Neighbour-joining tree of uncorrected p-distances for a 395 bp alignment of 12s for Blattidae only. Bootstrap values >50% shown on branch nodes, new specimens highlighted in yellow. Species designations for S3 shown on branch.



Appendix 6. Hemiptera (Meenoplidae and Cixiidae) Molecular Genetic Analyses. Dr. T. Finston 13-Oct-2009.

Methods:

- Two specimens of Meenoplidae S1, one specimen of Fulgoroidea
- 417 bp fragment of 12s
- Uncorrected p-distances
- Neighbour-joining tree
- 500 bootstrap replicates
- Genbank vouchers: *Geisha distinctissima*, Fulgoroidea, Flatidae; *Olarius* sp, Fulgoroidea, Cixiidae; *Tachycixius pilosus*, Fulgoroidea, Cixiidae
- 357 bp fragment of 12s final alignment including outgroups

Results:

The two Minister's North specimens identified as Meenoplidae S1 (MN0013, EXR1147) shared the same haplotype. The two specimens fell into the previously recognised clade of Meenoplidae containing specimens from a wide geographical area (Figure 1). The two specimens differed from other haplotypes in the clade by 2.7 to 5.4% sequence divergence (Table 1). The range of sequence divergence across the entire clade was 0 to 6.6%.

The third Minister's North specimen (EXR0597), identified as belonging to the order Fulgoroidea, formed a well-supported clade with two of the outgroup voucher sequences belonging to the Fulgoroidea genera *Olarius* and *Tachycixius* in the family Cixiidae (Figure 1). The haplotype for EXR0597 differed from the two Cixiidae genera by 15.6 to 17.9% sequence divergence (Table 1).

Conclusions:

The Minster's North specimens identified as Meenoplidae S1 belong to a common widespread species found also at Yarrie, Packsaddle, Area C, Jinaryi, Boundary Ridge, Jimblebar, and OB18.

The Minster's North specimen identified as Fulgoroidea shows affinities to voucher specimens of Cixiidae, but the high level of sequence divergence suggests it is a distinct species.

Table 1. Pair-wise uncorrected p-distances for a 357 bp fragment of 12s.

	1	2	3	4	5	6	7	8
[1] MN0013								
[2] EXR1147	0.000							
[3] EJUNK07	0.054	0.054						
[4] EJUNK07	0.054	0.054	0.000					
[5] LB040	0.054	0.054	0.000	0.000				
[6] WJR001	0.054	0.054	0.004	0.004	0.004			
[7] CU0307A	0.054	0.054	0.004	0.004	0.004	0.000		
[8] CA0023A	0.054	0.054	0.008	0.008	0.008	0.004	0.004	
[9] CA0023B	0.054	0.054	0.008	0.008	0.008	0.004	0.004	0.000
[10] CA0023A	0.054	0.054	0.008	0.008	0.008	0.004	0.004	0.000
[11] PSA0231	0.031	0.031	0.062	0.062	0.062	0.062	0.062	0.062
[12] PSA0231R	0.031	0.031	0.062	0.062	0.062	0.062	0.062	0.062
[13] PSA0231R	0.031	0.031	0.062	0.062	0.062	0.062	0.062	0.062
[14] PSE0036R	0.039	0.039	0.062	0.062	0.062	0.062	0.062	0.062
[15] EXR0728	0.054	0.054	0.000	0.000	0.000	0.004	0.004	0.008
[16] PSA0092R	0.054	0.054	0.000	0.000	0.000	0.004	0.004	0.008
[17] PSA0069R	0.054	0.054	0.000	0.000	0.000	0.004	0.004	0.008
[18] PSA0069R	0.054	0.054	0.000	0.000	0.000	0.004	0.004	0.008
[19] CA0023	0.054	0.054	0.008	0.008	0.008	0.004	0.004	0.000
[20] PSD0034R	0.039	0.039	0.062	0.062	0.062	0.062	0.062	0.062
[21] FG678	0.054	0.054	0.004	0.004	0.004	0.000	0.000	0.004
[22] JIN0098R	0.047	0.047	0.054	0.054	0.054	0.054	0.054	0.054
[23] JIN0282R	0.051	0.051	0.066	0.066	0.066	0.066	0.066	0.066
[24] GC0099R	0.031	0.031	0.054	0.054	0.054	0.054	0.054	0.054
[25] EJR0235	0.054	0.054	0.004	0.004	0.004	0.000	0.000	0.004
[26] EJR0219	0.054	0.054	0.000	0.000	0.000	0.004	0.004	0.008
[27] GF0094R	0.027	0.027	0.051	0.051	0.051	0.051	0.051	0.051
[28] EXR0892	0.054	0.054	0.004	0.004	0.004	0.000	0.000	0.004
[29] JIN0036R	0.054	0.054	0.000	0.000	0.000	0.004	0.004	0.008
[30] EXR0597	0.195	0.195	0.222	0.222	0.222	0.222	0.222	0.222
[31] Olarius_sp	0.222	0.222	0.233	0.233	0.233	0.233	0.233	0.233
[32] Tachycixius	0.198	0.198	0.195	0.195	0.195	0.195	0.195	0.195
[33] Geisha	0.436	0.436	0.436	0.436	0.436	0.432	0.432	0.436

Table 1, cont'd.

	9	10	11	12	13	14	15	16
[1]								
[2]								
[3]								
[4]								
[5]								
[6]								
[7]								
[8]								
[9]								
[10]	0.000							
[11]	0.062	0.062						
[12]	0.062	0.062	0.000					
[13]	0.062	0.062	0.000	0.000				
[14]	0.062	0.062	0.039	0.039	0.039			
[15]	0.008	0.008	0.062	0.062	0.062	0.062		
[16]	0.008	0.008	0.062	0.062	0.062	0.062	0.000	
[17]	0.008	0.008	0.062	0.062	0.062	0.062	0.000	0.000
[18]	0.008	0.008	0.062	0.062	0.062	0.062	0.000	0.000
[19]	0.000	0.000	0.062	0.062	0.062	0.062	0.008	0.008
[20]	0.062	0.062	0.039	0.039	0.039	0.000	0.062	0.062
[21]	0.004	0.004	0.062	0.062	0.062	0.062	0.004	0.004
[22]	0.054	0.054	0.047	0.047	0.047	0.058	0.054	0.054
[23]	0.066	0.066	0.051	0.051	0.051	0.062	0.066	0.066
[24]	0.054	0.054	0.027	0.027	0.027	0.039	0.054	0.054
[25]	0.004	0.004	0.062	0.062	0.062	0.062	0.004	0.004
[26]	0.008	0.008	0.062	0.062	0.062	0.062	0.000	0.000
[27]	0.051	0.051	0.027	0.027	0.027	0.035	0.051	0.051
[28]	0.004	0.004	0.062	0.062	0.062	0.062	0.004	0.004
[29]	0.008	0.008	0.062	0.062	0.062	0.062	0.000	0.000
[30]	0.222	0.222	0.202	0.202	0.202	0.222	0.222	0.222
[31]	0.233	0.233	0.222	0.222	0.222	0.230	0.233	0.233
[32]	0.195	0.195	0.202	0.202	0.202	0.210	0.195	0.195
[33]	0.436	0.436	0.444	0.444	0.444	0.436	0.436	0.436

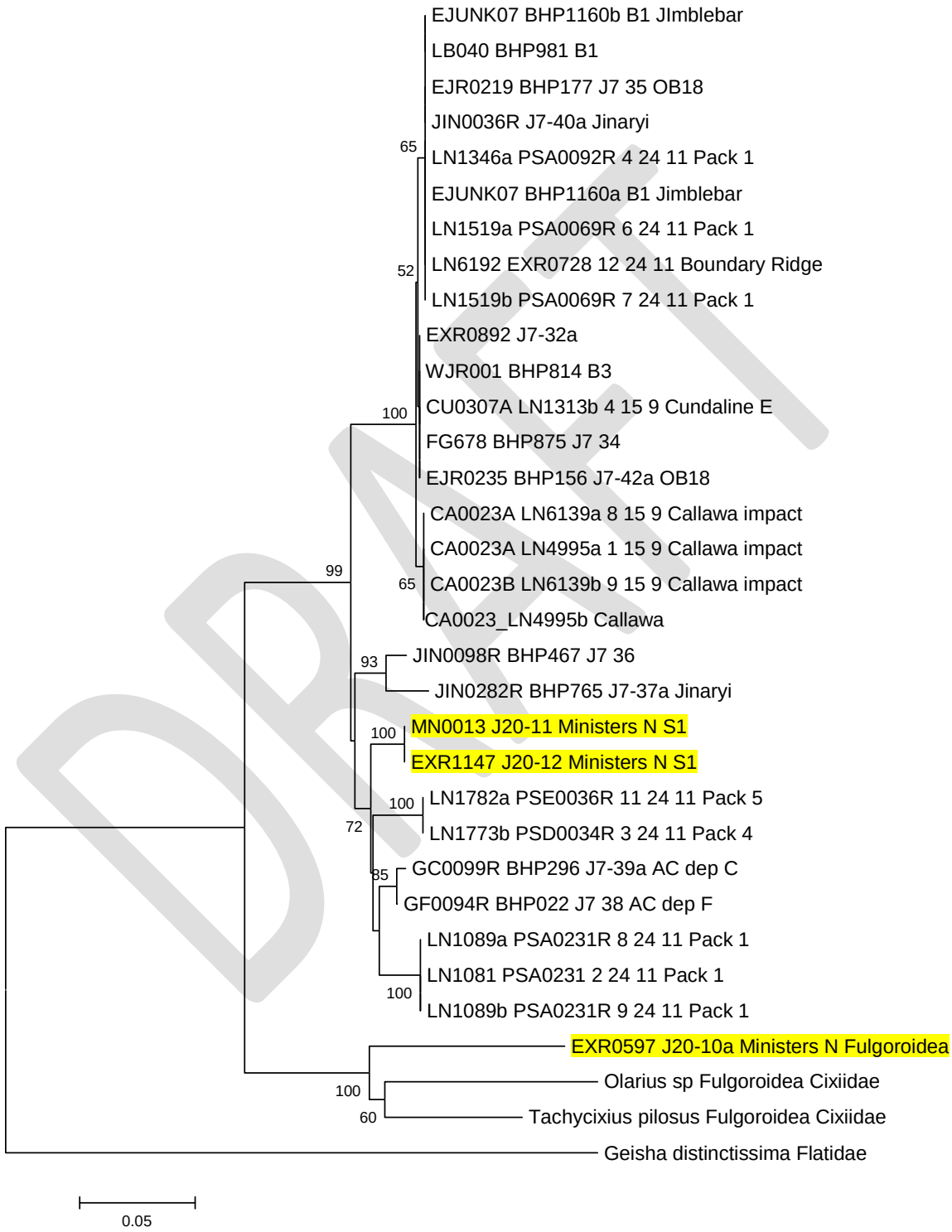
Table 1, cont'd.

