Koala Genetics Study Project

Inland Rail Program

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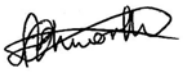
27 June 2024

Koala Genetics Study Project

Inland Rail Program



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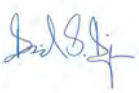

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Acronyms and Abbreviations

Name	Description
AEP	Annual Exceedance Probability
ALA	Atlas of Living Australia
ARTC	Australian Rail Track Corporation
N2N	Narromine to Narrabri
N2NS	Narrabri to North Star
NS2B	North Star to Border
B2G	Border to Gowrie
ELA	Eco Logical Australia
G2H	Gowrie to Helidon
H2C	Helidon to Calvert
C2K	Calvert to Kagaru
K2AB	Kagaru to Acacia Ridge/Bromelton
DAWE	Department of Agriculture, Water and Environment
DCCEEW	Department of Climate Change, Energy, the Environment and Water (formerly Department of Agriculture, Water and Environment)
DESI	Department of Environment, Science and Innovation
DOEMP	Draft Outline Environmental Management Plan
DTMR	Department of Transport and Main Roads
EIS	Environmental Impact Statement
EMP	Environmental Management Plan
FFJV	Future Freight Joint Venture
GHD	GHD Group Pty Ltd
IRP	Inland Rail Program
KMP	Koala Management Plan
MNES	Matters of National Significance
OCG	Office of the Coordinator-General
RFI	Request for Information
SAT	Spot Assessment Technique
SEQ	Southeast Queensland
ul	micro litre
UniSC	University of Sunshine Coast
WSP	Williams Sale Partnership

EXECUTIVE SUMMARY

Environmental Resources Management Australia Pty Ltd (ERM) was commissioned by Australian Rail Track Corporation (ARTC) to conduct studies into koala genetics, habitat, occurrence, and utilisation across sections of the Inland Rail Program (IRP) from Narromine to Acacia Ridge/Bromelton (note: Kagaru to Acacia Ridge/Bromelton section (K2ARB) is no longer a part of the IRP) (the project area). The study was conducted along these sections of the IRP as per the scope outlined by ARTC. A collaboration has been undertaken with the University of the Sunshine Coast (UniSC) Detection Dogs for Conservation (DDC) Team to obtain genetic information from koalas across the project area. This report presents the findings and recommendations of this collaborative study project to support koala management throughout the design, construction, and operation of the IRP.

The project area for this study included properties with existing land access agreements (LAA) in place with ARTC and other publicly accessible land (road reserves, state forests and travelling stock reserves) within the IRP alignment and is situated across central-north New South Wales (NSW), from the township of Narromine north to the border, and south-east Queensland, from the border north to Toowoomba and then east to the Brisbane suburbs of Acacia Ridge and Bromelton. The final section of the rail from Kagaru to Acacia Ridge and Bromelton (K2ARB) has since been removed from the IRP, however, remains included in this study in order to provide a more robust analysis of koala genetics. The IRP involves the construction of 500km of new tracks in northern New South Wales and south-east Queensland and the upgrade of existing tracks along an additional 1600km throughout the existing inter-state line.

The IRP was separated into 13 sections at the time of the study (now reduced to 12 in response to Inland Rail review, 2022), being delivered through the planning and approvals stages as distinct projects. As per the scope of works in the study proposal, the genetics study focuses on eight (8) sections between the town of Narromine and Brisbane and here after these eight sections will be referred to as the IRP for the purpose of this report. Land use histories vary considerably across the eight sections of the IRP with extensive clearing for agriculture present, particularly west of the Great Dividing Range, while large areas of state forests also remain. The IRP also extends through heavily urbanised areas including the regional cities and townships of Toowoomba, Narrabri, Moree and Narromine. ARTC has identified the need to provide appropriate identification, assessment, and management of key environmental issues associated with the IRP. One such environmental issue is the presence of koalas and key areas of habitat and connectivity within the IRP and the need for innovative approaches for baseline assessment and ongoing monitoring. This forms the rationale for undertaking this koala genetic study, focusing on understanding koala occurrence and habitats, and important connectivity across the landscape, to assist in identifying key opportunities for koala management at the local and landscape scale.

Using desktop reviews and learnings from previous surveys throughout the IRP, key threats have been identified to koala populations within the IRP. These include:

- Habitat loss and degradation (including habitat fragmentation, edge effects and barrier effects);
- Injury or mortality from vehicle strike;
- Injury or mortality from train strike;
- Injury or mortality from predators, including domestic dogs, foxes and dingoes;
- Prevalence of disease (as well as increased susceptibility); and
- Extreme weather events (drought, flood, heat stress).

To more effectively identify important areas of koala habitat within the IRP, hotspots of potential koala activity were established using a combination of existing koala presence data, sourced from the Atlas of Living Australia, community groups and previous IRP surveys, and mapped areas of known koala habitat. Field verification of koala hotspots was undertaken by a range of specialists for ARTC.

Koala detection dogs provide reliable, efficient, and accurate methods for surveying large areas of the alignment. Survey locations for this study were informed by pre-fieldwork ‘hotspots’ of known koala

activity to maximise detection of fresh koala scats.
A total of 120 detection dog surveys were undertaken throughout the IRP at 118 locations. Of these, fresh scats and individual koalas were found at forty-one (41) sites with detections made in northern NSW and southeast Queensland. Across these sites, seventy-three (73) fresh koala scats were detected and thirty-six (36) unique individuals identified. Samples were separated into two distinct groups: coastal (those samples located east of the Great Dividing Range - Toowoomba) and inland (those samples located west of the Great Dividing Range). Twenty samples were categorised as coastal while the remaining 13 as inland.

The most common habitat type that was sampled in the study area consisted of poor to moderate quality eucalypt open forest and woodland dominated by *Eucalyptus populnea, E. crebra and E. camaldulensis* with a mixture of other notable canopy species identified, but with less consistency across sites. Other prominent habitat types were dominated by *Allocasuarina luehmannii, Casuarina cunninghami, Casuarina cristata* and *Callitris glaucophylla.* Based on a review of koala habitat assessment criteria and methods undertaken by the Australian National University (2021), many of the dominant species identified throughout the IRP are also identified as locally important koala trees within the koala management bioregions relevant to the various stages of the IRP. Important ancillary habitat trees were also present. The type of habitats that surveys were completed in were consistent with DCCEEW (previously known as DAWE) classifications of koala habitat (forests or woodlands, higher proportion of feed trees, remnant or non-remnant vegetation, and roadside or railway vegetation) (DAWE, 2022). In low koala density areas, obtaining adequate samples for analysis can be challenging. For this study, where access was limited to those properties where land access agreements were in place , comparisons have been made with other genetic data from other studies across Queensland and New South Wales, using data held in UniSC databases. Some samples were pooled with IRP samples to form three groups along the Inland Rail alignment, while other groups were included to help contextualise the population status along the IRP alignment.

Overall, the three groups from which samples were collected in this study, compared to other groups in the larger landscape had:

- High levels of heterozygosity: Higher levels of heterozygosity equate to larger genetic diversity within a population(s), which is positive as genetic diversity is linked to both individual health and at population level, evolutionary potential; with Group 3 (B2G + G2H) higher than the other groups;
- Low levels of inbreeding and of relatedness between individuals: this suggests gene flow is currently occurring within the groups and the potential for negative impacts due to excessive inbreeding is minimal;
- Relatively small population size, with Group 3 (B2G + G2H) having a larger population size relative to other groups. This results in overall lower densities of koalas across the landscape;
- No concerns regarding sex ratio, but high chlamydia prevalence, except Group 3 which is medium prevalence (however the sample size remain very low). The ratio of males to females is not of concern for genetic health although chlamydia prevalence is high. Caution should be taken when interpreting these findings as the sample size is small; and
- Diet analysis results indicate that the diet of koalas varies between individuals and varies alongside geographic location. With a key conclusion being, that in some cases koalas were eating species that they were not recorded as using for daytime roosting indicating that factors other than food choice influence the selection of trees to roost in.

There appears to be reasonable connectedness of koala populations within the landscape, and so it is imperative that ongoing design and management measures support the ability for koalas to traverse the landscape through the maintenance of habitat connectivity. Although scat detections throughout the IRP were made across a linear geographic area with access lead by ARTC, further investigation of koala genetics at a broader landscape scale will be beneficial for overcoming these limitations. This approach will also enable a greater understanding of koala genetics at a broader scale and inform effective koala management and impact mitigation measures within the IRP.

1. INTRODUCTION AND PURPOSE

1.1 Background

Environmental Resources Management Australia Pty Ltd (ERM) has been engaged by Australian Rail Track Corporation (ARTC) to conduct a study into koala occurrence and genetics for the Inland Rail Program (IRP) to better manage this species during delivery of the IRP. In doing so, a collaboration has been undertaken with the University of the Sunshine Coast (UniSC) Detection Dogs for Conservation (DDC) Team to obtain genetic information from these koala populations across the IRP.

This Report provides a description of survey effort and discussion of results from the field survey effort, including the results of the genetic analysis of sampled scats and a summary of habitats sampled. The results of the field surveys have been assessed to identify areas of koala hotspots and locations where the IRP traverses landscapes where connectivity for koala should be focused to maintain habitat connectivity for the species. It is noted that these results were initially refined with pre-fieldwork Koala hotspots, using Queensland and NSW state koala habitat mapping and historic koala presence data from ALA, BioNet and WildNet and previous surveys for the IRP.

1.2 Objectives

This study aims to provide an understanding of, and contribute new knowledge to, the current status of inland koala populations. Doing so will provide valuable contributions to scientific knowledge while also aligning with the objectives of state and federal koala recovery plans to identify priority areas for threat reduction, opportunities for collaborative studying and identifying priority areas of koala habitat for protection and restoration. Following previous field assessments throughout the IRP, this study aims to extend what is currently known about koalas in the project area and results will contribute to the design and application of koala management strategies that are tailored to the local regions of the IRP. To achieve this, two primary objectives have been identified to guide study efforts:

- Provide an assessment of koala genetics for individuals belonging to the *combined QLD and NSW populations* present along the IRP alignment, identifying genetic diversity, gene flow and population structure along the alignment. A diet analysis is included as part of this assessment; and
- Identify health characteristics such as the presence of disease, particularly Chlamydia (*Chlamydia pecorum*), in the koalas inhabiting the IRP alignment. Although the prevalence of koala retrovirus is also an important population characteristic that informs conservation management, sampling for koala retrovirus was deemed to be beyond the scope of this genetics research as sampling fresh scat material for koala retrovirus was not thought to have provided robust and meaningful results that could positively inform koala management within the IRP.

1.3 Ecology and Existing Knowledge of the Koala

The koala, *Phascolarctos cinereus*, is a tree dwelling marsupial and is known as one of Australia's most iconic and recognisable animals. Identifiable by their stocky bodies, residual tails, and muscular limbs with sharp climbing claw, koalas will typically weigh between 4 and 15 kilograms, with males weighing up to 50% more than females (Nowak, 2005). Body weight in known to fluctuate throughout their range, with individuals in more southern extents typically weighing up to twice that of those in the north (DCCEEW 2022).

Koalas are distributed across eastern and southern mainland Australia, particularly in forests on more fertile soils or with easily accessible water sources. Predominantly solitary, koalas will maintain home ranges of highly variable sizes depending on the quality of habitat available. Known estimates range from 135 ha (Ellis et al. 2002), to as little as 1 ha (Davies, et al., 2013). In the agricultural landscapes of SEQ, home ranges are known to vary between 5.3 ha and 91.4 ha (Davies, et al., 2013). Although generally considered sedentary, males are known to disperse over longer distances than females (DES 2022).

Koalas occupy a range of forest and woodland habitats however their presence is dependent on the availability of appropriate food trees. Koalas possess a highly specialised diet with only a small number of species proving appropriate food resources. These trees belong almost exclusively to the genus *Eucalyptus*, however some *Corymbia, Angophora* and *Lophostemon* species are also known (Youngentob, Marsh, & Skewes, 2021). While not important as food resources, ancillary tree species provide important structural habitat features that are used for thermoregulation, resting, movement and protection (Youngentob, Marsh, & Skewes, 2021). Due to the low caloric content of their diet, koala's will typically rest up to 22 hours a day (Johnson 2018), with the majority of activity being crepuscular or nocturnal. The toxicity found in eucalypt leaves is not palatable to most herbivores and so koalas maintain little to no competition for food resources (Johnson 2018).

Koalas are capable of breeding annually when conditions are favourable but is heavily influenced by seasonality, population density, food quality and availability, and climate. Reproduction will fail or not occur when conditions are unsuitable or when individuals exhibit poor physical condition (DCCEEW 2022). Like most marsupials, gestation is relatively short in koalas at approximately 30 – 36 days. Joeys are dependent on their mothers for the first 12 months of life and will remain with them until sexual maturity at 2 years old. The life expectancy for a koala is between 13 and 18 years although males may have a slightly lower survival rate than females (DCCEEW 2022).

1.3.1 Key Threatening Processes

The Southeast Queensland bioregion supports some of the largest populations of koalas in the state and habitat identified as important koala habitat extends over 6 million hectares (ha) (Fowler, Houlden, Hoeben, & Timms, 2001). On a similar note, the Northern Tablelands Koala Management Area (KMA 4) and NSW North-Coast Koala Management Area (KMA 1) are additionally important areas for koala populations. These areas pertain small koala populations that are fragmented throughout, however still provide important habitat for the species. The importance of habitat is acknowledged by the Southeast Queensland Koala Conservation Strategy 2020-2025 (DES 2020), which outlined the top two actions as Habitat Protection and Habitat Restoration, followed by threat management, improved mapping, monitoring, studying and reporting, community engagement, and partnerships and strategic co-ordination. The Conservation Advice for the koala combined populations of Queensland, New South Wales and the Australian Capital Territory (12 February 2022), references a study by Adams-Hosking et al. in 2016 reflecting that the population in the Southeast Queensland region to be 15,821. This study provides an estimation of a 53% decline over three preceding generations (15-21 years).

A number of key threats to koala populations within the proposed IRP are present and have the potential to be exacerbated by its development and operation. These are:

- Habitat loss and degradation (including habitat fragmentation, edge effects and barrier effects);
- Injury or mortality from vehicle strike;
- Injury or mortality from train strike;
- Injury or mortality from predators, including domestic dogs, foxes, and dingoes;
- Prevalence of disease (as well as increased susceptibility); and
- Extreme weather events (drought, flood, heat stress).

Stressors to the environment, including heatwaves, droughts, bushfires, and floods are known to cause high mortality in koalas, as well as this, post extreme event recovery can be impaired by other threatening processes (DCCEEW 2022). The 2019-2020 "Black Summer" bushfires effected koala populations from south-east Queensland down to eastern Victoria. Literature estimates that the catastrophic event had immense impacts to koala populations along the east coast of Australia (Dunstan et al., 2021, Cristescu et al., 2021, Phillips et al., 2021). These bushfire events were part of the reasoning behind the Koala being reclassified as Endangered under the EPBC act.

Although the black summer fires had devastating effects to koala populations, recent studies have shown in some areas within three months of the fire event koalas are able to live wholly within a burnt habitat, being able to disperse through a previously burnt area that was severely impacted from fire (Matthews et al., 2007, M. Lane and K. Marsh). This information was recorded from GPS trackers and observations of fresh scat in burnt habitat. Overall, scientific literature suggest that scale and severity of fire has a large influence of the effects to both koala individuals / populations and their respective habitat, however, even high severity fire does not necessarily render a landscape as unusable habitat to a koala (Law et al., 2022b).

Koala populations have experienced significant declines in recent decades following wide scale deforestation and habitat fragmentation. (Seabrook, McAlpine, Phinn, Callaghan, & Mitchell, 2003). Agriculture, mining and urbanisation are significant drivers of this change in land use and in the Southeast Queensland bioregion large areas of remnant forests have been removed since 2000 (de Oliveira, Murray, de Villiers, & Baxter, 2014). Within the area covered by the IRP, historic land clearing, largely for agricultural purposes has been undertaken. Within the IRP, the vegetation quality varies considerably between remnant forests to low-lying cleared flood plains.

Vehicle related mortality and domestic and wild dog attacks pose significant impact to koala populations (de Oliveira, Murray, de Villiers, & Baxter, 2014). Mortality from these occurrences typically remove healthy breeding individuals, posing a threat to juvenile koala dispersal post-weaning (occurring in both males and females). Mature males are at higher risk due to larger home ranges and increased activity during the breeding season, with males typically dispersing more frequently and over larger distances than females. Although males have a higher risk of mortality and will disperse more frequently, losses of any individual to this trauma have the potential to negatively affect or even disrupt gene flow (DCCEEW 2022).

Disease can be a major contributor to population decline and population viability, with wild populations carrying disease pathogens (DCCEEW 2022). Quigley and Timms (2020) identifies the two major pathogens affecting koalas as *Chlamydia pecorum,* leading to chlamydial disease and koala retrovirus (KoRV). The significant impact of disease, particularly from Chlamydia and KoRV, is routinely highlighted in koala literature (Grogan, et al., 2017; McCallum, Kerlin, Ellis, & Carrick, 2017; Polkinghorne, Hanger, & Timms, 2013) therefore presenting a need to include it as a threat that needs to be mitigated for the IRP. Narayan (2019) reflects on the presence of population instability in the face of human-induced environmental changes, which may lead to reduced genetic variability of wild koalas and increases in population vulnerability to diseases. While it is not known to what extent these diseases currently impact the koala population within the IRP, it can be assumed that the risks associated with disease transmission remain high for the population.

1.4 Assessing Koala Genetics to Inform Management

Current understanding of koala abundance at local scales is limited across their distribution and the differentiation of distinct populations of koalas is difficult due to limited genetic data. A lack of consistent monitoring methodologies has also led to a high level of uncertainty at a bioregional scale that limits opportunities to implement effective management strategies (Adams-Hoskins, et al., 2016). Understanding population genetics at the local scale provides valuable insight into population health, patterns of dispersal and gene flow, and population stability in the face of current and future threatening processes. While it is beyond the scope of this study to identify distinct populations of koalas along the IRP alignment, this study will provide a locally specific investigation of genetic diversity that is important for informing future management for koalas along the IRP.

2. PROJECT AREA DESCRIPTION

The IRP will provide a service for the interstate freight market that is reliable and competitive in price and availability relative to road transport. The Australian Government has engaged ARTC to deliver the 1600 km long IRP through enhancements and upgrades of the existing inter-state line, and 500 km of new track in northern New South Wales and south-east Queensland.

The focus of this Report is on the sections of IRP from Narromine, New South Wales to Acacia Ridge/ Bromelton, Queensland. This portion of the IRP is broken up into eight sections: Narromine to Narrabri (N2N), Narrabri to North Star (N2NS), North Star to Border (NS2B), Border to Gowrie (B2G), Gowrie to Helidon (G2H), Helidon to Calvert (H2C), Calvert to Kagaru (C2K), Kagaru to Acacia Ridge and Bromelton (K2ARB). Within this program the sections of rail cross the Brigalow Belt South Bioregion and the South-East Queensland Bioregion. The Brigalow Belt bioregion is characterised by predominantly mixed eucalypt woodland with areas of brigalow (*Acacia harpophylla)* scrubs and open Mitchell grasslands. The South-East Queensland Bioregion is one of the most biodiverse bioregions in Australia. The bioregion is characterised by eucalypt forests and woodlands, with rainforests on mountain slopes and in stream valleys and Wallum heaths near the coasts. Majority of the IRP lies within the Brigalow Belt from N2N to the end of B2G, with G2H to K2ARB lying within South-East Queensland. Each of the sections of the IRP crosses varying landscape and as such can be elaborated on below and shown in Figure 2-1.

These IRP sections were selected for the koala genetic study as the alignment traverses areas of known koala populations in NSW and Queensland, where the species is listed as endangered under the Commonwealth.

2.1 QLD Inland Rail Project Sections

Kagaru to Acacia Ridge and Bromelton (K2ARB)

The K2ARB was the last section of the IRP, however the planned 49 kilometres of existing track upgrades through Kagaru to Acacia Ridge and Bromelton is no longer a part of the IRP. The former K2ARB section of the IRP is still able to provide meaningful insights into koala genetics and critical habitat and so data collected from this section has been included in this koala genetics study. It is the section of the previous IRP most urbanised with pockets of vegetation consisting of nature/wildlife refuges, and road parcels having wildlife crossings or bridges to uphold connectivity for koala activity. To note it is in proximity to the Greenbank Military Reserve, the Glider forest and the Parkinson Bushland Reserve.

Calvert to Kagaru (C2K)

Calvert to Kagaru covers 53-kilometres of the IRP and it possesses more koala hotspots across the Teviot Range, Peak Crossing and Ebenezer localities. Outside of these biodiversity corridors the IRP passes through more highly fragmented agricultural land, dominated by pasture grasses with isolated trees or woody regrowth.

Helidon to Calvert (H2C)

The Helidon to Calvert section traverses 47 kilometres of the IRP and two koala hotspot areas occur within this area. One is found within the mountain range between Grandchester and Laidley, and the other is found in a southern remnant patch of the Lockyer state forest. Remaining portions of land are largely utilised for agriculture and grazing, with several small urban nodes sprawled across the area.

Gowrie to Helidon (G2H)

Gowrie to Helidon is the shortest section of the IRP being of 28 kilometres long yet holds significance to the IRP, being intersected by the Toowoomba Range, a known koala hotspot and biodiversity

corridor. Outside of this mountain range native viable habitat is highly fragmented and compromised by grazing lands.

Border to Gowrie (B2G)

Border to Gowrie, a 217-kilometre section (including 9 km of North Star to Border (NS2B)) of the IRP moving through South-East Queensland, with the landscape dominated by agricultural practises and urbanised land, with extensive grazing areas intersected by State Forests. Riparian zones, notably the Condamine River, are of high value for koala activity.

2.2 NSW Inland Rail Project Sections

North Star to Border (NS2B)

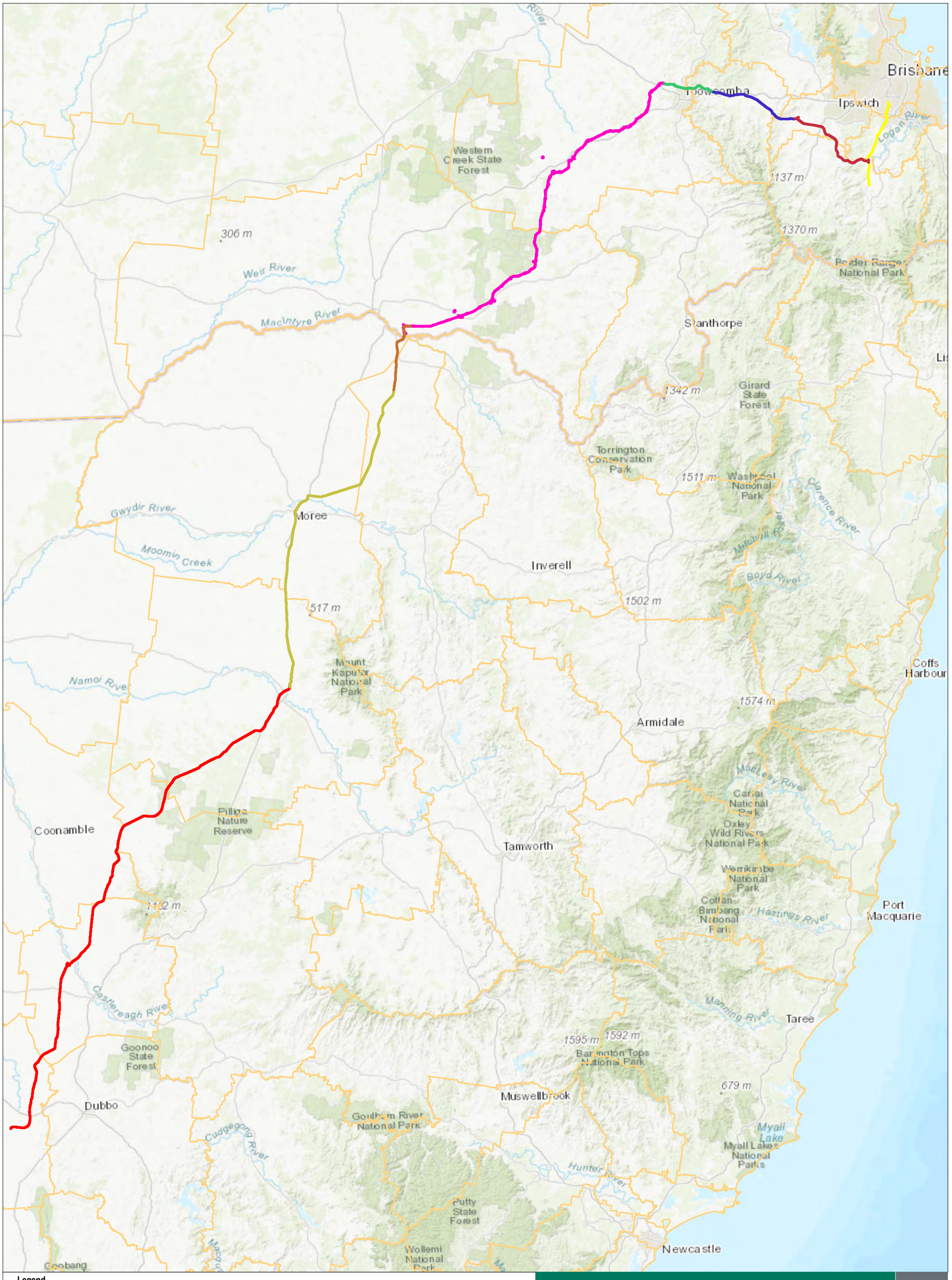
North Star to Border, a 39-kilometre stretch (30 km in NSW and 9 km in QLD) reaching the border of NSW and Queensland. This section of the IRP also is defined by land that has been modified for agricultural practises with large tracts of remnant vegetation rare and most native vegetation in isolated woodland re-growth pockets and in a degraded state. Koala activity is linked to riparian habitat of drainage lines and on the banks of the Macintyre River.

Narrabri to North Star (N2NS) (Phase 2)

Narrabri to North Star section is 187 kilometre long, however, this study is only concerned with Phase 2 upgrading 13-kilometres of existing railway between Moree and Camurra, then building upon 2-kilometres of new track across the Mehi-Gwydir floodplain. The rail corridor and surrounding area has been transformed extensively by agricultural activities with ‘patches’ of native woodland vegetation existing sporadically. Koala sightings have especially been associated with riparian corridors including the Mehi River and Gwydir River, Traveling Stock Reserves (TSRs) and isolated road parcel trees.

Narromine to Narrabri (N2N)

Narromine to Narrabri is a 306 km stretch of the IRP in Northern NSW. Much of this portion of the IRP is fragmented woodland habitat altered for agricultural purposes like running livestock or growing crops. The most northern section is dominated with state forests of The Pilliga, with historic records indicating koala presence by Etoo Creek.



Legend

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- C2K - Calvert to Kagaru
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert
- K2AB - Kagaru to Acacia Ridge/Bromelton
- N2N - Narromine to Narrabri
- N2NS - Narrabri to North Star
- NS2B - North Star to Border
- LGA Boundary

Data Source:
ESRI Would Topographic Map

ARTC Koala Genetics Study IRP Area

F2-1

Drawing No:	0628339_N2AB_TM_G001_R2.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 10 20 30Km

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ERM

3. SURVEY LOCATIONS

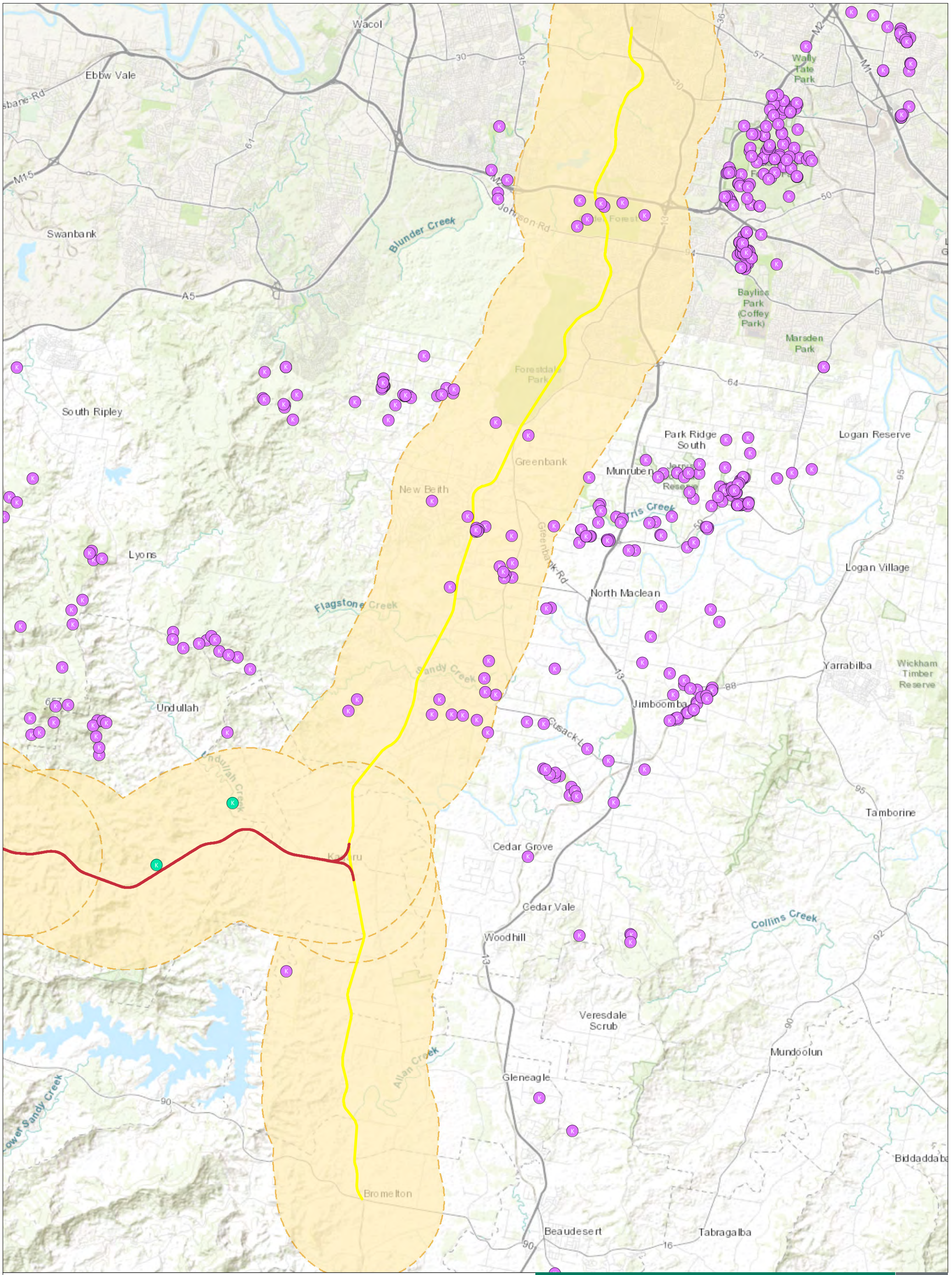
As the ARTC IRP operates across a wide geographic area, koala 'hotspots' were selected throughout the alignment and searching for scat was undertaken at each location. This provides suitable coverage across the entire IRP, while maximising the time spent in the field to collect samples.

Using Queensland and NSW state koala habitat mapping and historic koala presence data from ALA, BioNet and WildNet and previous surveys for the IRP (Figure 3-1), potential koala hotspots were identified. Hotspots were defined as those areas containing both a high abundance of historic presence records and potentially suitable habitat for either koala foraging or dispersal. These hotspots were used to identify important locations to be ground-truthed through this genetics study. The desktop and field-based investigations were confined to sites lying within the IRP linear corridor and, as guided by ARTC, had to have held active Land Access Agreements at time of survey.

Identifying these pre-fieldwork 'hotspots' of activity was based on collating previous records of koala individuals and records of signs of presence, rather than only sampling suitable habitat types, was chosen to maximise detectability while also providing adequate coverage of IRP. The delineation of koala hotspots is based on triangulating; known areas of high koala activity, previous data acquired from Environmental Impact Statements across the IRP and quality of habitat at each site. The table below outlines pre-fieldwork 'hotspot' locations for each section of the IRP and thus survey effort required. The location of these survey points is shown across the entire IRP in Figure 3-1.

Table 3-1 Pre-fieldwork koala hotspot locations and number per project

Project and length	Number of Pre-fieldwork hotspot locations per Inland Rail Project
Narromine to Narrabri (306km)	7
Narrabri to North Star (187km)	9
North Star to Border (39km)	4
Border to Gowrie (217km)	10
Gowrie to Helidon (28km)	3
Helidon to Calvert (47km)	2
Calvert to Kagaru (53km)	4
Kagaru to Acacia Ridge and Bromelton (49km)	3
Program-wide combined Narromine to ARB	**42**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Swanbank
Blunder Creek
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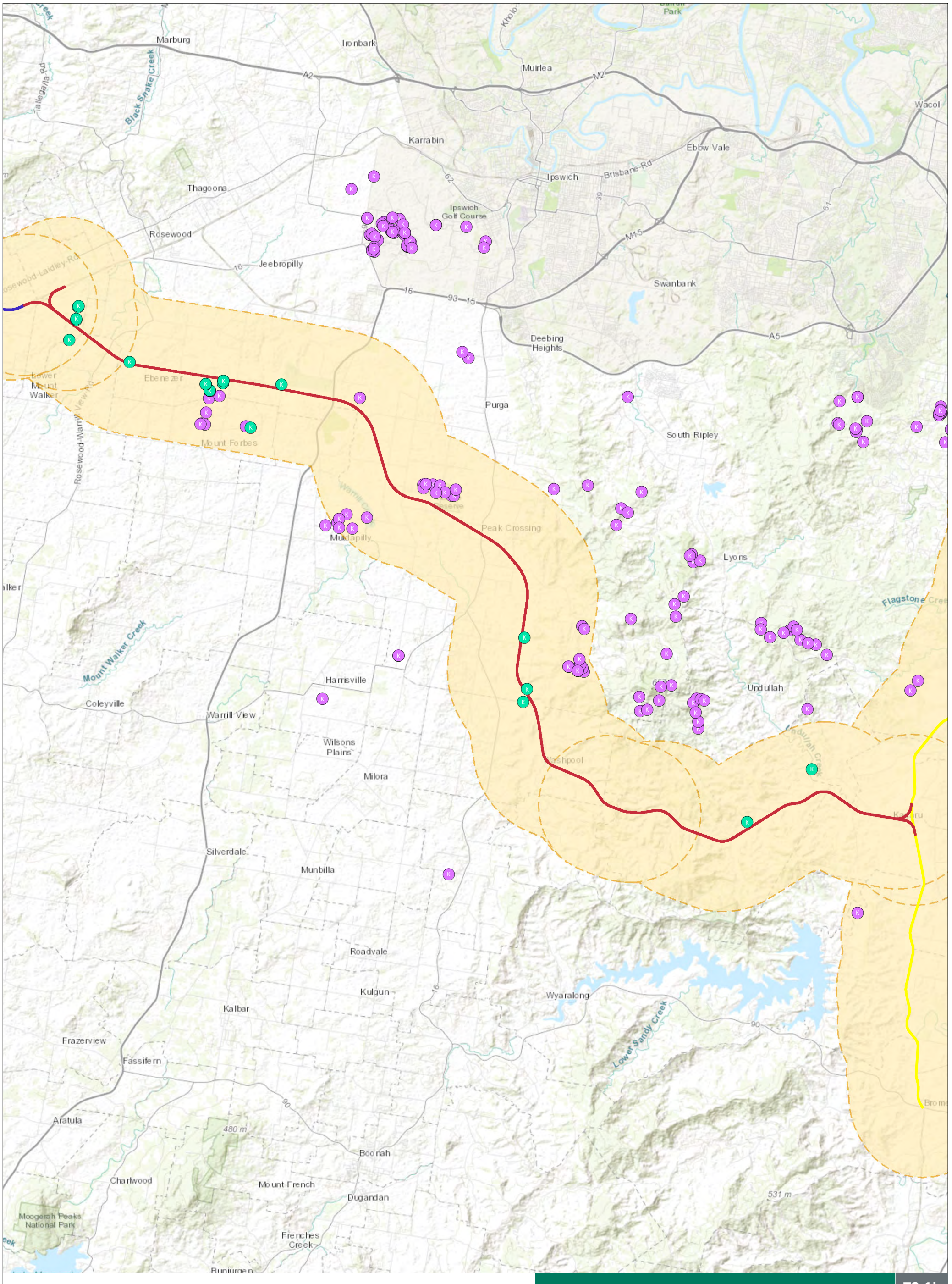
Wally Tate Park
M2
M1
M1-5
A5
Bayliss Park (Coffey Park)
Marsden Park
Forestdale Park
South Ripley
Park Ridge South
Logan Reserve
Munruben
New Beith
Greenbank
Lyons
Flagstone Creek
North Maclean
Logan Village
Sandy Creek
Undullah
Jimboomba
Yarrabilba
Wickham Timber Reserve
Cusack Ln
Tamborine
Cedar Grove
Cedar Vale
Collins Creek
Woodhill
Veresdale Scrub
Mundoolun
Gleneagle
Allan Creek
Bromelton
Beaudesert
Tabragalba
Legend
3km Buffer Along The IRP Aligment
Koala Records (Australian Living Atlas)
C2K Koala Observations (FFJV)
Inland Rail Alignment
C2K - Calvert to Kagaru
K2AB - Kagaru to Acacia Ridge/Bromelton
Data Source:
ESRI Would Topographic Map
Location of Koala Records across IRP Area
F3-1a
Drawing No: 0628339_N2AB_TM_G002_R3.mxd
Date: 14/02/2024
Drawing Size: A4
Drawn By: VN/CB
Reviewed By: MD
Coordinate System: GDA2020 MGA Zone 56
0 2 4Km
N
Koala Genetics Study Project – Inland Rail Program (NSW and QLD)
Client: ARTC
This figure may be based on third party data or data which has not been verified by ERM and it may not be to scale. Unless expressly agreed otherwise, this figure is intended as a guide only and ERM does not warrant its accuracy.
ERM


Marburg
Ironbark
Muirlea
Wacol
Karrabin
Ebbw Vale
Ipswich
Brisbane Rd
Thagoona
Ipswich Golf Course
Rosewood
Jeebropilly
Swanbank
Deebing Heights
Purga
South Ripley
Peak Crossing
Lyons
Undullah
Harrisville
Coleyville
Warrill View
Wilsons Plains
Milora
Silverdale
Munbilla
Roadvale
Kulgun
Kalbar
Wyaralong
Frazerview
Fassifern
Aratula
Boonah
Charlwood
Mount French
Dugandan
Moogerah Peaks National Park
Frenches Creek
Mount Walker
Lower Mount Walker
Ebenezer
Mount Forbes
Peak Crossing Reserve
Mutdapilly
Mount Walker Creek
Black Snake Creek
Flagstone Creek
Lower Sandy Creek
480 m
531 m
A2
M2
M1
A5
Legend
3km Buffer Along The IRP Aligment
Koala Records (Australian Living Atlas)
C2K Koala Observations (FFJV)
Inland Rail Alignment
C2K - Calvert to Kagaru
H2C - Helidon to Calvert
K2AB - Kagaru to Acacia Ridge/Bromelton
Data Source:
ESRI Would Topographic Map
Location of Koala Records across IRP Area
F3-1b
Drawing No: 0628339_N2AB_TM_G002_R3.mxd
Date: 14/02/2024
Drawing Size: A4
Drawn By: VN/CB
Reviewed By: MD
Coordinate System: GDA2020 MGA Zone 56
0 2 4Km
N
Koala Genetics Study Project – Inland Rail Program (NSW and QLD)
Client: ARTC
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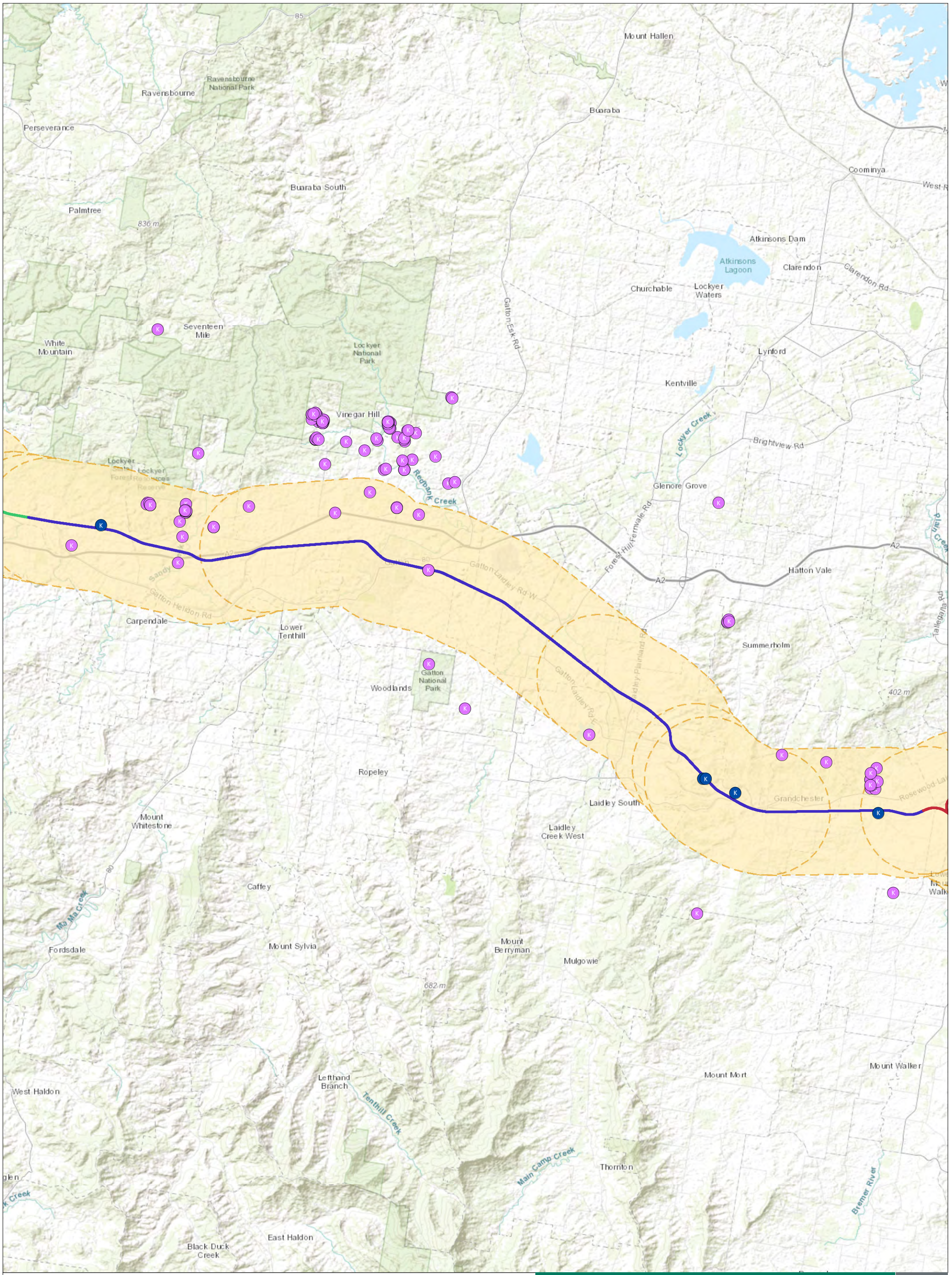
ERM



Legend

- 3km Buffer Along The IRP Aligment
- Koala Records (Australian Living Atlas)
- H2C Koala Observations (FFJV)

Inland Rail Alignment

- C2K - Calvert to Kagaru
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert

Data Source:
ESRI Would Topographic Map

Location of Koala Records across IRP Area

F3-1c

Drawing No:	0628339_N2AB_TM_G002_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

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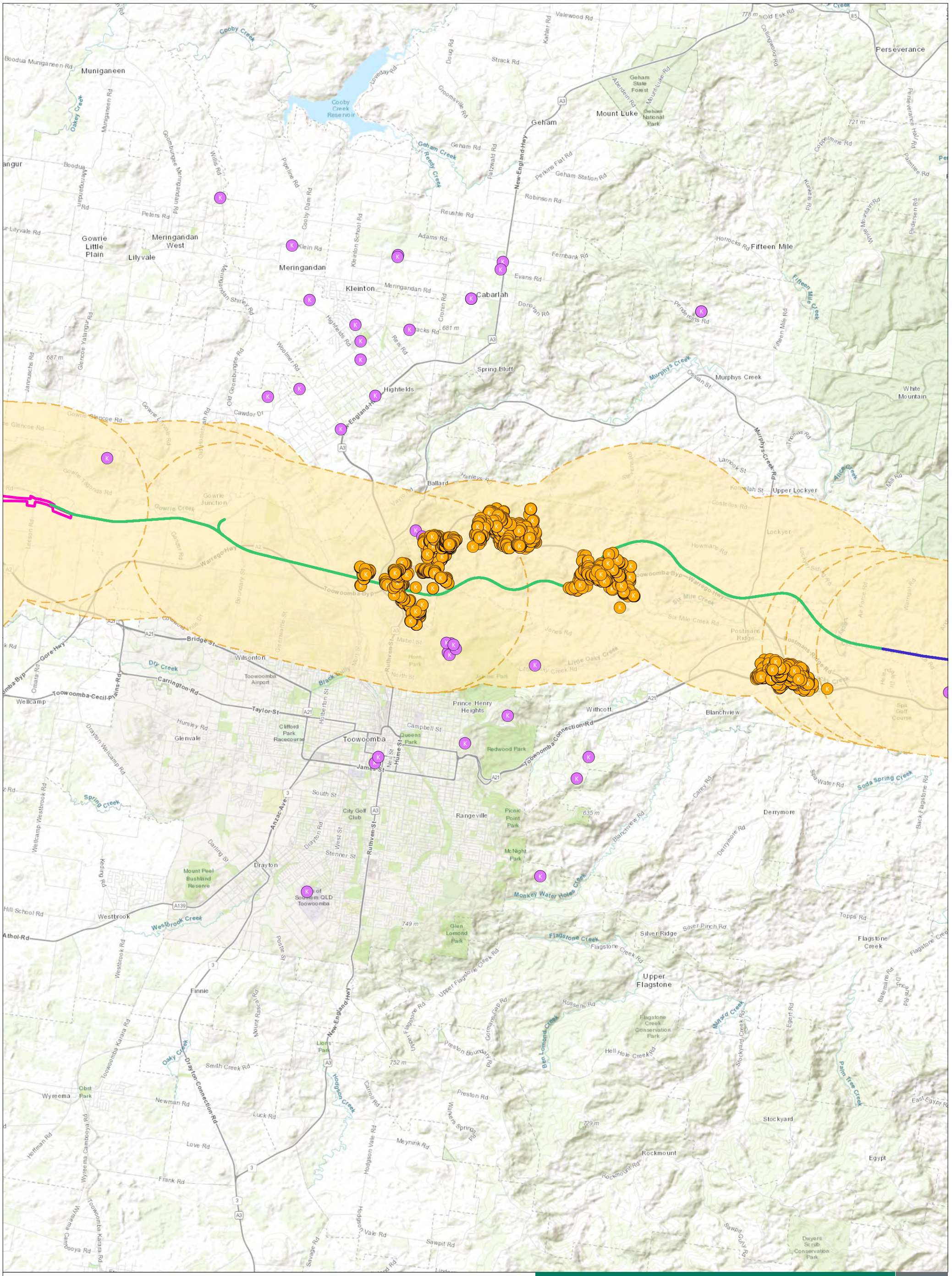
ERM



Legend

- 3km Buffer Along The IRP Aligment
- Koala Records (Australian Living Atlas)
- G2H TSRC Koala Tracking (DTMR)

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert

Data Source:
ESRI Would Topographic Map

Location of Koala Records across IRP Area

F3-1d

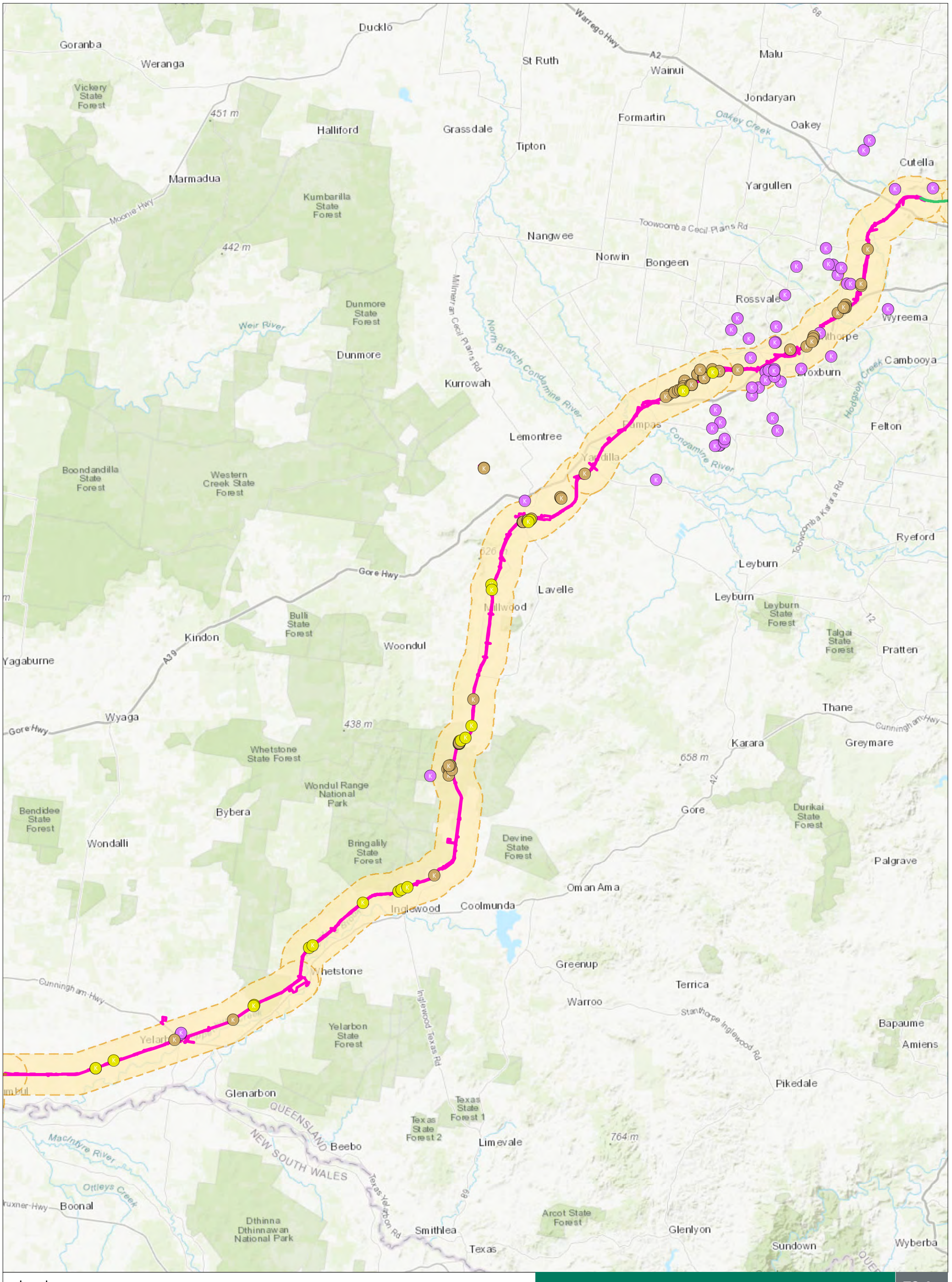
Drawing No:	0628339_N2AB_TM_G002_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

N

ERM



Legend

- 3km Buffer Along The IRP Aligment
- Koala Records (Australian Living Atlas)
- B2G Koala Observations (Ausecology)
- B2G Koala Observations (Cardno)

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- G2H - Gowrie to Helidon
- NS2B - North Star to Border

Data Source:
ESRI Would Topographic Map

Location of Koala Records across IRP Area

F3-1e

Drawing No:	0628339_N2AB_TM_G002_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

