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:	***2014.006b-eD***	(to be completed by ICTV officers)

Short title: Establish the genus *Cyclovirus* in the family *Circoviridae*; creating 28 new species in the family
(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** ☒

Author(s):

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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)	Circoviridae Study Group

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

Date first submitted to ICTV:	June 15, 2015
Date of this revision (if different to above):	June 29, 2015

ICTV-EC comments and response of the proposer:

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

Code	***2014.006bD***	(assigned by ICTV officers)

To create 28 new species within:

Genus:	***Cyclovirus* (new)**
Subfamily:	
Family:	***Circoviridae***
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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Bat cyclovirus	bat cyclovirus (BaCyV)	HQ738637
Bat faeces associated cyclovirus 1	bat faeces associated cyclovirus 1 (BaFCyV-1)	HM228874
Bat faeces associated cyclovirus 2	bat faeces associated cyclovirus 2 (BaFCyV-2)	JF938079
Bat faeces associated cyclovirus 3	bat faeces associated cyclovirus 3 (BaFCyV-3)	JF938081
Bat faeces associated cyclovirus 4	bat faeces associated cyclovirus 4 (BaFCyV-4)	JF938082
Bovine cyclovirus	bovine cyclovirus (BoCyV)	HQ738634
Chimpanzee faeces associated cyclovirus	chimpanzee faeces associated cyclovirus (ChmFCyV)	GQ404849
Dragonfly cyclovirus 1	dragonfly cyclovirus 1 (DfCyV-1)	JX185419
Dragonfly cyclovirus 2	dragonfly cyclovirus 2 (DfCyV-2)	JX185422
Dragonfly cyclovirus 3	dragonfly cyclovirus 3 (DfCyV-3)	JX185424
Dragonfly cyclovirus 4	dragonfly cyclovirus 4 (DfCyV-4)	JF938080
Dragonfly cyclovirus 5	dragonfly cyclovirus 5 (DfCyV-5)	JX185426
Dragonfly cyclovirus 6	dragonfly cyclovirus 6 (DfCyV-6)	KC512918
Dragonfly cyclovirus 7	dragonfly cyclovirus 7 (DfCyV-7)	KC512919
Dragonfly cyclovirus 8	dragonfly cyclovirus 8 (DfCyV-8)	KC512920
Florida wood cockroach cyclovirus	Florida wood cockroach cyclovirus (FWCasCyV)	JX569794
Gallus cyclovirus	gallus cyclovirus (GaCyV)	HQ738643
Goat cyclovirus	goat cyclovirus (GoCyV)	HQ738636
Human cyclovirus 1	human cyclovirus 1 (HuCyV-1)	KF031466
Human cyclovirus 2	human cyclovirus 2 (HuCyV-2)	KC771281
Human cyclovirus 3	human cyclovirus 3 (HuCyV-3)	KF726984
Human faeces associated cyclovirus 1	human faeces associated cyclovirus 1 (HuFCyV-1)	GQ404847
Human faeces associated cyclovirus 2	human faeces associated cyclovirus 2 (HuFCyV-2)	GQ404844
Human faeces associated cyclovirus 3	human faeces associated cyclovirus 3 (HuFCyV-3)	GQ404846
Human faeces associated cyclovirus 4	human faeces associated cyclovirus 4 (HuFCyV-4)	GQ404858
Human faeces associated cyclovirus 5	human faeces associated cyclovirus 5 (HuFCyV-5)	GQ404845
Human faeces associated cyclovirus 6	human faeces associated cyclovirus 6 (HuFCyV-6)	GQ404854
Human faeces associated cyclovirus 8	human faeces associated cyclovirus 8 (HuFCyV-8)	GQ404855

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

We have analyzed all the full genomes of cycloviruses available in public databases using SDT v1.2 (Muhire et al., 2014). Our analysis (Figures 1-3) shows that 80% pairwise identity species cut off is best suited for cycloviruses (a similar one is proposed for circoviruses) and maintains a uniform classification in the family *Circoviridae*. Hence viruses with <80% pairwise identities coupled with phylogenetic support (Figures 2) should be considered as new species.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2014.006cD***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Circoviridae***
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	***2014.006dD***	(assigned by ICTV officers)

To name the new genus: *Cyclovirus*

Assigning the type species and other species to a new genus

Code	***2014.006eD***	(assigned by ICTV officers)

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

Human cyclovirus 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:**

28

Reasons to justify the creation of a new genus:

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

The genus *Cyclovirus* will accommodate newly discovered viruses whose replication associated proteins (Rep) are most closely related to those of circoviruses (Figures 4-6); yet they have distinct genome organization and characteristics (Rosario et al. 2012b).

Both circoviruses and cycloviruses have ambisense genomes and phylogenetically similar Reps; however, phylogenetic analyses show that circoviruses and cycloviruses form distinct clades and there are consistent differences in the length of the intergenic region located at the 3’ end of the major ORFs (Figure 5). Also, in contrast to circoviruses, the Rep of cycloviruses is not encoded on the same strand as the canonical nonanucleotide motif.

