Canine Scent Detectives Promoting Koala Population Health in Bellingen - Coffs Harbour LGAs.

Report prepared by:
CANINES FOR WILDLIFE

Report prepared for:

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December 2024

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Acknowledgements

Canines for Wildlife koala detection dog scat surveys were conducted on the traditional lands of the Gumbaynggirr people, custodians of Dunggirr (koala). We pay our respects to all Elders past, present and emerging, their ongoing connection and stewardship of country.

Funding for the projects that contribute to this report were provided to Jaliigirr Biodiversity Alliance (JBA) and Canines for Wildlife (CFW) by the Australian Government (Aus. Govt), Great Eastern Ranges Initiative (GER), World Wide Fund for Nature – Australia (WWF), North Coast Local Land Services (NCLLS), City of Coffs Harbour (COHCC), Coffs Harbour and District Local Aboriginal Land Council (CH+DLALC) and Transgrid.

The genetic analysis was funded through Jaliigirr Biodiversity Alliance. Thanks to Romane Cristescu, Ajith Horane Karayalgage and Katrin Hohwieler, University of Sunshine Coast Detection Dogs for Conservation Laboratory (UniSC) and Diversity Arrays Technology for their collaboration (DaRT).

We thank the private landholders, NSW National Parks and Wildlife Service and the Bellingen and Coffs Harbour Councils for their support and access to sites. A number of people provided information on where koalas had been seen enabling collection of scat material, and WIRES Coffs Harbour provided scats from koalas in care.

In particular, thanks to Justin Couper (project manager, JBA) for his coordination and support in implementing the projects. John Turbill (DCCEEW), Tim Scanlan, Martin Smith (NPWS), Melanie Craig (Coffs Harbour Regional Landcare), Mathew Smith and Darren Skinner (CH+DLALC), Sally Whitelaw (COHCC) and Ashley Goodwill (NCLLS) and Brad Nesbitt Canines for Wildlife provided guidance in determining priority areas and negotiating access.

A big thank you to Max (English Springer Spaniel) and handlers Lynn Baker and Jack Nesbitt, Poa (English Springer Spaniel) and handler Lily Alvarez for their hard work and commitment to the project, many kilometres travelled, and koala scats found. Thanks to Brad Nesbitt for assistance in the field and report preparation.

Lynn Baker and Max

Lily Alvarez and Poa

Jack Nesbitt and Max

Citation: This document should be cited as:

Canines for Wildlife (2024). Canine Scent Detectives Promoting Koala Population Health in Bellingen - Coffs Harbour LGAs. Prepared for Jaliigirr Biodiversity Alliance.

Executive Summary

One hundred and fifteen sites within the Bellingen-Coffs Harbour LGAs were surveyed by the Canines for Wildlife detection dog team for koala scats between June 2020 and January 2024.

Over 700 koala scat locations were recorded, and 155 samples were collected. The samples were processed to have the DNA extracted from koala epithelial cells attached to the external surface of the scat. The DNA extracted allows researchers to understand the level of relatedness between koalas, as well as the disease status of the koalas.

Ninety-two koalas were uniquely identified across the project area. The Bellingen-Coffs Harbour koala population is shown to be of high genetic diversity with a low presence of Chlamydia disease.

The study also showed that koalas in Bongil Bongil National Park, on the eastern side of the Pacific Highway, and koalas in Gleniffer, on the western side of the Pacific Highway are closely related. This emphasises the importance of the overpass and underpasses of the Pacific Highway and the importance of having connected koala habitat along corridors. It also highlights the significantly important contribution private lands are making to connecting and preserving koala habitat.

In some of the study sites, several individual koalas separated by 5-13kms were found to be related, showing that individuals are using these habitat corridors and crossing land tenures to interact. Some koalas are moving across the urban areas of Coffs Harbour. Maintaining these habitat connections is important in maintaining a genetically robust population.

The location of a group of Chlamydia free koalas in the Fernbrook cluster is interesting and could highlight a healthy group of koalas of high conservation value. More koala scats need to be found in this area for analysis.

Despite the loss of habitat in the Bellingen-Coffs Harbour LGAs, a healthy population of koalas seems to persist. This study shows the importance of the remaining wildlife corridors, and by extension, hints at the risk to this population should those corridors be broken.

Canine Scent Detectives Promoting Koala Population Health in the Bellingen- Coffs Harbour LGA's.

Project Background

The Bellingen-Coffs Harbour LGA's are known to support koalas and important areas of koala habitat. The objective of the combined projects was to map koala activity in priority locations in the two LGA's, and to assess the genetic diversity and population structure, genetic relatedness of individuals and disease (C*hlamydia pecorum)* status.

In 2023, Canines for Wildlife (CFW) received funding from the Australian Government (Aust. Govt.) Koala Conservation and Protection- Community Grants Round 1, Great Eastern Ranges (GER) and World Wide Fund for Nature - Australia (WWF) Cores, Corridors and Koalas Phase 2 and North Coast Local Land Services (NCLLS) Koala ARKS to undertake koala scat detection dog surveys and collect fresh scat material for genetic analysis. In addition, CFW were contracted by North Coast Local Land Services to undertake koala scat detection surveys as part of the Koala ARKS project on private properties in the Bellingen and Coffs Harbour regions from 2022-2024.

The University of Sunshine Coast Detection Dogs for Conservation Laboratory (UniSC) were contracted by Jaliigirr Biodiversity Alliance (JBA) to prepare samples and analyse the results obtained through genotyping provided by Diversity Arrays Technology (DArT).

JBA and CFW had previously received funding from City of Coffs Harbour (COHCC) through Environmental Levy Grants, Coffs Harbour and District Local Aboriginal Local Land Council (CH+DLALC) and Transgrid in 2020-2022 to survey for koala scats and undertake genetic analysis. The results from the survey and genetic analysis were reported by CFW and UniSC in 2022 (see references). The survey data and genetic analysis from the five projects have been combined within this report.

Introduction

The CFW detection dog team undertook surveys to locate and record koala activity through scat detection, to improve knowledge of koala distribution, genetic health, priority areas and habitat usage.

Priority locations were selected by discussion with stakeholders including NPWS, COHCC, DCEEW, NCLLS, JBA and CFW.

Koala scat detection dog surveys were conducted on private and public lands within the Bellingen and Coffs Harbour region from 2020 to January 2024. Koala scat locations and associated feed trees were mapped, significant food trees identified, and fresh scats collected and stored for genetic and health analysis. Fresh koala scats were transported to the UniSC laboratory where the scats were processed and samples prepared for genotyping which was conducted by DArT.

Survey methodology

The detection dog team systematically searched each site for koala scats using 400m x 50m belt transects. The number of transects per site depended on size of the property, koala habitat and access. The scent detection dogs worked off leash, controlled by voice, whistle and hand signals and worked closely with their handlers, coursing in zig zag fashion using their nose to locate the presence of koalas and their scats. When a scat was located the dogs indicated with a passive response. The location and condition of scats were recorded and suitable scats collected for genetic analysis.

At each site one or more surveys were undertaken depending on the presence of suitable habitat and access. In addition to recording koala scat locations along the survey transects, associated tree species and significant food trees were identified. The significant food trees were determined through the presence of multiple aged scats and trees that had associated scat presence over multiple visits.


Max


Poa

The detection dogs wore an identifying harness as well as a GPS tracking collar. The handler used a Garmin GPS to record the detection dog search tracks and record GPS locations of the koala scats.

The koala scats were aged based on the characteristics identified in Table 1. Categories 1-3 were collected for scat analysis.

Table 1. General guide used to age koala scats in the field (Karayalage et al. 2024)

Scat age categories	Age	Characteristics
1	One day old or less	Very fresh (covered in mucus, wet)
2	Couple of days old	Fresh (shine and smell)
3	Couple of weeks old	Medium fresh (shine or smelly when broken)
4	Months old	Old (no shine, no smell)
5	More than a few months old	Very old and discoloured

The rate of scat decay can vary significantly based on factors such as ground layer and litter structure and moisture, rainfall history over sampling period, invertebrate and vertebrate mammal activity e.g. bandicoot diggings, burrows (Rhodes et al 2011, Cristescue et al 2018, OWAD 2020). Surveys were suspended after heavy rain due to scat degradation and to provide time for new material to accumulate.

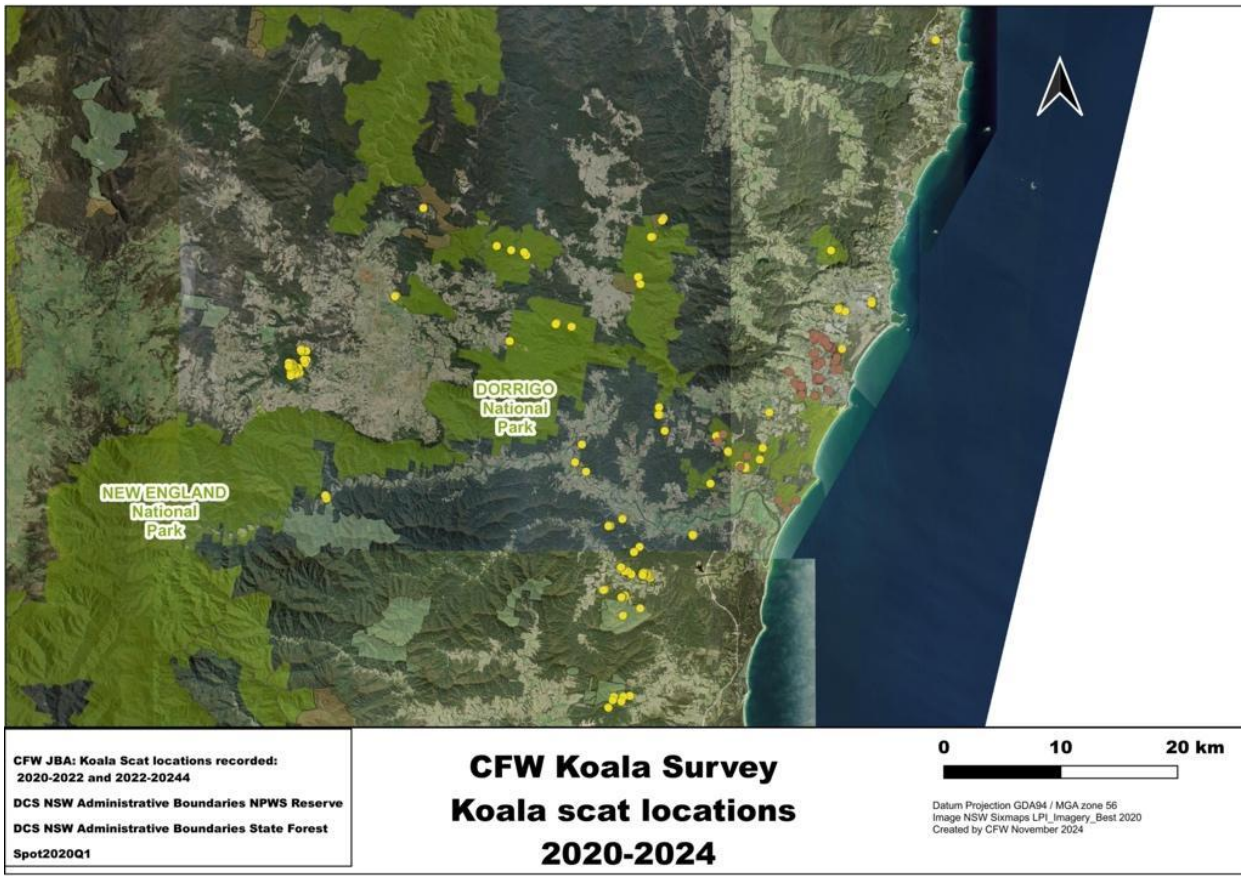
Koala scats were carefully collected using clean gloves or gently rolling them with the lid into a clean vial to minimise abrasion and contamination of the scat. The scats samples were labelled and placed in a cold esky until they were transferred to a freezer and stored at -20C. The frozen koala scat samples were recorded in a spreadsheet and transported to the UniSC laboratory in February 2024. The Coffs Harbour samples had previously been delivered to UniSC in 2022.

