SCENIC RIM 2024 KOALA POPULATION STUDY

Prepared by OWAD Environment
in collaboration with
WildDNA | Federation University Australia

For Scenic Rim Regional Council

Study funded by the Australian Government

SCENIC RIM REGIONAL COUNCIL

Australian Government

Acknowledgements

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The local community is thanked and congratulated for their enthusiastic collaboration with Scenic Rim Regional Council officers and Watergum staff to perform this highly successful non-invasive Koala genetic sampling program in record time.

Scenic Rim Regional Council also thanks the Department of Defence and the Koala Research and Monitoring Team from Queensland's Department of Environment, Tourism, Science and Innovation for their help in collecting scat samples for this study.

Watergum staff surveying for Koalas and scat samples during this study. Photo credit: Watergum Community Inc.

‘Elvin’, male Koala (sample code SCE061) from Tamborine Mountain. Photo credit: Jens Sohnrey.

OWAD training Scenic Rim Regional Council and Watergum in non-invasive Koala DNA sampling.
Photo credit: Lara Solyma.

DOCUMENT CONTROL SHEET

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Project Title:	Scenic Rim 2024 Koala population study
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Project Summary:	This report presents the results of a non-invasive Koala population study in the Local Government Area of the Scenic Rim, Southeast Queensland. The genetic sampling program was designed and coordinated by Scenic Rim Regional Council, with scat samples from wild Koalas collected by the local community across the study area. DNA isolated from the scat samples was then subjected to a selected suite of molecular tests to provide insights into the population structure, genetic health, pathogens, and diet of the Scenic Rim Koalas. This data can be used to inform improved strategies aimed at conserving and recovering the species.
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TERMS, ABBREVIATIONS AND DEFINITIONS

Allele	Each of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome. **Private alleles** are alleles that are found only in a single genetic group of individuals among a broader collection of groups. (Allelic: relating to alleles.)
C. pecorum	*Chlamydia pecorum* is a species of bacterium from the family Chlamydiaceae. Chlamydiosis is considered the most important infectious disease of Koalas as it is the most common and the most pathogenic *Chlamydia* species infecting Koalas. In the Koala, it can cause urinary tract disease, reproductive disease, infertility and death.
DNA	Deoxyribonucleic acid encodes genetic information. A DNA molecule is made up of a great number of smaller molecules called nucleotides. DNA governs the production of proteins and other molecules essential to cell function. Mammals generally have two sets of DNA: one half inherited from the individual's mother, the other half from the father.
Epithelial cells	Mucous-type cells that line the internal and external surfaces of the body.
Gene	The basic physical and functional unit of heredity. Genes are made up of DNA and act as instructions to make protein molecules. (Genetic/genetically: relating to genes.)
Genetic drift	Genetic drift is a random change in allele frequencies in a group of individuals over generations due to chance events, rather than natural selection. It has the most significant effects in small, isolated groups of Koalas, where genetic diversity can be rapidly lost. Genetic drift can limit the ability of Koalas to adapt to environmental changes, disease pressures, and climate variability.
Gene flow	Refers to the transfer of genes from one group of individuals to another, that occurs via individuals migrating, mixing and reproducing, facilitating genetic exchange and reducing genetic differentiation between these groups.
Genotype	A genotype is the complete set of genetic information (alleles) an organism carries at a particular gene or across its entire genome. It determines the hereditary traits of an individual and influences characteristics such as physical traits, disease susceptibility, and evolutionary adaptations.
KoRV	Koala Retrovirus (KoRV) is a retrovirus that infects Koalas with 13 described subtypes (KoRV-A to KoRV-M). Some variants are associated with increased susceptibility to cancers, metabolic disorders, and immunodeficiencies. Similar to the effects of HIV in humans, immunosuppression caused by certain KoRV subtypes increase Koalas' vulnerability to infectious diseases such as (but not limited to) *Chlamydia pecorum*. **KoRV-A is endogenous** in Queensland and New South Wales, meaning it is permanently integrated into the Koala genome and inherited vertically, i.e. from parent to offspring. All other known subtypes **KoRV-B to KoRV-M are exogenous** and transmitted horizontally (between individuals) and/or vertically (from parent to offspring).
LGA	Local Government Area.
Microsatellite	A short, repetitive sequence of DNA consisting of 1–6 base pairs that is repeated multiple times in a row. These sequences, also known as short tandem repeats (STRs), are highly variable in length between individuals, making them useful genetic markers for population genetics, forensic analysis, and species conservation.

1.0 EXECUTIVE SUMMARY

Scenic Rim Regional Council (SRRC) engaged OWAD Environment to support the delivery of a non-invasive Koala genetic study led by the community, aimed at understanding the population dynamics and health of Koalas in the Scenic Rim Local Government Area (LGA) of Southeast Queensland. This project was financed by Australian Government grant funding.

SRRC designed the sampling program and coordinated local community participation in its execution, facilitating the collection of Koala scats (faecal pellets) across the region. DNA was isolated from the scat samples and subjected to molecular testing, allowing for:

- The generation of individual Koala genetic profiles to assess population structure and connectivity; and
- The detection of key Koala pathogens, including *Chlamydia pecorum* and all known subtypes of the Koala Retrovirus (KoRV).

The genetic data obtained in this study, combined with a substantial number of reference samples from wild Koalas non-invasively sampled across Southern Queensland over the last 10 years, were analysed to assess population connectivity and the prevalence of *C. pecorum* and KoRV across the region.

Results indicate that habitat loss and fragmentation since European settlement has resulted in significant genetic differentiation among groups of Koala across Southeast Queensland. The analysis identified five distinct population clusters within the Scenic Rim LGA, highlighting a fragmented population structure within the study area. Gene flow between these five clusters exhibited a highly asymmetric pattern, with most migration occurring unidirectionally toward the SEQ-03 cluster, suggesting limited reciprocal exchange between the clusters. This imbalance may contribute to increased genetic isolation and reduced genetic diversity in other clusters, potentially heightening their vulnerability to environmental pressures and disease.

Both *C. pecorum* and Koala Retrovirus (KoRV) were detected across the study area. *C. pecorum* was found in 40% of individuals tested, while four KoRV subtypes were identified: KoRV-A (100%), KoRV-B (3%), KoRV-D (31%), and KoRV-F (12%). The presence of these pathogens, particularly in fragmented population clusters, underscores the importance of ongoing disease surveillance and management strategies to mitigate potential health impacts on already isolated Koala population clusters.

Habitat loss and fragmentation poses significant challenges for the long-term viability of Koalas in the Scenic Rim. Isolated population clusters face an increased risk of local extinction due to several compounding factors including: reduced genetic diversity which can lead to genetic drift, inbreeding depression, and higher impacts from environmental threats (vehicle strikes, diseases, dog attacks) and stochastic events (e.g. prolonged droughts, fires).

Further, the lack of reciprocal migration between clusters, as detected in this study, suggests that natural recolonization of declining population clusters is unlikely to occur without targeted intervention. If genetic isolation continues to intensify, some Koala clusters may become genetically and demographically unsustainable, resulting in their local extirpation.

To mitigate the risks of local extinction associated with the genetic differentiation and isolation observed within the Scenic Rim, an effective conservation strategy should prioritize:

1. Restoring connectivity between the population clusters in targeted areas, to promote gene flow and population cluster stability.
2. Securing remaining habitats and increasing habitat within the population clusters, to facilitate natural dispersal and recolonization.
3. Targeted translocations or genetic rescue efforts in critically isolated clusters.
4. Ongoing genetic monitoring to scientifically evaluate the effectiveness of conservation actions implemented.

Addressing genetic fragmentation and isolation in Koalas is essential for their long-term survival. This study highlights the critical role of local communities, including the local government and 'citizen scientists', in enabling large-scale, non-invasive genetic monitoring of across the Scenic Rim region. By collecting scat samples, local community members contributed valuable data on genetic diversity, population structure, and disease prevalence. Combining the unique DNA profiles of the Koalas sampled in this study with the large and growing number of wild Koalas already non-invasively sampled across the region, allowed to obtain insights into some of the vital ecological and genetic processes the species relies on for survival. It is highlighted that many other species above and beyond the Koala, including some keystone species[1] as well as some other threatened species, rely on those same vital processes for their own survival.

The success of this initiative underscores the value of continued investment in community-led science which provides a cost-effective, scalable approach to tracking population trends, habitat connectivity, and emerging health threats. Empowering local communities ensures that Koala conservation efforts remain sustainable, data-driven, and adaptable to future challenges.

[1] A keystone species is an organism that has a large impact on its ecosystem relative to its abundance. Keystone species can be animals, plants, fungi, or bacteria. They play a central role in maintaining the structure, health and function of an ecosystem by e.g. regulating populations of other species, creating / regenerating / or changing habitats, providing food and shelter for other species, etc. Keystone species are vital for the stability and resilience of ecosystems. If they are lost/removed, it can cause dramatic shifts in the ecosystem.

2.0 INTRODUCTION

2.1 PURPOSE OF THIS REPORT

This report provides a crucial foundation for developing an effective conservation plan for Koalas in the Scenic Rim region of Southeast Queensland. By leveraging the genetic data presented, the plan can establish measurable Key Priority Indicators (KPIs) such as genetic diversity parameters, population connectivity (gene flow and direction), and pathogen prevalence, which serve as benchmarks to measure the effectiveness of measures implemented. Ongoing monitoring through repeat non-invasive genetic sampling, using scat collection and analysis, is both cost-effective and practical. The use of microsatellite markers allows for the collection and analysis of degraded scats stored at ambient temperature, enabling broad sample collection by anyone, without the need to capture or disturb wild Koalas. Community engagement is a key component, as local residents can play an active role in scat collection, increasing sample size and geographic coverage. Regular analysis of genetic and pathogenic data directly informs management, providing insights into population health, gene flow, and disease prevalence. The strategy should be adaptive, with periodic evaluation of its effectiveness against the KPIs, allowing for adjustments based on the latest scientific findings. Ultimately, the plan must include efforts to establish effective wildlife corridors to reconnect fragmented habitats, ensuring long-term genetic exchange and population resilience. This approach will not only safeguard the Scenic Rim Koalas but can also serve as a model for broader regional conservation efforts.

2.2 STUDY AREA

The study area is the Scenic Rim Regional Council LGA, located to the southeast of Brisbane, Queensland (see **Figure 2.1**).

With a surface area of 4,250km^2, the SRRC represents 12% of Southeast Queensland.

Since European settlement, approximately 70% of its native vegetation cover has been cleared. Most of the clearing occurred between the late 1800s and the 1960s, mainly for agricultural purposes; land use that largely persists today. As a result of the recent clearing and ongoing land uses, today the forest cover of the LGA consists largely of a matrix of small, disconnected forest patches – for the exception of a strip of relatively connected forest in the range that runs along the fringes of the LGA to the east, south and west.

2.3 BACKGROUND

Koala conservation in Eastern Australia faces serious challenges due to habitat loss and fragmentation, human activities and changing weather patterns, which have led and continue to lead to declining populations. Without a comprehensive understanding of any given region's Koala genetic health, population dynamics, and disease prevalence, these challenges will likely intensify. Reduced genetic diversity makes Koalas more susceptible to a range of threats and environmental changes, limiting their ability to adapt and recover.

Without accurate data on population trends and disease, conservation efforts may be ineffective or misdirected, potentially resulting in further population declines, local extinctions, and the irreversible loss of this iconic species. Despite significant investments since 2012 when the

Koala was first listed on the EPBC Act[2], including over $300 million committed since 2022 alone[3], the species continues to decline over much of its eastern range. Accurate data on Koala population trends and disease across its range is currently lacking yet is urgently needed to guide effective investment decisions which are necessary if the species is to have a chance at persisting, perhaps even being recovered.

To address this issue, WildDNA and OWAD began collaborative Koala surveys in 2015, collecting Koala scats from which DNA is extracted. Data derived from scat is crucial for assessing Koala genetic health, population dynamics, and disease prevalence, thereby providing the foundation for more effective conservation strategies; and achieves this at a limited cost.

Dr Faye Wedrowicz (WildDNA) pioneered the extraction of viable DNA from degraded Koala scat in the early 2010s. Building on her work, in 2014 Olivia Woosnam and Alex Dudkowski (OWAD), both Certified Environmental Practitioners[4], invested in the first professional field detection canines for Koala DNA sampling. Investing in these highly specialised canines enabled OWAD to fast-track the optimisation of all aspects of the sample collection process, with the objective of making non-invasive Koala genetic sampling easily accessible to everyone as quickly as possible, including (but not limited to) local communities under a 'citizen-science' model. Indeed, obtaining the data required across the species' range is a sizable task that can only be achieved in a timely and cost-effective manner if the methods are robust while also being inexpensive, rapid, and made easily accessible to anyone wishing to contribute to the concerted effort required.

By 2021, the process was sufficiently optimised to involve large-scale citizen-scientist participation. This culminated in the Australian Government funding several community-led projects in 2022, then subsequently funding this study, where the genetic sampling was conducted by local communities.

The data collected feeds into Australia's largest non-invasive Koala genetic database that currently spans from Victoria to Queensland. This resource not only supports academic research but is also paramount to reduce costs and eliminates animal ethics concerns associated with invasive sampling methods (i.e., avoids unnecessary stress to the animals caused by locating, capturing, handling and sedating wild Koalas to extract blood/tissue). Further, this resource provides data that can directly inform improved strategies and guide effective investment decisions – ultimately advancing Koala conservation on a national scale.

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2 Australian Government *Environment Protection and Biodiversity Conservation Act 1999*.

3 See https://www.dcceew.gov.au/sites/default/files/documents/annual-report-2023-national-recovery-plan-koala.pdf.

4 The CEnvP Scheme assesses the experience, skills and ethical conduct of environmental and social professionals across Australia, New Zealand and the globe. It recognises leading practitioners and gives confidence to clients and the community. Supported by legislation and government recommendations in Australia and New Zealand, CEnvP certification is the marker for trusted professionals.

LEGEND

Scenic Rim Regional Council Local Government Area

FIGURE 2.1

Location of study area

Scenic Rim
2024 Koala population study

3.0 SUMMARY OF METHODS

All methods used in this study – scat selection, collection, and storage techniques, combined with the sample condition scoring system and the use of microsatellite genetic markers – are particularly well suited to a citizen-science model because:

1. **Scats do not require refrigeration or freezing**: they are stored at ambient temperature, allowing for easy transportation via Australia Post.
2. **Scats do not need to be 'fresh'**: using microsatellite markers, scats can remain viable for genetic analysis for up to several months after being deposited by a Koala.

These factors mean that a vast number of potential Koala genetic samples are available for collection in the wild wherever the species persists, even at low densities. Since an individual Koala produces approximately 150 pellets every 24 hours (Ellis *et al* 1998) – in other terms approximately 1,000 pellets per Koala per week – and that scats can remain viable for many weeks when using microsatellite markers, there is ample material in the natural environment wherever the species persists.

Importantly, anyone can collect samples without any prior experience, without any kind of specialised detection tools or equipment, and the samples can be conveniently mailed via Australia Post, thereby removing all barriers that typically prevent local communities from participating in Koala genetic research.

