

Supplementary Information for:

Host ecology and phylogeny shape the temporal dynamics of social bee viromes

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Supplementary Method 1: Primers and protocols used to differentiate the *B. terrestris*/*B. lucorum* complex and *B. hortorum*/*B. ruderatus* complex.

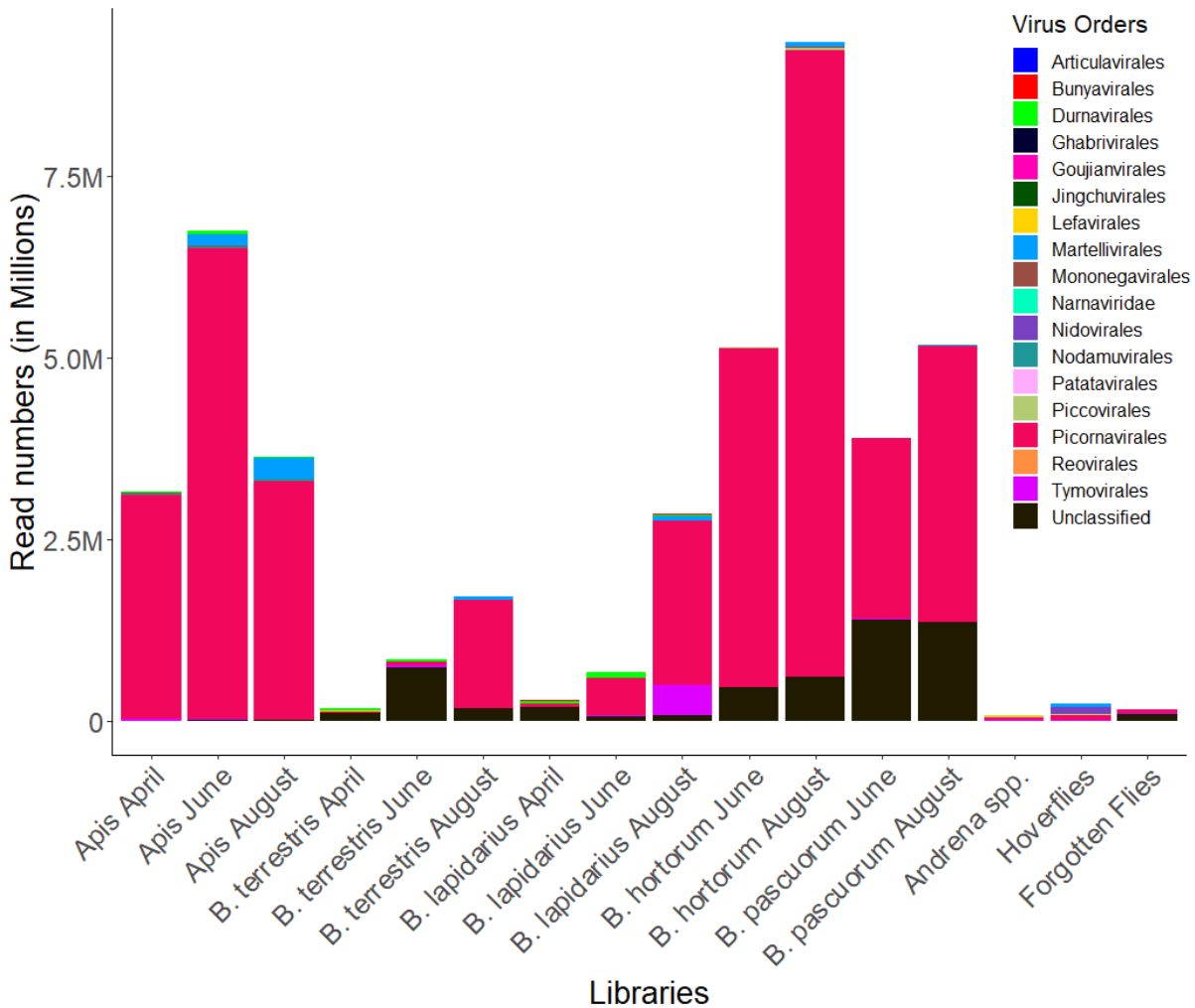
Target	Primer name	Sequence	Amplification program	Amplicon size (bp)	References
<i>B. terrestris</i> / <i>B. lucorum</i> complex	BBM1IGSF BBM1IGSR	GGAGCAATAATTTCAATAAATAG AARTTCAAAGCACTAATCTGC	15s at 95°C 15s at 55°C 45s at 72°C ×38 cycles	180 210	R. Schmid-Hempel (pers. comm.), Manley <i>et al.</i> 2023
<i>B. hortorum</i> / <i>B. ruderatus</i> complex	CYTBF CYTBR	TTCAGCAATTCCATATATTGGAC ATTACACCTCCTCATTATTAGG	30s at 94°C 30s at 48°C 60s at 72°C ×35 cycles	~500	Ellis <i>et al.</i> 2005

References:

Ellis, J.S., Knight, M.E. & Goulson, D. Delimiting species for conservation using molecular markers: the taxonomic status of *Bombus ruderatus* (Fabricius) and *Bombus hortorum* (Linnaeus) (Hymenoptera: Apidae). *Journal of Insect Conservation* **9**, 75-83 (2005).

Manley, R. *et al.* Conservation measures or hotspots of disease transmission? Agri-environment schemes can reduce disease prevalence in pollinator communities. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **378**, 20220004 (2023).

Supplementary Figure 1: Stack plot showing normalized viral read numbers categorized by virus orders in each meta-transcriptome library. Magenta color shows the high prominence of picornavirales in most samples. The bottom dark color represents unclassified viral species. Source data are provided as a Source Data file.



Supplementary Figure 2: Coverage plots of insect virus sequence assemblies with vsiRNA reads.

Sample collection time points are represented in different colors, with April collection data in green, June collection data in red, blue collection data in blue, and mixed time point data in grey (i.e. for mining bees, hoverflies and the 'Forgotten flies'). Coverage depth is represented by number of reads (y axis) along the sequence (x axis) for each virus with detected vsiRNA reads above the defined threshold, in (2a) *Apis mellifera*, (2b) *Bombus terrestris*, (2c) *Bombus lapidarius*, (2d) *Bombus hortorum*, (2e) *Bombus pascuorum*, (2f) mining bees *Andrena* spp., (2g) hoverflies, and (2h) the 'Forgotten flies'. Abbreviations: ABPV = Acute bee paralysis virus, AhaemNLV = *Andrena haemorrhoa* nege-like virus, ARV = *Apis* rhabdovirus, BQCV = Black queen cell virus, CBPV = Chronic bee paralysis virus, DWV = Deformed wing virus, LSV = Lake Sinai virus, SBV = Sacbrood virus, SBPV = Slow bee paralysis virus, VDV-2 = *Varroa destructor* virus-2, VOV-1 = *Varroa orthomyxovirus*-1. Viral assemblies and mapping outputs are available at <https://doi.org/10.6084/m9.figshare.27888378>.