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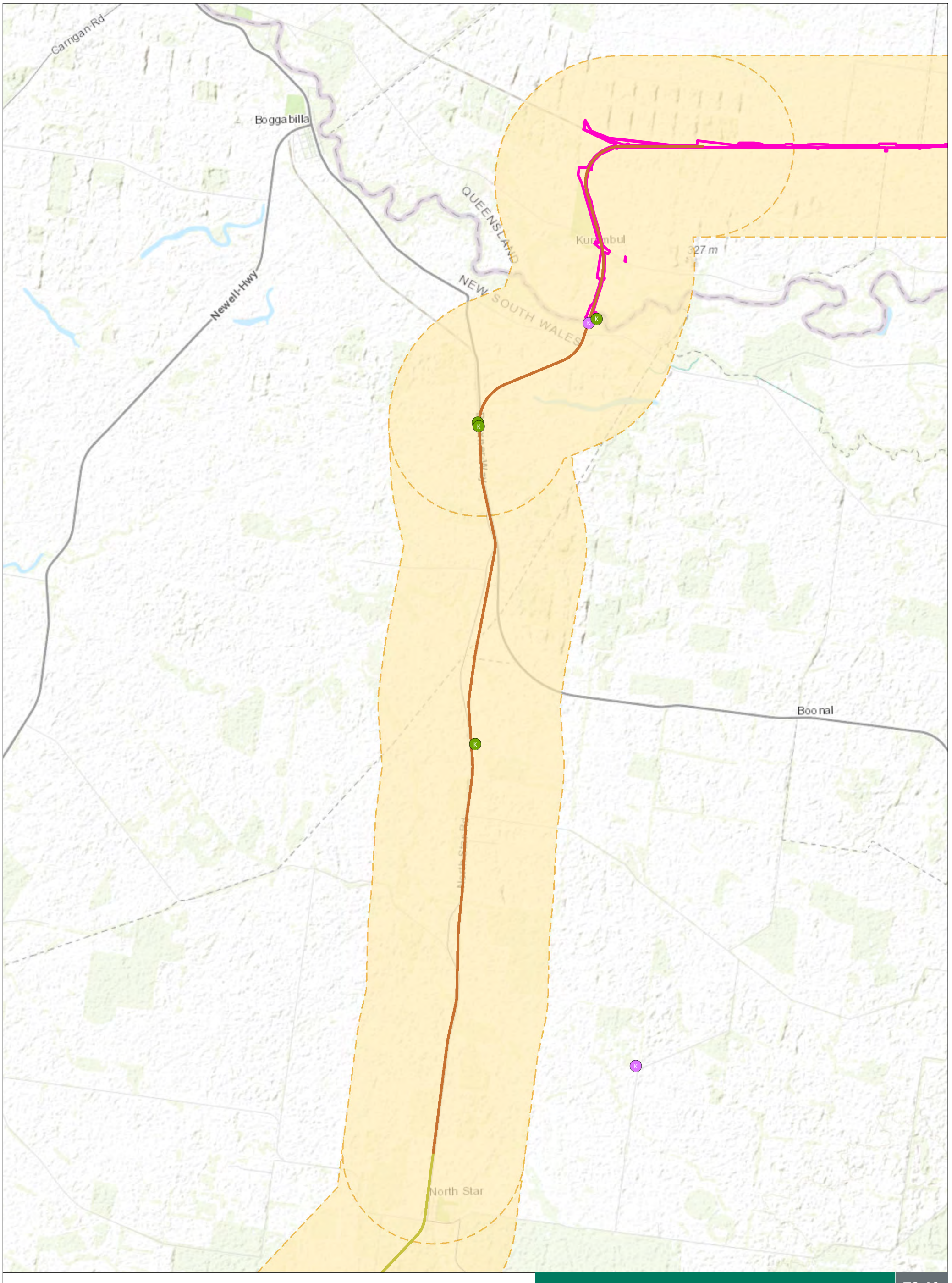


Legend

- 3km Buffer Along The IRP Aligment
- Koala Records (Australian Living Atlas)
- NS2B Koala Observations (FFJV)

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- N2NS - Narrabri to North Star
- NS2B - North Star to Border

Data Source:
ESRI Would Topographic Map

Location of Koala Records across IRP Area

F3-1f

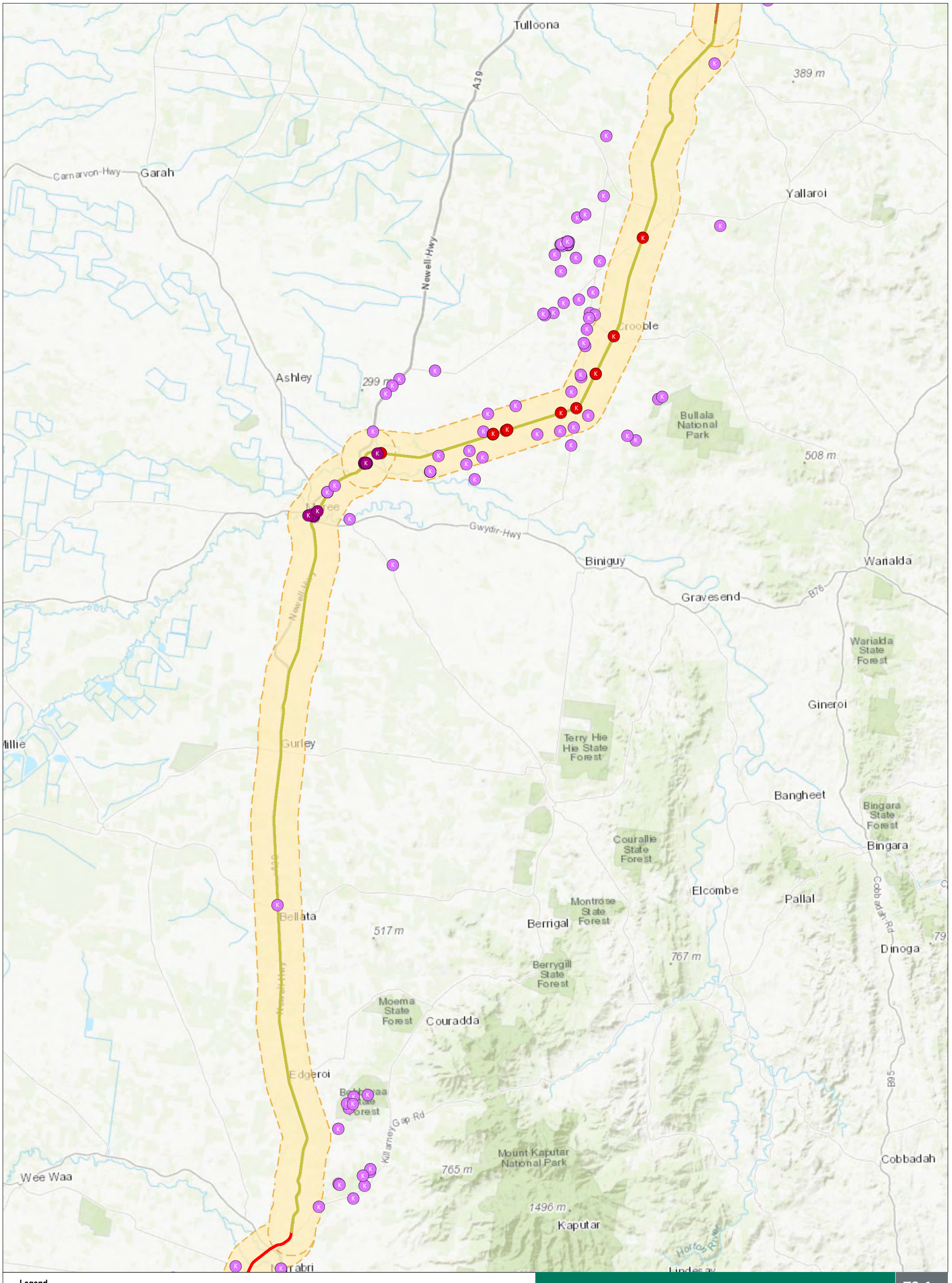
Drawing No:	0628339_N2AB_TM_G002_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

N

ERM


Tulloona
A39
389 m
Carnarvon Hwy
Garah
Newell Hwy
Yallaroi
Crooble
Ashley
299 m
Bullala National Park
508 m
Gwydir Hwy
Biniguy
Warialda
Gravesend
B76
Warialda State Forest
Gineroi
Terry Hie Hie State Forest
Bangheet
Bingara State Forest
Bingara
Courallie State Forest
Elcombe
Pallal
Montrose State Forest
Berrigal
517 m
767 m
Berrygill State Forest
Moema State Forest
Couradda
Dinoga
B95
Edgeroi
Killarney Gap Rd
Mount Kaputar National Park
765 m
Cobbadah
Wee Waa
1496 m
Kaputar
Narrabri
Legend
3km Buffer Along The IRP Aligment
Koala Records (Australian Living Atlas)
N2NS SP2 Observed Koala Sightings (BDAR)
N2NS Koala Records (Umwelt/GHD)
Inland Rail Alignment
N2N - Narromine to Narrabri
N2NS - Narrabri to North Star
NS2B - North Star to Border
Data Source:
ESRI Would Topographic Map
Location of Koala Records across IRP Area
F3-1g
Drawing No: 0628339_N2AB_TM_G002_R3.mxd
Date: 14/02/2024
Drawing Size: A4
Drawn By: VN/CB
Reviewed By: MD
Coordinate System: GDA2020 MGA Zone 56
0 2 4Km
N
Koala Genetics Study Project – Inland Rail Program (NSW and QLD)
Client: ARTC
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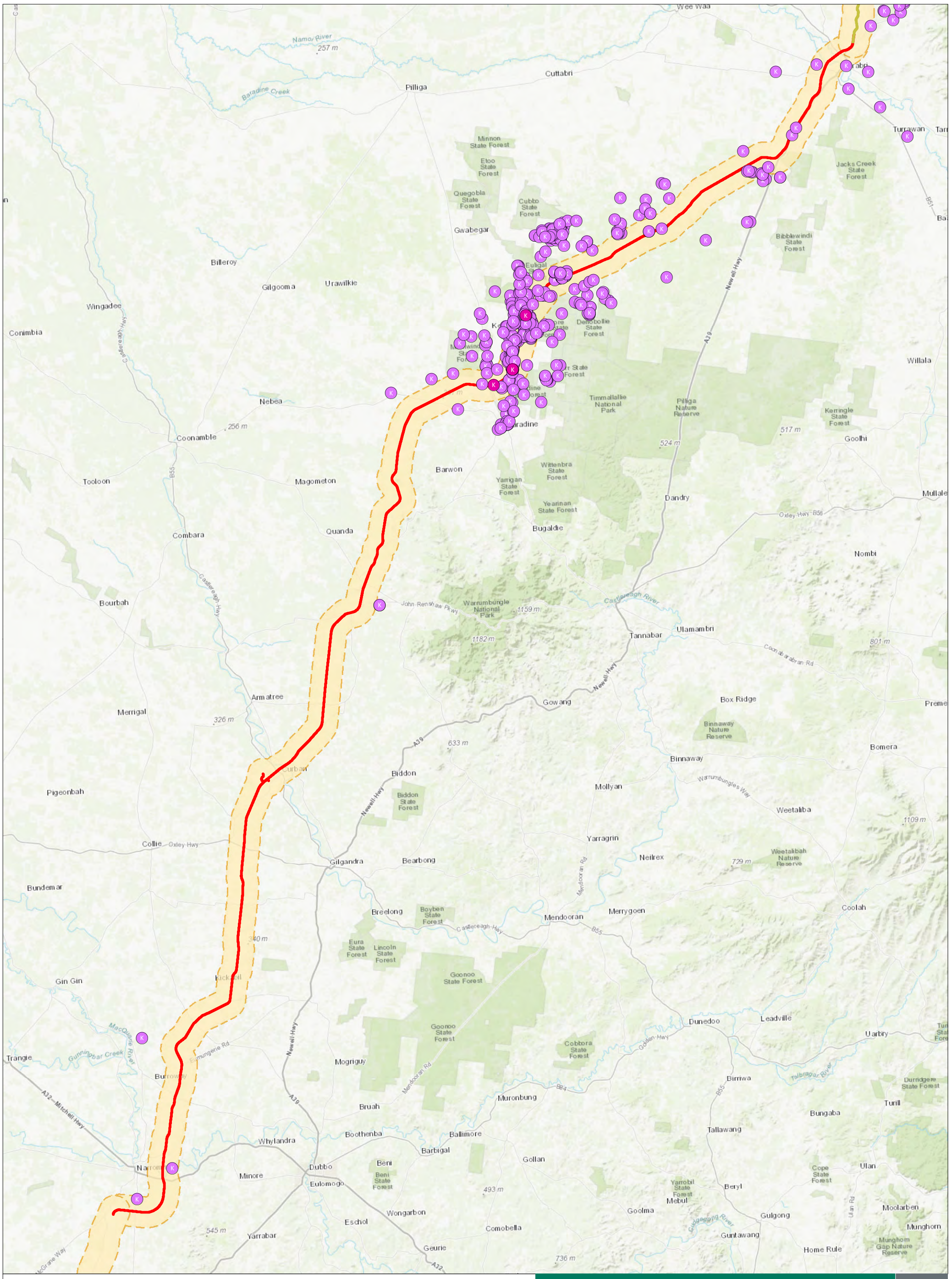
ERM



Legend

- 3km Buffer Along The IRP Aligment
- Koala Records (Australian Living Atlas)
- N2N Koala Observations (GHD)

Inland Rail Alignment

- N2N - Narromine to Narrabri
- N2NS - Narrabri to North Star

Data Source:
ESRI Would Topographic Map

Location of Koala Records across IRP Area

F3-1h

Drawing No:	0628339_N2AB_TM_G002_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

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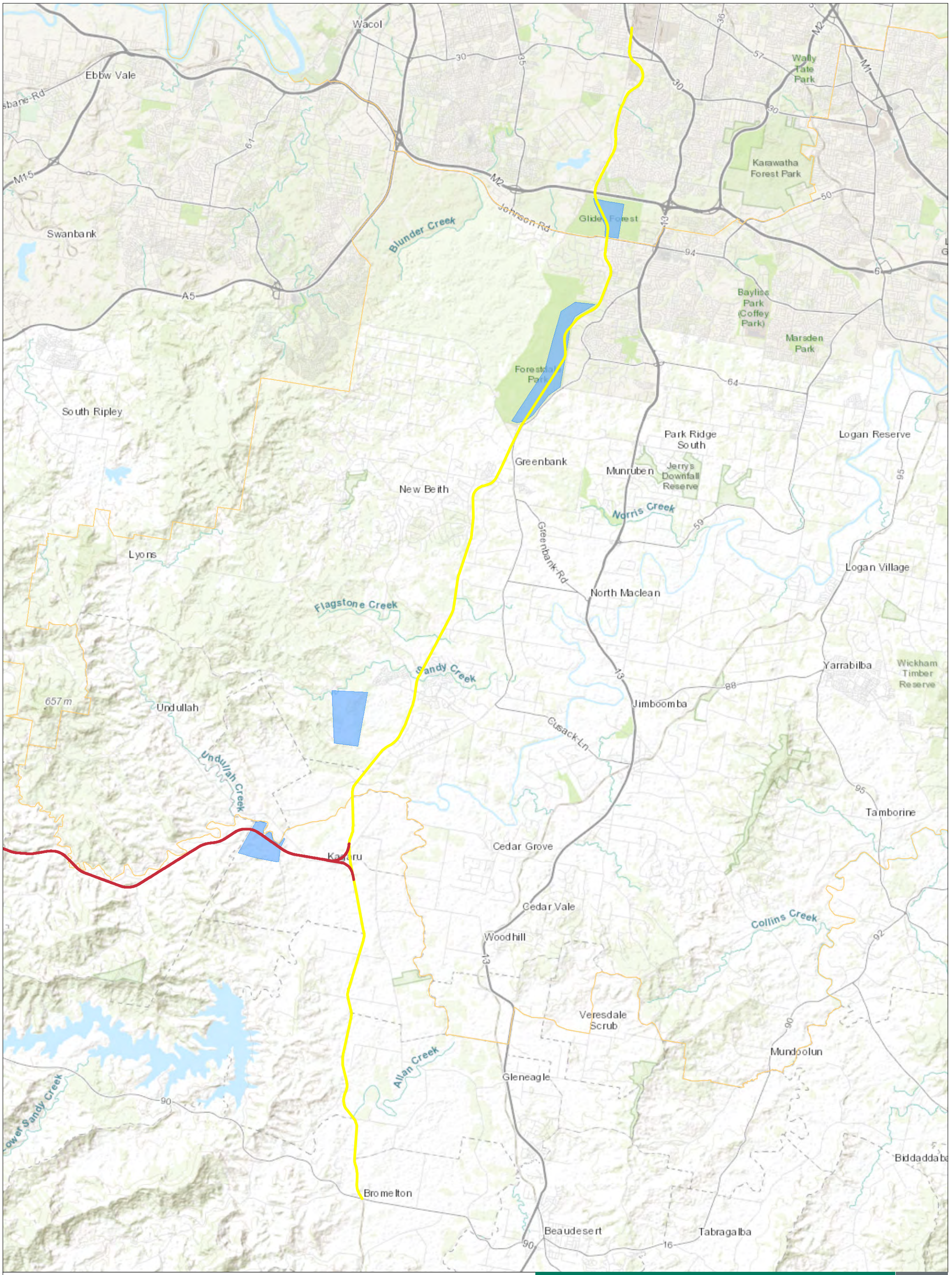
ERM



Legend

- Pre-fieldwork Hotspots

Inland Rail Alignment

- C2K - Calvert to Kagaru
- K2AB - Kagaru to Acacia Ridge/Bromelton
- LGA Boundary

Data Source:
ESRI Would Topographic Map

Location of Pre-Fieldwork Koala Hotspots across IRP Area

F3-2a

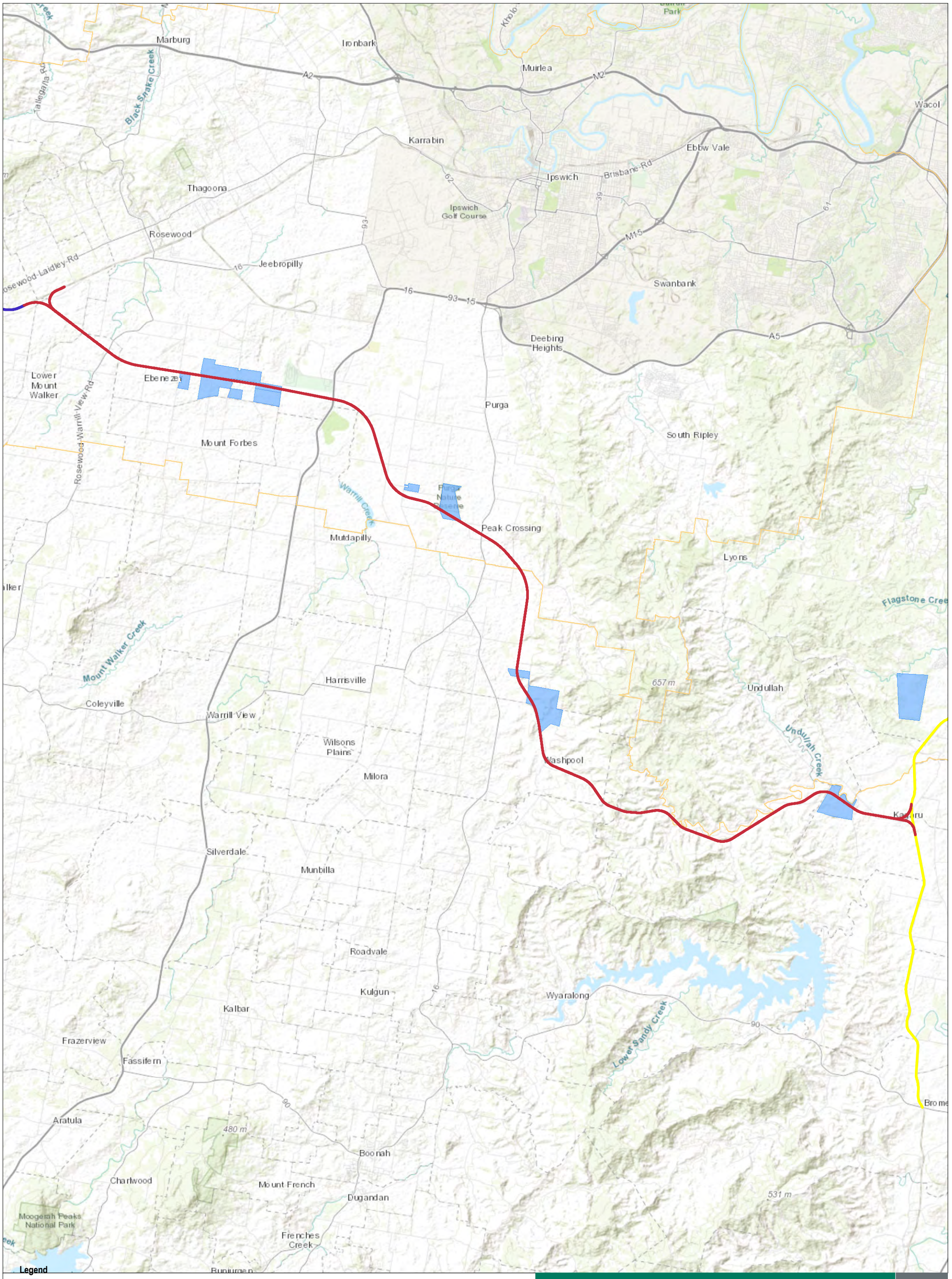
Drawing No:	0628339_N2AB_TM_G003_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

N

ERM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Ironbark
Muirlea
A2
M2
Wacol
Tallegalla Rd
Black Snake Creek
Karrabin
Ebbw Vale
Ipswich
Brisbane Rd
Thagoona
Ipswich Golf Course
62
39
61
93
M1-5
Rosewood
Jeebropilly
16
Rosewood-Laidley Rd
Swanbank
93
15
Deebing Heights
A5
Lower Mount Walker
Ebenezer
Rosewood-Warrill View Rd
Purga
Mount Forbes
South Ripley
Warrill Creek
Purga Nature Reserve
Peak Crossing
Mutdapilly
Lyons
Flagstone Cree
Mount Walker Creek
Harrisville
657 m
Undullah
Coleyville
Warrill View
Undullah Creek
Wilsons Plains
Milora
Washpool
Silverdale
Munbilla
Roadvale
Kulgun
Kalbar
Wyaralong
Lower Sandy Creek
90
Frazerview
Fassifern
Aratula
480 m
Boonah
Charlwood
Mount French
Dugandan
531 m
Moogerah Peaks National Park
Frenches Creek
Bunjurgen
Legend
Pre-fieldwork Hotspots
Inland Rail Alignment
C2K - Calvert to Kagaru
H2C - Helidon to Calvert
K2AB - Kagaru to Acacia Ridge/Bromelton
LGA Boundary
Data Source:
ESRI Would Topographic Map
Location of Pre-Fieldwork Koala Hotspots across IRP Area
F3-2b
Drawing No: 0628339_N2AB_TM_G003_R3.mxd
Date: 14/02/2024
Drawing Size: A4
Drawn By: VN/CB
Reviewed By: MD
Coordinate System: GDA2020 MGA Zone 56
0 2 4Km
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Koala Genetics Study Project – Inland Rail Program (NSW and QLD)
Client: ARTC
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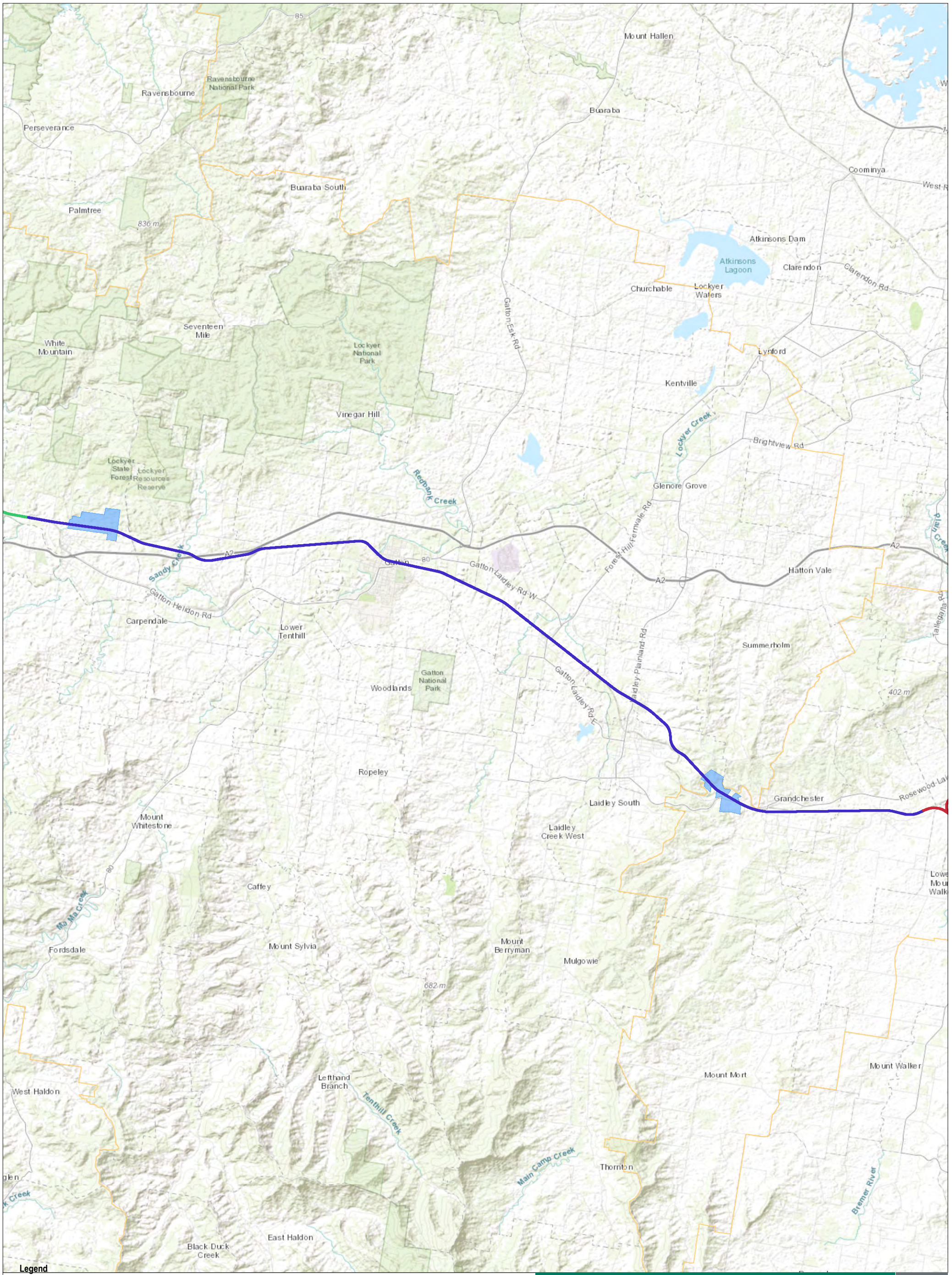
ERM



Legend

- Pre-fieldwork Hotspots

Inland Rail Alignment

- C2K - Calvert to Kagaru
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert
- LGA Boundary

Data Source:
ESRI Would Topographic Map

Location of Pre-Fieldwork Koala Hotspots across IRP Area

F3-2c

Drawing No:	0628339_N2AB_TM_G003_R3.mxd		
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	

Coordinate System: GDA2020 MGA Zone 56

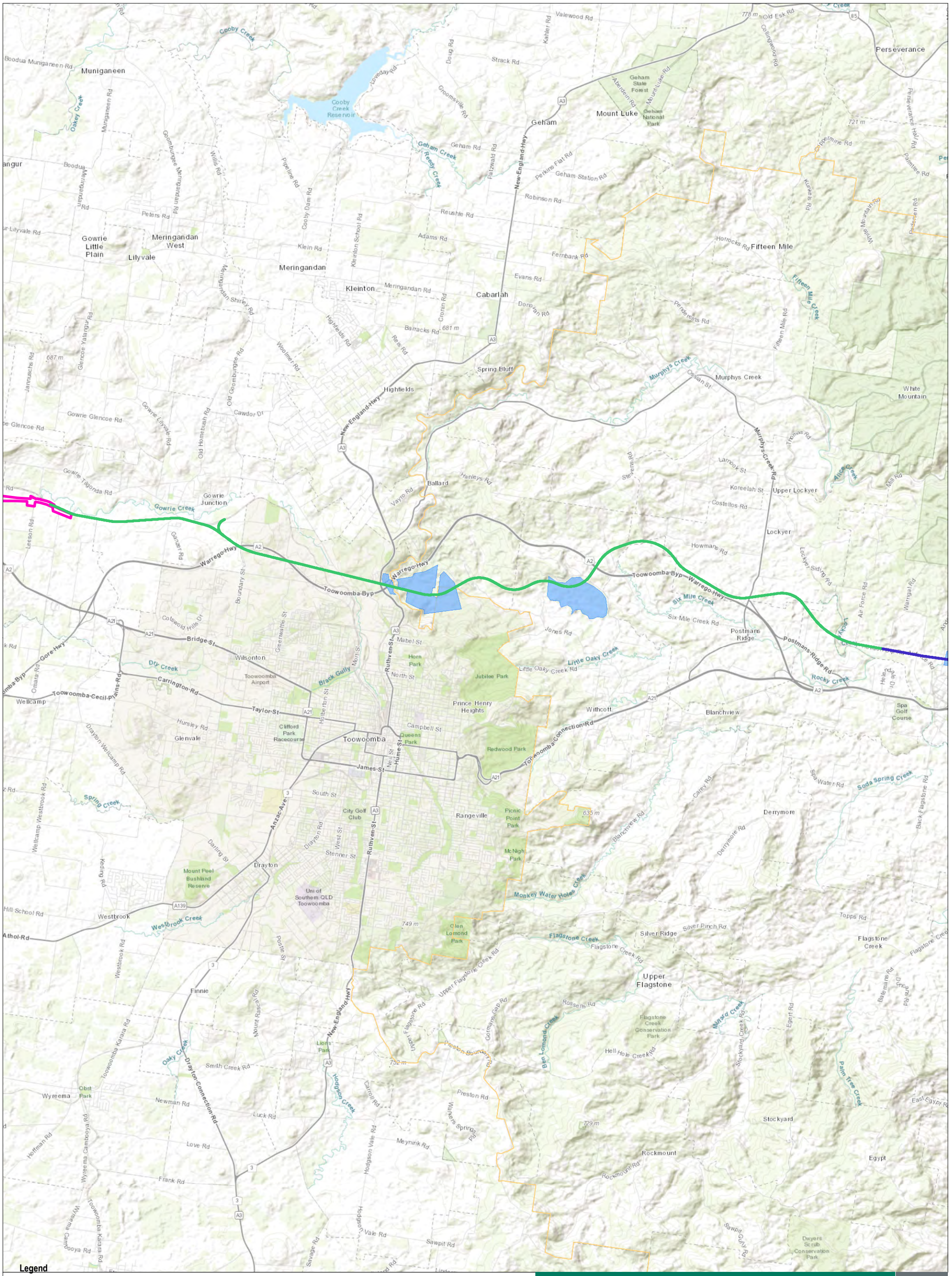
0 2 4Km

N

Koala Genetics Study Project – Inland Rail Program (NSW and QLD)

Client: ARTC

ERM



Legend

- Pre-fieldwork Hotspots

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert
- LGA Boundary

Data Source:
ESRI Would Topographic Map

Location of Pre-Fieldwork Koala Hotspots across IRP Area

F3-2d

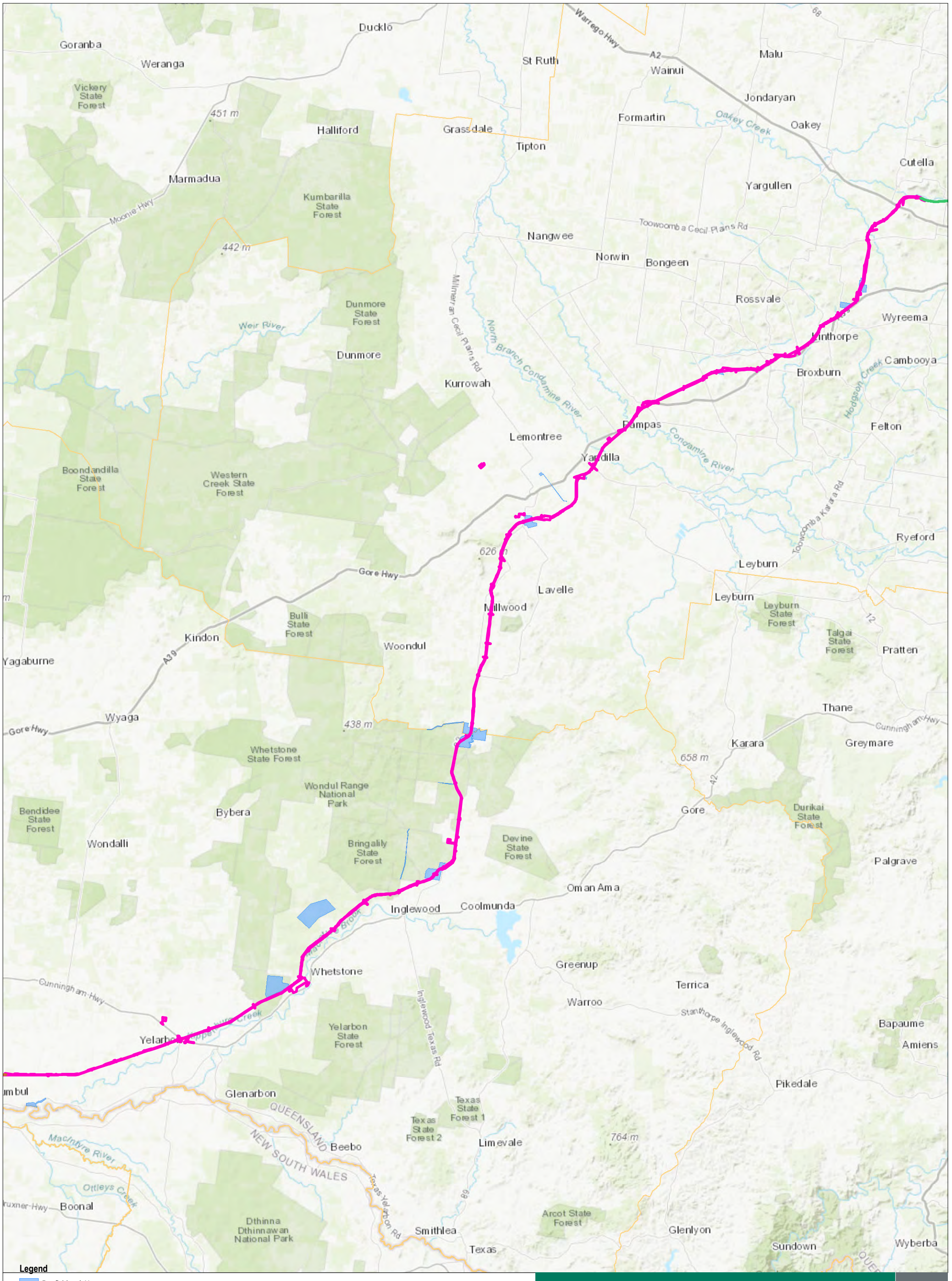
Drawing No:	0628339_N2AB_TM_G003_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

N

ERM



Legend

- Pre-fieldwork Hotspots

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- G2H - Gowrie to Helidon
- NS2B - North Star to Border
- LGA Boundary

Data Source:
ESRI Would Topographic Map

Location of Pre-Fieldwork Koala Hotspots across IRP Area

F3-2e

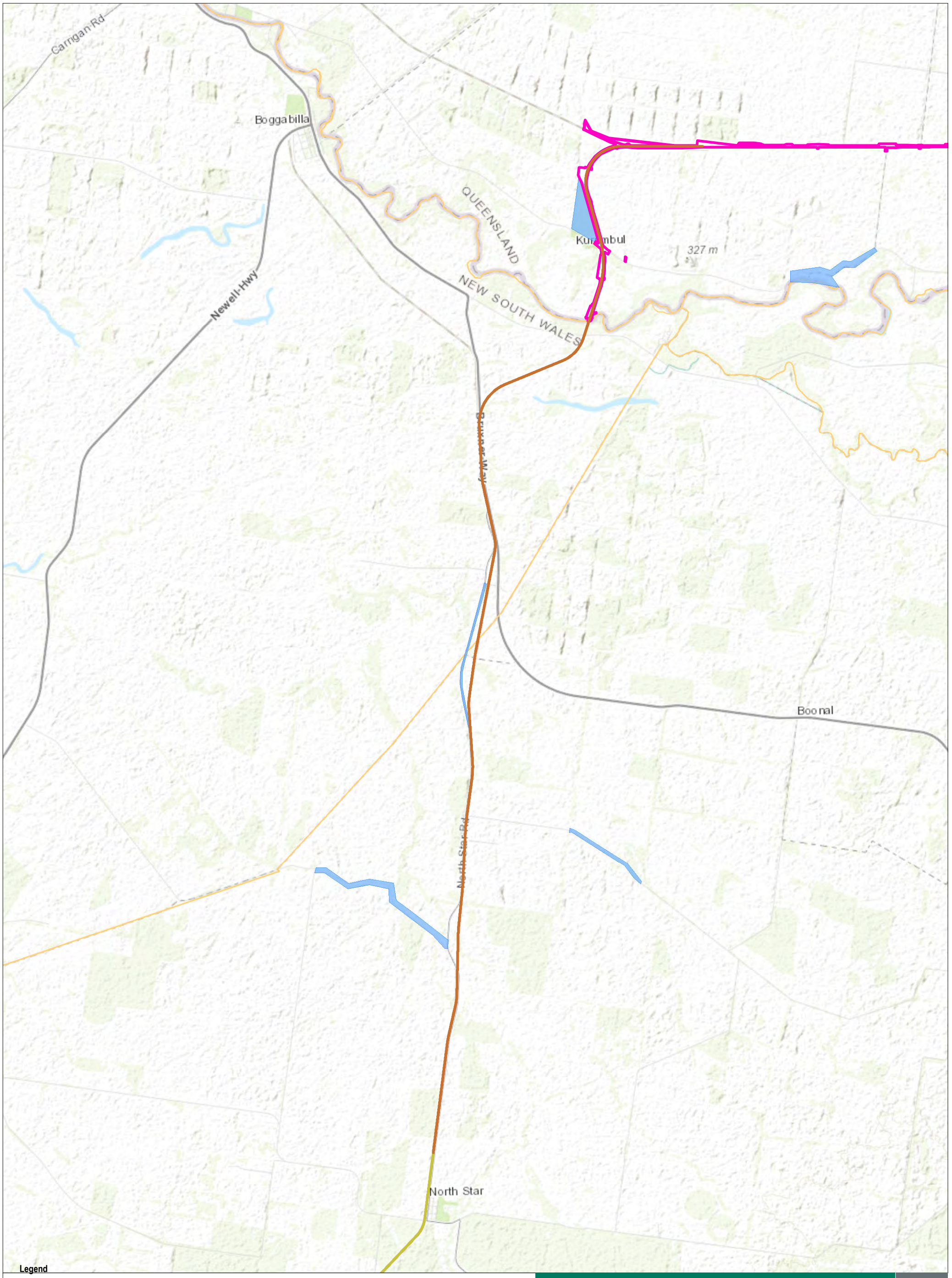
Drawing No:	0628339_N2AB_TM_G003_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

N

ERM



Legend

- Pre-fieldwork Hotspots

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- N2NS - Narrabri to North Star
- NS2B - North Star to Border
- LGA Boundary

Data Source:
ESRI Would Topographic Map

Location of Pre-Fieldwork Koala Hotspots across IRP Area

F3-2f

Drawing No:	0628339_N2AB_TM_G003_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

N

ERM

Legend

Pre-fieldwork Hotspots

Inland Rail Alignment

- N2N - Narromine to Narrabri
- N2NS - Narrabri to North Star
- NS2B - North Star to Border

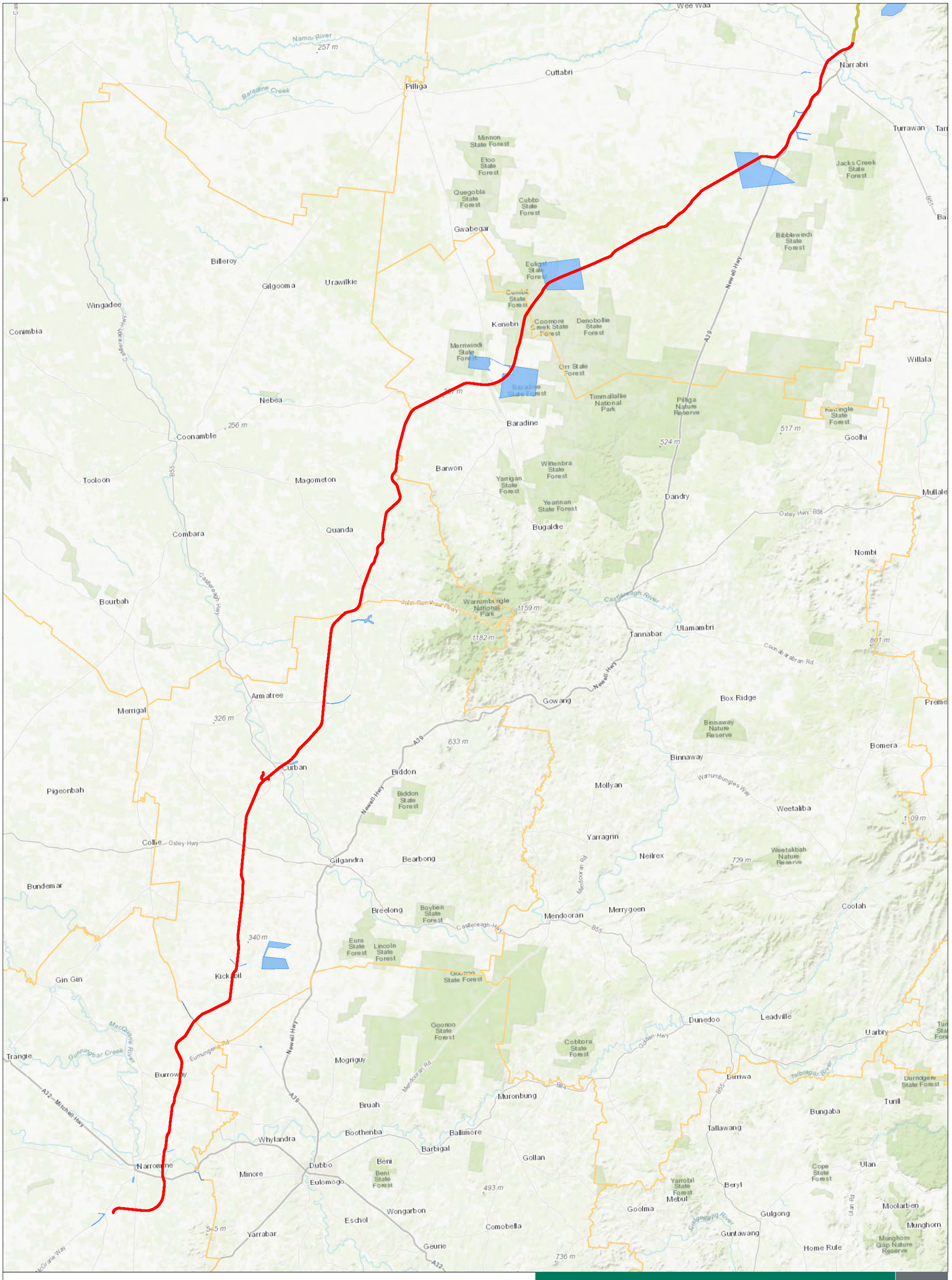
Data Source:
ESRI Would Topographic Map

Location of Pre-Fieldwork Koala Hotspots across IRP Area

F3-2g

Drawing No:	0628339_N2AB_TM_G003_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC
Coordinate System: GDA2020 MGA Zone 56 0 2 4Km		N	This figure may be based on third party data or data which has not been verified by ERM and it may not be to scale. Unless expressly agreed otherwise, this figure is intended as a guide only and ERM does not warrant its accuracy.

ERM



Legend

Pre-fieldwork Hotspots

Inland Rail Alignment

N2N - Narromine to Narrabri

N2NS - Narrabri to North Star

Data Source:
ESRI Would Topographic Map

Location of Pre-Fieldwork Koala Hotspots across IRP Area

F3-2h

Drawing No:	0628339_N2AB_TM_G003_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

N

ERM

4. METHODOLOGY

4.1 Previous Survey Effort

Prior to the field surveys conducted by ERM, several field surveys have been completed to inform various stages of the IRP development process. A summary of these additional surveys can be seen in Table 4-1 below. Koalas and koala scats and tracks were confirmed throughout the IRP. Critical habitat has also been verified with ground-truthed vegetation surveys undertaken for the developmental process.

Table 4-1 Summary of previous survey effort

Inland Rail Section	Consultant	Timing of Surveys	Survey types	Scat Detection	Koala detection
N2N	GHD	■ September and Novermber 2018; ■ March, August, September, October 2019; ■ February 2022	■ Active searches; ■ Spotlighting; ■ Call Playback; ■ Thermal Drone imaging	Yes	Yes
N2NS (Phase 2)	NGH	■ December 2015; ■ December 2019; ■ February 2020	■ Koala SAT searches; ■ Spotlighting	Yes	Yes
NS2B	FFJV	■ Between October 2018 and October 2019.	■ Call Playback; ■ Spotlight searches; ■ Active searches	No	Yes
B2G	ELA	■ April -October 2016	■ Koala habitat assessments; ■ Koala SAT searches; ■ Incidental fauna observations	Yes	No
		■ June 2018- May 2019	■ Koala habitat assessments; ■ Koala SAT searches; ■ Incidental fauna observations	Yes	No
	Cardno	■ 19 October- 13 November 2020 ■ 15 February to 27 March 2021	■ Koala habitat assessments; ■ Koala SAT searches; ■ Incidental fauna observations	Yes	Yes
	Ausecology	■ 30 August- 30 December 2021 ■ 15- 17 March 2022	■ Koala habitat assessments; ■ Koala SAT searches; ■ Incidental fauna observations	Yes	Yes
G2H	Arup	■ March 2017	■ Spotlighting; ■ Active Searches; ■ Koala SAT searches	Yes	No

Inland Rail Section	Consultant	Timing of Surveys	Survey types	Scat Detection	Koala detection
	EMM	■ September 2018-May 2019	■ Spotlighting; ■ Active Searches; ■ Koala SAT searches	Yes	No
	Eco logical Australia	■ December 2018-April 2019	■ Spotlighting; ■ Active Searches; ■ Koala SAT searches	Yes	Yes
H2C	Arup/SMEC	■ March-June 2016	■ Active Searches; ■ Koala SAT searches	Yes	No
	EMM	■ September-November 2018	■ Koala habitat assessments; ■ Incidental fauna observations	Yes	No
	ELA	■ December 2018-April 2019	■ Koala habitat assessments; ■ Koala SAT searches; ■ Incidental fauna observations	Yes	No
C2K	Jacobs-GHD	■ March-May 2016	■ Koala habitat assessments; ■ Koala SAT surveys; ■ Incidental fauna observations	Yes	Yes
	GHD	■ December 2016 – February 2017	■ Active Searches	Yes	No
	EMM	■ September-November 2018	■ Incidental fauna observations	No	Yes
	FFJV	■ December – April 2019; ■ May 2019; ■ June – July 2019	■ Koala habitat assessments; ■ Koala SAT surveys; ■ Incidental fauna observations	Yes	No
K2ARB	WSP	■ May-June 2016	■ Koala habitat assessments; ■ Koala SAT surveys; ■ Incidental fauna observations	Yes	No
	ERM	■ February-May 2018 ■ January 2020	■ Koala habitat assessments; ■ Koala SAT surveys; ■ Incidental fauna observations		

4.2 Current Field Survey Effort

A total of forty (40) field days have been undertaken throughout the IRP across five (5) separate field surveys between April and December of 2022.

Table 4-2 Survey Effort for Genetics Study

Inland Rail Section	Survey effort
K2ARB	4 days
C2K	5 days
H2C G2H	6 days
B2G	8 days
NS2B	5 days
N2NS	5 days
N2N	7 days
TOTAL DAYS	**40**

4.3 Koala Detection Dog Surveys

The application of trained detection dogs in threatened species conservation has been identified as a highly efficient, non-invasive survey technique that consistently outperforms alternative methods while also minimising risk of additional disturbance and issues surrounding detectability. Previous studies have proven the methods capacity to accurately detect scats with dogs able to distinguish between individuals within a species, as well as closely related non-target species (Bennett, et al., 2022; Cristescu, Miller, & Frère, 2020).

The field surveys for detecting and collecting koala scats for genetic analysis were completed by a UniSC dog handler and ERM ecologist with experience in fauna surveys.

Each hotspot site was distinguished using field collected data including location name, survey unique identifying number including for each sample, GPS coordinates. A purpose-built koala database developed for the Study was used field data collection. The detection dog was fitted with a GPS collar to ensure that the survey effort for each sampling location was quantifiable. Any ecological characteristics that may have influenced the detectability and decay of scats was also recorded (example: wet areas will increase decay rates; therefore scats will be detectable for a shorter amount of time, fire also decrease scat survival time).

Surveys were performed using a fresh scat detection dog, an accurate and fast way to collect genetic material in areas where koala presence has been confirmed. This method is designed to maximise the chance of detecting fresh scat for genetic analyses in the minimum amount of time and allows for coverage of large areas. Surveys were undertaken within predefined koala hotspots where land access agreements could be established by the client. Koalas/scats detected were photographed, and fresh scat material was collected and refrigerated for genetic analysis. Any opportunistic observations of koala scats, scratches or individual koalas made on foot or from the vehicle while traveling between survey locations were also recorded. Anecdotal and historic observations made by the members of the public and property owners were collected by the DDC team.

Upon detection of a koala, three characteristics were observed using binoculars. (1) The sex of the koala, (2) any external signs of Chlamydia infection (ocular infection/conjunctivitis known as pink eyes, or urinary tract infections known as wet bottom), and (3) The presence of an ear tag showing evidence of previous veterinary care.

4.4 Diet Preference and Vegetation Assessment

Variations in nutritional value and palatability is common in koala food trees, even those species considered to be important for populations in other areas. The development of DNA profiles of local food tree species was undertaken using single nucleotide polymorphism panels for comparison with DNA extracted from koala scats – informing food tree preference to guide targeted future rehabilitation efforts.

Leaf samples were taken from established trees throughout the IRP to ensure adequate spatial coverage. Only species identified as locally important koala trees were included in the study. Two samples were collected from each tree and included mature and immature leaves, provided that adequate confidence in species identification was possible. Samples were collected from all trees where a koala or koala scat was detected as well as additional samples from areas where no detections were made.

To ensure geographic coverage throughout the range, each species was sampled from a minimum of three sites. Samples were collected from four individuals of each species present at each site. Where abundant and widespread species were present, samples were only taken to ensure accurate coverage of geographic range. This approach allowed for the prioritisation of rare or restricted species, where present, without impacting on landscape level genetic diversity.

4.5 Data Analysis

The UniSC team has developed innovative and koala specific genetic analysis techniques. The use of microsatellites for analysis is now surpassed by next generation genotyping, providing thousands of markers, increasing the definition of genetic analyses for more accurate results. Our method targets both coding and non-coding regions of the DNA and enables more reliable assessments of genetic diversity.

The development of molecular tools was built on Dr Romane Cristescu's (our nominated Genetic Study Lead) proven track record in conservation genetics, especially in developing markers for koalas, as published in the peer reviewed literature:

- *Cristescu RH, Sherwin WB, Handasyde K, Cahill V & DW Cooper (2010). Detecting bottlenecks using BOTTLENECK 1.2.02 in wild populations: the importance of the microsatellite structure. Conservation Genetics, 11:1043-1049 (IF : 2.0); and*
- *Cristescu RH, Cahill V, Sherwin WB, Handasyde K, Carlyon K, Whisson D, Herbert CA, Carlsson BJ, Wilton AN & DW Cooper (2009). Inbreeding and testicular abnormalities in a bottlenecked population of koalas (Phascolarctos cinereus). Wildlife Research, 36: 299-308 (IF: 0.7).*

Genetic information was obtained from non-invasively collected koala scats detected using a detection dog trained to detect fresh koala scat. These non-invasive survey methods are valuable for detecting low density species, avoiding unnecessary disturbance to individuals, and for efficiently and cost effectively surveying large areas (Bennett, et al., 2022).

Landscape genetic analysis provides information on the health of koala populations in terms of inbreeding, genetic diversity and landscape genetic connectivity. High levels of inbreeding puts individuals at risk of decreased fitness and may lead to increased disease susceptibility. Landscape genetic analysis can also provide unique insights into how populations are connected or isolated. Gene flow reflects not just the ability of animals to move through the landscape (i.e. landscape permeability), but also survival that contributes to the next generation. Our experienced team has demonstrated experience in the collection and analysis of genetic data, and importantly interpretation of data that will provide important insights to the IRP.

The following sections provide detailed descriptions of the data analysis methodology conducted by the UniSC team.

4.5.1 Genetic Analysis

The best DNA quality is found in very fresh koala scats (Schultz, Cristescu et al. 2018). Therefore, scats were only collected if they were estimated to be less than one week old (categories 1 and 2, Table 4-3), still presenting a shiny mucus layer and a strong smell. Scats were collected in a sterile 50 ml falcon tube without direct skin contact to avoid contamination and further degradation or loss of DNA. Samples were transferred to a -20°C freezer at the end of each survey. A total of 73 samples were collected for genetic and disease analyses from dog surveys and opportunistic koala sightings.

Table 4-3 Guide used to age koala scats in the field

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

4.5.1.1 DNA Extraction

All samples were processed for extraction of both koala and *Chlamydia* DNA. The protocol of Schultz et al. (2018) was followed to isolate DNA from koala scats. The QIAamp PowerFecal Pro DNA Kit (Qiagen) was used following the manufacturer's instructions. An additional one-hour incubation step (65° C) after adding the buffer to the faecal sample was added and samples were vortexed for seven minutes at maximum speed using Genie 2 Vortex Mixer (Scientific Industries). Final DNA isolates were eluted in 200 ul of elution buffer and concentrated down to a volume of ~ 30 ul. The amount of DNA present in extracted samples (both koala and foreign) was determined using the Thermo Scientific NanoDrop 2000 Spectrometer (Thermo Fisher Scientific, Victoria). Extracted DNA was stored at -80°C until being shipped to Diversity Arrays Technology (DArT, Canberra).