The koala scat samples were held frozen at the UniSc laboratory until processed and sent to the DArT Laboratory for genotyping. The UniSC laboratory analysed the results received from DArT and prepared the reports (Hohweiler et al. 2022 and Karayalage et al. 2024).

Survey Results

One hundred and fifteen sites were surveyed for koala activity within the Bellingen-Coffs Harbour LGAs between June 2020 and January 2024 (Figure 1).

Figure 1: CFW survey sites with koala scats (2020-2024)
Yellow- 2022-2023, Red 2020-2022



Of the 115 sites surveyed, 83% had koala activity recorded, and 730 scat locations were recorded across the project area (Table 2).

Table 2: Summary of CFW Koala detection dog surveys results Bellingen-Coffs Harbour LGAs from 2020-2023

PROJECT	YEAR	GENERAL LOCATIONS	NO. SITES SURVEYED	NO. SCAT LOCATIONS	NO. SITES NIL SCAT
COHCC	2020-2022	Bongil Bongil-Boambee-Sawtell-Toormina	30	232	5
CH+DLALC	2020-2022	Mylestom, Kalang	2	75	0
Opportunistic surveys	2022	Bindarri, Lowanna	3	3	
NCLLS Koala Arks	2022-2023	Repton, Brierfield, Bellingen, Valery, Orara, Karangi, Coramba, Lowana, Ulong, Nana Glen, Bostobrick, Darkwood	24	130	5
JBA Koala Corridors Project	June 2023-Jan 2024	Bonville, Bongil, Valery, Bellingen, Brierfield, Martells, Woolgoolga, Sherwood, Coffs Harbour, Bindari, Fernbrook, Dorrigo plateau, Bollanolla.	56	290	9
TOTALS			**115**	**730**	**19**

One hundred and fifty-five samples were collected in vials, labelled and stored. Locations of the scats collected in 2020-2022 in the peri-urban Coffs Harbour area are shown in Figure 2. Locations of all scat samples collected in 2020-2024 are shown in Figure 3.

The samples were processed to have the DNA extracted from koala epithelial cells attached to the external surface of the scat. Not all scats have epithelial cells attached and not all the others have sufficient cells to yield enough DNA or DNA of sufficient quality for analysis (R. Cristescu pers comm. 2024). The processes for individual genetic fingerprinting, gender, *Chlamydia* status, genetic

diversity and population structure require different quality thresholds and so the number of individual samples included in each case varied (Karayalage et al. 2024).

Figure 2: Koala scat samples collected by CFW in Coffs Harbour 2020-2022 (Hohwieler et al. 2022)

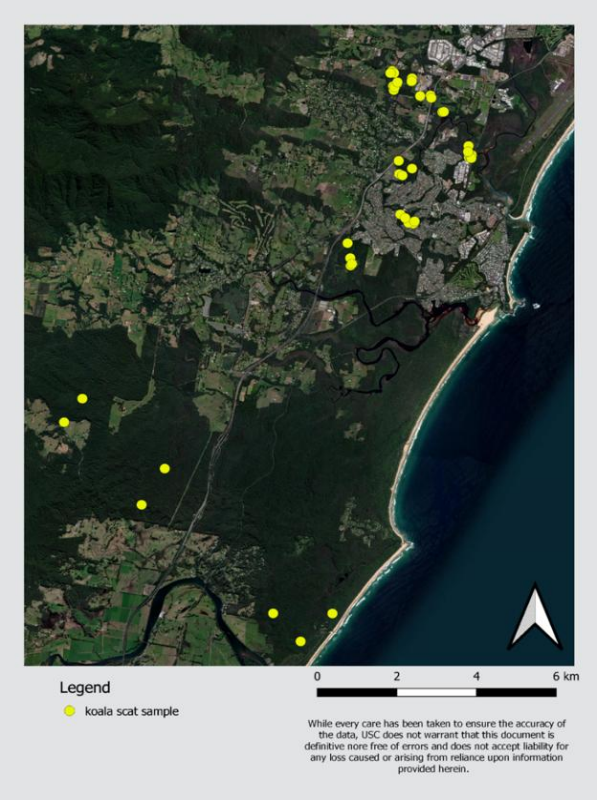
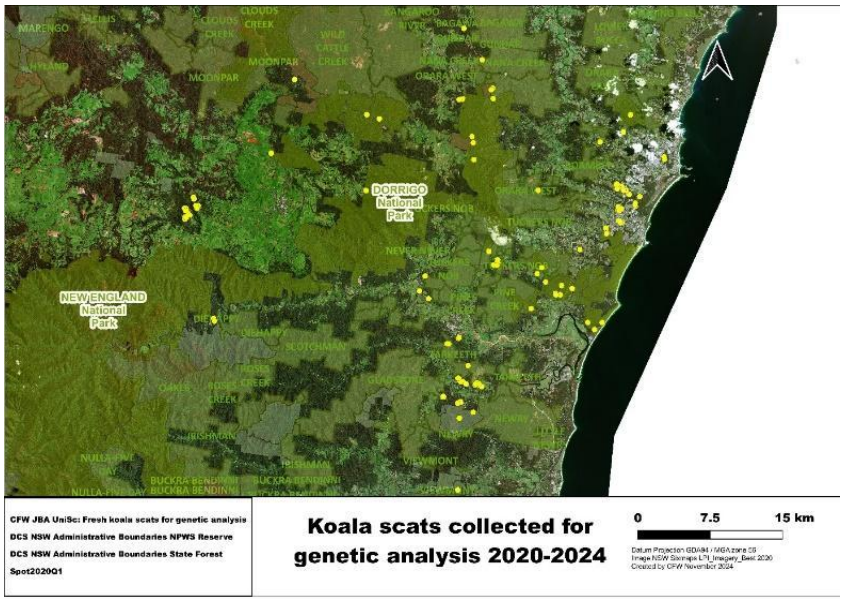


Figure 3: All koala scat samples collected by CFW 2020-2024



A total of 155 samples were collected for genetic analysis of which:

- 115 samples were of suitable quality for DNA extraction
- Duplicate samples and samples that failed quality control thresholds were not included
- Only unique koalas are used for estimating genetic relatedness, sex ratio, *Chlamydia* prevalence, and population genetic parameters
- 92 koalas were uniquely identified across the project area (Figure 4)
- The gender analysis identified 47 males and 39 females, resulting in a sex ratio of 1.0:0.83 male to female, showing a slight male bias in the population (Karayalage et al. 2024) (Figure 5).

Figure 4: Location of the 92 individual koalas recorded across the Bellingen-Coffs Harbour region.

