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Using non-invasive sampling methods to determine the prevalence and distribution of *Chlamydia pecorum* and koala retrovirus in a remnant koala population with conservation importance

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Abstract

Context. Pathogenic infections are an important consideration for the conservation of native species, but obtaining such data from wild populations can be expensive and difficult. Two pathogens have been implicated in the decline of some koala (*Phascolarctos cinereus*) populations: urogenital infection with *Chlamydia pecorum* and koala retrovirus subgroup A (KoRV-A). Pathogen data for a wild koala population of conservation importance in South Gippsland, Victoria are essentially absent.

Aims. This study uses non-invasive sampling of koala scats to provide prevalence and genotype data for *C. pecorum* and KoRV-A in the South Gippsland koala population, and compares pathogen prevalence between wild koalas and koalas in rescue shelters.

Methods. *C. pecorum* and KoRV-A provirus were detected by PCR of DNA isolated from scats collected in the field. Pathogen genetic variation was investigated using DNA sequencing of the *C. pecorum ompA* and KoRV-A *env* genes.

Key results. *C. pecorum* and KoRV-A were detected in 61% and 27% of wild South Gippsland individuals tested, respectively. KoRV-A infection tended to be higher in shelter koalas compared with wild koalas. In contrast with other Victorian koala populations sampled, greater pathogen diversity was present in South Gippsland.

Conclusions. In the South Gippsland koala population, *C. pecorum* is widespread and common whereas KoRV appears less prevalent than previously thought. Further work exploring the dynamics of these pathogens in South Gippsland koalas is warranted and may help inform future conservation strategies for this important population.

Implications. Non-invasive genetic sampling from scats is a powerful method for obtaining data regarding pathogen prevalence and diversity in wildlife. The use of non-invasive methods for the study of pathogens may help fill research gaps in a way that would be difficult or expensive to achieve using traditional methods.

Additional keywords: *Chlamydia ompA* genotyping, KoRV *env* genotyping, non-invasive sampling, *Phascolarctos cinereus*, scats.

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Introduction

Infectious diseases in wildlife have the potential to contribute to population decline (McCallum 2012). Pathogenic organisms are therefore an important consideration in conservation biology and for the prediction of extinction risk (Gerber *et al.* 2005; McCallum 2012). Impacts of a specific pathogen on an individual are easily assessed, but determining whether the pathogen has a significant impact at the population level is more difficult (McCallum *et al.* 2017). Methods that enable the rapid and inexpensive assessment of infection prevalence in wild populations would be extremely useful for determining wide-scale impacts of pathogen infection.

The koala (*Phascolarctos cinereus*) is an arboreal marsupial inhabiting eucalypt forests of Australia's east, from Queensland (Qld) in the north, through New South Wales (NSW) to Victoria (Vic.), and South Australia (SA) in the south (Martin and Handasyde 1999). In Victoria, koala populations have a long history of anthropogenic interference. In the late 1800s, koala populations were established on Phillip and French Islands in Westernport Bay using small numbers of koalas from the South Gippsland region (Lewis 1954; Wedrowicz *et al.* 2017). By the early 1900s, habitat clearance for agriculture, increased frequency and intensity of fires and hunting for the koala fur trade had led to the extirpation of most mainland koala

populations in Victoria and South Australia (Lewis 1954; Menkhorst 2008), while koala populations on Phillip and French Islands had grown to the point of overpopulation. Island koalas were subsequently reintroduced widely across

- 5 mainland Victoria (and also South Australia), so most koala populations in these states are descendants of the few individuals moved to Phillip and/or French Island in the late 1800s (Martin 1989; Menkhorst 2008). The exception is the koala population in South Gippsland, Victoria, which is
- 10 a genetically unique, remnant population not derived from individuals released as part of the translocation program (Houlden *et al.* 1999; Lee *et al.* 2012; Wedrowicz *et al.* 2018a). There is significantly greater genetic diversity in the South Gippsland koala population, compared with other
- 15 Victorian koala populations, making the South Gippsland population a high conservation priority (Lee *et al.* 2012; Wedrowicz *et al.* 2018a).

- Koalas in Queensland, New South Wales and the Australian Capital Territory are listed as Vulnerable under the
- 20 *Environmental Protection and Biodiversity Conservation Act 1999* (EPBC Act; EaCRC 2012) due to recent population declines. In contrast, koala populations in Victoria and South Australia were excluded from EPBC Act listing (EaCRC 2011a, 2011b) due to their relative abundance and overpopulation
- 25 in some areas, but the genetic homogeneity of these koala populations may increase their vulnerability.

- Two pathogens, *Chlamydia pecorum* and koala retrovirus (KoRV), infect koalas and are often cited as major contributors to population decline (EaCRC 2011a), despite a limited
- 30 understanding of their role in host population dynamics (Grogan *et al.* 2017; McCallum *et al.* 2017). Large-scale population level studies of koala pathogens are lacking in the literature (but see Nyari *et al.* 2017; Quigley *et al.* 2018). This may be due, in part, to the challenges associated with koala

- 35 capture, which can be stressful to koalas, potentially resulting in death (Radford *et al.* 2006; Phillips 2011). To minimise stress, prolonged examination and invasive sampling often require the administration of anaesthetic. A 2006 study involving the capture and anaesthetisation of koalas reported the average cost
- 40 of capture and veterinarian teams as AUD1362 per koala caught (Radford *et al.* 2006). Koala capture is therefore time consuming and expensive, which can limit the ability to survey widely for pathogens in koala populations. Because sampling wild populations is difficult, sick or injured koalas from wildlife
- 45 shelters are often used for pathogen studies and to obtain prevalence data. However, the potential bias associated with this type of sampling for estimations of prevalence, and an inability to obtain comparative data from the wild population, can limit the conclusions that may be drawn from such studies.
- 50 Differences in prevalence between wild and morbid animals can provide useful information regarding a pathogen's impact on a population, and may indicate whether the pathogen is associated with mortality (McCallum *et al.* 2017).