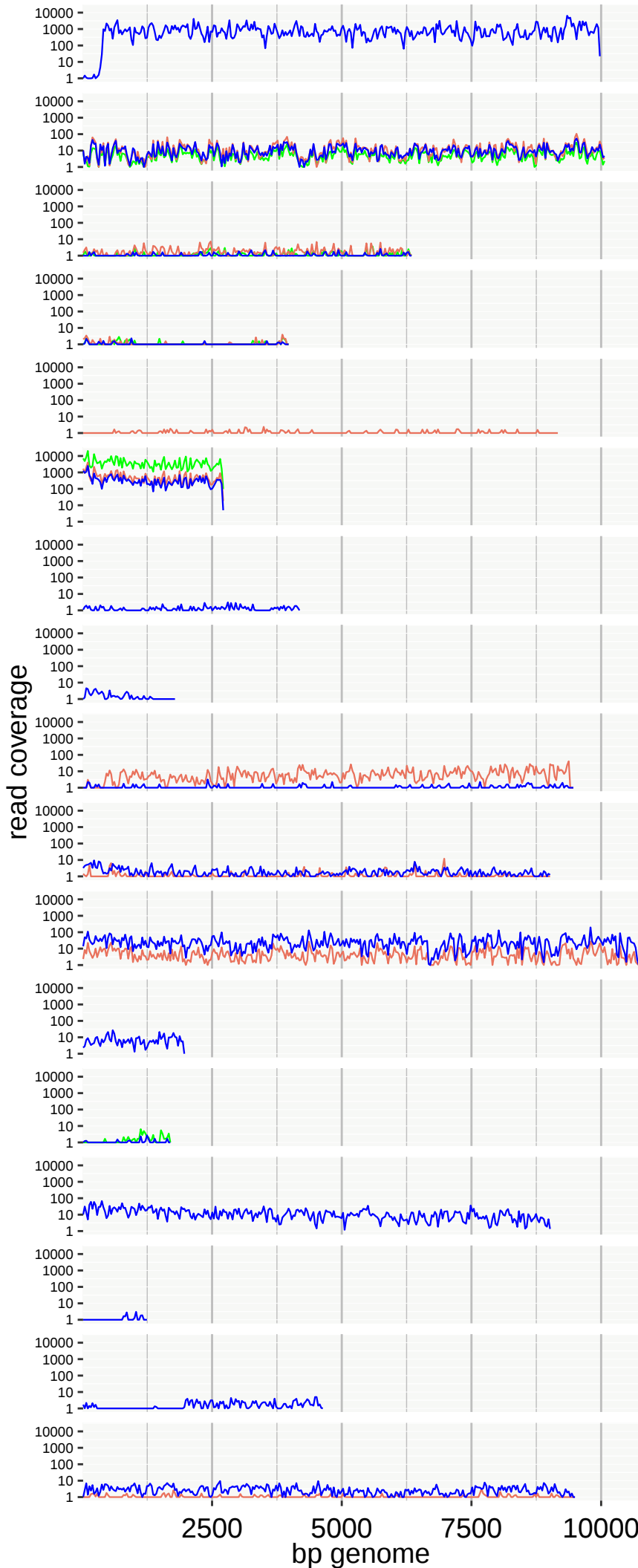
Supplementary Figure 2a

A.mellifera



Supplementary Figure 2b

B. terrestris



ABPV *

Allermuir Hil virus 1 *

Allermuir Hil virus 3

B. cryptarum densovirus bcry3

Black Hill Virus

Castleton Burn Virus

Cnoc Mor Virus

Densovirus Den2

Mayfield Virus 1 bee strain *

Mayfield Virus 2 *

Mill Lade Virus *

New *B. hortorum* Dumyat-like virus

New *B. terrestris* densovirus

New Picornavirales 6 *

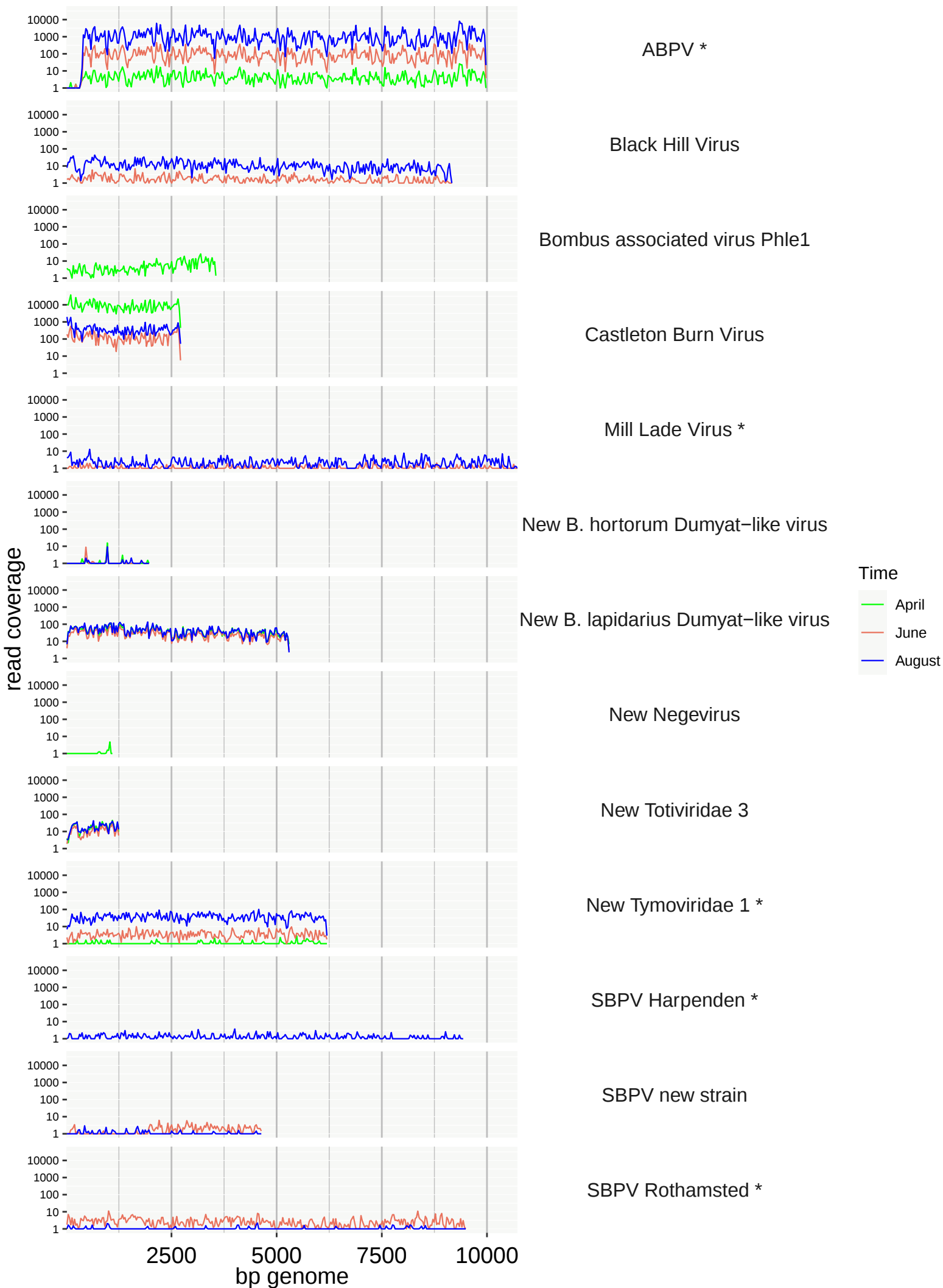
New Totiviridae 3

SBPV new strain

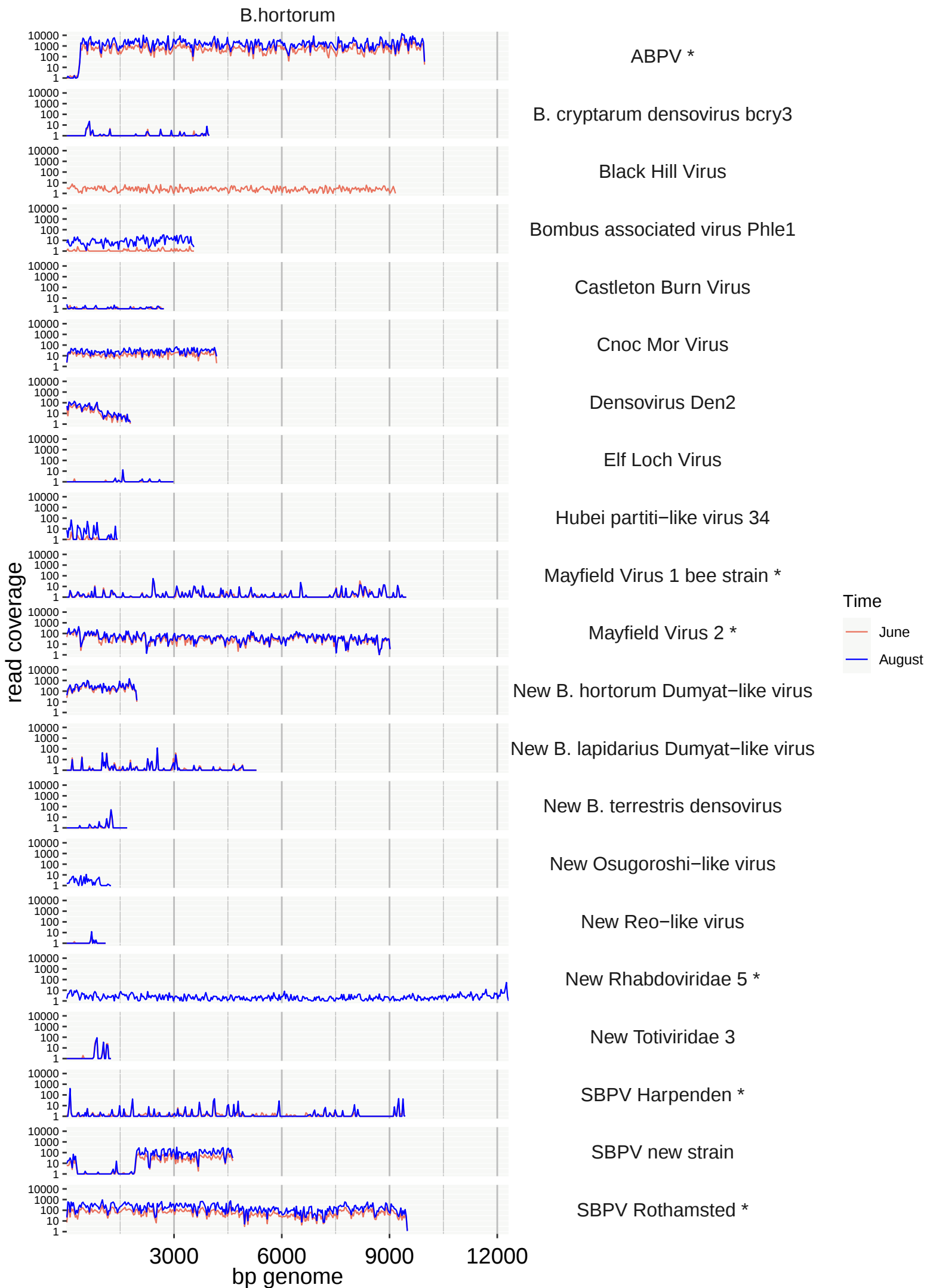
SBPV Rothamsted *

Supplementary Figure 2c

B.lapidarius

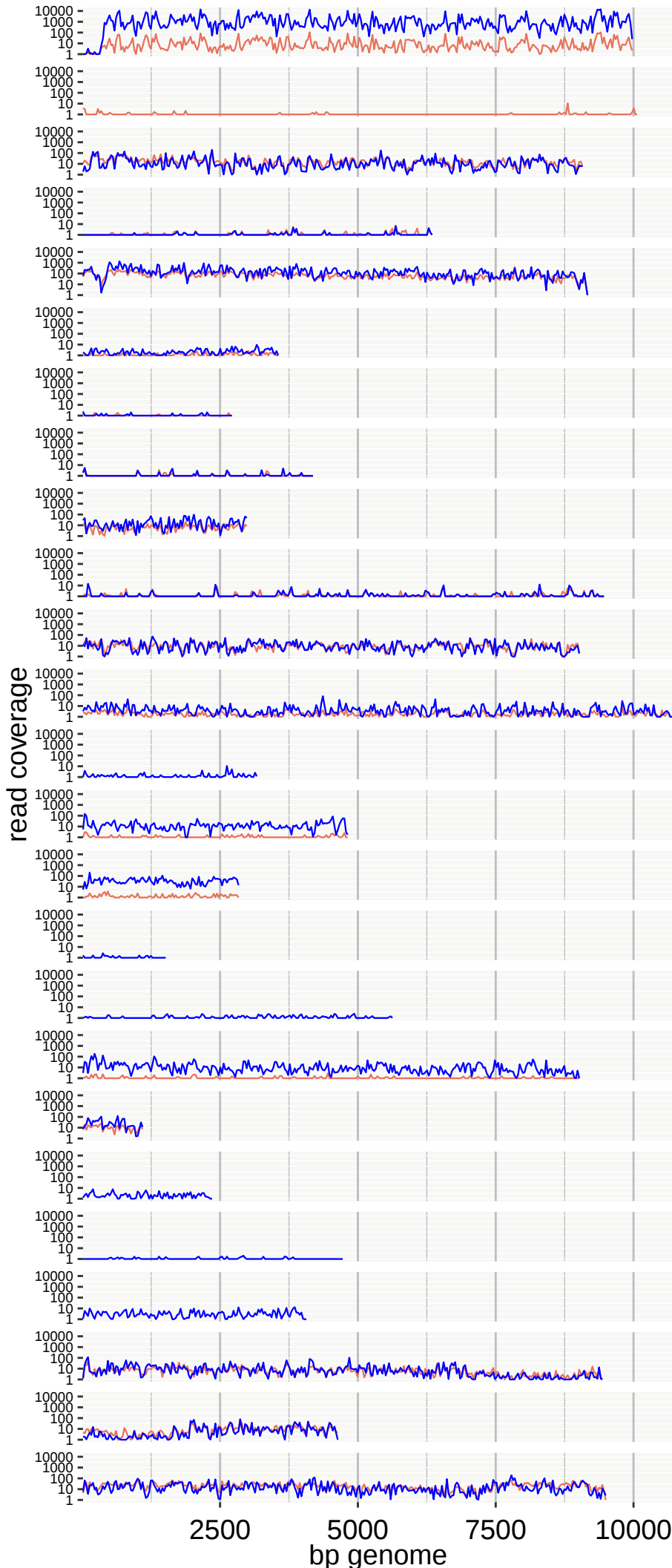


Supplementary Figure 2d



Supplementary Figure 2e

B.pascuorum



ABPV *

Allermuir Hil virus 1 *

Allermuir Hil virus 2

Allermuir Hil virus 3

Black Hill Virus

Bombus associated virus Phle1

Castleton Burn Virus

Cnoc Mor Virus

Elf Loch Virus

Mayfield Virus 1 bee strain *

Mayfield Virus 2 *

Mill Lade Virus *

New Bee Iflavirus 1

New Bee Iflavirus 2

New Castleton Burn-like virus

New Jingchuvirales

New Mayfield-like bee virus 1

New Picornavirales 6 *

New Reo-like virus

New Rhabdoviridae 4

New Totiviridae 2

New Virgaviridae 2

SBPV Harpenden *

SBPV new strain

SBPV Rothamsted *

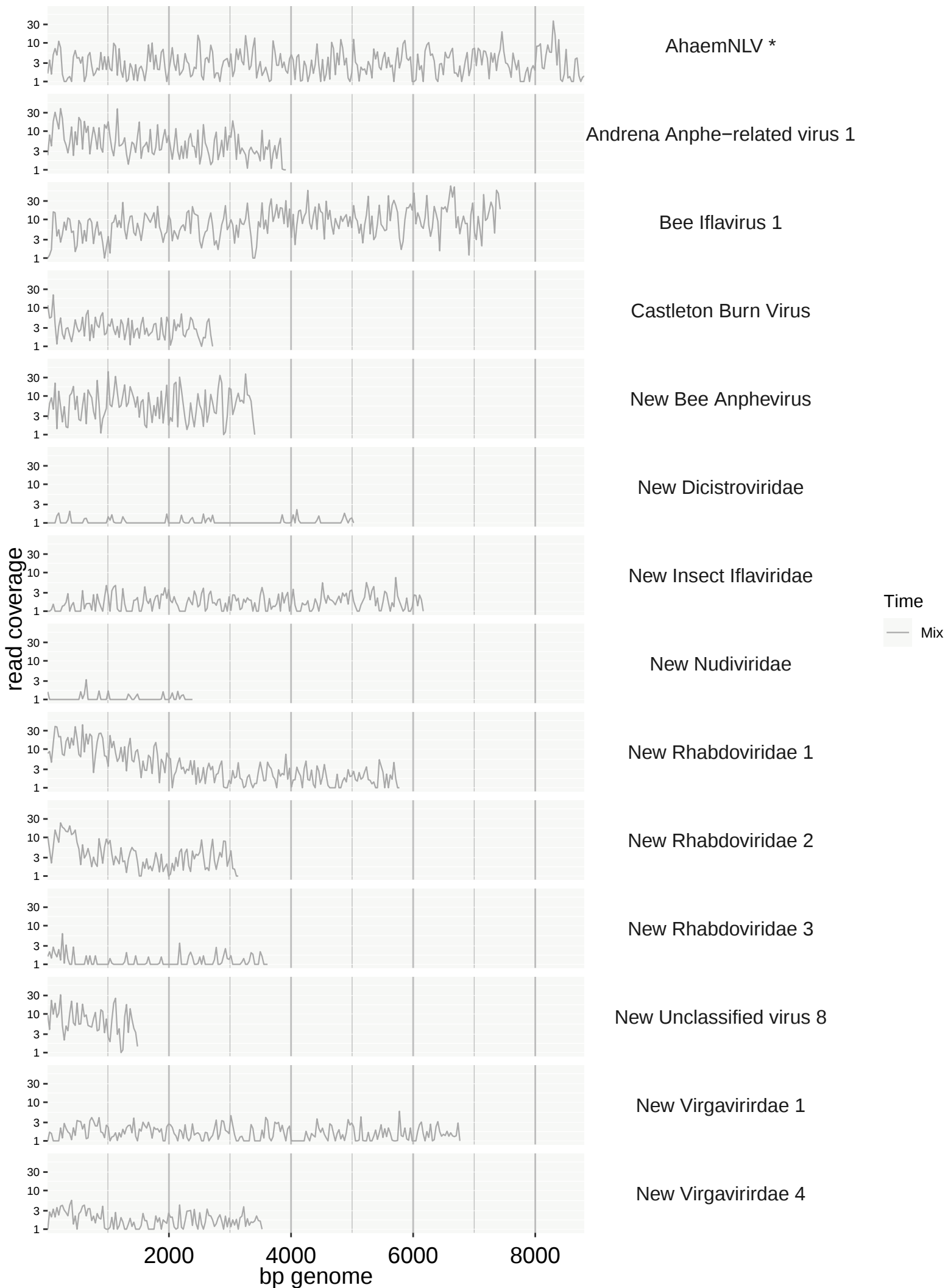
Time

June

August

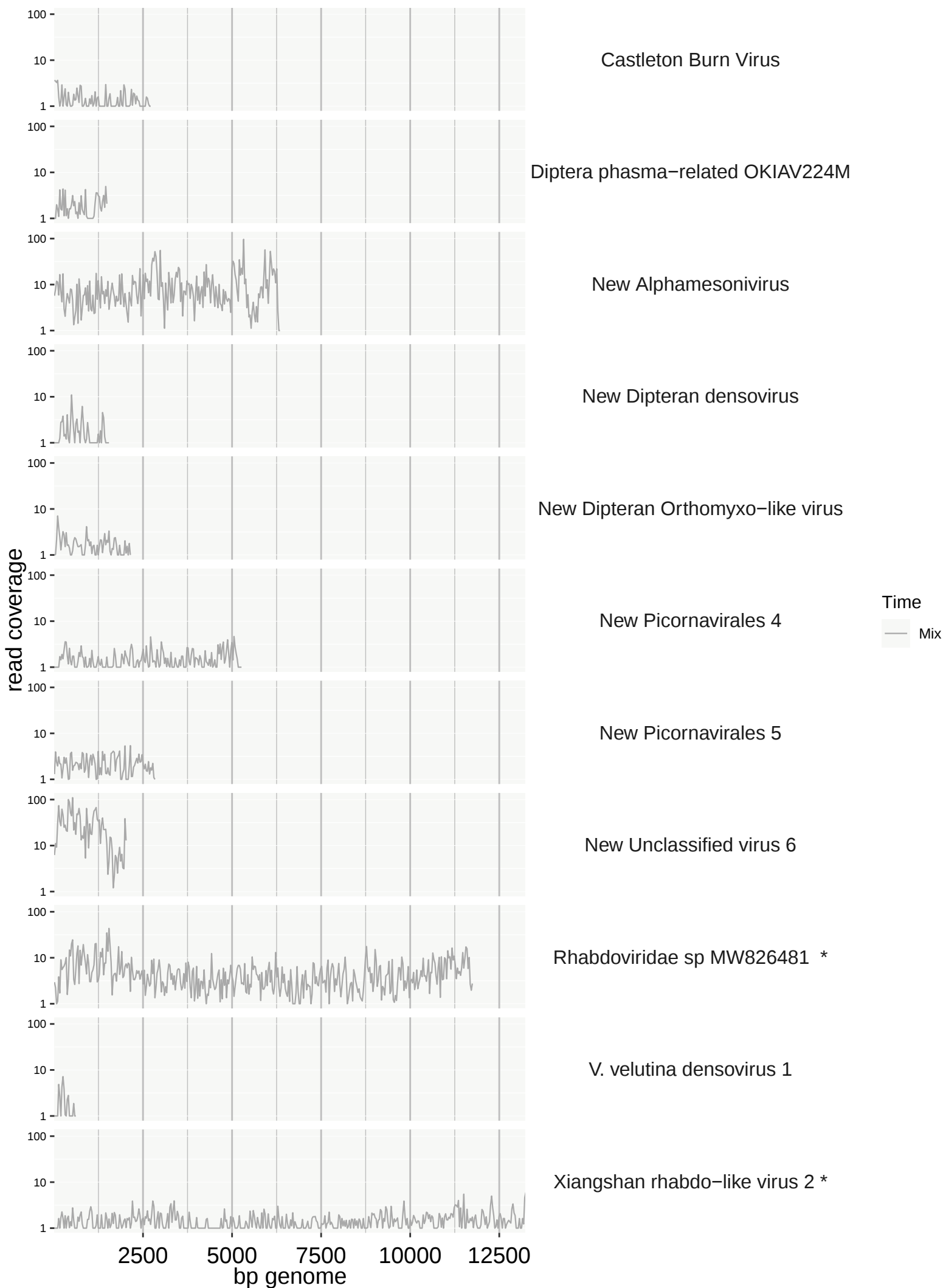
Supplementary Figure 2f

Andrena



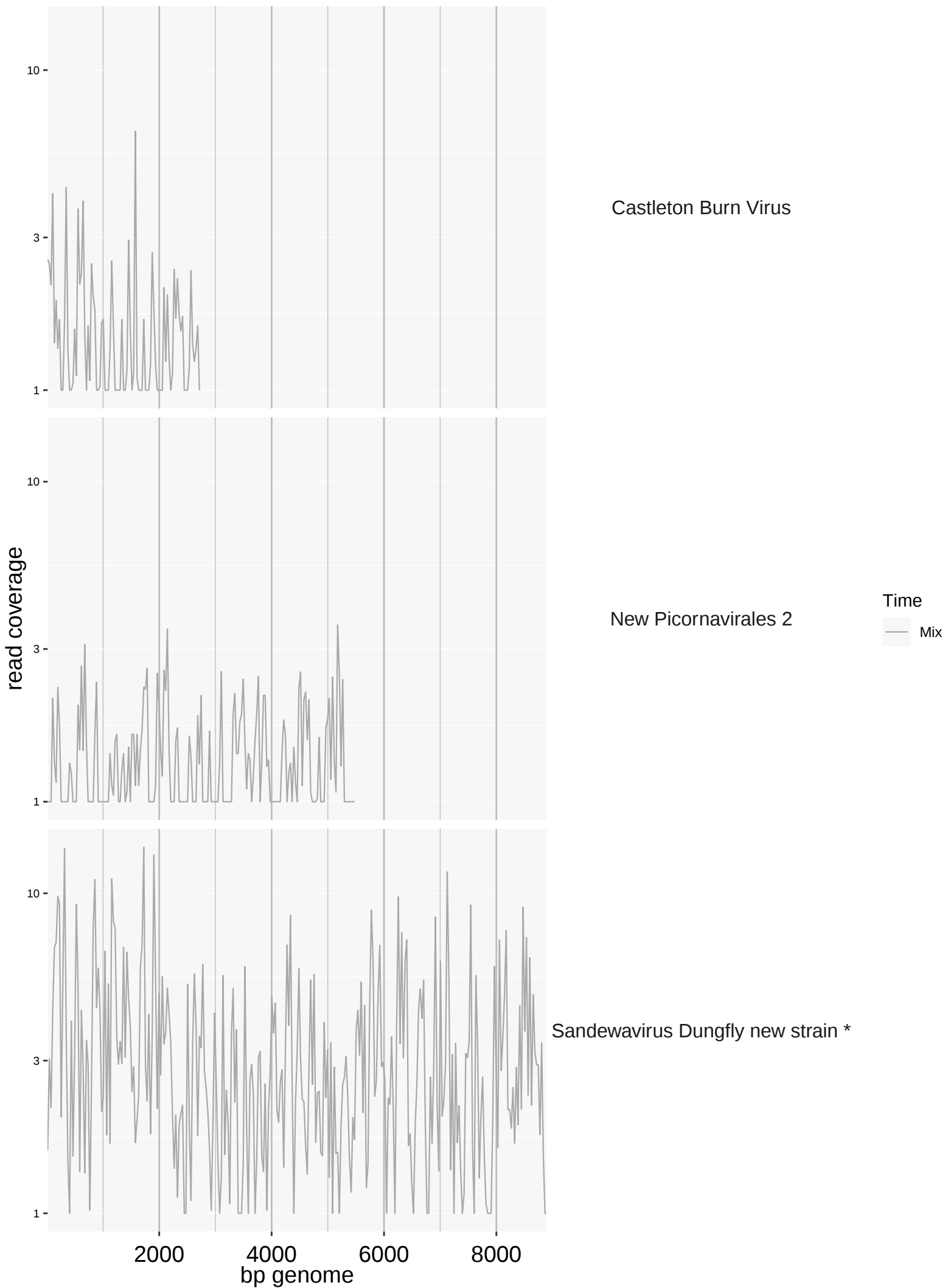
Supplementary Figure 2g

Hoverflies



Supplementary Figure 2h

ForgottenFlies



Supplementary Figure 3: Viral small interfering RNAs (vsiRNA) profiles showing insect and plant viruses positive and negative strand reads stacked by size, ordered by sample: (3.1 – 3.4) vsiRNA profiles from pooled *Apis mellifera* RNA from April collection, (3.5 – 3.9) vsiRNA profiles from pooled *Apis mellifera* RNA from June collection, (3.10 – 3.12) vsiRNA profiles from pooled *Apis mellifera* RNA from August collection, (3.13 – 3.14) vsiRNA profiles from pooled *Bombus