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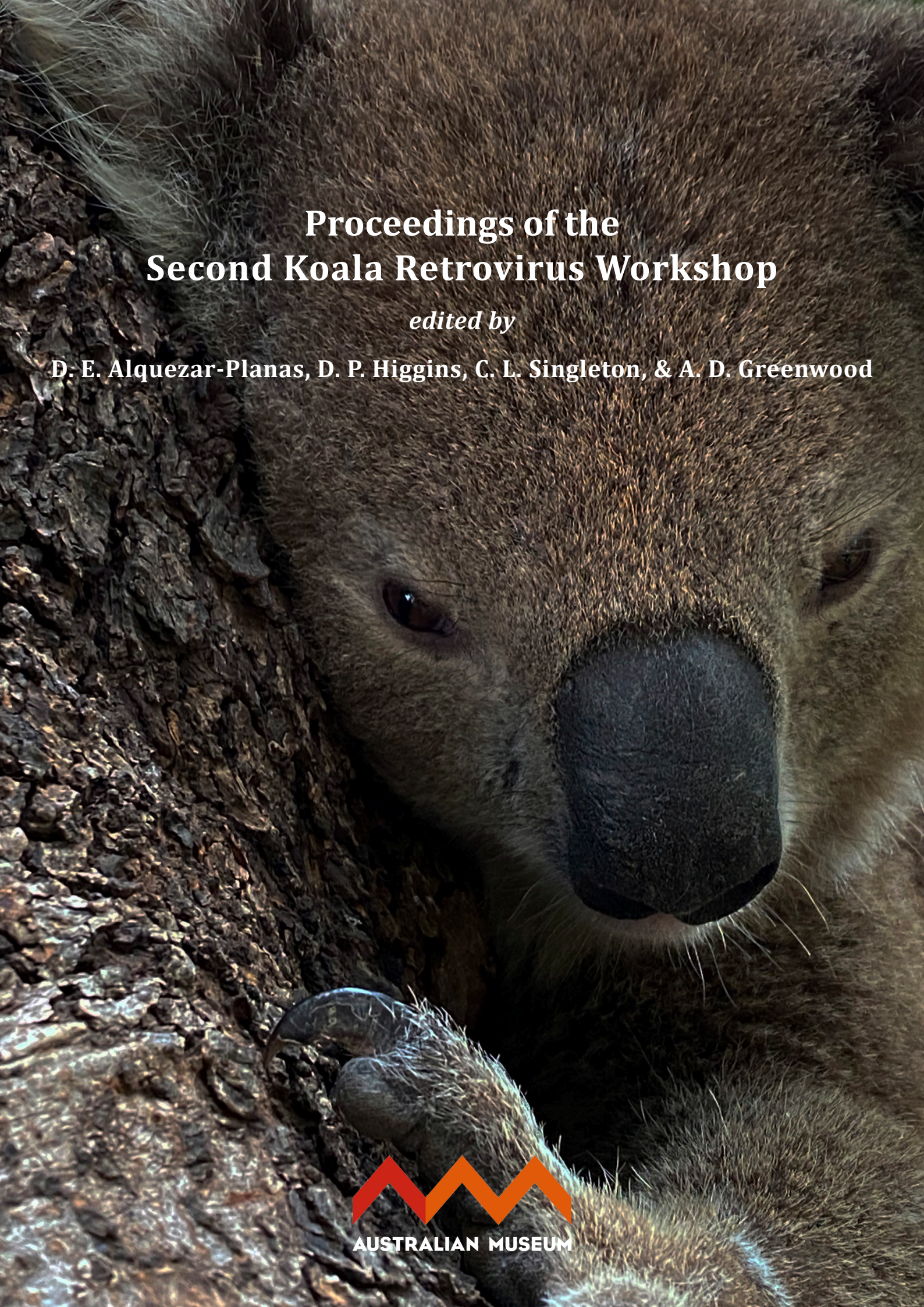

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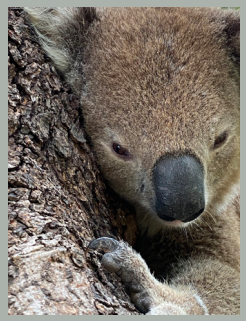

Cover photo
by Damien P. Higgins

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Endogenous and Exogenous Koala Retrovirus Patterns in Wild Koalas across Australia

