

Supplemental Information for:

Viral diversity and co-evolutionary dynamics across the ant phylogeny

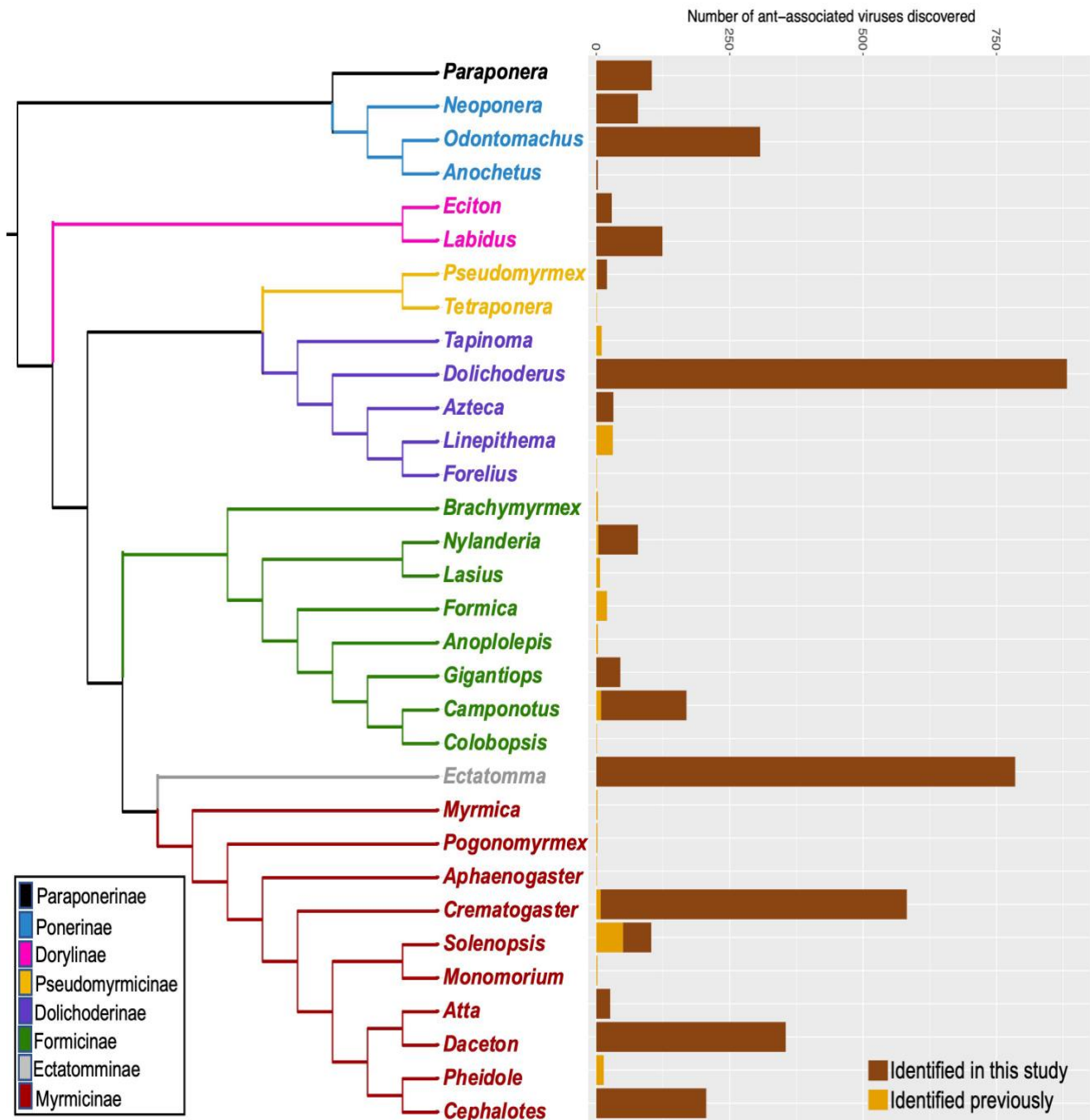
Peter J. Flynn and Corrie S. Moreau

Table of Contents:

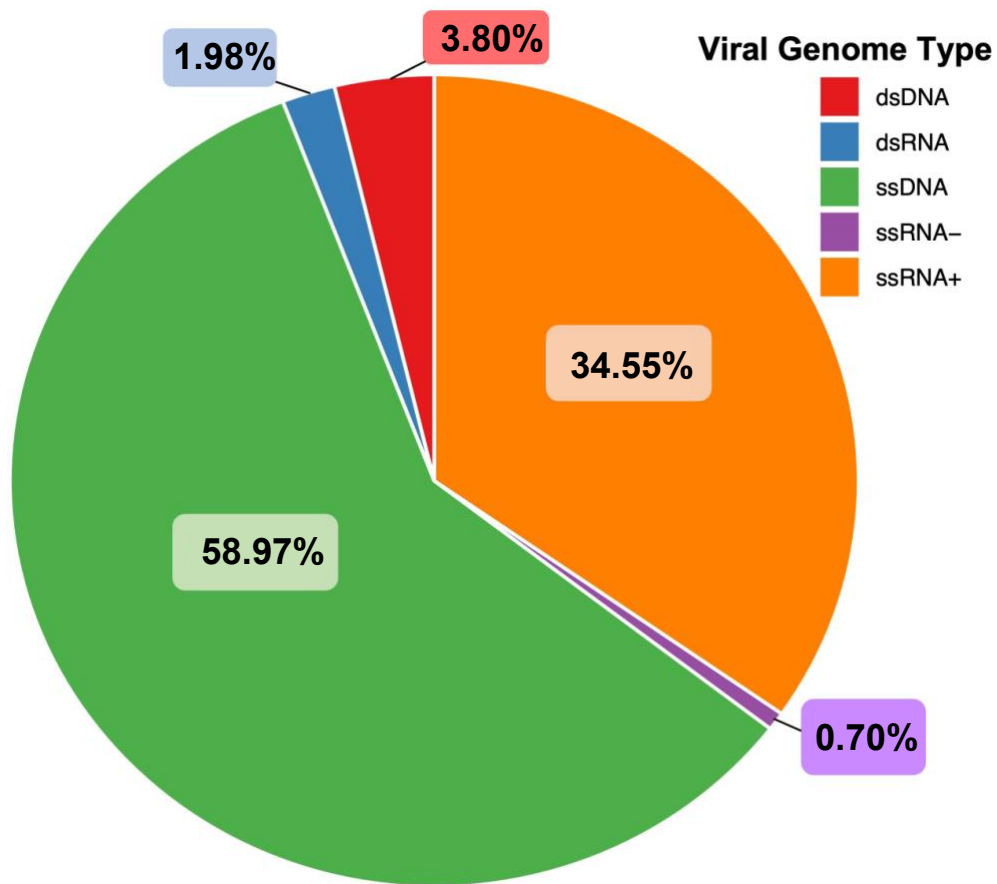
Supplementary Fig. 1	Page 2
Supplementary Fig. 2	Page 3
Supplementary Fig. 3	Page 4
Supplementary Fig. 4	Page 5
Supplementary Fig. 5	Page 6
Supplementary Fig. 6	Page 7
Supplementary Fig. 7	Page 8
Supplementary Table 1	Page 9-12
Supplementary Table 2	Page 13
Supplementary Table 3	Page 14-15
Supplementary Table 4	Page 16
Supplementary Table 5	Page 17-18
Dataset S1	Separate File



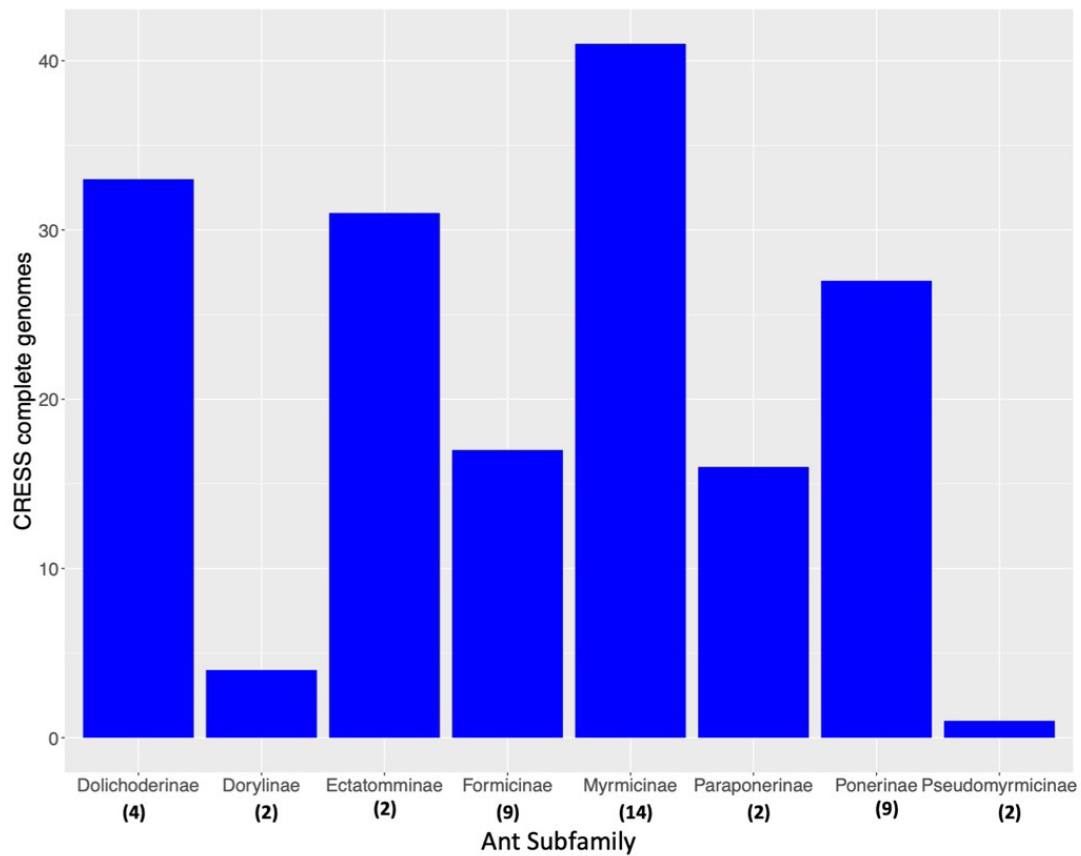
Supplementary Figure 1. Sampling scheme for ant colony collection sites. (A) Four rainforest sampling locations within Nouragues Reserve, French Guiana and (B) Four urban sampling locations within Cayenne, French Guiana.