terrestris* RNA from April collection, (3.15 – 3.16) vsiRNA profiles from pooled *Bombus terrestris* RNA from June collection, (3.17 – 3.19) vsiRNA profiles from pooled *Bombus terrestris* RNA from August collection, (3.20 – 3.22) vsiRNA profiles from pooled *Bombus lapidarius* RNA from April collection, (3.23 – 3.24) vsiRNA profiles from pooled *Bombus lapidarius* RNA from June collection, (3.25 – 3.26) vsiRNA profiles from pooled *Bombus lapidarius* RNA from April collection, (3.27 – 3.30) vsiRNA profiles from pooled *Bombus hortorum* RNA from June collection, (3.31 – 3.33) vsiRNA profiles from pooled *Bombus hortorum* RNA from August collection, (3.34 – 3.37) vsiRNA profiles from pooled *Bombus pascuorum* RNA from June collection, (3.38 – 3.40) vsiRNA profiles from pooled *Bombus pascuorum* RNA from August collection, (3.41 – 3.43) vsiRNA profiles from pooled mining bees (*Andrena spp.*) RNA, (3.44 – 3.45) vsiRNA profiles from pooled hoverflies RNA, and (3.46) vsiRNA profiles from pooled ‘Forgotten flies’ RNA. Abbreviations: ABPV = Acute bee paralysis virus, AhaemNLV = *Andrena haemorrhoa* nege-like virus, ArMV = *Arabis* mosaic virus, ARV = *Apis* rhabdovirus, BQCV = Black queen cell virus, CBPV = Chronic bee paralysis virus, CLRV = Cherry leaf roll virus, CVA = Cherry virus A, CYV = Caraway yellows virus, DWV = Deformed wing virus, LRV = *Lychnis* ringspot virus, LSV = Lake Sinai virus, PDV = *Prunus* dwarf virus, PVF = *Prunus* virus F, RCNVA = Red clover nepovirus A, RRV = Raspberry ringspot virus, SBV = Sacbrood virus, SBPV = Slow bee paralysis virus, VDV-2 = *Varroa destructor* virus-2, VOV-1 = *Varroa orthomyxovirus*-1. Viral assemblies and mapping outputs are available at <https://doi.org/10.6084/m9.figshare.27888378>.

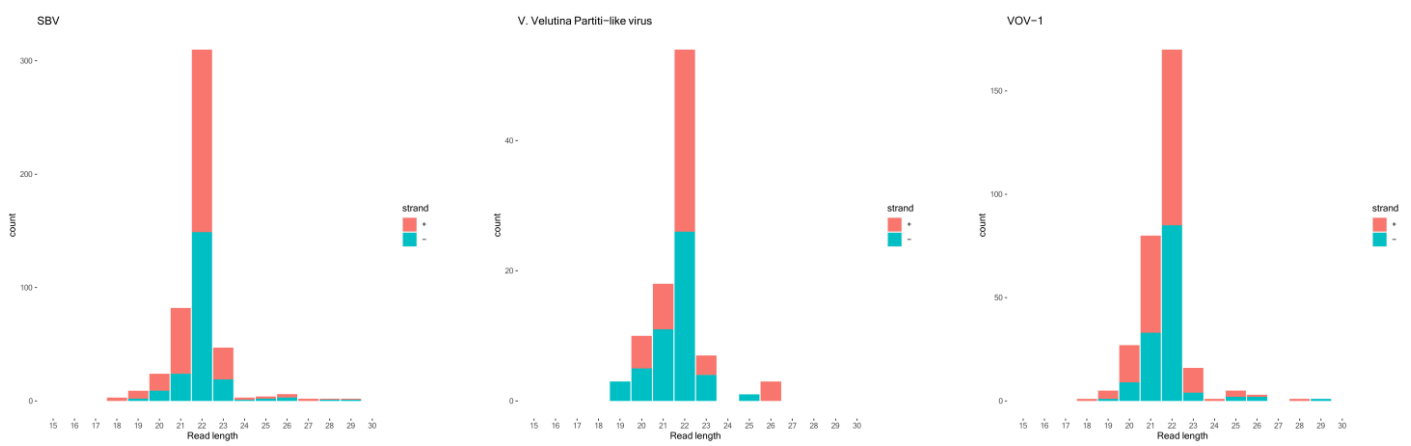
Supplementary Figure 3.1 : *Apis mellifera* – April – insect virus vsiRNA profiles



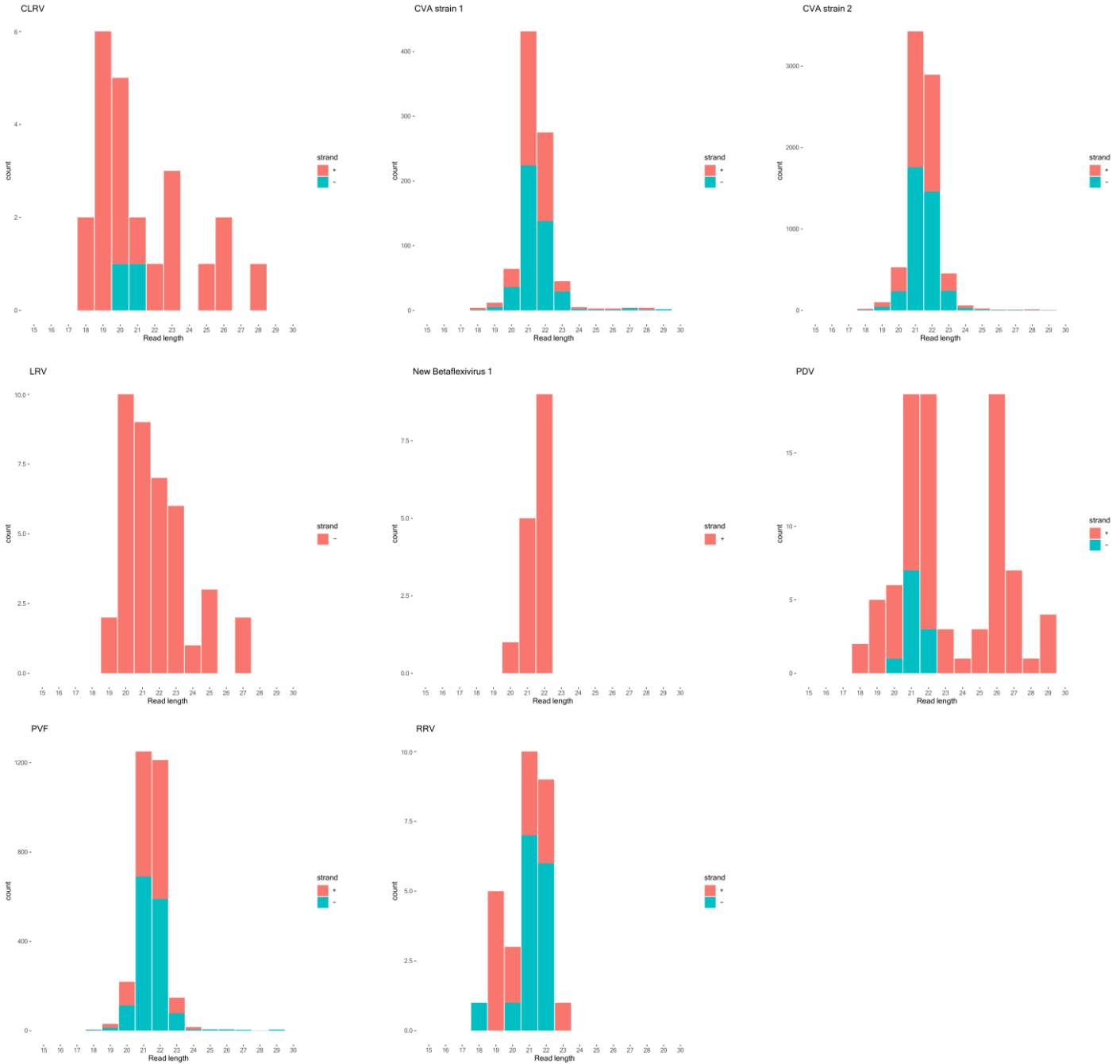
Supplementary Figure 3.2 : *Apis mellifera* – April – insect virus vsiRNA profiles



