



This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	2016.016aP	(to be completed by ICTV officers)				
Short title: Create <i>Actinidia chlorotic ringspot-associated emaravirus</i> as a new species in the genus <i>Emaravirus</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)						
Modules attached (modules 1 and 10 are required)	1 <input checked="" type="checkbox"/>	2 <input type="checkbox"/>	3 <input type="checkbox"/>	4 <input type="checkbox"/>	5 <input type="checkbox"/>	
	6 <input type="checkbox"/>	7 <input type="checkbox"/>	8 <input type="checkbox"/>	9 <input type="checkbox"/>	10 <input checked="" type="checkbox"/>	

Author(s):

Zheng Y., Navarro B., Wang G., Wang Y., Yang Z., Xu W., Zhu C., Wang L., *Di Serio F., and *Hong N.

Corresponding author with e-mail address:

Ni Hong: whni@mail.hzau.edu.cn,
 Francesco Di Serio: francesco.diserio@ipsp.cnr.it

List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)

Emaravirus study group

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV: June 2016
 Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be “unassigned” within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	2016.016aP	(assigned by ICTV officers)
To create 1 new species within:		
Genus:	<i>Emaravirus</i>	Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “ (new) ” after its proposed name. • If no genus is specified, enter “ unassigned ” in the genus box.
Subfamily:		
Family:		
Order:		
Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
<i>Actinidia chlorotic ringspot-associated emaravirus</i>	Actinidia chlorotic ringspot associated virus HN-6	KT861481 to KT861485

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
 - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria.**
 - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Species demarcation criteria for the genus *Emaravirus* are:

1. Differences in relevant gene product sequences of more than 25%
2. Differences in host ranges
3. Differences in vector specificities

The molecular investigation conducted on a kiwifruit plant HN-6 affected by leaf chlorotic ringspot symptoms showed the presence of a novel virus, named actinidia chlorotic ringspot-associated virus (AcCRaV), with typical features of emaraviruses [i.e. fig mosaic virus (FMV, Elbeaino *et al.*, 2009a, 2009b, 2012); rose rosette virus (RRV, Laney *et al.*, 2011); raspberry leaf blotch virus (RLBV, McGavin *et al.*, 2012); pigeonpea sterility mosaic virus (PPSMV, Elbeaino *et al.*, 2014; Kumar *et al.*, 2003), High Plains wheat mosaic virus (HPWMoV), (Tatineni *et al.*, 2014; Skare *et al.*, 2006); and European mountain ash ringspot-associated virus (EMARaV, Mielke-Ehret & Mühlbach, 2007). Other tentative emaraviruses are: redbud yellow ringspot-associated virus (RYRSaV, Laney *et al.*, 2010; Di Bello *et al.*, 2016), pigeonpea sterility mosaic virus 2 (PPSMV-2, Elbeaino *et al.*, 2015) and woolly burdock yellow vein virus (WBYVV, (Bi *et al.*, 2012)].

Characterization of AcCRaV showed that: (i) it is mechanically transmissible to *N. benthamiana* plants; (ii) shape of the virus particle (Double-Membraned Bodies, DMB) is similar to that of all emaraviruses; (iii) five RNA segments compose its genome; (iv) each of the five RNAs encodes a single protein on the negative-sense strand; (v) the first 13 nucleotides at both 5' and 3' termini of all RNA segments are almost complementary to each other and and

identical to those reported in all known emaraviruses (Elbeaino *et al.*, 2009b, 2014; Laney *et al.*, 2011; Mielke & Mühlbach, 2007); (vi) all RNA-encoded proteins of AcCRaV, i.e. RNA-dependent RNA polymerase (RdRp, RNA-1), putative glycoprotein precursor (GP, RNA-2), putative nucleocapsid (NC, RNA-3), putative movement protein (MP, RNA-4), P5 (unknown function, RNA-5), share high sequence identity with orthologs of emaraviruses and in particular with those of RYRSaV and EMARaV (Table 1).

All the above-listed properties and similarities with emaraviruses, support the classification of AcCRaV-HN6 as a representative of a new species in the genus *Emaravirus* (Mielke-Ehret N. & Mühlbach H.P., 2012).

