

TROPICANA GOLD PROJECT ADDITIONAL INFORMATION ON MYGALOMORPH SPIDERS & DNA STUDY

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TROPICANA GOLD PROJECT

Additional information on Mygalomorph Spiders & DNA Study

Tropicana Joint Venture





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EXECUTIVE SUMMARY

The Tropicana Joint Venture (TJV) is currently working through the relevant State and Federal Environmental Assessment processes associated with the proposed Tropicana Gold Project (Project), which is centred on the Tropicana and Havana gold prospects. The proposed TGP Operational Area is located approximately 330 km east north-east of Kalgoorlie, and 15 km west of the Plumridge Lakes Nature Reserve, on the western edge of the Great Victoria Desert biogeographic region of Western Australia.

Ecologia Environment (ecologia) was contracted in 2006 to conduct surveys for short range endemic invertebrates (SRE) in the proposed Operational Area. Trapping conducted in 2006 and 2008 and foraging conducted in 2009 located all but one species (Kwonkan sp. 2) outside the Operational area footprint. Another species of mygalomorph spider (Aganippe sp. 4) has only bee located within 800 m of the Operational area footprint, therefore is potentially exposed to indirect impact from the Project.

During autumn 2009, further work was conducted with the aim of collecting the aforementioned species. This work focused on mapping their geographic distribution within and around the Operational Area and defining their preferred habitat. A broadened DNA study of all collected specimens of the genus *Aganippe* and *Anidiops* was also conducted using the mitochondrial gene for cytochrome c oxidase 1 (COI) in order to unravel their phylogenetic relationships and provide additional information for a comparison with morphological taxonomy.

In the absence of live specimens of *Kwonkan* sp. 2, focus has been given to the identification of suitable habitat of the species and its geographic distribution. The habitat was identified using information from 2006 survey, published literature and comparisons with other species located onsite. Three habitat types were identified as suitable for *Kwonkan* sp. 2, of which two occurred both inside and outside the Operational footprint and one that occurs fully outside the footprint. None of these habitats were classified as 'island habitats' (isolated from similar types of soil and vegetation by environment deemed inhospitable to the species). The habitats identified were fully overlapping with the habitat of *Kwonkan* sp. 1 and partially overlapping with habitats of *Aganippe* sp. 2/7 and a new species, *Swolnpes darwini*. Given this similarity, the spatial distribution of *Kwonkan* sp. 2 is most likely to follow the same patterns as those of *Kwonkan* sp. 1, *Aganippe* sp. 2/7 and *Swolnpes darwini*. It was, therefore, likely that *Kwonkan* sp. 2 will be only partially impacted by the proposed Project, and this impact will not be significant to the species.

In order to ensure that the habitat of *Aganippe* sp. 4 was available further away from the impact area, transects were walked west and north-west from the site where it was previously recorded (T16). The results showed that the habitat extended a minimum of 2 km beyond the Operational footprint. Due to it's proximity to the operational area, and to ensure the species is not subject to indirect impact, DEC suggested that a monitoring programme for this habitat is designed (K. Brennan, DEC, pers. comm.). Three monitoring sites of the same habitat type are suggested for the monitoring:

- 1. The original site of *Aganippe* sp. 4 (T16), 800 m from the proposed Operational footprint,
- 2. A site north of original site of *Aganippe* sp. 4, approx 2 km outside the western boundary of the Operational footprint, and



3. A site south of original site of *Aganippe* sp. 4, approx. 2 km south of the southern boundary of the proposed Operational footprint

The monitoring programme will consist of:

- (i) Annual sensus of all mygalomorph burrows present (details to be discussed with Prof. B.Y. Main and DEC)
- (ii) Vegetation monitoring (such as. remote sensing of vegetation or a quadrat vegetation assessment; details to be discussed with DEC)
- (iii) Ground cover monitoring (i.e. quadrat leaf litter assessment; details to be discussed with DEC)



1.0 INTRODUCTION

1.1 Project Background

The Tropicana Joint Venture (TJV) is currently working through the relevant State and Federal Environmental Assessment processes associated with establishing the Tropicana Gold Project (TGP), which is centred on the Tropicana and Havana gold prospects. The proposed TGP Operational Area is located approximately 330 km east north-east of Kalgoorlie, and 15 km west of the Plumridge Lakes Nature Reserve, on the western edge of the Great Victoria Desert biogeographic region of Western Australia (

Figure 1). The Tropicana and Havana prospects represent the first gold resource discovered in this remote portion of Western Australia. Drilling to date suggests that the resource represents a multi-million ounce discovery within a new Greenfields gold province. The Project is a joint venture between AngloGold Ashanti Australia Limited (70% stakeholder and Manager) and the Independence Group NL (30% stakeholder).

The TGP consists of three main components (

Figure 1):

- operational area this area contains the mine, processing plant, aerodrome, village and other associated infrastructure;
- water supply area two basins have been investigated, the Minigwal Trough and Officer Basin; and
- infrastructure corridor two options are under consideration (Tropicana/Transline and Pinjin Road options).

After a series of surveys for short range endemic invertebrates conducted in 2006, 2008 and 2009, one mygalomorph spider species, *Kwonkan sp.* 2 (Figure 2), remains to be located outside the proposed Operational Area footprint, and one mygalomorph spider species, *Aganippe* sp. 4, was located within 800 m of the proposed Operational footprint and thus may be subject to some indirect impact.

Ecologia Environment (ecologia) was commissioned to conduct further work in 2009 with the aim of locating the aforementioned species, mapping their geographic distribution within and around the Operational Area and defining their preferred habitat. A broadened DNA study of all hand-collected specimens (including specimens from a regional area) was also conducted using the mitochondrial gene for cytochrome c oxidase 1 (COI) in order to unravel their phylogenetic relationships and provide additional information for a comparison with morphological taxonomy. Part A of the report summarises results of field surveys undertaken in 2006, 2008 and 2009, including the analysis of the suitable habitat, and Part B describes the DNA study of the hand-collected specimens

1.2 Survey Objectives

The objectives of the Environmental Protection Authority (EPA) with regards to invertebrate fauna management are to:

• maintain the abundance, species diversity and geographical distribution of short range endemic terrestrial invertebrate fauna; and



• protect Specially Protected (Threatened) fauna, consistent with the provisions of the *Wildlife Conservation Act 1950*.

Hence, the purpose of this survey was to provide sufficient information to allow the EPA to assess the impact of the TGP located within the operational area on *Kwonkan* sp. 2 and *Aganippe* sp. 4.



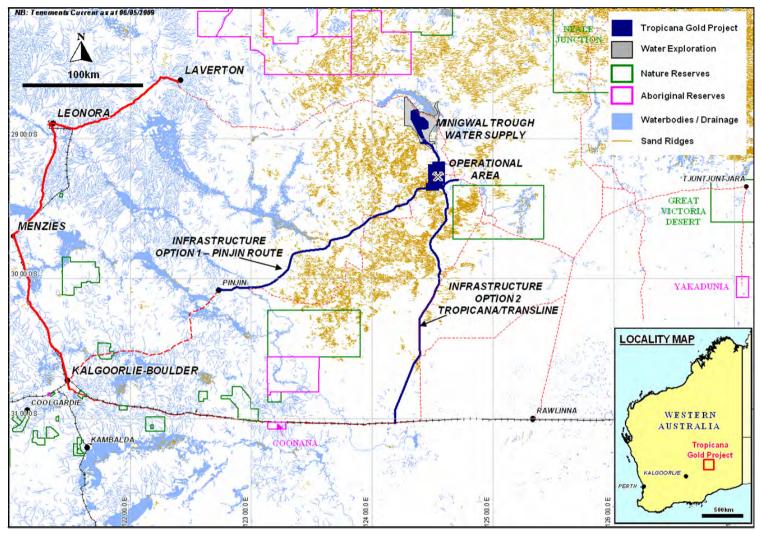


Figure 1 Location Plan of the Tropicana Gold Project in Western Australia





Figure 2 Kwonkan sp. 2, female



2.0 BIOPHYSICAL ENVIRONMENT

2.1 Climate

The operational area is located 330 km east-north-east of Kalgoorlie and 220 km east of Laverton. Average weather conditions in the area can be interpreted from data collected at weather stations in Laverton (to the north-west) and Balgair (to the south-east). A summary of the data is provided in Figure 3 and Table 1).

The climate is described as arid, with summer and winter rain averaging 100 –180 mm (Barton *et al.* 2001b, a). The majority of rainfall occurs during the summer months between January and April and is generally associated with cyclonic rainfall extending inland.

