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VIROLOGY DIVISION NEWS

Cryspovirus: a new genus of protozoan viruses in the family *Partitiviridae*

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Abstract The family *Partitiviridae* includes plant and fungal viruses with bisegmented dsRNA genomes and isometric virions in which the two genome segments are packaged separately and used as templates for semiconservative transcription by the viral polymerase. A new genus, *Cryspovirus*, has been approved for this family. Its name is based on that of the host genus, *Cryptosporidium*, which encompasses several species of apicomplexan parasites that infect a wide range of mammals, birds, and reptiles, and are a major cause of human diarrheal illness worldwide. The type species of the new genus is *Cryptosporidium parvum virus 1*. Distinguishing characteristics include infection of a protozoan host, a smaller capsid protein than found in other members of the family *Partitiviridae*, and sequence-based phylogenetic divergence.

Introduction

The family *Partitiviridae* encompasses a wide range of viruses characterized by isometric virions ~35 nm in diameter and a bisegmented dsRNA genome coding for only an RNA-dependent RNA polymerase (RdRp) on one segment and a capsid protein (CP) on the other [8, 9]. The two genome segments are packaged in separate virions [3] and used as templates for semiconservative transcription by the virion-associated RdRp molecule(s) [4, 30]. Viruses of the >30 species in this family recognized as of spring 2008 by the International Committee on Taxonomy of Viruses (ICTV) persistently infect plant or fungal hosts and are largely if not wholly ineffective at extracellular transmission. Until recently, only three genera were recognized: *Partitivirus*, *Alphacryptovirus*, and *Betacryptovirus* [8, 9]. Viruses infecting fungi were placed in the genus *Partitivirus*, and those infecting plants in the latter two genera.

Apicomplexan parasites in the genus *Cryptosporidium* infect a wide range of mammals, birds, and reptiles, and are a major cause of human diarrheal illness worldwide [34]. A bisegmented dsRNA virus associated with human stools containing *Cryptosporidium* oocysts was first reported in 1995 [7]. This virus was noted to be similar to previously described picobirnaviruses obtained from the feces of humans, other mammals, and birds, though with a smaller total genome length (3,000–3,500 vs. 4,000–4,500 bp). Subsequent nucleotide sequencing identified substantial differences between this "atypical" picobirnavirus from human feces containing *Cryptosporidium* oocysts and a more typical picobirnavirus from rabbits [10]. In the meantime, another laboratory group discovered a similar bisegmented dsRNA virus in propagated sporozoites from several isolates of *C. parvum*, but not (at that time) in those of other *Cryptosporidium* species [17]. Nucleotide sequencing and other studies from this second laboratory clarified that the *C. parvum* virus (specifically that from *C. parvum* KSU-1) shares a closer relationship with partitiviruses than with picobirnaviruses [15, 17]. As such, this virus would represent the only member of the family

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Partitiviridae to be yet identified from a protozoan host. Like other members of this family, the *C. parvum* virus appears to be largely if not wholly ineffective at extracellular transmission [15].

The distinguishing characteristics of the *C. parvum* virus, which we now call Cryptosporidium parvum virus 1 and abbreviate CSpV1, led us to propose a new genus in the family *Partitiviridae* to accommodate it. Establishment of this genus, named *Cryspovirus* to reflect the host genus *Cryptosporidium*, was considered positively by the ICTV Executive Committee at its 2008 meeting in Istanbul, Turkey, and then approved by that committee in June 2009.

Taxonomic structure

Order: Unassigned
Family: *Partitiviridae*
Genus: *Cryspovirus*
Type species: *Cryptosporidium parvum virus 1*

Despite being discovered a number of years ago, CSpV1 had not been formally recognized or classified by the ICTV. In association with proposing the genus *Cryspovirus* in the family *Partitiviridae*, we also proposed *Cryptosporidium parvum virus 1* as the type species of this genus. High levels of partial sequence identity among CSpV1-like dsRNAs from multiple human or bovine stool samples containing *C. parvum* or *C. hominis* [10, 13, 15, 17, 21–24, 38] suggest that most of these dsRNAs represent different variants of the type species. On the other hand, some CSpV1-like small (CP-encoding) dsRNAs from human stool samples containing *C. hominis*, *C. felis,* or *C. meleagridis* show somewhat greater sequence divergence [21, 22] and therefore might represent additional, yet-to-be-formally recognized species in the genus *Cryspovirus*. Criteria for distinguishing such new species would include the host species in which they naturally occur, the sizes of their genome segments and products, and the extent of amino acid sequence identity, as suggested for other members of the family *Partitiviridae* [8].