Virus properties

- Double-membrane virus-like particles from 95 to 110 nm in diameter observed at EM (Fig. 1) (Zheng *et al.*, 2016)
- Genome composed of five segments of negative sense-ssRNA: RNA-1, 7061 nt; RNA-2, 2267 nt; RNA-3, 1678 nt; RNA-4, 1664 nt; RNA-5, 1476 nt (Fig. 2) (in order from RNA-1 to RNA-5, the accession numbers are KT861481 to KT861485).
- Virus-encoded proteins: RdRp (p1), 226.9 kDa; GP (p2), 75 kDa; NC (p3), 34.6 kDa; MP (p4), 43.6 kDa; and p5, 26.5 kDa (Fig. 2).
- Consistent clustering of AcCRaV with other emaraviruses, in particular with RYRSaV and EMARaV, in a separate clade, in phylogenetic trees constructed with p1, p2 and p3 amino acid sequences (Fig. 3).
- High amino acids sequence identity with emaraviruses (Table 1).
- Mechanically transmissible to *N. benthamiana* plants.
- Unknown vector.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

- Bi Y., Tugume A.K., Valkonen, J.P., 2012. Small-RNA deep sequencing reveals *Arctium tomentosum* as a natural host of *Alstroemeria virus X* and a new putative *Emaravirus*. *Plos One*, 7: e42758.
- Di Bello P.L., Laney A.G., Druciarek T., Ho T., Gergerich R.C., Keller K.E., Martin R.R., Tzanetakis I.E., 2016. A novel emaravirus is associated with redbud yellow ringspot disease. *Virus Research* 222: 41-47.
- Elbeaino T., Digiario M., Alabdullah A.K., De Stradis A., Minafra A., Mielke N., Castellano M.A., Martelli G.P., 2009a. A multipartite negative-sense single-stranded RNA virus is the putative agent of fig mosaic disease. *Journal of General Virology*, 90 (5): 1281-1288.
- Elbeaino T., Digiario M., Martelli G.P., 2009b. Complete nucleotides sequence of four viral RNAs segments of fig mosaic virus. *Archives of Virology*, 154 (11): 1719-1727.
- Elbeaino T., Digiario M., Martelli G.P., 2012. RNA-5 and -6, two additional negative-sense RNA segments associated with Fig mosaic virus. *Journal of Plant Pathology*, 94 (2): 421-425.
- Elbeaino T., Digiario M., Uppala M., Sudini H., 2014. Deep sequencing of Pigeonpea sterility mosaic virus discloses five RNA segments related to emaraviruses. *Virus Research*, 188: 27–31.
- Elbeaino T., Digiario M., Uppala M., Sudini H., 2015. Deep-sequencing of dsRNAs recovered from mosaic-diseased pigeonpea (*Cajanus cajan* L.) revealed the presence of a novel emaravirus: Pigeonpea sterility mosaic virus 2 (PPSMV2). *Archives of Virology*, 160:2019–2029.
- Kumar P.L., Jones A.T., Reddy D., 2003. A novel mite-transmitted virus with a divided RNA genome

additional material in support of this proposal

References:

- closely associated with pigeonpea sterility mosaic disease. *Phytopathology*, 93, 71-81.
- Laney A.G., Gergerich R., Keller K., Martin R., Tzanetakis I., 2010. Rose rosette and redbud yellow ringspot are caused by two new emaraviruses. *Phytopathology*, 100, S67.
- Laney A.G., Keller K.E., Martin R.R., Tzanetakis I.E., 2011. A discovery 70 years in the making: characterization of the Rose rosette virus. *Journal of General Virology*, 92: 1727-1732.
- McGavin W.J., Mitchell C., Cock P.J.A., Wright K.M., MacFarlane S.A., 2012. Raspberry leaf blotch virus, a putative new member of the genus *Emaravirus*, encodes a novel genomic RNA. *Journal of General Virology*, 93: 430–437.
- Mielke N., Muehlbach H.P., 2007. A novel, multipartite, negative-strand RNA virus is associated with the ringspot disease of European mountain ash (*Sorbus aucuparia* L.). *Journal of General Virology*, 88: 1337–1346.
- Mühlbach H.P., Mielke-Ehret N., 2012. Emaravirus, p. 767–769. In King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds.). *Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses*, Elsevier-Academic Press, London UK.
- Skare J.M, Wijkamp I, Denham I, Rezende J.A.M., Kitajima E.W., Park J.W., Desvoyes B., Rush C.M., Michels G., Scholthof K.B.G., Scholthof H.B., 2006. A new eriophyid mite-borne membrane-enveloped virus-like complex isolated from plants. *Virology*, 347: 343–353.
- Tatineni S., McMechan A.J., Wosula E.N., Wegulo S.N., Graybosch R.A., French R., Hein G.L., 2014. An eriophyid mite-transmitted plant virus contains eight genomic RNA segments with unusual heterogeneity in the nucleocapsid protein. *Journal of Virology*, 88: 11834-11845.
- Zheng Y., Navarro B., Wang G., Wang Y., Yang Z., Xu W., Zhu C., Wang L., Di Serio F., Hong N., 2016. Actinidia chlorotic ringspot-associated virus: a novel emaravirus infecting kiwifruit plants. *Molecular Plant Pathology*: DOI: 10.1111/mpp.12421.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

