UniSC Australia | Detection Dogs for Conservation

Koala genetic monitoring of Redlands Coast mainland

FINAL REPORT

Prepared for Redland City Council

by Detection Dogs for Conservation, University of the Sunshine Coast

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List of abbreviations

Abbreviation	Meaning
CI	Confidence Interval
DDC	Detection Dogs for Conservation
DArT	Diversity Arrays Technology
H_O	Observed heterozygosity: the calculated level of heterozygosity from the allele frequencies of the population under study
H_E	Expected heterozygosity: the level of heterozygosity that could be expected based on observed allele frequencies if the population was at the Hardy-Weinberg equilibrium
H_S	Expected heterozygosity adjusted for small sample size
F_{IS}	Inbreeding coefficient: the proportion of the variance in the subpopulation contained in an individual
F_{ST}	Fixation index: measures the degree of genetic differentiation between genetic clusters
MAF	Minor allele frequency
RCC	Redland City Council
SNPs	Single Nucleotide Polymorphisms
UniSC	University of the Sunshine Coast

Executive summary

This project builds on previous landscape, fine-scale, data collected in 2018 and 2020/2021 in Redlands Coast and helps further inform koala genetic diversity and connectivity, as well as *Chlamydia* prevalence, to empower decision makers to effectively manage koalas. In particular, we focused on determining:

1) Redlands Coast koala population dynamics

A total of 223 scats were included for analyses. We conducted a total of 90 detection dog surveys across Redlands Coast mainland, leading to the collection of 112 scats. All 112 scat samples were extracted and genotyped, together with 27 scats collected during detection dog surveys and 75 scats collected after thermal-imaging drone surveys, as part of the Koala Sentinel Sites project by Redland City Council, as well as nine scats from monitoring ambassador koalas for the Koala Safe Neighbourhood (KSN) monitoring program, also supported by Redland City Council.

After filtering of genetic data and identification of duplicates, 97 unique individuals were identified. All results from 2023/2024 were compared to results from analyses using data collected in 2018 (N = 124) and 2020/2021 (N = 116). Of the 2023/2024 unique individuals, 11 had already been sampled during past surveys, with five koalas having samples dating back to 2018.

Male to female sex ratio across genetically identified unique individuals was 1:1.76, with the 80% confidence interval for the proportion of males in the population between 30% and 43%, indicating a female bias, which had not been previously recorded in 2018 and 2020/2021 – however, CIs of 2023/2024 and 2018 overlapped, indicating no statistically significant changes were detected since the start of the monitoring program.

2) Structure in the Redlands Coast koala population

Population structure analyses confirmed previous results from 2018 and 2020/2021 that the Redlands Coast mainland koala population is one single, connected population. These

analyses also identified samples originating from Minjerribah translocated koalas, both within 2024 samples and samples across 2018 – 2024. Low levels of genetic mixing between Minjerribah and mainland koalas were found. This indicates that translocated Minjerribah koalas have successfully reproduced with mainland koalas. If mixing between Minjerribah and mainland clusters continues over time, it could aid in increasing genetic diversity of the mainland population.

3) Health parameters of Redlands Coast koalas

Chlamydia infection prevalence across genetically identified koalas was 25%, with 80% confidence interval between 19% and 31%. Infection prevalence was similar for males and females, with seven *Chlamydia*-infected females, resulting in 27% prevalence (80% CI: 17% to 39%), and eight *Chlamydia*-infected males, resulting in 38% prevalence (80% CI: 26% to 52%). Overall *Chlamydia* prevalence was lower in 2023/2024 than 2020/2021, but comparable to levels found in 2018, based on the overlap of 80% confidence intervals. Future monitoring should aim to confirm the existence of a declining trend in infection prevalence. While the proportion of *Chlamydia*-infected koalas with active chlamydial disease remains unknown, *Chlamydia* infection is high, suggesting that disease continues to be a threat to Redlands Coast koalas.

Filtering for genetic diversity analyses resulted in the retention of 46 individuals and 1,279 SNPs. Similarly to previous years, observed heterozygosity (H_O) was lower than expected heterozygosity (H_S, the expected heterozygosity adjusted for small sample size), leading to a certain level of inbreeding. This is to be expected, as Redlands Coast koalas belong to what is commonly referred to as the Koala Coast population, which is thought to have been relatively isolated from the rest of SEQ, resulting in lower genetic diversity. Importantly, across the three Redlands Coast genetics surveys to date, no decrease in genetic diversity has been detected.

Finally, the effective population size (N_e) for Redlands Coast mainland koalas was 88.4 (90% CI: 86.9 – 89.8), remaining unchanged since 2018.

Overall, these results did not detect any concerning changes for the Redlands Coast mainland koala population, but highlight *Chlamydia* remains prevalent.

Executive summary table: Overview of findings from 2023/2024 in comparison to 2018 and 2020/21 data, as well as recapitulation of changes in 2020/21 compared to 2018. Improved measures are presented in green, deteriorating measures in red (e.g. for some measures, an increase is a positive sign, while for others it is a negative sign. For instance, an increase in inbreeding measures is not positive for the koala population, whilst an increase in effective population size is).

Measure	**Change 2020/21 compared to 2018**	**Change 2023/24 compared to 2020/21**	**Change 2023/24 compared to 2018**
Sex ratio	No change	Decrease in males	No change
Observed Heterozygosity	No change*	Increase#	Increase#
Expected Heterozygosity	No change*	No change	No change
Chlamydia positive koalas	No change*	Decrease	No change
Effective population size N_e	Increase	No change	Increase

** Changes are based on CIs (this is the difference from last reporting)*

 # Smaller sample size remained post filtering for these calculations, which may impact results

1 Introduction

1.1 Background

The koala (*Phascolarctos cinereus*) is a highly specialised, folivorous, arboreal marsupial, recognised internationally as one of Australia's most iconic species (Martin and Handasyde 1990). Bringing more than three billion dollars to the Australian economy each year (Conrad 2014, Markwell 2021), the koala is a culturally significant species that serves as an ecological umbrella for a wide range of wildlife inhabiting forest ecosystems (Ward, Rhodes et al. 2020). In recent decades, koala populations have experienced dramatic declines which legislation has so far failed to reverse (McAlpine, Lunney et al. 2015, Tisdell, Preece et al. 2017). Following the 2019-2020 Black Summer megafire, the species was listed as Endangered in Queensland, New South Wales and the Australian Capital Territory under the Australian Environment Protection and Biodiversity Conservation (EPBC) Act 1999 (Commonwealth of Australia 2022).

In addition to threats linked to climate change (such as the 2019-2020 Black Summer megafires), reasons for koala population declines include: 1) habitat loss and fragmentation, 2) diseases such as those caused by the bacterial pathogen *Chlamydia*, leading to blindness, sterility and potential death, and 3) the risks associated with koala movements in human-altered landscapes, including dog attacks and car strikes (Melzer, Carrick et al. 2000, McAlpine, Rhodes et al. 2006, Rhodes, Ng et al. 2011, Polkinghorne, Hanger et al. 2013, Burton and Tribe 2016, Gonzalez-Astudillo, Allavena et al. 2017, Beyer, de Villiers et al. 2018, Shabani, Shafapourtehrany et al. 2023, Vitali, Reiss et al. 2023). However, evidence about how these threats are impacting specific populations in real time are often not available to decision makers. Effective environmental planning requires fine-scale temporal and spatial information about 1) koala distribution, 2) koala density, 3) connectivity between koalas across the landscape, and 4) population health across the landscape. Generating these data has often been prohibitively costly (in time and financial resources), as traditionally this has required catching, sampling and monitoring live animals. Non-invasive methodologies aim to provide an alternative to these traditional survey methods and, due to their relative ease

(compared to catching animals), open the possibility of finer resolution monitoring e.g. more frequently (so as to enable more responsive adaptative management) and at the spatial scale where management decisions are made.

In 2018, the University of the Sunshine Coast's (UniSC) Detection Dogs for Conservation (DDC) team conducted a landscape survey across the whole Redland City Council's area (hereafter, Redlands Coast) using non-invasive methods to create a molecular dataset from koala scats (faecal pellets) to provide detailed information on the Redlands Coast koala population and its genetics. This was the first high resolution landscape scale genetic study for koalas using only non-invasive samples and it provided valuable insights into the Redlands Coast koala population. These surveys were repeated in 2020/2021 (mainland only), and now in 2023/2024 (mainland only), as part of a long-term monitoring program, to strengthen the scientific robustness of inferences and detect temporal trends in the Redlands Coast mainland koala population.

1.2 Scope of works

1.2.1 Survey 1 - 2018

In 2018, Redland City Council (RCC) contracted the DDC team to conduct a genetic study based on koala scat surveys in the Redlands Coast. The aims of this study were to better understand koala population characteristics and inform koala management plans. These surveys, conducted on the mainland and on Minjerribah (North Stradbroke Island), covered both urban and bushland sites. The method used was the deployment of detection dogs trained to detect koala scats, and the surveys provided baseline data on:

- genetic characteristics e.g. diversity and connectivity
- presence and prevalence of a major koala threat, the *Chlamydia* pathogen.

1.2.2 Survey 2 - 2020/2021

Survey data and analysis of scat samples collected in 2018 laid the foundation for better understanding koala population dynamics (also called genetic monitoring (Schwartz, Luikart

et al. 2007)) across the region. The mainland koala scat collection surveys were repeated, using detection dogs, in 2020/2021, with the objectives to investigate:

- genetic characteristics e.g. genetic diversity and connectivity
- presence and prevalence of the *Chlamydia* pathogen
- changes in koala genetics and *Chlamydia* prevalence over time.

1.2.3 Survey 3 - 2023/2024

The next repeat of the Redlands Coast koala genetic monitoring program was conducted on the mainland in 2023/2024. It combined koala scat collection using detection dogs for landscape genetic surveys, with scats collected through other koala programs of Council, namely sentinel sites (thermal imaging drones and detection dogs) and samples secured through radiotracking ambassador koalas. The objectives of the 2023/2024 surveys were to continue describing and monitoring changes in koala genetics and *Chlamydia* over time, with the aim to inform long-term conservation strategies and adaptive management practices.

Altogether, the koala genetic surveys provide an important measure of the status of koala populations across the Redlands Coast mainland. By utilising non-invasive, cost-effective and scalable methods to measure key koala health indicators, these surveys are contributing to building a large scale, scientifically robust dataset on koala genetic diversity, connectivity, and *Chlamydia* prevalence.

Using molecular analyses of collected scats, we aimed to assess changes in koala populations across the three survey periods, and investigate:

- sex ratio
- genetic structure
- presence and prevalence of chlamydial infection
- genetic diversity
- effective population size
- changes of these indicators over time.

This study aims to deliver insights to guide ongoing management efforts, aimed at ensuring the long-term viability of Redlands Coast koalas. The findings also aim to inform and support RCC in achieving its broader koala conservation objectives and deliverables by 2027.

2 Methods

2.1 Landscape scale koala scat collection surveys

2.1.1 Site selection

Survey sites in 2018 were selected based on habitat suitability and accessibility, and were primarily located within conservation areas, recreational areas (e.g. parks), rehabilitation areas, wildlife corridors and one National Park (Venman National Park), augmented by an appeal to private landowners to provide the survey teams access to their properties. In urban areas, sites were selected in areas where recent koala activity had been recorded (sighting records), either through the *Atlas of Living Australia* website, or from records collected by the Redlands Coast community-based Koala Action Group. In 2020/2021, surveys were repeated at the same sites as 2018; however, 1) only the mainland was included and 2) some sites could not be accessed due to construction, development or restrictions on private properties.

The 2023/2024 survey sites were selected to repeat visits of sites accessed in 2018 and 2020/2021, maximise geographic coverage of the mainland and enable the collection of as many fresh scat samples as possible within the project timeframe and budget. Site selection was refined based on accessibility (e.g. tenure) and efficiency, such as proximity to roads. Sites on private properties were included on a voluntary basis. Survey areas situated away from samples collected by DDC through other Council programs (see below) were prioritised for detection dog surveys (e.g. 2018 and 2020/2021 sites located within current sentinel monitoring sites (plus a 500 m buffer) were mostly excluded from 2023/2024 surveys because koalas present there had likely already been sampled).

In conjunction with the detection dog landscape survey genetic samples, additional samples were included from 1) the koala sentinel sites program (designed to enable the monitoring of population density trends, through the use of either drone-mounted thermal imaging or detection dogs), and 2) the ambassador koalas of the Koala Safe Neighbourhood program. Results of the sentinel site and ambassador koala surveys are presented in a separate report,

however genetic samples that were collected through these surveys are included in the genetic analyses of this report.

2.1.2 Detection dog surveys

At each site, a trained detection dog was used to locate koala scats. Detection dog surveys are more accurate and efficient than human surveys to locate koala scats (Cristescu, Foley et al. 2015) and can be adapted to a range of koala survey types, including genetic sampling (Cristescu, Miller et al. 2019).

The DDC team has developed a survey methodology known as the 'casual koala scat survey'. During casual surveys, the dog is encouraged to follow its nose and roam over an area of up to two hectares for approximately 30 minutes, or until the handler determines that the site has been thoroughly searched. This method, which allows for coverage of larger areas, is ideal for detecting fresh koala scats for genetic sampling.

During casual surveys, as the dog is free to determine where to search and follow the target scent, the search paths can vary based on individual and environmental factors, such as wind direction. Because of this, casual surveys can be difficult to replicate, making it challenging to compare results over time or space, and therefore are often not the preferred method to compare occupancy between areas or trends over time within an area. Occupancy was of course not the aim of this study, however we still aimed to maximise comparability between surveys. Therefore, the dog was fitted with a GPS collar to record the search track, and efforts were made to conduct surveys in as similar a manner as possible to past surveys. At each site, we aimed to cover a similarly sized area and, where possible, began casual searches at the same starting points used in the 2018 and 2020/2021 surveys. Location data from the dog GPS collars allowed us to map dog search tracks and assess any differences between surveys at a given site.