Origin of the new genus name:

"Cyclo” is derived from Latin “cyclus” and relates to the circular nature of the DNA genome

Reasons to justify the choice of type species:

Isolated from human cerebrospinal fluid of patients with acute central nervous system infections

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.

Analysis of the percentage pairwise identity of the genome sequences (one minus Hamming distances of pairwise aligned sequences with pairwise deletion of gaps, Figure 1) revealed a gap located at approximately 76-85%. We have used a similar approach to the one used for geminiviruses, in particular the *Mastrevirus*, *Curtovirus*, *Becurtovirus* and *Turncurtovirus* genera (Muhire et al. 2012; Varsani et al. 2014a; 2014b). Based on genome-wide pairwise identities isolates that share <80% genome wide pairwise nucleotide identity coupled with phylogenetic support (Figure 2) are considered distinct species in the genus *Cyclovirus*. We note that this threshold holds true for pairwise comparisons of either the capsid protein gene sequences or the replication associated protein gene sequences. However, only full genomes of cycloviruses will be considered for assignment of new species in the event that share <80% pairwise identity to any classified cyclovirus.

MODULE 10: **APPENDIX**: supporting material

Table 1: Details of identified cycloviruses

Species	Acronym	GenBank Accession #	Isolation source	Reference
bat cyclovirus	BaCyV	HQ738637	bat muscle	Li et al., 2011
bat faeces associated cyclovirus 1	BaFCyV-1	HM228874	bat faeces	Li et al., 2010
bat faeces associated cyclovirus 2	BaFCyV-2	JF938079	bat faeces	Li et al., 2010
bat faeces associated cyclovirus 3	BaFCyV-3	JF938081	bat faeces	Li et al., 2010
bat faeces associated cyclovirus 4	BaFCyV-4	JF938082	bat faeces	Li et al., 2010
bovine cyclovirus	BoCyV	HQ738634	cow muscle	Li et al., 2011
bovine cyclovirus	BoCyV	HQ738635	goat muscle	Li et al., 2011
chimpanzee faeces associated cyclovirus	ChmFCyV	GQ404849	chimpanzee faeces	Li et al., 2010
chimpanzee faeces associated cyclovirus	ChmFCyV	GQ404850	chimpanzee faeces	Li et al., 2010
dragonfly cyclovirus 1	DfCyV-1	HQ638058	*Pantala flavescens*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	JX185420	*Pantala flavescens*	Rosario et al., 2012a
dragonfly cyclovirus 1	DfCyV-1	JX185421	*Pantala flavescens*	Rosario et al., 2012a
dragonfly cyclovirus 1	DfCyV-1	HQ638065	*Pantala flavescens*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638066	*Pantala flavescens*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638067	*Pantala flavescens*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638069	*Tholymis tillarga*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	JX185419	*Pantala flavescens*	Rosario et al., 2012a
dragonfly cyclovirus 1	DfCyV-1	HQ638068	*Diplacodes bipunctata*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638049	*Tholymis tillarga*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638061	*Diplacodes bipunctata*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638062	*Diplacodes bipunctata*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638063	*Diplacodes bipunctata*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638064	*Diplacodes bipunctata*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638053	*Diplacodes bipunctata*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638054	*Diplacodes bipunctata*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638050	*Tholymis tillarga*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638051	*Tholymis tillarga*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638052	*Pantala flavescens*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638055	*Pantala flavescens*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638059	*Pantala flavescens*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638056	*Pantala flavescens*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638060	*Diplacodes bipunctata*	Rosario et al., 2011
dragonfly cyclovirus 1	DfCyV-1	HQ638057	*Diplacodes bipunctata*	Rosario et al., 2011
dragonfly cyclovirus 2	DfCyV-2	JX185422	*Pantala flavescens*	Rosario et al., 2012a
dragonfly cyclovirus 2	DfCyV-2	JX185423	*Anax junius*	Rosario et al., 2012a
dragonfly cyclovirus 3	DfCyV-3	JX185424	*Erythemis simplicicollis*	Rosario et al., 2012a
dragonfly cyclovirus 4	DfCyV-4	JF938080	bat faeces	Ge et al., 2011
dragonfly cyclovirus 4	DfCyV-4	JN377566	bat faeces	Ge et al., 2011
dragonfly cyclovirus 4	DfCyV-4	JX185425	*Somatochlora meridionalis*	Rosario et al., 2012a
dragonfly cyclovirus 4	DfCyV-4	KC512916	*Rhionaeschna multicolor*	Dayaram et al., 2013
dragonfly cyclovirus 4	DfCyV-4	KC512917	*Rhionaeschna multicolor*	Dayaram et al., 2013
dragonfly cyclovirus 5	DfCyV-5	JX185426	*Erythrodiplax umbrata*	Rosario et al., 2012a
dragonfly cyclovirus 5	DfCyV-5	JX185427	*Erythrodiplax umbrata*	Rosario et al., 2012a
dragonfly cyclovirus 6	DfCyV-6	KC512918	*Rhionaeschna multicolor*	Dayaram et al., 2013
dragonfly cyclovirus 7	DfCyV-7	KC512919	*Xanthocnemis zealandica*	Dayaram et al., 2013
Dragonfly cyclovirus 8	DfCyV-8	KC512920	*Orthetrum sabina*	Dayaram et al., 2013
Florida wood cockroach cyclovirus	FWCasCyV	JX569794	*Eurycotis floridana*	Padilla Rodriguez et al., 2013
gallus cyclovirus	GaCyV	HQ738643	chicken muscle	Li et al., 2011
gallus cyclovirus	GaCyV	HQ738644	chicken muscle	Li et al., 2011
goat cyclovirus	GoCyV	HQ738636	goat	Li et al., 2011
human cyclovirus 1	HuCyV-1	KF031471	human	Tan Ve et al., 2013
human cyclovirus 1	HuCyV-1	KF031470	human	Tan Ve et al., 2013
human cyclovirus 1	HuCyV-1	KF031469	human	Tan Ve et al., 2013
human cyclovirus 1	HuCyV-1	KF031467	human	Tan Ve et al., 2013
human cyclovirus 1	HuCyV-1	KF031465	human	Tan Ve et al., 2013
human cyclovirus 1	HuCyV-1	KF031468	human	Tan Ve et al., 2013
human cyclovirus 1	HuCyV-1	KF031466	human	Tan Ve et al., 2013
human cyclovirus 2	HuCyV 2	KC771281	human	Smits et al., 2013
human cyclovirus 3	HuCyV 3	KF726984	human	Phan et al., 2014
human cyclovirus 3	HuCyV 3	KF726985	human	Phan et al., 2014
human cyclovirus 3	HuCyV 3	KF726987	human	Phan et al., 2014
human cyclovirus 3	HuCyV 3	KF726986	human	Phan et al., 2014
human faeces associated cyclovirus 1	HuFCyV-1	GQ404847	human faeces	Li et al., 2010
human faeces associated cyclovirus 2	HuFCyV-2	GQ404844	human faeces	Li et al., 2010
human faeces associated cyclovirus 3	HuFCyV-3	GQ404846	human faeces	Li et al., 2010
human faeces associated cyclovirus 3	HuFCyV-3	GQ404848	human faeces	Li et al., 2010
human faeces associated cyclovirus 4	HuFCyV-4	GQ404858	human faeces	Li et al., 2010
human faeces associated cyclovirus 4	HuFCyV-4	GQ404857	human faeces	Li et al., 2010
human faeces associated cyclovirus 5	HuFCyV-5	GQ404845	human faeces	Li et al., 2010
human faeces associated cyclovirus 6	HuFCyV-6	GQ404854	human faeces	Li et al., 2010
human faeces associated cyclovirus 8	HuFCyV-7	GQ404855	human faeces	Li et al., 2010

