Targeted survey for the bare-rumped sheath-tailed bat in the South of Embley Project area, near Weipa, Queensland

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Contents

SUMMARY

RTA Weipa Pty Ltd (RTA) has identified significant bauxite reserves south of the Embley River near Weipa, Cape York peninsula, Queensland, that could sustain a mining operation for around 40 years. This planned 'South of Embley' (SoE) Project is defined by a mining lease extends in a forested strip c. 15 km wide along the coast between the Embley River and the Aboriginal Community of Aurukun. RTA submitted Environmental Impact Statements for this project to both the Queensland State Government (Rio Tinto Alcan 2011, 2012) and the Commonwealth Government (Rio Tinto Alcan 2013), which included studies on threatened species. This report describes two field surveys and an acoustic analysis on one of those species of conservation significance, the bare-rumped sheath-tailed bat *Saccolaimus saccolaimus nudicluniatus,* listed currently as 'Critically Endangered' under the *Environmental Protection and Biological Conservation Act 1999 (Cth).*

Survey methods and effort were consistent with that suggested by the Commonwealth's "Survey guidelines for Australia's threatened bats" (DEWHA 2010), and included representation in the dominant *Eucalyptus tetrodonta* closed forest habitat as well as riparian vegetation communities, and representation across the large Project area both inside and outside the planned infrastructure footprints. Survey methods were designed to maximise the likelihood of detection of this rare, high flying species of bat, and included trapping with harp traps and mist nets in the forest canopy, and acoustic surveys based on full spectrum (high quality) recordings with ultrasonic recorders ('bat detectors').

The present study represents a good benchmark for targeted surveys for *S. saccolaimus* in a large project area such as South of Embley. It included what we believe is the greatest targeted survey effort for this species (20 capture nights, with multiple traps/nets deployed per night) and the most effort with an appropriate capture technique (rope-mounted mist nets in the tree canopy). The survey did not capture any *S. saccolaimus* but had the largest capture return of two other *Saccolaimus* spp. (74 individuals) ever recorded in Australia. In these ways, the study was unprecedented. In addition, the total deployment of 110 full nights of recording with full spectrum detectors is also one of the largest acoustic surveys conducted in a single targeted survey programme in Australia, and has associated with it the largest reference echolocation call dataset from *Saccolaimus* that has been compiled to date. The effort compares well with that recommended in the Commonwealth Government's "Survey guidelines for Australia's threatened bats" (DEWHA 2010), and provides what we believe is the first comprehensive demonstration of an appropriate level of effort consistent with the guidelines for this species, at least in a large project area. Finally, the study also provided

the first quantitative analysis of the acoustic differences in signature echolocation calls amongst the three species of *Saccolaimus* in Australia with a novel multivariate statistical method, and pointed to where the analysis of a large datasets might be both useful and limited.

Briefly, the main conclusions of the study were:

1. Two species of sheath-tailed bat *Saccolaimus* spp. were confirmed unambiguously from the South of Embley Project area: the yellow-bellied sheath-tailed bat *S. flaviventris* and the Papuan sheath-tailed bat *S. mixtus*, based on examination of external morphology and confirmed subsequently with DNA barcoding.
2. There was no unambiguous evidence of the occurrence of the bare-rumped sheath-tailed bat *S. saccolaimus* in the South of Embley Project area. No captures were made, and while there were limitations in the acoustic analysis, there was no indication of presence from recordings of bat echolocation.
3. The rates of both capture and acoustic recordings of the two *Saccolaimus* species from across the SoE Project area suggested that the *Eucalyptus tetrodonta*-dominated forest south of Weipa represented suitable roosting and foraging habitat for these species, but particularly *S. mixtus*.
4. The higher number of echolocation calls recorded on the October 2012 survey compared to the June 2012 survey suggested either higher activity or higher local abundance of the two *Saccolaimus* species present at that time.
5. Vertical arrays of mist nets hoisted into the canopy were a highly effective method of capturing *Saccolaimus* species, as well as several other species of bat.
6. The study presents the first quantitative comparison of the echolocation calls of the three Australian species of *Saccolaimus* using multivariate statistics. Bulk amounts of putative bat pulses from unattended ultrasonic recordings could be tested for association with distinct confidence regions for each species based on a novel implementation of the formula for a predictive confidence ellipse. This allowed a very large amount of acoustic data to be analysed in a reasonable timeframe.
7. Based on acoustic data, it was clear that a large population of *S. saccolaimus* was not present, and if it was present in low numbers, these were such that it was undetectable given the significant survey expended effort and contemporary methodology used.
8. The acoustic methods provide good scope for long term monitoring of *S. mixtus* in the Project area, as a relatively abundant indicator species that depends on the *E. tetrodonta* forest for suitable habitat.

1.0 INTRODUCTION

1.1 Project background

RTA Weipa Pty Ltd (a subsidiary of Rio Tinto Aluminium Ltd, formerly Comalco Aluminium Ltd) has been mining bauxite since 1963 from the Weipa area, north of the Embley River on Cape York Peninsula, Queensland. These reserves are gradually being depleted and continuing demand for bauxite has encouraged exploration further afield. Extensive drilling by RTA on mining lease ML7024 has identified significant reserves south of the Embley River that could sustain a mining operation for around 40 years. Development of this reserve would involve the construction and operation of a bauxite mine and associated processing facilities, barge and ferry terminals, a port and shipping activities. This planned 'South of Embley' (SoE) Project (the Project) will involve a staged increase in production up to 50 million dry product tonnes per annum, though initially 22.5 million dry product tonnes per annum. The mining lease extends in a forested strip c. 15 km wide along the coast between the Embley River and the Aboriginal Community of Aurukun (Rio Tinto Alcan 2011, 2013).

The SoE Project was declared a 'significant project' by the Queensland State Government and a 'controlled action' by the Commonwealth Government, both of which required the preparation of an Environmental Impact Statement (EIS) (Rio Tinto Alcan 2011, 2012, 2013). Listed threatened species and communities (sections 18 and 18A of the *Environmental protection and Biological Conservation Act 1999 (Cth); (EPBC Act 1999)*) were relevant controlling provisions for the Commonwealth EIS (Rio Tinto Alcan 2013). One of those species of conservation significance was the bare-rumped sheath-tailed bat *Saccolaimus saccolaimus nudicluniatus.* This species is listed currently as 'Critically Endangered' under the *EPBC Act 1999* (SEWPaC 2013), is listed as 'Endangered' under Queensland's *Nature Conservation Act 1992*, and is ranked as a 'high priority' under the Queensland Department of Environment and Heritage Protection 'Back on Track' species prioritisation framework. The potential for its occurrence in the SoE Project area was acknowledged based on several observations:

1. Records from the eastern side of Cape York in Iron Range and north of Coen (Murphy 2002; Reardon et al. 2010);
2. A predicted distribution in the authoritative guide to Australian mammals (Hall et al. 2008; but see Schulz and Thomson 2007) that includes the most north-western parts of Cape York;

3. Its use of woodland habitats and riparian forest, which are represented at SoE;
4. The detection of one or more species of *Saccolaimus* during AnaBat-based acoustic surveys in the Project area between 2007 and 2009 (Rio Tinto Alcan 2013:page 6-98);
5. The general lack of surveys for bats on the western side of Cape York.

The present survey builds on the work undertaken by Balance! Environmental Pty Ltd who undertook the initial bat survey work using AnaBat acoustic detectors and harp traps in the Project area. The survey effort and trapping outcomes (but not the acoustic survey) from two 2012 dry season surveys, as described in the present document have been included in the Commonwealth EIS (Rio Tinto Alcan 2013).

1.2 Bare-rumped sheath-tailed bat

The bare-rumped sheath-tailed bat *S. saccolaimus* is represented by two population isolates in Australia: the north-west and around the Roper River area of the Northern Territory (various authors represent the extent of the Northern Territory distribution differently), and a relatively narrow coastal strip c. 40 km wide extending from Iron Range to just south of Townsville in north-eastern Queensland (Schulz and Thomson 2007; Csorba et al. 2008; Churchill 2008; Hall et al. 2008; **Figure 1**). The species was first described from Java (Temminck 1838; see Mahoney and Walton 1988), and it has a scattered distribution as five name-bearing entities (at either species or subspecies level depending on taxonomic predilection) between the west coast of India to Guadalcanal in the Solomon Islands (Simmons 2005; Csorba et al 2008). The Queensland population is referred to the trinomial *Saccolaimus saccolaimus nudicluniatus* (De Vis, 1905) that was first collected at Gowrie Creek near Cardwell, Queensland (Mahoney and Walton 1988). The Northern Territory population is not referred to any subspecific name under Territory legislation. Previously, Milne et al. (2009) found the two Australian population isolates to be genetically very similar based on a short mitochondrial DNA marker. Further taxonomic studies in progress (K.N. Armstrong unpublished) are investigating whether it is referrable to the nominate subspecies *S. s. saccolaimus* by placing both populations into a regional context.

In Queensland, *S. saccolaimus* is known from 23 museum specimens (21 specimens deposited with the Queensland Museum; two specimens deposited with Museum Victoria) and around eight observational records in the Queensland Government's WildNet database. All the records are within 50 km of the east coast, and are spread through three IBRA Bioregions (Cape York Peninsula, Wet Tropics, Brigalow Belt North; Thackway and Cresswell 2006) between Iron Range and just south of Townsville (**Figure 1**). Where habitat data is

available, most specimens have been collected or observed in *Eucalyptus platyphylla* woodland and riverine vine forest with sclerophyll elements. Within the last decade or so, roost sites have been discovered in Iron Range National Park (Murphy 2002) and Cairns (three trees in the Cairns Botanic Garden, and one elsewhere in Cairns). The roost discovered in Iron Range was located in the trunk of a dead *E. tetrodonta* in *E. tetrodonta*-dominated savanna woodland, with gallery forest containing rainforest tree species within 100 m. Two of the roosts in the Cairns Botanic Gardens are in *Melaleuca* sp. (G. Ford and K.N. Armstrong pers. obs.). Most roosts observed have been in broken tree trunks rather than branches. Given the widespread nature of these eucalypt woodlands and forests across northern Australia, the potential for roosting appears to be high, and its apparent absence in similar habitats between the east coast of Cape York and Darwin might be a perception based on lack of survey effort.

In the past, obtaining an unambiguous identification of the bare-rumped sheath-tailed bat has been complicated by practical and safety constraints in remote northern areas. Its echolocation calls are similar to those of other *Saccolaimus,* and also bats in the genera *Chaerephon* and *Mormopterus*, it flies high and is thus difficult to capture, and the typical practice of netting bats over waterways where they might fly lower is prevented by the risks posed by estuarine crocodiles. The present study sought to overcome these practical limitations in light of the Commonwealth survey guidelines for this species (DEWHA 2010).

There are two other species of *Saccolaimus* on Cape York – the yellow-bellied sheath-tailed bat *S. flaviventris* and the Papuan sheath-tailed bat *S. mixtus*, the latter being listed as 'Near Threatened' under Queensland's *Nature Conservation Act 1992*, and the former as 'Least Concern'. The known distribution of *S. mixtus* is the very northern area of Cape York, including parts of the Weipa Plateau near the west coast (Churchill 2008). It is thought to roost in trees like the other *Saccolaimus* in Australia, though one was observed in a limestone cave in Papua New Guinea (Tate 1941), and foraging was observed over tall open forests and woodlands. Bonaccorso (1998) lists only four records from Papua New Guinea. It is smaller in size than the other two Australasian *Saccolaimus*, with white belly fur and dark dorsal fur with white tips that gives it a grizzled appearance (Churchill 2008). The echolocation calls of *S. mixtus* have only recently been recorded and were commented to be distinguishable from those of *S. flaviventris*, though possibly similar to those of *S. saccolaimus* (Reardon et al. 2010), however no analysis was provided.

In contrast to the other two *Saccolaimus*, *S. flaviventris* has a wide distribution across northern Australia, and it migrates to the southern areas of eastern Australia between January

and April. Large tree hollows are their favoured roost sites, and they forage over a wide variety of habitats. *S. flaviventris* is of similar size to *S. saccolaimus*, with pale to yellow belly fur and shiny black fur on the dorsal surface (Churchill 2008). The mean characteristic frequency of their echolocation call is marginally lower in the Pilbara region of Western Australia compared to the Northern Territory (McKenzie and Muir 2000; McKenzie and Bullen 2009; Reinhold et al. 2001; Milne 2002;), but until recently when full spectrum detectors allowed the inspection of harmonic components of pulses its call could not always be distinguished readily from that of the northern free-tailed bat *Chaerephon jobensis* (McKenzie and Muir 2000; McKenzie and Bullen 2009; K.N. Armstrong unpublished data).

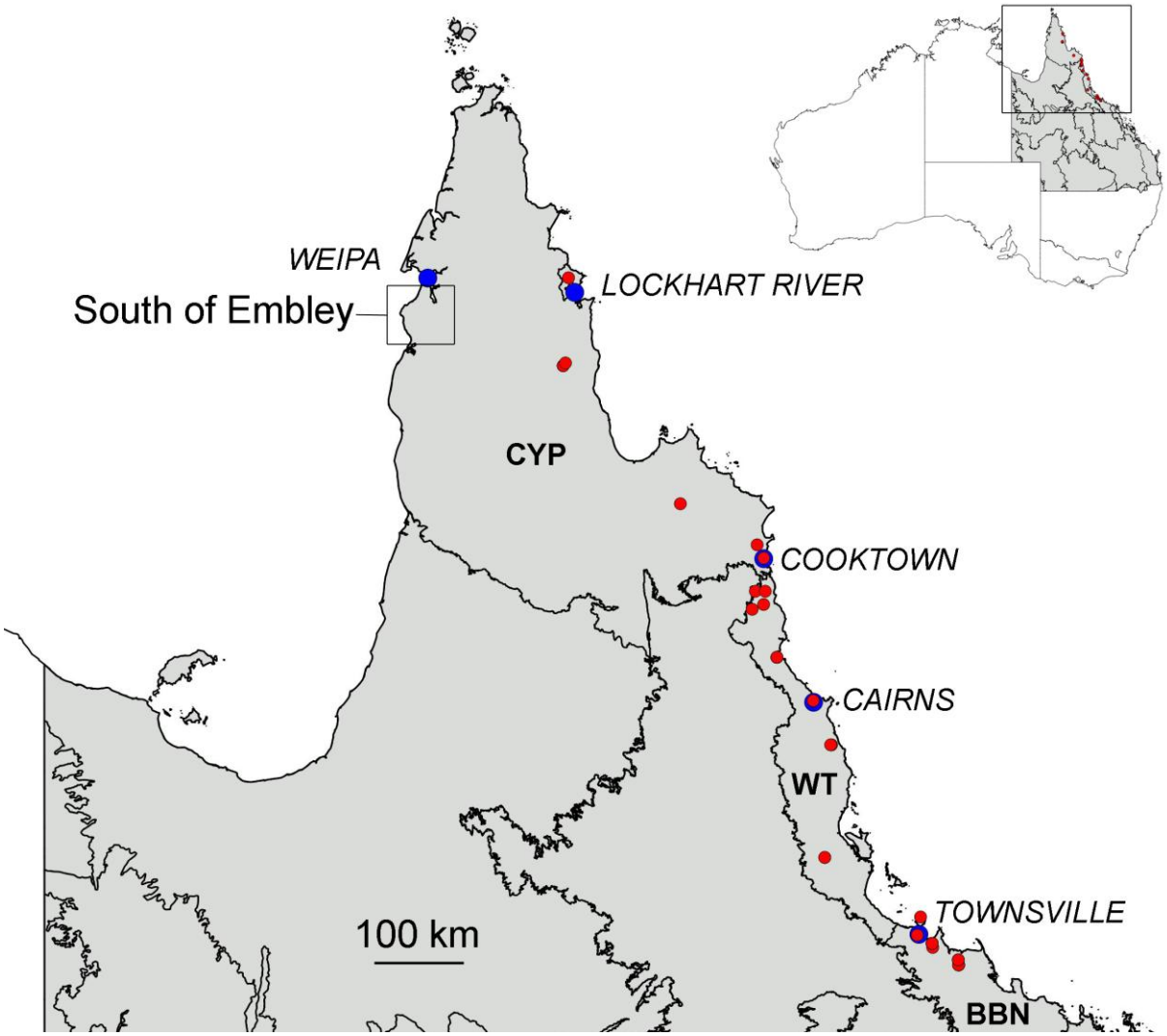

Figure 1. Queensland records of the bare-rumped sheath-tailed bat *S. saccolaimus* in near-coastal habitats between Lockhart River and near Townsville, and the location of the South of Embley Project area (blue dots: towns; red dots: records from WildNet and museums; includes IBRA Bioregion boundaries from DEHP 2013; CYP: Cape York Peninsula; WT: Wet Tropics; BBN: Brigalow Belt North).

1.3 South of Embley Project area

The SoE Project area falls within the Weipa Plateau Subregion (*sensu* Sattler and Williams 1999) of the Cape York Bioregion (Thackway and Cresswell 2006). This subregion covers a large area of the central and north-western part of the Cape York Peninsula, and includes the extensive bauxite plateau landscape from the Wenlock River basin south to Oyala Thumotang National Park (previously Mungkan Kandju National Park) and extending c. 70 km from the east coast (Rio Tinto Alcan 2011; DEHP 2013). It is relatively homogenous in vegetation and landform, and is characterised by extensive areas of Darwin stringybark *Eucalyptus tetrodonta* open forest and woodland, which are dissected by smaller areas of riparian vegetation, patches of vine thicket and paperbark swamps. Vegetation units have been defined previously by Godwin (1985) and Gunness et al. (1987), which have been classified into a series of 'Regional Ecosystems' (RE) (Sattler and Williams 1999). The Darwin stringybark woodland ("*E. tetrodonta, Corymbia nesophila* tall woodland on deeply weathered plateaus and remnants"; RE 3.5.2) comprises 87% of the Project area and 99% of the disturbance area for the Project (Rio Tinto Alcan 2011). Given that the bare-rumped sheath-tailed bat has been observed roosting in the trunks of tall trees in eucalypt woodland, both the stringybark woodland and other riparian REs were regarded as having the potential to be used by this species.