Supplementary Figure 3.3 : *Apis mellifera* – April – insect virus vsiRNA profiles



Supplementary Figure 3.4 : *Apis mellifera* – April – plant virus vsiRNA profiles



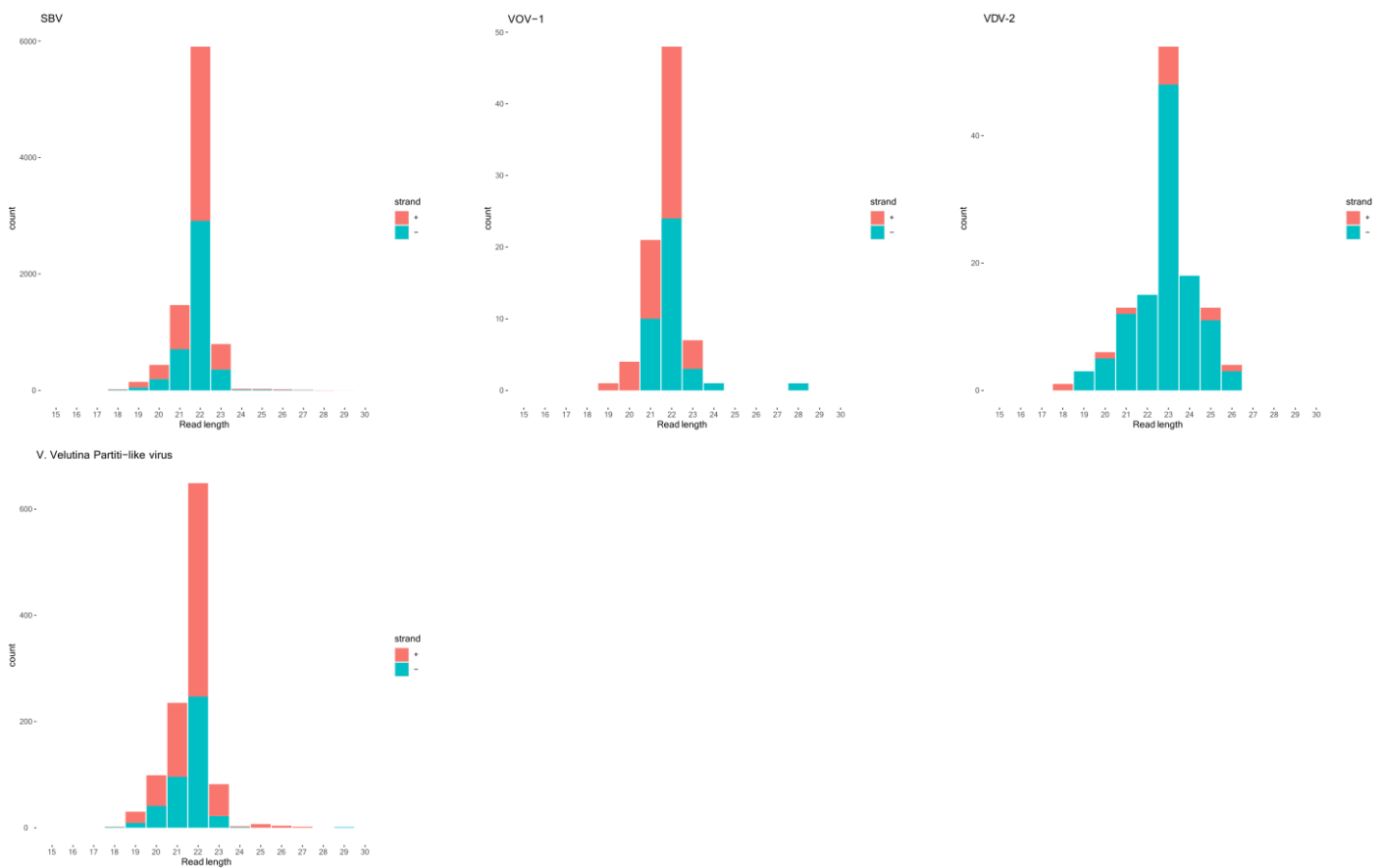
Supplementary Figure 3.5 : *Apis mellifera* – June – insect virus vsirNA profiles



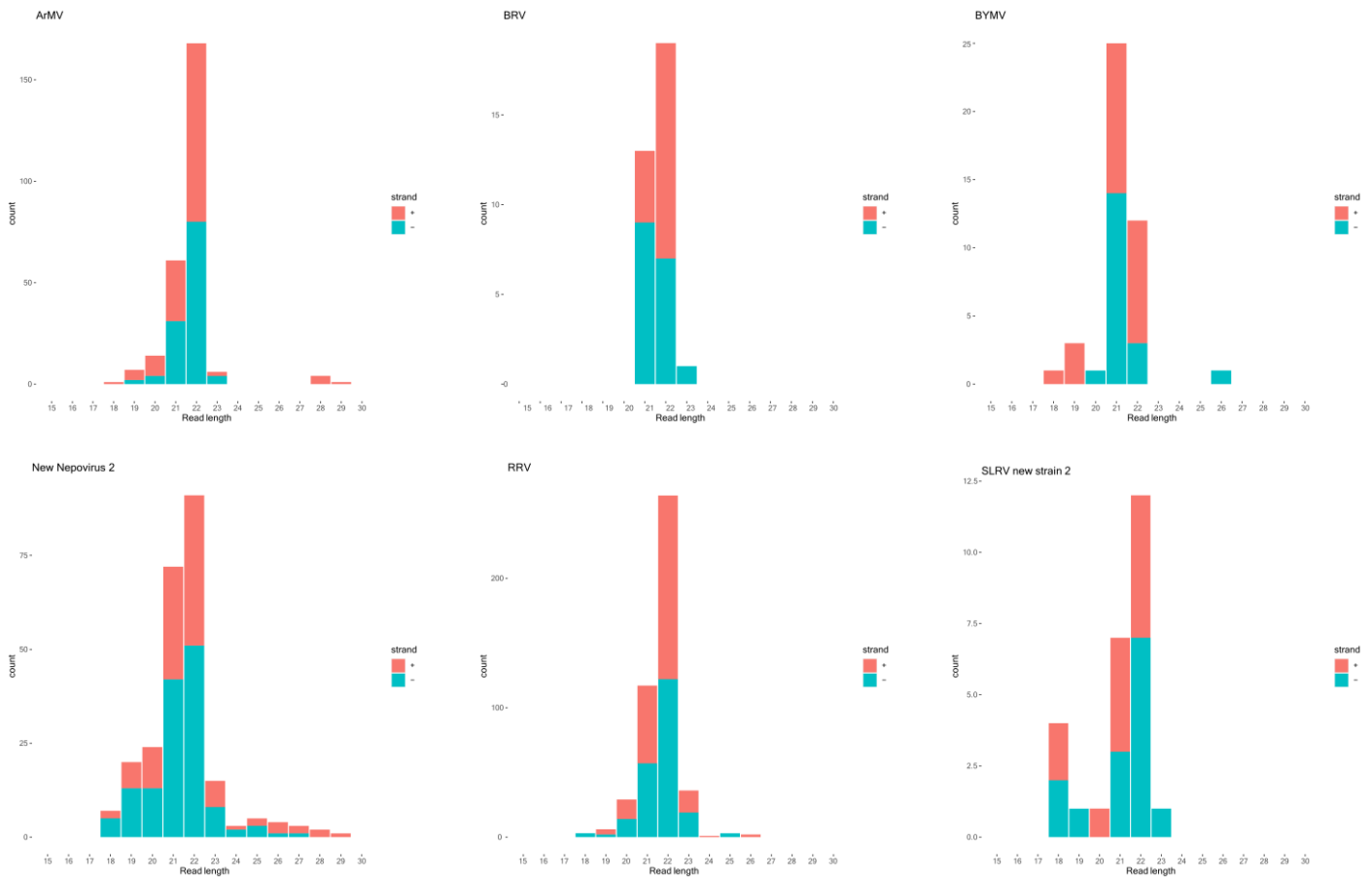
Supplementary Figure 3.6 : *Apis mellifera* – June – insect virus vsiRNA profiles



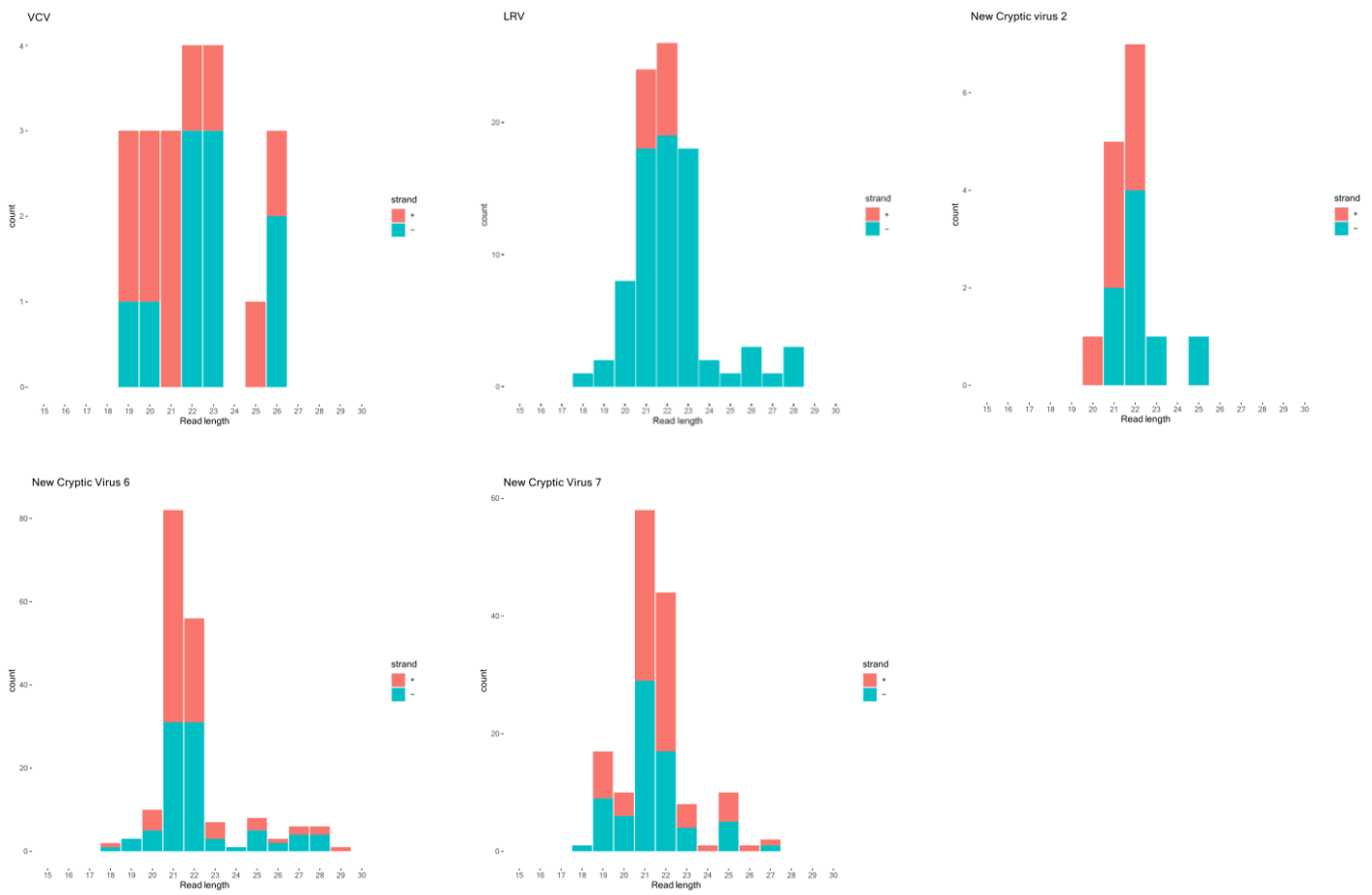
Supplementary Figure 3.7 : *Apis mellifera* – June – insect virus vsRNA profiles



