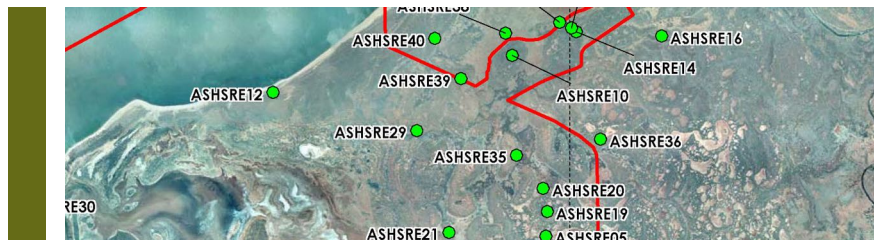




Ashburton Salt Project Level 2 Seasonal Fauna Survey



Prepared for K+S Salt Australia

October 2022

Biota
Environmental
Sciences



© Biota Environmental Sciences Pty Ltd 2020
ABN 49 092 687 119
Level 1, 228 Carr Place
Leederville Western Australia 6007
Ph: (08) 9328 1900 Fax: (08) 9328 6138

Project No.: 1261A

Prepared by: Dan Kamien

Document Quality Checking History

Version:	2.1	Peer review:	Jacinta King
	Rev 0	Director review:	Garth Humphreys
	Rev 0	Format review:	Garth Humphreys

Approved for issue: Garth Humphreys

This document has been prepared to the requirements of the client identified on the cover page and no representation is made to any third party. It may be cited for the purposes of scientific research or other fair use, but it may not be reproduced or distributed to any third party by any physical or electronic means without the express permission of the client for whom it was prepared or Biota Environmental Sciences Pty Ltd.

This report has been designed for double-sided printing. Hard copies supplied by Biota are printed on recycled paper.

Ashburton Salt Level 2 Fauna Survey

Contents

1.0	Summary	9
1.1	Background	9
1.2	Methodology	9
1.3	Results	9
1.4	Conservation Significance	10
2.0	Introduction	13
2.1	Project Background	13
2.2	Scope and Objectives	13
3.0	Methodology	15
3.1	Desktop Review	15
3.2	Survey Timing and Weather	16
3.3	Fauna Sampling	18
3.4	Identification and Data Analysis	27
3.5	Study Limitations	29
4.0	Desktop Assessment	30
4.1	Regional Context of the Study Area	30
4.2	Previous Fauna Surveys in the Locality	35
5.0	Survey Results	42
5.1	Fauna Habitats	42
5.2	Vertebrate Fauna	47
5.3	Short-Range Endemic Invertebrates	64
6.0	Assemblage Analysis	73
6.1	Species Accumulation Analysis	73
6.2	Contextual Analysis	74
6.3	Interpretation of Analyses	77
7.0	Conservation Significance	79
7.1	Vertebrate Fauna of Conservation Significance	79
7.2	SRE Invertebrates of Conservation Significance	90
8.0	Glossary	91
9.0	References	93

Appendix 1

Vertebrate Database Search Results and Previous Surveys in the Locality

Appendix 2

Threatened Fauna Statutory Framework

Appendix 3

DBCA Regulation 17 Permit

Appendix 4

Western Australian Museum Arachnid, Myriapod and Mollusc Database
Search Results

Appendix 5

Invertebrate Species Molecular Report

Appendix 6

Location of Recorded Conservation Significant Species

Appendix 7

Conservation Significant Species Considered Unlikely to Occur in Study Area

Tables

Table 1.1: Landscapes and landforms present in the study area.	9
Table 3.1: Criteria used to assign the likelihood of occurrence of a species within the study area.	16
Table 3.2: Summary of personnel conducting the survey.	17
Table 3.3: Weather at Onslow Airport during the Phase 1 survey period 2018.	17
Table 3.4: Weather at Onslow Airport during the Phase 2 survey period 2019.	17
Table 3.5: Trap location and effort during the survey.	20
Table 3.6: Systematic avifauna censuses undertaken at each fauna site within the study area during Phase 1.	21
Table 3.7: Systematic avifauna censuses undertaken at each fauna site within the study area during Phase 2.	21
Table 3.8: Remote camera locations and effort.	22
Table 3.9: Bat sampling locations and effort.	22
Table 3.10: Phase 1 SRE site locations and search effort.	24
Table 3.11: Phase 2 SRE site locations and search effort.	25
Table 3.12: Criteria used to determine SRE status.	28
Table 4.1: Land systems intersected by the study area.	30
Table 4.2: Geological units occurring in the study area (Geological Survey of WA (1995)).	32
Table 4.3: Soil units occurring in the study area.	32
Table 4.4: Previous relevant surveys conducted within 50 km of the study area.	36
Table 4.5: Vertebrate species identified from the desktop review.	39
Table 4.6: Vertebrate taxa of conservation significance previously recorded or potentially occurring in study area.	39
Table 4.7: Known or potential SRE taxa returned from database searches.	41
Table 5.1: Landscapes and landforms present in the study area.	42
Table 5.2: Systematic site descriptions and photographs.	44
Table 5.3: Vertebrate fauna recorded during the survey.	47
Table 5.4: Herpetofauna recorded in the study area.	50
Table 5.5: Avifauna recorded in the study area.	54
Table 5.6: Ground-dwelling mammals recorded in the study area.	61
Table 5.7: Bats recorded in the study area.	63
Table 5.8: Summary of potential SRE invertebrate fauna recorded from the study area	64

Table 6.1: Comparison of observed vertebrate species richness with estimator predictions.	74
Table 6.2: Herpetofauna species richness by study.	74
Table 6.3: Avifauna species richness by study.	75
Table 6.4: Ground mammals species richness by study.	76
Table 7.1: Potentially occurring conservation significant species from the desktop review that were not recorded in the study area.	87
Table 7.2: Likely and occurring conservation significant species' habitat preference within the study area.	89

Figures

Figure 2.1: Location of the study area.	14
Figure 3.1: Climate and weather graph depicting long-term averages and 2018 data.	18
Figure 3.2: Layout of pitfall and funnel traps.	19
Figure 3.3: Vertebrate trapping and sampling sites.	19
Figure 3.4: SRE sampling sites.	26
Figure 4.1: Land systems of the study area.	31
Figure 4.2: Surface geology of the study area.	33
Figure 4.3: Soils of the study area.	34
Figure 4.4: Previous relevant surveys intersecting the study area.	38
Figure 5.1: Vertebrate fauna landscapes of the study area.	43
Figure 5.2: Invertebrate SRE specimens collected within the study area.	65
Figure 6.1: Herpetofauna species accumulation curve.	73
Figure 6.2: Avifauna species accumulation curve.	73
Figure 6.3: Ground-dwelling mammals species accumulation curve.	74
Figure 6.4: nMDS plot of herpetofauna assemblage by study.	75
Figure 6.5: nMDS plot of avifauna assemblage by study.	76
Figure 6.6: nMDS plot of ground mammal assemblage by study.	77
Figure 7.1: Conservation significant vertebrate species recorded from the study area.	80

Plates

Plate 5.1: <i>Idiommata</i> sp. B38.	66
Plate 5.2: <i>Conothele</i> sp. C26.	66
Plate 5.3: <i>Conothele</i> sp. C26 closed burrow.	66
Plate 5.4: <i>Conothele</i> sp. C26 open burrow.	66
Plate 5.5: <i>Conothele</i> sp. C27.	67
Plate 5.6: <i>Conothele</i> sp. C27 closed burrow.	67
Plate 5.7: <i>Conothele</i> sp. C27 open burrow.	67
Plate 5.8: <i>Euoplos</i> sp. I67.	67
Plate 5.9: <i>Euoplos</i> sp. I67 closed burrow.	67
Plate 5.10: <i>Euoplos</i> sp. I67 open burrow.	67
Plate 5.11: <i>Aganippe</i> sp. I69.	68
Plate 5.12: <i>Aganippe</i> sp. I69 closed burrow.	68
Plate 5.13: <i>Aganippe</i> sp. I69 open burrow.	68
Plate 5.14: <i>Aname ellenae</i> female.	68
Plate 5.16: <i>Aname ellenae</i> male.	68

Plate 5.15: <i>Aname ellenae</i> burrow (photo source: WAM).	68
Plate 5.17: <i>Aname</i> sp. N57.	69
Plate 5.18: <i>Aname</i> sp. N57 burrow.	69
Plate 5.19: <i>Aname</i> sp. N141.	69
Plate 5.20: <i>Aname</i> sp. N141 closed burrow.	69
Plate 5.21: <i>Aname</i> sp. N141 open burrow.	69
Plate 5.22: <i>Aname</i> sp. N142.	70
Plate 5.23: <i>Aname</i> sp. N142 burrow.	70
Plate 5.24: <i>Aname</i> sp. N146.	70
Plate 5.25: <i>Aname</i> sp. N146 burrow.	70
Plate 5.26 <i>Succinea</i> sp.	71
Plate 5.27 <i>Rhagada convicta</i> .	71

1.0 Summary

1.1 Background

K+S Salt Australia Pty Ltd (K+S) is evaluating the possibility of developing a greenfield solar salt project (the proposed Ashburton Salt Project), located on the Western Australian coast approximately 40 km southwest of Onslow. Biota Environmental Sciences (Biota) was commissioned by K+S to undertake a two-phase, Level 2 terrestrial fauna survey to document the vertebrate fauna assemblage, fauna of conservation significance and Short-range Endemic (SRE) invertebrate fauna of the proposed project area (hereafter the study area). The purpose of this report is to provide information on the terrestrial fauna of the study area to inform the environmental impact assessment (EIA) of the proposal.

1.2 Methodology

A desktop review of relevant literature, databases, and past survey reports from the locality was undertaken in order to identify known features of conservation significance and compile a predicted faunal assemblage for the study area.

The field survey was conducted over two phases from October 30th to November 11th 2018 (Phase 1) and April 8th to 18th 2019 (Phase 2), in accordance with relevant Environmental Protection Authority policy and Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* guidance. Sampling effort within the study area included:

- systematic trapping at 14 locations within defined landforms, comprising 12 pit trapping transects with associated funnel traps, and two dedicated Elliott trapping transects with a total trap effort of 2,905 trap nights;
- a total of 57 avifauna censuses at the systematic trapping sites, totaling over 28 dedicated census hours;
- deployment of infrared motion cameras at 13 locations for a combined total of 61 nights;
- deployment of SongMeter echolocation call recorders targeting the bat assemblage at five locations for a total of 48 recording nights;
- non-systematic survey activities targeting vertebrate fauna, including ground foraging and identification of secondary signs; and
- a total of over 92 person hours dedicated to SRE fauna searches at 45 locations.

1.3 Results

1.1.1 Vertebrate Fauna Habitats

Five landscapes and associated landforms (fauna habitats) were determined for vertebrate fauna (Table 1.1).

Table 1.1: Landscapes and landforms present in the study area.

Landscapes	Landforms	Area (ha)
LANDSCAPE 1: Mainland remnants	<ul style="list-style-type: none"> • Longitudinal dune • Sand plain • Clay loam plain • Clay pan 	4,877
LANDSCAPE 2: Mud flats	<ul style="list-style-type: none"> • Hypersaline mudflats • Intertidal mudflats 	27,415

Landscape	Landforms	Area (ha)
LANDSCAPE 3: Inland dunes and plains	<ul style="list-style-type: none"> • Sand plain • Clay loam plain • Gilgai plain • Longitudinal dune • Clay pan • River bank • Creeklines and drainage 	18,271
LANDSCAPE 4: Coastal strand and dune	<ul style="list-style-type: none"> • Coastal dune • Beach 	1,690
LANDSCAPE 5: Mangroves	<ul style="list-style-type: none"> • Mangrove 	506

1.1.2 Vertebrate Species

The survey recorded a total of 171 vertebrate species, comprising 54 herpetofauna species, 97 avifauna species, 13 ground-dwelling mammal species and seven bat species.

The herpetofauna recorded represent over 51% of all herpetofauna species recorded from the locality based on database records and previous surveys. The recorded assemblage comprised four frog species (belonging to the families Pelodyadidae and Limnodynastidae), eight gecko species (families Carphodactylidae, Gekkonidae and Diplodactylidae), four legless lizard species (Pygopodidae), three dragon species (Agamidae), 18 skink species (Scincidae), four monitor species (Varanidae), four blind snake species (Typhlopidae), one python species (Pythonidae) and eight front-fanged snake species (Elapidae).

Bird species from 40 families were recorded from the study area, comprising 36 passerine and 61 non-passerine species. These represent 46% of all bird species recorded from the locality based on database records and previous surveys.

Thirteen ground-dwelling mammals were recorded from the study area, comprising one echidna (Tachyglossidae) four carnivorous marsupial species (Dasyuridae), one kangaroo species (Macropodidae), four rodent species (Muridae), three introduced predator species from two families (Canidae and Felidae). The species total represents 52% of all ground-dwelling mammal species recorded from the locality based on database records and previous surveys.

Seven bat species were recorded, comprising four species from the family Molossidae, and three species of Vespertilionidae.

1.1.3 Potential SRE Invertebrates

Mygalomorph spiders and land snails were the only taxonomic groups recorded in the study area with the potential to include SRE species. Of the 12 invertebrate taxa collected during the survey, eight mygalomorph spider taxa from four families are considered to be potential SREs. The remaining taxa have been demonstrated to not be SREs.

1.4 Conservation Significance

1.1.4 Fauna Habitat

Based on examination of aerial imagery and land systems mapping, none of the fauna habitats identified during the survey are confined to the study area, as they are common throughout the mainland east of Exmouth Gulf. Although their attributes are typical of similar habitat types in the wider locality, beach habitat, mangrove habitat (mangal) and the Ashburton River represent the habitat of highest fauna value in the study area, representing core habitat for the majority of conservation significant species recorded, likely to occur or potentially occurring within the study area.

Migratory shorebirds were also recorded throughout the habitats of the study area. The value of the study area in respect to migratory shorebirds is discussed in a separate report (Biota 2019a).

1.1.5 Vertebrates

The following 13 species of conservation significance¹ were recorded from the study area during the survey:

- Fork-tailed Swift, *Apus pacificus* (Migratory);
- Eastern Osprey, *Pandion cristatus* (Migratory);
- Common Sandpiper, *Actitis hypoleucos* (Migratory);
- Common Greenshank, *Tringa nebularia* (Migratory);
- Red-necked Stint, *Calidris ruficollis* (Migratory);
- Common Tern, *Sterna hirundo* (Migratory);
- Little Tern, *Sternula albifrons* (Migratory);
- Gull-billed Tern, *Gelochelidon nilotica* (Migratory);
- Caspian Tern, *Hydroprogne caspia* (Migratory);
- White-winged Black Tern, *Chlidonias leucopterus* (Migratory);
- Crested Tern, *Thalasseus bergii* (Migratory);
- Peregrine Falcon, *Falco peregrinus* (Other Specially Protected Fauna);
- Northern Coastal Free-tailed Bat, *Ozimops cobourgianus* (Priority 1).

Additionally, the Fairy Tern, *Sternula nereis* (Migratory) has previously been recorded within the study area, but not during the current survey. No reptiles or ground mammals of conservation significance were recorded from the study area.

1.1.6 SRE Invertebrates

Of the 12 invertebrate taxa collected during the survey, eight mygalomorph spider taxa from four families are considered to be potential SREs. Of these, five are known solely from the study area, comprising: *Idiommata* sp. B38; *Conothele* sp. C26; *Conothele* sp. C27; *Aname* sp. N142; and *Aname* sp. N146.

Although it is possible that these putative species exhibit highly localised distributions, they all occur on fauna habitats that are represented outside the study area. Additionally, the *Conothele* and *Aname* taxa occur in locations where analogous landscapes extend contiguously beyond the study area. Given this, it is unlikely that these taxa are restricted to the study area.

Although *Idiommata* sp. B38 was recorded on one occasion on mainland remnants, the biogeographical history of the study area and the distribution of other mygalomorph spiders in the study area indicate that this taxon is also likely to be more widespread than survey results indicate.

¹ Records of migratory shorebird species are limited to those recorded under the scope of this study, and migratory shorebirds have been further addressed in a separate study (Biota 2019a).

This page intentionally left blank.

2.0 Introduction

2.1 Project Background

K+S Salt Australia Pty Ltd (K+S) is evaluating the possibility of developing a greenfield solar salt project (the proposed Ashburton Salt Project), located on the Western Australian coast approximately 40 km southwest of Onslow. A development envelope has been identified to accommodate the solar salt evaporation ponds, crystallisation ponds and associated infrastructure. This area, combined with an associated access road corridor, is hereafter referred to as the study area (Figure 2.1).

Biota Environmental Sciences (Biota) was commissioned by K+S to undertake a two-phase, Level 2 terrestrial fauna survey to document the vertebrate fauna assemblage, fauna of conservation significance and Short-range Endemic (SRE) invertebrate fauna of the study area.

2.2 Scope and Objectives

The scope of this study was to undertake a two-phase Level 2 vertebrate fauna survey of the study area to meet Environmental Protection Authority (EPA) guidance. Specific objectives included:

- preparation of a desktop assessment including database and literature searches, in order to consolidate all available and relevant existing data for contextual comparison;
- assessment of the likelihood of occurrence of fauna of conservation significance, or their preferred habitat, within the study area;
- assessment and description of any habitats deemed significant for supporting known or potential populations of fauna of conservation significance; and
- documentation of the vertebrate and SRE invertebrate fauna assemblage within the study area using established sampling techniques.

This purpose of this report is to provide information on the terrestrial fauna of the study area to inform the environmental impact assessment (EIA) of the proposal.

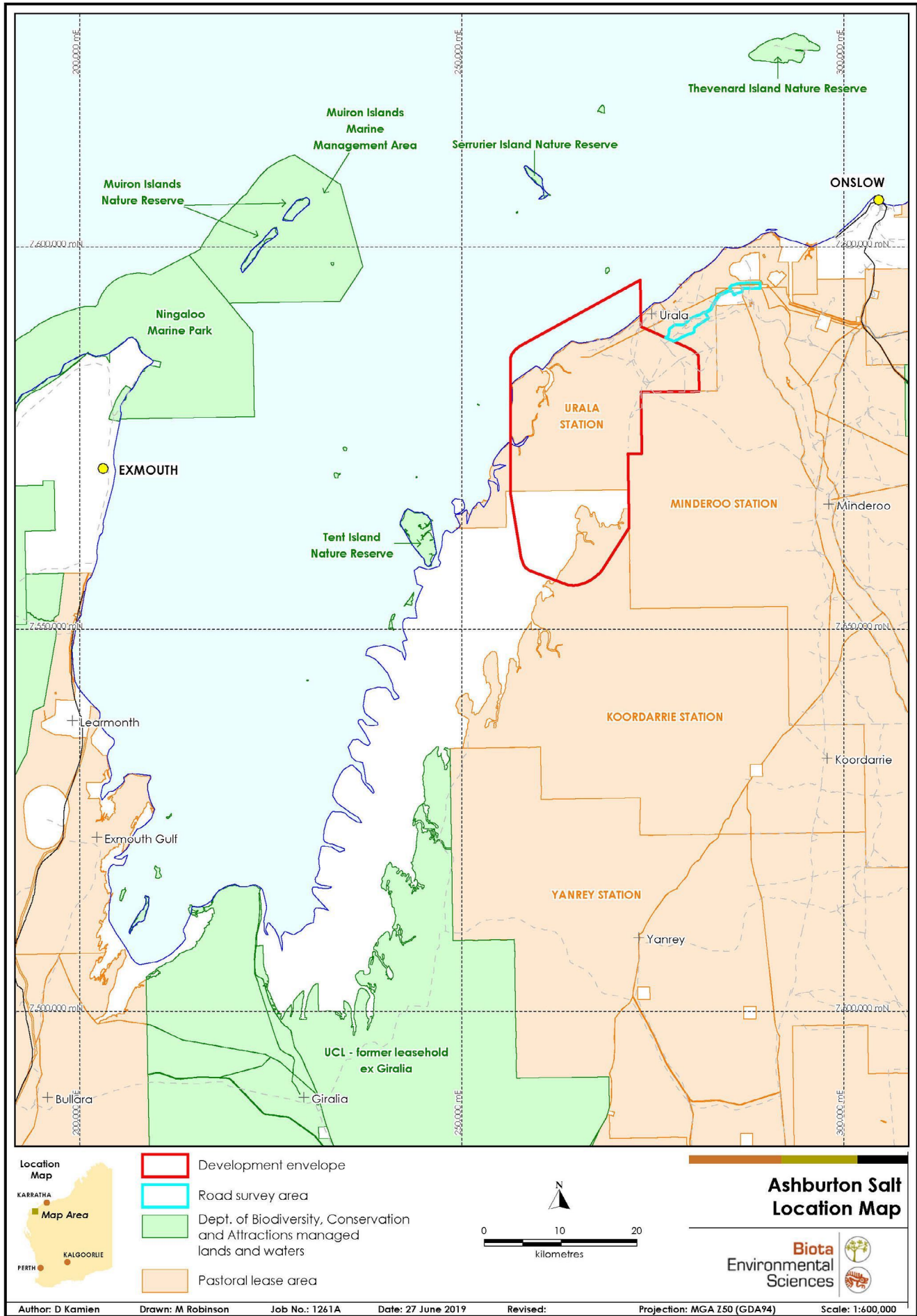


Figure 2.1: Location of the study area.

3.0 Methodology

3.1 Desktop Review

3.1.1 Literature Review

The literature review comprised:

- a summary of the Interim Bioregionalisation for Australia (IBRA) region and subregion (DSEWPaC 2012); and,
- a review of relevant biological surveys previously completed within the locality (50 km) of the study area.

The results of the literature review are summarised in Section 4.2.1 and detailed results are provided in Appendix 1.

3.1.2 Database Searches

The following databases were searched as part of the desktop assessment:

1. NatureMap (<https://naturemap.dpaw.wa.gov.au>) is a joint project of the Department of Biodiversity, Conservation and Attractions (DBCA) and the Western Australian Museum (WAM). This database represents the most comprehensive source of information on the distribution of Western Australia's flora and fauna, comprising records from the WA Threatened Fauna Database, Fauna Survey Returns Database (managed by the DBCA), the WAM Specimen Database, and the BirdLife Australia Atlas of Australian Birds. NatureMap was searched primarily to identify records of conservation significant fauna known from the locality of the study area.
2. The Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act 1999) Protected Matters Search Tool was searched to identify Federally listed fauna species and any other Matters of National Environmental Significance (MNES) that are known to or may occur in the locality.
3. WAM database, searched specifically in regard to SRE records; and
4. Biota's internal database from the locality.

Due to the size of the study area, two separate searches were conducted for the NatureMap and the EPBC Protected Matters Databases, using a buffer of 40 km centred at two different points within the study area: 22.0116° S, 114.7108° E and 21.8700° S, 114.7997° E. WAM database searches were based on a square polygon using the following coordinates (top left: 21.3463° S, 114.2099° E; bottom right: 22.5149° S, 115.4602° E), with search results requested from a minimum 40 km buffer from the study area boundary.

3.1.3 Assessment of Likelihood of Occurrence in the Study Area

Results from the literature review and database searches were used to compile a list of potential terrestrial fauna species that had previously been recorded from the study area locality using the rankings and criteria provided in Table 3.1. The likelihood that each taxon would occur in the study area was then assessed in consideration to:

- the documented distribution of the species;
- the proximity of the study area to known populations; and
- preferred habitat.

Habitats were preliminarily defined according to vegetation units, landforms apparent on aerial imagery, and taking into account existing information regarding the environment. The term 'close proximity' is defined as being within 20 km of the study area, while the broader 'locality' comprises the area up to 50 km from the study area.

Table 3.1: Criteria used to assign the likelihood of occurrence of a species within the study area.

Rank	Criteria
Recorded	1. The species has been previously recorded in the study area.
Likely to occur	1. There are existing records of the species in close proximity to the study area (within 20 km); and the species is strongly linked to a specific habitat, which is present in the study area; or the species has more general habitat preferences, and suitable habitat is present.
May potentially occur	1. There are existing records of the species from the locality (within 50 km), however the species is strongly linked to a specific habitat, of which only a small amount is present in the study area; or the species has more general habitat preferences, but only some suitable habitat is present. 2. There is suitable habitat in the study area, but the species is recorded infrequently in the locality.
Unlikely to occur	1. The species is linked to a specific habitat, which is absent from the study area; or 2. Suitable habitat is present, however there are no existing records of the species from the locality despite reasonable previous search effort in suitable habitat; or 3. There is some suitable habitat in the study area, however the species is very infrequently recorded in the locality or the only records are historic (>40 years ago).
Would not occur	The species is strongly linked to a specific habitat, which is absent from the study area; or The species' range is very restricted and does not include the study area; or The species is not considered extant in the locality.

3.1.4 Preliminary Habitat Mapping

Vertebrate fauna landscapes and landforms of the study area were identified prior to the survey based on Biota's fauna landscape approach (Blandford 2012), which identifies functional landforms within a broader landscape. Here, available digital aerial imagery and contour mapping was considered in combination with overlain vegetation mapping and regional land systems mapping.

3.1.5 Nomenclature

Consistent with EPA (2016a) technical guidance, species nomenclature for herpetofauna and mammals follows the standards of the WAM fauna taxonomic checklist, which is revised and released every six months, or as necessary. Avifauna nomenclature is in accordance with Christidis and Boles (2008).

3.1.6 Threatened Fauna Statutory Framework

Native fauna species that are rare, threatened with extinction, or have high conservation value, are specially protected by law under either or both of the State *Biodiversity Conservation Act 2016* and the Commonwealth *EPBC Act 1999*. The DBCA also maintains a list of Priority species that have not been assigned statutory protection under the *Biodiversity Conservation Act 2016*. Appendix 2 details the categories of conservation significance recognised under these three frameworks.

3.2 Survey Timing and Weather

3.2.1 Survey Team

Each of the field survey phases was conducted by a team of four Biota zoologists (Table 3.2). Phase 1 was completed over a 13 day period from October 30th to November 11th 2018, and Phase 2 was completed over an 11 day period from 8th to 18th April 2019. The survey was completed under "Licence to Take Fauna for Scientific Purposes" No. 08-002903-1 issued to Dan Kamien (Appendix 3).

Table 3.2: Summary of personnel conducting the survey.

Name	Position at Biota	Qualification	Years of Experience	Phase	Survey Role
Dan Kamien	Principal Zoologist	BSc. Hons	20	1 & 2	Project Manager Field Team Leader Field survey (Vertebrates, SREs, Avifauna) Data analysis and reporting
Penny Brooshooff	Senior Zoologist	BSc. Hons	9	1 & 2	Field survey (Vertebrates and SREs) Data analysis
Michael Greenham	Senior Zoologist	BSc.	18	1 & 2	Field survey (Vertebrates, SREs, Avifauna)
David Keirle	Zoologist	BSc. Hons	9	1	Field survey (Vertebrates, SREs, Avifauna)
Joshua Keen	Graduate Zoologist	BSc. Hons	3	2	Field survey (Vertebrates, SREs, Avifauna)

3.2.2 Daily Weather Observations

Weather data were obtained from the Bureau of Meteorology weather station at Onslow Airport (No. 5017), located approximately 18 km northeast of the study area. Phase 1 weather conditions were warm and dry with temperatures ranging from a minimum of 18.3°C to a maximum of 37.2°C, with no rainfall recorded locally within the study area during the survey (Table 3.3 and Figure 3.1). Weather during the Phase 2 survey was also warm, but with some rain falling mid-survey (Table 3.4 and Figure 3.1).

Table 3.3: Weather at Onslow Airport during the Phase 1 survey period 2018.

	30/10	31/10	01/11	02/11	03/11	04/11	05/11	06/11	07/11	08/11	09/11	10/11	11/11	Mean/ Total
Maximum temperature (°C)	32.6	30.8	31	31.2	32.3	35.5	37.1	37.2	36.6	32.9	33.5	29.4	31.8	33.2
Minimum temperature (°C)	20.4	19.8	19.8	18.3	19.0	18.6	18.6	19.4	20.8	20.8	22.6	21.7	18.8	19.9
Rainfall (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0

Table 3.4: Weather at Onslow Airport during the Phase 2 survey period 2019.

	8/4	9/4	10/4	11/4	12/4	13/4	14/4	15/4	16/4	17/4	18/4	Mean/ Total
Maximum temperature (°C)	35.7	33.1	34.4	34.1	35.9	32.4	32.2	34.5	33.5	34.4	32.7	33.9
Minimum temperature (°C)	23.6	26.4	27.9	28.4	24.6	24.4	26.3	22.2	21.9	21.3	22.5	24.5
Rainfall (mm)	0	0	0	0	0	5.8	0.8	0	0	0	0	6.6

3.2.3 Climate

Long-term climate data were obtained from the Onslow Airport weather station (No. 5017). Figure 3.1 illustrates the average monthly minimum and maximum temperatures and rainfall for the year preceding the survey as compared with the long-term averages. Temperatures in the year preceding the survey were mostly consistent with long-term averages.

Little rainfall was received four months preceding the Phase 1 survey, but this was also in accordance with the long-term average. Although over 62 mm of rainfall was received in the two months prior to the Phase 2 survey, the long-term average for this period is over 186 mm. In summary, survey conditions were in accordance with that expected for the Phase 1 dry season survey, but dryer than expected during the Phase 2 wet season survey.

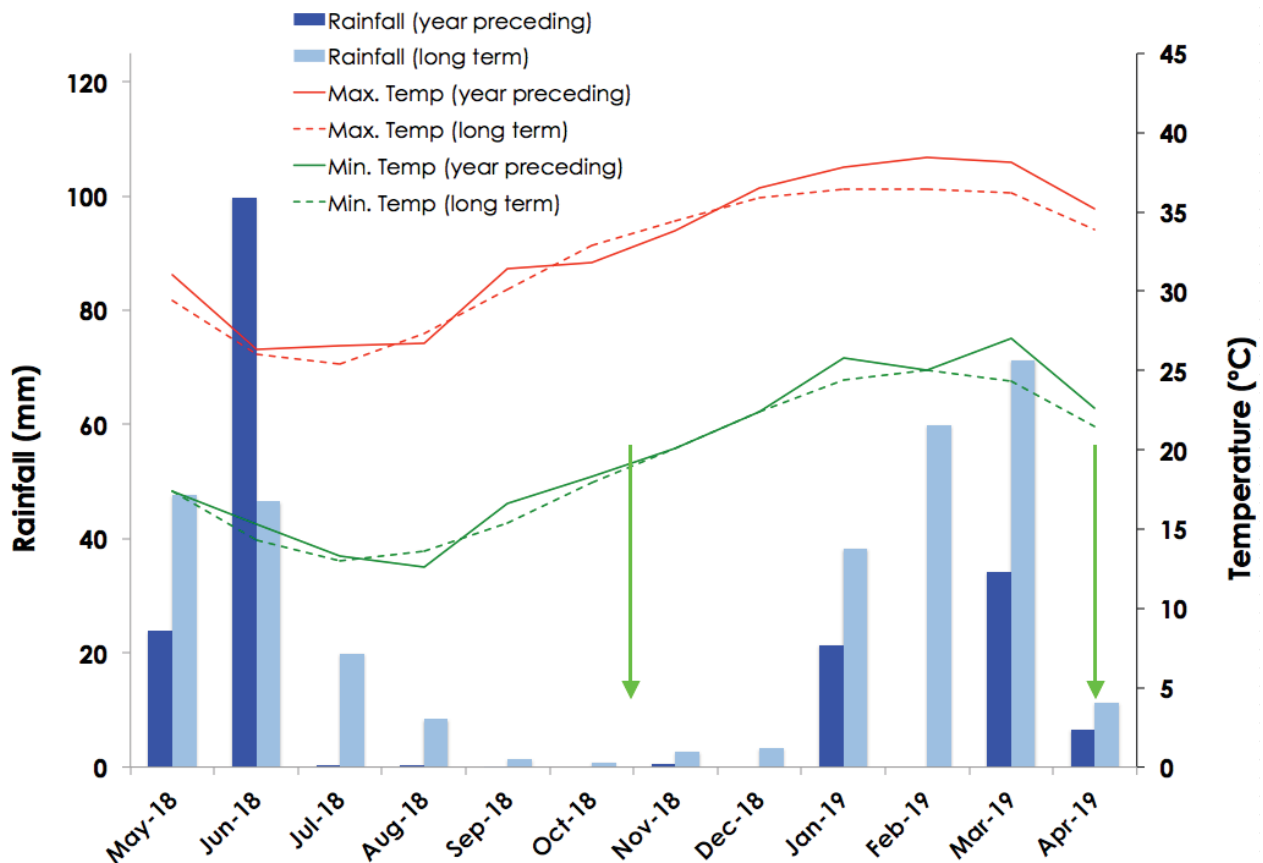


Figure 3.1: Climate and weather graph depicting long-term averages and 2018 data. (Long-term data rainfall and temperature data 1940-2018, green arrows indicate survey timing).

3.3 Fauna Sampling

Survey methodology and approach were undertaken with consideration of the following compliance and regulatory documents:

- Technical Guidance – Sampling Methods for Terrestrial Vertebrate Fauna (EPA 2016a);
- Technical Guidance – Terrestrial Fauna Surveys (EPA 2016b);
- Technical Guidance – Sampling of Short-range Endemic Invertebrate Fauna (EPA 2016c);
- Survey Guidelines for Australia's Threatened Birds (DEWHA 2010); and
- Survey Guidelines for Australia's Threatened Mammals (DSEWPaC 2011).

The vertebrate survey consisted of a combination of systematic trapping and non-systematic opportunistic and targeted searching (Sections 3.3.1.1 to 3.3.1.5). The invertebrate survey consisted of targeted searches undertaken for specific groups of invertebrates known to include SRE species (see Section 3.3.2).

Preliminary site selection was determined through assessment of aerial photography and thematic layers including land systems, geology and Beard's vegetation mapping (see Section 4.1). Sampling sites were located within representative land systems intersected by the study area (see Section 4.1.2). Further site assessments were conducted in the field while driving, flying in a helicopter and traversing on foot through the study area.

3.3.1 Terrestrial Vertebrates

3.3.1.1 Fauna Trapping

The systematic census component of the fauna survey consisted of 14 trapping transects, each located within a defined landform (see Section 5.1):

- Twelve dry pitfall trapping transects consisting of a single row of 10 pitfall traps arranged as alternating 20 litre buckets and 150 mm diameter x 600 mm high PVC tubes, spaced at 10 m intervals and connected with a 90 m length of 300 mm high fly wire fence. These transects also included three pairs of funnel traps (Figure 3.2).
- Two trapping transect consisted of a series of medium Elliott box traps spaced at approximately 10 –15 m intervals. Traps were baited with a universal bait mixture of peanut butter and oats.

A summary of trapping effort is provided in Table 3.5. In total, 2,905 trap days of systematic sampling were completed as part of this study. Figure 3.3 illustrates the locations of systematic trapping, remote cameras and bat sampling.

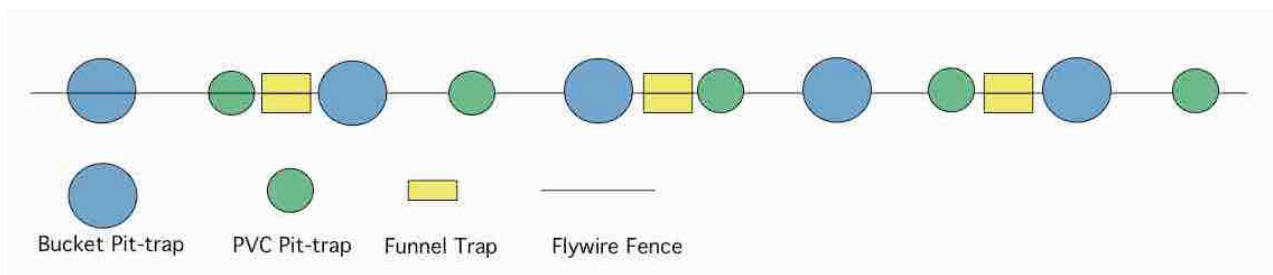


Figure 3.2: Layout of pitfall and funnel traps.

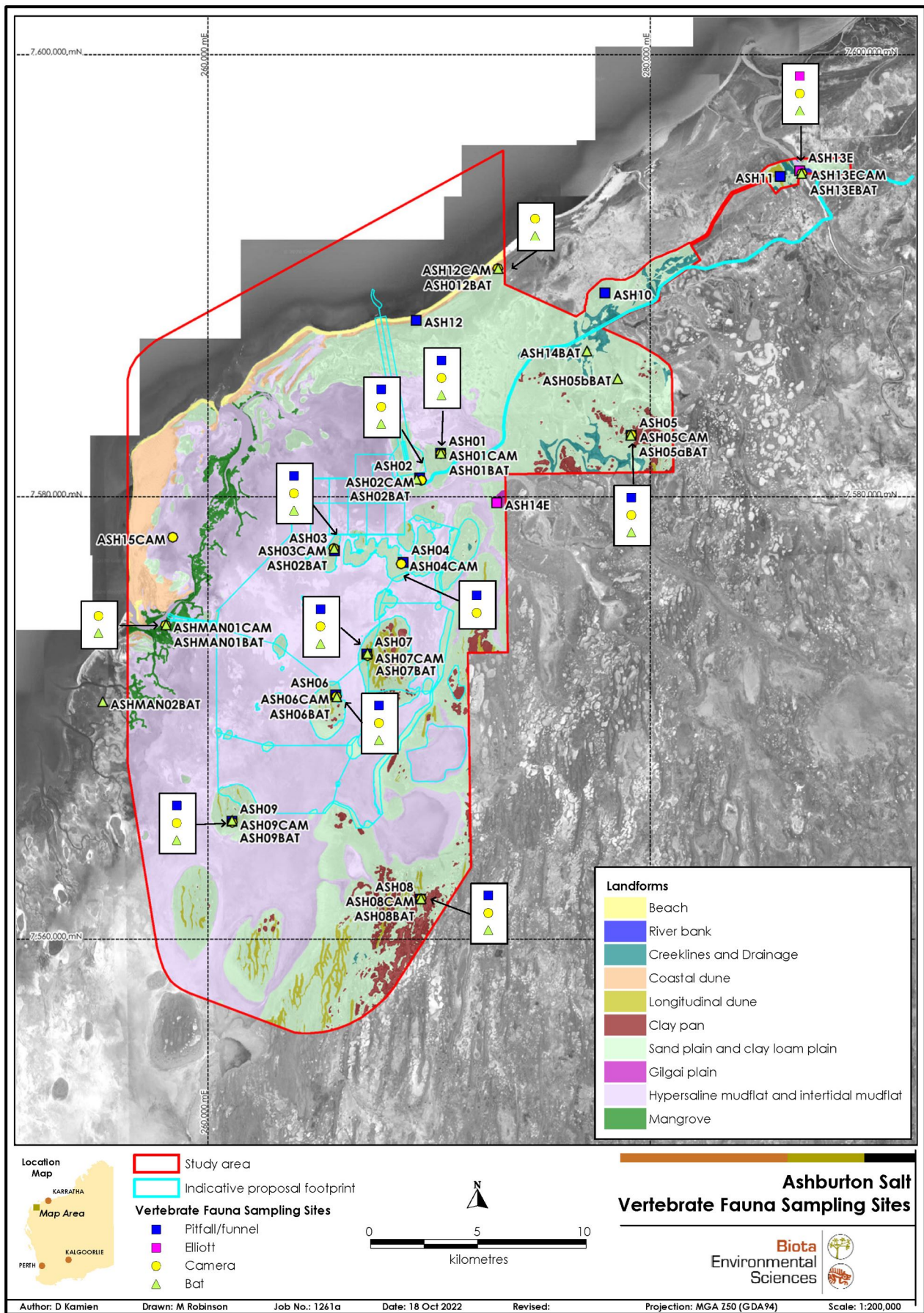


Figure 3.3: Vertebrate trapping and sampling sites.

Table 3.5: Trap location and effort during the survey.

Site	Easting (mE)	Northing (mN)	Habitat	Trap Type	Phase 1		Phase 2		Total Days Opened	Number of Traps	Trap Effort	
					Date Opened	Date Closed	Date Opened	Date Closed				
ASH01	270521	7581970	Clay loam plain	Pitfall/Funnel	31/10/18	07/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH02	269562	7580847	Clay loam plain	Pitfall/Funnel	01/11/18	08/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH03	265724	7577572	Sand plain	Pitfall/Funnel	01/11/18	08/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH04	268825	7577035	Longitudinal dune	Pitfall/Funnel	01/11/18	08/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH05	279137	7582784	Longitudinal dune	Pitfall/Funnel	03/11/18	10/11/18	8/4/19	16/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH06	265780	7571042	Clay loam plain	Pitfall/Funnel	02/11/18	09/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH07	267172	7572900	Longitudinal dune	Pitfall/Funnel	31/10/18	07/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH08	269609	7561818	Longitudinal dune	Pitfall/Funnel	02/11/18	09/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH09	261079	7565343	Longitudinal dune	Pitfall/Funnel	02/11/18	09/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH10	277955	7589222	Sand plain	Pitfall/Funnel	02/11/18	09/11/18	8/4/19	16/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH11	285879	7594496	Longitudinal dune	Pitfall/Funnel	03/11/18	10/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH12	269413	7587983	Coastal dune	Pitfall/Funnel	02/11/18	09/11/18	9/4/19	17/4/19	14	P: 10 F: 6	P: 140 F: 84	
ASH13E	286758	7594750	River bank	Elliott	03/11/18	10/11/18	–	–	7	E: 25	E: 175	
ASH14E	273060	7579732	Gilgai plain	Elliott	–	–	9/4/19	12/4/19	3	E: 14	E: 42	
										Total Pit Effort		1,680
										Total Funnel Effort		1,008
										Total Elliott Effort		217

3.3.1.2 Avifauna Sampling

Avifauna sampling was conducted using a combination of techniques including:

- unbounded area searches (30 minutes duration) conducted at systematic trapping sites;
- unbounded area searches at opportunistic locations containing habitats likely to support previously unrecorded species;
- opportunistic observations of birds recorded in the study area during the survey; and
- observations of coastal avifauna species that do not represent migratory shorebirds. These were recorded during migratory shorebird surveys (Biota 2019a) and are presented as opportunistic records in the results section of this report.

Fifty-seven unbounded area searches were completed at 14 locations (Table 3.6 and Table 3.7). Individual censuses were confined to discrete landforms, typically corresponding to vegetation type at each trapping site. Censuses were conducted between 6:11 am and 11:16 am (census start times indicated in Table 3.6 and Table 3.7), with a total of over 28 hours dedicated to systematic avifauna censusing during both survey phases.

Table 3.6: Systematic avifauna censuses undertaken at each fauna site within the study area during Phase 1.

Site	2/11/18	3/11/18	4/11/18	5/11/18	6/11/18	7/11/18	8/11/18	9/11/18	Total (minutes)
ASH01	7:44			7:42					60
ASH02				7:06					30
ASH03	8:55	9:27							60
ASH04	9:05			6:15					60
ASH05			8:37	7:49					60
ASH06			7:02		7:02				60
ASH07			6:15		6:18				60
ASH08		6:15	7:51	10:46					90
ASH09		7:51	8:42						60
ASH10		8:28		8:38				9:12	90
ASH11			7:14	6:33					60
ASH12		6:53			7:07				60
ASH13E			6:30		6:28		6:11		90
								Total	840

Table 3.7: Systematic avifauna censuses undertaken at each fauna site within the study area during Phase 2.

