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Final Report:

Koala Population Genetic Assessment Project

Prepared for Redland City Council

By the University of the Sunshine Coast, Detection Dogs for Conservation

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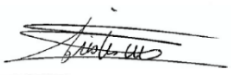
Signature:	
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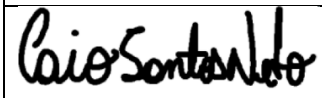
Signature:	

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Acronyms and glossary

These acronyms and glossary have been compiled from many sources and are given here to facilitate the flow of the report each term defined below will be followed by * when first mentioned in the text, to alert the reader this term is explained here.

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

DDC: Detection Dogs for Conservation at the University of the Sunshine Coast.

DES: State Department of Environment and Science

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 1995a, 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 et al. 2008, IUCN 2012, Frankham 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 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 Redland 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).

RCC: Redland City Council

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.

SD: Standard Deviation

SE: Standard Error

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.

SEQ: South East Queensland

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 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 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.

USC: University of the Sunshine Coast

Executive summary

Note. All genetic terms and concepts are defined in the "**Acronyms and glossary**" section of this report – genetic and other terms needing explanations are followed by * at first encounter to alert the reader that this is a term present in the "Acronyms and glossary".

Purpose

This project is a collaboration between Redland City Council (RCC) and the University of the Sunshine Coast's Detection Dogs for Conservation (DDC). In 2018, koala scat surveys were delivered using detection dogs paired with powerful next-generation genotyping. The project reported here aimed to repeat the 2018 koala scat surveys on Redlands Coast mainland in 2020, two years after the initial surveys. The ongoing project aims to better understand population characteristics that can inform efficient and effective management. In 2018, specific aims were to gain information on the distribution of koalas across Redlands Coast mainland, sex ratios*, gene flow across the landscape, and the investigation of genetic and physiological health parameters. The aim of the second study in 2020 was to repeat surveys and analyses done in 2018 and compare results between the two years. Furthermore, analyses could be repeated with data from both years pooled to increase overall sample size. Conservation genetics is a field in exponential growth and rapid development, so we also added new and improved analyses not available in 2018. Emphasis was placed on gathering data to inform:

1) Redlands Coast mainland koala population dynamics

In particular the:

a) distribution of koalas and potential temporal shifts,
b) number of genetically identified koalas in each year and between years,
c) number of females, males and therefore sex ratio and its change between years.

2) Dynamics of gene flow* in the Redlands Coast mainland koala population

In particular:

a) the extent of gene flow across the landscape,
b) the spatial distribution of closely related* koalas in close proximity across the landscape,
c) fine-scale population structure* assessment and dynamics thereof.

3) Health parameters of Redlands Coast mainland koalas in 2020/21 and comparison to data collected in 2018

In particular the:

a) levels of genetic diversity* and its dynamic across years,
b) levels of inbreeding* and its dynamic across years,
c) effective population size* and its dynamic across years,
d) presence of the Chlamydia pathogen and its dynamic across years.

Limitations

Koala presence surveys 2020/21

Sites were surveyed on only one occasion; therefore, the results presented here provide a snapshot of the population during the indicated survey period and it should be noted that evidence of koalas is likely to change seasonally (as koala movements vary with time). “The presence of absence does not equal the absence of presence” – to infer true absence, multiple surveys are generally necessary, from this survey, only presence can be confidently ascertained. In addition, most of the surveys were done with dogs trained to only detect very fresh scats (a few days old), therefore, absence of detection reflects that koalas were not present at the site in the last few days only.

In 2018, the southern areas of the Redlands Coast mainland were not surveyed as extensively as the northern areas, due to the high proportion of private properties with restricted access.

Council parks and bushlands were readily accessible. Because the DDC revisited survey sites from 2018, this bias still exists, despite efforts to identify and survey gaps by including new additional sites. This means the distribution of koalas in the southern areas of the Redlands Coast mainland could still be underestimated. This disproportion in sampling effort coincides with the division between urban and non-urban areas and, therefore, such comparisons are subject to this bias. However, wherever necessary, subsets were drawn to ensure minimal bias related to unequal sampling size.

Genetic analyses

Compared to high quality samples (e.g. biopsies/swabs), scat DNA* is degraded and presents multiple extraction difficulties (due to inhibitors present from the koala dietary component of the scat). However, here we were able to alleviate most of these limitations by using a genotyping method (DArTcap, see methods) appropriate for degraded DNA, which enabled the genotyping of numerous loci* (>1000). Still, the reliability of the results from genetic analyses relies heavily on the quality of the sample DNA, which requires the exclusion of low-quality samples which impacts the final sample size.

When analyses conducted could have been affected by uneven sample size, we randomly subsampled individuals for equal sample size and re-ran analyses to ensure significant patterns were valid.

While we are continuously improving our understanding of the links between Chlamydia presence (qualitative), Chlamydia load (quantitative) and clinical Chlamydia disease in koalas, we are still working towards verifying preliminary results (qualitative and quantitative) and the implications these results have in terms of health impacts on the koalas.

Koalas within Redlands Coast combine with parts of Brisbane and Logan Councils to form a population known as the “Koala Coast”*. It would be beneficial to koala conservation to sample, as a coordinated project, across the whole of the Koala Coast. However, management is more often than not constrained by administrative, not ecological or genetic, boundaries. Notably, although genetic sampling in this study was constrained by administrative boundaries, rather than ecological or genetic boundaries, some of the koalas sampled within Redlands Coast

(close to the borders of neighbouring Councils), would likely be using both Redlands Coast and neighbouring Councils areas and, therefore, representing genetic characteristics of koalas that extend beyond the boundaries of Redlands Coast.

Data set from 2018 genetic surveys and comparison

Since 2018, the genetic data generation and analysis has evolved. Technologies and methods have improved and, therefore, differences between 2018 results presented here might differ to the results presented in the 2018 Redlands Koala report. Therefore, all analyses using samples collected in 2018 were repeated, ensuring consistency and enabling comparison between the two years.

While the start points for the surveys were chosen to be the same as, or very close to, the starting points of the 2018 surveys, the direction of the survey track might differ between years, as the detection dogs were allowed to search freely. Therefore, two surveys, even starting from the same spot, can never be exactly the same, though the DDC aimed to cover a similar area as in 2018. It has to further be noted that the sampling in 2020/21 (August 2020 - April 2021) occurred in a different season to the sampling in 2018 (April 2018 - August 2018).

Not all survey sites from 2018 could be revisited in 2020/21 due to private access restrictions, construction activity or flooding events.

Findings

> ***Note.*** Specific mapped results per locality are given in Appendix 2.

Koala genetic sampling surveys 2020/21

A total of **262 surveys** were conducted on Redlands Coast mainland. Of those surveys, 114 of the survey sites (44%) had the detection dogs identifying koala scat presence, with **238 instances of scat detection** (old and fresh scats), where **242 fresh scat** samples were collected

and extracted for DNA (note that scats at one location were collected separately if they presented different characteristics as they can come from a different individual – i.e., from two koalas in one tree). During the scat surveys, a total of **48 live adult koalas** were spotted, of which seven showed signs of Chlamydia and 21 unknown health. Five were identified as females with a joey. One dead koala was found as well as two koala skulls.

Genotyping of 2020/21 koala scat samples

Of the fresh scat samples, **158 were successfully genotyped***. Following quality control and the removal of duplicate samples, **116 individuals were identified**. Overall, we found a sex ratio* of 59 males and 57 females (male to female ratio = 1:0.97). A total of 11 individuals that were sampled in 2018 were re-sampled in 2020/21.

Population structure and connectivity

Both in 2018 and in 2020/21, broad-scale population genetic assessment tools identified the Redlands Coast mainland koalas to belong to one single breeding population. However, we found strong evidence of fine-scale spatial and cryptic* structuring within the population. We were able to investigate genetic differentiation of koalas in a selection of suburbs (based on sample size) and found significant differentiation. Whilst the degrees of genetic differentiation between these locations are small, they could have long-term consequences if they persist / deteriorate further (i.e. populations could become isolated in the future, with all the negative genetic consequences of isolated populations).

We identified a cryptic genetic pattern that divides the Redlands Coast mainland koala population between north and south, with the division coinciding with three roads that are part of State Route 21 (namely, Mount Cotton Road, Duncan Road and Boundary Road). However, the role of the road in this finding remains to be assessed and the true impacts of roads, urbanisation and habitat shifts may not be fully appreciated until we sample future generations.

At an even finer scale, koalas in Redlands Coast mainland were frequently found in close proximity to related individuals. While this occurred more frequently in 2020/21 than in 2018,

these koalas were predominantly found in the northern urbanised suburbs in both years. This indicates that the increasing urban footprint on Redlands Coast mainland may be resulting in restricted dispersal opportunities and therefore gene flow between locations.

Population health

To assess the genetic vulnerability of a population requires us to think of a combination of 1) observed genetic diversity versus expected genetic diversity, 2) inbreeding and 3) effective population size. Together, these will be indicative of risk associated with inbreeding depression* and reduced evolutionary potential*. With koalas sampled in Redlands Coast mainland in 2020/21, we found:

1) lower levels of observed genetic diversity than expected genetic diversity compared to other koala populations across the koala's natural distribution,
2) multiple signs of high levels of inbreeding, with measures F_{IS} and IR found to be higher in 2020/21 than in 2018
3) low effective population size

These results fall in line with results from 2018. Overall, despite only two years between the two sampling periods, most measures indicate further degraded genetic diversity and inbreeding. Only effective population size has improved, however, only marginally so. Note that effective population size is important for population genetics and conservation biology of populations because effective population size, not census population size, is required to predict the rate of inbreeding and loss of genetic variation in the wild.

Interestingly, 11 individuals have been genetically re-sampled in 2020/21, which presents <9% of all individuals sampled in 2018. All these individuals were found in proximity to their 2018 locations.

Chlamydia was present and widely spread across Redlands Coast mainland. The levels of Chlamydia detected (any Chlamydial sequences detected) were 38% in 2020/2021. This is only a slight increase (3%) from 2018, when 35% of the sampled population tested positive for

Chlamydia. Overall, when data from both sampling periods were pooled, more females than males were found to be Chlamydia positive.

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Table 1: Overview of numbers and findings from 2020/21 in comparison to 2018 data. Improved measures are presented in green, deteriorating measures in red.

Measure	*Change**	*By %*
Number of surveys	*Increase*	*15.00%*
Number of samples	*Increase*	*2.50%*
Internal relatedness	*Increase*	*11.80%*
Inbreeding F_{IS}	*Increase*	*19.80%*
Urban F_{IS}	*Increase*	*9.40%*
Non-urban F_{IS}	*Increase*	*15.60%*
Observed Heterozygosity	*Decrease*	*-5%*
Expected Heterozygosity	*Decrease*	*-1.50%*
Chlamydia positive koalas	*Increase*	*3%*
Effective population size N_e	*Increase*	*19.80%*

**Note: For some measurements, an increase is a positive sign, for some a negative. For instance, an increase in inbreeding measures is not good for the koala population, whilst an increase in effective population size is. The colour code helps to see which changes are good (green) and which changes are not good (red).*

1. Introduction

1.1 Scope of works

In 2018, Redland City Council (RCC) contracted the University of the Sunshine Coast's Detection Dogs for Conservation (DDC) team to conduct a genetic study based on koala scat surveys across Redlands Coast mainland with the aim to better understand population characteristics to ultimately inform efficient management plans. Specifically, we aimed to gain:

- up-to-date information on koala presence,
- genetic diversity and connectivity of koala populations,
- Chlamydia distribution and frequency.

To further refine knowledge gained from the 2018 study, RCC contracted the DDC to repeat the study in 2020, i.e. revisit the same survey sites and collect samples for genetic analyses. Specifically, we aimed to understand:

- short-term changes in population dynamics (comparing the two survey periods) and update information on koala presence,
- dynamics of genetic and physical health aspects across time,
- if genetic parameters remain similar enough across years to be pooled, with the purpose of having a larger sample size for more detailed analyses.

Therefore, surveys to find genetic material from koalas (scats) were conducted and genetic data was analysed using samples collected in 2020 and 2021 (hereafter 2020/21). Results were compared to the analyses from 2018. Where we deemed it appropriate, the data were pooled and analyses were repeated using a larger sample size.

1.2 Background

The scale and speed at which habitat loss and fragmentation are affecting the landscape are critically reducing the evolutionary adaptive potential of most species (Frankham et al. 2017). Habitat loss and fragmentation through urbanisation have far reaching ecological consequences for wildlife (Newbold et al. 2015). Over the past 35 years alone, for instance, habitat

fragmentation has reduced species biodiversity to as little as 25% of its pre-industrial value across five continents (Haddad et al. 2015). This is because habitat loss and fragmentation reduce the amount of habitable space for wildlife and restrict the movement of animals - and their genes - between populations (Segelbacher et al. 2003). It is well established that the creation of small, isolated populations (see small population*) with reduced migration, causes a range of genetic consequences, including loss of heterozygosity*, increased inbreeding and inbreeding depression (Cristescu et al. 2009, Frère et al. 2010), increased genetic drift* and decrease in effective population size (N_e), all of these can be deleterious and increase extinction probability (Lacy 1997, Frankham et al. 2010, Frankham et al. 2017), especially if they remain unnoticed at first (Margan et al. 1998). More specifically, decreased genetic variation can result in reduced reproductive success, reduced disease resistance and decreased ability to adapt to changing environmental pressures (O'Brien et al. 1985, O'Brien and Evermann 1988, Sherwin et al. 2000). This is why the International Union for Conservation of Nature recognises the maintenance of genetic diversity and connectivity as a major objective of biodiversity conservation (McNeely et al. 1990, IUCN 2012).

Australia has the highest mammal extinction rate of any country in the world (Woinarski et al. 2015), therefore developing more effective conservation and monitoring is critical. Koalas (*Phascolarctos cinereus*) for instance, are, despite their iconic status and economic value (potentially $3.2 billion per annum (Conrad 2014)), experiencing an alarmingly sharp decline in the northern and eastern parts of their range i.e. Queensland, New South Wales and the Australian Capital Territory (Government 2012, Rhodes 2015). In Queensland, land area occupied by koalas has contracted by an estimated 31% over the past century (Gordon et al. 2006) and the number of koalas has declined by approximately 43% (39-46% range) over a period of 20 years (McAlpine et al. 2015). In some areas of the state, documented declines have been even greater, including an 80% decline in the Mulga Lands bioregion over 14 years (Seabrook et al. 2011) and, within the South-East Queensland bioregion, declines of 80% and 54% within the Koala Coast and Pine Rivers populations respectively (Rhodes et al. 2015). Of even further concern is evidence that the rates of decline in these areas are worsening over time (Rhodes et al. 2015). It should be noted that all these estimates stem from a time prior to the 2019-2020 mega bushfires, which further impacted koala populations (Phillips et al. 2021). The species is listed as vulnerable under the Australian *Environment Protection and*

Biodiversity Conservation Act in these areas (McAlpine et al. 2015) and under the IUCN Red List (IUCN 2012). However, after the loss of koalas in the mega bushfires, the species now meets criteria for IUCN to be uplisted to endangered (Wallis et al. 2020).

In addition to threats linked to climate change (such as the mega bushfires), the reasons for population declines are: 1) habitat loss and fragmentation (which reduces genetic diversity and connectivity), 2) infectious disease caused by the bacterial pathogen, Chlamydia (which causes blindness, sterility and potential death), and 3) the risks associated with koala movements in human-altered landscapes (including dog attacks and car strikes) (Rhodes et al. 2011b, Polkinghorne et al. 2013, Burton and Tribe 2016). However, evidence about how these threats are impacting specific populations are often not available to decision makers, and this stands for the Redlands Coast. To enable environmental planning, what is needed is temporal and fine-scale information about 1) koala distribution, 2) connectivity between koala populations, and 3) population health across the landscape. Generating this level of data has often been prohibitively costly (in time and financial resources), as traditionally these data often required catching, sampling and monitoring live animals. In 2018, the DDC conducted a Council-wide survey with specially trained koala detection dogs, to gather and provide detailed information on the Redlands Coast koala population and its genetics. This was the first fine-scale, yet landscape wide, genetic study for koalas using only non-invasive samples and provided valuable insights into the Redlands Coast koala population. However, the robustness of these findings can be strengthened by repeating the survey to gather more temporal data and to also increase overall sample size.

Here, we built on findings from the 2018 study and again used non-invasive and cost-effective methodologies to find koala scats (conservation detection dogs (Cristescu et al. 2015a) and genetically analyse the scats (Schultz et al. 2018a)). Thanks to the high repeatability of these methods, we were able to replicate the study and compare results between years. However, we are continuously re-assessing and improving our methods so as to provide the best possible data/outcomes. Since our initial study, molecular techniques have further evolved and lead to a much-improved genetic data set, both in quantity and quality. Given that we want to use the best data available, but also ensure comparability, we re-analysed the improved 2018 data set

and provide this data here. Furthermore, we replaced and adjusted some analyses with new and more informative ones.

Overall, this project builds on previous fine-scale data collected in 2018 and helps to further inform koala genomic diversity, disease and connectivity to empower decision makers to effectively manage their koalas. In particular, we focused on determining:

1) Redlands Coast koala population dynamics

Here, we conducted a total of **262 surveys** across Redlands Coast mainland, collected and extracted 242 koala scat samples and successfully genotyped 158 of them. After filtering of genetic data and identification of duplicates, 116 unique individuals were found.

We analysed the survey and genetic data to assess the:

a) distribution of koalas,
b) number of individuals genetically identified in each year and between years,
c) number of females, males and therefore sex ratio and its change between years.

All results from 2020/21 (N = 116) were compared with results from analyses using data collected in 2018 (N = 124). Whenever appropriate, data were pooled to increase the overall sample size and thus robustness of results.

2) Dynamics of gene flow in the Redlands Coast koala population

Here, we used the genetic data to estimate population genetic structure across the Redlands Coast mainland to assess:

a) the extent of gene flow across the landscape,
b) the spatial distribution of related koalas in close proximity across the landscape,
c) fine-scale population structure and dynamics thereof.

All results from 2020/21 were compared with results from analyses using data collected in 2018. Whenever appropriate, data were pooled to increase the overall sample size and thus robustness of results.

3) Health parameters of Redlands Coast koalas in 2018 and 2020/21

Here, we estimated a suite of genetic and health traits to assess the extent to which Redlands Coast mainland koalas may be vulnerable to local extinction. These included the:

a) levels of genetic diversity,
b) levels of inbreeding,
c) effective population size,
d) presence of the Chlamydia pathogen.

All results from 2020/21 were compared with results from analyses using data collected in 2018. Whenever appropriate, data were pooled to increase the overall sample size and thus robustness of results.

Together, and building on 2018 results, these genetic and health traits allow us to further refine the current status and make predictions about future risks associated with inbreeding depression and, as such, assess the evolutionary potential of koalas across the Redlands Coast mainland.

2. Methodology

2.1 Revisiting 2018 survey sites to understand presence and dynamics of the Redlands Coast koala populations

2.1.1 Site selection / sampling design

The DDC revisited the 2018 survey sites and aimed to begin the survey at the same start points (see limitations). In 2018, survey sites were mainly located in conservation areas, recreational areas (e.g. parks), rehabilitation areas, wildlife corridors and National Parks (Venman National Park). Sites in private properties were added, following media promotion of the surveys, on a voluntary basis. Generally, sites were not random, but were selected based on accessibility (tenure), efficiency (i.e. access roads) and to achieve a good geographical spread within our project timeframe. In urban areas, sites were specifically targeted when recent koala activities were recorded (either through “Atlas of Living Australia”, the Government hospital database,

"Koala Tracker" or Koala Action Group records). Additionally, new locations were incorporated to ensure a satisfactory coverage of ecological, geographical and social aspects of the Council area. For example, ecological corridors, regions where koalas are less frequently sighted, the area of the future Heinemann Road Sporting Complex, survey sites used by the Biolink Ecological Consultants in 2018, permanent State monitoring sites, urban zones and roads were added to the final design.

2.1.2 Survey types for koala scat detection

The DDC has developed survey methodology that is called '***casual koala scat survey***'. In the casual surveys, the dog is not constrained by the handler and is allowed to follow its nose roaming over an area of up to a couple of hectares within an approximate 30 minutes or up to when the handler deemed the search to have covered the site extensively. Casual surveys are a fast way to determine whether koala scats are present at a specific site. This method is designed to maximise the chance of detecting koala scat presence in the minimum amount of time. It also allows for coverage of larger areas. Finally, this is the best method to detect fresh koala scats for genetic sampling.

Nonetheless, casual surveys cannot be repeated to 100% in a revisiting survey, as the dog is freely making decisions about where to survey, especially based on wind direction, each time – therefore, the surveys are challenging to compare in time or space. For the Redlands Coast mainland surveys, we exclusively utilised casual koala scat surveys because our aims were 1) to maximise area coverage and 2) to maximise genetic sampling. As indicated in the limitations, it is not possible to fully recreate the same survey track or effort but we aimed to start a 2020/21 survey at the same start point as the equivalent 2018 survey and to cover a similar area. Note that detection dogs are fitted with a GPS collar to document the areas searched. This allowed us to provide detailed maps showing the tracks of the scat surveys but also a comparison showing the differences that may occur when revisiting a survey site using casual detection dog surveys. Dog survey effort was supplemented with drone surveys that occurred in Redlands Coast mainland as part of the Koala Safe Neighbourhood project within the same sampling period.

2.1.3 Dogs utilised for koala scat detection

We deployed mainly the following dogs during the koala scat surveys in Redlands Coast:

- Billie-Jean, trained on only very fresh scats,
- Baxter and Maya, trained on scats of all ages,
- Bear, trained on living koalas.

2.1.4 Scat identification

When a detection dog signalled that a koala scat was found, the handler visually confirmed the scat identification, recorded the location with a hand-held GPS and classified it by age (Table 2) to help estimate how recently a koala had utilised this area.

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);
- even-sized and especially fine particles;
- absence of insect parts (koalas do not eat insects); and
- very compact.

Table 2: Scat age categories

Scat Age Categories	Characteristics – approximate age
1	Extremely fresh (covered in mucus) – 1 day old or less
2	Fresh (shiny, smelly) – few days old
3	Medium fresh (shine, or smells when broken) – weeks old
4	Old (no shine, no smell) – months old
5	Very old and discoloured – many months to years old

Note: It has been estimated that koala scats can persist in the environment for up to four years (Rhodes et al. 2011a)

Figure 1: Koala scats, freshest (Category 1) on the right

2.1.5 Koala sightings

The dog handlers conducting the surveys were also looking for koalas in trees, especially after very fresh scats of age category 1 were found. However, since koala sightings were not the focus of this project, a maximum of 10 minutes was spent spotting before continuing the survey.

The dog handlers were also always on the lookout for opportunistic/incidental sightings of koalas. These can happen in the following manner: on foot or in the car while moving between survey locations; information passed on to DDC researchers by members of the public, property owners or passers-by. The general public is always considered as a source of local knowledge and individuals were questioned on koala presence, past and present, whenever possible.

When koalas / koala scats were located during opportunistic surveys, photographs of the animals / scats were taken, and fresh koala scats were collected for further analysis. Koalas were observed with binoculars to try to ascertain: (1) koala sex, (2) external signs of Chlamydial disease, often referred to as pink eyes (for ocular infection / conjunctivitis) and wet bottom (for urinary tract infection), and (3) presence of a joey. In the case of finding a sick or injured koala, the RCC wildlife ambulance was contacted.

2.2 Assessing how genetically connected populations of koalas are

Note. All genetic terms and concepts are defined in the "**Acronyms and glossary**" section of this report – genetic terms needing explanations are followed by * at first encounter to alert the reader that this is a term present in the "Acronyms and glossary".

2.2.1 Creation of the genetic dataset

Fresh scats (mainly, age categories 1 and 2) found during the surveys were collected for genetic analysis. Scats were collected in a sterile tube without direct skin contact to avoid potential contamination and loss of koala DNA. Tubes were kept on ice until they were stored in a -20 degrees Celsius freezer. DNA was extracted using the method described in Schultz et al. (2018b). DNA extractions were genotyped using a next-generation sequencing protocol for detecting Single Nucleotide* Polymorphism* or SNP* (Kilian et al. 2012) using specific probes that were designed for this project in 2018 to increase the percentage of SNPs replicated across most samples, and therefore enhance all downstream genetic analyses.

In order to achieve a subset of representative SNPs, we filtered loci in a stepwise manner to maximise both quality and quantity of the retained data. For each individual sample, loci with coverage* below 5 were assigned as missing data, as those SNPs are prone to be called erroneously. We then filtered further to only include SNPs showing at least a call rate of 80% and a minor allele* frequency (MAF) of 1%. We also removed samples with more than 60% missing data. We also removed loci that occurred on the same fragment as another locus and were likely linked and removed all monomorphic loci that. Filtering was done in R studio using the R package dartR (Gruber et al. 2018).

The identification of duplicate samples, which leads to the identification of individuals in the data set, is a crucial step when using non-invasively collected samples from the wild. Duplicate samples (i.e., samples that were collected from the same koala) were identified as pairs of samples which matched at >80% of SNP calls. After filtering, we investigated how many SNPs two samples had in common and how many were mismatched. We used known duplicate

genotypes and known mother and joey pairs to investigate how many SNPs are likely to be mismatched between two samples of the same individual (duplicate samples) versus highly related individuals. We determined that shared SNPs of duplicate samples match at 80% or more. Two samples that show >20% mismatch likely stem from different individuals and were considered distinct. Once identified, duplicate samples were removed from all further analyses.

Koala sex was identified from our samples by a set of 30 sex specific probes. Further details of all molecular methods are provided in Appendix 1.

2.2.2 Calculating genetic population differentiation

Broad-scale genetic population structure

We used two commonly used methods to estimate population structure. We used a simple Principal Coordinate Analysis (PCoA*) that clusters individuals based on genetic distance (genetic dissimilarities). Furthermore, we used the Bayesian clustering approach implemented in the fastSTRUCTURE software (Raj et al. 2014). In order to identify the most likely number of ancestral populations (K) in Redlands Coast mainland, we tested several potential values (K = 1 to 5).

Fine-scale genetic population differentiation

Genetic differentiation between suburbs was estimated by calculating F_{ST} (see F-statistics*) using the pooled data from 2018 and 2020/21, to reach the recommended minimum population size of 20, for a subset of suburbs. These suburbs were then included in a pairwise F_{ST} analysis using the AMOVA function in GeneAlex (Peakall and Smouse 2006), running 999 permutations to estimate *P*-values.

2.2.3 Cryptic population structure*

We assessed cryptic population structure using spatial principal component analysis (sPCA*), a spatially explicit multivariate method that explores non-random spatial distribution of genetic variation and is implemented in the R package *adegenet* (Jombart 2008, Jombart et al. 2008). A sPCA can identify spatial patterns by including both genetic variability and spatial

autocorrelation (Moran's I) into the calculations of principal components. We used a *neighbourhood by distance* approach with a minimum distance of d1 = 0 meters since different individuals can be found in the same area, and maximum distance was set to d2 = 10,000 meters, which reflects the expected maximum dispersal distance (Dique et al. 2003). The Eigenvalues give information on global and local signs of structure, with highly positive eigenvalues indicating strong variance and highly positive spatial autocorrelation (i.e., global structure). We tested whether global and/or local structure was significant using the *spca_randtest* function included in the *adegenet* package (Montano and Jombart 2017).

2.2.4 Fine-scale spatial distribution of related individuals in close proximity

Using relatedness calculated for each pair of individuals, we visualised where related individuals were found in close proximity to each other, i.e. within a distance < 600 metres. The radius was chosen to represent the average distance that a male and female koala would move for mating. This was calculated from known breeding pairs for which home ranges were calculated from GPS-collar data. Visualisation was done by running a model that is used to assess spatial autocorrelation, called INLA (Integrated Nested Laplace Approximation, Rue et al. (2017)) based on an adapted version of the LandRel method (Norman et al. 2017). We followed Norman et al. (2017) and Lindgren and Rue (2015) to build our INLA model using the R package R-INLA (Rue et al. 2009). We built a two-dimensional mesh that accounted for the shoreline to the east of Redlands. We used a spatial stochastic partial differential equation (SPDE) model for the interpolations (Lindgren et al. 2011). We used the average relatedness of a focal individual to non-focal individuals within a radius of 600 meters (hereafter "range relatedness"). This approach reduced the sample size to 92 individuals for 2020/21 and 90 for 2018. Individuals had on average 3.8 ± 2.7 and 2.9 ± 2.0 individuals within that range, respectively. For this analysis, we chose to use the Lynch & Ritland (Lynch and Ritland 1999) as our pairwise relatedness estimator, following Norman et al. (2017). Range relatedness values were interpolated on a lattice grid of 300 x 300 for 2020/21 and 2018. We created maps using the raster package in R (Hijmans et al. 2015) and QGIS (version 3.12.0). To facilitate visual comparisons between our two data sets, we used the same settings for minimum and maximum values for the colour scale of range relatedness when plotting the interpolations. For many

individuals in the southern part of the Redlands, we did not have samples from another individual within 600 m radius, therefore, landscape relatedness estimates for the southern part are underrepresented (for both 2020/21 and 2018).

2.3 Assessing how healthy Redlands Coast koalas are

2.3.1 Genetic bottleneck

We tested whether any population has recently undergone a genetic bottleneck using the software BOTTLENECK (v1.2.02; Luikart et al. 1998). We specified 100 iterations and used Wilcoxon sign rank tests to assess significance. BOTTLENECK v1.2.02 provides results for three models of the generation of new alleles; the stepwise mutation model (SMM), the infinite allele model (IAM) and the two-phased model of mutation (TPM). These models are discussed in Cornuet and Luikart (1996). *This will be completed and reported in the final report.*

2.3.2 Genetic diversity measures

Patterns of genetic diversity across Redlands Coast were assessed using GeneAlEx 6.5 (Peakall and Smouse 2006) using the following genetic diversity measures:

- Shannon's information index* (I)
- Expected heterozygosity (H_E)
- Observed heterozygosity (H_O)

2.3.3 Inbreeding and internal relatedness*

At the population level, the inbreeding coefficient (F_{IS}) was calculated using observed (H_O) and expected (H_E) heterozygosity [i.e., $F_{IS} = (H_E - H_O) / He$].

At the individual level, inbreeding was measured per scat by calculating the internal relatedness (IR) measure (Amos et al. 2001) in GENHET in R (Coulon 2010). Several measures are available to infer inbreeding from heterozygosity data without requiring pedigrees, as pedigrees are difficult to obtain in wild populations. Internal relatedness (IR) is currently the most widely

used index and its main strength is that allele frequency is incorporated into the measure (Aparicio et al. 2006). This measure is calculated as follows:

$$IR = (2H - \Sigma fi) / (2N - \Sigma fi),$$

where H is the number of loci that are homozygous, N is the number of loci and fi is the frequency of the i^{th} allele contained in the genotype.

2.3.4 Effective population size

Effective population size (N_e) was calculated based on the linkage disequilibrium method using LNDe v1.31 (Waples and Do 2008). For successful conservation strategies, it is important to have an understanding of the effective population size as this provides an indication of the number of individuals contributing their genes to the next generation. Effective population size, more so than the census population size, is closely linked to the rate at which allele frequency changes in the population, and will reflect the loss of genetic diversity, inbreeding, or genetic drift. Even though effective population size is recognised as one of the most important parameters in both conservation and evolutionary biology, it is not a trivial measure to estimate in the natural world (Waples and Do 2010). Recent advances have improved the linkage disequilibrium method, and the estimates are more precise when hundreds of SNPs (such as the present study) can be used (Luikart et al. 2010).

2.3.5 Chlamydia

Presence of the Chlamydia pathogen was assessed based on Chlamydia specific SNPs, and a koala was classified as chlamydial positive above a specific threshold (>9 SNPs detected out of the 30 Chlamydia-specific probes). Note that the presence and load of Chlamydia do not necessarily mean koalas are sick, as they can be passive carriers of the bacteria, or have recovered (Robbins et al. 2019).

2.3.6 Urban and non-urban populations in Redlands Coast mainland

We calculated and compared genetic diversity measures, inbreeding coefficient and internal relatedness (as outlined above) for urban and non-urban koalas across the Redlands Coast mainland population in order to assess the impact that urbanisation has on levels of genetic diversity. Urban and non-urban areas were defined using the regional land use categories in south-east Queensland defined by Queensland government and available from QSpatial (the Queensland Spatial Catalogue provided by the Queensland Government and available at: http://qldspatial.information.qld.gov.au/catalogue/custom/index.page).

As in 2018, we collected substantially more samples from urban areas than non-urban areas (2018: urban: N = 82; non-urban: N = 42 and 2020/21: urban: N = 78; non-urban: N = 38, see limitations). As a result, to compare relatedness between urban and non-urban areas, we randomly subsampled individuals from urban areas to reach an even sample size and ran analyses on these data. This was done to ensure that significant differences in our results were not a consequence of uneven sampling.

2.3.7 Temporal assessment using tissue samples collected in 2006

To facilitate the interpretation of contemporary genetic measures, it is valuable to investigate and compare the current genetic situation to previous studies or historic samples. This enables the results to be contextualised e.g. how is the current trend comparing with initial population characteristics? A true baseline would require samples from pre-European settlement of the same population. Acquiring such a sample set is done through museum collections, however our investigations determined that not enough samples from the Redlands are available in museums. Therefore, we investigated the existence of samples from a few generations prior to our surveys. The DDC secured access to a unique set of koala ear biopsies that were collected from sick and injured koalas at two major wildlife hospitals in the area (Moggill Koala Hospital (Moggill, Queensland) and the Australian Zoo Wildlife Hospital (Beerwah, Queesnland)). In this collection were a total of 261 ear tissue samples from Redlands koalas that were admitted between 2006 and 2007, i.e. about two generations prior to DDC surveys. DDC was able to secure external funding to analyse this dataset.

We extracted the DNA and genotyped the individuals using DArTcap. Ear biopsies were stored in 70% ethanol at room temperature until extraction. The hospital data did not provide geolocations but only street addresses of the location where individual koalas were found. Therefore, we geocoded sample locations as the centroid of the street using the R package ggmap (Kahle and Wickham 2013).

To compare this data with our recent measures, we analysed fine scale population structure, F_{ST} values, F_{IS}, and effective population size. Wherever appropriate, a subset of 100 individuals was used to minimize differences due to sample size alone and thus ensure comparability.

2.4 Limitations

2.4.1 Fieldwork

The sites were surveyed on only one occasion in each survey period; therefore, the presence / absence results presented here provide a snapshot of the population during this period and it should be noted that evidence of koalas found within the study areas is likely to change seasonally [as koala movements vary with time (Ellis et al. 2009)]. Note that the two survey periods occurred in different seasons, therefore we cannot exclude that this has impacted any difference found in presence/absence.

Detection dogs are a powerful method to study koala presence/absence and its use could greatly improve our ability to protect and conserve the koala. However, results of accuracy and efficiency of detection dogs will vary with both the dogs' and the handlers' abilities. Constant training and testing are required, as conducted by the DDC handlers and dogs.

The rate at which scats decay may also vary significantly between sites due to varying ground layer structure, composition, moisture, sunlight, local weather events and invertebrate activity (Rhodes et al. 2011a, Cristescu et al. 2012). Decomposed scats may lose their unique scent mark and the dog may no longer detect it – however this has not yet been proven to occur (Cristescu et al. 2015b).

Failure to detect koala scats in an area is not necessarily conclusive. Failure to detect koala scats may suggest either of the following:

- First, as most of the surveys were done with dogs trained to only detect very fresh scats (a few days old), the absence of detection reflects that koalas were not present at the site in the last few days only, but should not be interpreted as that koalas are not present at all.
- Koalas are not present in the area (i.e. true absence) at the time of the survey. Note that true current absence does not infer that the site has not been used in the past, or could not be used in the future, i.e. it could still be potential koala habitat.
- Koalas occur in the area, however 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" – to infer true absence, multiple surveys are generally necessary (MacKenzie and Royle 2005), from this survey, only presence can be confidently ascertained. In addition, for this particular survey, we mainly used a fresh scat detection dog. Therefore, the absence of fresh scats merely means that no koala was present recently.

In this project, survey effort in each area can be assessed by the track log of dog searches (provided in Figure 2 with a comparison to 2018 efforts, see details per locality in Appendix 2). This was complemented with incidental koala spotting between locations.

A large proportion of samples were collected from localities in the northern part of the Redlands Coast (i.e. all localities except Sheldon, Mount Cotton and Redland Bay). This bias is mainly a result of revisiting survey sites from 2018, where site selection was largely driven by accessibility (large proportion of southern Redlands Coast bushland is locked in private properties). Furthermore, urban areas were a priority for this work, as defined in the original scope of work ("*Provision of detailed data on Redlands urban population*") and further discussions with RCC.

A small number of sites could not be revisited due to access restrictions on private properties or due to construction activities on site. Furthermore, spring and summer 2020/21 experienced

heavy rainfalls and flooding due to La Nina events. This caused some delays in fieldwork but likely impacted scat detectability of older scats that could have been washed away in flooding events. After each heavy rainfall and flood episode, the DDC stopped surveys for 2-3 days to allow the ground to dry and to increase the likelihood of finding fresh scats that are suitable for DNA extraction (as rain can wash cells/DNA off scats).

Similar to the 2018 survey, we were able to detect more live koalas in urban environments (localities in the northern part of the Redlands Coast) due to a potentially higher detectability rate compared to natural bushlands of non-urban areas. In this hypothesis, which we have personally observed (but not measured / tested), koalas stand out more in urban areas because there are less trees to search, the trees are more clearly separated from one another, and road vegetation is thin, often offering a clear sky background - all of these factors making a koala easier to spot. Note also that samples were included from the Koala Safe Neighbourhood project, where spotting of koalas in certain suburbs was the focus.

It should further be noted that, while the start points for the surveys were chosen to be the same or very close to the staring points of the 2018 surveys, the direction of the survey track might differ between years. The scent detection depends on current koala movement but also wind direction and decisions made by the dog, which cannot and should not be forced to be recreated. Therefore, two surveys, starting from the same spot, can never be the exact same. We aimed, however, to cover a similar area as in 2018, to our best ability. It has to further be noticed that the sampling in 2020 (August 2020- April 2021) occurred in a different season to the sampling in 2018 (April 2018-August 2018). We are currently lacking information on seasonal movement and changes in physiological requirements of koalas.

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 have been underestimated.

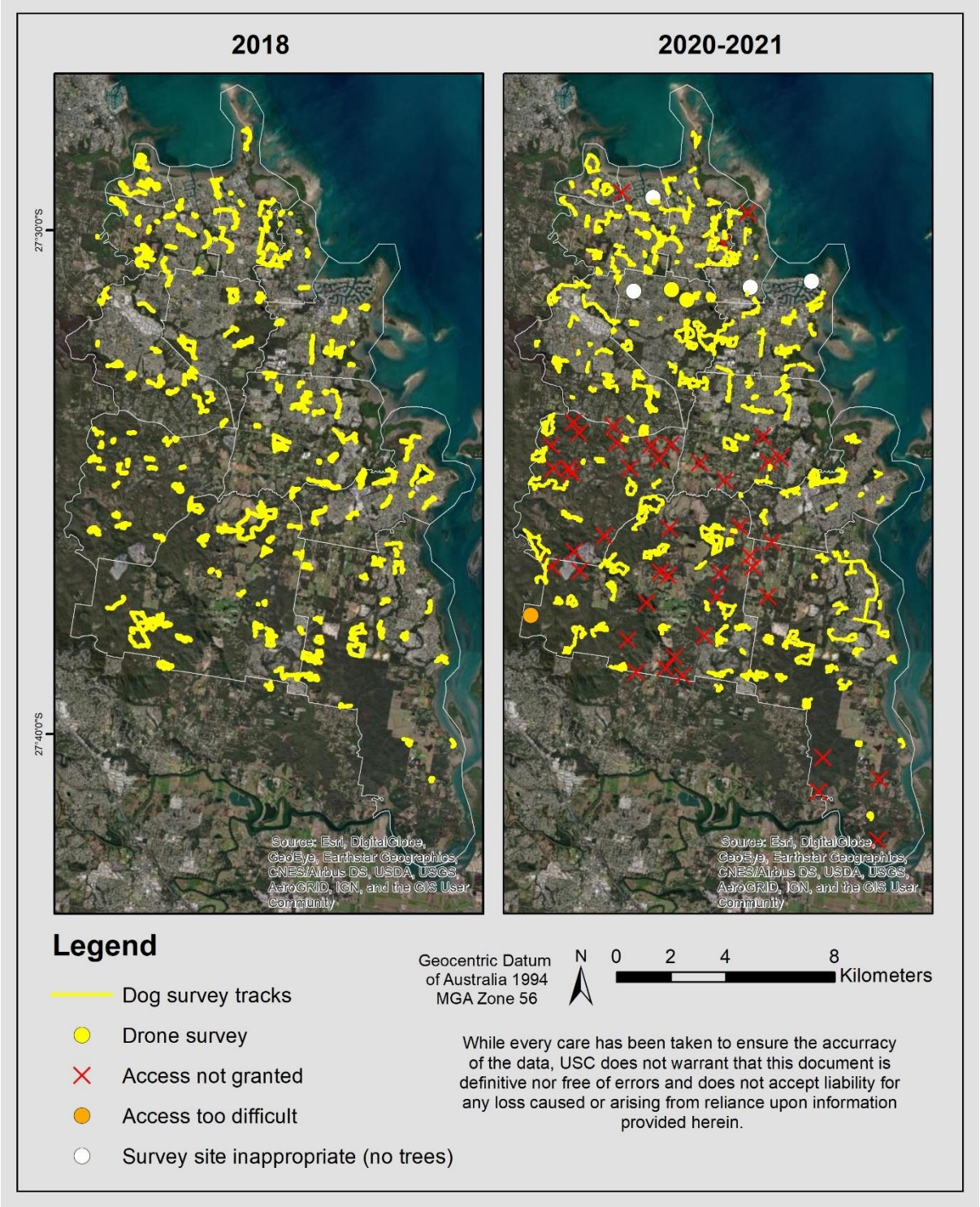

Figure 2: Dog tracks recorded during the surveys, as an indication of search effort across the Redlands Coast mainland, with both 2018 and 2020/21 presented. Note that in many instances, handlers also performed visual searches between sites that therefore

are not represented in the map. It is furthermore indicated where survey sites could not be revisited due to restricted access (red crosses and orange points) and where proposed new survey sites were unsuitable (white points). Dog surveys were supplemented with a few drone surveys presented here as yellow points.

2.4.2 Genetics

Genotyping was conducted non-invasively from material contained in the surface of koala scats (both koala and bacterial DNA). This allows for large scale, relatively cheap, unbiased sampling of DNA compared to more widely used methods (catching, anaesthetizing and collecting biopsy/swab, or relying on Hospital samples). However, compared to high quality samples (biopsies/swabs), scat DNA is degraded and presents multiple extraction difficulties (due to inhibitors present from the koala dietary component of the scat). However, we were able to alleviate these limitations by designing a new genotyping method in 2018 (DArTcap, see methods), which enabled the genotyping of numerous loci (>1000). In addition to the sequencing and genotyping methods, the analytical methods have since improved, meaning data has improved in both quality and quantity. To ensure consistency and comparability between 2018 and 2020/21 results, we re-analysed the 2018 data set.

It is important to note that comparisons of genetic diversity cannot be made across studies unless the set of genetic markers used are identical. An important comparison, however, that can be made is estimates of inbreeding (F_{IS}). This is because F_{IS} represents the ratio of the absolute difference between expected and observed heterozygosity, divided by the expected levels of heterozygosity.

Note that the genetic sampling in this study was constrained by administrative boundaries, rather than ecological or genetic boundaries - i.e. koalas within Redlands Coast, with parts of Brisbane and Logan Council areas, form a population known as the “Koala Coast” (Lee et al. 2010). This issue is partly compensated for by the fact that some of the koalas sampled in Redlands Coast were close to the borders of neighbouring Councils, with these individuals likely using both Redlands Coast and neighbouring Council areas. Therefore, these individuals likely represent genetic characteristics of koalas that extend beyond the boundaries of Redlands Coast.

It is known that koalas from Minjerribah (North Stradbroke Island) have been released on the Redlands Coast mainland in recent years, and in particular since 2018. It is possible that these introduced individuals have been unknowingly sampled and are included in the 2020/21 analysis. Because Minjerribah koalas form a separate population (see 2018 report) that has been identified to be genetically different to Redlands Coast mainland koalas, such "different" genetic compositions may affect diversity measures and other genetic parameters. The DDC did not identify any outliers in the 2020/21 data set but will continue to scan for Minjerribah koalas in future genetic collections.

Another important point is that, to date, no study has been able to fully understand the links between Chlamydia presence in a koala, Chlamydia load and clinical Chlamydial disease. This is an area of active research, but until these links are fully understood, results in terms of presence of chlamydia pathogen need to be viewed with caution (i.e. they might not reflect the level of disease threat).

2.4.3 Data set from 2018 genetic surveys

Since 2018, the genetic data generation and analysis has evolved. Technologies and methods have improved and, therefore, differences between 2018 results presented here might differ to the results presented in the 2018 Redlands Koala report. Therefore, all analyses using samples collected in 2018 were repeated to be consistent and allow data comparison between the two years.

2.4.4 Ear tissue samples from 2006/2007 for comparison

The ear tissue samples were stored in ethanol; however, some had already dried out when we handled them. Data quality was good overall, but DNA that has been stored for many years can be degraded. We filtered the data to minimise any effects thereof.

Note that there are differences in terms of sampling design that could have an effect on the results: 1) the tissue samples were collected year-round for two years, which makes this dataset different to our "snapshot" survey design, 2) the samples stem from sick or injured koalas

which means that healthy koalas are not included which could introduce a bias, and 3) geolocations have been estimated from addresses which means that the coordinates are only an approximation.

While we try to minimize biases, the fact that we are comparing data from scats and tissue needs to be highlighted. We ensured that the data quality was comparable, that only the same exact set of SNPs was used and that settings in analyses were consistent. Furthermore, research conducted within the DDC showed that genetic data from tissue and scat can be comparable as long as the scats are fresh (Schultz et al. 2018). Therefore, to the best of our current knowledge, the results presented from this analysis are robust.

3. Results

Note. This main body of the report focuses on giving general trends and analyses, and to preserve the flow, specific mapped or graphed results per locality are not included here, but given in Appendix 2.

3.1 Establishing where Redlands Coast koala populations are

3.1.1 Scat survey results

Koala scat surveys were undertaken between July 2020 and May 2021. Two to three teams consisting of a detection dog and a handler were deployed in parallel. Teams consisted mainly of: Caio Santos Neto with fresh scat detection dog Billie Jean, Dr Katrin Hohwieler with (all age) scat detection dog Baxter, Dr Romane Cristescu and Russell Miller with (all age) scat detection dog Maya and live koala detection dog Bear.

The detection dogs were worked by their handlers independently from each other and 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 worked on leash for safety reasons.

The Detection Dog Teams conducted a total of **262 surveys** across Redlands Coast mainland (Figure 3, see Appendix 2 for breakdown per locality). A total of 114 of the survey sites had the detection dogs identifying koala scat presence, with **238 instances of scat detection** (old and fresh scat, Figure 4, see Appendix 2 for breakdown per locality). Like in 2018, Thorneside was the only locality of the Redlands Coast where no survey detected signs of koalas in 2020/21. This should not be interpreted as a statement that koalas have been extirpated from Thorneside. Lack of detection may be due to any of the following: a) no fresh scats were present for the fresh scat detection dog to find, b) the areas covered by the scat detection dog had no scat present due to seasonal variation, or c) that areas of Thorneside currently used by koalas were not surveyed. However, due to the same result across the two survey years, there is increasing evidence that koalas are rare in this area of the Redlands. Note that a resident of Thorneside reported a koala sighting a couple of months prior to the surveys in that area.

Unlike 2018, the DDC could not find koala scats or other signs of koalas in the area of GJ Walter Park/Shore Street East/ Wharf Street in the 2020/21 survey period. In 2018, at least seven koalas, including mother-offspring pairs, were identified in this area. Therefore, it was surprising not to find evidence of (recent) koala presence there in 2020/21. However, members of the Koala Action Group (KAG) have informed the DDC about the presence of a female koala in this park that was likely taken to Australia Zoo Wildlife Hospital for treatment. Members of KAG and members of the public have observed a decreasing number of koalas and koala sightings in this area and, therefore, this area should be closely monitored. Regular monitoring of the area should be considered to investigate potential causes for this shift.

Similarly, no koalas/koala scats could be detected in Venman National Park in 2020/21, where four individual koalas were detected (genetically) in 2018. However, koalas in this bushland are likely to move more than in urban areas and might have not been detected in the 2020/21 surveys due to chance alone.

When scats were found, they were described in terms of age categories (Figure 4), allowing to differentiate between areas used very recently (within a few days, age categories 1 and 2), recently (within weeks, age category 3) or in the more distant past (months, age categories 4 and 5). A total of **242 samples of fresh scats** (note that multiple different fresh scat samples can be collected at the same scat detection location) were collected and extracted for DNA

(Figure 5), of which **158 scats were successfully genotyped for genetic analysis**. Almost all scats collected for genetic analysis were age category 1 or 2, which indicates the sites were used the same day as the survey (age category 1) or within the past few days (age category 2).

Comparison. A total of 262 surveys for koala scats were conducted in 2020/21. A total of 114 survey sites (44%) were positive for koala scat presences, with 242 samples of fresh scats collected for DNA extraction and genotyping. In comparison, in 2018 a total of 228 surveys were conducted on Redlands Coast mainland, of which 128 (56%) were positive for koala scats. A total of 329 scat samples collected for extraction, of which 236 were sent for genotyping. No koala scats were detected in GJ Walter Park area and Venman National Park in 2020/21, where scats were found in 2018.

Implications for Conservation. Koalas are still readily found in urban areas, where threats are heightened by the likelihood of interactions with vehicles and domestic dogs, as well as the lack of habitat connectivity (i.e., both at the canopy and forest levels), potentially forcing koalas to spend more time moving on the ground. Areas in which koalas were found in 2018, but not in 2020/21, may have experienced a decline in koala occupancy but it could also be explained by natural seasonal/temporal fluctuations.

Recommendation. Similar to recommendations in 2018, the protection of koalas in the Redlands Coast needs to include a strategic urban koala plan, as it appears not an insignificant proportion of the koala population is currently found in urban areas. Furthermore, areas where koala scats were found in 2018 but not in 2020/21 should be monitored more frequently to assess dynamics of koala occupancy in this area. If signs of koalas in these areas remain absent, investigations should be initiated to determine causes of the loss of koalas in these areas.

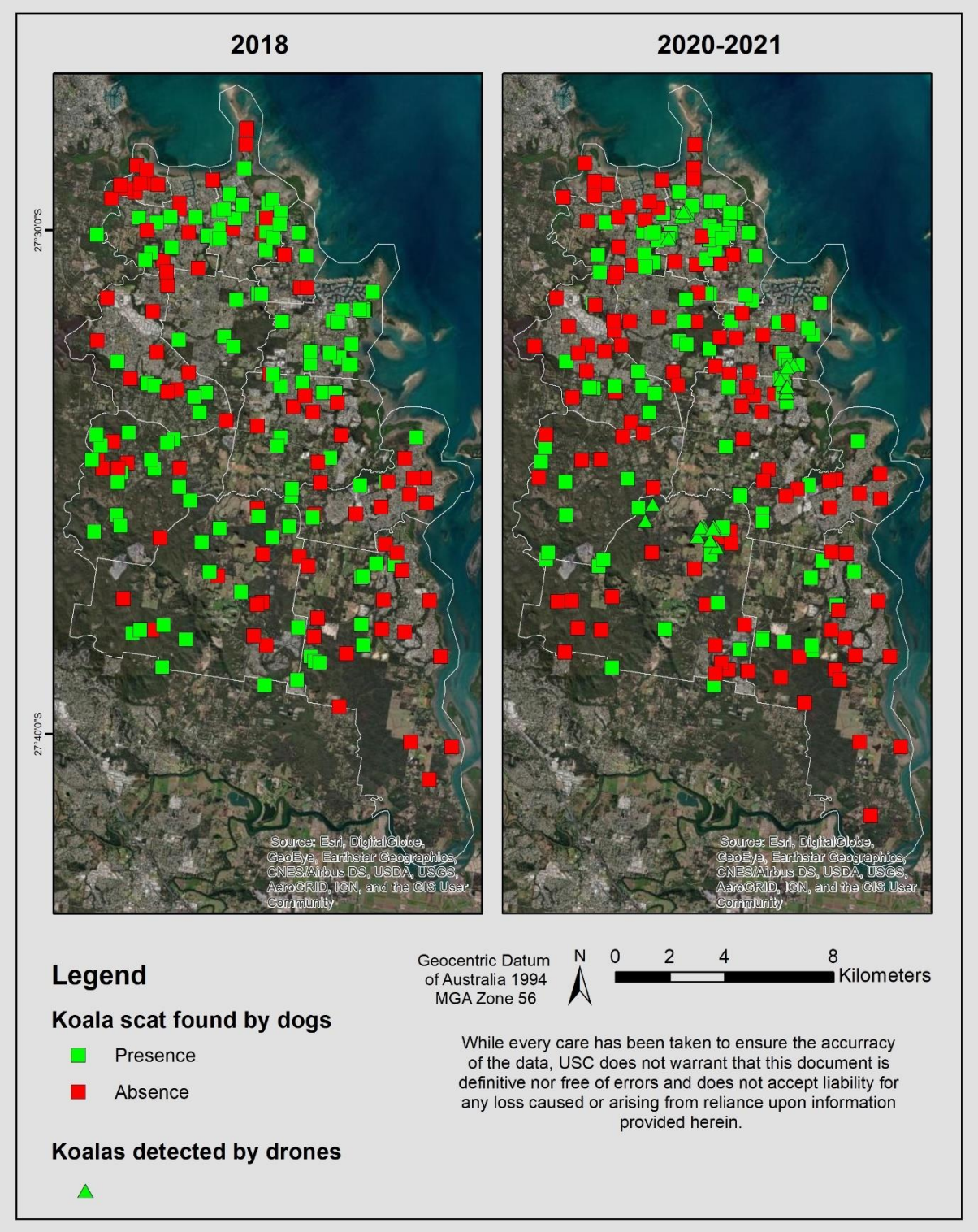

Figure 3: Sites where the Detection Dogs for Conservation teams surveyed across Redlands Coast mainland (N = 262 in 2020/21; N = 228 in 2018). Green points represent positive sites where koala scats were found. Red points indicate sites where koala presence could not be confirmed.

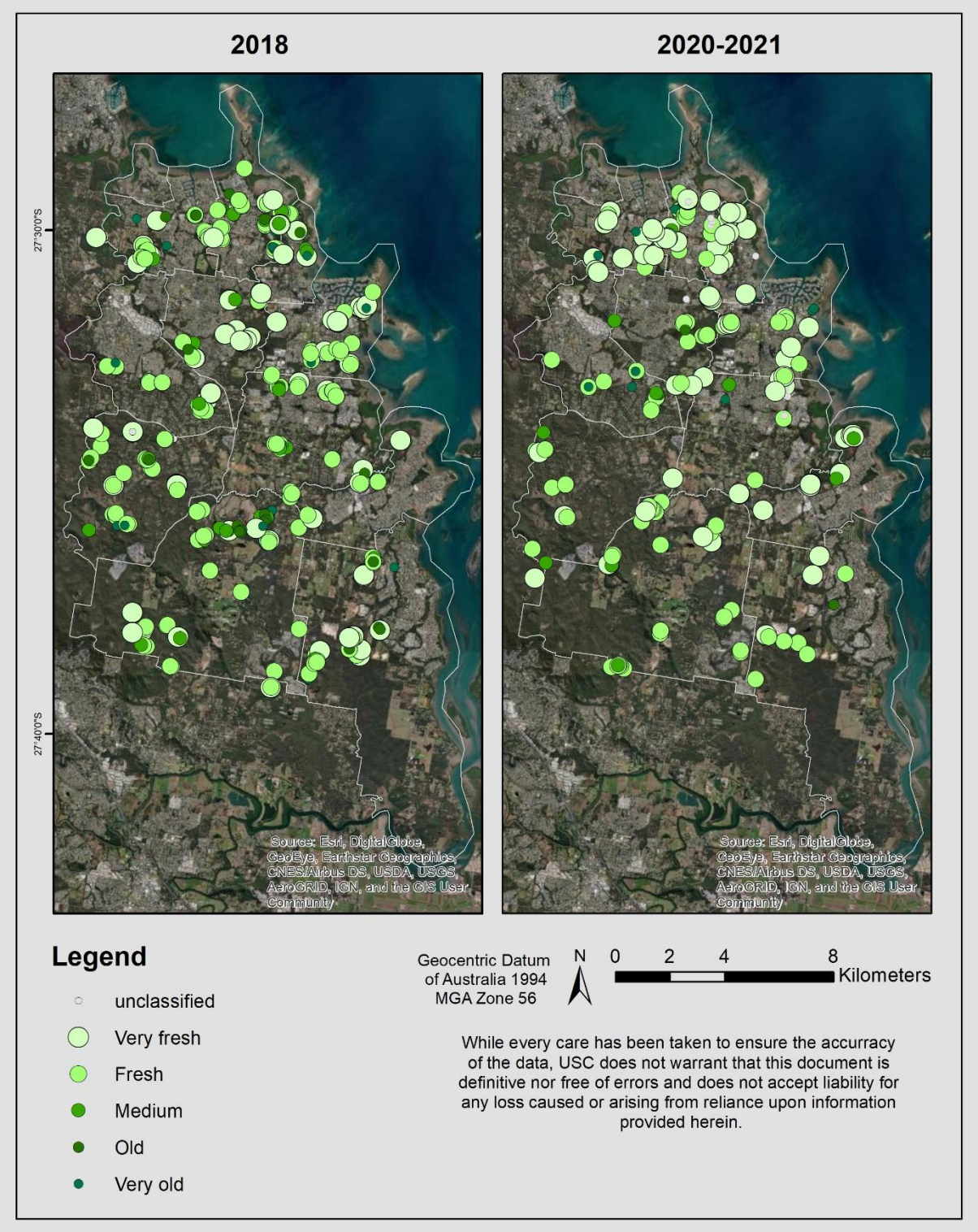

Figure 4: Map showing the instances of koala scat detection (broken down per scat age category) across Redlands Coast mainland in 2018 and 2020/21.