Supplementary Figure 3.8 : *Apis mellifera* – June – plant virus vsRNA profiles



Supplementary Figure 3.9 : *Apis mellifera* – June – plant virus vsiRNA profiles



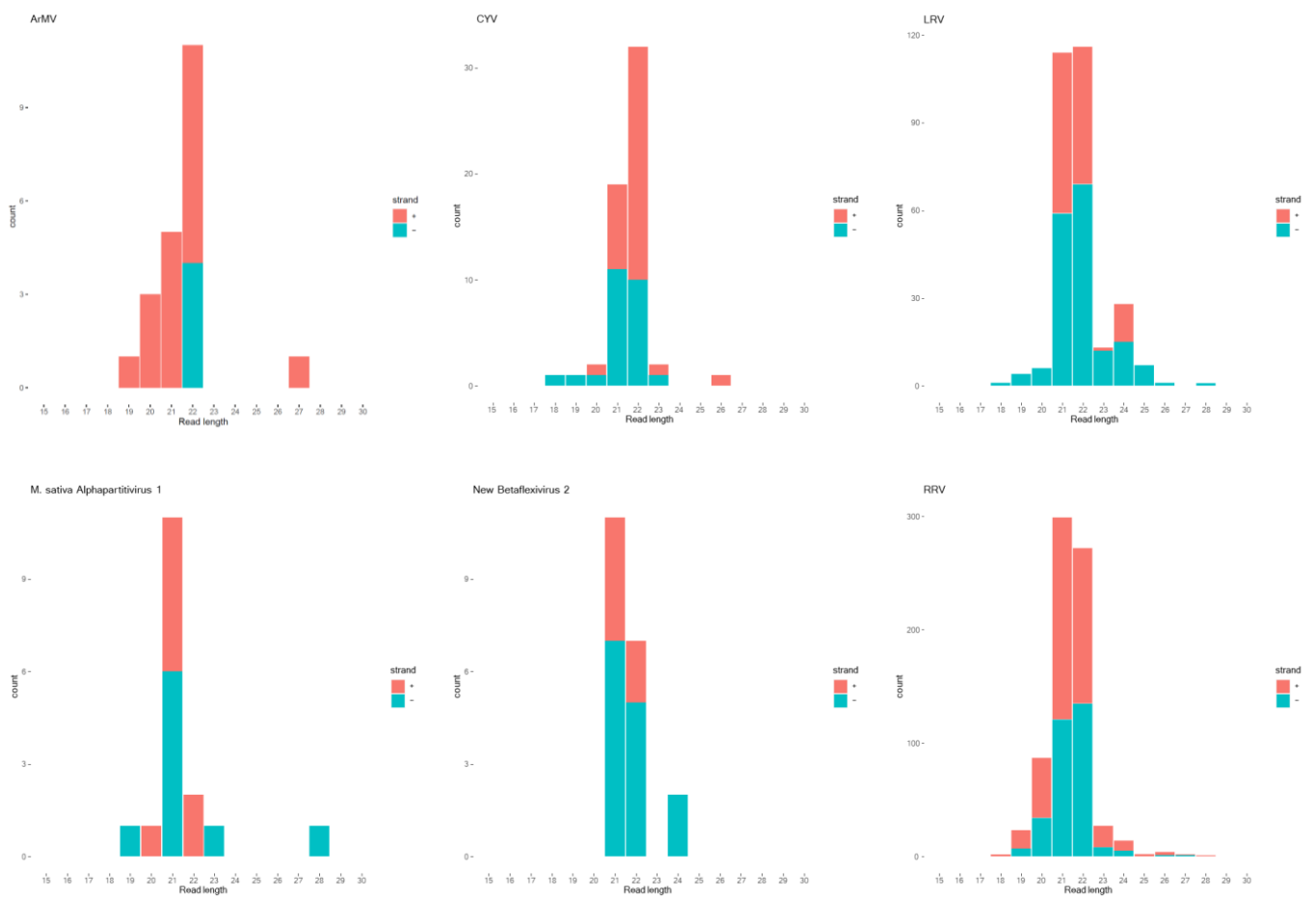
Supplementary Figure 3.10 : *Apis mellifera* – August – insect virus vsRNA profiles



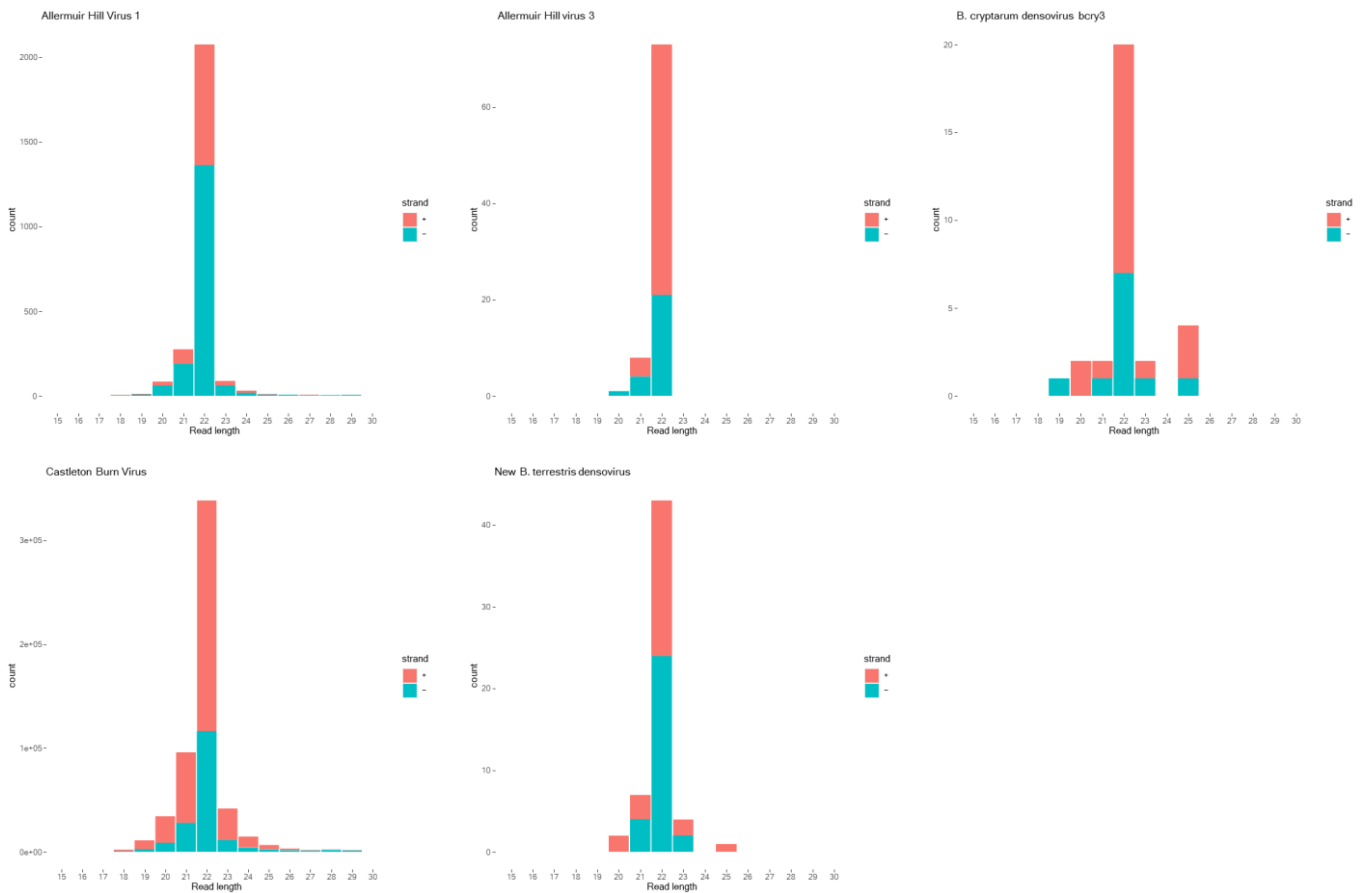
Supplementary Figure 3.11 : *Apis mellifera* – August – insect virus vsRNA profiles



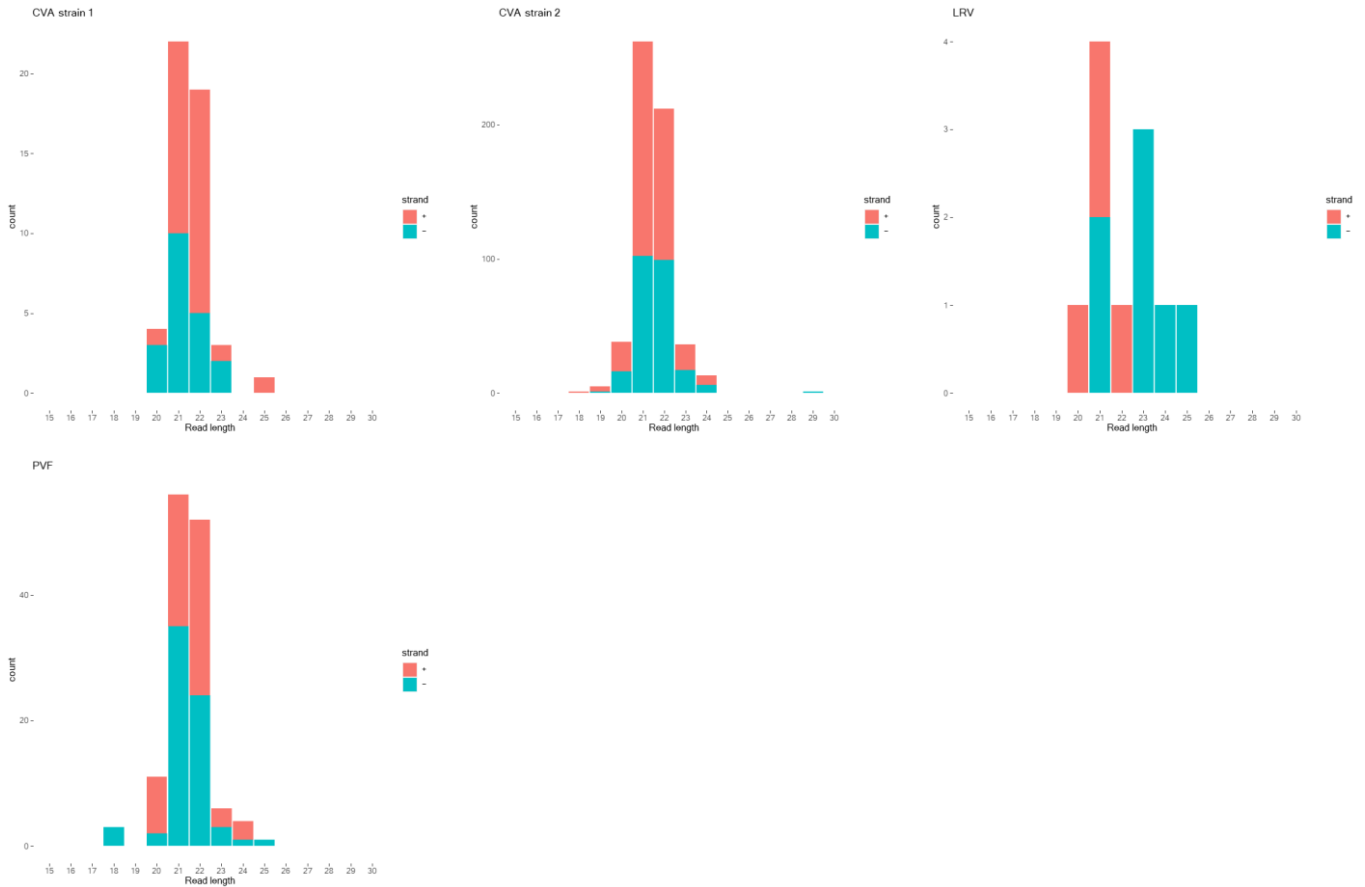
Supplementary Figure 3.12 : *Apis mellifera* – August – plant virus vsiRNA profiles



Supplementary Figure 3.13 : *Bombus terrestris* – April – insect virus vsRNA profiles



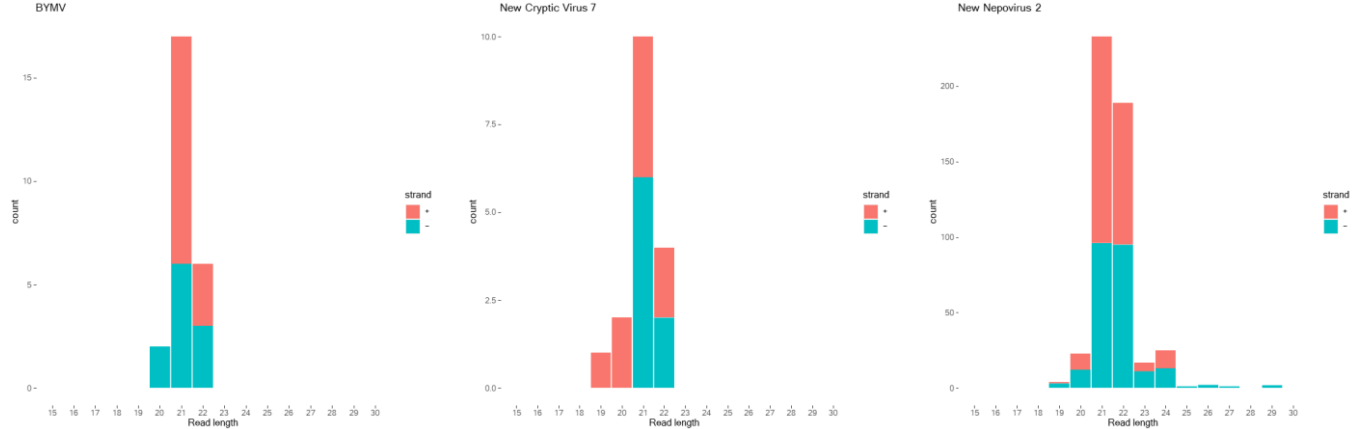
Supplementary Figure 3.14 : *Bombus terrestris* – April – plant virus vsRNA profiles



Supplementary Figure 3.15 : *Bombus terrestris* – June – insect virus vsRNA profiles