Site	9/4/19	10/4/19	11/4/19	12/4/19	13/4/19	14/4/19	15/4/19	16/4/19	Total (minutes)
ASH01		7:39					8:43		60
ASH02		7:52					9:24		60
ASH03		9:51						7:34	60
ASH04		8:59						6:52	60
ASH05			8:20				6:59		60
ASH06		11:06	8:54						60
ASH07		10:11	9:35						60
ASH08			7:21	7:38					60
ASH09			8:08	8:19					60
ASH10				7:35			8:36		60
ASH11			7:08			7:40			60
ASH12			9:29	10:04					60
ASH13E*	8:03								90
ASH14E		8:53		8:56					60
								Total	870

* Two additional censuses were conducted during targeted migratory shorebird work (5/4/19 and 6/4/19).

3.3.1.3 Remote Cameras

Thirteen remote infrared motion cameras were deployed primarily to target conservation significant species such as the Northern Quoll (*Dasyurus hallucatus*; Endangered).

Cameras were located in either longitudinal dune, clay loam plain, river bank or mangrove habitat where the targeted species might be expected to utilise. A bolus of universal bait was placed on the ground in the camera's field of view to attract animals. Motion cameras were deployed for a total of 61 trap nights (Table 3.8).

Table 3.8: Remote camera locations and effort.

Site	Easting (mE)	Northing (mN)	Habitat	Phase	Start Date	End Date	Sampling Nights
ASH01CAM	270530	7581963	Clay loam plain	1	2/11/18	7/11/18	5
ASH02CAM	269647	7580758	Clay loam plain	2	10/4/19	13/4/19	3
ASH03CAM	265689	7577664	Clay loam plain	2	10/4/19	13/4/19	3
ASH04CAM	268742	7576972	Longitudinal dune	1	2/11/18	7/11/18	5
ASH05CAM	279150	7582797	Longitudinal dune	2	10/4/19	17/4/19	7
ASH06CAM	265808	7570969	Clay loam plain	1	2/11/18	7/11/18	5
ASH07CAM	267207	7572818	Longitudinal dune	2	10/4/19	13/4/19	3
ASH08CAM	269644	7561825	Longitudinal dune	1	5/11/18	8/11/18	3
				2	10/4/19	17/4/19	7
ASH09CAM	261100	7565276	Longitudinal dune	1	2/11/18	7/11/18	5
ASH12CAM	273113	7590334	Coastal dune	1	3/11/18	8/11/18	5
ASH13ECAM	286874	7594638	River bank	1	3/11/18	8/11/18	5
ASHMAN01CAM	258077	7574202	Mangrove	1	5/11/18	8/11/18	3
ASH15CAM	258403	7578179	Coastal dune	2	8/4/19	10/4/19	2
Total							61

3.3.1.4 Bat Echolocation Recordings

Bat sampling was conducted within the study area using SM2BAT and SM4BAT SongMeters, which detect and record ultrasonic echolocation calls emitted during bat flight. For SM2 units, the selectable filters, triggers, jumper and audio settings used followed the manufacturers recommendations for bat detection (Wildlife Acoustics 2010).

Sampling was undertaken at 14 locations, with SongMeters deployed for a total of 48 recording nights (Table 3.9). The SongMeters were placed predominantly in locations considered likely to provide records for a range of species, including at river bank, mangrove and longitudinal dune habitats and where water was available for bats to drink.

Table 3.9: Bat sampling locations and effort.

Site	Easting (mE)	Northing (mN)	Habitat	Phase	On Date	Off Date	Nights Active
ASH01BAT	270531	7581976	Clay loam plain	2	10/4/19	13/4/19	3
ASH02BAT	269439	7580806	Longitudinal dune	1	2/11/18	5/11/18	3
ASH03BAT	265697	7577667	Clay loam plain	2	10/4/19	13/4/19	3
ASH05aBAT	279119	7582790	Longitudinal dune	2	10/4/19	13/4/19	3
ASH05bBAT	278530	7585348	Clay loam plain (cattle trough)	1	6/11/18	8/11/18	2
				2	14/4/19	16/4/19	2
ASH06BAT	265827	7570953	Clay loam plain	1	2/11/18	5/11/18	3
ASH07BAT	267216	7572918	Longitudinal dune	1	2/11/18	5/11/18	3
ASH08BAT	269635	7561831	Longitudinal dune	1	5/11/18	8/11/18	3
ASH09BAT	261084	7565370	Longitudinal dune	1	2/11/18	5/11/18	3
ASH012BAT	273130	7590323	Coastal dune (dam)	1	3/11/18	6/11/18	3
				2	11/4/19	14/4/19	3
ASH13EBAT	286854	7594631	River bank	1	5/11/18	8/11/18	3
				2	10/4/19	13/4/19	3

Site	Easting (mE)	Northing (mN)	Habitat	Phase	On Date	Off Date	Nights Active
ASH14BAT	277137	7586595	Clay loam plain	2	14/4/19	16/4/19	2
ASHMAN01BAT	258104	7574208	Mangrove	1	5/11/18	8/11/18	3
ASHMAN02BAT	255233	7570734	Mangrove	1	5/11/18	8/11/18	3
Total							48

Bat echolocation call analysis was conducted by Mr Dan Kamien of Biota using Kaleidoscope Pro software (Version 4.3.2), and following methods recommended by the Australasian Bat Society (2006) in conjunction with available reference data (Churchill 2008, McKenzie and Bullen 2009). Only sequences containing good quality search phase calls were considered for identification.

3.3.1.5 Other Non-Systematic Sampling

A range of non-systematic fauna observation techniques was used to supplement the systematic trapping data and to investigate additional habitats or microhabitats identified during the course of the survey. These activities included:

- habitat-specific searches for Threatened fauna;
- searches of microhabitats for reptiles, frogs and small mammals not commonly recorded via trapping (e.g. by raking leaf litter and turning rocks and logs);
- identification of secondary signs (where possible) including tracks, scats, skins, mounds, hollows, nests and diggings; and
- identification of road kill and other animal remains.

3.3.2 Short-Range Endemic Invertebrates

SRE invertebrates are taxonomic groups of invertebrates that exhibit naturally small distributions, (less than 10,000 km²; Harvey 2002) Certain groups of invertebrates are pre-disposed to short-range endemism through particular life history traits such as poor dispersal capabilities, confinement to disjunct habitats, slow reproduction and low fecundity (Harvey 2002, Ponder and Colgan 2002). Given the importance of short-range endemism to the conservation of biodiversity (EPA 2016c), the assessment of such invertebrate taxa is a potentially important component of impact assessment.

Prior to the survey, the WA Museum was consulted as to the most likely potential SRE groups for the survey, consistent with EPA guidance. This, plus Biota's extensive knowledge base on SRE fauna in the Pilbara, suggested that the mostly likely groups to be present in the study area setting and landscape types were mygalomorph spiders, millipedes and land snails. Other habitat types that may harbour other SRE fauna groups elsewhere, such as rockpiles, south-facing gorges, escarpments and plateaus, vine thickets and woodlands, were not present in the survey area, which was essentially a landscape of undulating sandplain and low clay depressions.

The taxonomic groups targeted during the survey therefore included:

- mygalomorph spiders (Mygalomorphae);
- terrestrial snails (Pulmonata); and
- millipedes (Diplopoda).

In total, over 92 person hours were dedicated to SRE fauna searches (Table 3.10 and Table 3.11), with a total of 45 SRE sites sampled (Table 3.10, Table 3.11 and Figure 3.4).

Mygalomorph spiders were targeted by visually locating burrows, and then excavating them. Some spiders were collected from pit traps targeting vertebrate fauna. Individuals were preserved in 70% ethanol to maintain specimen integrity for morphological description. Two legs were removed and placed in 100% ethanol to preserve DNA for molecular analysis.

Aestivating land snails were targeted by digging underneath hummock grasses and in drainage gullies. Millipedes were searched for under leaf litter and logs where present.

Table 3.10: Phase 1 SRE site locations and search effort.

Site	Easting (mE)	Northing (mN)	Personnel	Method	Habitat	Minutes Searched	Effort (minutes)
ASHSRE01	270519	7581981	2	Burrow search	Longitudinal dune	34	68
ASHSRE02	269570	7580866	2	Burrow search	Longitudinal dune	30	60
ASHSRE04a	268775	7577017	2	Burrow search	Longitudinal dune	36	72
ASHSRE04b	268821	7577043	1	Burrow search	Longitudinal dune	18	18
ASHSRE05	279162	7582791	2	Burrow search	Clay loam plain	36	72
ASHSRE07	267197	7572862	2	Burrow search	Longitudinal dune	61	122
ASHSRE08	269651	7561823	2	Burrow search	Sand plain	54	108
ASHSRE09	261066	7565361	2	Burrow search	Longitudinal dune	46	92
ASHSRE10	277949	7589257	4	Burrow search, Hummock turning	Sand plain	57	228
ASHSRE12	269417	7587939	2	Burrow search	Coastal dune	59	118
ASHSRE13	281621	7597000	2	Burrow search	Coastal dune	34	68
ASHSRE14	280236	7590105	3	Burrow search	Sand plain	32	96
ASHSRE15	280073	7590236	4	Burrow search, Leaf litter raking, Snail search	Sand plain	119	476
ASHSRE16	283290	7589946	2	Burrow search	Clay loam plain	45	90
ASHSRE17	281614	7596999	2	Burrow search, Rock turning	Clay loam plain	19	38
ASHSRE18	287660	7595111	4	Burrow search	Clay loam plain	29	116
ASHSRE19	279204	7583661	2	Burrow search	Clay pan	23	46
ASHSRE20	279059	7584492	2	Burrow search	Clay pan	12	24
ASHSRE21	275698	7582931	2	Burrow search	Clay loam plain	28	56
ASHSRE22	284619	7596675	4	Burrow search	Clay loam plain	42	168
ASHSRE23	258803	7562921	2	Burrow search	Longitudinal dune	53	106
ASHSRE24	262557	7560773	2	Burrow search	Clay pan	41	82
ASHSRE25	262283	7560904	2	Burrow search	Sand plain	15	30
ASHSRE26	272923	7579846	2	Burrow search	Clay loam plain	30	60
ASHSRE27	271355	7573746	2	Burrow search, Snail search	Clay loam plain	25	50
ASHSRE28	258743	7574283	2	Burrow search, Snail search	Sand plain	47	94
ASHSRE29	274555	7586570	2	Burrow search, Snail search	Clay loam plain	62	124
ASHSRE30	260185	7583925	2	Burrow search, Snail search	Coastal dune	54	108
ASHSRE31	259522	7581273	2	Burrow search, Snail search	Coastal dune	49	98
ASHSRE32	287676	7596673	3	Burrow search, Snail search	Clay loam plain	44	132
						Total	3,020

Table 3.11: Phase 2 SRE site locations and search effort.

Site	Easting (mE)	Northing (mN)	Personnel	Method	Habitat	Minutes Searched	Effort (minutes)
ASHSRE33	282628	7591554	4	Burrow search	Clay loam plain	37	148
ASHSRE34	279651	7590447	4	Burrow search	Clay loam plain	51	204
ASHSRE35	278105	7585690	2	Burrow search	Clay loam plain	61	122
ASHSRE36	281115	7586258	4	Burrow search	Clay loam plain	48	192
ASHSRE37	281420	7596260	3	Burrow search	Clay loam plain	122	366
ASHSRE38	277721	7590048	4	Burrow search	Clay loam plain	24	96
ASHSRE39	276126	7588422	4	Burrow search	Clay loam plain	27	108
ASHSRE40	275188	7589863	4	Burrow search	Clay loam plain	17	68
ASHSRE41	259546	7555455	2	Burrow search	Clay pan	69	138
ASHSRE42	262621	7554689	2	Burrow search	Clay pan	58	116
ASHSRE42	262621	7554689	2	Burrow search	Clay pan	39	78
ASHSRE43	282762	7594295	3	Burrow search	Longitudinal dune	82	246
ASHSRE44	259263	7556191	3	Burrow search	Clay loam plain	98	294
ASHSRE45	274698	7565295	3	Burrow search	Clay pan	75	225
ASHSRE46	265888	7571214	3	Burrow search	Clay loam plain	43	129
						Total	2,530

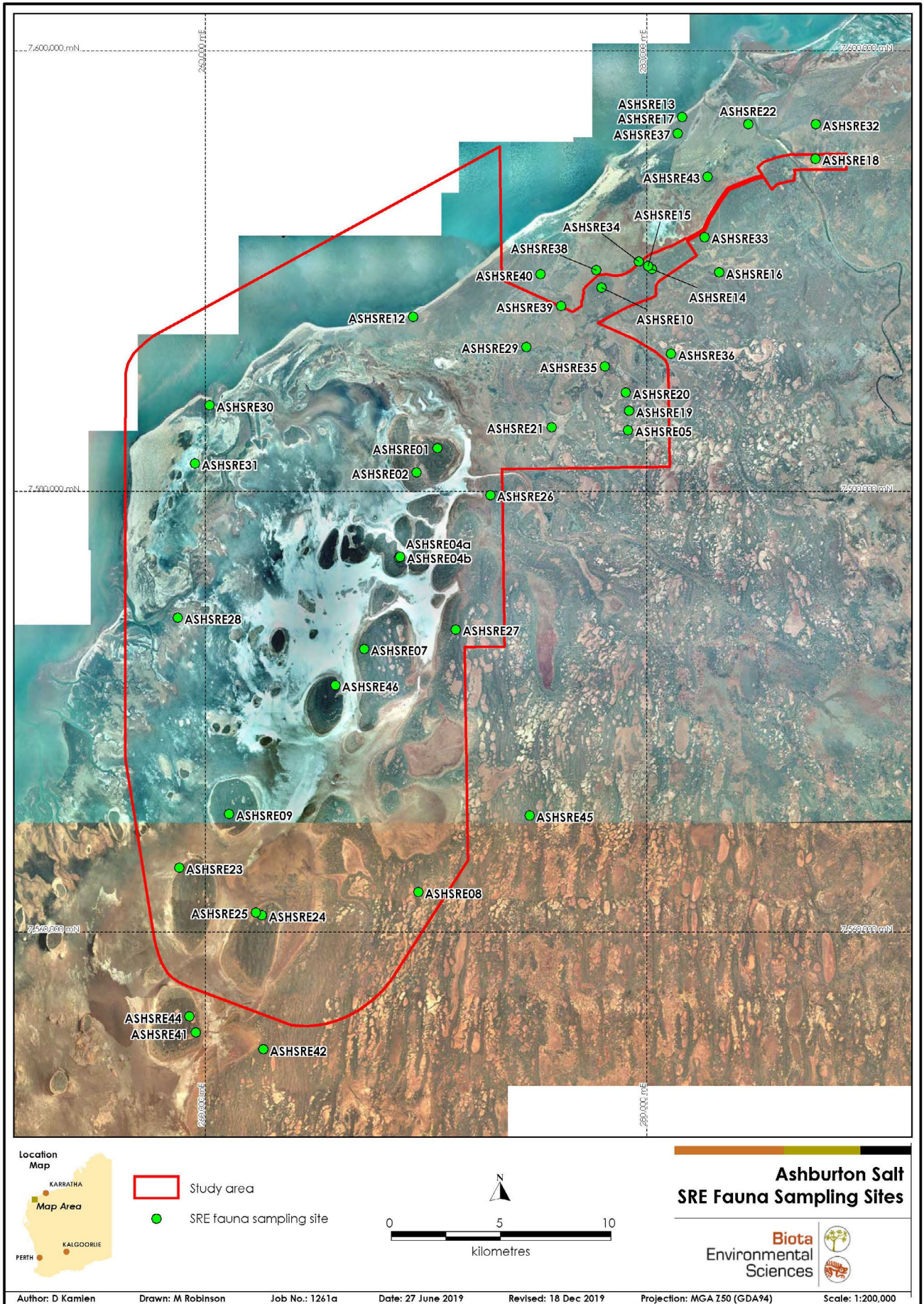


Figure 3.4: SRE sampling sites.

3.4 Identification and Data Analysis

3.4.1 Contextual Assemblage Analysis

The vertebrate fauna data obtained from both the study area and studies conducted in the locality were analysed using PRIMER v6.1 (Clarke and Gorley 2006) in order to place the study area into appropriate context. Data used for contextual analysis was limited to studies in which abundance data was available.

Data were analysed separately for each main fauna group (i.e. herpetofauna, avifauna and ground dwelling mammals). A resemblance matrix was then constructed using the Bray-Curtis similarity index, which produces a similarity value for all pairs of sites based on species representation.

Records were screened to remove data with the potential to bias the results. The following records were excluded from the analyses:

- opportunistic records that represented chance events and were not derived from equivalent sampling methods across sites;
- records from Elliott traps for herpetofauna analysis, as these traps typically target specific mammal species and only a small subset of reptile species; and
- bat records determined by echolocation recordings, which cannot be used to calculate abundance.

A Similarity Profile Routine (SIMPROF) was used to assess significant cut-off values at the 5% level (Clarke and Gorley 2006), thereby informing assemblage groupings. The resemblance matrix was then used to generate a non-metric Multi-Dimensional Scaling (nMDS) plot to assist with visualising the groupings amongst the sites based on faunal composition. The consolidated outputs of this approach were then used to define and compare fauna assemblages in the locality.

Survey factors for each site were imported into PRIMER, which comprised:

- landscape (as documented in the field by the survey zoologists and verified via aerial imagery);
- landform (as documented in the field by the survey zoologists);
- study area (survey).

Analysis of similarities (ANOSIM) routines were then conducted to statistically test whether there is a significant difference between fauna assemblages based on the above factors.

3.4.2 Species Accumulation

Plots of species accumulation curves and trends in the resultant curves over time can be used to assess sampling adequacy. When an adequate proportion of the fauna community has been surveyed, the curve should plateau and approach asymptote. PRIMER v6.1 was again used to calculate smoothed species accumulation curves based on 999 random permutations of the species and abundance data. Actual observed accumulation curves were also plotted.

Species accumulation curves alone cannot be used reliably to extrapolate predicted species richness for future biological sampling. In order to estimate asymptotic richness, the following asymptotic estimators were used (Clarke and Gorley 2006):

- Bootstrap estimator;
- Chao 1 richness estimator; and
- Jack-knife richness estimator.

Bat records were excluded from species accumulation analysis, as these were primarily determined by echolocation recordings and cannot be meaningfully used to calculate abundance.

3.4.3 SRE Fauna

3.4.3.1 Genetic Analysis

Mygalomorph spiders, land snails and millipedes are commonly recorded in the Pilbara bioregion, however very few mygalomorph spiders have been formally described (source: Biota internal database). Additionally, it is often not possible to identify mygalomorph spiders and snails through morphological characters. This report therefore makes use of DNA sequencing in combination with previous genetic studies to help infer putative species boundaries and better identify distributions.

Helix Molecular Solutions (Helix) carried out the relevant molecular analysis comparing sequences of specimens collected during the survey to those available in Helix's database and the publicly available GenBank database. From this, taxa were assigned to putative species based on Bayesian analysis of COX1 haplotypes. Previous assessments have been provided based on combining samples into species if they show less than 9.5% sequence divergence (Biota 2012). This value was discussed with Dr Terrie Finston (Helix) and Dr Mark Harvey (WAM) as a suitably conservative threshold for identifying likely species.

3.4.3.2 Determining SRE Status

The SRE status of species is based on their geographic distributions, which are described by two summary statistics. The first is the 'maximum spanning distance', which is the maximum linear distance between the two most widely separated records. The second statistic is the 'minimum spanning area', which is the area of the smallest polygon that can be drawn around all known records. The minimum spanning area can be used as a means for objectively establishing SRE status by comparison against the 10,000 km² criterion established by Harvey (2002).

Table 3.12 details the criteria used to determine the SRE status of putative species for the purposes of this report.

Table 3.12: Criteria used to determine SRE status.

SRE Status	Defining Criteria
Known SRE	Species, morphotype or genetic type has a documented distribution of <10,000 km ² . Species, morphotype or genetic type is well collected with numerous specimens typed and habitat preference understood.
Potential SRE	Species, morphotype or genetic type has a documented distribution of <10,000 km ² but is poorly sampled. Specimen may not be formally described or assigned to a morphotype / genetic type. Short-range endemism may be common in genus or family. May have been collected from restricted, refugia or isolated habitats.
Unlikely to be an SRE	Species, morphotype or genetic type has a documented distribution of <10,000 km ² but is poorly sampled. Specimen may not be formally described or assigned to a morphotype / genetic type. Short-range endemism is not common in genus or family. Taxon was not collected from restricted, refugia or isolated habitats. Few other individuals of the taxon collected, but records are separated by long distances (>100 km).
Not an SRE	Specimen formally described or assigned to a morphotype / genetic type. Species, morphotype or genetic type has a documented distribution of >10,000 km ² .
Undetermined	Taxa where there is insufficient taxonomic framework available to provide any informed comment on the species-level distribution of the fauna or, therefore, the risk of small-scale spatial restrictions.

3.5 Study Limitations

3.5.1 Environmental Impact Assessment

This report constitutes a baseline survey highlighting conservation significant findings only. Potential project impacts and management recommendations in regard to fauna habitats and assemblages are not presented in this report.

3.5.2 Vertebrate Fauna

- Not every section of the study area was ground-truthed or systematically sampled. Systematic fauna sampling was, however, completed at sites considered to represent the range of habitats present in the study area.
- Data collected using a combination of sampling methods (pit traps, Elliott traps, funnel traps and non-systematic sampling) were used to generate species accumulation curves. Inherent biases in individual sampling methods may have potential to skew analyses and bias species richness estimates. For example, Elliott trapping is typically unreliable in sampling herpetofauna, and funnel traps are unreliable in sampling mammals. However, the main aim is to present the fauna assemblage and to estimate sampling adequacy, which is accomplished by the inclusion of all species via all sampling methods.
- Data used for contextual analysis was limited to those obtained from sites in the study area locality and from studies in which abundance data was available.
- Records of migratory shorebird species are limited to those recorded under the scope of this study, and migratory shorebirds have further been addressed in a separate study (Biota 2019a).

3.5.3 Short-Range Endemic Invertebrates

- Not all sections of the study area were equally ground-truthed or sampled for fauna. However, SRE fauna searches and sampling were conducted in habitats considered to be representative of the range of units present within the study area.
- Many taxa are difficult to sample adequately. For example, mygalomorph spiders are time-consuming to locate, often cryptic, and morphological identification requires adult male specimens, which are often in low abundance and emerge from their burrow during selective, specific conditions such as following rain or during humid nights.

4.0 Desktop Assessment

4.1 Regional Context of the Study Area

4.1.1 IBRA Bioregion and Subregion

The Interim Biogeographic Regionalisation of Australia (IBRA7) recognises 89 bioregions for Australia (Department of Agriculture Water and the Environment 2020). The study area lies within the Cape Range subregion of the Carnarvon bioregion (CAR). It is also situated near the western boundary of the Roebourne subregion of the Pilbara bioregion (PIL).

4.1.2 Land Systems

Land systems mapping covering the study area has been prepared by Agriculture WA (Payne et al. 1987). The study area intersects four land systems (Dune, Littoral, Onslow and Yankagee), as summarised in Table 4.1 and shown in Figure 4.1. These four land systems are widespread in the locality.

Table 4.1: Land systems intersected by the study area.

Land System	Description	Total Area of Land System in the Cape Range subregion (ha)	Area of Land System in the Study Area (ha)	Percentage of Total Land System within Bioregion that occurs in the Study Area (%)
Dune (RGEDUN)	Dune fields supporting soft spinifex grasslands	37,467	3,965	10.6
Littoral (RGELIT)	Bare coastal mudflats with mangroves on seaward fringes, samphire flats, sandy islands, coastal dunes and beaches	142,055	29,979	21.1
Onslow (RGEONS)	Undulating sandplains, dunes and level clay plains supporting soft spinifex grasslands and minor tussock grasslands.	56,733	15,965	28.1
Yankagee (RGEYAN)	Plains with dunes and numerous claypans, soft spinifex and snakewood shrublands; in the west of the area.	110,310	2648	2.4

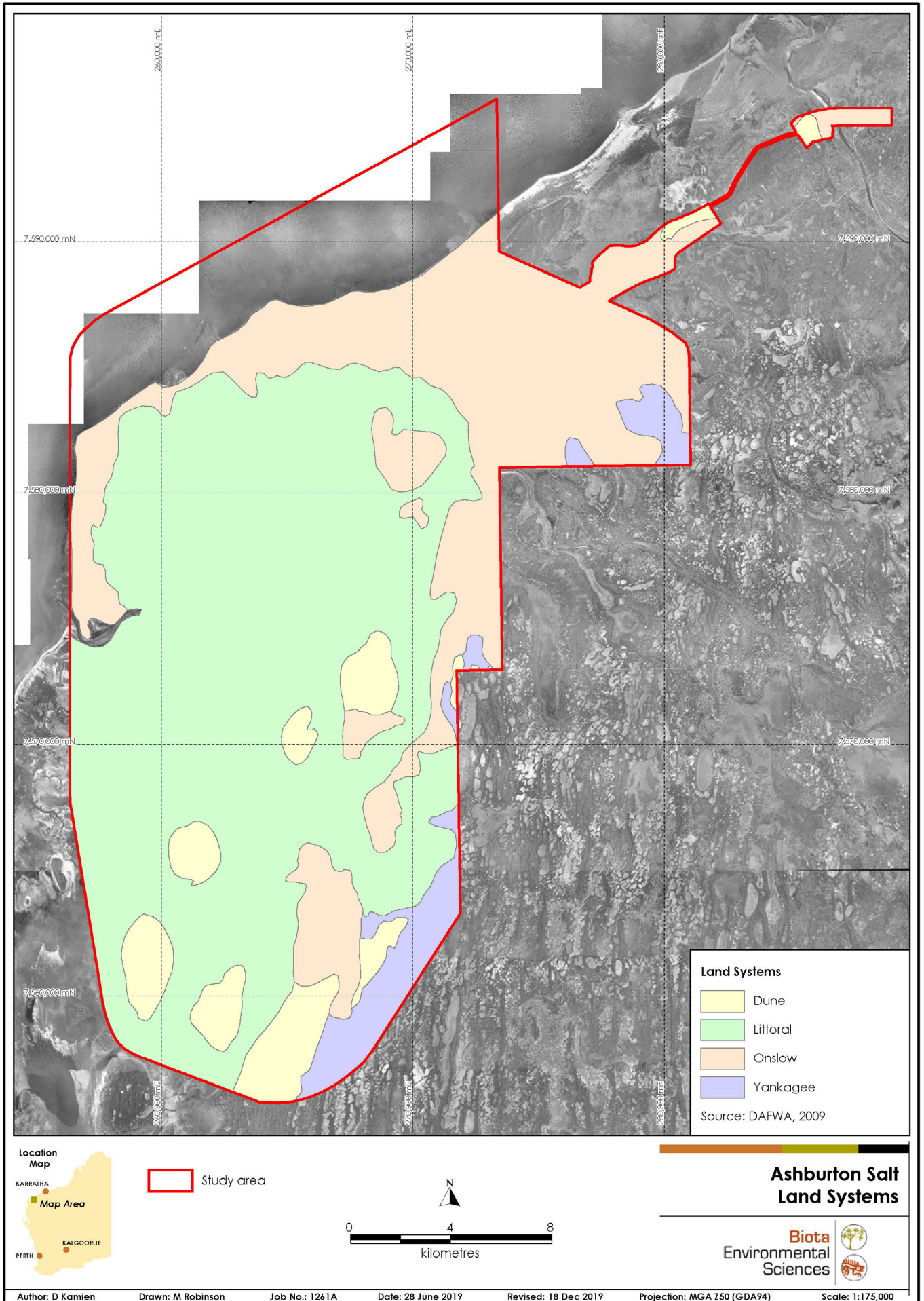


Figure 4.1: Land systems of the study area.

4.1.3 Surface Geology and Soils

The study area encompasses seven geological units (see Table 4.2). These units were mapped at a scale of 1:100,000 by the Geoscience Australia (2008) (Figure 4.2).

Table 4.2: Geological units occurring in the study area (Geological Survey of WA (1995)).

Geological Period	Unit Code	Geological Description	Area (ha)
–	Ocean	Ocean	7,616
Quaternary	Qa	Channel and flood plain alluvium; gravel, sand, silt, clay, locally calcreted	89
	Qd	Dunes: may include numerous interdune claypans; residual and aeolian sand with minor silt and clay	6,451
	Qdc	Coastal dunes: Beach sand, sand dunes, coastal dunes, beaches, and beach ridges	7,275
	Qe	Estuarine and delta deposits: Coastal silt and evaporite deposits	24,863
	Qrc	Colluvium: sheetwash, clay-silt-sand with sheet and nodular kankar; alluvial and aeolian sand-silt-gravel in depressions; local calcrete, reworked laterite	6,727
	Qt	Lake deposits: residual mud, clay, silt and sand, commonly gypsiferous and/or saline; playa and claypan, deposits	2,005
Tertiary	Czs	Sand plain: sand or gravel plains; quartz sand sheets commonly with ferruginous pisoliths or pebbles, minor clay; local calcrete, laterite, silcrete, silt, clay, alluvium, colluvium, aeolian sand	5,077

Four soil units were also mapped at a scale of 1:2,000,000 for the study area (Agriculture Western Australia 1967), as presented in Table 4.3 and Figure 4.3.

Table 4.3: Soil units occurring in the study area.

Unit Code	Soil Description	Area (ha)
Jw1	Low-lying coastal plains with some sand dunes	2,406
My57	Extensive plains with parallel sand dune formation	2,666
Oc58	Broad alluvial plains with a few clay pans and red sand dunes	5,945
SV8	Salt flats, tidal swamps, and coastal dune sands	40,991

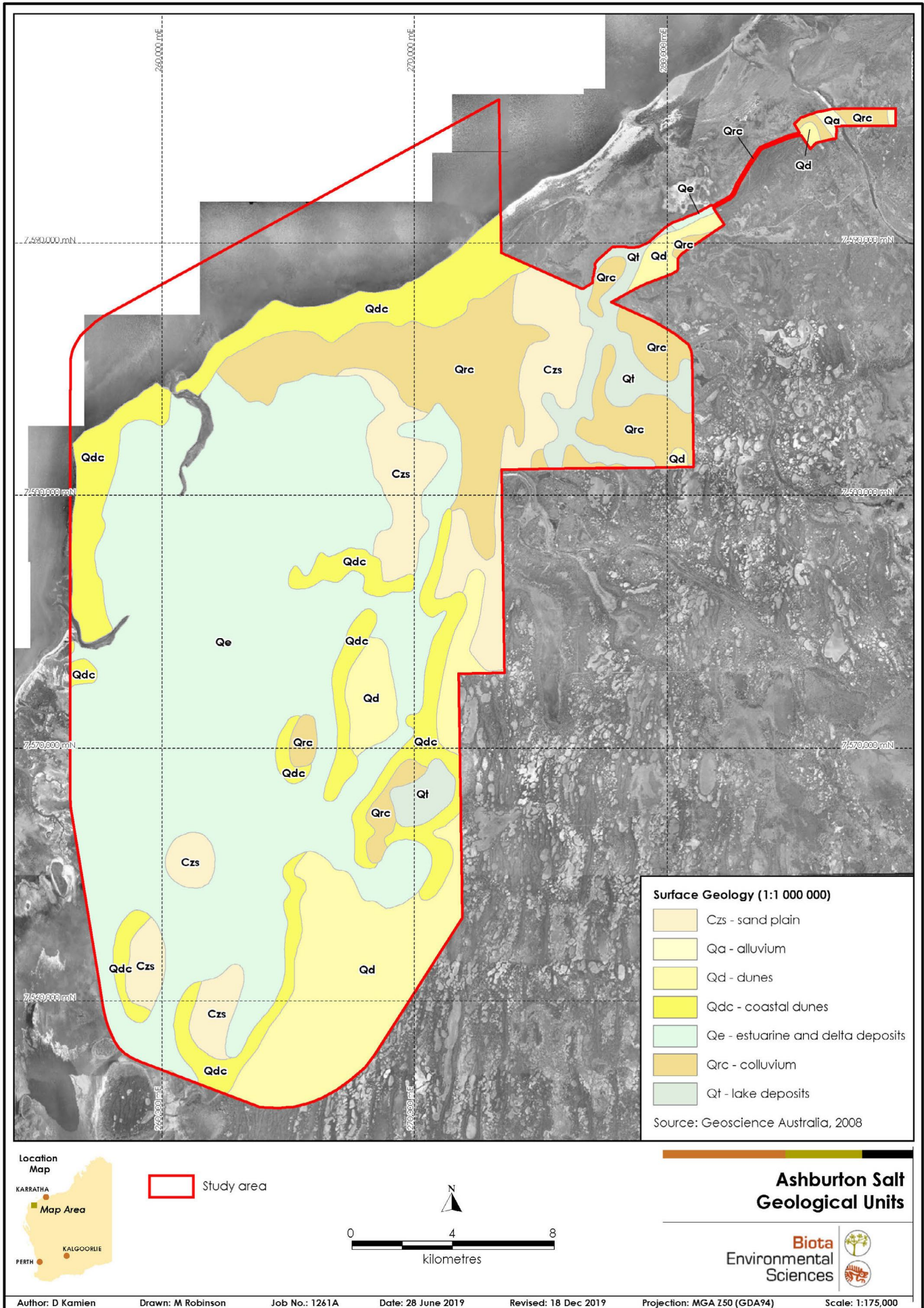


Figure 4.2: Surface geology of the study area.

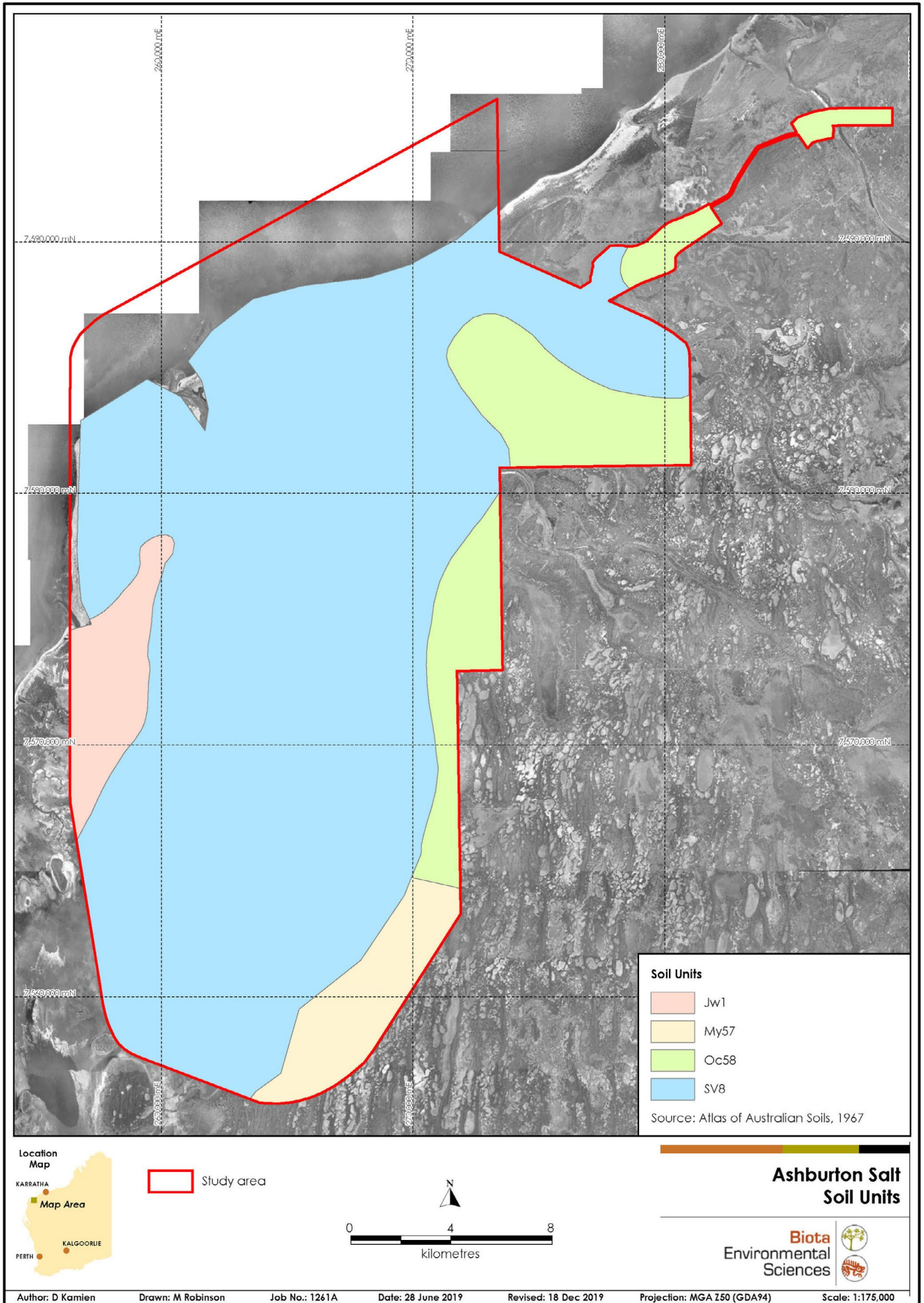


Figure 4.3: Soils of the study area.

4.1.4 Beard's Regional Vegetation Mapping

The study area lies within the Fortescue Botanical District of the Eremaean Botanical Province as defined by Beard (1975). The vegetation of this province is typically open, and frequently dominated by spinifex, wattles and occasional eucalypts. Based on Beard's (1975) descriptions and vegetation mapping of the vegetation of the Carnarvon Bioregion at a scale of 1:1,000,000, the following vegetation units occur within the study area:

- Cape Yannare Coastal Plain _43 (mangrove): Thicket; mangroves.
- Cape Yannare Coastal Plain _117: (t1Hi): Hummock grasslands, grass steppe; soft spinifex.
- Cape Yannare Coastal Plain _127: (fl): Bare areas; mud flats.
- Cape Yannare Coastal Plain _589: (xGc/t1Hi): Mosaic: Short bunch grassland - savanna / grass plain (Pilbara) / Hummock grasslands, grass steppe; soft spinifex.
- Cape Yannare Coastal Plain _670: (xSr.t2Hi): Hummock grasslands, shrub steppe; scattered shrubs over *Triodia basedowii*.
- Cape Yannare Coastal Plain _676: (k3Ci): Succulent steppe; samphire.
- Cape Yannare Coastal Plain _1271 (clay): Bare areas; claypans.

These vegetation units are widespread in the Cape Range subregion and have been subject to only minor clearing. However, given the broad scale of Beard's mapping, these units provide only limited information about the vegetation occurring in the study area.

A flora and vegetation survey was commissioned by K+S as part concurrent to this survey (Biota 2019b). This survey identified 18 vegetation types (excluding mangroves) within the study area (Biota 2019b).

4.1.5 Conservation Reserves in the Locality

The Tent Island Nature Reserve is the closest conservation reserve to the study area, situated 12 km to the southwest.

Six other reserves occur within 40 km of the study area – Cane River Conservation Park (including ex Giralia, ex Mt Minnie), Serrurier Island Nature Reserve (A-class), Thevenard Island Nature Reserve (A-class), Murion Islands Marine Management Area and the World Heritage listed Ningaloo Marine Park (A-class).

4.2 Previous Fauna Surveys in the Locality

Fauna surveys targeting both vertebrate fauna and SRE invertebrate fauna relevant to this study, conducted within the locality (50 km) of the study area since 2004 are summarised in Table 4.4 and Figure 4.4.

Table 4.4: Previous relevant surveys conducted within 50 km of the study area.

