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:	***2009.015a-kP***	(to be completed by ICTV officers)

Short title: New ssRNA virus genus infecting diatoms: Bacillarnavirus
(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:

Yuji Tomaru (tomaruy@affrc.go.jp), Keizo Nagasaki (nagasaki@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	***2009.015aP***	(assigned by ICTV officers)

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

Rhizosolenia setigera 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	***2009.015bP***	(assigned by ICTV officers)

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

Genus:	***Bacillarnavirus (new)***
Subfamily:	***unassigned***
Family:	***unassigned***
Order:	***Picornavirales***

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:
- 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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Rhizosolenia setigera RNA virus 01 (RsetRNAV01) is a recently isolated ssRNA virus which is infectious to the bloom-forming diatom *Rhizosolenia setigera*. It was previously referred to under the abbreviation RsRNAV (*Rhizosolenia setigera* RNA virus) in Nagasaki *et al.* (2004). The genome is a linear positive-stranded RNA with a polyA tail at the 3'-end. The Genbank accession number of its full genome sequence is AB243297.

In "Virus Taxonomy: the 8th Report of the International Committee on Taxonomy of Viruses", there are currently no reports of diatom-infecting ssRNA viruses. Since then, the full genome sequences of three diatom-infecting ssRNA viruses (RsetRNAV01, CtenRNAV01 and CsfrRNAV01; see below) have been elucidated and the viruses have been characterized. The phylogenetic tree constructed based on the RNA-dependent RNA polymerase (RdRp) amino acid sequences showed that they form a monophyly supported by a bootstrap value of 100% (annexes Fig. 6). The clade is apparently distinct from the cluster of *Dicistroviridae*, *Iflavirus*, *Comoviridae, Sequiviridae, Picornaviridae* and *Caliciviridae*. The clade is related to *Heterosigma akashiwo RNA virus SOG 263* (HaRNAV-SOG263: family *Marnaviridae,* order *Picornavirales*) and to AuRNAV01 (a proposed new species suggested to constitute a separate genus *Labyrnavirus* unassigned in the order *Picornavirales*). However, the clade of the three diatom-infecting viruses constitutes a distinct sub-branch in the tree. Based on the results, we propose a new diatom-infecting ssRNA virus genus "*Bacillarnavirus*" unassigned within the order *Picornavirales* that will contain three new species; RsetRNAV01 is proposed as the type species of the genus *Bacillarnavirus*.

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	***2009.015cP***	(assigned by ICTV officers)

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

Chaetoceros tenuissimus 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	***2009.015dP***	(assigned by ICTV officers)

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

Genus:	***Bacillarnavirus (new)***
Subfamily:	***unassigned***
Family:	***unassigned***
Order:	***Picornavirales***

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:
- 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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Chaetoceros tenuissimus RNA virus 01 (CtenRNAV01) is a recently-isolated ssRNA virus which is infectious to the bloom-forming diatom *Chaetoceros tenuissimus*. The genome is a linear positive-stranded RNA with a polyA tail at the 3'-end. The genome sequence is registered as a Genbank accession number AB375474. Phylogenetic analysis of the RdRp sequence of CtenRNAV01 revealed that the virus is closely related to (but distinct from) RsetRNAV01, the type species of the genus *Bacillarnavirus*. Based on the results, here we propose a new virus species *"Chaetoceros tenuissimus RNA virus"* in the newly proposed genus "*Bacillarnavirus*".

The RdRp amino acids sequence identity between RsetRNAV01 and CtenRNAV01 is 64.5 %, which is below the species demarcation criteria currently in use for most genera in the order *Picornavirales*. Another important difference between the two diatom-infecting virus species is their host organisms which differ at genus-level. Further, the SDS-PAGE patterns (indicating the molecular weight of major capsid proteins) show noticeable distinctiveness.