Chlamydia pecorum

- 55 The genus *Chlamydia* comprises gram-negative, intracellular bacteria associated with a range of diseases in their hosts, which include humans, ruminants, marsupials and birds

(Rank and Yeruva 2014). In koalas, *C. pecorum* infects both ocular and urogenital tissues, with significant pathological outcomes (Blanshard and Bodley 2008). The bacteria can rapidly spread within populations, with potential negative consequences for koala fecundity and health (Santamaria and 5 Schlagloth 2016). In particular, *C. pecorum* infections of the urogenital tract (UGT) can lead to sterility in females (Obendorf and Handasyde 1990), potentially contributing to population decline. Animals not previously exposed to *C. pecorum* may be more susceptible to severe infections (Martin and Handasyde 10 1990). The faecal–oral route is the main mode of *Chlamydia* transmission in most animal hosts (Rank and Yeruva 2014), but sexual transmission is considered to be the primary route of transmission in koalas. It is possible that *C. pecorum* is also transmitted via the faecal–oral route in koalas (Waugh *et al.* 15 2016), but this is yet to be demonstrated.

Genetic variants (or strains) of *C. pecorum* are classified according to differences in the nucleotide sequence of the *ompA* gene, which encodes the major outer membrane protein. In total, 14 *C. pecorum* *ompA* genotypes have been 20 reported in koalas to date, designated by the letters A to N (Table 1) (Jackson *et al.* 1997; Higgins *et al.* 2012; Kollipara *et al.* 2013; Legione *et al.* 2016b). Different strains of *C. pecorum* may have varying levels of pathogenicity, and immune responses may be specific to the infecting strain 25 (Mohamad *et al.* 2008, 2014). The introduction of unfamiliar strains of *C. pecorum* to a population could have negative health impacts (Waugh *et al.* 2016) and may be of concern for koala populations, since koalas are commonly translocated for management purposes (Menkhorst 2008; Santamaria and 30 Schlagloth 2016) or released by wildlife carers after rehabilitation in shelters (Guy and Banks 2012).

Koala retrovirus

KoRV was first detected in koalas in the year 2000 (Hanger *et al.* 2000), but analysis of archival specimens suggests that 35 KoRV has been present in Queensland and northern New South Wales koalas since at least the late 1800s (Ávila-Arcos *et al.* 2013). KoRV may be either exogenous or endogenous (Tarlinton *et al.* 2005; Simmons *et al.* 2012). Endogenous infection results from the insertion of KoRV provirus into 40 germ cells so that it is transmitted vertically, to offspring, via Mendelian inheritance (Tarlinton *et al.* 2005; Simmons *et al.* 2012). Integrated virus is then capable of producing infectious virus, so inherited KoRV can also be transmitted horizontally, between individuals (Tarlinton *et al.* 2005). To date, nine 45 genetic variants (A, B/J, C–I) of KoRV have been identified (Chappell *et al.* 2016), with koala retrovirus subgroup A (KoRV-A) the most widespread.

The effects of KoRV infection on koala health are currently not clear. Gibbon ape leukaemia virus (which shares 50 a close phylogenetic relationship with KoRV) causes leukaemia in gibbons (Hanger *et al.* 2000) while, in cats, the feline leukaemia virus is associated with the development of tumours, immunodeficiency and haematopoietic disorders, the most common symptom at initial presentation being anaemia 55 (Hartmann 2012). In koalas there is evidence that KoRV also causes immunosuppression and cancers such as lymphoma

Table 1. List of *Chlamydia pecorum ompA* genotypes described to date in koalas

The origins of the koalas in which the genotype was detected are also listed along with the GenBank accession number where available. The approximate number of base pairs in the *C. pecorum ompA* gene utilised in each study were: Jackson *et al.* (1997), 400 base pairs (bp); Higgins *et al.* (2012), 700 bp; Kollipara *et al.* (2013), ~1120 bp; Legione *et al.* (2016b), 1170 bp and for this study, 1050 bp. Owing to differences in the size of the *ompA* fragment analysed by the different studies, some genotypes based on a smaller portion of the *ompA* gene may not be able to be differentiated from genotypes based on a longer portion of the gene

<i>ompA</i> genotype	Locations detected	GenBank accession number/s	Reference/s
A	Qld: Mutdapilly, Redland Bay, Lone Pine Koala Sanctuary, Currumbin Sanctuary. NSW: Tanilba Bay	KF150132	Jackson <i>et al.</i> (1997); Kollipara <i>et al.</i> (2013)
A ⁰ Ko1 ^A	Qld: South-east region. NSW: Gunnedah	JF309281	Higgins <i>et al.</i> (2012)
A ⁰ 1	Qld	KY913821	This study
B	SA: Adelaide Hills	KF150133	Kollipara <i>et al.</i> (2013)
B ⁰ 1	Vic.: Raymond Island, Healesville, Strathbogie	JF309282; KU214248; KY913822	Higgins <i>et al.</i> (2012); Jackson <i>et al.</i> (1997); Legione <i>et al.</i> (2016b); this study ²
B ⁰ 2	Vic.: South West Coast (Cape Otway region)	KU214249	Legione <i>et al.</i> (2016b)
B ⁰ 3	Vic.: Far Western Victoria	KU214251	Legione <i>et al.</i> (2016b)
B ⁰ 4	Vic.: Raymond Island	KY913823	This study
B ⁰ 5	Vic.: South Gippsland	KY913824	This study
B ⁰ 6	Vic.: South Gippsland	KY913825	This study
C	Vic.: koala originally from Victoria but located at Featherdale Wildlife Park, NSW; Greater Gippsland	KU214245	Jackson <i>et al.</i> (1997); Legione <i>et al.</i> (2016b)
C ⁰ Ko3	Vic.: Strathbogie	JF309283	Higgins <i>et al.</i> (2012)
C ⁰ 1	Vic.: South Gippsland	KY913826	This study
C ⁰ 2	Vic.: South Gippsland	KY913827	This study
D	Australia	NA	Jackson <i>et al.</i> (1997)
E	NSW: Emerald Beach, Port Macquarie, Featherdale Wildlife Park	NA	Jackson <i>et al.</i> (1997)
E ⁰	Qld: Narangba, Lower Beechmont	KF15013	Kollipara <i>et al.</i> (2013)
F	Qld: St Bees Island, Brendale, North Stradbroke Island, East Coomera, Lower Beechmont, Elanora. NSW: Byron Bay, Port Macquarie, Tanilba Bay	KF150135; KY913828	Kollipara <i>et al.</i> (2013); this study
F ⁰ 1	NSW: Port Macquarie	KF150136	Kollipara <i>et al.</i> (2013)
F ⁰ 2	Vic.: Greater Gippsland	KU214246	Legione <i>et al.</i> (2016b)
F ⁰ 3	Vic.: South Gippsland	KY913829	This study
F ⁰ 4	Vic.: South Gippsland	KY913830	This study
F ⁰ 5	Vic.: South Gippsland	KY913831	This study
F ⁰ 6	Vic.: South Gippsland	KY913832	This study
F ⁰ 7	Vic.: South Gippsland	KY913833	This study
F ⁰ Ko5a	Qld: South-east region. NSW: Port Macquarie, Anna Bay, Lismore	JF309285	Higgins <i>et al.</i> (2012)
F ⁰ Ko5b	NSW: Port Macquarie	JF309286	Higgins <i>et al.</i> (2012) ^B
F ⁰ Ko5c	NSW: Port Macquarie	JF309288	Higgins <i>et al.</i> (2012)
F ⁰ Ko5d	NSW: Port Macquarie	JF309289	Higgins <i>et al.</i> (2012)
F ⁰ Ko5e	Qld: South-east region	JF309290	Higgins <i>et al.</i> (2012)
F ⁰ Ko5f	NSW: Port Macquarie	JF309291	Higgins <i>et al.</i> (2012)
F ⁰ Ko5fi	NSW: Port Macquarie	JF309292	Higgins <i>et al.</i> (2012)
F ⁰ Ko5 g	NSW: Port Macquarie	JF309293	Higgins <i>et al.</i> (2012)
G	Qld: South-east region, Brendale, East Coomera. NSW: Tanilba Bay. SA: Adelaide Hills	JF309284; KF150137	Higgins <i>et al.</i> (2012); Kollipara <i>et al.</i> (2013)
H	Qld: East Coomera, Lower Beechmont	KF150138	Kollipara <i>et al.</i> (2013)
I	NSW: Tanilba Bay	KF150139	Kollipara <i>et al.</i> (2013)
I ⁰	Vic.: South Gippsland	KY913834	This study
J	NSW: Port Macquarie, Tanilba Bay	KF150140	Kollipara <i>et al.</i> (2013)
K	NSW: Tanilba Bay	KF150141	Kollipara <i>et al.</i> (2013)
L	Vic.: Cape Otway National Park	KU214250; KY913835	Legione <i>et al.</i> (2016b); this study
M	Vic.: Greater Gippsland, South Gippsland	KU214247; KY913836	Legione <i>et al.</i> (2016b); this study