4.5.1.2 Genotyping

DNA aliquots were genotyped using a next-generation sequencing protocol for detecting single nucleotide polymorphisms (SNPs) by DArT (Kilian, Wenzl et al. 2012). For samples collected during this study, DArTag was used as a targeted approach involving a specifically designed molecular probe panel (koala specific capture probes) to select small target regions containing sequence variants. A total of 4,393 koala SNPs were genotyped. Sex and *Chlamydia* specific markers were also genotyped as an extra step.

To increase sample size and improve our ability to conduct more broad-scale genetic analyses, we increased our dataset by including data from samples genotyped in previous studies using the DArTcap method. DArTcap is a selective step after complexity reduction to genotype specific markers from DArTseq representations with the help of capture probes, which is a cost-efficient method that combines DArTseq and sequence capture techniques. The DArTcap method differs from DArTag in that genotyping is selective and restricted to specific markers selected from the DArTseq representation, but only producing approximately 2,000 SNPs for koalas. In comparison, DArTag also targets many markers, but is not restricted to markers identified by the DArTseq platform. Because SNPs generated by DArTcap and DArTag are from corresponding regions, we were able to co-analyse DArTag data generated during this study with DArTcap data generated in previous studies. The improved dataset allowed for more robust genetic analyses.

4.5.1.3 Filtering of genetic data

When working with non-invasive scat samples, genotyped data is filtered to improve the quality of the dataset by removing samples with too little data (low *individual call rate*) as well as SNP loci that were not called across most samples (low *locus call rate*). The thresholds for these filters can be adjusted depending on the type of analysis, e.g., only few loci are needed for identifying unique individuals, however many high-quality loci are needed for measuring genetic diversity. Therefore, different filtering regimes are applied for different analyses. Constant thresholds are also applied to remove potentially erroneous loci. This includes filtering for allele read depth (minimum threshold of 5) and minor allele frequency (MAF, minimum threshold of 0.01) and loci appearing on the same contig as another (*secondary loci*). Given that filtering can result in previously polymorphic loci becoming monomorphic, a filter to remove all monomorphic loci is also applied. All filtering was done using the R package *dartR* (Gruber, Unmack et al. 2018). Further details of specific filtering are presented in the respective results sections (Section 6).

4.5.1.4 Genetic fingerprinting and sexing

Genetic fingerprinting allows for each scat sample to be allocated to an individual. This adds value by avoiding double-counting of the same individual sampled in different locations or at different times. Duplicate samples were removed by using filtered data to estimate pairwise relatedness values which range from 0 (no kinship relationship) to 1 (a duplicate individual, 100% relatedness – i.e., to be removed).

Seven different relatedness estimators currently exist and several were used to investigate and remove duplicates. Results were primarily reported from the dyadml estimator which performed best in estimating pairwise relatedness (Milligan 2003). All relatedness analyses were conducted in R using the *related* package (Pew, Muir et al. 2014).

Koala sex can be determined through sex-linked genetic markers. When using non-invasive sampling techniques such as scat, low quality DNA can impact sex identification confidence. With this limitation in mind, this Report provides the sex call from SNP genetic markers i.e., male versus female (M /F). Sex ratio is the relationship between the number of males to the number of females. Typically, the sex ratio in natural, healthy populations is expected to be 1:1. Risks of extinction are increased if population sex ratios significantly deviate from 1:1. However, a small bias of sex ratio towards females can sometimes be desirable, especially in very small or rapidly declining populations. Sex ratio can only be calculated for a site if a sufficient proportion of random individuals are sampled, or for landscape surveys where sampling has achieved high coverage of the area.

4.5.1.5 Measuring genetic diversity

Genetic diversity was calculated using GenAlEx v. 6.5 (Peakall and Smouse 2012). We calculated three values:

- Observed Heterozygosity, H_O - the calculated level of heterozygosity from the allele frequencies of the population under study;
- Expected Heterozygosity, H_E (adjusted for small sample size) - the level of heterozygosity that could be expected based on observed allele frequencies if the population was at the Hardy-Weinberg equilibrium (panmictic population with constant genetic variation across generations). This is relevant as it allows the calculation of the inbreeding coefficient below; and
- Inbreeding coefficient, F_{IS} - the proportion of the variance in the subpopulation contained in an individual, it can range from -1 to 1 (the closer to 1, the higher the degree of inbreeding). Note that inbreeding can not only result from non-random mating but also from small, isolated populations, where all individuals are more closely related than large populations.

4.5.1.6 Chlamydia detection

The prevalence of *Chlamydia* (percentage of koalas with the pathogen) is an important population characteristic for informing conservation management. It has been shown that the *Chlamydia* pathogen can be detected in koala scats using SNP genotyping (Cristescu, Miller et al. 2019). However, pathogen prevalence does not equal disease, as the presence and severity of the disease varies greatly between individual koalas, as well as between populations (Ellis, Girjes et al. 1993, Waugh, Hanger et al. 2016). Notably, individual koalas can shed large numbers of *Chlamydia* organisms without clinical signs of disease (Wan, Loader et al. 2011) and populations can have high *Chlamydia* prevalence (infection) with low noticeable health impact (disease). For example, 90% of koalas in the Mt Lofty ranges were *Chlamydia* positive but had a low prevalence of clinical disease (Weigler, Girjes et al. 1988, Polkinghorne, Hanger et al. 2013). Therefore, quantifying *Chlamydia* prevalence is only the first part of understanding the threat that this pathogen presents to an individual and a population.

4.5.1.7 Koala Retrovirus Detection

Much like the prevalence of *Chlamydia,* the prevalence of koala retrovirus is in important population characteristic that informs conservation management. Unlike *Chlamydia* detection, the methodology for sampling koala retrovirus from fresh scat material is noted to retain issues regarding the specificity and sensitivity of testing. While remaining an active area of research, the reliability of results was not thought to have provided robust and meaningful results that could positively inform koala management within the IRP. As such, sampling for koala retrovirus was deemed to be beyond the scope of this genetics research.

4.5.2 Genetic co-analyses for DArTcap and DArTag data

To increase the number of samples used to calculate genetic measures, and therefore increase the robustness of comparisons, samples previously genotyped for other projects were co-analysed. As genetic technology is progressing rapidly, some of the data was created using a previous method (DArTcap), while some samples used the current method (DArTag). Only markers that are common for both methods were used for these analyses.

Genotype data developed through DArTcap and DArTag procedures were merged together for the samples collected from sites in NSW and QLD. A total of 594 koala samples and a total of 1162 koala SNPs were available for the analyses. Genotyped data were filtered following the methods described in Section 1.2.3 (Filtering of genetic data) to improve the quality of the data set. A total of 327 koala samples and 902 koala SNPs were retained after filtering for quality. Relatedness among the koala samples was estimated following the methods described in Section 4.5.1.4 (Genetic fingerprinting and sexing) to identify duplicate samples.

4.5.2.1 Genetic structure

The number of ancestral genetic clusters (*K*) was assessed using fastStructure (Raj, Stephens et al. 2014) and STRUCTURE software V 2.3 (Pritchard, Stephens et al. 2000). fastStructure utilises an algorithm for approximate inference of the population structure from large SNP genotype data sets using a variational Bayesian framework, hierarchical priors and heuristic scores to identify the number of populations and weak population structures within data. STRUCTURE analyses differences in the distribution of genetic variants amongst samples with a Bayesian iterative algorithm by placing samples into groups whose members share similar patterns of variation. STRUCTURE is powerful but comparatively slow for large datasets. Although fastStructure is less powerful it is much faster and provides a reasonable alternative for large datasets (Stift, Kolář et al. 2019). Hence, herein we report the results from both fastStructure and STRUCTURE analyses.

The results generated by the STRUCTURE program were collated using STRUCTURE HARVESTER web-based program to detect the most likely number of genetic clusters that best fit the data using the ΔK method (Earl and VonHoldt 2012).

The number of genetic clusters (*K*) was set to vary between 1 to 10 and the STRUCTURE program was set to run five independent iterations with the length of burn-in period of 10,000, and 100,000 MCMC (Markov Chain Monte Carlo) repetitions after burn-in, using admixture ancestry model with correlated allele frequencies among populations without prior sample location information. In addition, principal component analysis (PCA) was conducted for the 255 samples from 24 locations (locations were assigned to each sample in ArcGIS based on Councils) using the *dartR* package (Gruber, Unmack et al. 2018). The first two principal components that explained the majority of the variation were used to plot the data.

4.5.2.2 Genetic diversity and population differentiation

To estimate the genetic diversity among koala populations, the 255 unique koala samples from 24 different locations were grouped into ten different groups based on their geographical proximity (Figure 4-1), noting that the number of samples available at some locations was very small (Appendix Table A2). Six samples were removed from the population genetic diversity indices estimates; three from Redland City Council (sample names: DR220116KM1A, DR220115KM1E and 221015MB1A) because they were located on North Stradbroke Island, and another three from Goondiwindi (sample names: 220623Bi1C, 220623Bi1B and 220623Bi2B) due to a very small sample size for the location and no other close sample locations that these could be grouped with. To quantify the population genetic diversity, observed heterozygosity H_O, expected heterozygosity H_E and inbreeding coefficient F_{IS} were calculated using GenAlEx software V6.5 (Peakall and Smouse 2012), as described in Section 4.5.1.4 (Measuring genetic diversity). In addition, the average internal relatedness (IR), a measure that reflects the parental relatedness (Amos, Worthington Wilmer et al. 2001) was measured across the individuals in each group using GENHET function (Coulon 2009) in R. Further, contemporary effective population size (N_E) and associated parametric 95% confidence intervals were estimated using NeEstimator v2 (Do, Waples et al. 2014), implementing linkage disequilibrium method with random mating model and 0.05 as the lowest allele frequency. To estimate the genetic differentiation between populations (i.e., 10 groups here), pairwise F_{ST} values were calculated using GenAlEx software V6.5 (Peakall and Smouse, 2012). Geneflow, a measure of movement of alleles between populations via migration or gametes, was estimated as effective number of migrants (N_m) per generation using the F_{ST} values calculated in GenAlEx software V6.5 (Peakall and Smouse, 2012). Note that these numbers reflect historical rates of gene flow and may not represent current gene flow.

4.5.2.3 Genomic relationship

Genomic relationship among individual koalas was estimated as an additive relationship matrix using the R package *dartR* (Gruber et al. 2018) for all 255 samples, and categorised by group (assigned in section 1.3.2, Genetic diversity and population differentiation). The additive relationship matrix is a theoretical framework for estimating a relationship matrix that is consistent with an approach to estimate the probability that the alleles at a random locus are identical in state (Endelman and Jannink 2012). Additive relationships carry information on genetic resemblance from common inheritance and are based on probabilities that gene pairs are identical by descent (Wright 1922).

4.5.2.4 Sexing

Sex of unique individual koalas was determined using sex-linked genetic markers for koalas used in co-analyses. Of the 255 samples available for co-analyses, 115 samples passed the genotyping quality control for sex determination. Of the 36 ARTC samples available for co-analyses, 31 samples passed the genotyping quality control for sex determination.

4.5.2.5 Chlamydia detection

The prevalence of *Chlamydia* (percent of koalas with the pathogen) was estimated in koala scats using SNP genotyping for the samples used in DArTcap and DArTtag co-analyses. Of the 255 samples available for co-analyses, 105 samples passed the genotyping quality control for *Chlamydia* detection. Of the 36 ARTC samples available for co-analyses, 30 samples passed the genotyping quality control for *Chlamydia* detection.

4.5.2.6 Tree genetic analysis

To cross reference the diet preference of koala from scat collected during the genetics study, a DNA library of potential feed tree species was undertaken alongside the koala scat analysis by researchers from the University of Queensland.

Referring to literature a list of candidate koala food trees species for Queensland was collated (utilising species from genera Eucalyptus, Corymbia and Angophora). With this list, leaf samples were taken from four individual trees per species per sampling site along the eastern edge of QLD and NSW with the specimens being prepared (air drying, freezing etc.) for DNA extraction. Existing tree DNA sequences were supplemented into the study from other projects as well.

After specimen collection and preparation the samples underwent DNA extraction via automated plate-based extraction protocol, by Diversity Arrays Technology P/L, Canberra, Australia (DArT) to compile (DNA fragments) and single nucleotide polymorphisms (SNPs). Following this, the now created panel underwent validation to remove unusable datasets, final product being able to discriminate most eucalypt species (exceptions being closely related species or subspecies).

Further detailed information for this research can be found in Appendix C.

Figure 4-1 Map provided by UniSC of sample groups included in co-analyses; (A) North and (B) South

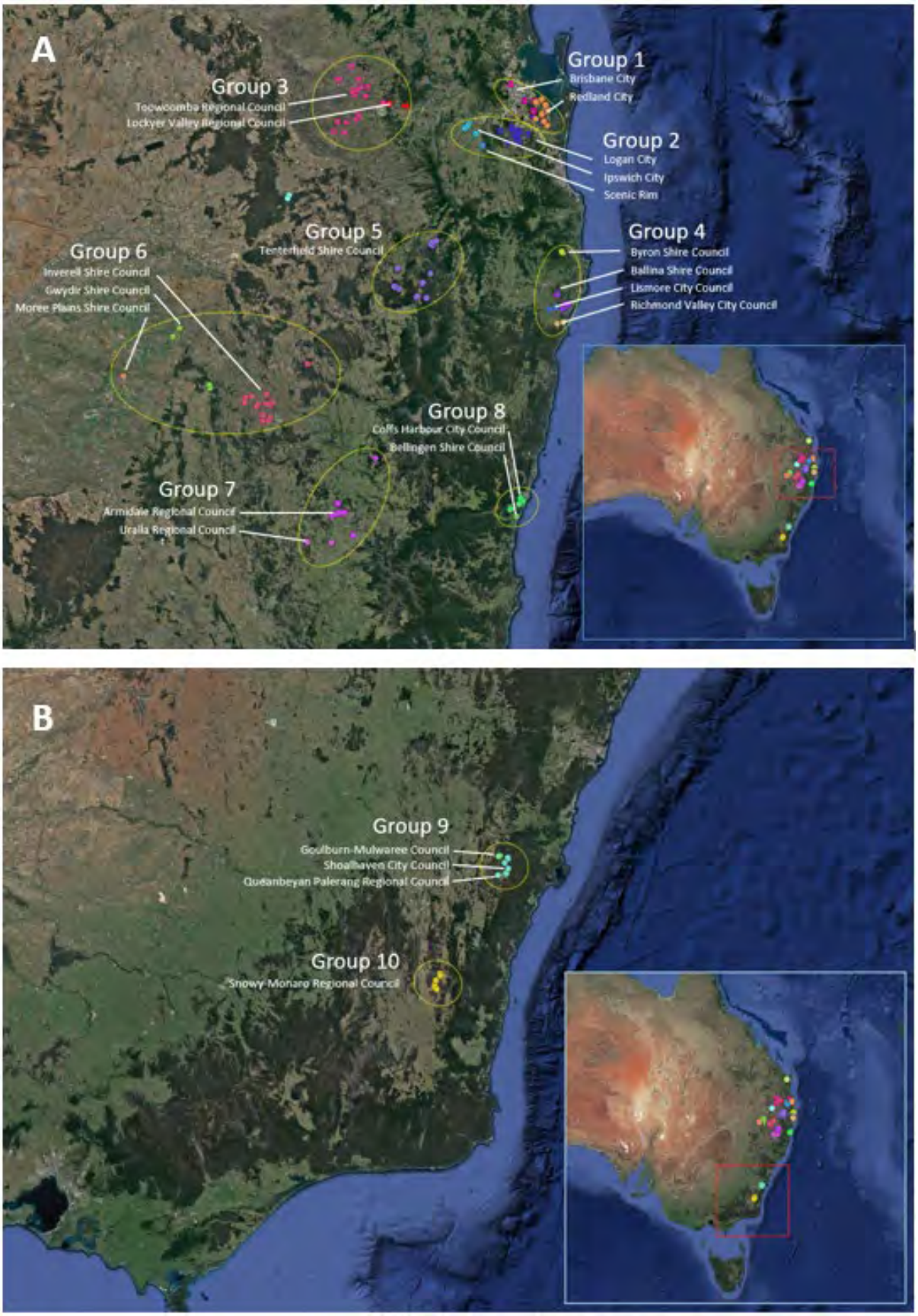


Table 4-4 Groupings of koala samples used for genetic diversity estimates

Group	Sample locations in the group (sample size)	Sample size per group	Number of ARTC Samples
Group 1	Brisbane City (05)	43	
	Redland City (37) (= Koala Coast)		
Group 2	Logan City (17)	24	4
	Ipswich City (06)		6
	Scenic Rim Regional (02)		2
Group 3	Lockyer Valley Regional (10)	34	10
	Toowoomba Regional Council (24)		5
Group 4	Byron Shire Council (02)	25	
	Ballina Shire Council (19)		
	Lismore City Council (02)		
	Richmond Valley Council (02)		
Group 5	Tenterfield Shire Council (31)	31	
Group 6	Inverell Shire Council (29)	36	
	Gwydir Shire Council (04)		2
	Moree Plains Shire Council (03)		3
Group 7	Armidale Regional Council (18)	21	
	Uralla Shire Council (03)		
Group 8	Coffs Harbour City Council (06)	08	
	Bellingen Shire Council (02)		
Group 9	Goulburn Mulwaree Council (07)	17	
	Shoalhaven City Council (09)		
	Queanbeyan–Palerang Regional Council (01)		
Group 10	Snowy Monaro Regional Council (10)	10	

4.5.3 Diet Analysis

4.5.3.1 Sequencing of potential food tree species

DNA was extracted from leaf samples using an automated plate-based extraction protocol, by Diversity Arrays Technology P/L, Canberra, Australia (DArT). The remaining reference leaves were extracted using CTAB extraction buffer with chloroform clean-up and ethanol precipitation. This protocol has been optimised for Eucalyptus by DArT in association with prior clients for Eucalyptus genotyping. Read counts for targets (DNA fragments) and single nucleotide polymorphisms (SNPs) within those targets were determined by DArT using their standard proprietary pipelines (Grewe et al. 2015, Kilian et al. 2012). Species belonging to the different genera and Eucalyptus subgenera were run through the DArT pipelines separately, to maximise the number of potentially useful loci identified. Read proportions for the reference and alternative SNP alleles at each locus were determined using custom code in R studio 1.2.5033.

4.5.3.2 Identification of species-specific SNPs and panel validation

Prior to identification of species-specific SNPs, a Principal Components Analysis was performed on the Hamming genetic distance matrix generated from the SNP data in GenAlEx 6.1 to confirm the species designations of the samples. Samples that did not cluster with their designated species or samples with one or more heterozygous loci were removed from the dataset. Additionally, for a small number of species that were taxonomically distinct from all other species in the datasets (i.e. *Araucaria cunninghamii and Acacia melanoxylon*), in sillico DArTs that were present only in the target species were also identified as potential species-specific genetic markers.

The list of species-specific SNPs identified from the DArTseq data was filtered to retain only a single SNP per unique DArT sequencing fragment to minimise the impact of linkage disequilibrium among potential markers. Up to 30 SNPs/in sillico DArTs were then selected per species for oligo design, using species-specific SNPs that were identified in the middle of the DArT target. Oligos were designed by identifying probe regions flanking the selected SNPs using primer design software in Genious (Drummond et al. 2009). The selected tags (SNPs and flanking regions) were run through a DArTag assay and were hybridised to denatured eucalyptus DNA before final amplification.

The products of DArTag assay were then sequenced on the HiSeq 2500 (Illumina), demultiplexed and targeted SNPs detected using DArT P/L's proprietary analytical pipeline (DArToolbox). There were four species (*E. prava, E. laevopinea E. andrewsii and Araucaria cunninghamii*) that were not included in the validation panel due to a lack of available samples. It was confirmed that these markers did not amplify in any non-target species, however, amplification in the target species could not be confirmed.

After validation 1122 markers were retained and the marker panel was able to discriminate most eucalypt species. There were some exceptions where closely related species or subspecies could not be distinguished, although DNA from one or more of the species in question was confirmed to be present. This was particularly notable for the ironbarks from the series Siderophloiae (*E. crebra, drepanophylla, fibrosa, rhombica, dura, cullenii, whitei, melanophloia*).

4.5.3.3 Diet determination from scats

DNA was extracted from the koala scats using CTAB extraction buffer with chloroform clean-up and ethanol precipitation (Healey et al. 2014). Those SNPs that were confirmed to be species-specific on the DArTag platform were amplified and sequenced from the faecal DNA extracts (as described above) to determine the composition of the koalas' diets. The sequencing read counts were filtered and removed if the average number of reads per detected marker was less than 3 or if fewer than two SNPs were detected in target species that had 3 or more validated markers to remove likely sequencing errors and low frequency contamination. The remaining filtered readers for each marker were normalised to account for differences among markers in amplification efficiency. The number of scaled reads for each target species was then averaged across those markers that were found in the highest proportion of target tree species individuals. The averaged scaled reads per target species were then converted to relative abundance by dividing by the total number of averaged scaled reads across all target species.

4.6 Limitations to Study

While this study helps address current knowledge gaps, focusing the study to within the boundary of the linear IRP and where landowner agreements could be established limited the ability to make profound conclusions for combined Queensland and New South Wales koala populations. In highly disturbed landscapes, such as those typical throughout the agricultural land of the IRP, koala home ranges are likely to be larger than in contiguous forest environments (Ellis, Melzer et al. 2002). Repeat surveys were not undertaken, therefore this does increase the potential of recording false absences for the study.

Additionally, surveys were unable to be extended to areas surrounding the IRP footprint (due to access limitations related to the large number of private landowners unrelated to the IRP), which would have allowed for greater geographic coverage and increased sample sizes, enabling more population-level comparisons. These factors constrained the number of samples that could be collected, limiting our ability to capture the broader landscape and seasonal context of koalas using the project footprint. The adoption of genetic co-analysis was used to address the above stated limitations. The results of this study should not be considered a thorough indication of state-wide koala population levels, however findings can be used as a proxy for koala health and population genetics at the local scale.

5. FIELD SURVEY RESULTS SUMMARY

A total of one hundred and twenty (120) detection dog surveys were undertaken throughout the IRP. Of these, fresh scats and individual koalas were found at forty-one (41) sites with detections made in northern NSW and Southeast Queensland (Figure 5-1). These detections were made within the N2NS, B2G, G2H, H2C, C2K, and K2AB sections of the IRP.

5.1 Scat Detections

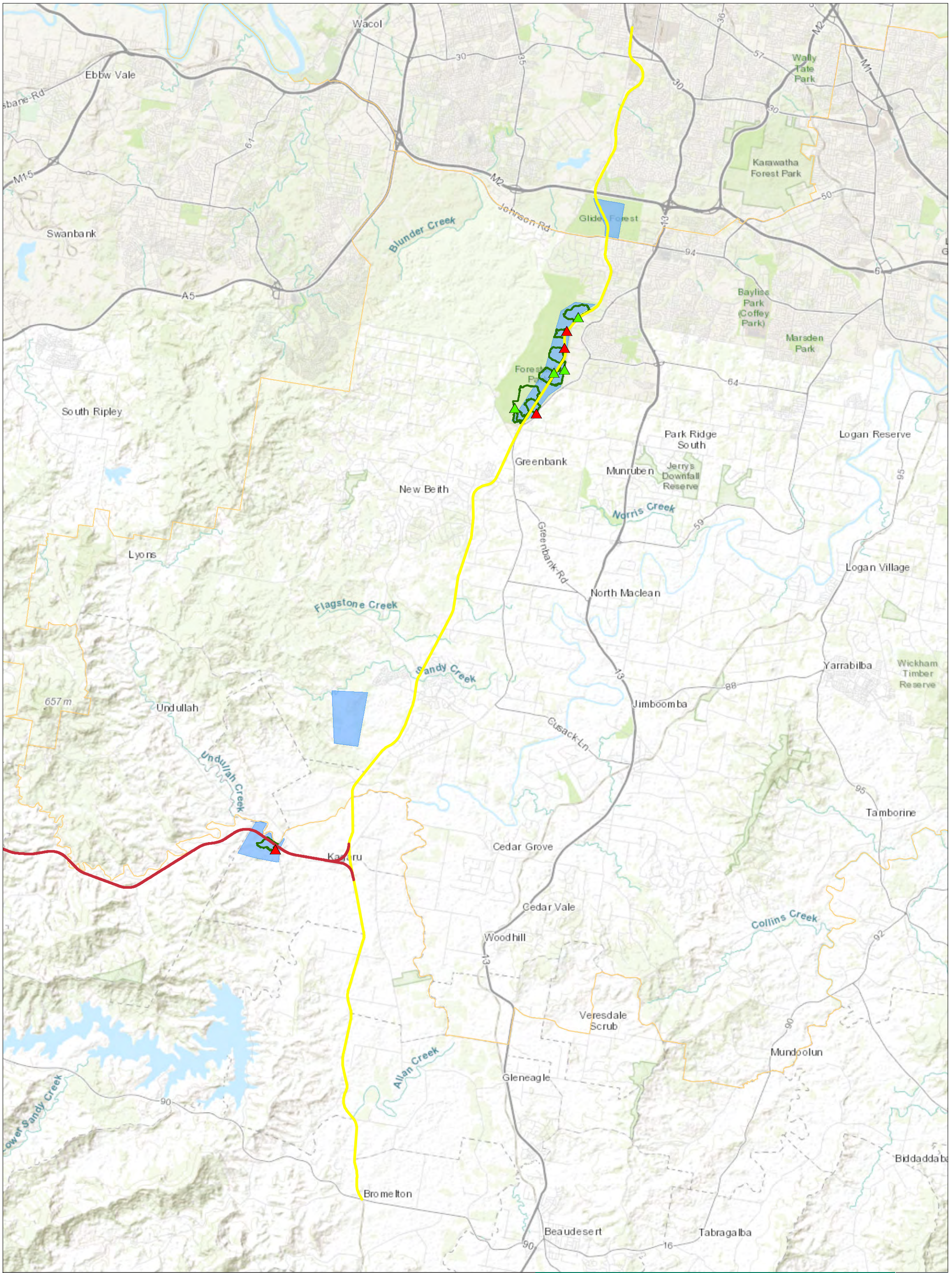
Koala detection dog surveys returned seventy-three (73) fresh scat detections throughout the IRP, resulting in thirty-six (36) unique individuals identified. The majority of these scat detections were found within QLD sections of the IRP. These were spread throughout the city council municipalities of Toowoomba, Logan City, Lockyer Valley Regional Council, City of Ipswich, Goondiwindi Region and Scenic Rim.

The remaining scats were identified in the two NSW council municipalities of Gwydir Shire and Moree Plains Shire in the townships of Croppa Creek and Moree.

5.2 Koala Detections

A total of eight (8) koala detections were made throughout the QLD sections of the IRP, within the city council municipalities of Toowoomba, Lockyer Valley Regional Council, City of Ipswich and Logan City. Koala detections were made incidentally in conjunction with the detection dog surveys, with the scat being traced to a physical individual and located visually by the handler. Five (5) individuals were identified as healthy with no signs of Chlamydia infection. Three (3) dead individuals were found, two (2) of which were identified only through bones. Cause of death could not be determined, however on one (1) dead female no obvious signs of chlamydia infection could be detected. Only one individual in the Lockyer Valley was found with ‘Wet Bum ‘, an indicator of chlamydia infection.

Koala detection dog surveys throughout the NSW sections of the IRP returned a single detection of an individual made in Moree on 22 July 2022. The individual was of unknown sex and no signs of Chlamydia infection could be observed. The individual was located in riparian vegetation along the edge of a river. No scat was found for collection within the vicinity.



Legend

Dog Surveys

- No Detection
- Detection
- Dog Track
- Pre-fieldwork Hotspots
- LGA Boundary

Inland Rail Alignment

- C2K - Calvert to Kagaru
- K2AB - Kagaru to Acacia Ridge/Bromelton

Data Source:
ESRI Would Topographic Map

Locations of Detection Dog Surveys

F5-1a

Drawing No:	0628339_N2AB_TM_G004_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

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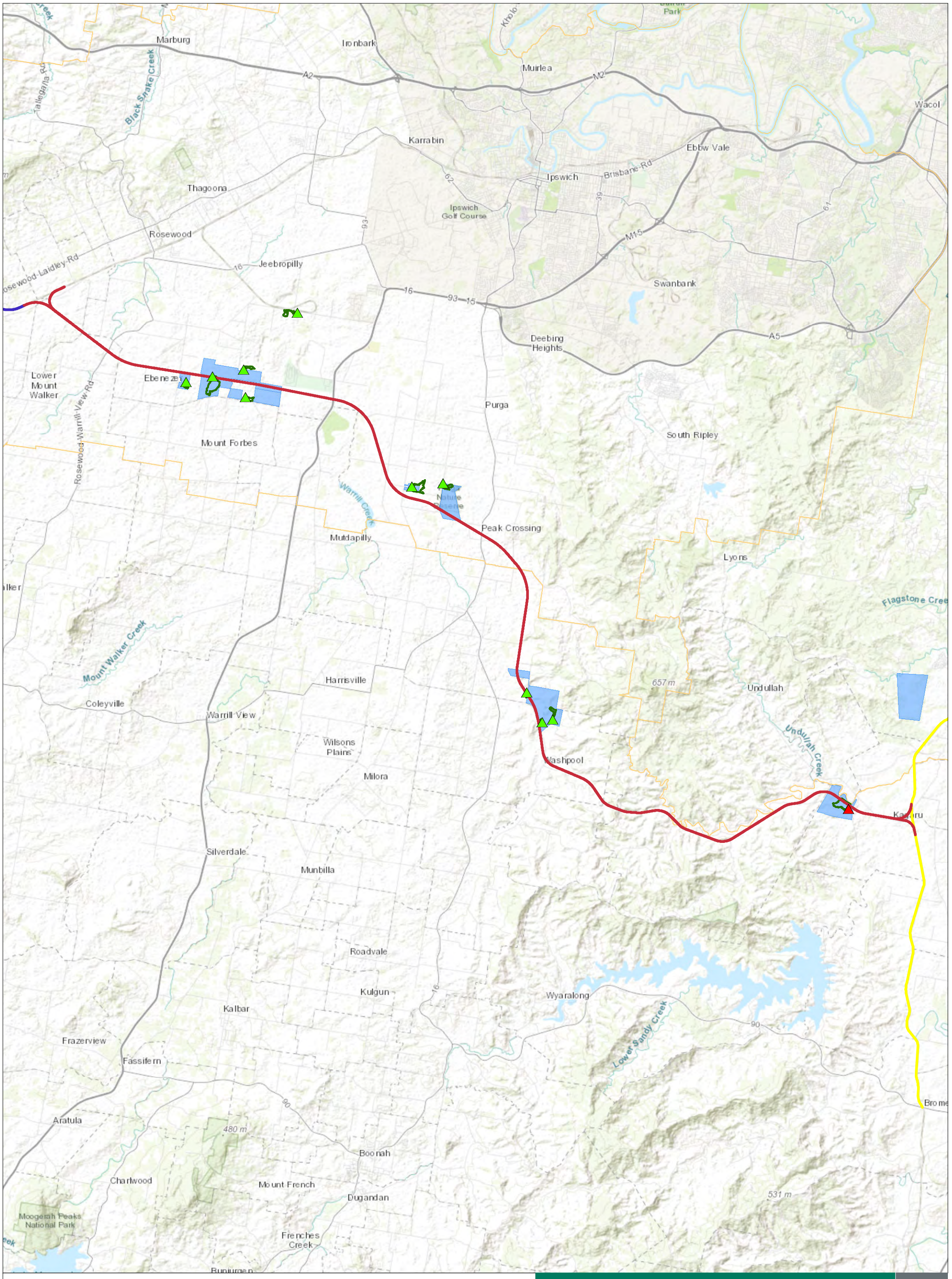


Legend

Dog Surveys
- No Detection
- Detection
- Dog Track
- Pre-fieldwork Hotspots
- LGA Boundary

Inland Rail Alignment
- C2K - Calvert to Kagaru
- H2C - Helidon to Calvert
- K2AB - Kagaru to Acacia Ridge/Bromelton

Data Source:
ESRI Would Topographic Map

Locations of Detection Dog Surveys

F5-1b

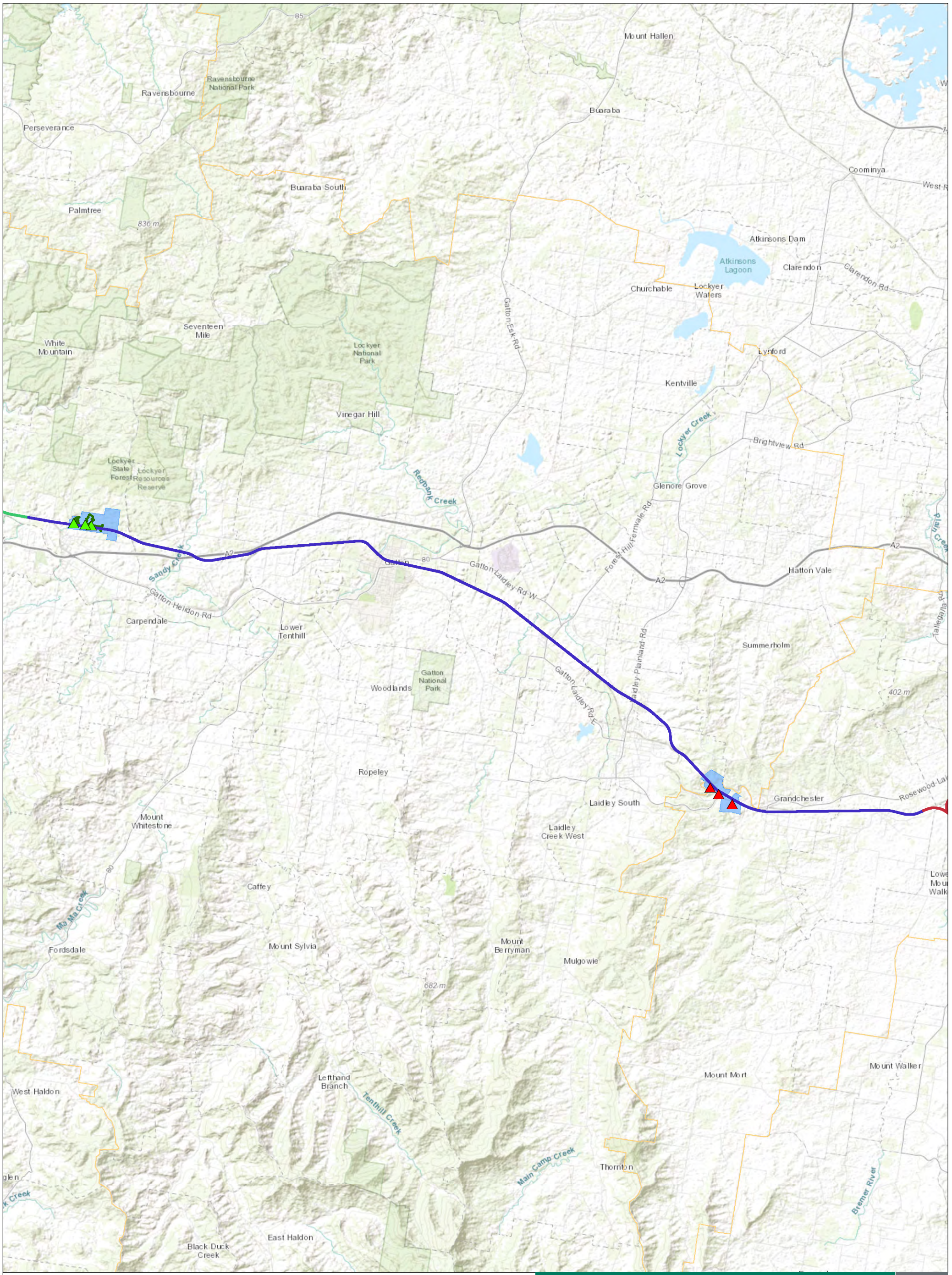
Drawing No:	0628339_N2AB_TM_G004_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

N

ERM



Legend

Dog Surveys
- No Detection
- Detection
- Dog Track
- Pre-fieldwork Hotspots
- LGA Boundary

Inland Rail Alignment
- C2K - Calvert to Kagaru
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert

Data Source:
ESRI Would Topographic Map

Locations of Detection Dog Surveys

F5-1c

Drawing No:	0628339_N2AB_TM_G004_R3.mxd		
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	

Coordinate System: GDA2020 MGA Zone 56

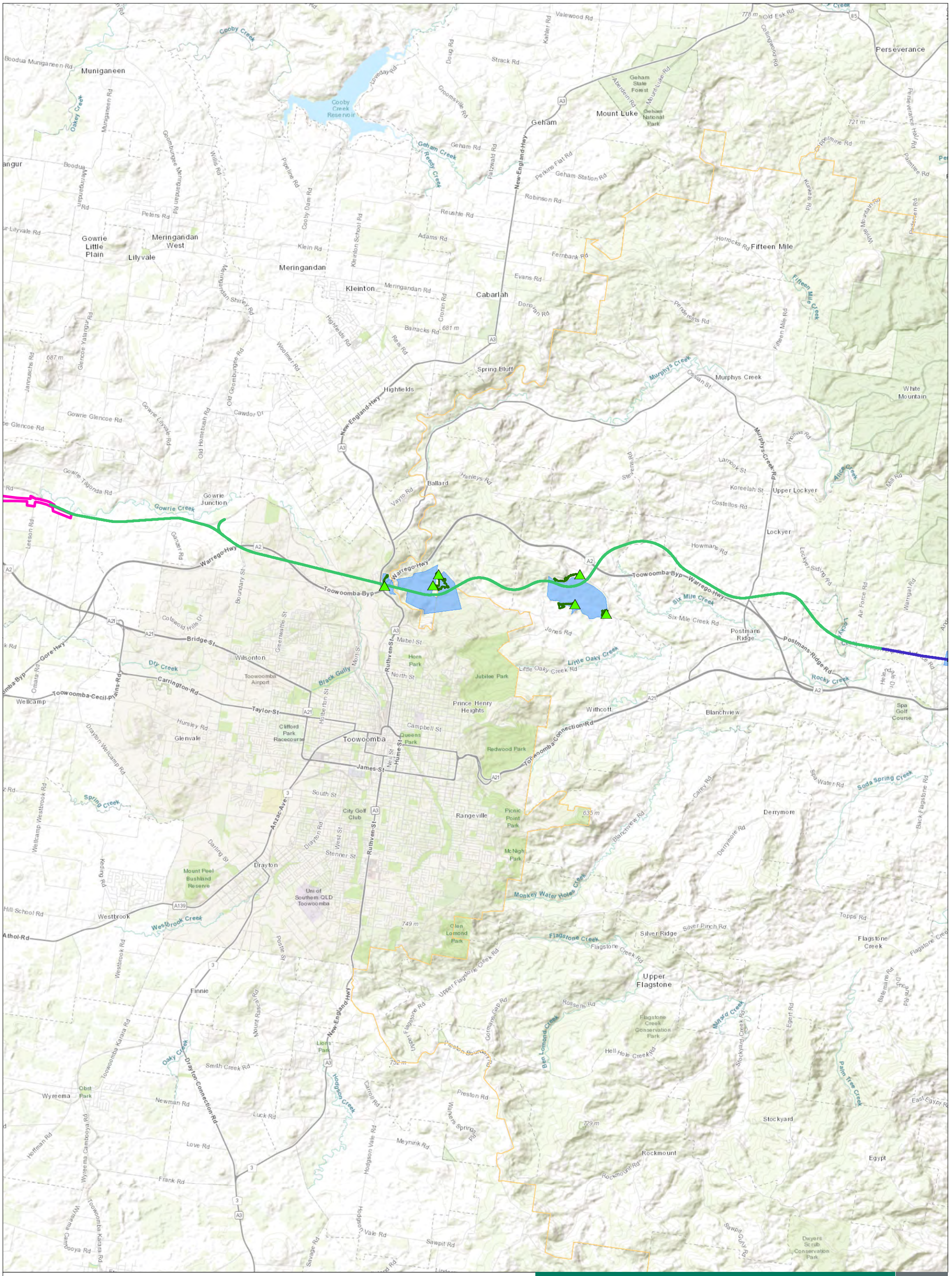
0 2 4Km

N

Koala Genetics Study Project – Inland Rail Program (NSW and QLD)

Client: ARTC

ERM



Legend

Dog Surveys
- Detection
- Dog Track
- Pre-fieldwork Hotspots
- LGA Boundary

Inland Rail Alignment
- B2G - NSW/QLD Border to Gowrie
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert

Data Source:
ESRI Would Topographic Map

Locations of Detection Dog Surveys

F5-1d

Drawing No:	0628339_N2AB_TM_G004_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

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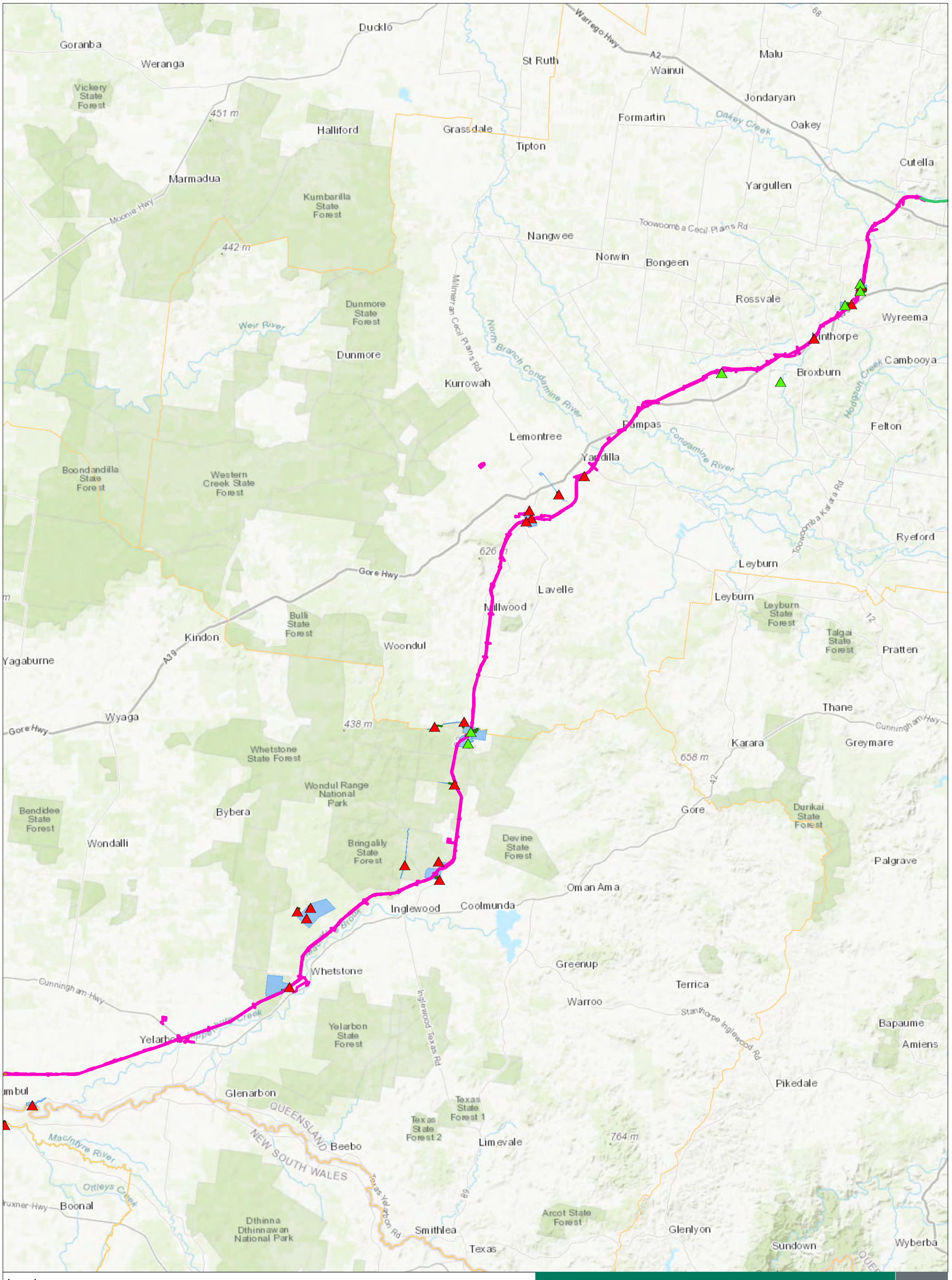

Legend

Dog Surveys
- No Detection
- Detection
- Dog Track
- Pre-fieldwork Hotspots
- LGA Boundary

Inland Rail Alignment
- B2G - NSW/QLD Border to Gowrie
- G2H - Gowrie to Helidon
- NS2B - North Star to Border

Data Source:
ESRI Would Topographic Map

Locations of Detection Dog Surveys

F5-1e

Drawing No:	0628339_N2AB_TM_G004_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

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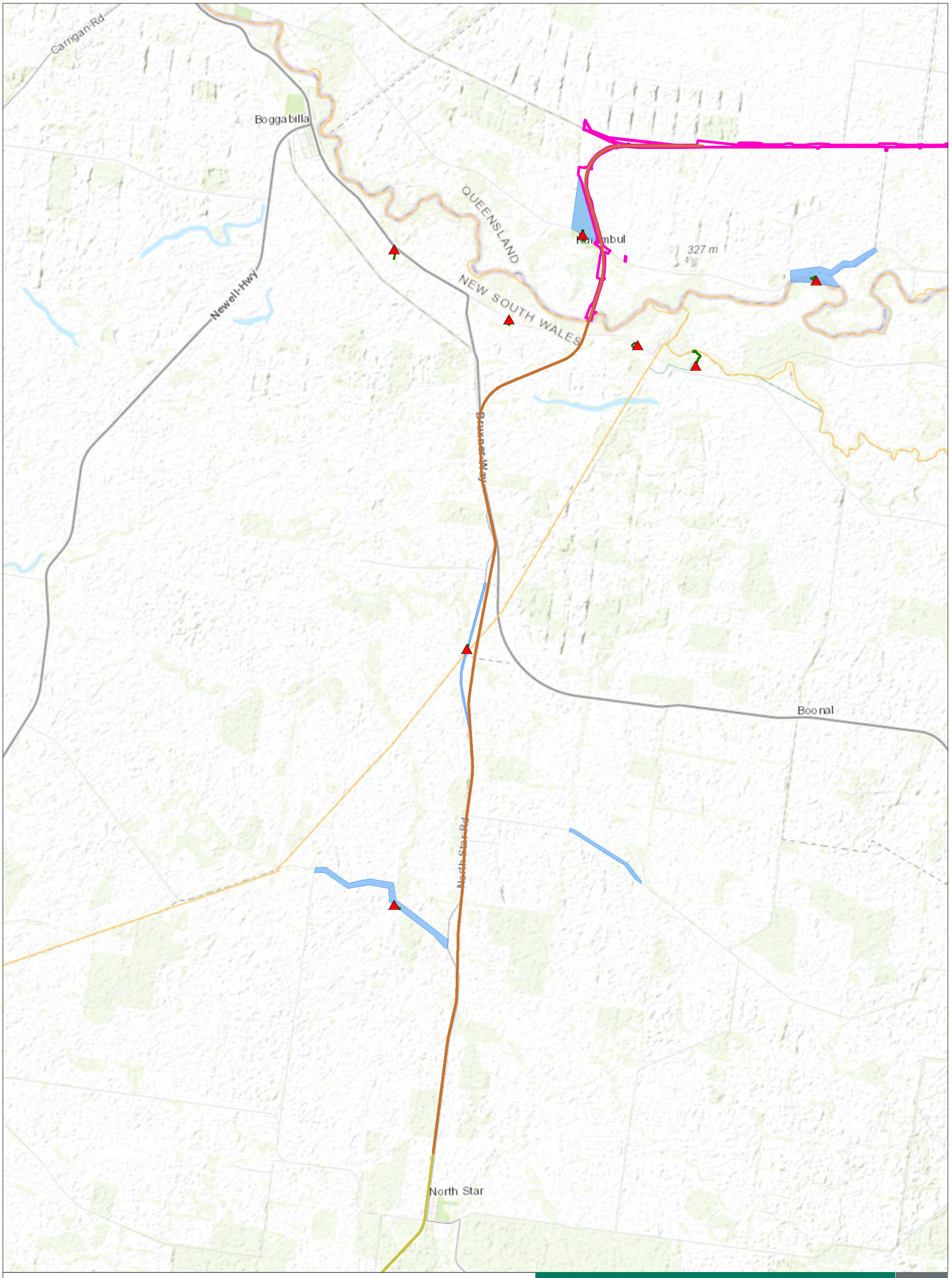

Legend

Dog Surveys
- No Detection
- Dog Track
- Pre-fieldwork Hotspots
- LGA Boundary

Inland Rail Alignment
- B2G - NSW/QLD Border to Gowrie
- N2NS - Narrabri to North Star
- NS2B - North Star to Border

Data Source:
ESRI Would Topographic Map

Locations of Detection Dog Surveys

F5-1f

Drawing No:	0628339_N2AB_TM_G004_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

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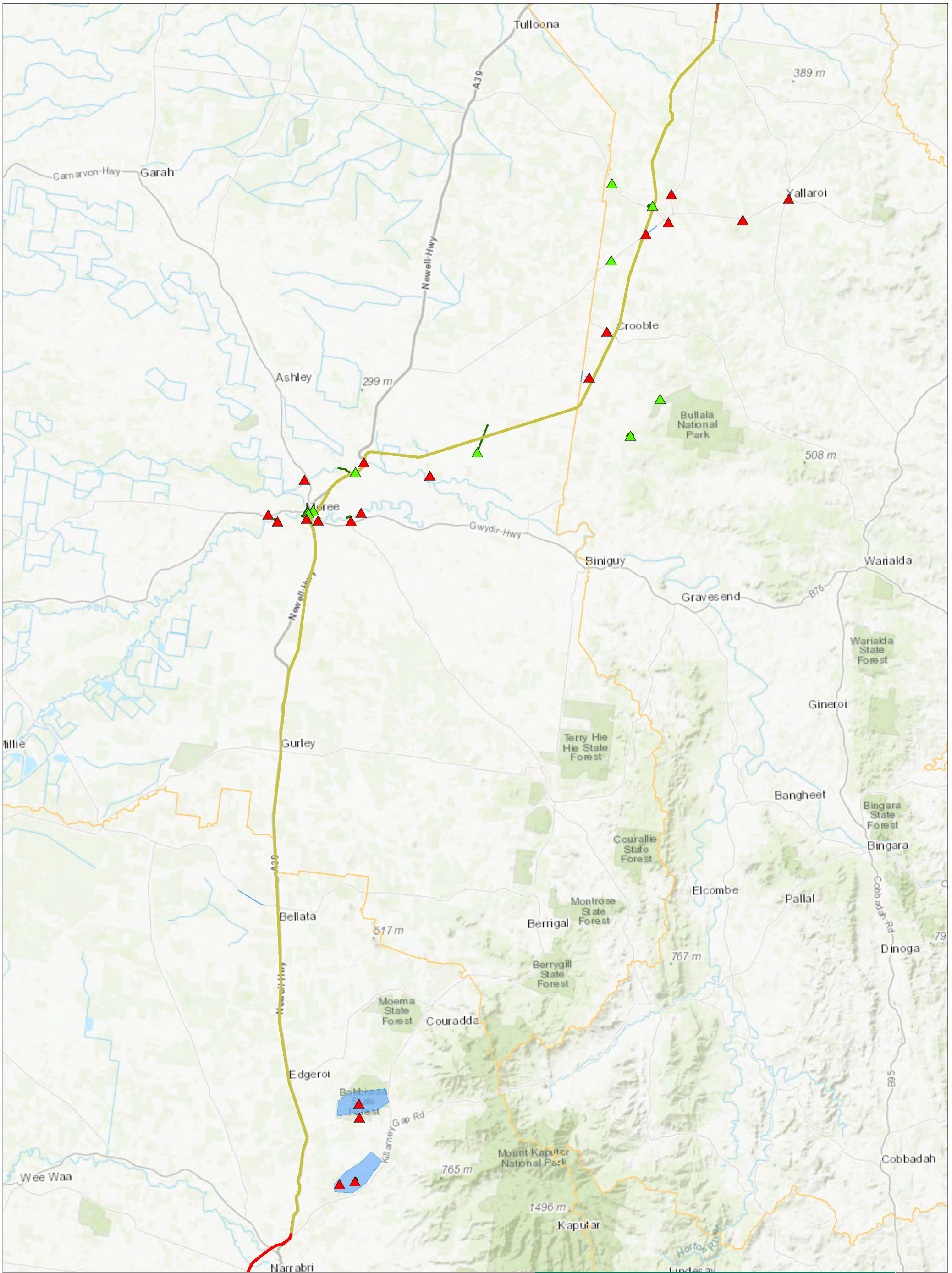

Legend

Dog Surveys
- No Detection
- Detection
- Dog Track
- Pre-fieldwork Hotspots

Inland Rail Alignment
- N2N - Narromine to Narrabri
- N2NS - Narrabri to North Star
- NS2B - North Star to Border

Data Source:
ESRI Would Topographic Map

Locations of Detection Dog Surveys

F5-1g

Drawing No:	0628339_N2AB_TM_G004_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

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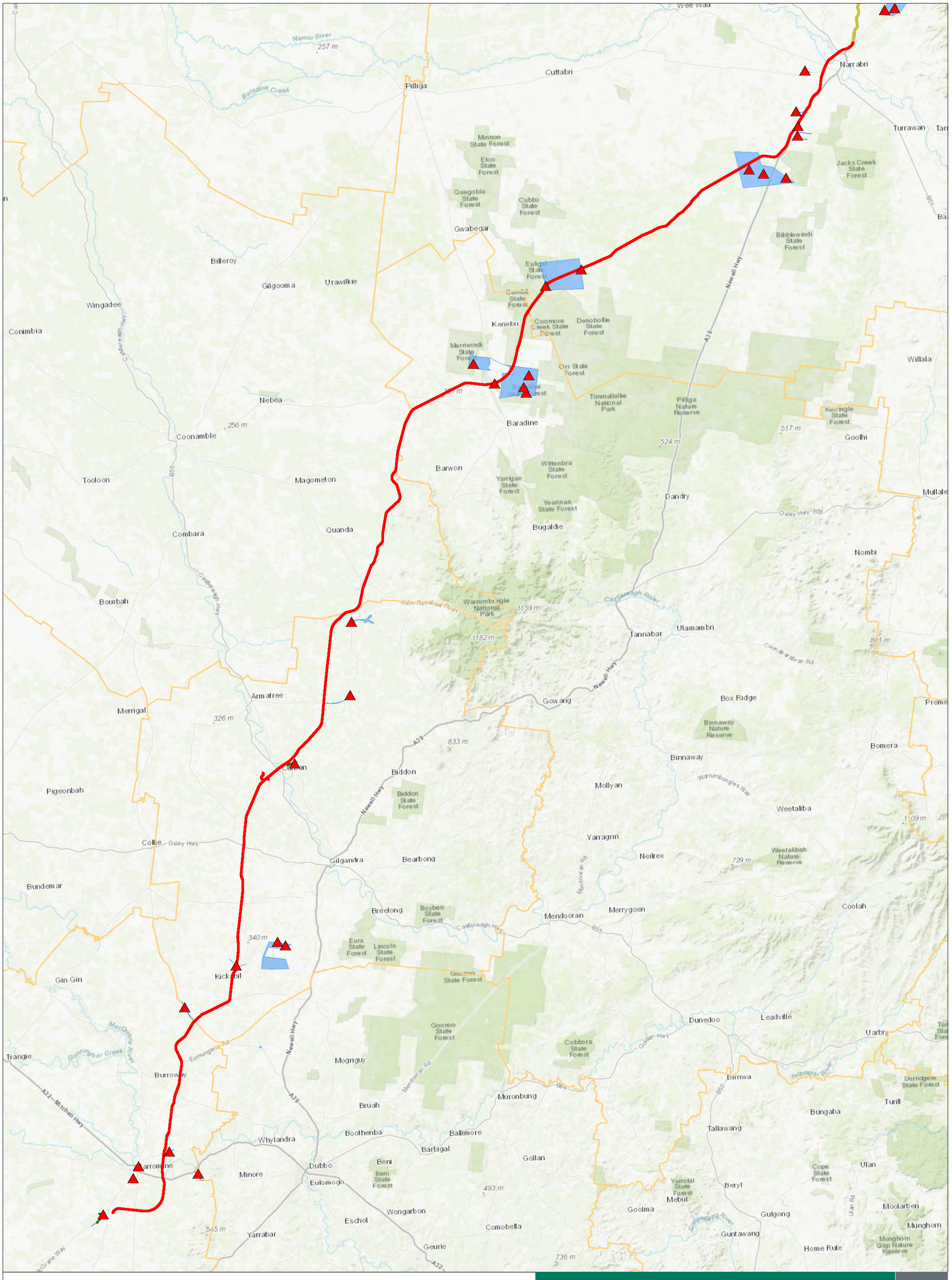

Legend

Dog Surveys
- No Detection
- Dog Track
- Pre-fieldwork Hotspots

Inland Rail Alignment
- N2N - Narromine to Narrabri
- N2NS - Narrabri to North Star

Data Source:
ESRI Would Topographic Map

Locations of Detection Dog Surveys

F5-1h

Drawing No:	0628339_N2AB_TM_G004_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

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ERM

5.3 Vegetation assessments

To gain a broad understanding of the available koala habitat within the IRP a total of ninety-one (91) vegetation assessments were completed alongside the detection dog surveys. See figure 5-2 for a representation of vegetation assessment survey effort.