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	***2009.015eP***	(assigned by ICTV officers)

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

Chaetoceros socialis f. radians 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	***2009.015fP***	(assigned by ICTV officers)

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

Genus:	***Bacillariovirus (new)***
Subfamily:	***unassigned***
Family:	***unassigned***
Order:	***Picornavirales***

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:
- 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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Chaetoceros socialis f. radians RNA virus 01 (CsfrRNAV01) is a recently-isolated ssRNA virus which is infectious to the bloom-forming diatom *Chaetoceros socialis* f. *radians*. The genome is a linear positive-stranded RNA; having a polyA tail at the 3'-end. The Genebank accession number of its full genome sequence is AB469874. Phylogenetic analysis of the RdRp sequence revealed that CsfrRNAV01 is closely related to (but distinct from) RsetRNAV01, the type species of the genus *Bacillarnavirus*. Based on the results, here we propose a new virus species *"Chaetoceros socialis f. radians RNA virus 01"* in the newly proposed genus "*Bacillarnavirus*".

The RdRp amino acids sequence identity between CsfrRNAV01 and RsetRNAV01 and between CsfrRNAV01 and CtenRNAV01 are 61.2 % and 46.0%, respectively. The most important difference among the three diatom-infecting virus species is their host organisms differing at genus-level; i.e., RsetRNAV01, CtenRNAV01 and CsfrRNAV01 are respectively infectious to *Rhizosolenia setigera*, *Chaetoceros tenuissimus* and *Chaetoceros socialis* f. *radians*. Further, their SDS-PAGE patterns (indicating the molecular weight of major capsid proteins) also show noticeable distinctiveness among RsetRNAV01 (41.5, 41.0, 29.5 kDa), CtenRNAV01 (33.5, 31.5, 30.0 kDa) and CsfrRNAV01 (32.0, 28.5, 25.0 kDa).

MODULE 3: **NEW GENUS**

creating and naming a new genus

Code	***2009.015gP***	(assigned by ICTV officers)

To create a new genus to contain the species listed below

Rhizosolenia setigera RNA virus 01
Chaetoceros tenuissimus RNA virus 01
Chaetoceros socialis f. radians RNA virus 01

Code	***2009.015hP***	(assigned by ICTV officers)

To name the new genus: *Bacillarnavirus*

assigning a new genus to higher taxa

Code	***2009.015iP***	(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:	***unassigned***
Order:	***Picornavirales***

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	***2009.015jP***	(assigned by ICTV officers)

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

Rhizosolenia setigera 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	***2009.015kP***	(assigned by ICTV officers)

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

- ***Chaetoceros tenuissimus RNA virus 01***
- ***Chaetoceros socialis f. radians RNA virus 01***

Reasons to justify the creation of a new genus:

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

In "Virus Taxonomy: 8th Report of ICTV", there are currently no reports of diatom-infecting ssRNA viruses. Since then, the full genome sequence of the three diatom-infecting viruses has been elucidated and the viruses have been characterized. The phylogenetic tree constructed based on the RdRp sequence showed that they form a monophyly supported by a bootstrap value of 100% (annexes Fig. 6). The clade is apparently distinct from the cluster of *Dicistroviridae*, *Iflavirus, Comoviridae, Sequiviridae, Picornaviridae* and *Caliciviridae*. As mentioned above, the clade is related to *Heterosigma akashiwo RNA virus SOG 263* (HaRNAV-SOG263), a virus in the genus *Marnavirus*, family *Marnaviridae,* order *Picornavirales* and to AuRNAV01, a newly-proposed species suggested to constitute a separate genus (*Labyrnavirus*) unassigned in the order *Picornavirales*. However, the clade of the three diatom-infecting viruses constitutes a distinct sub-

branch in the tree. Based on the results, we propose a new diatom-infecting ssRNA virus genus "*Bacillarnavirus*" unassigned in the order *Picornavirales* that will contain three new species; RsetRNAV01 is proposed as the type species of the genus *Bacillarnavirus*.

The structural and non-structural proteins' amino acid sequences of the three diatom-infecting ssRNA viruses mentioned above showed some similarities to those of HaRNAV-SOG263 and AuRNAV01 (see above); still, it may be too rough-and-ready to determine the family to which the new genus *Bacillarnavirus* belongs (data not shown).

Origin of the new genus name:

ss*RNA* virus infecting *Bacilla*riophytes → Bacillarnavirus

Reasons to justify the choice of type species:

RsetRNAV01 is the first-isolated diatom-infecting ssRNA virus and the most intensively-studied virus within the genus *Bacillarnavirus*.