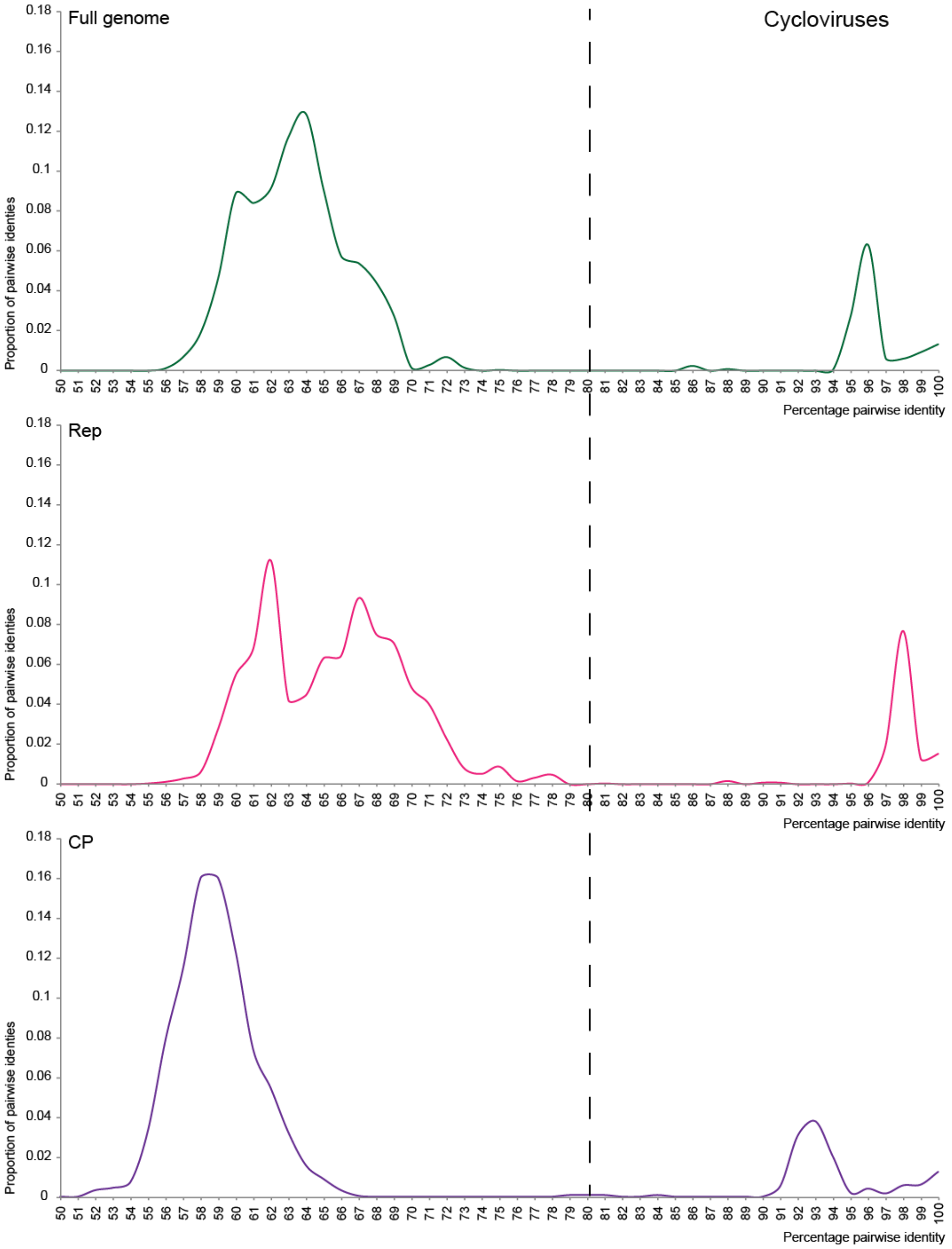

Figure 1: Distribution pairwise identity of cyclovirus genomes. The pairwise identities were calculated using SDT V1.2 (Muhire et al., 2014) with MUSCLE alignment algorithm (Edgar, 2004). Rep = replication associated protein gene; CP = capsid protein gene.

Within the cyclovirus genus we have assigned 28 species (Table 1) based on the 80% species threshold coupled with phylogenetic support (Figure 5). We have retained the original name of the virus sequence deposited by researchers where possible. We have also doubled checked to make sure that variants within a species share >80% pairwise identity (Figure 6).

