This form should be used for all taxonomic proposals. Please complete all those modules that are applicable.

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.

Part 1: **TITLE, AUTHORS, etc**

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| **Code assigned:** | ***2017.016B*** | | | | (to be completed by ICTV officers) |
| **Short title: To create six (6) new species in the genus *Cystovirus*** | | | | | |
| **Modules attached**  (Modules 1, 4 and either 2 or 3 are required. | | **1**  **2  3  4** | | | |
| **Author(s):** | | | | | |
| Minna M. Poranen, University of Helsinki (Finland)  Sari Mäntynen, University of Jyväskylä (Finland)  Jens H. Kuhn, National Institute of Allergy and Infectious Diseases (USA)  Evelien M. Adriaenssens, University of Liverpool (UK)  Andrew M. Kropinski, University of Guelph (Canada) | | | | | |
| **Corresponding author with e-mail address:** | | | | | |
| Minna M. Poranen, [minna.poranen@helsinki.fi](mailto:minna.poranen@helsinki.fi) | | | | | |
| **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 (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal) | | | **ICTV Bacterial and Archaeal Viruses Subcommittee** | | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | | | |
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| Date first submitted to ICTV: | | | | June 8, 2017 | |
| Date of this revision (if different to above): | | | |  | |

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| **ICTV-EC comments and response of the proposer:** |
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**Part 2**: **PROPOSED TAXONOMY**

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| Present the proposed new taxonomy on accompanying spreadsheet |
| **Name of accompanying spreadsheet:** **2017.016B.N.v1.Cystovirus\_6sp** |

Please display the taxonomic changes you are proposing on the accompanying spreadsheet module 2017\_TP\_Template\_Excel\_module. Submit both this and the spreadsheet to the appropriate ICTV Subcommittee Chair.

**Part 4:** **APPENDIX**: supporting material

| additional material in support of this proposal |
| --- |
| **References:** |
| Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36 (Web Server issue):W465-9.  Gottlieb P, Potgieter C, Wei H, Toporovsky I (2002a) Characterization of φ12, a bacteriophage related to φ6: nucleotide sequence of the large double-stranded RNA. Virology 295: 266-271.  Gottlieb P, Wei H., Potgieter C, Toporovsky I (2002b) Characterization of φ12, a bacteriophage related to φ6: nucleotide sequence of the small and middle double-stranded RNA. Virology293: 118-124.  Hoogstraten D, Qiao X, Sun Y, Hu A, Onodera S, Mindich L (2000) Characterization of φ8, a bacteriophage containing three double-stranded RNA genomic segments and distantly related to φ6. *Virology* 272: 218-224.  Jäälinoja HT, Huiskonen JT, Butcher SJ (2007) Electron cryomicroscopy comparison of the architectures of the enveloped bacteriophages φ6 and φ8. Structure15: 157-167.  Mindich L, Qiao X, Qiao J, Onodera S, Romantschuk M, Hoogstraten D (1999) Isolation of additional bacteriophages with genomes of segmented double-stranded RNA. J Bacteriol181: 4505-4508.  Mäntynen S, Laanto E, Kohvakka A, Poranen MM, Bamford JKH, Ravantti JJ (2015) New enveloped dsRNA phage from freshwater habitat. Journal of General Virology. 96: 1180-1189.  Qiao X, Qiao J, Onodera S, Mindich L (2000) Characterization of φ13, a bacteriophage related to φ6 and containing three dsRNA genomic segments. Virology275: 218-224.  Qiao X, Sun Y, Qiao J, Di Sanzo F, Mindich L (2010) Characterization of φ2954, a newly isolated bacteriophage containing three dsRNA genomic segments. BMC Microbiol10: 55-2180-10-55.  Wei H, Cheng RH, Berriman J, Rice WJ, Stokes DL, Katz A, Morgan DG, Gottlieb P (2009) Three-dimensional structure of the enveloped bacteriophage phi12: an incomplete T=13 lattice is superposed on an enclosed T=1 shell. PLoS One 4(9):e6850.  Yang Y, Lu S, Shen W, Zhao X, Shen M, Tan Y, Li G, Li M, Wang J, Hu F, Le S (2016) [Characterization of the first double-stranded RNA bacteriophage infecting *Pseudomonas aeruginosa*.](https://www.ncbi.nlm.nih.gov/pubmed/27934909) Sci Rep 6:38795. |