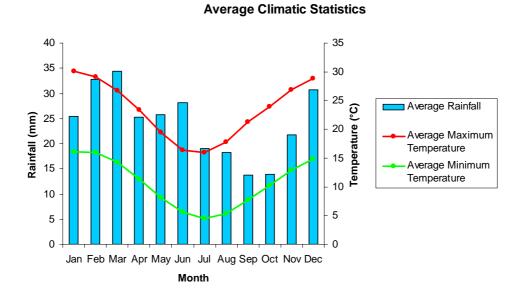


Figure 3 Combined Average Climatic Statistics for Balgair and Laverton 1983 – 2009 (http://www.bom.gov.au/)

 Table 1
 Climatic Statistics for Balgair and Laverton

Statistic	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Average Monthly Records Balgair (1983 – 2009)												
Max Temp (°C)	32.9	31.9	29.2	26.1	22.4	19.1	18.6	20.5	24.2	26.8	29.2	30.9
Min Temp (°C)	16.3	16.6	14.7	11.9	9	6.2	5.1	5.9	8.2	10.5	12.8	14.7
Rainfall (mm)	20.2	27.3	29.9	21.4	21.6	25.3	17	18.3	16	16.2	23.9	36.2
Average Month	Average Monthly Records Laverton (1983 – 2009)											
Max Temp (°C)	35.8	34.8	31.9	27.2	22.1	18.5	17.8	20	24.5	28	32.1	34.9
Min Temp (°C)	20.5	20	18	13.9	9.5	6.6	5.2	6.4	9.5	12.8	16.6	19.3
Rainfall (mm)	24.4	30	30.4	22.8	23.5	23.9	16.4	13.5	8.1	8.3	14.1	17.6
Combined Aver	Combined Average Records for Balgair and Laverton											
Max Temp (°C)	34.35	33.35	30.55	26.65	22.25	18.8	18.2	20.25	24.35	27.4	30.65	32.9
Min Temp (°C)	18.4	18.3	16.35	12.9	9.25	6.4	5.15	6.15	8.85	11.65	14.7	17
Rainfall (mm)	22.3	28.65	30.15	22.1	22.55	24.6	16.7	15.9	12.05	12.25	19	26.9



2.2 Biogeography

The operational area of the TGP is located within the Great Victoria Desert bioregion, as defined by Thackway and Cresswell in the Interim Biogeographic Regionalisation of Australia (IBRA) Version 6.1. On a finer scale, it is located on the northern border of the Central and Shield sub-regions of the Great Victoria Desert.

The Central region of the Great Victoria Desert is described as an:

"arid active sand-ridge desert with extensive dune fields of deep Quaternary aeolian sands overlying Permian strata of the Gunbarrel Basin. Landforms consist of salt lakes and major valley floors with lake derived dunes. Sand plains with extensive seif dunes running east west, occasional outcropping (breakaways) and quartzite hills provide minor relief" (Barton et al. 2001a).

The subregion covers an area of 14,286,995 ha, of which 9.11 % is vested in conservation estates.

2.3 Flora and Vegetation

The operational area is situated in the Helms Botanical District, near the border of the Great Victoria Desert and the Nullarbor Plain, within the Eremaean Botanical Province. At a broad scale, Beard (1975) described three distinct vegetation units within close proximity to and including the Tropicana Gold Exploration Area:

- 1. Acacia aneura (mulga) low woodland between sand ridges;
- 2. Tree (*Eucalyptus gongylocarpa, E. youngiana*) and shrub steppe between sand hills with hummock grassland (*Triodia basedowii*); and
- 3. Acacia aneura / Casuarina cristata (C. pauper) woodland (Mulga and sheoak).

The survey area is primarily located within the Mulga between sandhill complexes with longitudinal sand ridges.



3.0 METHODS

The survey methods adopted by the *ecologia* biologist have been developed in consultation with Prof. B.Y. Main, The University of Western Australia.

3.1 Sampling Methods

Two wet pitfall trapping surveys were conducted in 2006 and 2008 and three foraging surveys were conducted in March, May and September 2009. In order to determine habitat preference of each of the targeted species, transects were walked through various habitats with two people walking in parallel lines approximately 10-15 m apart. If a spider burrow was found, photographs were taken of the habitat and the burrow exterior. Some specimens were excavated for morphological identification and DNA analysis, in which case the burrow lid and food debris were also collected as these could be useful for taxonomic identification and information on behaviour. Apart from the target species, specimens of other *Aganippe* species (e.g. *Aganippe* sp. 7) were also collected to enable comparisons of habitat and DNA divergence.

3.2 External Support

Professor Barbara York Main, an expert taxonomist for mygalomorph spiders, was involved in the September 2009 field work (Figure 4) and in the morphological identification of all collected specimens.

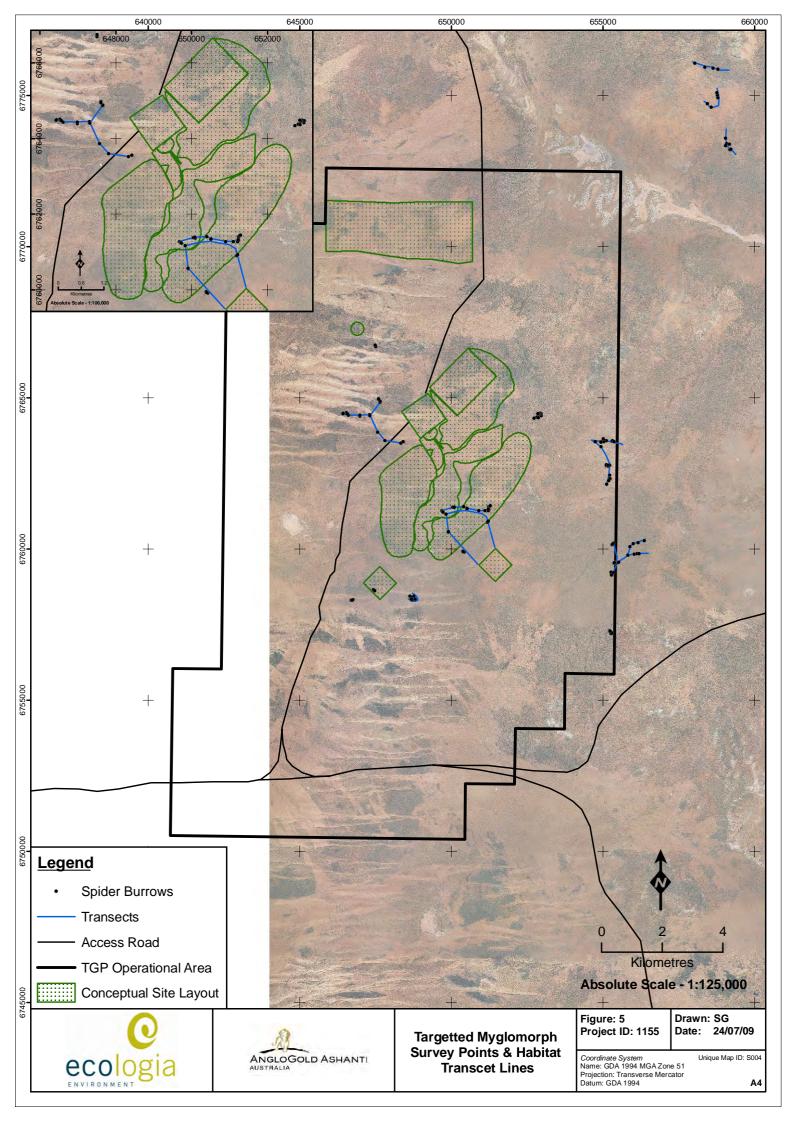


Figure 4 Prof. B.Y. Main (UWA) and Peter Bouteloup (AAGA) at Tropicana in September 2009

3.3 Site Selection and Habitat Mapping

Locations of transects were selected based on the data from the 2006 and 2008 surveys (see the main SRE report) and in the field. All transects walked are shown in Figure 5. Locations of all habitats and relevant species identified were overlaid on a vegetation map so connectivity of habitats could be assessed.