	17	18	19	20	21	22	23	24
[1]								
[2]								
[3]								
[4]								
[5]								
[6]								
[7]								
[8]								
[9]								
[10]								
[11]								
[12]								
[13]								
[14]								
[15]								
[16]								
[17]								
[18]	0.000							
[19]	0.008	0.008						
[20]	0.062	0.062	0.062					
[21]	0.004	0.004	0.004	0.062				
[22]	0.054	0.054	0.054	0.058	0.054			
[23]	0.066	0.066	0.066	0.062	0.066	0.027		
[24]	0.054	0.054	0.054	0.039	0.054	0.047	0.051	
[25]	0.004	0.004	0.004	0.062	0.000	0.054	0.066	0.054
[26]	0.000	0.000	0.008	0.062	0.004	0.054	0.066	0.054
[27]	0.051	0.051	0.051	0.035	0.051	0.043	0.047	0.004
[28]	0.004	0.004	0.004	0.062	0.000	0.054	0.066	0.054
[29]	0.000	0.000	0.008	0.062	0.004	0.054	0.066	0.054
[30]	0.222	0.222	0.222	0.222	0.222	0.198	0.202	0.206
[31]	0.233	0.233	0.233	0.230	0.233	0.210	0.218	0.226
[32]	0.195	0.195	0.195	0.210	0.195	0.191	0.198	0.198
[33]	0.436	0.436	0.436	0.436	0.432	0.447	0.436	0.444

Table 1, cont'd.

	25	26	27	28	29	30	31	32	33
[1]									
[2]									
[3]									
[4]									
[5]									
[6]									
[7]									
[8]									
[9]									
[10]									
[11]									
[12]									
[13]									
[14]									
[15]									
[16]									
[17]									
[18]									
[19]									
[20]									
[21]									
[22]									
[23]									
[24]									
[25]									
[26]	0.004								
[27]	0.051	0.051							
[28]	0.000	0.004	0.051						
[29]	0.004	0.000	0.051	0.004					
[30]	0.222	0.222	0.202	0.222	0.222				
[31]	0.233	0.233	0.222	0.233	0.233	0.179			
[32]	0.195	0.195	0.195	0.195	0.195	0.156	0.152		
[33]	0.432	0.436	0.440	0.432	0.436	0.498	0.525	0.471	

Figure 1. Neighbour-joining tree of uncorrected p-distances for a 357 bp fragment of 12s. Numbers on branch nodes correspond to bootstrap support. New specimens highlighted in yellow.



Appendix 7. Diplopoda (Polyxenida) Molecular Genetic Analyses. Dr. T. Finston 28-Oct-2009.

Methods:

- Three specimens of polyxenid from three bores were sequenced, corresponding to three morphospecies: S6, S7, S8
- 395 bp fragment 12s, 420 bp fragment 28s
- Uncorrected p-distances
- Neighbour joining trees
- 500 bootstrap replicates
- Genbank voucher sequences: *Monographis* sp, *Polyxenus lagurus*, Polyxenidae sp.

Results:

At 12s, the Minister's North specimens fell into the S1 clade, but were distributed in two separate sub-clades, C and D (Figure 1). Specimens EXR0601 (morphotype S6) and EXR0599 (morphotype S8) had similar haplotypes, differing by less than 1% sequence divergence (Table 1), but they differed from the EXR0597 (morphotype S7) haplotype by 3.8 to 4.1% sequence divergence (Table 1).

While not all samples were sequenced for 28s, the same overall pattern was apparent in both genes. The 28s tree showed distinct clades for S1, S5, and the surface specimen (Figure 2), however, the structure within S1 detected at 12s was not apparent at 28s. The 28s S1 clade contained specimens from 12s sub-clades A, B, C, and D, all of which shared the same haplotype at 28s.

Conclusions:

It is likely that there is a single species of polyxenid at Minister's North, species S1. Two of the specimens, EXR0601 and EXR0599, occurred in sub-clade D, while EXR0597 occurred in sub-clade C. The level of sequence divergence is relatively low between the four 12s sub-clades (<5%; see Table 1), however, the distinctiveness of the clades are well-supported by bootstrapping. The occurrence of specimens from different clades at the same site (e.g. Minister's North specimens occur in clades C and D; Jimblebar and Hashimoto specimens occur in clades A and D), suggests a complex history involving current or historically limited gene flow between clades. However, given the low levels of variation at 12s and the lack of differentiation between 12s clades at 28s, it is likely that 12s clades A-D represent geographic variants of a single widespread species.

Table 1. Sequence divergence (uncorrected p-distances) between haplotypes of polyxenids. Divergences between haplotypes from the same clade are highlighted in yellow. Unshaded cells represent distances between clades.

	1	2	3	4	5	6	7
[1] EXR0421_Jimblebar_W							
[2] EXR1642R_Jimblebar_W	0.000						
[3] EA0060RT_OB_24	0.000	0.000					
[4] EXR1010R_Jimblebar_W	0.000	0.000	0.000				
[5] EJUNK02_Jimblebar_E	0.000	0.000	0.000	0.000			
[6] ECP0702_OB_25	0.003	0.003	0.003	0.003	0.003		
[7] LB022_Hashioto	0.005	0.005	0.005	0.005	0.005	0.008	
[8] EXR0524_OB21_OB22	0.005	0.005	0.005	0.005	0.005	0.008	0.011
[9] GA0136R_MAC_dep_A	0.022	0.022	0.022	0.022	0.022	0.024	0.022
[10] EEX0078_OB_14	0.022	0.022	0.022	0.022	0.022	0.024	0.022
[11] GSR0020_Southern_Flank	0.027	0.027	0.027	0.027	0.027	0.030	0.027
[12] JIN0092R_OB13	0.024	0.024	0.024	0.024	0.024	0.027	0.024
[13] EEX0096_OB_13	0.022	0.022	0.022	0.022	0.022	0.024	0.022
[14] GSR0017_Southern_Flank	0.022	0.022	0.022	0.022	0.022	0.024	0.022
[15] GB0003R_MAC_dep_B	0.022	0.022	0.022	0.022	0.022	0.024	0.022
[16] EXR0597R_J20-24_Ministers_N_S7	0.016	0.016	0.016	0.016	0.016	0.019	0.022
[17] EXR0884_Alligator_Jaws	0.030	0.030	0.030	0.030	0.030	0.033	0.035
[18] PSA0505A_J3-9_Pack_1W_S1	0.041	0.041	0.041	0.041	0.041	0.043	0.041
[19] PSD0055_J3-11_Pack_4_S1	0.035	0.035	0.035	0.035	0.035	0.038	0.035
[20] CU0061_J3-14_Cundaline_S1	0.033	0.033	0.033	0.033	0.033	0.030	0.033
[21] BEV012_MAC_dep_D	0.030	0.030	0.030	0.030	0.030	0.027	0.030
[22] HH003_Hashimoto	0.030	0.030	0.030	0.030	0.030	0.027	0.030
[23] EJUNK03_Jimblebar_E	0.033	0.033	0.033	0.033	0.033	0.030	0.033
[24] RP081_MAC_dep_R	0.038	0.038	0.038	0.038	0.038	0.035	0.038
[25] EXR0601_J20-23_Ministers_N_S6	0.035	0.035	0.035	0.035	0.035	0.033	0.035
[26] EXR0599_J20-25_Ministers_N_S8	0.033	0.033	0.033	0.033	0.033	0.030	0.033
[27] MCM0116_Hashimoto	0.033	0.033	0.033	0.033	0.033	0.030	0.033
[28] MCM0114_Hashimoto	0.033	0.033	0.033	0.033	0.033	0.030	0.033
[29] PSE0019R_Packsaddle_5	0.033	0.033	0.033	0.033	0.033	0.030	0.033
[30] FG985_Jimblebar	0.033	0.033	0.033	0.033	0.033	0.030	0.033
[31] PI003_Hashimoto	0.033	0.033	0.033	0.033	0.033	0.030	0.033
[32] PSF0050R_LN2122_J5_7_Pack_6	0.223	0.223	0.223	0.223	0.223	0.223	0.228
[33] EXR0984_J7_14_Jimblebar	0.250	0.250	0.250	0.250	0.250	0.250	0.255

Table 1, cont'd.