During review of the current proposal by the ICTV Executive Committee, a question arose as to whether the genus name *Gammacryptovirus* might be preferable to *Cryspovirus*, since the genus names *Alphacryptovirus* and *Betacryptovirus* already exist in the family *Partitiviridae* [8, 9]. Historically, the genera *Alphacryptovirus* and *Betacryptovirus* were created under the family *Cryptoviridae* of "cryptic" (asymptomatic) plant viruses. Because of similarities between members of these two genera of plant viruses and fungal viruses classified under the genus *Partitivirus* in the family *Partitiviridae* [8, 9], these three genera were later combined under the family *Partitiviridae,* and the family *Cryptoviridae* was dropped. We hence did not propose the name *Gammacryptovirus* for this new genus because, based on past and current precedents, it could erroneously imply that the virus being considered is a plant virus. We did entertain the genus name *Cryptosporidiumvirus*, but opted for *Cryspovirus* for brevity.

Picobirnaviruses, another group of small, bisegmented dsRNA viruses [7, 10, 35], as introduced above, were recently assigned to the family *Picobirnaviridae* by the ICTV. Picorbirnaviruses and partitiviruses share structural similarities in their capsids, though distinguishing features are also evident [6, 28, 29]. Other characteristics of picobirnaviruses that argue against that new family also encompassing CSpV1 include: (1) infection of vertebrate hosts, (2) probable capacity for effective extracellular transmission, (3) probable copackaging of both genome segments in the infectious virion, (4) possession of a second open reading frame (ORF) in the CP-encoding genome segment, (5) autoproteolytic maturation of the CP [6], and (6) phylogenetically distinct CP and RdRp sequences.

The abbreviation CPV has been used at times for Cryptosporidium parvum virus [12, 15]. Unfortunately, this same abbreviation has also been used for several other viruses, including cytoplasmic polyhedrosis virus, canine parvovirus, and cowpox virus. To avoid confusion, we have introduced the unique abbreviation CSpV for the virus(es) from *C. parvum*.

Biological properties

Like other members of the family *Partitiviridae* [8, 9], CSpV1 seems to be transmitted largely if not wholly by intracellular means: vertically during cell division (asexual reproduction) of *Cryptosporidium* host cells to produce merozoites and sporozoites, as well as during gamete fusion (sexual reproduction) preceding encystment and sporogony. Horizontal, extracellular transmission to previously uninfected *Cryptosporidium* cells has not been demonstrated and seems unlikely to occur with any regularity based on available observations [15]. CSpV1 is disseminated in nature within *Cryptosporidium* oocysts, which are produced and shed in great profusion.

Also like other members of the family *Partitiviridae* [8, 9], CSpV1 is probably associated with persistent, largely avirulent infections of its hosts [15]. Some authors have thus referred to *C. parvum* as having a "viral symbiont" [18]. Although *Cryptosporidium* species are pathogens of humans and other vertebrates [34], there are so far no clear examples in which parasite pathogenicity is either positively or negatively modulated by infection with CSpV1 or another CSpV1-like virus. A recent study may spur interest in this subject, however, by showing a correlation between

levels of CSpV1-like dsRNAs in two *C. parvum* isolates and parasite fecundity in terms of oocyst excretion from infected calves [11].

The cell biology of CSpV1 infection, including subcellular localizations of replication and assembly complexes, remains largely unexplored. Host factors that are required for, modulate, inhibit, or are affected by CSpV1 infection are also unknown.

