

### **TROPICANA GOLD PROJECT**

### Targeted Mygalomorph Survey & DNA Study

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# **TROPICANA GOLD PROJECT**

Targeted Mygalomorph Survey & DNA Study

**Tropicana Joint Venture** 





29 July 2009

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1025 Wellington Street WEST PERTH WA 6005 Phone: 08 9322 1944 Fax: 08 9322 1599 Email: *admin@ecologia.com.au* 

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## EXECUTIVE SUMMARY

The Tropicana Joint Venture (TJV) is currently undertaking a pre-feasibility study and is working through the relevant State and Federal Environmental Assessment processes associated with the proposed 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.

*Ecologia* Environment (*ecologia*) was contracted to conduct a literature review of short-range endemism in Western Australia, and to undertake surveys for short-range endemic invertebrates (SRE) in the proposed operational area. Trapping conducted in 2006 and 2008 found three species of mygalomorph spider (*Aganippe* sp. 1, *Aganippe* sp. 4 and *Kwonkan* sp. 2) that were fully impacted by the proposed TGP mining and infrastructure footprint.

During autumn 2009, targeted surveys were conducted with the aim of collecting the aforementioned species, mapping their geographic distribution within and around the TGP Operational Area and defining their preferred habitat. In addition, a DNA study of all collected specimens of the genus *Aganippe* was 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.

The results of the targeted survey showed direct and/or indirect evidence of the occurrence of all three species outside the proposed TGP Operational footprint.

It was concluded that *Aganippe* sp. 4 was no longer impacted by the proposed TGP as the outline of the infrastructure footprint was modified to exclude the habitat of the species. In addition, suitable habitat was also found available further west from the proposed Operational footprint.

Specimens of *Aganippe* sp. 1 have been collected outside the infrastructure footprint and the low genetic divergence indicated that they are part of the same population as specimens inside the infrastructure footprint. Moreover, following further taxonomic examination, *Aganippe* sp. 8 was synonymised with *Aganippe* sp. 1, thus the geographic range of *Aganippe* sp. 1 was further expanded. *Aganippe* sp. 1 would be, therefore, only partially impacted by the TGP, and this impact would be insignificant to the species.

Specimens of *Aganippe sp.* 7 (non-target species) were used for comparison with the target species. In addition, the species was also subjected to further taxonomic examination and it was found identical with *Aganippe sp.* 2. Coupled with the synonymisation of *Aganippe* sp. 1 and *Aganippe* sp. 8, the taxonomic revisions reduced the eight *Aganippe* species listed in the main SRE report to six species.

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 sampling results, published literature and comparisons with other species at TGP. Three habitat types were identified suitable for *Kwonkan* sp. 2, of which two occurred both inside and outside the Operational footprint and one occurred outside the footprint only. The habitats were partially overlapping with suitable habitats of *Aganippe* sp. 2/7 and a new TGP species, *Swolnpes darwinii*. Given this similarity, the spatial distribution of *Kwonkan* sp. 2 is most likely to follow the same patterns as those of *Aganippe* sp. 2/7 and *Swolnpes darwini*. It was, therefore, concluded



that *Kwonkan* sp. 2 would be only partially impacted by the Operational footprint, and this impact would be insignificant to the species.



# 1.0 INTRODUCTION

### 1.1 Project Background

The Tropicana Joint Venture (TJV) is currently undertaking a pre-feasibility study and is working through the relevant State and Federal Environmental Assessment processes associated with the proposed on the viability of 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).

During a short-range endemic surveys in 2006 and 2008, three mygalomorph spider species, *Aganippe* sp.1 (Figure 2), *Aganippe* sp. 4 and *Kwonkan sp.* 2 (Figure 2), were found to be fully impacted by the proposed TGP mining and infrastructure footprint. *Ecologia* Environment (*ecologia*) was contracted to conduct a targeted survey in 2009 with the aim of collecting the aforementioned species, mapping their geographic distribution within and around the TGP and defining their preferred habitat. In addition, a DNA study of all collected specimens was to be 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 describes the field survey undertaken during March and May 2009, including the analysis of the suitable habitat, and Part B describes the DNA study of the 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 Shortrange 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 the *Aganippe* sp.1, *Aganippe* sp. 4 and *Kwonkan* sp. 2.



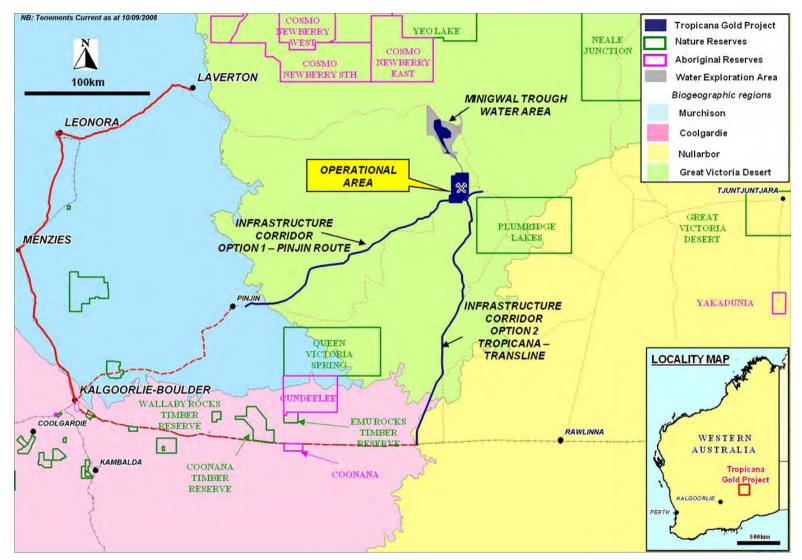


Figure 1 Location Plan of the Tropicana Joint Venture Operational Area in Western Australia







Figure 2 Aganippe sp. 1 (left) and Kwonkan sp. 2 (right)

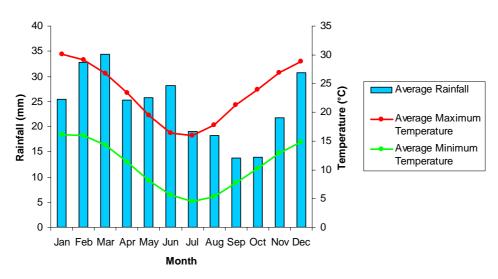


# 2.0 BIOPHYSICAL ENVIRONMENT

# 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 **Error! Reference source not found.**)

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.



**Average Climatic Statistics** 

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

| Statistic   | Jan      | Feb     | Mar       | Apr      | May   | Jun  | Jul  | Aug   | Sep   | Oct   | Nov   | Dec  |
|---|----------|---------|-----------|----------|-------|------|------|-------|-------|-------|-------|------|
| Average Month                                     | ly Recor | ds Balg | air (198: | 3 – 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 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 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 |

 Table 1
 Climatic Statistics for Balgair and Laverton



### 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 foraging surveys were conducted in March and May 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 burrows 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 was 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.

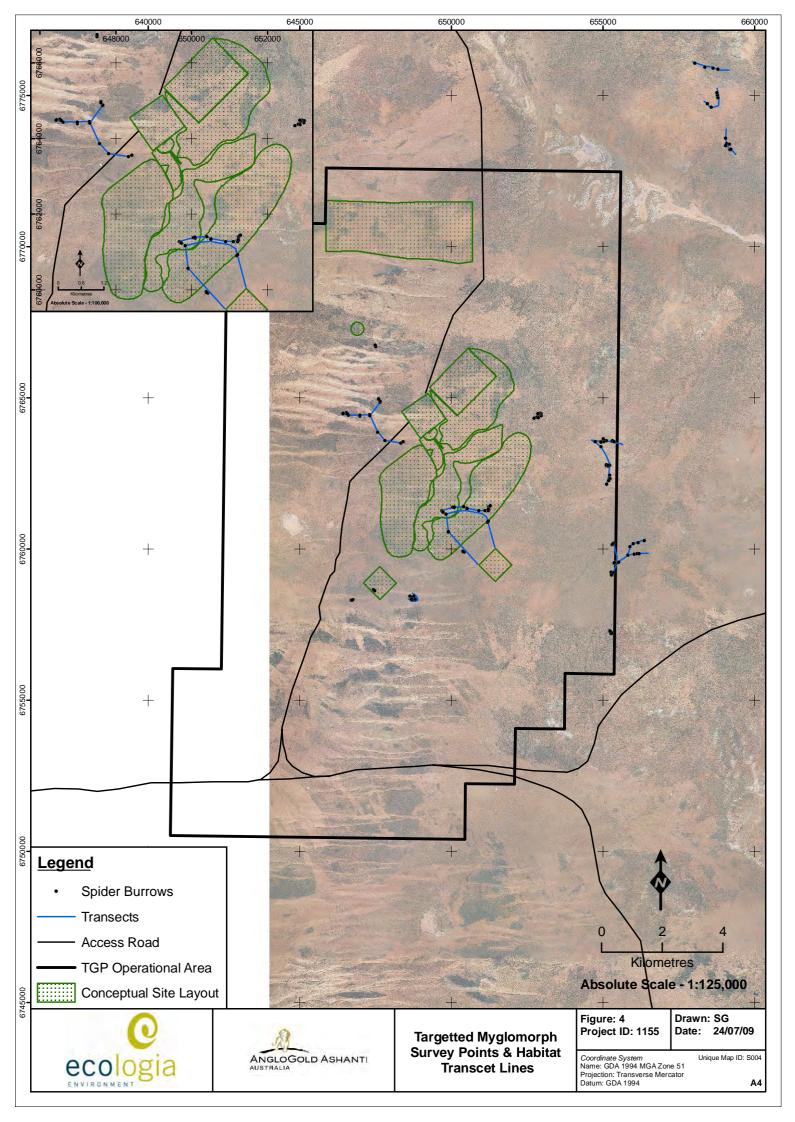
# 3.2 Site Selection

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 4.