PART A - SPECIES AND HABITAT ASSESSMENT



4.0 RESULTS AND DISCUSSION

4.1 Overview

Following surveys in 2006 and 2008, a total of 90 spider burrows were located in 2009, of which 25 were excavated and spiders were collected (including burrow tops and food debris). All 25 collected specimens were identified by traditional morphological characters, and 21 of the 25 specimens were analysed for DNA. The remaining 66 spiders were not disturbed in order to avoid negative impact on existing populations, but they were identified using burrow characteristics. Table 2 shows the identification and location of spiders that were hand collected in 2009, while Figure 7, Figure 8 and Appendix 2 show compilation of all locations of relevant species known to date, including data from 2006 and 2008 surveys. With a single exception, each point in Figure 7 (and Figure 8) represents a record of multiple specimens (2-7 specimens). It is also important to note that, unlike species in other parts of WA (e.g. mid-west, south-west, Pilbara), none of the species at Tropicana formed tight family clusters. The highest number of individuals found in immediate proximity to each other was two. Intraspecific burrows of twig-lining species (i.e. Aganippe sp. 2/7, Anidiops sp.) were separated by distances of 10-100 m, which indicates that spiderlings of these species are capable of dispersing over these relatively large distances. The typical distances between non-twiglining Aganippe (i.e. Aganippe sp. 1/8) were shorter (1-20 m), indicating that spiderlings of this species disperse less. However, these distances are still in stark contrast to other aganipines such as Aganippe occidentalis, Aganippe cupulifex, Idiosoma nigrum or Gaius villosus, whose spiderlings usually disperse few centimetres only.

Interestingly, a single specimen of a new species, *Yilgarnia* sp. from the family Nemesidae, was collected outside the operational footprint (654945E, 6763554N) during the targeted survey in September 2009. This species was not collected during surveys in 2006 and 2008, however it is not impacted by the Project.

4.2 Habitat Preference

Kwonkan sp. 2

Despite the repeated efforts during the trapping survey in 2008 and foraging surveys in March, May and September 2009, no live *Kwonkan* sp. 2 specimens could be collected. The following summary is based on literature, information from Prof. B.Y. Main, habitat assessment and similarities with other species found within the Operational Area.

There are currently six described species of *Kwonkan* in Australia: *K. wonganensis*, *K. anatolion*, *K. eboracum*, *K. goongarriensis*, *K. moriartii*, and *K. silvestris* (Main 1983). Only one of these species, *K. wonganensis*, prefers open ground of pebbly loam soils and constructs a turret of pebbles and soil at entrance resembling burrows of wolf spiders. The remaining species are found in loamy to sandy soils within the leaf litter of open Eucalyptus woodlands or heath (Main 1983). Burrows comprise a stocking-like silk tube loosely adhering to a shallow burrow wall. The opening forms a collapsible tube often with attached debris which, if in leaf litter, is extremely difficult to see. Only a handful of *Kwonkan* burrows have been recorded to date in WA due to their extremely cryptic nature, with many found accidentally while searching for burrows of other mygalomorph spiders (B. Y. Main, pers. comm.).



The site where the *Kwonkan* sp. 2 was collected in 2006 (T4) is located within the larger vegetation unit of low woodland; *Acacia aneura* (Beard 1975). On a small scale, however, *Acacia aneura* is interspersed with open low woodland of *Eucalyptus gongylocarpa*, over *Eucalyptus trivalvis* sparse mallee, over open low shrubs and *Triodia basedowii* moderately dense hummock grassland, or occasional *Casuarina* sp., with moderate leaf litter, mainly under trees and sparse wood litter, and with orange sand soils. For the purpose of this report, the habitat types were called:

- sandy, eucalypts;
- sandy, acacias; and
- sandy, she-oak & eucalypts (Table 3).

Two of these habitat types occurred both inside and outside the proposed Operational footprint and one occurred only outside the proposed Operational footprint. Transects walked from the original site T4 north-east, east, and south-east, across the proposed eastern waste landform and out of the proposed footprint, showed that pockets of these habitats extend a minimum of 6 km beyond the impact area (Figure 9, Figure 10 and Appendix 1). None of these habitats were classified as 'island habitats' (isolated from similar types of soil and vegetation by environment deemed inhospitable to the species). The habitats were fully overlapping with the habitat of *Kwonkan* sp. 1 and partially overlapping and interspersed with pockets of the open low-lying areas subject to sheet-flooding dominated by *Triodia* that is preferred by the *Aganippe sp.* 1/8, and also by open *Casuarina sp.* and/or *Acacia aneura* woodland over open low shrubs and *Triodia basedowii* that is preferred by *Aganippe* sp. 2/7.

The low genetic diversity of both *Aganippe sp.* 1/8 (0.0-0.3%) and *Aganippe sp.* 2/7 (0.0-1.7%) (see part B) within this area presents evidence that no barriers to dispersal exist across these habitat pockets and that the individual spiders belong to a larger population extending over a minimum of several kilometres well beyond the proposed Operational Area footprint. It is, therefore, reasonable to assume that the population of *Kwonkan* sp. 2 in this area is subject to the same natural conditions and that it follows the same patterns of distribution and dispersal, both inside and outside the proposed Operational Area footprint.

This assumption is supported by the data on a geographic distribution of another species from the family Nemesidae, *Swolnpes darwini* gen. nov. sp. nov. (B.Y. Main, pers. comm. 2009) found at Tropicana in 2008 (Figure 7 and Figure 6 and further details in the original report on 2006 and 2008 surveys). This species, which was until recently unknown to science, prefers pockets of habitat with open *Casuarina* woodland and it inhabits areas outside the Proposed Operational footprint over a distance of 12 km (Figure 7, Figure 8 and Appendix 1).

In summary, the targeted survey of the habitat preferred by *Kwonkan* sp. 2 suggests that the species is not restricted to the proposed Operational Area footprint. The population of *Kwonkan* sp. 2 is, therefore, expected to be partially impacted by the proposed Project, however this impact is likely to be insignificant to the species.





Figure 6 Swolnpes darwini gen. nov. sp. nov collected at TGP (A – dorsal view, B – ventral view); scale bar 3 mm



Aganippe sp. 4

Aganippe sp. 4 was collected in 2006 at the original site T16, which was, at the time located within the proposed Operational Area footprint. The proposed footprint of the western waste landform was modified in 2009 in such a way that the site T16 is now located approximately 800 m west of its nearest boundary (Figure 11). The species Aganippe sp. 4 is therefore no longer directly impacted by the TGP. Given the proximity of the site to the infrastructure border, it was suggested that an additional habitat assessment should be conducted in case the site T16 is impacted indirectly by the project.

On the large scale, the site T16 lies within a low woodland *Acacia aneura* (mulga) vegetation unit (Beard 1975). On a small scale, twig-lined Idiopidae burrows (currently identified as *Anidiops* sp., although genetically closer to *Aganippe*, refer to Part B) were located within pockets of *Acacia aneura* thickets with loamy to sandy soils, surrounded by open woodland and / or low sand dunes. For the purpose of this report, this habitat was defined as:

• sandy, acacia thicket (Table 3)

In order to ensure that the habitat of this site was available further away from the proposed operational area, transects were walked west and north-west from the site T16. The results showed that the habitat extended a minimum of 2 km beyond the TGP mining and infrastructure footprint area (Figure 9, Figure 10 and Appendix 1).

Due to it's proximity to the operational area, DEC suggested that monitoring programme for this habitat is designed (K. Brennan, DEC pers. comm.). Three monitoring sites of the same habitat type were selected:

- Site 1 the original site of *Aganippe* sp. 4, 800 m from the proposed operational area footprint,
- Site 2 north of original site of *Aganippe* sp. 4, approx 2 km outside the western boundary of the proposed operational area footprint, and
- Site 3 south of original site of *Aganippe* sp. 4, ~2 km south of the southern boundary of the proposed operational area footprint

The monitoring programme will consist of:

- 1. Annual sensus of all mygalomorph burrows present (details to be discussed with Prof. B.Y. Main and DEC)
- 2. Vegetation monitoring (such as. remote sensing of vegetation or a; details to be discussed with DEC)
- 3. Ground cover monitoring (i.e. quadrat leaf litter assessment; details to be discussed with DEC)



