



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:	2016.011a-dB	(to be completed by ICTV officers)			
Short title: To create three (3) new species in one (1) new genus, <i>Demosthenesvirus</i> , within the family <i>Siphoviridae</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 11 are required)	1 <input checked="" type="checkbox"/> 6 <input type="checkbox"/>	2 <input checked="" type="checkbox"/> 7 <input type="checkbox"/>	3 <input checked="" type="checkbox"/> 8 <input type="checkbox"/>	4 <input type="checkbox"/> 9 <input type="checkbox"/>	5 <input type="checkbox"/> 10 <input checked="" type="checkbox"/>

Author(s):

Evelien M. Adriaenssens—University of Pretoria (South Africa)
Jens H. Kuhn—NIH/NIAID/IRF-Frederick, Maryland (USA)
Andrew M. Kropinski—University of Guelph (Canada)

Corresponding author with e-mail address:

Andrew M. Kropinski Phage.Canada@gmail.com

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)

ICTV Bacterial and Archaeal Viruses
Subcommittee

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV:

June 2016

Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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	2016.011aB	(assigned by ICTV officers)	
To create 3 new species within:			
Genus:	<i>Demosthenesvirus</i>	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.	
Subfamily:			
Family:	<i>Siphoviridae</i>		
Order:	<i>Caudovirales</i>		
Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)	
<i>Gordonia virus Demosthenes</i>	Gordonia phage Demosthenes	KU998242.1	
<i>Gordonia virus Kvothe</i>	Gordonia phage Kvothe	KU998243.1	
<i>Gordonia virus Katyusha</i>	Gordonia phage Katyusha	KU963258.1	

Reasons to justify the creation and assignment of the new species: <ul style="list-style-type: none"> Explain how the proposed species differ(s) from all existing species. <ul style="list-style-type: none"> 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 11
<p>We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. The members of each of the proposed species differ from those of other species by more than 5% at the DNA level as confirmed with the BLASTN algorithm.</p>

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	2016.011bB	(assigned by ICTV officers)
To create a new genus within:		
Subfamily:		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
Family:	<i>Siphoviridae</i>	
Order:	<i>Caudovirales</i>	

naming a new genus

Code	2016.011cB	(assigned by ICTV officers)
To name the new genus: <i>Demosthenesvirus</i>		

Assigning the type species and other species to a new genus

Code	2016.011dB	(assigned by ICTV officers)
To designate the following as the type species of the new genus		
<i>Gordonia virus Demosthenes</i>		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:		
3		

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 11

These phages were isolated as part of the Phage Hunters Integrating Research and Education or Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science programs using *Gordonia terrae* 3612 as the host bacterium. These lytic phages were assigned to the DA Cluster based upon DNA sequence similarity.

BLASTN (Fig. 1), CoreGenes (Table 1) [2], phylogenetic analyses (Fig. 2) [3], and progressiveMauve analysis (Fig. 3) all indicate that the proposed genus, *Demosthenesvirus*, is cohesive and distinct from the other genera of viruses. On average, the genomes of members of this genus are 74.6 kb in length (59.4 mol% G+C), and encode 97 proteins and 0 tRNAs. The genomes possess 1172 bp direct terminal repeats.

Origin of the new genus name:

Named after first sequenced member of this genus, *Gordonia virus Demosthenes*.

Reasons to justify the choice of type species:

This was the first sequenced member of this group of viruses.

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.

We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. The members of each of the proposed species differ from those of other species by more than 5% at the DNA level as confirmed with the BLASTN algorithm.

additional material in support of this proposal

References:

1. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010; 5(6):e11147.
2. Turner D, Reynolds D, Seto D, Mahadevan P. CoreGenes3.5: a webserver for the determination of core genes from sets of viral and small bacterial genomes. BMC Res Notes. 2013; 6:140. doi: 10.1186/1756-0500-6-140.
3. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008; 36(Web Server issue):W465-9.
4. Agren J, Sundström A, Håfström T, Segerman B. Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. PLoS One. 2012;7(6):e39107.

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.

Fig. 1. Electron micrograph of negatively stained *Gordonia* phage Kvothe (<http://phagesdb.org/phages/Kvothe/>) - Limited permission was granted by The Actinobacteriophages Database, funded by the Howard Hughes Medical Institute, to use this electron micrograph for this taxonomy proposal; it cannot be reused without permission of The Actinobacteriophages Database.