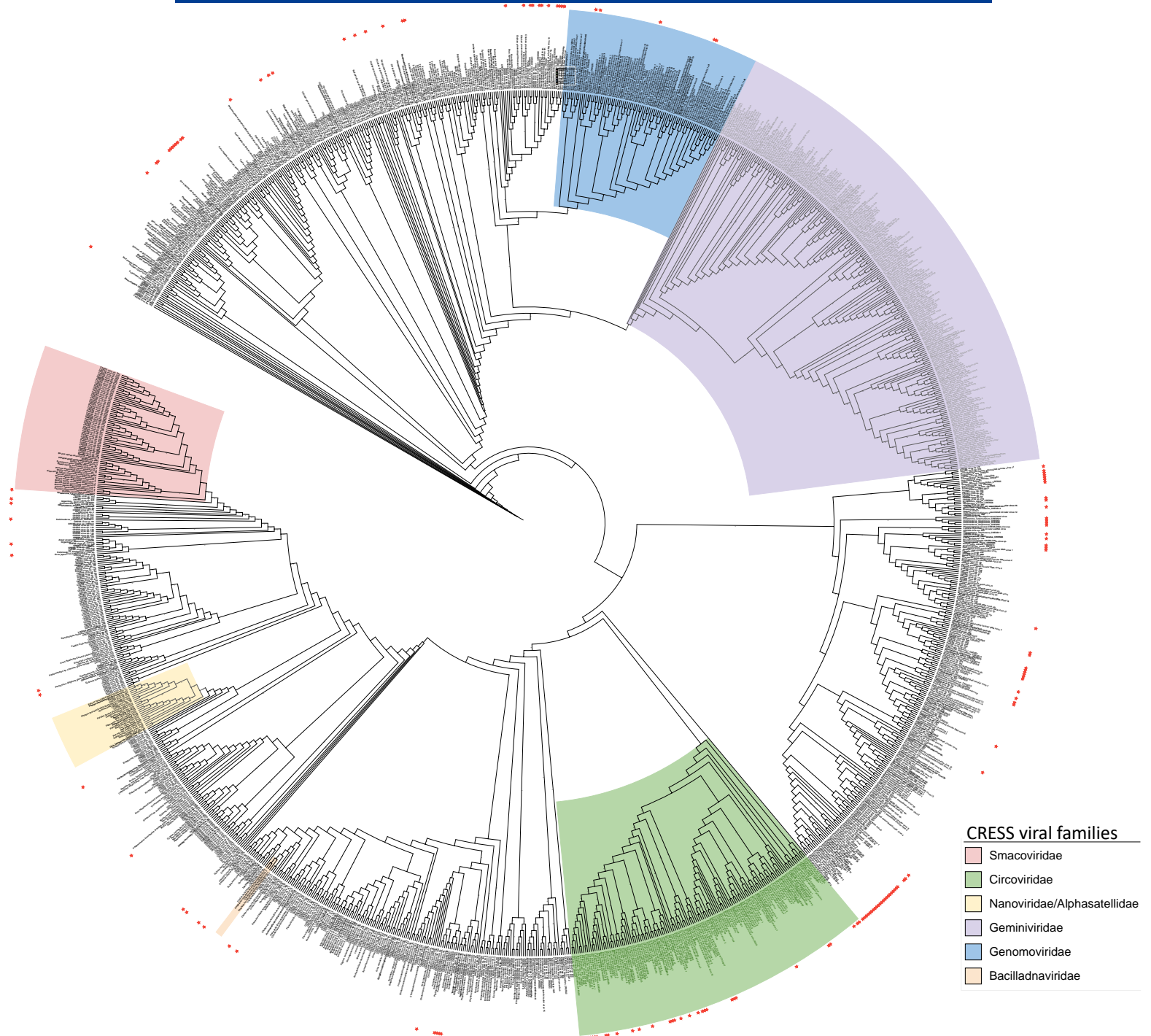
Supplementary Figure 2. Identification of ant-associated viruses across divergent viral clades within widespread ant host genera. Bars represent number of ant-associated viruses in each viral clade. Brown and yellow colors represent current and previously identified ant-associated viruses, respectively. Phylogeny modified from Nelsen et al. (2018).