The landscape throughout the survey sites was dominated by fragmented and disturbed remnant or advanced regrowth vegetation found along roadsides, ephemeral creek lines and publicly accessible areas (i.e., state forest, council parklands).

Overall, three Eucalypts presented as the main dominant canopy species: *Eucalyptus populnea, E. crebra* and *E. camaldulensis*. Each of the regions in the study area displayed varying quality and abundance of koala habitat. Accordingly, the habitat can be broken up into three different sections of the IRP:

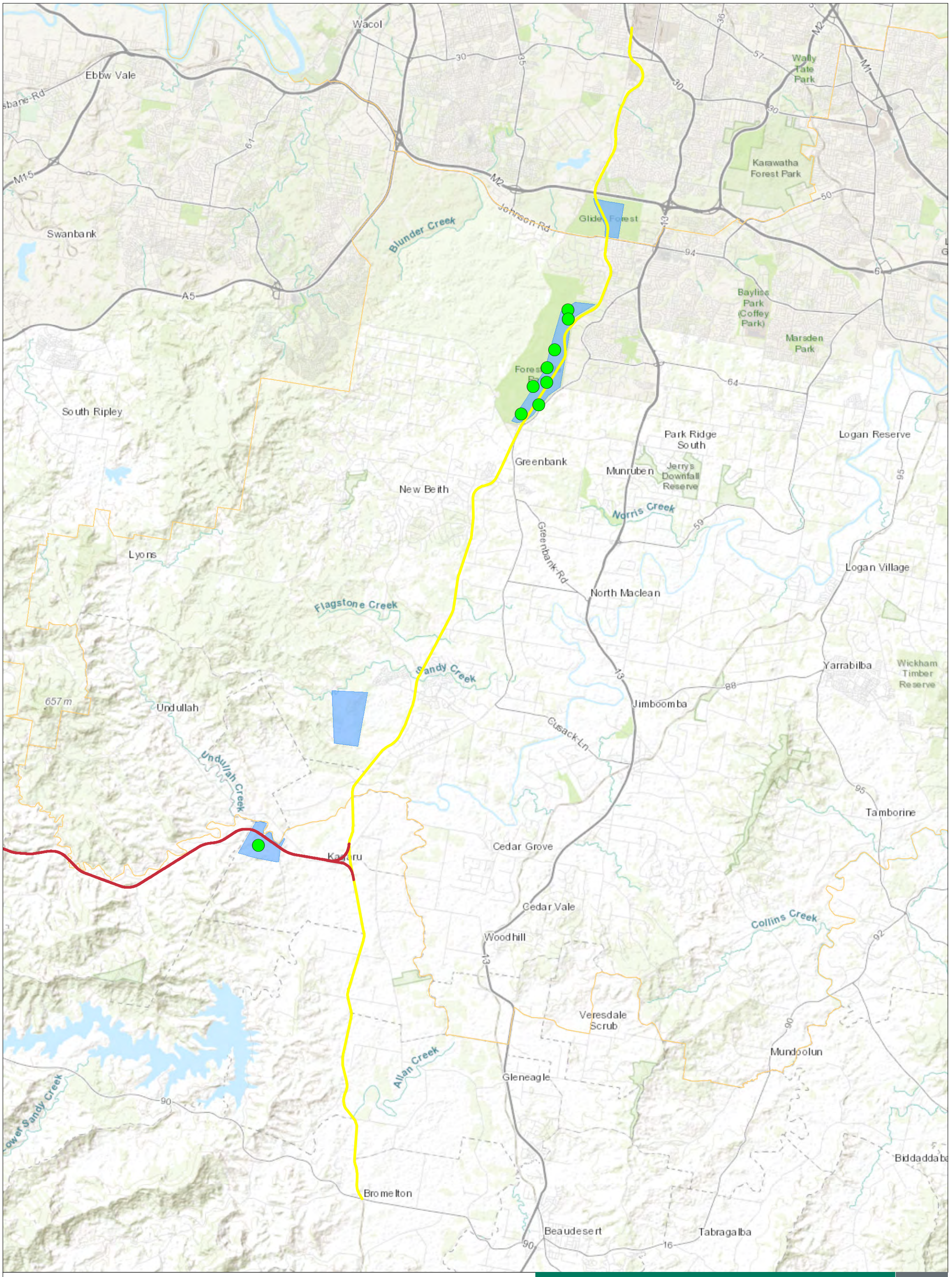
- East of the Toowoomba range (K2ARB, C2K, H2C and G2H);
- West of the Toowoomba range to the Queensland-NSW border (B2G); and
- New South Wales (NS2B, N2NS and N2N).

East of the Toowoomba Range vegetation surveys showed canopy species being dominated by *Eucalyptus crebra, E. citriodora and Corymbia intermedia*.

West of the Toowoomba region found canopy species being dominated by *E. populnea, E. camaldulensis, E. chloroclada.*

The NSW regions found canopy species being dominated by *E. camaldulensis, E. populnea and E. crebra.*

A further breakdown of koala food-tree availability across the projects of the IRP is found in Section 9 Koala Habitats in the Study Area.



Legend

- Quaternary Assessment
- Pre-fieldwork Hotspots
- LGA Boundary
- Inland Rail Alignment

Data Source:
ESRI Would Topographic Map

Vegetation Assessments Across the IRP

F5-2a

Drawing No:	0628339_N2AB_TM_G005_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

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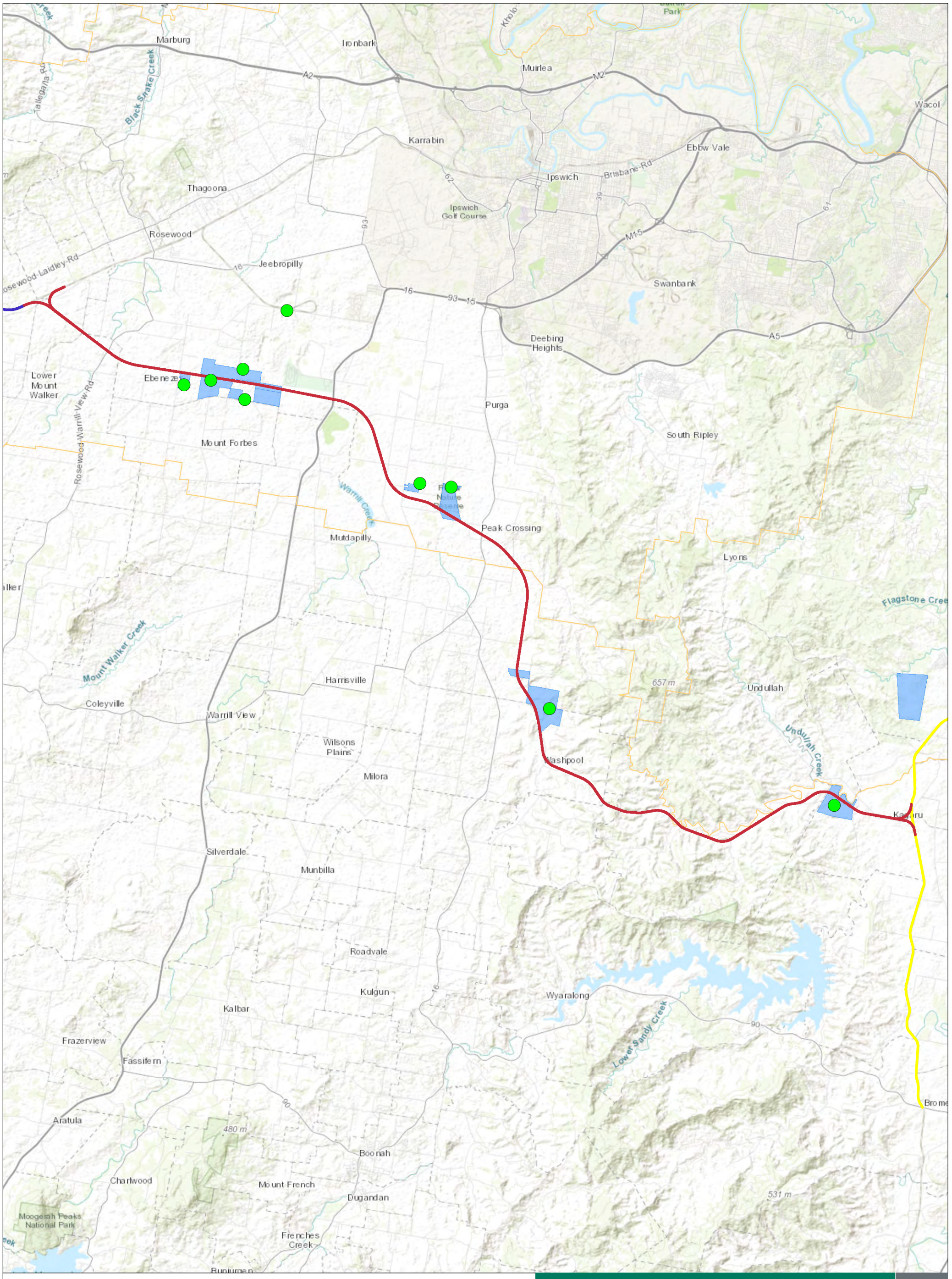

Legend

- Quaternary Assessment
- Pre-fieldwork Hotspots
- LGA Boundary
- **Inland Rail Alignment**

Data Source:
ESRI Would Topographic Map

Vegetation Assessments Across the IRP

F5-2b

Drawing No:	0628339_N2AB_TM_G005_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

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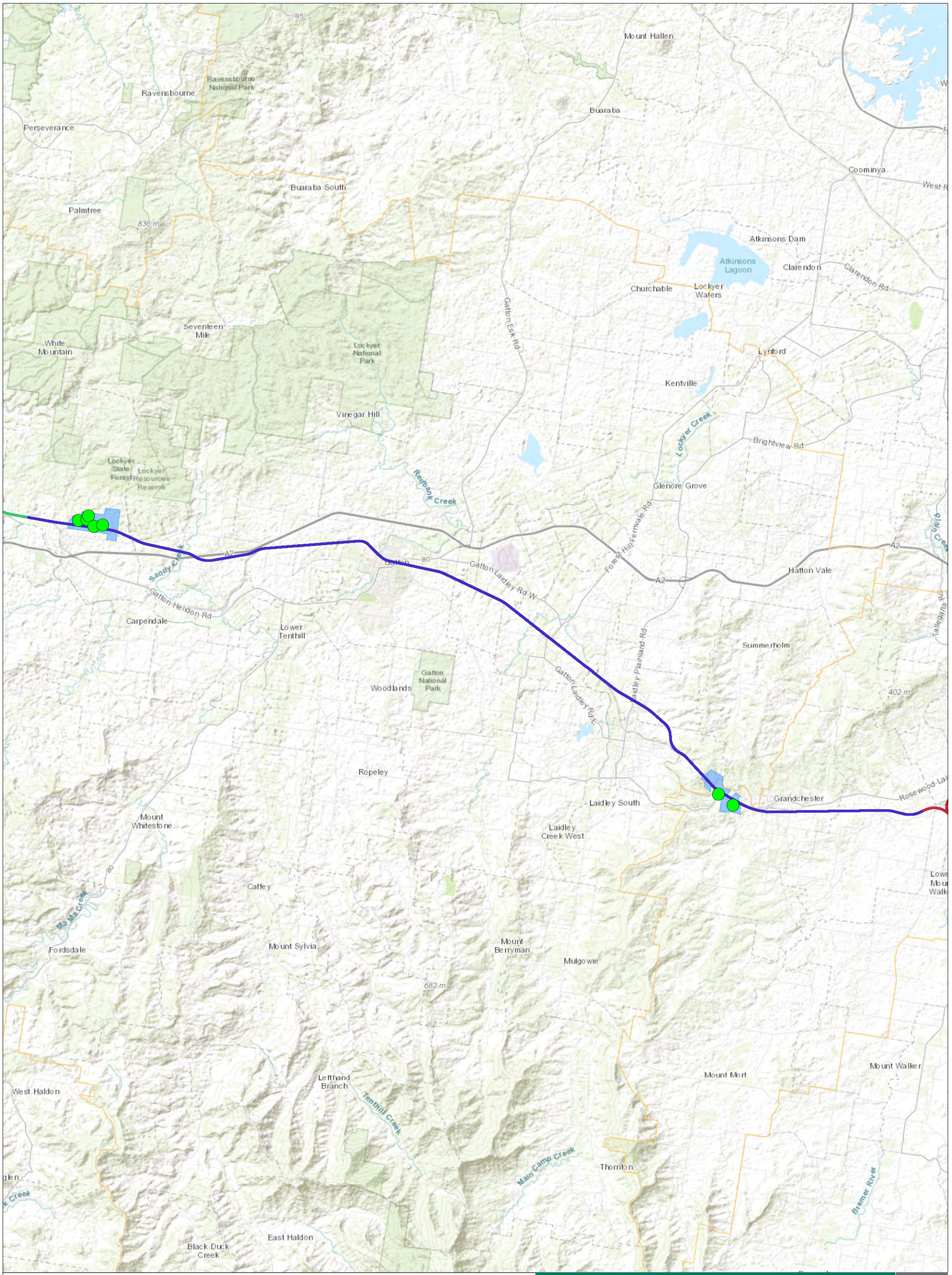

Legend

- Quaternary Assessment
- Pre-fieldwork Hotspots
- LGA Boundary
- **Inland Rail Alignment**

Data Source:
ESRI Would Topographic Map

Vegetation Assessments Across the IRP

F5-2c

Drawing No:	0628339_N2AB_TM_G005_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC
Coordinate System: GDA2020 MGA Zone 56			This figure may be based on third party data or data which has not been verified by ERM and it may not be to scale. Unless expressly agreed otherwise, this figure is intended as a guide only and ERM does not warrant its accuracy.

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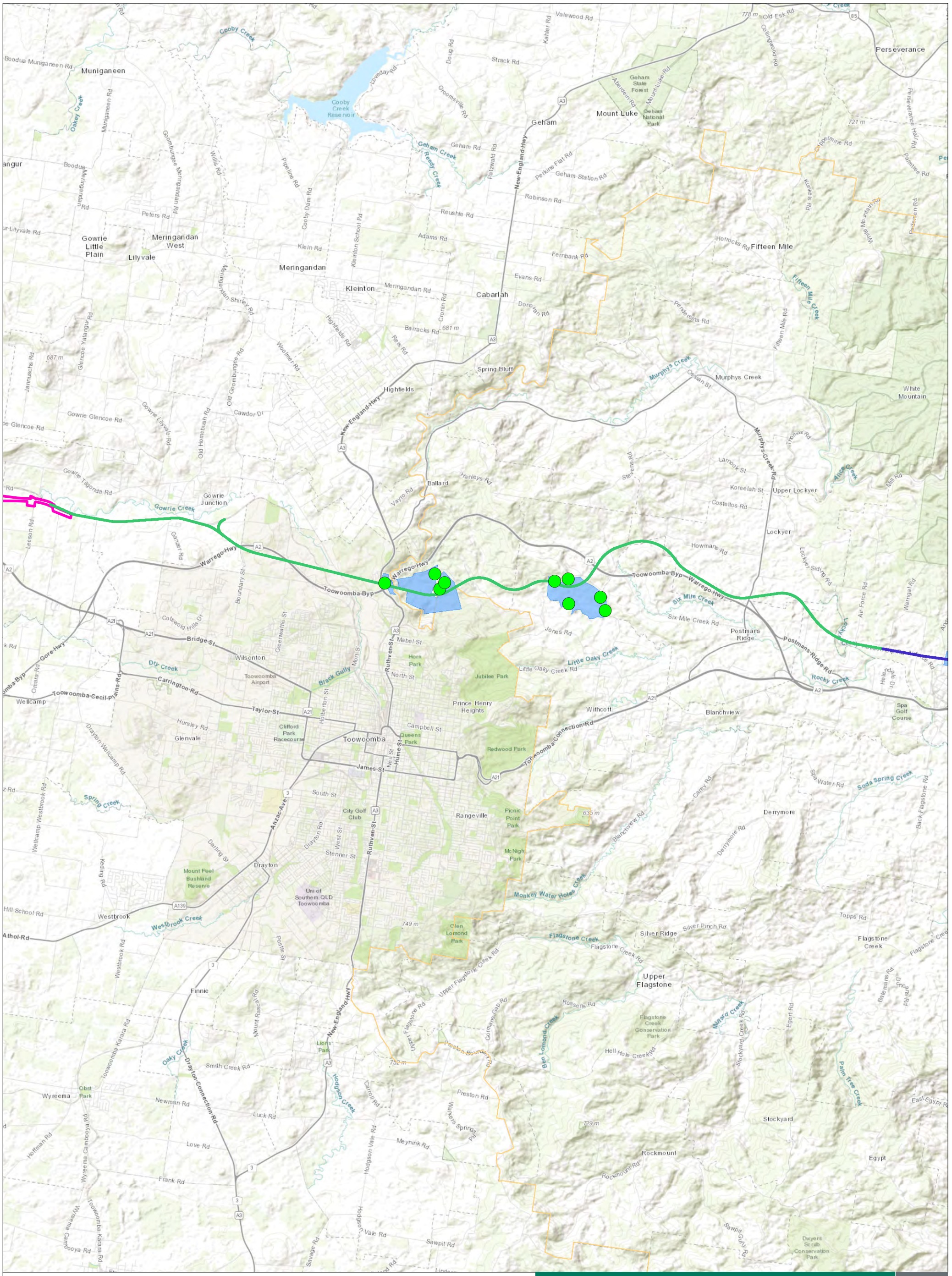
Cooby Creek
Boodua Muniganeen Rd
Muniganeen
Oakey Creek
Muniganeen Rd
Goombungee Meringandan Rd
Willis Rd
Cooby Creek Reservoir
Loveday Rd
Doug Rd
Strack Rd
Kahler Rd
Valewood Rd
Groomsville Rd
Geham Creek
Reedy Creek
Geham Rd
Patzwald Rd
Geham
New England Hwy
Mount Luke
Geham State Forest
Aberdeen Rd
Mount Luke Rd
Geham National Park
778 m
Old Esk Rd
Collingwood Rd
Perseverance
721 m
Perseverance Haul Rd
Palmtree Rd
Carpetmine Rd
Kunkels Rd
Mountain Rd
Pedersen Rd
Perkins Flat Rd
Geham Station Rd
Robinson Rd
Reushle Rd
Peters Rd
Pipeline Rd
Cooby Dam Rd
Kleinton School Rd
Adams Rd
Klein Rd
Gowrie Little Plain
Lilyvale
Meringandan West
Meringandan
Meringandan Shirley Rd
Kleinton
Meringandan Rd
Cabarlah
Cronin Rd
Fernbank Rd
Evans Rd
Horrocks Rd
Fifteen Mile
Fifteen Mile Creek
Fifteen Mile Rd
Donovan Rd
Penderests Rd
Barracks Rd
681 m
Highfields Rd
Reis Rd
Woolmer Rd
Spring Bluff
Murphys Creek
Ossian St
White Mountain
Highfields
New England Hwy
Old Goombungee Rd
Cawdor Dr
Gowrie Glencoe Rd
Gowrie Lilyvale Rd
Old Homebush Rd
Glencoe Yalangur Rd
Jannuschs Rd
687 m
Tronos Rd
Gowrie Tilgonda Rd
Gowrie Junction
Gowrie Creek
Leeson Rd
Ganzer Rd
Warrego Hwy
Toowoomba Byp
Ballard
Vayro Rd
Hanleys Rd
Stevens Rd
Larnook St
Koreelah St
Upper Lockyer
Alice Creek
Mill Rd
Costellos Rd
Lockyer
Howmans Rd
Lockyer Siding Rd
Air Force Rd
Warrigal Rd
Lockyer Creek
Six Mile Creek
Six Mile Creek Rd
Postmans Ridge
Postmans Ridge Rd
Rocky Creek
Jones Rd
Little Oaky Creek
Little Oaky Creek Rd
Withcott
Blanchview
Spa Golf Course
Toowoomba Connection Rd
Wellcamp
Toowoomba Cecil Plains Rd
Gore Hwy
Omara Rd
Dry Creek
Carrington Rd
Taylor St
Bridge St
Wilsonton
Cotswold Hills Dr
Greenwattle St
Boundary St
Toowoomba Airport
Black Gully
Hopetoun St
Mort St
Ruthven St
Mabel St
Horn Park
North St
Jubilee Park
Prince Henry Heights
Clifford Park Racecourse
Hursley Rd
Glenvale
Toowoomba
Campbell St
Queens Park
Hume St
Neil St
James St
Redwood Park
Drayton Wellcamp Rd
Spring Creek
Wellcamp Westbrook Rd
South St
Anzac Ave
City Golf Club
Rangeville
Picnic Point Park
635 m
Darling St
Drayton Rd
West St
Stenner St
Ruthven St
Drayton
Mount Peel Bushland Reserve
Kerry Rd
Uni of Southern QLD Toowoomba
Monkey Water Hole Creek
Soda Spring Creek
Spa Water Rd
Carey Rd
Blanchview Rd
Derrymore
Derrymore Rd
Back Flagstone Rd
Hill School Rd
Westbrook
Westbrook Creek
Athol Rd
Westbrook Rd
Finnie
Mount Rascal Rd
Toowoomba Karara Rd
Oaky Creek
Drayton Connection Rd
Smith Creek Rd
Lions Park
752 m
Glen Lomond Park
749 m
Flagstone Creek
Flagstone Creek Rd
Silver Ridge
Silver Pinch Rd
Topps Rd
Upper Flagstone
Upper Flagstone Creek Rd
Flagstone Rd
Germans Gap Rd
Rossells Rd
Ben Lomond Creek
Flagstone Creek Conservation Park
Hell Hole Creek Rd
Stockyard Creek Rd
Egert Rd
Batemans Rd
Orchard Rd
Palm Tree Creek
Preston Boundary Rd
Preston Rd
Walkers Springs Rd
Meynink Rd
Hodgson Creek
Carroll Rd
Hodgson Vale Rd
Sawpit Rd
Savage Rd
Wyreema
Obst Park
Newman Rd
Luck Rd
Love Rd
Frank Rd
Heffman Rd
Wyreema Cambooya Rd
Toowoomba Karara Rd
Stockyard
729 m
Rockmount
Rockmount Rd
Egypt
Dwyers Scrub Conservation Park
Sawpit Gully Rd
Data Source:
ESRI Would Topographic Map
Legend
Quaternary Assessment
Pre-fieldwork Hotspots
LGA Boundary
Inland Rail Alignment
Vegetation Assessments Across the IRP
F5-2d
Drawing No: 0628339_N2AB_TM_G005_R3.mxd
Date: 14/02/2024
Drawing Size: A4
Drawn By: VN/CB
Reviewed By: MD
Coordinate System: GDA2020 MGA Zone 56
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Koala Genetics Study Project – Inland Rail Program (NSW and QLD)
Client: ARTC
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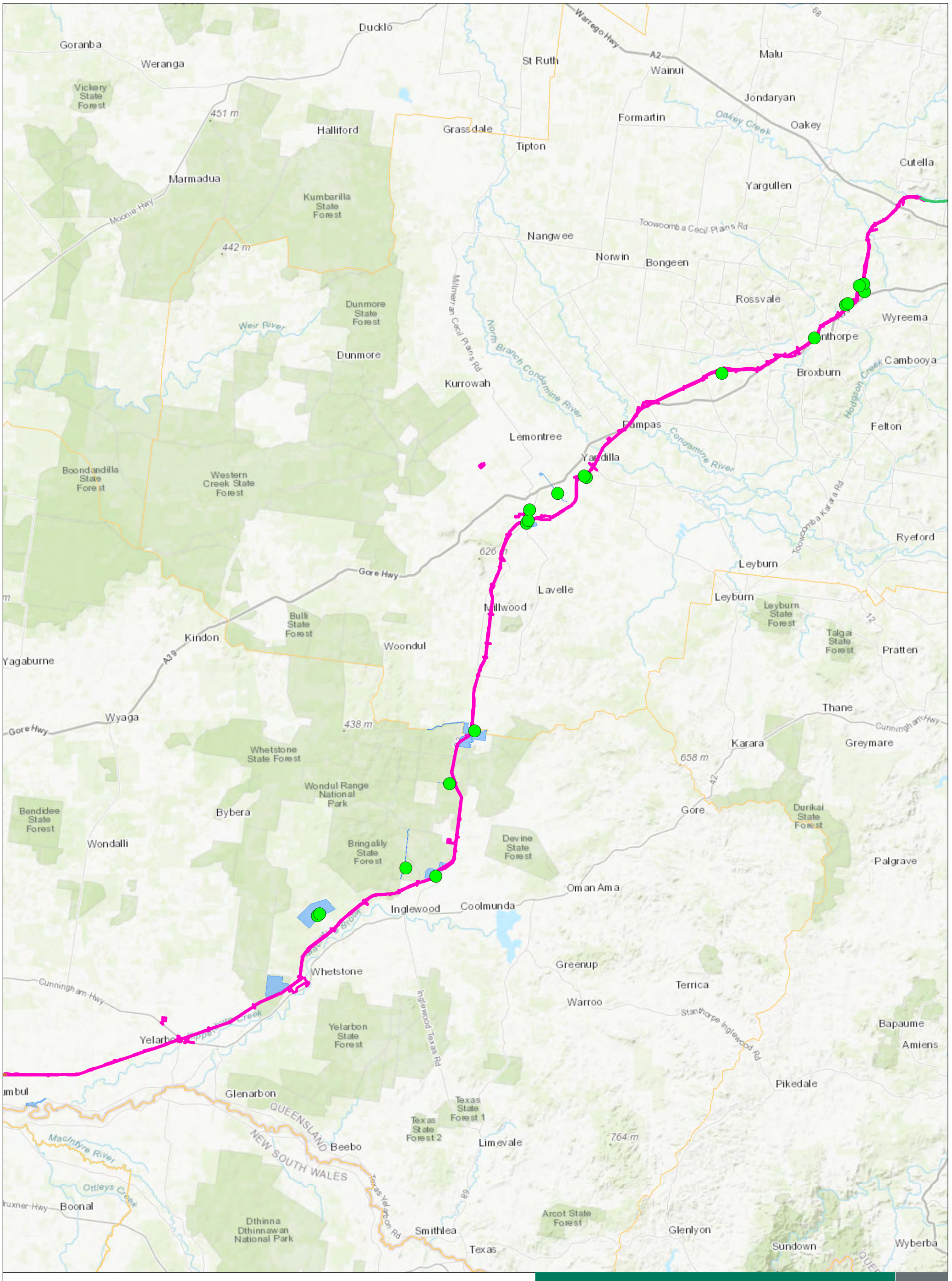

Legend

- Quaternary Assessment
- Pre-fieldwork Hotspots
- LGA Boundary
- **Inland Rail Alignment**

Data Source:
ESRI Would Topographic Map

Vegetation Assessments Across the IRP

F5-2e

Drawing No:	0628339_N2AB_TM_G005_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
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Coordinate System: GDA2020 MGA Zone 56

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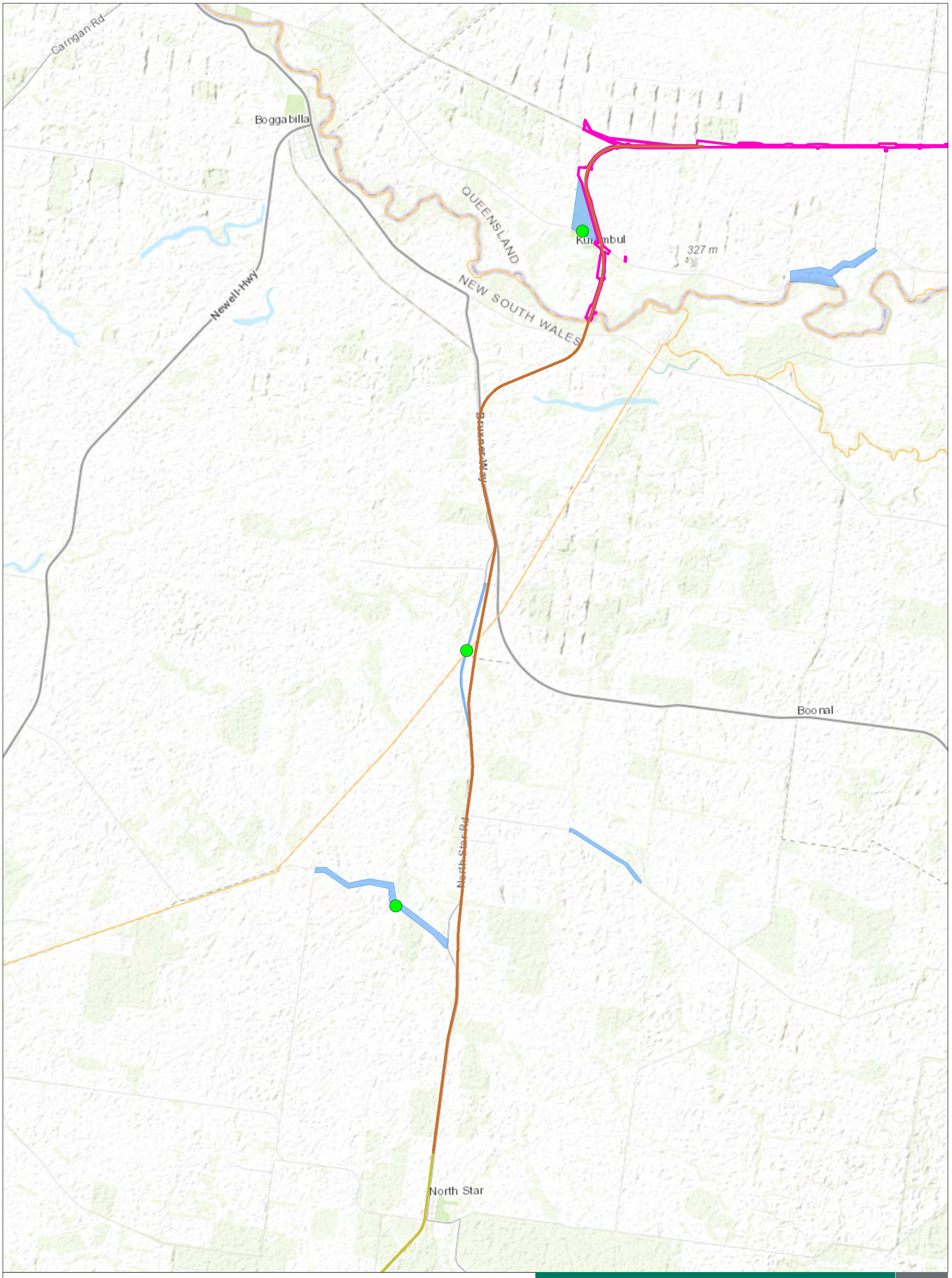

Legend

- Quaternary Assessment
- Pre-fieldwork Hotspots
- LGA Boundary
- **Inland Rail Alignment**

Data Source:
ESRI Would Topographic Map

Vegetation Assessments Across the IRP

F5-2f

Drawing No:	0628339_N2AB_TM_G005_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
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Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

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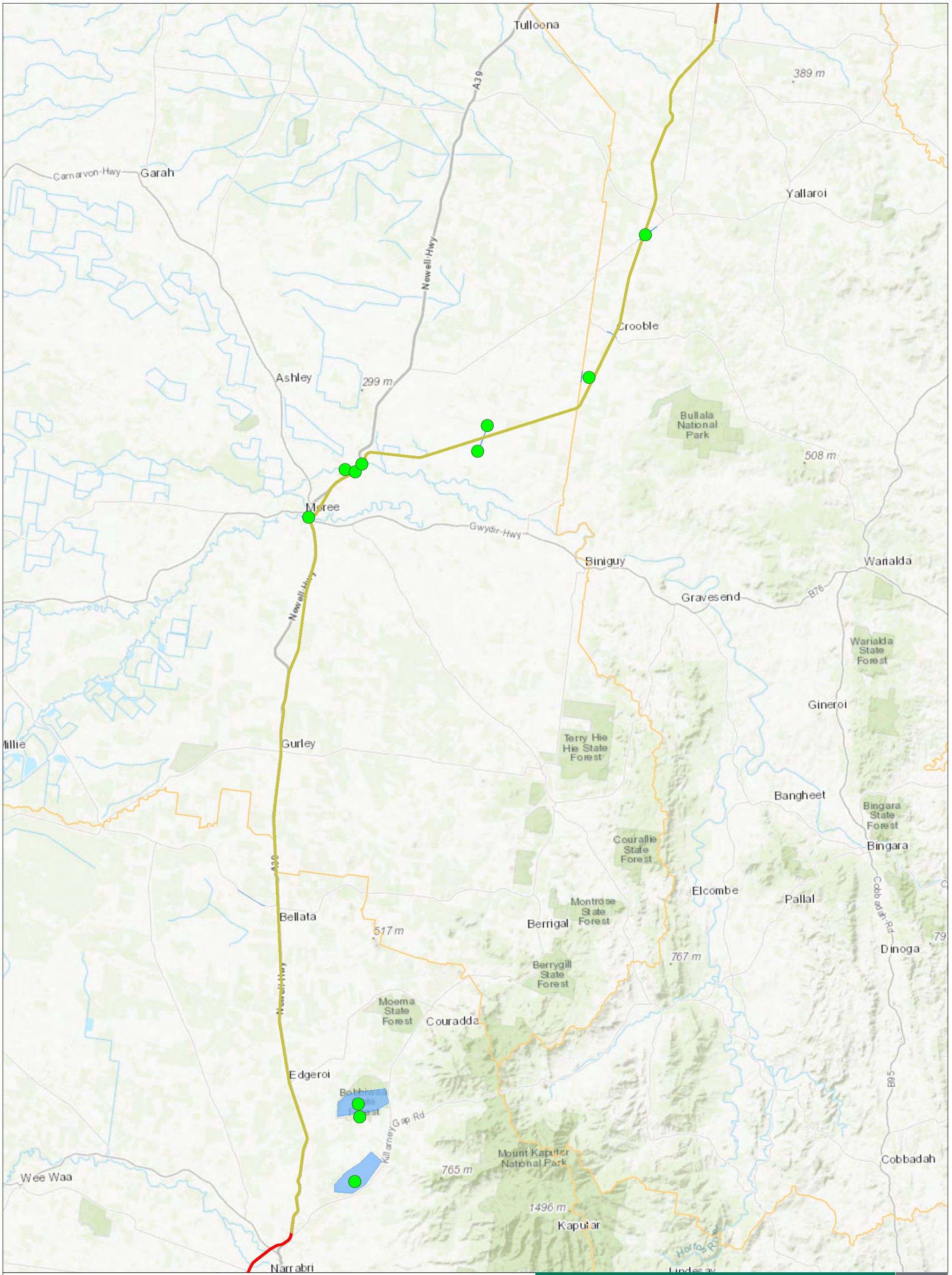

Legend

- Quaternary Assessment
- Pre-fieldwork Hotspots
- **Inland Rail Alignment**

Data Source:
ESRI Would Topographic Map

Vegetation Assessments Across the IRP

F5-2g

Drawing No:	0628339_N2AB_TM_G005_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
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Drawn By:	VN/CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

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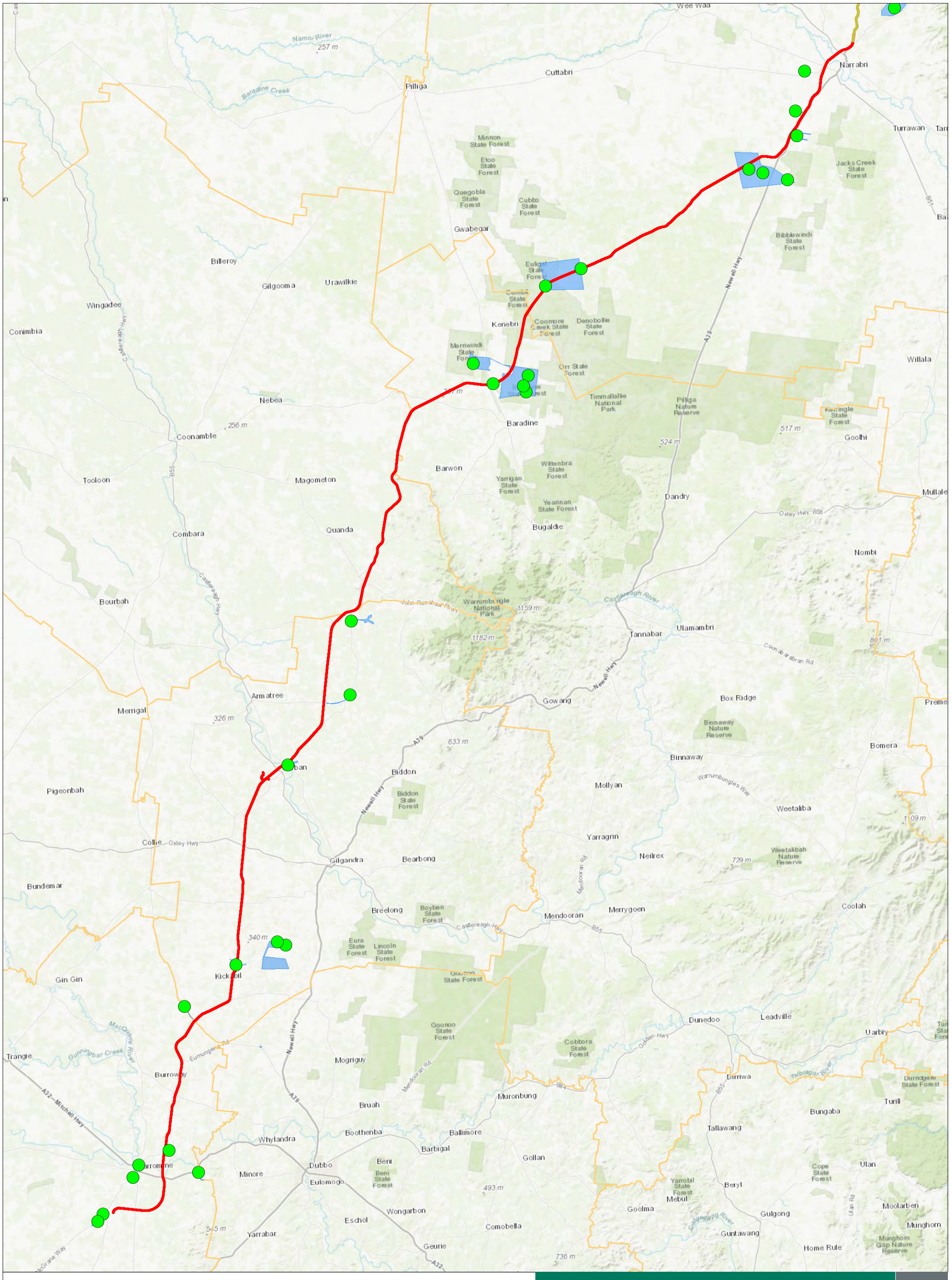

Legend

Quaternary Assessment

Pre-fieldwork Hotspots

Inland Rail Alignment

Data Source:
ESRI Would Topographic Map

Vegetation Assessments Across the IRP

F5-2h

Drawing No:	0628339_N2AB_TM_G005_R3.mxd	**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4
Drawn By:	VN/CB	Reviewed By: MD
Coordinate System: GDA2020 MGA Zone 56		Client: ARTC

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ERM

6. GENETIC ANALYSIS RESULTS AND DISCUSSION

Genetic analysis of koala scats detected using detection dog surveys was completed by the Detection Dogs for Conservation research group at the University of the Sunshine Coast. This section provides a compilation of the analysis and findings written by the team from UniSC, with the draft technical outputs from UniSC included in Appendix A.

6.1 Extraction, quality control unique individuals

A total of 73 fresh koala scat samples were collected for genetic analyses. All samples were genotyped using DArTag, however, as is typical in genotyping, particularly with non-invasive samples, genotypes differed in DNA quality. Therefore, some samples were excluded from subsequent analyses due to insufficient data. Figure 2-3 in Appendix A shows the location of all genetic samples and also which samples were used in subsequent analyses (successful genotyping), samples that were duplicates of the same individual koala (successful genotyping but removed due to duplication) and samples with poor DNA quality (insufficient genotype data). For further details see Appendix A Table A3.

Only approximately 200 SNPs are required for identifying unique individuals (Schultz et al. 2018); therefore, the focus was on maximising the number of individuals that could be used while retaining sufficient high-quality SNPs. In addition to the constant filters described in the methods, we applied a stepwise increasing locus call rate filtering, from 0.2 to 0.9, resulting in only retaining SNPs with at least 90% data. Samples were also filtered for individual call rate using an increasing call rate filtering from 0.1 to 0.2, resulting in only retaining individuals with at least 20% data. This resulted in the removal of 22 samples due to insufficient data, with 51 genotyped samples left for this analysis. A total of 230 loci were retained with 8.4% missing data.

Further genetic analyses revealed that there were 25 samples had at least one duplicate sample (scats collected from the same individual; see Appendix A Table A4). Sixteen of the duplicates were removed from the data set, as they did not represent unique individuals. This resulted in a total of 36 unique individual koalas successfully sampled. We have therefore genetically identified the minimum number of unique individuals present at the time of survey, but not the maximum, due to samples that did not meet our data quality thresholds being excluded.

6.1.1 Measures of Genetic Diversity

For detailed genetic analyses, such as determining genetic diversity, a different filtering process is required. For these analyses, the focus was on maximising the quality of the data while trying to maintain as many individuals as possible. In addition to the constant filters, we applied a stepwise increasing locus call rate filtering, from 0.2 to 0.8, resulting in retaining only SNPs with at least 80% data. Samples were also filtered for individual call rate through another stepwise increasing individual call rate filter, from 0.2 to 0.5, resulting in retaining only SNPs from individuals with at least 50% data. This resulted in the removal of 31 samples due to insufficient data, with 42 genotyped samples remaining for this analysis. A total of 2,805 loci were retained with 6.94% missing data. Thirteen duplicate samples (from 10 individuals) were identified in this dataset and subsequently removed from further analyses, leaving 32 individual koalas for examination of genetic diversity.

From these 32 individual koalas, we calculated three values of genetic diversity: observed Heterozygosity H_O, expected Heterozygosity H_E (adjusted for small sample size), and inbreeding coefficient F_{IS}. The 32 individual koalas from the footprint were spread across a large geographical area, therefore we grouped the samples between Coastal and Inland, splitting the samples between east (Coastal: N = 20) and west (Inland: N = 12) of Toowoomba (Table 6-1).

Table 6-1 Heterozygosity and inbreeding for Inland versus Coastal samples collected as part of the IRP project.

	H_O *(Higher is better)*	H_E *(Higher is better)*	F_{IS} *(Lower is better)*
Coastal (N = 20)	0.275	0.319	0.138
Inland (N = 12)	0.295	0.349	0.157

Caution should be taken when interpreting this geographically split data, as the sample sizes were low (≤ 20), which decreased the robustness of these estimates.

These results are best interpreted by comparing them with other populations where diversity measures were calculated using similar methods. For example, using similar methods, the UniSC team estimated diversity measures for NSW koalas in the Northern Tablelands (N = 76) to be H_O = 0.225, H_E = 0.279 and F_{IS} = 0.190; and for Redland City Council (mainland), Southeast Queensland koalas (N = 227) to be H_O = 0.237, H_E = 0.320 and F_{IS} = 0.259. Additional examples are provided in Table 6-2 and; all used the same method as in this report and indicate that the Inland and Coastal groups have high heterozygosity compared to other populations and average inbreeding. It is important to note, however, that samples grouped as Inland and Coastal are widely spread and geographically distant koalas were artificially pooled together. The comparison would have been improved by having a larger sample size over smaller geographic areas.

Table 6-2 Observed heterozygosity for Inland versus Coastal samples collected as part of this study compared to other populations (note that for heterozygosity, higher values are better).

Population	Ho
*ARTC Inland (N = 12)	0.295
*ARTC Coastal (N = 20)	0.275
Coastal NSW (Port Macquarie Hospital group 1) (35)	0.265
Northern Tablelands (20)	0.258
Coastal NSW (Port Macquarie Hospital group 2) (25)	0.247
Coffs Harbour (8)	0.235
Redlands mainland (44)	0.215

** Caution should be exercised when interpreting these results, as samples were obtained across a wide geographic range and may not be comparable with restricted geographic areas.*

Table 6-3 Inbreeding for Inland versus Coastal samples collected as part of the project compared to other populations (note that for inbreeding, lower values are better.

Population	F_{IS}
Northern Tablelands (20)	0.083
Northern Rivers (18)	0.096
Coffs Harbour (8)	0.099
Coastal NSW (Port Macquarie Hospital group 1) (35)	0.113
Redlands mainland (44)	0.117
*ARTC Coastal (N = 20)	0.138
Redlands Minjerribah urban areas (52)	0.154
*ARTC Inland (N = 12)	0.157
Coastal NSW (Port Macquarie Hospital group 2) (25)	0.178
Redlands Minjerribah (73)	0.196
Snowy Mountains (41)	0.235

** Caution should be exercised when interpreting these results, as samples were obtained across a wide geographic range and may not be comparable with results obtained from more restricted geographic areas.*

Further comparisons can be made by consulting from Kjeldsen et al. (2016). Here, measures are presented from other wild koala populations across Queensland, New South Wales and Victoria, using SNPs obtained through double digest restriction-associated (DArTseq), which differed from our koala specific DArTag panel (a panel we developed to increase non-invasive data recovery and consistency).

Here again, comparison with the Inland and Coastal groups suggest that these groups may have healthy observed heterozygosity and inbreeding averages; however, as noted above, the ARTC samples are spread over a vast geographical range and are compared to populations that are likely more geographically restricted (the difference in geographic spread could not be ascertained from Kjeldsen's paper).

Table 6-4 Genetic diversity established through double digest restriction-associated SNP sequencing (DArTseq)

State	Location	n	H_O	H_E	F_{IS}
QLD	St Bees Island	19	0.29	0.35	0.23
QLD	St Lawrence	19	0.26	0.30	0.20
QLD	Koala Coast	24	0.22	0.30	0.32
QLD	Ipswich	23	0.27	0.31	0.19
NSW	Port Macquarie	45	0.23	0.28	0.21
NSW	Campbelltown	9	0.27	0.33	0.27
VIC	South Gippsland	19	0.24	0.30	0.27
VIC	Cape Otway	13	0.24	0.25	0.11

Note: Wild koala populations across QLD, NSW and Victoria (note that the geographic spread of the samples for this study is unknown). n = sample size, HO = observed heterozygosity, HE = expected heterozygosity, FIS = inbreeding coefficient. Table taken from Kjeldsen et al. (2016).

6.1.2 Sexing

Applying quality control for genotype data, locus call rate of 0.9 and individual call rate of 0.8, retained 31 unique individuals for sexing from the 36 ARTC samples. Of the 31 samples tested for sexing, 15 were female, and 16 were male (Appendix Table A3).

6.1.3 Chlamydia detection

Quality control of genotype data for locus call rate and individual call rate of 0.8 retained 30 unique individuals for *Chlamydia* pathogen detection, of which 19 (63.3%) had *Chlamydia* pathogen present. Samples that tested positive for the *Chlamydia* pathogen were evenly distributed across the ARTC footprint. It is important to note, however, that presence of the *Chlamydia* pathogen does not always equate to clinical disease.

6.2 Genetic co-analyses for DArTcap and DArTag data

From the 327 samples retained after the basic filtrations, 72 samples were identified as duplicates. After removing the 72 duplicates, a total of 255 unique koala samples were retained for further genetic analyses (Appendix Table A1). Of the 255 samples used in DArTcap and DArTag co-analyses, 101 samples were genotyped using DArTcap methodology and 154 samples were genotyped using DArTag methodology. The samples were collected across multiple projects; of the 255 samples used in this report, 36 were collected for the ARTC project.

6.2.1 Genetic structure

To identify possible genetic clusters, all 255 samples were analysed together without prior population information using fastStructure and STRUCTURE software. fastStructure predicted the presence of two to six (i.e., K = 2 - 6) genetic clusters (Figure 6-1), while STRUCTURE results indicated the presence of three genetic clusters (i.e. K = 3, Figure 6-2), as inferred by the ΔK which peaked at K = 3 (Figure 6-3).

Samples from Brisbane and Redland City had membership proportions different to other QLD and Northern NSW samples (Figure 6-2). Similarly, samples from Inverell were different from the other samples, although marginally similar to samples from Armidale, Uralla, Coffs Harbour and Bellingen (Figure 6-2). The samples from the most Southern NSW locations, such as Goulburn-Mulwaree, Shoalhaven City, Queanbeyan–Palerang and Snowy Monaro were genetically distinct from all other sample locations (Figure 6-2).

A similar pattern of membership proportions was evident in fastStructure plots (K3 plot in Figure 6-3). Further increasing the number of predicted genetic clusters to *K* = 4-6 showed similar genetic structure of the samples from neighbouring locations (K4 to K6 plots in Figure 6-1). Overall, samples from closer geographic proximities showed similar genetic structure, which supported grouping samples for further genetic analyses.

Figure 6-1 The number of genetic clusters as predicted from fastStructure form K = 2 to K = 6. Each bar represents an individual koala and its membership proportions to defined clusters.