# 3.3 External Support

Professor Barbara York Main, an expert taxonomist for mygalomorph spiders, was involved in the identification of all collected specimens.





# PART A - SPECIES AND HABITAT ASSESSMENT



# 4.0 RESULTS AND DISCUSSION

### 4.1 Overview

A total of 89 spiders were found, of which 24 were collected and identified. Table 2 and Figure 5 show the identification and location of all collected spiders.

## 4.2 Habitat Preference

### Aganippe sp. 1

Seven specimens of *Aganippe* sp. 1 were collected from four sites, one of which was near the original site T5 inside the TGP mining and infrastructure footprint where the first specimen was collected in 2006, and three were located outside the TGP mining and infrastructure footprint (Figure 5). 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 dump and out of the TGP mining and infrastructure footprint showed that these pocket habitats extend a minimum of 6 km beyond the TGP mining and infrastructure footprint area (Figure 6 and Appendix 1).

In addition, further taxonomic examination showed that *Aganippe* sp. 1 and *Aganippe* sp. 8 were identical species (B.Y. Main, pers. comm. 2009). The species is henceforth coded as *Aganippe* so. 1/8 and its geographic range now includes previous records of *Aganippe* sp. 1 as well as Aganippe sp. 8 from the 2008 survey at sites T30 and T47 (for details see the main SRE report).



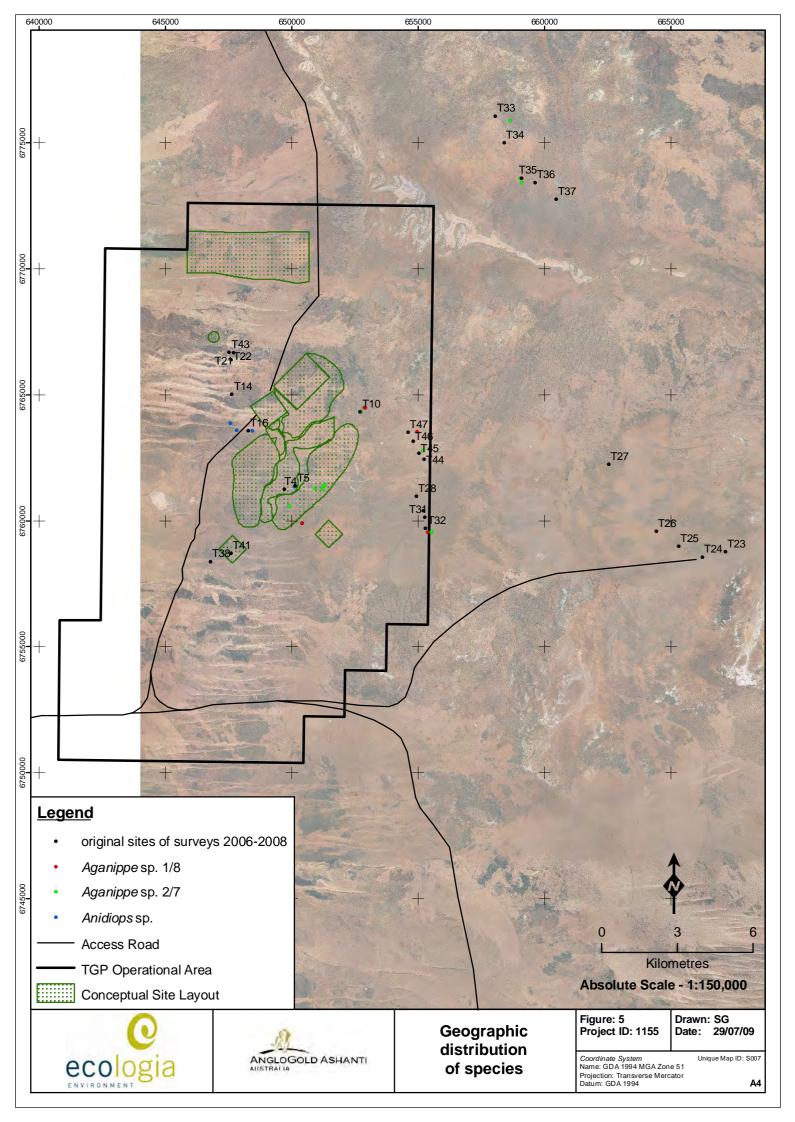
| Species          | Burrow     | DNA | Site code | Habitat type                         | Area                       | Zone | Easting  | Northing |
|------------------|------------|-----|-----------|--------------------------------------|----------------------------|------|----------|----------|
| Aganippe 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 |     | MDT01     | sandy, eucalypts & acacias           | SE havana, TROP 5          | 51J  | 650098.3 | 6761371  |
| Anidiops sp.     | twig-lined |     | SCT13     | sandy acacia thicket                 | W waste dump NE of TROP 16 | 51J  | 648418.1 | 6763565  |

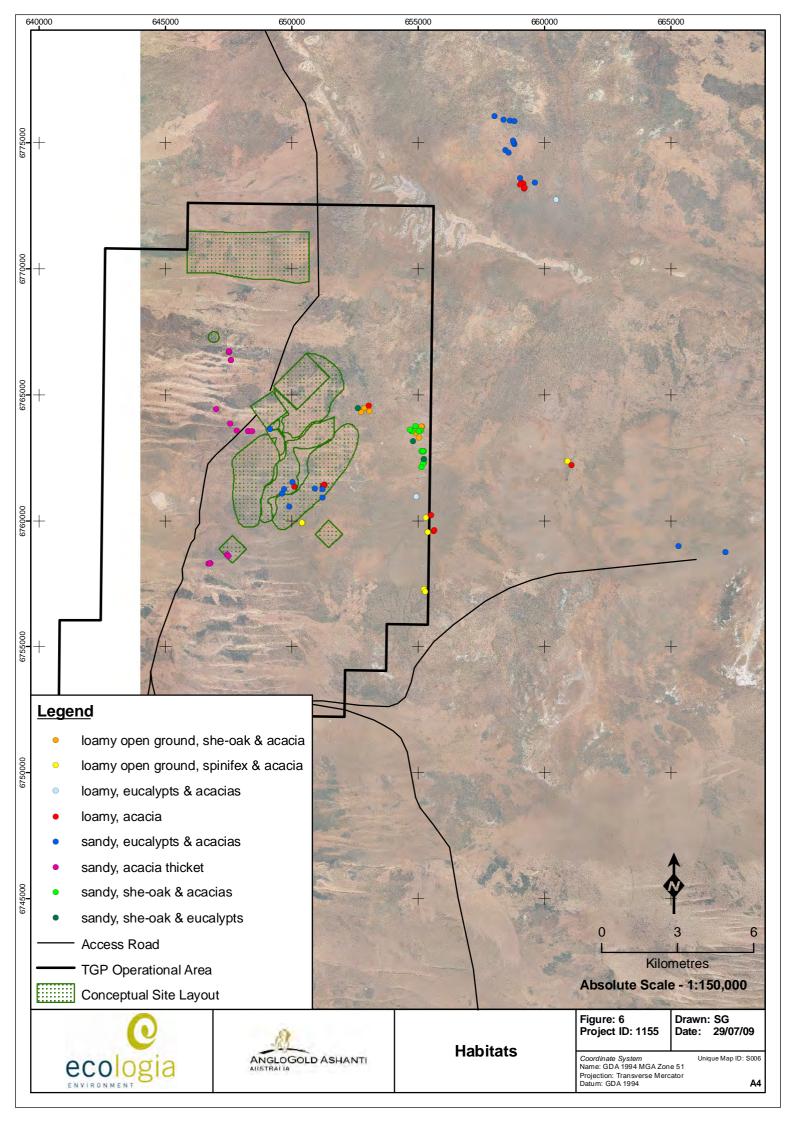
### **Table 2**A list of all specimens collected during the targeted surveys in March 2009 and May 2009

### **Table 3** Association between species and habitats (colours as in Figure 6)

| yellow      | loamy open ground, spinifex & acacia | Aganippe sp. 1/8 |                       |                  |               |                   |
|-------------|--------------------------------------|------------------|-----------------------|------------------|---------------|-------------------|
| orange      | loamy open ground, she-oak & acacia  | Aganippe sp. 1/8 |                       |                  |               |                   |
| red         | loamy, acacia                        |                  |                       | Aganippe sp. 2/7 |               |                   |
| purple      | sandy, acacia thicket                |                  | <i>Aganippe</i> sp. 4 | Aganippe sp. 2/7 | Kwonkan sp. 2 |                   |
| dark blue   | sandy, eucalypts (& acacias)         |                  |                       | Aganippe sp. 2/7 | Kwonkan sp. 2 | Swolnpes darwinii |
| pale blue   | loamy, eucalypts & acacias           |                  |                       |                  |               | Swolnpes darwinii |
| light green | sandy, she-oak & acacias             |                  |                       | Aganippe sp. 2/7 |               |                   |
| dark green  | sandy, she-oak & eucaypts            |                  |                       |                  | Kwonkan sp. 2 | Swolnpes darwinii |







### Aganippe sp. 4

*Aganippe* sp. 4 was collected in 2006 at the original site T16, which was, at the time located within the TGP mining and infrastructure footprint. The outline of the proposed western waste dump was modified in 2009 in such a way that the site T16 is now located approximately 800 m west of its nearest boundary (Figure 7). The species *Aganippe* sp. 4 is therefore no longer directly impacted by the TGP.