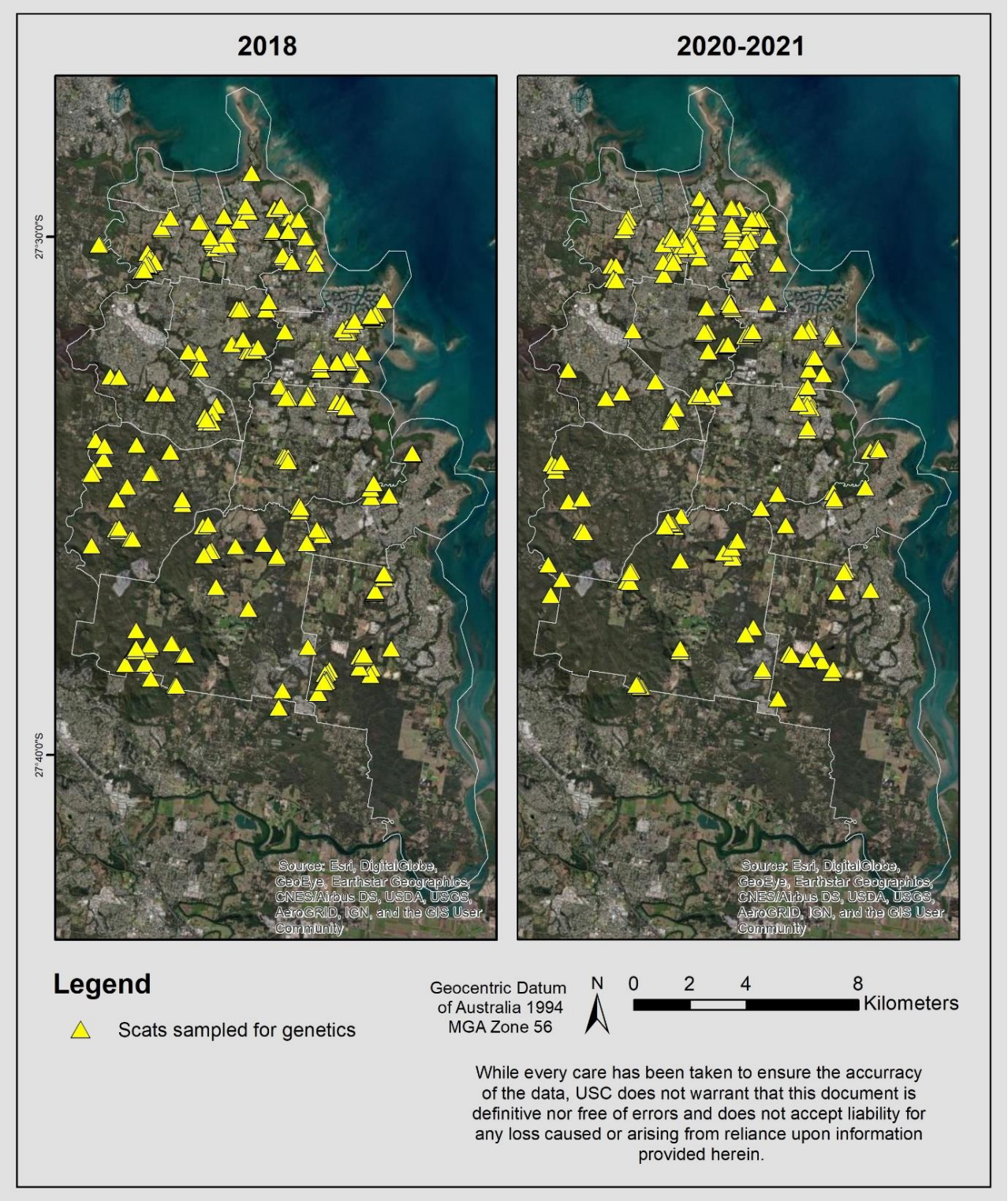

Figure 5: Map of genetic samples (N = 242 in 2020/21; N = 236 in 2018) collected by the Detection Dogs for Conservation teams across Redlands Coast mainland

3.1.2 Koala sightings

The handlers opportunistically spotted a total of **48 live adult koalas** during the surveys (Figures 6 and 7, see Appendix 2 for breakdown per locality) of which seven showed signs of Chlamydia (eye infection or wet bottom, Figure 8). Of the 48 koalas spotted, five were confirmed as females with a joey. One koala was found dead (cause unknown) and two skulls were found and identified as koala.

Most koalas were spotted in the northern localities of Redlands Coast mainland. No live koalas were spotted during the surveys in Capalaba or Sheldon despite finding scats, or Thorneside. This should not be interpreted as a statement that there are less koalas in these localities, as the survey was not designed to detect and count koalas (in addition, see Limitations regarding the unbalanced sampling effort between north versus south Redlands Coast). Two koalas were sighted during Mount Cotton surveys, which were both males with signs of Chlamydia infection (wet bottom). In Sheldon, one koala was found dead with the cause of death unknown and another two dead koalas were identified from skulls found during a survey. One koala sighting occurred in the north of Redland Bay (Giles Road Conservation Area). Multiple koala sightings occurred in Victoria Point, Thornlands, Cleveland, Birkdale, Wellington Point, Ormiston and Alexandra Hills.

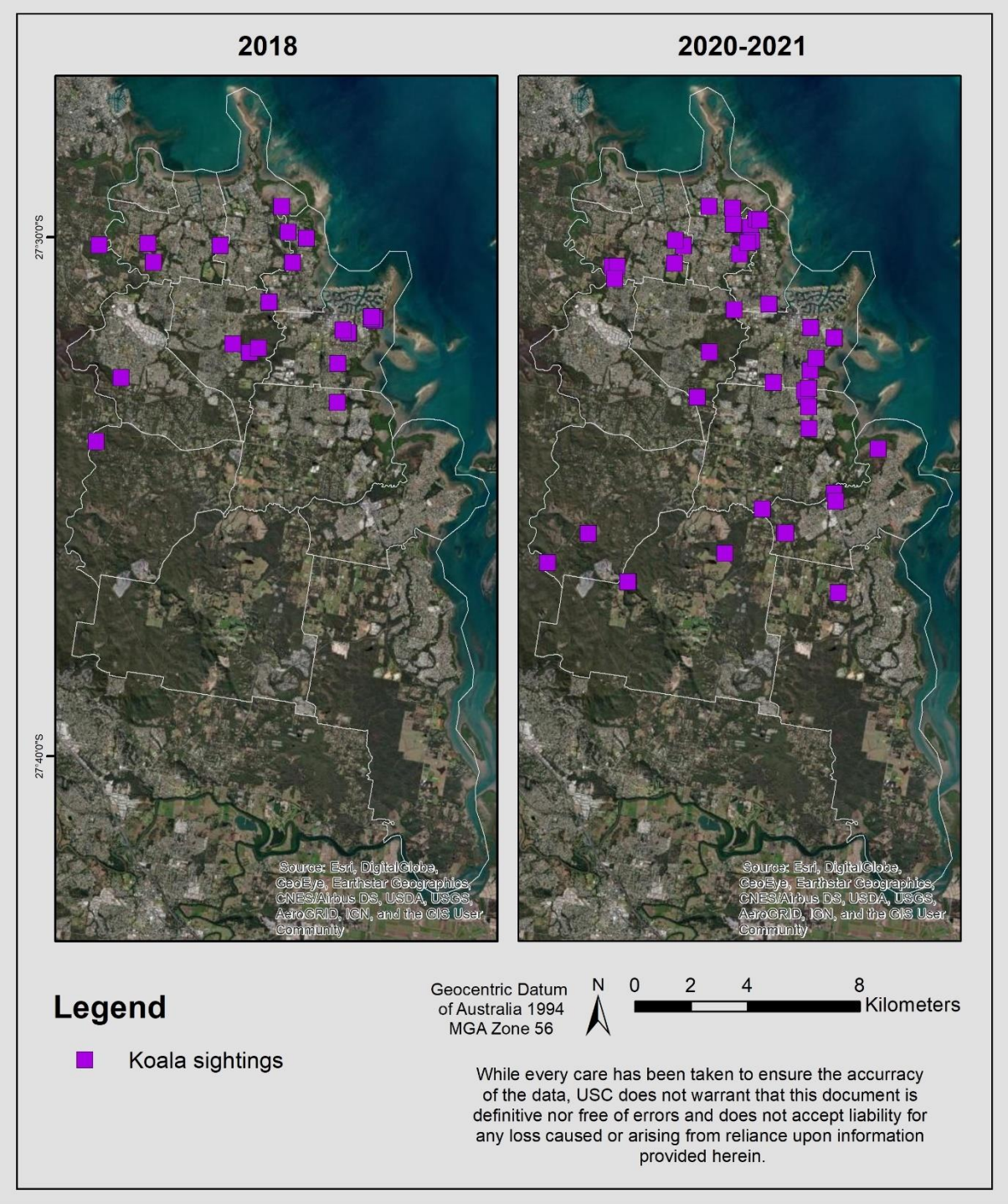

Figure 6: Map of koala (dead, alive and skulls) sightings (N = 48 in 2020/21; N = 26 in 2018), spotted by the Detection Dog Teams across Redlands Coast mainland

Figure 7: Healthy koala spotted in the field

Figure 8: Koala presenting signs of *Chlamydia* (conjunctivitis)

3.2 Koalas sampled both in 2018 and in 2020/2021

We genetically resampled 11 individuals (~9%) that were first sampled in 2018. Each resampled individual was sampled close to where it was sampled in 2018, as the following maps show. The maps in Appendix 3 show the positions of koalas sampled in 2018 and genetically recaptured in 2020/21.

3.3 Assessing how genetically connected populations of koalas are

Within the Redlands Coast mainland, **a total of 242 scat samples** were sent for genetic analyses of which 158 were successfully genotyped (Table 3). After three quality filtering steps (1st filtering done during quality check in the laboratory, 2nd filtering step done by genotyping provider, and 3rd filtering step done during SNP quality control, see Table 3, methods and detailed molecular methods in Appendix 1), we checked the remaining 144 scats for duplicates. Of these samples, 49 were found to be duplicated samples of 21 unique individuals. Following the removal of duplicate samples, **116 unique individuals remained for use** in all subsequent analyses (Table 3). Of these 116 individuals, 59 were found to be male and 57 were found to be female (Figure 9, see Table 3 for breakdown per locality). This resulted in a sex-ratio of 1:0.97 (male to female ratio). In comparison, in 2018, 124 individuals were successfully genotyped of which 55 were males and 69 were females. Therefore, the sex-ration of koalas on Redlands Coast mainland in 2018 was 1:1.25. We considered this a good sex ratio, as in natural population, a balanced sex ratio is good, however, a small bias of sex ratio towards female can sometimes be desirable, especially in very small or rapidly declining populations (Wedekind 2012). The observed change in sex-ratio is not worrying as it might simply be due to chance variation in sampling, however, population monitoring should continue to ensure that no real increase in male bias is actually occurring as a trend. Furthermore, it is important to monitor the sex of koalas that are killed, for example, by vehicle strikes or euthanised due to Chlamydia or reproductive disease. A sex bias in these events could have consequences for the Redlands Coast mainland koala population and, if found, should be investigated.

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for Conservation

Table 3: Table of sample sizes, at each location, for total number of scat samples successfully genotyped and final number of samples used for subsequent analyses (after quality filtering and duplicate identification), including their sex.

		Number of individuals after quality filtering and removing duplicates		
Locality	*Total scats successfully genotyped*	*Males*	*Females*	*Total*
Alexandra Hills	*14*	5	5	10
Birkdale	*28*	10	11	21
Capalaba	*8*	3	3	6
Cleveland	*14*	5	5	10
Mount Cotton	*28*	12	6	18
Ormiston	*13*	4	6	10
Redland Bay	*5*	3	2	5
Sheldon	*6*	1	3	4
Thornlands	*11*	5	4	9
Victoria Point	*9*	6	2	8
Wellington Point	*22*	5	10	15
Total	*158*	59	57	116

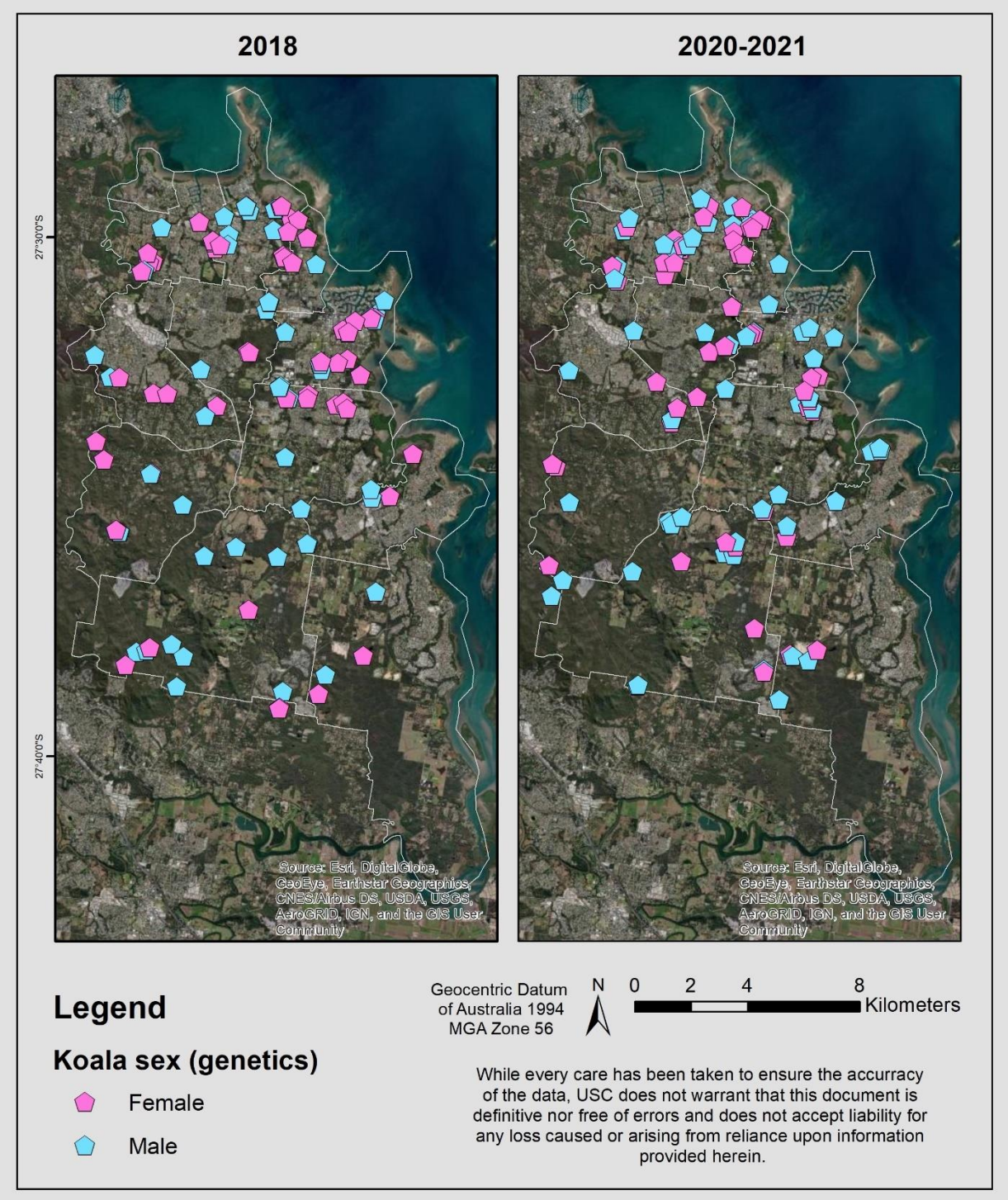


Figure 9: Individual koalas identified through scats, and their sex in 2018 (left, N = 55 males, 69 females) and 2020/21 (right, N = 59 males, 57 females).

3.3.1 Broad-scale genetic population differentiation

Using both a PCoA and a Bayesian clustering model implemented in fastSTRUCTURE, we found that the Redlands Coast mainland koala population is one single continuous population. This finding agrees with the previous results reported in 2018.

For comparison purposes, the analysis of the data collected in 2018 was redone using the same methods. The population was confirmed as one continuous population. For this reason, data from 2018 and 2020/21 were pooled and the same methods were applied to this pooled dataset to increase the robustness of this analysis. Again, the analysis showed one continuous population.

This is indicated by the PCoA which shows minor scattering of points (Figure 10A for 2020/21 and 10B for 2018), which means that there is minor differentiation but not significantly, as the sum of the two Principal coordinates is <10. Furthermore, when analysed with the data being pooled, there are no significant differences between individuals sampled in 2018 and 2020/21 (Figure 10C).

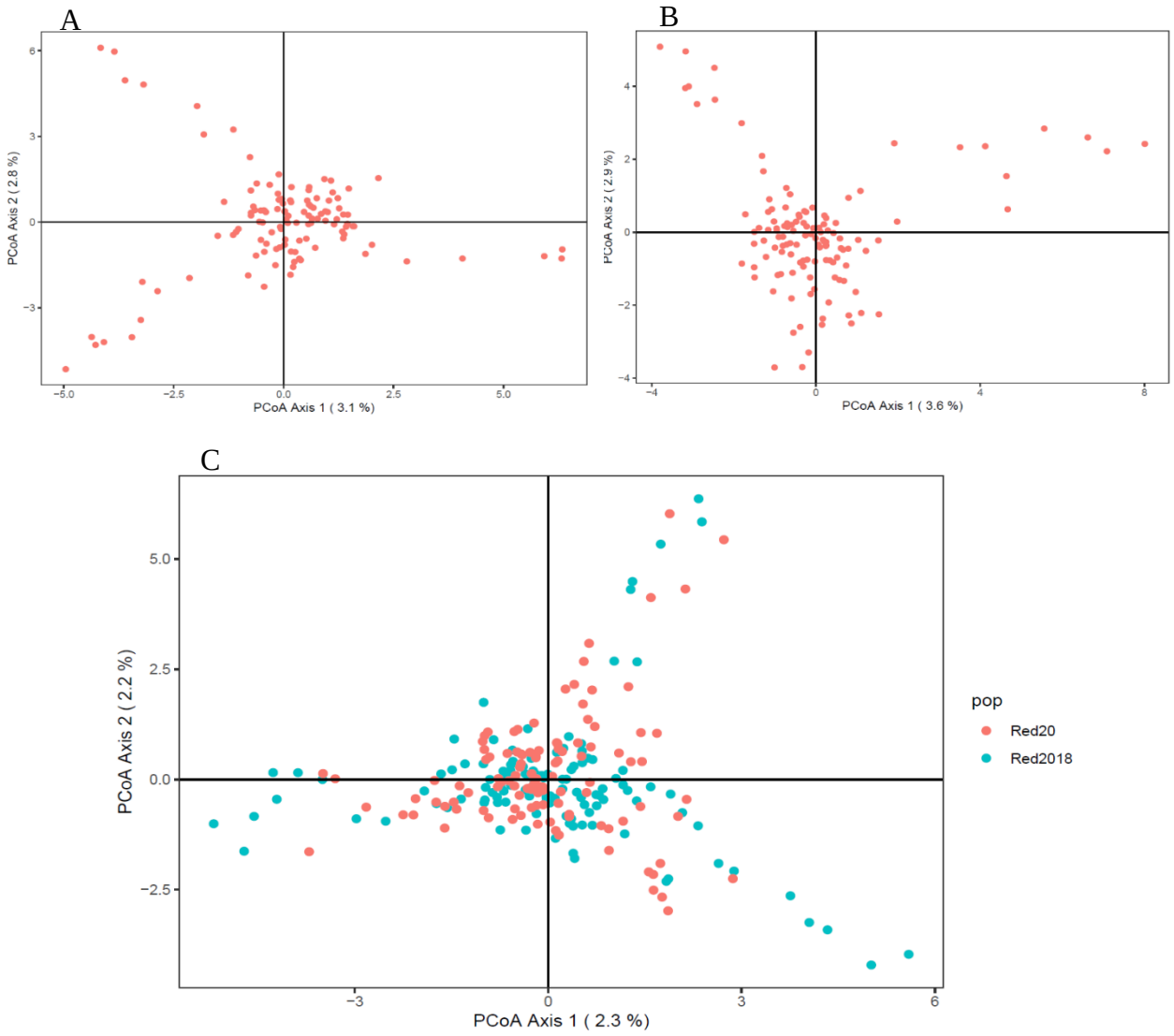
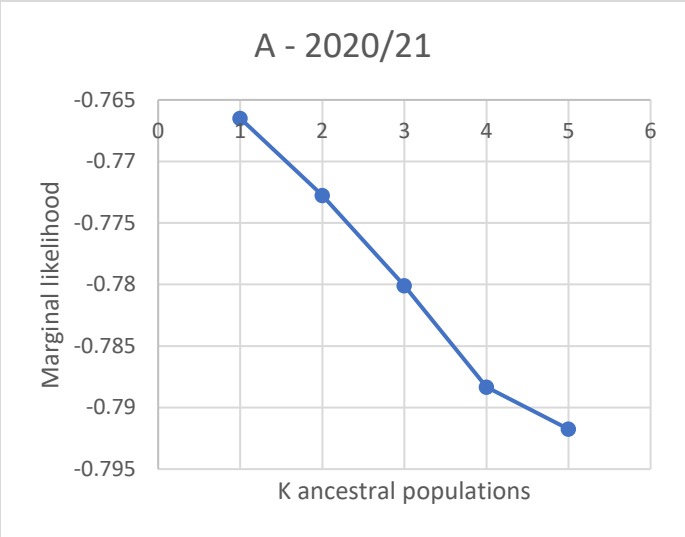
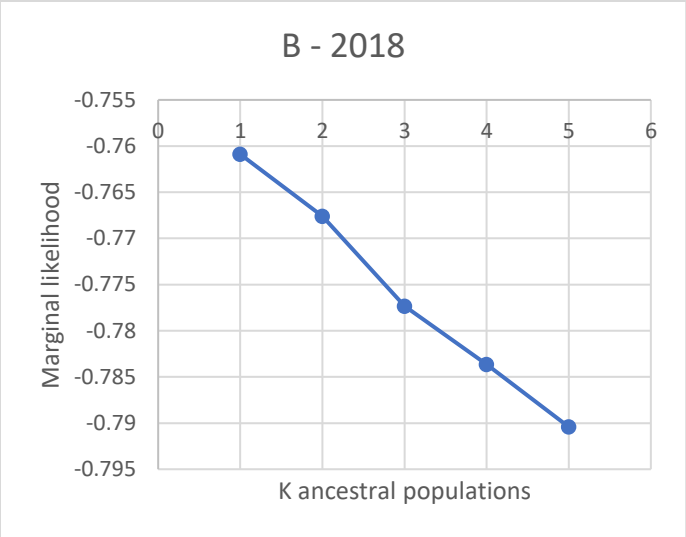


Figure 10: Scatter plot showing results of the PCoA, where population structure is shown by the distribution of points along the axes. Results are presented for the 2020/21 samples (A) as well as for 2018 (B) and the pooled data (2018 and 2020/21 together, C). Because the sum of the two axes is consistently <10, the PCoA results indicate one continuous breeding population.

The results from the structure analysis using fastSTRUCTURE are represented as a line plot that shows the likelihood for the population to be differentiated into *k* potential ancestral populations (Figure 11). We tested k to be from one to five. If there are significant ancestral structure in the population, the line (and therefore the likelihood) would peak at a given k. However, here, in all cases, the lines decline mostly continuously without peaking, indicating one continuous population.





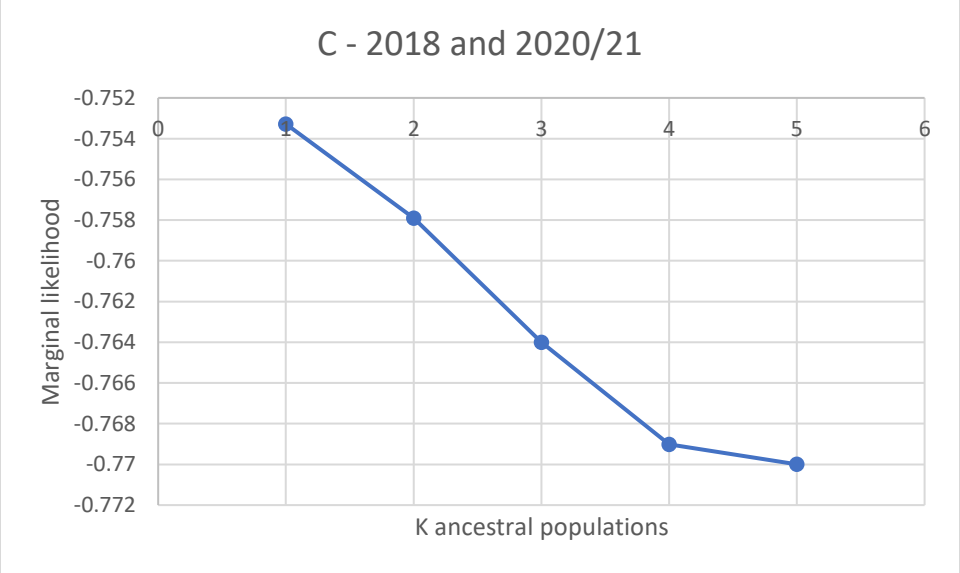


Figure 11: Line graph result from fastSTRUCTURE analysis, where population structure was tested for five potential populations (maximum likelihood K = 5). Results are presented for the 2020/21 samples (A) as well as for 2018 (B) and the pooled data (C - 2018 and 2020/21 together). If population structure existed, the line (and therefore the marginal likelihood) would peak at the most likely number of ancestral populations, which does not occur in this result.

Comparison. The broad scale population genetic characteristic of the Redlands Coast mainland koala population has been preserved over the past three years. Individuals sampled in 2018 and 2020/21 fall into the same genetic cluster when analysed together, so there has not been any differentiation between "generations" across years.

Implication for conservation. Not finding differentiation does not mean that koala genetic diversity has not changed, but that they have retained genetic similarity, which would be expected with sampling less than one generation apart.

Recommendation. Individuals sampled in 2018 and 2020/21 are likely from the same generation or would have a strong generation overlap. Therefore, pooling the data to increase sample size for some sample size sensitive analyses can be beneficial. This was done when no difference was detected between the two sampling periods, especially because pooling samples allows robust analyses at a smaller geographical scale (i.e. between localities, see below).

To get a full understanding of broad-scale population genetic patterns, however, sampling outside of the Redland City Council to include the whole of the Koala Coast would increase our understanding of gene flow in and out of the Koala Coast. This would be especially beneficial to investigate population source-sink dynamics.

3.3.2 Fine-scale genetic population differentiation and patterns

Using multiple methodologies, we found evidence for fine-scale and cryptic spatial genetic structuring in Redlands koalas. Although we propose some hypotheses as to why the fine-scale structure might exist, it must be noted that the genetic results can only detect structure, not explain its causes (i.e. causes are hypothetical only). In reality, what causes an effective barrier to gene flow, and the levels of permeability of different features across the landscape, are not clear for koalas. Although it is known that koalas can swim, travel across the ground through open areas, navigate urban landscapes, and cross multiple-lane highways - all would come at a cost to survival and therefore gene flow (Cristescu, unpublished data). The extent to which it does, however, is largely unknown.

Restricted gene flow on a local scale

Fine-scale genetic analyses requires the comparison of groups of individual koalas and, here, the groups were determined by locality boundaries. The analyses presented below can determine whether these artificial boundaries translate into genetic structuration of koalas into sub-populations. Even though these boundaries are artificial (man-made rather than ecological), these boundaries do reflect management boundaries and therefore are usually of relevance to decision makers.

This analysis depends on sample size and, because previous results reported here have shown that there are no major differences in population structure between individual koala samples in 2020/21 and 2018, we decided to use a pooled dataset for this analysis. A minimum sample size of N = 20 is usually recommended for calculating F_{ST} between populations. Therefore, we aimed to only include suburbs that had a sample size >20 after pooling the data.

We found significant genetic differentiation between all locations that were included in the analysis (Table 4), though the F_{ST} value in itself was consistently small. Furthermore, the number of migrants (Nm) between each pairwise comparison indicated that gene flow does exist across

each suburb of Redlands Coast mainland. Overall, these significant but small genetic differentiation measures highlight that gene flow exists but might experience restrictions between Birkdale, Cleveland, Mount Cotton and Wellington Point. Interestingly, Birkdale, Cleveland and Wellington Point are geographically in close proximity. Finding significant differentiation in those norther suburbs could indicate that events, such as the increasing urban footprint in the north of Redlands Coast mainland, may be resulting in restricted gene flow. However, analysis should be repeated with all suburbs included, provided that a sufficient sample size can be used.

Table 4: Significant pairwise genetic differentiation (F_{ST}) measures and its associated P value, and the number of migrants (Nm) between pairwise locations on Redlands Coast mainland using a minimum of 24 individuals per population and 1158 loci. Note that populations here are based on locality boundaries and do not reflect identified genetic entities.

Population 1	*Population 2*	*F_{ST}*	*P value*	*Nm*
Birkdale	*Cleveland*	*0.019*	*0.002*	*13.13*
Birkdale	*Mount Cotton*	*0.015*	*0.001*	*16.73*
Birkdale	*Wellington Point*	*0.013*	*0.011*	*18.94*
Cleveland	*Mount Cotton*	*0.012*	*0.002*	*19.95*
Cleveland	*Wellington Point*	*0.017*	*0.001*	*14.79*
Mount Cotton	*Wellington Point*	*0.015*	*0.008*	*16.97*

Cryptic genetic patterns of population differentiation

Whilst the Redlands Coast mainland population is one continuous breeding population (i.e. no broad scale genetic structuring found through PCoA or fastSTRUCTURE analysis), we did find evidence of cryptic spatial structuring based on the sPCA analysis. The sPCA for koalas in Redlands Coast mainland showed one significant (P = 0.001) *global* component with the

eigenvalues abruptly decreasing after the first component, indicating a significant contribution of the first PC to spatial structure. No significant *local* structure was identified.

The sPCA plot of the lagged scores of the first global principal component divides the koala population into two genetic clusters (Figure 12A). This division could have resulted from natural or historical circumstances. The separation is coinciding with State Route 21 (namely, Mount Cotton Road, Duncan Road and Boundary Road) but further investigation is needed to account for all landscape variables, e.g. creek lines that could explain this separation. Traffic along this particular road, however, has increased significantly over the past decades, as indicated by average annual daily traffic recorded in Redlands Coast mainland (Appendix 4 Figure 1). Data on vehicle-koala collisions from the wildlife hospital database, from 1997-2017, show that this particular road is a hotspot for such incidences in the Redlands (Appendix 4 Figure 2).

The same cryptic structure can be found when using samples collected in 2018 (Figure 12B), which indicates that this pattern is consistent. Furthermore, this allowed us to pool data from 2018 and 2020/21 to include more individuals in this analysis (Figure 12C).

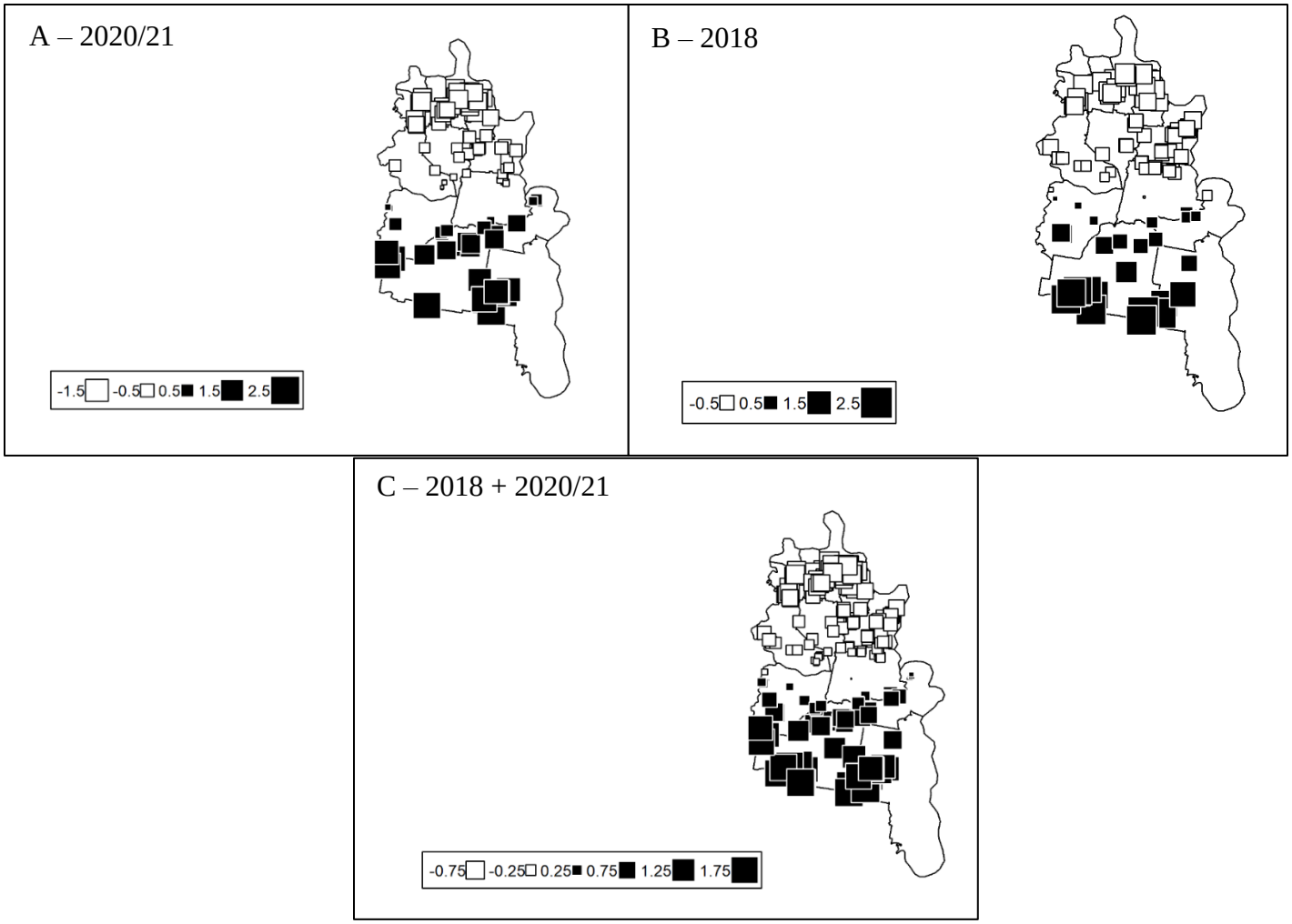


Figure 12: Map of Redlands City Council showing sPCA plot of the first global principal component, dividing the koala population into two genetic clusters – depicted in white vs black squares. Each square represents an individual koala and large black squares are most differentiated from large white squares, but small squares indicate less differentiation. Results are shown for individuals sampled in 2020/21 (A), 2018 as a comparison (B) and both years combined (C).

Fine-scale spatial distribution of related koalas in close proximity across Redlands Coast mainland

Results of average relatedness amongst koalas within a 600 metres radius are depicted in Figure 13 as a heatmap. In both 2020/21 and 2018, there are clusters of related individuals in close proximity that are significantly more related than neighbouring koalas in other parts of the landscape. Overall range relatedness in 2020/21 was higher, with a mean of 0.30 (SD* = 0.12), compared to 2018, with mean of 0.18 (SD = 0.23) (Figure 13). It has to be noted that this analysis is subject to variation as, by chance, more pre-dispersal mum-joey pairs could be sampled in one year compared to another year or seasonally. Therefore, differences are expected when comparing outputs from different years. However, overall trends can become apparent when repeated over multiple sampling periods. Pooling data is discouraged for this analysis, as it would not reflect koalas present in the landscape at the same time.

While the maps presenting range relatedness look different between years, in both 2018 and 2020/21, the northern and more urbanised suburbs contain a larger number of clusters with highly related individuals. This can be a signal for restricted dispersal opportunities through the urban matrix. Note that interpolations can only occur where sufficient data points are available. Due to underrepresented sampling in the southern area of Redlands Coast mainland, drawing conclusions on these areas is more challenging. However, this bias is consistently present in both 2018 and 2020/21. Despite the results being influenced by sampling density, the comparison of this analysis to the data from 2006 shows a clear increase in overall relatedness and patterns (Figure 14).

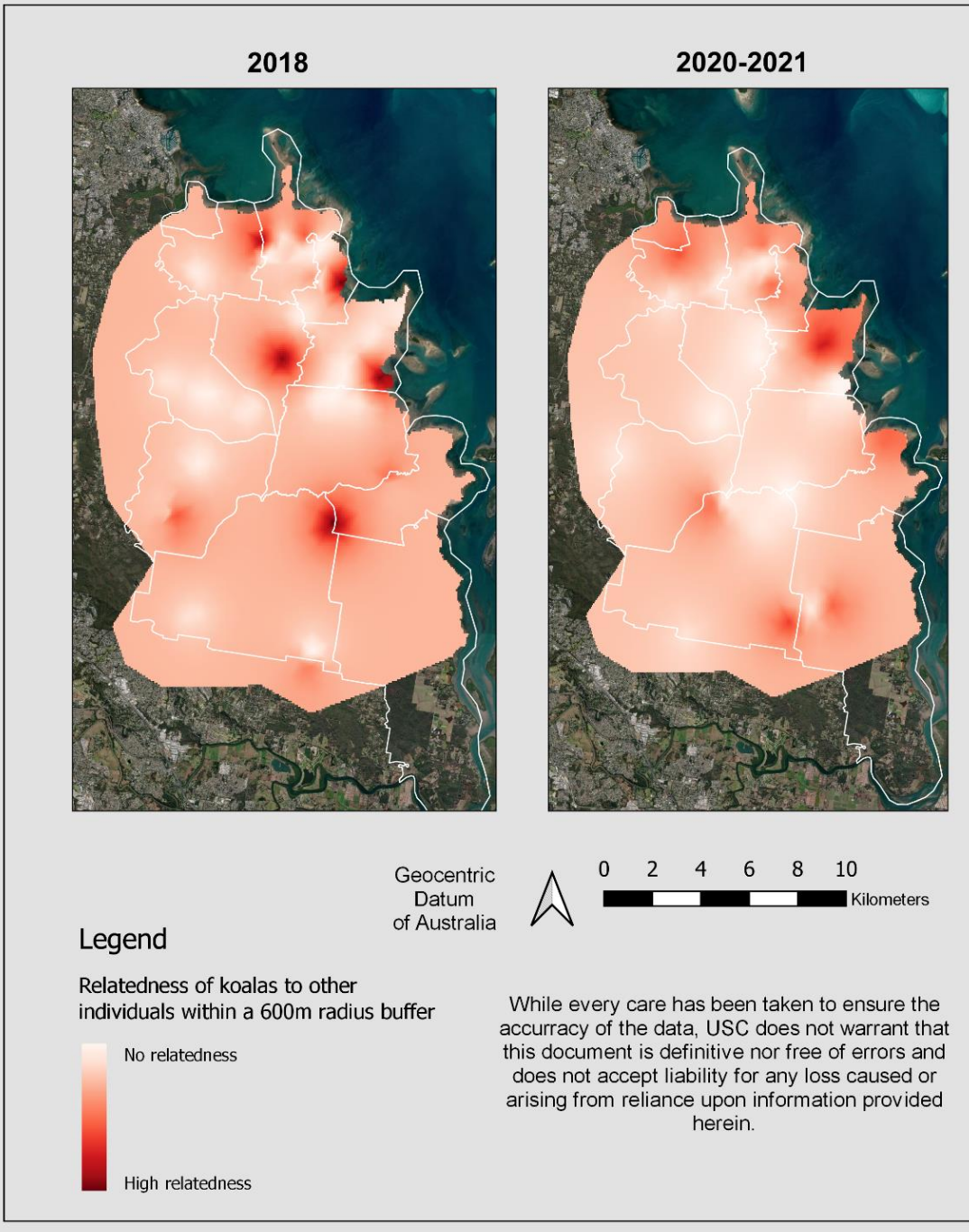

2018
2020-2021
Geocentric
Datum
of Australia
0 2 4 6 8 10
Kilometers
Legend
Relatedness of koalas to other
individuals within a 600m radius buffer
No relatedness
High relatedness
While every care has been taken to ensure the accurracy of the data, USC does not warrant that this document is definitive nor free of errors and does not accept liability for any loss caused or arising from reliance upon information provided herein.

Figure 13: Map of Redlands City Council showing results from the INLA analysis where the average relatedness of a focal individual to non-focal individuals within a buffer with a 600 metres radius was assessed. Results are interpolated across the landscape. Dark red areas contain individuals that are more related to individuals in their surrounding than would be expected in a randomly breeding population.

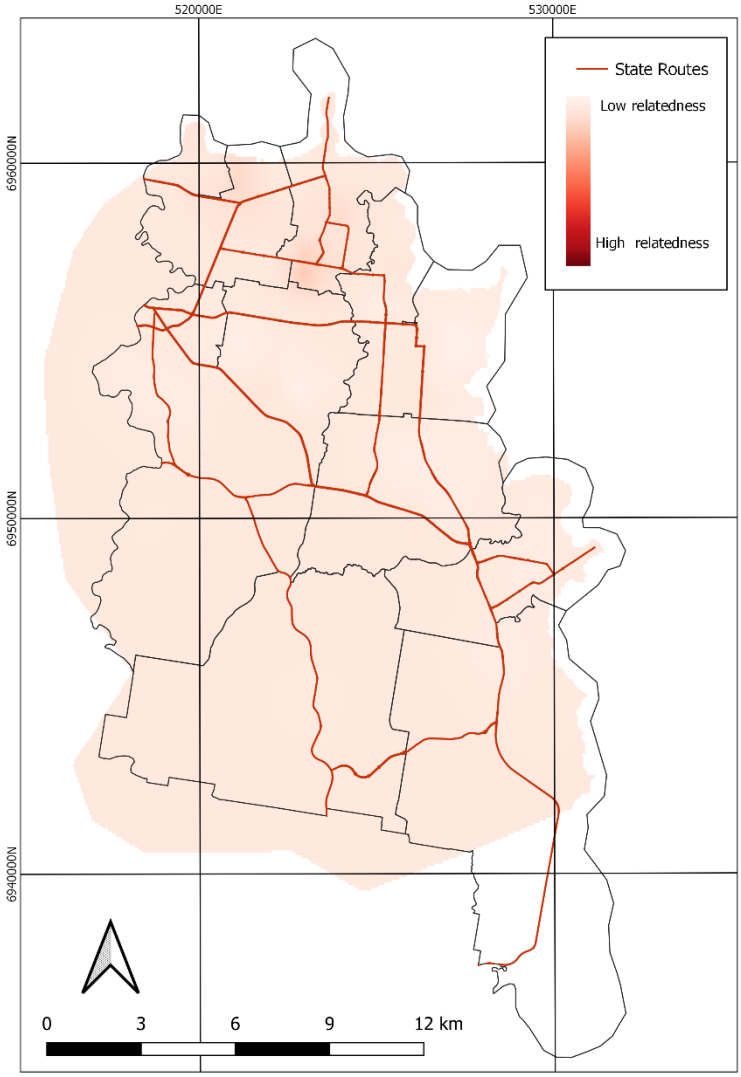


Figure 14: Map of Redlands City Council showing results from the INLA analysis using genetic samples from 2006 and 2007. Here, the average relatedness of a focal individual to non-focal individuals within a buffer with a 600 metres radius was assessed. Results are interpolated across the landscape. Dark red areas contain individuals that are more related

to individuals in their surrounding than would be expected in a randomly breeding population.

Comparison. Fine-scale genetic patterns are present in the Redlands Coast mainland koalas. Thanks to the repeat sampling in 2020/21, we have sufficient samples to pool data for a fine-scale assessment of genetic differentiation (F_{ST}) between suburbs, indicating existing but limited gene flow between suburbs. Cryptic structure exists consistently across years, with an apparent division of the population that coincides with State Route 21. Distribution of koalas with highly related individuals in their proximity differs between years but, in both years, such clusters appear predominantly in the more urbanised suburbs (Cleveland, Ormiston, Wellington Point, and Birkdale).

Implications for Conservation. Where dispersal and gene flow are restricted, the risk of inbreeding is heightened. Together with the results from the genetic diversity analysis (see below), there is concern that koalas in Redlands Coast mainland might experience ongoing inbreeding events which would decrease their future evolutionary potential and, with that, the ability to adapt to a changing environment.

Recommendation. Maintaining and improving connectivity will be key to preventing the identified high risk of inbreeding depression, especially within the urban footprint. Assessing the extent of roads as a barrier to gene flow could help to better understand the genetic patterns found here, also enabling mitigation strategies to be targeted where most effective.

3.4 Assessing how healthy Redlands Coast mainland koalas are

3.4.1 Genetic diversity

An overview of genetic diversity measures and results is presented in Table 5. Overall, and similar to other koala genetic studies (Kjeldsen et al. (2018), Table 6), we found that observed heterozygosity (H_O) was lower than expected heterozygosity (H_E). However, whilst observed heterozygosity was found to be ~9% lower than expected heterozygosity in Kjeldsen et al. (2018), here, with the individuals sampled in 2020/21 we found observed heterozygosity to be ~38% lower than expected heterozygosity (Table 5). This large difference between observed and expected heterozygosity is likely due to inbreeding and genetic drift occurring within the population. In comparison, the same analysis using individuals that were sampled in 2018 resulted in an expected heterozygosity of ~33% higher than observed heterozygosity.

Analyses were repeated separately, for each sampling period. Additionally, for the assessment of measures per suburb, samples from both sampling periods were pooled to increase sample size. Heterozygosity calculations are also more accurate the more samples are included; therefore, we ran the genetic diversity analysis including all samples together and found that overall, expected heterozygosity was ~35% higher than observed heterozygosity.

This population, part of what is commonly referred to as the Koala Coast population, is thought to have been relatively isolated from the rest of SEQ (Lee 2009). As a whole, the Koala Coast population has lower genetic diversity than the rest of SEQ mainland (Lee 2009, Lee et al. 2010).

Table 5: Measures of genetic diversity. N = number of samples used for genetic analyses, I = Shannon's information index, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, N_e = effective population size, IR = internal relatedness. SE = standard error, SD = standard deviation. To aid interpretation, under each genetic measure, we explain whether for genetic health, a higher or lower figure is better.

Population	*N*	*I (SE)*	*H_O (SE)*	*H_E (SE)*	*F_{IS}*	*N_e*	*IR (SD)*
		Higher is better	*Higher is better*	*Higher is better*	*Lower is better*	*Higher is better*	*Lower is better*
Redlands 2020/21	*116*	*0.481 (0.005)*	*0.229 (0.003)*	*0.316 (0.004)*	*0.275*	*90.9 (89.3 – 92.1)*	*0.313 (0.264)*
Redlands 2018	*124*	*0.486 (0.006)*	*0.241 (0.004)*	*0.321 (0.005)*	*0.249*	*75.9 (74.9 – 77.2)*	*0.280 (0.269)*
Redlands 2018 & 2020/21	*227*	*0.486 (0.005)*	*0.237 (0.003)*	*0.320 (0.004)*	*0.259*	*108.8 (107.7 – 110.1)*	*0.293 (0.268)*
Alexandra Hills	*14*	*0.460 (0.006)*	*0.258 (0.005)*	*0.305 (0.005)*	*0.153*	*NA*	*0.21 (0.20)*
Birkdale	*35*	*0.456 (0.006)*	*0.227 (0.004)*	*0.301 (0.005)*	*0.246*	*NA*	*0.34 (0.29)*
Capalaba	*13*	*0.444 (0.007)*	*0.206 (0.005)*	*0.295 (0.005)*	*0.300*	*NA*	*0.40 (0.26)*
Cleveland	*37*	*0.463 (0.006)*	*0.223 (0.005)*	*0.307 (0.005)*	*0.305*	*NA*	*0.37 (0.27)*
Mount Cotton	*36*	*0.478 (0.006)*	*0.265 (0.004)*	*0.315 (0.004)*	*0.161*	*NA*	*0.22 (0.25)*
Ormiston	*15*	*0.433 (0.007)*	*0.207 (0.005)*	*0.288 (0.005)*	*0.281*	*NA*	*0.39 (0.27)*
Redland Bay	*9*	*0.437 (0.007)*	*0.232 (0.005)*	*0.290 (0.005)*	*0.201*	*NA*	*0.30 (0.26)*
Sheldon	*13*	*0.463 (0.006)*	*0.249 (0.005)*	*0.307 (0.005)*	*0.190*	*NA*	*0.28 (0.27)*
Thornlands	*17*	*0.462 (0.006)*	*0.271 (0.005)*	*0.305 (0.005)*	*0.112*	*NA*	*0.18 (0.26)*
Victoria Point	*14*	*0.445 (0.007)*	*0.238 (0.005)*	*0.295 (0.005)*	*0.192*	*NA*	*0.26 (0.29)*
Wellington Point	*24*	*0.449 (0.006)*	*0.244 (0.005)*	*0.296 (0.005)*	*0.176*	*NA*	*0.27 (0.25)*

Table 6: Genetic diversity established through DArTseq genotyping technology in wild koala populations across eastern-Australia. n = sample size, Ho = observed heterozygosity, He = expected heterozygosity, %PL = percent of polymorphic loci, FIS = inbreeding coefficient and IR = internal relatedness. Table taken from Kjeldsen et al. (2018). Note that these measures cannot be directly compared with the measures presented in Table 5, but are given to enable the relative comparison of the Koala Coast (which encompasses Redlands Coast mainland) to other koala populations across Australia

Population	*n*	Bioregion	He (corr) ± SE	Ho ± SE	% PL	Fis ± SE	IR ± SD
Magnetic Island (MI)	20	BBN	0.14 ± 0.00	0.14 ± 0.00	47.8%	0.01 ± 0.00	0.57 ± 0.03
St Bees Island (SB)	21	CMC	0.14 ± 0.00	0.14 ± 0.00	53.8%	−0.03 ± 0.00	0.55 ± 0.14
St Lawrence (SL)	18	CMC	0.18 ± 0.00	0.16 ± 0.00	60.7%	0.07 ± 0.00	0.55 ± 0.10
Maryborough (M)	14	SEQ	0.15 ± 0.00	0.14 ± 0.00	45.2%	0 ± 0.00	0.57 ± 0.04
Moreton Bay (MB)	8	SEQ	*	*	*	*	*
Koala Coast (KC)	20	SEQ	0.17 ± 0.00	0.16 ± 0.00	59.6%	0.03 ± 0.00	0.55 ± 0.06
Ipswich (I)	22	SEQ	0.19 ± 0.00	0.17 ± 0.00	68.9%	0.07 ± 0.00	0.50 ± 0.07
Lismore (LI)	77	SEQ	0.17 ± 0.00	0.15 ± 0.00	74.5%	0.11 ± 0.00	0.55 ± 0.05
Woolgoolga (W)	9	NNC	*	*	*	*	*
Gunnedah (GD)	57	BBS	0.16 ± 0.00	0.15 ± 0.00	64.6%	0.06 ± 0.00	0.49 ± 0.10
Port Macquarie (PM)	85	NNC	0.18 ± 0.00	0.17 ± 0.00	80.9%	0.06 ± 0.00	0.58 ± 0.19
Blue Mountains (BM)	19	SYB	0.20 ± 0.00	0.18 ± 0.00	68.6%	0.1 ± 0.00	0.63 ± 0.11
Campbelltown (CT)	119	SYB	0.15 ± 0.00	0.14 ± 0.00	82.5%	0.03 ± 0.00	0.53 ± 0.11
Southern Highlands (SH)	25	SYB	0.18 ± 0.00	0.15 ± 0.00	64.0%	0.08 ± 0.00	0.56 ± 0.05
South Gippsland (SG)	17	SCP	0.11 ± 0.00	0.1 ± 0.00	37.7%	−0.01 ± 0.00	0.70 ± 0.09
Strzelecki (SZ)	19	SCP	0.11 ± 0.00	0.11 ± 0.00	39.4%	−0.01 ± 0.00	0.68 ± 0.09
French Island (FI)	39	SCP	0.10 ± 0.00	0.11 ± 0.00	49.1%	0.09 ± 0.00	0.80 ± 0.15
Cape Otway (CO)	28	SCP	0.12 ± 0.00	0.11 ± 0.00	53.7%	0.08 ± 0.00	0.75 ± 0.04
Hamilton (H)	4	VIM	**	**	**	**	**
Mt Lofty (ML)	23	EYB	0.13 ± 0.00	0.12 ± 0.00	60.2%	0.01 ± 0.01	0.76 ± 0.12
Kangaroo Island (KI)	14	KAN	0.13 ± 0.00	0.09 ± 0.00	44.6%	0.19 ± 0.01	0.83 ± 0.11

3.4.2 Effective population size

We found low effective population sizes (N_e) compared to conservation recommendations (Mace and Lande 1991, IUCN 2012, Frankham et al. 2014) for Redlands Coast mainland in both 2020/21 (Ne = 90.9, 95% CI = 89.3 – 92.1) and 2018 (Ne = 75.9, 95% CI = 74.9 – 77.2, Table 5). When

data from both years were pooled, Ne reached 108.8 (95% CI = 107.7 – 110.1). When compared to Kjeldsen et al. (2016; Table 7), the effective population sizes found across the Redlands Coast mainland and across years were still very low. It is important to note that the N_e for Koala Coast (which includes Redlands Coast mainland) represented in Kjeldsen et al. (2016) (921-infinite) is not directly comparable to the N_e found here because it has a different extent both in space (again, Koala Coast includes parts of Brisbane and Logan Councils) and time (koala samples in Kjeldsen et al. (2016) were opportunistically sampled, likely over many years).

Small effective population sizes do heighten the risk of extinction – it means that, all things being equal, these populations are more vulnerable and need to be treated with more caution. 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, it is recommended that minimum effective population sizes of at least 100 be maintained (Mace and Lande 1991, Frankham et al. 2014). Because the genetically effective population size is frequently <10% of the actual number of individuals in a population (Frankham 1995b), 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).

A final comment on the estimates of effective population size given here is that the method used, called linkage disequilibrium method, can produce an over-estimate of N_e for 1 or 2 generations if

the population is experiencing a current steep decline – this is because linkage disequilibrium can require several generations to decay and therefore current estimate can reflect population effective size of the past (Luikart et al. 2010). The DDC was able to source historical samples through collaboration, and genotyped 266 samples from 2006-2007 Redlands Coast mainland koalas. Results will be presented in final report.

Table 7: Genetic diversity established through double digest restriction-associated SNP sequencing in wild koala populations across Queensland, NSW and Victoria. n = sample size, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = inbreeding coefficient, IR = internal relatedness and Ne_{LD} = effective population size calculated using linkage equilibrium. Table taken from Kjeldsen et al. (2016).

State	Location	n	Ho	He	Fis ($P < 0.01$)	IR (±SD)	Ne_{LD} (95 %CI)
QLD	St Bees Island	19	0.29	0.35	0.23	0.29 (±0.15)	Infinite (∞)
QLD	St Lawrence	19	0.26	0.30	0.20	0.21 (±0.11)	Infinite (∞)
QLD	Koala Coast	24	0.22	0.30	0.32	0.42 (±0.29)	Infinite (921.20–∞)
QLD	Ipswich	23	0.27	0.31	0.19	0.26 (±0.16)	Infinite (∞)
NSW	Port Macquarie	45	0.23	0.28	0.21	0.25 (±0.15)	116.8 (109.8–124.6)
NSW	Campbelltown	9	0.27	0.33	0.27	0.34 (±0.27)	2.7 (2.4–3.2)
VIC	South Gippsland	19	0.24	0.30	0.27	0.31 (±0.34)	Infinite (∞)
VIC	Cape Otway	13	0.24	0.25	0.11	0.20 (±0.11)	46.7 (40.8–54.4)

3.4.3 Inbreeding coefficient

Aligning with the IUCN's predictions about low effective population sizes (outlined in introduction), we found a high inbreeding coefficient for the Redlands Coast mainland population in 2020/21 (F_{IS} = 0.275, Table 5). In 2018, F_{IS} was estimated to be 0.249 and thus slightly lower than in the latest sampling period. Overall, inbreeding coefficients for Redlands Coast mainland koalas were similar to those found in wild koala populations across eastern-Australia (Kjeldsen et

al. 2018). We found the inbreeding coefficient of urban koalas to be 30.6% higher than that of non-urban koalas (F_{IS} = urban footprint: 0.290; non-urban footprint: 0.222). This difference between urban and non-urban koalas has decreased compared to 2018, where F_{IS} was ~38% higher in urban koalas compared to non-urban koalas (F_{IS} = urban footprint: 0.265; non-urban footprint: 0.192). This does not reflect an improvement, as F_{IS} of koalas in both the urban and non-urban footprint have increased from 2018 to 2020/21, just that inbreeding coefficients between urban and non-urban have become more similar.

3.4.4 Internal relatedness

The IR values found across the Redlands Coast mainland in 2020/21 (IR = 0.313, SD = 0.264, Table 5) are lower than those values previously reported in Kjeldsen et al. (2018) (Table 6). While IR values of individuals sampled in 2020/21 were overall higher than in 2018 (IR = 0.280, SD = 0.269), other analyses indicated that there is little genetic difference between koalas sampled in 2020/21 and those sampled in 2018. Therefore, pooling them provided a larger sample size to calculate IR across both years (IR = 0.293, SD = 0.268). While the average IR is still higher than would be expected in a randomly breeding population, it is much lower than previously reported in Kjeldsen et al. (2018). The slight increase found from 2018 to 2020/21 (+11.8%) indicates that we might be seeing an increase in inbreeding, which is further supported by the increase in F_{IS} in those 2-3 years. The population was sampled within less than a generation, therefore, this might be an incidental fluctuation. Nonetheless, this finding gives reason to continue monitoring this population and increase efforts to promote geneflow to prevent genetic erosion.

In 2020/21, IR was 19% higher in koalas in the urban footprint of Redlands compared to those in non-urban areas (IR: urban footprint = 0.331, non-urban footprint = 0.278). This result is consistent with findings in 2018 (IR: urban footprint = 0.265, non-urban footprint = 0.192), though the difference was higher, with 38% higher IR in urban compared to non-urban koalas. This result is consistent with the previously reported F_{IS} results: overall values are increased for both urban and non-urban measures but more for non-urban measures, which decreased the relative difference.

3.4.5 Chlamydia

Chlamydia status of each individual was assigned using a threshold (>9 SNPs detected out of the 30 Chlamydia-specific probes) to classify an individual as having the pathogen detected. Of the 116 individuals that were sampled in the 2020/21 surveys, the Chlamydia pathogen was detected for 44 (38%), of which 26 were female and 18 were male koalas (Figure 15). This is a slight increase compared to the results from 2018, where the Chlamydia pathogen was detected in 43 out of 124 individuals (35%). Details provided in Table 8 show the number of individuals for which Chlamydia pathogen was detected in each suburb of Redlands Coast mainland, with data pooled for 2018 and 2020/21. Overall, the pathogen was more frequently detected for females than males. Although not tested statistically, an interesting observation is that highly urbanised suburbs seem to have lower Chlamydia prevalence than less urbanised suburbs. If confirmed, this could have multiple reasons, one being a potential bias towards people finding, and therefore treating, sick koalas in urban areas. This matter is further reviewed in the discussion.