	8	9	10	11	12	13	14	15
[1]								
[2]								
[3]								
[4]								
[5]								
[6]								
[7]								
[8]								
[9]	0.016							
[10]	0.016	0.005						
[11]	0.022	0.011	0.005					
[12]	0.019	0.005	0.005	0.011				
[13]	0.016	0.003	0.003	0.008	0.003			
[14]	0.016	0.003	0.003	0.008	0.003	0.000		
[15]	0.016	0.003	0.003	0.008	0.003	0.000	0.000	
[16]	0.022	0.022	0.024	0.030	0.024	0.022	0.022	0.022
[17]	0.035	0.035	0.038	0.043	0.038	0.035	0.035	0.035
[18]	0.041	0.035	0.038	0.043	0.038	0.035	0.035	0.035
[19]	0.035	0.030	0.033	0.038	0.033	0.030	0.030	0.030
[20]	0.033	0.030	0.030	0.030	0.030	0.027	0.027	0.027
[21]	0.030	0.030	0.033	0.033	0.033	0.030	0.030	0.030
[22]	0.030	0.030	0.033	0.033	0.033	0.030	0.030	0.030
[23]	0.033	0.033	0.035	0.035	0.035	0.033	0.033	0.033
[24]	0.038	0.038	0.041	0.041	0.038	0.038	0.038	0.038
[25]	0.041	0.041	0.043	0.043	0.041	0.041	0.041	0.041
[26]	0.033	0.033	0.035	0.035	0.033	0.033	0.033	0.033
[27]	0.033	0.033	0.035	0.035	0.033	0.033	0.033	0.033
[28]	0.033	0.033	0.035	0.035	0.033	0.033	0.033	0.033
[29]	0.033	0.033	0.035	0.035	0.033	0.033	0.033	0.033
[30]	0.033	0.033	0.035	0.035	0.033	0.033	0.033	0.033
[31]	0.033	0.033	0.035	0.035	0.033	0.033	0.033	0.033
[32]	0.228	0.231	0.228	0.231	0.228	0.228	0.228	0.228
[33]	0.247	0.250	0.255	0.255	0.253	0.253	0.253	0.253

Table 1, cont'd.

	16	17	18	19	20	21	22	23	24
[1]									
[2]									
[3]									
[4]									
[5]									
[6]									
[7]									
[8]									
[9]									
[10]									
[11]									
[12]									
[13]									
[14]									
[15]									
[16]									
[17]	0.024								
[18]	0.035	0.033							
[19]	0.030	0.027	0.014						
[20]	0.038	0.046	0.046	0.041					
[21]	0.035	0.043	0.043	0.038	0.003				
[22]	0.035	0.043	0.043	0.038	0.003	0.000			
[23]	0.038	0.046	0.046	0.041	0.005	0.003	0.003		
[24]	0.041	0.049	0.049	0.043	0.011	0.008	0.008	0.011	
[25]	0.041	0.049	0.054	0.049	0.014	0.011	0.011	0.014	0.014
[26]	0.038	0.046	0.046	0.041	0.005	0.003	0.003	0.005	0.005
[27]	0.038	0.046	0.046	0.041	0.005	0.003	0.003	0.005	0.005
[28]	0.038	0.046	0.046	0.041	0.005	0.003	0.003	0.005	0.005
[29]	0.038	0.046	0.046	0.041	0.005	0.003	0.003	0.005	0.005
[30]	0.038	0.046	0.046	0.041	0.005	0.003	0.003	0.005	0.005
[31]	0.038	0.046	0.046	0.041	0.005	0.003	0.003	0.005	0.005
[32]	0.231	0.231	0.231	0.234	0.220	0.223	0.223	0.226	0.226
[33]	0.255	0.253	0.264	0.258	0.250	0.250	0.250	0.250	0.247

Table 1, cont'd.

	25	26	27	28	29	30	31	32	33
[1]									
[2]									
[3]									
[4]									
[5]									
[6]									
[7]									
[8]									
[9]									
[10]									
[11]									
[12]									
[13]									
[14]									
[15]									
[16]									
[17]									
[18]									
[19]									
[20]									
[21]									
[22]									
[23]									
[24]									
[25]									
[26]	0.008								
[27]	0.008	0.000							
[28]	0.008	0.000	0.000						
[29]	0.008	0.000	0.000	0.000					
[30]	0.008	0.000	0.000	0.000	0.000				
[31]	0.008	0.000	0.000	0.000	0.000	0.000			
[32]	0.226	0.223	0.223	0.223	0.223	0.223	0.223		
[33]	0.258	0.250	0.250	0.250	0.250	0.250	0.250	0.307	

Figure 1. Neighbour-joining tree of sequence divergence (uncorrected p-distances) for a 390 bp fragment of the 12s gene for the Polyxenida. Numbers on branches represent bootstrap support >50%, based on 500 iterations. The new sequences from Minister's North are highlighted in yellow.

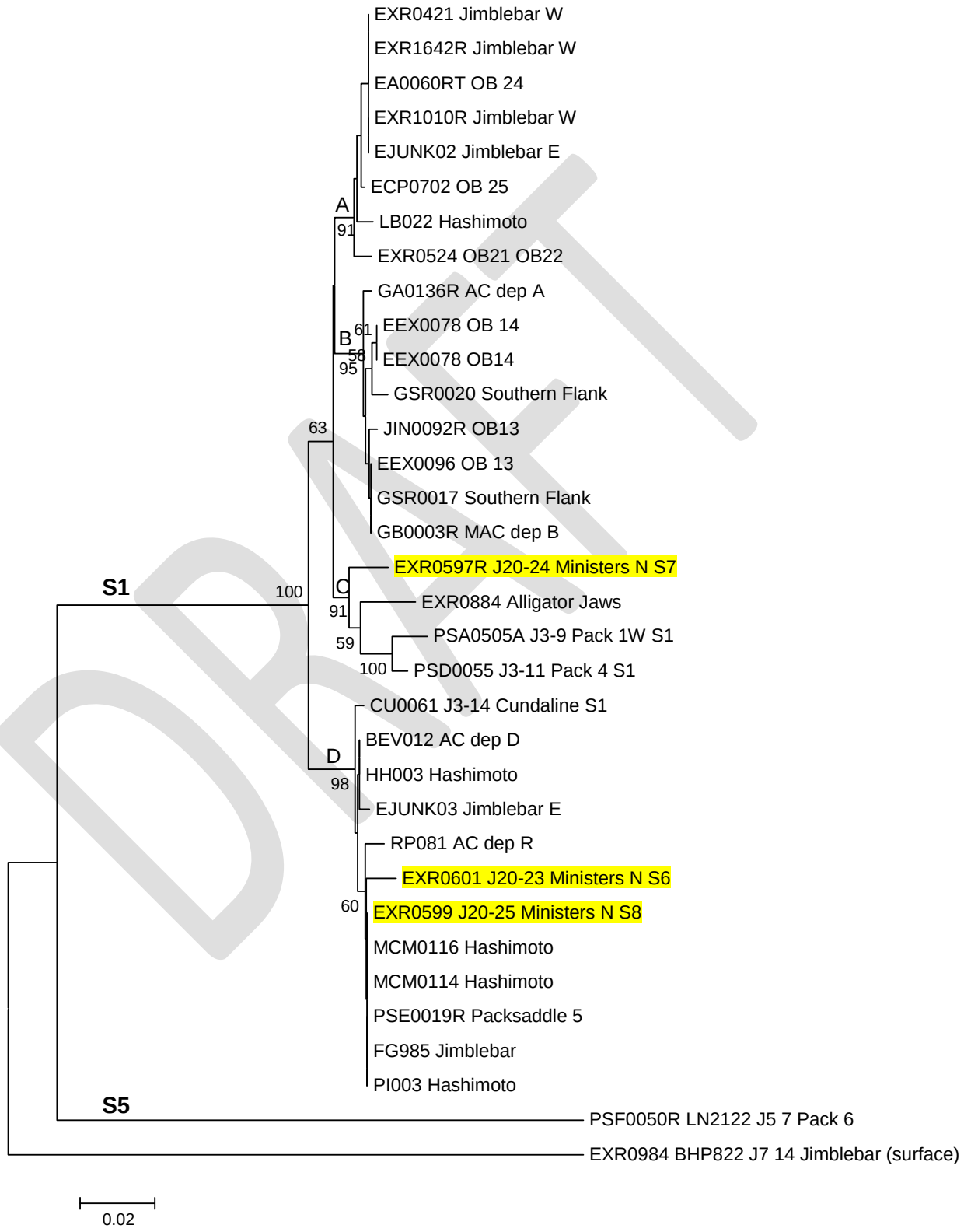
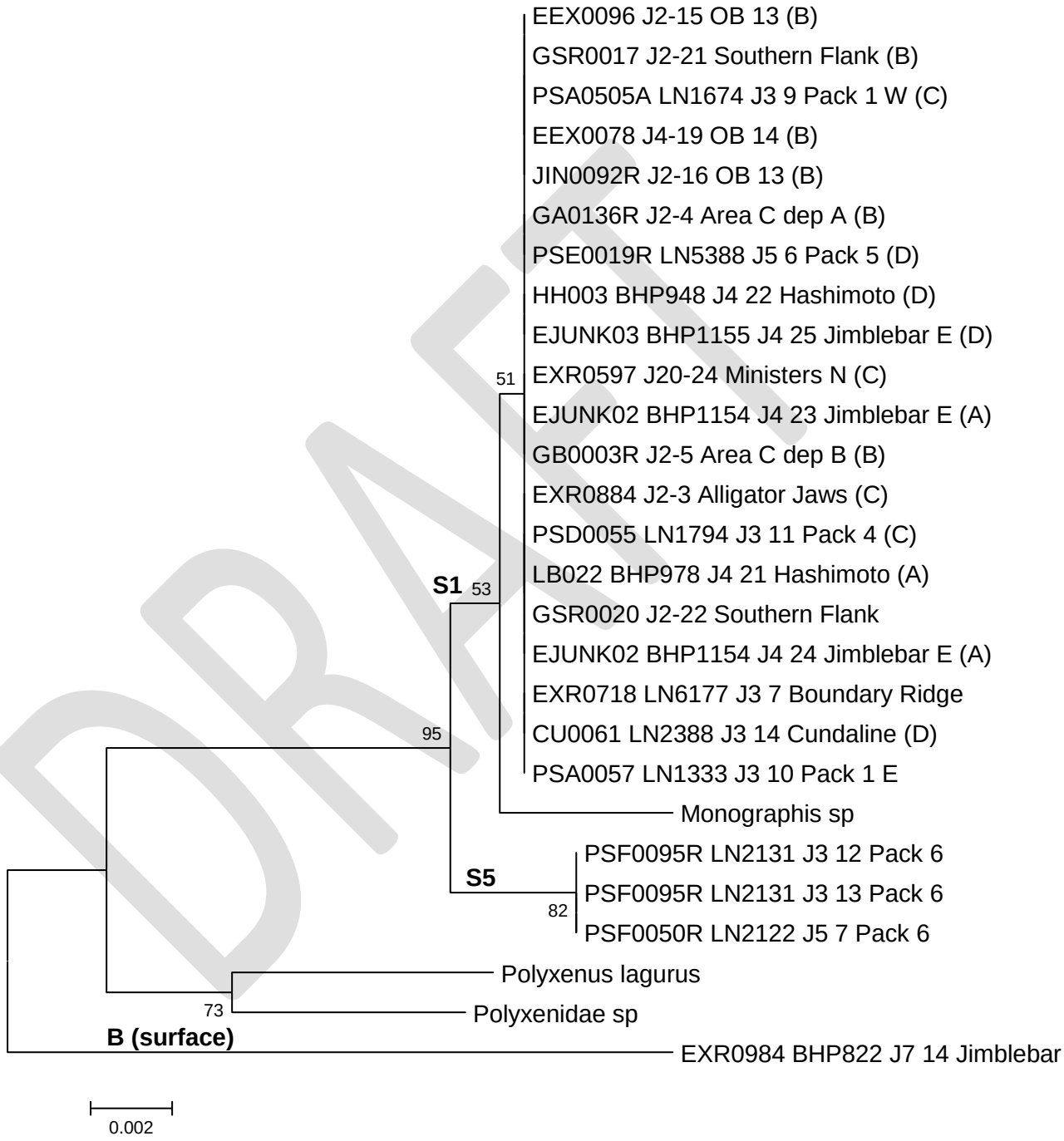