Report (Author)	Proximity to Study Area	Type of Survey	Survey Timing	Taxonomic Groups Documented	Survey Methods	Survey Limitations	Significant Findings
Yannarie Salt Project Fauna Survey (Biota 2005a)	0 km (overlaps) 175,580 ha	Level 2 single-phase survey	15 – 24/08/2004	<ul style="list-style-type: none"> • Terrestrial avifauna • Mammals • Reptiles • Amphibians • SRE Invertebrates 	<ul style="list-style-type: none"> • Pitfall trapping • Opportunistic records • Hand foraging • Bark peeling • Rock turning • Burrow searching • Night-time spotlighting • Recording secondary sign 	<ul style="list-style-type: none"> • Not all areas were ground-truthed or equally sampled for fauna • Single phase survey only • Terrestrial invertebrate sampling was largely opportunistic 	<ul style="list-style-type: none"> • 8 Federally listed conservation significant avifauna species
Yannarie Salt Project Mangrove and Coastal Ecosystems Study (Biota 2005b)		Targeted mangrove and migratory fauna survey	2 – 9/09/2004	<ul style="list-style-type: none"> • Migratory avifauna • Mangrove avifauna 	<ul style="list-style-type: none"> • Avifauna counts 	<ul style="list-style-type: none"> • Not all sections of study area ground-truthed • Further survey work may add additional species 	<ul style="list-style-type: none"> • 26 State listed conservation significant avifauna species • 40 Federally listed conservation significant avifauna species
Chevron Domgas Project Onslow Fauna Assessment (Validus 2008)	12 km northeast 100 ha	Level 2 single-phase survey	29/05 – 09/06/2008	<ul style="list-style-type: none"> • Terrestrial avifauna • Mammals • Bats • Reptiles • Amphibians 	<ul style="list-style-type: none"> • Pitfall trapping • Funnel trapping • Elliott trapping • Echolocation call recording • Opportunistic searches • Hand foraging • Rock turning • Burrow searching • Nocturnal spotlighting 	<ul style="list-style-type: none"> • Single phase survey only 	<ul style="list-style-type: none"> • 2 State listed conservation significant avifauna species • 17 Federally listed conservation significant avifauna species

Report (Author)	Proximity to Study Area	Type of Survey	Survey Timing	Taxonomic Groups Documented	Survey Methods	Survey Limitations	Significant Findings
West Pilbara Iron Ore Project Onslow Rail Corridor Terrestrial Fauna Survey (Biota 2009a)	0 km (overlaps) 100,651 ha	Level 2 single-phase survey	24/10 – 1/11/2008	<ul style="list-style-type: none"> • Terrestrial avifauna • Mammals • Bats • Reptiles • Amphibians • SRE Invertebrates 	<ul style="list-style-type: none"> • Pitfall trapping • Elliott trapping • Systematic bird sampling • Echolocation call recording • Burrow searching • Nocturnal spotlighting • Recording secondary sign • Opportunistic sightings 	<ul style="list-style-type: none"> • Single phase survey only 	<ul style="list-style-type: none"> • 9 Federally listed conservation significant avifauna species
Wheatstone Project Terrestrial Fauna Survey (Biota 2010)	1 km east 9,824 ha	Level 2 single-phase survey	14 – 23/04/2009	<ul style="list-style-type: none"> • Terrestrial avifauna • Mammals • Bats • Reptiles • Amphibians • SRE Invertebrates 	<ul style="list-style-type: none"> • Pitfall trapping • Funnel trapping • Elliott trapping • Recording secondary sign • Opportunistic records 	<ul style="list-style-type: none"> • Not all areas were ground-truthed or equally sampled for fauna • Single phase survey only 	<ul style="list-style-type: none"> • 2 State listed conservation significant mammal species • 2 State listed conservation significant avifauna species • 16 Federally listed conservation significant avifauna species
Onslow Solar Saltfield Terrestrial Fauna Survey (Biota 2004)	10 km northeast	Habitat comparison	9-15/09/2003	<ul style="list-style-type: none"> • Mammals • Reptiles • Amphibians 	<ul style="list-style-type: none"> • Pitfall trapping • Funnel trapping • Elliott trapping • Recording secondary sign • Opportunistic records 	<ul style="list-style-type: none"> • Dedicated study to examine weed infested (Buffel) communities versus unaffected. 	<ul style="list-style-type: none"> • 1 State listed conservation significant mammal species

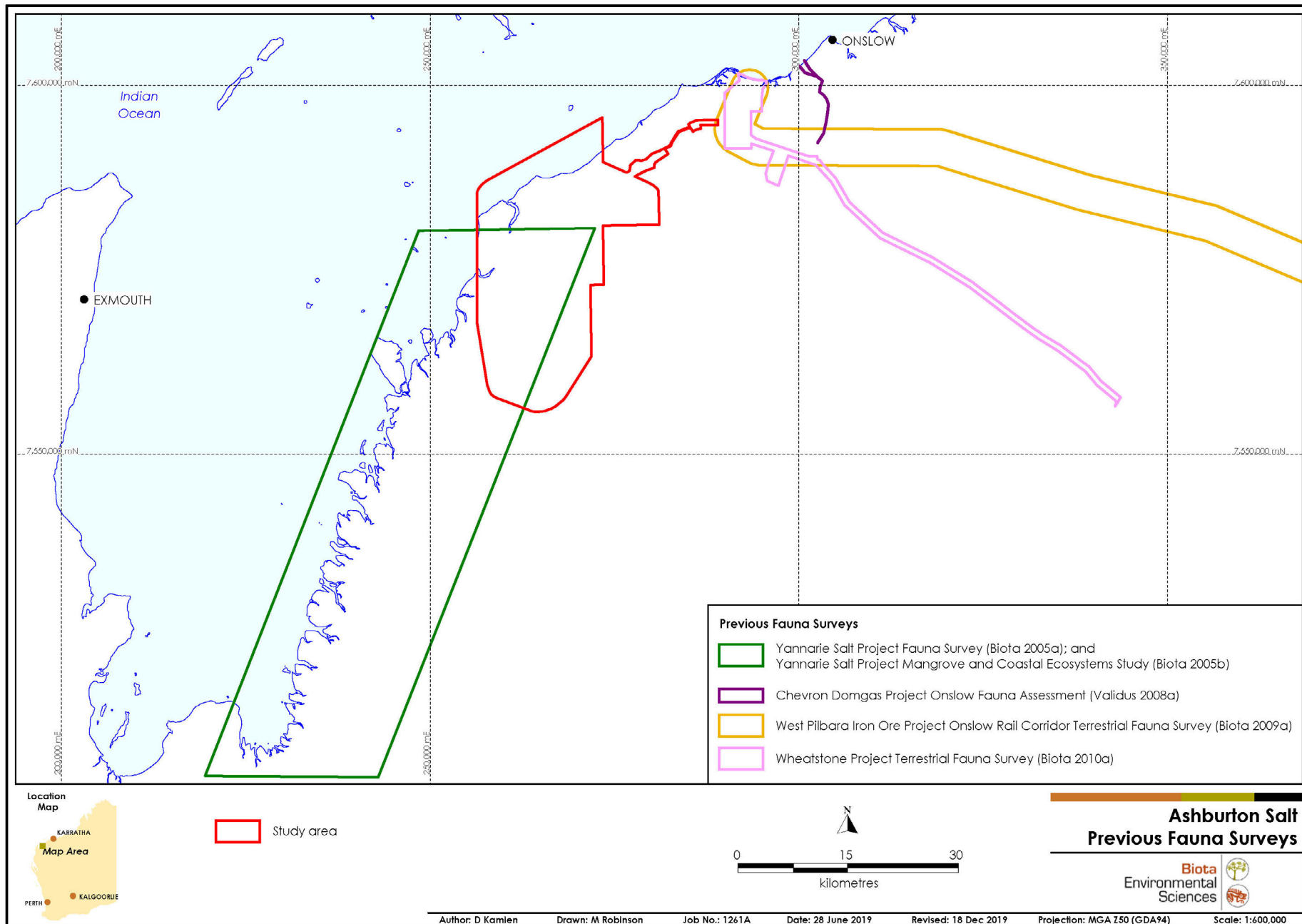


Figure 4.4: Previous relevant surveys intersecting the study area.

4.2.1 Vertebrate Fauna

Seven amphibian species, 81 reptile species, 210 bird species and 34 mammal species were identified as potentially occurring in the study area, based on the results of the desktop assessment (Table 4.5 and Appendix 1).

Table 4.5: Vertebrate species identified from the desktop review.

Fauna Group	Number of Species
Amphibians	7
Reptiles	81
Avifauna	210
Native Ground Mammals	17
Bats	9
Introduced Mammals	8
Total	332

Of these, 59 are State and/or Commonwealth listed conservation significant fauna species, as presented in Table 4.6. The potential occurrence of these species within the study area was assessed taking into account the results of this survey. This assessment is presented in Section 7.1.

Table 4.6: Vertebrate taxa of conservation significance previously recorded or potentially occurring in study area.

Family	Species Name	Common Name	Conservation Status	
			State	Commonwealth
Herpetofauna				
Pythonidae	<i>Liasis olivaceus barroni</i>	Pilbara Olive Python	Vulnerable	Vulnerable
Avifauna				
Apodidae	<i>Apus pacificus</i>	Fork-tailed Swift	Migratory	Migratory
Oceanitidae	<i>Oceanites oceanicus</i>	Wilson's Storm-Petrel	Migratory	Migratory
Diomedeidae	<i>Thalassarche melanophris</i>	Black-browed Albatross	Endangered/ Migratory	Vulnerable/ Migratory
Procellariidae	<i>Macronectes giganteus</i>	Southern Giant-Petrel	Migratory	Endangered
	<i>Ardenna pacifica</i>	Wedge-tailed Shearwater	Migratory	Migratory
	<i>Calonectris leucomelas</i>	Streaked Shearwater	Migratory	Migratory
	<i>Macronectes giganteus</i>	Southern Giant-Petrel	Migratory	Endangered
Fregatidae	<i>Fregata ariel</i>	Lesser Frigatebird	Migratory	Migratory
Threskiornithidae	<i>Plegadis falcinellus</i>	Glossy Ibis	Migratory	Migratory
Accipitridae	<i>Pandion cristatus</i>	Eastern Osprey	Migratory	Migratory
Falconidae	<i>Falco peregrinus</i>	Peregrine Falcon	Other Specially Protected Fauna	–
	<i>Falco hypoleucos</i>	Grey Falcon	Vulnerable	–
Charadriidae	<i>Pluvialis fulva</i>	Pacific Golden Plover	Migratory	Migratory
	<i>Pluvialis squatarola</i>	Grey Plover	Migratory	Migratory
	<i>Charadrius mongolus</i>	Lesser Sand Plover	Endangered/ Migratory	Endangered/ Migratory
	<i>Charadrius leschenaultii</i>	Greater Sand Plover	Migratory	Vulnerable/ Migratory
	<i>Charadrius veredus</i>	Oriental Plover	Migratory	Migratory
Scolopacidae	<i>Limosa limosa</i>	Black-tailed Godwit	Migratory	Migratory
	<i>Limosa lapponica menzbieri</i>	Bar-tailed Godwit	Vulnerable Migratory	Critically Endangered/ Migratory
	<i>Numenius minutus</i>	Little Curlew	Migratory	Migratory
	<i>Numenius phaeopus</i>	Whimbrel	Migratory	Migratory

Family	Species Name	Common Name	Conservation Status	
			State	Commonwealth
	<i>Numenius madagascariensis</i>	Eastern Curlew	Critically Endangered/ Migratory/	Critically Endangered/ Migratory/
	<i>Xenus cinereus</i>	Terek Sandpiper	Migratory	Migratory
	<i>Actitis hypoleucos</i>	Common Sandpiper	Migratory	Migratory
	<i>Tringa brevipes</i>	Grey-tailed Tattler	Priority 4 Migratory	Migratory
	<i>Tringa nebularia</i>	Common Greenshank	Migratory	Migratory
	<i>Tringa stagnatilis</i>	Marsh Sandpiper	Migratory	Migratory
	<i>Tringa glareola</i>	Wood Sandpiper	Migratory	Migratory
	<i>Arenaria interpres</i>	Ruddy Turnstone	Migratory	Migratory
	<i>Calidris tenuirostris</i>	Great Knot	Critically Endangered/ Migratory	Critically Endangered/ Migratory
	<i>Calidris canutus</i>	Red Knot	Endangered/ Migratory	Endangered/ Migratory
	<i>Calidris alba</i>	Sanderling	Migratory	Migratory
	<i>Calidris ruficollis</i>	Red-necked Stint	Migratory	Migratory
	<i>Calidris subminuta</i>	Long-toed Stint	Migratory	Migratory
	<i>Calidris melanotos</i>	Pectoral Sandpiper	Migratory	Migratory
	<i>Calidris acuminata</i>	Sharp-tailed Sandpiper	Migratory	Migratory
		<i>Calidris ferruginea</i>	Curlew Sandpiper	Critically Endangered/ Migratory
Glareolidae	<i>Glareola maldivarum</i>	Oriental Pratincole	Migratory	Migratory
Stercorariidae	<i>Stercorarius pomarinus</i>	Pomarine Jaeger	Migratory	Migratory
	<i>Anous stolidus</i>	Common Noddy	Migratory	Migratory
	<i>Onychoprion anaethetus</i>	Bridled Tern	Migratory	Migratory
	<i>Sternula albifrons</i>	Little Tern	Migratory	Migratory
	<i>Sternula nereis</i>	Fairy Tern	Vulnerable	Vulnerable
	<i>Gelochelidon nilotica</i>	Gull-billed Tern	Migratory	Migratory
	<i>Hydroprogne caspia</i>	Caspian Tern	Migratory	Migratory
	<i>Chlidonias leucopterus</i>	White-winged Black Tern	Migratory	Migratory
	<i>Sterna dougallii</i>	Roseate Tern	Migratory	Migratory
	<i>Sterna hirundo</i>	Common Tern	Migratory	Migratory
	<i>Thalasseus bergii</i>	Crested Tern	Migratory	Migratory
Psittacidae	<i>Pezoporus occidentalis</i>	Night Parrot	Critically Endangered	Critically Endangered
Hirundinidae	<i>Hirundo rustica</i>	Barn Swallow	Migratory	Migratory
Motacillidae	<i>Motacilla tschutschensis</i>	Eastern Yellow Wagtail	Migratory	Migratory
	<i>Motacilla cinerea</i>	Grey Wagtail	Migratory	Migratory
Mammals				
Dasyuridae	<i>Dasyurus hallucatus</i>	Northern Quoll	Endangered	Endangered
Peramelidae	<i>Perameles bougainville</i>	Shark Bay Bandicoot or Little Marl	Vulnerable	Endangered
Molossidae	<i>Ozimops cobourgianus</i>	Northern Coastal Free-tailed Bat	Priority 1	–
Muridae	<i>Leggadina lakedownensis</i>	Short-tailed Mouse	Priority 4	–
	<i>Pseudomys chapmani</i>	Western Pebble-mound Mouse	Priority 4	–

4.2.2 SRE Invertebrate Fauna

Although taxonomic groups known to contain SRE species have been targeted within the study area locality (Table 4.4 and Appendix 4), none of the collected taxa are listed as conservation significant. The consolidated data from the database searches yielded a total of 17 species-level taxa recorded from the locality, that potentially represent SREs, comprising:

- 57 Mygalomorphae (mygalomorph spiders), representing up to seven nominal species from two families; and
- 152 Gastropoda (snail) records, representing up to 10 nominal species from three families.

Of the 17 invertebrate taxa belonging to relevant groups retrieved from database searches, only three represent known or potential SREs (Table 4.7). These taxa may potentially occur or are likely to occur within the study area, based on the criteria outlined in Section 3.1.3 (see Appendix 4).

Table 4.7: Known or potential SRE taxa returned from database searches.

SRE Group	Family	Nominal Species	Distance to Study Area (km)	Habitat from which specimens were collected	Likelihood of Occurrence in Study Area
Mygalomorph spider	Nemesiidae	<i>Aname</i> `MYG102`	48	Sand plain; clay loam plain	May potentially occur
		<i>Aname</i> `sp.`	20	Sand plain; clay loam plain	Likely to occur
		<i>Kwonkan</i> `MYG090`	43	Sand plain	May potentially occur

5.0 Survey Results

5.1 Fauna Habitats

Five different landscapes (broad fauna habitats) were determined on the basis of the approach outlined in Section 3.1.4, in combination with on-ground habitat assessment and vegetation mapping conducted by Biota (2019b) (Table 5.1 and Figure 5.1). Sampling sites were selected based on fauna landscapes and landforms of the study area. Descriptions and photos of systematic sampling sites are presented in Table 5.2.

Table 5.1: Landscapes and landforms present in the study area.

Landscapes	Landforms	Area (ha)
LANDSCAPE 1: Mainland remnants	<ul style="list-style-type: none"> • Longitudinal dune • Sand plain • Clay loam plain • Clay pan 	4,877
LANDSCAPE 2: Mud flats	<ul style="list-style-type: none"> • Hypersaline mudflats • Intertidal mudflats 	27,415
LANDSCAPE 3: Inland dunes and plains	<ul style="list-style-type: none"> • Sand plain • Clay loam plain • Gilgai plain • Longitudinal dune • Clay pan • River bank • Creeklines and drainage 	18,271
LANDSCAPE 4: Coastal strand and dune	<ul style="list-style-type: none"> • Coastal dune • Beach 	1,690
LANDSCAPE 5: Mangroves	<ul style="list-style-type: none"> • Mangrove 	506

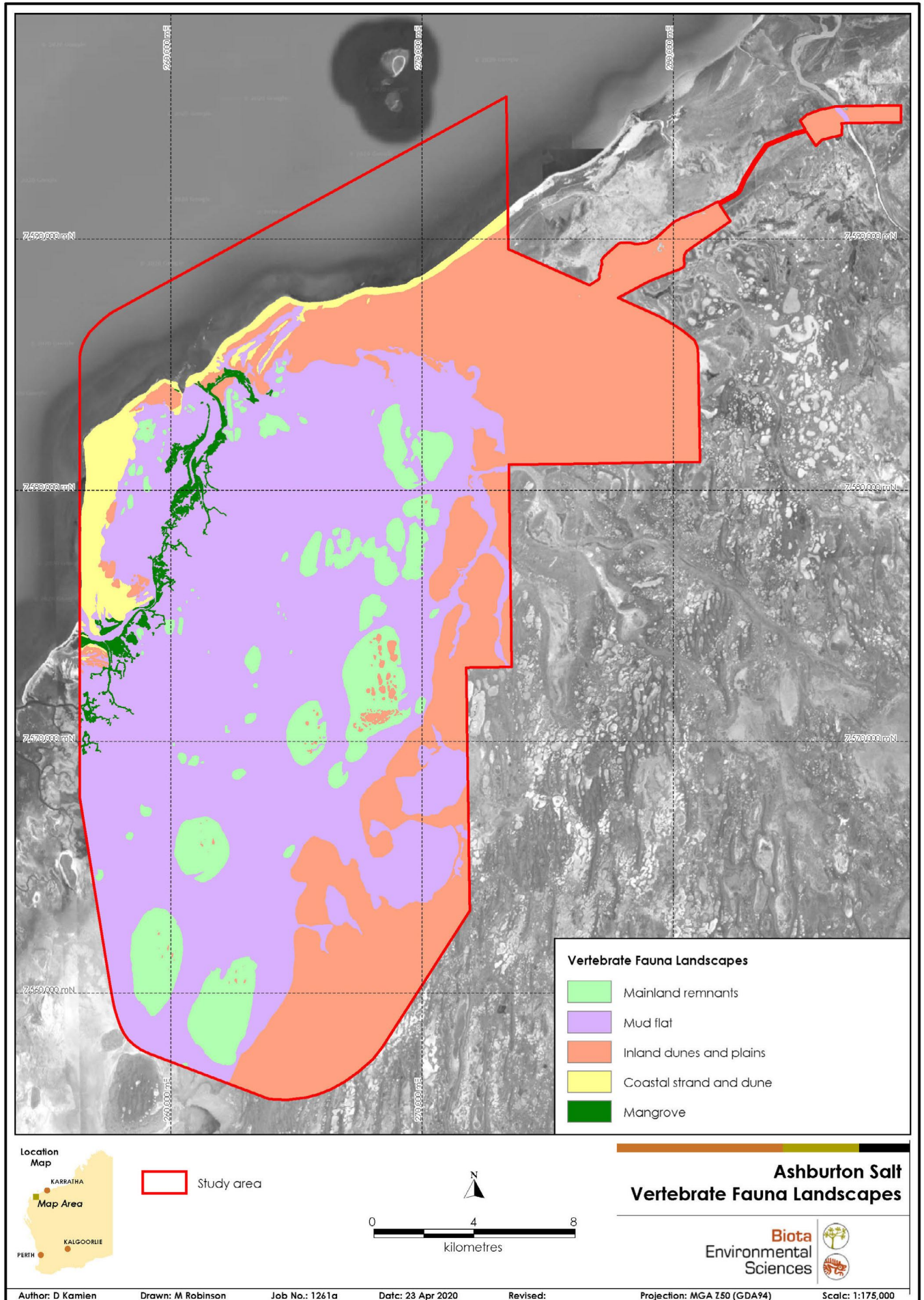
















Figure 5.1: Vertebrate fauna landscapes of the study area.

Table 5.2: Systematic site descriptions and photographs.

Site	Description	Photo
ASH01	<p>LANDSCAPE 1: Mainland remnants</p> <p>Landform: Clay loam plain</p> <p>Substrate: Clay loam</p> <p>Vegetation: <i>Acacia</i> spp. low open shrubland, <i>Triodia epactia</i> hummock grassland and *<i>Cenchrus ciliaris</i> very open tussock grassland</p> <p>Land system: Onslow</p> <p>Vegetation condition: Very good</p>	
ASH02	<p>LANDSCAPE 1: Mainland remnants</p> <p>Landform: Clay loam plain</p> <p>Substrate: Sandy clay loam</p> <p>Vegetation: <i>Acacia</i> spp. low open shrubland, over <i>Triodia epactia</i> hummock grassland and *<i>Cenchrus ciliaris</i> very open tussock grassland and <i>Tecticornia</i> spp. (samphire).</p> <p>Land system: Onslow</p> <p>Vegetation condition: Very good</p>	
ASH03	<p>LANDSCAPE 1: Mainland remnants</p> <p>Landform: Sand plain</p> <p>Substrate: Sandy loam</p> <p>Vegetation: <i>Acacia</i> spp. scattered low shrubs, over <i>Triodia epactia</i> hummock grassland and *<i>Cenchrus ciliaris</i> open tussock grassland</p> <p>Land system: Littoral</p> <p>Vegetation condition: Very good</p>	
ASH04	<p>LANDSCAPE 1: Mainland remnants</p> <p>Landform: Longitudinal dune</p> <p>Substrate: Sandy loam</p> <p>Vegetation: <i>Acacia</i> spp and <i>Hakea</i> sp. shrubland, over <i>Triodia epactia</i> hummock grassland and *<i>Cenchrus Ciliaris</i> open tussock grassland.</p> <p>Land system: Littoral</p> <p>Vegetation condition: Very good</p>	

Site	Description	Photo
ASH05	<p>LANDSCAPE 3: Inland dunes and plains</p> <p>Landform: Longitudinal dune</p> <p>Substrate: Sand</p> <p>Vegetation: <i>Grevillia stenobotrya</i> shrubland, over <i>Acacia</i> spp. low shrubland over <i>Triodia schinzii</i> hummock grassland and *<i>Cenchrus ciliaris</i> tussock grassland</p> <p>Land system: Yankagee</p> <p>Vegetation condition: Very good</p>	
ASH06	<p>LANDSCAPE 1: Mainland remnants</p> <p>Landform: Clay loam plain</p> <p>Substrate: Clay loam</p> <p>Vegetation: <i>Acacia</i> spp. very open shrubland, over <i>Triodia epactia</i> hummock grassland and *<i>Cenchrus ciliaris</i> very open tussock grassland.</p> <p>Land system: Dune</p> <p>Vegetation condition: Very good</p>	
ASH07	<p>LANDSCAPE 1: Mainland remnants</p> <p>Landform: Longitudinal dune</p> <p>Substrate: Sandy loam</p> <p>Vegetation: <i>Acacia</i> spp. shrubland, over <i>Triodia epactia</i> hummock grassland and *<i>Cenchrus ciliaris</i> open tussock grassland</p> <p>Land system: Dune</p> <p>Vegetation condition: Very good</p>	
ASH08	<p>LANDSCAPE 3: Inland dunes and plains</p> <p>Landform: Longitudinal dune</p> <p>Substrate: Sand</p> <p>Vegetation: <i>Grevillea</i> sp. scattered shrubs, over <i>Triodia epactia</i> closed hummock grassland and *<i>Cenchrus ciliaris</i> very open tussock grassland</p> <p>Land system: Yankagee</p> <p>Vegetation condition: Very good</p>	

Site	Description	Photo
ASH09	<p>LANDSCAPE 1: Mainland remnants</p> <p>Landform: Longitudinal dune</p> <p>Substrate: sand</p> <p>Vegetation: <i>Corymbia</i> sp. low scattered trees, over <i>Acacia</i> spp. very open shrubland, over <i>Triodia epactia</i> closed hummock grassland</p> <p>Land system: Dune</p> <p>Vegetation condition: Excellent</p>	
ASH10	<p>LANDSCAPE 3: Inland dunes and plains</p> <p>Landform: Sand plain</p> <p>Substrate: Clay sand</p> <p>Vegetation: <i>Acacia</i> spp. open shrubland over <i>Triodia schinzii</i> hummock grassland</p> <p>Land system: Onslow</p> <p>Vegetation condition: Very good</p>	
ASH11	<p>LANDSCAPE 3: Inland dunes and plains</p> <p>Landform: Longitudinal dune</p> <p>Substrate: Sand</p> <p>Vegetation: <i>Grevillea</i> sp. and <i>Acacia</i> spp. open shrubland, over <i>Triodia epactia</i> hummock grassland and *<i>Cenchrus ciliaris</i> open tussock grassland</p> <p>Land system: Dune</p> <p>Vegetation condition: Very good</p>	
ASH12	<p>LANDSCAPE 4: Coastal strand and dune</p> <p>Landform: Coastal dune</p> <p>Substrate: Sand</p> <p>Vegetation: <i>Acacia</i> spp. open heath, over <i>Triodia epactia</i> open hummock grassland and *<i>Cenchrus ciliaris</i> tussock grassland</p> <p>Land system: Onslow</p> <p>Vegetation condition: good</p>	

Site	Description	Photo
ASH13E	<p>LANDSCAPE 3: Inland dunes and plains</p> <p>Landform: River bank</p> <p>Substrate: Clay loam</p> <p>Vegetation: <i>Eucalyptus victrix</i> scattered trees over *<i>Parkinsonia</i> sp. tall open shrubland over *<i>Cenchrus ciliaris</i> open tussock grassland</p> <p>Land system: Onslow</p> <p>Vegetation condition: Poor</p>	
ASH14E	<p>LANDSCAPE 3: Inland dunes and plains</p> <p>Landform: Gilgai plain</p> <p>Substrate: Light clay</p> <p>Vegetation: <i>Acacia</i> spp. scattered low shrubs, over <i>Eriachne</i> sp. and *<i>Cenchrus ciliaris</i> open tussock grassland</p> <p>Land system: Onslow</p> <p>Vegetation condition: Good</p>	

5.2 Vertebrate Fauna

5.2.1 Vertebrate Fauna Overview

The survey recorded a combined total of 171 vertebrate species. Table 5.3 provides a summary of the number of species recorded from each major vertebrate group.

Table 5.3: Vertebrate fauna recorded during the survey.

Vertebrate Fauna Group	Number of Species
Amphibians	4
Reptiles ²	50
Birds	97
Native ground-dwelling Mammals	10
Introduced ground-dwelling Mammals	3
Bats	7
Total	171

The fauna recorded during the survey represent 52% of the total of 331 species identified from the locality of the study area (Appendix 1 and Section 4.0).

² This represents the minimum number of species recorded due to *Gehyra* spp. in the Pilbara having recently undergone extensive taxonomic revision (see Table 5.4).

5.2.2 Herpetofauna

The survey yielded a total of 54 herpetofauna species (Table 5.3 and Table 5.4). This represents over 61% of all herpetofauna species recorded from the locality based on database records and previous surveys (n=88) (Appendix 1).

Records comprised four frog species (belonging to the families Pelodyadidae and Limnodynastidae), eight gecko species (families Carphodactylidae, Gekkonidae and Diplodactylidae), four legless lizard species (Pygopodidae), three dragon species (Agamidae), 18 skink species (Scincidae), four monitor species (Varanidae), four blind snake species (Typhlopidae), one python species (Pythonidae) and eight front-fanged snake species (Elapidae).

Recorded herpetofauna abundance in Phase 1 was moderate to high, as expected in the late spring months following the dry season. However, abundance was lower than expected during Phase 2 following the wet season. The skinks *Lerista bipes* (n=176) and *Ctenotus pantherinus* (n=84), and the geckos *Heteronotia binoei* (n=91), *Gehyra pilbara* (n=47) and *Nephrurus levis* (n=58), were the most abundant herpetofauna species recorded during the survey.

No herpetofauna species of conservation significance were recorded in the study area during the survey.

This page intentionally left blank.

Table 5.4: Herpetofauna recorded in the study area.

Family/ Species	ASH01		ASH02		ASH03		ASH04		ASH05		ASH06		ASH07		ASH08		ASH09		ASH10		ASH11		ASH12		ASH 13E	ASH 14E	Opp.		Total	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2		
Pelodyridae																														
<i>Cyclorana maini</i>										2						2				1									5	
Limnodynastidae																														
<i>Neobatrachus albipes</i>										1																			1	
<i>Neobatrachus aquilonius</i>						1				14					2				9		20		2						48	
<i>Notaden nicholli</i>										4											15		14						33	
Carphodactylidae																														
<i>Nephrurus levis</i>	2				6	1	3	3	4		1	1	9	3	2		4	2			7	1	7	2					58	
Diplodactylidae																														
<i>Diplodactylus bilybara</i>	18	1	3	3		1													1										27	
<i>Lucasium stenodactylum</i>	2		1	1			1						1																6	
<i>Strophurus strophurus</i>							2		2				5								1	1	3						14	
Gekkonidae																														
<i>Gehyra pilbara</i>	13	3	3	4	2														11	11									47	
<i>Gehyra punctata*</i>	3																												3	
<i>Gehyra variegata*</i>	1		1		3		4		1			1				1						2							14	
<i>Heteronotia binoei</i>	10	6	11	10	2	1	5	5		4	3	1	1	1	2	5	4	2	6	6	1	2	1	2					91	
Pygopodidae																														
<i>Delma butleri</i>																		1											1	
<i>Delma pax</i>										1																				1
<i>Lialis burtonis</i>		1							1	1					1						2		5	3					14	
<i>Pygopus nigriceps</i>				1				2						1							1	2	1						8	
Agamidae																														
<i>Ctenophorus femoralis</i>																									3				3	
<i>Ctenophorus isolepis</i>							1																						1	
<i>Gowidon longirostris</i>																					1								1	
Scincidae																														
<i>Carlia munda</i>																1													1	
<i>Ctenotus duricola</i>	1		2				1																						4	
<i>Ctenotus grandis</i>					1		4			3			1	1	1		2			1	1	1							16	
<i>Ctenotus hanloni</i>	2				3	1		2					3			2	2	2		2		1							20	
<i>Ctenotus helenae</i>													1		1														2	
<i>Ctenotus iapetus</i>	2						1				2				4				1				1						11	
<i>Ctenotus maryani</i>		1																											1	
<i>Ctenotus pantherinus</i>	7	1	6	9	4		8	1			7	1	9	1		4	5	2	7	4	5	2	1						84	
<i>Ctenotus rufescens</i>																						1							1	
<i>Ctenotus saxatilis</i>			2										2		1	1		1			1		3	2					13	
<i>Eremiascincus pallidus</i>										1												2		3	1				7	
<i>Lerista bipes</i>		1			5	4		1	30	11	2		5	3	19	7	15	16	5	3	22	5	16	6					176	
<i>Lerista clara</i>					3		1			4	2	2	2	1	4		1	2	4	1	3								30	
<i>Lerista elegans</i>											1				1		1												3	
<i>Lerista onsloviana</i>															1						2		4	2					9	
<i>Menetia greyii</i>					1	1	1		3		3	3		1	2		4					2	1						22	
<i>Notoscincus ornatus</i>																	1	1											2	
<i>Tiliqua multifasciata</i>									1			1																	2	
Varanidae																														
<i>Varanus acanthurus</i>					1														2	1									4	
<i>Varanus brevicauda</i>	9		1		1		1		1		2				3		3			1									22	
<i>Varanus eremius</i>			2		3		1			1			2	1	1		2		3		4	1							21	
<i>Varanus gouldii</i>			1							1														1			1		4	
Typhlopidae																														
<i>Anilius ammodytes</i>							1															2	3						6	

Family/ Species	ASH01		ASH02		ASH03		ASH04		ASH05		ASH06		ASH07		ASH08		ASH09		ASH10		ASH11		ASH12		ASH 13E	ASH 14E	Opp.		Total	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2		
<i>Anilius grypus</i>	1	3		2	1			2	1	1		1	2	2	1	1			4	4	4		3	1						34
<i>Anilius hamatus</i>	2	4		2		1		2		1			1						3	2	2		1						21	
<i>Anilius pilbarensis</i>	1																												1	
Pythonidae																														
<i>Antaresia stimsoni</i>																				1										1
Elapidae																														
<i>Demansia psammophis</i>				1											1															2
<i>Demansia rufescens</i>																		1												1
<i>Furina ornata</i>	1							1												2										4
<i>Pseudechis australis</i>	1																					1						1		3
<i>Pseudonaja mengdeni</i>																					2	2								4
<i>Simoselaps anomalus</i>									1	1													4	3						9
<i>Suta punctata</i>	2																													2
<i>Ephalophis greyae</i>																												1		1
Total number of records	78	21	33	33	36	11	35	19	46	49	24	11	43	16	42	27	46	29	45	49	65	59	56	44	0	0	1	2	920	
Total number of species	21		15		17		21		21		13		16		20		16		18		24		20		0	0	3		54	

*Gehyra in the Pilbara has recently undergone extensive taxonomic revision, with several species sharing morphological characters that are similar or variable (Doughty et al. 2018, Kealley et al. 2018). As a result, they are not readily distinguished based on morphology alone, and require molecular sequencing to accurately identify them (Doughty et al. 2018, Kealley et al. 2018). Consequently, specimens attributed to *G. punctata* could also be *G. punctulata*. Similarly, specimens attributed to *G. variegata* could also be *G. crypta*.

5.2.3 Avifauna

The survey yielded a total of 98 bird species (Table 5.3 and Table 5.5). This represents over 46% of all bird species recorded from the locality based on database records and previous surveys (n=209) (Appendix 1).

Species from 40 families were recorded (Table 5.5), with the total comprising 36 passerine and 62 non-passerine species. Laridae (terns and gulls) was the most speciose family recorded during the survey, with nine species observed. The family Accipitridae (eagles, kites and other birds of prey) was also speciose with eight species recorded. The most abundant bird species were the Cockatiel, *Nymphicus hollandicus* (n=2,185 records) and the Zebra Finch, *Taeniopygia guttata* (n=1,157). These two species accounted for 59% of all bird observations in the study area during the survey.

Eleven bird species of elevated conservation significance were recorded in the study area:

- Fork-tailed Swift, *Apus pacificus* (Migratory). Recorded opportunistically within the study area on four occasions;
- Eastern Osprey, *Pandion cristatus* (Migratory). Recorded on ten occasions at site ASH03, ASH13E at the Ashburton River and opportunistically within the study area;
- Peregrine Falcon, *Falco peregrinus* (Other Specially Protected Fauna). Recorded on two occasions opportunistically within the study area;
- Common Sandpiper, *Actitis hypoleucos* (Migratory). Recorded on three occasions on the Ashburton River (excluding records presented in (Biota 2019a));
- Common Greenshank, *Tringa nebularia* (Migratory). Recorded on seven occasions on the Ashburton River (excluding records presented in (Biota 2019a));
- Red-necked Stint, *Calidris ruficollis* (Migratory). Recorded on six occasions on the Ashburton River (excluding records presented in (Biota 2019a));
- Little Tern, *Sternula albifrons* (Migratory). Recorded on 149 occasions within the study area on beaches, mangroves and on mudflats;
- Gull-billed Tern, *Gelochelidon nilotica* (Migratory). Recorded on 17 occasions from sites ASH03, ASH06, ASH07, ASH09, ASH12 and ASH13E, representing coastal dune, longitudinal dune, clay loam plain and river bank habitat;
- Caspian Tern, *Hydroprogne caspia* (Migratory). Recorded on 30 occasions within the study area on beaches and in mangroves;
- White-winged Black Tern, *Chlidonias leucopterus* (Migratory). Recorded on 19 occasions within the study area on beaches and intertidal mudflats;
- Crested Tern, *Thalasseus bergii* (Migratory). Recorded on 35 occasions within the study area on beaches and on intertidal mudflats.

This page intentionally left blank.

Family /Species	Common name	Conservation Status		ASH01		ASH02		ASH03		ASH04		ASH05		ASH06		ASH07		ASH08		ASH09		ASH10		ASH11		ASH12		ASH13E		ASH 14E	ASHMAN CAM01	Opportunistic		Total	
		State	Commonwealth	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2				
<i>Cladorhynchus leucocephalus</i>	Banded Stilt																												1				5	6	
Charadriidae																																			
<i>Charadrius ruficapillus</i>	Red-capped Plover																													4		24			28
<i>Eseyornis melanops</i>	Black-fronted Dotterel																														4	3		7	
<i>Erythrogonyx cinctus</i>	Red-kneed Dotterel																											6					6		
<i>Vanellus tricolor</i>	Banded Lapwing												4																				4		
Scolopacidae																																			
<i>Actitis hypoleucos</i>	Common Sandpiper	Migratory	Migratory																										3					3	
<i>Tringa nebularia</i>	Common Greenshank	Migratory	Migratory																										7					7	
<i>Calidris ruficollis</i>	Red-necked Stint	Migratory	Migratory																									6					6		
Turnicidae																																			
<i>Turnix velox</i>	Little Button-quail							2	1					3		1	1																	11	
Laridae																																			
<i>Sternula albifrons</i>	Little Tern	Migratory	Migratory																													46	78	124	
<i>Gelochelidon nilotica</i>	Gull-billed Tern	Migratory	Migratory						1					1		3				1							5	3	1		5	21	52	93	
<i>Hydroprogne caspia</i>	Caspian Tern	Migratory	Migratory																													11	13	24	
<i>Chlidonias hybrida</i>	Whiskered Tern																												2				80	82	
<i>Chlidonias leucopterus</i>	White-winged Black Tern	Migratory	Migratory																														19	19	
<i>Sterna hirundo</i>	Common Tern	Migratory	Migratory																														5	5	
<i>Thalasseus bengalensis</i>	Lesser Crested Tern																																35	35	
<i>Thalasseus bergii</i>	Crested Tern	Migratory	Migratory																												2	8	10		
<i>Chroicocephalus novaehollandiae</i>	Silver Gull															25												1		2		44	72		
Cacatuidae																																			
<i>Eolophus roseicapillus</i>	Galah			6	4		5			8		4					2				17	100	10		101		14			5		16	292		
<i>Cacatua sanguinea</i>	Little Corella					3					60										21	6	212		100		22		8			1	433		
<i>Nymphicus hollandicus</i>	Cockatiel				137		113		133		493		56			16		65				133		15		134		13	498		1	378	2,185		
Psittacidae																																			
<i>Barnardius zonarius</i>	Australian Ringneck																																4	4	
<i>Melopsittacus undulatus</i>	Budgerigar											20																	4			15	39		
Cuculidae																																			
<i>Chalcites basal</i>	Horsfield's Bronze-Cuckoo																						1									1	2		
<i>Cacomantis pallidus</i>	Pallid Cuckoo																												1			1	2		
Halcyonidae																																			
<i>Todiramphus sanctus</i>	Sacred Kingfisher																																12	12	
<i>Todiramphus chloris</i>	Collared Kingfisher																													1		3	4		
Meropidae																																			
<i>Merops ornatus</i>	Rainbow Bee-eater										2			1															3			1	7		
Maluridae																																			
<i>Malurus leucopterus</i>	White-winged Fairy-wren				3	13	3	4	5	9	5	2		3	5							1				1	4					36	94		
<i>Malurus lamberti</i>	Variagated Fairy-wren					5				3			4	4							8	4	10	3	29	4	3					3	80		
<i>Stipiturus ruficeps</i>	Rufous-crowned Emu-wren				4																												4		
Acanthizidae																																			
<i>Gerygone tenebrosa</i>	Dusky Gerygone																																28	28	
<i>Acanthiza uropygialis</i>	Chestnut-rumped Thornbill								2																								2		
Pardalotidae																																			
<i>Pardalotus rubricatus</i>	Red-browed Pardalote																												1			1	2		
<i>Pardalotus striatus</i>	Striated Pardalote																															7	7		
Meliphagidae																																			
<i>Lichenostomus virescens</i>	Singing Honeyeater			1	1	4		2	13	2	5	6	6		2	1	4	1	2			3	4	3	4	2						19	85		

Family /Species	Common name	Conservation Status		ASH01		ASH02		ASH03		ASH04		ASH05		ASH06		ASH07		ASH08		ASH09		ASH10		ASH11		ASH12		ASH13E		ASH 14E	ASHMAN CAM01	Opportunistic		Total
		State	Commonwealth	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2			
<i>Lichenostomus penicillatus</i>	White-plumed Honeyeater																											6				6	12	
<i>Manorina flavigula</i>	Yellow-throated Miner																						1	12			2				2	17		
<i>Sugomel niger</i>	Black Honeyeater									1								1													1	3		
<i>Lichmera indistincta</i>	Brown Honeyeater																						10				2				7	19		
Pomatostomidae																																		
<i>Pomatostomus temporalis</i>	Grey-crowned Babbler																															1	1	
Campephagidae																																		
<i>Coracina novaehollandiae</i>	Black-faced Cuckoo-shrike				1		1				1												10	1	2	1	4			2		1	24	
<i>Lalage sueurii</i>	White-winged Triller											2												4				1				7		
Pachycephalidae																																		
<i>Pachycephala melanura</i>	Mangrove Golden Whistler																														1	1		
<i>Pachycephala lanioides</i>	White-breasted Whistler																														23	23		
Artamidae																																		
<i>Artamus leucorhynchus</i>	White-breasted Woodswallow								2				4													7	2	2	9		15	41		
<i>Artamus personatus</i>	Masked Woodswallow															8			6	9	3			3	100							129		
<i>Artamus cinereus</i>	Black-faced Woodswallow					1		2	1																		1					5		
<i>Cracticus nigrogularis</i>	Pied Butcherbird				1	1		2																1							2	7		
Rhipiduridae																																		
<i>Rhipidura phasiana</i>	Mangrove Grey Fantail																												2		27	29		
<i>Rhipidura leucophrys</i>	Willie Wagtail				1	2		1		3			2												4		1			7	21			
Corvidae																																		
<i>Corvus bennetti</i>	Little Crow										1	2		2	15							70		2		8		6			4	110		
<i>Corvus orru</i>	Torresian Crow											2						1						2	1		3			1	10			
Monarchidae																																		
<i>Grallina cyanoleuca</i>	Magpie-lark																	1									2	2			2	7		
Alaudidae																																		
<i>Mirafra javanica</i>	Horsfield's Bushlark																							7			1	2			4	14		
Megaluridae																																		
<i>Cincloramphus mathewsi</i>	Rufous Songlark																										2				7	9		
<i>Cincloramphus cruralis</i>	Brown Songlark					2		1					1														1				4	9		
<i>Eremiornis carteri</i>	Spinifexbird							1					2		1											1			2	5	12			
Timaliidae																																		
<i>Zosterops luteus</i>	Yellow White-eye																												3		83	86		
Hirundinidae																																		
<i>Cheramoeca leucosterna</i>	White-backed Swallow											3	4																	1	8			
<i>Petrochelidon ariel</i>	Fairy Martin																													6	6			
<i>Petrochelidon nigricans</i>	Tree Martin																										6			28	34			
Estrildidae																																		
<i>Taeniopygia guttata</i>	Zebra Finch				26		78	2	18	20	244	8	62		30		111		35	2	28	4	27	21	26	84	124	4	27	61	2	5	108	1,157
Motacillidae																																		
<i>Anthus novaeseelandiae</i>	Australasian Pipit																											2			33	35		
Total number of records				13	177	13	230	9	170	55	758	29	242	15	39	21	183	12	102	13	39	146	281	287	65	468	284	103	119	574	65	110	1,472	6,094
Total number of species				10		17		15		15		19		12		13		10		6		18		21		22		45		8		15	82	98

OS= Other Specially Protected Fauna

This page intentionally left blank.

5.2.4 Ground-Dwelling Mammals

Thirteen ground-dwelling mammal species were recorded from the study area (Table 5.3 and Table 5.6). These represent over 52% of all ground-dwelling mammal species recorded from the locality (n = 25) based on database records and previous surveys (Appendix 1).

The species total comprised one echidna (family Tachyglossidae), four carnivorous marsupial species (Dasyuridae), one kangaroo species (Macropodidae), four rodent species (Muridae), and three introduced predator species from two families (Canidae) and (Felidae)

No ground-dwelling mammals of conservation significance were recorded during the survey.

This page intentionally left blank.

Table 5.6: Ground-dwelling mammals recorded in the study area.