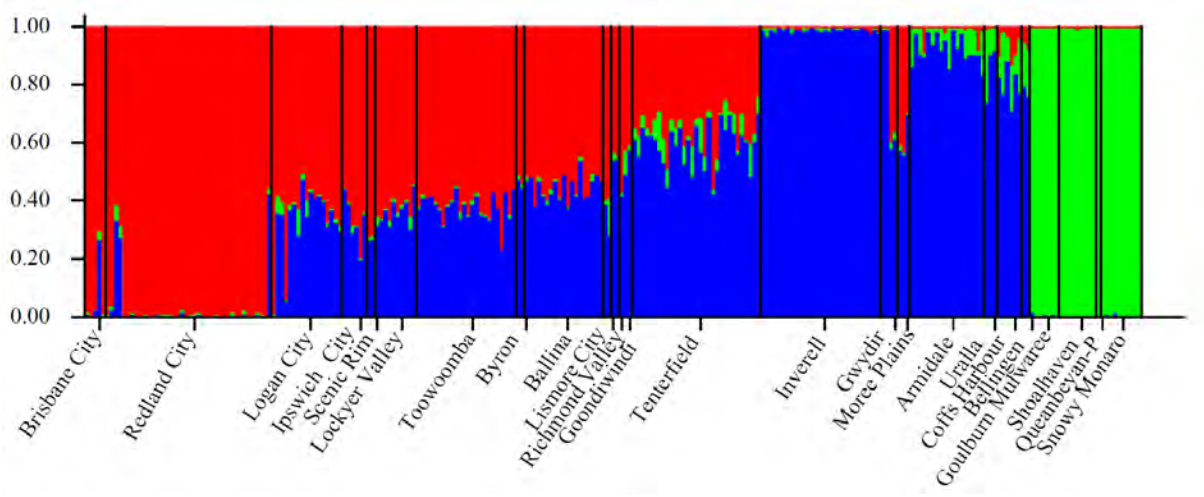


Figure 6-2 STRUCTURE plot showing the ancestral membership proportions defined by STRUCTURE results at K = 3 for 255 koala samples from 24 locations.

Each bar represents individual koala and its membership proportions to defined clusters. The details of individual koala and location information are given in Appendix A Table A1 in the order as it appears in Figure 3.

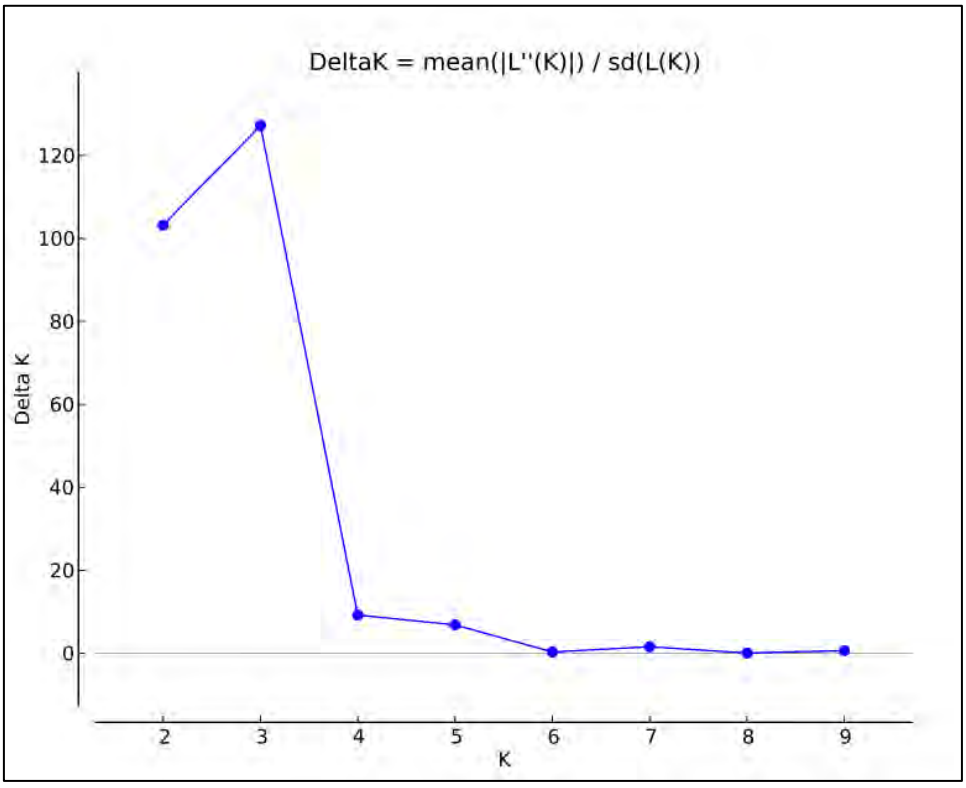


Figure 6-3 Delta K value generated for the STRUCTURE result at different K, which indicates the optimal number of ancestral clusters (K = 3).

PCA analysis of the 255 samples indicated that the samples from Goulburn-Mulware, Shoalhaven City, Queanbeyan–Palerang and Snowy Monaro formed a single cluster that was distinct from all other locations (Figure 6-4 and Appendix A Figure A2). Samples from Brisbane City and Redland City also formed a cluster that was somewhat separated from all other samples. Apart from the two clusters, the remaining samples from all other locations did not form easily separated clusters.

6.2.2 Genetic diversity between koala groups

Observed heterozygosity was broadly similar between koala groups (Table 6-5), with the exception of the two most Southern NSW groups, which had lower values for observed heterozygosity than other groups (i.e., Group 9 and Group 10). Observed heterozygosity was less than the expected heterozygosity in all koala groups. The lowest level of inbreeding was observed for Group 10 (samples from Snowy Monaro Regional Council), followed by Group 7 (samples from Armidale and Uralla).

Internal relatedness, which describes inbreeding at an individual level, was averaged across individuals and compared at population level and showed the highest averages in Groups 9 and 10 (Table 6-5). IR values can vary between 1 and -1, where maximum value of 1 indicates when all loci are homozygotes regardless allelic frequencies and the minimum value -1 is only reachable when all loci present only two alleles and the individual is heterozygote for all loci (Aparicio, Ortego et al. 2006). Overall, the average IR of populations observed in the present report is comparable to past studies (e.g., Kjeldsen et al., 2016; presented here in Table 6-6 for ease of comparison).

The estimated effective population size (N_E) varied between different koala groups and was lowest for Group 6 (samples from Inverell, Gwydir and Moree Plains). Groups 7, 8, 9 and 10 had N_E over 100 (Table 6-5).

Figure 6-4 Principal component analysis (PCA) plot for 255 unique koalas from 24 sample locations.

Each location has its own 95% CI ellipse and each dot represents an individual koala. A duplicate figure with location name next to the ellipse is given in Appendix A Figure A2 and the details of individual koala and location information are given in Appendix A Table A1.

Table 6-5 Genetic diversity indices for ten groupings of koalas from 24 separate locations, divided into 10 geographic groupings.

Details of grouping by location are given in Appendix Table A2. ARTC samples fall into groups 2, 3 and 6 (Group 2: Logan City + Ipswich City + Scenic Rim Regional; Group 3: Lockyer Valley Regional + Toowoomba Regional Council; and Group 6: Inverell Shire Council + Gwydir Shire Council + Moree Plains Shire Council).

Group	Sample size	H_O ± SE	H_E ± SE	F_{IS} ± SE	IR ± SD	N_E (CI)
Group_1	42	0.219 ± 0.005	0.256 ± 0.006	0.144 ± 0.008	0.276 ± 0.146	79.9 (76.6 – 84.1)
Group_2	25	0.245 ± 0.005	0.298 ± 0.005	0.178 ± 0.008	0.215 ± 0.159	69.1 (64.1 – 72.5)
Group_3	34	0.261 ± 0.005	0.299 ± 0.005	0.125 ± 0.007	0.170 ± 0.129	170.1 (155.6 - 187.9)
Group_4	25	0.215 ± 0.006	0.251 ± 0.006	0.144 ± 0.009	0.288 ± 0.134	48.7 (46.0 - 51.5)
Group_5	31	0.253 ± 0.005	0.283 ± 0.005	0.106 ± 0.008	0.153 ± 0.114	156.0 (137.2 – 165.9)
Group_6	36	0.228 ± 0.006	0.260 ± 0.006	0.124 ± 0.007	0.230 ± 0.102	43.8 (42.5 – 45.4)
Group_7	21	0.224 ± 0.006	0.245 ± 0.006	0.084 ± 0.013	0.242 ± 0.107	108.7 (97.3 – 129.8)
Group_8	08	0.202 ± 0.007	0.233 ± 0.006	0.132 ± 0.009	0.312 ± 0.124	151.5 (94.4 – 499.5)
Group_9	17	0.160 ± 0.006	0.191 ± 0.006	0.162 ± 0.013	0.451 ± 0.095	120.8 (97.2 – 167.4)
Group_10	10	0.171 ± 0.007	0.177 ± 0.006	0.037 ± 0.009	0.412 ± 0.078	210.4 (116.5 – 1481.9)

Note - H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, IR = internal relatedness and N_E = effective population size. SE: standard error, SD: standard deviation, CI: 95% confidence interval.

Table 6-6 Genetic diversity of wild koala populations across QLD, NSW and Victoria (from Kjeldsen et al., 2016).

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

6.2.3 Genetic differentiation among groups

Significant genetic differentiation was observed among the 10 koala groups (F_{ST} = 0.117, *P* < 0.001, F'_{ST} = 0.166). Pairwise comparison of F_{ST} values indicated significant differences between all koala groups (Table 6-7), where Group 9 and 10 (samples from most Southern NSW) indicated high level of genetic differentiation to all other groups, although the pairwise F_{ST} value is very small for between group 9 and 10 (Table 6-7).

Table 6-7 Pairwise FST values for ten groupings.

All values are statistically significant (P < 0.001) for each comparison. Details of grouping by location are given in Appendix A Table A2. Lower values indicate higher level of genetic similarity.

	Group_ 1	**Group_ 2**	**Group_ 3**	**Group_ 4**	**Group_ 5**	**Group_ 6**	**Group_ 7**	**Group_ 8**	**Group_ 9**
Group_2	0.059								
Group_3	0.086	0.035							
Group_4	0.132	0.075	0.099						
Group_5	0.092	0.035	0.047	0.08					
Group_6	0.153	0.092	0.098	0.134	0.055				
Group_7	0.166	0.105	0.119	0.151	0.072	0.072			
Group_8	0.142	0.086	0.107	0.130	0.058	0.088	0.066		
Group_9	0.222	0.170	0.183	0.214	0.147	0.18	0.179	0.164	
Group_10	0.23	0.181	0.188	0.226	0.154	0.188	0.192	0.185	0.066

Gene flow between koala groups was tested as the number of effective migrants (N_m) between groups. A high number of effective migrations was observed between Group 2 and Group 3 (i.e. between Logan City + Ipswich City + Scenic Rim Regional Council populations and Lockyer Valley + Toowoomba Regional Council populations,), as well as between Group 2 and Group 5 (i.e. between Logan City + Ipswich City + Scenic Rim Regional Council populations and Tenterfield population, Table 10). Overall, N_m > 1 was observed for all comparisons, except those involving Group 1 (Brisbane + Redland City) vs Group 9, as well as Group 10 (samples from two groups in most Southern NSW) and Group 4 (samples from Byron, Ballina, Lismore and Richmond Valley) vs Group 9 and Group 10 (samples from two groups in most Southern NSW, Table 10).

Table 6-8 Number of migrants (Nm) between koala groups as estimated using FST values.

	Group_1	Group_2	Group_3	Group_4	Group_5	Group_6	Group_7	Group_8	Group_9
Group_2	3.985								
Group_3	2.649	6.766							
Group_4	1.651	3.076	2.257						
Group_5	2.458	6.966	5.007	2.864					
Group_6	1.381	2.470	2.301	1.618	4.293				
Group_7	1.255	2.132	1.849	1.410	3.241	3.208			
Group_8	1.506	2.672	2.053	1.671	4.093	2.584	3.513		
Group_9	0.875	1.226	1.109	0.919	1.454	1.140	1.151	1.281	
Group_10	0.839	1.131	1.073	0.860	1.372	1.080	1.051	1.105	3.522

6.2.4 Genomic relationship

The genomic relationship heatmap indicates higher relatedness of koalas within the same location or to neighbouring locations in comparison to the distal locations (Figure 6-5). Overall, the genomic relationship heatmap indicated a relatedness pattern that supported the observed genetic structure analyses (Section 6.2.1).

6.2.5 Sexing

The 115 samples that passed quality control for sex determination belonged to the following groups: Group 1 (n = 27), Group 2 (n = 10), Group 3 (n = 14), Group 4 (n = 23), Group 5 (n = 20), Group 6 (n = 5), Group 9 (n = 11) and Group 10 (n = 2). Two samples from Goondiwindi and another sample from North Stradbroke Island are not presented here, due to being geographically separated from the koala location groups. Koala sex by group is indicated in Table 6-9.

Table 6-9 Koala sex by group as estimated using genotyping of sex markers.

ARTC samples fall into Group 2, 3 and 6 (Group 2: Logan City + Ipswich City + Scenic Rim Regional Council; Group 3: Lockyer Valley Regional Council + Toowoomba Regional Council; and Group 6: Inverell Shire Council + Gwydir Shire Council + Moree Plains Shire Council).

Group	Male	Female	Sex ratio
1	8	19	1:2.4
2	6	4	1:0.7
3	7	7	1:1
4	11	12	1:1.1
5	13	7	1:0.5
6	2	3	1:1.5
9	5	6	1:1.2
10	1	1	1:1

6.2.6 Chlamydia detection

From the 105 samples that passed quality control for *Chlamydia* analysis, 57 samples (54.3%) were positive for *Chlamydia*. *Chlamydia* prevalence in each group is indicated in Table 6-10. Two samples from Goondiwindi and another sample from North Stradbroke Island were removed from the analyses.

Table 6-10 Chlamydia prevalence for each group as indicated by the genotyping of Chlamydia markers for 105 samples.

ARTC samples fall into Groups 2, 3 and 6 (Group 2: Logan City + Ipswich City + Scenic Rim Regional Council; Group 3: Lockyer Valley Regional Council + Toowoomba Regional Council; and Group 6: Inverell Shire Council + Gwydir Shire Council + Moree Plains Shire Council).

Group	Positive	Negative	Prevalence
1	16	8	67%
2	8	2	80%
3	7	7	50%
4	9	11	45%
5	6	12	33%
6	3	1	75%
9	6	4	60%
10	1	1	50%

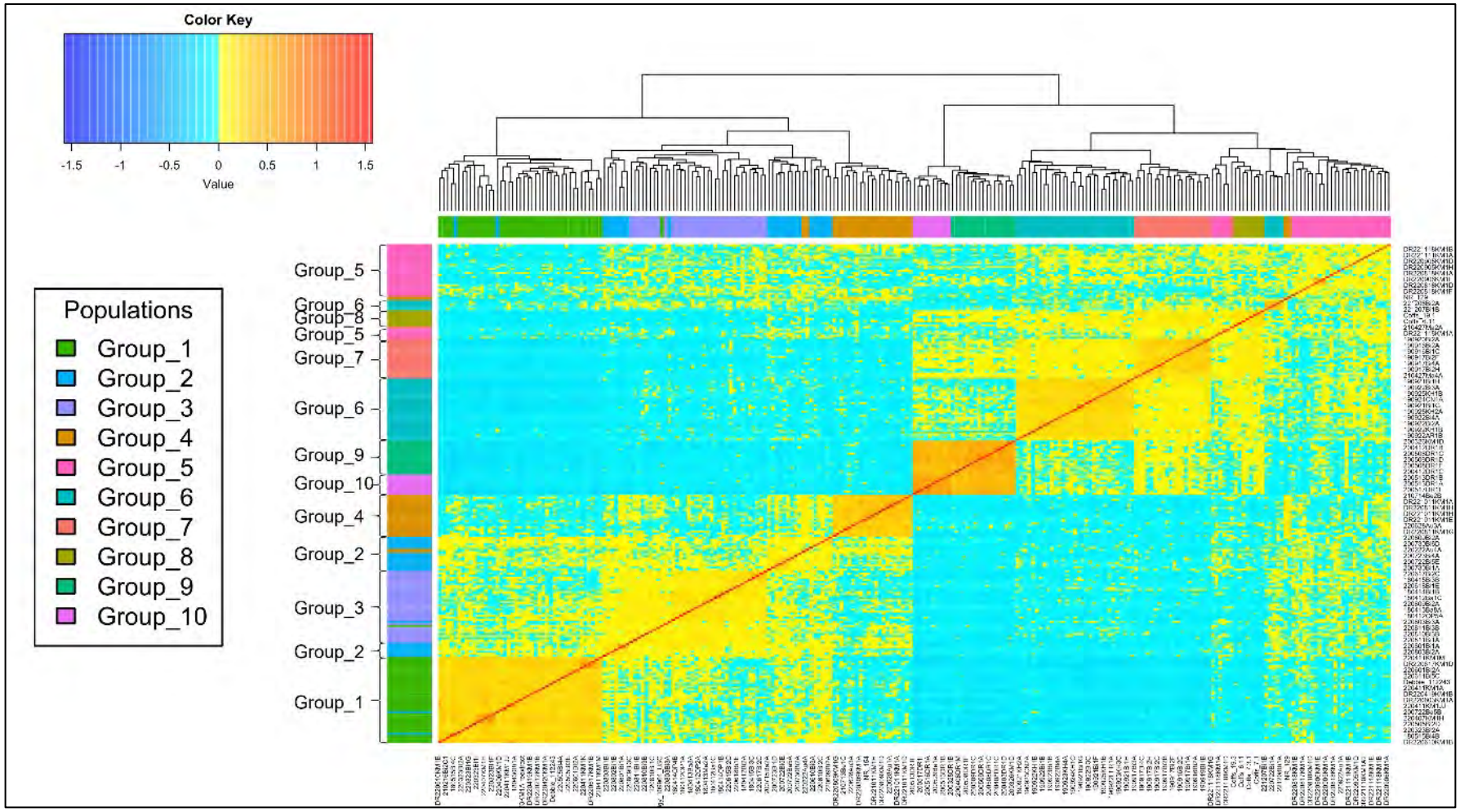


Figure 6-5 Genomic relationship matrix heat map for 10 geographic groupings as indicated in Table 2. Relatedness varies from high (red) to low (blue) as indicated by the colour key.

6.3 Diet Analysis

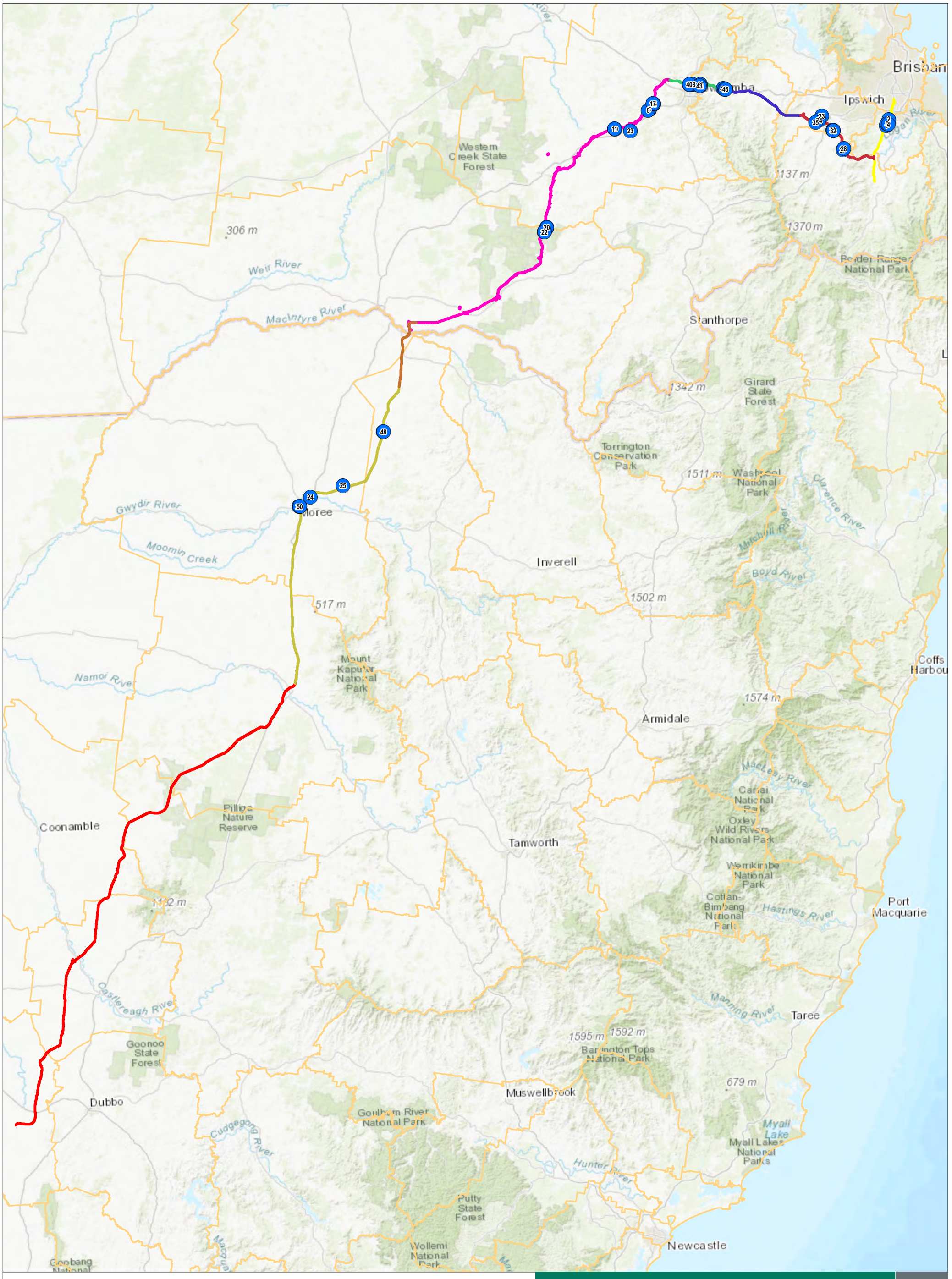
Of the 55 scat samples sequenced in this study, 49 returned reads for the validated diet markers after quality control filtering. Across all samples, between 1 and 7 tree species were detected per sample (average = 3.35). Thirty-seven individual species were detected across the 49 validated samples with several other species potentially present as indicated by the presence of markers for groups of target species. The most frequently detected species were *Eucalyptus chloroclada* (detected in 18 samples), *E. tereticornis* (17 samples), *E. amplifolia subsp. sessiliflora* (16 samples) and *E. camaldulensis* (16 samples). These species were also often found at high relative abundance within samples (range of average relative abundances: 24% to 44%). *E. prava* was detected in a single sample (IR21: 220623BI1B), however, as the markers for this species have not been validated it could not be included in the relative abundance calculations. The diet species detected differed between geographic regions and between individuals (Figure 6-6). See Appendix B for a breakdown of sample number identification and location.

In the Brisbane region *Eucalyptus seeana* was detected in three out of four samples collected in the south at an average relative abundance of 48.35%. *E. tereticornis* was detected in seven out of nine samples collected west of Brisbane at an average relative abundance of 62.6% (Figure 6-7, Table 6-11).

In the Toowoomba region, the tree species detected varied considerably between samples. *E. amplifolia subsp. sessiliflora subsp. sessiliflora, E. tereticornis, E. chloroclada* and *E. melliodora* were all detected in four or more samples (from 11) at relative abundances between 10.3% and 34.6% (Figure 6-8, Table 6-12).

In the region to the north-east of Goondiwindi *E. camaldulensis* was found in all six samples at an average relative abundance of 39.7%, while *E. amplifolia subsp. sessiliflora* and *E. tereticornis* were also found at high relative abundance in two individuals each (Figure 6-9, Table 6-13).

In the Moree Region, five out of seven samples contained *E. camaldulenis* with an average relative abundance of 35.2%, however the abundances ranged from 1% to 100%. While *E. populnea* and *E. chloroclada* were also found at high relative abundance (64.6% and 58.7%, respectively) in 3 and 2 samples, respectively (Figure 6-10, Table 6-14).



Legend

Diet Samples

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- C2K - Calvert to Kagaru
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert
- K2AB - Kagaru to Acacia Ridge/Bromelton
- N2N - Narromine to Narrabri
- N2NS - Narrabri to North Star
- NS2B - North Star to Border
- LGA Boundary

Data Source:
ESRI Would Topographic Map

Map Of Diet Sample Locations Across The Entire Study Area

F6-6

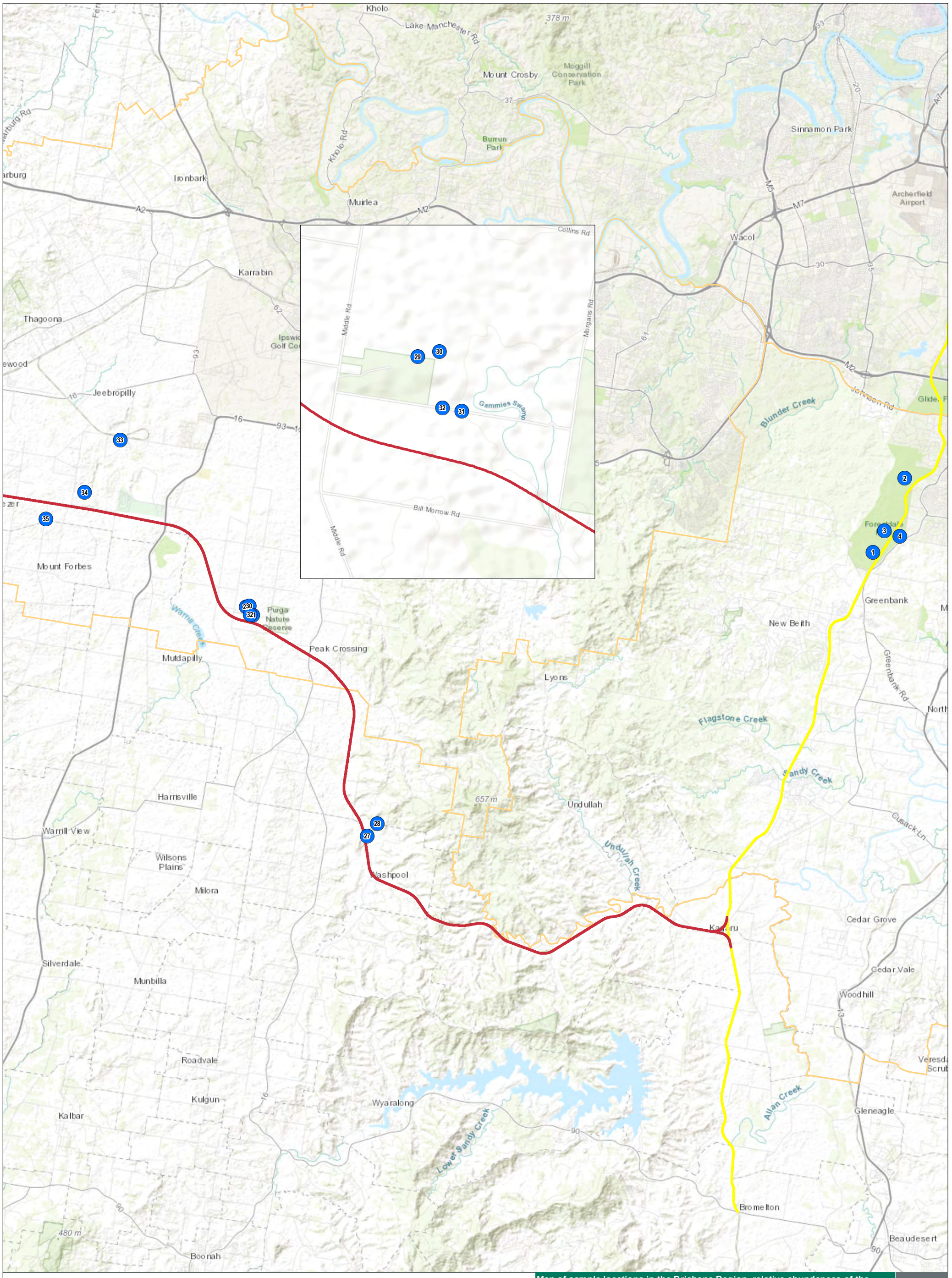
Drawing No:	0628339_N2AB_TM_G011_R0.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 10 20 30Km

N

ERM



Legend

Diet Samples

Inland Rail Alignment

C2K - Calvert to Kagaru

K2AB - Kagaru to Acacia Ridge/Bromelton

LGA Boundary

Data Source:
ESRI Would Topographic Map

Map of sample locations in the Brisbane Region, relative abundances of the different dietary tree species detected in each sample as indicated in Table 6-11

F6-7

Drawing No:	0628339_N2AB_TM_G012_R0.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	15/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 1 2 3Km

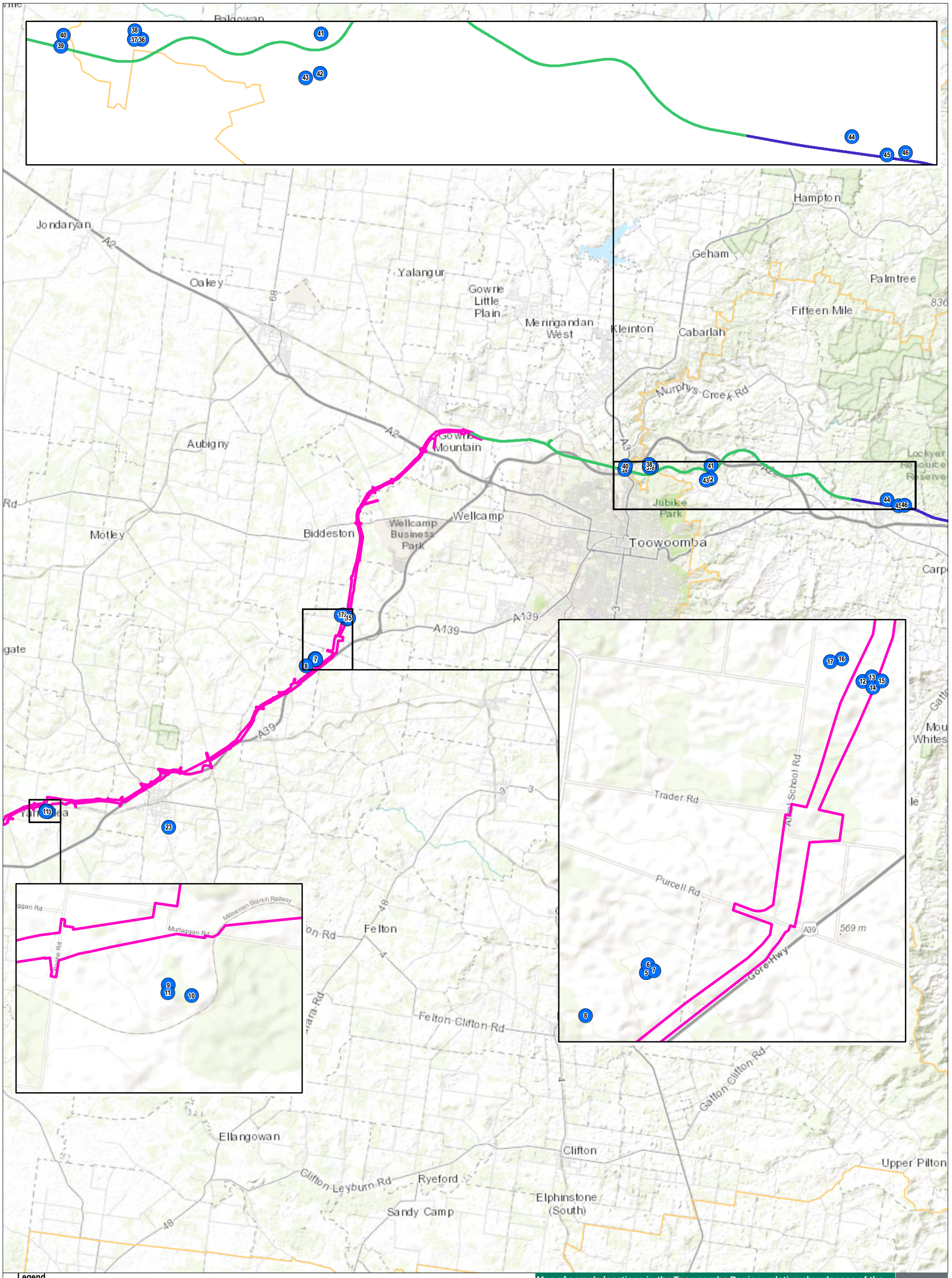
N

ERM

Table 6-11 Relative abundances of the different dietary tree species detected in each sample in the Brisbane region, each abundance is given in a percentage of the sample

	Sample Number												
Species	**1**	**2**	**3**	**4**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
Angophora leiocarpa			56.1										
Corymbia citriodora					6.7							8.3	
Corymbia variegata					15.6								
Eucalyptus amplifolia subsp. sessiliflora			4.6	4.7				58.2	43.4		0.2		
Eucalyptus camaldulensis								21.0	3.5				
Eucalyptus cambageana				1.2									
Eucalyptus chloroclada				1.6	2.1		100		2.1		2.9	4.7	
Eucalyptus crebra and/or elegans												4.8	
Eucalyptus crebra, elegans and/or fibrosa					5.6								
Eucalyptus exserta								0.2				0.2	
Eucalyptus platyphylla									1.2				
Eucalyptus populnea											1.3		
Eucalyptus punctata			10.2										
Eucalyptus melanophloia											22.6		
Eucalyptus melanophloia and/or whiteii													4.2
Eucalyptus resinifera											14.3		
Eucalyptus rhombica					13.0								
Eucalyptus seeana	66.5	100	26.9										
Eucalyptus tereticornis	33.5		2.3		57.2	100		20.5	49.8	100	58.7	82.0	95.8

Eucalyptus tereticornis and/or robusta				92.4									



Legend

Diet Samples

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert
- LGA Boundary

Data Source:
ESRI Would Topographic Map

Map of sample locations in the Toowoomba Region, relative abundances of the different dietary tree species detected in each sample as indicated in Table 6-12

F6-8

Drawing No:	0628339_N2AB_TM_G013_R0.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	CB	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 1 2 3Km

N

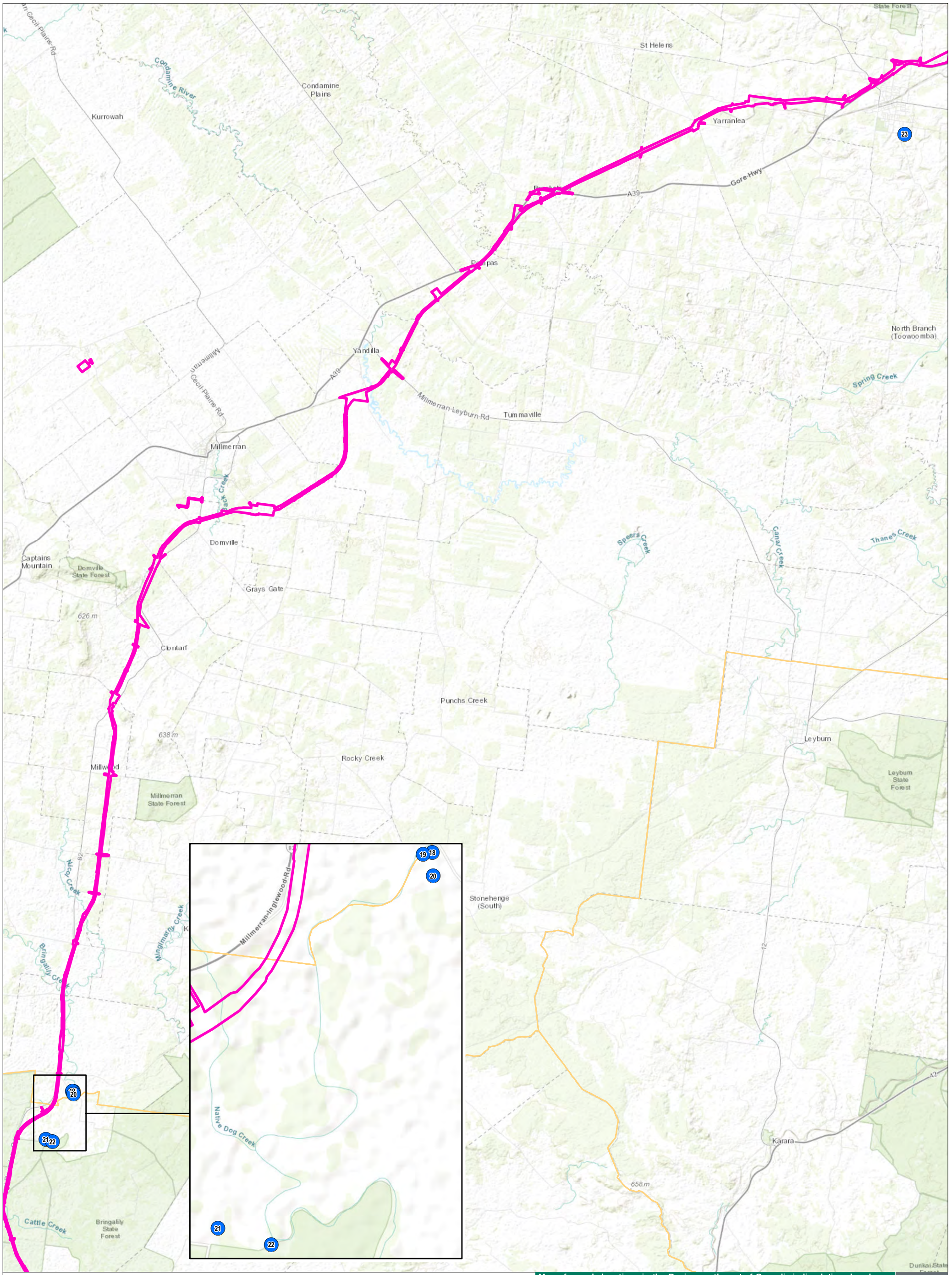
ERM

Table 6-12 Relative abundances of the different dietary tree species detected in each sample in the Toowoomba region, each abundance is given in a percentage of the sample

	Sample Number																						
Species	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**
Corymbia maculata																							48.3	21.7
Eucalyptus albens								5.2	0.2										37.7					
Eucalyptus amplifolia subsp. sessiliflora			68.6	58.6												11.1			0.9		55.4	100	18.4	22.2
Eucalyptus blakelyi			6.0											59.7										
Eucalyptus brownii					84.2		2.1																	
Eucalyptus camaldulensis		24.4		41.4																8.9				
Eucalyptus carnea														38.2										
Eucalyptus chloroclada	26.5	61.3	7.2						4.5					2.1			49.0		1.3	17.6				
Eucalyptus conica								6.1																
Eucalyptus coolabah							0.1																	
Eucalyptus crebra, elegans and/or fibrosa	33.0								8.1		11.9													40.1
Eucalyptus cullenii and/or whitteii							8.0																	
Eucalyptus dura					15.8	21.1																		

	Sample Number																					
Species	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**
Eucalyptus dura and/or siderophloia															100									
Eucalyptus dura, fibrosa and/or rhombica												7.8												
Eucalyptus eugenioides																		0.4						
Eucalyptus exserta		3.1																			12.1			
Eucalyptus fibrosa													5.7											
Eucalyptus granitica and/or whitteii									19.1	38.6	68.9													
Eucalyptus longirostrata																73.5	2.4	18.0						
Eucalyptus macta	40.5		10.5																					
Eucalyptus melanophloia												85.8	74.2							50.1				
Eucalyptus melanophloia and/or whiteii								70.7																
Eucalyptus melliodora									2.4	1.0						15.4	15.7	2.3	7.7					
Eucalyptus microcarpa						5.0																		
Eucalyptus orgadophila						53.7	4.7	14.9	64.8	60.5	18.1		10.4											
Eucalyptus platyphylla							0.0	0.3																
Eucalyptus populnea						20.2	85.0	2.9																

	Sample Number																					
Species	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**
Eucalyptus punctata																		79.3						
Eucalyptus rhombica									0.9		1.1													
Eucalyptus siderophloia													4.9											
Eucalyptus tereticornis		11.2															9.9			23.4	32.4		33.3	16.0
Eucalyptus tereticornis and/or robusta																			52.4					
Eucalyptus tindaliae			7.7																					
Eucalyptus whiteii												6.4	4.8											



Legend

Diet Samples

Inland Rail Alignment

B2G - NSW/QLD Border to Gowrie

LGA Boundary

Data Source:
ESRI Would Topographic Map

Map of sample locations in the Region north-east of Goondiwindi, relative abundances of the different dietary tree species detected in each sample as indicated in Table 6-13

F6-9

Drawing No:	0628339_N2AB_TM_G014_R0.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 1 2 3Km

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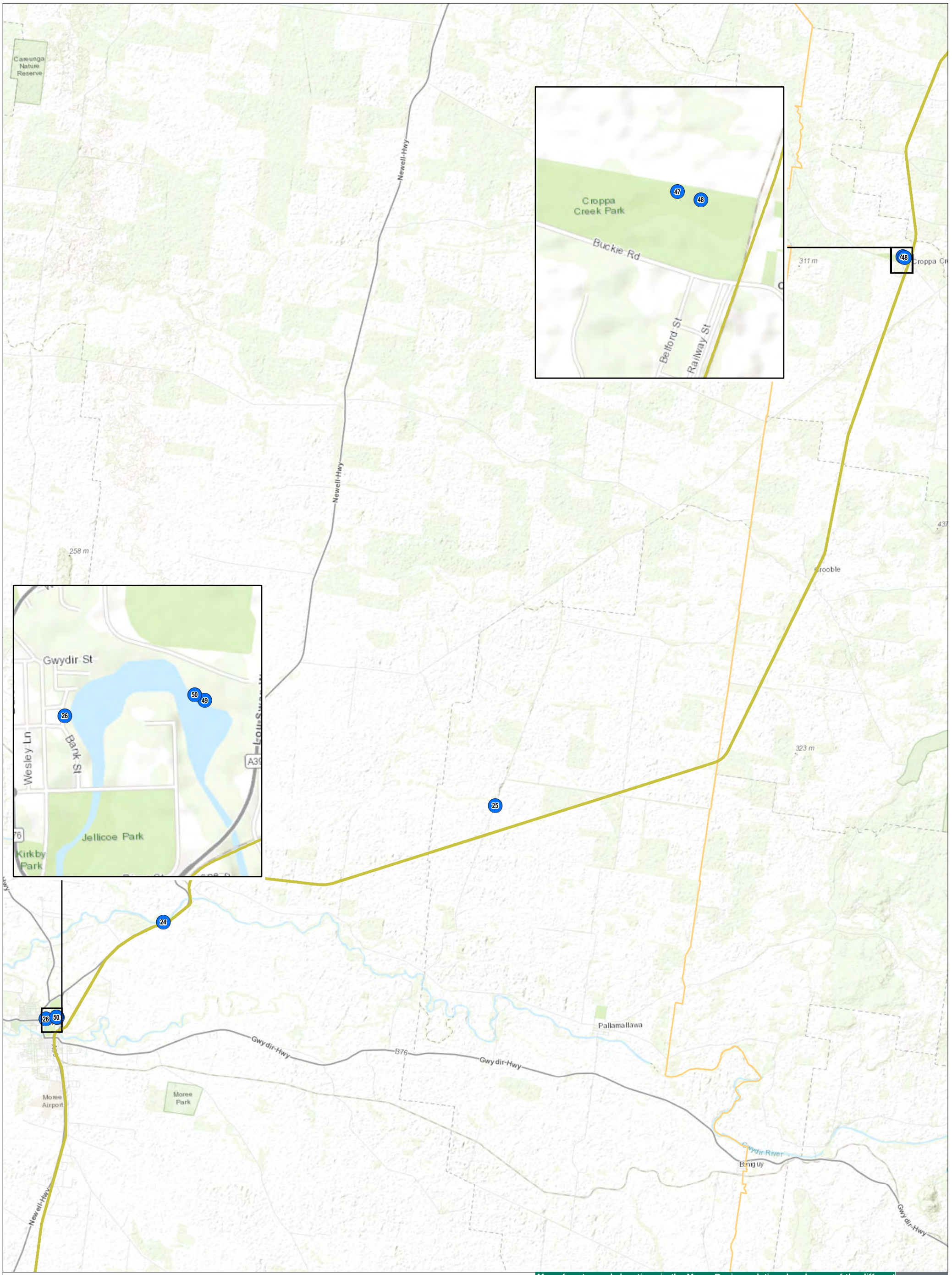
ERM

Table 6-13 Relative abundances of the different dietary tree species detected in each sample in the region north-east of Goondiwindi, each abundance is given in a percentage of the sample

	Sample number					
Species	**18**	**19**	**20**	**21**	**22**	**23**
Eucalyptus amplifolia subsp. sessiliflora		13.4	63.3			
Eucalyptus blakelyi					29.5	
Eucalyptus camaldulensis	39.0	21.9	28.1	17.6	57.2	74.7
Eucalyptus chloroclada		15.7	8.7		12.2	
Eucalyptus conica	61.0	6.3				
Eucalyptus drepanophylla				21.3		
Eucalyptus major		14.7				
Eucalyptus platyphylla						25.3
Eucalyptus tereticornis		28.1		61.1		
Eucalyptus woollsiana					1.2	



Legend

Diet Samples

Inland Rail Alignment

N2NS - Narrabri to North Star

LGA Boundary

Data Source:
ESRI Would Topographic Map

Map of scat sample locations in the Moree Region, relative abundances of the different dietary tree species detected in each sample as indicated in Table 6-14

F6-10

Drawing No:	0628339_N2AB_TM_G015_R0.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 1 2 3Km

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Table 6-14 Relative abundances of the different dietary tree species detected in each sample in the Moree region, each abundance is given in a percentage of the sample

	Sample Number						
Species	**24**	**25**	**26**	**47**	**48**	**49**	**50**
Eucalyptus amplifolia subsp. sessiliflora						51.5	
Eucalyptus blakelyi	47.4						
Eucalyptus camaldulensis	4.7		1.0	22.5		48.0	100
Eucalyptus chloroclada	18.3		99.0				
Eucalyptus melliodora					6.9		
Eucalyptus platyphylla						0.4	
Eucalyptus populnea		23.2		77.5	93.1		
Eucalyptus tereticornis and/or robusta	29.6						
Eucalyptus rhombica		35.4					
Eucalyptus woollsiana		41.5					

7. KOALA GENETIC ANALYSIS AND CONCLUSIONS

The IRP transects the Queensland and New South Wales landscape, and as part of the genetics study, data collected by UniSC from the broader landscape has been utilised for further analysis and therefore a deeper understanding of how the koalas in close proximity to the IRP play compare to other koala populations in the broader New South Wales and Queensland regions.

A key priority for IRP is to preserve current landscape connectivity for movement of koalas. However, along the footprint, and within the accessible area for sampling, the number of individual koalas sampled presents a challenge for connectivity analyses across the IRP. Indeed, for genetic samples to be collected, a koala needs to have been present at the survey site in the few days prior to a site visit. In low koala density or large home range areas, a koala might only be present at a specific site for a very narrow window throughout the year.

On this basis, some samples were pooled from other studies with IRP samples to form groups along the IRP, while other groups were included to help contextualise the population status associated with the IRP and include.

- Narrabri to North Star (Group 6);
- Border to Gowrie and Gowrie to Helidon (Group 3); and
- Calvert to Kagaru and Kagaru to Acacia Ridge and Bromelton (Group 2).

We further calculated genetic differentiation between groups 2,3 and 6, and then to the other groupings (1, 4, 5, 7, 8, 9, 10 ,11, 12) to add context of the genetic variation of the project koalas to the broader landscape of QLD and NSW koalas. How similar or dissimilar the groups are to one another reveals the extent of gene flow. Group 2 and 3 values showed low differentiation between Border to Helidon and Calvert to Acacia Ridge.

Overall the three groups which included IRP samples, compared to other groups in the larger landscape had:

- High levels of heterozygosity: Higher levels of heterozygosity equate to larger genetic diversity within a population(s), which is positive as genetic diversity is linked to both individual health and at population level, evolutionary potential; with Group 3 (B2G + G2H) higher than the other groups;
- Low levels of inbreeding and of relatedness between individuals: this suggests gene flow is currently occurring within the groups and the potential of negative impacts due to excessive inbreeding is minimal;
- Relatively small population size, resulting in overall lower densities of koalas across the landscape. It is noted however that group 3 (B2G + G2H) has a larger population size relative to other groups.; and
- No concerns regarding sex ratio, but high chlamydia prevalence, except Group 3 which is medium prevalence (however the sample size remain very low). The ratio of males to females is not of concern for genetic health although chlamydia prevalence is high. Caution should be taken when interpreting these findings as the sample size is small.

To overcome current limitations, future studies should attempt to sample across a broader geographic range than just the IRP. This is likely to assist in overcoming the geographic gaps in detected samples while also enabling more robust assessments of koala genetics and current threats at a landscape level. Repeat surveys are also likely to assist in increasing the rate of detection of individuals across the IRP.

8. KOALA DIET ANALYSIS AND CONCLUSIONS

A key priority for IRP is to preserve the foraging variability and options for koalas. The relative abundances of the tree species detected in each scat sample analysed by UniSC were provided to offer insight into the dominant and minor components of the diet to identify preferred foraging trees.

Overall, four species should be considered to be the main preferred foraging trees: *Eucalyptus chloroclada, E. tereticornis, E. amplifolia subsp. sessiliflora,* and *E. camaldulensis*. This may be a result of local availability of species, that is, some individuals may only have access to woodlands dominated by *E. tereticornis* and as a result their diet is also dominated by this species. Each of the regions in the study area showed significant differences in the diet preferences of koalas. Accordingly, we will examine the preferred foraging trees in three different sections of the IRP:

- East of the Toowoomba range;
- West of the Toowoomba range to the Queensland-NSW border; and
- New South Wales.

The Brisbane region had the lowest variety of tree species identified, with the southern samples dominated by *E. seeana*, and the western samples dominated by *E. tereticornis.* However, a total of 20 species or species groups were recorded in the 13 samples, although at lower abundances.

The Toowoomba region had the greatest number of samples and also the largest variety of tree species identified (total 35 species or species groups), featuring 4 main species at relatively equal abundances: *E. amplifolia subsp. sessiliflora, E. tereticornis, E. chloroclada* and *E. melliodora.* The region north-east of Goondiwindi added an additional three species to the large variety of species identified in Toowoomba: *E. drepanophylla, E. major,* and *E. woollsiana*. However, these were found in lower abundances as *E. camaldulensis* was the dominant species in all samples. This makes this region the most diverse for the foraging preferences of the individuals.

The diet analysis in the NSW region near Moree was also dominated by *E. camaldulensis*, however, *E. populnea* and *E. chloroclada* were also identified as important foraging species. There were 10 total species identified in the diet analysis.

Section 5.3 reveals the results from the in-field vegetation analysis supports the diet preference further with vegetation samples in the Brisbane region being dominated by *E. crebra, E. citriodora and Corymbia intermedia*, the Toowoomba region being dominated by *E. populnea, E. camaldulensis and E. chloroclada*, and the NSW regions being dominated by *E. camaldulensis, E. populnea and E. crebra.* Noting there was a difference in field observed canopy dominance versus the scat analysis of the same regions.

For example, the Toowoomba region scat analysis indicated feed tree preferences due to results lacking the three dominant tree species that were observed for that region. The most common species detected in scat samples for this region were *E. amplifolia subsp. sessiliflora, E. tereticornis, E. chloroclada* and *E. melliodora*. This supports the idea that while a eucalypt species is dominating a region, the individual koala will seek out its desired food tree. Appendix C also agrees stating *'The diet species detected differed between geographic regions and between individuals'.*

The spatial variability in the preferred diet trees of koalas indicates that any replacement planting should be done in accordance with the pre-clearance Regional Ecosystems. Preferred food trees should be planted, however care should be taken to not develop a monoculture that would result in individual diets becoming dominated by one or two species, therefore reducing the nutritional value to be gained.

As with the genetic analysis, future studies should attempt to cover a broader range across the IRP to provide a more robust analysis. Repeat surveys may also assist in increasing the rate of detection of individuals across the IRP as well as account for seasonal variation.

9. KOALA HABITATS IN THE STUDY AREA

The IRP comprising of eight sections, being of eight distinct projects, reflects considerably varying histories across the IRP. Land use varies from agricultural areas (largely cleared and utilised for intensive cultivation through to livestock grazing), to large areas of state forests and protected areas as well as extending through heavily urbanised areas particularly the suburbs of southern Brisbane and regional cities and townships of Toowoomba, Narrabri, Moree and Narromine.

To overcome the limitations of a small sample size from this study, a landscape approach was taken that incorporated historic koala observation data and ground-truthed koala habitat assessments to evaluate the potential suitability of habitat and opportunities to maintain connectivity of key areas within the IRP and surrounding landscape. This section (Sections 8.1.1-8.1.8) therefore presents a discussion based on the findings of this study including:

- areas of high density of koala records, identified as koala hotspot region;
- areas where maintenance of connectivity are important (post-identification of survey hotspots);
- key areas to undertake ongoing koala monitoring to assess the performance of proposed mitigation measures determined to maintain or improve genetic diversity in identified koala populations;
- a project-specific habitat connectivity strategy to maintain and improve habitat connectivity to avoid and minimise impacts to koala; and
- how knowledge from this study can inform impact assessment and offsetting of significant impacts resulting from the IRP.

Using a combination of approaches including results from this study and desktop assessments of historic koala records and habitat availability, koala hotspot regions within each project were defined. as:

- A high density of historic koala records is known;
- Suitable koala habitat is present; and
- Detection dog surveys returned positive detections of koala occupancy.

9.1 Kagaru to Acacia Ridge and Bromelton

The K2ARB is no longer apart of the IRP, however data from the study associated in the areas within the K2ARB section will still provide beneficial data on important koala populations, habitat areas and identification of genetic diversity.