Figure 2. Neighbour-joining tree of p-distances for a 420 bp fragment of 28s. Numbers on branch nodes indicate bootstrap support over 500 iterations. The 12s clades to which haplotypes of S1 belong are indicated in parentheses.



Appendix 8. Thysanura (*Atelurodes*) Molecular Genetic Analyses. Dr. T. Finston 14-Oct-2009.

Methods:

- Specimen of *Atelurodes* from Minister's North, assigned to S3
- 357 bp fragment of 12s
- Uncorrected p-distances
- Neighbour-joining tree
- 500 bootstrap replicates
- Outgroup sequences A32, NB1, KCRC0107

Results:

The specimen from Minister's North fell into the clade containing haplotypes of Ateluriinae from a widespread geographical range (clade 1; Figure 1). Within clade 1 are geographical clusters of haplotypes (sub-clades A-C). The Minister's North specimen formed a well-supported sub-clade with haplotypes of *Atelurodes* S2 from Area C and Packsaddle, and was most similar to haplotypes from Packsaddle 5 and 6 (sub-clade C; Figure 1). The Minister's North specimen differed from the Packsaddle 5 and 6 haplotypes by 1.5 to 3.7% sequence divergence, while variation within sub-clade C ranged from 0.3 to 4.6% (Table 1). Variation in clade 1 ranged from 0 to 8.5% (Table 1). Clade 2, containing specimens from Alligator Jaws and Mudlark, was more divergent, differing by 7.3 to 10.7% from haplotypes in clade 1 (Table 1).

Conclusions:

The Minister's North specimen of *Atelurodes* shows affinities to specimens from Packsaddle and Area C, and is likely to belong to a widespread species whose distribution ranges from Packsaddle to Hashimoto. The species shows moderate genetic variation over this range (up to 8.5%).

Table 1. Pair-wise uncorrected p-distances for a 357 bp fragment of 12s. Sub-clades A-C and clade 2 highlighted in yellow. Unshaded cells represent between clade distances.

Clade				1				
Sub-clade				A				
	1	2	3	4	5	6	7	8
[1] PSA0318R								
[2] GBRS0196	0.009							
[3] GF0268R	0.009	0.012						
[4] GD0041R	0.009	0.012	0.006					
[5] GC0108R	0.018	0.021	0.015	0.009				
[6] GA0116R	0.018	0.015	0.015	0.009	0.012			
[7] GA0011R	0.021	0.024	0.018	0.012	0.015	0.009		
[8] GA0272R	0.015	0.018	0.012	0.006	0.009	0.003	0.006	
[9] EXR0966	0.030	0.034	0.027	0.021	0.030	0.024	0.027	0.021
[10] PSF0113R	0.021	0.018	0.018	0.012	0.021	0.021	0.024	0.018
[11] PSE0019R	0.043	0.046	0.040	0.034	0.037	0.043	0.046	0.040
[12] PSF0006R	0.027	0.030	0.024	0.018	0.027	0.027	0.030	0.024
[13] EEX0078	0.061	0.058	0.064	0.058	0.067	0.055	0.064	0.058
[14] JIN0418R	0.061	0.058	0.064	0.058	0.067	0.055	0.064	0.058
[15] WRKRC148	0.052	0.049	0.055	0.049	0.058	0.052	0.061	0.055
[16] JIN0109	0.061	0.058	0.064	0.058	0.067	0.055	0.058	0.058
[17] EA0310R	0.055	0.052	0.058	0.052	0.055	0.049	0.052	0.052
[18] EA0118R	0.052	0.049	0.055	0.049	0.052	0.052	0.055	0.055
[19] EAP0338	0.052	0.049	0.055	0.049	0.052	0.046	0.049	0.049
[20] MCM0109	0.055	0.052	0.058	0.058	0.061	0.055	0.064	0.058
[21] EXR0791	0.052	0.049	0.055	0.049	0.052	0.046	0.055	0.049
[22] EJ055R	0.052	0.049	0.055	0.049	0.052	0.046	0.055	0.049
[23] FG678	0.052	0.049	0.055	0.049	0.052	0.046	0.055	0.049
[24] EXR1386	0.095	0.091	0.098	0.091	0.095	0.082	0.079	0.085
[25] EXR1139	0.088	0.085	0.091	0.085	0.088	0.076	0.073	0.079

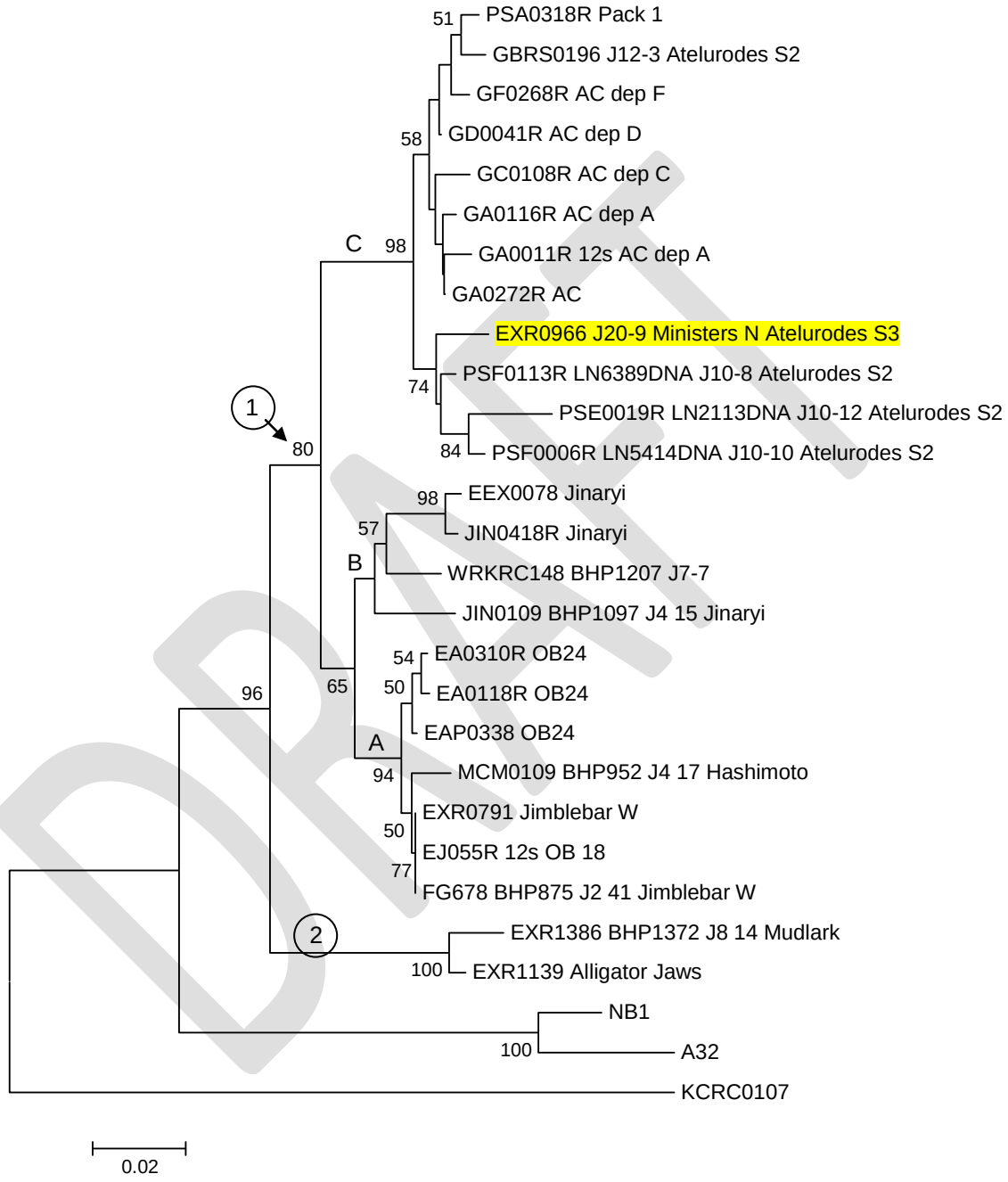
Table 1, cont'd.