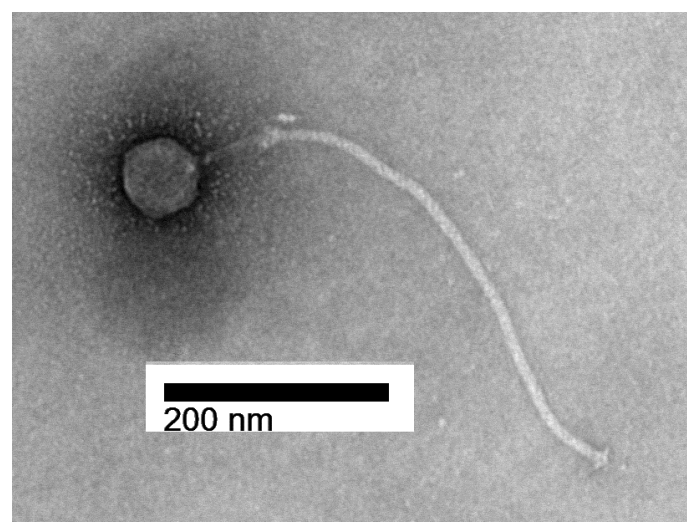


Table 1. Properties of the three phages belonging to the genus *Demosthenesvirus*

Gordonia phage	GenBank Accession No.	Terminal repeats	Genome length (kb)	Genome (mol% G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	Protein (% sequence identity)**
Demosthenes	KU998242.1	1172 bp TR	74.07	59.3	95	0	100	100
Kvothe	KU998243.1	1172 bp TR	74.46	59.5	99	0	86	95.8
Katyusha	KU963258.1	1172 bp TR	75.38	59.5	98	0	86	95.8

* Determined using BLASTN; ** Determined using CoreGenes [2]; Gordonia phage Benczkowski14 (KU963262.1) should be considered a strain of Gordonia phage Katyusha within this genus.

Fig. 1. Gegenees BLASTN (Top) and TBLASTX (Bottom) analysis in accurate mode (fragment size: 200 bp; step size: 100 bp) of bacteriophages which possess genomes > 200kb. The phages boxed in black on the left diagram should be considered as strains within the same species.

Fig. 2. Phylogenetic analysis of the (A) major capsid protein, and (B) large subunit terminase proteins of *Gordonia* and related phages constructed using “one click” at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline is already set up to run and connect programs recognized for their accuracy and speed (MUSCLE for multiple alignment and PhyML for phylogeny) to reconstruct a robust phylogenetic tree from a set of sequences." It also includes the use of Gblocks to eliminate poorly aligned positions and divergent regions. "The usual bootstrapping procedure is replaced by a new confidence index that is much faster to compute. See: Anisimova M., Gascuel O. Approximate likelihood ratio test for branches: A fast, accurate and powerful alternative (Syst Biol. 2006;55(4):539-52.) for details. **Red** = *Demosthenesvirus*.

