Limited overlap in RNA virome composition among rabbits and their ectoparasites reveals barriers to virus transmission

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Running title: Comparison of rabbit and ectoparasite viromes

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Abstract

Ectoparasites play an important role in virus transmission among vertebrates. However, little is known about the extent and composition of viruses that pass between invertebrates and vertebrates. In Australia, flies and fleas support the mechanical transmission of viral biological controls against wild rabbits - rabbit haemorrhagic disease virus (RHDV) and myxoma virus. We compared virome structure and composition in rabbits and these associated ectoparasites, sequencing total RNA from multiple tissues and gut contents of wild rabbits, fleas collected from these rabbits, and flies trapped sympatrically. Meta-transcriptomic analyses identified 50 novel viruses from multiple RNA virus families. Rabbits and their ectoparasites were characterised by markedly different viromes: although viral contigs from six virus families/groups were found in both rabbits and ectoparasites, none were vertebrate-associated. A novel calicivirus and picornavirus detected in rabbit caecal content were vertebrate-specific: the newly detected calicivirus was distinct from known rabbit caliciviruses, while the novel picornavirus clustered with the *Sapeloviruses*. Several *Picobirnaviridae* were also identified, falling in diverse phylogenetic positions suggestive of an association with co-infecting bacteria. The remaining viruses found in rabbits, and all those from ectoparasites, were likely associated with invertebrates, plants and co-infecting endosymbionts. While no full genomes of vertebrate-associated viruses were detected in ectoparasites, suggestive of major barriers to biological transmission with active replication, small numbers of reads from rabbit astrovirus, RHDV and other lagoviruses were present in flies. This supports the role of flies in the mechanical transmission of RHDV and implies that they may assist the spread of astroviruses.

Keywords: virus, ectoparasites, transmission, calicivirus, meta-transcriptomics, evolution

Introduction

Ectoparasites act as vectors for many notable viral pathogens of vertebrates, including Zika virus, dengue virus, and tick-borne encephalitis virus (Boyer, Calvez, Chouin-Carneiro, Diallo, & Failloux, 2018; Lindquist & Vapalahti, 2008; Rodhain, 2015). Transmission can occur "biologically", with active virus replication in the ectoparasite, or "mechanically" without ectoparasite replication (Chihota, Rennie, Kitching, & Mellor, 2001; Kuno & Chang, 2005; McColl et al., 2002; Rodhain, 2015). Both mechanisms enable viruses to spread across spatial or ecological barriers that might inhibit direct transmission (Rosenberg & Beard, 2011). Ectoparasites are predominantly arthropods, including such animals as lice and fleas, as well as intermittent ectoparasites such as mosquitos, ticks and blowflies (Hopla, Durden, & Keirans, 1994).

The European rabbit (*Oryctolagus cuniculus*) has been profoundly impacted by ectoparasite-mediated viral transmission. As rabbits are a pest species in Australia, two virus biological controls - rabbit haemorrhagic disease virus (RHDV; single-stranded RNA) and myxoma virus (MYXV; double-stranded DNA) - were deliberately introduced to control wild rabbit populations in the 1950s and 1990s, respectively (Cooke & Fenner, 2002). Blowflies (Calliphoridae) and bushflies (Muscidae) are associated with the transmission of RHDV, while two species of rabbit fleas (*Spilopsyllus cuniculi* and *Xenopsylla cunicularis*) aid MYXV transmission (and mosquitos are potentially involved in the subsidiary transmission of both viruses) (Asgari, Hardy, Sinclair, & Cooke, 1998; Cooke & Fenner, 2002; Hall, Huang, Roberts, & Strive, 2019; McColl et al., 2002; Merchant et al., 2003; Sobey & Conolly, 1971). As viral replication is not believed to occur in insect tissue, transmission is entirely mechanical. RHDV is ingested by flies during feeding on carcasses and viable virus excreted in fly spots (Asgari et al., 1998), while fleas transmit MYXV through contaminated

mouthparts (Fenner, Day, & Woodroofe, 1952). Although RHDV is transmissible directly by the faecal-oral route, flies facilitate transmission between isolated populations (Schwensow et al., 2014). Indeed, before it's official release, RHDV escaped quarantine from Wardang island, South Australia, purportedly due to fly-vectored transmission (Asgari et al., 1998; McColl et al., 2002). MYXV can also be transmitted via direct contact, although biting insect vectors enhance transmission and as such, rabbit fleas were also deliberately introduced into Australia (Merchant et al., 2003; Sobey & Conolly, 1971).

Despite the importance of the ectoparasite-vector system in virus transmission and evolution, little is known about the composition of virus communities in both host types. Metagenomic studies of arthropod vector species such as mosquitoes and ticks have revealed an unexpectedly rich virus diversity, most of which likely do not infect vertebrates (Harvey, Rose, Eden, Lo, et al., 2019; Shi et al., 2017). Hence, it is not known what proportion of the viruses present in invertebrates pass to vertebrates and vice versa, although such information is central to understanding the evolution of vector-borne transmission and determining whether some viruses have more liberal host preferences than others.

The advent of bulk RNA sequencing ("meta-transcriptomics") has revolutionized our perception of viral diversity and host range (Shi et al., 2016; Shi, Zhang, & Holmes, 2018), revealing large numbers of seemingly benign viruses (Shi, Lin, et al., 2018). The invertebrate meta-transcriptomic studies undertaken to date include various species of ectoparasite, such as mosquitos, ticks and fleas, revealing abundant and complex viromes (Harvey, Rose, Eden, Lawrence, et al., 2019; Harvey, Rose, Eden, Lo, et al., 2019; Shi et al., 2017). Herein, by comparing the viromes of Australian wild rabbits alongside associated rabbit fleas and sympatric flies, we present the first joint study of virome composition in vertebrates and

100 their associated ectoparasites. Our aim was to determine whether and how virome
101 composition differed between rabbits and the ectoparasites sampled on or near these
102 rabbits, and whether some types of virus were common to both types of host such that they
103 are involved in either biological or mechanical transmission.
104

105 ## Materials and Methods

106 ### *Tissue Sampling*

107 Sampling was performed at two sites within the Australian Capital Territory (ACT), Australia:
108 site 1 was at CSIRO Crace (-35.22, 149.12), Gungahlin (GUN), a suburb of Canberra, while
109 site 2 was at Gudgenby Valley (-35.74, 148.98) in Namadgi National Park (Gudg). At site 1,
110 rabbits were trapped in carrot baited cages and killed by cervical dislocation. Trapping
111 occurred over 3-5 consecutive nights for two separate weeks of the 2016/2017 southern
112 hemisphere summer (18^{th} – 22^{nd} December 2016, 8^{th} – 11^{th} January 2017). A total of 20
113 rabbits were sampled, with weights ranging between 0.27 kg and 1.95 kg (mean 0.82 kg). At
114 site 2, rabbits were killed by shooting on 2^{nd} February 2017. Eighteen rabbits were collected,
115 weighing between 0.52 kg and 2.2 kg (mean 1.49 kg). Blood (in EDTA tubes), lung, liver,
116 duodenum, and caecal content were collected from each rabbit. Where fleas were present
117 on rabbits, they were collected and grouped by rabbit. Tissues and fleas were stored below
118 -80ºC immediately after collection.
119

120 Commercially available fly traps (Envirosafe™) were placed at the same locations in the
121 same weeks as rabbit sampling. Traps were baited with rabbit tissue/gut content and/or
122 chicken necks, and bait was physically separated from flies to prevent contamination. Fly
123 traps were left out for periods of up to 24 hours. Only live flies were taken from traps to
124 ensure fresh samples. Live flies were chilled at 4ºC or -20ºC for periods of 5-10 mins to

allow initial visual identification of fly species, before being frozen at -80°C. From site 1 (GUN), 149 flies representing 5 species were collected, while 22 flies from 2 species were collected from site 2 (Gudg) (Table 1).

All work was carried out according to the Australian Code for the Care and Use of Animals for Scientific Purposes with approval from the institutional animal ethics committee (Permit CWLA-AEC#16-02).

RNA extraction

RNA was extracted separately for each sample; from 20 mg of rabbit tissue or bone marrow, 75 µl of rabbit blood, individual whole flies or groups of at least 5 fleas from individual rabbits. RNA was extracted using the Maxwell 16 LEV simplyRNA tissue kit in combination with the Maxwell nucleic acid extraction robot (Promega, WI, USA), according to manufacturer's instruction, including DNase treatment.

Library construction and sequencing

Rabbit RNA was pooled by tissue type and collection site, with up to 20 individuals per pool, while insect RNA was pooled by species and collection site, with pool sizes ranging from 2 – 10 individuals (Table 1). Where large numbers of flies of the same species were collected, RNA from a maximum of 10 flies were pooled together. Liver RNA required further DNase treatment after pooling, using Invitrogen TURBO DNase (Thermofisher Scientific). All pooled RNA was further purified using the RNeasy MinElute clean-up kit (Qiagen, Hilden, Germany) and quantified using the Qubit RNA Broad-range Assay with the Qubit Fluorometer v3.0 (Thermofisher Scientific). RNA pools were assessed for quality using the Agilent RNA 6000 Nano kit and Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA).

Library construction and sequencing was performed at the Australian Genomic Research Facility. Libraries were constructed using the TruSeq Total RNA Library Preparation protocol (Illumina, CA, USA) and rRNA was removed using the Illumina Ribo-Zero Gold rRNA removal kit (Epidemiology). Paired-end (100 bp) sequencing of each RNA library was performed on the HiSeq 2500 sequencing platform (Illumina, CA, USA).

Assembly and genome annotation

De novo assembly of reads into contigs was performed using Trinity (Grabherr et al., 2011) following trimming with Trimmomatic (Bolger, Lohse, & Usadel, 2014). The RSEM tool (B. Li, Ruotti, Stewart, Thomson, & Dewey, 2010) in Trinity was used to calculate the relative abundance of each contig (expected counts). BLASTn and DIAMOND BLASTx were then used to compare Trinity contigs to the NCBI nucleotide (nt) database (*e*-value cut-off 1×10^{-10}) and non-redundant protein (nr) database (*e*-value cut-off 1×10^{-5}), respectively. Results were filtered so that only contigs that had a viral hit (excluding endogenous viruses/retroviruses) from each BLAST search were retained.

Equivalent BLAST analyses were performed on individual reads to detect viruses at low abundance, with *e*-value cut-offs of 1×10^{-4} for BLASTx and 1×10^{-10} for BLASTn. A conservative approach was taken such that only reads that had a virus result in both the BLASTn and BLASTx analyses were considered as legitimate hits. Ectoparasite library read-mapping to specific virus reference sequences or rabbit viral contigs was conducted using Bowtie2 (Langmead & Salzberg, 2012).

To remove residual host rRNA sequences, all reads were mapped to host rRNA using Bowtie2 (Langmead & Salzberg, 2012). The rabbit host rRNA target index was generated

175 from a complete *O. cuniculus* 18S rRNA reference sequence obtained from GenBank
176 (accession NR_033238) and a near complete *O. cuniculus* 28S rRNA sequence obtained
177 from the SILVA high quality ribosomal database (Quast et al., 2013) (accession
178 GBCA01000314). The arthropod rRNA target index was generated from 18S and 28S
179 GenBank sequences from *Spilopsyllus cuniculi* and multiple *Chrysomya*, *Calliphora*,
180 *Sarcophaga*, and *Musca* species. The total number of reads that did not map to host rRNA
181 for each library were used as the denominator to calculate the percentage of reads mapped
182 to viral contigs.
183
184 The Geneious assembler (Kearse et al., 2012) was used to extend viral contigs where
185 possible. Open reading frames of viral contigs were identified using the online GeneMark
186 heuristic approach to gene prediction tool (Besemer & Borodovsky, 1999), while conserved
187 domains were identified using RSP-TBLASTN v2.6.0, a variant of PSI-BLAST (Altschul et al.,
188 1997).
189
190 ***Phylogenetic analyses***
191 Reference RNA-dependent RNA polymerase (RdRp) amino acid sequences for each virus
192 family were downloaded from NCBI and aligned with viral contigs using MAFFT v7.271
193 (Katoh & Standley, 2013). Where necessary, large data sets were condensed to a more
194 manageable size using CD-HIT version 4.8.1 (W. Li & Godzik, 2006). Poorly and
195 ambiguously aligned sites were removed using trimAl v1.2rev59 (Capella-Gutierrez, Silla-
196 Martinez, & Gabaldon, 2009). Alignments were visualized in Geneious (Kearse et al., 2012).
197 Maximum likelihood trees of each alignment were inferred using PhyML (Guindon et al.,
198 2010) employing the LG amino acid replacement model selected by IQTree (Nguyen,
199 Schmidt, von Haeseler, & Minh, 2015), using a combination of NNI (Nearest Neighbour

Interchange) and SPR (Subtree Pruning and Regrafting) branch-swapping. Branch supports were estimated with the Shimodaira-Hasegawa (SH)-like approximate likelihood ratio test (Guindon et al., 2010). The size and length of each alignment is provided in Table S1 and details of viral contigs included in phylogenies are provided in Table S2.

Screening PCRs for detection of rabbit calicivirus and picornavirus

Primer sets were designed to amplify a small region of each of the novel rabbit calicivirus and picornavirus genomes for the detection of these viruses in individual caecal content samples. The calicivirus primer set GRC_F5.6 (5'-TTA CTC AGA GCG ACC AAG TGC-3', positive sense) and GRC_R5.9 (5'-CCA GTT CTC GCC TGT ATC CAG-3', negative sense) amplified a 278 bp region, while the picornavirus primer set GRP_F6.5 (5'-GAT CTT ATC CCA CCC AAT CGT GA-3', positive sense) and GRP_R6.9 (5'-ATA GCC TCT TCT CCA TAA CCA AGC-3', negative sense) amplified a 401 bp region. RT-PCRs were conducted using the QIAGEN® OneStep Ahead RT-PCR Kit according to the manufacturer's directions with 1 µl of RNA (diluted 1:10 in nuclease-free water) in a 10 µl reaction volume with 0.25 µM of each primer. PCR conditions included 10 cycles of touchdown PCR, with the annealing temperature decreasing by 0.5 °C each cycle from a starting temp of 60 °C, and a further 30 cycles with annealing temperature at 55 °C. Representative amplicons were Sanger sequenced to confirm their legitimacy.