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

Virus species within the genus *Bacillarnavirus* are infectious to diatoms and harbor a single-stranded linear RNA genome with a polyA tail at the 3'-end. Genomic sequences of the genus *Bacillarnavirus* are registered with Genbank accession numbers AB243297 (RsetRNAV01), AB375474 (CtenRNAV01), and AB469874 (CsfrRNAV01).

Although the phylogenetic tree constructed based on the RdRp sequence showed that the three diatom-infecting ssRNA viruses form a monophyly supported by a bootstrap value of 100%, they are different in the host diatom species which they infect. Identity in RdRp amino acid sequence among RsetRNAV01, CtenRNAV01 and CsfrRNAV01 is only 46.0~64.5 %. Further, their SDS-PAGE patterns (reflecting the molecular weight of major capsid proteins) also show their noticeable distinctiveness.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Tomaru Y, Takao Y, Suzuki H, Nagumo T, Nagasaki K (2009) Isolation and characterization of a single-stranded RNA virus infecting the bloom forming diatom Chaetoceros socialis. Appl. Environ. Microbiol. 75: 2375-2381.
2. 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.
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. 2008.7
4. Nagasaki, K. (2008) Dinoflagellates, diatoms and their viruses. J. Microbiol. 46(3): 235-243.
5. Shirai, Y., Tomaru, Y., Takao, Y., Suzuki, H., Nagumo, T., Nagasaki, K. (2008) Isolation and characterization of a single-stranded RNA virus infecting the marine planktonic diatom Chaetoceros tenuissimus Meunier. Appl. Environ. Microbiol. 74(13): 4022-4027.
6. Shirai, Y., Takao, Y., Mizumoto, H., Tomaru, Y., Honda, D., Nagasaki, K. (2006) Genomic and phylogenetic analysis of a single-stranded RNA virus infecting Rhizosolenia setigera (Stramenopiles: Bacillariophyceae). J. Mar. Biol. Ass. U.K., 86: 475-483.
7. Nagasaki, K., Tomaru, Y., Katanozaka, N., Shirai, Y., Nishida, K., Itakura, S., Yamaguchi, M. (2004) Isolation and characterization of a novel single-stranded RNA virus infecting the bloom-forming diatom Rhizosolenia setigera. Appl. Environ. Microbiol., 70: 704-711.
8. Takao, Y., Mise, K., Nagasaki, K., Okuno, T., Honda, D. (2006) Complete nucleotide sequence and genome organization of a single-stranded RNA virus infecting the marine fungoid protist Schizochytrium sp. J. Gen. Virol. 87: 723-733.
9. Takao, Y., Nagasaki, K., Mise, K., Okuno, T., Honda, D. (2005) Isolation and characterization of a novel single-stranded RNA Virus infectious to a marine fungoid protist, Schizochytrium sp. (Thraustochytriaceae, Labyrinthulea). Appl. Environ. Microbiol. 71: 4516-4522.
10. Lang, A. S., Culley, A. I., Suttle, C. A. (2004) Genome sequence and characterization of a virus (HaRNAV) related to picorna-like viruses that infects the marine toxic bloom-forming alga Heterosigma akashiwo. Virology 320: 206-217.
11. Tai, V., Lawrence, J. E., Lang, A. S., Chan, A. M., Culley, A. I., Suttle, C. A. (2003) Characterization of HaRNAV, a single-stranded RNA virus causing lysis of Heterosigma akashiwo (Raphidophyceae). J. Phycol. 39: 343-352.

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, above-mentioned three viruses (RsetRNAV01, CtenRNAV01 and CsfrRNAV01) belonging to the proposed genus *Bacillarnavirus* are introduced.

RsetRNAV01 is an icosahedral virus (32 nm in diameter) (previously reported as RsRNAV in Nagasaki *et al*. 2004) (Fig.1). Virus particles' accumulation is observed in the host cytoplasm.