At each site, photographs were taken and any ecological characteristics that might have influenced the detectability or degradation of scats (e.g. wet areas or recent fire (Cristescu, Goethals et al. 2012)) were recorded.

All surveys are authorised under UniSC animal ethics (ANA23217) and DDC research permits (P-PTUKI-100534527-1, P-PTC -100534544-2).

2.1.3 Detection dogs utilised for koala scat surveys

The following detection dogs were deployed for the koala scat surveys:

- Bear - trained to detect live koalas and fresh koala scats
- Billie-Jean, Summer and Loki - trained to detect fresh koala scats only
- Austin - trained to detect koala scats of all ages.

Data collected during casual surveys was used to map the detection of koalas and/or koala scats at each site. It is important to note that surveys conducted with Bear, Billie-Jean, Loki and Summer can be used to determine koala presence; however, they cannot determine koala absence, as these dogs have been trained to ignore koala scats that are more than a few days old.

2.1.4 Identifying and characterising koala scats

When a detection dog indicated that a koala scat was present, the field team visually confirmed the scat identification – for this reason, it is critical that an ecologist or experienced koala researcher assists in scat identification. In fact, scat identification presents challenges even to experienced ecologists (Harrington, Harrington et al. 2010). Typical koala scats (**Figure 1**) have the following characteristics (Triggs 1996):

- Symmetrical and bullet-shaped (not jelly-bean shaped)
- Generally, about 1.5 cm long by 0.5 cm wide (adult koala scat size)
- Evenly-sized, fine particles
- Absence of insect parts (koalas do not eat insects).

Figure 1. Typical characteristics of koala scats.

Differences in scat size and shape were also observed, as this can sometimes indicate the presence of multiple individuals using one tree, such as an adult female and her joey. In this case, scats of different size and shape were collected in separate tubes. Location was determined using a hand-held GPS (Alpha ® 100, Garmin Ltd., Olathe, USA) and recorded. Scats were classified by age (**Error! Reference source not found.**) to help estimate how recently a koala had used the area and determine whether scats were sufficiently fresh for molecular analyses. Fresh scats were collected and stored at –20 °C in the field for subsequent genetic analyses.

Table 1. Guide used to age koala scats in the field

Scat age categories	Estimated age	Characteristics
1	Less than one day old	Very fresh (covered in mucus, wet)
2	Up to a week old	Fresh (shine and obvious odour)
3	Weeks old	Medium fresh (shine or odour when broken)
4	Months old	Old (no shine, no odour)
5	More than several months old	Very old and discoloured

2.1.5 Koala sightings

During the surveys, the field team visually searched for koalas, especially where very fresh scats were found. However, as koala sightings were not the focus of this project, a maximum of 10 minutes was spent visually searching before continuing with the scat survey.

The field team also observed and recorded any opportunistic/incidental sightings of koalas between survey locations or based on information passed on by members of the public or property owners. The general public was considered a valuable source of local knowledge and, whenever possible, individuals were consulted about koala sightings, past and present, to maximise sample collection.

When a koala was located, photographs were taken and visual observations were made using binoculars to determine: 1) koala sex, 2) reproductive status (presence of a joey), and 3) external signs of chlamydial disease, such as inflammation, crusting or redness of the eyes and/or wetness and staining of the rump. If a koala showed signs of injury or illness, the Redlands After-hours Wildlife Ambulance (RAWA) was contacted.

2.2 DNA extraction and genotyping

Typically, DNA quality is highest when acquired from very fresh koala scats (Schultz et al. 2018). Fresh scats, estimated to be less than one week old (**Error! Reference source not found.**, categories 1 and 2) present a shiny mucus layer and a strong smell. To maximise the quality of genetic data, we aimed to collect fresh scats during casual dog surveys. Scats were collected without direct skin contact (to avoid loss of DNA) and stored at -20°C until they could be processed for DNA extractions.

Scat samples were processed for DNA extraction of a single scat's outer layer, yielding both koala and *Chlamydia* DNA. We followed a similar protocol to Schultz, Cristescu et al. (2018) to isolate DNA from koala scats. However, instead of scraping the outer layer from the scat, we used a lysis wash to rinse the DNA from the surface of the scat. We used the QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol with minor modifications. An additional one-hour incubation step (65 ^{o}C) was performed after

adding the buffer to the faecal sample, and samples were vortexed for 7 minutes at maximum speed using Genie 2 Vortex Mixer (Scientific Industries, New York, USA). Final DNA isolates were eluted in 200 µl of elution buffer and concentrated to a volume of ~30 µl using a Vacufuge Concentration Plus Concentrator (Eppendorf, Hamburg, Germany) set at 65 °C for 45 minutes. Concentrated DNA extracts were stored at –80 °C until being shipped to Diversity Arrays Technology (DArT, Canberra, Australia) for genotyping, to establish a genetic identity – or genetic fingerprint – for each sample.

DNA extracts were genotyped by DArT using a next-generation sequencing protocol for detecting Single Nucleotide Polymorphisms (SNPs) (Jaccoud, Peng et al. 2001, Kilian, Wenzl et al. 2012). Scat analysis is a non-invasive genetic sampling method and can be conducted at a lower cost compared to the use of tissue or blood samples which require catching and anaesthetising koalas. In contrast to high-quality blood/biopsy samples, however, DNA present in scats is lower in quantity and quality, which yields fewer genetic markers. We were able to address some of these limitations by designing new genotyping methods (first DArTcap and then, in 2022, DArTag). These methods target specific loci by using an exclusively designed, koala specific molecular capture probe that selects small target regions containing sequence variants. This method increased both genotyping success and data quality; however, data quality improvement is still limited, and some samples yield too little genetic data to be included in further analyses. While 2018 and 2020/2021 samples were genotyped with a different method (DArTcap), overlap of SNPs between the two products aims to ensure comparability between years. However, to confirm this, genotyping was repeated, this time using DArTcap. Because DArTcap resulted in lower genotyping success, only genetic diversity measures are reported using these data, due to better comparability with measures from previous years (genetic diversity measurements are more sensitive to changes in SNP calling methodologies) while all other measures are reported using DArTag data.

2.3 Data analyses

All sample collection locations were mapped using ArcGIS/Pro v10.2. Genetic data were analysed using the R package *dartR* (Gruber, Unmack et al. 2019) in the R environment using R v4.4.2 (R Core Team 2018), as specified below.

2.3.1 Filtering of genetic data

Genotyped data were filtered to improve the quality of the dataset by removing samples with too little data (low *individual call rate*) as well as SNP loci that were not called across most samples (low *locus call rate*). The thresholds for these filters were adjusted depending on the type of analysis. For identifying unique individuals, only about 200 high-quality loci are required (Schultz, Cristescu et al. 2018). Therefore, we aimed to maximise the number of individuals that could be used while retaining sufficient high-quality SNPs. To achieve this, we retained loci with a call rate ≥0.8 – resulting in only retaining SNPs with at least 80% data. Samples were also filtered for individual call rate ≥0.3, so that only samples with at least 30% data were kept for analyses.

Higher quality genetic data are required for population structure and genetic diversity analyses. Therefore, when filtering for these analyses, we focused on getting many high-quality SNPs (>1000), while retaining fewer samples. This was achieved by maintaining a locus call rate threshold of ≥0.8 and increasing the individual call rate threshold to ≥0.5, thereby retaining only samples with at least 50% data..

Other constant thresholds (i.e. kept the same across both individual identification and genetic diversity analyses) were applied to remove potentially erroneous loci. This included filtering for allele read depth (≥5) and minor allele frequency (MAF ≥0.01) and excluding loci appearing on the same contig as another (*secondary loci*). Because filtering can result in previously polymorphic loci becoming monomorphic, all monomorphic loci were removed after filtering routines.

2.3.2 Genetic fingerprinting

Genetic fingerprinting allowed allocation of scat samples to individual koalas. Individual identification improves the accuracy of population estimates for *Chlamydia* prevalence, sex ratio and genetic diversity by enabling identification and elimination of samples originating from the same individual koala (scats from the same individual deposited in different locations at different times). Duplicate samples were identified using a relatedness estimate, calculated using the Dyadic Maximum Likelihood estimator in the *related* package in R (Pew, Muir et al. 2015). We classified samples with a genetic relatedness >0.75 as duplicates. When duplicate samples were identified, only the highest-quality sample was retained for further analyses.

2.3.3 Sex determination

Sex was determined using sex-linked genetic markers that were developed and integrated into the DArTag panel. Typically, the sex ratio in natural, healthy populations is expected to be 1:1. Risk of extinction is increased if population sex ratios significantly deviate from 1:1, although a small bias of sex ratio towards females can sometimes be desirable, especially in very small or rapidly declining populations (Wedekind 2012). We calculated the confidence interval for the proportion of males within the sampled population using the R package *DescTools* (Signorell 2025).

2.3.4 Measuring population structure

To identify the possible presence of genetic structure/clusters within the dataset, a population genetic structure analysis was conducted. We explored the genetic structure across sampled individuals by investigating the possible presence of genetic clusters (k). Genetic structure was first assessed using a principal component analysis (PCA) within the *dartR* package. We then explored the presence of genetic clusters using a Discriminant Analysis of Principal Components within the *adegenet* package in R (Jombart 2008, Jombart, Devillard et al. 2010). This multivariate method uses K-means and synthetic variables to create and select models to explain genetic variability within a population. We then further confirmed the population structure and investigated the flow of genes between clusters using

the software sNMF to estimate admixture coefficients within the LEA package in R (Frichot, Mathieu et al. 2014, Frichot and François 2015). This uses non-negative matrix factorization algorithms to produce least-squares estimates of ancestry proportions. Here, we set the number of clusters (k) to vary between 1 and 10, calculated cross-entropy criterion, set the number of repetitions for each k to 100 and the alpha regularisation parameter also to 100. The degree of genetic differentiation between clusters was assessed using the fixation index (F_{ST}), using the *dartR* package and 100 bootstraps.

2.3.5 *Chlamydia* detection

Detection of the *Chlamydia* pathogen from scats was conducted using the same DNA extracts, but using *Chlamydia*-specific probes developed and integrated into the DArTag panel to detect the presence of *Chlamydia* DNA. We calculated the 80% confidence interval for the proportion of *Chlamydia*-positive individuals within the sampled population using the R package *DescTools*. In 2018 and 2020/2021 *Chlamydia* infection was assessed using similar *Chlamydia*-specific probes through the DArTcap platform (i.e. >9 SNPs detected out of the 30 Chlamydia-specific probes).

Please note, the prevalence of *Chlamydia* (percent of koalas with the pathogen detected) is an important population characteristic for informing conservation management. However, the presence and severity of the disease varies greatly between individual koalas, as well as between populations (Ellis, Girjes et al. 1993, Waugh, Hanger et al. 2016). Notably, individual koalas can shed large numbers of *Chlamydia* pathogen without clinical signs of disease (Wan, Loader et al. 2011), and populations can have high *Chlamydia* prevalence (infection) with low noticeable health impact (disease). For example, Polkinghorne et al. (2013) found that 90% of koalas in the Mt Lofty ranges were *Chlamydia* positive but had a low prevalence of clinical disease (see also Weigler et al. 1988). Therefore, quantifying *Chlamydia* prevalence is only the first step towards understanding the threat that this pathogen presents to both an individual and a population.

2.3.6 Measuring genetic diversity

Genetic diversity was calculated using *dartR*. We calculated the following heterozygosity values:

- **Observed heterozygosity H_O** - the calculated level of heterozygosity from the allele frequencies of the population under study
- **Expected heterozygosity H_S** (adjusted for small sample size) - the level of heterozygosity that could be expected based on observed allele frequencies if the population was at the Hardy-Weinberg equilibrium (panmictic population with constant genetic variation across generations). To calculate expected heterozygosity, *dartR* uses the unbiassed estimates in Nei (1987).

We are currently investigating ways to reliably calculate the inbreeding coefficient F_{IS} (the proportion of the variance in the subpopulation contained in an individual) according to the current advances in the field. Indeed, there has been recent concerns raised in the scientific literature about issues calculating F_{IS} from SNPs only, where sample size produces a bias of estimates (Schmidt, Jasper et al. 2021).

2.3.7 Measuring effective populations size (Ne)

Generally, the number of individuals that effectively participates in producing the next generation is named as effective population size. Contemporary effective population size (Ne) and associated parametric 95% confidence intervals were estimated using NeEstimator v2 (Do, Waples et al. 2014), implementing linkage disequilibrium method with random mating model and 0.05 as the lowest allele frequency.

2.4 Constraints and limitations

2.4.1 Fieldwork

The sites were surveyed on only one occasion in each survey period; therefore, the koala occupancy results presented here provide a snapshot of the population during this period. It is important to note that the landscape genetic sampling in 2024 (August 2024-November 2024) and in 2020/2021 (August 2020-April 2021) occurred in a different season to the

sampling in 2018 (April 2018-August 2018), therefore it is possible that some differences in koala occupancy were partly due to seasonal patterns. It should be noted that evidence of koalas found within the study areas is likely to change over time, as koala movements vary with season (Ellis et al. 2009).

Detection dogs are a powerful method to study koala presence/absence, and greatly improve our ability to protect and conserve the koala. However, survey accuracy and efficiency will vary with dogs' and handlers' abilities. Ongoing training and testing are required and undertaken by DDC handlers and dogs.