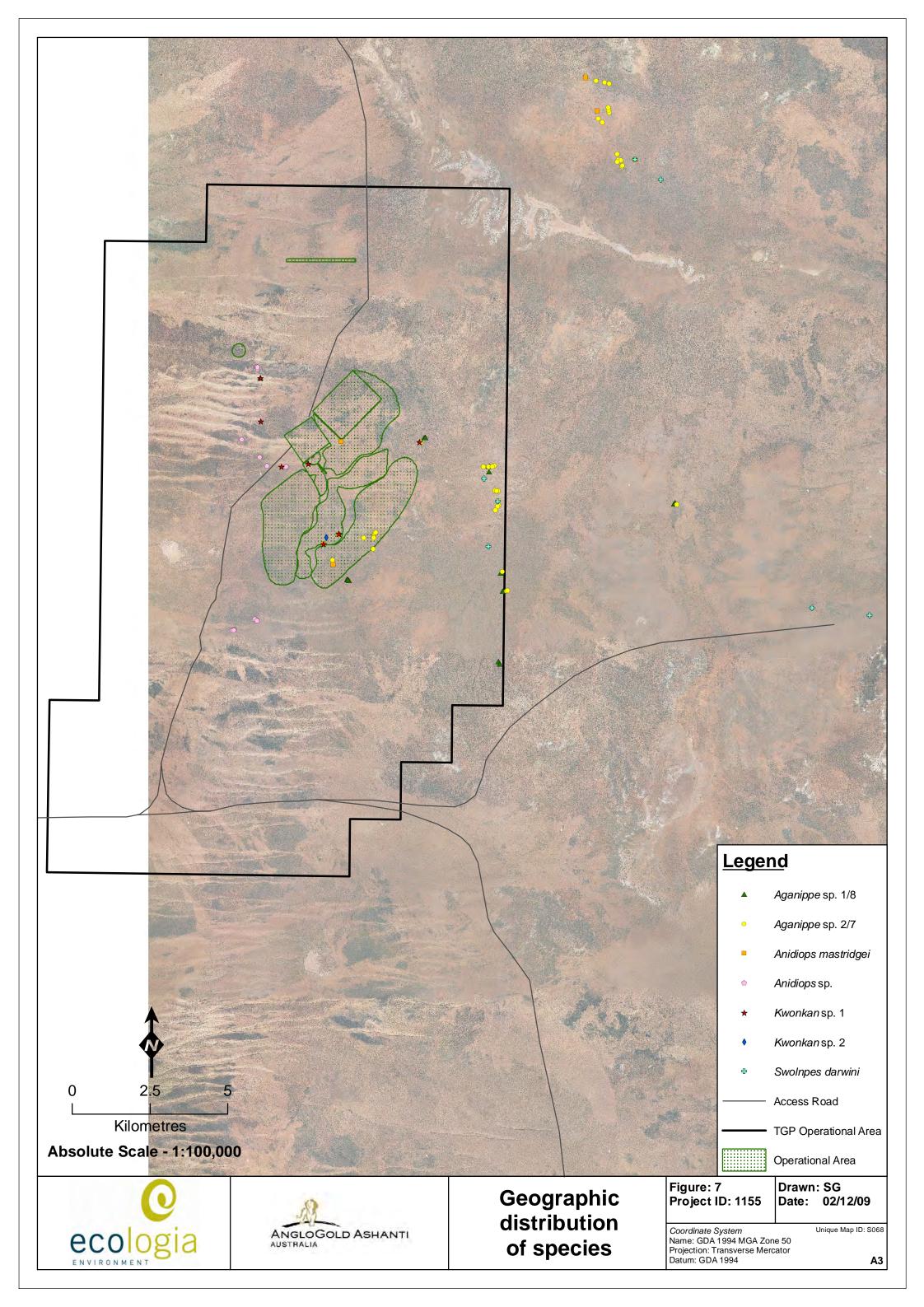
 Table 2
 A list of all specimens collected during the targeted surveys in March - September 2009

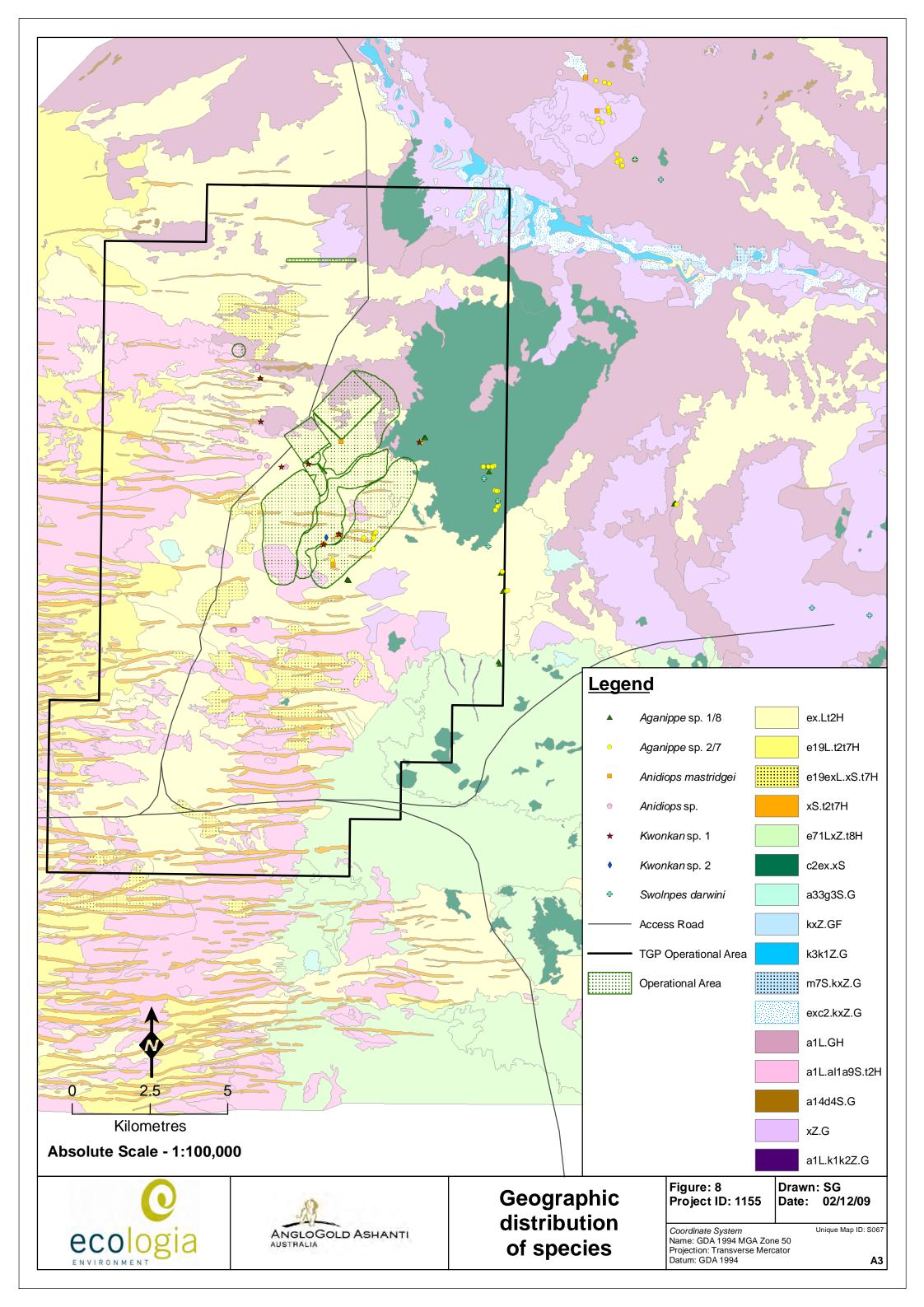
Aganippe sp. 2/7	twig-lined	Α	MDT05	sandy, eucalypts & acacias	E wastedump E of TROP 5	51J	650902.1	6761281
Aganippe sp. 2/7	twig-lined	Α	MDT08	loamy, acacia	E wastedump E of TROP 5	51J	651245.4	6761393
Aganippe sp. 2/7	twig-lined	Α	MDT12	sandy, eucalypts & acacias	E wastedump E of TROP 5	51J	649906.3	6760570
Aganippe sp. 2/7	twig-lined	Α	MDT20A	loamy, acacia	E wastedump E of TROP 5	51J	655502.9	6759556
Aganippe sp. 2/7	twig-lined	Α	MKT03	sandy, eucalypts & acacias	E wastedump E of TROP 5	51J	651224.3	6761273
Aganippe sp. 2/7	twig-lined	Α	MKT108	sandy, she-oak & acacias	TROP 45	51J	655176	6762783
Aganippe sp. 2/7	twig-lined	Α	MKT115	loamy open ground, she-oak & acacia	TROP 10 (Hat trick)	51J	652737.8	6764341
Aganippe sp. 2/7	twig-lined	Α	SCT01	loamy, acacia	E wastedump E of TROP 5	51J	651294.5	6761436
Aganippe sp. 2/7	twig-lined	Α	SCT06	loamy, acacia	E wastedump E of TROP 5	51J	655525.9	6759576
Aganippe sp. 2/7	twig-lined	В	MDT41	sandy, eucalypts & acacias	N of OA close to TROP 33	51J	658645.3	6775912
Aganippe sp. 2/7	twig-lined	В	MDT48	sandy, eucalypts & acacias	N of OA close to TROP 35	51J	659087.6	6773427
Aganippe sp. 2/7	twig-lined	В	SCT23	sandy, eucalypts & acacias	N of OA close to TROP 35	51J	659054.3	6773603
Aganippe sp. 2/7	twig-lined		MDT130	sandy, she-oak & acacias	TROP45	51J	655122.4	6762772
Aganippe sp. 1/8	mud-door	С	MDT114	loamy open ground, she-oak & acacia	TROP 10 (Hat trick)	51J	652887.2	6764485
Aganippe sp. 1/8	mud-door	С	MDT128	loamy open ground, she-oak & acacia	TROP 47	51J	654945.1	6763554
Aganippe sp. 1/8	mud-door	С	MDT14	loamy open ground, spinifex & acacia	E wastedump E of TROP 5	51J	650404.7	6759924
Aganippe sp. 1/8	mud-door	С	MDT17	loamy open ground, spinifex & acacia	Silhouette (SE of camp)	51J	655378.1	6759549
Aganippe sp. 1/8	mud-door	С	MDT54	loamy open ground, spinifex & acacia	Silhouette (SE of camp)	51J	655394.4	6759554
Aganippe sp. 1/8	mud-door	С	MKT114	loamy open ground, she-oak & acacia	TROP 10 (Hat trick)	51J	652861.3	6764488
Aganippe sp. 1/8	mud-door	С	SCT02	loamy open ground, spinifex & acacia	E wastedump E of TROP 5	51J	650403.1	6759919
Anidiops sp.	twig-lined	D	SCT14	sandy acacia thicket	W waste dump NE of TROP 16	51J	647814.2	6763597
Anidiops sp.	twig-lined	Е	MDT30	sandy acacia thicket	W waste dump NE of TROP 16	51J	647568.3	6763874
Anidiops sp.	twig-lined		SCT13	sandy acacia thicket	W waste dump NE of TROP 16	51J	648418.1	6763565
Anidiops mastridgei	twig-lined		MDT01	sandy, eucalypts & acacias	SE havana, TROP 5	51J	650098.3	6761371
Yilgarnia sp.	open		MDT201	loamy open ground, she-oak & acacia	TROP 47	51J	654945.1	6763554

