




Smacoviridae: a new family of animal-associated single-stranded DNA viruses

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Abstract

Smacoviruses have small (~2.3–2.9 kb), circular single-stranded DNA genomes encoding rolling circle replication-associated proteins (Rep) and unique capsid proteins. Although smacoviruses are prevalent in faecal matter of various vertebrates, including humans, none of these viruses have been cultured thus far. Smacoviruses display ~45% genome-wide sequence diversity, which is very similar to that found within other families of circular Rep-encoding single-stranded (CRESS) DNA viruses, including members of the families *Geminiviridae* (46% diversity) and *Genomoviridae* (47% diversity). Here, we announce the creation of a new family *Smacoviridae* and describe a sequence-based taxonomic framework which was used to classify 83 smacovirus genomes into 43 species within six new genera, *Bovismacovirus* (n=3), *Cosmacovirus* (n=1), *Dragsmacovirus* (n=1), *Drosmacovirus* (n=3), *Huchismacovirus* (n=7), and *Porprismacovirus* (n=28). As in the case of genomoviruses, the species demarcation is based on the genome-wide pairwise identity, whereas genera are established based on the Rep amino acid sequence identity coupled with strong phylogenetic support. A similar sequence-based taxonomic framework should guide the classification of an astonishing diversity of other uncultured and currently unclassified CRESS DNA viruses discovered by metagenomic approaches.

With the advent of metagenomics approaches, a large diversity of unknown viruses has been uncovered in various environmental, plant, and animal samples [23]. Sampling of animal faecal matter has proved to be particularly efficient for the discovery of a wide variety of novel viral types, in particular those with small DNA genomes. Until recently, the circular replication-initiation protein encoding single-stranded (CRESS) DNA viruses associated with eukaryotic

hosts have been classified by the International Committee on Taxonomy of Viruses (ICTV) into four families, namely *Circoviridae*, *Genomoviridae*, *Geminiviridae* and *Nanoviridae*. In 2018, the ICTV created two new families for classification of CRESS DNA viruses, *Bacilladnaviridae* and *Smacoviridae*. The family *Bacilladnaviridae*, which includes viruses infecting diatoms, a major group of unicellular algae widespread in aquatic habitats, has been described elsewhere [7]. Here, we introduce the family *Smacoviridae* (*smaco*-stands for *small* circular DNA viruses) and describe the ICTV-approved sequence-based taxonomic framework for classification of these viruses.

Smacoviruses [15, 17], previously also referred to as chipoviruses [22, 24], have been identified in faecal matter of various vertebrates, including humans, as well as in the abdomina of dragonflies of two species (Table 1). Thus far, none of these viruses have been cultured or found in animal tissue sample. Nonetheless, the viruses have been discovered using viral metagenomics approaches and for the majority of the smacoviruses, the validity of genome sequences has been verified by either PCR amplification using abutting primers followed by Sanger sequencing of these products or by amplification, cloning and Sanger sequencing of the recombinant plasmids [1, 3, 4, 9, 15, 22, 24].

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Table 1 Summary of taxa that are part of the family *Smacoviridae*