Failure to detect koala scats in an area does not necessarily mean that koalas are not present. Failure to detect koala scats may suggest one of the following:

- Koalas were not present at the site recently. Note that this does not indicate true absence, particularly given that most of the surveys were conducted using dogs trained to only detect very fresh scats (a few days old).
- Koalas were not present (i.e. true absence). Note that true absence does not infer that the site has not been used by koalas in the past or could not be used in the future.
- Koalas occur in the area, but scats were not detected (false negative) because:
    - Scats were present at some stage but decayed and disappeared from the environment before the survey was conducted;
    - The dog did not detect the scat; and/or,
    - The dog indicated the presence of a scat, but it was too decayed to be confirmed.

The presence of absence does not equal the absence of presence. Confirming true absence typically requires multiple surveys (MacKenzie and Royle 2005) and only presence can be reliably ascertained from these surveys. In addition, fresh scat detection dogs were used for a majority of these surveys, and the absence of fresh scats merely means that koalas may not have been present in the preceding few days/weeks.

In this project, survey effort in each area could be assessed using the track log of dog searches (provided in **Figure 2**). This was supplemented with incidental visual koala searches between locations.

A large proportion of samples were collected from localities in the northern part of the Redlands Coast (all localities except Sheldon, Mount Cotton and Redland Bay). This bias is mainly a result of revisiting survey sites from 2018 and 2021/2022, where site selection was largely driven by accessibility (a large proportion of southern Redlands Coast bushland lies within private properties). A number of sites from previous surveys could not be revisited in 2024, due to access restrictions on private properties or due to budget limitation. However, we added thermal imaging drone sentinel sites in large natural areas in the southern end of the Redlands Coast.

Heavy rainfalls caused some fieldwork delays. After each heavy rainfall and flood episode, the DDC stopped surveys for two to three days to allow the ground to dry and increase the likelihood of finding fresh scats suitable for genetic analysis (as rain can wash cells/DNA off from scats).

Similar to the 2018 and 2021/2022 surveys, we detected more live koalas in urban environments (localities in the northern part of the Redlands Coast), possibly due to higher detectability rates in comparison to natural woodlands or non-urban areas. Although this has not been researched, the DDC field team have observed that koalas are often more easily seen in urban areas, as the typically sparser, more widely spaced vegetation in urban settings may make it easier to spot koalas against a clear sky background. Note also that samples were included from koalas in the Koala Safe Neighbourhood program, where visually locating and observing koalas in certain localities was the primary focus.

Further, while the start points for the surveys were chosen to be the same or very close to the staring points from the 2018 and 2021/2022 surveys, the direction of the survey track might differ between years. Scent detection can be influenced by current wind direction and thus orientation decisions made by the dog. Therefore, a survey cannot be exactly replicated,

even when starting from the same location. Nonetheless, we aimed to replicate previous surveys as closely as possible.

Despite these limitations, the DDC surveys provide an extensive overview of koala presence and genetic characteristics across Redlands Coast. While reading the report, the reader should keep these limitations in mind, particularly the likelihood that the distribution of koalas in the southern areas of the Redlands Coast mainland may be underestimated – however, the thermal imaging drone surveys have increased the coverage of the southern part of the Redlands Coast mainland compared to previous years.

2.4.2 Genetic analyses

Genetic genotyping is a rapidly advancing field, which strives to continue to develop, test and improve the methods utilised e.g. from originally six microsatellites to now the availability of ~ 700 full koala genomes in less than three decades (Houlden, England et al. 1996, McLennan, Kovacs et al. 2025). This means that there are differences in the methodologies employed across years. Here, we use SNP, and an overlap across SNP panels was ensured to enable the comparison of the genetic characteristics of the Redlands Coast population across years of monitoring. Given the lack of total overlap across SNP panels utilised, longitudinal comparison of genetic profiles (i.e. identification of resamples individuals) required the use of a smaller subsample of SNPs, e.g. those which overlap across panels. Moreover, staying at the cutting edge of advances of the genetic field comes with higher risks, as new tools are tested and validated in real time.

3 Results and discussion

3.1 Field surveys

3.1.1 Koala scat surveys

Landscape scale surveys for koala scats were undertaken between the 21st of August and the 8th of November 2024. One to two field teams, consisting of a detection dog, a handler and an assistant were deployed independently from each other. The detection dogs were directed to search for koala scats mostly off-leash, with the handlers guiding them to maximise site coverage. Whenever a site was close to a road (e.g. road reserves or small parks), the dogs were on leash for safety reasons.

The detection dog teams conducted a total of 90 surveys across Redlands mainland, and koala scats were detected during 55 of these surveys, leading to the detection of scats (all ages) at 145 locations (**Figure 3**), and the collection of 112 fresh scats at 49 sites. While surveys in 2018 and 2020/2021 had found no signs of koalas in Thorneside, surveys in 2024 were able to detect koala scats in one location within this locality. This confirms that, while koalas are rare in this locality, they do occur, as supported by a sighting in 2021 by a Thorneside resident. Other sites that were previously negative for fresh scat detection but positive in 2024 include: Hilliard's Creek Corridor (Francis St section), [redacted] in Sheldon (private property), South Street Conservation Area, Greater Glider Conservation Area (positive in 2018, negative in 2020/2021, positive this time) and Frank Street Bushland Refuge.

There are also areas where fresh koala scats were detected previously but not during this survey. These include: Hilliards Creek Platypus Corridor, Coolnwynpin Nature Reserve, Kingfisher Rd, Victoria Point, [redacted] in Sheldon (private property), Counihan St Park and German Church Rd. A special note was made by ecologists that this third survey confirms results from 2020/2021 survey in the GJ Walter Park and surrounds. In 2018, seven koalas were detected, whereas repeat surveys in 2020/2021 and 2023/2024 found few koalas or scats. GJ Walter Park was a locally known koala hotspot (Koala Action Group, *personal*

communication), which was still confirmed in the 2018 genetic surveys, but seem to no longer be presenting multiple scats/sightings.

In addition to the 112 scat samples collected during the landscape scale genetic surveys, we included 75 scat samples from 11 thermal imaging drone sentinel site surveys, 27 scat samples from three detection dog sentinel surveys, and nine scat samples from monitoring ambassador koalas for the Koala Safe Neighbourhood (KSN) monitoring program. Thus, a total of 223 samples of fresh koala scats were collected and processed for genetic analyses (**Figure 4**, **Table 2**). The drone sites, especially in Redlands Bay, increased our samples in the most southern parts of Redlands Coast (southern half of Redland Bay).

Comparison. A total of 90 koala scat surveys using detection dogs were conducted in the 2024 genetic landscape surveys, of which 55 were positive for koala scats (63%). In comparison, 44% of sites were positive for koala scats in 2020/2021, and 56% were positive for koala scats on Redlands Coast mainland in 2018. Note that these results are not to be compared with common occupancy or presence/absence data (e.g. scat surveys), as most of the surveys only focused on recently deposited (fresh) koala scats. Increased efforts in scat collection occurred in the southern of Redlands Coast, with also more koalas sighted in these areas compared to the other two surveys in this monitoring program. A special area of concern is the GJ Walter Park and surrounds, which continues to show low koala signs compared to 2018 and local knowledge (as was already found in 2020/2021).

Implications for Conservation. Koala signs are still readily found in the Redlands Coast, including in urban areas, where threats are heightened by the likelihood of interactions with vehicles and domestic dogs, as well as a lower habitat connectivity (i.e. both at the canopy and patch levels), potentially forcing koalas to move longer distances on the ground.

Recommendation. Similar to previous recommendations in 2018 and in 2020/2021, the protection of koalas in the Redlands Coast needs to include a strategic urban koala plan, as it

appears that not an insignificant proportion of the koala population is currently found within urban areas.

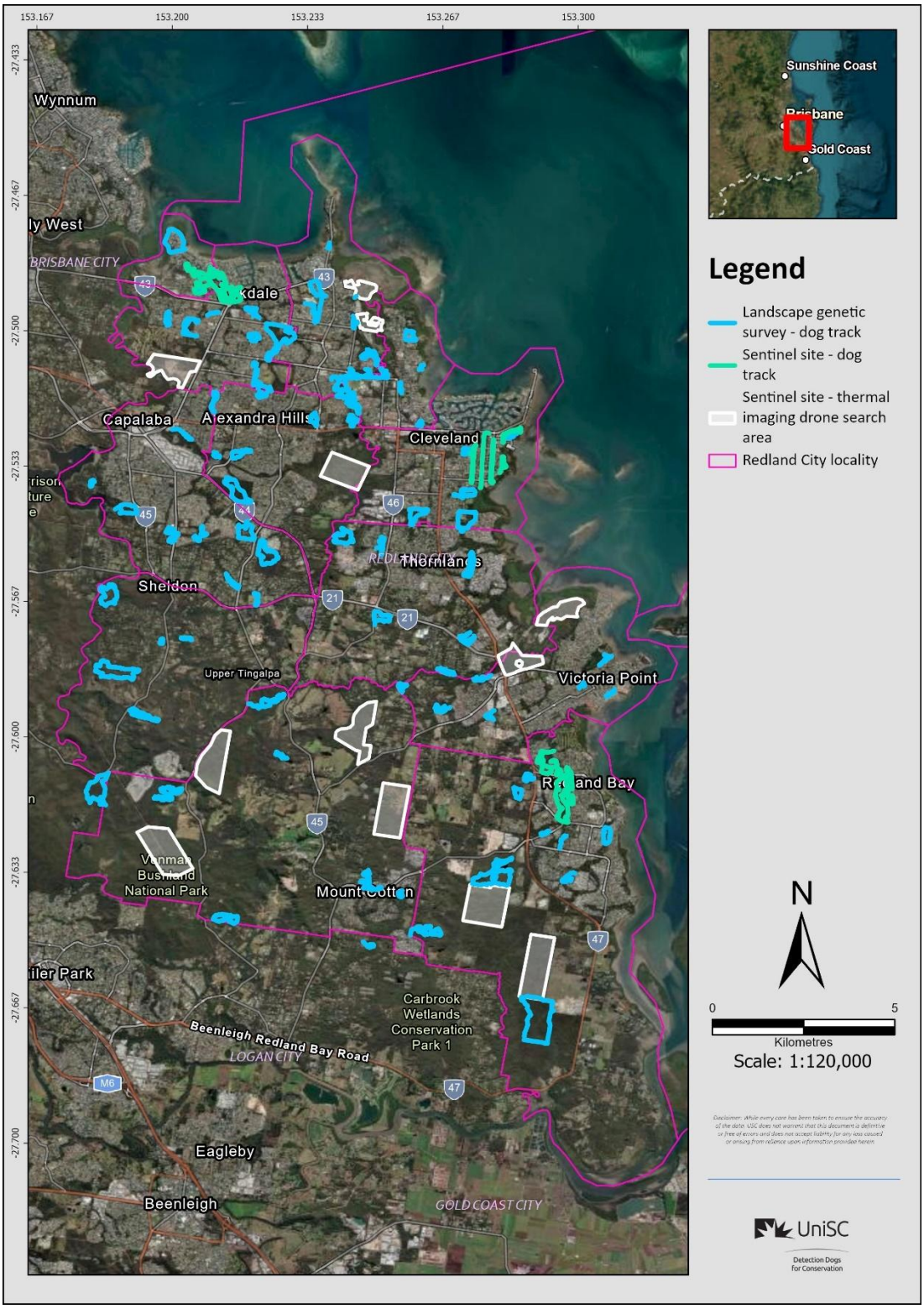

Figure 2. Dog tracks recorded during the landscape genetic surveys, as an indication of search effort across the Redlands Coast mainland in 2024. Note that in many instances, the field team also performed visual searches between sites that therefore are not represented in the map. Dog surveys were supplemented with drone surveys, presented here as white polygons.