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| **Annex:**  **Introduction:**  The *Cystoviridae* family currently includes one genus *Cystovirus* and one species, *Pseudomonas virus phi6.* Six additional, cystovirus-related phages with dsRNA genomes have been isolated and completely sequenced (Table 1; Mindich *et al*., 1999; Hoogstraten *et al*., 2000; Qiao *et al*., 2000, 2010; Gottlieb *et al*., 2002a,b; Mäntynen *et al*., 2015; Yang *et al*., 2016).  **Species demarcation**: We have chosen 95% RNA sequence identity as the criterion for demarcation of species in the *Cystovirus* genus. The members of each of the proposed species differ from those of other species by more than 5% at the RNA level as confirmed with the Clustal Omega Multiple Alignment (Table 2).  **Reasons to justify the assignment of the new species to the existing genus *Cystovirus*:**  All six new isolates share similar genome and virion organizations with Pseudomonas phage phi6. Their genomes are composed of three linear dsRNA segments (S-, M- and L-segments) (Fig. 1) and similar genes can be identified in similar order in each segment (Fig. 1). The genome lengths (12.7 kb – 15.0 kb) and GC contents (53.4–58.8%) are also similar (Table 1). Phylogenetic analyses were done separately for each genome segment using Clustal Omega Multiple Alignment (Fig. 2) and “One Click” at Phylogeny.fr (Fig. 3). Due to the size of the L-segments (over 6000 bp), the phylogenetic analysis was done only for the S- and M-segments with “One Click” (Dereeper *et al*., 2008). The clustering was different in each tree, suggesting that there has been reassortment of segments between members of the proposed species. Also, exchange of genome segments between the different isolates has been experimentally shown in a few cases (Mindich *et al*., 1999; Qiao *et al*., 2000; Qiao *et al*., 2010).  The virion organization of members of the proposed species, if described, resembles that of members of the type species (Jäälinoja *et al*., 2007; Wei *et al*., 2009). Virions are enveloped and their genomes are enclosed in icosahedrally symmetric protein shells. |

