DETECTION DOGS FOR CONSERVATION
Sniffing out solutions usc.edu.au/ddc
University of the Sunshine Coast

Final Report:

Redland Coast Koala Population Assessment Project

(Q-1864-17_18-ERE)

Prepared for Redland City Council

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

Dr Romane Cristescu, Katrin Hohwieler, Dr Kasha Strickland, Dr Bethan Littleford-Colquhoun and Dr Celine Frere

November 2018


DETECTION DOGS
FOR CONSERVATION
Sniffing out solutions usc.edu.au/ddc
University of the Sunshine Coast

Acknowledgments

The authors are grateful for the opportunity and privilege to work on Quandamooka Country. Especially, the authors would like to thank the Quandamooka Yoolooburrabee Aboriginal Corporation (QYAC) and all Rangers (NBD, EHP, IAS, FFS and QPWS) for sharing their experience and for their support in the field. In particular, we thoroughly enjoyed benefiting from the experience and knowledge in the field, of Darren Burns, Kathryn Crouch, Natia Burns, Claudia Engelbrecht, Callum Coghill, Elijah Addley, Jayden Brown-Engelbrecht, Jimmy Murray, Shara Beard and Isaac Burns.

The authors would like to thank Redland City Council for funding and supporting this research. In particular, Cathryn Dexter, for her unfailing support and energy in organising and coordinating the many aspects of this research to ensure a smooth delivery. We are also grateful for the openness, willingness to share and opportunity to work collaboratively, throughout this project, with Christine Hosking (Global Change Institute, The University of Queensland) and Biolink Ecological Consultants. We are thankful for Redland City Council staff who promoted the research and helped us recruit private properties to survey. IndigiScapes personnel, Lisa Bailey, Jenny Davis and Maree Manby, as well as Deidre de Villiers, gracefully shared their extensive knowledge of the Redlands Coast koalas. Deidre de Villiers provided biopsy samples, historical from the Redlands Coast and widely distributed across SEQ, that have both enabled this research and will enhance our understanding of the Redlands Coast koalas, and the importance of this cannot be overstated – we are extremely indebted.

Citizen scientists, and in particular Debbie Pointing, Ken Rawlins and the rest of the Koala Action Group as well as Jan Aldenhoven, helped increase further genetic sample collection by their local knowledge, help in the field and collecting scats for the Detection Dogs for Conservation.

We are grateful to all landowners who granted us access to their properties. In particular, Sibelco who welcomed us on mining lease, Dan Carter for supporting and facilitating our research, as well as enabling a successful Yarraman sampling, and Lisa Karklis for sharing GIS layers.

Disclaimer

This report was prepared in accordance with the scope of work agreed with Redland City Council (the Client) and is subject to the specific time, cost and other constraints as defined by the scope of work.

To prepare this report, USC relied on information supplied by the Client, and does not accept responsibility for the accuracy or completeness of this information. USC also relied on information gathered at particular times and under particular conditions, and does not accept responsibility for any changes or variances to this information which may have subsequently occurred. Accordingly, the authors of the report provide no guarantee, warranty or representation in respect to the accuracy, adequacy or completeness of the information, whether generally or for use or reliance in specific circumstances. To the extent permitted by law, the authors exclude any liability, including any liability for negligence, for any loss, damage, injury, illness howsoever caused, including (with limitation) by the use of, or reliance upon, the information, and whether arising from errors or omissions or otherwise.

Table of Contents

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 genetic 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.

Bottleneck: an event that drastically reduces the size of a population. The bottleneck may have various anthropogenic or natural causes, including environmental disasters, hunting / overexploitation, habitat destruction, or diseases. Population bottlenecks produce a decrease in the gene pool of the population, as many alleles that were present in the original population are lost, therefore the remaining population has a lower level of genetic diversity. Following a population bottleneck, the remaining (smaller) population also faces a higher level of genetic drift and further decrease in genetic diversity. Indeed, in small populations, infrequently occurring alleles face a greater chance of being lost, which further decreases the gene pool.

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

DES: Department of Environment and Science (Queensland)

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 only indirectly linked to the census size of a population, instead they are directly dependent on the genetically effective population size. 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 as the population under study. Although for natural populations, the effective to census population size ratio (N_e/N) 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 other 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).

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 (Mace et al. 2008).

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 (Lande 1998).

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.

Extinction vortex: describes the likely adverse interaction between human impacts, inbreeding, and demographic fluctuations that result in a reinforcing feedback loop and spiral downwards in population size towards extinction.

Evolutionarily significant units (ESU): a term used to define a population worth protecting and managing on its own, based on geographical and historical isolation. The recognition of ESUs is primarily relevant to long-term management issues, that is, defining conservation priorities and setting strategy, although in the short term it may be prudent to avoid translocating individuals between ESUs. Simulation studies suggest that it takes about $4N_e$ generations from the time that two populations separate for there to be a high probability of being independent ESUs (therefore Redlands mainland and Minjerribah / North Stradbroke Island could be considered independent ESUs).

Founder effect: a phenomenon that occurs when a small group of individuals becomes isolated from a larger population. Regardless of what the original population looked like, the new population will resemble only the individuals that founded the smaller, distinct population. This small population size means that the colony may have:

- reduced genetic variation from the original population,
- a non-random sample of the genes in the original population.

F-statistics (fixation index)**:** is the basic method used to measure the amount of subdivision in populations, and consists of three measures, $\mathbf{F_{IS}}$, $\mathbf{F_{ST}}$, and the less commonly used $\mathbf{F_{IT}}$. 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 FST (produced by dividing FST 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 closest 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 purge: an increased efficiency of natural selection to eliminate deleterious alleles because of inbreeding (which increases the number of homozygous loci thereby preventing deleterious alleles to "hide" in heterozygous animals). This means that a population that has survived a long period of time at a relatively small size (e.g. koalas on Minjerribah / North Stradbroke Island) is less at risk from further inbreeding or population reduction than a population that was large for a long time and has experienced a recent population decrease (e.g. Redlands mainland).

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:

> $\mathbf{H_O}$ = observed heterozygosity, the calculated level of heterozygosity from the allele frequencies of the population under study,
>
> $\mathbf{H_E}$ = 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.

LGA: Local Government Areas

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).

Outbreeding depression: a decrease in fitness in offspring resulting from mating between individuals that are genetically dissimilar (potentially, individuals from populations that have been isolated for a long time). This can be due to the offspring having genes from a parent that are not adapted to the local conditions (local adaptation), or to breaking gene complexes that are co-adapted.

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).

QYAC: Quandamooka Yoolooburrabee Aboriginal Corporation

qPCR (quantitative, or real-time, PCR): a molecular technique based on PCR (see above) that allows for the quantification of DNA in real time.

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
Grand-parents 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).

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 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:

- **Large-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 no to very low gene flow. The software usually tests for 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 Fst, 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 Fst).

- 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 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, a collaboration between Redland City Council (RCC) and the University of the Sunshine Coast's Detection Dogs for Conservation (DDC), delivered koala scat surveys using detection dogs paired with powerful next-generation genotyping to better understand current population characteristics that can inform efficient and effective management. Specific aims were to gain information, across both Redlands mainland and Minjerribah / North Stradbroke Island, on:

1) Where Redlands Coast koala populations are

In particular:

a) the distribution of koalas,
b) the number of genetically identified koalas,
c) the number of females, males and therefore sex ratio.

2) How connected populations of koalas are (e.g. gene flow*)

In particular:

a) the extent of gene flow across the landscape,
b) the spatial distribution of closely related koalas across the landscape (cryptic population genetic structure*).

3) How healthy Redlands Coast koalas are

In particular:

a) whether populations have experienced a genetic bottleneck*,
b) the levels of genetic diversity*,
c) the levels of inbreeding*,
d) the effective population size*,
e) the presence of the Chlamydia pathogen.

Findings

Note. Specific results per locality are given in Appendix 3.

Koala presence / absence surveys

A total of **531 surveys** were conducted, 303 on Minjerribah / North Stradbroke Island and 228 on the mainland. Out of the 531 surveys, 343 of the survey sites had the detection dogs identifying koala scat presence, with **977 instances of scat detection (old and fresh scats)**. Cleveland, on the mainland, and all townships on Minjerribah / North Stradbroke Island, were very high for koala scat presence (more than 90% survey sites returned presence of koalas). During the scat surveys, a total of **116 live adult koalas** were spotted, of which four showed severe signs of Chlamydia and ten were identified as females with a joey. All koalas were spotted in the northern half of the mainland or on Minjerribah /North Stradbroke Island.

Implications for Conservation. Koalas are widespread across the Redlands Coast, and koalas and koala scats were easy to find doing the surveys. Koalas are readily found in urban areas in particular, where threats are heightened by the density of vehicles and domestic dogs, as well as a lack of habitat connectivity, both at the canopy (between trees) and forest (between patches) levels. This could theoretically drive koalas to spend more time travelling across the ground (although more research into fine scale koala movement is needed).

Recommendation. Protection of koalas in the Redlands Coast needs to include a strategic urban koala plan, as it seems a non-negligible part of the koala population on the mainland is currently found in urban areas.

It should be noted, however, that survey effort could be increased to the southern part of the Redlands mainland (Sheldon, Mount Cotton, Redland Bay, see Limitations).

Genotyping

A total of **689 samples of fresh scats** were collected, of which **383 scats were processed for genetic analysis.** Following quality control and the removal of duplicate samples, 193 individuals were identified, categorised as follows: N = 99 on the mainland and N = 94 on Minjerribah / North Stradbroke Island. Overall, we found a good sex ratio* of 89 males and 104 females (male to female ratio = 1:1.2).

Population structure and connectivity

We found evidence of two ancestral populations across the Redlands Coast: Redlands mainland and Minjerribah / North Stradbroke Island, with no further genetic differentiation of populations within either. Importantly, genetic analyses revealed that both Redlands mainland and Minjerribah / North Stradbroke Island populations are each one continuous breeding population (i.e. we did not find isolated populations within each of the two populations of Redlands mainland and Minjerribah / North Stradbroke Island).

However, we found strong evidence of fine-scale spatial structuring within each population. We identified, for instance, a potential barrier to gene flow between Cleveland and Wellington Point / Birkdale individuals on Redlands mainland, as well as between Amity / Point Lookout and the rest of the island on Minjerribah / North Stradbroke Island. Whilst the degrees of genetic differentiation between these aforementioned locations are small, they are likely to 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). This

indicates that events such as fire on Minjerribah / North Stradbroke Island and the increasing urban footprint on Redlands mainland may be resulting in restricted gene flow between locations. However, the true impacts of these events / habitat shifts may not be fully appreciated until we sample future generations.

At an even finer scale, within Redlands mainland, individuals were significantly more closely related than expected by chance within a smaller range (up to 350 m) than they were on Minjerribah / North Stradbroke Island. This indicates that island koalas disperse further than those on the Redlands mainland. Furthermore, koalas within the Redlands mainland urban footprint were significantly more related to each other than those found in the non-urban footprint of Redlands mainland.

Implications for Conservation. We found evidence of continuous breeding populations in both Redlands mainland and Minjerribah / North Stradbroke Island, which shows gene flow, and thus koala dispersal, still exist at this scale - a very positive result.

Nonetheless, we found evidence of fine-scale spatial structuring within each population, especially on Redlands mainland. These fine-scale spatial structuring results are concerning (i.e. this is not a positive situation for koala genetic conservation) as they provide evidence that koalas on the mainland:

1. Do not disperse as much as koalas on Minjerribah / North Stradbroke Island
2. Are surrounded by close relatives

This, combined with the existing low permeability of the urban matrix for dispersal, will only further increase the potential risk of inbreeding depression*.

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*. In both Redlands mainland and Minjerribah / North Stradbroke Island we found:

1) much 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,
3) strong evidence of past genetic bottlenecks,
4) low effective population sizes (Redlands mainland: 85.7; Minjerribah / North Stradbroke Island: 92.9).

Note that the effective population size is the number of individuals that, if they behaved in the manner of an ideal population, would result in the same loss of genetic diversity, inbreeding, or genetic drift* than the studied population. 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. The estimate of effective population size is not constrained by the number of individuals sampled – i.e. 20 individuals can produce an infinite effective population size [see Table 6 of this report from Kjeldsen et al. (2016)].

Chlamydia was present and widely spread across Redlands Coast. The levels of Chlamydia detected (any Chlamydial sequences detected) were higher on the mainland (56%) and lower on Minjerribah / North Stradbroke Island (21%).

Implications for Conservation. Together these results, combined, form a concerning picture and should be taken as a call for action. Our results provide evidence that koalas on the mainland and Minjerribah / North Stradbroke Island have:

1. Strong evidence of past genetic bottlenecks
2. High inbreeding both at population ($H_E > H_O$, high F_{IS}*, see F-statistics*) and individual (high IR*) levels
3. Small effective population size (N_e)

In addition, koalas on the mainland are experiencing:

1. Higher Chlamydia infection rate
2. Higher population inbreeding (F_{IS}) in the urban footprint

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. (Please see background for these recommendations and uncertainties surrounding these numbers in the Acronym and glossary section of this report)

Using these guidelines, both Redlands mainland and Minjerribah / North Stradbroke Island populations fall short of the recommended effective population size and are therefore at high risk of inbreeding depression and decreased evolutionary potential. This, combined with high F_{IS}, high IR and high Chlamydia infection rate for mainland koalas, indicates the vulnerability of koalas across the Redlands Coast and a heightened vulnerability of the mainland population.

As it stands, genetically, Redlands mainland and Minjerribah / North Stradbroke Island koalas fit within the Critically Endangered category of the IUCN Red List. It is important to note that this analysis is limited to administrative boundaries (on the mainland in particular). We do not suggest that the IUCN would classify the Redlands mainland and Minjerribah / North Stradbroke Island populations as critically endangered, but we are underlining that their genetic characteristics have reached a level that should concern decision makers in charge of preserving the koala population in the Redlands Coast for future generations. Although the administrative boundaries of the Redlands Coast are artificial and irrelevant to koala ecology, most management decisions will be constrained by these boundaries. Ultimately, the accountability for ensuring the survival of the Redlands Coast koalas (rightly or not) will be attributed to local government.

Recapitulative table of all positive (green), negative (red) and neutral (orange) findings from this project. Note that comments on the presence of koalas are qualitative only, as the surveys were not designed to compare presence across areas (i.e. surveys were not random nor standardised).

		Redlands mainland	Minjerribah / North Stradbroke Island
Presence results (qualitative assessment only)			
	1	wide geographic spread of koala presence	wide geographic spread of koala presence
	2	large koala presence within the urban footprint	large koala presence within the urban footprint
	3	large number of koalas sighted	large number of koalas sighted
	4	large number of fresh scats collected	large number of fresh scats collected
Genetic results			
Large-scale structure	**5**	one continuous population / a single lineage	one continuous population / a single lineage
	6	gene flow maintained across the whole area	gene flow maintained across the whole area
Fine-scale structure	**7**	evidence of sub-structure / decrease gene flow	evidence of sub-structure / decrease gene flow
	8	closely related individual nearby / low dispersal	closely related individuals able to disperse
	9	individuals within urban footprint more related than outside urban footprint	
Genetic health	**10**	small bias of sex ratio towards female (1:1.4)	small bias of sex ratio towards male (1:0.9)
	11		lower genetic diversity / polymorphism (founder effect) than the mainland, but still higher than other island koalas
	12	high level of inbreeding at the population level	high level of inbreeding at the population level
	13	high level of inbreeding at the individual level	high level of inbreeding at the individual level
	14	evidence for genetic bottleneck	evidence for genetic bottleneck
	15	effective population size lower than recommendations of >100 to prevent inbreeding	effective population size lower than recommendations of >100 to prevent inbreeding
	16	effective population size lower than recommendations of >1000 to maintain evolutionary potential	effective population size lower than recommendations of >1000 to maintain evolutionary potential
	17	potentially still linked to other populations outside Redlands Coast	population potentially more resistant to inbreeding through genetic purging
Disease results			
	18	high level of chlamydia	low level of chlamydia

Recommendations

Altogether, we found that the Redlands Coast, which historically was known to harbour a large koala population, still had evidence of widespread koala presence, and koalas and their fresh scats were readily found. Each separate geographical entity, Redlands mainland and Minjerribah / North Stradbroke Island, formed one koala population where gene flow has been maintained.

To ensure the viability of Redland Coast koala populations for the future, Redland City Council should focus efforts on tackling the concerning emerging trends identified. Protecting remaining habitat, as well as maintaining and improving connectivity, will be key to preventing the identified high risk of inbreeding depression, especially within the urban footprint. An increase in habitat loss and / or fragmentation will result in small, enclosed populations where further inbreeding and loss of genetic diversity is unavoidable.

The existing gene flow occurring within Redlands mainland and Minjerribah / North Stradbroke Island is the life line of Redlands Coast koala populations – this will need to be monitored closely in the future so that any disruption to gene flow will be quickly detected, and swift management measures implemented where required.

In the event that future monitoring detects an increased deterioration in gene flow, Redlands City Council might have to carefully assess and consider potential use of more intensive management strategies (e.g. artificial inseminations, translocation of genetically dissimilar males into genetically poor, enclosed populations). This will require additional work to develop a genetic breeding rescue program suited to the Redland Coast koalas.

1. Introduction

1.1 Scope of works

Redland City Council (RCC) contracted the University of the Sunshine Coast's Detection Dogs for Conservation (DDC) team to conduct koala scat surveys across RCC with the aim to better understand current population characteristics to 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.

In particular, the following were within the scope of the project:

1. Undertake comprehensive koala scat surveys of Council's nominated sites or areas (in both urban and bushland settings) – nominated sites / areas were to be provided to USC prior to survey start (but see limitations)
2. Undertake comprehensive collection and storage of koala scats in preparation for DNA population sampling
3. Provision of detailed data on urban koala populations
4. Provision of presence/absence data (through scats) in bushland areas to increase knowledge of koala distribution across the Redlands Coast, targeting knowledge gaps (i.e. areas with low / no information or old records)
5. Provision of next generation genotyping for the genetic analysis of koala scats to provide fine scale understanding of urban and bushland koala populations (i.e. sex ratio, population structure and genetics, inbreeding and disease in particular)

1.2 Background

While subdivision of populations has been commonplace throughout evolutionary history and is one of the keystone drivers of speciation (Coyne et al. 2004), the scale and speed at which this is now occurring is critically reducing the evolutionary adaptive potential of most species which inhabit human impacted landscapes (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. 2009b, 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. 2010b, 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 effective conservation and monitoring is of urgent priority. Koalas (*Phascolarctos cinereus*) for instance, are, despite their iconic status and economic value (potentially $3.2 billion per annum (Conrad 2014)), experiencing an alarming sharp decline in the northern and eastern parts of their range (Queensland, New South Wales and the Australian Capital Territory), where populations have diminished by 68% in less than 20 years (just three koala generations) (Government 2012, Rhodes 2015). The species is now listed as vulnerable under the Australian Environment Protection and Biodiversity Conservation Act in these areas (McAlpine et al. 2015), and therefore in the Redland City Council area.

The reasons for these declines are well known: 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), but evidence about how these factors are impacting specific populations is often not available to decision makers, and this stands for the Redlands Coast. To enable environmental planning, what is needed is fine-scale information about 1) where koala populations are, 2) how connected versus isolated they are, 3) how healthy they are, and 4) how they move in the landscape. Until recently, however, generating this level of data has often been prohibitively expensive (as traditionally these data required catching, sampling and monitoring live animals).

Here, we deployed proven non-invasive and cost-effective methodologies (conservation detection dogs (Cristescu et al. 2015b) and genetic analyses of scats (Schultz et al. 2018a)) to collect and measure these required fine-scale key health indicators for koalas across the Redlands Coast. As such, this project challenged the largest single barrier to effective koala conservation: lack of scientifically-robust and large scale data on koala genomic diversity, disease and connectivity to empower decision makers to effectively manage their koalas. In particular, we focused on determining:

1) **Where Redlands Coast koala populations are**

Here, we conducted a total of **531 surveys** across Redland City Council [Redlands mainland (N = 228) and Minjerribah / North Stradbroke Island (N = 303)] to assess:

a) the distribution of koalas (presence/absence),
b) the number of genetically identified koalas,
c) the number of females and males.

2) **How connected populations of koalas are (e.g. gene flow)**

Here, we estimated the extent of population genetic structure across the Redland City Council [Redlands mainland (N = 99) and Minjerribah / North Stradbroke Island (N= 94)] to assess:

c) the extent of gene flow across the landscape,
d) the spatial distribution of closely related koalas across the landscape (cryptic population genetic structure).

This enables us to measure whether koalas in the Redlands Coast are a single random-mating population (fragmented spatially, but connected by gene flow), partially connected fragments and meta-populations, or completely isolated subpopulations. Each of these scenarios will have different genetic consequences in relations to genetic drift, inbreeding, fitness and extinction risks (Frankham et al. 2010b).

3) How healthy Redlands Coast koalas are

Here, we estimated a suite of genetic and health traits to assess the extent to which Redlands Coast koalas [Redlands mainland (N = 99) and Minjerribah / North Stradbroke Island (N = 94)] may be vulnerable to local extinction. These included:

f) whether populations have experienced a genetic bottleneck,
g) the levels of genetic diversity,
h) the levels of inbreeding,
i) the effective population size,
j) the presence of Chlamydia.

Together, these genetic and health traits allow us to estimate 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. For instance, it has been shown that a 5% increase in inbreeding can increase population extinctions risk from 25% to 69% [*Clarkia pulchella* plants (Newman and Pilson 1997)]. These values represent intolerably high inbreeding depression in the wild. Further, IUCN recommendations state that effective population size (N_e) need to be ≥ 100 to avoid inbreeding depression and ≥ 1000 to maintain evolutionary potential of species (Mace et al. 2008, IUCN 2012, Frankham et al. 2014).

2. Methodology

2.1 Establishing where Redlands Coast koala populations are

2.1.1 Site selection / sampling design

Survey sites were located in conservation areas, recreational areas (e.g. parks), rehabilitation areas, wildlife corridors and National Parks (Venman National Park, Naree Budjong Djara 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 the greatest possible 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).

2.1.2 Survey types for koala scat detection

The DDC has developed and regularly uses two survey methodologies with detection dogs. The first survey methodology is called '***systematic koala scat survey***' where 30 trees are systematically searched (mirroring the standard human survey method) allowing for comparisons across space and time. The second survey methodology is called '***casual koala scat survey***' where we leave the dog to search more freely.

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 time limit or up to when the handler deemed the search to have covered the site extensively.

The casual surveys are an excellent and fast way to determine whether koala scats are present at a specific site. For example, this survey type is widely used to inform or test koala habitat mapping. This method is indeed 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.

The 'casual surveys' are more efficient and can sometimes be more accurate (the dog is free to follow its nose), however they lack the repeatability that 'systematic surveys' offer – therefore, the survey results might not be comparable in time or space. For the RCC surveys, we exclusively utilised casual koala scat surveys because our aims were 1) to maximise area coverage and 2) to maximise genetic sampling. Note that detection dogs are fitted with a GPS collar to document the areas searched.

2.1.3 Dogs utilised for koala scat detection

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

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

Teams mostly worked in parallel, however, at sites where knowledge of habitat use by koalas was sparse, an all-scat dog was deployed first to identify presence or absence of koalas. If presence was identified by this first dog, the fresh scat detection dog was deployed to find fresh scats (if not already found).

Surveys conducted with all-scat dogs are used to map presence/absence of koalas at a given site. In contrast, surveys conducted with Billie-Jean exclusively indicate presence when fresh scats are found but cannot be classified as koala absence when no scats are found, as Billie-Jean is trained to ignore old koala scats.

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 1) 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 1: 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 infection, often referred to as pink eyes (for ocular infection / conjunctivitis) and wet bottom (for urinary tract infection), and (3) presence of a joey. In case of finding a sick or injured koala, the RCC wildlife ambulance was contacted.

2.1.6 Citizen scientists

Scats were collected by citizen scientists when they saw koalas. Date and coordinates were recorded and the scats were stored in their household freezer until collected by the USC team.

Limitations

Koala presence surveys

Most sites were surveyed on only one occasion; therefore, the results presented here provide a snapshot of the population during this 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.

Due to external constraints (e.g., study design provided to USC after the survey starting date), the southern areas of the Redlands mainland were not surveyed as extensively as the northern areas or Minjerribah / North Stradbroke Island. This means the distribution of koalas in the southern areas of the Redlands mainland could be underestimated. Therefore, at this stage, some of the associated findings and implications remain to be consolidated.

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 these limitations by designing a new genotyping method (DArTcap, see methods), which enabled the genotyping of numerous loci* (>900).

Substantially more samples were collected from urban areas than non-urban areas on Redlands mainland (urban: N = 83; non-urban: N = 16), this was due to the study design being provided only after the USC team had been in the field for a few weeks. USC started in urban areas because this was a known priority area (from discussion with RCC) and then added more field work days at the end of the fieldwork time to increase the survey effort in the south. However, by then, samples could no longer be included in the analyses (because of time and budget constraints). When analyses conducted could have been affected by this uneven sample size, we randomly subsampled (ten times) individuals from urban areas and re-ran analyses to ensure significant patterns were valid. It is important to note that the DDC collected many more samples than budgeted, many of which are from non-urban areas on the mainland. These samples are available for future analyses which would further alleviate this potential limitation (detailed in Table 2).

We are still in the process of fully understanding the links between Chlamydia presence (qualitative), Chlamydia load (quantitative) and clinical Chlamydia disease in koalas and we

are currently working towards elucidating what DArTCap Chlamydia results (qualitative and quantitative) mean in terms of health impacts on the koalas.

It should be noted that koalas in Redlands Coast combine with part of Brisbane and Logan Councils to form a population known as the "Koala Coast", therefore it would have been more relevant 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. In addition, although genetic sampling in this study was constrained by administrative boundaries, rather than ecological or genetic boundaries, some of the koalas sampled in Redlands Coast close to the borders of neighbouring Councils would be using both Redlands Coast and neighbouring Councils, therefore representing genetic characteristics of koalas that extend beyond the boundaries of Redlands Coast.

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 designed for this project 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 using the following criteria (Kjeldsen et al. 2018). First, we included only SNP showing at least a call rate of 70% and a minor allele* frequency (MAF) of 3%. We checked for linkage disequilibrium between SNPs using PLINK (Purcell et al. 2007) and removed any SNPS which showed signs of linkage. We also removed samples with more than 50% missing data. Finally, we removed SNPs with a fixation index of greater than 0.7.

Duplicate samples (i.e., samples that were collected from the same koala) were identified as pairs of samples which had a relatedness value of greater than 0.65. Relatedness between genotyped samples was estimated using the Queller and Goodnight estimate (Queller and Goodnight 1989) in the RELATED R package (Pew et al. 2015). 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 differentiation

We used the Bayesian clustering approach implemented in the TESS3R R package within the R statistical environment to calculate assignment probabilities and assess genotypic clustering across the Redlands Coast (Caye et al. 2016). This approach incorporates spatial information about each individual when assessing population structure. In order to identify the number of ancestral populations (K) across the Redlands Coast, we used several values (K = 1 to 8). The default parameters of TESS3R were used, except we increased the tolerance of identifying genetic clusters to 1 x 10^{-14} and increased the maximum number of iterations for each K to 1 x 10^{7}. The optimal number of ancestral populations corresponded to the maximum of the cross-entropy criterion (assessed using a cross-entropy criterion graph; included in Appendix 2) across the range K = 1 to 8.

Two measures of population differentiation were calculated; F_{ST} (see F-statistics*), and F'_{ST}. These values were calculated using 999 permutations in GeneAIex (Peakall and Smouse 2006).

In addition, the number of migrants (Nm) was also calculated in GeneAIex using 999 permutations.

2.1.1 Fine-scale spatial distribution of related individuals

Spatial autocorrelation analyses were conducted in GeneAlEx 6.5 (Peakall and Smouse 2006) using a genetic distance matrix, representing the total genetic distance over 905 loci, and a geographic distance matrix. These analyses identify distances at which individuals are more related than expected if there was no fine-scale spatial structure of related individuals. We used sequentially increasing distance classes ranging between 50 metres and 11,500 metres.

Using relatedness calculated for each pair of individuals (calculated separately for Redlands mainland and Minjerribah / North Stradbroke Island), we visualised spatial patterns of pairwise relatedness by separating relatedness into three categories: 1) closely related (full and half siblings, parent/offspring), 2) related (cousins etc) and 3) not related (see Appendix 3 for breakdown per locality).

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).

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)

Observed (H_O) and expected (H_E) heterozygosity was calculated at each loci and population to test for possible departure from Hardy-Weinberg equilibrium*, using the Markov chain method in GeneAlEx 6.5.

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 LDNe 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 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). However, 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

Chlamydia status of koalas was determined through DArTCapture using 50 probes designed for Chlamydia. The probe design used to enrich for Chlamydia linked detection sequences used two approaches. Approach 1 used sequences previously discovered in standard DArTseq assays involving koalas with known infections of Chlamydia. Approach 2 involved an in-silico study of the various sequenced strains of Chlamydia and an extended search of previous koala DArTseq data looking for possible Chlamydia sequences that could be amplified in the Koala DArTseq library. In this way, a proportion of the probes were designed to enrich for sequences already tested and a proportion were designed to enrich for new sequences. Enriching for specific sequences in the sequencing was used to increase the detection sensitivity. Overall the DArTcap enrichment process was able to pull out more sequences with specific BLAST hits assigned to the Chlamydia genome from the huge number of more common bacterial sequences and give a higher signal to noise for Chlamydia detection compared to DArTseq data previously obtained from scat extracted DNA.

Chlamydia status of each individual was assigned using two different classifications:

1) A qualitative approach (presence / absence): if any chlamydial sequences were detected in an individual, it was assigned a positive result.
2) A quantitative approach (potential disease): a threshold (>9 SNPs detected out of the 30 probes) was used to classify an individual as chlamydial positive. This measure is, therefore, more conservative and may be more indicative of individuals who present chlamydial disease, not just chlamydial infection.

Note that presence and load of Chlamydia do not necessarily mean koalas are sick, as they can be passive carriers of the bacteria, or have recovered.

2.3.6 Urban and non-urban populations in Redlands 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 mainland population in order to assess the impact 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 a result of several limitations of this study, we unintentionally collected substantially more samples from urban areas than non-urban areas on Redlands mainland (urban: n = 83; non-urban: n = 16). As a result, to compare relatedness between urban and non-urban areas, we randomly subsampled individuals from urban areas and ran analyses on these data. This was done to ensure that results were not a consequence of uneven sampling. We repeated the random subsampling 10 times.

2.4 Limitations

2.4.1 Fieldwork

The sites were surveyed on only one occasion; 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)].

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. 2015a).

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

- 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 this project, survey effort in each area can be assessed by the track log of dog searches (provided in Figure 2, see details per locality in Appendix 3). This was complemented with koala spotting between locations. This was particularly intense on North Stradbroke Island, as off-leash dogs were identified as a risk to the DDC dogs, and therefore, especially in Dunwich and Amity where koalas are easily spotted due to their high density, most of the survey effort did not rely on detection dog surveys.

On the mainland, a large proportion of samples were collected from localities in the northern part of the Redlands Coast (i.e. all localities but Sheldon, Mount Cotton and Redland Bay).

This bias is partly due to a large proportion of southern Redlands Coast bushland being locked in private properties, as well as urban areas being 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. Therefore, surveys immediately started in urban areas in the northern region of the Redlands Coast, while the study design with priority areas for the bushland part of the RCC surveys (mainly covering the southern areas of the Redlands Coast) was delayed until the 09/05/18. The DDC added extra days, after the island survey, in August and September 2018, to increase coverage of the southern areas (Figure 3). Indeed, this increased the samples collected, however, due to timeframe and budget constraints, these samples were not processed in time to be included here. Had the study design, along with priority areas, been available prior to the start of the field surveys in April, the DDC could have achieved a more balanced distribution of the genotyped samples within budget (i.e. decrease the number of samples from urban areas analysed). Alternatively, to increase sample balance across the Redlands mainland, the extra samples in the south should be analysed as a priority.

Additionally, more koalas could have been detected in urban environments (localities in the northern part of the Redlands Coast) due to a potentially higher detectability rate compared to natural woodlands 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 making a koala easier to spot.

Despite these limitations (low number of private properties available as survey sites, delay in establishing priority areas for bushland surveys in southern part of the Redlands Coast, potential difference in koala detectability rate), 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 mainland have been underestimated. Therefore, at this stage, some of the associated findings and implications remain to be consolidated.

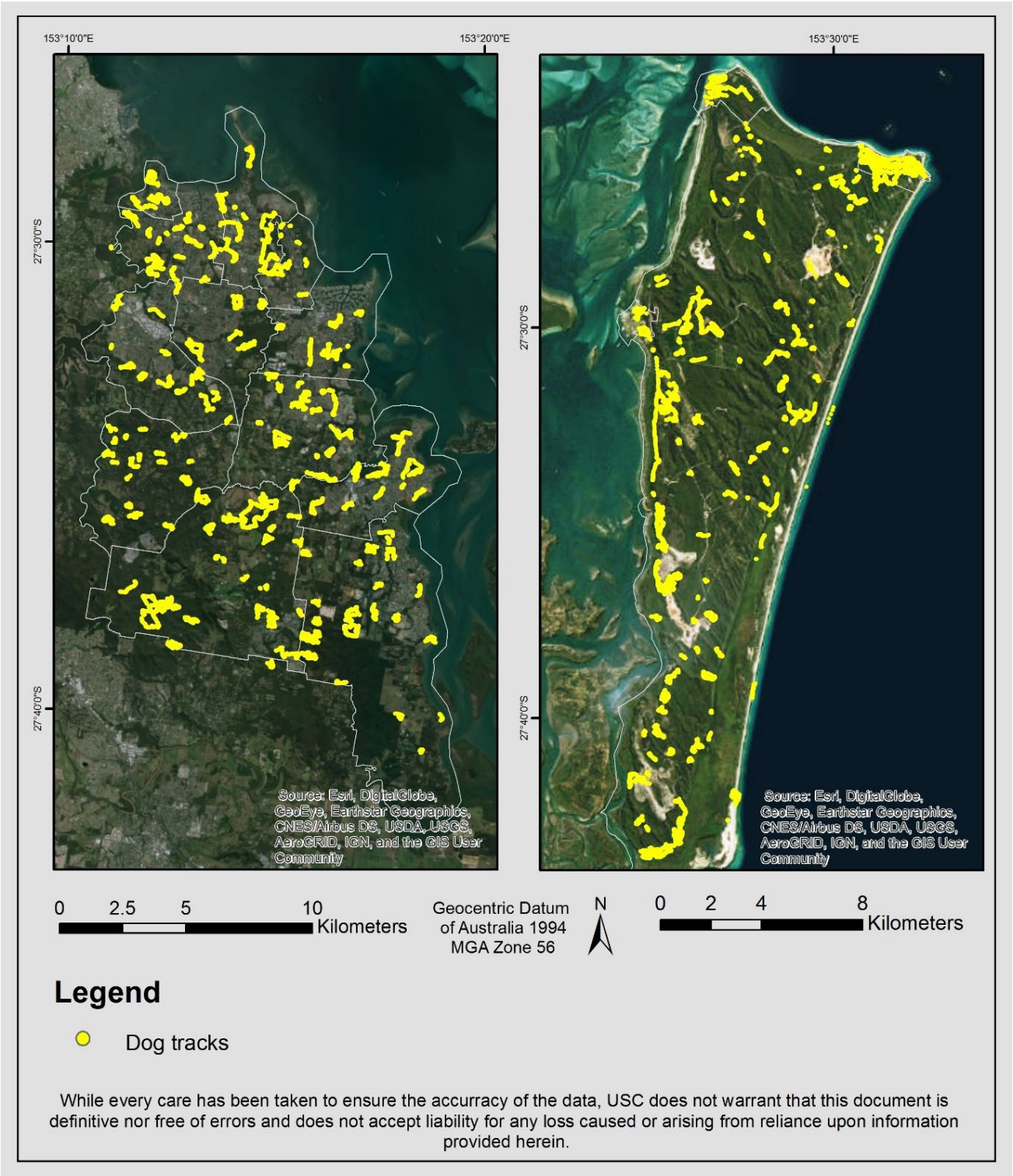


Figure 2: Dog tracks recorded during the surveys, as an indication of search effort across the Redlands Coast, note that in many instances, handlers also performed visual searches between sites, that therefore are not represented in the map

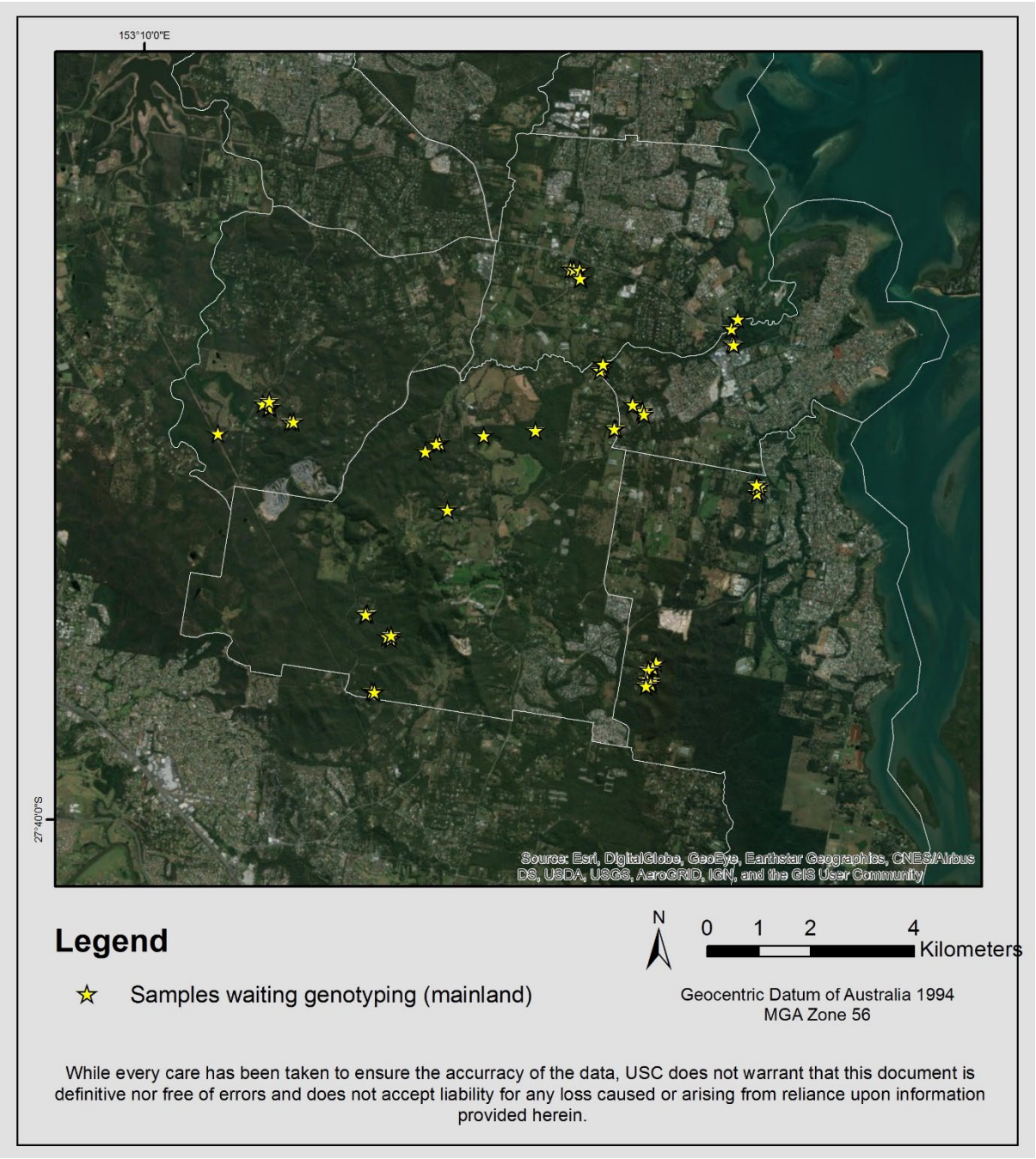


Figure 3: Extra samples collected in the southern (non-urban) part of Redlands Coast at the end of the survey period and waiting for genotyping funds

2.3.1 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, for the first time, we were able to alleviate these limitations by designing a new genotyping method (DArTcap, see methods), which enabled the genotyping of numerous loci (>900).

We collected substantially more samples from urban areas than non-urban areas on Redlands mainland (urban: n = 83; non-urban: n = 16). To alleviate this limitation, when comparing relatedness between urban and non-urban areas, we randomly subsampled individuals from urban areas and ran analyses on these data. This was done to ensure that results were not a consequence of uneven sampling. We repeated the random subsampling 10 times. To avoid subsampling, further genotyping needs to be done on non-urban samples (samples from the southern half of Redlands mainland), however, we are currently waiting for additional funding in order to do these analyses.

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 in Redlands Coast with part of Brisbane and Logan Councils, form a population known as the “Koala Coast” (Lee et al. 2010), therefore it would have been more relevant 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. 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 represent genetic characteristics of koalas that extend beyond the boundaries of Redlands Coast.

An 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 Chlamydia disease. We are currently working towards elucidating how DArTCap Chlamydia results and clinical signs are linked.

3. Results

Note. This main body of the report focuses on giving general trends and analyses, and to preserve the flow, specific results per locality are not included here, but given in Appendix 3.

3.1 Establishing where Redlands Coast koala populations are

3.1.1 Scat survey results

Koala scat surveys were undertaken between the 24th of April and the 3rd of September 2018. Two to three teams consisting of a detection dog and a handler were deployed in parallel: Katrin Hohwieler with fresh scat detection dog Billie Jean, Nicola Kent with (all age) scat detection dog Baxter, Dr Romane Cristescu with (all age) scat detection dog Maya, with two extra handlers / koala spotters supporting the field work effort as required (Russell Miller, Kye McDonald).

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 **531 surveys** across Redlands Coast (Figure 4, see Appendix 3 for breakdown per locality), 303 on Minjerribah / North Stradbroke Island and 228 on Redlands mainland. Out of the 531 surveys, 343 of the survey sites had the detection dogs identifying koala scat presence, with **977 instances of scat detection** (old and fresh scat, Figure 5, see Appendix 3 for breakdown per locality). Thorneside was the only locality of the Redlands Coast where no survey detected signs of koalas (note that this cannot be interpreted as a statement that koalas have been extirpated from Thorneside. It could be that no fresh scats were present for the fresh scat detection dog to find, or that the areas covered by the scat detection dog had no scat present due to seasonal variation, or that areas of Thorneside currently used by koalas were not surveyed etc.). Cleveland, on the mainland, and all townships on Minjerribah / North Stradbroke Island, were very high for koala scat presence (more than 90% survey sites returned presence of koalas).

When scats were found, they were described in terms of age categories (Figure 5), 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 **689 samples of fresh scats** were collected (Figure 6), of which **383 scats were processed for genetic analysis** (this was due to limited current funding; scats have been extracted and are ready to be genotyped once more funding is available). 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). In 5.8% of cases, no fresher scats could be found, and scats of age category 3 were collected.

Note again that the sites were surveyed on only one occasion; therefore, the presence / absence results presented here provide a snapshot of the population during the survey period and that evidence of koalas found within the study areas is likely to change seasonally [as koala movements can vary with time (Ellis et al. 2009)].

Implications for Conservation. Koalas are readily found in urban areas, where threats are heightened by the density of vehicles and domestic dogs, as well as the lack of habitat

connectivity (i.e., both at the canopy and forest levels) which could potentially force koalas to spend more time moving on the ground.

Recommendation. Protection of koalas in the Redlands Coast needs to include a strategic urban koala plan, as it seems a non-negligible part of the koala population is currently found in urban areas.

It must be noted, however, that survey effort was more limited in the southern part of the Redlands Coast (see Limitations), and additional surveys might be required in non-urban areas.

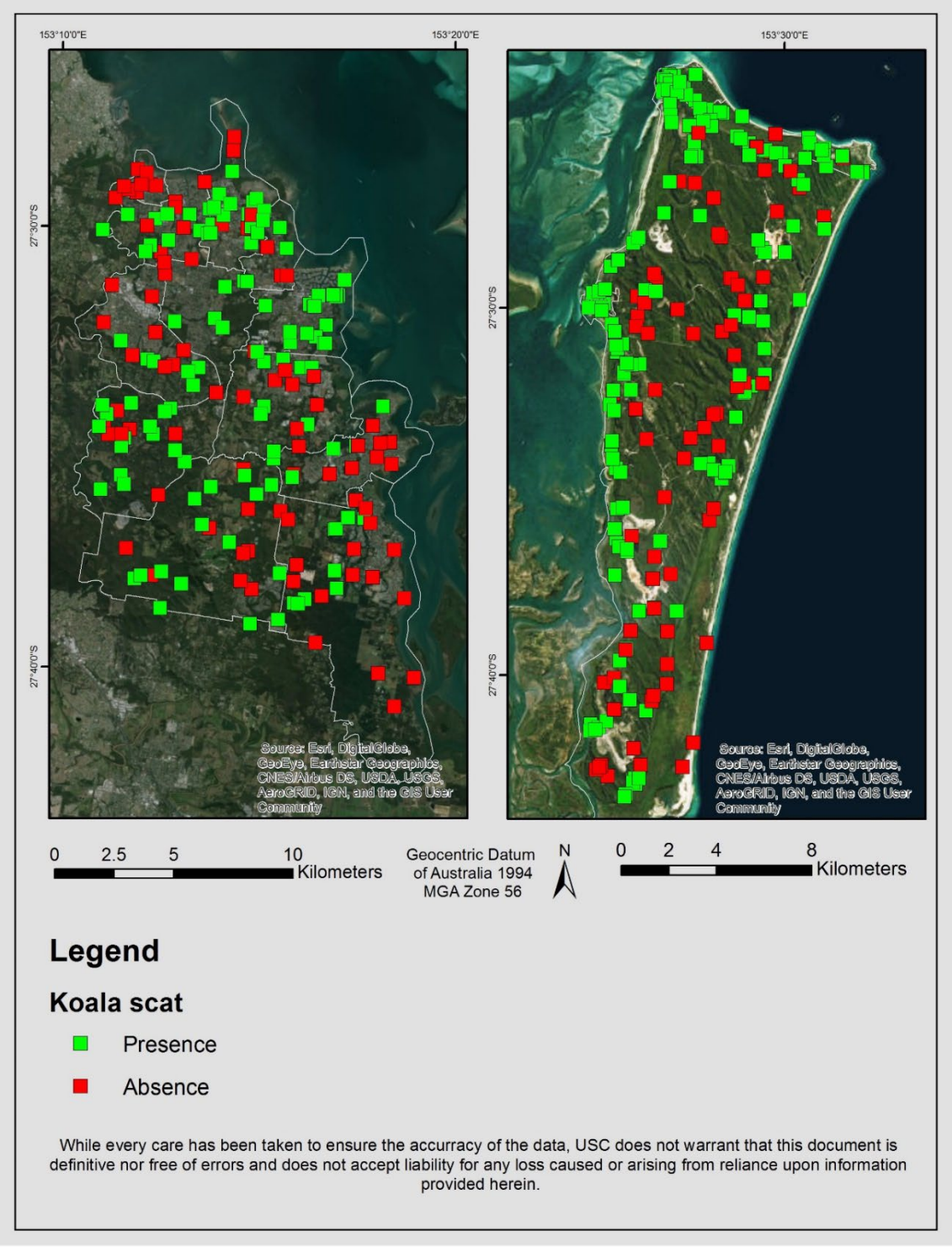


Figure 4: Sites where the Detection Dogs for Conservation teams surveyed across Redland City Council. Green points represent positive sites where koala scats were found. Red points indicate sites where koala presence could not be confirmed. A total of 531 surveys were conducted of which 343 revealed koala presence

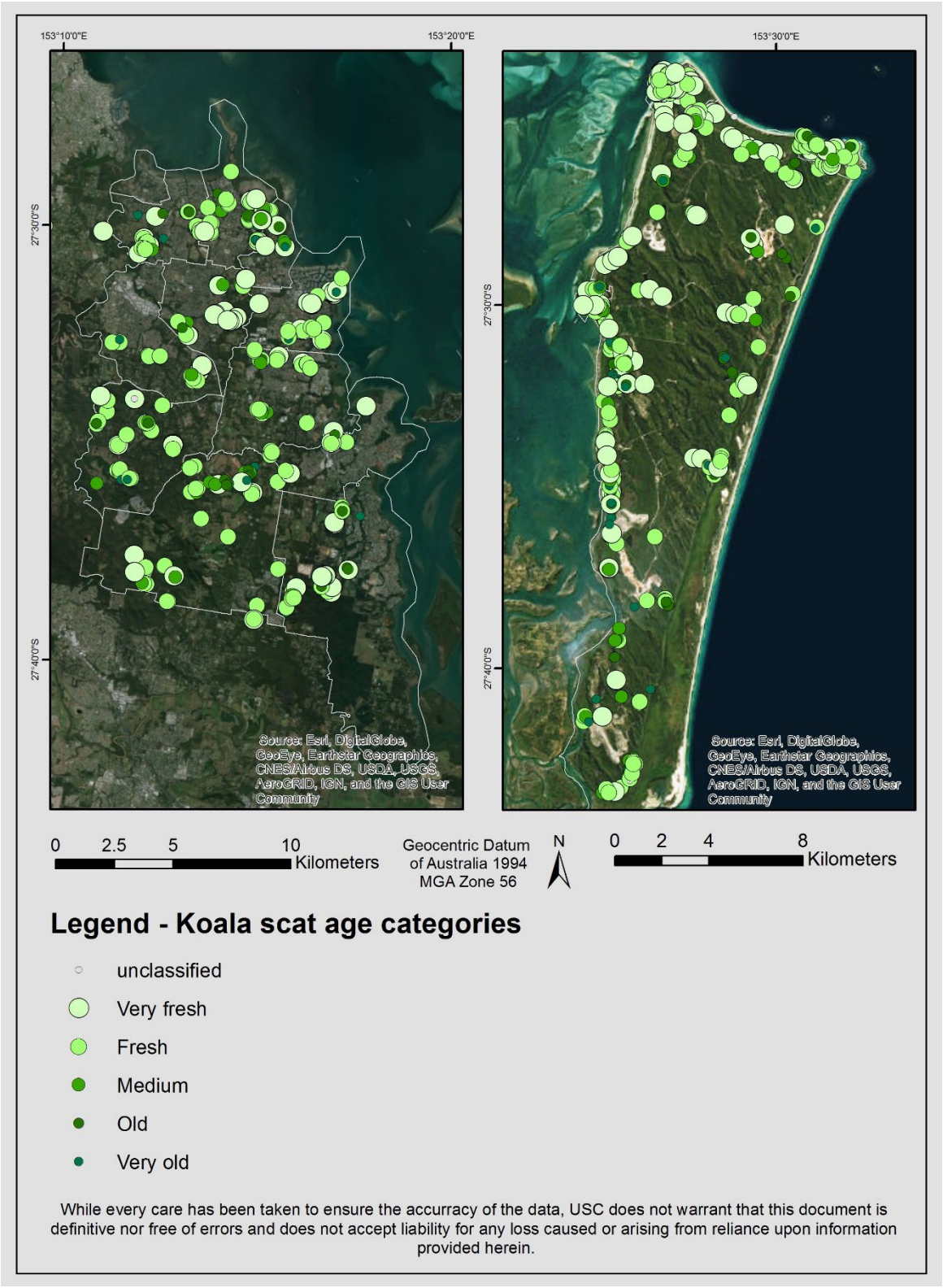


Figure 5: This map shows the 977 instances of scat detection (broken down per scat age category) across Redland City Council

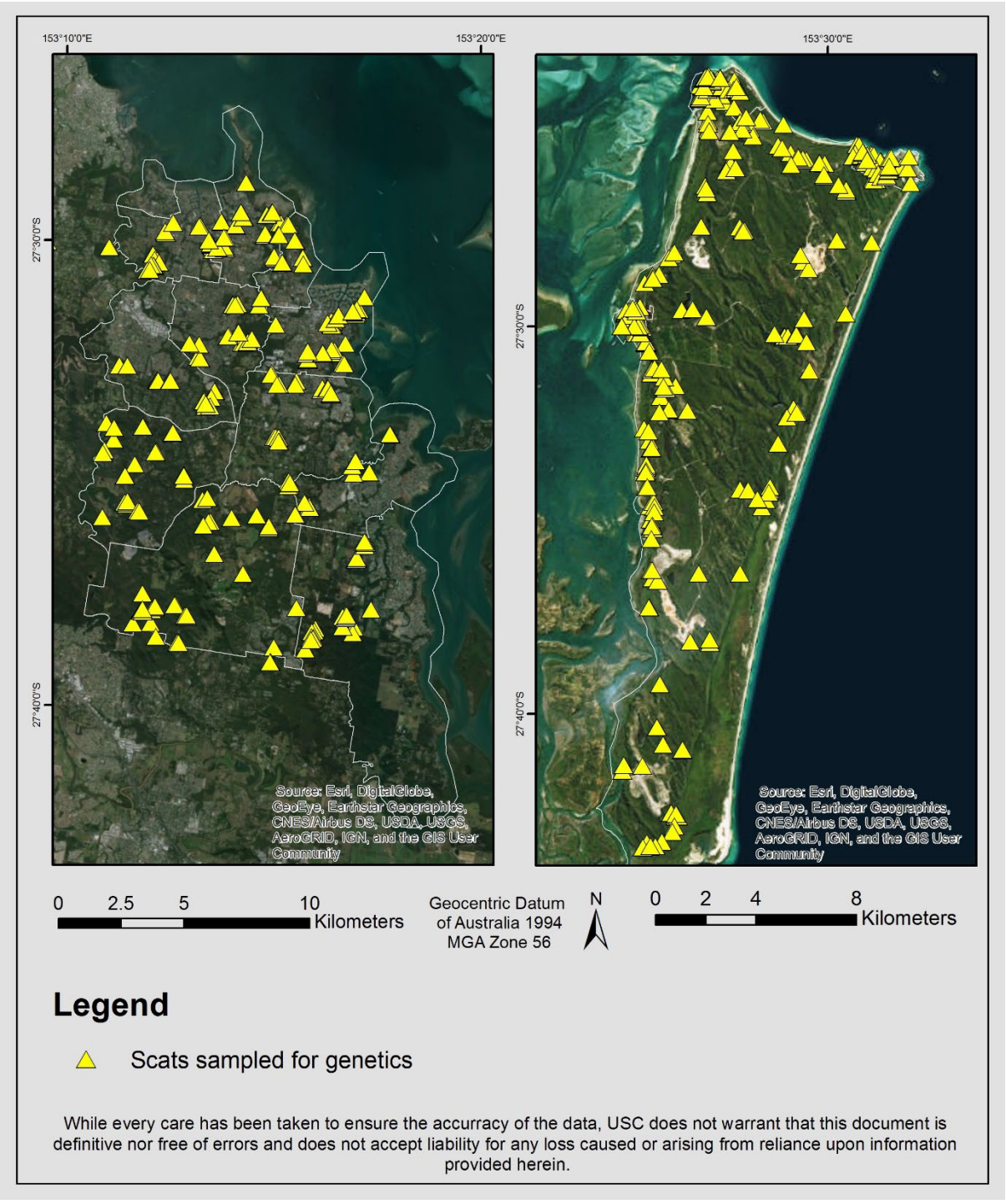


Figure 6: Map of genetic samples (N=695) collected by the Detection Dogs for Conservation teams across Redland City Council

3.1.2 Koala sightings

The handlers spotted a total of **116 live adult koalas** during the surveys (Figures 7 and 8, see Appendix 3 for breakdown per locality) of which four showed severe signs of Chlamydia (eye infection or wet bottom, Figure 9). Of the 116 koalas spotted, 10 were confirmed as females with a joey.

All koalas were spotted in the northern localities of Redlands Coast or Minjerribah / North Stradbroke Island. Seven of the koalas sighted were found in Cleveland in close proximity to each other (Wharf Street/Middle Street/GJ Walter Park). A large number of koalas were spotted in Amity (N = 36), and no koalas were spotted during the surveys in either Mount Cotton, Redland Bay, Thorneside or Victoria Point. 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 for the unbalanced sampling effort between north versus south).

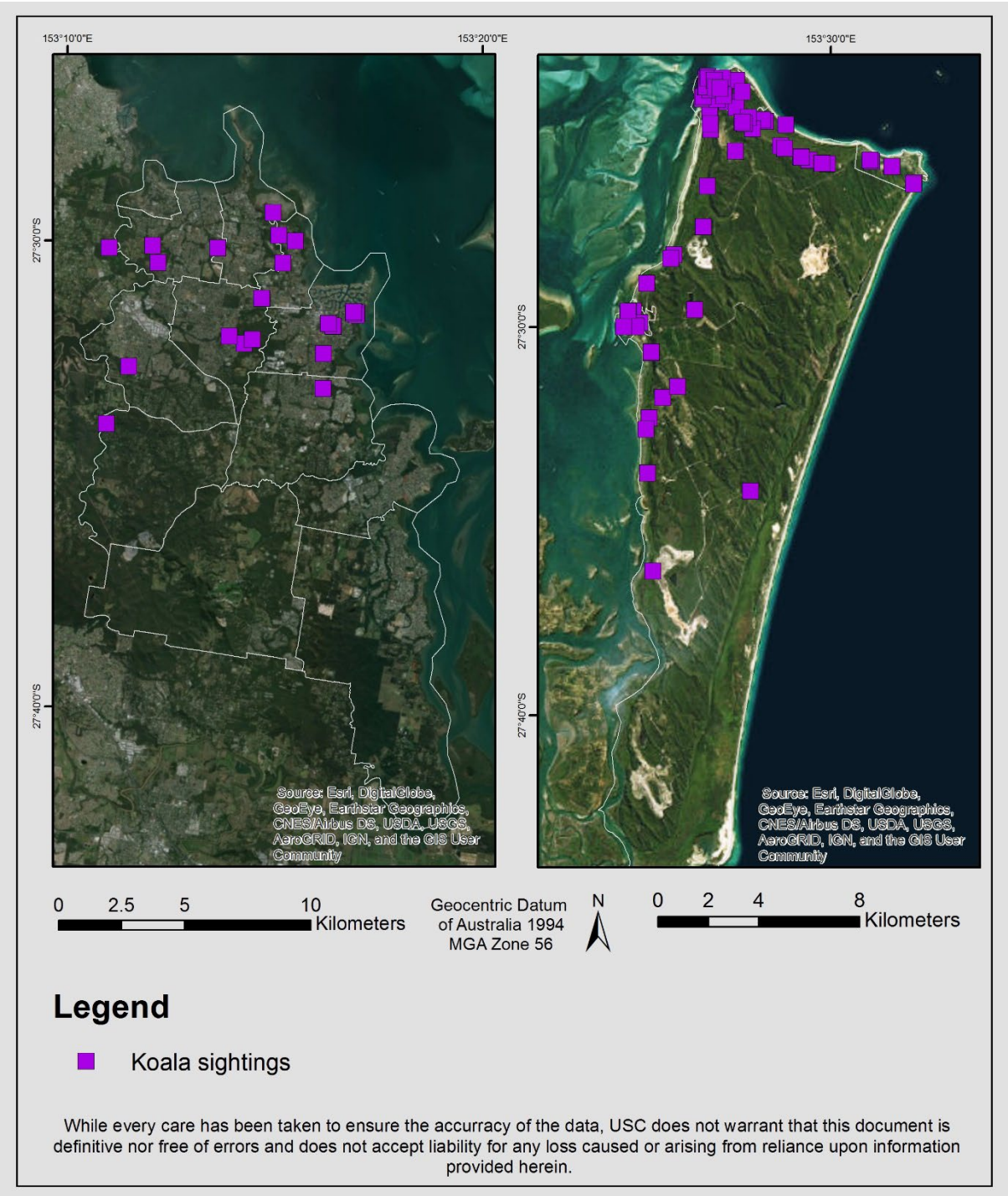


Figure 7: Map of koala sightings (N = 116), spotted by the Detection Dog Teams across Redland City Council

Figure 8: Healthy male koala spotted in the field wearing an ear tag

Figure 9: Koala presenting severe signs of *Chlamydia* ("wet bottom")

3.2 Assessing how genetically connected populations of koalas are

Within the Redlands Coast, **a total of 383 scat samples** were sent for genetic analyses based on available funds (Table 2). After two quality filtering steps (1st filtering done during library construction and 2nd filtering step done during SNP quality control, see Table 2, methods and detailed molecular methods in Appendix 1), we checked the remaining 279 scats for duplicates (Table 2). Of these samples, 86 were found to be duplicated samples of 61 unique individuals. Following the removal of duplicate samples, 193 individuals remained for use in all subsequent analyses. Of these 193 individuals, 89 were found to be male and 104 were found to be female

(Figure 10, see Table 2 for breakdown per locality). This resulted in a sex-ratio of 1:1.2 (male to female ratio). 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). Note that the ratio for Redlands mainland is more biased toward females, at 1:1.4, while Minjerribah / North Stradbroke Island has a small male biased sex ratio at 1:0.9. This could be that males experience higher mortality on the mainland, possibly due to increased risks (compared to the island) related to their increased movements (compared to females) during the breeding season.

Table 2: Table of sample sizes for each location for total number of scats collected, number of samples sent for genotyping, number of samples that were successfully genotyped, the number of samples that were used in analyses once duplicated samples (samples belonging to one individual koala) had been removed and the number of samples waiting for funding in order to be analysed.

						Number of individuals after removing duplicates				
Population	*Locality*	*Total scats sent for genotyping*	*Samples after filtering (step 1)*	*Samples after filtering (step 2)*	*Number of duplicates*	*Males*	*Females*	*Total*	*Samples waiting for funding*	*Total scats collected*
North Stradbroke Island	*Amity Point*	39	39	35	9	12	14	26	44	83
	Dunwich	12	11	10	3	4	3	7	19	31
	NSI (non-urban)	76	68	50	2	24	24	48	119	195
	Point Lookout	20	18	17	4	9	4	13	31	51
	All	147	137	112	18	49	45	94	213	360
Redlands mainland	*Alexandra Hills*	*20*	*15*	6	1	3	2	5	3	23
	Birkdale	*31*	*31*	29	13	5	11	16	11	42
	Capalaba	*17*	*15*	10	4	2	4	6	2	19
	Cleveland	*65*	*61*	55	29	17	9	26	1	66
	Mount Cotton	*17*	*13*	13	5	5	3	8	20	37
	Ormiston	*14*	*12*	11	4	2	5	7	5	19
	Redland Bay	*9*	*9*	4	1	1	2	3	16	25
	Sheldon	*19*	*12*	6	1	2	3	5	7	26
	Thornlands	*20*	*19*	15	6	3	6	9	6	26
	Victoria Point	*2*	*2*	2	0	0	2	2	18	20
	Wellington Point	*22*	*18*	16	4	9	3	12	4	26
	All	*236*	*208*	167	68	40	59	99	93	329
Total		*383*	*345*	279	86	89	104	193	306	689

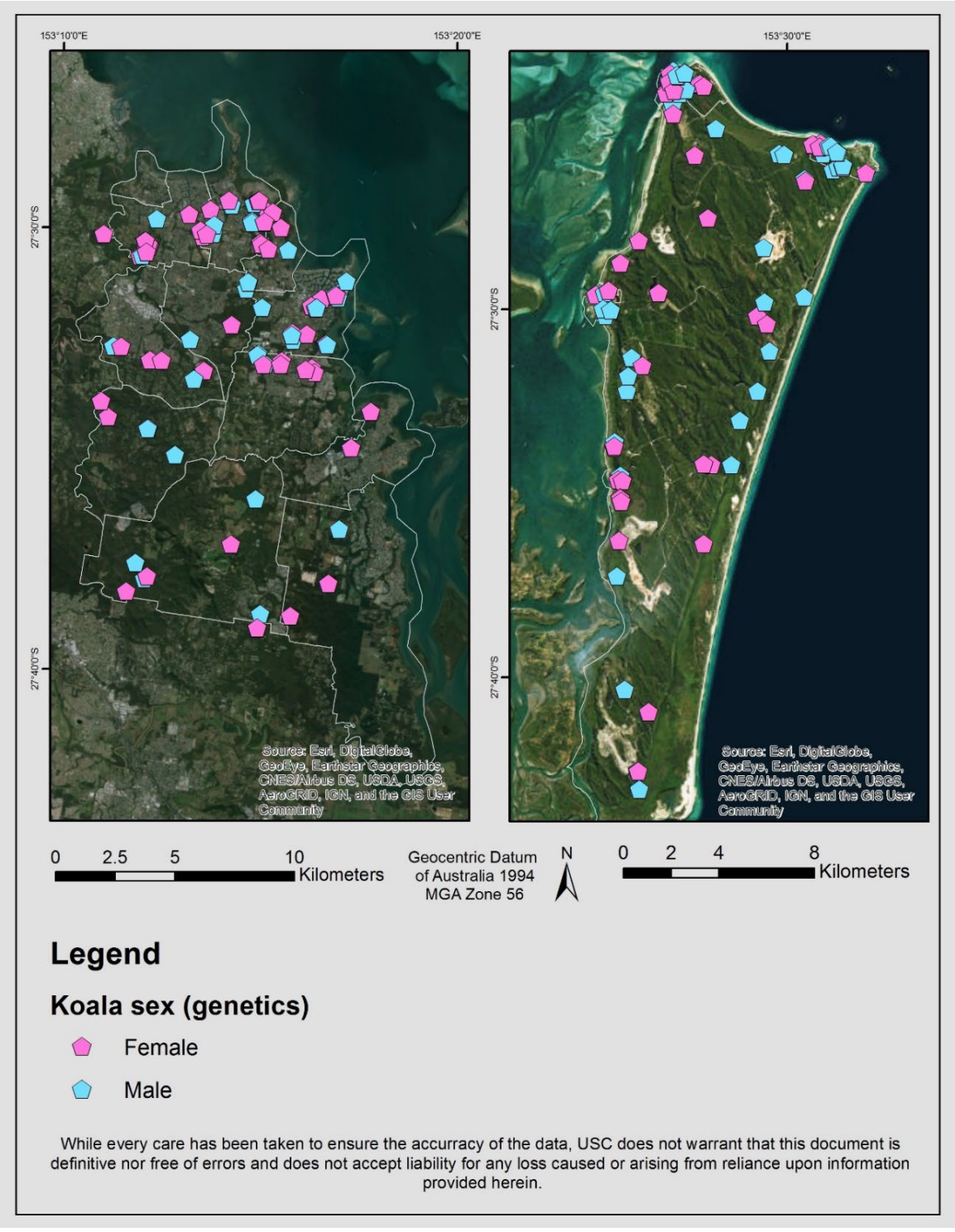


Figure 10: Individual koalas identified through scats, and their sex

3.2.1 Genetic structuring

Using TESS, a program for estimating spatial population structure based on geography, we found evidence for two ancestral populations across the Redlands Coast which, based on their geographic locations, are referred to as Redlands mainland and Minjerribah / North Stradbroke Island (Figure 11, Appendix 2). Unsurprisingly, we found significant and extensive genetic differentiation between these two populations (F_{ST} = 0.193, *P* value = 0.001; F'_{ST} = 0.305).

These results are in agreement with previous, less extensive (six microsatellites, N = 36), genetic surveys of Minjerribah / North Stradbroke Island where the island koala population was found to be isolated from Redlands mainland and actually genetically more similar to the Gold Coast koalas than to Redlands mainland (Cristescu et al. 2011). Because Redlands mainland and Minjerribah / North Stradbroke Island have been isolated for a long time (approximately 8000 years, so >$4N_e$), and they are geographically discrete, they may need to be considered "evolutionary significant units*" and translocation avoided (Moritz 1994, Sherwin et al. 2000).

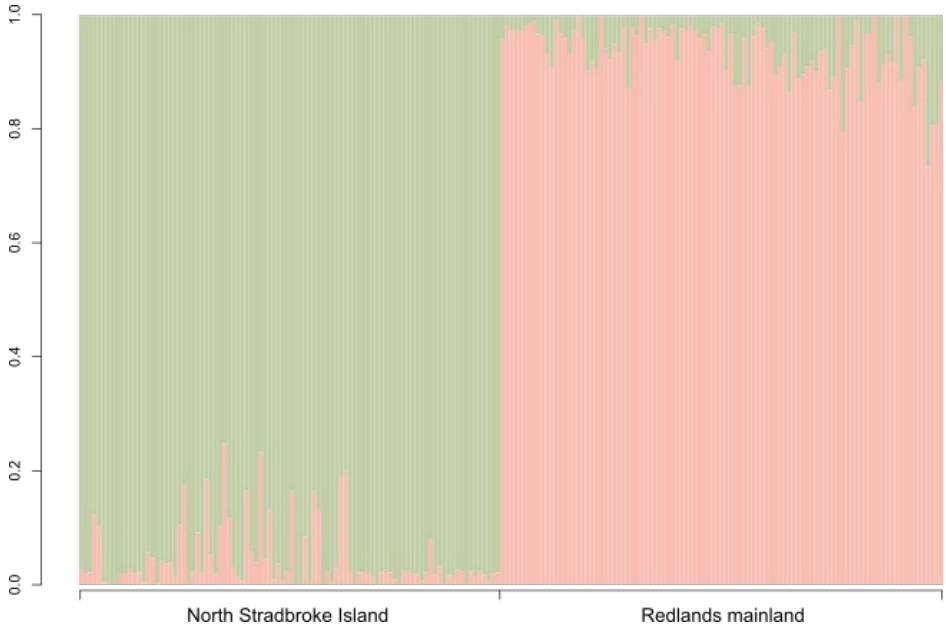


Figure 11: Stacked bar chart from the results of the TESS analysis for two populations (maximum likelihood K = 2). Each individual is represented by a single bar showing the degree of admixture. Individuals are grouped by populations referred to Minjerribah / North Stradbroke Island (green, n = 94) and Redlands mainland (red, n = 99)

Whilst two ancestral populations were found across the Redlands Coast (Redlands mainland and Minjerribah / North Stradbroke Island), TESS did not identify any broad scale genetic structuring within each of these populations (Appendix 2). Therefore, and within the legislative boundaries of Redlands Coast, each of these populations can be considered as one continuous population (i.e. each separate population is made up of a single lineage and can be considered a single breeding population). Additionally, we found no evidence that genetic differentiation increases with increasing geographic distance (genetic isolation by distance) for either Redlands mainland or Minjerribah / North Stradbroke Island populations (Figure 12). This further supports the fact that Redlands mainland and Minjerribah / North Stradbroke Island populations are each one continuous

breeding population. Because this survey's sampling stopped at the council boundaries, we do not know how far the mainland population extends into neighbouring Councils. Past genetic studies at the SEQ level found that Redlands mainland clustered with part of Brisbane City Council (south of the Brisbane River), and Logan City Council (east of the M1 Motorway) to form what is known as the Koala Coast (Lee et al. 2010). If further fragmentation of the habitat has not stopped gene flow in the past ten years, we can assume Redlands mainland to still be part of the Koala Coast genetic cluster. Koalas do not recognise administrative boundaries and therefore, we can safely infer that koala scats close to the Council boundaries belong to koalas using both Redlands mainland and neighbouring Councils. This ensures that genetic characteristics of koalas described here as the "Redlands mainland" population encompass a larger extent than the Redland City Council boundaries.

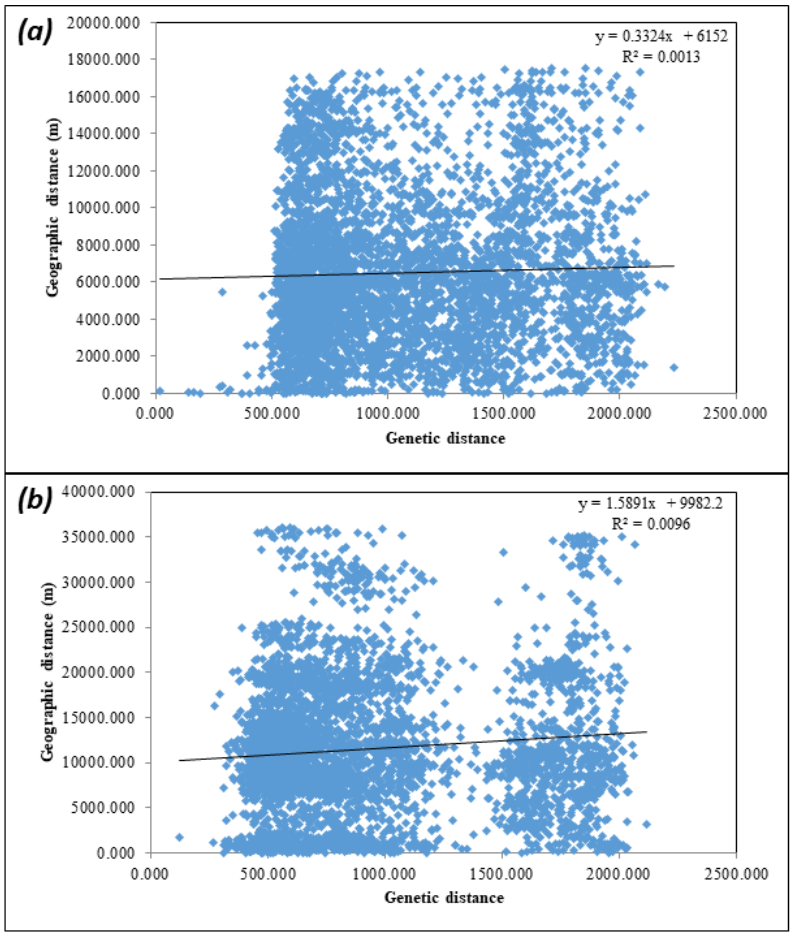


Figure 12: Scatterplots showing the relationship between genetic and geographic distance for Redlands mainland (a) and Minjerribah / North Stradbroke Island (b)

Implications for Conservation. Redlands mainland and Minjerribah / North Stradbroke Island koalas are genetically distinct and should at least be considered as independent conservation management units and potentially, given their history, evolutionary significant units. This is because the two populations have been naturally isolated for potentially 8000 years and there might be local adaptations to island life jeopardised by any translocations. This is termed an "outbreeding depression*", where local adaptations are lost or disrupted, and the introduction of new genes is

deleterious. Outbreeding depression is possible for populations that have not exchanged genes for 500 years (Frankham et al. 2011).

Recommendation. Translocation of koalas between Redlands mainland and Minjerribah / North Stradbroke Island should be avoided. In addition to the genetic differentiation of the two populations, translocations might introduce undetected pathogens - for example, Chlamydia can be dormant and not detectable for many months (Rank and Yeruva 2014). Chlamydial risk associated with koala translocation has been underlined (Waugh et al. 2016).

3.2.2 Fine-scale spatial structuring

Whilst Redlands mainland and Minjerribah / North Stradbroke Island populations are each one continuous breeding population (i.e. no broad scale genetic structuring found), we did find evidence of fine-scale spatial structuring within each population. As these fine-scale genetic analyses rely on comparing groups of individual koalas, 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. 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 elucidate 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 elucidated for koalas. Although it is known that koalas can swim, travel on the ground extensively 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

When looking at localities within Redlands mainland and Minjerribah / North Stradbroke Island with >9 individuals, we found significant genetic differentiation between several locations (indicated by an asterisks (*) in Table 3). Whilst the number of migrants (Nm) between each pairwise comparison indicated that gene flow does exist across each population, the gene flow per generation has been sufficiently restricted which, over time, has generated a strong positive signal of local spatial genetic structure within Redlands mainland and Minjerribah / North Stradbroke Island. Overall, these significant genetic differentiation measures highlight that a potential barrier to gene flow exists between Cleveland and Wellington Point/Birkdale individuals on Redlands mainland, as well as between Amity/Point Lookout and the rest of the island on Minjerribah / North Stradbroke Island (Figure 13). Whilst the degree of genetic differentiation between these aforementioned locations are small (F_{ST} = 0.015-0.026), they are significant (P value = 0.003-0.006). This indicates that events such as fire on Minjerribah / North Stradbroke Island and the increasing urban footprint on Redlands mainland may be resulting in restricted gene flow between locations. However, we may not see the true impact of these events / habitat shifts until we sample future generations.

Table 3: Pairwise genetic differentiation (F_{ST}) measures and its associated P value, and the number of migrants (Nm) between pairwise locations on Redlands mainland and Minjerribah / North Stradbroke Island using 905 loci. Significant genetic differentiation measures are shown in bold and asterisks (*). NSI = bushland areas of Minjerribah / North Stradbroke Island (i.e. outside of the three townships). Note that populations here are based on locality boundaries.

Population 1	*Population 2*	*F_{ST}*	*P value*	*Nm*
Birkdale	***Cleveland***	***0.018***	***0.003****	***13.27***
Birkdale	*Thornlands*	*0.002*	*0.399*	*151.79*
Cleveland	*Thornlands*	*0.010*	*0.109*	*25.00*
Birkdale	*Wellington Point*	*0.009*	*0.125*	*26.68*
Cleveland	***Wellington Point***	***0.026***	***0.004****	***9.45***
Thornlands	*Wellington Point*	*0.013*	*0.130*	*19.40*
Amity	***NSI***	***0.015***	***0.003****	***16.94***
Amity	*Point Lookout*	*0.009*	*0.091*	*27.37*
NSI	***Point Lookout***	***0.020***	***0.006****	***12.42***

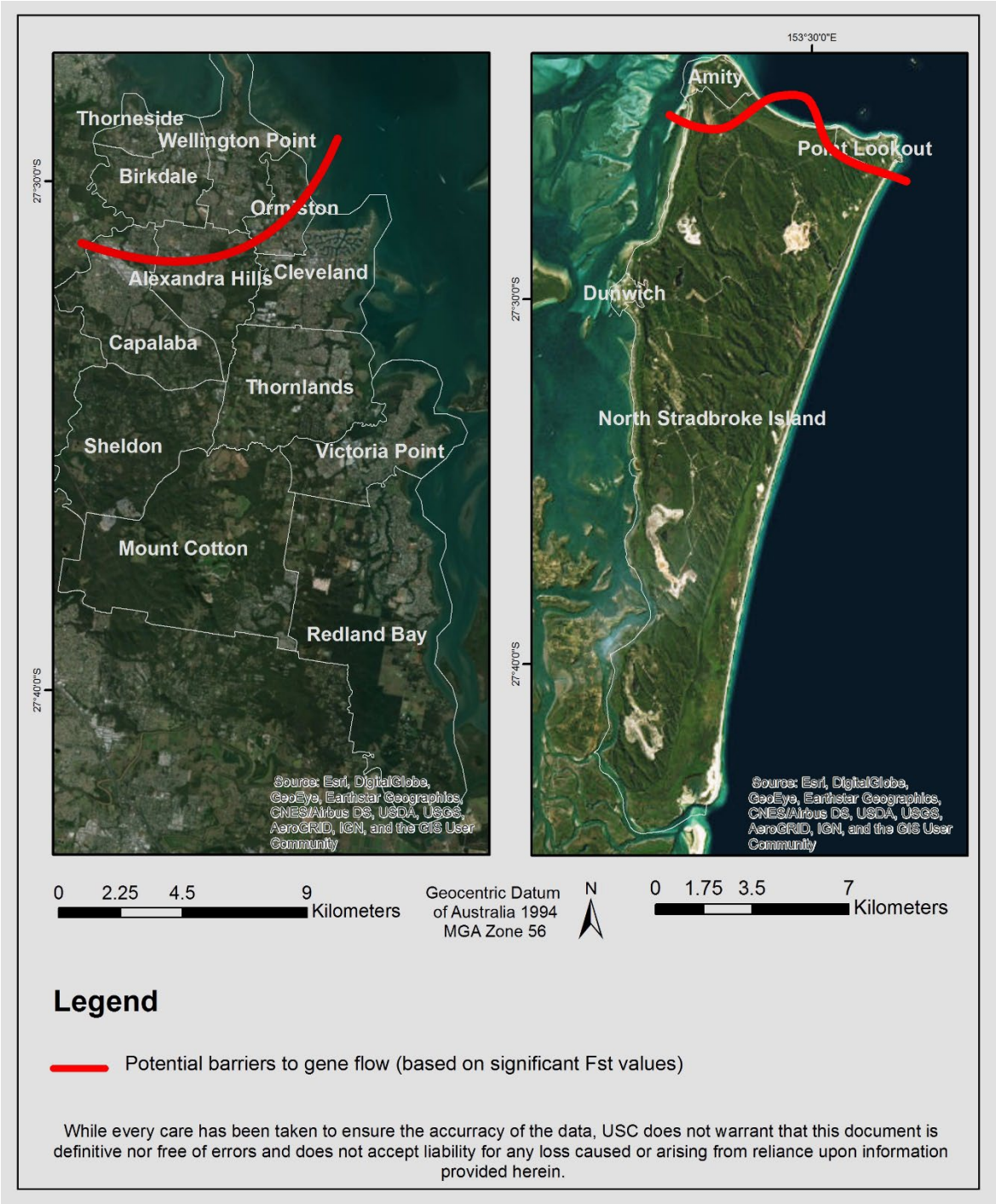


Figure 13: Potential barriers to gene flow in the Redlands Coast

Spatial autocorrelation

Spatial autocorrelation analyses within Redlands mainland and Minjerribah / North Stradbroke Island detected strong differences in local genetic structuring (Figure 14). On the Redlands mainland, individuals were found to be more closely related than expected by chance from zero to 350 meters of each other. In contrast, Minjerribah / North Stradbroke Island individuals were found to be more closely related than expected by chance at distances from 450 meters to >1.25 kilometres (1250m). These patterns indicate that Minjerribah / North Stradbroke Island individuals disperse further than those on the Redlands mainland. Since dispersal is key to avoiding inbreeding depression (reduced fitness), shorter dispersal distances found for Redlands mainland koalas may put them at greater risk of inbreeding depression in the future. Note that relatedness was similar in Redlands mainland and Minjerribah / North Stradbroke Island (Appendix 2, Figure 4).

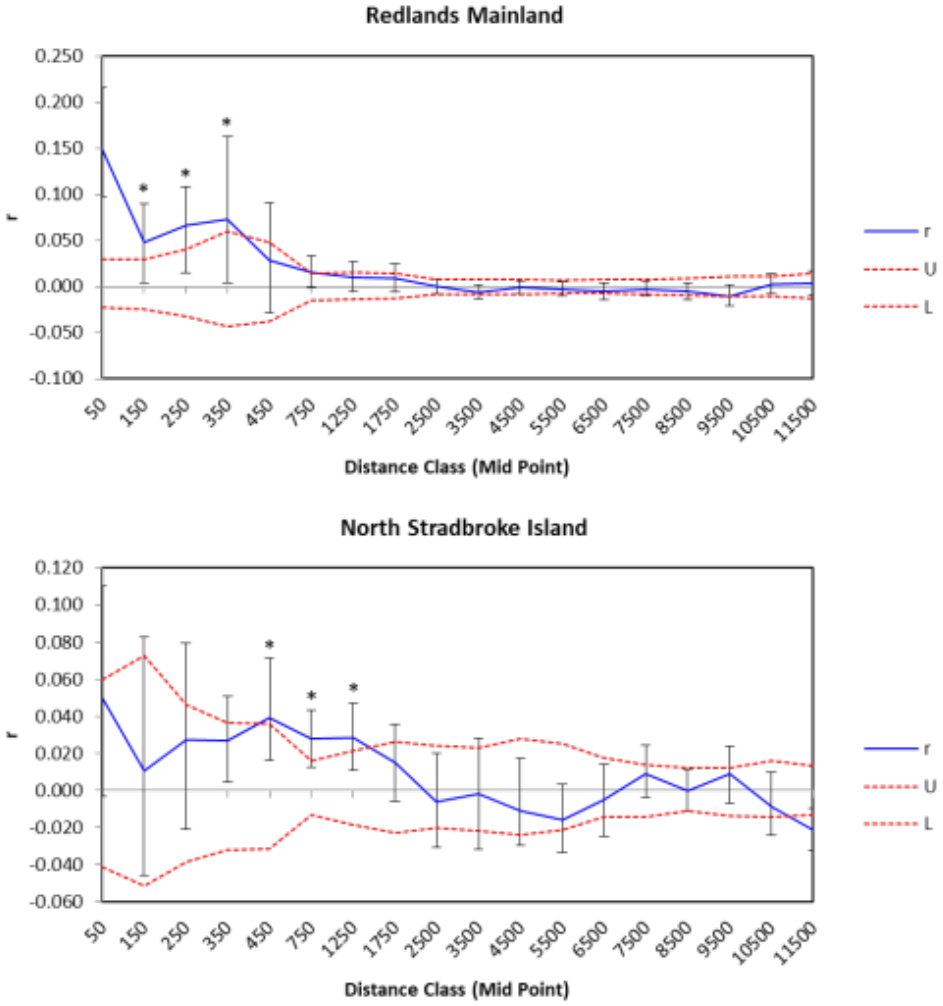


Figure 14: Relatedness (r) correlograms for a) Redlands mainland generated from 905 loci genotypes of 99 individuals, and b) Minjerribah / North Stradbroke Island generated from 905 loci genotypes of 94 individuals over a distance of 11500m. In both plots, error bars around the autocorrelation r values are from 1000 bootstrap iterations and 999 permutations. From 999 random shuffles (plus the observed value as the 1000th permutation), the values of the 25th and 975th ranked permutated r values are taken to define the upper (U) and lower (L) bounds of the 95% confidence interval. If the calculated r-value falls outside this confidence belt, significant spatial genetic structure is inferred.

Relatedness on Redlands mainland

In addition to finding that individuals on Redlands mainland do not disperse as far as those individuals on Minjerribah / North Stradbroke Island, we found that individuals within the Redlands mainland urban footprint were significantly more related to each other than those found in the non-urban footprint of Redlands mainland (ANOVA: F value = 104.7, *P* value = <0.001, Figures 14 and 15). These patterns were shown to persist even when we randomly subsampled individuals from the urban footprint to ensure that results were not a consequence of uneven sample sizes (random subsampling was repeated 10 times). This significant result is driven by the large presence of relatedness outliers (shown by dot points outside of the interquartile range; Figure 15) within the urban footprint. These outliers indicate that there are numerous pairs of koalas within the urban footprint that are more related to one another than is usually found (shown by the interquartile range).

Some of the related individuals in Figure 16 are large distances apart, for example up to 25 km on Minjerribah / North Stradbroke Island. Koalas are known to be able to disperse long distances (up to 10 km in Dique et al. 2003). When the landscape permits it (i.e. in terms of connectivity and levels of anthropogenic threats), they can also travel far. For example, a radio-tracked koala on Minjerribah / North Stradbroke Island travelled 6.6 km in 2.5 months (Cristescu 2011). Finally, koalas are sometimes (accidentally or otherwise) released away from their point of capture after veterinary treatment, and this possibility cannot be excluded.