3.1 OVERVIEW OF SAMPLING METHOD

3.1.1 Sampling design

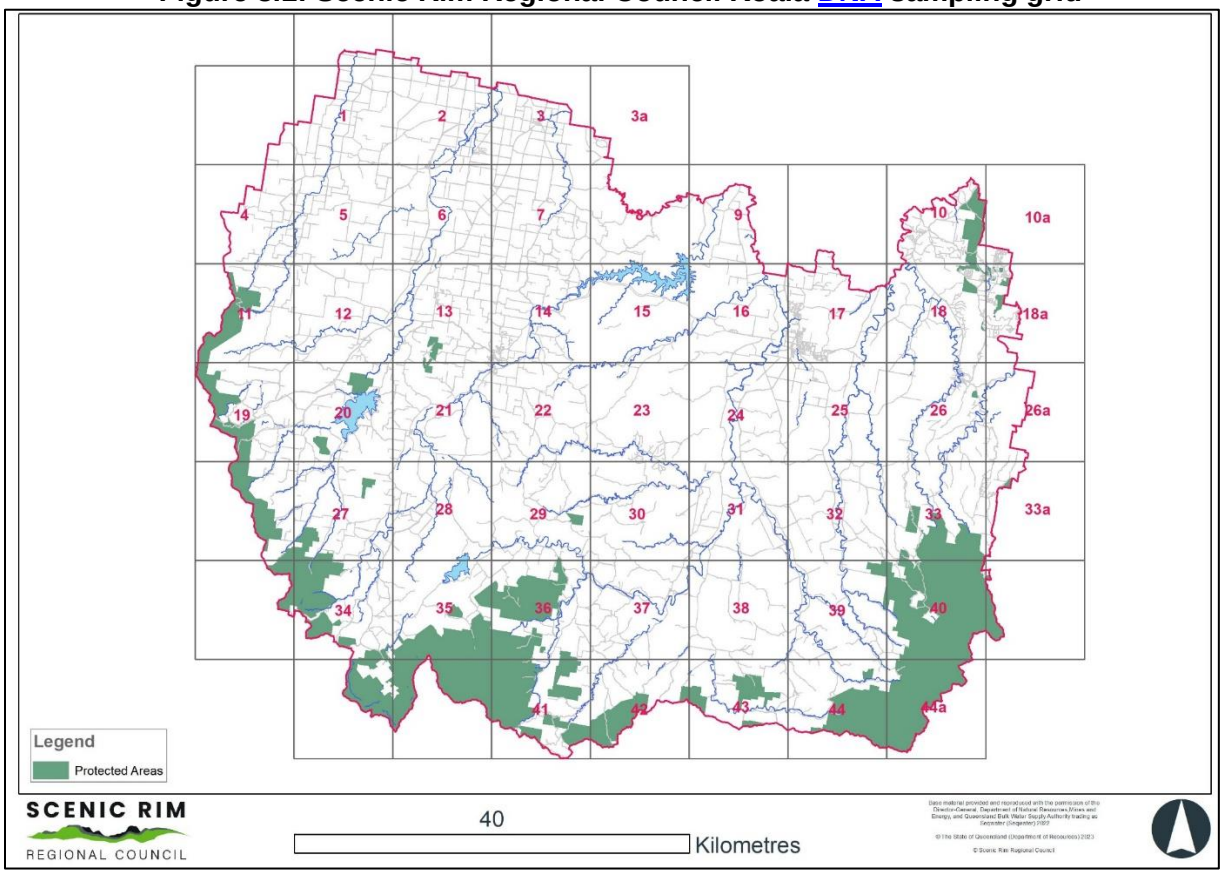
SRRC developed the sampling design for this project. SRRC opted for a systematic design whereby a 10km x 10km grid was overlaid over the LGA, for a total of 59 grid cells (see **Figure 3.1**, next page).

44 of these grid cells were the initial focus, with the balance being small portions of the LGA or areas hard to access.

SRRC staff then identified a number of potentially interested parties who may wish to participate in this project, including (but not limited to) Land For Wildlife program members, whom SRRC engaged in a targeted manner.

As the sampling progressed and community interest increased, the sampling effort in the eastern portions of the LGA was expanded to include further grid cells (10a, 18a, 26a, 33a and 44a – see **Figure 3.1**).

Figure 3.1: Scenic Rim Regional Council Koala DNA sampling grid



3.1.2 Collection of samples

Prior to the Australian Government funding this project, OWAD had already trained key staff from both SRRC and the Watergum community group in the non-invasive sampling of wild Koalas. Staff trained by OWAD in 2023, then disseminated the knowledge to local community participants for this project. The training consisted of the following key elements:

1. **Identify Koala scat:** Participants were shown how to recognise Koala scat and distinguish it from that of other species. They were also taught how to recognise potentially viable Koala scat samples through visual cues of scat appearance.
2. **Collection method**: Participants collected Koala scats using toothpicks, carefully handling each sample to maintain its integrity and avoid contamination.
3. **Preservation technique**: After collecting scats, the toothpicks holding each scat were embedded into foam. This foam was then placed in a section of downpipe to safeguard the samples during storage and transportation (see **Figure 3.2**).
4. **Recording details**: To ensure traceability, each sample was tagged with the collection date and specific coordinates on a pre-affixed label, allowing for precise geolocation of each sample's origin.
5. **Individual identification**: By observing the scat size/shape/colour found at a given location, collectors attempted to distinguish whether scats from multiple Koalas might be present. If multiple size/shape/colour of scats were observed at a given location, scats were collected in separate sample kits.

Figure 3.2: Koala DNA sample kit
Made of PVC downpipe cut to size, foam insert, and toothpicks for collecting scats.

SRRC staff coordinated the collection of samples gathered by the community, and regularly posted these (via Australia Post) to the OWAD office for assessment.

3.2 SAMPLE CONDITION SCORING

When new samples were received by OWAD, these underwent close visual inspection following the 'Koala DNA sample condition scoring system' developed and optimised in 2021 thanks to funding provided by Brisbane City Council. An overview of this sample scoring system is provided in **Appendix 1**.

Samples that passed this vetting process constituted a potentially viable DNA sample, were attributed a condition score, were entered electronically, and submitted to WildDNA via Australia Post.

Note that this vetting process also serves to remove any scats collected that clearly do not originate from the Koala as evidenced by simple physical examination. However, any material that has any chance of potentially originating from the Koala, is retained (provided it meets the minimum criteria). Indeed, in about 10-15% of instances the Koala can produce material that does not fit the typical physical characteristics of Koala scat – be it because it is pap (a specialised form of faeces produced by mothers with joeys), or due to old age, or pathogens, or other circumstances.

In instances where atypical material is received that potentially originates from the Koala, this material is retained provided it meets the minimum criteria (see **Appendix 1**). If any scat or pap submitted is not Koala scat or pap, this is identified by the Laboratory Quality Control process (see **Section 3.3.2**) and is automatically reported on and flagged 'not Koala'.

3.3 OVERVIEW OF LABORATORY METHODS

WildDNA (Federation University) received all samples for this project, coordinated all Koala genetic and pathogenic molecular tests, and performed all downstream analyses.

3.3.1 Surface wash

Scat samples received by WildDNA were processed immediately upon arrival. Epithelial cells on the outer surface of the scat were removed and captured in liquid. Surface washes were stored frozen until the sampling program was completed for this project.

3.3.2 DNA isolation and Laboratory Quality Control

Upon completion of the sampling program, DNA was isolated from the surface washes of each sample. The quantity and quality of the target DNA were then assessed, a process referred to as Laboratory Quality Control (QC). Each sample was assigned a QC result of either 'pass', 'low quality pass', or 'fail'.

For downstream molecular tests, only the isolate with the highest DNA concentration and a QC rating of 'pass' or 'low quality pass' was utilised.

The video below shows parts of the surface-washing and DNA isolation processes.

Video: Sneak peek into the lab
(Click on image to play – internet connection required)

3.3.3 Molecular tests

Koala genetic and pathogenic tests

All 'pass' or 'low quality pass' samples were then tested for Koala DNA profiling, sexing, and pathogen identification tests for detection of *Chlamydia pecorum* and variants of Koala retrovirus.

Tree species genotyping test

In addition to the above tests that were planned at the inception of this project, some scats from a subset of Koalas sampled in this project also underwent genotyping to identify some of the tree species contained in those scats. The purpose was to obtain an additional dataset to provide an insight into the diet of Koalas in the study area.

3.3.4 Population analyses

Using the Koala genetic and pathogenic data obtained in this study, as well as that from reference samples from wild Koalas previously sampled and profiled in the region, a range of analyses and bioinformatics[5] were then performed to unveil key population dynamics processes in the study area and the greater landscape.

[5] Bioinformatics is an interdisciplinary field of science that develops methods and software tools for understanding biological data, especially when the datasets are large and complex.

4.0 SUMMARY OF RESULTS

4.1 LABORATORY QUALITY CONTROL

The community-led sampling program was completed in 2.5 months between 11 April and 29 June 2024.

During that time, the community gathered a total of 117 kits which contained material that passed the minimum condition assessment (see **Section 3.2**) hence constituted potentially viable putative Koala DNA samples. These were surface-washed immediately upon reception by WildDNA (see **Section 3.3.1**) and stored until the sampling phase was completed.

Once the sampling phase was completed, to remain within budget for this study a selection of 103 samples was retained to undergo Laboratory Quality Control (see **Section 3.3.2**).

84 of the 103 samples passed Laboratory Quality Control, or an 82% lab QC success. The 19 samples that failed lab QC originate from the Koala; however, these contained an insufficient amount and/or quality of Koala DNA to proceed to downstream molecular testing.

- **100%** of samples were confirmed to originate from the Koala; and
- **82%** of samples contained sufficient quantity/quality of Koala DNA to proceed to downstream molecular testing.

This is an excellent lab QC success rate that is comfortably above the 70% mark that was hoped to be achieved at project inception.

These very high success rates highlight the excellent execution by SRRC at coordinating the community-led collection of quality Koala DNA samples, as well as the efficacy of the support systems developed specifically to facilitate Koala genetic sampling under a citizen-science model. What's more, SRRC completed the sampling program over a period of only 2.5 months, which is remarkable.

4.3 DNA PROFILING SUCCESS

98% of the samples that passed lab QC (82/84) provided reliable Koala DNA profiles.

This high DNA profiling success rate reaffirms the effectiveness of the laboratory quality control (QC) process in prioritizing samples with a high probability of yielding reliable Koala DNA profiles.

4.4 OVERALL STUDY SUCCESS

Overall, 82 of the 103 samples provided reliable Koala DNA profiles, resulting in an **overall study success of 80%**.

This is a very high success rate for the non-invasive genetic sampling of any animal. It is even more commendable given that the sampling was conducted by the community, what's more in record time (only 2.5 months) and at minimal cost to the taxpayer. The success of this initiative, alongside several recent studies employing the same model, demonstrates that these methods are not only highly effective but also optimally designed for community-led Koala research and conservation.

This study reinforces that, with the right methodologies and support systems in place, local communities are not only highly capable of leading applied Koala research but, in many cases, are the most effective entities for delivering timely, cost-efficient, and large-scale Koala conservation outcomes.

Effectiveness and accessibility of non-invasive Koala genetic sampling

The Koala scat sampling method used in this study is able to:

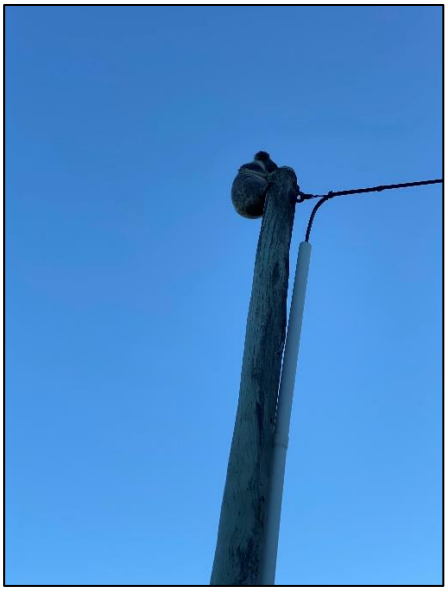
1. Achieve high genotyping success rates;
2. Provide a comprehensive suite of key genetic and pathogen test results essential for informing effective conservation strategies;
3. Allow broad participation and community engagement;
4. Deliver robust genetic data within a short timeframe; and
5. Offer equivalent cost-effectiveness in applied conservation research.

Community-led Koala research as a strategic conservation tool

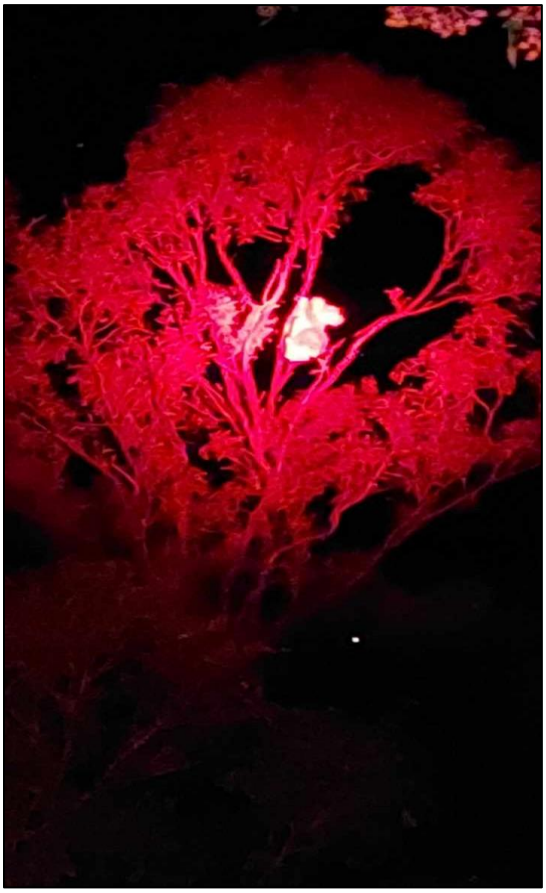
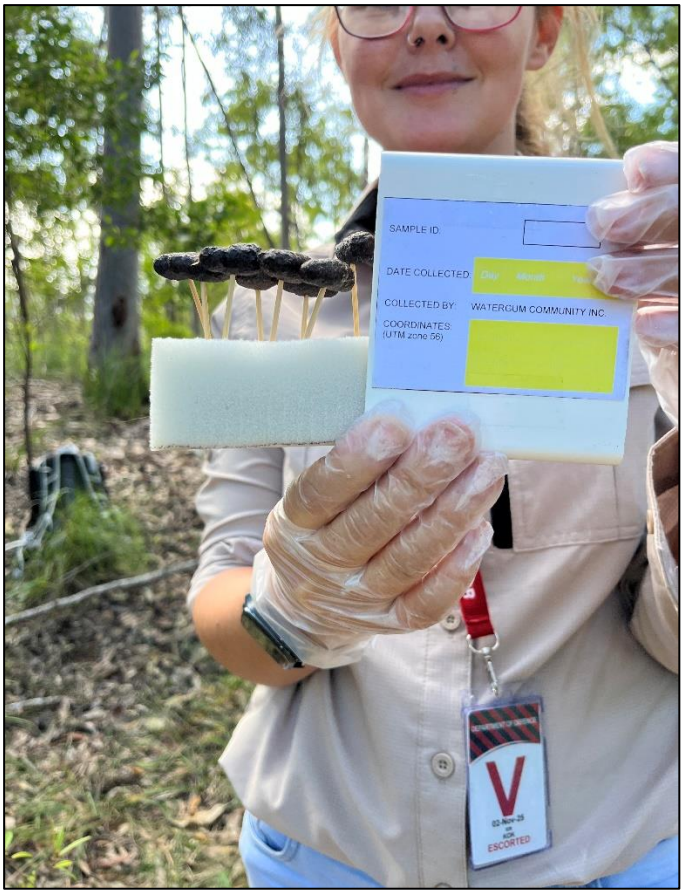
Empowering local communities to actively participate in applied Koala research provides significant strategic and practical advantages, including:

1. **Scientific efficacy** – Community-led sampling has been demonstrated to produce high-quality, reliable data.
2. **Cost efficiency** – The approach significantly reduces research costs compared to 'traditional' expert-driven field surveys. The resulting major reduction in costs traditionally associated with Koala research, allows those savings in public funding to be redirected toward the large-scale tangible conservation efforts required to secure the species (e.g. securing and restoring habitats, building fully vegetated fauna overpasses over roads, etc).
3. **Timely data collection** – Non-invasive sampling driven by local communities enables rapid and large-scale assessments, critical for conservation decision-making. This is urgently needed across Eastern Australia to secure the species.
4. **Transparency in public funding allocation** – Ensures accountability in the use of government and grant funding for Koala research.
5. **Open access to genetic data** – Enhances transparency and facilitates data-driven conservation efforts.
6. **Bridging the gap between public institutions and local communities** – Strengthens collaboration between **government agencies/academia, and the public**, fostering a more inclusive and engaged conservation framework.