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| **Table 1.** Properties of the proposed members of the *Cystoviridae* family. | | | | | | | |
| **Pseudomonas phage** | | **RefSeq No** | **GenBank**  **accession No** | **Genome length (kb)** | **GC%** | **No. CDS**  **(in positive strand)** | **% RNA sequence similarity to the type species\*** |
| Pseudomonas phage phi6 (member of the type species) | | NC\_003715; NC\_003716; NC\_003714 | M17461; M17462; M12921 | 13.4 | 55.8 | 13 | 100 |
| Pseudomonas phage phi8 | | NC\_003299; NC\_003300; NC\_003301 | AF226851; AF226852; AF226853 | 15 | 54.5 | 19 | 42.4, 44.0, and 44.0 for L, M and S segments |
| Pseudomonas phage phi12 | | NC\_004173; NC\_004175: NC\_004174 | AF408636; AY039807; AY034425 | 13.2 | 55.2 | 15 | 43.8, 45.7 and 42.9 for L, M and S segments |
| Pseudomonas phage phi13 | | NC\_004172; NC\_004171; NC\_004170 | AF261668; AF261667; AF261666 | 13.7 | 57.7 | 13 | 50.0, 45.6 and 44.5 for L, M and S segments |
| Pseudomonas phage phi2954 | | NC\_012091; NC\_012092; NC\_012093 | FJ608823; FJ608824; FJ608825 | 12.7 | 53.4 | 14 | 44.9, 44.2 and 43.6 for L, M and S segments |
| Pseudomonas phage phiNN | |  | KJ957164; KJ957165; KJ957166 | 13.3 | 54.7 | 13 | 78.7, 52.1 and 82.8 for L, M and S segments |
| Pseudomonas phage phiYY | |  | KX074201; KX074202; KX074203 | 13.5 | 58.8 | 14 | 50.1, 43.7 and 43.2 for L, M and S segments |
|  | \*Determined using EMBOSS Needle Pairwise Sequence Alignment  **Table 2.** Nucleotide sequence similarities (%) between the genome segments, determined using Clustal Omega Multiple Alignment. Color code: >95 % = red, >75 % = green, >50 % = yellow.   |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | | **Nucleotide sequence similarities (%) between the L-segments** | | | | | | | | | **Phage** | **phi6** | **phi8** | **phi12** | **phi13** | **phi2954** | **phiNN** | **phiYY** | | **phi6** | 100 | 38.47 | 36.42 | 48.84 | 36.01 | 79.46 | 48.46 | | **phi8** | 38.47 | 100 | 35.8 | 38.96 | 34.82 | 38.51 | 38 | | **phi12** | 36.42 | 35.8 | 100 | 35.98 | 51.13 | 36.07 | 35.48 | | **phi13** | 48.84 | 38.96 | 35.98 | 100 | 34.9 | 48.62 | 62.93 | | **phi2954** | 36.01 | 34.82 | 51.13 | 34.9 | 100 | 35.74 | 34.76 | | **phiNN** | 79.46 | 38.51 | 36.07 | 48.62 | 35.74 | 100 | 47.84 | | **phiYY** | 48.46 | 38 | 35.48 | 62.93 | 34.76 | 47.84 | 100 | | **Nucleotide sequence similarities (%) between the M-segments** | | | | | | | | |  | **phi6** | **phi8** | **phi12** | **phi13** | **phi2954** | **phiNN** | **phiYY** | | **phi6** | 100 | 38.17 | 38.99 | 40.34 | 39.2 | 51.18 | 35.34 | | **phi8** | 38.17 | 100 | 38.47 | 39.75 | 38.75 | 36.48 | 35.91 | | **phi12** | 38.99 | 38.47 | 100 | 61.26 | 37.46 | 39.15 | 38.32 | | **phi13** | 40.34 | 39.75 | 61.26 | 100 | 37.5 | 40.66 | 39.43 | | **phi2954** | 39.2 | 38.75 | 37.46 | 37.5 | 100 | 37.32 | 34.89 | | **phiNN** | 51.18 | 36.48 | 39.15 | 40.66 | 37.32 | 100 | 37.25 | | **phiYY** | 35.34 | 35.91 | 38.32 | 39.43 | 34.89 | 37.25 | 100 | | **Nucleotide sequence similarities (%) between the S-segments** | | | | | | | | |  | **phi6** | **phi8** | **phi12** | **phi13** | **phi2954** | **phiNN** | **phiYY** | | **phi6** | 100 | 38.41 | 34.74 | 40.48 | 37.39 | 83.42 | 40.79 | | **phi8** | 38.41 | 100 | 33.3 | 37.13 | 34.02 | 37.7 | 37.11 | | **phi12** | 34.74 | 33.3 | 100 | 33.22 | 46.43 | 33.48 | 38.57 | | **phi13** | 40.48 | 37.13 | 33.22 | 100 | 34.45 | 40.23 | 37.1 | | **phi2954** | 37.39 | 34.02 | 46.43 | 34.45 | 100 | 36.41 | 41.64 | | **phiNN** | 83.42 | 37.7 | 33.48 | 40.23 | 36.41 | 100 | 40.18 | | **phiYY** | 40.79 | 37.11 | 38.57 | 37.1 | 41.64 | 40.18 | 100 |     **Fig. 1**. Genome maps of the segments S (A), M (B) and L (C) of the proposed members of the *Cystoviridae*. Open reading frames (ORFs) of the predicted positive strands are depicted and amino acid sequence similarities (%) between corresponding ORFs are indicated. Comparisons were conducted with EMBOSS Needle Pairwise Sequence Alignment. The order of the genome segments follows the clustering in the phylogenetic trees presented in Fig. 2.  A B    C    **Fig. 2.** Phylogenetic trees showing relationships between proposed members of the *Cystoviridae* based on nucleotide sequence comparisons of the segments S (A), M (B) and L (C),constructed using Clustal Omega Multiple Alignment**.**  A B    **Fig. 3.** Phylogenetic trees showing relationships between proposed members of the *Cystoviridae* based on nucleotide sequence comparisons of the segments S (A), M (B) and L (C),constructed using “One Click” at Phylogeny.fr. | | | | | | |