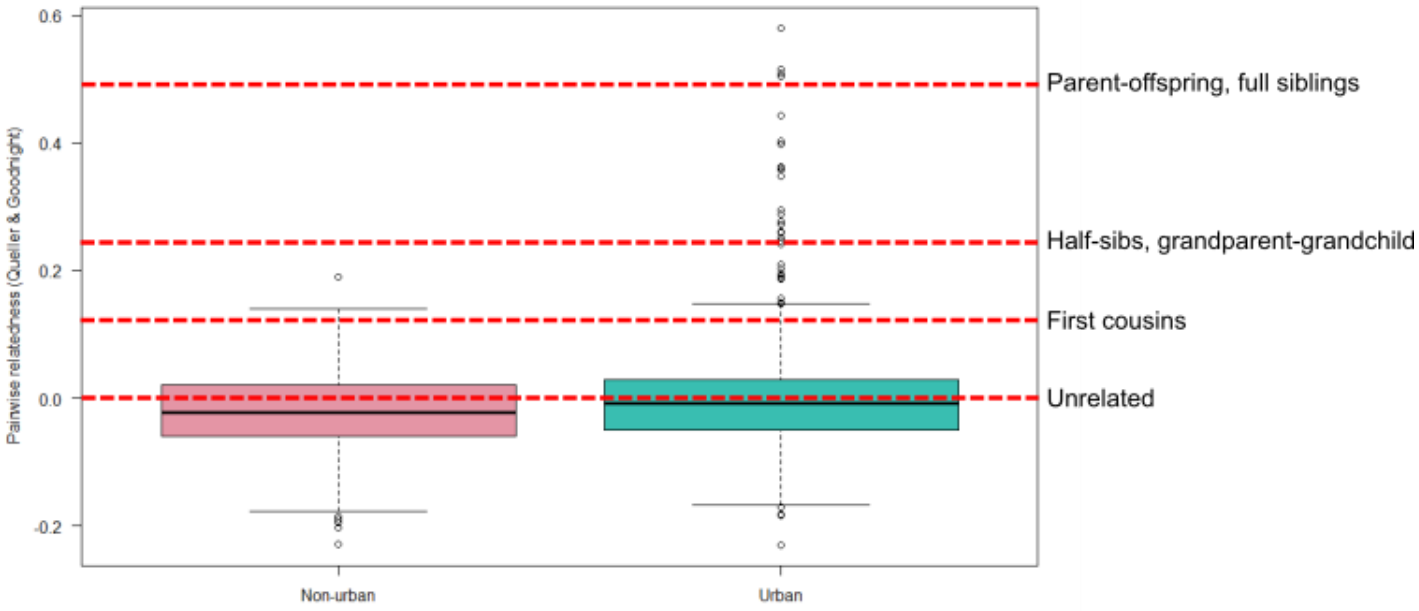


Figure 15: Boxplot showing differences in pairwise relatedness in the Redlands mainland urban and non-urban footprint. Boxes show median internal relatedness and interquartile range. Note that negative values indicate that the relatedness between the pair was less than that expected between two random individuals (Queller and Goodnight 1989).

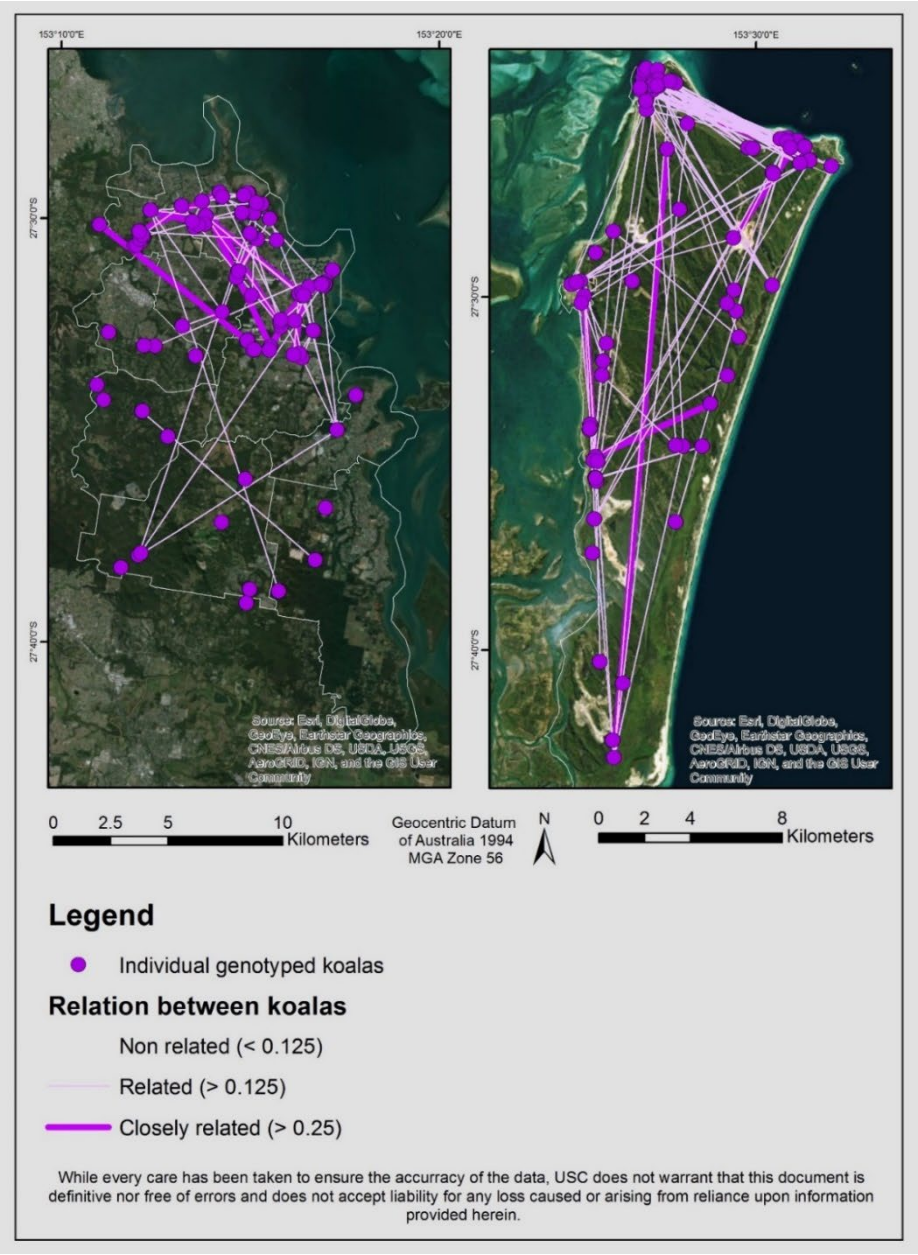


Figure 16: Relatedness between pairs of individuals in Redlands mainland and Minjerribah / North Stradbroke Island (0.5 equals full siblings and parent/offspring, 0.25 half siblings, grand-parents, uncle/aunt, 0.125 first cousin). Please note that Redlands mainland and Minjerribah / North Stradbroke Island have similar average relatedness.

Implications for Conservation. Together these results are of high concern. Our results provide evidence that koalas on the mainland:

1. Do not disperse as much as koalas on Minjerribah / North Stradbroke Island
2. Are surrounded by close relatives

This combined with the existing low permeability of the urban matrix for dispersal will only further increase the potential risk of inbreeding depression.

Recommendation. Maintaining and improving connectivity will be key to preventing the identified high risk of inbreeding depression, especially within the urban footprint.

3.3 How healthy Redlands Coast koalas are

3.3.1 Genetic diversity

Overall, and similar to other koala genetic studies (Kjeldsen et al. 2018), we found that observed heterozygosity (H_O) was lower than expected heterozygosity (H_E). However, whilst expected heterozygosity was found to be ~9% higher than observed heterozygosity in Kjeldsen et al. (2018), here, we found expected heterozygosity to be ~31% higher than observed heterozygosity (Table 4). This large difference between observed and expected heterozygosity is likely due to high levels of population inbreeding.

Levels of genetic diversity were higher in Redlands mainland than on Minjerribah / North Stradbroke Island (H_E = Redlands mainland: 0.298 ± 0.006; North Stradbroke Island: 0.22 ± 0.007, see Table 4 for details). In addition, we also found that the percentage of polymorphic loci on Minjerribah / North Stradbroke Island was 22% lower than on Redlands mainland (Redlands mainland = 96.2%, North Stradbroke Island = 73.8%). The lower genetic diversity and polymorphism of Minjerribah / North Stradbroke Island compared to Redlands mainland could be due to a founder effect*, i.e. the colonisation of the area by a low number of individuals, originally

from the Gold Coast (Cristescu et al. 2011), followed by a small population size for multiple generations. Minjerribah / North Stradbroke Island is thought to be one of only few natural koala island populations, and its level of genetic diversity has been found to be higher than introduced island populations (Cristescu et al. 2009a, Cristescu et al. 2011). Redlands mainland population has been shown to be connected to part of Brisbane City Council and Logan City Council, and therefore potentially had a larger population size for longer than Minjerribah / North Stradbroke Island (Lee 2009). This population, known as the Koala Coast population, however, is thought to have been relatively isolated from the rest of SEQ as well as from Minjerribah / North Stradbroke Island (Lee 2009). The Koala Coast population, as a whole, has lower genetic diversity than the rest of SEQ mainland (Lee 2009, Lee et al. 2010).

Table 4: 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, %PL = percent of polymorphic loci. 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)* *Higher is better*	*H_O (SE)* *Higher is better*	*H_E (SE)* *Higher is better*	*F_{IS}* *Lower is better*	*N_e* *Higher is better*	*IR (SD)* *Lower is better*	*%PL* *Higher is better*
Redlands mainland	*99*	*0.451 (0.007)*	*0.229 (0.004)*	*0.298 (0.006)*	*0.232*	*85.7 (83.8 - 87.7)*	*0.333 (0.239)*	*96.24*
Minjerribah / NSI	*94*	*0.328 (0.010)*	*0.166 (0.005)*	*0.220 (0.007)*	*0.245*	*92.9 (88.5 - 97.8)*	*0.450 (0.186)*	*73.81*
Alexandra Hills	*23*	*0.399 (0.009)*	*0.270 (0.008)*	*0.266 (0.006)*	*-0.014*	*NA*	*0.214 (0.249)*	*76.69*
Birkdale	*42*	*0.423 (0.008)*	*0.226 (0.006)*	*0.280 (0.006)*	*0.192*	*NA*	*0.336 (0.236)*	*85.19*
Capalaba	*19*	*0.408 (0.009)*	*0.252 (0.007)*	*0.273 (0.006)*	*0.077*	*NA*	*0.292 (0.279)*	*76.80*
Cleveland	*66*	*0.426 (0.008)*	*0.224 (0.005)*	*0.282 (0.006)*	*0.204*	*NA*	*0.336 (0.225)*	*87.40*
Mount Cotton	*21*	*0.429 (0.008)*	*0.272 (0.007)*	*0.284 (0.006)*	*0.043*	*NA*	*0.214 (0.249)*	*83.43*
Ormiston	*19*	*0.373 (0.009)*	*0.198 (0.007)*	*0.250 (0.006)*	*0.209*	*NA*	*0.415 (0.247)*	*69.50*
Redland Bay	*15*	*0.331 (0.010)*	*0.208 (0.009)*	*0.226 (0.007)*	*0.082*	*NA*	*0.418 (0.258)*	*56.02*
Sheldon	*19*	*0.376 (0.009)*	*0.206 (0.007)*	*0.251 (0.006)*	*0.180*	*NA*	*0.408 (0.269)*	*69.50*
Thornlands	*21*	*0.420 (0.008)*	*0.257 (0.007)*	*0.279 (0.006)*	*0.077*	*NA*	*0.261 (0.254)*	*82.32*
Victoria Point	*4*	*0.278 (0.010)*	*0.207 (0.010)*	*0.192 (0.007)*	*-0.078*	*NA*	*0.352 (0.200)*	*45.41*
Wellington Point	*26*	*0.390 (0.009)*	*0.191 (0.006)*	*0.260 (0.006)*	*0.266*	*NA*	*0.448 (0.239)*	*75.36*
Amity Point	*26*	*0.320 (0.010)*	*0.177 (0.006)*	*0.214 (0.007)*	*0.173*	*NA*	*0.399 (0.176)*	*65.08*
Dunwich	*7*	*0.282 (0.010)*	*0.174 (0.007)*	*0.189 (0.007)*	*0.078*	*NA*	*0.438 (0.224)*	*53.04*
NSI (non-urban)	*48*	*0.326 (0.010)*	*0.156 (0.005)*	*0.220 (0.007)*	*0.288*	*NA*	*0.494 (0.196)*	*66.19*
Point Lookout	*13*	*0.299 (0.010)*	*0.177 (0.007)*	*0.200 (0.007)*	*0.118*	*NA*	*0.394 (0.106)*	*58.12*

Table 5: 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 4, but are given to enable the relative comparison of the Koala Coast (which encompasses Redlands 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

Using a program which detects recent effective population size reductions using SNP allele frequencies (BOTTLENECK), we found strong evidence for genetic bottlenecks in both Redlands mainland and Minjerribah / North Stradbroke Island populations using three different allele models (*P* values = 0.000). It is interesting to note that previous work using microsatellite markers on 36

koalas failed to detect a bottleneck on Minjerribah / North Stradbroke Island (Cristescu et al. 2011), however the SNP panel used in this study has more power than microsatellites and is therefore better suited to detect bottlenecks (Chikhi and Bruford 2005).

3.3.3 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 both Redlands mainland and Minjerribah / North Stradbroke Island (N_e = Redlands mainland: 85.7; Minjerribah / North Stradbroke Island: 92.9, Table 4). When compared to Kjeldsen et al. (2016; Table 6), the effective population sizes found across the Redlands Coast were extremely low. It is important to note that the N_e for koala Coast (which includes Redlands mainland) is not directly comparable to the N_e found here because it has a different extent both in space (again, Koala Coast includes part of Brisbane and Logan Councils) and time (koala samples in Kjeldsen et al. (2016) were opportunistically sampled, perhaps over many years).

The effective population size for Minjerribah / North Stradbroke Island might have been low for several generations as a consequence of its isolation, a small population size and founder effect. Potentially, a small effective population size on the island is of lower risk than the mainland because populations that have been small for longer have less risk of having many highly deleterious alleles, a phenomenon known as genetic purge*. The current effective population size for Minjerribah / North Stradbroke Island, however, could also be small as a result of the 2013/2014 fire that burnt a large proportion of the island and had an unknown impact on the koala population.

However, small effective population sizes do heighten the risk of extinction in the near future – 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 is currently working on estimating effective population sizes through other methods, but these require two samples collected at different points in time (temporal method). Luckily, the DDC was able to source historical samples through collaboration, and genotyping of 2006-2007 Redlands Coast samples is under way (see section "Future steps and monitoring recommendations"). However, the linkage disequilibrium method is more powerful than the temporal method for early detection of bottlenecks or fragmentation, therefore both methods have their strengths (Luikart et al. 2010).

Table 6: Genetic diversity established through double digest restriction-associated SNP sequencing in wild koala populations across QLD, 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 calculate 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)

3.3.4 Inbreeding coefficient

Aligning with the IUCN's predictions about low effective population sizes (outlined in introduction), we found high inbreeding coefficients for both Redlands mainland and Minjerribah / North Stradbroke Island populations (F_{IS} = Redlands mainland: 0.232; Minjerribah / North Stradbroke Island: 0.245, Table 4). Here, we found higher inbreeding coefficients for all Redlands Coast koalas than those found in wild koala populations across eastern-Australia (Kjeldsen et al. 2018). This concurs with previous findings that the Koala Coast, being isolated from the rest of SEQ mainland, had lower genetic diversity than other SEQ koala populations (Lee et al. 2010). Interestingly, we found higher inbreeding coefficients (Table 4) for locations that were also found to have high genetic differentiation measures (Table 3). This suggests that these locations are becoming genetically isolated from one another, resulting in increased localised inbreeding. Moreover, within the Redlands mainland population, we found the inbreeding coefficient of urban

koalas to be 34% higher than that of non-urban koalas (F_{IS} = urban footprint: 0.236; non-urban footprint: 0.176). This further highlights that urban koalas on Redlands mainland are extremely vulnerable to inbreeding depression.

3.3.5 Internal relatedness

In line with the results of inbreeding at the population level, we found that internal relatedness of individuals was significantly higher on Minjerribah / North Stradbroke island than on Redlands mainland (IR = Redlands mainland: 0.333, North Stradbroke Island: 0.450; ANOVA: F value = 14.56, *P* value = < 0.001; Table 4, Figures 17 and 18). However, it should be noted that the IR values found across the Redlands Coast are lower than those values previously reported in Kjeldsen et al. (2018). A significantly higher internal relatedness on Minjerribah / North Stradbroke Island is likely due to founder effect and/or genetic bottlenecks that have occurred while koalas colonised the island.

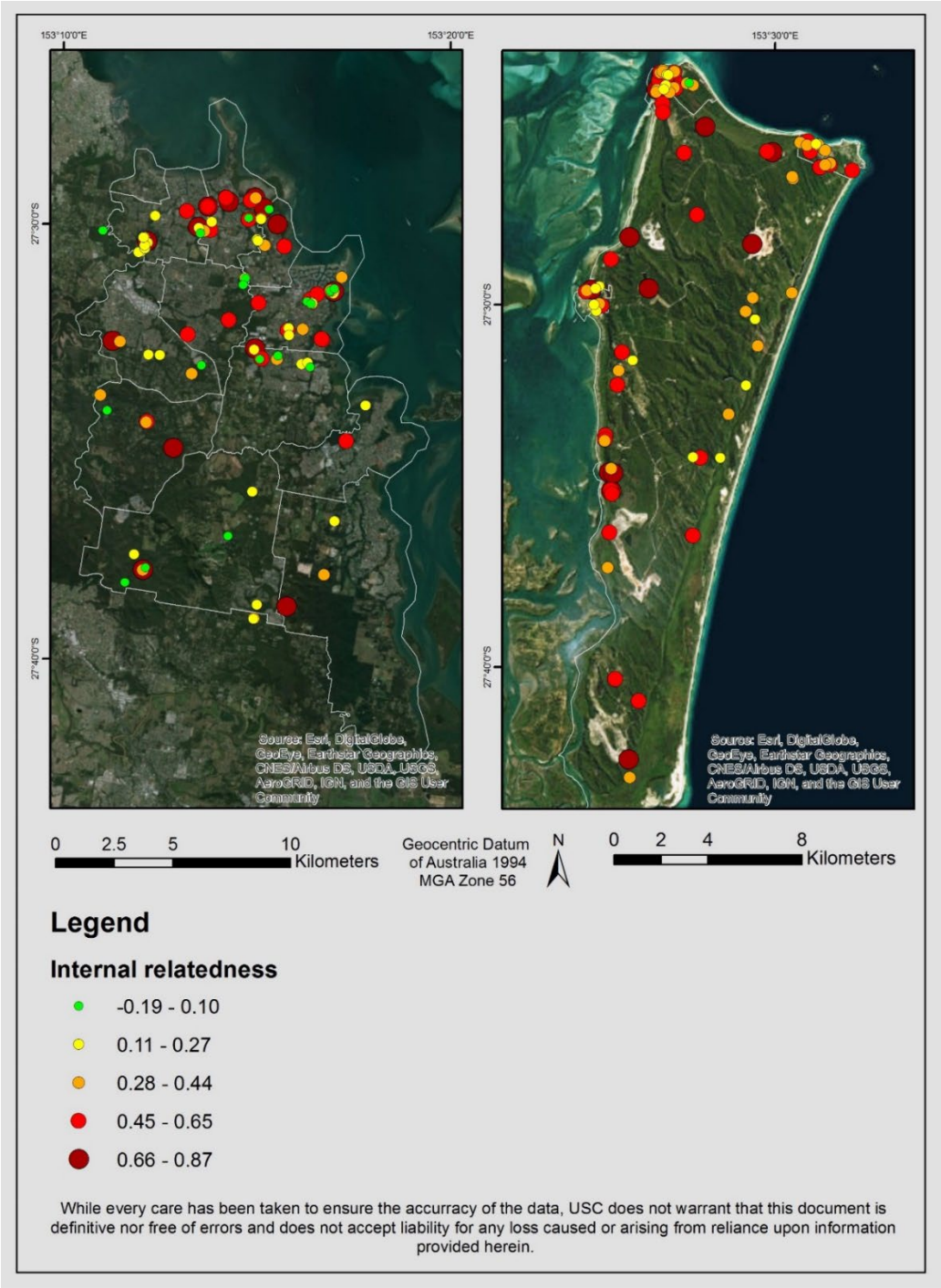


Figure 17: Internal relatedness, a widely used measure of inbreeding at the individual level (the higher the internal relatedness, the more inbred)

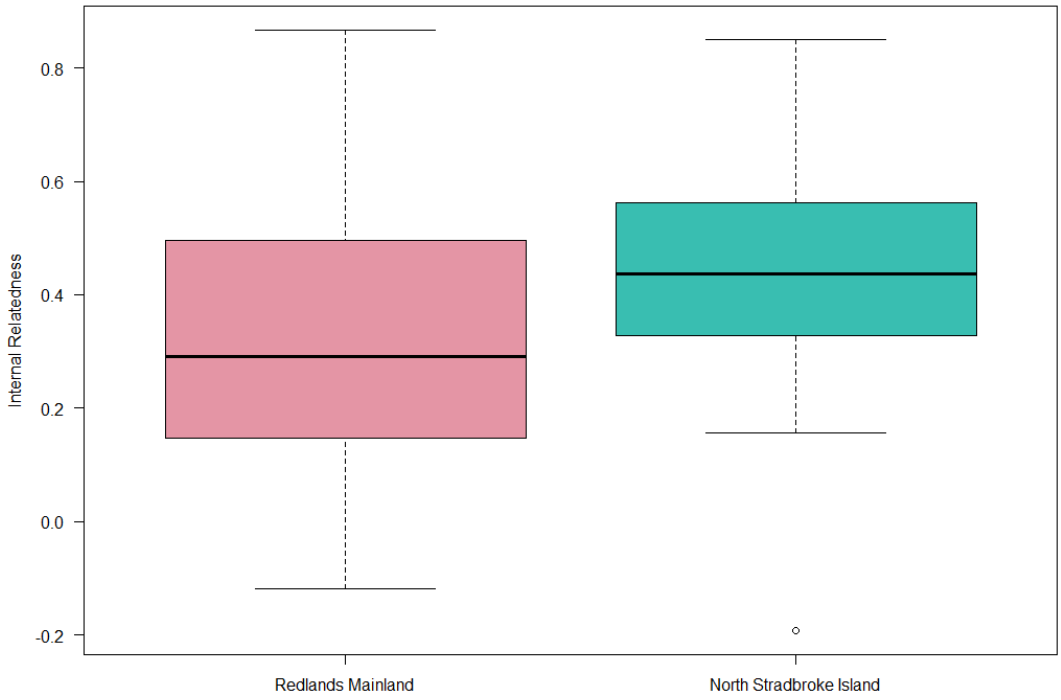


Figure 18: Boxplot showing differences in internal relatedness in Redlands mainland and Minjerribah / North Stradbroke Island. Boxes show the interquartile range, thick lines are the median values (i.e. the value such that a number is equally likely to fall above or below it) for internal relatedness, whiskers show minimum and maximum values. This boxplot shows lower internal relatedness in the mainland

3.3.6 Chlamydia

Using molecular techniques (outlined in methods) we used two different classifications of Chlamydial status for Redlands Coast koalas:

3) A qualitative approach: if any Chlamydial sequences were detected in an individual, it was assigned a positive result.

4) A quantitative approach: a threshold (>9 SNPs detected out of the 30 probes) was used to classify an individual as Chlamydial positive. This measure is, therefore, more conservative and may be more indicative of individuals who present viable Chlamydia pathogens, not just Chlamydial DNA traces (this is currently unknown and an area of active research).

Qualitative Chlamydial results (presence / absence)

Of the 193 identified koalas used for genetic analysis across the Redlands Coast, we found a total of 76 individuals (39%) where Chlamydia (regardless of load) was detected (outlined in methods). Chlamydia was widely distributed across Redlands Coast. Of these 76 individuals, 20 individuals were found on Minjerribah / North Stradbroke Island, whereas 56 individuals were found in Redlands mainland (Table 7 and Figure 19). This translates to 21% of the koalas sampled on the island and 56% of the koalas sampled in Redlands mainland having Chlamydia detected. Overall, Chlamydia was detected in 57% more females than males (females = 47%, males = 30%).

Quantitative Chlamydial results (potential disease)

Of the 193 identified koalas, we found a total of 29 individuals (15%) that were positive for Chlamydial disease using a Chlamydia load threshold (outlined in methods). Of these 29, only one individual was found on Minjerribah / North Stradbroke Island, whereas 28 individuals were found in Redlands mainland (Table 7 and Figure 19). This translates to 1% of the koalas sampled on the island and 28% of the koalas sampled in Redlands mainland having Chlamydia detected. Overall, Chlamydial infection over the threshold was found in 42% more females than males (females = 17%, males = 12%).

Table 7: Table detailing the number of individuals that were identified as Chlamydia positive in each population and locality.

		Quantitative approach			*Qualitative approach*		
Population	***Locality***	***Total***	***Males***	***Females***	***Total***	***Males***	***Females***
North Stradbroke Island	*Amity Point*	1	1	0	7	4	3
	Dunwich	0	0	0	2	1	1
	NSI (non-urban)	0	0	0	11	4	7
	Point Lookout	0	0	0	0	0	0
	All	1	1	0	20	9	11
	Tested	94	49	45	94	49	45
	Percent	1%	2%	0%	21%	18%	24%
Redlands mainland	*Alexandra Hills*	2	1	1	2	1	1
	Birkdale	5	2	3	13	4	9
	Capalaba	4	1	3	4	1	3
	Cleveland	5	4	1	16	7	9
	Mount Cotton	3	0	3	4	1	3
	Ormiston	0	0	0	4	0	4
	Redland Bay	1	0	1	1	0	1
	Sheldon	3	1	2	3	1	2
	Thornlands	4	0	4	5	1	4
	Victoria Point	0	0	0	1	0	1
	Wellington Point	1	1	0	3	2	1
	All	28	10	18	56	18	38
	Tested	99	40	59	99	40	59
	Percent	28%	25%	30%	56%	45%	64%
Redlands Coast	*All*	29	11	18	76	27	49
	Tested	193	89	104	193	89	104
	Percent	15%	12%	17%	39%	30%	47%

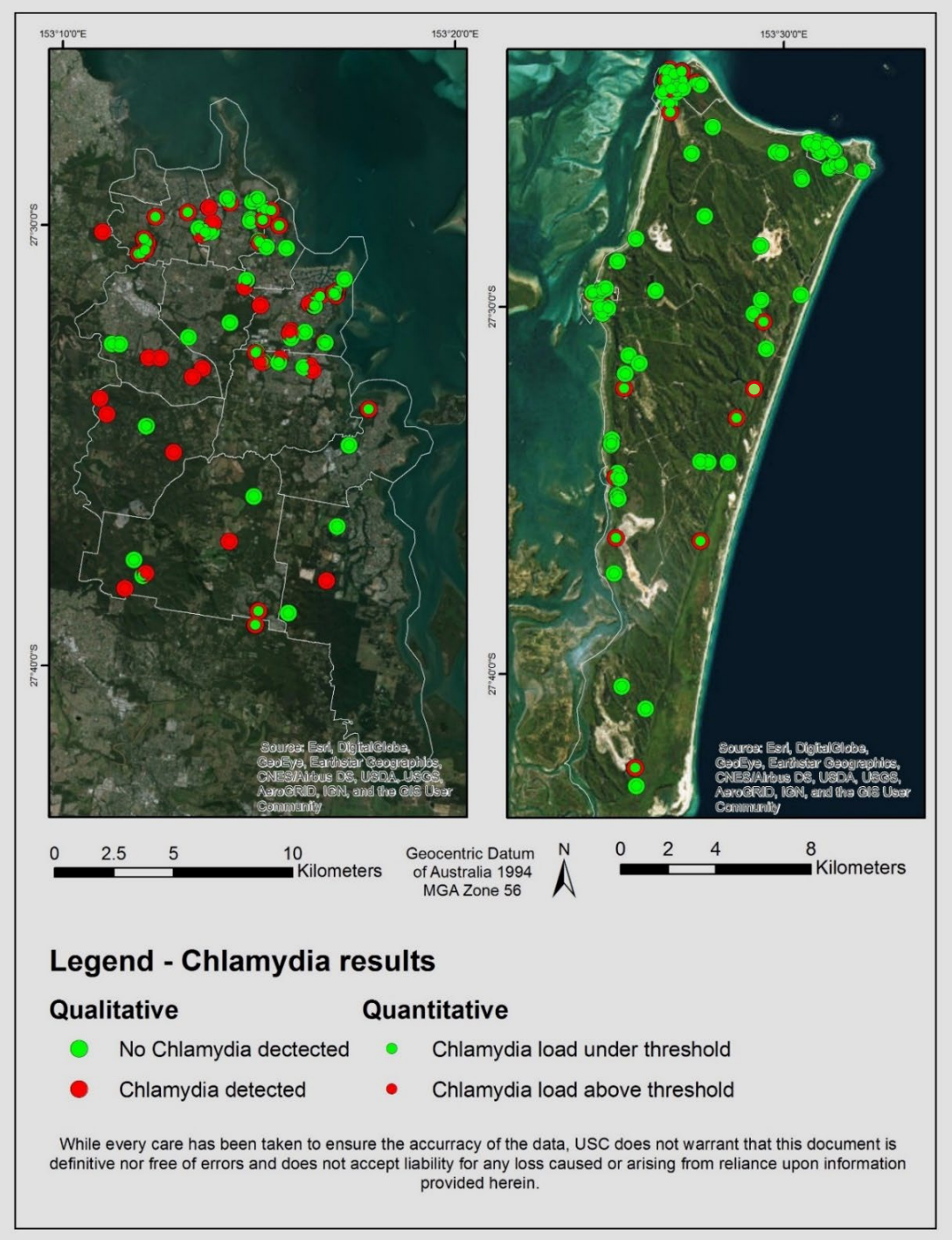


Figure 19: Map of Chlamydia presence (Note that green dots are negative for both tests and red dots are positive for both tests)

Implications for Conservation. Together, these results are of high concern. Our results provide evidence that koalas on Redlands mainland and Minjerribah / North Stradbroke Island have:

1. Experienced bottlenecks
2. High inbreeding both at the population (H_E>>H_O, high F_{IS}) and at the individual (IR) levels
3. Small effective population size (N_e)

In addition, koalas on Redlands mainland are experiencing:

1. Higher Chlamydia infection rate
2. Higher population inbreeding (F_{IS}) in the urban footprint

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, both Redlands mainland and Minjerribah / North Stradbroke Island populations fall short of the recommended effective population size and are therefore at high risk of inbreeding depression. This combined with high F_{IS}, high IR and high Chlamydia infection rate for mainland koalas indicate the vulnerability of koalas across the Redlands Coast, and a heightened vulnerability of the mainland population.

Recommendation. Maintaining and / or increasing connectivity in Redlands mainland will be key to preventing the identified high risk of inbreeding depression, especially within the urban footprint.

4. Discussion

Insights into koala distribution

The Detection Dogs for Conservation teams conducted a total of 531 surveys across the Redlands Coast, of which 343 revealed koala presence, with 977 instances of scat detection recorded (scats of all ages). A total of 689 samples (fresh scats) were collected for genetic analysis of which 383 were processed for genotyping (while a further 306 have been extracted but await further funding). These genetic samples were collected from all but one locality (Thorneside).

There was a larger number of incidental koala spotting events in the more urbanised part of the Redlands mainland and on Minjerribah / North Stradbroke Island. While sparse, presence of fresh scat indicates that the southern part of the mainland is also being used by koalas, and further surveys in these areas would likely result in a higher number of presence records. Nonetheless, it is worth noting that urban koala populations, where threats are high, could potentially be at greater density (this could be due to the higher nutrient coastal soils resulting in koala habitat with greater koala carrying capacity) – making them a difficult, yet critical, management priority.

Quantify the chlamydial disease threat

Chlamydial infection (presence of the pathogen) was widespread across the mainland, however it was relatively limited (yet still present) on Minjerribah / North Stradbroke Island. Because we developed a new (very sensitive) method to assess the chlamydial status of koalas, we had access to:

1) Qualitative measures of Chlamydia (presence / absence of Chlamydial DNA traces), and
2) Quantitative measures of Chlamydia, potentially more related to viable Chlamydial DNA.

When comparing qualitative measures of Chlamydia (presence / absence) from scat samples, Chlamydia detection rates were similar between Redlands mainland koalas (56% Chlamydia

detection) and a recent survey of the Brisbane City Council koalas (55% chlamydial infection rate was found (OWAD Environment 2017). Note that this was from qPCR* methods that have a potential for overestimating Chlamydial prevalence (Cristescu et al. 2018). It is also compatible with a dataset derived from wildlife hospitals in southeast Queensland (Gonzalez-Astudillo et al. 2017). However, when comparing quantitative measures of Chlamydia (potentially more linked to diseased individuals), we found that 28% of Redlands mainland koalas were positive for Chlamydia, whereas results from Australia Zoo Wildlife Hospital for koalas admitted to the hospital between 2013-2016, were much higher (Table 8). For the hospital koala dataset, it should be noted that it; 1) encompasses both urogenital and ocular chlamydial disease, whereas molecular methods from scat samples can only detect urogenital infections and 2) relies entirely on humans to find koalas, introducing a large bias. However, the percentage of koalas that were admitted to the hospital with Chlamydia (either as the main reason for admission, or admitted for another reason but having Chlamydia disease as well) compared to the total number of admitted koalas still gives an indication of comparative disease prevalence. Here, we are not interested in absolute numbers of koalas, but in the percentage of diseased koalas between Councils. Although Australia Zoo Wildlife Hospital is only one of the facilities that receives wild koalas for treatment, there is no reason to think that the proportion of koalas affected with disease is not representative of other facilities (i.e. there is no reason to assume more diseased koalas are sent to a particular veterinary facility). Finally, hospital data from the period 2002-2009 combining all facilities (sourced from the QLD Department of Environment at the time) comparing Redlands mainland to other SEQ Councils (i.e. Brisbane City, Caboolture Shire, Ipswich City, Logan City, Pine Rivers) also showed that Redlands mainland had, in percentage, more disease aetiology (i.e. cystitis and conjunctivitis) than other Councils (Cristescu et al. 2011). Therefore, we present here the Australia Zoo Wildlife Hospital dataset, but this is with the understanding that the reader keeps in mind this is a subset of all wild koalas admitted for treatment in SEQ.

Table 8: Percentage of diseased koalas admitted to Australia Zoo Wildlife Hospital (2013-2016) showing again that Redland City Council has a high level of disease

	Diseased koalas	Total koalas admitted	Percent of diseased koalas
Redlands City Council	**82**	**133**	**62%**
Sunshine Coast Regional Council	45	78	58%
Moreton Bay Regional Council	407	742	55%
Gold Coast City	59	112	53%
Logan City Council	34	65	52%
Brisbane City Council	36	77	47%

A major issue with understanding Chlamydia results is the lack of clarity surrounding how presence (qualitative measure) of a chlamydial infection translates into disease (potentially quantitative) and, in turn, koala mortality and population decline. This is partly because the severity of the disease varies greatly between individual koalas as well as populations (Ellis et al. 1993, Waugh et al. 2016). Notably, individual koalas can shed large numbers of chlamydial organisms without clinical signs (Wan et al. 2011) and populations can have high chlamydial prevalence (infection) with low noticeable health impact [90% of koalas in the Mt Lofty ranges were Chlamydia positive but had a low prevalence of clinical disease (Polkinghorne et al. 2013) see also Weigler *et al.* (1988)]. Recent studies have found that potentially, some specific Chlamydia strains, could be linked to Chlamydia virulence, while others found that plasmids, not strains, might explain virulence (Jelocnik et al. 2014, Phillips et al. 2018). In any case, the fact that some koalas presented severe signs of clinical disease in this study, supported by Australia Zoo Wildlife Hospital data showing Redlands Coast presents high levels of disease, suggests Chlamydia is likely a threat to Redlands Coast koala populations on the mainland. It is also worth noting that there is a clear link between low genetic diversity / high inbreeding and susceptibility to disease (Frankham et al. 2010a). Therefore, genetic health needs to be actively maintained to avoid further risk of increased disease susceptibility.

It is interesting that Chlamydia prevalence and signs of chlamydial disease are low on Minjerribah / North Stradbroke Island. The reasons for this are unknow, however, three main hypotheses can be formed: 1) the Chlamydia strain is not as virulent on the island as on the mainland, 2) Minjerribah / North Stradbroke Island koalas are more resistant, or able to fight more efficiently, the bacteria, for example thanks to better genes or better diet, 3) environmental stressors that precipitate disease are lower on the island than on the mainland. Disease expression has been linked to stress, this could be mediated via the impact of stress on the immune system (McEwen and Stellar 1993, Maddock and Pariante 2001). This is debated for the specific case of Chlamydia in koalas, and whether stressed koalas potentially are at higher risk of progressing to disease state remains unproven (Ellis et al. 1993). From the three explanations for the low impact of Chlamydia Minjerribah / North Stradbroke Island koalas, if indeed the Chlamydia strain on the island is benign, disease surveillance and quarantine might be well advised. More research into Chlamydia on the island also should be of priority.

DDC has independently funded threat mapping (road density, urbanisation, clearing, see Appendix 4) across the Redlands Coast and will combine these with the chlamydial dataset to further analyse threats to Redlands Coast koalas. This will be published in the future.

Avoid inbreeding depression

Inbreeding depression is the decline in fitness (such as survival and reproductive success) as a direct consequence of breeding with closely related individuals (Ralls et al. 1988). This can happen when available mates are mostly closely related, which is what we found for koalas in the Redlands mainland.

Any increase in inbreeding will usually have some adverse fitness effects. For instance, a 5% increase in inbreeding can increase population extinction risk by 45% in other species (Newman and Pilson 1997). We detected high levels of inbreeding in both Redlands mainland and Minjerribah / North Stradbroke Island. This is of high concern, but to make matters worse, we

measured a 34% increase in the inbreeding coefficient for urban koalas compared to non-urban koalas on the Redlands mainland.

In addition to the concerns around high levels of inbreeding, effective population sizes for both Redlands mainland (N_e = 85.7) and Minjerribah / North Stradbroke Island (N_e = 92.9) are below the recommended threshold from Frankham et al. (2014). To avoid inbreeding depression, recommendations are that effective population size (N_e) should be greater than 100 (Frankham et al. 2014). In particular, the long-term persistence of populations with $N_e < 100$ is compromised.

It is important to note that inbreeding depression is usually greater in stressful, rather than benign, environments (Frankham et al. 2010a). Indeed, there are often adverse interactions between human impacts, inbreeding, and demographic fluctuations. This results in a reinforced feedback loop and downwards spiral in population size towards extinction, referred to as the extinction vortex* (Gilpin and Soulé 1986).

It is also important to note, however, that Minjerribah / North Stradbroke Island is a natural koala population that has been existing at potentially low population size for thousands of generations, and has survived to this date. The population currently shows no noticeable negative signs of inbreeding [such as high susceptibility to disease, low breeding rate, and physical abnormalities including cryptorchidism (Cristescu et al. 2009a, Cristescu et al. 2011)]. This potentially indicates that this population, despite of (and because of) inbreeding, could have gone through a period of genetic purging, and now may be more genetically resilient, at least to further inbreeding.

Maintain evolutionary potential

Wild populations are constantly faced with new challenges, both natural (e.g. new diseases, natural disasters) and anthropogenic (e.g. introduced predators, road mortality). The ability of a population to persist relies on its ability to evolve in order to cope with these challenges. This phenomenon is captured in the term ‘evolutionary potential’, and is directly linked to genetic diversity and, ultimately, effective population size (N_e) which allows diversity to be maintained.

Indeed, genetic variation for fitness in a closed, random-mating population is maintained by the balance between mutation (adding it), drift (removing it) and selection (either removing or retaining variation). When effective population size is too small, drift predominates and loss of genetic diversity (i.e. genetic stochasticity*) ensues. To preserve genetic diversity that sustains the evolutionary potential of wild populations, Frankham et al. (2014) recommends that effective population size (N_e) should be greater than 1000 individuals.

Populations such as Redlands mainland or Minjerribah / North Stradbroke Island, with $N_e < 1000$, are not doomed to extinction in the short to medium term, but their ability to evolve to cope with environmental change will erode with time and this could reduce their long-term viability (Frankham et al. 2014).

Fragmented populations and connectivity

Both Redlands mainland and Minjerribah / North Stradbroke Island are each currently one random-mating population (each fragmented spatially but connected by gene flow). While this is a positive result, given the detected high levels of inbreeding and low effective population sizes in the Redlands Coast koalas, any continuing decrease in gene flow in the future would have dire consequences.

The existing gene flow occurring within Redlands mainland and Minjerribah / North Stradbroke Island is the life line of Redlands Coast koala populations – this will need to be monitored closely in the future so that any disruption to gene flow will be quickly detected and swift management measures implemented where required.

In the event that future monitoring detects increased deterioration in gene flow, Redlands City Council might have to carefully assess and consider potential use of more intensive management strategies (e.g. artificial inseminations, translocation of genetically dissimilar males into genetically poor, enclosed, populations). This will require additional work to develop a genetic breeding rescue program suited to the Redland Coast koalas.

As it stands, genetically, with effective population sizes <100 individuals representing a risk of both inbreeding depression and reduced evolutionary potential, Redlands Coast koalas fit within IUCN Red List Criterion for a Critically Endangered population. It is important to note that this analysis is limited to the administrative boundaries (on the mainland in particular). We do not suggest that the IUCN would classify the Redlands mainland and Minjerribah / North Stradbroke Island populations as critically endangered, but we are underlining that their genetic characteristics have reached a level that should concern decision makers in charge of preserving the koala population in the Redlands Coast for future generations. Although the administrative boundaries of the Redlands Coast are artificial and irrelevant to koala ecology, most management decisions will be constrained by these boundaries. Ultimately, the accountability for ensuring the survival of the Redlands Coast koalas (rightly or not) will be attributed to local government.

Discussion summary (see Table 8). Altogether, we found that the Redlands Coast, which historically was known to harbour a large koala population, still had evidence of wide spread koala presence, and koalas and their fresh scats were readily found. Each separate geographical entity, Redlands mainland and Minjerribah / North Stradbroke Island, formed one koala population where gene flow has been maintained.

However, we found that several genetic measures used to assess population health and survival potential, as well as disease prevalence, were not encouraging. These results, combined, form a concerning picture and should be taken as a call for action. The Redlands Coast potentially has, in the DDC's experience, a large koala population, and it is showing signs of genetic degradation. Management actions taken now should be efficient (i.e. the population is not that critical that it cannot be improved) and ensure the Redlands Coast can boast a healthy koala population for generations to come.

Table 8: Recapitulative table of all positive (green), negative (red) and neutral (orange) findings from this project. Note that comments on the presence of koalas are qualitative only, as the surveys were not designed to compare presence across areas (i.e. surveys were not random nor standardised).

		Redlands mainland	Minjerribah / North Stradbroke Island
Presence results (qualitative assessment only)			
	1	wide geographic spread of koala presence	wide geographic spread of koala presence
	2	large koala presence within the urban footprint	large koala presence within the urban footprint
	3	large number of koalas sighted	large number of koalas sighted
	4	large number of fresh scats collected	large number of fresh scats collected
Genetic results			
Large-scale structure	**5**	one continuous population / a single lineage	one continuous population / a single lineage
	6	gene flow maintained across the whole area	gene flow maintained across the whole area
Fine-scale structure	**7**	evidence of sub-structure / decrease gene flow	evidence of sub-structure / decrease gene flow
	8	closely related individual nearby / low dispersal	closely related individuals able to disperse
	9	individuals within urban footprint more related than outside urban footprint	
Genetic health	**10**	small bias of sex ratio towards female (1:1.4)	small bias of sex ratio towards male (1:0.9)
	11		lower genetic diversity / polymorphism (founder effect) than the mainland, but still higher than other island koalas
	12	high level of inbreeding at the population level	high level of inbreeding at the population level
	13	high level of inbreeding at the individual level	high level of inbreeding at the individual level
	14	evidence for genetic bottleneck	evidence for genetic bottleneck
	15	effective population size lower than recommendations of >100 to prevent inbreeding	effective population size lower than recommendations of >100 to prevent inbreeding
	16	effective population size lower than recommendations of >1000 to maintain evolutionary potential	effective population size lower than recommendations of >1000 to maintain evolutionary potential
	17	potentially still linked to other populations outside Redlands Coast	population potentially more resistant to inbreeding through genetic purging
Disease results			
	18	high level of chlamydia	low level of chlamydia

Future steps and management considerations

Our recommendations for future steps and monitoring stem from the results of this report and includes both genetic and non-genetic approaches. We use the term monitoring, defined in Schwartz et al. (2007), as the repetition of surveys in time that enables comparison of a current situation with baseline data, and the term assessment as the initial collection of baseline data.

It is our belief that Council should only be implementing monitoring if:

1) the results of the monitoring will be used to choose between alternative management strategies, i.e. if the same management outcome will be implemented no matter what the monitoring results are, then funds are not well invested in monitoring. Monitoring for the sake of monitoring provides very interesting data, but might be more suited to research institutions than governments. This ensues that monitoring programs need to include decision trees on appropriate management actions, and thresholds for these actions. These need to involve science but are ultimately political decisions.

2) the budget allocated to monitoring is of sufficient level to provide the sensitivity and precision required to calculate whether the threshold for action is met. This means that if the budgeted method can only confidently detect a population decrease of 50%, then the threshold call for action cannot be a 20% decrease in population size. Note that the levels chosen as examples are not exaggerated levels, as change in population size is difficult to measure with any degree of precision: Plumptre (2002) calculated that changes in population size of up to 50% were unlikely to be detected by standard line transect surveys in tropical forest. Confidence intervals for estimates of population size of approximately 15% (i.e. population size = estimate ± 15% of estimate) have been described as "impressively small" – and, in reality, confidence intervals this small are hardly ever obtained (Luikart et al. 2010). Thresholds for action are not trivial to establish either, and depend on political will, budget, ethical considerations (e.g. animal welfare), and sometimes even philosophical standpoints (e.g. what population decrease is one willing to tolerate?).

Genetic monitoring, defined as the use of multiple genetic assessments in time, is still somewhat underutilised by decision makers, despite the multiple benefits genetic monitoring can provide for conservation and management (Schwartz et al. 2007). As underlined in this report, genetic parameters are essential for understanding long-term population survival potential and informing management strategies. Genetic monitoring also has the added benefit of being able to provide more traditional parameters [such as population distribution or disease prevalence, as in this report and see also Figure 20, adapted from Schwartz et al. (2007)].

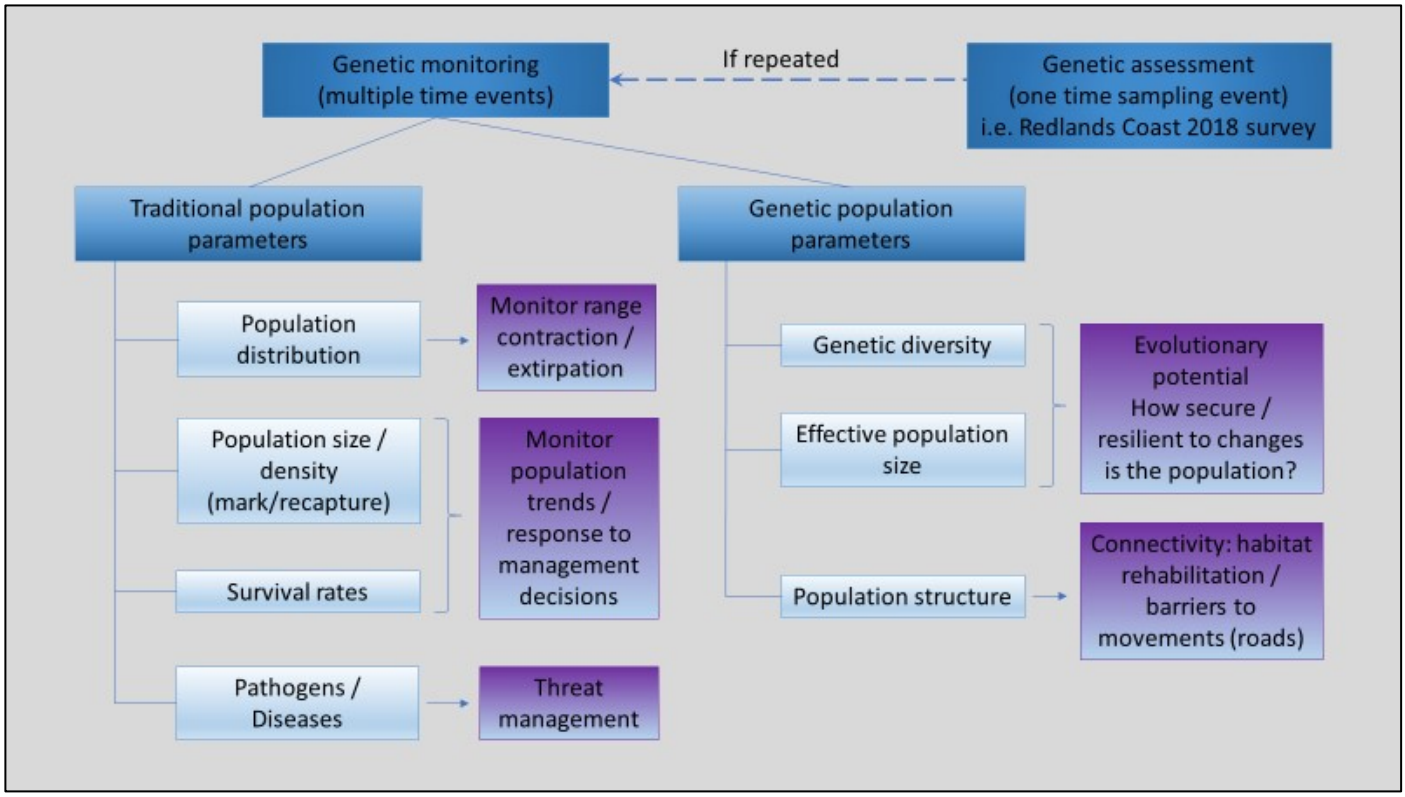


Figure 20: Examples of information gained from genetic monitoring, as well as how these can inform decision makers on how secure their koala populations are, and the effectiveness of their management strategies adapted from Schwartz et al. (2007)

Monitoring recommendations

The koala populations of Redlands mainland and Minjerribah / North Stradbroke Island are naturally isolated and are facing different challenges. Monitoring, therefore, should be tailored to each population, their current and potential future threats as well as management strategies implemented for each.

Redlands mainland

Koala distribution across the Redlands Coast is widespread and koala numbers, reflected by how readily available both koalas and fresh koala scats were during this project, are relatively numerous (this is a qualitative comment only and made in comparison to other field work areas covered by DDC both in QLD and NSW). The Koala Coast population might have been isolated for some time, as reflected in a low effective population size, as well as low genetic diversity compared to other SEQ populations [from (Lee 2009)]. Connectivity and gene flow are negatively impacted in the urban footprint. Chlamydia prevalence is high, and disease risk might be exacerbated by high inbreeding and low genetic diversity. While these are concerning trends, the identified high presence of koala scats across the Redlands Coast and the noteworthy numbers of koalas that were spotted indicate that the development and implementation of conservation actions for the Redlands Coast koalas is timely.

Aim 1: Complete the assessment of koala presence in the southern half of the mainland (Sheldon, Mount Cotton and Redland Bay)

Survey effort could be increased in the southern half of the mainland, but at minimum, all remaining samples from Sheldon, Mount Cotton and Redland Bay should be genotyped, and all genetic analyses in this report re-run, and an annexe report added to the present report.

Aim 2: Monitor genetic structure in urban areas to detect any further loss of connectivity

We detected finer-scale structure (not large-scale), and found that gene flow still exists, but is degrading, within urban areas. In areas where fine scale structure has been detected (particularly around Cleveland), considerations must be given to ways to increase gene flow, and in areas where no fine scale structure was detected, gene flow needs to be maintained.

As it is critical that gene flow is preserved, monitoring the trends through additional genetic analyses every one to two years might be required to detect any further deterioration of gene flow. If isolation is detected, and no remediation is possible (i.e. no additional koala tree plantings possible within the urban areas of concern, no further reduction in dog / vehicle koala casualties) artificial insemination might have to be considered. We would recommend insemination above koala translocation as insemination respects the role of maternal transmission – of gut microbiome adapted to local food trees, of landscape (safe paths) and of the ecological and social environment.

Aim 3: Place current genetic trends in historical context trough comparison with past genetic health of Redlands Coast koalas

DDC currently has access to 1676 historical Redlands Coast samples (collected between 1997 and 2013) and we strongly recommend that carefully chosen samples be genotyped (for example, from one and two koala generations ago). This will enable a clearer picture of what koala genetic diversity, inbreeding and effective population sizes were previously, allowing us to determine the speed of the genetic erosion* of Redlands Coast koala populations. Only by looking at trends can we properly assess how rapid the genetic deterioration of Redlands Coast koalas is, and the relative urgency of the situation.

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

As part of the DDC research, the DDC has commissioned a connectivity analyses of the Redlands Coast to overlay genetics and landscape connectivity and attempt to elucidate what prevents / enhances gene flow. The result of this research will be communicated to RCC. This might inform strategies to increase koala connectivity in terms of gene flow and to identify and correct barriers.

Aim 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

Urban mainland koalas have been identified as particularly vulnerable in this report. Urban koala threats are mainly anthropogenic (e.g. the cutting of backyard trees, vehicle collisions, dog attacks) – and although Council must lead programs to combat urban threats to koalas, halting or decreasing these threats will ultimately only be possible with community support.

Aim 6: Establish methodological calibration in collaboration with State Government (if possible)

Redlands Coast was surveyed by the State Government for many years to establish population trends (Rhodes 2015), this involved strip transect visual searches at specific locations in the Redlands Coast. Re-surveying (a subset of) these sites using the same method would enable:

1/ some survey overlap for methodological calibration with thermal drone and detection dog survey results,

2/ RCC to use already available historical data, calibrated / updated through this methodological comparison, to continue monitoring population trends.

This survey could be used as a final sign-off on past State methodology towards a transition to other more efficient monitoring methods. Otherwise, RCC might risk delays in establishing a new baseline using new methodologies and then potentially require many additional years of monitoring data to build up a new case of population decline. The newly established koala monitoring team at the State Department of Environment and Science (DES) has indicated they will be reverting to visual koala searchers (at a recent LGA meeting on December 4th, 2018). DES should therefore be approached to confirm if they will indeed be conducting these surveys, in which case RCC and USC should coordinate surveys using alternate technologies at close dates to the DES surveys.

Minjerribah / North Stradbroke Island

Connectivity is already high on the island, and potentially cannot be easily increased. Indeed, the sub-structure found in this report on the island could be resulting from the 2013-2014 fires that burnt approximately 70% of the island. Other barriers to koala movements could include open areas and artificial ponds from sand mining and the inhospitable vegetation types in the centre (high, old dunes) of the island. Levels of inbreeding are high, however, due to the natural long-term isolation of the island koalas, their resilience to inbreeding may be higher than the mainland population (through genetic purging). The genetic diversity on the island is lower than the mainland, but is the highest of all island koala populations studied to date (Cristescu et al. 2011). There is very little evidence that Chlamydia is a current threat on the island (Cristescu et al. 2011), and it is critical it remains so. Dog attacks have been problematic on the island for some time and, despite Council increasing dog control measures, remain a source of koala injury and death (Cristescu, unpublished data). Clearing vegetation to increase the urban footprint is a threat, although the extent of this will be dictated by future urban planning controlled by Council / QYAC.

Aim 1: Determine Chlamydia strain(s) present

Chlamydia strains on the island have never been described (previous to this report, the main information came from Sibelco research: "Understanding the movement and behaviour of koalas on North Stradbroke Island", Ellis and FitzGibbon). Molecular description of Chlamydia strain(s) present on the island is necessary to inform whether current quarantine of sick / injured koalas taken for treatment on the mainland is appropriate. There are different strains of Chlamydia that can affect koalas – some might be introduced, some native to the koala, and they might vary in their virulence (Jelocnik et al. 2013, Jelocnik et al. 2014). This is an area of active research and the precautionary principle might have to be invoked here.

If Chlamydia strain(s) present on the island are numerous and present in the mainland, quarantine is of lower concern. However, if strain(s) are different, or only a subsection of what is present on the mainland, quarantine needs to be maintained / upgraded (e.g. at private wildlife carers).

Aim 2: Monitor population size in remote bushland areas to the extent that it enables the early detection of population decrease, so that impacts from hidden threats (dogs / disease) are detected early

Potential measures to control risks to koalas in island bushland are possibly limited and / or more difficult for Council (fire, disease, dog attacks). Fire management is on-going on the island. Dogs and diseases acting in the bushland are problematic, because impacts from these potential threats can remain hidden to residents and Council.

For example, a very low number of feral dogs can have a large impact on koala populations (Beyer et al. 2018). It is hard to monitor koala mortality – usually it requires collaring koalas and being able to locate freshly dead (often, within a day) koalas for necropsy to establish cause of death (including samples collection and analyses). To reach meaningful proportions for different causes of death, a substantial part of the population needs to be monitored. Therefore, monitoring threats

is difficult, and monitoring causes of death is expensive, so it might be more efficient to broadly monitor population size and investigate exact causes only if a decline is detected.

Estimating wildlife population size (and density) is one of the hardest survey tasks in ecology (Luikart et al. 2010). Efficient, accurate and affordable methods to estimate population size are not currently available for koalas – however, the DDC is in the process of testing two methodologies: 1) thermal imagery from a drone using a strip transect search method and 2) mark/recapture from genetic fingerprinting using fresh scat detection dogs. These methods might prove sufficiently accurate and affordable to provide meaningful and timely evidence of population decline, which could then be followed up with 1) investigation of causes of decline and 2) remedial action.

Aim 3: Ensure Council urban annual count records external signs of Chlamydia disease and monitor prevalence of these signs

In urban areas, koalas are easily spotted by members of the public and Council already organises an intensive survey across the three townships of the island annually. These provide opportunities for visual monitoring of external signs for Chlamydia. External signs of disease can be monitored through either 1) the community (champions might be recruited with this specific task), 2) the annual survey, or 3) from wildlife rescuers or the local veterinarian giving most sick / injured koalas a first examination before dispatch to the mainland (see Wildlife Rescue Minjerribah contact). If an increase in signs of disease is noted, decision makers should have an action plan ready. This might have to include catching and treating koalas – an option most likely be more costly than prevention, see Aim 1.

5. References

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology **215**:403-410.

Amos, W., J. W. Wilmer, K. Fullard, T. Burg, J. Croxall, D. Bloch, and T. Coulson. 2001. The influence of parental relatedness on reproductive success. Proceedings of the Royal Society of London B: Biological Sciences **268**:2021-2027.

Aparicio, J. M., J. Ortego, and J. Cordero P. 2006. What should we weigh to estimate heterozygosity, alleles or loci? Molecular Ecology **15**:4659-4665.

Beyer, H., L., D. deVilliers, J. Loader, A. Robbins, M. Stigner, N. Forbes, J. Hanger, and M. Gonz á lez - Su á rez. 2018. Management of multiple threats achieves meaningful koala conservation outcomes. Journal of Applied Ecology.

Burton, E., and A. Tribe. 2016. The rescue and rehabilitation of koalas (Phascolarctos cinereus) in Southeast Queensland. Animals **6**:1-10.

Caye, K., T. M. Deist, H. Martins, O. Michel, and O. François. 2016. TESS3: fast inference of spatial population structure and genome scans for selection. Molecular Ecology Resources **16**:540-548.

Chikhi, L., and M. Bruford. 2005. 21 Mammalian Population Genetics and Genomics. Mammalian genomics:539.

Conrad, E. 2014. The economic value of the koala.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics **144**:2001-2014.

Couch, A. J., P. J. Unmack, F. J. Dyer, and M. Lintermans. 2016. Who's your mama? Riverine hybridisation of threatened freshwater Trout Cod and Murray Cod. PeerJ **4**:e2593.

Coulon, A. 2010. GENHET: an easy - to - use R function to estimate individual heterozygosity. Molecular Ecology Resources **10**:167-169.

Courtois, B., A. Audebert, A. Dardou, S. Roques, T. Ghneim- Herrera, G. Droc, J. Frouin, L. Rouan, E. Gozé, A. Kilian, N. Ahmadi, and M. Dingkuhn. 2013. Genome-wide association mapping of root traits in a Japonica rice panel. PLoS ONE **8**:e78037; DOI: 78010.71371/journal.pone.0078037.

Coyne, J. A., H. Allen Orr, and M. A. Sunderland. 2004. Speciation. Sinauer Associates.

Cristescu, R. 2011. Fauna recolonisation of mine rehabilitation through the example of arboreal marsupials, with a particular focus on the koala *Phascolarctos cinereus*. University of New South Wales, Sydney.

Cristescu, R., V. Cahill, K. Handasyde, K. Carlyon, D. Whisson, G. Johnson, C. Herbert, A. N. Wilton, B. L. J. Carlsson, and D. Cooper. 2009a. Inbreeding and testicular abnormalities in a bottlenecked population of koalas, *Phascolarctos cinereus*. Wildlife Research **36**:299-308.

Cristescu, R., V. Cahill, W. B. Sherwin, K. Handasyde, K. Carlyon, D. Whisson, C. A. Herbert, B. L. J. Carlsson, A. N. Wilton, and D. W. Cooper. 2009b. Inbreeding and testicular

abnormalities in a bottlenecked population of koalas (Phascolarctos cinereus). Wildlife Research **36**:299-308.

Cristescu, R., K. Goethals, P. B. Banks, F. Carrick, and C. Frère. 2012. Persistence and detectability of fecal pellets in different environment and the implication for pellet based census of fauna. International Journal of Zoology **2012, Article ID 631856**:doi:10.1155/2012/631856.

Cristescu, R. H., W. Ellis, D. d. Villiers, K. Lee, O. Woosnam-Merchez, C. Frere, P. Banks, D. Dique, S. Hodgkison, H. Carrick, D. Carter, P. Smith, and F. Carrick. 2011. North Stradbroke Island: An island ark for Queensland's koalas population? Proceedings of the Royal Society of Queensland **117**:309-334.

Cristescu, R. H., E. Foley, A. Markula, G. Jackson, D. Jones, and C. Frère. 2015a. Accuracy and efficiency of detection dogs: a powerful new tool for koala conservation and management. Scientific Reports **5**:DOI: 10.1038/srep08349.

Cristescu, R. H., E. Foley, A. Markula, G. Jackson, D. Jones, and C. Frère. 2015b. Accuracy and efficiency of detection dogs: a powerful new tool for koala conservation and management. Scientific Reports **5**:8349.

Cristescu, R. H., R. L. Miller, A. J. Schultz, L. Hulse, D. Jaccoud, S. Johnston, J. Hanger, R. Booth, and C. H. Frère. 2018. Developing non-invasive methodologies to assess koala population health through detecting Chlamydia from scats. Molecular Ecology Resources **submitted**.

Cruz, V. M. V., A. Kilian, and D. A. Dierig. 2013. Development of DArT marker platforms and genetic diversity assessment of the U.S. collection of the new oilseed crop Lesquerella and related species. PLoS ONE **8**:e64062.

Dique, D. S., J. Thompson, H. J. Preece, D. L. de Villiers, and F. N. Carrick. 2003. Dispersal patterns in a regional koala population in south-east Queensland. Wildlife Research **30**:281-290.

Donnellan, S. C., R. Foster, C. Junge, C. Huveneers, P. Rogers, A. Kilian, and T. Bertozzi. 2015. Fiddling with the proof: the Magpie Fiddler Ray is a colour pattern variant of the common Southern Fiddler Ray (Rhinobatidae: *Trygonorrhina*). Zootaxa **3981**:367-384.

Ellis, W., A. Melzer, and F. Bercovitch. 2009. Spatiotemporal dynamics of habitat use by koalas: The checkerboard model. Behavioral Ecology and Sociobiology **63**:1181-1188.

Ellis, W. A. H., A. A. Girjes, F. N. Carrick, and A. Melzer. 1993. Chlamydial infection in koalas under relatively little alienation pressure. Australian Veterinary Journal **70**:427-428.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology **14**:2611-2620.

Frankham, R. 1995a. Effective population size/adult population size ratios in wildlife: a review. Genetics Research **66**:95-107.

Frankham, R. 1995b. Inbreeding and Extinction - a Threshold Effect. Conservation Biology **9**:792-799.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2010a. Introduction to Conservation Genetics, 2nd ed. . Cambridge University Press, Cambridge, U.K.

Frankham, R., J. D. Ballou, M. D. B. Eldridge, R. C. Lacy, K. Ralls, M. R. Dudash, and C. B. Fenster. 2011. Predicting the Probability of Outbreeding Depression. Conservation Biology **25**:465-475.

Frankham, R., J. D. Ballou, K. Ralls, M. D. B. Eldridge, M. R. Dudash, C. B. Fenster, and P. Sunnucks. 2017. Genetic Management of Fragmented Animal and Plant Populations. Oxford University Press, Oxford, UK.

Frankham, R., C. J. A. Bradshaw, and B. W. Brook. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation **170**:56-63.

Frankham, R. F., J. D. Ballou, and D. A. Briscoe. 2010b. Introduction to conservation genetics. Cambridge University Press.

Frère, C. H., M. Krützen, A. M. Kopps, J. Mann, and W. B. Sherwin. 2010. Too Close to Home: Inbreeding Avoidance or Tolerance in Wild Bottlenose Dolphins (*Tursiops* sp.)? Proceedings of the Royal Society of London, Biological Sciences **277**:2667-2673.

Gilpin, M. E., and M. E. Soulé. 1986. Minimum viable populations: processes of extinction. Pages 19–34 *in* M. E. Soulé, editor. Conservation Biology: The Science of Scarcity and Diversity Sinauer Associates, Sunderland, MA.

Gonzalez-Astudillo, V., R. Allavena, A. McKinnon, R. Larkin, and J. Henning. 2017. Decline causes of Koalas in South East Queensland, Australia: a 17-year retrospective study of mortality and morbidity. Scientific Reports **7**:42587.

Government, Q. 2012. Koala Coast Koala Population Report 2010., DERM.

Haddad, N. M., L. A. Brudvig, J. Clobert, K. F. Davies, A. Gonzalez, R. D. Holt, T. E. Lovejoy, J. O. Sexton, M. P. Austin, C. D. Collins, W. M. Cook, E. I. Damschen, R. M. Ewers, B. L. Foster, C. N. Jenkins, A. J. King, W. F. Laurance, D. J. Levey, C. R. Margules, B. A. Melbourne, A. O. Nicholls, J. L. Orrock, D.-X. Song, and J. R. Townshend. 2015. Habitat fragmentation and its lasting impact on Earth's ecosystems. Science Advances **1**.

IUCN. 2012. IUCN Red List of Threatened Species. Version 2012.1. www.iucnredlist.org, 23 July 2012.

Jelocnik, M., F. D. Frentiu, P. Timms, and A. Polkinghorne. 2013. Multi-locus sequence analysis provides insights into the molecular epidemiology of Chlamydia pecorum infections in Australian sheep, cattle and koalas. Journal of Clinical Microbiology:JCM. 00992-00913.

Jelocnik, M., E. Walker, Y. Pannekoek, J. Ellem, P. Timms, and A. Polkinghorne. 2014. Evaluation of the relationship between Chlamydia pecorum sequence types and disease using a species-specific multi-locus sequence typing scheme (MLST). Veterinary Microbiology **174**:214-222.

Kilian, A., P. Wenzl, E. Huttner, J. Carling, L. Xia, H. Blois, V. Caig, K. Heller-Uszynska, D. Jaccoud, C. Hopper, M. Aschenbrenner-Kilian, M. Evers, K. Peng, C. Cayla, P. Hok, and G. Uszynski. 2012. Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms. Pages 67-89 *in* F. Pompanon and A. Bonin, editors. Data Production and Analysis in Population Genomics: Methods and Protocols. Humana Press, Totowa, NJ.

Kjeldsen, S. R., H. W. Raadsma, K. A. Leigh, J. R. Tobey, D. Phalen, A. Krockenberger, W. A. Ellis, E. Hynes, D. P. Higgins, and K. R. Zenger. 2018. Genomic comparisons reveal biogeographic and anthropogenic impacts in the koala (Phascolarctos cinereus): a dietary-specialist species distributed across heterogeneous environments. Heredity.

Kjeldsen, S. R., K. R. Zenger, K. Leigh, W. Ellis, J. Tobey, D. Phalen, A. Melzer, S. FitzGibbon, and H. W. Raadsma. 2016. Genome-wide SNP loci reveal novel insights into koala (Phascolarctos cinereus) population variability across its range. Conservation Genetics **17**:337-353.

Lacy, R. C. 1997. Importance of genetic variation to the viability of mammalian populations. Journal of Mammalogy **78**:320-335.

Lande, R. 1998. Anthropogenic, ecological and genetic factors in extinction and conservation. Researches on Population Ecology 40:259– 269.

Lee, K. 2009. Conservation genetics of the koala (*Phascolarctos cinereus*) in Queensland and Northeast New South Wales. The University of Queensland, Brisbane.

Lee, K. E., J. M. Seddon, S. W. Corley, W. A. H. Ellis, S. D. Johnston, D. L. d. Villiers, H. J. Preece, and F. N. Carrick. 2010. Genetic variation and structuring in the threatened koala populations of Southeast Queensland. Conservation Genetics **11**:2091-2103.

Luikart, G., F. Allendorf, J. Cornuet, and W. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity **89**:238-247.

Luikart, G., N. Ryman, D. A. Tallmon, M. K. Schwartz, and F. W. Allendorf. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conservation Genetics **11**:355-373.

Mace, G. M., N. J. Collar, K. J. Gaston, C. Hilton-Taylor, H. R. Akcakaya, N. Leader-Williams, E. J. Milner-Gulland, and S. N. Stuart. 2008. Quantification of Extinction Risk: IUCN's System for Classifying Threatened Species. Conservation Biology **22**:1424-1442.

Mace, G. M., and R. Lande. 1991. Assessing Extinction Threats: Toward a Reevaluation of IUCN Threatened Species Categories. Conservation Biology **5**.

MacKenzie, D. I., and J. A. Royle. 2005. Designing occupancy studies: general advice and allocating survey effort. Journal of Applied Ecology **42**:1105-1114.

Maddock, C., and C. M. Pariante. 2001. How does stress affect you? An overview of stress, immunity, depression and disease. Epidemiology and Psychiatric Sciences **10**:153-162.

Margan, S. H., R. K. Nurthen, M. E. Montgomery, L. M. Woodworth, E. H. Lowe, D. A. Briscoe, and R. Frankham. 1998. Single large or several small? Population fragmentation in the captive management of endangered species. Zoo BIology **17**:467–480

McAlpine, C., D. Lunney, A. Melzer, P. Menkhorst, S. Phillips, D. Phalen, W. Ellis, W. Foley, G. Baxter, D. de Villiers, R. Kavanagh, C. Adams-Hosking, C. Todd, D. Whisson, R. Molsher, M. Walter, I. Lawler, and R. Close. 2015. Conserving koalas: a review of the contrasting regional trends, outlooks and policy challenges. Biological Conservation **192**:226-236.

McEwen, B. S., and E. Stellar. 1993. Stress and the individual: mechanisms leading to disease. Archives of Internal Medicine **153**:2093-2101.

McNeely, J. A., K. R. Miller, W. V. Reid, R. A. Mittermeier, and T. B. Werner. 1990. Conserving the world's biological diversity. International Union for Conservation of Nature and Natural Resources.

Moritz, C. 1994. Defining 'evolutionarily significant units' for conservation. Trends in Ecology & Evolution **9**:373-375.

Newbold, T., L. N. Hudson, S. L. L. Hill, S. Contu, I. Lysenko, R. A. Senior, L. Borger, D. J. Bennett, A. Choimes, B. Collen, J. Day, A. De Palma, S. Diaz, S. Echeverria-Londono, M. J. Edgar, A. Feldman, M. Garon, M. L. K. Harrison, T. Alhusseini, D. J. Ingram, Y. Itescu, J. Kattge, V. Kemp, L. Kirkpatrick, M. Kleyer, D. L. P. Correia, C. D. Martin, S. Meiri, M. Novosolov, Y. Pan, H. R. P. Phillips, D. W. Purves, A. Robinson, J. Simpson, S. L. Tuck, E. Weiher, H. J. White, R. M. Ewers, G. M. Mace, J. P. W. Scharlemann, and A. Purvis. 2015. Global effects of land use on local terrestrial biodiversity. Nature **520**:45-50.

Newman, D., and D. Pilson. 1997. Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. Evolution **51**:354-362.

O'Brien, S. J., and J. F. Evermann. 1988. Interactive influence of infectious disease and genetic diversity in natural populations. Trends in Ecology & Evolution **3**:254-259.

O'Brien, S. J., M. E. Roelke, L. Marker, A. Newman, C. A. Winkler, D. Meltzer, L. Colly, J. F. Evermann, M. Bush, and D. E. Wildt. 1985. Genetic basis for species vulnerability in the cheetah. Science **227**:1428-1434.

OWAD Environment. 2017. Brisbane City Council Koala detection dog surveys.

Palstra, F. P., and D. J. Fraser. 2012. Effective/census population size ratio estimation: a compendium and appraisal. Ecology and Evolution **2**:2357-2365.

Palstra, F. P., and D. E. Ruzzante. 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Molecular Ecology **17**:3428-3447.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Pew, J., P. H. Muir, J. Wang, and T. R. Frasier. 2015. related: an R package for analysing pairwise relatedness from codominant molecular markers. Molecular Ecology Resources **15**:557-561.

Phillips, S., A. Robbins, J. Loader, J. Hanger, R. Booth, M. Jelocnik, A. Polkinghorne, and P. Timms. 2018. Chlamydia pecorum gastrointestinal tract infection associations with urogenital tract infections in the koala (Phascolarctos cinereus). PLOS ONE **13**:e0206471.

Plumptre, A. J. 2002. Monitoring mammal populations with line transect techniques in African forests. Journal of Applied Ecology **37**:356-368.

Polkinghorne, A., J. Hanger, and P. Timms. 2013. Recent advances in understanding the biology, epidemiology and control of chlamydial infections in koalas. Veterinary Microbiology **165**:214-223.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945-959.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. R. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. W. de Bakker, M. J. Daly, and P. C. Sham. 2007. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. The American Journal of Human Genetics **81**:559-575.

Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution **43**:258-275.

Ralls, K., J. Ballou, and A. Templeton. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology **2**:185-193.

Raman, H., R. Raman, A. Kilian, F. Detering, J. Carling, N. Coombes, S. Diffey, G. Kadkol, D. Edwards, M. McCully, P. Ruperao, I. A. P. Parkin, J. Batley, D. J. Luckett, and N. Wratten. 2014. Genome-wide delineation of natural variation for pod shatter resistance in *Brassica napus*. PLoS ONE **9**:e101673.

Rank, R. G., and L. Yeruva. 2014. "Hidden in plain sight:" Chlamydial gastrointestinal infection and its relevance to "persistence" in human genital infections. Infection and Immunity.

Rhodes, J. R., Beyer, H. L., Preece, H.J. and McAlpine, C.A. . 2015. South East Queensland koala population modelling study. Page 88 *in* UniQuest, editor., Brisbane.

Rhodes, J. R., D. Lunney, C. Moon, A. Matthews, and C. A. McAlpine. 2011a. The consequences of using indirect signs that decay to determine species' occupancy. Ecography **34**:141-150.

Rhodes, J. R., C. F. Ng, D. L. d. Villiers, H. J. Preece, C. A. McAlpine, and H. P. Possingham. 2011b. Using integrated population modelling to quantify the implications of multiple threatening processes for a rapidly declining population. Biological Conservation **144**:1081–1088.

Schultz, A., R. Cristescu, B. Littleford-Colquhoun, D. Jaccoud, and C. H. Frère. 2018a. Fresh is best: Accurate SNP genotyping from koala scats. Ecology and Evolution.

Schultz, A., J., R. Cristescu, H., B. Littleford‐Colquhoun, L., D. Jaccoud, and C. Frère, H. 2018b. Fresh is best: accurate SNP genotyping from koala scats. Ecology and Evolution **8**:3139-3151.

Schwartz, M. K., G. Luikart, and R. S. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology & Evolution **22**:25-33.

Segelbacher, G., J. Höglund, and I. Storch. 2003. From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. Molecular Ecology **12**:1773-1780.

Shaffer, M. L. 1981. Minimum population sizes for species conservation. Bioscience **31**:131-134.

Sherwin, W. B., P. Timms, J. Wilcken, and B. Houlden. 2000. Analysis and conservation implications of koala genetics. Conservation Biology **14**:639-649.

Triggs, B. 1996. Tracks, scats and other traces: A field guide to Australian mammals. Oxford University Press, South Melbourne.

Wan, C., J. Loader, J. Hanger, K. W. Beagley, P. Timms, and A. Polkinghorne. 2011. Using quantitative polymerase chain reaction to correlate *Chlamydia pecorum* infectious load with ocular, urinary and reproductive tract disease in the koala (*Phascolarctos cinereus*). Australian Veterinary Journal **89**:409-412.

Waples, R. S., and C. Do. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources **8**:753-756.

Waples, R. S., and C. Do. 2010. Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evolutionary Applications **3**:244-262.

Waugh, C., J. Hanger, P. Timms, and A. Polkinghorne. 2016. Koala translocations and Chlamydia: Managing risk in the effort to conserve native species. Biological Conservation **197**:247-253.

Wedekind, C. 2012. Managing population sex ratios in conservation practice: how and why? Topics in conservation biology. InTech.

Weigler, B. J., A. A. Girjes, N. A. White, N. D. Kunst, F. N. Carrick, and M. F. Lavin. 1988. Aspects of the epidemiology of *Chlamydia psittaci* infection in a population of koalas (*Phascolarctos cinereus*) in southeastern Queensland, Australia. Journal of Wildlife Diseases **24**:282-291.

Woinarski, J. C. Z., A. A. Burbidge, and P. L. Harrison. 2015. Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement. Proceedings of the National Academy of Sciences **112**:4531-4540.

6. Appendices

Appendix 1: Detailed molecular methods

DNA extraction Koala intestinal epithelial cells were extracted from the surface of collected scats using the QIAamp DNA Stool Mini Kit (Qiagen) following the "Isolation of DNA from Stool for Human DNA Analysis" manufacturer protocol, with modification. 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). Finally, all extracted samples had 2µL Qiagen RNase A (Qiagen) added, and incubated at 56º C for 2 minutes, to remove RNA contamination. 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).

Sequences identified during the DArTseq™ process were run through the National Center for Biotechnology Information's (NCBI) BLAST (basic local alignment search tool) (Altschul et al. 1990) to investigate possible dietary or disease-related DNA that was included in scats.

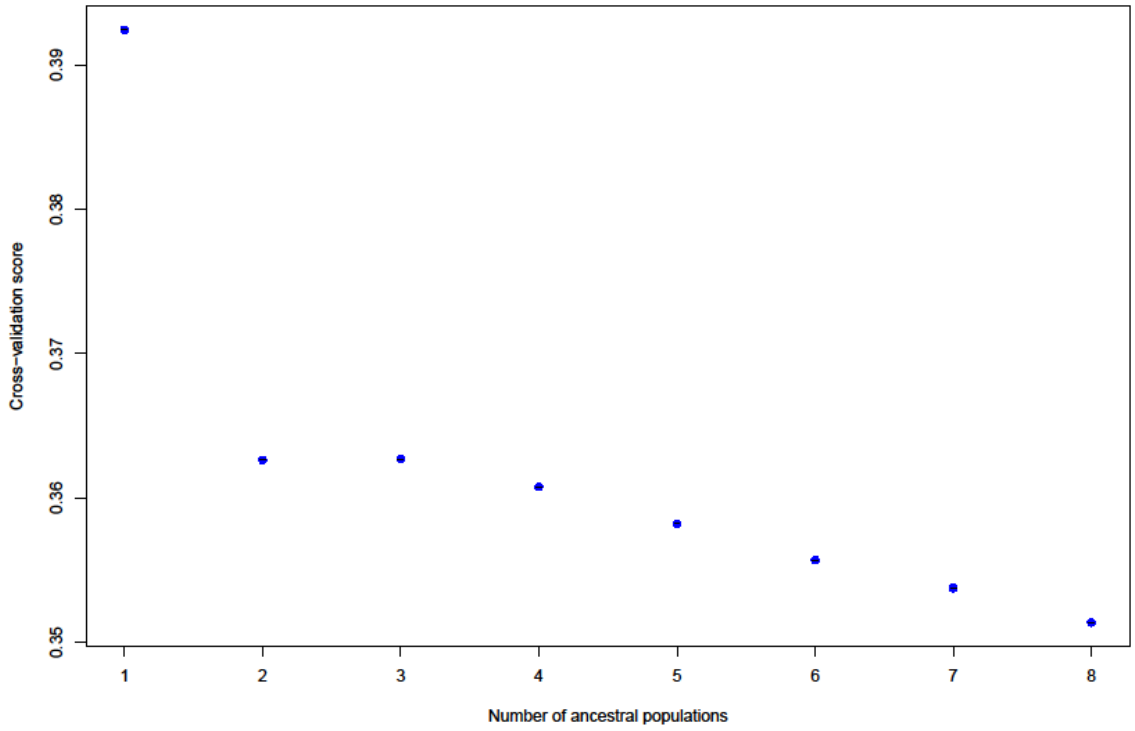
Appendix 2: Additional results

Identification of ancestral populations

We used the Bayesian clustering approach implemented in the TESS3R R package within the R statistical environment to calculate assignment probabilities and assess genotypic clustering (Caye et al. 2016). This approach incorporates spatial information about each individual when assessing population structure. We examined K = 1 – 8 genetic clusters (tolerance = 1×10^{-14}, max. iterations = 1×10^{-7}). We used the ΔK (Evanno et al. 2005) method and the (Ln(Pr(X|K)) method (Pritchard et al. 2000) to infer the number of genetic groups.

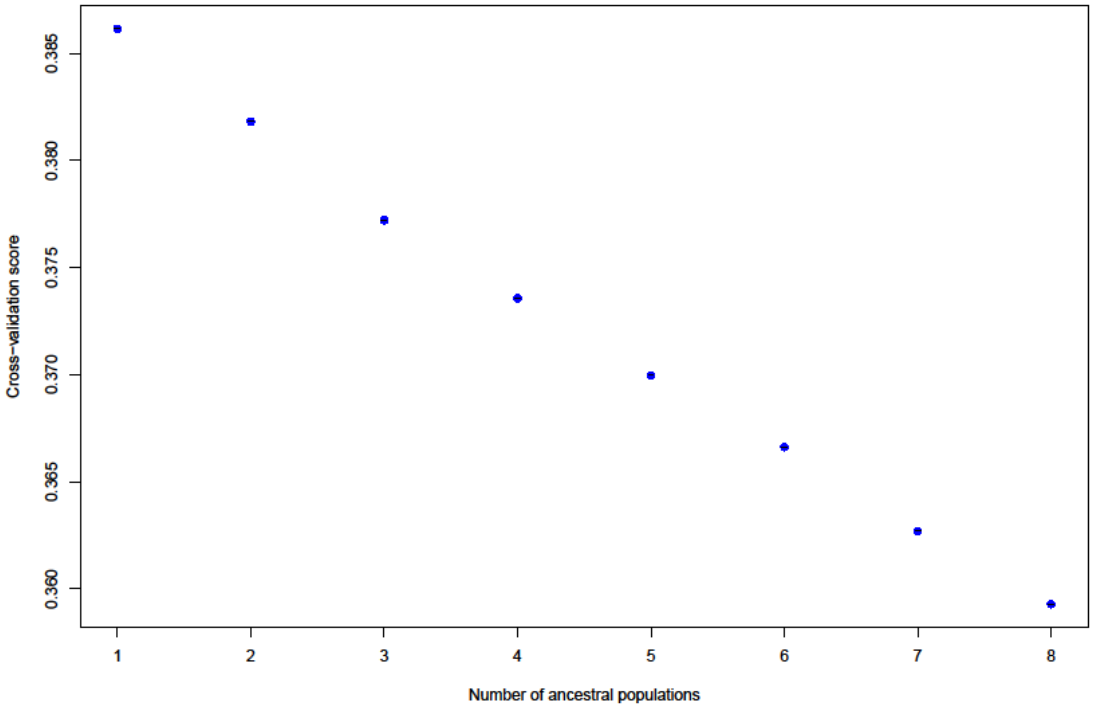
Figures below show the plots used to infer the number of ancestral population present across 1) all samples, 2) Redlands mainland and 3) Minjerribah / North Stradbroke Island using cross validation scores. What these graphs represent is the likelihood of several scenarios in terms of number of populations. If we observe a severe drop in the graph, this means the likelihood of one scenario is much higher than others. If no drop is observed (points form a straight downward line), then the most likely scenario is one population. TESS identified two ancestral populations across the entire Redland shire; one ancestral population on Redlands mainland and one ancestral population on North Stradbroke Island.