9.1.1.1 Historic Koala Records

The 2016, 2018 and 2020 field investigations did not record any sightings of koalas. There are records for this species within 10 km of Beaudesert Road, Bromelton Loop, Middle Road/Greenbank Loop and Kagaru Loop Enhancement Work Areas. As a part of the environmental investigations for K2ARB a search of the Queensland Government Wildlife database returned koala records within the Glider Forest (in 2018), as well as near the southern part of the Greenbank Military Training Area (in 2015), closest to the Middle Road/Greenbank Loop Enhancement Work Area. Since 2011, there have been 10 records of koalas within 5 km of the Disturbance Footprint utilised for the previous investigations. The infrequent and low number of records indicate that koalas occur within the Study Area, and at very low densities. As a part of the environmental investigations for K2ARB potential koala scratch marks were found on *Eucalyptus propinqua* trees and likely koala scats were observed within the Glider Forest, close to the Project Area.

ALA records of koala presence show a high density of recent records throughout the K2ARB, particularly within Karawatha Forest, Glider Forest and Parkinson Bushland Reserve, and throughout patches of retained vegetation and creeks adjacent to and surrounded by heavily urbanised areas. It should be noted that the proximity to urban areas may influence the number of sightings that have historically been made of individuals in relation to the size and quality of habitat.

From 2014-2018, OWAD Environment undertook surveys with detection dogs to determine koala distribution, genetics and infection status across selected areas (protected areas, forests, and reserves) in the Brisbane City Council (BCC) and Logan City Council (LCC) Local Government Areas (LGAs). The results of the BCC Koala Detection Dog Study showed a decline in koala genetic diversity from 2016 to 2018 (most noticeable in the Larapinta, Karawatha and Larapinta locations which make up part of the broader Flinders Karawatha Bioregional Corridor) and was concluded to be a result of habitat fragmentation due to industrial and residential developments in the area (OWAD Environment, 2018a). The LCC LGA Detection Dog Studies found koala scats at 63% of the 50 parks sampled in the initial work across the LCC LGA (OWAD Environment, 2014), koala scats at 11 of the 21 properties surveyed in the following survey (OWAD Environment, 2015), and KoRV-A detected in 28% of the individuals identified during 2018 surveys across 84 locations (OWAD Environment, 2018b). The results of these surveys identified three main genetic populations across LCC LGA and BCC LGA including:

- Northern cluster within the BCC LGA;
- Central cluster within predominately the BCC LGA; and
- South-western cluster which is within the LCC LGA.

When comparing these populations, the results of these surveys indicated that the northern and central clusters of koala populations in the BCC LGA were less genetically diverse than those in the south-western cluster found in LCC LGA, demonstrating a greater level of permeability across the landscape in LCC LGA (OWAD Environment, 2018b). It also noted that koala movement and dispersal in the LCC LGA was largely constrained by geographical areas, showing four main populations within this LGA (OWAD Environment, 2018b).

9.1.1.2 Findings from the Study

A single hotspot was identified in K2ARB and was located inside the Greenbank Military Reserve. Four koala scat detections were made following detection dog surveys and numerous historic koala records are present. The hotspot is defined as extending from the Logan Motorway south to the edge of contiguous forest and Teviot Brook. A few key forests and reserve provide. These include The Glider Forest, Parkinson Bushland Reserve and Karrawatha Forest which both contain historic records of koalas, provide important corridors within the hotspot connecting the surrounding landscape. Retained vegetation in riparian zones is likely to be of high importance for maintaining connectivity.

Table 9-1 Koala Hotspot Region within K2ARB

Hotspot Number	Hotspot Extent	Local Habitat Values	Corridors	Reason for Hotspot
1	Inclusive of New Beith to Glider Forest (Hilcrest)	Remnant vegetation and high-value regrowth vegetation patches, and remnant vegetation lining creeks, both dominated by Koala food trees.	The Glider Forest Parkinson Bushland Reserve Greenbank Military Training Area	Koala records within intact remnant vegetation with linkage to other vegetated area.

9.1.1.3 Available Koala Habitat

In line with the environmental investigations for the K2ARB, extensive ground-truthing and field surveys were undertaken to determine the extent of koala habitat for the K2ARB. Survey effort was conducted in-line with state and federal guidelines undertaken by WSP in May-June 2016, and ERM in February-May 2018 and January 2020. This consisted of active searches and Spot Assessment Technique (SAT) searches.

The environmental investigations for K2ARB identified that this Project Area is characterised by urbanisation, notably adjacent to the Greenbank Military Training Area, with patches of vegetation within the Project Area being frequently managed (cleared) for existing general rail operations. Koala habitat is available in the form of regrowth woodlands and some wildlife corridors of remnant-to-advanced-regrowth vegetation dominated by eucalypt species common across the Project Area.

Typical koala food tree species found in this region include *Corymbia intermedia, E. seena* and *E. tereticornis*, see Table 8-2 for a breakdown of canopy species identified on the Koala Detection Dog Surveys.

Table 9-2 Available Koala Habitat within K2ARB

Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
84	■ *Melaleuca quinquinervia* ■ *Corymbia intermedia* ■ *Lophestemon suaveolens* ■ *Eucalyptus seeana*	Intact remnant vegetation	Located in Greenbank Military Reserve, adjacent to heavily urbanised landscape and major roads
85	■ *Eucalyptus tereticornis* ■ *Eucalyptus crebra* ■ *Corymbia intermedia* ■ *Lophostemon suaveolens*	Intact remnant vegetation	Located in Greenbank Military Reserve, adjacent to heavily urbanised landscape and major roads
86	■ *Eucalyptus tereticornis* ■ *Corymbia intermedia*	Intact remnant vegetation	Located in Greenbank Military Reserve, adjacent to heavily urbanised landscape and major roads
87	■ *Lophostemon suaveolens* ■ *Eucalyptus seeana* ■ *Melaleuca quinquinervia* ■ *Corymbia intermedia*	Intact remnant vegetation	Located in Greenbank Military Reserve, adjacent to heavily urbanised landscape and major roads
88	■ *Eucalyptus tereticornis* ■ *Lophostemon suaveolens* ■ *Melaleuca quinquinervia* ■ *Corymbia intermedia*	Intact remnant vegetation	Located in Greenbank Military Reserve, adjacent to heavily urbanised landscape and major roads

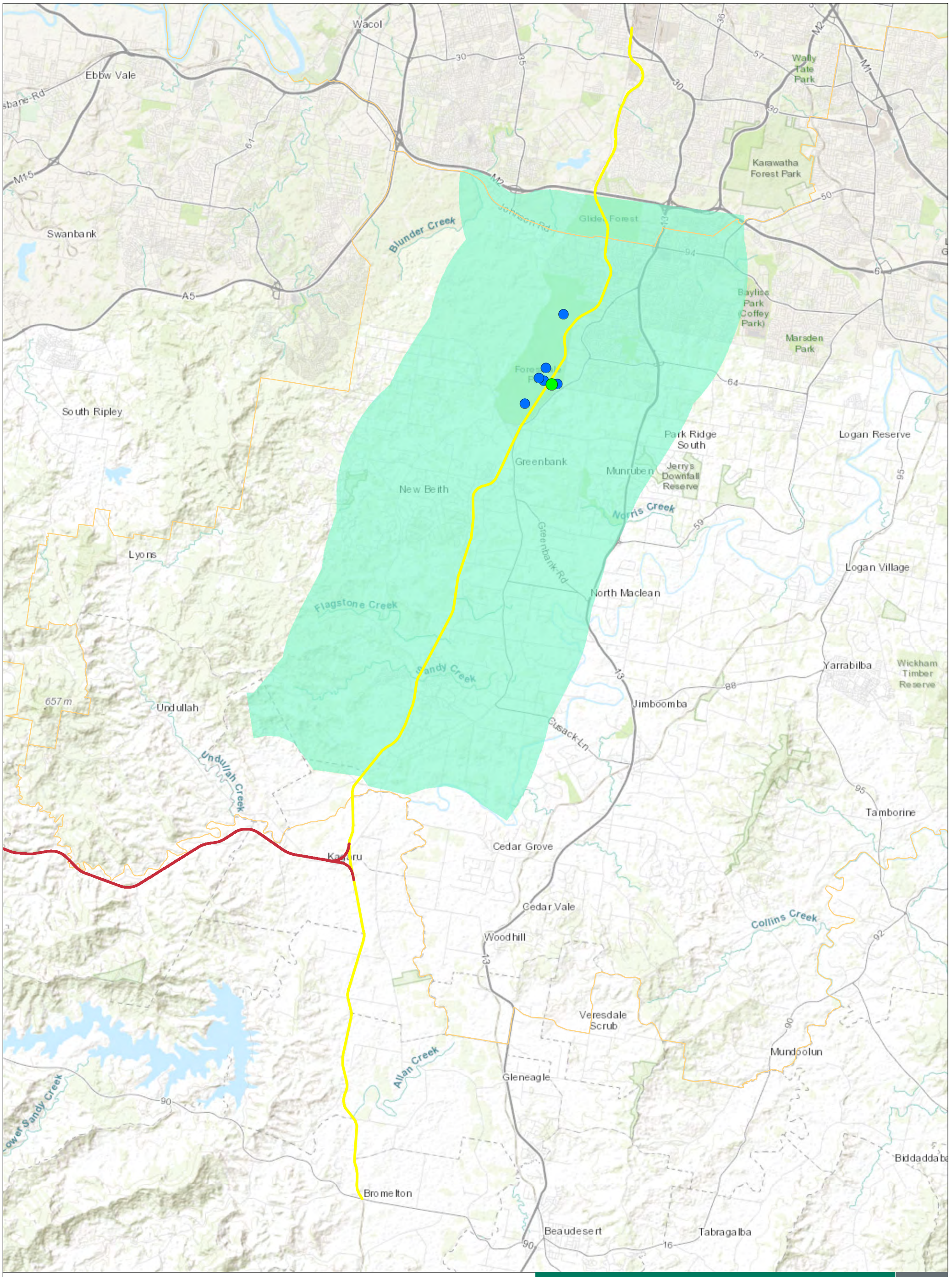
Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
89	■ *Eucalyptus seeana* ■ *Melaleuca quinquinervia* ■ *Corymbia intermedia*	Intact remnant vegetation	Located in Greenbank Military Reserve, adjacent to heavily urbanised landscape and major roads
90	■ *Corymbia citriodora* ■ *Lophostemon suaveolens* ■ *Melaleuca quinquinervia* ■ *Corymbia intermedia*	Intact remnant vegetation	Located in Greenbank Military Reserve, adjacent to heavily urbanised landscape and major roads
91	■ *Corymbia citriodora* ■ *Lophostemon suaveolens* ■ *Melaleuca quinquinervia*	Intact remnant vegetation	Located in Greenbank Military Reserve, adjacent to heavily urbanised landscape and major roads

9.1.1.4 Opportunity for Connectivity

Locations considered important for maintaining connectivity include the eucalypt dominated woodland of the Glider Forest as well as the vegetation of the Greenbank Military Training Area, existing as a fauna corridor in contrast to the surrounding predominately urban landscape. Both forested areas are important components of the broader Flinders-Karawatha Biodiversity Corridor which is notable as one of the largest and last remaining stretches of continuous forest within SEQ (McGregor & Jones, 2016). The full extent of this corridor extends from Karawatha Forest to Flinders Peak to Brisbane's south and west to the southern side of Ipswich (McGregor & Jones, 2016). The continued connectivity of suitable habitat provided by the corridor will be critical for koalas within the SEQ extent and the surrounding landscape. Maintenance of connectivity through utilising existing crossing structures should be prioritised. Crossing structures should be in line with the koala Sensitive Design Guidelines (2022) and consider the best practice design of structures recommended in the WSP Preliminary Fauna Connectivity Strategy for the B2G Project, however within this section of the IRP the proponent was able to demonstrate that no additional fauna connectivity structures were required for KARB for the EPBC referral application.

Where offsetting is required for significant residual impacts to the koala, priority should be given to potential offset sites within 5km of the alignment, and with vegetation connected to, or in close proximity to, the hotspot region and containing koala food trees important for the K2ARB such as *E. tereticornis, E. crebra* and *Lophostemon* and *Corymbia spp*.

See Figure 9-1 for hotspot region associated within K2ARB.



Legend

- Koala sightings
- Scat detections - East
- Active Koala Hotspots
- LGA Boundary

Inland Rail Alignment

- C2K - Calvert to Kagaru
- K2AB - Kagaru to Acacia Ridge/Bromelton

Data Source:
ESRI Would Topographic Map

Active Koala Hotspots with K2AB

F9-1

Drawing No:	0628339_N2AB_TM_G006_R3.mxd		Koala Genetics Study Project – Inland Rail Program (NSW and QLD)
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

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9.2 Calvert to Kagaru

9.2.1.1 Historic Koala Records

Studies undertaken for the C2K draft EIS confirmed koala presence throughout the Project Area. Observations of nine individuals and scat/scratch marks were found in Ebenezer, Peak Crossing and Washpool localities. ALA database showed several records throughout Project Area and even more in the greater surroundings. These records were largely concentrated in patches of protected forest, particularly across the ranges. The majority of these records are recent and were made post 2010.

9.2.1.2 Findings from the Study

Two hotspots have been identified within C2K and have been ranked in terms of priority for mitigation and management actions. Hotspot one is inclusive of the towns of Ebenezer to Peak Crossing and incorporates a mosaic of cleared agricultural land, advanced regrowth, and remnant vegetation. Riparian zones and protected nature reserves offer important corridors to koala dispersal within this hotspot, with prominence of Warrill Creek and the eucalypt dominated woodland of Purga Nature Reserve. Six scat detections were made following detection dog surveys and were noticeably within proximity to clusters of historic koala records.

Hotspot two is within proximity to hotspot one and is separated by a narrow section of cleared agricultural land. Due to the absence of historic records in between these hotspots, and the inability to survey in these areas, these hotspots have been delineated for the purpose of this study. It is possible that dispersal between hotspots occurs through the use of paddock trees and riparian vegetation, however this cannot be confirmed through this study. Hotspot two is inclusive of sandy creek south of Peak Crossing to the town of Washpool. Sandy Creek, Purga Creek and the eucalypt dominated woodlands across the Tevoit Range that extends beyond the IRP offer the primary corridors for koala movement within this hotspot and likely enables connectivity to suitable habitat in the surrounding area. Three koala scat detections were made just north of Washpool following detection dog surveys.

Table 9-3 Koala Hotspot Regions within C2K

Hotspot Number	Hotspot Extent	Local Habitat Values	Corridors	Reason for Hotspot
1	Inclusive of Ebenezer to Peak Crossing	Remnant vegetation and high-value regrowth vegetation patches, and remnant vegetation lining creeks, both dominated by Koala food trees.	Warrill Creek Purga Nature Reserve	Koala records within intact remnant vegetation with linkage to other larger vegetated area.
2	Inclusive of Sandy Creek (Peak Crossing) to Washpool.	Remnant vegetation of the Mountain Range, and remnant vegetation lining creeks, both dominated by Koala food trees.	Sandy Creek Purga Creek Teviot Range	Koala records within intact remnant vegetation with linkage to other larger vegetated area.

9.2.1.3 Available Koala Habitat

In line with the C2K draft EIS extensive ground-truthing and field surveys were undertaken to determine the extent of koala habitat for C2K. Survey effort was conducted in-line with state and federal guidelines undertaken by Jacobs-GHD in March-May 2016, GHD in December-February 2017, EMM September-November 2018 and FFJV December-April, May-July 2019. This consisted of habitat assessments, active searches, SAT searches and incidental observations.

The C2K draft EIS studies undertaken identified that this Project Area has remnant vegetation associated with sloping and steep topography or is highly fragmented with majority of the IRP dominated by pasture grassland, isolated trees and areas of advanced regrowth; by-products of historic land clearing. Remnant eucalypt vegetation is associated with the Peak Crossing, Teviot Range and Ebenezer areas (To the North and South of the Project Area). Most koala records are associated with riparian habitat near Western Creek, Warrill Creek and Purga Creek including its associated floodplains.

Typical koala food tree species found in this region include *E. crebra, C. citriodora and C. tessellaris*, see Table 9-4 for a breakdown of canopy species identified on the koala detection dog surveys.

Table 9-4 Available Koala Habitat within C2K

Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
75	■ *Corymbia citriodora* ■ *Eucalyptus crebra*	Likely remnant	Edge of cleared pastoral land
76	■ *Corymbia citriodora* ■ *Eucalyptus crebra*	Advanced regrowth/remnant	Edge of cleared pastoral land
77	■ *Eucalyptus tereticornis* ■ *Callitris glaucophylla*	Advanced regrowth/remnant	Purga Nature Reserve
78	■ *Eucalyptus crebra* ■ *Eucalyptus tereticornis* ■ *Corymbia tessellaris*	Advanced regrowth/remnant	Purga Nature Reserve
79	■ *Corymbia tessellaris* ■ *Eucalyptus crebra* ■ *Corymbia citriodora*	Advanced regrowth/remnant	Connectivity with local nature reserved edging pastoral land
80	■ *Eucalyptus woollsiana*	Advanced regrowth/remnant	Connectivity with local nature reserved edging pastoral land
81	■ *Corymbia tessellaris* ■ *Eucalyptus crebra* ■ *Eucalyptus tereticornis* ■ *Lophostemon suaveolens*	Advanced regrowth/remnant	Connectivity with local nature reserved edging pastoral land
82	■ *Corymbia citriodora* ■ *Eucalyptus crebra*	Advanced regrowth/remnant	Connectivity with local nature reserved edging pastoral land
83	■ *Eucalyptus crebra* ■ *Corymbia tessellaris*	Advanced regrowth/remnant	Connectivity with local nature reserved edging pastoral land

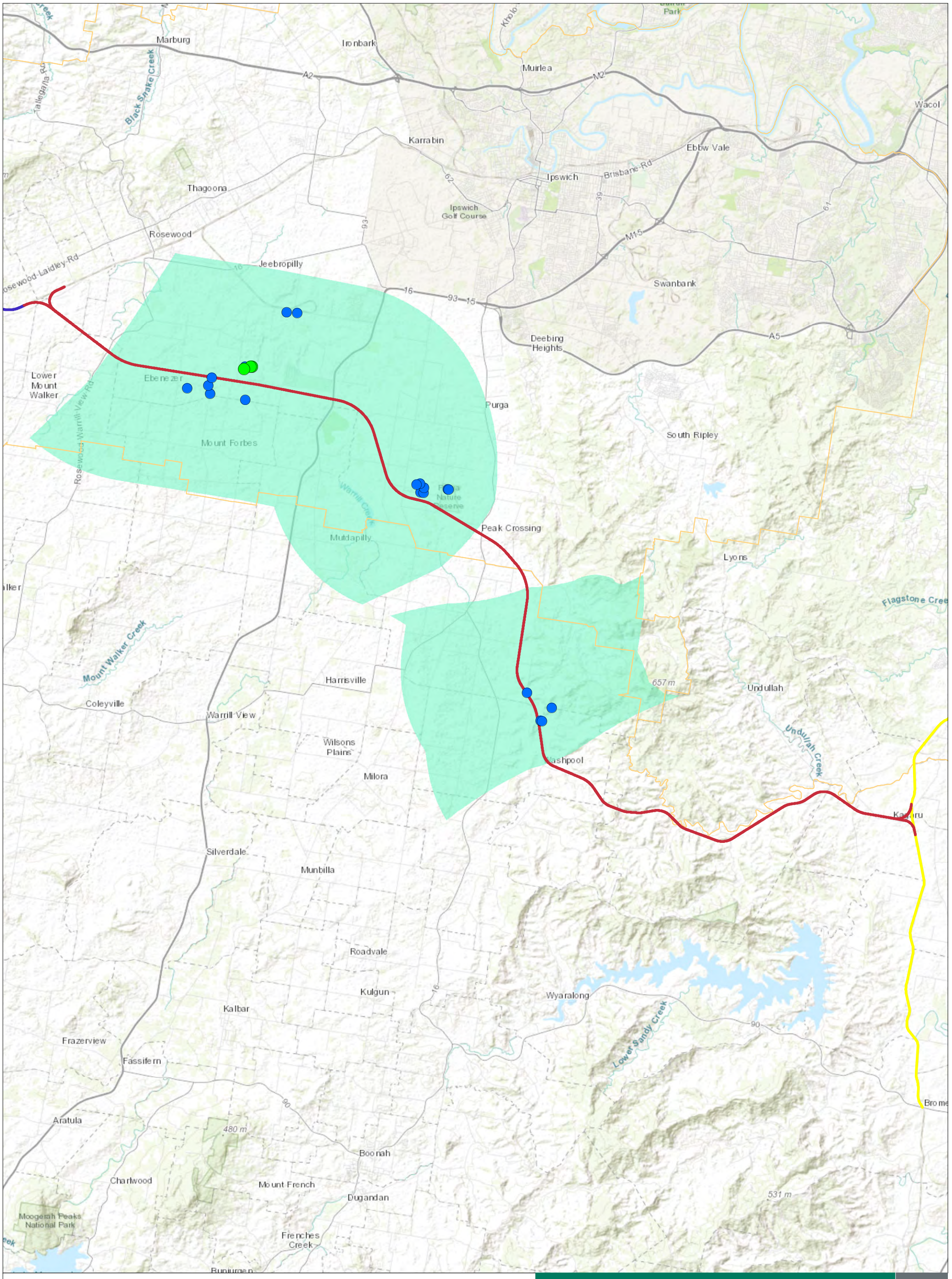
9.2.1.4 Opportunity for Connectivity

Locations considered important for maintaining connectivity include the eucalypt dominated woodland of the Purga Nature Reserve as well as the riparian vegetation along Purga, Sandy and Warrill creeks. These provide important fauna corridors throughout otherwise heavily cleared agricultural land. Connecting fragmented eucalypt vegetation to the contiguous forest of the Tevoit Ranges will also be important for improving connectivity. Maintenance of connectivity through utilising existing crossing structures should be prioritised. Crossing structures should be in line with the Koala Sensitive Design Guidelines (2022) and consider the WSP Preliminary Fauna Connectivity Strategy for the B2G Project.

The eastern edge of C2K overlaps with the Flinders-Karawatha Biodiversity Corridor. In addition to providing important continuous koala habitat, this corridor is critical for facilitating extensive connectivity across the broader landscape. In particular, this corridor is likely to provide connectivity between the second hotspot of C2K and the active hotspot identified within K2ARB. As such, any efforts to maintain or improve connectivity will be beneficial to local koalas across the landscape, beyond the extent of the IRP.

Where offsetting is required for significant residual impacts to the koala, priority should be given to potential offset sites within 5km of the alignment, and with vegetation connected to, or in close proximity to, the hotspot region and containing koala food trees important for the C2K such as *E. tereticornis, E. crebra,* and *Lophostemon* and *Corymbia spp*.

See Figure 9-2 for hotspot locations associated within the Project Area.



Legend

- Koala sightings
- Scat detections - East
- Active Koala Hotspots
- LGA Boundary

Inland Rail Alignment

- C2K - Calvert to Kagaru
- H2C - Helidon to Calvert
- K2AB - Kagaru to Acacia Ridge/Bromelton

Data Source:
ESRI Would Topographic Map

Active Koala Hotspots with C2K

F9-2

Drawing No:	0628339_N2AB_TM_G006_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

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9.3 Helidon to Calvert

Studies undertaken for the H2C draft EIS confirmed koala presence throughout the IRP. Observations of five individuals and scat/scratch marks localised to between Helidon and Gatton as well as between Laidley to Calvert. One was recorded near Helidon, three between Laidley and Grandchester and one near Calvert. ALA database showed several records throughout Project Area and even more in the greater surroundings.

9.3.1.1 Findings from the Study

Two hotspots have been identified within H2C following desk top assessments and detection dog surveys and have been ranked in terms of priority for intervention and mitigation measures. Hotspot one is inclusive of Helidon, Lockyer State Forest and Ringwood and is likely to connect to the Adjacent hotspot in G2H. Remnant eucalypt vegetation is still dominant, particularly across the range and in riparian creek vegetation. Historic koala records are numerous surrounding the town of Helidon and three scat detections were made following detection dog surveys.

A considerable gap between hotspot one and hotspot two is present in H2C with the landscape dominated primarily by cleared agricultural land and urban areas with minimal records of koala presence. Hotspot two is bordered by the town of Laidley and is inclusive of the remnant forest surrounding the town of Grandchester and Western Creek on the outskirts of Calvert. Historic koala records are present within the area defined as the hotspot but no detections were made following detection dog surveys. Consultation with community members and local organisations have confirmed that anecdotal observations of koalas within the hotspot is known with sightings and known instances of koala fatalities presented in community consultation workshops for the IRP.

Table 9-5 Koala Hotspot Regions within H2C

Hotspot Number	Hotspot Extent	Local Habitat Values	Corridors	Reason for Hotspot
1	Inclusive of Helidon, Lockyer State Forest to Ringwood.	Remnant vegetation of the Mountain Range and lining creeks, dominated by Koala food trees.	Lockyer State Forest Helidon Hills	Koala records within intact remnant vegetation with linkage to other larger vegetated area.
2	Inclusive of Laidley to Western Creek (Calvert).	Remnant vegetation of the Mountain Range and lining creeks, dominated by Koala food trees.	Little Liverpool Range	Koala records within intact remnant vegetation with linkage to other larger vegetated area.

9.3.1.2 Available Koala Habitat

In line with the H2C draft EIS, extensive ground-truthing and field surveys were undertaken to determine the extent of koala habitat for the Helidon to Calvert Project. Survey effort was conducted in-line with state and federal guidelines undertaken by Arup/SMEC in March-June 2016, EMM in September-November 2018 and ELA December-April 2019. This consisted of habitat assessments, active searches, SAT searches and incidental observations. Studies undertaken to support the H2C draft EIS identified that remnant vegetation was fragmented and typically occurred in areas characterised by rocky ranges and sloping topography such as the Lockyer Valley, Helidon Hills and the Little Liverpool Range. Regrowth of varying quality is also present along roadsides and in isolated patches adjoining pastoral land and urban areas.

Typical koala food tree species found in this region include *E. crebra* and *C. citriodora*, see Table 8-6 for a breakdown of canopy species identified on the koala detection dog surveys.

Table 9-6 Available Koala Habitat within H2C

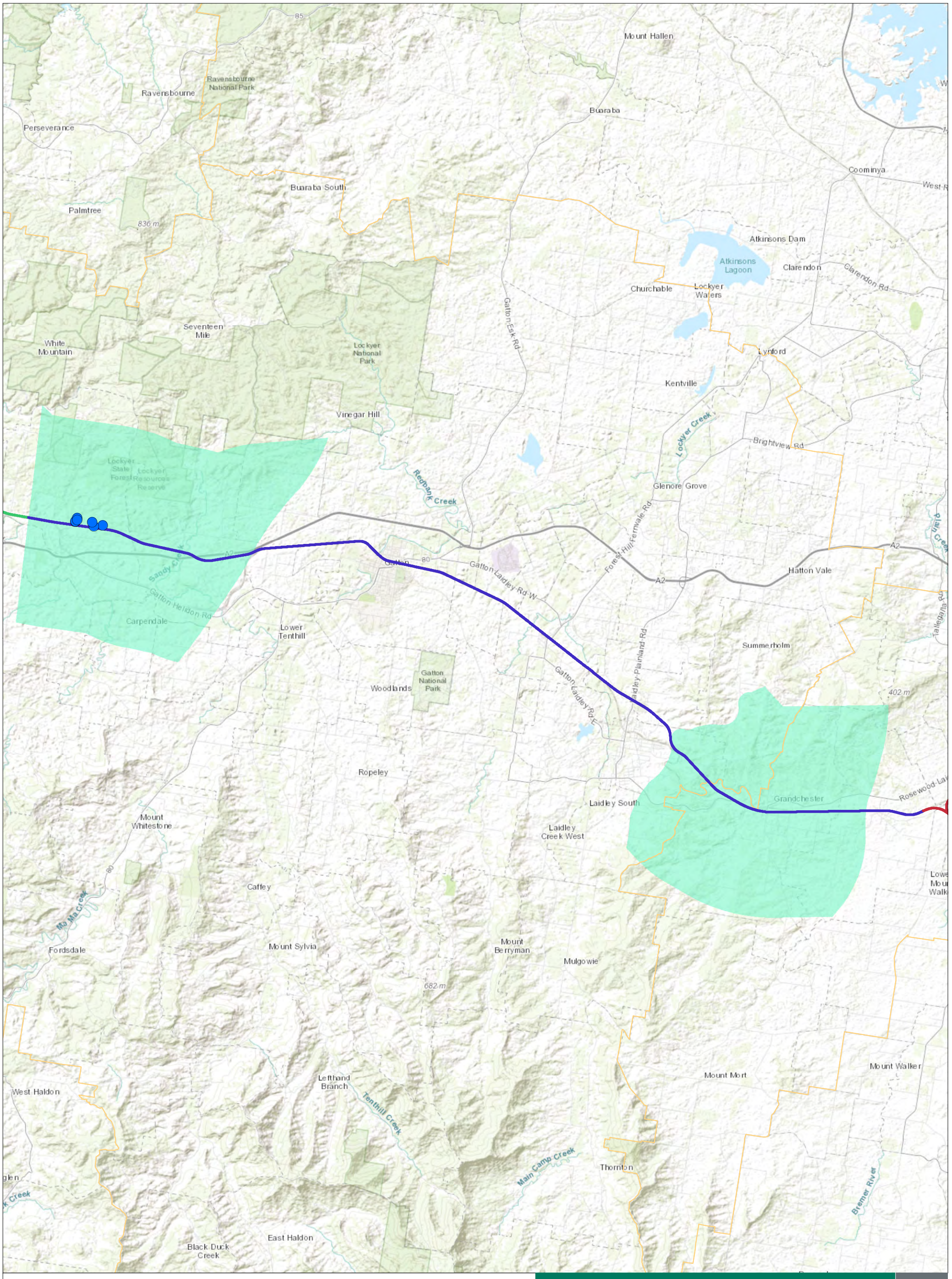
Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
68	■ *Eucalyptus crebra* ■ *Corymbia citriodora*	Advanced Regrowth	Riparian zone adjacent to cleared agricultural land
69	■ *Eucalyptus crebra* ■ *Corymbia citriodora*	Advanced regrowth	Cleared pastoral land
70	■ *Corymbia citriodora* ■ *Corymbia gummifera* ■ *Lophostemon suaveolens*	Remnant	Riparian zone in continuous forest
71	■ *Corymbia citriodora*	Remnant	Continuous forest
72	■ *Lophostemon suaveolens* ■ *Corymbia citriodora*	Remnant	Continuous forest
73	■ *Corymbia citriodora*	Remnant	Continuous forest
74	■ *Corymbia citriodora*	Remnant	Continuous forest

9.3.1.3 Opportunity for Connectivity

Locations considered important for maintaining connectivity include the eucalypt dominated woodland of the Lockyer State Forest as well as the Little Liverpool Range and Helidon Hills. These provide important fauna corridors throughout otherwise heavily cleared agricultural land. Also of note for potential connectivity are the riparian vegetation along the Lockyer Creek and Gatton National Park. Maintenance of connectivity through utilising existing crossing structures should be prioritised. Crossing structures should be in line with the Koala Sensitive Design Guidelines (2022) and consider the WSP Preliminary Fauna Connectivity Strategy for the B2G Project.

Where offsetting is required for significant residual impacts to the koala, priority should be given to potential offset sites within 5km of the alignment, and with vegetation connected to, or in close proximity to, the hotspot region and containing koala food trees important for the H2C such as*, E. crebra* and *Lophostemon* and *Corymbia spp*.

See Figure 9-3 for hotspot locations associated with the Project Area.



Legend

- Scat detections - East
- Active Koala Hotspots
- LGA Boundary

Inland Rail Alignment

- C2K - Calvert to Kagaru
- G2H - Gowrie to Helidon
- H2C - Helidon to Calvert

Data Source:
ESRI Would Topographic Map

Active Koala Hotspots with H2C

F9-3

Drawing No:	0628339_N2AB_TM_G006_R3.mxd		Koala Genetics Study Project – Inland Rail Program (NSW and QLD)
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

0 2 4Km

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ERM

9.4 Gowrie to Helidon

9.4.1.1 Historic Koala Records

High densities of koala records are present within G2H with the vast majority recorded between 2010 and 2021. These records occur within the eucalypt woodland between Postmans Ridge and Toowoomba, particularly adjacent to the Toowoomba bypass. Records are present within the more heavily urbanised Toowoomba however these are less frequent. Rocky creek and the adjacent vegetation contain many records between Helidon Spa and Postmans ridge all of which are recent. Many of these records are likely to be in association with the recent infrastructure upgrades, namely the development of the Toowoomba bypass.

9.4.1.2 Findings from the Study

A single hotspot of koala activity was identified within G2H and is inclusive of Mt Kynoch on the outskirts of Toowoomba to Helidon. Extensive historic records are present and numerous detections were made following detection dog surveys. The hotspot also possesses key koala habitat corridors across the Toowoomba range, which is heavily dominated by remnant forest, and the Lockyer Creek. The hotspot is also likely to overlap with adjoining hotspots within H2C.

Table 9-7 Koala Hotspot Region within G2H

Hotspot Number	Hotspot Extent	Local Habitat Values	Corridors	Reason for Hotspot
1	Inclusive of Mt Kynoch to Helidon.	Remnant vegetation of the Mountain Range and lining creeks, dominated by Koala food trees.	Lockyer Creek Toowoomba Range	Koala records within intact remnant vegetation with linkage to other larger vegetated area.

9.4.1.3 Available Koala Habitat

In line with the G2H draft EIS, extensive ground-truthing and field surveys were undertaken to determine the extent of koala habitat for the Gowrie to Helidon Project. Survey effort was conducted in-line with state and federal guidelines undertaken by Arup in March 2017, EMM in September-May 2019 and ELA December-April 2019. This consisted of habitat assessments, active searches, SAT searches and incidental observations.

The majority of G2H is dominated by cleared pasture and cropping land with considerable barriers to koala movement. Of the available eucalypt vegetation, the majority is highly fragmented regrowth of mixed quality with remnant mature eucalypt open forest isolated to the central portion of G2H. Notably the Toowoomba Bypass is a major existing barrier to koalas in the region. Suitable koala habitat is found in the Helidon Hills and Toowoomba Ranges areas as well as being characterised by riparian habitat, notably Gowrie Creek.

Typical koala food tree species found in this region include Narrow-leaved ironbark *E. crebra* and *C. citriodora*, see Table 8-8 for a breakdown of canopy species identified on the Koala Detection Dog Surveys.

Table 9-8 Available Koala Habitat within G2H

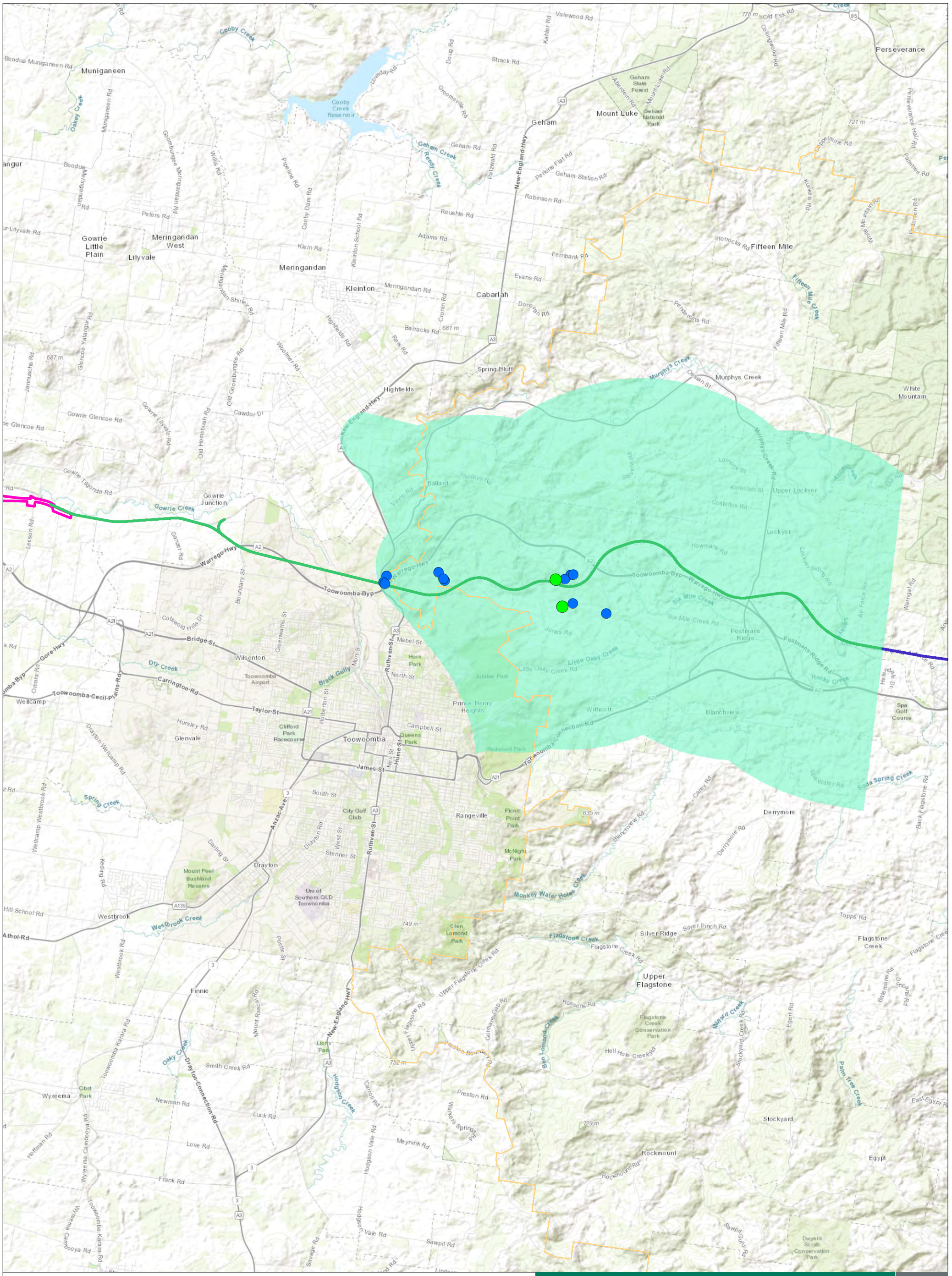
Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
59	■ *Eucalyptus crebra* ■ *Corymbia citriodora*	Remnant	Continuous forest
60	■ *Eucalyptus crebra* ■ *Corymbia*	Remnant	Continuous forest
61	■ *Eucalyptus crebra*	Remnant	Continuous forest with powerline track
62	■ *Eucalyptus citriodora*	Remnant	Continuous forest with access tracks
63	■ *Eucalyptus moluccana* ■ *Corymbia citriodora*	Remnant/Advanced regrowth	Continuous forest
64	■ *Corymbia citriodora* ■ *Eucalyptus crebra*	Remnant	Continuous forest
65	■ *Eucalyptus tereticornis* ■ *Corymbia citriodora*	Remnant	Continuous forest
66	■ *Eucalyptus tereticornis* ■ *Corymbia sp*	Remnant	Road corridor
67	■ Lophostemon confertus	Remnant	Continuous forest adjacent to urban infrastructure

9.4.1.4 Opportunity for Connectivity

Locations considered important for maintaining connectivity include the eucalypt dominated woodland of the Toowoomba range and the remnant riparian vegetation of the Lockyer Creek. These areas provide important fauna corridors across a landscape that has been heavily modified for both agriculture and urban development. Smaller creeks such as Rocky Creek also provide valuable corridors between remnant woodland and retained advanced regrowth. Major roads, such as the Toowoomba bypass, offer significant barriers to koala movement within G2H. Crossing structures should be in line with the Koala Sensitive Design Guidelines (2022) and consider the WSP Preliminary Fauna Connectivity Strategy for the B2G Project.

Where offsetting is required for significant residual impacts to the koala, priority should be given to potential offset sites within 5km of the alignment, and with vegetation connected to, or in close proximity to, the hotspot region and containing koala food trees important for the G2H such as*, E. crebra, E. moluccana, E. tereticornis* and *Lophostemon* and *Corymbia spp*.

See Figure 9-4 for hotspot locations associated with the Project Area.


Cooby Creek
Boodua Muniganeen Rd
Muniganeen
Oakey Creek
Muniganeen Rd
Cooby Creek Reservoir
Loveday Rd
Doug Rd
Strack Rd
Kahler Rd
Valewood Rd
778 m
Old Esk Rd
Collingwood Rd
Perseverance
Geham State Forest
Aberdeen Rd
Mount Luke Rd
Mount Luke
Geham National Park
Geham
721 m
Perseverance Haul Rd
Palmtree Rd
Geham Rd
Geham Creek
Reedy Creek
Patzwald Rd
New England Hwy
Perkins Flat Rd
Geham Station Rd
Robinson Rd
Reushle Rd
Adams Rd
Klein Rd
Peters Rd
Boodua
Muniganeen Rd
Goombungee Meringandan Rd
Willis Rd
Pipeline Rd
Cooby Dam Rd
Kleinton School Rd
Gowrie Little Plain
Lilyvale
Meringandan West
Meringandan
Kleinton
Meringandan Rd
Cabarlah
Fernbank Rd
Evans Rd
Horrocks Rd
Fifteen Mile
Fifteen Mile Creek
Fifteen Mile Rd
Penderests Rd
Donovan Rd
Cronin Rd
Barracks Rd
681 m
Highfields Rd
Reis Rd
Meringandan Shirley Rd
Woolmer Rd
Old Goombungee Rd
Glencoe Yalangur Rd
Jannuschs Rd
687 m
Spring Bluff
Highfields
Murphys Creek
Ossian St
White Mountain
Gowrie Glencoe Rd
Gowrie Lilyvale Rd
Old Homebush Rd
Cawdor Dr
Gowrie Tigronda Rd
Gowrie Junction
Gowrie Creek
Leeson Rd
Ganzer Rd
Warrego Hwy
Toowoomba Byp
Boundary St
Ballard
Hanleys Rd
Vayne Rd
Larnook St
Koreelah St
Upper Lockyer
Costellos Rd
Lockyer
Howmans Rd
Lockyer Siding Rd
Six Mile Creek
Six Mile Creek Rd
Jones Rd
Postmans Ridge
Postmans Ridge Rd
Rocky Creek
Little Oaky Creek
Little Oaky Creek Rd
Jubilee Park
Withcott
Blanchview
Spa Golf Course
Toowoomba Connection Rd
Redwood Park
Prince Henry Heights
Colsworld Hills Dr
Bridge St
Gleerwattle St
Wilsonton
Toowoomba Airport
Dry Creek
Carrington Rd
Taylor St
Black Gully
Mort St
Mabel St
Horn Park
North St
Ruthven St
Wellcamp
Toowoomba Cecil Plains Rd
Omara Rd
Clifford Park Racecourse
Hursley Rd
Glenvale
Holberton St
Toowoomba
Campbell St
Queens Park
Neil St
Hume St
James St
Drayton Wellcamp Rd
Spring Creek
Wellcamp Westbrook Rd
South St
City Golf Club
Anzac Ave
Rangeville
Picnic Point Park
635 m
Soda Spring Creek
Spa Water Rd
Carey Rd
Derrymore
Derrymore Rd
Blanchview Rd
Back Flagstone Rd
Darling St
Drayton Rd
West St
Stenner St
Ruthven St
Drayton
Mount Peel Bushland Reserve
Keating Rd
Uni of Southern QLD Toowoomba
McNight Park
Monkey Water Holes Creek
Hill School Rd
Westbrook
Westbrook Creek
Athol Rd
Glen Lomond Park
749 m
Silver Ridge
Silver Pinch Rd
Topps Rd
Flagstone Creek
Flagstone Creek Rd
Upper Flagstone Creek Rd
Upper Flagstone
Rossells Rd
Flagstone Creek Conservation Park
Ben Lomond Creek
Hell Hole Creek Rd
Germans Gap Rd
Preston Rd
Stockyard Creek Rd
Stockyard Creek
Egert Rd
Batemans Rd
Orchard Rd
Palm Tree Creek
East Egypt Rd
Finnie
Mount Rascal Rd
Toowoomba Karara Rd
Oaky Creek
Drayton Connection Rd
Smith Creek Rd
Lions Park
752 m
Hodgson Creek
Carroll Rd
Wyreema
Obst Park
Newman Rd
Luck Rd
Love Rd
Frank Rd
Heffman Rd
Wyreema Camooya Rd
Hodgson Vale Rd
Meynink Rd
Walkers Springs Rd
729 m
Stockyard
Rockmount
Rockmount Rd
Egypt
Sawpit Rd
Savage Rd
Sawpit Gully Rd
Dwyers Scrub Conservation Park
Legend
Koala sightings
Scat detections - East
Active Koala Hotspots
LGA Boundary
Inland Rail Alignment
B2G - NSW/QLD Border to Gowrie
G2H - Gowrie to Helidon
H2C - Helidon to Calvert
Data Source:
ESRI Would Topographic Map
Active Koala Hotspots with G2H
F9-4
Drawing No: 0628339_N2AB_TM_G006_R3.mxd
Date: 14/02/2024
Drawing Size: A4
Drawn By: VN
Reviewed By: MD
Coordinate System: GDA2020 MGA Zone 56
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Koala Genetics Study Project – Inland Rail Program (NSW and QLD)
Client: ARTC
This figure may be based on third party data or data which has not been verified by ERM and it may not be to scale. Unless expressly agreed otherwise, this figure is intended as a guide only and ERM does not warrant its accuracy.
ERM

9.5 Border to Gowrie

9.5.1.1 Historic Koala Records

Historic koala records accessed from ALA are present in high densities throughout the B2G project with a particularly dense cluster of records surrounding Toowoomba. Noticeably, a high proportion of these records are recent (between 2010 and 2021). Large areas of intact forest are present adjacent to B2G, yet records are also common throughout more heavily cleared agricultural land. These are largely located in linear stretches of roadside vegetation however riparian zones and fragmented patches of regrowth and remnant vegetation throughout the landscape also appear to provide a degree of habitat.

Active searches and SAT searches undertaken during surveys for the EIS have returned detections of koala scat and individuals within the B2G IRP.

9.5.1.2 Findings from the Study

Three hotspots were identified in B2G and have been provided with an initial ranking of priority for management and mitigation measures. Hotspot one is inclusive of Pittsworth to Athol and it largely dominated by isolated regrowth vegetation surrounded by cleared or semi-cleared pastoral land. Patches of isolated roadside vegetation also are present. No major corridors for koala movement have been identified, however connectivity may be provided by the paddock trees and small isolated remnant vegetation and regrowth that is present. Koala records are known from both historic presence data and detection dog surveys within the IRP. Extensive anecdotal evidence of koala activity has also been provided by local organisations and community members following community consultations.

Hotspot two is inclusive of Bringalily, Whetstone State Forests within the IRP and is noted to provide extensive corridors of suitable koala habitat. While slightly adjacent to the IRP, the Yelarbon State Forest and Devine State Forest also offer important areas of protected koala habitat. Corridors are maintained between these state forests through remnant roadside vegetation, paddock trees and riparian vegetation along key creeks and waterways. Historic koala records and detection dog surveys both confirm the presence of koalas within the IRP and is likely to be an important area for koala persistence and dispersal in the landscape.

The third hotspot is inclusive of Domville State Forest, Millmerran and the Condamine River. While relatively few records are known from historic data records or from detection dog surveys, numerous anecdotal evidence was provided by local organisations and community members. Of note within this hotspot is the presence of mining outside Domville. Existing riparian vegetation is likely to still provide corridors for connectivity and critical koala habitat, however existing land use practices may establish barriers to connectivity.

Table 9-9 Koala Hotspot Regions within B2G

Hotspot Number	Hotspot Extent	Local Habitat Values	Corridors	Reason for Hotspot
1	Inclusive of Pittsworth to Athol.	Isolated regrowth vegetation dominated by koala food trees surrounded by pastoral land.		Patches of isolated and roadside vegetation with Koala records.
2	Inclusive of Bringalily State Forest, Whetstone State Forest, and Yelarbon State Forest	Intact remnant vegetation in the State Forests is dominated by koala food trees.	Bringalily State Forest, Whetstone State Forest, and Canning Creek	Being of continuous remnant vegetation forming part of a larger protected vegetation area. Riparian habitat with Koala records.
3	Inclusive of Domville State Forest, Millmerran, to the Condamine River.	Isolated regrowth vegetation dominated by koala food trees surrounded by pastoral land. Remnant vegetation in both the State Forest and lining creeks, dominated by koala food trees. Surrounded by pastoral land.	Condamine River and floodplain Domville State Forest	Isolated riparian habitat and roadside vegetation with Koala records, and intact remnant vegetation with linkage to other larger vegetated area.

9.5.1.3 Available Koala Habitat

In line with the B2G draft EIS extensive ground-truthing and field surveys were undertaken to determine the extent of koala habitat for the Border to Gowrie Project. Survey effort was conducted in-line with state and federal guidelines undertaken by ELA from April to October 2016 and June 2018 to May 2019. This consisted of active searches, SAT searches, call playback and spotlighting.

The B2G draft EIS studies found the Project Area has been significantly modified by the clearing of native vegetation, overall being heavily fragmented by agriculture and urbanised land use. Suitable koala habitat was revealed to be within the riparian corridor of the Condamine River and associated floodplain and in regrowth and isolated vegetation around Pittsworth and surrounding regions in the north of the Project Area. Notably this Project is characterised by its proximity to Durakai and Bringalily State Forests.

Typical koala food tree species found in this region include *Eucalyptus camaldulensis*, *Eucalyptus populnea* and *Eucalyptus chloroclada*, see Table 9-10 for a breakdown of canopy species identified on the koala detection dog surveys.

Table 9-10 Available Koala Habitat within B2G

Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
41	■ *E. populnea* ■ *E. camaldulensis*	Historically cleared pastoral land with advanced regrowth	Isolated vegetation amongst pastoral land
42	■ *E. populnea* ■ *E. camaldulensis*	High dieback Historically cleared pastoral land with advanced regrowth	Isolated vegetation amongst pastoral land
43	■ *Eucalyptus camaldulensis* ■ *Corymbia erythrophloia*	Historically cleared pastoral land with advanced regrowth	Riparian zone
44	■ *E. populnea* ■ *E. camaldulensis* ■ *E. tereticornis* ■ *Casuarina cristata*	Historically cleared pastoral land with advanced regrowth	Isolated vegetation amongst pastoral land
45	■ *E. tereticornis* ■ *E. populnea* ■ *Acacia salicina*	Historically cleared pastoral land with advanced regrowth Significant dieback	Isolated vegetation amongst pastoral land
46	■ *E. tereticornis* ■ *Casuarina cunninghamiana* ■ *Acacia salicina*	Remnant/advanced regrowth Some dieback	Isolated patch of trees surrounded by cleared agricultural land
47	■ *E. chloroclada* ■ *E. melanophloia* ■ *Callistris glaucophylla*	Historically cleared agricultural land with some remnant/advanced regrowth	Adjacent to ephemeral creek
48	■ *E. crebra*	Historically cleared with moderate dieback along agricultural land	Adjacent to Creekline surrounded by cleared pastoral land
49	■ *E. melanophloia* ■ *E. crebra*	Narrow linear strip of remnant vegetation	Isolated roadside vegetation surrounded by cleared pastoral land
50	■ *E. crebra* ■ *E. chloroclada*	Historically cleared Creekline on pastoral land	Isolated vegetation surrounded by cleared pastoral land
51	■ *E. melanophloia* ■ *E. chloroclada*	Advanced regrowth along potential Creekline	Isolated vegetation surrounded by cleared pastoral land
52	■ *E. melanophloia* ■ *E. chloroclada*	Mature paddock trees along Creekline with cleared understory	Adjacent to mine site and surrounded by cleared pastoral land
53	■ *E. chloroclada* ■ *Corymbia erythrophloia* ■ *E. melanophloia*	Advanced regrowth with occasional mature trees	Along ephemeral creek Pastoral land connected to Devine State Forest and Bringalily State Forest
54	■ *E. populnea* ■ *Eucalyptus camaldulensis*	Remnant	Road corridor through state forest

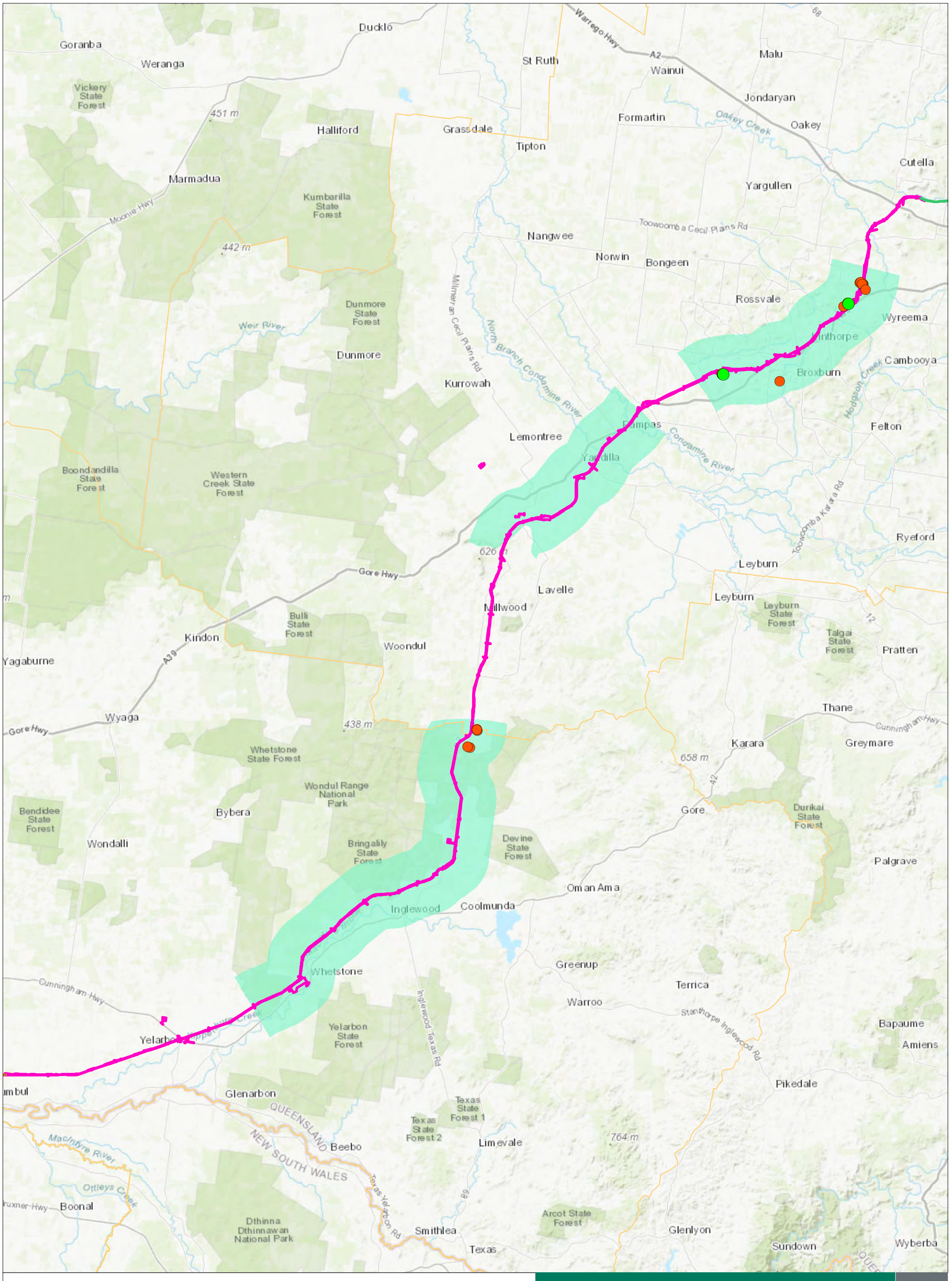
Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
55	■ *E. populnea* ■ *Eucalyptus camaldulensis*	Remnant	Road corridor through state forest
56	■ *Eucalyptus camaldulensis* ■ *Corymbia erythrophloia*	Advanced regrowth with occasional mature trees	Cleared pastoral land within close proximity to state forest
57	■ *E. populnea* ■ *Eucalyptus camaldulensis* ■ *E. tereticornis* ■ *Casuarina cristata*	Advanced regrowth and evidence of historic fires	Adjacent to quarry and connected to state forest
58	■ *E. tereticornis* ■ *E. populnea* ■ *Acacia salicina*	Advanced regrowth and evidence of historic fires	Adjacent to quarry and connected to state forest

9.5.1.4 Opportunity for Connectivity

Locations considered important for maintaining connectivity include the eucalypt dominated woodland of the Bringalily, Whetstone and Domville State Forests, which provide important corridors to more contiguous forests outside of the IRP, including Devine and Yelabon State Forests and Wondul Range National Park. Riparian zones are also important corridors for fauna connectivity with the Canning Creek and the Condamine River and associated floodplains providing critical connectivity across otherwise cleared agricultural land. Crossing structures should be in line with the Koala Sensitive Design Guidelines (2022) and the WSP Preliminary Fauna Connectivity Strategy for the B2G Project.