Genus	Species	Accession #	Isolate	Isolation source			
				Host	Host species	Country	Reference
<i>Bovismacovirus</i>	<i>Bovine associated bovismacovirus 1</i>	JN634851	CP11-49-3	Domestic cow	<i>Bos taurus</i>	South Korea	[9]
	<i>Bovine associated bovismacovirus 2</i>	KT862222	48_Fec5_cow	Domestic cow	<i>Bos taurus</i>	New Zealand	[24]
	<i>Dragonfly associated bovismacovirus 1</i>	KM598409	OdasCV-21-US-1679SC3-12	Red-faced Dragonlet	<i>Erythrodiplax fusca</i>	USA	[4]
<i>Drosmacovirus</i>	<i>Camel associated drosmacovirus 1</i>	KM573769	DcSCV_c1359	One-humped camel	<i>Camelus dromedarius</i>	United Arab Emirates	[26]
	<i>Camel associated drosmacovirus 2</i>	KM573774	DcSCV_c1330	One-humped camel	<i>Camelus dromedarius</i>	United Arab Emirates	[26]
	<i>Bovine associated drosmacovirus 1</i>	KT862224	48_Fec9_cow	Domestic cow	<i>Bos taurus</i>	New Zealand	[24]
<i>Huchismacovirus</i>	<i>Bovine associated huchismacovirus 1</i>	KT862223	48_Fec59973_cow	Domestic cow	<i>Bos taurus</i>	New Zealand	[24]
	<i>Bovine associated huchismacovirus 2</i>	KT862229	GP3_46075_cow	Domestic cow	<i>Bos taurus</i>	New Zealand	[24]
	<i>Chicken associated huchismacovirus 1</i>	KY086301	RS/BR/2015/2	Domestic chicken	<i>Gallus gallus</i>	Brazil	[13]
	<i>Chicken associated huchismacovirus 2</i>	KY086300	RS/BR/2015/3	Domestic chicken	<i>Gallus gallus</i>	Brazil	[13]
	<i>Human associated huchismacovirus 1</i>	KP233180	Oregon/6/2011/GottageGrove/5A1	Human	<i>Homo sapiens</i>	USA	[15]
		KP233181	Oregon/6/2011/GottageGrove/B3	Human	<i>Homo sapiens</i>	USA	[15]
		KP233182	Oregon/6/2011/GottageGrove/B45	Human	<i>Homo sapiens</i>	USA	[15]
		KP233183	Oregon/8/2011/Portland/D56	Human	<i>Homo sapiens</i>	USA	[15]
		KP233185	Virginia/2/2012/Albemarle/5117	Human	<i>Homo sapiens</i>	USA	[15]
		KP233186	Virginia/2/2012/Chesapeake/J23	Human	<i>Homo sapiens</i>	USA	[15]
		KP233188	Virginia/12/2011/Albemarle/G16	Human	<i>Homo sapiens</i>	USA	[15]
		KP233193	Orgeon/8/2011/Portland/D53	Human	<i>Homo sapiens</i>	USA	[15]
	<i>Human associated huchismacovirus 2</i>	KP264964	France/2/2008/2548	Human	<i>Homo sapiens</i>	France	[15]
		KP264966	France/12/2008/3454	Human	<i>Homo sapiens</i>	France	[15]
		KP264969	France/6/2008/2871	Human	<i>Homo sapiens</i>	France	[15]
KY086299		RS/BR/2015/4	Domestic chicken	<i>Gallus gallus</i>	Brazil	[13]	
KP233174		France/8/2008/2444	Human	<i>Homo sapiens</i>	France	[15]	

Table 1 (continued)

Genus	Species	Accession #	Isolate	Isolation source			
				Host	Host species	Country	Reference
Porprismacovirus	Human associated huchismacovirus 3	KP233175	France/1/2008/2610	Human	<i>Homo sapiens</i>	France	[15]
		KP233176	France/8/2008/2449	Human	<i>Homo sapiens</i>	France	[15]
		KP233177	France/4/2009/4265	Human	<i>Homo sapiens</i>	France	[15]
		KP233184	Virginia/1/2012/Mecklenburg/H19	Human	<i>Homo sapiens</i>	USA	[15]
		KP233187	Virginia/2/2012/Middlesex/I22	Human	<i>Homo sapiens</i>	USA	[15]
		KP264965	France/3/2008/2623	Human	<i>Homo sapiens</i>	France	[15]
		KP264967	France/12/2008/3454	Human	<i>Homo sapiens</i>	France	[15]
		KP233178	France/1/2009/3664	Human	<i>Homo sapiens</i>	France	[15]
		KP233179	France/1/2009/3663	Human	<i>Homo sapiens</i>	France	[15]
	KP264968	France/3/2009/4191	Human	<i>Homo sapiens</i>	France	[15]	
	KT862218	23_Fec30587_cow	Domestic cow	<i>Bos taurus</i>	New Zealand	[24]	
	KM573772	DcSCV_c1378	One-humped camel	<i>Camelus dromedarius</i>	United Arab Emirates	[26]	
	KM573770	DcSCV_c1072	One-humped camel	<i>Camelus dromedarius</i>	United Arab Emirates	[26]	
	KM573771	DcSCV_c1345	One-humped camel	<i>Camelus dromedarius</i>	United Arab Emirates	[26]	
	KM573775	DcSCV_c1358	One-humped camel	<i>Camelus dromedarius</i>	United Arab Emirates	[26]	
	GQ351272	DP152	Common chimpanzee	<i>Pan troglodytes</i>	Cameroon	[1]	
	GQ351275	GM510	Common chimpanzee	<i>Pan troglodytes</i>	Tanzania	[1]	
	GQ351273	GM495	Common chimpanzee	<i>Pan troglodytes</i>	Tanzania	[1]	
	GQ351274	GM476	Common chimpanzee	<i>Pan troglodytes</i>	Tanzania	[1]	
GQ351276	GM488	Common chimpanzee	<i>Pan troglodytes</i>	Tanzania	[1]		
GQ351277	GM415	Common chimpanzee	<i>Pan troglodytes</i>	Tanzania	[1]		
KY086298	RS/BR/2015/1	Domestic chicken	<i>Gallus gallus</i>	Brazil	[13]		
KP233191	SF3	Western gorilla	<i>Gorilla gorilla</i>	USA	[15]		
KP233192	SF4	Western gorilla	<i>Gorilla gorilla</i>	USA	[15]		

Table 1 (continued)