1. All samples



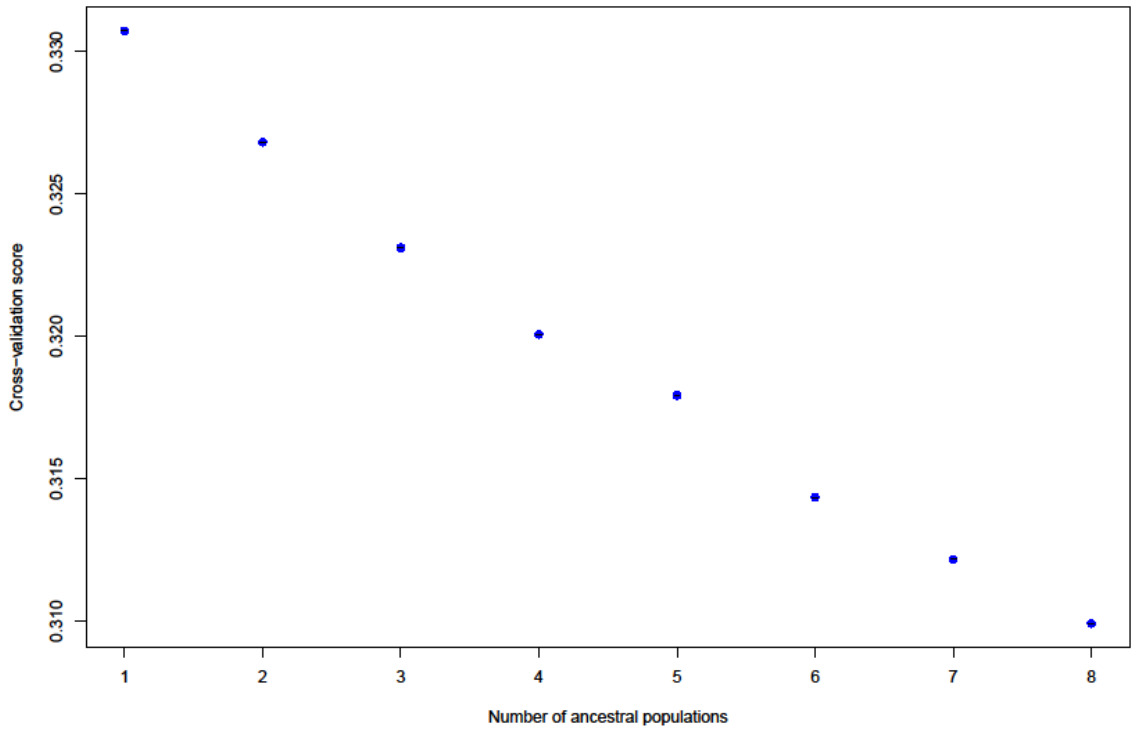
Appendix 2 Figure 1: Plots used to infer the number of ancestral population present across all samples. This identifies two ancestral populations.

2. Redlands mainland



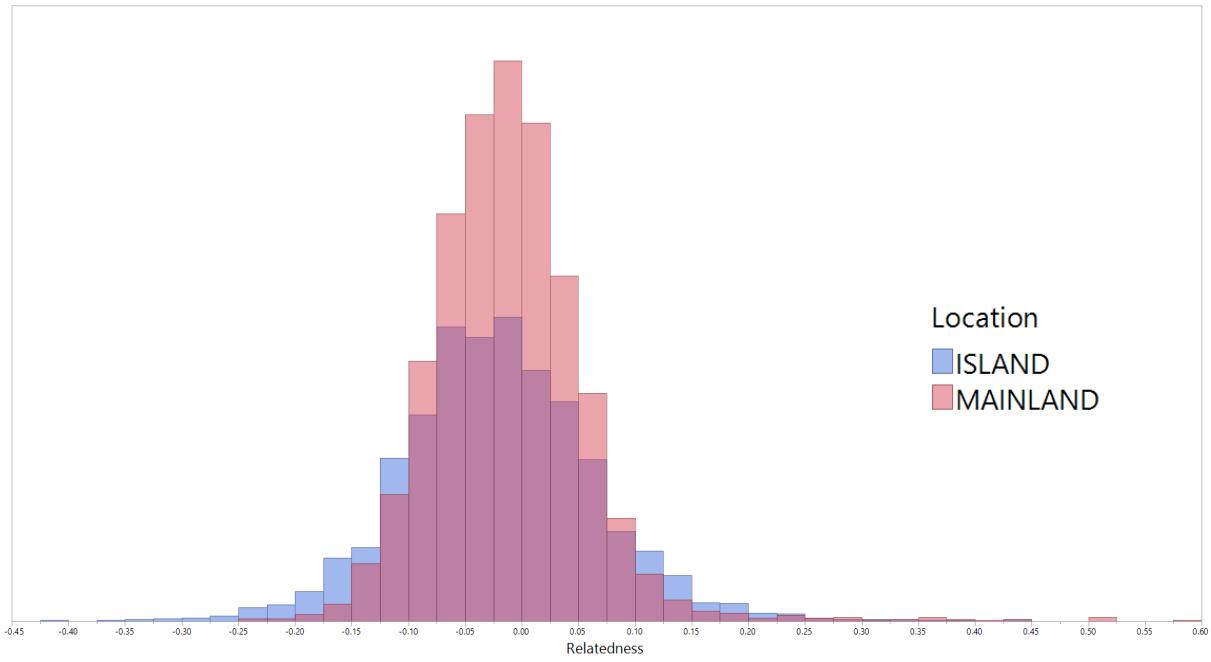
Appendix 2 Figure 2: Plots used to infer the number of ancestral population present across Redlands mainland. This identifies one ancestral population.

3. North Stradbroke Island



Appendix 2 Figure 3: Plots used to infer the number of ancestral population present across Minjerribah / North Stradbroke Island. This identifies one ancestral population.

Relatedness on Redlands mainland and Minjerribah / North Stradbroke Island



Appendix 2 Figure 4: Distribution of the relatedness found for koalas on Redlands mainland and Minjerribah / North Stradbroke Island, showing that both have all relatedness coefficients represented, and neither has higher levels of relatedness.

Appendix 3: Detailed results per locality

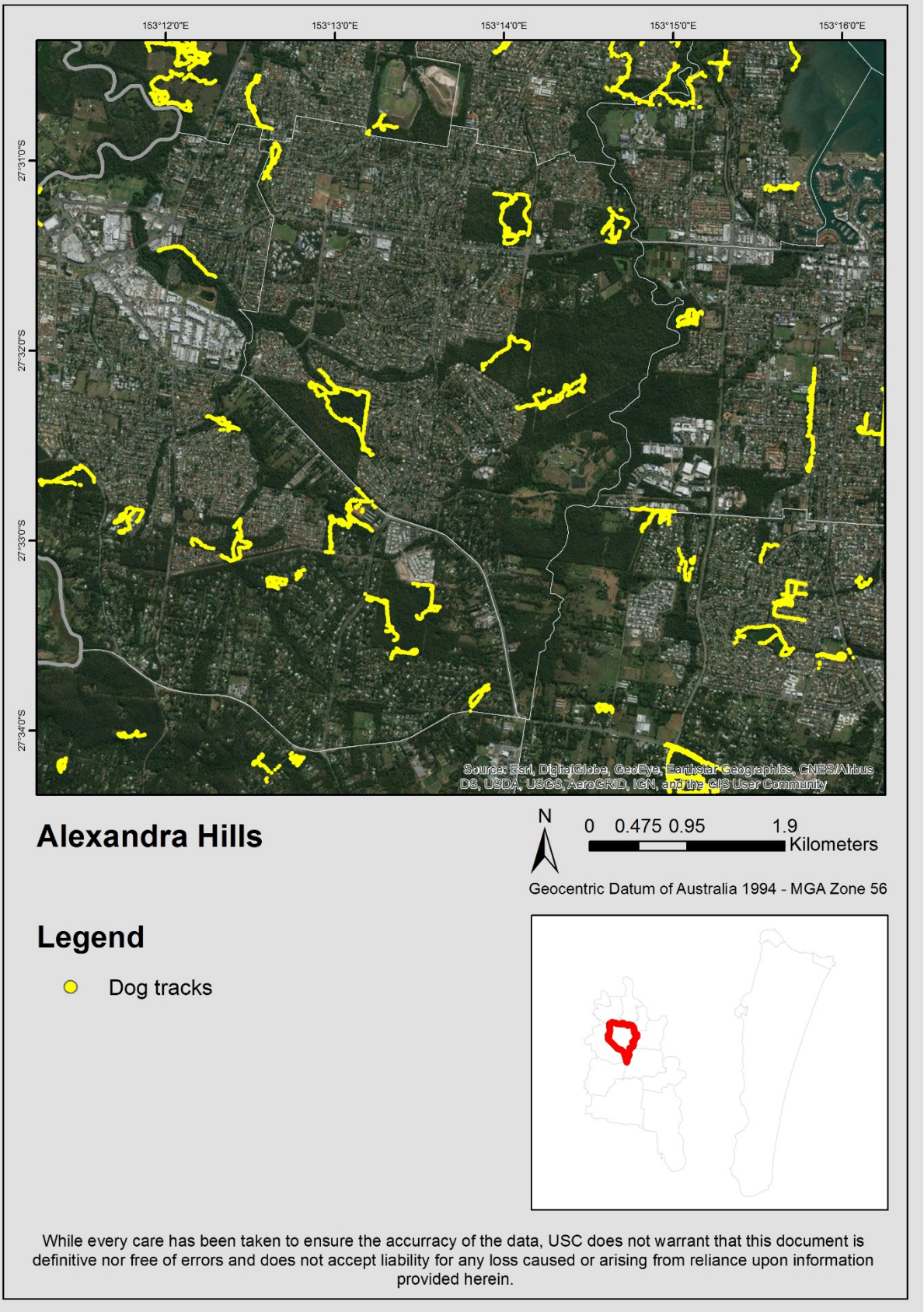
This Appendix details, per locality, results that have been presented at a broader scale in the main part of the report. The surveys were not designed to establish or compare occupation rates (or percent of sites used), as the surveys were not random, and the survey effort was not standardised, nor equal at each location or per locality. While looking at the following table 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.

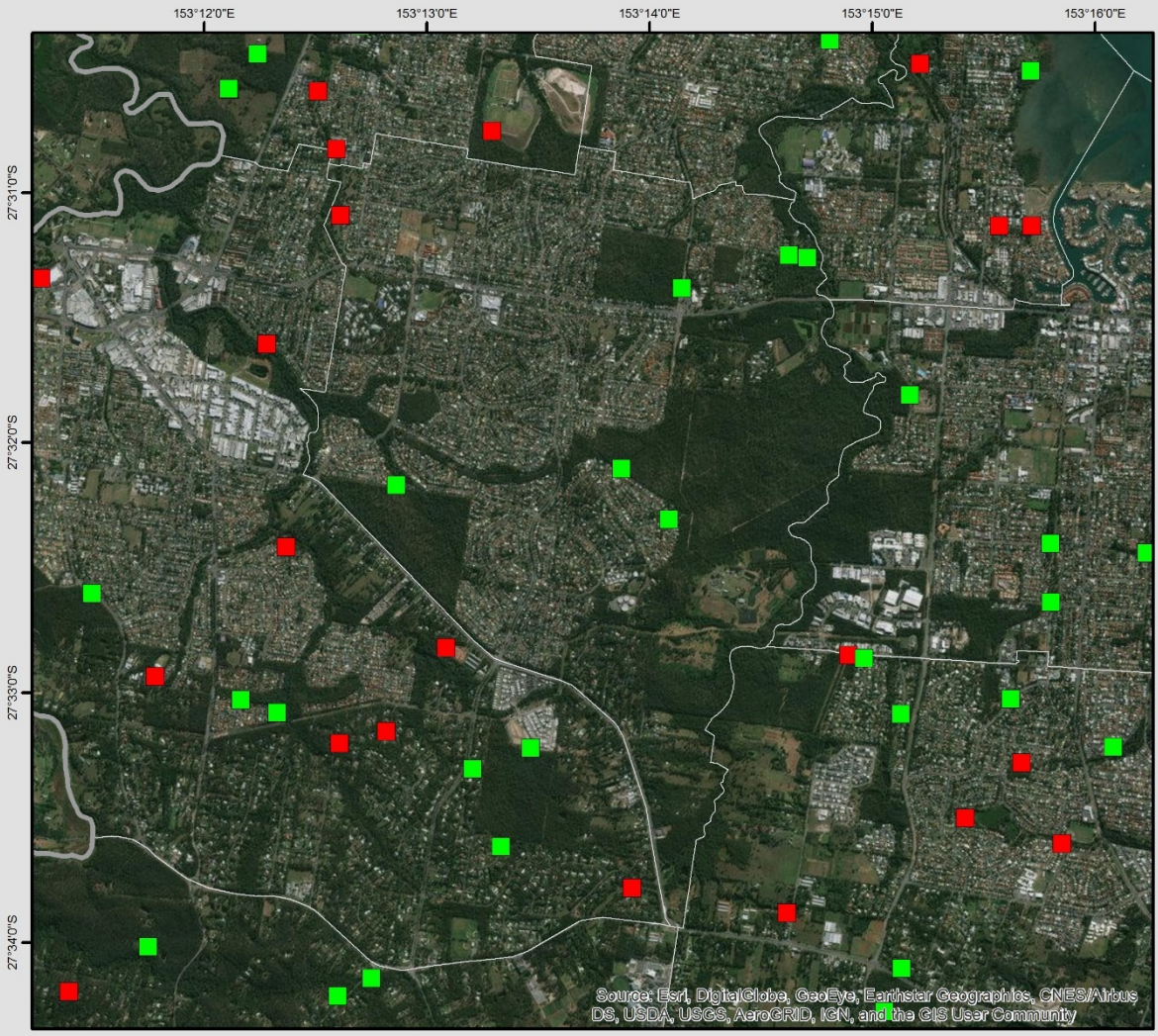
The number of surveys and the maps of their locations encompass both dog surveys and opportunistic koala spotting (survey effort however only accounts for dog searches). Presence of koala scats means the location had been used by koalas, however, absence of scats does not mean the location had not been 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. Relatedness maps give the relatedness of individuals found within a locality (at the exclusion of relatedness to those outside of the locality). Chlamydia maps present qualitative and quantitative results: qualitative = detection of any Chlamydial DNA (even if only one copy was present) and quantitative = detection of Chlamydia is above threshold. 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.

	Number of surveys	Positive surveys	Number of koala sighted	Joeys observed	Number individuals genotyped	Males (genetics)	Females (genetics)	Chlamydia detected	Chlamydia above threshold	Average IR (SD)
Alexandra Hills	7	6	5	No	5	3	2	2	2	0.21 (0.22)
Amity	33	33	36	Yes	26	12	14	9	1	0.39 (0.17)
Birkdale	25	15	3	No	16	5	11	13	5	0.33 (0.22)
Capalaba	15	6	1	Yes	6	2	4	4	4	0.29 (0.25)
Cleveland	21	21	11	Yes	26	17	9	16	5	0.33 (0.22)
Dunwich	14	14	9	Yes	7	4	3	2	0	0.41 (0.20)
Mount Cotton	28	17	0	NA	8	5	3	4	3	0.21 (0.23)
Ormiston	11	8	3	No	7	2	5	4	0	0.41 (0.22)
Point Lookout	13	12	8	Yes	13	9	4	0	0	0.39 (0.10)
Redland Bay	28	10	0	NA	3	1	2	1	1	0.41 (0.23)
Sheldon	24	17	1	No	5	2	3	3	3	0.40 (0.24)
Thorneside	8	0	0	NA	NA	NA	NA	NA	NA	NA
Thornlands	22	11	1	No	9	3	6	5	4	0.26 (0.23)
Victoria Point	18	5	0	NA	2	0	2	1	0	0.35 (0.14)
Wellington Point	18	12	1	No	12	9	3	3	1	0.44 (0.22)

Alexandra Hill


153°12'0"E
153°13'0"E
153°14'0"E
153°15'0"E
153°16'0"E
27°31'0"S
27°32'0"S
27°33'0"S
27°34'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Alexandra Hills
N
0 0.475 0.95 1.9
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
While every care has been taken to ensure the accuracy 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.



Alexandra Hills

N

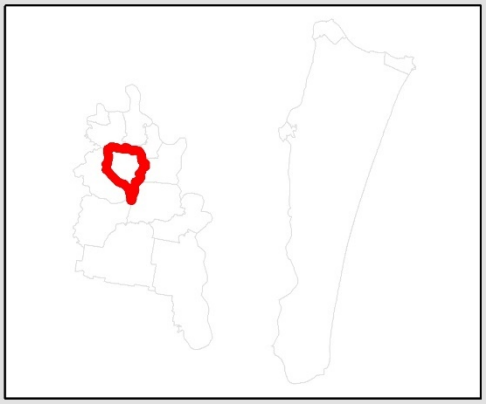
0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

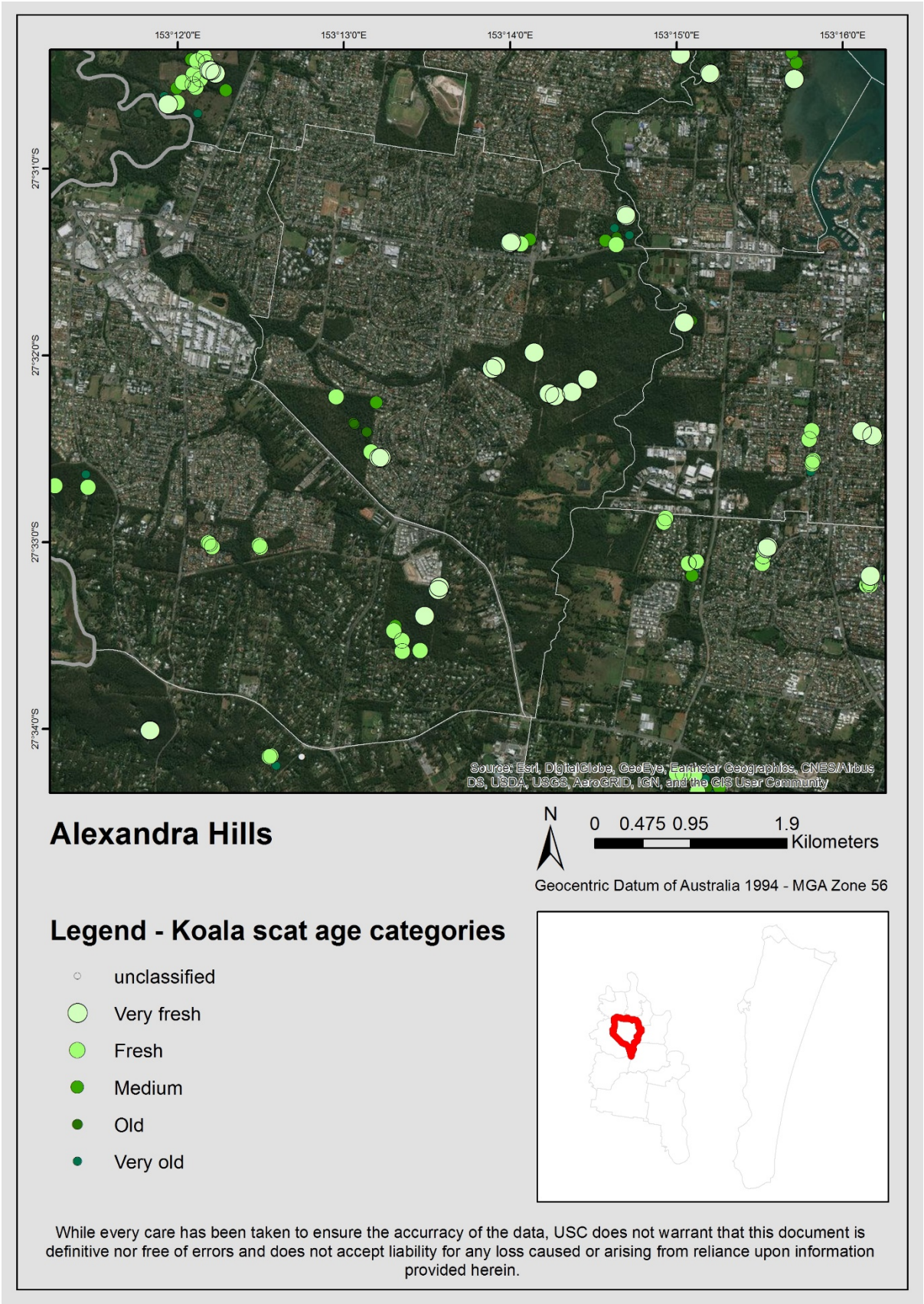
Legend

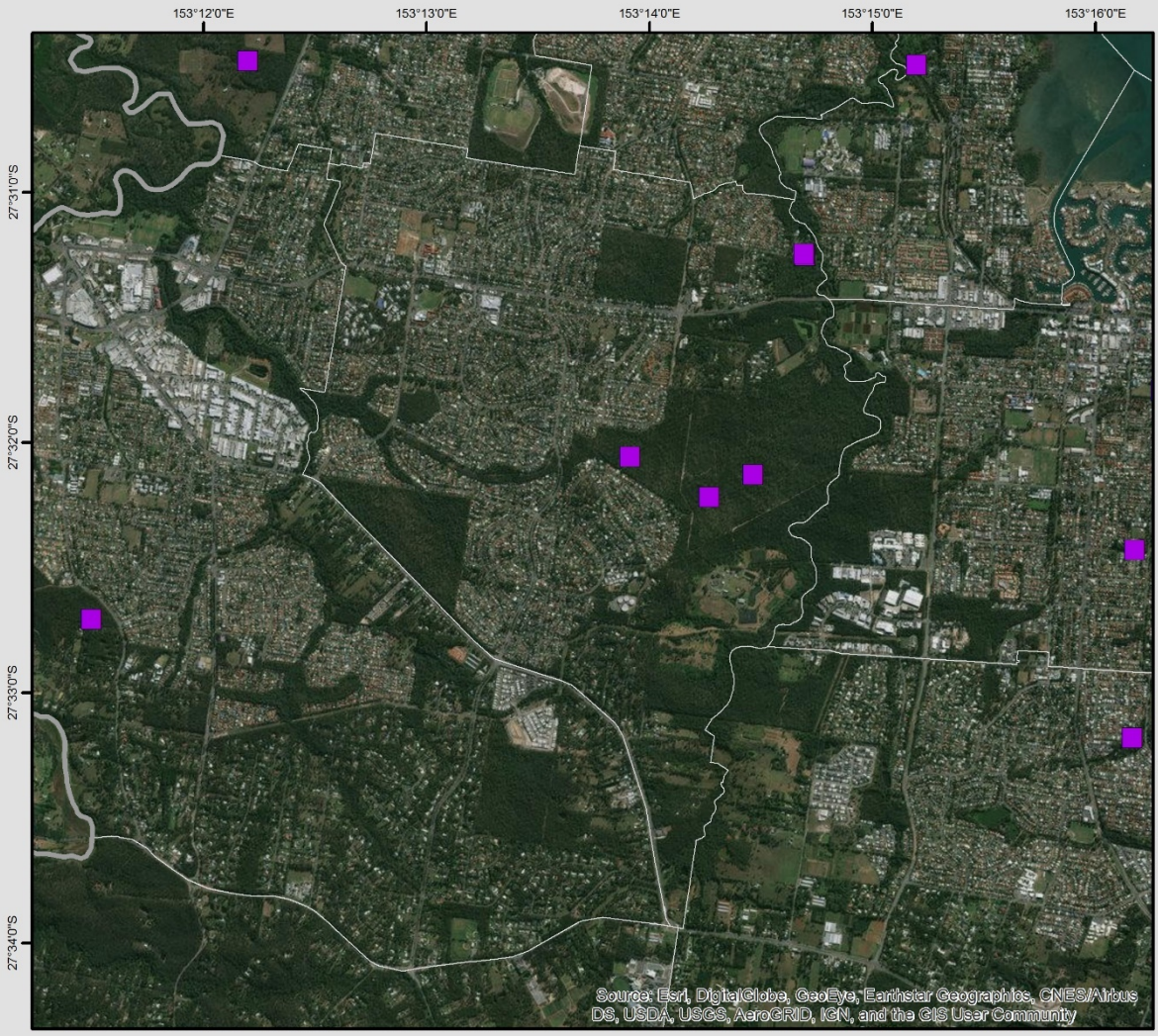
Koala scat

- Presence
- Absence



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.


153°12'0"E
153°13'0"E
153°14'0"E
153°15'0"E
153°16'0"E
27°31'0"S
27°32'0"S
27°33'0"S
27°34'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Alexandra Hills
N
0 0.475 0.95 1.9
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend - Koala scat age categories
unclassified
Very fresh
Fresh
Medium
Old
Very old
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.



Alexandra Hills

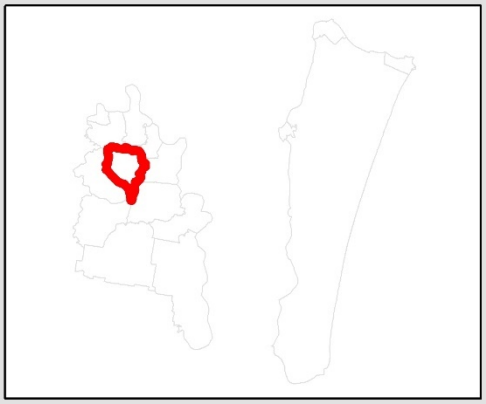
N

0 0.475 0.95 1.9
Kilometers

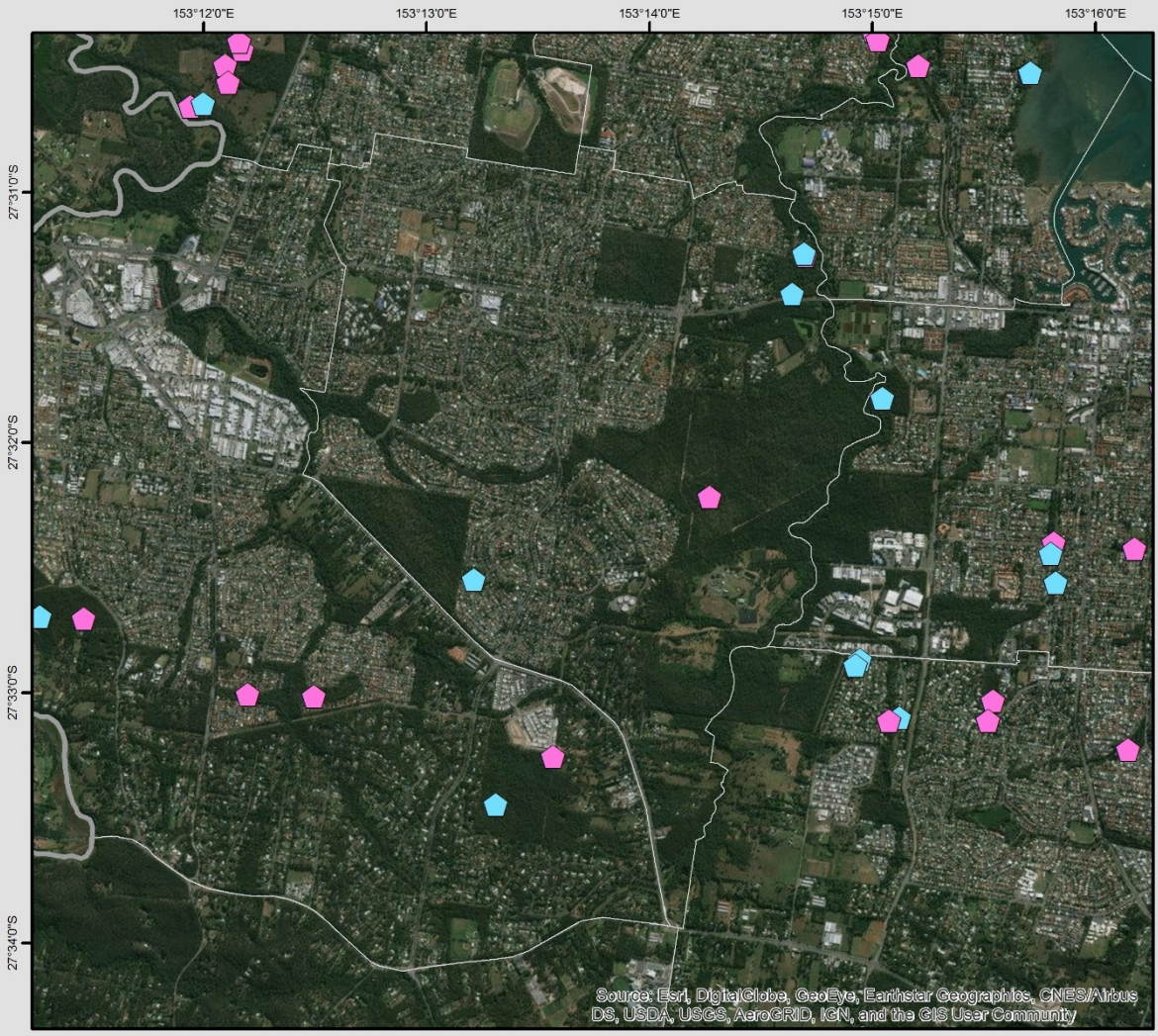
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Alexandra Hills

N

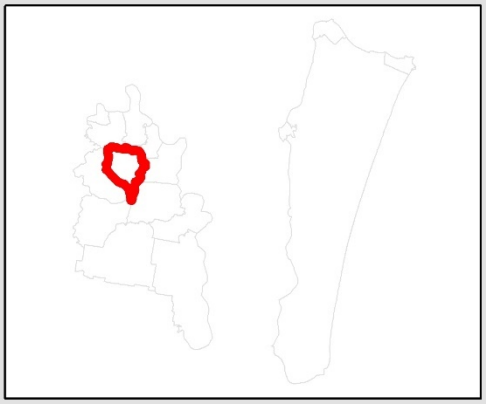
0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

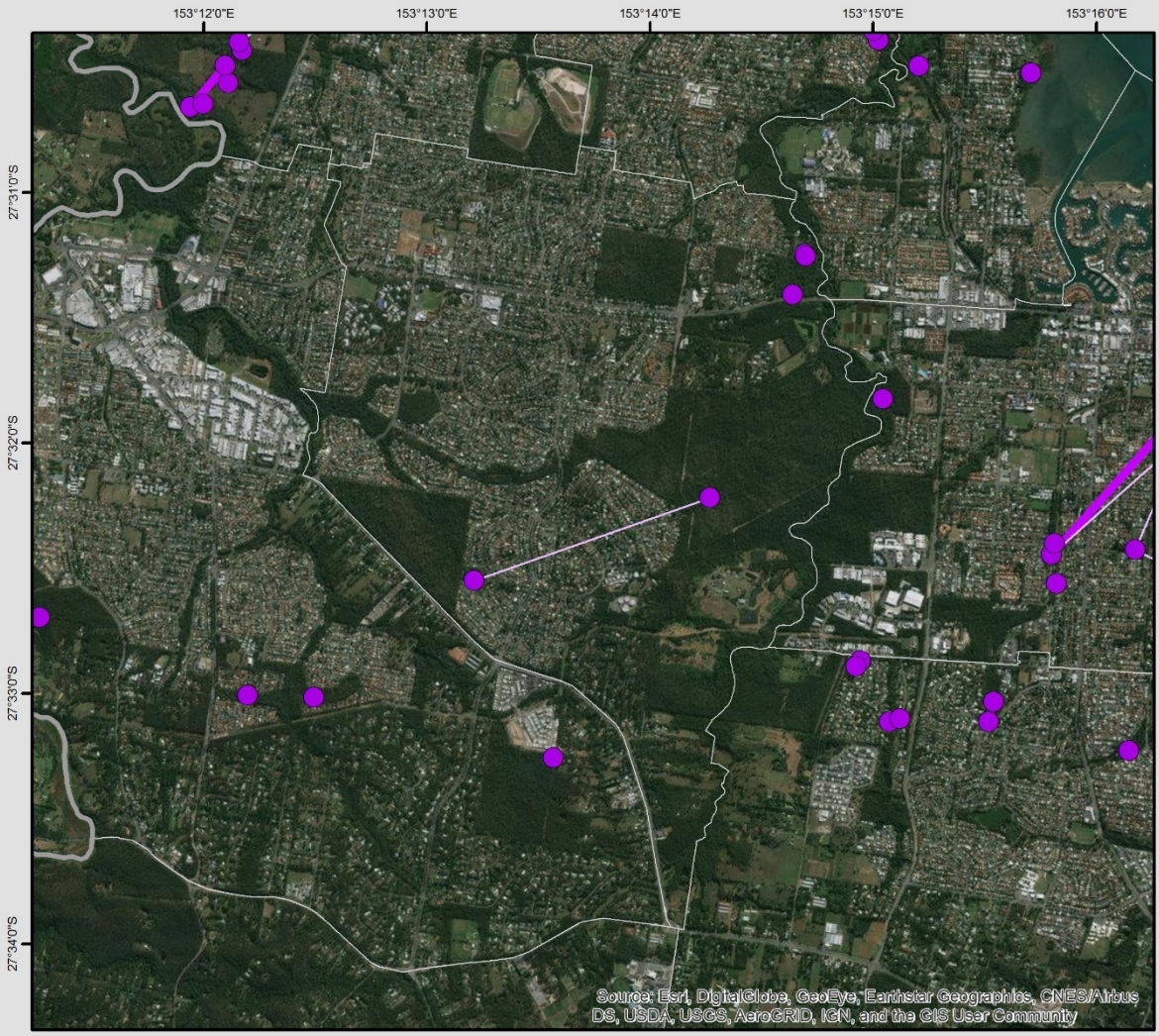
Legend

Koala sex (genetics)

- Female
- Male



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.



Alexandra Hills

N

0 0.475 0.95 1.9
Kilometers

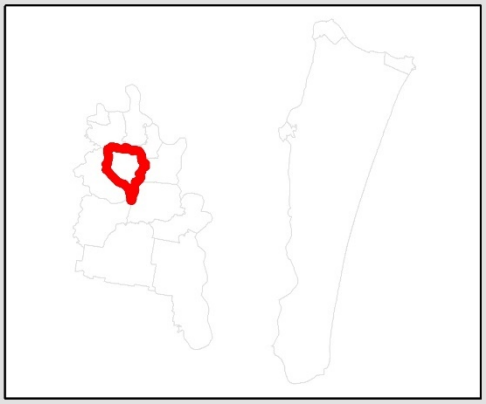
Geocentric Datum of Australia 1994 - MGA Zone 56

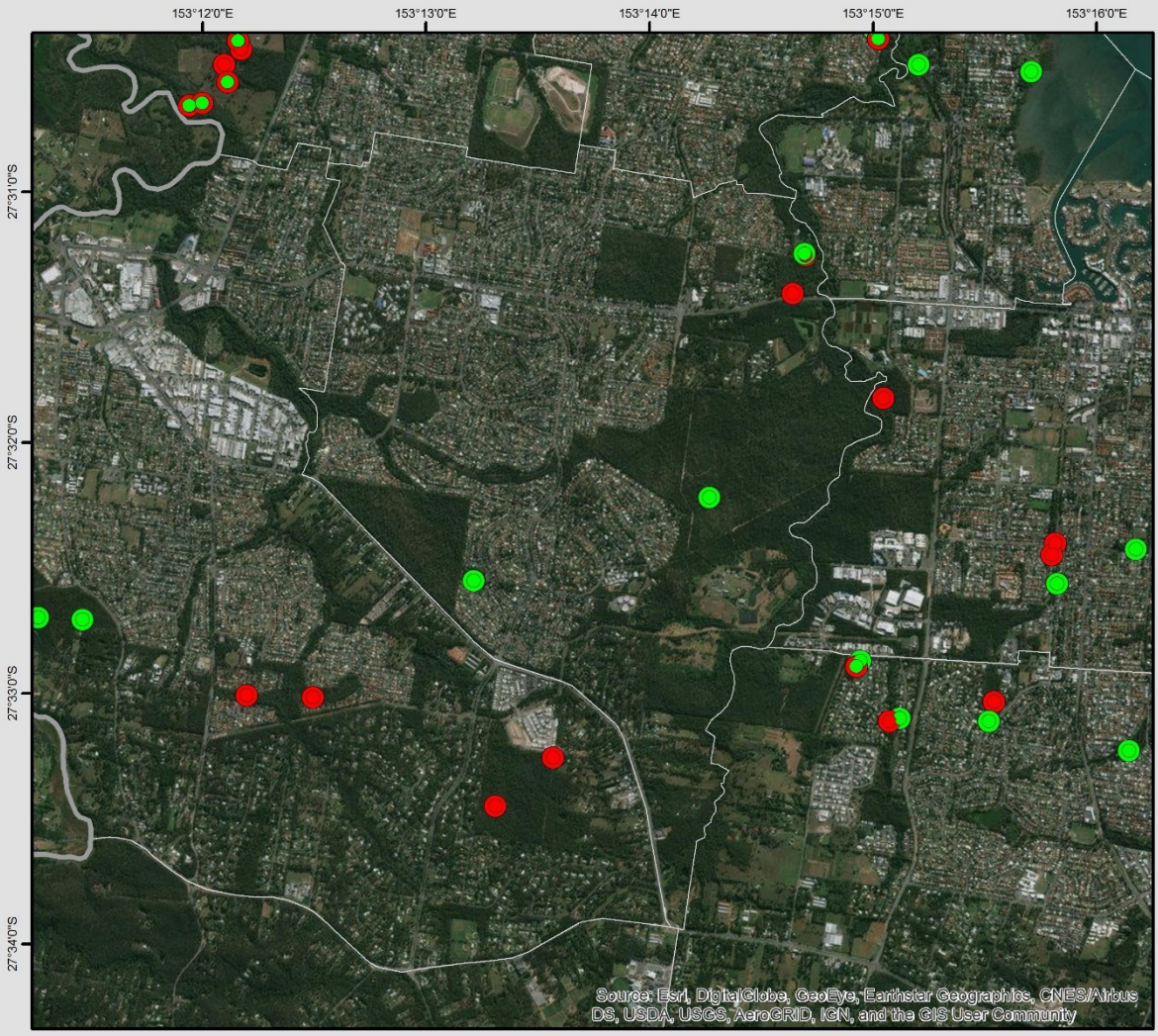
Legend

- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)





Alexandra Hills

N

0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

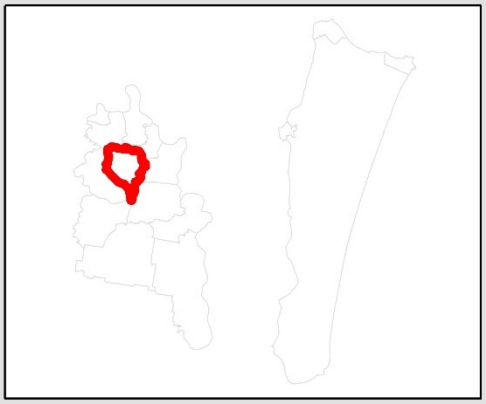
Legend - Chlamydia results

Qualitative

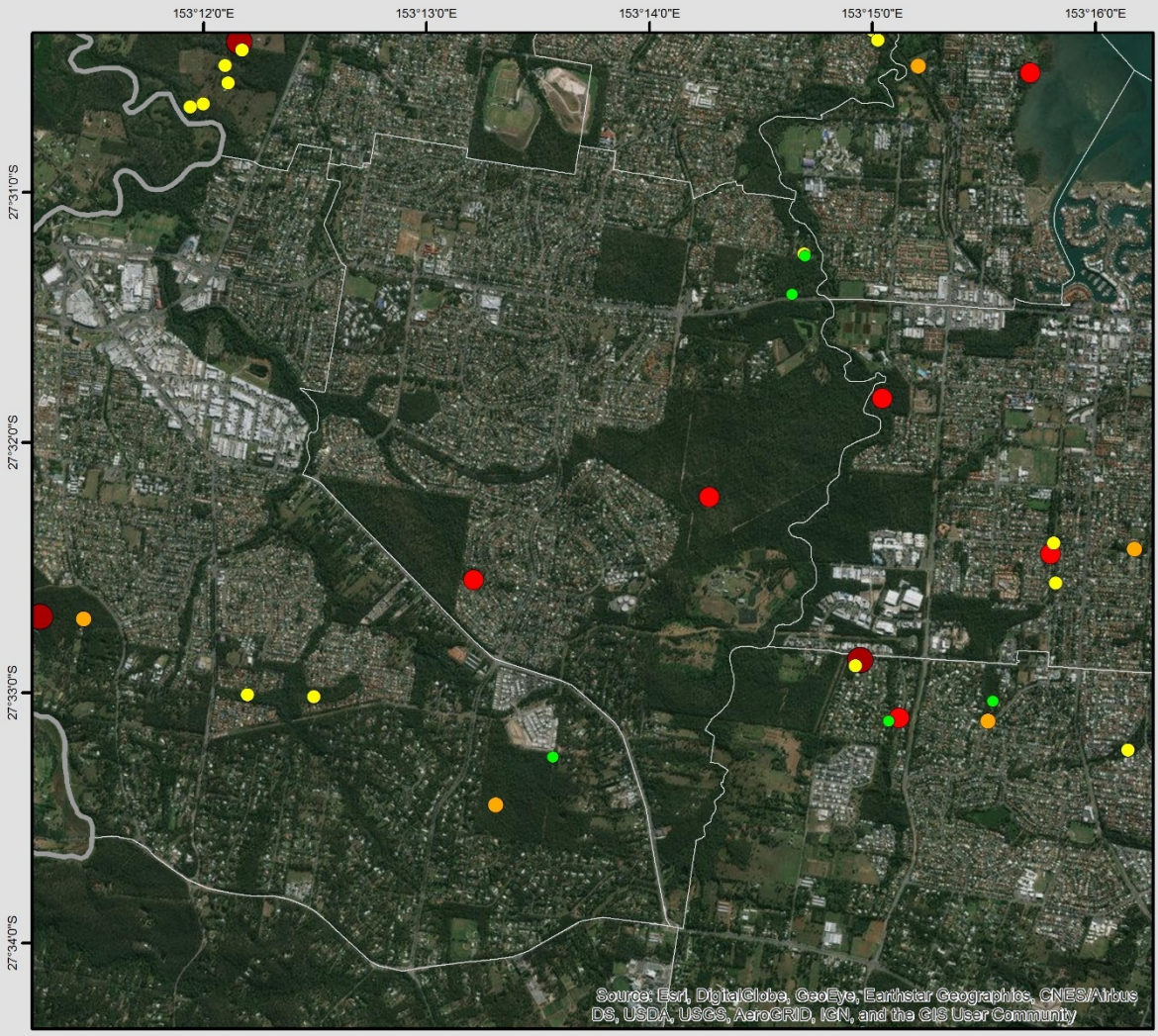
- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold



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.



Alexandra Hills

N

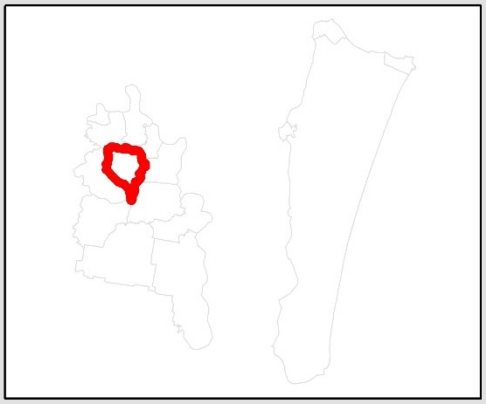
0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

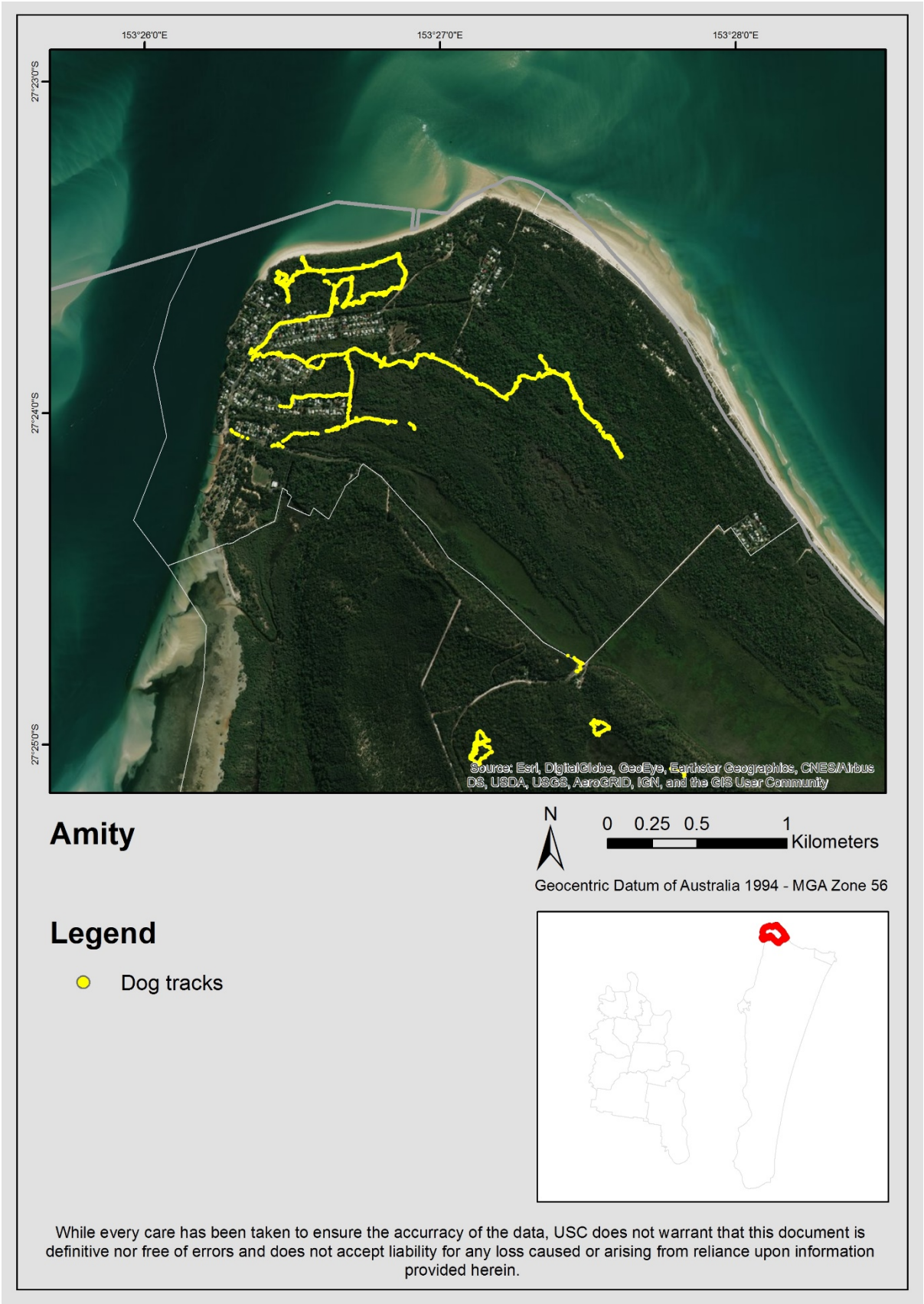
Internal relatedness

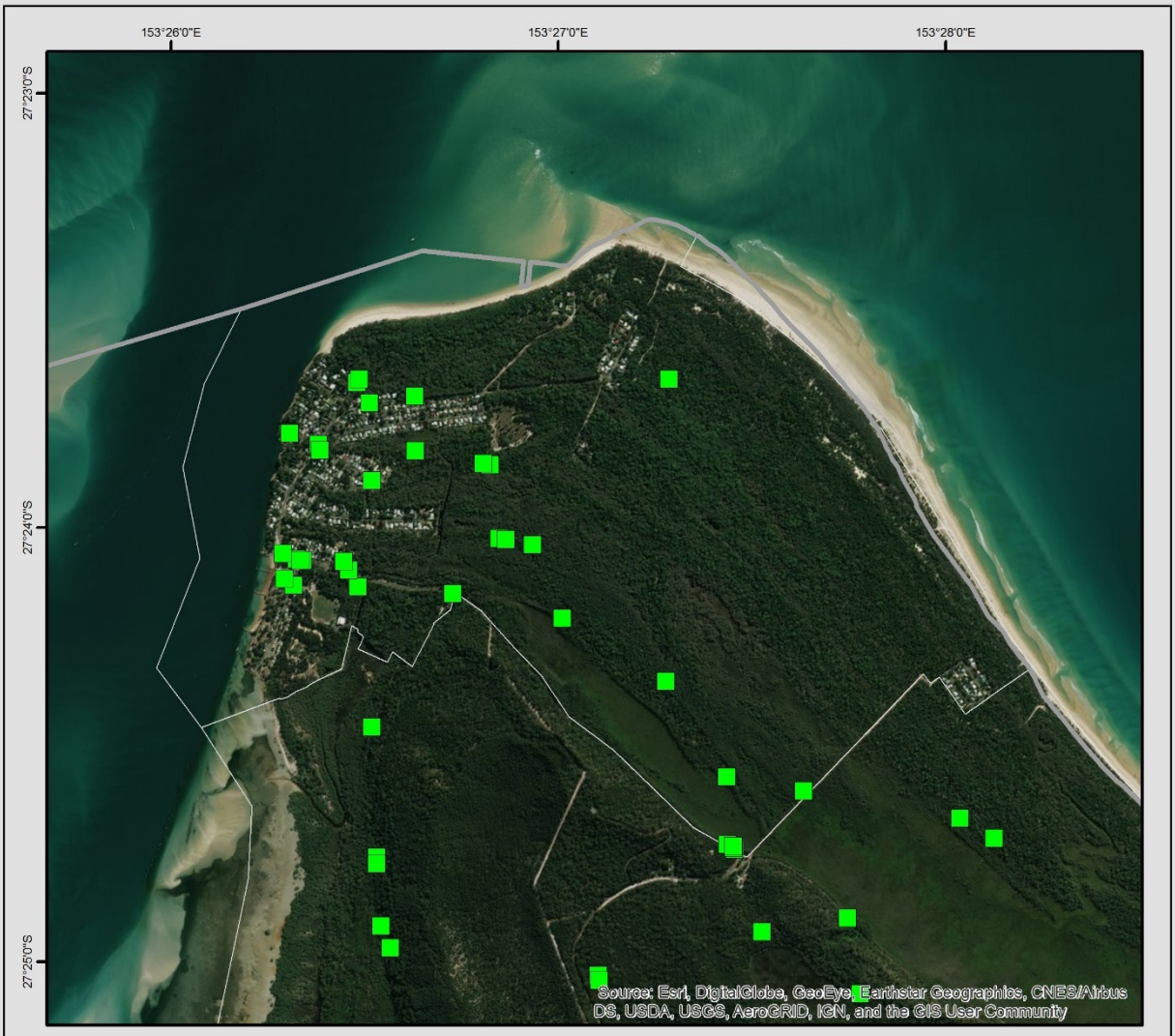
- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87




DETECTION DOGS
FOR CONSERVATION
Sniffing out solutions usc.edu.au/ddc
University of the Sunshine Coast

Amity


153°26'0"E
153°27'0"E
153°28'0"E
27°23'0"S
27°24'0"S
27°25'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Amity
N
0 0.25 0.5 1
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
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.



Amity

N

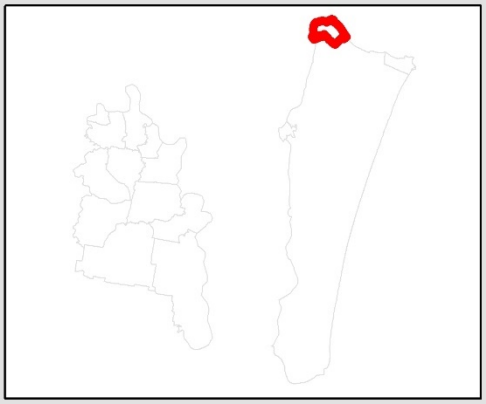
0 0.25 0.5 1
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

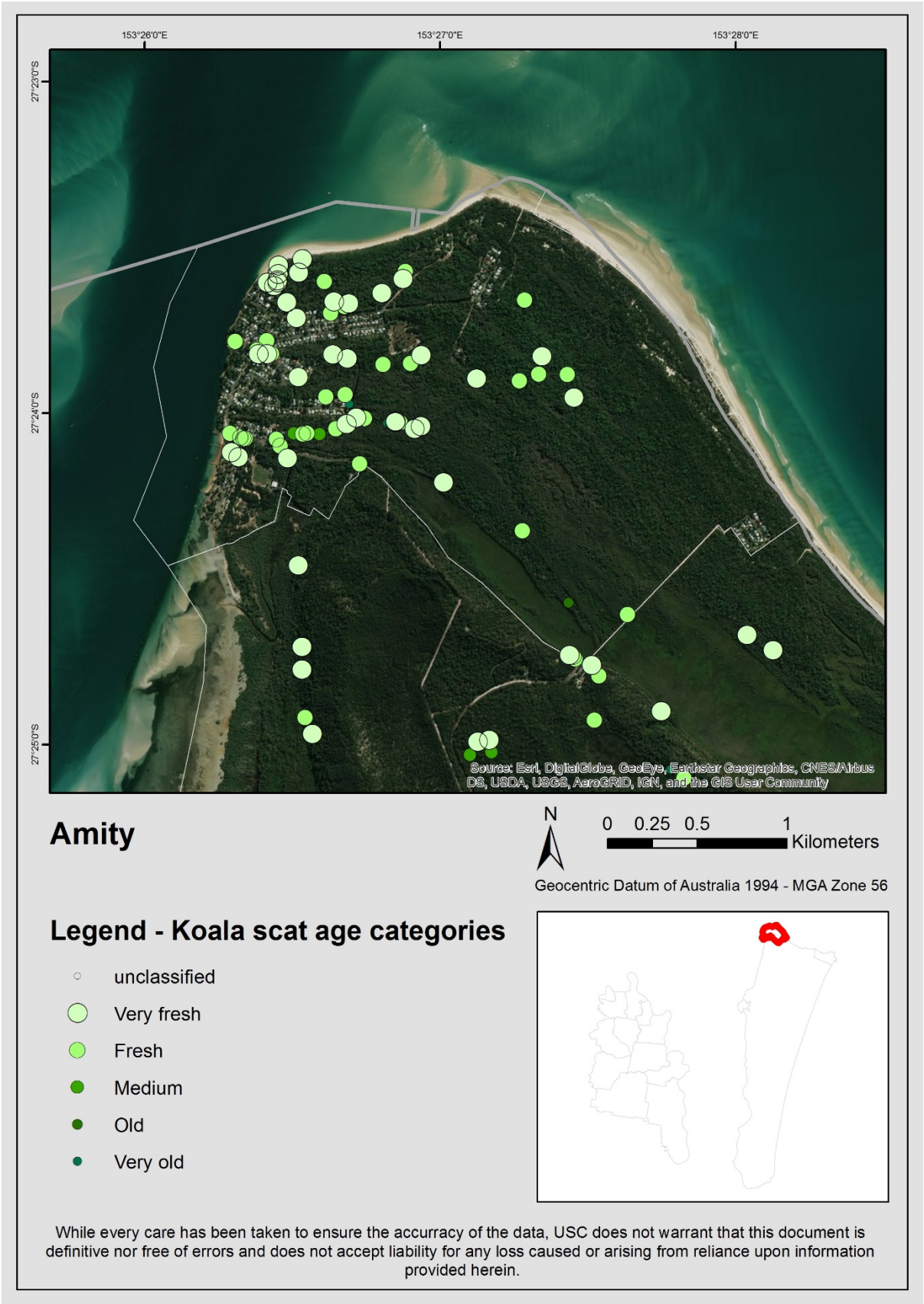
Legend

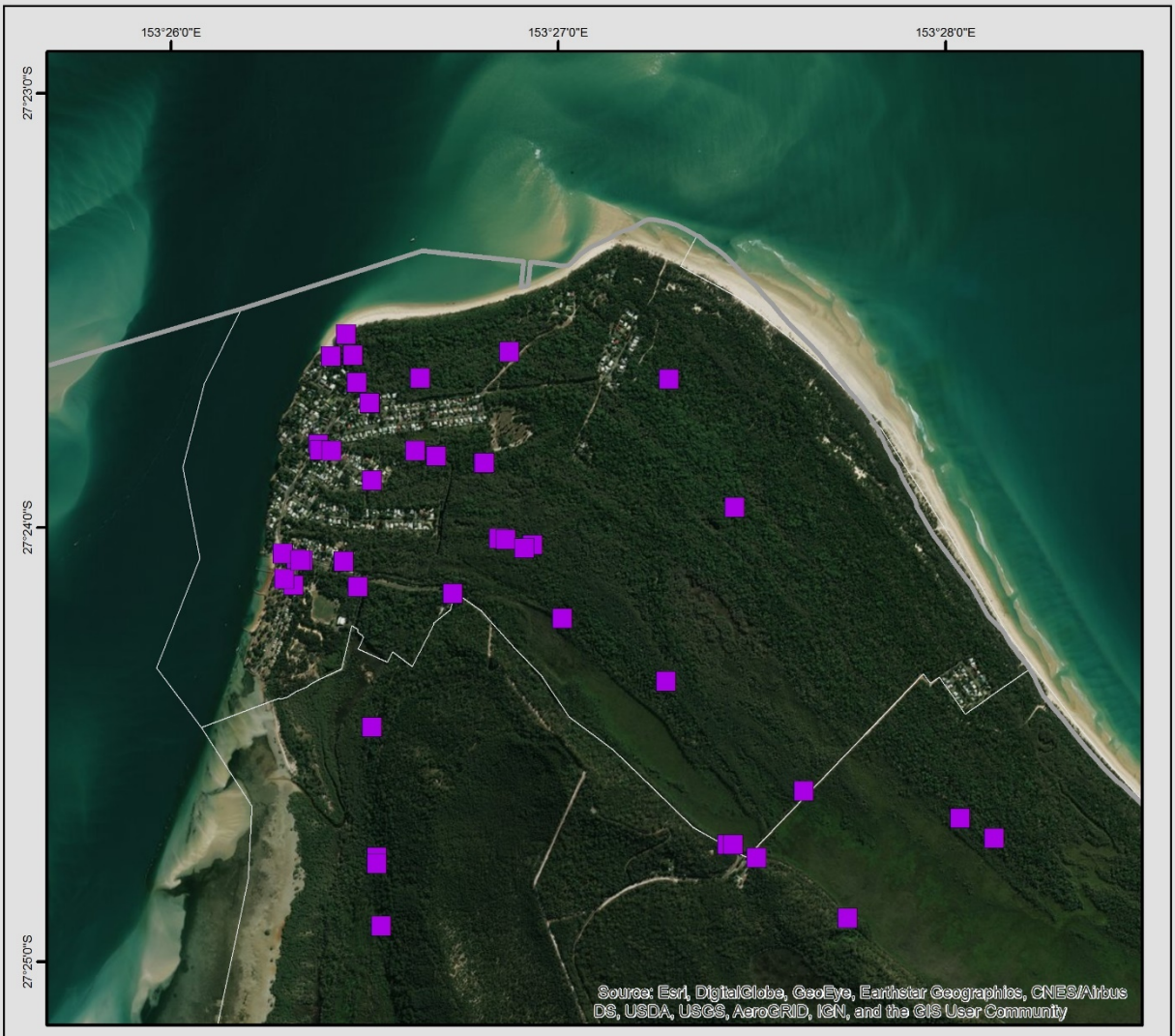
Koala scat

- Presence
- Absence



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.


153°26'0"E
153°27'0"E
153°28'0"E
27°23'0"S
27°24'0"S
27°25'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Amity
N
0 0.25 0.5 1
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend - Koala scat age categories
unclassified
Very fresh
Fresh
Medium
Old
Very old
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.



Amity

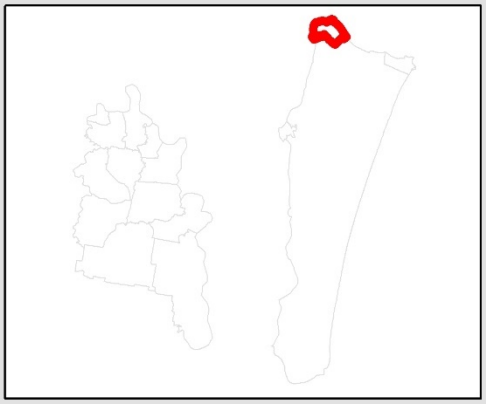
N

0 0.25 0.5 1
Kilometers

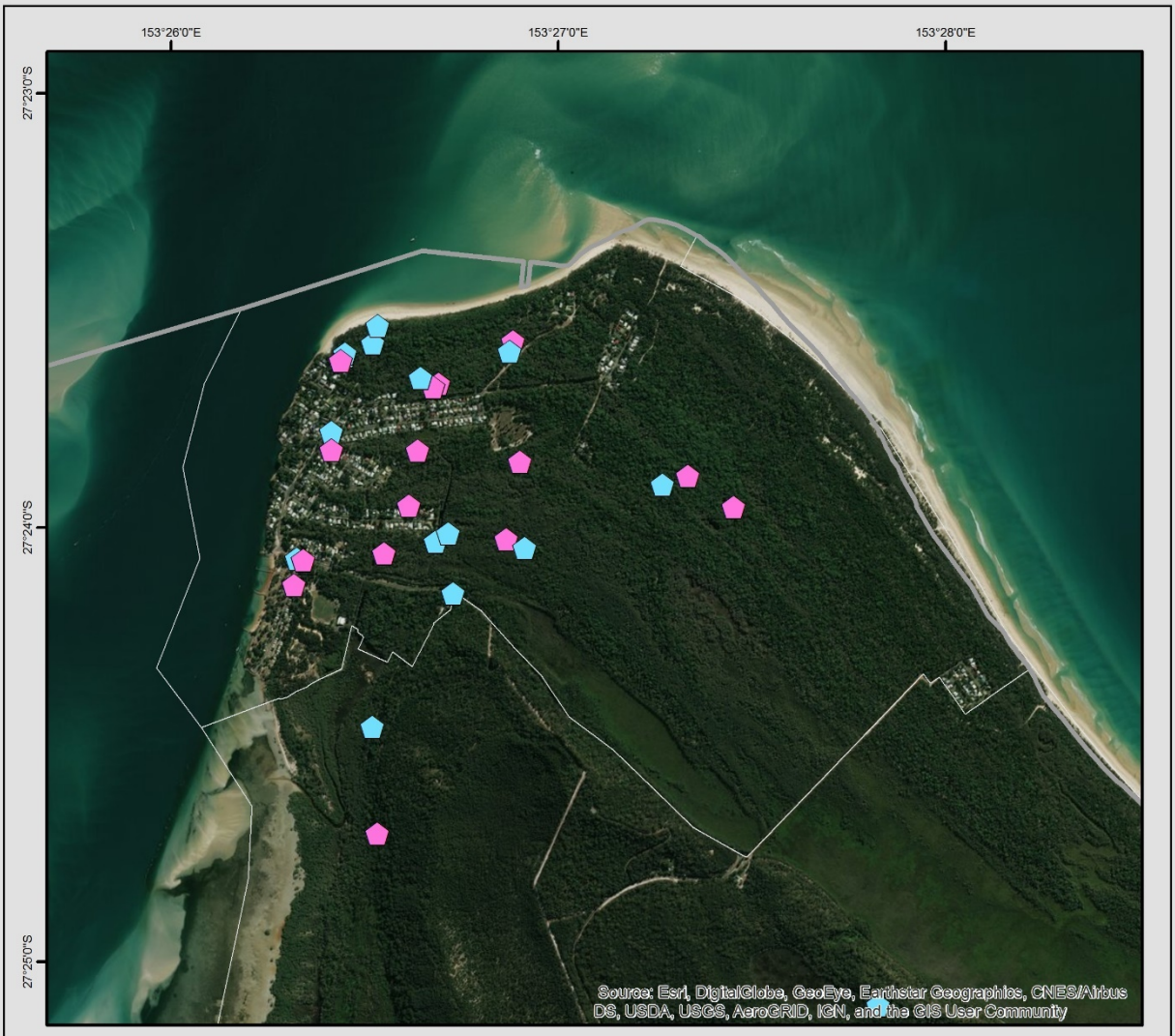
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Amity

N

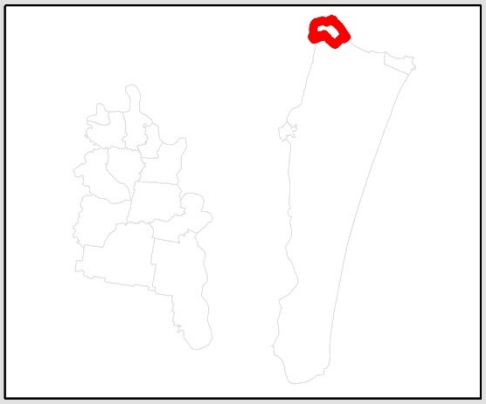
0 0.25 0.5 1
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

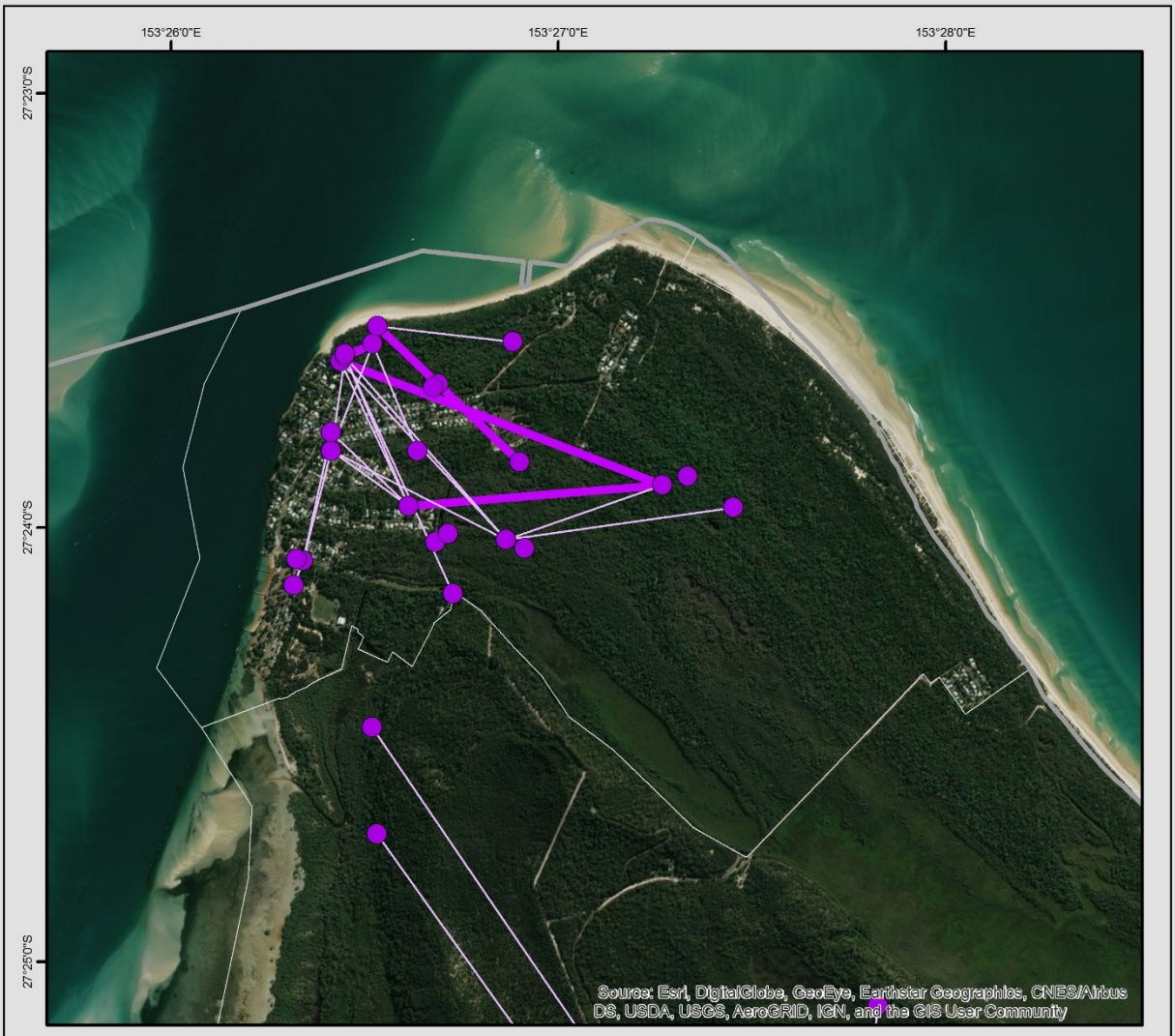
Legend

Koala sex (genetics)

- Female
- Male



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.



Amity

N

0 0.25 0.5 1 Kilometers

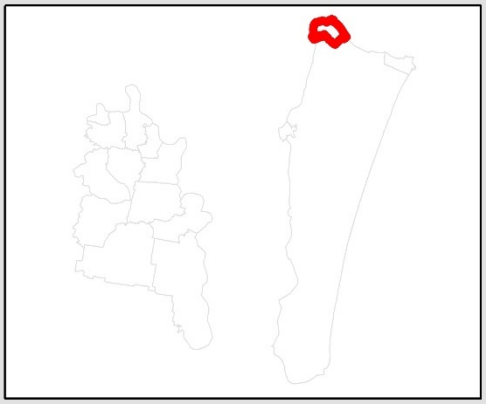
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

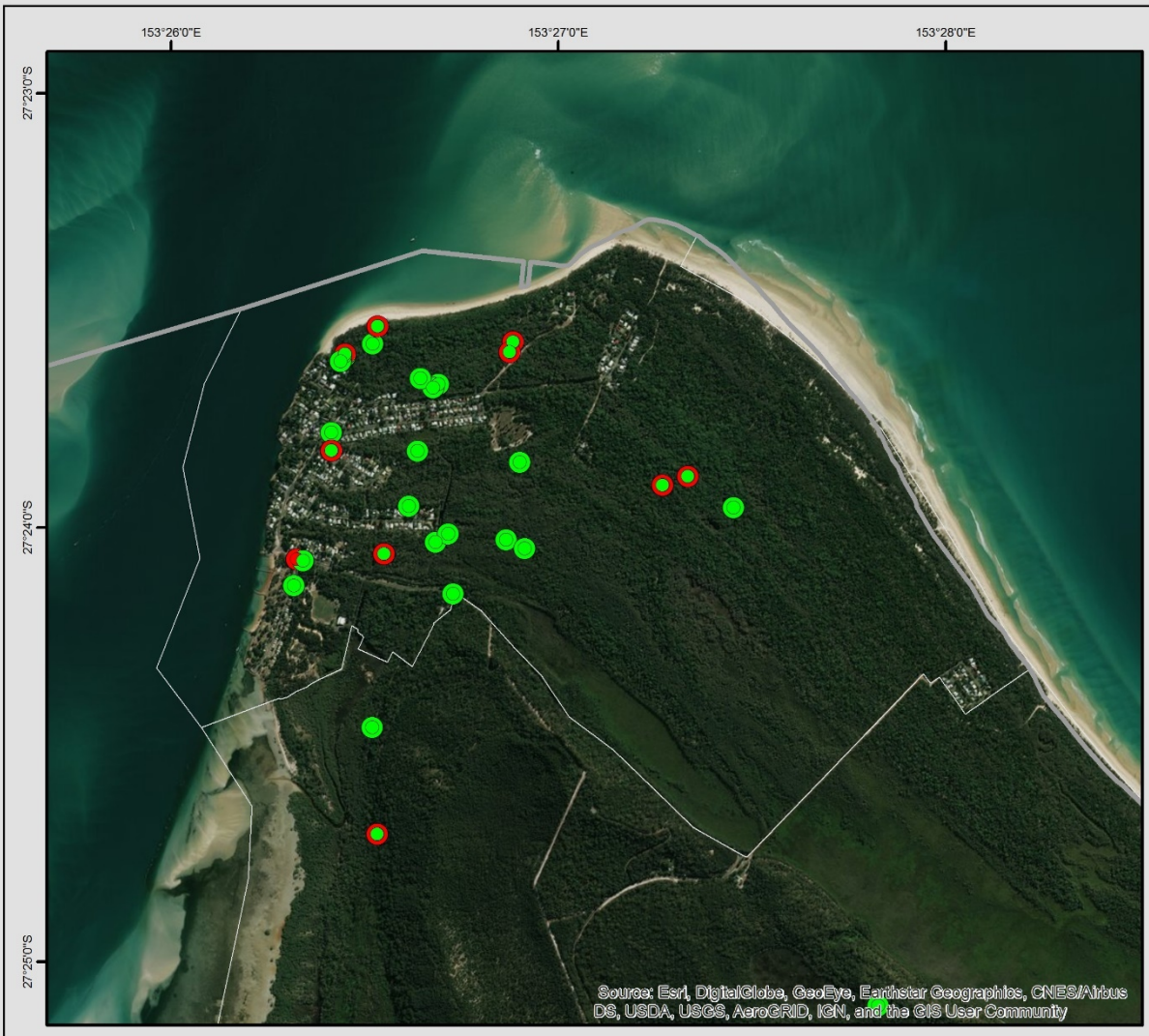
- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)



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.



Amity

N

0 0.25 0.5 1
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

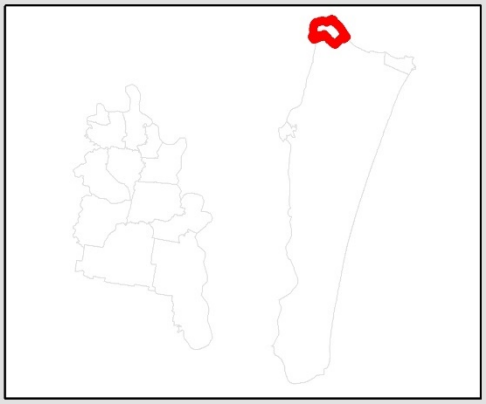
Legend - Chlamydia results

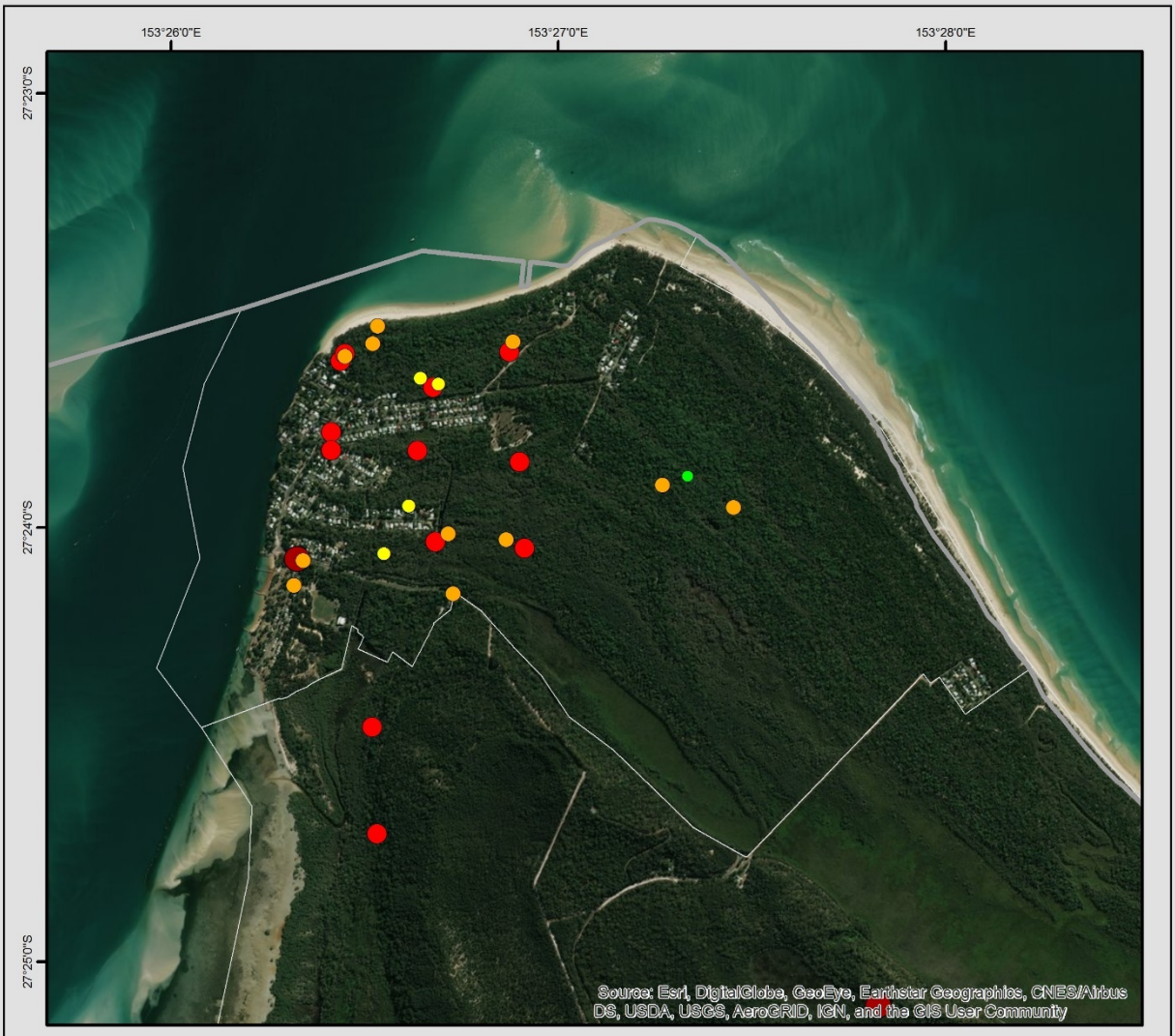
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold





Amity

N

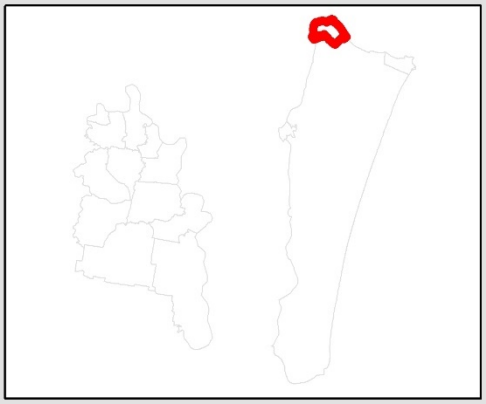
0 0.25 0.5 1
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

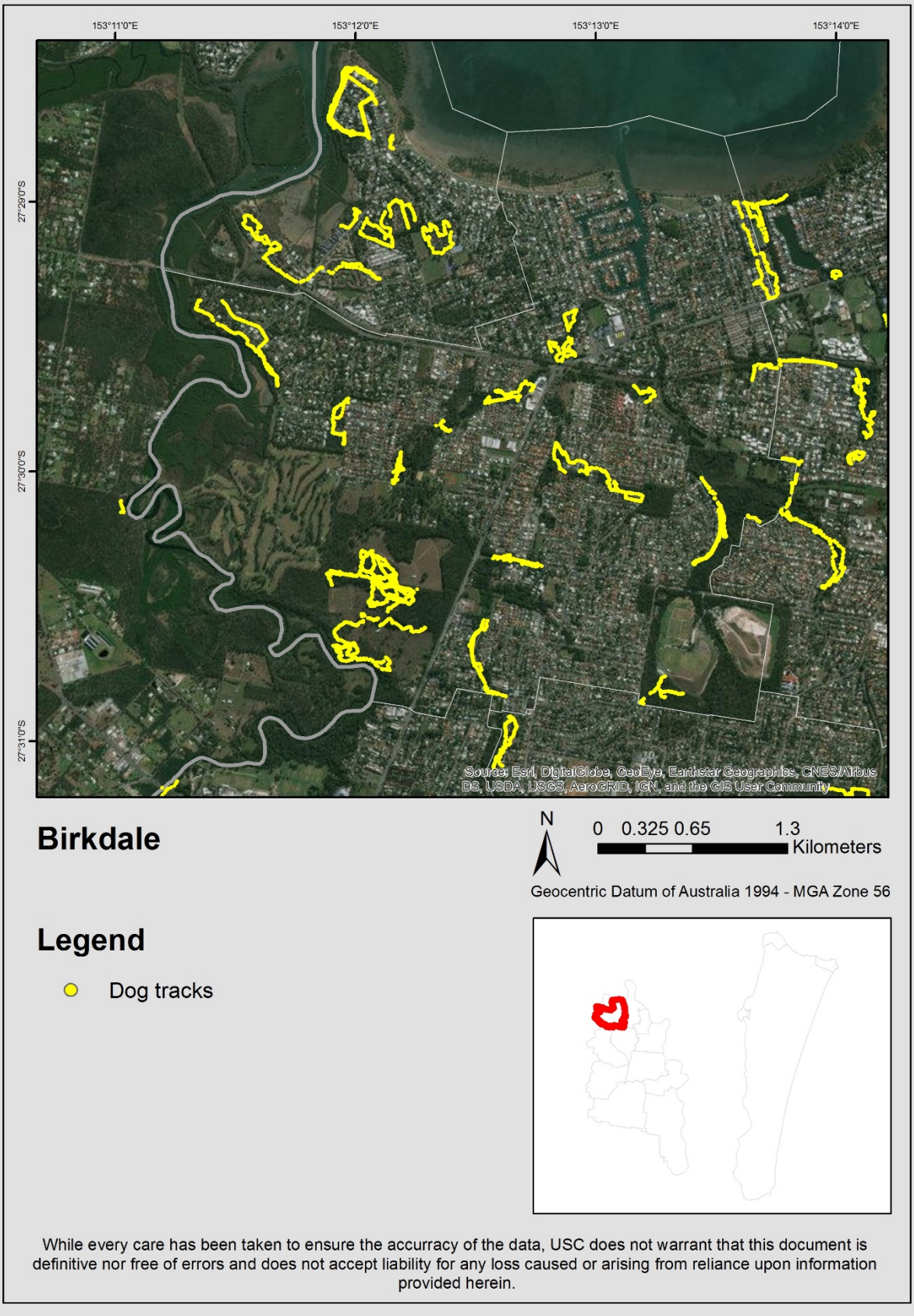
Legend

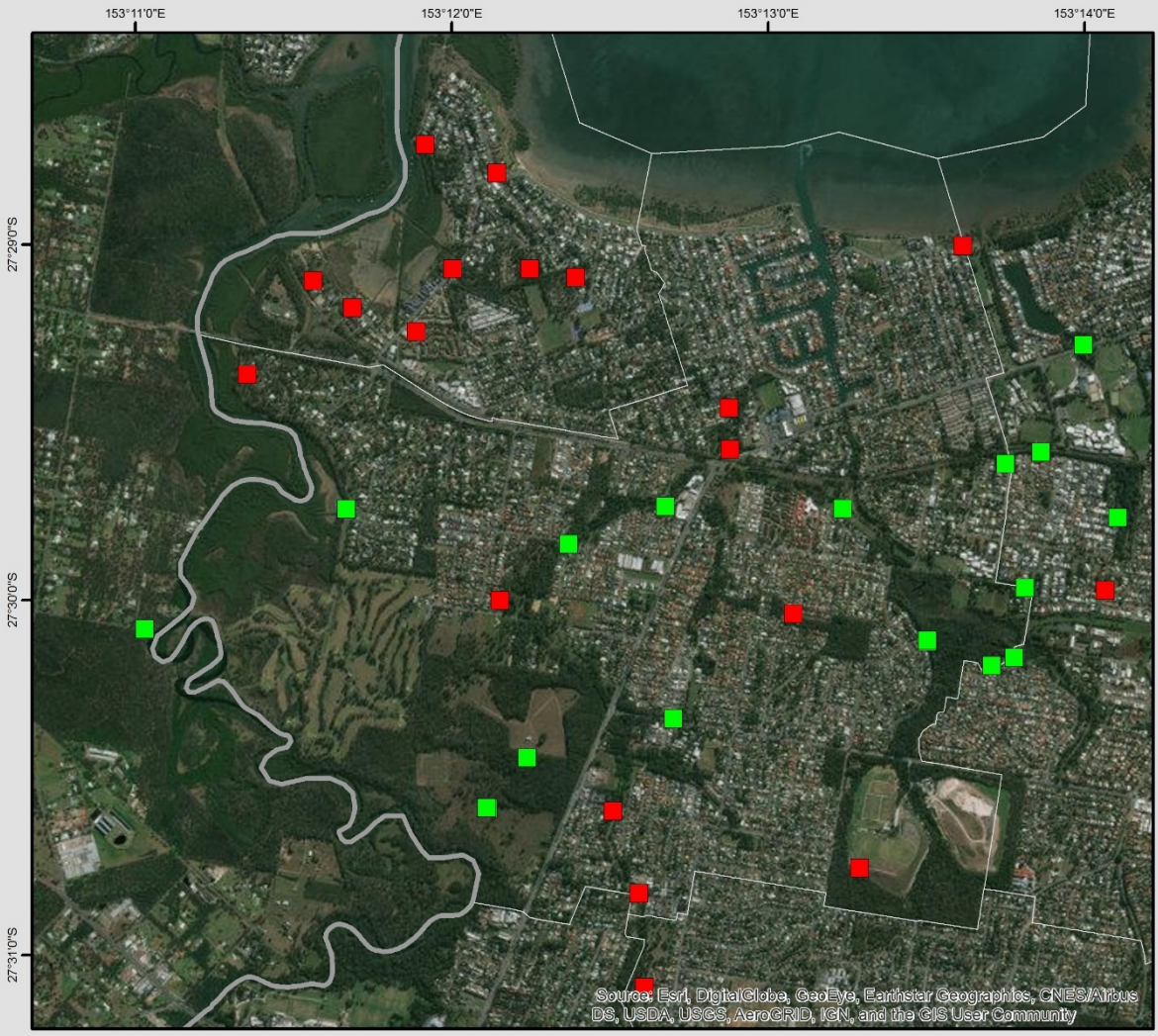
Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Birkdale


153°11'0"E
153°12'0"E
153°13'0"E
153°14'0"E
27°29'0"S
27°30'0"S
27°31'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Birkdale
N
0 0.325 0.65 1.3
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
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.