(continued next page)

Table 1. (continued)

ompA genotype	Locations detected	GenBank accession number/s	Reference/s
N	Vic.: French Island	KU214244	Legione <i>et al.</i> (2016a); (Legione <i>et al.</i> 2016b)
O	Vic.: South Gippsland	KY913837	This study

^AA⁰Ko1 may be equivalent to genotypes A or J.

^BF⁰Ko5a may be equivalent to E⁰2, F, F⁰1 or F⁰2.

and leukaemia (Tarlinton *et al.* 2005; Denner and Young 2013). Koalas with clinical chlamydiosis, a disease sometimes associated with immunosuppression, tend to have higher KoRV loads (Tarlinton *et al.* 2005), and an increased propensity to urogenital tract disease has been noted in female koalas co-infected with *C. pecorum* and KoRV (Legione *et al.* 2017).

KoRV appears to be endogenous in the north of the koala's range, with all koalas sampled in northern New South Wales and Queensland testing positive for KoRV provirus (Simmons *et al.* 2012). In New South Wales, there are no published KoRV prevalence data for populations south of Port Macquarie (north-east NSW; Fig. 1b). In Victoria and South Australia, the reported prevalence of KoRV is highly variable among populations, ranging from 0% to 82% (mean 51%; Simmons *et al.* 2012). However, these data are difficult to interpret because both wild and shelter animals were included in the Simmons *et al.* (2012) study. A recent study, testing 648 wild Victorian koalas for KoRV provirus (Legione *et al.* 2017), reported prevalence rates ranging from 17–40% (mean 25%). To date, genotypic analysis has detected only KoRV-A in Victorian koalas; KoRV-B has not been detected in Victorian populations (Legione *et al.* 2017).

The number of proviral KoRV copies detected per cell also varies greatly between koalas from Queensland and Victoria, with an average (per individual) of 165 copies per cell in Queensland, 1.5 copies per cell in some Victorian koalas and fewer than 0.001 copies per cell in other Victorian koalas (Simmons *et al.* 2012). This suggests that KoRV is exogenous, or early in the process of endogenisation, in Victorian koalas, because endogenously acquired KoRV is expected to result in at least one proviral copy per cell (Simmons *et al.* 2012). In Victoria, infection with KoRV-A has been found to be significantly associated with low body condition scores and the presence of 'wet bottom', resulting from chronic cystitis (Legione *et al.* 2017), indicating that KoRV-A is likely having a negative impact on the health of wild Victorian koala populations.

Detection of *C. pecorum* and KoRV using DNA from scats

Non-invasive genetic sampling overcomes many of the difficulties associated with studying pathogens in wild populations. Both *C. pecorum* and KoRV-A provirus can be detected in DNA isolated from scats (Wedrowicz *et al.* 2016), thereby providing a tool that facilitates the systematic sampling of large numbers of koalas at a reduced cost.

Using real time PCR, previous work comparing the detection of *C. pecorum* in paired DNA samples isolated from scats and urogenital swabs showed that *C. pecorum* can be reliably and

consistently detected in different scat samples from the same individual (Wedrowicz *et al.* 2016). A lower limit of eight *C. pecorum* DNA copies was detectable with the real time PCR assay used by Wedrowicz *et al.* (2016). The same study showed that KoRV-A provirus was able to be detected in DNA isolated from scats, with high concordance between standard and real time PCR assays (Wedrowicz *et al.* 2016). On average, it was found that the copy number of KoRV-A provirus was 30 times greater in DNA isolated from scat samples compared with ear tissue samples (Wedrowicz *et al.* 2016), suggesting that scats are a particularly good sample type for the detection of KoRV-A provirus in Victorian koalas.

The remnant koala population in South Gippsland, not derived from the translocation of island individuals, is genetically diverse and unique in Victoria (Lee *et al.* 2012; Wedrowicz *et al.* 2018a). The prevalence of *C. pecorum* and KoRV-A in this koala population is not well known. This study of South Gippsland's wild remnant koala population used non-invasive genetic sampling to: (1) obtain baseline data for *C. pecorum* and KoRV-A prevalence and distribution; (2) identify *C. pecorum* and KoRV-A genotypes present; and (3) compare prevalence between wild sampled koalas and sick or injured koalas admitted to a local wildlife shelter.