1.4 Acoustic surveys for bats

The present survey for the bare-rumped sheath-tailed bat incorporated acoustic recordings as a means of potentially identifying the species across the Project area from numerous sites. The ultrasonic echolocation calls of bats, which are produced for orientation in darkness and prey detection and localisation in flight, are useful for species identification because each produces a unique and distinguishable (in most cases) signal type. Analysis of the recordings made using electronic 'bat detectors' can reveal echolocating bat diversity at sampling sites with minimal effort, and usually with much greater rates of encounter than trapping. Bat detectors are placed and left to record unattended over a full night at the maximum number of sites in a project area. This is termed 'passive detection' and results in the recording of 'anonymous' calls that need to be attributed to a species.

Detectors such as the Wildlife Acoustics SM2BAT record in full spectrum (high quality) bitstream format that captures the harmonic structure of calls, which can be particularly useful for discriminating the Molossidae from the Emballonuridae in northern Australia (K.N.

Armstrong unpublished data). The SM2BAT microphone also has a wide zone of reception and is suited to recording high flying species of bat.

The first step in any analysis of acoustic recordings of bats is attributing the recorded 'anonymous' call types to individual species. This is usually done with the help of a 'reference library' of good quality calls recorded from confidently identified bats. There are potentially three species of sheath-tailed bat *Saccolaimus* sp. in the SoE Project area: *S. flaviventris*, *S. mixtus* and *S. saccolaimus*. Distinguishing these three species acoustically is not straightforward, despite some recent literature and conference presentations that have pointed to subtle but diagnostically useful characters and sequence patterns (Milne et al. 2009; Corben 2010; Coles et al. 2012; Ford et al. 2012). These include patterns of alternating characteristic frequency in successive pulses, distinctive feeding buzzes and triplets of pulses with a unique patterns and harmonic components. However, a comprehensive attempt to separate the three Australian *Saccolaimus* based on acoustic characters has not been published, and simply attempting to identify these diagnostic features in calls attributable to *Saccolaimus* sp. is difficult because of the unknown degree of intra-species call variation and the overlap in acoustic parameters amongst them. Addressing the shortfall in knowledge about acoustic discrimination was a major aim of the present study.

2.0 AIMS

1. To determine if the bare-rumped sheath-tailed bat *Saccolaimus saccolaimus* was present in the South of Embley Project area using a combination of capture and acoustic recording techniques;

2. If the bare-rumped sheath-tailed bat is present, determine its degree of commonness, vegetative habitat affinity and the location and character of roost sites in trees;

3. To determine if other *Saccolaimus* species (Papuan sheath-tailed bat *Saccolaimus mixtus* and the yellow-bellied sheath-tailed bat *S. flaviventris*) were present in the South of Embley Project area;

4. To capture all species of bat, but particularly species of *Saccolaimus*, and record reference echolocation calls that will assist with their discrimination in acoustic surveys;

5. To develop a means of distinguishing and identifying species of *Saccolaimus* using acoustic recordings;

6. To determine which methods are appropriate for the survey of *Saccolaimus* species.

3.0 METHODS

3.1 Survey locality, timing and approach

The present survey was undertaken in the South of Embley Project area, south of Weipa, Cape York Peninsula, in northern Queensland (**Figure 1**). The area contains an extensive gridded system of tracks cleared for mining exploration and pre-mining surveys. Prior to the present survey, relevant tracks were cleared of debris to allow access to targeted RE habitats, and these are shown on the various maps in this report. Track access in the Project area is severely restricted by waterlogging in the wet season, so the investigation was limited to the dry season only. Two separate dry season surveys were conducted to maximise the potential to encounter the target species: 16 to 26 June, and 9 to 20 October 2012, representing the early and late dry season.

The approach included two main methods – capture using mist nets and harp traps, and making acoustic recordings with full spectrum bat detectors. The capture of bats was particularly important not only for providing confidence in their identification (*see Section 3.3),* but also provided the opportunity to collect reference calls linked to a voucher (in this case a DNA barcode and sets of morphological characters from released individuals) to help identify the anonymous calls recorded on bat detectors deployed around the SoE Project area. The acoustic component of this survey was not reported in the Commonwealth Environmental Impact Statement (Rio Tinto Alcan 2013), as the analysis was still being undertaken. Thus, only the survey effort and trapping success were included in Rio Tinto Alcan (2013) and a full account of all survey results is presented in the current document.

The surveys conducted between 2007 and 2009 (as summarised in Rio Tinto Alcan 2013) preceded the release of the Commonwealth Government's "Survey guidelines for Australia's threatened bats" (DEWHA 2010), which provides a guide to the methods and survey effort deemed sufficient to demonstrate whether this species is present during an environmental impact assessment. They also preceded the availability of field deployable full spectrum bat detectors, which provide greater potential for identifying and discriminating some species of bat based on their echolocation call. Thus, the present survey had a wider range of techniques, equipment and guidelines available to it than the initial surveys between 2007 and 2009.

Prior to the present survey, indicative sampling sites for both trapping and acoustic recordings were chosen across the study area to ensure that effort was represented both within and outside of infrastructure footprints, and which also included the RE habitats most likely to provide either roosting or foraging habitat for sheath-tailed bats. Most effort was directed to the dominant stringybark woodland (RE 3.5.2), but the riparian areas around Dam C were also a priority (**Figure 2**). The approach was discussed with Commonwealth Government representatives in the Department of Sustainability, Environment, Water, Population and Communities regarding the design of the survey, mostly in terms of methods and survey effort.

All GPS location coordinates presented in this report are given in datum GDA94, zone 54L, and GIS mapping was undertaken in QUANTUM GIS version 1.8.0 software.

3.2 Trapping

Bats were trapped using both mist nets and harp traps. With the exception of pole-mounted mist nets, the equipment was hoisted into the tree canopy in order the maximise the likelihood of encountering high flying bat species such as *Saccolaimus* spp.

Harp traps are large aluminium frames that suspend vertically arranged sets of taut fishing line over a bag (Constantine 1958; Tidemann and Woodside 1978) and can be left unattended over a night and checked in the morning for captures. 'Triplebank' (i.e. a set of three vertical line sets; cf. 'doublebank') harps were used at all sites (except site H05) to maximise the likelihood of bat entanglement and capture. Potential bat flyways were identified in the open spaces along the tracks, and harps were suspended from branches with rope in the path of these flyways. In one case, two harp traps were positioned side by side to adequately cover a potential path (**Figure 3**). The elevation of traps varied between c. 10 and 25 m at their highest point, depending on the height of vegetation in certain habitats (lower near riparian areas). Harp traps were left overnight and checked in the early morning for captures. They were not used on the October survey, in favour of extra rope-mounted canopy mist nets that were found to be more successful.

Fine monofilament mist nets were used in two ways: 1. tensioned between telescopic poles (12 m length; maximum 7 m height), and 2. hoisted into the canopy on a rope frame suspended between two trees (modified after Sedgeley et al. 2012). The pole-mounted nets were positioned mainly across tracks and track intersections in open spaces where high-flying

bats were more likely to come closer to the ground. The rope-mounted net consisted of a single piece of rope suspended over the upper branches of two trees spaced c. 15 – 20 m apart, plus two large loops of rope 15 m apart that dropped from the frame between the two trees. Mist nets (three 15 m monofilament nets) were stacked vertically between the loops, and could be hoisted up or down to recover captures and reset the apparatus (**Figure 4**). Mist nets were attended at all times, and left open from dusk until 11.00 pm. On one occasion, a rope-mounted net was run for an entire night (site M03 on 19/6/2012). The placement of all equipment (trapping, acoustic recorders) is mapped in **Figure 5**, and survey effort is summarised in **Table 1** (see also **Appendices 1** and **2**).

Figure 2. Selected representative habitats in the South of Embley Project area. Clockwise from top left: *Eucalyptus tetrodonta* closed forest (RE 3.5.2); *E. tetrodonta* with *Banksia* (RE 3.3.21); *Melaleuca*; primary freshwater stream (RE 3.3.9), Centre: coastal freshwater lagoon at Pera Head (adjacent RE 3.2.3).

Figure 3. Trapping equipment employed on the survey. Clockwise from left: triple-stacked 15 metre monofilament mist nets suspended on a rope frame; monofilament mist net suspended between two telescopic poles; triple-bank harp traps suspended c. 25 m above ground from *E. tetrodonta*; detail of the harp traps showing their position in an anticipated bat 'flyway'.

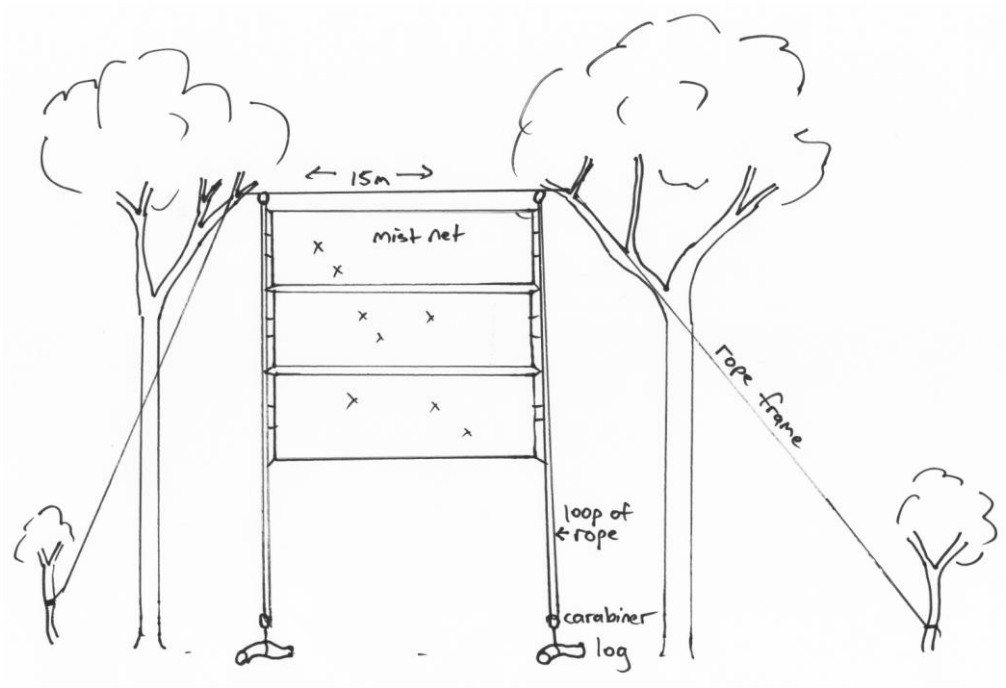

Figure 4. Schematic diagram of the rope-mounted mist net apparatus (modified after Sedgeley et al. 2012).

Table 1. Summary of survey effort from trapping and acoustic recording activities. See Appendix 1 for full details of each location.

	June 2012		
	No. sites	**No. trap nights**	**No. trap hours**
Harp traps	11	43	516
Pole mounted mist nets	4	4	20
Rope mounted mist nets	6	8	30
SM2BAT detectors	54	54	648
	October 2012		
	No. sites	**No. trap nights**	**No. trap hours**
Harp traps	0	0	0
Pole mounted mist nets	15	15	53
Rope mounted mist nets	20	20	72
SM2BAT detectors	56	56	616

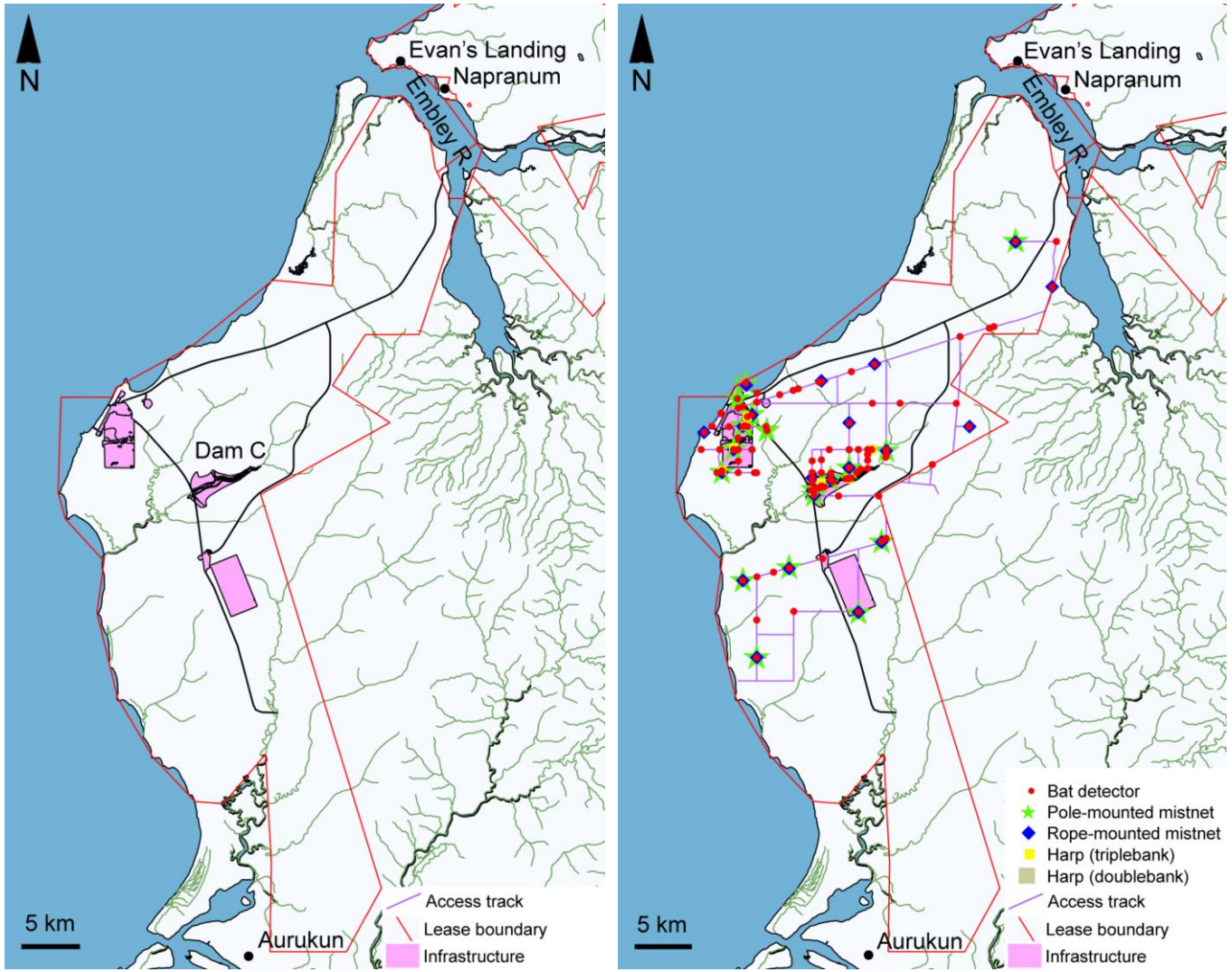

Figure 5a. Location of the ML7024 lease boundary (south of the Embley River), planned infrastructure footprints, and total equipment deployment throughout the SoE Project area during both 2012 surveys.

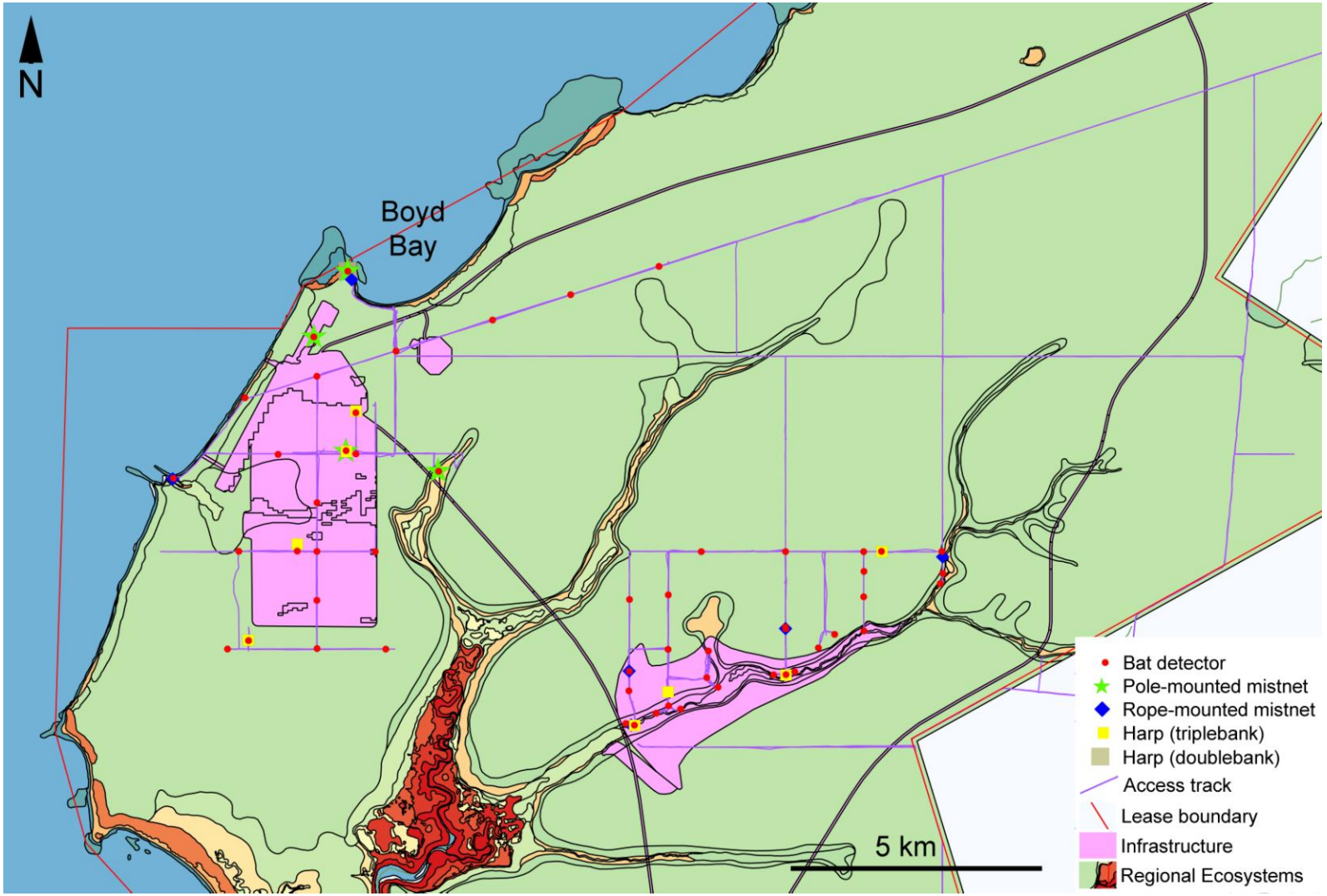

Figure 5b. Equipment deployment throughout the SoE Project area during the June 2012 survey, zoomed to the extent of site access.