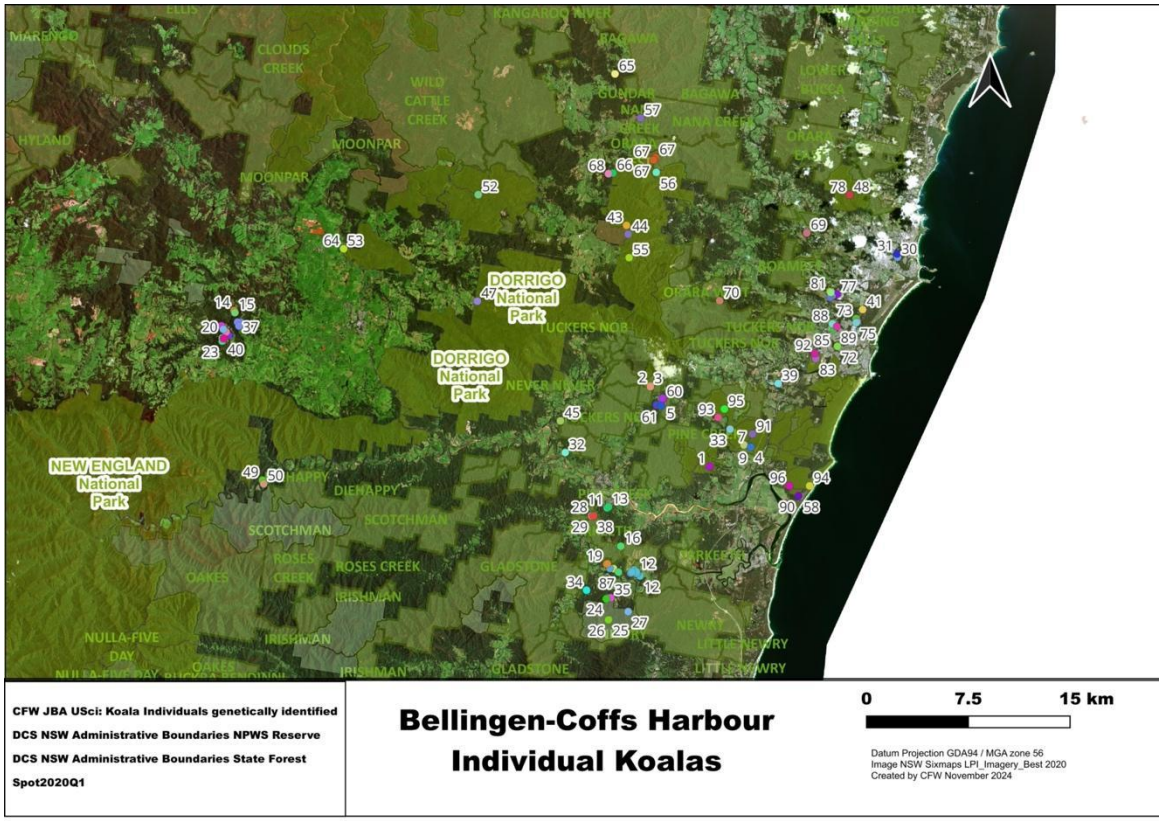
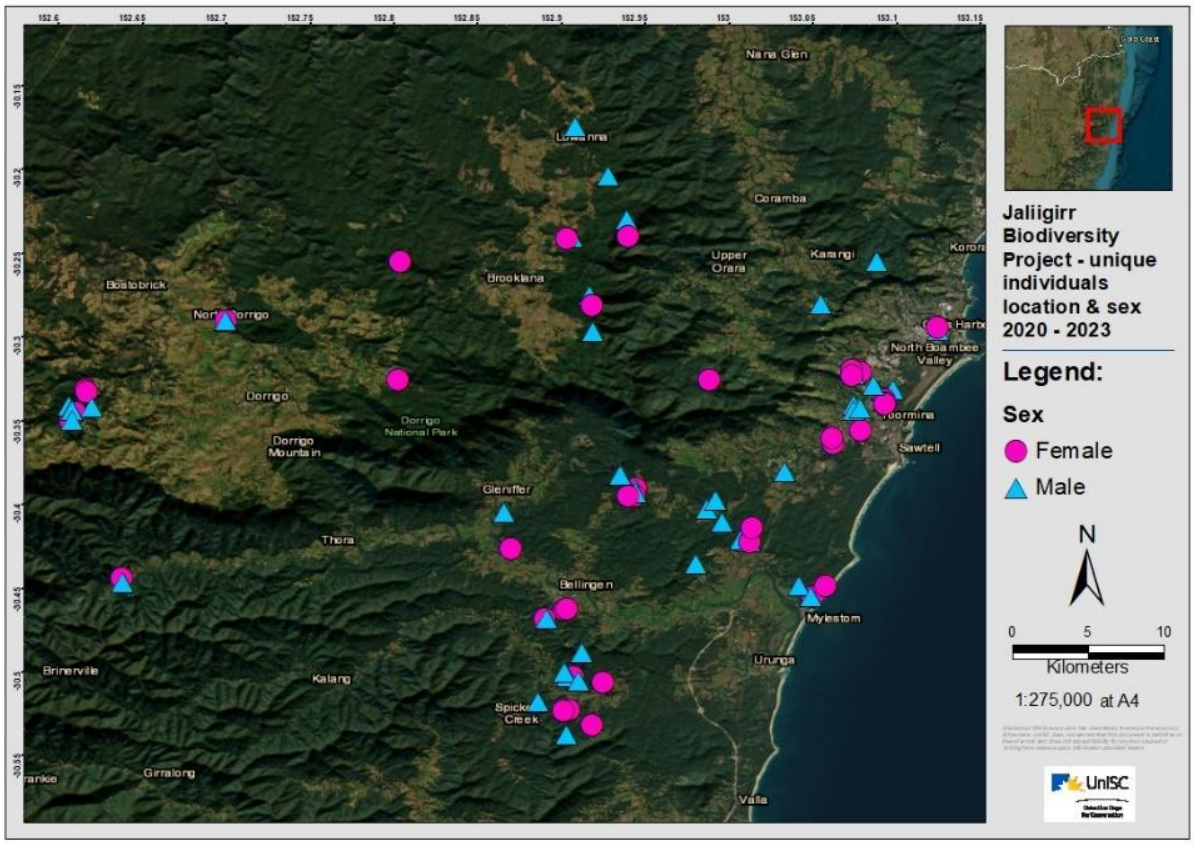
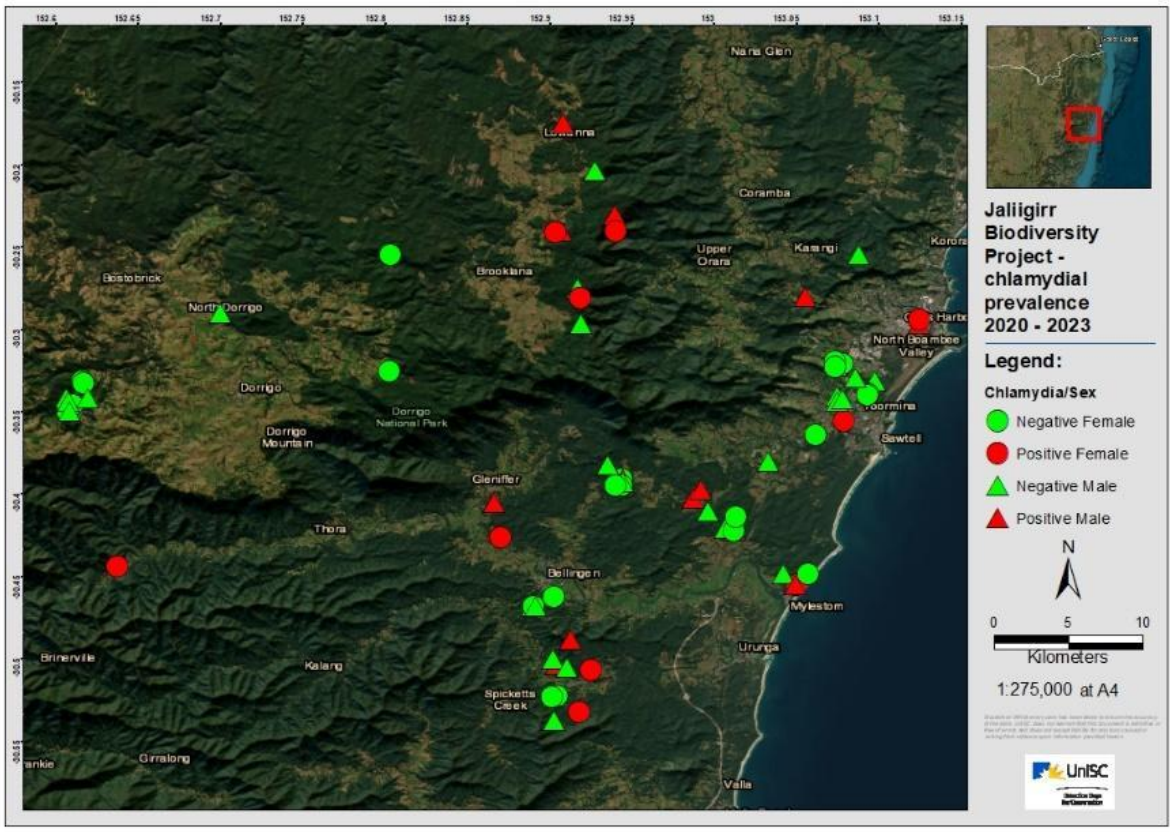


Figure 5 Distribution of male and female individual koalas (N=86) (Karayalage et al. 2024)



Of the 78 samples tested for *Chlamydia pecorum,* infection was detected in 27 individuals contributing to a population prevalence of 32.9 %. Of note, all 10 koalas surveyed in the Fernbrook group were *Chlamydia* free. (Figure 6).

Figure 6: Location and *Chlamydia* status of unique individuals by gender (Karayalage et al. 2024)



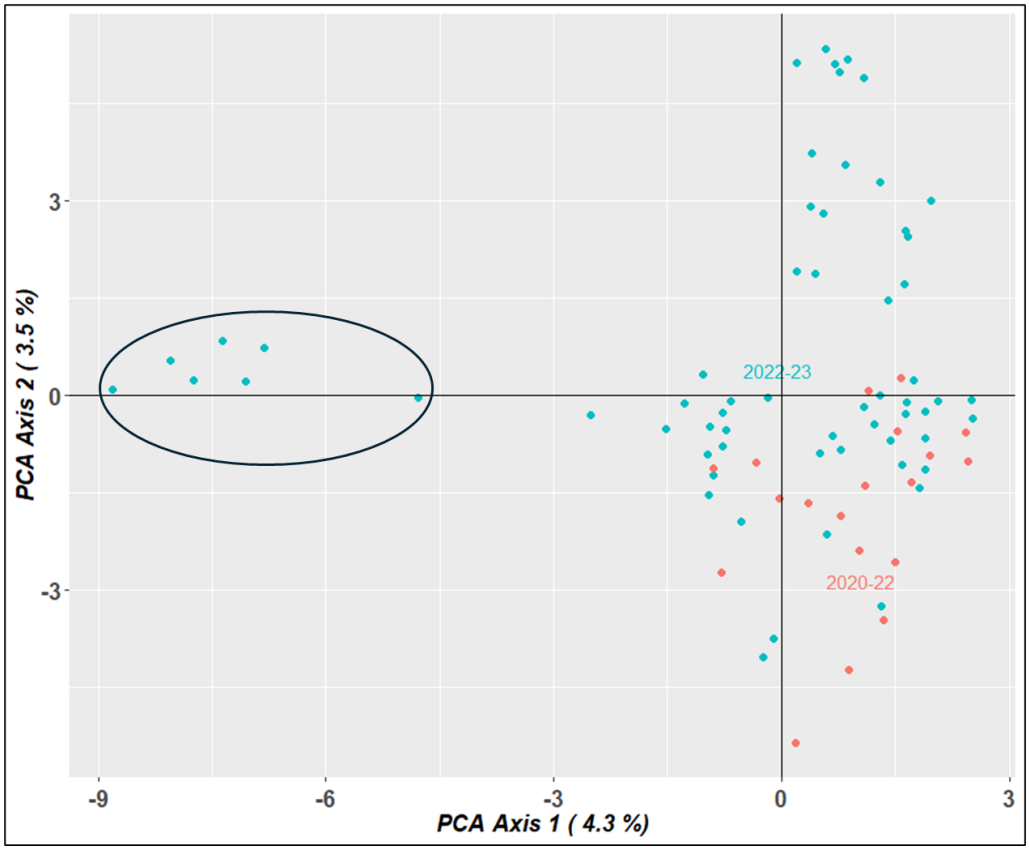
It is important to note that this does not necessarily reflect their chlamydial disease status. For instance, some koalas could have recovered from disease but were still carrying *Chlamydia* pathogen in their gastrointestinal tracts and others could be carrying the pathogen without any clinical signs. When *Chlamydia* infection does however progress into disease, it can cause infertility and overall increased morbidity and mortality. This can have a large negative impact on the population and its long-term persistence (Karayalage et al. 2024).

