MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	***2015.002a-gF***	(to be completed by ICTV officers)

Short title: Create one genus and one family for classification of Sclerotinia sclerotiorum hypovirulence associated DNA virus 1.
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)	1 ☒ 2 ☒ 3 ☒ 4 ☐ 5 ☒ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☒

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

Date first submitted to ICTV:	June 11, 2015
Date of this revision (if different to above):	July 30, 2015

ICTV-EC comments and response of the proposer:

Fungal and Protist Viruses Subcommittee Chair: June 11 proposal approved for submission. Question whether engagement with Geminiviridae SG might be warranted. Fungal and Protist Viruses Subcommittee Chair: July 30 revision approved for submission. Suggestions from EC47 have been adequately addressed.

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	*2015.002aF*		(assigned by ICTV officers)

To create 1 new species within:

Genus:	*Gemycircularvirus* **(new)**	Fill in all that apply.
Subfamily:		• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Family:	*Genomoviridae* **(new)**	• If no genus is specified, enter "**unassigned**" in the genus box.
Order:		

Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
Sclerotinia gemycircularvirus 1	Sclerotinia sclerotiorum hypovirulence associated DNA virus 1 (SsHADV-1)	GQ365709

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

Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1) is the first — and thus far the only — single stranded DNA virus known to infect fungi (all other fungal viruses contain RNA genomes) [7, 18]. The virus was isolated from a plant-pathogenic fungus *Sclerotinia sclerotiorum* [19]. Another unique property of SsHADV-1, not described for other fungal viruses, is its ability to establish infection when applied extracellularly in the form of purified viral particles [20]. Virions can infect the hyphae of virus-free *S. sclerotiorum* directly when applied to hyphae or sprayed on leaves of *Arabidopsis thaliana* and *Brassica napus* infected with *S. sclerotiorum*. When applied to *S. sclerotiorum* infected leaves, the virus can suppress the development of *S. sclerotiorum* induced lesions. SsHADV-1 has a narrow host range and beside *S. sclerotiorum* can infect related species *S. minor* and *S. nivalis*, but not other relatively closely related fungi, such as *Botrytis cinerea* [20]. Furthermore, SsHADV-1 has been identified in New Zealand and USA in environmental samples and insects, however, this is not surprising given the near global distribution of *S. sclerotiorum* [4, 8].

SsHADV-1 virions are non-enveloped, isometric, 20-22 nm in diameter and constructed from one capsid protein [19]. The genome is circular ssDNA molecule of 2,166 nucleotides and contains two genes – for capsid and replication initiation proteins (Module 10; Figure 1). The prediction of the capsid protein gene has been validated by N-terminal sequencing of the capsid protein purified from the virions. The large intergenic region contains a potential stem-loop structure with a nonanucleotide TAATATTAT motif at its apex, which is likely to be important for rolling-circle replication. The capsid protein of SsHADV-1 is not recognizably similar to the corresponding proteins from viruses in other taxa.

MODULE 3: **NEW GENUS**

Code	*2015.002bF*	(assigned by ICTV officers)

To create a new genus within:

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

Code	*2015.002cF*	(assigned by ICTV officers)

To name the new genus: *Gemycircularvirus*

Code	*2015.002dF*	(assigned by ICTV officers)

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

Sclerotinia gemycircularvirus 1	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

Although SsHADV-1 remains the only isolated member of the group, genomes of more than 50 SsHADV-1-like putative viruses have been reported (see Module 10; Table 1). These genomes have been sequenced from different environmental samples and many were isolated from fecal matter of various animals [2-5, 9-12, 14-17]. The hosts of these putative viruses remain unknown, however. Although metagenomic virus sequences without isolates are currently not classified by the ICTV, their diversity suggests that SsHADV-1-like viruses are abundant and widespread in the environment.

All putative SsHADV-1-like viruses encode homologous Rep and capsid proteins, and in phylogenetic analyses form monophyletic clades with SsHADV-1 (Module 10; Figures 2 and 3). Their genomes are of similar size, in the range of 2,089-2,290 nucleotides. Structural and genomic features of SsHADV-1 differ considerably from those of all other currently classified viruses. Particularly, the capsid protein is not recognizably similar to the corresponding proteins from other viruses, whereas the Rep protein in phylogenetic analyses forms a monophyletic clade, which is distinct from the Rep proteins of other ssDNA viruses (see a more detailed justification in Module 5). Therefore, classification of SsHADV-1 (and related putative viruses) calls for creation of a new genus.