Table 8: Table detailing the number of individuals where Chlamydia was detected in each population and locality.

	Chlamydia positive individuals 2018 & 2020/21			*Percentage of Chlamydia positive out of individuals sampled*
Locality	***Total***	***Males***	***Females***	
Alexandra Hills	9	5	4	9/14 (64%)
Birkdale	9	4	5	9/35 (25%)
Capalaba	6	4	2	6/13 (46%)
Cleveland	8	4	4	8/37 (22%)
Mount Cotton	17	7	10	17/36 (47%)
Ormiston	4	2	2	4/15 (27%)
Redland Bay	4	1	3	4/9 (44%)
Sheldon	10	3	7	10/13 (77%)
Thornlands	8	4	4	8/17 (47%)
Victoria Point	4	2	2	4/14 (29%)
Wellington Point	5	1	4	5/24 (21%)

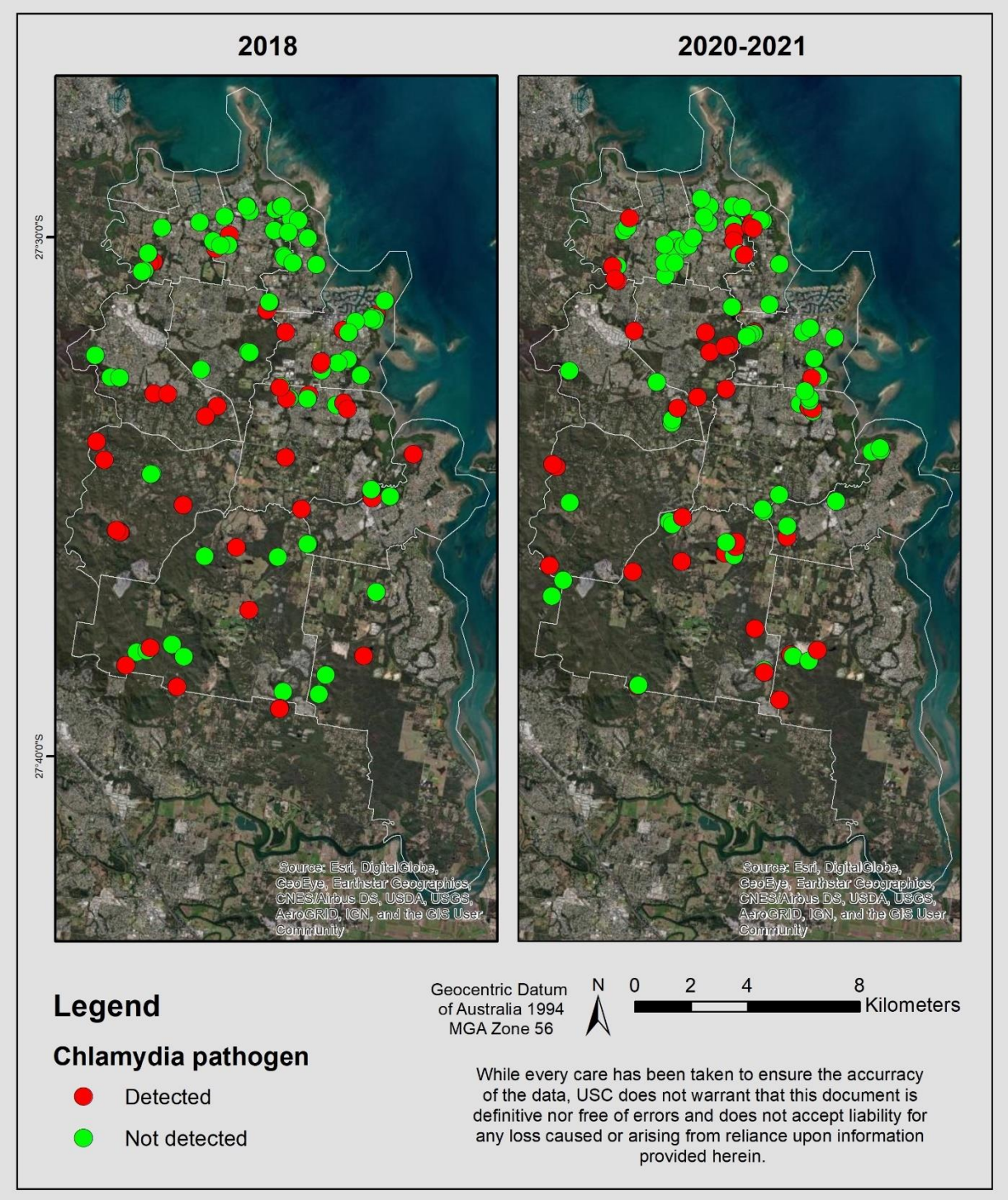


Figure 15: Map of Chlamydia presence in 2018 and 2020/21 (green points represent Chlamydia negative individuals; red points represent Chlamydia positive individuals)

Comparison. Our 2020/21 survey results generally confirm 2018 survey results and provide evidence that koalas on Redlands Coast mainland have:

1. increased inbreeding both at the population (H_E>>H_O, high F_{IS}) and at the individual (IR) level

2. small effective population size (N_e) in 2020/21, however, slightly larger than in 2018 and N_e>100 when survey periods were pooled

3. higher population inbreeding (F_{IS}) in the urban than in the non-urban footprint, but less so compared to values measured in 2018

4. high prevalence of chlamydia

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 et al. 2008, IUCN 2012, Frankham et al. 2014). Using these guidelines, the Redlands Coast mainland population falls short of the recommended effective population size when assessed per year, which is a sign for an increased risk of inbreeding depression. When data for both years are pooled, N_e lies at ~ 108, only just above the recommended effective population size to avoid inbreeding. This, combined with increasing F_{IS}, increasingly high IR and Chlamydia infection rates for Redlands Coast mainland koalas, indicate the vulnerability of the mainland population. While the difference between F_{IS} in urban and non-urban areas has decreased, it is because the non-urban F_{IS} levels are now more similar to urban levels than it was in 2018.

Recommendation. Regular monitoring, re-assessment and re-analysis of measures of genetic diversity, inbreeding, effective population size and Chlamydia infection rate for Redlands Coast mainland koalas will help to further understand the vulnerability of this population. Furthermore, continuous monitoring will help differentiate a true trend from fluctuation. Conservation efforts should continue to focus on establishing and maintaining connectivity of this population across the landscape.

3.4.6 Comparison to samples collected in 2006

Because there are only about two generations between the koalas sampled in 2006 and the koalas sampled by the DDC, it is not surprising that the cryptic structure identified through the sPCA can already be found in 2006 (Figure 16). When we compare the magnitude of the first positive (and significant) eigenvalue, it increased from 2006 to 2018, which indicates that this pattern of differentiation seems to be strengthening over time (Larroque et al. 2019). This might be further supported by the increase in F_{ST} between koalas north and south of the State route 21 which was not significant in 2006 with $F_{ST} = 0.003$ (*p*-value = 0.135) and $F'_{ST} = 0.004$. In 2018, however, F_{ST} was significant, though small with $F_{ST} = 0.008$ (*p*-value = 0.028) and $F'_{ST} = 0.012$. Table 9 shows how H_o, H_e, F_{IS}, N_e and sex ratio in 2006 compare to results from 2018 and 2020/21.

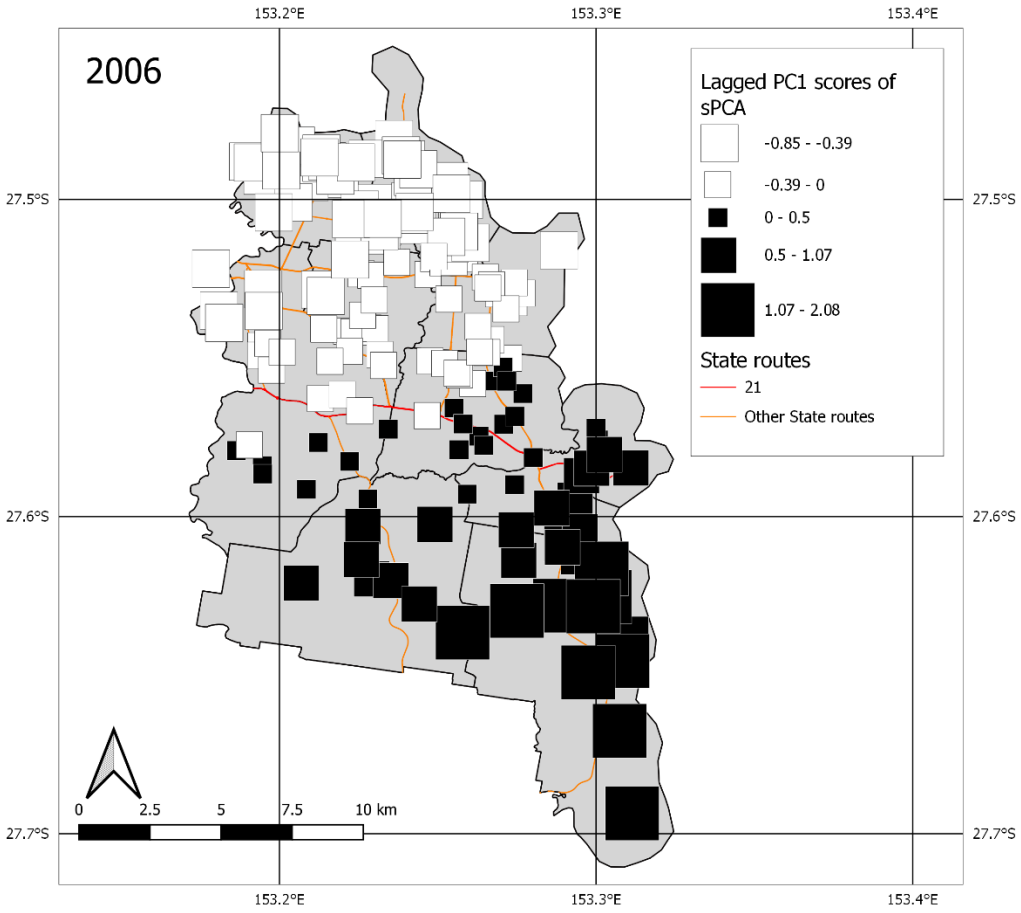


Figure 16: This map shows cryptic spatial genetic structure, the result of a spatial Principal Component Analysis. It represents results from 261 genetic samples of koalas from across Redlands Coast mainland that were admitted to wildlife hospital in 2006-2007. Squares represent individual koalas, black squares represent positive, white squares negative values. The size of the squares reflects the magnitude of the value and the strength of the effect.

Table 9: Comparison of genetic characteristics of the Redlands population at three points in time. Please note that sex ratio can be biased due to the data coming from hospital data (see Gonzalez-Astudillo et al. 2019), as for 2006/2007.

Measure	***2006/2007***	***2018***	***2020/21***
Observed heterozygosity (H_o)	0.357	0.241	0.229
Expected heterozygosity (H_e)	0.329	0.321	0.316
F_{IS}	-0.05	0.249	0.275
Effective population size (N_e)	393.2	75.9	90.9
Sex ratio (M:F)	1:0.88	1:1.25	1:0.97

NB For all measures but the sex ratio, a random subset of 100 individuals was used to calculate measures to ensure comparability.

Overall, this analysis shows that values of genetic diversity have decreased since 2006. While expected heterozygosity remains fairly stable across years, it is the observed heterozygosity that is decreasing strongly. Within 14 to 15 years, observed heterozygosity, that is the proportion of actual heterozygous loci, decreased by almost 36%. In 2006, H_o was indeed higher than H_e, leading to a negative F_{IS} value. This is usually the case in slightly more outbred populations, but being close to zero, F_{IS} in 2006 shows a population that is close to the Hardy-Weinberg-Equilibrium. Because of the increasing discrepancy between H_e and H_o, F_{IS} is much higher in 2018 and 2020/21, meaning that the population is no longer in equilibrium and effects of events such as inbreeding and genetic drift have affected the genetic diversity of the population. Most striking however, is the decreased effective population size. Taking the mean of 2018 and 2020/21 (83.4), N_e decreased by 78.8% since 2006-2007. Therefore, while the results with the combined dataset showed a Ne of >100, this analysis shows that it is much lower than what it was 12 to 15 years ago.

The Redlands Coast has experienced an increase in many threats and pressures during this period. Data shows that the human population on the mainland grew by ~19.9%, while the average annual daily traffic (downloaded from https://www.data.qld.gov.au/dataset/traffic-census-for-the-queensland-state-declared-road-network on 22/05/2020) increased by 16.6%. Some measure points along State Route 21 show an increase in average annual daily traffic of between 24.4% -

64.4%. Data from the KoalaBase further indicates a large loss of koalas since 1997, a decrease in population size that would affect genetic measures.

Overall, these findings give an insight into how koala genetic diversity might have looked in the Redlands a decade ago. Given that these measures only go back two generations, and that Redlands was already highly fragmented then, it can be assumed that historic levels would have been even higher to those measured in 2006. When interpreting these results, we need to keep in mind that there is a time lag for most genetic measures (Landguth et al. 2010). Values such as genetic diversity and F_{IS} from current samples likely reflect more accurately on levels from previous generations rather than on the contemporary population. We are able to overcome some of these restrictions, for example by looking at "live" measures such as relatedness and more cryptic population structure, but some limitations remain. The existence of the 2006 data set enables us to make assumptions that baseline values would have been significantly higher than what we currently find.

4. Discussion

The aim of this project was to repeat surveys that were conducted in 2018 to collect scats from koalas for genetic analyses. Through this approach, we are able to track changes in genetic diversity, sex ratio and health. The repeat of surveys in the same, or similar, areas of the initial surveys in 2018 provided an opportunity to assess how the occupancy at these sites is changing throughout time.

4.1 Koala occupancy across Redlands Coast mainland

The repeat surveys allowed us to compare presence and absence at survey sites between 2018 and 2020/21. In cases where we recorded koalas or fresh scats in 2018, but not in 2020/21, this may indicate a decrease in koala density or, at worst, extirpation (koalas having disappeared from a localised area). Alternatively, this can also be the result of seasonal or temporal fluctuation of occupancy. Note that the goal, and therefore the design, of this survey was not to determine distribution or density, but to collect genetic samples. Long-term monitoring design, with repeats

of the same survey points using methods developed specifically for this purpose (e.g. all scat or "habitat" detection dogs), would help to better understand changes in occupancy.

In 2020-21 again, no fresh koala scats were detected in the suburb of Thorneside. We can say with certainty that occupancy in this suburb is very low, however, a reported koala sighting a few months prior to the survey indicates that there is some infrequent koala activity in the area. This emphasizes the importance of citizen scientists and community awareness and engagement. Particularly in areas with low koala density, reporting of occasional public sightings can support Council in getting a more complete picture of koala presence, including during times and seasons outside of DDC survey periods.

Similarly, no (fresh) koala scats could be located during the 2020-21, nor the 2018, surveys in the south of the Redland Bay suburb. This is surprising, as this area comprises of five designated conservation areas (Figure 17) without any apparent barriers that would restrict movement. Koala scats were found in both survey periods in the northern part of Bayview Conservation Area and Days Road Conservation Area, including Kindilan Adventure Camp. However, in both survey periods, no scats were found in either Kidd Street, Serpentine Creek or Native Dog Road Conservation Area. There are only very few koala records entered in the Atlas of Living Australia (ALA), which could potentially be biased by a low number of visitors. In 2019, i.e. between the two DDC survey periods, a koala sighting was recorded through the ALA in Orchard Road Redland Bay, which is just outside the conservation areas. However, altogether, the low number of koala sightings gives further reason to believe that the habitat in this particular area, despite being mapped as koala habitat (Department of Environment and Science, 2021, Figure 18), either does not meet the requirements to carry many koalas or has experienced an event of high mortality (e.g. heat wave, drought, dog predation). It would be of interest to investigate this area for threats (e.g. past weather data or dog density), as well as botanical, soil and water indicators to better understand what the limiting factor could be. Parts of this area have been proposed to be monitored as sentinel sites for drone surveys.

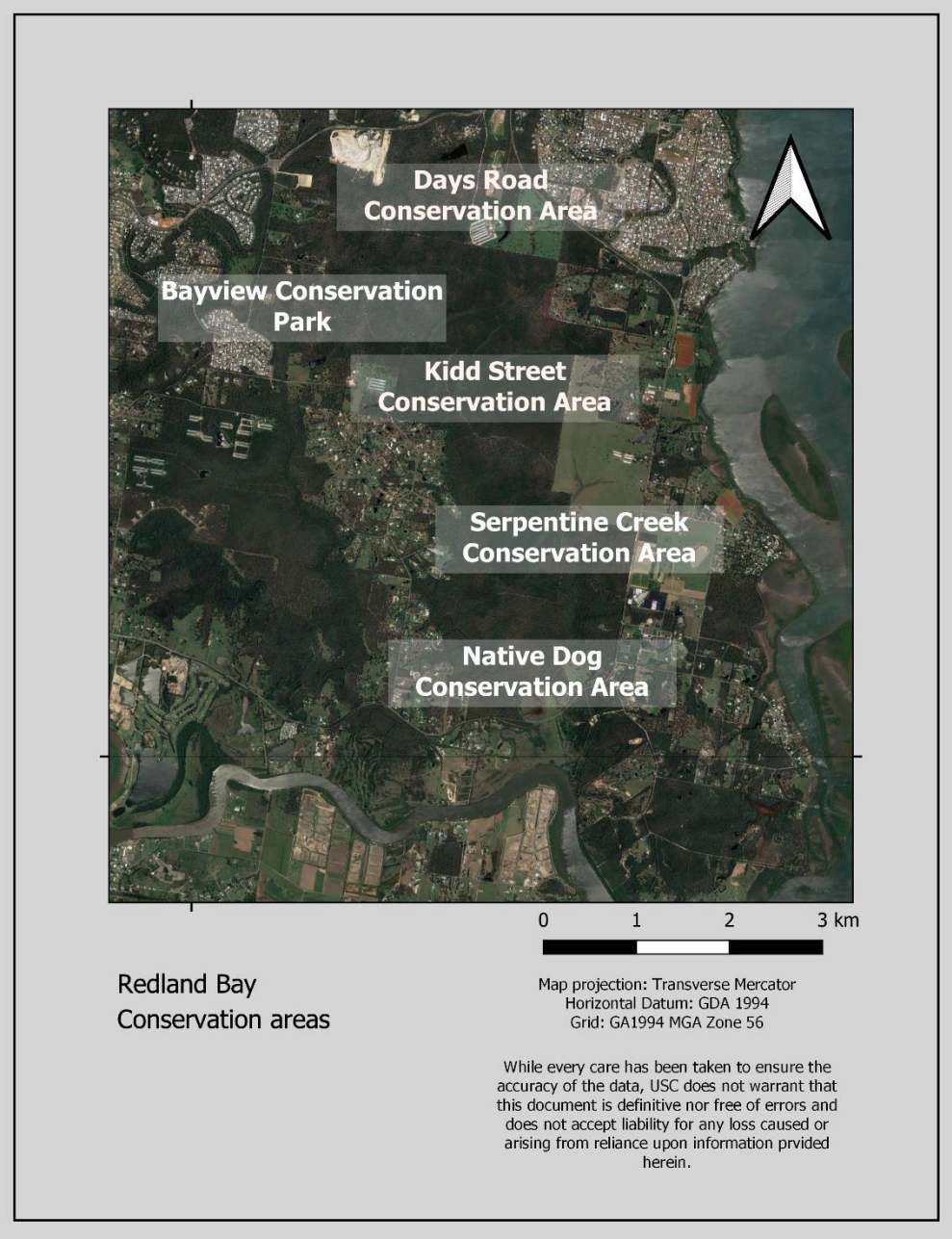


Figure 17: The southern area of Redlands Coast Council comprising the Redland Bay suburb. The figure shows the five conservation areas and parks in this suburb: Days Road Conservation Area, Bayview Conservation Park, Kidd Street Conservation Area, Serpentine Creek Conservation Area, and Native Dog Creek Conservation Area.

Two areas with good quality koala habitat (Department of Environment and Science, 2021, Figure 18) and positive survey sites in 2018 did not follow suit in 2020/21. In 2018, at least seven koalas, including mother-offspring pairs, were inhabiting the area around GJ Walter Park, Shore Street East, and Wharf Street, Cleveland. Community groups and members of the public recorded that a female koala with her joey was taken to Australia Zoo Wildlife Hospital for Chlamydia treatment just prior to our surveys in the area. However, they reported a general decline in koala sightings in this particular area. Seasonal fluctuations are possible, and it is unknown to what extent the above average rainfall might have influenced koala movements in the general area and the specific use of this site. Nonetheless, it is concerning to see such a decline in an area that is highly urbanised, widely recognised as a koala hub, and therefore closely monitored by the public. In such areas, more frequent formal surveys, either through community groups, community events or detection dogs, would be beneficial to gain better understanding of changes as they occur, and enable further investigation of their causes.

The second area with no koala scat detection in 2020/21 was Venman National Park. As the only National Park in Redlands Coast, this area is invaluable to koala conservation. The large continuous habitat means that there is a lot of area to cover and it is possible that previously identified koalas were not picked up during this round of detection dog surveys but are still in the area. Scat detection in this protected area was low in 2018, where only four individuals were identified through genetics. Therefore, we believe that the survey effort in this park should be increased to get a more robust understanding of the number of koalas present. Drone surveys with thermal imaging could help quantify koala numbers in Venman National Park and is therefore nominated as one of the proposed sentinel sites. Understanding the current population status, the presence of threats and the ability of the park to support koalas (through nutritional value and carrying capacity) could inform future management actions (if specific threats need to be addressed).

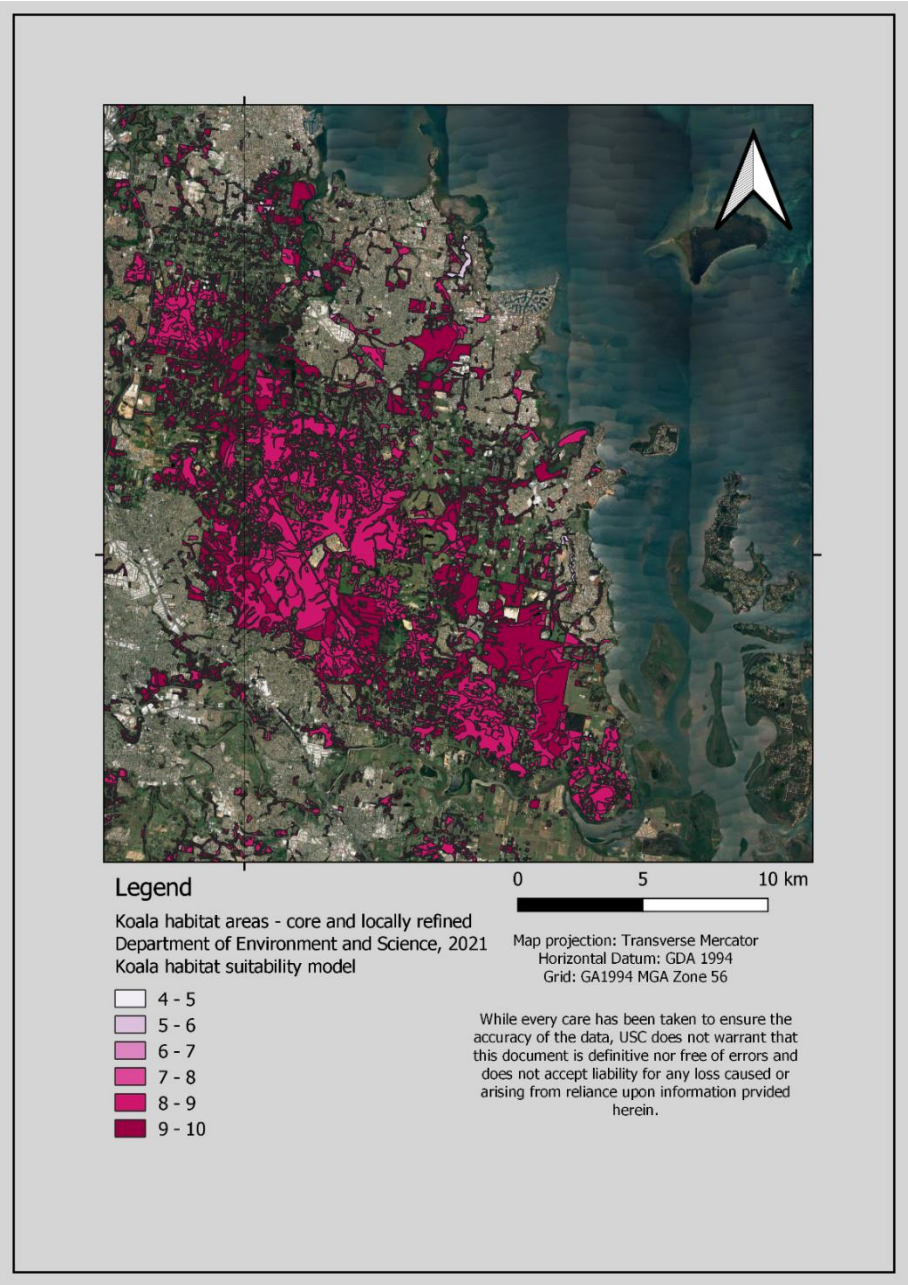


Figure 18: This map shows identified koala habitat in Redlands Coast Council mainland. Both core habitat and locally refined habitat are depicted. Data was taken from the Department of Environment and Science (https://environment.des.qld.gov.au/wildlife/animals/living-with/koalas/mapping/koalamaps).

4.2 Genetic connectivity and diversity

Similar to results reported in 2018, the genetic analysis of population structure identified one continuous breeding population. However, as we recommended in 2018, 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).

We first investigated whether there were genetic differences between samples collected in 2018 and 2020/21, but found none, and therefore pooled the data for some of the analyses. This allowed us to increase sample size and thus the robustness of results. For each data set - 2018, 2020/21 and pooled - we detected a fine-scale genetic pattern that divides the Redlands Coast koala population equally into two sections – north and south. While this pattern could have resulted from landscape or historic circumstances, the boundary coincides with parts of the State Route 21 (namely, Mount Cotton Road, Duncan Road and Boundary Road). This cryptic structure shows that there is likely a barrier to gene flow between koalas in the north and the south, and while it is still currently cryptic, this could lead to a long-term fragmentation of the population. Roads not only pose a major threat to koalas, but also fragment populations genetically (Lee at al. 2009, Dique et al 2003). Bespoke koala corridor mapping for Redlands Coast mainland was undertaken by the DDC in collaboration with Sally Chudleigh as one of the recommendations emerging from the previous report (Appendix 5). This mapping shows that many habitat corridors lead to State Route 21, but it is likely that individuals would then have to cross the road to get to the other side i.e. that there is no infrastructure for safe crossing (fencing and underpasses). Further on-ground investigation could help to better understand why there could be a barrier in this particular area. The decreased gene flow could be a result from increased mortality while crossing (barrier through direct mortality), decreased crossing due to inappropriate number/location of underpasses, or other structural problems. Future investigation could include, for instance, interviews of property owners about their experience with koalas in the area. Traffic analyses could identify problems such as frequency of vehicles speeding and peak traffic hours. If koalas have to pass through private

properties, an assessment of fences and dogs on those properties would also be valuable. Maintaining and improving connectivity and safe road crossing will continue to be key to prevent further genetic fragmentation.

The assessment of gene flow between suburbs showed that while differentiation was significant it was also very low and the number of migrants indicated that gene flow does exist between suburbs. For this analysis, we included many more samples in 2021 than in 2018, which explains some differences in results. However, consistent with 2018 results, Birkdale – Cleveland and Birkdale – Wellington Point resulted in significant differentiation. When assessing these suburbs and their connectivity through the koala corridor mapping (Appendix 5), it appears that there are no identified movement corridors between Birkdale and Wellington Point. Several mapped movement corridors do exist between Birkdale and Cleveland, however, the distance to overcome is substantial. Distance might also partly explain the significant F_{ST} between Mount Cotton and Cleveland/Wellington Point. Overall, it is likely that significant F_{ST}'s in the northern part of Redlands Coast are attributed to the dangerous and difficult urban matrix.

In 2020/21, both observed and expected heterozygosity were slightly lower than in 2018. Whether this is a real trend or simply variability due to random sampling will be easier to determine after a third period of genetic sampling in 2022. Whether ongoing loss of koalas due to Chlamydial infections, vehicle collisions, dog attacks and other threats are continuing the decline in population size on the Koala Coast (Rhodes et al. 2015), and therefore further decline in genetic diversity, is unknown. The bi-annual genetic monitoring surveys will help better understand genetic population trends in Redlands Coast.

Comparing our scat survey results to tissue samples that were collected in Redlands between 2006 and 2007, we find a loss in observed heterozygosity, resulting in an increased F_{IS} and a reduced effective population size. We know that the koala population in Redlands Coast mainland has decreased significantly over the past decades (Rhodes et al. 2015), so a loss in genetic diversity was to be expected given the reduced gene pool. While we are unable to provide a historic baseline

(i.e. prior to European settlement), this still allows us to put current measures of genetic diversity into context.

These results confirm the importance of monitoring the genetics of the Redlands koala population. The population has declined rapidly and, with that, its evolutionary adaptive potential has also declined. While it is difficult to pinpoint at which threshold genetic diversity is "enough", the proportion lost is likely an indicator of the potential genetic consequences. Populations with low genetic diversity are likely more susceptible to large impacts from emerging diseases and other environmental changes in the future. Stabilising and increasing koala population size is key. From data collected from the Koala Safe Neighbourhood project, breeding is still occurring in the Redlands, which means the focus should be on decreasing mortality.

Releases of koalas from Minjerribah (North Stradbroke Island) in the Redlands Coast mainland, after being orphaned or having recovered from injury, have the potential to introduce new genetic variants to the mainland. Observations of how well these individuals acclimatise to the mainland and its threats are ongoing. Successful reproduction could, in theory, be beneficial for the mainland population (Frankham et al. 2015, Bell et al. 2019, Weeks et al. 2017) and this should be investigated in future genetic monitoring.

4.3 Chlamydia disease poses largest threat to Redlands koala population

Chlamydial infection was only slightly higher in 2020/21 compared to 2018. Combining chlamydia results from 2018 and 2020/21 showed that the pathogen was, overall, detected more frequently in females than in males. Furthermore, some suburbs had alarmingly high numbers of chlamydia positive individuals, in particular Alexandra Hills (64%) and Sheldon (77%), while other suburbs such as Birkdale, Ormiston or Wellington Point showed much lower infection rates (25%, 27% and 21%, respectively). However, the comparably lower infection rate in some suburbs compared to others could be biased by how exposed koalas are to the public eye which makes it more likely for sick koalas to be rescued and treated. Another bias could have been introduced by the Koala Safe Neighbourhood program in the suburbs of Ormiston, Cleveland, Wellington Point,

Thornlands and Mount Cotton. Through this program, the DDC identified several sick koalas which were either euthanised based on welfare grounds or presented to AZWH for treatment.

It is of continuing concern that, of koalas that were brought to wildlife hospitals from Redlands mainland in 2018 and 2019 (N= 203), over 50% (110) were diagnosed with either cystitis or conjunctivitis, independent of the cause of their hospital admission (taken from KoalaBase, last updated November 18 2020, https://www.data.qld.gov.au/dataset/koala-hospital-data/resource/7c6f7da8-ef7a-48e4-bf4e-c449a885e46d). Of the 203 recorded admissions, 35 individuals were hit by vehicles and six were hit by trains. The fate of most koalas is not indicated in the database. However, 38 koalas were recorded as dead on arrival and 51 were reported as released back into the wild. Altogether, our results and the hospital data combined, paint a picture of chlamydial disease representing a major threat to Redlands Coast mainland koala population.

4.4. Discussion summary

Overall, the results of the repeat genetic study show that, while some measures have slightly changed, the general genetic characteristics (and potential issues arising from them) remain the same. While measures of genetic diversity were poorer this year, only ongoing genetic monitoring can help to determine whether it is a true trend or simply variance. Nevertheless, comparison of the current population with the population in 2006-2007, demonstrates a substantial decline in genetic measures, likely due to the decline in population size over past decades. Chlamydia remains a key threat for Redlands Coast mainland's koalas and this requires urgent immediate attention. Investigations of how the observed high chlamydia infection rates affect fertility and reproduction, and monitoring for trends in population sex ratio should be continued. Management recommendations in this report should be considered in addition to those from the report published in 2018.

5. Future steps and recommendations

Our recommendations for future steps and monitoring are based on the results of this report and include both genetic and non-genetic approaches. The order of recommendations is random and does not reflect importance.

Recommendation 1: Continue genetic monitoring of the Redlands Coast mainland koala populations to confirm trends

Results presented in this report are based on the second genetic survey of the Redlands koala population, two years after the first survey. Therefore, we now have genetic information for two points in time. To distinguish stochastic variance from a true trend, multiple time points are needed. Ongoing periodic monitoring will enable modelling trends, investigating correlations with other factors, and support more robust predictions. We recommend repeating the genetic survey in two years for the period 2022-2023.

Recommendation 2: Creating maps of wildlife fencing and road crossing aids across Redlands Coast

It is crucial to have accurate geographic information available that provides locations of wildlife fencing and road crossing aids. For in depth analysis of potential barriers and the efficiency of koala corridors, this information needs to be available as input for the models.

Recommendation 3: Assess habitat quality in areas of low density and identify potential for future koala carrying capacity in Redlands Coast

Multiple conservation areas that have been mapped as high-quality koala habitat (Department of Environment and Science, 2021) show signs of low occupancy. These areas could be assessed on the ground to confirm habitat quality and/or identify reasons for low or no occupancy. It is

important to understand limitations to potential koala density, especially whether it relates to threats that can be addressed or to ecological limiting factors, as this could inform management actions e.g. threat management and no-go areas for future relocations.

Recommendation 4: Continue to collect and analyse data from Koala Safe Neighbourhood program to understand breeding success, mortality, demography and other population dynamics

The already established Koala Safe Neighbourhood program is providing valuable data to transfer trends on the rest of the population. So far, breeding success, demography and other population dynamics are not well understood in the Redlands koala population. In addition, Sentinel Sites across the Redlands Coast should be established, which would provide further insights into population density and trends.

Recommendation 5: Analyse where corridors do and do not translate into genetic connectivity (true connectivity) to identify dysfunction and barriers

We established that safe movement and migration are important to maintain and increase population size. Movement corridors have now been mapped which will enable us to better understand how koalas are likely to move and disperse across the landscape. As different corridor data exist, all should be assessed together for functionality, i.e. if/which corridors are being utilised by koalas. This can be done by looking at kinship across the landscape, gene flow or available GPS data, and can help identify dysfunctional corridors and barriers that present opportunities for improvement.

Recommendation 6: Continue to advocate for increased funding to wildlife hospitals

The rehabilitation costs of one sick or injured koala is about 10,000 AUD (pers. comm. from Rosemary Booth, AZWH). In the past year alone, AZWH has received more than 26 koalas from

Redlands, mostly with Chlamydiosis, caught by members of the DDC. The influx has been increasing as the DDC team is spending many hours in the field and acting promptly when sick koalas are found. Additionally, the introduction of any disease management program will likely increase the number of koalas being sent to wildlife hospitals from Redlands. Therefore, it is important that RCC continues to advocate to the State Government's Department of Environment and Science to provide support funding to the wildlife hospitals for Redlands koalas.

Recommendation 7: Continue advocating to share monitoring programs or samples across the whole Koala Coast

We recommend to continue efforts to sample the whole of the Koala Coast to put the Redlands population into broader context. Alternatively, samples and data should be shared across Koala Coast LGAs.

Recommendations from the 2018 Koala Population Assessment Project Report and how they have been addressed

There were six recommendations for the Redlands mainland stated in the 2018 report. Below is a summary of the aims and how RCC and the DDC have addressed them:

A= Aim: AD= Addressed: R= Recommendation

1. **Complete the assessment of koala presence in the southern half of the mainland** (Sheldon, Mount Cotton, Redland Bay).

   A: Remaining samples collected in this area after the finalisation of the 2018 report were genotyped and added to the 2018 data presented in this report. In 2020/21, increased effort was made to gain more access to areas in the south of the Redlands.

   AD: Predominantly private land tenure again restricted many areas from being surveyed, despite RCC sending letters to residents in target areas seeking permission to access.
2. **Monitor genetic structure in urban areas to detect further loss of connectivity**

A: This aim suggested to monitor trends and status of gene flow through genetic monitoring every two years can help detect deterioration of gene flow.

AD: With the fieldwork and report delivered in 2020/21, this aim has been achieved two years after the first survey and report in 2018. It is recommended to maintain genetic monitoring in this rhythm to clearly separate true trend from variance.

3. **Place current genetic trends in historical context through comparison with past genetic health of Redlands koalas.**

   A: The DDC had access to tissue samples collected through wildlife hospitals across SEQ. A total of 266 tissue samples were collected in 2006, 12 years prior to the 2018 survey, and 14 years prior to the 2020/21 survey.

   AD: We genotyped these samples and analysed the data, which is presented in this report as an in-kind contribution.

4. **Study habitat connectivity to understand reasons for the observed fine-scale genetic structure / disrupted gene flow between some of the Redlands Coast localities**.

   AD: The DDC commissioned a connectivity analysis of the Redlands Coast which is presented in this report as an in-kind contribution (Appendix 5). This data helped to shed light into potential reasons for genetic differentiation and barriers, however detailed analyses remain to be conducted to determine significant correlations of landscape and genetics.

   R: The DDC can provide the Redland City Council with layers and raw data that was used for this analysis.

5. **Increase protection of koalas found in urban areas by developing a better understanding of fine scale koala movement and enabling the community to be more involved in koala protection within the urban footprint**

   AD: RCC has led many campaigns to tackle this particular aim. As part of this, the DDC has initiated the Koala Safe Neighbourhood program. This program involved designating areas where citizen scientists can monitor urban koalas. Koalas were fitted with a GPS and closely monitored for movement and disease. They were further equipped with a trial

Bluetooth ear tags which allows interaction between koalas and researchers and also citizen scientists.

6. **Establish methodological calibration in collaboration with State Governmen**t (if possible)

   A: Here, the DDC suggested to create a survey overlap between strip transect visual searches, thermal drone surveys and detection dog surveys to analyse the results for a methodological calibration.

   AD: The DDC has conducted numerous thermal drone/detection dog surveys which have been analysed, however, strip transects have so far not be included. This would be a valuable study for RCC and koala conservation outcomes across its range.

   The calibration and testing of drone survey methods to monitor koala population trends has been ongoing at the DDC.

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7. Appendix

Appendix 1: Detailed molecular methods

DNA extraction

Koala intestinal epithelial cells were extracted from the surface of collected scats. For samples collected in 2018, DNA extraction were performed using the QIAamp DNA Stool Mini Kit (Qiagen) following the "Isolation of DNA from Stool for Human DNA Analysis" manufacturer protocol, with modification. This extraction kit was discontinued in 2019 and after testing the best kit alternative, we continued extractions with the QIAamp PowerFecal Pro DNA Kit (Qiagen) with an additional one-hour incubation step after adding the buffer to the fecal sample. The amount of DNA present in extracted samples (both koala and foreign) was determined using the Thermo Scientific NanoDrop 1000 Spectrometer (Thermo Fisher Scientific, Victoria). Extracted DNA samples were stored at -80 ºC.

SNP Genotyping

SNP genotyping of DNA extracted from koala scat followed the general methodology outlined in Schultz et.al. (2018b). SNP genotyping was conducted by Diversity Arrays Technology, Canberra, using proprietary DArTseq™ technology. DArTseq™ represents a combination of DArT complexity reduction methods and next-generation sequencing platforms (Kilian et al. 2012, Courtois et al. 2013, Cruz et al. 2013, Raman et al. 2014). Specifically, SNP genotyping was conducted using DArTcap, which is a targeted application of DArTseq™ technology allowing for the sequencing of targeted markers. DArTcap is used in similar applications as DArTseqLD, but it applies a selective step after complexity reduction to genotype specific markers from DArTseq representations. This selection is achieved with the use of the nucleic acid "capture probes" that bind to restriction fragments in the representations carrying the specific DArTseq markers. Capture probes were designed by Diversity Arrays using DNA extracted from 189 tissue samples of koala

was used to target restriction fragments from koala DNA. The samples used to design the capture probes were ear punches collected by Deidre de Villiers in south-east Queensland in the last 15 years (the large geographical spread to avoid ascertainment bias).

DNA samples were processed in digestion/ ligation reactions (Kilian et al. 2012), ligating two adaptors corresponding to the combination of RE overhangs. For DNA extracted from koala scat, the combination of PstI and SphI restriction enzymes (RE) performed better in polymorphism detection efficiency. The PstI-compatible adapter includes the barcode. The reverse adapter contained the SphI-compatible overhang sequence.

The PstI-SphI fragments were amplified by adapter-mediated PCR* as follows: initial denaturation of 94ºC for 1 min, followed by 30 cycles of denaturation (94ºC for 20 s), annealing (58ºC for 30 s), and extension (72ºC for 45 s), with final extension phase of 72ºC for 7 min. The PCR primers were designed to add the required sequences for enabling sequencing in a single-read Illumina flowcell. Equimolar amounts of amplification products from each sample were bulked and applied to c-Bot (Illumina) bridge PCR followed by 77 cycles of single-read sequencing on Illumina Hiseq2500 (Illumina).

The resulting sequences generated were processed using proprietary DArT analytical pipelines. The primary pipeline filtered out poor quality sequences, while applying more stringent selection criteria to the barcode region. In this way, assignment of sequences to specific samples was very reliable. Identical sequences were then collapsed into “fastqcol” files for use in secondary pipeline analysis, using DArT PL's proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14).

For SNP calling, all tags from all libraries included in the DArTsoft14 analysis are clustered using DArT PL's C++ algorithm at the threshold distance of 3, followed by parsing of the clusters into separate SNP loci using a range of technical parameters, especially the balance of read counts for the allelic pairs. Additional selection criteria were added to the algorithm based on analysis of approximately 1,000 controlled cross populations. Testing for Mendelian distribution of alleles in these populations facilitated selection of technical parameters discriminating well true allelic

variants from paralogous sequences. In addition, multiple samples were processed from DNA to allelic calls as technical replicates, and scoring consistency was used as the main selection criteria for high quality/low error rate markers. Calling quality was assured by high average read depth per locus. This process is similar to that used in published literature using DArTseq™ SNPs from animal genetic samples (e.g. Donnellan et al. 2015, Couch et al. 2016).

Appendix 2: Detailed results per locality of koala scat surveys conducted in 2020/21

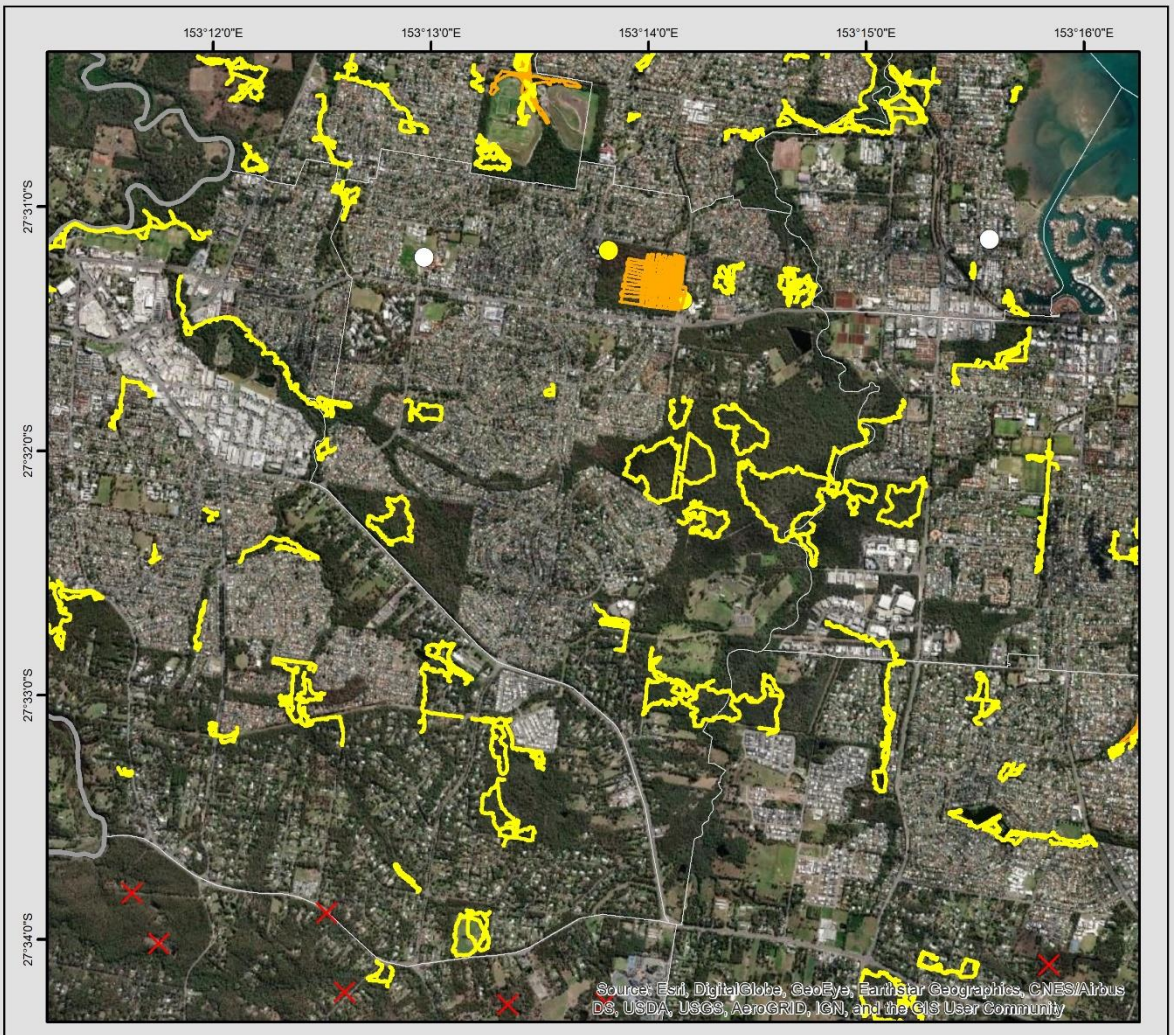
This Appendix details, per locality, results that have been presented at a broader scale in the main part of the report. The surveys were not designed to establish or compare occupation rates (or percent of sites used), as the surveys were not random, and the survey effort was not standardised, nor equal at each location or per locality. While looking at the following tables and maps, readers need to keep at the forefront of their mind that the goal of the DDC surveys was to collect genetic samples only. Presence of koala signs, and percent of surveys with koala presence per locality should not be used to calculate percent of occupancy and compare localities, as again, the survey design is not fit for this purpose. These results and maps per locality are provided as interesting additional information to the main genetic aim of this report.

The number of surveys and the maps of their locations encompass both dog surveys and opportunistic koala spotting (survey effort however only accounts for dog searches) as well as few drone flight surveys. Presence of koala scats means the location had been used by koalas, however, absence of scats does not mean the location had not been used, only that no scat, or fresh scat, was found on the day of the surveys (see limitations for detailed explanation).

Koala sighting maps show locations where the team spotted a koala, note that the same koala could have been spotted more than once (on different survey dates). Maps of koala sexes (male / female) are based on genetic results from scat collection. Chlamydia maps present the detection of any Chlamydial DNA (even if only one copy was present). Note again that presence and threshold of Chlamydia do not necessarily mean koalas are sick, they can be passive carriers of the bacteria, or have recovered. Internal relatedness in the table below is given as an average per locality, whereas maps represent each individual's internal relatedness separately

USC
Detection Dogs for Conservation

Alexandra Hill



Alexandra Hills

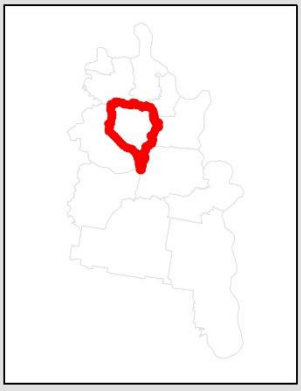
N

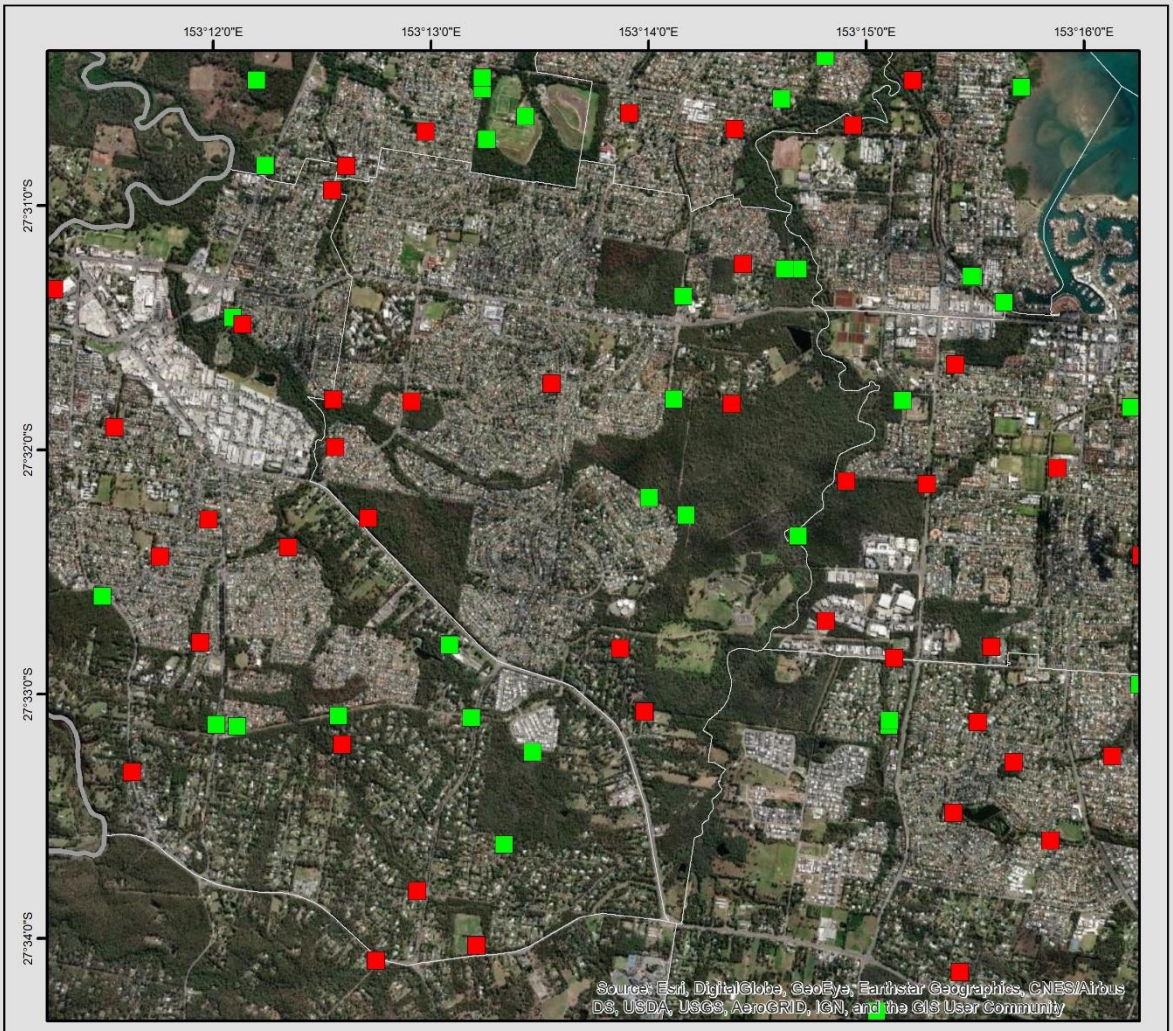
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog survey tracks
- Drone flights
- Drone survey
- Access not granted
- Survey site inappropriate (no trees)





Alexandra Hills

N

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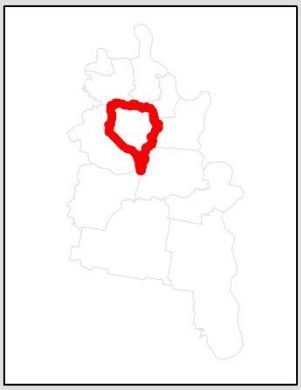
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence



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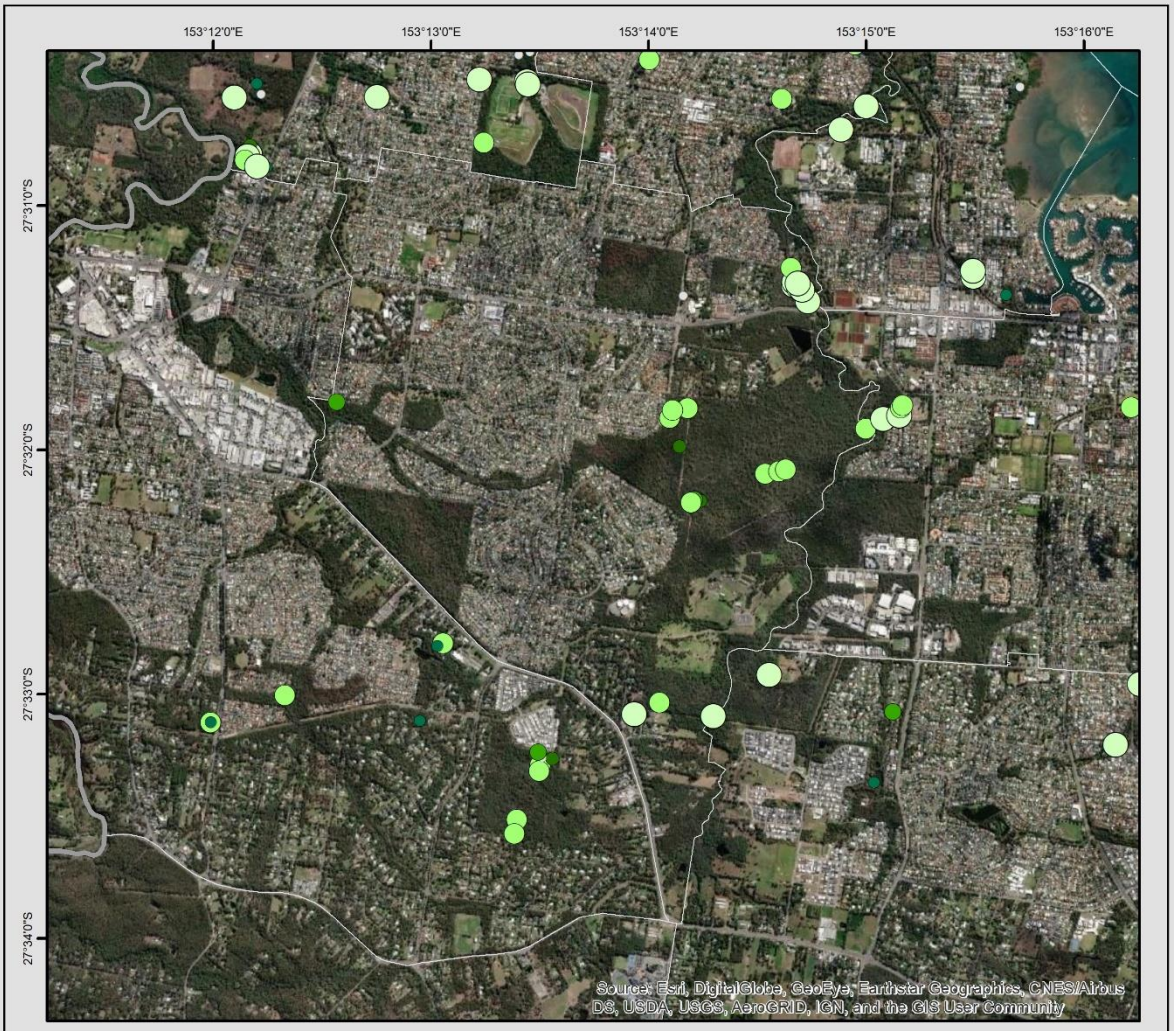


Alexandra Hills

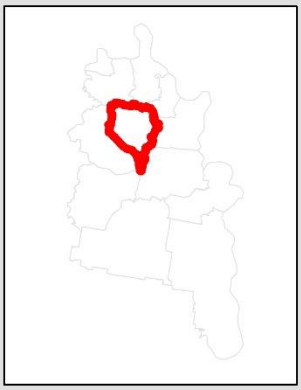
N

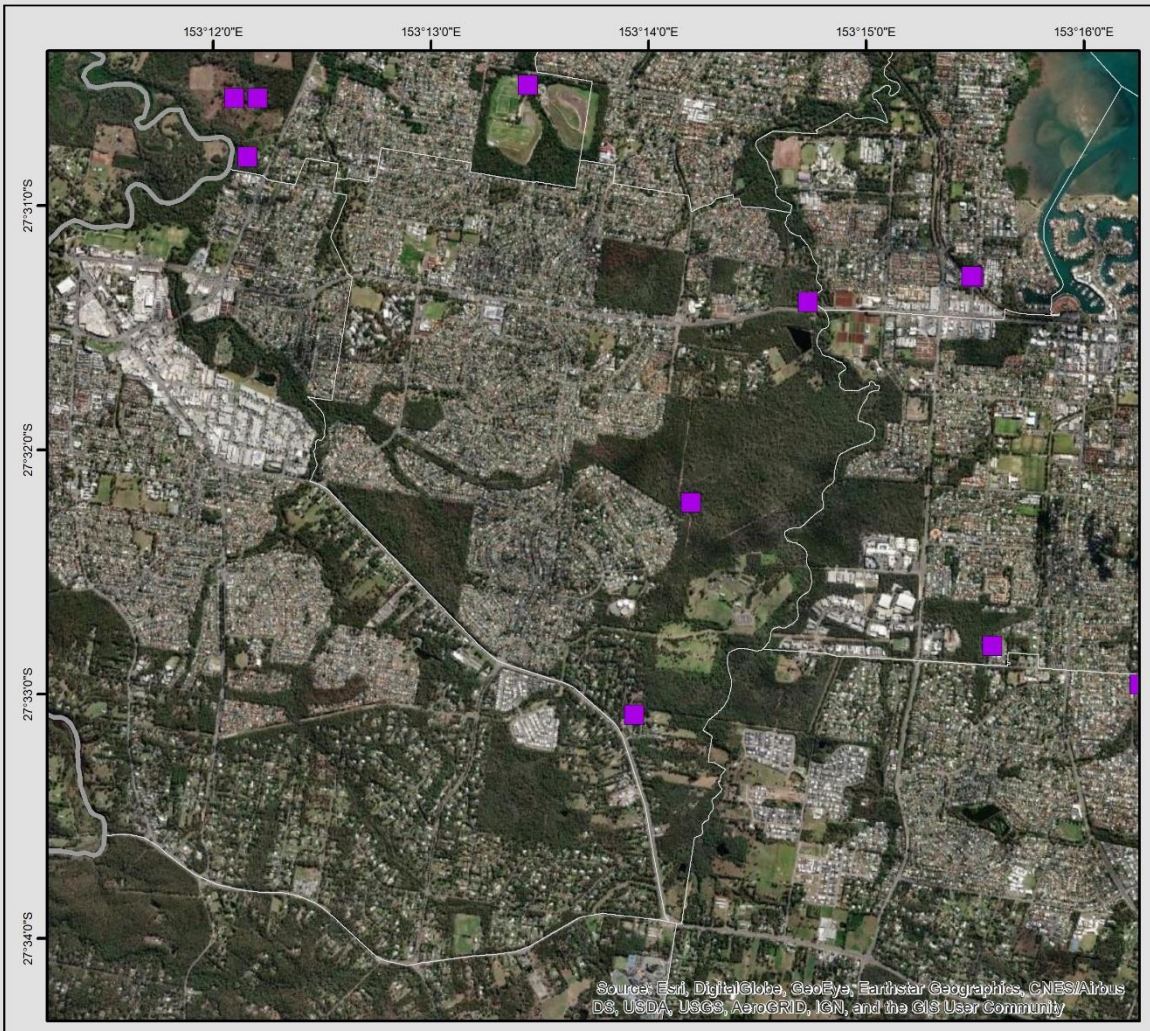
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Old
- Very old