Given the demonstrated success and efficiency of this model, continued investment in community-led, non-invasive Koala genetic research is strongly recommended to support evidence-based conservation strategies, enhance data accessibility, and improve policy implementation.

Above, left: Community members learn to collect Koala DNA samples in Beaudesert. Photo credit: Watergum Community Inc. **Above, right**: 'Lina Power', female Koala (sample code SCE121) before her rescue from Canungra in June 2024. She was treated for chlamydiosis (displayed cystitis and conjunctivitis) and released in August 2024. Photo credit: Phil Campbell and The Canungra Times.

Above, left: Spotlighting in Frazerview,11 April 2024. This male Koala (SCE022) contributed the first community-led scat sample collected for this study. Photo credit: Lara Solyma. **Above, right**: Sample SCE059 collected by Department of Defence staff on 30 May 2024 from Kokoda Barracks. Sample code SCE060 was also collected at the same location. These two samples collected from that location were confirmed to originate from two different male Koalas.

4.3 SUMMARY OF INDIVIDUAL KOALA RESULTS

DNA profile matching revealed that the 82 reliable profiles obtained belong to **81 unique Koalas**. Only one individual was sampled twice in this study, which is excellent since the goal was to minimise the instances where a particular Koala would be sampled more than once.

The sex was reliably identified for 79/81 individuals, and the KoRV genotyping test was successful for 75/81 individuals.

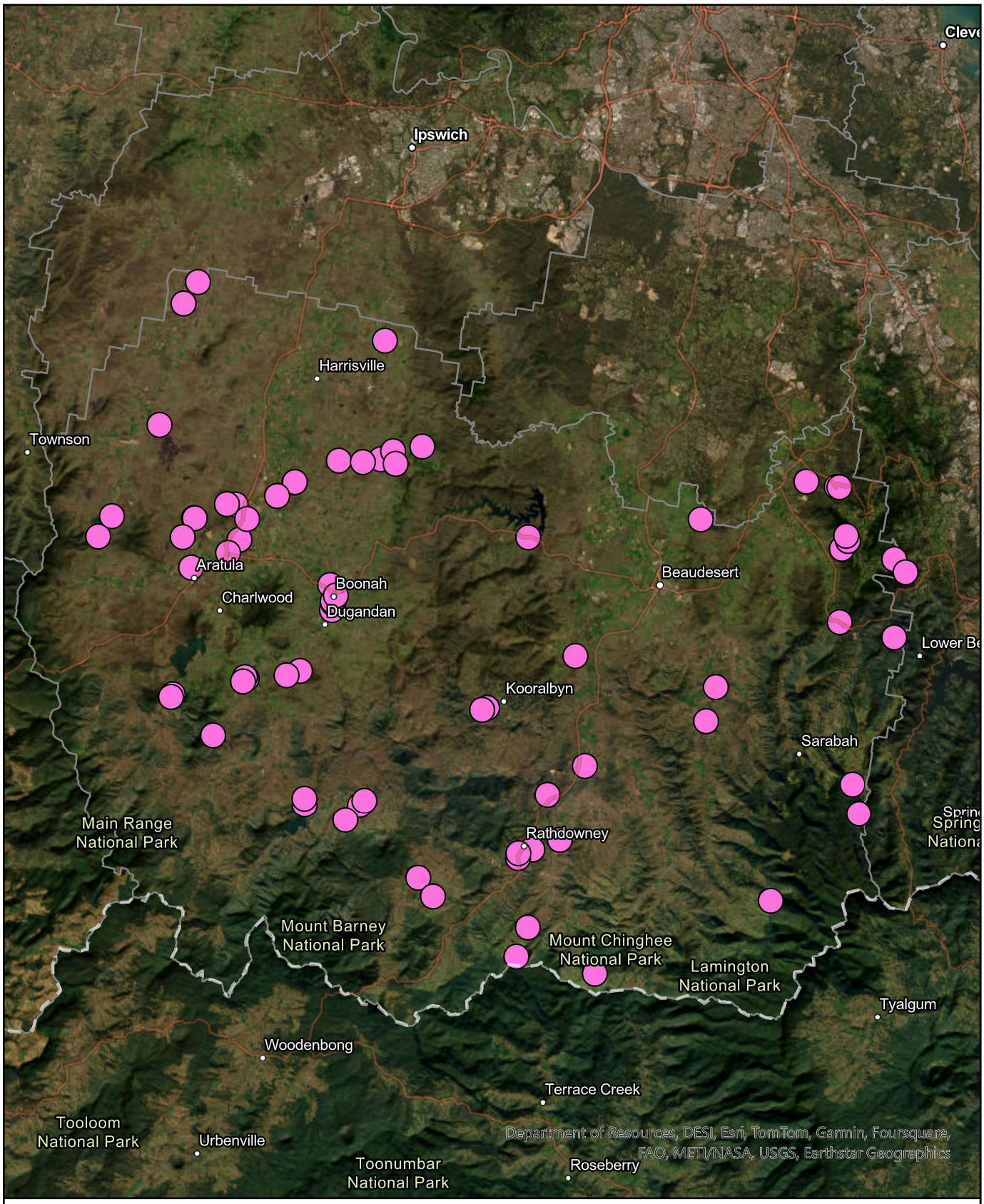
An overview of the individual results for the 81 unique Koalas successfully profiled in this study is provided in **Table 4.1** below.

Table 4.1: Summary of individual Koala results

Sex ratio	56% males 44% females
Proportion with *C. pecorum* detected	40%
Proportion with endogenous KoRV-A	100%
Forms of exogenous KoRV detected (and % of individuals with the form)	KoRV-B (3%) KoRV-D (31%) KoRV-F (12%)

Figure 4.1 (next page) shows the geographic locations where these 81 Koalas were sampled from in the study area. (Note that the Koala that was sampled twice, is only shown once on this map and assigned sample code SCE074 – the first time it was sampled. This female was sampled four days apart <100 meters apart by two different participants.) Note that **Figure 4.1** shows the sampling locations only, regardless of whether one or more unique Koalas were sampled and profiled from that location. In this study, there were five instances where two samples were collected at a location – when the participants suspected that scats observed at that location may belong to two distinct Koalas. These educated guesses were all proven correct: all five locations where two samples were collected, were confirmed to originate from distinct individuals. This further highlights the efficacy of the community training in this study.

Appendix 2 provides details of the individual test results for the 81 unique Koalas profiled in this study. Discussion regarding the pathogenic results in included in **Section 6**.



LEGEND

- Sampling locations of Koalas profiled
- LGA boundaries

FIGURE 4.1

Sampling locations of individual Koalas profiled

Scenic Rim
2024 Koala population study

5.0 POPULATION GENETIC ANALYSIS

5.1 POPULATION STRUCTURE AND TYPES OF GENETIC GROUPS

A population is a group of genetically similar individuals living in the same geographic area with the potential for random mating. These genetic groups can exist as either:

(1) A single large, genetically connected **population**; or
(2) A **metapopulation** consisting of multiple **subpopulations** that are separated by space but interact and remain reasonably well genetically connected through the regular exchange of individuals; or
(3) Small, fragmented, isolated population **'clusters'** that are disconnected from one another, with limited to no exchange of individuals between them leading to increasing genetic differentiation. (Note: exchange requires *reciprocated* migration.)

For Koalas, conservation is most effective when they exist in a single large, connected population, or as a metapopulation – how the species is naturally designed to occur, and how it occurred across Eastern Australia prior to European settlement. Koalas face the greatest risk of extinction when they occur in small, fragmented, isolated clusters.

A key threat to the survival of the Koala both in the short and long terms, is genetic erosion[6] and extirpation[7] ('local extinction') caused by habitat loss and fragmentation, which fragments the Koala population structure: once-connected large groups are divided into a series of disconnected clusters. Understanding the current population structure in any region is essential for assessing the Koala's status and determining the appropriate management actions needed to preserve or restore the vital processes the species relies on for survival. The effectiveness of Koala conservation hinges entirely on how swiftly and successfully these critical population processes are maintained or restored.

5.2 IMPORTANCE OF POPULATION STRUCTURE ANALYSIS

5.2.1 Historic population structure

Prior to European settlement, the Koala across Southeast Queensland and Northern New South Wales existed as a metapopulation consisting of likely **four subpopulations** that experienced varying degrees of genetic exchange (Neeves et al 2016). These subpopulations were weakly differentiated genetically, meaning there was some level of gene flow between them, but not enough to completely homogenise the species into one single population across the broad region. This structure was likely shaped by natural factors such as landscape features like rivers, mountain ranges and habitat preference (Houlden et al 1999).

5.2.2 Current population structure

Understanding how historical Koala population structure compares to the current structure is essential for assessing the impact of recent habitat modification (post European settlement) on

[6] Genetic erosion is a process where the limited gene pool of an endangered species diminishes even more when reproductive individuals die off before reproducing with others in their endangered low population.
[7] Extirpation is the local extinction of a species, where they cease to exist in a particular area but continue to exist elsewhere.

key population processes critical for the survival of the species. Effective Koala conservation requires a rapid, evidence-based response to maintain or restore vital processes such as genetic diversity, gene flow, and resilience to environmental change. Identifying the present-day population structure is essential to enable:

- Assessment of population connectivity and isolation, informing effective habitat management strategies;
- Detection of genetic bottlenecks and erosion, which threaten long-term viability; and
- Development of targeted conservation interventions to preserve or restore genetic diversity and connectivity.

By integrating these insights into conservation planning, management strategies can be proactively designed to prevent further population decline and enhance the long-term sustainability of Koalas across a region.

5.3 METHODS USED

To investigate the contemporary Koala population structure in the study area, population genetic analysis methods were applied. Genotypic data from this project, combined with data from ~1,000 wild Koalas from various other LGAs in the Southeast Queensland bioregion previously sampled and profiled over the last 10 years, including data from other recent citizen-science initiatives conducted in the Burnett and Darling regions[8], were used in this analysis. The genetic software package GENELAND[9] (Guillot *et al* 2008) was used to infer Koala population structure. It uses clustering methods that incorporate both genetic data and spatial information, making it particularly suitable for studies where geography may play a role in the structuring of populations. Migration rates were inferred between population clusters using BayesAss software[10].

5.4 RESULTS

5.4.1 Current population structure

Genetic analysis of Koalas from the Scenic Rim and surrounding areas in Southern Queensland conducted in this study revealed strong genetic differentiation across the region. This structuring is likely driven by recent habitat loss resulting from urbanization, agriculture and other anthropogenic activities. Within the Scenic Rim, **five differentiated population clusters** were identified (see **Figure 5.1** and **Figure 5.2**):

- **SEQ-03** in the northwest – also detected to date in large parts of Ipswich LGA, and parts of Brisbane and Logan LGAs;
- **SEQ-04** in the northeast – also detected to date in parts of Logan LGA;
- **SEQ-05** in the centre – also detected to date in parts of Logan LGA;
- **SEQ-06** in the eastern parts; and
- **SEQ-07** detected in two pockets in the west and south – also detected to date in and around Toowoomba city.

[8] Darling Downs 2024 Koala population study report and Inland Burnett 2024 Koala population study report.
[9] See Geneland Homepage for further information.
[10] See BayesAss - Bayesian Inference of Recent Migration Using Multilocus Genotype for further information.