Virions and replication

CSpV1 has isometric virions, with a buoyant density of ~1.42 g/cm^3 on CsCl gradients and a diameter of ~31 nm as visualized by negative staining and transmission electron microscopy [15, 16]. The capsids appear single layered and thin, with a suggestion of short protrusions on their surfaces.

Genome-deduced protein sequences [17, 22] have revealed that the CP of CSpV1 is identically sized, at 319 aa or ~37 kDa, to those encoded by CSpV1-like small dsRNAs from three other *Cryptosporidium* species. The CPs of other members of family *Partitiviridae* are consistently larger than this (see Table 1 for examples), suggesting that the CSpV1 CP may approach a minimal size for forming the type of 120-subunit $T = 1$ (so-called "$T = 2$") capsid recently shown to be characteristic of this family [28, 29]. The next smallest CP in the family is that of Fusarium solani virus 1, at 413 aa [27]. CSpV1 CP migrates with a relative molecular weight of 37,000–40,000 in denaturing gels [15] and is thus presumably full length (i.e., not proteolytically processed). An additional, 30,000-sized form of CSpV1-like CP has also been reported [18], but is likely a degradation product.

Genome-deduced protein sequences [17] have revealed that the RdRp of CSpV1 comprises 524 aa or ~62 kDa, approaching the RdRp size of certain members of the family *Partitiviridae* such as Penicillium stoloniferum virus F (Table 1). In line with other members of the family *Partitiviridae* [4], as few as one copy of the CSpV1 RdRp is thought to be packaged per virion, probably by being noncovalently anchored inside the capsid. This virion-associated RdRp molecule is expected to mediate synthesis of the CSpV1 RNAs during transcription and replication [14, 15].

The genome of CSpV1 comprises two distinct molecules of dsRNA, sized near 1,700 bp (dsRNA1, large segment) and 1,400 bp (dsRNA2, small segment) in nondenaturing gels [17]. Whether these dsRNAs are 5′-capped at either end remains unknown, but results indicate that they are unlikely to be 3′-polyadenylylated [17, 22]. Analyses performed with CSpV-1 virions separated on CsCl density gradients indicate that the large and small genome segments are packaged in separate particles [15], as is true for other members of the family *Partitviridae* [3, 8, 9]. In some members of this family, satellite or defective RNAs can be packaged and replicated in parallel with the two essential genome segment(s), although their significance to infection is generally unknown [8, 9]. To date, satellite or defective dsRNAs have not been reported in CSpV1.

Like those of other dsRNA viruses, the RdRp-containing particles of CSpV1 are expected to be "nanomachines" for RNA synthesis. Transcription by CSpV1 virions is asymmetric (producing only plus-strand products) and semiconservative (meaning that the parental plus strand is released while the newly synthesized plus strand is retained as part of the duplex template, that is, until the next round of transcription when it represents the parental plus strand) [14, 15]. The primary products of transcription appear to be

Table 1 Genome segment and protein lengths for CSpV1 and representative viruses from family *Partitiviridae*

Virus	GenBank accession nos.		Genome segment lengths (bp)		Protein lengths (aa)	
	dsRNA1	dsRNA2	dsRNA1	dsRNA2	RdRp[a]	CP[b]
CspV1	U95995	U95996	1,786	1,374	524	319[c]
PsV-F	AY738336	AY738337	1,677	1,500	538	420
FsV1	D55668	D55669	1,645	1,445	519	413
PsV-S	AY156521	AY156522	1,754	1,582	539	434
AhV	L39125	L39126	2,180	2,135	665	652
WCCV1	AY705784	AY705785	1,955	1,708	616	487

CSpV1, Cryptosporidium parvum virus 1; PsV-F, Penicillium stoloniferum virus F; FsV1, Fusarium solani virus 1; PsV-S, Penicillium stoloniferum virus S; AhV, Atkinsonella hypoxylon virus; WCCV1, White clover cryptic virus 1