Alexandra Hills

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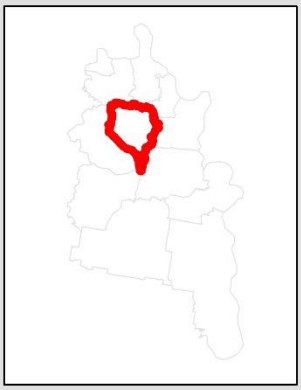
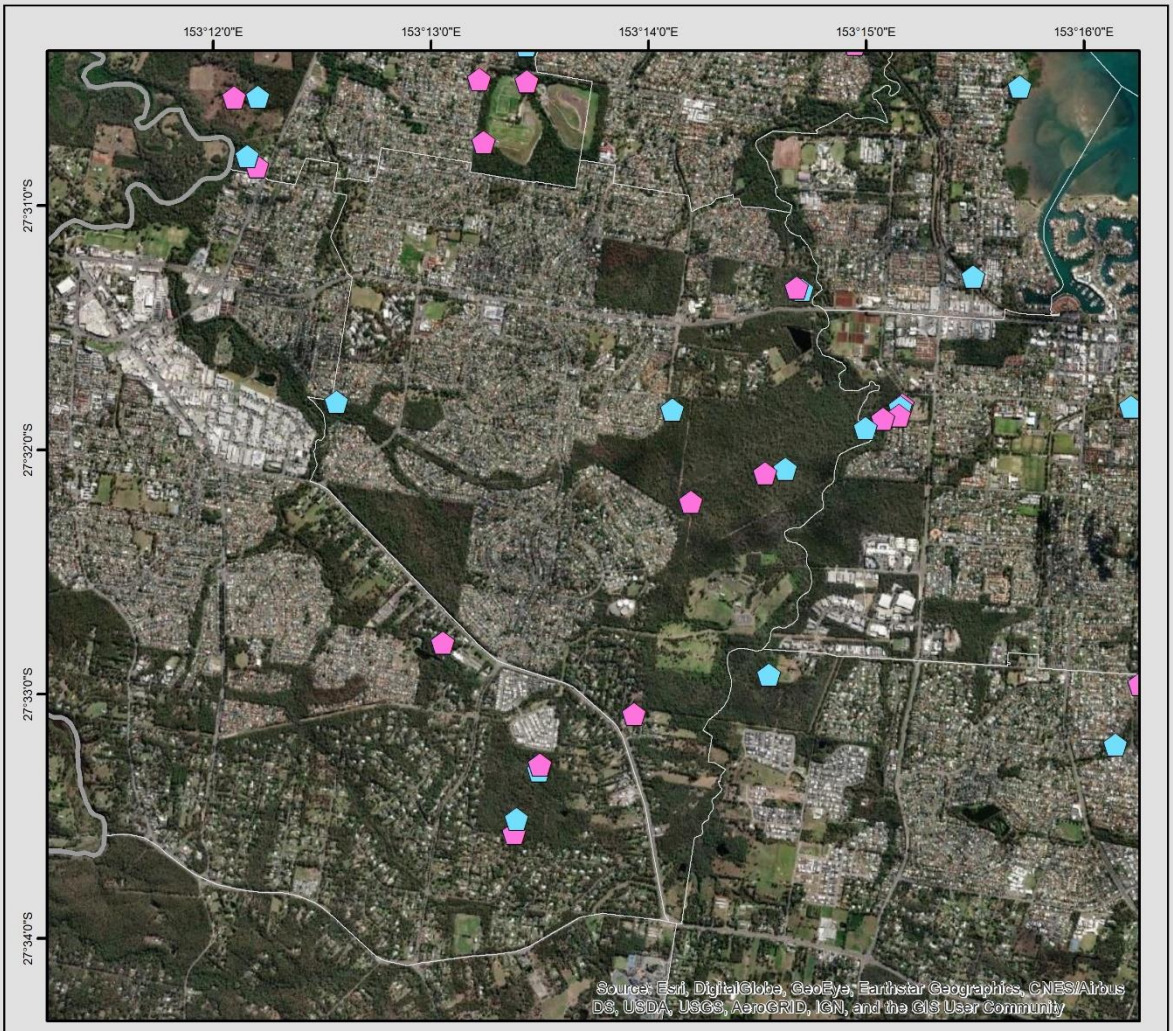
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings





Alexandra Hills

N

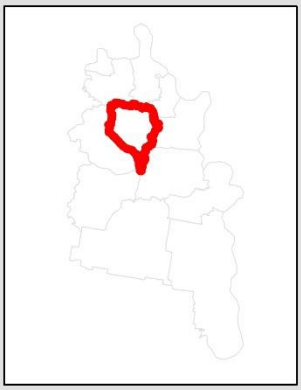
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male



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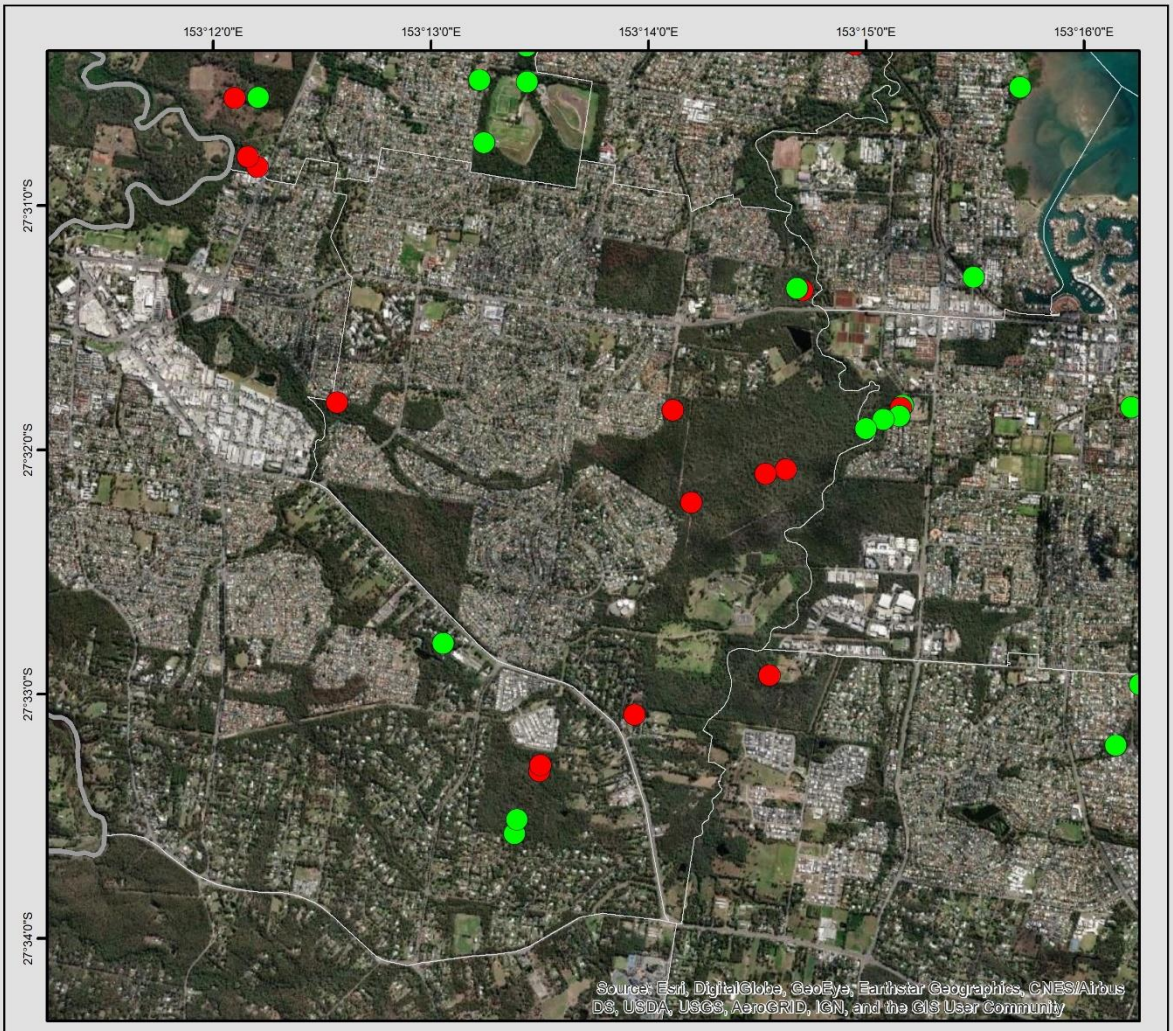


Alexandra Hills

N

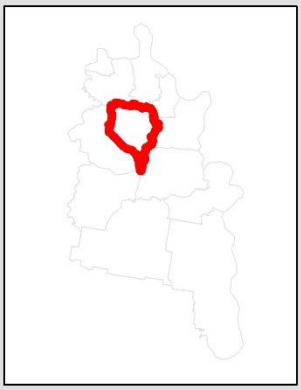
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Chlamydia pathogen

- Detected
- Not detected



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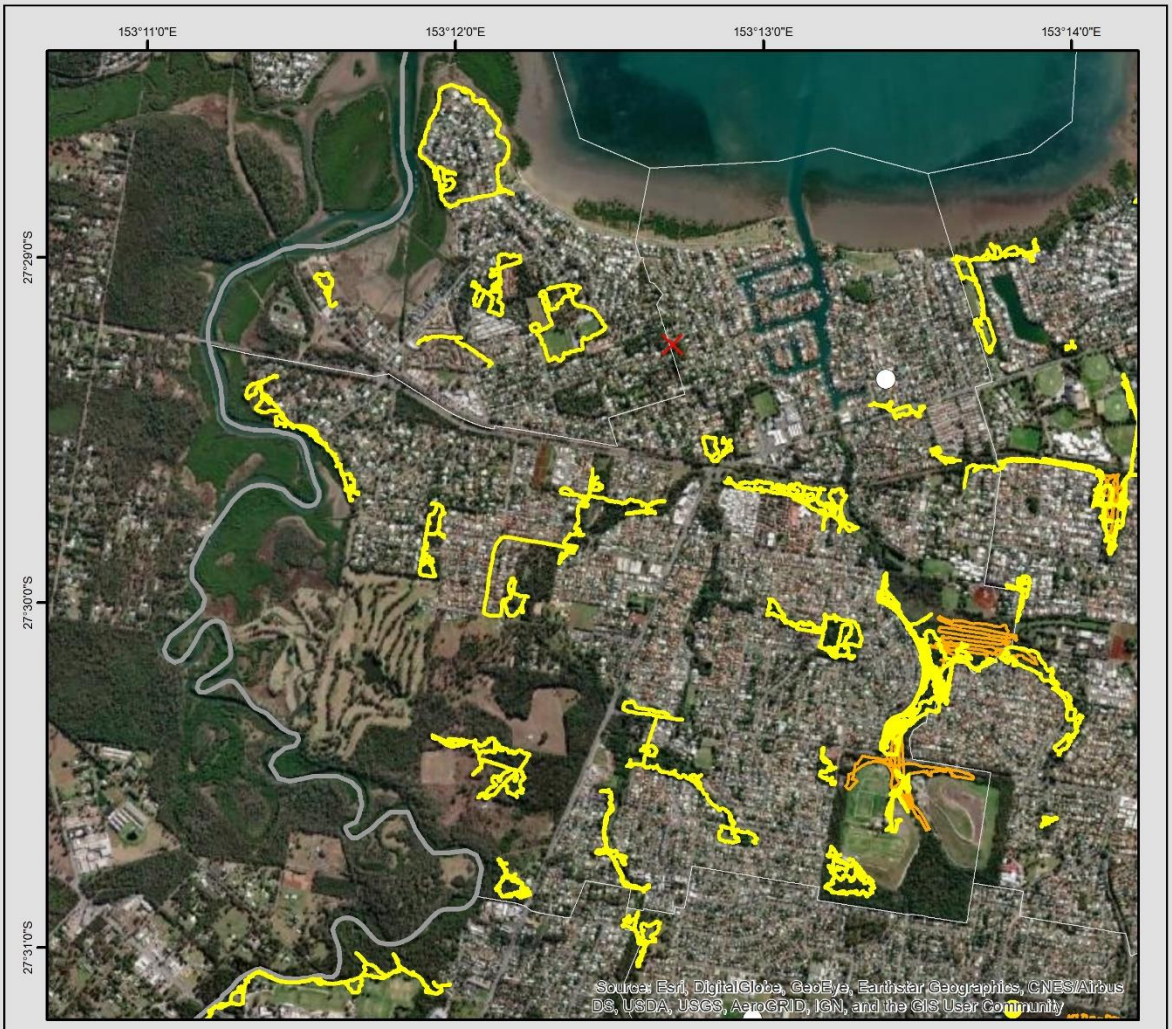
Birkdale



Birkdale

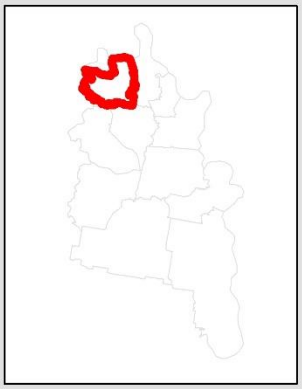
N

0 0.35 0.7 1.4
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog survey tracks
- Drone flights
- Drone survey
- Access not granted
- Survey site inappropriate (no trees)



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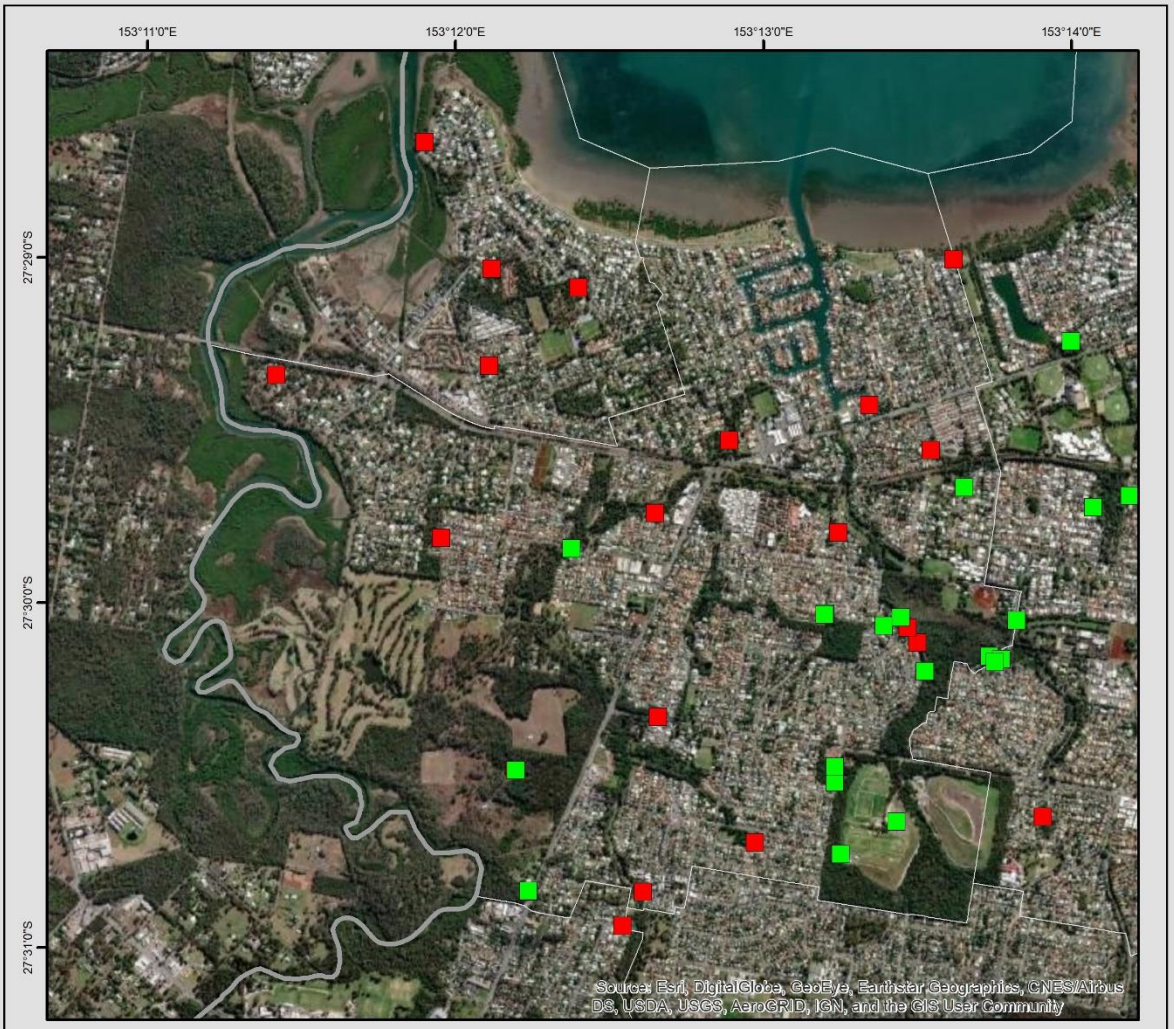


Birkdale

N

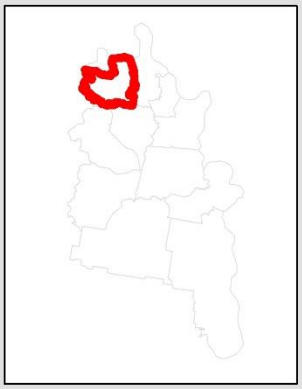
0 0.35 0.7 1.4
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence



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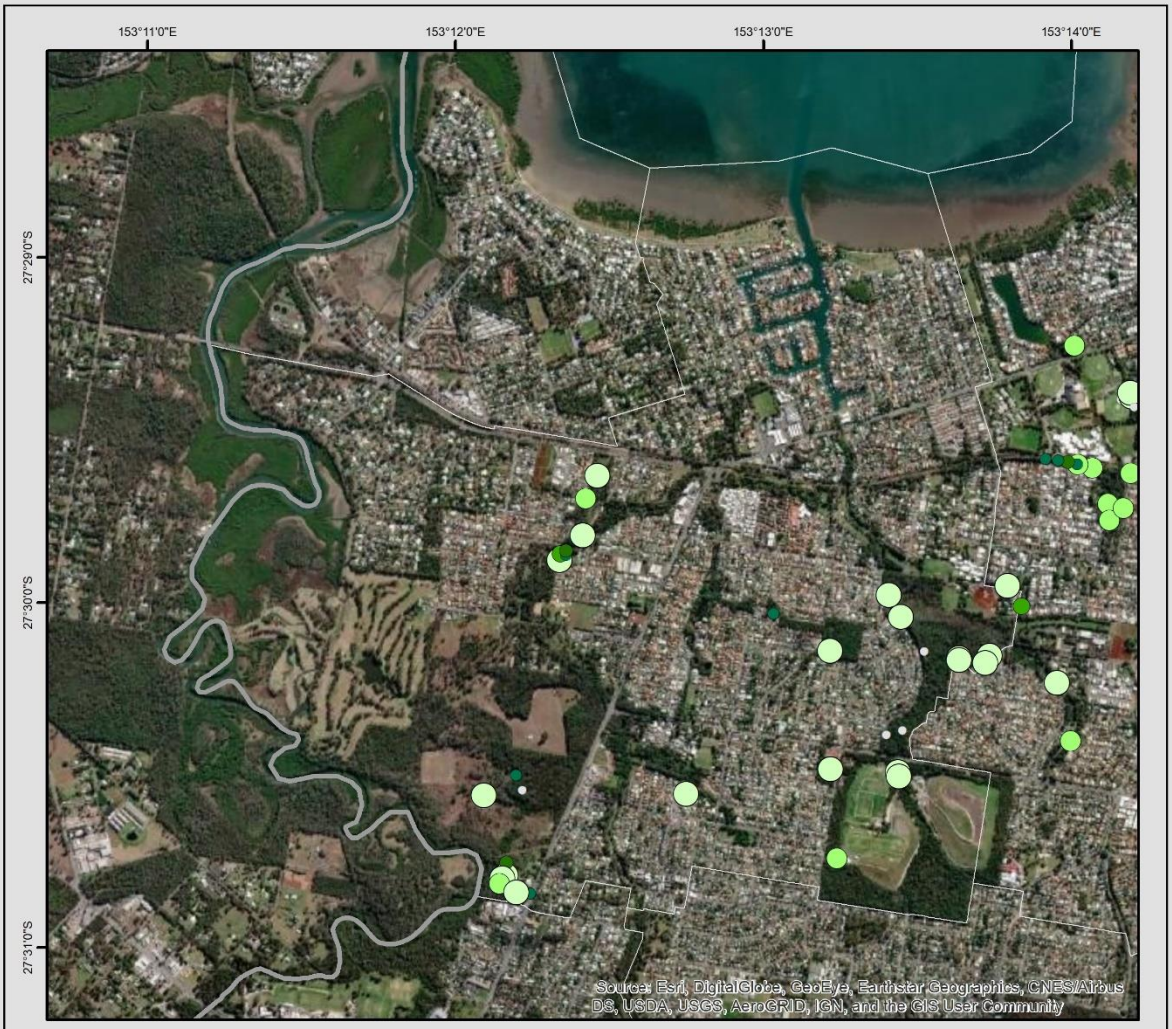


Birkdale

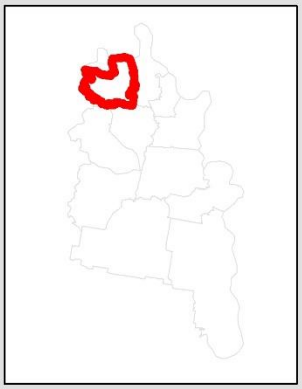
N

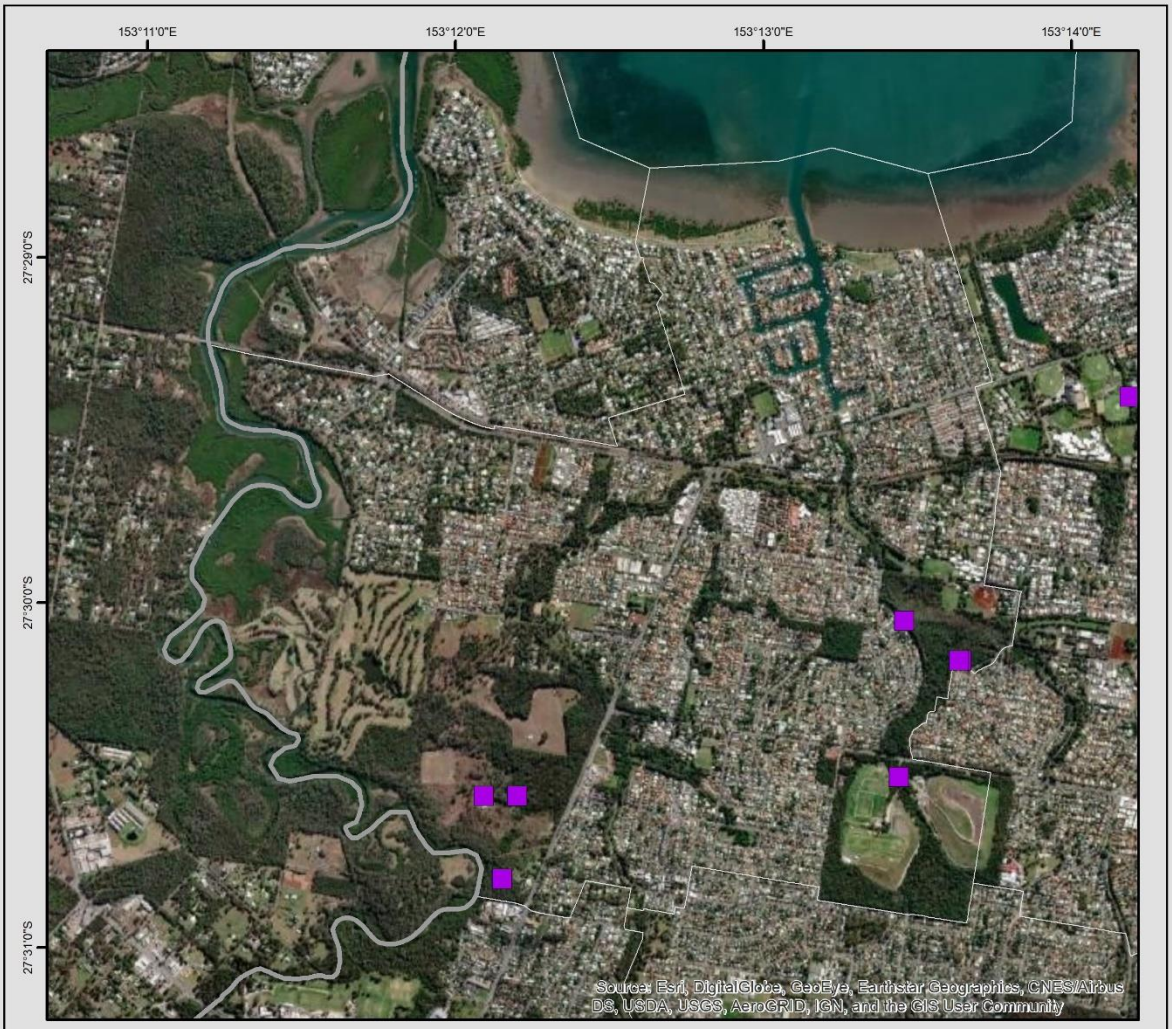
0 0.35 0.7 1.4 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Old
- Very old





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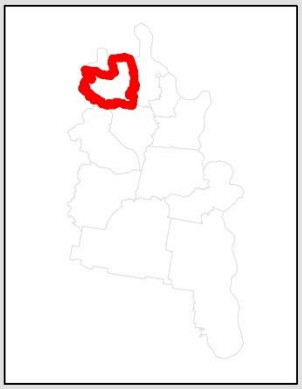
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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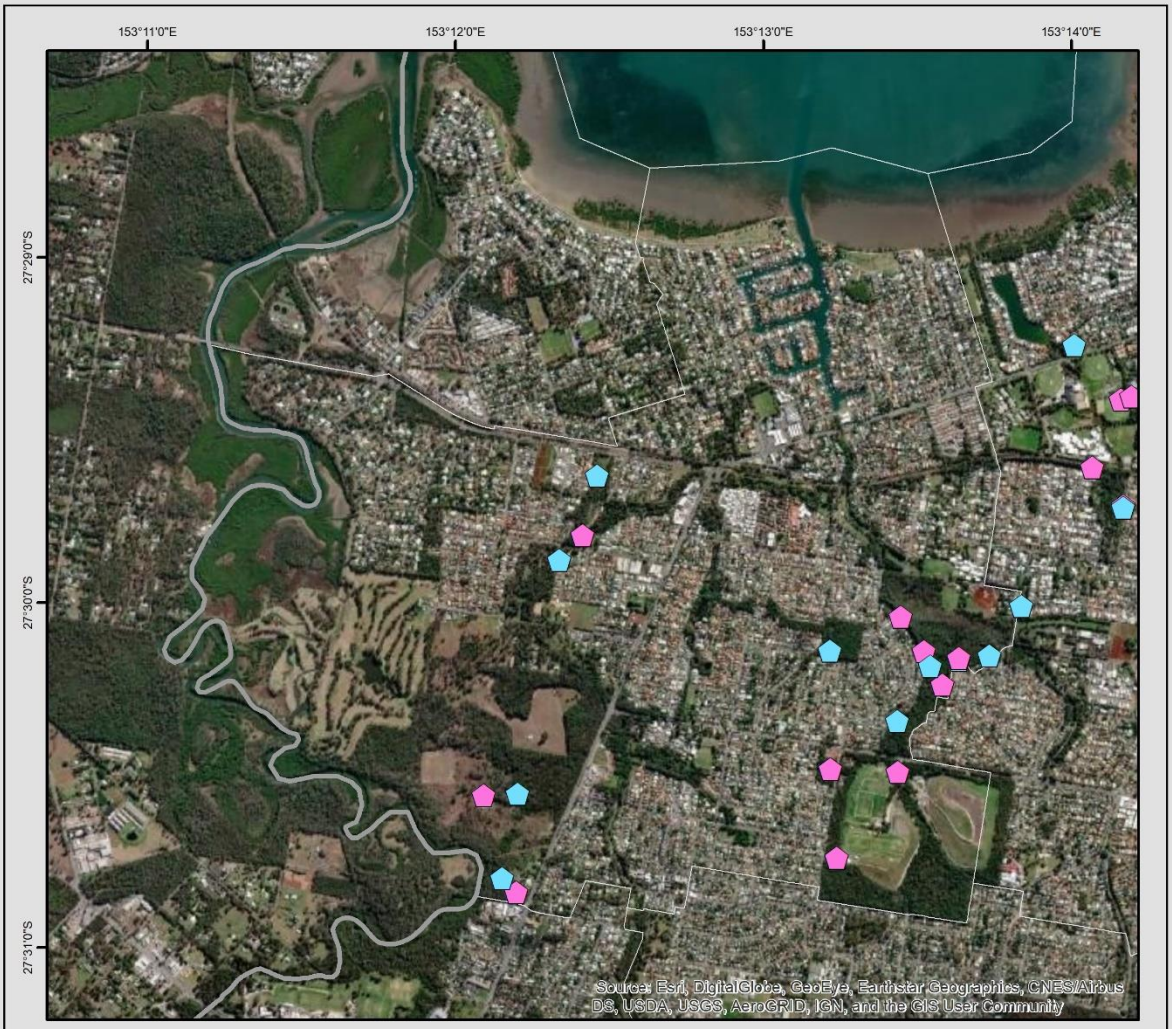


Birkdale

N

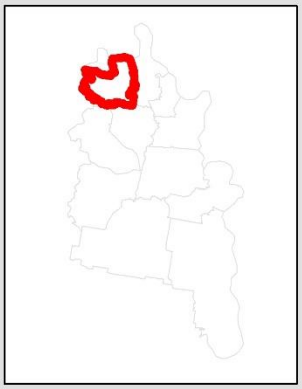
0 0.35 0.7 1.4
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male



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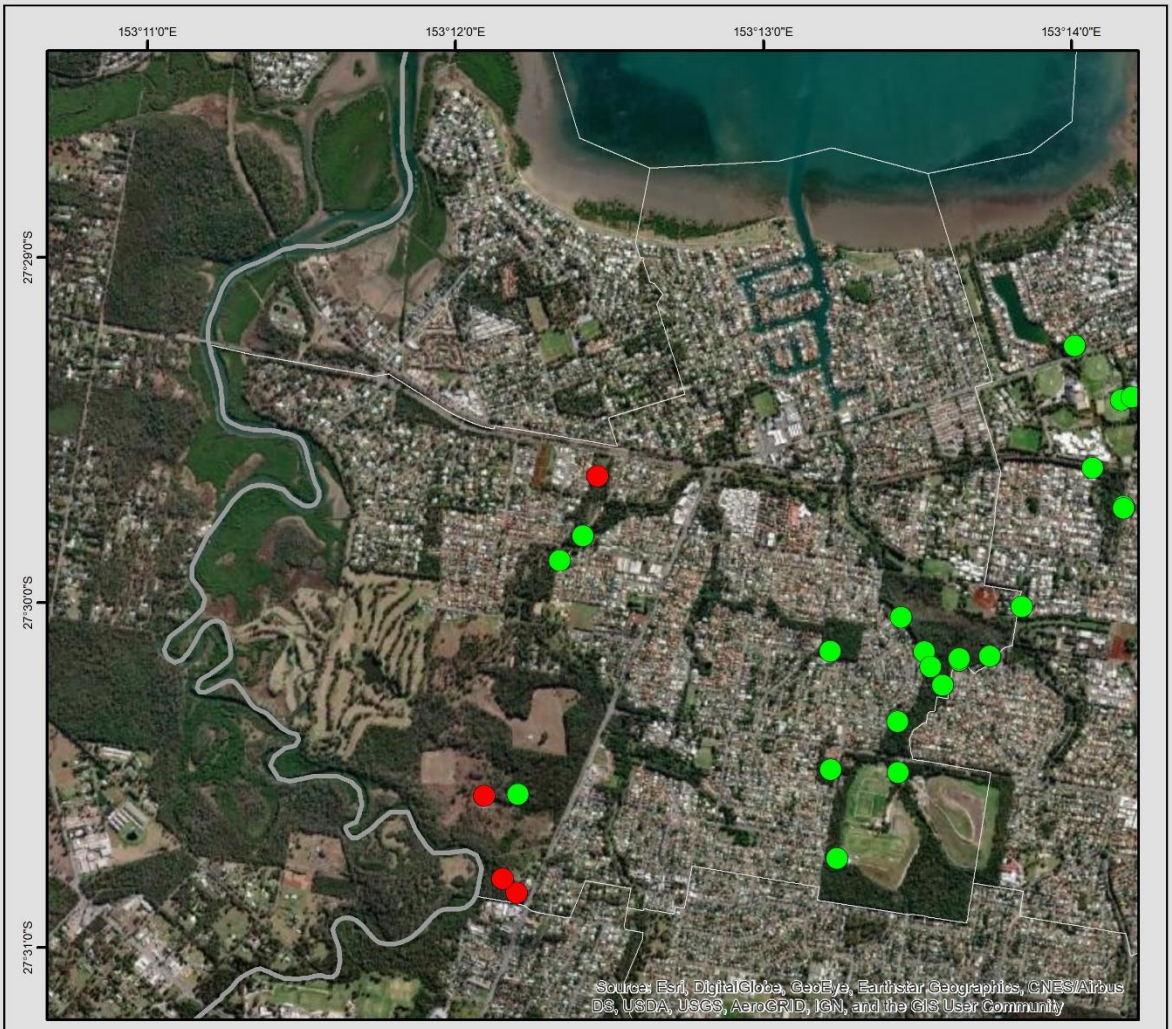

Birkdale

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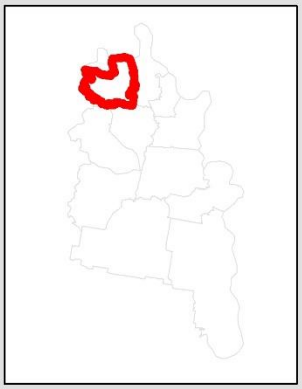
0 0.35 0.7 1.4
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Chlamydia pathogen

- Detected
- Not detected



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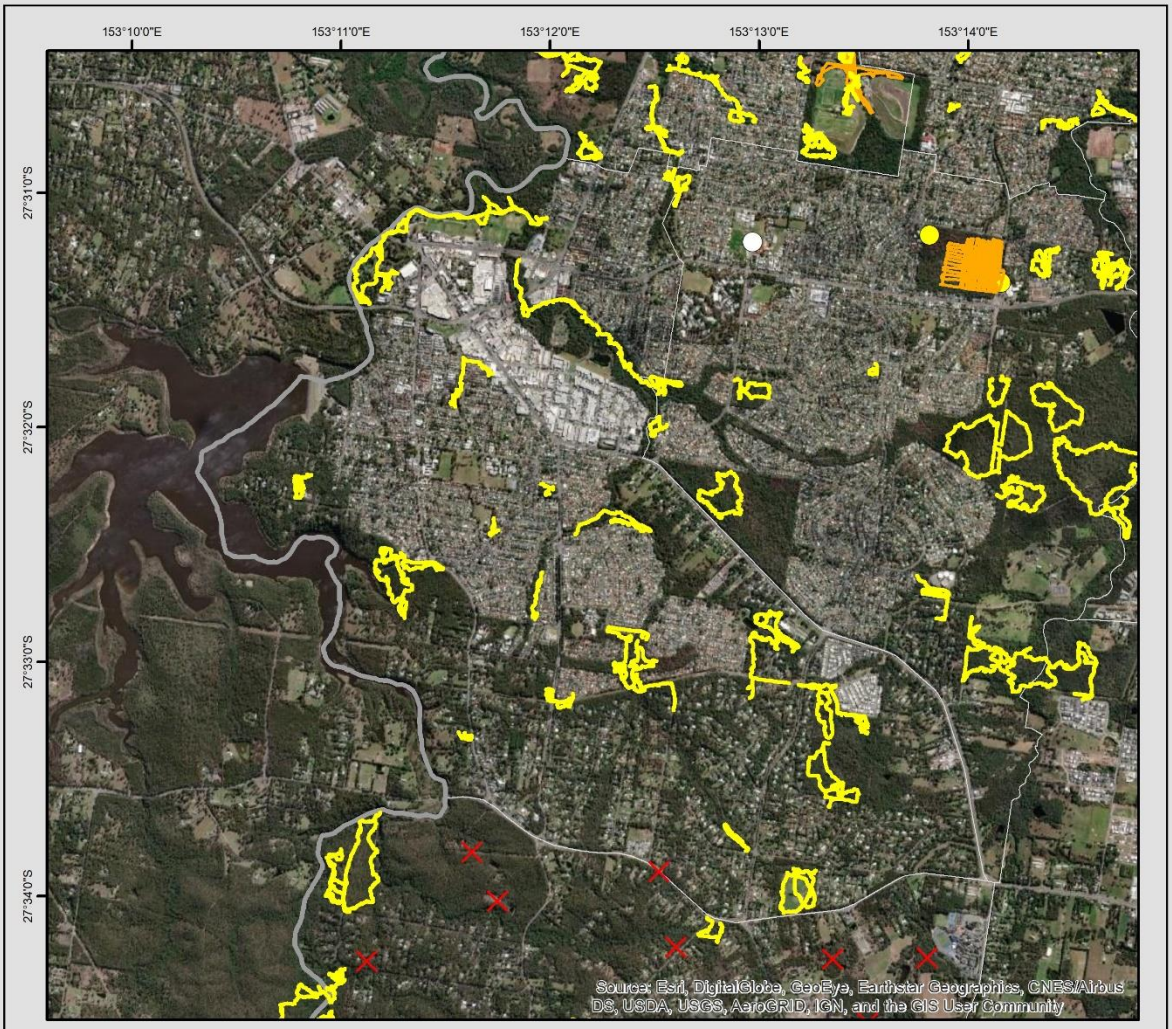
Capalaba



Capalaba

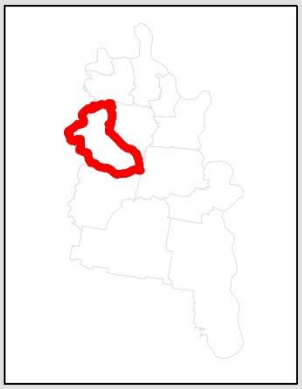
N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog survey tracks
- Drone flights
- Drone survey
- Access not granted
- Survey site inappropriate (no trees)



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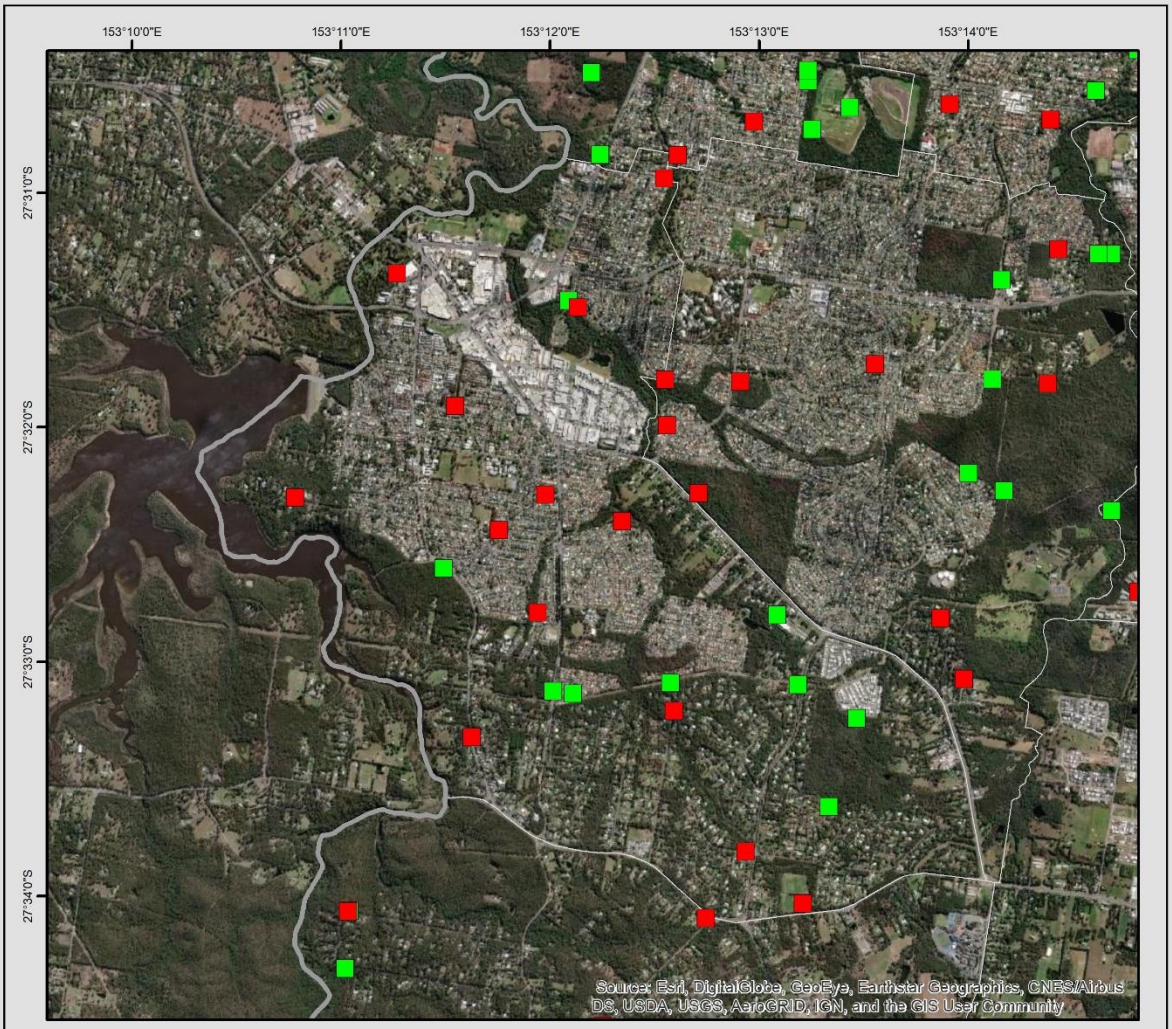


Capalaba

N

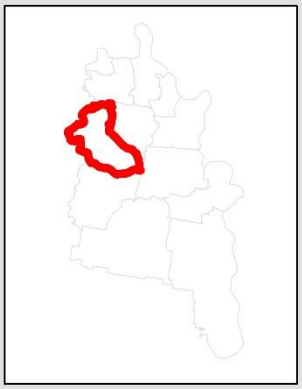
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence



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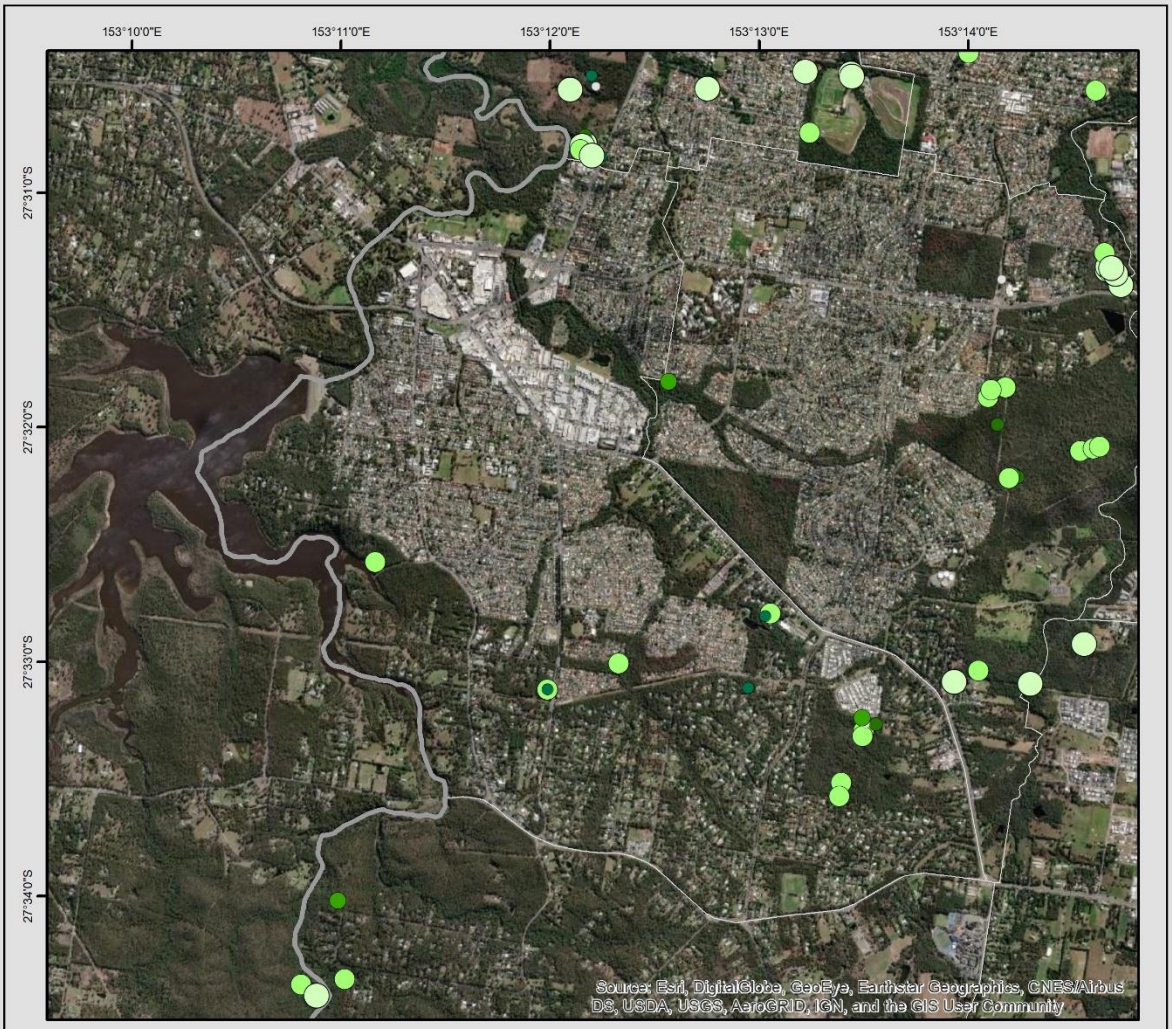


Capalaba

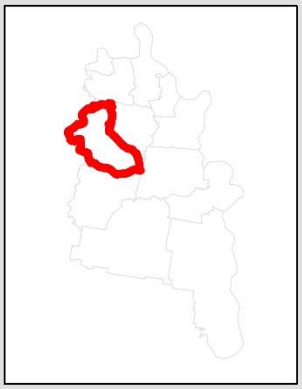
N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Old
- Very old



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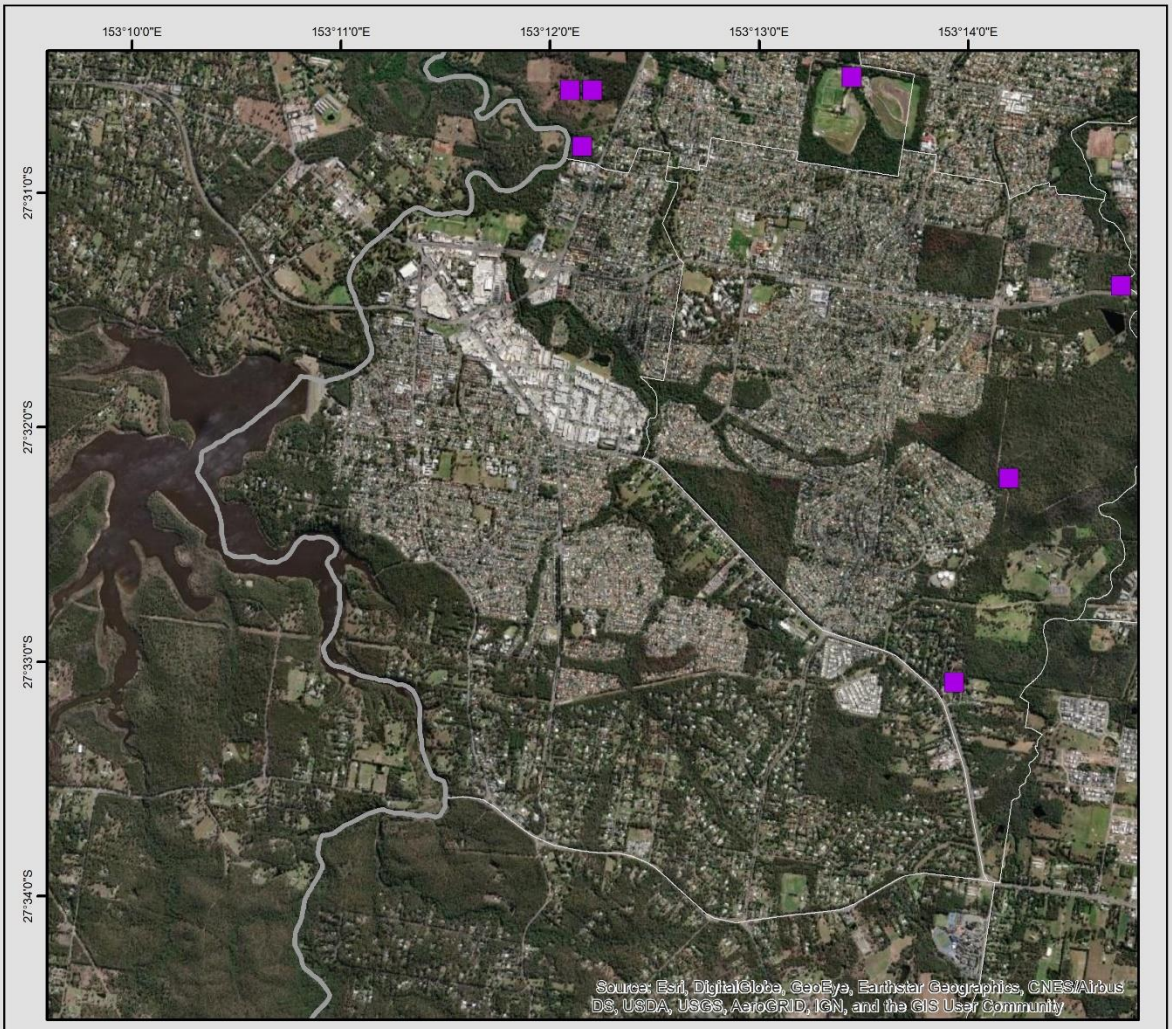


Capalaba

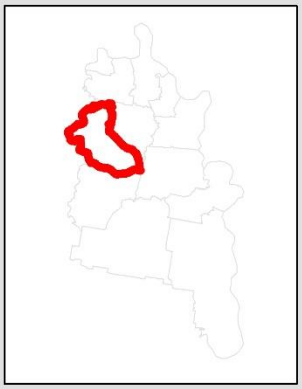
N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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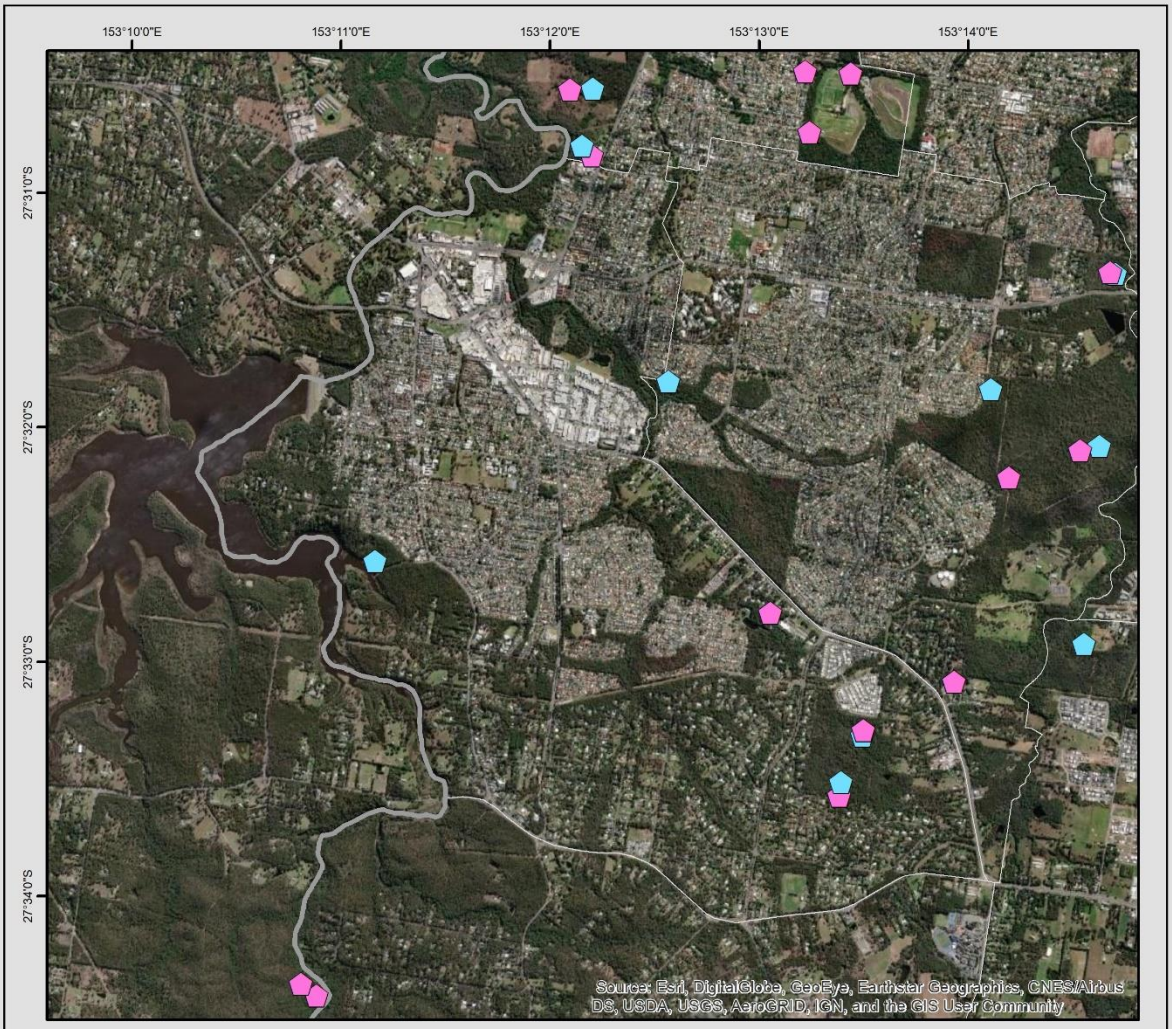


Capalaba

N

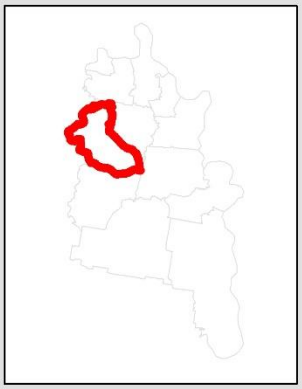
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male