Figure 5.1: Southeast Queensland Koala population structure

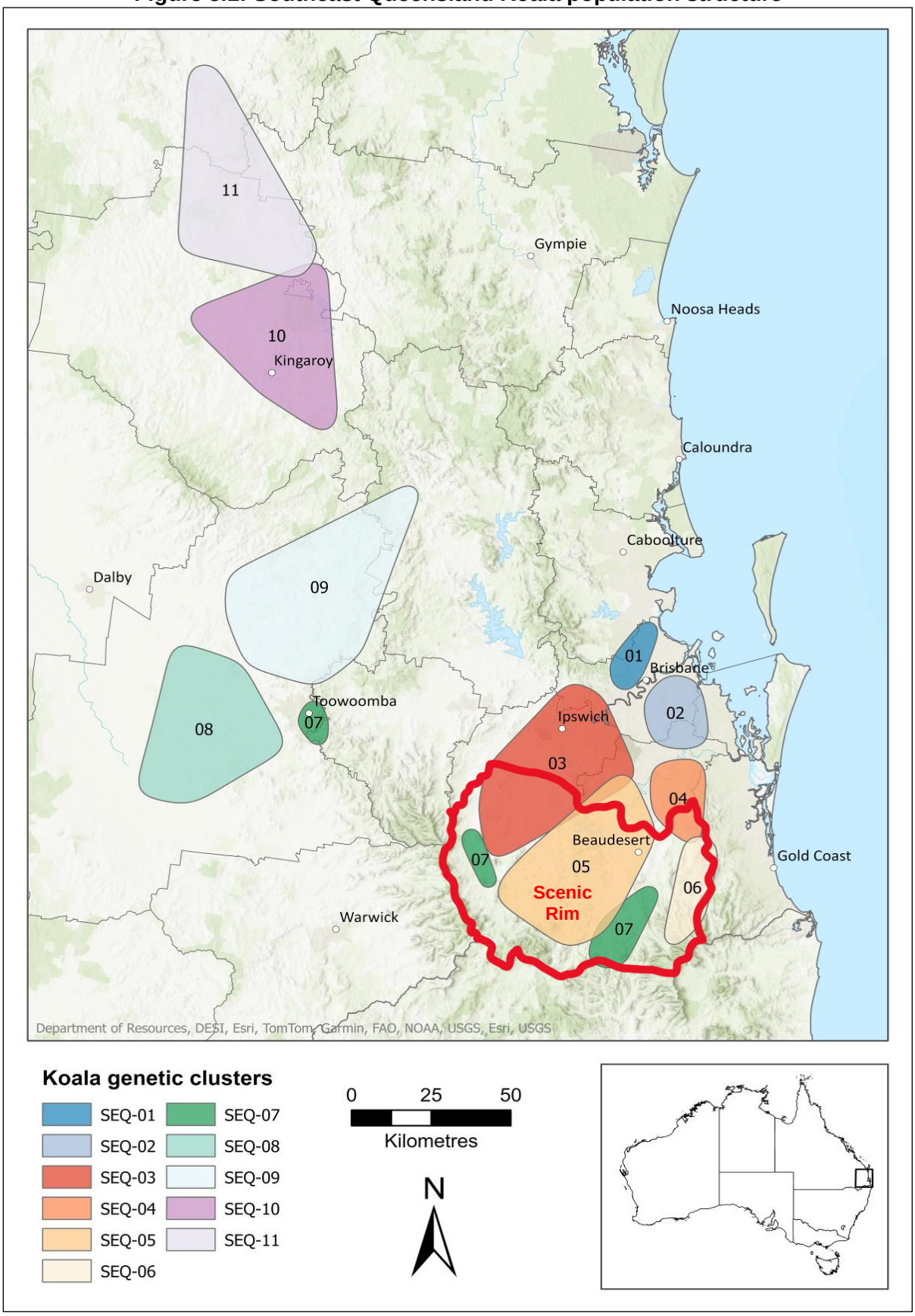
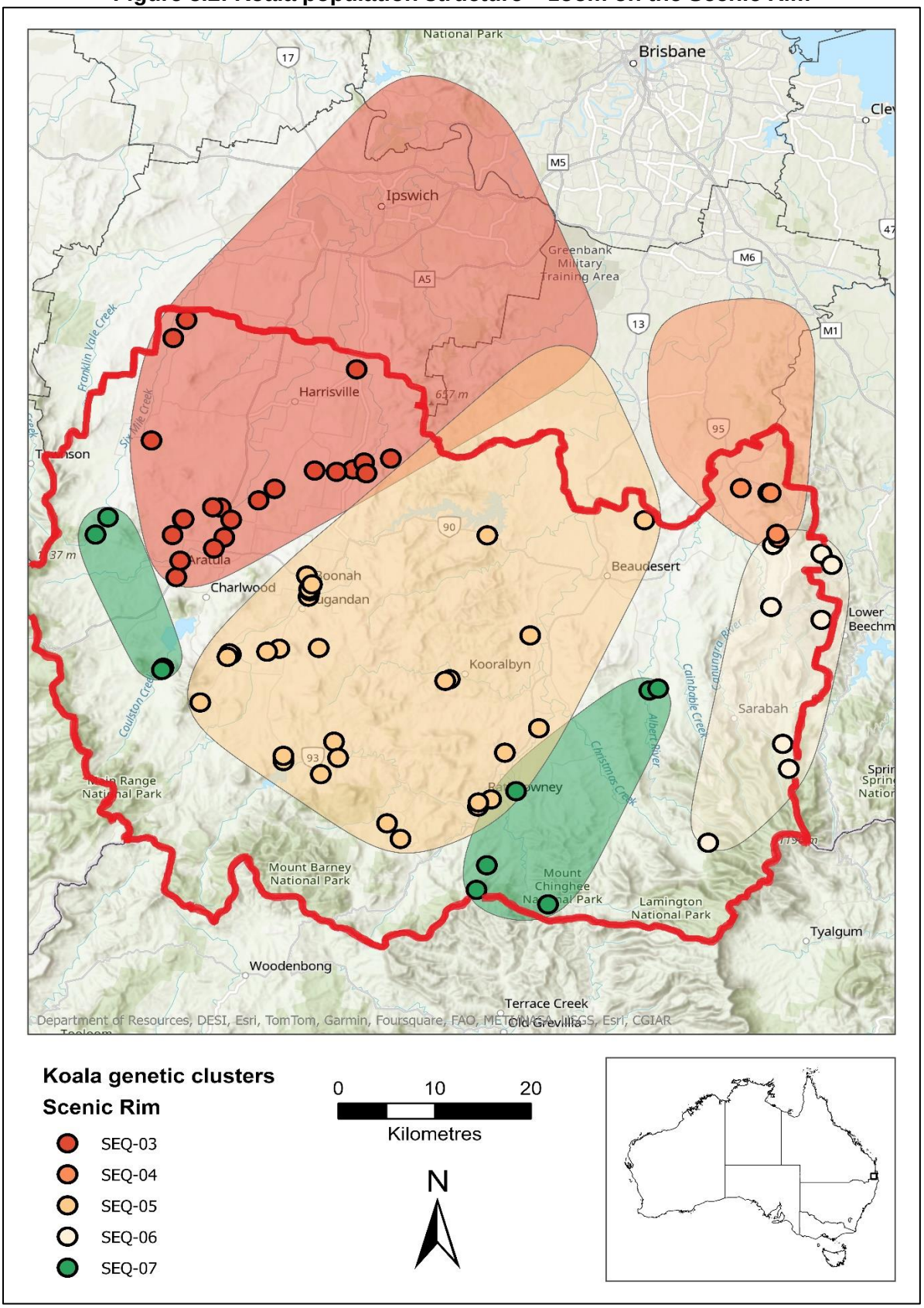


Figure 5.2: Koala population structure – zoom on the Scenic Rim



Note: the figure above shows the sampling locations of the Koalas profiled as part of this study only.

The identification of five genetically differentiated population clusters within the Scenic Rim LGA highlights a concerning level of fragmentation among Koalas in the study area. The presence of such a high number of genetically distinct groups in such close geographic proximity indicates limited gene flow across the LGA.

Even though limited gene flow can be driven by natural processes such as geographic barriers (e.g. mountain ranges, large rivers), there are no such natural barriers within the Scenic Rim that would explain the extent of genetic structuring observed today. The mountainous range which runs along the southern and western boundaries of the LGA, is the only potential natural barrier. However, genetic evidence suggests that this geographic feature does not impede Koala movement since individuals belonging to the SEQ-07 cluster have been detected in multiple locations, including western and southern parts of the Scenic Rim and around Toowoomba City (See **Figure 5.1**). This suggests that Koalas continue to maintain connectivity across this range.

Rather, the significant genetic differentiation observed within the Scenic Rim is a consequence of habitat loss and fragmentation since European settlement. The disruption of natural habitat (extent and connectivity) has significantly restricted movement and gene flow in the region, leading to the species now being 'split' into five distinct population clusters within the LGA. This is a major concern for the survival of the species in the region.

Reconnecting the five population clusters is the single most pressing management priority to secure the species in the Scenic Rim.

5.4.2 Genetic diversity

This section summarises essential findings regarding the genetic diversity of Southeast Queensland Koalas, with a focus on the Scenic Rim. It focuses on three main indicators: (1) allelic richness, (2) private alleles, and (3) percentage of total alleles. These metrics are critical for understanding the conservation value of different Koala genetic groups (see **Section 5.1**) and help guide effective management and prioritisation strategies.

1. **Allelic richness**:
   - Allelic richness reflects genetic diversity within a group. Higher allelic richness is associated with larger, well-connected groups where individuals can move and interbreed freely. This is essential for maintaining genetic health and resilience against environmental changes, threats and disease.
   - Reduced allelic richness often signals habitat fragmentation and isolation, which restricts gene flow and lowers diversity. This highlights areas where conservation efforts should focus on improving habitat connectivity to support natural migration and breeding patterns.
2. **Private alleles**:
   - Private alleles are unique genetic markers that are only found (or only persist) in a specific group. The presence of private alleles signifies a group's unique genetic contribution to the species.
   - If a group with private alleles were to disappear, these unique genes would be lost, reducing the genetic diversity of the species. Groups with high numbers of private alleles are therefore particularly valuable for conservation, as they represent irreplaceable genetic diversity.
3. **Percentage of total alleles**:
   - This metric indicates the proportion of the region's overall genetic diversity that exists within each group. A higher percentage reflects greater conservation significance, as it

means the group harbors a substantial portion of the genetic diversity of the Koala species in that area.
- Groups with a high percentage of total alleles play a critical role in sustaining regional genetic diversity, reinforcing their importance in prioritising conservation efforts and investments.

Table 5.1 below provides a summary of these three key genetic diversity values for the 11 Koala genetically differentiated population clusters identified to date across Southeast Queensland, with the results from the five clusters detected within the Scenic Rim shown in bold (SEQ-03, 04, 05, 06 and 07).

Table 5.1: Summary of genetic diversity results
(The colours for each population cluster reflect those on **Figure 5.1**)

	SEQ-01	*SEQ-02*	**SEQ-03**	**SEQ-04**	**SEQ-05**	**SEQ-06**	**SEQ-07**	*SEQ-08*	*SEQ-09*	*SEQ-10*	*SEQ-11*
Allelic richness	*4.8*	*4.9*	**6.7**	**5.1**	**6.9**	**5.3**	**5.8**	*4.9*	*6.4*	*5.7*	*5.1*
Private Alleles	*0*	*0*	**3**	**0**	**7**	**2**	**1**	*1*	*4*	*5*	*2*
Percent of total alleles (%)	*43*	*51*	**74**	**46**	**71**	**46**	**61**	*50*	*69*	*55*	*47*

Genetic analysis of Scenic Rim Koalas indicates relatively high overall genetic diversity, though notable variations exist among the five differentiated clusters identified in the study area.

Significant differences **in allelic richness** between these clusters suggest that some are likely to have experienced greater reductions in genetic variation than others. Indeed, Koalas within SEQ-04 and SEQ-06 exhibit notably lower allelic richness compared to the other three clusters, which may indicate a reduction in genetic diversity. In contrast, clusters **SEQ-03 and SEQ-05** show higher allelic richness, with the **highest values found to date** in Southern Queensland.

Private alleles — which indicate genetic uniqueness within a group — were detected in four of the five Scenic Rim clusters (SEQ-03, SEQ-05, SEQ-06, and SEQ-07). The presence of private alleles highlights the genetic significance of these clusters, as they contribute to the broader gene pool and may play a role in long-term species resilience. Notably, **SEQ-05** had seven private alleles, the **highest number recorded to date** in Southern Queensland.

Variations were also observed in the **percentage of total alleles** across clusters. SEQ-04 and SEQ-06 exhibited relatively lower values, while **SEQ-03 and SEQ-05 stand out for their higher values**, reinforcing patterns seen in the two other parameters.

When considering all 11 population clusters identified to date in Southern Queensland, clusters **SEQ-05 and SEQ-03 stand out for their high conservation significance**: SEQ-05 has the highest allelic richness and the highest number of private alleles, while SEQ-03 exhibits the highest percentage of total alleles and the second-highest allelic richness.

In contrast, SEQ-01, SEQ-02, and SEQ-04 showed no private alleles and lower allelic richness. These findings align with expectations, as these clusters are located in areas of Southeast Queensland where habitats are more extensively fragmented and modified due to human-related infrastructure and activities.

5.4.3 Movement of Koalas across the Scenic Rim

Gene flow involves the movement of genetic material between groups (via individuals migrating, dispersing and breeding) and plays a crucial role in maintaining genetic diversity, resilience, and long-term species survival. Understanding gene flow between groups is essential to assess connectivity between groups and evaluate each group's viability or vulnerability to local extinction.

Gene flow primarily occurs through immigration (arrival of new individuals into an area) and emigration (departure of individuals from the area). Maintaining genetic connectivity between areas such that the Koalas in those areas do not become differentiated and isolated from one another, requires *reciprocated* migration, i.e. a balance between immigration and emigration whereby the areas regularly *exchange* individuals/and genes. High and reciprocated migration rates between groups of individual Koalas are essential for ensuring that the species maintains vital function as part of a larger, interconnected metapopulation.

When habitat fragmentation restricts the movements of individuals, the Koala population structure itself can be fragmented: once connected metapopulations become 'split' into a series of small, disconnected, clusters (see **Section 5.1**). This leads to each cluster becoming increasingly smaller, isolated, and genetically differentiated, which in turn leads to genetic bottlenecks, inbreeding, and an elevated risk of local extinction via significantly increased vulnerability to a range of threatening processes.

Analysis of gene flow between the five Scenic Rim clusters revealed a highly asymmetric pattern: all statistically significant **migration was directed into SEQ-03**, with very low migration rates (1%–4%) in all other directions (**Table 5.2** and **Figure 5.3**). This pattern indicates very limited reciprocated exchange (of genes and of individuals), which is a significant concern for the long-term viability of the species in the study area. This lack of bidirectional gene flow suggests that **individuals are not dispersing evenly across the Scenic Rim**, leading to increasing genetic isolation and differentiation among clusters outside of SEQ-03. This finding highlights that the primary issue in the Scenic Rim region is the lack of reciprocal migration, with gene flow largely unidirectional toward SEQ-03. This indicates a **severe deficiency in genetic exchange, which must be addressed urgently** to prevent further genetic differentiation, inbreeding, and localised decline or loss of the species.

The population clusters must be urgently reconnected with one another to restore reciprocated exchange (of genes and of individuals – one cannot occur without the other) between the clusters. This requires urgently reconnecting the landscape.

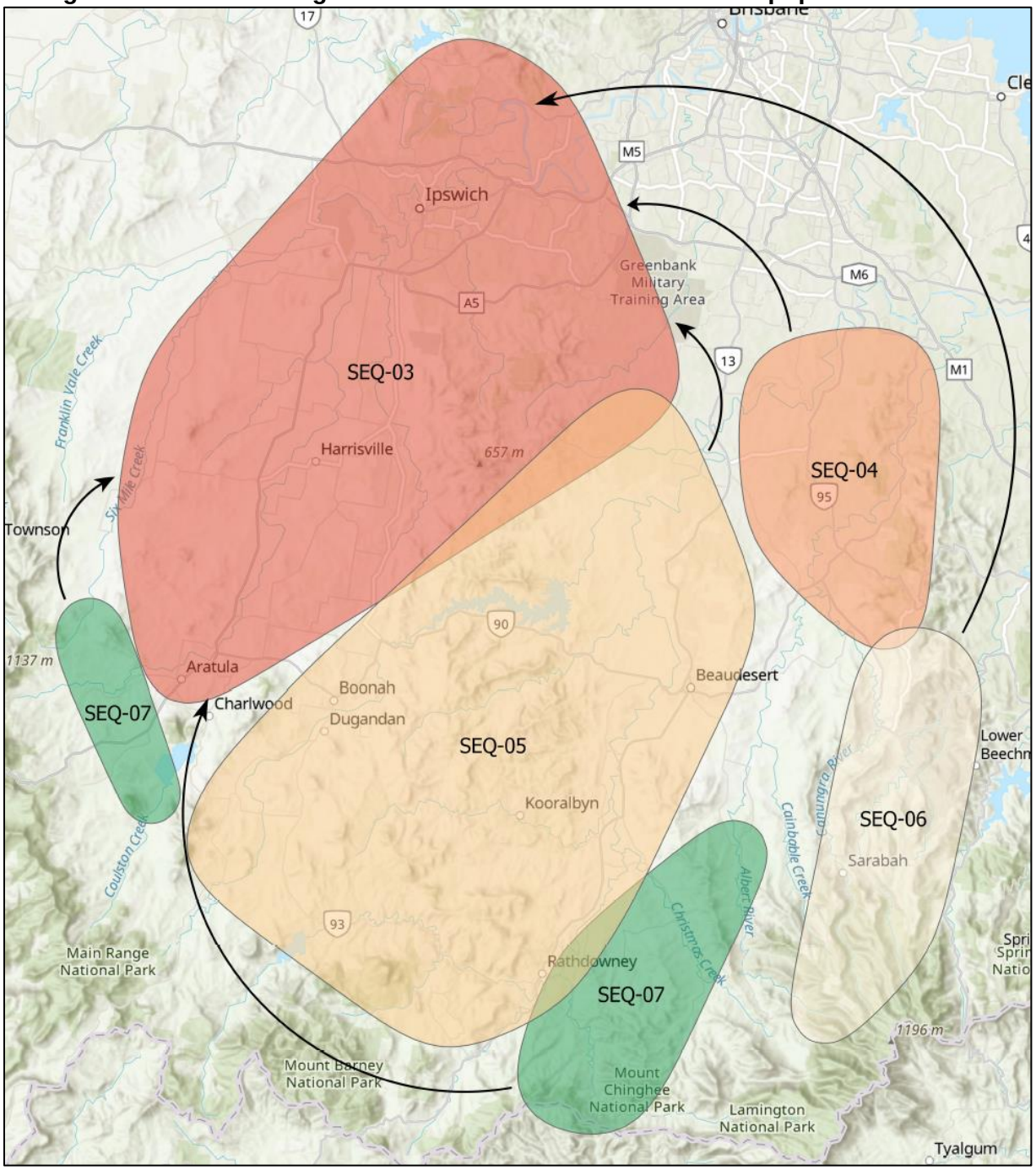
Table 5.2: Migration rates between the Scenic Rim Koala population clusters

From		To (% of migrants)
SEQ-05	→	**SEQ-03** (26%)
SEQ-07	→	**SEQ-03** (25%)
SEQ-06	→	**SEQ-03** (23%)
SEQ-04	→	**SEQ-03** (13%)
All other clusters	→	All other clusters (1% to 4%)

Highest

Lowest

Figure 5.3: Relevant migration between the Scenic Rim Koala population clusters



5.5 SUMMARY OF KEY FINDINGS ON MIGRATION AND GENETIC CONNECTIVITY

Genetic analysis of Koalas in the Scenic Rim has identified significant asymmetries in migration rates and connectivity. The observed patterns suggest that migration is not driven by habitat preference but by necessity, as Koalas navigate an increasingly fragmented and degraded landscape. Limited availability of safe, large and connected habitat, and the presence of multiple anthropogenic threats, likely influence these movements.