Origin of the new genus name:

Gemycircularvirus stands for <u>Ge</u>mini-like <u>my</u>co-infecting <u>circular virus</u>. The genus name has been proposed in [15] and is already widely used in the literature.

Reasons to justify the choice of type species:

Sclerotinia sclerotiorum hypovirulence associated DNA virus 1 is the first isolated representative of the proposed 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 applicable; the proposed genus includes a single species.

creating and naming a new family

Code	*2015.002eF*	(assigned by ICTV officers)

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

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	*2015.002fF*	(assigned by ICTV officers)

To name the new family: *Genomoviridae*

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	*2015.002gF*	(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

Gemycircularvirus **(new)**

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):**

0

Reasons to justify the creation of the new family:
Additional material in support of this proposal may be presented in the Appendix, Module 9

The replication-initiation protein (Rep) of SsHADV-1 is most closely related to the corresponding proteins of members of the family *Geminiviridae*. The Rep shares with geminiviral proteins two conserved domains, namely geminivirus Rep catalytic domain (Gemini_AL1; PF00799) and geminivirus Rep protein central domain (Gemini_AL1_M; PF08283) with conserved motifs for rolling-circle replication. However, Rep-based phylogenetic analysis shows that SsHADV-1 and other related putative viruses form a well-supported, monophyletic clade, which branches as a sister group to geminiviruses (Module 10; Figure 2). Unlike the Rep protein, the capsid protein of SsHADV-1 (and related viruses) does not display any recognizable sequence similarity to proteins of geminiviruses or any other group of known viruses. Furthermore, all geminiviruses possess distinctive geminate virions constructed from two incomplete T=1 icosahedra [1, 21], whereas the virion of SsHADV-1 is

isometric [19]. Finally, the number of genes and size of the genome differ considerably between SsHADV-1-like viruses and geminiviruses. In particular, all putative SsHADV-1-like viruses lack the movement protein which is essential for the plant geminiviruses.

Origin of the new family name:

Ge- for geminivirus-like, *nomo-* no movement protein

additional material in support of this proposal

References:

1. Böttcher B, Unseld S, Ceulemans H, Russell RB, Jeske H (2004) Geminate structures of African cassava mosaic virus. J Virol 78:6758-6765
2. Conceicao-Neto N, Zeller M, Heylen E, Lefrere H, Mesquita JR, Matthijnssens J (2015) Fecal virome analysis of three carnivores reveals a novel nodavirus and multiple gemycircularviruses. Virol J 12:79
3. Dayaram A, Opong A, Jaschke A, Hadfield J, Baschiera M, Dobson RC, Offei SK, Shepherd DN, Martin DP, Varsani A (2012) Molecular characterisation of a novel cassava associated circular ssDNA virus. Virus Res 166:130-135
4. Dayaram A, Potter KA, Pailes R, Marinov M, Rosenstein DD, Varsani A (2015) Identification of diverse circular single-stranded DNA viruses in adult dragonflies and damselflies (Insecta: Odonata) of Arizona and Oklahoma, USA. Infect Genet Evol 30:278-287
5. Du Z, Tang Y, Zhang S, She X, Lan G, Varsani A, He Z (2014) Identification and molecular characterization of a single-stranded circular DNA virus with similarities to Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1. Arch Virol 159:1527-1531
6. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307-321
7. Jiang D, Fu Y, Guoqing L, Ghabrial SA (2013) Viruses of the plant pathogenic fungus Sclerotinia sclerotiorum. Adv Virus Res 86:215-248
8. Kraberger S, Stainton D, Dayaram A, Zawar-Reza P, Gomez C, Harding JS, Varsani A (2013) Discovery of Sclerotinia sclerotiorum Hypovirulence-Associated Virus-1 in Urban River Sediments of Heathcote and Styx Rivers in Christchurch City, New Zealand. Genome Announc 1
9. Kraberger S, Arguello-Astorga GR, Greenfield LG, Galilee C, Law D, Martin DP, Varsani A (2015) Characterisation of a diverse range of circular replication-associated protein encoding DNA viruses recovered from a sewage treatment oxidation pond. Infect Genet Evol 31:73-86
10. Kraberger S, Farkas K, Bernardo P, Booker C, Arguello-Astorga GR, Mesleard F, Martin DP, Roumagnac P, Varsani A (2015) Identification of novel Bromus- and Trifolium-associated circular DNA viruses. Arch Virol 160:1303-1311
11. Lamberto I, Gunst K, Muller H, Zur Hausen H, de Villiers EM (2014) Mycovirus-like DNA virus sequences from cattle serum and human brain and serum samples from multiple sclerosis patients. Genome Announc 2
12. Ng TF, Chen LF, Zhou Y, Shapiro B, Stiller M, Heintzman PD, Varsani A, Kondov NO, Wong W, Deng X, Andrews TD, Moorman BJ, Meulendyk T, MacKay G, Gilbertson RL, Delwart E (2014) Preservation of viral genomes in 700-y-old caribou feces from a subarctic ice patch. Proc Natl Acad Sci U S A 111:16842-16847
13. Pei J, Grishin NV (2014) PROMALS3D: multiple protein sequence alignment enhanced with evolutionary and three-dimensional structural information. Methods Mol Biol 1079:263-271
14. Phan TG, Mori D, Deng X, Rajindrajith S, Ranawaka U, Fan Ng TF, Bucardo-Rivera F, Orlandi P, Ahmed K, Delwart E (2015) Small circular single stranded DNA viral genomes in unexplained cases of human encephalitis, diarrhea, and in untreated

