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:	***2013.003a-hB***	(to be completed by ICTV officers)
Short title: Create the family *Spiraviridae*, comprising the new genus *Alphaspiravirus* and one new species. (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:

David Prangishvili david.prangishvili@pasteur.fr

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)	Archaeal virus study group

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

Date first submitted to ICTV: June 2013
Date of this revision (if different to above):

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	***2013.003aB***	(assigned by ICTV officers)

To create new species within:

Genus:	***Spiravirus*** (new)
Subfamily:	
Family:	***Spiraviridae*** (new)
Order:	

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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Aeropyrum coil-shaped virus	HE681887.1

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

Aeropyrum coil-shaped virus in its virion morphology and genome sequence significantly differs from any known virus (see Module 9, Annex) and is the sole species is the genus.

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2013.003bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Spiraviridae*** (new)
Order:	

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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2013.003cB***	(assigned by ICTV officers)

To name the new genus: *Spiravirus*

Assigning the type species and other species to a new genus

Code	***2013.003dB***	(assigned by ICTV officers)

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

Aeropyrum coil-shaped virus

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

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

1

Reasons to justify the creation of a new genus:

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

We propose classifying the *Aeropyrum coil-shaped virus* APBV1 of the hyperthermophilic archaeaon *Aeropyrum pernix* as a first representative of a new genus because of the unique combination of its morphological and genomic properties. The virion morphology is clearly distinct from that of any known archaeal, bacterial, or eukaryotic virus. The linear, non-enveloped ASPV virion is a hollow, coil-shaped particle (Module 9, Annnex - Figure 1). It is formed from a coiling fiber, which consists of two intertwining halves of a single circular nucleoprotein (Module 9, Annnex - Figure 4).

The virus ACV is also exceptional for its genomic properties. It is the only virus with a single-stranded (ss) DNA genome among the known hyperthermophilic archaeal viruses. Moreover, the size of its circular genome, 24,893 nt, is double that of the largest known ssDNA genome (Module 9, Annex - Figure 5). The genome content of ACV is in line with its unique morphology and confirms that ACV is not closely related to any known virus.

Origin of the new genus name:

From Latin *spira*, for "coil"

Reasons to justify the choice of type species:

This is the only species in the genus

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.

Not relevant

NEW FAMILY

creating and naming a new family

Code	***2013.003eB***	(assigned by ICTV officers)
To create a new family containing the subfamilies and/or genera listed below within 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.		

Code	***2013.003fB***	(assigned by ICTV officers)
To name the new family: *Spiraviridae*		

assigning subfamilies, genera and unassigned species to a new family

Code		(assigned by ICTV officers)
To assign the following subfamilies (if any) 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	***2013.003gB***	(assigned by ICTV officers)
To assign the following genera 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		
Spiraviridae		
The new family will also contain any other new species created and assigned to it (Module 3) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of unassigned species that the family will contain (those NOT within any of the genera or subfamilies listed above):**		
none		

Reasons to justify the creation of the new family

We propose classifying the *Aeropyrum coil-shaped virus* APBV1 of the hyperthermophilic archaeaon *Aeropyrum pernix* as a first representative of a new family because of the unique combination of its morphological and genomic properties. The virion morphology is clearly distinct from that of any known archaeal, bacterial, or eukaryotic virus. The linear, non-enveloped ASPV virion is a hollow, coil-shaped particle (Module 9, Annnex - Figure 1). It is formed from a coiling fiber, which consists of two intertwining halves of a single circular nucleoprotein (Module 9, Annnex - Figure 4).

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

Origin of the new family name:

From Latin *spira*, for "coil"

APPENDIX: supporting material

additional material in support of this proposal

References:

Mochizuki, T., Krupovic, M., Pehau-Arnaudet, G., Sako, Y., Forterre, P., and Prangishvili, D. (2012). Archaeal virus with exceptional virion architecture and the largest single-stranded DNA genome. *Proc. Natl. Acad. Sci. USA* **109**, 13386-13391.

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

Structure of the ACV virion

Negatively stained virions of ACV analyzed by transmission electron microscopy (TEM) appear as rigid cylindrical particles of 220±10 x 28±2 nm with appendages of 20±2 nm protruding from both termini at 45° angles to the axis of the cylinder (Figure 1A). The virion surface demonstrated a clear periodic pattern with 40 turns of the putative helix readily distinguishable (Figure 1B). ACV virion is non-enveloped, as revealed by cryo-EM observationq of the virions embedded in vitreous ice (Figure 1C,D). The ice-embedded virions are more flexible than the negatively stained virions and measure 230±10 x 19±1 nm. Periodic, higher density regions caused by helical organization of the virion are visible along the sides of the cylindrical particles (Figure 1D). Because cryo-EM allows for the observation of samples in their natural conformation, avoiding possible artifacts caused by dehydration due to negative staining, the images of particles embedded in ice should be closer to the authentic structure of the ACV virion. The flexible coil of the native virion could indeed be prone to contraction and stiffening upon dehydration due to uranyl acetate staining. This process would explain the observed differences in virion appearance under the two conditions.

