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:	***2009.016a-iP***	(to be completed by ICTV officers)

Short title: New ssRNA virus family infecting dinoflagellates: Alvernaviridae
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 9 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☒
	6 ☐	**7** ☐	**8** ☐	**9** ☒	

Author(s) with e-mail address(es) of the proposer:

Keizo Nagasaki (nagasaki@affrc.go.jp), Yuji Tomaru (tomaruy@affrc.go.jp)

Has this proposal has been seen and agreed by the relevant study group(s)? Please select answer in the box on the right	**Yes**

ICTV-EC or Study Group comments and response of the proposer:

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

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.
If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	***2009.016aP***	(assigned by ICTV officers)

To create 1 new species with the name(s):

Heterocapsa circularisquama RNA virus 01

Part (b) assigning new species to higher taxa
All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	***2009.016bP***	(assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

Genus:	***Dinornavirus (new)***
Subfamily:	***unassigned***
Family:	***Alvernaviridae (new)***
Order:	***unassigned***

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.

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

Heterocapsa circularisquama RNA virus 01 (HcRNAV01) is the only characterized single-strand RNA virus that infects dinoflagellate. The particles are polyhedral (approximately 30 nm in diameter). The genome sequence of the single 4.4 kb RNA has been elucidated, revealing unique properties compared to other characterized viruses. It encodes a serine proteinase, which is distantly related to that of a member of the plant genus *Sobemovirus*. The RNA-dependent RNA-polymerase is unique with distant affinities with those of members of the families *Luteoviridae*, the genus *Sobemovirus* and the family *Barnaviridae*. The genomic organization of the virus is distinct from those of members of the families *Luteoviridae* and *Barnaviridae* and the genus *Sobemovirus* with the presence of two open reading frames and a stem-loop structure at the 3'-end. Because of these unique properties, we propose to create a new genus (*Dinornavirus*) and a new family (*Alvernaviridae*) for this virus. Because relations with other taxa are only distant, we propose to leave the new family as unassigned.

MODULE 3: **NEW GENUS**

creating and naming a new genus

Code	***2009.016cP***	(assigned by ICTV officers)

To create a new genus to contain the species listed below

Heterocapsa circularisquama RNA virus 01

Code	***2009.016dP***	(assigned by ICTV officers)

To name the new genus: *Dinornavirus*

assigning a new genus to higher taxa

Code	***2009.016eP***	(assigned by ICTV officers)

To assign the new genus as follows: Ideally, a genus should be placed within a higher taxon, but if not, write "**unassigned**" in the box below.

Subfamily:	***unassigned***
Family:	***Alvernaviridae (new)***
Order:	***unassigned***

If any of these taxa has yet to be created (in module 4, 5 or 6) please write "**(new)**" after its proposed name.

assigning type species and other species to a new genus

Code	***2009.016fP***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Heterocapsa circularisquama RNA virus 01

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

Code		(assigned by ICTV officers)

To assign the following as additional species of the new genus:

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

HcRNAV01 is the first characterized ssRNA virus with a dinoflagellate (*Heterocapsa circularisquama*) as a host. The genome is linear, positive-stranded and has a stem-loop structure at the 3'-end which differs in sequence among clones. The phylogenetic tree constructed based on the RdRp sequence showed HcRNAV01 is distinct from the members of other recognized taxa. Hence, it is appropriate to create a new genus for this dinoflagellate-infecting ssRNA virus.

Origin of the new genus name:

ss*RNA virus* infecting *dino*flagellates → *Dinornavirus*

Reasons to justify the choice of type species:

HcRNAV01 is the only ssRNA virus characterized that infects dinoflagellate (*Heterocapsa*

circularisquama). The entire genome sequence has been elucidated and some of its biological properties have been characterized (see annexes).

Species demarcation criteria in the new genus:

- If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences of new species

Two strains of HcRNAV01 have been intensively studied (HcRNAV01 strain 34 and strain 109); full genome sequences of these two virus strains were registered with Genbank accession numbers AB218608 and AB218609, respectively.