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Abstract. Our understanding of koala retrovirus (KoRV) has advanced dramatically in recent years. Cross-sectional studies examining hundreds of wild koalas (*Phascolarctos cinereus*) from populations across their natural Australian range (Queensland–New South Wales–Victoria) have shed new light on KoRV abundance and diversity in the wild. A single strain of KoRV (the originally characterized Hanger strain from 2000) appears to be the dominant KoRV strain within koalas, endogenous in northern populations and the predominant exogenous strain in southern populations. Alongside this strain are potentially exogenous variants representing both intact and defective versions of some of the many recognized KoRV subtypes (KoRV-A to KoRV-M). The patterns of these may suggest a transition from endogenous KoRV in the north to exogenous KoRV in the south, occurring in southern New South Wales. They also highlight how actively the hypervariable region of the envelope gene of KoRV is diversifying, with fragmented koala populations across the country containing unique and distinctive KoRV proviral profiles. As more koala populations are examined with increasingly sensitive and specific genetic tools, our understanding of KoRV is poised to continue to evolve as quickly as the virus itself.

Introduction

Koala retrovirus (KoRV) is known to exist both endogenously and potentially exogenously in koalas (*Phascolarctos cinereus*) (Hanger *et al*., 2000; Quigley & Timms, 2020). At some point in the last 49,900 years, KoRV began endogenizing or permanently incorporating its provirus into koala germline genomes in the northern Australian koala population (Tarlinton *et al*., 2006; Ishida *et al*., 2015). In parallel, within almost all koala populations across Australia, potentially exogenous strains of KoRV have continued to diversify into 13 recognized subtypes (KoRV-A to -M, based on differences in the receptor binding domain region of the envelope gene (Shojima *et al*., 2013; Xu *et al*., 2013; Xu *et al*., 2015; Chappell *et al*., 2017; Blyton *et al*., 2021). Targeted studies of both endogenous and exogenous KoRV strains in recent years have led to impressive advances in our understanding of this virus across the natural koala range in Australia (Table 1).

Endogenous KoRV-A

KoRV-A is the original and most prevalent subtype of KoRV detected across Australia (Hanger *et al*., 2000; Chappell *et al*., 2017; Quigley & Timms, 2020). Genetic analysis identified KoRV-A provirus to be present in northern Australian koalas in a pattern consistent with it being endogenously incorporated into their genomes (Tarlinton *et al*., 2006). Additional studies have supported this endogenous status with quantified KoRV provirus within Queensland and northern New South Wales koala cells at levels at or above one copy per cell, with the majority of provirus being KoRV-A (Simmons *et al*., 2012; Hobbs *et al*., 2017; Sarker *et al*., 2020; Quigley, Wedrowicz, *et al*., 2021).

Recent examination of KoRV proviral strains across Australia has revealed that every KoRV positive koala examined, from anywhere in Australia, contained a single dominant KoRV proviral sequence, identical to the originally published Hanger *et al.* (2000) KoRV sequence (accession

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Table 1. Summary of endogenous and exogenous koala retrovirus (KoRV) across Australia.

	Endogenous KoRV-A	Exogenous KoRV-A	Exogenous other subtypes (B to M)
General	Hanger *et al*., 2000 strain AF151794.2, represents 8–96% of KoRV provirus detected in positive koalas	KoRV-A variants, containing both intact and defective envelope genes, also detected in KoRV positive koalas. Defective variants appear uniformly abundant across Australia.	Generally represent ≤ 2% of total provirus detected in positive koalas, with each subtype detected at << 0.1 proviral copies/cell
Queensland and northern NSW	All koalas tested KoRV-A positive, provirus detected at ≥ 1 copies/cell	Intact non-Hanger KoRV-A strains represent < 0.1% of KoRV-A	Greatest diversity of subtype strains detected within individual koalas
Southern New South Wales	All koalas tested KoRV-A positive, but provirus levels not suggestive of endogenization (KoRV-A provirus detected at ~0.2 copies/cell)	Non-Hanger KoRV-A strains becoming more abundant	
Victoria	Not all koalas KoRV-A positive, KoRV-A provirus detected at << 0.01 copies/cell, indicating lack of endogenization	Intact non-Hanger KoRV-A strains represent up to 20% of KoRV-A	Least diversity of subtype strains detected with individual koalas

number AF151794.2) (Quigley, Wedrowicz, *et al*., 2021). This strain, which contains an attenuated CETAG Env protein motif (Oliveira *et al*., 2007), was detected as 87–96% of all the KoRV provirus in Queensland and northern New South Wales koalas (Quigley, Melzer *et al*., 2021; Quigley, Wedrowicz, *et al*., 2021). This strongly suggested that the Hanger KoRV-A strain is the endogenous strain in Queensland and northern New South Wales koala populations.