The 32.9% level for *chlamydia* infection in this population is an important assessment / index of disease status which makes it possible to compare this population's infection prevalence with other populations in NSW and to provide a baseline for future monitoring of the infection in the Bellingen-Coffs Harbour koala population.

Population structure and genetic diversity estimates were assessed based on 80 individuals and these koalas were assessed as:

- Panmictic Population (i.e. belonging to one population)
- Having a high level of genetic diversity and low inbreeding coefficient, indicating a genetically healthy population of koalas
- The Fernbrook group of koalas are part of the one population but showed a level of genetic differentiation which warrants further investigation.

Figure 7. Results of the principal component analysis (PCA) for the unique koalas in 2020–22 (red dots) and 2022–23 (blue dots) sample collections. Seven individuals from Fernbrook (JBAKG_20.0, JBAKG_21.0, JBAKG_23.1, JBAKG_26.0, JBAKG_27.0, JBAKG_31.0 and JBAKG_37.0) are shown inside the circle (Karayalage et al. 2024).



Genetic Relatedness

The genetic relatedness analysis assessed the inter-relatedness of individuals.

The two highest value groups showed:

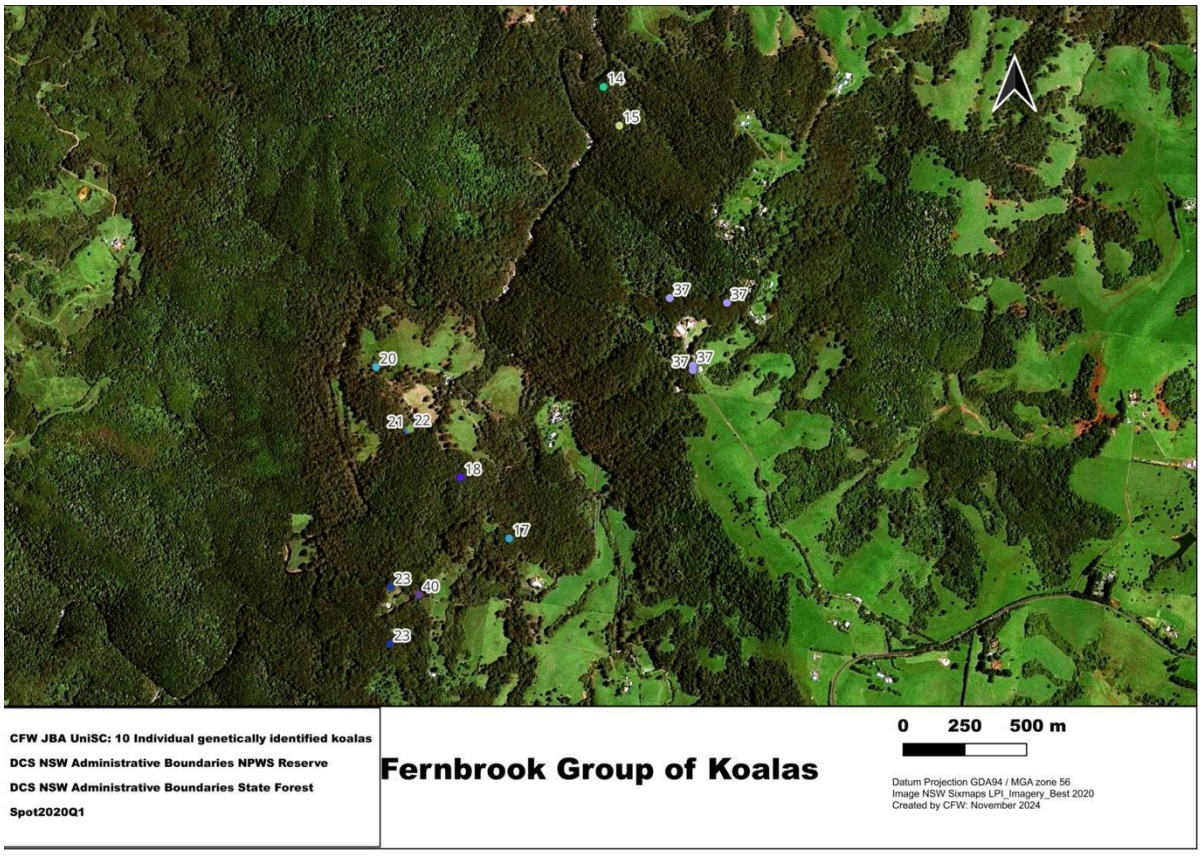
- 21 individual koalas with high relatedness values indicating parent-offspring or full-sibling relationships
- 36 individuals showed moderate relatedness indicating half-sibling, grandparent-grand-offspring or aunt/uncle/niece/nephew relationships
- The two levels of relatedness identified strong relationships between individuals within discrete areas including:
    - within the Fernbrook cluster of properties on the Dorrigo Plateau
    - the corridor between the coastal section of Bongil Bongil and Bindarri National Parks
    - the corridor from Bellingen-Tarkeeth along the Fernmount ridge
    - across highly developed sections of urban Coffs Harbour
    - between Junuy Juluum and Cascade National Parks on the Dorrigo Plateau

1. Fernbrook Group of Koalas (Dorrigo Plateau)

Ten individual koalas were identified in the Fernbrook cluster of properties (Map 7).
The Fernbrook group of koalas showed some differentiation of individuals from the rest of the population, though they were still considered to be part of the one panmictic population (Figure 7). The implications of this difference are not clear; however, they may reflect some potentially recent constraints to geneflow/koala movements in and out of the Fernbrook area. Further investigation of the level of differentiation of this population and surrounding areas would be of conservation interest, and it would be important to determine whether the group has become isolated or is connected to other yet unsampled koala locations.

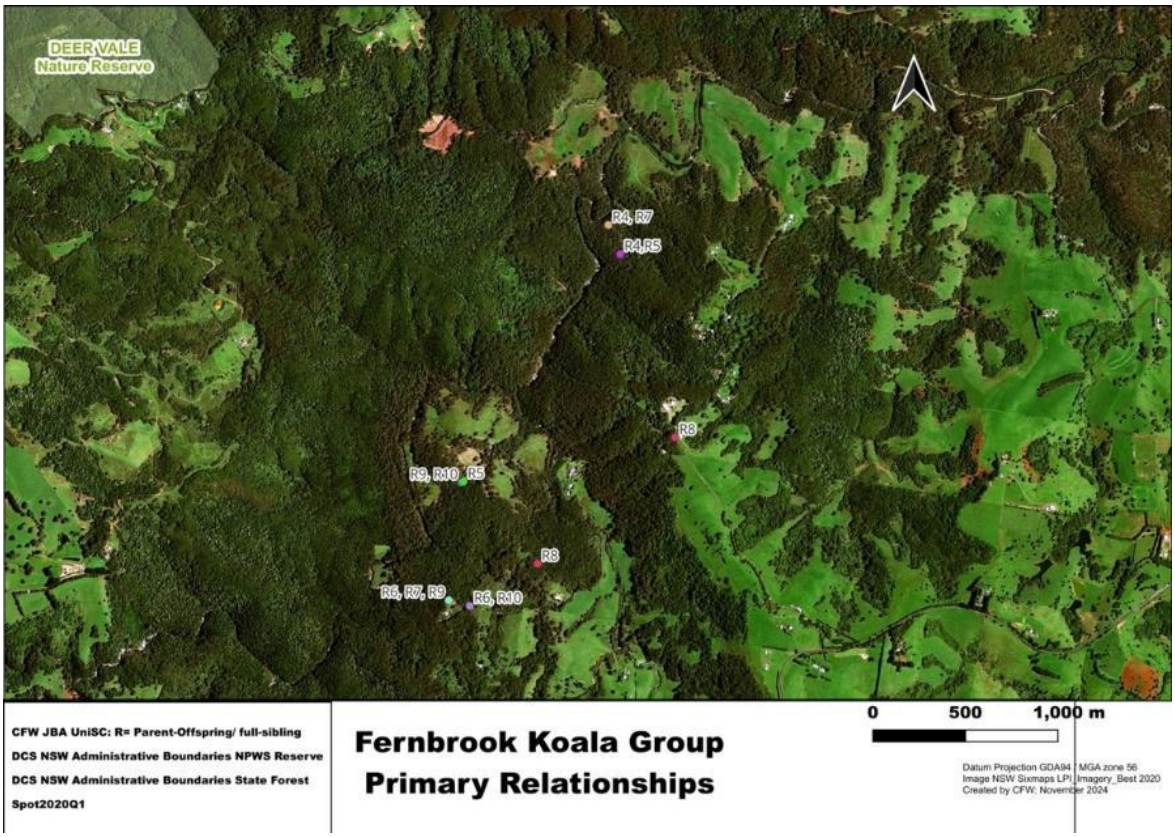
The *Chlamydia* analysis did not detect any chlamydial infection in individuals from the Fernbrook region (Karayalage et al. 2024). This is an important finding as discrete groups of chlamydia free koalas are uncommon. The Fernbrook group was a sample size of 10 individuals and so further sampling of other koalas in the area would be valuable to confirm the *Chlamydia* free status. If there is further confirmation of *Chlamydia* free status, then this would indicate a healthy group of koalas of high conservation value, and it would be important to consider appropriate steps to safeguard this group of koalas from exposure to the disease.