Given the proximity of the site to the infrastructure border, however, additional habitat assessment was conducted in case the site T16 might be subject to indirect impact.

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 (*Anidiops* sp.) 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 TGP, 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 6 and Appendix 1).

### Aganippe sp. 7

The species *Aganippe* sp. 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. 7 were collected both inside and outside the TGP mining and infrastructure footprint (Figure 5). On the large scale, the species was found in two distinct vegetation units (Beard 1975):

- 3. Low woodland; Acacia aneura (mulga)
- 4. 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 TGP mining and infrastructure footprint showed that these pocket habitats extended a minimum of 12 km beyond the TGP mining and infrastructure footprint. The comparison of specimens collected along Laverton track north of lake Rason palaeodrainage (Figure 6 and Appendix 1) 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.

*Aganippe sp.* 7 was also subjected to further taxonomic examination and the species was found identical with *Aganippe sp.* 2 (B. Y. Main, pers. comm., 2009). The species is henceforth referred to as *Aganippe* sp. 2/7.

Importantly, coupled with the synonymisation of *Aganippe* sp. 1 and *Aganippe* sp. 8, the taxonomic revisions reduced the eight *Aganippe* species listed in the main SRE report to six species.

### *Kwonkan* sp. 2

Despite the repeated efforts during the surveys in March and May 2009, no live *Kwonkan* sp. 2 specimens could be collected. The following summary is based on literature information, habitat assessment and similarities with other species found at TGP.

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, constructing cryptic burrows with small funnel entrance (Main 1983).

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).

Transects walked from the original site T4 north-east, east, and south-east, across the proposed eastern waste dump and out of the TGP mining and infrastructure footprint, showed that pockets of this habitat extend a minimum of 6 km beyond the TGP mining and infrastructure footprint (Figure 6 and Appendix 1). This habitat is interspersed and partially overlapping 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 beyond the TGP mining and infrastructure footprint. It is, therefore, reasonable to assume that the population of *Kwonkan* sp. 2 in this area is

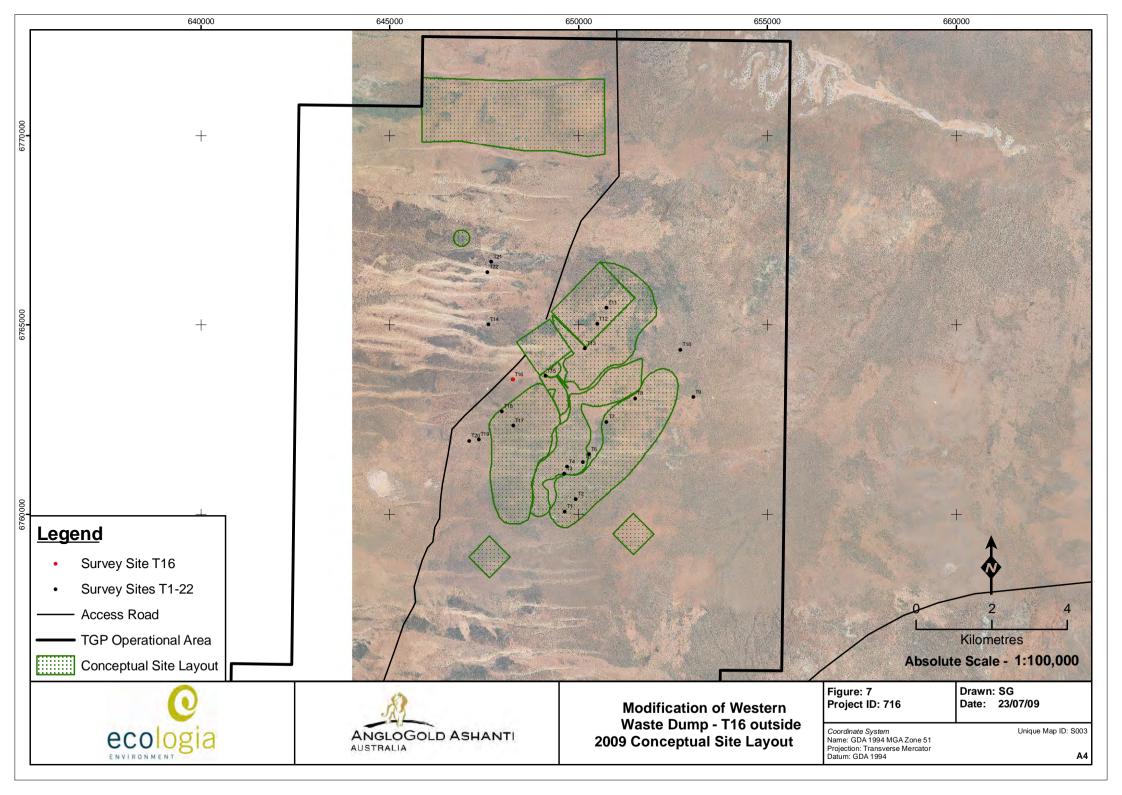


subject to the same natural conditions and that it follows the same patterns of distribution and dispersal, both inside and outside the proposed TGP mining and infrastructure footprint.

This assumption is supported by the data on a geographic distribution of another species from the family Nemesidae, *Swolnpes darwinii* gen. nov. sp. nov. (B.Y. Main, pers. comm. 2009) found at Tropicana in 2008 (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 TGP mining and infrastructure footprint over a distance of 12 km (Figure 6 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 TGP mining and infrastructure footprint area. The population of *Kwonkan* sp. 2 is, therefore, expected to be partially impacted by the proposed TGP, however this impact is likely to be insignificant to the species.





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# PART B - DNA STUDY





# Helix Molecular Solutions

|                   | ology The University | of Western Australia | PO Box 155                   |  |
|-------------------|----------------------|----------------------|------------------------------|--|
|                   | Io. 4 Hackett Drive  | Crawley WA 6009      | Leederville WA 6903          |  |
| t: (08) 6488 4509 | f. (08) 6488 1029    | abn: 32 133 230 243  | w. www.helixsolutions.com.au |  |

21 August 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 and Anidiops

Dear Magdalena,

Following is a summary of the results of the *Aganippe* study. Our data support the morphological designations as assigned by Dr. York Main, however, the data have also revealed divergence, associated with geographical locations, within *Aganippe* sp. 2/7. In addition, our data suggest the two specimens of *Anidiops* belong to two different species.

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

Sincerely,

Terrie Finston and Oliver Berry Helix Molecular Solutions

### Objective

The trapdoor spider fauna in WA is diverse, (Main, 1957; 1982), and many new species continue to be recovered (Schmidt, 2007). Morphological taxonomy can be difficult in this group of spiders especially where only one sex or adults have the diagnostic characters (Paquin & Hedin, 2004). Because morphological characters may not always capture true species diversity, DNA methodologies provide a rapid approach to this problem and are increasingly being employed. DNA sequencing allows a rapid assessment of genetic diversity and methods are readily transferred among taxa.

Twenty-three specimens of mygalomorph trap-door spiders collected from the Tropicana Gold Project (TGP; approximately 80 km east of Payne's Find, W.A.), and belonging to two genera (Aganippe and Anidiops), were sequenced for variation at the mitochondrial DNA gene cytochrome oxidase subunit 1 (COI). The samples were sequenced with the aim of providing complementary information to existing morphological identifications to validate the number of species/genera present in the collection, assess genetic diversity within assigned species, and assess relationships of the TGP specimens with other mygalomorphs from the region.

### Executive summary:

- Twenty-one specimens of trap-door spiders from the Tropicana Gold Project were sequenced for variation at the Cytochrome Oxidase subunit I (COI) gene. These included specimens assigned by morphology to Anidiops, Aganippe sp. 1/8 and Aganippe sp. 2/7.
- Four strongly divergent genetic lineages were recovered. These lineages were of sufficient divergence to classify as four distinct species.
- Morphological species assignments for Anidiops, Aganippe sp. 1/8 and Aganippe sp. 2/7 were supported by the genetic data.
- Genetic variation within Aganippe sp. 1/8 was low (<1% divergence between any pair of individuals).
- Genetic variation within Aganippe sp. 2/7 was moderate (approximately 7-8% divergence between any pair of individuals), with two divergent lineages corresponding to geographic locations approximately 20 km apart
- The observed moderate genetic divergence over 20 km likely reflects poor dispersal ability of these spiders, and in particular females. This level of divergence suggests the two lineages of Aganippe sp. 2/7 have been evolving independently for several million years
- The two Anidiops specimens had highly divergent haplotypes and are likely to represent two distinct species.

### Methods

Twenty-three specimens of mygalomorph spiders belonging to two genera (Aganippe and Anidiops) were sequenced for variation at the COI gene using primers M200 (GGAGGATTTGGA AATTGATTAGTTCC) and M205 (ACTGTAAATATGATGAGCTCA) (Simon et al. 1994) (Table 1). Genetic distances between unique genetic types (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotides different between sequences). Maximum parsimony (MP) analysis and neighbour-joining (NJ) of uncorrected p-distances were used to construct phylogenetic trees. The robustness of the branching pattern was assessed using 100 and 500 bootstrap iterations, respectively. The funnel web spider *Euagrus chisoseus* was used as an outgroup.

A second phylogenetic tree was constructed as above, using voucher specimens of Gaius and Aganippe from Goldfield and Wheatbelt sites (Mt. Jackson, Mt. Keith, Albion Downs, Mt. Gibson, Grasspatch, Koolyanobbing). As well as providing information on the relationships of the Tropicana specimens to other mygalomorphs of the region, this was done to test the validity of the Anidiops assignments.

