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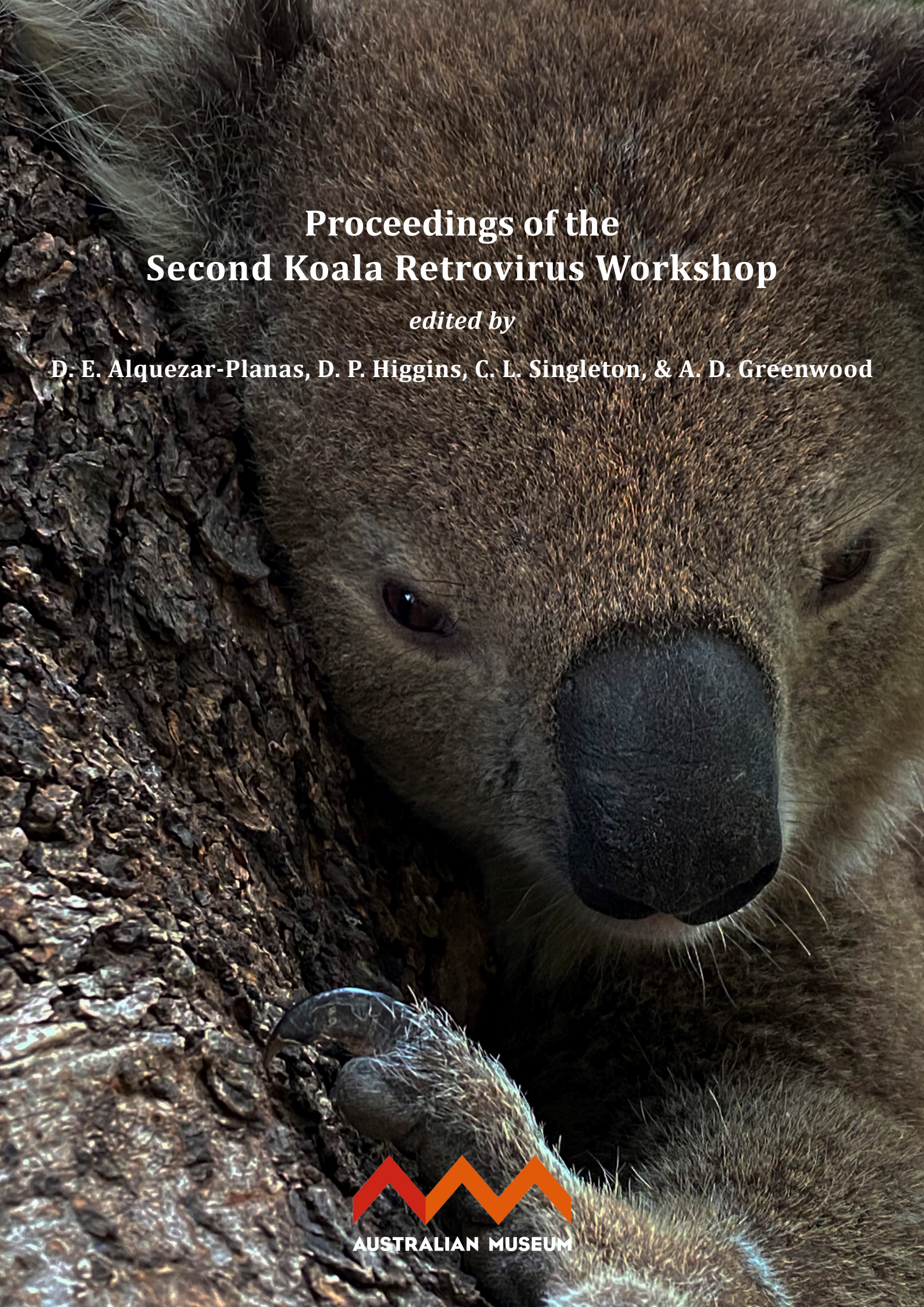