The sections below summarise the key findings, as well as some management priorities specific to some of the clusters identified within the Scenic Rim.

1. **Unidirectional migration into SEQ-03**
   - The majority of migration detected was directed *into* SEQ-03 *from* SEQ-04, SEQ-05, SEQ-06 and SEQ-07.
   - Low reciprocal migration (1%–4%) suggests that gene flow is largely one-way, raising concerns about depletion of emigrating clusters.
   - The geographic location of SEQ-03 (receiving immigration) is an already highly fragmented landscape that is facing increasing urban and industrial development pressures, particularly in the Ipswich and Logan regions.
   - Ensuring the long-term viability of SEQ-03 will require a coordinated conservation effort involving the Scenic Rim, Ipswich, Brisbane, and Logan local governments.

2. **High conservation significance of SEQ-03 and SEQ-05**
   - SEQ-03 is identified as a genetic reservoir of high conservation significance, making its protection a high priority for Koala conservation in Southeast Queensland.
   - Further investigations are warranted to identify the full extent of SEQ-03, as it may persist westward or northward beyond currently sampled areas (e.g., Lockyer Valley, Somerset).
   - SEQ-05, another genetic reservoir, is experiencing high unreciprocated migration (26%) into SEQ-03, indicating a potential decline in Koala numbers due to insufficient incoming gene flow.
   - SEQ-03 must be closely monitored via repeat non-invasive genetic sampling, including obtaining scientifically accurate estimates of the number of Koalas in this cluster via ongoing non-invasive ‘genetic tagging’. This will allow to closely monitor the numbers in real-time, and if required prompt targeted management interventions before this population cluster of high conservation significance is lost – this is necessary to preserve the private alleles present in this cluster.

3. **Limited migration into SEQ-07**
   - SEQ-07 exhibits significant outward migration, particularly into SEQ-03.
   - A similar pattern has been detected in the Darling Downs, where Koalas from cluster SEQ-07 in Toowoomba are migrating into SEQ-08 and SEQ-09 with limited reciprocal movement.
   - It is suspected that some level of connectivity persists through the Main Range/Border Ranges, facilitating movement between Toowoomba to Mount Chinghee National Park. However, further genetic sampling would be required to confirm this hypothesis.
   - The lack of any significant incoming migration into SEQ-07 detected to date, raises concerns about potential depletion in Koala numbers within this cluster. This would also require additional monitoring to confirm (and if confirmed, would require a targeted management response to secure this cluster).

6.0 CURRENT STATUTORY MAPPING

When reviewing the current extent of the Queensland Government 'Koala Priority Areas' and 'Koala Habitat Areas' (see **Figure 6.1**) a major issue becomes apparent:

At present, none or almost none of the habitats corresponding to the identified extents of Koala population clusters SEQ-03, SEQ-05 and SEQ-07 are mapped in the extent of 'Koala Priority Areas' within the Scenic Rim under the *South East Queensland Koala Conservation Strategy.*

Under this strategy:

- The clearing of natural habitat is prohibited only within an area identified as a 'Koala Priority Areas'.
- Therefore at present, the clearing of habitat is not prohibited in all parts of the Scenic Rim where clusters SEQ-03, SEQ-05 and SEQ-07 have been identified, with the exception of small fringes along the northern border of the LGA and a small part of the southern extent of cluster SEQ-05 (Mount Barney National Park).

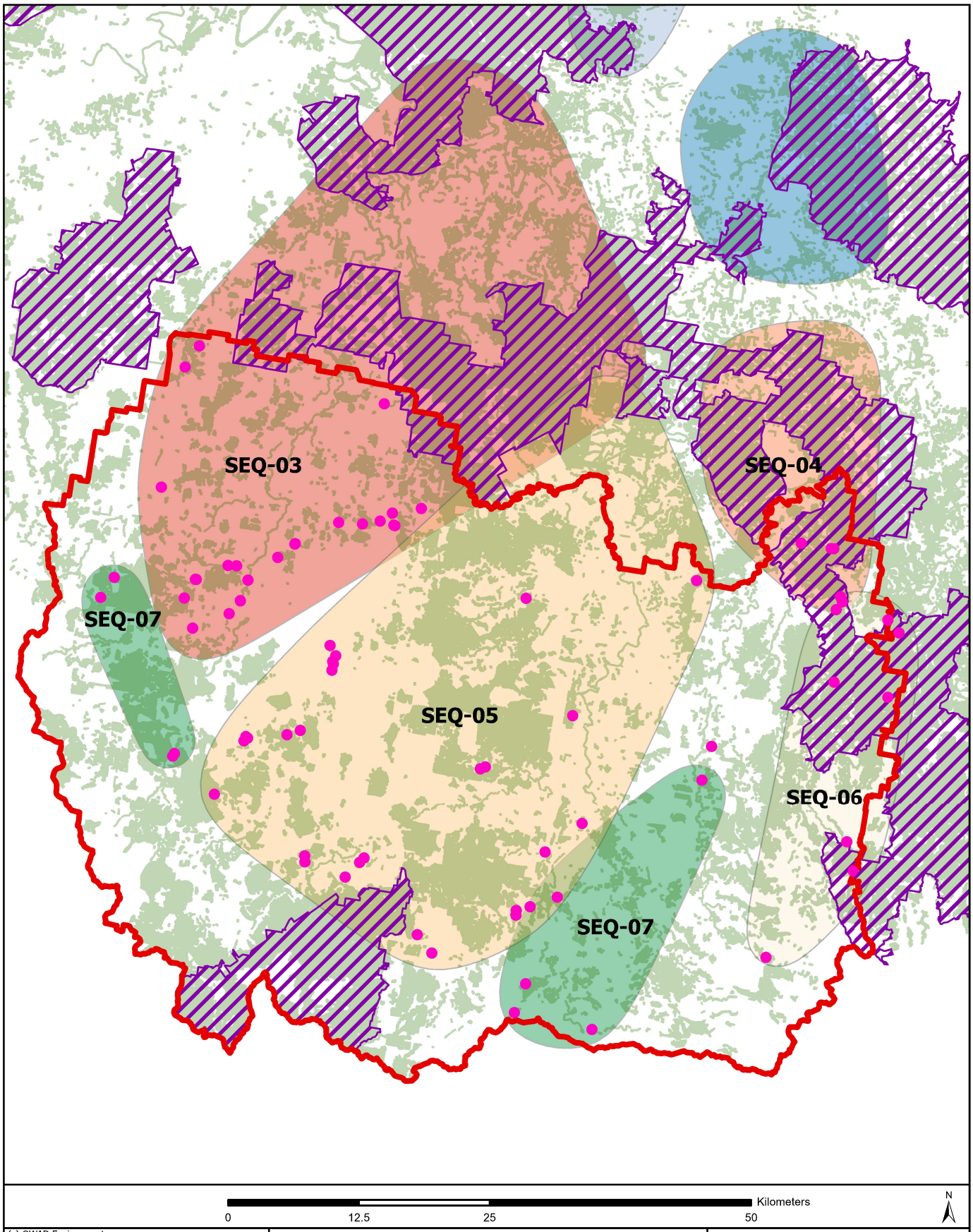
This is highly concerning, especially for clusters **SEQ-03 and SEQ-05 that are genetic reservoirs of high conservation significance**.

When reviewing the current extent of the Queensland Government 'Koala Habitat Areas', a significantly larger extent of remaining habitat is currently mapped within the Scenic Rim (see **Figure 6.1**). However, the clearing of habitat is not prohibited in KHAs under the *South East Queensland Koala Conservation Strategy.*

At present, none or almost none of the little habitats that remain for three of the Scenic Rim Koala population clusters are protected under State Government legislation, including the two clusters that are genetic reservoirs of national significance for the species.

Remaining habitats must be urgently protected, then swiftly reconnected, if the species is to persist across the Scenic Rim region.

Whether the Koala survives in the long-term, or continues to erode further than it already is (hence further reducing the chances of securing the species), hinges entirely on how quickly and effectively remaining habitats are protected and reconnected across the region.



LEGEND

- Scenic Rim Regional Council
- Koala Priority Areas
- Koala Habitat Areas
- Locations of Koalas profiled in this study

FIGURE 6.1

Current Koala Priority Areas and Koala Habitat Areas

Scenic Rim
2024 Koala population study

7.0 HEALTH OF SCENIC RIM KOALAS

In this section, we discuss the two key pathogens affecting Koalas, *Chlamydia pecorum* and Koala retrovirus:

1. *Chlamydia pecorum*, a bacterium of the Chlamydia family believed to have been introduced by European settlers and transmitted to Koalas via livestock. Among Koalas, the bacterium is transmissible via sexual intercourse but also from mother to joey, and potentially also via other forms of close contact between individuals. *C. pecorum*, is the primary bacterial species causing Chlamydial infection. When disease occurs, it classically triggers ocular conjunctivitis and/or urinary tract disease (often referred to as 'wet bottom'), but can also cause severe pneumonia, polyarthritis, severe gastrointestinal problems, reproductive disease which may cause infertility[11], many of which can be fatal. All these symptoms are typically painful, and without human intervention/medical assistance are often lethal if the animal's immune system cannot fight the infection.

2. Koala retrovirus, or KoRV, a group of viruses specific to Koalas. A well-known retrovirus affecting humans is HIV / AIDS. Some forms of KoRV are associated with cancers and immunosuppression, leaving affected Koalas at decreased ability to cope with infections – including the bacterium *C. pecorum.* Not unlike AIDS in humans, where prior to the effective medications that exist today, a simple common cold could escalate and become life-threatening. The current understanding of the various forms of KoRV suggests that subtype B (known as KoRV-B) may be particularly pathogenic and cause particularly severe immunosuppression, leading to particularly high death rates when a Koala infected with KoRV-B contracts any kind of infection including (but not limited to) *C. pecorum*.

Even though the *C. pecorum* and KoRV results of this study are presented in two separate sections, the two are intrinsically linked due to the immunosuppression now known to be caused by some forms of KoRV.

7.1 CHLAMYDIA PECORUM

In this study, *Chlamydia pecorum* (*C. pecorum*) was detected in 40% (32/81) of Koalas, with an equal distribution between sexes (16 females (50%) and 16 males (50%)).

This represents a relatively high prevalence compared to other regions in Southeast Queensland:

- **Brisbane region: 21%** post-control (*initially 40%, reduced to 21% within three years following targeted control measures by Brisbane City Council*).
- **Logan region: 28%**
- **Darling Downs region: 28%**
- **Burnett region: 44%**

The findings indicate that *C. pecorum* prevalence in the Scenic Rim is higher than in Logan and Darling Downs but lower than in Burnett, suggesting that targeted disease management

[11] In both females (e.g. inflammation and fibrosis of the reproductive tract, ovarian bursal cysts) and males (e.g. inflammation of the prostate gland which can prevent normal secretion of prostatic fluid during ejaculation).

strategies, such as those implemented in Brisbane, may be beneficial in reducing infection rates in this region.

In considering these *C. pecorum* test results, it is very important to note that:

- The presence of *C. pecorum* in scat does not necessarily indicate that the animal is sick or clinically diseased due to the bacterium. An individual can carry the bacterium yet be asymptomatic, i.e. not exhibit clinical symptoms of chlamydial disease.

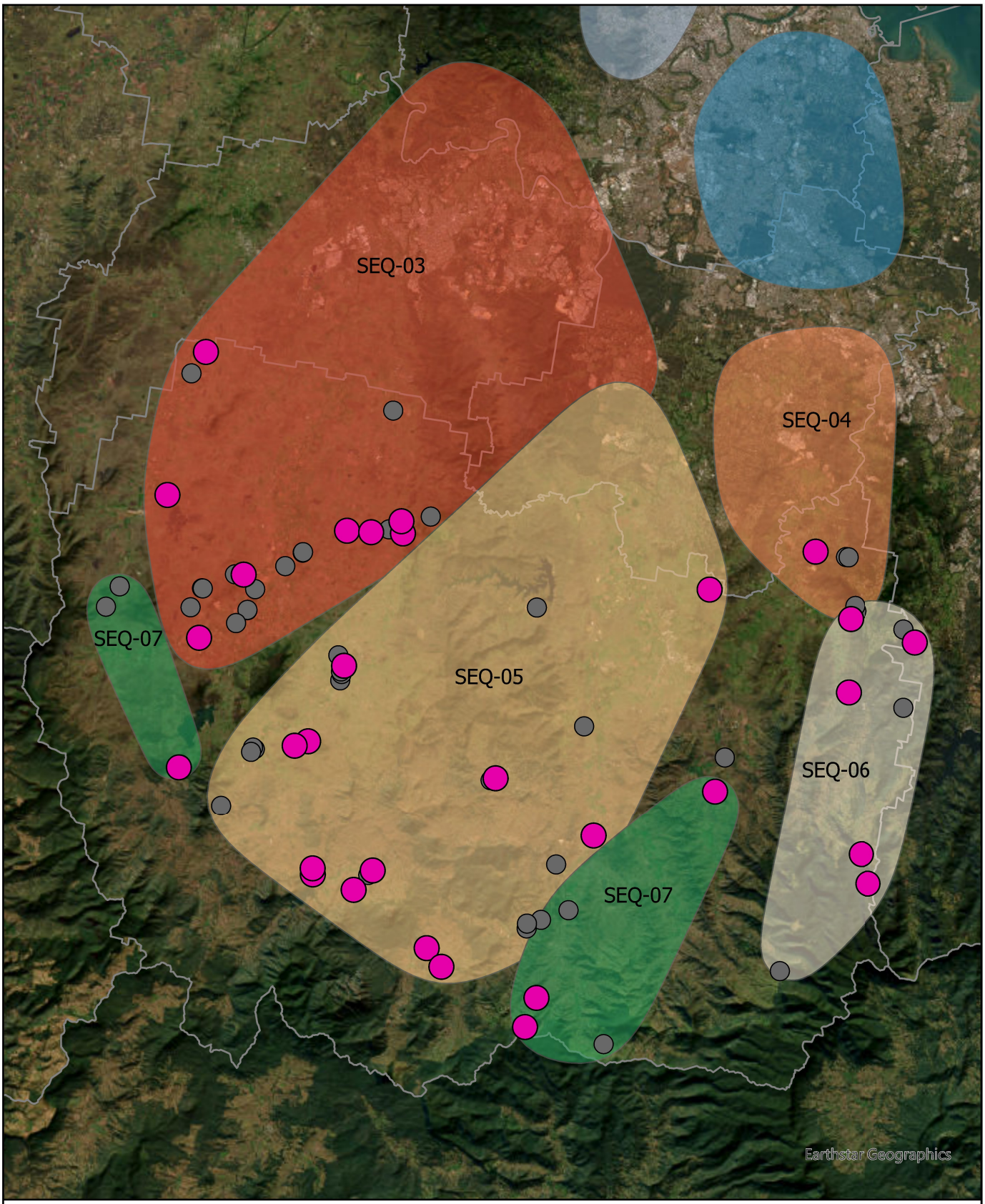
- A negative *C. pecorum* test result from scat does not necessarily indicate that the individual is free of the bacterium. An individual may carry the bacterium without it having spread to the gastrointestinal tract, in which case it is not detectable in its scat.[12]

Clinical symptoms associated with *C. pecorum* infection were historically thought to be triggered by environmental stressors such as habitat loss, climate change, and habitat modification (McAlpine *et al* 2017). While this may still hold true, recent evidence suggests that certain forms of KoRV can cause significant immunosuppression, severely compromising the immune system and reducing the individual's ability to combat infections, including but not limited to *C. pecorum* (Quigley and Timms, 2020).