Figure 1. Ultrastructure of kiwifruit leaves infected with AcCRaV. (A) Virions with surrounding double membrane in the cytoplasm of a mesophyll cell. (B) Close up of what presented in panel A. (C) and (D) Virions near endoplasmic reticulum in the cytoplasm of AcCRaV-infected cells. V, virion; Vc, vacuole; ER, endoplasmic reticulum; M, mitochondria; N, nucleus; Ch, chloroplast; Cw, cell wall.

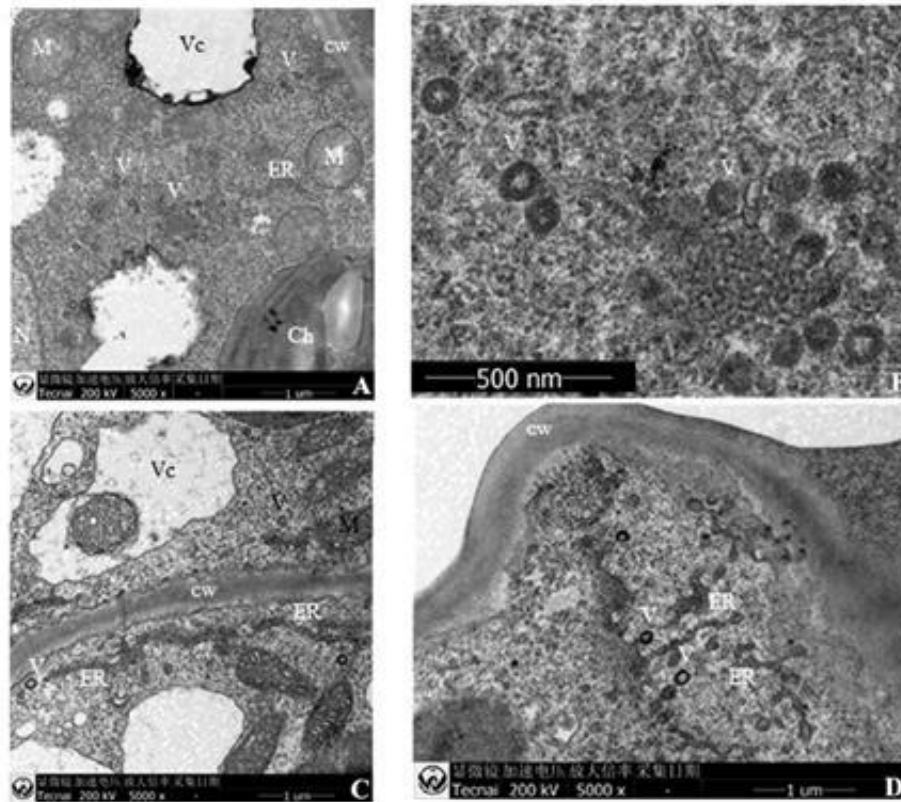


Figure 2 Schematic representation of the organization of the AcCRaV genome. The terminal 13 nucleotides conserved at the 5' and 3' termini are indicated as black boxes on each segment. Expression product of each of the five genomic RNAs is shown as a grey box containing information on aa length, estimated molecular weight (kDa) and putative functions of the predicted proteins.