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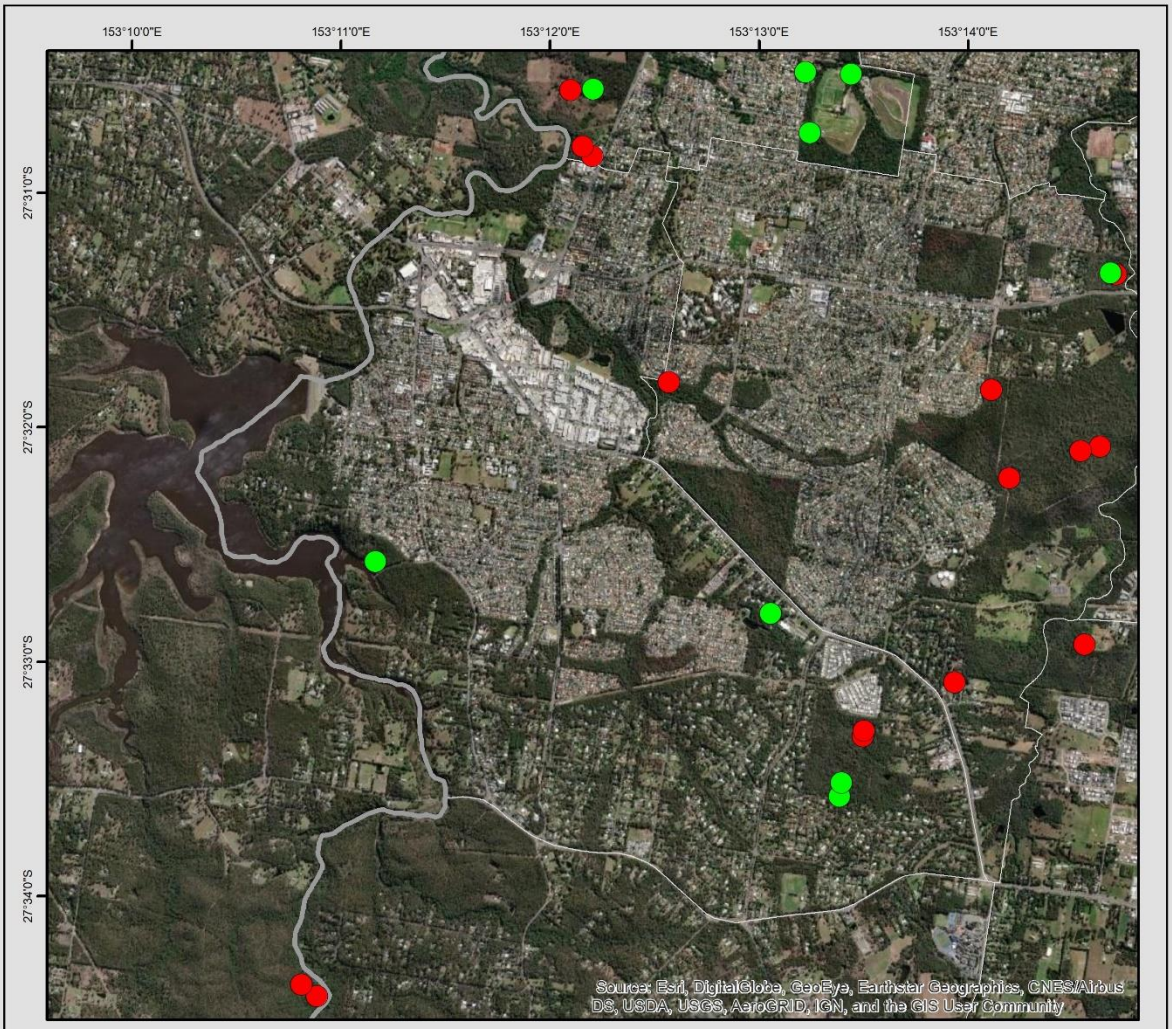


Capalaba

N

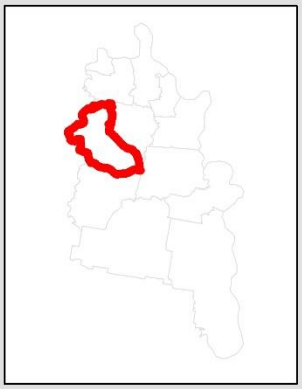
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Chlamydia pathogen

- Detected
- Not detected



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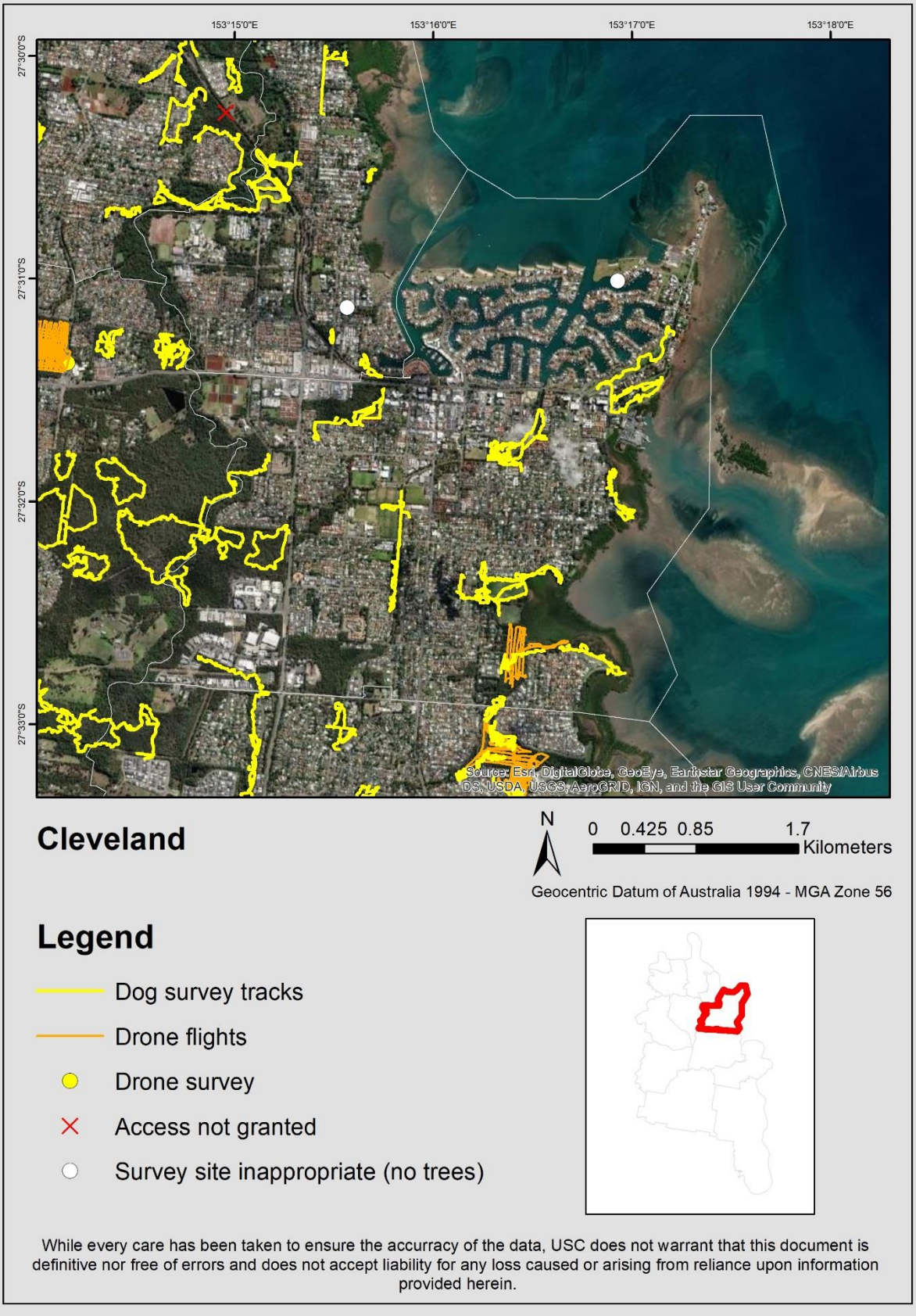
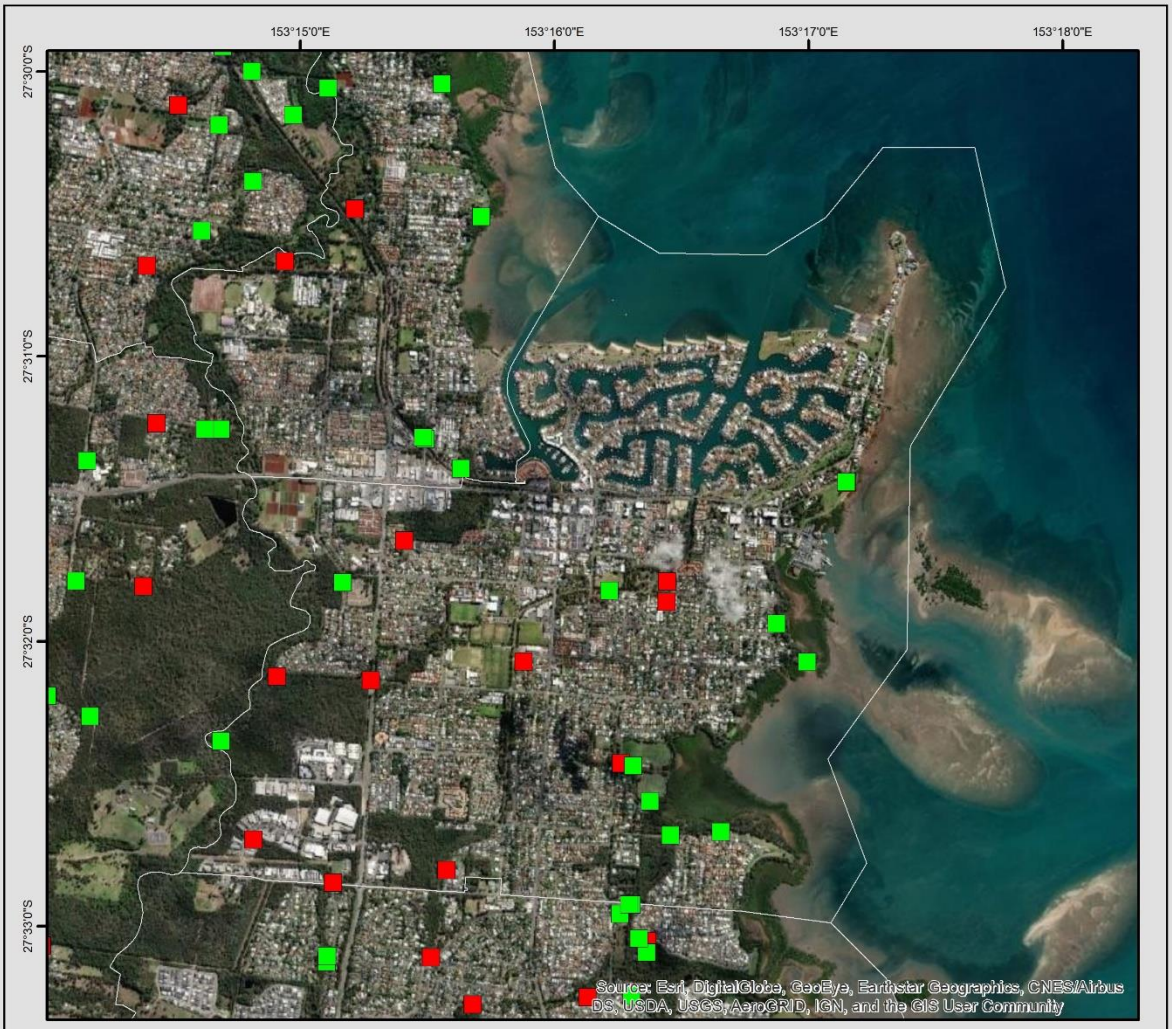
Cleveland





Cleveland

N

0 0.425 0.85 1.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence

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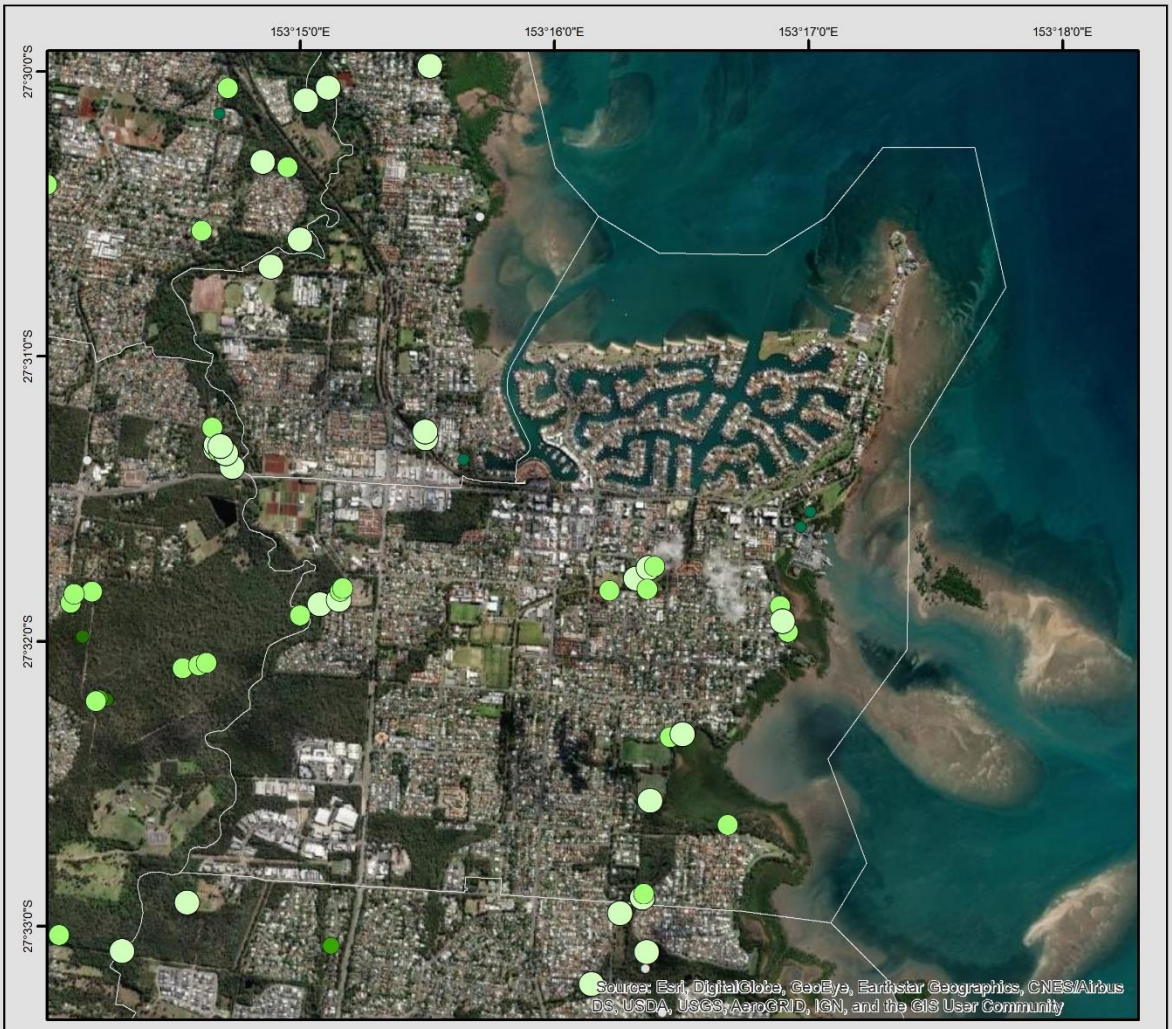

Cleveland

N

0 0.425 0.85 1.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Old
- Very old

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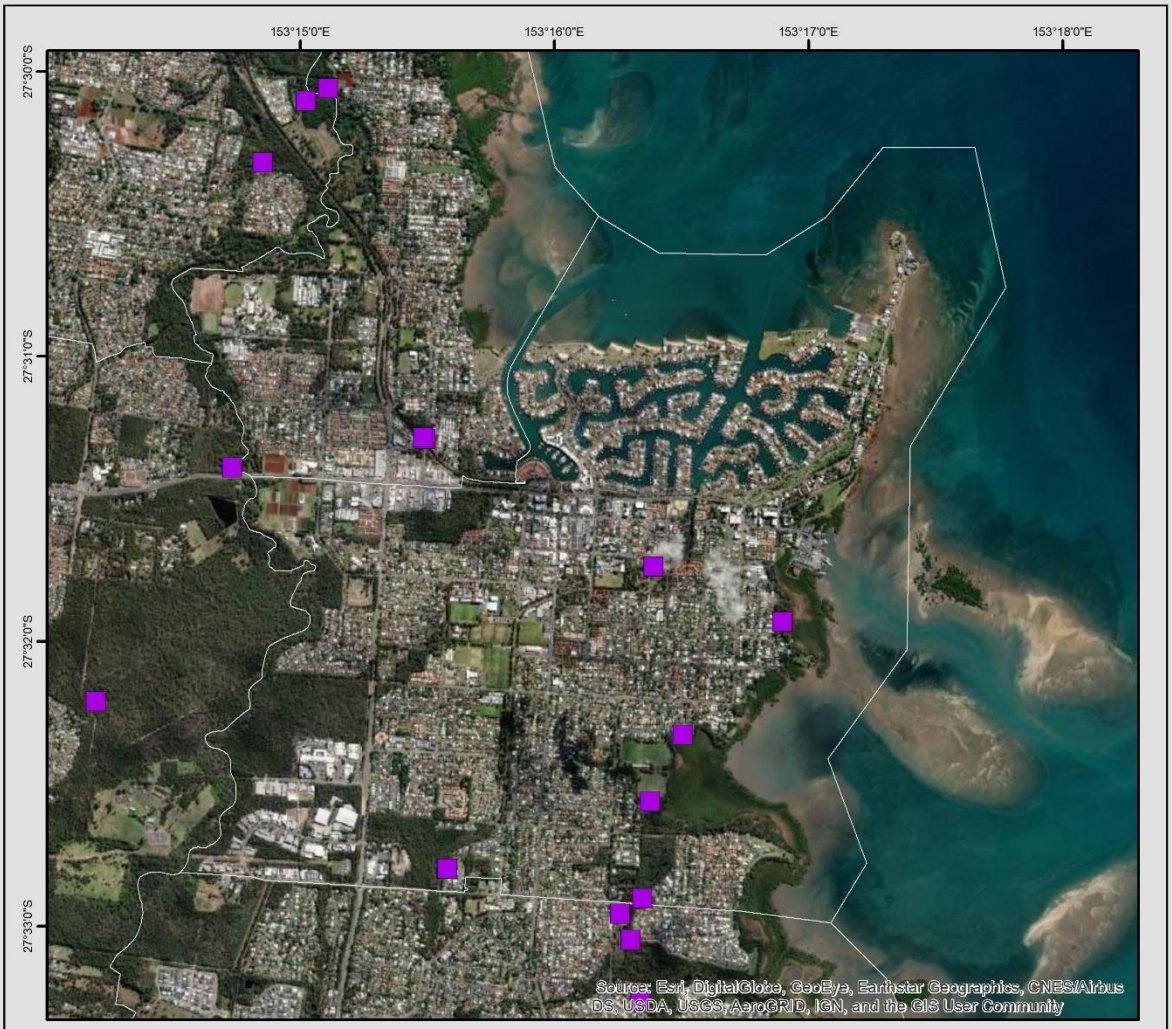

Cleveland

N

0 0.425 0.85 1.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings

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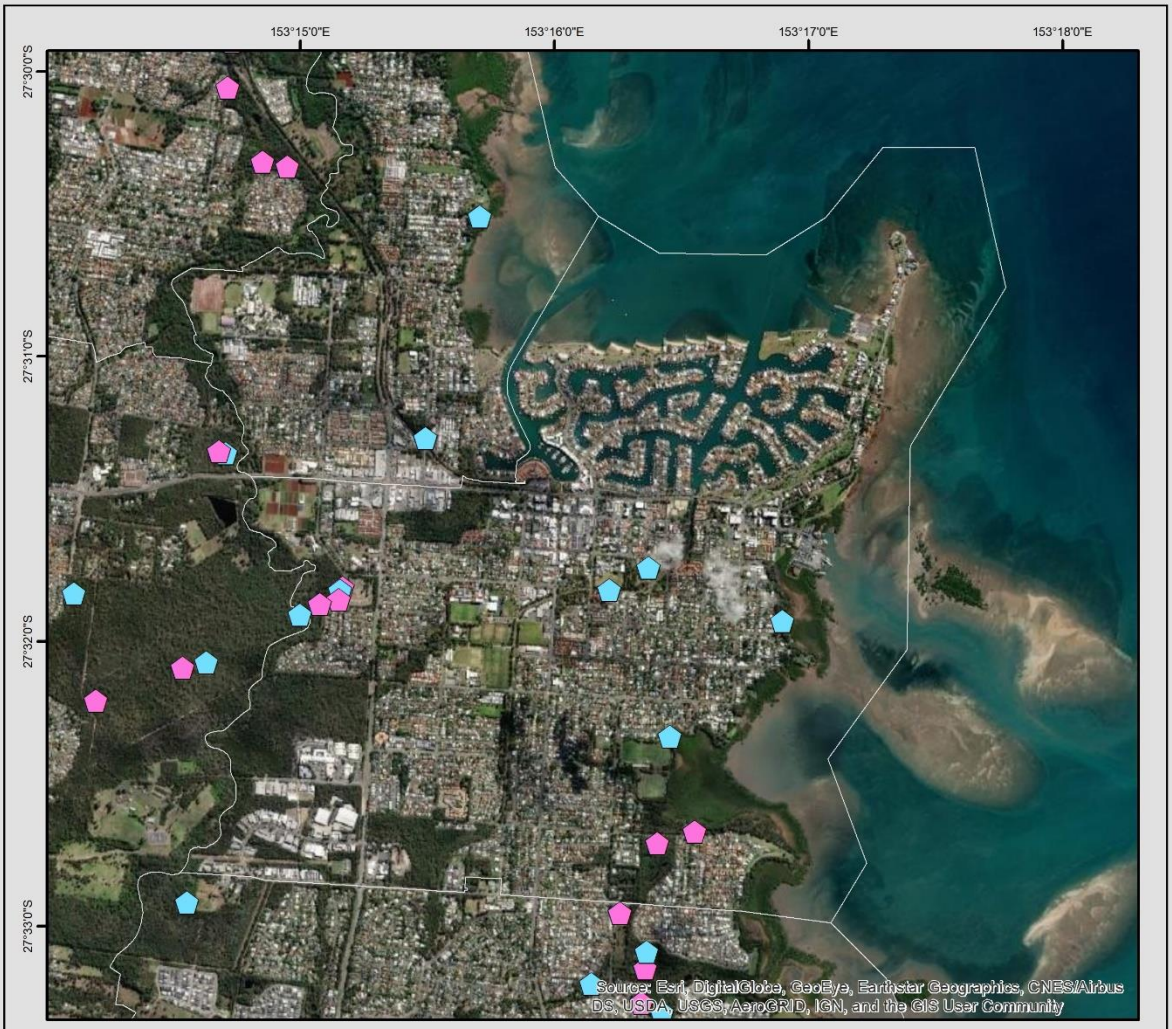

Cleveland

N

0 0.425 0.85 1.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male

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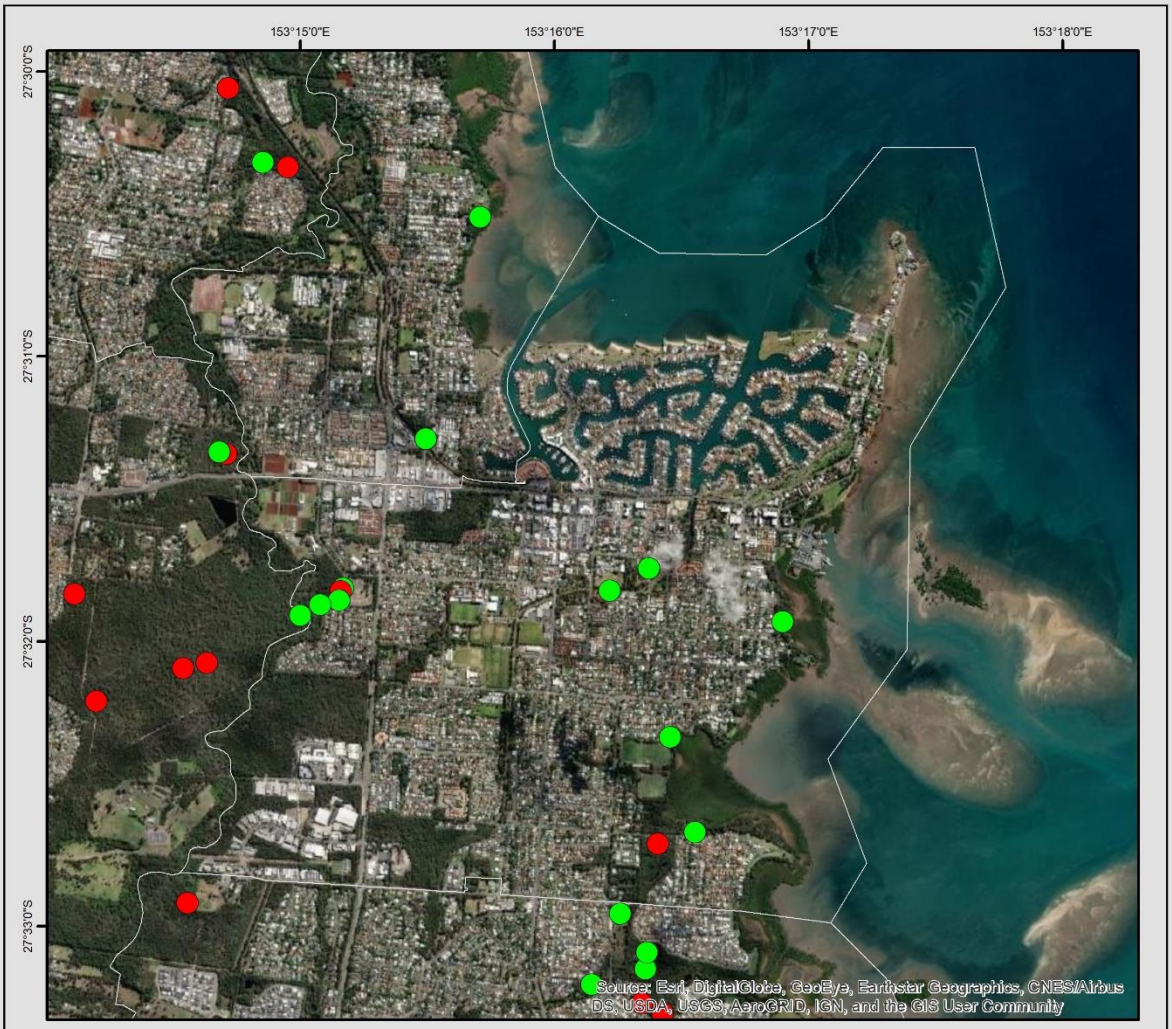

Cleveland

N

0 0.425 0.85 1.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Chlamydia pathogen

- Detected
- Not detected

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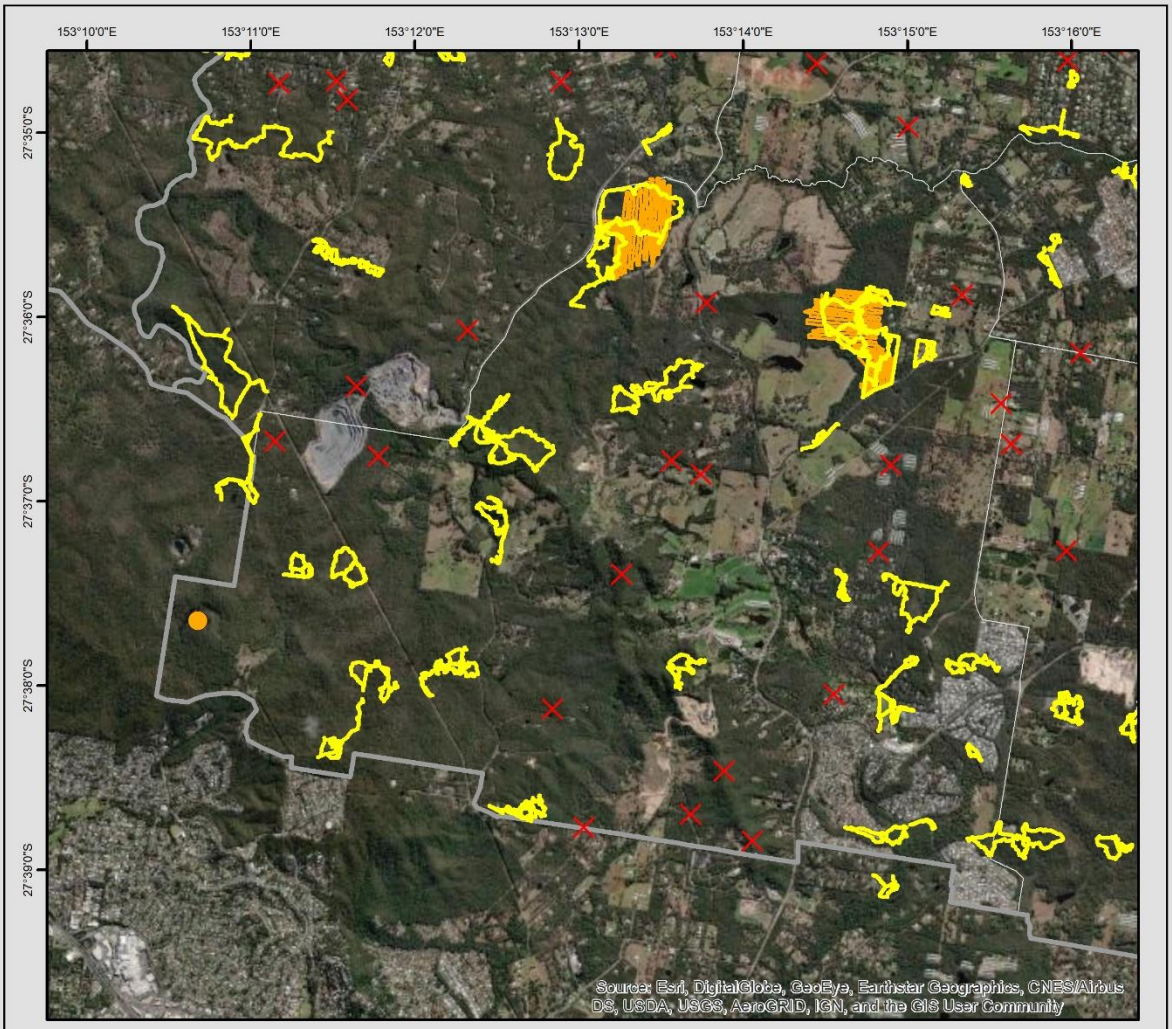
Mount Cotton



Mount Cotton

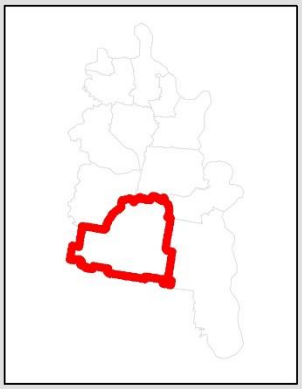
N

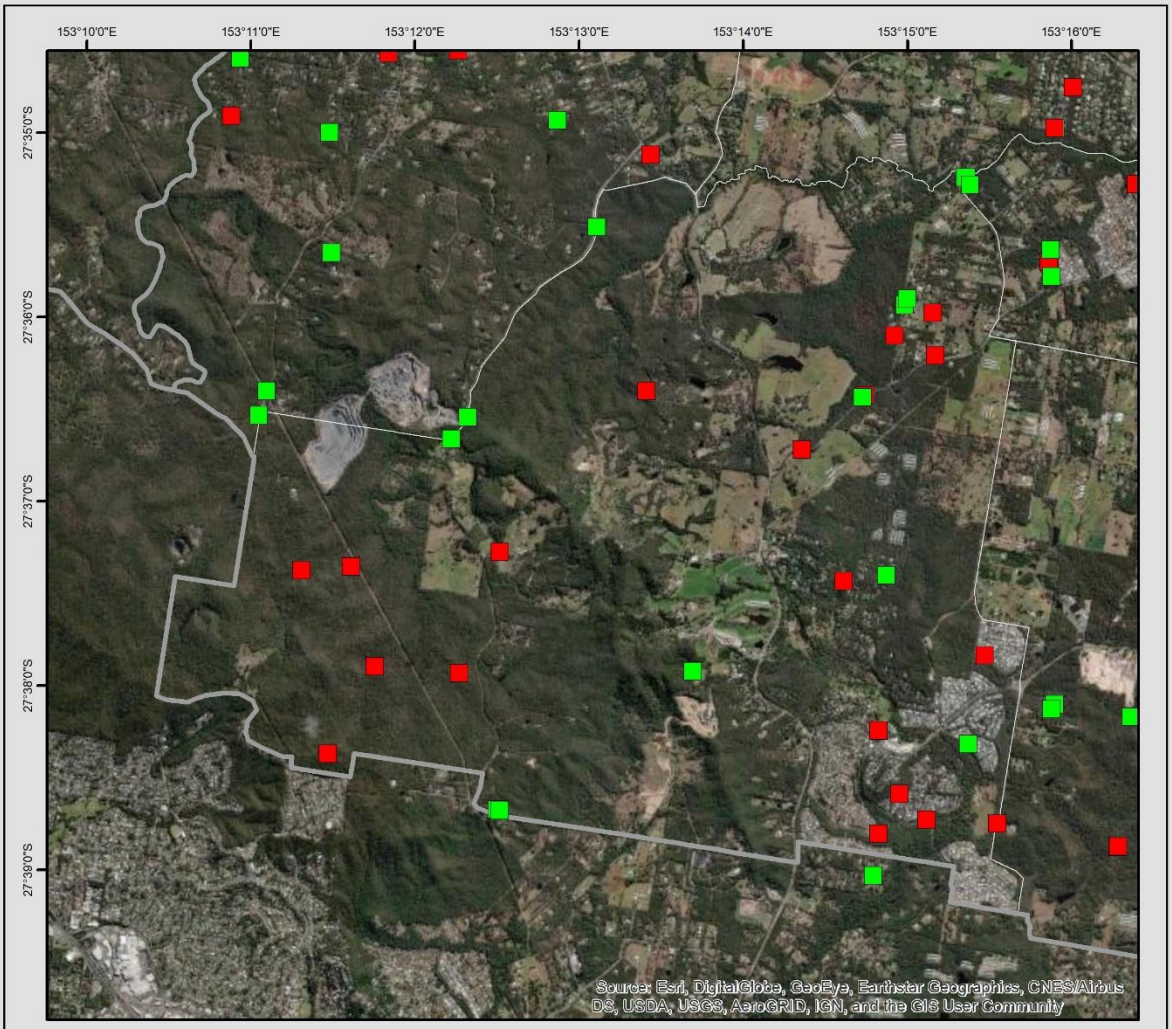
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog survey tracks
- Drone flights
- Access not granted
- Access too difficult





Mount Cotton

N

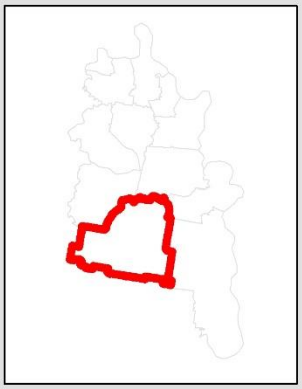
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence



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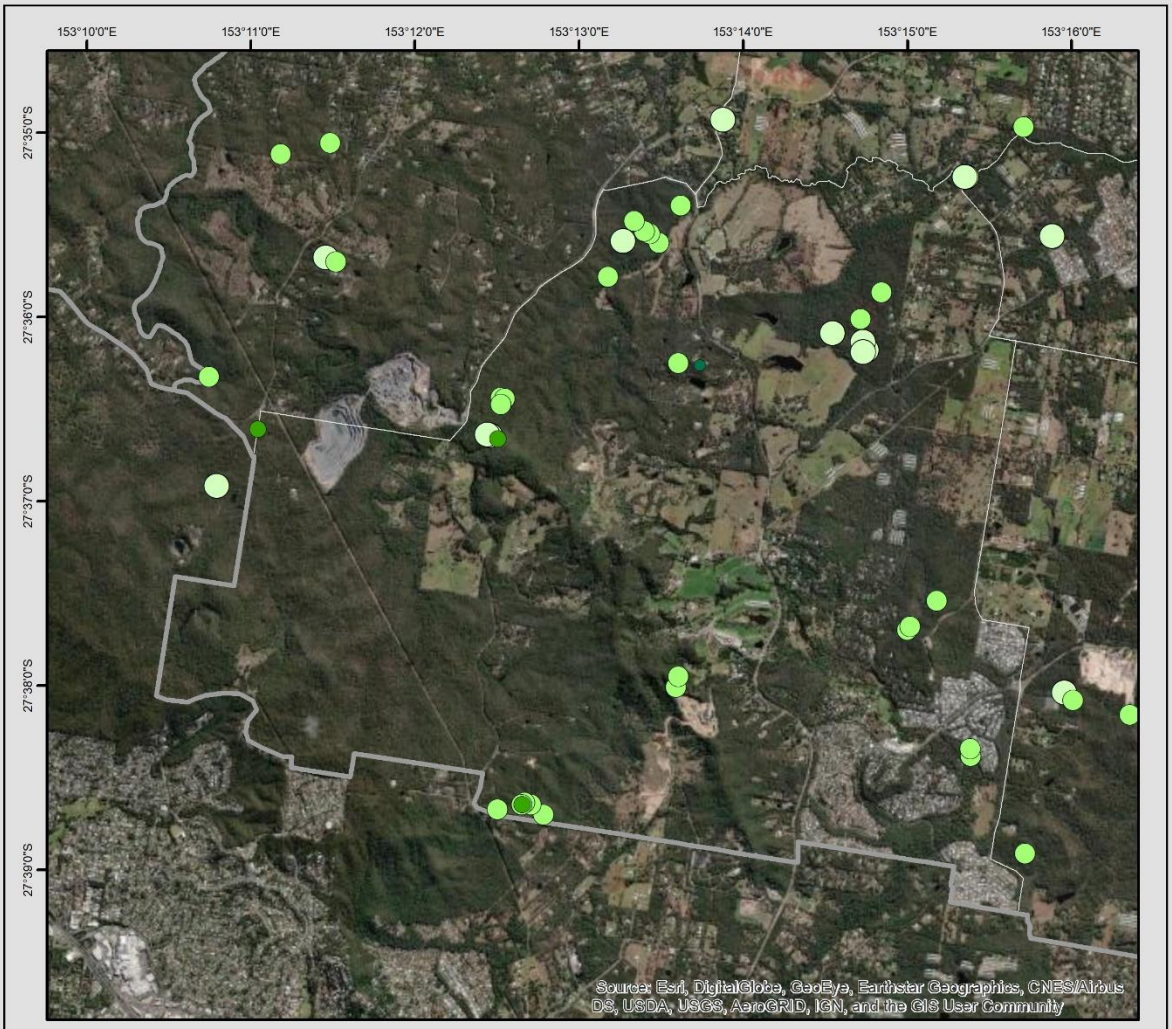


Mount Cotton

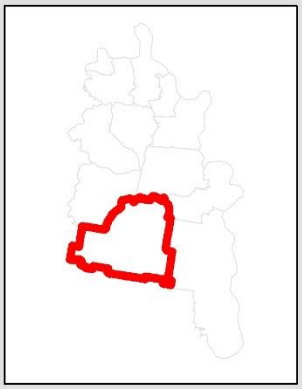
N

0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Very old



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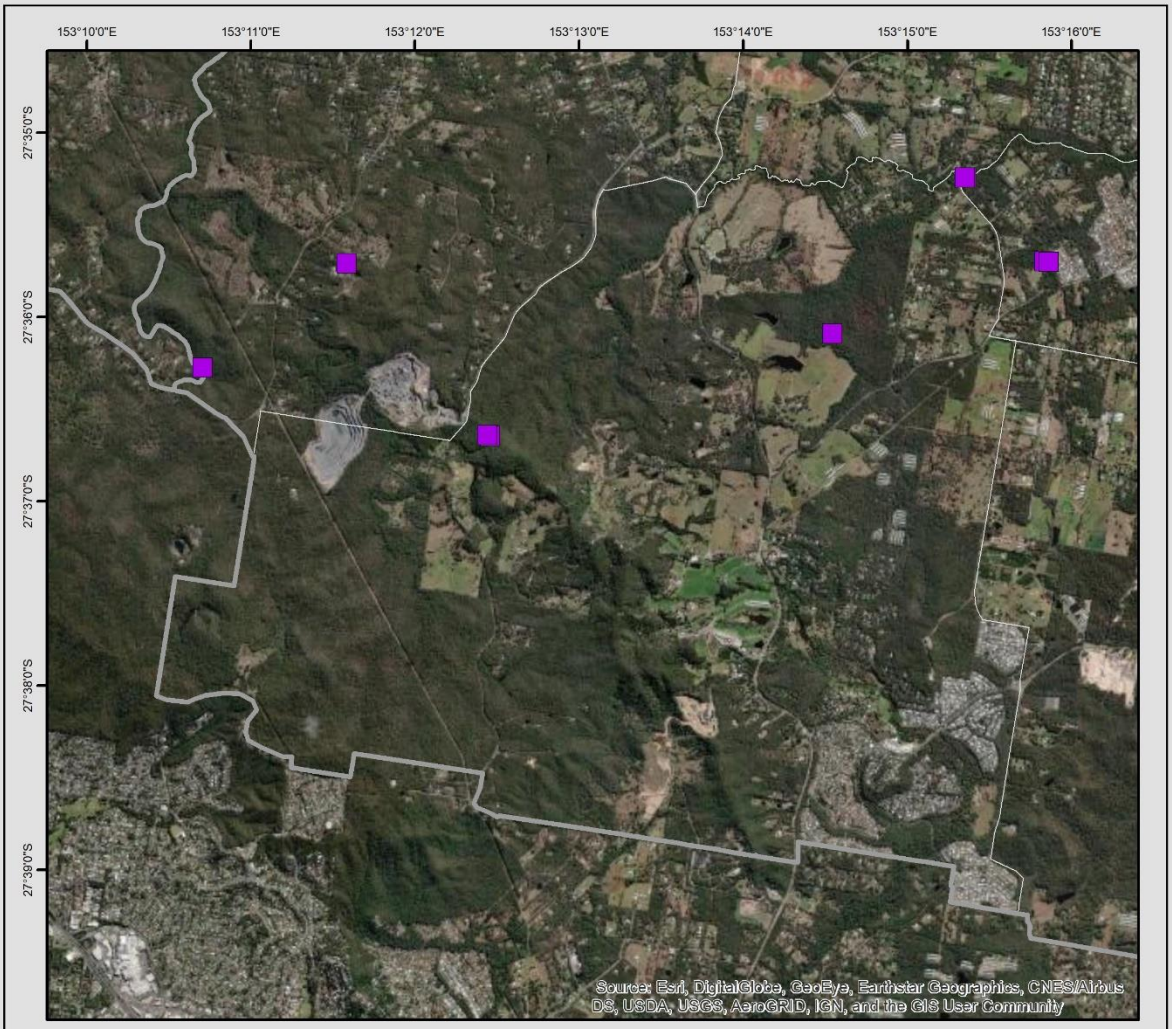


Mount Cotton

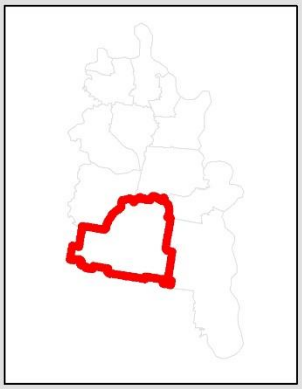
N

0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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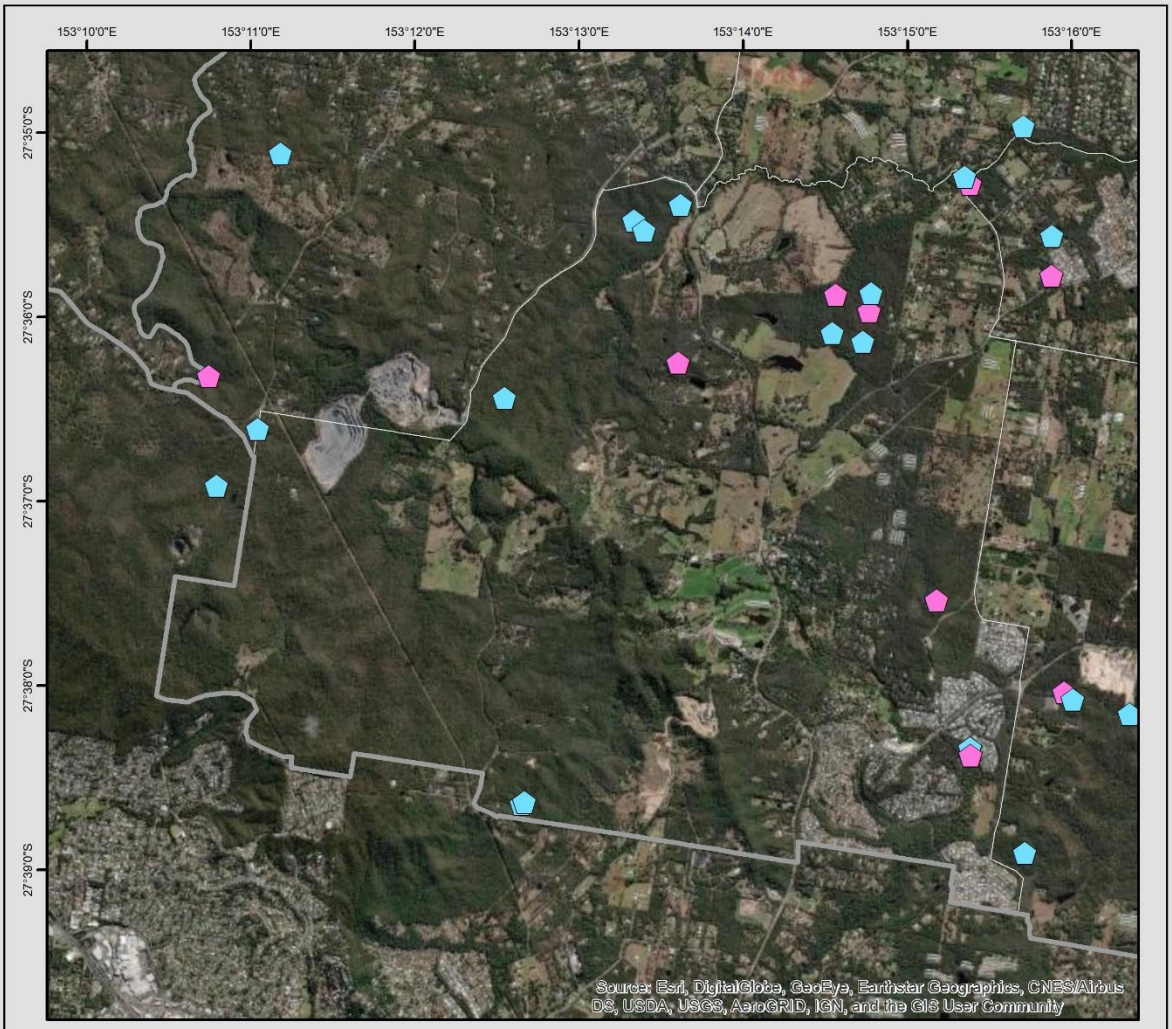


Mount Cotton

N

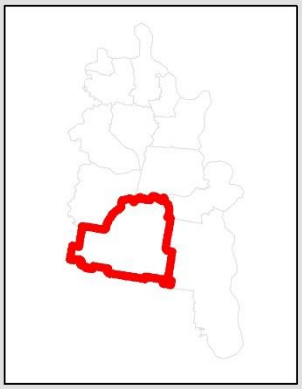
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male



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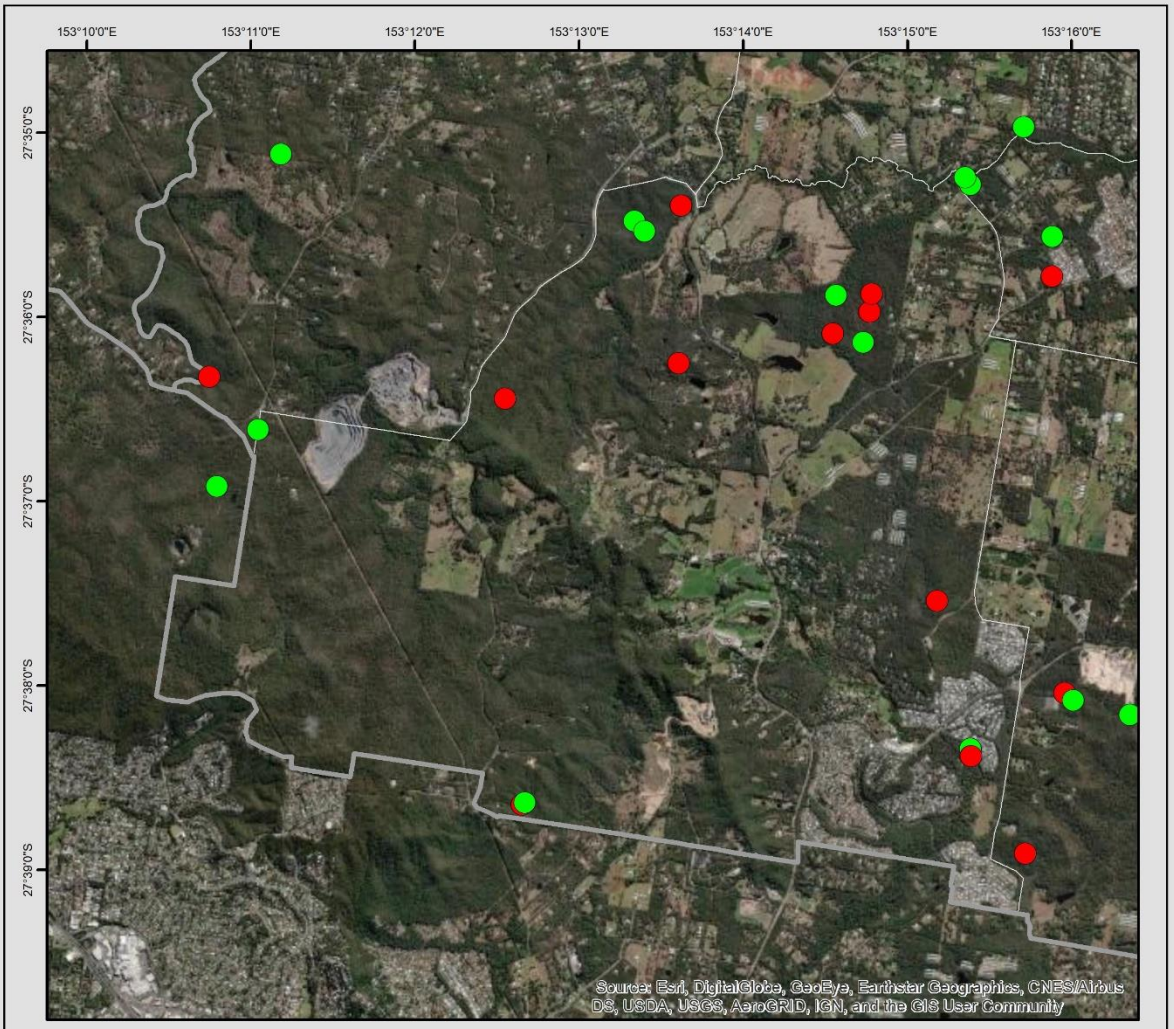


Mount Cotton

N

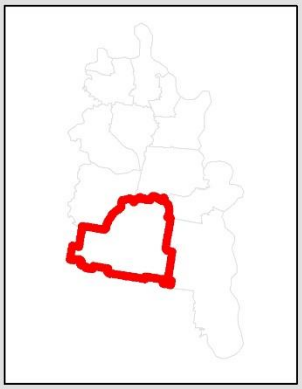
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Chlamydia pathogen

- Detected
- Not detected



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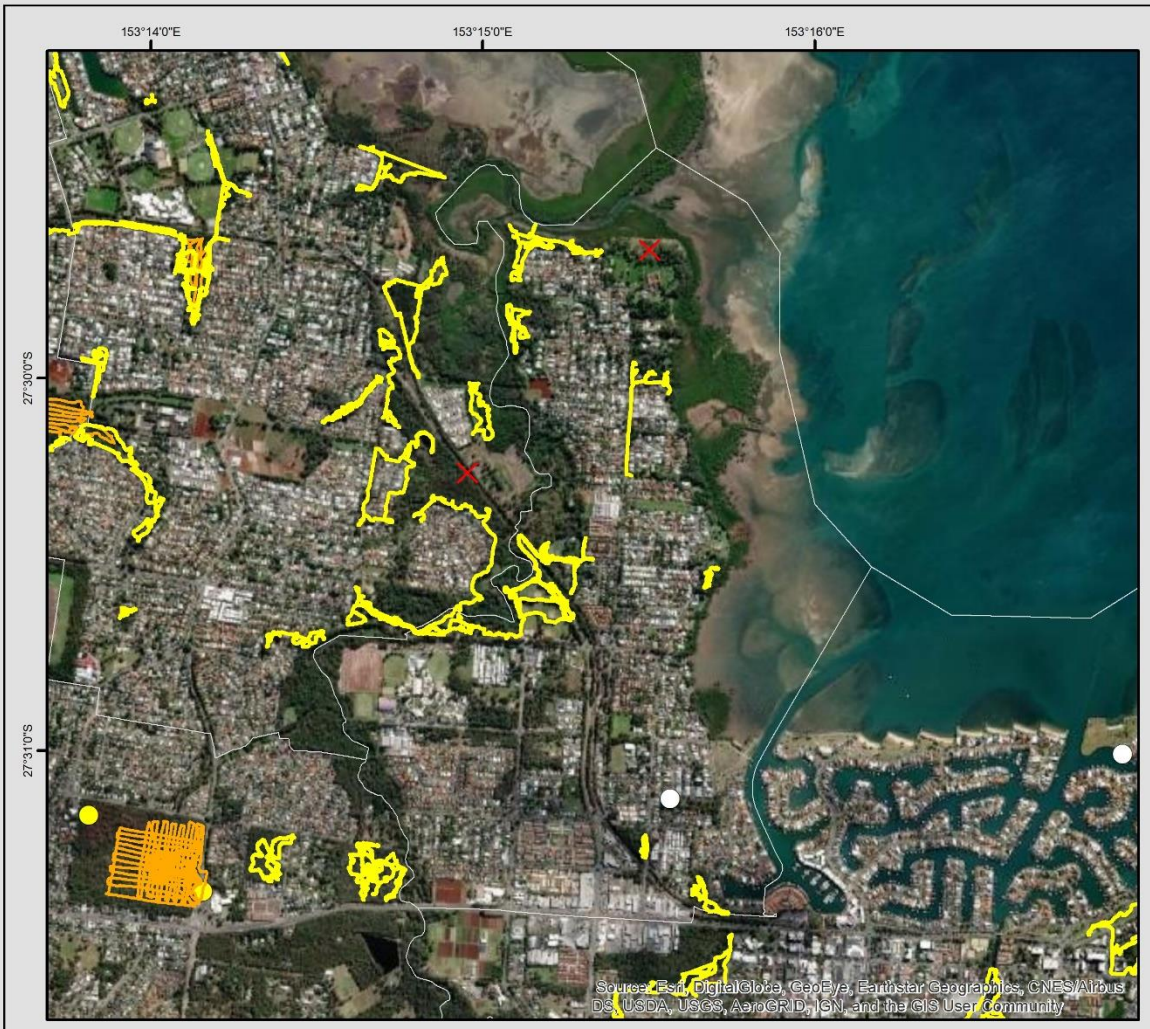
Ormiston



Ormiston

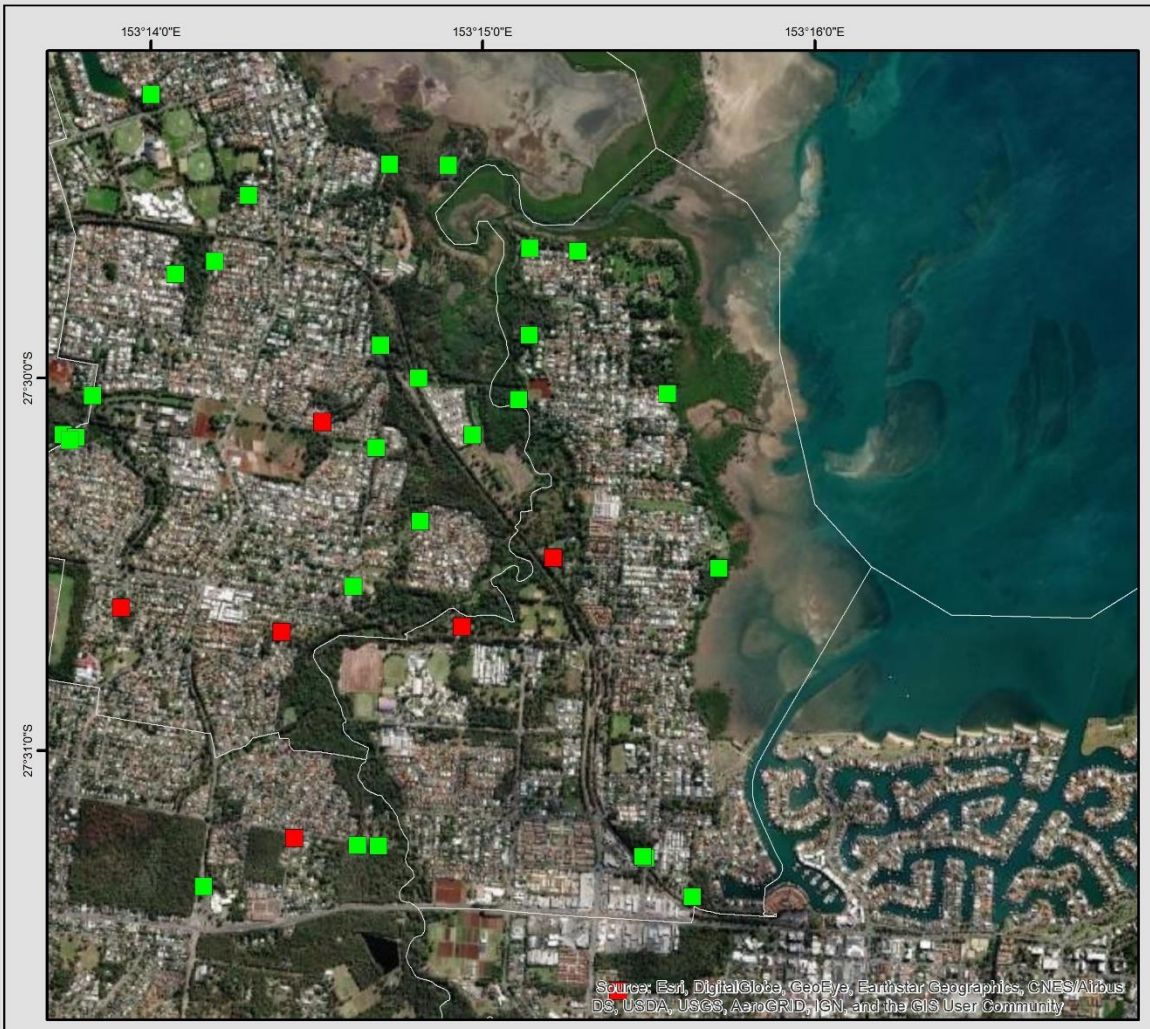
N

0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog survey tracks
- Drone flights
- Drone survey
- Access not granted
- Survey site inappropriate (no trees)