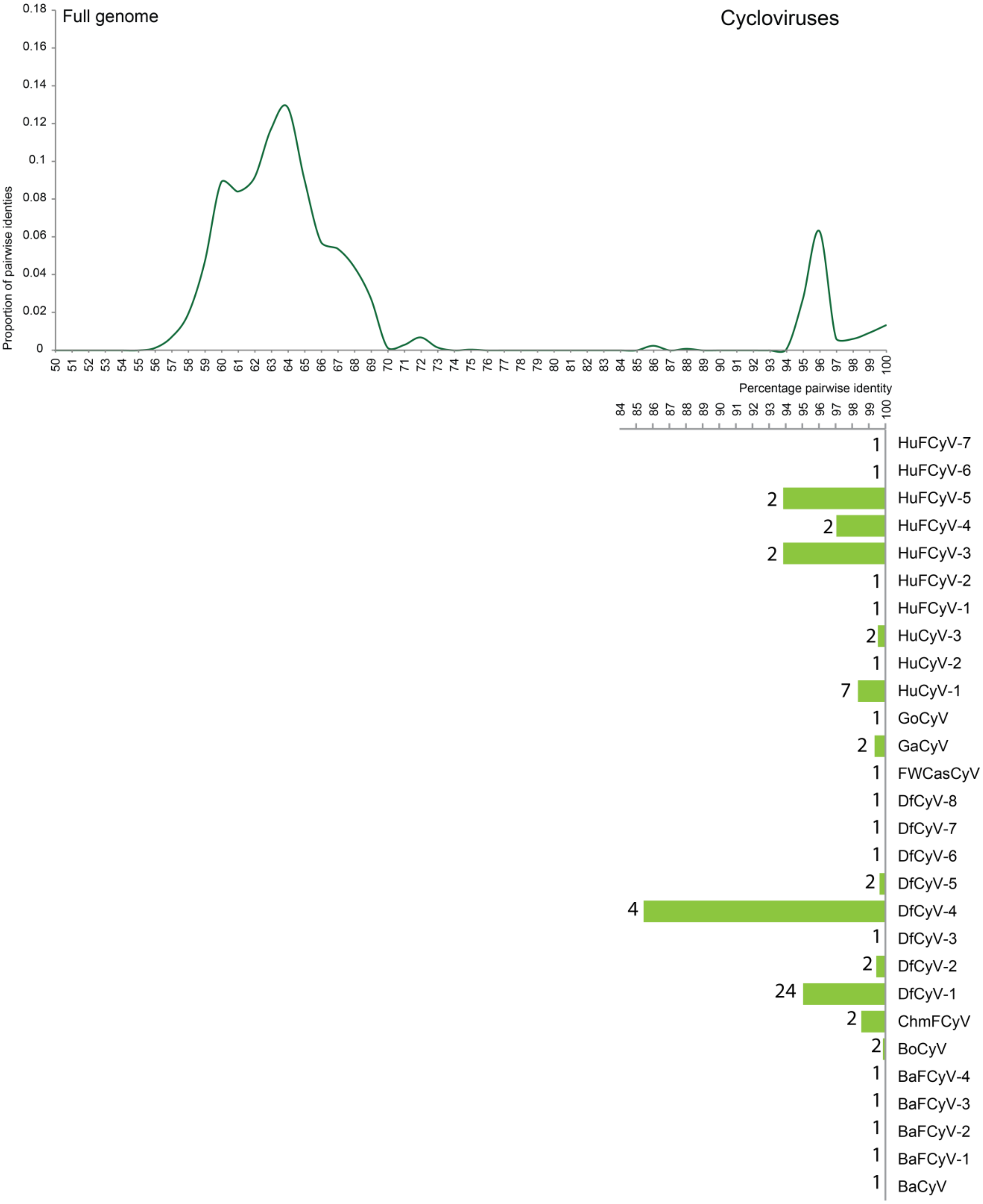

Figure 2: Diversity of variants within each assigned species in the genus cyclovirus. Numbers associated with bars refer to number of isolates within the species.