[a] Encoded by the larger genome segment, dsRNA1, in each listed virus

[b] Encoded by the smaller genome segment, dsRNA2, in each listed virus

[c] Full-length CspV1-like CPs of identical length to CSpV1 CP have been reported from each of three other *Cryptosporidium* species—*C. hominis, C. felis,* or *C. meleagridis*—with GenBank accession numbers DQ193518, DQ193519, and DQ193520

full-length copies of the genomic plus strand [14], which serve for translation by host ribosomes and also for incorporation into newly assembling virions. The CSpV1 RdRp is also presumed to mediate one round of minus-strand synthesis (replication) to generate the duplex genome molecule in newly assembled virions before switching to transcription mode. Since CSpV1 is likely to be regularly transmitted only by intracellular routes, its virions are likely to lack the machinery for cell entry.

Genomic and coding properties

Full-length nucleotide sequences have been reported for the small segment, dsRNA2, of CSpV1 [17] as well as the CSpV1-like small dsRNAs from three other *Cryptosporidium* species [22]. All have lengths of 1,374–1,502 bp, somewhat smaller than those of other members of family *Partitiviridae* (see examples in Table 1). A full-length nucleotide sequence of the large dsRNA segment, dsRNA1, has been reported only for CSpV1. It has a length of 1,786 bp, also at the small end of the range for members of family *Partitiviridae* (Table 1).

The genomic plus strand of each CSpV1-like dsRNA contains only one long ORF (Fig. 1). The ORF in dsRNA1 encodes RdRp, and that in dsRNA2 encodes CP. In CSpV1 dsRNA2 and the three other CSpV1-like small dsRNAs for which full-length sequences have been reported, the 5′ untranslated region (UTR) of the plus strand varies fairly widely in length, from 247 nt in CSpV1 to 345–355 nt in the others. The dsRNA2 sequence of CSpV1 [17] is missing ~100 nt at its 5′ terminus relative to that of the others [22], possibly due to a sequencing artifact. The 3′ UTR of the dsRNA2 plus strand is more consistently sized among these sequences, from 167 to 187 nt. The 5′ and 3′ UTRs of CSpV1 dsRNA1 are substantially shorter than those of its dsRNA2: 133 and 78 nt, respectively. The genomic minus strand of each appears to have little or no coding potential, consistent with its expected lack of access to host ribosomes during the viral replication cycle.

Several observations suggest that the CSpV1 CP may be translated via a noncanonical mechanism. (1) The 5′ UTR

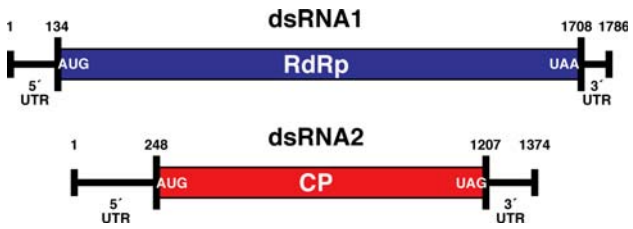

Fig. 1 Genome organization of CSpV1. The plus strand of each of the two genome segments (dsRNA1 and dsRNA2) includes one large ORF, encoding either RdRp or CP as indicated

of the plus strand of CSpV1 dsRNA2 (and even more so those of the other, putative CSpV1-like viruses) is unusually long. (2) The CP start codon is in a suboptimal context for translation initiation: YYYAUGA, versus consensus AARAUGR in a set of 81 nonviral *C. parvum* genes retrieved from GenBank. (3) The translating plus strands are likely not 3′-polyadenylylated, and may not be 5′-capped. The 5′ UTR of the plus strand of CSpV1 dsRNA1 is shorter, and the RdRp start codon is in a more optimal context (GAAAUGA), making it less clear whether its translation mechanism may also be noncanonical.

Conserved sequences have been noted at the plus-strand 3′ ends of CSpV1 dsRNAs 1 and 2, including the 3′-terminal nonanucleotide GGGAAGCCU [14]. These sequences have been proposed to be involved in recognition by the viral RdRp during replication (minus-strand synthesis) and/or packaging into virions. Interestingly, these conserved sequences are also found in the three other CSpV1-like small dsRNAs for which full-length sequences have been reported [22], but starting 60–80 nt before the 3′ terminus of each. Whether this represents real variation or sequencing artifacts in one or the other study remains uncertain.