Birkdale

N

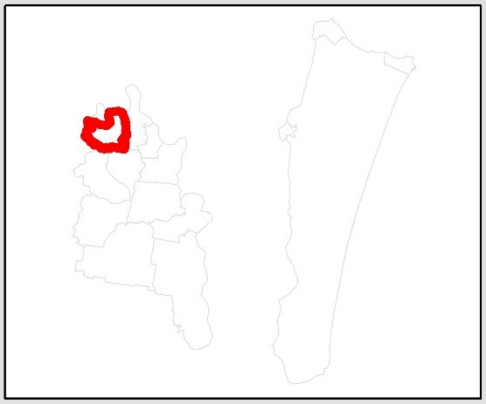
0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

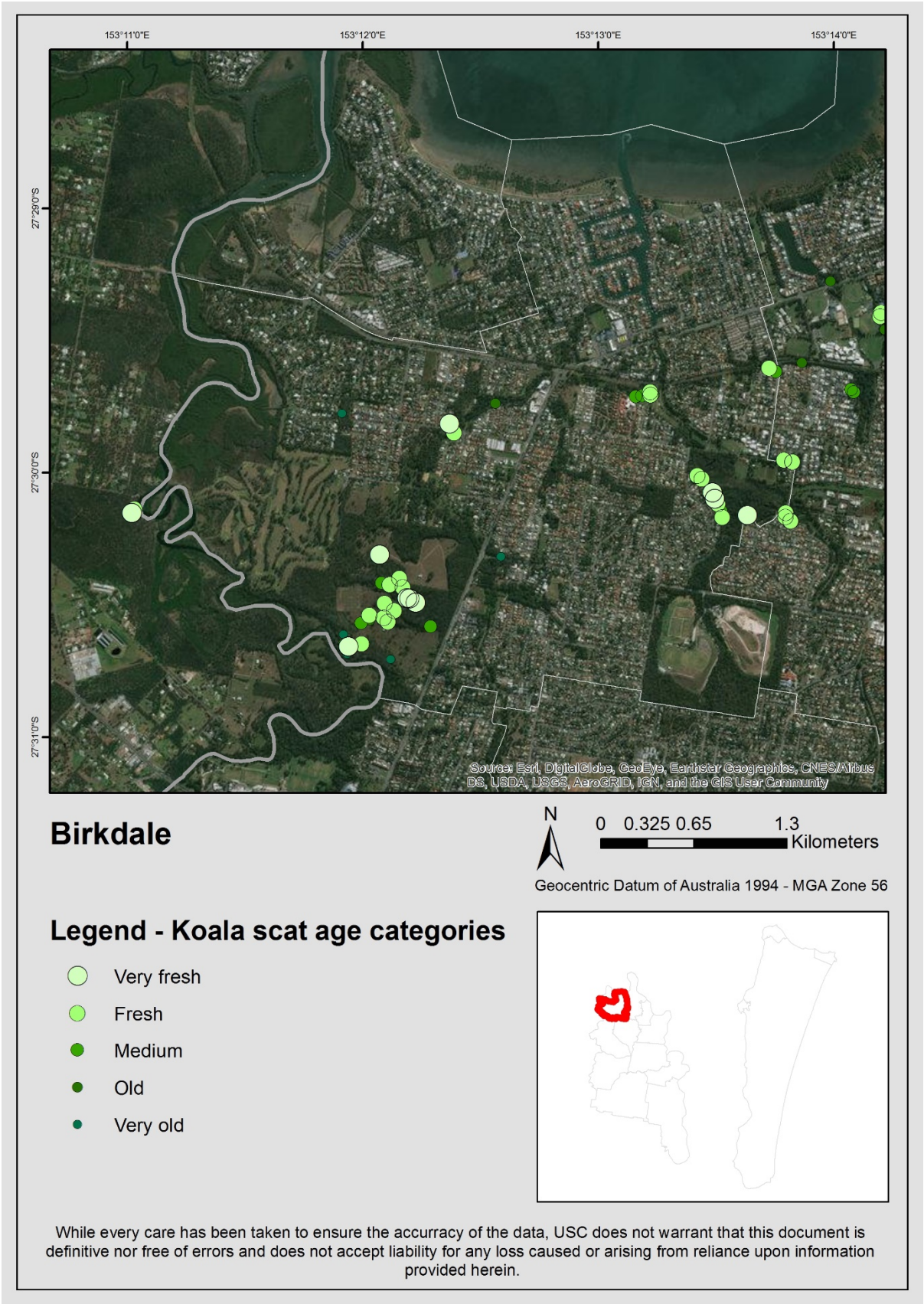
Legend

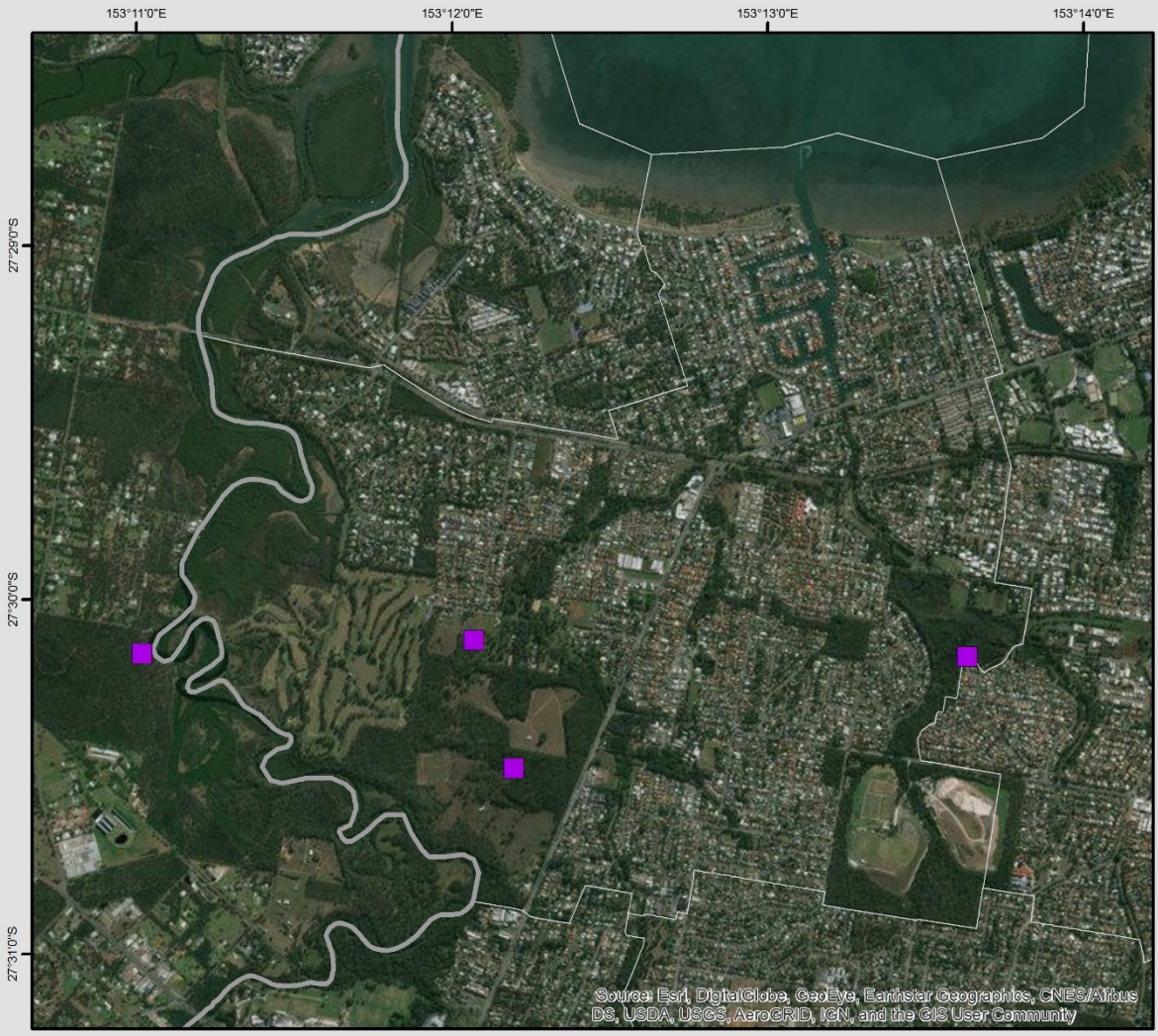
Koala scat

- Presence
- Absence



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.


153°11'0"E
153°12'0"E
153°13'0"E
153°14'0"E
27°29'0"S
27°30'0"S
27°31'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Birkdale
N
0 0.325 0.65 1.3
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend - Koala scat age categories
Very fresh
Fresh
Medium
Old
Very old
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.



Birkdale

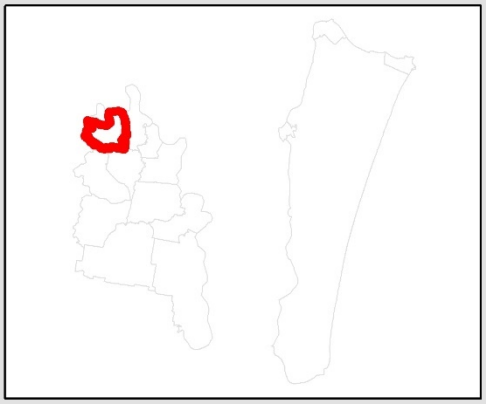
N

0 0.325 0.65 1.3
Kilometers

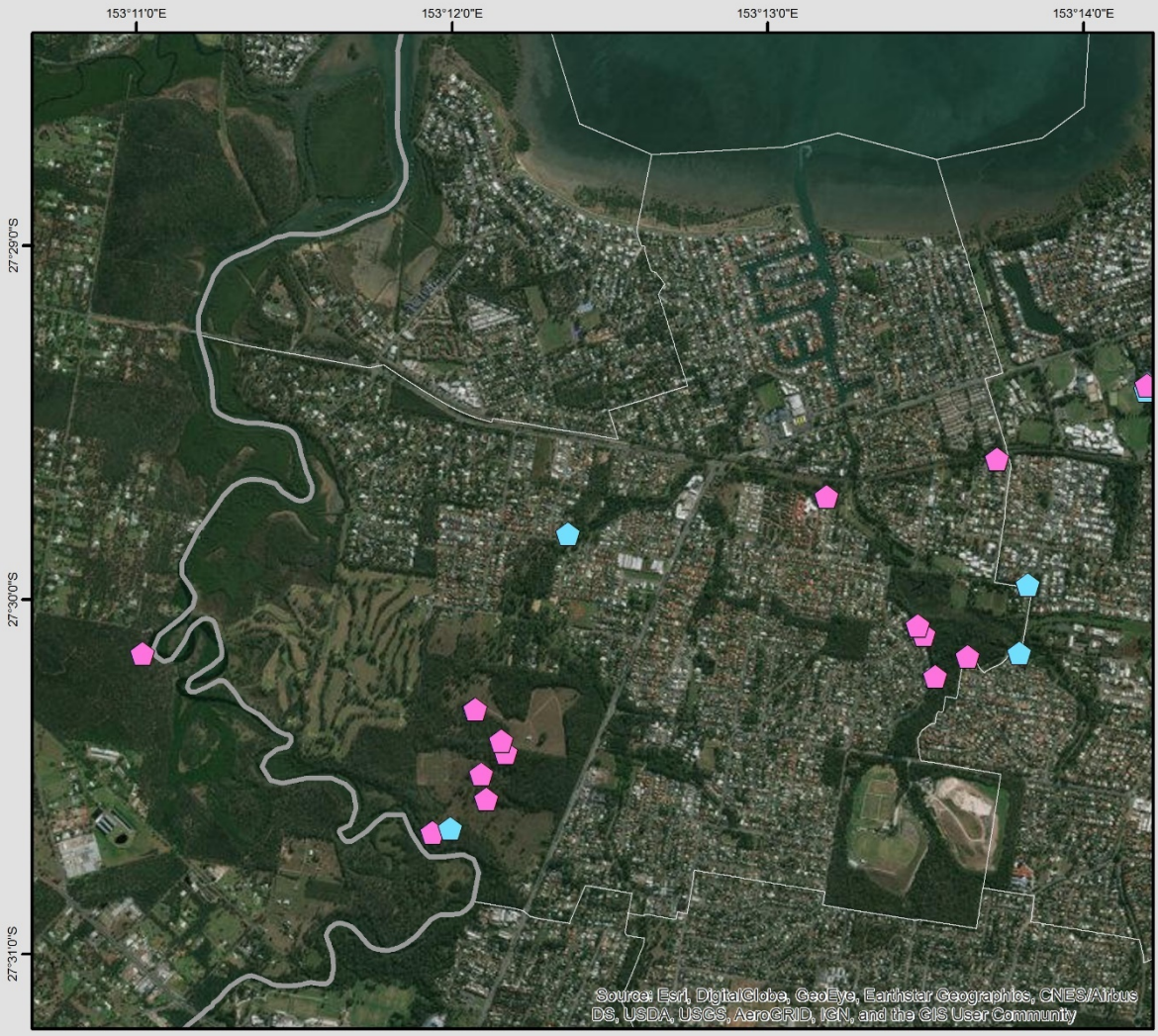
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Birkdale

N

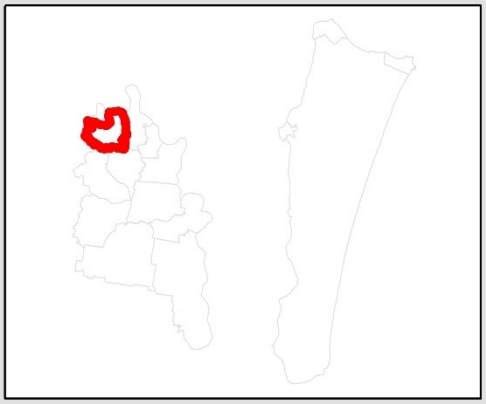
0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

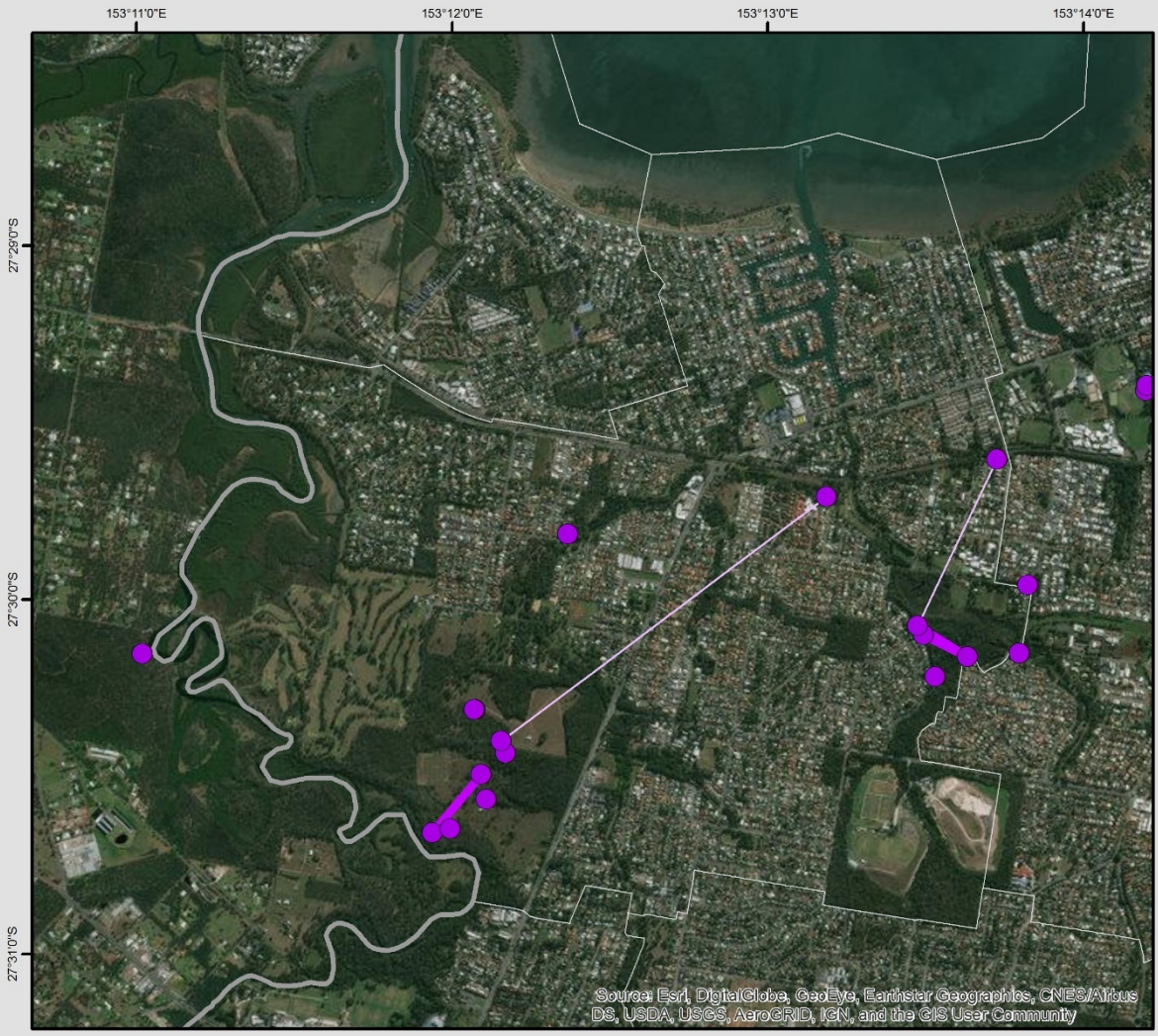
Legend

Koala sex (genetics)

- Female
- Male



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.



Birkdale

N

0 0.325 0.65 1.3
Kilometers

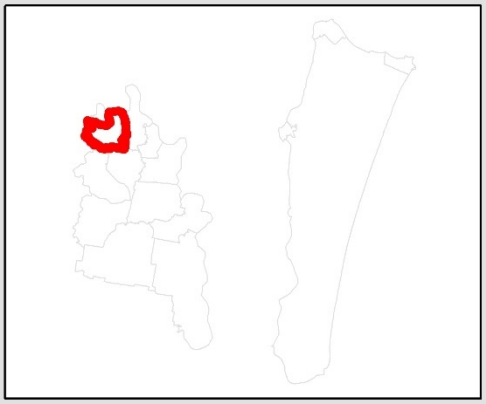
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

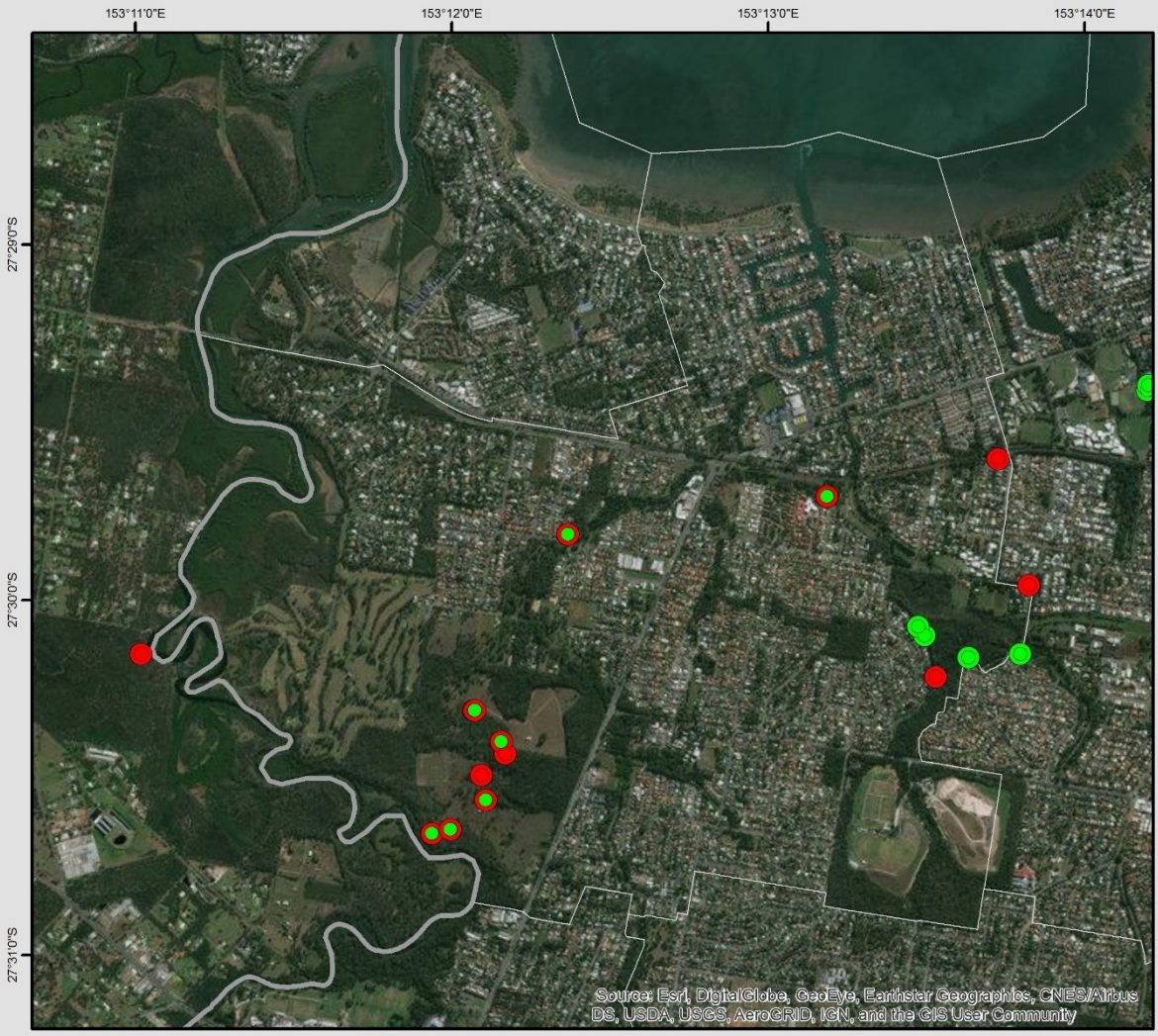
- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)



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.



Birkdale

N

0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

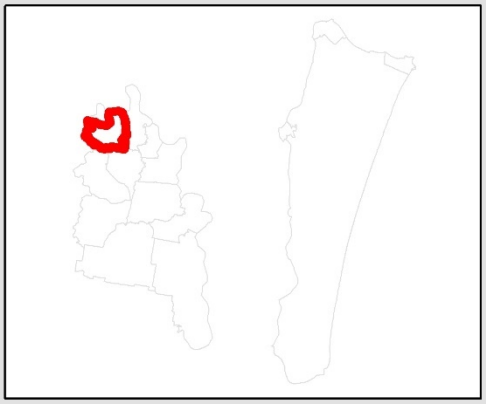
Legend - Chlamydia results

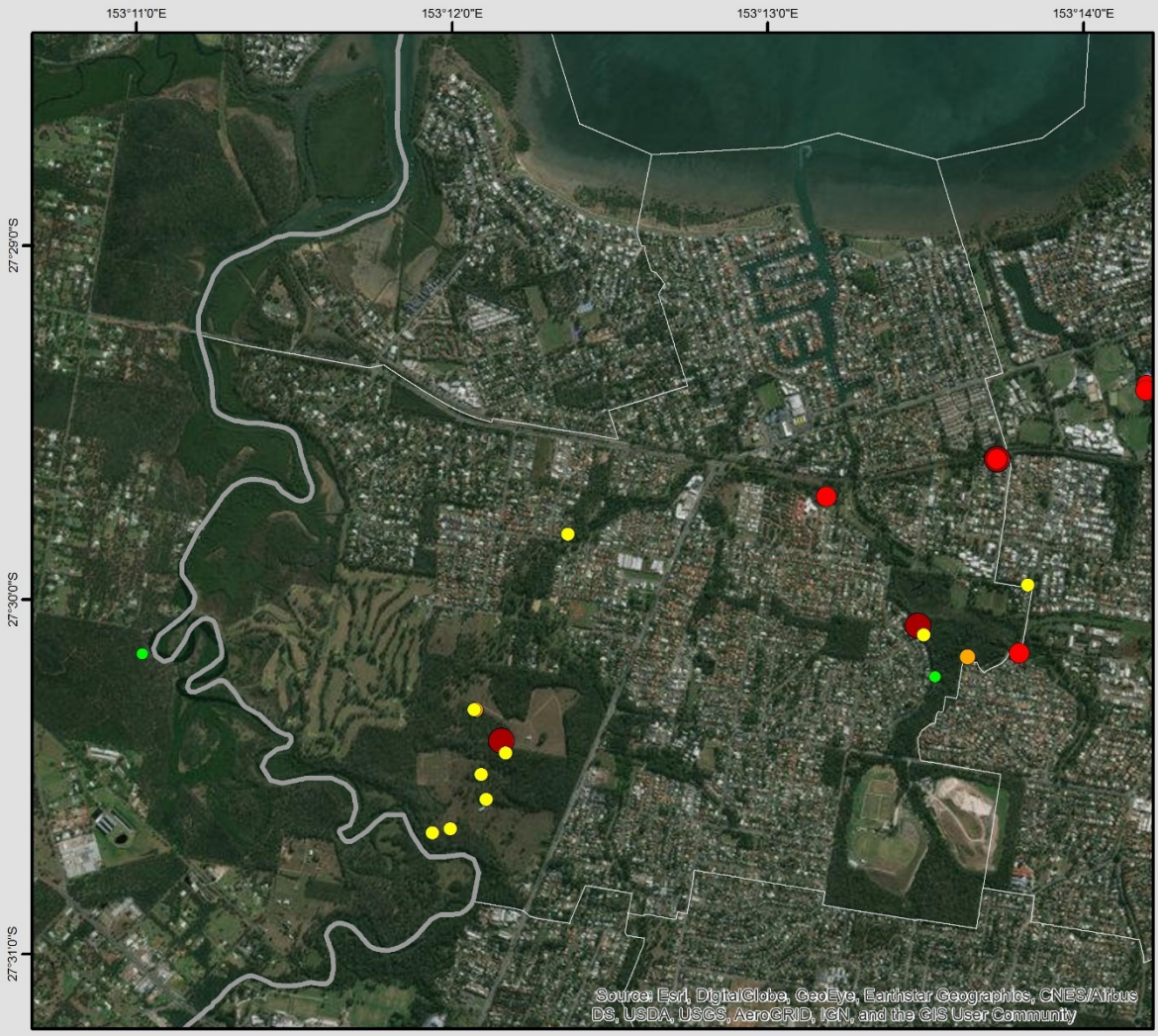
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold





Birkdale

N

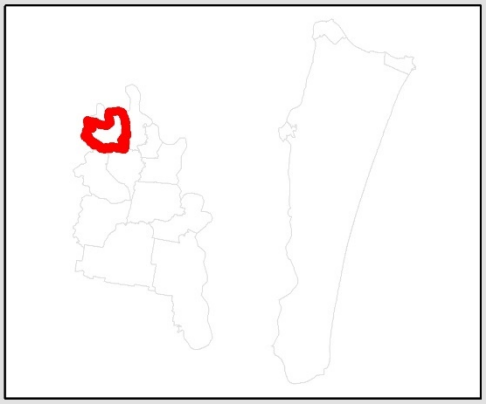
0 0.325 0.65 1.3
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

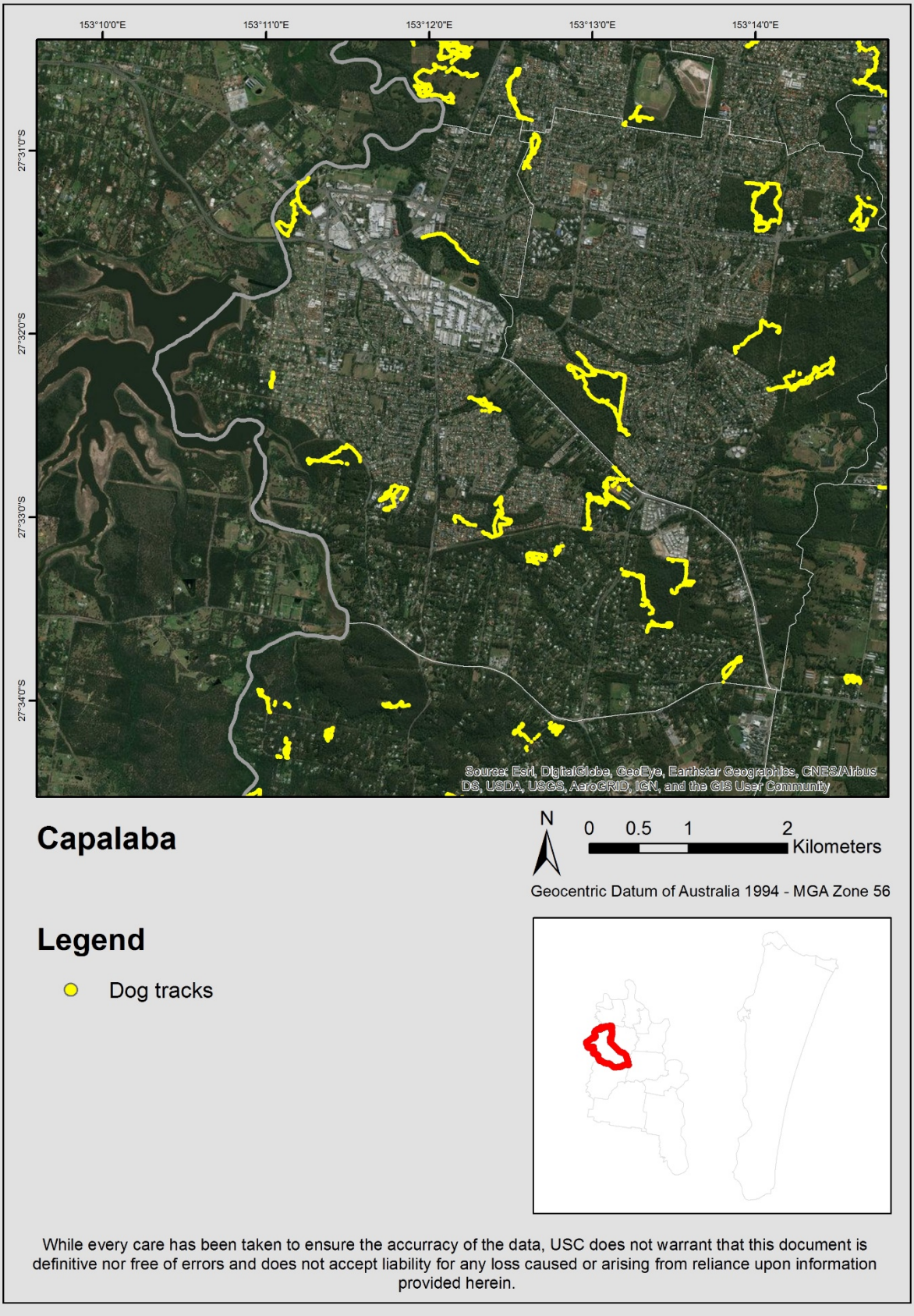
Legend

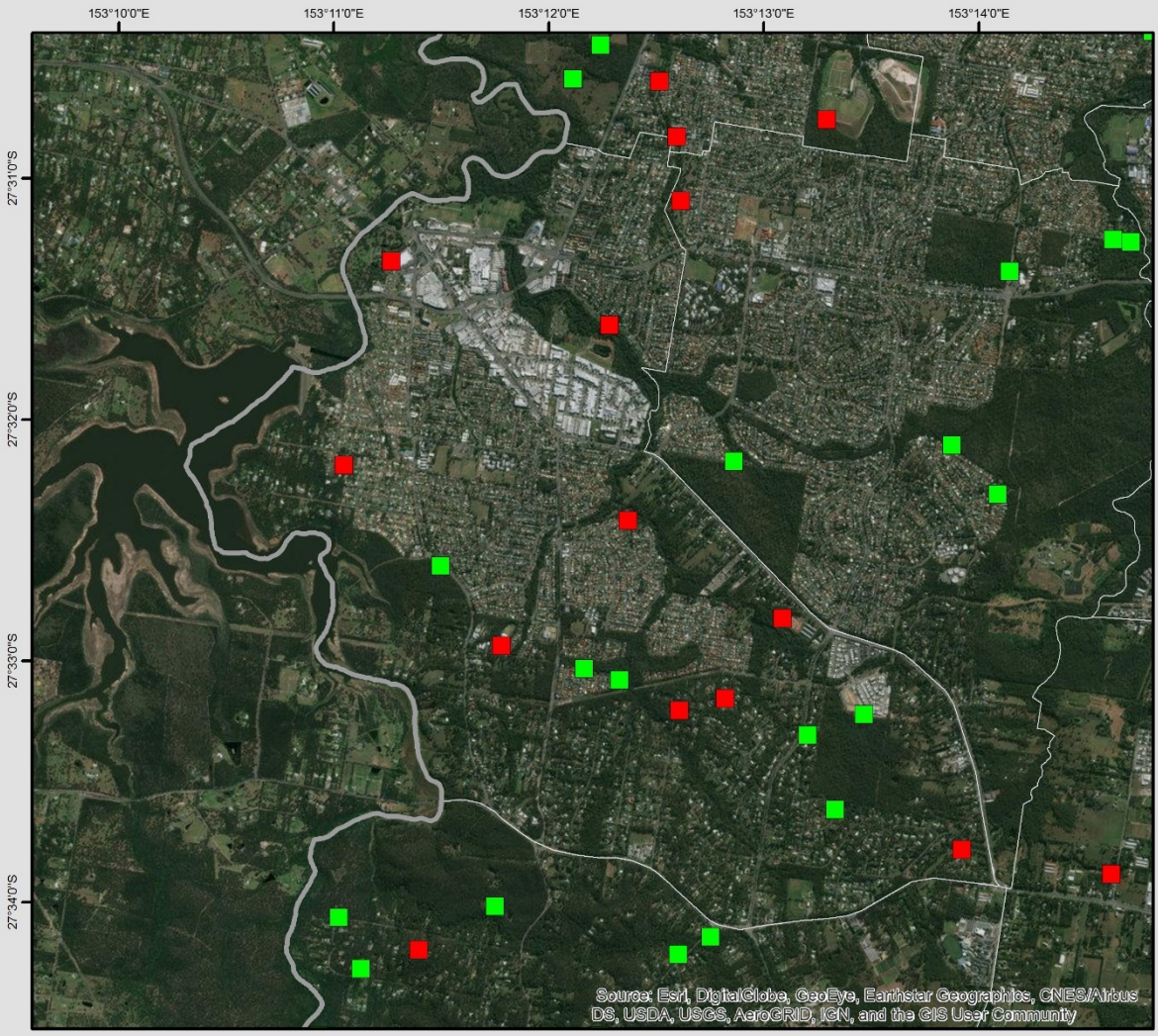
Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Capalaba


153°10'0"E
153°11'0"E
153°12'0"E
153°13'0"E
153°14'0"E
27°31'0"S
27°32'0"S
27°33'0"S
27°34'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Capalaba
N
0 0.5 1 2
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
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.



Capalaba

N

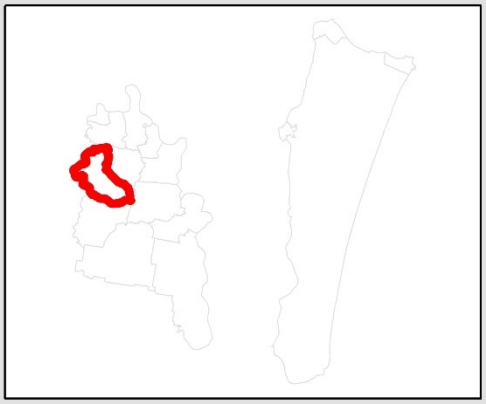
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

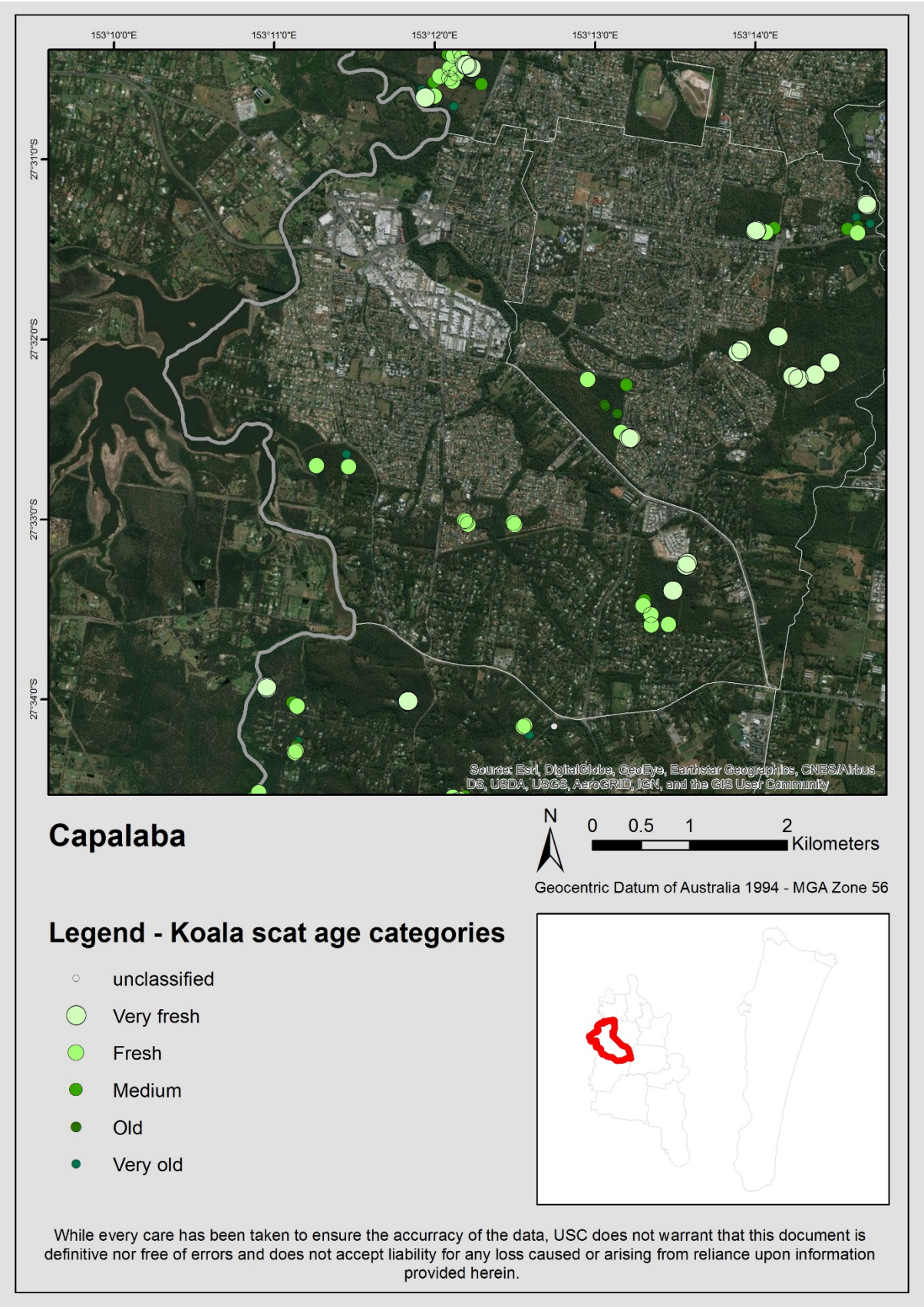
Legend

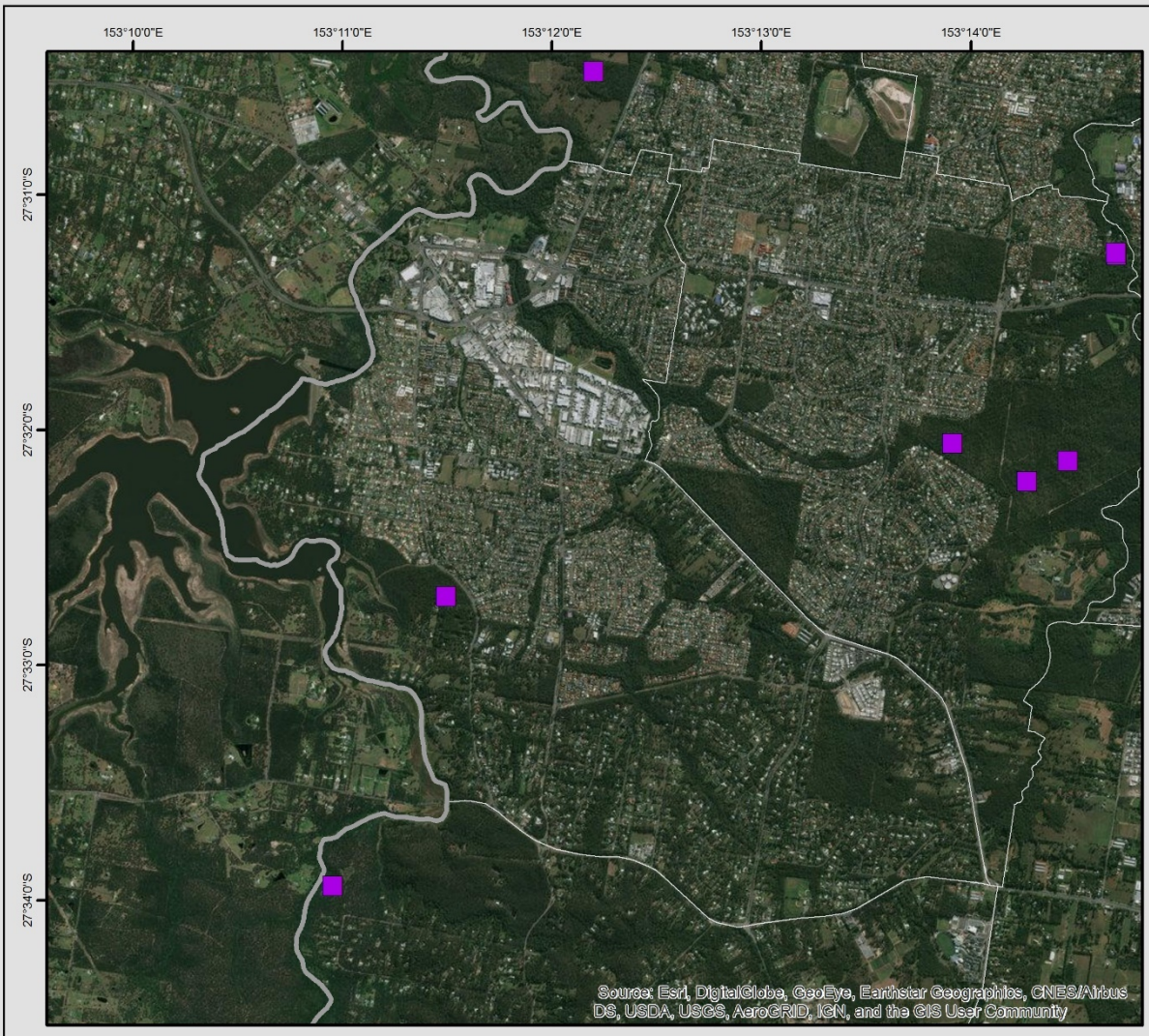
Koala scat

- Presence
- Absence



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.


153°10'0"E
153°11'0"E
153°12'0"E
153°13'0"E
153°14'0"E
27°31'0"S
27°32'0"S
27°33'0"S
27°34'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
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
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.



Capalaba

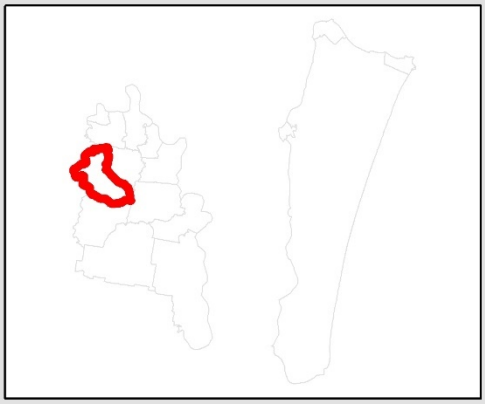
N

0 0.5 1 2 Kilometers

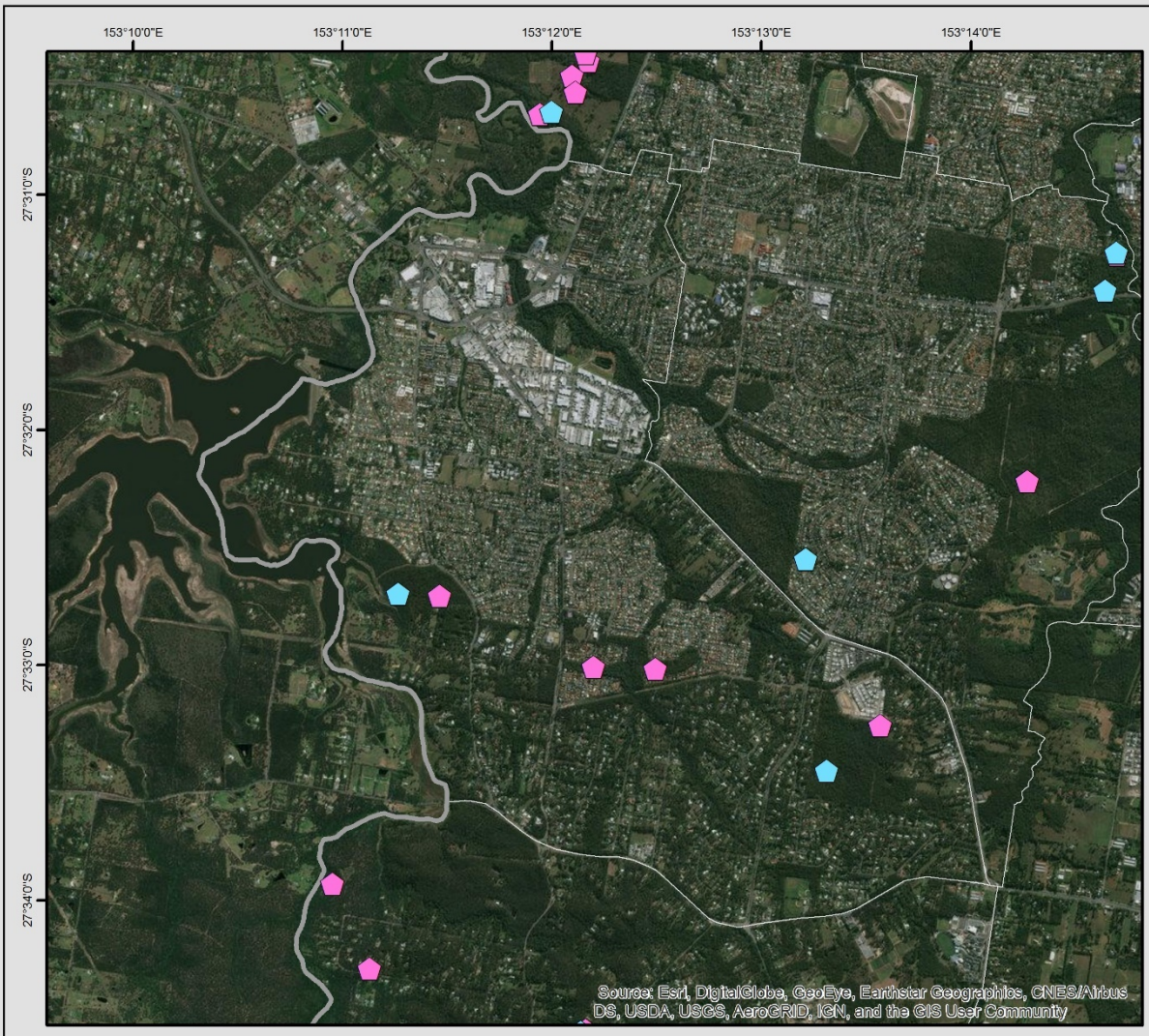
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Capalaba

N

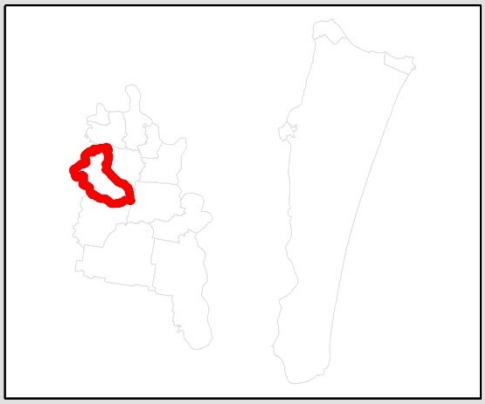
0 0.5 1 2 Kilometers

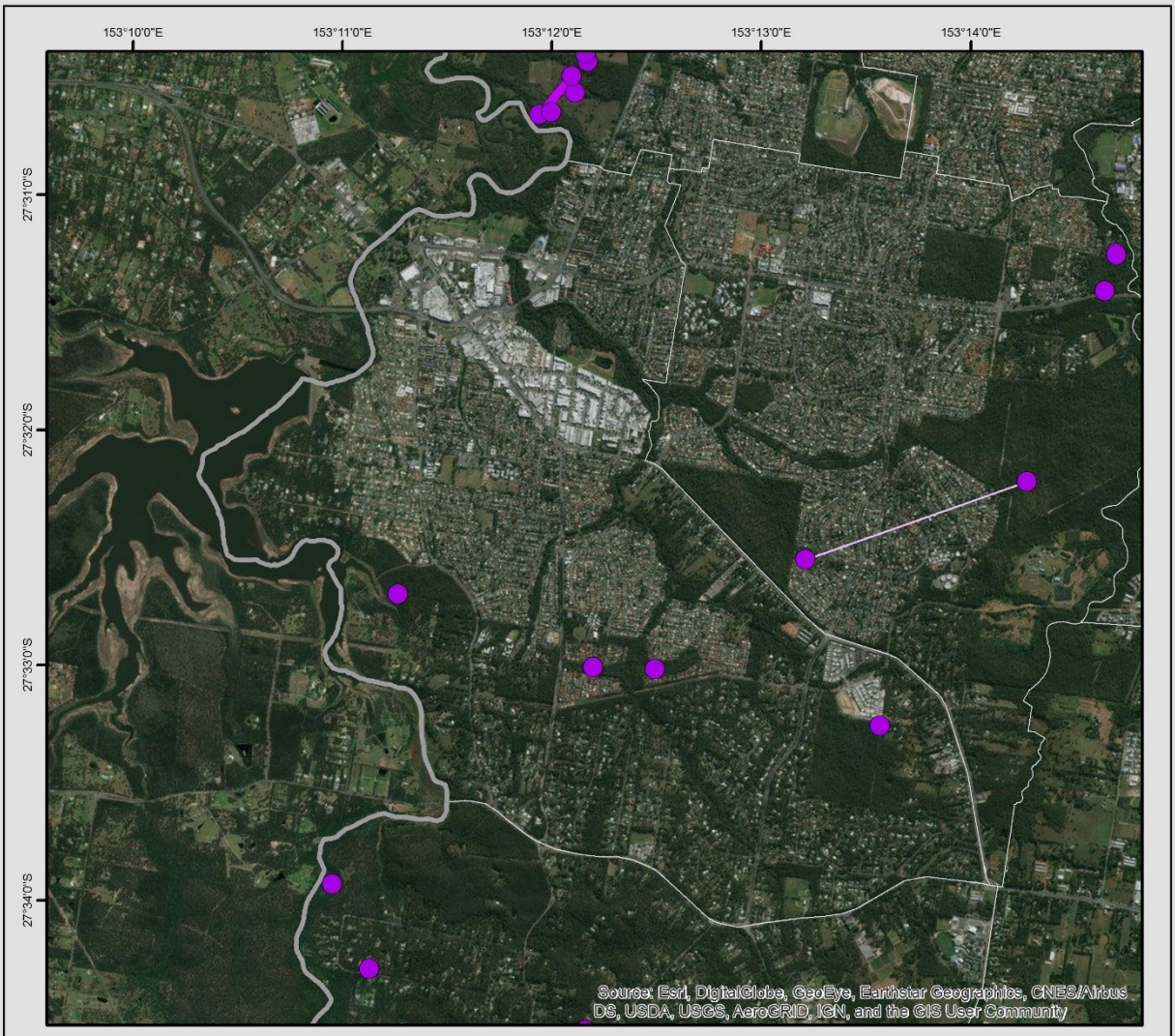
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male





Capalaba

N

0 0.5 1 2 Kilometers

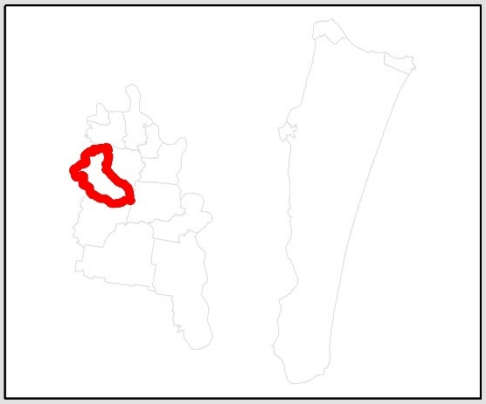
Geocentric Datum of Australia 1994 - MGA Zone 56

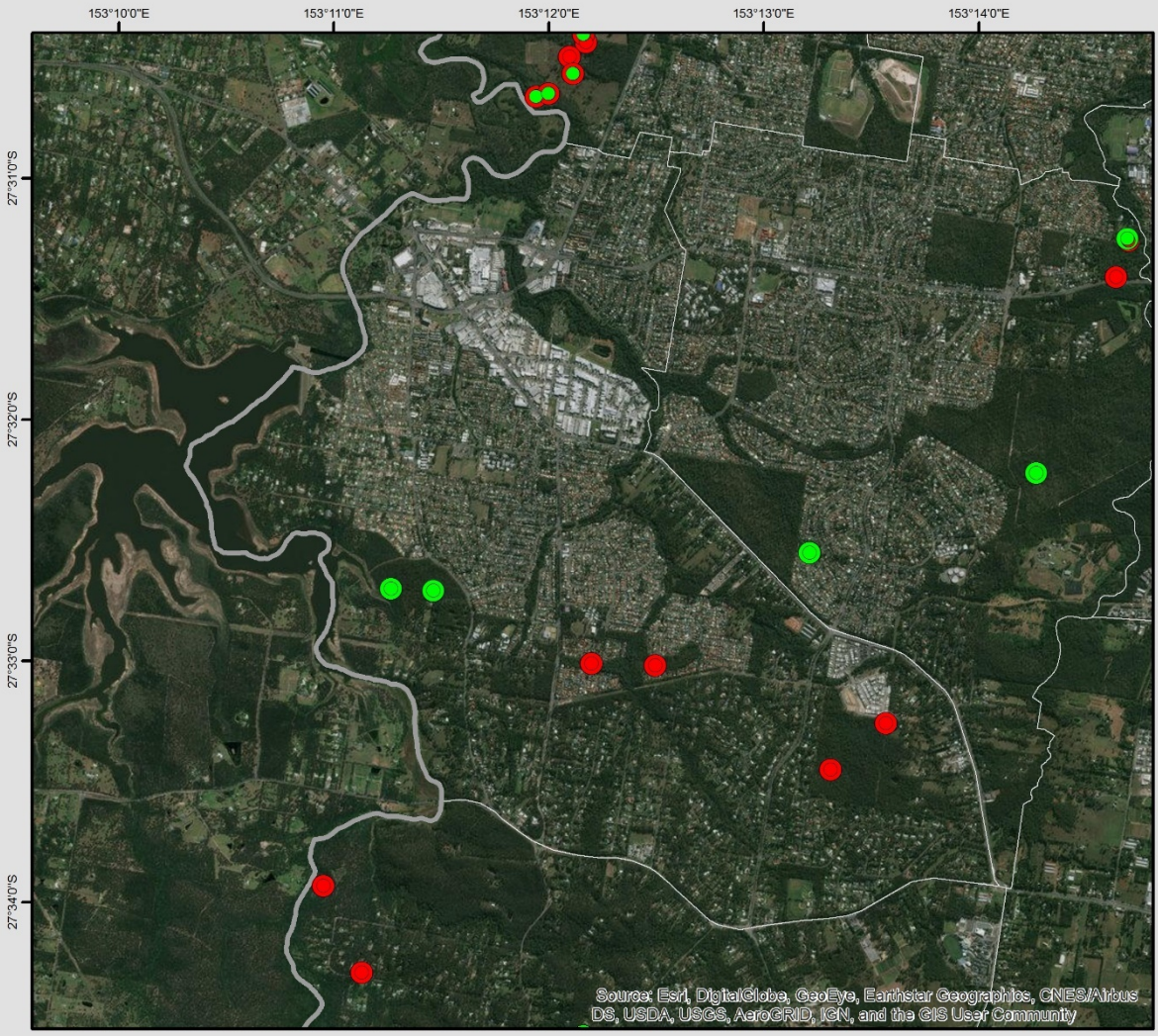
Legend

- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)





Capalaba

N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

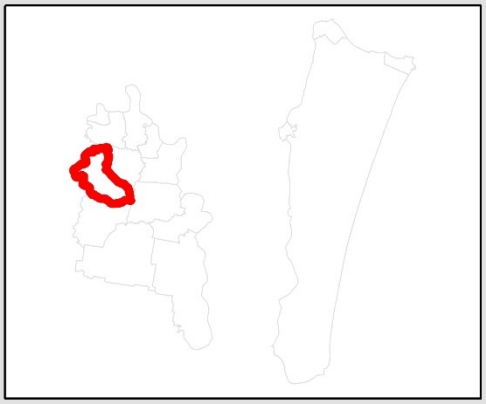
Legend - Chlamydia results

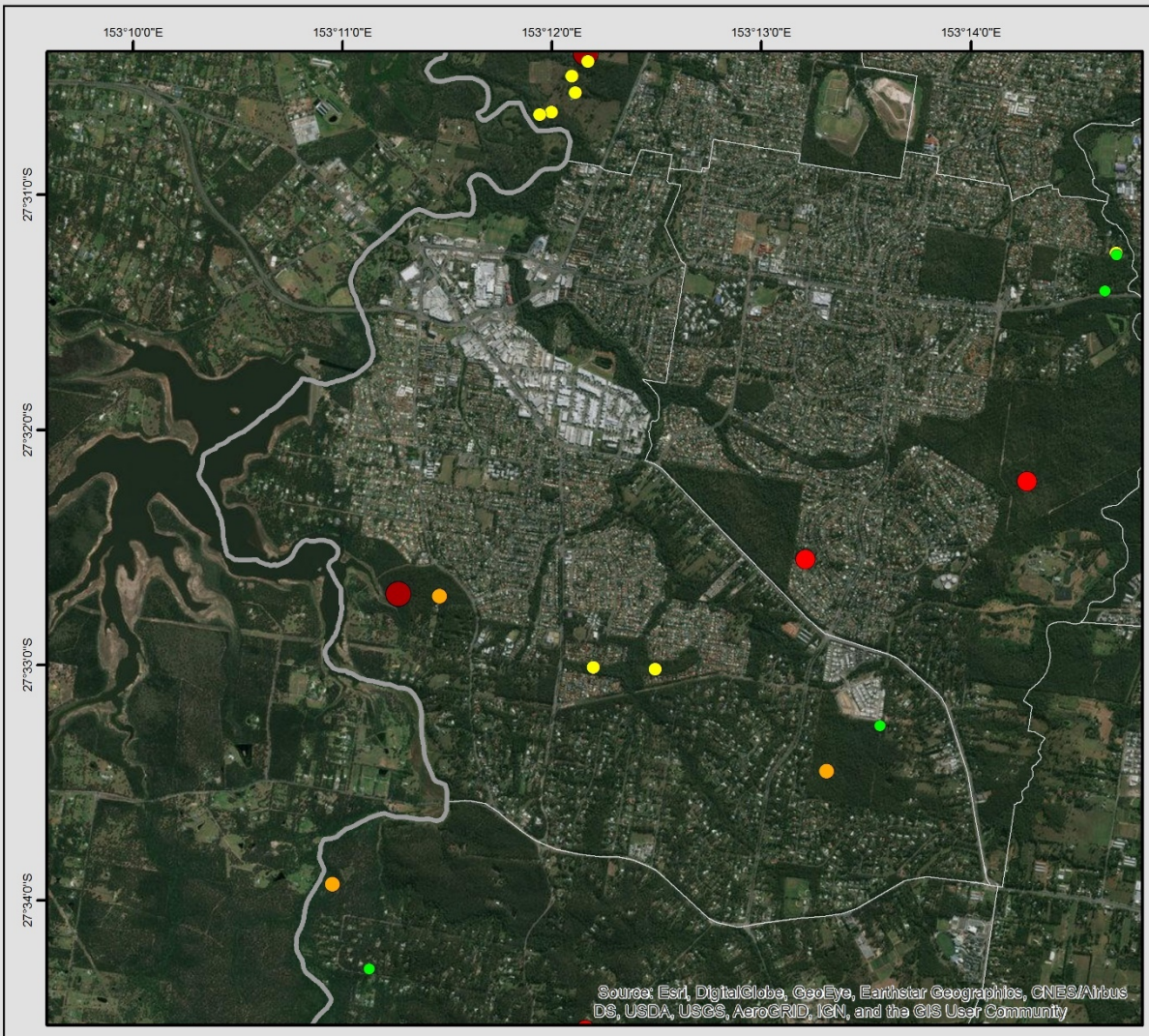
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold





Capalaba

N

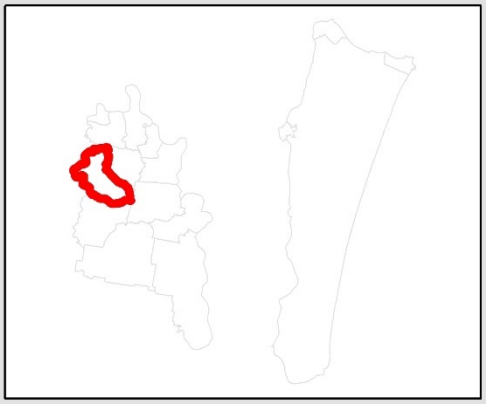
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

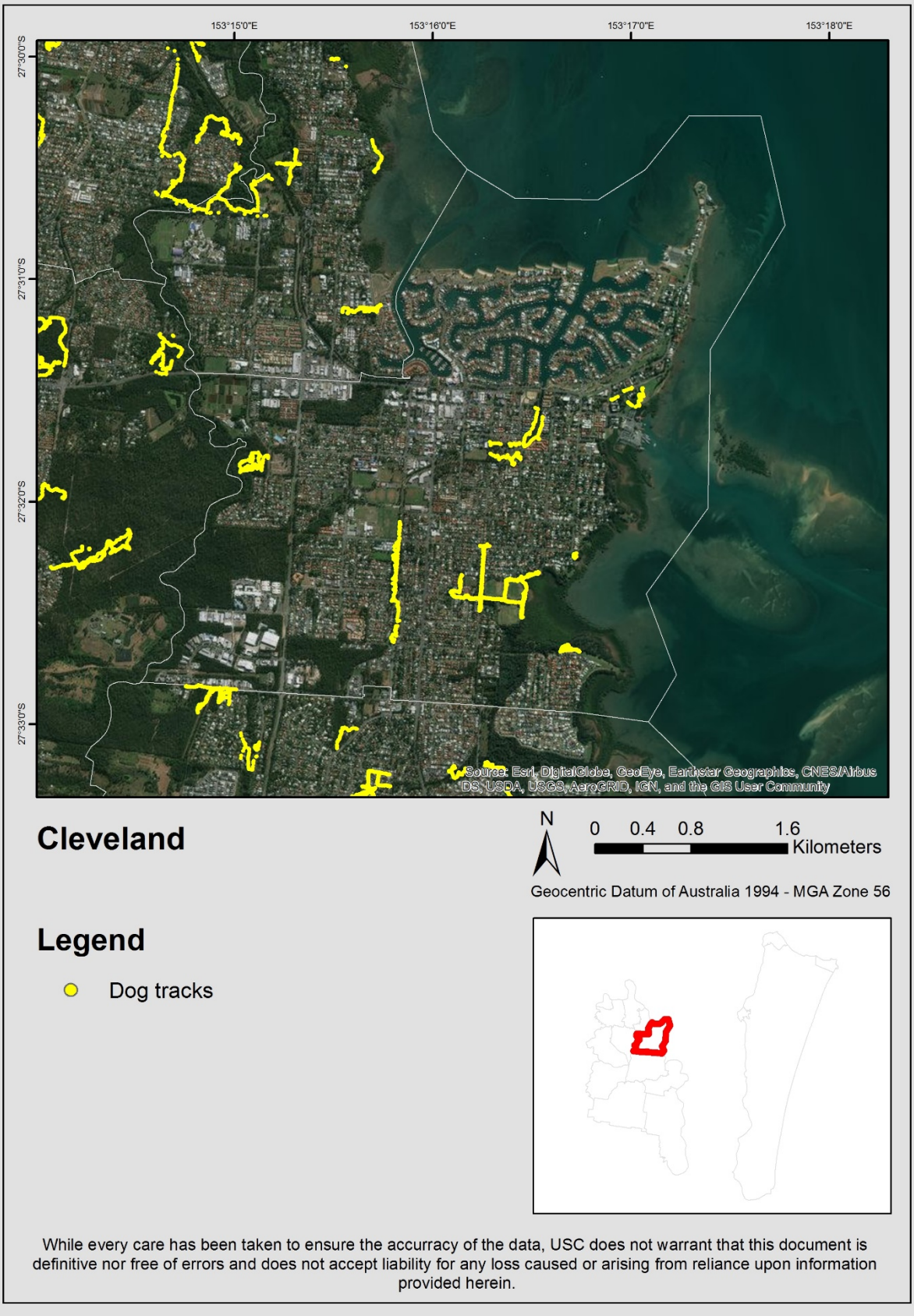
Legend

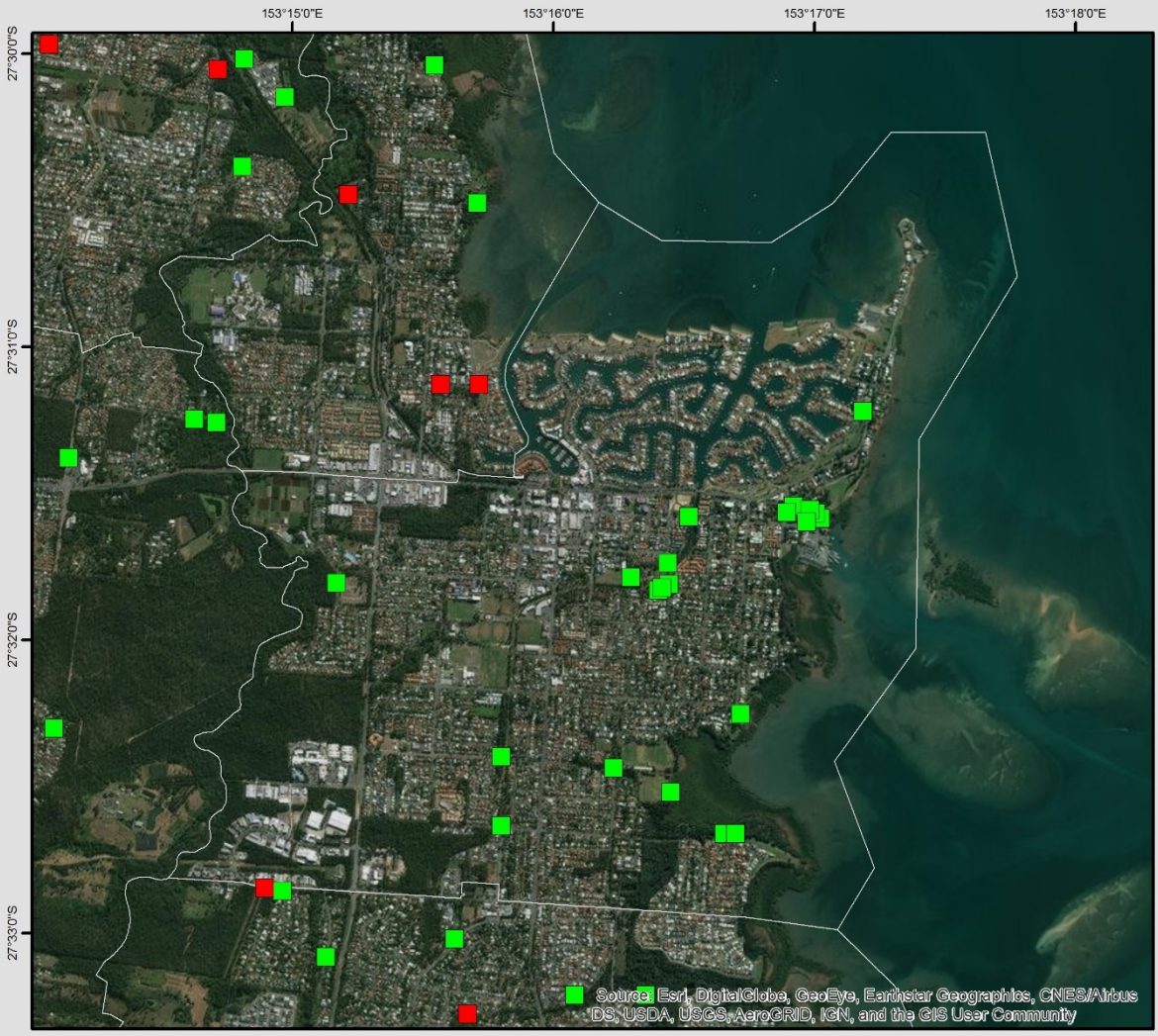
Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Cleveland


153°15'0"E
153°16'0"E
153°17'0"E
153°18'0"E
27°30'0"S
27°31'0"S
27°32'0"S
27°33'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Cleveland
N
0 0.4 0.8 1.6
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
While every care has been taken to ensure the accuracy 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.



Cleveland

N

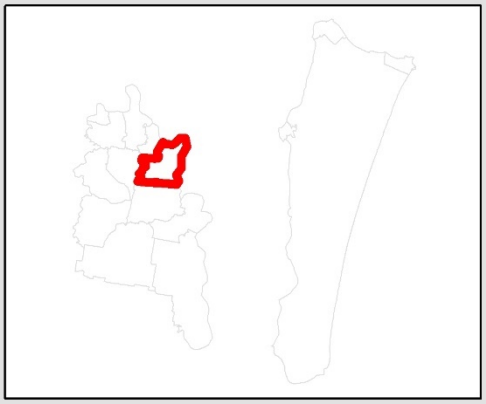
0 0.4 0.8 1.6
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

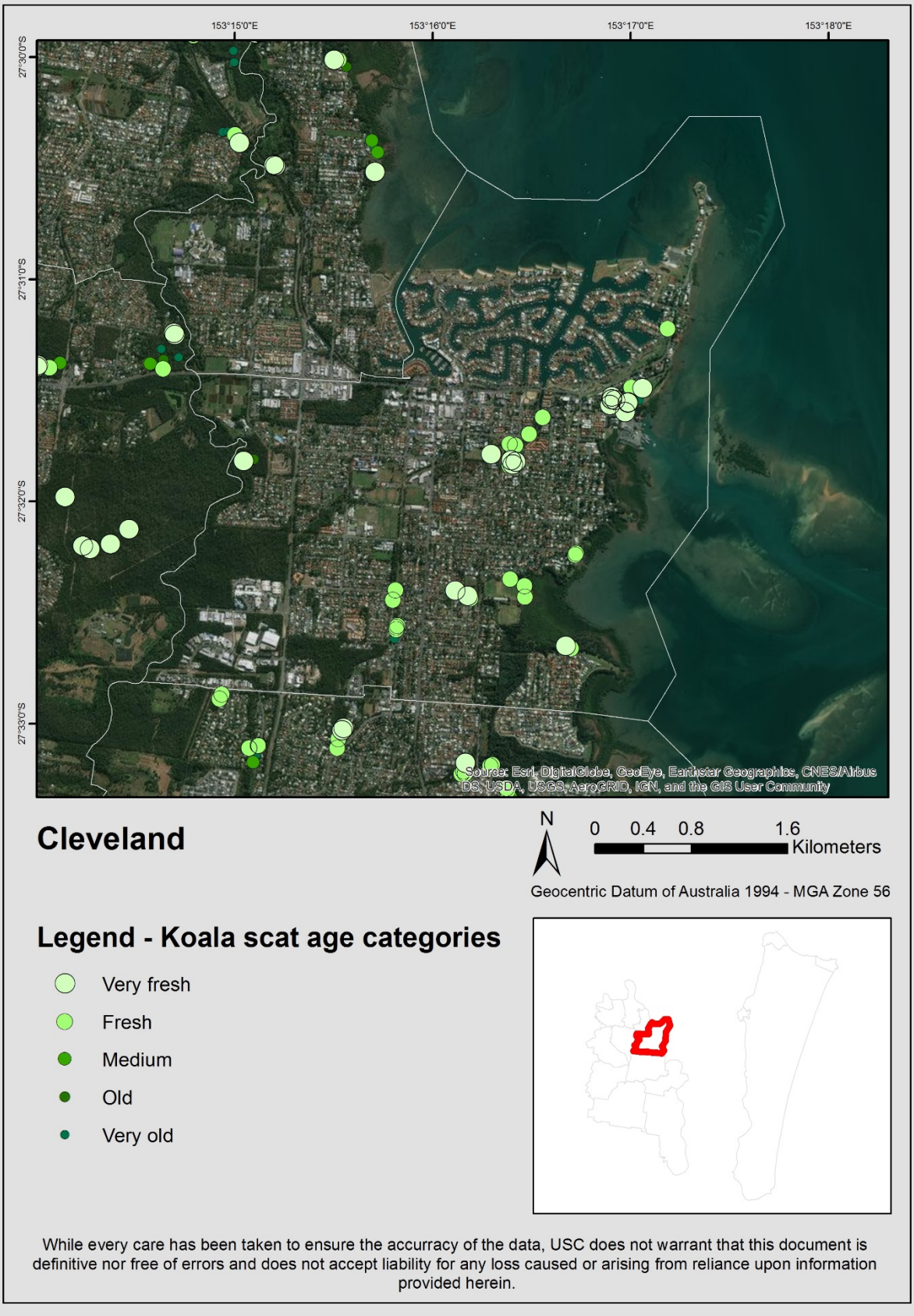
Legend

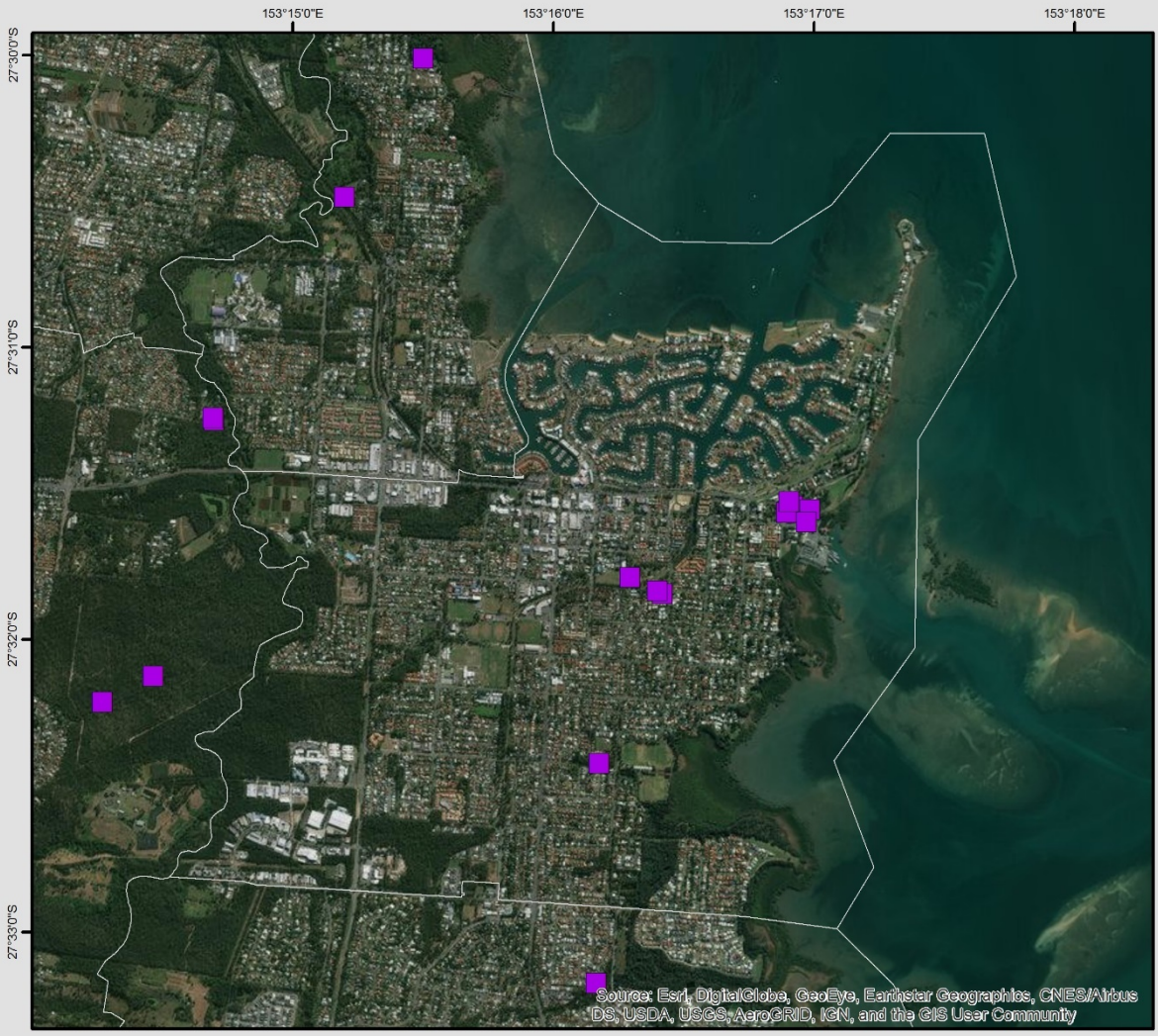
Koala scat

- Presence
- Absence



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.


153°15'0"E
153°16'0"E
153°17'0"E
153°18'0"E
27°30'0"S
27°31'0"S
27°32'0"S
27°33'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Cleveland
N
0 0.4 0.8 1.6
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend - Koala scat age categories
Very fresh
Fresh
Medium
Old
Very old
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.



Cleveland

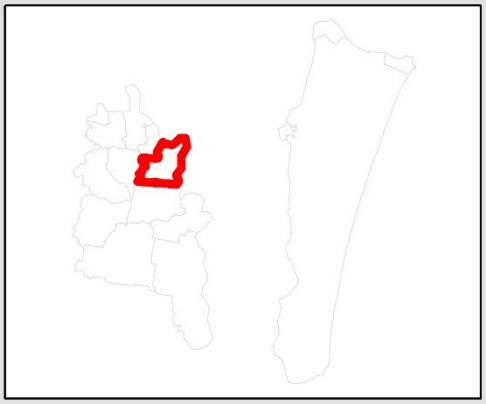
N

0 0.4 0.8 1.6
Kilometers

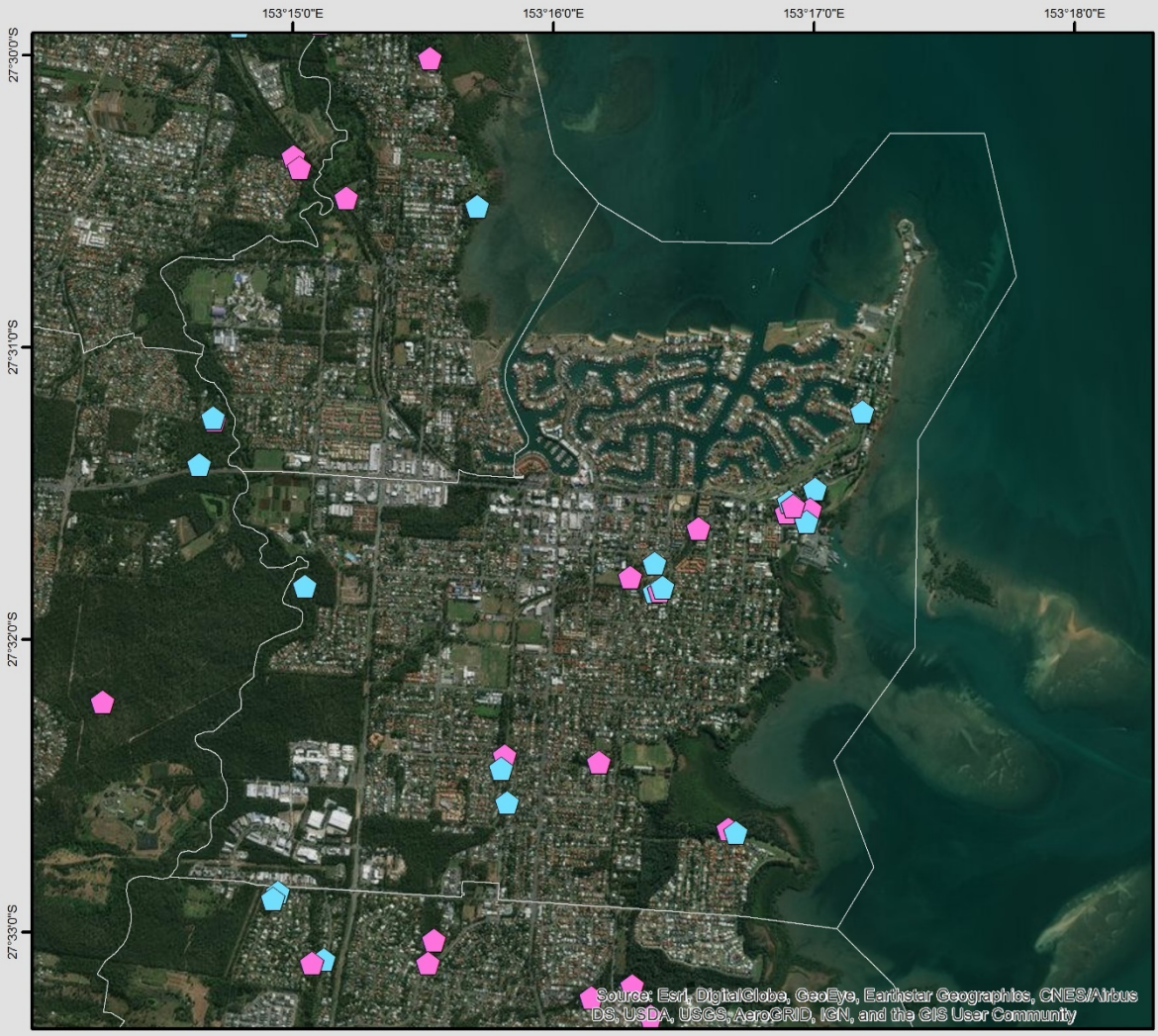
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Cleveland

N

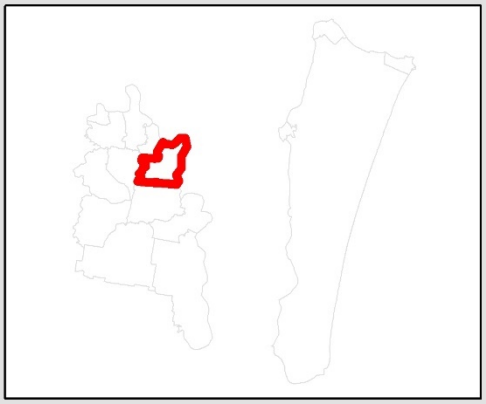
0 0.4 0.8 1.6
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

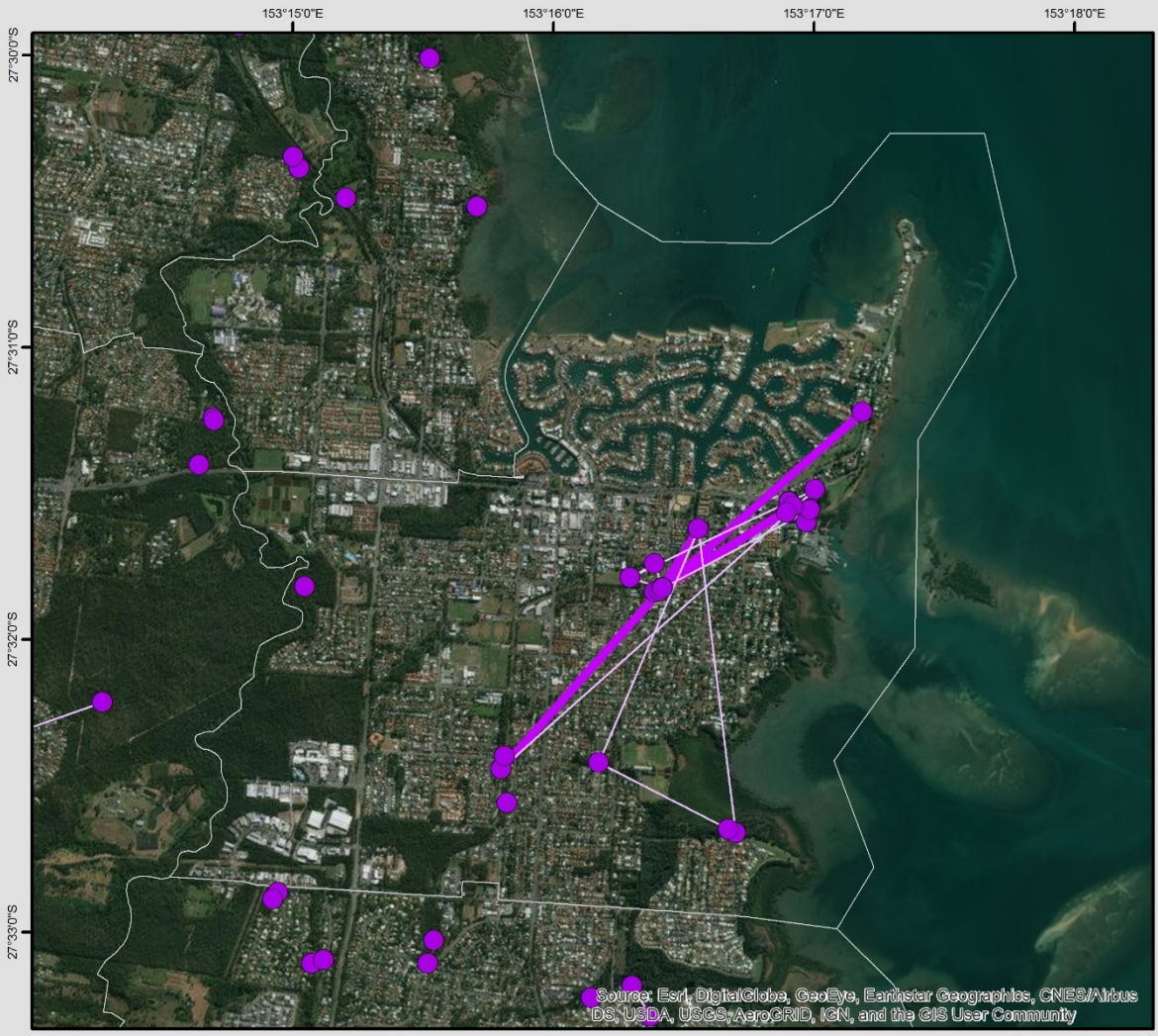
Legend

Koala sex (genetics)

- Female
- Male



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.