Species name	Common name	ASH01		ASH02		ASH03		ASH04		ASH05		ASH06		ASH07		ASH08		ASH09		ASH10		ASH11		ASH12		ASH13E	ASH14E	Opportunistic		Total
		P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2			
Tachyglossidae																														
<i>Tachyglossus aculeatus</i>	Short-beaked Echidna																		1										1	
Dasyuridae																														
<i>Dasykaluta rosamondae</i>	Kaluta											1	1		2		1		1										6	
<i>Planigale ingrami</i>	Long-tailed Planigale	10		2				1									1			4	1								19	
<i>Sminthopsis macroura</i>	Froggatt's Stripe-faced Dunnart							1							1														2	
<i>Sminthopsis youngsoni</i>	Lesser Hairy-footed Dunnart					7	1	2	3		2	5		3		1					2								26	
Macropodidae																														
<i>Osphranter robustus</i>	Euro, Biggada															1													1	
Muridae																														
<i>Notomys alexis</i>	Spinifex Hopping-mouse															1							1						2	
<i>Pseudomys delicatulus</i>	Delicate Mouse																								1				1	
<i>Pseudomys desertor</i>	Desert Mouse	1						1						1	1			1			1								6	
<i>Pseudomys hermannsburgensis</i>	Sandy Inland Mouse			3	1			2	1					1		1	1			1									11	
Canidae																														
* <i>Canis familiaris</i>	Dog/Dingo							1																			1		1	3
* <i>Vulpes vulpes</i>	Red Fox		1																										1	
Felidae																														
* <i>Felis catus</i>	Cat	1																										1	2	4
Total number of records		12	1	5	1	7	3	7	3	0	2	6	1	5	3	4	3	2	2	5	2	2	1	0	1	0	1	1	3	83
Total number of species		4		2		2		6		1		2		4		6		4		3		2		1		0	1	2	13	

This page intentionally left blank.

5.2.5 Bats

Seven bat species were recorded from the study area, comprising four species from the family Molossidae, and three species of Vespertilionidae (Table 5.7). The most commonly encountered species were the Gould's Wattled Bat, *Chalinolobus gouldii* (recorded at eight locations) and the Northern Free-tailed Bat, *Ozimops lumsdenae* (recorded at seven locations).

Northern Coastal Free-tailed Bat *Ozimops cobourgianus*, a Priority 1 species and mangrove specialist, was recorded within mangrove habitat both inside and outside of the study area.

Table 5.7: Bats recorded in the study area.

Family/Species	Common Name	Conservation Status		Phase 1							Phase 2								
		State	Federal	ASH02	ASH05	ASH06	ASH07	ASH08	ASH09	ASH12	ASH13E	ASHMAN01	ASHMAN02	ASH01	ASH03	ASH05B	ASH12	ASH13E	ASH14E
Molossidae																			
<i>Austronomus australis</i>	White-striped Free-tailed Bat	-	-								X			X				X	
<i>Chaerephon jobensis</i>	Greater Northern Free-tailed Bat	-	-		X							X							
<i>Ozimops cobourgianus</i>	Northern Coastal Free-tailed Bat	P1	-									X	X						
<i>Ozimops lumsdenae</i>	Northern Free-tailed Bat	-	-								X	X	X	X	X				X
Vespertilionida																			
<i>Chalinolobus gouldii</i>	Gould's Wattled Bat	-	-		X			X			X			X	X	X	X	X	X
<i>Nyctophilus geoffroyi</i>	Lesser Long-eared Bat	-	-							X	X		X					X	
<i>Scotorepens greyii</i>	Little Broad-nosed Bat	-	-		X					X	X					X		X	X
Total number of species				0	3	0	0	1	0	2	6	2	3	3	2	3	1	5	3

5.3 Short-Range Endemic Invertebrates

Two higher-order taxonomic groups with the potential to include SRE species were recorded within the study area, comprising the mygalomorph spiders and land snails. Sections 5.3.1 and 5.3.2 provide accounts of each group and detail the SRE status of each species (where known). Table 5.8 summarises the records by sites and habitat, while collection locations are provided in Figure 5.2.

Table 5.8: Summary of potential SRE invertebrate fauna recorded from the study area
(n=number of specimens).

Invertebrate Group Family	Taxon	n	Habitat	Sites Recorded	SRE Status	Previously Recorded?	Known Outside Study Area?
Mygalomorph Spiders							
Barychelidae	<i>Idiommata</i> sp. B38	1	Longitudinal dune	ASH09	Potential SRE	No	No
Ctenezidae	<i>Conothele</i> sp. C26	2	Clay loam plain	ASHSRE15 ASHSRE29	Potential SRE	No	No
	<i>Conothele</i> sp. C27	3	Longitudinal dune; Clay loam plain; Gilgai plain	ASHSRE09 ASHSRE26 ASH09	Potential SRE	No	No
Idiopidae	<i>Euoplos</i> sp. I67	5	Longitudinal dune; Clay loam plain	ASHSRE01 ASHSRE02 ASHSRE04 ASHSRE26 ASHSRE46	Not an SRE	Yes	Yes
	<i>Aganippe</i> sp. I69	2	Clay loam plain	ASHSRE34 ASHSRE36	Potential SRE	No	Yes
Nemesiidae	<i>Aname</i> sp. N5 <i>Aname ellenae</i>	5	Clay loam plain	ASHSRE01 ASHSRE08	Not an SRE	Yes	Yes
	<i>Aname</i> sp. N57	18	Clay loam plain Sand Plain	ASHSRE05 ASHSRE14 ASHSRE16 ASHSRE23 ASH05	Potential SRE	Yes	Yes
	<i>Aname</i> sp. N141	10	Longitudinal dune; Coastal dune	ASHSRE12 ASHSRE31 ASHSRE37 ASH11 ASH12	Potential SRE	No	Yes
	<i>Aname</i> sp. N142	3	Clay loam plain	ASHSRE10 ASHSRE21	Potential SRE	No	No
	<i>Aname</i> sp. N146	2	Clay loam plain	ASHSRE01 ASHSRE26	Potential SRE	No	No
Land Snails							
Succineidae	<i>Succinea</i> sp.	13	Clay loam plain	ASHSRE01 ASHSRE27 ASHSRE32	Not an SRE	Indet.	Yes
Camaenidae	<i>Rhagada convicta</i>	2	Habitat generalist	ASHSRE15	Not an SRE	Yes	Yes

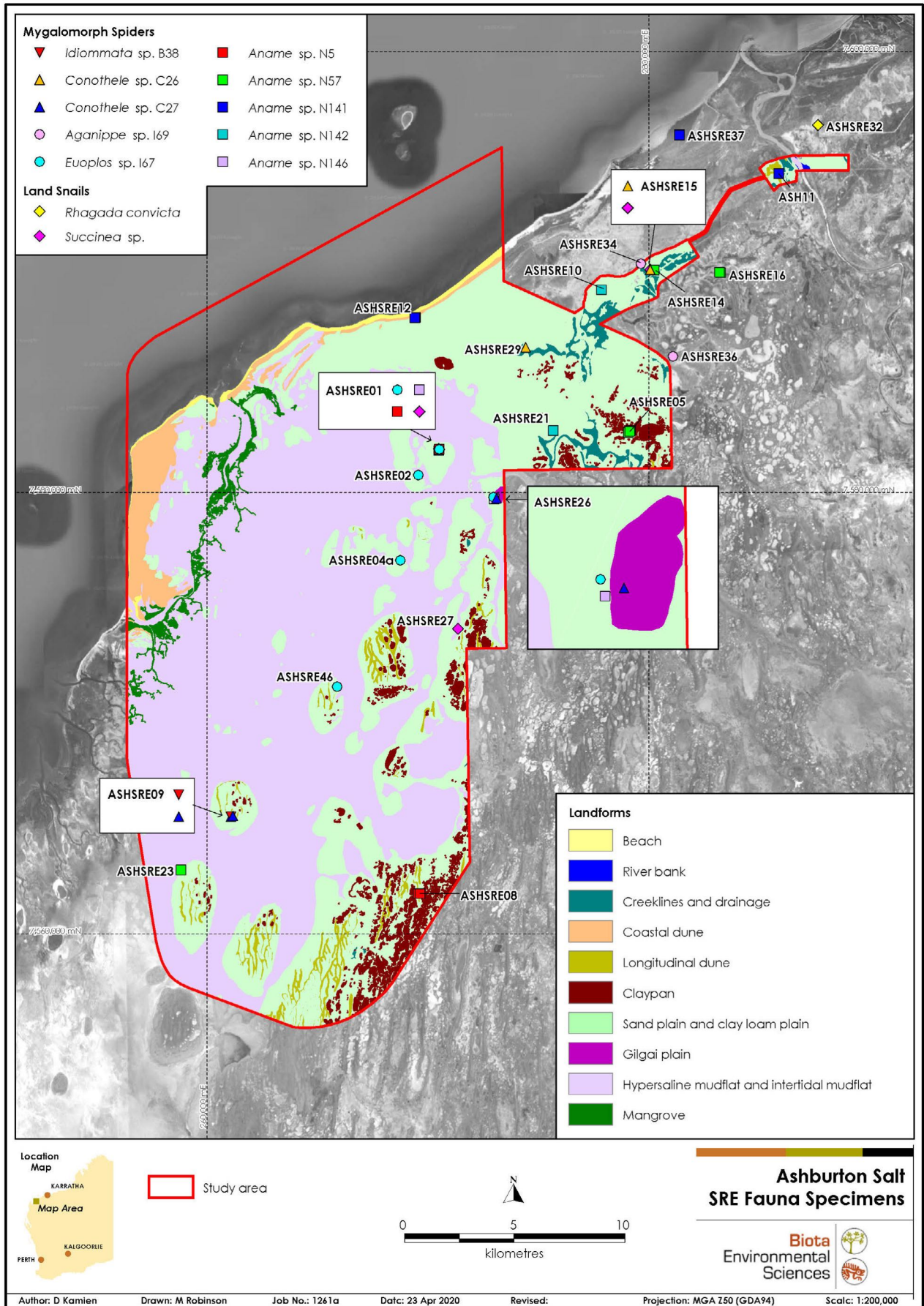


Figure 5.2: Invertebrate SRE specimens collected within the study area.

5.3.1 Mygalomorph Spiders

5.3.1.1 Barychelidae

Idiommata sp. B38

Distribution

One specimen of *Idiommata* sp. B38 (Plate 5.1) was recorded from within the study area at pitfall site ASH09 (Table 5.8, Figure 5.2 and Appendix 5). This is the first and only record of this putative species. This taxon was recorded from a pit trap. The fact that no burrows were located may indicate that its burrow is likely to be highly cryptic. This is common in many Barychelidae taxa and may be the primary reason as to why only one record was noted during the survey.

Habitat

Idiommata sp. B38 was recorded from a pit trap on a longitudinal dune. As this specimen represents the only record, any habitat specificity cannot be determined.

SRE Status

As this putative species is known from one location, it qualifies as a potential SRE.



Plate 5.1: *Idiommata* sp. B38.

5.3.1.2 Ctenezidae

Conothele sp. C26

Distribution

Two specimens of *Conothele* sp. C26 (Plate 5.2) were recorded from within the study area at sites ASHSRE15 and ASHSRE29 (Table 5.8, Figure 5.2 and Appendix 5). *Conothele* sp. C26 did not show a close affinity to any other previously sequenced specimens from Helix's database, or from GenBank, and as a result represents a new putative species.

Habitat

Both specimens were recorded from clay loam plain. Its burrow is characterised by a cryptic papery lid (Plate 5.3 and Plate 5.4).

SRE Status

As this putative species is known from two locations separated by only 7 km, it qualifies as a potential SRE.



Plate 5.2: *Conothele* sp. C26.



Plate 5.3: *Conothele* sp. C26 closed burrow.

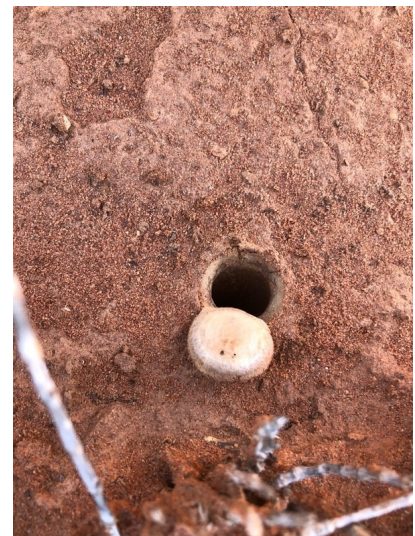


Plate 5.4: *Conothele* sp. C26 open burrow.

***Conothele* sp. C27**Distribution

Three specimens of *Conothele* sp. C26 (Plate 5.5) were recorded from within the study area at sites ASHSRE09, ASHSRE26 and pitfall site ASH09 (Table 5.8, Figure 5.2 and Appendix 5). Similar to C26, *Conothele* sp. C27 did not show a close affinity to any other previously sequenced specimens from Helix's database, or from GenBank and as a result, represents a new putative species.

Habitat

Specimens were recorded from longitudinal dunes, clay loam plain and gilgai plain. Its burrow is characterised by a cryptic papery lid (Plate 5.6 and Plate 5.7).

SRE Status

Specimens are separate by a maximum spanning distance of approximately 20 km and therefore this species qualifies as a potential SRE.



Plate 5.5: *Conothele* sp. C27.



Plate 5.6: *Conothele* sp. C27 closed burrow.



Plate 5.7: *Conothele* sp. C27 open burrow.

5.3.1.3 Idiopidae***Euoplos* sp. I67**Distribution

Five specimens of *Euoplos* sp. I67 (Plate 5.8) were recorded from four sites within the study area (Table 5.8, Figure 5.2 and Appendix 5). Eight specimens have previously been recorded on Barrow Island and in the Pannawonica locality; a minimum spanning area of approximately 10,100km².

Habitat

Within the study area, *Euoplos* sp. I67 is known from longitudinal dunes and clay loam plains. Previous records from beyond the study area were also recorded on clay loam soil. A cryptic clay lid characterises its burrow (Plate 5.9 and Plate 5.10).

SRE Status

As this putative species has a known distribution greater than 10,000 km² it is not a SRE.



Plate 5.8: *Euoplos* sp. I67.



Plate 5.9: *Euoplos* sp. I67 closed burrow.



Plate 5.10: *Euoplos* sp. I67 open burrow.

Aganippe sp. 169

Distribution

Two specimens of *Aganippe* sp. 169 (Plate 5.11) were recorded from within the study area at sites ASHSRE34 and ASHSRE36 (Table 5.8, Figure 5.2 and Appendix 5). These records represent the only known specimens of *Aganippe* sp. 169 and as a result, represent a new putative species.

Habitat

Both specimens were recorded from clay loam plain habitat. Its burrow is characterised by a cryptic papery lid (Plate 5.12 and Plate 5.13).

SRE Status

As this putative species is known from two locations separated by less than 5 km, it qualifies as a potential SRE.



Plate 5.11: *Aganippe* sp. 169.



Plate 5.12: *Aganippe* sp. 169 closed burrow.



Plate 5.13: *Aganippe* sp. 169 open burrow.

5.3.1.4 Nemesiidae

***Aname ellенаe* (*Aname* sp. N5)**

Distribution

Aname ellенаe (Plate 5.14 and Plate 5.15) was recorded on three occasions from within the study area at sites ASHSRE01 and ASHSRE08 (Table 5.8, Figure 5.2 and Appendix 5). This species is known from a further 32 records that have previously been recorded from 12 sites up to 700 km east of the study area. The documented minimum spanning area of this species is approximately 120,000 km².

Habitat

Specimens came from clay loam plain. Its burrow is characterised by an open hole (Plate 5.16).

SRE Status

As this species has a known distribution greater than 10,000 km² it does not represent a SRE taxon.



Plate 5.14: *Aname ellенаe* female.



Plate 5.15: *Aname ellенаe* male.



Plate 5.16: *Aname ellенаe* burrow (photo source: WAM).

***Aname* sp. N57**Distribution

This putative species was recorded on 18 occasions at five sites located inside and outside the study area (Plate 5.17), (Table 5.8, Figure 5.2 and Appendix 5). This species is represented by one other previously sequenced specimen from an unknown location. Based on the records obtained during the current study, the minimum spanning area of this putative species is approximately 119 km².

Habitat

Aname sp. N57 was recorded from sand plain and clay loam plain habitats. Its burrow is characterised by an open hole (Plate 5.18).

SRE Status

As this species has a known distribution less than 10,000 km² it represents a potential SRE taxon.



Plate 5.17: *Aname* sp. N57.



Plate 5.18: *Aname* sp. N57 burrow.

***Aname* sp. N141**Distribution

This putative species was recorded on 10 occasions at five sites located inside and outside the study area (Plate 5.19) (Table 5.8, Figure 5.2 and Appendix 5). *Aname* sp. N141 represents a new putative species that has not been recorded prior to this study. Based on the records obtained during the current study, the minimum spanning area of this putative species is approximately 109 km².

Habitat

Aname sp. N141 was recorded only on longitudinal dune and coastal dune habitat. Its burrow is characterised by an open hole, sometimes with a light covering of silk (Plate 5.20 and Plate 5.21).

SRE Status

As this species has a known distribution less than 10,000 km² it represents a potential SRE taxon.



Plate 5.19: *Aname* sp. N141.



Plate 5.20: *Aname* sp. N141 closed burrow.



Plate 5.21: *Aname* sp. N141 open burrow.

***Aname* sp. N142**

Distribution

This putative species was recorded on three occasions from two sites located inside the study area (Plate 5.22) (Table 5.8, Figure 5.2 and Appendix 5). *Aname* sp. N142 represents a new putative species that has not been recorded prior to this study. Based on the records obtained during the current study, the maximum spanning distance of this species is approximately 7.5 km.

Habitat

Aname sp. N142 was recorded on clay loam plains only. Its burrow is characterised by a small mound of dirt that when brushed away reveals a silk sock (Plate 5.23).

SRE Status

As this species has a known distribution less than 10,000 km² it represents a potential SRE taxon.



Plate 5.22: *Aname* sp. N142.



Plate 5.23: *Aname* sp. N142 burrow.

***Aname* sp. N146**

Distribution

This taxon was recorded on two occasions from two sites located inside the study area (Plate 5.24) (Table 5.8, Figure 5.2 and Appendix 5). *Aname* sp. N146 represents a new putative species that has not been recorded prior to this study. Based on the records obtained during the current study, the maximum spanning distance of this putative species is approximately 3.5 km.

Habitat

Aname sp. N146 was recorded from clay loam plains only. Its burrow is characterised by an open hole with a hooded lip. (Plate 5.25).

SRE Status

As this species has a known distribution less than 10,000 km² it represents a potential SRE taxon.



Plate 5.24: *Aname* sp. N146.

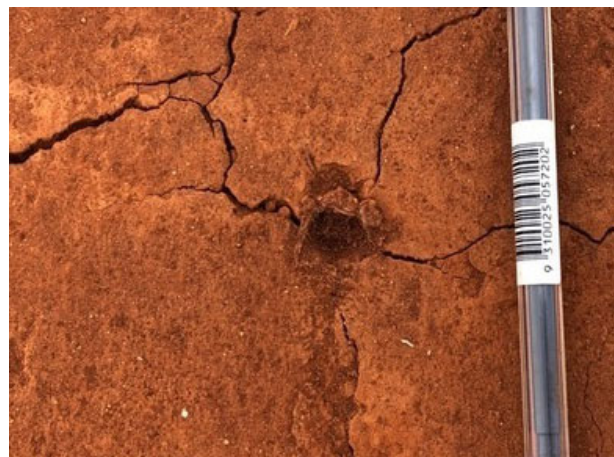


Plate 5.25: *Aname* sp. N146 burrow.

5.3.2 Land Snails

5.3.2.1 Succineidae

Succinea sp.

Distribution

This taxon was recorded on 13 occasions from three sites located both inside and outside the study area (Plate 5.26, Figure 5.2 and Table 5.8). However, no live specimens were recorded.

Habitat

Succinea sp. was recorded from clay loam plains.

SRE status

Although the genus *Succinea* is taxonomically poorly known in Australia, all known species are considered to be widespread (Whisson 2012).



Plate 5.26 *Succinea* sp.

5.3.2.2 Camaenidae

Rhagada convicta

Distribution

In the study area *Rhagada convicta* was recorded from a single site on clay loam plain habitat (Plate 5.27, Figure 5.2 and Table 5.8).

Habitat

This species had been recorded in a variety of habitats in the Pilbara, typically where large spinifex occurs, offering shade during aestivation.

SRE status

Rhagada convicta occurs from Exmouth to Port Hedland and inland as far as Pannawonica (Hamilton 2015, 2018). As this species has a known distribution greater than 10,000 km² it is not an SRE.



Plate 5.27 *Rhagada convicta*.

This page intentionally left blank.

6.0 Assemblage Analysis

6.1 Species Accumulation Analysis

Species accumulation curves for herpetofauna, avifauna and ground-dwelling mammals are presented in Figure 6.1, Figure 6.2 and Figure 6.3 respectively. Randomised curves for each of the three taxonomic groups approach asymptote, indicating that relatively few additional species are likely to be recorded with additional sampling effort. This indicates that the vertebrate assemblages were adequately documented during the survey period.

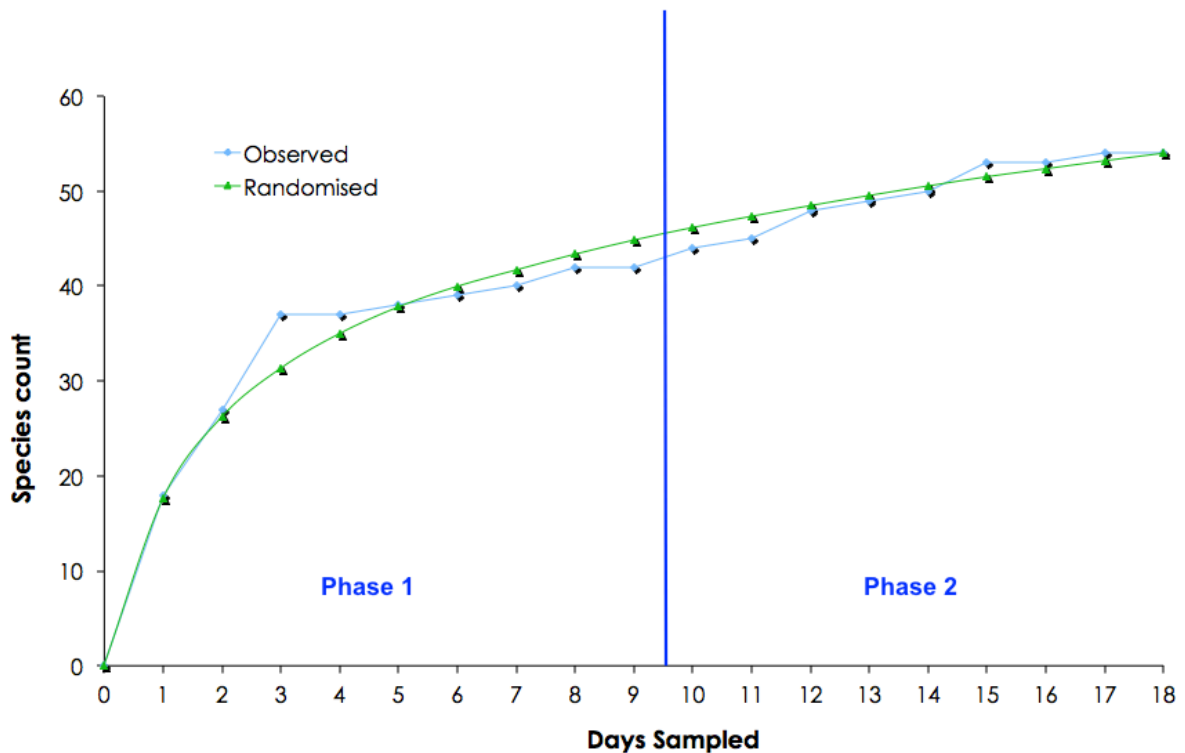


Figure 6.1: Herpetofauna species accumulation curve.

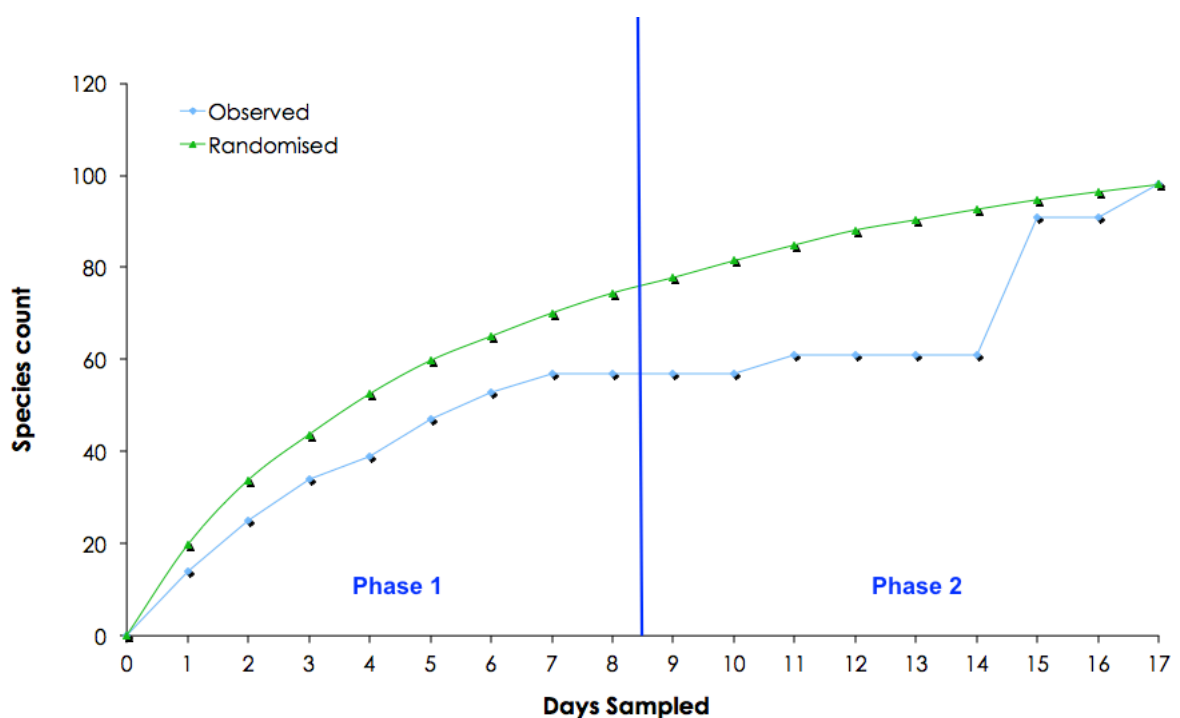


Figure 6.2: Avifauna species accumulation curve.

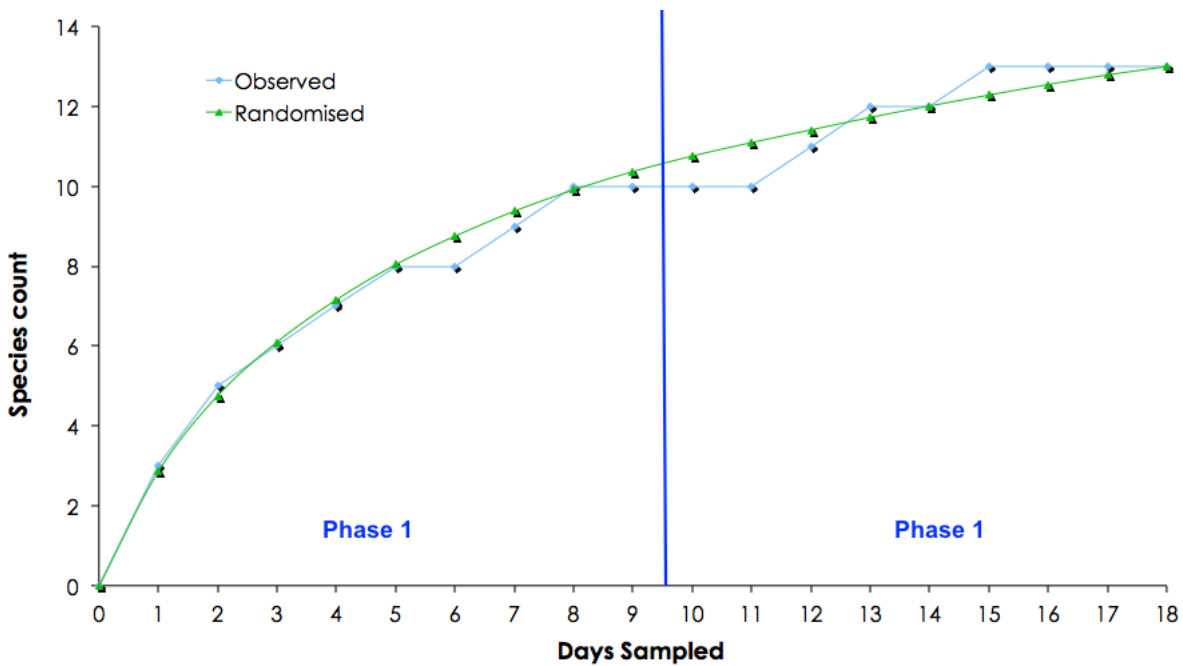


Figure 6.3: Ground-dwelling mammals species accumulation curve.

Observed species richness for each vertebrate group was only slightly below that predicted by the nonparametric estimators (Table 6.1). Excluding bats, the total of 164 species recorded represents approximately 84% of the mean estimate of total species richness, which indicates that on average 197 vertebrate species may be recorded in the study area using the same methods.

Table 6.1: Comparison of observed vertebrate species richness with estimator predictions.

Group	Observed	Estimators			
		Chao 1	Jackknife 1	Bootstrap	Mean
Herpetofauna	54	68	67	60	65
Avifauna	97	111	124	112	116
Ground mammals	13	17	17	15	16
Total	*164	196	208	187	197

*Note: accounting for recorded bat species, the total observed species count is 171 (see Section 5.2.5). As bat data are not quantitative, they could not be used in the species accumulation analysis.

6.2 Contextual Analysis

6.2.1 Herpetofauna

Herpetofauna species richness documented during the survey was above average compared to those documented from previous surveys in the locality (Table 6.2).

Table 6.2: Herpetofauna species richness by study.

Study	Herpetofauna Species Richness
Ashburton (this study)	54
Wheatstone	54
Onslow Solar Salt	23
Yannarie Salt	34
Average Previous studies	37

SIMPROF tests for herpetofauna assemblages documented by comparable studies in the locality showed significant group structure ($P_i=3.459$; $p<0.05$). That is, significant differences in species assemblages were noted between some of the studies (Actual $P_i >$ simulated P_i). This is apparent in the nMDS plot, which shows assemblages recorded at each study area formed relatively discrete clusters (Figure 6.4).

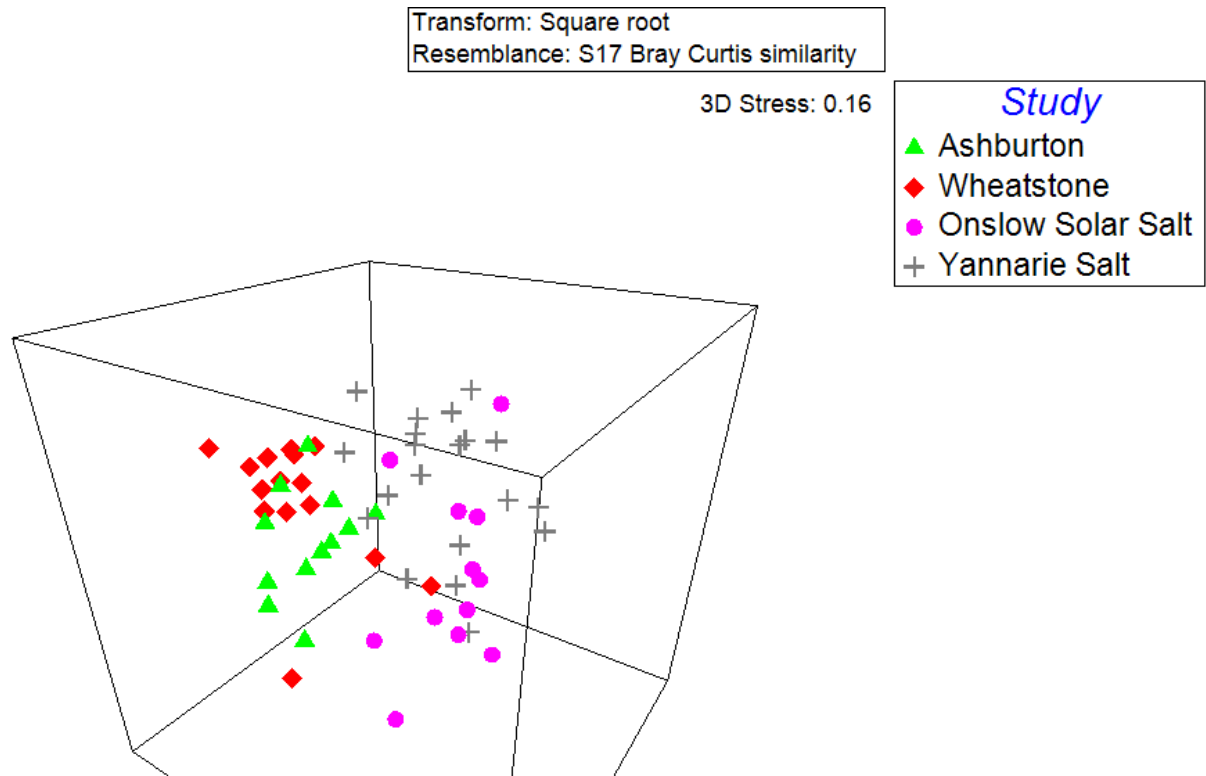


Figure 6.4: nMDS plot of herpetofauna assemblage by study. (Individual points represent one sampling site).

Two-way crossed ANOSIM results showed that the fauna landscape is a greater determinate of species assemblage than landform (see Table 5.1 (landscape Global $R = 0.441$, $p < 0.05$; landform Global $R = 0.144$, $p < 0.5$)). Furthermore, individual surveys have a greater influence on species assemblage than fauna landscape (study Global $R = 0.399$, $p < 0.05$; landscape Global $R = 0.234$; $p < 0.05$).

Across fauna landscape groups, pairwise tests reveal a significant difference in herpetofauna assemblage recorded at Ashburton compared with those of Onslow Solar Salt and Yannarie ($R = 0.456$, $p < 0.05$; $R = 0.484$, $p < 0.05$ respectively). However, the Ashburton herpetofauna assemblage is not significantly different to that of the assemblage recorded at Wheatstone ($R = 0.251$, $p > 0.05$).

6.2.2 Avifauna

Comparisons of the avifauna species richness³ of the current study with other surveys is presented in Table 6.3.

Table 6.3: Avifauna species richness by study.

Study	Avifauna Species Richness
Ashburton (this study)	97
Wheatstone	61

SIMPROF tests for avifauna assemblages documented by comparable studies in the locality resulted in evidence of significant group structure ($P_i = 2.795$; $p < 0.05$). That is, significant differences in species assemblages were noted between the two studies (Actual $P_i >$ simulated P_i). This is apparent in the nMDS plot that shows assemblages recorded at each of the two study areas formed two discrete clusters (Figure 6.5).

³ Yannarie Salt avifauna data is excluded as that was a targeted migratory shorebird survey. The Onslow Solar Salt study did not incorporate avifauna censusing.

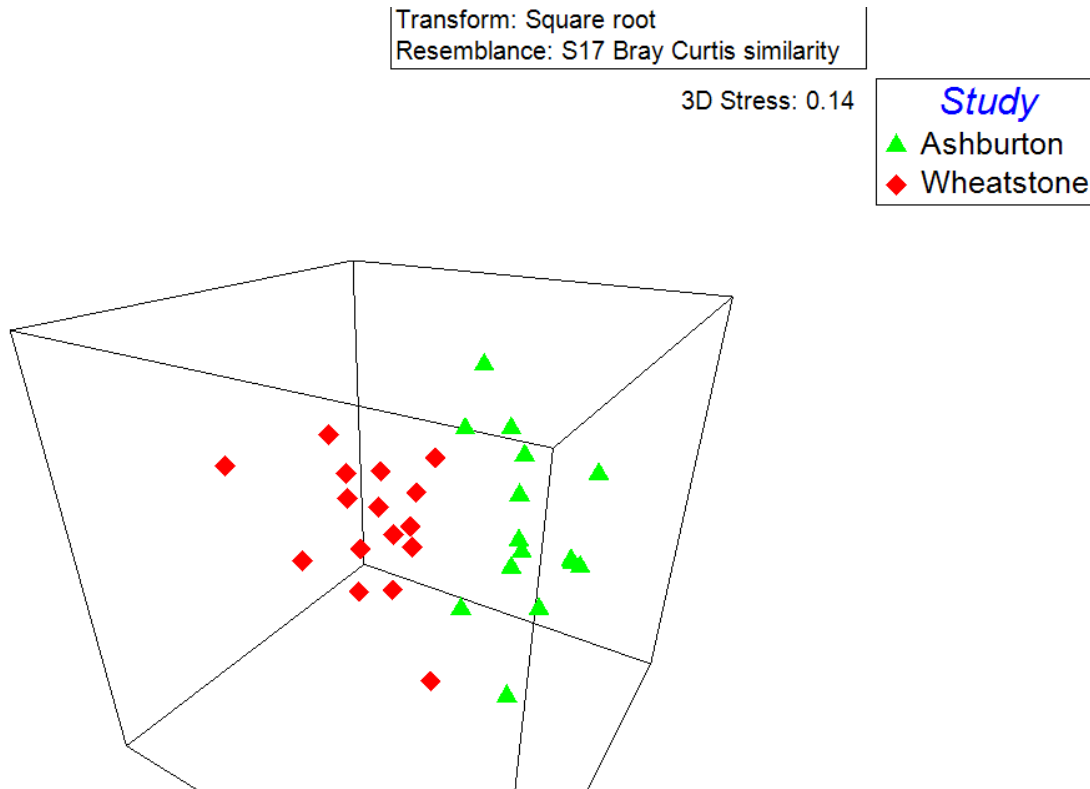


Figure 6.5: nMDS plot of avifauna assemblage by study. (Individual points represent one sampling site).

Although the relationship was weak, two-way crossed ANOSIM results showed that fauna landscape is a greater determinate of species assemblage than landform (landscape Global R =0.248, $p < 0.05$; landform Global R=0.089, $p > 0.5$). Significant difference in avifauna assemblage were recorded at Ashburton compared with those of Wheatstone. Furthermore, individual surveys have a greater influence on species assemblage than fauna landscape with significant difference in avifauna assemblage recorded at Ashburton compared with those of Wheatstone (study Global R = 0.655, $p < 0.05$; landscape Global R=0.225; $p > 0.05$).

6.2.3 Ground Mammals

Ground mammal species richness documented during the survey was above average compared to those documented from previous surveys in the locality (Table 6.4).

Table 6.4: Ground mammals species richness by study.

Study	Mammal Species Richness
Ashburton (this study)	13
Wheatstone	11
Onslow Solar Salt	8
Straits Salt	10
Average Previous studies	9.7

SIMPROF tests for mammal assemblages documented by comparable studies in the locality showed no evidence of significant group structure ($P_i = 1.009$; $p > 0.05$). That is, no significant differences in species assemblages were noted between any of the studies (Actual P_i = simulated P_i). This is also evident in the lack of discrete clusters in the nMDS plot shown in Figure 6.6.

Two-way crossed ANOSIM results showed that both fauna landscape and landform are poor determinates of species assemblage (landscape Global R =0.118, $p < 0.05$; landform Global R=-0.013, $p > 0.5$). There is a significant influence of individual surveys (across landscape groups) on species assemblage, but the amount of assemblage variability explained by this factor is low (Global R = 0.199, $p < 0.05$).

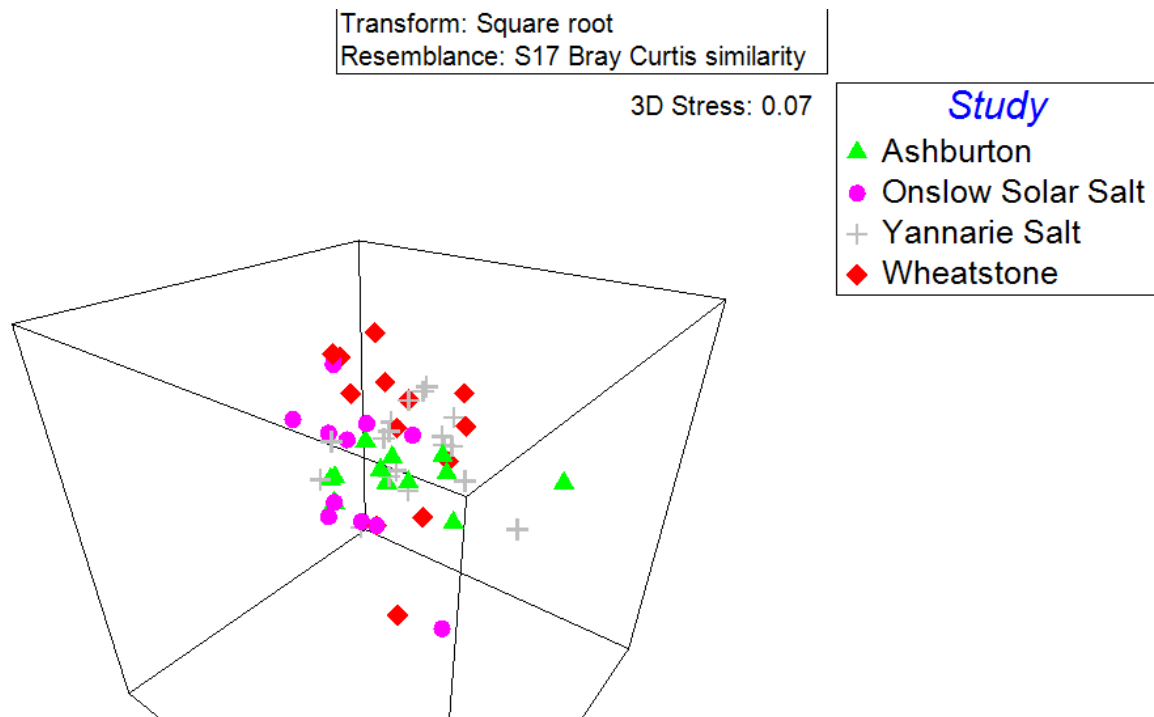


Figure 6.6: nMDS plot of ground mammal assemblage by study.
(Individual points represent one sampling site).

6.3 Interpretation of Analyses

6.3.1 Effectiveness of Sampling

Not all of the species potentially occurring within the study area (as compiled in the desktop review) were recorded. This is expected, as the study area contains only a subset of the habitats previously sampled in the locality. In addition, any given survey offers only a snapshot of an otherwise dynamic assemblage, which is influenced by many ecological and stochastic variables.

Despite this, randomised curves for each of the three taxonomic groups approach asymptote, indicating that the vertebrate assemblages were effectively documented during the survey period. This was supported by the non-parametric estimates, which indicated that on average 84% of the total fauna assemblage is likely to have been documented via the methods used during the survey period.

6.3.2 Fauna Assemblage Context

Although discernible separation of clusters for herpetofauna were observed, pairwise tests indicate that the Ashburton herpetofauna assemblage is not unique as it is not significantly different to that recorded at Wheatstone. Conversely, the avifauna assemblage recorded at Ashburton is significantly different to that recorded at Wheatstone (the only other comparable study in the locality). As both survey areas are adjacent habitats are analogous, the observed assemblage difference is likely to be a result of weather or other stochastic variables, rather than a true indication of the uniqueness of the assemblage. Regarding ground mammals, there was no significant assemblage difference between studies nor was there a large influence of fauna landscape or landform. This indicates that mammal assemblages are consistent with other studies previously conducted in the locality. Although fauna landscape was a factor for herpetofauna and avifauna species assemblage, other factors clearly had an influence also. Likely factors include:

- survey timing;
- climate preceding each survey;
- number of survey phases;
- size of each study area; and
- sampling effort.