MODULE 5: **NEW FAMILY**

creating and naming a new family

Code	***2009.016gP***	(assigned by ICTV officers)

To create a new family containing the subfamilies and/or genera listed below

genus *Dinornavirus*

Code	***2009.016hP***	(assigned by ICTV officers)

To name the new family: *Alvernaviridae*

assigning the new family to an order

Code		(assigned by ICTV officers)

To assign the new family created to the order: *unassigned*	If there is no Order, write "**unassigned**" here. If the Order has yet to be created (in Module 6) please write "**(new)**" after the proposed name.

assigning subfamilies, genera and unassigned species to a new family

Code		(assigned by ICTV officers)

subfamilies (if any) assigned to the new family:

You may list several subfamilies here. For each subfamily, please state whether it is new or existing.

- If the subfamily is new, it must be created in Module 4
- If the subfamily already exists, please complete Module 7 to 'REMOVE' it from its existing family

Code	***2009.016iP***	(assigned by ICTV officers)

genera assigned to the new family:

You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

genus *Dinornavirus (new)*

Code		(assigned by ICTV officers)

unassigned species in the new family (i.e. within the family but not assigned to any genus):

You may list several species here. For each species, please state whether it is new or existing.

- If the species is new, it must be created in Module 2
- If the species already exists, you should 'REMOVE' it from its current position by completing Module 7

Reasons to justify the creation of the new family:

Additional material in support of this proposal may be presented in the Appendix, Module 9

See modules 2 and 3 above for the justification to create a new species and a new genus in a new family, respectively. At present, HcRNAV01 is the only virus infecting Alveolates; the proposed family "Alvernaviridae" should also include future-isolated ssRNA virus infecting

Alveolates such as cilliates, apicomplexa, and other dinoflagellates.
Origin of the new family name:
ss*RNA virus* infecting *Alve*olates → *Alvernaviridae*

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Koonin, E.V., Wolf, Y. I., Nagasaki, K., Dolja, V. V. (2008) The big bang of picorna-like virus evolution antedates the radiation of eukaryotic supergroups. Nat. Rev. Microbiol. 6: 925-939.
2. Mizumoto, H., Tomaru, Y., Takao, Y., Shirai, Y., Nagasaki, K. (2008) Diverse responses of the bivalve-killing dinoflagellate Heterocapsa circularisquama to infection by a single-stranded RNA virus. Appl. Environ. Microbiol. 74(10): 3105-3111.
3. Nagasaki, K., Brussaard, C. P. D. (2008) Algal viruses. In: "Encyclopedia of Virology, Third Edition" ed. Mahy, B., Regenmortel, M. V., Elsevier, Oxford, UK. Vol.1, p.87-95.
4. Nagasaki, K. (2008) Dinoflagellates, diatoms and their viruses. J. Microbiol. 46(3): 235-243.
5. Tomaru, Y., Hata, N., Masuda, T., Tsuji, M., Igata, K., Masuda, Y., Yamatogi, T., Sakaguchi, M., Nagasaki, K. (2007) Ecological dynamics of the bivalve-killing dinoflagellate Heterocapsa circularisquama and its infectious viruses in different locations of western Japan. Environ. Microbiol. 9(6): 1376–1383.
6. Mizumoto, H., Tomaru, Y., Takao, Y., Shirai, Y., Nagasaki, K. (2007) Intraspecies host specificity of a single-stranded RNA virus infecting a marine photosynthetic protist is determined at the early steps of infection. J. Virol., 81(3): 1372-1378.
7. Nagasaki, K., Tomaru, Y., Shirai, Y., Takao, Y., Mizumoto, H. (2006) Dinoflagellate-infecting viruses. J. Mar. Biol. Ass. U.K., 86: 469-474.
8. Nagasaki, K., Shirai, Y., Takao, Y., Mizumoto, H., Nishida, K., Tomaru, Y. (2005) Comparison of genome sequences of single-stranded RNA viruses infecting the bivalve-killing dinoflagellate Heterocapsa circularisquama. Appl. Environ. Microbiol., 71(12): 8888-8894.
9. Tomaru, Y., Tanabe, H., Yamanaka, S., Nagasaki, K. (2005) Effects of temperature and light on stability of microalgal viruses, HaV, HcV and HcRNAV. Plankton Biol. Ecol., 52(1): 1-6.
10. Tomaru, Y., Nagasaki, K. (2004) Widespread occurrence of viruses lytic to the bivalve-killing dinoflagellate Heterocapsa circularisquama in the western coast of Japan. Plankton Biol. Ecol., 51(1): 1-6.
11. Nagasaki, K., Tomaru, Y., Nakanishi, K., Hata, N., Katanozaka, N., Yamaguchi, M. (2004) Dynamics of Heterocapsa circularisquama (Dinophyceae) and its viruses in Ago Bay, Japan. Aquat. Microb. Ecol., 34(3): 219-226.
12. Tomaru, Y., Katanozaka, N., Nishida, K., Shirai, Y., Tarutani, K., Yamaguchi, M. Nagasaki, K. (2004) Isolation and characterization of two distinct types of HcRNAV, a single-stranded RNA virus infecting the bivalve-killing microalga Heterocapsa circularisquama. Aquat. Microb. Ecol., 34(3): 207-218.