Map 7: Fernbrook Group of Koalas – 10 Individuals genetically identified



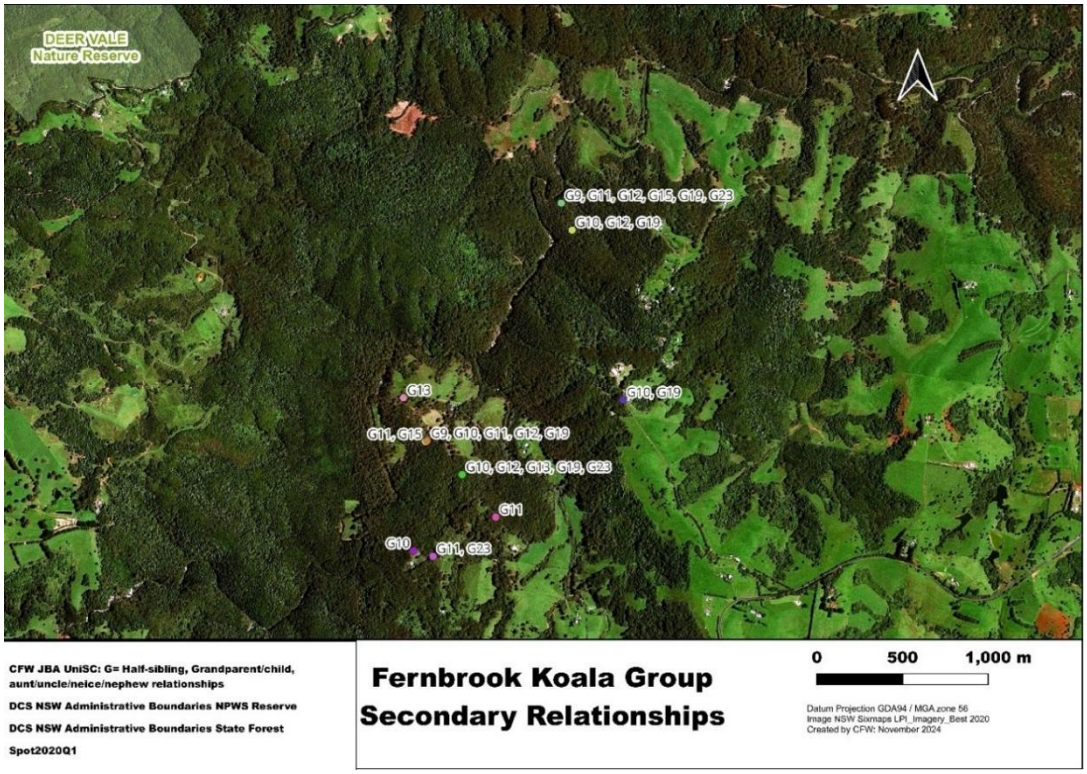
Of the 10 individuals genetically identified in Fernbrook group, 7 individuals showed close genetic relatedness (Map 8).

Map 8: Fernbrook Group of Koalas - Close genetic relationships



Map 9 shows the secondary relationships of half sibling, grandparent-grand offspring/aunt/uncle/nephew/niece relationships.

Map 9: Fernbrook Group of Koalas - Secondary genetic relationships

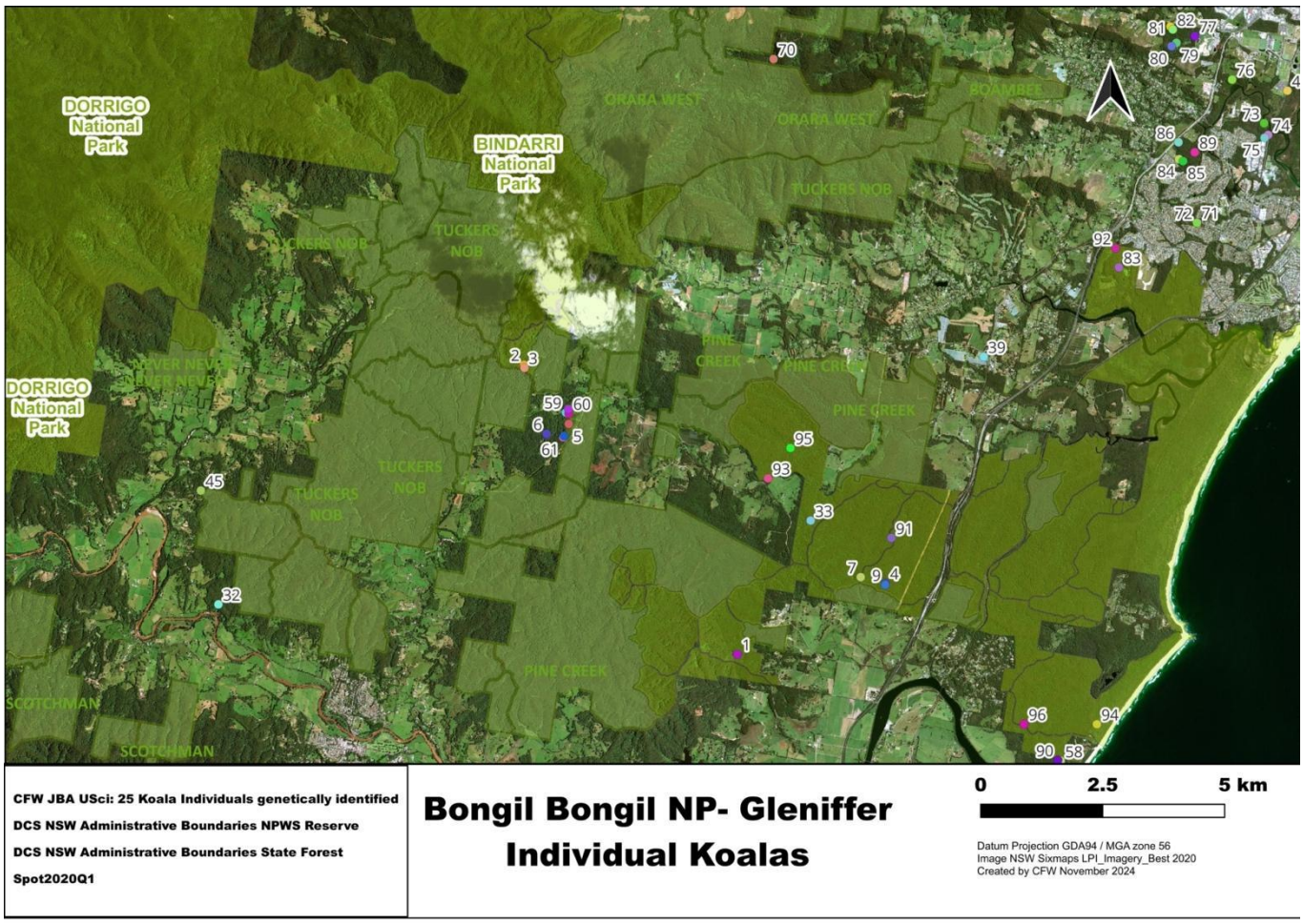


2. Bongil Bongil National Park to Gleniffer koala habitat corridor

A combination of National Park, State Forest and private properties form a vegetated east-west corridor to the north of the Bellinger River. Surveys were conducted in the two National Parks and on private properties (at Mylestom, Valery and Gleniffer).

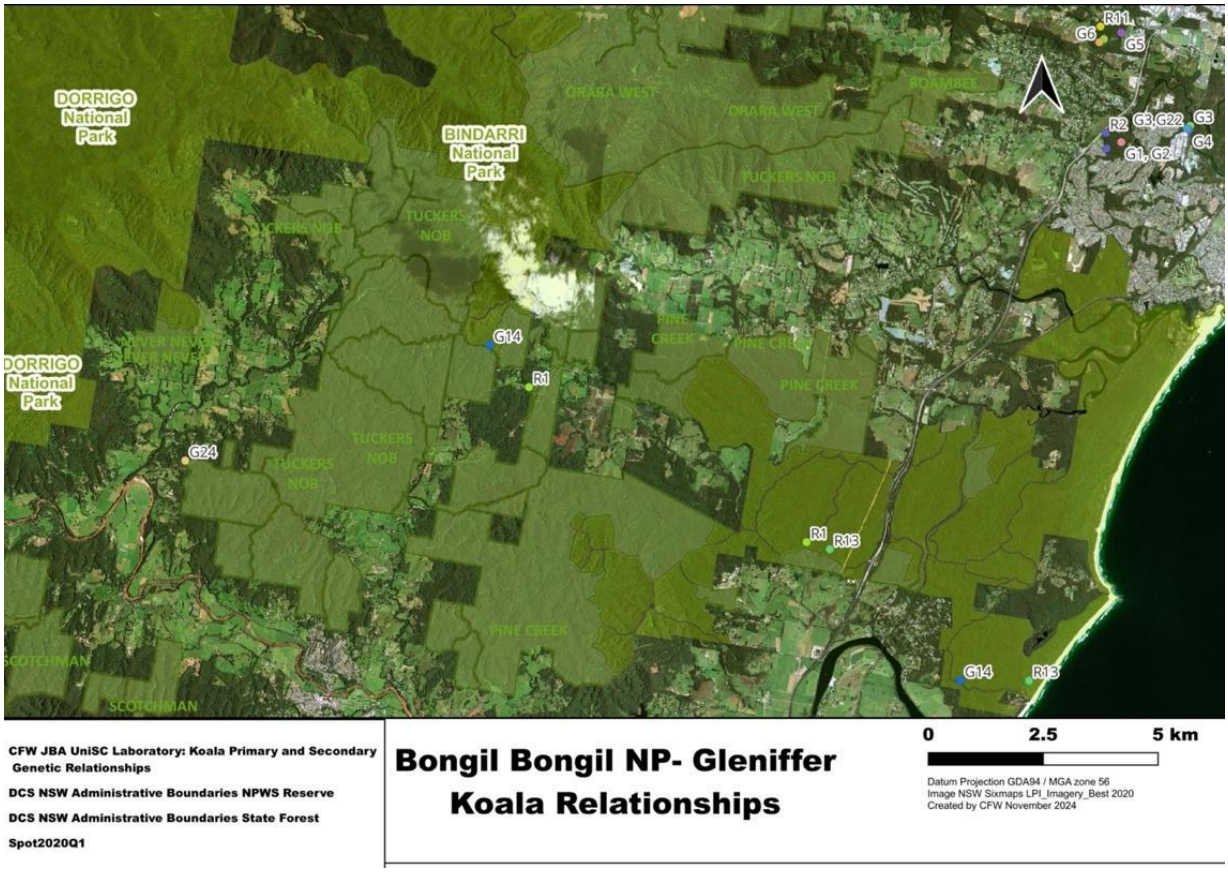
Twenty-four individual koalas were genetically identified within the corridor and seven individual koalas were recorded on just one private property situated between Pine Creek and Tuckers Nob State Forests and Bindarri NP (Map 10).