Extension/confirmation of 3' end of novel calicivirus genome

First strand cDNA synthesis was conducted using the Invitrogen Superscript IV reverse transcriptase system (Thermofisher Scientific, MA, USA), with 5 µl of RNA and 0.5 µM of GV270 gene-specific primer (Eden, Tanaka, Boni, Rawlinson, & White, 2013) in a 20 µl reaction volume. PCR was conducted using the Invitrogen Platinum Taq Polymerase High

Fidelity kit according to the manufacturer's protocol with specifically designed forward primer GRC_F6.2 (5'-CAG AGA ATG AGC TCA ACC GAC A-3'), and reverse primer GV271 (Eden et al., 2013). Reaction volumes of 40 µl included 2.5 µl of cDNA template and 1 µM of each primer. PCR was conducted for 45 cycles, with the annealing temperature starting at 65 °C and decreasing by 0.5 °C each cycle. The positive amplicon was approximately 500 bp (includes polyA tail) and was Sanger sequenced for confirmation.

Detection of lagoviruses in flies and rabbit carcasses

RNAs from individual flies and from the bone marrow of rabbit carcasses found near Gungahlin fly traps were screened for the presence of pathogenic lagoviruses using the multiplex RT-PCR described previously (Hall et al., 2018).

Results

Genetic identification of unknown arthropods

The majority of arthropods analysed in this study were identified to the species level through visual inspection. The remainder were characterised using the RNA-Seq data. Fleas were confirmed to be *Spilopsyllus cuniculi* (rabbit fleas) based on the presence of several highly abundant contigs of *Spilopsyllus cuniculi* rRNA and EF1a genes and the absence of any other *Spilopsyllus* species genes. A library of unidentified *Chrysomya* species (GUNChsp library) was determined to be *Chrysomya rufifacies* or *albiceps* (these two species are potentially the same) based on EF1a and rRNA genes. An unknown *Sarcophaga* species was most likely *Sarcophaga impatiens* based 28S rRNA identity.

247

248 ***Fly species trapped***

249 A wider diversity of flies were trapped at site 1, a suburb of Canberra (n = 5 species), than at
250 site 2, in Namadgi National Park (n = 2 species, Table 1). Species from the genera
251 *Calliphora*, *Chrysomya* (both Calliphoridae) and *Sarcophaga* (Sarcophagidae) were collected
252 from site 1, while species from *Calliphora* and *Musca* (Muscidae) were isolated from site 2.
253 *Calliphora augur* was the only species trapped at both sites (Table 1).

254

255 ***Virus contigs in ectoparasites***

256 A large number of RNA viral contigs were assembled from the flea and fly libraries. Of the
257 invertebrate species, *Calliphora vicina* had the highest virus abundance (as a proportion of
258 non-rRNA reads), with almost 2% of non-rRNA reads being viral, while *Chrysomya* species
259 had viral abundances of only 0.013 – 0.019% (Figure 1). Each ectoparasite species had
260 virus contigs from between 6 and 14 different RNA virus families. While viruses from several
261 different families were detected in fleas (10 – 14 families), viruses in both flea libraries largely
262 belonged to the *Iflaviridae* and Sobemo-like viruses (Figure 1). Of the fly species, the
263 *Calliphora augur* libraries harboured the highest number of virus families, although diversity
264 did not differ extensively between libraries. Although only fleas and *Calliphora augur* were
265 sampled from both sites, the viral diversity of these two species at each site suggests that
266 viral composition was associated with host species rather than collection location (Figure 1).
267 The fly results also suggest that there is a trend in viral composition at the genus level
268 (GUNCaA, GudgCaA and GUNCaV are genus *Calliphora*, while GUNChsp and GUNChV are
269 *Chrysomya*), with decreasing similarity in viral composition at the family level and beyond
270 (Figure 1). Members of the *Partitiviridae* and *Phenuiviridae* were detected in all invertebrate
271 species, although it is possible that some of the low abundance viruses (as low as 46 and

78 reads, respectively) represent cross-contamination since all flies were caught in the same trap at each location.

To establish the diversity and potential host of newly defined viruses, family level (in some cases super-family level) phylogenetic trees were estimated using the virus RdRp (Figure 2). While many of the highly diverse phylogenies had poorly resolved topologies, we identified at least 25 diverse viruses that likely constitute new species. The majority of viruses found in invertebrate species clustered with invertebrate-associated viruses in the *Dicistroviridae, Iflaviridae, Nodaviridae,* Flavi-like, *Solemoviridae*/Sobemo-like*,* Virga-like*, Orthomyxoviridae*, *Mononegavirales, Reoviridae, Phasmaviridae (Bunyavirales)* and unclassified bunyavirales groups. Additionally, many of the viruses found in insects, particularly fleas, were potentially viruses of fungi, protozoa or algae, being present in the *Hypoviridae, Narnaviridae, Partitiviridae,* the *Totiviridae-Chrysoviridae* group and certain *Phenuiviridae (Bunyavirales)*. The *Bromoviridae* virus identified in Gungahlin fleas clusters firmly among plant viruses, and with an abundance of only 0.002% it likely represents a plant virus carried by fleas rather than a virus of fleas themselves. Indeed, care must be taken in assigning viruses to hosts on the basis of metagenomic data alone. The *Iflaviridae* flea viruses found in this study clustered most closely with Watson virus, detected in fleas (*Pygiopsylla*) from Australian marsupials (Figure 2).

Virus contigs in rabbits

No viral contigs could be assembled from the rabbit liver, duodenum, or lung libraries. A small number of viral contigs were found in the Gudgenby blood library, but these were potential contaminants since (i) rabbits from Gudgenby were shot, occasionally resulting in perforation of the caecum which would contaminate blood in the body cavity, (ii) all viruses

297 detected in the blood were also detected in the caecum (including plant viruses unlikely to
298 be in blood), and (iii) no viruses were found in the blood of rabbits from the Gungahlin site
299 where there was no body cavity contamination.
300
301 In contrast, the caecal content for rabbits from both sites contained many viruses, with 8
302 and 11 RNA viral families detected in the Gudgenby and Gungahlin rabbits, respectively
303 (Figure 1), including over 25 likely new viral species. The viral composition of rabbit caecal
304 content was less consistent between the two sites than for the invertebrates. This may be a
305 consequence of sampling only small sections of caecal content, but could also reflect
306 differences in diet at each site (predominantly introduced pastures at site 1 versus more
307 subalpine native grassland plants at site 2). Regardless, *Narnaviridae* and *Virgaviridae* were
308 both highly abundant in the caecal content of rabbits from both locations, while
309 *Tombusviridae* was also a major component of the caecal virome of Gudgenby rabbits
310 (Figure 1). These three virus families, that make up more than 70% of the total viral
311 abundance in rabbit caecal content at each site, likely represent viruses of the rabbit diet
312 (plants) and commensal/parasitic organisms such as fungi and protists. Although the
313 *Tombusviridae* were traditionally associated with plants, recent studies have found many
314 tombus-like viruses in invertebrates (Shi et al., 2016) and these group with the caecal
315 content viruses determined here. Hence, these tombus-like viruses may in fact infect
316 commensal or parasitic microorganisms such as protists or fungi, or rabbits may be
317 incidentally eating invertebrates.
318
319 Although less abundant, diverse novel viruses from two vertebrate viral families - the
320 *Caliciviridae* and the *Picornaviridae* - and one potentially vertebrate-associated viral family,
321 the *Picobirnaviridae,* were detected in rabbit caecal content at both sites: all three at
322 Gungahlin, and the *Picobirnaviridae* at Gudgenby. Two related *Caliciviridae* contigs were

assembled, with 77.8% nucleotide identity in the genome and 90.8% identity at the RdRp protein level. They clustered most closely - although distantly - with a pig calicivirus and marmot norovirus (Figure 3), sharing 52 - 54% identity in the RdRp. Such a divergent phylogenetic position suggests that the new calicivirus contigs represents a new viral species (Figure 3), which we have termed *Racaecavirus*. After Sanger sequencing to extend the 3' end, one of the racaecavirus contigs encompassed a near complete genome, missing only the 5' UTR. *Racaecavirus* exhibited a classic calicivirus-like genome organization, with two open reading frames (ORF), one encoding a polyprotein including RdRp and capsid domains, and the second encoding a small protein of unknown function (Figure 3). Oddly, there appears to be only 1 nucleotide in the 3' UTR of this genome sequence, which was confirmed by Sanger sequencing.

Similarly, the entire coding region was obtained for a novel member of the *Picornaviridae*. This contained one large ORF, typical of the *Picornaviridae*, with multiple capsid proteins preceding non-structural proteins (Figure 3). The sequence also contained a 5' (478 nt) and 3' (74 nt) UTR, although it is not clear if these are complete. The novel virus clusters, with strong support, with members of the *Enterovirus* and *Sapelovirus* genera (Figure 3). The closest sequenced relatives were feline picornavirus, bat picornavirus 3, Apodemus agrarius picornavirus, and marmot sapelovirus 2, that share an identity of 61 - 64% with the novel rabbit picornavirus in the RdRp protein (Figure 3). This level of divergence and phylogenetic position would define this virus as a new species within the genus *Sapelovirus* or a newly defined sapelovirus-like genus (Figure 3) (Zell, 2018). Accordingly, we propose the name *Orycavirus*.

Since RNA-sequencing was conducted on pools of 18-20 samples, specific RT-PCRs for the novel racaecavirus and orycavirus identified here were designed to determine their

frequency in individual animals. As these two viruses were only found in the Gungahlin caecal content library, only Gungahlin samples were screened. Racaecavirus was detected in 4/20 samples, while orycavirus was detected in 10 of the 20 samples tested.

Finally, several picobirnaviruses were identified in rabbit caecal content, all of which clustered strongly in the supposedly vertebrate-specific genogroup 1 clade (Figure 4). Based on individual species sharing <75% amino acid similarity in the RdRp alignment, these data likely contain nine novel picobirnaviruses (although defined species demarcation criteria for this family are lacking). Consistent with naming conventions adopted for most picobirnavirus species, the tentative new viruses were named *Rabbit picobirnavirus* 1-9. Importantly, these viruses did not form a monophyletic group, but were distributed throughout genogroup 1. This pattern is typical of the *Picobirnaviridae* that show limited host structure in the RdRp phylogeny (Figure 4), and is compatible with the idea that these are in fact bacterial-associated viruses (Krishnamurthy & Wang, 2018). The RdRp segments (segment 2) were predicted to have one ORF, consistent with other members of this family. While pairing segments was difficult, several longer picobirnavirus segments with at least one large ORF, likely encoding the capsid, were identified in both caecal content libraries.

Virus families present in both insect and rabbit libraries

Viral contigs from the *Virgaviridae*/*Bromoviridae*/Virga-like (plant/invertebrate-associated), *Solemoviridae*/Sobemo-like (plant/invertebrate-associated), *Narnaviridae* (fungi/parasites/invertebrate-associated), *Partitiviridae* (plant/invertebrate/fungi/vertebrate faeces-associated), *Tombusviridae* (plant/invertebrate-associated) and *Toti-Chryso* (parasites/invertebrates/fungi-associated) groups were assembled from rabbit caecal content as well as from both fleas and flies (Figure 1 and Figure 5). As noted above, the

374 viruses assembled from rabbit caecal content in these virus families were unlikely to be
375 actively replicating in rabbits. In addition, where viruses of the same family were assembled
376 from arthropods as well as rabbits, they did not cluster together (Figure 2).
377
378 To further investigate the viral overlap between rabbits and ectoparasites, reads from
379 ectoparasite libraries were mapped to the viral contigs from the rabbit caeca. A total of 58
380 viral reads mapped to rabbit virus contigs, all associated with the viral groups described
381 above, and hence were likely mapping to conserved regions. Taken together, these results
382 show that no abundant viral species were shared between host and ectoparasites, such
383 that there was no strong evidence of biological vector transmission.
384
385 ***Low abundance vertebrate-associated viruses in ectoparasite libraries***
386 If the ectoparasites studied here were involved in mechanical transmission, viruses may not
387 be sufficiently abundant to be assembled into contigs. To detect vertebrate viruses at low
388 abundance we subjected individual reads from the flea and fly libraries to BLASTn and
389 BLASTx analyses. Accordingly, small numbers of reads were detected for two known
390 rabbit-specific viruses (Figure 6): Lagoviruses (RHDV and related viruses) of the *Caliciviridae*
391 family and rabbit astroviruses. The lagovirus reads detected included RHDV, rabbit
392 haemorrhagic disease virus 2 (RHDV2), and the benign rabbit calicivirus Australia-1 (RCV-
393 A1). Because of recombination between RHDV, RHDV2, and RCV-A1 (Hall et al., 2018),
394 classification of these viruses based on small numbers of reads is difficult. However, the
395 presence of reads mapping to the non-structural gene segments of RHDV and the RCV-A1-
396 like viruses, as well as the structural gene segments of RHDV2, suggests the presence of at
397 least two RHDV-like viruses in these fly libraries - a recombinant RHDV/RHDV2 and
398 recombinant RCV-A1-like/RHDV2. Equivalent read BLAST analyses were conducted on

399 rabbit libraries: two reads from RHDV2 recombinants were found in each of the Gudgenby
400 liver, Gudgenby lung and Gungahlin blood libraries. Since they were at very low abundance,
401 these viruses were not likely to be actively replicating in these rabbits, although they may
402 represent the early hours of infection or a cleared infection. No vertebrate-specific virus
403 reads were detected in the flea libraries or Gudgenby fly libraries. Due to the difficulty in
404 confirming the legitimacy of viral reads, only those that had a virus result for both BLASTx
405 and BLASTn analyses were included. Hence, this method will have necessarily led to a
406 conservative estimate and the omission of diverse virus reads since these are unlikely to be
407 detected in a BLASTn analysis.
408
409 Since some viruses were represented by as little as a single read per library, we confirmed
410 the presence of RHDV-like viruses in invertebrates by RT-PCR. Importantly, several
411 individual flies from all three libraries with RHDV-like reads were positive by RT-PCR despite
412 each library having only 2 – 10 reads. In addition, bone marrow from rabbit carcasses
413 collected during the same time and location as fly trapping at the Gungahlin site were also
414 positive for RHDV2 recombinants by RT-PCR. This, and the presence of lagoviruses in
415 rabbits and flies in the wider region at that time (Hall et al., 2019), suggests that pathogenic
416 lagoviruses were circulating at the time of sampling and the small number of reads in fly
417 libraries were *bona fide*. In contrast, no legitimate mapping occurred when ectoparasite
418 reads were mapped to a MYXV reference genome (NC_001132.2). This is consistent with
419 the absence of visible clinical signs of myxomatosis in the sampled rabbits. In addition, no
420 viruses with known pathogenic potential in humans were detected in fleas or flies.
421

Discussion

A rapidly changing climate increases the potential for ectoparasite-mediated pathogen transmission (Ogden, 2017). A key question is what proportion of the viruses detected in ectoparasites are potentially transmissible to their vertebrate hosts and vice versa, through either the biological or mechanical transmission routes. Similarly, it is important to determine whether some viruses have a greater propensity for mechanical transmission, or a greater capacity to productively infect both vertebrates and invertebrates. The answers to these questions will help reveal the barriers that prevent viruses from evolving vector-borne transmission.