This page intentionally left blank.

7.0 Conservation Significance

7.1 Vertebrate Fauna of Conservation Significance

7.1.1 Conservation Significant Vertebrate Fauna Recorded from the Study Area

The following 13 species of conservation significance were recorded from the study area during the current survey:

- Fork-tailed Swift, *Apus pacificus* (Migratory);
- Eastern Osprey, *Pandion cristatus* (Migratory);
- Common Sandpiper, *Actitis hypoleucos* (Migratory);
- Common Greenshank, *Tringa nebularia* (Migratory);
- Red-necked Stint, *Calidris ruficollis* (Migratory);
- Common Tern, *Sterna hirundo* (Migratory);
- Little Tern, *Sternula albifrons* (Migratory);
- Gull-billed Tern, *Gelochelidon nilotica* (Migratory);
- Caspian Tern, *Hydroprogne caspia* (Migratory);
- White-winged Black Tern, *Chlidonias leucopterus* (Migratory);
- Crested Tern, *Thalasseus bergii* (Migratory);
- Peregrine Falcon, *Falco peregrinus* (Other Specially Protected Fauna);
- Northern Coastal Free-tailed Bat, *Ozimops cobourgianus* (Priority 1).

Additionally, the Fairy Tern, *Sternula nereis* (Migratory) has previously been recorded within the study area, but not during the current survey.

The locations of the above records are provided in Appendix 6 and illustrated in Figure 7.1. Detailed descriptions of the species are provided below.

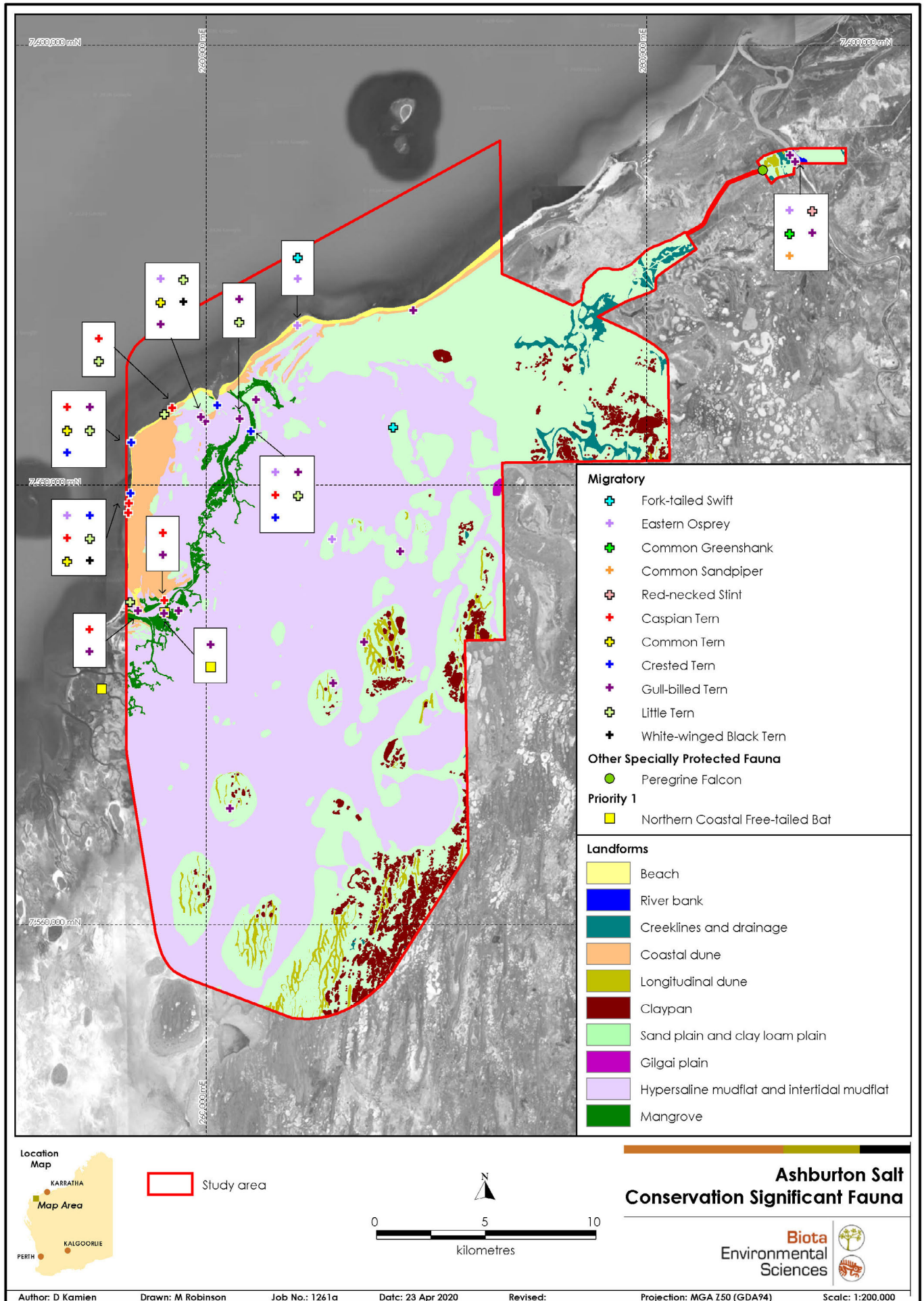


Figure 7.1: Conservation significant vertebrate species recorded from the study area.

7.1.2 Conservation Significant Vertebrate Fauna Occurring in the Study Area

Fork-tailed Swift, *Apus pacificus*

Migratory under the *WA Biodiversity Conservation Act 2016* and the *Commonwealth EPBC Act 1999*.

Distribution: The Fork-tailed Swift occurs across much of the Australian continent from September to April, particularly in the northern half of the continent. In general, the species is most common closer to the coast, but occurs over much of the Pilbara.

Ecology: The species is a non-breeding migrant to Australia and is generally present from September to April. In Australia, the species is entirely aerial in habits, foraging for flying insects and even sleeping on the wing. The species is highly mobile, often occurring in association with unsettled weather and low pressure systems (Johnstone and Storr 1998).

Occurrence: The species was recorded opportunistically at two locations within the study area in clay loam plain, and beach habitat (typically within mainland remnant and coast landscapes) (Figure 7.1 and Appendix 6).

Eastern Osprey, *Pandion cristatus*

Migratory under the *WA Biodiversity Conservation Act 2016* and the *Commonwealth EPBC Act 1999*.

Distribution: The Eastern Osprey occurs on the coast islands over much of Australia. It occasionally ranges inland along rivers, though mainly in the north of the country.

Ecology: The species feeds mainly on fish and breeds mainly on islands (Johnstone and Storr 1998).

Occurrence: Recorded at sites ASH03, ASH13E and opportunistically at five locations within the study area on clay loam plains, beach, mangrove and mudflat habitat (Figure 7.1 and Appendix 6).

Common Sandpiper, *Actitis hypoleucos*

Migratory under the *WA Biodiversity Conservation Act 2016* and the *Commonwealth EPBC Act 1999*.

Distribution: The Common Sandpiper occurs on all Australian coasts, many islands and much of the interior (Johnstone and Storr 1998).

Ecology: Preferred habitat includes edge of sheltered waters such as mudflats, estuaries, mangroves, river pools and claypans.

Occurrence: Three records were noted on the Ashburton River at site ASH13E (Figure 7.1 and Appendix 6).

Common Greenshank, *Tringa nebularia*

Migratory under the *WA Biodiversity Conservation Act 2016* and the *Commonwealth EPBC Act 1999*.

Distribution: The Common Greenshank occurs on all Australian coasts, being a casual or vagrant on many islands and much of the interior (Johnstone and Storr 1998).

Ecology: Preferred habitat includes shallow freshwaters and salt waters such as mudflats, estuaries, mangroves, lakes and samphire flats.

Occurrence: Seven records were noted on the Ashburton River at site ASH13E (Figure 7.1 and Appendix 6).

Red-necked Stint, *Calidris ruficollis*

Migratory under the *WA Biodiversity Conservation Act 2016* and the *Commonwealth EPBC Act 1999*.

Distribution: Occurs on most coasts and many islands in Australia (Johnstone and Storr 1998).

Ecology: Preferred habitat includes edges of sheltered salt, brackish or fresh waters, predominantly estuaries, beaches and salt lakes (Johnstone and Storr 1998).

Occurrence: Six records were noted on the Ashburton River at site ASH13E (Figure 7.1 and Appendix 6).

Common Tern, *Sterna hirundo*

Migratory under the WA *Biodiversity Conservation Act 2016* and the Commonwealth *EPBC Act 1999*.

Distribution: In Western Australia, this species occurs coastally north of Carnarvon (Johnstone and Storr 1998).

Ecology: Inhabits sheltered seas, including estuaries (Johnstone and Storr 1998).

Occurrence: A total of five individuals were recorded at three locations within the study area, on intertidal mudflat and beach habitat (Figure 7.1 and Appendix 6).

Little Tern, *Sternula albigifrons*

Migratory under the WA *Biodiversity Conservation Act 2016* and the Commonwealth *EPBC Act 1999*.

Distribution: In Western Australia, the Little Tern occurs in coastal regions north of Shark Bay (Simpson and Day 2004).

Ecology: Occurs mainly in sheltered seas, estuaries and mangrove creeks (Johnstone and Storr 1998).

Occurrence: A total of 124 individuals were recorded at 12 locations within the study area, on mudflat, mangrove and beach habitat (Figure 7.1 and Appendix 6).

Gull-billed Tern, *Gelochelidon nilotica*

Migratory under the WA *Biodiversity Conservation Act 2016* and the Commonwealth *EPBC Act 1999*.

Distribution: Occurs coastally in Western Australia, but extends inland where well watered flatlands occur (e.g. Murchison and Gascoyne rivers).

Ecology: Habitat includes shallow sheltered seas, close to land (in the north), estuaries, tidal creeks, claypans and watercourse (Johnstone and Storr 1998).

Occurrence: A total of 93 observations were recorded at 21 locations within the study area on mudflat, mangrove, beach and clay loam plain habitat within the study area (Figure 7.1 and Appendix 6).

Caspian Tern, *Hydroprogne caspia*

Migratory under the WA *Biodiversity Conservation Act 2016* and the Commonwealth *EPBC Act 1999*.

Distribution: Occurs on most coasts and many islands Australia wide (Johnstone and Storr 1998).

Ecology: Habitat includes shallow sheltered seas, estuaries, tidal creeks (Johnstone and Storr 1998).

Occurrence: There were a total of 24 observations recorded across 13 locations within the study area, on mudflat and beach habitat (Figure 7.1 and Appendix 6).

White-winged Black Tern, *Chlidonias leucopterus*

Migratory under the WA *Biodiversity Conservation Act 2016* and the Commonwealth *EPBC Act 1999*.

Distribution: In Western Australia, this species occurs predominantly on the northern and western coasts and coastal plains south of Busselton (Johnstone and Storr 1998).

Ecology: Habitat in the north includes shallow sheltered seas and estuaries. In the south this species typically inhabits fresh water lakes and swamps (Johnstone and Storr 1998).

Occurrence: A total of 19 observations were recorded at two locations within the study area, on mudflat and beach habitat (Figure 7.1 and Appendix 6).

Fairy Tern, *Sternula nereis*

Migratory under the WA Biodiversity Conservation Act 2016 and the Commonwealth EPBC Act 1999.

Distribution: Occurs on coasts and estuaries south of Port Hedland (Simpson and Day 2004).

Ecology: Breeds on sandy beaches and sand spits (Simpson and Day 2004).

Occurrence: Not recorded during the recent survey, but recorded within the study area in 2018 on hypersaline mudflats (NatureMap record) (Figure 7.1 and Appendix 6).

Crested Tern, *Thalasseus bergii*

Migratory under the WA Biodiversity Conservation Act 2016 and the Commonwealth EPBC Act 1999.

Distribution: Occurs on most coasts and many islands, Australia wide (Johnstone and Storr 1998).

Ecology: Inhabits mainly blue water seas in addition to estuaries and tidal creeks (Johnstone and Storr 1998).

Occurrence: A total of 10 individuals were recorded at five locations within the study area, on mudflat and beach habitat (Figure 7.1 and Appendix 6).

Peregrine Falcon, *Falco peregrinus*

Other Specially Protected Fauna under the WA Biodiversity Conservation Act 2016

Distribution: The Peregrine Falcon has an almost cosmopolitan distribution, but is absent from most deserts and the Nullarbor Plain (Johnstone and Storr 1998).

Ecology: This species inhabits a wide range of habitats including forest, woodlands, wetlands and open country (Pizzey and Knight 2007). Individuals maintain a home range of up to 30 km², and nest in recesses of cliff faces, tree hollows and along rivers (Johnstone and Storr 1998). However, the Peregrine Falcon, like other birds of prey, is a relatively long-lived species, with low reproductive rates and low population density. These factors, combined with the fact that it is a top-end predator and limited by prey availability, make it particularly vulnerable to human impact.

Occurrence: One individual was recorded opportunistically during the survey over clay loam plain habitat.

Northern Coastal Free-tailed Bat, *Ozimops cobourgianus*

DBCA listed Priority 4 species

Distribution: Endemic to Australia with distribution in Western Australian coastal areas from Exmouth Gulf to Broome (Churchill 2008).

Ecology: This species is a mangrove specialist, restricted to mangrove forests, adjacent areas of monsoon forest along larger waterways (Churchill 2008).

Occurrence: Recorded both inside and outside of the study area in intertidal mangrove habitat.

7.1.3 Conservation Significant Vertebrate Fauna Potentially Occurring in the Study Area

Twenty-one conservation significant species (excluding migratory shorebirds) were not recorded inside the study area, but based on the desktop review, have some likelihood of occurrence (see Section 4.2.1). The likelihood of occurrence of each species was further determined giving consideration to known distributions, availability of preferred habitat within the study area and last known records.

Fourteen species were considered unlikely to occur, or would not occur (Appendix 7). Detailed descriptions of the seven species that are likely to occur or may potentially occur are provided below and summarised in (Table 7.1).

Pilbara Olive Python, *Liasis olivaceus barroni*

Vulnerable under the WA *Biodiversity Conservation Act 2016* and Vulnerable under the Commonwealth *EPBC Act 1999*.

Distribution: The Pilbara Olive Python has a known distribution that coincides roughly with the Pilbara bioregion (DSEWPaC 2012). Resident populations occur in four broad areas: Pannawonica, Millstream, Tom Price and the Burrup Peninsula. At some of these sites, the species is considered stable and occurs in sizeable numbers (DotE 2018).

Ecology: Preferred habitat for the Pilbara Olive Python includes gorges, escarpments, rocky outcrops and water holes where it may find suitable prey (DotE 2018). It seeks shelter in caves, beneath boulders, in pools of water and occasionally in trees overhanging water (Bush and Maryan 2011). It is often associated with ephemeral or permanent water, but may also be recorded in rocky habitats some distance from these features (Biota 2009b), demonstrating that the species can have a large home range (estimated between 88 ha and 449 ha) (DotE 2018).

Likelihood of occurrence: Not previously recorded in the study area, but was recorded 2 km south of the study area, adjacent to Ashburton River. It is likely to occur where the study area intersects the Ashburton River.

Pomarine Jaeger, *Stercorarius pomarinus*

Migratory under the WA *Biodiversity Conservation Act 2016* and the Commonwealth *EPBC Act 1999*.

Distribution: An oceanic species, typically seen in the northern coast of Western Australia and in the southwest of the state (Simpson and Day 2004).

Ecology: The Pomarine Jaeger breeds in the Arctic and is an uncommon visitor to Australian inshore seas and bays. It feeds on fish, carrion, smaller birds up to the size of common gull and rodents (Johnstone and Storr 1998).

Likelihood of occurrence: Recorded approximately 22 km southwest of the study area on a beach. This species may potentially occur in the study area on occasion.

Roseate Tern, *Sterna dougallii*

Migratory under the WA *Biodiversity Conservation Act 2016* and the Commonwealth *EPBC Act 1999*.

Distribution: In Western Australia, this species occurs in waters, islands and coasts north of Bunbury (Simpson and Day 2004).

Ecology: Breeds in colonies on coasts and islands and feeds on surface fish.

Likelihood of occurrence: Recorded approximately 4 km northeast of the study area. It is likely to occur within the study area, but is unlikely to breed there.

Grey Falcon, *Falco hypoleucos*

Vulnerable under the WA *Biodiversity Conservation Act 2016*.

Distribution: The Grey Falcon occurs in small numbers across much of arid inland Australia, including the Pilbara.

Ecology: The Grey Falcon occurs mainly on lightly wooded plains and along major watercourses in arid Australia (Johnstone et al. 2013), and breeds in taller trees such as river red gums, or on isolated man-made structures such as communications towers. It is an active hunter, feeding on birds, reptiles and occasionally large insects.

Likelihood of occurrence: Recorded in 2000, approximately 80 km southwest of the study area in riparian vegetation. This species may potentially occur on occasion, particularly where the Ashburton River intersects the study area.

Barn Swallow, *Hirundo rustica*

Migratory under the WA *Biodiversity Conservation Act 2016* and the Commonwealth *EPBC Act 1999*.

Distribution: Occurs in northern Australia, near the coast.

Ecology: An aerial insectivore that typically inhabits open country with low vegetation, such as pasture, meadows and farmland, preferably with nearby water (Simpson and Day 2004).

Likelihood of occurrence: Nearest record located approximately 15 km east of the study area close to Onslow. Given the proximity of this record, this species may potentially occur in the study area.

Northern Quoll, *Dasyurus hallucatus*

Endangered under the WA *Biodiversity Conservation Act 2016* and Endangered under the Commonwealth *EPBC Act 1999*.

Distribution: The Northern Quoll formerly occurred across much of northern Australia but is now restricted to six main areas. Two of these areas are in Western Australia: the northwest Kimberley and Pilbara regions (Braithwaite and Griffiths 1994).

Ecology: The species is most abundant in open, rocky habitat and also commonly utilises gorges, breakaways and hills, particularly for denning purposes (van Dyck and Strahan 2008). It also occurs near creek lines and drainage lines, where adjacent plains and vegetated areas provide habitats for foraging and dispersal of young (van Dyck and Strahan 2008). Northern Quoll populations fluctuate on both annual and inter-annual cycles. This variability is driven by both the reproductive biology of individuals and longer-term cycles in response to regional stochastic processes such as rainfall, fire and related changes of prey populations (How et al. 2009).

Likelihood of occurrence: This species was recorded in 2012 and 2013 approximately 5 km southeast of the study area, 3 km east of the Ashburton River. As a result, it is likely to occur on occasion, particularly where the Ashburton River intersects the study area.

Short-tailed Mouse, *Leggadina lakedownensis*

DBCAs Priority 4 species

Distribution: In Western Australia, the distribution of this species includes the Pilbara and Kimberley bioregions (van Dyck and Strahan 2008).

Ecology: The Short-tailed Mouse occupies a diverse range of environments from monsoon tropical coast to semiarid climates, including stony spinifex and tussock grasslands, samphire and sedgeland (van Dyck and Strahan 2008). Records suggest that the primary mainland habitat comprises areas of native grassland, often associated with cracking clay and adjacent habitats, although this species has also been recorded from hilltops and *Cymbopogon* sp. grassland on sandy coastal areas near Onslow (NatureMap database).

Likelihood of occurrence: Although not recorded during the current survey, it was previously recorded 5 km southeast and northeast of the study area on repeated dunes and interdune plains landscape. Small patches of cracking clay were observed in the study area (eg. site ASH14E) which means this species is likely to occur within the study area.

This page intentionally left blank.

Table 7.1: Potentially occurring conservation significant species from the desktop review that were not recorded in the study area.

Species Name	Common Name	Conservation Status		Preferred Habitat	Habitat Available in Study Area	Occurrence in Locality	Likelihood of Occurrence in Study Area
		State	Commonwealth				
Herpetofauna							
<i>Liasis olivaceus barroni</i>	Pilbara Olive Python	Vulnerable	Vulnerable	Preferred habitat includes gorges, free faces, rocky outcrops and water holes where it may find suitable prey.	✓	Recorded 2 km south of the study area, adjacent to Ashburton River.	Likely to occur
Avifauna							
<i>Stercorarius pomarinus</i>	Pomarine Jaeger	Migratory	Migratory	Uncommon visitor to inshore seas. Breeds in Arctic.	x	Recorded approximately 22 km SW of study area in beach.	May potentially occur
<i>Sterna dougallii</i>	Roseate Tern	Migratory	Migratory	Occurs in waters, islands and coasts north of Bunbury.	✓	Recorded approximately 4 km NE of study area	Likely to occur
<i>Falco hypoleucos</i>	Grey Falcon	Vulnerable	–	Wide range of habitats in the arid zone but appears to be least rare in lightly wooded coastal and riverine plains.	✓	Nearest record located approximately 80 km SW of study area.	May potentially occur
<i>Hirundo rustica</i>	Barn Swallow	Migratory	Migratory	Open, low vegetation with nearby water.	✓	Nearest record located approximately 15km E of study area close to Onslow.	May potentially occur
Mammals							
<i>Dasyurus hallucatus</i>	Northern Quoll	Endangered	Endangered	Open rocky habitat, gorges, breakaways, free faces and rock piles particularly for denning purposes (van Dyck and Strahan 2008). Also occurs near creek lines and drainage lines, where adjacent plains and vegetated areas provide habitats for foraging and dispersal of young.	✓	Recorded 5km southeast of study area, approximately 3 km E of Ashburton River.	Likely to occur
<i>Leggadina lakedownensis</i>	Short-tailed Mouse	Priority 4	–	Primarily in areas of cracking clay (gilgai) and adjacent habitats.	✓	Recorded 5 km SE and northeast of study area.	Likely to occur

This page intentionally left blank.

7.1.4 Fauna Habitat Conservation Value

Based on examination of aerial imagery and land systems mapping, none of the fauna habitats identified during the fauna survey are confined to the study area, as they are common throughout the mainland adjoining Exmouth Gulf.

When assessing the value of habitat in the study area, it is prudent to consider the core habitat of individual species of conservation significance occurring or potentially occurring. Core habitat for species of conservation significance equates to "habitat critical to the survival of a species" (DotE 2013). Such habitats include those that are known, or are likely, to be utilised by listed species for key ecological activities such as denning, roosting, breeding, refugia and important foraging areas. As a result, it is assumed that some proportion of this habitat must be maintained across the species' range to ensure the persistence of the species in the region. Secondary habitats may be used for lesser foraging or on a transitory, dispersing, or occasional basis, but do not represent core habitat.

In short, when considering the faunal value of habitats within the study area, the following criteria can be used to assess areas of higher habitat value. These are habitats that:

- support fauna of conservation significance;
- support unique fauna assemblages; and/or
- are uncommon in the region.

Using these criteria, in combination with threatened species' habitat preferences, mangrove, river bank and beach habitats represent high value in the study area in that they represent core habitat for many migratory bird species. These habitats are not restricted to the study area and occur within the wider locality.

For the other conservation significant species recorded, likely to occur or potentially occurring within the study area, the Ashburton River represents potential core habitat (Table 7.2).

Table 7.2: Likely and occurring conservation significant species' habitat preference within the study area.

Species	Core Habitat	Secondary Habitat
Herpetofauna		
Pilbara Olive Python	River bank	River bank
Avifauna		
Pomarine Jaeger	Not present	Beach
Common Tern	Not present	Beach
Little Tern	Mangrove	Beach
Gull-billed Tern	Intertidal mudflats; River	Beach; Mangrove
Caspian Tern	Not present	Beach; Mangrove
White-winged Black Tern	Not present	Beach; Mangrove
Fairy Tern	Beach	–
Crested Tern	River bank	Beach
Bridled Tern	Not present	Beach
Roseate Tern	Not present	Beach
Eastern Osprey	Beach; Mangrove; Intertidal mudflats; River bank	–
Peregrine Falcon	River bank	–
Grey Falcon	River bank	–
Barn Swallow	River bank	–
Fork-tailed Swift	Not present	–
Mammals		
Northern Quoll	Not present	River bank
Northern Coastal Free-tailed Bat	Mangrove	–
Short-tailed Mouse	Gilgai plain	–

7.2 SRE Invertebrates of Conservation Significance

7.2.1 SRE Fauna Habitat

Typically, leaf litter accumulation, rock fractures, elevated topography and deep soil profiles offer conditions that are conducive to the occurrence of microhabitats important for SRE fauna. These features provide refugia for potential SRE invertebrate fauna (that are absent from the surrounding landscape). Deep soil profiles, such as on alluvial plains and floodplains, are the only microhabitats of relevance to SREs in the study area, and provide a suitable substrate for invertebrates such as mygalomorph spiders to burrow and thereby create their own microhabitats. Effectively, potential SRE invertebrates may occur throughout the study area with the exception of the hypersaline mudflats, intertidal mudflats, mangrove and beach habitats.

7.2.2 Potential SREs

Of the 12 invertebrate taxa collected during the survey, eight mygalomorph spider taxa from four families are considered to be potential SREs. Of these, five are known solely from the study area. Specifically, these nominal species comprise: *Idiommata* sp. B38; *Conothele* sp. C26; *Conothele* sp. C27; *Aname* sp. N142 and *Aname* sp. N146.

Although it is possible that these putative species exhibit highly localised distributions, they all occur on fauna habitats that are represented outside the study area. Additionally, *Conothele* sp. C26; *Conothele* sp. C27; *Aname* sp. N142 and *Aname* sp. N146 occur in locations where analogous landscapes extend contiguously beyond the study area. Given this, it is unlikely that these taxa are restricted to the study area.

Idiommata sp. B38 is known from a single mainland remnant in longitudinal dune habitat, at a location where analogous habitat does not extend contiguously beyond the study area. It may be argued that the isolated nature of this remnant may indicate that *Idiommata* sp. B38 is restricted at small scale. However, these remnants were contiguous with the mainland during the last glacial period commencing in the Pleistocene epoch, 110,000 years before present and continuing to the early Holocene epoch, 10,000 years before present (DEWHA 2008). The Holocene marked the onset of marine transgression resulting in formation of the mainland remnants less than 10,000 years before present (DEWHA 2008), a time period likely to be insufficient for speciation to occur.

That is, the mainland remnant from which *Idiommata* sp. B38 was recorded was recently part of the mainland habitat and as a result this nominal species is also likely to occur on the mainland and on other mainland remnants. Additionally, *Conothele* sp. C27; *Aname* sp. N142; and *Aname* sp. N146 occur both on mainland remnants and inland areas, which gives credibility to this hypothesis.

8.0 Glossary

Biota	Biota Environmental Sciences.
DBCAs	Department of Biodiversity, Conservation and Attractions.
DotE	The then Department of the Environment (now Agriculture Water and the Environment).
EIA	Environmental Impact Assessment.
EPA	Environmental Protection Authority of Western Australia.
EPBC Act	Commonwealth <i>Environment Protection and Biodiversity Conservation Act 1999</i> .
IBRA	Interim Biogeographic Regionalisation for Australia.
Landform	A geomorphological unit that is largely defined by its surface form and location in the study area.
Maximum spanning distance	The maximum linear distance between two records.
Minimum spanning area	The area of the smallest polygon that can be drawn around all location records for a taxon. Can be used as a means for objectively establishing SRE status by comparison against the 10,000 km ² criterion established by Harvey (2002).
MNES species	Species that are listed as Matters of National Environmental Significance under the EPBC Act.
SM2Bat and SM4Bat	SongMeter 2 or 4 acoustic bat call recorder.
sp. (plural: spp.)	Abbreviation of "species".
SRE	Short-range Endemic.
Study Area	Development envelope has been identified to include the solar salt evaporation and crystallisation ponds and associated infrastructure.
Systematic sampling	Sampling using trapping transects (including pitfall traps, Elliott traps or funnel traps) and avifauna censuses in defined habitats.
Taxon (plural: taxa)	A taxonomic entity, typically at species level or below.
WAM	Western Australian Museum.

This page intentionally left blank.

9.0 References

- Agriculture Western Australia (1967). Atlas of Australian Soils for Western Australia. CSIRO, Melbourne.
- Australasian Bat Society (2006). Recommendations of the Australasian Bat Society Inc for reporting standards for insectivorous bat surveys using bat detectors. *The Australian Bat Society Newsletter* 27:6–9.
- Beard, J. S. (1975). Vegetation Survey of Western Australia 1:1,000,000 Vegetation Series. Map Sheet 5 - Pilbara. University of Western Australia Press, Western Australia.
- Biota (2004). Onslow Solar Saltfield Terrestrial Fauna Survey. Unpublished report prepared for Onslow Salt Pty Ltd, Biota Environmental Sciences, Western Australia.
- Biota (2005a). Yannarie Salt Project Fauna Survey - Fauna and Fauna Assemblage Survey. Unpublished report prepared for Straits Salt Pty Ltd, September 2005, Biota Environmental Sciences, Western Australia.
- Biota (2005b). Yannarie Salt Project Mangrove and Coastal Ecosystem Study - Baseline Ecological Assessment. Unpublished report prepared for Straits Salt Pty Ltd, September 2005, Biota Environmental Sciences, Western Australia.
- Biota (2009a). West Pilbara Iron Ore Project Onslow Rail Corridor Terrestrial Fauna Survey. Unpublished report prepared for API Management, July 2009, Biota Environmental Sciences, Western Australia.
- Biota (2009b). A Two-Phase Fauna Survey of the West Turner Syncline Area. Unpublished report prepared for Rio Tinto Iron Ore, Biota Environmental Sciences, Western Australia.
- Biota (2010). Wheatstone Project Terrestrial Fauna Survey. Unpublished report prepared for URS Australia Pty Ltd and Chevron Australia Pty Ltd, January 2010, Biota Environmental Sciences, Western Australia.
- Biota (2012). A Molecular Survey of Mygalomorph Spiders from the Pilbara Bioregion, and Greater Western Australia. Unpublished internal report, Biota Environmental Sciences, Western Australia.
- Biota (2019a). Ashburton Salt Project Migratory Shorebird Assessment. Unpublished report prepared for K+S Salt Pty Ltd, Biota Environmental Sciences, Western Australia.
- Biota (2019b). Ashburton Salt Project Detailed Flora and Vegetation Survey: Phase 1 Interim Report. Biota Environmental Sciences.
- Blandford, D. C. (2012). Functional landform systems and the characterisation of fauna landscapes. An introductory training course prepared for Biota Environmental Sciences Pty Ltd, DC Blandford & Associates.
- Braithwaite, R. W., and A. D. Griffiths (1994). Demographic variation and range contraction in the northern quoll, *Dasyurus hallucatus* (Marsupialia: Dasyuridae). *Wildlife Research* 21(2):203–217.
- Bush, B., and B. Maryan (2011). *Field Guide to Snakes of the Pilbara, Western Australia*. Western Australia Museum.
- Christidis, L., and W. E. Boles (2008). *Systematics and Taxonomy of Australian Birds*. CSIRO Publishing, Melbourne.
- Churchill, S. K. (2008). *Australian Bats*, 2nd edition. Allen and Unwin, Australia.
- Clarke, K., and R. Gorley (2006). *Primer v6: User Manual/Tutorial*. PRIMER-E Ltd, Plymouth, UK.

- Department of the Environment and Energy (2019). Australia's bioregions (IBRA) [WWW Document]. Retrieved from <https://www.environment.gov.au/land/nrs/science/ibra>.
- DEWHA (2008). Sedimentology and geomorphology of the North-west Marine Region: A Spatial Analysis. Retrieved from <https://parksaustralia.gov.au/marine/management/resources/scientific-publications/sedimentology-and-geomorphology-north-west-marine-region/>.
- DEWHA (2010). Survey Guidelines for Australia's Threatened Birds. Department of Sustainability, Environment, Water, Population and Communities.
- DotE (2013). Matters of National Environmental Significance - Significant Impact Guidelines 1.1 *Environment Protection and Biodiversity Conservation Act 1999*. Department of the Environment, Canberra, Australia.
- DotE (2018). *Liasis olivaceus barroni* - Olive Python (Pilbara subspecies) SPRAT Profile [WWW Document]. Retrieved from http://www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=66699.
- Doughty, P., A. M. Bauer, M. Pepper, and J. S. Keogh (2018). Spots before the eyes: revision of the saxicoline geckos of the *Gehyra punctata* species complex in the Pilbara region of Western Australia. *Records of the Western Australian Museum* 33:1–50.
- DSEWPaC (2011). Survey Guidelines for Australia's Threatened Mammals. Department of Sustainability, Environment, Water, Population and Communities, Canberra. Retrieved from <http://www.environment.gov.au/epbc/publications/threatened-mammals.html>.
- DSEWPaC (2012). Interim Biogeographic Regionalisation for Australia (IBRA), Version 7 (Subregions) - States and Territories. Department of Sustainability, Environment, Water, Population and Communities, Canberra. Retrieved from <http://www.environment.gov.au/topics/land/national-reserve-system/science-maps-and-data/australias-bioregions-ibra>.
- EPA (2016a). *Technical Guidance: Sampling Methods for Terrestrial Vertebrate Fauna*. Environmental Protection Authority, Western Australia.
- EPA (2016b). *Technical Guidance: Terrestrial Fauna Surveys*. Environmental Protection Authority, Western Australia.
- EPA (2016c). *Technical Guidance: Sampling of Short Range Endemic Invertebrate Fauna*. Environmental Protection Authority, Western Australia.
- Geological Survey of Western Australia (1995). 1:100 000 geological map - Rocklea (2352). Government of Western Australia Department of Mines and Petroleum.
- Geoscience Australia (2008). Surface geology of Australia 1:1,000,000 scale, Western Australia [Digital Dataset]. Retrieved from <http://www.ga.gov.au>.
- Hamilton, Z. R. (2015). DNA barcoding reveals a contact zone in cryptic lineages of *Rhagada* land snails (Pulmonata: Camaenidae) in an ancient landscape. *in prep*.
- Hamilton, Z. R. (2018). Patterns and Processes of Evolution in *Rhagada* (Gastropoda: Camaenidae) Snails from the Pilbara Riong, Western Australia.
- Harvey, M. S. (2002). Short-Range Endemism Among the Australian Fauna: Some Examples from Non-Marine Environments. *Invertebrate Systematics* 16:555–570.
- How, R. A., P. B. Spencer, and L. H. Schmitt (2009). Island populations have high conservation value for northern Australia's top marsupial predator ahead of a threatening process. *Journal of Zoology* 278:206–217.

- Johnstone, R. E., A. H. Burbidge, and J. C. Darnell (2013). Birds of the Pilbara region, including seas and offshore islands, Western Australia: distribution, status and historical changes. *Records of the Western Australian Museum Supplement* 78:343–441.
- Johnstone, R. E., and G. M. Storr (1998). *Handbook of Western Australian Birds Volume I - Non-Passerines (Emu to Dollarbird)*. Western Australian Museum, Perth.
- Kealley, L., P. Doughty, M. Pepper, J. S. Keogh, M. Hillyer, and J. Huey (2018). Conspicuously concealed: revision of the arid clade of the *Gehyra variegata* (Gekkonidae) group in Western Australia using an integrative molecular and morphological approach, with the description of five cryptic species. *PeerJ* 6:e5334. doi: 10.7717/peerj.5334.
- McKenzie, N. L., and R. Bullen (2009). The echolocation calls, habitat relationships, foraging niches and communities of Pilbara microbats. *Records of the Western Australian Museum Supplement* 78:123–155.
- Menkhorst, P., and F. Knight (2011). *A Field Guide to the Mammals of Australia*, 3rd edition. Oxford University Press, Australia.
- Payne, A. L., P. J. Curry, and G. F. Spencer (1987). *Technical Bulletin No. 73: An inventory and condition survey of rangelands in the Carnarvon Basin, Western Australia*. Western Australian Department of Agriculture, South Perth WA.
- Pizzey, G., and F. Knight (2007). *The Field Guide to the Birds of Australia*. Page (P. Menkhorst, Ed.), 8th edition. Harper Collins Publishers, Sydney.
- Ponder, W. F., and D. J. Colgan (2002). What makes a narrow-range taxon? Insights from Australian freshwater snails. *Invertebrate Systematics* 16:571–582.
- Simpson, K., and N. Day (2004). *Field Guide to the Birds of Australia*, 7th edition. The Penguin Group, Victoria.
- Validus (2008). Chevron Domgas Project Onslow Fauna Assessment. Unpublished report prepared for Sinclair Knight Mertz, Validus Group.
- van Dyck, S., and R. Strahan (Eds.) (2008). *The Mammals of Australia*, 3rd edition. Reed New Holland, Sydney.
- Whisson, C. S. (2012). Land snails from the Mount Richardson area (Western Australia). Unpublished report WAMTS074 for Biota Environmental Sciences, 7 September 2012, Western Australian Museum.
- Wildlife Acoustics (2010). *Song Meter User Manual, Model SM2, with Song Meter SM2BAT 192kHz Stereo or 384kHz Mono Ultrasonic Recorders addendum*.

Appendix 3

DBCAs Regulation 17 Permit





Wildlife Conservation Act 1950
REGULATION 17

Regulation 17 – Licence to take fauna for scientific purposes (Regulation 17 - Standard)

The undermentioned person may take fauna for research or other scientific purposes and where authorised, keep it in captivity, subject to the following and attached conditions, which may be added to, suspended or otherwise varied as considered fit.

Director General

Conditions

- 1 The licensee must comply with the provisions of the Wildlife Conservation Act 1950, Wildlife Conservation Regulations 1970 and any Notices in force under this legislation.
- 2 The licensee shall take fauna only in the manner stated on the endorsed Regulation 17 licence application form and endorsed related correspondence.
- 3 Unless specifically authorised in the conditions of this Licence or otherwise in writing by the Director General, species of fauna declared as likely to become extinct, rare or otherwise in need of special protection shall not be taken.
- 4 Any by-catch of fauna, which is declared to be rare, likely to become extinct, or otherwise in need of special protection shall be released immediately at the point of capture. Where such fauna taken under this licence is injured or deceased, the licensee shall contact the Department's Wildlife Licensing Section for advice on disposal. Records must be kept of any such fauna so captured and details are to be included in the report required under further condition below.
- 5 Any interaction involving Gazetted Threatened Fauna that may be harmful to the fauna and/or invasive may require approval from the Commonwealth Department of the Environment ph 02 6274 1111. Interaction with such species is controlled by the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 and Environment Protection and Biodiversity Conservation Regulations 2000 as well as the Wildlife Conservation Act 1950 and Wildlife Conservation Regulations 1970.
- 6 No fauna shall be taken in areas where it would impinge on pre-existing scientific research programs.
- 7 Except in the case of approved lethal traps, the licensee shall ensure that measures are taken in the capture and handling of fauna to prevent injury or mortality resulting from that capture or handling. Where traps or other mechanical means or devices are used to capture fauna these shall be deployed so as to prevent exposure of trapped animals to ants and debilitating weather conditions and inspected at regular intervals throughout each day of their use. At the conclusion of research all markers used, and signs and structures erected by the licensee shall be removed and the environment returned to its original condition.
- 8 Not more than ten specimens of any one protected species of fauna shall be taken and removed from any location less than 20km apart. Where exceptional circumstances make it necessary to take a larger number of specimens from a particular location in order to obtain adequate statistical data, the collector must proceed with circumspection and justify their actions to the Director General in advance.
- 9 The licensee shall not release any fauna or their progeny in any area where it does not naturally occur, nor hand such fauna over to any other person or authority unless approved by the Director General, nor dispose of the remains of such fauna in any manner likely to confuse the natural or present day distribution of the species.
- 10 Bioprospecting involving the removal of sample aquatic and terrestrial organisms for chemical extraction and bioactivity screening shall not be conducted without specific written approval by the Director General.
- 11 No fauna is to be taken from any CALM land, as defined in the Conservation and Land Management Regulations 2002, without prior written approval of the Director General. No fauna is to be taken from any public land without the prior written approval of the Government Authority managing that land.
- 12 The licensee must not enter upon any private property or pastoral lease for the purposes of this licence, nor take any fauna from any private land or pastoral lease without the prior consent in writing of the owner or occupier. Similarly, in the case of Aboriginal lands, the licensee must not enter upon or take fauna from such lands without the written approval of the Department of Aboriginal Affairs and/or the relevant native title holders or applicants.
- 13 Copies of this licence and any written approval or consent required by conditions of this licence must be carried by the licensee and any person/s authorised under the licence at all times when conducting activities relevant to the licence

DEPARTMENT OF PARKS AND WILDLIFE



Department of
Parks and Wildlife



Enquiries: 17 DICK PERRY AVE, KENSINGTON, WESTERN AUSTRALIA
Telephone: 08 9219 9000
Facsimile: 08 9219 8242
Web Site: <https://wildlifelicensing.dpaw.wa.gov.au>
Correspondance: **Locked Bag 30**
Bentley Delivery Centre WA 6983

PAGE 2
NO. 08-002903-1

and must be presented to an authorised officer of the Department upon request.

- 14 All holotypes and syntypes and a half share of paratypes of species or subspecies permitted to be permanently taken under this licence shall be donated to the Western Australian Museum. Duplicates (one pair in each case) of any species collected, which represents a significant extension of geographic range shall upon request be donated to the Western Australian Museum.
- 15 To prevent any unnecessary collecting in this State, all specimens and material taken and retained under the authority of this license shall, upon request, be loaned to the Western Australian Museum. Any unused portion or portions of any specimen collected under the authority of this license shall be offered to the Western Australian Museum for inclusion in its collection or made available to other scientific workers if so required.
- 16 Within one month of the expiration of this licence, the holder shall submit an electronic return into the department's Wildlife Licensing System, detailing the locality, site, geocode, date and number of each species of fauna captured, sighted or vouchered during the currency of the licence. A copy of any paper, report or thesis resulting from the research shall upon completion be lodged with the Director General.

Purpose

Ashburton Salt Project terrestrial vertebrate fauna surveys using Elliott, dry pit and funnell traps, and by foraging and raking; and short range endemic (SRE) invertebrate fauna surveys using dry pit traps, and by foraging, raking, sieving and excavation. Captured vertebrate fauna may have morphometric and physical condition details recorded prior to release at capture site. A subset of invertebrate fauna and non-conservation listed vertebrate fauna species may be retained for further study and voucher specimens.

Locations

K plus S Salt Australia Pty Ltd proposed Ashburton Salt Project area, approximately 40 km southwest of Onslow, Pilbara Region.