Cleveland

N

0 0.4 0.8 1.6 Kilometers

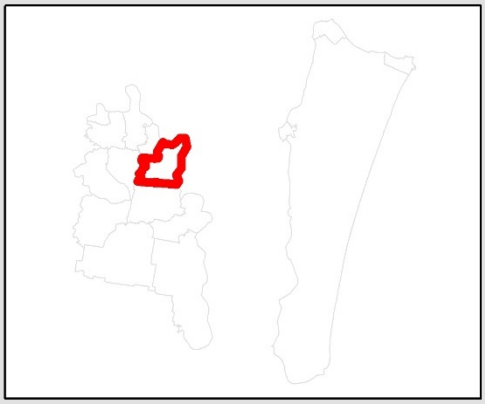
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

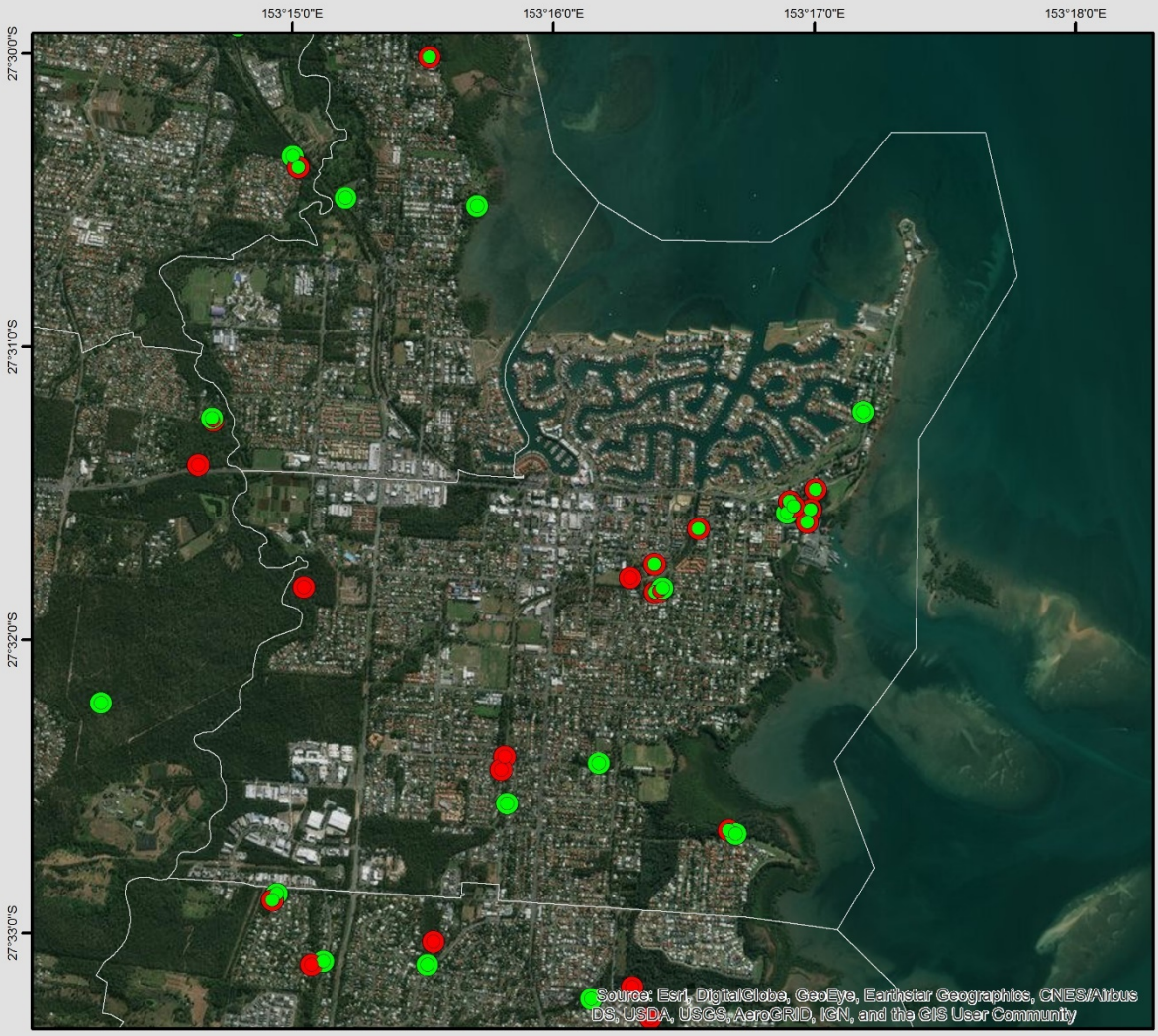
- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)



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.



Cleveland

N

0 0.4 0.8 1.6
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

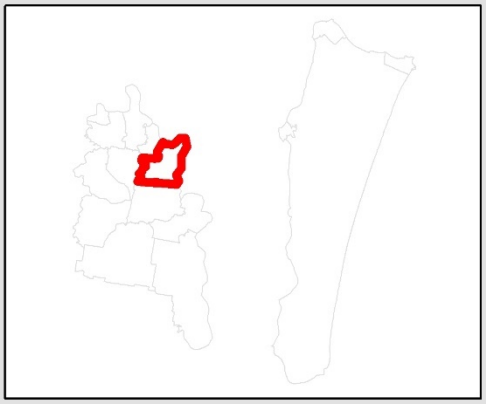
Legend - Chlamydia results

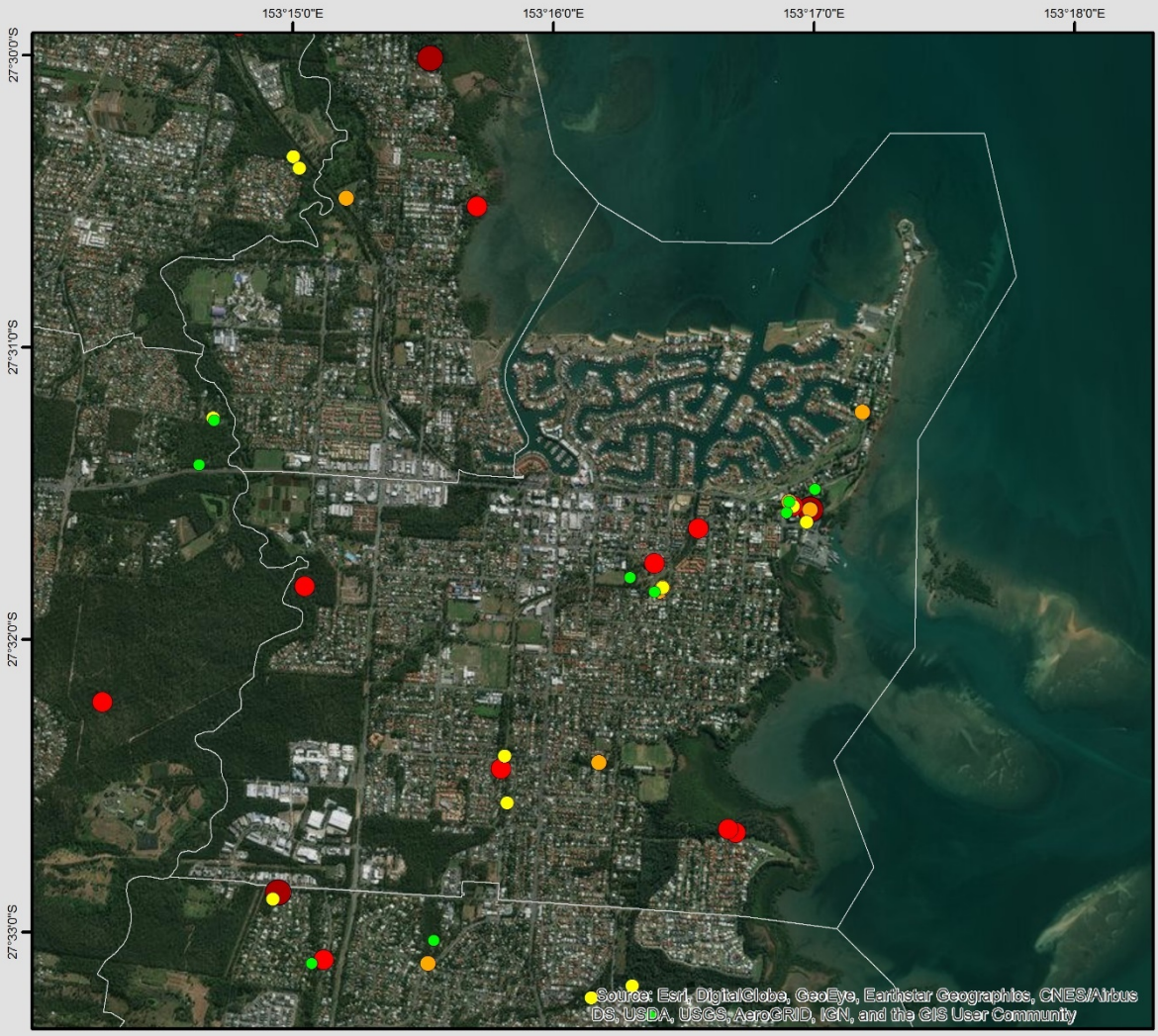
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold





Cleveland

N

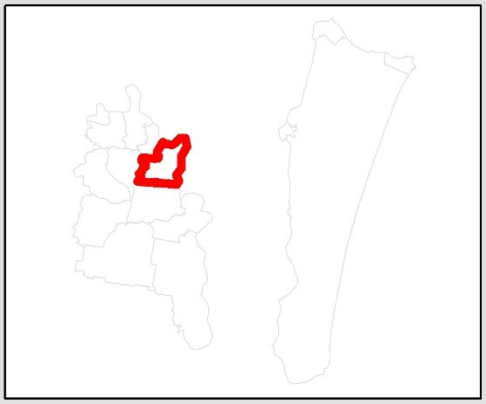
0 0.4 0.8 1.6
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

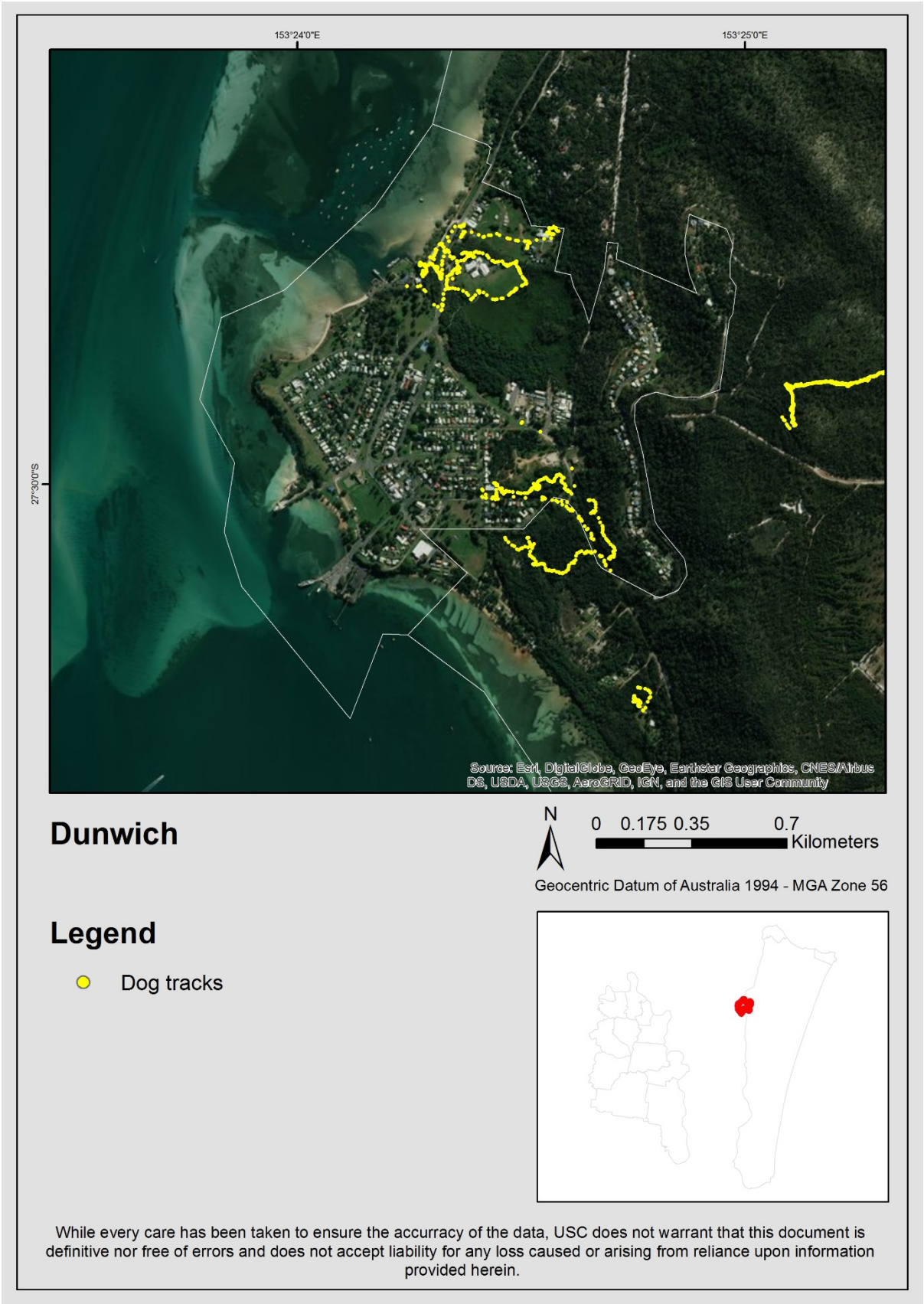
Legend

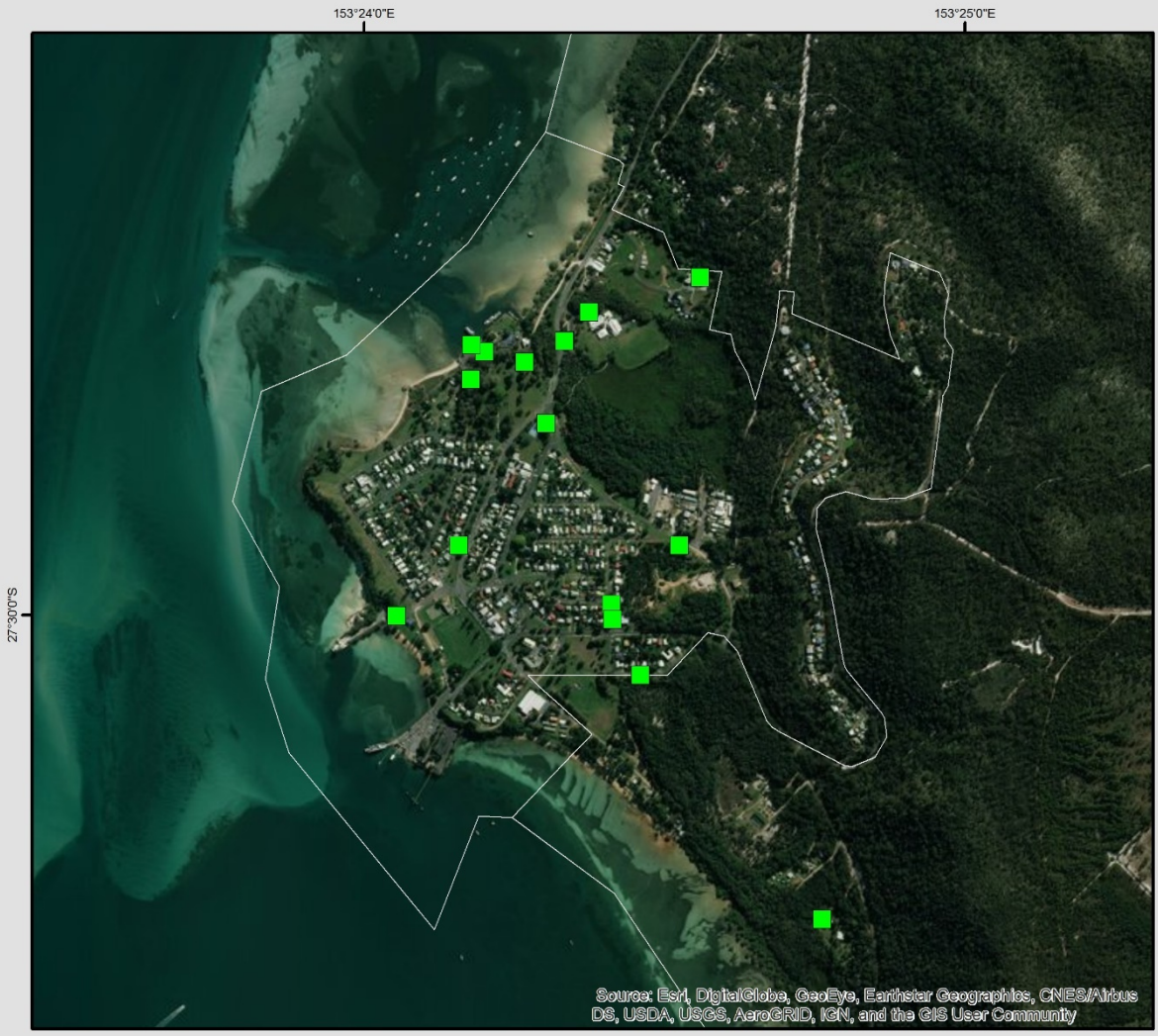
Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Dunwich


153°24'0"E
153°25'0"E
27°30'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Dunwich
N
0 0.175 0.35 0.7
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
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.



Dunwich

N

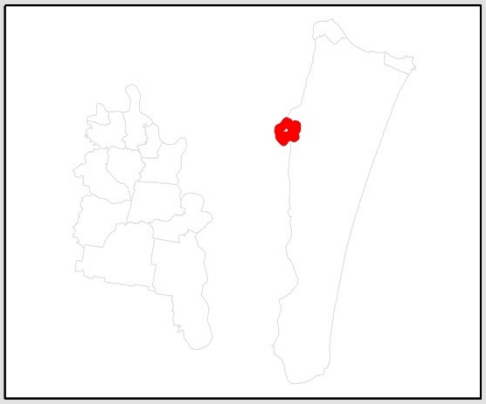
0 0.175 0.35 0.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

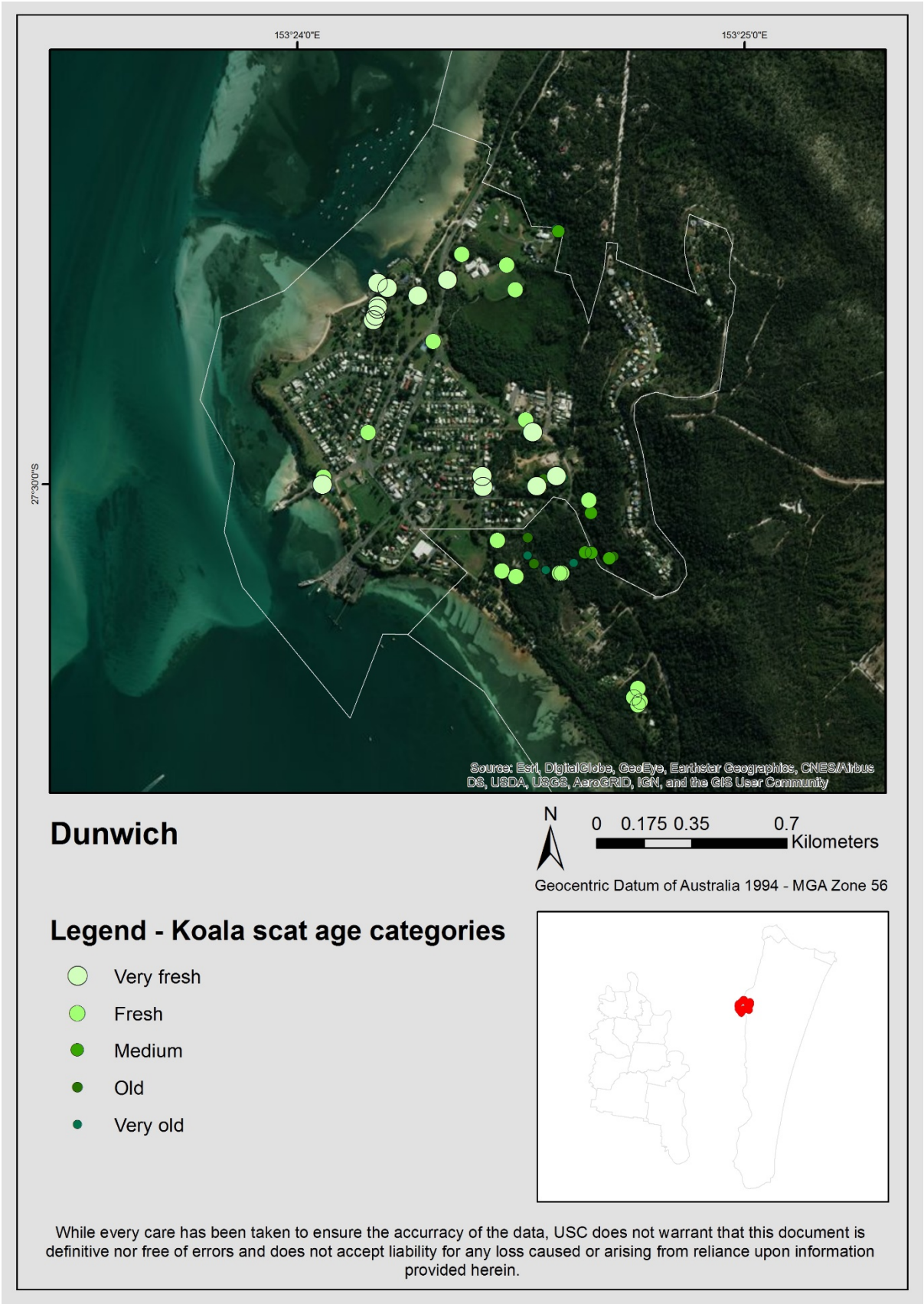
Legend

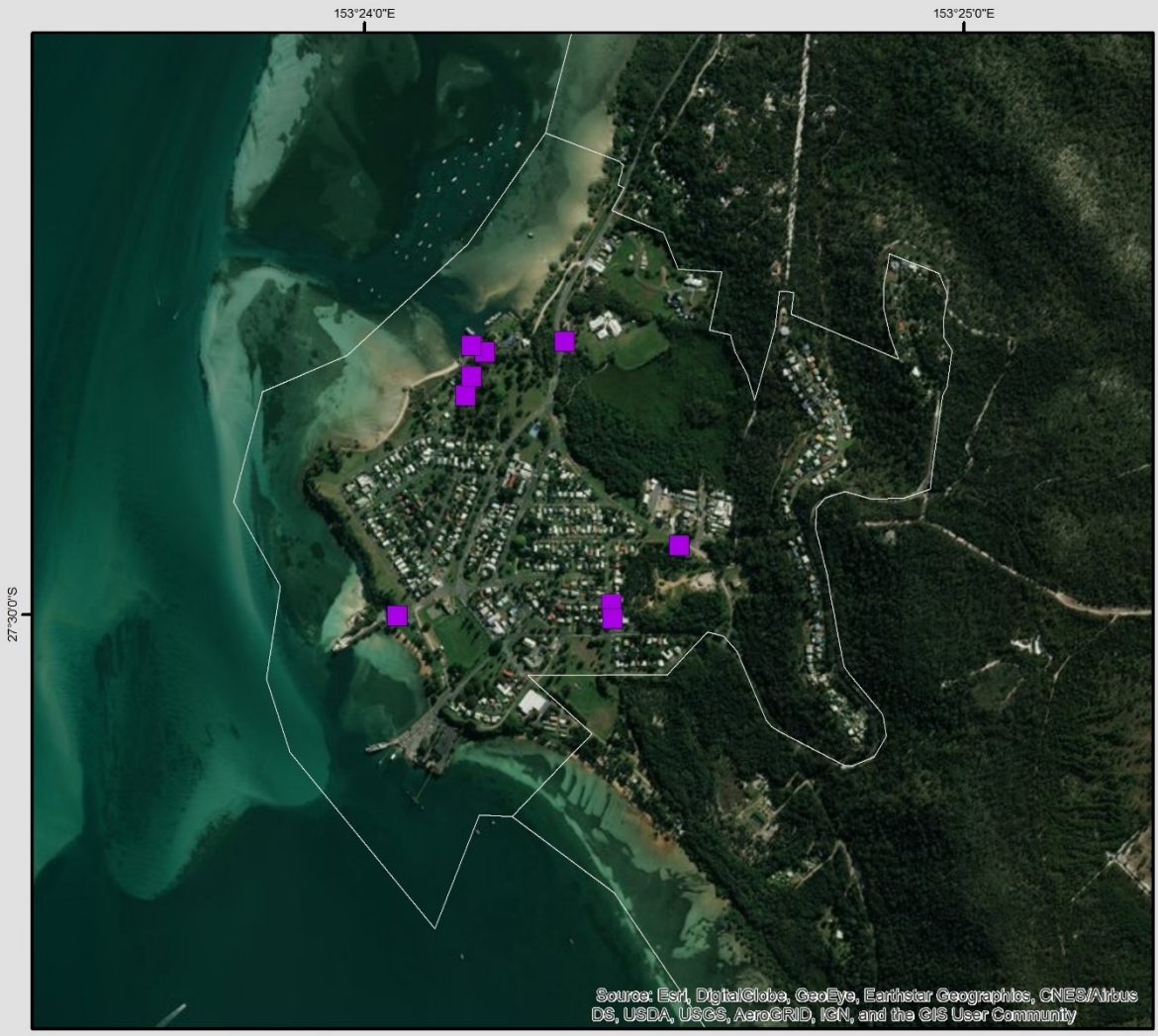
Koala scat

- Presence
- Absence



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.


153°24'0"E
153°25'0"E
27°30'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Dunwich
N
0 0.175 0.35 0.7
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend - Koala scat age categories
Very fresh
Fresh
Medium
Old
Very old
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.



Dunwich

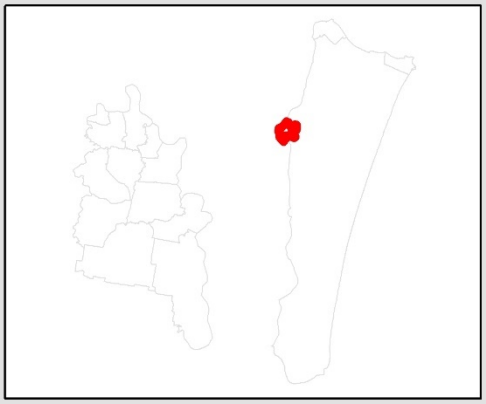
N

0 0.175 0.35 0.7
Kilometers

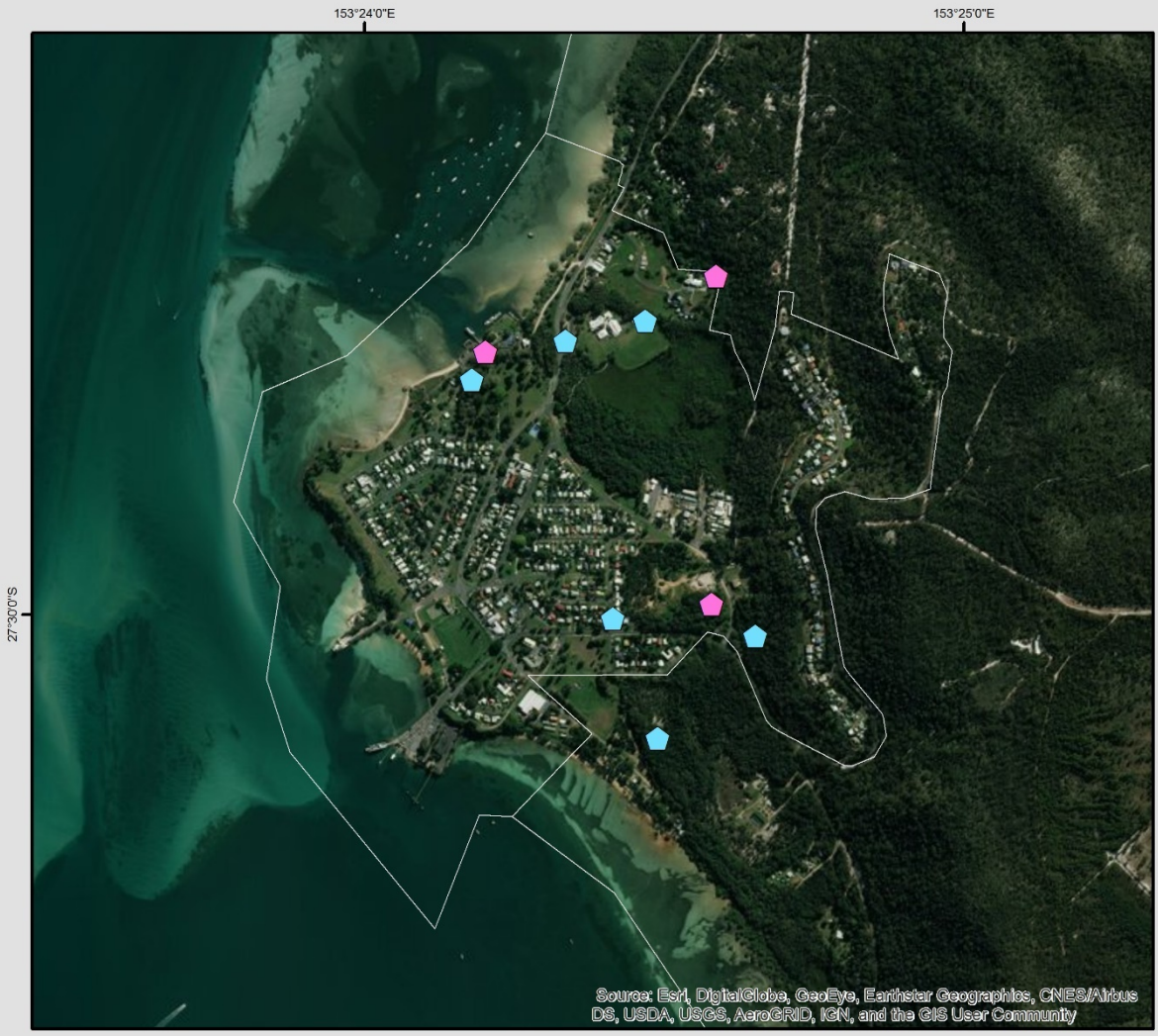
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Dunwich

N

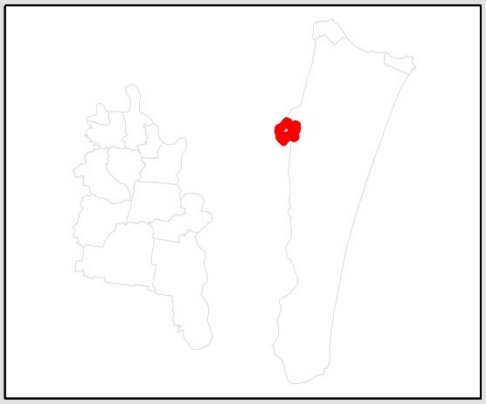
0 0.175 0.35 0.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

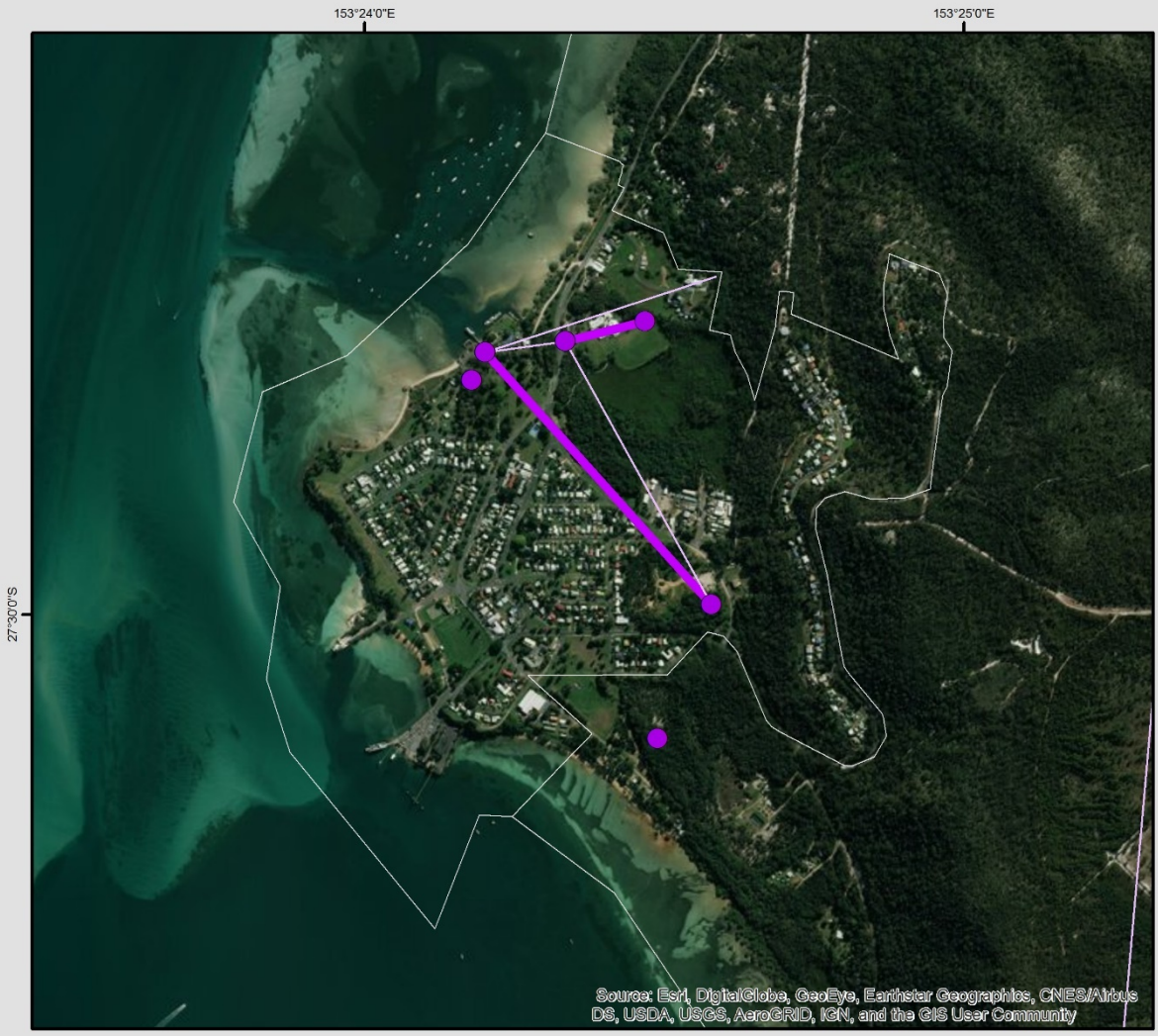
Legend

Koala sex (genetics)

- Female
- Male



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.



Dunwich

N

0 0.175 0.35 0.7
Kilometers

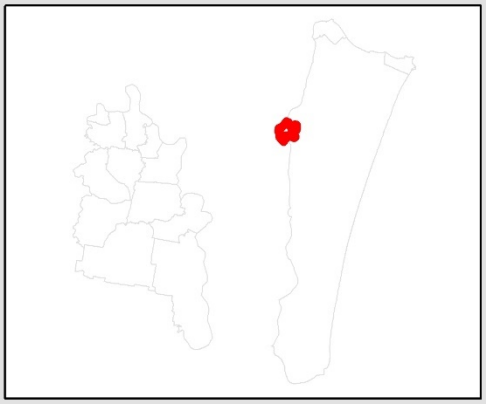
Geocentric Datum of Australia 1994 - MGA Zone 56

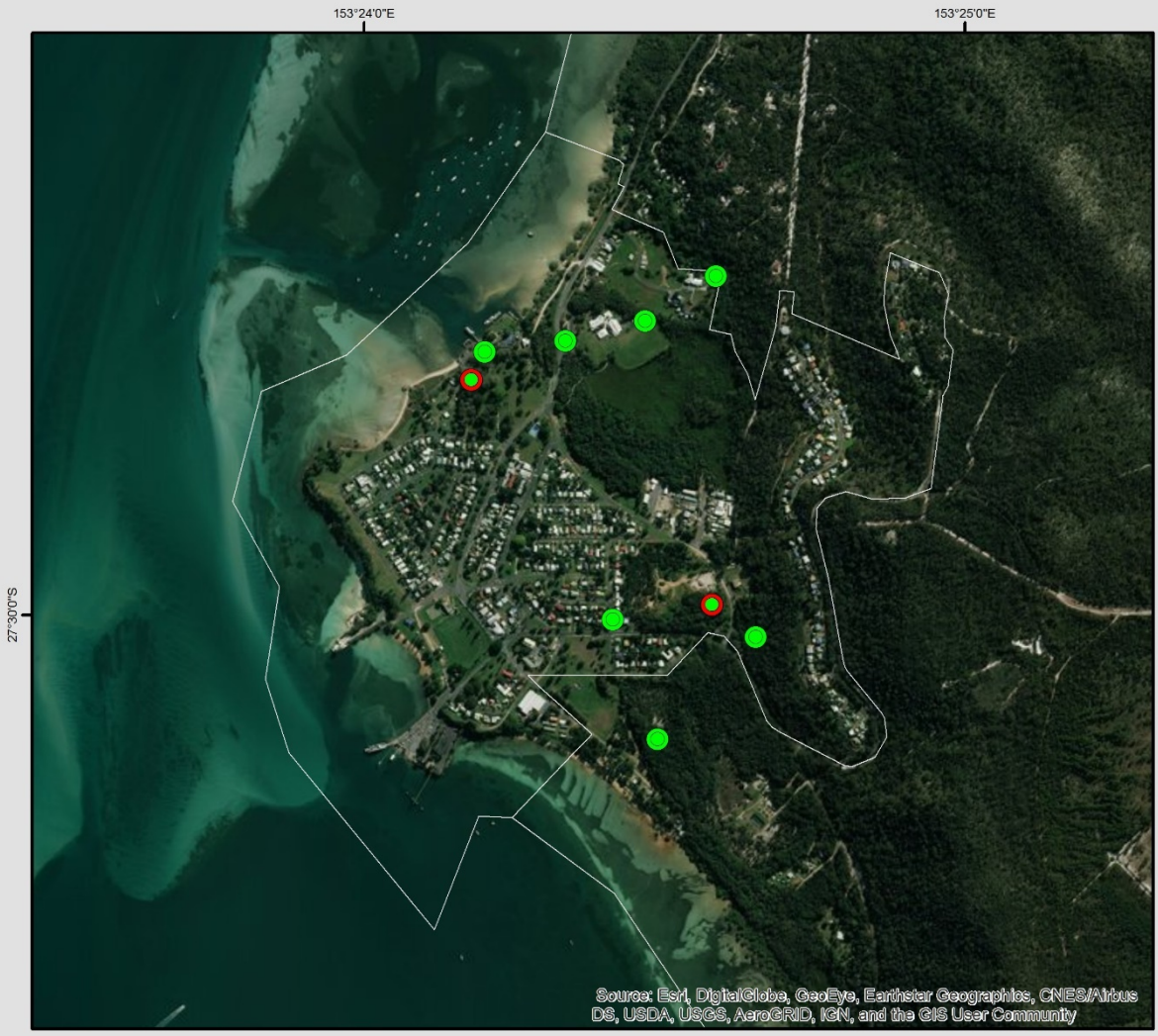
Legend

- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)





Dunwich

N

0 0.175 0.35 0.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

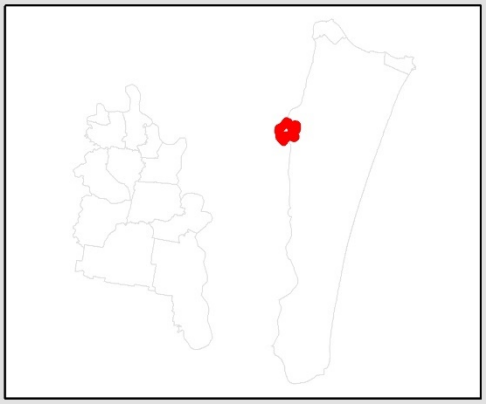
Legend - Chlamydia results

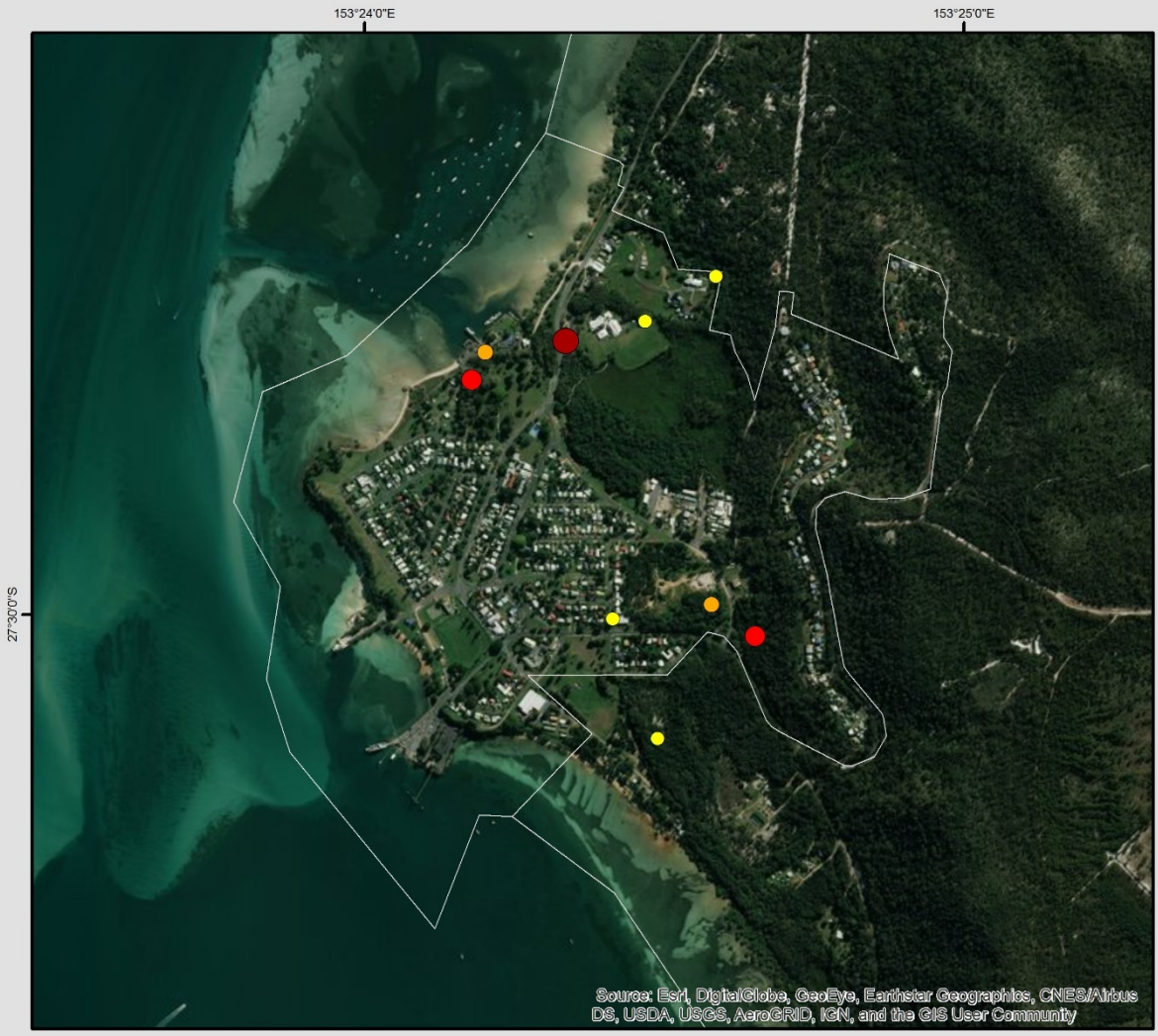
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold





Dunwich

N

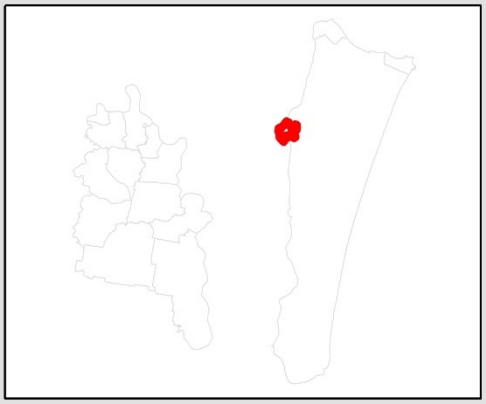
0 0.175 0.35 0.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

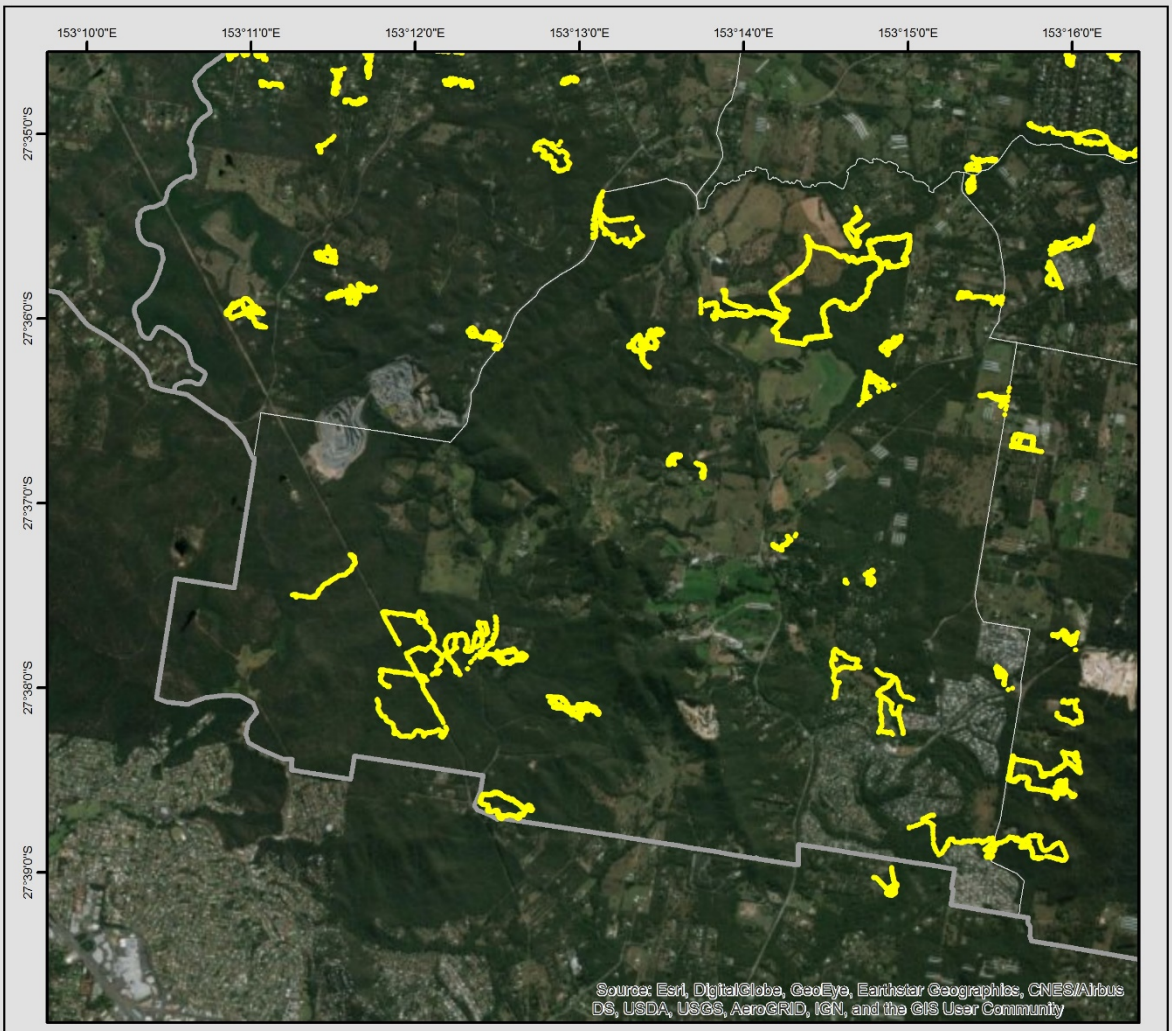
Legend

Internal relatedness

- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Mount Cotton



Mount Cotton

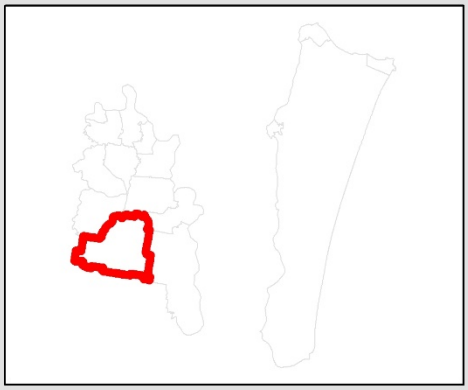
N

0 0.5 1 2
Kilometers

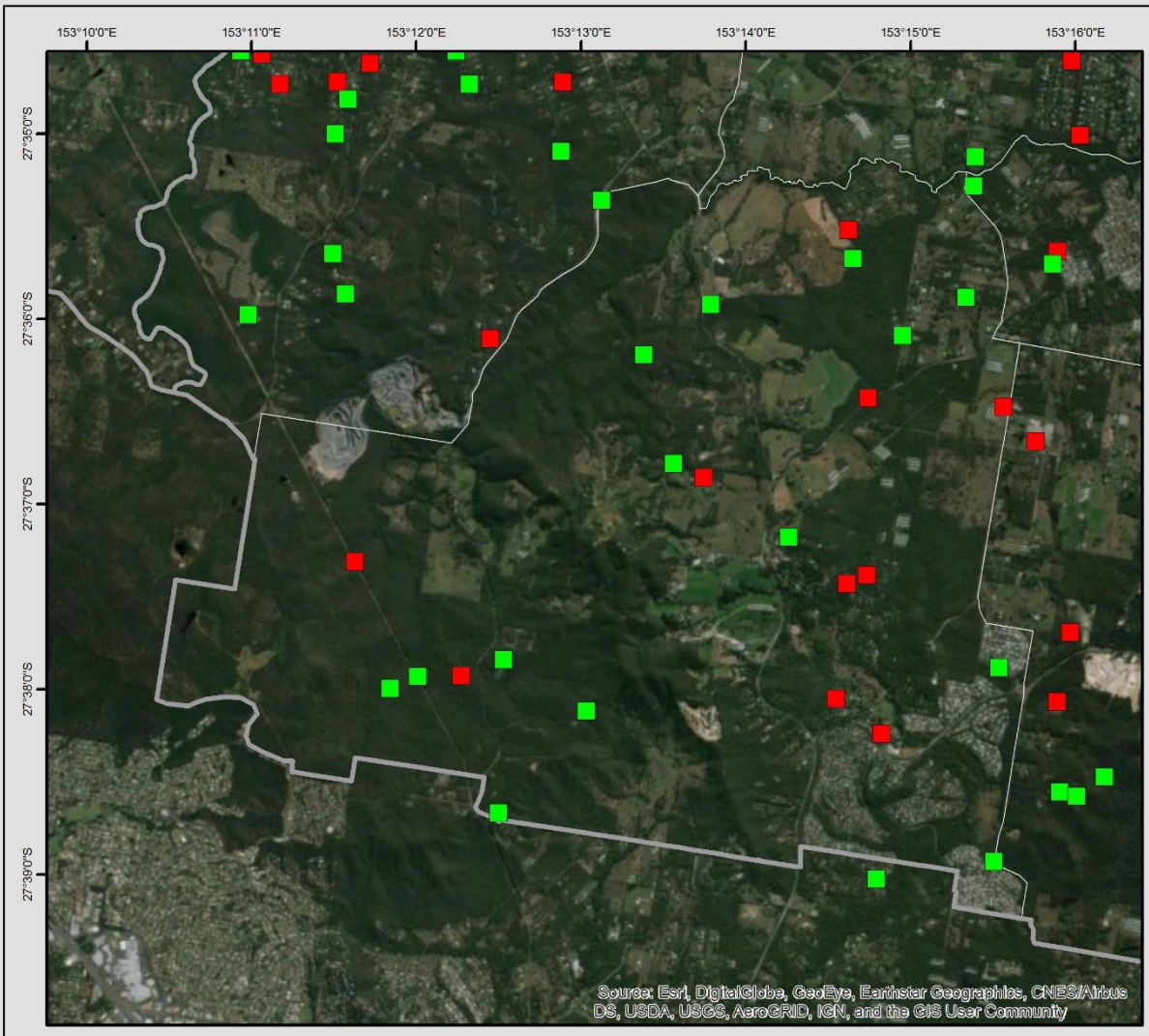
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog tracks



While every care has been taken to ensure the accuracy 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.



Mount Cotton

N

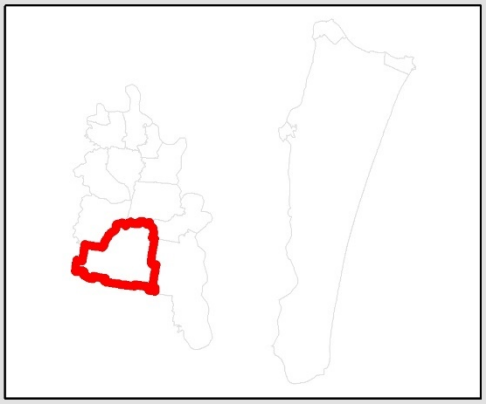
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

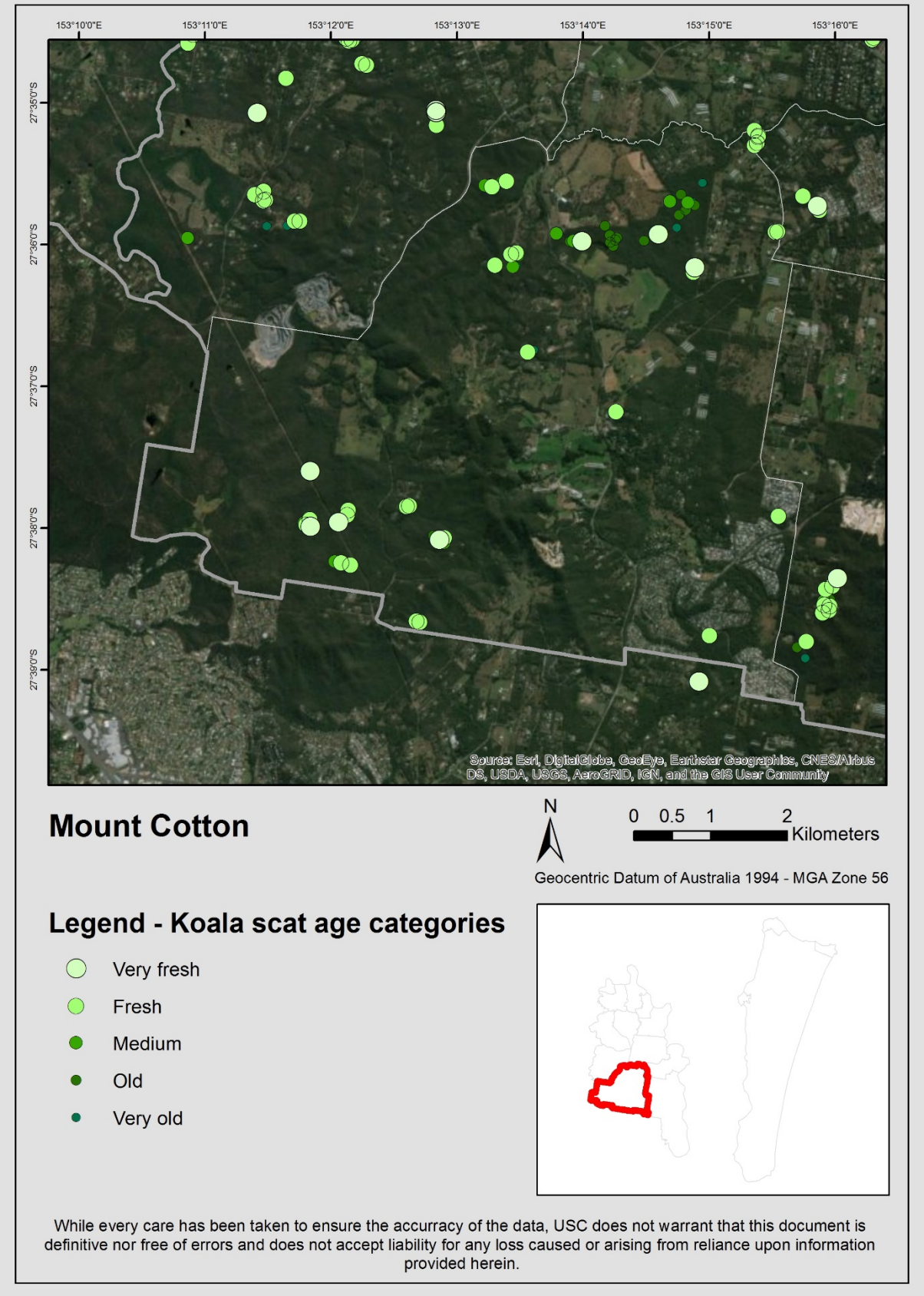
Legend

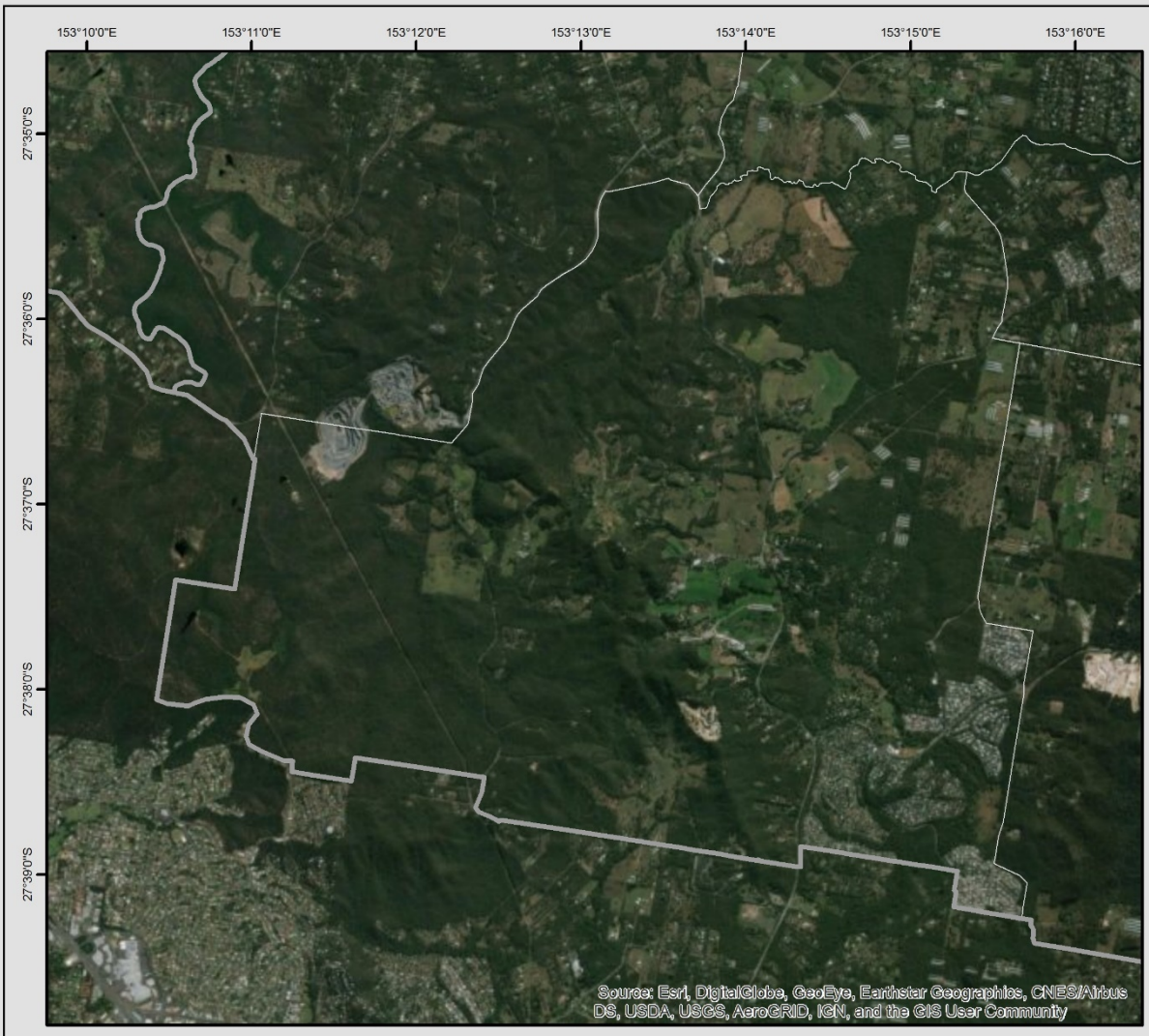
Koala scat

- Presence
- Absence



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.


153°10'0"E
153°11'0"E
153°12'0"E
153°13'0"E
153°14'0"E
153°15'0"E
153°16'0"E
27°35'0"S
27°36'0"S
27°37'0"S
27°38'0"S
27°39'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Mount Cotton
N
0 0.5 1 2
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend - Koala scat age categories
Very fresh
Fresh
Medium
Old
Very old
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.



Mount Cotton

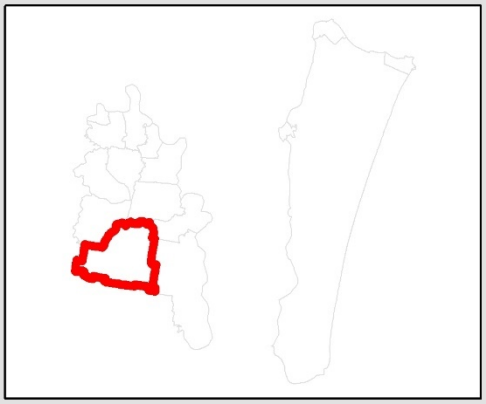
N

0 0.5 1 2
Kilometers

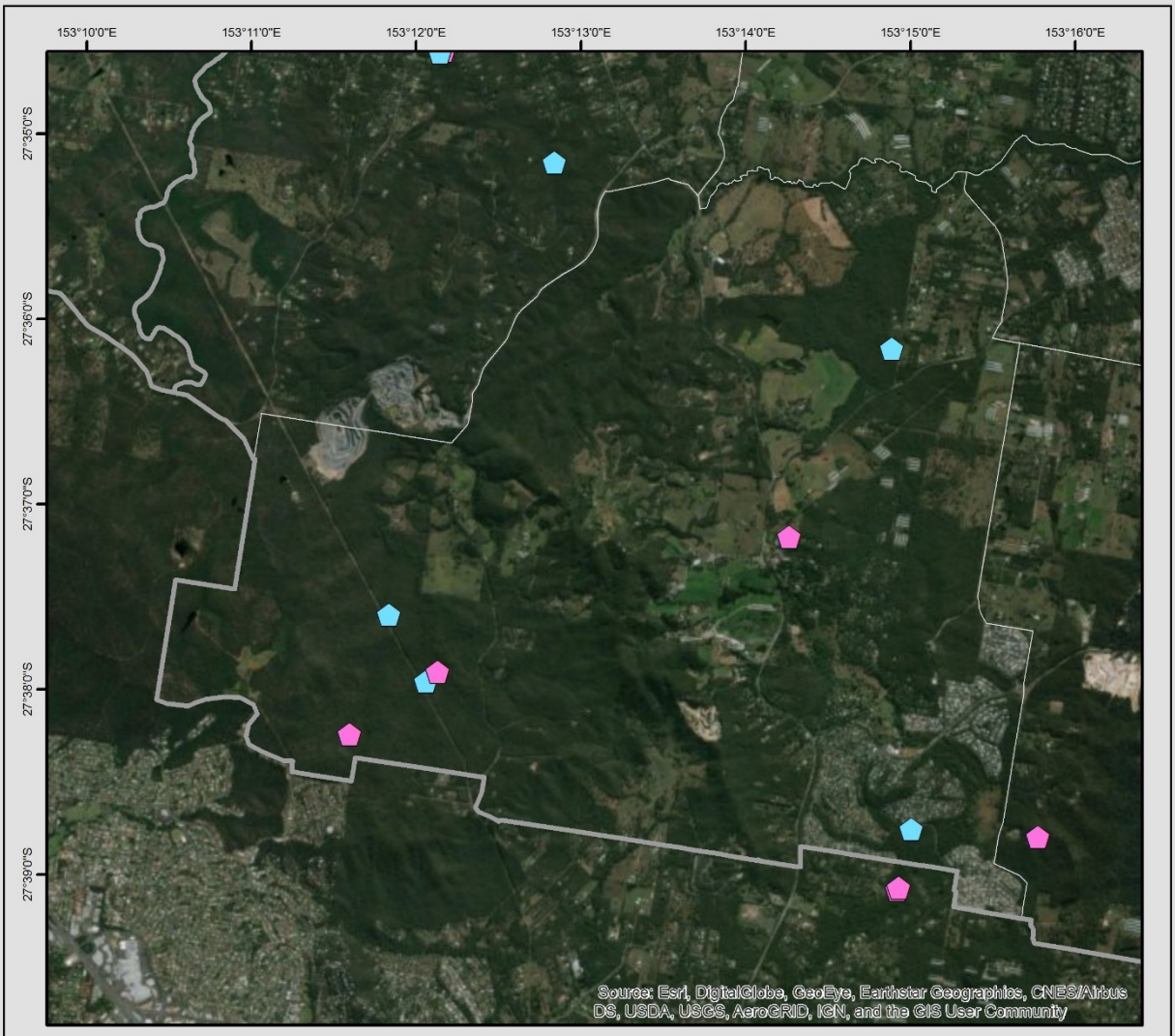
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Mount Cotton

N

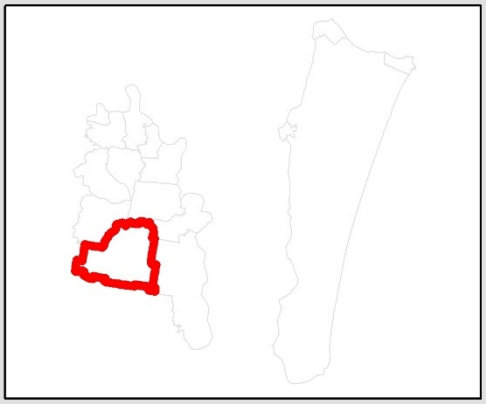
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

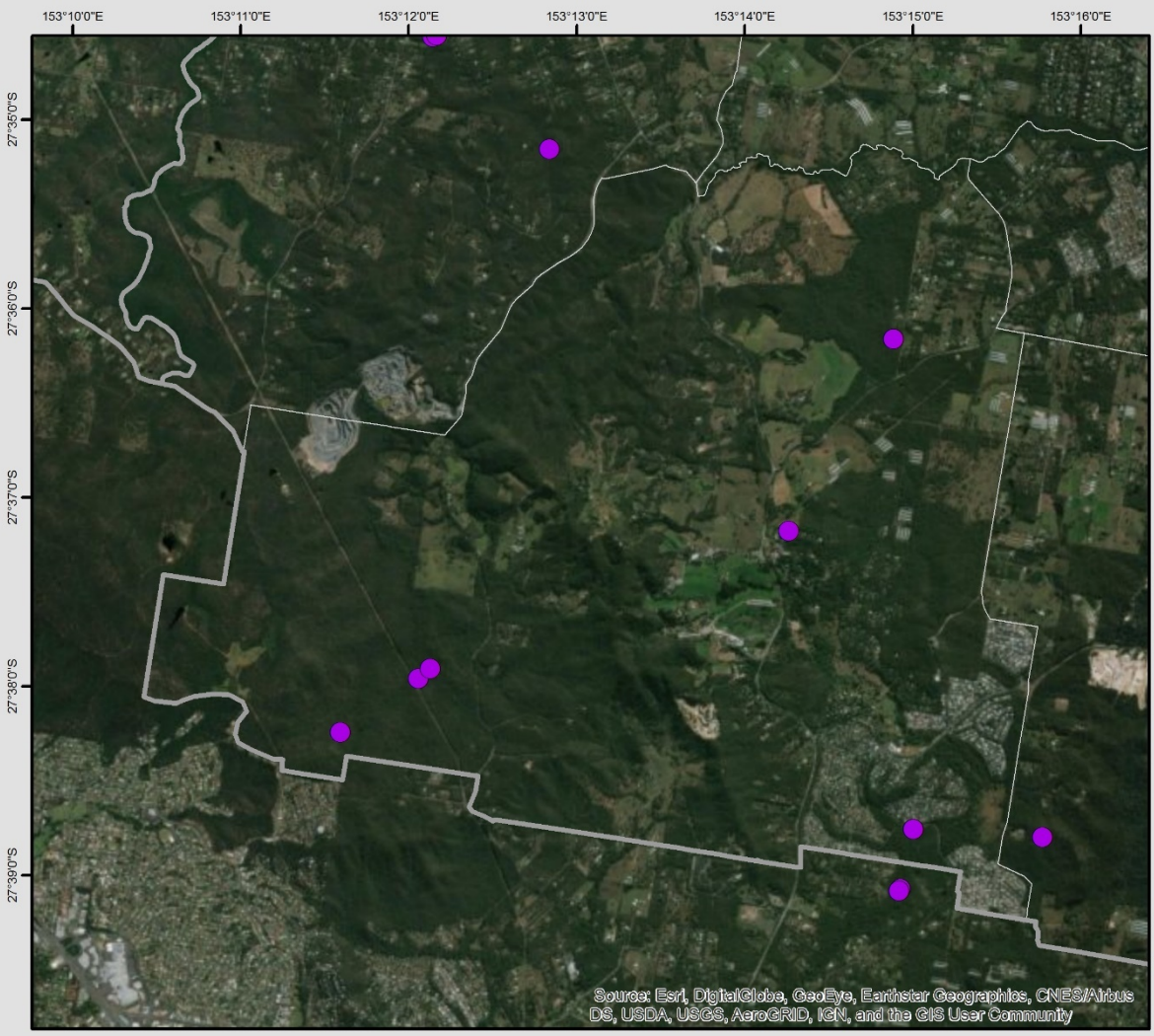
Legend

Koala sex (genetics)

- Female
- Male



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.



Mount Cotton

N

0 0.5 1 2
Kilometers

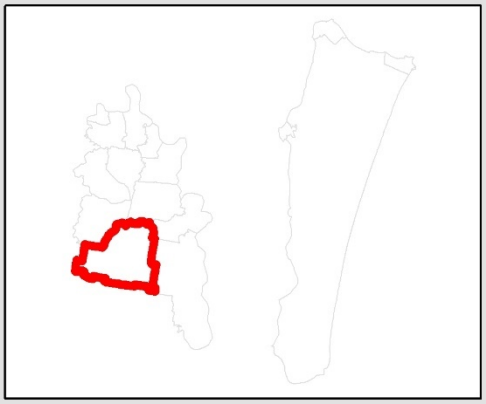
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

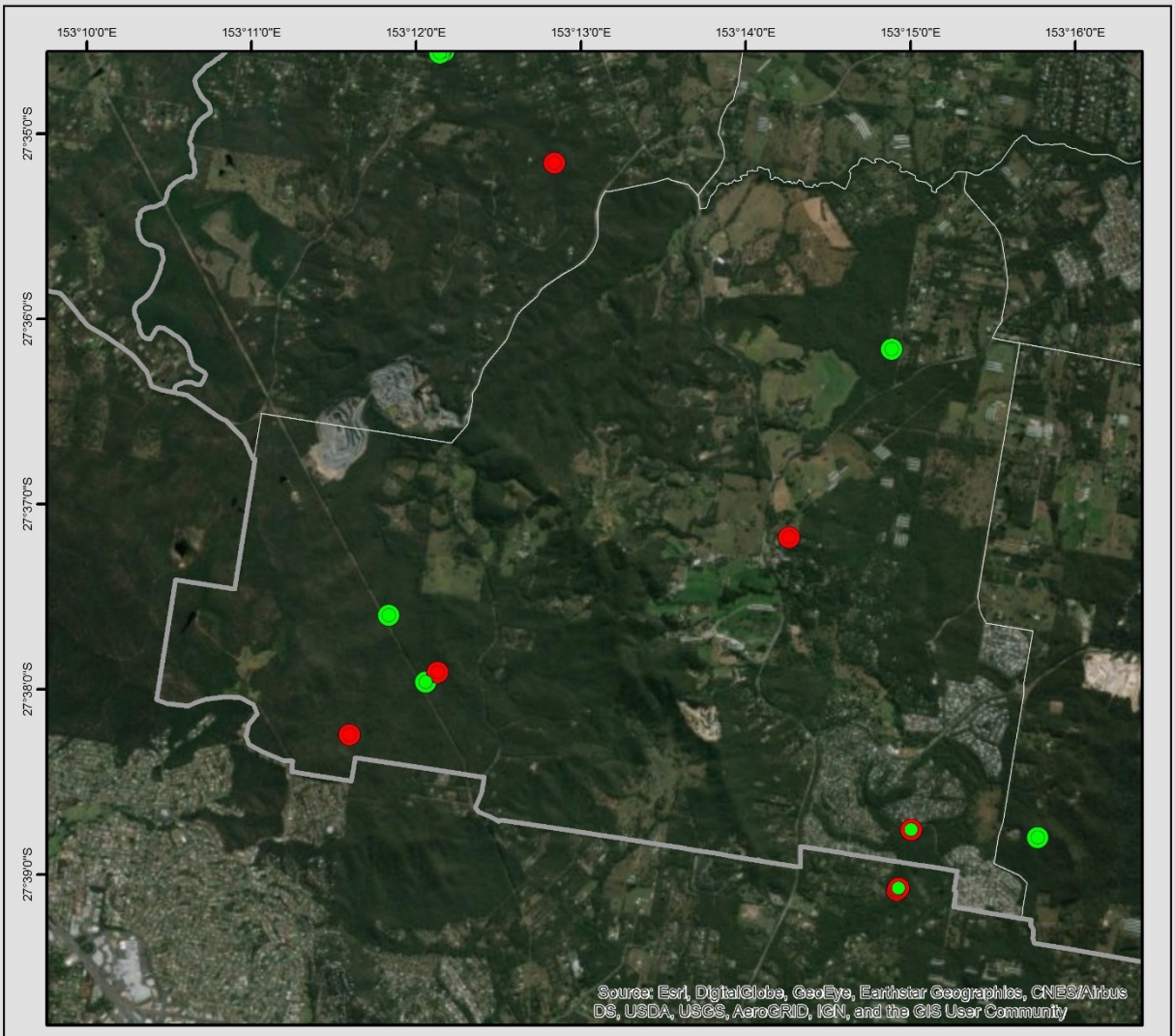
- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)



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.



Mount Cotton

N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

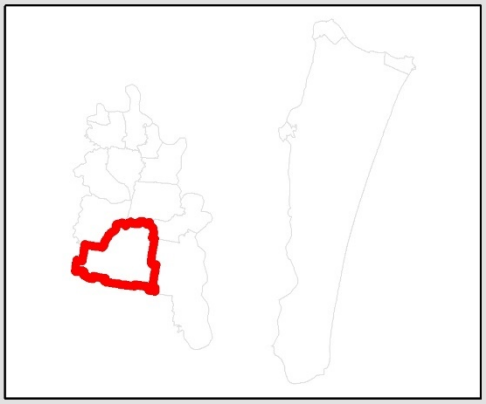
Legend - Chlamydia results

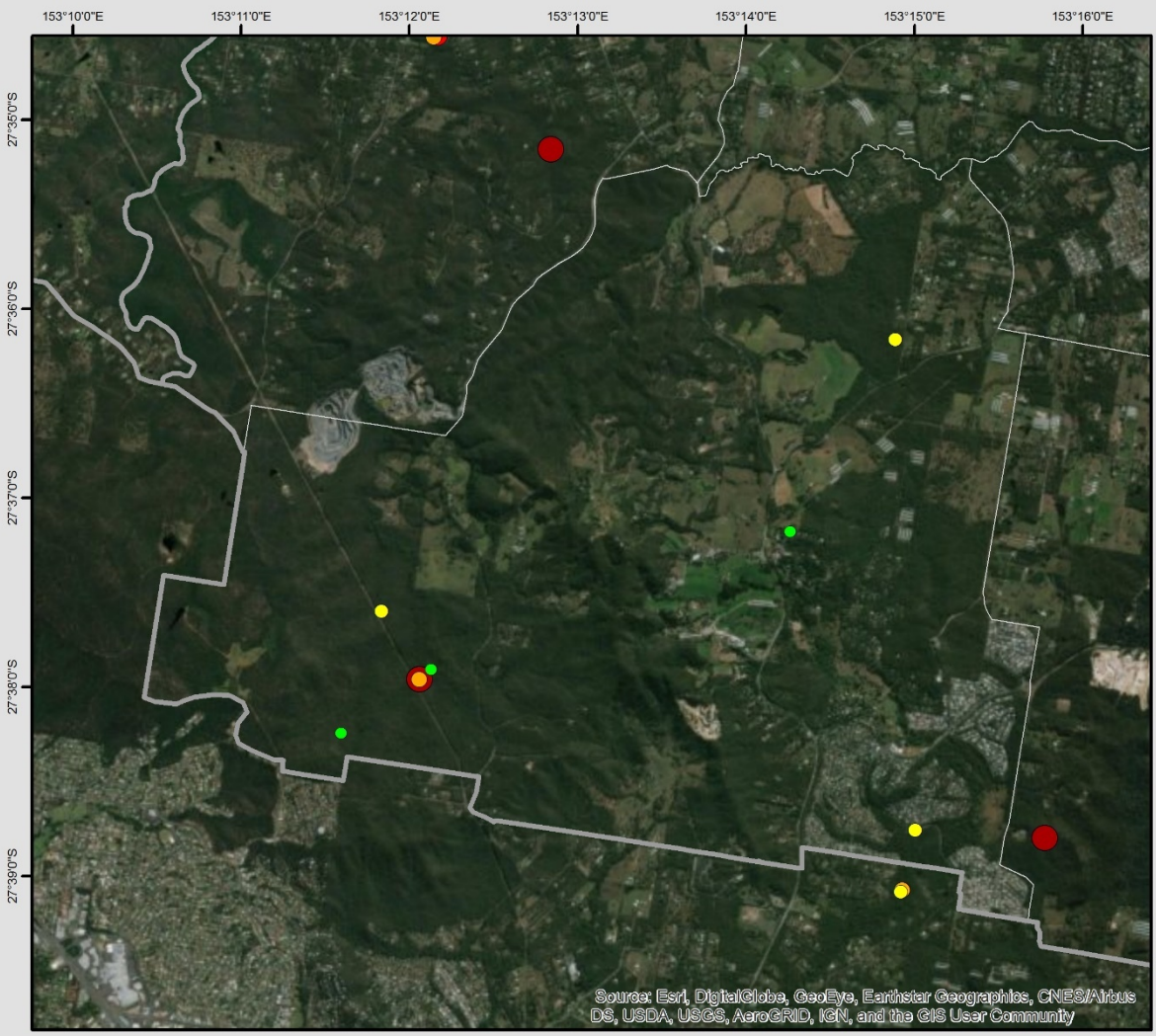
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold





Mount Cotton

N

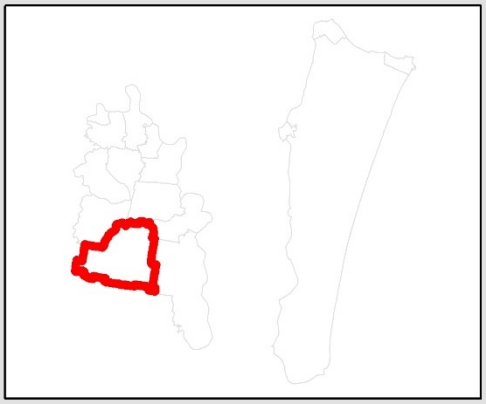
0 0.5 1 2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

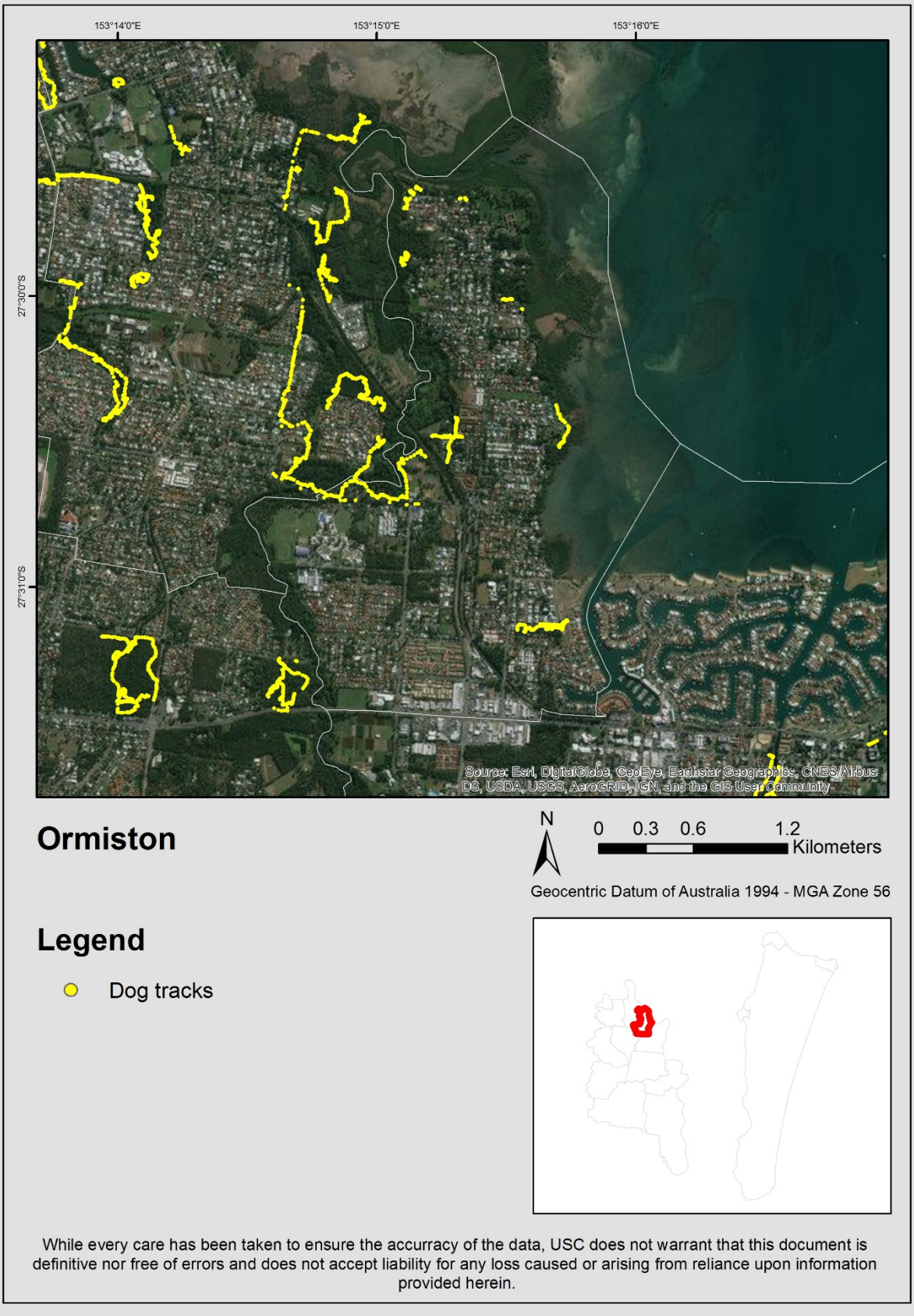
Legend

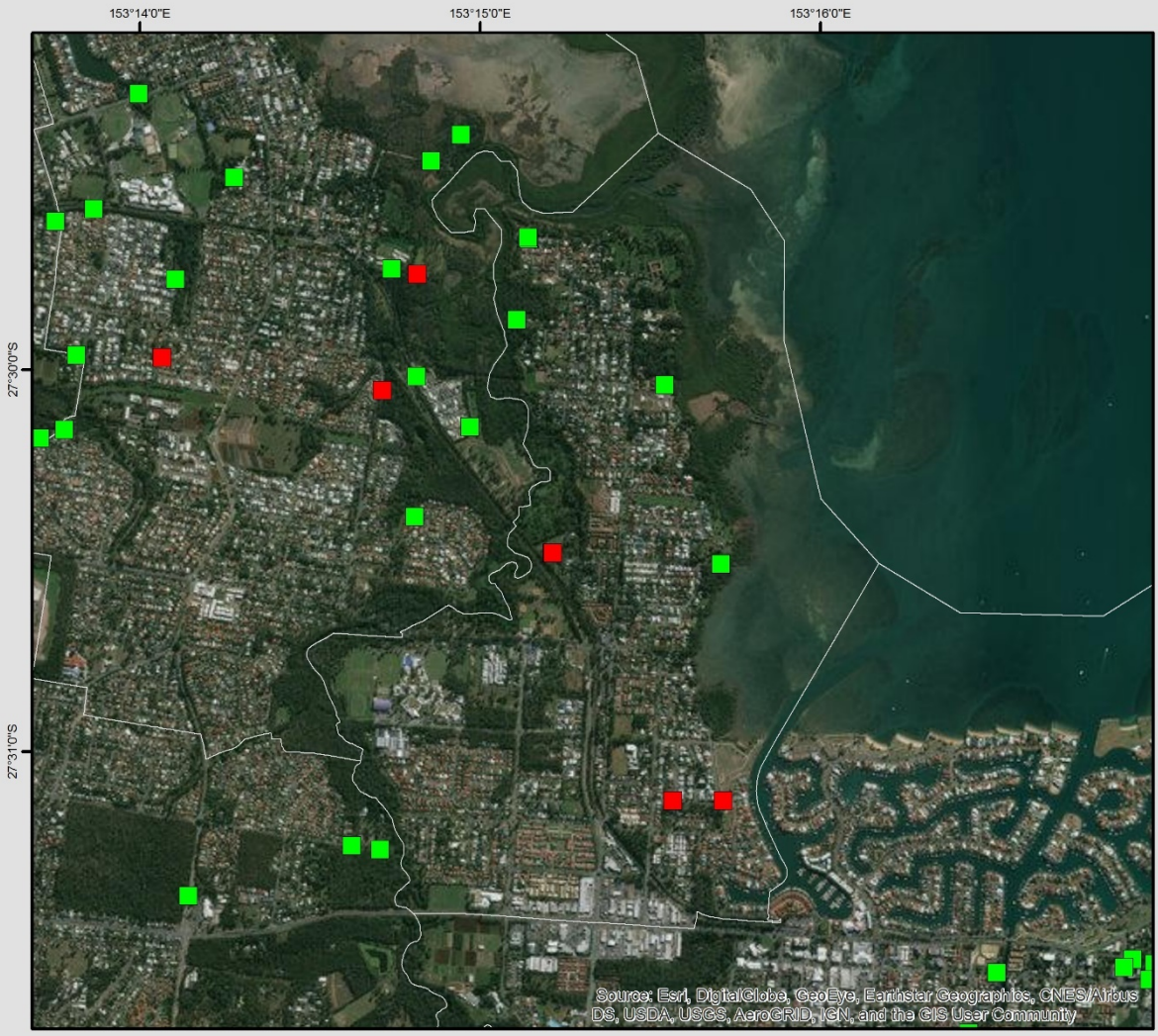
Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Ormiston


153°14'0"E
153°15'0"E
153°16'0"E
27°30'0"S
27°31'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Ormiston
N
0 0.3 0.6 1.2
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
While every care has been taken to ensure the accuracy 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.