This virus was first isolated from water samples of Ariake Sound in western Japan in April 2002. The latent period and burst size of RsetRNAV01 are 48 hours and 1,100-3,000 infectious units per host cell, respectively. The infection specificity of this virus is strain-specific rather than species-specific. The major structural proteins of RsetRNAV01 are 41.5, 41.0, and 29.5 kDa. RsetRNAV01 genome is an ssRNA which is 8,877 nt long (excluding polyA tail), polyadenylated, lacking a cap structure, and has two major open reading frames (ORFs): ORF-1 (4,818 nt), coding for replicases, e.g. RNA helicase, RNA-dependent RNA polymerase (RdRp); and ORF-2 (2,883 nt), coding for the above mentioned structural proteins. The ORFs are separated by a 323 nt intergenic region (IGR), flanked by a 624 nt 5'-untranslated region (UTR) and a 229 nt 3'-UTR (Shirai et al. 2006) (Fig. 2).

CtenRNAV01 causes the lysis of the bloom-forming marine diatom *Chaetoceros tenuissimus* Meunier (Shirai et al. 2008). CtenRNAV01 was first isolated form water samples of Ariake Sound in western Japan during June 2004. This virus is an icosahedral virus (31 nm in diameter) (Fig. 3). Virus particles are accumulated in the host cytoplasm with crystalline array formations (Fig. 4). The latent period and the burst size of CtenRNAV01 are <24 hours and ~10^4 infectious units per host cell, respectively. It harbors a ssRNA genome that is 9,431 nt (excluding a poly-A tail region) including two ORFs: ORF-1 (5,211 nt) and ORF-2 (2,646 nt). The major structural proteins are 33.5, 31.5, and 30.0 kDa in molecular mass.

CsfrRNAV01 causes lysis of the bloom-forming diatom species, *Chaetoceros socialis* Lauder f. *radians* (Schütt) Proschkina-Lavrenko (Tomaru et al. 2009). This virus was first isolated from water samples of Hiroshima Bay, western Japan in April 2005. CsfrRNAV01 is a very small polyhedral virus (22 nm in diameter) (Fig. 5). Virus particles accumulate in the host cytoplasm. The latent period and burst size of CsfrRNAV01 are <48 hours and 66 infectious units per host cell, respectively (maybe the burst size is underestimated). CsfrRNAV01 harbors an ssRNA genome and encodes at least three polypeptides of 32.0, 28.5 and 25.0 kDa. By RNA sequencing analysis, the genome was revealed to be 9,467 nt excluding a poly-A tail; genome structure is almost similar to that of RsetRNAV01.

Although the phylogenetic tree constructed based on the RdRp sequence showed that these three diatom-infecting ssRNA viruses form a monophyly supported by a bootstrap value of 100% (Fig. 6), they are different in the host diatom species which they infect. Identity in RdRp amino acid sequence among RsetRNAV01, CtenRNAV01 and CsfrRNAV01 is only 46.0~64.5 %. Further, their SDS-PAGE patterns (reflecting the molecular weight of major capsid proteins) also show their noticeable distinctiveness as mentioned above.

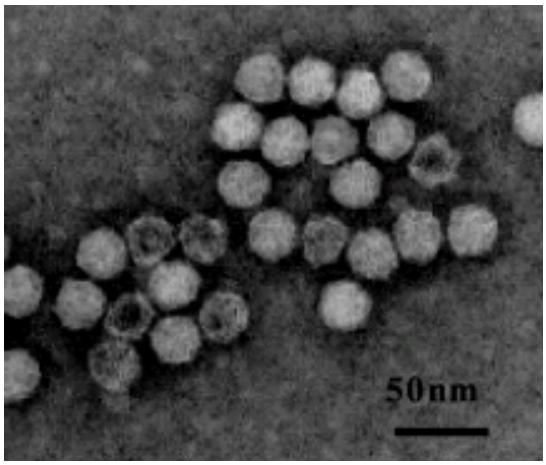


Fig. 1. Negatively-stained virions of RsetRNAV01. (reprinted with copyright permission from American Society for Microbiology: Nagasaki, K. et al. **70:** 704-711 [published in February 2004])