Exogenous KoRV-A

In contrast to northern koalas, the evidence to date suggests that endogenization of KoRV-A is absent or at least very rare in southern koalas. Both presence/absence and quantification studies of KoRV in southern New South Wales and Victorian koala populations have detected koalas that appear to be KoRV negative, with KoRV proviral levels much less than one copy per cell when present (Simmons *et al*., 2012; Wedrowicz *et al*., 2016; Legione *et al*., 2017; Quigley, Wedrowicz, *et al*., 2021).

Detailed examination of these southern koala populations continues to find the Hanger *et al.,* 2000 KoRV-A strain to be the dominant KoRV strain present in KoRV positive koalas (Quigley, Wedrowicz, *et al*., 2021). However, the proportion of total provirus represented by this strain drops from an average of ≥ 87% in the north to only 67% of provirus per koala in the south (Quigley, Wedrowicz, *et al*., 2021). The KoRV proviral load within examined southern koalas notably contained a KoRV-A variant (A3003, accession number MN931401.1) with 15 single nucleotide polymorphisms (SNPs) when compared to the Hanger *et al.,* 2000 KoRV-A strain (Quigley, Wedrowicz, *et al*., 2021). This resulted in five non-synonymous amino acid changes in the Env protein, returning the KoRV-A A3003 variant to the more virulent CETTG motif (Oliveira *et al*., 2007)). While the KoRV-A A3003 variant was detectable in all KoRV positive koalas across Australia, A3003 abundance increased dramatically from an average of < 0.1% of provirus per koala in the north to ~21% of provirus per koala in the south (Quigley, Wedrowicz, *et al*., 2021). This data, coupled with proviral quantifications suggesting less than one in five cells per koala in southern New South Wales and less than one in a hundred koala cells per koala in Victoria contain KoRV provirus (Quigley, Wedrowicz, *et al*., 2021), is supportive though not definitive evidence that KoRV remains exogenous in these southern regions.

These detailed KoRV genetic analyses also revealed that defective KoRV variants are detectable in koalas across Australia and their abundance appears independent of endogenization status. A defective KoRV-A variant (A3002, accession number MN931400.1), which has a two base pair insertion when compared to the Hanger *et al.,* 2000 strain, creating a frameshift/stop codon in the envelope gene, was identified in every KoRV positive koala studied. Interestingly, this defective KoRV-A strain represented between 3–10% of all KoRV proviral reads detected in any koala from any part of Australia (Quigley, Wedrowicz, *et al*., 2021; Quigley, Melzer, *et al*., 2021).

Other potentially exogenous KoRV subtypes

Targeted proviral analysis continues to detect KoRV proviral variation falling under the identified subtypes KoRV-B to -M. Quantification of KoRV-B, KoRV-D, and KoRV-F proviral levels confirmed that these subtypes are present at much less than one copy per 10 koala cells, when detectable at all (Quigley, Wedrowicz, *et al*., 2021). Despite these variants composing only a small fraction (usually < 2%) of the total KoRV provirus present in any individual koala, they represent an impressive range of diversification among the koala populations studied (Quigley, Melzer, *et al*., 2021). Comparing koala populations separated by habitat fragmentation for as little as 90 years, distinct population shifts in their KoRV proviral diversity suggested that lineage diversification of KoRV is still an active process (Quigley, Melzer, *et al*., 2021).

Conclusions

As more koala populations across Australia are studied with increasingly sensitive and specific genetic tools, our understanding of KoRV will continue to evolve. Presently, it appears that most of the KoRV provirus load in koalas can be traced back to a single, dominant KoRV strain (the originally identified Hanger *et al.,* 2000 KoRV-A strain) that has endogenized into koala genomes in northern Australia and continues to circulate in southern Australia. However,

that individual strain itself may represent as many as seven distinct genome colonization events as determined by LTR variation identified (Ishida *et al*., 2015). Other defective and intact KoRV variants, encompassing all the recognized KoRV subtypes (A-M), vary in their distribution among koala populations across the country. Continued KoRV research will not only improve our understanding of this retrovirus for better koala conservation, but also expand our knowledge about the active process of diversification and endogenization of retroviruses in real time.

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