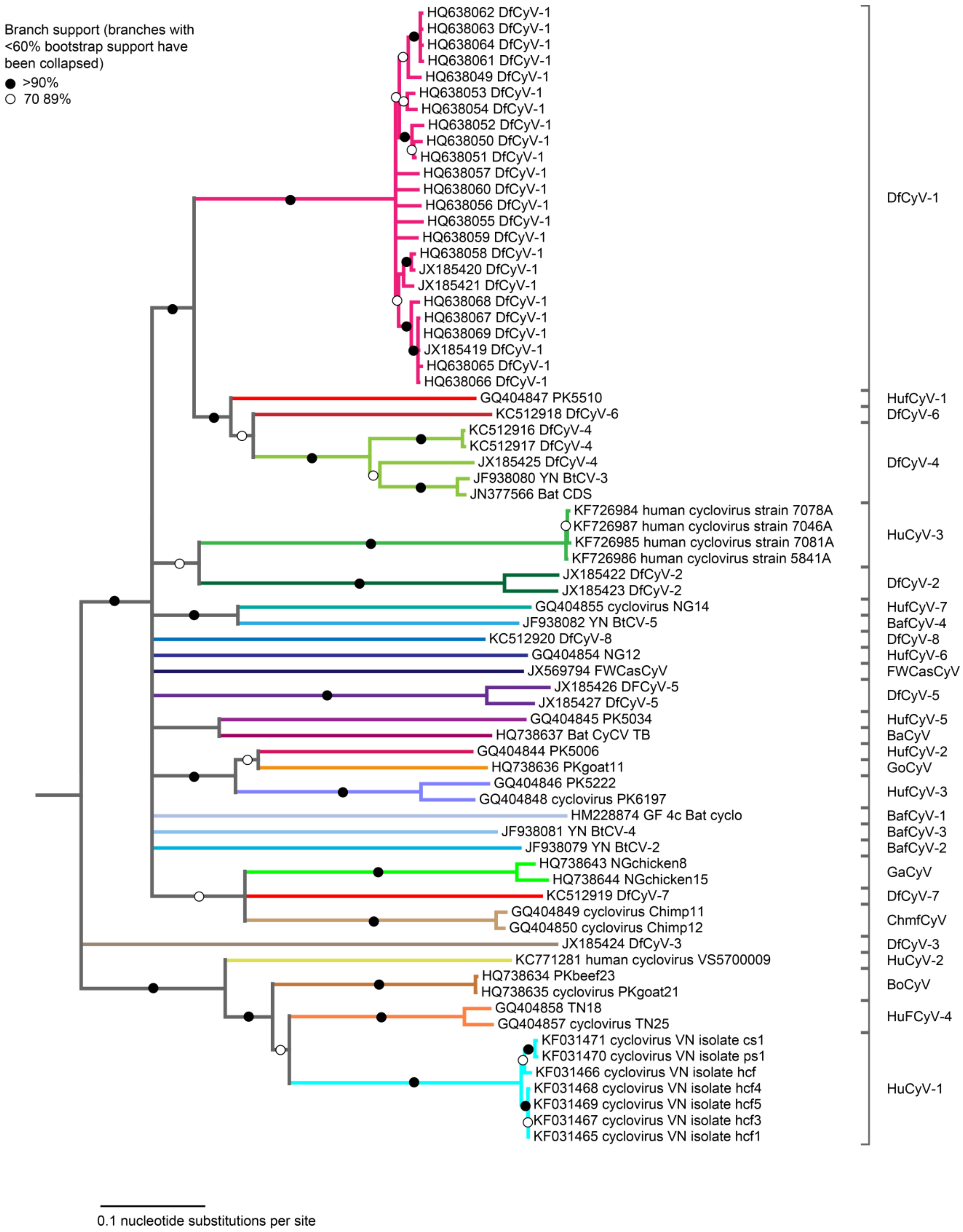

Figure 3: Neighbor-joining (with Jukes-Cantor model) phylogenetic tree (1000 bootstrap) inferred from full genome alignments. Branches with <60% support have been collapsed.