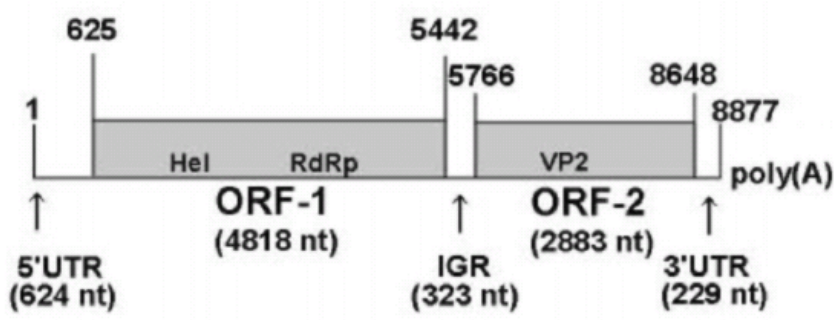


Fig. 2. Schematic genome structure of RsetRNAV01. Numbers indicate base positions from the 5'-terminus in the nucleotide sequence. Hel, RNA helicase domain; RdRp, RNA-dependent RNA polymerase domain. (reprinted with copyright permission from the Cambridge University Press: Shirai et al. **86:** 469-474 [published in June 2006])

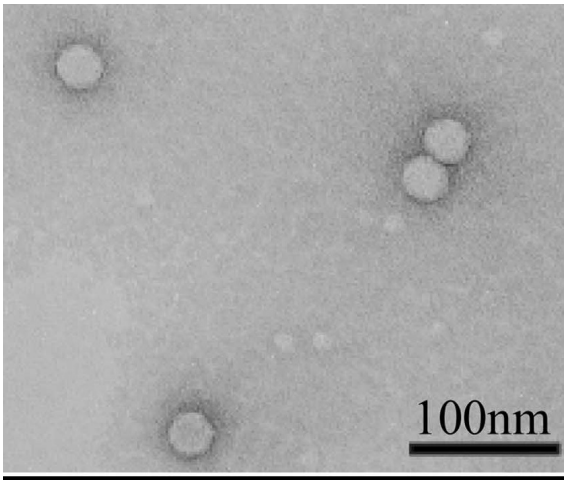


Fig. 3. Negatively-stained virions of CtenRNAV01. (reprinted with copyright permission from the American Society for Microbiology: Shirai, Y. et al. *Appl. Environ. Microbiol.* **74:** 4022-4027. [published in July 2008])