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.

Here, the only member within the proposed genus *Dinornavirus* in the proposed family *Alvernaviridae* (*Heterocapsa circularisquama RNA virus 01*: HcRNAV01) is introduced. HcRNAV01 is a polyhedral virus ~30 nm in diameter (Fig. 1) harboring a single-stranded (+) RNA genome ca. 4.4 kb long. The virus replicates in the cytoplasm of *H. circularisquama* (Fig. 2). The latent period and the burst size were respectively estimated at 24-48 h and 3,400-21,000 infectious units cell^{-1}. Both the host and virus populations are composed of multiple types; i.e., by a cross-reactivity test, HcRNAV01 clones were roughly divided into two types (UA and CY), which showed complementary strain-specific infectivity. Hence, the host strains were also divided into two types; type C hosts were only sensitive to type UA virus, and type B hosts were only sensitive to type CY virus (Tomaru *et al.*, 2004). To summarize, multiple types of *H. circularisquama* and HcRNAV01 (i.e., at least two independent host-virus systems) were considered to coexist in a given geographical area. The genomic RNA of typical strains of type CY virus and UA virus (strain 34 and strain 109 of HcRNAV01, respectively) were fully sequenced and compared (Genbank accession numbers are AB218608 and AB218609, respectively). The genome structures of these two virus strains are shown in Fig. 3. They were ~97.0 % similar to each other at the nucleotide sequence level. The genome has two open reading frames (ORF-1 and -2) (Nagasaki *et al*., 2005). The 3'ends of the viral genomes lacked a poly(A) tail but a stem-loop structure was predicted. ORF-1 is assumed to be a replicase polyprotein gene encoding a serine protease and an RNA-dependent RNA polymerase (RdRp). The best fit BLAST comparison of the serine protease domain of HcRNAV01 was for the Lucerne transient streak virus, a plant virus belonging to the genus *Sobemovirus* (BLAST e-value based on 2008 database = 4e-4). The RdRp domain was related to that of the *Mushroom bacilliform virus,* a member of the genus *Barnavirus* in the family *Barnaviridae* (BLAST e-value: 5e-11), and of *Poinsettia cryptic virus* (1e-10) and *Poinsettia latent virus* (7e-10), two viruses currently classified as a tentative member of the genus *Alphacryptovirus* in the family *Partitiviridae* but also related to the family *Luteoviridae* and the genus *Sobemovirus* (Nagasaki *et al*., 2005). These low BLAST hits show that *HcRNAV01* is evolutionarily quite distant from any other characterized land or aquatic viruses. Considering that HcRNAV01 is the only ssRNA virus infecting "Alveolates", these results are probably not surprising. The phylogenetic tree constructed based on the RdRp amino acid sequence confirmed that HcRNAV01 is apparently distinct from members of other known taxa (Fig. 4).