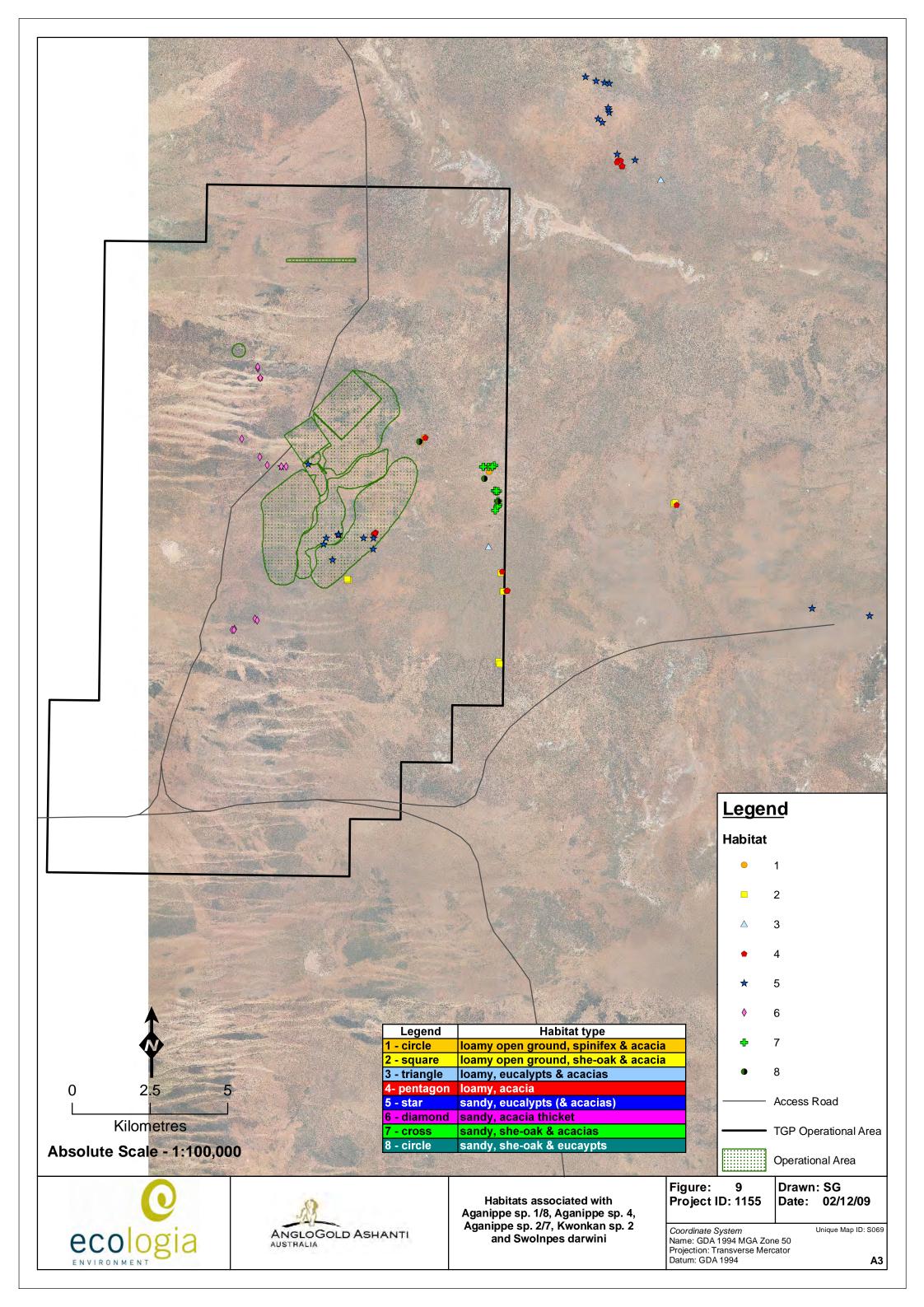
 Table 3
 Association between species and habitats (legend as in Figures 9 and 10)