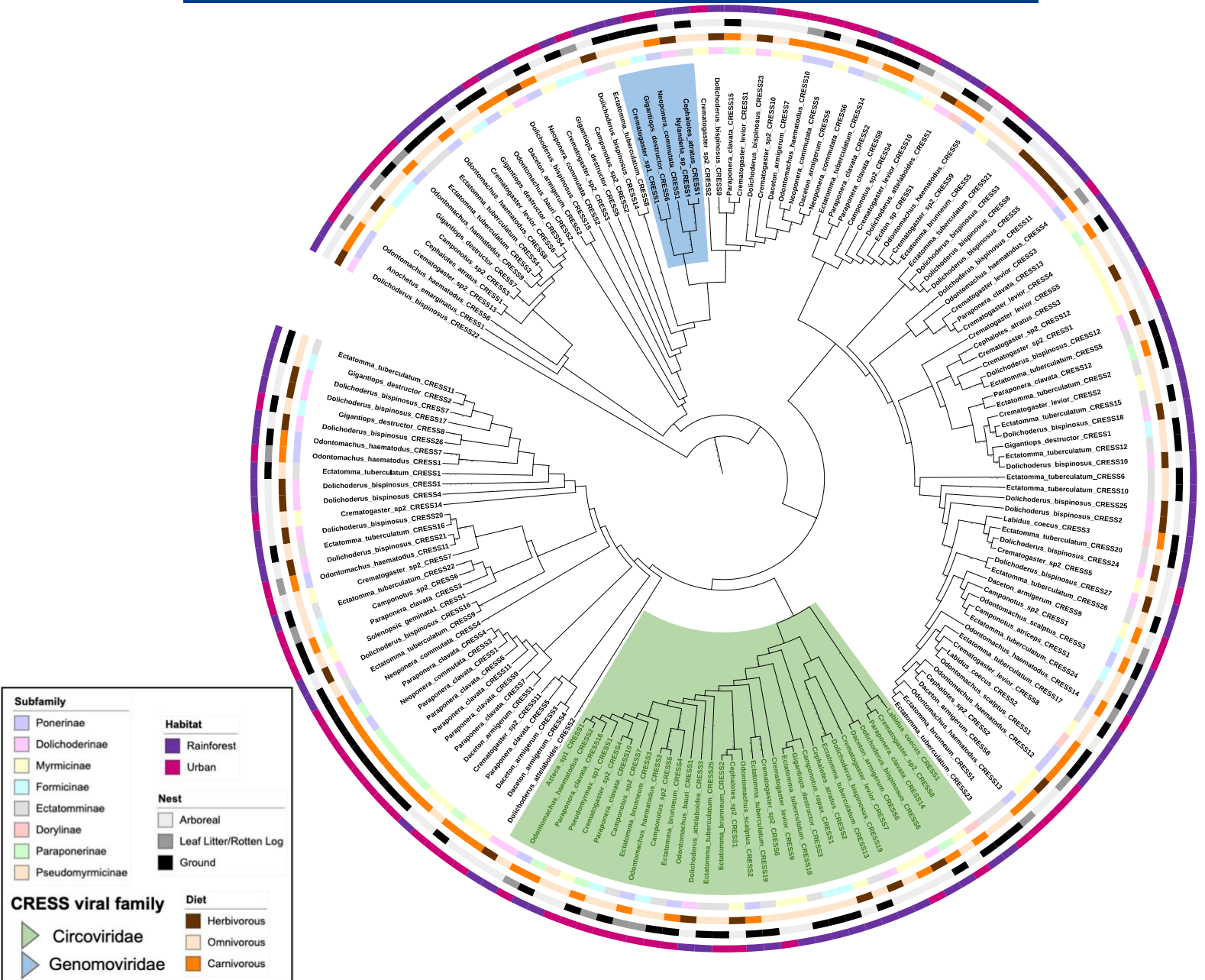
Supplementary Figure 3. Genome Type Pie chart. Chart represents distribution of viral genome fragments identified in this study by viral genome type.



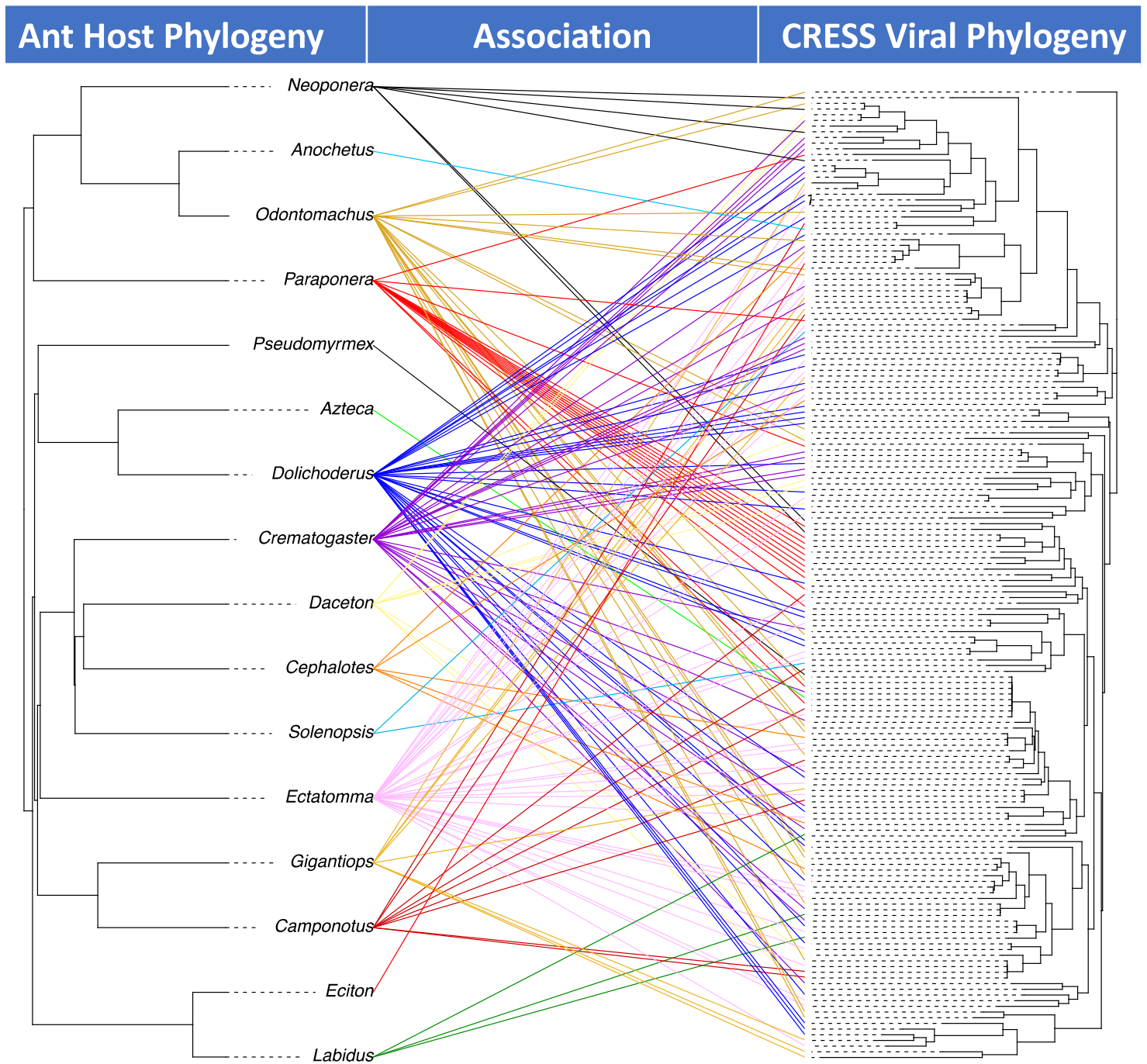
S study. Sorted by ant host subfamily. Number in parenthesis below ant subfamily is number of samples per subfamily. **S**