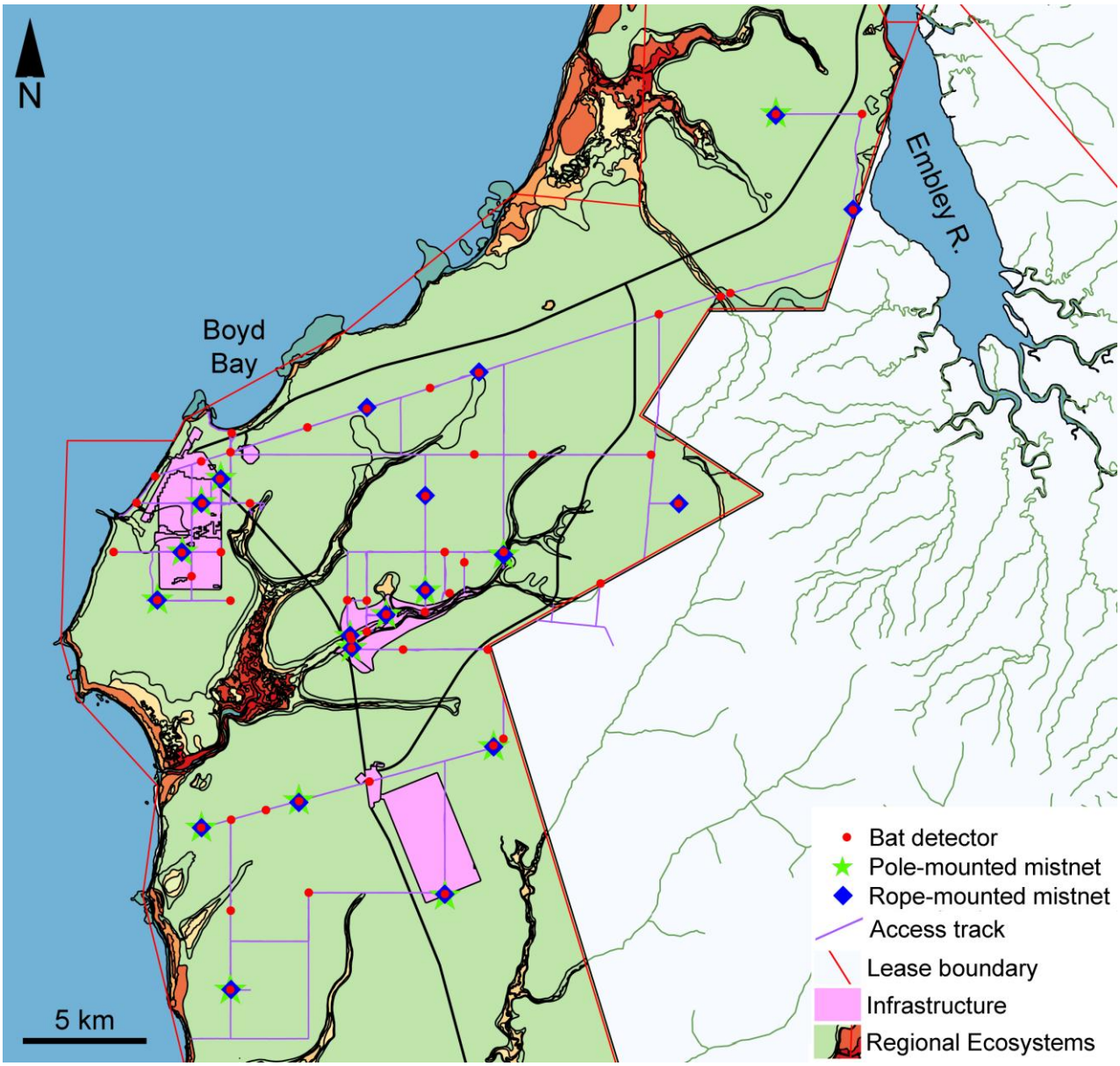

Figure 5c. Equipment deployment throughout the SoE Project area during the October 2012 survey, zoomed to the extent of site access.

3.3 Identification of Saccolaimus species

The Commonwealth's "Survey guidelines for Australia's threatened bats" (DEWHA 2010) states that:

> "*Saccolaimus saccolaimus* and the Yellow-bellied Sheathtail-bat *S. flaviventris* can be difficult to distinguish, and in some cases can only be identified by genetic analysis (Milne et al 2009)."

Given this, species of *Saccolaimus* captured during the survey were identified based on two approaches: observing diagnostic external morphological characters, and DNA barcoding. Based on descriptions in the published literature and the author's (K.N. Armstrong) examinations of these species as part of current taxonomic investigations, the three species can be distinguished readily using the following combination of characters: forearm length, body weight, presence/absence of wing and throat pouches and pelage colour (Churchill 2008), plus the shape of lamdoidal crest as palpated in live individuals (ball-like in *S. saccolaimus* and *S. mixtus*; linear ridge in *S. flaviventris*; K.N. Armstrong unpublished observations). Summary information from captures was noted for cross reference with the results from DNA barcoding.

A subset of *Saccolaimus* individuals captured had a biopsy sample removed from the wing membrane with a sterile 4 mm dermal punch. The tissue was preserved in 20% Dimethyl sulphoxide/saturated saline solution (Worthington Wilmer and Barratt 1996). DNA was extracted using a salting-out method followed by precipitation in isopropanol. Polymerase Chain Reaction (PCR) was conducted using primers that amplified a fragment (positions 1–801) of the mitochondrial *cytochrome-b* gene plus 45 bases of the adjacent *tRNAglu* region (M1226 forward: 5'-AATGACATGAAAAATCACCGTTGT-3' and M40 reverse: 5'-AAATAGGAARTATCAYTCTGGTTTRAT-3'), Promega Amplitaq Gold® Taq DNA Polymerase in 20 uL total volumes, and an annealing temperature of 50 °C. The resulting sequences were edited and aligned manually in BIOEDIT version 7.1.3 software (Hall 1999).

Sequences of *cytochrome-b* available on GenBank from the study of Milne et al. (2009), plus that of Anwarali and Baker (2008 unpublished) and Bastian et al. (2008 unpublished), were downloaded and added to the alignment of sequences. Given that the sequences from Milne et al. (2009) were relatively short (154 bp, representing bases 175–328 of the *cytochrome-b* region), a second sequence alignment was made corresponding to this abbreviated section of the gene only. A distance-based phylogram was produced using the

Neighbour Joining method in PAUP* version 4.10b software (Swofford 2002), with 2000 bootstrap replicates to assess support for the resulting clade membership. Given that the taxonomy of the three Australian *Saccolaimus* species is well resolved, and despite the absence of publically available genetic sequences for *S. mixtus*, it was anticipated that each would be represented as genetically well-separated monophyletic clades (i.e. homogeneous groups with membership exclusive of the other species). Any misidentification of field-collected individuals would then show up as incorrect clade membership. The resulting phylogenetic tree was represented and edited in FIGTREE version 1.4.0 (Rambaut 2006-2012).

3.4 Field deployment of bat detectors

The SM2BAT acoustic detectors were deployed over a total of 10 nights in the periods between 16 and 26 June 2012, and 9 and 20 October 2012. A total of 54 'unattended' recording sites were established in June 2012, with half of the total each in the 'infrastructure' and 'Dam C' areas. Recordings were made from a total of 56 sites during the October 2012 survey, and were spread across the Project area (**Table 1**; **Appendix 3**).

The following settings were chosen using the manufacturer's instruction manuals (Wildlife Acoustics 2007-2010, 2007-2011, 2009-2011a,b) as a guide and the authors' previous practical experience, and saved to .SET files in the Song Meter Configuration software version 3.1.2: automated start and end of recording based on calculated daily sunset and sunrise times, respectively; sampling frequency of 384 kHz; WAC0 file format (lossless compression) in 30-minute blocks; trigger level for recording start 6 dB above background and continuing for 1 sec following the end of the trigger event; High Pass Filter setting fs/24 (signals above c. 16 kHz) or fs/64 (signals above c. 6 kHz); Low Pass Filter off; microphone amplification gain 48 dB. Each unit was checked before deployment for updated firmware version and internal clock setting, and correct function of the automated programme mode via a flashing LED light.

At the recording site microphones were oriented upward at a 45 degree angle as standard, and attached to thin saplings or posts around 1 m above the ground. The detectors were waterproofed in plastic boxes, and microphones were placed in a piece of PVC with the end of the microphone flush with the rim of the pipe so that the zone of reception was not affected significantly. For each recording site a GPS position was recorded and associated with the serial number of the recording unit and deployment date, and notes were made regarding the habitat in front of the unit. Recordings were made over a full night in each case.

3.5 Recording reference calls from captured bats

Reference echolocation calls were recorded from bats captured during the survey (especially species of *Saccolaimus*) so that anonymous calls recorded on unattended SM2BAT detectors could be identified following comparison with recordings made from bats with a verifiable species identification. Reference recordings were made with an Echo Meter EM3 (Wildlife Acoustics). Bats were recorded upon their release back to the forest after they were identified, measured and biopsy sampled (in most cases). Each individual was recorded for as long as possible after they were released in a relatively open area of the forest (usually an intersection between two major tracks). Tracking of the released individual was aided by the attachment of small chemi-luminescent tags (Glowstix Australia). Tags were attached with magic tape to the belly fur, and were observed to fall off after a minute or two if the individual stayed within detecting range. Some individuals stayed in the area for several minutes. The 'release method' is not always ideal for recording high quality search phase calls because mouth-emitting bats that produce modulated calls typically switch to shorter duration calls with a greater bandwidth within the first minute or so after their release. However, it represented the best way available to obtain calls of these bats flying in relatively open areas where they were originally captured, and where most bat detectors were deployed.

3.6 Analysis of acoustic recordings

3.6.1 Developing an analysis approach

Within the last few years, the availability of new ultrasonic recording technology (bat detectors) has provided new opportunities for identifying and discriminating bat species using their echolocation calls, and several manufacturers have in addition released numerous updates to both hardware and the associated software for format conversions and analysis. This has required the development of completely new approaches to analysing acoustic data in many instances (Specialised Zoological unpublished reports). In the present case, the goals were to first develop a method for acoustically discriminating the three *Saccolaimus* species (and the northern free-tailed bat *Chaerephon jobensis*) with the available reference call recordings, and subsequently use this to identify the species of *Saccolaimus* at each of the 110 unattended recording sites containing 'anonymous' bat pulses.

The accumulation of many gigabytes of data from 110 nights of full spectrum acoustic recordings presented several challenges for analysis:

1. A robust and comprehensive determination of whether the three Australian *Saccolaimus* species could be distinguished acoustically had not been undertaken elsewhere, and there was no guarantee that unambiguous identifications of *S. saccolaimus* could be made from echolocation calls in areas of sympatry with other *Saccolaimus* species.

2. No set of high quality full spectrum or AnaBat Zero Crossings format reference calls of any species of *Saccolaimus* was available at the beginning of the study.

3. The unattended recordings dataset comprised around 585 gigabytes of compressed (WAC0) computer memory, and almost 200,000 Zero Crossings format sound files of 15 seconds duration or less. Equivalent numbers of WAV format files were potentially required (converted from WAC0) in order to separate *Saccolaimus* spp. from *C. jobensis* based on harmonic patterns.

4. Automated analysis of acoustic data has limitations in terms of the recognition of faint (low amplitude) pulses and an unknown rate of returning a false positive identification of a target species. Some types of automated analysis require reference and anonymous recordings to be of the same quality (i.e. recorded at the same sampling rate).

Given the amount of data, an analysis method that relied on computational power rather than manual inspection of spectrograms and observer objectivity was sought. Several automated processes were considered and tested initially, and they are described briefly here as justification for the choice of approach that was made ultimately.

1. Automated identification of the target species based on power envelope comparisons of full spectrum WAV format files. Full spectrum recordings contain potentially more information that can be used to discriminate species. A powerful approach based on WAV format data is implemented in SoundID software (Boucher and Jinnai 2013), which relies on matching the power envelope (derived from the Linear Predictive Coding algorithm) between reference and anonymous signals, and assigns an identification based on an acoustically appropriate distance metric (Geometric Distance, GD; Jinnai et al. 2009, 2010). This approach has been used successfully on the Pilbara leaf-nosed bat (Specialised Zoological unpublished reports). The intention in the present study was to record reference calls of all *Saccolaimus* species (plus *C. jobensis*) at a standardised sampling frequency of 384 kHz (the highest resolution

available on SM2BAT detectors), with a visit planned to a recently discovered colony of the bare-rumped sheath-tailed bat in the Cairns Botanic Gardens. While reference calls of the yellow-bellied sheath-tailed bat and Papuan sheath-tailed bat became available from the Project area, the colony of the bare-rumped sheath-tailed bat was unfortunately absent on several occasions when it was visited. A generous donation of calls recorded on previous occasions was later made available to this study (G. Ford, unpublished data from Cairns Botanic Garden; R. Coles, unpublished data), however they had been recorded at a different sampling frequency (256 kHz, 500 kHz) and could therefore not be used in SoundID for matching reference and anonymous calls. The reference calls were then used in another approach, but it was instructive to first determine whether the call sets in full spectrum from each species could be separated based on Geometric Distance and other parameters. Pairwise comparisons showed little overlap between calls of the bare-rumped sheath-tailed bat and the other two species of *Saccolaimus*, suggesting the possibility of discriminating them using another method (**Figure 6**).

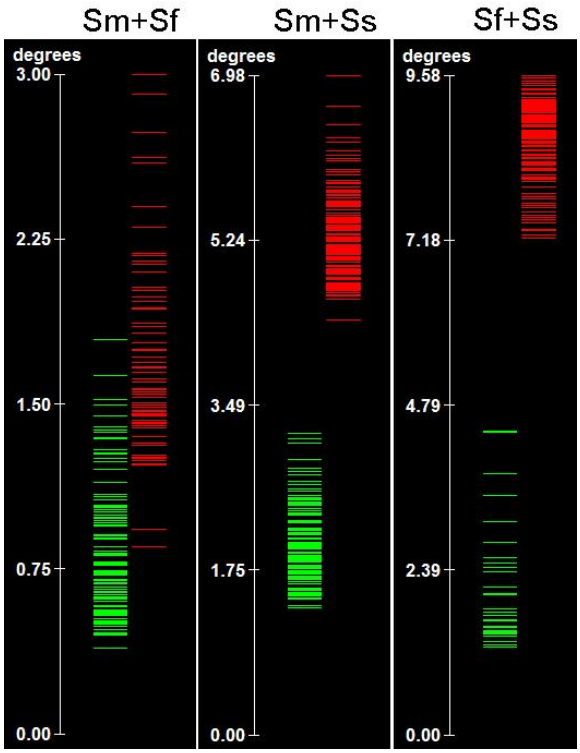

Figure 6. Three sets of pairwise comparisons of the power curves of pulses among species of *Saccolaimus* using Geometric Distance (in degrees; based on an individually optimised value for the spread of the weighting curve). In each case, bars on the left hand side in green represent within-species comparisons between single pulses, and the bars on the right hand side in red represent between-species comparisons (Sf: *S. flaviventris*; Sm: *S. mixtus*; Ss: *S. saccolaimus*).

2. Classification by Discriminant Function Analysis of automated measurements of pulses from full spectrum WAV format files. The software SonoBat version 3.05p provides the ability to derive a large list of variables measured from signals it identifies as 'bat echolocation pulses' in an automated process. Following measurement of all good quality pulses from the reference calls (from the Project area and the reference set from Cairns donated by G. Ford), a Discriminant Function Analysis (DFA) was performed to determine which combination of variables could be used to best separate the clusters representing each of the three species. The analysis, as undertaken in SPSS version 11.5 software, showed that it was indeed possible to separate the three species acoustically (**Tables 2** and **3**; **Figure 7**). The intention was to measure automatically all pulses in full spectrum WAV format and use SPSS to classify each pulse according to the discriminant functions and Mahalanobis distance to determine group membership. However, the procedure of measurement in SonoBat was much too slow to process the large anonymous call dataset in a reasonable timeframe, so the approach was abandoned. However, it reinforced the previous finding based on Geometric Distance that there is sufficient information in WAV format files to discriminate the four bat species echolocating with a characteristic frequency between 15 and 25 kHz.

Table 2. Eigenvalues of each discriminant function based on the WAV format reference call dataset.

Function	Eigenvalue	% of Variance	Canonical Correlation
1	13.94	78.93	0.97
2	3.08	17.44	0.87
3	0.64	3.63	0.63

Table 3. Unstandardised Canonical Discriminant Function Coefficients from the WAV dataset (from which discriminant functions can be constructed).

