GOVERNMENT OF WESTERN AUSTRALIA

Department of **Biodiversity, Conservation and Attractions**

Biodiversity and Conservation Science

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**Genotyping of bilby scats collected from Hillside Station, Western Australia.**

Martin Dziminski and Fiona Carpenter

**Background and field sampling**

Spectrum Ecology provided three scat samples to the Department of Biodiversity, Conservation and Attractions for genotyping, all of which were confirmed to be greater bilby (*Macrotis lagotis*) scats (Table 1). Samples were opportunistically collected on the 15th June and 2nd August 2019. Samples were stored dry, at room temperature, in 30ml tubes, approximately 1/3-filled with silica gel beads and tissue, until DNA extraction was undertaken.

**Laboratory analyses**

DNA extractions were undertaken following the protocol in Carpenter and Dziminski (2017). Genomic DNA was extracted from scats using the Qiagen QIAamp Fast DNA Stool Mini Kit with some modifications from Piggott and Taylor (2003) to the recommended procedures included in the kit. DNA was screened using eight highly polymorphic microsatellite markers (Table 2). These were multiplexed into two polymerase chain reactions (PCR) using the Qiagen Multiplex PCR Plus Kit. PCR amplification was performed using cycling conditions modified from the Qiagen Multiplex PCR Plus Kit. The PCR product was then analyzed on an ABI3730XL Sequencer, sized using Genescan-500 LIZ internal size standard, and genotyped using Genemapper Software 5.

**Genotyping**

Of the three supplied samples, two samples yielded DNA and amplified at enough loci to include in identity analysis (Table 3). Genotyping using the eight loci identified one distinct individual (Table 3).

Alleles were compared manually against genotypes previously obtained from samples collected from Hillside Station, the Fortescue Metals Group Rail site and Turner and Shaw Rivers. The individual identified this year from Hillside Station has not been recorded previously during survey or monitoring at any of these sites.

**Recommendations**

Since the samples were collected opportunistically across a limited area, abundance analyses were unable to be completed. Maximum likelihood spatially explicit capture-recapture analyses (SECR: Efford 2004) are used to calculate densities and numbers of animals within activity areas or across larger tracts of occupied habitat selected for survey. These analyses require systematically collected samples from within delineated activity areas or a selected defined portion of land that is spatially continuously occupied. Two previous studies (Dziminski *et al.* 2018; Dziminski *et al.* 2019) are attached as examples should abundance need to be sampled in the future.

Sincerely,

Dr Martin Dziminski
Research Scientist

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Table 1. Bilby scat samples provided by Spectrum Ecology.

| Low Ecol ID | DBCA ID | Latitude | Longitude | Date Collected |
|---|---|---|---|---|
| Scat 1 | BIL0803 | -21.62673 | 119.36973 | 15/08/2019 |
| Scat 2 | BIL0804 | -21.62639 | 119.3684 | 2/08/2019 |
| MM Scat | BIL0805 | -21.62493 | 119.3673 | 2/08/2019 |

Table 2. Microsatellite markers used in PCR.

| Locus | Fluorescent label | Reference |
|---|---|---|
| Multiplex 1 | | |
| B02 | 6-FAM | Moritz et al. (1997) |
| B17 | VIC | Moritz et al. (1997) and Smith et al. (2009) |
| B56 | PET | Moritz et al. (1997) and Smith et al. (2009) |
| B66 | NED | Moritz et al. (1997) |
| Multiplex 2 | | |
| B55 | 6-FAM | Moritz et al. (1997) |
| B22 | VIC | Moritz et al. (1997) |
| B41 | PET | Moritz et al. (1997) and Smith et al. (2009) |
| B63 | NED | Moritz et al. (1997) |

Table 3. Individuals identified from scat samples.

| Individual | Low Ecol ID | Latitude | Longitude |
|---|---|---|---|
| 1 | Scat 2 | -21.62639 | 119.3684 |
| 1 | MM Scat | -21.62493 | 119.3673 |

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