Clade			1					
Sub-clade			A		B			
	9	10	11	12	13	14	15	16
[1]								
[2]								
[3]								
[4]								
[5]								
[6]								
[7]								
[8]								
[9]								
[10]	0.015							
[11]	0.037	0.027						
[12]	0.021	0.012	0.021					
[13]	0.061	0.058	0.079	0.064				
[14]	0.067	0.064	0.085	0.070	0.006			
[15]	0.064	0.055	0.076	0.061	0.027	0.027		
[16]	0.067	0.064	0.079	0.064	0.037	0.030	0.034	
[17]	0.061	0.058	0.064	0.058	0.043	0.037	0.052	0.030
[18]	0.064	0.055	0.061	0.055	0.046	0.040	0.049	0.034
[19]	0.058	0.055	0.064	0.055	0.040	0.034	0.049	0.027
[20]	0.067	0.064	0.070	0.064	0.043	0.037	0.052	0.037
[21]	0.058	0.055	0.064	0.055	0.034	0.027	0.043	0.027
[22]	0.058	0.055	0.064	0.055	0.034	0.027	0.043	0.027
[23]	0.058	0.055	0.064	0.055	0.034	0.027	0.043	0.027
[24]	0.088	0.085	0.107	0.091	0.088	0.095	0.098	0.082
[25]	0.082	0.079	0.101	0.085	0.076	0.082	0.091	0.076

Table 1, cont'd.

Clade	1		2						
Sub-clade	C								
	17	18	19	20	21	22	23	24	25
[1]									
[2]									
[3]									
[4]									
[5]									
[6]									
[7]									
[8]									
[9]									
[10]									
[11]									
[12]									
[13]									
[14]									
[15]									
[16]									
[17]									
[18]	0.003								
[19]	0.003	0.006							
[20]	0.012	0.015	0.015						
[21]	0.009	0.012	0.006	0.009					
[22]	0.009	0.012	0.006	0.009	0.000				
[23]	0.009	0.012	0.006	0.009	0.000	0.000			
[24]	0.088	0.091	0.085	0.101	0.091	0.091	0.091		
[25]	0.076	0.079	0.073	0.088	0.079	0.079	0.079	0.015	

Figure 1. Neighbour-joining tree of uncorrected p-distances for a 357 bp fragment of 12s. Numbers on branch nodes correspond to bootstrap support. New specimen highlighted in yellow. Circled numbers refer to major clades, letters refer to geographical sub-clades.



Appendix 9. Schizomida (nr. *Draculoides*) Molecular Genetic Analyses. Dr. T. Finston 23-Oct-2009.

Methods:

- Two specimens of schizomid species S4 from Minister's North
- 423 bp alignment of 12s
- Uncorrected p-distances
- neighbour-joining tree
- 500 bootstrap replicates
- Genbank voucher sequences: *Draculoides mesozeirus*, *D. julianneae*, *D. vinei*, *Paradraculoides* sp., *P. gnorphicola*, *P. anachoretus*, *P. bythius*, *P. kryptus*.
- Alignment including sequence vouchers 348 bp

Results:

There were four well-supported clades of schizomid haplotypes (A-D; Figure 1). The two Minister's North specimens formed their own clade (clade B, Figure 1), having similar haplotypes that differed by 1.1% sequence divergence (Table 1). They formed a well-supported clade with clade A, which contained haplotypes from Packsaddle 6 and Area C, deposits A, B, and F. Clade A and clade B differed by 8.6 to 9.8% sequence divergence (Table 2). Distances between the other clade comparisons ranged from 13.2 to 18.7% sequence divergence (Table 2). Distances within clades were lower, ranging from 0.3 to 7.8% sequence divergence (Table 1).

The Minister's North specimens did not show close affinities to any of the voucher specimens of *Draculoides* and *Paradraculoides* (Figure 2). They do not appear to belong to *Paradraculoides*, which forms a distinct clade from all remaining haplotypes (Figure 2). The genus *Draculoides* is paraphyletic in this analysis (all *Draculoides* specimens do not share the same most recent ancestor). The Minister's North specimens share the same major clade with *D. julianneae*, and *D. vinei* but the relationship is not well-supported (Figure 2).

Conclusions:

The Minister's North specimens show a strong relationship with specimens from Packsaddle 6 and Area C. However, based on strong bootstrap support and moderately high genetic divergence (8.6 to 9.8%), the Minister's North specimens are likely to represent a distinct species from those at Packsaddle 6 and Area C. By comparison, for the same dataset, *P. bythius* and *P. kryptus* differ by 2.7 to 3.0% sequence divergence.

The Minister's North specimens show some affinities to the genus *Draculoides*, but the relationship is not well supported, thus generic status cannot be unequivocally determined using the present genetic data

Table 1. Pair-wise genetic distances (uncorrected p-distances) between haplotypes of schizomids, based on a 348 bp fragment of 12s.

	1	2	3	4	5	6	7	8	9	10
[1] PSF203_P6										
[2] PSF138_P6	0.006									
[3] GB0045R	0.055	0.060								
[4] GA0340R	0.066	0.072	0.049							
[5] GA0039R_2	0.072	0.078	0.055	0.006						
[6] GA0039R	0.066	0.072	0.049	0.000	0.006					
[7] GF0130R	0.075	0.075	0.055	0.057	0.063	0.057				
[8] GF0415R	0.075	0.075	0.055	0.057	0.057	0.057	0.006			
[9] EXR0973	0.092	0.092	0.095	0.086	0.092	0.086	0.098	0.098		
[10] EXR0597	0.092	0.092	0.095	0.086	0.092	0.086	0.098	0.098	0.011	
[11] PSA0391_P1W	0.187	0.187	0.172	0.167	0.172	0.167	0.164	0.164	0.172	0.170
[12] PSA0067_P1E	0.181	0.181	0.167	0.161	0.167	0.161	0.158	0.158	0.172	0.164
[13] PSA0108_P1E	0.178	0.178	0.164	0.158	0.164	0.158	0.155	0.155	0.170	0.161
[14] PSC0123	0.181	0.181	0.181	0.178	0.184	0.178	0.178	0.178	0.164	0.164
[15] PSD017_P4	0.172	0.172	0.167	0.164	0.170	0.164	0.161	0.161	0.152	0.152
[16] PSC0058_P3	0.178	0.178	0.172	0.170	0.175	0.170	0.167	0.167	0.158	0.158
[17] PSC0276_P3	0.178	0.184	0.172	0.170	0.175	0.170	0.172	0.172	0.164	0.164
[18] PSE002_P5	0.181	0.181	0.175	0.172	0.178	0.172	0.170	0.170	0.161	0.161
[19] PSD0083_P4	0.178	0.178	0.172	0.170	0.175	0.170	0.167	0.167	0.158	0.158
[20] PSD41	0.181	0.181	0.175	0.172	0.178	0.172	0.170	0.170	0.161	0.161

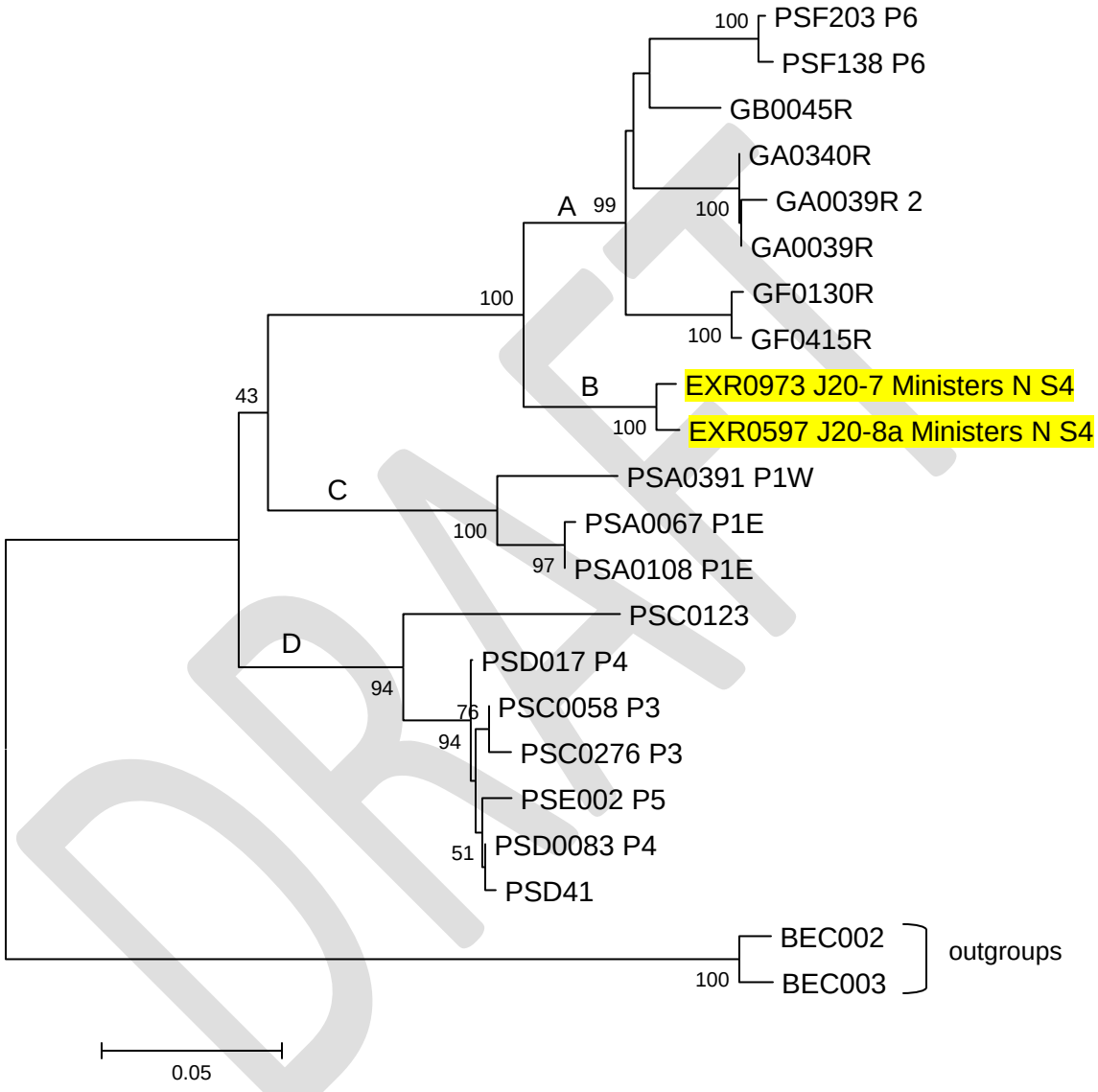
Table 1, cont'd.