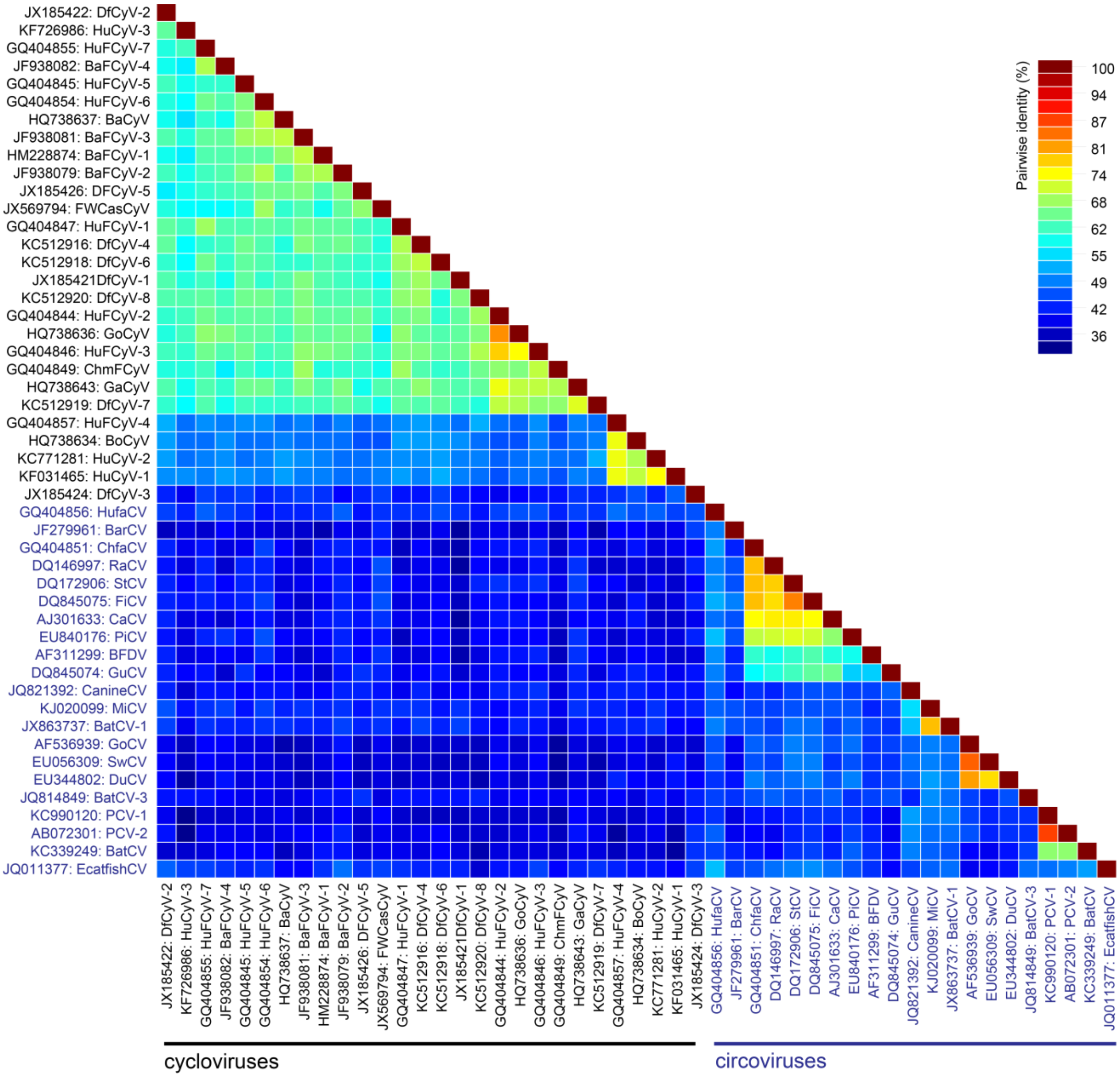

Figure 4: Two directional pairwise identity matrix of the replication associated protein amino acid sequences of representative circoviruses and cycloviruses.

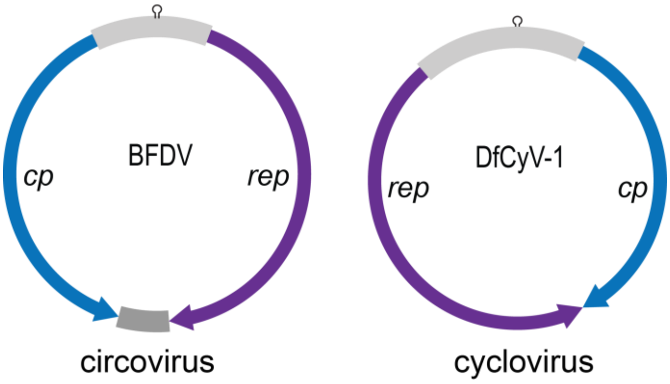

Figure 5: Genome organisation of circoviruses (e.g., beak and feather disease virus; BFDV) and cycloviruses (e.g., dragonfly cyclovirus 1; DfCyV-1). Replication-associated protein (*rep*) and capsid protein (cp) genes as well as intergenic regions (highlighted in grey) are shown.