Where offsetting is required for significant residual impacts to the koala, priority should be given to potential offset sites within 5km of the alignment, and with vegetation connected to, or in proximity to, the hotspot region and containing koala food trees important for the B2G such as*, E. crebra, E. tereticornis, E. Camaldulensis, E. populnaea, E. melanophloia,* and *Lophostemon, Angophora,* and *Corymbia spp*.

See Figure 9-5 for hotspot locations associated within the Project Area.



Legend

- Koala sightings
- Scat detections - West
- Active Koala Hotspots
- LGA Boundary

Inland Rail Alignment

- B2G - NSW/QLD Border to Gowrie
- G2H - Gowrie to Helidon
- NS2B - North Star to Border

Data Source:
ESRI Would Topographic Map

Active Koala Hotspots with B2G

F9-5

Drawing No:	0628339_N2AB_TM_G006_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

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9.6 North Star to Border

9.6.1.1 Historic Koala Records

Historic koala records accessed from ALA are present in the Project Area, with records seen near heavily cleared agricultural land. These are largely located in linear stretches of roadside vegetation in the North Star locality. Riparian zones and fragmented patches of regrowth and remnant vegetation throughout the landscape also appear to provide a degree of habitat with records from the Macintyre River. It should be noted that the proximity to urban areas may influence the number of sightings that have historically been made of individuals in relation to the size and quality of habitat.

Previous active searches and SAT searches undertaken in 2018 returned detections of one individual along the Macintyre River as well as scat and scratch marks found in 2021 across the Project Area.

9.6.1.2 Findings from the Study

No historic koala presence records are known across NS2B and field surveys did not return detections. N2S2B is mostly comprised of highly modified agricultural land with very little available habitat that could support koalas or aid in dispersal. No hotspots have been identified within NS2B. Further field surveys have been proposed for July 2023.

9.6.1.3 Available Koala Habitat

In line with the NS2B draft EIS extensive ground-truthing and field surveys were undertaken to determine the extent of koala habitat for the North Star to Border Project. Survey effort was conducted in-line with state and federal guidelines undertaken by FFJV in October 2018 to October 2019 and in 2021. This consisted of active searches, SAT searches, call playback and spotlighting.

The NS2B draft EIS studies found the Project Area has been significantly modified by agricultural land use, where the clearing of native vegetation has been extensive. Large tracts of remnant vegetation are rare within the Project Area, with most of the remaining native vegetation occurring in small fragments, often in a highly degraded state. Koala habitat is available in the form of riparian vegetation along waterways or drainage lines, with an individual identified within the riparian vegetation of the Macintyre River. Typical koala food tree species found in this region include Poplar box (*Eucalyptus populnea)*, see Table 9-11 for a breakdown of canopy species identified on the Koala Detection Dog Surveys.

Table 9-11 Available Koala Habitat within NS2B

Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
38	■ *Eucalyptus populnea* ■ *Casuarina cristata*	Some mature trees and advanced regrowth with cleared understory and limited recruitment	Isolated patch of vegetation surrounded by cleared pastoral and cropping land
39	■ *Eucalyptus populnea*	Narrow linear roadside vegetation with some mature trees and advanced regrowth	Isolated patch of vegetation surrounded by cleared pastoral and cropping land
40	■ *Eucalyptus populnea*	Narrow linear roadside vegetation with some mature trees and advanced regrowth	Isolated patch of vegetation surrounded by cleared pastoral and cropping land

9.6.1.4 Opportunity for Connectivity

No hotspots of koala activity have been identified within the NS2B owing to its relatively small size and a lack of koala detections. However, the Macintyre River offers important opportunities for maintaining connectivity and improving available habitat and, across the broader landscape, can provide some connection to Yelarbon State Forest, albeit over large geographic distances. Smaller creeks and drainage lines throughout the cleared agricultural land may also provide opportunities for improving connectivity across the landscape. Crossing structures should be in line with the best practice designs and aim to support the objectives of the Koala Habitat Protection Guidelines (2019) and State Environmental Planning Policy (SEPP) (Biodiversity and Conservation) (Chapter 4 Koala habitat protection) 2021. The design and best practice recommendations provided by the WSP Preliminary Fauna Connectivity Strategy and NSW Koala Strategy (Environment and Heritage Department of Planning and Environment, 2022) for the B2G Project will also provide valuable learnings for the implementation of suitable koala crossing structure.

9.7 Narrabri to North Star (Phase 2)

9.7.1.1 Historic Koala Records

Numerous historic records sourced from ALA exist within N2NS. These records are largely more recent than those of N2N being made between 2010 and 2021. These records are noticeable concentrated around the town of Moree with records present within the riparian vegetation of the Mehi river as well as roadside vegetation across the broader landscape. Spotlighting and SAT searches undertaken in 2015 returned positive detections of both koala individuals and scats.

Another cluster of historic records is present surrounding the town of Crooble. Records made before 2010 exist in the nearby Bullala National Park. More recent records are present but appear to be restricted to linear strips of riparian vegetation and roadsides with minimal connectivity.

As presented in the N2NS draft EIS (2017), an additional fourteen individuals have been sighted across the project. These detections were made at fauna survey sites 15 and 16, with an additional four being made opportunistically along the project. Two additional scats were found along the project.

9.7.1.2 Findings from the Study

Desk-top assessments of historic presence data and field surveys have identified one koala hotspot within N2NS. This hotspot extends from the Mehi River in Moree to the town of Croppa Creek. Riparian vegetation along the Mehi River and Gwydir River, as well as along roadside vegetation in the surrounding landscape are likely to be important corridors for koala movement and surveys returned detections of scats and individual koalas.

Table 9-12 Koala Hotspot Region within N2NS

Hotspot Number	Hotspot Extent	Local Habitat Values	Corridors	Reason for Hotspot
1	Inclusive of Moree to Croppa Creek.	Isolated regrowth vegetation dominated by koala food trees surrounded by pastoral land. Remnant vegetation lining a creek dominated by koala food trees. Surrounded by pastoral land.	Mehi River Gwydir River	Isolated riparian habitat and roadside vegetation with Koala records.

9.7.1.3 Available Koala Habitat

In line with the N2NS draft EIS extensive ground-truthing and field surveys were undertaken to determine the extent of koala habitat for the Narrabri to North Star Project. Survey effort was conducted in-line with state and federal guidelines undertaken by NGH in December of 2015 and 2019, and February 2020 within Phase 2. This consisted of active searches, SAT searches and spotlighting.

The N2NS draft EIS studies revealed majority of the Project Area has been heavily modified by past and ongoing disturbances associated with the rail corridor and surrounding agricultural activities. Patches of koala habitat were determined to exist sporadically within the Project Area and are typically associated with riparian corridors, Travelling Stock Reserves (TSRs), road reserves or remnant to regrowth vegetation skirting farmland. Typical koala food tree species found in this region include *Eucalyptus tereticornis*, *Eucalyptus camaldulensis* and *Eucalyptus populnea*, see Table 9-13 for a breakdown of canopy species identified on the Koala Detection Dog Surveys.

The southern end of the IRP is located immediately north of Narrabri on an embankment above the Namoi River. Of significance, the alignment traverses the Gwydir River floodplain. The northern end of the Project Area at North Star is located south of the Macintyre River.

The implementation of dedicated wildlife culverts is considered best practice for koala sensitive design however other options are also available to ensure connectivity is maintained. These include refuge poles and koala rails within culverts are also proven to still be useful.

Table 9-13 Available Koala Habitat within N2NS

Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
26	■ *Eucalyptus camaldulensis*	Remnant vegetation lining a creek. Surrounded by pastoral land.	Creek line
27	■ *Eucalyptus camaldulensis*	Remnant vegetation lining a creek. Surrounded by pastoral land.	Creek line
28	■ *Eucalyptus camaldulensis*	Remnant vegetation lining a creek. Surrounded by pastoral land.	Creek line
29	■ *Eucalyptus populnea* ■ *Casuarina cristata*	Isolated regrowth patch of vegetation surrounded by pastoral land	Road corridor
30	■ *Eucalyptus populnea* ■ *Casuarina cristata*	Isolated regrowth patch of vegetation surrounded by pastoral land	Road corridor
31	■ *Eucalyptus crebra,* ■ *Eucalyptus tereticornis* ■ *Eucalyptus amplifolia subsp. sessiliflora* ■ *Callitris glaucophylla*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest

Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
32	■ *Eucalyptus tereticornis* ■ *Allocasuarina costata* ■ *Eucalyptus melanophloia* ■ *Eucalyptus amplifolia subsp. sessiliflora* ■ *Casuarina glaucophylla*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest
33	■ *Eucalyptus tereticornis* ■ *Corymbia tessellaris* ■ *Allocasuarina luehmannii*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest
34	■ *Eucalyptus moluccana* ■ *Allocasuarina luehmannii* ■ *Callitris glaucophylla*	Remnant vegetation lining a creek. Surrounded by pastoral land.	Creek line
35	■ *Angophora floribunda* ■ *Eucalyptus. sp* ■ *Casuarina glaucophylla* ■ *Allocasuarina luehmannii*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest
36	■ *Casuarina cristata*	Isolated regrowth patch of vegetation surrounded by pastoral land	Road corridor
37	■ *Eucalyptus populnea* ■ *Casuarina cristata*	Isolated regrowth patch of vegetation surrounded by pastoral land	Road corridor

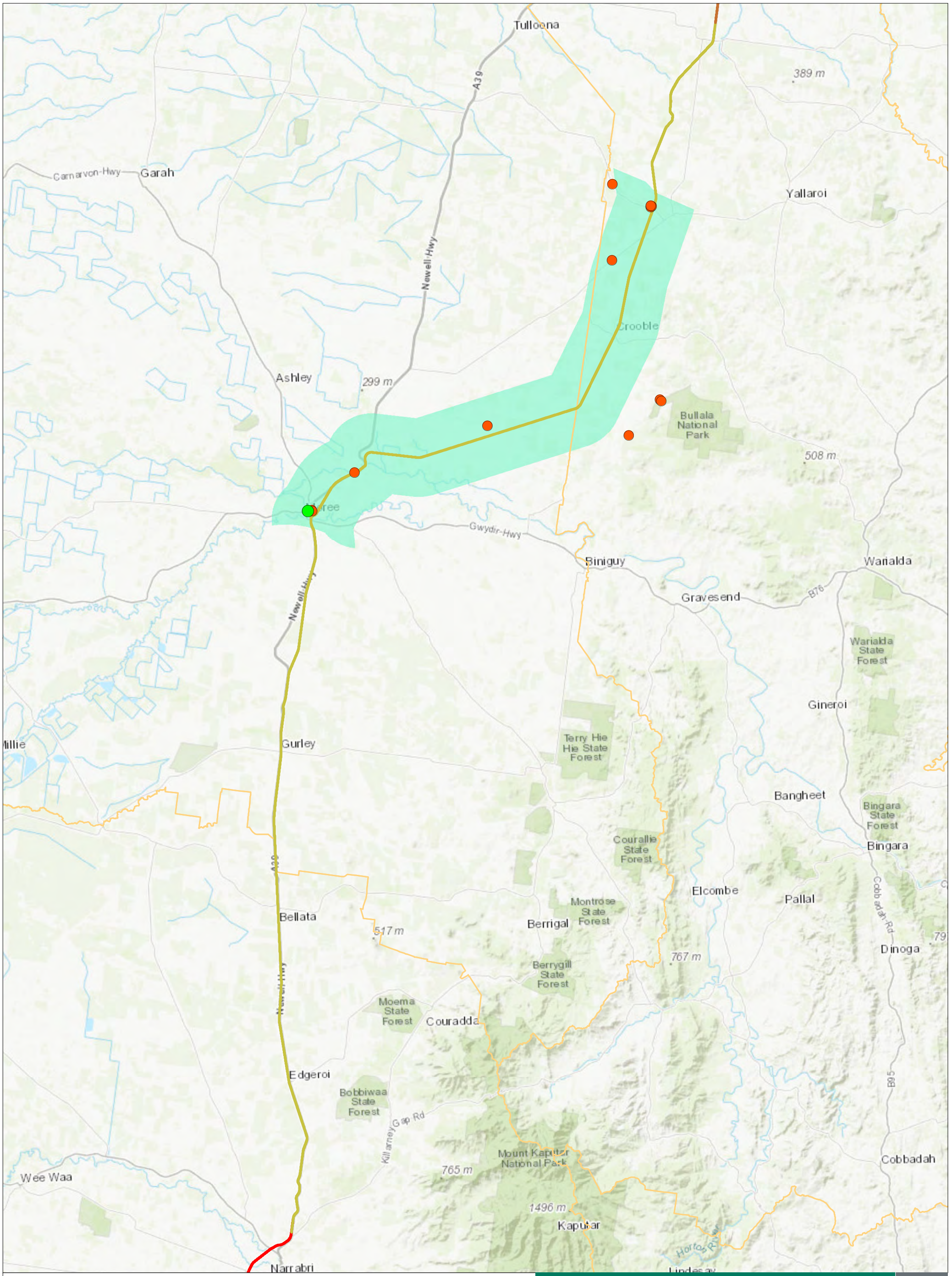
9.7.1.4 Opportunity for Connectivity

Focus should be placed on sections of the Project Area which cross riparian habitat and therefore could have the potential to form a barrier. Locations of importance would include Mehi River and Gwydir River. Refuge poles will likely be beneficial where canopy and mature trees are limited.

Culverts and koala

The landscape of N2NS (Phase 2) is largely heavily cleared agricultural land with little in the way of contiguous remnant forest. Riparian zones are considered important for maintaining fauna corridors within N2NS and of particular importance is Mehi and Gydir Rivers. Riparian vegetation does provide opportunities to maintain connections with remnant forest outside of the IRP, such as Bullala CCA Zone 1 National Park, and other smaller retained vegetation. Maintenance of connectivity through utilising existing crossing structures should be prioritised. Crossing structures within N2NS (phase 2) should be in line with the best practice designs and aim to support the objectives of the Koala Habitat Protection Guidelines (2019) and State Environmental Planning Policy (SEPP) (Biodiversity and Conservation) (Chapter 4 Koala habitat protection) 2021. Design will also be in line with The Koala Sensitive Design Guidelines (2022) and consider the WSP Preliminary Fauna Connectivity Strategy for the B2G Project.

See Figure 9-6 for hotspot locations associated within the Project Area.



Legend

- Koala sightings
- Scat detections - West
- Active Koala Hotspots

Inland Rail Alignment

- N2N - Narromine to Narrabri
- N2NS - Narrabri to North Star
- NS2B - North Star to Border

Data Source:
ESRI Would Topographic Map

Active Koala Hotspots with N2NS

F9-6

Drawing No:	0628339_N2AB_TM_G006_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC
Coordinate System: GDA2020 MGA Zone 56			This figure may be based on third party data or data which has not been verified by ERM and it may not be to scale. Unless expressly agreed otherwise, this figure is intended as a guide only and ERM does not warrant its accuracy.

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ERM

9.8 Narromine to Narrabri

9.8.1.1 Historic Koala Records

Historic koala records sourced from ALA throughout the N2N alignment of the IRP are heavily concentrated around the Pillaga Nature Reserve with additional records located sporadically towards the south. Presence records are noticeably absent between the town of Kickabil and Tenandra State Forest, an approximately 54km stretch of the IRP. While pockets of vegetation do exist in the form of roadside vegetation and small reserves, suitable habitat is largely absent from the landscape, with the bulk of surrounding land cleared pastoral and/or cropping land. The bulk of these records were recorded before 2010. Some records made between 2012 and now exist however are themselves not recent with the most recent record made in 2016. Post 2010 records within N2N are isolated to the Pillaga Nature Reserve with no recent records made anywhere else along the project.

During previous survey work along the IRP, koala scats were detected at Coolangala Creek and Etoo Creek following surveys in 2018 and 2019. The age of these scats was not known, and no individuals were observed. While this is an indication that some individuals were at least present at the time, it is unknown of these areas provided permanent habitat or were merely used for dispersal.

Findings from the Study

With a combination of desktop reviews of historic presence data and ground-truthing field surveys, a single hotspot has been identified within N2N. This hotspot is located south of Narrabri and is inclusive of the Pillaga Nature Reserve. While koala detections were not made within this hotspot following the field surveys for the IRP, the high density of historic records and the presence of potentially suitable habitat were identified as being suitable for inclusion as an area of priority for the IRP.

Table 9-14 Koala Hotspot Region within N2N

Hotspot Number	Hotspot Extent	Local Habitat Values	Corridors	Reason for Hotspot
1	South of Narrabri inclusive of The Pilliga Nature Reserve.	Remnant vegetation dominated by koala food trees.	The Pilliga Nature Reserve	Being of continuous remnant vegetation forming part of a larger protected vegetation area.

9.8.1.2 Available Koala Habitat

In line with the N2N draft EIS extensive ground-truthing and field surveys were undertaken to determine the extent of koala habitat for the Narrabri to Narromine Project. Survey effort was conducted in-line with state and federal guidelines undertaken by GHD in September and November of 2018 as well as March, August, September, October of 2019. This consisted of active searches, drone surveys, spotlighting and call playback.

Results revealed much of the southern and central portion of the Project Area is located in land cleared for agriculture. In the northern end of the Project Area, large sections are in areas dominated by vegetation associated with state forests of the Pilliga National Park (state forest sections). Typical koala food tree species found in this region include *Eucalyptus camaldulensis, Eucalyptus crebra* and *Eucalyptus microcarpa*. A detailed assessment of canopy species identified along the IRP is presented in Table 9-15.

It was determined that for the N2N Project riparian vegetation is likely to provide habitat and dispersal corridors for this species, with Etoo Creek (near the Aloes picnic area in the Pilliga), identified as a key site for observing koalas from both anecdotal and the GHD survey data. There is a record from 2019 associated with roadside vegetation south of Narromine.

The N2N draft EIS stated that evidence provided to the recent inquiry in koala populations in NSW noted that the Pilliga koala population was 'completely unviable' or already extinct (Legislative Council Portfolio Committee 7 2020) and this is supported by *Lunney et. al.* in 2017, which found decline of over 80 per cent in both the distribution and activity of koalas. These findings support those of this study that suggests that any koalas still present within the Pillaga are in extremely low densities and population viability is likely to be in decline.

Table 9-15 Available Koala Habitat within N2N

Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
1	■ *Eucalyptus tereticornis*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest creek line
2	■ *Eucalyptus microcarpa* ■ *Eucalyptus dwyerii* ■ *Callitris glaucophylla*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest
3	■ *Eucalyptus crebra* ■ *Callitris glaucophylla* ■ *Allocasuarina luehmannii*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest
4	■ *Eucalyptus camaldulensis* ■ *Eucalyptus crebra* ■ *Angophora floribunda* ■ *Allocasuarina luehmannii* ■ *Callitris glaucophylla*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest creek line
5	■ *Eucalyptus camaldulensis* ■ *Callitris glaucophylla* ■ *Brachychiton sp.* ■ *Eucalyptus populnea*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest
6	■ *Eucalyptus camaldulensis* ■ *Callitris glaucophylla* ■ *Allocasuarina luehmannii* ■ *Angophora floribunda*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest creek line
7	■ *Eucalyptus chloroclada* ■ *Eucalyptus crebra* ■ *Callitris glaucophylla*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest
8	■ *Eucalyptus crebra* ■ *Allocasuarina leuhmanii*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest
9	■ *Eucalyptus camaldulensis*	Remnant vegetation lining a creek. Surrounded by pastoral land.	Ephemeral creek line
10	■ *Eucalyptus camaldulensis* ■ *Callitris glaucophylla*	Remnant vegetation lining a creek. Surrounded by pastoral land.	Ephemeral creek line
11	■ *Eucalyptus populneus* ■ *Eucalyptus microcarpa*	Advanced regrowth, historically cleared	Road corridor
12	■ *Eucalyptus microcarpa* ■ *Callitris glaucophylla*	Advanced regrowth, historically cleared	Road corridor

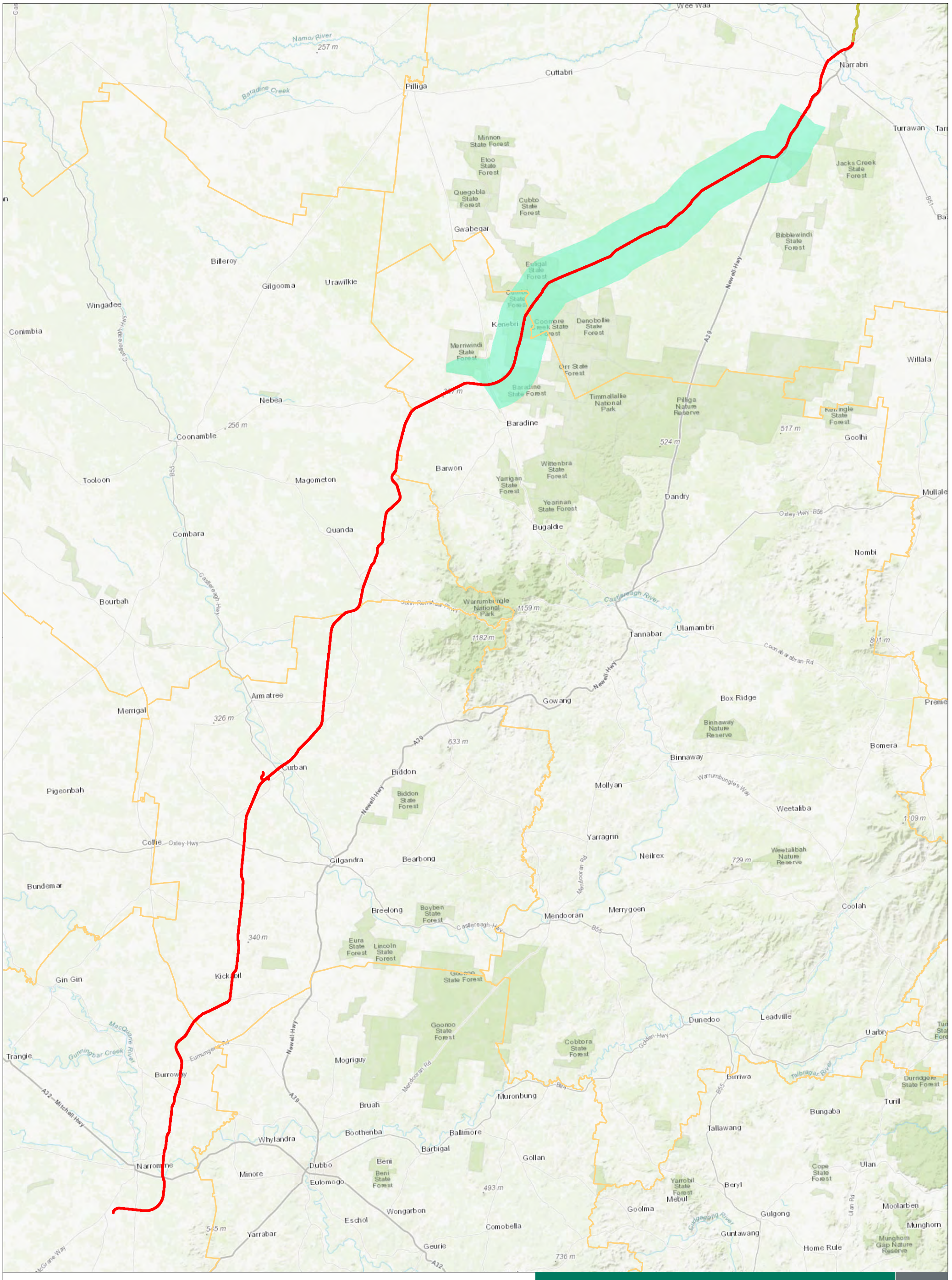
Quaternary Number	Dominant Canopy Species	Vegetation Quality	Landscape Context
13	■ *Eucalyptus tereticornis* ■ *Eucalyptus populnea* ■ *Casuarina glauca*	Advanced regrowth, planted canopy species	Managed Parkland
14	■ *Eucalyptus camaldulensis*	Remnant canopy, understory cleared	Riparian habitat edging urbanised area
15	■ *Eucalyptus populneus*	Remnant canopy, understory cleared	Road corridor
16	■ *Eucalyptus dealbata* ■ *Callitris glaucophylla*	Isolated remnant patch of vegetation surrounded by pastoral land	Road corridor
17	■ *Eucalyptus dealbata* ■ *Eucalyptus microcarpa* ■ *Callitris glaucophylla* ■ *Allocasuarina luehmannii*	Isolated remnant patch of vegetation surrounded by pastoral land	Road corridor
18	■ *Eucalyptus woollsiana* ■ *Eucalyptus dealbata* ■ *Callitris glaucophylla* ■ *Allocasuarina luehmannii*	Isolated advanced regrowth patch of vegetation surrounded by pastoral land	Road corridor
19	■ *Eucalyptus woollsiana* ■ *Eucalyptus crebra* ■ *Allocasuarina luehmannii* ■ *Callitris glaucophylla*	Isolated remnant patch of vegetation surrounded by pastoral land	Managed land/State Forest
20	■ *Eucalyptus crebra* ■ *Callitris glaucophylla* ■ *Allocasuarina luehmannii*	Isolated remnant patch of vegetation surrounded by pastoral land	Managed land/State Forest
21	■ *Eucalyptus camaldulensis* ■ *Casuarina Cunninghamiana* ■ *Callitris glaucophylla*	Remnant vegetation lining a creek. Surrounded by pastoral land.	Ephemeral creek line
22	■ *Eucalyptus moluccana* ■ *Allocasuarina luehmannii* ■ *Callitris glaucophylla*	Remnant vegetation as a part of a larger protected vegetation area.	Managed land/State Forest
23	■ *Eucalyptus tereticornis*	Remnant vegetation lining a creek. Surrounded by pastoral land.	Ephemeral creek line
24	■ *Eucalyptus blakelyii* ■ *Callitris glaucophylla*	Remnant vegetation lining a creek. Surrounded by pastoral land.	Ephemeral creek line
25	■ *Eucalyptus microcarpa* ■ *Eucalyptus dwyerii* ■ *Callitris glaucophylla*	Isolated advanced regrowth patch of vegetation surrounded by pastoral land and managed land/state forest	Road corridor

9.8.1.3 Opportunity for Connectivity

Despite the lack of recent koala records in this section, focus should be placed on sections of the Project Area which cross riparian habitat and therefore could have the potential to form a barrier. Locations of importance would include Coolangala Creek and Etoo Creek.

The Pillaga Nature reserve is considered important for maintaining connectivity. While no koala detections were made in N2N and historic records are largely from before 2010, the Pillaga and surrounding state forests still offers an extensive network of suitable eucalypt woodland that extends across the broader landscape beyond the IRP. Riparian vegetation is noted to be of particular importance for connecting smaller state forests and retained vegetation towards the south of the IRP. Crossing structures should be in line with the Koala Sensitive Design Guidelines (2022) and consider the WSP Preliminary Fauna Connectivity Strategy for the N2N Project.

See Figure 9-7 for hotspot locations associated within the Project Area.



Legend

Active Koala Hotspots **Inland Rail Alignment**

N2N - Narromine to Narrabri

N2NS - Narrabri to North Star

Data Source:
ESRI Would Topographic Map

Active Koala Hotspots with N2N

F9-7

Drawing No:	0628339_N2AB_TM_G006_R3.mxd		**Koala Genetics Study Project – Inland Rail Program (NSW and QLD)**
Date:	14/02/2024	Drawing Size: A4	
Drawn By:	VN	Reviewed By: MD	Client: ARTC

Coordinate System: GDA2020 MGA Zone 56

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10. CONCLUSION AND RECOMMENDATIONS

This study aimed to help support already existing knowledge of the koala in the IRP regions, and to dive deeper into the movement of the species across the vast landscape. The new information gathered from this study can further support decisions made to maintain and/or improve habitat connectivity and help acknowledge key areas for ongoing koala monitoring to ensure measures put in place are effective.

A key priority for IRP is to preserve current landscape connectivity for movement of koalas. Due to low koala density and a low number of koala's sampled across the IRP, previous samples were pooled from other studies to form groups along the IRP in order to perform connectivity analysis across the IRP. Other groups outside the IRP were also formed for comparison and to help contextualise the population status associated with the IRP.

The genetic diversity results from this study indicated 10 geographical groupings of koalas, three of which Included IRP ARTC samples:

- Calvert to Kagaru and Kagaru to Acacia Ridge and Bromelton (Group 2);
- Border to Gowrie and Gowrie to Helidon (Group 3); and
- Narrabri to North Star (Group 6).

Overall, these three groups compared to the other groups in the larger landscape had:

- High levels of heterozygosity: Higher levels of heterozygosity equate to larger genetic diversity within a population(s), which is a positive indicator as genetic diversity is linked to both individual health and, at population level, evolutionary potential (with Group 3 (B2G + G2H) showing higher heterozygosity than the other groups);
- Low levels of inbreeding and of relatedness between individuals: This suggests gene flow is currently occurring within the groups along the IRP and the potential of negative impacts due to excessive inbreeding is minimal;
- Relatively small population size, with Group 3 (B2G + G2H) having a larger population size relative to other groups. This results in overall lower densities of koalas across the landscape; and
- No concerns regarding sex ratio, but high chlamydia prevalence, except Group 3 which is medium prevalence (however the sample size remain very low). The ratio of males to females is not of concern for genetic health although chlamydia prevalence is high. Caution should be taken when interpreting these findings as the sample size is small.

When compared with the results of the vegetation assessments, the analysis of eucalypt forage species from the sampled scats indicates some variability between the dominant eucalypt species measured and the eucalypts detected in the scats (Table 10-1). The spatial variability in the preferred diet trees of koalas indicates that any replacement planting should be done in accordance with the bioregion. Preferred food trees as detected in the scat analysis should be planted, however care should be taken to not develop a monoculture that would result in individual diets becoming dominated by one or two species, therefore reducing the nutritional value to be gained.

Table 10-1 Summary of dominant eucalypt detected in samples and vegetation assessments

Locality	Dominant eucalypt detected in scat samples	Dominant canopy trees from vegetation assessments
Brisbane region (east of the divide)	*E. seeana, E tereticornis*	*E. tereticornis C. citriodora, C. intermedia, E. crebra*
Toowoomba region (west of the divide)	*E. amplifolia subsp. sessiliflora, E. tereticornis, E. chloroclada* and *E. melliodora*	*E. crebra, E. citriodora and C. intermedia*
Moree region	*E. camaldulenis, E. populnea, E. chloroclada*	*E. camaldulensis, E. populnea and E. crebra.*

Based on these findings, the following recommendations should be prioritised throughout the IRP:

1. This genetics study provides a baseline assessment of koala genetics within the IRP; however it is recommended that additional surveys be completed beyond the IRP in order to increase the robustness of genetic analysis and allow for further analysis to identify distinct population groups of koalas within the IRP.
2. It is evident that there is considerable movement/dispersal of koalas along the Study Area, therefore it is imperative to adhere to the objectives and recommendations of the draft Koala Management Plans (KMPs) (and with due consideration of B2G's Preliminary Fauna Connectivity Strategy, Appendix P of the draft revised EIS)to ensure adequate corridors and/or ways for koalas to be able to move through the IRP respectively.
3. To further the effectiveness of this study in identifying distinct koala population groups within the IRP and beyond, an effort to enable surveys to be conducted in all landholder properties along the IRP and beyond should be prioritized as this would enable true representation of the movements and dispersal ranges of koala groups within each area.
4. Forage tree preference indicates that koalas may seek out preferred forage trees that are not part of the dominant tree species within a landscape. Although the current study represents a small sample size, the eucalypt species detected in the sampled scats did not always contain the dominant species as measured from vegetation assessments.

This genetics study revealed that mitigation measures are necessary to maintain current koala genetic diversity by allowing gene flow along and across the Inland Rail alignment, supporting the objectives of the Draft KMPs, preliminary Fauna Connectivity Strategy, and Draft Outline Environmental Management Plan developed for the IRP. The KMPs will ensure that these mitigation measures are successful by monitoring measurable outcomes and adopting an adaptive management approach.

This study provides data to support assessments of koalas along the IRP alignment and forms part of the baseline for ongoing monitoring as a part of the draft KMPs. To overcome the current limitations of the study, additional surveys across areas unable to be sampled and beyond the IRP will likely provide more accurate assessments of priority areas for koala mitigation and management measures within the IRP.

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APPENDIX A UNISC GENETICS METHODS AND RESULTS

Note – Results presented here are preliminary as the genetic analyses were somewhat limited by the small sample size; caution should be exercised when interpreting these results. Additional work will be conducted to increase the data set, including re-extraction of samples that failed quality control and further detection dog surveys to locate and collect additional koala scat samples. Once this is complete, the combined genetic data set will be re-analysed, which may alter the results.

Acronyms and glossary

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

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

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

DNA: Deoxyribonucleic acid, a molecule carrying genetic information.

Effective population size (N_e): Broadly, this refers to the number individuals in a population who are able to contribute to the next generation's genetic material and is usually a subset of the census population. More specifically, N_e is the size of an idealised population that would lose genetic diversity at the same rate as the actual population. This is one of the most important parameters in population genetics and conservation biology because the genetic 'health' of a population is directly dependent on the effective population size, and indirectly linked to the census size of a population. The likelihood that a gene will be sampled as the next generation is produced is affected by the breeding structure of the population. In an 'ideal population':

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

In an ideal population, all individuals have an equal opportunity to pass on their genes and the effective population size should be equal to the census population. In reality, this does not occur because not all members within a population mate, the ratio of males to females is rarely equal, mating isn't random, and generations overlap. Due to these factors, the effective population size is never as large as the census population. It is important to note that N_e is particularly sensitive to an unequal number of males and females in the population.

In short, the effective population size translates the census size of a real population into the size of an idealised population showing the same rate of loss of genetic diversity, inbreeding, or genetic drift for a population under study. In a natural population, the ratio of effective population size to census population size (N_e /N) is typically 0.1 (Frankham 1995, Palstra and Ruzzante 2008). The relationship between these two measures is complex and it is important to refrain from making inferences about census population size based on effective population size (Palstra and Fraser 2012).

Current recommendations for the genetic conservation of species in the wild are that:

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

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

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

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

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

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

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

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

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

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

Genetic stochasticity: genetic consequences of small populations, including inbreeding, loss of genetic diversity due to genetic drift and chance fixation of deleterious mutations that reduce

fitness and can drive a population or species towards extinction (often in combination with other factors).

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

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

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

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

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

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

IR: Internal Relatedness is a measure of inbreeding at the individual level (as opposed to population level, such as F_{IS}). It is calculated from heterozygosity data and does not require a pedigree, which is difficult to obtain in wild populations. Internal relatedness is currently the most widespread used index for inbreeding and its main strength is that allele frequencies are incorporated into the measure.

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

Inbreeding depression: reduction in fitness due to inbreeding.

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

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

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

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

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

SD: Standard Deviation

SE: Standard Error

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

SEQ: South East Queensland

Small populations: the fact that small, isolated, populations are more prone to extinction (or extirpation) is well established, and therefore a goal in conservation is to avoid species being fragmented into small populations. In general, there are four sources of stochasticity that can cause small population to go extinct (from Shaffer 1981):

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

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

Structure: within a species, genetic structure exists because not all individuals are able to breed with all other individuals of the same species, due to geographic proximity (even if a species distribution is continuous). Simplistically, this reflects that individuals that live close to each other have a higher chance of breeding than individuals further apart. Population structure (i.e., the genetic differentiation of local populations) is increased by mutation, genetic drift (due to finite population size) and natural selection favouring adaptations to local environmental conditions; but is decreased by gene flow (the movement of gametes, or individuals). Population structure is higher when gene flow between populations is lower, and so population structure is increased by habitat fragmentation and isolation.

Population structure can be studied through allele frequencies and can only be inferred with a sample size large enough to calculate robust allele frequencies. Therefore, sample size dictates the unit of comparison and the scale at which genetic structure can be examined.

Genetic structure can be hierarchically described:

- **Broad-scale structure** (often studied through a Bayesian statistic programs) usually defines 'populations' as independent breeding units, with each population descending from a different lineage and with very little to no gene flow. The software usually tests whether distinct populations can be inferred **without any *a priori* geographic information** and identifies migrants (individuals belonging genetically to one population, but geographically to another one), and admixed individuals, that are offspring of migrants between populations.
- **Fine-scale structure** (often calculated through F_{ST}, see F-statistics) usually describes sub-populations (also called local populations or demes) where gene flow exists but is restricted. Genetic structure at this level is studied by comparing allele frequencies between artificially constructed populations (e.g. between countries, between states, between councils) and then testing whether the populations should be considered one or multiple, and how similar the populations are to one another (pairwise F_{ST}).
- Finally, the distribution of related individuals in space, an even finer structure that can be referred to as "cryptic", can be described through autocorrelation measures, where distances between all individuals and their genetic relatedness are compared.

Table A1. The list of 255 unique samples available from 24 sample locations for genetic data analysis with DArTcap and DArTag genotype data.

Sample Name	Sample Location	Latitude	Longitude	ARTC sample
180430Bi1A	Brisbane City	-27.5023	153.1839	No
210216Bi2B1	Brisbane City	-27.5738	153.1815	No
210216Bi2C1	Brisbane City	-27.5729	153.1803	No
Kidgy_109964_USC	Brisbane City	-27.382	152.993	No
Debbie_112243	Brisbane City	-27.5006	153.1067	No
180515Bi4B	Redland City	-27.6511	153.249	No
201127Bi1B1	Redland City	-27.6153	153.1799	No
DR220115KM1E	Redland City	-27.4139	153.4706	No
DR220116KM1A	Redland City	-27.4297	153.4988	No
220411KM1P	Redland City	-27.5944	153.2478	No
220411KM1JJ	Redland City	-27.5982	153.2424	No
220323BI2A	Redland City	-27.5683	153.2966	No
220323BI1F	Redland City	-27.4899	153.246	No
220406KM1D	Redland City	-27.5083	153.1974	No
220411KM1A	Redland City	-27.6024	153.2472	No
220323BI1G	Redland City	-27.4887	153.2458	No
220411KM1N	Redland City	-27.596	153.2463	No
DR220212KM1B	Redland City	-27.6788	153.2893	No
220323BI1I	Redland City	-27.4897	153.2448	No
220406KM1G	Redland City	-27.5108	153.1986	No
220411KM1M	Redland City	-27.5974	153.2444	No
220407KM1H	Redland City	-27.4903	153.2481	No
DR220420KM1A	Redland City	-27.4909	153.2449	No
DR220623KM1A	Redland City	-27.6302	153.1963	No
DR220603KM1A	Redland City	-27.6037	153.2448	No
220411KM1K	Redland City	-27.5985	153.2405	No
220505BI3E	Redland City	-27.5353	153.2785	No
DR220419KM1B	Redland City	-27.5916	153.2487	No
DR220617KM1D	Redland City	-27.595	153.2443	No
DR220603KM1A-reextract	Redland City	-27.6037	153.2448	No
DR220610KM1C	Redland City	-27.6504	153.264	No
220505BI2A	Redland City	-27.5315	153.2815	No
DR220610KM1D	Redland City	-27.6488	153.2611	No
DR220617KM1B	Redland City	-27.6003	153.2433	No
DR220420KM1D	Redland City	-27.4899	153.2458	No
DR220610KM1B	Redland City	-27.6491	153.2644	No
DR220622KM1H	Redland City	-27.489	153.2448	No
220505BI2D	Redland City	-27.5331	153.2821	No
220505BI4A	Redland City	-27.5251	153.2777	No

DR220610KM1A	Redland City	-27.6464	153.2667	No
220601Bi5F	Redland City	-27.5289	153.2728	No
220611Bi5C	Redland City	-27.5299	153.2742	No
220601BI2A	Redland City	-27.5318	153.2815	No
220601Bi5C	Redland City	-27.5347	153.2737	No
221015MB1A	Redland City	-27.401	153.4382	No
180515Bi4C	Redland City	-27.6513	153.2488	No
200722Bi5E	Logan City	-27.7406	153.0629	No
200723Bi4A	Logan City	-27.8013	152.9727	No
200722Ba5B	Logan City	-27.7388	153.063	No
200730Bi2A	Logan City	-27.7858	153.0442	No
200723Bi1D	Logan City	-27.7236	153.0661	No
200722Ba5A	Logan City	-27.7416	153.0625	No
200730Bi1A	Logan City	-27.79	153.0616	No
200730Bi6D	Logan City	-27.7381	153.133	No
200714Bi5D	Logan City	-27.7544	153.029	No
200722Ba3A	Logan City	-27.7124	153.0506	No
200715Ba2A	Logan City	-27.7491	153.022	No
200730Bi8B	Logan City	-27.7162	152.9054	No
220610Bi2C	Logan City	-27.7047	152.9959	Yes
220610Bi3A	Logan City	-27.7058	152.9989	Yes
220610Bi2A	Logan City	-27.7004	152.9966	Yes
220609Bi2A	Logan City	-27.6827	153.0032	Yes
220803Bi3A	Ipswich City	-27.6984	152.6182	Yes
220803Bi2A	Ipswich City	-27.6878	152.6358	Yes
220803Bi1A	Ipswich City	-27.6672	152.6562	Yes
220803Bi1B	Ipswich City	-27.667	152.6517	Yes
220802Bi1B	Ipswich City	-27.7334	152.7094	Yes
220802Bi1E	Ipswich City	-27.7368	152.7097	Yes
220801Bi1A	Scenic Rim Regional	-27.8256	152.7624	Yes
220801Bi2A	Scenic Rim Regional	-27.8204	152.7666	Yes
220811Bi1B	Lockyer Valley Regional	-27.5365	152.1315	Yes
220810Bi1B	Lockyer Valley Regional	-27.5163	152.0096	Yes
220811Bi3B	Lockyer Valley Regional	-27.5398	152.1436	Yes
220809Bi1B	Lockyer Valley Regional	-27.5163	151.9722	Yes
220809Bi2A	Lockyer Valley Regional	-27.5141	151.9703	Yes
220810Bi1D	Lockyer Valley Regional	-27.5151	152.0113	Yes
220811Bi1A	Lockyer Valley Regional	-27.5374	152.1315	Yes
220810Bi1C	Lockyer Valley Regional	-27.5164	152.0067	Yes
220811Bi1D	Lockyer Valley Regional	-27.5380	152.1306	Yes
220810Bi3B	Lockyer Valley Regional	-27.5238	152.0087	Yes
180415Bi3B	Toowoomba Regional	-27.6558	151.6588	No
180412Bi2A	Toowoomba Regional	-27.4432	151.7337	No
180413Ba6A	Toowoomba Regional	-27.5165	151.9525	No

180415Bi3C	Toowoomba Regional	-27.6562	151.6586	No
180412Ba1A	Toowoomba Regional	-27.4246	151.737	No
180413Ba8A	Toowoomba Regional	-27.3973	151.8326	No
180410Ma5A	Toowoomba Regional	-27.4585	151.7289	No
180412Ba1C	Toowoomba Regional	-27.4247	151.7387	No
180414OP1B	Toowoomba Regional	-27.3427	151.6049	No
180415Ba3A	Toowoomba Regional	-27.6506	151.5874	No
180412OP1A	Toowoomba Regional	-27.4242	151.7428	No
180414OP4B	Toowoomba Regional	-27.2503	151.6978	No
180412OP2A	Toowoomba Regional	-27.4217	151.7436	No
180414Bi1B	Toowoomba Regional	-27.347	151.59	No
180413Ma1A	Toowoomba Regional	-27.4364	151.7059	No
180415OP2B	Toowoomba Regional	-27.4032	151.7768	No
180412OP5A	Toowoomba Regional	-27.3463	151.7582	No
180413Ma2A	Toowoomba Regional	-27.4665	151.8157	No
220624Bi1A	Toowoomba Regional	-27.7306	151.6415	Yes
220617Bi2C	Toowoomba Regional	-27.7205	151.5597	Yes
220809Bi3C	Toowoomba Regional	-27.5149	151.9543	Yes
220615Bi2C	Toowoomba Regional	-27.6296	151.7422	Yes
220618Bi1E	Toowoomba Regional	-27.6033	151.7614	Yes
220615Bi2E	Toowoomba Regional	-27.634	151.736	Yes
220222Au4A	Byron Shire Council	-28.5877	153.415	No
220222Au5A	Byron Shire Council	-28.5794	153.4059	No
210713Be1C	Ballina Shire Council	-28.8837	153.3723	No
210714Be2B	Ballina Shire Council	-28.9826	153.4146	No
220628Au1A	Ballina Shire Council	-28.9609	153.4544	No
DR221011KM1E	Ballina Shire Council	-28.9468	153.4462	No
DR220811KM1H	Ballina Shire Council	-28.9676	153.4453	No
DR220809KM1E	Ballina Shire Council	-28.959	153.4544	No
DR221011KM1G	Ballina Shire Council	-28.9544	153.4574	No
DR220809KM1D	Ballina Shire Council	-28.9585	153.454	No
DR221011KM1A	Ballina Shire Council	-28.9494	153.4349	No
DR220811KM1G	Ballina Shire Council	-28.9646	153.4473	No
DR220809KM1G	Ballina Shire Council	-28.9604	153.4539	No
220627Au3B	Ballina Shire Council	-28.954	153.4565	No
DR221011KM1D	Ballina Shire Council	-28.9482	153.4431	No
DR220811KM1C	Ballina Shire Council	-28.9703	153.4334	No
DR220809KM1F	Ballina Shire Council	-28.965	153.432	No
DR220809KM1A	Ballina Shire Council	-28.9608	153.4334	No
220628Au3A	Ballina Shire Council	-28.9667	153.4336	No
DR220809KM1B	Ballina Shire Council	-28.955	153.4547	No
DR221011KM1H	Ballina Shire Council	-28.9524	153.4589	No
NR_154	Lismore City Council	-28.9895	153.3078	No
NR_39	Lismore City Council	-28.9747	153.3596	No

NR_129	Richmond Valley Council	-29.0794	153.42	No
NR_A_14	Richmond Valley Council	-29.0913	153.3699	No
220623Bi1C	Goondiwindi Regional	-28.1784	151.1934	Yes
220623Bi1B	Goondiwindi Regional	-28.1771	151.1927	Yes
220623Bi2B	Goondiwindi Regional	-28.2007	151.1819	Yes
DR220905KM1A	Tenterfield Shire Council	-28.6983	152.0688	No
DR220906KM1H	Tenterfield Shire Council	-28.8251	152.0438	No
DR220818KM1E	Tenterfield Shire Council	-28.5297	152.3489	No
DR220906KM1D	Tenterfield Shire Council	-28.8292	152.0557	No
DR221111KM1B	Tenterfield Shire Council	-28.9091	152.3083	No
DR221118KM1B	Tenterfield Shire Council	-28.8098	152.2742	No
DR220905KM1H	Tenterfield Shire Council	-28.6974	152.0775	No
DR220906KM1A	Tenterfield Shire Council	-28.834	152.0568	No
DR220818KM1A	Tenterfield Shire Council	-28.5282	152.3476	No
DR220906KM1E	Tenterfield Shire Council	-28.8244	152.0448	No
DR221111KM1A	Tenterfield Shire Council	-28.9046	152.3108	No
DR221118KM1E	Tenterfield Shire Council	-28.8045	152.2778	No
DR220905KM1B	Tenterfield Shire Council	-28.7008	152.0724	No
220922Au1A	Tenterfield Shire Council	-28.8442	152.0843	No
DR220818KM1D	Tenterfield Shire Council	-28.5263	152.3457	No
DR221110KM1B	Tenterfield Shire Council	-28.897	152.2338	No
DR221118KM1A	Tenterfield Shire Council	-28.8115	152.2722	No
DR220818KM1F	Tenterfield Shire Council	-28.5265	152.3441	No
DR221118KM1D	Tenterfield Shire Council	-28.8026	152.2715	No
DR220817KM1A	Tenterfield Shire Council	-28.5379	152.356	No
DR220819AS1A	Tenterfield Shire Council	-28.5121	152.3574	No
DR221110KM1A2	Tenterfield Shire Council	-28.9013	152.225	No
DR221118KM1C	Tenterfield Shire Council	-28.8026	152.2715	No
DR221110KM1C	Tenterfield Shire Council	-28.8968	152.2332	No
DR221119KM1A	Tenterfield Shire Council	-28.7362	152.3421	No
DR220818KM1B	Tenterfield Shire Council	-28.5278	152.3476	No
DR220906KM1I	Tenterfield Shire Council	-28.8267	152.0451	No
DR221116KM1A	Tenterfield Shire Council	-28.5031	152.3896	No
DR221118KM1G	Tenterfield Shire Council	-28.8005	152.2794	No
DR220905KM1D	Tenterfield Shire Council	-28.7021	152.0767	No
DR220906KM1B	Tenterfield Shire Council	-28.8351	152.0573	No
190921Bi1N	Inverell Shire Council	-29.6137	150.8406	No
190923KH4A	Inverell Shire Council	-29.779	151.0259	No
190925KH2A	Inverell Shire Council	-29.655	150.9435	No
190922KH1B	Inverell Shire Council	-29.6584	151.0015	No
190921Bi1B	Inverell Shire Council	-29.617	150.8299	No
190923KH3C	Inverell Shire Council	-29.779	150.9793	No
190922Bi3C	Inverell Shire Council	-29.606	150.8912	No
190923Bi1B	Inverell Shire Council	-29.7376	150.9701	No

190921Bi1A	Inverell Shire Council	-29.6181	150.8296	No
190921Bi1H	Inverell Shire Council	-29.6161	150.8273	No
190923CN2A	Inverell Shire Council	-29.7796	150.9835	No
190922KH2A	Inverell Shire Council	-29.6594	151.0253	No
190922AR1A	Inverell Shire Council	-29.6469	151.0615	No
190922Bi1B	Inverell Shire Council	-29.661	151.0434	No
190922AR1B	Inverell Shire Council	-29.6469	151.0615	No
190922FFR1A	Inverell Shire Council	-29.6467	151.0623	No
190923Bi1F	Inverell Shire Council	-29.7344	150.9826	No
190925KH1A	Inverell Shire Council	-29.6762	150.9315	No
190921KH2A	Inverell Shire Council	-29.7517	151.0413	No
190921Bi3A	Inverell Shire Council	-29.6512	150.8315	No
190925KH1B	Inverell Shire Council	-29.6762	150.9315	No
190922Bi2A	Inverell Shire Council	-29.666	151.0463	No
190922Bi4C	Inverell Shire Council	-29.5895	151.0128	No
190922Bi4A	Inverell Shire Council	-29.5896	151.0133	No
190921Bi1F	Inverell Shire Council	-29.6167	150.8281	No
190921Bi1G	Inverell Shire Council	-29.6162	150.8276	No
190923KH3A	Inverell Shire Council	-29.779	150.9793	No
190922Bi3A	Inverell Shire Council	-29.6062	150.8913	No
210310Ba6A1	Inverell Shire Council	-29.3755	151.3444	No
190924KH1C	Gwydir Shire Council	-29.5238	150.5468	No
190924CN1A	Gwydir Shire Council	-29.5462	150.554	No
221207Bi1B	Gwydir Shire Council	-29.1218	150.3052	Yes
221207Bi7A	Gwydir Shire Council	-29.183	150.2515	Yes
220722Bi1A	Moree Plains Shire Council	-29.4626	149.844	Yes
221208Bi2A	Moree Plains Shire Council	-29.4621	149.85	Yes
221208Bi1A	Moree Plains Shire Council	-29.4619	149.8443	Yes
190916Bi4A	Armidale Regional Council	-30.4223	151.6029	No
190917Bi1B	Armidale Regional Council	-30.4455	151.543	No
190917Bi2B	Armidale Regional Council	-30.3613	151.5843	No
190916Bi1B	Armidale Regional Council	-30.4177	151.6346	No
190920Bi2A	Armidale Regional Council	-30.5776	151.714	No
190916Bi1C	Armidale Regional Council	-30.4172	151.6337	No
190917Bi2J	Armidale Regional Council	-30.3578	151.5898	No
190919Bi1A	Armidale Regional Council	-30.4327	151.5658	No
190916Bi2A	Armidale Regional Council	-30.4193	151.645	No
190917Bi2C	Armidale Regional Council	-30.3608	151.5833	No
190917Bi2H	Armidale Regional Council	-30.3524	151.5855	No
190916Bi2C	Armidale Regional Council	-30.4212	151.6466	No
190919Bi1B	Armidale Regional Council	-30.4322	151.5659	No
190916Bi3B	Armidale Regional Council	-30.4276	151.598	No
190917Bi1A	Armidale Regional Council	-30.4455	151.543	No
190917Bi2F	Armidale Regional Council	-30.3576	151.5825	No

210427Ma2A	Armidale Regional Council	-30.0395	151.8967	No
210427Ma4A	Armidale Regional Council	-30.5796	151.7139	No
190917Bi3A	Uralla Shire Council	-30.6313	151.5375	No
190917Bi4C	Uralla Shire Council	-30.626	151.3416	No
190917Bi4A	Uralla Shire Council	-30.6259	151.3393	No
Coffs_1.7	Coffs Harbour City Council	-30.3371	153.0927	No
Coffs_6.11	Coffs Harbour City Council	-30.3193	153.0727	No
Coffs_19.3	Coffs Harbour City Council	-30.3972	152.9917	No
Coffs_2.1	Coffs Harbour City Council	-30.3393	153.0935	No
Coffs_7.3.1	Coffs Harbour City Council	-30.3602	153.061	No
Coffs_7.1	Coffs Harbour City Council	-30.3638	153.0617	No
Coffs_19.1	Bellingen Shire Council	-30.4028	152.9869	No
Coffs_22.1	Bellingen Shire Council	-30.4482	153.0416	No
200508DR1C	Goulburn Mulwaree Council	-34.8999	150.0571	No
200509DR1D	Goulburn Mulwaree Council	-34.9078	150.0369	No
200509DR1C	Goulburn Mulwaree Council	-34.9112	150.0392	No
200509DR1A	Goulburn Mulwaree Council	-34.9049	150.0494	No
200508DR1F	Goulburn Mulwaree Council	-34.9028	150.0569	No
200508DR1A	Goulburn Mulwaree Council	-34.8958	150.0528	No
200507DR1B	Goulburn Mulwaree Council	-34.9081	150.0517	No
200412DR1A	Shoalhaven City Council	-34.9711	150.1017	No
200403DR1D	Shoalhaven City Council	-35.0656	150.1231	No
200412DR1E	Shoalhaven City Council	-35.0214	150.1472	No
200408DR1M	Shoalhaven City Council	-34.9204	150.142	No
200328DR1B	Shoalhaven City Council	-35.07	150.1299	No
200326KM1D	Shoalhaven City Council	-35.0645	150.1256	No
200412DR1B	Shoalhaven City Council	-34.9716	150.1039	No
200408DR1C	Shoalhaven City Council	-34.9326	150.1458	No
200412DR1C	Shoalhaven City Council	-34.9702	150.1058	No
200511DR1A	Queanbeyan-Palerang Regional Council	-35.0853	150.0286	No
200516DR1D	Snowy Monaro Regional Council	-36.1089	149.3072	No
200517DR1M	Snowy Monaro Regional Council	-36.0637	149.3187	No
200516DR1B	Snowy Monaro Regional Council	-36.1278	149.3076	No
200517DR1I	Snowy Monaro Regional Council	-36.066	149.3144	No
200516DR1A	Snowy Monaro Regional Council	-36.1317	149.3037	No
200529Be1A	Snowy Monaro Regional Council	-36.1445	149.3298	No
200513DR1B	Snowy Monaro Regional Council	-36.0099	149.3721	No

200513DR1F	Snowy Monaro Regional Council	-36.002	149.3663	No
200527DR1B	Snowy Monaro Regional Council	-36.0217	149.3727	No
200513DR1A	Snowy Monaro Regional Council	-36.0088	149.3719	No

Table A2. Groupings of koala samples used for genetic diversity estimates.