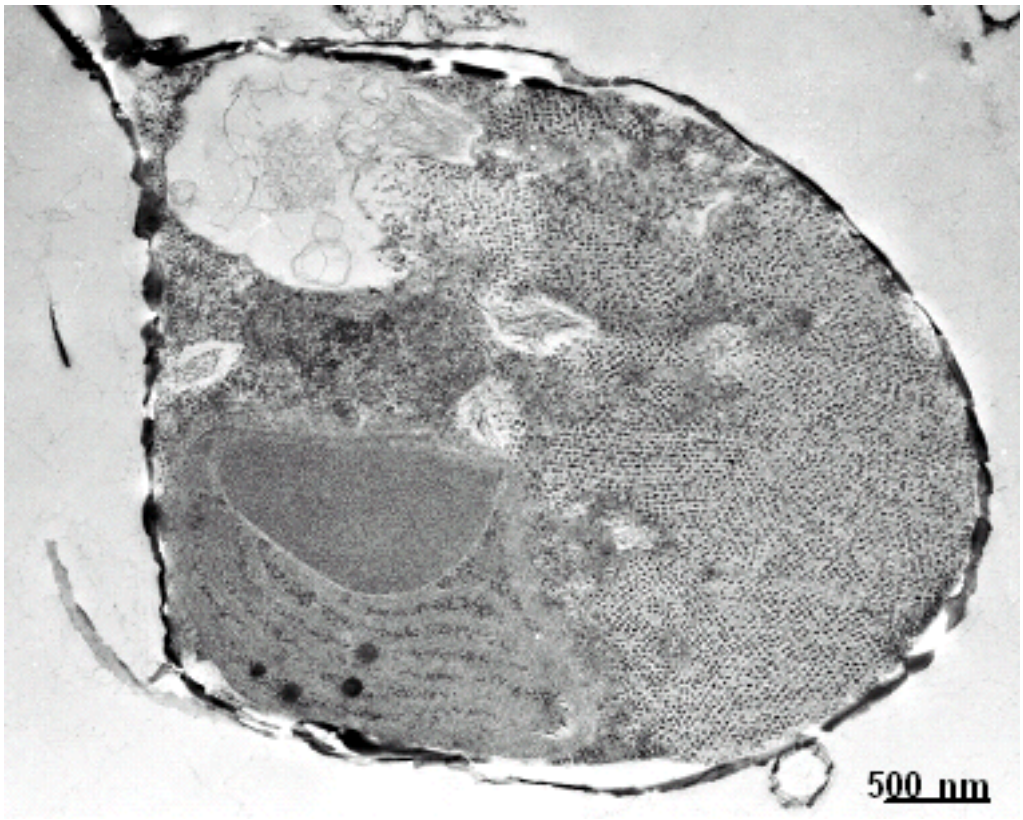


Fig. 4. Transmission electron micrograph of thin section of a CtenRNAV01-infected *Chaetoceros tenuissimus* cell at 48 hours postinoculation. Virions accumulate in the host cytoplasm forming a crystalline array.

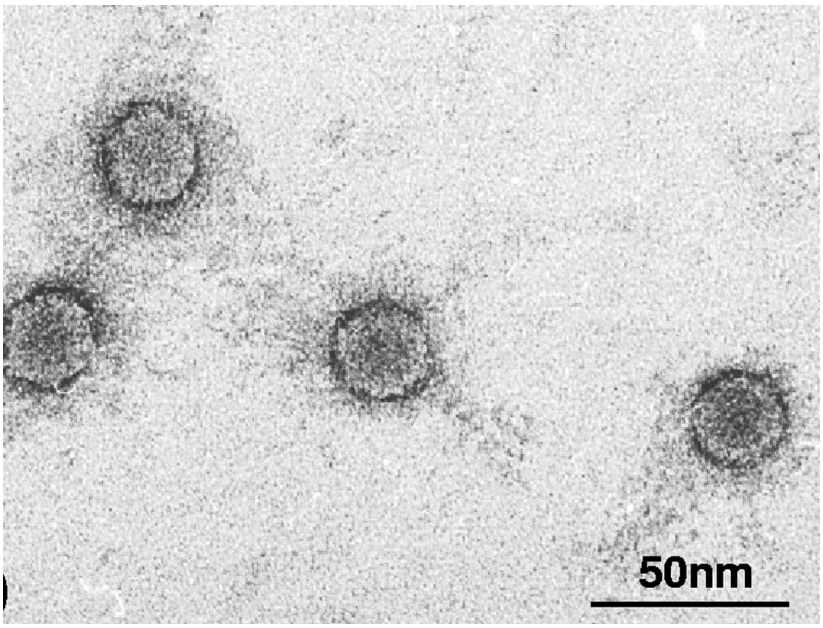
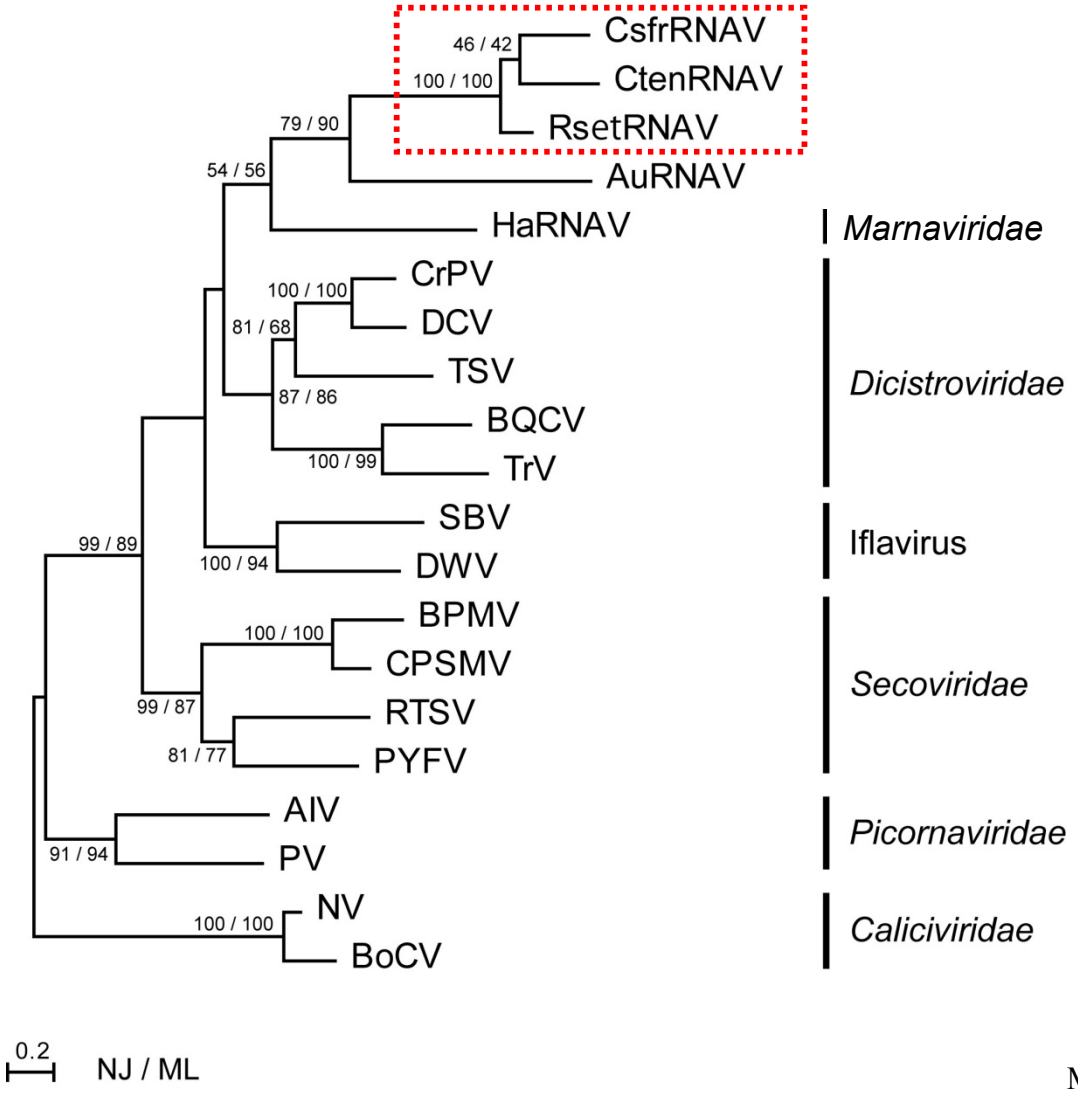