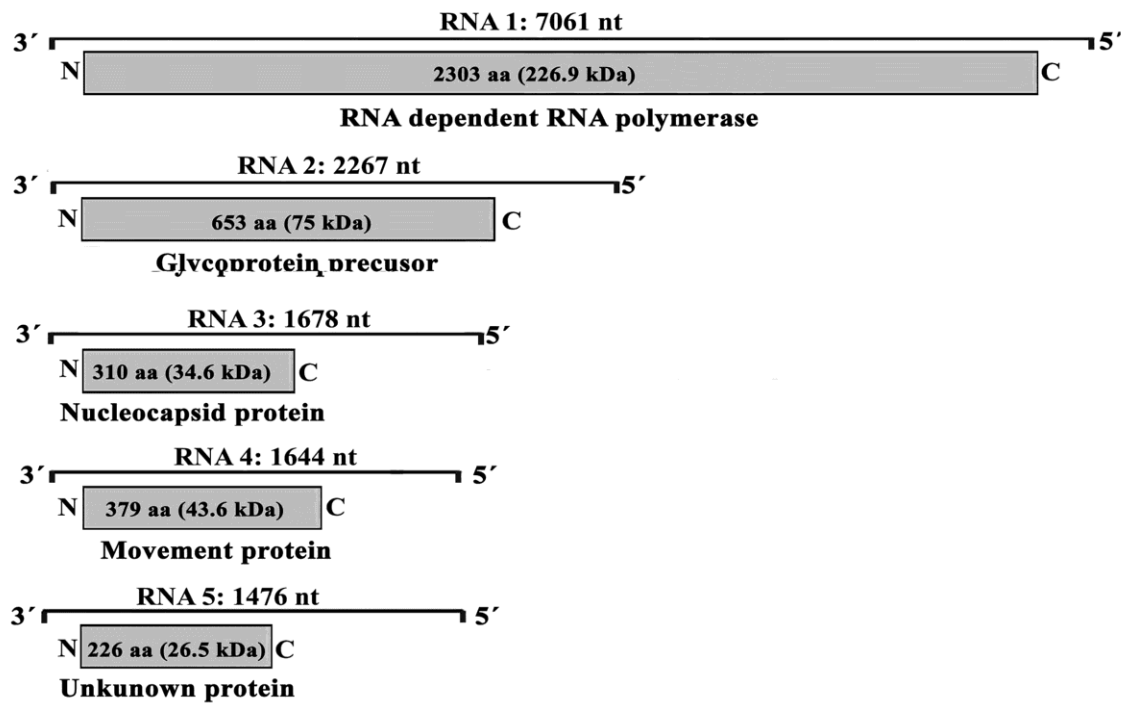


Figure 3. Phylogenetic trees constructed on multiple alignments of amino acid sequences of AcCRaV P1, P2 and P3 proteins with emaraviruses and the selected bunyaviruses. Trees were constructed in MEGA 5.1 (Tamura et al., 2011) by the neighbor-joining method with 1,000 bootstrap replicates. GenBank accession numbers of proteins used for phylogenetic analyses are reported alongside virus acronyms: Bunyamwera virus (BUNV); Dugbe virus (DUGV); Puumala virus (PUUV); Rift Valley fever virus (RVFV); tomato spotted wilt virus (TSWV); and a tenuivirus, rice grassy stunt virus (RGSV). Sonchus yellow net virus (SYNV), a member of the genus *Nucleorabdovirus*, was used as an outgroup species in all trees. Note that AcCRaV formed a separate clade with RYRSaV and EMARaV from other members of the genus *Emaravirus*. The bar represents the number of amino acid replacements per site.