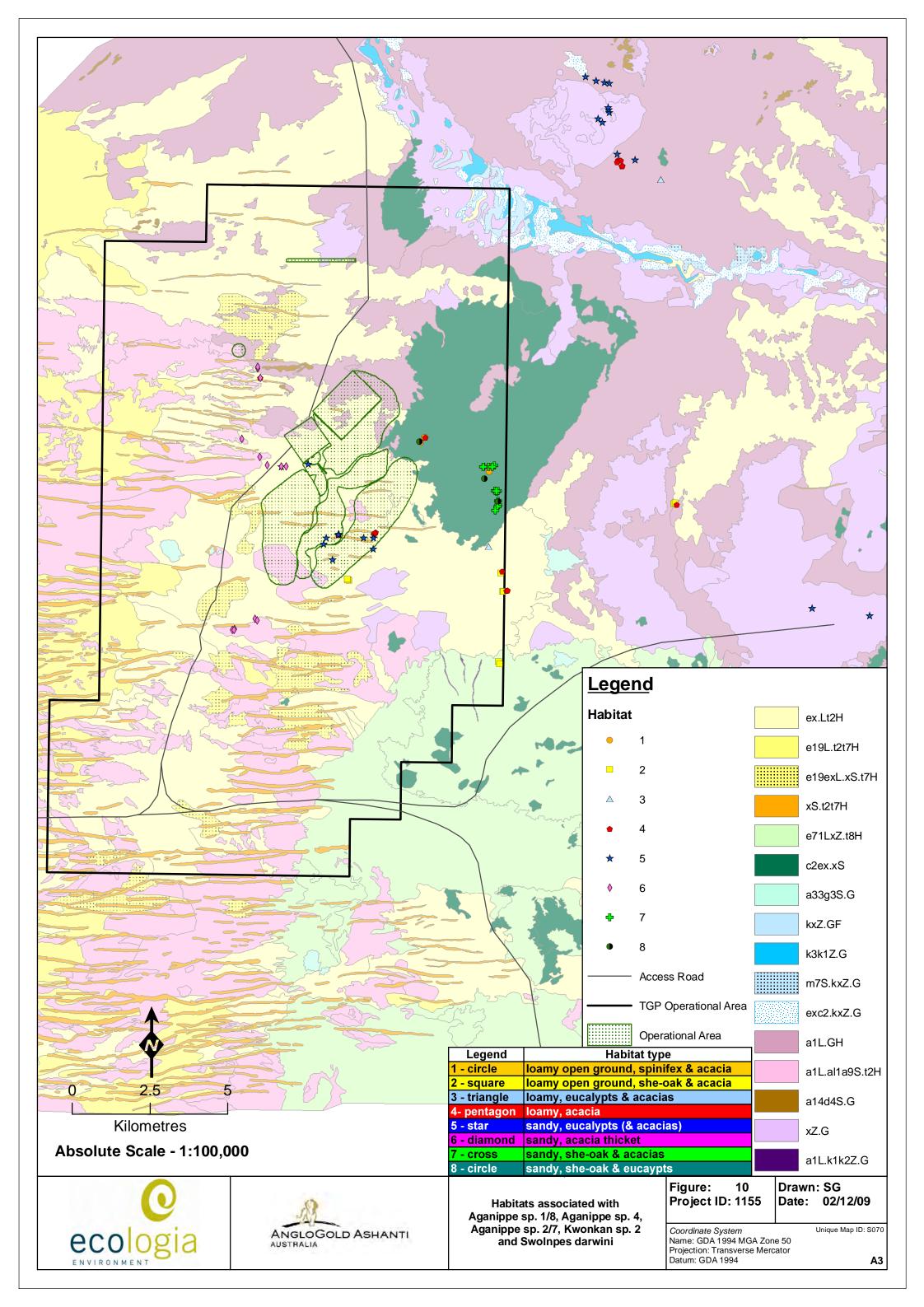
legend	habitat type	Aganippe sp. 1/8	Aganippe sp. 4	Aganippe sp. 2/7	Kwonkan sp. 2	Swolnpes darwinii
1 - circle	loamy open ground, spinifex & acacia	Aganippe sp. 1/8				
2 - square	loamy open ground, she-oak & acacia	Aganippe sp. 1/8				
3 - triangle	loamy, eucalypts & acacias					Swolnpes darwinii
4- pentagon	loamy, acacia			Aganippe sp. 2/7		
5 - star	sandy, eucalypts (& acacias)			Aganippe sp. 2/7	Kwonkan sp. 2	Swolnpes darwinii
6 - diamond	sandy, acacia thicket		Aganippe sp. 4	Aganippe sp. 2/7	Kwonkan sp. 2	
7 - cross	sandy, she-oak & acacias			Aganippe sp. 2/7		
8 - circle	sandy, she-oak & eucaypts				Kwonkan sp. 2	Swolnpes darwinii

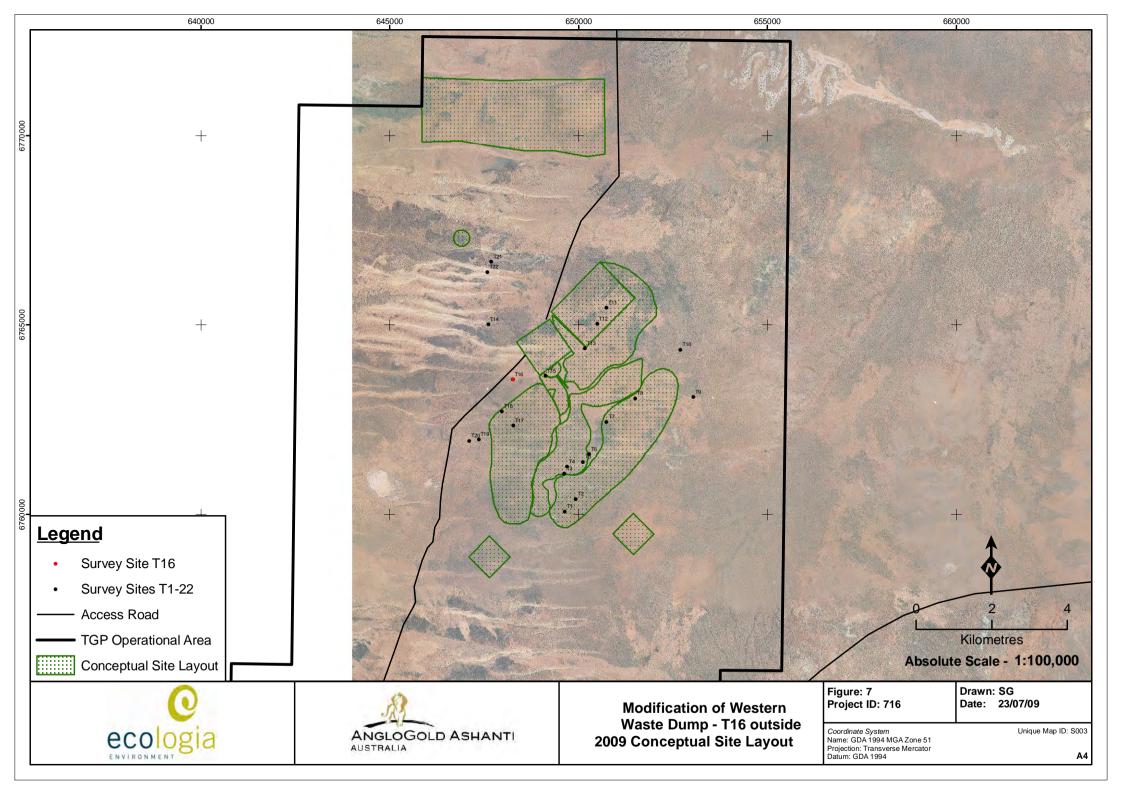












4.3 Habitat Preference – other species

Aganippe sp. 1/8

Seven specimens of *Aganippe* sp. 1/8 were collected from four sites in 2009, one of which was near the original site T5 inside the proposed Operational Area footprint where the first specimen was collected in 2006, and three were located outside (Table 2 and Figure 7 and Figure 8). On the large scale, the species was found in two distinct vegetation units (Beard 1975):

- 1. Low woodland; Acacia aneura (mulga)
- 2. Hummock grasslands, open low tree steppe; *Acacia aneura* (mulga), *Casuarina pauper* (sheoak) [syn. C. cristata] over *Triodia basedowii* between sand ridges

On a small scale within the two vegetation units, however, the species was always associated with pockets of low-lying open ground subject to sheet-flooding and dominated by *Triodia*, with a surface crust on clay and loamy soils. For the purpose of this report, this habitat type was defined as either:

- loamy open ground, spinifex & acacia; and
- loamy open ground, she-oak & acacia (Table 3).

Transects walked from the original site T5 east and north-east across the proposed eastern waste landform and out of the proposed Operational Area footprint showed that these pocket habitats extend a minimum of 6 km beyond proposed footprint (Figure 9, Figure 10) and Appendix 1).

The DNA comparison with specimens collected at Neale Junction Nature Reserve (175 km north of TGP) revealed two taxa (probably different species) from Neale Junction related to *Aganippe* sp. 1/8 at Tropicana, which indicates that allopatric speciation occurred due to the species geographic separation (for details see Part B).

Aganippe sp. 2/7

The species *Aganippe* sp. 2/7 was not one of the target species of this survey, however data on its distribution and genetic divergence were compiled in order to enable comparison with the target species. Twelve specimens of *Aganippe* sp. 2/7 were collected in 2009 both inside and outside the proposed Operational Area footprint (Table 2). On the large scale, the species was found in two distinct vegetation units (Beard 1975):

- 1. Low woodland; Acacia aneura (mulga)
- 2. Hummock grasslands, open low tree steppe; *Acacia aneura* (mulga), *Casuarina pauper* (sheoak) [syn. C. cristata] over *Triodia basedowii* between sand ridges

On a small scale within the two vegetation units, however, the species was associated with pockets of habitat types which were defined for the purpose of this report as:



- sandy, eucalypts & acacias;
- sandy, she-oak & acacias;
- loamy open ground, she-oak & acacia; and
- loamy, acacia.