Genus	Species	Accession #	Isolate	Isolation source			
				Host	Host species	Country	Reference
	<i>Howler monkey associated porprismacovirus 1</i>	KP233189	SF1	Black howler	<i>Alouatta caraya</i>	USA	[15]
	<i>Human associated porprismacovirus 1</i>	KT600068	SmaCV2_ID31	Human	<i>Homo sapiens</i>	Peru	[17]
	<i>Human associated porprismacovirus 2</i>	KP233190	SF2	Common chimpanzee	<i>Pan troglodytes</i>	USA	[15]
		KT600069	SmaCV3_ID16	Human	<i>Homo sapiens</i>	Peru	[17]
		KX838317	BWA1115	Human	<i>Homo sapiens</i>	Botswana	-
		KX838318	BWA7684	Human	<i>Homo sapiens</i>	Botswana	-
	<i>Lemur associated porprismacovirus 1</i>	KP233194	SF5	Ring-tailed lemur	<i>Lemur catta</i>	USA	[15]
	<i>Porcine associated porprismacovirus 1</i>	JX274036	Cass	Domestic pig	<i>Sus scrofa domesticus</i>	New Zealand	[22]
		KF193403	J481	Domestic pig	<i>Sus scrofa domesticus</i>	South Korea	[8]
		KT862226	56_Coc3310_hare	European hare	<i>Lepus europaeus</i>	New Zealand	[24]
		KT862227	59_Coc3310_possum	Common brush-tail	<i>Trichosurus vulpecula</i>	New Zealand	[24]
	<i>Porcine associated porprismacovirus 2</i>	KC545226	f	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[2]
		KJ577818	TP3	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[3]
		KC545227	3L7	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[2]
	<i>Porcine associated porprismacovirus 3</i>	KC545228	4L13	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[2]
		KC545229	4L5	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[2]
		KC545230	L2T	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[2]
		KJ577810	DP2	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[3]
	<i>Porcine associated porprismacovirus 4</i>	KJ577811	DP3	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[3]
	<i>Porcine associated porprismacovirus 5</i>	KJ577819	XP1	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[3]
	<i>Porcine associated porprismacovirus 6</i>	KJ577812	EP2-A	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[3]
		KJ577813	EP2-B	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[3]
		KJ577814	EP3-C	Domestic pig	<i>Sus scrofa domesticus</i>	USA	[3]

Table 1 (continued)

Genus	Species	Accession #	Isolate	Isolation source			
				Host	Host species	Country	Reference
		KJ577815	EP3-D	Domestic pig	<i>Sus scrofa domestica</i>	USA	[3]
	<i>Porcine associated porprismacovirus 8</i>	KJ577817	GP2	Domestic pig	<i>Sus scrofa domestica</i>	USA	[3]
	<i>Porcine associated porprismacovirus 9</i>	KJ577816	FP1	Domestic pig	<i>Sus scrofa domestica</i>	USA	[3]
	<i>Porcine associated porprismacovirus 10</i>	KT862225	49_Fec25_pig	Domestic pig	<i>Sus scrofa domestica</i>	New Zealand	[24]
	<i>Rat associated porprismacovirus 1</i>	KP860906	KS/11/0577	Brown rat	<i>Rattus norvegicus</i>	Germany	[21]
		KP860907	Mu/10/1799	Brown rat	<i>Rattus norvegicus</i>	Germany	[21]
		KP860908	KS/11/0582	Brown rat	<i>Rattus norvegicus</i>	Germany	[21]
	<i>Sheep associated porprismacovirus 1</i>	KT862220	47_Fec58729_sheep	Sheep	<i>Ovis aries</i>	New Zealand	[24]
	<i>Sheep associated porprismacovirus 2</i>	KT862221	47_Fec60415_sheep	Sheep	<i>Ovis aries</i>	New Zealand	[24]
	<i>Sheep associated porprismacovirus 3</i>	KT862219	47_Fec58091_sheep	Sheep	<i>Ovis aries</i>	New Zealand	[24]
	<i>Turkey associated porprismacovirus 1</i>	KF880727	TuSCV	Wild turkey	<i>Meleagris gallopavo</i>	Hungary	[19]
<i>Cosmacovirus</i>	<i>Bovine associated cosmacovirus 1</i>	KT862228	GP3_45917_cow	Domestic cow	<i>Bos taurus</i>	New Zealand	[24]
<i>Dragsmacovirus</i>	<i>Dragonfly associated dragsmacovirus 1</i>	KM598410	OdasCV-5-US-1683LM1-12	Four-spotted skimmer	<i>Libellula quadrimaculata</i>	USA	[4]

The genomes of currently identified smacoviruses are ~2300-2900 nucleotides-long, contain two major open reading frames (ORF), encoding the rolling circle replication-associated protein (Rep) and capsid protein (CP; Figure 1). The two ORFs in all 83 smacovirus genomes are bidirectionally organised, separated by two intergenic regions. Similar to other CRESS DNA viruses, smacoviruses contain a conserved nonanucleotide sequence located at a putative stem-loop structure at the origin of replication (Figure 1), where nicking of the dsDNA replicative intermediate is predicted to occur. The Reps of smacoviruses are homologous but phylogenetically distinct from those of classified CRESS DNA viruses (Figure 2). Phylogenetic analysis and comparison of the conserved sequence motifs suggest a closer evolutionary relationship between the smacovirus and nanovirus Reps [7].