Supplementary Figure 5. Phylogenetic tree containing all CRESS viruses within the lineage available in GenBank and newly identified ant-associated CRESS viruses. An amino acid maximum likelihood phylogeny of the *Rep* protein is shown. Newly identified complete ant-associated CRESS viruses from this study are marked with a red dot. Color shaded regions refer to currently described CRESS viral families.



Supplementary Figure 6. Phylogenetic tree of ant-associated complete CRESS genomes identified in this study. An amino acid maximum likelihood phylogeny of the *Rep* protein is shown. The ant host species name is given for each discovered CRESS virus. The inner circle refers to ant subfamily, inner middle circle refers to the diet of the ant host species (herbivorous, omnivorous, or carnivorous), outer middle circle refers to the nest type of ant host species (arboreal, ground, or leaf litter/rotten log), outer circle refers to environment the ant was sampled from (urban or rainforest). Color shaded regions refer to complete ant-associated CRESS viruses from this study which fell within a described CRESS viral family.



Supplementary Figure 7. Tanglegram with ant host phylogeny by genus on left and discovered complete ant-associated CRESS viral phylogeny on right. Colors of association are randomly prescribed for each of the ant genera.

Supplementary Table 1. Ant Colony Sample Information. From Left to Right: Genus, Species, and Subfamily information about each ant sampled; Diet: H= herbivorous, C= Carnivorous, O = Omnivorous; Nest: A= Arboreal, LL= leaf litter/rotten log, G = ground; Habitat: R = rainforest, U = urban; bacterial abundance (qPCR levels): L=low, M=Medium, H=high; No. ants is the number of ants collected of that colony and pooled for sequencing; Colony sampling ID.

Genus	species	subfamily	die t	nes t	habit at	bacteri al	No. ant s	ID
<i>Anochetus</i>	<i>emarginatus</i>	Ponerinae	C	A	R	L	14	CSM3712
<i>Atta</i>	<i>cephalotes</i>	Myrmicinae	H	G	R	L	18	CSM3738
<i>Atta</i>	<i>cephalotes</i>	Myrmicinae	H	G	U	M	23	CSM3740
<i>Atta</i>	<i>colombica</i>	Myrmicinae	H	G	U	L	30	PJF12
<i>Azteca</i>	<i>chartifex</i>	Dolichoderinae	O	A	U	H	27	PJF11
<i>Azteca</i>	<i>sp1</i>	Dolichoderinae	O	A	R	L	27	CSM3696
<i>Camponotus</i>	<i>atriceps</i>	Formicinae	O	LL	R	H	30	CSM3697
<i>Camponotus</i>	<i>atriceps</i>	Formicinae	O	LL	U	M	22	PJF13
<i>Camponotus</i>	<i>femoratus</i>	Formicinae	O	LL	R	L	16	CSM3695a
<i>Camponotus</i>	<i>rapax</i>	Formicinae	O	G	R	M	5	CSM3684

MOLECULAR ECOLOGY

<i>Camponotus</i>	<i>sp1</i>	Formicinae	O	G	U	M	15	CSM367 2
<i>Camponotus</i>	<i>sp2</i>	Formicinae	O	LL	U	H	31	PJF16
<i>Cephalotes</i>	<i>atratus</i>	Myrmicinae	H	A	U	H	5	PJF17
<i>Cephalotes</i>	<i>atratus</i>	Myrmicinae	H	A	R	H	19	CSM371 6
<i>Cephalotes</i>	<i>minutus</i>	Myrmicinae	H	A	U	M	18	CSM367 1
<i>Cephalotes</i>	<i>sp1</i>	Myrmicinae	H	A	R	H	23	CSM373 3
<i>Crematogaster</i>	<i>levior</i>	Myrmicinae	O	A	R	L	7	CSM369 5b
<i>Crematogaster</i>	<i>sp1</i>	Myrmicinae	O	G	R	L	20	CSM370 1
<i>Crematogaster</i>	<i>sp2</i>	Myrmicinae	O	A	U	L	45	PJF24
<i>Daceton</i>	<i>armigerum</i>	Myrmicinae	C	A	R	H	11	CSM371 7
<i>Daceton</i>	<i>armigerum</i>	Myrmicinae	C	A	U	M	28	CSM373 9
<i>Dolichoderus</i>	<i>attelaboideus</i>	Dolichoderinae	H	A	R	H	18	CSM369 1
<i>Dolichoderus</i>	<i>bispinosus</i>	Dolichoderinae	H	A	R	L	6	CSM368 8
<i>Eciton</i>	<i>sp</i>	Dorylinae	C	G	R	L	47	CSM370 7