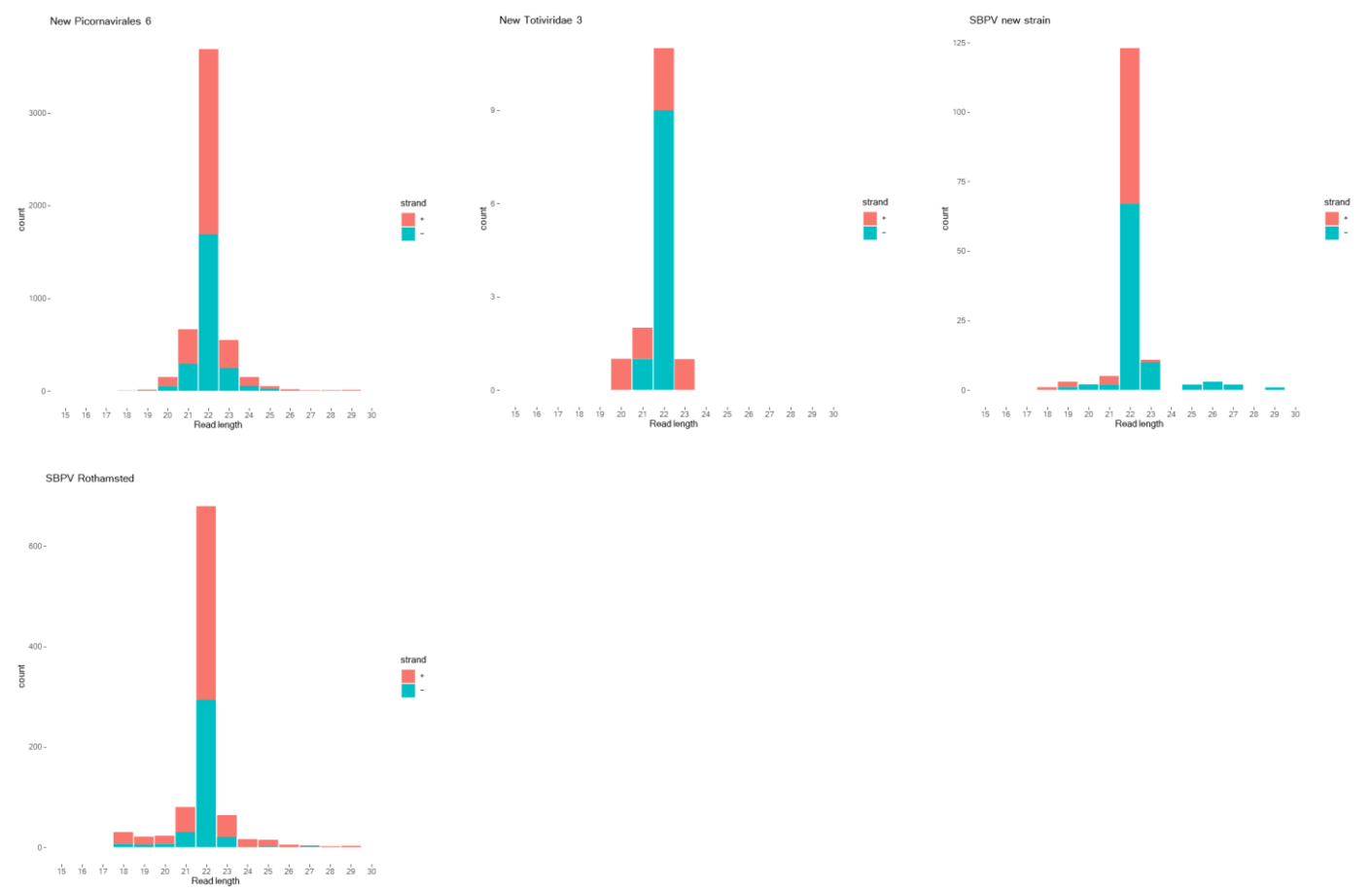
Supplementary Figure 3.16 : *Bombus terrestris* – June – plant virus vsRNA profiles



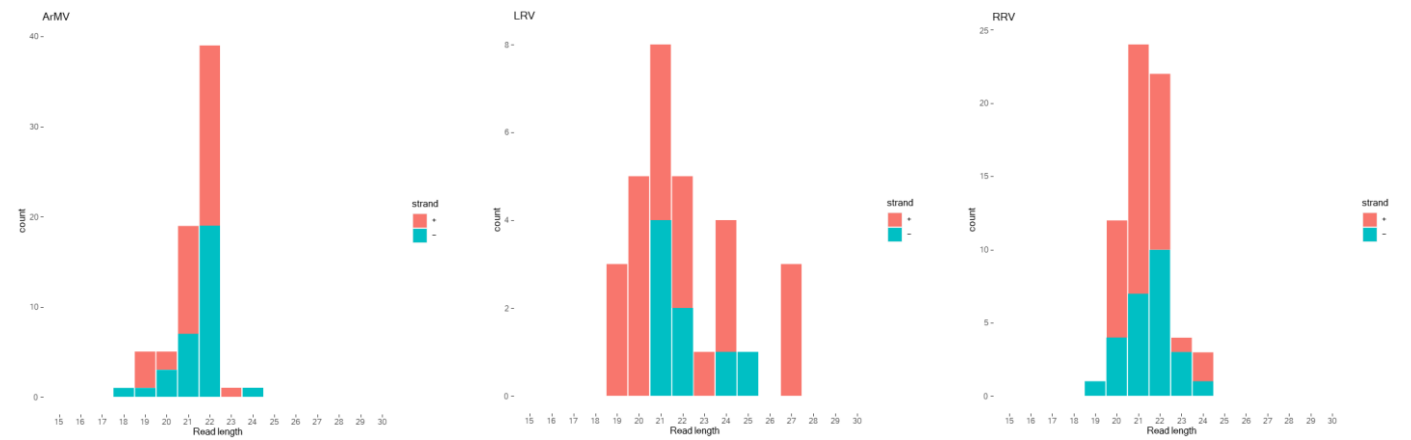
Supplementary Figure 3.17 : *Bombus terrestris* – August – insect virus vsRNA profiles



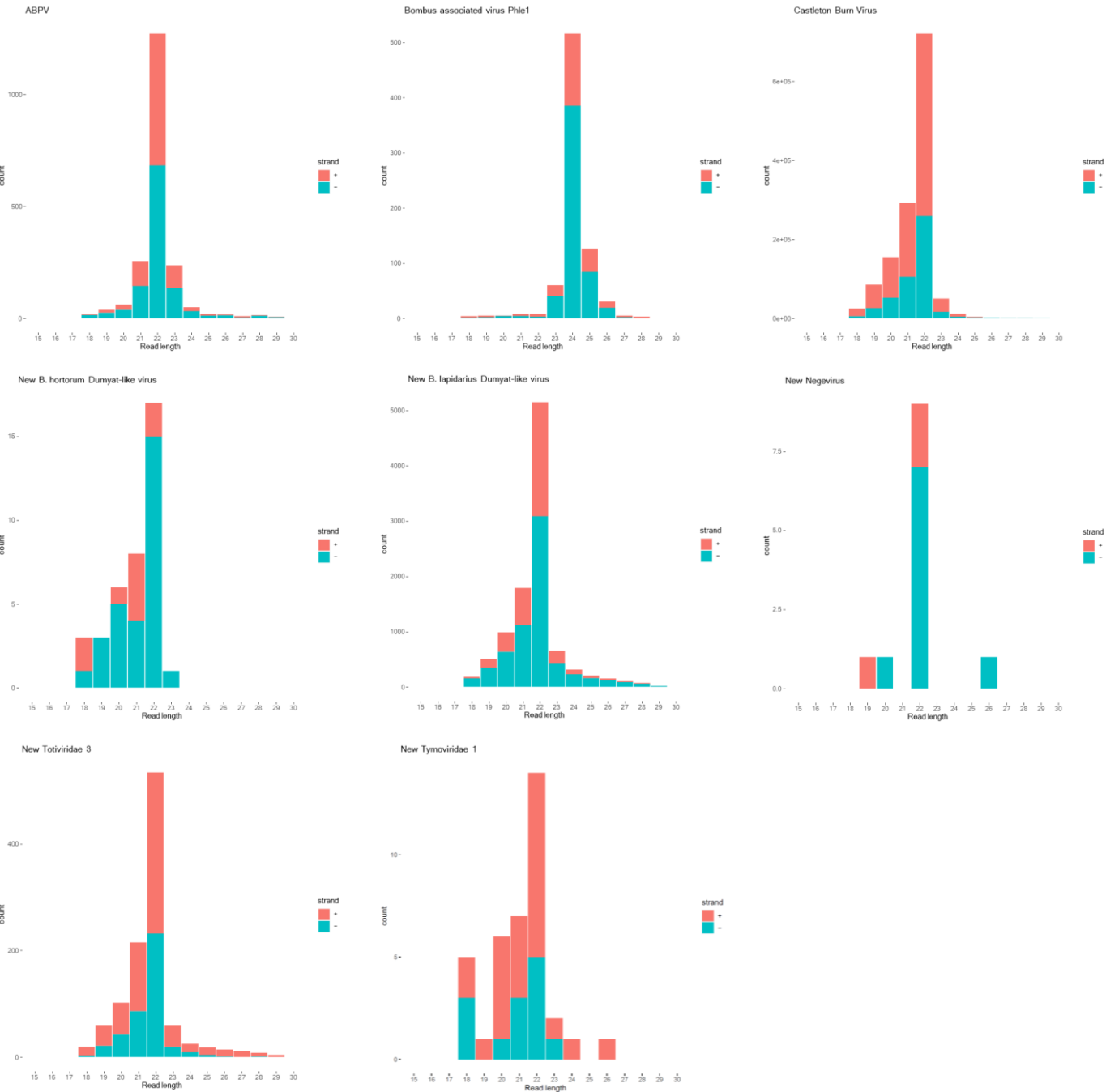
Supplementary Figure 3.18 : *Bombus terrestris* – August – insect virus vsRNA profiles



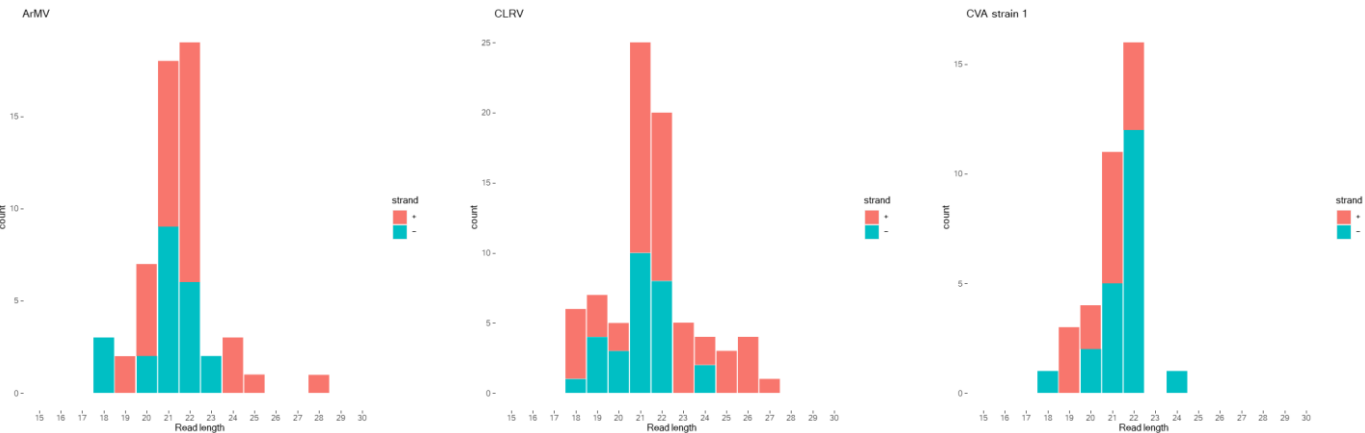
Supplementary Figure 3.19 : *Bombus terrestris* – August – plant virus vsRNA profiles



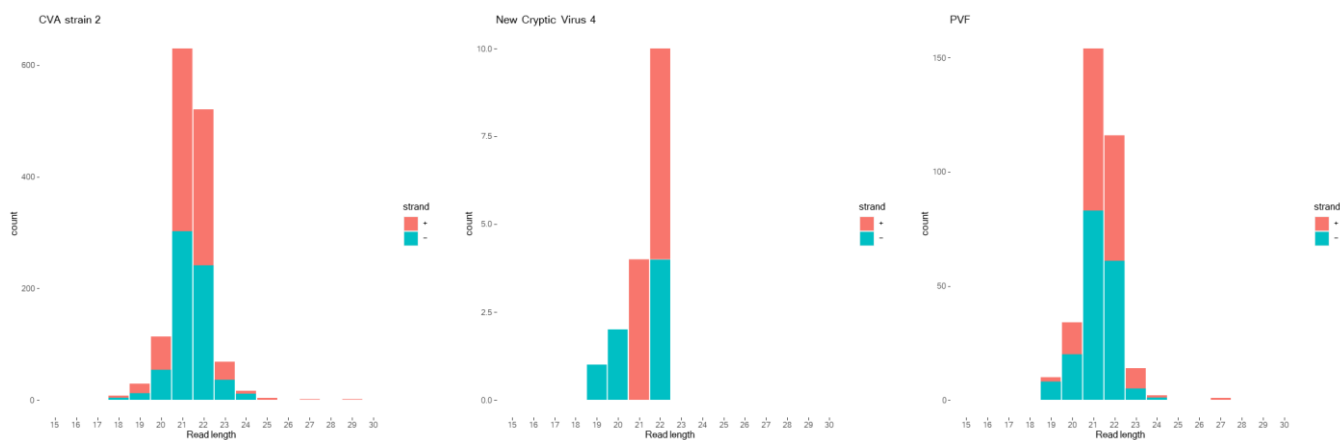
Supplementary Figure 3.20 : *Bombus lapidarius* – April – insect virus vsiRNA profiles



Supplementary Figure 3.21 : *Bombus lapidarius* – April – plant virus vsiRNA profiles



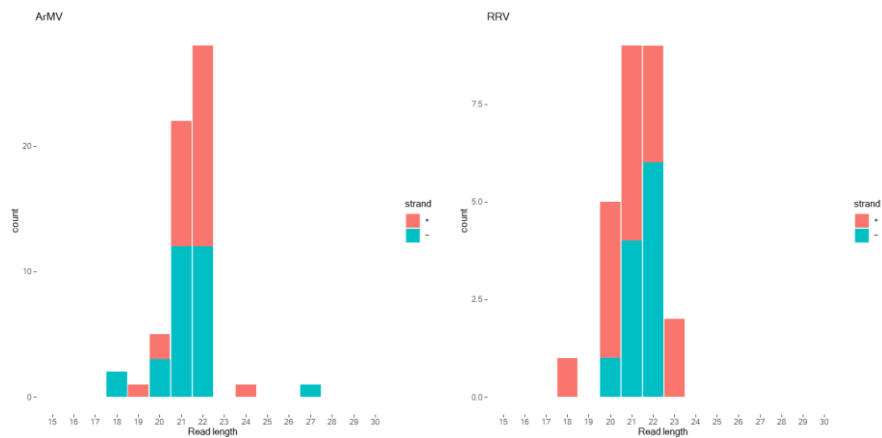
Supplementary Figure 3.22 : *Bombus lapidarius* – April – plant virus vsiRNA profiles