Fig. 5. Negatively-stained virions of CsfrRNAV01. (reprinted with copyright permission from the American Society for Microbiology: Nagasaki, K. et al. *Appl. Environ. Microbiol.* **75:** 2375–2381. [published in April 2009])



Marnaviridae

Fig. 6. ML tree based on deduced amino acid sequences of the RdRp whole domain. ML bootstrap values (%) from 100 samples are shown at the nodes, followed by bootstrap values based on the NJ analysis (%) from 100 samples. The ML distance scale bar is shown. The amino acid sequences used for comparison in the analyses are as follows with the NCBI accession numbers: Aichi virus (AIV), AB010145; Aurantiochytrium single-stranded RNA virus 01 (AuRNAV), BAE47143; Bovine enteric calicivirus (BoCV), AJ011099; Bean pod mottle virus (BPMV), AF394608; Black queen cell virus (BQCV), AF183905; Chaetoceros socialis f. radians RNA virus 01 (CsfrRNAV), AB469874; Chaetoceros tenuissimus RNA virus 01 (CtenRNAV), AB375474; Cowpea severe mosaic virus (CPSMV), M83830; Cricket paralysis virus (CrPV), M21938; Drosophila C virus (DCV), AF014388; Deformed wing virus (DWV), AY292384; Heterosigma akashiwo RNA virus (HaRNAV), AY337486; Norwalk virus (NV), M87661; Human poliovirus 1 Mahoney (PV), V01149; Parsnip yellow fleck virus (PYFV), D14066; Rhizosolenia setigera RNA virus 01 (RsetRNAV), BAE79742; Rice turgo spherical virus (RTSV), AAA66056; Sacbrood virus (SBV), AF092924; Triatoma virus (TrV), AF178440; and Taura syndrome virus (TSV), AF277675. The cluster of diatom-infecting viruses is highlighted in red. Fig. 5. Negatively-stained virions of CsfrRNAV01. (reprinted with copyright permission from the American Society for Microbiology: Nagasaki, K. et al. *Appl. Environ. Microbiol.* **75:** 2375–2381. [published in April 2009])