Map 10: Bongil Bongil – Gleniffer Corridor - 25 Individual koalas identified



Map 11 shows the primary and secondary genetic relationships between koalas from east of the Pacific Highway and west of the Pacific Highway and relationships from the eastern section of the corridor and the west.

Map 11: Bongil to Gleniffer corridor - Primary and Secondary genetic relationships



There were close genetic relationships identified within the Bongil Bongil NP – Gleniffer corridor including:

- R 13 – Full sibling or parent-offspring relationship between an individual at Tuckers Rock east of the Pacific Highway and Mailmans Track, west of the Highway (approximately 5kms separation)
- R1 – Full Sibling or parent-offspring relationship between an individual in Bongil Bongil NP and private property near Bindarri NP, approximately 7km separation
- G14- Half-sibling/grandparent-grandoffspring/ aunt/uncle/niece/nephew relationship between two individuals: one east of the Pacific Highway and the other in Bindarri NP, over 12 kms separation
- Three individual koalas were identified west of Tuckers Nob SF on private land. Two of these were male koalas at Roses Rd in Gleniffer which are related in secondary relationships and both using the same property and significant food tree.

These relationships demonstrate that the koalas are genetically interacting along the entire corridor. There are important genetic connections between east and west of the highway emphasising the importance of the overpass and underpasses of the Pacific Highway and the importance of having

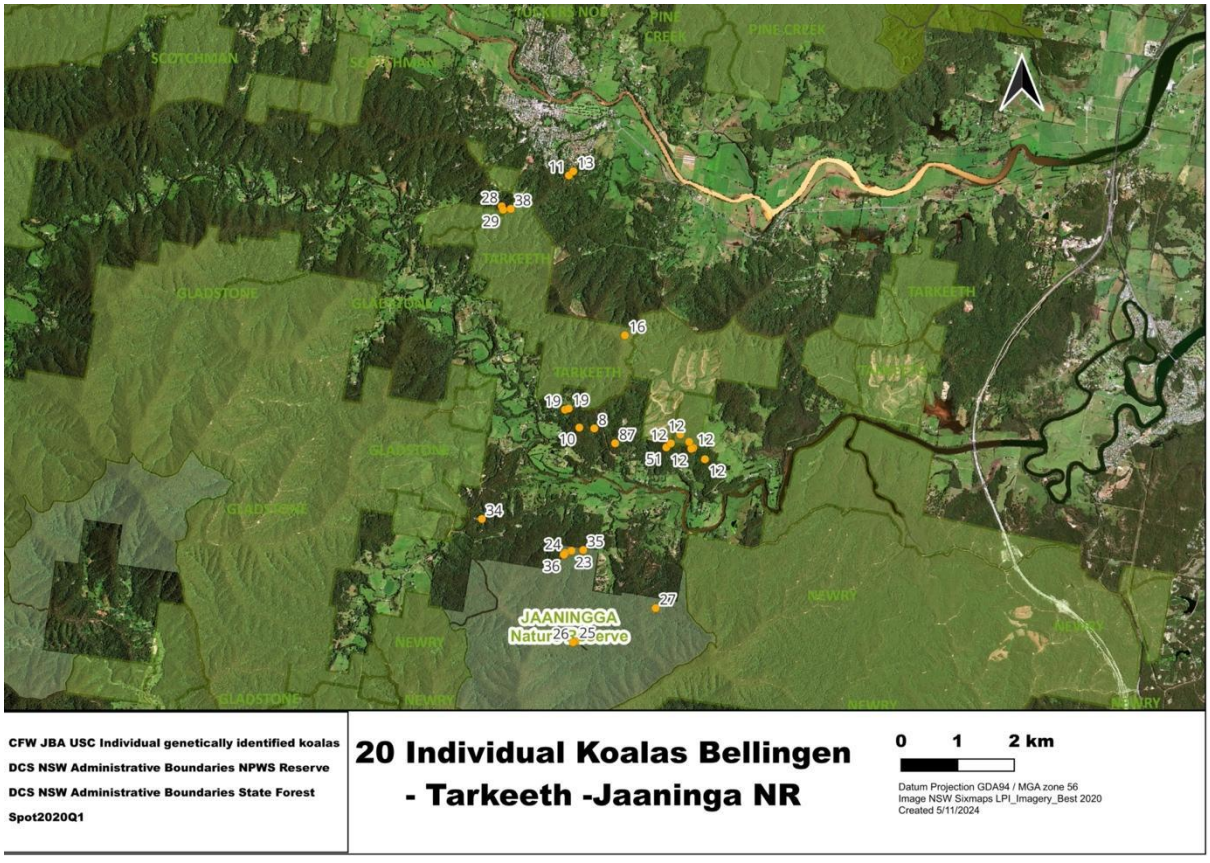
connected koala habitat along the corridor. It also highlights the significantly important contribution private lands are making to connecting and preserving koala habitat.

3. South Arm Road Tarkeeth to Bellingen and Jaaninga NR to Brierfield Habitat Corridors

Two vegetated corridors run east-west on the north and south side of the Kalang River in the Bellingen LGA. The northern corridor runs parallel to South Arm Road and along the Fernmount Ridge. The land tenure is public land Tarkeeth State Forest and private landholdings. Twelve individual koalas were genetically identified (Map 12).

The southern corridor includes public land managed by NPWS and State Forest and private landholdings. Eight individual koalas were identified between Jaaningga NR and private property on Martells Road Brierfield (Map 12).

Map 12: Lower Kalang River Corridors - 20 Individual koalas: 12 on the northern corridor and 8 on the southern corridor.

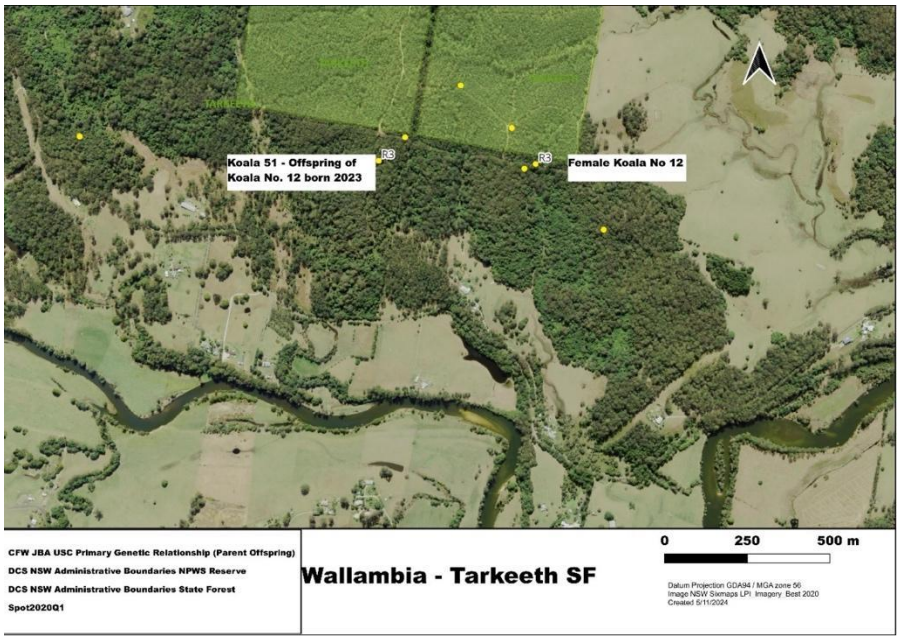


Map 13 shows a primary relationship R3 between two koalas, known to be parent-offspring utilising private properties and public land (Tarkeeth SF). No. 12 is a female koala resident in habitat on private property and adjacent to Tarkeeth SF. Her movements around her home range have been recorded by scat records over a three-year period. No 51 is her offspring, born in November 2023 and recorded within the same area of habitat.

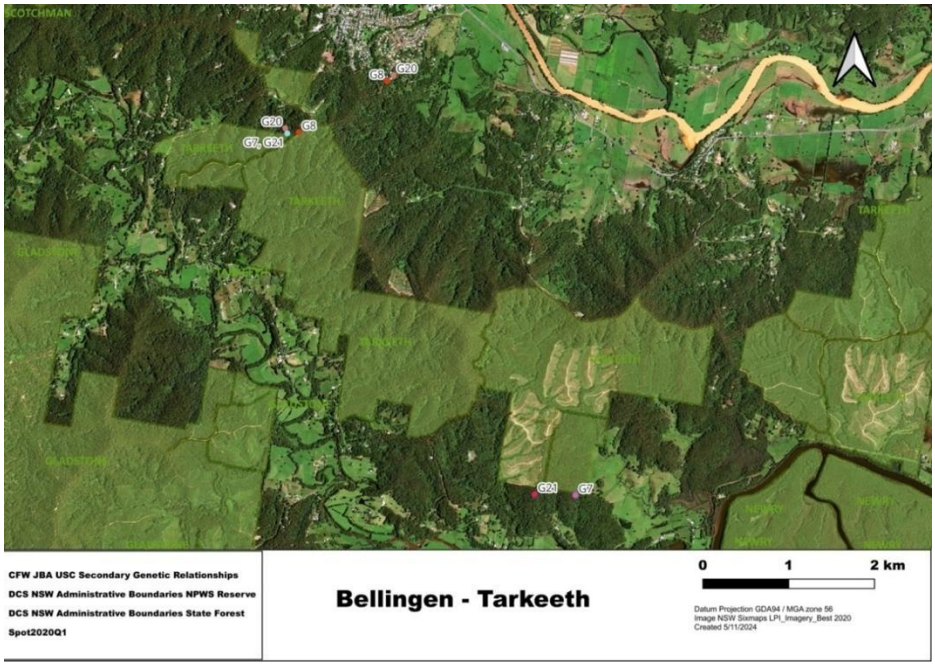
This pair were shown to have secondary relationships with koalas over 6km away in forest behind the Bellingen Waste Transfer Station. There were also two secondary relationships between koalas in the forest behind the Waste Transfer Station and urban Bellingen (Map 14).