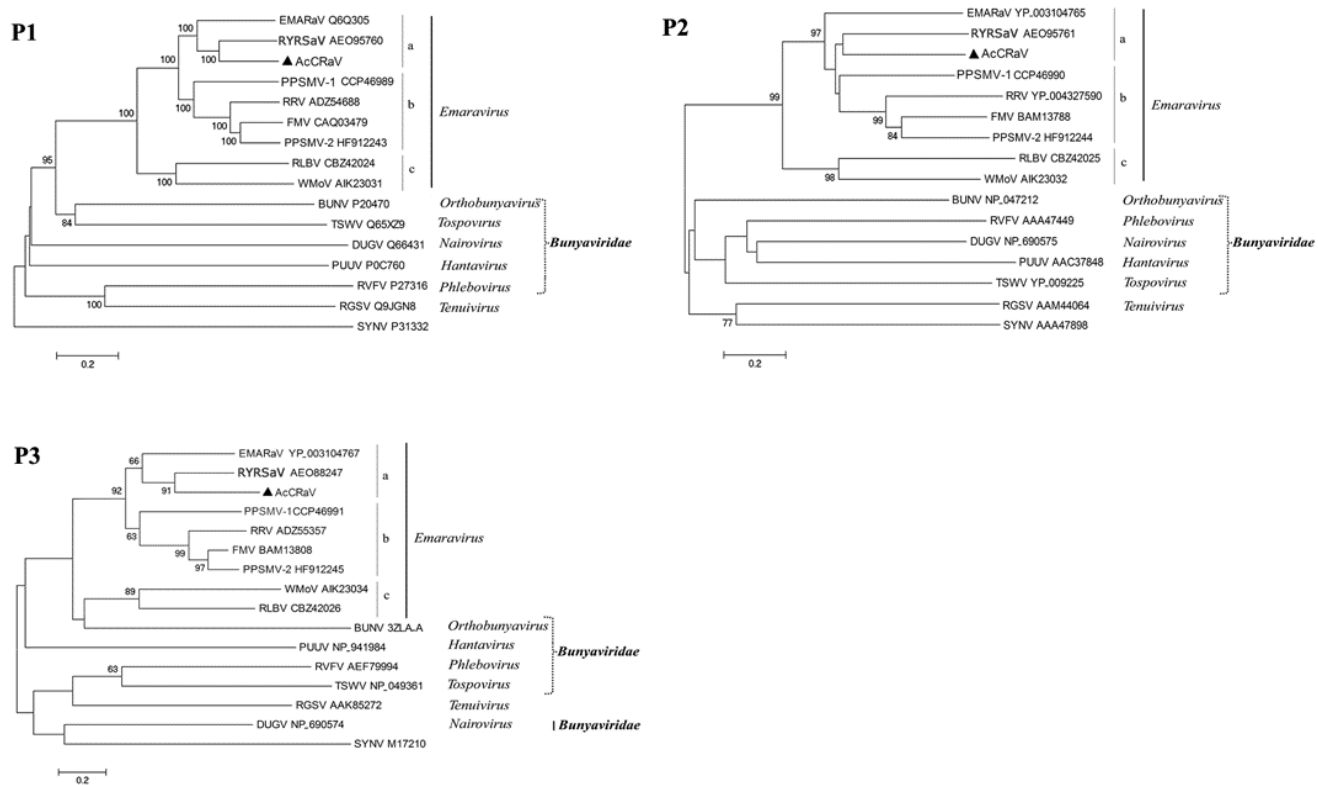


Table 1. Nucleotide and amino acid sequence identities (%) between AcCRaV and other emaraviruses.