References:

sewage. Virology 482:98-104

15. Rosario K, Dayaram A, Marinov M, Ware J, Kraberger S, Stainton D, Breitbart M, Varsani A (2012) Diverse circular ssDNA viruses discovered in dragonflies (Odonata: Epiprocta). J Gen Virol 93:2668-2681

16. Sikorski A, Massaro M, Kraberger S, Young LM, Smalley D, Martin DP, Varsani A (2013) Novel myco-like DNA viruses discovered in the faecal matter of various animals. Virus Res 177:209-216

17. van den Brand JM, van Leeuwen M, Schapendonk CM, Simon JH, Haagmans BL, Osterhaus AD, Smits SL (2012) Metagenomic analysis of the viral flora of pine marten and European badger feces. J Virol 86:2360-2365

18. Xie J, Jiang D (2014) New insights into mycoviruses and exploration for the biological control of crop fungal diseases. Annu Rev Phytopathol 52:45-68

19. Yu X, Li B, Fu Y, Jiang D, Ghabrial SA, Li G, Peng Y, Xie J, Cheng J, Huang J, Yi X (2010) A geminivirus-related DNA mycovirus that confers hypovirulence to a plant pathogenic fungus. Proc Natl Acad Sci U S A 107:8387-8392

20. Yu X, Li B, Fu Y, Xie J, Cheng J, Ghabrial SA, Li G, Yi X, Jiang D (2013) Extracellular transmission of a DNA mycovirus and its use as a natural fungicide. Proc Natl Acad Sci U S A 110:1452-1457

21. Zhang W, Olson NH, Baker TS, Faulkner L, Agbandje-McKenna M, Boulton MI, Davies JW, McKenna R (2001) Structure of the Maize streak virus geminate particle. Virology 279:471-477

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.

Table 1. Genome sequences of putative SsHADV-1-like viruses.

GenBank accession #	Virus genome name
KP263543	Badger feces-associated gemycircularvirus strain 588t
KM510192	Bromus-associated circular DNA virus 3 isolate
KJ938717	Caribou feces-associated gemycircularvirus
JQ412056	Cassava associated cicular DNA virus isolate G14
JX185430	Dragonfly-associated circular virus 1 isolate FL1-2X-2010
JX185429	Dragonfly-associated circular virus 2 isolate FL2-5X-2010
JX185428	Dragonfly-associated circular virus 3 isolate TO-DFS3B2-2010
KF371643	Faecal-associated gemycircularvirus 1a
KF371642	Faecal-associated gemycircularvirus 1b
KF371641	Faecal-associated gemycircularvirus 1c
KF371640	Faecal-associated gemycircularvirus 2
KF371639	Faecal-associated gemycircularvirus 3
KF371638	Faecal-associated gemycircularvirus 4