### Results

Phylogenetic analysis and morphotype associations

A 616 base-pair (bp) fragment of COI was isolated from 21 of the 23 specimens. Sequences for each specimen are shown in Appendix 1. Of the 616 bp alignment, 406 characters were constant, 83 were variable but parsimony-uninformative, and 127 were parsimony informative. Both tree building methods produced similar trees (Figure 1; only the NJ tree is shown). Five wellsupported genetic lineages were identified (A-E, Figure 1), and these corresponded to morphological designations. Lineages A and B formed a well-supported monophyletic clade, corresponding to Aganippe sp. 2/7 (hereafter referred to as Aganippe sp. 2/7-A and Aganippe sp. 2/7-B). Lineage C was also well-supported, and corresponded to Aganippe sp. 1/8. Lineages D and E corresponded to Anidiops morphotypes, but they did not form a monophyletic clade. While there was broad agreement between morphological designations and the genetic data, the DNA showed further distinctions within the morphological designations (see below).

#### Divergence between lineages

Genetic divergence between the five lineages was moderate to high, ranging from 7.0% between lineages A and B to 18.1% between lineages C and E (Table 2). It should be noted that that p-distance is a conservative estimate, in that it doesn't account for mutational saturation. Hence these distances should be considered minimum estimates and could in fact be higher. Lineages D and E (Anidiops sp.) were highly divergent, differing from one another by 17.4% sequence divergence.

#### Divergence within morphotypes

Haplotype diversity within morphotypes and lineages was substantially lower than divergences between morphotypes and lineages (Figure 2). Diversity within morphotypes ranged from low (<1% - Aganippe sp. 1/8) to moderate (up to nearly 8% - Aganippe sp. 2/7). The observed divergence within Aganippe sp. 2/7 was a result of the variation between lineages A and B, which ranged from 7.0 to 7.8% (Table 3; Figure 2). Haplotype diversity within the two lineages of Aganippe sp. 2/7 was substantially lower, ranging from 0 to 1.7% in lineage A and from 0.2 to 1.2% in lineage B. Haplotype diversity within Aganippe sp. 1/8 ranged from 0 to 0.3% sequence divergence (Table 3). All pair-wise divergences between haplotypes are shown in Appendix 2.

#### Geographic distribution of lineages

The lineages had relatively well-defined geographical distributions. Lineages A (Aganippe sp. 2/7-A), C (Aganippe sp. 1/8), D and E (Anidiops sp.) are found clustered at the southern sites, with lineages A and C having overlapping distributions, and lineages D and E being found west of the A/C group. Lineage B (Aganippe sp. 2/7-B) is found approximately 20 km to the northeast (Figure 3).

### Relationships of TGP fauna to other mygalomorph groups

Both the TPG Aganippe and Anidiops were contained within a well-supported clade containing Aganippe castellum and other Aganippe species from Wheatbelt and Goldfields sites (Figure 4). In particular, Aganippe sp. 1/8 and the Anidiops specimen from SCT14 formed a clade with Aganippe sp. 'MG014' from Albion Downs, with the relationship between Anidiops sp. and Aganippe sp. 'MG014' being particularly well-supported. Aganippe sp. 2/7 formed a clade with specimens of Aganippe from Mt. Jackson, Koolyanobbing and Forrestainia, although the relationship was not particularly well-supported. The haplotypes of Gaius formed a separate, well-supported clade.

### Conclusions

Correspondence between morphology and genetics provides a robust foundation for the identification of distinct species. Morphotypes Aganippe sp.1/8 and Aganippe sp. 2/7 form monophyletic clades that are highly divergent. This reflects a long history of evolutionary isolation and thus their status as distinct species is supported by this study. Similarly, while specimens SCT14 and MDT30 were both identified as *Anidiops*, genetic differentiation between them was high (17.4%). This level of divergence suggests that they are unlikely to belong to the same species.

Variation within Aganippe sp. 2/7 requires further consideration. Sequence divergence between lineages A and B is substantially higher (approximately 7-8%) than within lineages (<2%), and the lineages are geographically separated from one-another. Further, the Lake Rason

paleodrainage separates the sites where the A and B lineages are found (M. Davis, pers. comm.). While this level of divergence is sometimes seen between distinct spider species (Barrett & Hebert, 2005), the biology of trapdoor spiders is conducive to producing variation at maternally-inherited mitochondrial genes such as COI. Female trapdoor spiders rarely leave their burrow; it is the male of the species that tends to disperse (Main, 1982). Furthermore, unlike many spider groups, juveniles do not disperse aerially (Main, 1995). Trap door spiders are thus a group likely to show genetic divergence over small spatial scales, which may not be reflected in morphological variation. Whether they are considered "species" or not, they have a long-independent evolutionary history, corresponding to several millions of years of isolation.

Aganippe sp. 1/8 and 2/7 showed affinities to other Agnaippe specimens from Goldfield and Wheatbelt sites, however the large genetic distances between genetic groups (represented by long branch lengths) suggests that Aganippe sp. 1/8 and 2/7 represent new species.

The taxonomic status of the Anidiops specimens needs further investigation. Both Anidiops haplotypes were contained within the Aganippe clade. It is believed that Aganippe evolved from Anidiops (B. York Main, pers. comm. to M. Davis) hence, this might explain the affinity of the Anidiops specimens to the genus Aganippe. However, without DNA sequences of Anidiops we cannot fully resolve the taxonomic status of the TGP specimens. Based on the current data set, the Anidiops specimens appear to have no close affinity to the genus Gaius.

#### Acknowledgements

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| Organism         | Code   | zone | Easting  | Northing |
|------------------|--------|------|----------|----------|
| Anidiops sp.     | MDT01  | 51 J | 650098.3 | 6761371  |
| Aganippe sp. 2/7 | MDT05  | 51 J | 650902.1 | 6761281  |
| Aganippe sp. 2/7 | MDT08  | 51 J | 651245.4 | 6761393  |
| Aganippe sp. 1/8 | MDT114 | 51 J | 652887.2 | 6764485  |
| Aganippe sp. 2/7 | MDT12  | 51 J | 649906.3 | 6760570  |
| Aganippe sp. 1/8 | MDT128 | 51 J | 654945.1 | 6763554  |

Table 1. Specimens sequenced for COI and their morphological assignments.

| 1                | 1      | 1   | i i      | i       |
|------------------|--------|-----|----------|---------|
| Aganippe sp. 1/8 | MDT14  | 51J | 650404.7 | 6759924 |
| Aganippe sp. 1/8 | MDT17  | 51J | 655378.1 | 6759549 |
| Aganippe sp. 2/7 | MDT20A | 51J | 655502.9 | 6759556 |
| Anidiops sp.     | MDT30  | 51J | 647568.3 | 6763874 |
| Aganippe sp. 2/7 | MDT41  | 51J | 658645.3 | 6775912 |
| Aganippe sp. 2/7 | MDT48  | 51J | 659087.6 | 6773427 |
| Aganippe sp. 1/8 | MDT54  | 51J | 655394.4 | 6759554 |
| Aganippe sp. 2/7 | MKT03  | 51J | 651224.3 | 6761273 |
| Aganippe sp. 2/7 | MKT108 | 51J | 655176   | 6762783 |
| Aganippe sp. 1/8 | MKT114 | 51J | 652861.3 | 6764488 |
| Aganippe sp. 2/7 | MKT115 | 51J | 652737.8 | 6764341 |
| Aganippe sp. 2/7 | SCT01  | 51J | 651294.5 | 6761436 |
| Aganippe sp. 1/8 | SCT02  | 51J | 650403.1 | 6759919 |
| Aganippe sp. 2/7 | SCT06  | 51J | 655525.9 | 6759576 |
| Anidiops sp.     | SCT13  | 51J | 648418.1 | 6763565 |
| Anidiops sp.     | SCT14  | 51J | 647814.2 | 6763597 |
| Aganippe sp. 2/7 | SCT23  | 51J | 659054.3 | 6773603 |

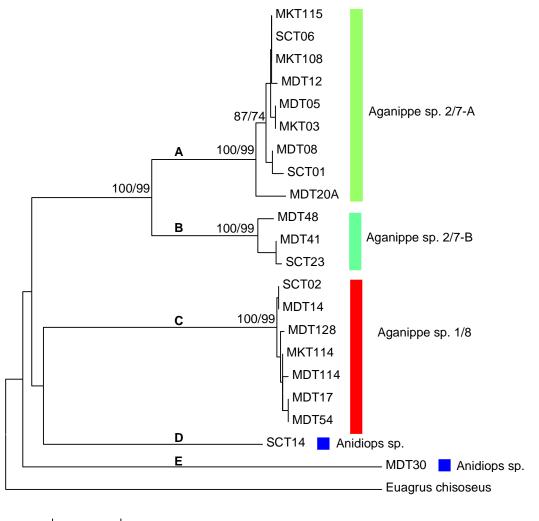
Table 2. Genetic diversity (uncorrected p-distances) between the major lineages A-E.

| Lineage | А         | В         | С         | D    | E |
|---------|-----------|-----------|-----------|------|---|
| А       | Х         |           |           |      |   |
| В       | 7.0-7.8   | Х         |           |      |   |
| С       | 13.9-15.1 | 13.9-14.4 | Х         |      |   |
| D       | 13.6-14.1 | 13.6-14.1 | 13.3-13.4 | Х    |   |
| E       | 16.6-18.4 | 16.6-17.1 | 17.9-18.1 | 17.4 | Х |

Table 3. Genetic diversity (uncorrected p-distances) within morphotypes.