Transects walked within and outside the proposed Operational Area footprint showed that these pocket habitats extended a minimum of 12 km beyond the footprint (Figure 9, Figure 10 and Appendix 1). The comparison of specimens collected along Laverton track north of lake Rason palaeodrainage and the group of specimens collected south of the palaeodrainage revealed a deep split of approx. 7-8% of genetic divergence, contrasting with the 0.0-1.7% divergence within each group (for details see Part B). This suggests that the two groups belong to two separate populations.



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PART B – UPDATED DNA STUDY





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30 November 2009

Dr. Magdalena Davis Manager Invertebrate Sciences **Ecologia** 1025 Wellington Street WEST PERTH WA 6005

Via email

Re. Report on the molecular systematics of the trapdoor spiders Aganippe including **Neale Junction**

Dear Magdalena,

Following is an updated phylogenetic analysis and distance matrix, which now include four specimens of Aganippe from Neale Junction. Our results suggest the presence of two species of Aganippe at Neale Junction, which show affinities to Aganippe sp. 1/8 from Tropicana. The moderately high genetic distances between the two sites, however, suggests the specimens are likely to belong to distinct species.

Please feel free to contact us if you have any questions or if you require further analyses.

Thanks again for the opportunity to collaborate with Helix, and we look forward to working with you again in future.

Sincerely,

Terrie Finston **Helix Molecular Solutions**



Objective

To add specimens of Aganippe from Neale Junction to the existing phylogeny and assess their relationship to specimens of Aganippe from the Tropicana collections.

Methods

Four specimens of mygalomorph spiders from the Neale Junction Nature Reserve (NJNR), belonging to the genus Aganippe were sequenced for variation at the COI gene using primers M200 (GGAGGATTTGGA AATTGATTAGTTCC) and M205 (ACTGTAAATATATGATGAGCTCA) (Simon et al. 1994). The NJNR is diverse in arid adapted flora and fauna and is approximately 172 km north of the AGAA exploration site at Tropicana Gold Project (M. Davis, pers. Comm.).

Genetic distances between unique genetic types (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotide differenences between sequences). Neighbour-joining (NJ) of uncorrected p-distances was used to construct a phylogenetic tree. The robustness of the branching pattern was assessed using 500 bootstrap iterations. The funnel web spider *Euagrus chisoseus* and the jumping spider *Phidippus* sp. were used as outgroups. The phylogenetic tree also included voucher specimens of *Gaius* and *Aganippe* from Goldfield and Wheatbelt sites (Mt. Jackson, Mt. Keith, Albion Downs, Mt. Gibson, Grasspatch, Koolyanobbing).

Results

The Neale Junction specimens occured in two clades (Figure 1) that differed by 8.9 to 9.6% sequence divergence (Table 1). The Neale Junction haplotypes formed a clade with the haplotypes of Aganippe sp. 1/8 from Tropicana. The relationship between the Neale Junction and Aganippe sp 1/8 haplotypes was well supported, as evidenced by high bootstrap values (Figure 1), but distances between haplotypes at the two sites ranged from 7.0 to 8.6% sequence divergence (Table 1).

Conclusions

There are likely to be two species of Aganippe present in the Neale Junction collections, due to the moderately high genetic divergence (approximately 9-10%) between the two observed clades. The occurrence of two divergent genetic groups at a single site further supports the notion of distinct species. This pattern is usually indicative of secondary contact between two previously isolated populations. Mating experiments or an analysis of nuclear genes would be necessary to confirm reproductive isolation.

The patterns shown by Aganippe sp. 1/8 and the Neale Junction Aganippe (occurrence in the same clade, but moderately high divergence between them) suggests it is likely that the Tropicana Aganippe sp. 1/8 is a distinct species from the two Aganippe species at Neale Junction. While genetic differentiation is not surprising given the geographic distance between the two sites, this level of divergence typically indicates distinct species in many spider groups (Barrett & Hebert, 2005). It is important to note that the poor dispersal ability of trapdoor spiders, particularly the females, who rarely leave their burrows, may result in elevated levels of variation at maternally-inherited mitochondrial genes such as COI.

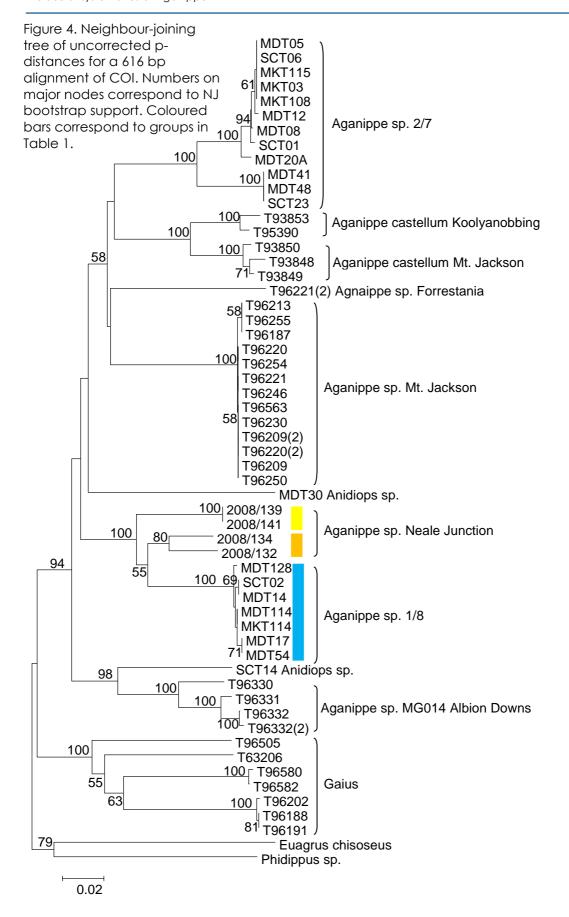
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Barrett, R.D.H. & Hebert, P.D.N. 2005. Identifying spiders through DNA barcodes. Canadian Journal of Zoology, 83, 481-491.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. Annals of the Entomological Society of America, 87, 651-701.

Table 1. Genetic diversity (uncorrected p-distances) between haplotypes in the *Aganippe* sp. 1/8 – Neale Junction clade. Shaded values represent within-group distances (yellow = Neale Junction, blue = *Aganippe* sp. 1/8). Unshaded values represent between-group distances.

	1	2 Neale J	3 unction	4	5 Tropic	6 cana
[1] 2008/139						
[2] 2008/141	0.000					
[3] 2008/134	0.089	0.089				
[4] 2008/132	0.096	0.096	0.044			
[5] MDT128	0.086	0.086	0.077	0.070		
[6] SCT02	0.086	0.086	0.082	0.075	0.005	
[7] MDT14	0.086	0.086	0.082	0.075	0.005	0.000
[8] MDT114	0.084	0.084	0.079	0.072	0.002	0.002
[9] MKT114	0.084	0.084	0.079	0.072	0.002	0.002
[10] MDT17	0.086	0.086	0.082	0.075	0.005	0.005
[11] MDT54	0.086	0.086	0.082	0.075	0.005	0.005
		-	Tropicana			
	7	8	9	10	11	
[1] 2008/139	•		·	. •		
[2] 2008/141						
[3] 2008/134						
[4] 2008/132						
[5] MDT128						
[6] SCT02						
[7] MDT14						
[8] MDT114	0.002					
[9] MKT114	0.002	0.000				
[10] MDT17	0.005	0.002	0.002			
[11] MDT54	0.005	0.002	0.002	0.000		



Appendix 1.

>2008/139

>2008/134

>2008/141

>2008/132

Appendix 1

Habitats





Type habitat of Aganippe sp. 1/8 inside TGP area

Site T5

Habitat type: loamy open ground, Spinifex & acacia

Description: Acacia jamesiana sparse medium shrubs, over *Triodia basedowii*. Sparse leaf litter, mainly under shrubs and sparse wood litter. Open ground; redorange sandy loam.

Type specimen (male)





Habitat of Aganippe sp. 1/8 located outside TGP

Burrow (right)

Female at the entrance of her burrow (below). Third left leg collected for DNA analysis.









SE of T5

Sites MDT13, MDT14, MDT15 and SCT02

Habitat type: loamy open ground, Spinifex & acacia

Description: Mixed mulga (*Acacia aneura*) open tall shrubland over *Triodia basedowii* open hummock grass.

Undulating plain. Sparse leaf litter, mainly under shrubs. Loose soil and surface crust

Red-orange sandy clay



T28 & T30-32 (Silhouette)

Sites MDT17, MDT54, MDT123, MDT135 and MDTA4

Habitat type: loamy open ground, Spinifex & acacia

Description: *Eucalyptus* sp. woodland with some *Acacia*. Mature Spinifex.