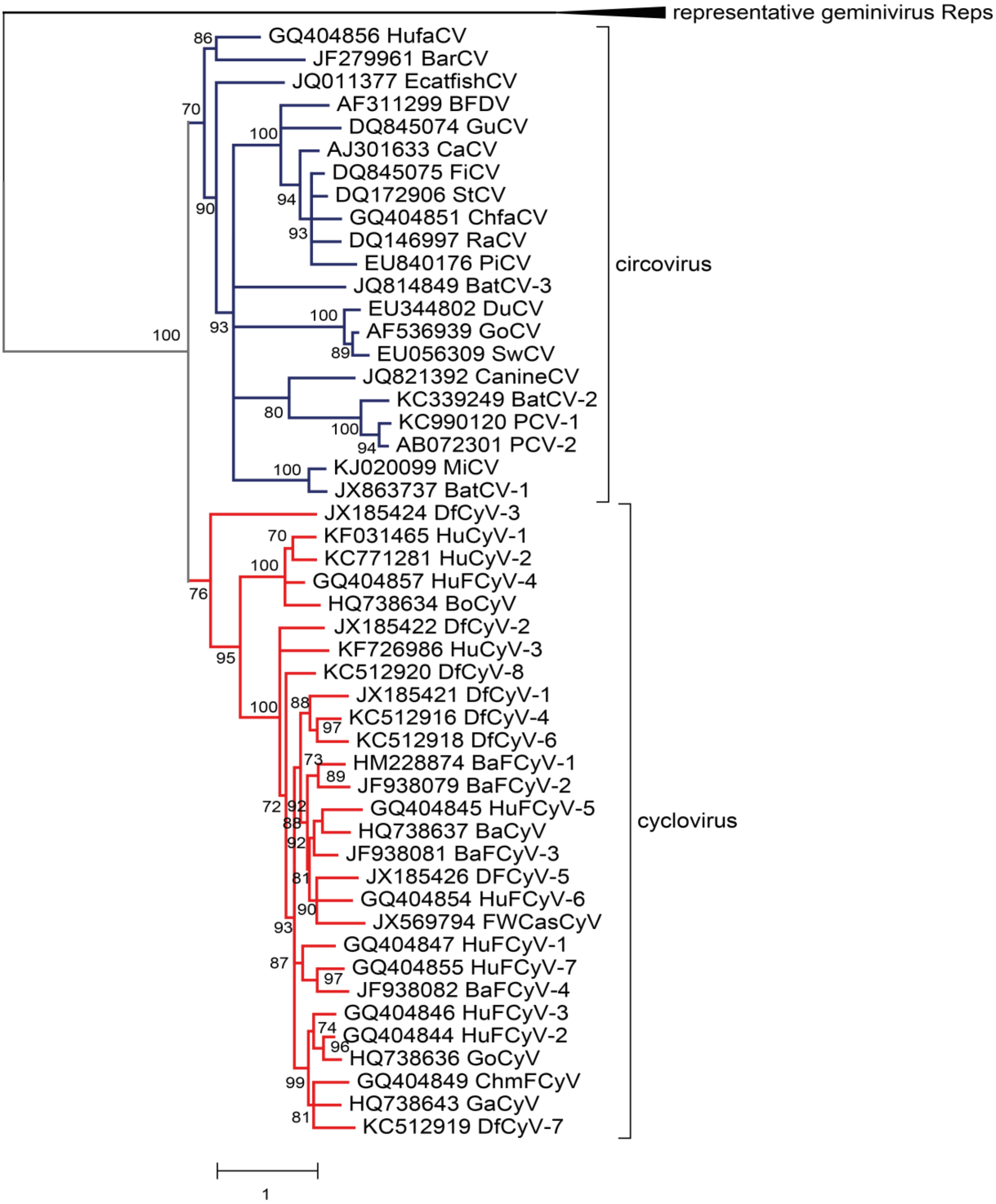

Figure 6: Rep amino acid maximum likelihood phylogenetic tree inferred using PHYML with aLRT branch support. Branches <70% support have been collapsed. The tree is rooted with Rep sequences of geminiviruses.

References

Biagini, P., Bendinelli, M., Hino, S., Kakkola, L., Mankertz, A., Niel, C., Okamoto, H., Raidal, S., Teo, C.G. & Todd, D. (2012). *Circoviridae*. In : Virus Taxonomy, IXth Report of the International Committee for the Taxonomy of Viruses (A.M.Q. King, M.J. Adams, E.B. Cartens, and E.J. Lefkowitz, eds), 343-349. Elsevier/Academic Press, London.