Measurement variable[1]	Fn 1	Fn 2	Fn 3
Bndw20dB	1.02	0.68	-0.07
FFwd5dB	-2.81	-0.77	1.75
FFwd20dB	2.71	0.74	-1.70
CummNmlzdSlp	-2.78	0.51	4.56
FreqCtr	-0.12	0.34	-0.04
HiFreq	0.13	0.48	-0.28
Bndwdth	0.27	-0.25	0.23
TotalSlope	2.59	-1.04	-3.89
StartSlope	0.08	0.15	0.15
Fc	-0.53	0.16	0.07
CallDuration	-0.11	0.07	0.01
Amp1stQrtl	-0.01	0.01	0.02
AmpGausR2	1.59	-0.34	-0.84
AmpEndLn60ExpC	0.28	-0.77	-0.07
Amp2ndMean	-1.94	1.64	6.96
DurOf15dB	-0.06	0.03	-0.02
(Constant)	13.38	-22.85	-4.52

[1] The meaning of variable names is available at URL: http://www.sonobat.com/SonoBat%20parameters.html

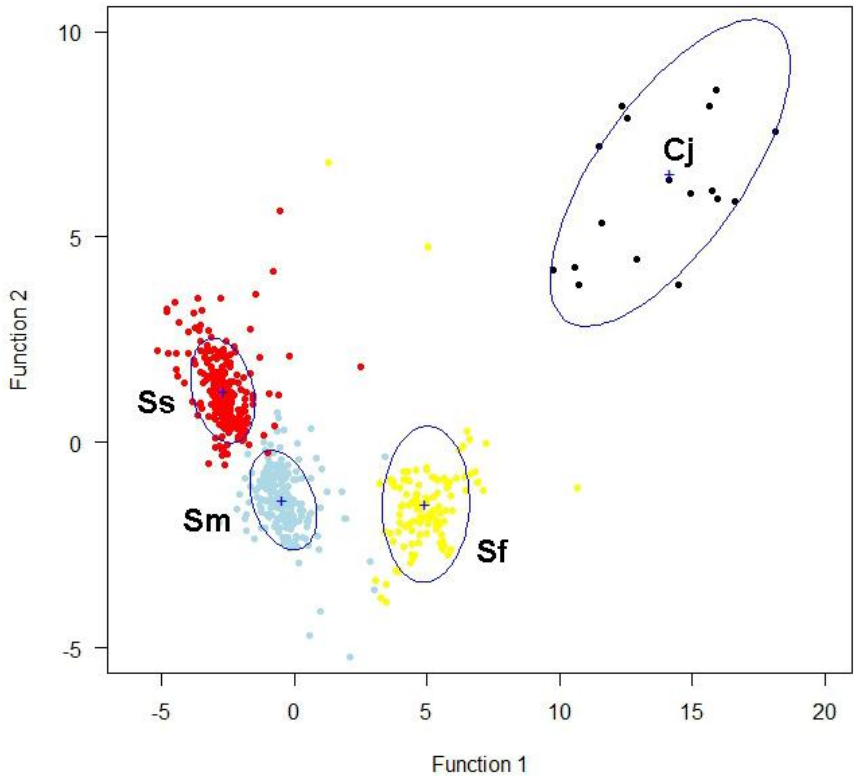

Figure 7. Discriminant Function Analysis plot showing the separation of species (Cj: *C. jobensis*; Sf: *S. flaviventris*; Sm: *S. mixtus*; Ss: *S. saccolaimus*), and confidence ellipses representing one Standard Deviation (confidence interval of 68%, equivalent to α=0.32) from the group centroids (+) (plotted using R).

3. Classification by Discriminant Function Analysis of automated measurements of pulses from Zero Crossings Analysis (ZCA) format files. The reference calls from the three *Saccolaimus* and *C. jobensis* was converted to ZCA format and the Discriminant Function Analysis was rerun in SPSS statistical software on variables measured from pulses using AnalookW version 3.9f software. As with the DFA performed on WAV files, there was adequate separation of each species based on their echolocation calls. Given that automated measurement of the entire unattended recordings dataset in ZCA was possible in a short amount of time, it was decided to proceed with an approach based only on ZCA format files and derived measurements. However, to identify the very large number of putative bat pulses, a sequence of steps was developed in R language. The full approach is described in more detail in the next section.

3.6.2 Defining the acoustic signature of each *Saccolaimus* species

Reference calls were available from a total of seven *C. jobensis*, 15 *S. flaviventris*, 21 *S. mixtus*, and an unknown number of individuals of *S. saccolaimus* recorded in flight near the Cairns tree roost. These were recorded in WAC0 compressed format and converted to WAV format with Kaleidoscope 1.0.0 software. Recordings from each individual were edited and clipped in Adobe Audition CS6 version 5.0.2 software to include only good quality pulses without other signals. The WAV files were then converted to ZCA format in Kaleidoscope, and opened in AnalookW version 3.9f software. Standard measurements were made automatically from each pulse and saved in text files.

A stepwise Discriminant Function Analysis was undertaken in SPSS version 11.5 software to define maximally separated clusters representing each species based on combinations of measurement variables from the echolocation pulses. The value of Wilks Lambda was highly significant ($p<0.001$) and three canonical variables derived from the analysis allowed for good separation of species groups (**Tables 4** and **5**, **Figure 8**).

Table 4. Eigenvalues of each discriminant function based on the ZCA format reference call dataset.

Function	Eigenvalue	% of Variance	Canonical Correlation
1	13.04	79.64	0.96
2	2.93	17.89	0.86
3	0.40	2.47	0.54

Table 5. Unstandardised Canonical Discriminant Function Coefficients from the ZCA dataset (from which discriminant functions can be constructed).

Measurement variable[1]	Fn 1	Fn 2	Fn 3
DUR	-0.16	0.17	0.60
FMAX	1.58	0.46	-0.07
FMIN	0.65	-0.09	1.19
FMEAN	-2.97	0.85	-1.36
TK	0.14	-0.65	0.72
FK	0.40	-1.05	1.23
TC	0.01	0.07	-0.56
FC	-0.10	0.69	-0.51
S1	0.00	0.00	0.01
SC	0.04	0.01	0.02
(Constant)	7.29	-20.89	-13.09

[1] DUR: pulse duration in msec, FMAX: maximum frequency of the pulse in kHz, FMIN: minimum frequency of the pulse in kHz, FMEAN: mean frequency over the whole pulse in kHz, TK: time at the knee (where the pulse slope has the greatest change in rate) in msec, FK: frequency at the knee in kHz, TC: time at the point of characteristic frequency in msec, FC: characteristic frequency (the frequency at the end of the flattest portion of the call) in kHz, S1: initial slope before the knee in octaves per sec, SC: slope at the flattest section of the call; see Gannon et al. 2004 for further details.

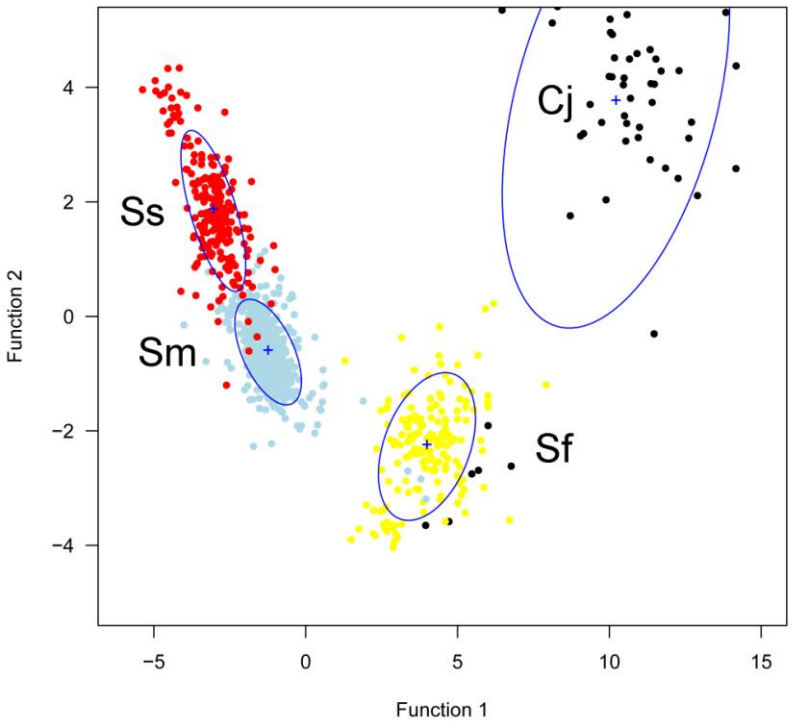

Figure 8. Scatterplot of the coordinates derived from the entering pulse measurements into the first and second discriminant functions, showing clear separation of the four species (Cj: *C. jobensis*; Sf: *S. flaviventris*; Sm: *S. mixtus*; Ss: *S. saccolaimus*), including confidence ellipses representing one Standard Deviation (confidence interval of 68%, equivalent to α=0.32) from the group centroids (+) (plotted using R).

While the variation of some species overlapped (e.g. *S. mixtus* and *S. saccolaimus*), there were subsets of each defined by confidence ellipse regions of one Standard Deviation that were mostly free of the calls of another species (**Figure 8**). Furthermore, while discrimination was not perfect (e.g. **Figure 8** shows that some calls of *S. saccolaimus* could clearly be misidentified as *S. mixtus*), the results demonstrated the potential for identifying some types of call from *S. saccolaimus* with a low likelihood of the misattribution of calls from another species.

3.6.3 Identification of anonymous acoustic signals using automated methods

Following adequate discrimination of species, the entire June and October WAC0 datasets were converted to ZCA format using Kaleidoscope software. The 'scan' function incorporating an 'all bats' filter in AnalookW was used to automatically measure all putative bat pulses in the recordings from each SM2BAT unit, and output a text file of 16 variables.

To attribute a species identification to each putative bat pulse, a novel analysis approach needed to be developed based on the Discriminant Function Analysis undertaken on the reference call dataset. In Microsoft Excel, *x* and *y* Cartesian coordinates were calculated for each anonymous putative bat pulse using the discriminant functions derived from the Unstandardised Canonical Discriminant Function Coefficients generated from the reference call dataset. From this point, the goal of the analysis was to determine whether points on a scatterplot derived from the *x* and *y* coordinates of each putative bat pulse fell within or outside one of the confidence ellipse regions associated with a particular species. Given that there was a total of 1,236,380 putative bat pulses, the most efficient means of doing this was to develop a series of steps in R language (Ihaka and Gentleman 1996; R Development Core Team 2003).

Before the R script could be written, and as one possible approach, the statistical algorithm for a predictive confidence ellipse needed to be converted into a geometric formula that *x* and *y* coordinates could be substituted into. This was preferred over an approach that gave a probability value for predicted group membership (e.g. based on Mahalanobis distance from a group centroid) because the desired outcome from each of the c. 2 million putative bat pulses was simply an indication of whether it fell within the range of reference values that minimised Type II error (misidentification), i.e. the 68% confidence ellipse region. This essentially discards much of the variation in the anonymous dataset, resulting in very high Type I errors (not detecting a species that was present), but the relative importance of providing an

unambiguous identification was greater than trying to determine the presence of the target in areas of the scatterplot where call variation was likely to overlap.

The formula for a predictive confidence ellipse is (after Johnson and Wichern, 1998: 264; see this reference for an explanation of symbols):

$$(Z-\bar{Z})'S^{-1}(Z-\bar{Z}) \leq \frac{p(n^2-1)}{n(n-p)}F_{p,n-p}(1-\alpha)$$

The process of converting the above to a geometric formula is currently unpublished, but will be submitted for publication in the future. The creation of the predictive ellipse for each species was aided by calculations in Microsoft Excel, and the resulting shape and location of each ellipse was checked against that produced in R by the 'ellipse' package (Murdoch et al. 2013) by drawing it in the software WinPlot version '13 September 2012' (Parris 2012).

The R script then tested a file of *x* and *y* coordinates from each SM2BAT detector unit against the predictive confidence ellipse of each of the four species of interest, and output the results. Given that pulse measurements were automated in AnalookW, and also that ZCA representations of pulses can include a smaller portion of the signal than that available in WAV format (including fragments resulting from split pulses), a large proportion of measured signals were expected to fall outside the confidence regions. In addition, because the reference call collection was derived from relatively few individuals calling in a situation that undoubtedly limited the expression of their signal repertoire, a much greater amount of variation was expected from the anonymously recorded signals.

Given this, identification was based on two sets of observations. Firstly, the output from the R script was examined to determine which pulses fell within confidence regions, and then correlated with site and Regional Ecosystem (RE) category. This left a large proportion of signals unallocated.

The second process took a more 'fuzzy' approach to identification. It was clear that the amount of variation in the anonymous call dataset was much greater than that in the reference call dataset. Thus, the carefully discriminated confidence regions were useful only for identifying a subset of the anonymous call variation. By looking at the clustering pattern of all points from either each SM2BAT unit (as an arbitrary group) or the June or October survey as a whole, it was a straightforward process to determine how many species were likely to be present commonly. Most importantly, since much of the variation seemingly associated with a large cluster representing *S. mixtus* overlapped partly into the confidence region of *S. saccolaimus*, it was decided to allocate an identification of the latter species only if discrete

clusters of points separate from the cluster of *S. mixtus* were present. The implication of this approach is twofold:

1. the error rates from false negatives (a Type I error; α: the probability that a species is present but is not identified) and false positives (a Type II error; β: the probability that a species is not present but is identified as such, i.e. misattributing a signal to the wrong species) are essentially ignored in favour of the interpretation of major patterns; and
2. a rare recording resulting from one or a very small number of passes of *S. saccolaimus* in front of the acoustic recording device would be missed unless they grouped distinctively outside of the variation attributable to *S. mixtus*.

Thus, unless *S. saccolaimus* was present in reasonable numbers, it was unlikely that the species would be identified with confidence from acoustic recordings.

4.0 RESULTS

4.1 Species recorded by capture

A total of 16 *S. mixtus* were captured in June 2012, but no other species of *Saccolaimus* was trapped. In October 2012, 40 *S. mixtus* were captured, with an additional 1 individual as a recapture, plus 17 *S. flaviventris*. Thus, a total of 73 *Saccolaimus* spp. (plus one recapture) were trapped, with around three quarters being *S. mixtus*. The external morphology of these two species is sufficiently distinctive to allow discrimination in the field, especially following the capture and inspection of so many examples (**Table 6**; **Figure 9**). No *S. saccolaimus*, as indicated from inspection and measurement of external morphology, were captured on either survey.

In addition to the *Saccolaimus* spp., eight other bat species were captured, representing four additional families (**Table 7**). Identifications were made based on external morphology, and through comparison with DNA barcodes collected from elsewhere on Cape York (K.N. Armstrong unpublished data, not shown). Reference calls were also collected. The total number of individuals from all species except *Saccolaimus* spp. was 40. While the emballonurids were the targeted focus of the survey, it is still unusual to have the relative representation of *Saccolaimus* as almost twice that of the other species combined.

Table 6. Summary of forearm measurements from captures of *Saccolaimus* spp. (Mean ± Standard Deviation, range, sample size; measurements in mm).

Yellow-bellied sheath-tailed bat	*S. flaviventris*	74.7 ± 1.6 73.1 – 76.4 n=7
Papuan sheath-tailed bat	*S. mixtus*	65.2 ± 1.5 62.0 – 68.0 n=27

Table 7. Species identified in the present survey from all sites combined, with total number of individuals captured.

EMBALLONURIDAE		
Yellow-bellied sheath-tailed bat	*Saccolaimus flaviventris*	17
Papuan sheath-tailed bat	*Saccolaimus mixtus*	56+1 recapture
MINIOPTERIDAE		
Eastern bent-winged bat	*Miniopterus oceanensis*	1
MOLOSSIDAE		
Northern free-tailed bat	*Chaerephon jobensis*	18
PTEROPODIDAE		
Little red flying-fox	*Pteropus scapulatus*	3 (plus 100s-1000s observed)
Eastern blossom bat	*Syconycteris australis*	2
VESPERTILIONIDAE		
Hoary wattled bat	*Chalinolobus nigrogriseus*	12
Eastern long-eared bat	*Nyctophilus bifax*	1
Forest pipistrelle	*Pipistrellus adamsi*	1
Northern broad-nosed bat	*Scotorepens sanborni*	2

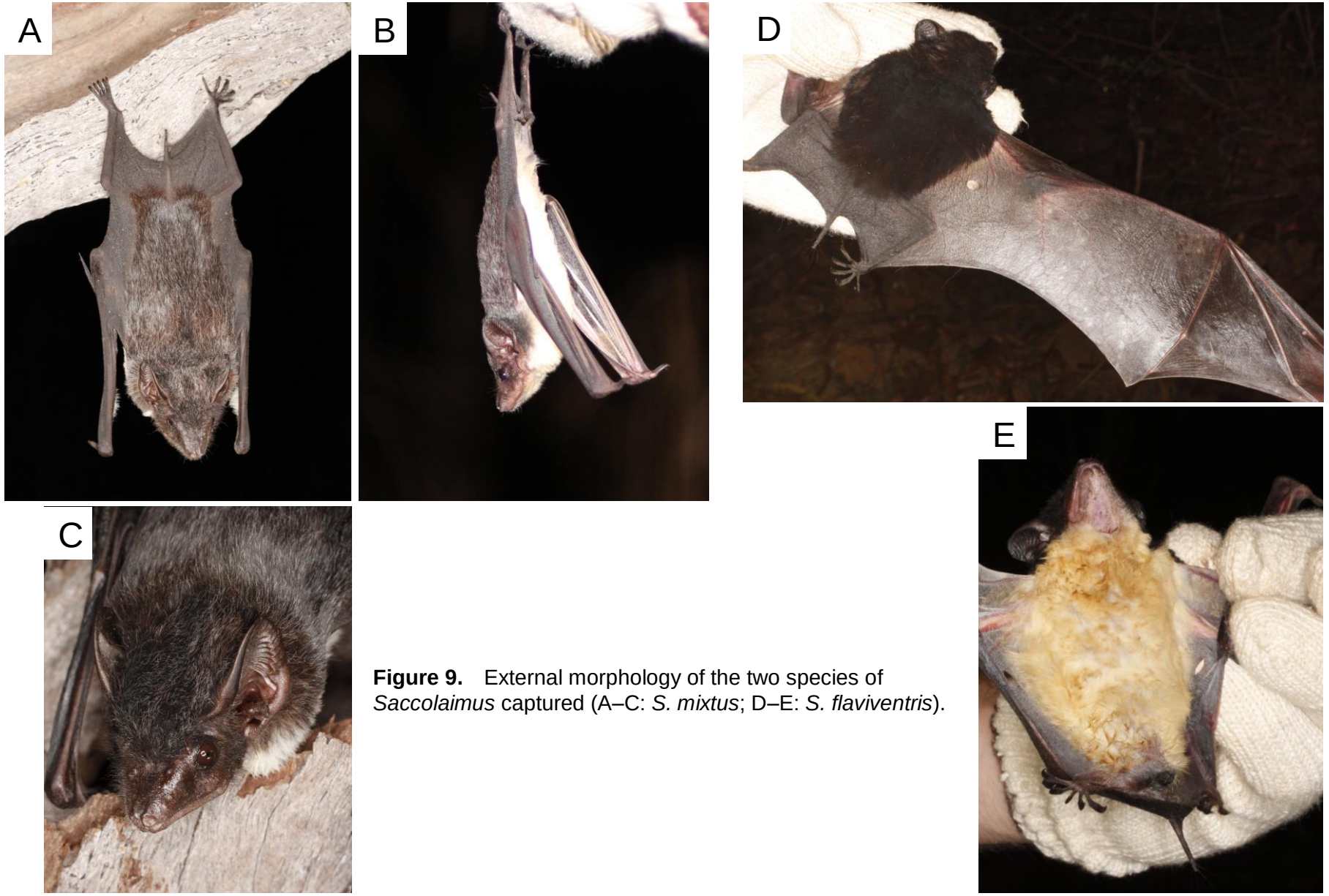

Figure 9. External morphology of the two species of *Saccolaimus* captured (A–C: *S. mixtus*; D–E: *S. flaviventris*).