To better understand these key components of vector transmission, we compared the viromes of apparently healthy Australian wild rabbits with those of associated fleas and sympatric flies known to be involved in the transmission of rabbit viruses (Asgari et al., 1998; Sobey & Conolly, 1971). No viruses were found in the lung, liver, duodenum or blood, suggesting the absence of an acute or chronic systemic infection in the wild rabbits sampled for this study. In contrast, considerable viral diversity was detected in the caecal content. This likely reflects the role this organ plays in the digestion of plant matter, such that it is rich in bacteria, other microorganisms, and semi-digested plant material (Forsythe & Parker, 1985; Velasco-Galilea et al., 2018). Based on phylogenetic position, most viruses identified in the caecal content are likely to be associated with the rabbit diet or other commensal microorganisms, such as fungi and protozoa (Figure 2). To our knowledge, equivalent viral meta-transcriptomics analyses on caecal content have not been reported, although an abundance of plant and microorganism-associated viruses is consistent with the faecal viromes of other herbivores (Guan et al., 2018; Woo et al., 2014; Zhang et al., 2017). Importantly, we identified diverse novel viruses in rabbits – *Racaecavirus* and

Orycavirus - that cluster with other vertebrate-associated viruses (in the *Caliciviridae* and the *Picornaviridae*, respectively) suggesting that the most likely hosts are the rabbits from which they were sampled. In addition, several novel picobirnaviruses were detected, although their true host is uncertain. Overall, the abundance of the potential vertebrate viruses detected in rabbits was relatively low: calicivirus 0.003%, picornavirus 0.025%, picobirnavirus 0.002-0.011%, although benign rabbit viruses have been previously shown to be present at low titre (Capucci, Fusi, Lavazza, Pacciarini, & Rossi, 1996; Strive, Wright, & Robinson, 2009). Furthermore, as these viruses were isolated from caecal content, we would not expect to have sampled a high proportion of rabbit cells and by extension, viruses replicating in these cells.

Members of the *Caliciviridae* and *Picornaviridae* are frequently detected in vertebrates (Shi, Lin, et al., 2018; Zell, 2018), with many cases of confirmed host association (Feinstone, Kapikian, & Purceli, 1973; Ohlinger & Thiel, 1991; Thornhill, Kalica, Wyatt, Kapikian, & Chanock, 1975; Wells & Coyne, 2019). The *Caliciviridae* can be associated with serious illnesses, such as gastroenteritis in humans (Dolin, 1978) and haemorrhagic disease in rabbits (Ohlinger, Haas, Meyers, Weiland, & Thiel, 1990), while the *Picornaviridae* are a diverse group of viruses associated with various diseases in humans and animals (Zell, 2018). Although there are two existing genera that include rabbit caliciviruses, rabbit *Vesivirus* and *Lagovirus,* the novel rabbit calicivirus identified here, *Racaecavirus*, clustered most closely with a pig calicivirus (*St-Valerian swine virus*) and *Marmot norovirus* (Figure 3), both sampled from the gut of healthy animals (L'Homme et al., 2009; Luo et al., 2018). *St-Valerian swine virus* is the only species within the newly classified genus *Valovirus* and the virus identified here (together with *Marmot norovirus*) likely belongs to this genus (L'Homme et al., 2009). The novel rabbit picornavirus we identified in caecal content, *Orycavirus*, was phylogenetically distinct to other rabbit picornaviruses, clustering with enteroviruses and

473 sapeloviruses/sapelo-like viruses (Figure 3). The genus *Enterovirus* includes important
474 human respiratory pathogens, as well as more serious symptoms such as acute flaccid
475 myelitis, meningitis, myocarditis and encephalitis (Wells & Coyne, 2019). Enteroviruses
476 primarily target the gastrointestinal tract and most infections are thought to be
477 asymptomatic (Wells & Coyne, 2019). The genus *Sapelovirus* was initially classified with
478 members from swine, primate and avian hosts, and an unclear link to pathogenicity (Tseng
479 & Tsai, 2007), although the creation of several new genera may now be appropriate (Zell,
480 2018). The closest relatives of orycavirus were isolated from faeces of apparently healthy
481 cats, bats, and marmots, as well as rodents with unknown disease status (Lau et al., 2011;
482 Lau et al., 2012; Luo et al., 2018). It is notable that the calicivirus and picornavirus detected
483 here cluster with other viruses isolated from the gut content of seemingly healthy vertebrate
484 hosts, tentatively suggestive of a cellular tropism specific to the lower intestinal tract.
485 Additionally, since the sampled rabbits were apparently healthy, the novel calici- and
486 picorna- viruses are likely non-pathogenic. Whether these viruses were present in the
487 founder population of rabbits first introduced into Australia or whether they were exotic
488 incursions awaits additional sampling from diverse locations.
489
490 Nine novel species of *Picobirnaviridae* were identified in the rabbit caecum. *Picobirnaviridae*
491 have been detected in several vertebrate species, including rabbits (Ganesh, Masachessi, &
492 Mladenova, 2014; Ludert, Abdul-Latiff, Liprandi, & Liprandi, 1995; Woo et al., 2016), as well
493 as invertebrates (Shi et al., 2016) and diatom colonies (Urayama, Takaki, & Nunoura, 2016).
494 The picobirnaviruses documented here all cluster with the highly diverse and seemingly
495 vertebrate-associated Genogroup 1. The new viruses do not form a monophyletic group by
496 host species (Figure 4), consistent with other members of this family, and diverse
497 picobirnaviruses are commonly found in a single species (Knox, Gedye, & Hayman, 2018;
498 Woo et al., 2016). Consistent with our detection of *Picobirnaviridae* in caecal content,

499 viruses of this family have commonly been isolated from stool samples or cloacal swabs of
500 vertebrates, either with no apparent symptoms or associated with diarrhea (Cummings et
501 al., 2019; Smits et al., 2011; Smits et al., 2012; Woo et al., 2019). Although it has been
502 suggested that these viruses are opportunistic pathogens (Ganesh et al., 2014), the
503 absence of host phylogenetic structure and lack of conclusive detection in solid tissues
504 suggests that vertebrates and invertebrates may not be the true hosts of this virus family.
505 Indeed, based on the presence of conserved prokaryotic ribosomal binding sites, it was
506 recently proposed that prokaryotes are the true hosts of *Picobirnaviridae* (Krishnamurthy &
507 Wang, 2018), which would accord with the lack of taxonomic structure in vertebrate hosts.
508
509 A large number of diverse viruses were discovered in fleas collected from rabbits and
510 Calliphoridae, Sarcophagidae and Muscidae flies trapped sympatrically (Figure 1 and Figure
511 5). Viral composition in ectoparasites varied according to host species (Figure 1) rather than
512 location, consistent with that seen in Australian mosquitos (Shi et al., 2017). The majority of
513 highly abundant viruses were invertebrate viruses, with the remainder likely representing
514 viruses of fungi, protozoa or other commensal microbes (Figure 2). Several viral
515 families/groups identified in rabbit flea libraries were also found in fleas collected from
516 Australian marsupials or rats, including the *Solemoviridae, Iflaviridae*, *Narnaviridae,*
517 *Phenuiviridae* and *Totiviridae* (Harvey, Rose, Eden, Lawrence, et al., 2019). Generally,
518 viruses from rabbit fleas did not cluster with viruses from other flea species (Figure 2), with
519 the exception of the *Iflaviridae* flea viruses most closely related to Watson virus, a virus of
520 *Pygiopsylla* fleas collected from an Australian marsupial (Harvey, Rose, Eden, Lawrence, et
521 al., 2019). Viruses from six different viral groups/families were identified in both
522 ectoparasites and rabbits (Figure 5), although the ectoparasite viruses were phylogenetically
523 distinct from those found in rabbit caeca (Figure 2). This, and that none of the overlapping
524 viral families were vertebrate-associated, suggests that there may be important barriers to

cross-species transmission. Indeed, no highly abundant vertebrate viruses were found in flies or fleas, suggesting that the species investigated here are not likely to be biological vectors for any vertebrate viruses, and that potential arboviruses are rare.

Carrion/bush flies and fleas have been implicated in the mechanical transmission of RHDV and MYXV in rabbits (Asgari et al., 1998; Hall et al., 2019; McColl et al., 2002; Sobey & Conolly, 1971). In these cases, no viral replication takes place within the ectoparasite, such that viral abundance would be very low and viral contigs may not be assembled. Accordingly, to detect viruses potentially associated with mechanical transmission, we also explored the low abundant viral reads from the invertebrate libraries (i.e. reads which were not assembled into contigs). This revealed evidence of RHDV and related lagoviruses (*Caliciviridae*) in three Calliphoridae fly species (Figure 6) - a family of flies associated with RHDV transmission (Asgari et al., 1998; Hall et al., 2019; McColl et al., 2002). Since the introduction of RHDV into Australia in 1995, several related viruses have been detected, including recombinants of the original RHDV and RHDV2, or RCV-A1 benign viruses and RHDV2 (Hall et al., 2015; Hall et al., 2018; Mahar, Read, et al., 2018). At least two RHDV2 variants were detected in fly reads (RHDV/RHDV2 and RCV-A1-like/RHDV2), both known to be circulating at that time (Hall et al., 2019; Hall et al., 2018; Mahar, Hall, et al., 2018), and were confirmed by RT-PCR. A small number of RHDV2 reads were also identified in rabbit libraries, and incidentally, RHDV2 was detected by RT-PCR in dead rabbits found synchronously at the study site. Since RHDV infection is generally acute and susceptible rabbits die rapidly (Cooke & Fenner, 2002), RHDV-like reads were likely detected from recovering animals, in which RHDV RNA is detectable for at least 15 weeks post-infection (Gall, Hoffmann, Teifke, Lange, & Schirrmeier, 2007). These results demonstrate that mechanically transmitted viruses can be detected concurrently in the vertebrate host and ectoparasite using a meta-transcriptomics approach, even in the case of highly virulent

551 viruses not known to cause persistent infections. Interestingly, rabbit astrovirus was also
552 detected in *Sarcophaga impatiens* and *Chrysomya varipes*, although no reads were
553 detected in rabbit material. This virus has been associated with enteric disease in rabbits,
554 but may be detected in the gut in the absence of symptoms (Martella et al., 2011). The
555 detection of rabbit astrovirus in flies is of interest as it suggests that astrovirus may be
556 present in Australian wild rabbit populations and must be shed at high titres if it was
557 acquired from faeces. However, as we did not detect any reads in healthy rabbits, more
558 work is clearly needed to establish whether rabbit astroviruses can be transmitted by
559 arthropods.
560
561 No viruses known to infect humans, or indeed any other vertebrates besides leporids, were
562 detected in the sampled flies. These fly species are attracted to carrion and faeces, a factor
563 that would promote the mechanical transmission of excreted viruses or those present in
564 carcasses (Norris, 1965). Due to their excessive numbers, rabbit carcasses and faeces are
565 not uncommon in rabbit-infested areas (like the sampling locations), whereas human
566 remains and faeces are hopefully rarer and less accessible. However, we may have
567 expected to find more viruses of livestock (site 1) and native vertebrate species (site 1 and
568 2), which are abundant in the sampling locations. Hence, vertebrate-associated viral
569 mechanical transmission by fly species may be uncommon, and factors such as high
570 prevalence and high virus load in carcasses or faeces - as seen for RHDV-like viruses - may
571 therefore be necessary for mechanical transmission (Mahar, Hall, et al., 2018; Neimanis,
572 Larsson Pettersson, Huang, Gavier-Widen, & Strive, 2018). In contrast to flies, no vertebrate
573 virus reads - including MYXV - were detected in fleas, although their behaviour of feeding on
574 vertebrate blood rather than carcasses and faeces may limit opportunities for mechanical
575 transmission to periods of acute systemic or viraemic infections. As such, ectoparasite

576 behaviour and host preference, alongside viral pathogenesis and prevalence, are likely
577 important for mechanical transmission.
578
579 In sum, while rabbits and ectoparasites carry viruses from some of the same viral families,
580 viruses from ectoparasites are phylogenetically distinct from viruses found in rabbit caecal
581 content, suggesting that major host barriers exist that prevent invertebrate viruses from
582 establishing productive replication cycles in vertebrates. Importantly, however, flies carried
583 a very low abundance of vertebrate viruses with pathogenic capacity in rabbits, including
584 RHDV for which fly-mediated mechanical transmission has already been demonstrated.
585 Hence, although biological transmission appears difficult to evolve, flies may serve as
586 important mechanical vectors for rabbit-associated viruses.
587

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595

596 **Data accessibility**

597 - Raw data: NCBI SRA BioProject XXXX.
598 - Viral contigs presented in phylogenies: GenBank accession numbers XXXXX-XXXX.

599

600 ## Author contributions

601 JE Mahar was involved in sample collection and research design, performed research,
602 analysed data, and wrote the paper. M Shi contributed analytical pipelines and was involved
603 in research design and manuscript editing. RN Hall was involved in sample collection, fly
604 identification, research design and manuscript editing. T Strive was involved in research
605 design, sample collection and manuscript editing. EC Holmes designed and obtained
606 funding for research, assisted with data analysis and manuscript writing.