These close genetic relationships confirm that the koalas are resident in habitat along the Fernmount range and this habitat is comprised of both private lands and State Forest. This genetic relationship emphasises the importance of the current connected corridor between the Tarkeeth State Forest (native regrowth and plantation) and the adjoining private lands.

Map 13: Wallambia to Tarkeeth SF- Primary Relationship

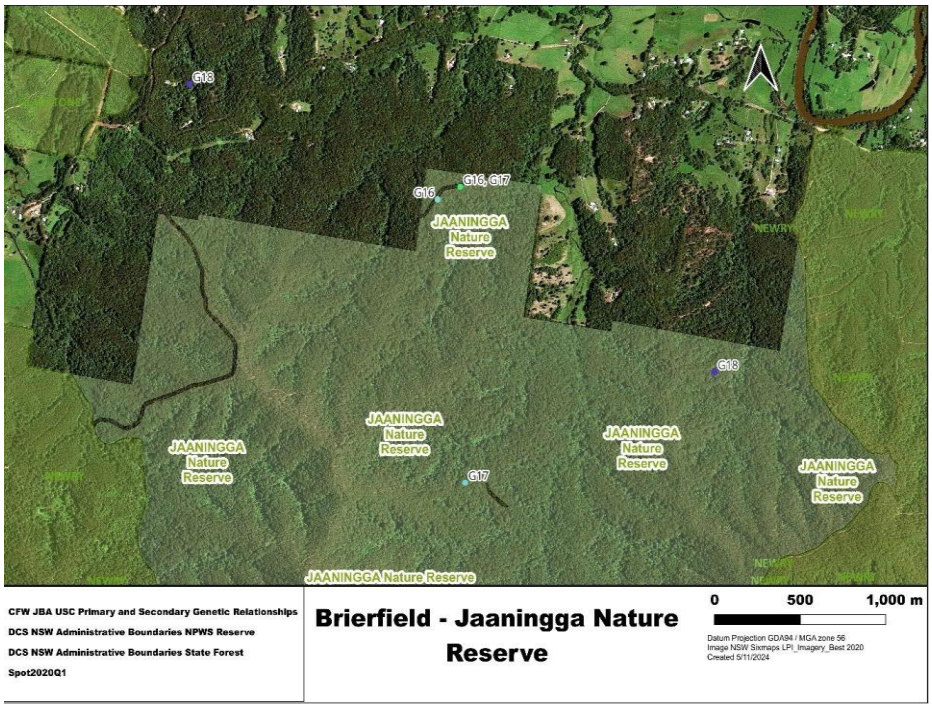


Map 14: Bellingen-Tarkeeth corridor - Secondary genetic relationships



Map 15 shows genetic linkages between individuals in Jaaningga NR and private land on Bowraville Road, approximately 3 kilometres away. These koalas are connected via a vegetated corridor of NPWS and private land tenure.

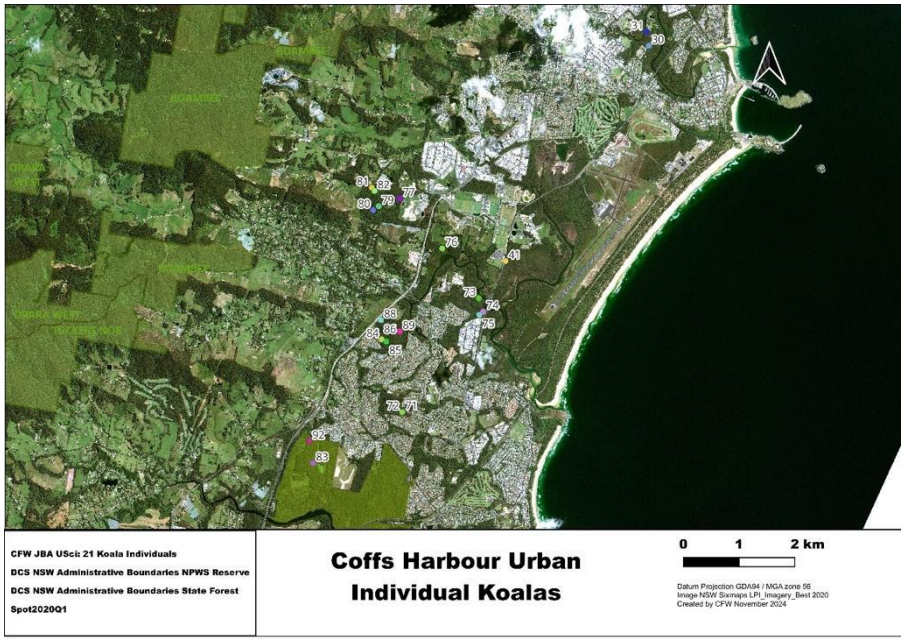
Map 15: Jaaningga Nature Reserve to Brierfield - primary and secondary relationships



4. City of Coffs Harbour

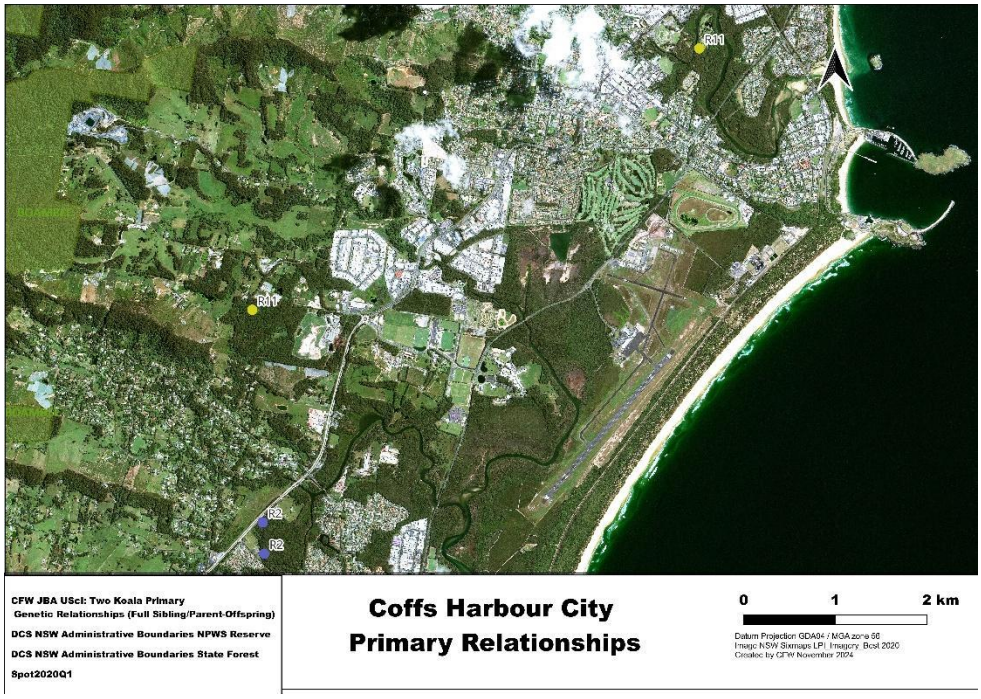
Twenty-one individual koalas were identified within the Coffs Harbour city and urban surrounds (Map 16).

Map 16: Coffs Harbour Urban – 21 Individual Koalas



There were some unexpected primary relationship linkages, for example, R11 between the Coffs Harbour Botanic Gardens and the Englands Road Waste Facility. These koalas had to navigate the city environment to interact between these locations (Map 17).

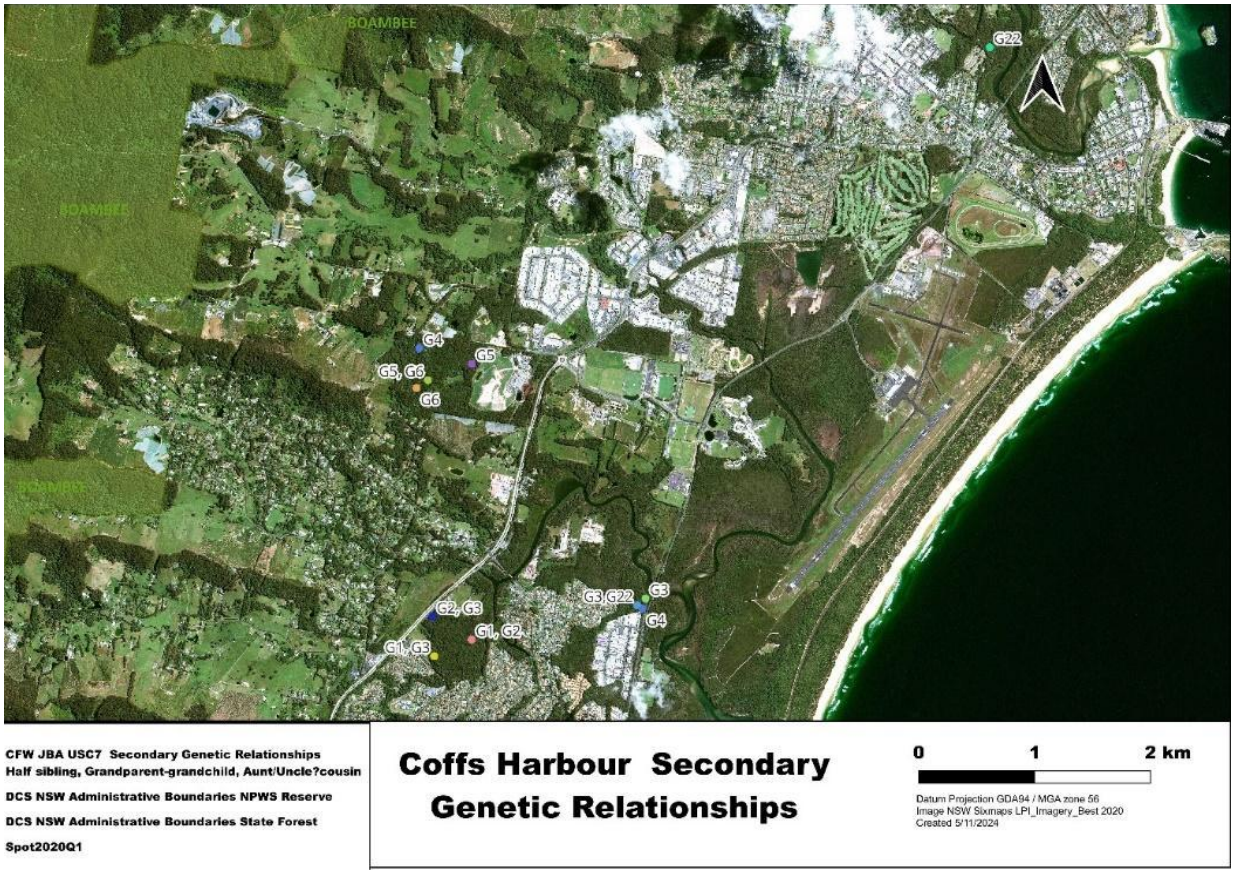
Map 17 Coffs Harbour - Primary Relationships