In this study, two of the Koalas sampled that tested positive for *C. pecorum* were observed to display the classic symptoms of chlamydiosis and were taken into care (individuals identified as SCE107 sampled in Anthony and SCE121 sampled in Canungra, see **Appendix 2**). All others either did not exhibit any observable clinical symptoms, or were not (/or could not be) seen by the person collecting their scat.

The sampling locations of the Koalas whose scat tested positive for *C. pecorum* in this study are shown in **Figure 7.1**. The bacterium was detected dispersed across the study area and found in all five population clusters, with no obvious cluster nor geographic area of the Scenic Rim appearing to be more affected than others.

[12] This is scientifically known as ‘false negative’.



LEGEND

Koalas where Chlamydia pecorum was:

Detected Not detected

LGA boundaries

FIGURE 7.1

Distribution of C. pecorum detected in the study area

Scenic Rim
2024 Koala population study

7.2 KOALA RETROVIRUS

The Koala retrovirus, or KoRV, is a group of viruses specific to Koalas.

Although the understanding of the various forms of KoRV and their roles in disease remains under investigation, knowledge has dramatically advanced in recent years (Quigley and Timms 2023). There are currently 13 forms of the virus described KoRV A - M:

- KoRV subtype A, or KoRV-A, is endogenous in Queensland and Northern New South Wales. This means it is embedded in the genome of all Koalas in those regions, where all joeys are born with it.[13]
- The remaining 12 forms (KoRV-B through to KoRV-M) are exogenous. This means these forms are not embedded in the genome of the Koala, and are transmissible from one individual to the next.

The endogenous form (KoRV-A) is transmitted vertically, i.e. from mother to joey. There is more uncertainty regarding how the exogenous forms are transmitted in the wild, however, evidence is emerging suggesting both vertical (mother to joey) as well as horizontal (individual to individual) transmission for at least some exogenous forms of the virus (Kayesh *et al* 2020).

Recent advances in KoRV research indicate that some exogenous forms are associated with high incidences of certain types of cancers, nervous systems dysfunctions, and immunosuppression. Evidence is also mounting that some forms likely play a key role in determining how a Koala's immune system copes with infections, including bacterial infections such as *C. pecorum*.

7.2.1 KoRV-A results

KoRV-A was detected in 100% of Koalas in this study, which is expected since this form is known to be endogenous in Queensland and most of New South Wales (Meers *et al* 2014, Chappell *et al* 2017). In these regions, all joeys are born with KoRV-A as it is integrated into the genome and inherited from their parents.

There are currently no known management measures for KoRV-A. Furthermore, since all Queensland Koalas carry KoRV-A, it is not a distinguishing factor in determining health outcomes in Queensland. More relevant for assessing health outcomes are the **exogenous forms** of KoRV, which are not integrated into the genome. These forms have a stronger association with disease severity and immunosuppression. Unlike KoRV-A, targeted management strategies for exogenous forms can be implemented, where warranted, to mitigate their impact on Koala health.

[13] KoRV-A is currently in the process of being fully integrated into the DNA of the species itself. As many readers may already be aware, what makes up the DNA of many species is ancient viral DNA that has been embedded and fully integrated into the species itself. This is a common natural process that typically occurs over tens of thousands to millions of years. KoRV-A is extensively studied worldwide as it presents a unique case study in real-time retroviral endogenization.

7.2.2 Exogenous forms of KoRV detected

Three exogenous forms of KoRV were detected in individual Koalas across the study area, at the following prevalence:

- KoRV-B: 3%
- KoRV-D: 31%
- KoRV-F: 12%

Their sampling locations are shown on **Figure 7.2**.

Overall, **40%** of individuals in this study carried at least one form of exogenous KoRV, of which 35% carried only one form and only 5% carried two forms. This is a **relatively low prevalence of exogenous KoRV** compared to other regions in Southern Queensland:

Region	Individuals carrying only one form of exogenous KoRV	Individuals carrying two or more forms of exogenous KoRV
Scenic Rim:	35%	5%
Logan:	7%	85%
Brisbane:	25%	46%
Darling Downs:	28%	43%
Burnett:	51%	10%

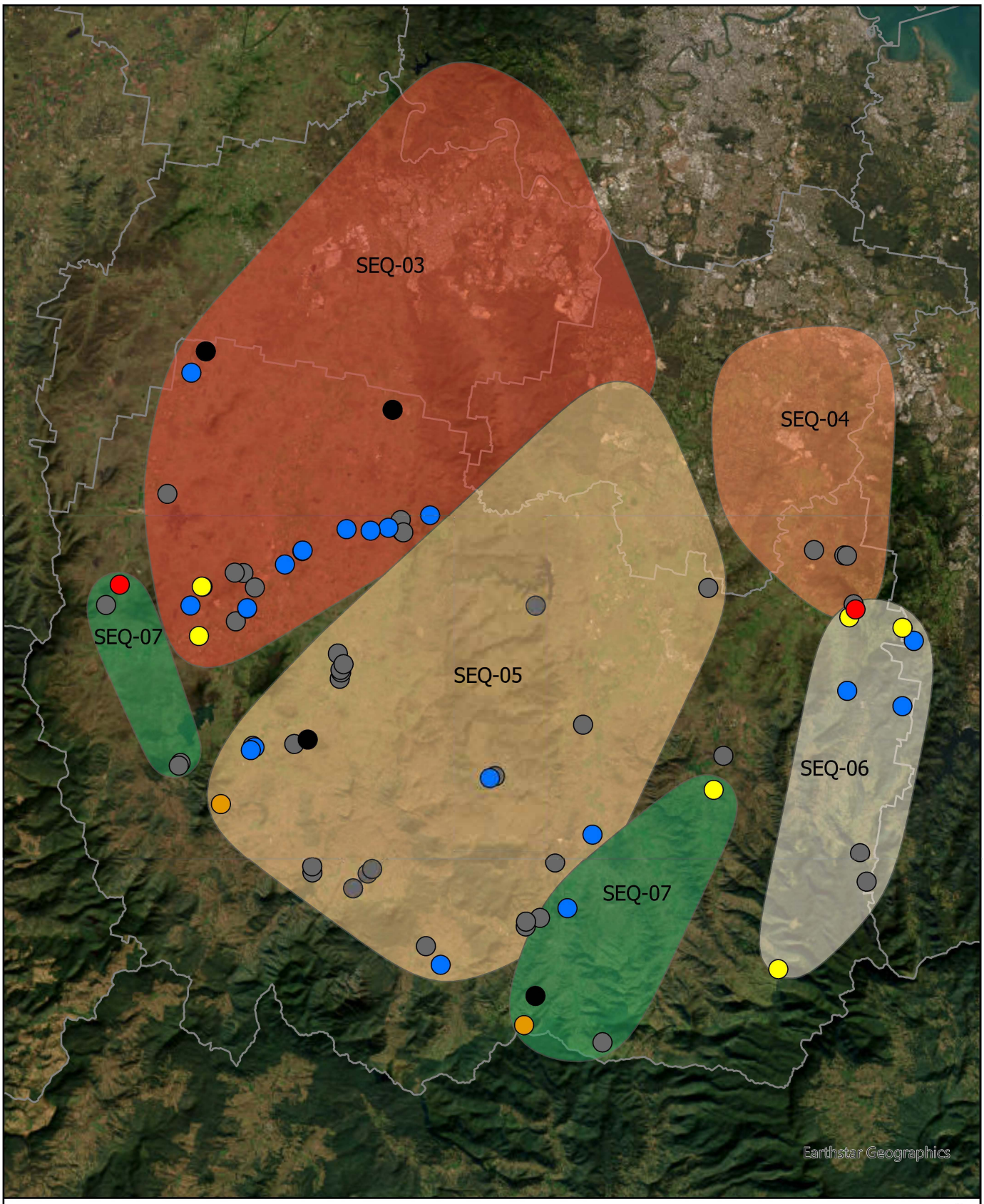
Interestingly, 31% of the Scenic Rim Koalas whose scat tested positive for *C. pecorum* had at least one form of exogenous KoRV (see **Appendix 2** for details) – this is lower than the average 40% *C. pecorum* prevalence found across the study area. This may be related to the fact that KoRV-D is the predominant exogenous form that was found in the study area.

The risk of false negatives associated with the *Chlamydia pecorum* detection test (see Section 7.1) does not apply to the KoRV genotyping test used in this study. All KoRV results obtained for the 75 individuals for which the test was successful represent their true KoRV status at the time of sampling in 2024.

The test was unsuccessful for six individuals, meaning their KoRV status could not be determined. However, for the 75 successfully genotyped individuals, the results accurately reflect their KoRV infection status at the time of sample collection.

It is important to note that KoRV transmission is dynamic, and additional transmission may have occurred since the 2024 sampling period. Therefore, these results provide a snapshot of KoRV prevalence at the time of collection rather than a definitive long-term status for each individual.

Figure 7.2 shows the exogenous KoRV results in the study area. The sections thereafter provide some discussion about the three forms of exogenous KoRV detected in the Scenic Rim.



LEGEND

Koalas with the following exogenous KoRV subtypes:

- KoRV-B + KoRV-D
- KoRV-D
- KoRV-D + KoRV-F
- KoRV-F
- None
- Unknown
- LGA boundaries

FIGURE 7.2

Exogenous KoRV subtypes detected in the study area

Scenic Rim
2024 Koala population study

KoRV-B

KoRV-B was detected in only two individuals in this study — one in the northeast and another in the west of the study area (see **Figure 7.2**).

While this represents a very low prevalence, it warrants further investigation as increasing evidence links KoRV-B with a high incidence of cancers (*Xu et al., 2013; Quigley et al., 2018*) and an elevated risk of chlamydial disease (*Waugh et al., 2017; Quigley & Timms, 2020*).

Moreover, research suggests that Koalas carrying KoRV-B are at increased risk of illness, and those co-infected with both KoRV-B and *Chlamydia pecorum* are highly likely to develop severe, often fatal chlamydial disease. The combination of these two pathogens has been associated with elevated mortality rates, particularly in small, isolated population clusters.

More detailed investigations regarding KoRV-B within the Scenic Rim is also warranted since the Scenic Rim shares a Koala population cluster (SEQ-07) with the Darling Downs – region where KoRV-B has was not detected, marking it as the only Southeast Queensland LGA investigated to date where this pathogen has not been detected.

KoRV-B is one of the forms that is transmissible both vertically (inherited in the womb) and horizontally (between individuals) (Kayesh *et al* 2020), hence it can spread relatively quickly. It is therefore imperative to withhold from performing any action that will increase habitat connectivity and hence increase the mixing and dispersal of individuals in the two parts of the Scenic Rim where KoRV-B has been detected in this study, until a better understanding of KoRV-B prevalence is obtained. Depending on the results obtained, it may be warranted to first control KoRV-B in affected areas before undertaking any action that will increase connectivity and the mixing of individuals, so as to not create new KoRV-B transmission pathways.

KoRV-D

This form is transmissible only horizontally between individuals, and not vertically (in the womb) (Kayesh *et al* 2020).

KoRV-D was detected in 31% of koalas in this study (see Figure 7.2). While the current understanding of KoRV-D remains limited, emerging research suggests that this subtype may be more frequently observed in healthy koalas that do not exhibit clinical symptoms of *Chlamydia pecorum* infection (*Quigley et al., 2019*).

This raises the possibility that KoRV-D could be associated with a stronger immune response or increased resistance to *C. pecorum*, though further research is needed to confirm this relationship.

- KoRV-D was the most commonly detected exogenous KoRV subtype in the Scenic Rim.
- Koalas infected with KoRV-D in this study has a lower-than-average incidence of *C. pecorum* infection (27% vs study average 40%).
- However, the two individuals in this study that displayed clinical signs of chlamydiosis and required veterinary care (SCE107 and SCE121) were both found to have KoRV-D.

KoRV-F

This form is also transmissible only horizontally between individuals (Kayesh *et al* 2020).

KoRV-F was detected in 12% of individuals in this study (see **Figure 7.2**). This form of KoRV is poorly understood, as it was initially identified in captive Koalas worldwide and has only recently been found in some wild Koalas.

KoRV-F detection in wild Koalas

- Quigley *et al* (2021) has detected KoRV-F in wild Koalas from:
  - St Bees Island (Moreton Bay)
  - Hidden Vale (Ipswich LGA)
  - Gold Coast
- KoRV-F was detected in wild Koala scat OWAD sampled between 2017 and 2021 from:
  - Brisbane LGA
  - Logan LGA

The authors of this report are not aware of KoRV-F having been detected in wild Koalas from any other regions yet.

Interestingly, KoRV-F was not detected in the scat of any of the ~200 wild Koalas recently tested from the Darling Downs, Burnett, and New South Wales Hunter regions. This may suggest that KoRV-F may have a limited geographic distribution; or is perhaps only emerging in certain regions.

Unclear role in Koala health

Scientific data on the pathogenic implications of KoRV-F remains limited, and its impact on Koala health is not yet well understood.

Unlike KoRV-B, which has been associated with disease susceptibility, and KoRV-D, which has been linked to potential disease resistance, KoRV-F has not been strongly correlated with either diseased or healthy Koalas.

8.0 INSIGHTS INTO DIET

Diet analysis from Koala scats offers valuable insights into the feeding habits and habitat use of Koalas, which is crucial for their conservation. By examining scat samples, species of trees Koalas consumed approximately one week prior can be determined, helping to identify food sources available to them. This information can be used to guide habitat restoration efforts, ensuring that food trees are protected or replanted in areas where Koalas are most at risk. Additionally, diet analysis can reveal changes in feeding behaviour that may be linked to environmental stressors such as habitat fragmentation, changing weather patterns, or food scarcity.