607 **Table 1. Rabbit and insect sampling and pooling details**

Library	Site[†]	Species	Sample Type	No. samples in RNA-Seq pool
Rabbit tissues				
GUN-Bl	1	*Oryctolagus cuniculus*	Blood	20
GUN-Li	1	*Oryctolagus cuniculus*	Liver	20
GUN-Lu	1	*Oryctolagus cuniculus*	Lung	20
GUN-Duo	1	*Oryctolagus cuniculus*	Duo	20
GUN-CC	1	*Oryctolagus cuniculus*	Caecal content	20
Gudg-Bl	2	*Oryctolagus cuniculus*	Blood	18
Gudg-Li	2	*Oryctolagus cuniculus*	Liver	18
Gudg-Lu	2	*Oryctolagus cuniculus*	Lung	18
Gudg-Duo	2	*Oryctolagus cuniculus*	Duo	18
Gudg-CC	2	*Oryctolagus cuniculus*	Caecal content	18
Arthropods				
GUN-F	1	*Spilopsyllus cuniculi*[‡]	Entire fleas grouped by rabbit	>70 fleas from 8 rabbits
GUN-ChV	1	*Chrysomya varipes*	Entire fly	10
GUN-Chsp	1	*Chrysomya rufifacies/albiceps*[‡]	Entire fly	10
GUN-CaV	1	*Calliphora vicina*	Entire fly	10
GUN-CaA	1	*Calliphora augur*	Entire fly	10
GUN-Sasp	1	*Sarcophaga impatiens*[‡]	Entire fly	2
Gudg-F	2	*Spilopsyllus cuniculi*[‡]	Entire fleas grouped by rabbit	>50 fleas from 7 rabbits
Gudg-CaA	2	*Calliphora augur*	Entire fly	10
Gudg-Un	2	*Musca vetustissima*	Entire fly	2

608 [†]Site 1 - CSIRO Crace, Gungahlin; Site 2 - Gudgenby Valley in Namadgi National Park.
609 [‡]Species designation based on RNA-Seq data.

610 **Figure legends**

611 **Figure 1. RNA virus abundance and composition in rabbit and invertebrate libraries.**
612 The top plot displays the abundance of viral reads (y-axis) in each library (x-axis) as a
613 proportion of total non-rRNA reads. The bottom plot shows the viral composition of each
614 library by virus family/group (shaded/grouped by superfamily). Potential vertebrate viruses
615 are indicated by asterisks within the shading. Only RNA viruses (with RdRp) are shown and
616 only virus families that had an abundance of at least 0.001% in at least one library are
617 presented. UC denotes unclassified viruses. Virus libraries are labelled as follows (collection
618 site - species): GUN_CC, Gungahlin - rabbit caecal content; Gudg_CC, Gudgenby - rabbit
619 caecal content; GUNF, Gungahlin - flea; GudgF, Gudgenby - flea; GUNCaA, Gungahlin -
620 *Calliphora augur*; GudgCaA, Gudgenby - *Calliphora augur*; GUNChsp, Gungahlin -
621 *Chrysomya rufifacies/albiceps*; GUNCaV, Gungahlin - *Calliphora vicina*; GUNChV, Gungahlin
622 - *Chrysomya varipes*; GUNSasp, Gungahlin - *Sarcophaga impatiens*; GudgUn, Gudgenby -
623 *Musca vetustissima*. Note that only the caecal content libraries from rabbits are included in
624 the plots since no viruses were found in the other libraries. A cladogram connecting the
625 libraries beneath the x-axis indicates the relationships between the sampled hosts in each
626 library, where tips represent host species and nodes from top-to-bottom represent the
627 levels of genus, family, super-family, order, class and kingdom.
628
629 **Figure 2. ML trees of the RdRp of likely non-vertebrate viruses**. The taxa name (and
630 branches in minimized trees) for sequences obtained in this study are bolded and coloured
631 red (rabbit caecal content), blue (flies), or purple (fleas), based on the animal from which
632 they were obtained, with relevant animal symbols adjacent to the names. Viruses that likely
633 constitute a new viral species are indicated by a pink star symbol adjacent to taxa names,
634 and a proposed virus species name is given as the taxa name (with strain name in
635 parentheses). For GenBank sequences, taxa names are coloured by the apparent host

636 group from which virus or viral sequence was reportedly isolated: black, invertebrate; teal,
637 fungi/mould/yeast; maroon, vertebrates; orange, Trypanosomatidae; pink, Stramenopiles
638 (microalgae(diatom)/Oomycetes); grey, other protozoa (Coccidia, *Trichomonas*, *Giardia*).
639 SH-like support values are represented by circles at the nodes if >0.7 and are sized
640 according to values where the largest circles represent an SH-like support of 1. For
641 sequences that are less than 80% of the alignment length, the sequence length in amino
642 acids (aa) and an asterisk is included in the taxa name.
643
644 **Figure 3. Phylogenetic analysis of the RdRp of vertebrate-specific viruses found in**
645 **rabbit caecum.** ML trees of the RdRp region of (A) the novel rabbit calicivirus –
646 *Racaecavirus* - and (B) the novel rabbit picornavirus – *Orycavirus* - together with
647 representative reference sequences for these virus families are shown. Taxa names of the
648 viruses discovered in this study are bolded with a black rabbit symbol adjacent. A pink star
649 symbol adjacent to taxa names indicates a novel virus species and the proposed virus
650 species name is given as the taxa name (with strain name in parentheses). GenBank
651 accession numbers are included in the taxa name and these names are colour-coded
652 according to host as specified by coloured symbols to the right of each tree. Clade labelling
653 indicates specific genera. SH-like approximate likelihood ratio branch support greater than
654 0.7 is indicated by circles at the nodes which are sized according to degree of support (SH-
655 like support of 1 has the largest size). Trees were midpoint rooted for clarity. The genome
656 structure and length of the isolated contigs is shown below each tree, with open boxes
657 representing ORFs, and green boxes indicating conserved protein domains: Polyprotein N-
658 term, N-terminal region of the polyprotein; Hel, helicase; RdRp, RNA-dependent RNA
659 polymerase; Calici coat, calicivirus capsid/coat protein; 3CPro, 3C proteinase.
660

Figure 4. Phylogenetic analysis of the RdRp of novel picobirnaviruses. ML tree of the RdRp region of novel rabbit picobirnaviruses and representative picobirnaviruses from GenBank. The novel rabbit picobirnavirus taxa names are bolded, coloured red and emphasised with a black rabbit symbol adjacent to the name. A pink star symbol adjacent to taxa names indicates a novel virus species and the proposed virus species name is given as the taxa name (with strain name in parentheses). The taxa names of GenBank sequences include accession numbers and are coloured according to the host taxa from with they were isolated (all invertebrate host taxa are coloured black). The host taxa associated with sequences in each clade are indicated with symbols to the right of the clade. The single picobirnavirus sequence isolated from a diatom colony is indicated with an arrow. SH-like approximate likelihood ratio branch support greater than 0.7 is indicated by circles at the nodes which are sized according to degree of support (SH-like support of 1 is maximum size). Trees were midpoint rooted for clarity.

Figure 5. Overlap of RNA viral families in rabbits and ectoparasites. The number of viral families/groups for which contigs were assembled from either rabbit caecal content libraries (red circle), fly libraries (blue circle) and flea libraries (purple circle), and the level of overlap for each host group are indicated by a Venn diagram. The viral families associated with each segment are listed with grey dotted lines connecting lists to segments of the Venn diagram. The abundance of each viral family for the three groups is indicated by the size of circles next to virus family names. Circles are colour-coded according to the rabbit, fly or flea group with which they are associated, and the circle sizes reflect the highest abundance of the relevant virus family within a single library in the rabbit, flea or fly group. UC=unclassified.

686 **Figure 6. Vertebrate-specific virus reads detected in ectoparasite libraries.** The number
687 of reads from vertebrate viruses (y-axis) detected in ectoparasite libraries (x-axis) is
688 presented as a stacked bar plot. Vertebrate virus reads that were detected include rabbit
689 astrovirus (RAstV), and RHDV-like viruses that include recombinant variants of rabbit
690 haemorrhagic disease virus (RHDV), and related viruses rabbit haemorrhagic disease virus 2
691 (RHDV2), and rabbit calicivirus Australia 1 (RCV-A1). In the legend, NS and capsid in
692 parentheses indicates reads mapping to non-structural genes or capsid gene, respectively.
693 Ectoparasite libraries are labelled as follows (location - species): GUNF, Gungahlin - flea;
694 GudgF, Gudgenby - flea; GUNCaA, Gungahlin - *Calliphora augur*; GudgCaA, Gudgenby -
695 *Calliphora augur*; GUNChsp, Gungahlin - *Chrysomya rufifacies/albiceps*; GUNCaV,
696 Gungahlin - *Calliphora vicina*; GUNChV, Gungahlin - *Chrysomya varipes*; GUNSasp,
697 Gungahlin - *Sarcophaga impatiens*; GudgUn, Gudgenby - *Musca vetustissima*.

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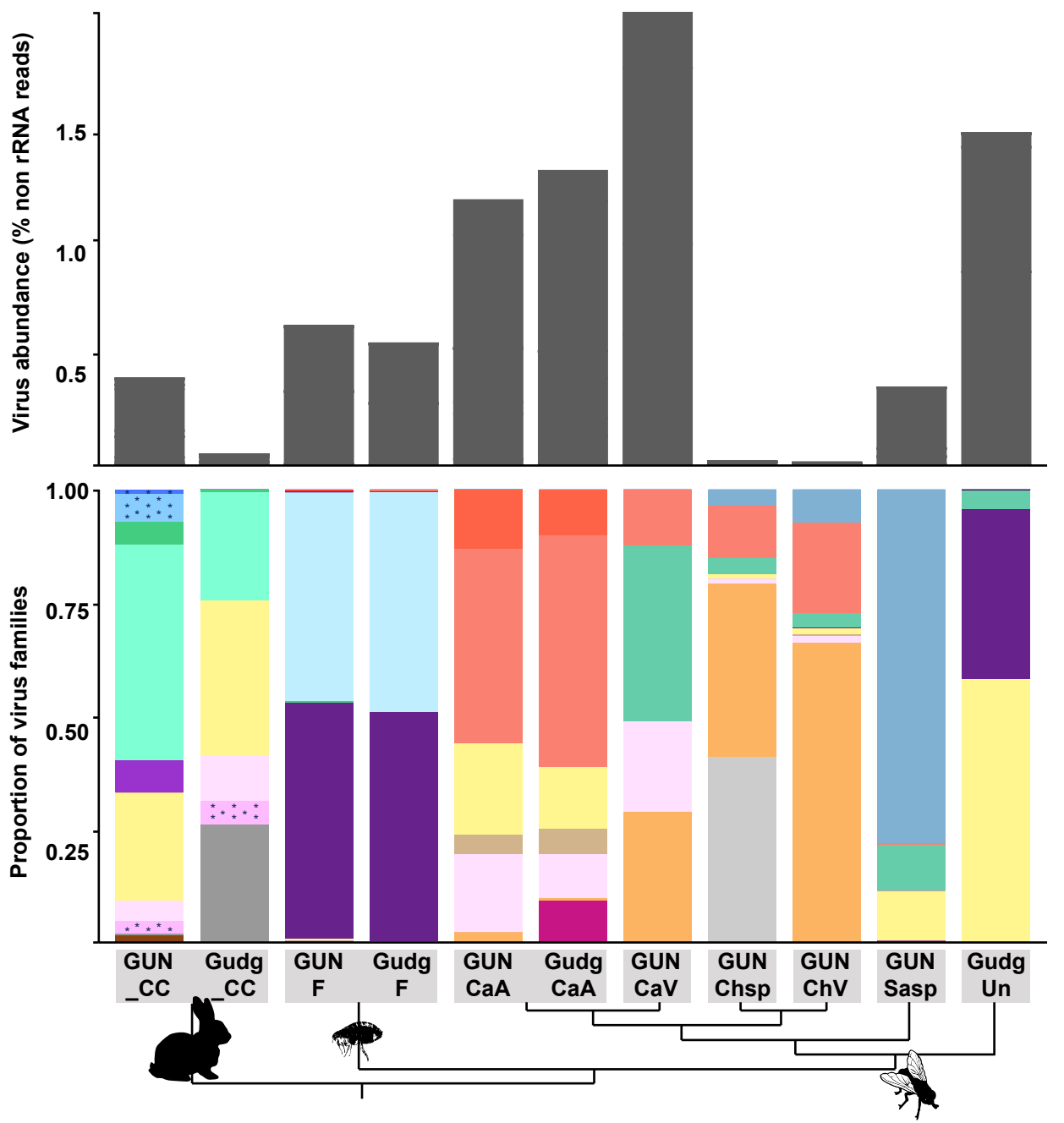
915