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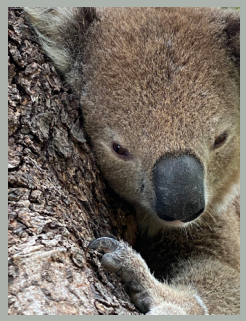

Cover photo
by Damien P. Higgins

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Bats or Rodents, Who Started it? Short History of the Gibbon Ape Leukaemia Virus–Koala Retrovirus Clade

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Abstract. The close genetic relationship between gibbon ape leukaemia virus (GALV) and koala retrovirus (KoRV) has puzzled scientists since its discovery. As the two hosts are separated geographically and taxonomically, it was hypothesized that cross-species transmission of an ancestor virus from another host into gibbons and koalas had occurred. The relatively recent introduction of KoRV into the koala genome and the apparent absence of GALV in wild gibbons suggest that this ancestor virus or a close relative may still be in circulation. Investigation into the nature of this ancestor virus may provide insights on the impact of KoRV on declining koala populations and will also broaden our understanding of host-virus coevolution. A variety of mammalian species have been identified to harbor GALV-like viruses, but the true host of the ancestral virus of KoRV and GALV remains uncertain. Here we provide a short history of the most prominent candidates: rodents and bats.

Introduction

The isolation of koala retrovirus (KoRV) in 2000 instigated one of the most intriguing mysteries in retrovirology (Hanger *et al.*, 2000). The virus had a very high sequence identity and phylogenetic relationship with gibbon ape leukaemia virus (GALV), which had been identified in captive white-handed gibbons (*Hylobates lar*) in the 1970s. The close relationship between these viruses indicated that cross-species transmission had likely occurred. However, the two species (koalas and gibbons) are evolutionarily and geographically distant (Fig. 1), thus the direct transmission of virus between the species seemed improbable. Researchers hypothesized that these viruses were introduced into each species via another host. The debates over identifying the precursor virus and the original reservoir host continue.

Based on published phylogenetic analysis of the GALV-KoRV clade, both bats and rodents are host to viruses in basal and crown positions (Greenwood *et al.*, 2018). However, what makes the rodent reservoir more prominent is the fact that 53% of all gammaretroviral-derived endogenous retroviruses (ERVs) are shown to have rodent origins (Hayward *et al.,* 2013). It has been proposed that while bats are highly capable recipients of cross-species retrovirus transmission events, rodents are more commonly the originator of these events (Cui *et al.,* 2015); for example, murine retrovirus transmission to porcine endogenous retrovirus (PERV) (Denner, 2007) and the likely tree shrew origin of *Rhinolophus ferrumequinum* retrovirus (RfRV) found in the greater horseshoe bat (Cui *et al.*, 2015).

Here we summarize the history of GALV and look into two prominent candidates for the "ancestor" of the GALV-KoRV clade: rodents and bats.