Group	Sample locations in the group (sample size)	Sample size per group	Number of ARTC Samples
Group 1	Brisbane City (05)	43	
	Redland City (37) (= Koala Coast)		
Group 2	Logan City (17)	24	04
	Ipswich City (06)		06
	Scenic Rim Regional (02)		02
Group 3	Lockyer Valley Regional (10)	34	10
	Toowoomba Regional (24)		06
Group 4	Byron Shire Council (02)	25	
	Ballina Shire Council (19)		
	Lismore City Council (02)		
	Richmond Valley Council (02)		
Group 5	Tenterfield Shire Council (31)	31	
Group 6	Inverell Shire Council (29)	36	
	Gwydir Shire Council (04)		02
	Moree Plains Shire Council (03)		03
Group 7	Armidale Regional Council (18)	21	
	Uralla Shire Council (03)		
Group 8	Coffs Harbour City Council (06)	08	
	Bellingen Shire Council (02)		
Group 9	Goulburn Mulwaree Council (07)	17	
	Shoalhaven City Council (09)		
	Queanbeyan–Palerang Regional Council (01)		
Group 10	Snowy Monaro Regional Council (10)	10	

Note. Three samples from Goondiwindi were collected for the ARTC project but do not appear in this table, as they were not included in the analyses.

Table A3. Overview of all ARTC samples, including sample name, whether samples were duplicates, data quality control (QC) for subsequent analyses, whether data was used to estimate number of unique individuals, whether data was used for genetic diversity analyses, Sex, Chlamydia detection, and geographical coordinates (DArTag analyses).

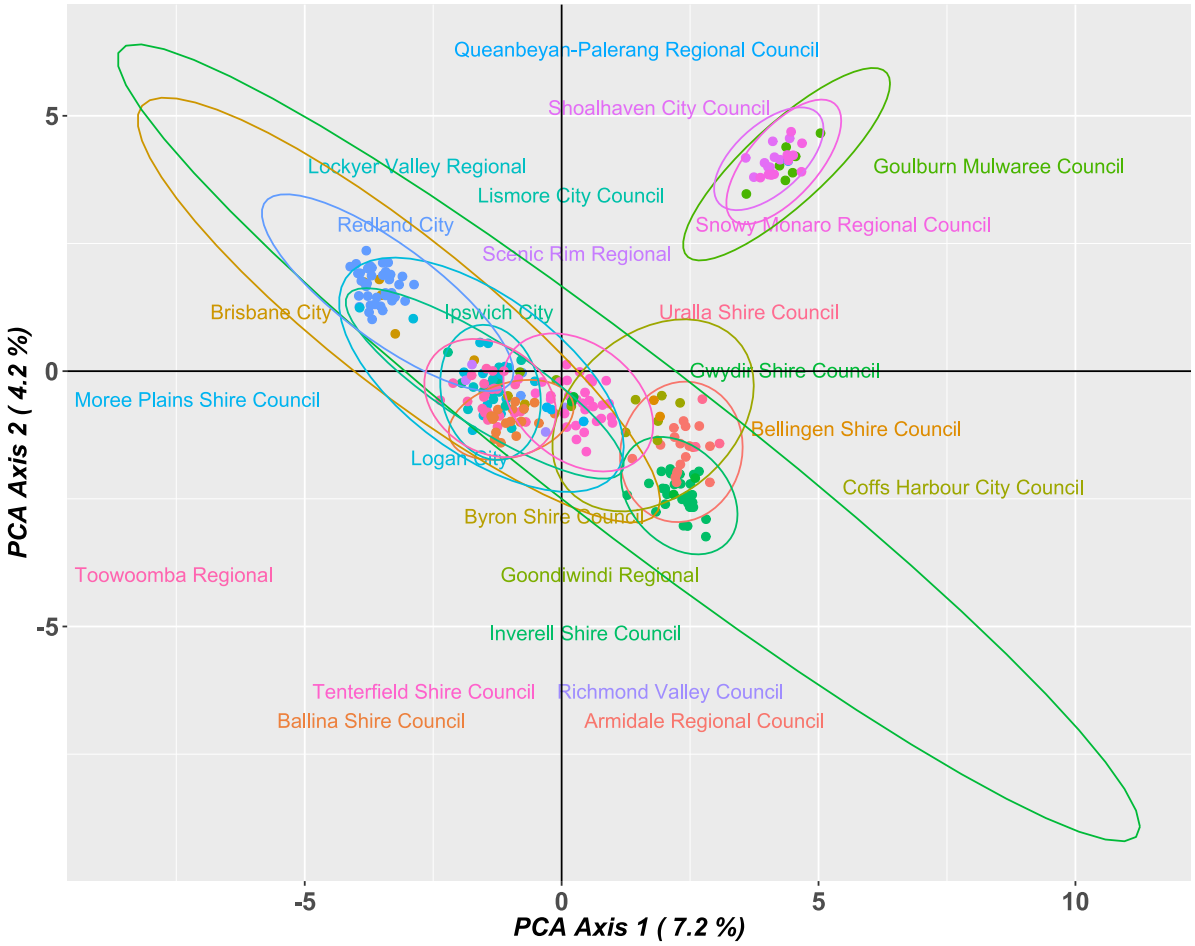
Sample ID	Duplicate	QC for Unique individual (Pass/Fail)	Unique individuals	Genetic diversity	QC for Sex (Pass/Fail)	Sex	QC for Chlamydia (Pass/Fail)	Chlamydia detection	Lat	Long
220609Bi1A	-	Failed	-	No	Failed	-	Failed	-	-27.7122	152.9889
220609Bi2A	No	Pass	Yes	Yes	Pass	F	Pass	Yes	-27.6827	153.0032
220610Bi2A	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-27.7004	152.9966
220610Bi2B	-	Failed	-	No	Failed	-	Failed	-	-27.7036	152.9941
220610Bi2C	No	Pass	Yes	Yes	Pass	F	Pass	Yes	-27.7047	152.9959
220610Bi3A	No	Pass	Yes	Yes	Pass	F	Pass	Yes	-27.7058	152.9989
220610Bi3B	-	Failed	-	No	Failed	-	Failed	-	-27.7058	153.001
220615Bi2A	-	Failed	-	No	Failed	-	Failed	-	-27.6307	151.7425
220615Bi2B	-	Failed	-	No	Failed	-	Failed	-	-27.6301	151.7423
220615Bi2C	No	Pass	Yes	Yes	Pass	F	Pass	No	-27.6296	151.7422
220615Bi2D	Yes	Pass	No	No	Failed	-	Failed	-	-27.6301	151.7427
220615Bi2E	Yes	Pass	No	No	Failed	-	Failed	-	-27.634	151.736
220617Bi2A	-	Failed	-	No	Failed	-	Failed	-	-27.7193	151.5579
220617Bi2B	Yes	Pass	No	No	Failed	-	Failed	-	-27.7198	151.5579
220617Bi2C	Yes	Pass	Yes	Yes	Pass	F	Pass	No	-27.7205	151.5597
220617Bi2D	Yes	Pass	No	No	Failed	-	Failed	-	-27.7203	151.5579
220618Bi1A	-	Failed	No	No	Failed	-	Failed	-	-27.6052	151.7634
220618Bi1B	-	Failed	No	No	Failed	-	Failed	-	-27.6058	151.7643
220618Bi1C	Yes	Pass	No	No	Failed	-	Failed	-	-27.6058	151.7644
220618Bi1D	-	Failed	-	No	Failed	-	Failed	-	-27.6052	151.7652
220618Bi1E	Yes	Pass	Yes	No	Pass	M	Pass	No	-27.6033	151.7614
220618Bi1F	Yes	Pass	Yes	Yes	Failed	-	Failed	-	-27.6035	151.7603
220623Bi1A	-	Failed	Yes	No	Failed	-	Failed	-	-28.1773	151.1926
220623Bi1B	No	Pass	Yes	Yes	Pass	F	Pass	No	-28.1771	151.1927
220623Bi1C	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-28.1784	151.1934
220623Bi2A	-	Failed	-	No	Failed	-	Failed	-	-28.1997	151.1782
220623Bi2B	Yes	Pass	No	No	Failed	-	Failed	-	-28.2007	151.1819
220624Bi1A	No	Pass	Yes	Yes	Pass	F	Pass	Yes	-27.7306	151.6415
220713Bi2A	-	Failed	-	No	Failed	-	Failed	-	-29.4195	149.9071
220713Bi4A	-	Failed	-	No	Failed	-	Failed	-	-29.3697	150.0833
220721Bi3A	Yes	Pass	No	No	Failed	-	Failed	-	-29.4632	149.8442
220722Bi1A	Yes	Pass	Yes	Yes	Pass	M	Pass	Yes	-29.4626	149.844

220801Bi1A	Yes	Pass	Yes	Yes	Pass	F	Pass	No	-27.8256	152.7624
220801Bi1B	Yes	Pass	No	No	Failed	-	Failed	-	-27.8252	152.7619
220801Bi2A	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-27.8204	152.7666
220802Bi1A	-	Failed	-	No	Failed	-	Failed	-	-27.7337	152.7079
220802Bi1B	-	Failed	-	No	Failed	-	Failed	-	-27.7334	152.7094
220802Bi1C	-	Failed	-	No	Failed	-	Failed	-	-27.735	152.7112
220802Bi1D	-	Failed	-	No	Failed	-	Failed	-	-27.737	152.711
220802Bi1E	Yes	Pass	No	No	Failed	-	Failed	-	-27.7368	152.7097
220802Bi3A	No	Pass	Yes	No	Failed	-	Failed	-	-27.6961	152.6082
220803Bi1A	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-27.6672	152.6562
220803Bi1B	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-27.667	152.6517
220803Bi2A	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-27.6878	152.6358
220803Bi3A	No	Pass	Yes	Yes	Pass	M	Pass	No	-27.6984	152.6182
220809Bi1A	-	Failed	-	No	Failed	-	Failed	-	-27.5159	151.9719
220809Bi1B	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-27.5163	151.9722
220809Bi2A	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-27.5141	151.9703
220809Bi3A	-	Failed	-	No	Failed	-	Failed	-	-27.5171	151.9536
220809Bi3B	No	Pass	Yes	No	Failed	-	Failed	-	-27.5167	151.9533
220809Bi3C	No	Pass	Yes	Yes	Pass	M	Pass	No	-27.5149	151.9543
220810Bi1A	-	Failed	-	No	Failed	-	Failed	-	-27.515	152.0122
220810Bi1B	No	Pass	Yes	Yes	Pass	M	Pass	No	-27.5163	152.0096
220810Bi1C	No	Pass	Yes	Yes	Pass	F	Failed	-	-27.5164	152.0067
220810Bi1D	No	Pass	Yes	Yes	Pass	F	Pass	No	-27.5151	152.0113
220810Bi3A	-	Failed	-	No	Failed	-	Failed	-	-27.523	152.0119
220810Bi3B	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-27.5238	152.0087
220811Bi1A	Yes	Pass	Yes	Yes	Pass	F	Pass	Yes	-27.5374	152.1315
220811Bi1B	No	Pass	Yes	No	Failed	-	Failed	-	-27.5365	152.1315
220811Bi1C	Yes	Pass	No	No	Failed	-	Failed	-	-27.5379	152.1305
220811Bi1D	Yes	Pass	Yes	Yes	Pass	F	Pass	No	-27.538	152.1306
220811Bi3A	Yes	Pass	No	No	Failed	-	Failed	-	-27.5402	152.1394
220811Bi3B	No	Pass	Yes	Yes	Pass	M	Pass	Yes	-27.5398	152.1436
221207Bi1A	Yes	Pass	No	No	Failed	-	Failed	-	-29.1214	150.3042
221207Bi1B	Yes	Pass	Yes	Yes	Pass	M	Pass	Yes	-29.1218	150.3052
221207Bi1C	Yes	Pass	No	No	Failed	-	Failed	-	-29.1228	150.3042
221207Bi5A	No	Pass	Yes	Yes	Failed	-	Failed	-	-29.0955	150.2545
221207Bi7A	No	Pass	Yes	Yes	Pass	F	Pass	No	-29.183	150.2515
221208Bi1A	No	Pass	Yes	Yes	Pass	F	Pass	Yes	-29.4619	149.8443
221208Bi1B	-	Failed	-	No	Failed	-	Failed	-	-29.4621	149.8443
221208Bi2A	Yes	Pass	Yes	No	Pass	F	Pass	Yes	-29.4621	149.85
221208Bi2B	Yes	Pass	No	No	Failed	-	Failed	-	-29.4619	149.8496
221208Bi2C	Yes	Pass	Yes	Yes	Failed	-	Failed	-	-29.4614	149.8493

Table A4. Pairwise table showing relatedness values between samples that are very likely from the same individual koala. Note that, on five instances, individuals were identified as the same individual with more than one other sample, whilst the other samples are duplicates. The asterisk indicates which sample was removed based on insufficient data.

Sample 1	Sample 2	Wang (2002)	Lynch and Ritland (1999)	Milligan (2003)
220615Bi2D	220615Bi2E	0.934	0.906	0.944
220615Bi2E	220623Bi1A	0.839	0.527	0.796
220623Bi1A	220623Bi2B	0.887	0.735	0.854
220623Bi2B	220802Bi1E	0.982	0.962	1
220802Bi1E	220615Bi2D	0.980	0.963	0.971
220615Bi2D	220623Bi1A	0.877	0.642	0.804
220615Bi2D	220623Bi2B	0.939	0.897	0.948
220615Bi2E	220623Bi2B	1	1	1
220615Bi2E	220802Bi1E	0.929	0.827	0.937
220623Bi1A	220802Bi1E	0.913	0.829	0.866
220617Bi2B*	220617Bi2C	0.687	0.798	1
220617Bi2C	220617Bi2D*	0.687	0.790	1
220617Bi2D*	220617Bi2B*	0.391	0.560	0.947
220618Bi1C*	220618Bi1E	0.908	0.898	0.966
220618Bi1E	220618Bi1F*	0.962	0.964	1
220618Bi1F*	220618Bi1C*	0.903	0.889	1
220721Bi3A	220722Bi1A	1	1	1.098
220801Bi1A	220801Bi1B	0.604	0.717	1.041
220811Bi1A	220811Bi3A*	0.995	0.994	1
220811Bi1C*	220811Bi1D	0.958	0.953	1
221207Bi1A*	221207Bi1B	0.905	0.913	1.036
221207Bi1B	221207Bi1C*	0.728	0.800	1.055
221207Bi1C*	221207Bi1A*	0.758	0.819	1.088
221208Bi2A*	221208Bi2B*	1	1	1
221208Bi2B*	221208Bi2C	1	1	1
221208Bi2C	221208Bi2A*	1	1	1.119

Figure A2. Principal component analysis (PCA) plot for 255 unique koalas from 24 locations. Each location has its 95% CI ellipse.



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APPENDIX B UNISC DIET STUDY SAMPLE ID

Table 11-1 Sample Id And Locations Taken From Unisc Koala Diet Analysis Data, Sample Number Was Assigned For The Purposes Of Mapping And Identification In This Report

Sample ID	Latitude	Longitude	Sample number	Sample ID	Latitude	Longitude	Sample number
220609Bi1A	-27.7122361	152.9889027	1	**220722Bi1A**	-29.4625715	149.8439588	26
220609BI2A	-27.6826527	153.0032046	2	**220801Bi1B**	-27.8252010	152.7619230	27
220610BI2B	-27.7036150	152.9941374	3	**220801Bi2A**	-27.8203614	152.7665640	28
220610BI3B	-27.7057817	153.0009636	4	**220802BI1A**	-27.7336759	152.7079343	29
220615BI2B	-27.6300514	151.7423274	5	**220802BI1B**	-27.7333631	152.7094265	30
220615BI2C	-27.6296256	151.7421600	6	**220802BI1D**	-27.7370225	152.7109793	31
220615BI2D	-27.6301272	151.7427218	7	**220802BI1E**	-27.7368031	152.7096508	32
220615BI2E	-27.6339930	151.7360492	8	**220803Bi1B**	-27.6669582	152.6516855	33
220617BI2B	-27.7198355	151.5579458	9	**220803Bi2A**	-27.6877892	152.6357785	34
220617BI2C	-27.7205485	151.5596708	10	**220803Bi3A**	-27.6983854	152.6182173	35
220617BI2D	-27.7203316	151.5579190	11	**220809BI1A**	-27.5159188	151.9718864	36
220618BI1A	-27.6052280	151.7633827	12	**220809BI1B**	-27.5163362	151.9721761	37
220618BI1B	-27.6058229	151.7642981	13	**220809BI2A**	-27.5141105	151.9702833	38
220618BI1C	-27.6057513	151.7643698	14	**220809BI3A**	-27.5170733	151.9536104	39
220618BI1D	-27.6051905	151.7652475	15	**220809BI3C**	-27.5148848	151.9542791	40
220618BI1E	-27.6032513	151.7613683	16	**220810Bi1A**	-27.5150194	152.0122013	41
220618BI1F	-27.6034761	151.7602613	17	**220810Bi3A**	-27.5230155	152.0119375	42
220623BI1A	-28.1773021	151.1926390	18	**220810Bi3B**	-27.5238411	152.0087000	43
220623BI1B	-28.1771044	151.1927035	19	**220811Bi1B**	-27.5365243	152.1315362	44
220623BI1C	-28.1784397	151.1933634	20	**220811Bi3A**	-27.5402114	152.1394267	45
220623BI2A	-28.1996994	151.1782250	21	**220811BI3B**	-27.5397950	152.1436227	46
220623BI2B	-28.2007315	151.1818646	22	**221207BI1A**	-29.1214000	150.3041700	47
220624BI1A	-27.7306276	151.6414796	23	**221207BI1B**	-29.1217500	150.3052200	48
220713Bi2A	-29.4194731	149.9071345	24	**221208BI2A**	-29.4621400	149.8500100	49
220713Bi4A	-29.3697201	150.0832739	25	**221208BI2B**	-29.4619100	149.8495700	50

APPENDIX C KOALA DIET ANALYSIS BY UQ

Koala Diet Analysis

Australian Inland Rail

December 2023

By Michaela Blyton

The University Of Queensland
AUSTRALIA

Scope and Limitations

This report details the tree species identified in koala scat samples through the detection of species-specific genetic markers (SNPs: single nucleotide polymorphisms) on the DarTag platform (Diversity Arrays Technologies) using a panel of 1029 validated and 93 unvalidated oligos covering 89 potential food tree species found in the state of Queensland. Only species included in the oligo panel can be detected using this method. As such, if other species not in the panel were eaten by the koalas sampled, then those species would not be identified.

Failure to detect a species in a sample does not guarantee that the species was not eaten, particularly where the total number of diet associated reads returned for that sample is low (< 50). As such, it is recommended that the total number of diet associated reads returned be taken into account when considering the results for a particular sample.

The relative abundances of the tree species detected in a sample are provided to offer insight into the dominant and minor components of the diet. However, it should be noted that at this time the method for assigning the relative abundance values has not been validated against data from koalas with known diets. Validation of the method is currently in process but until it is completed the relative abundance values should be treated with caution.

Methods

Queensland Diet DarTag Oligo Panel

Potential food tree species sampling: A list of candidate koala food trees species for Queensland was assembled with reference to numerous published lists of koala food and shelter trees for Queensland and New South Wales. The list included not only eucalypts (the genera Eucalyptus, Corymbia and Angophora), but also a number of non-eucalypts, including species from the genera Melaleuca, Acacia, Allocasuarina, Casuarina and Lophostemon.

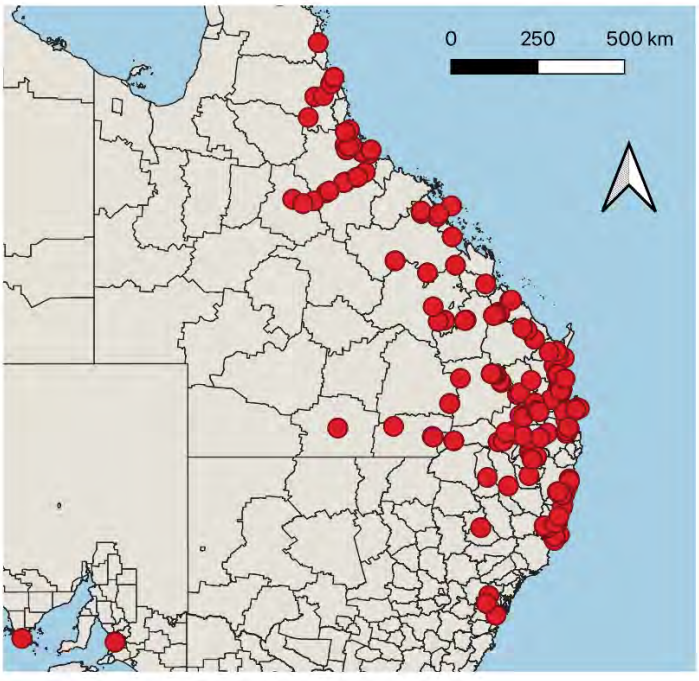

Figure 1. Map illustrating the geographic distribution of sites where leaf samples were collected from candidate koala food tree species.

The locations of all trees from which genetic material was included in the development of the Queensland panel can be seen in *Figure 1*. A more detailed, interactive, map can also be viewed on the "Our Methods" page of our website www.whatdokoalaseat.org and we encourage the reader to view this map to better understand our collections. The viewer can click on individual points to learn more about the samples included and to access links to websites providing information about each species. A complete list of tree taxa and number of replicates included in the development of the Queensland panel is listed in Table 1. Most leaf samples were collected specifically for this project by Dean Nicolle, of Currency Creek Arboretum, in June 2021. Dr Nicolle is Australia's pre-eminent eucalypt expert. During these collections, 4 individual trees were sampled per species/site combination, for 73 taxa from 135 locations, for a total of 144 unique site-species combinations and 576 leaf samples. Sequencing was performed on combined DNA extracts from two individual trees from each site/species combination, so that these collections contributed 288 sequence representations.

Existing tree sequence data from other projects was also included. This included the red gum species *E. tereticornis* and *E. blakelyi*, which were collected by John Whale at locations throughout NSW and Queensland as part of his PhD research into the genetics of this group. It also included numerous ironbark species from the series Siderophloiae, collected by Ben Moore during a previous research project in Queensland, and some species that had been included in a previous study of koala diet composition in the forests of northeast NSW. These were included to increase the range of geographic and thereby genetic variation included in our collection, and in some instances trees from beyond Queensland were also included.

After collection in the field, leaf samples were either air-dried immediately, dried on silica immediately, or returned frozen to a university and subsequently freeze-dried prior to extraction of DNA.

Table 1: List of candidate koala food tree taxa for which gene sequences were included in the development of the Queensland panel. Note that most sequencing wells included DNA from two individuals.

Taxon	Number of Sequencing Wells
Acacia melanoxylon	2
Allocasuarina littoralis	10
Allocasuarina sp.	5
Allocasuarina torulosa	9
Angophora costata	3
Angophora floribunda	4
Angophora leiocarpa	2
Araucaria cunninghamii	2
Casuarina glauca	4
Casuarina sp.	2
Corymbia citriodora	6
Corymbia clarksoniana	2
Corymbia dallachiana	2
Corymbia erythrophloia	4
Corymbia gummifera	5
Corymbia intermedia	10
Corymbia maculata	6
Corymbia tessellaris	8
Corymbia trachyphloia subsp. trachyphloia	8
Corymbia variegata	4
Eucalyptus acmenoides	8
Eucalyptus albens	6
Eucalyptus amplifolia subsp. sessiliflora	2

Eucalyptus andrewsii	2
Eucalyptus bancroftii	4
Eucalyptus blakelyi	8
Eucalyptus brownii	2
Eucalyptus brunnea	2
Eucalyptus camaldulensis	5
Eucalyptus camaldulensis subsp. acuta	4
Eucalyptus camaldulensis subsp.camaldulensis	2
Eucalyptus cambageana	4
Eucalyptus campanulata	2
Eucalyptus carnea	6
Eucalyptus chloroclada	2
Eucalyptus citrodora	2
Eucalyptus conica	4
Eucalyptus coolabah	8
Eucalyptus crebra	10
Eucalyptus cullenii	4
Eucalyptus drepanophylla	8
Eucalyptus dunnii	2
Eucalyptus dura	4
Eucalyptus elegans	4
Eucalyptus eugenioides	6
Eucalyptus exserta	7
Eucalyptus fibrosa	4
Eucalyptus fibrosa nubilla	4
Eucalyptus grandis	7
Eucalyptus granitica	4
Eucalyptus haemostoma	2
Eucalyptus intertexta	2
Eucalyptus laevopinea	4
Eucalyptus largiflorens	2
Eucalyptus latisinensis	2
Eucalyptus longirostrata	4
Eucalyptus macta	2
Eucalyptus major	4
Eucalyptus melanophloia	2
Eucalyptus melanophloia subsp. melanophloia	6
Eucalyptus melliodora	4
Eucalyptus microcarpa	4
Eucalyptus microcorys	8
Eucalyptus moluccana	6
Eucalyptus nobilis	2
Eucalyptus ochrophloia	2
Eucalyptus orgadophila	6
Eucalyptus pellita	4
Eucalyptus pilularis	8
Eucalyptus platyphylla	8
Eucalyptus populnea	8
Eucalyptus portuensis	2
Eucalyptus prava	3
Eucalyptus propinqua	8
Eucalyptus punctata	6

Eucalyptus racemosa	7
Eucalyptus resinifera	3
Eucalyptus resinifera subsp. hemilampra	4
Eucalyptus rhombica	2
Eucalyptus robusta	8
Eucalyptus saligna	8
Eucalyptus scoparia	2
Eucalyptus seeana	4
Eucalyptus siderophloia	8
Eucalyptus sideroxylon	2
Eucalyptus signata	2
Eucalyptus taurina	4
Eucalyptus tereticornis	18
Eucalyptus tindaliae	5
Eucalyptus whitei	5
Eucalyptus woollsiana	2
Lophostemon confertus	6
Lophostemon suaveolens	2
Melaleuca quinquenervia	2
Syncarpia glomulifera	2
Syncarpia glomulifera subsp. glomulifera	4
Grand Total	447

Sequencing of potential food tree species: DNA was extracted from leaf samples collected by Dean Nicolle using an automated plate-based extraction protocol, by Diversity Arrays Technology P/L, Canberra, Australia (DArT). The remaining reference leaves were extracted using CTAB extraction buffer with chloroform clean-up and ethanol precipitation. DNA extracts were sequenced at high density (returning approximately 2.5 million reads per sample) on the DArTseq platform by Diversity Arrays Technology P/L, Canberra, Australia (DArT). The *Eucalyptus* Dartseq (1.0) product was selected that uses the PstI/HpaII restriction enzyme combination, which returns approximately 50 000, 64 bp loci with an average read depth for the reference allele of > 20. This protocol has been optimised for *Eucalyptus* by Diversity Arrays Technologies in association with prior clients for *Eucalyptus* genotyping. Read counts for targets (DNA fragments) and single nucleotide polymorphisms (SNPs) within those targets were determined by DArT using their standard proprietary pipelines [1, 2]. As DArT's pipelines are optimised to identify loci that are present and variable across the majority of the samples in the dataset, loci with private alleles may not be retained if the species in the dataset are too taxonomically distinct. As such, species belonging to the different genera and *Eucalyptus* subgenera were run through the pipelines separately, to maximise the number of potentially useful loci identified. Read proportions for the reference and alternative SNP alleles at each locus were determined using custom code in R studio 1.2.5033 [3].

Identification of species-specific SNPs and panel validation: Prior to identification of species-specific SNPs, a Principal Components Analysis was performed on the Hamming genetic distance matrix generated from the SNP data in GenAlEx 6.1 to confirm the species designations of the samples. Samples that did not cluster with their designated species were then removed from the dataset. Loci for which one or more individuals were heterozygous were also removed from the dataset as these loci could not contain fixed private alleles. Species-specific SNPs were identified in the remaining loci using custom written code in R studio 1.2.5033 [3]. Additionally, for a small number of species that were taxonomically distinct from all other species in the datasets (i.e. *Araucaria cunninghamii* and *Acacia melanoxylon*), in sillico darts that were present only in the target species were also identified as potential species-specific genetic markers.

The list of species-specific SNPs identified from the DArTseq data was filtered to retain only a single SNP per unique DArT sequencing fragment to minimise the impact of linkage disequilibrium among potential markers. Up to 30 SNPs/in sillico darts were then selected per species for oligo design. Of the suitable SNPs, those that were fixed, had higher DArTseq read counts and higher reproducibility were preferentially selected. Where SNPs from DArT targets that were only present in some individual trees had to be included, those that were present in a higher proportion of the individuals were given preference. Oligos were designed for the selected SNPs in house by DArT by identifying probe regions flanking the selected SNPs using primer design software in Genious [4]. Of the candidate species-specific SNPs only those that were found in the middle of the DArT target were suitable for oligo design.

The selected tags (SNPs and flanking regions) were then amplified and sequenced using the designed oligos from generally four individual trees of each species using the DArTag platform to establish that they remained species specific on the different platform. Briefly, the pooled species-specific oligos were hybridized to denatured eucalyptus DNA, then the targeted SNPs were copied and amplified with simultaneous addition of demultiplexing primers. The products of DArTag assay were then sequenced on the HiSeq 2500 (Illumina), demultiplexed and targeted SNPs detected using DArT P/L's proprietary analytical pipeline (DArToolbox). There were four species (*E. prava, E. laevopinea E. andrewsii* and *Araucaria cunninghamii*) that were not included in the validation panel due to a lack of available samples. As such, the SNPs/in sillico darts for these species could not be fully validated. We confirmed that these markers did not amplify in any non-target species, however, we could not confirm amplification in the target species.

After validation we retained 1122 markers with the number of markers identified per taxon/species shown in Table 2. The marker panel demonstrated the ability to discriminate most eucalypt species, with some exceptions where closely related species or subspecies could not be distinguished, even though we could be confident that DNA from one or more of the species in question was present. This was particularly notable for the ironbarks from the series Siderophloiae (*E. crebra, drepanophylla, fibrosa, rhombica, dura, cullenii, whitei, melanophloia*).

Table 2: List of DarTag markers in the Queensland panel for candidate koala food tree taxa

Taxon	Number of DarTag Markers	
Acasia melanoxylon	28	
Allocasuarina	10	
Allocasuarina littoralis	19	
Allocasuarina torulosa	0	
Angophora	24	
Angophora costata	7	
Angophora floribunda	6	
Angophora leiocarpa	17	
Araucaria cunninghamii	60	unvalidated
Casuarina glauca	15	
Casuarina sp1	9	
Casuarina sp2	18	
Corymbia citriodora	8	
Corymbia clarkoniana	17	
Corymbia dallachiana	16	
Corymbia erythrophloia	14	
Corymbia gummifera	8	
Corymbia gummifera and/or *intermedia*	1	
Corymbia intermedia	5	
Corymbia maculata	10	
Corymbia tessellaris	5	

Corymbia trachyphloia	11	
Corymbia variegata	15	
Eucalyptus acmenoides	9	
Eucalyptus albens	14	
Eucalyptus amplifolia	14	
Eucalyptus andrewsii	13	unvalidated
Eucalyptus bancroftii	19	
Eucalyptus bancroftii and/or *seeana*	8	
Eucalyptus blakelyi	7	
Eucalyptus brownii	13	
Eucalyptus brunnea	12	
Eucalyptus camaldulensis	35	
Eucalyptus cambageana	15	
Eucalyptus campanulata	12	
Eucalyptus carnea	9	
Eucalyptus chloroclada	17	
Eucalyptus conica	11	
Eucalyptus coolabah	16	
Eucalyptus crebra	3	
Eucalyptus crebra and/or *elegans*	1	
Eucalyptus crebra and/or *drepanophylla*	1	
Eucalyptus crebra, elegans and/or *fibrosa*	1	
Eucalyptus cullenii	5	
Eucalyptus cullenii and/or *crebra*	1	
Eucalyptus cullenii and/or *melanophloia*	1	
Eucalyptus cullenii and/or *whitteii*	1	
Eucalyptus drepanophylla	2	
Eucalyptus drepanophylla and/or *dura*	1	
Eucalyptus dunnii	13	
Eucalyptus dura	7	
Eucalyptus elegans	3	
Eucalyptus eugenioides	13	
Eucalyptus exserta	11	
Eucalyptus fibrosa	4	
Eucalyptus fibrosa and/or *taurina*	1	
Eucalyptus fibrosa and/or *whiteii*	1	
Eucalyptus grandis	13	
Eucalyptus granitica	12	
Eucalyptus granitica and/or *whitteii*	1	
Eucalyptus haemastoma	5	
Eucalyptus intertexta	10	
Eucalyptus laevopinea	9	unvalidated
Eucalyptus largiflorens	11	
Eucalyptus latisinensis	9	
Eucalyptus longirostrata	19	
Eucalyptus macta	13	
Eucalyptus macta and/or *resinifera*	1	
Eucalyptus major	20	
Eucalyptus melanophloia	6	
Eucalyptus melliodora	12	
Eucalyptus microcarpa	10	
Eucalyptus microcorys	14	

Eucalyptus moluccana	5	
Eucalyptus nobilis	12	
Eucalyptus ochrophloia	11	
Eucalyptus orgadophila	9	
Eucalyptus pellita	17	
Eucalyptus pilularis	6	
Eucalyptus platyphylla	15	
Eucalyptus populnea	13	
Eucalyptus portuensis	6	
Eucalyptus prava	11	unvalidated
Eucalyptus propinqua	15	
Eucalyptus punctata	11	
Eucalyptus racemosa	7	
Eucalyptus resinifera	12	
Eucalyptus rhombica	18	
Eucalyptus robusta	6	
Eucalyptus saligna	13	
Eucalyptus scoparia	17	
Eucalyptus seeana	13	
Eucalyptus siderophloia	4	
Eucalyptus sideroxylon	17	
Eucalyptus signata	8	
Eucalyptus taurina	2	
Eucalyptus tereticornis	8	
Eucalyptus tindaliae	15	
Eucalyptus whiteii	4	
Eucalyptus woollsiana	9	
Eucalytpus dura and/or *siderophloia*	1	
Eucalytpus dura, fibrosa and/or *rhombica*	1	
Eucalytpus dura, fibrosa and/or *taurina*	1	
Eucalytpus fibrosa and/or *rhombica*	1	
Eucalytpus melanophloia and/or *whiteii*	1	
Eucalytpus tereticornis and/or *robusta*	2	
Lophostemon confertus	6	
Lophostemon suaveolens	12	
Melaleuca glutaraldeyde	17	
Syncarpia glomulifera	14	
Grand Total	**1122**	

Diet Determination from Scats

DNA was extracted from the koala scats using CTAB extraction buffer with chloroform clean-up and ethanol precipitation [5]. 100 mg of material taken from the centre of each scat sample was ground into a fine powder using the TissueLyser II (Qiagen). Each sample was bead beaten three times at 30 Hz for 1 minute after snap freezing in liquid nitrogen. The samples were then digested in 1000 µl (scats) of extraction buffer (0.1 M Tris-HCL, 1.4 M NaCl, 0.02M EDTA, 20 $g.l^{-1}$ of cetyltrimethyl ammonium bromide and 0.3 % β-mercaptoethanol) in a ThermoMixer (Eppendoff) at 65 °C for 1 hour with mixing at 1000 rpm. The digested samples were centrifuged for 10 minutes at 16 000 g. The supernatant was then washed twice in one volume of chloroform:isoamyl alcohol (24:1). The DNA was then precipitated by addition of a half volume of 5M NaCl to the separated aqueous phase, followed by 3 volumes of cold 95% ethanol and incubation at -20 °C for 1 hour. The samples were then centrifuged for 10 minutes at 16 000 g. The DNA pellet was washed with 700 µl of 70% ethanol followed by 700 µl of 95% ethanol. The DNA was dried for

30 minutes at 65 °C and resuspended in 40 µl of TE buffer. Inhibitors were removed from the DNA extracts using the OneStep PCR Inhibitor Removal Kit (Zymo) according to the manufacturer's instructions.

Those SNPs that were confirmed to be species-specific on the DArTag platform were amplified and sequenced from the faecal DNA extracts (as described above) to determine the composition of the koalas' diets. The resulting sequencing read counts by sample table was then filtered to remove likely sequencing errors and low frequency contamination. For each target species, sequencing reads were pruned from the dataset if the average number of reads per detected marker was less than 3 or if fewer than two SNPs were detected in target species that had 3 or more validated markers. The remaining filtered readers for each marker were then divided by the average number of reads returned for that marker in the target tree species to account for differences among markers in amplification efficiency. The number of scaled reads for each target species was then averaged across those markers that were found in the highest proportion of target tree species individuals (e.g if three markers were detected for a target species in a sample with two of those found in all trees sequenced for that species while the last marker was only found in half the target trees, then the scaled reads would be averaged across to two fixed markers while the reads from the last marker were not considered). The averaged scaled reads per target species were then converted to relative abundance by dividing by the total number of averaged scaled reads across all target species.

Results

Of the 55 scat samples sequenced on the DarTag platform using the Queensland diet oligo panel, 49 returned reads for the validated diet markers after quality control filtering. An average of 333.59 filtered diet reads were returned per sample (range: 3 to 1591) with less than 50 filtered reads returned for three samples.

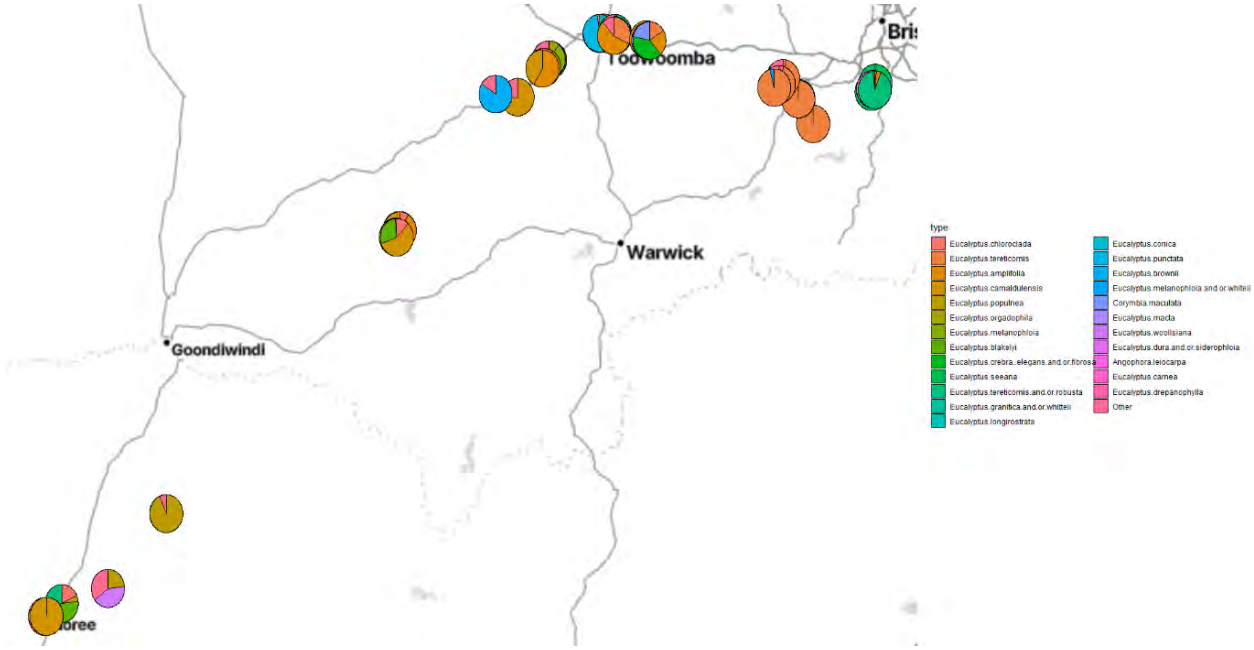

Figure 2. Map of scat sample locations with pie charts illustrating the relative abundance of the different dietary tree species detected in each sample.

Across all samples, between 1 and 7 tree species were detected per sample (average = 3.35). Thirty-seven individual species were detected across the 49 samples with several other species potentially present as indicated by the presence of markers for groups of target species. The most frequently detected species were *Eucalyptus chloroclada* (detected in 18 samples), *E. tereticornis* (17 samples), *E. amplifolia* (16 samples) and *E. camaldulensis* (16 samples). These species were also often found at high relative abundance within samples (range of average relative abundances: 24% to 44%). *E. prava* was detected in a single sample (IR21: 220623BI1B), however, as the markers for this species have not been validated it

could not be included in the relative abundance calculations. Please see a breakdown of the results by sample in the excel workbook "ARTC diet analysis results". The diet species detected differed between geographic regions and between individuals (*Figure 2*).

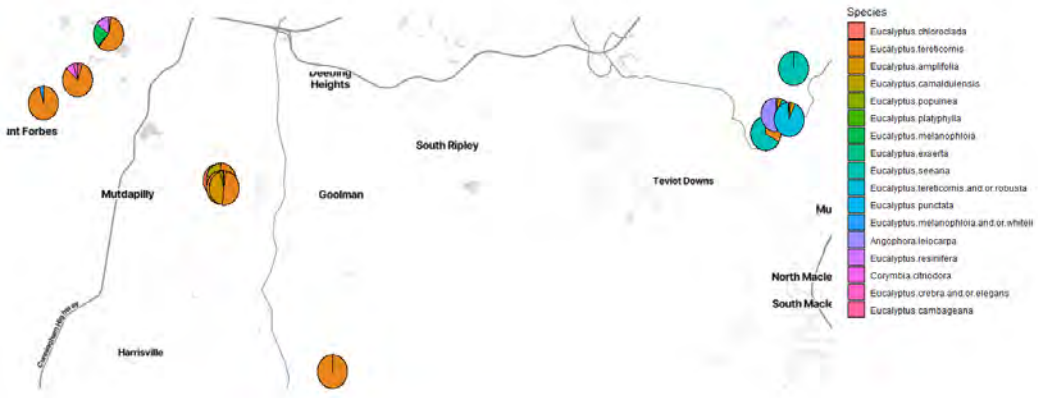

*Figure 3. Map of scat sample locations in the **Brisbane Region** with pie charts illustrating the relative abundance of the different dietary tree species detected in each sample.*

In the Brisbane region *Eucalyptus seeana* was detected in 3/4 samples collected in the south at an average relative abundance of 64.5%, while *Eucalyptus tereticornis* was detected in 7/8 samples collected west of Brisbane at an average relative abundance of 72.4% (*Figure 3*).

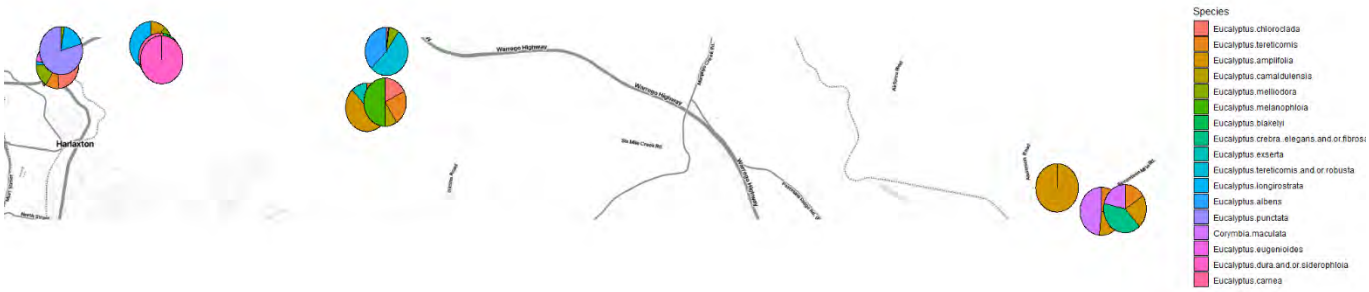

Figure 4. Map of scat sample locations in the **Toowoomba Region** with pie charts illustrating the relative abundance of the different dietary tree species detected in each sample.

In the Toowoomba region, the tree species detected varied considerably between samples. *E. amplifolia*, *E. tereticornis*, *E. chloroclada* and *E. melliodora* were all detected in four or more samples (from 11) at relative abundances between 10.3% and 34.6% (*Figure 4*).

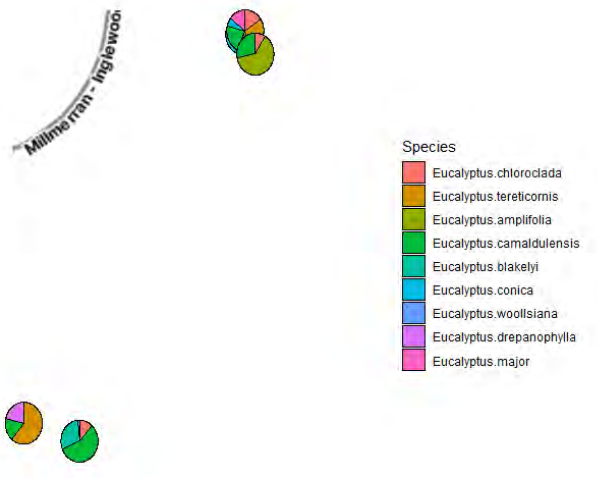

Figure 5. Map of scat sample locations in the **Region to the north-east of Goondiwindi** with pie charts illustrating the relative abundance of the different dietary tree species detected in each sample.

In the region to the north-east of Goondiwindi *E. camaldulensis* was found in all five samples at an average relative abundance of 32.7%, while *E. amplifolia* and *E. tereticornis* were also found at high relative abundance in two individuals each (*Figure 5)*.

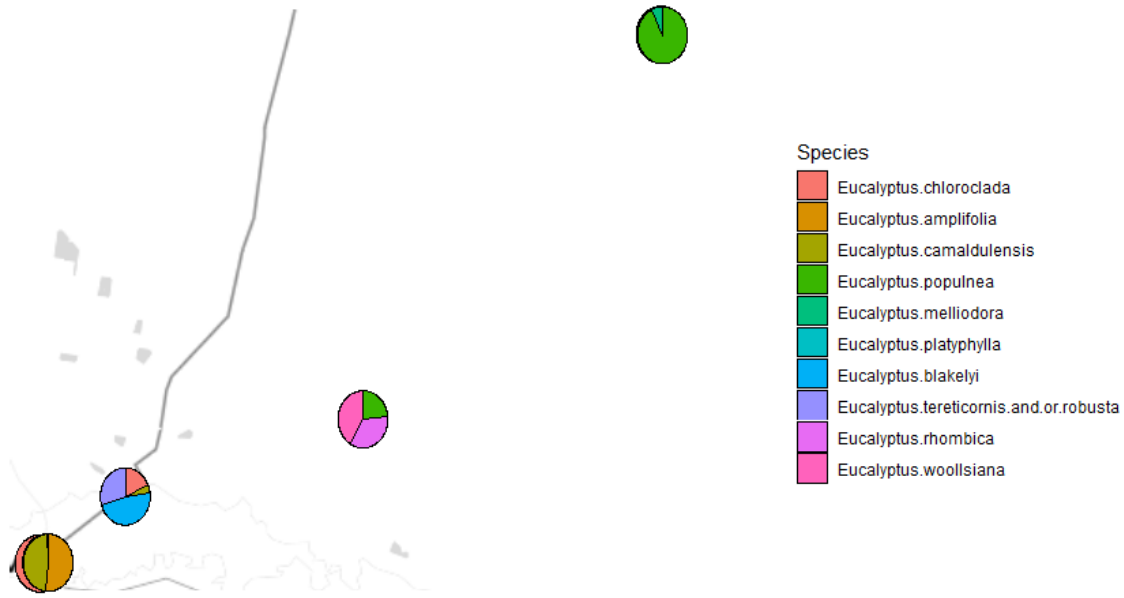

Figure 6. Map of scat sample locations in the **Moree Region** with pie charts illustrating the relative abundance of the different dietary tree species detected in each sample.

In the Moree Region, 5/7 samples contained *E. camaldulenis* with an average relative abundance of 35.2%, while *E. populnea* and *E. chloroclada* were also found at high relative abundance (64.6% and 58.7%, respectively) in 3 and 2 samples, respectively (*Figure 6*).

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