MOLECULAR ECOLOGY

<i>Ectatomma</i>	<i>brunneum</i>	Ectatomminae	O	G	U	L	9	CSM3676
<i>Ectatomma</i>	<i>tuberculatum</i>	Ectatomminae	O	G	R	L	11	CSM3686
<i>Gigantiops</i>	<i>destructor</i>	Formicinae	O	G	R	L	21	CSM3715
<i>Gigantiops</i>	<i>destructor</i>	Formicinae	O	G	U	M	6	PJF18
<i>Labidus</i>	<i>coecus</i>	Dorylinae	C	G	R	L	39	CSM3718
<i>Neoponera</i>	<i>commutata</i>	Ponerinae	C	G	R	H	11	CSM3709
<i>Neoponera</i>	<i>commutata</i>	Ponerinae	C	G	U	M	4	PJF19
<i>Nylandaria</i>	<i>sp</i>	Myrmicinae	O	LL	U	L	45	PJF14
<i>Odontomachus</i>	<i>bauri</i>	Ponerinae	C	G	U	M	19	PJF15
<i>Odontomachus</i>	<i>haematodus</i>	Ponerinae	C	LL	U	M	9	CSM3670
<i>Odontomachus</i>	<i>haematodus</i>	Ponerinae	C	LL	U	M	18	CSM3741
<i>Odontomachus</i>	<i>haematodus</i>	Ponerinae	C	G	R	M	19	CSM3719
<i>Odontomachus</i>	<i>hastatus</i>	Ponerinae	C	A	R	H	17	CSM3681
<i>Odontomachus</i>	<i>scalptus</i>	Ponerinae	C	G	R	L	15	CSM3687

MOLECULAR ECOLOGY

<i>Paraponera</i>	<i>clavata</i>	Paraponerinae	C	G	U	L	10	CSM3673
<i>Paraponera</i>	<i>clavata</i>	Paraponerinae	C	G	R	L	12	CSM3708
<i>Pseudomyrmex</i>	<i>gracilis</i>	Pseudomyrmecinae	O	A	R	H	7	CSM3713
<i>Pseudomyrmex</i>	<i>sp1</i>	Pseudomyrmecinae	O	A	R	L	10	CSM3711
<i>Solenopsis</i>	<i>geminata</i>	Myrmicinae	O	G	U	L	75	PJF10
<i>Solenopsis</i>	<i>virulens</i>	Myrmicinae	O	LL	R	M	19	CSM3700

Supplementary Table 2. Table of Viral Genome Isolates. Complete viral genomes within this dataset. Viral genome was considered an isolate if had >80% genome-wide pairwise identity to classified viral isolate.

Viral Genome Name	Isolate Name	query cover	e-value	% identity	Query length	Query accession
Pseudomyrmex ant CRESS virus 1	Dragonfly associated cyclovirus 5	65%	0	91.61%	1787	JX185427.1
Crematogaster ant CRESS virus 4	Dragonfly associated cyclovirus 5	60%	0	91.61%	1787	JX185427.1
Paraponera clavata CRESS virus 10	Dragonfly associated cyclovirus 5	67%	0	91.45%	1787	JX185427.1
Odontomachus scalptus CRESS virus 2	Dragonfly associated cyclovirus 5	60%	0	91.61%	1787	JX185427.1
Azteca ant CRESS virus 1	Dragonfly associated cyclovirus 5	66%	0	91.61%	1787	JX185427.1
Odontomachus haematodus CRESS virus 3	Dragonfly associated cyclovirus 5	66%	0	91.61%	1787	JX185427.1
Camponotus ant CRESS virus 5	Dragonfly associated cyclovirus 5	60%	0	91.61%	1787	JX185427.1
Crematogaster ant CRESS virus 4	Dragonfly associated cyclovirus 5	59%	0	91.61%	1787	JX185427.1
Crematogaster levior CRESS virus 7	Dragonfly associated cyclovirus 5	67%	0	91.61%	1787	JX185427.1
Neoponera commutata CRESS virus 2	Miniopterus associated gemycircularvirus 1	100%	0	97.35%	2193	NC_038480.1
Crematogaster ant CRESS virus 8	Pacific flying fox associated cyclovirus-3	99%	0	97.59%	1838	KT732788.1
Nylanderia ant CRESS virus 1	Pteropus associated gemycircularvirus 5	100%	0	92.62%	2205	NC_038488.1
Cephalotes atratus CRESS virus 1	Pacific flying fox faeces associated gemycircularvirus-7	99%	0	79.34%	2217	NC_038502.1
Gigantiops destructor CRESS virus 6	Pacific flying fox faeces associated gemycircularvirus-7	98%	0	78.00%	2234	MZ556209.1
Dolichoderus bispinosus CRESS virus 19	Bat circovirus POA/2012/II	98%	0	91.62%	1755	NC_025791.1

Supplementary Table 3. Phylogenetic Tests of virus-host co-divergence. (A) Bayesian tip-association significance testing (BaTS), (B) Procrustean Approach to Cophylogeny (PACo), and (C) JANE, co-phylogenetic reconstruction. Red p-values indicate significance.