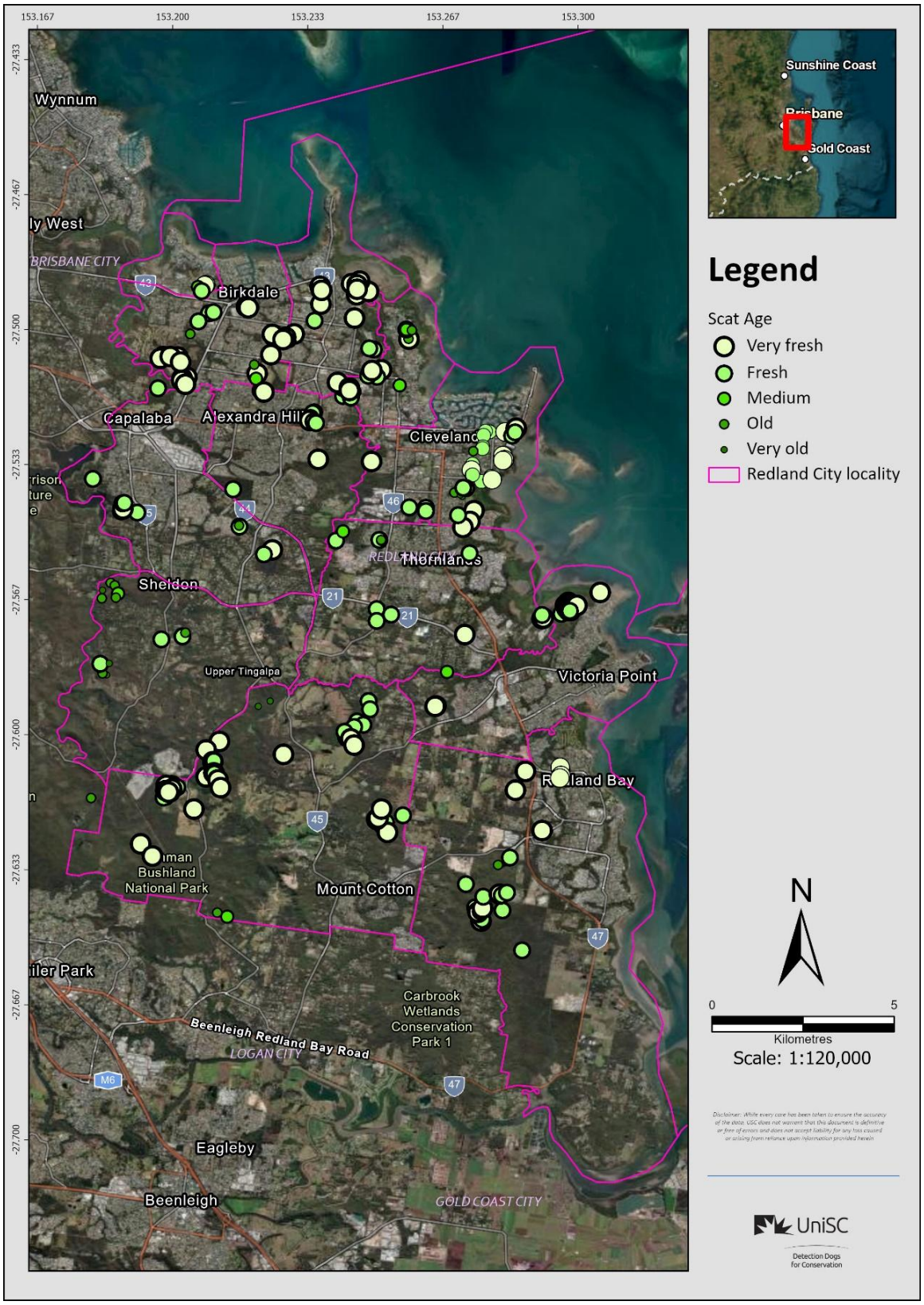

Figure 3. Koala scat detections (by scat age category, Table 1) across Redlands Coast mainland in 2024.

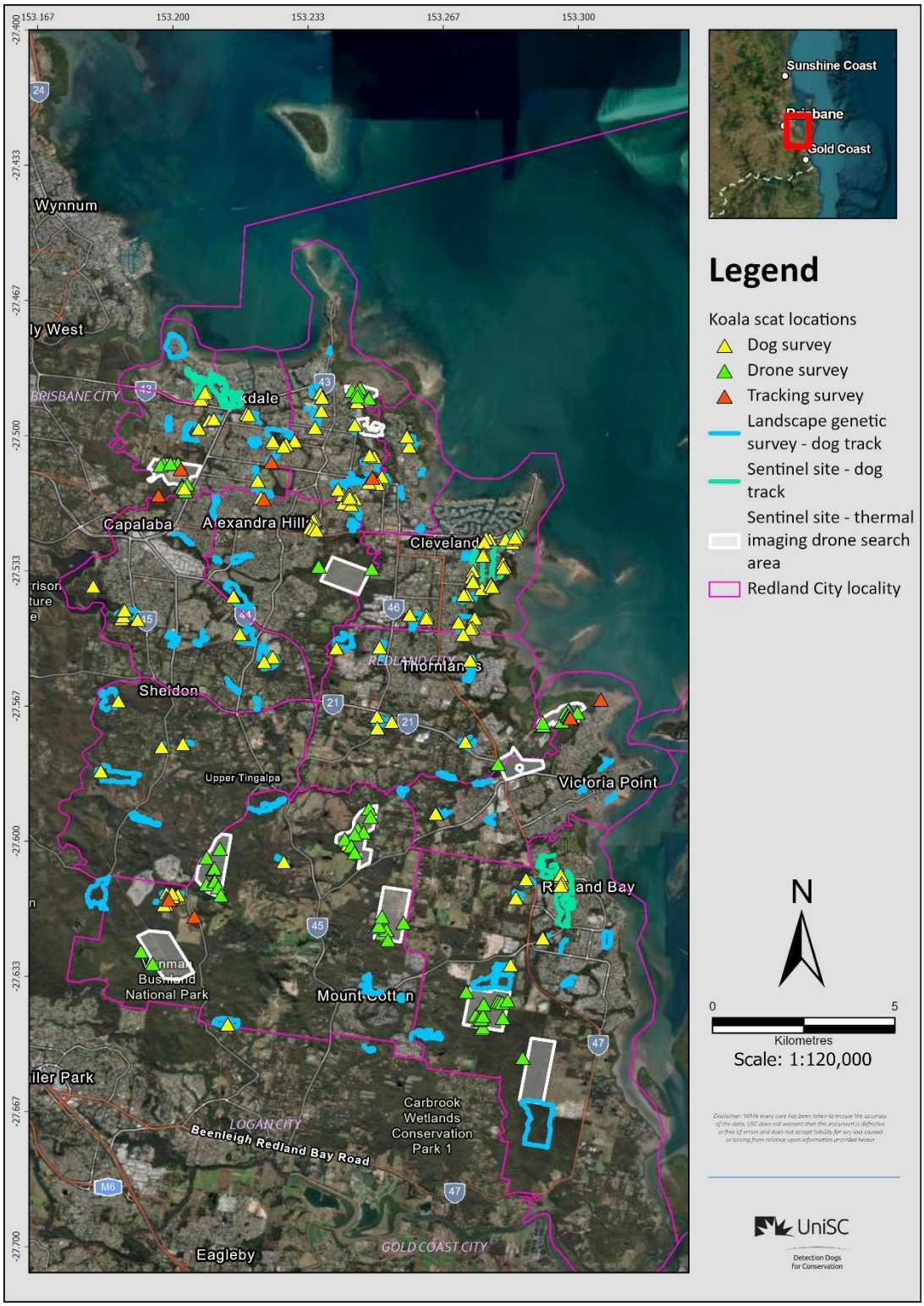

Figure 4. Locations of koala scat samples analysed from 2024 surveys. Landscape genetic survey samples N = 112, sentinel (drone) survey samples N = 75, sentinel (detection dogs) survey samples N = 27, ambassador koala samples N = 9).

3.1.2 Koala sightings

Field teams sighted a total of eight adult koalas during scat surveys using detection dogs, none of which presented observable signs of chlamydial infection (**Figures 5, 6**). A further 18 koalas were sighted during post-drone surveys, of which two showed signs of chlamydial disease. Please note that the number of koalas visually observed during post-drone surveys is only the number of koalas detected by the field team the following day and not the total number of koalas detected using the thermal imaging drone.

Koala sightings in the surveys conducted here, which aim to collect genetic samples, are only opportunistic e.g. the team spends more time looking for scats on the ground than looking in the canopy for koalas. Thus, opportunistic koala sightings is not a systematic survey, and not be the most robust method to compare trends between years.

Figure 5. Female koala and her joey sighted during surveys on 22/08/2024 within Henry Ziegenfusz Park, Cleveland.

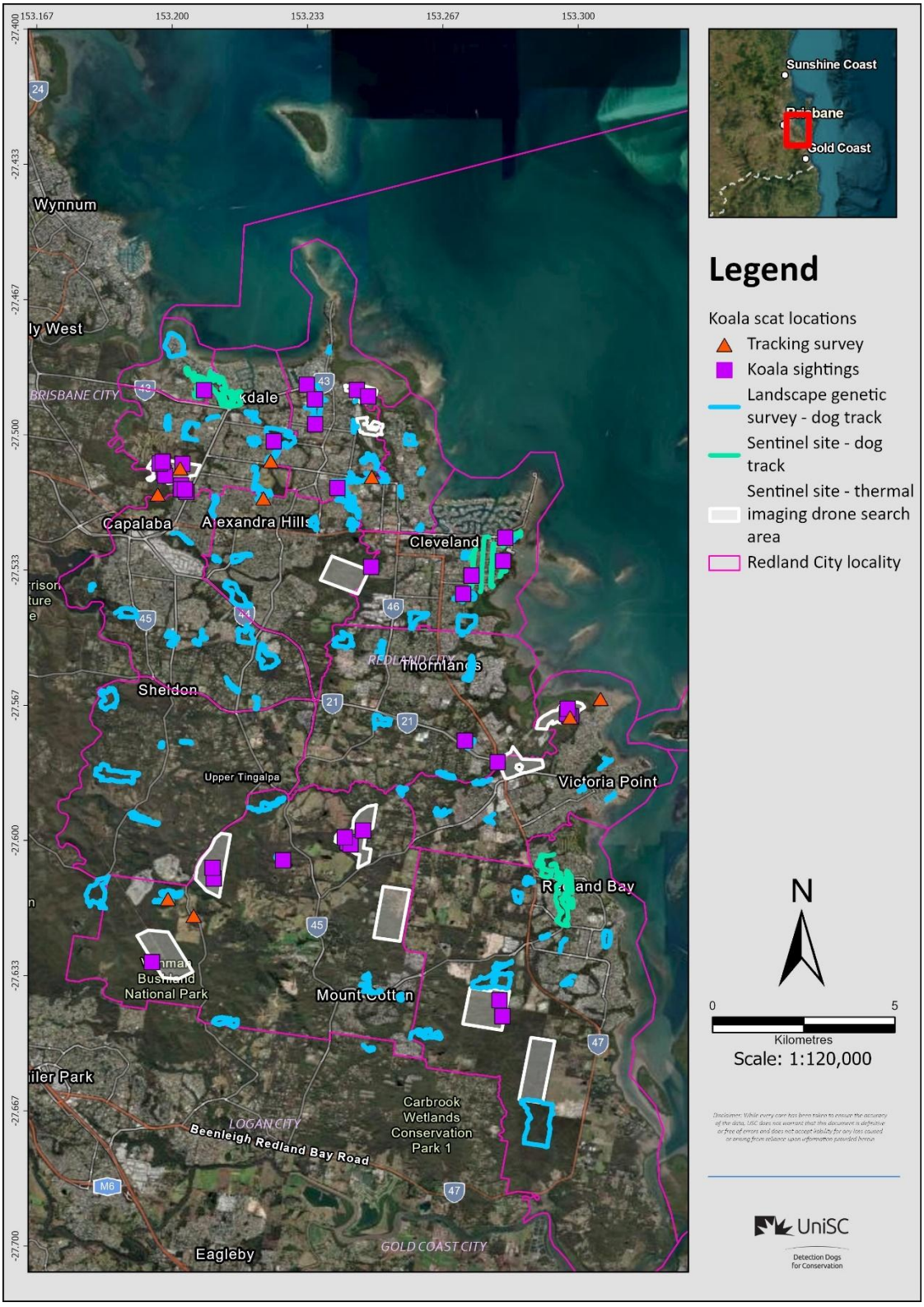

Figure 6. Koala sightings (dog surveys N = 8, post-drone verifications surveys N = 18) across Redlands Coast mainland during the scat surveys, and locations of radio-tracked koalas when their scat was collected for this study.

3.2 Koala scat genetic analyses

3.2.1 Quality control, genotyping and unique individuals

A total of 223 samples from 2023/2024 were genotyped; however, as expected with non-invasive samples, they differed in DNA quality. Here, 122 samples passed quality control when filtering for individual koala identification, resulting in 211 SNPs with average missing data of 12.2%. Genotyping success varied with scat age and survey method (**Table 2**), where post-drone surveys resulted in higher quality samples due to the ability to identify the location of koalas and, subsequently, the freshest scats.

Table 2. Genotyping success (whether samples were of sufficient genetic quality to pass filtering for individual identification) for different scat age categories (Table 1) and survey methodology.

	Group	Number of samples	Genotyping success
Scat age category	1	116	75%
	2	103	35%
	3	4	25%
Survey methodology	Post-drone surveys	75	71%
	Dog-only surveys	139	38%
	KSN monitoring surveys	9	44%
Total		223	55%

Genetic analyses resulted in the identification of 97 unique koalas within the 2023/2024 samples, of which 94 koalas were successfully analysed for sex determination (reported immediately below) and for *Chlamydia* presence (reported in section 3.2.4). Of 97 uniquely identified koalas, we were able to genetically compare 79 koalas to those previously identified during 2018 and 2020/2021 surveys. Of these, 11 (14%) had already been sampled in the past surveys (**Figure 7**). Five koalas had samples dating back to 2018. As a comparison, we genetically resampled 11 individuals (~9%) in 2020/2021 that were first sampled in 2018.

Most of these resampling events show the koalas stayed very close to the previous surveys (2 to 6 years), except one koala i6 having moved about 5 km (**Figure 7**).

Figure 7. Location of resampled koalas across years.

3.2.2 Sex ratio

The 94 identified koalas which were successfully analysed for sex determination included 34 males and 60 females, resulting in a male to female sex ratio of 1:1.76. From these samples, we estimate that the 80% confidence interval for the proportion of males within the population is between 30% and 43%, indicating a female bias. **Table 3** shows the proportion of males at each locality, along with 80% confidence intervals. Based on the upper range of 80% CI, Victoria Point, Capalaba and Birkdale are likely the most female-biased.

Table 3. Number of individuals genetically sexed at each locality, with observed percentage of males and estimated 80% confidence interval (CI). Note that estimated localities with less individuals have larger CIs due to small sample size

Locality	Number of surveys	Number of sexed individuals	Percentage of males	80% CI
Alexandra Hills	3	4	25%	8%-57%
Birkdale	5	13	23%	12%-41%
Capalaba	3	3	0%	0%-35%
Cleveland	4	9	33%	17%-55%
Mount Cotton	10	28	50%	38%-62%
Ormiston	2	2	0%	0%-45%
Redland Bay	3	12	50%	33%-67%
Sheldon	2	2	50%	16%-84%
Thornside	1	1	0%	0%-62%
Thornlands	5	5	40%	18%-67%
Victoria Point	3	6	50%	27%-73%
Wellington Point	7	9	11%	3%-31%

When compared to 2020/2021 , the overall Redlands Coast koala sex ratio across 2023/2024 samples was more biased towards females, based on the lack of overlap with 80% CI, while it may be similar to 2018 results (**Table 4**).