Supplementary Figure 3.23 : *Bombus lapidarius* – June – insect virus vsiRNA profiles



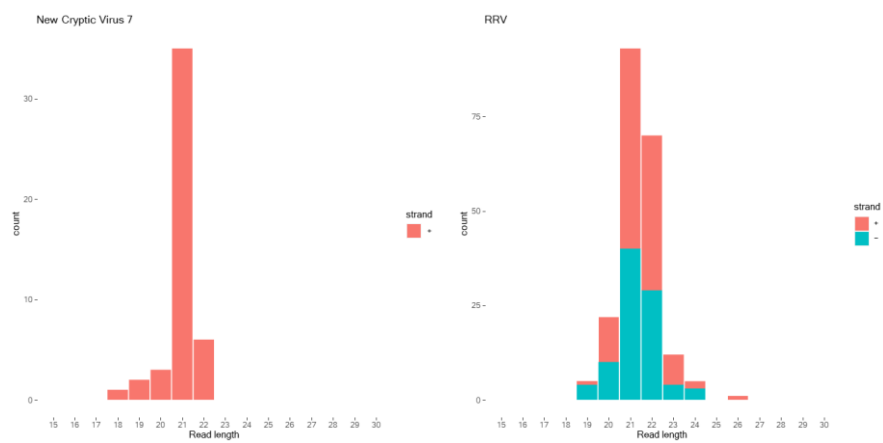
Supplementary Figure 3.24 : *Bombus lapidarius* – June – plant virus vsRNA profiles



Supplementary Figure 3.25 : *Bombus lapidarius* – August – insect virus vsiRNA profiles



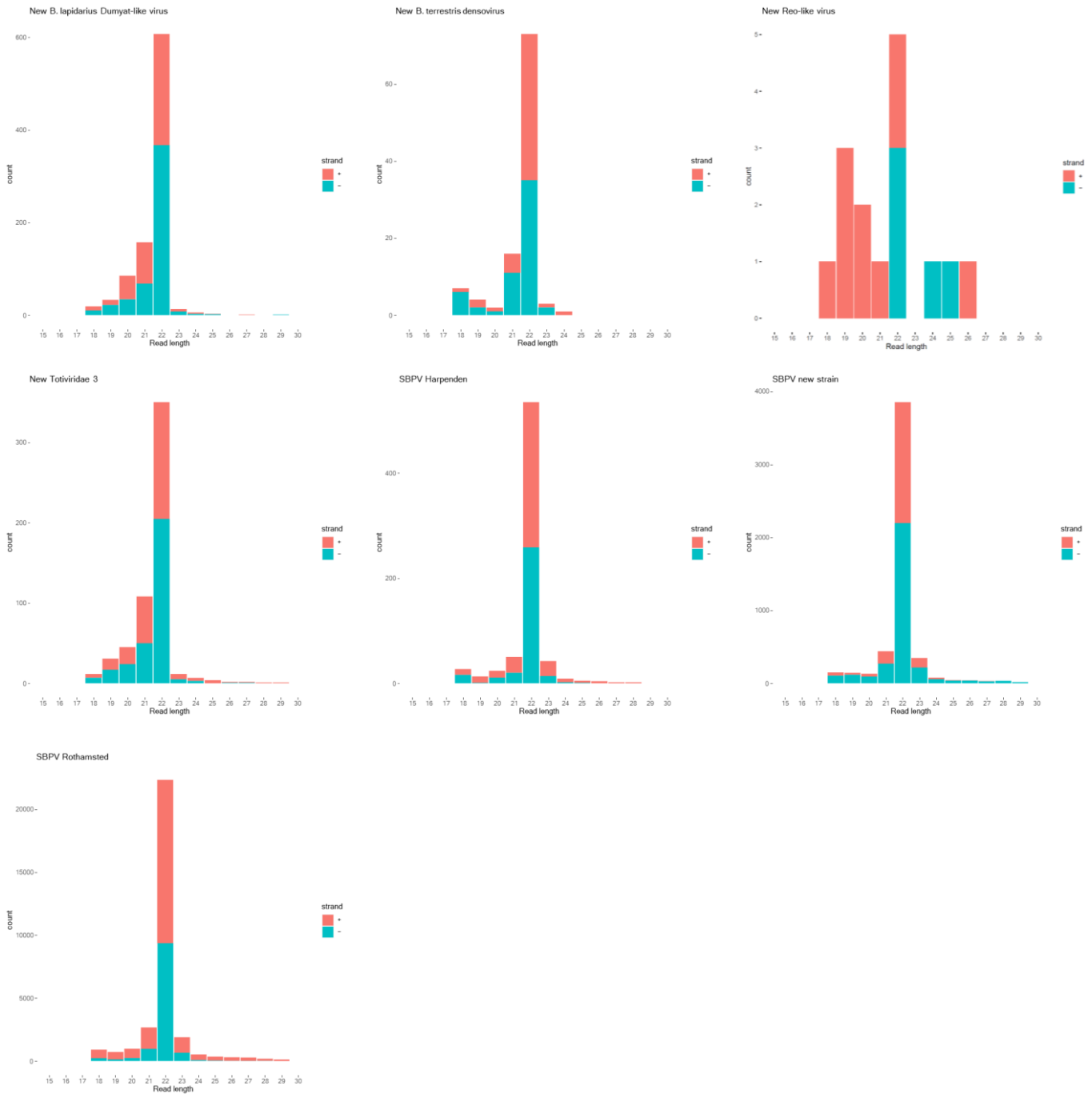
Supplementary Figure 3.26 : *Bombus lapidarius* – August – plant virus vsRNA profiles



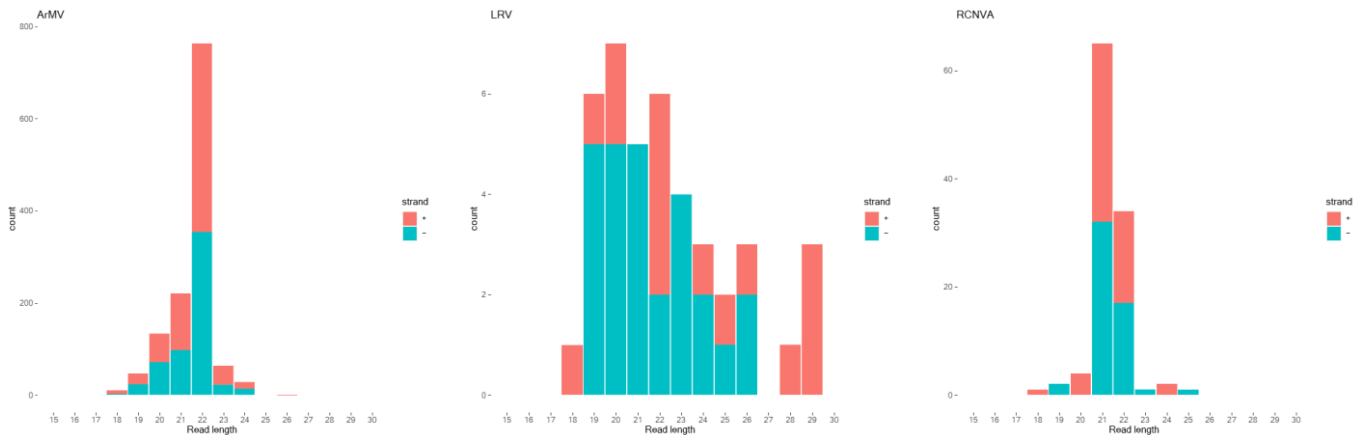
Supplementary Figure 3.27 : *Bombus hortorum* – June – insect virus vsiRNA profiles



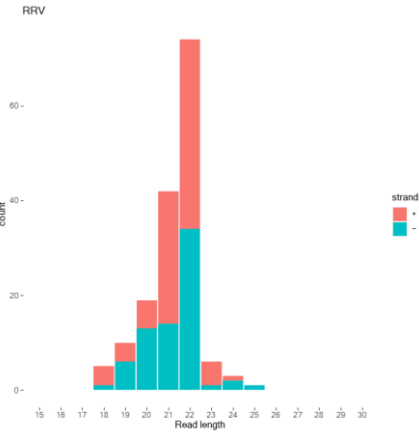
Supplementary Figure 3.28 : *Bombus hortorum* – June – insect virus vsRNA profiles



Supplementary Figure 3.29 : *Bombus hortorum* – June – plant virus vsRNA profiles



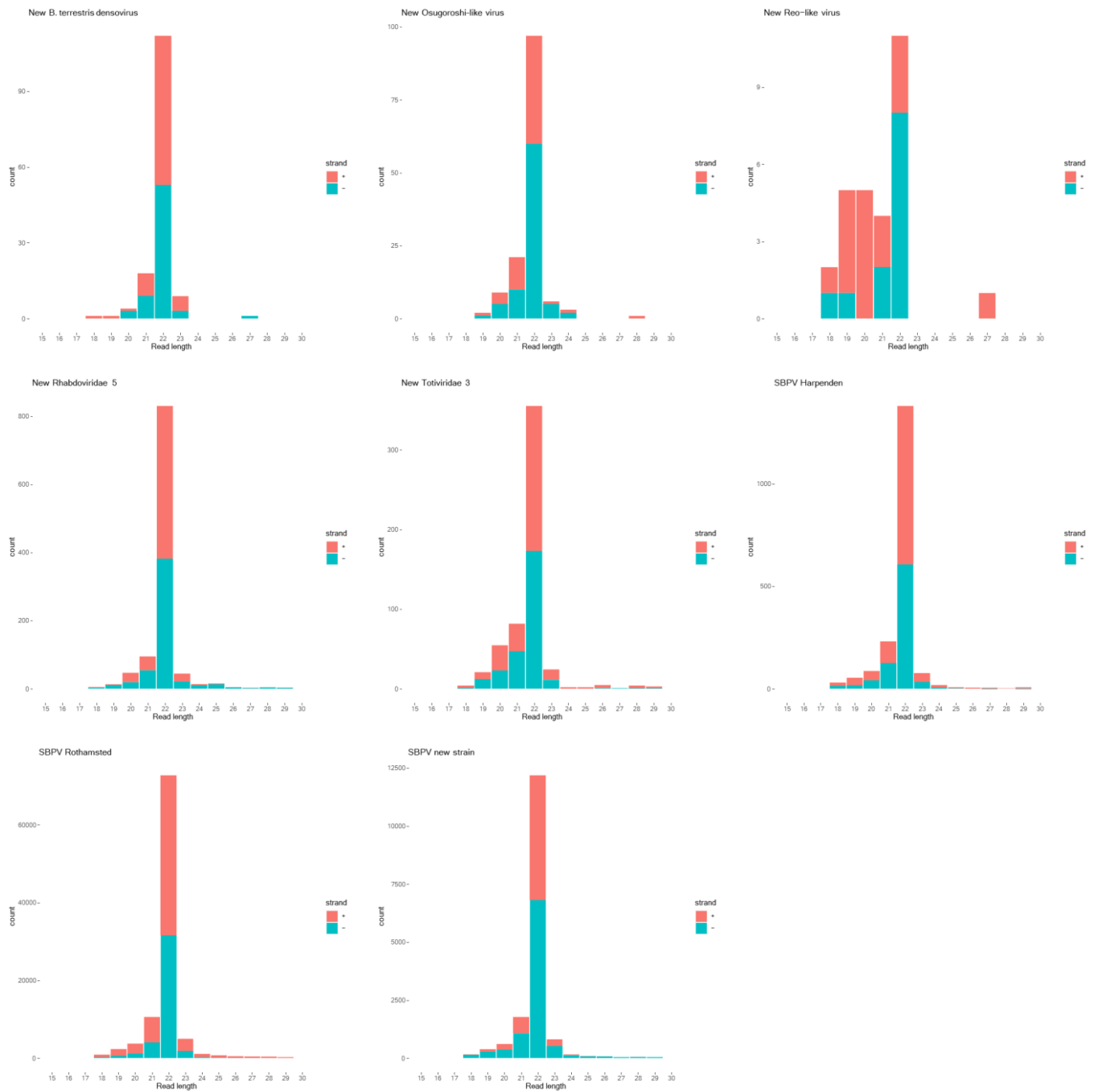
Supplementary Figure 3.30 : *Bombus hortorum* – June – plant virus vsiRNA profile



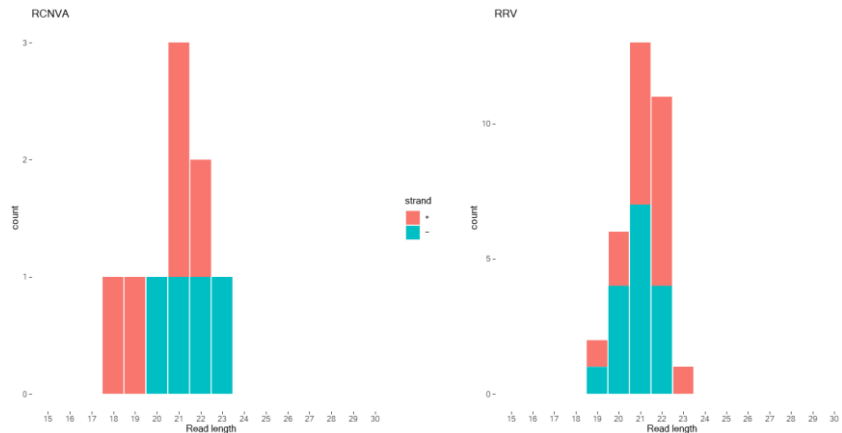
Supplementary Figure 3.31 : *Bombus hortorum* – August – insect virus vs iRNA profiles



Supplementary Figure 3.32 : *Bombus hortorum* – August – insect virus vsRNA profiles