Phylogenetic relationships

Species in the family *Partitiviridae* recognized by the ICTV as of its 2008 meeting in Istanbul, Turkey, represent >30 plant and fungal viruses. Eighteen of these species have been characterized in the literature by full-length sequences of both viral genome segments, allowing phylogenetic comparisons of the, respectively, encoded proteins, RdRp and CP (Fig. 2). A clade of mostly plant viruses assigned to the genus *Alphacryptovirus* is evident in both phylogenetic trees, as has been previously described [1, 2]. Genus *Betacryptovirus* is not represented in Fig. 2 because of the complete lack of sequences in the databases. A clade of fungal viruses assigned to the genus *Partitivirus* is also evident in both trees, although in this case two divergent subclades are clearly seen, as has been repeatedly noted in previous reports [1, 2, 5, 9, 25, 31–33]. Fungal viruses in the family *Partitiviridae* thus appear to be polyphyletic, and the genus *Partitivirus* may warrant future partition into two or more genera reflecting these subclades [9]. CSpV1 is well separated from each of these other clades in both phylogenetic trees (Fig. 2), supporting the conclusion that it should reside in a separate genus.

To which other group of viruses in the family *Partitiviridae* is CSpV1 most closely related? Although the answer is not yet definitive because of low bootstrap-support values in Fig. 2, the results to date suggest that CSpV1 is most closely related to the subclade of the genus *Partitivirus* containing Penicillium stoloniferum viruses F