Methods

Sample collection

The focal study area was the South Gippsland region in Victoria, Australia, which covers an area of around 6000 km² (Figs 1, 2). Within this area, around 700 km² is covered by vegetation types likely to contain eucalypts preferred by the local koala population (DELWP 2017), but this habitat is extremely fragmented. Koala scats were also sampled at other sites in Victoria, including Raymond Island (koalas of Phillip Island origin), Cape Otway (koalas of French Island origin), Mallacoota (koalas of French Island origin) and interstate koala populations from south-east New South Wales (SENSW), north-east New South Wales (NENSW) and south-east Queensland (SEQld) (Fig. 1). Population density of both the Raymond Island and Cape Otway koala populations is considered to be high (Menkhorst 2008; Whisson *et al.* 2016).

Shelter animals admitted to the Southern Ash Wildlife Shelter (SAWS) in Gippsland (for a variety of reasons, e.g. road trauma, dog attacks and illness) were also sampled. Shelter koalas included individuals originating from both South Gippsland and Central Gippsland. The history of the Central Gippsland koala population is not well known, but koala translocations from French and Snake (koalas of both

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Fig. 1. Prevalence of infection with (a) *Chlamydia pecorum* and (b) koala retrovirus subgroup A (KoRV-A) provirus detected in DNA isolated from koala scats. The distribution of the koala is shaded (adapted from Department of the Environment 2015).

French and Phillip Island origin) Islands to the area are known to have occurred (Martin 1989; Emmin 1996). The population is most probably supplemented by individuals released or escaping from the local wildlife shelter, which takes in koalas 5 from across Victoria.

Scat samples were collected following the protocol described by Wedrowicz *et al.* (2013), where scats that were deemed sufficiently fresh (i.e. scats were intact, had a shiny outer coating and were unlikely to have been exposed to rain) 10 were collected and stored on toothpicks inserted into the side of the scat. Spatial data for scat samples collected from wild

populations were recorded using a handheld GPS. Tissue samples ($n = 44$, collected by SAWS) from individuals which had been euthanased, usually without being admitted, were also analysed for the presence of KoRV-A.

DNA isolation

The surface of each scat was washed in phosphate-buffered saline and DNA was isolated from the wash using either the QIAamp DNA Stool Mini Kit (for some samples; Qiagen, www.qiagen.com) as described in Wedrowicz *et al.* (2013), or the

Fig. 2. Prevalence of (a) *Chlamydia pecorum* and (b) koala retrovirus subgroup A (KoRV-A) provirus in South Gippsland according to koala population cluster, as inferred by Wedrowicz *et al.* (2018a). Genotype-determined population clusters made up of more than six individual koalas are numbered from 1 to 6. Population clusters 4 and 5 are in areas of relatively high koala density (one koala per 4 ha), while surrounding areas have much lower (but undefined) koala densities. Points on the map represent the location of wild sampled koalas. Unshaded areas of the map represent regions not sampled.

AxyPrep MAG Soil, Stool and Water DNA Kit (for others; Axygen, www.fisherbiotec.com.au) as described in Wedrowicz *et al.* (2018b). DNA was isolated from tissue samples using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol.

Microsatellite genotyping of koala DNA

Microsatellite genotype data collected as part of another study (Wedrowicz *et al.* 2018a) were used to identify koalas that were sampled more than once. Duplicate samples from the same individual were removed so that each DNA isolate used 5

here for pathogen screening represented an individual koala. To minimise the chance of false negatives due to poor DNA quantity and/or quality in samples, only DNA isolates producing a microsatellite genotype with at least eight positive loci were used for pathogen screening.

Sample sets for C. pecorum and KoRV-A screening

After selection of high quality samples and removal of duplicate individuals, 336 samples were screened for *C. pecorum* and 263 samples screened for KoRV-A. The two sample sets were discrete, but contained some overlap: 121 samples (individual koalas) from the wild South Gippsland population were screened for the presence of both pathogens.

The *C. pecorum* study involved isolates from 336 unique individuals, comprising 176 wild South Gippsland koalas and 160 koalas from reference populations. Victorian reference samples were obtained from Raymond Island ($n = 26$), Cape Otway ($n = 41$), Mallacoota ($n = 5$) and shelter individuals from South Gippsland ($n = 22$). Interstate reference samples were obtained from south-east New South Wales ($n = 12$) and south-east Queensland ($n = 13$) (Fig. 1a).

The KoRV-A study involved isolates from 281 unique individuals, including 142 wild South Gippsland koalas. Victorian reference samples were obtained from Raymond Island ($n = 18$), Cape Otway ($n = 11$), Mallacoota ($n = 3$) and shelter individuals from Central Gippsland ($n = 17$) and South Gippsland ($n = 61$), while interstate populations sampled included coastal and inland regions of south-east New South Wales ($n = 12$) and shelter individuals from north-east New South Wales ($n = 17$) (Fig. 1b).

Detection of C. pecorum and classification of ompA sequences

DNA isolates were screened for the presence of *C. pecorum* using a real time PCR assay targeting a 76 base pair (bp) region of the *Chlamydia* outer membrane protein A gene (*ompA*; Pantchev *et al.* 2010), including TaqMan Exogenous Internal Positive Control (IPC) Reagents (Applied Biosystems, www.thermofisher.com). Amplification was carried out using a presence-absence protocol on the Applied Biosystems Step One Plus instrument (Wedrowicz *et al.* 2016). For a randomly selected subset of positive isolates ($n = 61$), ~1140 bp of the *C. pecorum ompA* gene was amplified and sequenced as described in Wedrowicz *et al.* (2016).

Sequences with greater than 1% nucleotide difference from previously described genotypes were considered a new genotype and designated a new letter as per Kollipara *et al.* (2013). If the *ompA* sequence had nucleotide differences of less than 1%, sequences were classified as the same genotype (using the same letter) followed by a prime symbol. When several genotypes were detected with less than 1% difference in nucleotide sequence, the genotype letter was designated with both a number and a prime symbol (e.g. B, B⁰1, B⁰2 etc.) and referred to as a genotype variant.