ORF-2 codes for the viral single major structural protein (Nagasaki *et al*., 2005). ORF-2 has four specific regions where amino acid substitutions (between type CY and type UA) are frequently found; many of the amino acid substitutions were located in regions predicted to be on the outside (surface side) of the viral capsid protein in a model of the tertiary structure (Fig. 5) (Nagasaki *et al.*, 2005). When the genome RNA of strain 109 (type CY virus) was injected into 'inappropriate' host *H. circularisquama* cells (type C host) using a particle bombardment method with a gene gun, the intracellular virus replication occurred at a lower efficiency. In contrast, no viral replication was detected when the strain 109 suspension was simply inoculated to the type C host culture (Mizumoto *et al*., 2007). Integrating these data, the type-specific host specificity of this virus is principally determined by nano-structures on the virus surface that may affect its binding affinity to the host cell. The less efficient viral replication in the host cell transfected with 'inappropriate' is presumably due to the compatibility between the viral genome and the intracellular components that are essential for intracellular viral replication.

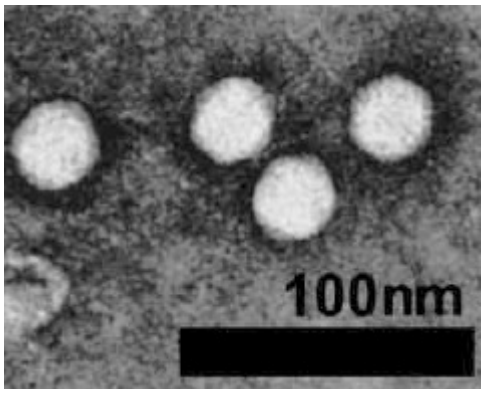

Fig. 1. Negatively stained HcRNAV01 particles. (reprinted with copyright permission from American Society for Microbiology: (reprinted with copyright permission from the Inter-Research: Tomaru, Y. et al. *Aquat. Microb. Ecol.* **34(3),** 207-218. [published in March 2004])

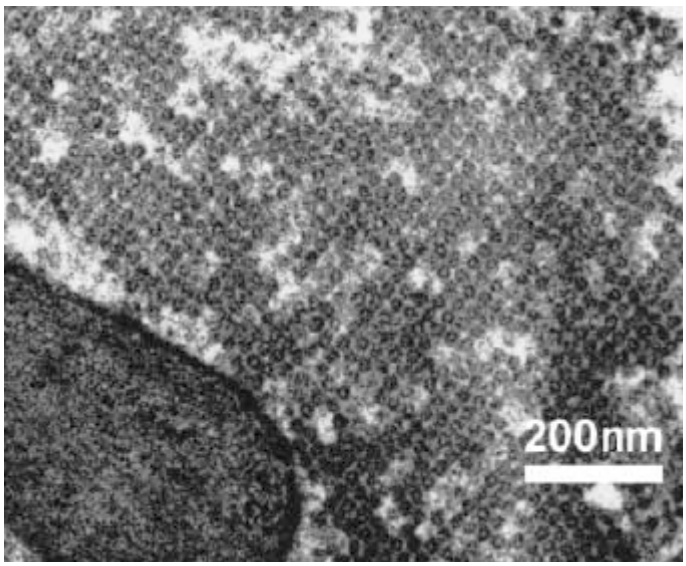

Fig. 2. Crystalline array of HcRNAV01 propagating in the cytoplasm of *Heterocapsa circularisquama*. (reprinted with copyright permission from American Society for Microbiology: (reprinted with copyright permission from the Inter-Research: Tomaru, Y. et al. (2004) *Aquat. Microb. Ecol.* **34(3),** 207-218. [published in March 2004])