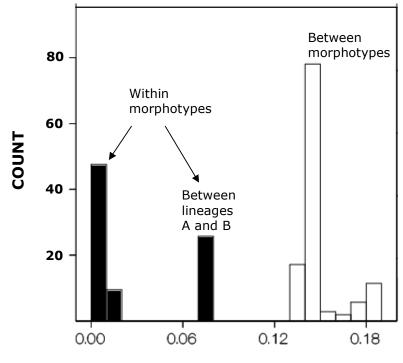
| Morphotype         | Range of genetic divergence |
|--------------------|-----------------------------|
| Aganippe sp. 2/7   | 0.0 - 7.8 %                 |
| Aganippe sp. 2/7-A | 0.0 - 1.7%                  |
| Aganippe sp. 2/7-B | 0.2 - 1.2%                  |
| Aganippe sp. 1/8   | 0.0 - 0.3%                  |
| Anidiops sp.       | 17.4%                       |

Figure 1. Neighbour-joining tree of uncorrected p-distances for a 616 bp alignment of COI. Numbers on major nodes correspond to NJ and MP bootstrap support, respectively Letters on branches correspond to lineages as referred to in the text. Morphological designations are indicated to the right of the haplotypes, and coloured bars correspond to map of lineages (Figure 2).



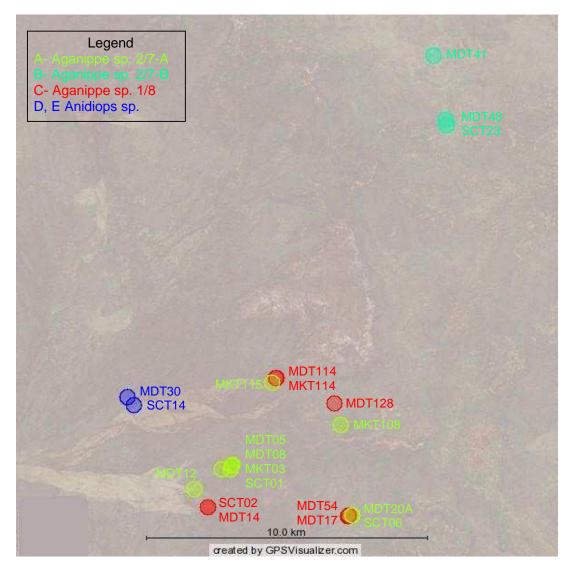
0.02

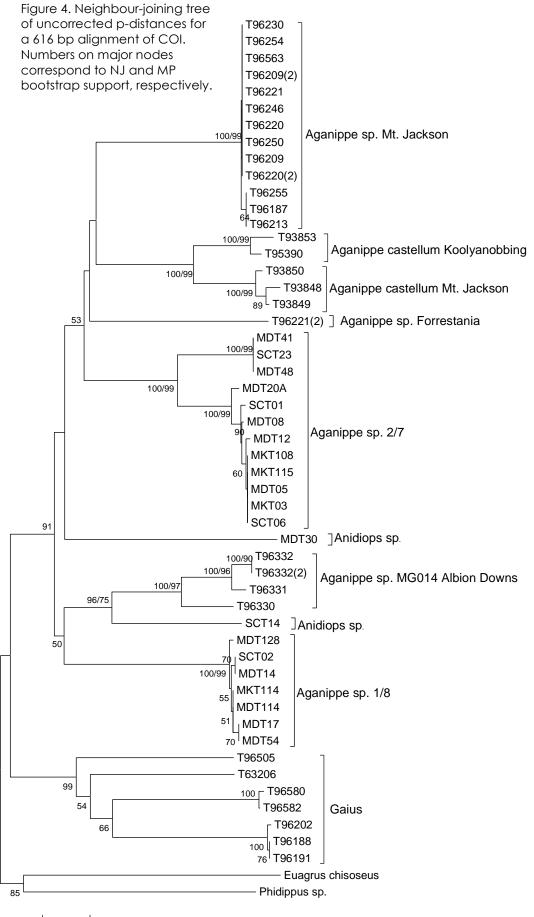
Figure 2. Histogram showing the distribution of pair-wise sequence divergence between haplotypes (uncorrected p-distances), highlighting differences within and between morphotypes.



Sequence Divergence

Figure 3. Distribution of genetic lineages as shown if Figure 1.





0.02

Appendix 1.Complete sequences for 21 specimens of mygalomorphs for a 616 bp fragment of COI.

MDT41

SCT02 MDT05 MDT08 **MDT114** MDT12 **MDT128** MDT14 MDT17 MDT20A MDT30 MDT48 MDT54 **MKT03 MKT108 MKT114 MKT115** SCT01 SCT06 SCT14 TGATTAGTTCCTTTAATGTTAGGGGCACCTGATATGGCTTTTCCTCGGATGAATAATTTGAGATTTTGATTGTTACCT SCT23 MDT41 SCT02 CCTTCTTTATTTTTTGTTATTATCTTCAGTAATTGAGGTTGGTGGGGGGCTGGGTGAACAATTTATCCTCCTTTG MDT05 CCTTCTTTGTTTTTTGATGGTTTCTTCTTTGATTGAGGTTGGGGTAGGGGCTGGTTGGACTATTTATCCTCCATTG MDT08 CCTTCTTTGTTTTTTGATGGTTTCTTCTTTGATTGAGGTTGGGGTAGGGGCTGGTTGGACTATTTATCCTCCATTG **MDT114** CCTTCTTTATTTTTTGTTATTATCTTCAGTAATTGAGGTTGGTGGGGGGGCTGGGTGAACAATTTATCCTCCTTTG MDT12 CCTTCTTTGTTTTTTGATGGTTTCTTCTTTGATTGAGGTTGGGGTAGGGGCTGGTTGGACTATTTATCCTCCATTG **MDT128** CCTTCTTTATTTTTGTTATTATCTTCAGTAATTGAGGTTGGTGTGGGGGGCTGGGTGAACAATTTATCCTCCTTTG MDT14 CCTTCTTTATTTTTTGTTATTATCTTCAGTAATTGAGGTTGGTGGGGGGCTGGGTGAACAATTTATCCTCCTTTG MDT17 CCTTCTTTATTTTTTTGTTATTATCTTCAGTAATTGAGGTTGGTGGGGGGCTGGGTGAACAATTTATCCTCCTTTG MDT20A CCTTCTTTGTTTTTTGATGGTTTCTTCTTTGATTGAGGTTGGGGTAGGGGCTGGTTGGACTATTTATCCTCCATTG MDT30 CCTTCTTTATTTTTTTAATGGTTTCTTCGTTGATACAGATTGGGGCTTGGGGTGGGATGAACCATTTATCCTCCTTTG MDT48 MDT54 CCTTCTTTATTTTTTGTTATTATCTTCAGTAATTGAGGTTGGTGGGGGGCTGGGTGAACAATTTATCCTCCTTTG MKT03 CCTTCTTTGTTTTTTTGATGGTTTCTTCTTTGATTGAGGTTGGGGTAGGGGCTGGTTGGACTATTTATCCTCCATTG **MKT108** CCTTCTTTGTTTTTTTGATGGTTTCTTCTTTGATTGAGGTTGGGGTAGGGGCTGGTTGGACTATTTATCCTCCATTG **MKT114** 