By contrast, although conserved among smacoviruses, the CPs do not display recognizable sequence similarity to the CPs of other known viruses.

Analysis of the genome-wide pairwise identities of the 83 smacoviruses (Figure 3) shows 45% diversity amongst these genomes, which is similar to values determined for members of the families *Geminiviridae* [27] and *Genomoviridae* [12, 25]. The plot of the distribution of pairwise identities shows a trough between 76 and 88%. Hence, for this group of viruses, 77% genome-wide pairwise identity is chosen as a species demarcation threshold. Using this approach, the 83 smacoviruses were assigned to 43 species (Table 1).

Maximum likelihood phylogenetic analysis of the Rep sequences of all 83 smacoviruses reveals four main clusters with >90% branch support and two singletons (Figure 4).

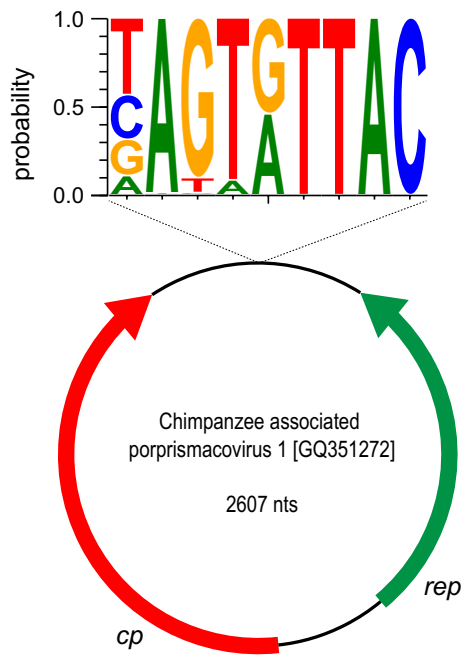


Fig. 1 Genome organization of a representative smacovirus (chimpanzee associated porprismacovirus 1 [GQ351272]) and a WebLogo of the nonanucleotide motif found in smacoviruses

Rep sequences within each of the four clades in general share >40% pairwise identity, whereas sequences from different phylogenetic clades show less than 40% identity to each other. We note that phylogenetic trees produced using complete genome (Figure 5) and CP (Figure 6) sequences are not congruent with the Rep phylogeny, presumably due to intra-familial recombination between different smacovirus genomes resulting in chimeric entities encoding Rep and CP with different evolutionary histories, as has been also observed for genomoviruses [25]. Given that smacovirus Reps are considerably more conserved than CPs (Figure 3) and due to the fact that Reps are the only proteins shared across all CRESS DNA viruses [11, 20], genera were established based on the phylogenetic analysis of the Rep sequences coupled with their pairwise sequence identity. Accordingly, 40% Rep amino acid sequence identity coupled with strong phylogenetic support is proposed as a genus level demarcation threshold.

The naming practice for smacoviruses and other uncultivated CRESS DNA viruses, such as genomoviruses [25], typically involves adoption of the name of an organism or material from which the virus genome has been sequenced. In the absence of evidence of actual infection, the word “associated” is usually added to the potential host name to