Virus Families - Grouped by super-family

Picorna-Calici
- Caliciviridae
- Picornaviridae
- Dicistroviridae
- Iflaviridae

Partiti-Picobirna
- Partitiviridae
- Picobirnaviridae

Narna-Levi
- Narnaviridae

Flavi
- UC Flavi−like

Orthomyxo
- Orthomyxoviridae

Mono-Chu
- UC Mononegavirales

Reo
- Reoviridae

Hepe-Virga
- Bromoviridae
- Virgaviridae
- Virga−like

Luteo-Sobemo
- Sobemovirus
- Sobemo−like

Bunya-Arena
- Phasmaviridae
- Phenuiviridae
- UC Bunyavirales

Tombus-Noda
- Tombusviridae
- Nodaviridae

Toti-Chryso
- Totiviridae

* * * Indicates potential vertebrate virus

Figure 1

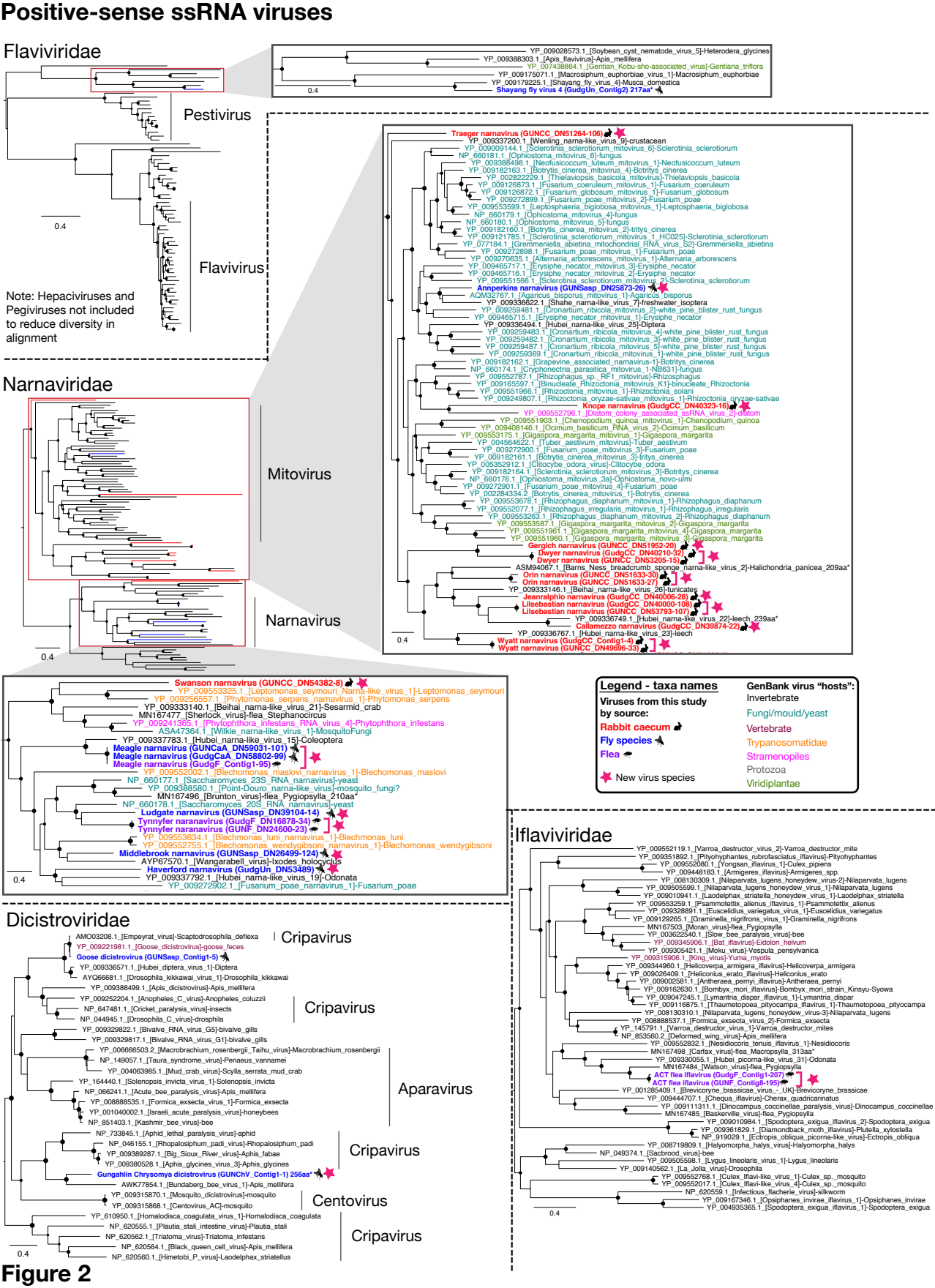

Positive-sense ssRNA viruses
Flaviviridae
Pestivirus
Flavivirus
Note: Hepaciviruses and Pegiviruses not included to reduce diversity in alignment
YP_009028573.1_[Soybean_cyst_nematode_virus_5]-Heterodera_glycines
YP_009388303.1_[Apis_flaviviruslike]-Apis_mellifera
YP_007438864.1_[Gentian_Kobu-sho-associated_virus]-Gentiana_triflora
YP_009175071.1_[Macrosiphum_euphorbiae_virus_1]-Macrosiphum_euphorbiae
YP_009179225.1_[Shayang_fly_virus_4]-Musca_domestica
Shayang fly virus 4 (GudgUn_Contig2) 217aa*
Narnaviridae
Mitovirus
Narnavirus
Traeger narnavirus (GUNCC_DN51264-106)
YP_009337200.1_[Wenling_narna-like_virus_9]-crustacean
YP_009009144.1_[Sclerotinia_sclerotiorum_mitovirus_6]-Sclerotinia_sclerotiorum
NP_660181.1_[Ophiostoma_mitovirus_6]-fungus
YP_009388498.1_[Neofusicoccum_luteum_mitovirus_1]-Neofusicoccum_luteum
YP_009182163.1_[Botrytis_cinerea_mitovirus_4]-Botrityscinerea
YP_009272899.1_[Fusarium_poae_mitovirus_2]-Fusarium_poae
YP_009553599.1_[Leptosphaeria_biglobosa_mitovirus_1]-Leptosphaeria_biglobosa
NP_660179.1_[Ophiostoma_mitovirus_4]-fungus
NP_660180.1_[Ophiostoma_mitovirus_5]-fungus
YP_009182160.1_[Botrytis_cinerea_mitovirus_2]-tritys_cinerea
YP_009121785.1_[Sclerotinia_sclerotiorum_mitovirus_1_HC025]-Sclerotinia_sclerotiorum
YP_077184.1_[Gremmeniella_abietina_mitochondrial_RNA_virus_S2]-Gremmeniella_abietina
YP_009272898.1_[Fusarium_poae_mitovirus_1]-Fusarium_poae
YP_009270635.1_[Alternaria_arborescens_mitovirus_1]-Alternaria_arborescens
YP_009465717.1_[Erysiphe_necator_mitovirus_3]-Erysiphe_necator
YP_009465716.1_[Erysiphe_necator_mitovirus_2]-Erysiphe_necator
YP_009551566.1_[Sclerotinia_sclerotiorum_mitovirus_2]-Sclerotinia_sclerotiorum
Annperkins narnavirus (GUNSasp_DN25873-26)
AQM32767.1_[Aparicus_bisporus_mitovirus_1]-Aparicus_bisporus
YP_009336622.1_[Shahe_narna-like_virus_7]-freshwater_isoptera
YP_009259481.1_[Cronartium_ribicola_mitovirus_2]-white_pine_blister_rust_fungus
YP_009465715.1_[Erysiphe_necator_mitovirus_1]-Erysiphe_necator
YP_009336494.1_[Hubei_narna-like_virus_25]-Diptera
YP_009259483.1_[Cronartium_ribicola_mitovirus_4]-white_pine_blister_rust_fungus
YP_009259482.1_[Cronartium_ribicola_mitovirus_3]-white_pine_blister_rust_fungus
YP_009259487.1_[Cronartium_ribicola_mitovirus_5]-white_pine_blister_rust_fungus
YP_009259369.1_[Cronartium_ribicola_mitovirus_1]-white_pine_blister_rust_fungus
YP_009182162.1_[Grapevine_associated_narnavirus-1]-Botrityscinerea
NP_660174.1_[Cryphonectria_parasitica_mitovirus_1-NB631]-fungus
YP_009552787.1_[Rhizophagus_sp._RF1_mitovirus]-Rhizosphagus
YP_009165597.1_[Binucleate_Rhizoctonia_mitovirus_K1]-binucleate_Rhizoctonia
YP_009551966.1_[Rhizoctonia_mitovirus_1]-Rhizoctonia_solani
YP_009249807.1_[Rhizoctonia_oryzae-sativae_mitovirus_1]-Rhizoctonia_oryzae-sativae
Knope narnavirus (GudgCC_DN40323-16)
YP_009552796.1_[Diatom_colony_associated_ssRNA_virus_2]-diatom
YP_009551903.1_[Chenopodium_quinoa_mitovirus_1]-Chenopodium_quinoa
YP_009408146.1_[Ocimum_basilicum_RNA_virus_2]-Ocimum_basilicum
YP_009553175.1_[Gigaspora_margarita_mitovirus_1]-Gigaspora_margarita
YP_004564622.1_[Tuber_aestivum_mitovirus]-Tuber_aestivum
YP_009272900.1_[Fusarium_poae_mitovirus_3]-Fusarium_poae
YP_009182161.1_[Botrytis_cinerea_mitovirus_3]-tritys_cinerea
YP_009352912.1_[Clitocybe_odora_virus]-Clitocybe_odora
YP_009182164.1_[Sclerotinia_sclerotiorum_mitovirus_3]-Botrityscinerea
NP_660176.1_[Ophiostoma_mitovirus_3a]-Ophiostoma_novo-ulmi
YP_009272901.1_[Fusarium_poae_mitovirus_4]-Fusarium_poae
YP_002284334.2_[Botrytis_cinerea_mitovirus_1]-Botrytis_cinerea
YP_009553678.1_[Rhizophagus_diaphanum_mitovirus_1]-Rhizophagus_diaphanum
YP_009552077.1_[Rhizophagus_irregularis_mitovirus_1]-Rhizophagus_irregularis
YP_009553263.1_[Rhizophagus_diaphanum_mitovirus_2]-Rhizophagus_diaphanum
YP_009553587.1_[Gigaspora_margarita_mitovirus_2]-Gigaspora_margarita
YP_009551961.1_[Gigaspora_margarita_mitovirus_4]-Gigaspora_margarita
YP_009551960.1_[Gigaspora_margarita_mitovirus_3]-Gigaspora_margarita
Gergich narnavirus (GUNCC_DN51952-20)
Dwyer narnavirus (GUNCC_DN40210-32)
Dwyer narnavirus (GUNCC_DN53205-15)
ASM94067.1_[Barns_Ness_breadcrumb_sponge_narna-like_virus_2]-Halichondria_panicea_209aa*
Orin narnavirus (GUNCC_DN51633-30)
Orin narnavirus (GUNCC_DN51633-27)
YP_009333146.1_[Beihai_narna-like_virus_26]-tunicates
Jeanralphio narnavirus (GudgCC_DN40006-28)
Lisebastian narnavirus (GudgCC_DN40000-108)
Lisebastian narnavirus (GUNCC_DN53793-107)
YP_009336749.1_[Hubei_narna-like_virus_22]-leech_239aa*
Callamezzo narnavirus (GudgCC_DN39874-22)
YP_009336767.1_[Hubei_narna-like_virus_23]-leech
Wyatt narnavirus (GudgCC_Contig1-4)
Wyatt narnavirus (GUNCC_DN49696-33)
Swanson narnavirus (GUNCC_DN54382-8)
YP_009553325.1_[Leptomonas_seymouri_Narna-like_virus_1]-Leptomonas_seymouri
YP_009256557.1_[Phytomonas_serpens_narnavirus_1]-Phytomonas_serpens
YP_009333140.1_[Beihai_narna-like_virus_21]-Sesarmid_crab
MN167477_[Sherlock_virus]-flea_Stephanocircus
YP_009241365.1_[Phytophthora_infestans_RNA_virus_4]-Phytophthora_infestans
ASA47364.1_[Wilkie_narna-like_virus_1]-MosquitoFungi
YP_009337783.1_[Hubei_narna-like_virus_15]-Coleoptera
Meagle narnavirus (GUNCaA_DN59031-101)
Meagle narnavirus (GudgCaA_DN58802-99)
Meagle narnavirus (GudgF_Contig1-95)
YP_009552002.1_[Blechomonas_maslovi_narnavirus_1]-Blechomonas_maslovi
NP_660177.1_[Saccharomyces_23S_RNA_narnavirus]-yeast
YP_009388580.1_[Point-Douro_narna-like_virus]-mosquito_fungi?
MN167496_[Brunton_virus]-flea_Pygiopsylla_210aa*
NP_660178.1_[Saccharomyces_20S_RNA_narnavirus]-yeast
Ludgate narnavirus (GUNSasp_DN39104-14)
Tynnyfer naranavirus (GudgF_DN16878-34)
Tynnyfer naranavirus (GUNF_DN24600-23)
YP_009553634.1_[Blechmonas_luni_narnavirus_1]-Blechmonas_luni
YP_009552755.1_[Blechomonas_wendygibsoni_narnavirus_1]-Blechomonas_wendygibsoni
Middlebrook narnavirus (GUNSasp_DN26499-124)
AYP67570.1_[Wangarabell_virus]-Ixodes_holocyclus
Haverford narnavirus (GudgUn_DN53489)
YP_009337792.1_[Hubei_narna-like_virus_19]-Odonata
YP_009272902.1_[Fusarium_poae_narnavirus_1]-Fusarium_poae
Legend - taxa names
Viruses from this study by source:
Rabbit caecum
Fly species
Flea
New virus species
GenBank virus "hosts":
Invertebrate
Fungi/mould/yeast
Vertebrate
Trypanosomatidae
Stramenopiles
Protozoa
Viridiplantae
Dicistroviridae
Cripavirus
Aparavirus
Centovirus
AMO03208.1_[Empeyrat_virus]-Scaptodrosophila_deflexa
YP_009221981.1_[Goose_dicistrovirus]-goose_feces
Goose dicistrovirus (GUNSasp_Contig1-5)
YP_009336571.1_[Hubei_diptera_virus_1]-Diptera
AYQ66681.1_[Drosophila_kikkawai_virus_1]-Drosophila_kikkawai
YP_009388499.1_[Apis_dicistrovirus]-Apis_mellifera
YP_009252204.1_[Anopheles_C_virus]-Anopheles_coluzzii
NP_647481.1_[Cricket_paralysis_virus]-insects
NP_044945.1_[Drosophila_C_virus]-drosphila
YP_009329822.1_[Bivalve_RNA_virus_G5]-bivalve_gills
YP_009329817.1_[Bivalve_RNA_virus_G1]-bivalve_gills
YP_006666503.2_[Macrobrachium_rosenbergii_Taihu_virus]-Macrobrachium_rosenbergii
NP_149057.1_[Taura_syndrome_virus]-Penaeus_vannamei
YP_004063985.1_[Mud_crab_virus]-Scylla_serrata_mud_crab
YP_164440.1_[Solenopsis_invicta_virus_1]-Solenopsis_invicta
NP_066241.1_[Acute_bee_paralysis_virus]-Apis_mellifera
YP_008888535.1_[Formica_exsecta_virus_1]-Formica_exsecta
YP_001040002.1_[Israeli_acute_paralysis_virus]-honeybees
NP_851403.1_[Kashmir_bee_virus]-bee
NP_733845.1_[Aphid_lethal_paralysis_virus]-aphid
NP_046155.1_[Rhopalosiphum_padi_virus]-Rhopalosiphum_padi
YP_009389287.1_[Big_Sioux_River_virus]-Aphis_fabae
YP_009380528.1_[Aphis_glycines_virus_3]-Aphis_glycines
Gungahlin Chrysomya dicistrovirus (GUNChV_Contig1-1) 256aa*
AWK77854.1_[Bundaberg_bee_virus_1]-Apis_mellifera
YP_009315870.1_[Mosquito_dicistrovirus]-mosquito
YP_009315868.1_[Centovirus_AC]-mosquito
YP_610950.1_[Homalodisca_coagulata_virus_1]-Homalodisca_coagulata
NP_620555.1_[Plautia_stali_intestine_virus]-Plautia_stali
NP_620562.1_[Triatoma_virus]-Triatoma_infestans
NP_620564.1_[Black_queen_cell_virus]-Apis_mellifera
NP_620560.1_[Himetobi_P_virus]-Laodelphax_striatellus
Iflaviviridae
YP_009552119.1_[Varroa_destructor_virus_2]-Varroa_destructor_mite
YP_009351892.1_[Pityohyphantes_rubrofasciatus_iflavirus]-Pityohyphantes
YP_009552080.1_[Yongsan_iflavirus_1]-Culex_pipiens
YP_009448183.1_[Armigeres_iflavirus]-Armigeres_spp.
YP_009830809.1_[Nilaparvata_lugens_honeydew_virus-2]-Nilaparvata_lugens
YP_009505599.1_[Nilaparvata_lugens_honeydew_virus_1]-Nilaparvata_lugens
YP_009010941.1_[Laodelphax_striatella_honeydew_virus_1]-Laodelphax_striatella
YP_009553259.1_[Psammotettix_alienus_iflavirus_1]-Psammotettix_alienus
YP_009328883.1_[Euscelidius_variegatus_virus_1]-Euscelidius_variegatus
YP_009129265.1_[Graminella_nigrifrons_virus_1]-Graminella_nigrifrons
MN167503_[Moran_virus]-flea_Pygiopsylla
YP_003622540.1_[Slow_bee_paralysis_virus]-bee
YP_009345906.1_[Bat_iflavirus]-Eidolon_helvum
YP_009305421.1_[Moku_virus]-Vespula_pensylvanica
YP_009315906.1_[King_virus]-Yuma_myotis
YP_009344960.1_[Helicoverpa_armigera_iflavirus]-Helicoverpa_armigera
YP_009026409.1_[Heliconius_erato_iflavirus]-Heliconius_erato
YP_009009818.1_[Antheraea_pernyi_iflavirus]-Antheraea_pernyi
YP_009162630.1_[Bombyx_mori_iflavirus]-Bombyx_mori_strain_Kinsyu-Syowa
YP_009047245.1_[Lymantria_dispar_iflavirus_1]-Lymantria_dispar
YP_009116875.1_[Thaumetopoea_pityocampa_iflavirus_1]-Thaumetopoea_pityocampa
YP_009030310.1_[Nilaparvata_lugens_honeydew_virus-3]-Nilaparvata_lugens
YP_008888537.1_[Formica_exsecta_virus_2]-Formica_exsecta
YP_145791.1_[Varroa_destructor_virus_1]-Varroa_destructor_mites
NP_853560.2_[Deformed_wing_virus]-Apis_mellifera
YP_009552832.1_[Nesidiocoris_tenuis_iflavirus_1]-Nesidiocoris
MN167498_[Carfax_virus]-flea_Macropsylla_313aa*
YP_009330055.1_[Hubei_picorna-like_virus_31]-Odonata
MN167484_[Watson_virus]-flea_Pygiopsylla
ACT flea iflavirus (GudgF_Contig1-207)
ACT flea iflavirus (GUNF_Contig8-195)
YP_001285409.1_[Brevicoryne_brassicae_virus_-_UK]-Brevicoryne_brassicae
YP_009444707.1_[Chequa_iflavirus]-Cherax_quadricarinatus
YP_009111311.1_[Dinocampus_coccinellae_paralysis_virus]-Dinocampus_coccinellae
MN167485_[Basskerville_virus]-flea_Pygiopsylla
YP_009010984.1_[Spodoptera_exigua_iflavirus_2]-Spodoptera_exigua
YP_009361829.1_[Diamondback_moth_iflavirus]-Plutella_xylostella
NP_919029.1_[Ectropis_obliqua_picorna-like_virus]-Ectropis_obliqua
YP_008719809.1_[Halyomorpha_halys_virus]-Halyomorpha_halys
NP_049374.1_[Sacbrood_virus]-bee
YP_009505598.1_[Lygus_lineolaris_virus_1]-Lygus_lineolaris
YP_009140562.1_[La_Jolla_virus]-Drosophila
YP_009552768.1_[Culex_Iflavi-like_virus_1]-Culex_sp._mosquito
YP_009552017.1_[Culex_Iflavi-like_virus_4]-Culex_sp._mosquito
NP_620559.1_[Infectious_flacherie_virus]-silkworm
YP_009167346.1_[Opsiphanes_invirae_iflavirus_1]-Opsiphanes_invirae
YP_009495365.1_[Spodoptera_exigua_iflavirus_1]-Spodoptera_exigua
0.4