4.2 DNA barcoding to confirm the identity of captures

To demonstrate competence in identifying and distinguishing the species of *Saccolaimus* based on external morphology in the field, wing biopsy tissue samples from a subset of the captures were DNA barcoded for a short section of the mitochondrial *cytochrome-b* gene and compared with sequences available on GenBank (http://www.ncbi.nlm.nih.gov/) and the study of Milne et al. (2009). In all cases, the field identification corresponded with that indicated by the DNA barcode (**Figure 10**). Only two *Saccolaimus* species were identified: *S. flaviventris* and *S. mixtus*. The genetic distance and high bootstrap support values amongst the monophyletic clades representing these three species is large enough to avoid ambiguity for identifications.

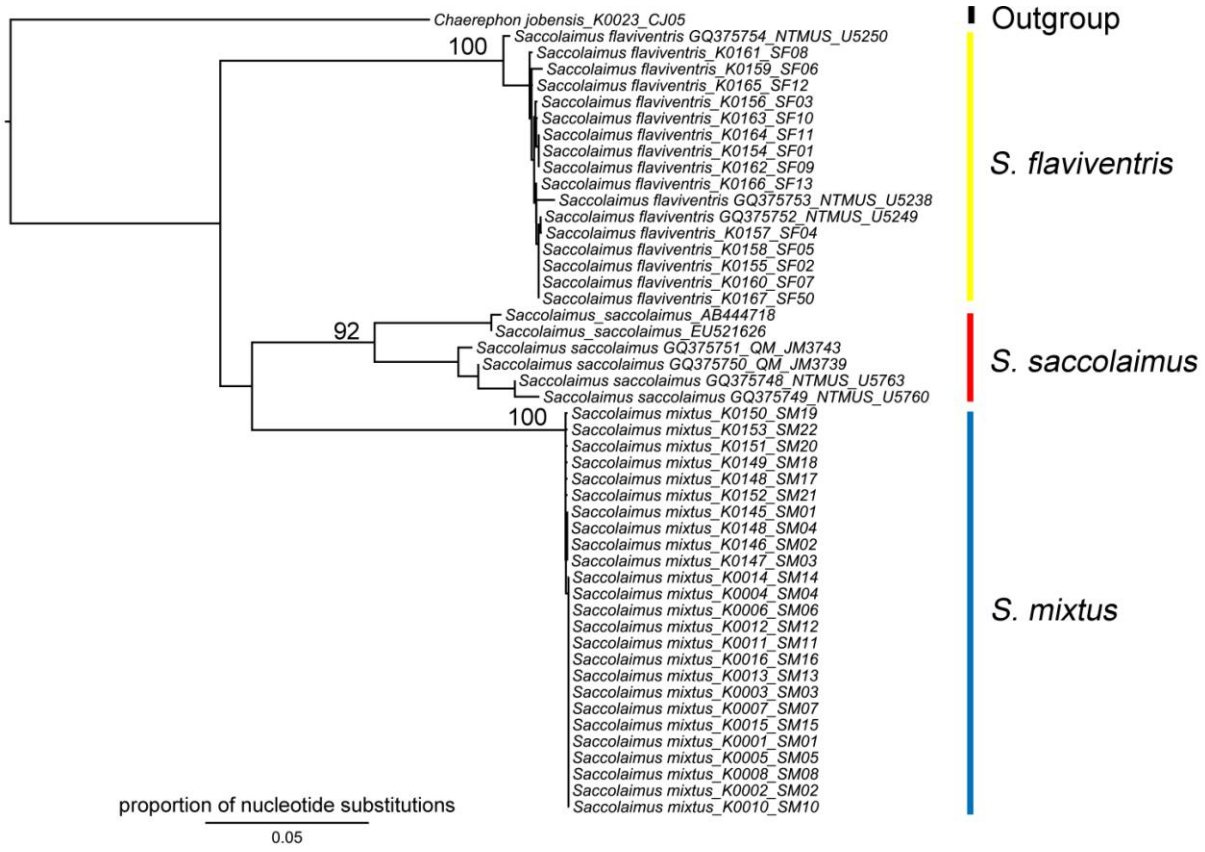

Figure 10. Distance phylogram showing the association of samples DNA barcoded from field captures at Weipa ('K' numbers) with those on GenBank and the study of Milne et al (2009) (all others including 'JM' numbers from specimens in the Queensland Museum and 'U' numbers for the Museum and Art Gallery of the Northern Territory). P-distances[1] amongst the three ingroup clades were as follows: *S. saccolaimus* with *S. mixtus* and *S. flaviventris*, respectively: 0.161 and 0.163; *S. flaviventris* with *S. mixtus*: 0.194.

[1] p-distance is the proportion (p) of nucleotide sites at which two sequences or groups of sequences being compared are different. It is obtained by dividing the number of nucleotide sites showing differences by the total number of nucleotides compared. It can be converted to % divergence by multiplying by 100.

4.3 Acoustic detection of bats

The analysis of acoustic data focussed on the identification of the three *Saccolaimus* and their discrimination from *C. jobensis*, and therefore a site by species summary of all bat species is not presented here. The collection of reference calls from the SoE Project area, when combined with other full spectrum reference recordings made by The University of Adelaide / South Australian Museum (K.N. Armstrong unpublished data) plus the ZCA format recordings of Reardon et al. (2010), represent the beginnings of a comprehensive echolocation call library from the Cape York Bioregion that will help with future acoustic monitoring of all echolocating bat species.

All three approaches explored in the preliminary analysis showed the possibility of separating the four species of bat that produce echolocation pulses with the characteristic frequency of the loudest harmonic between 15 – 25 kHz (**Tables 2 – 5; Figures 7** and **8**). Even the dataset subject to processes that reduce complexity (the ZCA format dataset) had sufficient information to allow discrimination of the four species, given the reference calls available. Representative pulse types are given in **Figure 11**, illustrating the relatively obvious differences between *C. jobensis* and *Saccolaimus* spp. based on the harmonic interval (c. 20 kHz in *C. jobensis*; c. 10 kHz in *Saccolaimus* spp.), and the wide variation in pulse shapes that all of the latter make in various situations.

The identification of putative bat pulses from anonymous recordings based on measurements entered into the discriminant functions, and then tested for inclusion into one of the available confidence ellipse regions showed that a very large proportion of putative bat pulses were within or very close to the confidence regions of *S. flaviventris* and *S. mixtus* (**Figures 12** and **13**). The variation of pulse measurements from anonymous pulses was significantly greater than the size of the confidence regions defined from a much smaller set of reference calls (127,895 out of 988,147 points fell inside the confidence region of a species of *Saccolaimus*), demonstrating that a very high proportion of pulses were not ascribed an identification, thus giving a correspondingly high Type I error rate.

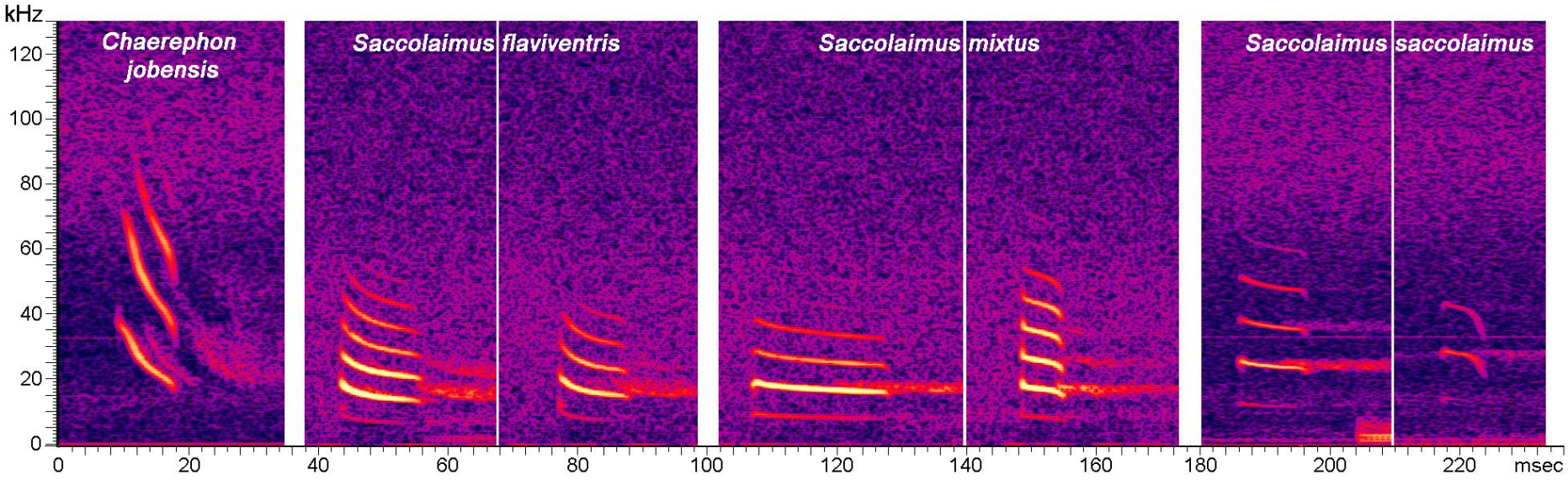

Figure 11. Representative pulses of bat species in the SoE Project area that have the characteristic frequency of the loudest harmonic between 15 – 25 kHz. Note that *C. jobensis* is distinguishable by inspection on the basis of harmonic patterning, and the three species of *Saccolaimus* produce pulses with a variety of shapes, many being similar amongst species, and those illustrated are not necessarily exclusive to the species named. The number of harmonics detected depends primarily on the distance between the bat and microphone, plus their relative orientation.

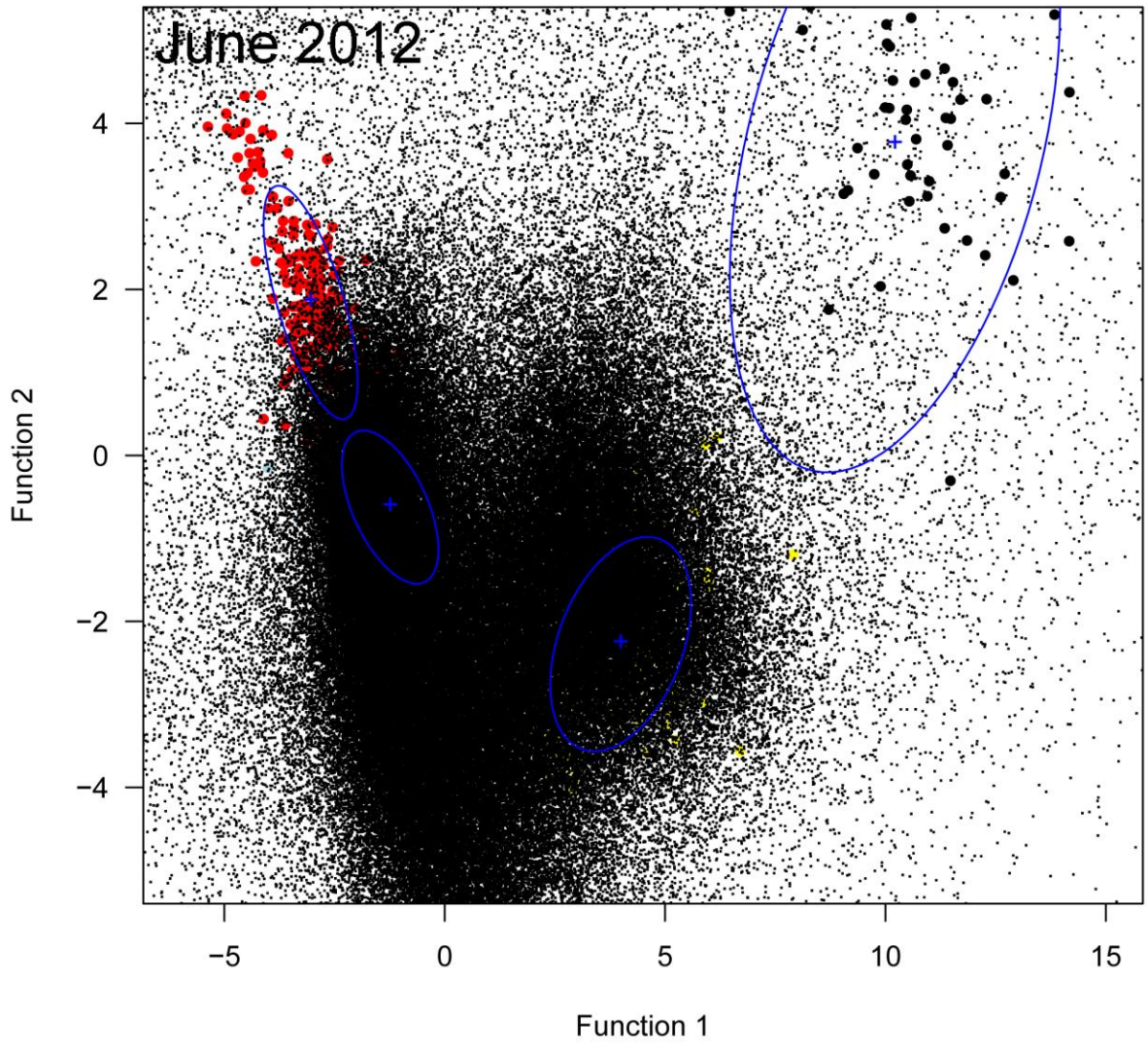

Figure 12. All measured pulses with a characteristic frequency less than 30 kHz in the June 2012 survey from all six bat detectors combined. See **Figure 8** for a clearer view of the clusters of each species apparent from the reference calls only, and **Appendix 4** for plots per bat detector.

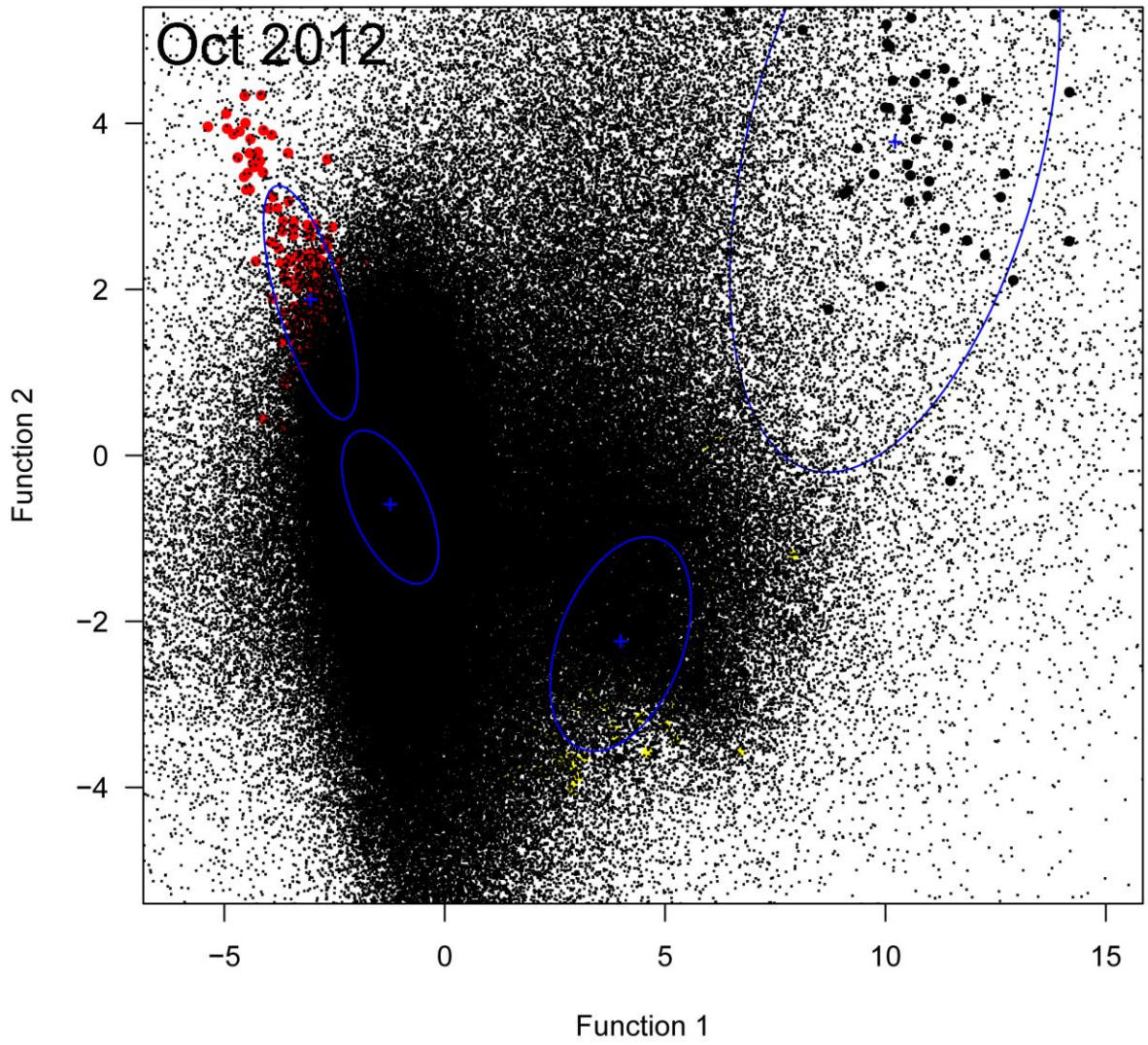

Figure 13. All measured pulses with a characteristic frequency less than 30 kHz in the October 2012 survey from all six bat detectors combined. See **Figure 8** for a clearer view of the clusters of each species apparent from the reference calls only, and **Appendix 4** for plots per bat detector.

4.4 Association of capture and acoustic records with REs and season

Species of *Saccolaimus* were captured all across the SoE Project area (**Figure 14**; **Appendices 1** and **2**). This included captures both inside and outside the planned infrastructure footprints, close to riparian vegetation at Dam C, and in both the June and October surveys. All captures were made in the *E. tetrodonta* habitat (RE 3.5.2), with the single exception of site M06 in RE 3.2.6 at Pera Head (where bats were present either foraging or commuting over a freshwater lake). This reflects the dominant representation of the *E. tetrodonta* closed forest in the Project area, and also the difficulty of finding good trapping sites in the lower, denser (i.e. lacking tracks and open flight spaces suitable for netting) riparian vegetation. However it simultaneously suggests the importance of the *E. tetrodonta* vegetation unit as a foraging resource for these species. It is also highly likely that roosts were present, give that some captures were made soon after dark.