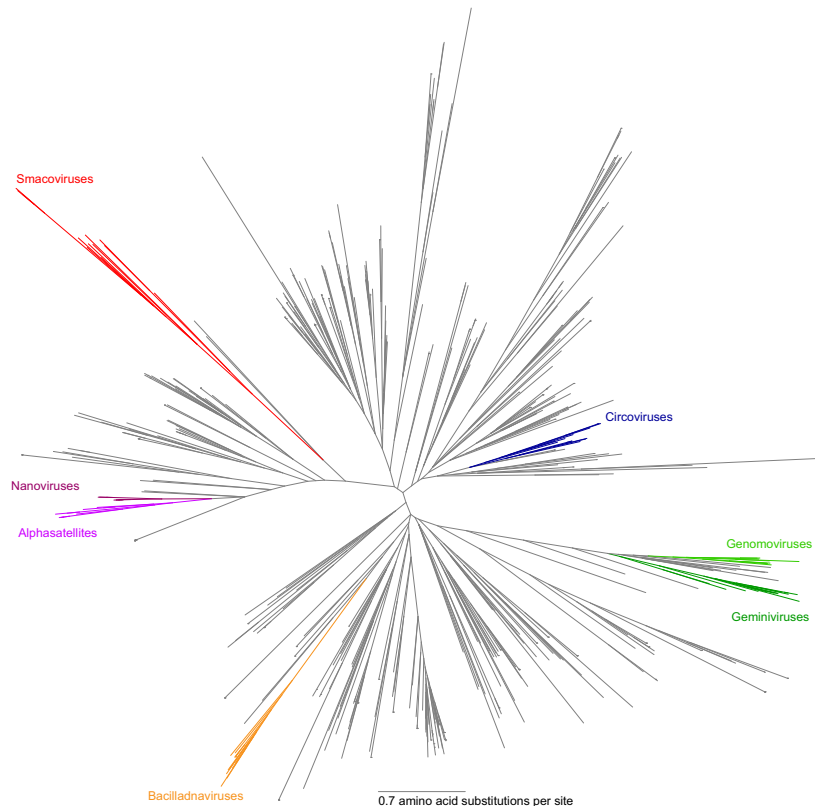


Fig. 2 Unrooted approximate maximum likelihood phylogenetic tree of Reps of CRESS DNA viruses inferred using FastTree [18]. Major groups of classified CRESS DNA viruses as well as alphasatellites associated with geminiviruses and nanoviruses are colour coded

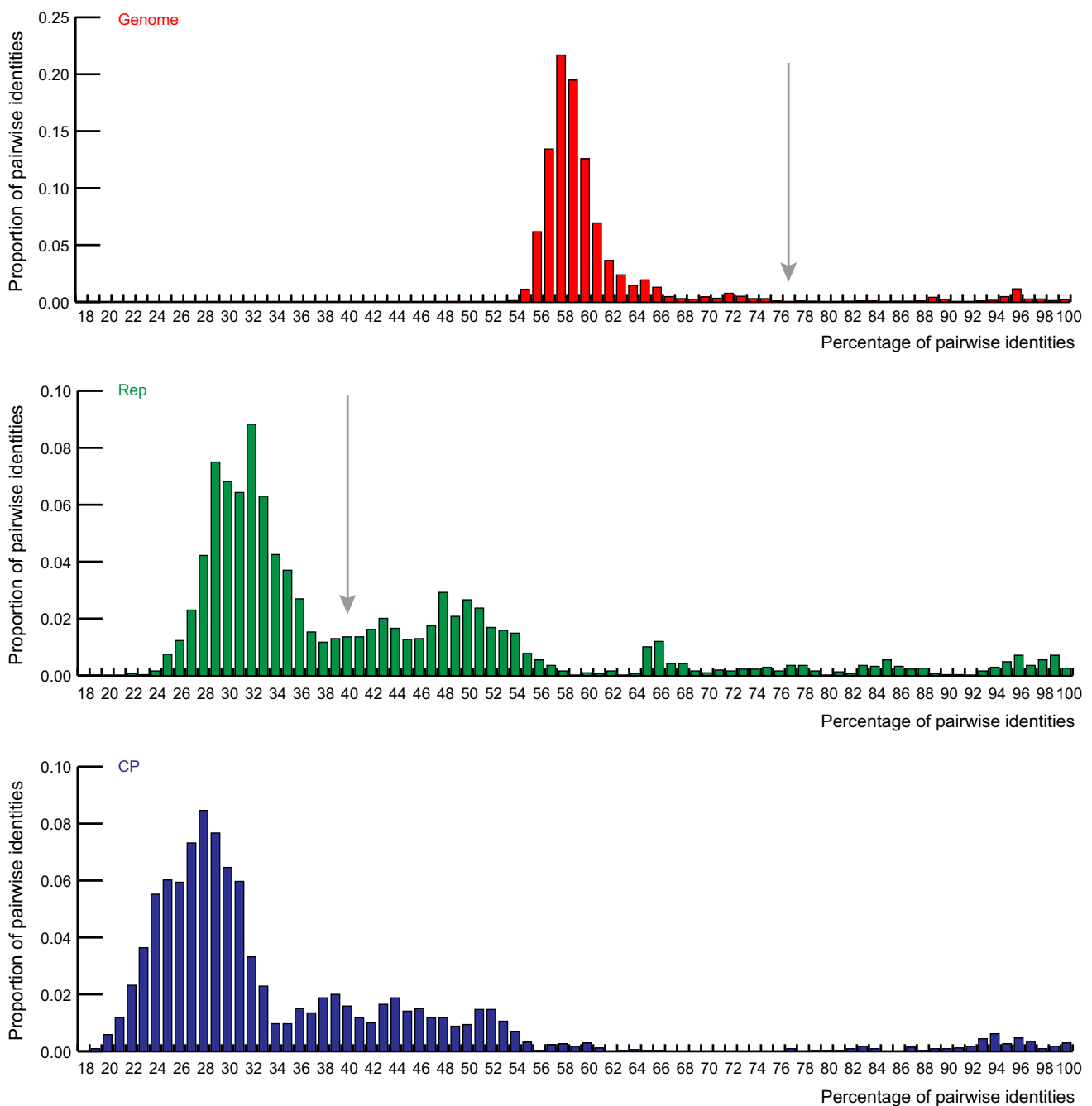


Fig. 3 Distribution of pairwise identities of the full genome (upper panel), the replication initiation protein (middle panel) and the capsid protein sequences determined using SDT v1.2 [14]. The arrows