Figure 2

Positive-sense ssRNA viruses continued

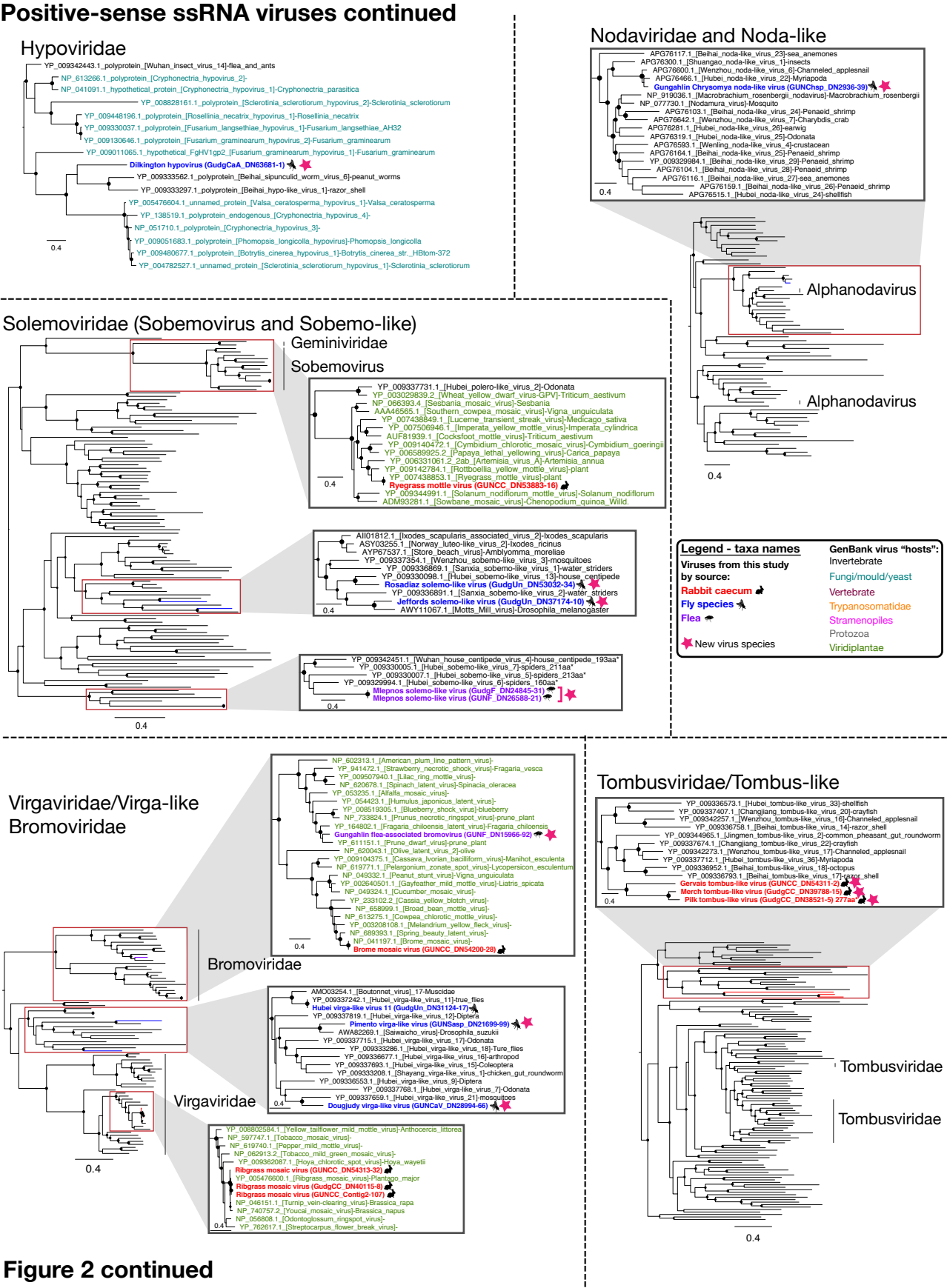


Figure 2 continued

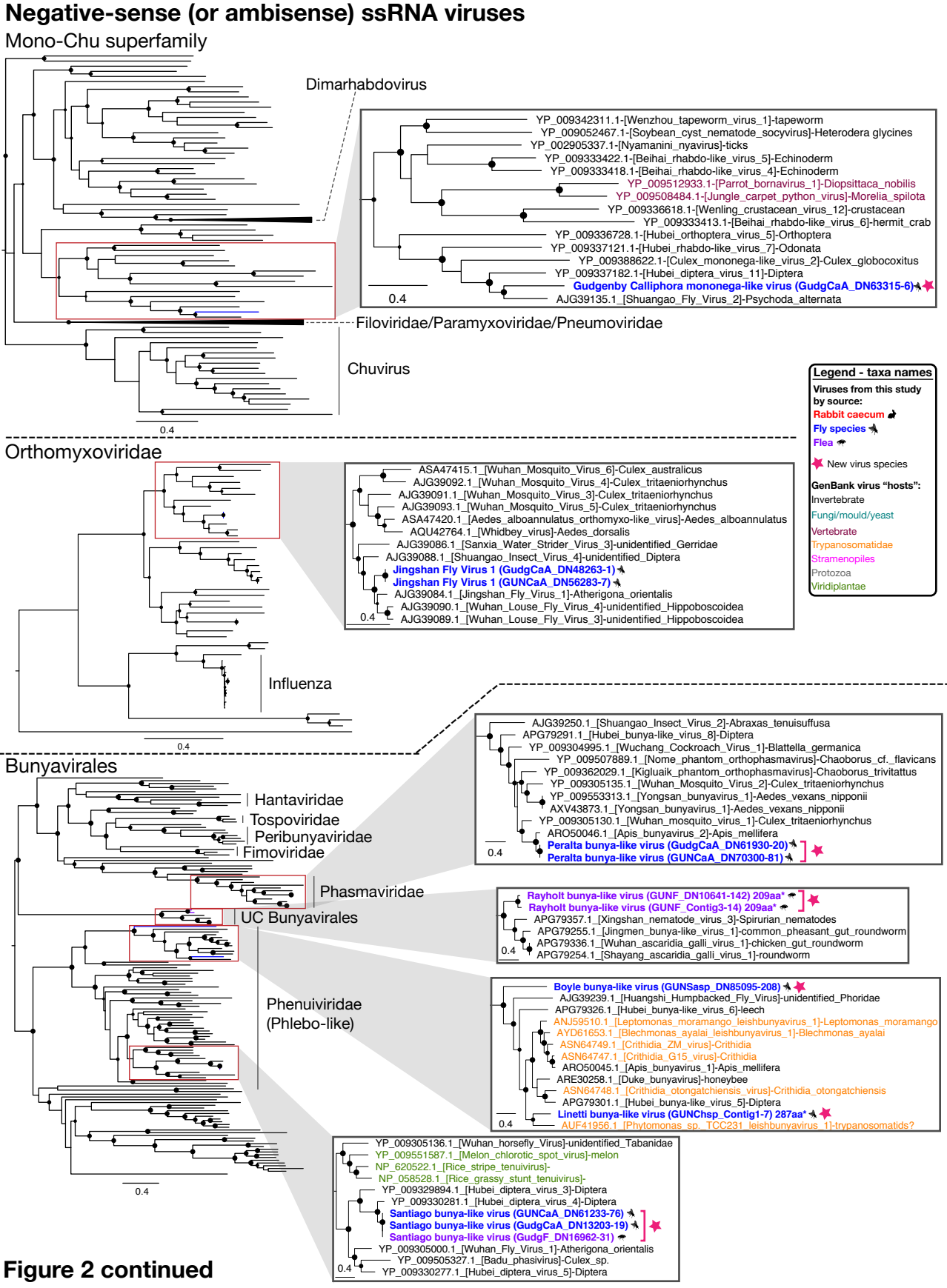


Figure 2 continued

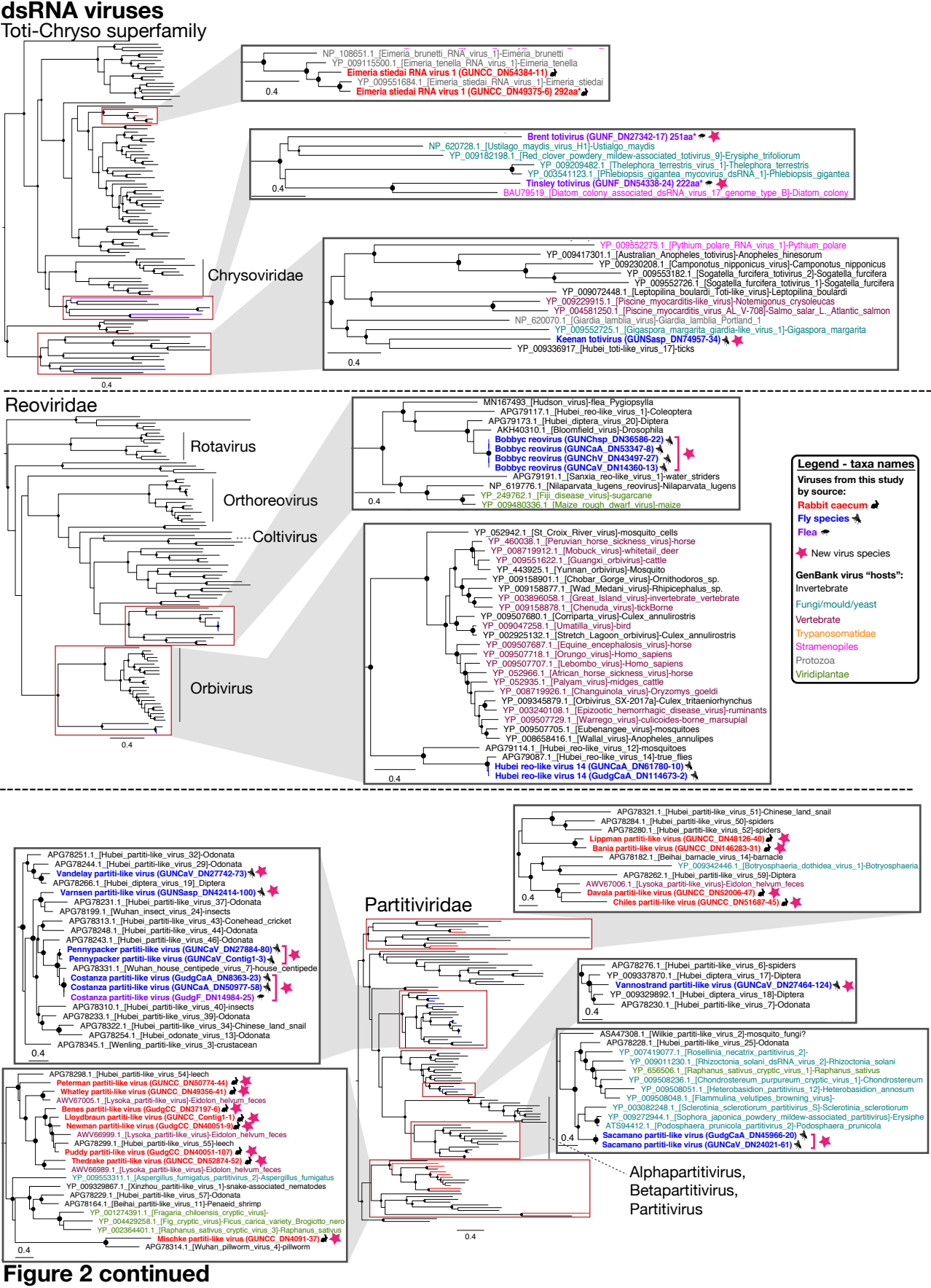


Figure 2 continued

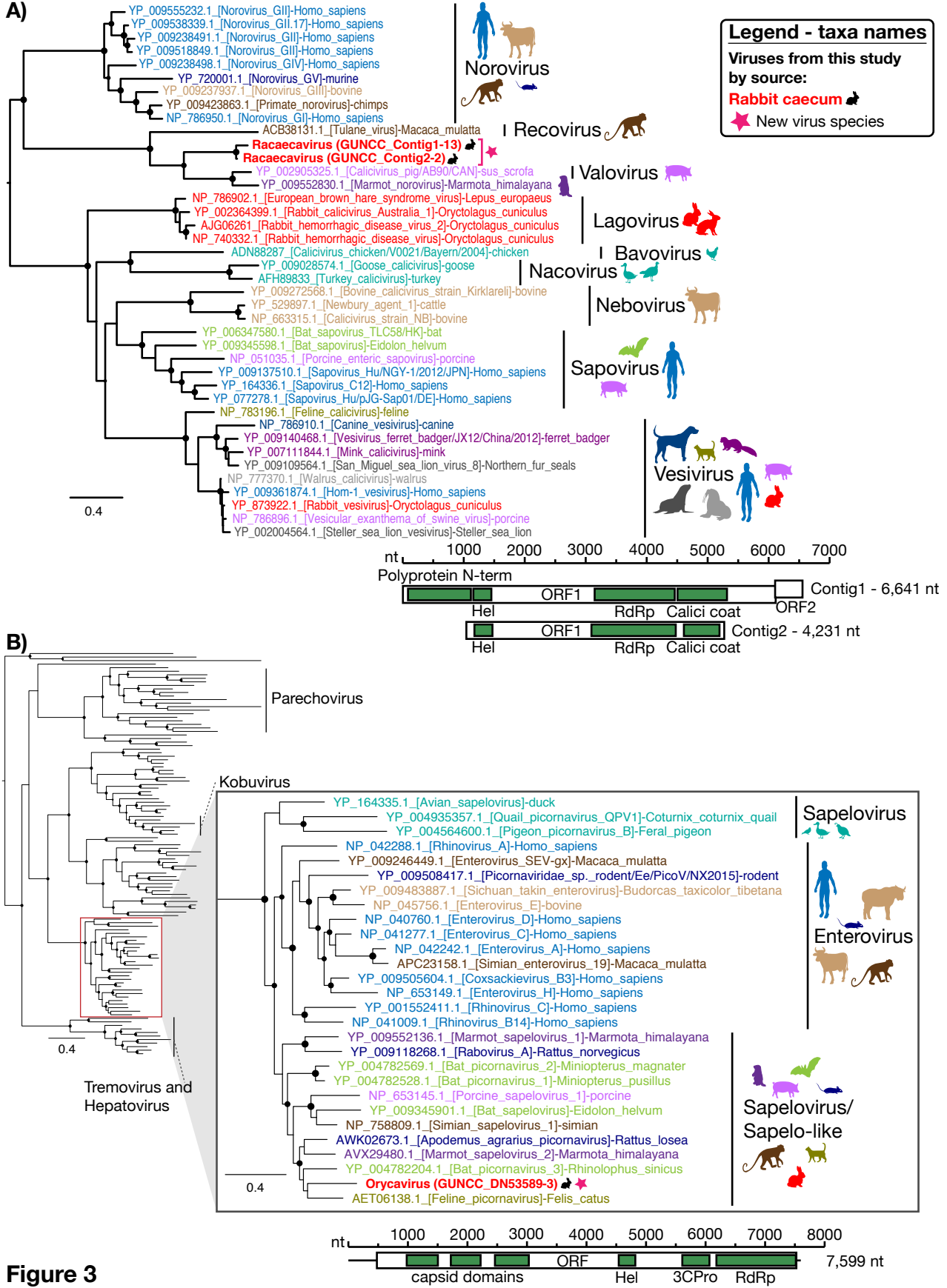

A)
YP_009555232.1_[Norovirus_GII]-Homo_sapiens
YP_009538339.1_[Norovirus_GII.17]-Homo_sapiens
YP_009238491.1_[Norovirus_GII]-Homo_sapiens
YP_009518849.1_[Norovirus_GII]-Homo_sapiens
YP_009238498.1_[Norovirus_GIV]-Homo_sapiens
YP_720001.1_[Norovirus_GV]-murine
YP_009237937.1_[Norovirus_GIII]-bovine
YP_009423863.1_[Primate_norovirus]-chimps
NP_786950.1_[Norovirus_GI]-Homo_sapiens
Norovirus
ACB38131.1_[Tulane_virus]-Macaca_mulatta
Recovirus
Racaecavirus (GUNCC_Contig1-13)
Racaecavirus (GUNCC_Contig2-2)
YP_002905325.1_[Calicivirus_pig/AB90/CAN]-sus_scrofa
YP_009552830.1_[Marmot_norovirus]-Marmota_himalayana
Valovirus
NP_786902.1_[European_brown_hare_syndrome_virus]-Lepus_europaeus
YP_002364399.1_[Rabbit_calicivirus_Australia_1]-Oryctolagus_cuniculus
AJG06261_[Rabbit_hemorrhagic_disease_virus_2]-Oryctolagus_cuniculus
NP_740332.1_[Rabbit_hemorrhagic_disease_virus]-Oryctolagus_cuniculus
Lagovirus
ADN88287_[Calicivirus_chicken/V0021/Bayern/2004]-chicken
Bavovirus
YP_009028574.1_[Goose_calicivirus]-goose
AFH89833_[Turkey_calicivirus]-turkey
Nacovirus
YP_009272568.1_[Bovine_calicivirus_strain_Kirklareli]-bovine
YP_529897.1_[Newbury_agent_1]-cattle
NP_663315.1_[Calicivirus_strain_NB]-bovine
Nebovirus
YP_006347580.1_[Bat_sapovirus_TLC58/HK]-bat
YP_009345598.1_[Bat_sapovirus]-Eidolon_helvum
NP_051035.1_[Porcine_enteric_sapovirus]-porcine
YP_009137510.1_[Sapovirus_Hu/NGY-1/2012/JPN]-Homo_sapiens
YP_164336.1_[Sapovirus_C12]-Homo_sapiens
YP_077278.1_[Sapovirus_Hu/pJG-Sap01/DE]-Homo_sapiens
Sapovirus
NP_783196.1_[Feline_calicivirus]-feline
NP_786910.1_[Canine_vesivirus]-canine
YP_009140468.1_[Vesivirus_ferret_badger/JX12/China/2012]-ferret_badger
YP_007111844.1_[Mink_calicivirus]-mink
YP_009109564.1_[San_Miguel_sea_lion_virus_8]-Northern_fur_seals