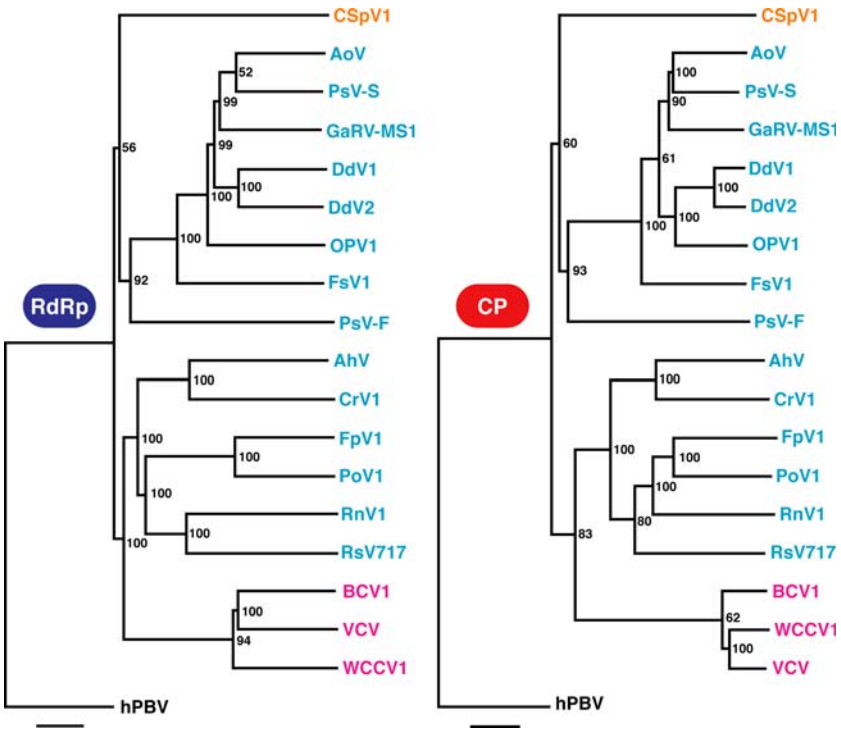

Fig. 2 Phylogenetic trees based on the complete RdRp (*left*) and CP (*right*) sequences of each analyzed virus. Sequences were multiply aligned using M-Coffee version 6.0.7 as implemented at http://www.tcoffee.org [26, 36]. Neighbor-joining trees were then generated using PAUP* (version 4.0b10) and plotted as phylograms using FigTree (version 1.1.2). Each tree was rooted by designating human picobirnavirus (hPBV) as outgroup. Respective consensus trees obtained from 2,000 bootstrap replicates showed identical topologies to the displayed phylograms, and the bootstrap percentage values are indicated at the nodes. *Scale bars* indicate distance in units of 0.1 substitutions per aa position. Virus names are color coded in the on-line version to reflect current genus assignments in the family *Partitiviridae* (see text for further discussion): genus *Cryspovirus* (top 1 virus, *orange*); genus *Partitivirus* (next 14 viruses, *cyan*); genus *Alphacryptovirus* (bottom 3 viruses other than hPBV, *magenta*). Certain of the virus abbreviations and GenBank accession numbers are defined in Table 1. Other ICTV-recognized members of family *Partitiviridae* included in the trees are: AoV, Aspergillus ochraceous virus (EU118277 and EU118278); PsV-S, Penicillium stoloniferm virus S (AY156521 and AY156522); GaRV-MS1, Gremmeniella abietina RNA virus MS1 (AY089993 and AY089994); DdV1, Discula destructiva virus 1 (AF316992 and AF316993); DdV2, Discula destructiva virus 2 (AY033436 and AY033437); OPV1, Ophiostoma partitivirus 1 (AM087202 and AM087203); FsV1, Fusarium solani virus 1 (D55668 and D55669); CrV1, Ceratocystis resinifera virus 1 (AY603051 and AY603052); FpV1, Fusarium poae virus 1 (AF015924 and AF047013); PoV1, Pleurotus ostreatus virus 1 (AY533036 and AY533038); RnV1, Rosellinia necatrix virus 1 (AB113347 and AB113348); RsV717, Rhizoctonia solani virus 717 (AF133290 and AF133291); BCV1, beet cryptic virus 1 (EU489061 and EU489062); and VCV, Vicia cryptic virus (AY751737 and AY751738). GenBank accession numbers for hPBV are AB186897 and AB186898

and S. Pairwise comparisons of the CP and RdRp sequences of CSpV1 with those of partitivirus Penicillium stoloniferum virus F (19 and 23% identity, respectively), partitivirus Atkinsonella hypoxylon virus (15 and 18% identity, respectively), and alphacryptovirus white clover cryptic virus 1 (16 and 20% identity, respectively) support this conclusion.

Practical uses for host studies

Because human infections by *C. parvum* or *hominis* are commonly linked to consumption of fecally oocyst-contaminated water, sensitive methods to detect the oocysts in potential sources are important. These methods include detection of CSpV1-like CPs by immunologic means such as dot blotting [12, 18, 19] and detection of CSpV1-like dsRNAs by reverse transcription and polymerase chain reaction [20]. Whether the oocysts detected in contaminated sources remain viable for infection is another important question, and a correlation between loss of parasite viability and loss of detectable CSpV1-like dsRNAs may be useful in this regard [16].

The association with contaminated water means that human infections by *C. parvum* or *hominis* can occur in epidemic-like outbreaks [34]. Detection is not, therefore, the only relevant issue, but also epidemiological tracking to link the specific parasite isolates in patients to those in potential sources. Sequences of CSpV1-like genome fragments amplified by reverse transcription and polymerase chain reaction have proven useful in this regard [21, 23, 24, 38]. CSpV1-like sequences have also been useful in

ongoing efforts to clarify the diversity and taxonomy of *Cryptosporidium* species, including the partitioning of *C. hominis* from *C. parvum* [13, 37].

Both *C. parvum* and *C. hominis* are also commonly associated with direct person-to-person transmission, and *C. parvum* with direct animal-to-animal transmission (calf to calf, cow to calf, etc.), again through oocyst-contaminated fecal material. For example, in cases of chronic cryptosporidiosis accompanying AIDS and other immunosuppressive illnesses [34], oocyst shedding over long periods raises particular concerns for transmission. Methods for detection and epidemiologic tracking of *C. parvum* and *hominis* are clearly important in such cases of direct transmission as well.

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