Capture rates between the June and October surveys are not comparable because much experience was gained on the first survey, where it was clear that rope-mounted mist nets hoisted into the canopy were more successful than harp traps and pole-mounted mist nets. As a result, greater effort was put into canopy netting in October.

An assumption that guided the distribution of trapping and acoustic recording effort in the design phase of the study was that *S. saccolaimus* was likely, if indeed present, to rely heavily on the riparian habitats. While it was not possible to confirm this given that the species was not detected, it was interesting to note how common and widespread the other two *Saccolaimus* species were in the Project area. This observation is supported by both the trapping results (20 of 27 canopy net sets) and by acoustic detection, since they were present flying over every sampled position in the Project area in both seasons (**Figure 14**; **Appendix 3**). The resulting implication is that these two *Saccolaimus* species are possibly catholic in their usage of the variety of vegetation communities available. However, a tally of the number of pulses (inside confidence regions only) for each RE in each species showed that *S. flaviventris* might have had a slight preference, given a greater proportional representation within riparian REs (**Table 8; Figure 15**).

There was also a pronounced seasonal difference in the amount of activity, with the number of pulses falling inside confidence ellipses of all *Saccolaimus* species together being around five times as high in October compared with June (21,010 pulses compared with 106,885; **Table 8**). Given that sampling effort was around the same (54 recordings sites in June, 56 sites in October), this suggests that bats were either more abundant or more active in October, possibly because of warmer weather and greater food availability. It might also have been partly due to the fact

that bats might change the shape of their call in June for some reason (i.e. flying at different distances to vegetation), with a greater proportion of pulses falling outside the confidence ellipses, or alternatively that less pulses are detected in June because bats fly higher over the canopy (and thus further away from the detectors). In addition, it was apparent that *S. mixtus* was more common in both months compared to *S. flaviventris*, based on the number of points inside the confidence ellipses (**Table 8**). However, making interpretations is difficult given that different species might fly at different heights, and bats may fly many kilometres in a night when commuting and foraging at multiple sites. At Pera Head, bats were observed flying in from the ocean over the forest in the early morning, and one *S. mixtus* was recaptured c. 10 km from a site where it was first encountered two nights previously. These various observations highlight how little we understand about nightly foraging activity and range in all Australian *Saccolaimus*.

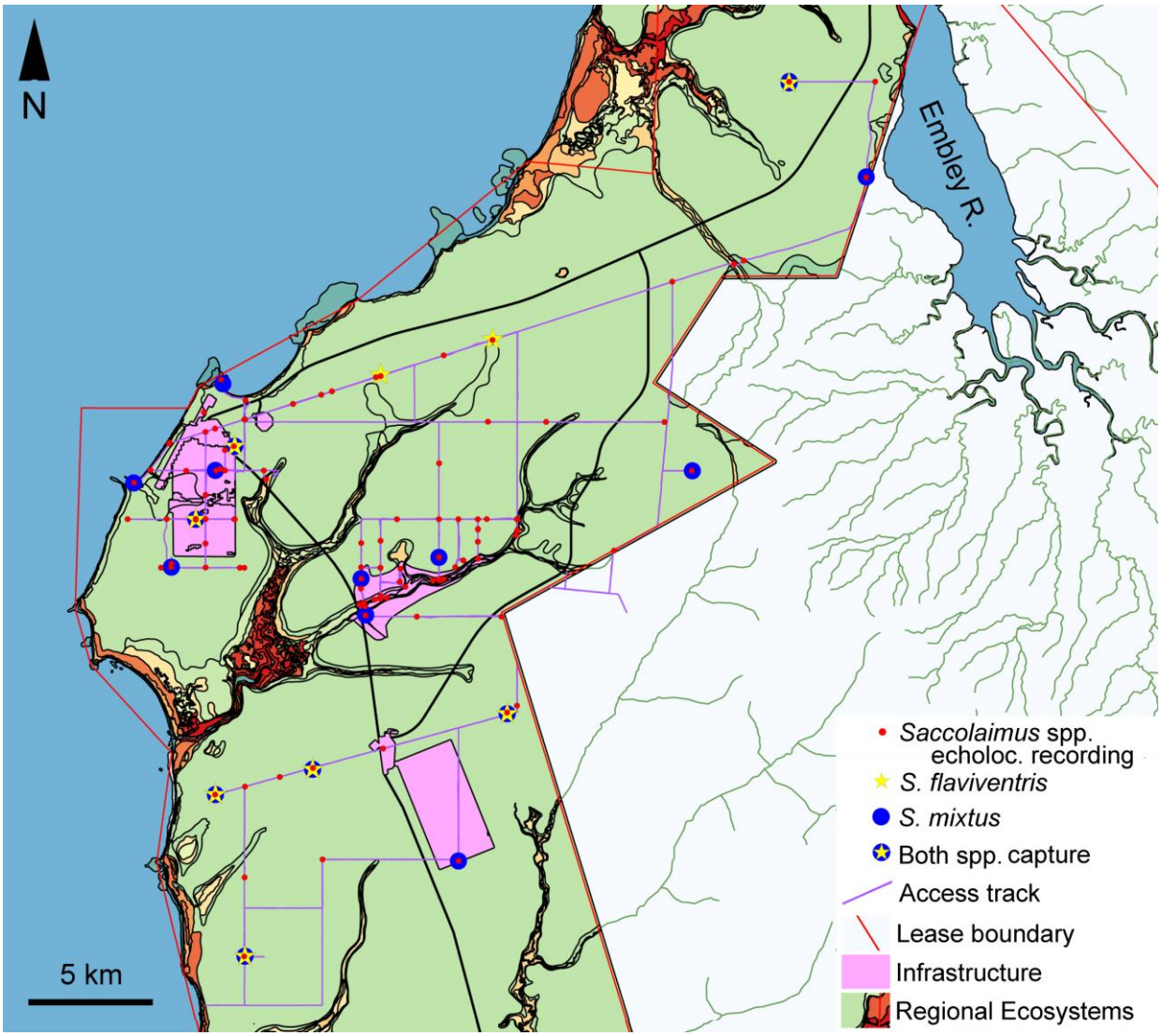

Figure 14. Distribution of *Saccolaimus* captures across the SoE Project area correlated with Regional Ecosystems, plus records of these species based on acoustic recordings.

Table 8. Tally of the number of putative bat pulses falling within the confidence region of each *Saccolaimus* species, with totals for each Regional Ecosystem (RE), and seasonal survey (n: the number of recording sites; see **Appendix 5** for explanation of the RE codes).

RE	*S. flaviventris*	*S. mixtus*	*"S. saccolaimus"*	n	All *Sacc.*	All pulses
3.2.2	224	196	18	1		
3.2.6	3,433	1,799	281	1		
3.3.21	755	1,428	247	6		
3.3.50a	84	146	20	1		
3.3.9	4,819	3,724	704	7		
3.5.2	15,668	86,464	9,158	74		
3.5.22c	452	1,008	101	6		
3.7.3	3,026	77	44	1		
June	7,126	11,887	1,997	46	21,010	236,439
October	15,002	83,195	8,688	51	106,885	751,708
Total	22,128	95,082	10,685	97	127,895	988,147

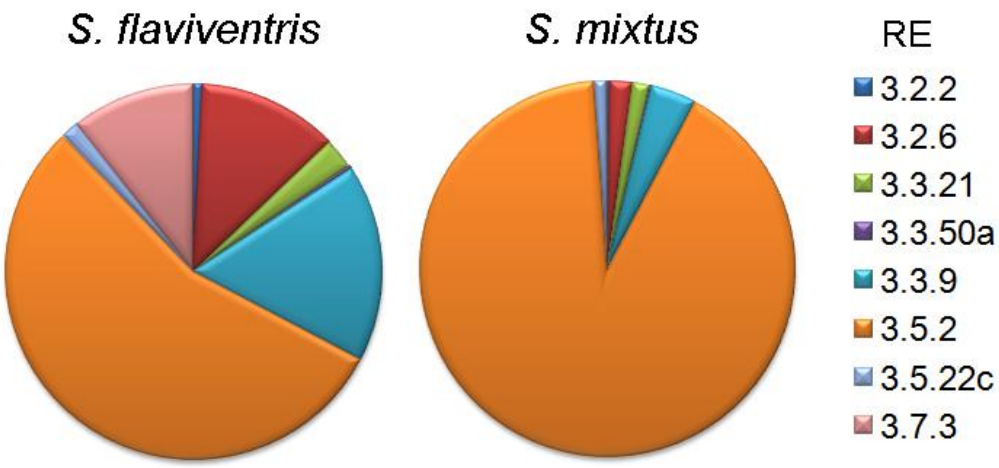

Figure 15. Representation of two *Saccolaimus* species in each Regional Ecosystsm (RE) for both seasonal surveys combined based on the total number of pulses within confidence regions (see **Appendix 5** for explanation of the RE codes).

5.0 DISCUSSION

The present study represents a good benchmark for targeted surveys for *S. saccolaimus* in a large project area such as South of Embley. It included what we believe to be the greatest targeted survey effort for this species (20 capture nights, with multiple traps/nets deployed per night), the most effort with an appropriate capture technique (rope-mounted mist nets in the tree canopy) and the largest capture return of *Saccolaimus* spp. (74 individuals) ever recorded in Australia. In these ways, the study was unprecedented. In addition, the total deployment of 110 full nights of recording with full spectrum detectors is also one of the largest acoustic surveys conducted in a single targeted survey programme in Australia, and has associated with it the largest reference echolocation call dataset from *Saccolaimus* that has been compiled to date. The effort compares well with that recommended in the Commonwealth Government's "Survey guidelines for Australia's threatened bats" (DEWHA 2010), and provides what we believe is the first comprehensive demonstration of an appropriate level of effort consistent with the guidelines for this species, at least in a large project area. Finally, the study also provided the first quantitative analysis of the acoustic differences in signature echolocation calls amongst the three species of *Saccolaimus* in Australia with a novel multivariate statistical method, and pointed to where the analysis of a large datasets might be both useful and limited.

5.1 The likelihood that S. saccolaimus is present at SoE

There was no ambiguous identification of any captured individual in the study, based on either external morphology or DNA barcodes. All *Saccolaimus* species captured could be attributed with high confidence to either *S. flaviventris* or *S. mixtus*. While we cannot say what level of capture effort and success would be sufficient to detect a rare species such as *S. saccolaimus*, the results from the present survey provide good evidence that it is either absent or at such low numbers as to be undetectable in the Project area. Given that only 3.7% of the *E. tetradonta* forest (RE 3.5.2) in the Cape York Bioregion will be cleared for the SoE Project (Rio Tinto Alcan 2012), and that riparian zones will be avoided, this provides some confidence that the potential impact of mining on this bat species will be limited in the worst case scenario. The area of forest to be retained is large and will contain an abundance of potential roost hollows. The relative impact on more common species of *Saccolaimus* is likely to be greater, though of unknown magnitude.

Two main approaches to identifying *S. saccolaimus* from acoustic recordings were undertaken. In the first, a strict determination of presence based on whether measurements of anonymously

recorded pulses could be placed into a narrow confidence region gave indications that the species was present. However, an inspection of the associated Discriminant Function Analysis plot clearly showed that potentially all of the points inside the confidence region of *S. saccolaimus* could be attributable to *S. mixtus*. It is possible that some calls of *S. saccolaimus* could have been present amongst the large amount of variation attributable to *S. mixtus*, especially considering that a small number of the reference pulses of *S. saccolaimus* occurred in the confidence region of *S. mixtus* (**Figure 8**). Thus, while the Discriminant Function Analysis based on reference calls provided a good indication that the calls of the three *Saccolaimus* could be distinguished, in reality the amount of variation from unattended recordings overwhelmed any ability to detect a rare occurrence, and testing of the allocation of individual putative bat pulses to confidence regions was not informative given the aim.

In the second, 'fuzzy', approach, the identification of *S. saccolaimus* was sought by inspection of clusters of points in the DFA plot distinct from the large amount of variation from *S. mixtus* and *S. flaviventris*. The variation in reference calls suggested there were some pulse types (based on characteristic frequencies and shape) unique to *S. saccolaimus*. Upon inspection of data combined from several nights of unattended recording (or the entire monthly dataset), no distinct clusters were present in this area of the DFA plot. While this does not provide information about rarely recorded sequences, or pulses with characteristics very similar to *S. mixtus*, it does provide evidence that a population of equivalent size to the other two *Saccolaimus* species is not present.

Unless *S. saccolaimus* was present in reasonable numbers, it was unlikely that the species would be identified from acoustic recordings. Only capture is likely to give an unambiguous identification, given the reference echolocation calls available to the analysis. It can be extremely resource intensive to provide proof of absence, so it is unfortunate that a non-invasive, and easily implemented method did not have the power detect a rare species (based on the reference echolocation data available to the study). However, the combined approach of capture and acoustic surveys represented the greatest and most contemporary approach to detecting this rare species in a very large project area, and the results did not suggest the presence of a significant population of *S. saccolaimus*. If it is indeed present, it is in such limited numbers as to be undetectable with the best available methods and significant effort.

5.2 The importance of SoE habitats for other bat species

The results suggested that the *E. tetrodonta* woodland south of the Embley River is a highly utilised and important habitat of *S. mixtus* and *S. flaviventris*, given both the number of captures, and also the large amount of calls recorded on the SM2BAT detectors. The acoustic data suggested that *S. mixtus* was the more common species, and further study would help to identify if it uses other habitats to the same degree within its range on Cape York. Given the relatively small distribution of this species in Australia, its Near Threatened status under Queensland State environmental legislation, and how little is known of its ecology and habitat preference, building on the observations from the present study is likely to be helpful for future environmental impact assessment work. Of particular relevance for this apparently abundant species in the local habitat in the SoE Project area is where it roosts, the density of roosts in the *E. tetrodonta* habitat, how often it might change roosts, and how breeding roosts might be used by bats and identified by investigators.

It is interesting that the more widespread species *S. flaviventris* was apparently at lower abundance in the Project area than *S. mixtus*. There is an obvious size difference between the two that suggests a different diet, and wing shape differences that might also point to differences in foraging strategy and flight space use. Of interest, both intrinsically and perhaps also in the context of environmental impact studies, is why the ranges of *S. mixtus* and *S. saccolaimus* are so small compared with the almost continental distribution of *S. flaviventris*. Understanding habitat associations of these three species might help to predict the relative impact of human land uses on Cape York on these bats.

Another significant observation made on the survey was the relative abundance of flying-foxes in June compared to an almost non-detectable presence in October. During the June survey, hundreds of little red flying-foxes *Pteropus scapulatus* (and possibly also *P. alecto*) were seen flying in a common direction at dusk, and many were seen feeding in the trees surrounding mist nets being attended at night. It was noted that the *E. tetrodonta* was flowering at the time of survey in June, and given that the bats pollinate this species, it is likely that flying-foxes represent key pollinators and thus keystone species in the extensive *E. tetrodonta* forest. This could be important to consider in the future management and rehabilitation of the forest.

5.3 Techniques for determining presence and roosts in trees

It is possible that other techniques not undertaken in the present survey programme might also be of use in the context of environmental impact studies. Initially, it was intended to make searches for roost sites, and keep watch at dusk trees with large hollows potentially containing *Saccolaimus* spp.. Upon familiarisation with the Project area, it was realised that searching for roosts in an extensive forest of literally millions of stems was a formidable task, it was impractical to safely inspect hollows above 20 m given the terrain, and keeping watch at one hollow of many potential roosts was simply not feasible in the context of an intensive trapping and recording programme. Instead, the best use of resources was directed to maximising the potential to capture individuals, and to discriminate the species of high flying bats acoustically.

5.4 Acoustic recordings for identification and monitoring

While this study showed that the amount of within-species acoustic variation can be very large compared to that available from reference call collections (and the collection of *Saccolaimus* calls in this study is certainly the largest ever recorded), the data from unattended bat detectors was useful for four main reasons:

1. It provided identifications of two of the three *Saccolaimus* species at most sites with a relatively high degree of accuracy given the separated confidence regions generated from reference calls. Thus, while much of the anonymously recorded data was eventually excluded, the identification to species came from that proportion of the variation most likely to be attributable to a particular species (i.e. within confidence regions). The implication is that the more data that are collected (i.e. the more unattended sampling sites that are established), the higher the rate of encounter will be for all species, and therefore the greater the basis for each species identification. With the relatively quick approach outlined here, a very large amount of data could be processed in a timeframe conducive to environmental impact assessments and more importantly, long term monitoring programmes.

2. The breadth of variation from the anonymously recorded calls that appeared to make up distinct clusters for each species was large, and demonstrated how much call variation might be needed from reference data to more confidently ascribe identifications, and calculate rates of false negative and false positive identifications. The implication is that

identifications made on the basis of manual inspection of echolocation sequences, as well as processes based on multivariate statistics and confidence regions without sufficient reference material, may have very high rates of misidentification or non-attribution of calls.

3. Given that it only requires relatively few good quality sequences of several pulses to fall within confidence ellipses to provide a species identification with reasonable confidence from a site, the relative abundance of a species can be estimated. This measure ignores the actual number of pulse identifications per site, and gives a measure of commonness based on the proportion of recording sites where a species is identified. With sufficient recording sites, combined with an efficient processing method for targeted species, this can be a useful comparative statistic for monitoring and ecological studies.

4. Similarly, comparative measures of activity can be derived from the tally of pulses that fall only within confidence ellipses, if there is sufficient replication of recording sites. In addition to simply comparing relative presence or commonness across a project area, activity can be a useful additional metric for non-invasive monitoring. While there may be factors other than actual abundance that contribute to the total number of pulses recorded by a bat detector, it may still be useful to indicate general patterns.

In the future, where a greater level of confidence might be required for the identification of *S. saccolaimus*, the identification of calls that are absolutely diagnostic of the species deserve consideration. Such calls that rely on full spectrum recordings have been mentioned by Coles et al. (2012), but no details have yet been published. Given the relative rarity of this species, the amount of acoustic recording effort needed to detect it is likely to be high, precluding an analysis approach based on manual inspection of many thousands of WAV files. However, while non-trivial to develop, an automated identification process using one of the available commercial software programmes or another customised approach might be helpful should additional reference recordings of diagnostic calls become available.