Map 18 shows secondary relationships again between a koala at the Coffs Harbour Botanic Gardens (G22) and council reserve on Boambee Creek, Hogbin Drive Sawtell, over 5kms away. Other interesting genetic relationships were between G4 at the Englands Rd Waste Facility west of the Pacific Highway and the council reserve at Boambee Creek (east of the Highway) and between the council reserve on Boambee Creek and the council reserve on Bruce King Drive Toormina (G3).

These relationships show that koalas are moving and interacting through the Coffs Harbour city and urban environments, highlighting the importance of council reserves and raising questions about how some of the connections between koalas are being navigated through heavily built-up areas.

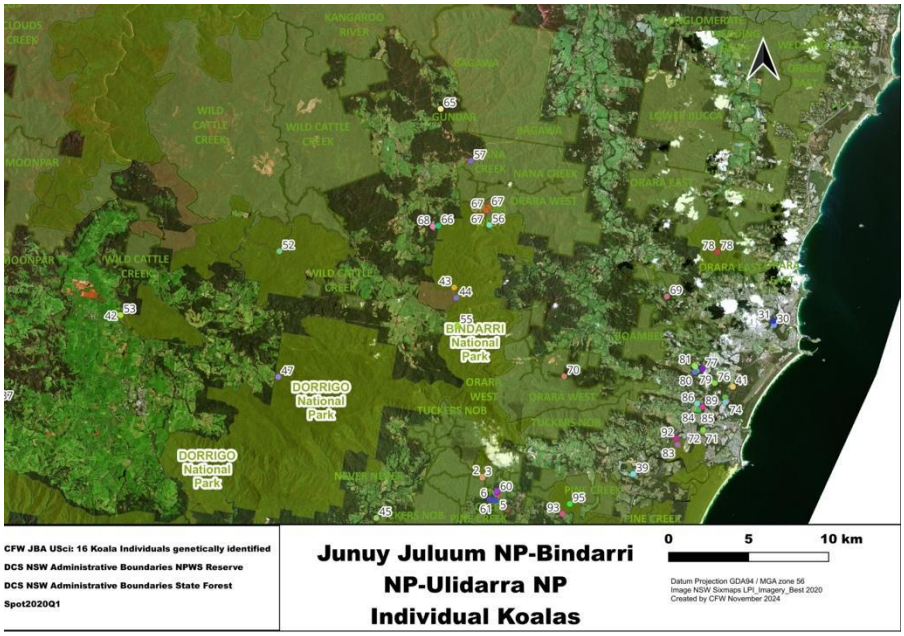
Map 18 Coffs Harbour secondary relationships



5. Coffs Harbour Hinterland – Dorrigo plateau koala habitat corridor

Koala scat samples collected on private lands and National Parks including Cascade, Junuy Juluum, Bindarri, Ulidarra and Dorrigo NPs to the west of Coffs Harbour and on the Dorrigo Plateau. Sixteen individual koalas were genetically identified 16 (Map 19).

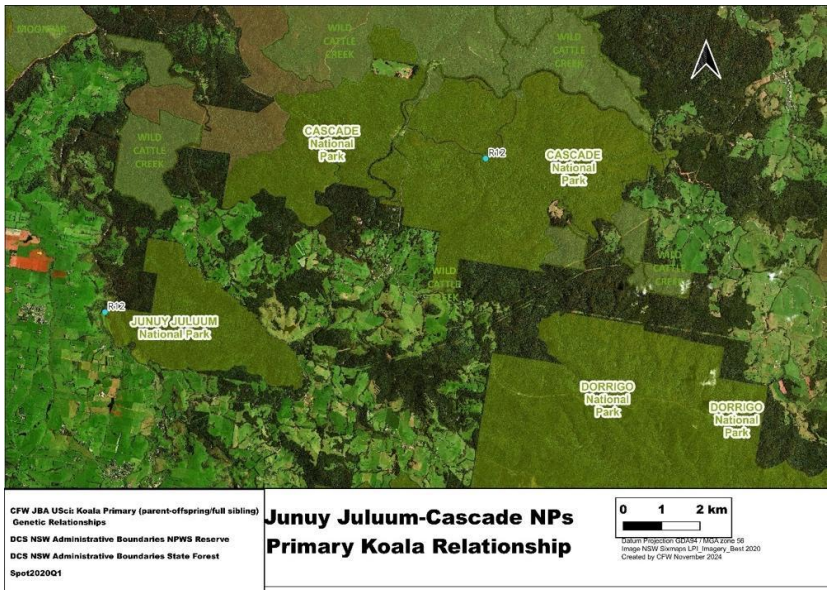
Map 19 Coffs Hinterland to Dorrigo Plateau – 16 Individual koalas



Three individuals were identified at Junuy Jullum and one individual in Cascade NP.

Of particular interest, was a primary relationship between two female koalas: one at Cascade NP and the other at Junuy Juluum NP. This pair is separated by approximately 10kms and it would be of interest to determine the corridor of movement between these two locations (Map 20).

Map 20: Junuy Juluum- Cascade NPs - primary koala relationships



Discussion

The results of the koala scat surveys and associated genetic analysis demonstrate that koalas are actively utilising habitat on public and private lands across the Bellingen-Coffs Harbour LGAs. A high proportion (83%) of the sites surveyed had koala scat present and 92 individual koalas were identified within the project area. The koalas identified were found to belong to one population.

The genetic analysis has highlighted the high genetic diversity and low inbreeding coefficient of the population. Karayalage et al. 2024 noted that measures of genetic diversity and inbreeding come with an associated time-lag and often, signs of decline in these measures only occur after the population has already experienced a major impact. The results from this project will provide an important baseline, but it will be important to monitor the population over time to assess any changes in the genetic health.

A group of koalas at Fernbrook showed some genetic differentiation to the rest of the population. Further investigation is recommended to establish whether these koalas are an isolated group or are connected to koalas in areas not yet surveyed. Because the number of individuals is small and they are closely related, it will be important to determine if they are connected to other koalas in the area.

The Bellingen-Coffs Harbour population was found to have a slight male bias and a relatively low *Chlamydia pecorum* status at 32.9%. While this currently presents a relatively healthy population, the situation requires monitoring to ensure that the male bias and increases in chlamydial infection do not affect the long-term reproductive viability of the population. The Fernbrook group appears to be *Chlamydia* free, and this is significant as discrete *Chlamydia* free groups of koalas are uncommon. It will be important to investigate further to determine if there are more koalas within this cluster and adjacent areas that are also *Chlamydia* free as this will have implications for future management of the group.

Important genetic linkages between individual koalas were identified across four habitat corridors/connections. A number of these relationships were between individuals at some distance (5-13kms) highlighting the importance of connected habitat for these koalas to interact within. The linkages between Tarkeeth and Bellingen, Bongil Bongil NP to Gleniffer, highlighted the important role of connected multi-tenure public and private lands in supporting koala habitat.

The east-west movement of koalas across the Pacific Highway in Bongil Bongil NP highlight the necessity to maintain the koala overpass and underpasses along the highway in good condition, to maintain the connection between the east and west groups of koalas.

The ability of koalas to navigate the heavily built sections of Coffs Harbour to interact with koalas over distances of more than 5kms, highlighted the importance of the council vegetated reserves, fencing of roads in high use areas to protect koalas from vehicle collisions, and making provision for the koalas to move safely through this landscape.

Conclusion

The Bellingen-Coffs Harbour koala population is of high genetic diversity and low *Chlamydia* disease status at this point of time. Genetic diversity is important to maintain koala health and resilience to disease and other threats. There is a time-lag in genetic health showing the impacts of major stresses including loss of habitat and disease, so it will be important to re-sample this population to ensure that the genetic and disease health of the population is being maintained. The population showed a slight male bias, where generally, the sex ratio of a healthy population is expected to be close to 1:1. The male bias in this population should be monitored in the long-term as an over-bias to males may negatively affect population growth (Karayalage et al. 2024).

The location of a group of *Chlamydia pecorum* free koalas in the Fernbrook cluster is interesting and could highlight a healthy group of koalas of high conservation value. This group is currently known from a small number of individuals, who are closely related, and if truly isolated, this could impact their genetic diversity in the long-term. It will be important to determine if there are other koala individuals in the group that were not sampled and determine their chlamydial status; and whether the group are linked to koalas in areas that have not been surveyed to date. These questions need to be resolved to be able to develop a management strategy to ensure the group are protected from introduction of the chlamydial infection and maintain their genetic diversity (Karayalage et al. 2024).

The survey results and subsequent analysis have confirmed the importance of habitat connectivity in supporting a genetically diverse and healthy population. The existing habitat corridors are comprised of public tenure (National Parks, State Forest and Council Reserves) and private land.

Several individual koalas separated by 5-13kms were found to have close genetic relationships, showing that individuals are using these habitat corridors and crossing land tenures to interact. Maintaining these habitat connections will be important in ensuring the gene flow and a robust population genetic structure.

This project has provided quality data to support decision making and to inform the protection and habitat management of this important koala population. Several areas of enquiry became apparent from the project and these areas would benefit from further survey work and genetic analysis. These include further investigation of the Fernbrook population and surrounding areas, and additional surveys and scat collections within identified gap areas.

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