To confirm the female sex ratio bias across the 2023/2024 samples, sample genotyping and genetic analyses were repeated using DArTcap, the method previously used in 2018 and 2020/2021, and from which the current method was developed, obtaining the same results. We further confirmed that the female biased sex ratio was not due to low quality of some samples (i.e. only male markers are identified, and samples with more missing data are more likely to not be genotyped for these male markers). To perform this verification, we increased stringency of filtering; however, even the best-quality sample set was female biased. As a final check, we examined Koalabase data from SEQW wildlife hospitals, for koalas rescued in Redlands Coast, and we also found a female bias.

Table 4. Comparison of the percentage of males sampled across years, with the resulting 80% Confidence Intervals (CIs) for the population, and sex ratio

Year	Number of individuals sexed	Proportion of males	80% CI	Sex Ratio
2018*	124	44%	39% - 50%	1:1.25
2020/2021	116	51%	45% - 57%	1:0.97
2023/2024	94	36%	30% - 43%	1:1.76

*2018 surveys included Minjerribah, but mainland results only are presented here

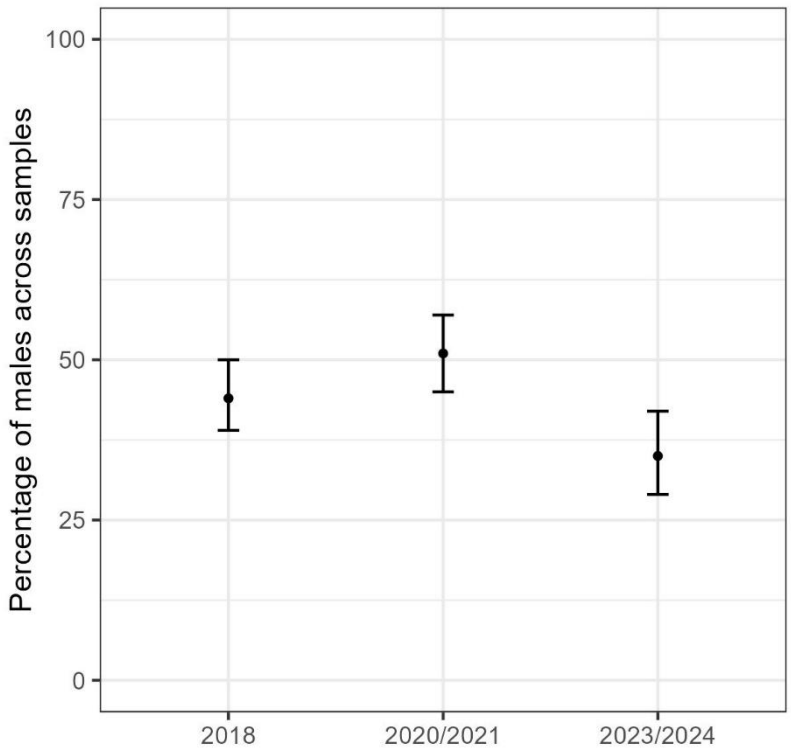

Figure 8. Comparison of the percentage of males sampled across years, with the resulting 80% Confidence Intervals (CIs) for the population.

The observed change in sex-ratio might be due to chance variation in sampling (i.e. at our statistical level of confidence, there is a ~20% chance that population levels are outside the 80% CIs), but if not, potential reasons behind a female bias should be investigated. It is important to analyse hospital and/or rescue records for the sex of koalas that are injured or killed. For example, there may be a male bias in koalas killed by vehicle strikes or dog attacks (particularly during the breeding season) or in koalas euthanised due to *Chlamydia* (even though this is usually female biased). A sex bias in any of these causes of mortality could have consequences for the Redlands Coast mainland koala population. A small sex bias toward females though is generally better than a male bias, as number of females is closely linked to number of joeys produced, and thus has a larger influence on population size trend.

3.2.3 Population structure

Both Discriminant Analysis of Principal Components and sNMF analysis confirmed previous results from 2018 and 2020/2021 that the Redlands Coast mainland koala population is a single, connected population (except for Minjerribah translocated koalas, which are not belonging to the same population). When samples from 2018 – 2024 were analysed together (N = 260), they belong to the same genetic cluster, showing there has not been differentiation between "generations" across years for the Redlands Coast mainland population.

Population structure analysis allowed the identification of samples originating from Minjerribah translocated koalas, both within 2024 samples and samples across 2018 – 2024 (N = 260). This was confirmed by including 222 samples (originating from 62 unique koalas) from Minjerribah, collected in 2021 for another project by DDC (**Figure 9** and **Figure 10**). We found low levels of mixing between translocated Minjerribah and mainland koalas, particularly of two koalas, one sampled in 2024 which is likely to be first descendant of a translocated Minjerribah koala, and one sampled in 2021, which may be second or third descendant of a translocated Minjerribah koala (**Figure 11** and **Figure 12**). This indicates that translocated Minjerribah koalas have successfully reproduced with mainland koalas. If mixing between Minjerribah and mainland clusters continues over time, it could aid in increasing genetic diversity of the mainland population.

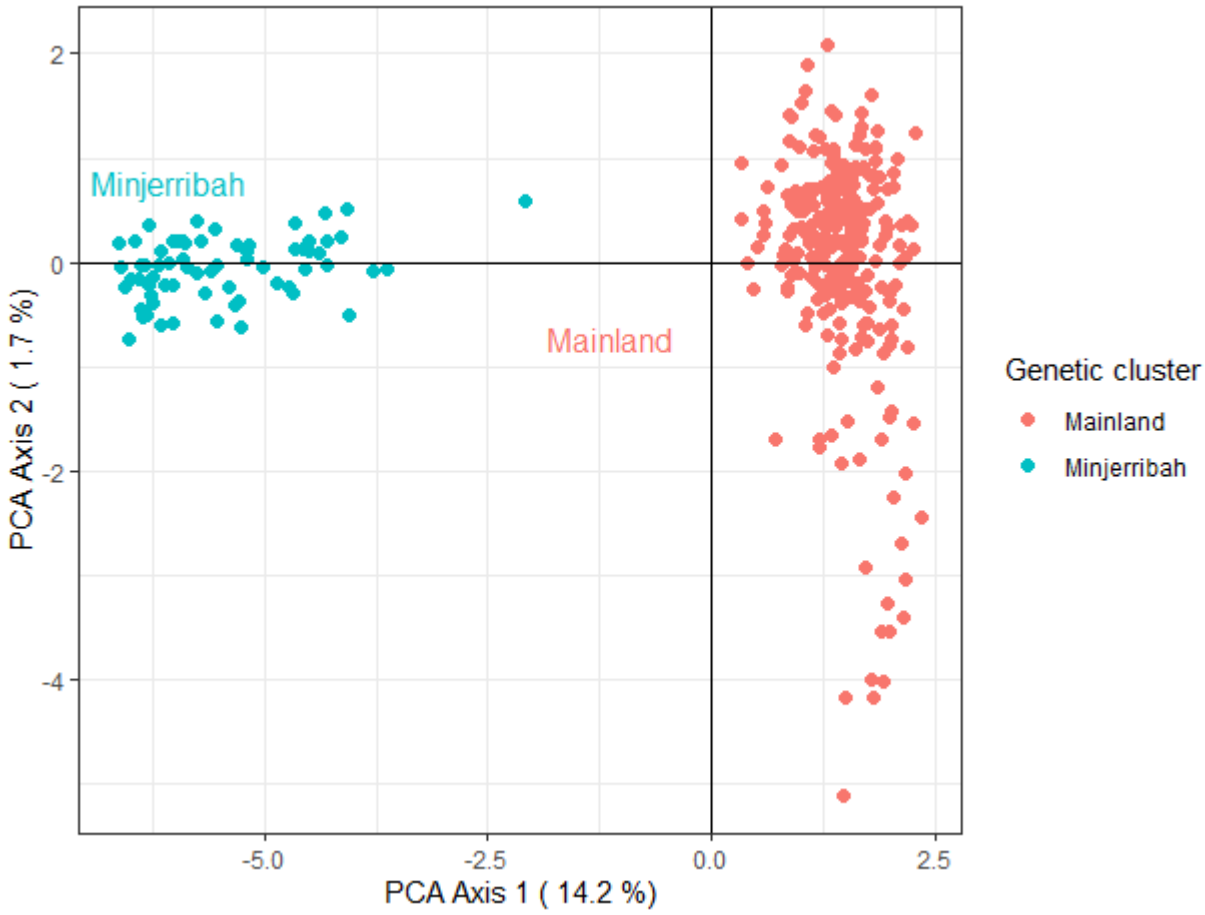

Figure 9. Genetic cluster allocation using sNMF analyses of Redlands Coast koala samples (*N* = 260, including some koalas identified as translocations from Minjerribah, see Figure 11) and an extra *N* = 62 koala samples from Minjerribah coincides with the results of a PCoA of genetic samples. Samples originated from uniquely identified koalas.

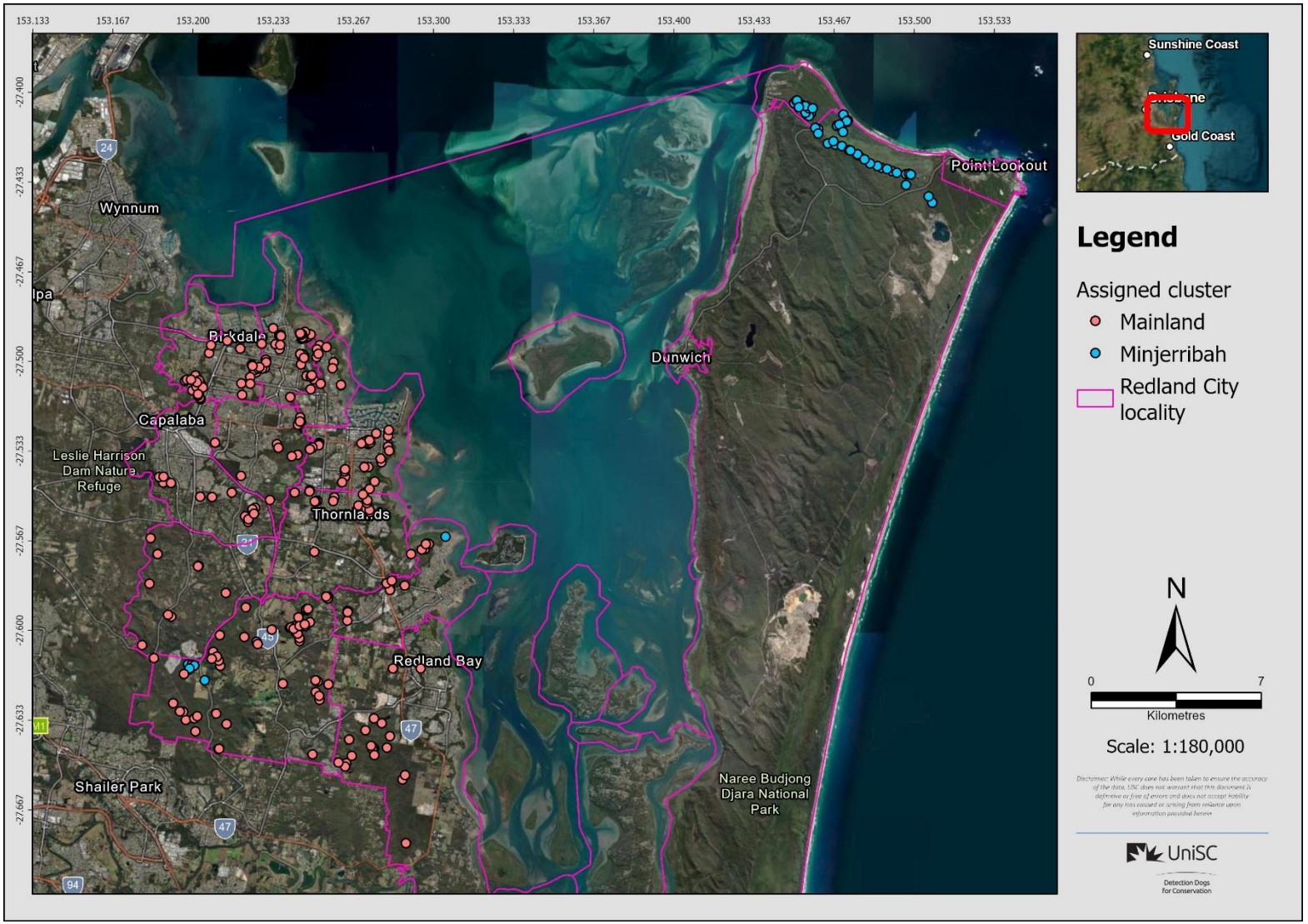

Figure 10. Genetic cluster allocation using sNMF analyses of Redlands Coast samples and an extra 62 individual koalas originating from Minjerribah. This analysis was able to identify Minjerribah translocated koalas (blue) amongst the mainland koalas (red).