Keywords: endogenous retrovirus (ERV), cross-species transmission, koala retrovirus (KoRV), gibbon ape leukemia virus (GALV), woolly monkey virus (WMV), flying fox retrovirus (FFRV)
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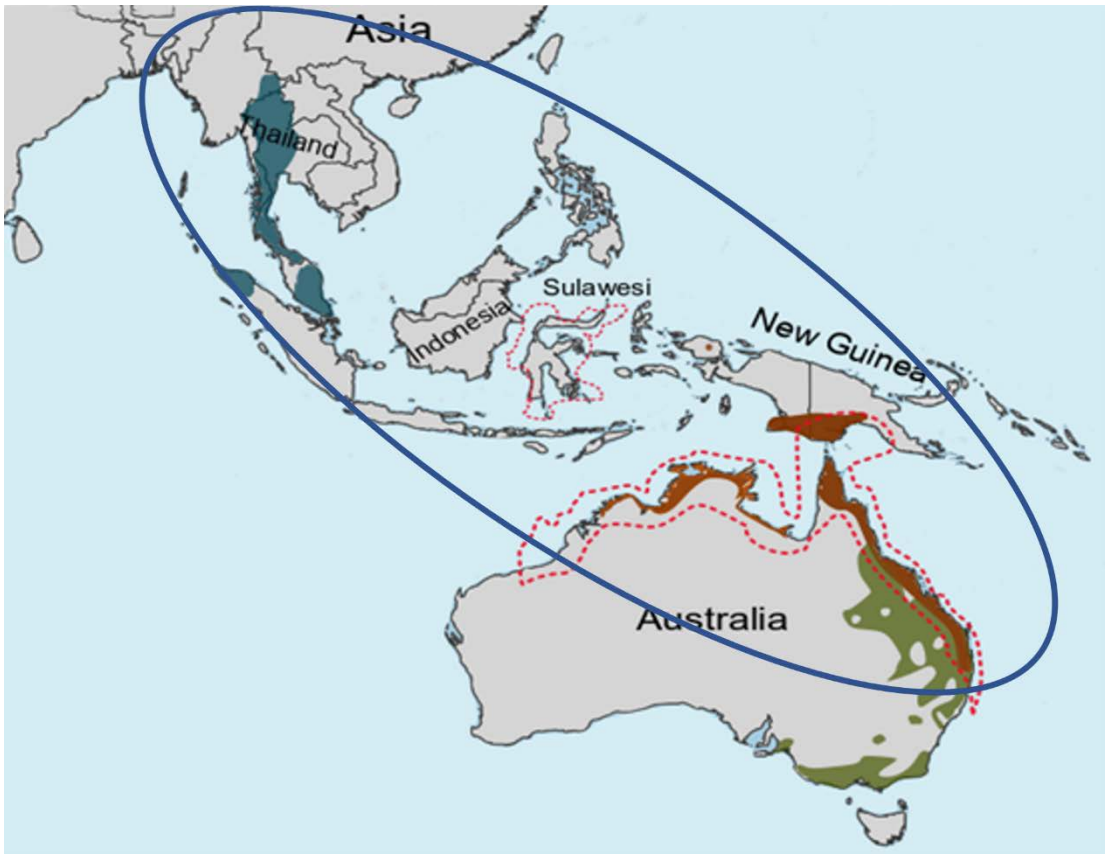

Figure 1. The approximate geographic distribution of white-handed gibbon (teal shade), *Melomys burtoni* (brown shade), koala (green shade), and *Pteropus alecto* (which includes *P. conspicillatus*) (red dotted line) that harbor GALV-SEATO, MbRV/MelWMV, KoRV and HPG/FFRV viruses, respectively. The distributions of other bat species harbouring KoRV/GALV-like viruses lie within the solid blue line, comprising *Synconycteris australis* (northern Australia, Papua New Guinea, Indonesia), *Macroglossus minimus* (northern Australia, PNG, Indonesia, SE Asia), *Hipposideros larvatus* (SE Asia, Indonesia). The distributions are based on the International Union for Conservation of Nature Red List of Threatened Species (https://www.iucnredlist.org/). The base image was generated using the free open source QGIS.

Gibbon ape leukaemia virus (GALV)

GALV is an exogenous retrovirus with oncogenic potential (Kawakami *et al.*, 1980). There are five recognized strains of GALV (Alfano *et al.*, 2016a) , including the initial isolates of GALV from cases of lymphoid neoplasia in captive gibbons at research facilities in Bangkok (GALV-SEATO) and in San Francisco (GALV-SF). The virus was subsequently detected in captive gibbons at other locations in the USA (GALV-Br) and in Bermuda (GALV-Hall's Island), and in cultured cells (GALV-X). Woolly monkey virus (WMV), which was isolated from a woolly monkey (*Legothrix lagothrica)* that had been housed with a GALV-infected gibbon, clusters phylogenetically with the five GALV strains (Alfano *et al.*, 2016a).