Ormiston

N

0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence

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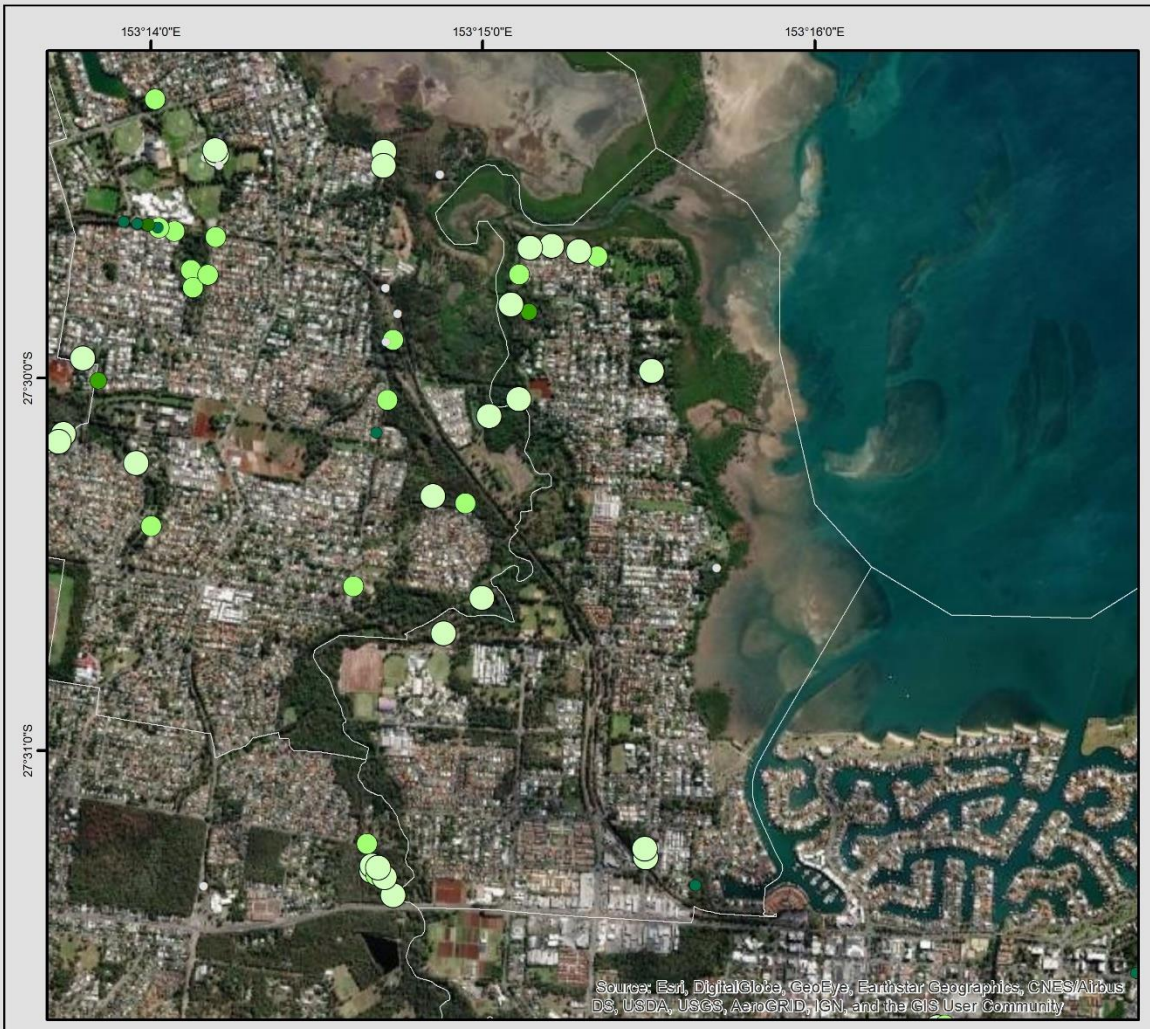

Ormiston

N

0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Old
- Very old

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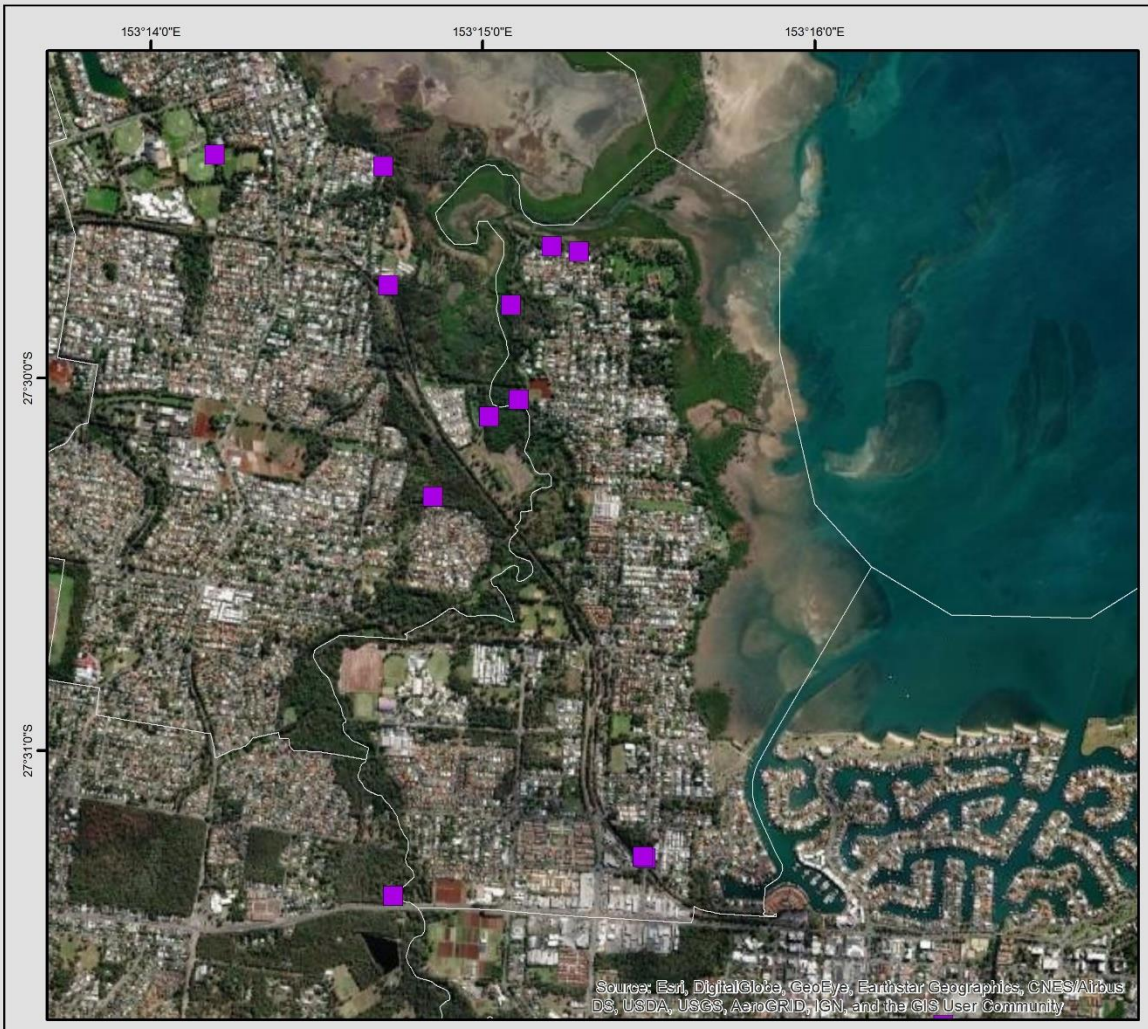


Ormiston

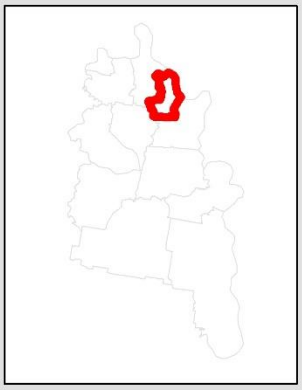
N

0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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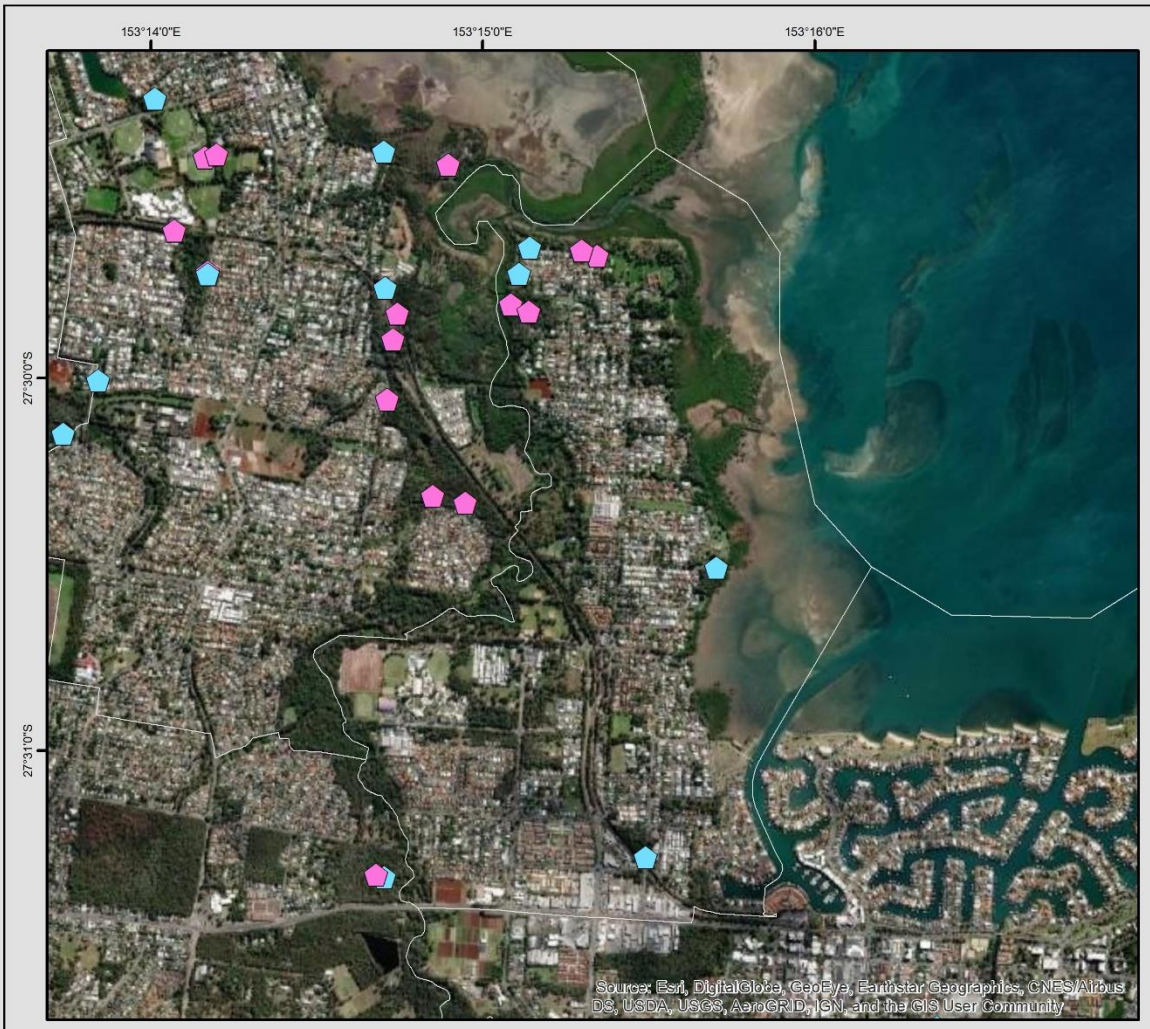

Ormiston

N

0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male

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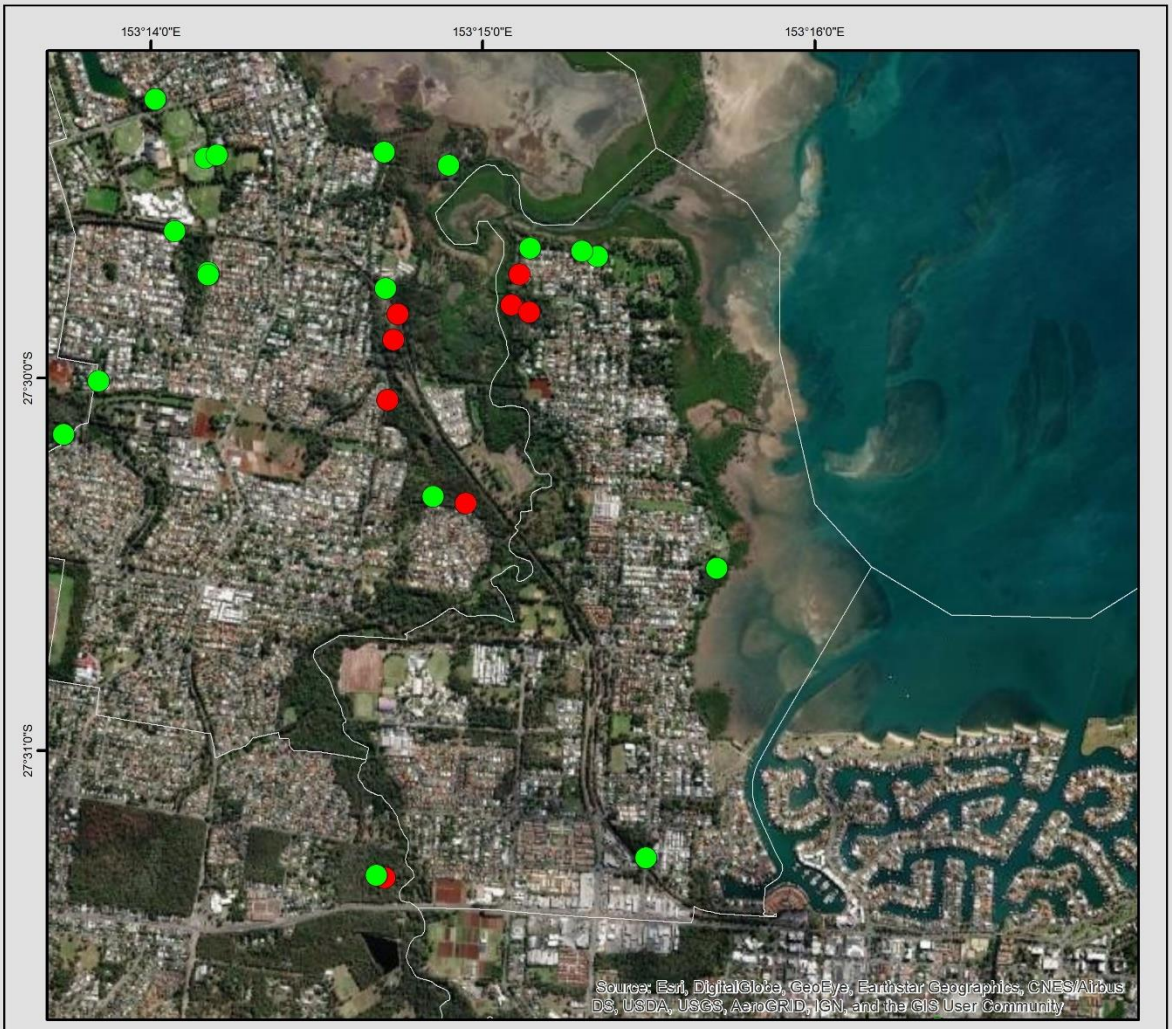


Ormiston

N

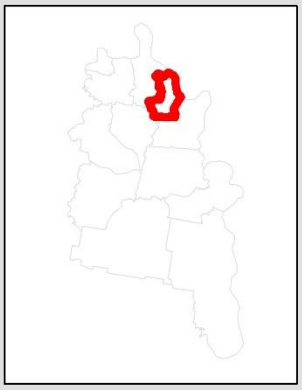
0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Chlamydia pathogen

- Detected
- Not detected



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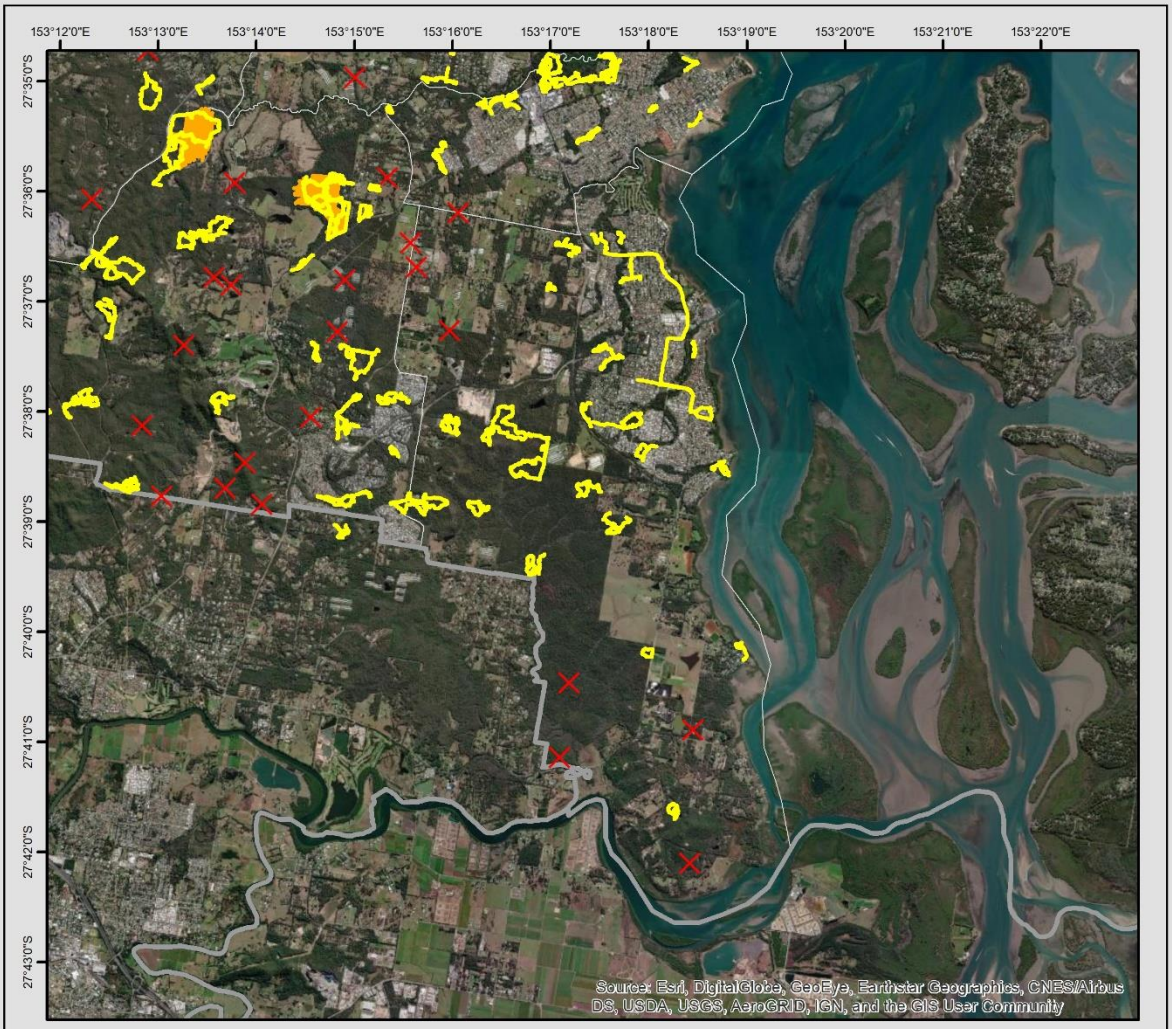
Redland Bay



Redland Bay

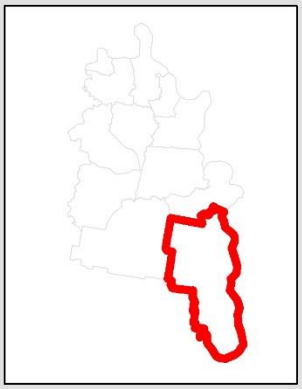
N

0 1 2 4 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog survey tracks
- Drone flights
- Access not granted



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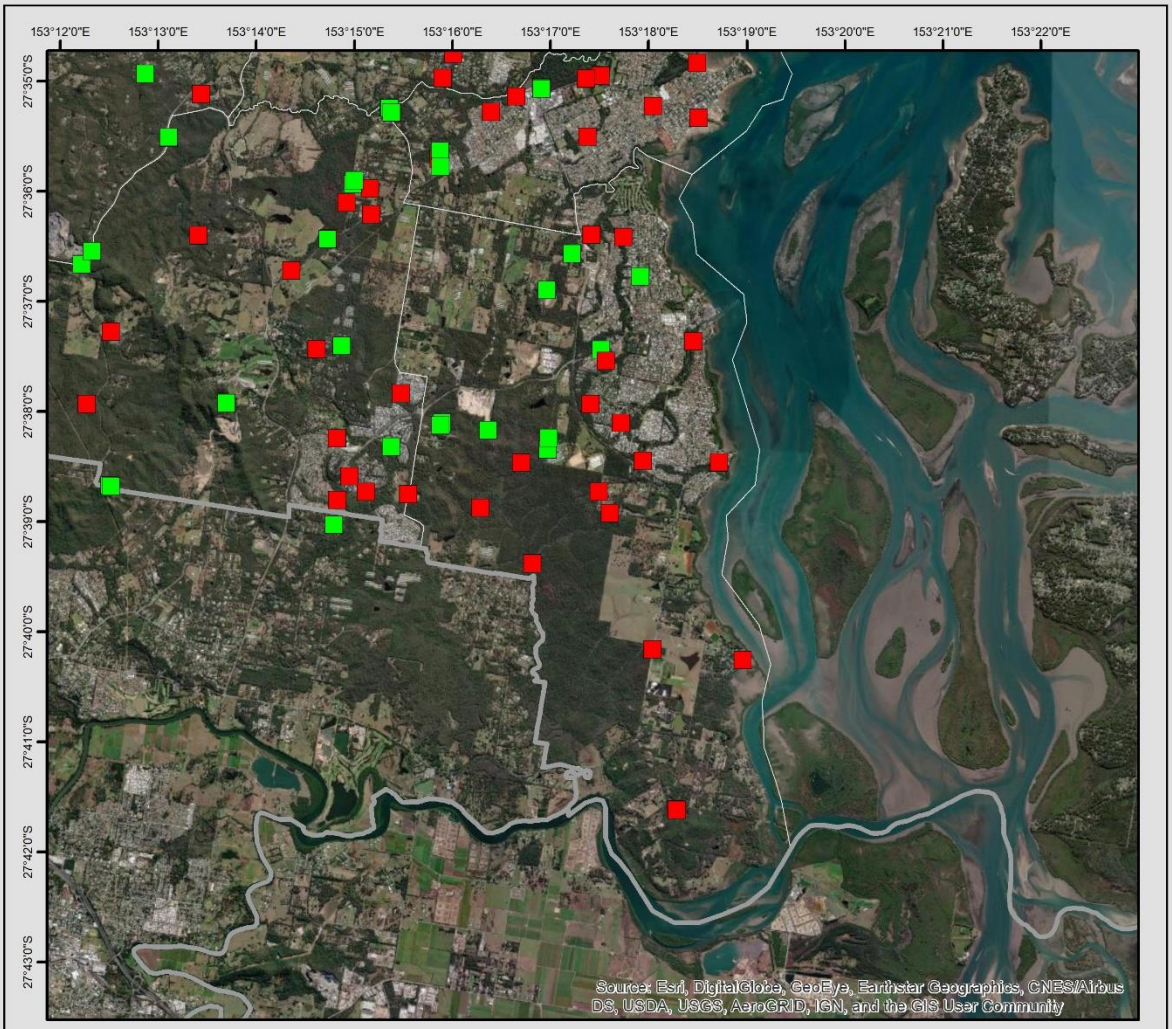


Redland Bay

N

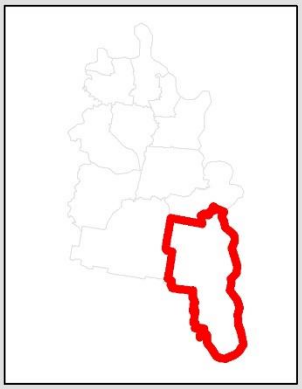
0 1 2 4 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence



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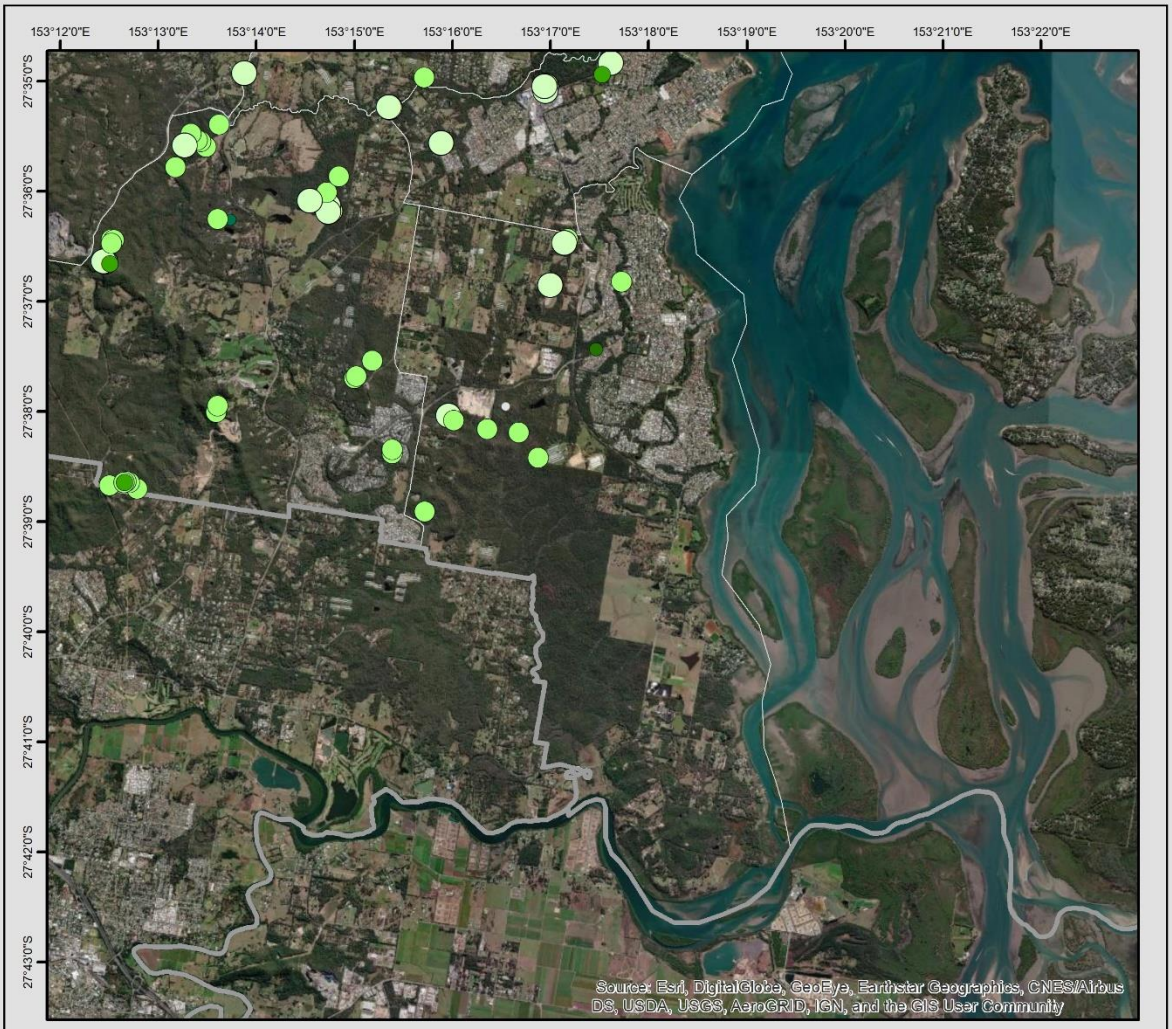


Redland Bay

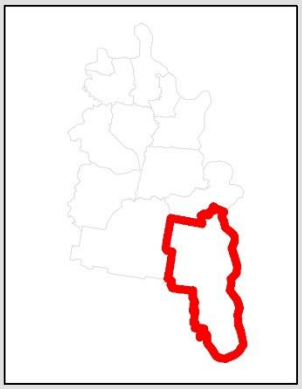
N

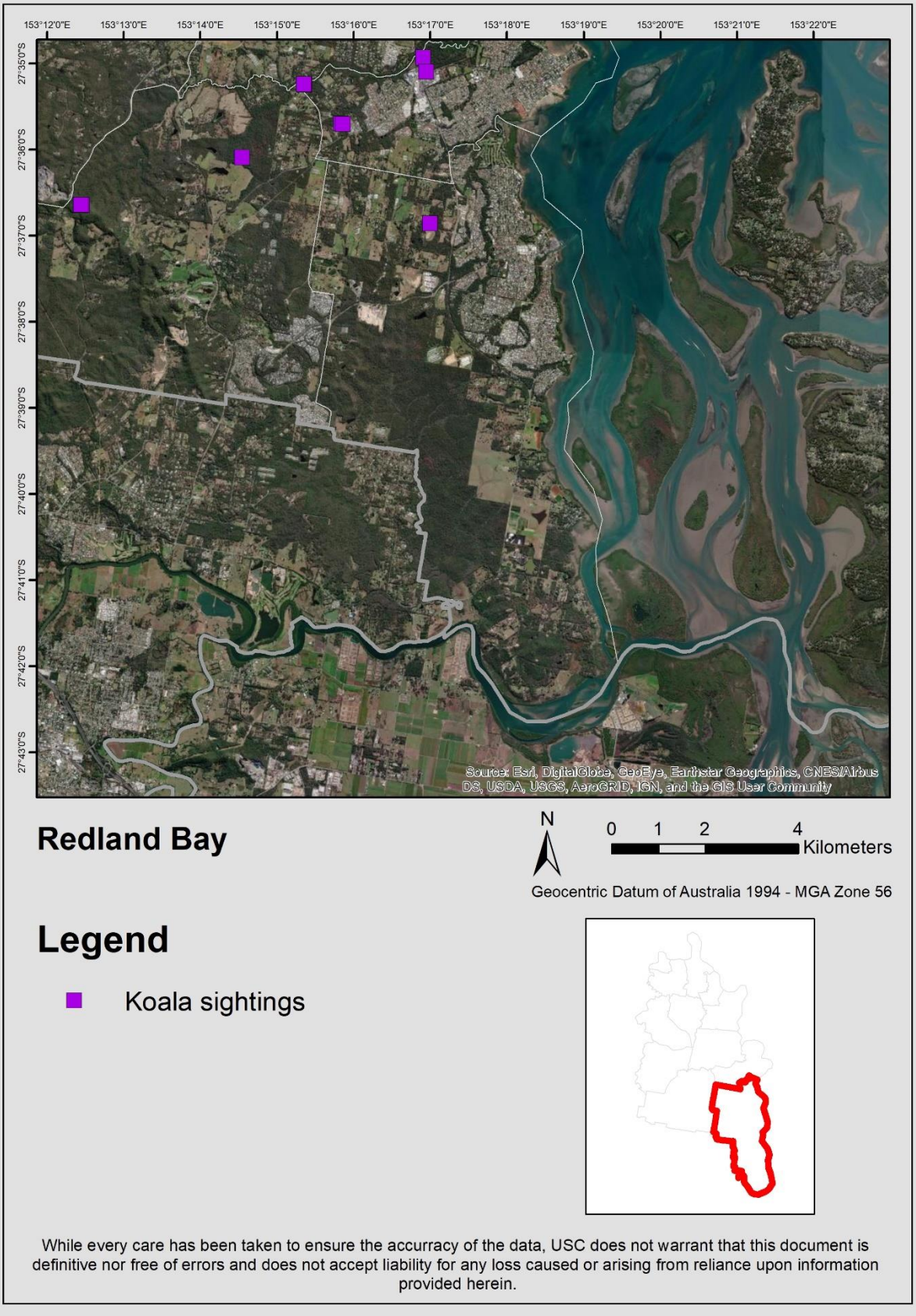
0 1 2 4 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Old
- Very old





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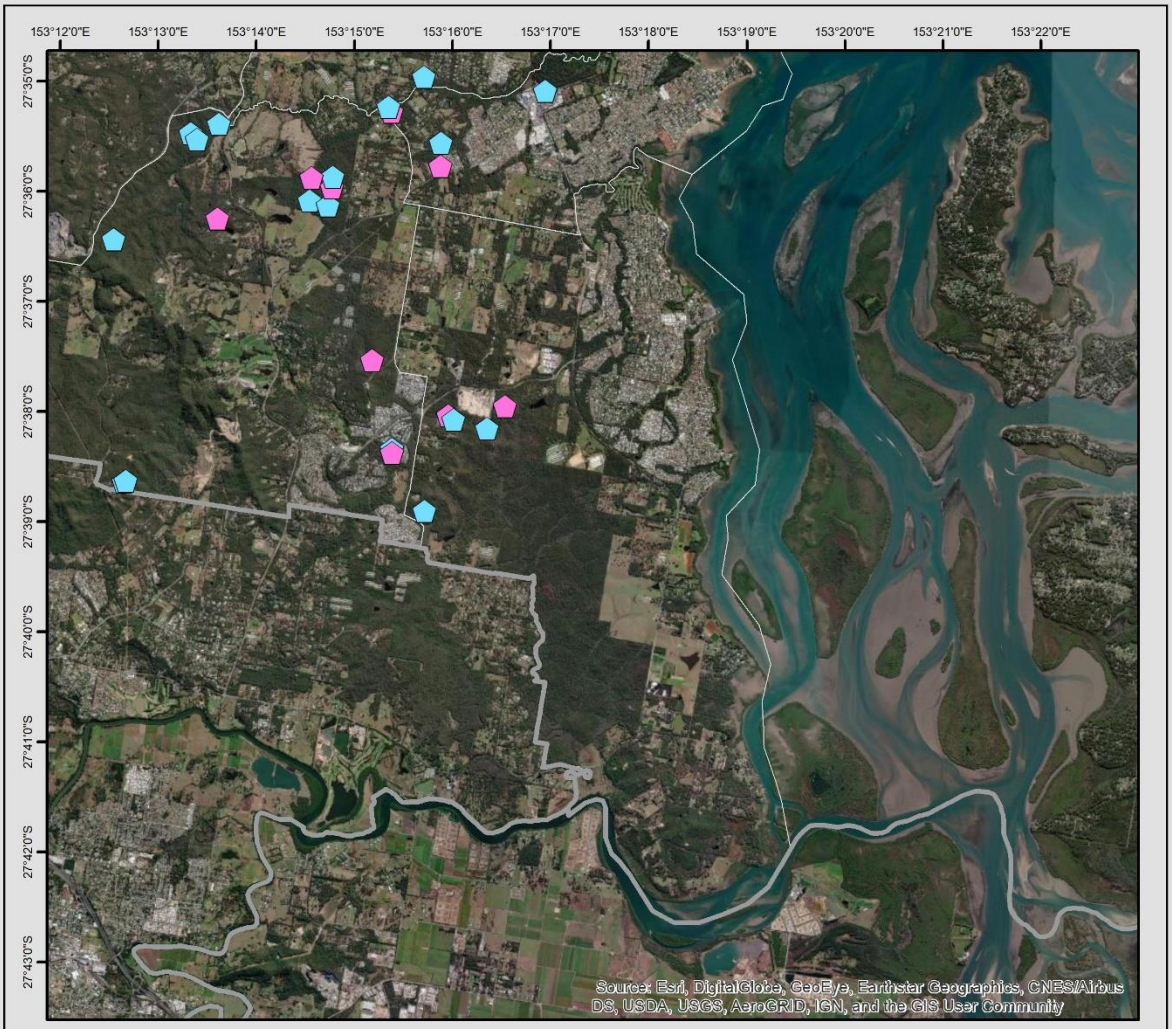


Redland Bay

N

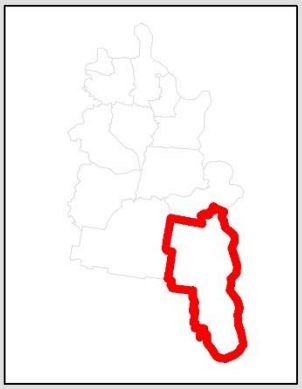
0 1 2 4 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male



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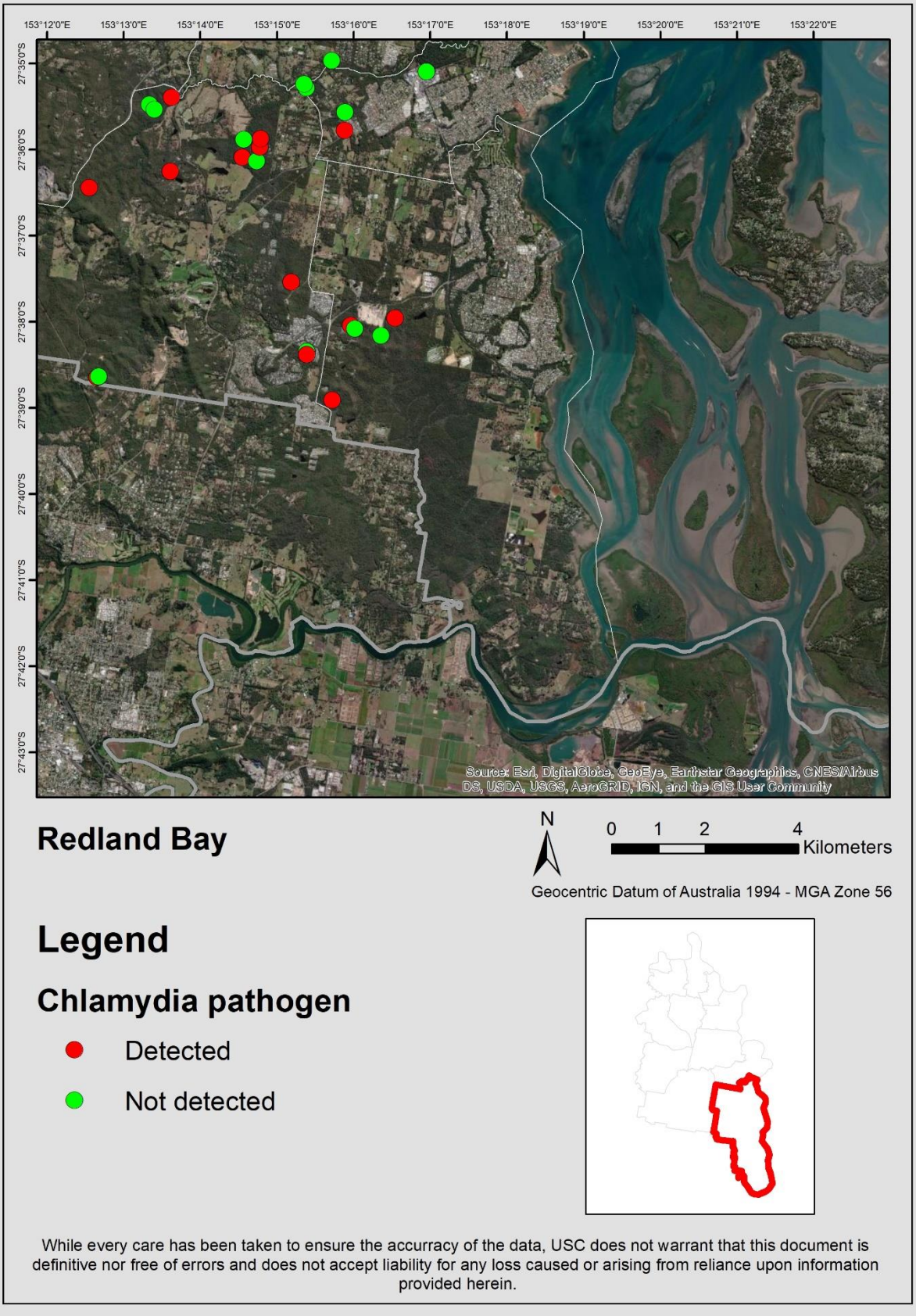

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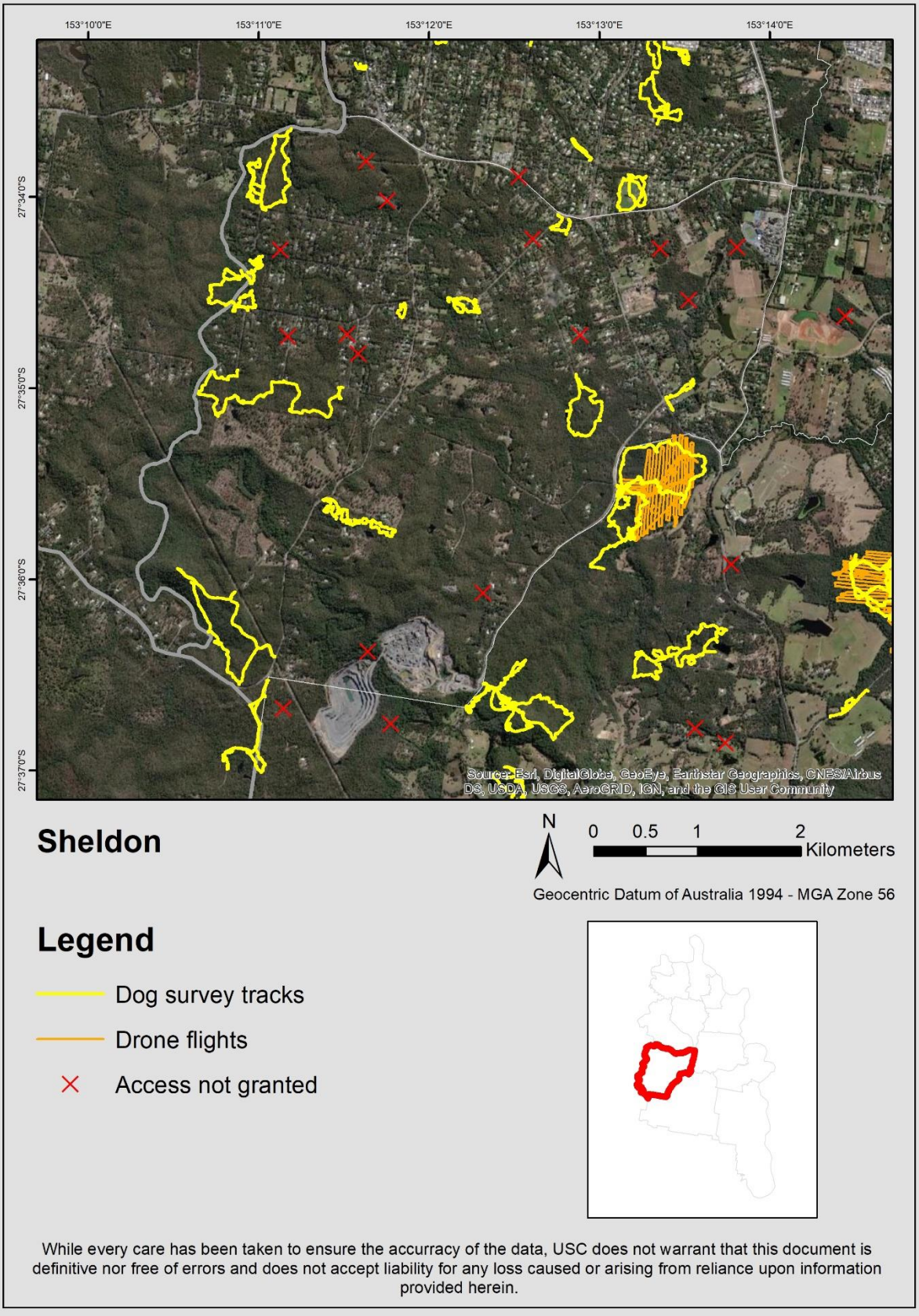
Sheldon



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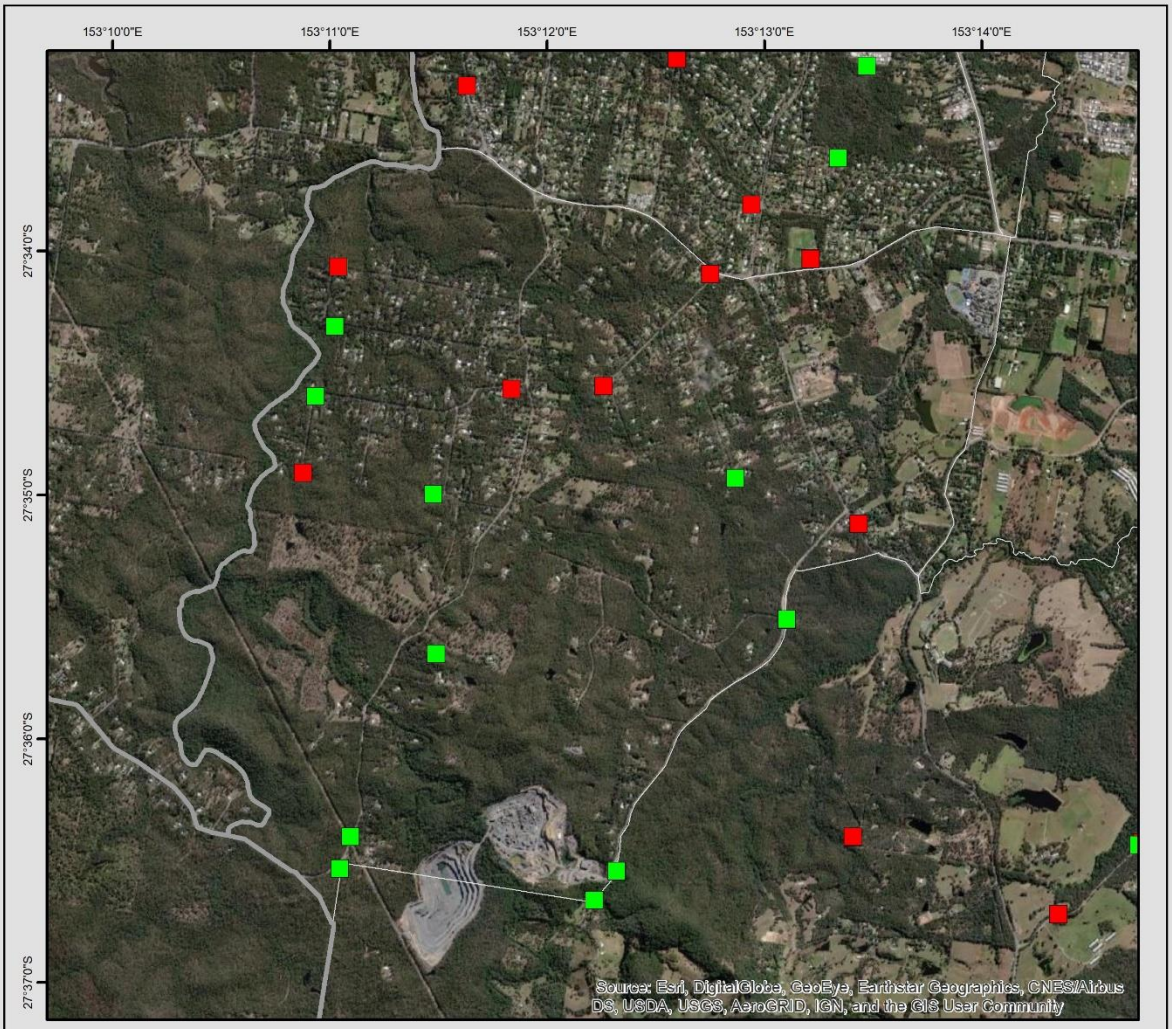


Sheldon

N

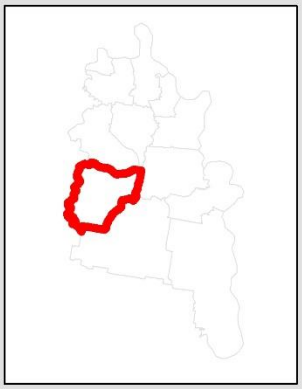
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence



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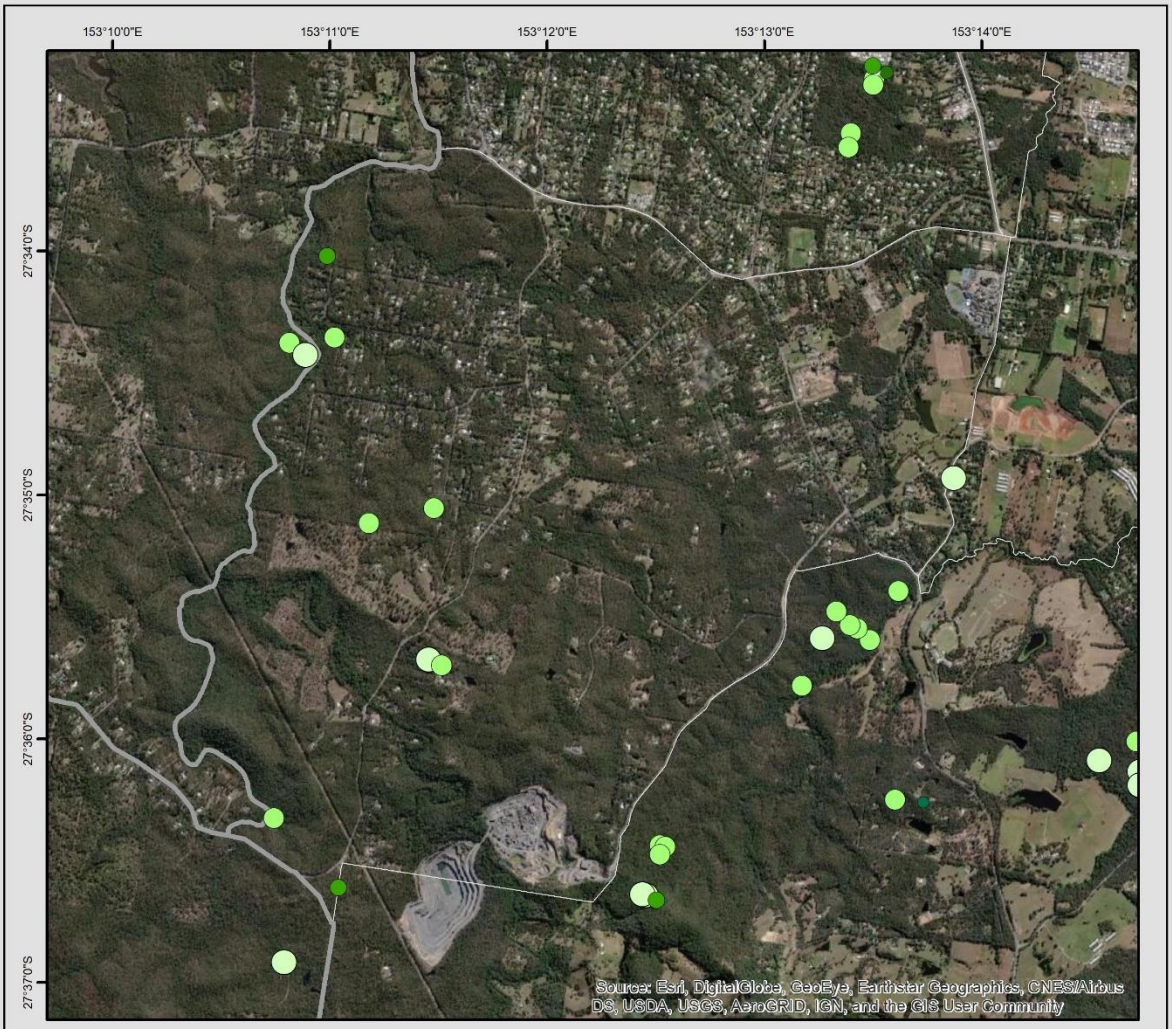


Sheldon

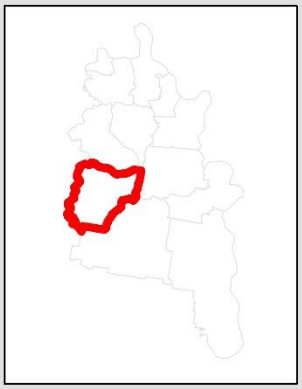
N

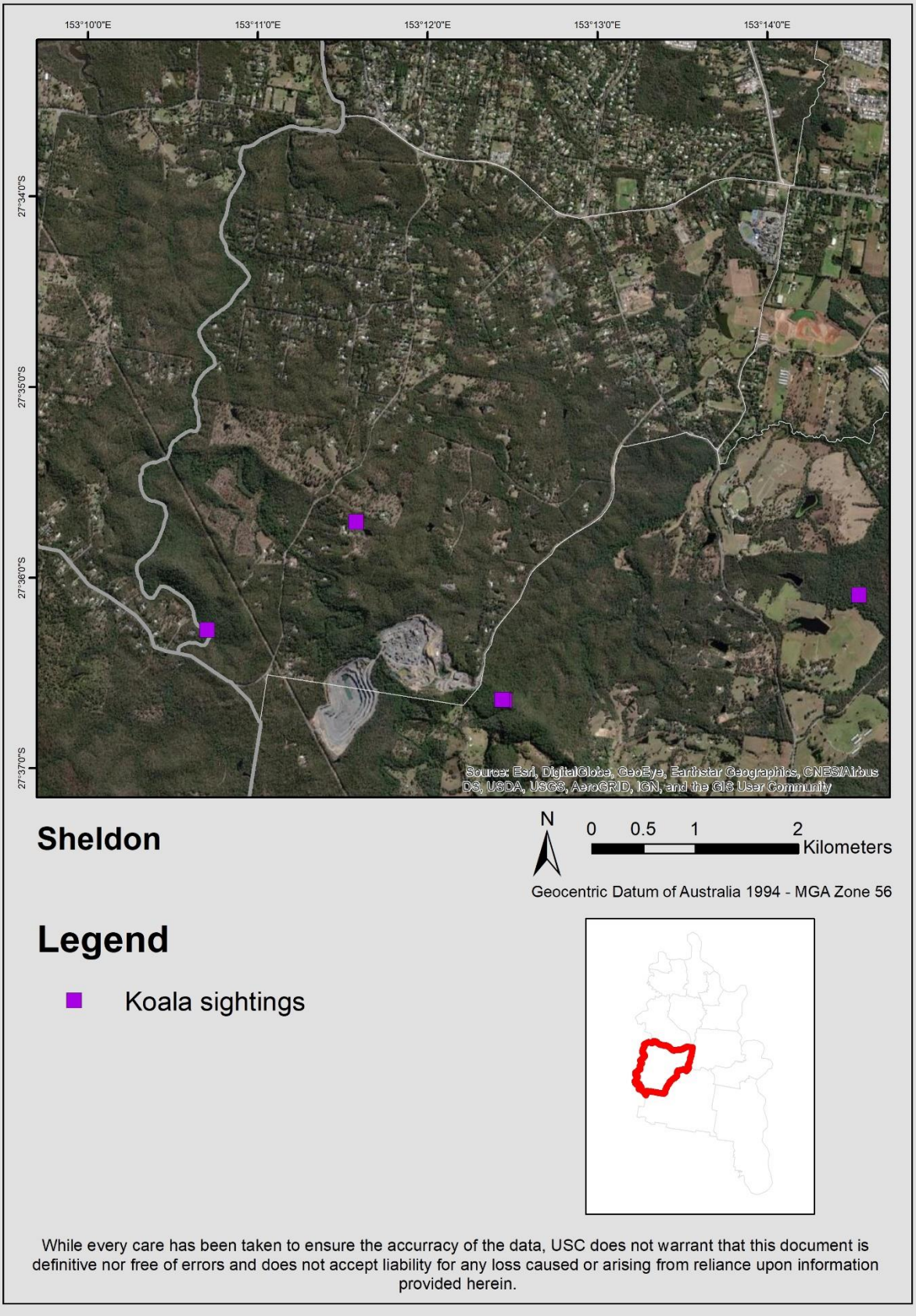
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Old
- Very old





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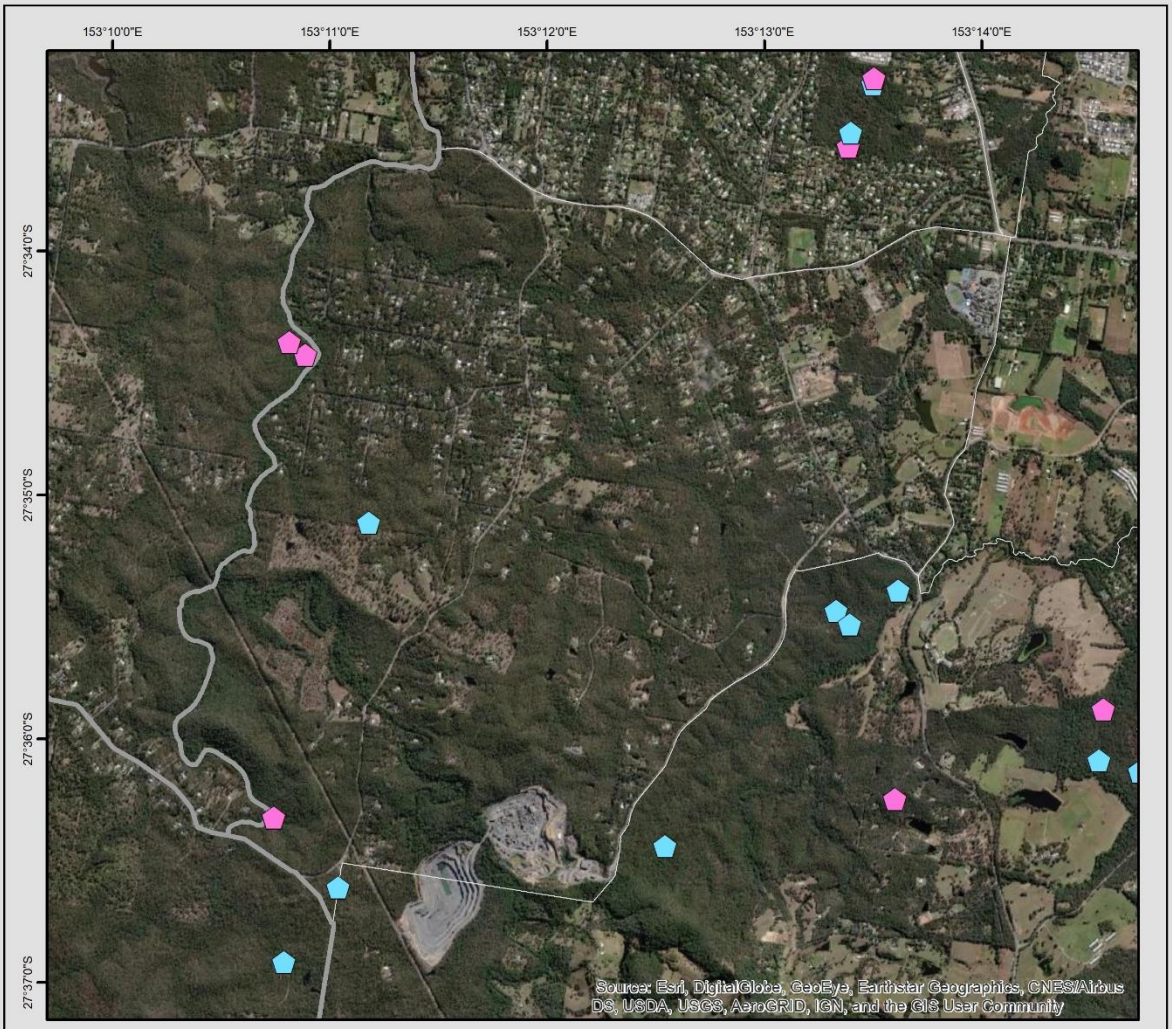