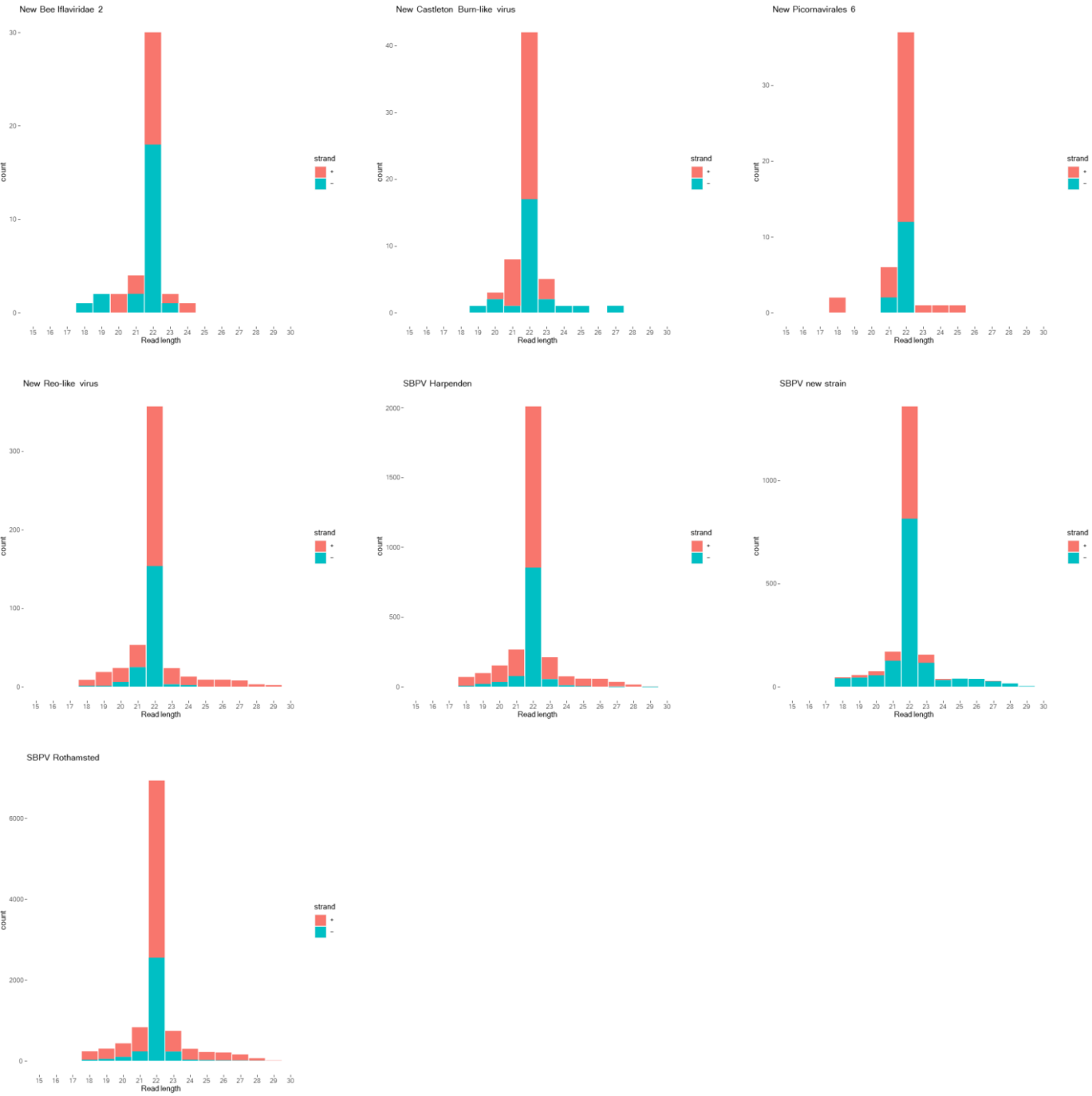
Supplementary Figure 3.33 : *Bombus hortorum* – August – plant virus vsRNA profiles



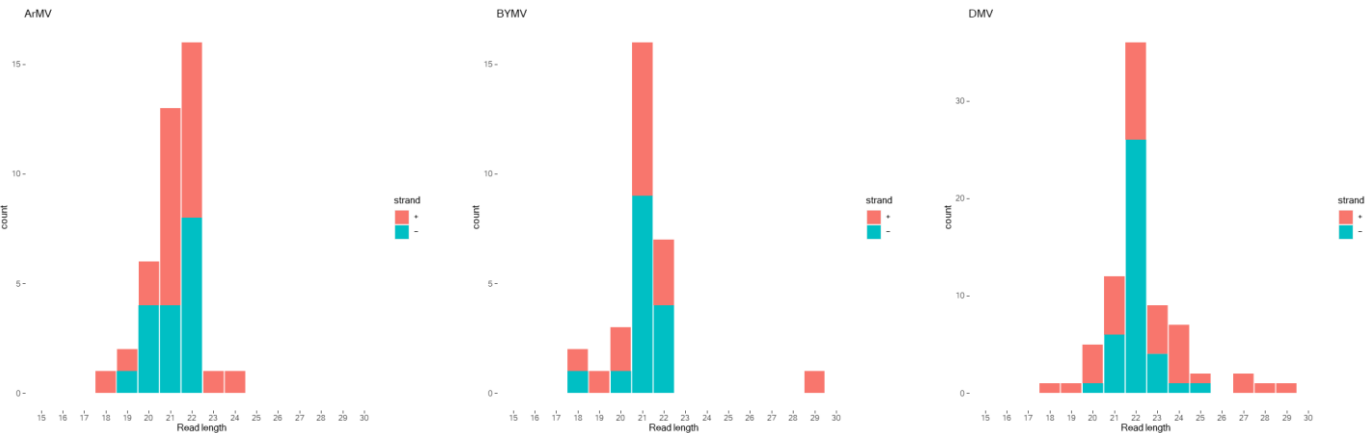
Supplementary Figure 3.34 : *Bombus pascuorum* – June – insect virus vs iRNA profiles



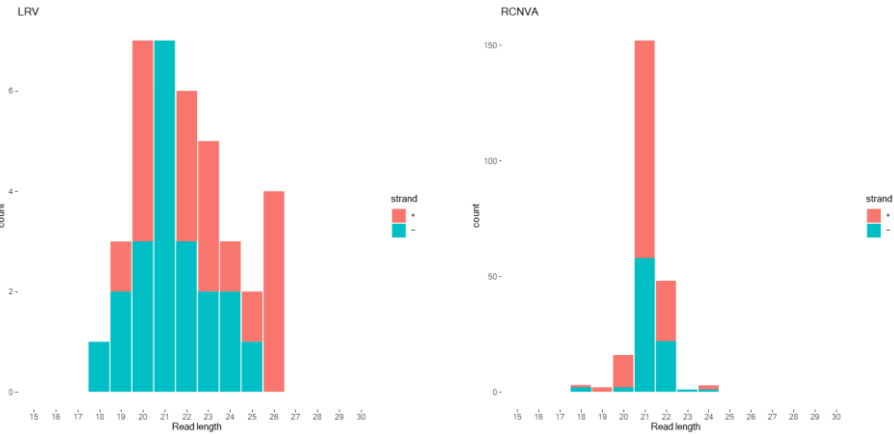
Supplementary Figure 3.35 : *Bombus pascuorum* – June – insect virus vsRNA profiles



Supplementary Figure 3.36 : *Bombus pascuorum* – June – Plant virus vsRNA profiles



Supplementary Figure 3.37 : *Bombus pascuorum* – June – plant virus vsiRNA profiles



Supplementary Figure 3.38 : *Bombus pascuorum* – August – insect virus vs iRNA profiles



Supplementary Figure 3.39 : *Bombus pascuorum* – August – insect virus vs iRNA profiles



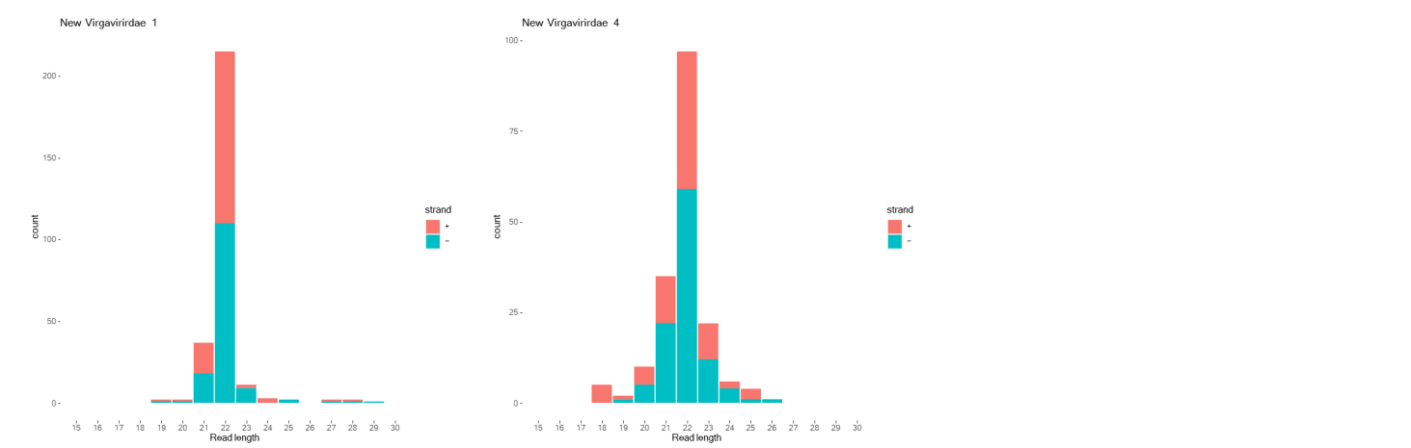
Supplementary Figure 3.40 : *Bombus pascuorum* – August – plant virus vsiRNA profiles



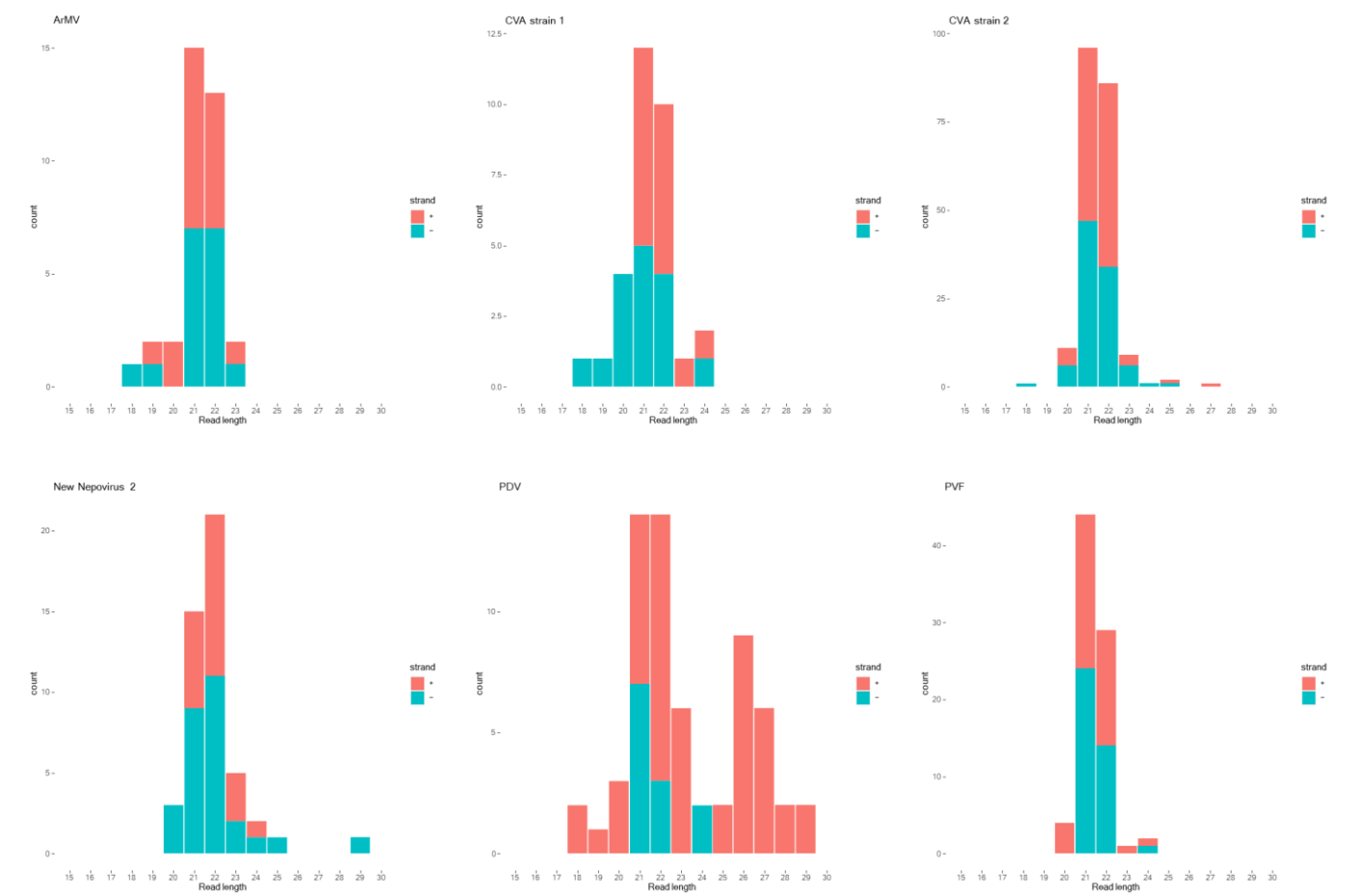
Supplementary Figure 3.41 : *Andrena spp.*– Mixed time points – insect virus vsiRNA profiles



Supplementary Figure 3.42 : *Andrena spp.*– Mixed time points – insect virus vsiRNA profiles



Supplementary Figure 3.43 : *Andrena spp.*– Mixed time points – plant virus vsiRNA profiles



Supplementary Figure 3.44 : Hoverflies – Mixed time points – insect virus vsiRNA profiles



Supplementary Figure 3.45 : Hoverflies – Mixed time points – plant virus vsiRNA profiles



Supplementary Figure 3.46 : Forgotten flies – Mixed time points – insect virus
vsiRNA profiles