NP_777370.1_[Walrus_calicivirus]-walrus
YP_009361874.1_[Hom-1_vesivirus]-Homo_sapiens
YP_873922.1_[Rabbit_vesivirus]-Oryctolagus_cuniculus
NP_786896.1_[Vesicular_exanthema_of_swine_virus]-porcine
YP_002004564.1_[Steller_sea_lion_vesivirus]-Steller_sea_lion
Vesivirus
0.4
Legend - taxa names
Viruses from this study by source:
Rabbit caecum
New virus species
nt
1000
2000
3000
4000
5000
6000
7000
Polyprotein N-term
ORF1
Hel
RdRp
Calici coat
ORF2
Contig1 - 6,641 nt
Contig2 - 4,231 nt
B)
Parechovirus
Kobuvirus
YP_164335.1_[Avian_sapelovirus]-duck
YP_004935357.1_[Quail_picornavirus_QPV1]-Coturnix_coturnix_quail
YP_004564600.1_[Pigeon_picornavirus_B]-Feral_pigeon
NP_042288.1_[Rhinovirus_A]-Homo_sapiens
YP_009246449.1_[Enterovirus_SEV-gx]-Macaca_mulatta
YP_009508417.1_[Picornaviridae_sp._rodent/Ee/PicoV/NX2015]-rodent
YP_009483887.1_[Sichuan_takin_enterovirus]-Budorcas_taxicolor_tibetana
NP_045756.1_[Enterovirus_E]-bovine
NP_040760.1_[Enterovirus_D]-Homo_sapiens
NP_041277.1_[Enterovirus_C]-Homo_sapiens
NP_042242.1_[Enterovirus_A]-Homo_sapiens
APC23158.1_[Simian_enterovirus_19]-Macaca_mulatta
YP_009505604.1_[Coxsackievirus_B3]-Homo_sapiens
NP_653149.1_[Enterovirus_H]-Homo_sapiens
YP_001552411.1_[Rhinovirus_C]-Homo_sapiens
NP_041009.1_[Rhinovirus_B14]-Homo_sapiens
YP_009552136.1_[Marmot_sapelovirus_1]-Marmota_himalayana
YP_009118268.1_[Rabovirus_A]-Rattus_norvegicus
YP_004782569.1_[Bat_picornavirus_2]-Miniopterus_magnater
YP_004782528.1_[Bat_picornavirus_1]-Miniopterus_pusillus
NP_653145.1_[Porcine_sapelovirus_1]-porcine
YP_009345901.1_[Bat_sapelovirus]-Eidolon_helvum
NP_758809.1_[Simian_sapelovirus_1]-simian
AWK02673.1_[Apodemus_agrarius_picornavirus]-Rattus_losea
AVX29480.1_[Marmot_sapelovirus_2]-Marmota_himalayana
YP_004782204.1_[Bat_picornavirus_3]-Rhinolophus_sinicus
Orycavirus (GUNCC_DN53589-3)
AET06138.1_[Feline_picornavirus]-Felis_catus
Sapelovirus
Enterovirus
Sapelovirus/
Sapelo-like
0.4
Tremovirus and
Hepatovirus
nt
1000
2000
3000
4000
5000
6000
7000
8000
ORF
capsid domains
Hel
3CPro
RdRp
7,599 nt