Sheldon

N

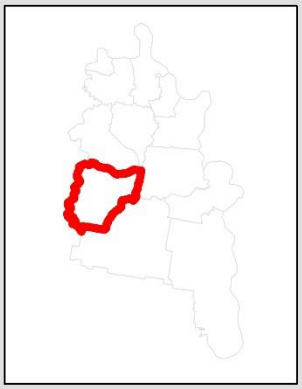
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male



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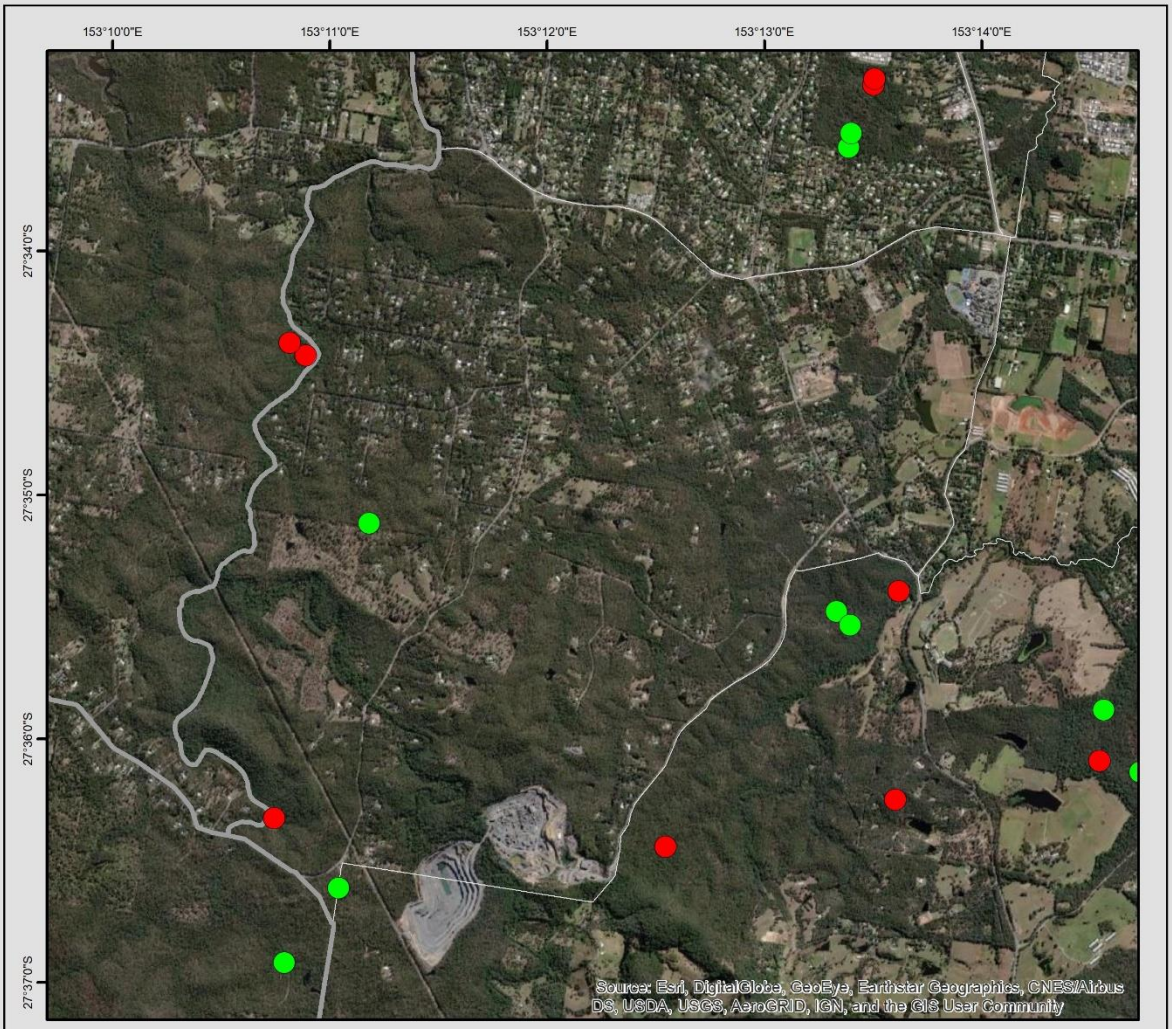


Sheldon

N

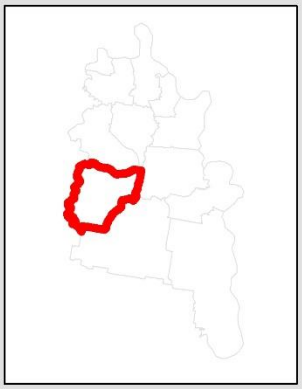
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Chlamydia pathogen

- Detected
- Not detected



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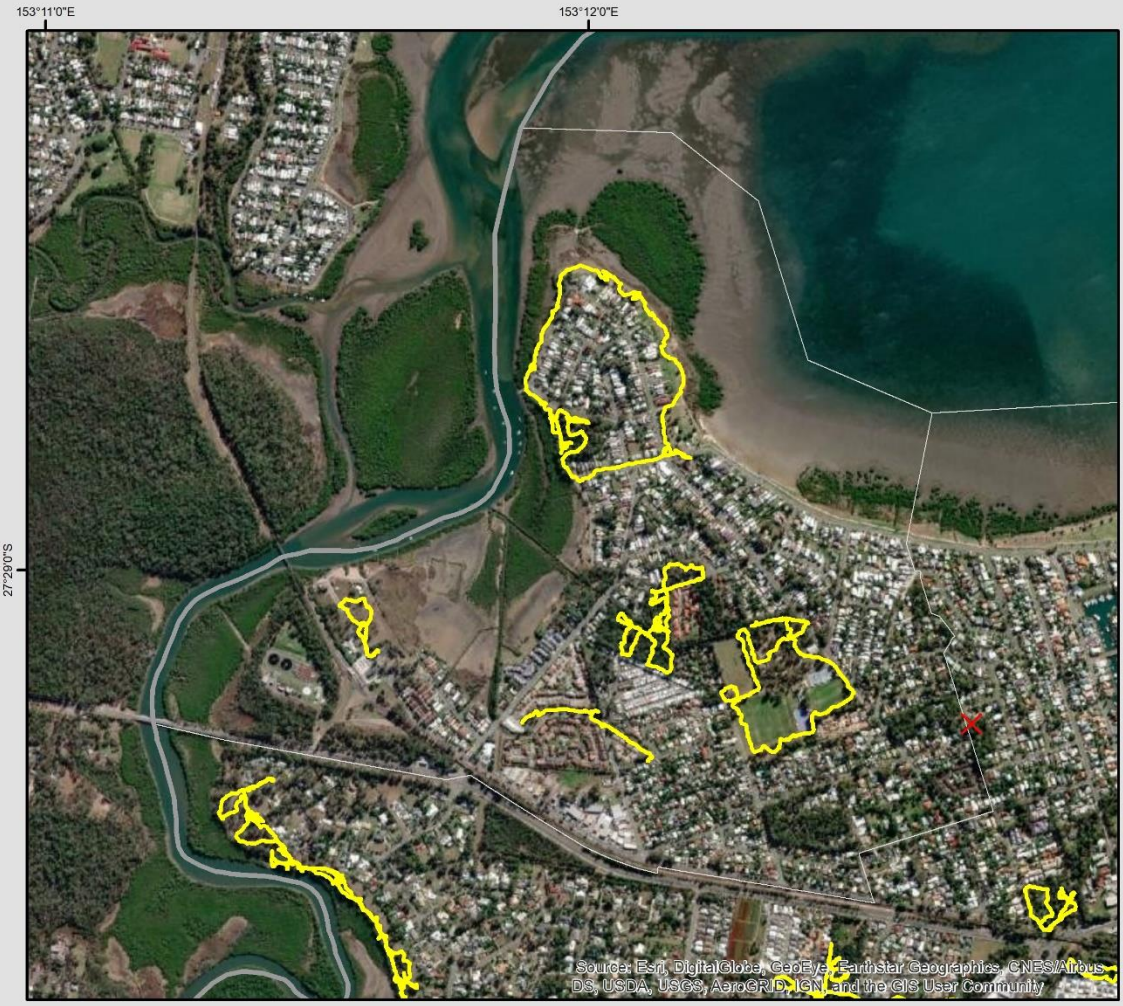
Thorneside



Thorneside

N

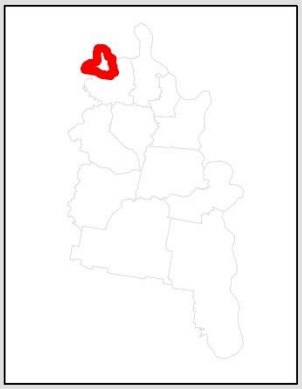
0 0.2 0.4 0.8 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Dog survey tracks

Access not granted



Thorneside

N

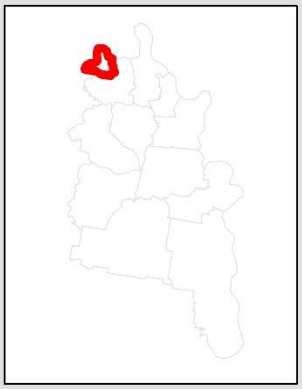
0 0.2 0.4 0.8
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Absence



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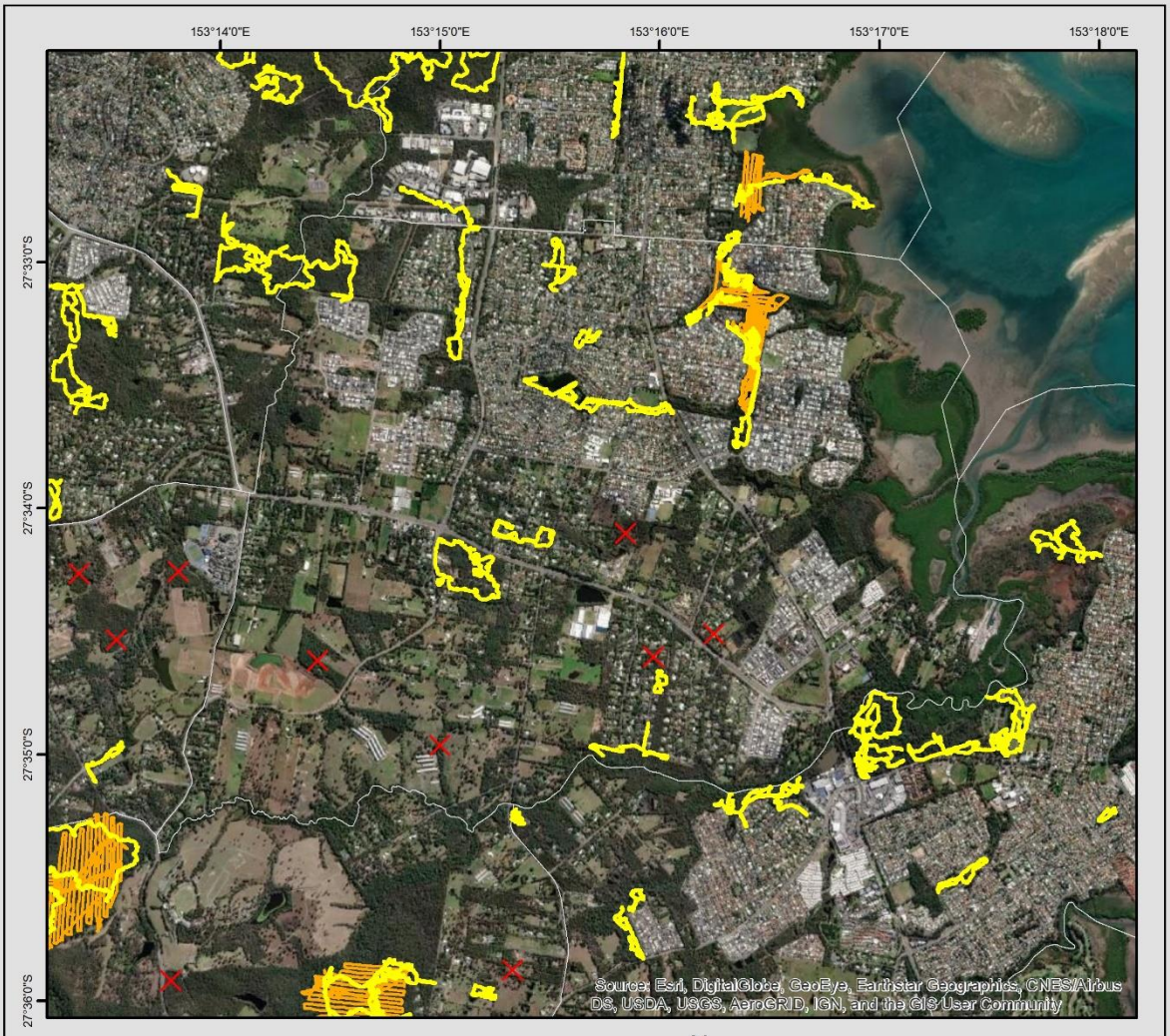
Thornlands



Thornlands

N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog survey tracks
- Drone flights
- Access not granted

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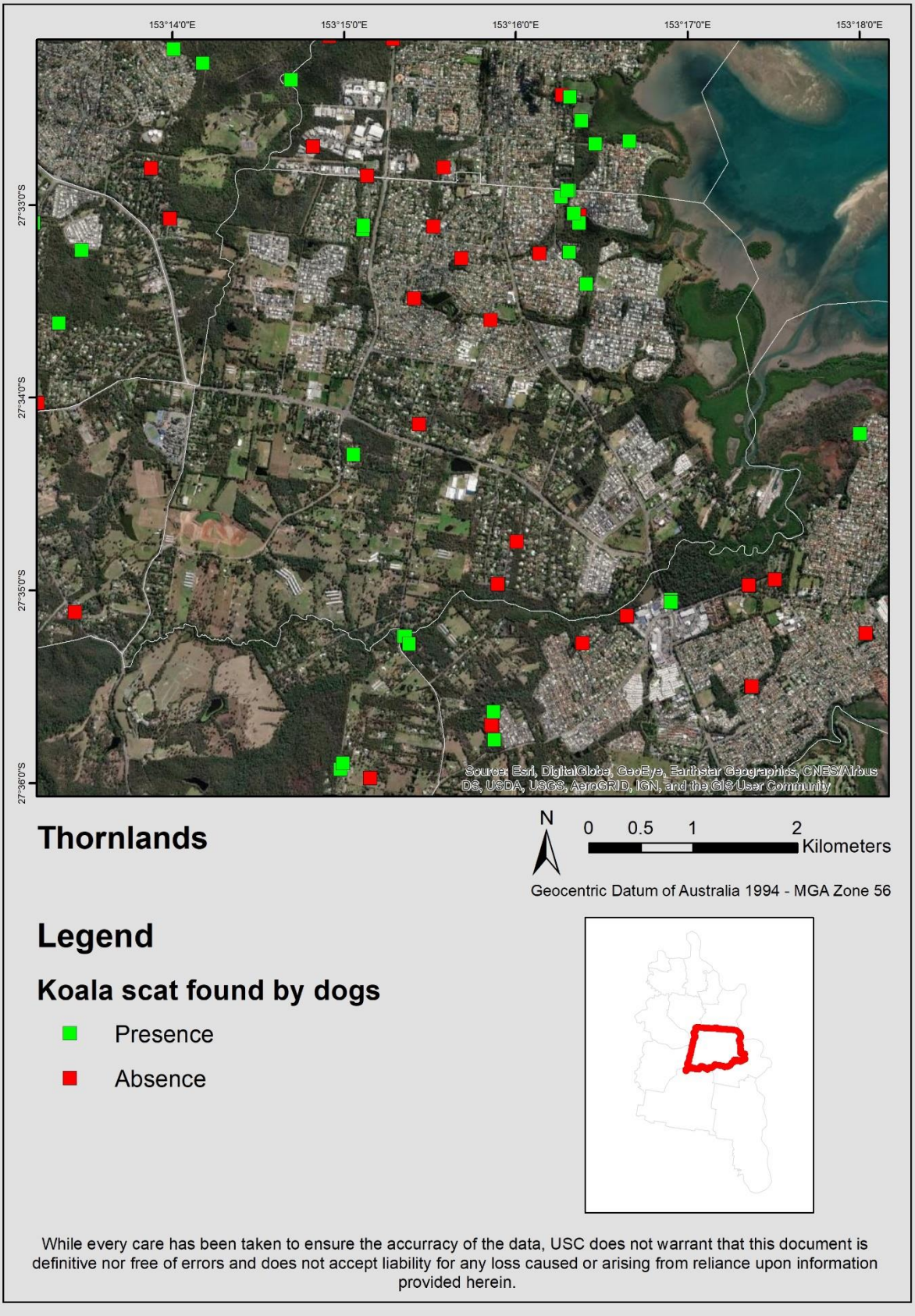

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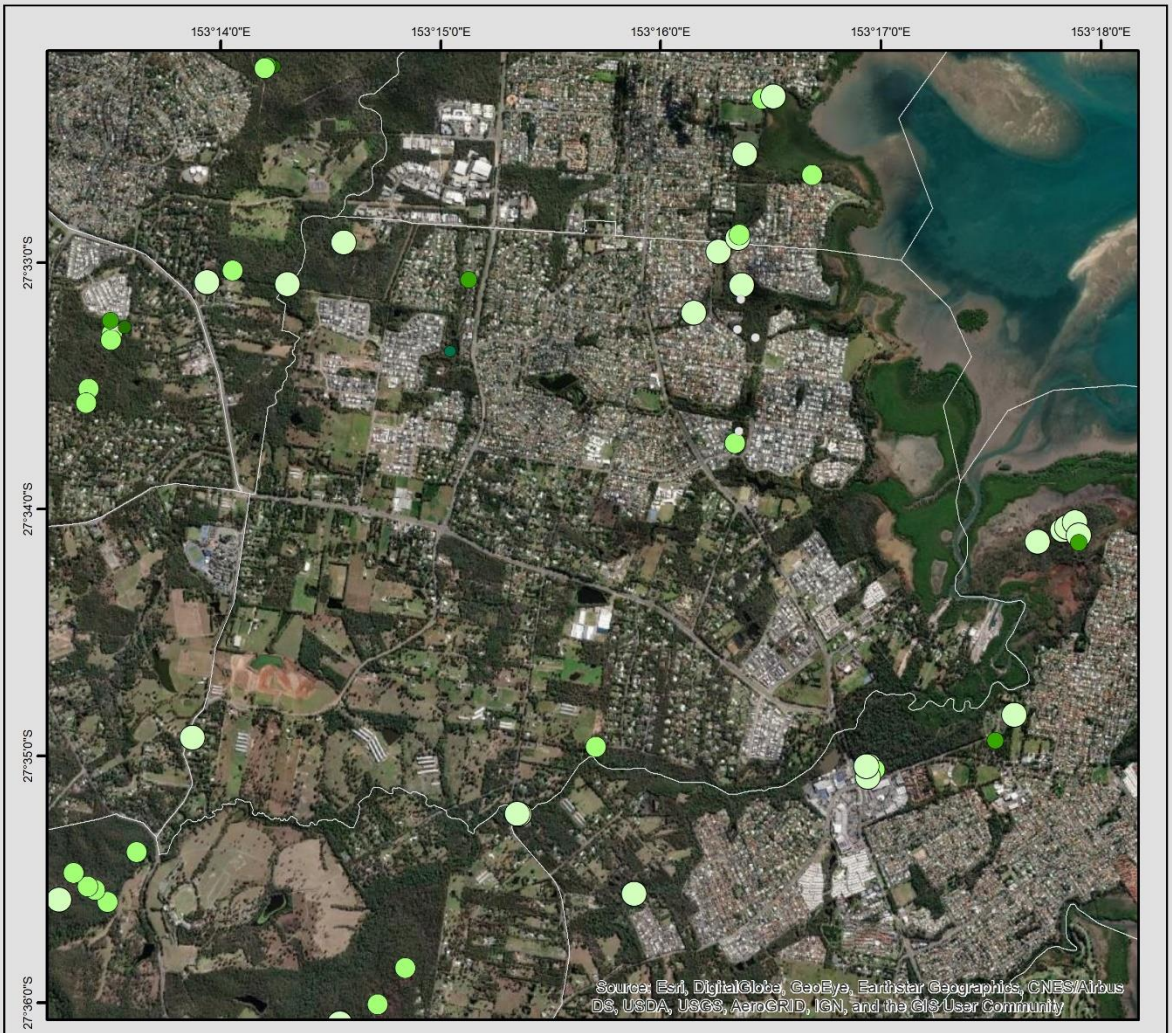


Thornlands

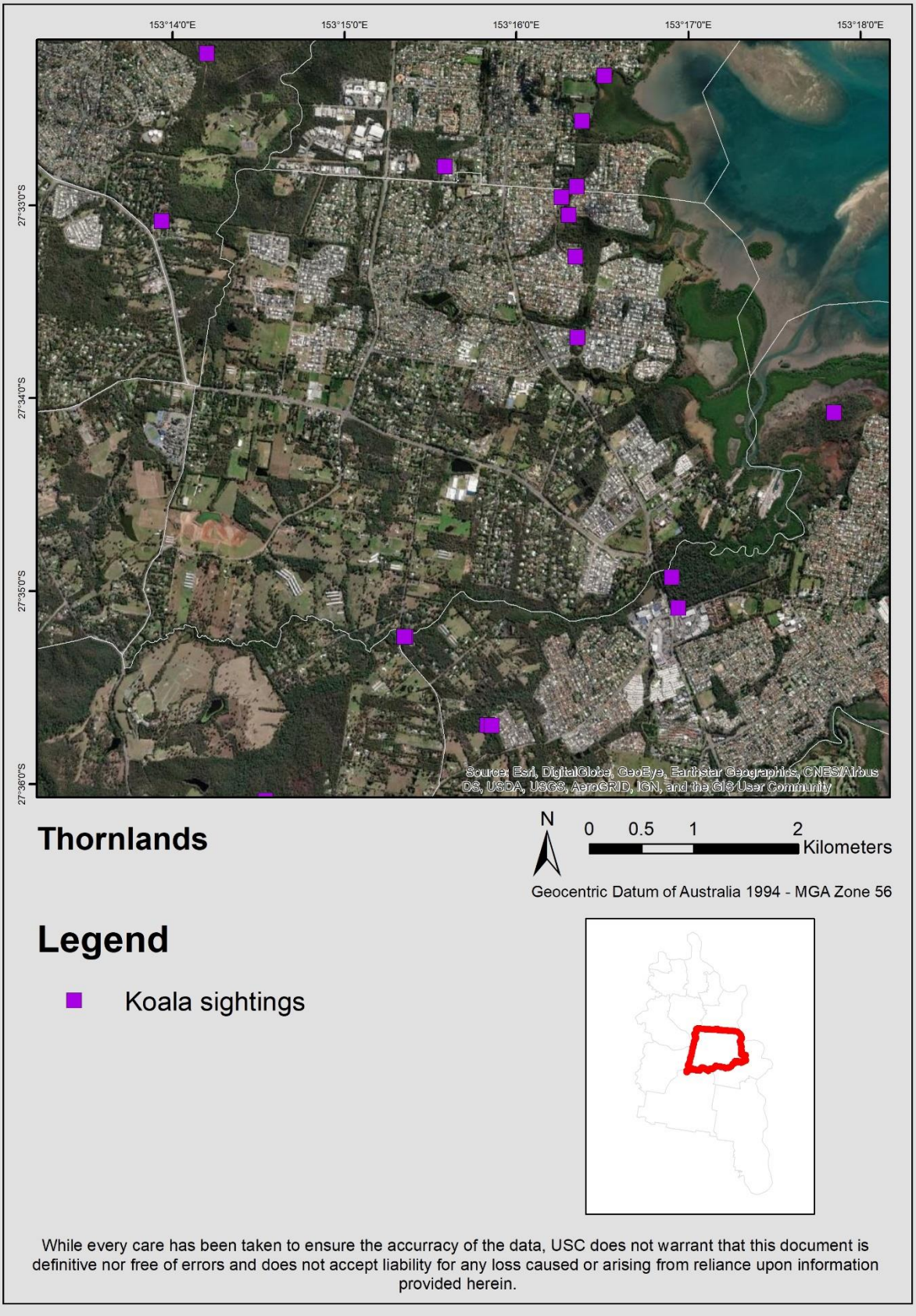
N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Old
- Very old



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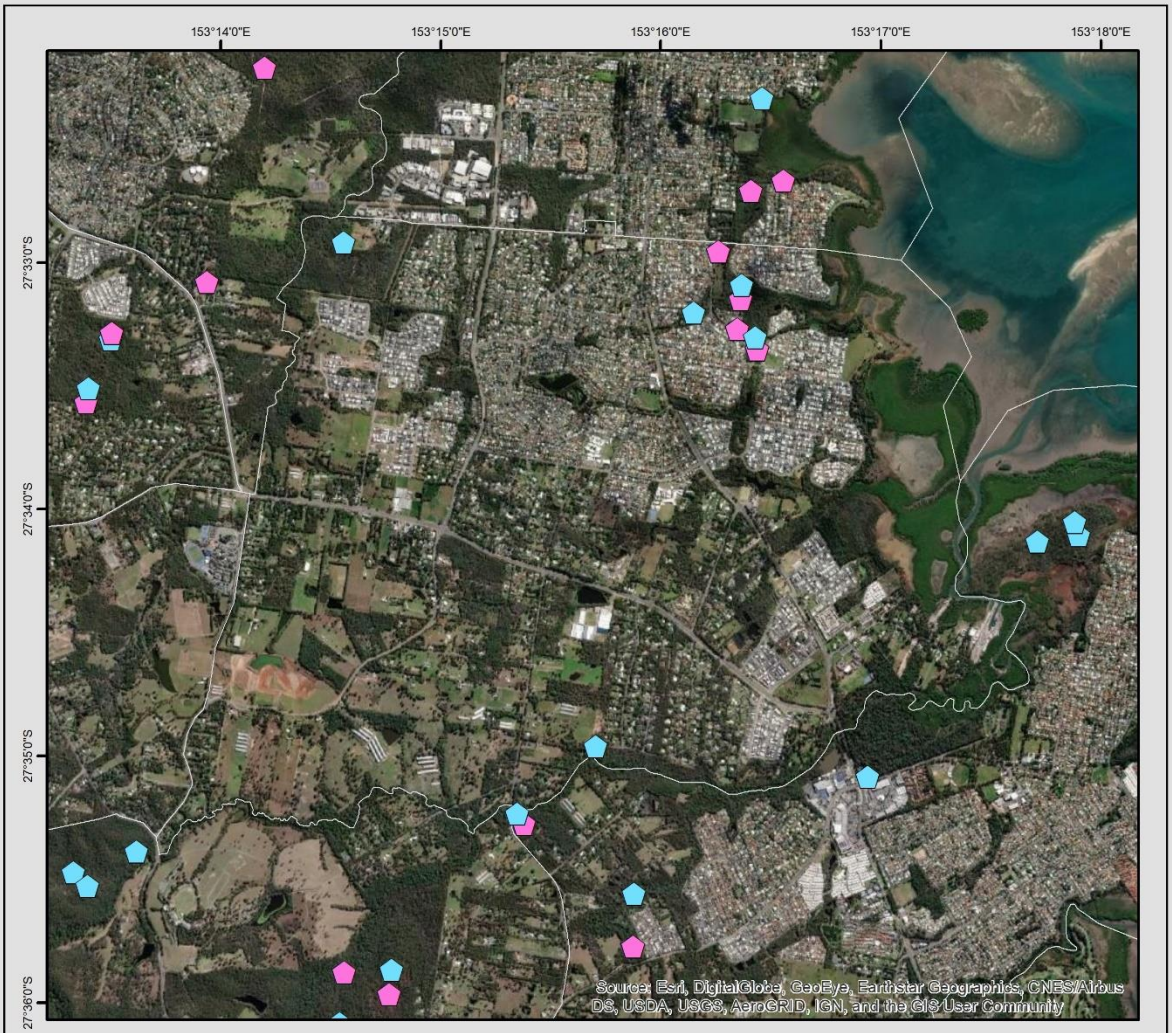

Thornlands

N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male

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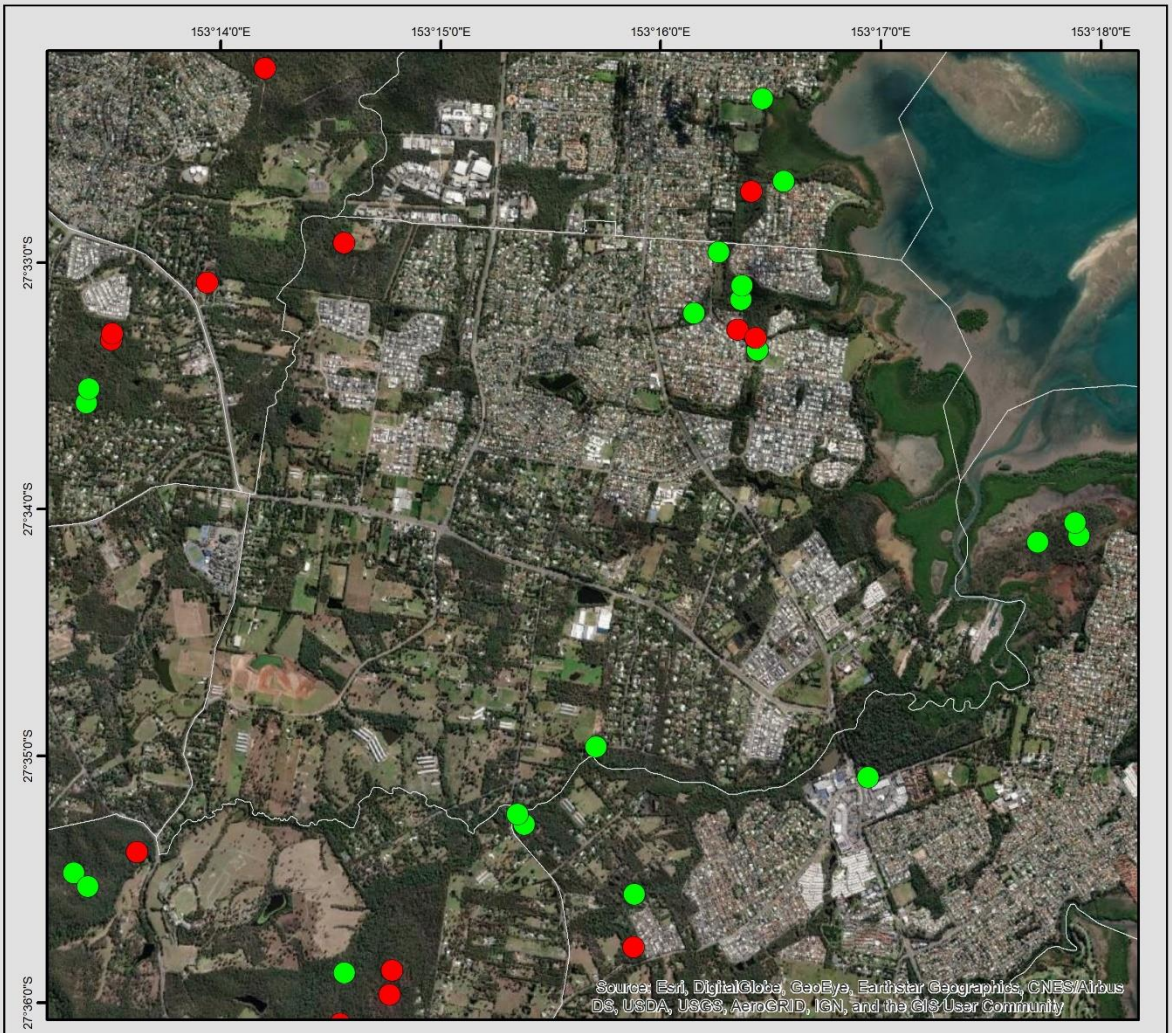


Thornlands

N

0 0.5 1 2 Kilometers

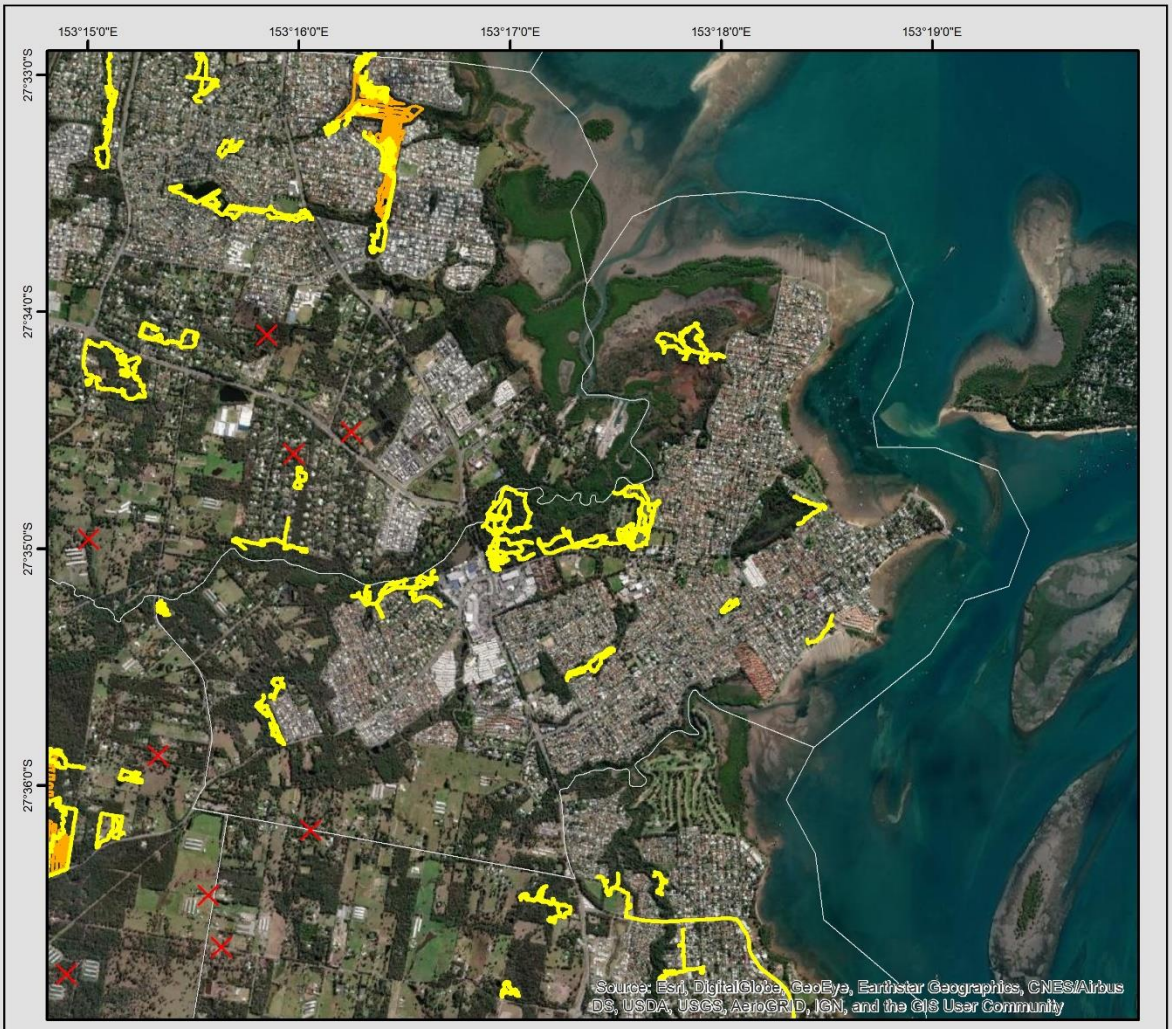
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Chlamydia pathogen

- Detected
- Not detected

Victoria Point



Victoria Point

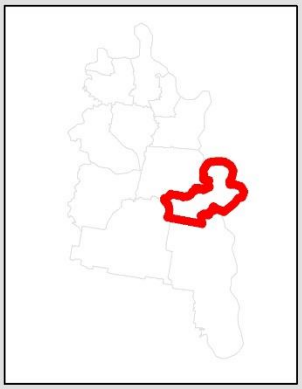
N

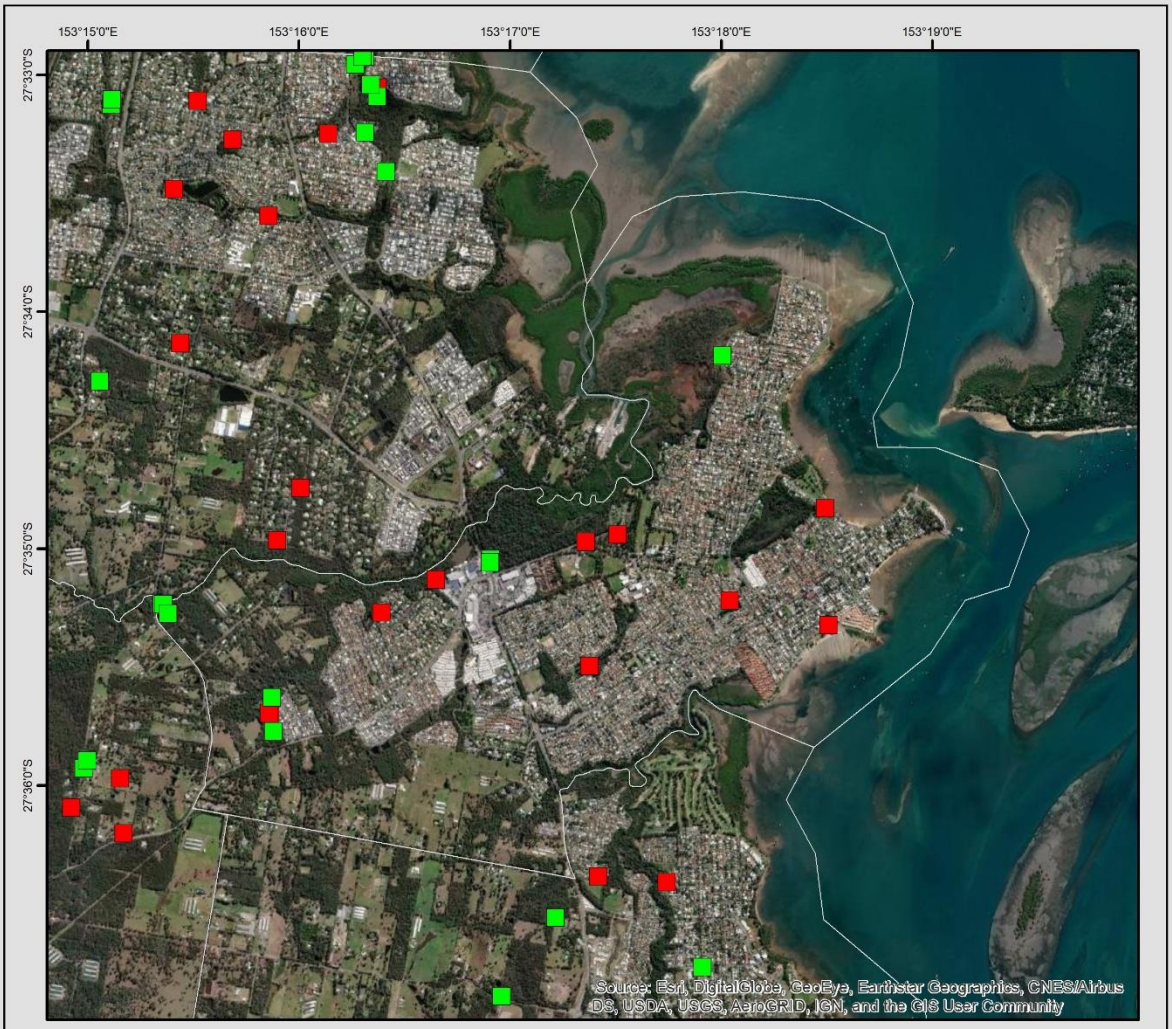
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog survey tracks
- Drone flights
- Access not granted





Victoria Point

N

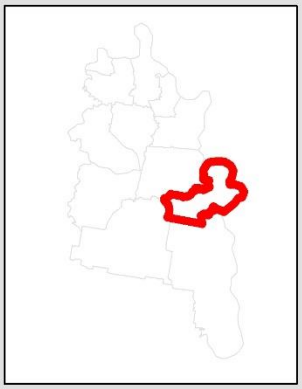
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence



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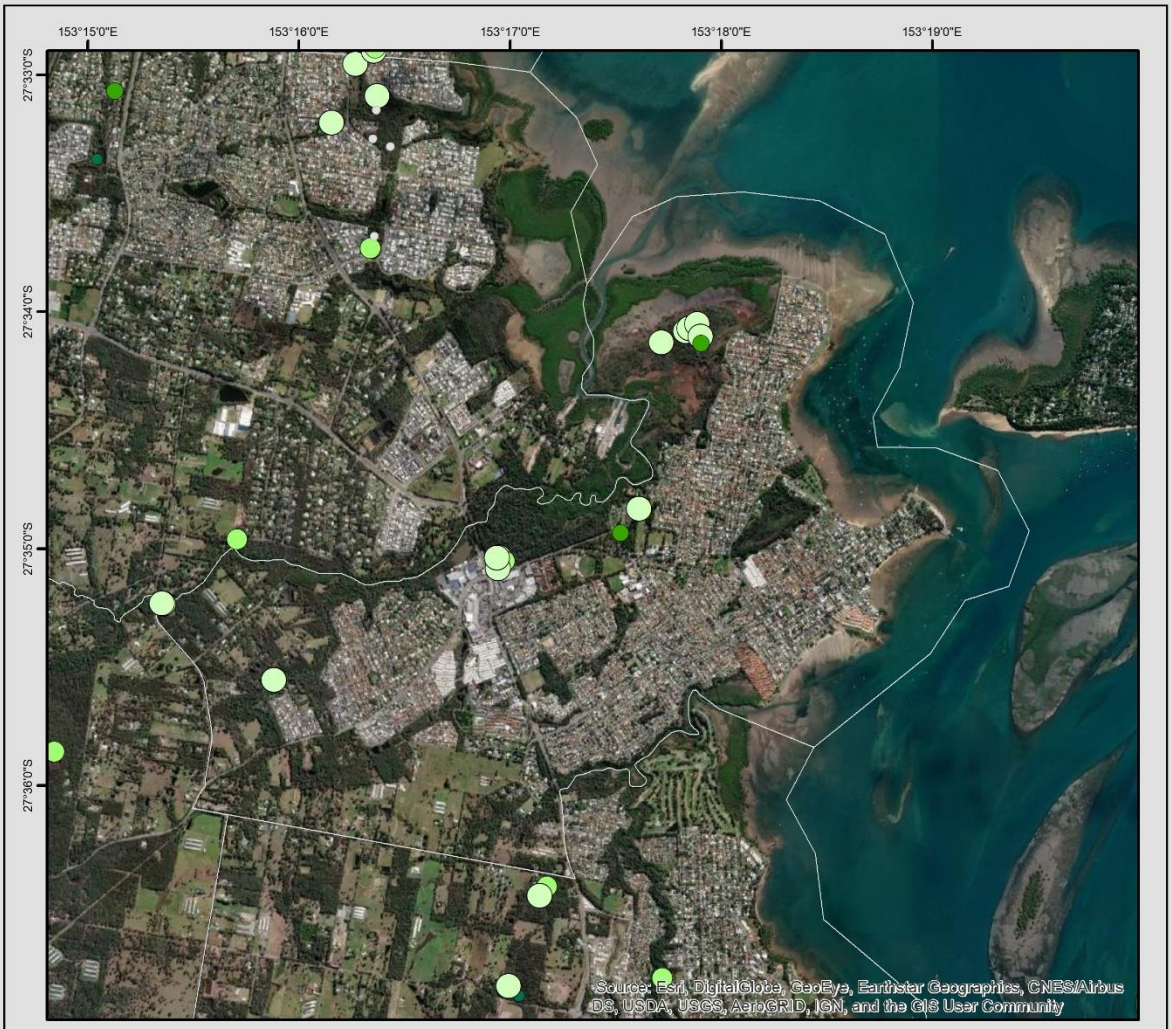


Victoria Point

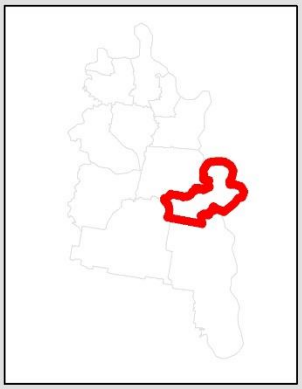
N

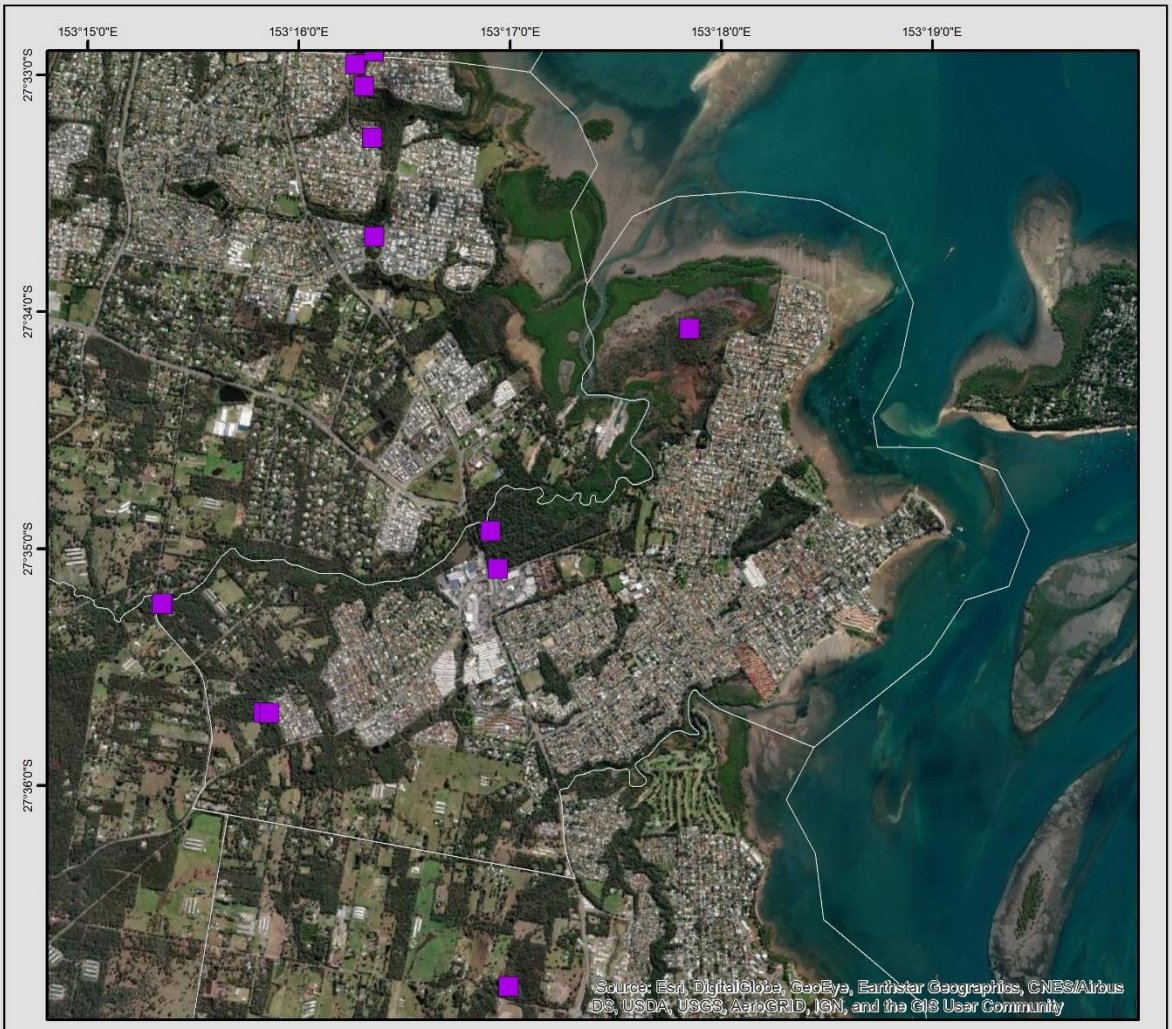
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Very old





Victoria Point

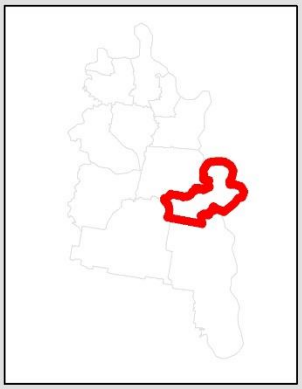
N

0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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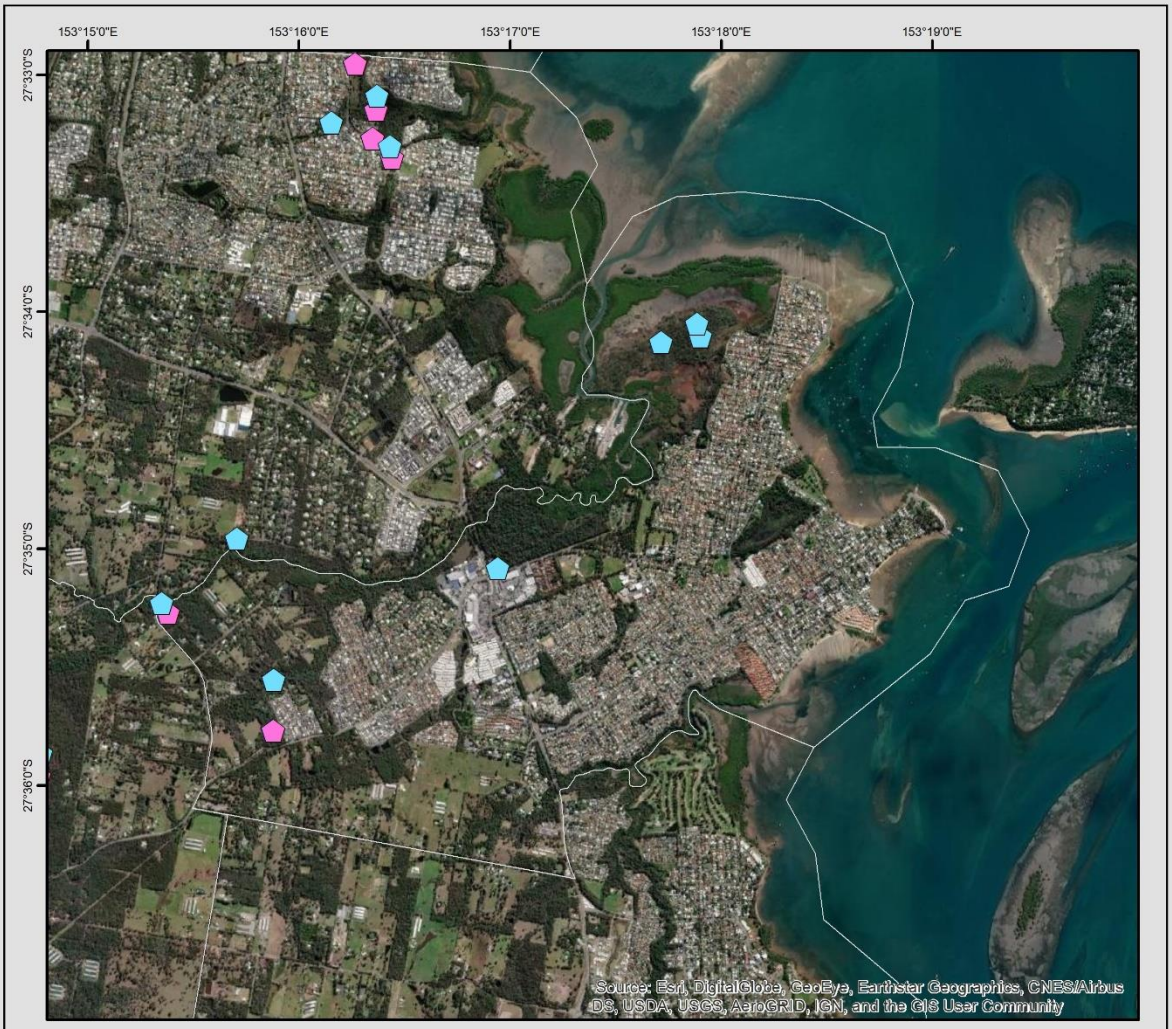


Victoria Point

N

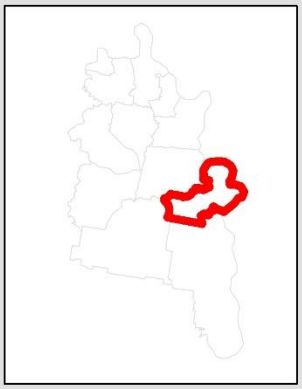
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male



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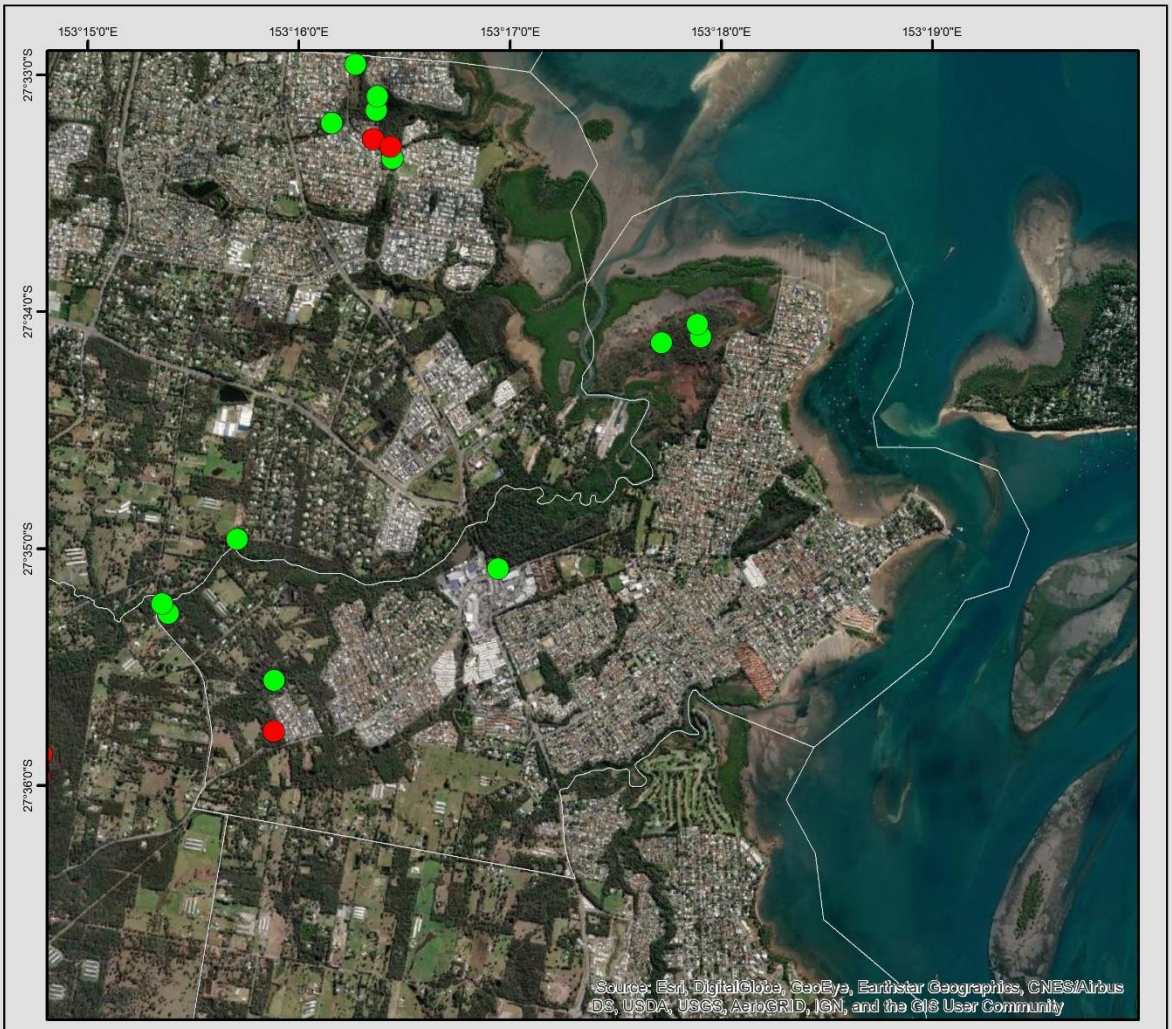


Victoria Point

N

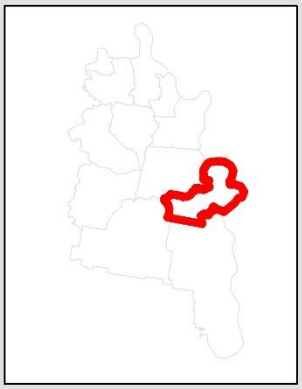
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Chlamydia pathogen