Authorised Person

Surname	Given name(s)
Humphreys	Garth
Ford	Stewart
Teale	Roy
Keirle	David
Greenham	Michael
King	Jacinta
Graff	John
Schmidt	Sylvie
Brooshooff	Penny
Keen	Joshua

DEPARTMENT OF PARKS AND WILDLIFE



Department of
Parks and Wildlife



Enquiries: 17 DICK PERRY AVE, KENSINGTON, WESTERN AUSTRALIA
Telephone: 08 9219 9000
Facsimile: 08 9219 8242
Web Site: <https://wildlifelicensing.dpaw.wa.gov.au>
Correspondance: **Locked Bag 30**
Bentley Delivery Centre WA 6983

PAGE 3
NO. 08-002903-1

Date of Issue 20/09/2018
Valid From 29/10/2018
Date of Expiry 30/06/2019

Licensee: Mr Daniel Kamien
Address Biota Environmental Sciences Pty Ltd
PO Box 155
Leederville WA 6903
Australia

Issued by a Wildlife Licensing Officer of the Department of Parks and Wildlife under delegation from the Minister for Environment pursuant to section 133(1) of the Conservation and Land Management Act 1984.

Appendix 4

Western Australian Museum Arachnid, Myriapod and Mollusc Database Search Results



SRE Group	Family	Species	NatureMap	EPBC Act	WAM	SRE Status	Distribution and Habitat in Search Locality	Land System	Suitable Habitat Available in Study Area?	Likelihood of Occurrence in Study Area
Mygalomorph spider	Barychelidae	<i>Idiommata</i> `MYG110`			<input type="checkbox"/>	Not an SRE	Recorded 70 km E of study area on sand plain	Uaroo	Yes	Unlikely to occur
	Nemesiidae	<i>Aname</i> `MYG034`			<input type="checkbox"/>	Not an SRE	Recorded to within 2 km of study area on sand plain and dune	Onslow Dune	Yes	Likely to occur
		<i>Aname</i> `MYG102`			<input type="checkbox"/>	Known SRE	Recorded to within 48 km of study area on sand plain and alluvial plain	Uaroo Cane	Yes	May potentially occur
		<i>Aname</i> `sp.`			<input type="checkbox"/>	Undetermined	Recorded within 20 E of study area on sand plain and alluvial plain	Onslow Cane	Yes	Likely to occur
		<i>Aname ellenae</i>			<input type="checkbox"/>	Not an SRE	Recorded within 2 km NE of study area on sand plain	Onslow	Yes	Likely to occur
		<i>Aname mainae</i>			<input type="checkbox"/>	Not an SRE	Recorded within the study area on vegetated patches adjacent to coastal mudflats	Litoral	Yes	Recorded
		<i>Kwonkan</i> `MYG090`			<input type="checkbox"/>	Known SRE	Recorded approximately 43 km E of study area on sand plain	Uaroo	Yes	May potentially occur
Land Snail	Camaenidae	<i>Rhagada convicta</i>			<input type="checkbox"/>	Not an SRE	Recorded within the study area on dunes. Also recorded on off-shore islands and adjacent to coastal mudflats	Dune Littoral	Yes	Recorded
		<i>Rhagada tescorum</i>			<input type="checkbox"/>	Not an SRE	Recorded 23 km NE of study area on sand plain. Also recorded on off-shore islands	Dune	Yes	May potentially occur
	Pupillidae	<i>Gastrocopta mussoni</i>			<input type="checkbox"/>	Not an SRE	Recorded 73 km E of study area on sand plain and also recorded on sand plain adjacent to granite hills	Uaroo Boolaloo	Yes	Unlikely to occur
		<i>Gastrocopta larapinta</i>			<input type="checkbox"/>	Not an SRE	Recorded 73 km E of study area on sand plain	Uaroo	Yes	Unlikely to occur
		<i>Pupoides beltianus</i>			<input type="checkbox"/>	Not an SRE	Recorded to within 18 km SE of study area on flood plain. Also recorded on sand plain	Uaroo Nanyarra	Yes	Likely to occur
		<i>Pupoides cf. eremicolus</i>			<input type="checkbox"/>	Not an SRE	Recorded to within 8 km NE of study area on sand plain	Onslow	Yes	Likely to occur
		<i>Pupoides contrarius</i>			<input type="checkbox"/>	Not an SRE	Recorded to within 19 km NE of study area on dune	Dune	Yes	Likely to occur
		<i>Pupoides lepidulus</i>			<input type="checkbox"/>	Not an SRE	Recorded 40 km NW of study area on sandy substrate of off-shore islands	-	Yes	Unlikely to occur
		<i>Pupoides</i> sp.			<input type="checkbox"/>	Not an SRE	Recorded within 20 km NE of study area on vegetated islands adjacent to coastal mudflats	Littoral	Yes	Likely to occur
	Succineidae	<i>Succinea</i> sp.			<input type="checkbox"/>	Not an SRE	Recorded 70 km E of study area on sand plains	Uaroo	Yes	Unlikely to occur

Appendix 5

Invertebrate Species Molecular Report





Helix

Molecular Solutions

School of Animal Biology The University of Western Australia
Hackett Entrance No. 4 Hackett Drive Crawley WA 6009

PO Box 155
Leederville WA 6903

t: [08] 6488 4509

f: [08] 6488 1029

abn: 32 133 230 243

w: www.helixsolutions.com.au

7th March 2019

Dan Kamien
Biota Environmental Sciences
Level 1 / 228 Carr Place
Leederville WA 6007

Via email

Re. Report on the molecular systematics of the K plus S Salt mygalomorph specimens from Ashburton.

Dear Dan,

Following is a summary of the results of the invertebrate molecular investigation we have completed for the Ashburton Salt Fauna survey. Results suggest that amongst the twenty-five successfully sequenced mygalomorph spider specimens, three belonged to two previously unrecorded species of *Conothele* (family Ctenizidae), one belongs to a distinct previously recorded species of *Euoplos* (family Idiopidae), four belonged to two previously unrecorded species of *Aname* (family Nemesiidae), there were also seventeen *Aname* specimens belonging to three previously recorded species. We were unable to obtain a good quality sequence from seven specimens, and therefore the placement of these suspected nemesiid specimens remains unresolved.

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

Sincerely,

Dr. Zoë Hamilton, Dr. Terrie Finston and Yvette Hitchen
Helix Molecular Solutions



Background and Objective

The infraorder of Arachnida, Mygalomorphae, includes trapdoor spiders and their kin, and they are frequently identified as short-range endemics (SREs) (e.g. Harvey *et al.*, 2011; Castalanelli *et al.*, 2014). Identification of species has traditionally been performed using morphological techniques, however, only males can be used in identification, as both females and juveniles lack the diagnostic characters used in identification, and furthermore there is a large backlog of undescribed taxa. DNA barcoding with the use of *COI* mtDNA has become a rapid, objective method aiding mygalomorph species identifications and their distributions, and is recognised as providing important information that regulatory authorities can use to assess environmental impacts of large-scale developments (Harvey *et al.*, 2008; Environmental Protection Authority, 2009; Castalanelli *et al.*, 2014). Extensive molecular work has been conducted on the trap-door spider fauna of Western Australia (Helix, 2009a & b, 2010, 2011a - l, 2012a - i, 2013a & b, 2014a - d, 2015a - e). The resulting dataset provides a molecular framework that can be used to provide regional context for localised sampling.

Thirty-two specimens of invertebrate fauna belonging to three families of mygalomorph spiders (Araneae: Mygalomorphae: Ctenizidae, Idiopidae & Nemesiidae) from the Ashburton Salt survey area that occurs predominantly in the Canarvon bioregion (CAR). The Canarvon bioregion contains two biological subregions, with the study area falling within the Cape Range subregion (CAR1). The thirty-two mygalomorph specimens were sequenced for variation at the mitochondrial cytochrome oxidase subunit I gene (*COI*). The twenty-five successful resulting molecular sequences were then assessed to determine the number of taxa present and compare these results to those sequences publically available on GenBank and those already in Helix's database for context.

Executive summary

- Thirty-two specimens of mygalomorph spiders belonging from the survey area were sequenced and assessed for variation at the *COI* mtDNA gene. The molecular data were then placed within an existing molecular taxonomic framework for each family, using *COI* mtDNA sequences from GenBank as well as all mygalomorph *COI* sequences in the Helix database.
- Three families (Ctenizidae, Idiopidae & Nemesiidae) were amongst the twenty-five successfully sequenced specimens. Eighteen haplotypes were amongst the twenty-five successfully sequenced individuals, with five haplotype shared amongst twelve individuals.
- Analyses place the three Ctenizidae specimens within the *Conothele* genus. Both these specimens belong to previously unrecorded lineages and hence both are likely to represent new species, based on molecular data.
- The single idiopid specimen belonged to the genus *Euoplos*, with the specimen representing a previously recorded species, based on the molecular data.
- Analyses place the twenty-one nemesiid specimens within the *Aname* genus, with two new species represented by four specimens, and the remaining seventeen specimens belonging to three previously recorded species.

Methods

Thirty-two mygalomorph spider specimens from sixteen sampling locations (Table 1) were sequenced for variation at the cytochrome oxidase subunit I gene (*COI*) using primers LCOI & HCO2 (Folmer *et al.*, 1994). Seven of these sequences were unable to be analysed, due to the sequence quality. The resulting twenty-five mygalomorph sequences comprised eighteen haplotypes (Table 2).

The sequences from the nine successfully sequenced individuals were edited using SEQUENCHER software (Gene Codes Corporation, Ann Arbor, MI, USA). Alignment was performed with CLUSTAL W (Thompson *et al.*, 1994) using default parameters. DNA nucleotide sequences were translated into protein sequences to ensure that the amplified sequences corresponded to the target mtDNA. The translated protein sequences were then checked for the presence of stop codons. All sequences were 'BLAST'ed (Basic Local Alignment Search Tool) with the NCBI (National Centre for Biotechnology Information). This program compares DNA nucleotide sequences with a library of sequences and identifies sequences

within the database that resemble the query sequences above a certain threshold. Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotides different between sequences). To account for polymorphism within lineages, the net genetic diversity of Nei (1987) was calculated to give a 'corrected' distance between lineages.

For phylogenetic analysis, likelihood ratio tests using the Bayesian Information Criterion were calculated in MEGA 6.06 (Tamura *et al.*, 2013) to determine the best-fit model of evolution. The phylogenetic analyses were calculated in MEGA 6.06 (Tamura *et al.*, 2013) using maximum likelihood (ML) with 1000 bootstrap replicates, based on the genetic distances with the best-fit model of evolution calculated for each family. For all families (Ctenizidae, Idiopidae & Nemesiidae), the best model of evolution was the General Time Reversible model with gamma distribution and invariant sites (GTR+G+I). For the Ctenizidae the parameter for the gamma distribution was 1.29. For the Nemesiidae the parameter for gamma distribution of 0.79, and for the Idiopidae it was 0.67)

The phylogenetic analysis performed separately for each mygalomorph family, and included the representative haplotypes for the twenty-five specimens from the survey area, as well as a total of one hundred and sixty-eight reference specimens (Ctenizidae n=37, Idiopidae n=8, Nemesiidae n=123) all within 15 % sequence divergence of the twenty-five specimens, obtained from both Helix's database and from GenBank (Appendix 1).

Results

Phylogenetic Analyses

A 676 base-pair (bp) fragment of *COI* was isolated for 24 of the 32 specimens. A 458 bp fragment was isolated for an additional individual. Because multiple specimens shared identical DNA sequences (haplotypes), the data set was reduced to include only unique haplotypes. Of the 25 specimens, thirteen had unique haplotypes.

Ctenizidae

The phylogenetic analysis for the three specimens, along with the 37 additional reference sequences (all included sequences showed ≤ 15 % sequence divergence) revealed the Ashburton Salt specimens to sit within the clade containing reference sequences belonging to the genus *Conothele* (see Figure 1). None of the ctenizid specimens showed a close affinity to any of the previously sequenced specimens from Helix's database, or from GenBank. Two specimens (OO04 & OO06) belong to a single *Conothele* species (CAN_C26), not previously sequenced, and the third specimen (OO05) to a second *Conothele* species also not previously recorded (CAO_C27). The C26 species was most closely related to the C27 species (10.2 % sequence divergence, Table 2). The closest relative of this C27 species, based on genetic distance, was a species in the Helix database CAM_C11_DW8 (9.9%, Table 2) recorded previously near Wittenoom.

Idiopidae

Phylogenetic analyses placed a single specimen within the genus *Euoplos*. This genus belongs to the subfamily Arbanitinae, and the tribe Euoplini (Rix *et al.*, 2017). It has recently been examined amongst the revision of the family with two described species in Western Australia. IN WA, there are three currently recognised species groups; *E. inornatus*, *E. mcmillani* and *E. hoggi*, all of which are currently being revised (Dr. Michael Rix pers. comm., 2019). Species of *Euoplos* from the Pilbara belong to the *E. hoggi*-group, although they are rare and a only a few species are thought to exist (Dr Michael Rix pers. comm, 2019). The single *Euoplos* Ashburton Salt specimen (OO02) showed affinity to specimens from both the Helix database and GenBank, with sequence divergences ranging from 4.6 % to 8.2 %. Two divergent clades with high bootstrap support are evident, indicating that these two clades have been separated for millions of years. Regardless of whether these two clades are considered separate species, the group undoubtedly requires further assessment. By applying the 9.5 % sequence divergence 'cut-off' that was tested by Castalanelli *et al.*, (2014), we believe this specimen belongs to a previously recorded but yet undescribed species (IBM_I67). If we consider the two divergent clades to be distinct evolutionary units at this stage, then the Ashburton specimen belongs to

the clade (Clade A in Figure 2) that has previously been recorded at south-west of Pannawonica, according to the Helix database and from three additional records according to the Genbank specimens. The second clade (Clade B, Figure 2) has been recorded from Barrow Island according to the Helix database and three more records according to the GenBank collections.

Nemesiidae

The family Nemesiidae is one of the most diverse and species-rich mygalomorph families in Australia, with 15 genera and 99 named species (Castalanelli *et al.*, 2014; 2017). Amongst the twenty-one Ashburton Salt nemesiid mygalomorph specimens, fourteen haplotypes exist, with five haplotypes shared amongst twelve individuals. Amongst these Ashburton Salt nemesiid specimens, two new species of *Aname* were recognised, namely *Aname* sp. NRT_N141 (n=1) and *Aname* sp. NRU_N142 (n=3). The seventeen remaining specimens were found to belong to previously recorded species including *Aname* sp. NCH_N82 (n=1), *Aname* sp. NAQ_N5 (n = 5), and *Aname* sp. NDW_N57 (n = 11). There are six records of the 'N5' species on Genbank. The Helix database records show that the 'N82' species has been recorded previously 80km E of Hyden, and the 'N5' species 15km S of Onslow. The close relationship with Onslow record is unsurprising given the geographical proximity. However the close affiliation with the specimen collected east of Hyden is an unexpected result considering the significant geographic distance between the specimens. This result could be explained by either a widespread distribution of the species or perhaps lab/field labelling mistakes.

Conclusions

The mtDNA gene cytochrome oxidase 1 (*COI*) is widely considered to show suitable variation to distinguish species (Hebert *et al.*, 2003a), and the use of this gene can be extremely effective for 'DNA barcoding' in taxa where clear differentiation exists between intra and interspecific levels of divergence (e.g. Hebert *et al.*, 2004a; 2004b). In a comparison of *COI* sequences for over 13,000 pairs of taxa, Hebert *et al* (2003b) found a mean of 11.1 % sequence divergence between distinct species. Nearly 80 % of these comparisons found that species pairs differed from one another by greater than 8 % sequence divergence. Despite its merits in barcoding however, a taxon by taxon approach, examining the amount of phylogenetic variation within and between taxa is the most widely accepted method of delineating species and their distributions, especially in areas where rapidly expanding mining operations outpace taxonomic treatment of unresolved taxa.

References

- Australian Faunal Directory.** Australian Government, Department of Sustainability, Environment, Water, Population and Communities
<http://www.environment.gov.au/biodiversity/abrs/online-resources/fauna/index.html>
- Brower AVZ. 1994.** Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences USA*. **91**: 6491-6495.
- Castalanelli MA, Teale RJ, Rix MG, Kennington JW, Harvey Ms. 2014.** Barcoding of mygalomorph spiders (Araneae: Mygalomorphae) in the Pilbara bioregion of Western Australia reveals a highly diverse biota. *Invertebrate Systematics* **28**: 375- 385. Doi: [10.1071/IS3058](https://doi.org/10.1071/IS3058)
- Castalanelli MA, Huey JA, Hillyer MJ, Harvey MS. 2017.** Molecular and morphological evidence for a new genus of small trapdoor spiders from arid Western Australia (Araneae: Mygalomorphae: Nemesiidae: Anaminae). *Invertebrate Systematics* **31**: 492-505.
- Environmental Protection Authority 2009.** Sampling of short range endemic invertebrate fauna for environmental impact assessment in Western Australia. In 'Guidance for the Assessment of Environmental Factors (in accordance with the Environmental Protection Act 1986). Vol. No. 20'. pp. 1-31.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994.** DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294-299.
- Harvey MS, Berry O, Edward KL, Humphreys G. 2008.** Molecular and morphological systematics of hypogean schizomids (Schizomida: Hubbardiidae) in semiarid Australia. *Invertebrate Systematics* **22**: 167-194.
- Harvey MS, Rix MG, Framenau VW, Hamilton ZR, Johnson MS, Teale RJ, Humphreys G, Humphreys WF. 2011.** Protecting the innocent: studying short-range endemic taxa enhances conservation outcomes. *Invertebrate Systematics* **25**: 1-10.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003a.** Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* **270**: 313-321.
- Hebert PDN, Ratnasingham S, deWaard JR. 2003b.** Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B (supplement)* **270**, S96-S99.
- Hebert, P.D.N., Penton EH, Burns JM, Janzen DH, Hallwachs W. 2004a.** Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *PNAS* **101**: 14812-14817.
- Hebert PDN, Stoeckle MY, Zemiak TS, Francis CM 2004b.** Identification of birds through DNA barcodes. *PLoS Biology* **2**: 1657-1663.
- Helix 2009a.** Report on the molecular systematics of *Aganippe castellum*. Unpublished report for Biota Environmental Sciences.
- Helix 2009b.** Report on the molecular systematics of wishbone spider *Aname*. Unpublished report for BHP Billiton.
- Helix 2010.** Report on the molecular systematics of mygalomorph spiders from Forestania. Unpublished report for Biota Environmental Sciences.
- Helix 2011a.** Report on the molecular systematics of *Galeosoma* from Forestania. Unpublished report for Biota Environmental Sciences.
- Helix 2011b.** Report on the molecular systematics of Mygalomorphae from Forrestania. Unpublished report for Biota Environmental Sciences.
- Helix 2011c.** Report on the molecular systematics of Mygalomorphae from Deception. Unpublished report for Biota Environmental Sciences.
- Helix 2011d.** Report on the molecular systematics of Mygalomorphae from South Parmelia. Unpublished report for Biota Environmental Sciences.
- Helix 2011e.** Report on the molecular systematics of Mygalomorphae from Jinidi. Unpublished report for Biota Environmental Sciences.

- Helix 2011f.** Report on the molecular systematics of Mygalomorphae from Southern Flank. Unpublished report for Biota Environmental Sciences.
- Helix 2011g.** Report on the molecular systematics of Mygalomorphae from Area C to Yandi. Unpublished report for Biota Environmental Sciences.
- Helix 2011h.** Report on the molecular systematics of Mygalomorphae from Hammersley Irrigation. Unpublished report for Biota Environmental Sciences.
- Helix 2011i.** Report on the molecular systematics of Mygalomorphae from Mudlark. Unpublished report for Biota Environmental Sciences.
- Helix 2011j.** Report on the molecular systematics of Mygalomorphae from Marillana. Unpublished report for Biota Environmental Sciences.
- Helix 2011k.** Report on the molecular systematics of Mygalomorphae from Jinidi to Manline. Unpublished report for Biota Environmental Sciences.
- Helix 2011l.** Report on the molecular systematics of Mygalomorphae from Eastern Deviation. Unpublished report for Biota Environmental Sciences.
- Helix 2012a.** Report on the molecular systematics of Mygalomorphae from the Pilbara. Unpublished report for Biota Environmental Sciences, 31 January.
- Helix 2012b.** Report on the molecular systematics of Mygalomorphae from West Turner Syncline. Unpublished report for Biota Environmental Sciences.
- Helix 2012c.** Report on the molecular systematics of Mygalomorphae from Koodaideri West Corridor. Unpublished report for Biota Environmental Sciences.
- Helix 2012d.** Report on the molecular systematics of Mygalomorphae from Koodaideri phase IV and Koodaideri South. Unpublished report for Biota Environmental Sciences.
- Helix 2012e.** Report on the molecular systematics of Mygalomorphae from Cape Lambert. Unpublished report for Biota Environmental Sciences.
- Helix 2012f.** Report on the molecular systematics of Mygalomorphae from Marra Mamba. Unpublished report for Biota Environmental Sciences.
- Helix 2012g.** Report on the molecular systematics of Mygalomorphae from Mt Richardson. Unpublished report for Biota Environmental Sciences.
- Helix 2012h.** Report on the molecular systematics of Mygalomorphae from West Turner Extension. Unpublished report for Biota Environmental Sciences.
- Helix 2012i.** Report on the molecular systematics of Mygalomorphae from Southern Flank to Jinidi. Unpublished report for Biota Environmental Sciences.
- Helix 2013a.** Report on the molecular systematics of Mygalomorphae from Mt Richardson phase I and II. Unpublished report for Biota Environmental Sciences.
- Helix 2013b.** Report on the molecular systematics of mygalomorphae from Koodaideri rail corridor. Unpublished report for Biota Environmental Sciences.
- Helix 2014a.** Molecular systematics of mygalomorphae from Kundip. Unpublished report for Biota Environmental Sciences.
- Helix 2014b.** Molecular systematics of mygalomorphae from Koodaideri northern extension. Unpublished report for Biota Environmental Sciences.
- Helix 2014c.** Molecular Systematics of mygalomorphae from Yandi Billiards. Unpublished report for Biota Environmental Sciences.
- Helix 2014d.** Molecular systematics of mygalomorphae from Yandi Billiards phase 2. Unpublished report for Biota Environmental Sciences.
- Helix 2015a.** Report on the molecular systematics of mygalomorphae from Bungaroo Valley. Unpublished report for Biota Environmental Sciences.
- Helix 2015b.** Report on the molecular systematics of mygalomorphae from Baby Hope Downs. Unpublished report for Biota Environmental Sciences.

- Helix 2015c.** Molecular systematics of mygalomorphae from Yandi Oxbow. Unpublished report for Biota Environmental Sciences.
- Helix 2015d.** Molecular systematics of mygalomorphae from Red Hill. Unpublished report for Biota Environmental Sciences.
- Helix 2015e.** Molecular systematics of mygalomorphae from the Buckland Hills Level 2 fauna survey. Unpublished report for Biota Environmental Sciences.
- Nei M. 1987.** DNA polymorphism within and between populations. In: *Molecular Evolutionary Genetics*. pp.254-286. Columbia University Press New York, NY.
- Rix MG, Raven RJ, Main BY, Harrison SE, Austin AD, Cooper SJB, Harvey MS. 2017.** The Australian spiny trapdoor spiders of the family Idiopidae (Mygalomorphae: Arbanitinae): a relimitation and revision at the generic level. *Invertebrate Systematics* **31**: 566-634.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013.** MEGA 6: Molecular Evolutionary Genetic Analysis using Maximum Likelihood, evolutionary distance, and Maximum Parsimony methods. *Molecular Biology and Evolution* **30**: 2725 – 2729.
- Thompson J, Higgins D, Gibson T. 1994.** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680. doi:10.1093/nar/ 22.22.4673.

Table 1. Mygalomorph spider specimens used in the present study, and the genetic lineage to which they belong. Coloured shading refers to colour-coding used to highlight species in Figures 1 to 3. Unshaded cells represent samples that failed to sequence.

Biota Specimen ID	Helix Lab ID	Family	Genetic Lineage/ Taxonomic ID
M20181106.ASHSRE05-03	OO01	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181104.ASHSRE01-01	OO02	Idiopidae	Previously record species <i>Euoplos</i> sp. IBM_I67
M20181104.ASHSRE02-01	OO03	Nemesiidae	no data
M20181104.ASHSRE15-01	OO04	Ctenizidae	New species <i>Conothele</i> sp. CAN_C26
M20181106.ASHSRE09-01	OO05	Ctenizidae	New species <i>Conothele</i> sp. CAO_C27
M20181110.ASHSRE29-02	OO06	Ctenizidae	New species <i>Conothele</i> sp. CAN_C26
M20181103.ASHSRE12-01	OO07	Nemesiidae	New species <i>Aname</i> sp. NRT_N141
M20181104.ASHSRE14-01	OO08	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181104.ASHSRE14-02	OO09	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181105.ASHSRE05-01	OO10	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181105.ASHSRE05-02	OO11	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181105.ASHSRE16-01	OO12	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181105.ASHSRE16-02	OO13	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181106.ASHSRE01-01	OO14	Nemesiidae	Previously recorded species <i>Aname</i> sp. NAQ_N5/ <i>Aname ellenae</i>
M20181106.ASHSRE08-01	OO15	Nemesiidae	Previously recorded species <i>Aname</i> sp. NAQ_N5/ <i>Aname ellenae</i>
M20181106.ASHSRE08-02	OO16	Nemesiidae	Previously recorded species <i>Aname</i> sp. NAQ_N5/ <i>Aname ellenae</i>
M20181106.ASHSRE10-01	OO17	Nemesiidae	New species <i>Aname</i> sp. NRU_N142
M20181106.ASHSRE10-02	OO18	Nemesiidae	New species <i>Aname</i> sp. NRU_N142
M20181107.ASHSRE05-01	OO19	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181107.ASHSRE18-01	OO20	Nemesiidae	no data
M20181107.ASHSRE18-02	OO21	Nemesiidae	no data
M20181107.ASHSRE18-03	OO22	Nemesiidae	no data
M20181108.ASHSRE01-01	OO23	Nemesiidae	Previously recorded species <i>Aname</i> sp. NAQ_N5/ <i>Aname ellenae</i>
M20181108.ASHSRE21-01	OO24	Nemesiidae	New species <i>Aname</i> sp. NRU_N142
M20181109.ASHSRE08-01	OO25	Nemesiidae	Previously recorded species <i>Aname</i> sp. NAQ_N5/ <i>Aname ellenae</i>
M20181109.ASHSRE23-01	OO26	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181109.ASHSRE23-02	OO27	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181109.ASHSRE23-03	OO28	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20181110.ASHSRE29-01	OO29	Nemesiidae	no data
M20181111.ASHSRE31-01	OO30	Nemesiidae	Previously recorded species <i>Aname</i> sp. NCH_N82
M20181111.ASHSRE32-01	OO31	Nemesiidae	no data
M20181111.ASHSRE32-02	OO32	Nemesiidae	no data

Table 2. Genetic p-distance between the eighteen mygalomorph haplotypes belonging to the twenty-five individuals, from three families (Ctenizidae, Idiopidae & Nemesiidae) sequenced from the Ashburton Salt survey area as shown in Figure 1. Specimens identified with '*' are those haplotypes used in analyses. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

	OO01	OO02	OO04	OO05	OO06	OO07	OO08	OO09	OO10	OO11	OO12	OO13	OO14	OO15	OO16	OO17	OO18	OO19	OO23	OO24	OO25	OO26	OO27	OO28	
OO01																									
OO02	0.241																								
OO04	0.227	0.252																							
OO05	0.23	0.244	0.121																						
OO06	0.227	0.246	0.011	0.115																					
OO07	0.173	0.234	0.214	0.229	0.204																				
OO08	0.036	0.255	0.210	0.221	0.214	0.182																			
OO09*	0.036	0.255	0.210	0.221	0.214	0.182	0.000																		
OO10	0.000	0.241	0.227	0.23	0.227	0.173	0.036	0.036																	
OO11	0.036	0.256	0.214	0.225	0.218	0.178	0.006	0.006	0.036	0.036															
OO12	0.036	0.256	0.214	0.225	0.218	0.178	0.006	0.006	0.036	0.036	0.000														
OO13*	0.165	0.222	0.209	0.203	0.211	0.173	0.173	0.173	0.165	0.165	0.175	0.175													
OO14*	0.175	0.218	0.211	0.200	0.205	0.182	0.177	0.177	0.175	0.175	0.179	0.179	0.043												
OO15*	0.175	0.218	0.211	0.200	0.205	0.182	0.177	0.177	0.175	0.175	0.179	0.179	0.043	0.000											
OO16*	0.232	0.230	0.234	0.234	0.226	0.223	0.223	0.223	0.224	0.224	0.224	0.201	0.195	0.195											
OO17	0.232	0.230	0.234	0.232	0.234	0.228	0.223	0.223	0.232	0.232	0.224	0.224	0.201	0.195	0.002										
OO18	0.000	0.241	0.227	0.23	0.227	0.173	0.036	0.036	0.000	0.000	0.036	0.036	0.165	0.175	0.232	0.232									
OO19	0.000	0.241	0.227	0.23	0.227	0.173	0.036	0.036	0.000	0.000	0.036	0.036	0.165	0.175	0.232	0.232	0.201	0.165							
OO23	0.232	0.228	0.234	0.23	0.234	0.228	0.223	0.223	0.232	0.232	0.224	0.224	0.201	0.195	0.009	0.008	0.232	0.201							
OO24	0.169	0.218	0.211	0.207	0.205	0.178	0.167	0.167	0.169	0.169	0.169	0.169	0.044	0.011	0.011	0.009	0.008	0.232	0.201	0.046	0.203				
OO25*	0.044	0.244	0.210	0.214	0.180	0.026	0.026	0.026	0.044	0.044	0.030	0.030	0.157	0.165	0.223	0.223	0.044	0.157	0.223	0.159					
OO26*	0.042	0.249	0.214	0.223	0.219	0.182	0.028	0.028	0.042	0.042	0.028	0.028	0.157	0.165	0.225	0.225	0.042	0.157	0.225	0.159	0.005				
OO27	0.041	0.247	0.212	0.221	0.217	0.180	0.026	0.026	0.041	0.041	0.026	0.026	0.156	0.163	0.223	0.223	0.041	0.156	0.223	0.158	0.003	0.002			
OO28	0.041	0.247	0.212	0.221	0.217	0.180	0.026	0.026	0.041	0.041	0.026	0.026	0.156	0.163	0.223	0.223	0.041	0.156	0.223	0.158	0.003	0.002			

Table 3. Genetic p-distance (below) and the associated standard error (above – blue text) between all Ashburton Salt mygalomorph (Ctenizidae) haplotypes shown in Figure 1. Two *Conothele* haplotypes, and associated genetic distances, shaded in pink and yellow, as per colour-coding in Figure 1. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

	OO05	OO06	OO07	OO08	OO09	OO10	OO11	OO12	OO13	OO14	OO15	OO16	OO17	OO18	OO19	OO23	OO24	OO25	OO26	OO27	OO28				
OO05																									
OO06	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO07	0.12	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO08	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO09	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO10	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO11	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO12	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO13	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO14	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO15	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO16	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO17	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO18	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO19	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO23	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO24	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO25	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO26	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO27	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OO28	0.10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Table 4. Genetic p-distance (below) and the associated standard error (above – blue text) between all *Euoplos* mygalomorph (family Idiopidae) haplotypes shown in Figure 2. The single Ashburton Salt *Euoplos* specimen, and associated genetic distances, shaded in blue, as per colour-coding in Figure 2. Uncorrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

	OO02	NDZ_N60_AD 13	NEA_N61_AD 538	KJ745208.1 MYG307 T54391	KY295272.1 WAM T54389	KJ745207.1 MYG307 T54389	KJ744727.1 MYG218 T110212	KY295257.1 WAM T110196_	KJ744723.1 MYG218 T110204
OO02		0.011	0.008	0.008	0.008	0.008	0.010	0.011	0.011
NDZ_N60_AD13	0.082		0.012	0.011	0.012	0.012	0.004	0.000	0.000
NEA_N61_AD538	0.048	0.096		0.003	0.000	0.000	0.011	0.012	0.012
KJ745208.1 MYG307 T54391	0.046	0.091	0.005		0.003	0.003	0.011	0.011	0.011
KY295272.1 WAM T54389	0.048	0.096	0.000	0.005		0.000	0.011	0.012	0.012
KJ745207.1 MYG307 T54389	0.048	0.096	0.000	0.005	0.000		0.011	0.012	0.012
KJ744727.1 MYG218 T110212	0.074	0.011	0.094	0.090	0.094	0.094		0.004	0.004
KY295257.1 WAM T110196_	0.082	0.000	0.096	0.091	0.096	0.096	0.011		0.000
KJ744723.1 MYG218 T110204	0.082	0.000	0.096	0.091	0.096	0.096	0.011	0.000	

Table 5. Genetic p-distance (below) and the associated standard error (above – blue text) between all Ashburton Salt *Aname* mygalomorph (family Nemesiidae) haplotypes and all reference nemesiid specimens for the colour shaded species depicted in Figure 3. Ashburton Salt specimens, and associated genetic distances, shaded in colour, as per colour-coding in Figure 3. Ashburton Salt labels in bold text. Within species p-distances outlined in bold borders. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance. For all p-distances between Nemesiidae (n=123) mygalomorph specimens see Table 6.

		N57					N5										N82		N141		N142	
		OO09	OO13	OO26	OO27	OO11	OO16	OO25	JQ772137.1 T98773	JQ772138.1 T98892	OO14	NAQ_NS_A D352	KJ745479.1 T98883	JQ772141.1 T98887	KJ745488.1 T98884	KJ745489.1 T98895	OO30	NCH_N82.1 H7	OO07	OO16	OO24	
N57	OO09		0.006	0.009	0.009	0.011	0.020	0.020	0.021	0.021	0.021	0.020	0.021	0.020	0.020	0.020	0.021	0.021	0.021	0.021	0.023	0.023
	OO13	0.012		0.010	0.009	0.011	0.020	0.020	0.021	0.021	0.021	0.021	0.021	0.021	0.020	0.020	0.021	0.021	0.021	0.021	0.023	0.023
	OO26	0.030	0.036		0.004	0.013	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.021	0.021	0.021	0.021	0.022	0.023
	OO27	0.030	0.030	0.006		0.012	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.021	0.021	0.021	0.021	0.022	0.023
	OO11	0.042	0.042	0.058	0.052		0.020	0.020	0.020	0.020	0.021	0.020	0.021	0.020	0.020	0.020	0.021	0.021	0.021	0.021	0.023	0.023
N5	OO16	0.161	0.164	0.155	0.152	0.161		0.005	0.011	0.011	0.010	0.010	0.010	0.010	0.010	0.010	0.021	0.021	0.021	0.022	0.022	
	OO25	0.152	0.155	0.152	0.148	0.156	0.009		0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.011	0.021	0.021	0.021	0.022	0.022	
	JQ772137.1 T98773	0.170	0.173	0.164	0.161	0.164	0.042	0.045		0.013	0.011	0.012	0.011	0.012	0.013	0.013	0.021	0.021	0.021	0.022	0.022	
	JQ772139.1 T98892	0.167	0.170	0.158	0.155	0.164	0.039	0.048	0.056		0.007	0.004	0.006	0.004	0.005	0.005	0.021	0.021	0.021	0.020	0.022	
	OO14	0.170	0.173	0.161	0.158	0.170	0.036	0.045	0.045	0.015		0.005	0.003	0.005	0.006	0.006	0.021	0.021	0.021	0.021	0.022	
	NAQ_NS_AD352	0.164	0.167	0.158	0.155	0.164	0.033	0.042	0.052	0.006	0.009		0.004	0.000	0.003	0.003	0.021	0.021	0.021	0.020	0.022	
	KJ745479.1 T98883	0.167	0.170	0.161	0.158	0.167	0.033	0.042	0.045	0.012	0.003	0.006		0.004	0.005	0.005	0.021	0.021	0.021	0.021	0.022	
	JQ772141.1 T98887	0.164	0.167	0.158	0.155	0.164	0.033	0.042	0.052	0.006	0.009	0.000	0.006		0.003	0.003	0.021	0.021	0.021	0.020	0.022	
	KJ745488.1 T98894	0.161	0.164	0.155	0.152	0.161	0.036	0.045	0.055	0.009	0.012	0.003	0.009	0.003		0.000	0.021	0.021	0.021	0.021	0.022	
	KJ745489.1 T98895	0.161	0.164	0.155	0.152	0.161	0.036	0.045	0.055	0.009	0.012	0.003	0.009	0.003	0.000		0.021	0.021	0.021	0.021	0.022	
N82	OO30	0.182	0.179	0.179	0.179	0.179	0.179	0.182	0.179	0.176	0.176	0.176	0.176	0.176	0.179	0.179		0.004	0.017	0.023	0.023	
	NCH_N82_H7	0.176	0.173	0.173	0.173	0.167	0.176	0.179	0.176	0.170	0.170	0.170	0.170	0.170	0.173	0.173	0.006		0.017	0.022	0.023	
N141	OO07	0.185	0.179	0.179	0.179	0.176	0.173	0.167	0.179	0.164	0.170	0.164	0.164	0.167	0.167	0.112	0.112		0.022	0.022	0.023	
N142	OO16	0.212	0.212	0.209	0.209	0.224	0.194	0.200	0.200	0.203	0.206	0.200	0.206	0.200	0.197	0.197	0.212	0.209	0.206		0.005	
	OO24	0.218	0.218	0.215	0.215	0.230	0.200	0.206	0.206	0.203	0.212	0.206	0.212	0.206	0.203	0.203	0.218	0.215	0.212	0.009		

Table 6. Genetic p-distance (below) and the associated standard error (above – blue text) between all Ashburton Salt *Aname* mygalomorph (family Nemesiidae) haplotypes and all reference nemesiid specimens depicted in Figure 3. Ashburton Salt specimens, and associated genetic distances, shaded in colour, as per colour-coding in Figure 3. Ashburton Salt labels in bold text. Within species p-distances outlined in bold borders. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

Table 6 attached separately due to size and detail of the p-distance matrix

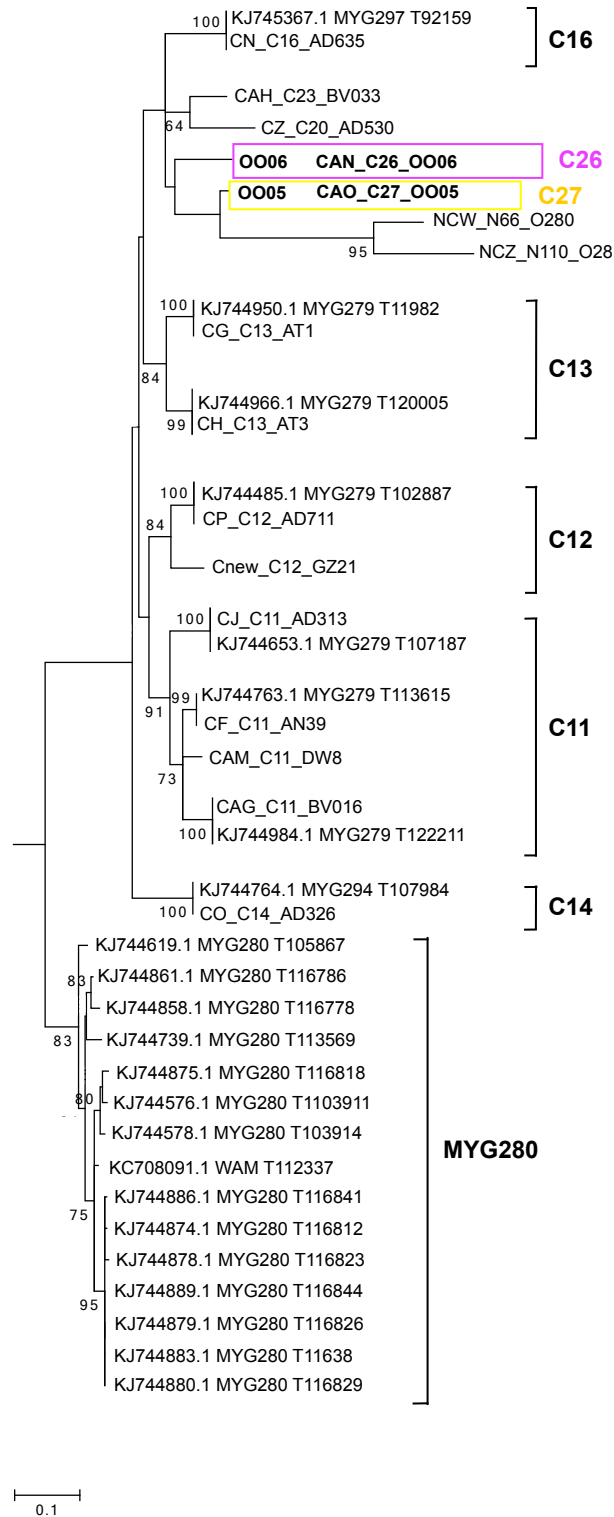


Figure 1. Maximum likelihood analysis of Ctenizidae COI mtDNA sequences, showing the placement of the Ashburton Salt mygalomorph specimens (in bold text) within the current taxonomic framework of the family Ctenizidae. Coloured boxes highlight the species to which the Ashburton Salt specimens belong. All sequences within 15 % sequence divergence are represented in the tree. 'C' numbers on tree refer to ctenizid species based on the 9.5 % species threshold tested in Castalanelli *et al.*, (2014). 'T' numbers refer to WA Museum registration code. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).

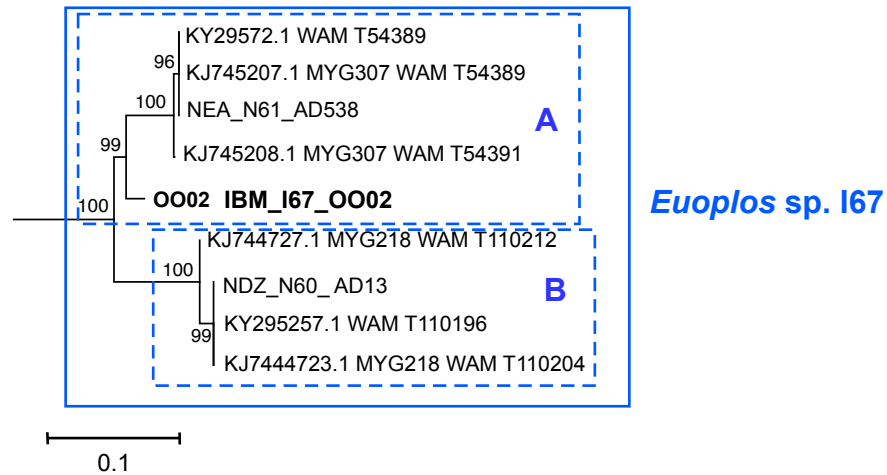


Figure 2. Maximum likelihood analysis of Idiopidae COI mtDNA sequences, showing the placement of the Ashburton Salt mygalomorph specimens (in bold text) within the current taxonomic framework of the genus *Euoplos* in Western Australia (family Idiopidae). The coloured box highlights the species to which the Ashburton Salt specimen belongs, based solely on the 9.5 % species 'threshold' tested in Castalanelli *et al.*, (2014). All reference sequences within 15 % sequence divergence are represented in the tree. The two inner boxes (dashed lines) represent the two highly divergent clades (A & B) of *Euoplos*. 'I' numbers on tree refer to idiopid species based on the 9.5 % species threshold tested in Castalanelli *et al.*, (2014). 'T' numbers refer to WA Museum registration code. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Terminal nodes are collapsed for species. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).