KoRV-A PCR and sequencing

Because previous screening for KoRV genotypes A and B across Victoria indicated that only KoRV-A was present

(Legione *et al.* 2017), samples were only screened for KoRV-A in this study. Infection with KoRV-A was determined using standard PCR as described in Wedrowicz *et al.* (2016). Standard PCRs utilised KoRV-A specific primers published in Xu *et al.* (2013) alongside koala β -actin primers (Markey *et al.* 2007) to confirm the presence of koala DNA. To investigate potential genetic variants of KoRV-A across broad sample areas, a 1115 bp region of the KoRV-A *env* gene was amplified for a subset of KoRV-A positive samples ($n = 17$), using primers KoRV-*env*1-F (5'-AGACGGGAAGTGTCTGTT TGG-3') and KoRV-*env*1-R (5'-GGGGGTGAGGCCAGAAT TAC-3') (see Wedrowicz *et al.* (2016) for PCR details). Sequences were subsequently aligned and compared using MEGA 6 (Tamura *et al.* 2013).

Pathogen prevalence between local population clusters

The fine-scale population structure of the South Gippsland koala population as inferred by Wedrowicz *et al.* (2018a) was used here to investigate potential differences in pathogen prevalence between genotype-determined koala population clusters within South Gippsland. The natural breaks (Jenks) method in ArcGIS (ESRI, Redlands, CA) was used to categorise the prevalence of infection for each population cluster into three categories.

Statistical analysis

Comparisons between rates of infection were conducted with Pearson's chi-square test for count data using the *stats* package within the R software environment (R Core Team 2014).

Results

Chlamydia pecorum

Prevalence of C. pecorum

C. pecorum was detected in nearly half (49%) of all individuals tested (166/336). In South Gippsland, *C. pecorum* was detected in 61% (107/176) of individuals sampled (Fig. 1a). The prevalence of infection in Raymond Island individuals, founded by Phillip Island stock, was high, 81% (21/26), whereas the prevalence of *C. pecorum* was much lower in populations founded by French island translocations: 4.9% (2/41) at Cape Otway and not detected in any of the five individuals sampled from Mallacoota. The prevalence of *C. pecorum* was 38% (5/13) and 27% (3/11) in NENSW and SEQld respectively, which was lower than the prevalence in South Gippsland, although sample sizes were small.

Association between koala density and C. pecorum infection in South Gippsland

The prevalence of infection according to fine-scale population structure in South Gippsland is shown in Fig. 2. Within South Gippsland there are two regions where population density is higher than in surrounding areas, having a population density of approximately one koala per 4 ha (Allen 2015; R. Appleton, HVP, pers. comm.). Elsewhere in South Gippsland, population densities are not well defined but are substantially lower (R. Appleton, HVP, pers. comm.). These areas of higher population density correspond to

population clusters 4 and 5 in Fig. 2. We grouped genotype-determined population clusters according to low or high koala density and compared prevalence. The prevalence of *C. pecorum* infection was significantly greater ($P = 0.0004$) in areas of high koala density (Fig. 2a, regions 4 and 5) compared with surrounding areas, where koala densities are lower (Fig. 2a, all other regions); infection rates were 77% (51/66) and 49% (52/106), respectively.

ompA diversity

ompA gene sequence data were obtained for 61 samples confirmed positive for *C. pecorum* using real time PCR.

In South Gippsland, six *ompA* genotypes were detected (Fig. 3a; Table 2): F (42%), B (23%), M (21%), C (9%), I (2%) and one novel genotype designated genotype O (2%). Previously unreported genotype variants were also found in South Gippsland. These included two variants of genotype C (C⁰1 and C⁰2) that differed from genotype C reported by Legione et al. (2016b) by one and two bases respectively (amino acid sequences of all three C genotypes were identical). Three group B genotype variants (B⁰4 B⁰6) and five F genotype variants (F⁰3 F⁰7) were also detected. All B⁰10 and F genotype variants had differing protein sequences, with three to six amino acid differences between the B variants and two to four amino acid differences between the F

Fig. 3. Distribution of genotypes for (a) *Chlamydia pecorum* (*ompA*) and (b) koala retrovirus subgroup A (KoRV-A) provirus (*env*) detected in this study.

variants. Another *ompA* sequence with seven base pair differences to genotype I (Kollipara *et al.* 2013) was detected and specified as genotype variant I⁰ (seven amino acid differences were also present). *C. pecorum ompA* sequence data generated in this study are available under GenBank accession numbers KY913821–KY913837.

The six *ompA* genotypes (B, C, F, I, M and O) and multiple genotype variants (within genotypes B, C and F) found in South Gippsland contrasted with the limited diversity detected

in other Victorian populations; all sequenced Raymond Island samples had *ompA* genotype B⁰1 or B⁰4 (which differ from *ompA* genotype B by two and one base pairs, respectively), while genotype L was detected in both positive Cape Otway samples (Fig. 3a).

15 KoRV

Prevalence of KoRV-A

The proportion of individuals testing positive for KoRV-A in sampled populations was variable (Fig. 1b). The incidence

of KoRV-A infection in wild Victorian populations ranged from 18% (2/11) at Cape Otway to 27% (39/142) in the South Gippsland population; these differences were not significant. All individuals tested in SENSW (12/12) were found to be KoRV-A positive (Fig. 1b), representing the most 5 southern koala population identified to date, with an apparent KoRV prevalence of 100%.

Animals entering shelters tended to have a higher prevalence of KoRV-A than wild animals from the same region (Fig. 1b). In South Gippsland, KoRV-A was detected in 41% (25/61) of 10 shelter animals, compared with 27% (39/142) of individuals sampled in the wild ($P = 0.08$), whereas 76% (13/17) of shelter koalas originating from Central Gippsland were KoRV-A positive (Fig. 1b). The difference in prevalence between shelter animals from South Gippsland (41%, $n = 61$) and 15 shelter animals from Central Gippsland (76%, $n = 17$) was significant ($P = 0.02$). Within Victorian shelter animals, KoRV-A was detected at similar rates in both scats (86% CG; 33% SG) and tissues (70% CG; 47% SG) from separate sets of individuals, indicating comparable detection rates between 20 sample types (CG: $\chi^2 = 0.03$, $P = 0.86$, SG: $\chi^2 = 0.67$, $P = 0.42$).