MKT115

CCTTCTTTGTTTTTTGATGGTTTCTTCTTTGATTGAGGTTGGGGTAGGGGGCTGGTTGGACTATTTATCCTCCATTG SCT01 CCTTCTTTGTTTTTTGATGGTTTCTTCTTTGATTGAGGTTGGGGTAGGGGCTGGTTGGACTATTTATCCTCCATTG SCT06 CCTTCTTTGTTTTTTGATGGTTTCTTCTTTGATTGAGGTTGGGGTAGGGGCTGGTTGGACTATTTATCCTCCATTG SCT14 CCTTCTTTGTTTTTTTTTGGTTTCGTCTTTGATTGAGGTAGGAGTGGGAGCAGGATGAACGATTTATCCTCCTTTG SCT23 CCTTCTTTGTTTTTTGATGGTTTCTTCTTTAATTGAGGTTGGGGTGGGGGGCTGGTTGAACTATTTATCCTCCATTG MDT41 SCT02 TCTTCAGGGGTAGGTCATAGAGGTGGAGGAATAGATTTTGTTGTGTTTTCTTTACATTTGGCGGGGGGCTTCTTCAATT MDT05 TCTTCAGGTGTCGGGCATAGAGATGGGGGGGATAGATTTTGTTGTTTTTTCTTTGCATTTAGCGGGTGCTTCTTCAATT MDT08 TCTTCAGGTGTTGGGCATAGAGATGGGGGGGATAGATTTTGTTGTTTTTTCTTTGCATTTAGCGGGTGCTTCTTCAATT **MDT114** TCTTCAGGGGTAGGTCATAGAGGTGGAGGAATAGATTTTGTTGTGTGTTTTCTTTACATTTGGCTGGGGCTTCTTCAATT MDT12 **MDT128** TCTTCAGGGGTAGGTCATAGAGGTGGAGGAATAGATTTTGTTGTGTTTTCTTTACATTTGGCTGGGGCTTCTTCAATT MDT14 TCTTCAGGGGTAGGTCATAGAGGTGGAGGAATAGATTTTGTTGTGTTTTCTTTACATTTGGCGGGGGGCTTCTTCAATT MDT17 TCTTCAGGGGTAGGTCATAGAGGTGGAGGAATAGATTTTGTTGTGTTTTCTTTACATTTGGCTGGGGCTTCTTCAATT MDT20A TCTTCAGGTGTTGGGCATAGAGATGGGGGGGATAGATTTTGTTGTTGTTTTTCTTTGCATTTAGCGGGTGCTTCTTCAATT MDT30 TCTTCTAGTGTTGACCATAGAGGTGGGGGG-ATAGATTTTGTTGTTTATTCTCTTCATTTAGCTGGTGCTTCTTCAATT MDT48 MDT54 TCTTCAGGGGTAGGTCATAGAGGTGGAGGAATAGATTTTGTTGTGTTTTCTTTACATTTGGCTGGGGCTTCTTCAATT MKT03 TCTTCAGGTGTCGGGCATAGAGATGGGGGGGATAGATTTTGTTGTTTTTTCTTTGCATTTAGCGGGTGCTTCTTCAATT **MKT108** TCTTCAGGTGTCGGGCATAGAGATGGGGGGGATAGATTTTGTTGTTTTTTCTTTGCATTTAGCGGGTGCTTCTTCAATT **MKT114** TCTTCAGGGGTAGGTCATAGAGGTGGAGGAATAGATTTTGTTGTGTTTTCTTTACATTTGGCTGGGGCTTCTTCAATT **MKT115** TCTTCAGGTGTCGGGCATAGAGATGGGGGGGATAGATTTTGTTGTTTTTTCTTTGCATTTAGCGGGTGCTTCTTCAATT SCT01 TCTTCAGGTGTTGGGCATAGAGATGGGGGGGATAGATTTTGTTGTTGTTTTTCTTTGCATTTAGCGGGTGCTTCTTCAATT SCT06 TCTTCAGGTGTCGGGCATAGAGATGGGGGGGATAGATTTTGTTGTTTTTTCTTTGCATTTAGCGGGTGCTTCTTCAATT SCT14 SCT23 MDT41 ATAGGAGCTATTAATTTTATTTCTACTGTAATTAATATGCGAACTAAGGGAATAGAGTTTGAACGCGTCCCTTTGTTT SCT02 ATAGGTGCTATTAATTTTATTTCTACTATTATAAACATACGAGCTTTAGGAATGAGGTTTGAGCGTGTTCCTTTGTTT MDT05 ATGGGGGCTATTAATTTTATTTCTACTGTAATTAATATGCGGACTAAGGGGATAGAGTTTGAACGGGTACCTTTGTTT MDT08 ATGGGGGCTATTAATTTTATTTCTACTGTAATTAATATGCGGACTAAGGGGATAGAGTTTGAACGGGTACCTTTGTTT **MDT114** ATAGGTGCTATTAATTTTATTTCTACTATTATAAACATACGAGCTTTAGGAATGAGGTTTGAGCGTGTTCCTTTGTTT MDT12 ATGGGGGCTATTAATTTTATTTCTACTGTAATTAATATGCGGACTAAGGGGATAGAGTTTGAACGGGTACCCTTGTTT **MDT128** ATAGGTGCTATTAATTTTATTTCTACTATTATAAACATACGAGCTTTAGGAATGAGGTTTGAGCGTGTTCCTTTGTTT MDT14 ATAGGTGCTATTAATTTTATTTCTACTATTATAAACATACGAGCTTTAGGAATGAGGTTTGAGCGTGTTCCTTTGTTT MDT17 ATAGGTGCCATTAATTTTATTTCTACTATTATAAACATACGAGCTTTAGGAATGAGGTTTGAGCGTGTTCCTTTGTTT MDT20A ATGGGGGCTATTAATTTTATTTCTACTGTAATTAATATGCGGACTAAGGGGATAGAGTTTGAACGCGTACCTTTGTTT MDT30 ATGGGCGCTATTAATTTCATTTCTACAATTATTAATATGCGAGCTATAGGAATGTTATTTGAGCGTGCACCATTGTTT MDT48 ATAGGAGCTATTAATTTTATTTCTACTGTAATTAATATGCGAACTAAGGGAATAGAGTTTGAACGCGTCCCTTTGTTT MDT54 ATAGGTGCCATTAATTTTATTTCTACTATTATAAACATACGAGCTTTAGGAATGAGGTTTGAGCGTGTTCCTTTGTTT

MKT03

ATGGGGGCTATTAATTTTATTTCTACTGTAATTAATATGCGGACTAAGGGGATAGAGTTTGAACGGGTACCTTTGTTT **MKT108** ATGGGGGCTATTAATTTTATTTCTACTGTAATTAATATGCGGACTAAGGGGATAGAGTTTGAACGGGTACCTTTGTTT **MKT114** ATAGGTGCTATTAATTTTATTTCTACTATTATAAACATACGAGCTTTAGGAATGAGGTTTGAGCGTGTTCCTTTGTTT **MKT115** ATGGGGGCTATTAATTTTATTTCTACTGTAATTAATATGCGGACTAAGGGGATAGAGTTTGAACGGGTACCTTTGTTT SCT01 ATGGGGGCTATTAATTTTATTTCTACTGTAATTAATATGCGGACTAAGGGGATAGAGTTTGAACGGGTTCCTTTGTTT SCT06 ATGGGGGCTATTAATTTTATTTCTACTGTAATTAATATGCGGACTAAGGGGATAGAGTTTGAACGGGTACCTTTGTTT SCT14 ATAGGGGCTATTAATTTTATTTCTACTATTGTAAATATACGAGCTATAGGGATGGTGTTTGAACGTGTTCCTTTGTTT SCT23 ATAGGAGCTATTAATTTTATTTCTACTGTAATTAATATGCGAACTAAGGGAATAGAGTTTGAACGCGTCCCTTTGTTT MDT41 GTATGGTCGGTGATGGTAACTGCTGTTTTATTATTGTTGTCTCTTCCTGTATTGGCTGGGGCCGATTACTATGTTATTG SCT02 GTTTGATCTGTTATAGTGACTACGGTGTTATTGTTATTATCTTTACCAGTATTGGCTGGGGCTATTACTATATTGTTG MDT05 GTATGGTCAGTAATGGTGACTGCTGTTTTATTATTGTTGTCTCTTCCTGTATTAGCTGGGGCAATTACTATATTATTG MDT08 GTATGGTCAGTAATGGTGACTGCTGTTTTATTATTGTTGTCTCTCCTGTATTAGCTGGGGCAATTACTATATTATTG **MDT114** GTTTGATCTGTTATAGTGACTACGGTGTTATTGTTATTATCTTTACCAGTATTGGCTGGGGGCTATTACTATATTGTTG MDT12 GTATGGTCAGTAATGGTGACTGCTGTTTTATTATTGTTGTCTCTTCCTGTATTAGCTGGGGCAATTACTATATTATTG **MDT128** GTTTGATCTGTTATAGTGACTACGGTGTTATTGTTATTATCTTTGCCAGTATTGGCTGGGGCTATTACTATATTGTTG MDT14 GTTTGATCTGTTATAGTGACTACGGTGTTATTGTTATTATCTTTACCAGTATTGGCTGGGGCTATTACTATATTGTTG MDT17 GTTTGATCTGTTATAGTGACTACGGTGTTATTGTTATTATCTTTACCAGTATTGGCTGGGGGCTATTACTATATTGTTG MDT20A GTATGGTCAGTAATGGTGACTGCTGTTTTATTATTGTTGTCTCTCTGTATTAGCTGGGGCAATTACTATATTATTG MDT30 GTATGGTCTGTAATAATTACTGCAATTTTATTGTTGTTGTCTCTTCCAGTATTGGCTGGTGCTATTACTATATTGTTA MDT48 GTATGGTCGGTGATGGTAACTGCTGTTTTATTGTTGTCTCTTCCTGTATTGGCTGGGGCGATTACTATGTTATTG MDT54 GTTTGATCTGTTATAGTGACTACGGTGTTATTGTTATTATCTTTACCAGTATTGGCTGGGGCTATTACTATATTGTTG MKT03 GTATGGTCAGTAATGGTGACTGCTGTTTTATTATTGTTGTCTCTCCTGTATTAGCTGGGGCAATTACTATATTATTG **MKT108** GTATGGTCAGTAATGGTGACTGCTGTTTTATTATTGTTGTCTCTTCCTGTATTAGCTGGGGCAATTACTATATTATTG **MKT114** GTTTGATCTGTTATAGTGACTACGGTGTTATTGTTATTATCTTTACCAGTATTGGCTGGGGCTATTACTATATTGTTG **MKT115** GTATGGTCAGTAATGGTGACTGCTGTTTTATTATTGTTGTCTCTTCCTGTATTAGCTGGGGCAATTACTATATTG SCT01 GTATGGTCAGTAATGGTGACTGCTGTTTTATTATTGTTGTCTCTTCCTGTATTAGCTGGGGCAATTACTATATTG SCT06 GTATGGTCAGTAATGGTGACTGCTGTTTTATTATTGTTGTCTCTTCCTGTATTAGCTGGGGCAATTACTATATTATTG SCT14 GTATGGTCTGTTATAGTAACTACAGTGTTGTTGCTATTATCTCTACCTGTGTTAGCTGGAGCTATTACTATGTTGTTA SCT23 GTATGGTCGGTGATGGTAACTGCTGTTTTATTATTGTTGTCTCTTCCTGTATTGGCTGGGGCGATTACTATGTTATTG MDT41 SCT02 MDT05 MDT08 **MDT114** ACTGATCGTAATTTTAATACTTCTTTTTTTGATCCGGCTGGTGGGGGGTGATCCTGTGTTATTTCAACATTTGTTTTGA MDT12 **MDT128** MDT14 ACTGATCGTAATTTTAATACTTCTTTTTTGATCCGGCTGGTGGGGGGGTGATCCTGTGTTATTTCAACATTTGTTTTGA MDT17 MDT20A 