A. Major capsid protein

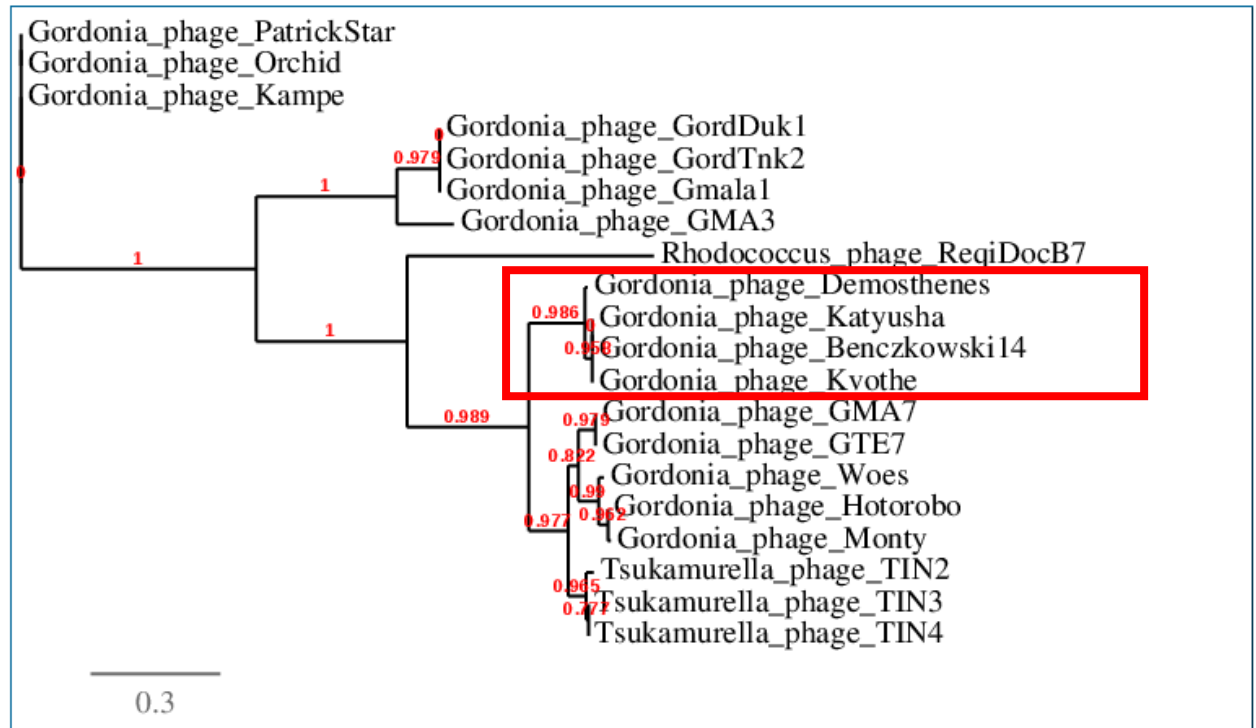


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

B. TerL protein

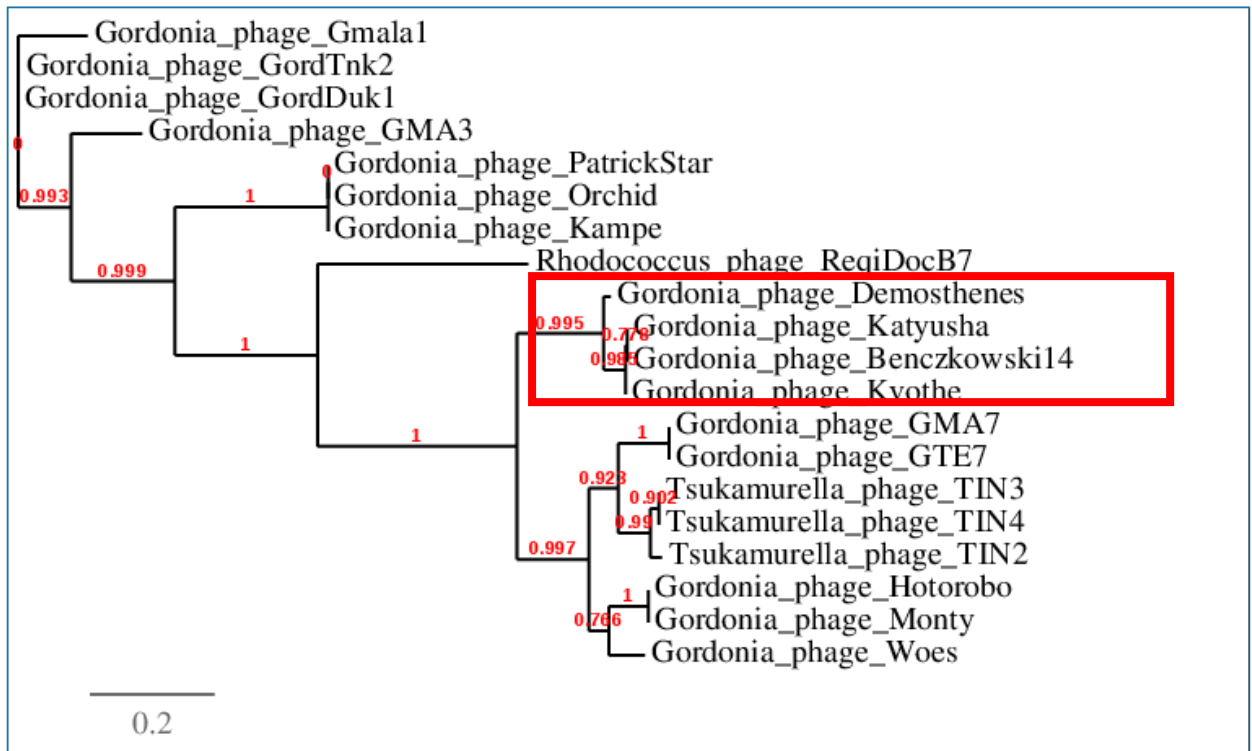


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

Fig. 3. progressiveMauve alignment [1] of the annotated genomes of members of the *Demosthenesvirus* genus – from top to bottom: Demosthenes, Katyusha, and Kvothe. Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within the colored blocks with the height of the plot proportional to the average nucleotide identity (Aaron Darling, personal communication).