Table 2. Summary of the *Chlamydia pecorum ompA* genotypes detected in this study

SG, South Gippsland; RI, Raymond Island; OTW, Cape Otway; SENSW, South East New South Wales; SEQld, South East Queensland. DNA sequences are available under GenBank accession numbers KY913821–KY913837

<i>ompA</i> genotype group	<i>ompA</i> genotype variant	SG	RI	OTW	SENSW	SEQld
A	A ⁰ 1	–	–	–	–	1
B	B ⁰ 1	–	10	–	–	–
	B ⁰ 4	–	2	–	–	–
	B ⁰ 5	9	–	–	–	–
	B ⁰ 6	1	–	–	–	–
C	C ⁰ 1	3	–	–	–	–
	C ⁰ 2	1	–	–	–	–
F	F	–	–	–	1	2
	F ⁰ 3	11	–	–	–	–
	F ⁰ 4	4	–	–	–	–
	F ⁰ 5	1	–	–	–	–
	F ⁰ 6	1	–	–	–	–
	F ⁰ 7	1	–	–	–	–
I	I ⁰	1	–	–	–	–
L	L	–	–	2	–	–
M	M	9	–	–	–	–
O	O	1	–	–	–	–

The association between koala density and KoRV-A infection in South Gippsland

The fine-scale distribution of KoRV-A infection in wild South Gippsland koala population clusters is demonstrated in 25 Fig. 2b. Unlike *C. pecorum*, KoRV-A prevalence in the wild

South Gippsland koala population was not strongly related to population density. KoRV-A prevalence was similar in areas of relatively high koala density (Fig. 2b, population clusters 4 and 5; 31% positive, $n = 55$) and areas of lower koala density 30 (Fig. 2b, all population clusters except 4 and 5; 26% positive, $n = 84$; $\chi^2 = 0.05$, $P = 0.82$).

KoRV-A env genotypes

DNA sequencing identified three KoRV-A *env* genotypes: KV01, previously identified by Hanger *et al.* (2000) (GenBank 35 AF151794.2), was detected in samples from northern NSW and in Victorian samples originating from both Central Gippsland ($n = 1$) and South Gippsland ($n = 2$); KV02 (*env* sequence one base pair different to that of KV01) was found exclusively in south-east NSW samples ($n = 4$); and KV03 40 (*env* sequence seven base pairs different to that of KV01) was found only in Victorian koalas ($n = 8$; Fig. 3b; Table 3). Sequences obtained from the KoRV-A *env* gene are available under GenBank accession numbers KY979231–KY979233.

Table 3. Variable sites in koala retrovirus subgroup A *env* sequences and the frequency with which each was detected by population SG, South Gippsland; CG, Central Gippsland; RI/OTW, Raymond Island/Cape Otway; SENSW, South East New South Wales; NENSW, North East New South Wales. DNA sequences are available under GenBank accession numbers KY979231–KY979233

	Nucleotide position								Frequency by sample group				
	46	52	82	349	403	499	613	974	SG	CG	RI/OTW	SENSW	NENSW
KV01	C	C	A	A	C	G	G	A	2/6	1/2	–	–	2/2
KV02	.	.	.	.	.	.	A	.	–	–	–	4/4	–
KV03	T	T	G	G	T	A	.	C	4/6	1/2	3/3	–	–

Prevalence of *C. pecorum* and KoRV-A by gender

In the wild South Gippsland koala population, *C. pecorum* was more common in females (74%, $n = 70$) compared with males (54%, $n = 92$; $\chi^2 = 5.9$, $P = 0.01$), while the rate of KoRV-A infection was similar between the sexes; 31% ($n = 58$) for females and 28% ($n = 72$) for males ($\chi^2 = 0.04$, $P = 0.83$).

For South Gippsland koalas admitted to the wildlife shelter, the prevalence of *C. pecorum* was similar between males (44%, $n = 9$) and females (46%, $n = 13$). Shelter males tended to have a higher rate of KoRV-A presence (48%, $n = 29$) than did shelter females (33%, $n = 27$), although the difference was not significant ($\chi^2 = 0.74$, $P = 0.39$).

Although not significant, wild females tended to have a higher prevalence of *C. pecorum* (74%, $n = 70$) than did shelter females (46%, $n = 13$, $\chi^2 = 2.9$, $P = 0.09$). *C. pecorum* was detected at similar rates for wild males (54%, $n = 92$) and shelter males (44%, $n = 9$; $\chi^2 = 0.05$, $P = 0.83$). Conversely, KoRV-A tended to be present more often in shelter males (48%, $n = 29$) compared with wild males (28%, $n = 72$), although the difference was also not significant ($\chi^2 = 3.0$, $P = 0.08$). Small shelter sample sizes may have limited the power of these comparisons.

Prevalence of individuals with both *C. pecorum* and KoRV-A

Of the 121 wild South Gippsland individuals that were tested for both *C. pecorum* and KoRV-A, neither infection was detected in 34 (28%) individuals. *C. pecorum* alone was detected in 52 (43%) individuals, KoRV-A only in 9 (7%) individuals and both *C. pecorum* and KoRV-A in 26 (21%) individuals. Rates of infection in these categories were similar for females and males, but males were more likely to be free of both infections than were females (40% and 14% respectively, $\chi^2 = 8.0$, $P = 0.005$).

Discussion

This study is one of few that assess the prevalence of *C. pecorum* and KoRV in a wild koala population. This is important because the high costs associated with traditional sampling make the study of pathogens in wild populations rare in the literature (e.g. Nyari *et al.* 2017; Quigley *et al.* 2018). This study also represents the first large-scale investigation of the prevalence and spatial distribution of *C. pecorum* and KoRV-A in the wild South Gippsland koala population, and was made possible by use of non-invasive DNA sampling from koala scats. The prevalence of infection, not the prevalence of disease, was estimated in this study. Whether individuals sampled were symptomatic or asymptomatic was not known or not recorded.

Pathogen prevalence

Kolipara *et al.* (2013) found *C. pecorum* prevalence to range between 20% and 61% in wild populations in Queensland and New South Wales, with genotype F being the most widespread. In Victoria, *C. pecorum* is present in all populations descended from Phillip Island individuals, while French Island koalas, and populations solely established by individuals translocated from French Island, are often considered 'Chlamydia free', although very low levels of infection have been reported (Emmin 1996; Legione *et al.* 2016a, 2016b). Legione *et al.* (2016b) found

C. pecorum prevalence ranged from 1% to 46%, in wild-caught Victorian koala populations, with genotype B the dominant strain in western Victoria and Raymond Island (Phillip Island-derived), and genotypes C and F most common in Gippsland. In South Australia, genotypes B and G have been detected in the Mount Lofty Ranges (Kolipara *et al.* 2013), with urogenital *C. pecorum* reported at 60% (Speight *et al.* 2016) and 90% (Polkinghorne *et al.* 2013). The prevalence of *C. pecorum* in the Kangaroo Island population of South Australia, initially established by French Island individuals, is reported to be 0% (Polkinghorne *et al.* 2013).