indicate the thresholds of the full genome (top panel) and Rep protein (middle panel) pairwise sequence identities used as the species and genus demarcation criteria, respectively

emphasize that the organism may or may not be the actual host. As a case in point, it has been recently suggested that dsRNA viruses of the family *Picobirnaviridae*, which for three decades were considered to infect eukaryotes [5], might instead replicate in bacteria that populate the enteric tract of animals [10]. Thus, utmost caution should be exercised when assigning viruses to potential hosts.

The following names for the six genera within the *Smacoviridae* have been adopted

Bovismacovirus: **Bovine smacovirus**

3 species (Table 1);

Drosmacovirus: **Dromedary smacovirus**

3 species (Table 1);

Fig. 4 Maximum likelihood phylogenetic tree of the Rep amino acid sequences of the smacoviruses inferred using PhyML [6] with the LG+G+I+F substitution model. The tree is rooted with the Rep sequences of nanoviruses

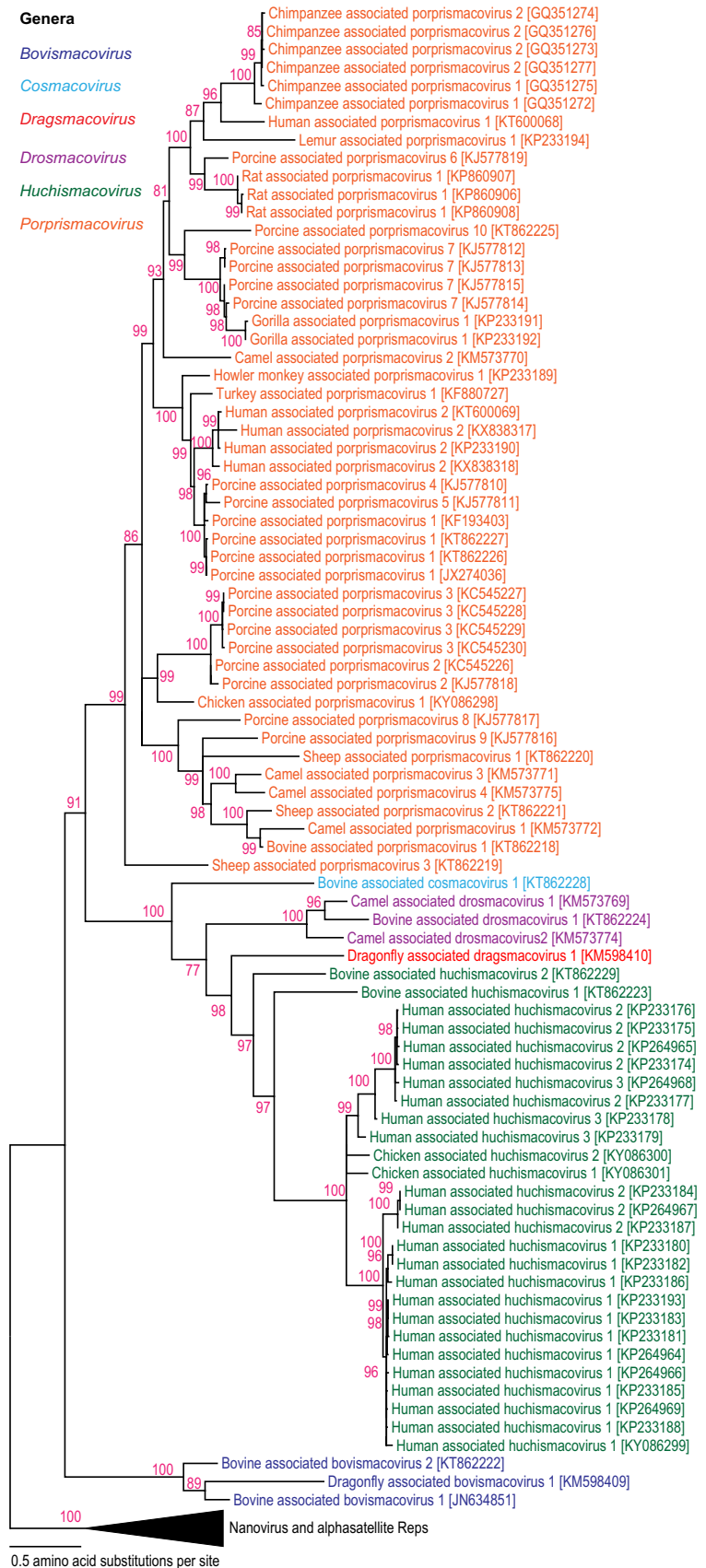
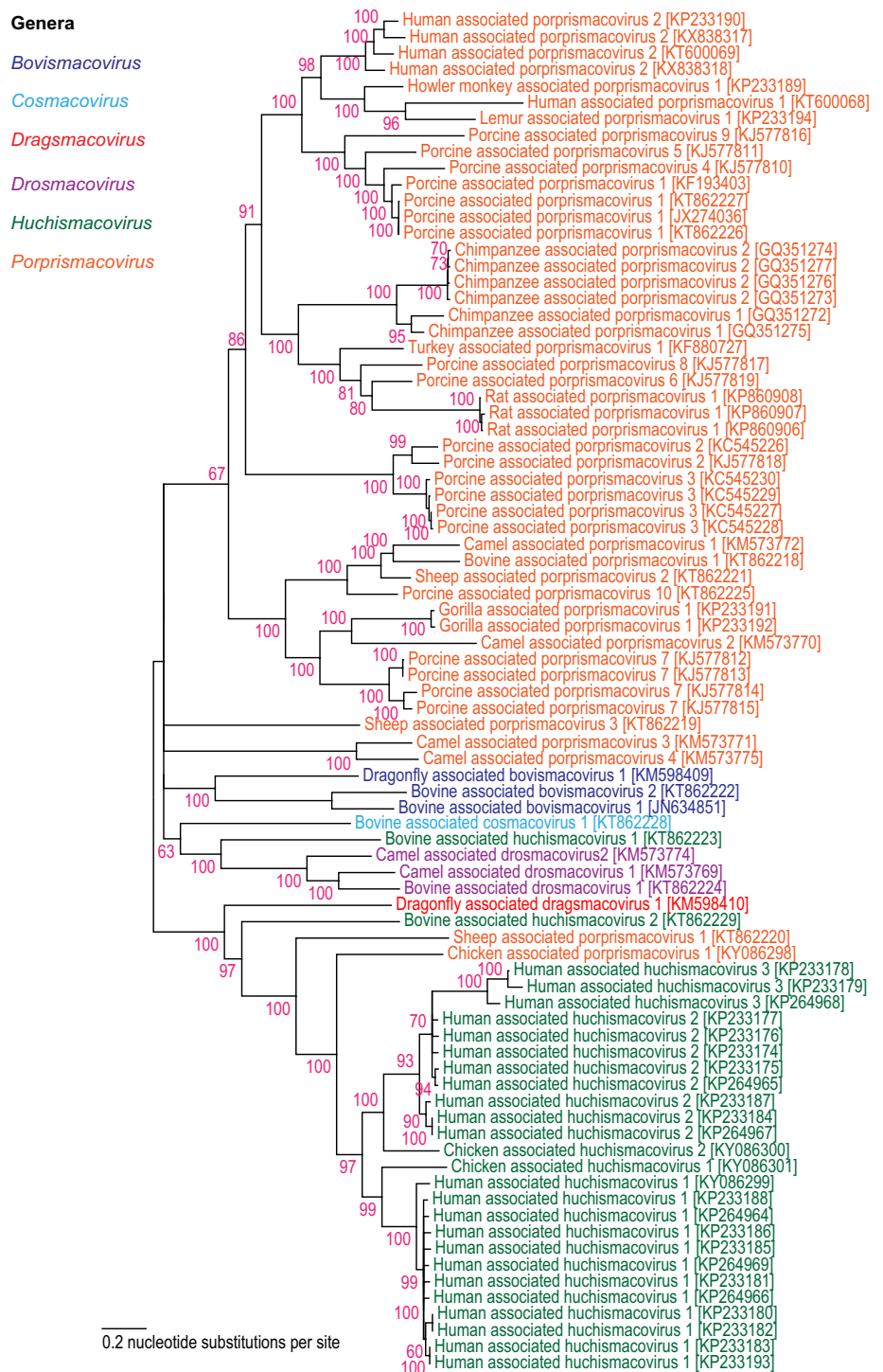


Fig. 5 Maximum likelihood phylogenetic tree of the genome sequences inferred using IQ-TREE [16] with K3Pu+I+G4 substitution model. Branches with <60% bootstrap support have been collapsed and the tree is mid-point rooted



Huchismacovirus: **H**uman and **ch**icken **smacovirus**

7 species (Table 1);

Porprismacovirus: **P**orcine and **pr**imate **smacovirus**

28 species (Table 1);