Comparison of the ACV virion with virions of other helical viruses, the tobacco mosaic virus (TMV) and the archaeal rudivirus SIRV2 (Figure 2) reveal that the pitch of the ACV virion helix, estimate to be ~4.8 nm, is nearly twice as wide as the pitch of the two other virions, ~2.3 nm. Considering the length of the virion and the measured pitch of the helix, the calculated number of helix turns is 45. Using these parameters and assuming the diameter to be equal to 28 nm, the helix-forming filament would be approximately 4000 nm in length.

The results of EM observations suggest an unusual nucleoprotein arrangement in the ACV virion. The observation of completely or partially unwound virions (Fig. 3) revealed that the nucleoprotein filament represents a rope-like helical fiber composed of two intertwined strands, which

are clearly visible in the insets in Figure 3B. Theses observations highlight that at least two levels of helical organization exist in the virion and that the filament, which forms the virion helix, can itself have a helical structure, being composed of two intertwining strands. Each of these strands is most likely a nucleoprotein containing a strand of ssDNA. Because the viral ssDNA is circular, the two strands likely represent the two halves of the same circular nucleoprotein. Based on these considerations, a tentative model for the structural organization of the ACV virion can be proposed (Fig. 4).

The virions carry two major proteins with molecular masses of ~ 23 and 18.5 kDa, and a few minor bands of proteins with molecular masses in the range of ~5–13 kDa.

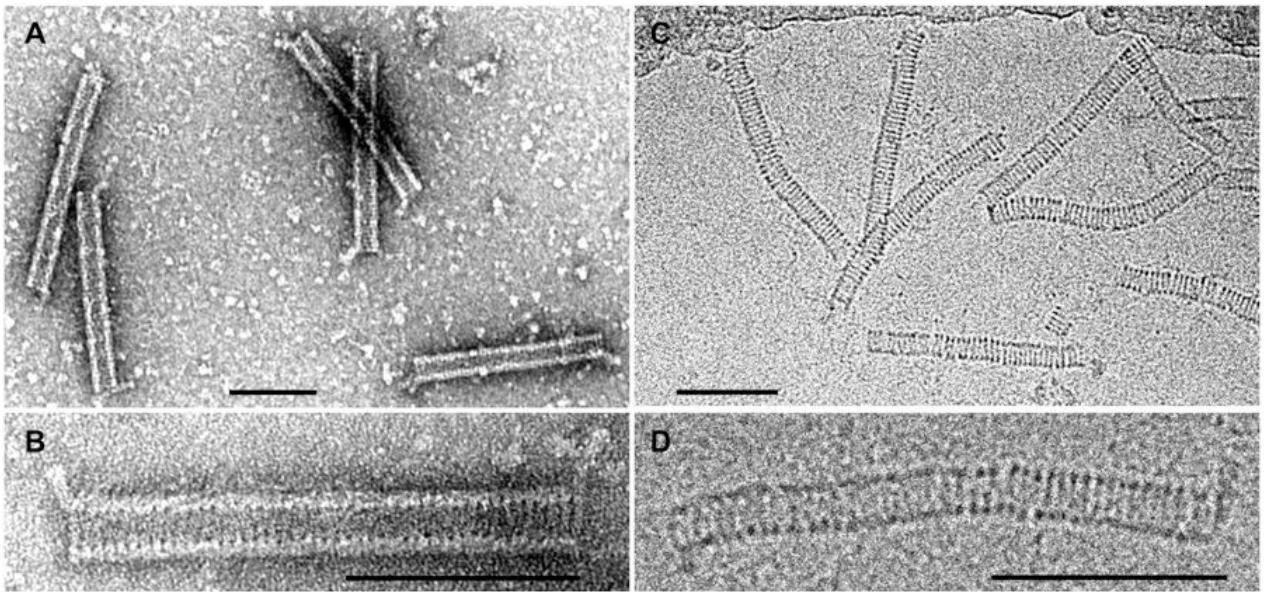

Figure 1. Electron micrographs of ACV virions. (A and B) Negatively stained with 2 % uranyl acetate. (C and D) Sample embedded in vitreous ice. Scale bars, 100 nm.