MDT30

MDT48 MDT54 MKT03 **MKT108** MKT114 ACTGATCGTAATTTTAATACTTCTTTTTTTGATCCGGCTGGTGGGGGGTGATCCTGTGTTATTTCAACATTTGTTTTGA **MKT115** SCT01 SCT06 SCT14 SCT23 MDT41 TTTTTTGGTCATCCTGAAGTTTATATTTTGATTTTGCCTGGGTTTGGTATGGTATCTCATATTGTTAGAGGTTCGGTA SCT02 TTCTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGGGTTTGGGATAGTGTCTCATATTGTTAGAGGTTCAGTT MDT05 TTTTTTGGTCATCCTGAGGTTTATATCTTGATTTTACCAGGGTTTGGTATAGTGTCTCATATCGTTAGAGGTTCGGTA MDT08 TTTTTTGGTCATCCTGAGGTTTATATCTTGATTTTACCAGGGTTTGGTATAGTGTCTCATATCGTTAGAGGTTCGGTA **MDT114** TTCTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGGGTTTGGGATAGTGTCTCATATTGTTAGAGGTTCAGTT MDT12 TTTTTTGGTCATCCTGAGGTTTATATCTTGATTTTACCAGGGTTTGGTATAGTGTCTCATATCGTTAGAGGTTCGGTA MDT128 TTCTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGGGTTTGGGATAGTGTCTCATATTGTTAGAGGTTCAGTT MDT14 TTCTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGGGTTTGGGATAGTGTCTCATATTGTTAGAGGTTCAGTT MDT17 TTCTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGGGTTTGGGATAGTGTCTCATATTGTTAGAGGTTCAGTT MDT20A TTTTTTGGTCATCCTGAGGTTTATATCTTGATTTTACCAGGGTTTGGTATAGTGTCTCATATCGTTAGAGGTTCGGTA MDT30 TTTTTTGGGCATCCTGAGGTTTACATTCTGATTTTGCC-----TGGTATCACATATTGTAAGAGGGTAGGTG MDT48 TTTTTTGGTCATCCTGAAGTTTATATTTTGATTTTGCCTGGGTTTGGTATGGTATCTCATATTGTTAGAGGTTCGGTA MDT54 TTCTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGGGTTTGGGATAGTGTCTCATATTGTTAGAGGTTCAGTT MKT03 TTTTTTGGTCATCCTGAGGTTTATATCTTGATTTTACCAGGGTTTGGTATAGTGTCTCATATCGTTAGAGGTTCGGTA **MKT108** TTTTTTGGTCATCCTGAGGTTTATATCTTGATTTTACCAGGGTTTGGTATAGTGTCTCATATCGTTAGAGGTTCGGTA **MKT114** TTCTTTGGTCATCCTGAGGTTTATATTTTAATTTTGCCAGGGTTTGGGATAGTGTCTCATATTGTTAGAGGTTCAGTT **MKT115** TTTTTTGGTCATCCTGAGGTTTATATCTTGATTTTACCAGGGTTTGGTATAGTGTCTCATATCGTTAGAGGTTCGGTA SCT01 TTTTTTGGTCATCCTGAGGTTTATATCTTGATTTTACCAGGGTTTGGTATAGTGTCTCATATCGTTAGAGGTTCGGTA SCT06 TTTTTTGGTCATCCTGAGGTTTATATCTTGATTTTACCAGGGTTTGGTATAGTGTCTCATATCGTTAGAGGTTCGGTA SCT14 TTTTTTGGTCATCCTGAGGTTTATATTTTGATTTTACCTGGTTTTGGAATAGTGTCTCATATTGTGAGAGGTTCGGTA SCT23 TTTTTTGGTCATCCTGAAGTTTATATTTTGATTTTGCCTGGGTTTGGTATGGTATCTCATATTGTTAGAGGTTCGGTA MDT41 GGCAAGCGAGAACCTTTTGGATTATTAGGAATGATTTATGCGATGGTTGGGATTGGCGGAATAGGGTTTG SCT02 MDT05 GGGAAGCGTGAACCTTTTGGGTTATTAGGAATGATTTATGCGATAGTTGGAATTGGTGGGATGGGATTTG MDT08 GGGAAGCGTGAACCTTTTGGGTTATTAGGAATGATTTATGCAATAGTTGGAATTGGTGGGATGGGATTTG **MDT114** MDT12 GGGAAGCGTGAACCTTTTGGGTTATTAGGAATGATTTATGCAATAGTTGGAATTGGTGGGATGGGATTTG **MDT128** MDT14 MDT17 GGGAGGCGTGAACCCTTTGGGTTATTAGGAATGATTTATGCAATAGTAGGAATTGGTGGGATGGGATTTG MDT20A MDT30 GGTAAGCAAGAACCTTTTGGGTCATTGGGGATAATTTATGCGATGGCTGGTATTGGGGGGGATAGGGTTTG MDT48 GGCAAGCGAGAACCTTTTGGATTATTAGGAATGATTTATGCGATGGTTGGAATTGTTGGTATAGGATATG MDT54 **MKT03** GGGAAGCGTGAACCTTTTGGGTTATTAGGAATGATTTATGCGATAGTTGGAATTGGTGGGATGGGATTTG

 Appendix 2. Pair-wise sequence divergence (uncorrected p-distances) for individual mygalomorph specimens for a 616 bp fragment of COI. Green shaded cells correspond to divergence within morphotype Aganippe sp. 2/7. Red shaded cells correspond to divergence within morphotype Aganippe sp. 1/8.

| lineage     | A     |       |       |       |       |       |       |       |       |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|             | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     |
| [ 1] MKT115 | х     |       |       |       |       |       |       |       |       |
| [2] SCT06   | 0.000 | х     |       |       |       |       |       |       |       |
| [ 3] MKT108 | 0.000 | 0.000 | х     |       |       |       |       |       |       |
| [ 4] MDT12  | 0.002 | 0.002 | 0.002 | Х     |       |       |       |       |       |
| [ 5] MDT05  | 0.002 | 0.002 | 0.002 | 0.003 | x     |       |       |       |       |
| [ 6] MKT03  | 0.002 | 0.002 | 0.002 | 0.003 | 0.000 | x     |       |       |       |
| [ 7] MDT08  | 0.003 | 0.003 | 0.003 | 0.005 | 0.005 | 0.005 | х     |       |       |
| [ 8] SCT01  | 0.007 | 0.007 | 0.007 | 0.008 | 0.008 | 0.008 | 0.003 | х     |       |
| [ 9] MDT20A | 0.013 | 0.013 | 0.013 | 0.015 | 0.015 | 0.015 | 0.013 | 0.017 | X     |
| [10] MDT48  | 0.071 | 0.071 | 0.071 | 0.073 | 0.070 | 0.070 | 0.071 | 0.073 | 0.075 |
| [11] MDT41  | 0.073 | 0.073 | 0.073 | 0.075 | 0.071 | 0.071 | 0.073 | 0.075 | 0.076 |
| [12] SCT23  | 0.075 | 0.075 | 0.075 | 0.076 | 0.073 | 0.073 | 0.075 | 0.076 | 0.078 |
| [13] SCT02  | 0.144 | 0.144 | 0.144 | 0.146 | 0.144 | 0.144 | 0.146 | 0.146 | 0.148 |
| [14] MDT14  | 0.144 | 0.144 | 0.144 | 0.146 | 0.144 | 0.144 | 0.146 | 0.146 | 0.148 |
| [15] MDT128 | 0.146 | 0.146 | 0.146 | 0.148 | 0.146 | 0.146 | 0.148 | 0.148 | 0.149 |
| [16] MKT114 | 0.146 | 0.146 | 0.146 | 0.148 | 0.146 | 0.146 | 0.148 | 0.148 | 0.149 |
| [17] MDT114 | 0.148 | 0.148 | 0.148 | 0.149 | 0.148 | 0.148 | 0.149 | 0.149 | 0.151 |
| [18] MDT17  | 0.148 | 0.148 | 0.148 | 0.149 | 0.148 | 0.148 | 0.149 | 0.149 | 0.151 |
| [19] MDT54  | 0.148 | 0.148 | 0.148 | 0.149 | 0.148 | 0.148 | 0.149 | 0.149 | 0.151 |
| [20] SCT14  | 0.138 | 0.138 | 0.138 | 0.139 | 0.139 | 0.139 | 0.141 | 0.141 | 0.136 |
| [21] MDT30  | 0.181 | 0.181 | 0.181 | 0.181 | 0.179 | 0.179 | 0.181 | 0.184 | 0.182 |