6.0 CONCLUSIONS

1. Two species of sheath-tailed bat *Saccolaimus* spp. were confirmed unambiguously from the South of Embley Project area: the yellow-bellied sheath-tailed bat *S. flaviventris* and the Papuan sheath-tailed bat *S. mixtus*. Identification was made firstly by examination of external morphology and confirmed subsequently with DNA barcoding.
2. There was no unambiguous evidence of the occurrence of the bare-rumped sheath-tailed bat *S. saccolaimus* in the South of Embley Project area. No captures were made, and while there were limitations in the acoustic analysis, there was no indication of presence from recordings of bat echolocation (110 full nights over a total of 4 weeks in June and October 2012).
3. The rates of both capture and acoustic recordings of the two species of *Saccolaimus* from across the SoE Project area suggested that the *Eucalyptus tetrodonta*-dominated woodland south of Weipa represented suitable foraging and roosting habitat for these species, but particularly *S. mixtus*.
4. The higher number of echolocation calls recorded on the October 2012 survey suggested either higher activity or higher local abundance of the two *Saccolaimus* species present at that time.
5. Vertical arrays of mist nets hoisted into the canopy were a highly effective method of capturing *Saccolaimus* species, as well as several other species of bat. This would be a good focus for effort on similar future surveys.
6. The study presents the first quantitative comparison of the echolocation calls of the three Australian species of *Saccolaimus* using multivariate statistics. Bulk amounts of putative bat pulses from unattended ultrasonic recordings could be tested for association with distinct confidence regions for each species based on a novel implementation of the formula for a predictive confidence ellipse. This allowed a very large amount of acoustic data to be analysed in a reasonable timeframe.
7. However, the variation in acoustic variables from unattended recordings of bats in flight over the habitat is much greater than that from reference collections, and a second approach was required to determine the presence of *S. saccolaimus* from acoustic recordings. Based on acoustic data, it was clear that a large population of *S. saccolaimus* was not present, and if it was present in low numbers, these were such that it was undetectable given the significant survey expended effort and contemporary methodology used.
8. The acoustic methods provide good scope for long term monitoring of *S. mixtus* in the Project area, as a relatively abundant indicator species that depends on the *E. tetrodonta* forest for suitable habitat.

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APPENDICES

Appendix 1a. Summary of harp trap deployments and captures in June 2012. Double bank harp traps have two sets of vertical fishing lines, triple banks have three. No harp traps were deployed in October 2012.

Site	Type	Easting (54L)	Northing	Dates (nights of)	Trap hours	Captures[1]	RE
H01	Triple bank	569095	8568717	16/06/2012–17/06/2012	24	—	3.5.2
H02	Triple bank	569097	8568711	16/06/2012–17/06/2012	24	—	3.5.2
H03, H04	Triple bank	569293	8569542	16/06/2012–19/06/2012	48	Cn(1), Mo(1), Nb(1)	3.5.2
H05	Double bank	569125	8572410	16/06/2012–25/06/2012	48	—	3.2.2
H06	Triple bank	568093	8566817	18/06/2012–19/06/2012	120	—	3.5.2
H07	Triple bank	567091	8564849	18/06/2012–19/06/2012	24	—	3.5.2
H08	Triple bank	575002	8563104	21/06/2012–25/06/2012	24	—	3.3.9
H09	Triple bank	575686	8563790	22/06/2012–25/06/2012	60	—	3.5.2
H10	Triple bank	578102	8564137	22/06/2012–25/06/2012	48	—	3.3.21
H11	Triple bank	580069	8566662	22/06/2012–25/06/2012	48	—	3.5.2

[1] Captures: Cn: hoary wattled bat *Chalinolobus nigrogriseus*; Mo: eastern bent-winged bat *Miniopterus oceanensis*; Nb: eastern long-eared bat *Nyctophilus bifax*.

Appendix 1b. Summary of mist net deployments and captures in June 2012. Pole mounted nets were erected at ground level; triple stacked nets were suspended on rope frames to a height at the top of c. 20 m.

Site	Type	Easting (54L)	Northing	Dates (night of)	Trap hours	Captures [1]	Tissue sample codes	RE
M01	pole mounted	569087	8568719	16/06/2012	5	—	—	3.5.2
M02	pole mounted	568430	8571060	16/06/2012	5	—	—	3.5.2
M03	triple stack	569206	8572231	17/06/2012, 19/06/2012	5	Cj(4), Cn(2), Sa(2), Sm(8)	CN01-CN02, SM06-SM13	3.5.2
M04	pole mounted	569125	8572410	17/06/2012	5	Cj(4)	—	3.2.2
M05	pole mounted	570978	8568310	18/06/2012	5	Cn(1)	—	3.3.50a
M06	triple stack	565542	8568152	18/06/2012	5	Cn(1), Pa(1), Ps(1), Sm(5)	CN03, SM01-SM05	3.2.6
M07	triple stack	574929	8563110	21/06/2012	5	—	—	3.3.9
M08	triple stack	581311	8566555	22/06/2012	5	—	—	3.5.22c
M10	triple stack	574889	8564217	23/06/2012, 24/06/2012	5	Sm(3), Ps(1)	SM14-SM16	3.5.2
M11	triple stack	578092	8565087	25/06/2012	5	—	—	3.5.2

[1] Captures: Cj: northern free-tailed bat *Chaerephon jobensis*; Cn: hoary wattled bat *Chalinolobus nigrogriseus*; Pa: forest pipistrelle *Pipistrellus adamsi*; Ps: little red flying-fox *Pteropus scapulatus*; Sa: eastern blossom bat *Syconycteris australis*; Sf: yellow-bellied sheath-tailed bat *Saccolaimus flaviventris*; Sm: Papuan sheath-tailed bat *Saccolaimus mixtus*; Sn northern broad-nosed bat *Scotorepens sanborni*.

Appendix 2a. Summary of pole-mounted mist net deployments and captures in October 2012. See Appendix 1a,b for an explanation of species identity acronyms.

Site	Easting (54L)	Northing	Dates (night of)	Trap hours	Captures	Tissue sample codes	RE
P01	592491	8584668	10/10/2012	4	Sm(1)	—	3.5.2
P02	575055	8562707	12/10/2012	3.5	—	—	3.5.2
P03	580898	8558662	12/10/2012	3.5	—	—	3.5.2
P04	578896	8552536	13/10/2012	3.5	—	—	3.5.2
P05	570084	8548665	13/10/2012	3.5	—	—	3.5.2
P06	572900	8556443	15/10/2012	3.5	—	—	3.5.2
P07	568900	8555322	15/10/2012	3.5	—	—	3.5.2
P08	567089	8564690	16/10/2012	3.5	—	—	3.5.2
P09	568114	8566664	16/10/2012	3.5	—	—	3.5.2
P10	568896	8568701	17/10/2012	3.5	—	—	3.5.2
P11	569694	8569696	17/10/2012	3.5	—	—	3.5.2
P12	581305	8566540	18/10/2012	3.5	—	—	3.5.22c
P13	578088	8565062	18/10/2012	3.5	—	—	3.5.2
P14	576482	8564092	19/10/2012	3.5	—	—	3.5.2
P15	575004	8563198	19/10/2012	3.5	—	—	3.3.21

Appendix 2b. Summary of rope-mounted canopy mist net deployments and captures in October 2012. See Appendix 1a,b for an explanation of species identity acronyms.

Site	Easting (54L)	Northing	Dates (night of)	Trap hours	Captures	Tissue samples	Comment	RE
T01	580300	8574057	9/10/2012	4	Sf(2)	SF02-SF03		3.5.2
T02	575697	8572602	9/10/2012	4	Sf(1)	SF01		3.5.2
T03	592485	8584609	10/10/2012	4	Sf(2), Sm(5)	SF04-SF05	With P01	3.5.2
T04	595660	8580742	10/10/2012	4	Cn(2), Sm(10), Sn(2)	SG01-SG02, SM17-SM20		3.5.2
T05	588510	8568654	11/10/2012	3.5	Sm(2)	—		3.5.2
T06	578093	8568986	11/10/2012	3.5	—	—		3.5.2
T07	575081	8562723	12/10/2012	3.5	Sm(1) recapture	—	With P02	3.5.2
T08	580898	8558662	12/10/2012	3.5	Sf(1), Sm(5)		With P03	3.5.2
T09	578894	8552605	13/10/2012	3.5	Cn(1), Sm(2)	—	With P04	3.5.2
T10	570115	8548688	13/10/2012	3.5	Sf(1), Sm(1)		With P05	3.5.2
T11	572899	8556376	15/10/2012	3.5	Cn(1), Sm(1), Sf(6)	SF06-SF11	With P06	3.5.2
T12	568902	8555337	15/10/2012	3.5	Cj(10), Cn(2), Sf(2), Sm(2)	CN20-CN21, SF12-SF13	With P07	3.5.2
T13	568093	8566658	16/10/2012	3.5	Sf(1), Sm(2)		With P09	3.5.2
T14	568899	8568670	17/10/2012	3.5	Sm(1)	—	With P10	3.5.2
T15	569697	8569664	17/10/2012	3.5	Sf(1), Sm(1)		With P11	3.5.2
T16	581309	8566552	18/10/2012	3.5	—	—	With P12	3.5.22c
T17	578089	8565093	18/10/2012	3.5	Sm(3), Ps(1)	—	With P13	3.5.2
T18	576474	8564089	19/10/2012	3.5	—	—	With P14	3.5.2
T19	575005	8563237	19/10/2012	3.5	—	—	With P15	3.5.22c
T20	567089	8564690	16/10/2012	3.5	Cn(1), Sm(4)	CN22	With P08	3.5.2

Appendix 3a. Summary of SM2BAT deployments in June 2012. Number of pulses attributed to each *Saccolamius* species are given.

Site	Date	Serial	Easting	Northing	Recording hours	RE	*S. flaviventris*	*S. mixtus*	*"S. saccolaimus"*
S01	16/06/2012	8066	569126	8572403	12	3.2.2	224	196	18
S02	16/06/2012	8048	567699	8568653	12	3.5.2	175	1963	439
S03	16/06/2012	8072	569291	8569505	12	3.5.2	192	493	68
S04	16/06/2012	8045	569293	8568663	12	3.5.2	40	274	44
S05	16/06/2012	8052	569091	8568729	12	3.5.2	311	146	6
S06	16/06/2012	8060	568430	8571059	12	3.5.2	204	510	129
S07	17/06/2012	8060	570114	8570769	12	3.5.2	8	5	0
S08	17/06/2012	8052	572091	8571404	12	3.5.2	*No data*		
S09	17/06/2012	8045	573690	8571922	12	3.5.2	33	289	15
S10	17/06/2012	8072	575500	8572502	12	3.5.2	138	170	4
S11	17/06/2012	8066	568494	8570252	12	3.5.2	282	54	2
S12	17/06/2012	8048	567020	8569811	12	3.5.2	681	1502	391
S13	18/06/2012	8048	570986	8568306	12	3.3.50a	84	146	20
S14	18/06/2012	8045	568497	8564676	12	3.5.2	188	49	6
S15	18/06/2012	8052	566663	8564667	12	3.5.2	*No data*		
S16	18/06/2012	8066	567094	8564840	12	3.5.2	13	4	0
S17	18/06/2012	8060	569905	8564665	12	3.5.2	21	147	7
S18	18/06/2012	8072	565555	8568163	12	3.2.6	3433	1799	281
S19	19/06/2012	8052	568496	8567663	12	3.5.2	*No data*		
S20	19/06/2012	8060	568494	8566666	12	3.5.2	78	59	13
S21	19/06/2012	8066	568493	8565665	12	3.5.2	27	146	10
S22	19/06/2012	8072	569689	8566662	12	3.5.2	90	31	1
S23	19/06/2012	8045	568092	8566672	12	3.5.2	*No data*		
S24	19/06/2012	8048	566895	8566668	12	3.5.2	105	384	20
S25	21/06/2012	8060	574996	8563107	12	3.3.9	83	280	69
S26	21/06/2012	8052	574884	8563811	12	3.5.2	*No data*		
S27	21/06/2012	8066	575686	8564660	12	3.5.2	61	231	33

Continued over ...

Appendix 3a. Summary of SM2BAT deployments in June 2012, *continued*.

Site	Date	Serial	Easting	Northing	Recording hours	RE	*S. flaviventris*	*S. mixtus*	*"S. saccolaimus"*
S28	21/06/2012	8072	578092	8566662	12	3.5.2	29	458	38
S29	21/06/2012	8045	579692	8566661	12	3.5.2	11	180	41
S30	21/06/2012	8048	581292	8566664	12	3.5.22c	33	43	14
S31	22/06/2012	8045	581313	8566214	12	3.5.22c	20	13	0
S32	22/06/2012	8052	580056	8566667	12	3.5.2	*No data*		
S33	22/06/2012	8066	579697	8566257	12	3.5.2	9	136	6
S34	22/06/2012	8060	578100	8564143	12	3.3.21	73	383	112
S35	22/06/2012	8072	576478	8564082	12	3.5.2	26	87	12
S36	22/06/2012	8048	575696	8563504	12	3.3.21	8	120	21
S37	23/06/2012	8045	581259	8566007	12	3.3.9	18	48	4
S38	23/06/2012	8052	579691	8565737	12	3.5.2	*No data*		
S39	23/06/2012	8066	579097	8564966	12	3.5.2	34	8	0
S40	23/06/2012	8060	577848	8564139	12	3.3.21	26	34	1
S41	23/06/2012	8072	576709	8563884	12	3.3.9	9	16	4
S42	23/06/2012	8048	575444	8563352	12	3.3.9	10	39	2
S43	24/06/2012	8060	578088	8565117	12	3.5.2	7	26	1
S44	24/06/2012	8072	576511	8564627	12	3.3.21	63	495	33
S45	24/06/2012	8048	574818	8563140	12	3.3.9	9	72	8
S46	24/06/2012	8066	578764	8564691	12	3.5.2	1	4	0
S47	24/06/2012	8052	579697	8565037	12	3.3.9	*No data*		
S48	24/06/2012	8045	574892	8564213	12	3.5.2	1	106	4
S49	25/06/2012	8045	574896	8565681	12	3.5.2	90	174	12
S50	25/06/2012	8072	575942	8563438	12	3.3.9	46	129	14
S51	25/06/2012	8048	575691	8565775	12	3.5.2	23	24	5
S52	25/06/2012	8060	578281	8564201	12	3.3.21	53	246	70
S53	25/06/2012	8052	578095	8565083	12	3.5.2	42	154	15
S54	25/06/2012	8066	576366	8566661	12	3.5.2	14	14	4

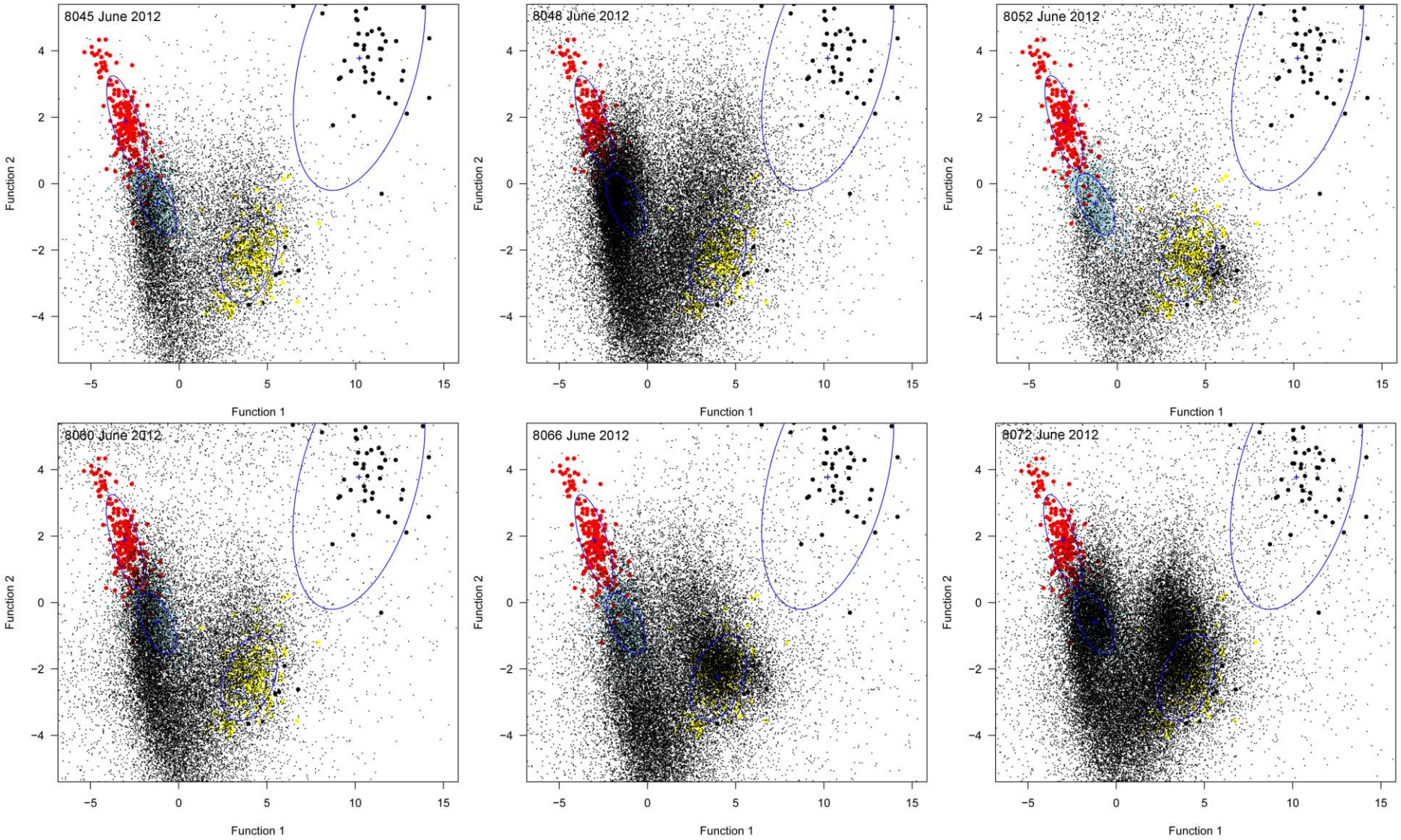
Appendix 3b. Summary of SM2BAT deployments in October 2012. Number of pulses attributed to each *Saccolamius* species are given.