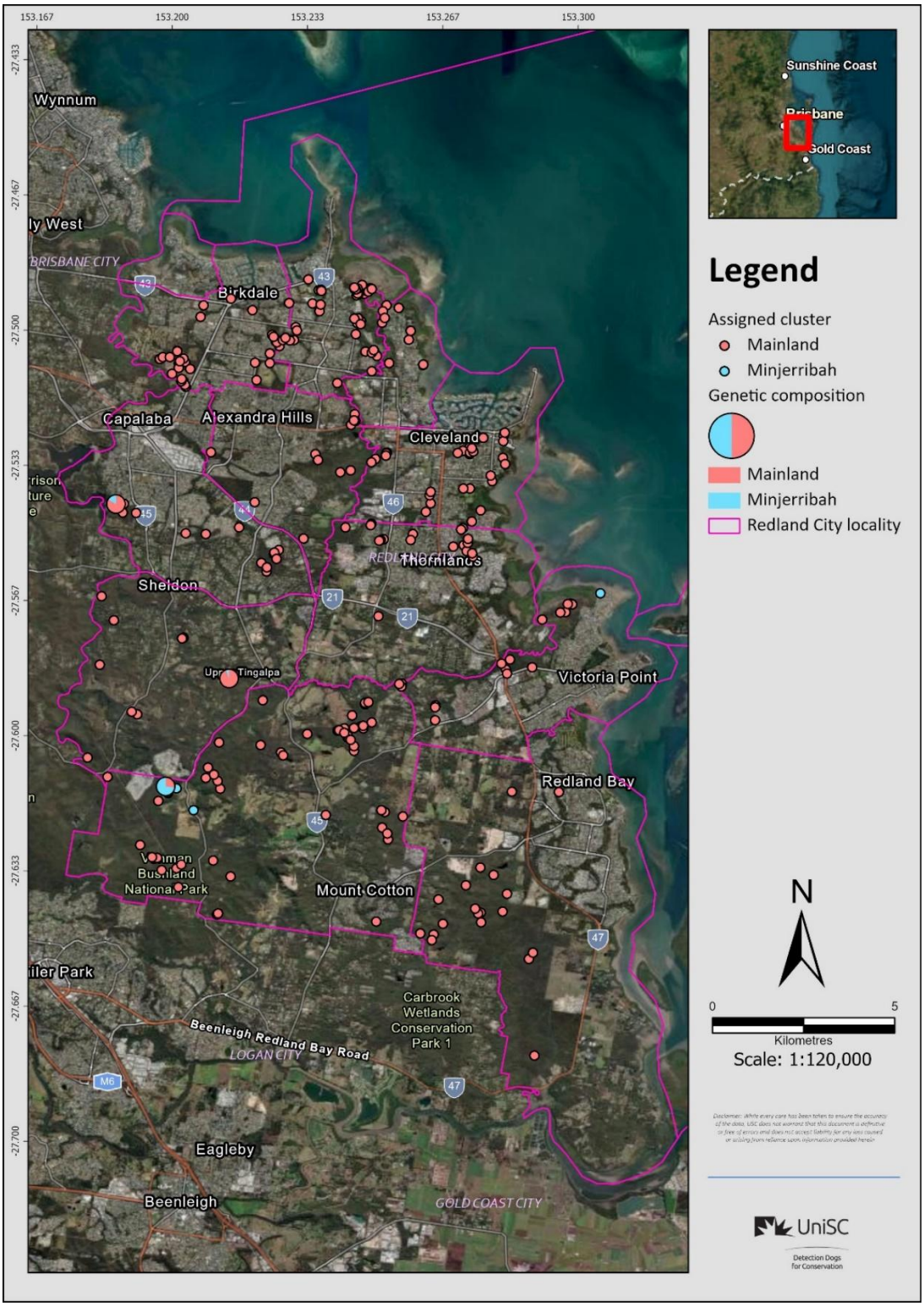

Figure 11. Genetic cluster contributions within each genetic sample for mainland koalas (circles in the above map). Mixed individuals (i.e. those including genetic make-up from both mainland (red) and Minjerribah (blue) clusters, see pie charts in the map) indicate that translocated Minjerribah koalas are breeding successfully with mainland koalas.

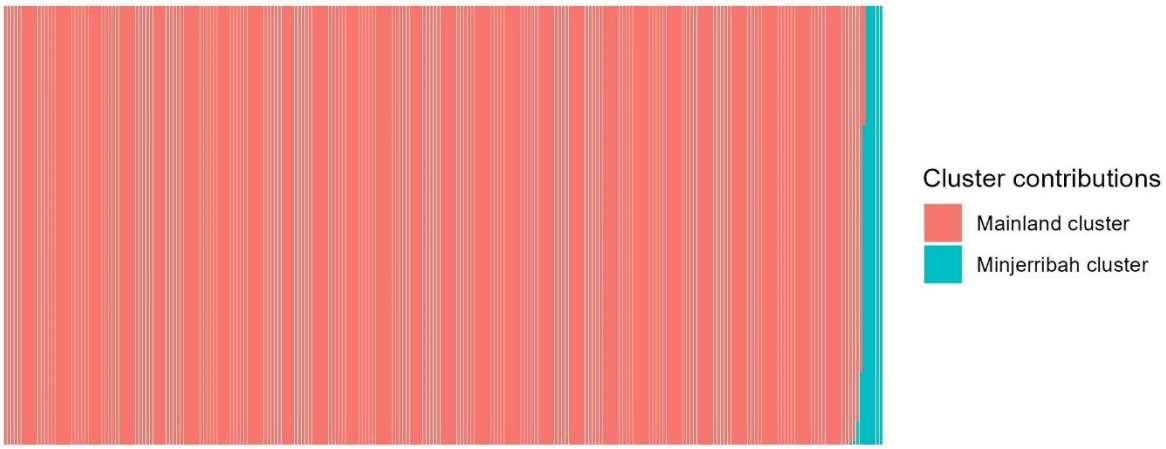

Figure 12. Genetic cluster contributions within each genetic sample for mainland koalas (columns in the above barplot). Mixed individuals (i.e. those including genetic make-up from both mainland (red) and Minjerribah (blue) clusters) indicate that translocated Minjerribah koalas are reproducing successfully with mainland koalas.

Comparison. The broad scale population genetic characteristics of the Redlands Coast mainland koala population have been preserved over the past six years. Individuals sampled in 2018 – 2024 fall into the same genetic cluster when analysed together, so there has not been any differentiation between sampling events. The exception to this is Minjerribah translocated koalas, which form a separate cluster to that of mainland koalas. Currently, some breeding exists between mainland and translocated Minjerribah koalas.

Implication for conservation. Not finding differentiation means that koalas have retained genetic similarity over the last 6 years, and they also have remained one breeding population. Mixing with Minjerribah koalas may provide benefits for increased diversity over time.

Recommendation. To gain a full understanding of broad-scale population genetic patterns, sampling outside of the Redlands Coast to include the whole of the Koala Coast (with part of Logan and Brisbane City Councils) would increase our understanding of gene flow in and out of the Koala Coast as well as within (e.g. whether the Koala Coast remains one breeding population). This would be especially beneficial to investigate population source-sink dynamics.

3.2.4 *Chlamydia* prevalence

We detected *Chlamydia* infection in 23 of the 94 analysed koalas in 2023/2024, resulting in a *Chlamydia* prevalence of 25% (**Figure 13**). Here, we estimate that the 80% confidence interval for *Chlamydia* prevalence within the sampled population is between 19% and 31%. Infection prevalence was similar for males and females, with seven *Chlamydia*-infected females, resulting in 27% prevalence (80% CI: 17% to 39%), and eight *Chlamydia*-infected males, resulting in 38% prevalence (80% CI: 26% to 52%). This indicates (at this level of confidence) that *Chlamydia* infection may not vary between koala sexes.

Chlamydia prevalence per locality, with 80% confidence intervals, is presented in **Table 5**. Overall, disease prevalence was similar across localities, with Victoria Point, Redland Bay and Cleveland likely having the lowest levels. No *Chlamydia*-infected koalas were found in Sheldon during the current round of monitoring; however, only two koalas were tested in this locality, providing a sample size too small for making useful comparisons. Interestingly, this locality had presented very high *Chlamydia* prevalence across pooled data from 2018 and 2020/2021 (77% prevalence, 80% CI: 59% - 88%, N = 13 koalas). Prevalence in Redland Bay (44%, 80% CI: 26% - 65%, N = 9) also declined compared to measures across 2018 and 2020/2021.

Table 5. Number of individuals genetically identified at each locality, with observed *Chlamydia* prevalence and estimated 80% confidence interval (CI). Note that estimated localities with less individuals have larger CIs due to smaller sample sizes

Locality	Number of surveys	Number of individuals	*Chlamydia* prevalence (%)	80% CI
Alexandra Hills	3	4	25	8%-57%
Birkdale	5	13	31	17%-49%
Capalaba	3	3	33	11%-68%
Cleveland	7	9	11	3%-31%
Mount Cotton	10	28	36	25%-48%
Ormiston	2	2	50	16%-84%
Redland Bay	4	12	8	3%-24%
Sheldon	2	2	0	0%-45%
Thorneside	1	1	0	0%-62%
Thornlands	5	5	20	6%-49%
Victoria Point	3	6	0	0%-21%
Wellington Point	7	9	33	17%-55%

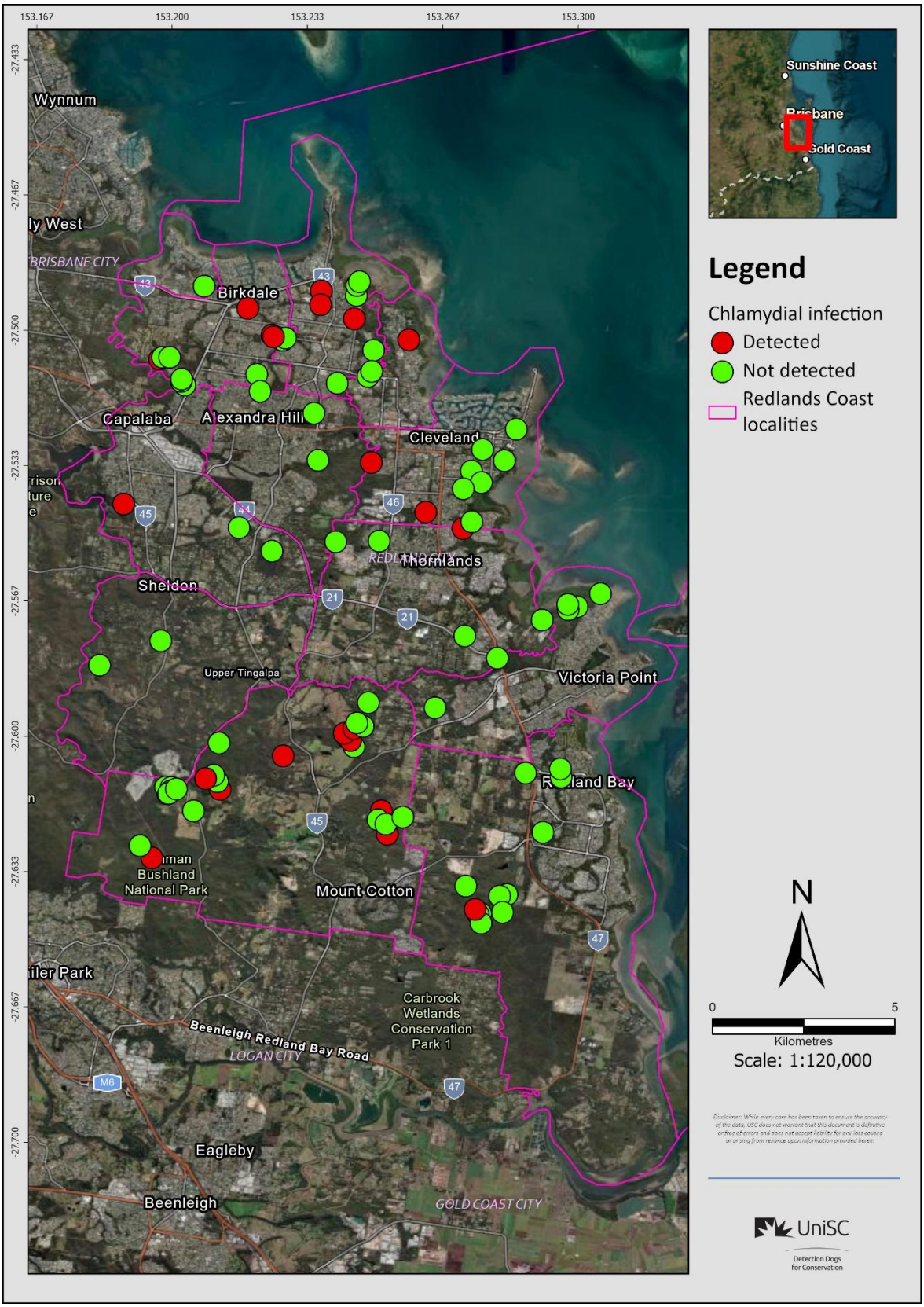

Figure 13. *Chlamydia* infection status of uniquely identified koalas in 2023/2024.

Overall, *Chlamydia* prevalence across Redlands Coast mainland was lower in 2023/2024 than 2020/2021, based on the lack of overlap with 80% CIs (and this was almost true in comparison to 2018 too; however, there was an overlap in CIs, **Table 6**, **Figure 14**). When looking at prevalence by koala sex, this decrease in *Chlamydia* prevalence appears to be linked to a decrease in female prevalence, but not male prevalence (**Table 6**) – care has to be taken while interpreting these results as they could be due, unfortunately, to euthanasia at wildlife hospitals, i.e. mortality. The proportion of *Chlamydia*-infected koalas with active chlamydial disease remains unknown. Assessing *Chlamydia* prevalence, however, is the first step in assessing the potential threat that disease represents in a population.