Aganippe sp. 2/7 Aganippe sp. 1/8

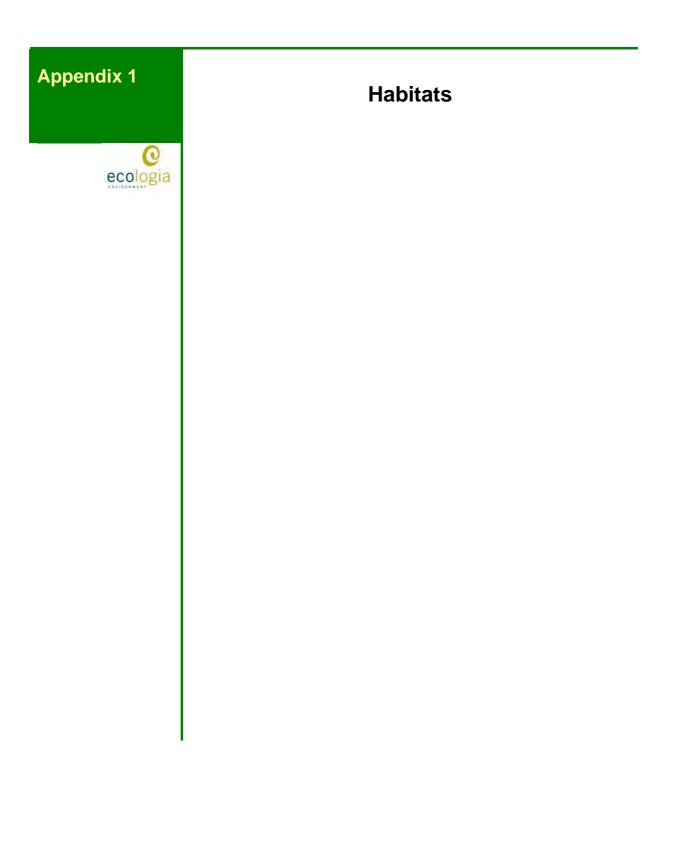
#### Appendix 1, continued

| В     |       |       | С     |       |       |       |       |       |       |  |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|
| 10    | 11    | 12    | 13    | 14    | 15    | 16    | 17    | 18    | 19    |  |
|       |       |       |       |       |       |       |       |       |       |  |
|       |       |       |       |       |       |       |       |       |       |  |
|       |       |       |       |       |       |       |       |       |       |  |
|       |       |       |       |       |       |       |       |       |       |  |
|       |       |       |       |       |       |       |       |       |       |  |
|       |       |       |       |       |       |       |       |       |       |  |
|       |       |       |       |       |       |       |       |       |       |  |
|       |       |       |       |       |       |       |       |       |       |  |
|       |       |       |       |       |       |       |       |       |       |  |
| х     |       |       |       |       |       |       |       |       |       |  |
| 0.010 | х     |       |       |       |       |       |       |       |       |  |
| 0.012 | 0.002 | x     |       |       |       |       |       |       |       |  |
| 0.141 | 0.139 | 0.141 | х     |       |       |       |       |       |       |  |
| 0.141 | 0.139 | 0.141 | 0.000 | X     |       |       |       |       |       |  |
| 0.143 | 0.141 | 0.143 | 0.003 | 0.003 | x     |       |       |       |       |  |
| 0.143 | 0.141 | 0.143 | 0.002 | 0.002 | 0.002 | Х     |       |       |       |  |
| 0.144 | 0.143 | 0.144 | 0.003 | 0.003 | 0.003 | 0.002 | х     |       |       |  |
| 0.144 | 0.143 | 0.144 | 0.003 | 0.003 | 0.003 | 0.002 | 0.003 | x     |       |  |
| 0.144 | 0.143 | 0.144 | 0.003 | 0.003 | 0.003 | 0.002 | 0.003 | 0.000 | х     |  |
| 0.136 | 0.139 | 0.141 | 0.134 | 0.134 | 0.134 | 0.133 | 0.134 | 0.134 | 0.134 |  |
| 0.171 | 0.166 | 0.167 | 0.181 | 0.181 | 0.179 | 0.179 | 0.181 | 0.181 | 0.181 |  |

Aganippe sp. 2/7 Aganippe sp. 1/8

#### Appendix 2, continued

| D          | E  |
|------------|----|
| 20         | 21 |
|            |    |
|            |    |
|            |    |
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|            |    |
|            |    |
|            |    |
|            |    |
|            |    |
|            |    |
|            |    |
|            |    |
| x<br>0.174 |    |
| 0.174      | Х  |





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

#### Site T5

Habitat type: loamy open ground, Spinifex & acacia

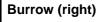
**Description:** Acacia jamesiana sparse medium shrubs, over *Triodia basedowii*. Sparse leaf litter, mainly under shrubs and sparse wood litter. Open ground; redorange sandy loam.

Type specimen (male)





### Habitat of Aganippe sp. 1/8 located outside TGP



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, she-oak & acacia

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

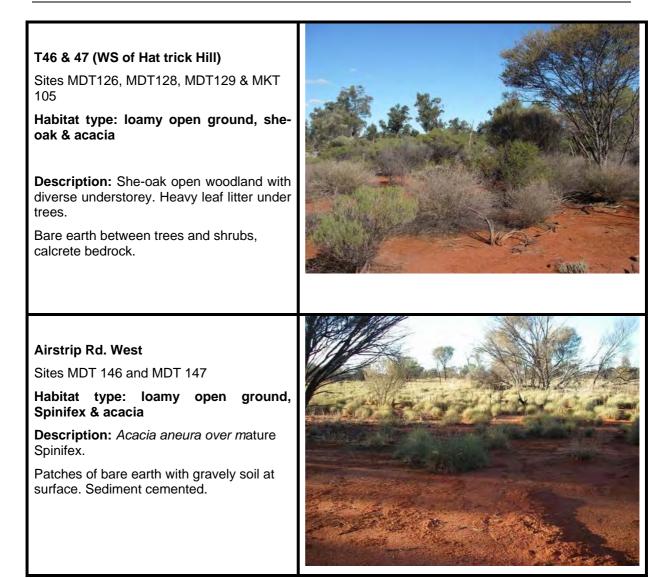
Open ground, red sandy clay, calcrete bedrock





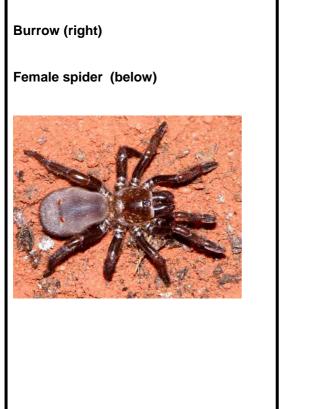








## Type habitat of *Aganippe* sp. 2/7 inside TGP





#### 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

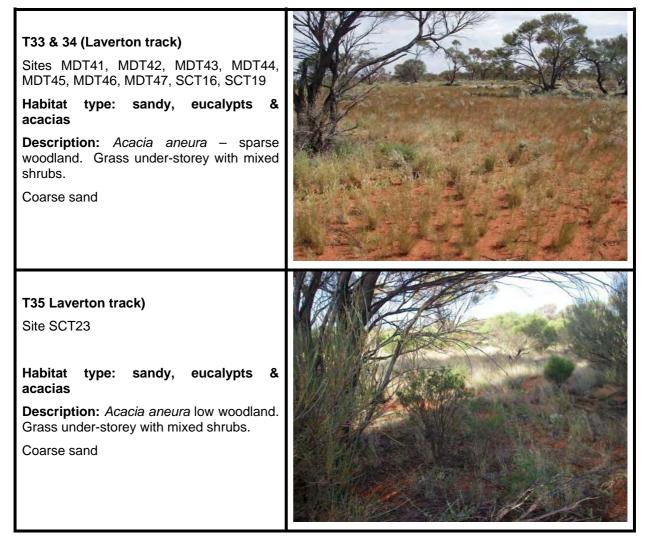
**Description:** *Eucalyptus gongylocarpa* open low woodland, over *Eucalyptus trivalvis* sparse mallee, over open low shrubs and *Triodia basedowii* moderately dense hummock grassland.

Moderate leaf litter, mainly under trees and sparse wood litter

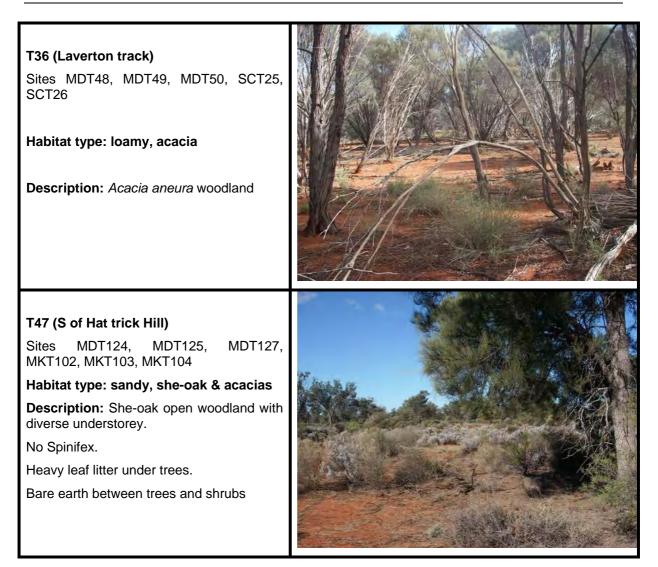
Orange sand



# Type habitat of Aganippe sp. 2/7 outside TGP







# 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, sheoak & 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 SwoInpes darwinii gen nov. sp. nov. (outside TGP)

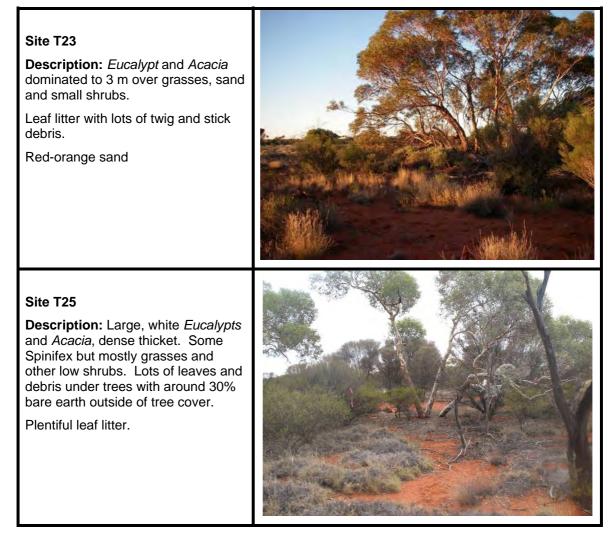
#### Site T 46

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

Lots of bare earth and calcrete



# Other habitat of SwoInpes darwinii gen nov. sp. nov. (outside TGP)





# 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