Cosmacovirus: **C**ow **smacovirus**

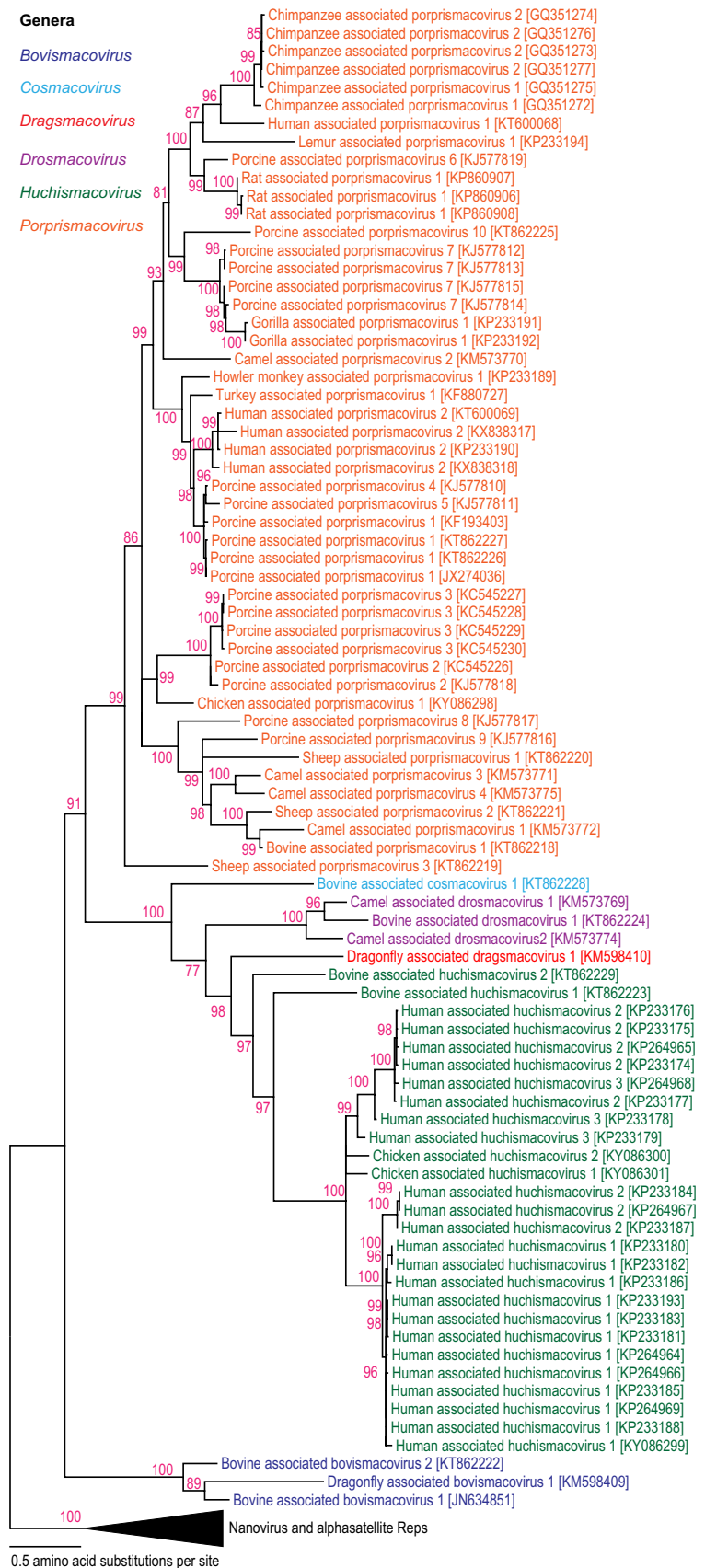
1 species (Table 1);

Dragmacovirus: **D**ragonfly **smacovirus**.

1 species (Table 1).

We would like to note that the species *Sheep associated porprismacovirus 3*, *Bovine associated huchismacovirus 1* and *Bovine associated huchismacovirus 2* have been tentatively assigned to genera *Porprismacovirus* and *Huchismacovirus*. It is highly likely that, as more sequences become available,

Fig. 6 Maximum likelihood phylogenetic tree of the CP amino acid sequences of the smacoviruses inferred using PhyML [6] with the LG+G+I+F substitution model. The phylogenetic tree is mid-point rooted



new genera will have to be created for these divergent smacoviruses (Figures 4 and 5).

Sequence based taxonomic framework employed here for smacoviruses and previously applied for genomoviruses [25] should guide the classification of an astonishing diversity of other uncultured CRESS DNA viruses described by metagenomic approaches.

Compliance with ethical standards

Conflict of interest The authors declare there are no conflicts of interest.

Research involving human participants and/or animals The research did not involve human participants or animals.

Informed consent The research did not involve human participants or animals.

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