Table 6. *Chlamydia* prevalence by year, presented overall across the Redlands Coast mainland, as well as separately for males and females. *N* = Number of individuals, CI = confidence interval

Year	*N*	*Chlamydia* prevalence	80% CI	Male *Chlamydia* prevalence	80% CI	Female *Chlamydia* prevalence	80% CI
2018*	124	35%	29% –40%	33%	25% – 41%	36%	29% –44%
2020/ 2021	116	38%	32% – 44%	31%	23% –39%	46%	37% –54%
2023/ 2024	94	25%	19% – 31%	38%	26% –52%	27%	17% – 39%

*2018 surveys included Minjerribah, but mainland results only are presented here

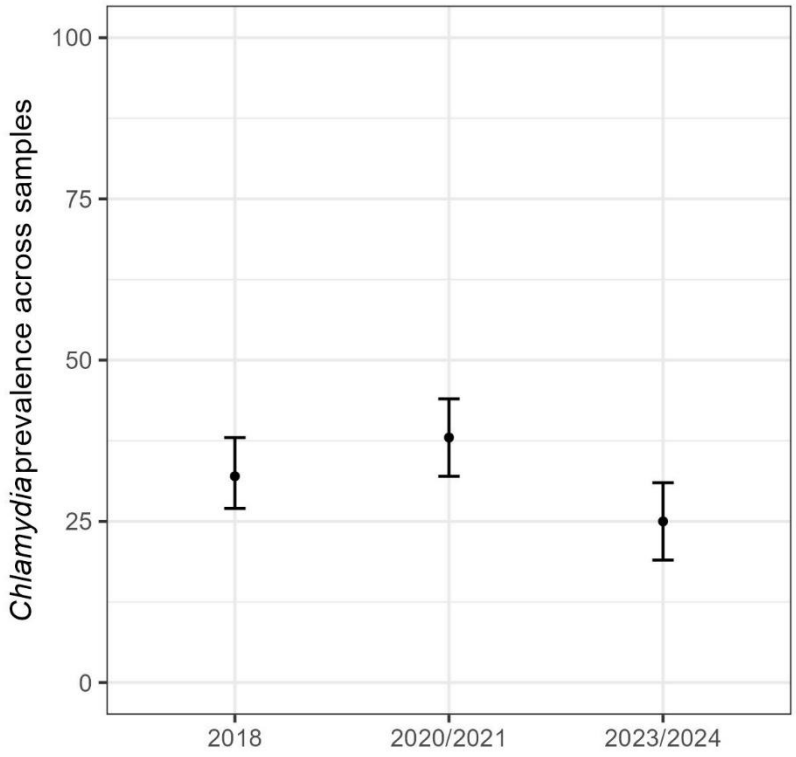

Figure 14. *Chlamydia* prevalence by year for the Redlands Coast mainland, with population 80% confidence intervals.

3.2.1 Genetic diversity

Filtering for genetic diversity analyses resulted in the retention of 46 individuals and 1,279 SNPs. Similarly to previous years, observed heterozygosity (H_O) was lower than (small sample size) expected heterozygosity (H_S) (**Table 7**). We found a slight increase in observed heterozygosity across the 2023/2024 data; however, this could be due to the lower sample size, as this is known to artificially increase heterozygosity estimates from SNPs (Schmidt, Jasper et al. 2021). Redlands Coast koalas belong to what is commonly referred to as the Koala Coast population, which is thought to have been relatively isolated from the rest of SEQ, resulting in lower genetic diversity (Lee 2009, Lee, Seddon et al. 2010). More recently, using full genomes, Redlands Coast was also found to have the lowest standardised heterozygosity across all included locations in QLD (McLennan, Kovacs et al. 2025). The same study underlined that all koalas east of the Pacific

Highway presented lower genetic diversity than geographically close koalas from the western side of the highway. Long-term monitoring of the Redlands Coast koalas will be able to identify any trends in potential changes to genetic diversity. Across the three city-wide genetics surveys to date, no concerning changes to genetic diversity have been detected.

Table 7. Measures of genetic diversity. *N* = number of samples used for genetic analyses, H_O = observed heterozygosity, H_S = expected heterozygosity (corrected for small sample size), SE = standard error. When interpreting these values, note that a higher value is better

Year	*N*	H_O (SE)	H_S (SE)
2018*	124	0.241 (0.004)	0.321 (0.005)
2020/2021	116	0.229 (0.003)	0.316 (0.004)
2023/2024	46	0.272 (0.004)	0.316 (0.004)

*2018 surveys included Minjerribah, but mainland results only are presented here

3.2.2 Effective population size

The estimated population size (N_e) for Redlands Coast mainland koalas in 2024 was similar to that reported previously in the Redlands Coast, sitting in between the values estimated from the samples in 2018 and 2021 (**Table 8**). These values are lower than those recommended to preserve the genetic health of species in conservation recommendations (Mace and Lande 1991, IUCN 2012, Frankham, Bradshaw et al. 2014), however, the koalas sampled for these repeat projects only represent part of the Koala Coast, a region identified as one isolated koala population that extends from Brisbane (south of the Brisbane River) through to Logan (east of the M1 Motorway) and Redlands Coast Local Government Areas. Minjerribah-translocated koalas were excluded from this analysis to avoid introducing a bias, and because the strong genetic differences were not handled by the N_e estimator. In addition, our estimates for N_e for Redlands Coast koalas are similar to that found for the whole genetic cluster of SEQ and northern NSW, of 108.9 (95% CI: 88.9–137.0, McLennan, Kovacs et al. 2025).

Table 8. Estimated effective population size ($\mathbf{N_e}$) across monitoring years, *N* = Number of individuals, CI = confidence interval

Year	*N*	N_e	90% CI
2018	124	75.9	74.9 – 77.2
2020/2021	116	90.9	89.3 – 92.1
2023/2024	46	88.4	86.9 – 89.8

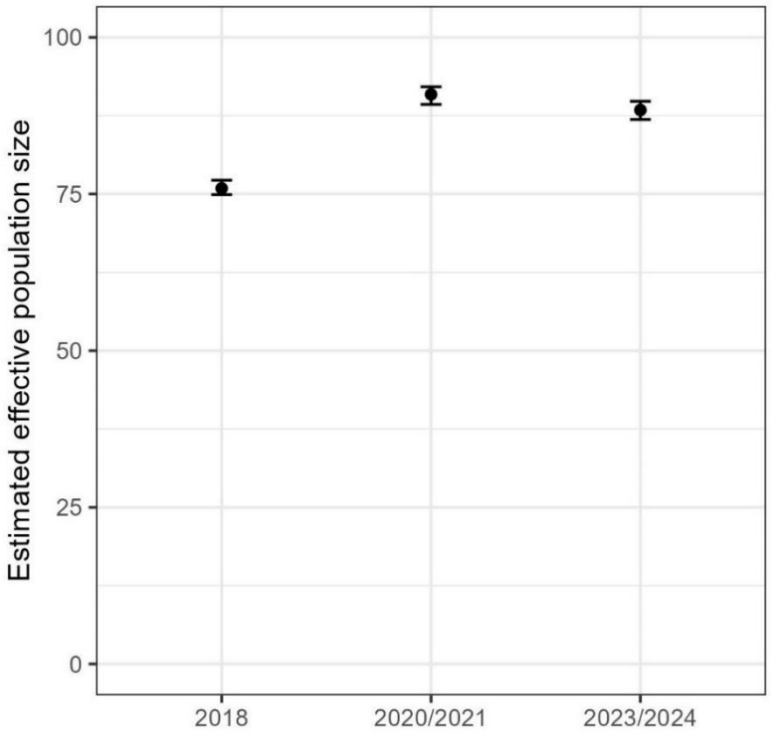

Figure 15. Estimated effective population size (Ne) across monitoring years with 90% confidence intervals.

Small effective population sizes can lead to increased risk of extinction. Small populations are more susceptible to demographic stochasticity, whereby random variations in birth and death rates can lead to extinction. In addition, small populations can suffer disproportionately from genetic effects, such as accumulation of recessive deleterious alleles under inbreeding, loss of quantitative characters that allow adaptation, accumulation of mildly deleterious mutations, and various other behavioural, social, and demographic factors. To safeguard genetic variability over hundreds of years, it is recommended that minimum effective population sizes of at least 100 be maintained (Mace and Lande 1991, Frankham, Bradshaw et al. 2014). Because the genetically effective population size is frequently <10% of the actual number of individuals in a population (Frankham 1995), this suggests an absolute minimum population of 1000 individuals is necessary

to avoid deleterious inbreeding. Even larger populations are needed to preserve quantitative trait variation: to maintain high levels (>90%) over thousands of years requires minimum effective population sizes of at least 5000 and to prevent the accumulation of mildly deleterious mutations over tens of thousands of years requires minimum effective population sizes of around 10,000-100,000. Because of difficulties in estimating key parameter values, these critical population sizes are best interpreted as guides to the relative importance of different characteristics rather than real thresholds for management (Mace and Lande 1991).

Comparison. These results generally confirm 2018 – 2021 survey results, and provide evidence that koalas on Redlands Coast mainland have:

1. retain similar (or potentially improved) genetic diversity and similar, albeit small, effective population size (N_e) in 2023/2024, however, similar to 2020/2021 which was slightly larger than in 2018

2. prevalence of *Chlamydia* could be declining, which could also be due to chlamydial disease reducing the koala population size.

Implication for conservation. The IUCN recommends that in order to avoid inbreeding depression, effective population size needs to be ≥ 100, and ≥ 1000 to maintain evolutionary potential of a species (Mace, Collar et al. 2008, IUCN 2012, Frankham, Bradshaw et al. 2014). Using these guidelines, the Redlands Coast mainland population falls short of the recommended effective population size. However, the Redlands Coast is part of the Koala Coast, and this would be the right unit of measurement (as an isolated population), and the three councils of the Koala Coast should work together to deliver the genetic monitoring at the level relevant to koalas.

While *Chlamydia* has decreased, its prevalence continues to be important in the Redlands Coast, with levels comparable to those found in 2018. This, combined with small effective population size, indicates the potential vulnerability of the Redlands Coast mainland koala population.

Recommendation. Regular monitoring, re-assessment and re-analysis of measures of genetic diversity, effective population size and *Chlamydia* infection rate for Redlands Coast mainland koalas will help monitor the evolution of this population, as some indicators seem to be improving while some are stabilising. Furthermore, continuous monitoring will help differentiate trends from fluctuations. Conservation efforts should continue to focus on establishing and maintaining connectivity of this population across the landscape, as well as continue efforts to mitigate the risk of *Chlamydia*.

4 Discussion highlights

This project continues the landscape genetic monitoring program started in 2018 and performed every three years. The aim of this program is to detect and track changes in genetic diversity, sex ratio and health of Redlands Coast mainland koalas.

4.1 Genetic connectivity and diversity

Similar to what has been previously found throughout this monitoring program, genetic population structure analyses identified one continuous breeding population across the Redlands Coast mainland. The exception to this was the detection of the translocated Minjerribah koalas, which formed a separate genetic cluster. Low levels of mixing could be detected between Minjerribah translocated individuals and mainland koalas. Translocation of koalas from Minjerribah, after being orphaned or having recovered from injury, to the Redlands Coast mainland, has the potential to introduce new genetic variants to the mainland. Observations of how well these translocated individuals acclimatise to the mainland and its threats are ongoing. The results presented here suggest that the translocated Minjerribah koalas are successfully breeding. This could, in theory, be beneficial for the mainland population, leading to increased genetic diversity (Frankham et al. 2015, Bell et al. 2019, Weeks et al. 2017). Future genetic monitoring should continue to track the effective breeding between Minjerribah translocated koalas and mainland koalas, as well as any resulting changes to genetic diversity, observed at the population level. While the current report identified a small increase in observed heterozygosity, this could be caused by a smaller sample size of this year's monitoring. Longitudinal genetic monitoring of the Redlands koala population will be able to identify any patterns in genetic diversity over time.

Furthermore, and as previously recommended, acquiring and including samples from outside the Redlands Coast boundaries would help understand genetic patterns at a more ecologically relevant scale (i.e. using natural boundaries instead of administrative ones).

4.2 *Chlamydia* stabilises, but continues to pose a major threat to Redlands Coast koala population

Chlamydial infection in Redlands Coast mainland koalas has been relatively high since the start of the monitoring program, in 2018, with the pathogen being detected in approximately one in three koalas. In 2020/2021, we detected an increase in *Chlamydia* prevalence, driven by high prevalence in females, where nearly half of the sampled females were infected with the pathogen. In 2023/2024, *Chlamydia* prevalence across sampled females decreased. *Chlamydia* prevalence decreased in localities where it was very high in 2021, such as Alexandra Hills and Sheldon, which could be that disease management is having an impact, or that these populations are losing diseased animals which decreases pathogen prevalence. Overall, *Chlamydia* prevalence continues to pose a major threat to the Redlands Coast mainland koala population because, even though there has not been an overall increase in prevalence, disease management actions have not yet been able to decrease levels of *Chlamydia* prevalence since 2018.

Overall, the results of this study show that the general genetic characteristics of Redlands Coast mainland koalas are similar since 2018. *Chlamydia* remains a key threat for Redlands Coast mainland koalas, where prevalence in 2023/2024 remains comparable to that of 2018, although a potential increase in female prevalence observed in 2020/2021 was no longer detected. About one in four Redlands Coast mainland koalas are infected with the *Chlamydia* pathogen. Management actions that aim to reduce prevalence of *Chlamydia* across the population are on-going and should be increased.