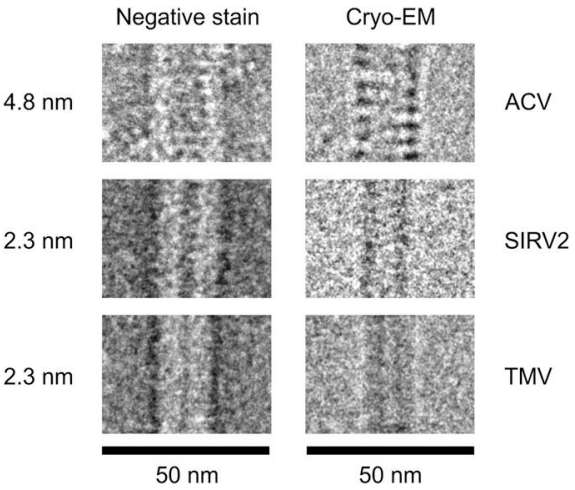

Figure 2. Comparison of ACV to other helical viruses. Portions of negatively stained virions of ACV, tobacco mosaic virus (TMV), and *Sulfolobus islandicus* rod-shaped virus 2 (SIRV2) are shown in the left column, whereas their cryo-EM images are in the right column. Original micrographs are shown in SI, Fig. S3. The helix pitch, determined by Fourier transformation of the negative stained images, is indicated.

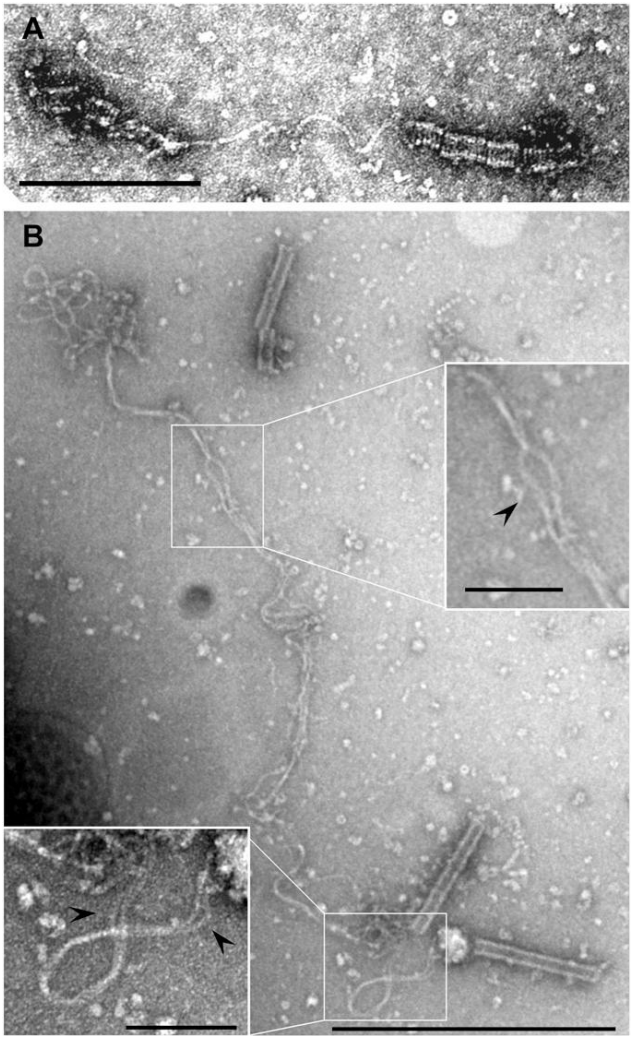

Figure 3. Transmission electron micrographs of disrupted ACV virions. (A) Fragments of partially disassembled virions connected by a twisted filament. (B) A completely unwound helix-forming filament; the regions where two constituent strands of the helix-forming filament are clearly distinguishable are shown in insets and indicated by arrows. Scale bars (A) 200 nm; (B) 500 nm, 100 nm in insets.

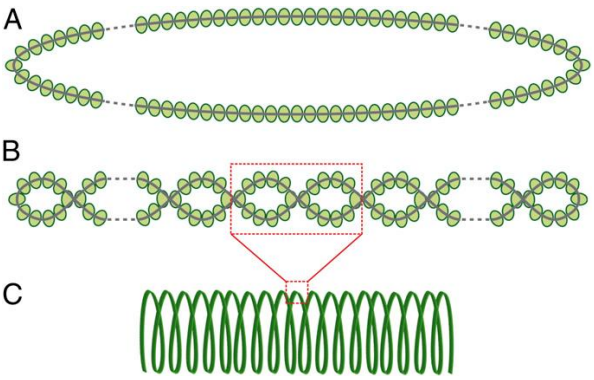

Figure 4. Schematic representation of different levels of organization of the circular ACV nucleoprotein. As described in the text, the two halves of the circular nucleoprotein (A) intertwine with each other and form a nucleoprotein filament (B), which is condensed into the helical coil of the virion (C).