Figure 3 attached separately due to size and detail of the phylogenetic tree

Figure 3. Maximum likelihood analysis of nemesiid COI mtDNA sequences, showing the placement of the Ashburton Salt mygalomorph specimens (highlighted within coloured boxes) within the current taxonomic framework of the family Nemesiidae. The coloured boxes indicate the species to which the Ashburton Salt specimens belong. All sequences within 15 % sequence divergence are represented in the tree. 'N' numbers on tree refer to nemesiid species based on the 9.5 % species threshold tested in Castalanelli *et al.*, (2014). 'T' numbers refer to WA Museum registration code. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).

Appendix 1. Mygalomorph spider specimens utilized during analysis in the present study, the source of the data, and the genetic lineage to which they belong. Ashburton Salt specimens shaded in grey.

Helix Code	Source	Genbank ID	Family	Genus	Helix species assignment	WAM Museum Registration No.
0005	This study	n/a	Ctenizidae	<i>Conothele</i>	C27	n/a
0006	This study	n/a	Ctenizidae	<i>Conothele</i>	C26	n/a
CAG C11 BV016	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C11	n/a
n/a	Helix Database & GenBank	KJ744950	Ctenizidae	<i>Conothele</i>	MYG279	T119982
n/a	Helix Database & GenBank	KJ744966	Ctenizidae	<i>Conothele</i>	MYG279	T120005
n/a	Helix Database & GenBank	KJ744763	Ctenizidae	<i>Conothele</i>	MYG279	T113615
n/a	Helix Database & GenBank	KJ744485	Ctenizidae	<i>Conothele</i>	MYG279	T102887
n/a	Helix Database & GenBank	KJ744984	Ctenizidae	<i>Conothele</i>	MYG279	T122211
n/a	Helix Database & GenBank	KJ745367	Ctenizidae	<i>Conothele</i>	MYG297	T92159
n/a	Helix Database & GenBank	KJ744653	Ctenizidae	<i>Conothele</i>	MYG279	T107187
n/a	Helix Database & GenBank	KJ744674	Ctenizidae	<i>Conothele</i>	MYG294	T107984
n/a	GenBank	KC708091	Ctenizidae	<i>Conothele</i>	n/a	T112337
n/a	Helix Database & GenBank	KJ744875	Ctenizidae	<i>Conothele</i>	MYG280	T116818
n/a	Helix Database & GenBank	KJ744889	Ctenizidae	<i>Conothele</i>	MYG280	T116844
n/a	Helix Database & GenBank	KJ744886	Ctenizidae	<i>Conothele</i>	MYG280	T116841
n/a	Helix Database & GenBank	KJ744879	Ctenizidae	<i>Conothele</i>	MYG280	T116826
n/a	Helix Database & GenBank	KJ744878	Ctenizidae	<i>Conothele</i>	MYG280	T116823
n/a	Helix Database & GenBank	KJ744874	Ctenizidae	<i>Conothele</i>	MYG280	T116812
n/a	Helix Database & GenBank	KJ744578	Ctenizidae	<i>Conothele</i>	MYG280	T103914
n/a	Helix Database & GenBank	KJ744576	Ctenizidae	<i>Conothele</i>	MYG280	T103911
n/a	Helix Database & GenBank	KJ744883	Ctenizidae	<i>Conothele</i>	MYG280	T116838
n/a	Helix Database & GenBank	KJ744880	Ctenizidae	<i>Conothele</i>	MYG280	T116829
n/a	Helix Database & GenBank	KJ744739	Ctenizidae	<i>Conothele</i>	MYG280	T113569
n/a	Helix Database & GenBank	KJ744619	Ctenizidae	<i>Conothele</i>	MYG280	T105867
n/a	Helix Database & GenBank	KJ744864	Ctenizidae	<i>Conothele</i>	MYG280	T116786
n/a	Helix Database & GenBank	KJ744858	Ctenizidae	<i>Conothele</i>	MYG280	T116778
CAH C23 BV033	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C23	n/a
CAM C11 DW8	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C11	n/a
CF C11 AN39	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C11	n/a
CG C13 AT1	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C13	n/a
CH C13 AT3	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C13	n/a
CJ C11 AD313	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C11	n/a
CN C16 AD635	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C16	n/a
Cnew C12 GZ21	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C12	n/a
CO C14 AD326	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C14	n/a
CP C12 AD711	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	C12	n/a
CZ C20 AD530	Helix Database	n/a	Ctenizidae	<i>Conothele</i>	N66	n/a
NCW N66 O280	Helix Database	n/a	Ctenizidae		N110	n/a
NCZ N110 O285	Helix Database	n/a	Ctenizidae			n/a
0002	This study	n/a	Idiopidae	<i>Euoplos</i>	I67	n/a
NDZ N60 AD13	Helix Database	n/a	Idiopidae	<i>Euoplos</i>	N60	n/a
NEA N61 AD538	Helix Database	n/a	Idiopidae	<i>Euoplos</i>	N61	n/a
n/a	Helix Database & GenBank	KJ745208	Idiopidae	<i>Euoplos</i>	MYG307	T54391
n/a	GenBank	KY295272	Idiopidae	<i>Euoplos</i>	n/a	T54389
n/a	Helix Database & GenBank	KJ745207	Idiopidae	<i>Euoplos</i>	MYG307	T54389
n/a	Helix Database & GenBank	KJ744727	Idiopidae	<i>Euoplos</i>	MYG218	T110212
n/a	GenBank	KY295257	Idiopidae	<i>Euoplos</i>	n/a	T110196
n/a	Helix Database & GenBank	KJ744723	Idiopidae	<i>Euoplos</i>	MYG218	T110204
0007	This study	n/a	Nemesiidae	<i>Aname</i>	N141	n/a
0009	This study	n/a	Nemesiidae	<i>Aname</i>	N57	n/a
0011	This study	n/a	Nemesiidae	<i>Aname</i>	N57	n/a
0013	This study	n/a	Nemesiidae	<i>Aname</i>	N57	n/a
0014	This study	n/a	Nemesiidae	<i>Aname</i>	N57	n/a
0016	This study	n/a	Nemesiidae	<i>Aname</i>	N5	n/a
0018	This study	n/a	Nemesiidae	<i>Aname</i>	N142	n/a
0024	This study	n/a	Nemesiidae	<i>Aname</i>	N142	n/a
0025	This study	n/a	Nemesiidae	<i>Aname</i>	N5	n/a
0026	This study	n/a	Nemesiidae	<i>Aname</i>	N57	n/a
0027	This study	n/a	Nemesiidae	<i>Aname</i>	N57	n/a
0030	This study	n/a	Nemesiidae	<i>Aname</i>	N82	n/a
CAB C2 BM41	Helix Database	n/a	Nemesiidae	<i>Aname</i>	C2	n/a
CAC C2 BM8	Helix Database	n/a	Nemesiidae	<i>Aname</i>	C2	n/a
CAM C11 DW8	Helix Database	n/a	Nemesiidae	<i>Aname</i>	C11	n/a
CD C2 AN5	Helix Database	n/a	Nemesiidae	<i>Aname</i>	C2	n/a
CH C13 AT3	Helix Database	n/a	Nemesiidae	<i>Aname</i>	C13	n/a
CS C9 AD765	Helix Database	n/a	Nemesiidae	<i>Aname</i>	C9	n/a
NAQ N5 AD352	Helix Database	n/a	Nemesiidae	<i>Aname</i>	N5	n/a
NAW N6 AD429	Helix Database	n/a	Nemesiidae	<i>Aname</i>	N6	n/a
NAY N9 AD453	Helix Database	n/a	Nemesiidae	<i>Aname</i>	N9	n/a
NAZ N6 AD457	Helix Database	n/a	Nemesiidae	<i>Aname</i>	N6	n/a
NBC N6 AD577	Helix Database	n/a	Nemesiidae	<i>Aname</i>	N6	n/a
NBF N11 AD592	Helix Database	n/a	Nemesiidae	<i>Aname</i>	N11	n/a
NBG N10 AD597	Helix Database	n/a	Nemesiidae	<i>Aname</i>	N10	n/a
NBM N4 AD678	Helix Database	n/a	Nemesiidae	<i>Aname</i>	N4	n/a

Helix Code	Source	Genbank ID	Family	Genus	Helix species assignment	WAM Museum Registration No.
NBR_N14_AD746	Helix Database	n/a	Nemesiidae	Aname	N14	n/a
NBU_N15_AD753	Helix Database	n/a	Nemesiidae	Aname	N14	n/a
NCH_N82_I17	Helix Database	n/a	Nemesiidae	Aname	N82	n/a
NCW_N66_O280	Helix Database	n/a	Nemesiidae	Aname	N66	n/a
NCX_N81_O281	Helix Database	n/a	Nemesiidae	Aname	N81	n/a
NCZ_N110_O285	Helix Database	n/a	Nemesiidae	Aname	N110	n/a
NDC_N84_O288	Helix Database	n/a	Nemesiidae	Aname	N84	n/a
NDG_N78_O304	Helix Database	n/a	Nemesiidae	Aname	N78	n/a
NDN_N80_O336	Helix Database	n/a	Nemesiidae	Aname	N80	n/a
NDQ_N99_O344	Helix Database	n/a	Nemesiidae	Aname	N99	n/a
NDW_N57_AD793	Helix Database	n/a	Nemesiidae	Aname	N57	n/a
NDX_N58_AD500	Helix Database	n/a	Nemesiidae	Aname	N58	n/a
NEE_N63_BN12	Helix Database	n/a	Nemesiidae	Aname	N63	n/a
NEH_N112_BI22	Helix Database	n/a	Nemesiidae	Aname	N112	n/a
NEN_N115_AD594	Helix Database	n/a	Nemesiidae	Aname	N115	n/a
NES_N119_AD715	Helix Database	n/a	Nemesiidae	Aname	N119	n/a
NGI_N131_CZ41	Helix Database	n/a	Nemesiidae	Aname	N131	n/a
NGN_N19_DC5	Helix Database	n/a	Nemesiidae	Aname	N19	n/a
NGQ_N134_DG3	Helix Database	n/a	Nemesiidae	Aname	N134	n/a
NIF_N19_GZ26	Helix Database	n/a	Nemesiidae	Aname	N19	n/a
NREF2_N19_AF1	Helix Database	n/a	Nemesiidae	Aname	N19	n/a
NREF4_N21_AF6	Helix Database	n/a	Nemesiidae	Aname	N21	n/a
NRF_N7_NN11	Helix Database	n/a	Nemesiidae	Aname	N7	n/a
NRG_N7_NN15	Helix Database	n/a	Nemesiidae	Aname	N7	n/a
NRH_N7_NN16	Helix Database	n/a	Nemesiidae	Aname	N7	n/a
NRI_N7_NN17	Helix Database	n/a	Nemesiidae	Aname	N7	n/a
NRJ_N7_NN18	Helix Database	n/a	Nemesiidae	Aname	N7	n/a
NRK_N7_NN20	Helix Database	n/a	Nemesiidae	Aname	N7	n/a
NRL_N7_NN21	Helix Database	n/a	Nemesiidae	Aname	N7	n/a
NRM_N7_NN37	Helix Database	n/a	Nemesiidae	Aname	N7	n/a
NRO_N7_NN38	Helix Database	n/a	Nemesiidae	Aname	N7	n/a
NRP_N138_NN35	Helix Database	n/a	Nemesiidae	Aname	N138	n/a
NX_N33_AR114	Helix Database	n/a	Nemesiidae	Aname	N33	n/a
NGW	Helix Database & GenBank	n/a	Nemesiidae	Aname	MYG161	T100057
NGW	Helix Database & GenBank	n/a	Nemesiidae	Aname	MYG161	T100058
NHP	Helix Database & GenBank	n/a	Nemesiidae	Kwonkan	MYG175	T101209
NHM	Helix Database & GenBank	n/a	Nemesiidae	Aname	MYG173	T101214
NHG	Helix Database & GenBank	n/a	Nemesiidae	Aname	MYG227	T112949
NHQ	Helix Database & GenBank	n/a	Nemesiidae	Aname	MYG250	T121597
NGZ	Helix Database & GenBank	n/a	Nemesiidae	Aname	MYG030	T96495
NHE	Helix Database & GenBank	n/a	Nemesiidae	Aname	MYG066	T96559
NHF	Helix Database & GenBank	n/a	Nemesiidae	Aname	MYG067	T96570
NHJ	Helix Database & GenBank	n/a	Nemesiidae	Aname	MYG034	T98900
n/a	Helix Database & GenBank	KJ744404	Nemesiidae	Aname	MYG173	T101214
n/a	GenBank	MH932613	Nemesiidae	Aname	n/a	T146058
n/a	Helix Database & GenBank	KJ745495	Nemesiidae	Aname	n/a	T99584
n/a	Helix Database & GenBank	MG800157	Nemesiidae	Aname	MYG411	WAMT94952
n/a	Helix Database & GenBank	KJ744911	Nemesiidae	Aname	MYG331	T118812
n/a	Helix Database & GenBank	KJ744914	Nemesiidae	Aname	MYG331	T118818
n/a	Helix Database & GenBank	KJ744909	Nemesiidae	Aname	MYG331	T118805
n/a	Helix Database & GenBank	KJ744901	Nemesiidae	Aname	MYG331	T118789
n/a	Helix Database & GenBank	KJ744902	Nemesiidae	Aname	MYG331	T118791
n/a	Helix Database & GenBank	KJ744733	Nemesiidae	Aname	MYG177	T112359
n/a	Helix Database & GenBank	KJ745296	Nemesiidae	Aname	MYG364	T85262
n/a	Helix Database & GenBank	KJ745251	Nemesiidae	Aname	MYG177	T76829
n/a	Helix Database & GenBank	KJ745249	Nemesiidae	Aname	MYG177	T76827
n/a	Helix Database & GenBank	KJ744910	Nemesiidae	Aname	MYG331	T118811
n/a	Helix Database & GenBank	KJ745494	Nemesiidae	Aname	MYG034	T98900
n/a	Helix Database & GenBank	KJ745287	Nemesiidae	Aname	MYG351	T82303
n/a	Helix Database & GenBank	KJ744610	Nemesiidae	Aname	MYG371	T105216
n/a	Helix Database & GenBank	KJ745257	Nemesiidae	Aname	MYG177	T77081
n/a	Helix Database & GenBank	KJ745245	Nemesiidae	Aname	MYG177	T76823
n/a	Helix Database & GenBank	KJ745244	Nemesiidae	Aname	MYG177	T76822
n/a	Helix Database & GenBank	KJ745242	Nemesiidae	Aname	MYG177	T76820
n/a	Helix Database & GenBank	KJ744648	Nemesiidae	Aname	MYG373	T107087
n/a	GenBank	KJ745479	Nemesiidae	Aname	n/a	T98883
n/a	GenBank	JQ772141	Nemesiidae	Aname	n/a	T98897
n/a	GenBank	KJ745488	Nemesiidae	Aname	n/a	T98894
n/a	GenBank	KJ745489	Nemesiidae	Aname	n/a	T98895
n/a	GenBank	JQ772139	Nemesiidae	Aname	n/a	T98892
n/a	GenBank	JQ772137	Nemesiidae	Aname	n/a	T98773
n/a	Helix Database & GenBank	KJ745375	Nemesiidae	Aname	MYG034	T93314
n/a	Helix Database & GenBank	KJ744649	Nemesiidae	Aname	MYG373	T107089
n/a	Helix Database & GenBank	KJ745232	Nemesiidae	Aname	MYG351	T74241
n/a	Helix Database & GenBank	KJ745231	Nemesiidae	Aname	MYG351	T74238
n/a	GenBank	JQ772136	Nemesiidae	Aname	n/a	T95404
n/a	Helix Database & GenBank	KJ745306	Nemesiidae	Aname	MYG365	T88603

Helix Code	Source	Genbank ID	Family	Genus	Helix species assignment	WAM Museum Registration No.
n/a	GenBank	KJ744651	Nemesiidae	Aname	n/a	T107182
n/a	Helix Database & GenBank	KJ745302	Nemesiidae	Aname	MYG365	T88597
n/a	Helix Database & GenBank	KJ745301	Nemesiidae	Aname	MYG365	T88596
n/a	Helix Database & GenBank	KJ745233	Nemesiidae	Aname	MYG351	T74247
n/a	Helix Database & GenBank	KJ745293	Nemesiidae	Aname	MYG351	T82309
n/a	Helix Database & GenBank	KJ745288	Nemesiidae	Aname	MYG351	T82304
n/a	GenBank	KJ744987	Nemesiidae	Aname	n/a	T122215
n/a	Helix Database & GenBank	MG800161	Nemesiidae	Aname	MYG102	T98767
n/a	Helix Database & GenBank	KJ745203	Nemesiidae	Aname	MYG342	T52181
n/a	GenBank	KJ744577	Nemesiidae	Aname	n/a	T103913
n/a	GenBank	JQ772152	Nemesiidae	Aname	n/a	T98433
n/a	GenBank	KJ745051	Nemesiidae	Aname	n/a	T122823
n/a	GenBank	KJ744571	Nemesiidae	Aname	n/a	T103906
n/a	GenBank	KJ744394	Nemesiidae	Aname	n/a	T101158
n/a	Helix Database & GenBank	KJ745299	Nemesiidae	Aname	MYG001	T86678
n/a	Helix Database & GenBank	KJ745239	Nemesiidae	Aname	MYG177	T74755
n/a	GenBank	KJ745365	Nemesiidae	Aname	n/a	T92127
n/a	GenBank	KJ745170	Nemesiidae	Aname	n/a	T126301
n/a	GenBank	KJ745093	Nemesiidae	Aname	n/a	T122870
n/a	GenBank	KJ745012	Nemesiidae	Aname	n/a	T122263
n/a	GenBank	KJ744781	Nemesiidae	Aname	n/a	T113636
n/a	GenBank	KJ744630	Nemesiidae	Aname	n/a	T105886
n/a	GenBank	KJ744611	Nemesiidae	Aname	n/a	T105851
n/a	GenBank	KJ744491	Nemesiidae	Aname	n/a	T103177
n/a	GenBank	KJ745371	Nemesiidae	Aname	n/a	T92456
n/a	GenBank	KJ745168	Nemesiidae	Aname	n/a	T126296
n/a	GenBank	KJ745398	Nemesiidae	Aname	n/a	T95402
n/a	GenBank	JQ772131	Nemesiidae	Aname	n/a	T95401
n/a	GenBank	KJ744982	Nemesiidae	Aname	n/a	T122207
n/a	GenBank	JQ772132	Nemesiidae	Aname	n/a	T95402
n/a	GenBank	KJ745370	Nemesiidae	Aname	n/a	T92455
n/a	GenBank	KJ745396	Nemesiidae	Aname	n/a	T95400



Helix

Molecular Solutions

School of Animal Biology The University of Western Australia
Hackett Entrance No. 4 Hackett Drive Crawley WA 6009

PO Box 155
Leederville WA 6903

t: (08) 6488 4509

f: (08) 6488 1029

abn: 32 133 230 243

w: www.helixsolutions.com.au

5 July 2019

Tobias Thöenelt
K plus S Salt Australia Pty Ltd
Level 27, St Martins Tower
44 St Georges Terrace
Perth WA 6000

Via email

Re. Helix Job 572 - Report on the molecular investigation of specimen M20181111.ASHSRE31.01 from Ashburton phase 1 sampling - re-sequencing of odd sample.

Dear Tobias,

Following is a summary of the results of the second DNA extraction and sequencing of a nemesiid mygalomorphae specimen (M20181111.ASHSRE31.01 = original Helix DNA extraction OO30) collected in Phase 1.

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

Sincerely,

Dr. Zoë Hamilton, Dr. Terrie Finston and Yvette Hitchen
Helix Molecular Solutions



Background and Objective

Analyses during phase 1 sequencing found one specimen (Helix ID OO30) showed affinity to a previously collected species from Forresteria (Helix, 2019a), an unexpected result considering the vast geographic distance between specimens. During the current investigation, tissue was subsampled from the original specimen, DNA re-extracted and sequenced for variation at the cytochrome oxidase subunit I gene (COI) using primers LCOI & HCO2 (Folmer *et al.*, 1994). The resulting sequence (Helix ID OO33) was then re-analysed along with the entire nemesiid data set used during phase 2 analyses, as per methods in phase 2 report (Helix, 2019b).

Results

Phylogenetic Analyses

A 436 base-pair (bp) fragment of COI was isolated and analysed for the specimen (OO33).

Nemesiidae

The repeat nemesiid specimen (OO33) from phase 1 sampling (Table 1) was analysed along with the phase 2 specimens (n=17) and one hundred and sixty-one reference specimens from the Helix database (n=74) and GenBank (n=87). The OO33 specimen showed 12.3 % sequence divergence from the specimen (OO30) sequenced from phase 1 that showed affinity with the Forresteria species *Aname* sp. NCH_N82 (Table 2). The OO33 specimen instead showed between 4.4 % and 4.7 % sequence divergence from the *Aname* sp. NRT_N141 species collected during both phases of sampling.

Contamination of samples of interest can occur during sampling or in the lab and will most often result in a 'messy' unusable sequence, as more than one set of DNA is amplified. At Helix steps are taken to minimise the potential for contamination by ensuring any specimens we receive are clearly labelled and these labels correspond to a spreadsheet containing specimen information including locality and collection details. Labelling in the lab is clear and consistent and used as a reference through all stages of analysis. Within the laboratory aseptic techniques including UV treatment of equipment, autoclaving of plastic ware and bleaching of the bench area are used to ensure a sterile and contamination-free work area. In addition, a PCR negative control (a blank containing PCR chemical reagents and sterile water in place of DNA) is used to ensure that cross contamination has not occurred. In the event of a false amplification (amplification within the negative control), all reagents would be changed and samples re-run. All results are critically assessed with reference specimens to ensure resulting relationships 'make sense'.

The original sequence produced for OO30 was a clean decipherable sequence, although analyses found the sequence to show very little affinity to other sequences from the project area. We believe that the initial result found for OO30 was the result of contamination, labelling, or sample mix-up prior to lodgement with Helix and subsequent DNA extraction. This investigation highlights the importance of critically assessing results. The initial results implied that the species had a distribution in excess of 1, 200 km, with very little molecular divergence, a pattern not previously documented for nemesiid mygalomorph spiders.

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

Sincerely,

Dr. Zoë Hamilton, Dr. Terrie Finston and Yvette Hitchen
Helix Molecular Solutions

References

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

Helix 2019a. Molecular systematics of the K plus S Salt mygalomorph specimens from Ashburton. Unpublished report for Biota Environmental Sciences.

Helix 2019b. Molecular systematics of the K plus S Salt mygalomorph specimens from Ashburton, phase 2 sampling. Unpublished report for Biota Environmental Sciences.

Table 1. Ashburton sample used during current molecular investigation.

Biota ID	Helix ID (phase 1)	Helix ID (current extraction)	Family	Genetic Lineage/Taxonomic ID
M20181111.ASHSRE31.01	OO30	OO33	Nemesiidae	Previously recorded species <i>Aname</i> sp. <u>NRT_N141</u>

Table 2. Genetic p-distance (below) and the associated standard error (above – blue text) between all Ashburton Salt *Aname* mygalomorph (family Nemesiidae) haplotypes shown in Figure 1. OO30 and OO33 specimens in bold text. Shaded specimens as per colour-coding for species in Helix 2019a & Helix 2019b. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

Table 2 attached separately as a PDF document due to the size and detail.

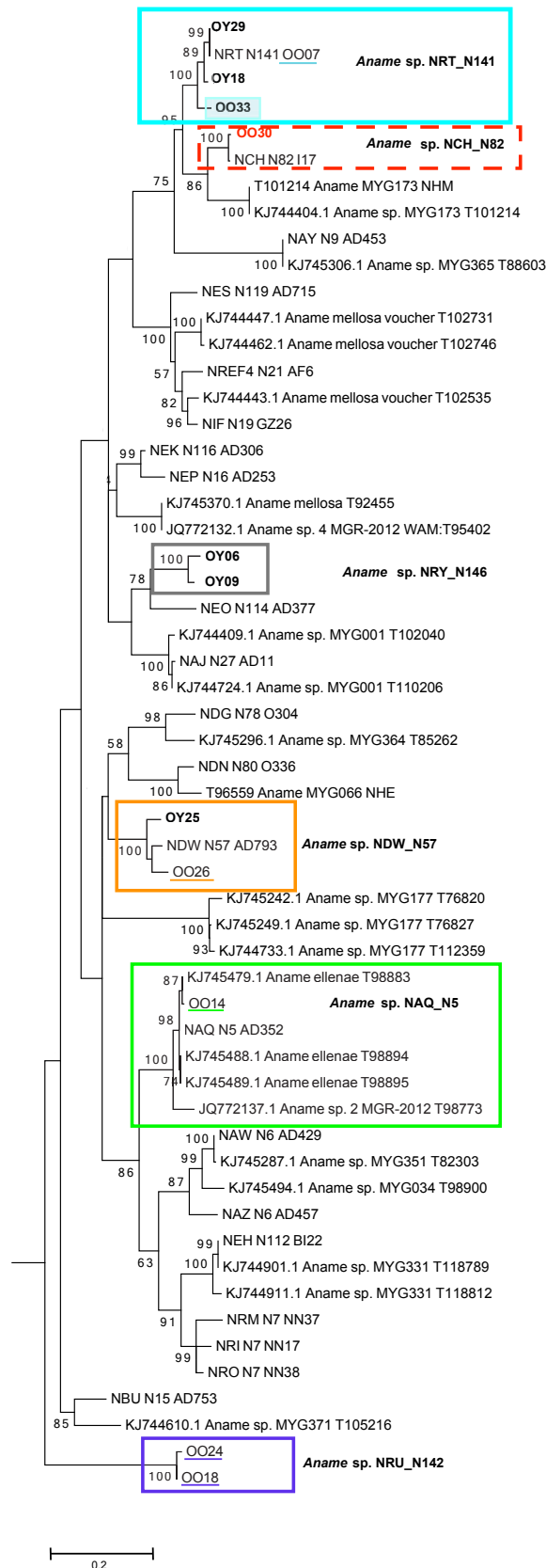


Figure 1. Maximum likelihood analysis of reduced nemesiid dataset of COI mtDNA sequences, showing the placement of the Ashburton Salt mygalomorph specimens (highlighted within coloured boxes) within the current taxonomic framework of the genus *Aname* (Nemesiidae). The outer coloured box outline indicates the species to which the Ashburton Salt specimens belong. Phase 1 specimens are underlined, phase 2 specimens in bold text. Phase 1 specimen in question in red bold text, repeat specimen shaded in turquoise. All sequences within 15 % sequence divergence are represented in the tree. 'N' numbers on tree refer to nemesiid species based on the 9.5 % species threshold tested in Castalanelli *et al.*, (2014). 'T' numbers refer to WA Museum registration code.



Helix

Molecular Solutions

School of Animal Biology The University of Western Australia
Hackett Entrance No. 4 Hackett Drive Crawley WA 6009

PO Box 155
Leederville WA 6903

t: [08] 6488 4509

f: [08] 6488 1029

abn: 32 133 230 243

w: www.helixsolutions.com.au

5 July 2019

Tobias Thöenelt
K plus S Salt Australia Pty Ltd
Level 27, St Martins Tower
44 St Georges Terrace
Perth WA 6000

Via email

Re. Helix Job 572-Report on the molecular systematics of the K plus S Salt mygalomorph specimens from Ashburton, phase 2 sampling.

Dear Tobias,

Following is a summary of the results of the invertebrate molecular investigation we have completed for the Ashburton Salt Fauna survey, Phase 2. Results suggest that amongst the twenty-six successfully sequenced mygalomorph spider specimens, one belonged to a previously unrecorded species of *Idiommata* (family Barychelidae), two belonged to a distinct previously recorded species of *Conothele* (family Ctenizidae), four belonged to a previously unrecorded species of *Euoplos* (family Idiopidae). There were also seventeen *Aname* specimens belonging to two previously recorded species, and one previously unrecorded species (family Nemesiidae). We were unable to obtain a good quality sequence from seven specimens, and therefore the placement of these suspected idiopid and nemesiid specimens remains unresolved.

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

Sincerely,

Dr. Zoë Hamilton, Dr. Terrie Finston and Yvette Hitchen
Helix Molecular Solutions



Background and Objective

The infraorder of Arachnida, Mygalomorphae, includes trapdoor spiders and their kin, and they are frequently identified as short-range endemics (SREs) (e.g. Harvey *et al.*, 2011; Castalanelli *et al.*, 2014). Identification of species has traditionally been performed using morphological techniques, however, only males can be used in identification, as both females and juveniles lack the diagnostic characters used in identification, and furthermore there is a large backlog of undescribed taxa. DNA barcoding with the use of COI mtDNA has become a rapid, objective method aiding mygalomorph species identifications and their distributions, and is recognised as providing important information that regulatory authorities can use to assess environmental impacts of large-scale developments (Harvey *et al.*, 2008; Environmental Protection Authority, 2009; Castalanelli *et al.*, 2014). Extensive molecular work has been conducted on the trap-door spider fauna of Western Australia (Helix, 2009a & b, 2010, 2011a - l, 2012a - i, 2013a & b, 2014a - d, 2015a - e). The resulting dataset provides a molecular framework that can be used to provide regional context for localised sampling.

Thirty-three specimens of invertebrate fauna belonging to four families of mygalomorph spiders (Araneae: Mygalomorphae: (Barychelidae, Ctenizidae, Idiopidae & Nemesiidae) from the Ashburton Salt survey area that falls within the Cape Range subregion (CAR1) of the Carnarvon bioregion (CAR) were sequenced for variation at the mitochondrial cytochrome oxidase subunit I gene (COI). The twenty-six successful resulting molecular sequences were then assessed to determine the number of taxa present and compare these results to those sequences from phase 1 sampling (n=25), and sequences publically available on GenBank, as well as those already in Helix's database for context.

Executive summary

- Thirty-three specimens of mygalomorph spiders belonging from the Ashburton Salt survey area (phase 2) were sequenced and assessed for variation at the COI mtDNA gene. The molecular data were then placed within an existing molecular taxonomic framework for each family, using COI mtDNA sequences from phase 1 sampling, sequences from GenBank, and mygalomorph COI sequences in the Helix database;
- Four families (Barychelidae, Ctenizidae, Idiopidae & Nemesiidae) were amongst the twenty-six successfully sequenced specimens. nineteen haplotypes were amongst the twenty-six successfully sequenced individuals, with one haplotype shared amongst seven individuals, and another shared amongst two individuals;
- The single specimen of Barychelidae belonged to the genus *Idiommata*, but showed no close affinity to previous sequenced specimens, and is hence likely to represent a new species (*Idiommata* sp. BBS_B38);
- Analyses place the two Ctenizidae specimens within the *Conothele* genus. Both specimens belong to the new species (*Conothele* sp. CAO_C27) previously recorded only during phase 1 sampling;
- Four idiopid specimens belong to the genus *Euoplos*, in the same species (*Euoplos* sp. IBM_167) as that recorded in phase 1;
- Two additional idiopid specimens belong to the genus *Aganippe*, with the specimen representing a previously unrecorded species, based on the molecular data (*Aganippe* sp. IBN_169);
- Analyses place the seventeen nemesiid specimens within the *Aname* genus, with one new species represented by two specimens (*Aname* sp. NRY_N146), and the remaining fifteen specimens belonging to two previously recorded species (*Aname* sp. NRT_N141 & *Aname* sp. NDW_N57), recorded only during phase 1;
- A total of eleven species were recorded from phase 1 and 2 sampling, with eight species recorded during phase 1 and an additional three species from phase 2. Four species recorded during phase 1 were also recorded during phase 2;
- Of the eleven species recorded from both phases of sampling, seven have not been recorded previously.

Methods

Thirty-three mygalomorph spider specimens from fifteen sampling locations (Table 1) were sequenced for variation at the cytochrome oxidase subunit I gene (COI) using primers LCOI & HCO2 (Folmer *et al.*, 1994). Seven of these sequences were unable to be analysed, due to the sequence quality. The resulting twenty-six mygalomorph sequences comprised nineteen haplotypes (Table 2).

The sequences from the twenty-six successfully sequenced individuals (COI) edited using Geneious version 6.1.8 software (<https://www.geneious.com>) performed within MEGA version 5.05 (Tamura *et al.*, 2011) using the built-in alignment tool using CLUSTAL W (Thompson *et al.*, 1994) using default parameters. DNA nucleotide sequences were translated into protein sequences to ensure that the amplified sequences corresponded to the target mtDNA. The translated protein sequences were then checked for the presence of stop codons, to ensure that pseudogenes hadn't been amplified. Pseudogenes have a DNA sequence that is similar to the functional gene (e.g. COI) however, they do not code for a functioning protein despite the shared ancestry with the functional gene. The presence of pseudogenes can complicate molecular analyses, producing odd results. DNA sequences were translated into proteins with EXPASy using the invertebrate genetic code. All sequences analysed were of high quality with no evidence of heterogeneous peaks. All resulting sequences were 'BLAST'ed (Basic Local Alignment Search Tool) with the NCBI (National Centre for Biotechnology Information). This program compares DNA nucleotide sequences with a library of sequences and identifies sequences within the database that resemble the query sequences above a certain threshold. Genetic distances between unique genetic sequences (haplotypes) were measured using uncorrected p-distances (total percentage of nucleotides different between sequences). To account for polymorphism within lineages, the net genetic diversity of Nei (1987) was calculated to give a 'corrected' distance between lineages.

For phylogenetic analysis, likelihood ratio tests using the Bayesian Information Criterion were calculated in MEGA 7.0 (Kumar *et al.*, 2016) to determine the best-fit model of evolution. The phylogenetic analyses were calculated in MEGA 7.0 (Kumar *et al.*, 2016) using maximum likelihood (ML) with 1000 bootstrap replicates, based on the genetic distances with the best-fit model of evolution calculated for each family. The tree was rooted using two outgroups, *Centruroides vittatus* (EU404114), and *Mesobuthus martensii* (JF700146) obtained from GenBank.

For all families (Barychelidae, Ctenizidae, Idiopidae & Nemesiidae), the best model of evolution was the General Time Reversible model with gamma distribution and invariant sites (GTR+G+I). For the Barychelidae the parameter for gamma distribution was 0.56, for the Ctenizidae the parameter for the gamma distribution was 1.29. For the Idiopidae it was 0.66 and for the Nemesiidae the parameter for gamma distribution of 0.71. For the separate analysis of the *Euoplos* specimens (also family Idiopidae), the best model of evolution was Tamura-Nei with gamma distribution (TN93+G, G=0.22).

The phylogenetic analysis performed separately for each mygalomorph family, and included the representative haplotypes for the twenty-six specimens from the survey area (phase 2 sampling), as well as a total of three hundred and eighteen reference specimens (Barychelidae n=11, Ctenizidae n=37, Idiopidae n=109, Nemesiidae n=161), in addition to the twenty-five specimens from phase 1 sampling, all within 15 % sequence divergence of the twenty-six specimens (phase 2), obtained from both Helix's database (n=190) and from GenBank (n=128) (Appendix 1).

For both the Idiopidae and the Nemesiidae a reduced dataset was secondarily analysed for simplification of the phylogenetic tree, due to the large number of samples examined. However, the full dataset was examined for relationships to reference specimens.

Results

Phylogenetic Analyses

A 683 base-pair (bp) fragment of COI was isolated for twenty-four of the twenty-six specimens. A 435 bp fragment was isolated for an additional two individuals. Because multiple specimens shared identical DNA sequences (haplotypes), the data set was reduced to include only unique haplotypes. Of the twenty-six specimens, seventeen had unique haplotypes.

Barychelidae

The phylogenetic analysis for the single specimen of Barychelidae (OY27), along with the eleven additional reference specimens (Genbank n=4, Helix database n=7) found the Ashburton barychelid to sit within a clade containing an individual of *Idiommata* from the Helix sequence database (BU_B18_AD535), and an *Idiommata* from GenBank (KJ74500.1) (Figure 1). The closest relative to the Ashburton specimen (OY27), based on genetic distance, was a specimen from the Helix database (BU_B18_AD535) collected 6km S of Williambury Homestead, at 10.7 % sequence divergence, followed next by a GenBank *Idiommata* specimen (KJ745100) at 11.5 % sequence divergence (Table 3). The barychelid sequenced during phase 2 sampling has not been previously recorded based on sequences from GenBank and the Helix database, and hence appears to be a newly recorded taxon (*Idiommata* sp. BBS_B38).

Ctenizidae

The phylogenetic analysis for the two specimens, along with the forty additional reference sequences, including those from phase 1 sampling (n=3), GenBank (n=23), and the Helix database (n=14) (Table 4) revealed the Ashburton Salt specimens to sit within the clade containing reference sequences belonging to the genus *Conothele* (see Figure 2). All included reference sequences showed ≤ 15 % sequence divergence. The phase 2 ctenizid specimens showed only 2.6 % sequence divergence from each other (Table 4) and were only 2.4 % divergent from the *Conothele* specimens, species *Conothele* sp. CAO_C27, sequenced during phase 1 sampling (Helix, 2019). The closest relative based on genetic distance was a *Conothele* species (CAN_C26, n=2) that showed 9.2 % divergence, also sequenced from phase 1 sampling (Table 4; Helix, 2019). The C27 species recorded during phase 2 did not show a close affinity to any other previously sequenced specimens from Helix's database, or from GenBank.

Idiopidae

Phylogenetic analyses placed four idiopid specimens, the lineage represented by a single haplotype (OY19), in the genus *Euoplos* (Figure 3). This genus belongs to the subfamily Arbanitinae, and the tribe Euoplini (Rix *et al.*, 2017). It has recently been examined amongst the revision of the family with two described species in Western Australia. The *Euoplos* are highly divergent from other Idiopidae and hence analyses were conducted on *Euoplos* separately to other Idiopidae specimens. Analyses on the *Euoplos* specimens from phase 2 sampling (n=4) were analysed along with those collected during phase 1 sampling (n=1), in addition to nine additional reference specimens (Helix database n= 2, GenBank n=6). The four *Euoplos* specimens, were represented by a single haplotype during analyses (OY19) and were only 0.5 % divergent from the *Euoplos* sequenced during phase 1 sampling (Table 5; Helix, 2019). The *Euoplos* recorded from P1 & P2 showed affinity to specimens from both the Helix database and from GenBank with sequence divergence ranging from 4.6 % to 8.0 % (Table 5) and by applying the 9.5 % sequence divergence 'cut-off' that was tested by Castalanelli *et al.*, (2014), as well as addressing the phylogenetic relationships (Figure 3) we believe this specimen belongs to a previously recorded but yet undescribed species, showing moderate differentiation among sites (*Euoplos* sp. IBM_I67). While divergence is greater than is typically observed between individuals of the same species (Hebert, 2003), the pattern of the phylogeny (each clade from a single common ancestor showing slightly higher distance from the next-nearest clade instead of separate clades showing long branch lengths between them) suggests an isolation by distance speciation model. We recommend further investigation into this closely related group. In addition to the phase 1 specimen, there were six specimens belonging to this species from GenBank (KJ745208.1, KJ295272.1, KJ745207.1, KJ744727.1, KY295257.1 & KJ744723.1), and two from the Helix database (NEA_N61AD538 collected 27 km SW of Pannawonica, and NDZ_N60_AD13 collected 105.2 km NNE of Onslow), all of which were included in analyses.

Phylogenetic analyses were performed on the two addition idiopid specimens along with one hundred and four reference specimens from the Helix database (n=79) and GenBank (n=25). The two idiopid specimens were represented by two haplotypes (OY07, OY13) and analyses placed them within the genus *Aganippe* (Figure 4, Table 6). The specimens showed 10.7 % sequence divergence from the nearest relative, which was from the Helix database (IBJ_116_AD505 collected 5.5km NW of Cossack). The two specimens represent a newly recorded species *Aganippe* sp. IBN_169, based on molecular data, and show 2.9 % intraspecific sequence divergence (Table 6).

Nemesiidae

The twenty-one nemesiid specimens from phase 1 sampling were analysed along with the phase 2 specimens (n=17) and one hundred and sixty-one reference specimens from the Helix database (n=74) and GenBank (n=87). Amongst the seventeen nemesiid specimens from phase 2, seven individuals belong to the previously recorded species *Aname* sp. NDW_N57 (Figure 5), eight specimens belong to the species *Aname* sp. NRT_N141, recorded previously only during phase 1 sampling. The remaining two specimens belong to a new distinct *Aname* species, not previously recorded (*Aname* sp. NRY_N146). Amongst the seventeen specimens from phase 2, ten haplotypes existed, with two haplotypes shared amongst nine individuals.

The species *Aname* sp. NDW_N57 showed up to 5.3 % intraspecific variation amongst individuals, with the closest relative to the phase 2 specimen (OY25) sharing the same haplotype (0.00 % sequence divergence), collected during phase 1 sampling (OO11) (Table 7). The closest relative to the species, based on genetic distance, was a specimen from GenBank (MG800157.1, WAMT94952) that showed 13.9 % sequence divergence from the phase 2 specimen (OY25).

The species *Aname* sp. NRT_N141 showed just 2.0 % intraspecific sequence divergence (table 7). The closest specimen to the species, based on genetic distance, was a specimen collected during phase 1 sampling (OY18) with 10.9 % sequence divergence, and a specimen from the Helix database NCH_N82_117 collected at Forrestania, also with 10.9 % sequence divergence.

The newly recorded species *Aname* sp. NRY_N146, recorded only during phase 2, showed 3.0 % intraspecific divergence between the two specimens (OY06 & OY09). The closest relatives, based on genetic distance, showed 12.6 % sequence divergence and were from both the helix database (NBA_N28_AD512 collected 67.1km WSW of Karratha), and GenBank (KJ745477.1, KJ745468.1 & KJ745474.1) (Table 7).

Conclusions

The mtDNA gene cytochrome oxidase 1 (*COI*) is widely considered to show suitable variation to distinguish species (Hebert *et al.*, 2003a), and the use of this gene can be extremely effective for 'DNA barcoding' in taxa where clear differentiation exists between intra and interspecific levels of divergence (e.g. Hebert *et al.*, 2004a; 2004b). In a study by Castalanelli *et al.*, (2014) comparing *COI* sequences (barcoding gene) for 1134 mygalomorph sequences from seven families, the diversity of species in the Pilbara region of Western Australia was analysed to test the genetic relationships between and within species, and assess molecular results against morphotype designations. The majority (92%) of the morphotypes that had been previously recognised based on adult male morphology were recovered using sequence data, showing the utility of barcoding in mygalomorph spiders (Castalanelli *et al.*, 2014). Despite its merits in barcoding however, a taxon by taxon approach, examining the amount of phylogenetic variation within and between taxa is the most widely accepted method of delineating species and their distributions, especially in areas where rapidly expanding mining operations outpace taxonomic treatment of unresolved taxa.

In summary, we detected a total of eleven species among the fifty-one successfully sequenced individuals detected from both phases of the study, with eight species recorded during phase 1 and an additional three species from phase 2. Four species recorded during phase 1 were also recorded during phase 2. Of the eleven species recorded from both phases of sampling, seven have not been recorded previously, based on the sequences available for comparison.