	11	12	13	14	15	16	17	18	19	20
[1] PSF203_P6										
[2] PSF138_P6										
[3] GB0045R										
[4] GA0340R										
[5] GA0039R_2										
[6] GA0039R										
[7] GF0130R										
[8] GF0415R										
[9] EXR0973										
[10] EXR0597										
[11] PSA0391_P1W										
[12] PSA0067_P1E	0.049									
[13] PSA0108_P1E	0.052	0.003								
[14] PSC0123	0.167	0.155	0.158							
[15] PSD017_P4	0.144	0.132	0.129	0.075						
[16] PSC0058_P3	0.144	0.132	0.129	0.075	0.006					
[17] PSC0276_P3	0.149	0.138	0.135	0.080	0.011	0.006				
[18] PSE002_P5	0.152	0.141	0.138	0.083	0.009	0.014	0.020			
[19] PSD0083_P4	0.144	0.132	0.129	0.075	0.006	0.006	0.011	0.009		
[20] PSD41	0.147	0.135	0.132	0.075	0.009	0.009	0.014	0.011	0.003	

Table 2. Range of pair-wise genetic distances (uncorrected p-distances) between major clades of schizomids, based on a 348 bp fragment of 12s.

	A	B	C	D
A	X			
B	8.6-9.8	X		
C	15.5-18.7	16.1-17.2	X	
D	16.1-18.4	15.2-16.4	13.2-15.8	X

Figure 1. Neighbour-joining tree of uncorrected p-distances between haplotypes of schizomids, based on a 423 bp fragment of 12s. Numbers on branch nodes represent bootstrap support. Letters on branches refer to the major clades as discussed in the text.



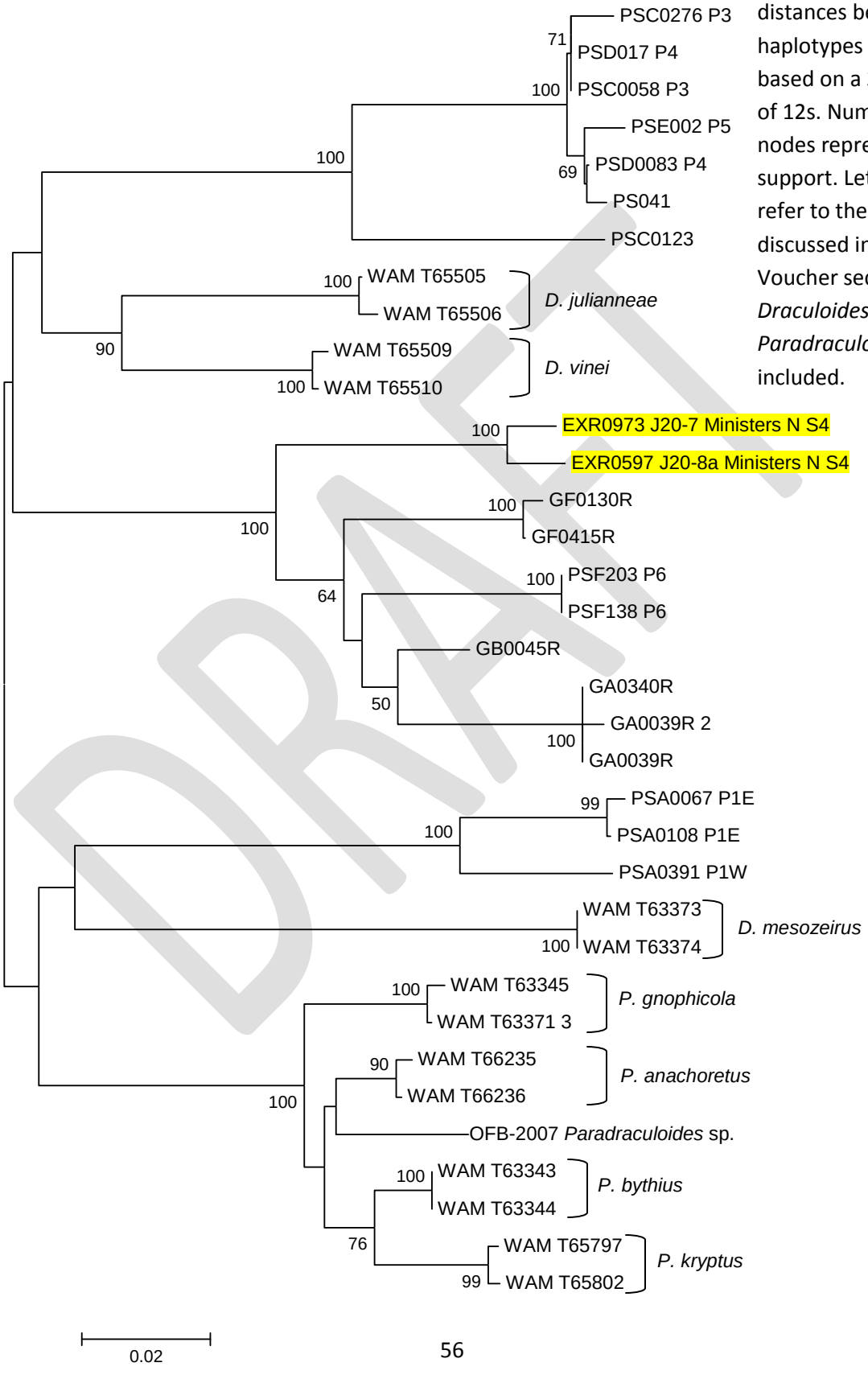


Figure 2. Neighbour-joining tree of uncorrected p-distances between haplotypes of schizomids, based on a 348 bp fragment of 12s. Numbers on branch nodes represent bootstrap support. Letters on branches refer to the major clades as discussed in the text. Voucher sequences of *Draculoides* and *Paradraculoides* are included.

Appendix 10. Araneae (Gnaphosidae, Linyphiidae, Symphytognathidae) Molecular Genetic Analyses. Dr. T. Finston 5-Oct-2009.

Methods:

- 372 bp fragment 12s
- Uncorrected p-distances
- Neighbour-joining tree
- 500 bootstrap replications
- Voucher sequence Gnaphosidae sp. from Packsaddle

Results:

The four new specimens from Minister's North fit into the pre-existing lineage B (Figure 1). Within lineage B, the Minister's North specimens fell into three minor lineages (Figure 1). Only one of the four showed close affinities to other haplotypes from previous collections. EXR1148 J20-3 clustered with specimens from Packsaddle 2, showing 4.3% sequence divergence (Table 1). These haplotypes differed from the remaining haplotypes in lineage B by 19.0 – 26.3% sequence divergence. Two of the Minister's North haplotypes were highly similar – EXR0968 and EXR1148 J20-4 differed by less than 1% (Table 1), but differed from the remaining haplotypes in lineage B by 10.8 – 25.4% sequence divergence. Finally, EXR0969 formed a distinct lineage that differed from the remaining haplotypes in lineage B by 24.6 – 31.0% sequence divergence.

The remaining haplotypes in lineage B fall into three groups, one containing specimens from Area C, Packsaddle and Jinaryi (lineage B3). Lineage B3 differs from the remaining haplotypes by 19.0 – 26.7% sequence divergence. Lineages B1 and B2 are both well-supported, but their relationship to one another is poorly supported. B1 and B2 differ by 14.7 – 15.9% sequence divergence.

Lineage A is composed of five minor lineages. Lineage A1, from Area C, differs from the remaining haplotypes in lineage A by 19.4 – 28.4% sequence divergence. Lineages A2 – A5 (found at OB18, Jimblebar, Hashimoto) form a well-supported group, but distances between haplotypes range from 15.5 – 28.4% sequence divergence.

Distances between lineages A and B ranged from 38.8 – 48.3% sequence divergence (Table 1).

Conclusions:

- It is likely that there are three species of araneomorphs in the four specimens from Minister's North:
    - Species 1: EXR0968 and EXR1148 J20-4 (lineage B4); form a distinct lineage differing from other haplotypes in lineage B by approximately 11 – 25%
    - Species 2: EXR1148 J20-3 (lineage B5); likely to belong to the same species as the specimens from PSC0165R (Packsaddle 2), but differs from other haplotypes in lineage B by approximately 19 – 26% sequence divergence.
    - Species 3: EXR0969 (lineage B6); differs from other haplotypes in lineage B by approximately 25 – 31% sequence divergence.

- The remaining haplotypes in lineage B fall into three groups that likely correspond to three species. B3 contains specimens from Area C, Packsaddle and Jinaryi (lineage B3), which show increasing genetic distance with geographic distance up to about 8.2% sequence divergence. This type of pattern is typical of limited gene flow between populations in which there are no clear geographical barriers to dispersal.
- Lineage A shows a similar pattern but with greater incremental increases in genetic distance, up to about 29%. We would regard this group as containing at least two species due to the large genetic distance between GDR0070 and the remaining haplotypes. Lineages A2 to A5 may also correspond to distinct species due to the large genetic distances between them over a relatively short geographic distance – the five lineages occur at Hashimoto, Jimblebar, and OB18. Particularly striking is the occurrence of lineages A3 to A5 at Jimblebar.
- To place these results in context, a comparison of 29 spider genera from 24 families showed that on average, species differed by 6.5 – 23.1 % sequence divergence at COI (Barrett & Hebert, 2005). Among 37 different spider species, intraspecific variation ranged from 0-7.4%. It is important to note that these measures were obtained from surface-dwelling spiders.

References

Barrett, R.D.H. and Hebert, P.D.N. (2005). Identifying spiders through DNA barcodes. *Canadian Journal of Zoology*, **83**: 481-491.