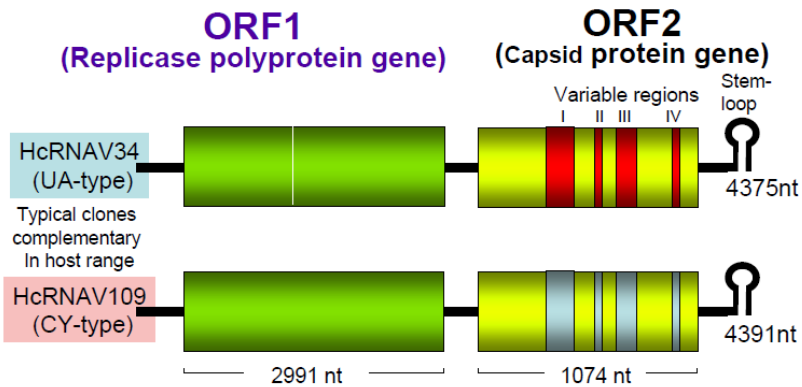

Fig. 3. Genome structures of two HcRNAV01 strains (strains 34 and 109) having complementary intraspecies host ranges. It is predicted that the four variable regions in ORF2 (capsid protein gene) determine the host specificity. (reprinted with copyright permission from the American Society for Microbiology: Nagasaki, K. et al. *Appl. Environ. Microbiol.* **71(12):** 8888-8894. [published in December 2005])