(A) BaTs

Viral Clade	observed mean	null mean	AI ratio	p-value
Bunya-Arena	0.80	1.08	0.74	0.19
Cruciviridae	3.21	3.32	0.97	0.61
Hepe-Virga	0.80	0.87	0.92	0.48
Lefavirales	0.25	0.25	1.00	1.00
Mono-Chu	0.16	0.29	0.53	0.26
Narna-Levi	2.19	2.84	0.77	0.05
Partiti-Picobirna	3.24	3.73	0.87	0.06
Parvoviridae	22.02	22.50	0.98	0.27
Permutotetra	2.99	3.02	0.99	0.47
Tombus-Noda	4.46	5.17	0.86	0.02
Toti-Chryso	2.76	2.83	0.98	0.40
Weivirus	0.43	0.51	0.86	0.21
Luteo-Sobemo	5.48	6.23	0.88	0.05
Picornal-Calici	10.15	12.85	0.79	0.00
Caudovirales	5.65	5.85	0.97	0.32
Microviridae	26.18	28.81	0.91	0.02
CRESS	16.28	17.91	0.91	0.00

(B) PACo

Viral Clade	Observed m ²	p-value	permutations
Bunya-Arena	272039.3	0.1261	10000
Cruciviridae	650587.3	0.3515	10000
Hepe-Virga	93929.39	0.0782	10000
Mono-Chu	46047.47	0.2514	10000
Narna-Levi	351130.2	0.00	10000
Partiti-Picobirna	561866	6.00E-04	10000
Parvoviridae	3852543	0.0014	10000
Permutotetra	530079.7	0.06	10000
Tombus-Noda	860428.7	7.00E-04	10000
Toti-Chryso	567961.8	0.0099	10000
Lefavirales	60963.08	0.2263	10000
Weivirus	72595.43	0.4971	10000
Luteo-Sobemo	1383172	2.00E-04	10000
Picornal-Calici	25435813	0.00	10000
Caudovirales	409277.54	0.4359	10000
Microviridae	455665	0.02	10000
CRESS	3932443	0.05	10000

(C) Jane

Viral Clade	Original Cost	Mean	p-value	Cospeciation	Duplications	Host Switch	Losses	Failure to Diverge
Bunya-Arena	24	24.76	0.367	1	12	6	0	0
Cruciviridae	52	51.03	0.768	5	9	19	5	0
Hepe-Virga	9	8.49	0.784	2	2	3	1	0
Mono-Chu	7	7.29	0.653	1	3	2	0	0
Narna-Levi	45	48.7	0.0460	4	15-16	13-14	2-3	0
Partiti-Picobirna	53	54.4	0.356	6	5	23	2	0

MOLECULAR ECOLOGY

Parvoviridae	232	230.58	0.670	24	26-27	95-96	14-15	0
Permutotetra	50	49.6	0.760	4	9	19	3	0
Tombus-Noda	68	70.97	0.0490	6	10	28	2	0
Toti-Chryso	48	48.85	0.430	2	13	17	1	0
Lefavirales	2	2	1	1	0	1	0	0
Weivirus	2	6	0.039	4	0	1	0	0
Luteo-Sobemo	108	110.79	0.194	6	21	42	3	0
Picorna-Calici	205	212.28	0.0470	12	35	82-85	2-8	0
Caudovirales	93	92.74	0.580	5	16	36	5	0
Microviridae	150	167.3	0	8	181	180	1	0
CRESS	267	274.28	0.01	9-15	98-104	55-57	5-11	0

Supplementary Table 4. Tests of association between ant host ecological traits and viral phylogenies with δ . δ statistic was used for categorical ecological traits for Diet Type, Nest Type, Bacterial Abundance, and Habitat Type. Red p-values indicate significance.

Viral Clade	diet	diet p-value	nest	nest p-value	bacterial	bacterial p-value	habitat	habitat p-value
Bunya_Arena	0.507	0.46	1.33	0.7	0.503	0.33	0.454	0.7
Cruciviridae	0.284	0.64	0.464	0.75	0.967	0.43	1.489	0.04
Hepe_Virga	0.0542	0.63	0.538	0.79	1.332	0.21	0.766	0.39
Mono_Chua	0.21	0.41	0	1	0.535	0.45	0.611	0.38
Narna_Levi	1.392	0.15	0.659	0.33	1.159	0.12	1.122	0.11
Partiti_Picobirna	0.538	0.83	0.968	0.16	0.745	0.5	1.062	0.41
Parvoviridae	0.553	0.88	0.71	0.27	1.461	0.42	2.439	0
Permutotetra	1.848	0.17	0.544	0.8	1.32	0.1	1.034	0.12
Tombus_Noda	1.385	0.05	0.966	0.34	0.89	0.26	0.941	0.28
Toti_Chryso	0.57	0.62	1.299	0.41	1.463	0.47	0.843	0.94
Lefavirales	0.464	0.2	0.513	0.14	0.513	0.12	0.435	0.33
Weivirus	0.765	0.23	0.234	0.89	0.812	0.46	0.387	0.56
Luteo_Sobemo	1.591	0.05	1.58	0.04	1.347	0.15	1.38	0.02
Picornia_Calici	1.357	0	0.909	0	0.694	0	0.545	0
Caudovirales	0.72	0.46	0.8	0.55	0.568	0.04	0.771	0.1
Microviridae	0.564	0	0.635	0.74	2.075	0.02	1.4	0
CRESS	0.242	0.83	0.538	0.47	1.103	0.22	0.511	0.05