The virion architecture suggested for ACV has not been reported for any known virus with either a DNA or an RNA genome. The only known filamentous viruses with ssDNA genomes, members of the *Inoviridae* family, have virions that differ significantly in structure from the ACV virion. The former have cylindrical protein shells encasing two antiparallel strands of circular ssDNA that are partly base-paired and adopt a conformation similar to classical A- and B-form dsDNA. By contrast, the protein shell of the ACV virion apparently encases a single strand of DNA, precluding pseudo double helix formation. Although the inoviral nucleoprotein filament has no further level of organization, the circular ACV nucleoprotein adopts two more levels of organization by intertwining the two halves of the circular molecule and arranging the resulting helix into a superhelix of higher order to produce the cylindrical helix of the virion (Fig. 5). The differences in structural design appear to be the major reason for the size differences between the virion types. The inovirus virions are approximately 7 nm in width, compared to ~28 nm for ACV virions. Additionally, the ~3700 nm-long virion of the inovirus Pf4 is nearly 17 times longer than the ACV virion, even though the ACV genome is double the size of the Pf4 genome. It is noteworthy that in inoviruses and all other known non-enveloped filamentous viruses (*Rudiviridae, Alfaflexiviridae, Betaflexiviridae, Gammaflexiviridae, Closteroviridae, Potyviridae, Virgaviridae*), the nucleoprotein helix does not adopt any additional levels of organization, thereby distinguishing ACV from these viruses. Organization of a nucleoprotein filament into a coil-shaped helical structure has been reported only for enveloped viruses, e.g. members of the order *Mononegavirales*, such as Ebola and Marburg viruses. However, the helical nucleocapsids of these enveloped visuses contain a single strand of an RNA genome and thus are radically different from the coil-forming filament of ACV, composed of two intertwining nucleoprotein strands (Fig.5).

ACV genome

The circular ssDNA genome of ACV consists of 24,893 nt and has a G+C content of 46.7 %. The genome contains 57 predicted open reading frames (ORFs) (Fig. 5). All but one of these ORFs are present on the DNA strand that is packaged into the ACV virions, indicating that the genome is positive sense.

The results of the genome analysis are in line with the unique morphology of the ACV virus and confirm that ACV is not closely related to any other characterized archaeal, bacterial or eukaryal virus. Notably, ACV is the first hyperthermophilic archaeal virus with a ssDNA genome. The unique architectural solution employed by ACV to pack its circular ssDNA does not impose a strict constraint on genome size. Indeed, ACV has the largest genome among the known ssDNA viruses, at twice the size (24,8 versus 12,4 kb) of the previous record holder, the inovirus Pf4. The functional complexity of the ACV genome is far greater than that observed for other known ssDNA—and even certain dsDNA—viruses. The ability to maintain a relatively large genome allows ACV to encode auxiliary functions that, although not typical for ssDNA viruses, are rather frequent in complex dsDNA viruses.

The virus ACV does not encode a potential Rep candidate that would share significant sequence homology with known RCR Rep proteins involved in rolling circle replication mechanism,

operating in all known ssDNA viruses replication. The virus ACV might employ a novel mechanism of genome replication.

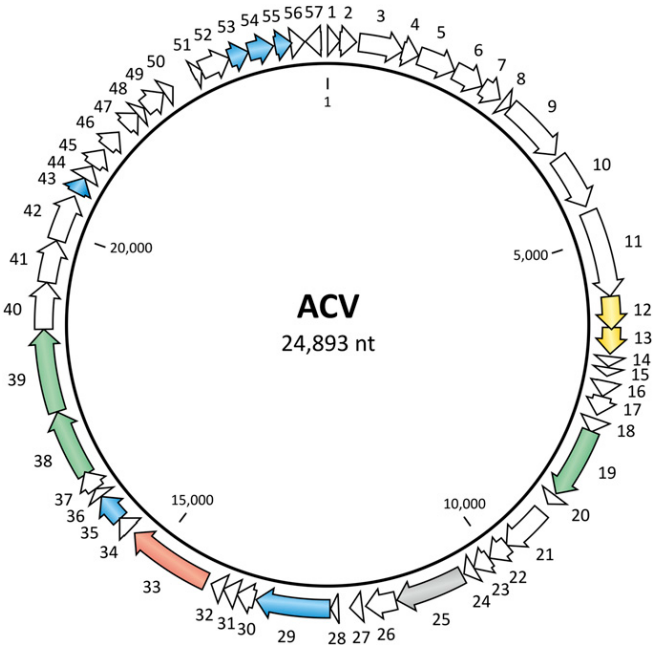

Figure 5. Circular genome map of ACV. The open reading frames (ORFs) are marked with arrows indicating the direction of transcription. The ORFs encoding putative proteins for which functions could be predicted are color-coded as follows: thioredoxins, yellow; carbohydrate metabolism, green; DNA-binding, blue; serine protease, gray; and recombinase, red.