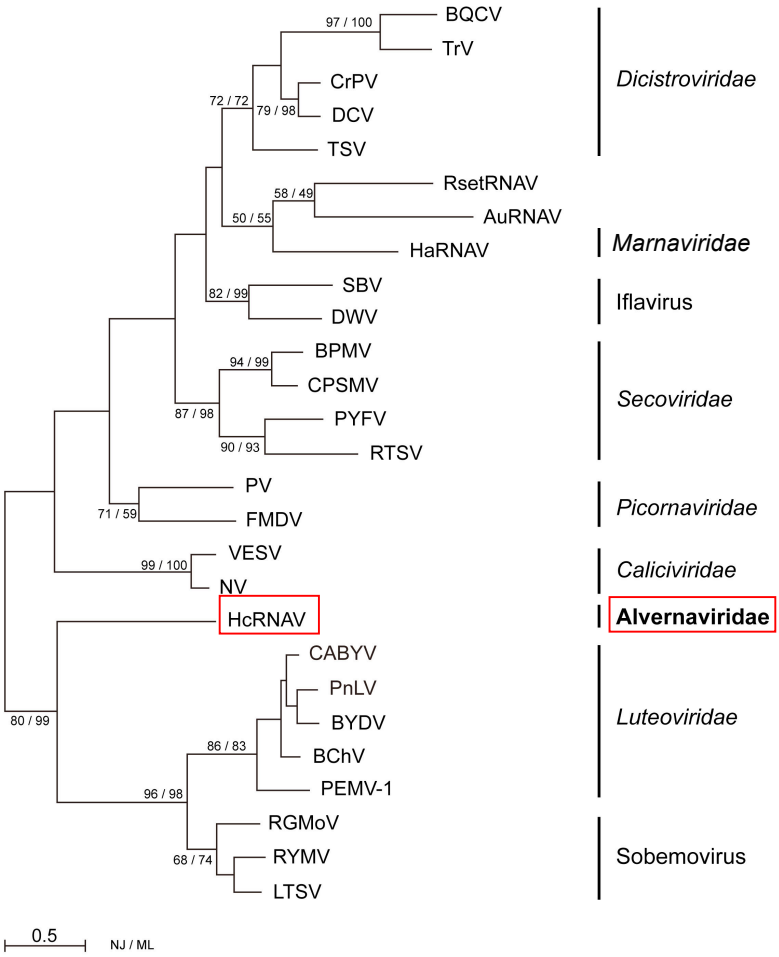

Fig. 4. Maximum likelihood tree calculated from confidently aligned regions of RNA-dependent RNA polymerase (RdRp) whole domain. ML bootstrap values (%) from 100 samples are shown at the nodes followed by bootstrap values based on neighbor-joining analysis (%) from 100 samples. The ML distance scale bars are shown. Amino acid sequences used for comparison in the analyses are as follows with the virus name, abbreviations in parentheses, and the database accession numbers (referring to the NCBI unless otherwise stated): Aurantiochytrium single-stranded RNA virus 01 (AuRNAV), BAE47143; Beet chlorosis virus (BChV), AAK49964; Bovine enteric calicivirus (BoCV), AJ011099; Bean pod mottle virus (BPMV), AF394608; Black queen cell virus (BQCV), AF183905; Barley yellow dwarf virus (BYDV), BAA01054; Cucurbit aphid-borne yellows virus (CABYV), CAA54251; Cowpea severe mosaic virus (CPSMV), M83830; Cricket paralysis virus (CrPV), M21938; Drosophila C virus (DCV), AF014388; Deformed wing virus (DWV), AY292384; Foot-and-mouth disease virus (FMDV), P03306; Heterosigma akashiwo RNA virus SOG 263 (HaRNAV), AY337486; Heterocapsa circularisquama RNA virus 01 (HcRNAV), AB218609; Lucerne transient streak virus (LTSV), U31286; Norwalk virus (NV), M87661; Pea enation mosaic virus 1 (PEMV-1), AAA72297; Poinsettia latent virus (PnLV), Human poliovirus 1 Mahoney (PV), V01149; CAI34771; Parsnip yellow fleck virus (PYFV), D14066; Ryegrass mottle virus (RGMoV), EF091714; Rhizosolenia setigera RNA virus 01 (RsetRNAV), DDBJ accession number AB243297; Rice turgo spherical virus (RTSV), AAA66056; Rice yellow mottle virus (RYMV), CAE81345; Sacbrood virus (SBV), AF092924; Triatoma virus (TrV), AF178440; and Taura syndrome virus (TSV), AF277675. (reprinted with copyright permission from the Cambridge University Press: Shirai et al. **86:** 469-474 [published in June 2006])

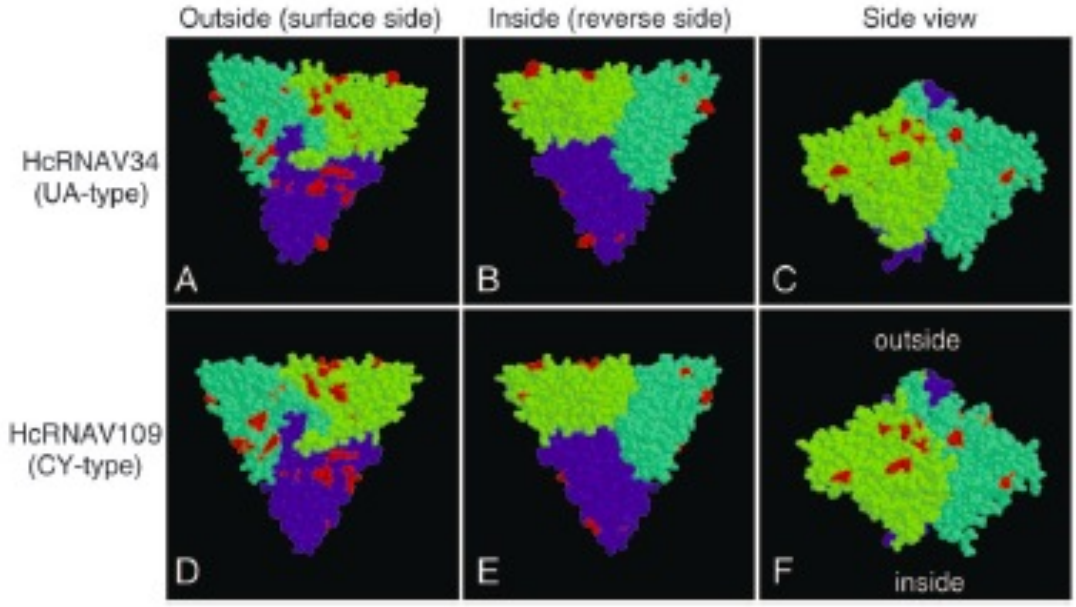

Fig. 5. Tertiary structures of the major capsid proteins of HcRNAV34 (A to C) and HcRNAV109 (D to F) predicted by computer modeling. Three monomers that make up the capsid trimers are indicated by different colors (green, blue, and yellow), and the amino acid molecules where complete substitution was observed between the UA type and the CY type are indicated by red. (A and D) Surface side view; (B and E) reverse side view; (C and F) side view. (reprinted with copyright permission from the American Society for Microbiology: Nagasaki, K. et al. *Appl. Environ. Microbiol.* **71(12):** 8888-8894. [published in December 2005])