Even though diet analysis from scats provides a non-invasive method to assess Koala health, monitor habitat quality, and assist conservation efforts, it must be stressed that what Koalas *can* eat in modified landscapes such as the study area, does not necessarily reflect *preferred* diet. It may only reflect the trees the animals can safely access, which is not necessarily those they would prefer or favour if given the choice. Obtaining a robust understanding of 'preferred' Koala food tree species in any given region, requires both identifying tree species from their scat (ideally ~50 scats per Koala collected across seasons; the larger the number of Koalas the better the representation of individual preferences), combined with botanical sampling of these tree species' leaves in the study region which are then subjected to nutritional value analysis. No botanical sampling was performed here, hence no nutritional value analysis was performed, and only one scat from a subset of Koalas from this study were analysed for tree species.

8.1 SUMMARY OF METHODS AND LIMITATIONS

One scat from a subset of 31 Koalas in this study were genotyped against a panel of 89 'potential Koala food tree species' commonly found in Southeast Queensland. Plant DNA extracted from the scats allowed for the identification of any of these 89 tree species present in the scats. Caution must be exercised when interpreting these results due to two key limitations:

- **Limited sample size and representation:** Only one scat from a limited number of Koalas was analysed, which does not represent the full spectrum of these individuals' diet at that time, nor of these individuals' daily or seasonal variation in diet. Moreover, the limited sample size of 31 Koalas is not necessarily representative of the diet of Scenic Rim Koalas in general.
- **Limited detection scope:** The analysis was restricted to a panel of 89 potential food tree species, which currently has a very heavy emphasis on Eucalyptus species (72%). As a result, any tree species not currently included in the panel cannot be detected in the scat, meaning that other food sources may have been consumed but could not be identified.

The dietary analysis conducted in this study provides an initial insight into the tree species consumed by Koalas from the Scenic Rim region. While this exercise represents only a partial snapshot of Koala foraging behaviour, it is still valuable in highlighting that the diet of wild Koalas remains poorly understood.

One of the primary reasons for this knowledge gap is that the ability to genotype tree species from scat samples has only recently become possible. This study is among the first applied studies to utilize this method, marking an important step toward better understanding the diet of wild Koalas.

Although the current genotyping test is still limited, the existing tree species panel for Southeast Queensland is sufficiently developed to begin providing useful ecological insights. With continued refinement, this approach has the potential to enhance knowledge of wild Koalas' diet, which could further inform habitat management and conservation strategies.

8.2 FINDINGS

Analysis of scat samples in this study identified at least 25 tree species consumed by 31 individual Koalas across the Scenic Rim region. **Table 8.1** presents these species in descending order based on the number of Koalas found to have ingested them.

It is important to note that these findings do not represent the proportion of these Koalas' diet that each tree species constitutes, nor do they necessarily indicate the nutritional value of these species for Koalas. The presence of a tree species in scat samples only confirms ingestion, not dietary preference or its relative contribution to overall nutrition.

Assessing the importance of these tree species for the Koalas of the Scenic Rim would require botanical sampling and nutritional analyses, which could provide further valuable insights for habitat protection and restoration initiatives.

Table 8.1: Tree species detected in the scats of a subset of 31 Koalas

Tree species	Number of Koalas that ingested the species
Eucalyptus tereticornis	19
Eucalyptus microcorys	10
Angophora species*	6
Lophostemon confertus	3
Eucalyptus crebra, elegans and/or fibrosa **	3
Eucalyptus chloroclada	3
Eucalyptus amplifolia	3
Eucalyptus propinqua	3
Eucalyptus grandis	3
Corymbia maculata	2
Melaleuca quinquenervia	2
Eucalyptus cullenii	2
Eucalyptus granitica and/or whitteii **	2
Eucalyptus populnea	1
Eucalyptus longirostrata	1
Eucalyptus platyphylla	1
Eucalyptus microcarpa	1
Eucalyptus dunnii	1
Eucalyptus melliodora	1
Eucalyptus elegans	1
Eucalyptus dura and/or siderophloia **	1
Eucalyptus tindaliae	1
Corymbia intermedia	1
Corymbia gummifera	1
Corymbia citriodora	1

* The panel currently has one single entry for Angophora species.
** The panel cannot currently distinguish between these closely related species.

8.3 DISCUSSION

Despite the limitations of this study, the detection of at least 25 tree species in scat samples suggests that Scenic Rim Koalas have a broad and varied diet. It is important to note that the tree species genotyping panel used in this exercise has known limitations, meaning that Koalas may be consuming additional species not currently represented in the panel.

Within individual scats, between one and up eight tree species were identified. Since a single scat represents only a portion of the tree species an individual Koala browsed on approximately one week prior, these findings suggest diverse daily feeding habits among Scenic Rim Koalas.

The tree species identified in this study can serve as a valuable reference for:

- Botanical sampling to analyse the nutritional value of Koala food trees in the Scenic Rim.
- Identifying preferred food species to inform:
    - Habitat protection priorities (e.g., increased conservation efforts for ecosystems containing tree species of high nutritional value).
    - Revegetation and restoration programs that ensure the availability of nutritionally important food trees.

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9.0 CONCLUSIONS AND RECOMMENDATIONS

9.1 SUMMARY OF KEY FINDINGS

Genetic testing conducted in this study identified 81 individual Koalas, and population genetic analysis revealed significant structuring with 11 genetically distinct population clusters identified to date across the broad Southeast Queensland region. This finding highlights a fragmented genetic landscape with limited gene flow between clusters, suggesting that groups of Koalas in Southeast Queensland are becoming increasingly isolated from one another.

High levels of genetic differentiation indicate that habitat fragmentation since European settlement has been a major driver of population isolation and genetic differentiation. The disruption of natural movement pathways has restricted genetic exchange, increasing the risk of inbreeding, genetic bottlenecks, and localised population declines.

Notably, the Scenic Rim harbours more than 50% of all private alleles detected to date across Southern Queensland, reinforcing its status as a critical genetic stronghold for Koalas. This high genetic diversity is essential for maintaining resilience and adaptive potential for the species, making the region a priority for long-term conservation efforts.

The Scenic Rim harbours 5 of the 11 genetically distinct Koala population clusters identified to date in Southern Queensland – including both of the region's most significant genetic reservoirs. This makes the Scenic Rim one of the most vital landscapes for the species' survival not just in Southeast Queensland, but nationally. Protecting and reconnecting these population clusters is critical to ensuring the long-term resilience of Koalas in Australia.

Despite its genetic significance, the current population structure and migration patterns observed in the Scenic Rim are cause for concern:

- Koalas in the region are increasingly fragmented into genetically distinct clusters due to habitat fragmentation.
- Limited gene flow between clusters suggests that some of these may be functionally isolated, increasing their risk of genetic bottlenecks and inbreeding.
- Without intervention, this genetic isolation could lead to local extinctions, further threatening the long-term viability of Koalas in the region.

9.2 PREREQUISITE TO AN EFFECTIVE KOALA CONSERVATION STRATEGY

For Koalas to persist in Southeast Queensland, including the Scenic Rim, a prerequisite to a conservation strategy being effective is that the vital ecological and genetic processes they rely on must not further degraded than they already are. Immediate action is required to:

- Safeguard remaining habitats to prevent further degradation of vital ecological and genetic processes.
- Restore and enhance connectivity in targeted areas, both within and between fragmented population clusters, to facilitate gene flow and reduce genetic isolation.

The latter may involve a range of measures such as (but not limited to) promoting natural regeneration of trees and forests, construction of effective fauna crossing infrastructure in targeted areas across e.g. existing or new roads, railways, etc. These measures, if adequately targeted and executed, will reduce mortality, facilitate safe dispersal, and improve gene flow across the region.

Without swift and effective intervention to safeguard and reconnect the fragmented landscape, the population clusters of the Scenic Rim will face further genetic erosion and decline, pushing them even closer to being lost.

9.3 EFFECTIVE KOALA CONSERVATION STRATEGY REQUIRED FOR THE SCENIC RIM

SRRC is strongly advised to develop a comprehensive Koala Conservation Strategy without delay, ideally in collaboration with other relevant government authorities to ensure holistic management and recovery of the population clusters that occur within the Scenic Rim. Implementing this strategy as soon as possible will be critical to ensuring the survival of the Koala in the region.

The key scientific findings in this report provide a foundation for designing, implementing, and monitoring the effectiveness of this strategy. Each distinct population cluster can be assigned its own set of Key Priority Indicators (KPIs), derived from the scientific values presented in this study, to address specific conservation needs and threats in a targeted and effective manner. This strategy, and the KPIs, should be adaptive so it can be adjusted or refined as new and/or further relevant data becomes available.

SRRC has already demonstrated exceptional leadership in community coordination and data collection, as evidenced by the high success rates achieved in this study. With this expertise, SRRC is well-positioned to coordinate the ongoing monitoring required to assess the effectiveness of the conservation strategy over time. By regularly re-sampling scat from the region and analysing new data, SRRC can directly measure progress against the defined KPIs, ensuring that timely adjustments and refinements are made as required to improve conservation outcomes; as well as detect emerging problems early so these can be addressed and controlled before they severely impact an entire group of Koalas.

With SRRC already leading community-led and participative Koala genetic research for conservation, SRRC is uniquely positioned to implement and sustain a proactive Koala conservation framework, in a timely and cost-effective manner, that has a high chance at effectively securing the Koala in the Scenic Rim region.

It is highlighted that the current understanding of the geographic distribution of the 11 Koala population clusters identified to date across Southeast Queensland, does not follow administrative boundaries. As a result, conserving and securing the species in the most efficient and cost-effective manner, requires coordinated management of all applicable planning authorities that share population clusters.

It is also highlighted that the current understanding of Koala population structure across the broad Southeast Queensland region, is based only the areas where sufficient data is available at the time of writing this report. As a result, there likely are more population clusters not yet identified, and the geographic boundaries of the 11 clusters already described only reflect where these have been detected at the time of writing this report. That is, their geographic distribution could extend further to areas where no or insufficient data is currently available.

9.4 How this study can be used to inform development applications

Entities involved in preparing or assessing development applications can directly reference the key scientific findings of this study to ensure evidence-based decision-making. If a proposed development site falls within the mapped extent of any of the 11 identified Koala population clusters, this study provides critical baseline data, including:

- Conservation value of the affected cluster
- Health status and disease prevalence
- Key ecological processes (e.g., gene flow and migration rates, connectivity, breeding dynamics)
- Major threats and challenges

This information can be used to assess the potential impacts of a proposed development on the relevant cluster, to ensure compliance with applicable local planning codes, State Government regulations and national environmental laws, including EPBC Act referral requirements, and to generally assist in guiding best-practice and well-informed decisions.

9.5 Benefits of community Koala scat sampling approach

The approach taken in this study demonstrates that the methods and support systems employed are highly suited to inform improved decision-making. By actively engaging local communities, this study achieved reliable results quickly and cost-effectively, highlighting the effectiveness of this model as a sustainable and scalable solution for ongoing Koala conservation. DNA from degraded Koala scat collected by the community can be analysed using microsatellite genetic markers, enabling the reliable identification of individual Koalas – essentially non-invasively 'tagging' each Koala based on its unique DNA profile. The genetic data derived from scat is very powerful and allows for numerous population analyses and insights, making it invaluable for conservation efforts and achieving a range of critical objectives including but not limited to:

- **Scientifically robust population estimates**: Using the unique DNA fingerprints obtained from Koala scat, a 'capture-recapture' framework can be applied. Each scat sample's genotype is compared across sampling rounds, revealing whether the sample comes from a previously identified individual or a new one, enabling robust population size estimates.
- **Population structure and connectivity**: Genotyping reveals patterns of genetic similarity/or difference between groups, indicating the degree of connectivity or isolation between them. Understanding population structure is crucial for identifying isolated groups where habitat restoration or connectivity measures must be prioritised.
- **Genetic diversity**: The genetic data from scat samples provides insights into overall genetic diversity and health, which are essential indicators of a group's resilience to threats like disease and environmental change. High genetic diversity typically signals a healthy group, while low diversity can indicate vulnerability.
- **Health and disease monitoring**: Genetic data from scat samples provides information on the prevalence of certain pathogens, like Koala Retrovirus, or susceptibility to diseases. Monitoring genetic markers associated with disease resistance helps assess the clinical health status of individuals and of different genetic groups.
- **Longitudinal population tracking**: Regularly sampling scats from the same group of Koalas enables tracking changes in group size, genetic diversity, and clinical health over time. This longitudinal monitoring supports trend analysis and allows for the evaluation of conservation efforts and environmental impacts.

In summary, the model used in this study not only identifies individual Koalas non-invasively, quickly and cost-effectively, but it also yields critical data on population size, structure, genetic diversity and health, and empowers local communities. The scalability, community engagement and high scientific success repeatedly demonstrated in this and other studies, makes it a highly effective model for supporting long-term Koala research for conservation which ensures data transparency and accountability, and aligns with broader national conservation objectives.

9.6 Alignment with the National Koala Monitoring Program

Beyond monitoring, this model aligns with broader goals in the National Recovery Plan by fostering collaboration across local communities, and the public in general. The citizen-science approach empowers community and fosters cooperation between residents and their local government, making Koala research a shared responsibility and a truly collaborative effort.

In this study, Scenic Rim Regional Council has exemplified how local expertise can be leveraged for ongoing applied Koala research and monitoring, creating a sustainable, community-driven framework that serves as a model for other regions.

The methods and framework applied in this study fulfill many of the major objectives of the National Koala Monitoring Program, offering:

- **Population estimates**: Non-invasive 'genetic tagging' of Koalas, via repeat sampling of their scat, enables scientifically robust estimates of 'population size'.
- **Baseline data for conservation impact**: The genetic and health data collected serve as reliable initial indicators to evaluate the impact of subsequent conservation efforts and inform investment effectiveness.
- **Threat and conservation status assessment**: Changes in key genetic and/or health indicators act as early signals for potential threats, supporting proactive conservation decisions.
- **Strategic conservation guidance**: Providing precise data on Koala population structure, size, health and distribution allows for informed prioritisation of conservation actions.
- **Data collation and gap analysis**: Real-time updates to the genetic sampling database highlight knowledge and monitoring gaps, ensuring that research and conservation efforts remain relevant and targeted.

In summary, the methods and support systems utilised in this study provide a robust foundation to inform effective Koala conservation, fulfilling essential monitoring needs while promoting widespread community engagement, ensuring data transparency, and fostering collaboration between government organisations and the public. This approach not only meets the current requirements for Koala monitoring but also offers a scalable, replicable model that aligns with national conservation goals.

Given the demonstrated high success and efficiency of the model used in this and other studies, continued investment in community-led, non-invasive Koala genetic research is strongly recommended to support evidence-based conservation strategies, enhance data accessibility, and improve policy implementation.

10.0 STUDY LIMITATIONS

10.1 CHLAMYDIA PECORUM DETECTION

A positive *C. pecorum* result from scat indicates that the individual carries the bacterium, however this does not necessarily mean that the individual displays clinical symptoms of chlamydial disease. Furthermore, while scat-based testing is a valuable non-invasive tool for detecting *C. pecorum*, it has limitations in sensitivity, infection stage detection, and bacterial shedding variability. Complementary health assessments (e.g., ocular and urogenital examinations, blood tests) or additional diagnostic methods (e.g., swabs from infected sites) can provide a more comprehensive understanding of infection status and disease impact in wild Koalas. However, these tests are invasive, less ethical and far more costly due to wild Koalas needing to be located, captured, sedated and handled to collect samples directly from the animals.