GALV infection (either virus or antibodies) has never been reported in wild gibbons. There has been no definitive evidence of GALV infection or GALV-induced disease in captive gibbons for almost 40 years (Brown & Tarlinton, 2017; McKee *et al.*, 2017), although a serological study in 2015 detected GALV antibodies in 21 out of 76 captive gibbons in North America (Siegal-Willott *et al.*, 2015). It has been suggested that the circulation of GALV in captive gibbons in the 1970s stemmed from an initial transmission event, mostly likely at SEATO in the mid to late 1960s, followed by transportation of gibbons from that region to research facilities in North America (Brown & Tarlinton, 2017; McKee *et al.*, 2017). The nature of the transmission event remains uncertain but was probably either iatrogenic inoculation of gibbons with material derived from humans and other species, or direct contact between gibbons and rodents, which were held in large collections at SEATO (Brown & Tarlinton, 2017).

Rodents as a plausible source or intermediate host to GALV-KoRV clade

Following the initial discovery of GALV, related retroviruses were detected in native Asian rodents, including *Mus caroli, Mus cervicolor*, *Vandeleuria oleacea* and *Mus dunni* (now *Mus terricolor*) (Lieber *et al.*, 1975; Callahan *et al.*, 1979). However, the techniques used at that time (serology and DNA hybridization) were of relatively low resolution. More recent work including sequencing, phylogenetic analysis and receptor usage of these rodent viruses has revealed that they cluster separately to the GALV-KoRV clade, and that although there is some relationship, they are not close enough to be considered the origin of GALV (Hayward *et al.*, 2013; Brown & Tarlinton, 2017).

In 2014, a novel virus sequence that clustered with GALV and KoRV was reported in a native Australian rodent, the grassland melomys (*Melomys burtoni*) (Simmons *et al.*, 2014). The *Melomys burtoni* retrovirus (MbRV) sequence was present in all 17 animals examined suggesting a likely endogenous virus. The unsuccessful attempts to isolate the virus in cell culture and the inability to detect expression of viral RNA in the animals provided further evidence of the endogenous nature of the virus.

After MbRV identification in Australia, Alfano *et al.* (2016b) screened 26 Southeast Asian rodent species. This

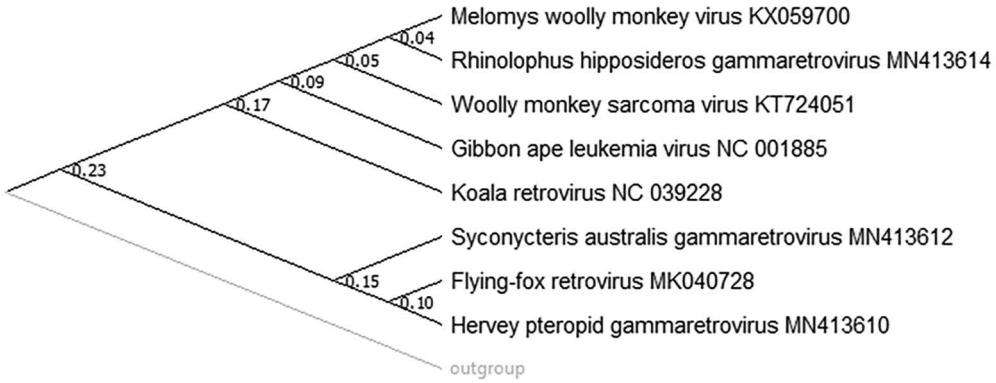

Figure 2. Time tree evolutionary analysis of representative KoRV and GALV-like retroviruses in the Australian and Asian regions. Generated using the RelTime method (Tamura *et al*., 2012). Divergence times for all branching points in the topology were calculated using the Maximum Likelihood method and JTT matrix-based model (Jones *et al*., 1992). The estimated log likelihood value of the topology shown is -7673.45. The tree is drawn to scale, with branch lengths measured in the relative number of substitutions per site. Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

study resulted in identification and characterization of Melomys woolly monkey virus (MelWMV) in another *M. burtoni* subspecies in Maluku Island of Indonesia that with 98% nucleotide similarity nested within GALVs as a subspecies of WMV (Alfano *et al.*, 2016b). The single integration event of MelWMV into *M. burtoni* subspecies, is a defective ERV that has endured large deletions in the *pol* (corresponding to reverse transcriptase domain), *env* and *gag* genes. This suggests MelWMV is no longer capable of producing viral particles nor re-integrating into the host genome without a helper virus, a classic characterization of an ERV.