Thick leaf litter under Eucalypts.

Patches of bare earth with gravely soil at surface. Sediment cemented.



T10 (W of Hat trick Hill)

Sites MDT114 and MKT114

Habitat type: loamy open ground, sheoak & acacia

Description: Acacia aneura over sparse Eucalyptus concinna mallee, over open Senna artemisioides subsp. petiolaris medium shrubs

Open ground, red sandy clay, calcrete bedrock





T46 & 47 (WS of Hat trick Hill)

Sites MDT126, MDT128, MDT129 & MKT 105

Habitat type: loamy open ground, sheoak & acacia

Description: She-oak open woodland with diverse understorey. Heavy leaf litter under trees

Bare earth between trees and shrubs, calcrete bedrock.



Airstrip Rd. West

Sites MDT 146 and MDT 147

Habitat type: loamy open ground, Spinifex & acacia

Description: Acacia aneura over mature Spinifex.

Patches of bare earth with gravely soil at surface. Sediment cemented.





Type habitat of Aganippe sp. 2/7 inside TGP

Burrow (right)

Female spider (below)







East of T4

Sites MDT08, MDT09, MDT10, MDT20A, MDT136, SCT01, SCT06

Habitat type: loamy, acacia

Description: Mixed mulga (*Acacia aneura*)

shrubland over Triodia basedowii

Abundant leaf litter, mainly under shrubs.

Loose soil and surface crust

Red-orange sandy clay





South-East of T4

Sites MDT05, MDT12 and MKT03

Habitat type: sandy, eucalypts &

acacias

Description: Eucalyptus gongylocarpa open low woodland, over Eucalyptus trivalvis sparse mallee, over open low shrubs and Triodia basedowii moderately dense hummock grassland.

Moderate leaf litter, mainly under trees and sparse wood litter

Orange sand



Type habitat of Aganippe sp. 2/7 outside TGP

T33 & 34 (Laverton track)

Sites MDT41, MDT42, MDT43, MDT44, MDT45, MDT46, MDT47, SCT16, SCT19

Habitat type: sandy, eucalypts & acacias

Description: Acacia aneura – sparse woodland. Grass under-storey with mixed shrubs.

Coarse sand



T35 Laverton track)

Site SCT23

Habitat type: sandy, eucalypts & acacias

Description: Acacia aneura low woodland. Grass under-storey with mixed shrubs.

Coarse sand





T36 (Laverton track)

Sites MDT48, MDT49, MDT50, SCT25, SCT26

Habitat type: loamy, acacia

Description: Acacia aneura woodland



T47 (S of Hat trick Hill)

Sites MDT124, MDT125, MDT127, MKT102, MKT103, MKT104

Habitat type: sandy, she-oak & acacias

Description: She-oak open woodland with

diverse understorey.

No Spinifex.

Heavy leaf litter under trees.

Bare earth between trees and shrubs



Other habitat of Aganippe sp. 2/7 outside TGP

T44 & 45 (S of Hat trick Hill)

Sites MDT131, MDT132, MDT133, MDT134, MKT106, MKT107, MKT108, MKT109, MKT110

Habitat type: sandy, she-oak & acacias

Description: She-oak open woodland with diverse understorey.

No Spinifex

Lots of bare earth and calcrete





T10 (b); SW of Hat trick Hill

Site MKT115

Habitat type: loamy open ground, she-

oak & acacia

Description: Acacia aneura and sparse Eucalyptus concinna mallee over open Senna artemisioides subsp. petiolaris medium shrubs

Undulating Plain. Moderate leaf litter, mainly under shrubs and sparse wood litter.

Red sandy clay, Calcrete bedrock



Habitat of Aganippe sp. 4 (outside TGP)

East of T16

Site SCT13

Description: Acacia aneura low shrubland, over *Triodia desertorum* open hummock grass.

Dune valley. Sparse leaf litter, mainly under shrubs and sparse wood litter.

Red-orange fine sand



West of T16

Sites SCT14 and MDT30

Description: Acacia aneura low shrubland; abundant leaf litter, mainly under shrubs and wood litter. Patches of open ground

Red-orange fine sand



NW of T16

Site SCT15

Description: Acacia aneura low shrubland; abundant leaf litter, mainly under shrubs and wood litter.

Red-orange fine sand





T21,22 &43

Sites MDT 115 and MDT116

Description: Acacia aneura low shrubland, over *mature Spinifex*

Dune valley. Sparse leaf litter, mainly under shrubs and sparse wood litter.

Red-orange fine sand



T38

Sites MDT118, MDT119, MKT101

Description: East-west facing dune.

Eucalypts present mid to peak. Diverse understorey of shrubs to 1.5m. Spinifex mature and sand very soft.

Leaf litter thick under *Eucalyptus* and *Acacia*.



T41

MDT 117 and MDT120

Description: North facing, gently sloping dune base.

Acacia over sparse shrubs and Triodia sp.

Red-orange fine sand.





Type habitat of Kwonkan sp. 2 inside TGP area

Site T4

Description: Eucalyptus gongylocarpa open low woodland, over Eucalyptus trivalvis sparse mallee, over open low shrubs and Triodia basedowii moderately dense hummock grassland.

Moderate leaf litter, mainly under trees and sparse wood litter

Orange sand



Potential habitat of Kwonkan sp. 2 outside TGP area

Site T10 (a)

Description: Casuarina pauper (sheoak) open medium woodland, over sparse Eucalyptus concinna mallee, over open Senna artemisioides subsp. petiolaris medium shrubs

Undulating Plain. Moderate leaf litter, mainly under shrubs and sparse wood litter

Red sandy clay, Calcrete bedrock



Site T14

Description: Post-fire: Eucalyptus ewartiana open medium mallee, over Codonocarpus cotinifolius and Callitris preissii trees-shrubs, over other shrubs such as Aluta maisonneuvei subsp. auriculata and Solanum plicatile, with Amphipogon caricinus and Triodia basedowii grasses.

Inter-dune swale. Red sand. Post-fire: Acacia acanthoclada subsp. acanthoclada and other mixed sparse to scattered low or dwarf shrubs and grasses. Linear dune crest and slope. Yellow sand





Sites T16 (a)

Description: Callitris columellaris scattered trees, over Eucalyptus youngiana sparse mallee, over Anthotroche pannosa / Pityrodia loricata open low shrubland, over Triodia desertorum open hummock grass.

Dune Crest. Sparse leaf litter, mainly under shrubs and sparse wood litter. Burnt a long time ago

Red-orange fine sand



Site T22 (a)

Description: Mixed *Acacia aneura* (mulga) mid-dense low woodland, over mid-dense tall shrubs of dominantly *Eremophila latrobei* subsp. *latrobei / Dodonaea rigida*, over *Triodia basedowii* hummock grass.

Rocky sandstone slope. Sparse leaf litter, mainly under shrubs and sparse wood litter

Pale orange sand.





Type habitat of *Swolnpes morganensis* gen. nov. sp. nov. (outside TGP)

Site T 46

Description: She-oak open woodland with diverse understorey with Spinifex starting to appear.

Lots of bare earth and calcrete



Other habitat of *Swolnpes morganensis* gen. nov. sp. nov. (outside TGP)

Site T23

Description: *Eucalypt* and *Acacia* dominated to 3 m over grasses, sand and small shrubs.

Leaf litter with lots of twig and stick debris.

Red-orange sand



Site T25

Description: Large, white *Eucalypts* and *Acacia*, dense thicket. Some Spinifex but mostly grasses and other low shrubs. Lots of leaves and debris under trees with around 30% bare earth outside of tree cover.

Plentiful leaf litter.





Site T28

Description: *Eucalypt* sp. woodland with some *Acacia*. Mature Spinifex.

Thick leaf litter under Eucalypts.

Patches of bare earth with gravely soil at surface. Sediment cemented.



Site T36

Description: Weeping, small 'Calistomen' and large Eucalypt to 6 m – white/salmon bark.

Variety of shrub understorey and grasses 70%.

Bare earth between trees.

100% leaf litter and debris under trees.

Course sands suggesting ancient river channel.



Site T37

Description: Dominated by mature Spinifex – 50%.

Bare sand over-storey, large *Acacia* sp. with accumulated leaf litter beneath.

Red sand and a few mature *Eucalyptus*.





Site T44

Description: She-oak open woodland with diverse understorey.

No Spinifex

Lots of bare earth and calcrete