Table 1. Pair-wise sequence divergence (uncorrected p-distances) for a 372 bp fragment of 12s. Distances within lineage B are highlighted in yellow, distances within lineage A are highlighted in turquoise. Unshaded areas represent distances between lineages A and B.

	1	2	3	4	5	6	7	8	9	10	11	12
[1] FG1100	x											
[2] WH051	0.017	x										
[3] EJR0247	0.159	0.155	x									
[4] EJP0232	0.151	0.147	0.009	x								
[5] JIN0070R	0.129	0.142	0.147	0.138	x							
[6] BP013	0.138	0.142	0.155	0.147	0.082	x						
[7] GD0080R	0.121	0.125	0.138	0.129	0.065	0.017	x					
[8] Gnaphosidae	0.121	0.125	0.138	0.129	0.065	0.017	0.003	x				
[9] EXR0968	0.159	0.155	0.147	0.138	0.125	0.125	0.108	0.108	x			
[10] EXR1148 J20-4	0.168	0.164	0.155	0.147	0.125	0.125	0.108	0.108	0.009	x		
[11] EXR1148 J20-3	0.241	0.237	0.259	0.250	0.228	0.237	0.220	0.220	0.190	0.190	x	
[12] PSC0165R_J11-11	0.254	0.250	0.263	0.254	0.233	0.241	0.224	0.224	0.203	0.203	0.043	x
[13] PSC0165R_J11-10	0.254	0.250	0.263	0.254	0.233	0.241	0.224	0.224	0.203	0.203	0.043	0.000
[14] PSC0165R_J11-12	0.254	0.250	0.263	0.254	0.233	0.241	0.224	0.224	0.203	0.203	0.043	0.000
[15] EXR0969	0.284	0.293	0.310	0.302	0.293	0.289	0.272	0.272	0.259	0.254	0.246	0.267
[16] GDR0070	0.401	0.401	0.414	0.405	0.388	0.405	0.388	0.388	0.392	0.388	0.392	0.401
[17] EJR0237	0.422	0.422	0.448	0.440	0.409	0.418	0.401	0.401	0.414	0.409	0.409	0.418
[18] EJ003R	0.422	0.422	0.448	0.440	0.409	0.418	0.401	0.401	0.414	0.409	0.409	0.418
[19] EJ0391R	0.414	0.414	0.448	0.440	0.401	0.409	0.392	0.392	0.409	0.405	0.409	0.418
[20] Ecowise	0.405	0.405	0.427	0.418	0.414	0.440	0.422	0.422	0.435	0.435	0.453	0.444
[21] EXR0793	0.409	0.409	0.461	0.453	0.431	0.435	0.418	0.418	0.457	0.453	0.444	0.453
[22] EXR0791	0.409	0.409	0.461	0.453	0.431	0.435	0.418	0.418	0.457	0.453	0.444	0.453
[23] EXR0980	0.414	0.414	0.466	0.457	0.435	0.440	0.422	0.422	0.461	0.457	0.453	0.457
[24] FG633	0.427	0.427	0.483	0.474	0.466	0.461	0.444	0.444	0.483	0.478	0.466	0.483
[25] MCM0120	0.427	0.427	0.474	0.466	0.440	0.461	0.444	0.444	0.470	0.466	0.453	0.466
[26] HH0118R	0.427	0.431	0.470	0.461	0.435	0.457	0.440	0.440	0.466	0.461	0.453	0.466

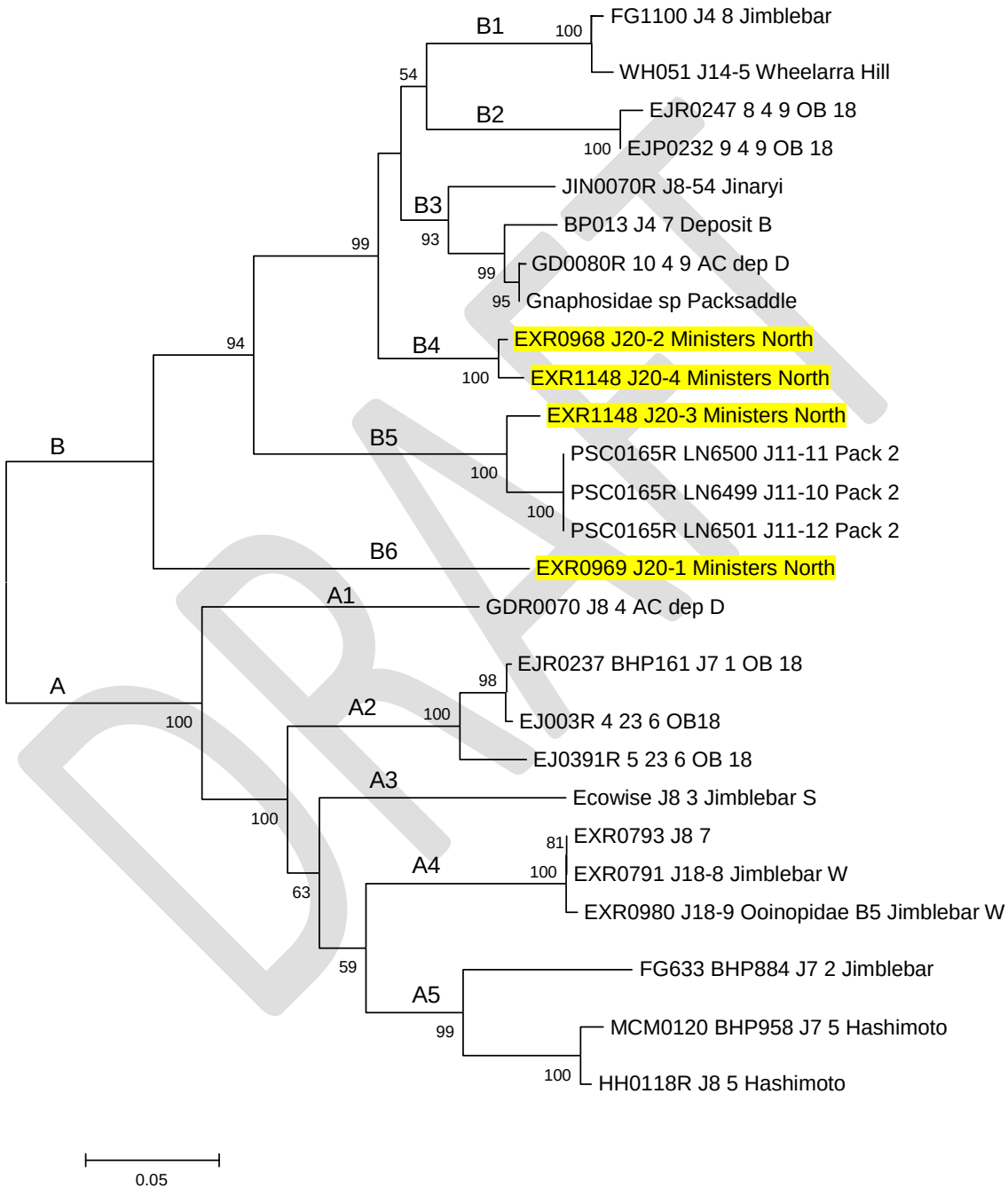
Table 1, cont'd.

	13	14	15	16	17	18	19	20	21	22	23
[1] FG1100											
[2] WH051											
[3] EJR0247											
[4] EJP0232											
[5] JIN0070R											
[6] BP013											
[7] GD0080R											
[8] Gnaphosidae											
[9] EXR0968											
[10] EXR1148 J20-4											
[11] EXR1148 J20-3											
[12] PSC0165R_J11-11											
[13] PSC0165R_J11-10	x										
[14] PSC0165R_J11-12	0.000	x									
[15] EXR0969	0.267	0.267	x								
[16] GDR0070	0.401	0.401	0.349	x							
[17] EJR0237	0.418	0.418	0.388	0.194	x						
[18] EJ003R	0.418	0.418	0.388	0.198	0.004	x					
[19] EJ0391R	0.418	0.418	0.392	0.211	0.043	0.039	x				
[20] Ecowise	0.444	0.444	0.435	0.280	0.224	0.228	0.228	x			
[21] EXR0793	0.453	0.453	0.401	0.233	0.159	0.164	0.177	0.211	x		
[22] EXR0791	0.453	0.453	0.401	0.233	0.159	0.164	0.177	0.211	0.000	x	
[23] EXR0980	0.457	0.457	0.405	0.237	0.168	0.172	0.185	0.211	0.009	0.009	x
[24] FG633	0.483	0.483	0.405	0.284	0.194	0.198	0.190	0.216	0.164	0.164	0.172
[25] MCM0120	0.466	0.466	0.405	0.259	0.190	0.194	0.198	0.190	0.155	0.155	0.155
[26] HH0118R	0.466	0.466	0.405	0.263	0.198	0.203	0.207	0.194	0.168	0.168	0.168

Table 1, cont'd.

	24	25	26
[1] FG1100			
[2] WH051			
[3] EJR0247			
[4] EJP0232			
[5] JIN0070R			
[6] BP013			
[7] GD0080R			
[8] Gnaphosidae			
[9] EXR0968			
[10] EXR1148 J20-4			
[11] EXR1148 J20-3			
[12] PSC0165R_J11-11			
[13] PSC0165R_J11-10			
[14] PSC0165R_J11-12			
[15] EXR0969			
[16] GDR0070			
[17] EJR0237			
[18] EJ003R			
[19] EJ0391R			
[20] Ecowise			
[21] EXR0793			
[22] EXR0791			
[23] EXR0980			
[24] FG633	x		
[25] MCM0120	0.112	x	
[26] HH0118R	0.134	0.022	x

Figure 1. Neighbour-joining tree of uncorrected p-distances for a 372 bp alignment of 12s. Letters on branches correspond to lineages as referred to in the text, and correspond to designations assigned in a previous report for consistency. Numbers on branches represent bootstrap support over 500 iterations. New specimens highlighted in yellow.



Appendix 11. Coleoptera (*Anillina*) Molecular Genetic Analyses. Dr. T. Finston 9-Nov-2009.