Ormiston

N

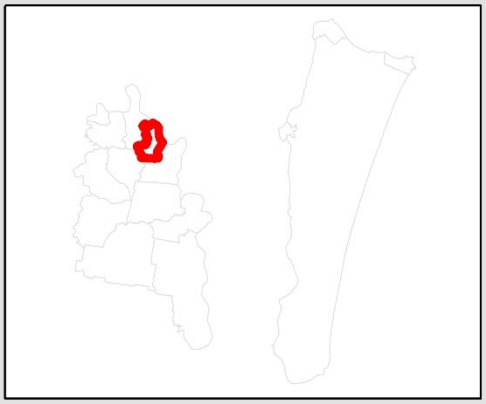
0 0.3 0.6 1.2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

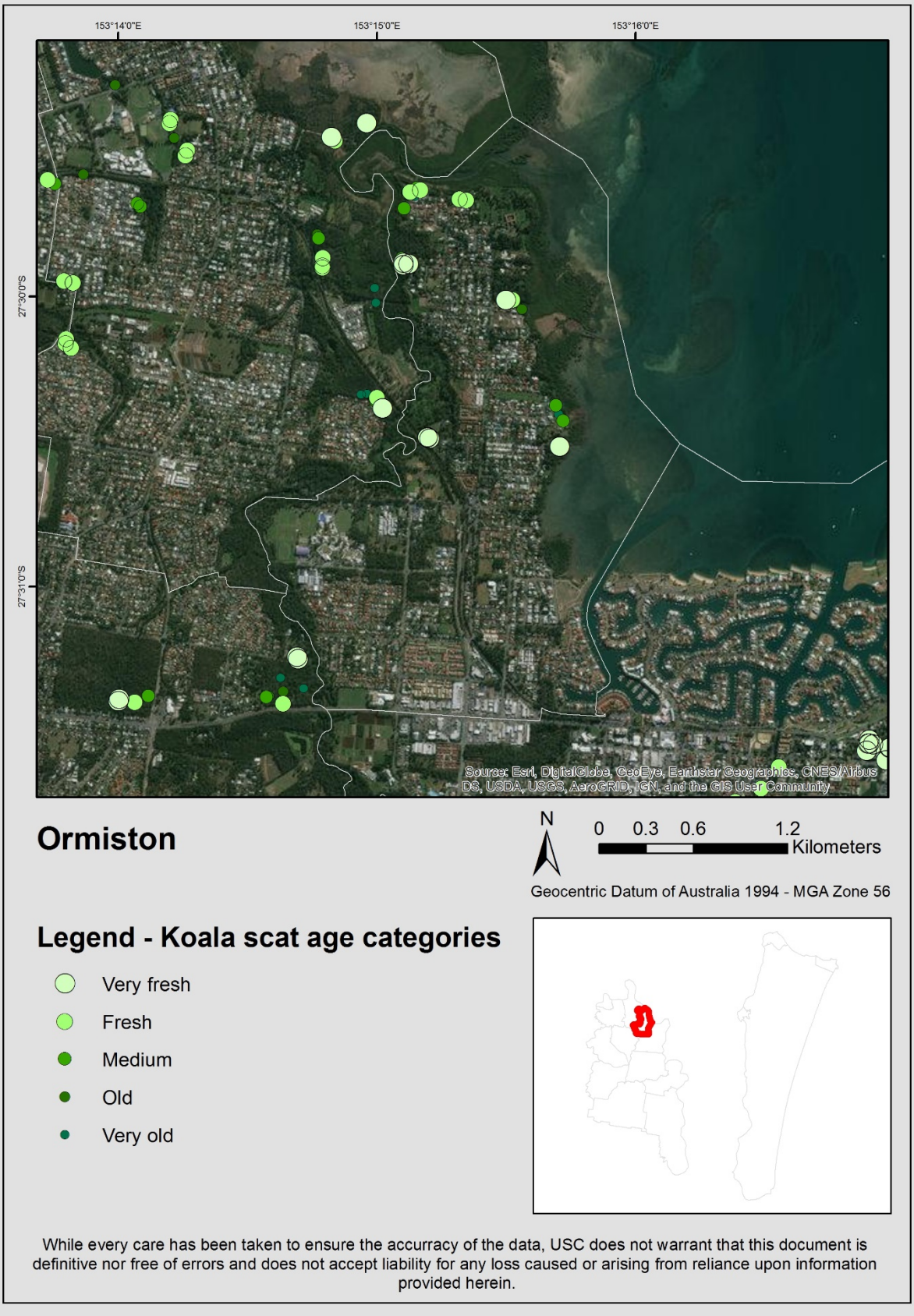
Legend

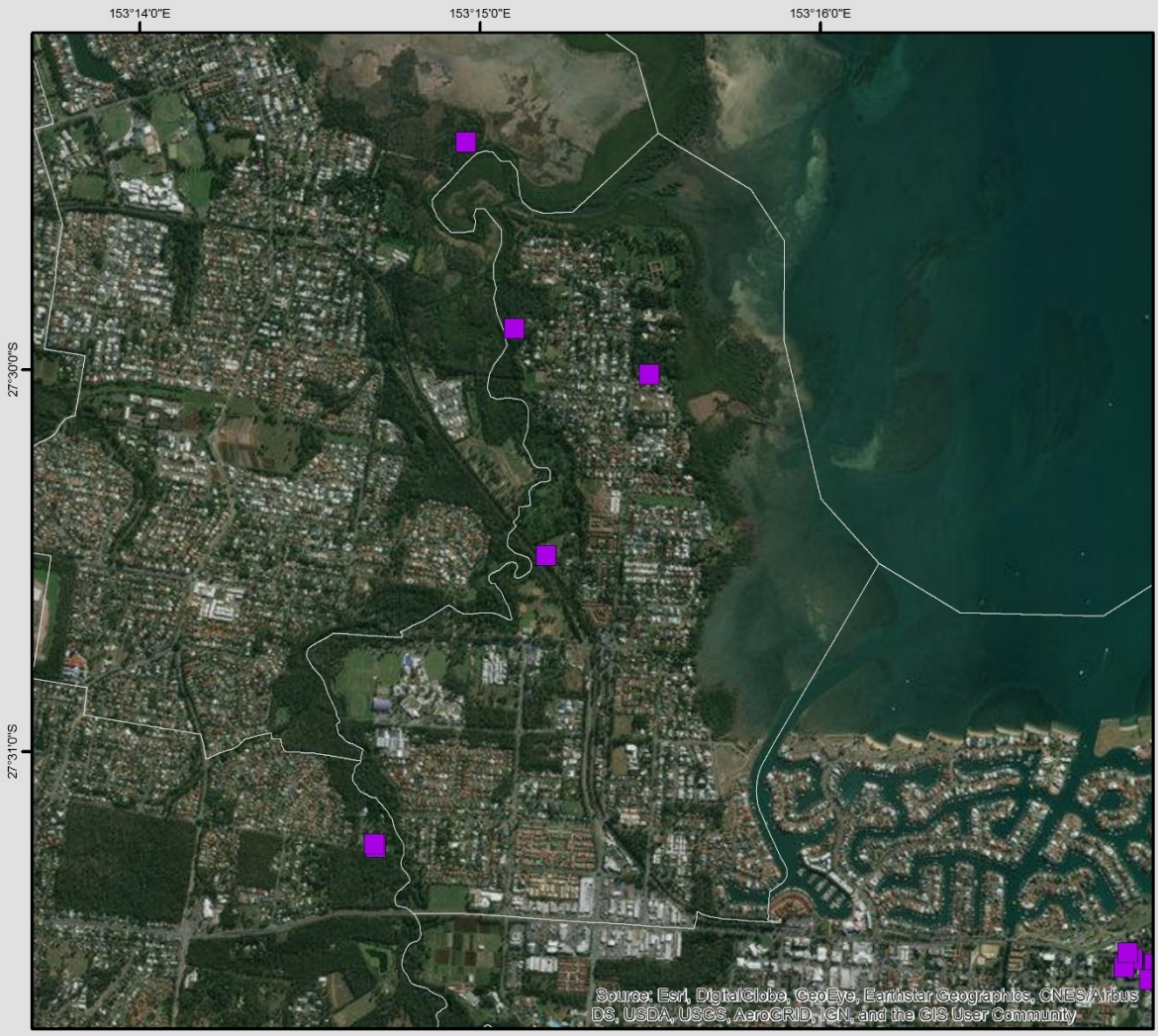
Koala scat

- Presence
- Absence



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.


153°14'0"E
153°15'0"E
153°16'0"E
27°30'0"S
27°31'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Ormiston
N
0 0.3 0.6 1.2
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend - Koala scat age categories
Very fresh
Fresh
Medium
Old
Very old
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.



Ormiston

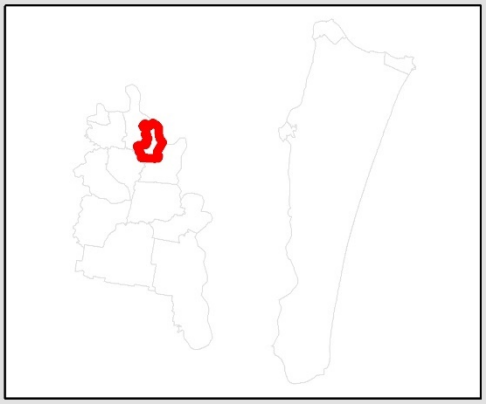
N

0 0.3 0.6 1.2 Kilometers

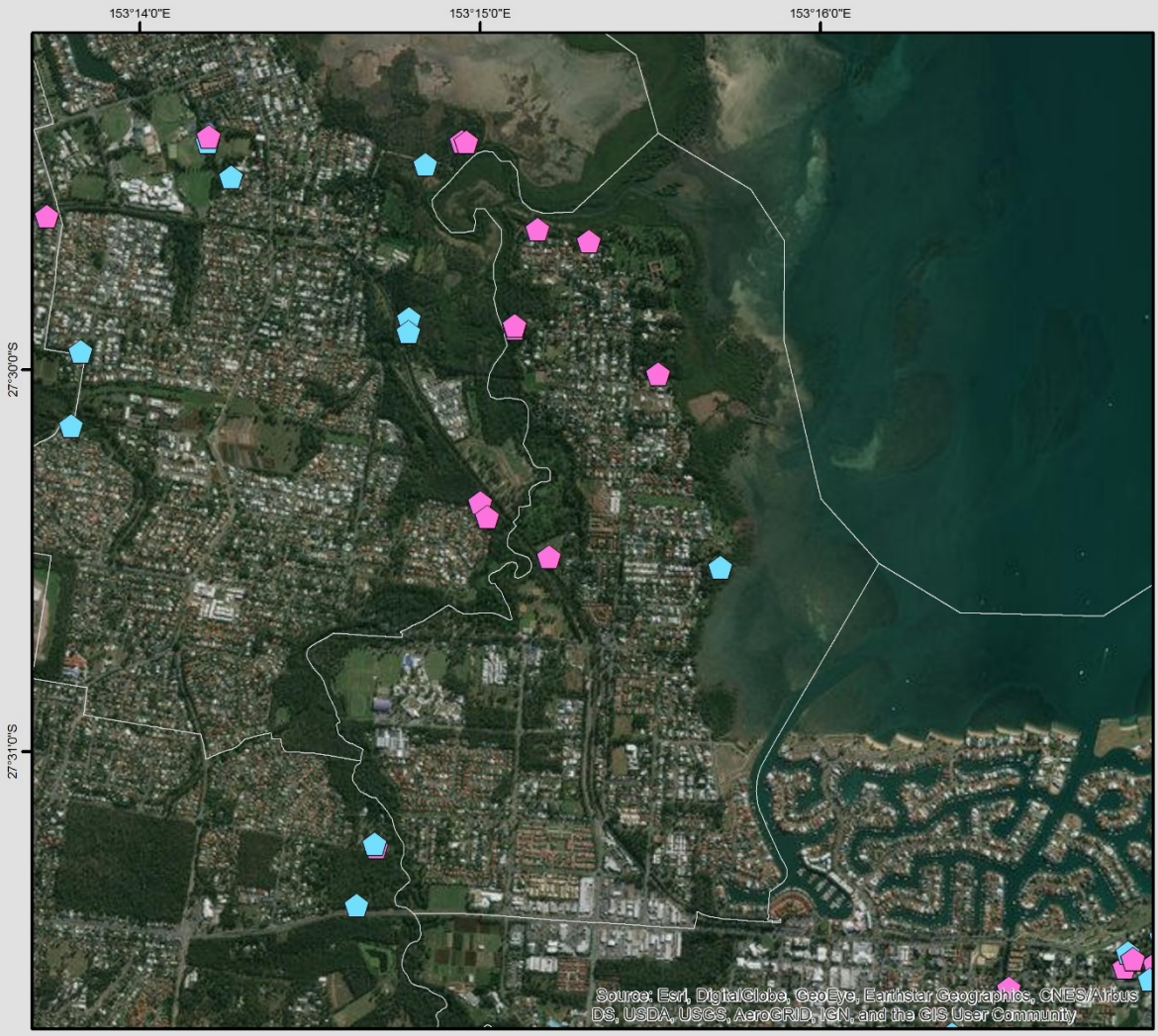
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Ormiston

N

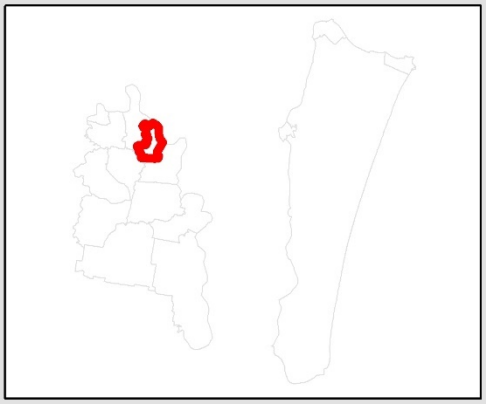
0 0.3 0.6 1.2 Kilometers

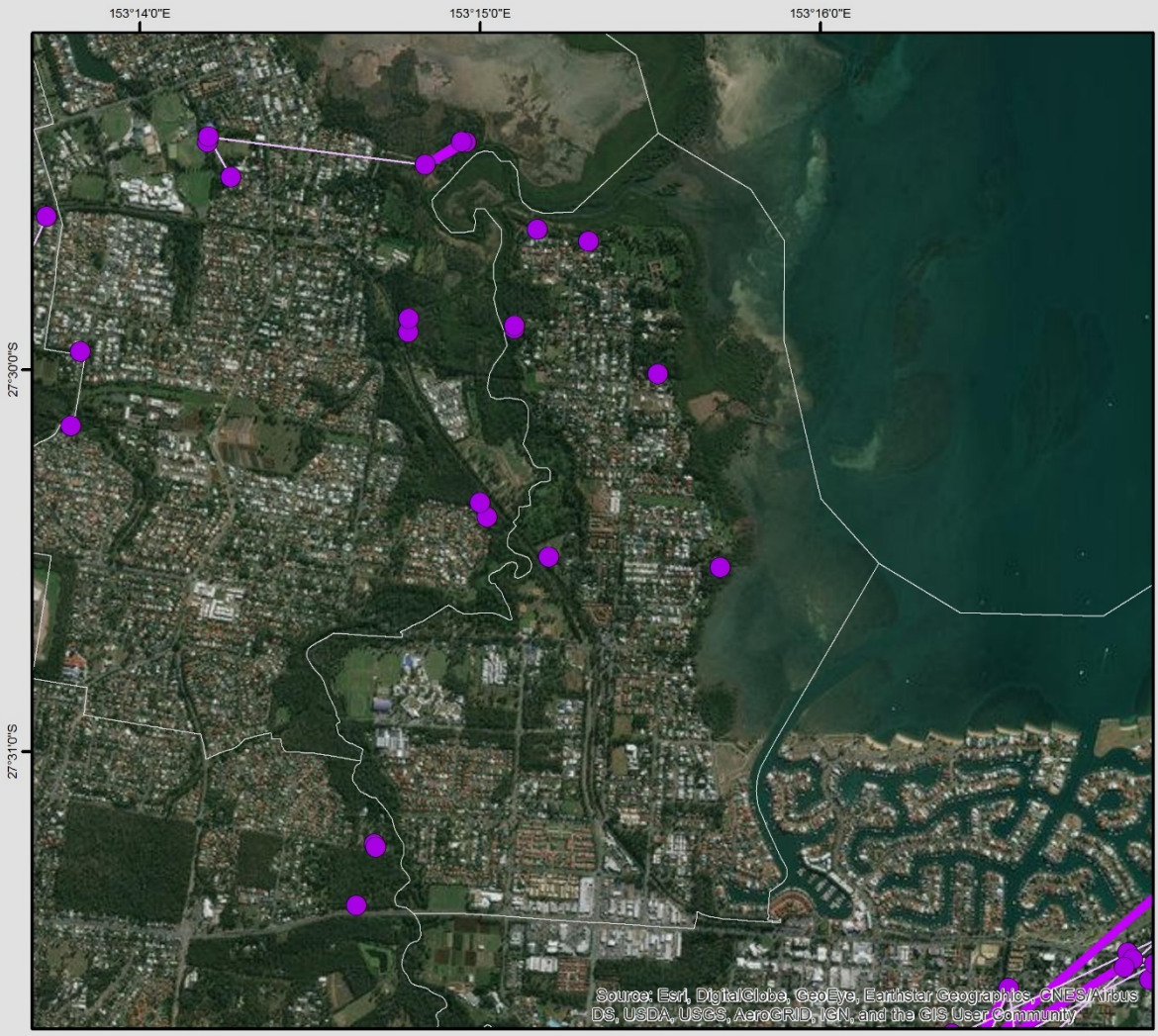
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male





Ormiston

N

0 0.3 0.6 1.2
Kilometers

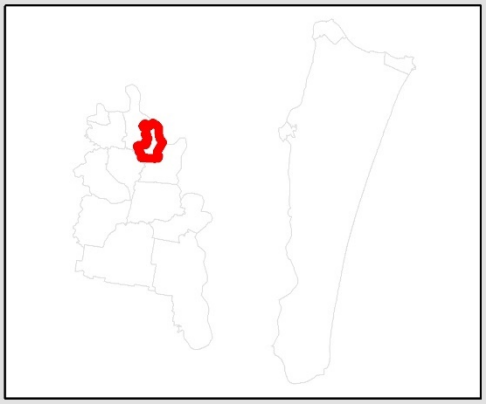
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

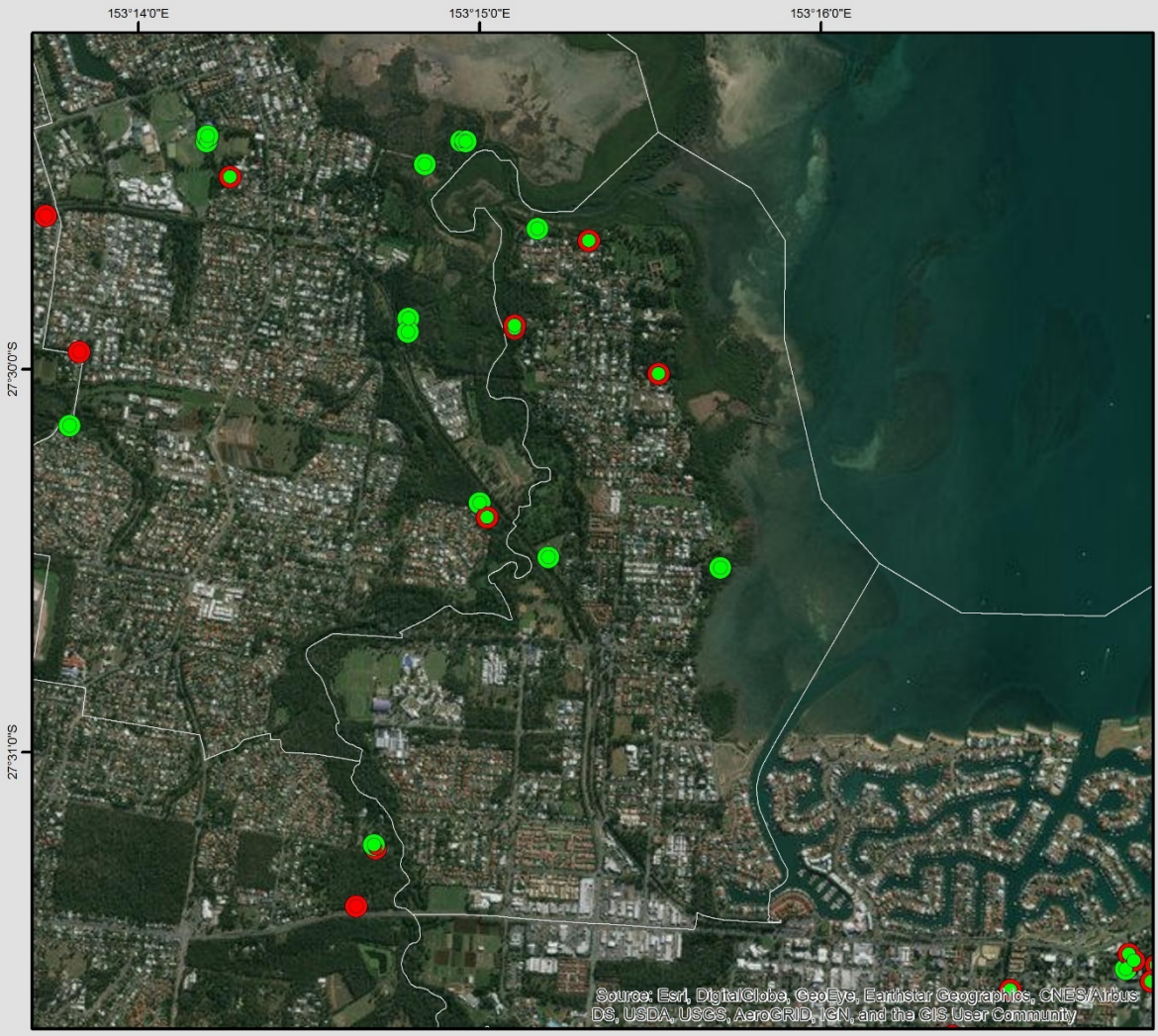
- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)



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.



Ormiston

N

0 0.3 0.6 1.2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

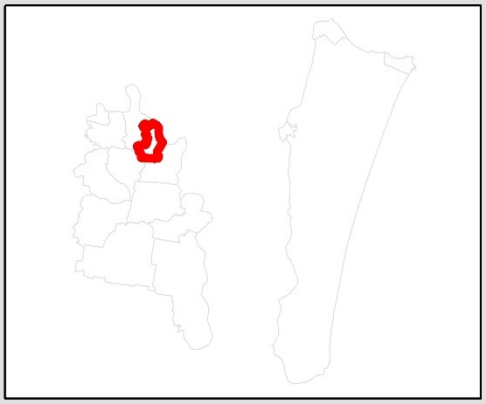
Legend - Chlamydia results

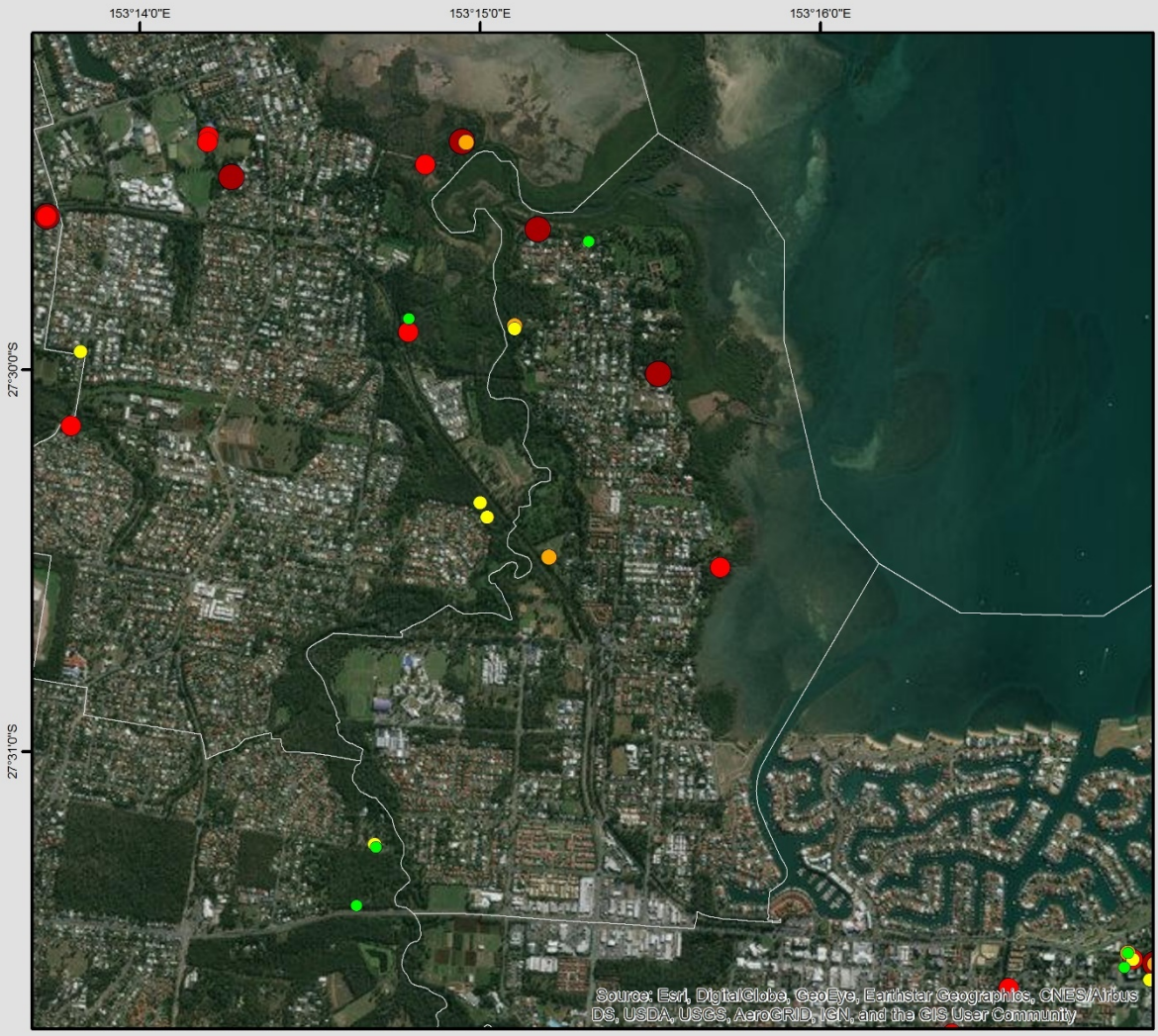
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold





Ormiston

N

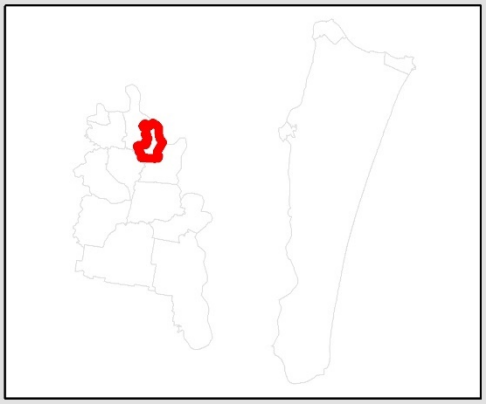
0 0.3 0.6 1.2
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

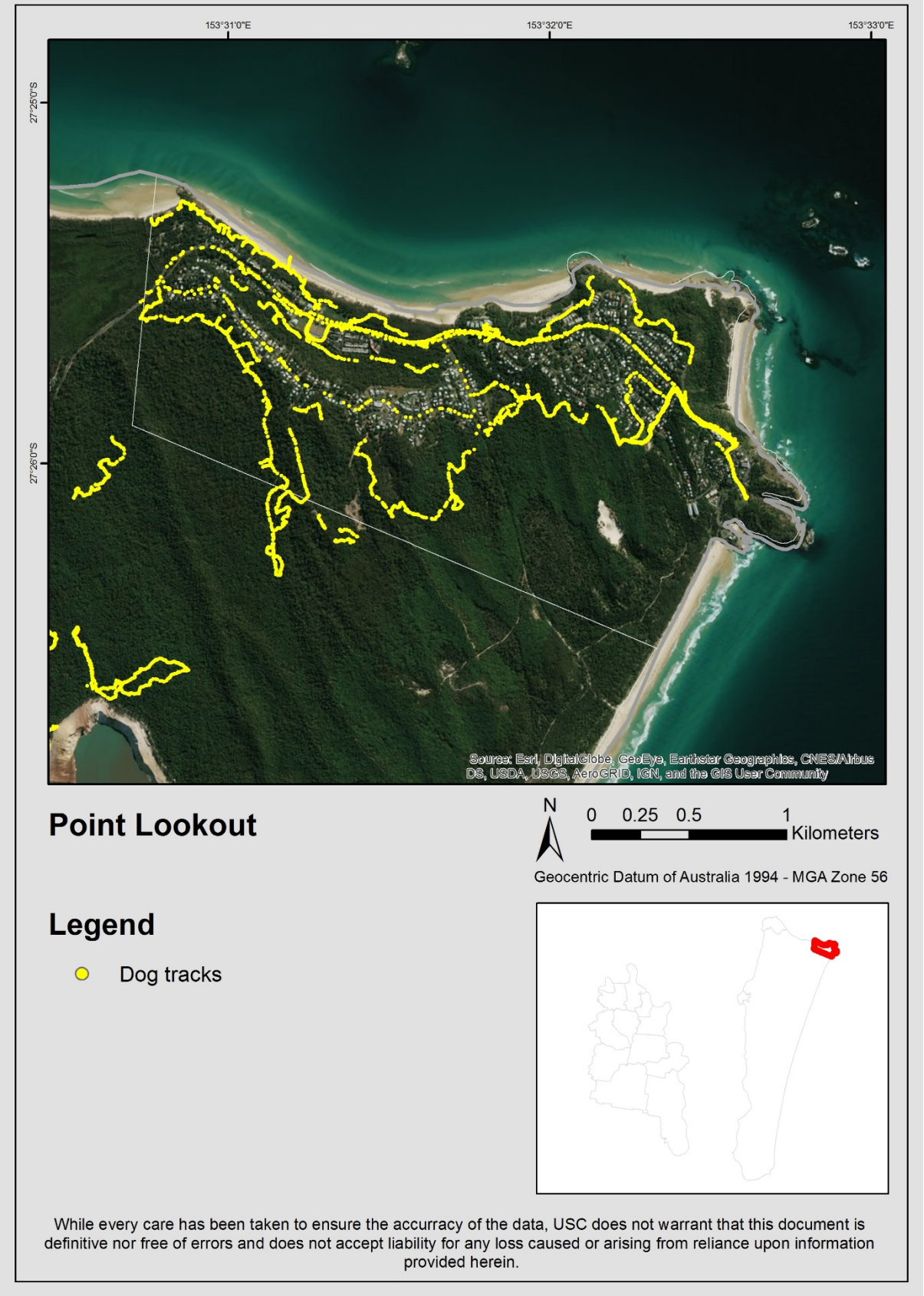
Legend

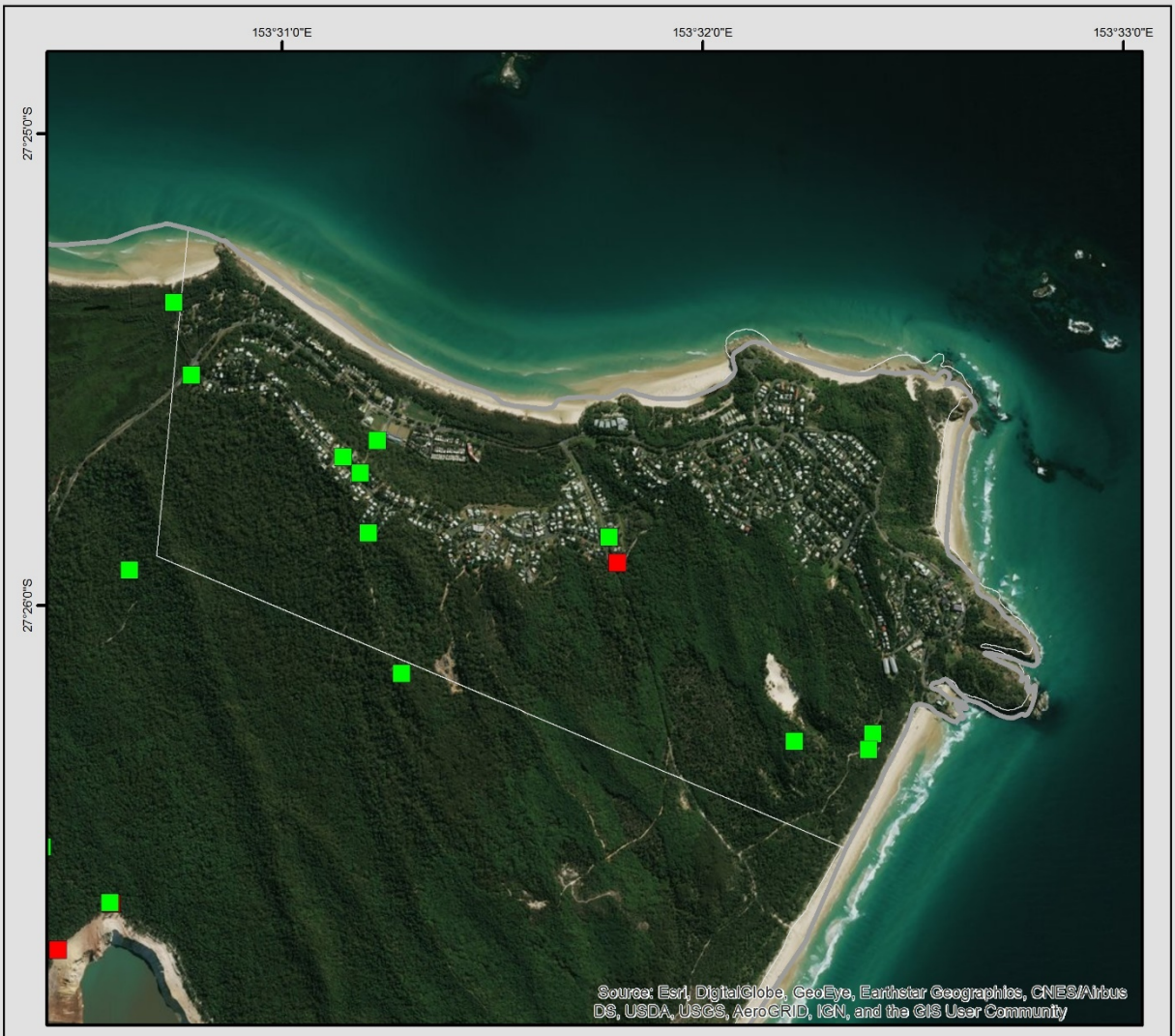
Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Point Lookout


153°31'0"E
153°32'0"E
153°33'0"E
27°25'0"S
27°26'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Point Lookout
N
0 0.25 0.5 1
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
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.



Point Lookout

N

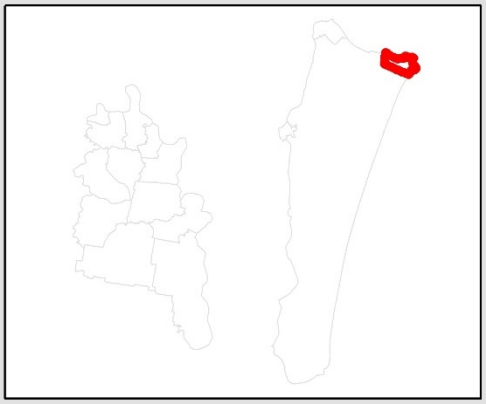
0 0.225 0.45 0.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

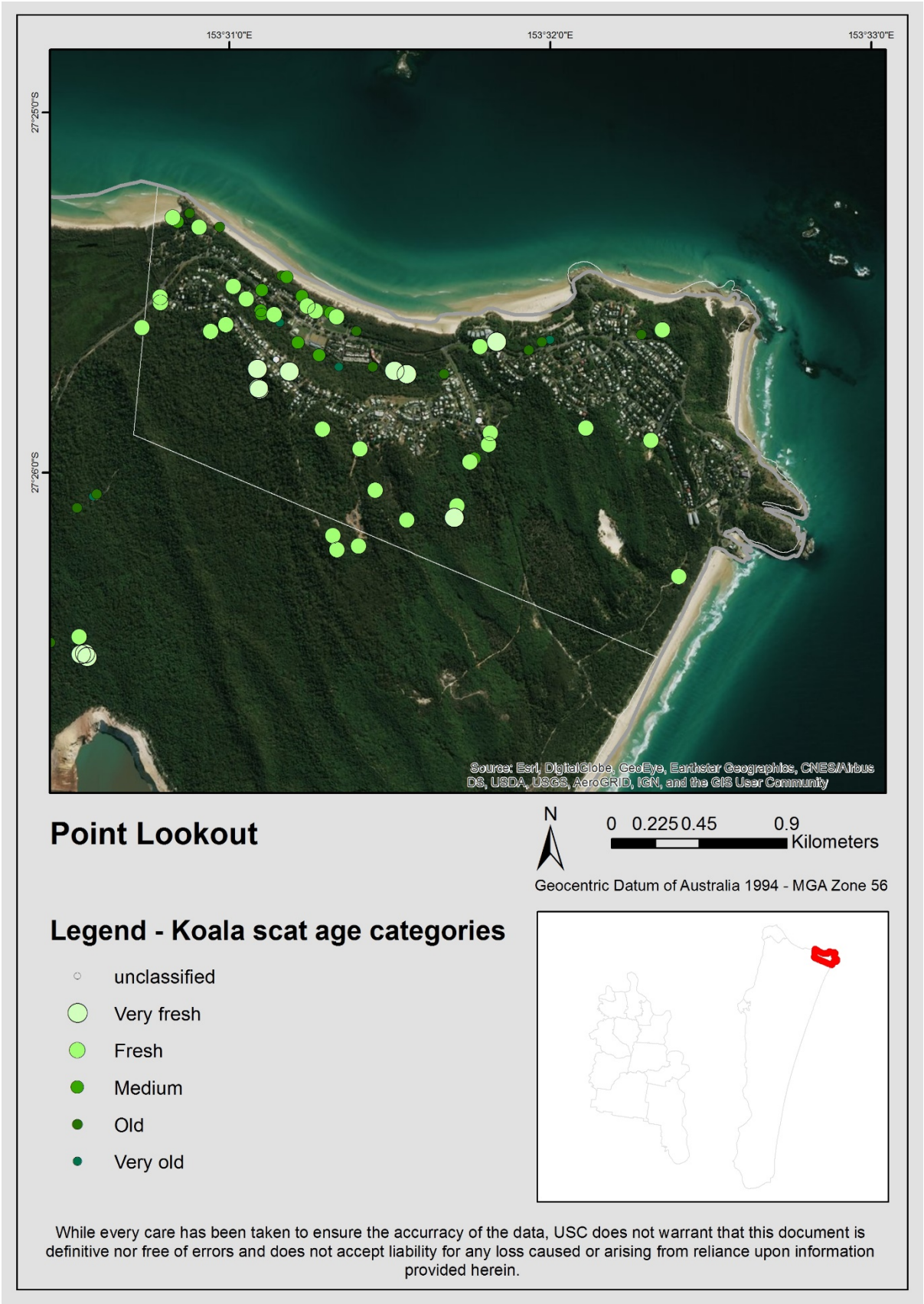
Legend

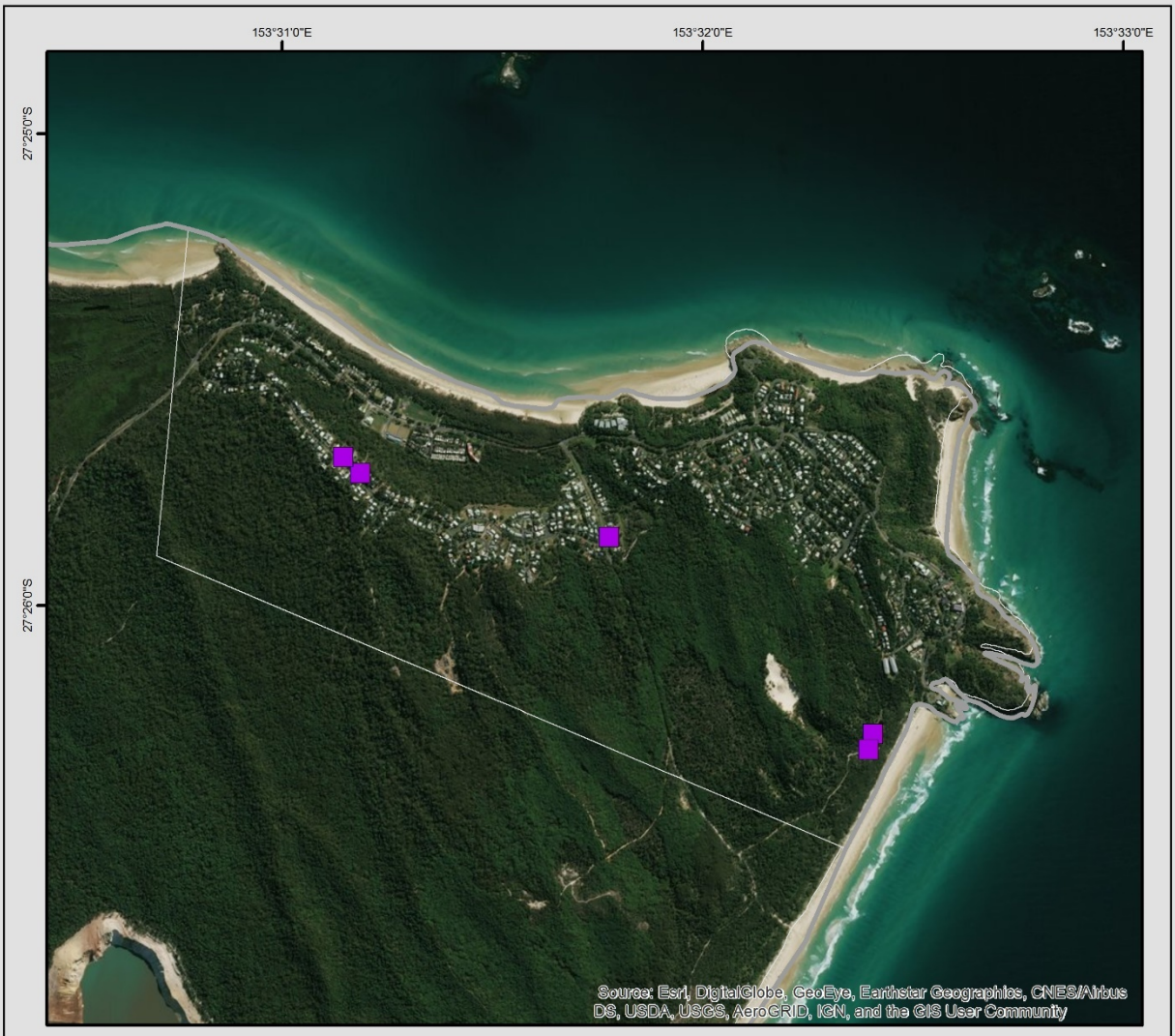
Koala scat

- Presence
- Absence



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.


153°31'0"E
153°32'0"E
153°33'0"E
27°25'0"S
27°26'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Point Lookout
N
0 0.225 0.45 0.9
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend - Koala scat age categories
unclassified
Very fresh
Fresh
Medium
Old
Very old
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.



Point Lookout

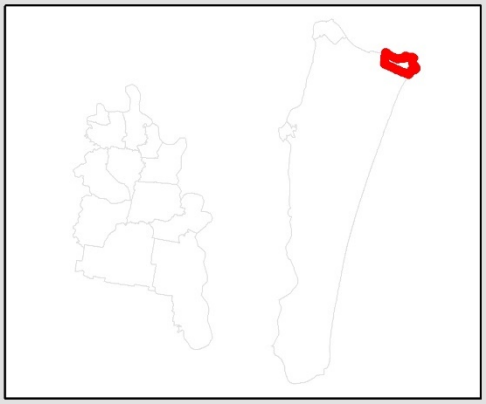
N

0 0.225 0.45 0.9
Kilometers

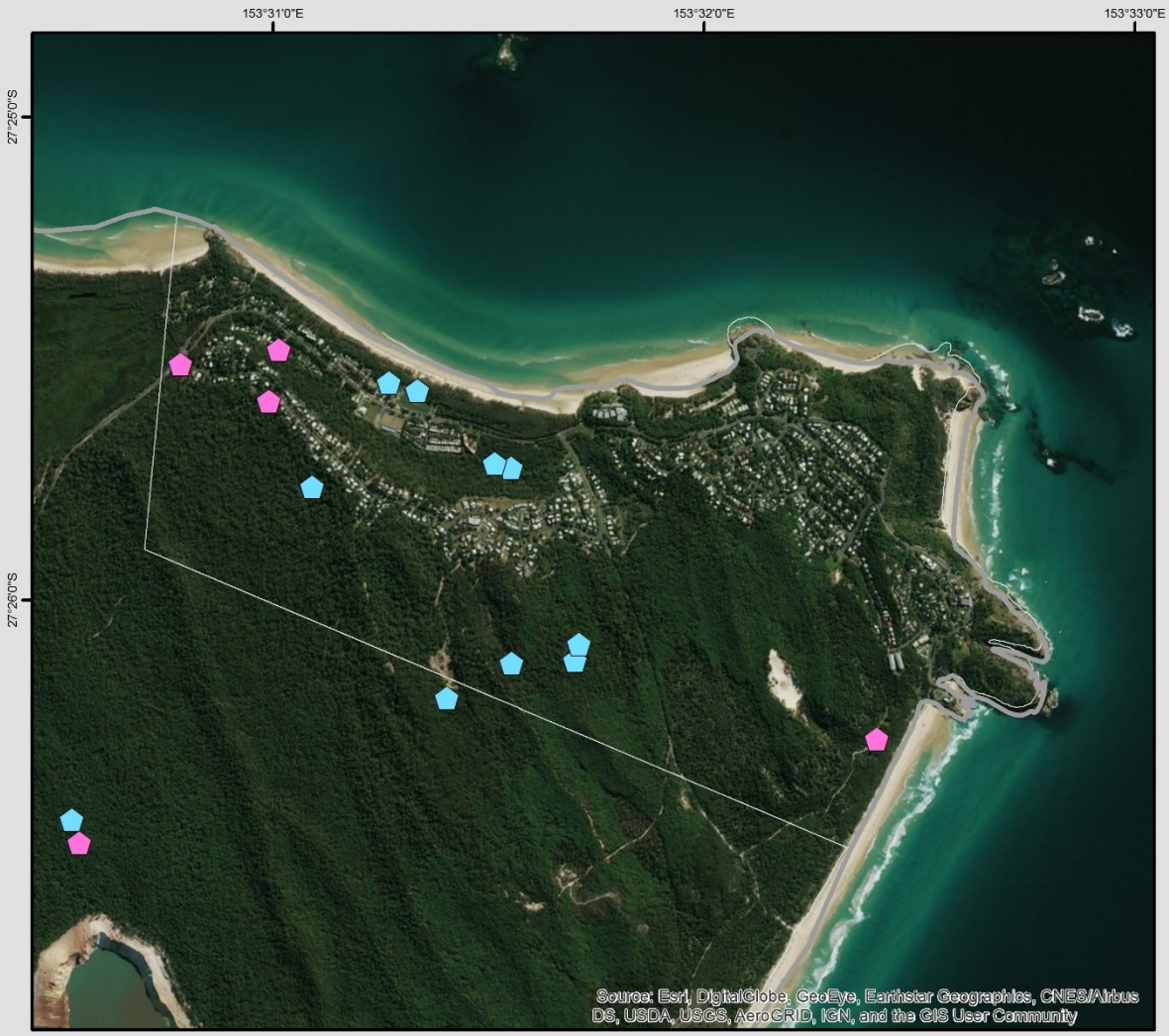
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Point Lookout

N

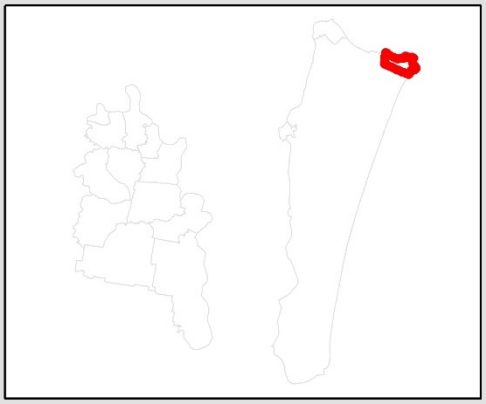
0 0.225 0.45 0.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

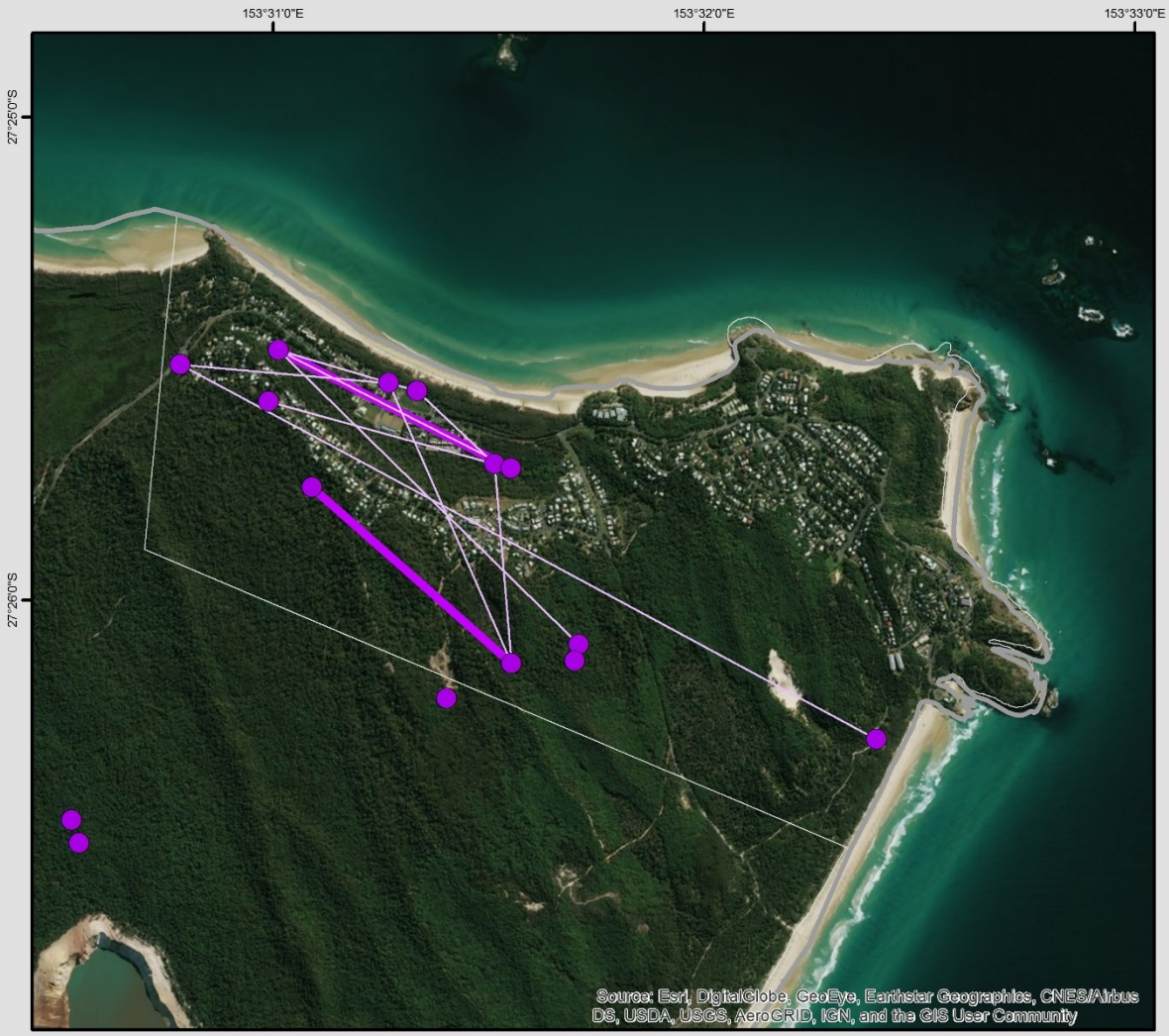
Legend

Koala sex (genetics)

- Female
- Male



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.



Point Lookout

N

0 0.225 0.45 0.9
Kilometers

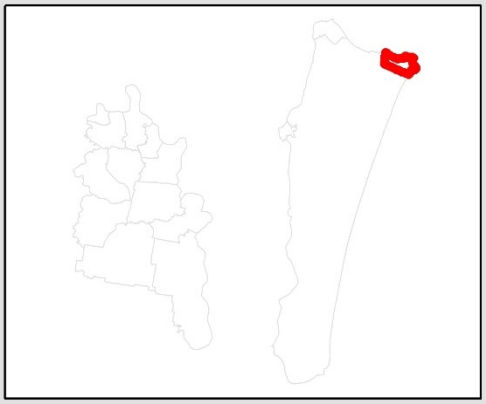
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

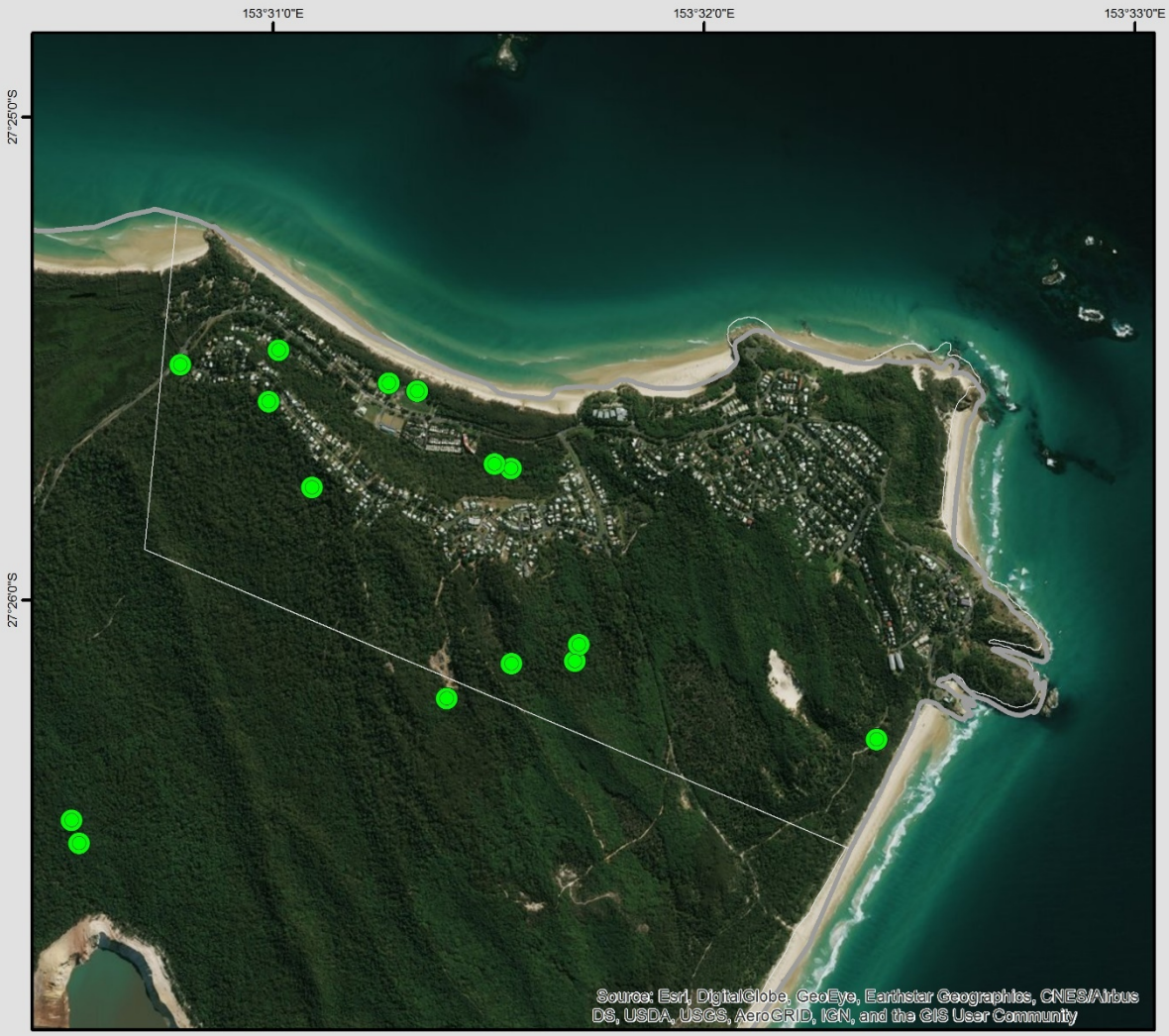
- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)



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.



Point Lookout

N

0 0.25 0.5 1 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

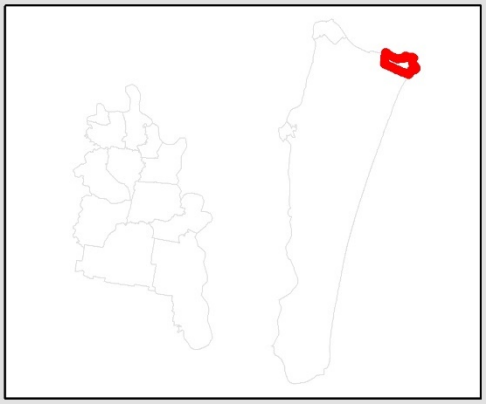
Legend - Chlamydia results

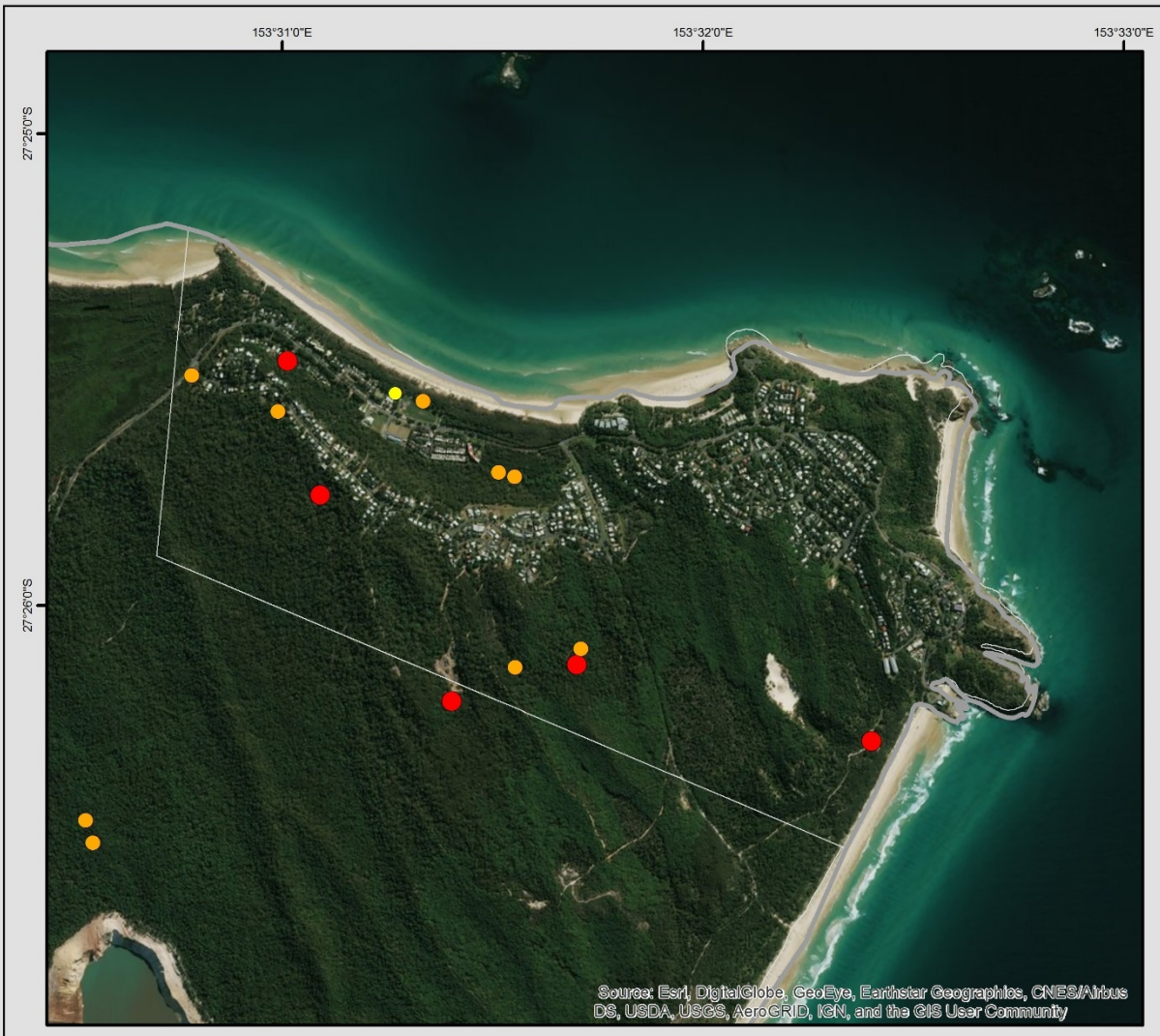
Qualitative

- No Chlamydia dectected

Quantitative

- Chlamydia load under threshold





Point Lookout

N

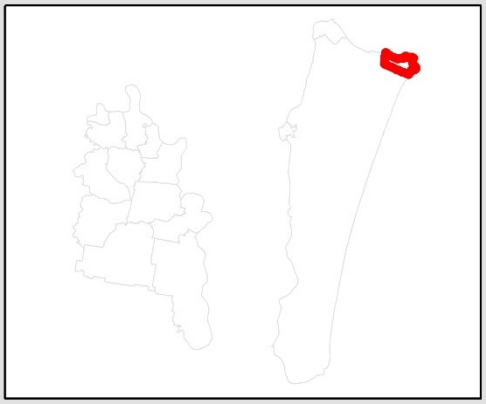
0 0.25 0.5 1 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

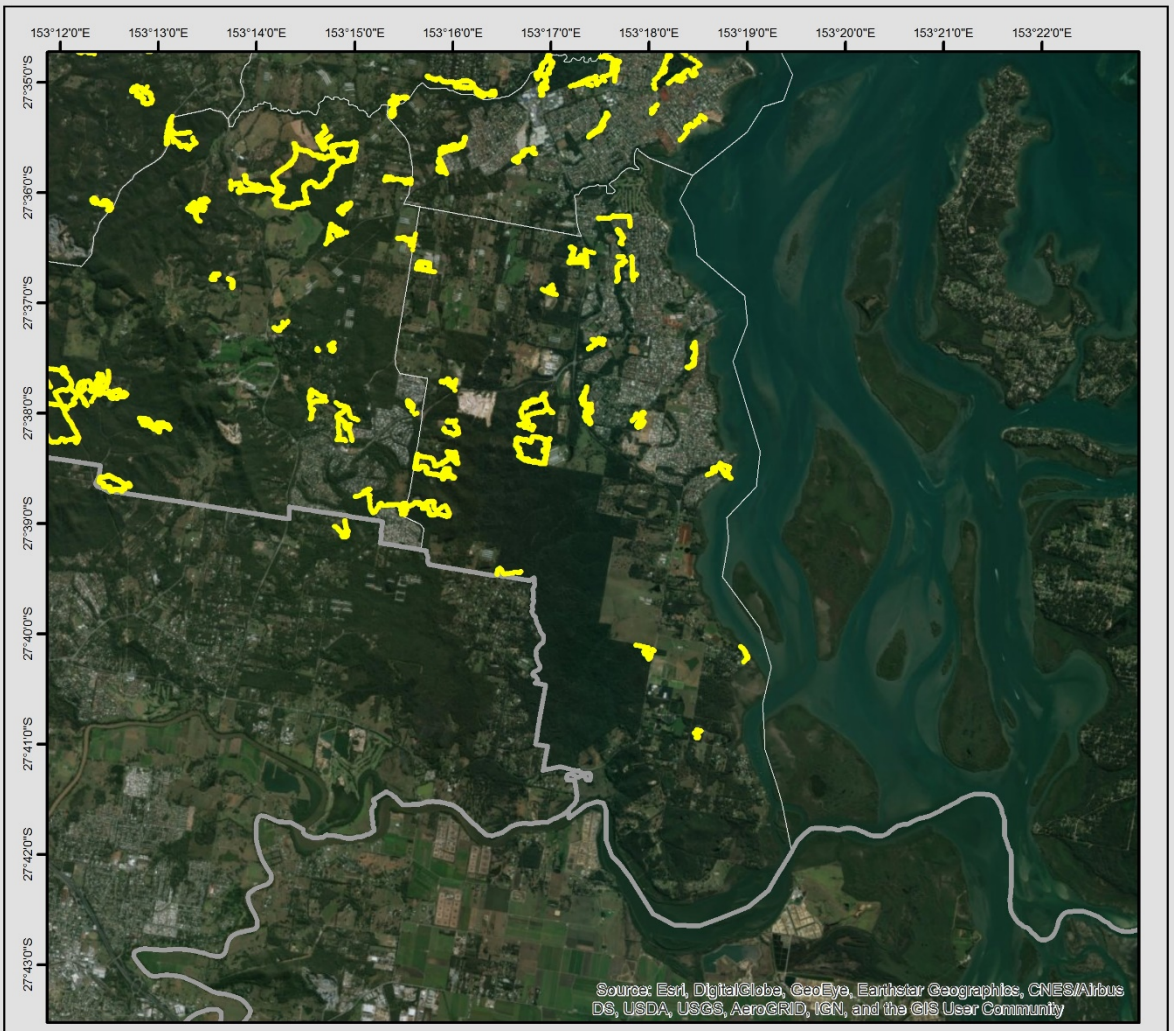
Legend

Internal relatedness

- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65



Redland Bay



Redland Bay

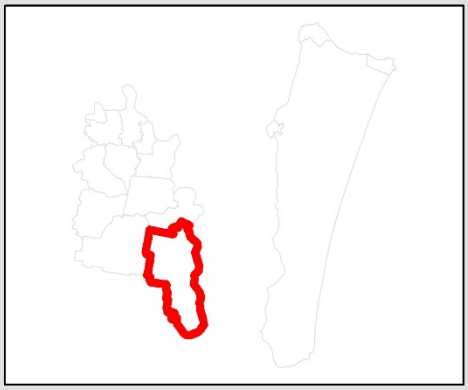
N

0 1 2 4 Kilometers

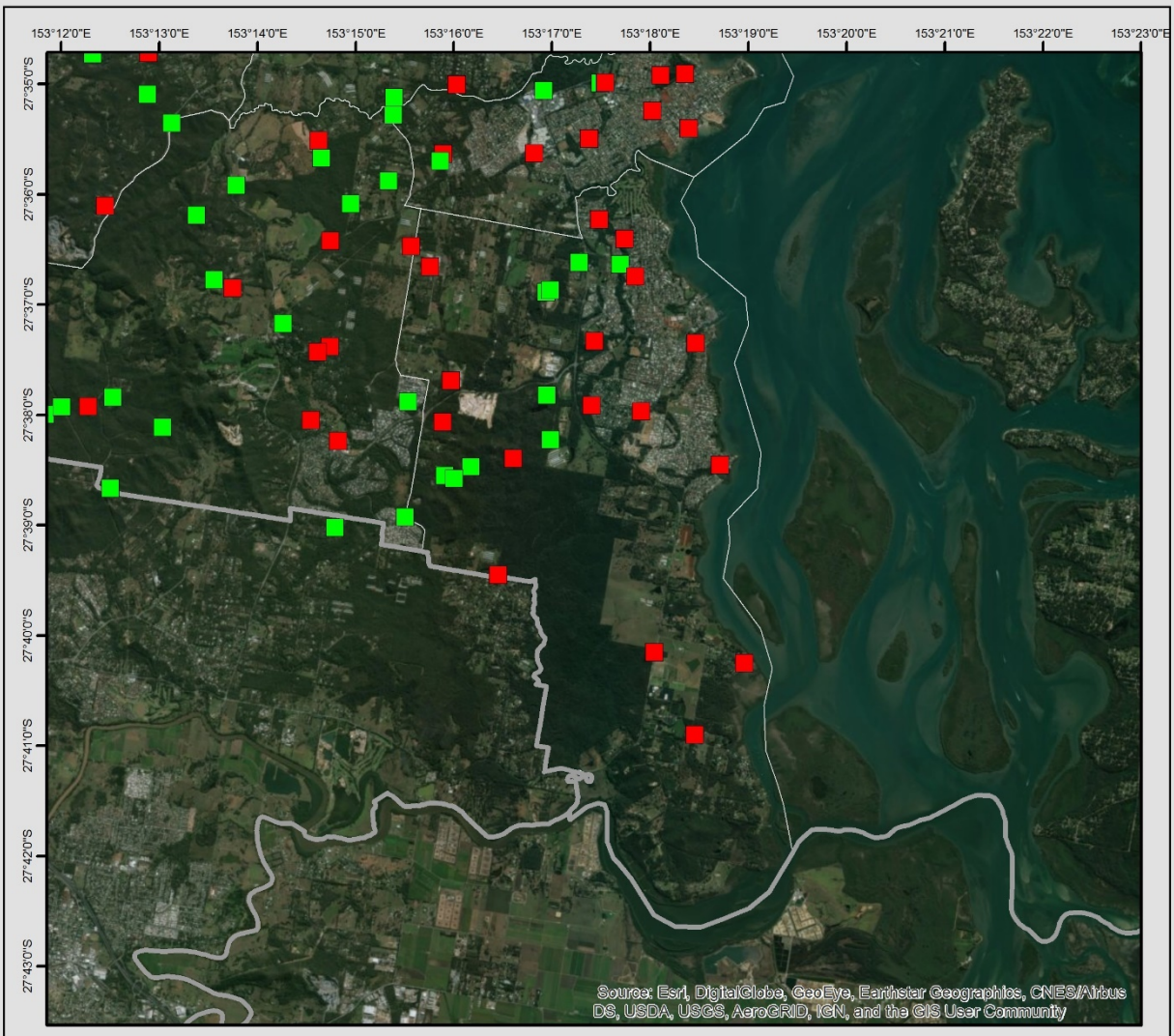
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog tracks



While every care has been taken to ensure the accuracy 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.



Redland Bay

N

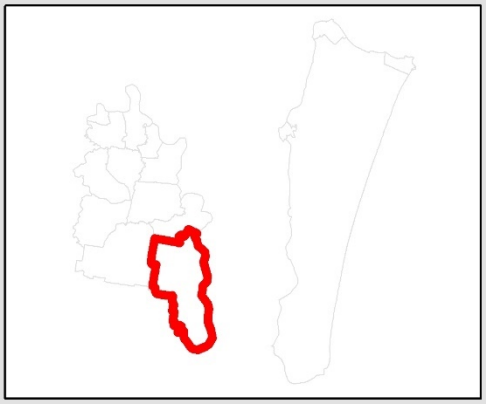
0 1 2 4 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

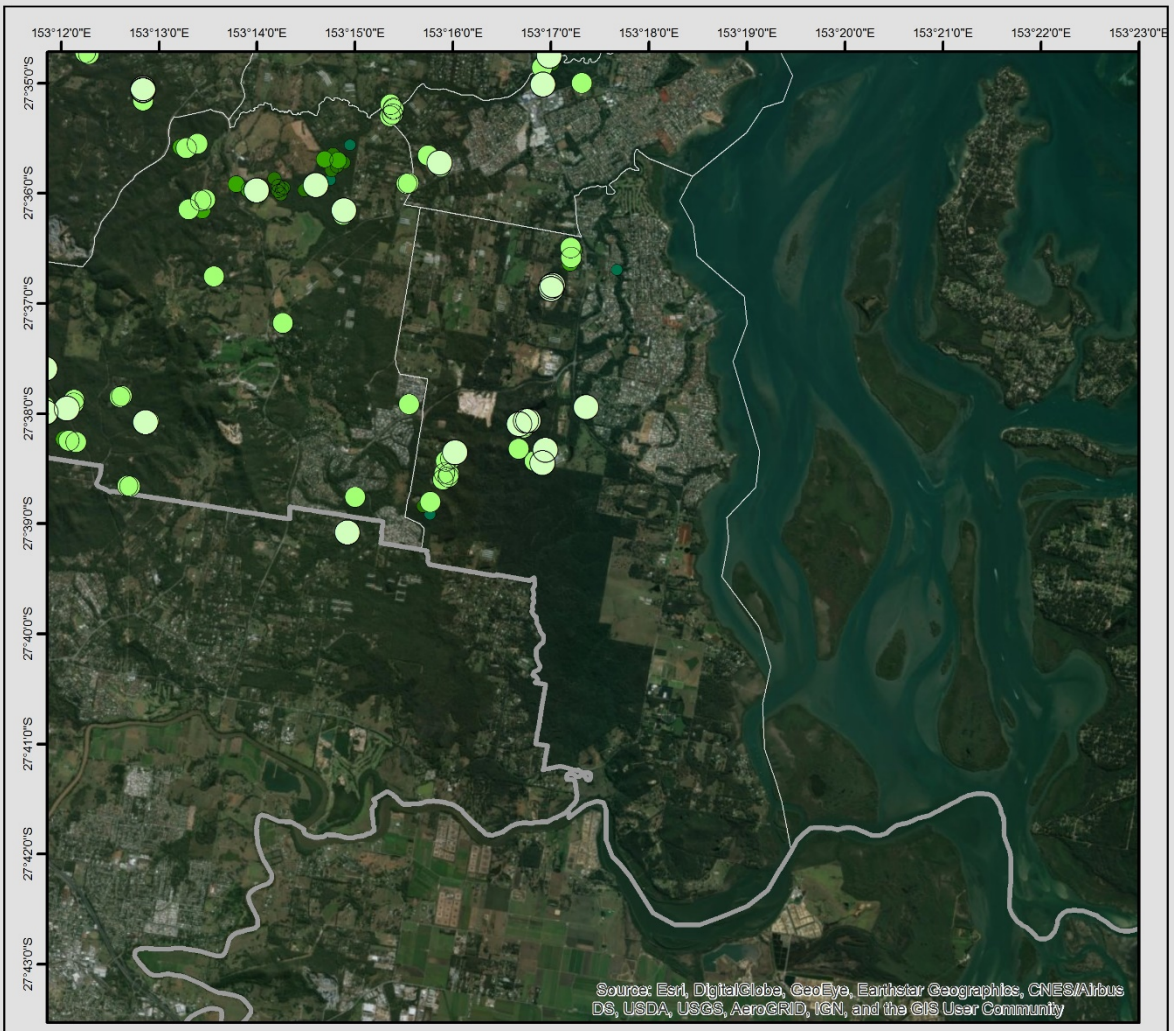
Legend

Koala scat

- Presence
- Absence



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.



Redland Bay

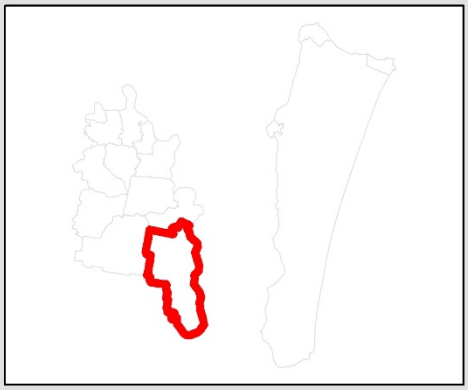
N

0 1 2 4 Kilometers

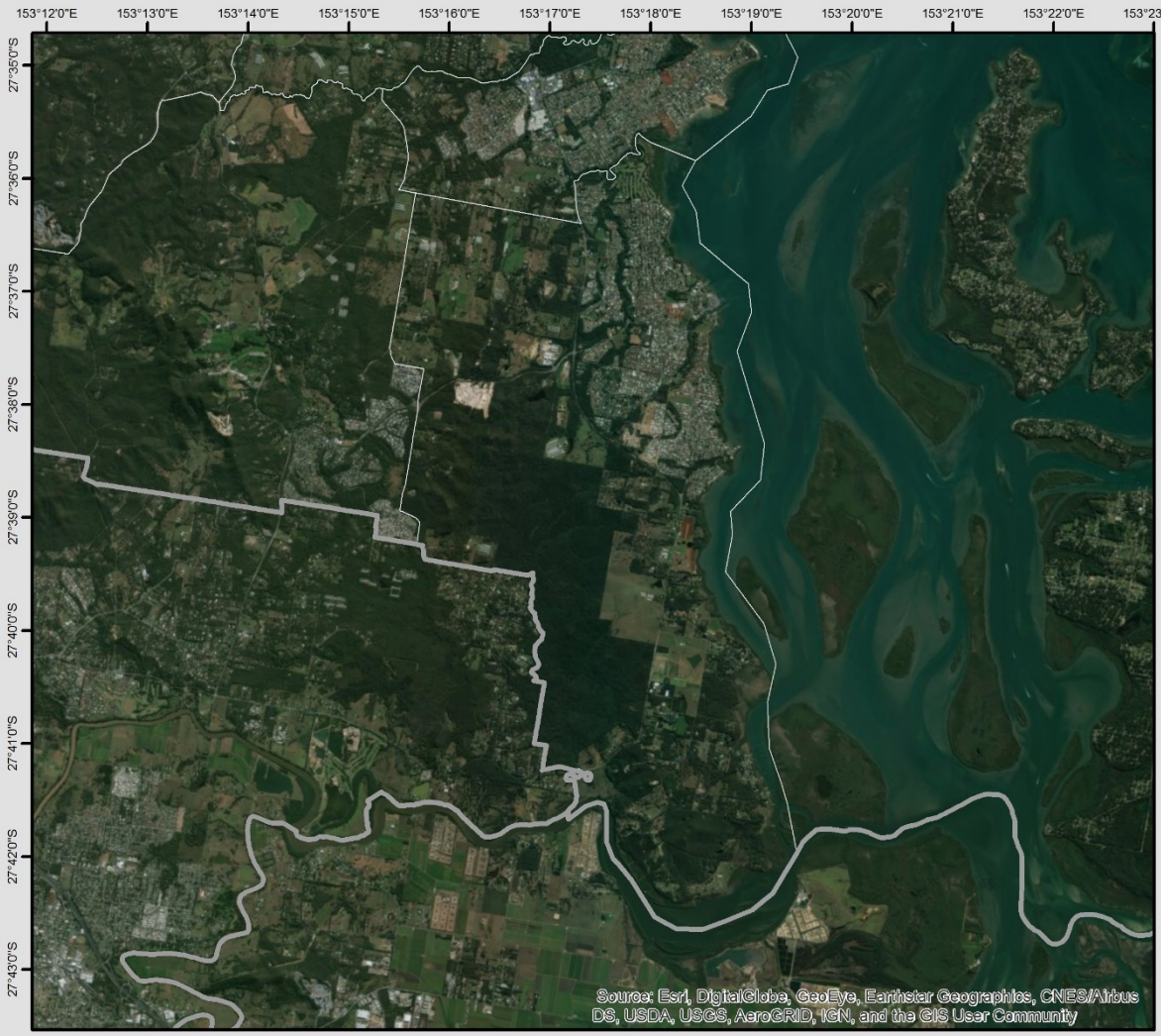
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

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



While every care has been taken to ensure the accuracy 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.



Redland Bay

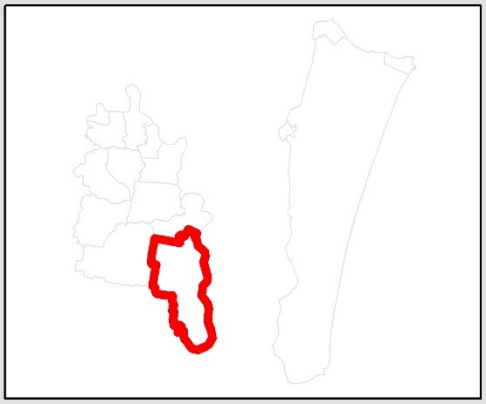
N

0 1 2 4 Kilometers

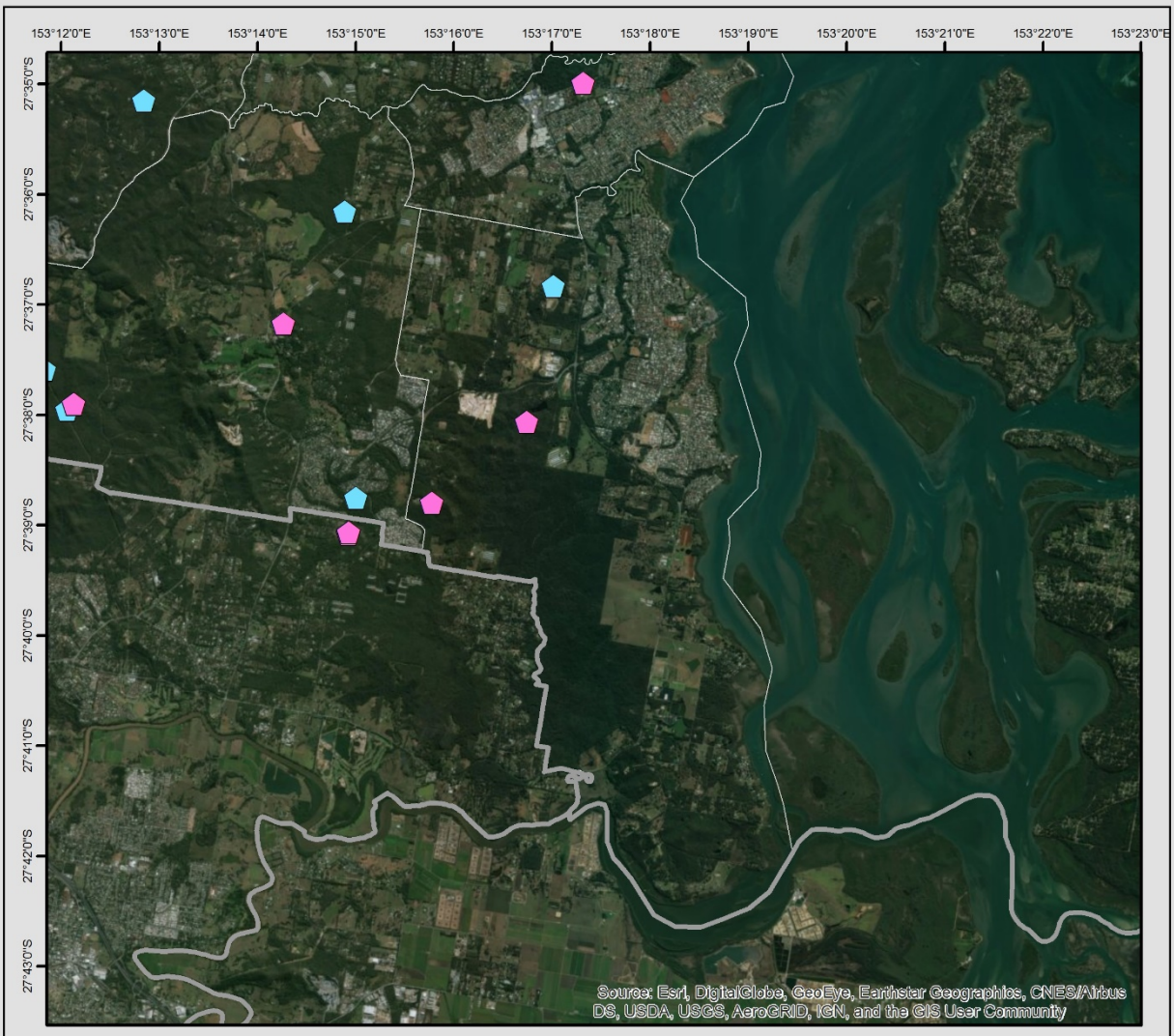
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Redland Bay

N

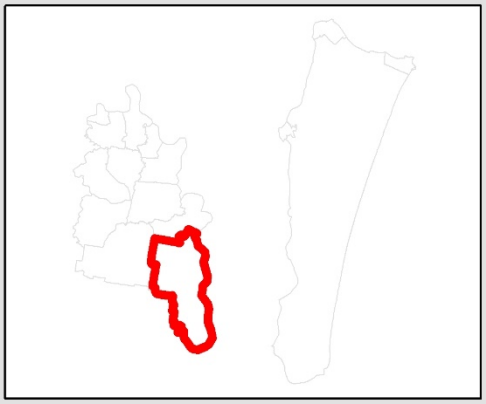
0 1 2 4 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

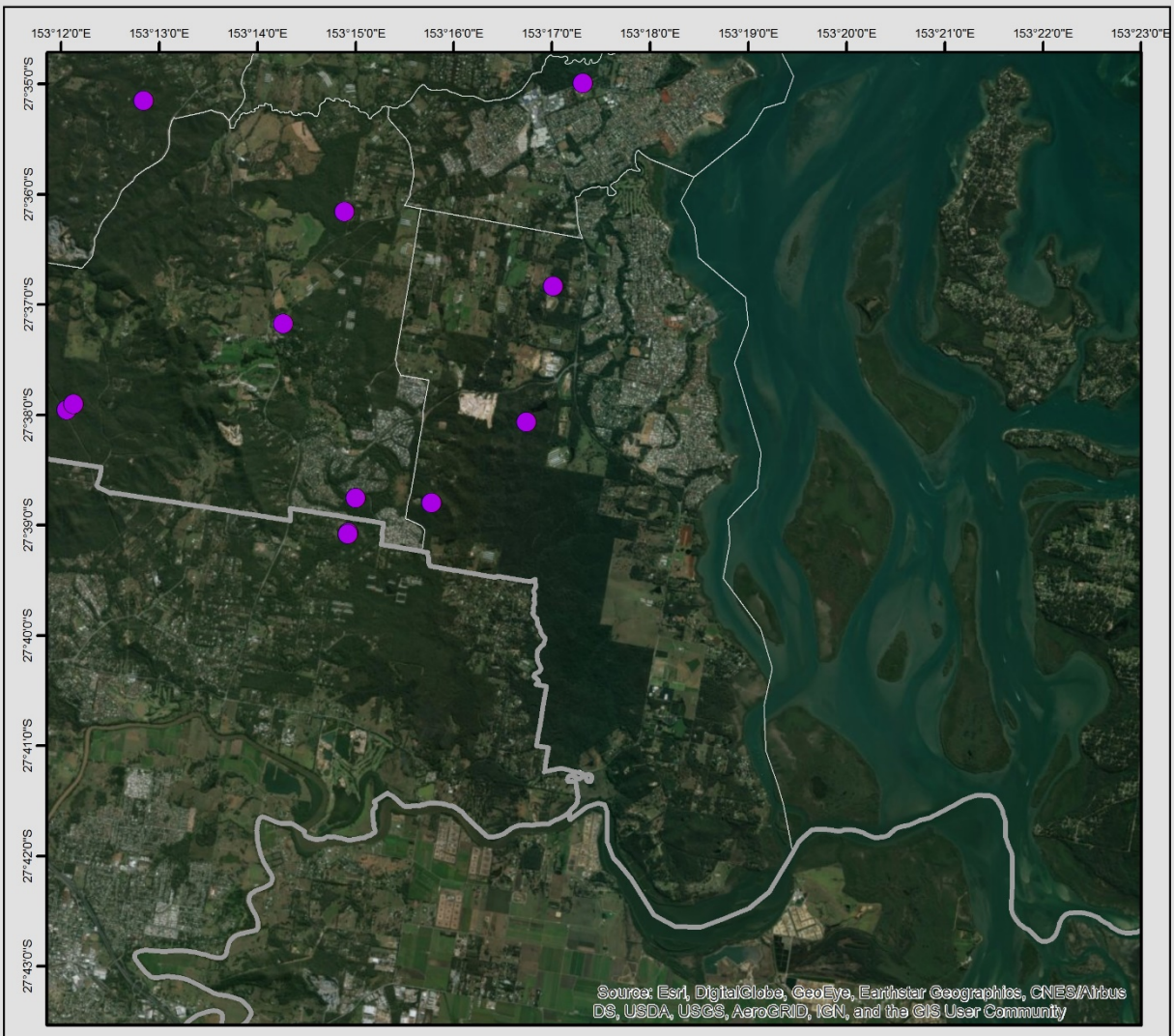
Legend

Koala sex (genetics)

- Female
- Male



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.