Dayaram, A., Potter, K.A., Moline, A.B., Rosenstein, D.D., Marinov, M., Thomas J.E., Britbart, M., Rosario, K., Arguello-Astorga, G.R. & Varsani, A. (2013). High global diversity of cycloviruses amongst dragonflies. Journal of General Virology 94, 1827-1840.

Delwart, E. & Linlin, L. (2012). Rapidly expanding genetic diversity and host range of the Circoviridae viral family and other Rep encoding small circular ssDNA genomes. Virus Res 164, 114-121.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32, 1792-1797.

Ge, X., Li, J., Peng, C., Wu, L., Yang, X., Wu, Y., Zhang, Y. & Shi, Z. (2011). Genetic diversity of novel circular ssDNA viruses in bats in China. Journal of General Virology 92, 2646-2653.

Kapoor, A., Dubovi, E. J., Henriquez-Rivera, J. A. & Lipkin, W. I. (2012). Complete genome sequence of the first canine circovirus. Journal of Virology 86, 7018-7018.

Li, L., Kapoor, A., Slikas, B., Bamidele, O. S., Wang, C., Shaukat, S., Masroor, M. A., Wilson, M. L., Ndjango, J. B. N. & Peeters, M. (2010). Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. Journal of Virology 84, 1674-1682.

Li, L., Shan, T., Soji, O. B., Alam, M. M., Kunz, T. H., Zaidi, S. Z. & Delwart, E. (2011). Possible cross-species transmission of circoviruses and cycloviruses among farm animals. Journal of General Virology 92, 768-772.

Muhire, B., Martin, D. P., Brown, J. K., Navas-Castillo, J., Moriones, E., Zerbini, F. M., Rivera-Bustamante, R., Malathi, V. G., Briddon, R. W. & Varsani, A. (2013). A genome-wide pairwise-identity-based proposal for the classification of viruses in the genus Mastrevirus (Geminiviridae). Archives of Virology. 158: 1411-1424

Muhire, B.M., Varsani, A. & Martin, D.P. (2014) SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One, 9, e108277.

Phan, T.G., Luchsinger, V., Avendano, L.F., Deng, X. & Delwart, E. (2014). Cyclovirus in nasopharyngeal aspirates of Chilean children with respiratory infections. Journal of General Virology 95, 922-927.

Rosario, K., Dayaram, A., Marinov, M., Ware, J., Kraberger, S., Stainton, D., Breitbart, M. & Varsani, A. (2012a). Diverse circular single-stranded DNA viruses discovered in dragonflies (Odonata: Epiprocta). Journal of General Virology 93, 2729-2739.

Rosario, K, Duffy S, & Breitbart M (2012b). A field guide to eukaryotic circular single-stranded DNA viruses: Insights gained from metagenomics. Archives of Virology. 157: 1851-1871.

Rosario, K., Marinov, M., Stainton, D., Kraberger, S., Wiltshire, E. J., Collings, D. A., Walters, M., Martin, D. P., Breitbart, M. & Varsani, A. (2011). Dragonfly cyclovirus, a novel single-stranded DNA virus discovered in dragonflies (Odonata: Anisoptera). Journal of General Virology 92, 1302-1308.

Rosario, K., Padilla-Rodriguez, M., Kraberger, S., Stainton, D., Martin, D. P., Breitbart, M. & Varsani, A. (2013). Discovery of a novel mastrevirus and alphasatellite-like circular DNA in dragonflies (Epiprocta) from Puerto Rico. Virus Research 171, 231-237.

Smits, S.L., Zijlstra, E.E., van Hellemond, J.J., Schapendonk, C.M., Bowedes, R., Schürch, A.C., Haagmans, B.L., Osterhaus, A.D. (2013). Novel Cyclovirus in human cerebrospinal fluid, Malawi, 2010-2011. Emerging Infectious Diseases 19, 1511-1513.

Tan le, V., van Doorn, H.R., Nghia, H.D., Chau, T.T., Tu le, T.P., de Vries, M., Canuti, M., Deijs, M., Jebbink, MF., Baker, S., Bryant, J.E., Tham, N.T., BKrong, N.T., Boni, M.F., Loi, T.Q., Phuong le, T., Verhoeven, J.T., Crusat, M., Jeeninga, R.E., Schultsz, C., Chau, N.V., Hien, T.T., van der Hoek, L., Farrar, J., & de Jong, M.D. (2013). Identification of a new Cyclovirus in cerebrospinal fluid of patients with acute central nervous system infections. MBio 4, e00231

Varsani, A., Navaz-Castillo, J., Moriones, E., Hernández-Zepeda, C., Idris, A., Brown, J.K., Zerbini, F.M., Martin, D.P. (2014) Establishment of three new genera in the family Geminiviridae: Becurtovirus, Eragrovirus and Turncurtovirus. Archives of Virology 159:2193-2203

Varsani, A., Martin, D.P., Navaz-Castillo, J., Moriones, E., Hernández-Zepeda, C., Idris, A., Zerbini, F.M., Brown, J.K. (2014) Revisiting the classification of curtoviruses based on genome-wide pairwise identity. Archives of Virology 159:1873–1882