RNA	Virus	Virus ID	Genome		5' UTR		ORF			3' UTR	
			Length ^a	% ^a	Length ^a	% ^a	Length ^a	% ^a	% ^b	Length ^a	% ^a
RNA-1 (RdRp)	AcCRaV	KT861481	7061	-	111	-	6912	-	-	38	-
	RYRSaV	JF795479	7049	66.1	109	59.4	6900	66.2	64.6	40	65.8
	EMARaV	NC_013105	7040	60.5	114	56.8	6882	60.4	54.8	44	76.3
	RRV	NC_015298	7026	58.0	107	57.1	6831	57.9	47.6	88	73.7
	PPSMV-1	HF568801	7022	58.3	88	59.1	6885	58.2	48.3	49	68.4
	PPSMV-2	HF912243	7009	55.7	45	64.4	6885	56.0	47.1	79	75.0
	FMV	AM941711	7039	58.3	106	58.5	6894	58.2	48.5	39	65.8
	RLBV	FR823299	7062	55.3	126	59.5	6888	55.0	35.5	48	78.9
	WMoV	KJ939623	6981	54.1	94	53.2	6819	53.7	33.4	68	76.3
RNA-2 (GP)	AcCRaV	KT861482	2267	-	251	-	1962	-	-	54	-
	RYRSaV	JF795480	2220	59.5	241	67.8	1929	58.3	48.3	50	75.5
	EMARaV	NC_013106	2335	57.0	336	60.0	1941	55.4	42.3	58	70.4
	RRV	NC_015299	2245	54.6	257	47.9	1938	53.8	39.6	50	73.5
	PPSMV-1	HF568802	2223	54.8	235	52.6	1947	53.9	41.9	41	80.5
	PPSMV-2	HF912244	2229	51.0	47	48.9	1950	52.2	39.0	232	62.3
	FMV	AB697829	2252	53.6	274	50.6	1926	54.0	41.8	52	76.9
	RLBV	FR823300	2135	49.3	133	52.6	1953	50.1	24.3	49	70.2
	WMoV	KJ939624	2211	50.9	128	48.0	2004	51.2	28.0	79	66.7
RNA-3 (NC)	AcCRaV	KT861483	1678	-	645	-	933	-	-	100	-
	RYRSaV	JF795481	1414	58.9	383	59.2	942	62.4	55.6	89	62.5
	EMARaV	NC_013107	1559	54.2	495	48.0	945	56.6	40.5	119	62.0
	RRV	HQ891893	1544	53.3	494	53.8	951	51.0	33.9	99	67.7
	PPSMV-1	HF568803	1442	52.2	413	53.0	927	52.0	36.0	102	52.0
	PPSMV-2	HF912245	1335	50.7	101	49.5	939	51.6	35.3	295	56.7
	FMV	AB697849	1491	56.6	444	59.0	948	55.6	38.3	99	56.8
	RLBV	FR823301	1365	51.9	433	57.7	879	49.1	19.9	53	67.3
	WMoV	KJ939626	1441	50.3	352	52.4	870	48.3	19.5	219	62.0
RNA-4 (MP)	AcCRaV	KT861484	1664	-	426	-	1140	-	-	98	-
	RYRSaV	JF795482	1513	61.2	283	62.1	1131	62.6	51.9	99	59.2
	EMARaV	NC_013108	1348	51.6	504	53.8	699	47.5	13.8	145	54.7
	RRV	HQ891882	1541	55.2	372	56.1	1086	52.7	31.3	83	55.4
	PPSMV-1	HF568804	1563	53.2	400	51.4	1086	51.0	29.6	77	61.3
	PPSMV-2	HF912246	1491	47.3	82	54.9	1086	49.8	27.6	323	53
	FMV	HQ703346	1472	57.4	305	54.9	1086	51.7	30.1	81	72.8
	RLBV	FR823302	1675	48.9	472	50.0	1122	48.8	20.4	81	61.7
	WMoV	KJ939627	1682	51.1	475	51.7	1095	49.2	22.2	112	59.2
RNA-5	AcCRaV	KT861485	1476	-	699	-	681	-	-	96	-
	PPSMV-1	HF945448	1801	51.5	295	49.5	1422	51.4	22.3	84	63.1
	PPSMV-2	HG939489	1833	38.7	105	39.7	1422	39.4	9.2	306	51.9
	PPSMV-2 ^c	HG939490	1194	40.4	68	55.9	717	40.5	15.3	409	54.7
	FMV	CCH27326	1752	49.2	186	51.6	1509	50.4	20.4	57	59.6
	EMARaV ^d	NC_013108	1348	51.3	504	54.4	699	51.3	18.7	145	59.4
	RRV ^e	KM007082	1402	41.2	523	43.0	702	41.8	9.6	67	55.9
	RLBV	FR823303	1718	51.7	220	54.1	1431	52.0	22.3	67	62.7
	WMoV	KJ939628	1715	50.4	158	57.4	1437	50.4	21.7	120	64.6
WMoV ^f	KJ939631	1339	48.8	715	50.1	531	49.5	16.7	93	59.8	

^a nucleotide

^b amino acid

^c PPSMV-2 RNA-6

^d EMARaV RNA-4

^eRRV RNA-6 and protein 6b

^f WMoV RNA-8