Although *Melomys* is currently confined to the Australo-Papuan region, this paraphyletic group along with 64 rodent species native to Australia, descends from a mixture of southeast Asian and Australo-Papuan "old endemic" rodents (Rowe *et al.*, 2008; Bryant *et al.*, 2011; Geffen *et al.*, 2011; Fabre *et al.*, 2018) (Fig. 1). Where there is no evidence of endogenization of GALV-KoRV-like sequences in bats, *Melomys* seem like a plausible source or intermediate host that shares a deep history with this viral group and their respective vertebrate hosts.

Bats and the evolution of GALV-KoRV like retroviruses

The recent characterization of novel gammaretroviruses with potential evolutionary relationships to GALV- and KoRV-like retroviruses in chiropteran species (bats) is not surprising, as bats are known reservoirs for many viruses, and the finding supports the suggestion that there may be several origins of retroviruses in bat species (Cui *et al.*, 2012a). Recent reports of gammaretroviruses in a variety of bat species inform investigations into the evolutionary origins of GALV and KoRV. These viruses include flying fox retrovirus (FFRV) variants in *Pteropus alecto* (McMichael *et al.*, 2019) and *P. conspicullatus* (McMichael *et al.*, unpublished data); Hervey Pteropid Gammaretrovirus (HPG), MmGRV and SaGRV from the Australian bat species *P. alecto*, *Macroglossus minimus* and *Syconycteris australis* respectively; and HlGRV and RhGRV, from the Asian bat species *Hipposideros larvatus* and *Rhinolophus hipposideros,* respectively (Hayward *et al.*, 2020).

The presence of intact open reading frames of FFRV (McMichael *et al.*, 2019) and the infectivity of HPG viral constructs (Hayward *et al.*, 2020) suggests that the flying fox retroviruses, FFRV and HPG, may be exogenous and infectious in nature. The hypothesis of "species jumping" of exogenous retroviruses closely related to GALV and KoRV (Simmons *et al.*, 2014; Greenwood *et al.*, 2018) suggests that the most likely candidate species of transmission are those species that transit between the Australian mainland and southeast Asia, with geographic ranges and feeding ecology that may result in close contact with both gibbons and koalas (Fig. 1). It has been suggested that bats that harboured distinct gammaretroviruses may have played an important role as reservoir hosts during the diversification of mammalian gammaretroviruses, and that bat retroviruses are not constrained by geographic barriers (Cui *et al.*, 2012a; Cui *et al.*, 2012b). Denner similarly suggests the hypothesis that retroviruses of bats are the origin of GALV and KoRV, which also deserve consideration (Denner, 2016).

Notwithstanding these hypotheses, molecular clock and phylogenetic analyses (Fig. 2), shows that the novel gammaretroviruses found in Australian megabat species from the genera *Pteropus*, *Macroglossus* and *Syconycteris*, are a divergent evolutionary lineage to that of GALV, KoRV and the KoRV-GALV-like Asian bat and rodent clades of gammaretroviruses. While the relationship between the KoRV and GALV-like gammaretroviruses is still unclear, it is likely that these retroviruses may have an unknown common ancestor. Thus, further investigation into the diversity of gammaretroviruses in Australian and Asian bat species may elucidate their evolutionary origins.

Conclusion and future aspects

Although retroviruses that are closely related to GALV and KoRV have been described in a variety of rodent and bat species, the definitive ancestral virus of both GALV and KoRV remains uncertain. Debate on the identity of the host of this ancestral virus continues, as does the question of whether this virus is still circulating in its host or has become a defective endogenous element.

The consensus for further retroviral screening of the rich

biodiversity within this region is clear, specifically *Melomys* and bat species across their biogeographical ranges. Different characteristics of these species, such as the short generation time of rodents and the unique intrinsic immunity of bats to viral infection, provide diverse opportunities to study the intriguing history of this group of viruses.

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