- Detected
- Not detected



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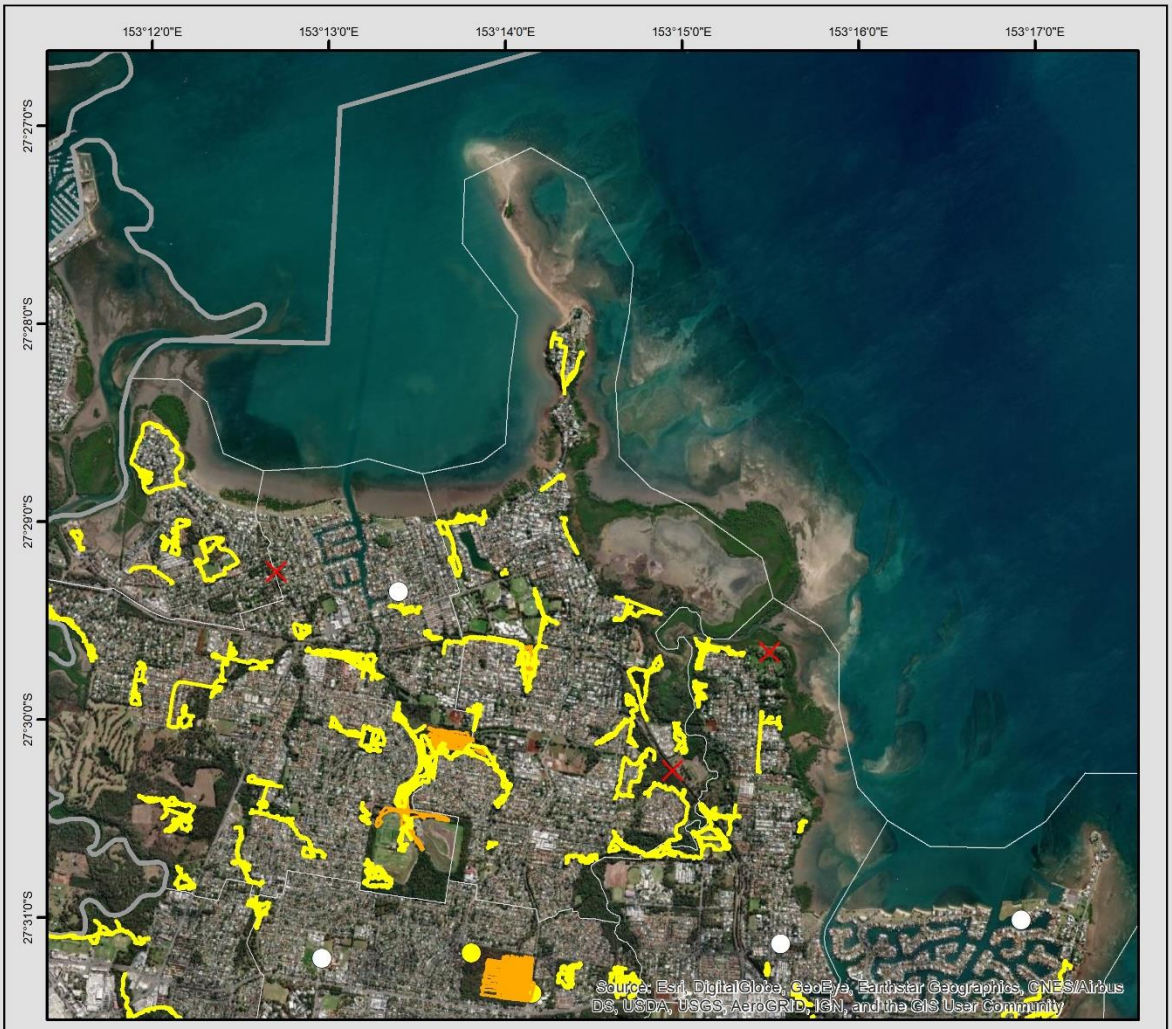
Wellington Point



Wellington Point

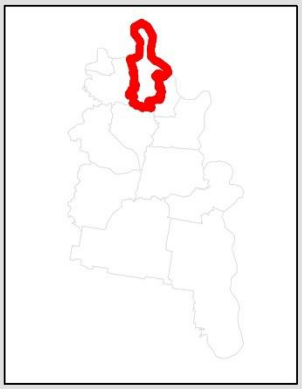
N

0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog survey tracks
- Drone flights
- Drone survey
- Access not granted
- Survey site inappropriate (no trees)



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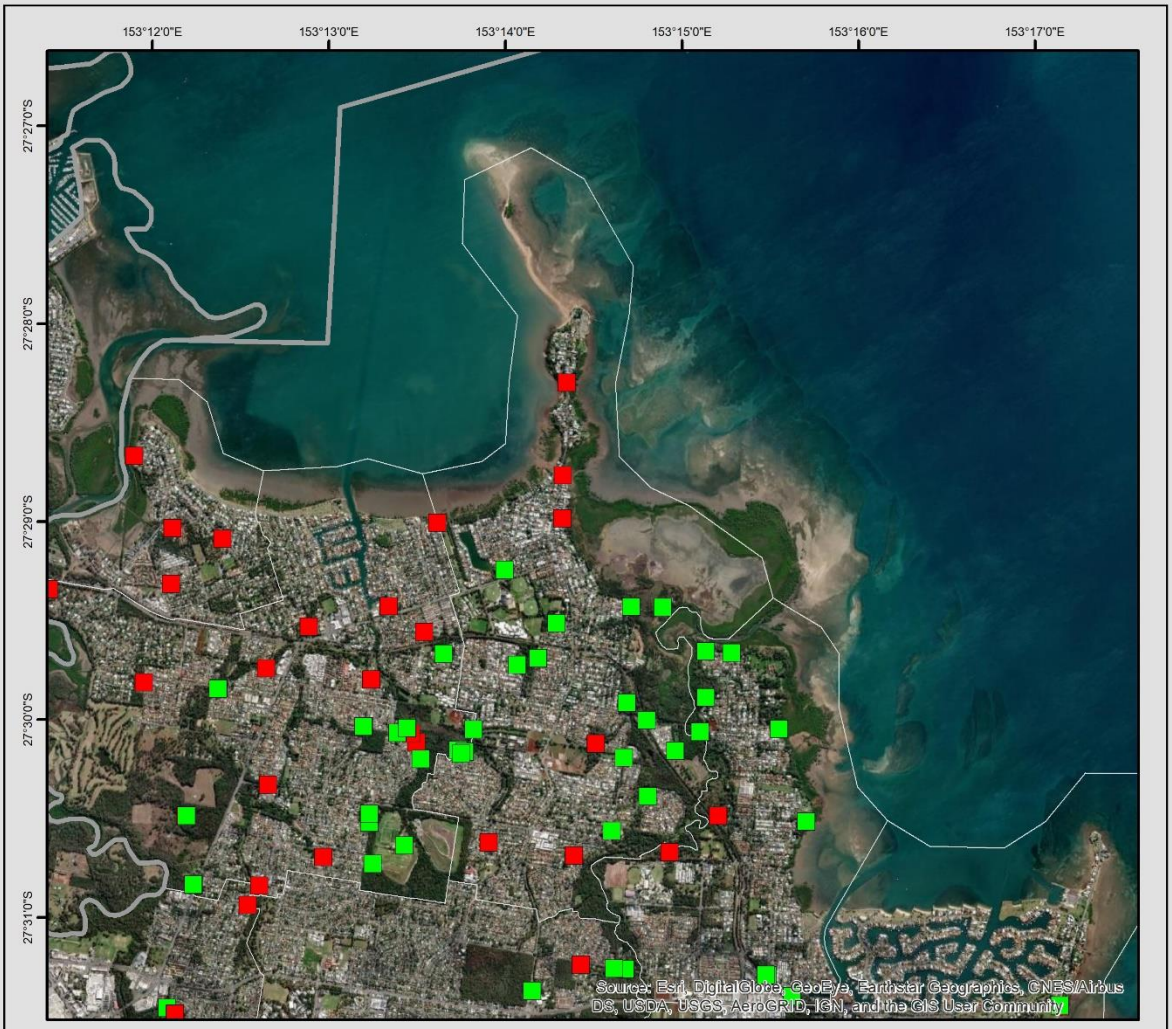


Wellington Point

N

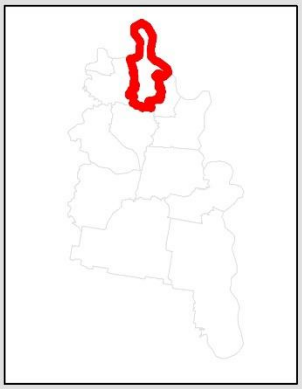
0 0.5 1 2
Kilometers

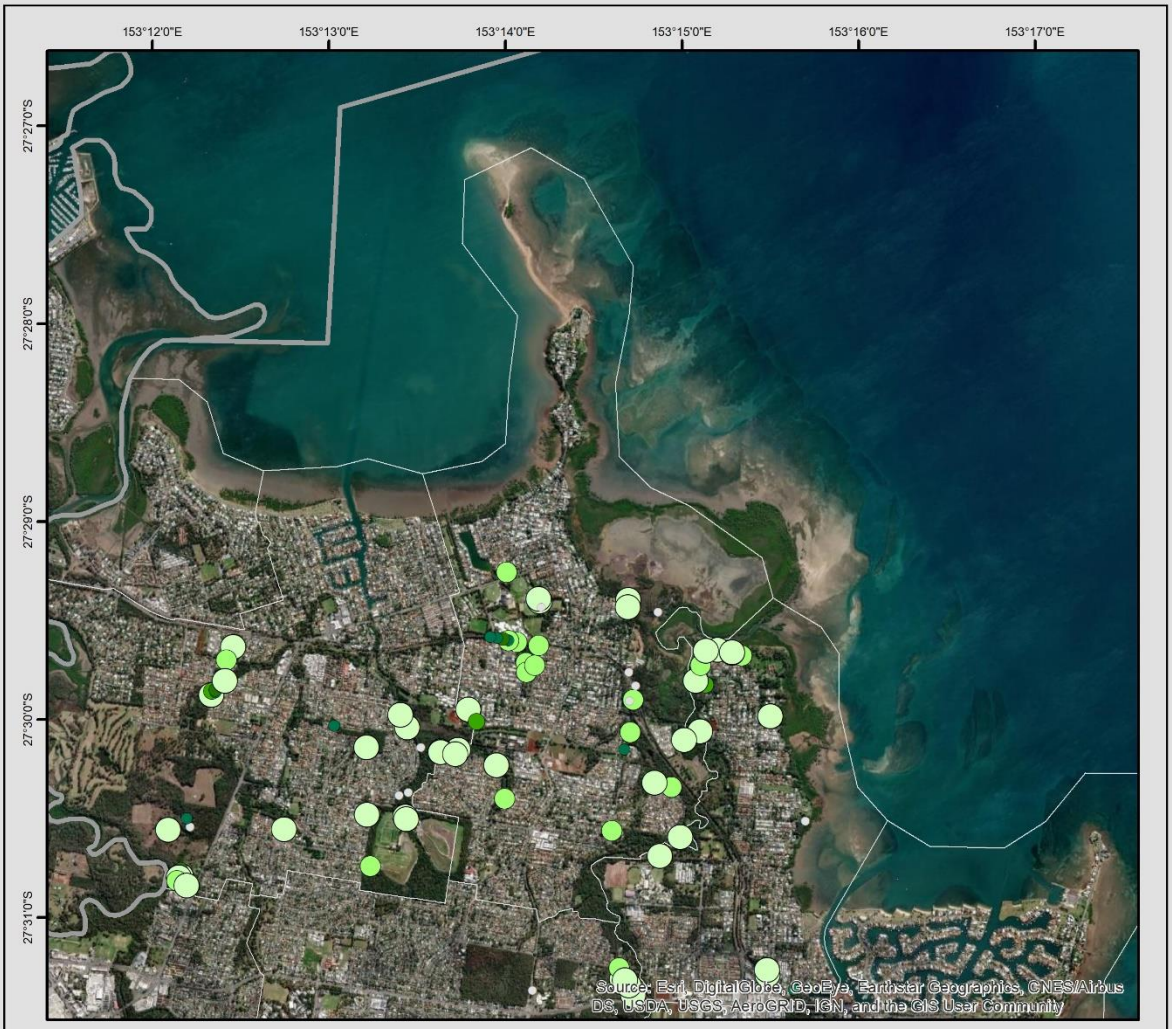
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala scat found by dogs

- Presence
- Absence





Wellington Point

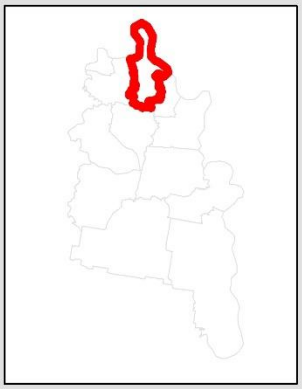
N

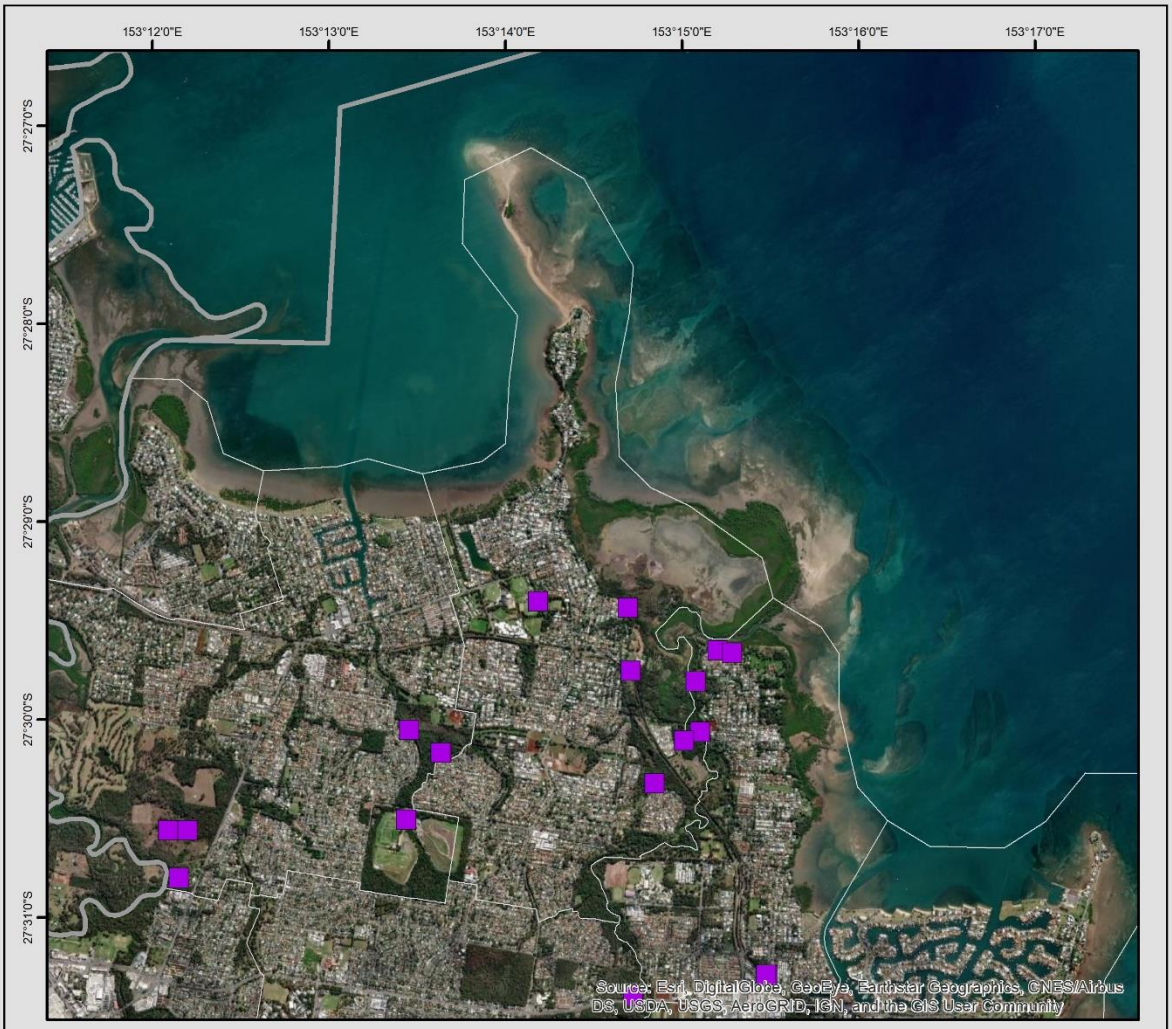
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

- unclassified
- Very fresh
- Fresh
- Medium
- Old
- Very old





Wellington Point

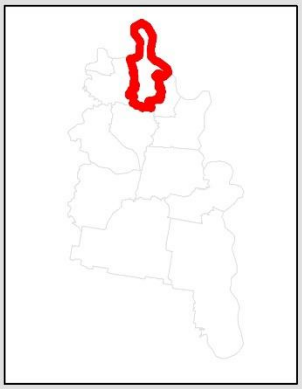
N

0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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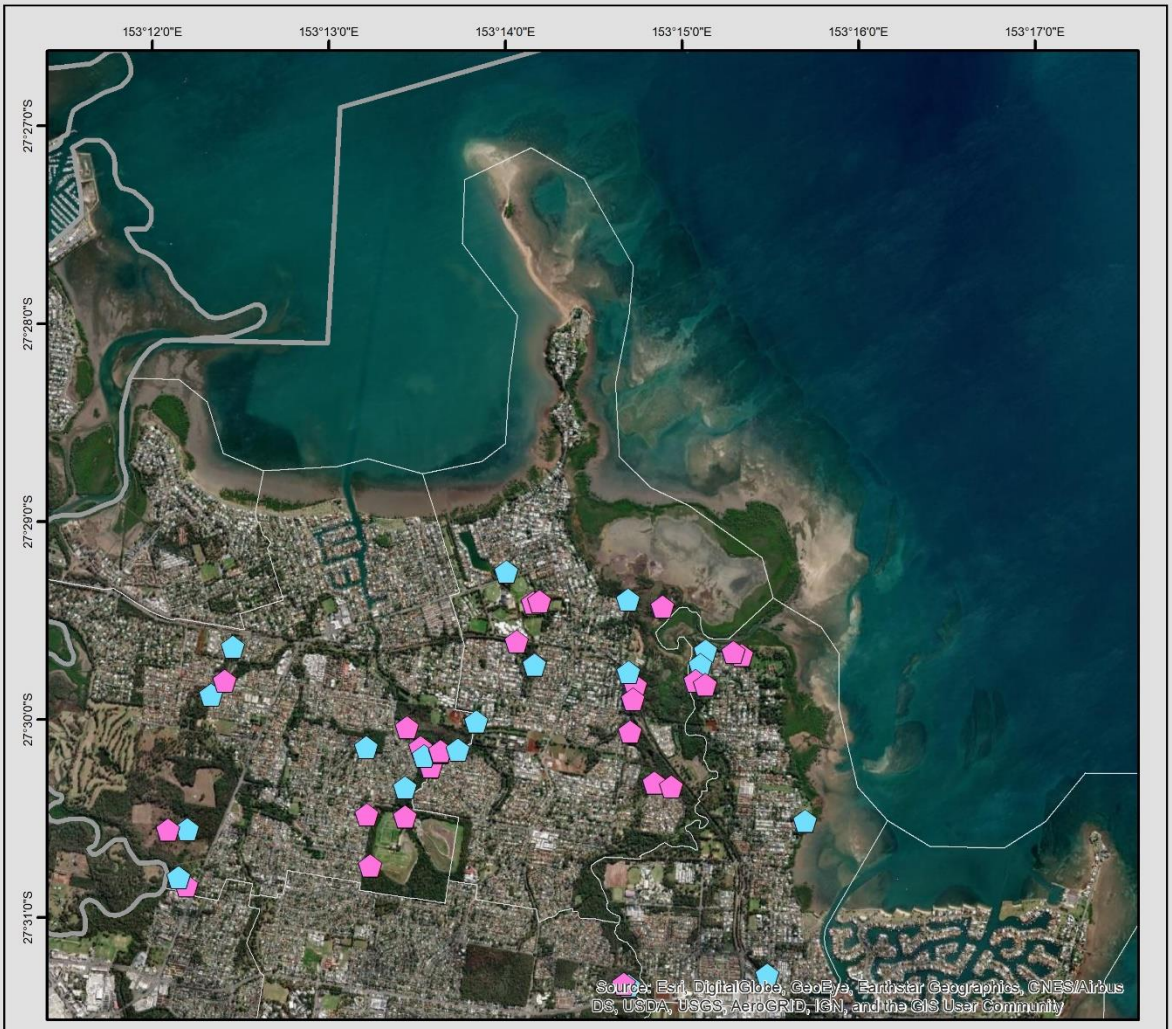


Wellington Point

N

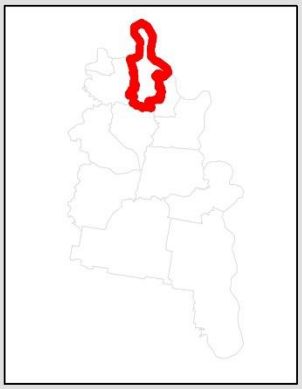
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male



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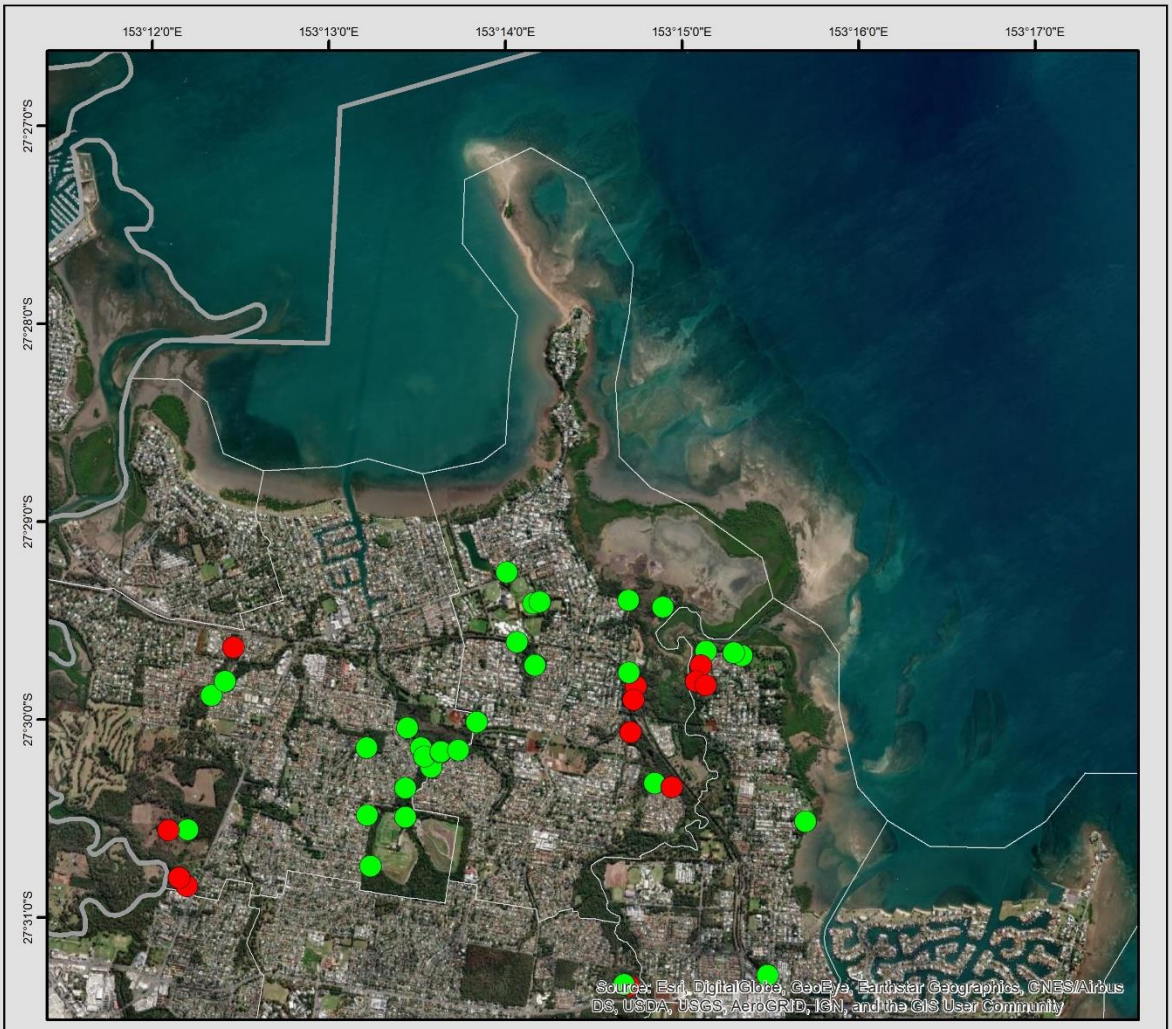


Wellington Point

N

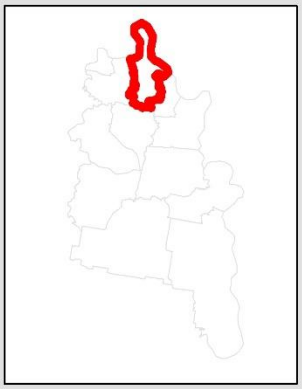
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

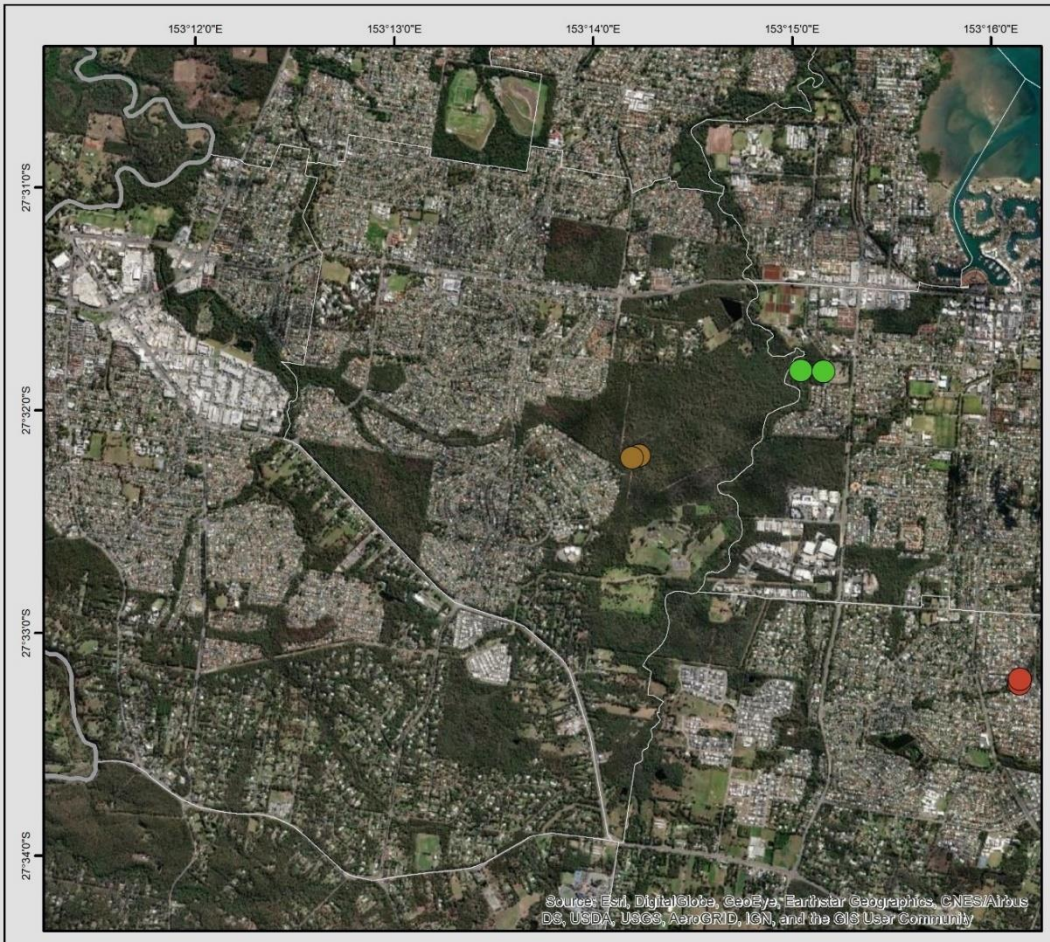
Chlamydia pathogen

- Detected
- Not detected



Appendix 3: Koalas sampled both in 2018 and in 2020/2021

We genetically resampled 11 individuals that were first sampled in 2018. Each resampled individual was sampled close to where it was sampled in 2018, as the following maps show. The maps show the positions of koalas sampled in 2018 and genetically recaptured in 2020/21. Each individual is shown in a different colour and individuals are numbered with numbers 1-11 – e.g. individual number 1 is shown in purple and was sampled in Wellington Point. Because the maps show bordering suburbs, some individuals appear in multiple maps.



Alexandra Hills

N

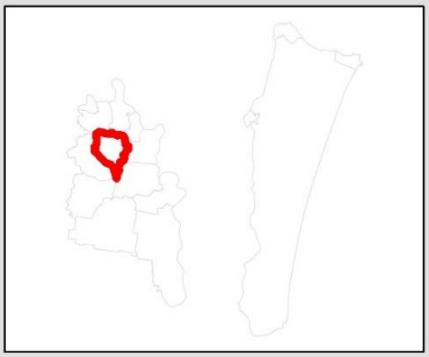
0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala position in 2018 and 2020/21

- 3
- 8
- 9



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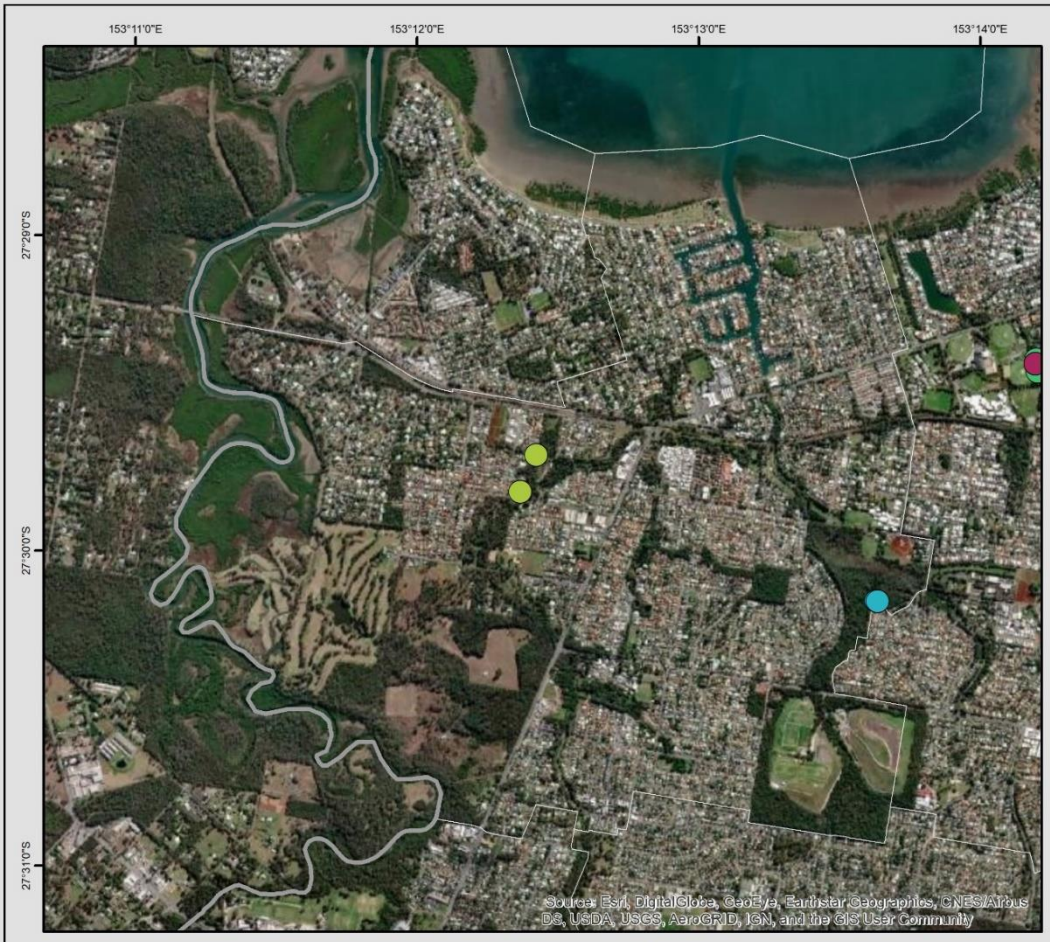


Birkdale

N

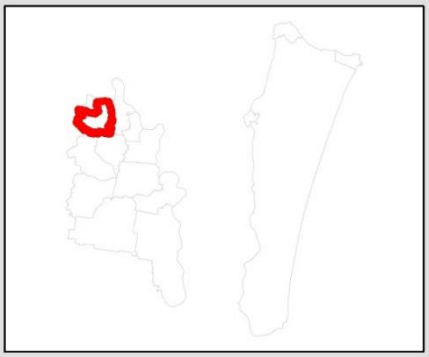
0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala position in 2018 and 2020/21

- 2
- 4
- 5
- 6



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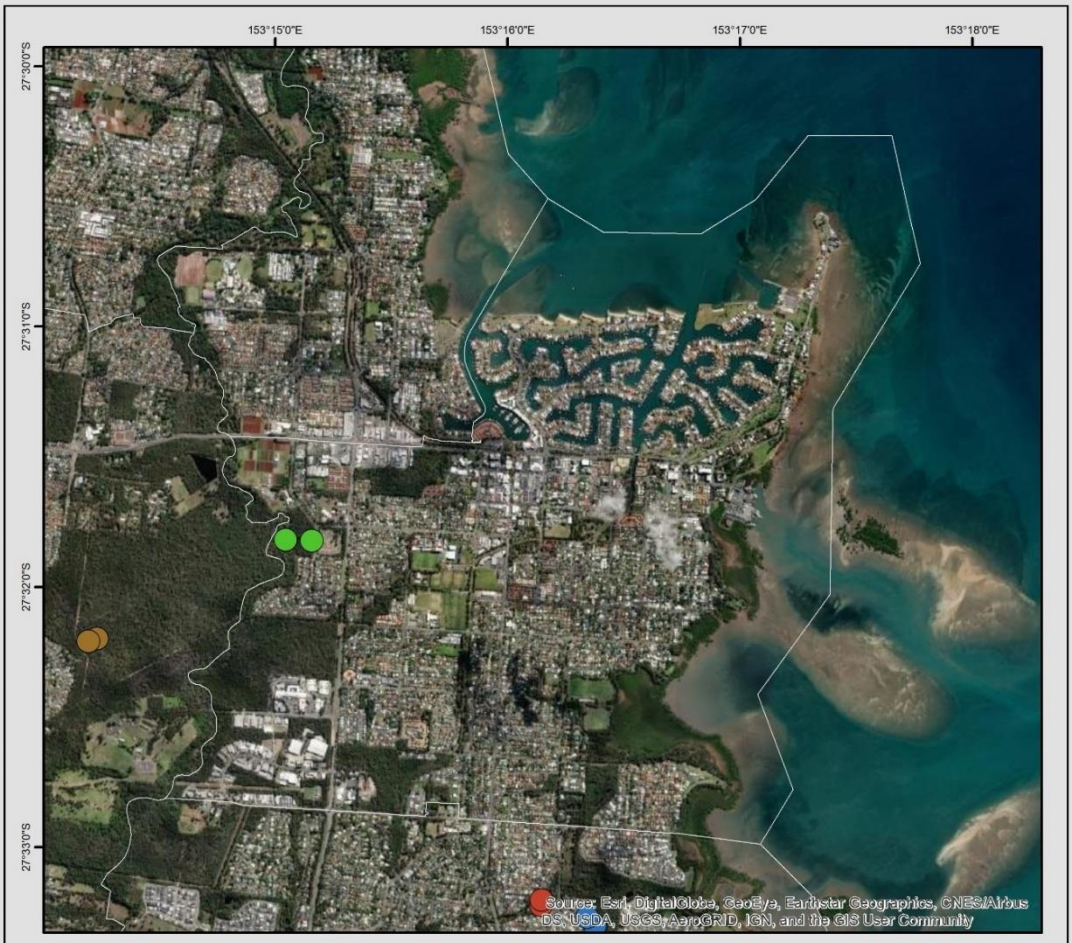


Cleveland

N

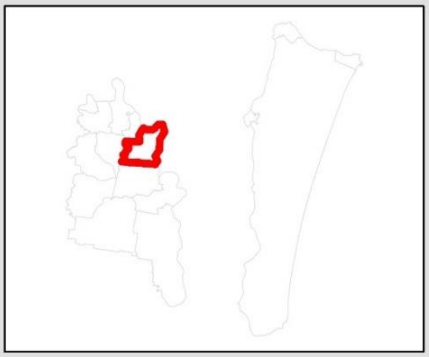
0 0.4 0.8 1.6 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala position in 2018 and 2020/21

- 3
- 7
- 8
- 9



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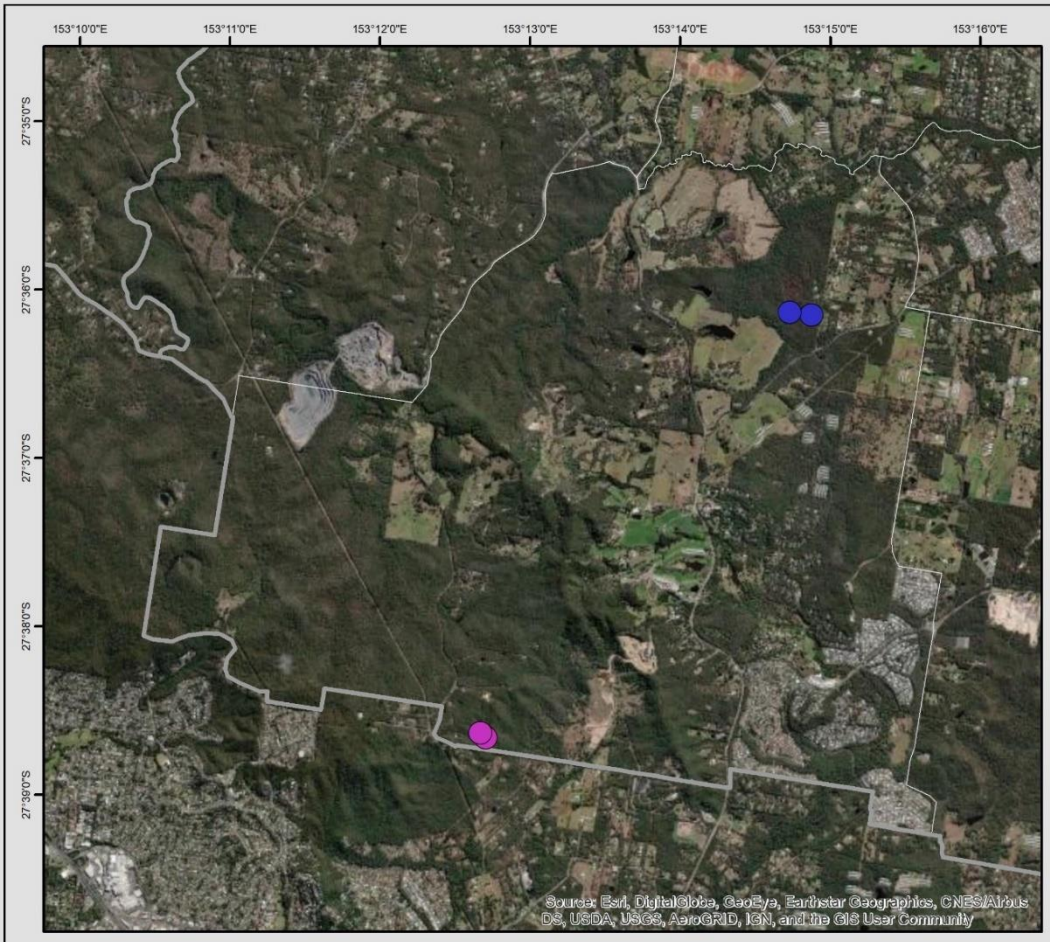


Mount Cotton

N

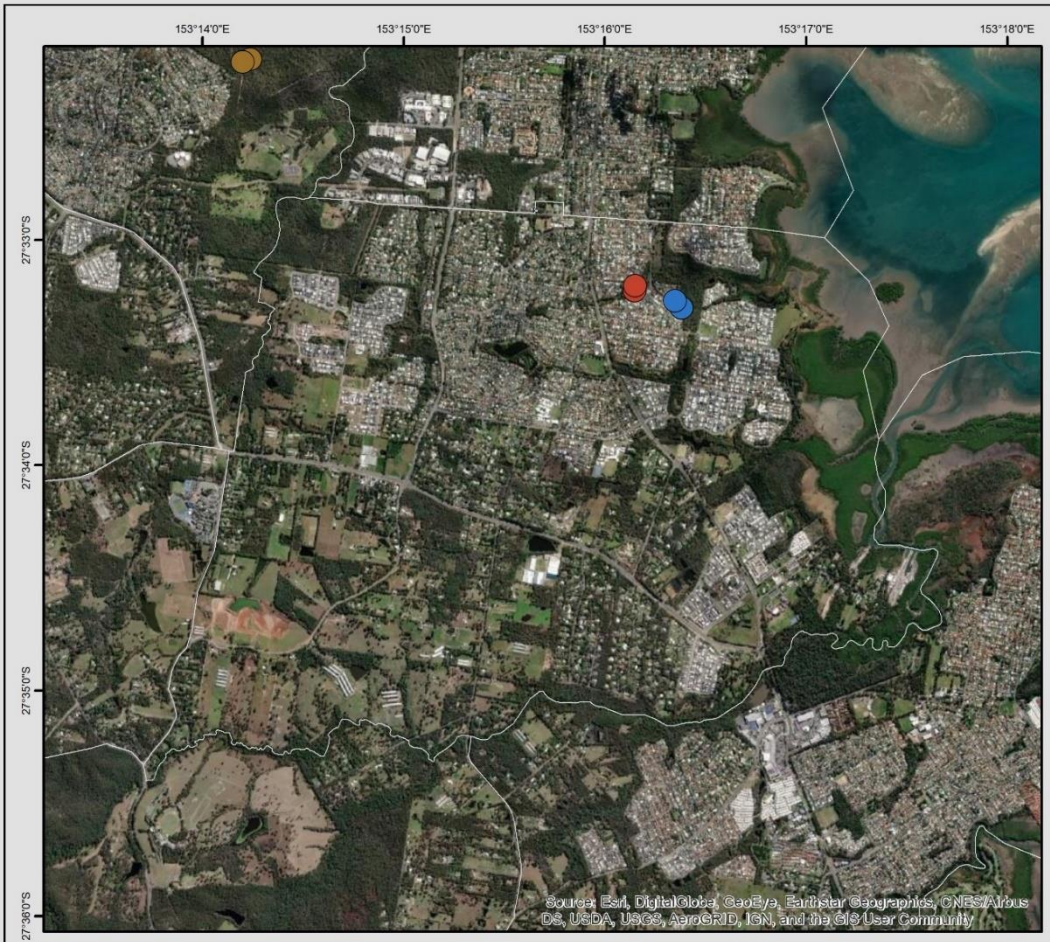
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala position in 2018 and 2020/21

- 10
- 11



Thornlands

N

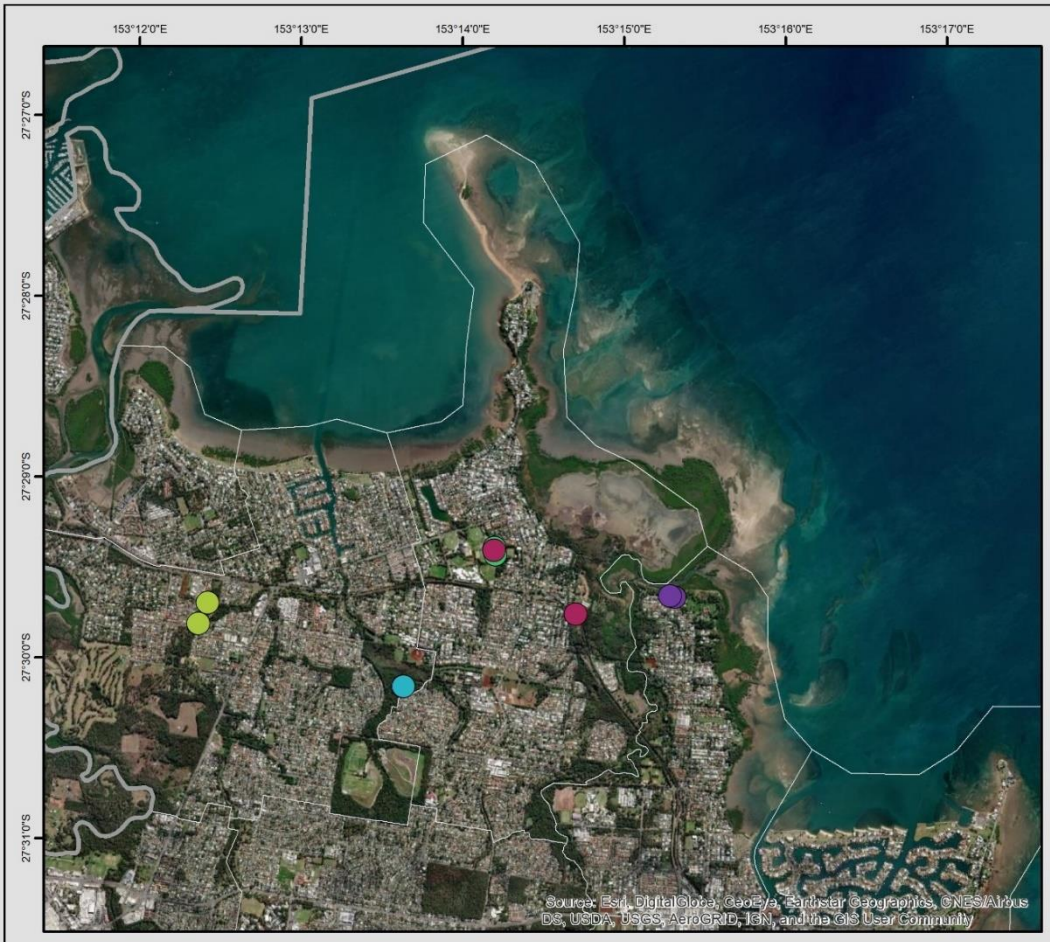
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala position in 2018 and 2020/21

- 3
- 7
- 8



Wellington Point

N

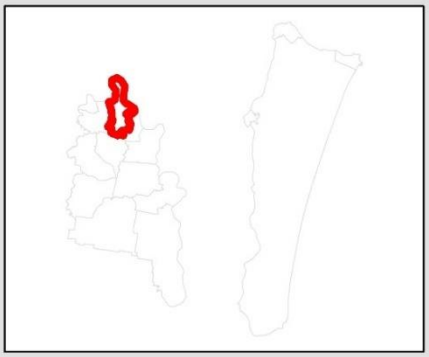
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala position in 2018 and 2020/21

- 1
- 2
- 4
- 5
- 6



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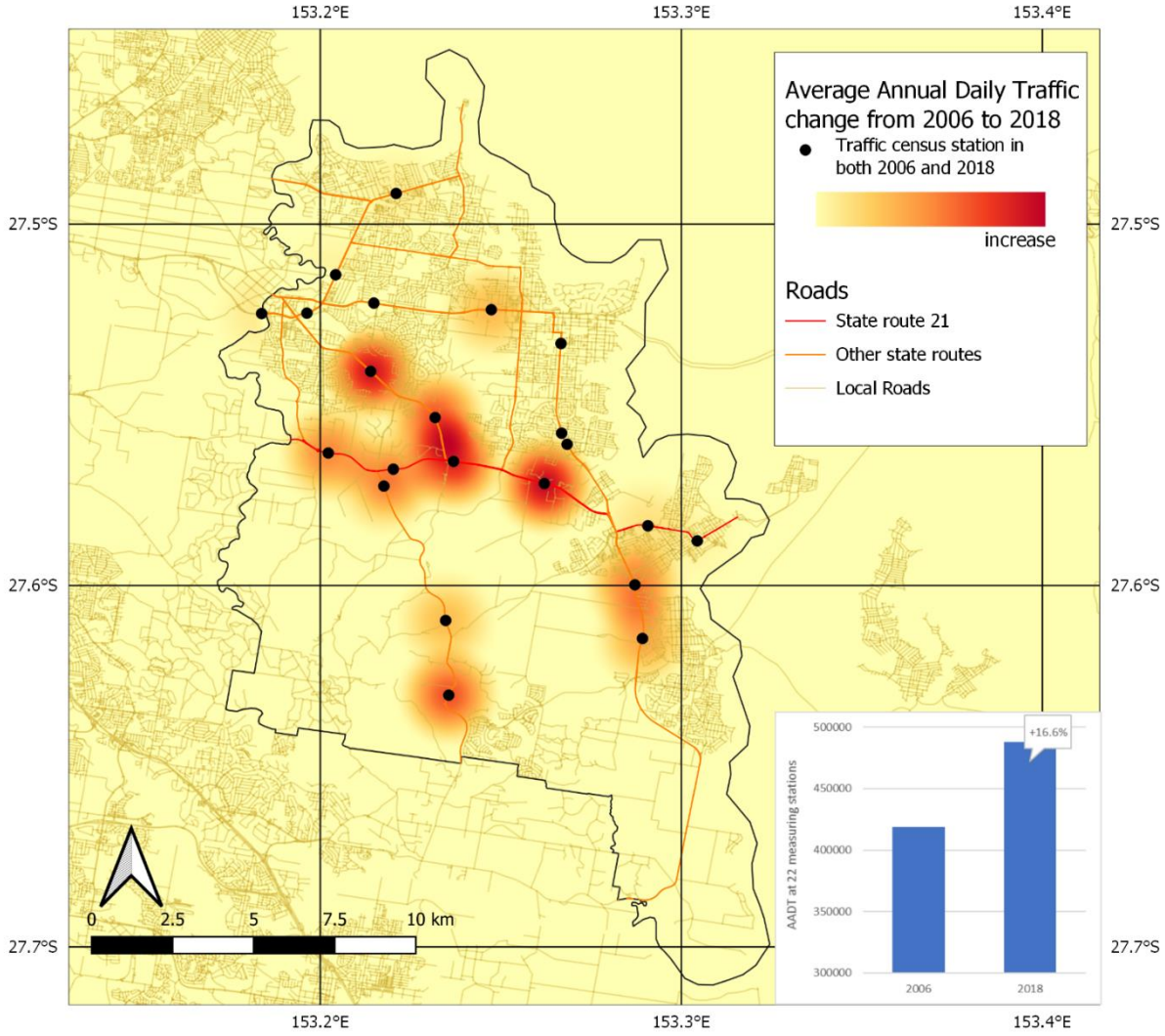
Appendix 4: Traffic development in Redlands Coast mainland from 2006 to 2018, hotspots of traffic increase and koala vehicle collision numbers

Overall, traffic on Redlands Coast mainland increased from a mean average traffic of 19,027.5 in 2006 to a mean average traffic of 22,187.3 in 2018 (or 16.6% increase). While a few roads measured a decrease in average traffic, most showed an increase (Appendix 4 Figure 1). Appendix 4 Figure 2 shows a heat map with census measure points in Redlands where traffic has increased from 2006 to 2018. It is important to note that only 22 measure points across Redlands were available and this result does not present census data for other roads in Redlands. However, these 22 measure points were located along larger roads and should therefore represent the most relevant ones. For instance, measure points along State Route 21 show an increase in average annual daily traffic of between 24.4% - 64.4%.



Appendix 4 Figure 1. We found an overall increase of 16.6% in average annual daily traffic across 22 census stations in Redland City Council. This heat map shows the increase in Average Annual Daily Traffic counts from 2006 to 2018. While at some stations average traffic decreased over the course of the 12 years, many other experienced an increase of up to 64.4%.

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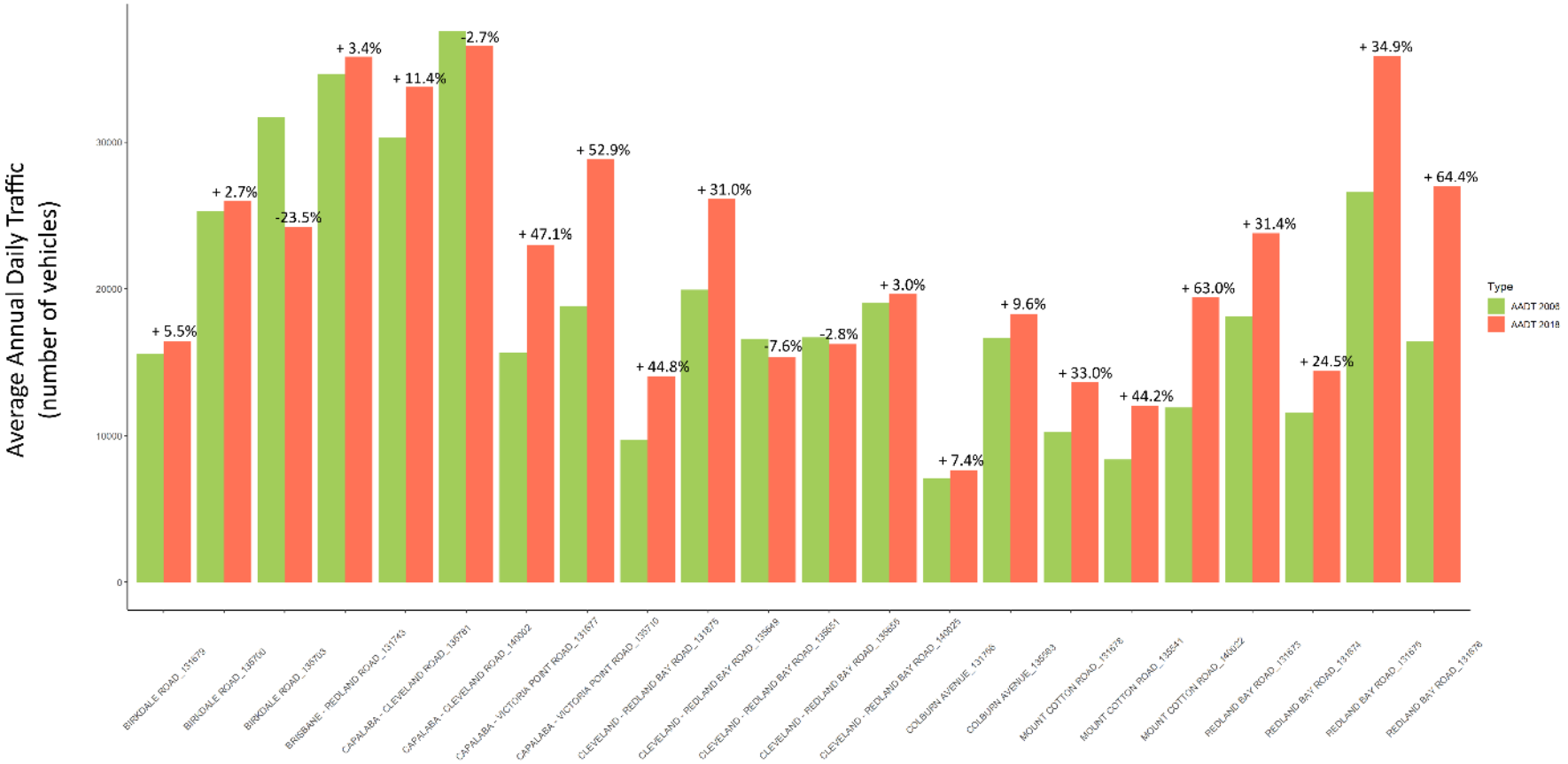

Appendix 4 Figure 2: Average traffic (Average Annual Daily Traffic) details for 22 census measuring stations across Redland City Council.

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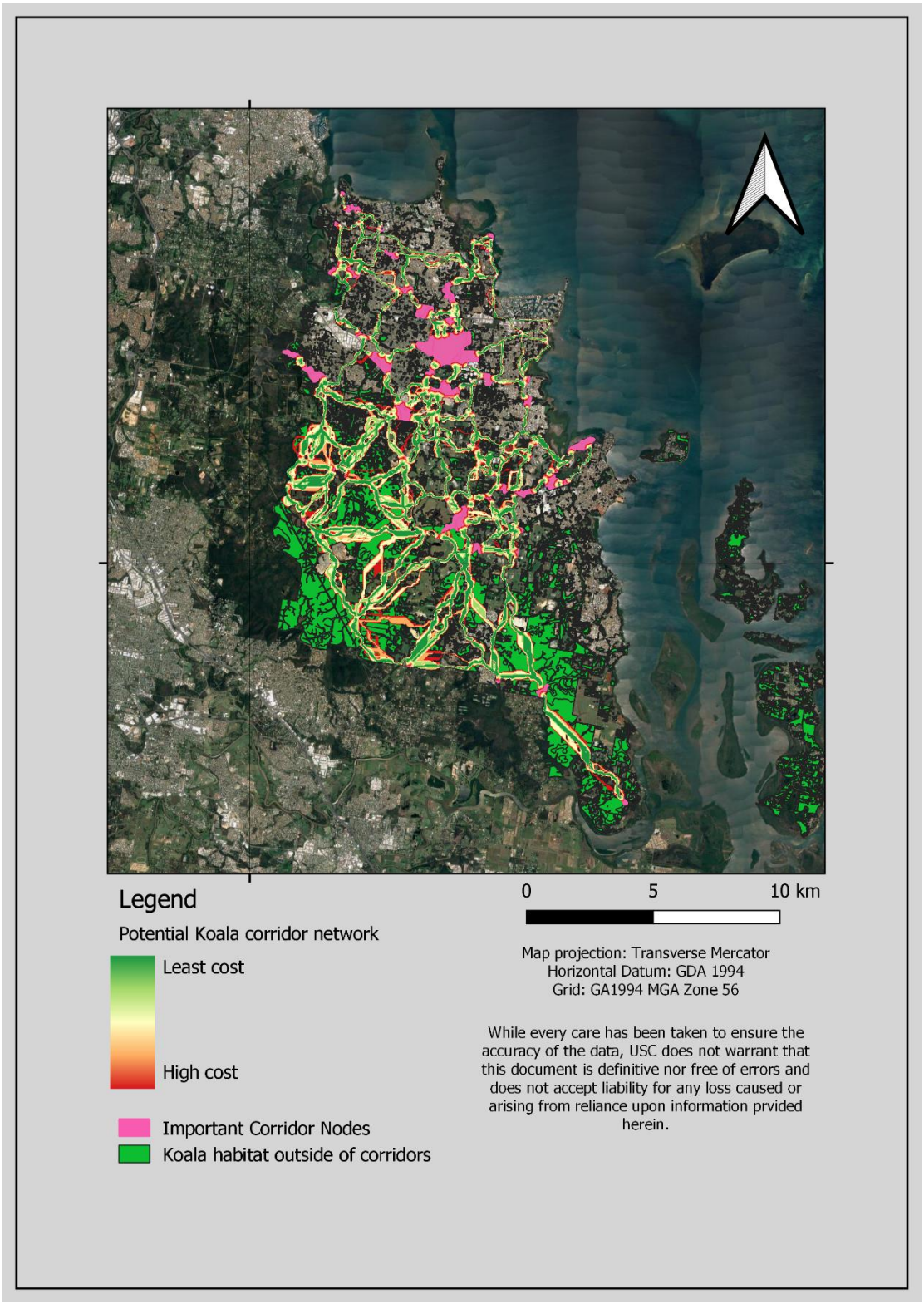
Appendix 5: Koala corridor mapping for Redlands Coast mainland provided by the DDC.



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End of report