Levels of infection with *C. pecorum* are high within South Gippsland koalas (overall 61%), ranging from 49% in areas of low koala density to 77% in areas of high koala density. Severe chlamydial disease is thought to be more common in koalas from Queensland and New South Wales than in those in Victoria and South Australia (EaCRC 2011a). Except for populations derived from French Island individuals (i.e. Cape Otway and Mallacoota in Victoria), where the prevalence of *C. pecorum* is very low (Emmin 1996; Legione *et al.* 2016a, 2016b), this does not appear to be due to a lower prevalence of infection. A recent study of 160 koalas in south-east Queensland found a *C. pecorum* infection rate of 31% (Nyari *et al.* 2017). This study shows that the prevalence of *C. pecorum* infection in some Victorian koala populations (South Gippsland and Raymond Island) is not lower than that in northern koala populations.

Koala populations in which all sampled animals test positive for KoRV-A provirus have been identified in northern NSW and Queensland (Simmons *et al.* 2012). Similarly, all koalas tested from populations in south-east NSW in this study were found to be KoRV-A positive. Rates of infection for free-ranging populations at Cape Otway and Raymond Island recorded in this study were low (18% and 22% respectively), in close agreement with those reported by Legione *et al.* (2017). The prevalence of KoRV-A determined in this study for the Raymond Island population using scats (4/18) is also comparable to that determined using blood samples from wild individuals by Simmons *et al.* (2012).

KoRV-A prevalence in South Gippsland was previously reported to be as high as 69% (Simmons *et al.* 2012), which is significantly higher than the overall prevalence determined for South Gippsland in this study (27%, $P < 0.0005$). However, Simmons *et al.* (2012) used samples collected opportunistically, primarily from dead animals, so this difference may be explained by the potential for KoRV-A positive individuals to be overrepresented in shelter or road-killed samples.

In South Gippsland, males and females were equally likely to be infected with either or both pathogens, whereas male koalas were more likely to be pathogen free than female koalas. This could suggest increased rates of mortality for male koalas infected simultaneously with both pathogens, resulting in a greater number of living males being free of both infections. Males from the South Gippsland shelter population also showed a tendency towards higher rates of KoRV-A infection compared with shelter females. One possible explanation for these observations is that in Victoria, KoRV-A may have greater virulence in males compared with females. Though transmission pathways for KoRV are not well known, it is suggested that

vertical transmission of KoRV to young joeys may also occur via ingestion of pap from the mother (Eiden and Taliaferro 2011). Modelling shows that for pathogens transmitted both horizontally and vertically (postnatally from the mother), the evolution of lowered virulence in female hosts is favoured (Úbeda and Jansen 2016). Further work will be required to explore the significance of these findings.

The effect of koala population density on *C. pecorum* and KoRV-A prevalence

- The prevalence of pathogenic infections in host populations is often related to population density (May and Anderson 1979). Koala population density appears to play a role in the prevalence of infection in the South Gippsland region; koalas in high-density areas are three times more likely to carry *C. pecorum* than those in lower density areas (Fig. 2a). An association with high population density could also explain the high proportion of individuals carrying *C. pecorum* on Raymond Island. In contrast, while the density of koalas at Cape Otway is also very high, estimated at around 18 individuals per hectare (Whisson *et al.* 2016), *C. pecorum* was detected in only two individuals from the 41 sampled. This may reflect a very recent introduction of the bacteria to the Cape Otway population, a difference in the strain's (genotype L) pathogenicity or mode of transmission and/or differences in host population susceptibility. Decreased susceptibility in the Cape Otway population to *C. pecorum* is unlikely because rapid dissemination of *Chlamydia* has been recorded in translocated French Island koalas (from which the Cape Otway population was established; Santamaria and Schlagloth 2016), suggesting that low pathogenicity of genotype L and/or its recent introduction are more probable. In the Santamaria and Schlagloth (2016) study, French Island individuals were translocated to an area where the resident population was *Chlamydia* positive. On release, all French Island koalas were *Chlamydia* free, but almost all (16/17) originally *Chlamydia*-negative animals tested positive for chlamydial antibodies after 19 months; the *ompA* genotype(s) present were not identified.

- In contrast to *C. pecorum*, the prevalence of KoRV-A did not appear to be influenced by population density in South Gippsland. Prevalence was similar in areas of relatively high koala density (Fig. 2b, population clusters 4 and 5; 31% positive, $n = 55$) and areas of lower koala density (Fig. 2b, all population clusters except 4 and 5; 26% positive, $n = 84$). This may suggest that exogenous KoRV-A found in Victorian koalas is not easily transmitted among individuals. This hypothesis is supported by observations that KoRV-A infection rates in Victorian koalas have remained stable over a period of about 3 years, with KoRV-A prevalence on French and Raymond Islands consistently reported at 20–30% (Simmons *et al.* 2012; Legione *et al.* 2017). Additionally, a study of exogenous KoRV-B infection in a wild Queensland koala population indicated a relatively low horizontal transmission rate of 3% per year (Quigley *et al.* 2018).

Pathogen genotypes

- Apart from one novel genotype (O), all other genotypes detected in this study are identical or near identical to genotypes previously reported from UGT samples (Kollipara *et al.* 2013;

Legione *et al.* 2016b) showing that the same strains of koala *C. pecorum* detected in UGT samples are detected in DNA isolated from scats.

In contrast to the single genotypes detected in the two island derived populations sampled for this study, six *C. pecorum ompA* 5 genotypes were detected in South Gippsland koalas (Fig. 3a). The single genotype (B) detected on Raymond Island may have in part resulted from the history of this population, having been subjected to a genetic bottleneck in the late 1800s (when the Phillip Island population was initially established), and again 10 with the introduction of individuals to Raymond Island. Similarly, the very low prevalence of *C. pecorum* in the Cape Otway koala population (which was established by translocated French Island koalas; Legione *et al.* 2016a), could be attributed to the transfer of *Chlamydia*-free individuals to French Island in the 15 late 1800s, resulting in a population free of the pathogen (Martin and Handasyde 1999).