Redland Bay

N

0 1 2 4 Kilometers

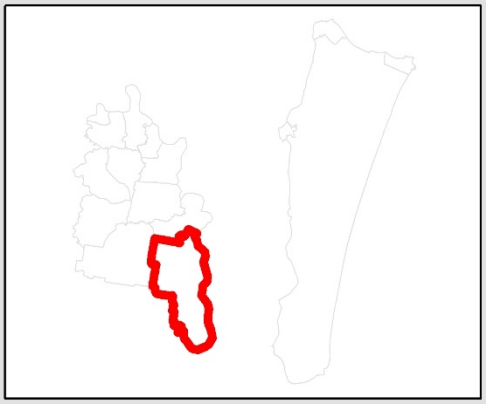
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

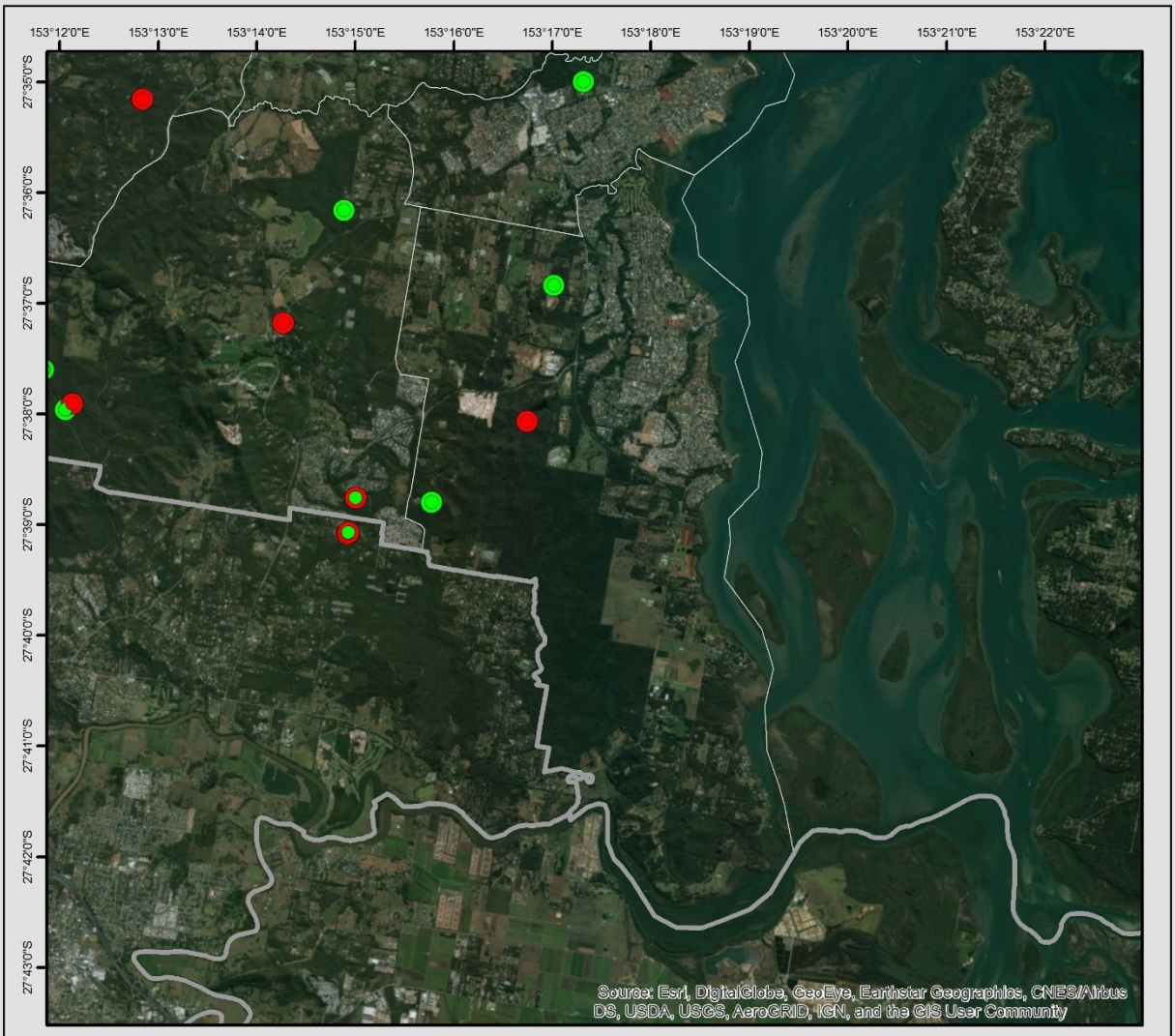
- Individual genotyped koalas

Relation between koalas

Non related (< 0.125)



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.



Redland Bay

N

0 1 2 4 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

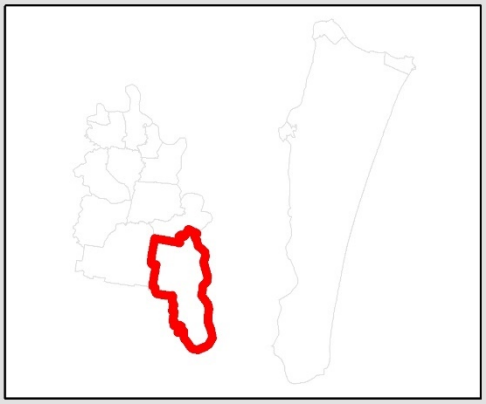
Legend - Chlamydia results

Qualitative

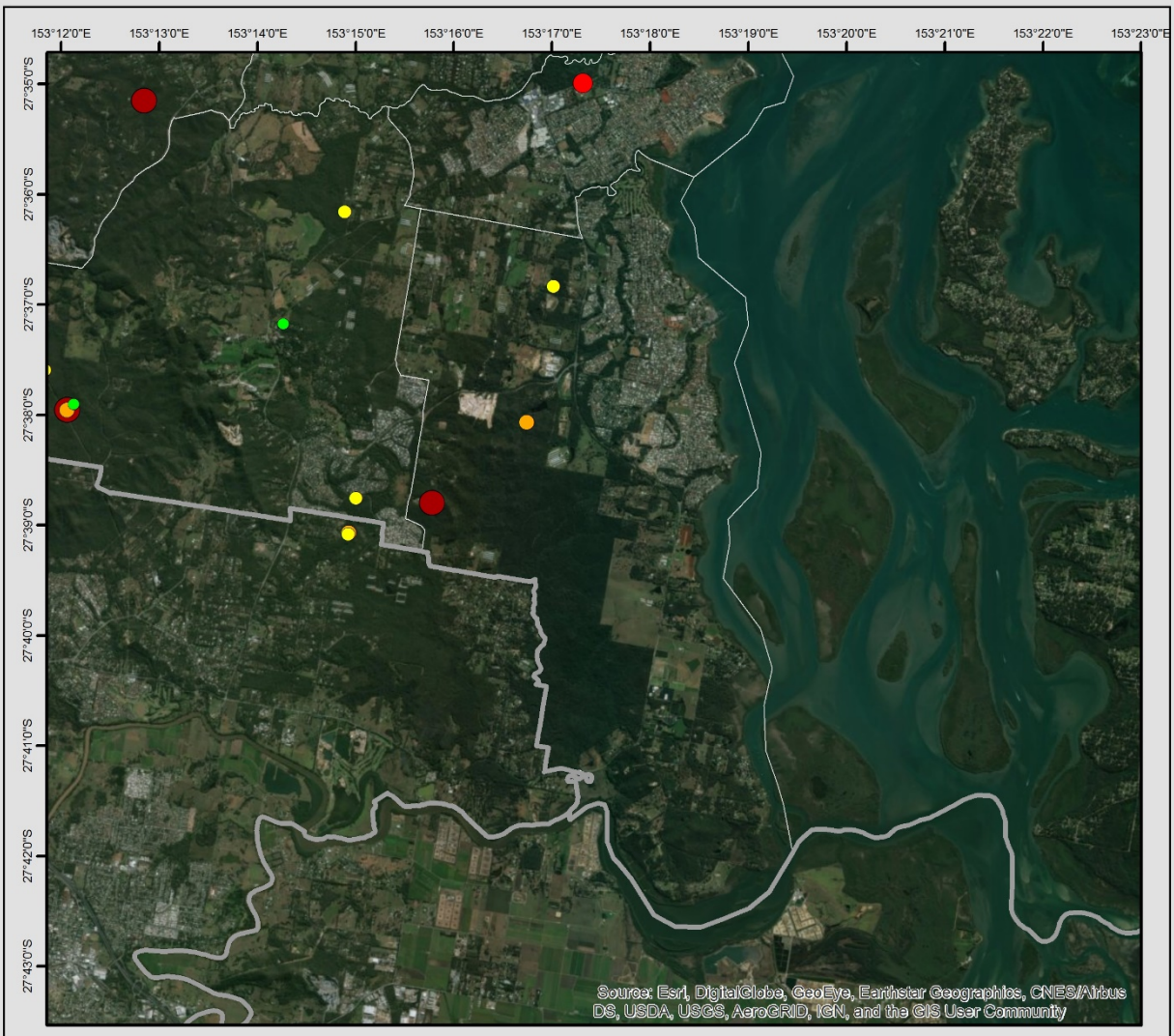
- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold



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.



Redland Bay

N

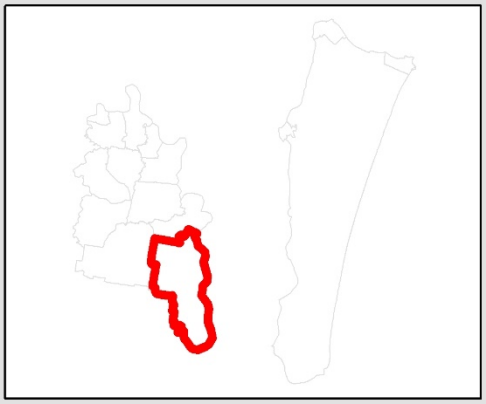
0 1 2 4 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

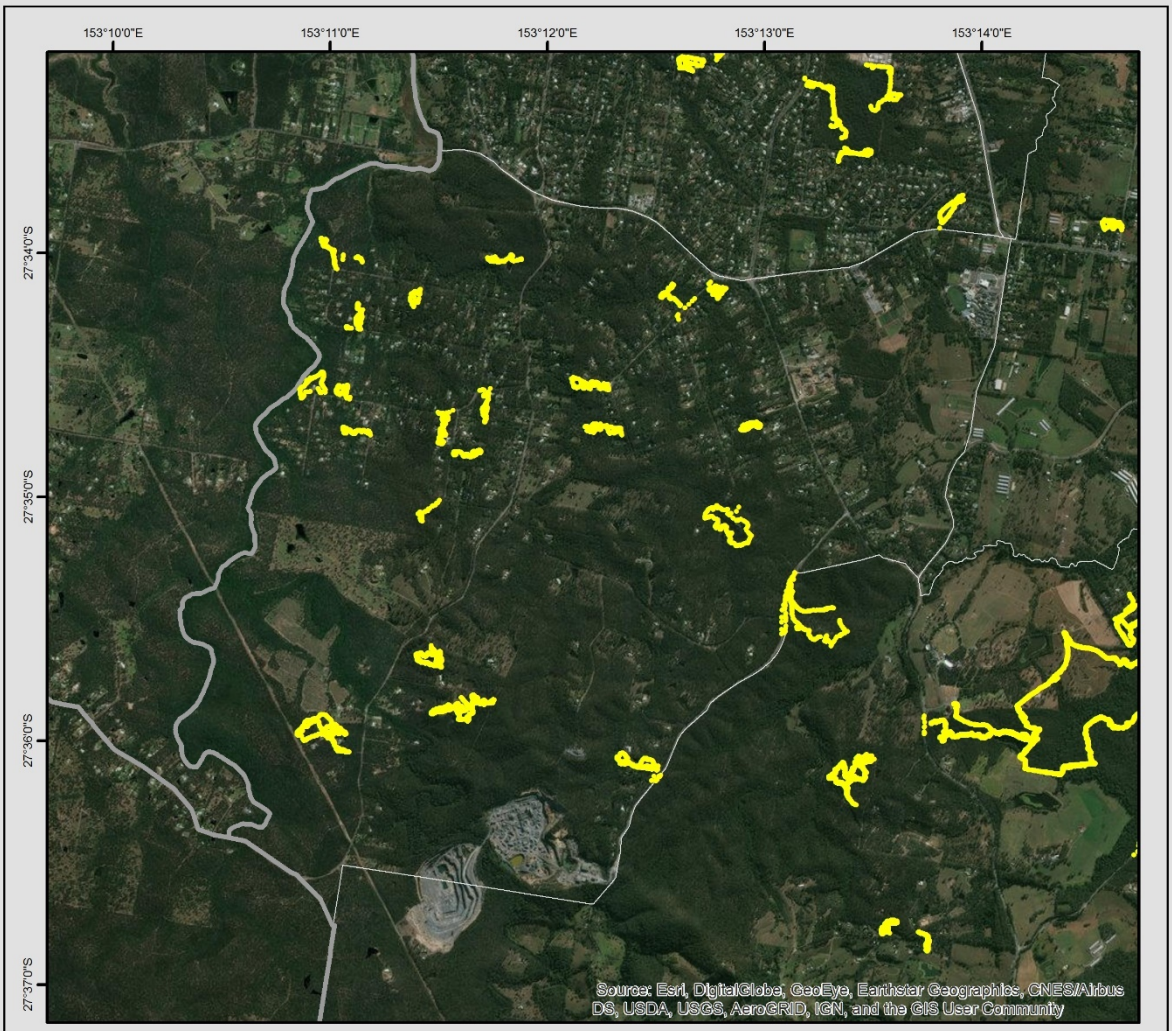
Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87




DETECTION DOGS
FOR CONSERVATION
Sniffing out solutions usc.edu.au/ddc
University of the Sunshine Coast

Sheldon



Sheldon

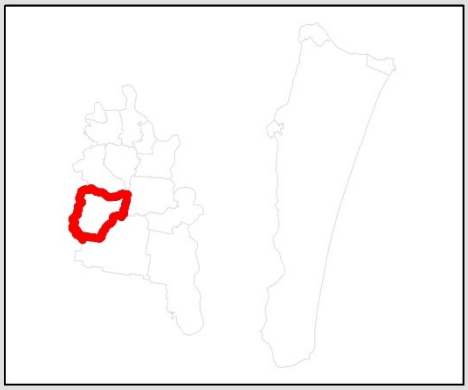
N

0 0.475 0.95 1.9
Kilometers

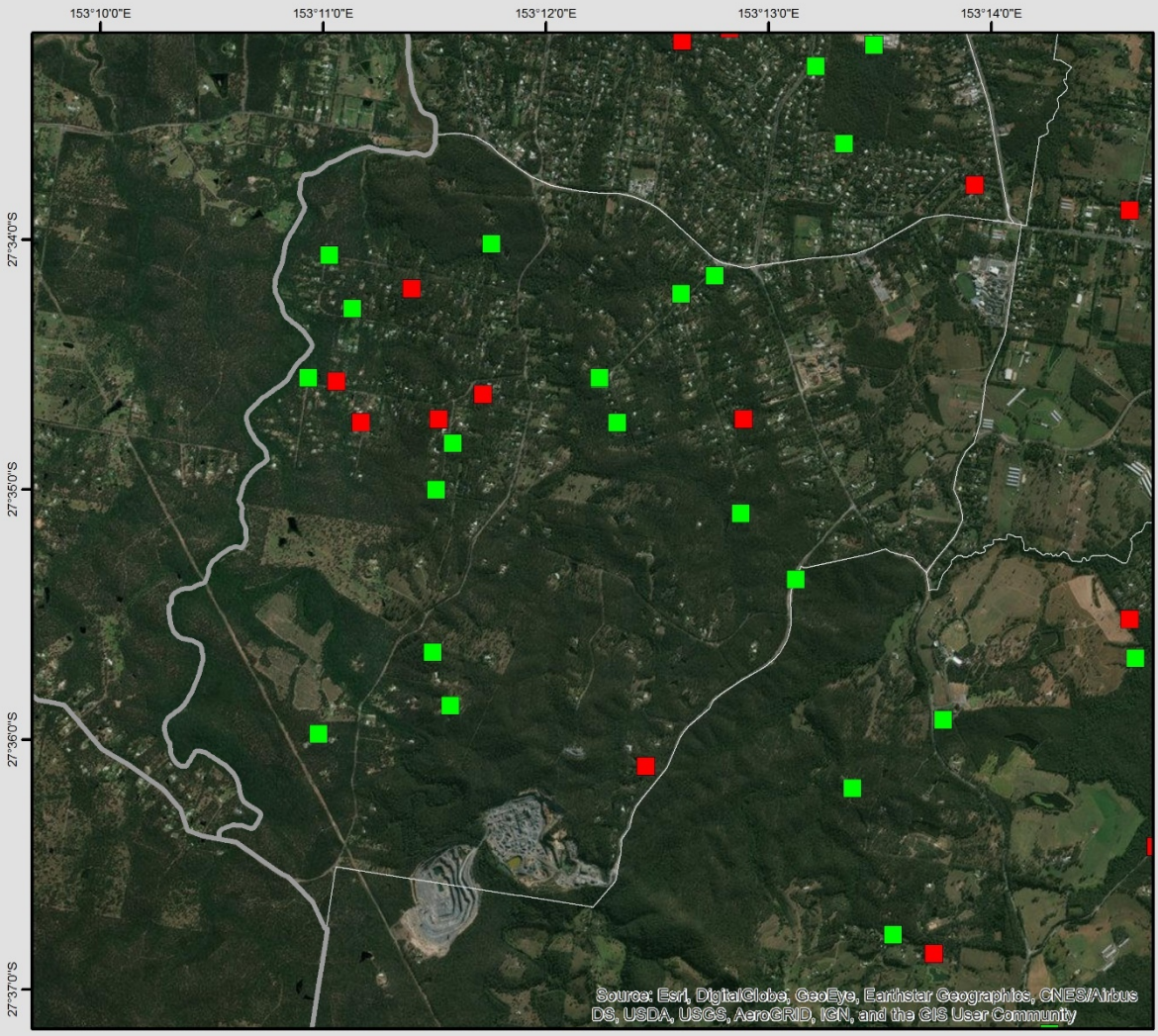
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog track



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.



Sheldon

N

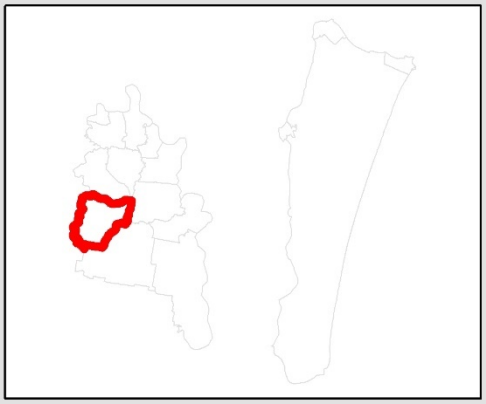
0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

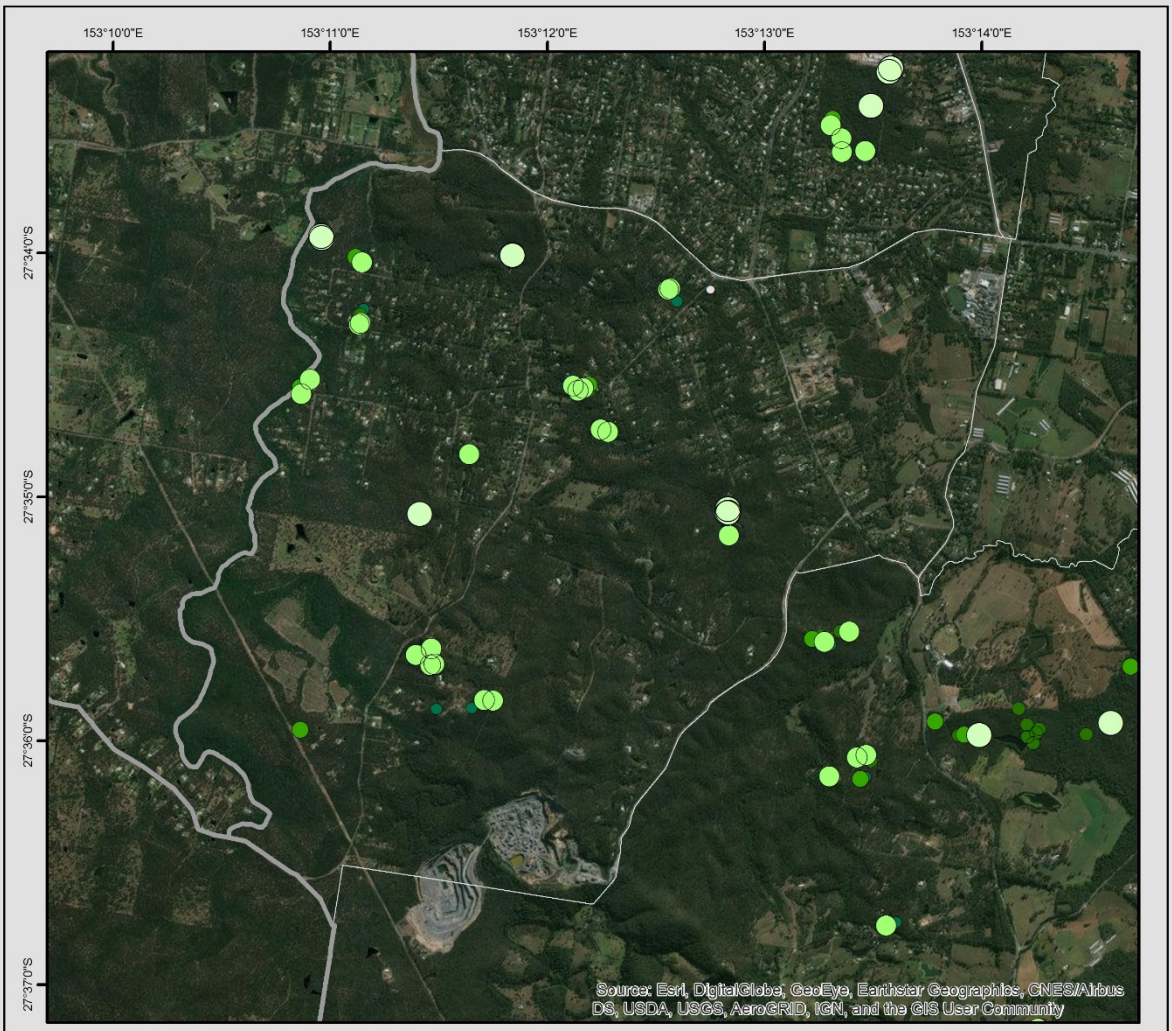
Legend

Koala scat

- Presence
- Absence



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.



Sheldon

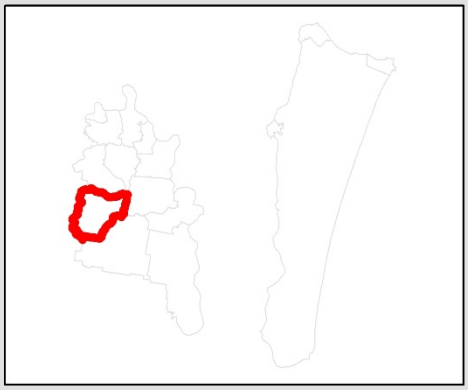
N

0 0.475 0.95 1.9
Kilometers

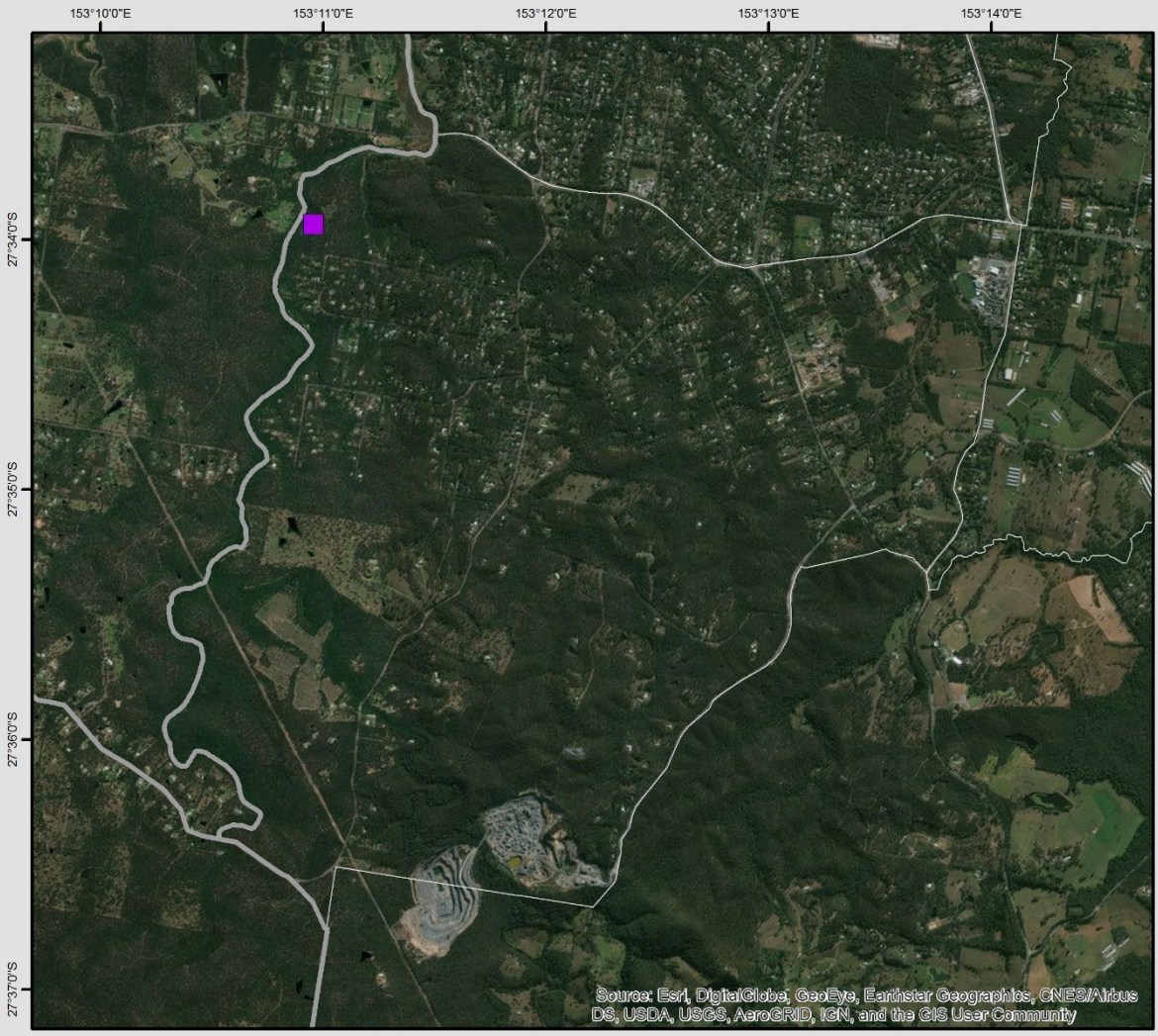
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend - Koala scat age categories

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



While every care has been taken to ensure the accuracy 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.



Sheldon

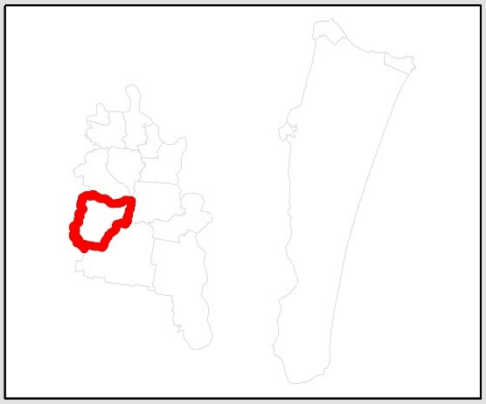
N

0 0.475 0.95 1.9 Kilometers

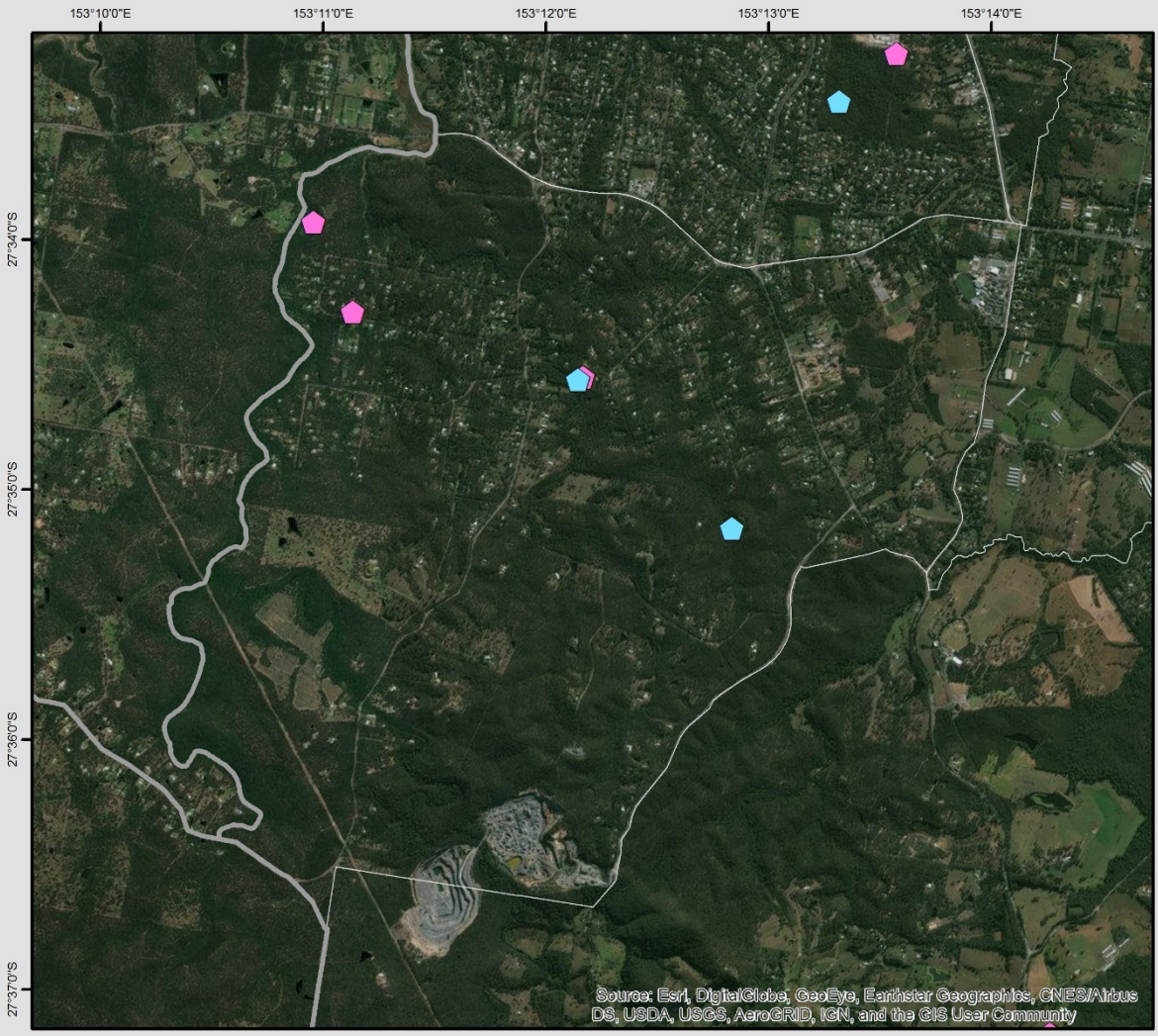
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



While every care has been taken to ensure the accuracy 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.



Sheldon

N

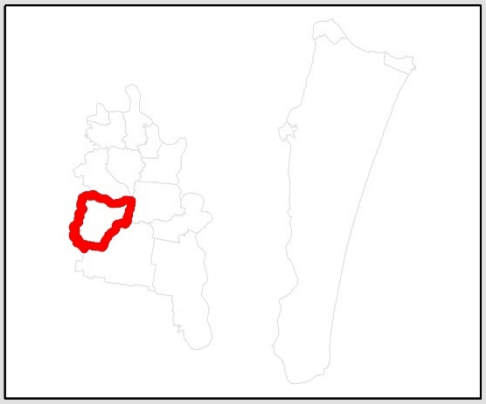
0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

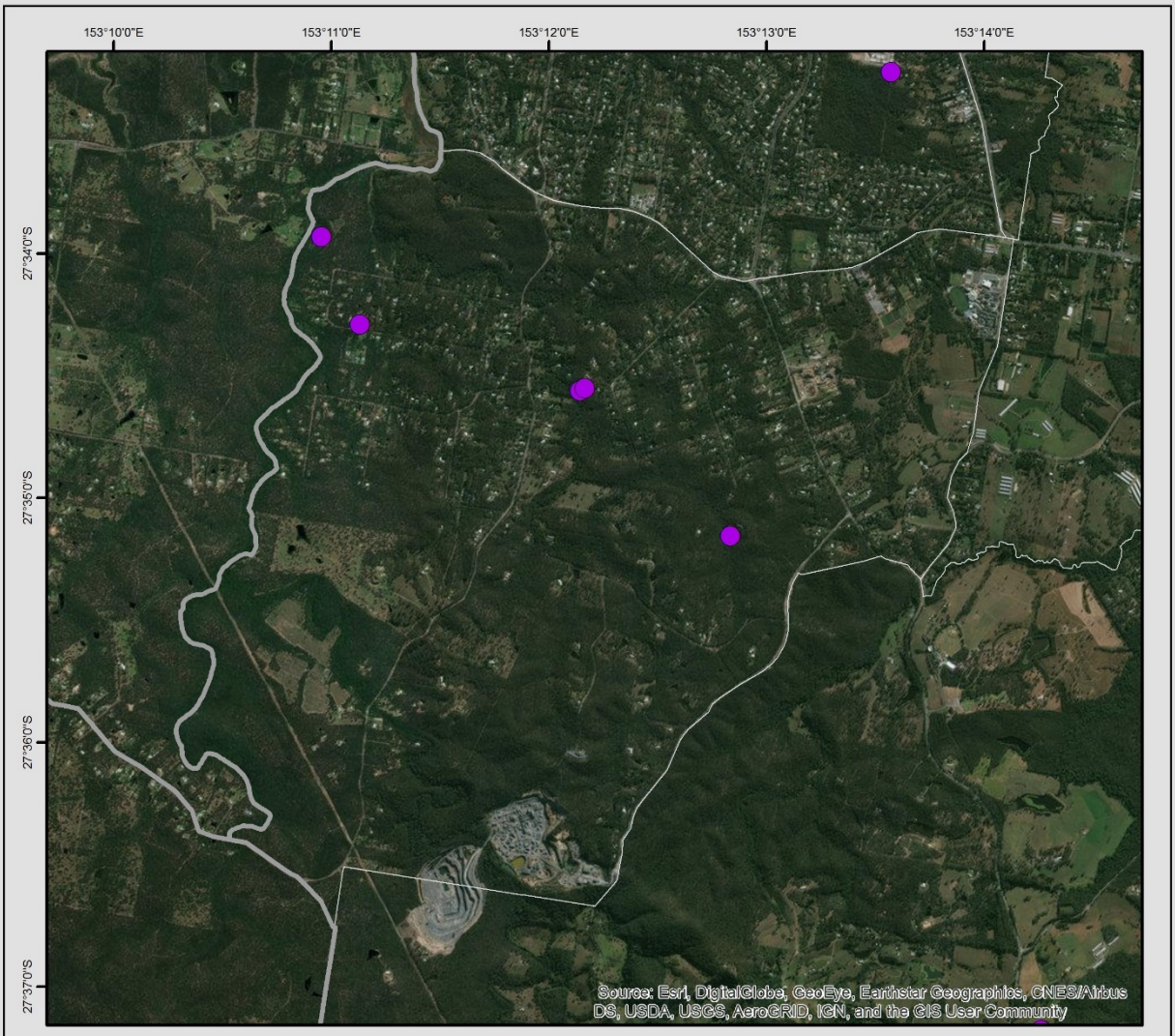
Legend

Koala sex (genetics)

- Female
- Male



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.



Sheldon

N

0 0.475 0.95 1.9
Kilometers

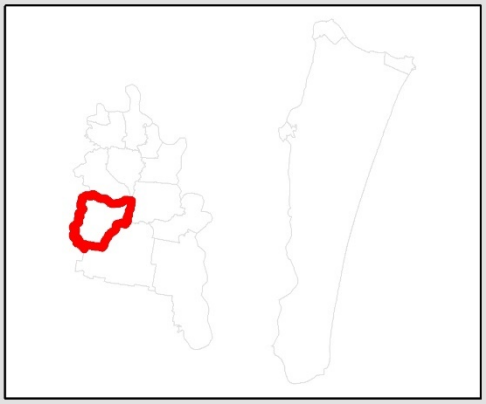
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

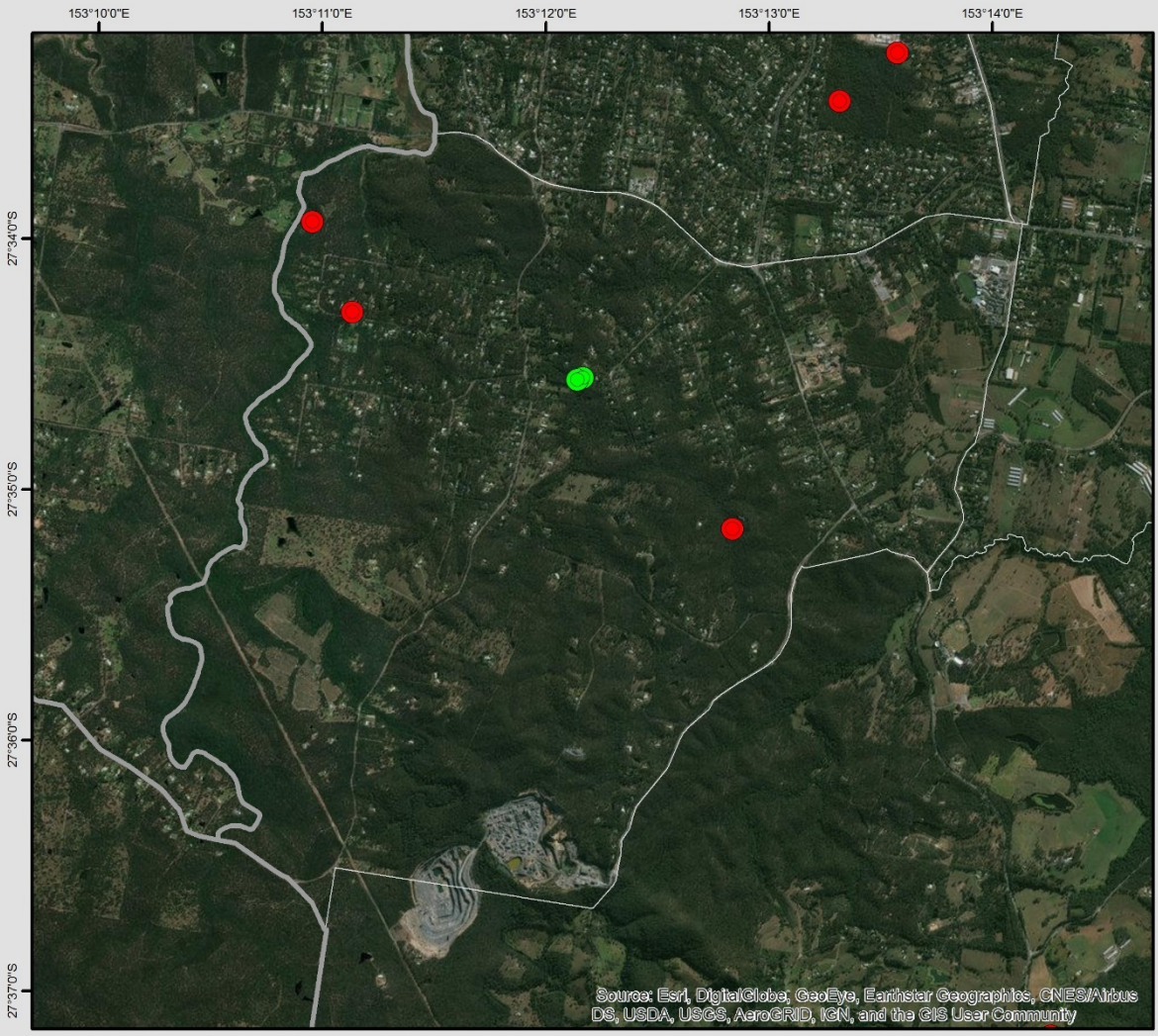
- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)



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.



Sheldon

N

0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

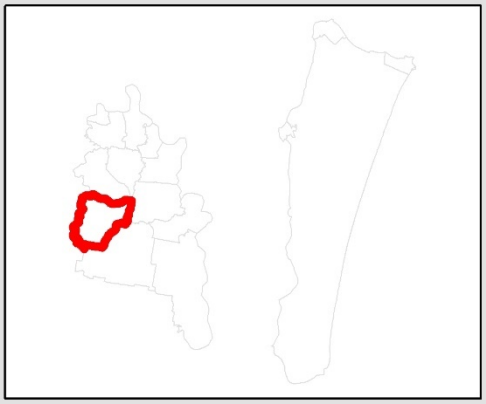
Legend - Chlamydia results

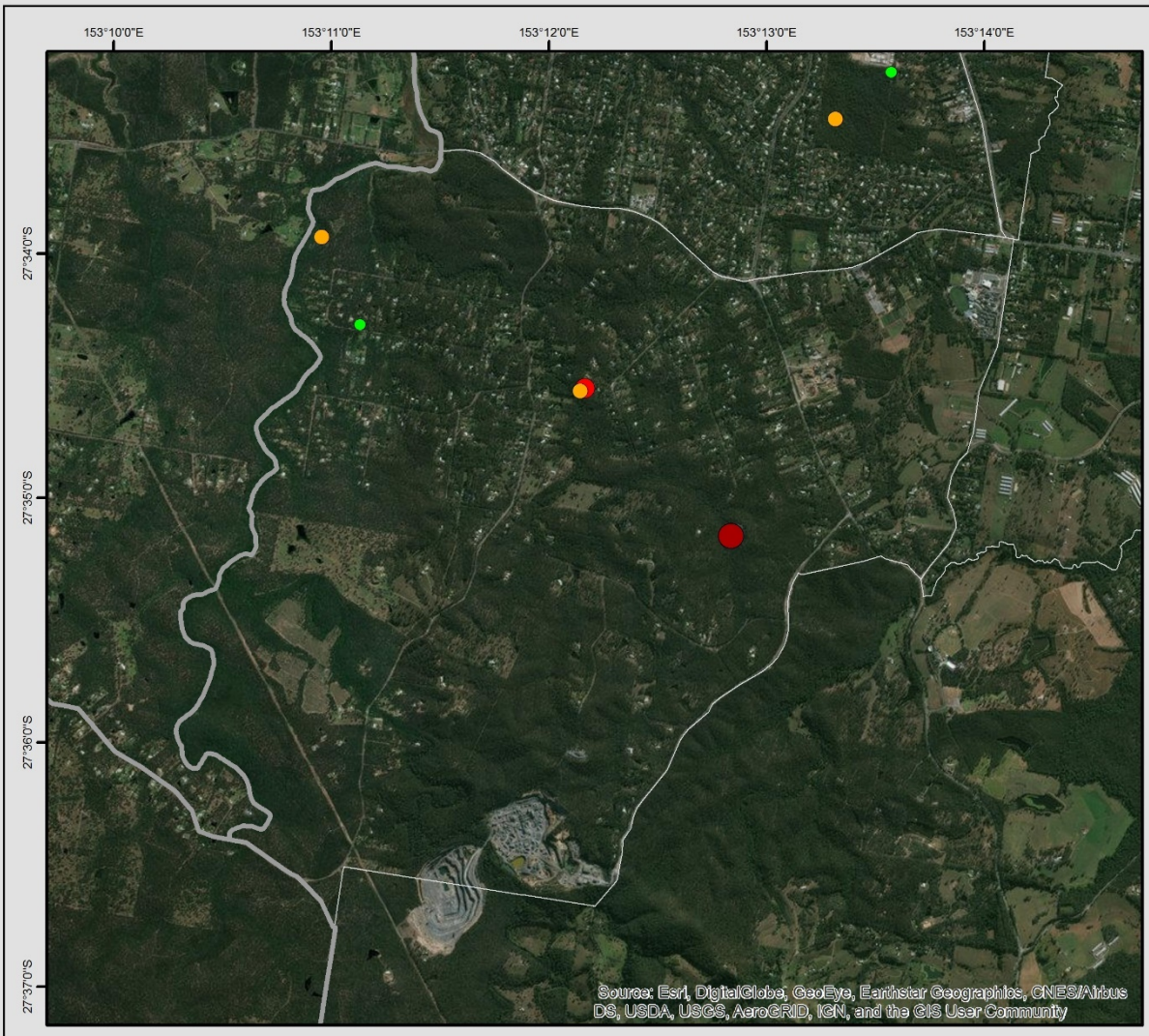
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold





Sheldon

N

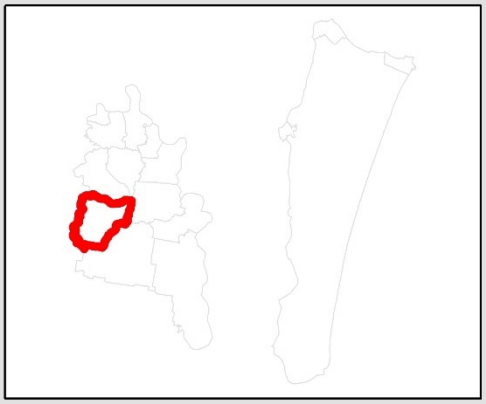
0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

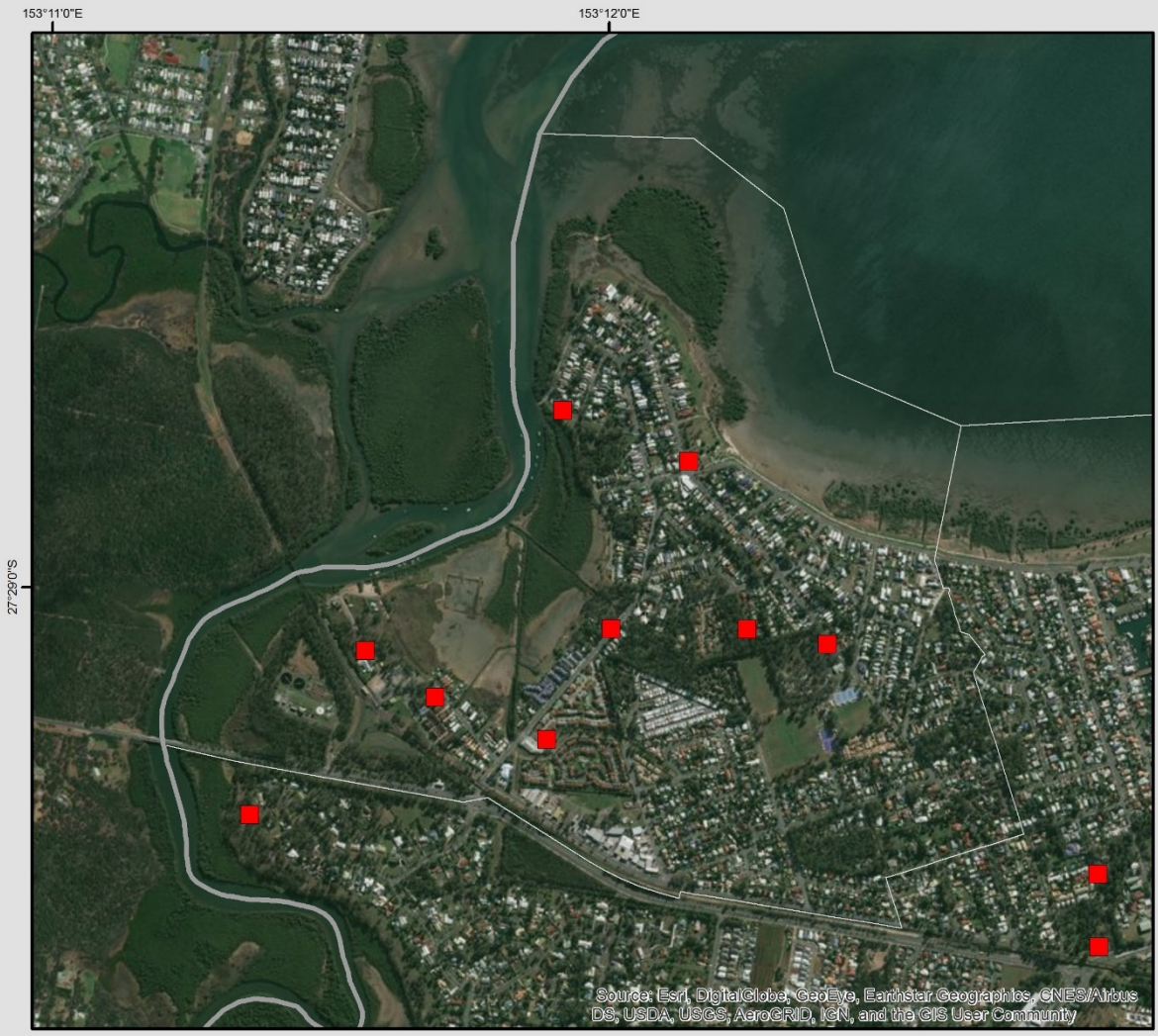
Legend

Internal relatedness

- -0.19 - 0.10
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Thorneside



Thorneside

N

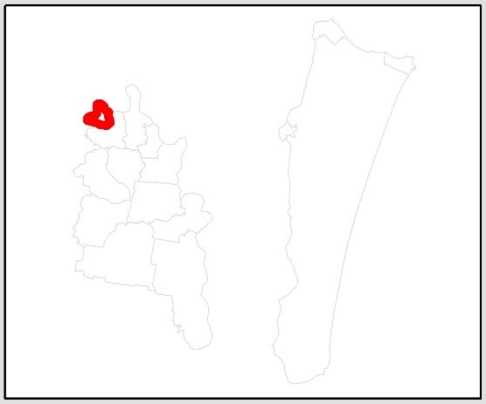
0 0.175 0.35 0.7
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

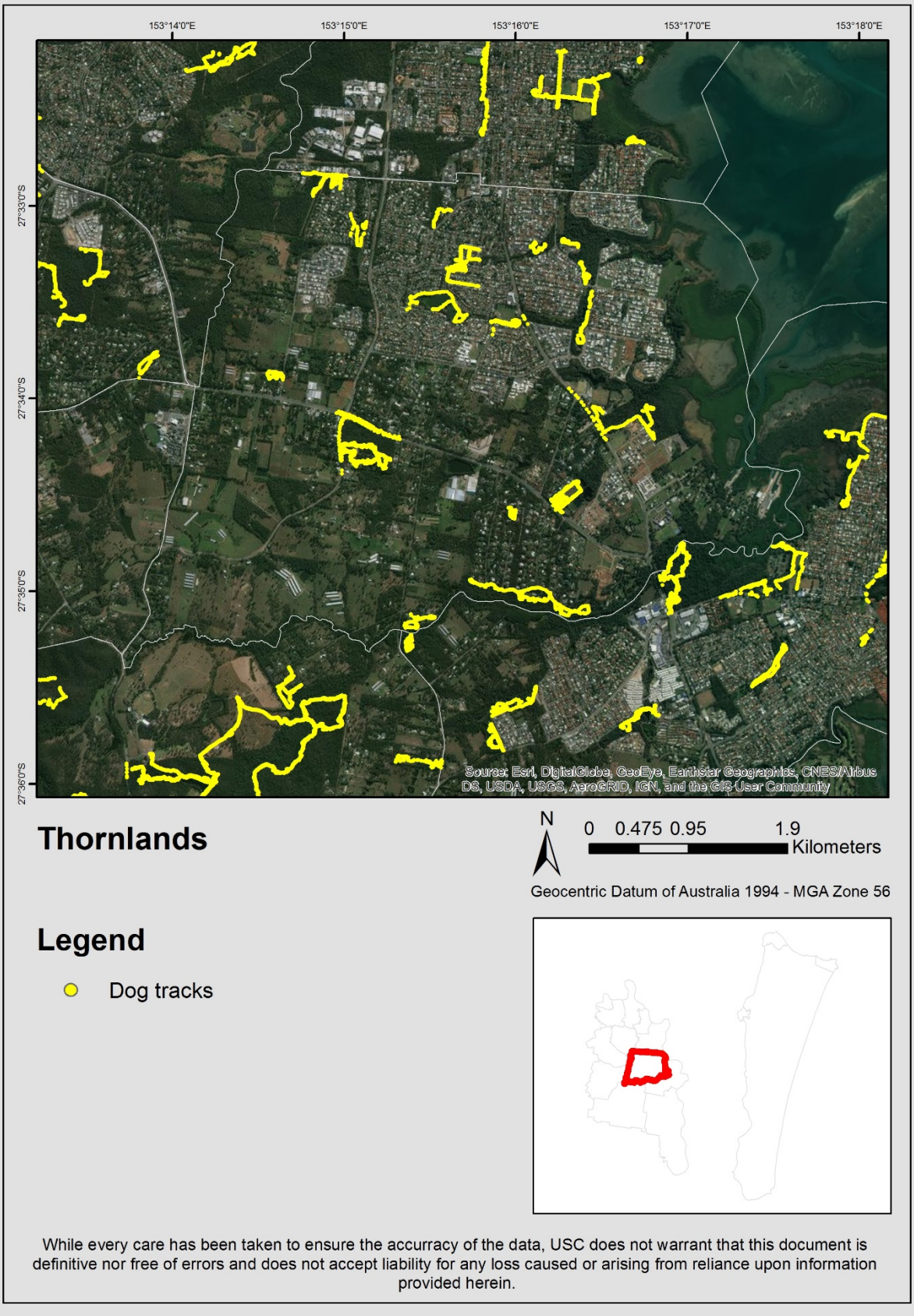
Koala scat

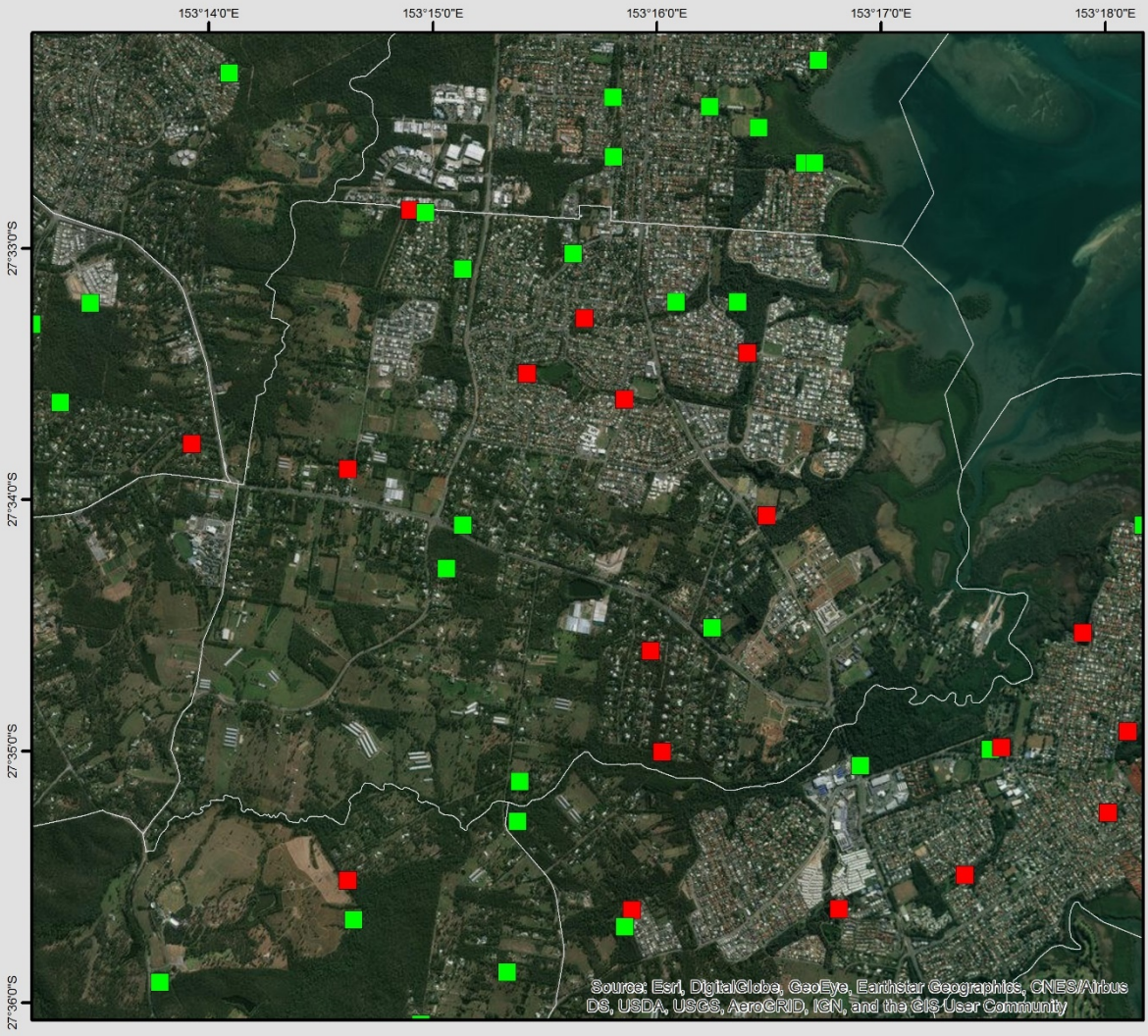
- Presence
- Absence



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.

Thornlands


153°14'0"E
153°15'0"E
153°16'0"E
153°17'0"E
153°18'0"E
27°33'0"S
27°34'0"S
27°35'0"S
27°36'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Thornlands
N
0 0.475 0.95 1.9
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
While every care has been taken to ensure the accuracy 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.



Thornlands

N

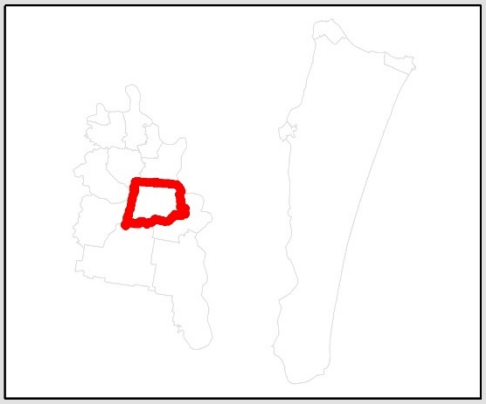
0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

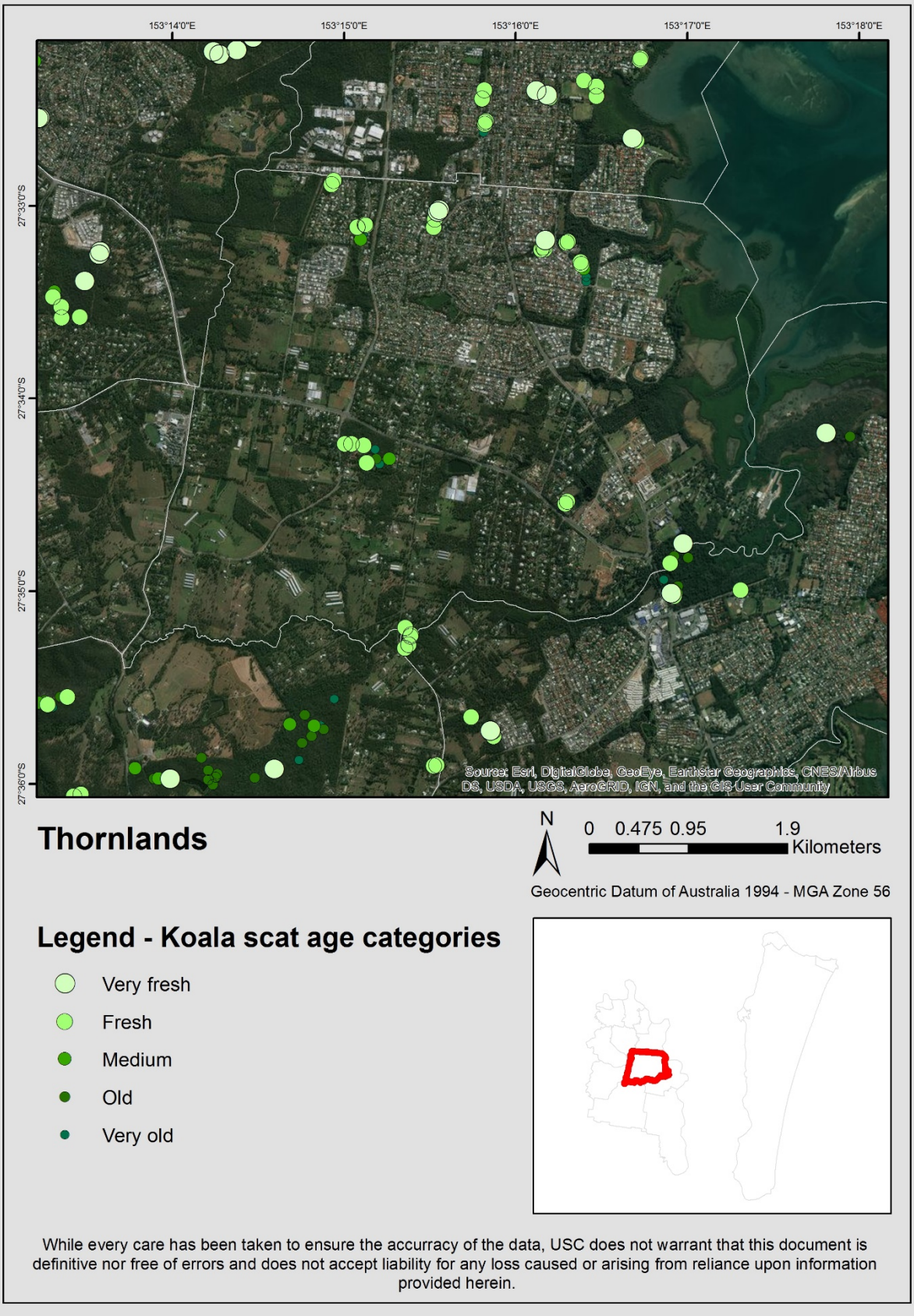
Legend

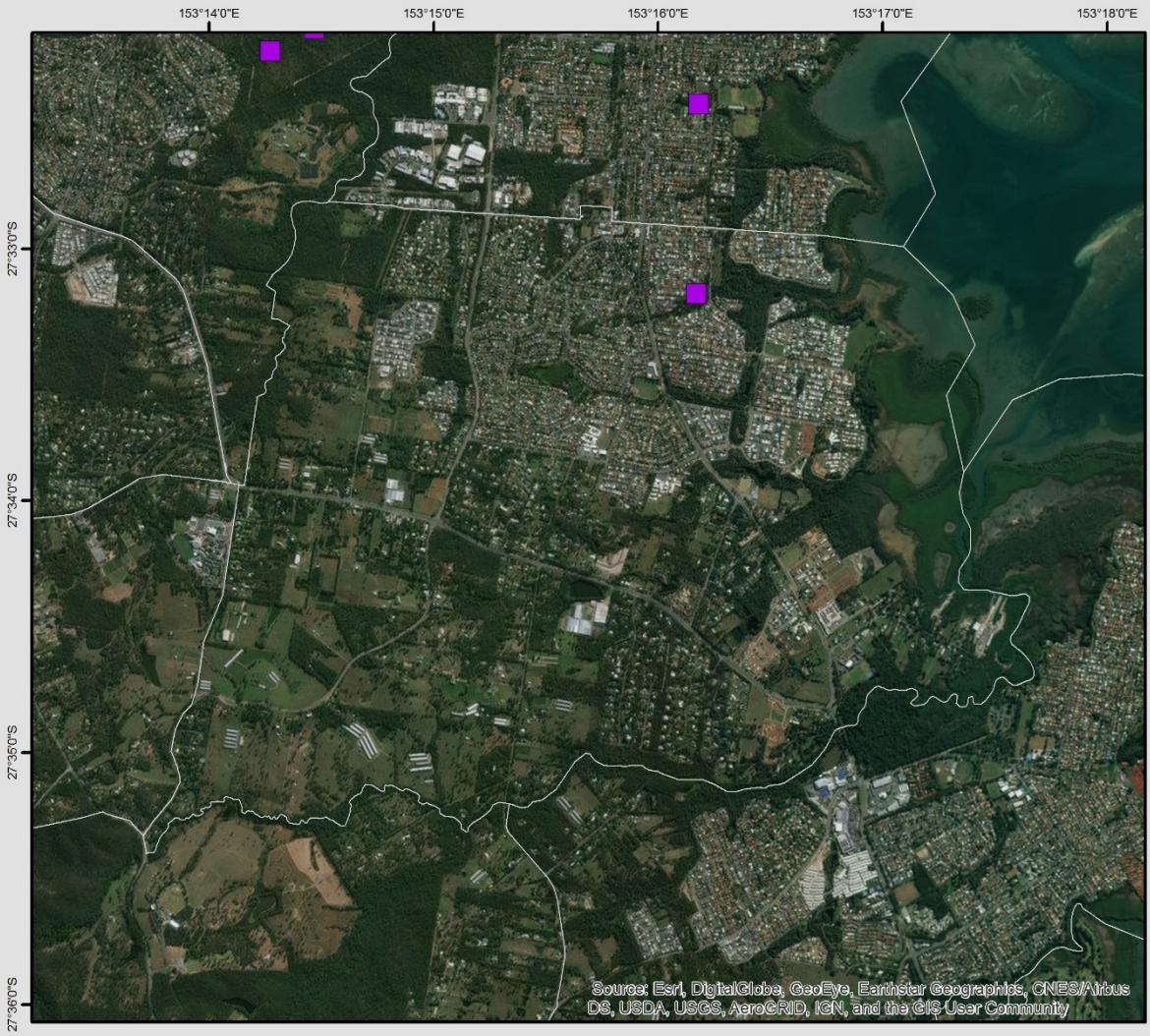
Koala scat

- Presence
- Absence



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.


153°14'0"E
153°15'0"E
153°16'0"E
153°17'0"E
153°18'0"E
27°33'0"S
27°34'0"S
27°35'0"S
27°36'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Thornlands
N
0 0.475 0.95 1.9
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend - Koala scat age categories
Very fresh
Fresh
Medium
Old
Very old
While every care has been taken to ensure the accuracy 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.



Thornlands

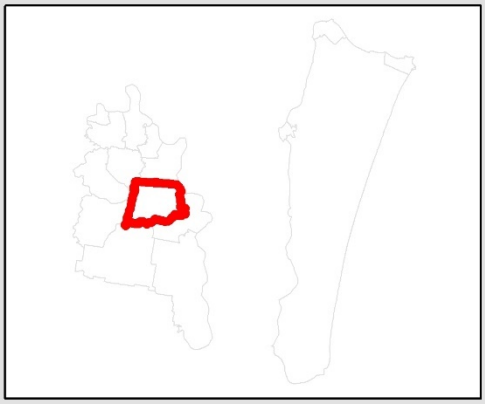
N

0 0.475 0.95 1.9
Kilometers

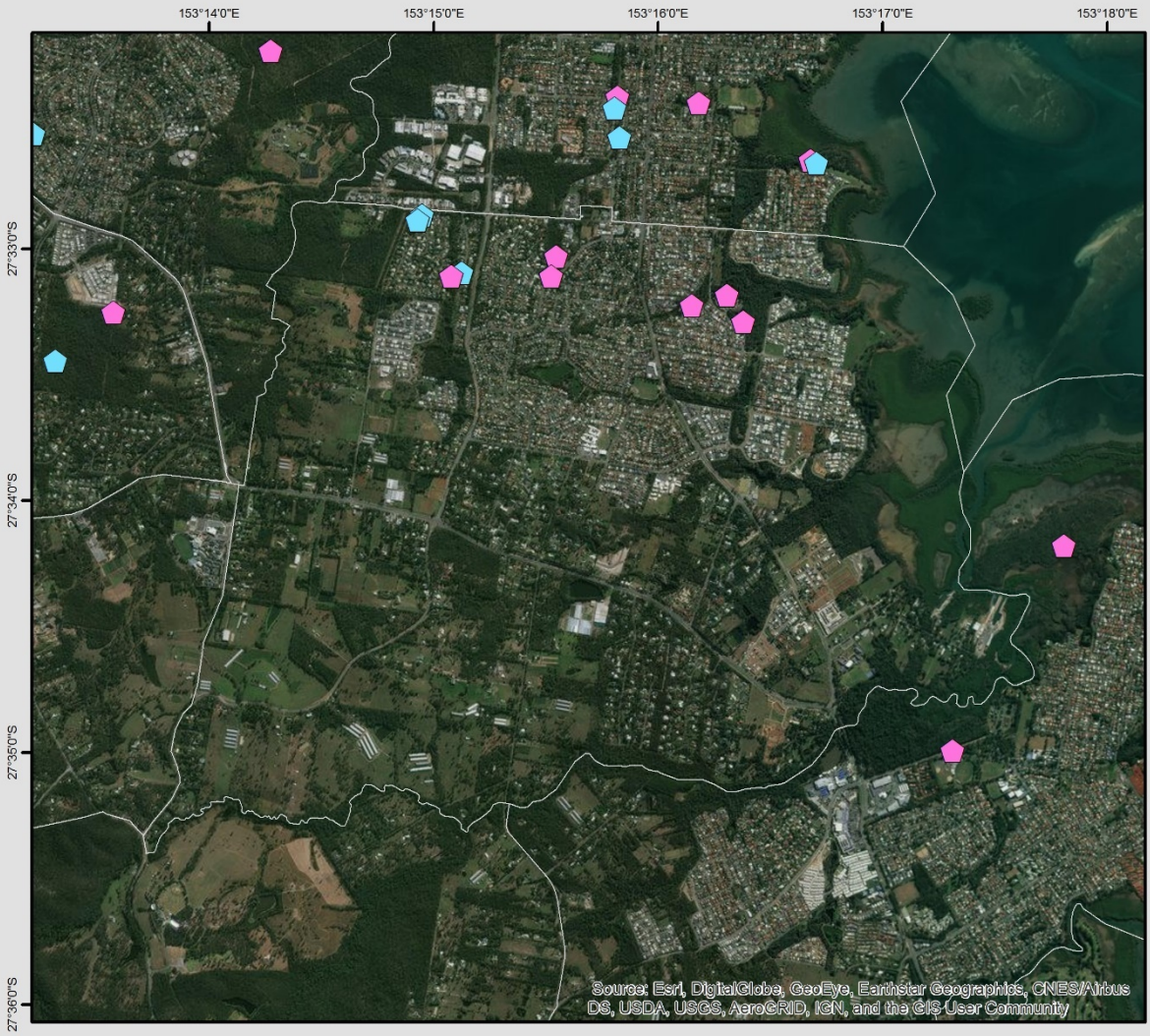
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Thornlands

N

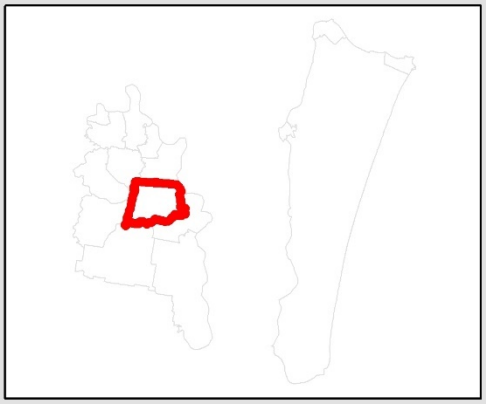
0 0.475 0.95 1.9
Kilometers

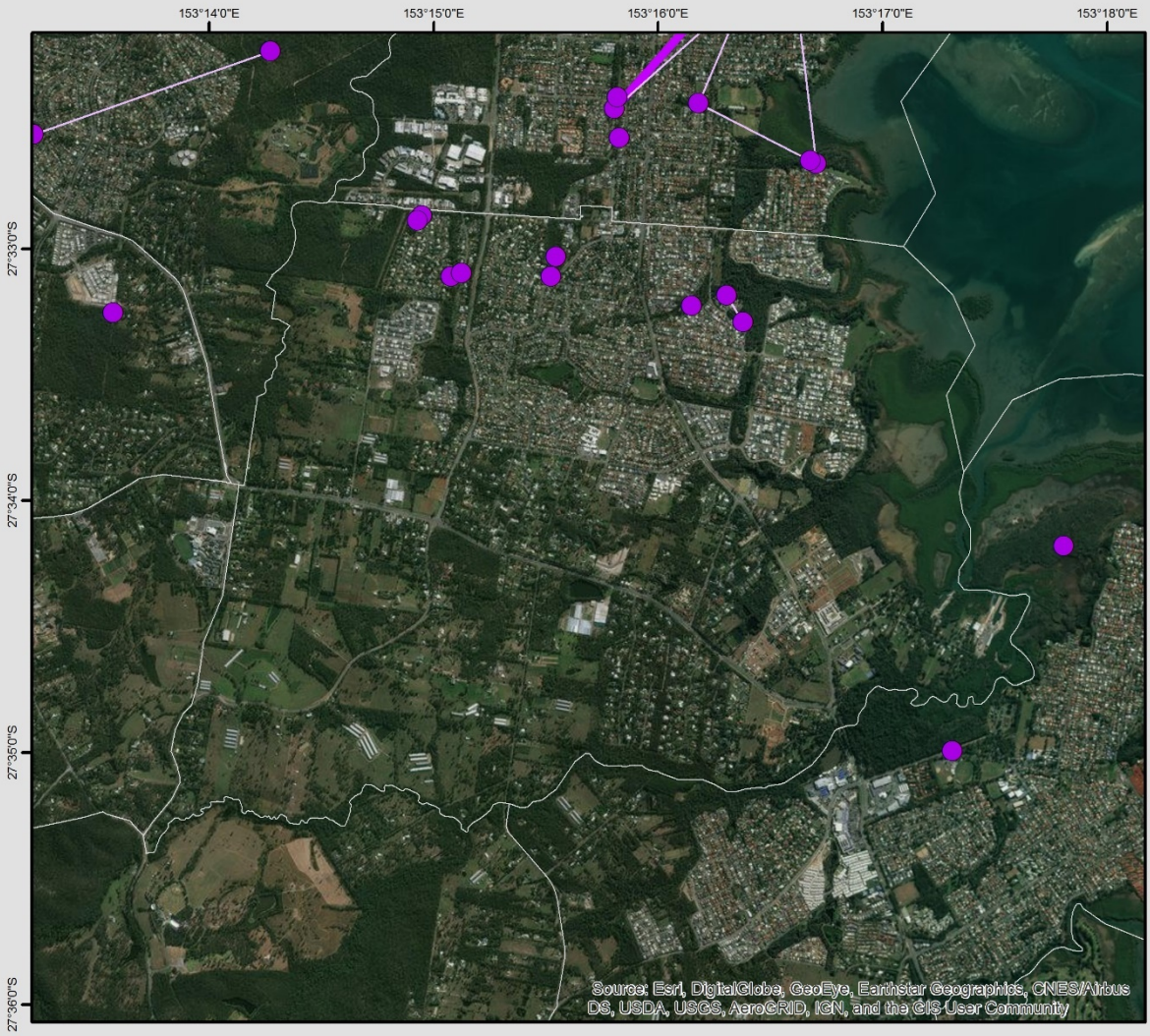
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Koala sex (genetics)

- Female
- Male





Thornlands

N

0 0.475 0.95 1.9
Kilometers

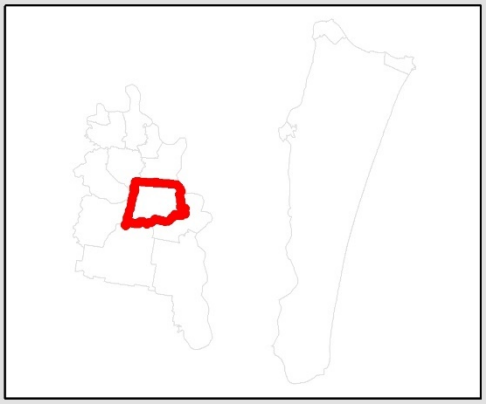
Geocentric Datum of Australia 1994 - MGA Zone 56

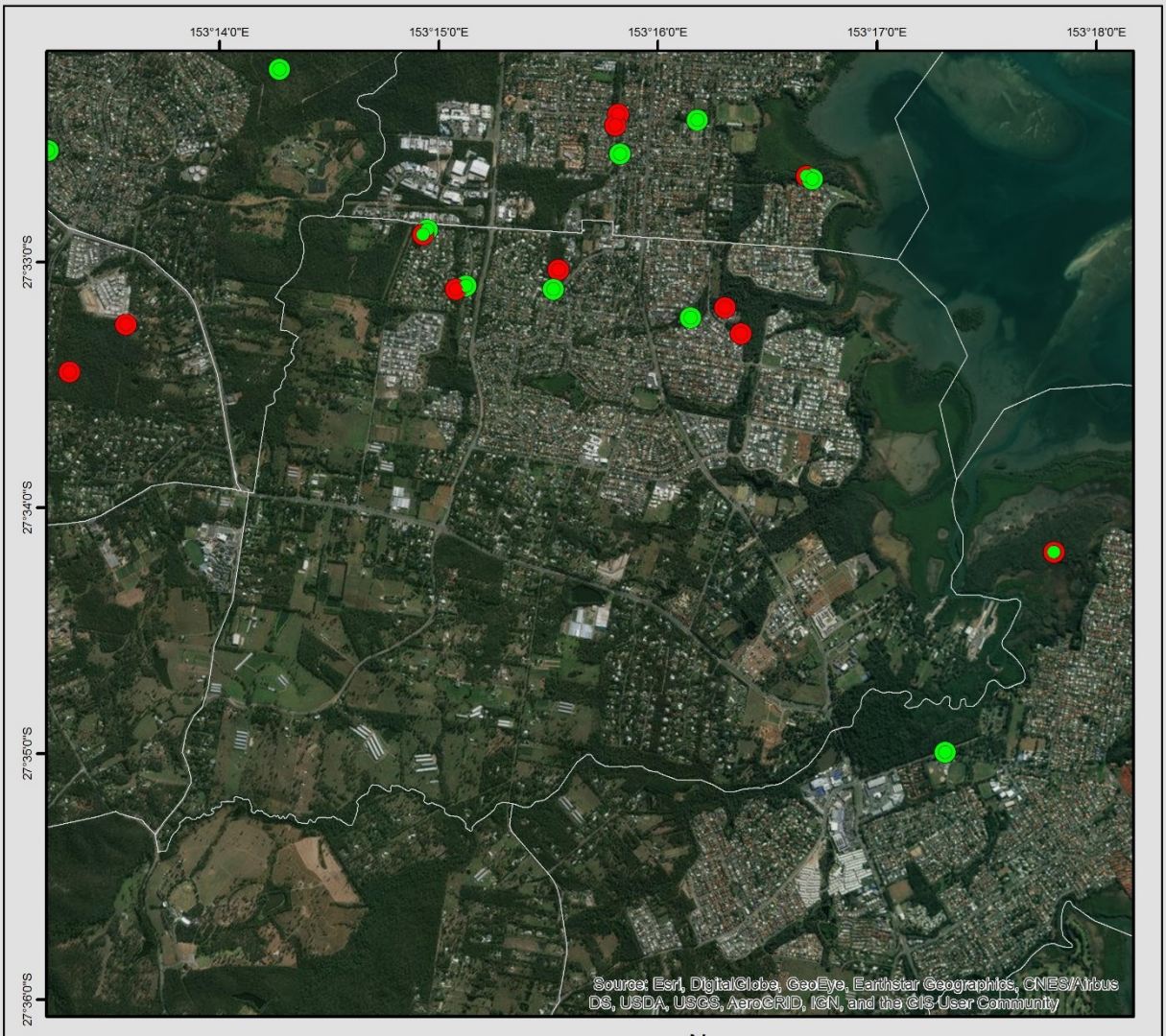
Legend

- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)





Thornlands

N

0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

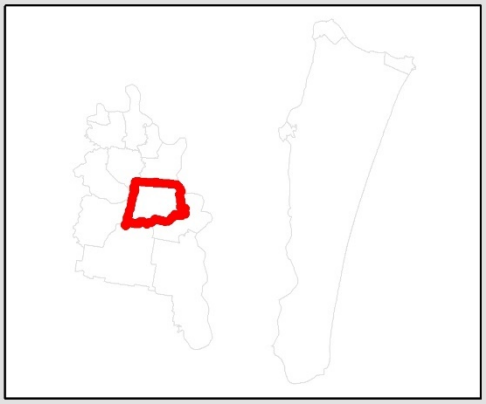
Legend - Chlamydia results

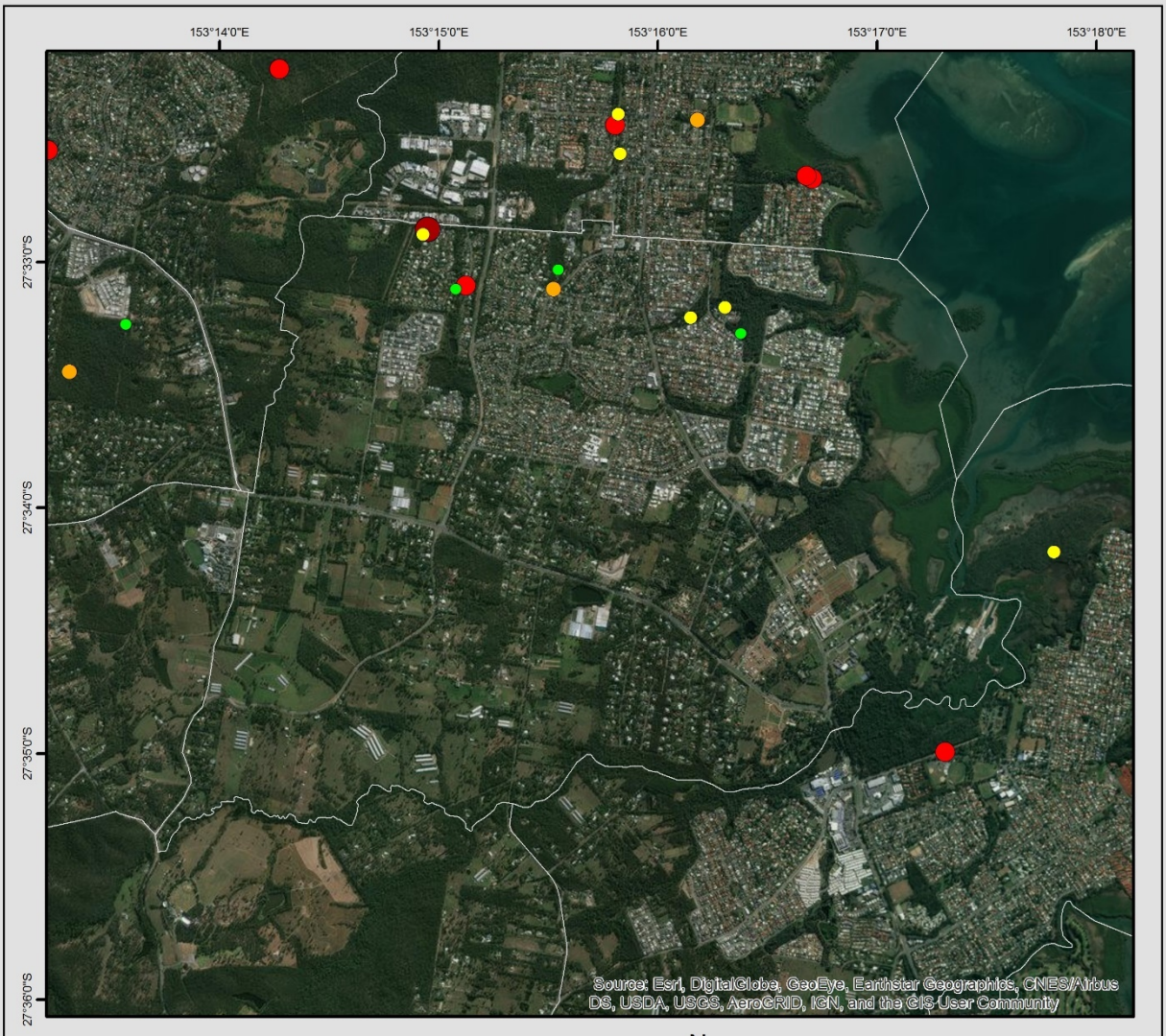
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold





Thornlands

N

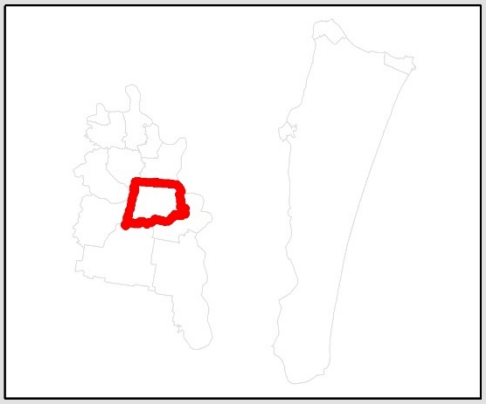
0 0.475 0.95 1.9
Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

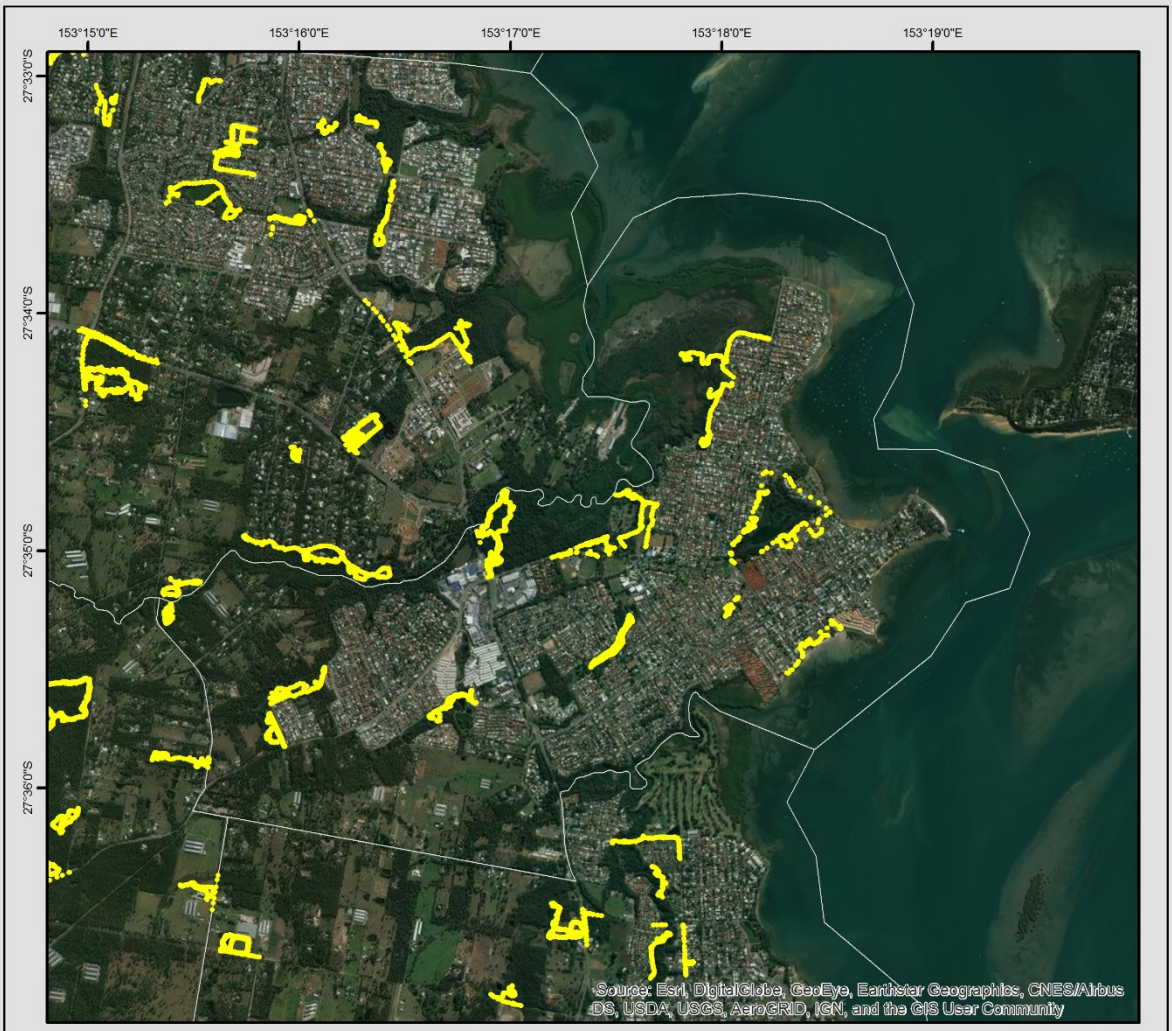
Legend

Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Victoria Point



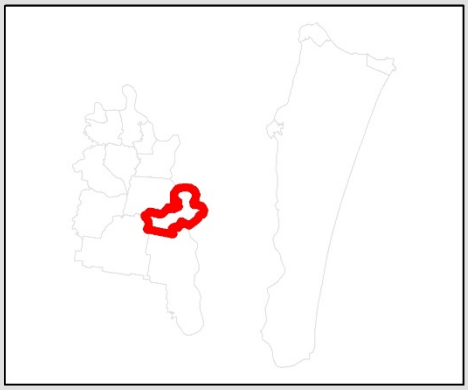
Victoria Point

N
0 0.5 1 2
Kilometers

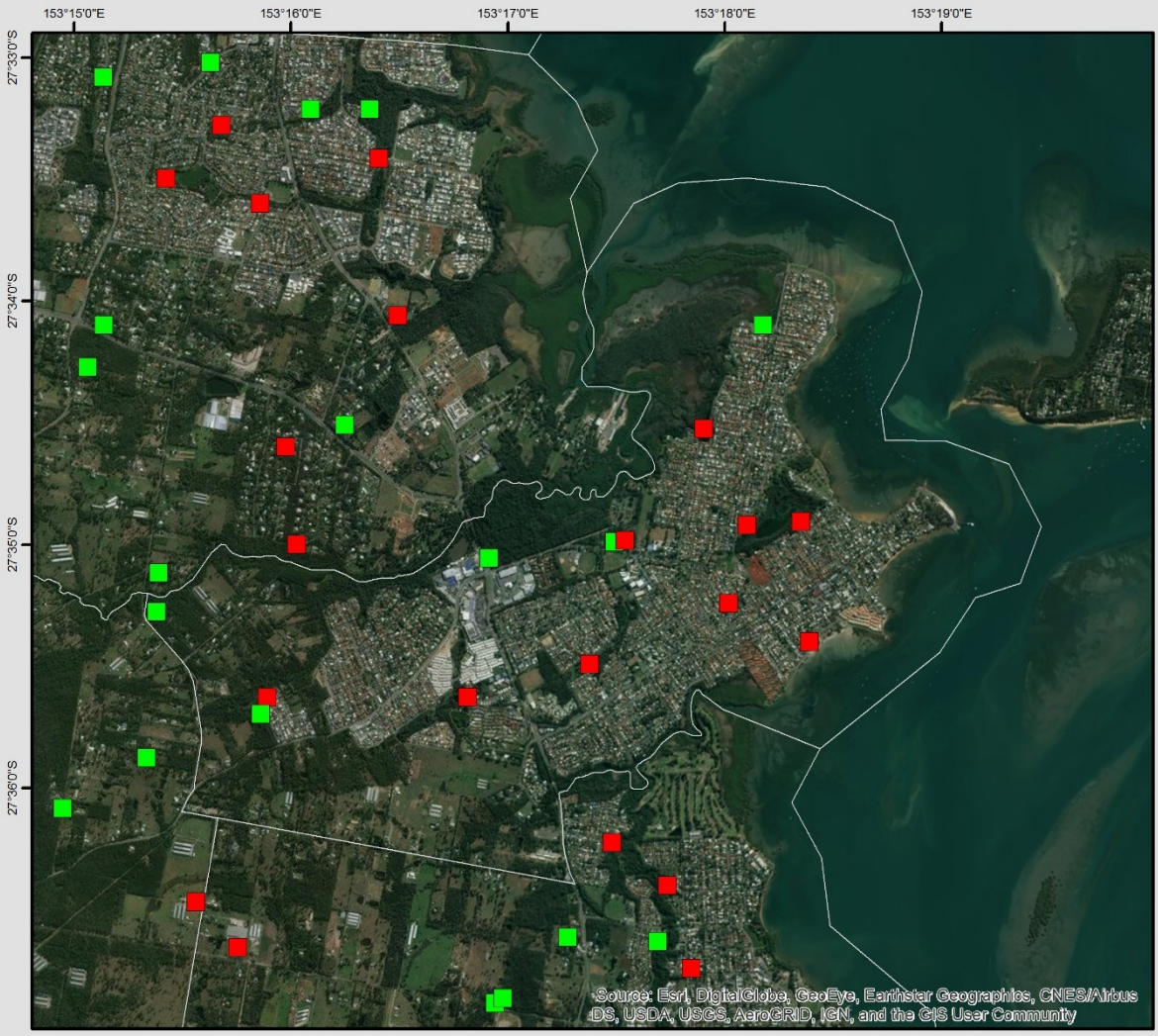
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Dog tracks



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.