Methods:

- 481 bp fragment 16s, 775 bp fragment COI
- Uncorrected p-distances
- Neighbour-joining tree
- 500 bootstrap replicates
- Genbank outgroup specimens:

Results:

The Pilbara specimens formed two clades that were distinct to all outgroup haplotypes. The Minister's North specimen clustered with the *Illaphanus* specimens from Yarrie and Quarry 8 in both 16s (Figure 1) and COI (Figure 2). The support for this grouping was moderate at 16s, and weaker at COI. The Minister's North specimen differed from the *Illaphanus* haplotypes by 11.7 to 14.7% at 16s (Table 1), and 16.3 to 16.7% at COI (Table 2). The haplotypes from Packsaddle and Area C differed from one another by 3.0% at 16s (Table 1). The Packsaddle and Area C haplotypes differed from the Minister's North *Anillina* specimen by 12.1 to 13.4% and from the *Illaphanus* haplotypes by 12.1 to 15.3% (Table 1).

Conclusions:

The specimen from Minister's North appears to represent a distinct species, given the relatively high level of genetic divergence between it and the other carabid haplotypes and the apparent morphological differences (the Minister's North specimen was identified as *Anillina*, whereas the Yarrie/Quarry 8 specimens were identified as *Illaphanus)*. Sampling from additional sites is required to fully assess the distribution of the species.

Table 1. Percent sequence divergence (uncorrected p-distances) among haplotypes for 16s.

	1	2	3	4	5	6	7
[1] Pamborus_brisbanensis							
[2] Pamborus_viridis	0.006						
[3] Pamborus_macleayi	0.009	0.006					
[4] Metius_malachiticus	0.089	0.085	0.087				
[5] Notaphus_sp	0.083	0.083	0.081	0.074			
[6] Lophyridia_chloris	0.130	0.128	0.123	0.143	0.130		
[7] Cicindela_togata	0.134	0.132	0.128	0.138	0.128	0.026	
[8] GFR010	0.132	0.126	0.126	0.117	0.123	0.147	0.145
[9] PSF0019R	0.126	0.119	0.119	0.111	0.109	0.136	0.136
[10] EXR1154	0.136	0.134	0.128	0.145	0.121	0.162	0.157
[11] LN1735_EXR1622	0.136	0.134	0.132	0.128	0.132	0.155	0.160
[12] LN6119_CA0021	0.157	0.155	0.157	0.134	0.136	0.160	0.162
[13] LN1368_CA0113	0.160	0.157	0.160	0.136	0.138	0.162	0.164

	8	9	10	11	12	13
[1] Pamborus_brisbanensis						
[2] Pamborus_viridis						
[3] Pamborus_macleayi						
[4] Metius_malachiticus						
[5] Notaphus_sp						
[6] Lophyridia_chloris						
[7] Cicindela_togata						
[8] GFR010						
[9] PSF0019R	0.030					
[10] EXR1154	0.153	0.145				
[11] LN1735_EXR1622	0.134	0.121	0.117			
[12] LN6119_CA0021	0.147	0.130	0.145	0.111		
[13] LN1368_CA0113	0.149	0.132	0.147	0.113	0.002	

Table 2. Percent sequence divergence (uncorrected p-distances) among haplotypes for COI.

	1	2	3	4	5	6	7
[1] CA0021							
[2] EXR1622	0.132						
[3] EXR1154RC	0.163	0.167					
[4] Metius malachiticus	0.146	0.151	0.154				
[5] Bembidion coxendix	0.160	0.160	0.130	0.112			
[6] Bembidion honestum	0.142	0.149	0.134	0.095	0.080		
[7] Lophyridia chloris	0.192	0.196	0.189	0.168	0.168	0.160	
[8] Notaphus sp	0.154	0.172	0.177	0.136	0.141	0.126	0.166

Figure 1. Neighbour-joining tree of uncorrected p-distances among haplotypes of Carabidae for 16s. Similar Genbank sequences included as outgroups. Numbers on branches show bootstrap support over 500 iterations.

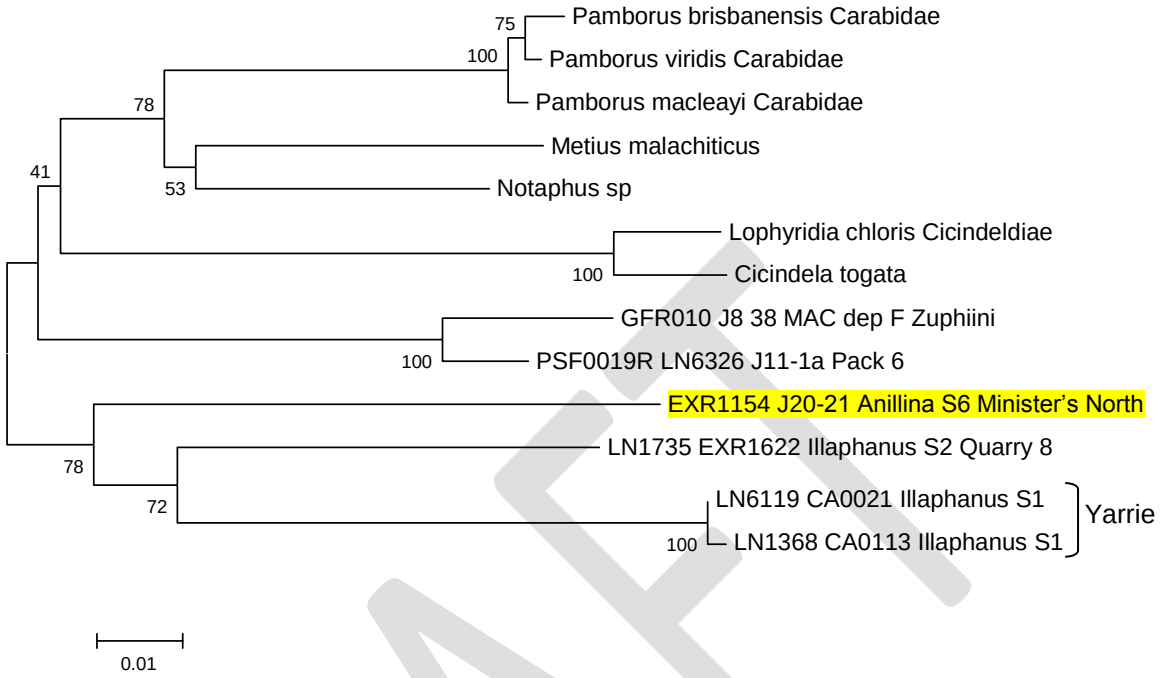
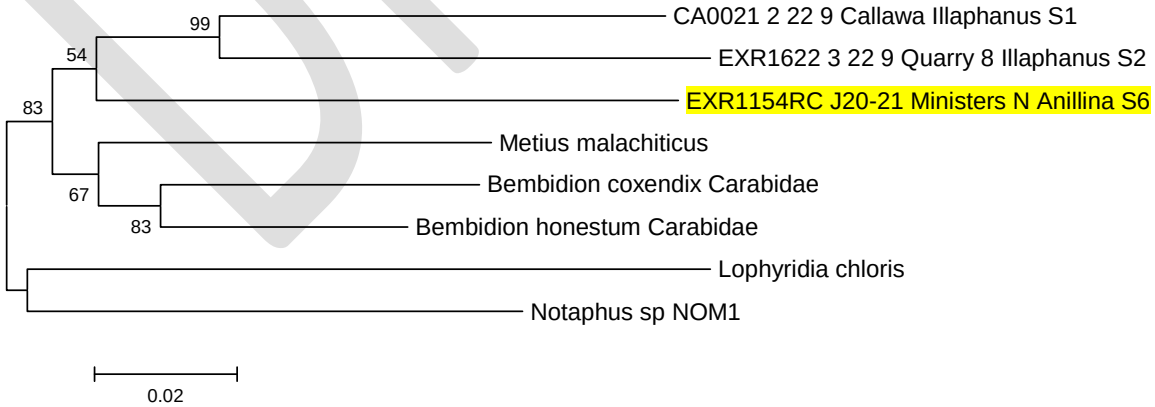


Figure 2. Neighbour-joining tree of uncorrected p-distances among haplotypes of Carabidae for COI. Similar Genbank sequences included as outgroups. Numbers on branches show bootstrap support over 500 iterations.



Appendix 12. Scolopendrida (Cryptopidae) Molecular Genetic Analyses. Dr. T. Finston 13-Oct-2009.

Methods:

- 372 bp fragment of 12s
- Uncorrected p-distances
- Neighbour-joining tree
- 500 bootstrap replicates

Results:

The new specimen of Scolopendrida from Minister's North occurred basally (externally) to a previously recognised clade (clade A; Figure 1). Bootstrap support for this relationship was high, however the haplotype differed from the remaining haplotypes in clade A by 15.7 to 16.9% sequence divergence (Table 1).

Conclusions:

The specimen from Minister's North, while showing affinities to specimens in clade A, is likely to be a distinct species, due to the high sequence divergence between it and the other haplotypes in clade A.

Table 1. Genetic divergence (uncorrected p-distances) between haplotypes of Scolopendromorpha for a 372 bp fragment of 12s.

	1	2	3	4	5	6	7	8	9
[1] PSE0019R	x								
[2] JIN0069R	0.047	x							
[3] LB035	0.160	0.171	x						
[4] MN0013R	0.160	0.169	0.157	x					
[5] PSA0382R	0.318	0.331	0.323	0.326	x				
[6] EA0310R	0.312	0.329	0.320	0.318	0.030	x			
[7] EXR729	0.367	0.359	0.359	0.351	0.251	0.251	x		
[8] EXR726	0.367	0.359	0.359	0.351	0.251	0.251	0.000	x	
[9] GA0167R	0.445	0.464	0.436	0.414	0.448	0.436	0.423	0.423	x

Figure 1. Neighbour-joining tree of uncorrected p-distances between haplotypes of Scolopendromorpha for a 372 bp fragment of 12s. Numbers on branch nodes represent bootstrap support >50%. New specimen highlighted in yellow.