In total, 15 *C. pecorum ompA* genotypes have been detected in koala populations across Australia to date (Table 1; Jackson *et al.* 1997; Higgins *et al.* 2012; Kollipara *et al.* 2013; Legione 20 *et al.* 2016b). Apart from *ompA* genotype F, which has been detected in koalas from Queensland, New South Wales and Victoria, and genotype G, which has been detected in both Queensland and South Australia, other *ompA* genotypes appear specific to a given region (Table 1). One possible 25 explanation for the observed distribution of *C. pecorum* genotypes could be the introduction of the pathogen from numerous separate sources. Some *ompA* genotypes are found to be highly similar to those of *C. pecorum* strains found in agricultural animals (Kollipara *et al.* 2013; Legione *et al.* 30 2016b). Multi-locus sequence typing of *C. pecorum* DNA has demonstrated a close relationship between certain *C. pecorum* strains infecting koalas and livestock, indicating the potential for cross-species transmission and the possibility that *C. pecorum* was originally (and possibly repeatedly) introduced to koala 35 populations from livestock (Jelocnik *et al.* 2013).

Knowledge of the different pathogen genotypes present in a population may be important when considering management actions such as animal translocation. Virulence of *C. pecorum* strains infecting koalas and other animal hosts may vary 40 (Mohamad *et al.* 2014; Nyari *et al.* 2017), and the severity of disease may differ depending on the health of the individual animal or the presence of environmental stressors that may impact health and therefore disease susceptibility (Timms 2005; Lunney *et al.* 2012; McAlpine *et al.* 2017). The 45 potential for exposure to new chlamydial strains that may have negative health impacts is an important consideration for proposed translocations. For this reason, it is important to return rehabilitated individuals to their location of origin. Quarantining animals from differing locations at wildlife 50 hospitals is also important to prevent transmission of pathogens among asymptomatic individuals, who may then disseminate foreign strains throughout their home range upon release.

The three KoRV *env* genotypes identified in this study corresponded broadly with sampling location, with KV01 55 being from northern NSW (but also found in Victoria), KV02 from southern NSW and KV03 from Victoria. All base changes between the three *env* genotypes were synonymous, except for nucleotide 974 in *env* genotype KV03 (Table 3).

The nucleotide change at site 974 from A to C resulted in an amino acid substitution from asparagine in K01 to histidine in K03, changing the amino acid sequence at sites 324–326 from asparagine–alanine–serine in K01 to histidine–alanine–serine in K03. The asparagine–alanine–serine motif is associated with *N*-linked glycosylation of the asparagine residue (Gavel and Heijne 1990); the asparagine–histidine substitution at this site might, therefore, have resulted in the loss of a glycosylated site in K03. Glycosylation plays a role in the pathogenesis of many viruses (Vigerust and Shepherd 2007), so this change may have altered the viral infectivity of KoRV-A in Victoria, and could potentially help to explain the differing prevalence rates observed between koala populations in Queensland and New South Wales (100% prevalence) and Victoria and South Australia (<100% prevalence).

Comparison of wild and shelter koalas

Almost all shelter individuals tested for *C. pecorum* originated from areas of low koala density, where habitat is highly fragmented and intersected by farmland, towns and major roads. *C. pecorum* was detected in 45% of the wildlife shelter koala population while prevalence for *C. pecorum* in the low density areas of South Gippsland was 49%, indicating a similar rate of infection in both shelter and wild populations. Similar prevalence of *C. pecorum* in both shelter koalas and wild koalas suggests that *C. pecorum* may not be a major cause of mortality in the South Gippsland koala population. This result should, however, be treated with caution because scat samples were only obtained from koalas deemed fit for rehabilitation by the wildlife shelter; euthanased individuals were not sampled. KoRV-A was detected in 41% of shelter individuals originating from South Gippsland and in 27% of wild individuals within South Gippsland. Higher prevalence of KoRV-A in shelter animals compared with wild koalas suggests that KoRV-A is a potential contributor to mortality in the South Gippsland koala population. Differences in KoRV-A prevalence between shelter and wild animals may reflect negative impacts of KoRV-A on koala health and, subsequently, an over-representation of KoRV-A positive individuals in Victorian koalas affected by road trauma, requiring veterinary treatment or admitted to shelters. This is supported by Legione *et al.* (2017), who found that koalas in poor health (with low body condition scores) were seven times more likely to be KoRV-A positive than healthy koalas. Further sampling of shelter koalas, along with collection of admission data, such as blood biochemistry, diagnosis and body condition score, would be useful to gain further insight into the effects of KoRV-A on Victorian koalas.

Future directions

McCallum *et al.* (2017) point out a lack of long-term population monitoring and suggest that the relationship between stress and clinical disease may only be clarified by monitoring koalas over time for levels of stress, infection and clinical disease. The ability to obtain a range of information from scat samples, including a unique identifying genotype for individual koalas (Wedrowicz *et al.* 2013) and infection status for *C. pecorum* and KoRV-A (Wedrowicz *et al.* 2016), provides

a relatively inexpensive means by which populations may be monitored long-term. Although a presence–absence protocol was used to detect *C. pecorum* and KoRV-A provirus in this study, future studies could be designed to quantify the relative amounts of pathogen for each individual sampled. Coupling the methods mentioned above with faecal cortisol estimations of stress (Narayan *et al.* 2013), as well as observational data such as fertility rates (e.g. counts of back young) or the presence of ‘wet bottom’, would minimise animal stress and potential risks to the animal and also reduce costs, potentially allowing the acquisition of larger datasets.

Conclusions

This study highlights the usefulness of non-invasive genetic sampling of koala scats, permitting a comprehensive survey of *C. pecorum* and KoRV-A prevalence in the South Gippsland koala population. Further investigations may indicate the level of impact of these pathogens on koala populations and facilitate the timely implementation of appropriate strategies to limit potential negative impacts. Given the high prevalence and strain diversity of *C. pecorum* throughout the South Gippsland region, and the likelihood of increasing environmental pressures in the future (e.g. climatic changes or habitat shifts; Adams-Hosking *et al.* 2011; González-Orozco *et al.* 2016), the incidence and/or severity of overt disease may increase in the region over the coming years. The indication that KoRV-A infection may be having a negative impact on koalas is also of concern. Due to the conservation importance of the diverse remnant koala population in South Gippsland, monitoring of these infections in the region over time is vital.

Data accessibility

Genotypic data used for this study can be accessed from figshare.com: https://figshare.com/articles/Koala_database_sample_details_and_genetic_data/5656120.

Conflicts of interest

The authors declare no conflicts of interest.

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5 Ethics Committee (AEC No. GIPP/2011/03).

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