Supplementary Table 5. Tests of association between ant host ecological traits and viral phylogenies. Bayesian tip-association significance testing (BaTS) was used for (A) Bacterial Abundance, (B) Diet Type, (C) Habitat Type, and (D) Nest Type. Red p-values indicate significance.

(A) Bacterial Abundance

Viral Clade	observed mean	null mean	AI ratio	p-value
Bunya-Arena	0.80	1.00	0.80	0.27
Cruciviridae	1.05	1.40	0.75	0.15
Hepe-Virga	0.27	0.40	0.68	0.28
Lefavirales	0.00	0.40	0.00	0.32
Mono-Chu	0.38	0.38	1.00	1.00
Narna-Levi	1.24	2.07	0.60	0.03
Partiti-Picobirna	2.26	2.36	0.96	0.57
Parvoviridae	6.82	8.38	0.81	0.04
Permutotetra	2.10	2.22	0.94	0.62
Tombus-Noda	2.54	3.37	0.75	0.05
Toti-Chryso	0.37	0.37	0.99	0.54
Weivirus	0.04	0.15	0.28	0.51
Luteo-Sobemo	3.64	3.96	0.92	0.28
Picorna-Calici	6.61	8.17	0.81	0.03
Caudovirales	2.74	3.93	0.70	0.02
Microviridae	9.53	11.77	0.81	0.01
CRESS	6.94	8.69	0.80	0.02

(B) Diet Type

Viral Clade	observed mean	null mean	AI ratio	p-value
Bunya-Arena	0.52	0.86	0.60	0.13
Cruciviridae	2.31	2.63	0.88	0.76
Hepe-Virga	0.87	0.87	1.00	1.00
Lefavirales	0.16	0.25	0.66	1.00
Mono-Chu	0.16	0.30	0.51	0.23
Narna-Levi	1.49	1.98	0.75	0.13
Partiti-Picobirna	1.90	2.43	0.78	0.11
Parvoviridae	12.83	13.11	0.98	0.60
Permutotetra	1.57	1.64	0.96	0.58
Tombus-Noda	3.13	3.63	0.86	0.17
Toti-Chryso	2.11	2.23	0.95	0.59
Weivirus	0.04	0.26	0.17	0.15
Luteo-Sobemo	3.44	3.94	0.87	0.20
Picorna-Calici	6.34	8.78	0.72	0.00
Caudovirales	4.12	3.86	1.07	0.66
Microviridae	24.41	24.98	0.98	0.36
CRESS	10.05	11.96	0.84	0.03

MOLECULAR ECOLOGY

(C) Habitat Type

Viral Clade	observed mean	null mean	AI ratio	p-value
Bunya-Arena	0.80	0.93	0.86	0.36
Cruciviridae	1.59	1.60	0.99	0.49
Hepe-Virga	0.50	0.62	0.80	0.88
Lefavirales	0.16	0.25	0.66	1.00
Mono-Chu	0.38	0.38	1.00	1.00
Narna-Levi	1.11	1.48	0.75	0.19
Partiti-Picobirna	1.19	1.32	0.91	0.65
Parvoviridae	4.72	8.97	0.53	0.00
Permutotetra	1.62	1.93	0.84	0.76
Tombus-Noda	1.60	2.48	0.64	0.04
Toti-Chryso	0.33	0.37	0.90	0.33
Weivirus	0.26	0.42	0.63	1.00
Luteo-Sobemo	3.06	3.35	0.91	0.69
Picornal-Calici	3.90	6.98	0.56	0.00
Caudovirales	3.28	3.36	0.98	0.45
Microviridae	10.00	15.02	0.67	0.00
CRESS	4.13	8.91	0.46	0.00

(D) Nest Type

Viral Clade	observed mean	null mean	AI ratio	p-value
Bunya-Arena	0.80	1.02	0.79	0.27
Cruciviridae	2.15	2.79	0.77	0.94
Hepe-Virga	0.68	0.87	0.78	0.81
Lefavirales	0.16	0.25	0.66	1.00
Mono-Chu	0.00	0.00	0.00	1.00
Narna-Levi	1.59	1.92	0.83	0.80
Partiti-Picobirna	1.69	2.10	0.80	0.18
Parvoviridae	13.32	14.86	0.90	0.92
Permutotetra	2.00	2.04	0.98	0.54
Tombus-Noda	2.60	2.74	0.95	0.59
Toti-Chryso	1.02	1.05	0.97	0.51
Weivirus	0.26	0.39	0.67	0.74
Luteo-Sobemo	3.16	3.78	0.84	0.15
Picornal-Calici	7.08	8.48	0.84	0.06
Caudovirales	3.16	3.64	0.87	0.22
Microviridae	20.48	20.72	0.99	0.42
CRESS	11.96	11.21	1.07	0.77