|	
KF371637	Faecal-associated gemycircularvirus 5
KF371636	Faecal-associated gemycircularvirus 6
KF371635	Faecal-associated gemycircularvirus 7
KF371634	Faecal-associated gemycircularvirus 8
KF371633	Faecal-associated gemycircularvirus 9
KF371632	Faecal-associated gemycircularvirus 10
KF371631	Faecal-associated gemycircularvirus 11
KP133075	Gemycircularvirus SL1
KP133076	Gemycircularvirus SL2
LK931483	HCBI8.215 virus
LK931484	HCBI9.212 virus
KJ413144	Human genital-associated circular DNA virus-1 isolate 349
KF413620	Hypericum japonicum associated circular DNA virus isolate VNHJ1W
JN704610	Meles meles fecal virus isolate VS4700006
KP263544	Mongoose feces-associated gemycircularvirus a strain 181a
KP263545	Mongoose feces-associated gemycircularvirus b strain 160b
KP263546	Mongoose feces-associated gemycircularvirus c strain 541c
KP263547	Mongoose feces-associated gemycircularvirus d strain 478d
HQ335086	Mosquito VEM virus SDBVL G
LK931485	MSSI2.225 virus
KM598385	Odonata associated gemycircularvirus-1 isolate OdaGmV-1-US-260BC-12
KM598386	Odonata associated gemycircularvirus-1 isolate OdaGmV-1-US-260SR1-12
KM598387	Odonata associated gemycircularvirus-2 isolate OdaGmV-2-US-1642KW-12
KM598388	Odonata associated gemycircularvirus-2 isolate OdaGmV-2-US-1634LM2-12
KM598389	Odonata-associated circular virus-6 isolate OdasCV-6-US-1642LM1-12
KJ547627	Sewage-associated circular DNA virus-3 isolate