References

- Australian Faunal Directory.** Australian Government, Department of Sustainability, Environment, Water, Population and Communities
<http://www.environment.gov.au/biodiversity/abrs/online-resources/fauna/index.html>
- Brower AVZ. 1994.** Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences USA*. **91**: 6491-6495.
- Castalanelli MA, Teale RJ, Rix MG, Kennington WG, Harvey MS. 2017.** Barcoding of mygalomorph spiders (Araneae: Mygalomorphae) in the Pilbara bioregion of Western Australia reveals a highly diverse biota. *Invertebrate Systematics* **28**: 375-385.
- Castalanelli MA, Huey JA, Hillyer MJ, Harvey MS. 2017.** Molecular and morphological evidence for a new genus of small trapdoor spiders from arid Western Australia (Araneae: Mygalomorphae: Nemesiidae: Anaminae). *Invertebrate Systematics* **31**: 492-505.
- Environmental Protection Authority 2009.** Sampling of short range endemic invertebrate fauna for environmental impact assessment in Western Australia. In 'Guidance for the Assessment of Environmental Factors (in accordance with the Environmental Protection Act 1986). Vol. No. 20'. pp. 1-31.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994.** DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294-299.
- Harvey MS, Berry O, Edward KL, Humphreys G. 2008.** Molecular and morphological systematics of hypogean schizomids (Schizomida: Hubbardiidae) in semiarid Australia. *Invertebrate Systematics* **22**: 167-194.
- Harvey MS, Rix MG, Framenau VW, Hamilton ZR, Johnson MS, Teale RJ, Humphreys G, Humphreys WF. 2011.** Protecting the innocent: studying short-range endemic taxa enhances conservation outcomes. *Invertebrate Systematics* **25**: 1-10.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003a.** Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* **270**: 313-321.
- Hebert PDN, Ratnasingham S, deWaard JR. 2003b.** Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. *Proceedings of the Royal Society of London B (supplement)* **270**, S96-S99.
- Hebert, P.D.N., Penton EH, Burns JM, Janzen DH, Hallwachs W. 2004a.** Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *PNAS* **101**: 14812-14817.
- Hebert PDN, Stoëckle MY, Zemiak TS, Francis CM 2004b.** Identification of birds through DNA barcodes. *PLoS Biology* **2**: 1657-1663.
- Helix 2009a.** Report on the molecular systematics of *Aganippe castellum*. Unpublished report for Biota Environmental Sciences.
- Helix 2009b.** Report on the molecular systematics of wishbone spider *Aname*. Unpublished report for BHP Billiton.
- Helix 2010.** Report on the molecular systematics of mygalomorph spiders from Forestania. Unpublished report for Biota Environmental Sciences.
- Helix 2011a.** Report on the molecular systematics of *Galeosoma* from Forestania. Unpublished report for Biota Environmental Sciences.
- Helix 2011b.** Report on the molecular systematics of Mygalomorphae from Forrestania. Unpublished report for Biota Environmental Sciences.
- Helix 2011c.** Report on the molecular systematics of Mygalomorphae from Deception. Unpublished report for Biota Environmental Sciences.
- Helix 2011d.** Report on the molecular systematics of Mygalomorphae from South Parmelia. Unpublished report for Biota Environmental Sciences.
- Helix 2011e.** Report on the molecular systematics of Mygalomorphae from Jinidi. Unpublished report for Biota Environmental Sciences.

- Helix 2011f.** Report on the molecular systematics of Mygalomorphae from Southern Flank. Unpublished report for Biota Environmental Sciences.
- Helix 2011g.** Report on the molecular systematics of Mygalomorphae from Area C to Yandi. Unpublished report for Biota Environmental Sciences.
- Helix 2011h.** Report on the molecular systematics of Mygalomorphae from Hammersley Irrigation. Unpublished report for Biota Environmental Sciences.
- Helix 2011i.** Report on the molecular systematics of Mygalomorphae from Mudlark. Unpublished report for Biota Environmental Sciences.
- Helix 2011j.** Report on the molecular systematics of Mygalomorphae from Marillana. Unpublished report for Biota Environmental Sciences.
- Helix 2011k.** Report on the molecular systematics of Mygalomorphae from Jinidi to Manline. Unpublished report for Biota Environmental Sciences.
- Helix 2011l.** Report on the molecular systematics of Mygalomorphae from Eastern Deviation. Unpublished report for Biota Environmental Sciences.
- Helix 2012a.** Report on the molecular systematics of Mygalomorphae from the Pilbara. Unpublished report for Biota Environmental Sciences, 31 January.
- Helix 2012b.** Report on the molecular systematics of Mygalomorphae from West Turner Syncline. Unpublished report for Biota Environmental Sciences.
- Helix 2012c.** Report on the molecular systematics of Mygalomorphae from Koodaideri West Corridor. Unpublished report for Biota Environmental Sciences.
- Helix 2012d.** Report on the molecular systematics of Mygalomorphae from Koodaideri phase IV and Koodaideri South. Unpublished report for Biota Environmental Sciences.
- Helix 2012e.** Report on the molecular systematics of Mygalomorphae from Cape Lambert. Unpublished report for Biota Environmental Sciences.
- Helix 2012f.** Report on the molecular systematics of Mygalomorphae from Marra Mamba. Unpublished report for Biota Environmental Sciences.
- Helix 2012g.** Report on the molecular systematics of Mygalomorphae from Mt Richardson. Unpublished report for Biota Environmental Sciences.
- Helix 2012h.** Report on the molecular systematics of Mygalomorphae from West Turner Extension. Unpublished report for Biota Environmental Sciences.
- Helix 2012i.** Report on the molecular systematics of Mygalomorphae from Southern Flank to Jinidi. Unpublished report for Biota Environmental Sciences.
- Helix 2013a.** Report on the molecular systematics of Mygalomorphae from Mt Richardson phase I and II. Unpublished report for Biota Environmental Sciences.
- Helix 2013b.** Report on the molecular systematics of mygalomorphae from Koodaideri rail corridor. Unpublished report for Biota Environmental Sciences.
- Helix 2014a.** Molecular systematics of mygalomorphae from Kundip. Unpublished report for Biota Environmental Sciences.
- Helix 2014b.** Molecular systematics of mygalomorphae from Koodaideri northern extension. Unpublished report for Biota Environmental Sciences.
- Helix 2014c.** Molecular Systematics of mygalomorphae from Yandi Billiards. Unpublished report for Biota Environmental Sciences.
- Helix 2014d.** Molecular systematics of mygalomorphae from Yandi Billiards phase 2. Unpublished report for Biota Environmental Sciences.
- Helix 2015a.** Report on the molecular systematics of mygalomorphae from Bungaroo Valley. Unpublished report for Biota Environmental Sciences.
- Helix 2015b.** Report on the molecular systematics of mygalomorphae form Baby Hope Downs. Unpublished report for Biota Environmental Sciences.

- Helix 2015c.** Molecular systematics of mygalomorphae from Yandi Oxbow. Unpublished report for Biota Environmental Sciences.
- Helix 2015d.** Molecular systematics of mygalopmorphae from Red Hill. Unpublished report for Biota Environmental Sciences.
- Helix 2015e.** Molecular systematics of mygalomorphae from the Buckland Hills Level 2 fauna survey. Unpublished report for Biota Environmental Sciences.
- Helix 2019.** Molecular systematics of the K plus S Salt mygalomorph specimens from Ashburton. Unpublished report for Biota Environmental Sciences.
- Kumar S, Stecher G, Tamura K. 2016.** MEGA 7: Molecular Evolutionary Genetic Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**: 1870-1874.
- Nei M. 1987.** DNA polymorphism within and between populations. In: *Molecular Evolutionary Genetics*. pp.254-286. Columbia University Press New York, NY.
- Rix MG, Raven RJ, Main BY, Harrison SE, Austin AD, Cooper SJB, Harvey MS. 2017.** The Australian spiny trapdoor spiders of the family Idiopidae (Mygalomorphae: Arbanitinae): a relimitation and revision at the generic level. *Invertebrate Systematics* **31**: 566-634.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013.** MEGA 6: Molecular Evolutionary Genetic Analysis using Maximum Likelihood, evolutionary distance, and Maximum Parsimony methods. *Molecular Biology and Evolution* **30**: 2725 – 2729.
- Thompson J, Higgins D, Gibson T. 1994.** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680. doi:10.1093/nar/ 22.22.4673.

Table 1. Mygalomorph spider specimens used in the present study (n=26), and the genetic lineage to which they belong. Coloured shading refers to colour-coding used to highlight species in Figures 1 to 5. Unshaded cells represent samples that failed to sequence.

Sample ID	Helix ID	Taxonomy	Genetic Lineage/Taxonomic ID
M20190409.ASH05-01	OY01	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20190409.ASHSRE026-01	OY02	Idiopidae	Previously recorded species <i>Euoplos</i> sp IBM_I67
M20190410.ASH05-01	OY03	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20190410.ASH05-02	OY04	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20190410.ASH05-03	OY05	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20190410.ASHSRE01-01	OY06*	Nemesiidae	New species <i>Aname</i> sp NRU_N146
M20190410.ASHSRE34-01	OY07*	Idiopidae	New species <i>Aganippe</i> sp. IBN_I69
M20190411.ASH12-01	OY08	Nemesiidae	Previously recorded species <i>Aname</i> sp NRT_N141
M20190411.ASHSRE026-01	OY09*	Nemesiidae	New species <i>Aname</i> sp NRU_N146
M20190411.ASHSRE026-02	OY10*	Ctenizidae	Previously recorded species <i>Conothele</i> sp. CAO_C27
M20190411.ASHSRE35-01	OY11	Nemesiidae	no data
M20190411.ASHSRE35-02	OY12	Nemesiidae	no data
M20190411.ASHSRE36-01	OY13*	Idiopidae	New species <i>Aganippe</i> sp. IBN_I69
M20190412.ASH09-01	OY14*	Ctenizidae	Previously recorded species <i>Conothele</i> sp. CAO_C27
M20190412.ASH12-01	OY15	Nemesiidae	Previously recorded species <i>Aname</i> sp NRT_N141
M20190412.ASH12-02	OY16	Nemesiidae	Previously recorded species <i>Aname</i> sp NRT_N141
M20190412.ASHSRE37-01	OY17	Nemesiidae	Previously recorded species <i>Aname</i> sp NRT_N141
M20190412.ASHSRE37-02	OY18*	Nemesiidae	Previously recorded species <i>Aname</i> sp NRT_N141
M20190412.ASHSRE46-1	OY19*	Idiopidae	Previously recorded species <i>Euoplos</i> sp IBM_I67
M20190413.ASH02-01	OY20	Nemesiidae	no data
M20190413.ASH12-01	OY21	Nemesiidae	Previously recorded species <i>Aname</i> sp NRT_N141
M20190413.ASHSRE34-01	OY22	Idiopidae	no data
M20190415.ASH02-01	OY23	Idiopidae	Previously recorded species <i>Euoplos</i> sp IBM_I67
M20190415.ASH05-01	OY24	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20190415.ASH05-02	OY25*	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20190415.ASH05-03	OY26	Nemesiidae	Previously recorded species <i>Aname</i> sp. NDW_N57
M20190415.ASH09-01	OY27*	Barychelidae	New species <i>Idiommata</i> sp. BBS_B38
M20190415.ASH11-01	OY28	Nemesiidae	Previously recorded species <i>Aname</i> sp NRT_N141
M20190415.ASH12-01	OY29*	Nemesiidae	Previously recorded species <i>Aname</i> sp NRT_N141
M20190415.ASHSRE04-1	OY30	Idiopidae	Previously recorded species <i>Euoplos</i> sp IBM_I67
M20190417.ASHSRE43-01	OY31	Idiopidae	no data
M20190418.ASHSRE44-01	OY32	Idiopidae	no data

Table 2. Genetic p-distance (below) and the associated standard error (above – blue text) between the fifty-one individuals, from four families (Barychelidae, Ctenizidae, Idiopidae & Nemesiidae) sequenced from the Ashburton Salt survey area (phases 1 & 2) as shown in Figures 1 to 5. Colour-coding corresponds to that used in Figures 1 to 5. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

Table 2 attached separately as a PDF document due to the size and detail.

Table 3. Genetic p-distance (below) and the associated standard error (above – blue text) between the Ashburton Salt mygalomorph (Barychelidae) haplotype (in bold text) shown in Figure 1, and all reference specimens. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

	1	2	3	4	5	6	7	8	9	10	11	12
1 OY27 (BBS_B38)		0.016	0.018	0.017	0.017	0.017	0.017	0.016	0.019	0.016	0.017	0.018
2 BAF_B30_AC6	0.120		0.016	0.016	0.017	0.016	0.017	0.016	0.019	0.016	0.016	0.018
3 BAN_B24_O110	0.141	0.118		0.017	0.017	0.017	0.018	0.016	0.020	0.017	0.018	0.017
4 BAQ_B22_O117	0.128	0.113	0.123		0.017	0.016	0.017	0.016	0.020	0.017	0.017	0.017
5 BBK_B19_CB32	0.123	0.130	0.133	0.130		0.016	0.017	0.017	0.019	0.018	0.017	0.017
6 BL_B17_AR106	0.123	0.120	0.136	0.120	0.110		0.013	0.016	0.019	0.017	0.011	0.016
7 BP_B17_AD111	0.136	0.136	0.146	0.128	0.138	0.066		0.016	0.019	0.017	0.013	0.016
8 BU_B18_AD535	0.107	0.110	0.120	0.107	0.136	0.115	0.110		0.019	0.013	0.016	0.018
9 KJ744742.1 <i>Selenotholus</i> sp. MYG381 T113579	0.164	0.174	0.189	0.189	0.164	0.174	0.169	0.166		0.020	0.020	0.019
10 KJ745100.1 <i>Idiommata</i> sp. MYG320 T123112	0.115	0.118	0.128	0.125	0.143	0.123	0.128	0.077	0.189		0.017	0.019
11 KJ744575.1 <i>Aureococrypta</i> sp. MYG315 T103910	0.125	0.120	0.146	0.123	0.128	0.049	0.077	0.115	0.184	0.128		0.016
12 KJ745481.1 <i>Aureococrypta</i> sp. MYG319 T98886	0.143	0.151	0.133	0.123	0.133	0.110	0.118	0.141	0.179	0.176	0.115	

Table 4. Genetic p-distance (below) and the associated standard error (above – blue text) between all Ctenizidae mygalomorph specimens (family Ctenizidae) haplotypes shown in Figure 2. Colour coding corresponds to Figure 2. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

Table 4 attached separately as a PDF document due to the size and detail.

Table 5. Genetic p-distance (below) and the associated standard error (above – blue text) between all *Euoplos* mygalomorph specimens (family Idiopidae) haplotypes shown in Figure 3. The phase 2 Ashburton Salt *Euoplos* specimen in bold text. All specimens belonging to the *Euoplos* sp. IBM_I67 species shaded in blue, as per colour-coding in Figure 3. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

Table 5 attached separately as a PDF document due to the size and detail.

Table 6. Genetic p-distance (below) and the associated standard error (above – blue text) between all remaining idiopid mygalomorph specimens (family Idiopidae) haplotypes shown in Figure 2. The two Ashburton Salt Phase 2 specimens in bold text. All specimens (n=2) belonging to the *Aganippe* sp. IBN_I69 species, shaded in pink, as per colour-coding in Figure 4. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

Table 6 attached separately as a PDF document due to the size and detail.

Table 7. Genetic p-distance (below) and the associated standard error (above – blue text) between all Ashburton Salt *Aname* mygalomorph (family Nemesiidae) haplotypes shown in Figure 5. Ashburton Salt Phase 2 specimens in bold text. Shaded specimens as per colour-coding for species in Figure 7. Un-corrected p-distances do not account for mutational saturation, which results from back mutations, and therefore provide a conservative estimate of genetic distance.

Table 7 attached separately as a PDF document due to the size and detail.

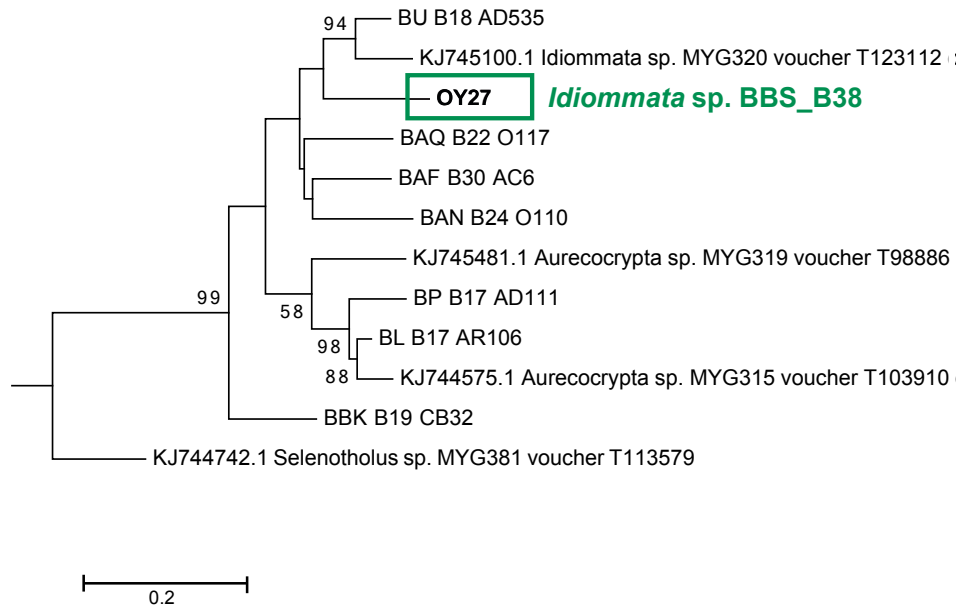


Figure 1. Maximum likelihood analysis of the single barychlid *COI* mtDNA sequence, showing the placement of the Ashburton Salt mygalomorph specimen (in bold text) within the current taxonomic framework of the family Barychelidae. Coloured boxes highlight the species to which the Ashburton Salt specimens belong. Phase 2 specimen in bold text. All sequences within 15 % sequence divergence are represented in the tree. 'B' numbers on tree refer to Barychelidae species based on the 9.5 % species threshold tested in Castalanelli *et al.*, (2014). 'T' numbers refer to WA Museum registration code. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).

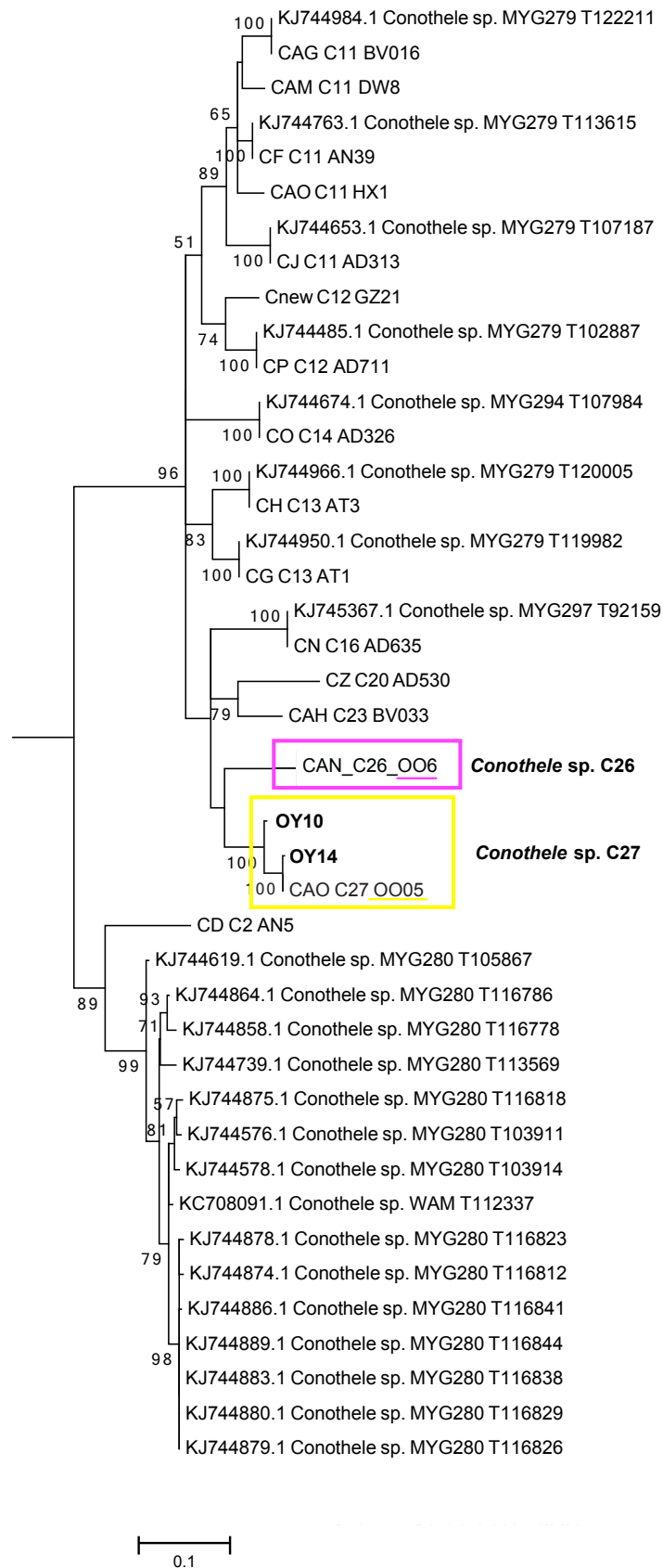


Figure 2. Maximum likelihood analysis of ctenizid COI mtDNA sequences, showing the placement of the Ashburton Salt mygalomorph specimens (in bold text) within the current taxonomic framework of the genus *Conothele* in Western Australia (family Ctenizidae). The coloured box highlights the species to which the Ashburton Salt specimens belong (both phases). Phase 1 specimens are underlined, phase 2 specimens in bold text. All sequences within 15 % sequence divergence are represented in the tree. 'C' numbers on tree refer to Ctenizidae species based on the 9.5 % species threshold tested in Castalanelli et

al., (2014). 'T' numbers refer to WA Museum registration code. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Terminal nodes are collapsed for species. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).

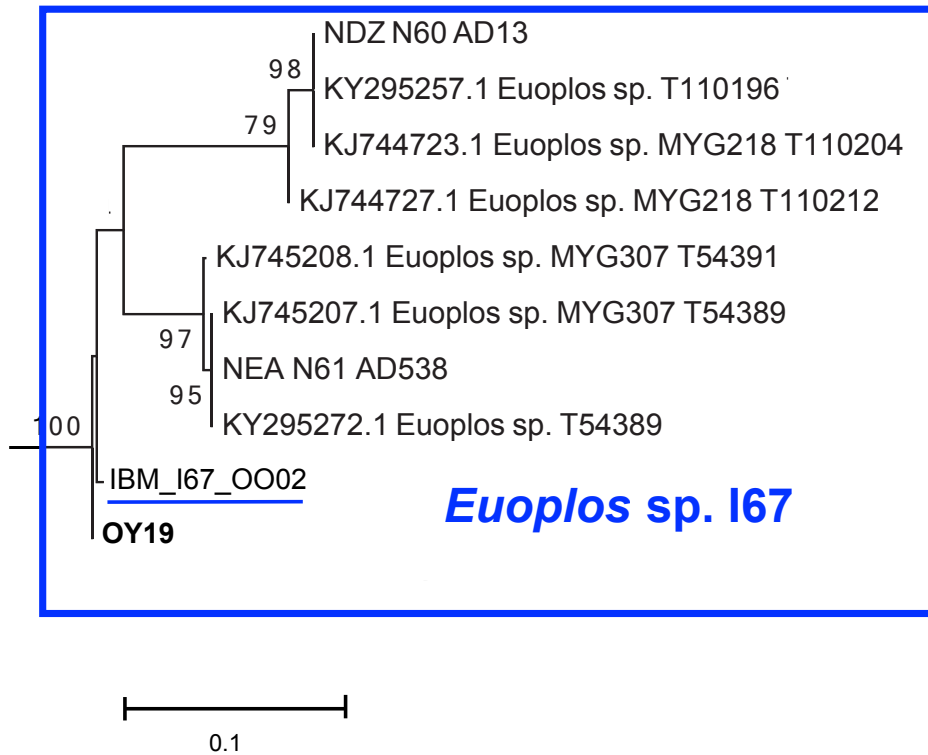


Figure 3. Maximum likelihood analysis of ten COI mtDNA sequences, showing the placement of the Ashburton Salt mygalomorphae specimens (highlighted within coloured boxes) within the current taxonomic framework of the genus *Euoplos* (Idiopidae). The outer coloured box outline indicates the species to which the Ashburton Salt specimens belong. Phase 1 specimens are underlined, phase 2 specimens in bold text. All sequences within 15 % sequence divergence are represented in the tree. 'I' numbers on tree refer to Idiopid species based on the 9.5 % species threshold tested in Castalanelli *et al.*, (2014). 'T' numbers refer to WA Museum registration code. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).

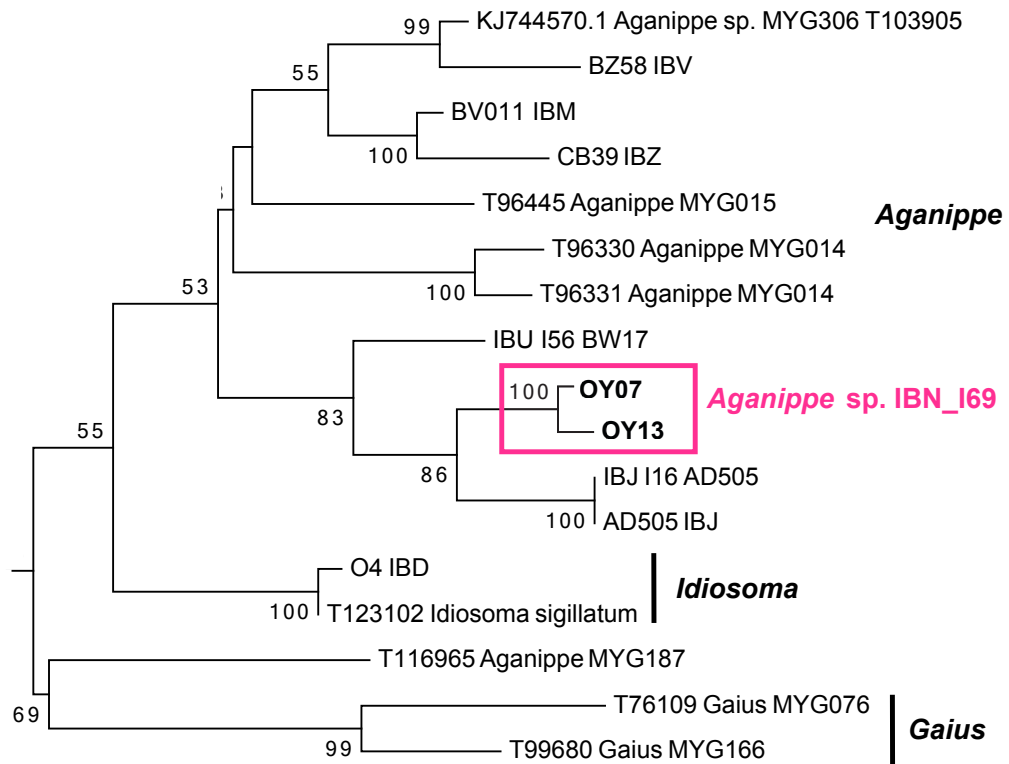


Figure 4. Maximum likelihood analysis of reduced idiopid dataset of seventeen *COI* mtDNA sequences, showing the placement of the Ashburton Salt mygalomorph specimens (highlighted within coloured boxes) within the current taxonomic framework of the genus *Aganippe* (Idiopidae). The outer coloured box outline indicates the species to which the Ashburton Salt specimens belong. Phase 2 specimens in bold text. All sequences within 15 % sequence divergence are represented in the tree. 'I' numbers on tree refer to Idiopid species based on the 9.5 % species threshold tested in Castalanelli *et al.*, (2014). 'T' numbers refer to WA Museum registration code. Numbers on nodes indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).

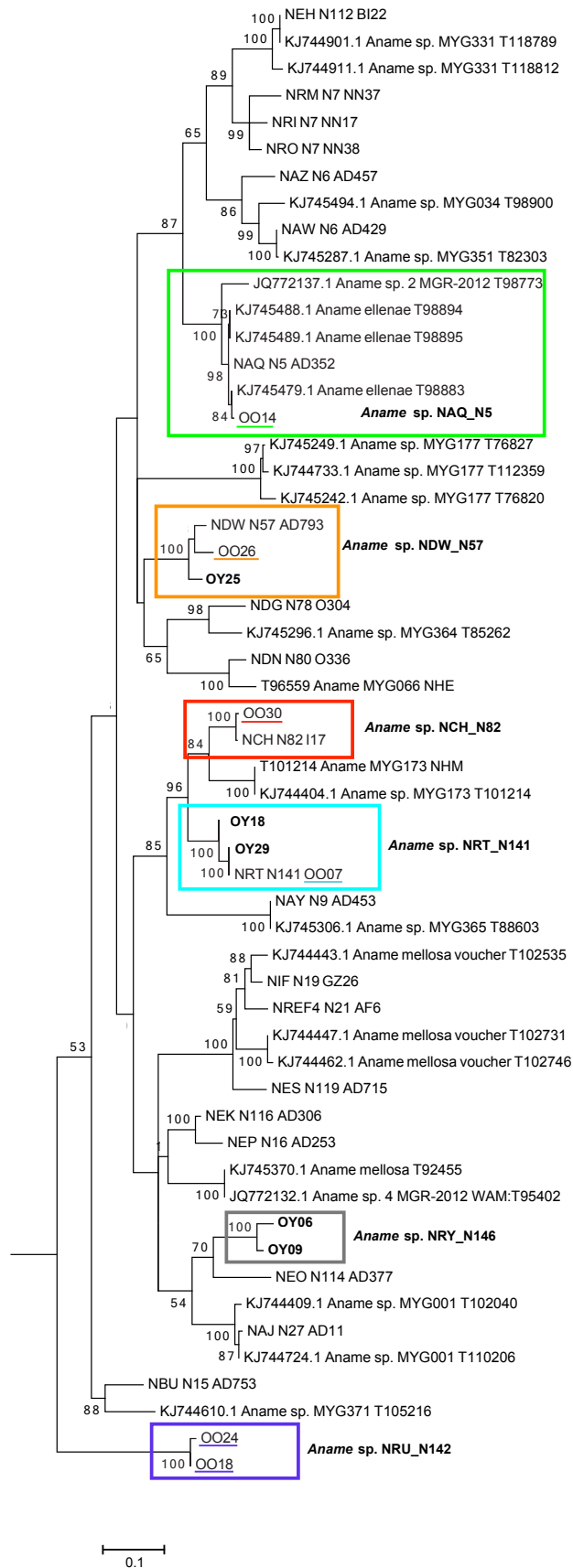


Figure 5. Maximum likelihood analysis of reduced nemesiid dataset of fifty-five *COI* mtDNA sequences, showing the placement of the Ashburton Salt mygalomorph specimens (highlighted within coloured boxes) within the current taxonomic framework of the genus *Aname* (Nemesiidae). The outer coloured box outline indicates the species to which the Ashburton Salt specimens belong. Phase 1 specimens are underlined, phase 2 specimens in bold text. All sequences within 15 % sequence divergence are represented in the tree. 'N' numbers on tree refer to nemesiid species based on the 9.5 % species threshold tested in Castalanelli *et al.*, (2014). 'T' numbers refer to WA Museum registration code. Numbers on nodes

indicate nodal support by means of maximum likelihood (ML) bootstrap values. Bootstrap values <60 are not shown. Scale indicates inferred evolutionary distance (substitutions/site).

Appendix 1. Mygalomorph spider specimens utilized during analysis in the present study, the source of the data, and the genetic lineage to which they belong. Phase 2 Ashburton Salt specimens in Bold Text.

Appendix 1 attached separately as a PDF document due to the size and detail.

Appendix 6

Location of Recorded Conservation Significant Species



Common Name, Species	Conservation Status	Site	Easting (mE)	Northing (mN)	Number	Habitat	Phase
Fork-tailed Swift, <i>Apus pacificus</i>	Migratory	Opportunistic	264152	7587309	2	Beach	Apr 2019
		Opportunistic	268502	7582661	2	Clay loam plain	Apr 2019
Eastern Osprey, <i>Pandion cristatus</i>	Migratory	ASH13E	286758	7594750	1	River bank	Nov 2018
		ASH03	265724	7577572	1	Clay loam plain	Apr 2019
		Opportunistic	264152	7587309	1	Beach	Apr 2019
		Opportunistic	262024	7582485	1	Mangrove	Mar 2019
		Opportunistic	262024	7582485	1	Beach	Mar 2019
		Opportunistic	259966	7582925	1	Intertidal mudflat	Apr 2019
		Opportunistic	256480	7579214	1	Beach	Dec 2019
Common Sandpiper, <i>Actitis hypoleucos</i>	Migratory	ASH13E	286758	7594750	3	River bank	Apr 2019
Common Greenshank, <i>Tringa nebularia</i>	Migratory	ASH13E	286758	7594750	7	River bank	Apr 2019
Red-necked Stint, <i>Calidris ruficollis</i>	Migratory	ASH13E	286758	7594750	6	River bank	Nov 2018
Common Tern, <i>Sterna hirundo</i>	Migratory	Opportunistic	259966	7582925	3	Intertidal mudflat	Apr 2019
		Opportunistic	256595	7581987	1	Beach	Dec 2018
		Opportunistic	256562	7579655	1	Beach	Mar 2019
Little Tern, <i>Sternula albifrons</i>	Migratory	Opportunistic	262024	7582485	5	Mangrove	Dec 2018
		Opportunistic	261515	7583056	2	Mangrove	Nov 2018
		Opportunistic	259966	7582925	2	Intertidal mudflat	Apr 2019
		Opportunistic	258446	7583559	22	Beach	Apr 2019
		Opportunistic	258097	7583262	4	Beach	Dec 2018
		Opportunistic	262024	7582485	5	Beach	Mar 2019
		Opportunistic	256606	7582019	4	Beach	Nov2018
		Opportunistic	256595	7581987	19	Beach	Dec 2018
		Opportunistic	256606	7582019	8	Beach	Nov2018
		Opportunistic	256562	7579655	39	Beach	Mar 2019
		Opportunistic	256480	7579214	2	Beach	Dec 2018
		Opportunistic	256452	7578782	10	Beach	Apr 2019
Gull-billed Tern, <i>Gelochelidon nilotica</i>	Migratory	ASH04	268825	7577035	1	Clay loam plain	Nov 2018
		ASH06	265780	7571042	1	Clay loam plain	Nov 2018
		ASH07	267172	7572900	3	Clay loam plain	Nov 2018
		ASH09	261079	7565343	1	Clay loam plain	Nov 2018
		ASH12	269413	7587983	3	Coastal Dune	Nov 2018
		ASH13E	286758	7594750	4	River bank	Nov 2018
		ASHMAN01CAM	258077	7574202	2	Mangrove	Nov 2018
		Opportunistic	286536	7595044	2	River bank	Nov 2018
		Opportunistic	286536	7595044	1	River bank	Dec 2018

Common Name, Species	Conservation Status	Site	Easting (mE)	Northing (mN)	Number	Habitat	Phase
		Opportunistic	262258	7583930	1	Clay loam plain	Nov 2018
		Opportunistic	262024	7582485	2	Mangrove	Dec 2018
		Opportunistic	261515	7583056	3	Mangrove	Nov 2018
		Opportunistic	260503	7583685	17	Intertidal mudflat	Apr 2019
		Opportunistic	259966	7582925	33	Intertidal mudflat	Apr 2019
		Opportunistic	259754	7583144	2	Intertidal mudflat	Apr 2019
		Opportunistic	256606	7582019	1	Beach	Nov 2018
		Opportunistic	256595	7581987	2	Beach	Dec 2018
		Opportunistic	256606	7582019	2	Beach	Nov 2018
		Opportunistic	258746	7574327	2	Clay loam plain	Nov 2018
		Opportunistic	258095	7574824	3	Intertidal mudflat	Dec 2018
		Opportunistic	256886	7574326	2	Beach	Nov 2018
Caspian Tern, <i>Hydroprogne caspia</i>	Migratory	Opportunistic	262023	7582485	2	Mangrove	Dec 2018
		Opportunistic	260503	7583685	3	Intertidal mudflat	Apr 2019
		Opportunistic	258446	7583559	1	Beach	Apr 2019
		Opportunistic	262024	7582485	1	Beach	Mar 2019
		Opportunistic	256606	7582019	2	Beach	Nov2018
		Opportunistic	256595	7581987	1	Beach	Dec 2018
		Opportunistic	256606	7582019	2	Beach	Nov2018
		Opportunistic	256562	7579655	3	Beach	Mar 2019
		Opportunistic	256480	7579214	2	Beach	Dec 2018
		Opportunistic	256452	7578782	3	Beach	Apr 2019
		Opportunistic	258104	7574802	1	Intertidal mudflat	Dec 2018
		Opportunistic	258104	7574802	2	Intertidal mudflat	March 2019
Opportunistic	256886	7574326	1	Beach	Nov 2018		
White-winged Black Tern, <i>Chlidonias leucopterus</i>	Migratory	Opportunistic	259966	7582925	7	Intertidal mudflat	Apr 2019
		Opportunistic	256562	7579655	12	Beach	Mar 2019
Crested Tern, <i>Thalasseus bergii</i>	Migratory	Opportunistic	260503	7583685	5	Intertidal mudflat	Apr 2019
		Opportunistic	262024	7582485	1	Beach	Mar 2019
		Opportunistic	256595	7581987	2	Beach	Dec 2018
		Opportunistic	256562	7579655	2	Beach	Mar 2019
Peregrine Falcon, <i>Falco peregrinus</i>	OS	Opportunistic	285292	7594314	1	Clay loam plain	Apr 2019
Northern Coastal Free-tailed Bat, <i>Ozimops cobourgianus</i>	Priority 1	ASHMAN01BAT	258104	7574208	C	Mangrove	Nov 2018
		ASHMAN02BAT	255233	7570734	C	Mangrove	Nov 2018

Note: some opportunistic records include birds other than migratory shorebird species recorded in Biota (2019a)

OS= Other Specially Protected Fauna.

C=echolocation calls

Appendix 7

Conservation Significant Species Considered Unlikely to Occur in Study Area



Species Name	Common Name	Conservation Status		Preferred Habitat	Habitat Available in Study Area	Occurrence in Locality	Likelihood of Occurrence in Study Area
		State	Commonwealth				
Herpetofauna							
<i>Ctenotus angusticeps</i>	Airlie Island Ctenotus	Priority 3	Vulnerable	Samphire shrubland or marine couch grassland, in the intertidal zone along mangrove.	✓	Recorded approximately 50 km NE of study area on Airlie Island.	Unlikely to occur
Avifauna							
<i>Oceanites oceanicus</i>	Wilson's Storm-Petrel	Migratory	Migratory	Oceanic species. Breeds in Antarctic and Subantarctic islands, but visits northwest waters during winter.	x	Recorded approximately 25 km west of the study area in the Indian Ocean.	Unlikely to occur
<i>Thalassarche impavida</i>	Campbell's Albatross	Vulnerable Migratory	Vulnerable Migratory	Oceanic species. Occurs in seas of south and west coast, typically as far north as 28° latitude.	x	Nearest NatureMap record is over 100 km S of the study area in offshore waters of Perth.	Unlikely to occur
<i>Thalassarche carteri</i>	Indian Yellow-nosed Albatross	Endangered	Vulnerable, Marine, Migratory	Oceanic species. May occur as far north as the study area	x	Nearest ALA record is 300 km S of the study area at Carnarvon.	Unlikely to occur
<i>Macronectes giganteus</i>	Southern Giant-Petrel	Migratory	Migratory	Oceanic species. Breeds in Antarctic and Subantarctic islands. May occur as far north as the study area.	x	Nearest NatureMap record is 300 km S of the study area at Carnarvon.	Unlikely to occur
<i>Ardenna pacifica</i>	Wedge-tailed Shearwater	Migratory	Migratory	Breeds on islands off Western Australian coast.	x	Recorded approximately 25 km NW of the study area in the Indian Ocean and at Serrurier Island	Unlikely to occur
<i>Limnodromus semipalmatus</i>	Asian Dowitcher	Migratory	Marine, Migratory	Common in coastal mudflats further north on the Pilbara coast.	✓	Nearest ALA record from offshore at Barrow Island, some 150 km north of the study area	Unlikely to occur
<i>Calonectris leucomelas</i>	Streaked Shearwater	Migratory	Migratory	Western seas south to 28° latitude. Breeds on sub-tropical islands of northwest Pacific. Uncommon.	x	Nearest record approximately 100 km NW of study area on Barrow Island. Records also exist in Carnarvon vicinity.	Unlikely to occur
<i>Fregata ariel</i>	Lesser Frigatebird	Migratory	Migratory	Common in tropical seas south to Dampier Archipelago. Breeding on remote islands.	x	Nearest ALA record from 100 km SW of study area on oceanic side of Northwest Cape.	Unlikely to occur
<i>Anous stolidus</i>	Common Noddy	Migratory	Migratory	Northern and western seas, south to Lancelin. Inhabits remote islands.	x	Recorded on Mackeral Islands approximately 20 km northwest of study area.	Unlikely to occur
<i>Phaethon lepturus</i>	White-tailed Tropic Bird	Migratory	Marine, Migratory	An oceanic species breeding on offshore tropical islands.	x	Offshore records north of Exmouth, some 60 km west of the study area	Unlikely to occur
<i>Onychoprion anaethetus</i>	Bridled Tern	Migratory	Migratory	Oceans, coasts and Islands throughout much of Western Australia.	✓	Recorded approximately 13 km W of study area	Unlikely to occur
<i>Pezoporus occidentalis</i>	Night Parrot	Critically Endangered	Critically Endangered	Arid or semi-arid spinifex grasslands with large, established and unburnt hummocks. Foraging habitat includes areas of samphire, bluebush and saltbush.	✓	Previously recorded in 1969 at Onslow (19 Km NE of study area), and approximately 40 km S of study area.	Unlikely to occur
<i>Motacilla tschutschensis</i>	Eastern Yellow Wagtail	Migratory	Migratory	Paddocks, marshes and grassy wetlands.	x	Nearest record located approximately 500 km E of study area at the Fortescue Marsh.	Would not occur
<i>Motacilla cinerea</i>	Grey Wagtail	Migratory	Migratory	Fast-flowing streams, often at high altitude. Outside of the breeding season it is found in greater variety of habitats.	x	Single record approximately 500km E of study area on Fortescue Marsh. Rare migrant to Australia, very rare south of Kimberley region.	Would not occur
Mammals							
<i>Perameles bougainville</i>	Shark Bay Bandicoot or Little Marl	Vulnerable	Endangered	Semi-arid shrubland	✓	Extinct on the mainland.	Would not occur
<i>Pseudomys chapmani</i>	Western Pebble-mound Mouse	Priority 4	–	Stony hillsides and plains with hummock grasslands.	✓	Recorded 14 km E of study area.	Unlikely to occur