Victoria Point

N

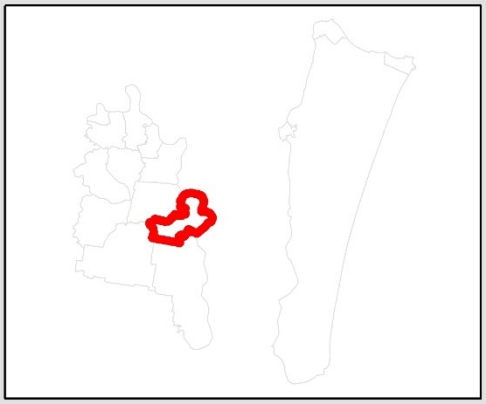
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

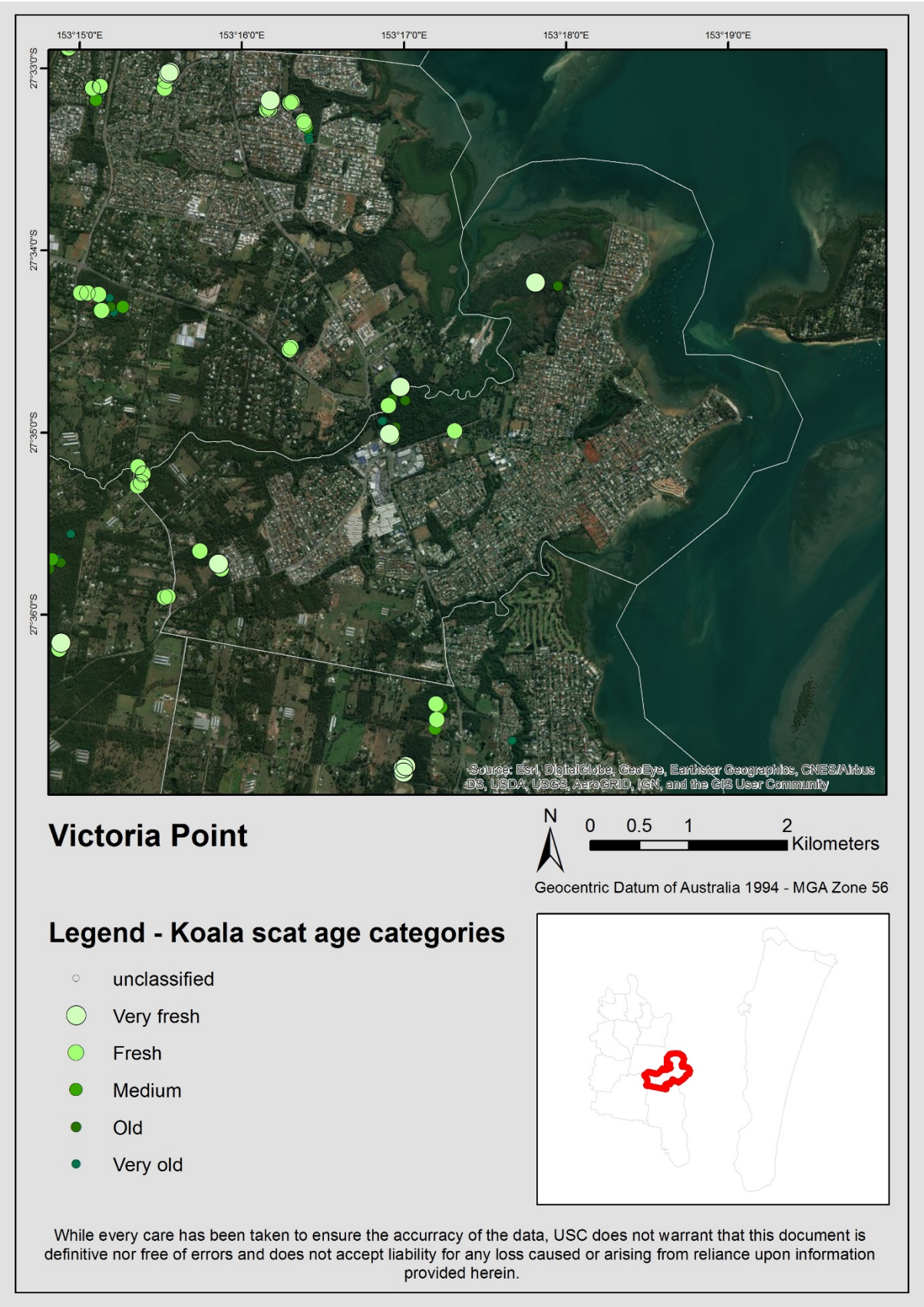
Legend

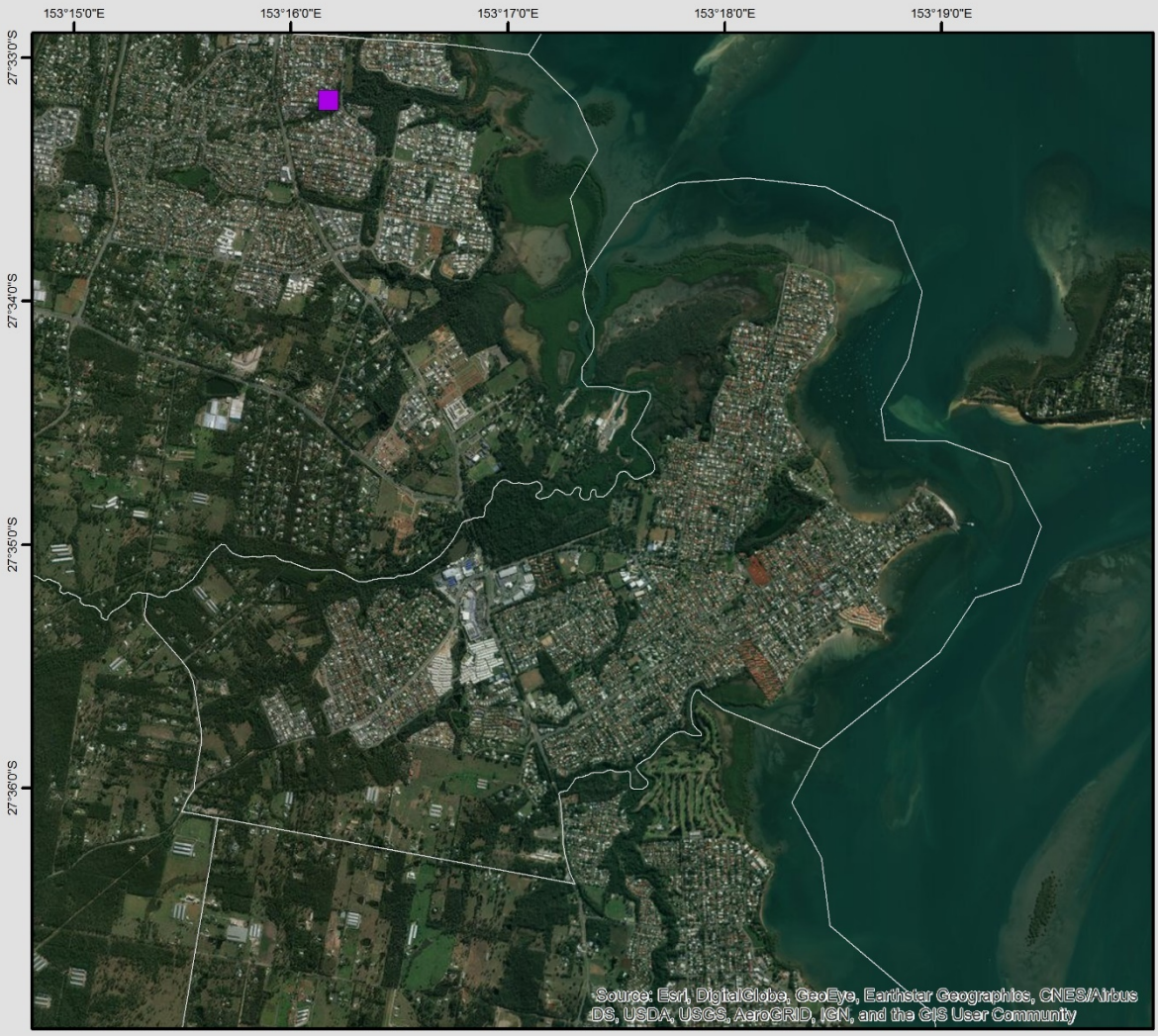
Koala scat

- Presence
- Absence



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.


153°15'0"E
153°16'0"E
153°17'0"E
153°18'0"E
153°19'0"E
27°33'0"S
27°34'0"S
27°35'0"S
27°36'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
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
Old
Very old
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.



Victoria Point

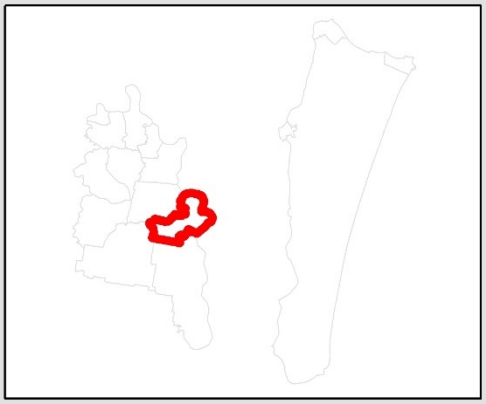
N

0 0.5 1 2 Kilometers

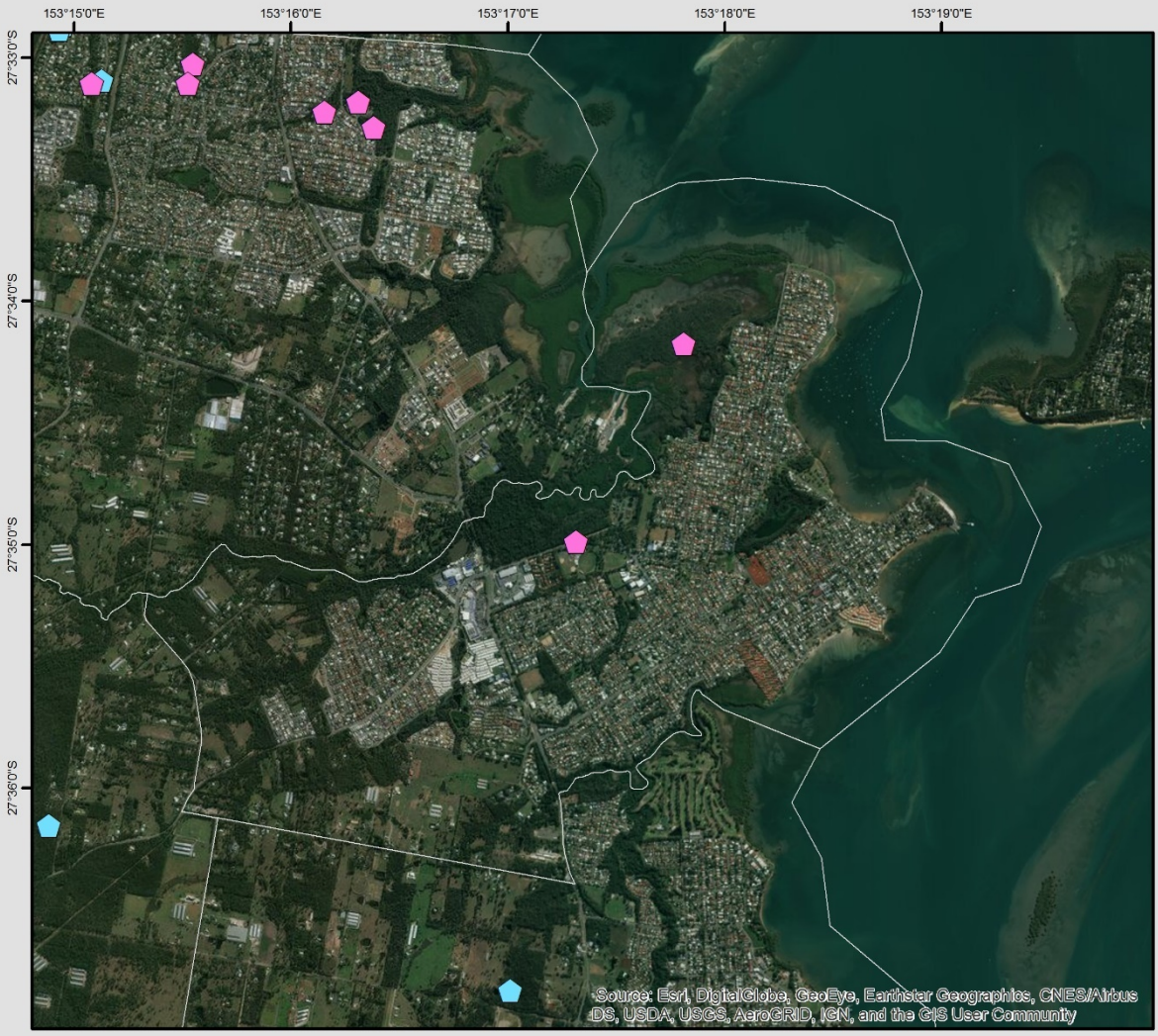
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Victoria Point

N

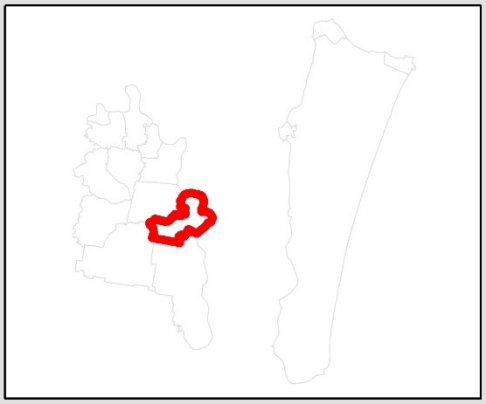
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

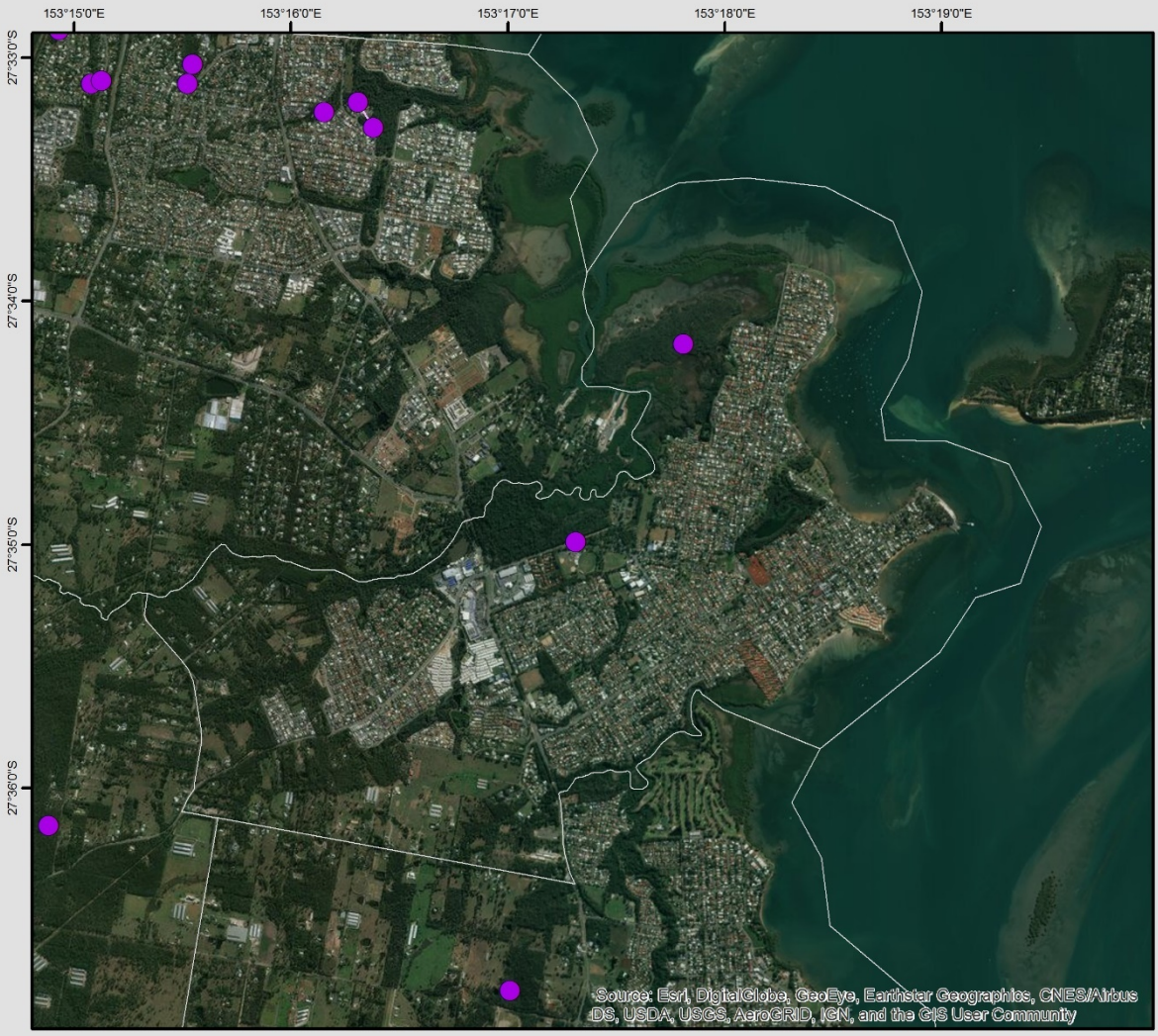
Legend

Koala sex (genetics)

- Female
- Male



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.



Victoria Point

N

0 0.5 1 2 Kilometers

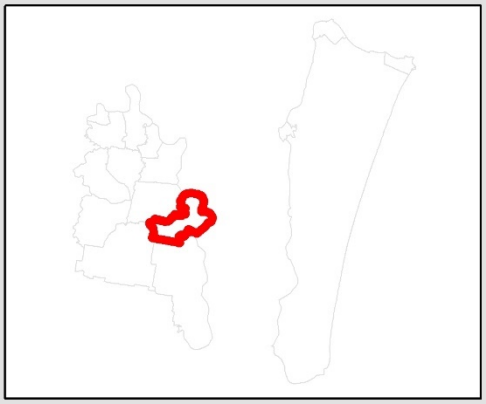
Geocentric Datum of Australia 1994 - MGA Zone 56

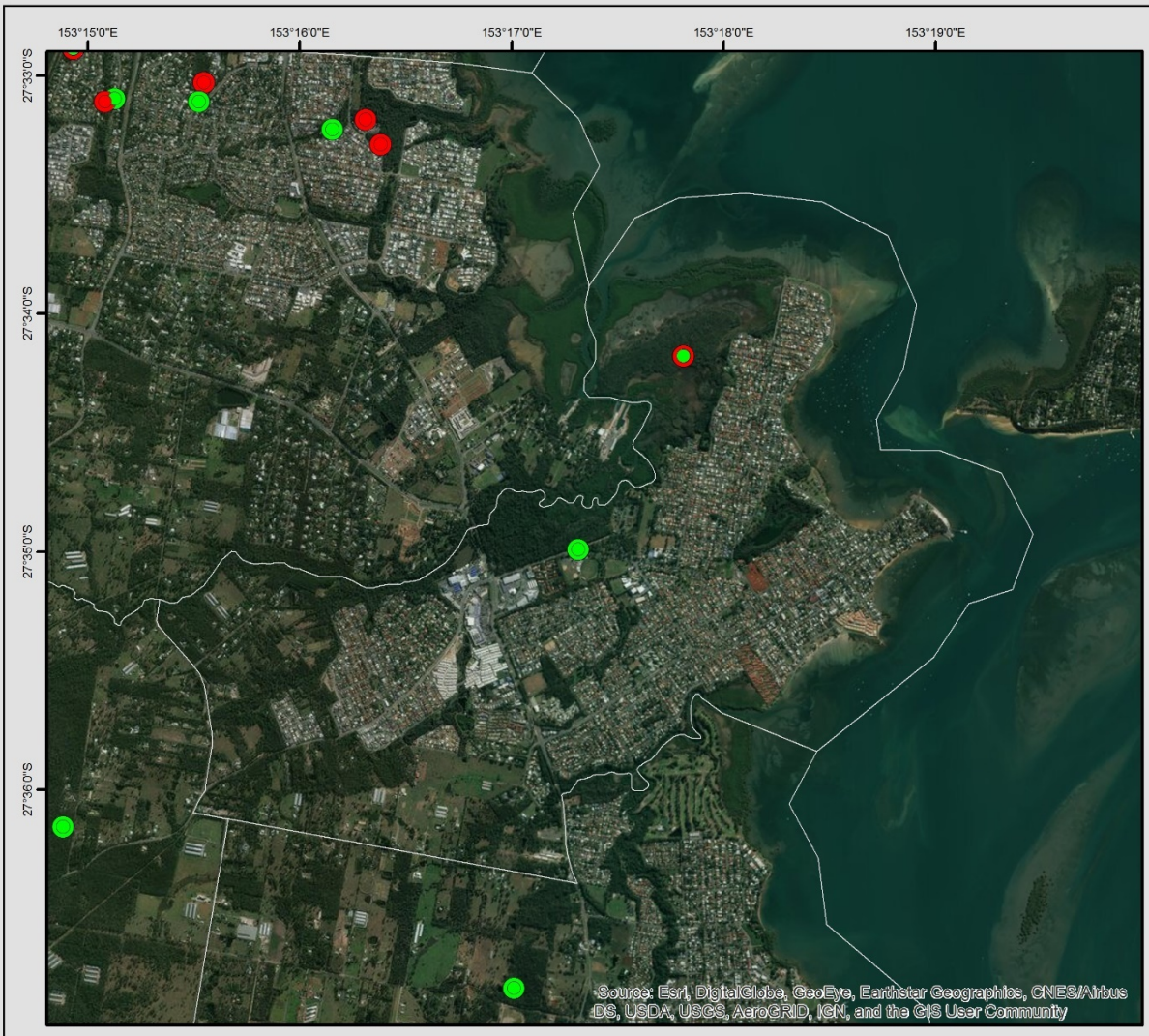
Legend

- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)





Victoria Point

N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

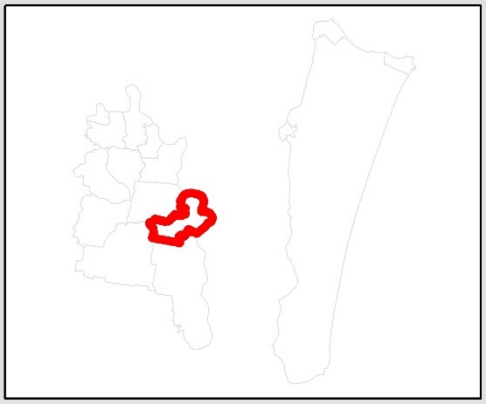
Legend - Chlamydia results

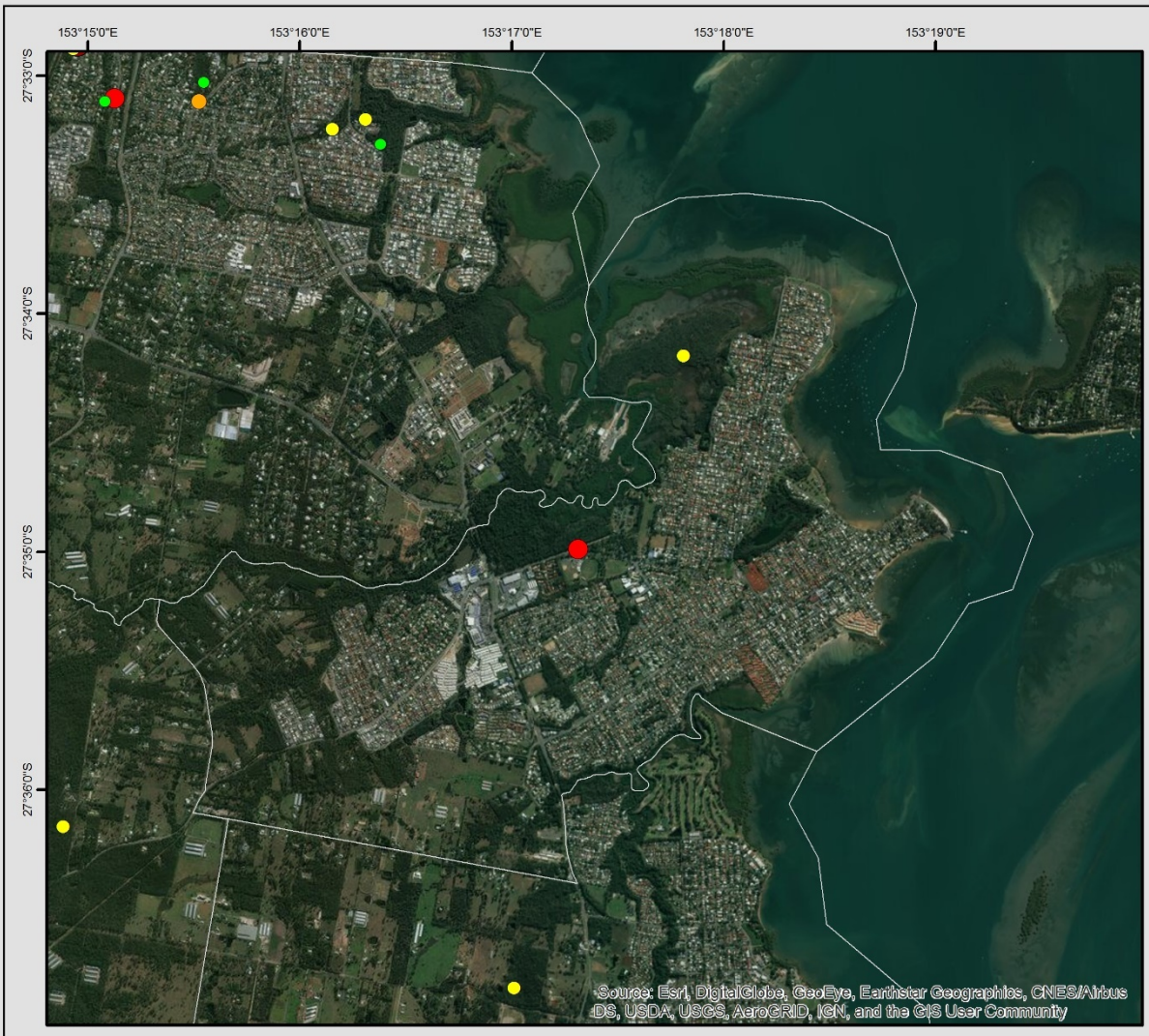
Qualitative

- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold





Victoria Point

N

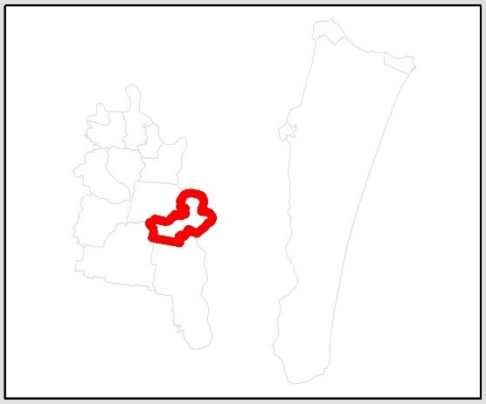
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

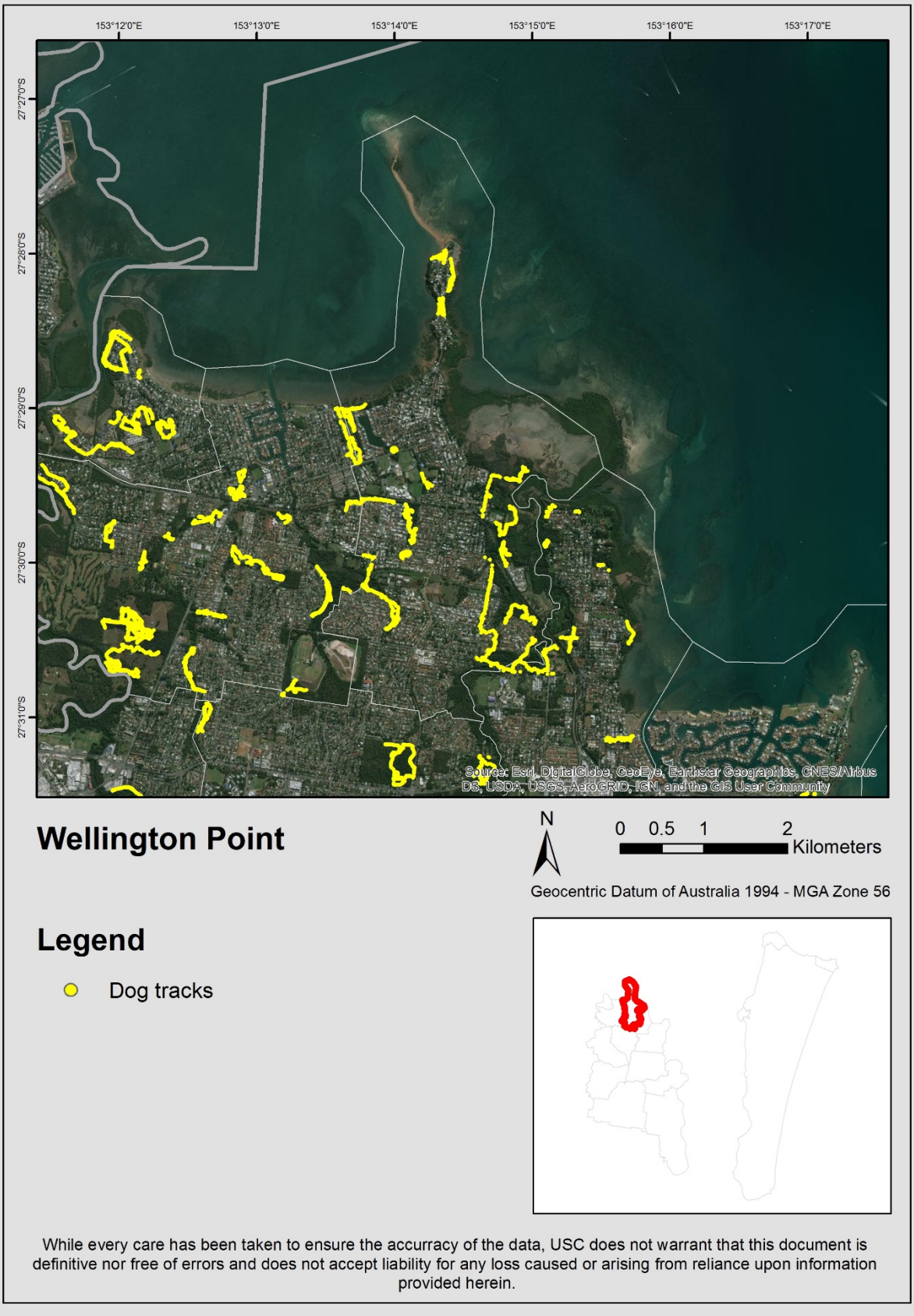
Legend

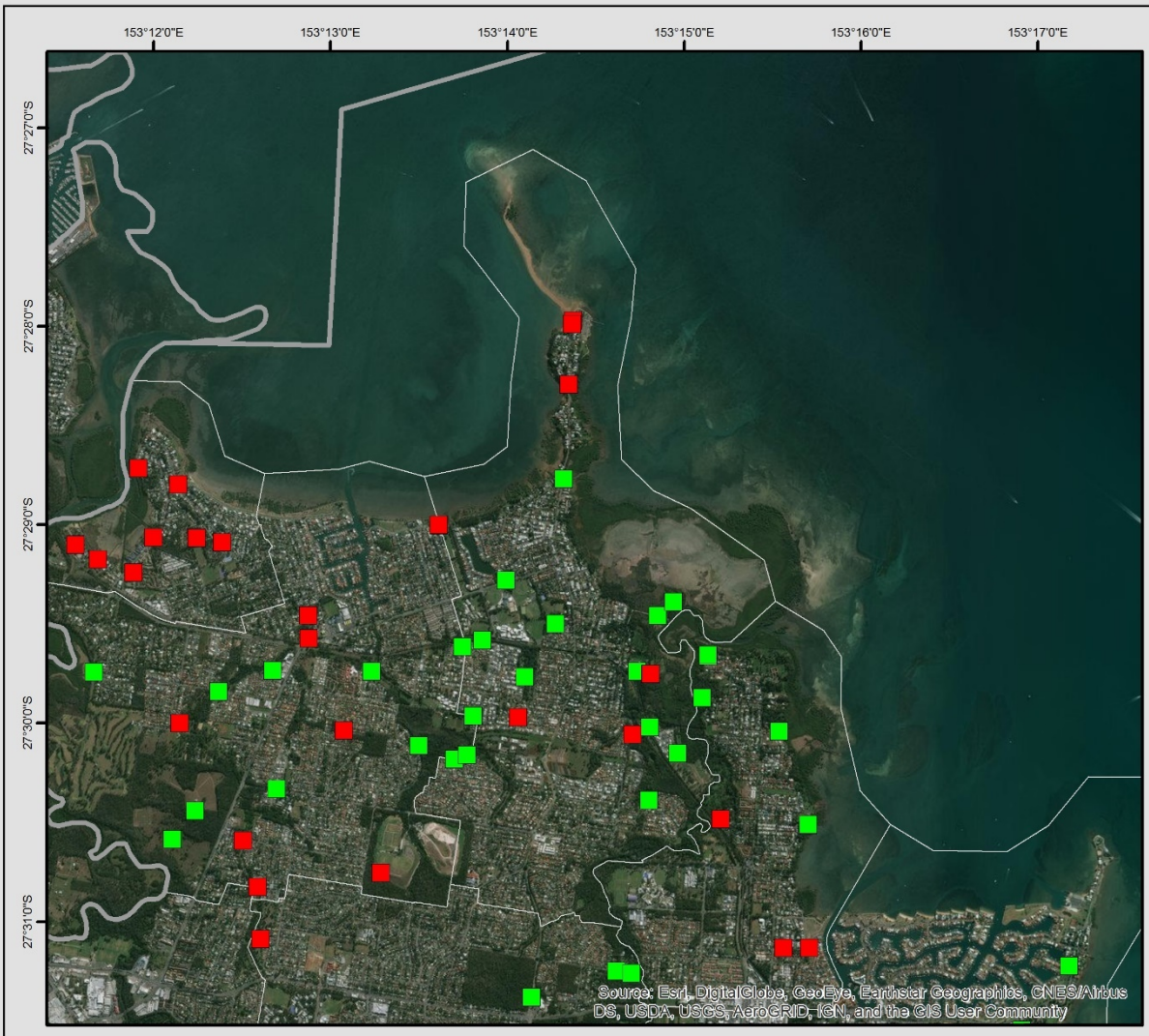
Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Wellington Point


153°12'0"E
153°13'0"E
153°14'0"E
153°15'0"E
153°16'0"E
153°17'0"E
27°27'0"S
27°28'0"S
27°29'0"S
27°30'0"S
27°31'0"S
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Wellington Point
N
0 0.5 1 2
Kilometers
Geocentric Datum of Australia 1994 - MGA Zone 56
Legend
Dog tracks
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.



Wellington Point

N

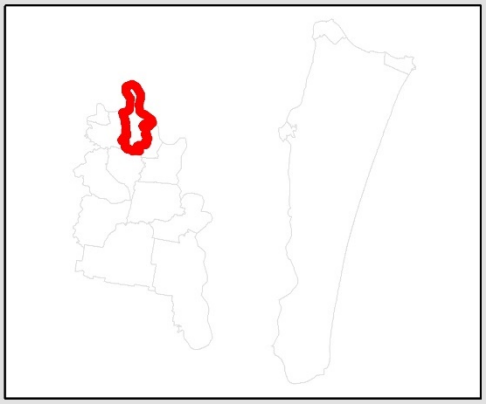
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

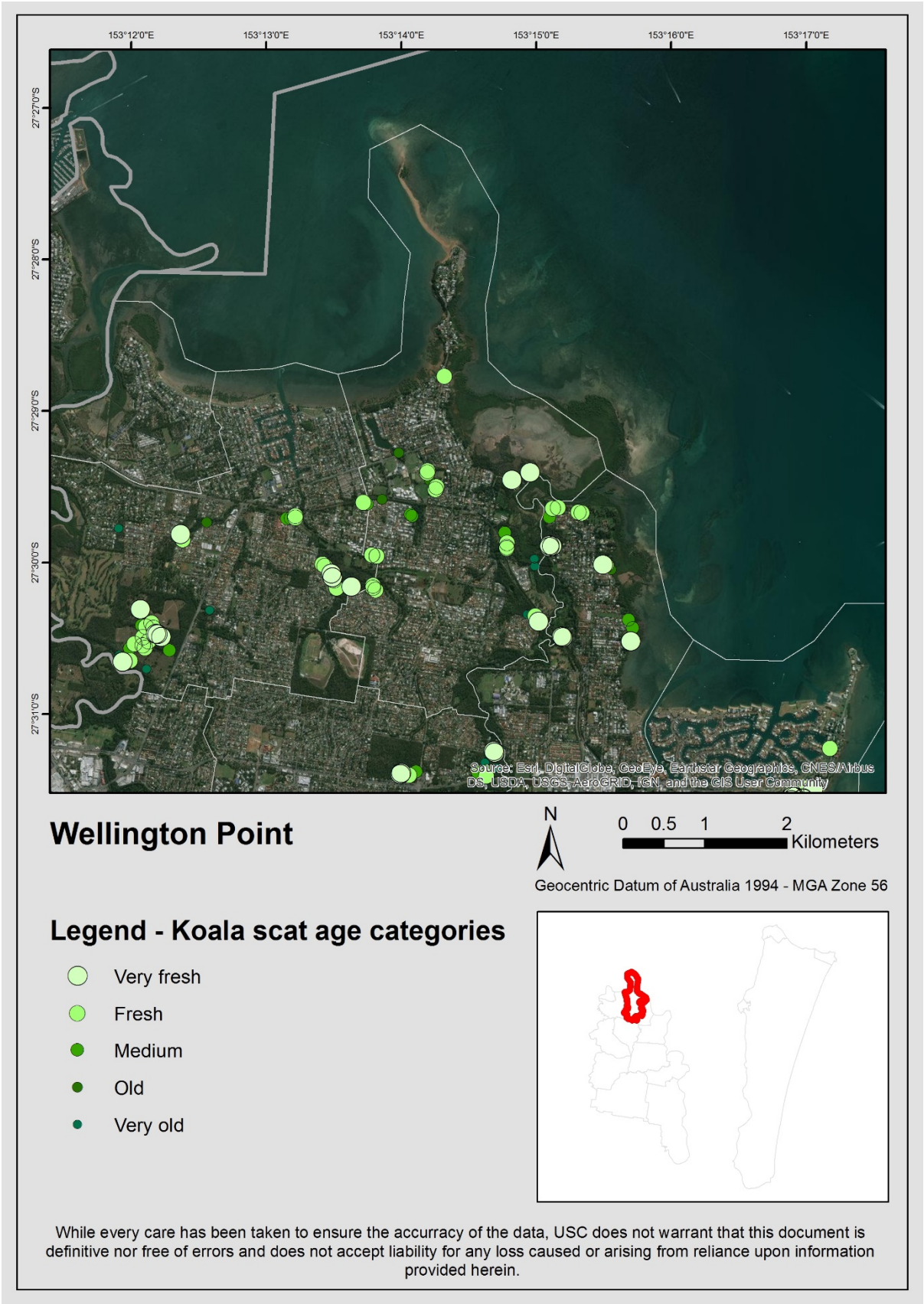
Legend

Koala scat

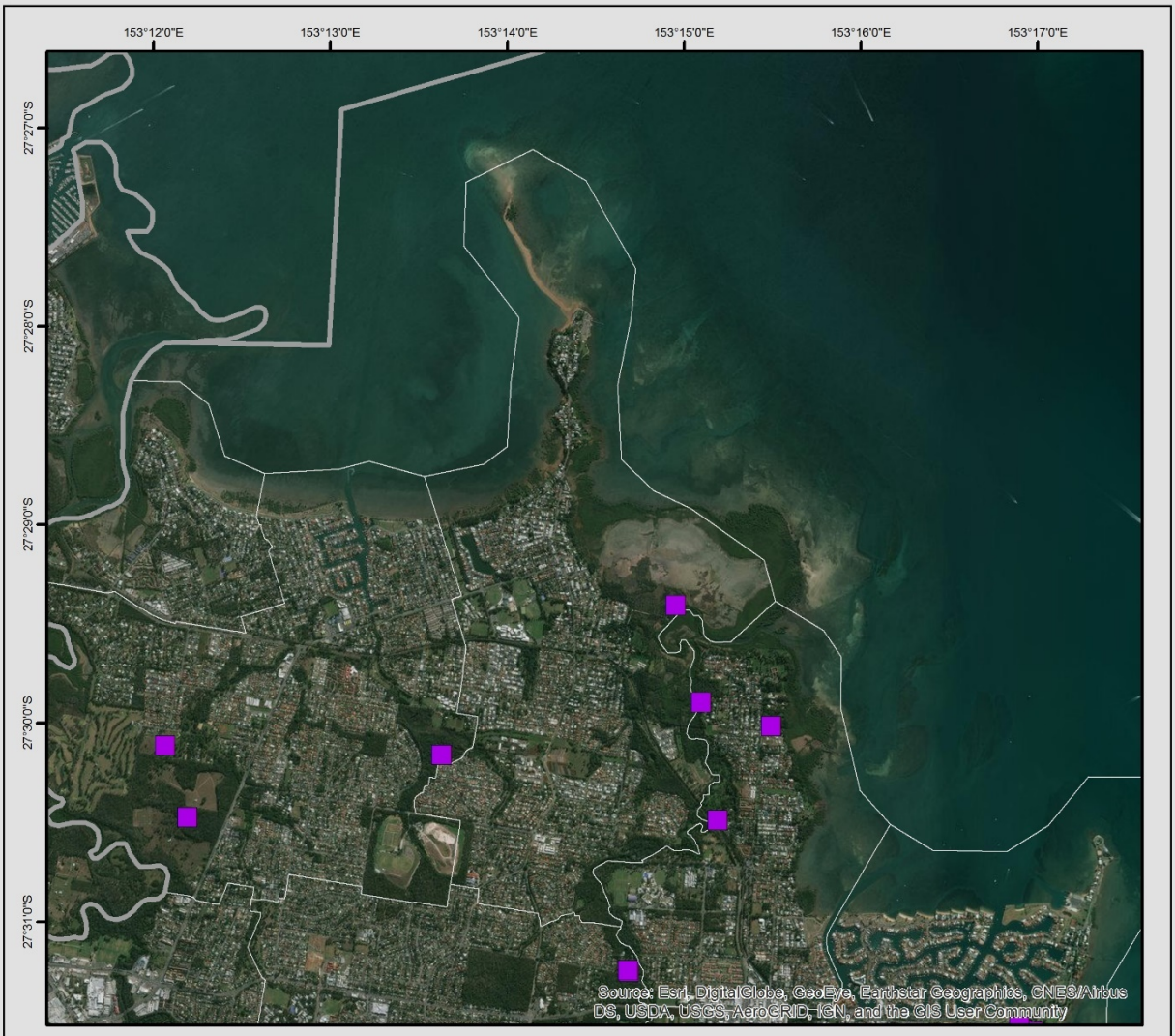
- Presence
- Absence



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.



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.



Wellington Point

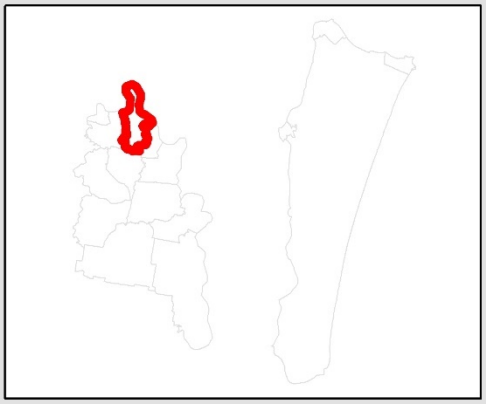
N

0 0.5 1 2 Kilometers

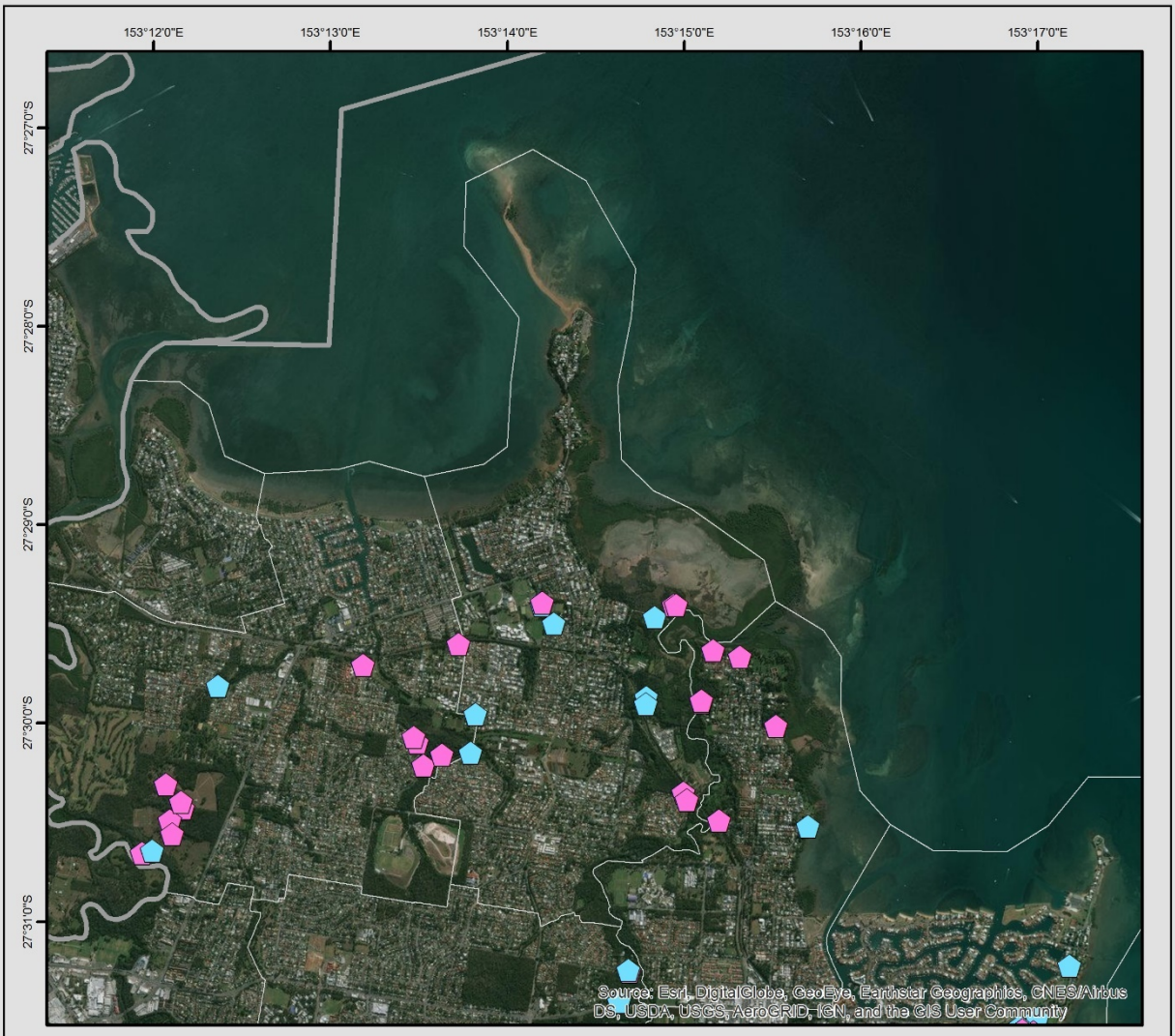
Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

- Koala sightings



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.



Wellington Point

N

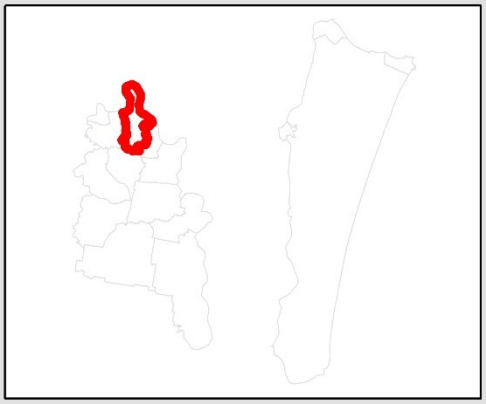
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

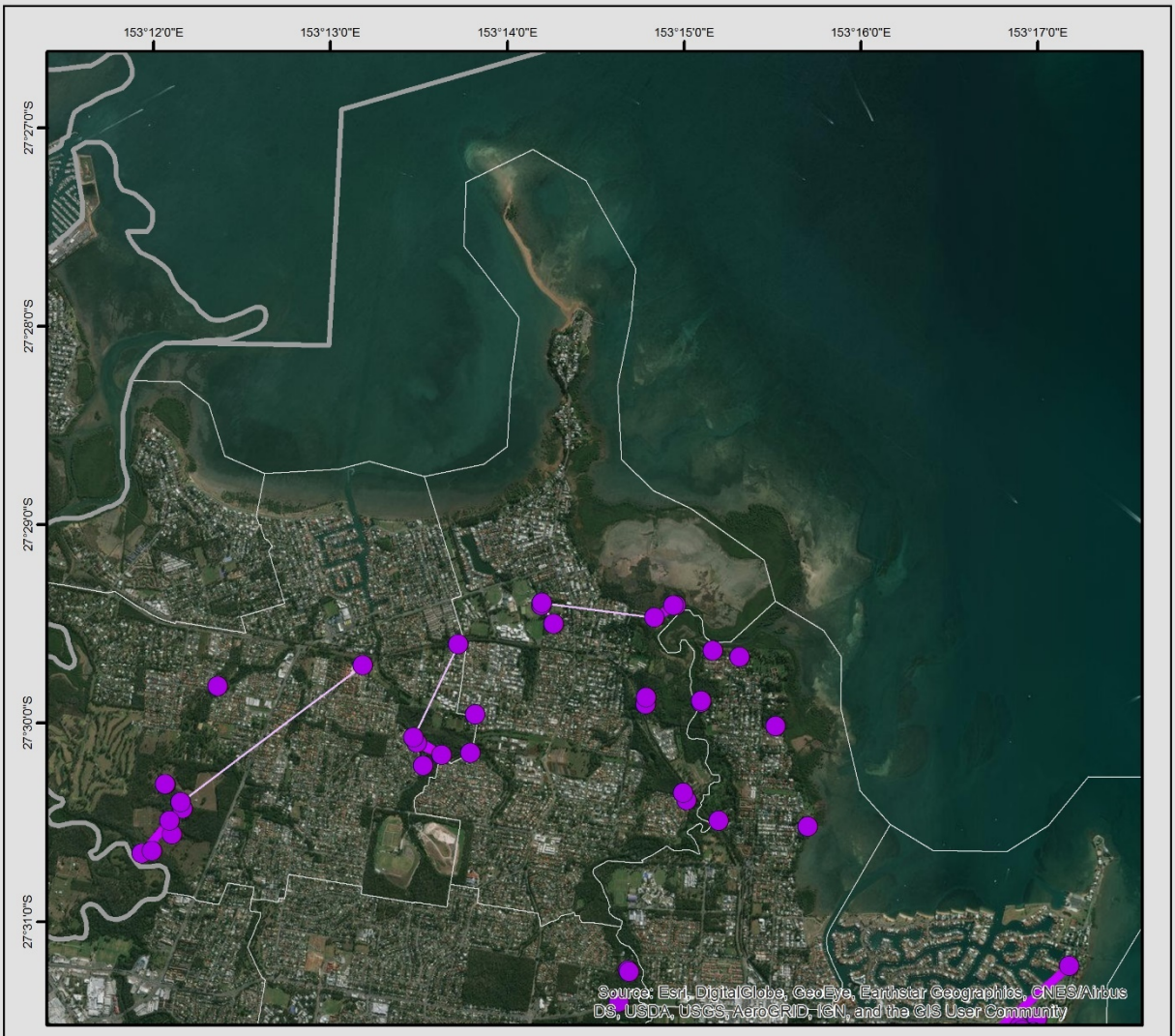
Legend

Koala sex (genetics)

- Female
- Male



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.



Wellington Point

N

0 0.5 1 2 Kilometers

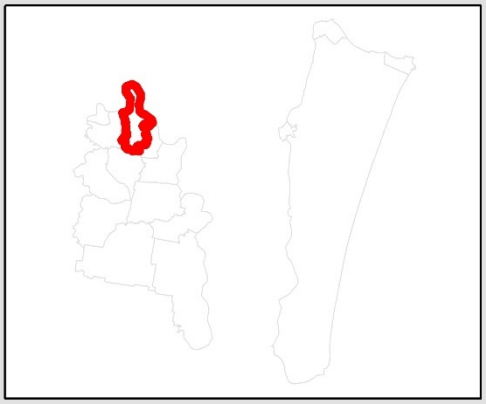
Geocentric Datum of Australia 1994 - MGA Zone 56

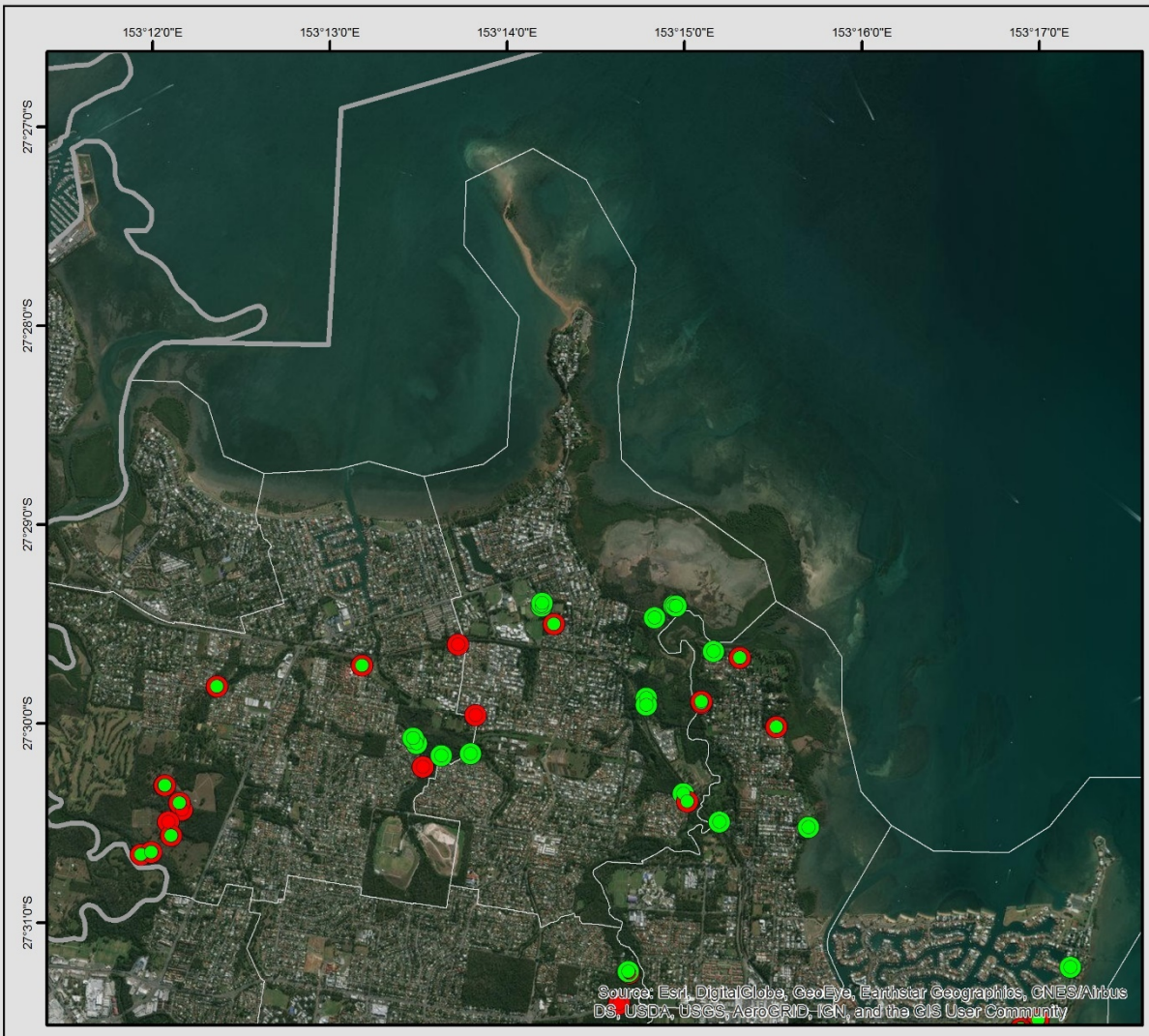
Legend

- Individual genotyped koalas

Relation between koalas

- Non related (< 0.125)
- Related (> 0.125)
- Closely related (> 0.25)





Wellington Point

N

0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

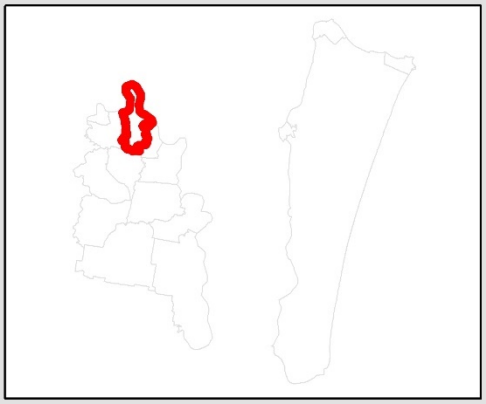
Legend - Chlamydia results

Qualitative

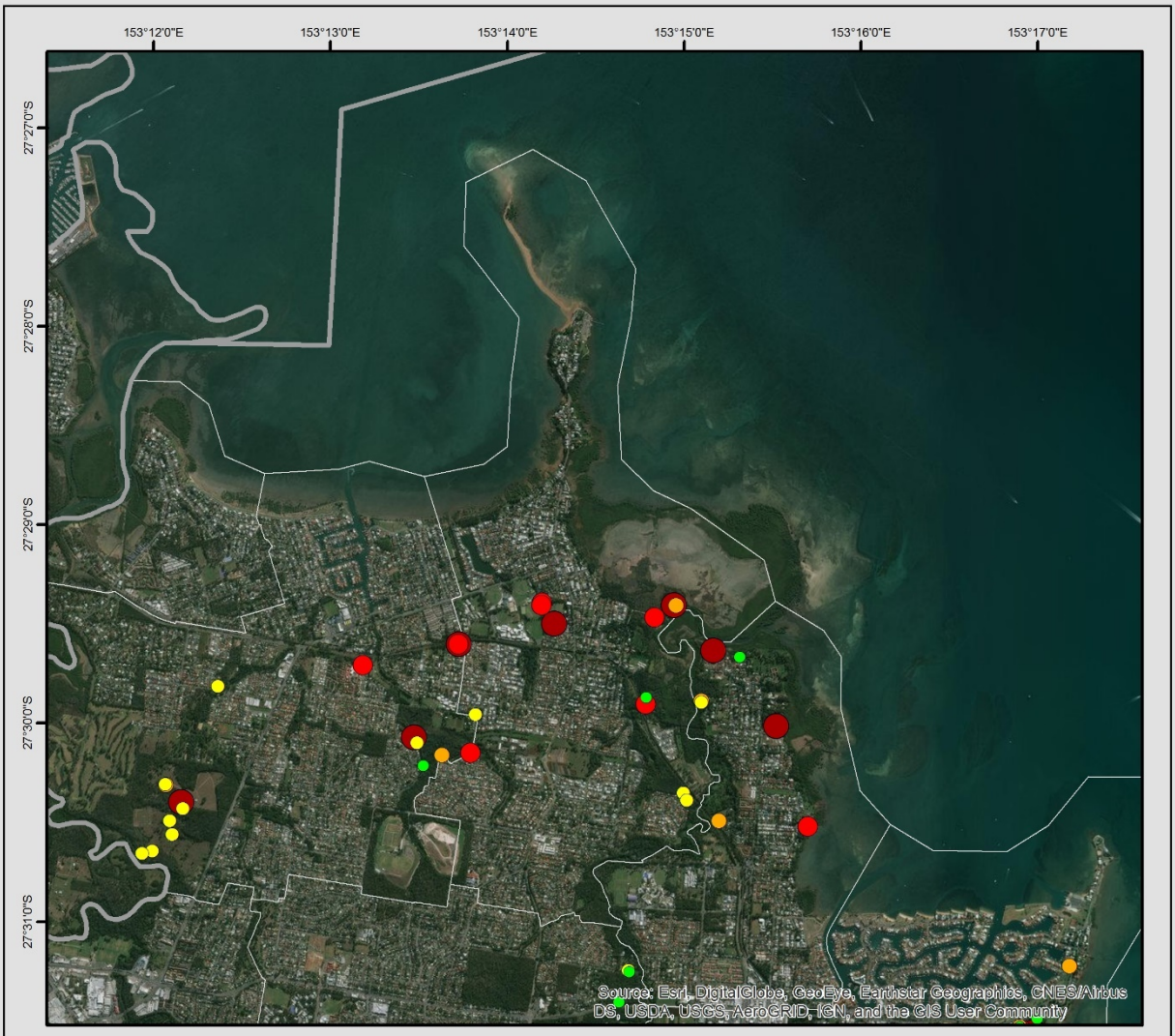
- No Chlamydia dectected
- Chlamydia detected

Quantitative

- Chlamydia load under threshold
- Chlamydia load above threshold



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.



Wellington Point

N

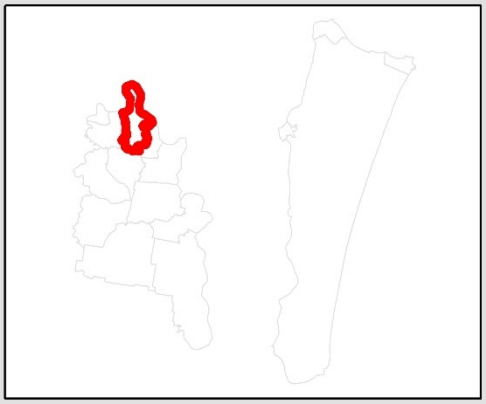
0 0.5 1 2 Kilometers

Geocentric Datum of Australia 1994 - MGA Zone 56

Legend

Internal relatedness

- -0.19 - 0.10
- 0.11 - 0.27
- 0.28 - 0.44
- 0.45 - 0.65
- 0.66 - 0.87



Appendix 4: Threat mapping commissioned by USC for USC research purpose, and that will be used to further analyse threats to koalas in the Redlands Coast

Brief methodology (based on Healthy Land and Water methodology and report to Noosa Council) outline into creating these maps:

Figure 1: Road Density (sealed roads only)

- Combination of SDRN (state digital road network) <= road type 5 intersecting sealed roads according to local government road data
- Line Density using 1000m radius
- Classified into 4 using natural breaks

Figure 2: Clearing Hotspots (2001-2010)

- Merged slats from 2001-2002 through to 2009-2010

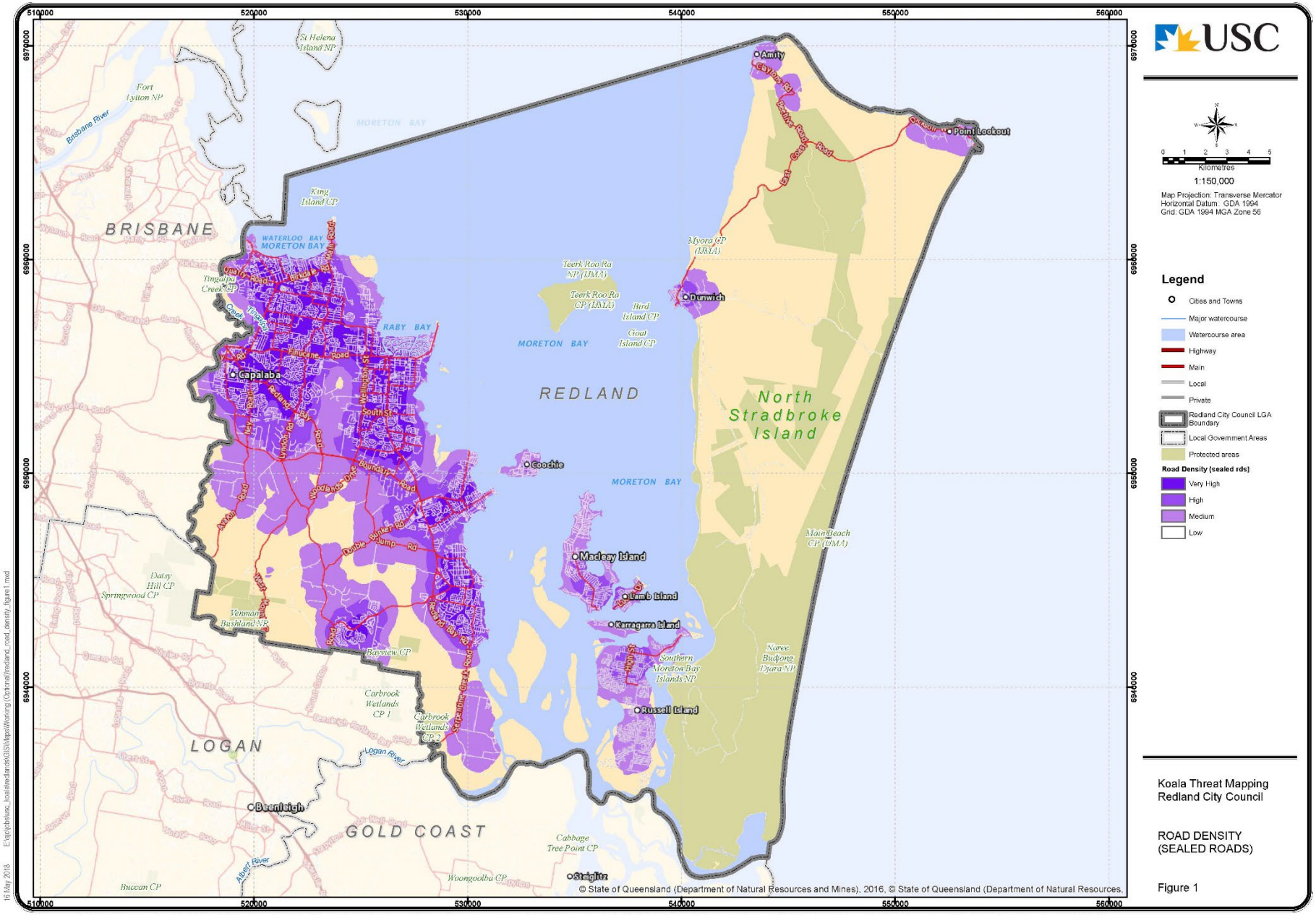
  Slats/clearing includes;

  - *Pasture*
  - *Infrastructure*
  - *Crop*
  - *Mine*
  - *Missed clearing in previous era*
  - *Settlement*
  - *Timber plantation*
- Clipped to a 1km buffer around each LGA to ensure boundary effects are taken into account
- Sum of Focal stats (250m circular radius)
- Classified into 4 using natural breaks

Figure 3: Small Lots/ Urbanisation

- Property lots <=8 hectares (roads excluded in analysis as these will be mapped in the roads threat layer)
- Clipped to a 1km buffer around each LGA to ensure boundary effects are taken into account
- Sum of Focal stats (250m circular radius)
- Classified into 4 using natural breaks

DETECTION DOGS
FOR CONSERVATION
Sniffing out solutions usc.edu.au/ddc
University of the Sunshine Coast



© State of Queensland (Department of Natural Resources and Mines), 2016, © State of Queensland (Department of Natural Resources.

USC

0 1 2 3 4 5
Kilometres
1:150,000

Map Projection: Transverse Mercator
Horizontal Datum: GDA 1994
Grid: GDA 1994 MGA Zone 56

Legend

- Cities and Towns
- Major watercourse
- Watercourse area
- Highway
- Main
- Local
- Private
- Redland City Council LGA Boundary
- Local Government Areas
- Protected areas

Road Density (sealed rds)

- Very High
- High
- Medium
- Low

Koala Threat Mapping
Redland City Council

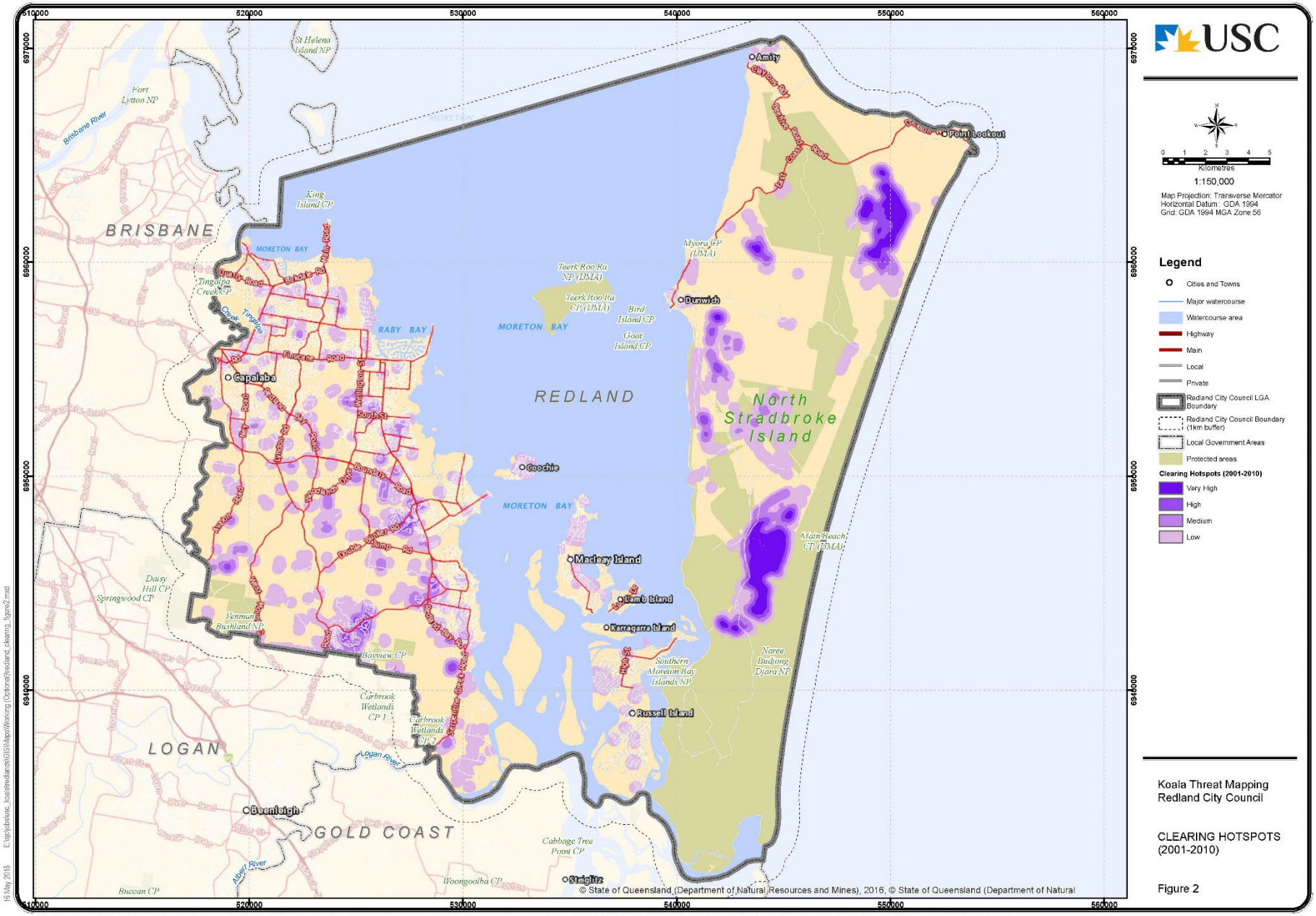
ROAD DENSITY
(SEALED ROADS)

Figure 1

16 May 2018 E:\sig\dds\usc_koala\redlands\GIS\Maps\Working (Ctrl\one\)\redland_road_density_figure1.mxd

Data source: DNRME: Local Government Boundaries QLD (Mar 2016), Rail Network QLD (Feb 2014), Baseline Roads and Tracks QLD (May 2018), Place name gazetteer (May 2018) Property Boundaries (May 2018) DES: Statewide landcover and trees study 2001-2010, Protected Areas (May 2018) RCC: Roads sealed (May 2018) © State of Queensland (DNRME, DES)

DETECTION DOGS
FOR CONSERVATION
Sniffing out solutions usc.edu.au/ddc
University of the Sunshine Coast



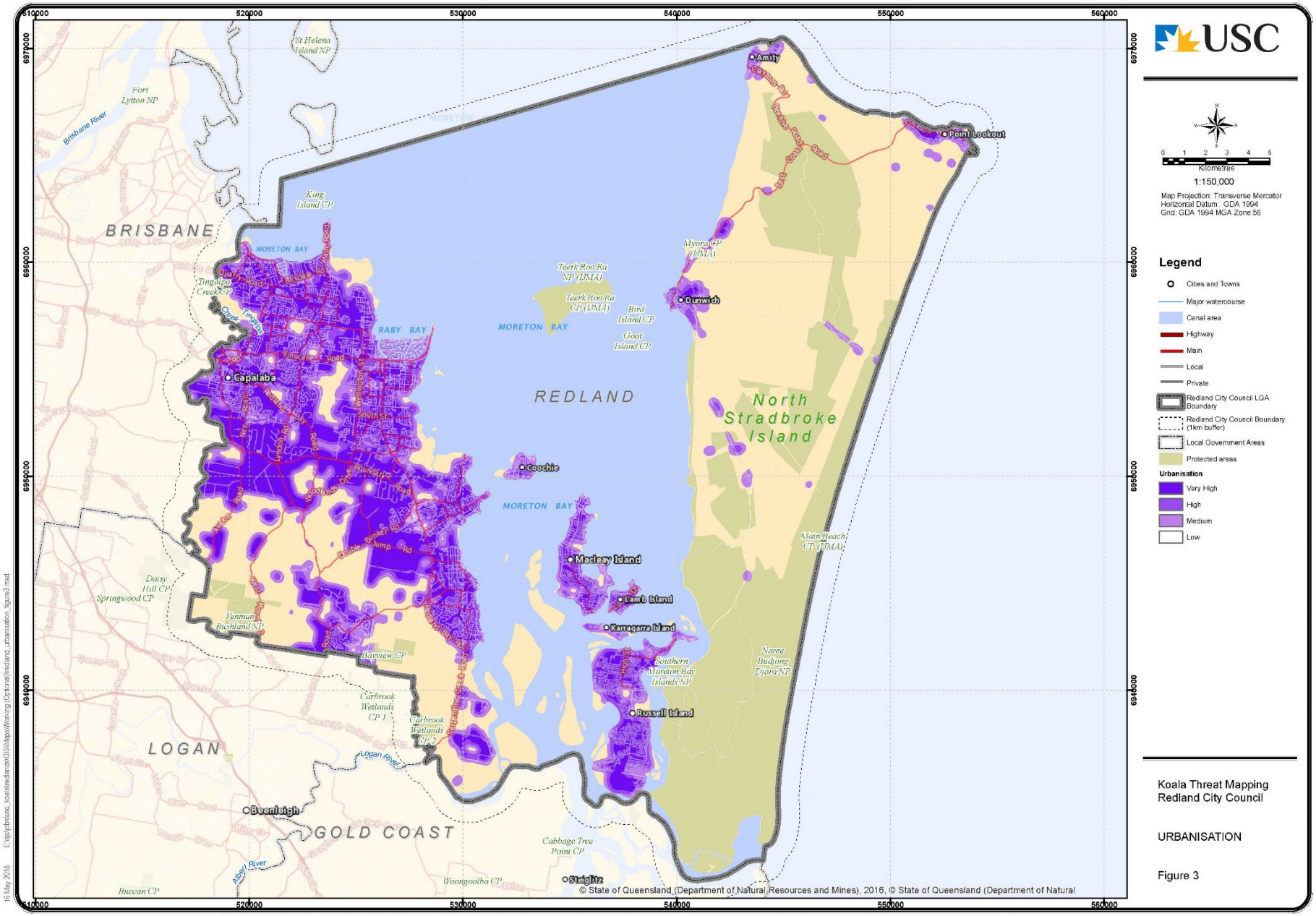
16 May 2018 E:\gis\jobs\usc_koala\redlands\GIS\Maps\Working (Optional)\redland_clearing_figure2.mxd

Data source: DNRME: Local Government Boundaries QLD (Mar 2016), Rail Network QLD (Feb 2014), Baseline Roads and Tracks QLD (May 2018), Place name gazetteer (May 2018) Property Boundaries (May 2018) DES: Statewide landcover and trees study 2001-2010, Protected Areas (May 2018) © State of Queensland (DNRME, DES)

DETECTION DOGS FOR CONSERVATION

Sniffing out solutions usc.edu.au/ddc

University of the Sunshine Coast



16 May 2018 E:\projects\usc_koala\redlands\GIS\Maps\Working (Optional)\redland_urbanisation_figure3.mxd

Data source: DNRME: Local Government Boundaries QLD (Mar 2016), Rail Network QLD (Feb 2014), Baseline Roads and Tracks QLD (May 2018), Place name gazetteer (May 2018) Property Boundaries DCDB Lite (May 2018), Major Watercourse lines (April 2014) DES: Protected Areas (May 2018) © State of Queensland (DNRME, DES)

QYAC rangers and DDC team on Minjerribah during the 2018 koala surveys

End of report