KM821747	Sewage-associated gemycircularvirus-1 isolate
KJ547642	Sewage-associated gemycircularvirus-2 isolate BS3911
KJ547643	Sewage-associated gemycircularvirus-3 isolate BS4149
KJ547634	Sewage-associated gemycircularvirus-4 isolate BS3913
KJ547635	Sewage-associated gemycircularvirus-5 isolate BS3963
KJ547636	Sewage-associated gemycircularvirus-6 isolate BS4014
KJ547637	Sewage-associated gemycircularvirus-7a isolate BS3939
KJ547640	Sewage-associated gemycircularvirus-7b isolate BS3972
KJ547638	Sewage-associated gemycircularvirus-8 isolate BS3917
KJ547639	Sewage-associated gemycircularvirus-9 isolate BS3970
KJ547644	Sewage-associated gemycircularvirus-10a isolate BS3980
KJ547645	Sewage-associated gemycircularvirus-10b isolate BS3849
KJ547641	Sewage-associated gemycircularvirus-11 isolate BS4117
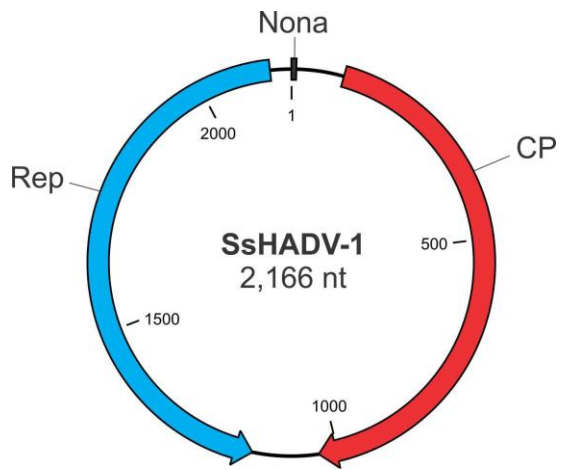
Figure 1. Genome map of SsHADV-1. Genes encoding the replication-initiation protein (Rep) and capsid protein (CP) are shown with blue and red arrows, respectively. The position of the nonanucleotide (Nona) important for rolling-circle replication is also indicated.
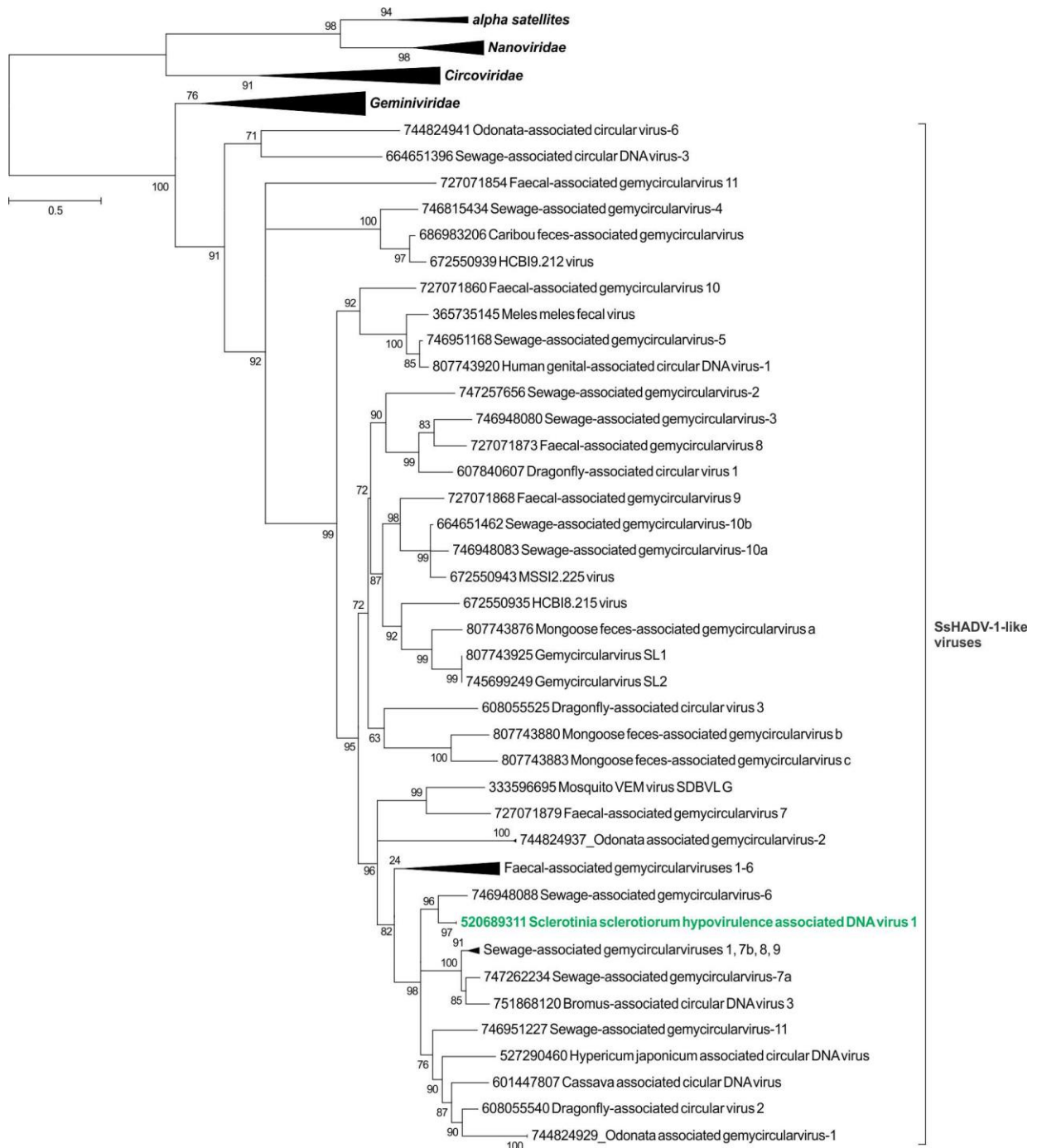
Figure 2. Phylogenetic analysis of the replication proteins of ssDNA viruses. The type species of the proposed genus *Gemycircularvirus* within the family *Genomoviridae* is highlighted in green. For phylogenetic analysis, protein sequences were aligned using PROMALS3D [13] and columns containing gaps were removed from the alignment. Maximum-likelihood phylogenetic analysis was carried out using PhyML 3.1 [6], with the Whelan and Goldman (WAG) model of amino acid substitutions, including a gamma law with four substitution rate categories. Numbers at the branch points represent SH (Shimodaira–Hasegawa)-like local support values. The scale bar represents the number of substitutions per site. For clarity, the tree was mid-point rooted. All taxa are indicated with the corresponding GenBank identifiers (GIs).
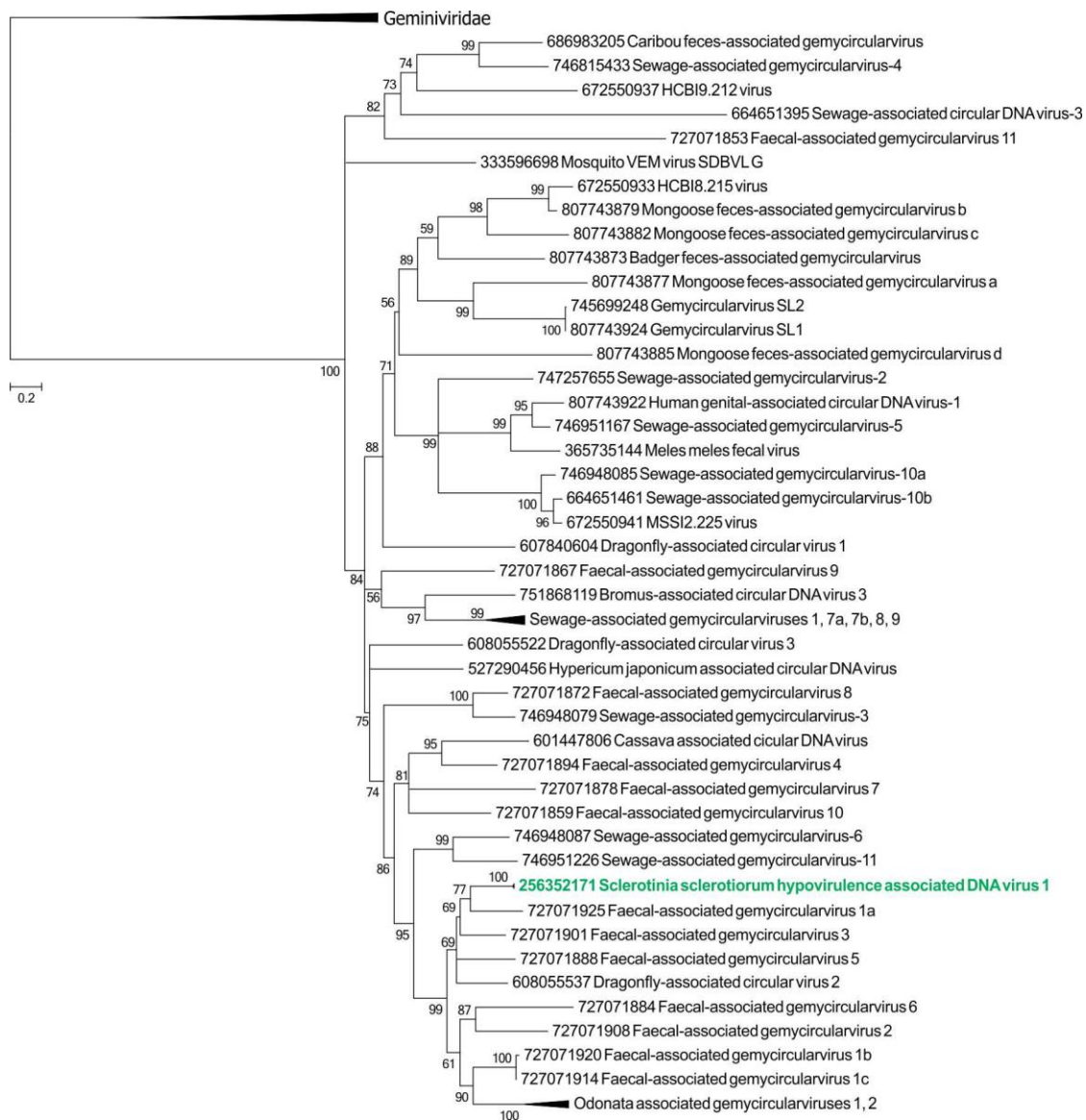
Figure 3. Phylogenetic analysis of the SsHADV-1-like capsid proteins. The type species of the proposed genus *Gemycircularvirus* within the family *Genomoviridae* is highlighted in green. For phylogenetic analysis, protein sequences were aligned using PROMALS3D [13] and columns containing gaps were removed from the alignment. Maximum-likelihood phylogenetic analysis was carried out using PhyML 3.1 [6], with the Whelan and Goldman (WAG) model of amino acid substitutions, including a gamma law with four substitution rate categories. Numbers at the branch points represent SH (Shimodaira–Hasegawa)-like local support values. The scale bar represents the number of substitutions per site. The tree was rooted with the capsid proteins of geminiviruses. All taxa are indicated with the corresponding GenBank identifiers (GIs).