Site	Date	Serial	Easting	Northing	Recording hours	RE	*S. flaviventris*	*S. mixtus*	*"S. saccolaimus"*
G01	9/10/2012	8060	580293	8574032	11	3.5.2	144	804	89
G02	9/10/2012	7983	578288	8573401	11	3.5.2	19	176	15
G03	9/10/2012	8072	575694	8572550	11	3.5.2	139	262	8
G04	9/10/2012	7849	573253	8571782	11	3.5.2	*No data*		
G05	9/10/2012	8048	570091	8570766	11	3.5.2	136	16	1
G06	9/10/2012	8066	570140	8571551	11	3.5.2	185	311	11
G07	10/10/2012	8048	592489	8584653	11	3.5.2	330	1417	200
G08	10/10/2012	8066	596028	8584660	11	3.5.2	7	495	31
G09	10/10/2012	8072	587684	8576431	11	3.5.2	95	5178	526
G10	10/10/2012	8060	590224	8577157	11	3.5.22c	11	546	64
G11	10/10/2012	7983	590621	8577299	11	3.7.3	3026	77	44
G12	10/10/2012	7849	595657	8580719	11	3.5.2	*No data*		
G13	11/10/2012	8072	580104	8570672	11	3.5.2	2588	10713	1687
G14	11/10/2012	8048	582498	8570665	11	3.5.2	9	268	24
G15	11/10/2012	8066	587364	8570659	11	3.5.2	3	31	5
G16	11/10/2012	8060	588484	8568656	11	3.5.2	161	3800	178
G17	11/10/2012	7983	578097	8568963	11	3.5.2	211	399	44
G19	12/10/2012	8060	585291	8565370	11	3.5.2	27	1746	94
G20	12/10/2012	7849	580658	8562653	11	3.5.2	*No data*		
G21	12/10/2012	8066	577180	8562654	11	3.5.2	1578	4283	465
G22	12/10/2012	8072	575063	8562710	11	3.5.2	409	678	42
G23	12/10/2012	7983	581301	8558991	11	3.5.2	243	3184	101
G24	12/10/2012	8048	580892	8558711	11	3.5.2	21	1308	60
G26	13/10/2012	8060	570084	8548682	11	3.5.2	372	1046	430
G27	13/10/2012	7983	573306	8552669	11	3.5.2	181	3683	479
G28	13/10/2012	8072	578902	8552613	11	3.5.2	116	559	77
G29	15/10/2012	8048	568888	8555335	11	3.5.2	460	2188	147

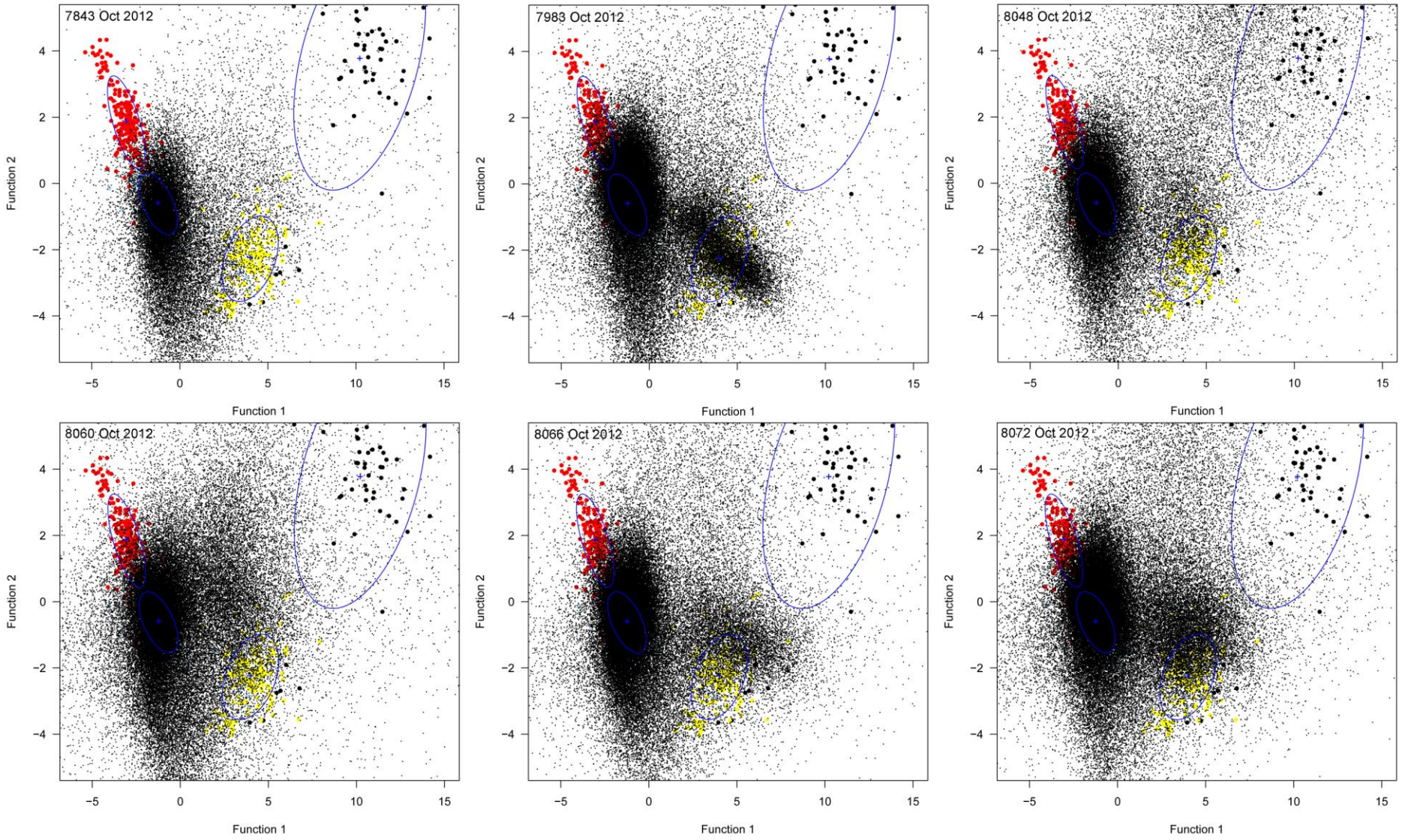
Continued over ...

Appendix 3b. Summary of SM2BAT deployments in October 2012, *continued*.

Site	Date	Serial	Easting	Northing	Recording hours	RE	*S. flaviventris*	*S. mixtus*	*"S. saccolaimus"*
G30	15/10/2012	8060	570117	8555663	11	3.5.2	277	1248	239
G31	15/10/2012	7849	570101	8551930	11	3.5.2	18	86	11
G32	15/10/2012	8072	571548	8556060	11	3.5.2	32	322	25
G33	15/10/2012	7983	572911	8556429	11	3.5.2	1392	606	57
G34	15/10/2012	8066	575798	8557232	11	3.5.2	41	156	23
G35	16/10/2012	8066	567089	8564689	11	3.5.2	14	265	16
G36	16/10/2012	7849	570090	8564666	11	3.5.2	*No data*		
G37	16/10/2012	8060	568492	8565665	11	3.5.2	51	1393	155
G38	16/10/2012	8072	568091	8566640	11	3.5.2	128	707	20
G39	16/10/2012	7983	565291	8566663	11	3.5.2	375	10070	1204
G40	16/10/2012	8048	569700	8566658	11	3.5.2	181	2739	604
G41	17/10/2012	8072	568890	8570387	11	3.5.2	24	77	5
G42	17/10/2012	8060	566995	8569775	11	3.5.2	420	1124	131
G43	17/10/2012	7849	566228	8568687	11	3.5.2	565	655	28
G44	17/10/2012	7983	568927	8568664	11	3.5.2	30	41	3
G45	17/10/2012	8048	569660	8569664	11	3.5.2	60	759	207
G46	17/10/2012	8066	570894	8568663	11	3.5.2	75	6600	593
G47	18/10/2012	8048	581312	8566665	11	3.5.22c	41	3962	113
G48	18/10/2012	8072	579694	8566239	11	3.5.2	36	269	4
G49	18/10/2012	8066	578890	8566662	11	3.5.2	269	6118	203
G50	18/10/2012	7983	579088	8564971	11	3.5.2	2	119	2
G51	18/10/2012	8060	578072	8564197	11	3.5.22c	22	61	27
G52	18/10/2012	7849	578091	8565107	11	3.5.2	46	1693	119
G53	19/10/2012	8060	574889	8564683	11	3.5.2	84	255	8
G54	19/10/2012	8066	575054	8563075	11	3.3.9	16	99	21
G55	19/10/2012	8072	575004	8563227	11	3.5.22c	2	10	3
G56	19/10/2012	8048	575687	8564667	11	3.5.2	284	281	32
G57	19/10/2012	7983	575691	8563388	11	3.3.21	46	312	13
G58	19/10/2012	7849	576487	8564097	11	3.5.2	*No data*		



Appendix 4a. Patterns over 10 recording nights in June 2012, grouped arbitrarily by the serial number of the recorder, showing the presence of both *S. mixtus* and *S. flaviventris*, and little or no evidence of *C. jobensis* or *S. saccolaimus*. Refer to **Figure 8** for a guide to the identity of confidence ellipses.



Appendix 4b. Patterns over 10 recording nights in October 2012, grouped arbitrarily by the serial number of the recorder, showing the presence of both *S. mixtus* and *S. flaviventris*, and little or no evidence of *C. jobensis* or *S. saccolaimus*. Refer to **Figure 8** for a guide to the identity of confidence ellipses.

Appendix 5. Details of the Regional Ecosystems (after Sattler and Williams 1999) where *Saccolaimus* spp. were detected by capture or acoustic recording.

Code	Description	Detail
3.5.2	*Eucalyptus tetrodonta, Corymbia nesophila* tall woodland on deeply weathered plateaus and remnants.	*Eucalyptus tetrodonta* (Darwin stringybark) predominates forming a distinct but discontinuous canopy (22-32m tall) with *Corymbia nesophila* (Melville Island bloodwood) present as a subdominant to codominant canopy species. Large *Erythrophleum chlorostachys* (Cooktown ironwood) trees may be present. These occur just below the canopy. A very sparse to sparse sub canopy layer (8-25m tall) is dominated by *Eucalyptus* spp. and *Grevillea glauca* (bushman's clothes peg). Scattered low trees (4-8m tall) are sometimes present. *Acacia* spp. and *Eucalyptus* spp. dominate the sparse to very sparse shrub layer (0.5-2m tall). The ground layer is usually sparse to mid-dense and dominated by the grasses, *Sarga plumosum* (plume sorghum), *Heteropogon triticeus* (giant speargrass), *Alloteropsis semialata* (cockatoo grass) and *Eulalia mackinlayi* (silky browntop). Occurs on deeply weathered plateaus and remnants. (BVG1M: 14a)
3.7.3	*Eucalyptus cullenii* ± *E. tetrodonta* woodland on erosional escarpments and plains. Occurs on erosional escarpments and plains on the edge of the bauxite plateaus.	*Eucalyptus cullenii* (Cullen's ironbark) dominates the sparse canopy (14-25m tall). Other *Eucalyptus* spp. or *Corymbia* spp. particularly *E. tetrodonta* (Darwin stringybark) and *Corymbia disjuncta* (cabbage gum) may be present and are occasionally subdominant. *Erythrophleum chlorostachys* (Cooktown ironwood) is also frequently subdominant. The very sparse to sparse sub canopy layer (4-10m tall) is composed most frequently of *Planchonia careya* (cocky apple), *C. disjuncta, Eucalyptus tetrodonta, Petalostigma banksii* (smooth-leaved quinine) and *Alphitonia pomaderroides* (soapwood). The sparse to very sparse shrub layer (0.5-3m tall) frequently includes *Croton arnhemicus* (hard cascarilla), *Erythrophleum chlorostachys*, *Decaschistia peninsularis*, *Corymbia disjuncta, C. nesophila* (Melville Island bloodwood) and *Planchonia careya* shrubs. The grasses *Heteropogon triticeus* (giant speargrass), *Sarga plumosum* (plume sorghum), *Eulalia mackinlayi* (silky browntop) and *Schizachyrium* spp. (fire grass) dominate the sparse to mid-dense ground layer. Occurs on erosional escarpments and plains on the edge of the bauxite plateaus. (BVG1M: 13a)

Continued over ...

Appendix 5. Details of the Regional Ecosystems, *continued*.

Code	Description	Detail
3.5.22c	*Corymbia clarksoniana* + *Erythrophleum chlorostachys*+ *Corymbia* spp. + *Eucalyptus* spp. woodland on plains.	*Corymbia clarksoniana* (Clarkson's bloodwood) dominates the sparse canopy. In the northern areas, this species is replaced by another bloodwood, *C. novoguinensis. Lophostemon suaveolens* (swamp mahogany), *Parinari nonda* (nonda) and less frequently *Erythrophleum chlorostachys* (Cooktown ironwood) are subdominant trees. The sparse sub canopy is most frequently dominated by *Melaleuca viridiflora* (broad-leaved teatree). *Livistona muelleri* (dwarf fan palm) and *Alphitonia pomaderroides* (soapwood) are also common components of this layer. *Antidesma ghaesembilla* (black currant) and *Flueggea virosa* subsp. *melanthesoides* (white currant) are characteristic species of the sparse shrub layer. The ground layer is sparse to dense and dominated by *Fimbristylis* sp., *Heteropogon triticeus* (giant speargrass), *Aristida* sp. (three-awned speargrass) and *Ischaemum* sp. Occurs on undulating rises and plains. (BVG1M: 9e)
3.2.2	Semi-deciduous vine thicket on coastal dunes and beach ridges.	The dense, uneven canopy (6-12m tall) is dominated by a mixture of deciduous and evergreen species with *Sterculia quadrifida*, *Canarium australianum* (scrub turpentine), *Cochlospermum gillivraei* (kapok), *Erythrina vespertilio* (batswing coral tree), *Ficus virens* (white fig), *Millettia pinnata* and *Terminalia muelleri* (Australian almond) the principal deciduous species. The evergreen species include *Neofabricia myrtifolia* (yellow teatree), *Syzygium suborbiculare* (Lady apple), *Celtis philippensis* var. *philippensis*, *Manilkara kauki, Polyalthia nitidissima* and *Thryptomene oligandra*. Occasional emergents up to 25 metres tall are present. The mid-dense sub canopy layer (2-8m tall) contains a variety of species with *Cupaniopsis anacardioides* the most frequent species. *Eugenia reinwardtiana, Exocarpos latifolius, Canthium* sp., *Ixora timorensis* and *Strychnos lucida* (strychnine bush) are the most commonly occurring shrubs in the very sparse to sparse shrub layer (0.5-1.5m tall). A number of thin vines such as *Cayratia cardiophylla* and *Cissus adnata* are present in both the canopy and low tree layer. The ground layer is very sparse, and composed of predominantly graminoids. Occurs on coastal dunes and beach ridges. (BVG1M: 3b)
3.2.6	*Casuarina equisetifolia* woodland. Occurs on foredunes.	No further description.

Continued over ...

Appendix 5. Details of the Regional Ecosystems, *continued*.

Code	Description	Detail
3.3.50a	*Melaleuca viridiflora* ± *Petalostigma pubescens* low open woodland on low plains (paperbark wetland).	Palustrine wetland (e.g. vegetated swamp). *Melaleuca viridiflora* (broad-leaved teatree) dominates a very sparse canopy (4-14m tall) with scattered emergent *Corymbia clarksoniana* (Clarkson's bloodwood) (8-18m tall) often present. Other *Corymbia* spp. or *Eucalyptus* spp. occur very occasionally as emergent trees. A very sparse sub canopy tree layer (2-9m tall) is present at most sites with *M. viridiflora* and *Petalostigma pubescens* (quinine) occurring at the greatest densities. A very sparse shrub layer (<0.5 m) dominated by *M. viridiflora* juveniles is present at most sites. The ground layer is sparse to mid-dense and dominated by grasses or sedges in wetter parts. *Schizachyrium* spp. (fire grass), *Aristida* spp. (three-awned spear grasses), *Eriachne* spp. (wanderrie grasses) and *Eremochloa bimaculata* (poverty grass) are common dominant species in this layer. Occurs on low-lying plains. (BVG1M: 21a)
3.3.9	*Lophostemon suaveolens* +/- *Melaleuca leucadendra* open forest. Occurs on streamlines, swamps and alluvial terraces.	*Lophostemon suaveolens* (swamp mahogany), *Xanthostemon crenulatus* and occasionally *Melaleuca leucadendra* (weeping teatree) dominate the sparse to mid-dense canopy which can range in height from 15 to 25 metres tall. *Dillenia alata* (golden guinea tree) is a common subdominant canopy species. A number of *Acacia* spp. (wattles), *Eucalyptus* spp. or *Corymbia* spp. and other tree species may be present in the canopy. A very sparse to sparse sub canopy layer (2-10m tall) of palms and other trees may be present in some situations. A very sparse to sparse shrub layer (0.5-2m tall) is usually present and may be dominated in some areas by either *Banksia dentata* (banksia) or *Melaleuca viridiflora* (broad-leaved teatree). The ground layer is sparse to mid-dense and composed of a variety of sedges, graminoids and ferns. Occurs on streamlines, swamps and alluvial terraces. (BVG1M: 22b)
3.3.21	*Corymbia clarksoniana* ± *Syzygium eucalyptoides* woodland. Lower slopes of sand ridges and in drainage depressions.	*Corymbia clarksoniana* (Clarkson's bloodwood) dominates the sparse canopy (8-18m tall). *Melaleuca viridiflora* (broad-leaved teatree) and *Syzygium eucalyptoides* subsp. *eucalyptoides* are subdominant trees. *M. viridiflora* occurs at high stem densities, particularly in the sparse sub canopy, (6-7m tall) where it is usually dominant. *Corymbia polycarpa* (long-fruited bloodwood) was recorded at one site. *Banksia dentata* (banksia), *Asteromyrtus symphyocarpa* (lineament tree) and *Neofabricia mjoebergii* (yellow teatree) are characteristic species of the sparse shrub layer (0.2-6m tall). The ground layer is mid-dense and dominated by *Schoenus sparteus*, *Fimbristylis* sp., *Scleria* sp., *Eriocaulon* sp. and *Cartonema parviflorum*. Lower slopes of sandridges and in drainage depressions. (BVG1M: 9e)