Figure 3

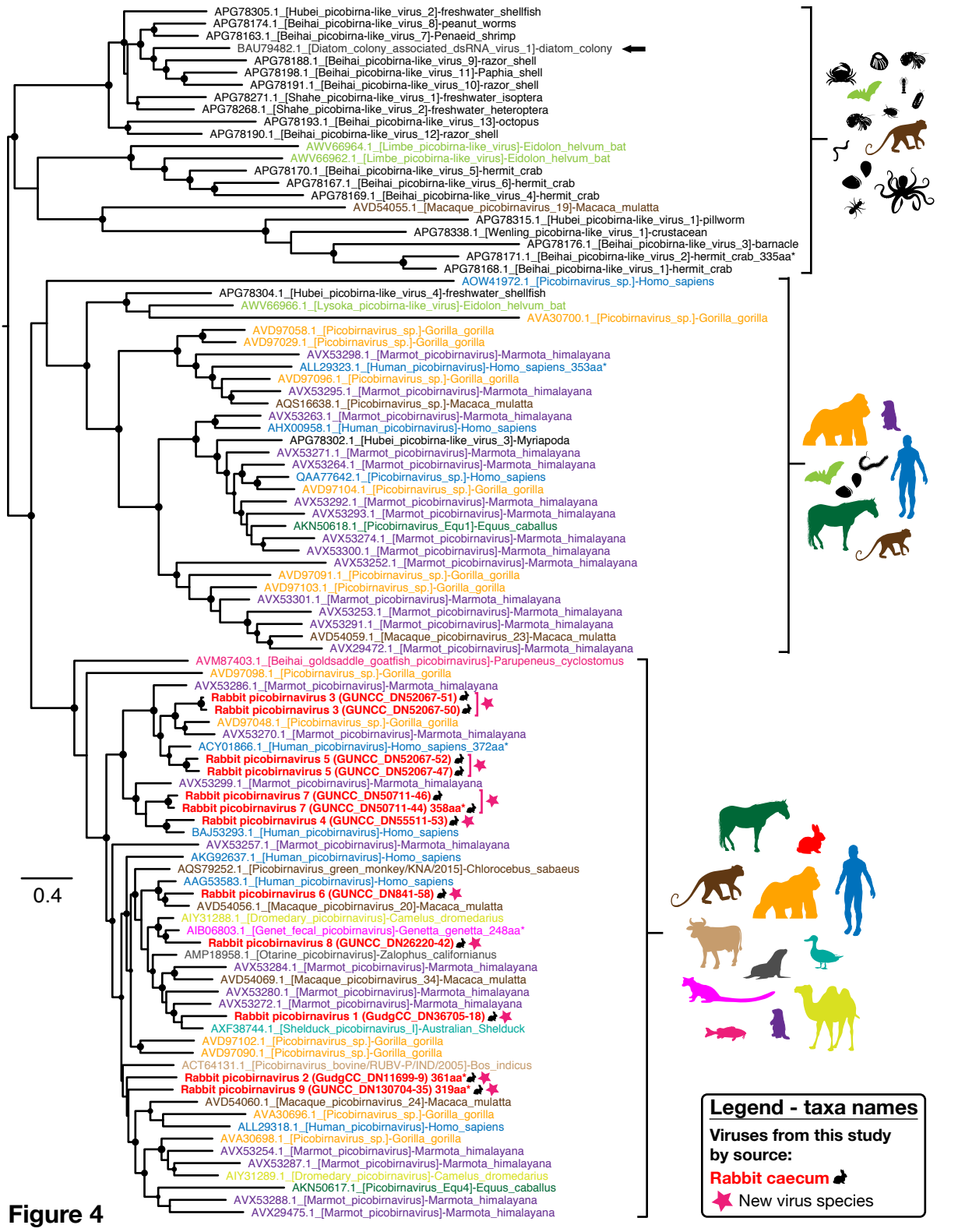

APG78305.1_[Hubei_picobirna-like_virus_2]-freshwater_shellfish
APG78174.1_[Beihai_picobirna-like_virus_8]-peanut_worms
APG78163.1_[Beihai_picobirna-like_virus_7]-Penaeid_shrimp
BAU79482.1_[Diatom_colony_associated_dsRNA_virus_1]-diatom_colony
APG78188.1_[Beihai_picobirna-like_virus_9]-razor_shell
APG78198.1_[Beihai_picobirna-like_virus_11]-Paphia_shell
APG78191.1_[Beihai_picobirna-like_virus_10]-razor_shell
APG78271.1_[Shahe_picobirna-like_virus_1]-freshwater_isoptera
APG78268.1_[Shahe_picobirna-like_virus_2]-freshwater_heteroptera
APG78193.1_[Beihai_picobirna-like_virus_13]-octopus
APG78190.1_[Beihai_picobirna-like_virus_12]-razor_shell
AWV66964.1_[Limbe_picobirna-like_virus]-Eidolon_helvum_bat
AWV66962.1_[Limbe_picobirna-like_virus]-Eidolon_helvum_bat
APG78170.1_[Beihai_picobirna-like_virus_5]-hermit_crab
APG78167.1_[Beihai_picobirna-like_virus_6]-hermit_crab
APG78169.1_[Beihai_picobirna-like_virus_4]-hermit_crab
AVD54055.1_[Macaque_picobirnavirus_19]-Macaca_mulatta
APG78315.1_[Hubei_picobirna-like_virus_1]-pillworm
APG78338.1_[Wenling_picobirna-like_virus_1]-crustacean
APG78176.1_[Beihai_picobirna-like_virus_3]-barnacle
APG78171.1_[Beihai_picobirna-like_virus_2]-hermit_crab_335aa*
APG78168.1_[Beihai_picobirna-like_virus_1]-hermit_crab
AOW41972.1_[Picobirnavirus_sp.]-Homo_sapiens
APG78304.1_[Hubei_picobirna-like_virus_4]-freshwater_shellfish
AWV66966.1_[Lysoka_picobirna-like_virus]-Eidolon_helvum_bat
AVA30700.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVD97058.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVD97029.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVX53298.1_[Marmot_picobirnavirus]-Marmota_himalayana
ALL29323.1_[Human_picobirnavirus]-Homo_sapiens_353aa*
AVD97096.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVX53295.1_[Marmot_picobirnavirus]-Marmota_himalayana
AQS16638.1_[Picobirnavirus_sp.]-Macaca_mulatta
AVX53263.1_[Marmot_picobirnavirus]-Marmota_himalayana
AHX00958.1_[Human_picobirnavirus]-Homo_sapiens
APG78302.1_[Hubei_picobirna-like_virus_3]-Myriapoda
AVX53271.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVX53264.1_[Marmot_picobirnavirus]-Marmota_himalayana
QAA77642.1_[Picobirnavirus_sp.]-Homo_sapiens
AVD97104.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVX53292.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVX53293.1_[Marmot_picobirnavirus]-Marmota_himalayana
AKN50618.1_[Picobirnavirus_Equ1]-Equus_caballus
AVX53274.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVX53300.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVX53252.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVD97091.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVD97103.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVX53301.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVX53253.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVX53291.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVD54059.1_[Macaque_picobirnavirus_23]-Macaca_mulatta
AVX29472.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVM87403.1_[Beihai_goldsaddle_goatfish_picobirnavirus]-Parupeneus_cyclostomus
AVD97098.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVX53286.1_[Marmot_picobirnavirus]-Marmota_himalayana
Rabbit picobirnavirus 3 (GUNCC_DN52067-51)
Rabbit picobirnavirus 3 (GUNCC_DN52067-50)
AVD97048.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVX53270.1_[Marmot_picobirnavirus]-Marmota_himalayana
ACY01866.1_[Human_picobirnavirus]-Homo_sapiens_372aa*
Rabbit picobirnavirus 5 (GUNCC_DN52067-52)
Rabbit picobirnavirus 5 (GUNCC_DN52067-47)
AVX53299.1_[Marmot_picobirnavirus]-Marmota_himalayana
Rabbit picobirnavirus 7 (GUNCC_DN50711-46)
Rabbit picobirnavirus 7 (GUNCC_DN50711-44) 358aa*
Rabbit picobirnavirus 4 (GUNCC_DN55511-53)
BAJ53293.1_[Human_picobirnavirus]-Homo_sapiens
AVX53257.1_[Marmot_picobirnavirus]-Marmota_himalayana
AKG92637.1_[Human_picobirnavirus]-Homo_sapiens
AQS79252.1_[Picobirnavirus_green_monkey/KNA/2015]-Chlorocebus_sabaeus
AAG53583.1_[Human_picobirnavirus]-Homo_sapiens
Rabbit picobirnavirus 6 (GUNCC_DN841-58)
AVD54056.1_[Macaque_picobirnavirus_20]-Macaca_mulatta
AIY31288.1_[Dromedary_picobirnavirus]-Camelus_dromedarius
AIB06803.1_[Genet_fecal_picobirnavirus]-Genetta_genetta_248aa*
Rabbit picobirnavirus 8 (GUNCC_DN26220-42)
AMP18958.1_[Otarine_picobirnavirus]-Zalophus_californianus
AVX53284.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVD54069.1_[Macaque_picobirnavirus_34]-Macaca_mulatta
AVX53280.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVX53272.1_[Marmot_picobirnavirus]-Marmota_himalayana
Rabbit picobirnavirus 1 (GudgCC_DN36705-18)
AXF38744.1_[Shelduck_picobirnavirus_I]-Australian_Shelduck
AVD97102.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVD97090.1_[Picobirnavirus_sp.]-Gorilla_gorilla
ACT64131.1_[Picobirnavirus_bovine/RUBV-P/IND/2005]-Bos_indicus
Rabbit picobirnavirus 2 (GudgCC_DN11699-9) 361aa*
Rabbit picobirnavirus 9 (GUNCC_DN130704-35) 319aa*
AVD54060.1_[Macaque_picobirnavirus_24]-Macaca_mulatta
AVA30696.1_[Picobirnavirus_sp.]-Gorilla_gorilla
ALL29318.1_[Human_picobirnavirus]-Homo_sapiens
AVA30698.1_[Picobirnavirus_sp.]-Gorilla_gorilla
AVX53254.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVX53287.1_[Marmot_picobirnavirus]-Marmota_himalayana
AIY31289.1_[Dromedary_picobirnavirus]-Camelus_dromedarius
AKN50617.1_[Picobirnavirus_Equ4]-Equus_caballus
AVX53288.1_[Marmot_picobirnavirus]-Marmota_himalayana
AVX29475.1_[Marmot_picobirnavirus]-Marmota_himalayana
0.4
Legend - taxa names
Viruses from this study by source:
Rabbit caecum
New virus species

Figure 4

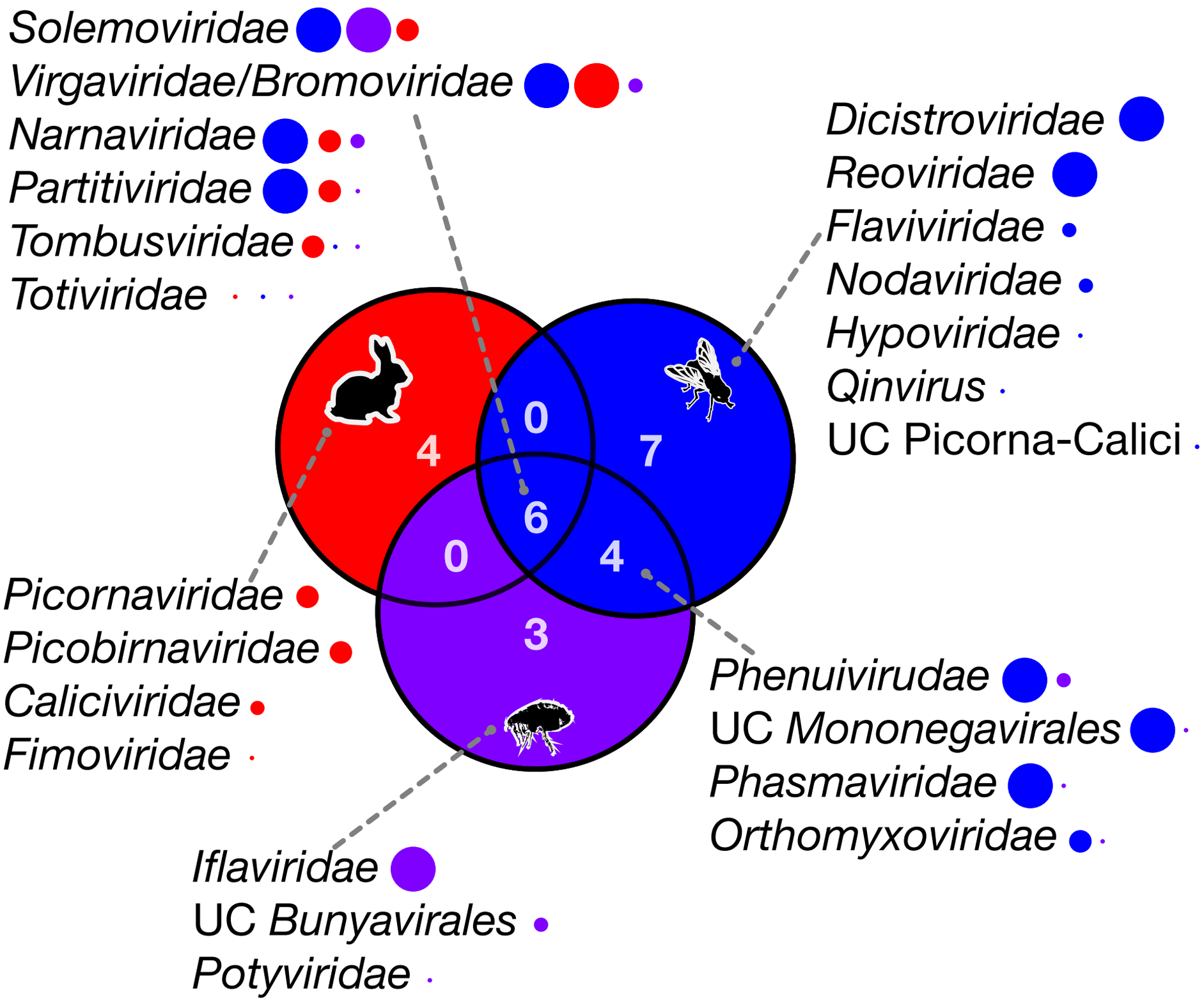

Solemoviridae
Virgaviridae/Bromoviridae
Narnaviridae
Partitiviridae
Tombusviridae
Totiviridae
Dicistroviridae
Reoviridae
Flaviviridae
Nodaviridae
Hypoviridae
Qinvirus
UC Picorna-Calici
4
0
7
6
0
4
3
Picornaviridae
Picobirnaviridae
Caliciviridae
Fimoviridae
Phenuivirudae
UC Mononegavirales
Phasmaviridae
Orthomyxoviridae
Iflaviridae
UC Bunyavirales
Potyviridae
Virus contig abundance (highest within single library)
>0.1%
0.01 - 0.1%
0.001 - 0.01%
<0.001%

Figure 5

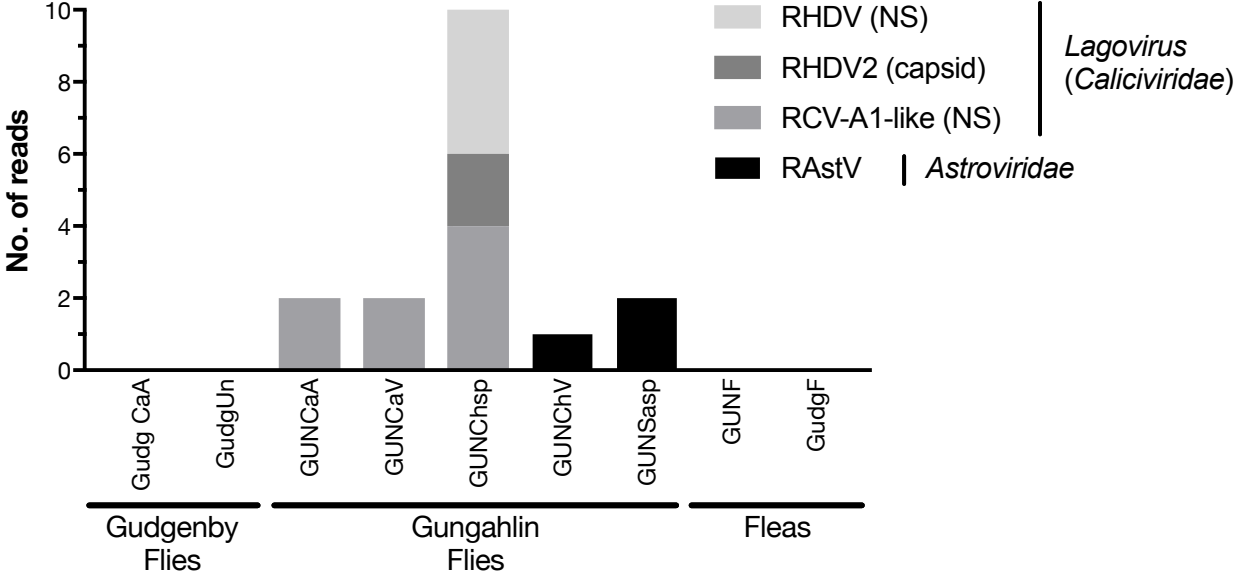

No. of reads
10
8
6
4
2
0
RHDV (NS)
RHDV2 (capsid)
RCV-A1-like (NS)
RAstV
Lagovirus (Caliciviridae)
Astroviridae
Gudg CaA
GudgUn
GUNCaA
GUNCaV
GUNChsp
GUNChV
GUNSasp
GUNF
GudgF
Gudgenby Flies
Gungahlin Flies
Fleas

Figure 6