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Appendix

Glossary

The glossary have been compiled from many sources and is given here to facilitate the flow of the report.

Allele: a variant of a gene. The size of an allele can vary in size (e.g. between one nucleotide to hundreds of nucleotides). At the population level, variation in alleles are used to estimate patterns of genetic diversity.

Coverage: also called read depth, describes the number of times that a given nucleotide in the genome has been read. In Next Generation sequencing methods such as used here, the genome is fragmented into short sections of base pairs. These are read individually and then assembled through bioinformatics. For this assemblage to work with minimal error, multiple individual reads are required per fragment and nucleotide to achieve a certain level of confidence for a SNP call.

Cryptic population structure: discrete and geographically coherent grouping of genetically similar individuals

DNA: Deoxyribonucleic acid, a molecule carrying genetic information.

Effective population size (N_e) is one of the most important parameters in population genetics and conservation biology. This is because potential genetic issues are indirectly linked to the census size of a population, and directly dependent on the genetically effective population size. For example, if a population has 100 members (the census population), the effective population size would only include the number of breeding adults in the population - since a population normally includes non-breeding adults and juveniles. However, for the theory of population genetics what matters is the chance that two copies of a gene will be sampled as the next generation is produced, and this is affected by the breeding structure of the population. Consider the effect of unequal numbers of mating males and females.

The term ideal population is used to describe a population that has the following characteristics:

- The number of breeding males equals the number of breeding females.
- Mating is random, and all of the organisms will produce offspring.
- One organism doesn't produce more offspring than another.
- The population of breeding organisms remains constant from one generation to the next.

When the population follows all of the criteria for an ideal population, the effective population size should be equal to the census population - as in an ideal population all individuals have an equal opportunity to pass on their genes. Obviously, it's pretty rare for this to occur because not all members within a population mate, the ratio of males to females is rarely equal, mating isn't random, and so on. N_e is particularly sensitive to unequal numbers of males and females in the population. So, in reality, the effective population size will never be as large as the census population.

In short, the effective population size translates the census size of a real population into the size of an idealised population showing the same rate of loss of genetic diversity, inbreeding, or genetic drift for a population under study. The effective to census population size ratio (N_e /N) for natural populations was found to be on average of 0.1 (Frankham 1995, Palstra and Ruzzante 2008), these two measures relate to each other in no simple relation and therefore researchers should probably refrain (or at minimum exert caution) from making inferences about census population size based on effective population size (Palstra and Fraser 2012).

Current recommendations for the genetic conservation of species in the wild (Mace, Collar et al. 2008, IUCN 2012, Frankham, Bradshaw et al. 2014) are that in order to:

- avoid inbreeding depression, effective population size needs to be ≥ 100, and
- maintain evolutionary potential of a species, effective population size needs to be ≥ 1000.

Effective population size recommendations are based on the Extinction Theory, as summarised in Mace et al. (2008). The explanations below are extensively drawn from Mace et al. (2008), for specific references see the original paper.

All things being equal, the probability of extinction is greater when a population size is small or its decline rate is high. Small populations are more susceptible to demographic stochasticity, whereby random variations in birth and death rates can lead to extinction even when the average population growth rate is positive. In addition, small populations can suffer disproportionately from genetic effects, such as accumulation of recessive deleterious alleles under inbreeding, loss of quantitative characters that allow adaptation, accumulation of mildly deleterious mutations, and various other behavioural, social, and demographic factors. To safeguard genetic variability over hundreds of years, originally it was recommended that minimum effective population sizes of at least 50 be maintained, this was recently revised to 100 (Frankham, Bradshaw et al. 2014).

Evolutionary potential: the ability of a population to evolve to cope with environmental changes. Often simplistically equated with genetic diversity (especially for quantitative characters such as fitness), but it is also influenced by N_e.

F-statistics (fixation index)**:** is the basic method used to measure the amount of subdivision in populations, and consists of three measures, **F_{IS}, F_{ST},** and the less commonly used **F_{IT}**. These measures relate to the amounts of heterozygosity at various levels of a population structure: individual (I), subpopulation (S) and total (T).

F_{ST} estimates the amount of structuring of a population into subpopulations, and can range from 0 to 1 (where 0 means complete sharing of genetic material and 1 means no sharing). In this report, F'_{ST}, the standardised F_{ST} (produced by dividing F_{ST} by the maximum value it can obtain, given the observed within-population diversity) was also calculated to enable comparisons of our results to other studies.

F_{IS}, also called inbreeding coefficient, is the proportion of the variance in the subpopulation contained in an individual and can range from -1 to 1 (the closer to 1, the higher the degree of inbreeding). Note that inbreeding can not only result from non-random matings (matings between cousins for example), but also from small isolated populations, where all individuals are more closely related than large populations.

Gene flow: movement of alleles between populations via migrants or gametes. Gene flow maintains genetic diversity and promotes evolution by spreading new genes and combinations of genes throughout a species' range, however it may also constrain evolution by preventing adaptation to local conditions (and therefore, animal translocations need to be carefully thought out).

Genetic diversity: The extent of genetic variation in a population (or species, or across a group of species), for example heterozygosity or allelic diversity.

Genetic drift: changes in the genetic composition of a population due to random sampling in finite populations.

Genetic erosion: inbreeding depression and loss of genetic diversity in small populations.

Genetic stochasticity: genetic consequences of small populations, including inbreeding, loss of genetic diversity due to genetic drift and chance fixation of deleterious mutations that reduce fitness and can drive a population or species towards extinction (often in combination with other factors).

Genotype: in diploid species (species with two sets of chromosomes - paternal and maternal copies), genotype is often used to refer to the particular pair of alleles that are carried by an individual. A genotype is described as homozygous if it features two identical alleles and as heterozygous if the two alleles differ. The process of determining a genotype is called genotyping.

Hardy-Weinberg Equilibrium: is a principle that is used to examine, based on observed genotype frequencies (see observed / expected heterozygosity), whether a population is experiencing forces such as natural selection, non-random mating, genetic drift, and gene flow. The Hardy-Weinberg Equilibrium states that in the absence of these forces, the genetic variation in a population will remain constant from one generation to the next. Therefore, if a population of interest is found not to be at the Hardy-Weinberg Equilibrium, underlying causes can be explored.

Heterozygosity: refers to the presence of two different alleles within a diploid individual, here it refers to the presence of two different nucleotides at a specific SNP locus. Commonly, at the population level, two measures of average heterozygosity (calculated for all SNP loci and all individuals) are reported:

> **H_O** = observed heterozygosity, the calculated level of heterozygosity from the allele frequencies of the population under study,
>
> **H_E** = expected heterozygosity, the level of heterozygosity that could be expected based on observed allele frequencies if the population was at the Hardy-Weinberg* equilibrium.

The comparison between observed and expected level of heterozygosity is a measure of interest:

- A lower observed heterozygosity compared to the expected heterozygosity can be a sign of inbreeding.
- A higher observed heterozygosity compared to the expected heterozygosity can be due to the mixing of two previously isolated populations.

IR: Internal Relatedness is a measure of inbreeding at the individual level (as opposed to population level, such as F_{IS}). It is calculated from heterozygosity data and does not require a pedigree (pedigrees are difficult to obtain in wild populations). Internal relatedness is

currently the most widespread used index for inbreeding and its main strength is that allele frequencies are incorporated into the measure.

Inbreeding occurs when individuals are more likely to mate with relatives than with randomly chosen individuals in the population. Inbreeding increases the probability that offspring are homozygous, which can lead to lower fitness, a phenomenon commonly referred to as inbreeding depression.

Inbreeding depression: reduction in fitness due to inbreeding.

Koala Coast: The Koala Coast is a region in southeast Queensland that extents from Brisbane (south of the Brisbane River) through to Logan (east of the M1 Motorway) and Redlands Coast Local Government Areas. The Koala Coast has been identified as one of the most important natural koala populations in Australia.

Locus (plural **loci**): refers to a specific position in the genetic material (such as in a chromosome), for example where a SNP is detected.

Nucleotide: A nucleotide is the basic structural unit and building block for DNA. These building blocks are hooked together to form a chain of DNA. There are four types of bases in DNA. They are called: Adenine (A), Cytosine (C), Guanine (G) and Thymine (T).

PCoA: Principal Coordinate Analysis, a method that attempts to represent the dissimilarities between samples in a low dimensional space (2-3 dimensions).

PCR: Polymerase Chain Reaction, a technique in molecular genetics that permits the analysis of any short sequence of DNA even in samples containing only minute quantities of DNA, such as scats.

Polymorphism: any difference in the nucleotide sequence between individuals. Here, we refer to polymorphic loci when, across the population, differences occur between individuals (the opposite situation is a monomorphic locus where all individuals in the population have the same DNA sequence).

Relatedness: in genetics, defines the degree of consanguinity (also referred to as coefficient of relationship) between individuals. Typically, offspring receive half of their DNA from each parent, and have therefore a coefficient of relatedness of 0.5 with them (see typical levels of relatedness for some common relationships in table below).

	Coefficient of relatedness
Parent-offspring	0.5
Full sibling (same mother, same father)	0.5
Half sibling (same mother, different father, or the opposite)	0.25
Avuncular (e.g. uncle/nephew)	0.25
Grandparents grand-offspring	0.25
First cousins	0.125
Unrelated	0

There are many (infinite) possible combinations between individuals, and in some distant past, all animals are related to each other, so coefficient of relatedness can take all levels between 0 and 1. In addition, because of recombination between chromosomes during the creation of gametes, DNA is not inherited in a perfect 0.5 from each parent manner, creating even more possible levels for coefficients of relatedness. Note that there are many ways to calculate relatedness, and some coefficients calculated from genetic markers (and not family trees), such as the Queller and Goodnight used in this report, can take negative values (when two individuals are less related than relatedness expected between two random individuals.

Sex ratio: the relationship between the number of males to the number of females. Typically, the sex ratio in natural populations is expected to be 1:1. Risks of extinction are increased if population sex ratios deviate from 1:1. However, a small bias of sex ratio towards females can sometimes be desirable, especially in very small or rapidly declining populations.

Shannon's information index (I): is commonly used to describe diversity at the genetic level because of its ability to be integrated and compared to community-level diversity data.

Small populations: the fact that small, isolated, populations are more prone to extinction (or extirpation) is well established, and therefore a goal in conservation is to avoid species being

fragmented into small populations. In general, there are four sources of stochasticity that can cause small population to go extinct (from Shaffer 1981):

- demographic stochasticity: chance events in the survival and reproductive success of a finite number of individuals,
- environmental stochasticity: due to temporal variation of habitat parameters and the populations of competitors, predators, parasites, and diseases,
- natural catastrophes: such as floods, fires, droughts,
- genetic stochasticity: resulting from changes in gene frequencies due to founder effect, random fixation, or inbreeding – all influencing survival.

SNP: Single Nucleotide Polymorphism is the most common type of genetic variation. Each SNP represents a difference in a single DNA building block, called a nucleotide (there are four nucleotides: A, C, T and G).

sPCA: spatial principal component analysis, a tool to investigate cryptic spatial patterns of genetic variability using georeferenced multilocus genotypes. Unlike in a normal PCA, both variance between individuals and well as their spatial autocorrelation is taken into account. Two different types of structures can be assessed: global structure, which displays positive spatial autocorrelation, differentiates between two spatial groups or find a cline between them; local structure, which displays negative spatial autocorrelation, and would find stronger genetic differences among neighbours than among random pairs of entities (see Jombart, Devillard et al. (2008) for further details).

Structure: within a species, genetic structure exists because not all individuals are able to breed with all other individuals of the same species (i.e. due to geographic proximity). This can occur even if a species distribution is continuous due to geographic isolation: simplistically, this reflects that individuals that live closely to each other have a higher chance to breed together than individuals further apart. Population structure, i.e. the genetic differentiation of local populations, is increased by mutation, genetic drift due to finite population size, and natural selection favouring adaptations to local environmental

conditions; but is decreased by gene flow (the movement of gametes, or individuals). Population structure is higher when gene flow between populations is lower, and so population structure is increased by habitat fragmentation and isolation.

Gene flow cannot be directly seen, but population structure can be studied through allele frequencies - this underlines a critical point, that structure can only be inferred with a sample size large enough to calculate robust allele frequencies. This means that sample size dictates, in any study, the unit of comparison and the scale at which the genetic structure can be examined – i.e. depending on the intensity of the sampling design, whether appropriate sample size is reached per park, locality, council or region. In this report, we could achieve fine-scale genetic structure comparisons between localities. In previous studies, Redlands Coast mainland was pooled with neighbouring regions under the name "Koala Coast" and this became the unit for comparison with other regions of Australia (Kjeldsen, Raadsma et al. 2018).

Genetic structure can be hierarchically described:

- **Broad-scale structure** (often studied through a Bayesian statistic programs) usually defines "populations", these are independent breeding units, each population coming from a different lineage, and with none to very low gene flow. The software usually tests whether distinct populations can be inferred **without any *a priori* geographic information** and identifies migrants (individual belonging genetically to one population, but geographically to another one), and admixed individuals, that are offspring of migrants between populations.
- **Fine-scale structure** (often calculated through F_{ST}, see F-statistics) usually describes sub-populations (also called local populations or demes) where gene flow exists but is restricted. Genetic structure here is studied by comparing allele frequencies **between artificially constructed populations** (e.g. between Countries, between States, between Councils) and then testing whether the populations should be considered one or multiple, and how similar the populations are to one another (pairwise F_{ST}).

- Finally, the distribution of related individual in space, an even finer structure that can be referred to as “cryptic”, can be described through autocorrelation measures, where distances between all individuals and their genetic relatedness are compared.

End of report