10.2 GENETIC STATISTICS

The genetic statistics presented in this report are based on genotypic data from the 81 unique Koalas profiled from the Scenic Rim region as part of this study, as well as a large number of 'reference samples' from wild Koalas previously sampled (solely via scat) over the last decade across the broad Southeast Queensland region, whose genotypic data were already available on the database.

10.3 TREE SPECIES GENOTYPING FROM KOALA SCAT

Only the tree species that are included in the tree panel used, can be detected from scat. As such, if other species not in the panel were eaten by the Koalas sampled, then those species cannot be identified.

Failure to detect a tree species (represented in the panel) in a scat sample, does not guarantee that the species was not present in the scat. Indeed, a tree species could have been present in a sample but was not successfully genotyped.

Only one scat from a limited number of Koalas were used for this exercise. This means that individual variation in diet is poorly represented, moreover, there is no temporal variation in diet in this study.

10.4 LIMITATIONS OF DATA INTERPRETATION

The interpretations provided in this report are only valid for the areas sampled during this study and reflect the conditions at the time of sampling. As environmental conditions, population dynamics, and disease prevalence may change over time, the findings presented in this report should be considered within this temporal and spatial context.

Additionally, the results and conclusions in this report should be read and interpreted alongside any future studies on the genetics and health of Koalas in the study area. Continued research and monitoring will be essential to track changes over time, validate these findings (or otherwise), and inform ongoing conservation efforts.

11.0 REFERENCES

Blyton MDJ, Pyne M and Chappell K (2022). Koala retrovirus load and non-A subtypes are associated with secondary disease among wild northern koalas. *PLoS pathogens* vol. 18,5 e1010513.

Chappell KJ, Brealey JC, Amarilla AA, Watterson D, Hulse L, Palmieri C, Johnston SD, Holmes EC, Meers J and Young PR (2017). Phylogenetic diversity of koala retrovirus within a wild koala population. *Journal of Virology* 91(3).

Ellis WAH, Sullivan BJ, Lisle QT and Carrick FN (1998). The spatial and temporal distribution of koala faecal pellets. *Wildlife Research* 25(6): 663-668.

Guillot G, Santos F, Estoup A (2008). Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. *Bioinformatics* 24, 1406-1407.

Kayesh MEH, Hashem MA, Tsukiyama-Kohara K (2020). Koala retrovirus epidemiology, transmission mode, pathogenesis, and host immune response in koalas (Phascolarctos cinereus): a review. *Archives of Virology* 165, 2409-2417.

McAlpine C, Brearley ., Rhodes J, Bradley A, Baxter G, Seabrook L, Lunney D, Liu Y, Cottin M, Smith AG and Timms P (2017). Time-delayed influence of urban landscape change on the susceptibility of koalas to chlamydiosis. *Landscape Ecology* 32, 663-679. doi: 10.1007/s10980-016-0479-2.

Meers J, Simmons G, Jones K, Clarke DTW and Young PR (2014). Koala retrovirus in free-ranging populations − prevalence. *Technical Reports of the Australian Museum, Online* (2014) 24: 15-17.

Neaves LE, Frankham GJ, Dennison S, Fitzgibbon S, Flannagan C, Gillett A, et al. (2016). Phylogeography of the Koala, (*Phascolarctos cinereus*), and Harmonising Data to Inform Conservation. PLoS ONE 11(9): e0162207.

Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945-959.

Quigley B, Ong V, Hanger J, Timms P (2018). Molecular dynamics and mode of transmission of Koala Retrovirus (KoRV) as it invades and spreads through a wild Queensland koala population. *Journal of Virology* 92(5).

Quigley B, Phillips S. Olagoke O, Robbins A, Hanger J, Timms P (2019). Changes in endogenous and exogenous koala retrovirus subtype expression over time reflect koala health outcomes. *Journal of Virology* 93(18).

Quigley B and Timms P (2020). Helping koalas battle disease – Recent advances in Chlamydia and koala retrovirus (KoRV) disease understanding and treatment in koalas. *FEMS Microbiology Reviews* 44(5): 583-605.

Quigley BL, Melzer A, Ellis W, Tzipori G, Nilsson K, Olagoke O, Robbins A, Hanger J, TimmsP. (2021). Koala retrovirus in northern Australia shows a mixture of stable endogenization and exogenous lineage diversification within fragmented koala populations. *Journal of Virology* 95: e02084-20.

Quigley B and Timms P (2023). Endogenous and exogenous koala retrovirus patterns in wild koalas across Australia. Technical Report, *Australian Museum Online* 38:7-9.

Waugh C, Hanger J, Loader J, King A, Hobbs M, Johnson R, Timms P (2017). Infection with koala retrovirus subgroup B (KoRV-B), but not KoRV-A, is associated with chlamydial disease in free-ranging koalas (Phascolarctos cinereus). *Scientific Reports* 7.

Wedrowicz F, Karsa M, Mosse J and Hogan FE (2013). Reliable genotyping of the koala (*Phascolarctos cinereus*) using DNA isolated from a single faecal pellet. *Molecular Ecology Resources* 13(4): 634-641.

Wedrowicz F, Saxton T, Mosse J, Wright W and Hogan FE (2016). A non-invasive tool for assessing pathogen prevalence in koala (*Phascolarctos cinereus*) populations: detection of *Chlamydia pecorum* and koala retrovirus (KoRV) DNA in genetic material sourced from scats. *Conservation Genetics Resources* 8(4): 511-521.

Wedrowicz F, Mosse J, Wright W and Hogan FE (2017). Validating the use of non-invasively sourced DNA for population genetic studies using pedigree data. *Web Ecology* (17): 9-18.

Wilson GA and Rannala B (2003). Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* 163(3): 1177-1191.

Xu W, Stadler C, Gorman K, Jensen N, Kim D, Zheng H, Tang S, Switzer W, Pye G, Eider M (2013). An exogenous retrovirus isolated from koalas with malignant neoplasias in a US zoo. *Proceedings of the National Academy of Science USA* 110:11547-52.

APPENDIX 1
SUMMARY OF SAMPLE CONDITION SCORING SYSTEM

Sample condition scoring system

OWAD developed a sample condition scoring system according to the appearance of putative Koala scats or pap.

Extensive testing by an external analytical laboratory has demonstrated that this system is reliable in predicting the viability of samples in producing a reliable Koala DNA profile.

A sample is attributed a score from 1 to 3, where 1 is excellent condition and 3 is poor condition.

It has been demonstrated that on average **88%**, **72%** and **40%** of scats that score **1**, **2** and **3** respectively, contain Koala DNA of sufficient quality *and* quantity to provide a reliable Koala DNA profile.

Condition score	Representative photographs	Comments
1		Material that is in **excellent** condition (1) These are often fresh scats/pap tending to be less than 1-2 weeks old when first removed from the natural environment. Typical visual characteristics: • Very high level of gloss around the entire scat surface **AND** • Absence of significant dust or debris **AND** • No sun or rain damage **AND** • No insect predation **AND** • Smooth and uncracked surface
2	 	Material that is in **good** condition (2) These are scats that have a decent amount of gloss on all or most of their surface, and typically are: • Fresh material (1-2 weeks old) that has suffered some albeit limited environmental damage **OR** • Older material up to several months old that, given how long it was in the natural environment, has suffered only limited environmental damage. Typical visual characteristics: • Decent level of gloss around all or most of the surface **AND/OR** • Limited dust or debris **AND/OR** • Limited sun or rain damage **AND/OR** • Limited insect predation **AND/OR** • Limited cracking of the surface
3		Material that is in **poor** condition (3) This material is typically distinctly duller and retains only a limited amount of gloss, **OR** has decent gloss but only on portions of the surface. This material is typically either: • Fresh material that has suffered significant environmental damage (e.g. fresh scats that fell directly on dirt, or were exposed to rain) **OR** • Older material up to several months old, that has suffered notable environmental damage and retains only limited gloss. Typical visual characteristics: • Limited level of gloss on all or only parts of the surface **AND/OR** • Notable dust or debris **AND/OR** • Notable sun or rain damage **AND/OR** • Notable insect predation **AND/OR** • Notable cracking of the surface

APPENDIX 2
INDIVIDUAL TEST RESULTS FOR THE UNIQUE KOALAS PROFILED IN THIS STUDY

Appendix 2: Individual test results for the unique Koalas profiled in this study

Sample code	Date collected	Coordinates (UTM zone 56)		Sex	*C. pecorum*	KoRV-A	Exogenous KoRV subtypes
		Easting	Northing				
SCE022	11-Apr-24	455501	6910516	Male	Not Detected	Detected	F
SCE023	10-Apr-24	503394	6910391	Female	Detected	Detected	
SCE024	12-Apr-24	516260	6913460	Male	Not Detected	Detected	
SCE025	12-Apr-24	516508	6913430	Female	Not Detected	Detected	
SCE027	12-Apr-24	513393	6913965	Female	Detected	Detected	
SCE028	12-Apr-24	516736	6907613	Female	Detected	Detected	F
SCE029	12-Apr-24	474461	6915683	Male	Detected	Detected	
SCE030	11-Apr-24	473148	6916070	Male	Not Detected	Detected	D
SCE034	06-May-24	521772	6906622	Female	Not Detected	Detected	
SCE035	14-May-24	473544	6927271	Female	Not Detected	Detected	Unknown
SCE037	13-May-24	459794	6908449	Female	Not Detected	Detected	Unknown
SCE038	26-May-24	460482	6895352	Male	Not Detected	Detected	D
SCE039	22-May-24	521763	6906628	Male	Not Detected	Detected	F
SCE040	25-May-24	503907	6891336	Male	Detected	Detected	F
SCE041	26-May-24	455228	6905847	Male	Detected	Detected	F
SCE042	26-May-24	459791	6908477	Female	Not Detected	Detected	D
SCE043	26-May-24	460317	6895495	Female	Not Detected	Detected	
SCE045	22-May-24	454512	6930782	Female	Not Detected	Detected	D
SCE046	23-May-24	459434	6911800	Female	Detected	Detected	
SCE047	28-May-24	457306	6889992	Male	Not Detected	Detected	D, F
SCE048	27-May-27	465000	6913874	Female	Not Detected	Detected	D
SCE050	27-May-24	465035	6913920	Female	Not Detected	Detected	D
SCE051	29-May-24	458717	6907232	Female	Not Detected	Detected	
SCE055	27-May-24	455876	6932800	Male	Detected	Detected	Unknown
SCE057	29-May-24	465960	6883433	Female	Detected	Detected	
SCE059	30-May-24	521757	6899243	Male	Not Detected	Detected	Unknown
SCE060	30-May-24	521757	6899243	Male	Not Detected	Detected	D
SCE061	30-May-24	522836	6905396	Male	Detected	Detected	D
SCE064	02-Jun-24	483201	6892594	Female	Detected	Detected	
SCE065	30-May-24	468542	6901831	Female	Not Detected	Detected	
SCE067	07-Jun-24	471190	6883380	Female	Not Detected	Detected	
SCE068	07-Jun-24	469809	6881974	Female	Detected	Detected	
SCE069	08-Jun-24	455573	6910501	Female	Not Detected	Detected	
SCE070	06-Jun-24	465515	6896084	Male	Detected	Detected	
SCE071	08-Jun-24	487483	6879142	Male	Not Detected	Detected	
SCE072	10-Jun-24	486128	6878333	Female	Not Detected	Detected	
SCE073	09-Jun-24	458612	6911833	Female	Not Detected	Detected	
SCE074 *	05-Jun-24	468365	6904194	Female	Not Detected	Detected	
SCE076	02-Jun-24	468676	6902431	Male	Not Detected	Detected	
SCE077	03-Jun-24	463358	6912603	Female	Not Detected	Detected	D
SCE078	05-Jun-24	471607	6883805	Male	Detected	Detected	
SCE079	06-Jun-24	465515	6896084	Female	Detected	Detected	Unknown
SCE080	09-Jun-24	504826	6894554	Female	Not Detected	Detected	
SCE081	05-Jun-24	468614	6902673	Female	Not Detected	Detected	
SCE083	07-Jun-24	468905	6903205	Female	Detected	Detected	
SCE084	16-Jun-24	491552	6897492	Male	Not Detected	Detected	
SCE086	29-May-24	517751	6885348	Female	Detected	Detected	
SCE087	11-Jun-24	487055	6871773	Male	Detected	Detected	Unknown
SCE090	09-Jun-24	485977	6869021	Male	Detected	Detected	D, F
SCE093	20-Jun-24	477086	6917273	Female	Not Detected	Detected	D
SCE094	20-Jun-24	474319	6916833	Female	Detected	Detected	
SCE095	17-Jun-24	465941	6884007	Female	Detected	Detected	

Sample code	Date collected	Coordinates (UTM zone 56)		Sex	*C. pecorum*	KoRV-A	Exogenous KoRV subtypes
		Easting	Northing				
SCE096	14-Jun-24	510019	6874300	Female	Not Detected	Detected	F
SCE097	14-Jun-24	510019	6874300	Female	Not Detected	Detected	F
SCE099	19-Jun-24	453487	6893876	Female	Not Detected	Detected	
SCE102	20-Jun-24	452244	6919315	Male	Detected	Detected	
SCE103	18-Jun-24	474560	6915640	Female	Not Detected	Detected	
SCE104	18-Jun-24	474560	6915640	Male	Not Detected	Detected	
SCE105	19-Jun-24	453322	6893627	Female	Detected	Detected	
SCE107	19-Jun-24	469186	6915940	Female	Detected	Detected	D
SCE108	18-Jun-24	486161	6878789	Male	Not Detected	Detected	
SCE109	21-Jun-24	518374	6882561	Male	Detected	Detected	
SCE110	22-Jun-24	464244	6895668	Male	Detected	Detected	
SCE114	16-Jun-24	471463	6915800	Male	Detected	Detected	D
SCE119	20-Jun-24	488912	6884376	Female	Not Detected	Detected	
SCE121	20-Jun-24	516534	6900688	Female	Detected	Detected	D
SCE123	22-Jun-24	460132	6895087	Female	Not Detected	Detected	D
SCE124	21-Jun-24	460519	6910431	Unknown	Not Detected	Detected	
SCE125	22-Jun-24	478090	6874711	Male	Detected	Detected	
SCE126	22-Jun-24	476697	6876471	Male	Detected	Detected	
SCE127	19-Jun-24	517323	6908357	Male	Not Detected	Detected	B, D
SCE128	25-Jun-24	517146	6908845	Male	Not Detected	Detected	
SCE129	26-Jun-24	446440	6908779	Unknown	Not Detected	Detected	
SCE130	25-Jun-24	447739	6910695	Male	Not Detected	Detected	B, D
SCE131	23-Jun-24	490079	6880056	Male	Not Detected	Detected	D
SCE132	25-Jun-24	492448	6887105	Male	Detected	Detected	D
SCE133	25-Jun-24	493392	6867407	Female	Not Detected	Detected	
SCE134	27-Jun-24	487094	6908698	Male	Not Detected	Detected	
SCE135	23-Jun-24	478090	6874711	Female	Detected	Detected	D
SCE136	27-Jun-24	482730	6892410	Male	Not Detected	Detected	D
SCE137	29-Jun-24	454427	6908721	Female	Not Detected	Detected	D

***** SCE074: denotes a female that was sampled twice by two collectors a few days apart <50m apart. The date and coordinates associated with the first time she was sampled are displayed in this table.